Draft for Open Consultation

The Amphibian Conservation Action Plan (ACAP): A status review and roadmap for global amphibian conservation

Author: IUCN SSC Amphibian Specialist Group

Date: July 2022

Editors: Sally Wren, Amaël Borzée, Ruth Marcec-Greaves and Ariadne Angulo

Document information

This document is a preprint that has been prepared for public consultation, to allow broad input from the whole amphibian conservation community to this ACAP update. As such, this is not the final document.

You can find information on how to provide feedback on this document, including a downloadable feedback form, as well as a recommended citation, at: https://www.iucn-amphibians.org/resources/acap/

Contents

Executive summary

As the most threatened vertebrate class on earth, amphibians are at the forefront of the biodiversity crisis, with the start of global amphibian declines and extinctions dating back several decades now. The Amphibian Conservation Action Plan (ACAP), the first taxonomic class-level plan of its kind, was first published in 2007 and then updated as a digital resource in 2015, with the goal of acting as a unified global strategy to save amphibians. However, although there have been resources allocated to amphibian conservation since the first ACAP, these have not been of the order of magnitude needed to adequately address the global amphibian crisis.

In an effort to help improve this situation the current ACAP is adopting a different strategy: the development of two complementary documents that work to 1) synthesise developments in major themes of amphibian conservation over the last 15 years (an academic status review – this document), and 2) summarise the key take-home messages and recommendations to a broader audience in a user-friendly way (a practitioner document that will follow the status review). The purpose is thus to provide the most up-to-date evidence on threats and approaches to amphibian conservation, and from there identify gaps and priorities that can then be disseminated and adopted by stakeholders across the globe.

Each chapter of this status review was developed by the matching Amphibian Specialist Group's (ASG) thematic working groups. Led by 1-3 working group chairs and supported by working group members, chapters have also had the input of professionals outside of ASG with expertise in given themes.

This document consists of two introductory chapters and twelve thematic chapters divided into three sections:

- Threats - Chapters 3-7 on climate change; ecotoxicology; habitat loss; infectious diseases; and trade and sustainable use
- Informing decision-making - Chapters 8-10 on communications and education; conservation planning; and surveys and monitoring
- Species management - Chapters 11-14 on conservation breeding; assisted reproductive technologies and biobanking; genomics; and translocations

In broad terms, each chapter covers the most important knowledge, technological and conceptual developments in a particular theme over the last decade and a half, highlighting knowledge gaps, challenges, needs and opportunities for future conservation action.

Key messages

1. As a whole there is an enormous deficit in information for most amphibian species, which hampers decision-making and evidence-based, conservation action. Increased collaborations both within and outside the amphibian conservation community are urgently needed to begin to bridge some of these information gaps. Integrating different approaches can help augment information and leverage additional support to amphibian conservation.
2. While this document is global in scope it is informed by local and regional realities. Not everything that is in this document will be transferable to every region. However, those aspects that are relevant to a region can be addressed accordingly, and these results can then feedback again into a global strategy, and be readapted in other regions to benefit from the shared experience. Translation from local to global and

back to local is crucial to ensure that regional experiences feed into a global framework and that this framework accurately reflects shared patterns and realities so that it can inform international conventions and organisations, especially in view of global environmental change.

3. Relative to the scope of amphibian declines and extinctions, adequate financial and human resources and necessary policy measures have largely lagged behind this decades-long crisis. Should this pattern persist, we can expect to continue losing amphibian populations and species in increasingly large numbers. It is therefore critical that amphibian conservation becomes both an integral and a conspicuous part of the biodiversity conservation agenda of international and national conservation organisations of all sizes, of national and subnational levels of government, of the various institutions that focus on biodiversity education and research, of funding entities, and of organised communities and media.

Resumen ejecutivo

Comprendiendo la clase de vertebrados más amenazada del mundo, los anfibios están en la primera línea de la crisis de biodiversidad, con el inicio de las declinaciones y extinciones de los anfibios remontándose ya varias décadas. El Plan de Acción de Conservación de los Anfibios (ACAP por sus siglas en inglés), el primer plan a nivel de clase taxonómica, fue publicado inicialmente en el 2007 y luego actualizado como un recurso digital en el 2015, con la meta de actuar como una estrategia global unificada para salvar a los anfibios. Sin embargo, aunque ha habido recursos dirigidos a la conservación de anfibios desde el primer plan, estos no han sido de la magnitud requerida para abordar la crisis global de los anfibios de forma adecuada.

En un esfuerzo por mejorar esta situación, este ACAP está adoptando una estrategia diferente: el desarrollo de dos documentos complementarios que de manera conjunta 1) sintetizan los acontecimientos y progreso en temas importantes para la conservación de los anfibios en los últimos 15 años (una revisión del estado académico – este documento), y 2) resumen los principales mensajes y recomendaciones dirigidos a una audiencia amplia de una manera accesible (un documento para implementadores que seguirá la revisión de estado). El propósito es, por ende, ofrecer la evidencia más actualizada acerca de las amenazas y abordajes en lo que refiere a la conservación de los anfibios, y a partir de ello identificar vacíos y prioridades que pueden luego ser diseminadas y adoptadas por actores relevantes a lo largo del planeta.

Cada capítulo de este documento fue desarrollado por el respectivo grupo temático del Grupo de Especialistas de Anfibios (ASG por sus siglas en inglés). Liderados por 1-3 presidentes de grupos temáticos y apoyados por miembros de los grupos de trabajo, los capítulos también han recibido el aporte de profesionales fuera del ASG con experiencia en determinados temas.

Este documento comprende dos capítulos introductorios y doce capítulos temáticos divididos en tres secciones:

- Amenazas - Capítulos 3-7 sobre cambio climático; ecotoxicología; pérdida de hábitat; enfermedades infecciosas; y comercio y uso sostenible
- Informando la toma de decisiones - Capítulos 8-10 sobre comunicaciones y educación; planificación de conservación; y muestreos y monitoreo
- Manejo de especies - Capítulos 11-14 sobre reproducción de conservación; tecnologías de reproducción asistida y biobancos; genómica; y translocaciones

En términos generales cada capítulo cubre el conocimiento y desarrollo tecnológico y conceptual más importantes de la última década y media, resaltando los vacíos de conocimiento, retos, necesidades y oportunidades para futuras acciones de conservación.

Mensajes principales

1. En su conjunto existe un enorme déficit de información para la mayoría de especies de anfibios, lo que dificulta el proceso de toma de decisiones y acciones de conservación basadas en evidencia. Es necesario incrementar las colaboraciones tanto dentro como fuera de la comunidad de conservación de anfibios, para así comenzar a zanjar algunos de los vacíos de información. La integración de abordajes distintos

puede ayudar a aumentar la información y catalizar apoyo adicional hacia la conservación de anfibios.

2. Aunque este documento es de alcance global está informado por realidades locales y regionales. No todo lo que está en él será transferible a cada región. No obstante, esos aspectos que sí son relevantes a cada región pueden ser abordados como corresponde, y esos resultados pueden luego retroalimentar una estrategia global y ser readaptados en otras regiones para lograr un beneficio a partir de la experiencia compartida. La traducción de lo local a lo global y vice-versa es crucial para asegurar que las experiencias regionales puedan alimentar un marco global y que este marco refleje los patrones y realidades compartidos, de manera que pueda informar convenciones y organizaciones internacionales, especialmente dado el cambio ambiental global.
3. En relación a la magnitud de las declinaciones y extinciones de anfibios, los recursos financieros y humanos y las medidas políticas necesarias vienen muy rezagados ante esta crisis de décadas de duración. Si este patrón persiste, podemos esperar la continua pérdida de poblaciones de anfibios y especies en números cada vez mayores. Es por ende crítico que la conservación de anfibios se vuelva un componente tanto integral como conspicuo de la agenda de las organizaciones de conservación internacionales y nacionales de todos los tamaños, de los distintos niveles de gobierno, de las varias instituciones que se enfocan en la investigación y educación de la biodiversidad, de entidades financieras, de comunidades organizadas, y de los medios de comunicación.

Résumé analytique

Etant la classe de vertébrés la plus menacée au monde, les amphibiens sont au premier plan de la crise de la biodiversité. Le début de leur déclin et de l'extinction de certaines espèces à l'échelle mondiale a commencé il y a plusieurs dizaines d'années. Le Plan d'Action pour la Conservation des Amphibiens (ACAP en Anglais) est le premier plan d'action pour la conservation des espèces au niveau du rang taxonomique des classes. Il a été publié pour la première fois en 2007, puis mis à jour en tant que ressource numérique en 2015 avec l'objectif de servir à la création d'une stratégie mondiale unifiée pour sauver les amphibiens. Cependant, bien que des ressources aient été allouées à la conservation des amphibiens depuis le premier ACAP, elles n'ont pas été de l'ordre de grandeur nécessaire pour faire face de manière adéquate à la crise mondiale de la disparition des amphibiens.

Pour aider à l'amélioration de cette situation, la version actuelle de l'ACAP adopte une stratégie différente: la préparation de deux documents complémentaires qui cherchent à 1) synthétiser les développements dans les principaux thèmes de la conservation des amphibiens au cours des 15 dernières années (une synthèse des développements académiques - ce document) , et 2) résumer les principaux messages et les recommandations pour le grand public d'une manière accessible (un document de mise en œuvre qui suivra la synthèse des développements). L'objectif est donc de fournir les preuves les plus récentes des menaces et les approches de la conservation des amphibiens, et à partir de là, d'identifier les lacunes et les priorités qui peuvent ensuite être diffusées et adoptées par les acteurs de la conservation à travers le monde.

Chaque chapitre de cette synthèse des développements en conservation a été élaboré par le groupe de travail thématiques travaillant sur le sujet et faisant partie du Groupe des Spécialistes des Amphibiens (ASG en Anglais). Chaque chapitre a été dirigé par 1 à 3 chefs de groupes de travail, en concert avec les membres du groupe. Les chapitres ont également bénéficié de la contribution de professionnels extérieurs à l'ASG et ayant une expertise correspondante.

Ce document se compose de deux chapitres introductifs et de douze chapitres thématiques divisés en trois sections :

- Menaces - Chapitres 3 à 7 sur le changement climatique ; l'écotoxicologie ; la destruction de l'habitat ; les maladies infectieuses ; le commerce et l'utilisation durable
- Prise de décisions informées - Chapitres 8 à 10 sur les communications et l'éducation ; la planification de la conservation ; les enquêtes et le suivi
- Gestion des espèces - Chapitres 11 à 14 sur l'élevage en captivité ; les technologies de procréation assistée et biobanques ; la génomique ; les translocations

En termes généraux, chaque chapitre couvre les connaissances et les développements technologiques et conceptuels les plus importants pour chaque thème développé au cours des quinze dernières années, en mettant en évidence les lacunes, les défis, les besoins et les opportunités pour les futures actions de conservation.

Messages principaux

1. Dans l'ensemble, il y a un extraordinaire manque d'informations pour la plupart des espèces d'amphibiens, ce qui entrave la prise de décisions et les actions de

conservation basées sur des faits scientifiques.. De nouvelles collaborations au sein de la communauté de conservation des amphibiens, mais aussi avec des personnes externes, sont nécessaires de toute urgence pour commencer à combler ces lacunes en matière d'information. L'intégration de différentes approches peut permettre d’acquérir les informations nécessaires et de tirer parti d'un soutien supplémentaire pour la conservation des amphibiens.

2. Bien que ce document ait une portée mondiale, il est dirigé par les réalités locales et régionales. Tous les points exposés dans ce document ne seront pas nécessairement transférables à toutes les régions. Cependant, les aspects qui sont pertinents pour une région peuvent être traités en conséquence, et ces résultats peuvent ensuite être réintégrés à la stratégie mondiale, et ensuite être réadaptés dans d'autres régions qui pourront bénéficier de ces expériences. La transformation du point de vue local au point de vue mondial et le retour au point de vue local, est cruciale pour garantir que les expériences régionales alimentent un cadre mondial et que ce cadre reflète correctement les modèles et réalités locales afin d’informer les conventions et organisations internationales, spécialement compte tenu des changements environnementaux mondiaux.

3. En comparaison avec l'ampleur du déclin et l'extinction des amphibiens, les ressources financières et humaines et les mesures politiques nécessaires sont en retard par rapport à cette crise qui dure depuis des douzaines d’années. Si ce schéma persiste, nous pouvons nous attendre à continuer de perdre de plus en plus de populations et d’espèces d'amphibiens. Il est donc essentiel que la conservation des amphibiens devienne à la fois une partie intégrante et visible du programme de

conservation de la biodiversité des organisations de conservation internationales et nationales de toutes tailles, mais aussi au niveau des gouvernements nationaux et régionaux, des institutions diverses qui se concentrent sur l'éducation et la recherche sur la biodiversité, des organismes de financement, des associations et des médias.

Acknowledgements

We are grateful to Detroit Zoological Society, the Amphibian Survival Alliance, funding from the IUCN Species Survival Commission (SSC) Internal Grant Program, supported by the Environment Agency-Abu Dhabi, Nanjing Forestry University and Re:wild, without whose financial and in-kind support this important update of the Amphibian Conservation Action Plan would not have been possible.

Development of this document would not have been possible without the following people, who generously gave their time and expertise during the conception and drafting of chapters: Aarón Josué Quiroz Rodríguez, Amir Hamidy, Amy Reaume, Andrés Merino-Viteri, Andrés Rymel Acosta Galvis, Andrew Cunningham, Andrew Snyder, Ángela M. Suárez Mayorga, Angelica Crottini, Anne Alexandre, Arvin C. Diesmos, Benedikt Schmidt, Bill Peterman, Blake Klocke, Brett Scheffers, Bryan Stuart, Claudio Azat, Cori Richards-Zawacki, Daniel Rodríguez, David B Wake, David Blackburn, Diego A. Gómez-Hoyos, Dion Kelly, Edgar Lehr, Felipe Toledo, Florina Stanescu, Francis Lemckert, Francois Becker, Gailmarie Kimmel, Gonçalo M. Rosa, Ian Little, Imadeddin Albaba, Iñigo Martinez-Solano, Ivelina Frantsova-Bauer, James Collins, James Lewis, Jean-Marc Hero, Jennifer Buchanan, Jenny Urbina, Johanna Rode-Margono, John Wilkinson, Joseph Doumbia, Karen Lips, Kaya Klop-Toker, Leida Dos Santos, Lily Rodriguez, Luis Alberto Rueda Solano, Luis Castillo Roque, Luis Fernando Marin, Luisa Arnedo, Lydia Johnson, Maria Forzan, Mark Wilkinson, Mark-Oliver Rödel, Maureen Donnelly, Mauricio Akmentins, Michelle Thompson, N'Guessan Emmanuel Assemian, Nicolas Heard, Nida Al-Fulaij, Nikki Roach, Nina Marshall , Ninad Amol Gosavi, Paul Crump, Paul Doughty, PK Malonza, Thomas Ziegler, Rachel Rommel, Rayna Bell, Reid Harris, Rob Stuebing, Robert Puschendorf, Robert Ward, Roberta Lecis, Ross Alford, S.C. Bordoloi, Segniagbeto H. Gabriel, Sherilyn Bos, Simon Mickleburgh, Stefano Doglio, Steven Allain, Tahar Slimani, Trent Garner, Valia Esther Herrera Alva, Vanessa K. Verdade, Vicky Flechas, Víctor Jassmani Vargas García and Vincenzo Ferri.

Further, we thank the following people who contributed to the development of chapters beyond those for which they are authors: Andrés Valenzuela-Sánchez, Antonio W. Salas, Ariel Rodríguez, Benjamin Tapley, Cynthia Paszkowski, Dan Cogalniceanu, David Bickford, Dede Olson, Federico Kacoliris, Franco Andreone, James Watling, Javiera Cisternas, Jeanne Tarrant, Jeff Dawson, Jess Hua, John Measey, Karthikeyan Vasudevan, Kevin Johnson, Luke Linhoff, Mark C Urban, Michael Lau, Michelle D Boone, Natalie Calatayud, Nicolas Urbina-Cardona, Richard Griffiths, Ted Kahn and Tiffany Kosch.

We also thank Soledad Delgado Oyarzún, Vishal Prasad Kumar and Claire Relton who developed figures for this document, helping to clearly illustrate complex ideas.

Debbie Bishop has provided support right from our initial discussions about what format this updated ACAP should take. We thank Debbie both for her insightful comments and her moral support throughout the whole process.

Finally, we would like to acknowledge the tireless efforts of the whole amphibian conservation community, whose research and work is represented in this document, and whose actions are what will make a difference for the amphibian species that need our support.

The ACAP Editorial Team:
Sally Wren, Amaël Borzée, Ruth Marcec-Greaves and Ariadne Angulo.

Acronyms and abbreviations

AArk	Amphibian Ark
ACAP	Amphibian Conservation Action Plan
ARLA	Amphibian Red List Authority
ASA	Amphibian Survival Alliance
ASG	IUCN SSC Amphibian Specialist Group
AZE	Alliance for Zero Extinction
Bd	*Batrachochytrium dendrobatidis*
Bsal	*Batrachochytrium salamandrivorans*
CBD	Convention on Biological Diversity
CITES	Convention on International Trade in Endangered Species of Wild Fauna and Flora
CNA	Conservation Needs Assessment
COVID-19	Coronavirus disease 2019
CPSG	IUCN SSC Conservation Planning Specialist Group
eDNA	Environmental DNA
EDGE	Evolutionarily Distinct and Globally Endangered
GAA	Global Amphibian Assessment
IPCC	Intergovernmental Panel on Climate Change
IUCN	International Union for Conservation of Nature
KBA	Key Biodiversity Areas
NGO	Non-Governmental Organisation
SSC	IUCN Species Survival Commission
USFWS	United States Fish and Wildlife Service

Chapter 1. Overview of amphibians and their conservation

Sally Wren[1,2], Ariadne Angulo[1], Jos Kielgast[1,3,4], Phil J. Bishop[1,2], Ruth Marcec-Greaves[1,5], Jennifer Luedtke[1,6] Janice Chanson[1] and Amaël Borzée[1,7]

[1] IUCN SSC Amphibian Specialist Group, 3701 Lake Shore Blvd W, P.O. Box 48586, Toronto, Ontario M8W 1P5, Canada

[2] Department of Zoology, University of Otago, 340 Great King Street, Dunedin 9016, New Zealand

[3] Section for Freshwater Biology, Department of Biology, University of Copenhagen, Universitetsparken 4, DK-2100, Denmark

[4] Center for Macroecology, Evolution and Climate, Natural History Museum of Denmark, Universitetsparken, 15, DK-2100, Denmark

[5] Honduras Amphibian Rescue and Conservation Center, 1200 SE Oak Ridge Drive, Oak Grove, Missouri, 64075, USA

[6] Re:Wild, P.O. Box 129, Austin, Texas, 78767, USA

[7] Laboratory of Animal Behaviour and Conservation, College of Biology and the Environment, Nanjing Forestry University, Nanjing, People's Republic of China

Abstract

Amphibians are extraordinary and diverse creatures that have roamed the earth for millions of years; yet, they are currently the most threatened vertebrate class on earth, with over 40% of species at risk of extinction. Herein we offer a brief overview of the amphibians, covering aspects such as broad taxonomic classification, their geographic distribution, natural history and ecology, their importance and evolutionary uniqueness, as well as their conservation

status and the global response to conserve them. We also discuss the background that informed this version of the Amphibian Conservation Action Plan and what is contemplated in it, as well as our aspirations for its adoption and implementation.

Introduction

Few creatures embody transformation and renewal in the human imagination collective like amphibians. They have had an important presence across cultures and time, and even in the present day, many of us have fond memories of watching tadpoles in ponds and listening to frogs and toads calling into the night. Of course, well above their significance to our species, amphibians are pillars of the food web, keeping the cycles of life going. Yet, they are at the bleeding edge of the modern biodiversity crisis, having experienced the worst population declines and highest risk of extinction at the vertebrate class level, and two global amphibian-specific pandemics in recent times (prompted by the batrachochytrid fungi *Batrachochytrium dendrobatidis* and *Batrachochytrium salamandrivorans*). Resilient as they have been over geological time (early amphibians emerged in the Late Devonian, around 350-360 million years ago; Alford, Richards & McDonald, 2013; Hime et al., 2021), The IUCN Red List of Threatened Species™ (IUCN Red List) has found that over 40% of amphibians are at risk of extinction (IUCN, 2022). Amphibians are in serious trouble, in no small measure because of humans, and we are in peril of losing some of the most emblematic and magnificent creatures to have roamed this earth.

So, who are the amphibians? What makes amphibians the incredible, awe-inspiring and extraordinary creatures that we know and love? In the sections and chapters that follow our community provides a synthesis of the status of amphibians, their importance, the challenges faced and the responses.

Classification

There are currently 8,455 recognised extant amphibian species (Frost, 2021, see also Figure 1.1); however, this number continues to grow at a rate of roughly one new species described every other day (AmphibiaWeb, 2021). Since the creation of the first comprehensive catalogue of amphibian species richness (Frost, 1985) the number of known amphibian species has more than doubled. This is an extraordinary rate of species discovery compared to that of other vertebrates (Vences & Köhler, 2007).

Amphibians occur in all sizes, shapes and colours, but are contained within three taxonomic orders: Anura (frogs and toads), Caudata (salamanders and newts) and Gymnophiona (caecilians). Anurans are the most ubiquitous and most species-rich of these orders both in terms of families and species, with 58 families and 7,471 species (Frost, 2021). They are followed by Caudata, with 9 families and 771 species, and Gymnophiona (10 families and 214 species; Frost, 2021).

Distribution

Anurans are the most widespread amphibians, occurring on all continents but Antarctica. The highest species richness is in tropical ecosystems, but they inhabit virtually all environments on the globe except the most extreme dry or cold (Figure 1.1). The salamanders and newts are less species-rich and have a markedly different distribution. They are largely restricted to the Northern Hemisphere (Duellman, 1999) with highest species richness in the temperate zone, in particular in the northeastern USA. Only a single but highly species-rich family (Plethodontidae, with 491 spp.) has radiated into Central and South America, occurring also in southern Europe and Korea (Frost, 2021). Fewer species live on the Eurasian continent and the order is completely absent in sub-Saharan Africa, Madagascar, the Arabian Peninsula, insular Southeast Asia and Oceania. Caecilians are by far the least species-rich order, and have a pantropical distribution (Duellman, 1999), known from the tropics of the Americas, Africa, Asia, Southeast Asia and the Seychelles (Stuart et al., 2008).

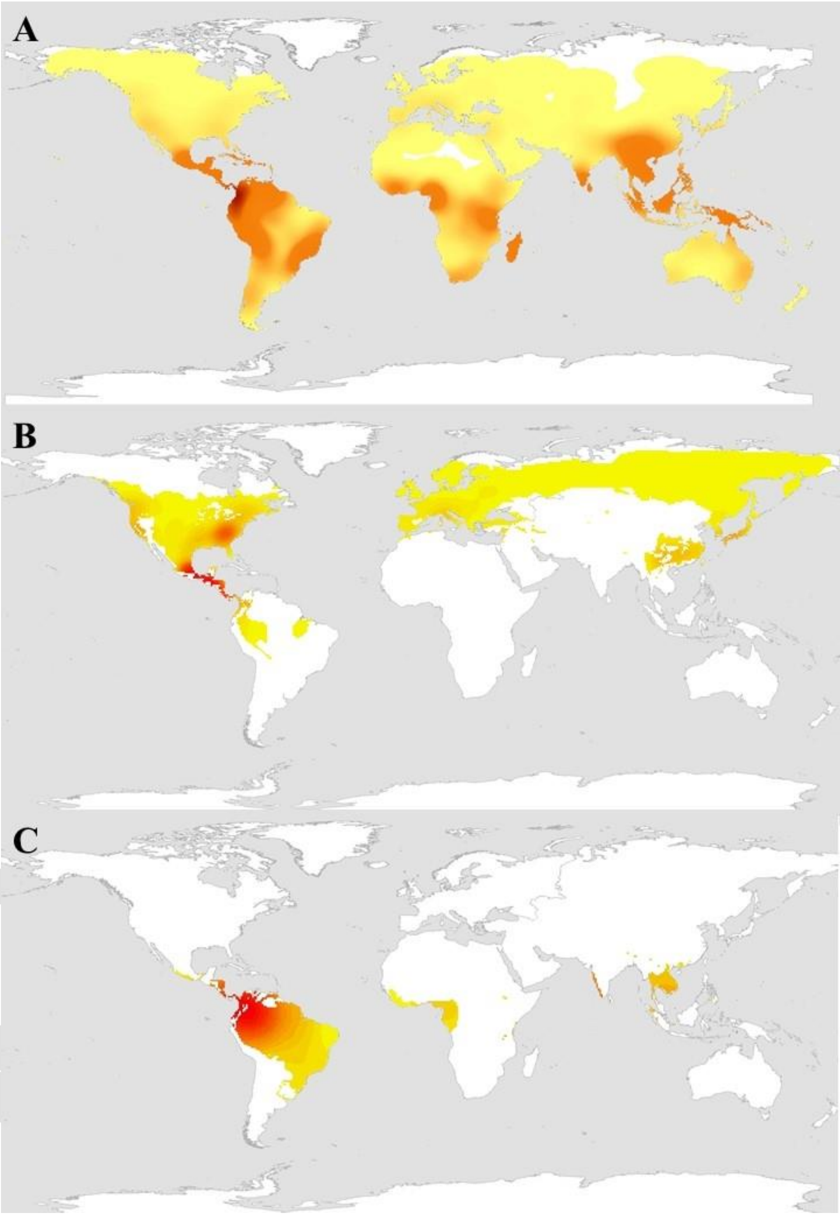


Figure 1.1: Map of global amphibian distribution, by Order. Warmer colours indicate higher species richness. (A) Anura, (B) Caudata, (C) Gymnophiona. Figure drawn by Vishal Prasad Kumar. Source: Amphibian distribution data downloaded from IUCN Red List website (https://www.iucnredlist.org/).

As with much of terrestrial biodiversity, amphibian species richness drastically increases towards the Equator (see e.g. Stuart et al., 2008). The Neotropics is by far the most species-rich zoogeographical zone, with Brazil having the highest number of species (1152), followed by Colombia (820 species), Ecuador (670 species) and Peru (662; AmphibiaWeb, 2022). Amazingly, some species occur far from the Equator, showing remarkable adaptations to cold environments. For example, the North American wood frog (*Lithobates sylvaticus)* tolerates complete freezing during hibernation (Sinclair et al., 2013), and the Siberian salamander (*Salamandrella keyserlingii*) tolerates even more extreme temperatures that can reach as low as -35 °C (Berman, Leirikh & Meshcheryakova, 2010). Others live with extreme weather patterns at high altitude. The record holders are the frog *Pleurodema marmoratum,* breeding at 5,348 m asl in Peru (Seimon et al., 2007) and the salamander *Pseudoeurycea gadovii* recorded up to 4,250 m asl in Mexico (Solano-Zavaleta, García-Vázquez & Mendoza-Hernández, 2009).

At the other end of the spectrum, several genera of anurans and salamanders have adapted to arid areas by burrowing into the ground and forming a protective cocoon around their body in order to aestivate (Secor & Lignot, 2010). In some cases, aestivation can last up to ten months, with one extreme case of five consecutive years suggested for the Australian water-holding frog *Ranoidea platycephala* (Secor & Lignot, 2010).

Natural history and ecology

It is difficult to capture the sheer array of amphibian natural history and ecology in just a few words. The impressive diversity in morphology, distribution, habitat use, physiology, mimicry, reproduction, behaviours, life stages, ecological attributes, and life histories easily merit several dedicated volumes and indeed a few brave souls have attempted this task. By necessity, we are obliged to select a handful of notable examples, but with the understanding that they are just the tip of the proverbial iceberg.

Amphibians occupy a diverse variety of terrestrial and freshwater environments. All three orders have species that live underground, that are fully aquatic, fully terrestrial and more or less everything in between. On the vertical axis they occur several metres underground (fossorial), up to the tallest tree canopies (arboreal). On the horizontal axis they are land or water dwellers (or alternate between both); in water, they occupy lentic and lotic habitats ranging from the world's largest lakes and rivers to the water captured in the leaf axils of plants, and even brackish waters of estuaries.

While the amphibian life cycle is most often pictured with eggs laid in water, which develop into tadpoles that metamorphose to land-living adults, the actual diversity of amphibian life histories is manifold and spectacular. Although most amphibians do have free-living aquatic larvae (i.e. tadpoles), an estimated 29% reproduce through direct development, which means their eggs hatch into miniature adults. At least 68 amphibian species evolved away from egg laying completely, giving birth to fully developed young (Sodhi et al., 2008). This reproductive diversity is also reflected in the vastly different fecundity and population dynamics among amphibians, requiring a variety of conservation strategies. For instance, a single Great Plains toad (*Anaxyrus cognatus*) has been documented as laying 45,000 eggs in one breeding season (Thibaudeau & Altig, 1999), while the Alpine salamander (*Salamandra atra*) gives live birth to only 1-2 young after 3 years of gestation (Häfeli, 1971). These two extremes capture but a few aspects of the 74 different reproductive modes that have been described by scientists to date (Nunes-de-Almeida, Haddad & Toledo, 2021). There are over

30 forms of parental care observed in amphibian species, ranging from basic egg guarding to very advanced behaviours (Schulte et al., 2020). Some species raise their young on their body, like the aquatic frogs *Pipa* spp. that hatch either tadpoles or fully developed young through the skin on their back (Rabb & Rabb, 1960). Others are marsupial, carrying their young until fully developed in a skin pouch on their backs, e.g. members of the treefrog genus *Gastrotheca* (Elinson et al., 1990). Perhaps even more mystifying are those cases where the eggs are incubated inside the body cavity and are orally "expectorated" as fully developed froglets, e.g. the vocal sac in Darwin's frogs (*Rhinoderma darwinii*) and the stomach in the now extinct gastric brooding frogs *Rheobatrachus* (McDiarmid, 1978). There is also a species, *Oophaga pumilio,* where females carry their tadpoles long distances on their backs to deposit them in the water of leaf axils of epiphytic plants and raise them exclusively on unfertilised eggs (Summers, McKeon & Heying, 2006). Still other amphibians make subterranean chambers, securing moisture for their eggs in desert sand dunes, e.g. *Breviceps macrops* (Minter, 2004). One final, fascinating example is the Taita caecilian (*Boulengerula taitana*), which nests underground and feeds its young the outermost layer of its own skin (Kupfer et al., 2006).

Amphibians are also diverse where body size is concerned. Measuring only 7.7 mm, the smallest recorded vertebrate is the frog *Paedophryne amauensis* from Papua New Guinea (Rittmeyer et al., 2012), whereas – at 32 cm – the largest anuran on record is the Goliath frog, *Conraua goliath* (Sabater-Pi, 1985). The smallest known salamander is *Thorius arboreus* from Mexico, with the largest known adult of this species measuring 20.0 mm snout-vent length (Hanken & Wake, 1994). The Chinese salamander *Andrias davidanus*, on the other hand, is the largest amphibian reaching up to 180 cm (AmphibiaWeb, 2022). Amongst the caecilians, growing to 151.5 cm is *Caecilia thompsoni*; whereas the smallest adult caecilian is *Idiocranium russeli* at 0.90 cm (AmphibiaWeb, 2022).

Our knowledge of amphibian longevity is spotty at best, but it appears that caudates are generally more long lived than anurans (Smirina, 1994). While many species are presumed to be relatively short-lived, with a lifespan of only a few years in the case of anurans and tropical species, there are some exceptions. Notably, the olm (*Proteus anguinus*), a small cave salamander, has a predicted maximum lifespan of over 100 years and an adult average lifespan of 68.5 years (Voituron et al., 2011).

Evolutionary uniqueness

Amphibians emerged around 350-360 million years ago (Alford, Richards & McDonald, 2013; Hime et al., 2021). While the early amphibian faunas differed dramatically from their modern counterparts, representatives of many of the currently recognised amphibian families were most likely already present when dinosaurs roamed our planet (Roelants et al., 2007). Some extant species are particularly isolated across deep evolutionary time, and 23 families have fewer than 10 species (Frost, 2021).

The Zoological Society of London (ZSL) Evolutionarily Distinct and Globally Endangered (EDGE) programme identifies these special species. Using a combined score of evolutionary distinctiveness (ED) and extinction risk (taken from the IUCN Red List), species are ranked based on their evolutionary history and how threatened they are (Safi et al., 2013). As of 2020, 863 amphibians from all three amphibian orders were listed as EDGE species. The top-ranked anuran, salamander and caecilian are Archey's frog (*Leiopelma archeyi*), the Chinese giant salamander (*A. davidianus*) and the Mount Oku caecilian (*Crotaphatrema lamottei*), respectively (ZSL, 2020).

Conservation status

Reports of amphibian declines began to emerge in the 1950s (see Bishop et al., 2012), but for a long time only as infrequent publications in the peer-reviewed literature. It was not until at the First World Congress of Herpetology, held in the United Kingdom in 1989, that the disparate observations of herpetologists from all over the world were shared, raising concerns that the scope and severity of these declines were beyond what anyone had previously thought (Bishop et al., 2012; Stuart, 2012). Alarmingly, catastrophic declines were documented even in pristine environments. For example, the two Australian gastric-brooding frogs (*Rheobatrachus* spp.) disappeared in less than a year, one in the late 1970s, the other in the mid-1980s, and the Costa Rican golden toad (*Incilius periglenes*) disappeared within two years and has not been seen since 1989 (Stuart, 2012). All three species are now considered Extinct and there are many others that have suffered a similar fate. An even larger number are considered Possibly Extinct because there are no known surviving subpopulations, but exhaustive surveys have yet to confirm their extinction.

In response to the widespread concerns, a global push began to better understand their causes and to determine the conservation actions that might halt the decline of amphibian populations (see Global response section in this chapter for details). In 2001, IUCN, Conservation International, and NatureServe began the Global Amphibian Assessment (GAA), the first-ever comprehensive extinction risk evaluation of all 5,743 species described amphibians at the time. The assessment results published in 2004 were devastating: amphibians were the most threatened vertebrate group, with 32.5% of species categorised as threatened on the 2004 IUCN Red List (Stuart et al., 2004). Furthermore, 22.5% of the species were classified as Data Deficient (DD), having too little or too uncertain data to make a reliable assessment. Employing the IUCN Red List best estimate approach, the same proportion of DD species was assumed to be threatened as the data sufficient species, which provided a total estimate of 40% of all amphibians threatened with extinction.

In addition to providing a snapshot of the current conservation status of amphibians, undertaking regular comprehensive updates of all species also provides an opportunity to monitor conservation status over time. The Red List Index (RLI) is an indicator developed by IUCN to illustrate the changing conservation status of a group of species based on genuine improvements or deteriorations in Red List category. This biodiversity indicator has become widely used to compare the status of various taxonomic groups, as well as, for example, a measure of progress towards the UN Convention on Biological Diversity targets (Butchart et al., 2004; Secretariat of the Convention on Biological Diversity, 2020). The first GAA also estimated what the Red List category would have been in 1980 based on current knowledge. Using these data, the RLI was calculated for 1980 and 2004. It showed a significant downward trend, equivalent to an increase of 30% of species listed in a higher threat category in 2004 compared to 1980 (Butchart et al., 2005). This highlighted amphibians as one of the most rapidly declining taxonomic groups (Secretariat of the Convention on Biological Diversity, 2020). An analysis using the RLI to assess the impact of conservation on amphibians, birds and mammals found that while conservation efforts were having an appreciable effect on the trend in conservation status for birds and mammals, this was not seen for amphibians (Hoffmann et al., 2010).

A Red List assessment for a species is considered to be current for 10 years from the date of the assessment, and is considered the minimum standard for providing the most up-to-date information to conservation efforts and tracking trends through the RLI at regular intervals. The ASG launched the second Global Amphibian Assessment (GAA2) in 2015, which is on track for completion in 2022, and now includes more than 8,000 species (see Chapter 2 for this and other related challenges). The GAA2 results available to date confirm that amphibians are still the most threatened vertebrate group, with 41% of species currently within threatened categories (Figure 1.2; IUCN, 2021). When complete, the GAA2 will also provide a third data point in time for the RLI, which will give an indication of the conservation status of amphibians as a group, whether they are improving or deteriorating, and if the latter is true, whether the rate of deterioration has slowed.

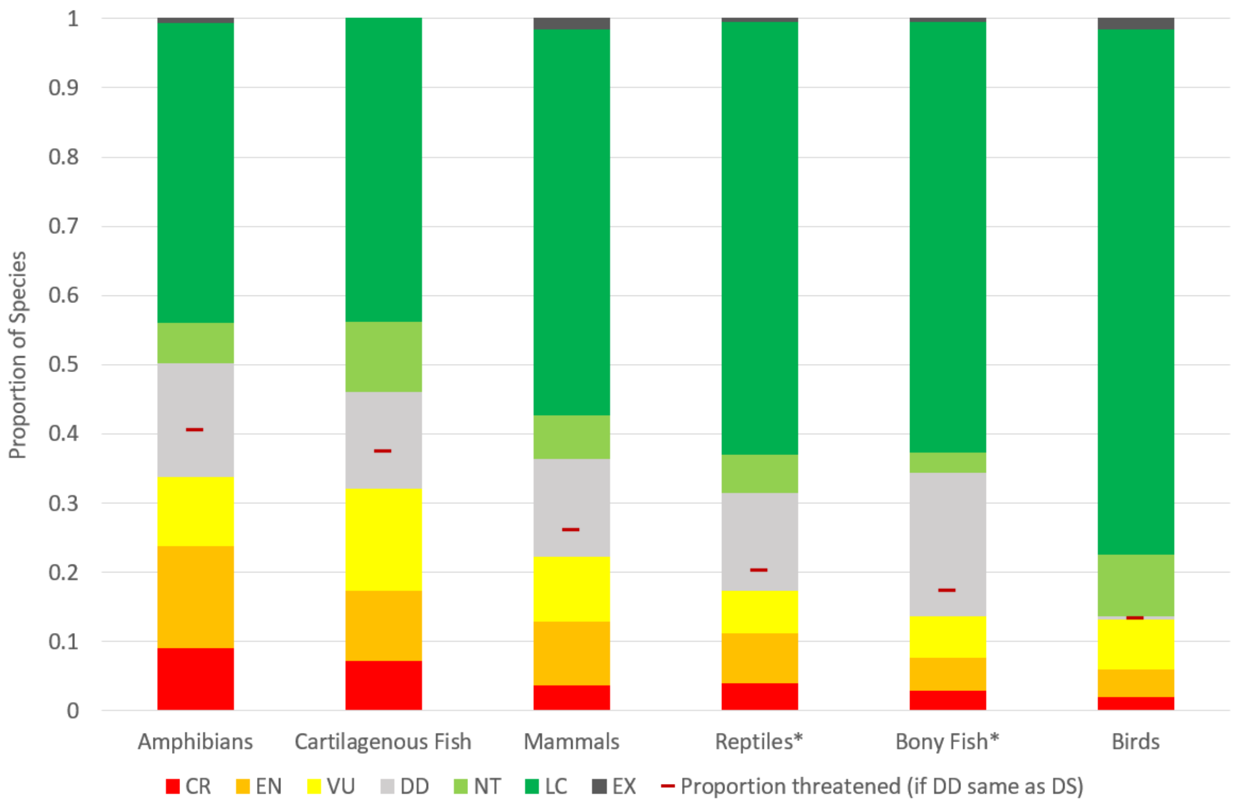


Figure 1.2. Proportion of species in each Red List category by vertebrate group. Red line indicates the estimated proportion of species classified as threatened if Data Deficient species

are threatened in the same proportion as data sufficient species. The category Extinct in the Wild was not included because numbers are very small and would not be visible on the chart. A further ~3,000 amphibian assessments will be added to the Red List in 2022, once the GAA2 is completed. Analysis of the GAA2 data is expected to show that some regions and taxa are disproportionately threatened, as was the case in the first GAA – an important consideration when planning where to focus conservation efforts. * An asterisk denotes groups where not all species have been assessed. Data Source: IUCN Red List version 2021-2.

There has been a huge amount of research on amphibians since the first GAA, some of which was no doubt spurred by the response of the herpetological community to the plight of amphibians highlighted on the IUCN Red List. This new research has provided sufficient information for many species to be comprehensively assessed and hence removed from their previous Data Deficient category.

However, it should be noted that some species will always be difficult to remove from the Data Deficient category. For example, species known only from the type specimen, the provenance of which is unknown, or where there is considerable taxonomic uncertainty, to the point that a species may not be valid.

As well as Data Deficient species, almost all amphibians would benefit from more information on their distribution, population, ecology, and threats. Thus, the recommendations of this publication will not only serve to direct the focus of conservation actions on the ground, but will also inform and improve conservation assessments. Each chapter specifies the research needed to inform these actions and inform future Global

Amphibian Assessments, completing the Assess-Plan-Act conservation cycle of the Species Survival Commission.

Vulnerability to threats

Amphibians can be particularly vulnerable to threats; they are often adapted to spend different parts of their life cycle in specific habitats, terrestrial and aquatic, and as a result they are vulnerable to changes in both environments. Many species, particularly those in tropical regions, have very small distributions, and large proportions of a population can be affected by changes to a relatively small area. The habitat-specific life stages also means that amphibians often consume different types of food as larvae and adults, increasing their potential for ingesting toxins and their exposure to parasites. In addition, amphibians are ectotherms, and are therefore sensitive to temperature changes, while their moist permeable skin leaves them exposed to desiccation and to pollutants in the water and air. Many species have low vagility and are unable to move to effectively escape environmental threats. As well as these threats, there are also indirect factors, including that they are not considered to be charismatic relative to mammals and birds, leading to amphibians receiving overall less attention from researchers, conservation practitioners, and conservation funding than these other taxa (see Chapter 2).

It is important to note that the diverse and ubiquitous threats that affect species currently assessed as threatened also affect non-threatened and Data Deficient species. More details on most of the threats and how they impact amphibians can be found in the relevant chapters throughout this document.

Importance of amphibians

The value of a species is often translated into the benefits it provides to humanity and interpreted in monetary or utilisation value. It is, however, important to look at the value of a species from a different point of view: the intrinsic value of a species. Here, we look at how human societies have relied on amphibians for their development and how we still rely on them, rather than how human societies can benefit from exploiting amphibians (Doak et al., 2013). This is one example of the viewpoints available, and even within the field of conservation alternatives can be found. For instance, some may focus on the evolutionary value of a given species, and others may focus on its representation and connectedness within the environment.

The history of humans and amphibians is more tightly linked than it is generally expressed or understood, in terms of culture, medical development, disease and pest control, and much more – acknowledging a cultural bias. Early human populations were attracted to wetlands and other fertile ecosystems due to their richness in primary producers, a preference generally matching with that of all vertebrates (Small & Cohen, 2004; Pérez, Schuster & Jofré, 2018), resulting in the establishment of human settlements in habitats also favoured by amphibians. Human societies have relied and continue to rely on amphibians as a food source (Mbaiwa, 2011), in hunting practices (Myers, Daly & Malkin, 1978), fishing or controlling water quality. For example, a Chilean giant frog (*Calyptocephalella gayi*) was placed inside water wells as a bioindicator of water quality in central Chile (personal communication A. V. Sánchez), and Ranidae and Bufonidae are used for a similar purpose in Indonesia (Mardiastuti et al., 2020).

A notable benefit derived from amphibians is their use in traditional and modern human healthcare (Clarke, 1997). These include, for instance, the crucifix frog *Notaden bennettii* from Australia, whose secretions are used for biological glues in human medicine (Zhu, Chuah & Wang, 2018). Another example is that of antibiotics developed from amphibian antimicrobial polypeptides, and the amphibian skin polypeptide Gaegurin 4 is effective against both gram-positive and gram-negative bacteria (Won, Kang & Lee, 2009).

Amphibians have also contributed to the foundations of some technologies. For instance, this file is available through the Internet, which most people access through a Wi-Fi connection. The algorithms used in Wi-Fi networks were developed with the help of the call properties of *Dryophytes japonicus*, a northeast Asian treefrog (Hernández & Blum, 2012). This species has also benefited human culture through technological advancements such as the distributed graph colouring theory (Hernández Pibernat, 2012) and medical analgesic developments (Zhu et al., 2014), and it may provide other benefits to human medicine as it is able to survive temperatures as low as -35°C (Berman, Meshcheryakova & Bulakhova, 2016) and its physiological adaptations have been studied in space (Yamashita et al., 1997). These examples based on a single species are a very short list of how humans need amphibians in their everyday lives.

Most of the planet's ecosystems are dependent on amphibians for the multiple ecological roles they provide (reviewed by Valencia-Aguilar, Cortés-Gómez & Ruiz-Agudelo, 2013). Of course, humans benefit directly from intact ecosystems in which amphibian populations are healthy and functional. Termed 'ecosystem services', direct benefits are generally divided into provisioning, regulating, cultural and supporting services (Millennium Ecosystem Assessment, 2005). Indirect benefits include regulating ecosystem services, such as pollination, seed dispersal, climate regulation, carbon sequestration, and the control of pests and diseases. Finally, their position at the base of the food chain means that their global population declines have significantly impacted the survival of their predators (Zipkin et al., 2020), and all subsequent levels of the food chain.

The important place of amphibians in human culture, both positive and negative, is explored in depth in Chapter 8 on Communication and Education. Some of the oldest examples include early Egyptian and Greek fertility symbols represented by frogs (Cooper, 1992). Another is “Jin Chan”, or the money toad, which is considered to bring prosperity and good fortune in some East Asian cultures. By contrast, treefrogs in Korea are linked to carelessness. Popular Korean tales tell the story of a young treefrog burying his dead mother by a riverside. Her actual wish was to be buried elsewhere. However, she was attempting to outsmart her son after a lifetime of his ignoring her advice. The plot twist is that this was the first time he ever listened to her, which resulted in her still getting the opposite of her wishes, even after her death. Divine power is also revealed through amphibians, such as the devastating plague of frogs in the Old Testament of the Bible.

Many contemporary human populations are less exposed to disease and pests thanks to chemical and medical advancements, but this was not the case a few centuries ago when natural control vectors against pest and disease were the only means of regulation and treatment (e.g. Mohneke, 2011). Amphibian predation on both adults and larvae mosquitoes and flies has been a form of vector control that decreases pathogen transmission to humans, including deadly diseases such as malaria and dengue (DuRant & Hopkins, 2008). An increase in the incidence of malaria was recently shown to have been associated with the collapse of amphibian communities in Central America - an unexpected occurrence in a century that has been characterised by widespread chemical control measures of arthropod vectors and successful disease eradication through vaccinations (Springborn et al., 2020). Amphibians also act as pest control in agricultural habitats as they are generalist feeders and ingest all types of invertebrates, including pests such as locusts (Attademo, Peltzer & Lajmanovich, 2005), thus naturally improving crop yields, especially rice (Teng et al., 2016) and soybean (Attademo, Peltzer & Lajmanovich, 2005).

Finally, the charisma of amphibians is of great value to conservation efforts. For instance, the Chile Darwin's frog is used as an emblem on local beer, clothing, toys, and a restaurant (personal communication A. V. Sánchez). In South Africa, amphibians are of interest to tourists and the revenue derived from ecotourism provides wages to guides and inspiration for locally sold handcraft (Loubser, Mouton & Nel, 2001).

The diversity of life modes, ecology and behaviours of amphibians makes them important nodes in food webs, both as prey and predators. The transition from primary to secondary consumers when metamorphosing also results in an energy flow in ecosystems (Davic & Welsh Jr, 2004). This is especially important for nutrients present in higher concentration in the aquatic habitat compared to the terrestrial one, e.g. nitrogen, but also in the other direction, when amphibians bring nutrient to water bodies when spawning (Earl et al., 2011; Semlitsch, O'Donnell & Thompson, 2014). In addition, due to their ectotherm physiology, amphibians use comparatively less energy than homeotherms, and thus convert more of their diet into organic biomass (Pough, 1980; Pough, 1983). The fact that amphibians make up the largest proportion of biomass in many temperate and tropical ecosystems, e.g. salamanders in North American forests (Burton & Likens, 1975) also means that all mechanisms of energy transfer related to amphibians are proportionately more important than that of other organisms. In addition, besides the flow of nutrients, amphibians also affect the composition of ecosystems by enhancing soil aeration and consequently soil productivity (Seale, 1980). As a result, they also benefit soil and water quality, an especially important factor in view of the need for water security. Finally, it is important to understand that the roles and functions of amphibians in the ecosystem are still not fully understood, and the roles that each species might play needs to be fulfilled as a small missing link could result in greater ecological deficits, threatening the ecosystems on which all species on Earth depend.

Global response

The universal importance of amphibians compels us to act to rectify their human-caused declines. Some actions can be targeted to specific local conditions and needs, and as such implemented at a local scale. Whereas a global response is required for others because the threats affecting amphibians are global in scope and nature – climate change, disease, trade, and invasive species all span across borders. Moreover, species ranges do not respect political boundaries and their survival is dependent on a coordinated collaborative international response.

The first Amphibian Conservation Action Plan (ACAP) was the output of the Amphibian Conservation Summit held in 2005. It was the amphibian conservation community's response to global amphibian declines, highlighted by the GAA, "*because it is morally irresponsible to document amphibian declines and extinctions without also designing and promoting a response to this global crisis*" (Gascon et al., 2007).

It is difficult to assess the impact of the first ACAP, since it was developed among a suite of actions, all parts of a global push to increase awareness of amphibian declines and to include amphibians in conservation priorities. The Summit prompted some major organisational changes. Firstly, the IUCN SSC Amphibian Specialist group was constituted, bringing together the existing IUCN Groups: the Declining Amphibian Population Task Force, the Global Amphibian Assessment team, and the Global Amphibian Specialist Group (Bishop et al., 2012). Subsequently, the Amphibian Ark (AArk) was formed in 2007 with the aim of supporting implementation of the ex situ goals in the ACAP (Amphibian Ark, 2012). Finally, the Amphibian Survival Alliance (ASA) was set up following the IUCN SSC's Amphibian Mini-Summit in 2009, with the aim of coordinating organisations working on amphibian conservation (Bishop et al., 2012).

These three organisations – ASG, AArk and ASA – work together on the global response under the shared Vision "*Amphibian thriving in nature*" (Figure 1.3). Meanwhile, the ACAP has certainly been widely cited to support amphibian conservation action, but the impact remains insufficient, as amphibians are still the most threatened vertebrate taxon (IUCN, 2021).

A second version of the ACAP was developed in 2015, moving to an online 'living document' format, with the aim of updating it in real time. However, after completion it became clear that this format was not as impactful as the ASG had envisioned; users found it difficult to navigate and it was particularly hard for those with unreliable internet connections. As such, when we set out to work on this third iteration of the ACAP, our desire was to be more strategic. A survey was conducted from mid-August to mid-September 2019 to understand how the amphibian conservation community used the existing versions of the ACAP, and how it might be improved to better inform conservation action. In terms of document format, the survey results clearly indicated a strong preference for a PDF, rather than the living document, as well as the need for an Executive Summary. In terms of content, respondents recommended more clearly linking evidence on the effectiveness of interventions to ACAP recommendations, and a clearer expression of how global priorities can be implemented as manageable projects. We have strived to answer these needs when working on this update.

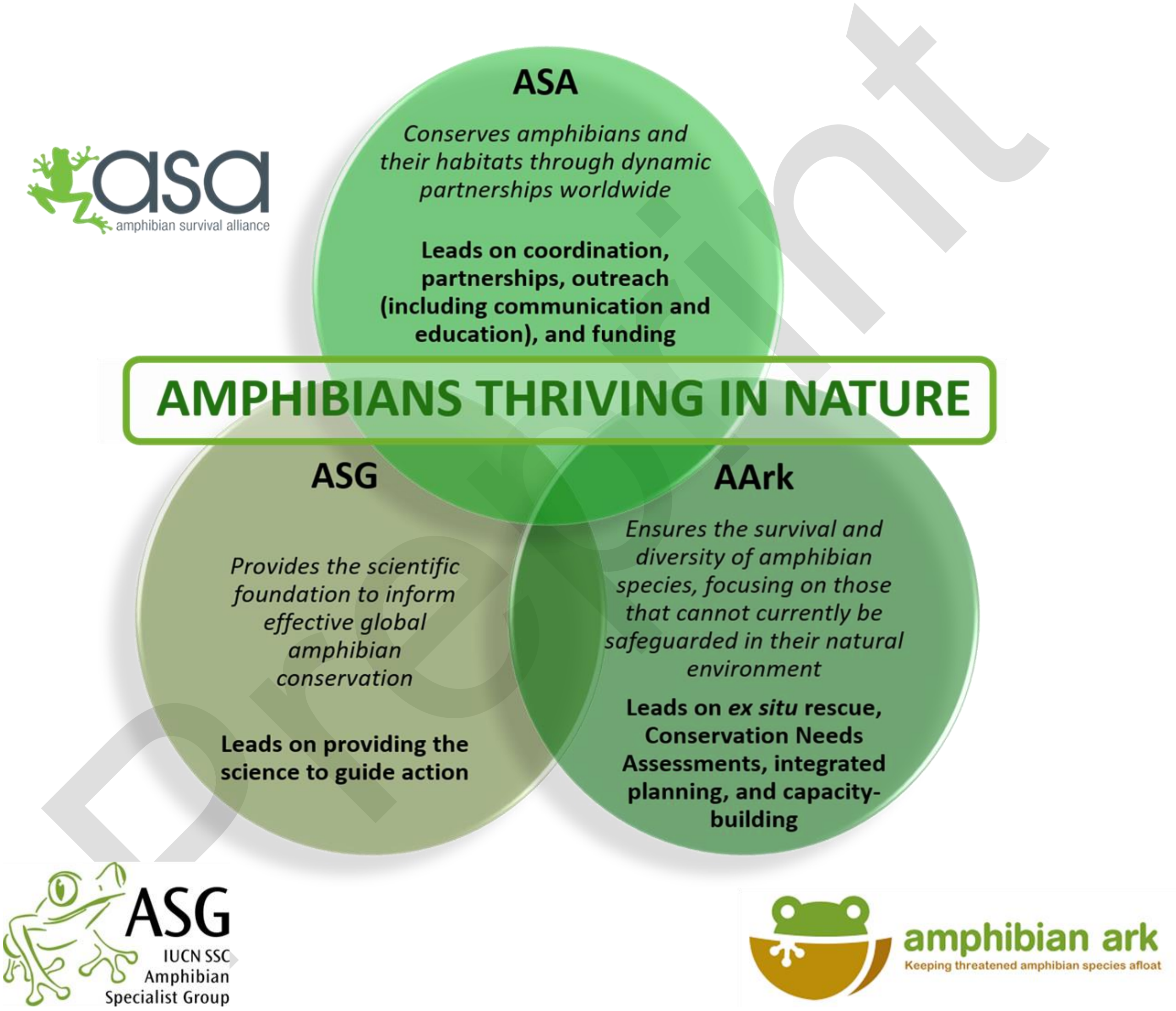


Figure 1.3. ASG, AArk and ASA work together on the global response to amphibian declines, under the shared Vision “Amphibian thriving in nature”.

Through the process of re-imagining what the ACAP could be, with the survey feedback in mind, we concluded that it was not possible for one document to answer all the needs of the amphibian community. Rather, it would be useful for ACAP to become two partner documents. The first being this document, which gathers the most up to date evidence on subjects related to amphibian conservation, providing a solid, citable, academic basis for action. The second is *The Bishop Guide to Amphibian Conservation*, an action-driven, more user-friendly source of practical solutions to be published after this document. Together, these documents form the third ACAP, covering the period of 2022-2032.

Our aim is for the two documents to guide and support amphibian conservation activities worldwide, being used as a framework for research and conservation, driving action and providing evidence-based advice to all involved in this sphere of work – conservation organisations, governments, funders and the general public– on how to address threats to amphibians and meet global conservation targets. While the scope of ACAP is global, many actions will need to be targeted at a local scale, and we have provided case studies throughout with examples of how global goals can be applied to a variety of contexts.

We were mindful that this version of the ACAP should be a product of the broader amphibian conservation community. Thus, we endeavoured to be as inclusive as possible in the status review. As such, individual chapters were drafted collaboratively by ASG's Thematic Working Groups, with introductory material drafted by members of the ASG Secretariat. This draft document is now available for open consultation with a request for feedback from the whole amphibian conservation community. Finally, it will be independently peer reviewed before publication. As such, we have aimed to develop a scientifically robust text, which resulted from a collaborative effort from the amphibian conservation community, and we are grateful to everyone who has and is supporting the process throughout.

This document consists of two introductory chapters and twelve thematic chapters divided into three sections:

1. **Threats** - Chapters 3-7 on climate change; ecotoxicology; habitat loss; infectious diseases; and trade and sustainable use
2. **Informing decision-making** - Chapters 8-10 on communications and education; conservation planning; and surveys and monitoring
3. **Species management** - Chapters 11-14 on conservation breeding; assisted reproductive technologies and biobanking; genomics; and translocations.

Amphibian conservation is a multi-faceted field and collaboration is critical to our success. In addition, overlap exists between these chapters as indeed many of them are interdisciplinary. Because of this, the chapters could be arranged in several different ways. Likewise, we appreciate the scope for additional subjects to be included within this document. For example, we have discussed creating new ASG thematic working groups to address invasive species and habitat restoration. However, during the timeframe of drafting this document expert groups were not yet available to cover these subjects. We look forward to their inclusion in future versions.

This document covers the ten-year period, 2022-2032. This timeframe was chosen based on the global scope of the ACAP, the time required to make progress on broad issues, and the resources required to update this document, balanced with likely advances in knowledge that will need to be incorporated into conservation decision-making.

As we have seen, evaluating the impact of previous ACAP versions is difficult. While still challenging to achieve with a document such as this, we aim to improve on measuring and communicating impact and use of this version. Academically, we can track citations in journals. The use of a digital object identifier (DOI) for this version will permit us to track online mentions of ACAP more widely, including in policy documents, news, blogs and social media. Recording mentions will allow us to measure how effectively we have communicated the existence of an updated ACAP document, and its academic use. However, while awareness of the updated ACAP is important, it is also necessary to assess the extent and type of use on the ground. The real challenge will be whether we can determine if the existence and use of ACAP results in positive outcomes and, ultimately, impacts the conservation status of individual species.

As a first step, assessing ACAP use by the conservation community will be achieved by obtaining feedback from the global community. Regional Groups are ideally positioned to report on local/regional advances, in a format which measures ASG's own adoption and on the ground implementation of ACAP recommendations. Similarly, a process to track implementation by our partner organisations – ASA and AArk – will be put in place. This can then be extended to the partners of these three organisations. As a final ambition, together we will also seek ways to understand whether the uptake and promotion of ACAP drives new resources to conservation initiatives (e.g. influences the priorities of funding mechanisms) and research.

The ultimate aim is to improve the conservation status of amphibian species, with fewer species classified as threatened. To track this, regular reassessments will be needed to identify and capture genuine improvements in the IUCN Red List. Eventually, this should result in an improving or stabilised Red List Index (Butchart et al., 2005). Changes such as this are unlikely to occur within the ten-year timeframe of this ACAP, due to the time necessary to improve species status and see this reflected on the Red List, but we believe this to be a worthy long-term vision for the conservation community and humankind as a whole. Our aim is that through implementation of this document, and future versions of ACAP, amphibians will no longer be the most threatened vertebrate taxon as threats will have decreased for all taxa, and we will see all amphibian species thriving in nature.

Acknowledgements

We are grateful to Andrés Valenzuela Sanchez and Jeanne Tarrant for their suggestions on this chapter.

References

Alford, R. A., Richards, S. J., & McDonald, K. R. (2013). Biodiversity of Amphibians. *Encyclopedia of Biodiversity* 1:169-178. doi:10.1016/B978-0-12-384719-5.00254-9

AmphibiaWeb. (2021). https://amphibiaweb.org. (2022). *https://amphibiaweb.org*. University of California: Berkeley, CA, USA.

Attademo, A., Peltzer, P., & Lajmanovich, R. (2005). Amphibians occurring in soybean and implications for biological control in Argentina. *Agriculture, Ecosystems & Environment* 106:389–394.

Berman, D., Meshcheryakova, E., & Bulakhova, N. (2016). The Japanese tree frog (*Hyla japonica*), one of the most cold-resistant species of amphibians. *Doklady Biological Sciences* 471(1):276-279.

Berman, D. I., Leirikh, A. N., & Meshcheryakova, E. N. (2010). The Schrenck newt (*Salamandrella schrenckii*, Amphibia, Caudata, Hynobiidae) is the second amphibian that withstands extremely low temperatures. *Doklady Biological Sciences* 431(1):131–134. doi:10.1134/S0012496610020171

Bishop, P. J., Angulo, A., Lewis, J. P., Moore, R. D., Rabb, G. B., & Garcia Moreno, J. (2012). The amphibian extinction crisis-what will it take to put the action into the Amphibian Conservation Action Plan? *S.A.P.I.E.N.S.* 5(2):97-111.

Burton, T. M., & Likens, G. E. (1975). Salamander populations and biomass in the Hubbard Brook experimental forest, New Hampshire. *Copeia* 3:541-546.

Butchart, S. H. M., Stattersfield, A. J., Baillie, J., Bennun, L. A., Stuart, S. N., Akçakaya, H. R. ... Mace, G. M. (2005). Using Red List Indices to measure progress towards the 2010 target and beyond. *Philosophical Transactions of the Royal Society B: Biological Sciences* 360:255–268. doi:10.1098/rstb.2004.1583

Butchart, S. H. M., Stattersfield, A. J., Bennun, L. A., Shutes, S. M., Akçakaya, H. R., Baillie, J. E. M. ... Mace, G. M. (2004). Measuring global trends in the status of biodiversity: Red List indices for birds. *PLoS biology* 2(12):e383. doi:10.1371/journal.pbio.0020383

Clarke, B. T. (1997). The natural history of amphibian skin secretions, their normal functioning and potential medical applications. *Biological Reviews* 72(3):365-379.

Cooper, J. (1992). *Symbolic and Mythological Animals*. New York, USA: HarperCollins.

Davic, R. D., & Welsh Jr, H. H. (2004). On the ecological roles of salamanders. *Annual Review of Ecology, Evolution, and Systematics* 35:405-434.

Doak, D. F., Bakker, V. J., Goldstein, B. E., & Hale, B. (2013). What is the future of conservation? *Trends in Ecology & Evolution* 29(2):77-81.

Duellman, W. E. (1999). *Patterns of distribution of amphibians: a global perspective*. Baltimore, USA: Johns Hopkins University Press.

DuRant, S. E., & Hopkins, W. A. (2008). Amphibian predation on larval mosquitoes. *Canadian Journal of Zoology* 86:1159–1164.

Earl, J. E., Luhring, T. M., Williams, B. K., & Semlitsch, R. D. (2011). Biomass export of salamanders and anurans from ponds is affected differentially by changes in canopy cover. *Freshwater Biology* 56:2473-2482.

Elinson, R. P., Del Pino, E. M., Townsend, D. S., Cuesta, F. C., & Eichhorn, P. (1990). A practical guide to the developmental biology of terrestrial-breeding frogs. *The Biological Bulletin* 179(2):163–177.

Frost, D. R. (1985). *Amphibian species of the world (Vol. 1)*. Lawrence, USA: Allen Press.

Frost, D. R. (2021). *Amphibian Species of the World: an Online Reference. Version 6.1. Electronic Database accessible at https://amphibiansoftheworld.amnh.org/index.php*. New York, USA. : American Museum of Natural History.

Gascon, C., Collins, J. P., Moore, R. D., Church, D. R., McKay, J. E., & Mendelson, J. R. I. (2007). *Amphibian conservation action plan: proceedings IUCN/SSC Amphibian Conservation Summit 2005*. Gland, Switzerland and Cambridge, UK: IUCN/SSC Amphibian Specialist Group.

Häfeli, H. P. (1971). Zur Fortpflanzungsbiologie des Alpensalamanders (Salamandra atra Laur [Reproductive biology of the alpine salamander (Salamandra atra Laur)]. *Revue Suisse de Zoologie* 78(2):235-293.

Hanken, J., & Wake, D. B. (1994). Five new species of minute salamanders, genus *Thorius* (Caudata: Plethodontidae), from Northern Oaxaca, Mexico. *Copeia* 3:573-590.

Hernández, H., & Blum, C. (2012). Distributed graph coloring: an approach based on the calling behavior of Japanese tree frogs. *Swarm Intelligence* 6(2):117-150.

Hernández Pibernat, H. (2012). *Swarm intelligence techniques for optimization and management tasks insensor networks.* (PhD). Universitat Politècnica de Catalunya, Barcelona, Spain.

Hime, P. M., Lemmon, A. R., Lemmon, E. C. M., Prendini, E., Brown, J. M., Thomson, R. C. ... Weisrock, D. W. (2021). Phylogenomics reveals ancient gene tree discordance in the amphibian tree of life. *Systematic Biology* 70(1):49-66.

Hoffmann, M., Hilton-Taylor, C., Angulo, A., Böhm, M., Brooks, T. M., Butchart, S. H. ... Veloso, A. (2010). The impact of conservation on the status of the world's vertebrates. *Science* 330(6010):1503–1509. doi:10.1126/science.1194442

IUCN. (2022). The IUCN Red List of Threatened Species. *Version 2021-3*:https://www.iucnredlist.org.

Kupfer, A., Müller, H., Antoniazzi, M. M., Jared, C., Greven, H., Nussbaum, R. A., & Wilkinson, M. (2006). Parental investment by skin feeding in a caecilian amphibian. *Nature* 440:926-929.

Loubser, G. J. J., Mouton, P. l. F. N., & Nel, J. A. J. (2001). The ecotourism potential of herpetofauna in the Namaqua

National Park, South Africa. *South African Journal of Wildlife Research* 31(1-2):13-23.

Mardiastuti, A., Masy'ud, B., Ginoga, L. N., Sastranegara, H., & Sutopo. (2020). Wildlife species used as traditional medicine by local people in Indonesia. *Biodiversitas Journal of Biological Diversity* 22(1):329-337. doi:10.13057/biodiv/d220140

Mbaiwa, J. E. (2011). Changes on traditional livelihood activities and lifestyles caused by tourism development in the Okavango Delta, Botswana. *Tourism management* 32(5):1050-1060.

McDiarmid, R. W. (1978). Evolution of parental care in frogs. In: G. M. Burghardt & M. Bekoff (Eds.), *The development of behavior: comparative and evolutionary aspects*. New-York, USA: Garland STPM Press.

Millennium Ecosystem Assessment. (2005). *Ecosystems and Human Well-Being: Wetlands and Water Synthesis*. Retrieved from Washington, DC.:

Minter, L. R. (2004). *Breviceps macrops* Boulenger, 1907. Pp. 180182. In: L. R. Minter, M. Burger, J. A. Harrison, H. H. Braack, P. J. Bishop, & D. Kloepfer (Eds.), *Atlas and Red Data Book of the Frogs of South Africa, Lesotho and Swaziland*. Smithsonian Institution, USA: SI/MAB Series No. 9.

Mohneke, M. (2011). *(Un) sustainable use of frogs in West Africa and resulting consequences for the ecosystem.* (PhD). Humboldt-Universität zu Berlin, Berlin, Germany.

Myers, C. W., Daly, J. W., & Malkin, B. (1978). A dangerously toxic new frog (*Phyllobates*) used by Emberá Indians of western Colombia, with discussion of blowgun fabrication and dart poisoning. *Bulletin of the American Museum of Natural History* 161(2):1-28.

Nunes-de-Almeida, C. H. L., Haddad, C. F. B., & Toledo, L. F. (2021). A revised classification of the amphibian reproductive modes. *Salamandra* 57(3):413-427.

Pérez, A. E., Schuster, V., & Jofré, D. P. (2018). Amphibiomorphic modeled and painted pottery from argentine patagonia and Central-Southern Chile. Functional interpretation and identification of species based on mimetic and aposematic traits. *Open Archaeology* 4:394-405.

Pough, F. H. (1980). The advantages of ectothermy for tetrapods. *The American Naturalist* 115:92-112.

Pough, F. H. (1983). Amphibians and reptiles as low-energy systems. In: W. P. A. a. S. I. Lustick (Ed.), *Behavioral energetics: the cost of survival in vertebrates* (pp- 141-188). Columbus, USA: Ohio State University Press.

Rabb, G. B., & Rabb, M. S. (1960). On the mating and egg-laying behavior of the Surinam toad, *Pipa pipa*. *Copeia* 4:271-276.

Rittmeyer, E. N., Allison, A., Gründler, M. C., Thompson, D. K., & Austin, C. C. (2012). Ecological guild evolution and the discovery of the world's smallest vertebrate. *PLoS One* 7(1):e29797.

Roelants, K., Gower, D. J., Wilkinson, M., Loader, S. P., Biju, S. D., Guillaume, K. ... Bossuyt, F. (2007). Global patterns of diversification in the history of modern amphibians. *Proceedings of the National Academy of Sciences* 104(3):887-892.

Sabater-Pi, J. (1985). Contribution to the biology of the giant frog (*Conraua goliath*, Boulenger). *Amphibia-Reptilia* 6(2):143-153.

Safi, K., Armour-Marshall, K., Baillie, J. E., & Isaac, N. J. (2013). Global patterns of evolutionary distinct and globally endangered amphibians and mammals. *PLoS One* 8(5):e63582. doi:10.1371/journal.pone.0063582

Schulte, L. M., Ringler, E., Rojas, B., & Stynoski, J. L. (2020). Developments in amphibian parental care research: history, present advances, and future perspectives. *Herpetological Monographs* 34(1):71-97. doi:10.1655/HERPMONOGRAPHS-D-19-00002.1

Seale, D. B. (1980). Influence of amphibian larvae on primary production, nutrient flux, and competition in a pond ecosystem. *Ecology* 61(6):1531-1550.

Secor, S. M., & Lignot, J.-H. (2010). Morphological plasticity of vertebrate aestivation. In: C. A. Navas & C. J. E. (Eds.), *Aestivation. Progress in molecular and subcellular biology* (Vol. 49, pp- 183-208). New York, USA: Springer.

Secretariat of the Convention on Biological Diversity. (2020). *Global Biodiversity Outlook 5*. Montreal, Canada: Convention on Biological Diversity.

Seimon, T. A., Seimon, A., Daszak, P., Halloy, S. R. P., Schloegel, L. M., Aguilar, C. A. ... Simmons, J. E. (2007). Upward range extension of Andean anurans and chytridiomycosis to extreme elevations in response to tropical deglaciation. *Global Change Biology* 13(1):288–299. doi:10.1111/j.1365-2486.2006.01278.x

Semlitsch, R., K. , O'Donnell, K., & Thompson, F. I. (2014). Abundance, biomass production, nutrient content, and the possible role of terrestrial salamanders in

Missouri Ozark forest ecosystems. *Canadian Journal of Zoology* 92:997-1004.

Sinclair, B. J., Stinziano, J. R., Williams, C. M., MacMillan, H. A., Marshall, K. E., & Storey, K. B. (2013). Real-time measurement of metabolic rate during freezing and thawing of the wood frog, *Rana sylvatica*: implications for overwinter energy use. *The Journal of experimental biology* 216:292-302. doi:10.1242/jeb.076331

Small, C., & Cohen, J. E. (2004). Continental Physiography, Climate, and the Global Distribution of Human Population. *Current Anthropology* 45(2):269-277.

Smirina, E. M. (1994). Age determination and longevity in amphibians. *Gerontology* 40(2-4):133-146. doi:10.1159/000213583

Sodhi, N. S., Bickford, D., Diesmos, A. C., Lee, T. M., Koh, L. P., Brook, B. W. ... Bradshaw, C. J. (2008). Measuring the meltdown: drivers of global amphibian extinction and decline. *PLoS One* 3(2):e1636.

Solano-Zavaleta, I., García-Vázquez, U. O., & Mendoza-Hernández, A. A. (2009). Notas sobre la distribución geográfica de las salamandras *Pseudoeurycea gadovii* y *Pseudoeurycea melanomolga* (Caudata: Plethodontidae). *Revista Mexicana de Biodiversidad* 80:575- 577.

Springborn, M. R., Weill, J. A., Lips, K. R., Ibáñez, R., & Ghosh, A. (2020). Amphibian collapses exacerbated malaria outbreaks in Central America. *medRxiv* 2020.12.07:20245613. doi:10.1101/2020.12.07.20245613

Stuart, S., Hoffmann, M., Chanson, J., Cox, N., Berridge, R., Ramani, P.,

& Young, B. (2008). *Threatened amphibians of the world*.

Stuart, S. N. (2012). Responding to the amphibian crisis: Too little, too late? *Alytes* 29(1–4):9–12.

Stuart, S. N., Chanson, J. S., Cox, N. A., Young, B. E., Rodrigues, A. S., Fischman, D. L., & Waller, R. W. (2004). Status and trends of amphibian declines and extinctions worldwide. *Science* 306(5702):1783-1786. doi:10.1126/science.1103538

Summers, K., McKeon, C. S., & Heying, H. (2006). The evolution of parental care and egg size: a comparative analysis in frogs. *Proceedings of the Royal Society of London B: Biological Sciences* 273(1587):687-692.

Teng, Q., Hu, X.-F., Luo, F., Cheng, C., Ge, X., Yang, M., & Liu, L. (2016). Influences of introducing frogs in the paddy fields on soil properties and rice growth. *Journal of soils and sediments* 16(1):51-61.

Thibaudeau, G., & Altig, R. (1999). Chapter 7. Endotrophic Anurans: Development and Evolution. In: R. W. McDiarmid & R. Altig (Eds.), *Tadpoles: The Biology of Anuran Larvae* (pp- 295–337). Chicago, USA: University of Chicago Press.

Valencia-Aguilar, A., Cortés-Gómez, A. M., & Ruiz-Agudelo, C. A. (2013). Ecosystem services provided by amphibians and reptiles in neotropical ecosystems. *International Journal of Biodiversity Science, Ecosystem Services and Management* 9:257–272.

Vences, M., & Köhler, J. (2007). Global diversity of amphibians (Amphibia) in freshwater. In: *Freshwater Animal Diversity Assessment*. New York, USA: Springer.

Voituron, Y., de Fraipont, M., Issartel, J., Guillaume, O., & Clobert, J. (2011). Extreme lifespan of the human fish (*Proteus anguinus*): a challenge for ageing mechanisms. *Biology Letters* 23(7):105-107. doi:10.1098/rsbl.2010.0539

Won, H. S., Kang, S. J., & Lee, B. J. (2009). Action mechanism and structural requirements of the antimicrobial peptides, gaegurins. *Biochimica et Biophysica Acta (BBA)-Biomembranes* 1788(8):1620-1629.

Yamashita, M., Izumi-Kurotani, A., Mogami, Y., Okuno, M., Naitoh, T., & Wassersug, R. J. (1997). The Frog in Space (FRIS) experiment onboard Space Station Mir: final report and follow-on studies. *Biological Sciences in Space* 11(4):313-320.

Zhu, W., Chuah, Y. J., & Wang, D.-A. (2018). Bioadhesives for internal medical applications: a review. *Acta biomaterialia* 74:1-16.

Zhu, Y., Li, Z., Liu, H., He, X., Zhang, Y., Jin, J. ... Lai, R. (2014). Novel analgesic peptides from the tree frog of *Hyla japonica*. *Biochimie* 99:38-43.

Zipkin, E. F., DiRenzo, G. V., Ray, J. M., Rossman, S., & Lips, K. R. (2020). Tropical snake diversity collapses after widespread amphibian loss. *Science* 367(6479):814-816.

ZSL. (2020). Edge of Existence

.

Chapter 2. Common themes and challenges

Ariadne Angulo[1], Sally Wren[1,2], Ruth Marcec-Greaves[1,3], Jos Kielgast[1,4,5], Jennifer Luedtke[1,6], Louise Hobin[1], Kelsey Neam[1,6], Janice Chanson[1], Luis Fernando Marin da Fonte[1,7] and Amaël Borzée[1,8]

[1] IUCN SSC Amphibian Specialist Group, 3701 Lake Shore Blvd W, P.O. Box 48586, Toronto, Ontario M8W 1P5, Canada

[2] Department of Zoology, University of Otago, 340 Great King Street, Dunedin 9016, New Zealand

[3] Honduras Amphibian Rescue and Conservation Center, 1200 SE Oak Ridge Drive, Oak Grove, Missouri, 64075, USA

[4] Section for Freshwater Biology, Department of Biology, University of Copenhagen, Universitetsparken 4, DK-2100, Denmark

[5] Center for Macroecology, Evolution and Climate, Natural History Museum of Denmark, Universitetsparken, 15, DK-2100, Denmark

[6] Re:Wild, P.O. Box 129, Austin, Texas, 78767, USA

[7] Amphibian Survival Alliance, Porto Alegre, 91770-000, Rio Grande do Sul, Brazil

[8] Laboratory of Animal Behaviour and Conservation, College of Biology and the Environment, Nanjing Forestry University, Nanjing, People's Republic of China

Abstract

In this chapter we provide a brief overview of the importance of taxonomy, extinction risk assessments and evidence-based decision-making for conservation work, highlighting key developments in each of these subjects, and suggested approaches to help address some of the

current challenges. It is important to bear in mind that, while working on specific amphibian conservation problems, we as a community also strive to make advances in these common themes, which are necessary for effective action worldwide.

Introduction

Taxonomy, extinction risk assessments, and evidence-based decision-making are key to informing virtually every aspect of conservation work. In previous versions of ACAP there were stand-alone chapters for Red List assessments (i.e. extinction risk; Chapter 9, Gascon et al., 2007) and taxonomy and systematics (Chapter 10, Gascon et al., 2007). However, since these subjects underpin all conservation actions, instead of having dedicated chapters in this document we briefly present them in this introductory chapter.

In terms of challenges, much of what is in this chapter was inspired by responses to a question asked to all IUCN SSC Amphibian Specialist Group (ASG) members when signing up to join the ASG in 2013-2016 and 2017-2020: “Other than funding, what is the single largest factor limiting effective conservation strategies for amphibians at global and regional levels?” (Note that we have included a section on “resourcing amphibian conservation” later in this chapter). These expert perceptions highlighted a number of obstacles which are almost ubiquitous to those working in amphibian conservation, including lack of coordination and collaboration, lack of government support, amphibians not being prioritised, and a lack of knowledge of species biology/ecology.

Taxonomy

The capacity to effect changes through conservation action is underpinned by accurately identified and delineated species (Angulo & Icochea, 2010). The discipline of taxonomy thus plays a fundamental role in species conservation (Mace, 2004 and references therein), and has a bearing on everything from surveys and monitoring, extinction risk assessments, prioritisation approaches (e.g. Evolutionarily Distinct and Globally Endangered (EDGE) rankings, Alliance for Zero Extinction (AZE) and Key Biodiversity Areas (KBA) sites), funding acquisition, and species conservation planning and implementation.

Taxonomy, however, is not static, and it undergoes change as a result of name changes and new species descriptions. Generally speaking, taxonomic change does not appear to have a consistent effect on conservation, although splitting taxa could lead to increased protection (Morrison III et al., 2009). Amphibian taxonomy has seen significant changes over the last two decades, both in terms of efforts to align higher-level taxonomic hierarchy with phylogenetic hypotheses (e.g. Frost et al., 2006; Pyron & Wiens, 2011), and in terms of new species descriptions, which have been occurring at a rate of about 100-150 species/year (Tapley et al., 2018; Streicher, Sadler & Loader, 2020; AmphibiaWeb, 2021). Amphibians as a clade still have many undescribed species and, while it is unclear exactly how many, conservative estimates by Giam et al. (2012) placed the number at over 3000 undescribed species when the study was published. This suggests there are approximately 900 additional species still left to be described at this time, and up to half of them could be threatened (Liu et al., 2022).

A limitation for conservation is that species are the basis for conservation assessments and species management (Mace, 2004), and if a species is not described it cannot be assessed for extinction risk. Or, if it is described but includes cryptic taxa, the assessment will not reflect the species' real extinction risk (see e.g. Angulo & Icochea, 2010). Furthermore, taxonomic splits are likely to result in range reduction, which are more likely to result in species being listed as threatened. Species are described primarily by taxonomists, and where there are few taxonomists and few resources to undertake taxonomic studies, species descriptions will likely lag behind, having a direct impact on our ability to identify threatened species in a timely manner. The term “taxonomic impediment” has come to be associated with this phenomenon (Raposo et al., 2021), with a suite of multi-faceted reasons accounting for this situation (see Engel et al., 2021).

Taxonomic uncertainty, or the uncertainty surrounding the delineation of a biological entity, is another challenge. Extinction risk assessments and conservation action recommendations are based on specific biological entities, but when our understanding of these entities is unclear or changes, then these assessments have to be modified accordingly (see examples in Angulo & Icochea, 2010). Furthermore, species whose taxonomic validity is in question (due to e.g. unknown provenance, lost type specimens, etc.) are typically assessed as Data Deficient.

Some suggestions that could be implemented to help advance both amphibian taxonomy and conservation include:

1. Taxonomic clarity list(s): there are many cases of species where taxonomy is a major issue to an adequate extinction risk assessment and subsequent decision-making. It would thus be helpful to identify, contribute to and maintain a list of those instances where taxonomic clarity is specifically needed for conservation decision-making, in particular, instances of species listed as Data Deficient due to taxonomic uncertainty (currently 414 of 7,296 species based on the IUCN Red List; ~5%). This is something that could be led from the taxonomic community.

2. Awareness-raising: obtaining funding for taxonomic work is extremely difficult, in certain instances perhaps even more so than obtaining funding for conservation. It is therefore important to raise awareness about the importance of taxonomy for conservation among funding entities, conservation organisations and the general public, and where possible and relevant include both aspects in fundraising efforts. Both taxonomists and conservationists could join forces in this endeavour.
3. Increase collaborations: certain parts of the world have a dearth of taxonomists and resources relative to their respective species richness. Creating a network to strengthen international collaborations may help advance taxonomic studies in these regions. This could be led from the amphibian taxonomic community, with support from the conservation community (e.g. establishing such a network within the ASG).

Updating the IUCN Red List assessments

The process and task of assessing the conservation status of amphibians for the IUCN Red List has evolved over time. The first Global Amphibian Assessment (GAA) completed the extinction risk assessment of the then-known 5,743 species between 2001-2004 (Stuart et al., 2004). Each species was evaluated against the IUCN Red List Categories and Criteria (IUCN, 2012) through a series of regional workshops to assess their global conservation status. Before it was dissolved, the GAA team made updates to the IUCN Red List in 2006 and 2008, adding new species and some re-assessments. Overall, the key challenges of the GAA included convening the global herpetological community to undertake a comprehensive assessment for the first time, and maintaining consistency in the application of the categories and criteria across all regions. More information on the GAA process is available on the ASG website (www.iucn-amphibians.org/wp-content/uploads/2019/03/Amphibians-Initiative-2008-webcontent-Downloaded-27Nov2018-1.pdf).

The ASG's Amphibian Red List Authority (ARLA) was established in 2009 to continue the work of adding newly described species to the IUCN Red List each year and to update GAA assessments as needed. By that time, more than 6,000 species had been described. The appointment of Regional ARLA Coordinators began in 2010 to support and guide this work, which was undertaken by short-term volunteers. After six years of continuous effort, the ARLA found that a large backlog of new species and out-of-date GAA assessments had accumulated. The strategy for maintaining the amphibian data on the Red List was reviewed at that time and identified several persistent challenges, including the increasing rate of taxonomic changes highlighted earlier in this chapter; the emergence and evolution of threats faced by amphibians; and the ARLA's reliance on volunteers. The amphibian assessments on the Red List were becoming outdated faster than the ARLA could update them.

In response to these challenges, the ARLA launched the second Global Amphibian Assessment (GAA2) in 2015. Replicating the approach of the first GAA, funding was gradually raised to employ a core global ARLA team to coordinate assessment projects for each ASG region in collaboration with Regional ARLA Coordinators, ASG Regional Chairs, and key experts in amphibian conservation and research. Due for completion in 2022, the GAA2 synthesises 18 years' worth of data, and has assessed the global conservation status of more than 8,000 species (~ 95% of currently known species) through a combination of in-person and virtual workshops of different sizes, internships and consultant contracts, and collaborations with national red list processes. In addition to the challenges mentioned above, the GAA2 built the case for a second assessment process to donors and partners; tackled the increasing data requirements for red listing; incorporated successive versions of the IUCN Red List Guidelines which required changes to methods such as the calculation of extent of occurrence (EOO); and shifted to an entirely virtual assessment process in 2020 due to the emergence of the COVID-19 pandemic.

The forthcoming third Global Amphibian Assessment (GAA3) will aim to update all 2009-2022 assessments and evaluate the global extinction risk of all newly described species by 2030, as per the IUCN Red List Strategic Plan 2021-2030. By that time, the number of valid amphibian species is expected to approach 10,000. Building on the GAA and GAA2 models and lessons learned during those initiatives, several technological and methodological innovations are being developed to improve the quality of assessment data and decrease the number of years required to undertake a global amphibian assessment.

The ARLA invites interested parties to provide information to the GAA3, collaborating on the following priorities:

- Publishing data relevant to Red List assessments in species descriptions and survey and expedition reports, where possible. Of particular interest is information regarding ecological traits that increase a species' vulnerability to specific threats; past and present habitat quality; current population status, and past/present/future threatening processes.
- Contacting the ARLA when a species urgently requires assessment or reassessment in light of significant emerging threats, and where new adequate information is available for the assessment of newly described Not Evaluated (NE) and Data Deficient (DD) species. In such instances the ARLA will endeavour to prioritise their extinction risk assessment in a timely manner.
- Improving the quality of distribution maps, including historical ranges.

- Increasing the consistency and accuracy with which certain threats are evaluated, including emerging diseases, trade, over-harvesting, and climate change.
- Undertaking Green Status of Species assessments alongside Red List assessments.
- Broadening participation in the assessment process to include the knowledge and experience of indigenous peoples, citizen science initiatives, managers of conservation projects, zoo and aquarium staff, members of governmental agencies, donors, etc.
- Strengthening linkages with national red list processes.
- Encouraging the use of Red List data – both the category and supporting information – in conservation planning, collaboration, and action at global and national levels.

Limited understanding of species ecology and biology

In an ideal scenario we would be able to gather and access the basic data that are needed to understand the conservation status and needs of all species. As this is not the case, we need to make conservation decisions based on existing information, which is often imperfect and incomplete. Following a precautionary approach, the IUCN Red List Categories and Criteria (IUCN, 2012) allows the use of a variety of types of data quality, including observed, estimated, projected, inferred, and suspected. In addition, the IUCN Red List differentiates between required and recommended data, so as to facilitate assessments in data-poor situations. These approaches enable the assessment of the extinction risk of species that have different types and amounts of data and different levels of uncertainty associated with the data. While IUCN Red List assessments are robust to missing data (Maes et al., 2015), the data needed to accurately assess all species against all the IUCN Red List criteria are currently crucially missing or too uncertain for 16% of amphibians listed as DD, which is higher than the 14% DD mammals and the 0.4% DD birds (IUCN, 2022). This is especially relevant as half of amphibian species listed as DD are estimated to be threatened (González-del-Pliego et al., 2019).

The data that are typically used to inform extinction risk assessments include geographic range, population status, population trend, habitat and ecology, threats, and conservation actions in place. These are covered in the IUCN Red List website (IUCN, 2022, see https://www.iucnredlist.org/assessment/supporting-information#Population). The Amphibian Ark Conservation Needs Assessment (CNA) compiles additional and complementary information derived from 26 questions, seeking to determine the conservation needs of any species (https://www.conservationneeds.org/Help/EN/QuestionsAnswers.htm). Together, both these resources list data that, if all available, would allow a comprehensive picture of the conservation status and needs of an amphibian species. However, not all types of data are equally available, and some are more resource-intensive and thus not as easy to obtain. Where extinction risk is concerned, the most commonly missing information is that relating to population status, trends, species-specific life history (much information is inferred from known congeners and used as a proxy for the lesser known species), certain types of threats, their synergies, and their relative contributions to any observed declines.

Only a fraction of these types of data become available for even the most studied species (Nori, Villalobos & Loyola, 2018). Furthermore, once a species is assessed, additional knowledge is required to plan appropriate conservation interventions, and understand and remedy the original causes of decline. This not only encompasses the target species, but also the habitats in which it thrives, the behaviours that need to be expressed and the ecological requirements to ensure that the environment provided is adequate for the conservation of the species (Conde et al., 2019).

The reasons behind the lack and paucity of data can be as varied as they are subtle. Based on the ASG's membership data, the most frequently mentioned challenge in amphibian conservation is lack of resources and investment, which can be extended to research on the subject matter. However, as can be seen in the "Resourcing amphibian conservation" section, while this is reflected in the figures that we have collated, there are also other important reasons to consider. There are not very many papers that cover this subject, so we offer some reflections based on our own collective experiences, some of the references that we could find, as well as some recommendations:

1. Geographic and thematic realities and biases: the highest amphibian species richness can be found in tropical regions, where there is still an undetermined number of undescribed species (Moura & Jetz, 2021). Taxonomy is thus a priority for many tropical herpetologists, who tend to develop their skills in this field. The distribution of threatened species also coincides with many amphibian species richness hotspots, so in a way threatened species compete for attention with the undescribed species. In addition, there are geographic and cultural aspects that may play a role, such as the availability of professional opportunities and the support, or lack thereof, to publish scientific papers (Young et al., 2001; Urbina-Cardona, 2008). Understanding what these realities are within an amphibian biologist's own region and community, as well as increasing international collaborations to advance amphibian taxonomy in regions with few taxonomists and resources to undertake taxonomic studies (see Taxonomy section), may help to take further steps to change the status quo.
2. Data ownership and data sharing: use of unpublished data can be a sensitive issue, especially among certain disciplines, career stages, cultural perspectives and stakeholders (e.g. consulting firms involved in environmental impact assessments;

von May et al., 2008). On the opposite end of the spectrum, some types of information that don't pertain to the immediate field of interest may not be prioritised for use (e.g. information on threats in a taxonomically-focused programme). Consideration of data sharing among the multiple stakeholders would be a valuable development for increased access to data and knowledge, as would developing and improving policies on how data would be used and contributors acknowledged (Tapley et al., 2018).

3. Data quality: where data are available there are sometimes questions regarding how they are collected. This is especially the case when the data are not published in peer-reviewed journals (see e.g. von May et al., 2008). In some instances and under certain conditions, it may be safer to use some types of data (e.g. occurrence data with specialist identification) over others (e.g. survey data that require standardised methodologies). Citizen science projects, such as the Amphibian BioBlitz run by the iNaturalist.org platform (https://www.inaturalist.org/projects/global-amphibian-bioblitz) or FrogID in Australia (https://www.frogid.net.au), have the potential to provide important occurrence data and in this way help bridge some knowledge gaps in light of the number of participants and data (more than 220,000 participants contributing data for more than 4,900 species in the iNaturalist Amphibian BioBlitz).
4. Capacity to fundraise: the ability to bring in financial resources for project work can be limited by the lack of familiarity with the process of writing and applying for grants, which may preclude amphibian biologists from applying or from presenting competitive proposals. Furthermore, limited fluency in English may be another constraint in countries that speak languages other than English, as most calls for proposals are in English. More training opportunities in fundraising would help build capacity in this regard, while multicultural collaborations could help with proposal

development in the English language (see the section on "Resourcing amphibian conservation" for more information). In addition, grant providers could also help overcome this issue by accepting applications in languages other than English.

5. Synthesis: new studies are constantly being published; however, the scientific literature tends to be dispersed across many journals, making it difficult to get an overview of the “big picture”. Thus, there is a need for studies that bring together the various sources of information into a cohesive body of work that may allow for a quicker identification of knowledge and gaps, which can in turn help inform what kind of data are still needed. Research communities would be well positioned to lead these sorts of studies.
6. Coordination: individual amphibian biologists are often comfortable working within their established sites and their networks, but in order to address knowledge gaps more effectively at a country or regional level, higher-level coordination is needed. Coordination requires dedicated effort and time, and unfortunately it is rarely contemplated outside of a specific project or organisation; yet, it is absolutely essential to increasing efficiencies and filling knowledge gaps. Because of this, higher-level coordination efforts would be best led by institutions such as government agencies, museums, NGOs and herpetological societies, and/or (depending on the scope) the ASG, Amphibian Survival Alliance (ASA) or the Atelopus Survival Initiative (ASI) when appropriately resourced.

Evidenced-based conservation action

Over the past two decades there has been a growing push for evidence-based conservation action, based on the example of evidence-based reforms in medicine and public health (Pullin & Knight, 2001). The aim of such initiatives is to close the gap between scientific knowledge and conservation action (Sutherland et al., 2004), avoid repetition of unsuccessful interventions, and more effectively use the limited funding that is available to achieve the biggest conservation impact.

However, making conservation decisions based on evidence relies on the relevant evidence being available to those making the decisions. Specifically, it requires monitoring and evaluation of conservation actions (Pullin & Knight, 2001) and reporting of what is found (both successes and failures) in a format that is freely available to others involved in conservation decision-making. This requires that the information be available in a language that can be understood by the decision-makers (Amano et al., 2021), and that there is not a significant delay in publishing relevant evidence, which needs to be available in a timely manner to have maximum impact on conservation action (Christie et al., 2021). Furthermore, some evidence will clearly help in making better decisions, particularly where the benefits of a specific approach have been well assessed, e.g. the removal of an invasive fish which preys on a threatened amphibian species (Sutherland et al., 2021). However, it may be more complex to apply evidence-based thinking to multi-dimensional issues, operating in context-specific situations, where directly relevant evidence is unavailable (Adams & Sandbrook, 2013).

While there has been an increase in effort to make results more freely available, for example the establishment and growth of the Conservation Evidence information resource (www.conservationevidence.com) and the open access journal Conservation Evidence (Sutherland et al., 2004; Sutherland et al., 2019), there are still significant biases in reported results. For instance, Christie et al. (2020), found that approximately 90% of the published evidence on amphibian conservation interventions in the Conservation Evidence journal is based on studies from North America, Western Europe and Australia. Furthermore, taxonomic bias was also clear, with only a single study on Gymnophiona. As such, extrapolating results to different taxa in tropical climates and habitats may not be appropriate. In addition, negative results are often underreported for a variety of reasons, such as difficulty to publish such results in peer-reviewed journals, and potential stigma when applying for future funding.

In order to increase the use of available evidence in amphibian conservation, with the wider aim of improving conservation outcomes, we encourage researchers and implementers to:

1. Review existing evidence-based resources: when planning conservation interventions, consult the available evidence-based literature and broader resources to inform your decision-making process. Some important resources include the Conservation Evidence website (www.conservationevidence.com), which currently gathers ca. 130 actions for amphibians, and the publications "Amphibian Conservation: Evidence for the effects of interventions" (https://www.conservationevidence.com/synopsis/download/7) and What Works in Conservation (https://www.openbookpublishers.com/product/1490).
2. Plan up front to report results: methodically record results of interventions that you are undertaking, so that you can report on results whether or not the action was successful.
3. Report your findings: communicate your results in a timely manner, and preferably in a format that will be freely available to others. This may be in an Open Access journal, or could be within a newsletter, bulletin, or magazine, such as the amphibian conservation community's publication, FrogLog. Also consider if it may be more useful to report your findings in a specific language, or multiple languages.

4. Strategically fill gaps in the current evidence-base: aim to specifically report on effectiveness of conservation actions outside Western Europe and North America, and with better representation of all taxa. This may be via publication of information already gathered, or strategically aiming to fill known gaps.

Resourcing amphibian conservation

Amphibians are the most threatened vertebrate class on earth (IUCN, 2022), yet, the level of global investment in amphibian conservation has not been commensurate with the amphibian extinction crisis, which has been known and publicised for several decades. Even within the often financially constrained world of conservation, chronic and severe underfunding has been a persistent issue (Bishop et al., 2012). Armed with the results of the first GAA, a group of amphibian experts were convened to the 2005 Amphibian Conservation Summit in Washington, USA to develop the first ACAP. It was estimated that implementing ACAP would cost over US$ 400 million over a period of five years (2006-2010; Gascon et al., 2007). As global fundraising for amphibians was not tracked it is unclear how much of these funds were raised; however, we know that it was nowhere near that target. There aren't many studies that examine amphibian conservation spending, but we know for example that in the United States amphibians receive just one-quarter of the Endangered Species Act (ESA) funding that other vertebrate classes do (Gratwicke, Lovejoy & Wildt, 2012). There are also documented instances of lost support. For example, the United States Fish and Wildlife Service (USFWS) managed the Amphibians in Decline Fund, which supported conservation efforts in 25 countries from 2010-2016. Unfortunately, the programme ended once funding dried up (Scott, 2021). The collective experience of amphibian-focused groups and organisations, including ASG, are very much in line with this finding.

This scenario, and the continuing difficulties in supporting amphibian conservation at a global scale, begs a couple of questions: 1) why is it so difficult to fundraise for amphibian conservation, and 2) when fundraising is successful, how much has been raised?

The first question is more complex as there are likely many aspects at play. To begin with, amphibians are not part of the charismatic megafauna that often get the most attention. It has been shown that factors such as charisma are often more important than ecological information or conservation status in driving individuals' willingness to pay for biodiversity conservation efforts, and that individuals often have preferences for species more similar to humans (Colléony et al., 2017; Martín-López, Montes & Benayas, 2007). The second question, however, is something that we can investigate more easily, especially when referring to project funding. In order to better understand the international financial support received by amphibian projects we wrote to established non-taxonomically focused biodiversity conservation funds, supporting organisations and donors. We approached twenty organisations that regularly provide grants, awards and materials for projects that support general biodiversity conservation and asked them about patterns of applications and funding for different taxa as well as perspectives on what changes may be needed.

Of the twenty organisations contacted, twelve replied positively. However, because of the focus of some of these organisations or how they organised their project support (for example, not explicitly by taxonomic group, or with different groupings), not all of the responses could be used in the same way. A summary for those organisations that did record the number of grants awarded by taxonomic group is provided in Figure 2.1. It is important to note that the data provided covered different timeframes or specific programmes, so we used proportions of what was reported to account for these differences. Several organisations funded multi-taxa projects or projects without a taxonomic focus (e.g. habitat-based), which may have been beneficial to amphibians. Most of the organisations surveyed have been providing grants for amphibian conservation for a decade or more.

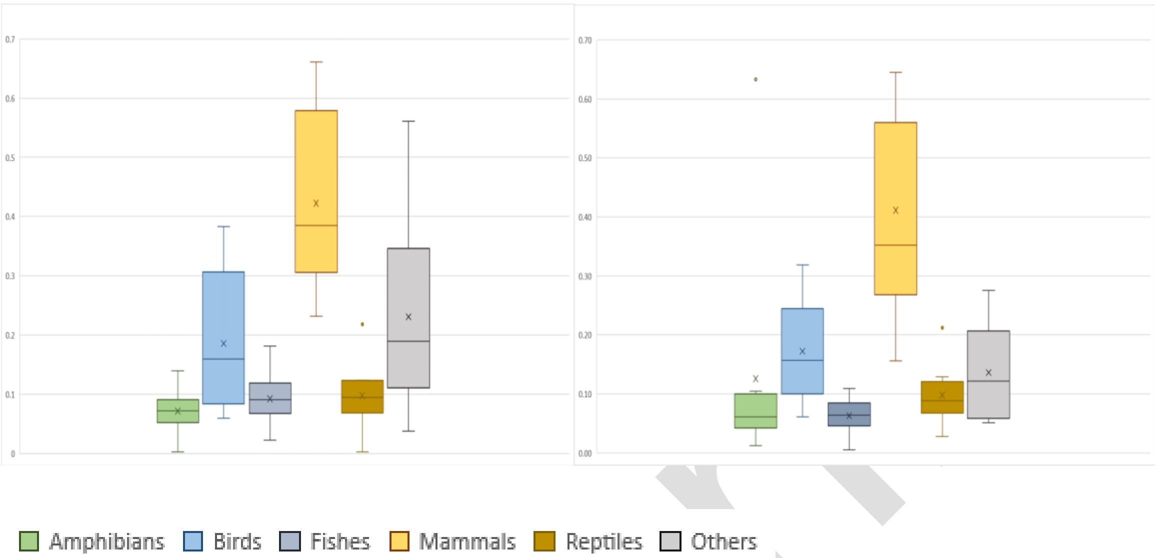


Figure 2.1: A sample of funding for amphibian conservation compared with other taxa, for which data were available; a) Focal taxa by proportion of funded projects (n = 9 funders), and b) Focal taxa by proportion of total dollar investment (n = 8 funders). Note: The ‘other’ category encompasses projects for plants, fungi and invertebrates, and those which are not taxon-specific. Source: A. Angulo & S. Wren, unpublished data.

One organisation had a grant programme specifically for amphibians, so over 60% of projects funded had amphibians as the focal taxon (seen as an outlier in Figure 2.1a). For the remainder of respondents, the proportion of funded proposals and proportion of dollar spending that focus on amphibian projects were relatively small, particularly when compared with those for birds and mammals. Mean dollar investment in amphibians was lower than that for all other vertebrate taxa.

Of those organisations that did keep track of accepted and submitted proposals, the proportions of amphibian applications that were funded were comparable to or higher than other taxonomic groups in their respective grant periods (14.3% - 29%). Most organisations did not have a policy for funding a specific number, proportion, or dollar amount for any given taxa; however, several responded that they do take into account, for example, the high proportion of threatened amphibian species, when reviewing applications.

While it is true that, of the data assessed, amphibian proposals receive less funding relative to their vertebrate counterparts, there are a few new pieces of information that can help us understand the funding shortfall in a different light and adjust our collective fundraising approach accordingly. To begin with, based on our limited survey figures and some of the feedback received, amphibian proposals seem to be submitted less frequently than those of other vertebrate groups, so it stands to reason that allocated funding would reflect this. Potential causes could be simply because the pool of prospective applicants is smaller relative to other taxonomic groups due to amphibians' perceived lack of charisma, or the lack of prestige in working on this taxon (Urbina-Cardona, 2008), or because of limited language or technical capacities, all of which result in a broad lack of capacity in amphibian conservation. This indicates that increased applications for amphibian-focused projects could result in increased funding being allocated to amphibian conservation. However, several organisations would like to see proposals that have a high degree of collaboration (for example, some organisations receive projects that are similar to each other and that would benefit from working together), that focus more on specific approaches (e.g. threat mitigation, instead of mostly collecting baseline data) or coming from locally-based parties in particular regions (e.g. Africa and Asia), so it is important that as a community we understand what are the priorities of funding organisations and that we address them accordingly.

Given our improved understanding of the nuances involved in resourcing amphibian conservation through projects, we suggest the following:

1. Increasing capacity for grant-writing and fundraising: there is a need for more high-quality amphibian proposals to be considered in the various granting mechanisms that are available to biodiversity conservation. Investing in developing this capacity should result in a higher number of quality applications and therefore in more amphibian conservation projects getting funded. The ASG has its Grant Writing Mentorship Programme, which pairs an experienced reviewer with an up-and-coming amphibian conservationist so that a proposal can be assessed prior to being submitted. Scaling up the programme, in addition to putting together resources that can complement it, should help increase grant-writing capacity.
2. Expanding approaches: obviously baseline data are essential to inform conservation action but these data alone may not be sufficient to qualify for a conservation grant. Most amphibian conservationists are formally trained researchers but are not necessarily trained in implementing conservation action, so a reassessment of scope would be advisable for applicants. Projects implementing actions aimed at mitigating a specific threat might have a higher chance of securing a grant.
3. Increasing collaborations and coordination: to reduce duplicity and internal competition within the amphibian conservation community it is important that researchers and conservationists who work on similar systems within the same geographic and thematic areas collaborate. In order to achieve this regional or national-level coordination is necessary. With appropriate resources, ASG would be well positioned to support this coordination via its regional groups, as would ASA and ASI via their respective partners.

Collaborations

Collaboration is key to conservation. When asked "Other than funding, what is the single largest factor limiting effective conservation strategies for amphibians at global and regional levels?", lack of coordination and collaboration within the amphibian conservation community was the third most common response among ASG members in both the 2013-2016 and 2017-2020 IUCN quadrennia. There are many forms of collaboration, and multiple forms are often needed to maximise conservation success.

In amphibian conservation, perhaps the first and most obvious form of collaboration is that between the persons implementing conservation projects and those conducting research. Interdisciplinary collaboration is vital to conservation success, as there is a vast diversity in competencies required for modern conservation, as is described in the chapters of this ACAP. In practical application, no one action described in the following chapters can be isolated from the others in terms of achieving successful conservation of amphibians. This explains the deliberate overlap of ACAP's chapters and why ASG highly recommends that collaborations be applied to conservation action. While collaboration may seem intuitive, interdisciplinary collaboration can be a challenge to execute, with challenges in communication and increases in complexity and length of projects (Lanterman & Blithe, 2019; Pannell et al., 2019). Many modern universities are promoting interdisciplinary training in the new generation of conservation implementers, but often their administrations have not yet determined how to effectively overcome the separation of disciplines and do not fully appreciate that this can take more time and effort to execute than single-discipline research (Andrade et al., 2014; Lanterman & Blithe, 2019; Pannell et al., 2019). The benefits of interdisciplinary action outweigh the challenges, and can be overcome by remaining openminded, using frequent communication among all stakeholders, and promoting collaborations as outputs to funding sources and administrators (Andrade et al., 2014; Lanterman & Blithe, 2019; Pannell et al., 2019). In addition to collaborations across conservation disciplines, partnering with others of the same discipline is encouraged for increased efficiency. Often several researchers in separate institutions will work in tandem on the same conservation goal and find themselves competing for funding and resources.

A second form of collaboration to emphasise is interdisciplinary collaboration with individuals who have skillsets outside of the conservation sciences (Aziz et al., 2013). Conservation is too often placed exclusively in the hands of scientists, and while science and research are paramount to understanding conservation needs and actions, participation from disciplines outside of conservation sciences is crucial to implement conservation. In the face of the extinction crisis and climate change, the urgent need for novel solutions and radical changes to how we live requires the engagement of all sectors in the conservation of nature. This means all skillsets are needed in the field of conservation. While this demand for collaborators with varied skill sets is recognised by many conservation scientists, it is still an area of great need.

The third form of collaboration, and most important for true conservation success, requires the collaboration of the community, may it be through non-governmental or governmental organisations. A community can be as small as a neighbourhood, or can be as large as a global community. While this is the most important form of collaboration, it can also be the most challenging to achieve and measure. Collaboration with local communities can lead to impacts such as habitat protection (Roach, Urbina-Cardona & Lacher Jr, 2020; O'Brien et al., 2021) and increase in positive behaviours toward species (Perry-Hill et al., 2014). Examples of collaboration in global amphibian conservation include not only the work of the ASG but also that of AArk and the ASA, which catalyse action by linking up partners with common or complementary interests and skills, respectively. Likewise, the ASI does this at a regional level for the genus *Atelopus*, seeking to nurture coordinated collaborative efforts.

Improving governance

There are multiple international conventions relevant to amphibian conservation - the Convention on Biological Diversity (CBD), Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), Ramsar Convention on Wetlands of International Importance Especially as Waterfowl Habitat, Convention concerning the Protection of the World's Cultural and Natural Heritage, and the United Nations Framework Convention on Climate Change (UNFCCC), to mention a few. However, inadequate governance – encompassing lack of legal support, lack of political will, and lack of enforcement of existing laws – is one of the obstacles to implementing amphibian conservation most frequently cited by ASG members (mentioned by 23% of respondents in 2013 and 34% of respondents in 2019, for the 2017-2020 quadrennium).

Even where obligations for implementation of such conventions are relatively clear, we have failed to meet the targets (Butchart et al., 2010; Butchart et al., 2015; Harrop & Pritchard, 2011). While there has been criticism that targets were unachievable given the timeframe (Collen et al., 2013), and could be framed better to support necessary actions (Butchart, Di Marco & Watson, 2016; Green et al., 2019), there seems to be a disconnect between governments' commitments to biodiversity conservation on the global stage, and implementation of the practical local-scale action through national regulatory frameworks required to achieve those goals (Atisa, 2020; Collen et al., 2013). It is clear that transformative change is required if we are to reverse the current trajectory of declines (Díaz et al., 2019; Leclère et al., 2020; Mace et al., 2018; Tickner et al., 2020).

Lack of government support, specifically for amphibian conservation actions, may also be linked to the reasons amphibians are often not prioritised compared with other taxa (see above). Nevertheless, most countries are parties to numerous international conventions and therefore have an obligation to act to reverse biodiversity declines, so how can we better increase governmental support - at a national and local level - for amphibian conservation action?

Rogalla von Bieberstein et al. (2019) suggest the following actions that can be taken to engage governments and contribute to improving implementation of policy:

1. Establish a science-policy platform to promote and facilitate the generation and use of best available knowledge.
2. Improve data gathering, reporting and monitoring, including building more effective mechanisms for managing, sharing and using data.
3. Develop indicators that adequately support implementation of national plans and strategies that can be used across all the biodiversity-related conventions.
4. Provide recommendations based on results accompanied with evidence for successful approaches and making biodiversity data more accessible for policy makers.

Changes to conservation in the face of COVID-19

Since the beginning of 2020 the global COVID-19 pandemic has had enormous consequences on just about every facet of human activity, including biodiversity conservation. In the early days of the initial mass lockdowns, there were many questions and few answers on the impacts of COVID. Shortly after the onset of these lockdowns we started seeing images of an assorted variety of wildlife in decidedly urban settings throughout the world, and there was a sense that the compulsory collective pause of much of human activity had been good news for nature. We began to see blogs, editorials and letters that wondered about conservation in the face of COVID (e.g. Evans et al., 2020; Pearson et al., 2020), and while there appeared to be some good news for the short term (e.g. reduction in noise, pollution and greenhouse gases, Corlett et al., 2020), there were also enormous and immediate negative impacts (reduced funding, cancellation of physical meetings and field work and classes, increase in waste and illegal harvesting, slowing the deployment of renewable energy, massive losses in ecotourism revenue critical to conservation, to mention a few). Years have now passed since those first lockdowns, and while we now have a better understanding of some of their most immediate impacts, it will take us some more time (and perhaps in some cases, we may never) to get a better sense of their reach. Furthermore, some human activities continue to be heavily impacted while others have resumed to some extent and/or been adapted; and modified lockdowns continue to take place as a result of subsequent COVID waves, so the pandemic is still affecting the world and will continue to do so in the foreseeable future.

The number of papers and editorials documenting the impact of this global pandemic on conservation is increasing at a steady pace, so this writing is by necessity a snapshot in time. Perhaps the most obvious impacts are:

1. On-the-ground conservation: with mobility restrictions and shrinking budgets, the protection of priority conservation areas or endemic and threatened species has been greatly affected by COVID. The hiatus in activities such as patrolling, enforcement, containment, treatment, and eradication of invasive species has led to an increase in deforestation, logging, poaching, mining and diseases (Bang & Khadakkar, 2020),

with further encroachment into natural habitats also increasing the risk for new human diseases (Morris et al., 2016; Di Marco et al., 2020). Indigenous communities that are conservation stewards are themselves highly vulnerable to both illicit activities and COVID, being constantly threatened on both fronts.

2. Education: the first and subsequent lockdown mobility restrictions disrupted routine academic and research activities such as classes, labs and exams. Professors and students had to quickly adapt to an online format for teaching and learning, without much prior experience with this format (Corlett et al., 2020). It was a bumpy start for most, and while the format may lend itself to some subjects it is challenging to implement for disciplines that have practical components, such as conservation (Corlett et al., 2020), where labs and field courses have been postponed or cancelled altogether. This also affects timelines for graduation and for young conservation professionals entering the workforce. The impacts of COVID on education may be even stronger in areas where internet access is not reliable or fully available.
3. Research: the pandemic has also impacted transportation, travel and entry into political jurisdictions at all scales, and while there has been some relaxation of travel restrictions at the local level and to some degree at the international level, the airline industry is still one of the hardest hit by COVID and travel remains reduced relative to its pre-pandemic levels. This has affected not only the ability to visit field study sites, but also loan of specimens, exchange of samples, and for those labs that depend on equipment and materials that need to be transported from somewhere else, putting lab work and experiments on hold or ending them abruptly. As travel and exchange restrictions loosen, highly targeted and serious consideration may need to be given to further enhancing capacity building in-country when the opportunity arises.

4. Networking and decision-making: physical meetings of all sizes have been either postponed or cancelled due to the pandemic. Beyond the obvious ability to meet and connect with prospective collaborators, employers or academic advisors, this is of immense consequence to large, international meetings where intergovernmental policies and international agreements are discussed and agreed upon, such as the Convention on Biological Diversity (CBD), the UN Climate Change Conference (Corlett et al. 2020), and the IUCN World Conservation Congress. Online meetings have allowed for smaller virtual gatherings, which work for more modestly-sized conferences although not for policy decision-makers.
5. Wildlife trade: the pandemic originated at the interface between wildlife, domestic animals and humans, and there was a rapid agreement at the international level that wildlife trade is among the vectors that enabled the pandemic. Some countries took rapid action to restrict or even ban the wildlife trade of some specific species, most notably mammals (Borzée et al., 2020). However, no such change was brought to the amphibian trade, despite the panzootics already impacting amphibians, and where the importance of human activities in its spread is not debated. Amphibian populations harvested for the trade, and especially those exported towards western countries or dedicated to high-end consumption would benefit from an update of amphibian trade regulation, and the COVID pandemic could be such trigger (Borzée et al., 2021).
6. Funding: resources for both operational costs and project work have been severely impacted by the pandemic. Non-profit organisations, inclusive of NGOs, zoos, aquaria and museums have all been significantly hurt by COVID, having had to cut hours, furlough, or let staff go altogether. Some government entities have also experienced cuts due to shifting priorities, and initiatives whose business models relied on ecotourism saw their primary source of income dry up overnight. Some

donor organisations have allowed for proposals to cover operational costs, which is helpful, but the need is still enormous, especially because other funding agencies have temporarily paused their funding programmes.

7. Professional opportunities: a combination of the issues outlined above means that the jobs available in conservation, an already scarcely resourced and highly competitive field, are even harder to come by, especially for non-charismatic biodiversity. What are the consequences to biodiversity when there are limited spaces for those who speak for biodiversity?

It is important to note that while some COVID-driven changes may appear to have had a positive impact on conservation, the overall impact is likely to be highly detrimental to conservation as a whole (see e.g. Lindsey et al., 2020). Given the points highlighted above, it is clear that there are major structural cracks that need to be addressed to help conservation through the pandemic crisis, but also with a view to longer-term changes leading to some sustainability. A concerted collective effort by the conservation community is needed to re-think how conservation is done and funded, to engage other sectors where environmental stewardship is a priority, and to be flexible but also plan strategically. The time to do so is now.

Acknowledgements

We are grateful to all the people and organisations who provided the valuable information that forms the basis of the Resourcing amphibian conservation section: Anne Alexandre (Prince Bernhard Nature Fund), Amy Reaume (Brevard Zoo), Gailmarie Kimmel (IDEAWILD), Johanna Rode-Margono and Ivelina Frantsova-Bauer (Stiftung Artenschutz – Species Conservation Foundation), Luisa Arnedo (National Geographic Society), Lydia

Johnson (Whitley Fund for Nature), Nicolas Heard (The Mohamed Bin Zayed Species Conservation Fund), Nida Al-Fulaij (People's Trust for Endangered Species), Nina Marshall (Critical Ecosystem Partnership Fund), Sherilyn Bos (Conservation Leadership Programme), Simon Mickleburgh (The Rufford Foundation).

References

Adams, W. M., & Sandbrook, C. (2013). Conservation, evidence and policy. *Oryx* 47(3):329–335. doi:10.1017/S0030605312001470

Amano, T., Berdejo-Espinola, V., Christie, A. P., Willott, K., Akasaka, M., Baldi, A. ... Sutherland, W. J. (2021). Tapping into non-English-language science for the conservation of global biodiversity. *PLoS biology* 19(10):1–29. doi:10.1371/journal.pbio.3001296

AmphibiaWeb. (2021). https://amphibiaweb.org.

Andrade, K., Corbin, C., Diver, S., Eitzel, M. V., Williamson, J., Brashares, J., & Fortmann, L. (2014). Finding your way in the interdisciplinary forest: notes on educating future conservation practitioners. *Biodiversity and Conservation* 23(14):3405-3423.

Angulo, A., & Icochea, J. (2010). Cryptic species complexes, widespread species and conservation: lessons from Amazonian frogs of the *Leptodactylus marmoratus* group (Anura, Leptodactylidae). *Systematics and Biodiversity* 8(3):357–370. doi:10.1080/14772000.2010.507264

Atisa, G. (2020). Policy adoption, legislative developments, and implementation: the resulting global differences among countries in the management of biological resources. *International Environmental Agreements: Politics, Law and Economics* 20(1):141–159. doi:10.1007/s10784-020-09467-7

Aziz, S. A., Clements, G. R., Rayan, D. M., & Sankar, P. (2013). Why conservationists should be concerned about natural resource legislation affecting indigenous peoples' rights: lessons from Peninsular Malaysia. *Biodiversity and Conservation* 22(3):639-656.

Bang, A., & Khadakkar, S. (2020). Biodiversity conservation during a global crisis: Consequences and the way forward. *Proceedings of the National Academy of Sciences* 117(48):29995-29999.

Bishop, P. J., Angulo, A., Lewis, J. P., Moore, R. D., Rabb, G. B., & Garcia Moreno, J. (2012). The amphibian extinction crisis-what will it take to put the action into the Amphibian Conservation Action Plan? *S.A.P.I.E.N.S.* 5(2):97-111.

Borzée, A., Kielgast, J., Wren, S., Angulo, A., Chen, S., Magellan, K. ... Bishop, P. J. (2021). Using the 2020 global pandemic as a springboard to highlight the need for amphibian conservation in eastern Asia. *Biological Conservation* 255:08973. doi:10.1016/j.biocon.2021.108973

Borzée, A., McNeely, J., Magellan, K., Miller, J. R. B., Porter, L., Dutta, T. ... Zhang, L. (2020). COVID-19 highlights the need for more effective wildlife trade legislation. *Trends in Ecology & Evolution* 35(12):1052-1055.

Butchart, S. H. M., Clarke, M., Smith, R. J., Sykes, R. E., Scharlemann, J. P. W., Harfoot, M. ... Burgess, N. D. (2015). Shortfalls and solutions for meeting national and global conservation area targets.

Conservation Letters 8(5):329-337. doi:10.1111/conl.12158

Butchart, S. H. M., Di Marco, M., & Watson, J. E. M. (2016). Formulating smart commitments on biodiversity: lessons from the Aichi Targets. *Conservation Letters* 9(6):457–468. doi:10.1111/conl.12278

Butchart, S. H. M., Walpole, M., Collen, B., Van Strien, A., Scharlemann, J. P. W., Almond, R. E. A. ... Watson, R. (2010). Global biodiversity: indicators of recent declines. *Science* 328(5982):1164–1168. doi:10.1126/science.1187512

Christie, A. P., Amano, T., Martin, P. A., Petrovan, S. O., Shackelford, G. E., Simmons, B. I. ... Sutherland, W. J. (2020). The challenge of biased evidence in conservation. *Conservation Biology* 35(1):249-262. doi:10.1111/cobi.13577

Christie, A. P., White, T. B., Martin, P. A., Petrovan, S. O., Bladon, A. J., Bowkett, A. E. ... Sutherland, W. J. (2021). Reducing publication delay to improve the efficiency and impact of conservation science. *PeerJ* 9:1–24. doi:10.7717/peerj.12245

Collen, B., Pettorelli, N., Baillie, J. E. M., & Durant, S. M. (2013). *Biodiversity monitoring and conservation: bridging the gaps between global commitment and local action. Biodiversity Monitoring and Conservation: Bridging the Gap between Global Commitment and Local Action*. Hoboken, USA: John Wiley & Sons.

Colléony, A., Clayton, S., Couvet, D., Saint Jalme, M., & Prévot, A. C. (2017). Human preferences for species conservation: Animal charisma trumps endangered status. *Biological Conservation* 206:263–

269. doi:10.1016/j.biocon.2016.11.035

Conde, D. A., Staerk, J., Colchero, F., Silva, R. d., Schöley, J., Baden, H. M. ... Vaupel, J. W. (2019). Data gaps and opportunities for comparative and conservation biology. *Proceedings of the National Academy of Sciences* 116(19):9658-9664.

Corlett, R. T., Primack, R. B., Devictor, V., Maas, B., Goswami, V. R., Bates, A. E. ... Rothp, R. (2020). Impacts of the coronavirus pandemic on biodiversity conservation. *Biological Conservation* 246:108571. doi:10.1016/j.biocon.2020.108571

Di Marco, M., Baker, M. L., Daszak, P., De Barro, P., Eskew, E. A., Godde, C. M. ... Ferrier, S. (2020). Sustainable development must account for pandemic risk. *Proceedings of the National Academy of Sciences* 117(8):3888-3892.

Díaz, S., Settele, J., Brondízio, E. S., Ngo, H. T., Agard, J., Arneth, A. ... Zayas, C. N. (2019). Pervasive human-driven decline of life on Earth points to the need for transformative change. *Science* 366(6471). doi:10.1126/science.aax3100

Engel, M. S., Ceríaco, L. M. P., Daniel, G. M., Dellapé, P. M., Löbl, I., Marinov, M. ... Zacharie, C. K. (2021). The taxonomic impediment: a shortage of taxonomists, not the lack of technical approaches. *Zoological Journal of the Linnean Society* 193(2):381–387. doi:10.1093/zoolinnean/zlab072

Evans, K. L., Ewen, J. G., Guillera-Arroita, G., Johnson, J. A., Penteriani, V., Ryan, S. J. ... Gordon, I. J. (2020). Conservation in the maelstrom of Covid-19 – a call to action to solve the challenges, exploit opportunities and prepare for the next pandemic. *Animal Conservation* in press. doi:10.1111/acv.12601

Frost, D. R., Grant, T., Faivovich, J. N., Bain, R. H., Haas, A., Haddad, C. L. F. B. ... Wheeler, W. C. (2006). The Amphibian Tree of Life. *Bulletin of the American Museum of Natural History* 297:1-291. doi:10.1206/0003-0090(2006)297[0001:TATOL]2.0.CO;2. hdl:2246/5781

Gascon, C., Collins, J. P., Moore, R. D., Church, D. R., McKay, J. E., & Mendelson, J. R. I. (2007). *Amphibian conservation action plan: proceedings IUCN/SSC Amphibian Conservation Summit 2005*. Gland, Switzerland and Cambridge, UK: IUCN/SSC Amphibian Specialist Group.

Giam, X., Scheffers, B. R., Sodhi, N. S., Wilcove, D. S., Ceballos, G., & Ehrlich, P. R. (2012). Reservoirs of richness: least disturbed tropical forests are centres of undescribed species diversity. *Proceedings of The Royal Society B* 279(1726):67–76. doi:10.1098/rspb.2011.0433

González-del-Pliego, P., Freckleton, R. P., Edwards, D. P., Koo, M. S., Scheffers, B. R., Pyron, R. A., & Jetz, W. (2019). Phylogenetic and trait-based prediction of extinction risk for data-deficient amphibians. *Current Biology* 29(9):1557-1563.e1553. doi:10.1016/j.cub.2019.04.005

Gratwicke, B., Lovejoy, T. E., & Wildt, D. E. (2012). Will amphibians croak under the endangered species act? *Bioscience* 62(2):197-202.

Green, E. J., Buchanan, G. M., Butchart, S. H. M., Chandler, G. M., Burgess, N. D., Hill, S. L. L., & Gregory, R. D. (2019). Relating characteristics of global biodiversity targets to reported progress. *Conservation Biology* 33(6):1360–1369. doi:10.1111/cobi.13322

Harrop, S. R., & Pritchard, D. J. (2011). A hard instrument goes soft: The implications of the Convention on Biological Diversity's current trajectory. *Global Environmental Change* 21(2):474–480. doi:10.1016/j.gloenvcha.2011.01.014

IUCN. (2012). *IUCN Red List categories and criteria, version 3.1, second edition*. Gland and Cambridge: IUCN.

IUCN. (2022). The IUCN Red List of Threatened Species. *Version 2021-3*:https://www.iucnredlist.org.

Lanterman, J. L., & Blithe, S. J. (2019). The benefits, challenges, and disincentives of interdisciplinary collaboration. *Commoning Ethnography* 19(2(1)):149-165.

Leclère, D., Obersteiner, M., Barrett, M., Butchart, S. H. M., Chaudhary, A., De Palma, A. ... Young, L. (2020). Bending the curve of terrestrial biodiversity needs an integrated strategy. *Nature* 585(7826):551–556. doi:10.1038/s41586-020-2705-y

Lindsey, P., Allan, J., Brehony, P., Dickman, A., Robson, A., Begg, C. ... Tyrrell, P. (2020). Conserving Africa's wildlife and wildlands through the COVID-19 crisis and beyond. *Nature ecology & evolution* 4:1300-1310.

Liu, J., Slik, F., Zheng, S., & Lindenmayer, D. B. (2022). Undescribed species have higher extinction risk than known species. *Conservation Letters* in press. doi:10.1111/conl.12876

Mace, G. M. (2004). The role of taxonomy in species conservation. *Philosophical Transactions of the Royal Society of London B* 359:711-719.

Mace, G. M., Barrett, M., Burgess, N. D., Cornell, S. E., Freeman, R., Grooten, M., & Purvis, A. (2018). Aiming higher to bend the curve of biodiversity loss. *Nature Sustainability* 1(9):448–451. doi:10.1038/s41893-018-0130-0

Maes, D., Isaac, N. J., Harrower, C. A., Collen, B., Van Strien, A. J., & Roy, D. B. (2015). The use of opportunistic data for IUCN Red List assessments. *Biological Journal of the Linnean Society* 115(3):690-706.

Martín-López, B., Montes, C., & Benayas, J. (2007). The non-economic motives behind the willingness to pay for biodiversity conservation. *Biological Conservation* 139(1–2):67–82. doi:10.1016/j.biocon.2007.06.005

Morris, A. L., Guégan, J.-F., Andreou, D., Marsollier, L., Carolan, K., Le Croller, M. ... Gozlan, R. E. (2016). Deforestation-driven food-web collapse linked to emerging tropical infectious disease, *Mycobacterium ulcerans*. *Science Advances* 2. doi:e1600387

Morrison III, W. R., Lohr, J. L., Duchen, P., Wilches, R., Trujillo, D., Mair, M., & Rener, S. S. (2009). The impact of taxonomic change on conservation: Does it kill, can it save, or is it just irrelevant? *Biological Conservation* 142:3201–3206. doi:10.1016/j.biocon.2009.07.019

Moura, M. R., & Jetz, W. (2021). Shortfalls and opportunities in terrestrial vertebrate species discovery. *Nature ecology & evolution* 5(5):631-639.

Nori, J., Villalobos, F., & Loyola, R. (2018). Global priority areas for amphibian research. *Journal of Biogeography* 45(11):2588-2594.

O'Brien, D., Hall, J. E., Miró, A., O'Brien, K., & Jehle, R. (2021). A co-development approach to conservation leads to informed habitat design and rapid establishment of amphibian communities. *Ecological Solutions and Evidence* 2(1):e12038.

Pannell, J. L., Dencer-Brown, A. M., Greening, S. S., Hume, E. A., Jarvis, R. M., Mathieu, C. ... Runghen, R. (2019). An early career perspective on encouraging collaborative and interdisciplinary research in ecology. *Ecosphere* 10(10):e02899.

Pearson, R. M., Sievers, M., McClure, E. C., Turschwell, M. P., & Connolly, R. M. (2020). COVID-19 recovery can benefit biodiversity. *Science* 368(6493):838-839.

Perry-Hill, R., Smith, J. W., Reimer, A., Mase, A. S., Mullendore, N., Mulvaney, K. K., & Prokopy, L. S. (2014). The influence of basic beliefs and object-specific attitudes on behavioural intentions towards a rare and little-known amphibian. *Wildlife Research* 41(287-299).

Pullin, A. S., & Knight, T. M. (2001). Effectiveness in conservation practice: Pointers from medicine and public health. *Conservation Biology* 15(1):50–54. doi:10.1046/j.1523-1739.2001.99499.x

Pyron, R. A., & Wiens, J. J. (2011). A large-scale phylogeny of Amphibia including over 2800 species, and a revised classification of extant frogs, salamanders, and caecilians. *Molecular Phylogenetics and Evolution* 61(2):543-583. doi:10.1016/j.ympev.2011.06.012

Raposo, M. A., Kirwan, G. M., Calijorne Lourenço, A. C., Sobral, G., Bockmann, F. A., & Stopiglia, R. (2021). On the notions of taxonomic ‘impediment’, ‘gap’, ‘inflation’ and ‘anarchy’, and their effects on the field of conservation. *Systematics and Biodiversity*

19(3):296-311. doi:10.1080/14772000.2020.1829157

Roach, N. S., Urbina-Cardona, N., & Lacher Jr, T. E. (2020). Land cover drives amphibian diversity across steep elevational gradients in an isolated neotropical mountain range: Implications for community conservation. *Global Ecology and Conservation* 22:e00968.

Rogalla von Bieberstein, K., Sattout, E., Christensen, M., Pisupati, B., Burgess, N. D., Harrison, J., & Geldmann, J. (2019). Improving collaboration in the implementation of global biodiversity conventions. *Conservation Biology* 33(4):821–831. doi:10.1111/cobi.13252

Scott, A. (2021). Proposed bill would aid global amphibian conservation.

Streicher, J. W., Sadler, R., & Loader, S. P. (2020). Amphibian taxonomy: early 21st century case studies. *Journal of Natural History* 54(1-4):1-13. doi:10.1080/00222933.2020.1777339

Stuart, S. N., Chanson, J. S., Cox, N. A., Young, B. E., Rodrigues, A. S., Fischman, D. L., & Waller, R. W. (2004). Status and trends of amphibian declines and extinctions worldwide. *Science* 306(5702):1783-1786. doi:10.1126/science.1103538

Sutherland, W. J., Dicks, L. V., Petrovan, S. O., & Smith, R. K. (2021). *What works in conservation*. Cambridge, UK: Open Book Publishers.

Sutherland, W. J., Pullin, A. S., Dolman, P. M., & Knight, T. M. (2004). The need for evidence-based conservation. *Trends in Ecology and Evolution* 19(6):305–308. doi:10.1016/j.tree.2004.03.018

Sutherland, W. J., Taylor, N. G., MacFarlane, D., Amano, T., Christie, A. P., Dicks, L. V. ...

Wordley, C. F. R. (2019). Building a tool to overcome barriers in research-implementation spaces: The conservation evidence database. *Biological Conservation* 238:108199. doi:10.1016/j.biocon.2019.108199

Tapley, B., Michaels, C. J., Gumbs, R., Böhm, M., Luedtke, J., Pearce-Kelly, P., & Rowley, J. J. (2018). The disparity between species description and conservation assessment: a case study in taxa with high rates of species discovery. *Biological conservation.* 220:209-214.

Tickner, D., Opperman, J. J., Abell, R., Acreman, M., Arthington, A. H., Bunn, S. E. ... Young, L. (2020). Bending the curve of global freshwater biodiversity loss: an emergency recovery plan. *Bioscience* 70(4):330–342. doi:10.1093/biosci/biaa002

Urbina-Cardona, J. N. (2008). Conservation of neotropical herpetofauna: research trends and challenges. *Tropical Conservation Science* 1(4):359–375. doi:10.1177/194008290800100405

von May, R., Catenazzi, A., Angulo, A., Brown, J. L., Carrillo, J., Chávez, G. ... Twomey, E. (2008). Current state of conservation knowledge on threatened amphibian species in Peru. *Tropical Conservation Science* 1(4):376-396.

Young, B. E., Lips, K. R., Reaser, J. K., Ibáñez, R., Salas, A. W., Cedeño, J. R. ... Romo, D. (2001). Population declines and priorities for amphibian conservation in Latin America. *Conservation Biology* 15:1213-1223

THREATS

Chapter 3. Climate change

David Bickford[1*] and Guinevere O.U. Wogan[2], Deanna H. Olson[3], K. S. Seshadri[4], Mark C. Urban[5] Ana Carnaval[6], John Measey[7], Jodi J. L. Rowley[8], Sean Rovito[9], Rudolf von May[10], and Susan C. Walls[11]

[1] National Taiwan University, International College, Taipei, Taiwan; rokrokbickford@gmail.com

[2] Department of Integrative Biology, Oklahoma State University, Stillwater, OK; gwogan@okstate.edu

[3] Pacific Northwest Research Station, U.S.D.A. Forest Service, Corvallis, OR 97331, USA,

[4] Centre for Ecological Sciences, Indian Institute of Science, Bangalore, India

[5] Ecology & Evolutionary Biology, Center of Biological Risk, University of Connecticut, Storrs, CT, USA

[6] Department of Biology, City College of New York, and Biology Ph.D. Program, The Graduate Center, The City University of New York, New York, USA

[7] Centre for Invasion Biology, Department of Botany & Zoology, Stellenbosch University, Stellenbosch, South Africa

[8] Australian Museum Research Institute, Australian Museum, Sydney, NSW, Australia; Centre for Ecosystem Science, School of Biological, Earth and Environmental Sciences, UNSW Sydney, Sydney, NSW, Australia

[9] Unidad de Genómica Avanzada (Langebio), Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional, Irapuato, Guanajuato, Mexico

[10] Biology Program, California State University Channel Islands, One University Drive, Camarillo, CA 93012, USA
[11] Wetland and Aquatic Research Center, U.S. Geological Survey, Gainesville, FL 32653 USA
* Current contact, World Congress of Herpetology, 4566 Live Oak Canyon Rd., La Verne, CA, USA

Abstract

Amphibian ecology and distribution are strongly correlated with climate. Regional patterns of amphibian biodiversity are intimately linked to temperature, evapotranspiration rate, and clines in humidity. While amphibians are and will continue to be adversely affected by recent and projected changes in climate, research suggests that adaptation may happen more slowly than the expected rate of environmental shifts. Here, we review conservation-relevant aspects of both realised and potential impacts of climate change, and make recommendations for amphibian conservation planning and management, focusing on research, action, outreach, and policy. Recent advances in our understanding of climate change impacts on amphibians have primarily stemmed from ecological modelling and direct assessment of climatic tolerances and dispersal capacities through physiological assays, landscape genetics, and dispersal tracking. Anthropogenic climate change has already altered amphibian assemblages and their impacts on ecosystem functioning and services. Because of known and hypothesised ecological tolerances, many amphibians might have reached or exceeded most limits in their ability to adapt to or tolerate further climate change, however the uncertainties are substantial. Conservation planning and action should be implemented to forestall severe impacts of environmental shifts. Scientific research and science-based decision-making and policy development have already lagged; conservation planning and action are happening too

slowly for effective identification of threats and mitigation. If we are to avert further loss of amphibian biodiversity and decay of ecosystem services, we must quickly change our response rate. The lack of basic field research in natural habitats continues to be an underlying challenge. We suggest priority areas of research to include the development of biologically realistic predictive models of amphibian response to climate change, field verification of model estimates and key parameters, population monitoring across multiple sites and taxa, and a combination of efforts within and across ecosystems to understand how impacts of climate change can be better mitigated.

Introduction

Anthropogenic climate change is affecting biodiversity, globally (Parmesan & Yohe, 2003; Rosenzweig et al., 2008; Scheffers et al., 2016; Walther et al., 2002) - with a particularly strong impact on amphibians (IPCC, 2014; Li, Cohen, & Rohr, 2013; also see Figure 3.1). Although these animals have adapted to and survived past changes to the Earth's climate (Fey et al., 2015), the contemporary rate of climate change is worrisome by being higher than those previously witnessed over evolutionary scales, with most amphibians in the "slow" ecological response rate category (Williams, Ordonez, & Svenning, 2021). All aspects of climate change - air and sea surface temperatures, solar radiation, UV, humidity, cloud cover, precipitation, extreme weather event frequency, and sea level rise- can affect amphibian biodiversity (see Figure 3.2). Making matters worse, many amphibian populations are under additional stress due to other drivers, such as disease and habitat loss, which amplify when acting in synergy (Alton & Franklin, 2017; Cordier, Lescano, Rios, Leynaud, & Nori, 2020; Velasco et al., 2021). Here, we identify key impacts of climate change on amphibians, possible biological response-to-climate-change scenarios, research gaps, and management strategies and policies best suited for real world conservation actions. We offer this review in the context of the larger ACAP 2022, offering a pluralistic overview of extinction drivers and real-world solutions.

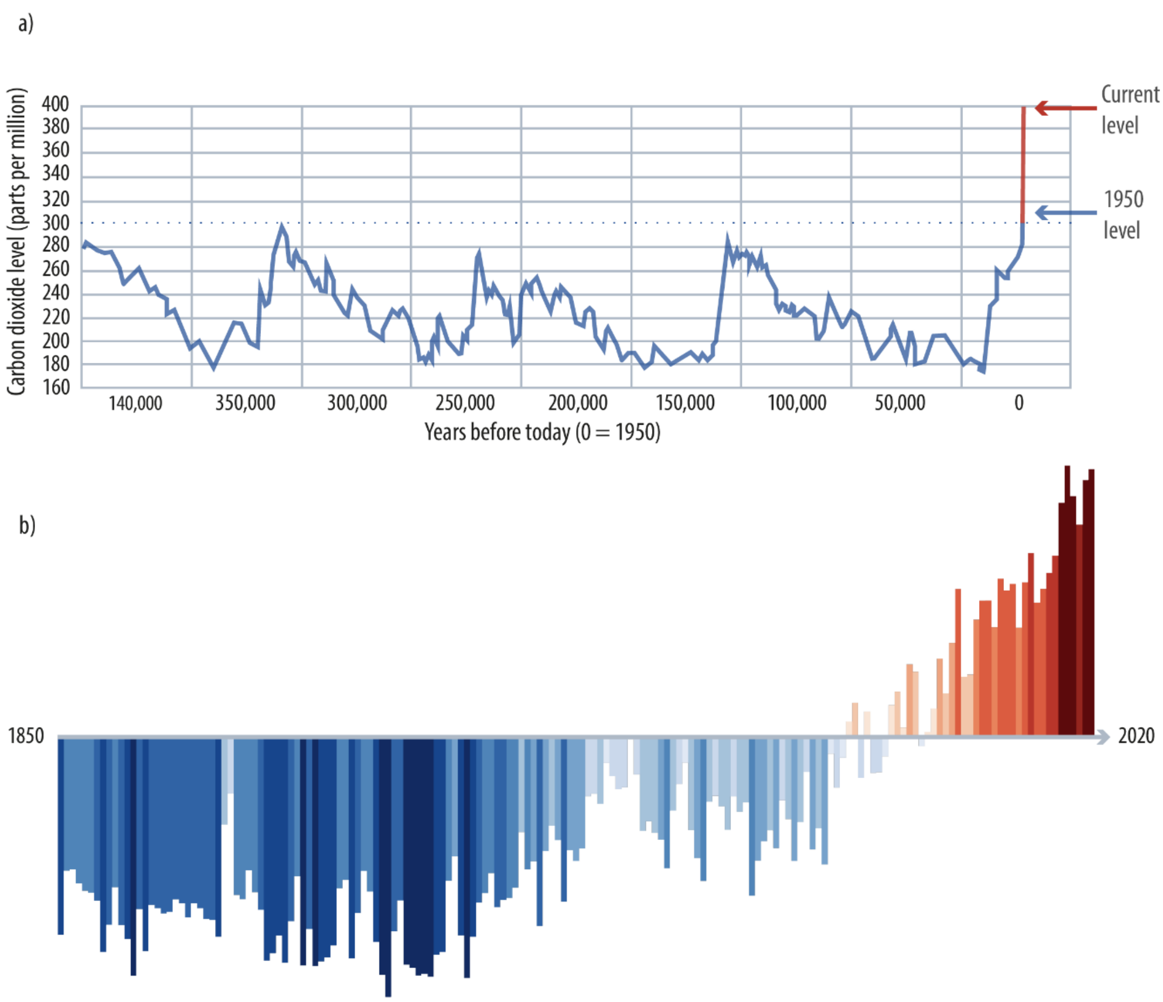


Figure 3.1. Trends in a) atmospheric carbon dioxide and b) global temperature. For centuries, atmospheric carbon dioxide had never been above the blue dottend line (a), and global temperatures have increased by over 1.2℃ (b).

Figure 3.2 Theoretical framework of direct and indirect drivers of extinction threat and decline risk to amphibians posed by climate change.

Status update

Observed impacts of climate change on amphibians

Observed population declines and changes in distribution

Despite very limited long-term data and ongoing surveys on amphibian populations, we have documented declines and potential increasing synergies of extinction drivers. Cahill et al.

(2013) evaluated local population extirpations with climate change or weather variation and found just two studies on amphibians. Since then, however, numerous studies have demonstrated similar population extirpations and range losses due to climate change. For example, in *Lithobates yavapaiensis* severe drought drove high mortality and population extirpation (Zylstra, Swann, Hossack, Muths, & Steidl, 2019), *Pseudophryne pengilleyi* lost 42% of its breeding sites following drought (Scheele, Driscoll, Fischer, & Hunter, 2012), and *Ambystoma talpoideum* populations were extirpated following drought and flooding (Walls, Barichivich, & Brown, 2013). Species Red List assessments which specifically reference climate change include 107 CR (Critically Endangered), 105 EN (Endangered), 35 VU (Vulnerable), and 19 NT (Near Threatened) assessed species, with drought, habitat shifts and alteration, storms, and flooding as the top three specific climate change threats (IUCN, 2020).

Observed extinctions

Of the 37 amphibian species classified by The IUCN Red List of Threatened Species (IUCN Red List) as Extinct or Extinct in the Wild, six implicate climate change as a causal threat, often through synergies with disease and habitat loss, but also more directly as a result of extreme weather, such as flooding and drought (IUCN, 2020). This allows us to contextualise contemporary amphibian extinctions due to climate change relative to the group's background extinction rate. Although efforts to quantify extinction rates among amphibians are complicated by the limited fossil record (particularly in the tropics), imprecise knowledge of the species richness, unknown life history traits of some clades, and imperfect detection, data from a single amphibian fossil assemblage suggested a background extinction rate of 5.2% per million years (Alroy, 2015). Estimates of contemporary extinction also vary: although IUCN reports 37 species as Extinct (EX) or Extinct in the Wild (EW) (IUCN, 2020), other estimates suggest at least 200 species of frogs alone have gone extinct in recent decades (Alroy, 2015), and contemporary extinction rates that are 211 times greater than background extinction rates (McCallum, 2007; Millennium Ecosystem Assessment, 2005).

Updating estimates from Barnosky et al. (2011) to reflect current IUCN Red List assessments of recognised extinctions and current species richness (AmphibiaWeb, 2020), we estimate an amphibian extinction rate of 9 extinctions per 1000 species per 1000 years (or million species-years) over the past 500 years. Estimating extinction at the same time scale, but limiting it to those species for which climate change has been implicated as a threat (currently 6 species categorised as Extinct or Extinct in the Wild), results in 1.5 extinctions per million species-years. This estimate jumps to 80 extinctions per million species-years due to climate change-related threats if we assume an extinction debt, that without human intervention, assumes species currently categorised as Vulnerable or Endangered will ultimately become Extinct.

Observed changes in phenotype and phenology

As ectotherms, amphibians are among the few taxa likely to respond strongly to changing climate (Buckley, Hurlbert, & Jetz, 2012). Determining the ability and extent that a species can undergo phenotypic adaptations or respond to phenological shifts because of climate change are among the key ongoing research questions (Radchuk et al., 2019). The recent focus on amphibian phenotypic responses provides nascent insights into expected trends with a warming climate, although more studies are needed to support or refute these hypotheses. One prediction is that amphibians will respond to warming climate by reducing body size (Sheridan & Bickford, 2011). Reductions in body size may affect reproductive output and demography (Hernández-Pacheco, Plard, Grayson, & Steiner, 2021). Studies have detected signatures in support of this prediction in several species of amphibians such as the *Plethodon* salamanders (Caruso, Sears, Adams, & Lips, 2014); worsening body condition in the California newt *Taricha torosa* (Bucciarelli et al., 2020), and the common toad *Bufo bufo* along with a confounding decrease in reproductive output (Reading, 2007). However, the predicted phenotypic response has not been recovered in all species analysed, such as North American wood frogs (*Lithobates sylvaticus*) and a mole salamander (*Ambystoma maculatum*) (Kirk, Galatowitsch, & Wissinger, 2019; Sheridan, Caruso, Apodaca, & Rissler, 2018). In contrast, the reverse trend has been observed in some species with body size increase in response to climate change, as observed over four decades in *Hynobius tokyoensis* (Okamiya, Hayase, & Kusano, 2021). A second prediction is that within colour polymorphic species, some morphs may have advantageous functional associations related to climatic conditions, although there is debate about the directionality of change (lighter or darker; Delhey, Dale, Valcu, & Kempenaers, 2020; Tian & Benton, 2020). This has been extensively studied in the eastern red-backed salamander (*Plethodon cinereus*), with studies of both the spatial and temporal distribution of morph frequencies (Evans, Forester, Jockusch, & Urban, 2018; Gibbs & Karraker, 2006); the effects of temperature on morph frequencies (Evans, Urban, & Jockusch, 2020); and the physiological differences between morphs (Moreno, 1989). Although the idea that morph frequencies can be used as bioindicators of climate change has come under scrutiny (Evans et al., 2018; Moore & Ouellet, 2015).

One of the most widely documented trends among amphibians is a pronounced shift to early breeding. On average, amphibian breeding phenology is advancing by 6.09 ± 1.65 d per decade with a range between 17.5 d delay to 41.9 d advance (Ge, Wang, Rutishauser, & Dai, 2015; Ovaskainen et al., 2013; Prodon et al., 2017; While & Uller, 2014). Many traits, both species-specific (e.g., reliance on temperature cues for timing of breeding, ability to track resources to be exploited) and more generalised characteristics relating to life history (e.g., body size, clutch size, number of clutches, early vs. late and/or explosive breeding, life span, etc.) influence phenological responses (While & Uller, 2014). In correspondence, frog species are also calling earlier in the year (Walpole, Bowman, Tozer, & Badzinski, 2012). Moreover, vocalisation which is a critical signal for mate choice, is impacted by climate change. For example, adult males of *Eleutherodactylus coqui,* have responded to increasing temperatures over a 23 year period by vocalising at higher frequencies and for shorter durations across an elevation gradient (Narins & Meenderink, 2014).

The ability of amphibians to compensate for phenological alterations varies and is species-specific. For example, development is disrupted in tadpoles of *Rana arvalis* when present in colder temperatures with scarce food resources (Burraco, Laurila, & Orizaola, 2021). Warming temperatures can also alter predator-prey dynamics, as observed when larger predatory *Ambystoma macrodactylum* larvae (benefiting from an earlier hatching and longer period of development) can significantly reduce survival rates of their smaller prey *Pseudacris regilla* if they do not undergo a similar phenological shift (Jara, Thurman, Montiglio, Sih, & Garcia, 2019). It remains to be seen if both predator and prey can develop behavioural responses to the changing climatic conditions. Overall, our understanding of the phenological responses to climate change among amphibians is increasing and points to shifts in most species studied. However, existing studies are strikingly skewed toward the northern hemisphere (Cohen, Lajeunesse, & Rohr, 2018).

Movement ecology and migration of amphibians

Movement is a fundamental yet poorly understood component of amphibian biology. The extent and ability of an organism to move within and across habitats affect gene flow, metapopulation dynamics, population viability, and species distributions, all of which also affect vulnerability to changing climate (Pittman, Osbourn, & Semlitsch, 2014). Amphibians move based on interactions between individuals or species, resource availability such as breeding ponds, and as a response to changes in the physical environment (Joly, 2019). Although there are numerous studies predicting the response of amphibian populations to changing climate, they are not yet validated because we know very little about dispersal abilities of amphibians and our insights into the fine-scale movement mechanisms are limited (Pittman et al., 2014).

Dispersal estimates that do exist for amphibians generally come from individual mark-recapture studies, telemetry studies or genetic estimates, and recent work shows that dispersal estimates from mark-recapture and genetic analyses are remarkably congruent (Wang & Shaffer, 2017). Telemetry studies, in particular, may be able to shed light on environmental cues that lead amphibians to disperse. For example, Henrique & Grant (2019) found that movement among *Leptodactylus latrans* was positively correlated with darker phases of the moon, higher temperatures, and increased precipitation, suggesting that there are both behavioural and environmental cues at work. Earlier studies using genetic data have shown a positive association of both dispersal distance and vagility with body size in several species of anurans and salamanders (Hillman, Drewes, Hedrick, & Hancock, 2014). In recent years, there has been an increasing emphasis on understanding movement behaviour and there has been much progress since a unifying framework for studying movement was proposed (Joly, 2019; Nathan et al., 2008). Models that include dispersal have been widely used in other organisms, but are only recently being applied to amphibians (e.g. Penner & Rödel, 2019).

Movement ecology research of amphibians allows potential to infer patterns and understand underlying processes of population dynamics and gene flow. It also provides insights into the adaptive significance of behaviours, and identifies physiological constraints of an organism in relation to fine-scale environmental variation. Future climate change research will benefit from technological advancements such as the miniaturization of GPS tags (Cagnacci, Boitani, Powell, & Boyce, 2010), harmonic direction finding (Pašukonis, Warrington, Ringler, & Hödl, 2014), passive infrared transponders (Cucherousset, Marty, Pelozuelo, & Roussel, 2008), and increasing accessibility of genome-wide sequencing techniques (McCartney-Melstad, Gidiş, & Shaffer, 2018). However, it may be impossible to use most of the tools except genomic techniques for studying the movement ecology of fossorial amphibians such as caecilians.

Amphibian responses to climate change – evidence of climate-tracking

Many of the studies of amphibian movement in regard to climate change have focused on mechanisms that enable tracking both *in situ* (through adaptation) and across space (through dispersal). Empirical research has characterised the thermal traits of many species, including tolerance to heat and cold, thermal breadth, and safety thermal margin (Brattstrom, 1968; Catenazzi, Lehr, & Vredenburg, 2014; Christian, Nunez, Clos, & Diaz, 1988; Mokhatla, Measey, & Smit, 2019; Navas, 1997; 2003; Nowakowski et al., 2018; von May et al., 2017). Niche divergence in physiological traits is both common and evolutionary labile (Nowakowski et al., 2018; von May et al., 2017), while thermal traits vary across sympatric species (von May et al., 2019), across parts of a species' range (Mittan & Zamudio, 2019), and even over an individual's lifetime, as plasticity and both short- and long-term acclimation are common (Gunderson & Stillman, 2015; Riddell, Odom, Damm, & Sears, 2018; Tejedo et al., 2010; Urban, Zarnetske, & Skelly, 2013; Valladares et al., 2014). However, acclimation to warm temperatures in one life stage (e.g., larvae) does not imply that other life stages (metamorphic, juvenile, adults) will retain increased tolerance to higher temperatures

(Enriquez-Urzelai et al., 2019). Other relevant physiological information available for amphibians include water loss, water uptake, ability to find water, type of development, and larval habitat (Cruz-Piedrahita, Navas, & Crawford, 2018; Madelaire et al., 2020; Nowakowski et al., 2018; Riddell & Sears, 2015; Scheffers et al., 2013; Sunday et al., 2014). Although less studied, it has been proposed that water balance may be a more critical process determining local adaptation and persistence relative to thermal tolerance (Cruz-Piedrahita et al., 2018).

Amphibian species can also track climate change by shifting along elevational and altitudinal climate gradients to remain within a suitable microhabitat. The degree that a species needs to disperse to remain in the same climatic niche depends on the velocity of climate change, reflecting the spatial gradient in climate (steep clines up mountains, shallow clines along latitude), and speed of local climate change (Loarie et al., 2009). Efficient climate tracking is expected for species that can disperse well, not only across natural landscapes but also in patchy and disturbed landscapes (Chen, Hill, Ohlemüller, Roy, & Thomas, 2011). For amphibians, dispersal varies by orders of magnitude with some species moving only metres and others moving kilometres (Semlitsch, 2008; Sinsch, 2014). Synergies with other processes known to impact survival - e.g. biotic interactions, disease dynamics (chytridiomycosis), and land use change (fire regimes) - are also known to interact with tracking (Moskwik, 2014; Seimon et al., 2017).

Local-scale inventories, resurveys, and monitoring, tied to measurements of environmental change on the ground, provide the best evidence of spatial climate tracking in amphibians. Resurveys in the Tsaratanana Massif, in Africa, detected significant changes in the altitudinal range of seven out of 19 species within a period of 10 years of documented warming

(Raxworthy et al., 2008). In North America, increasing air temperatures have been statistically correlated with upslope movement of a hybrid zone in *Plethodon* salamanders (Walls, 2009).

Insights from modelling

There is a tremendous need for developing effective conservation strategies as more species become more vulnerable to extinction and population declines from climate change (Foden et al., 2019). Understanding the range of impacts and mechanisms that amphibians face both physiologically and ecologically (see species interactions below), is a critical step to preventing extinctions, although there is increasing urgency to mitigate loss since the effects of climate change are already impacting amphibian species at a global scale. Our understanding of future changes to amphibian distributions and extinction risk has been informed by a variety of different types of models. These include vulnerability assessments that incorporate correlative, specific trait-based, mechanistic, and combined models. These models can be used both to predict future responses to climate change scenarios as well as to develop mitigation strategies to prevent losses.

Modelling: Vulnerability assessments

It is important to select appropriate climate change vulnerability assessment (CCVA) approaches for quantifying vulnerability and there have been four basic ways to do it to date: trait-based, correlative, mechanistic, and combined approaches. One considerable caveat in these endeavours is that we lack species-specific data for most taxa, and the best available data are often inadequate to do a comprehensive assessment (Urban et al., 2016). Unreliable or even misleading results can make conservation situations worse (e.g. Kroll, Runge, & MacCracken, 2009). Since the best available data are usually insufficient, Williams et al.

(2008) suggest relying on closely related species' relevant traits. For amphibians, there are a few important traits regarding species' vulnerability to climate change: mountaintop distribution, direct development (Nowakowski et al., 2018; Scheffers et al., 2013; von May et al., 2019), and lowland or coastal distribution are traits that seem to incur extreme vulnerability to amphibians but there is high variability across amphibian species' vulnerability. Since amphibians have physiological responses that are relatively easy to identify (e.g., to hydroperiod, available moisture and relative humidity, seasonality, etc.), many have small geographic ranges, and many populations are declining, there are important considerations for CCVAs that are unique to each species and/or geographic assemblage. For example, species-level estimates of tolerances to heat and cold are essential for inferring species' vulnerability to climate change (Nowakowski et al., 2018; Sunday et al., 2014), as are obtaining accurate measures or estimates of physiological traits and microclimates (Storlie et al., 2014).

Models of species' ranges under future climatic conditions are powerful tools to predict where the impact of climate change on amphibians may be greatest. Current models are supported by a large number of global climate, microhabitat, and species occurrence data (Table 3.1). Two primary approaches, correlative and mechanistic, have been used to forecast geographic ranges under future climates. Correlative models of species distributions infer species-specific environmental suitability based on climatic descriptions of known occupancy (with voucher specimens and/or observations) (Nix, 1986; Phillips, Anderson, & Schapire, 2006; Venables & Dichmont, 2004) and often forecast pervasive distribution shifts in response to anthropogenic climate change (Milanovich, Peterman, Nibbelink, & Maerz, 2010; Urban et al., 2016). Correlative models are relatively simple to fit with presence-only data, applicable across spatial scales, and perform well across a relatively short time window (e.g.,

< 20 breeding seasons). They also provide useful insights and testable hypotheses about demographic, range size, and species richness trends, especially for data-limited species, as is the case for most species, and especially those in hyper-diverse regions like the tropics (see Box 3.2). Moreover, correlative models that have been projected into the past, particularly back to the Pleistocene and Holocene, have been successfully validated with genetic data describing past amphibian population trends (Amaro, Rodrigues, Yonenaga-Yassuda, & Carnaval, 2012; Carnaval, Hickerson, Haddad, Rodrigues, & Moritz, 2009), as well as patterns of endemism (Carnaval et al., 2014).

Table 3.1: Abundant datasets enable scientists to monitor and model the potential impacts of climate change on amphibian distribution.

Example	Description	Source
Environmental data		
WorldClim 2	High resolution interpolated monthly temperature and precipitation	(Fick & Hijmans, 2017)
Climatologies at High Resolution for the Earth's Land Surface Areas (CHELSA)	High resolution interpolated monthly temperature and precipitation	(Karger et al., 2017)
Global surface water	High resolution data on water bodies	(Pekel, Cottam, Gorelick, & Belward, 2016)
Gridden temperature and precipitation climate extremes indices (GHCNDEX)	Gridded data on climate extremes (e.g. temperature and precipitation)	(Donat et al., 2013)
Microclimate data		
NicheMapR	R package; integrates terrain and atmospheric forcing data; generates hourly time-series of microclimatic conditions, above and below ground	(Kearney, Gillingham, Bramer, Duffy, & Maclean, 2020)

MICROCLIMA	R package; estimates microclimatic details from global data with high accuracy	(Maclean, 2020)
Species distribution data		
Global Biodiversity Information Facility (GBIF)	International network and data infrastructure; open access to occurrence data of all types of life on Earth	www.gbif.net
FrogID	National citizen science project; aids amphibian monitoring in Australia	https://www.frogid.net.au
iNaturalist	Citizen science-led database of species identity and locality records	https://www.inaturalist.org

However, correlative models are neither completely nor perfectly explanatory. Since they are based on environmental suitability inferred from species occurrence and usually neglect other mechanisms, such as species interactions, correlative models may fail to describe species' fundamental niches (Godsoe & Harmon, 2012; Higgins, Larcombe, Beeton, Conradi, & Nottebrock, 2020). Additionally, correlative models of species distributions projected onto future climates depend on the degree to which dispersal is parameterised. Since many amphibians are poor dispersers, limiting the future range of a species to a subset of the regions that it currently occupies may be biologically realistic. Studies that assume no dispersal typically predict larger range contractions than those in which dispersal is explicitly included (Lawler, Shafer, Bancroft, & Blaustein, 2010; Zellmer, Slezak, & Katz, 2020). The lack of estimates of direct dispersal capacity for most amphibian species limits application of correlative modelling results.

Mechanistic models include key biological processes that enhance predictive accuracy for climate change responses (Gilman, Urban, Tewksbury, Gilchrist, & Holt, 2010; Hoffmann & Sgró, 2011; Urban et al., 2016)—namely physiology, demography, dispersal, species interactions, evolution, and other responses to environmental variation (Urban et al., 2016). Despite requiring significantly more data, they likely approximate the fundamental niche of a species more closely than correlative models and may be more informative about causal factors affecting geographic range changes. The most commonly used mechanistic models for amphibians, biophysical models, predict areas where species can maintain a positive energy balance and incorporate physiological parameters (such as metabolic rate, thermal maxima, and behavioural limitations to foraging time) with environmental data (e.g., relative humidity, soil moisture, and ground-level temperature) to predict the timing and efficiency of foraging, and energy assimilation and expenditure (Kearney & Porter, 2004; Peterman & Gade, 2017;

Riddell et al., 2018). Mechanistic models also allow both behavioural and physiological plasticity, such as avoidance of extreme temperatures and metabolic rate acclimation to increasing temperatures, which can have dramatic effects on prediction of future ranges for amphibians relative to correlative models (Lyons & Kozak, 2020; Riddell et al., 2018). Despite these advantages, mechanistic models remain underutilised, often because of a dearth of necessary data, even for the best-studied species (see Urban et al., 2016). Furthermore, while some parameter values may need to be estimated from incomplete data, small changes in parameter values can have major effects on model results (Peterman & Gade, 2017).

Modelling amphibian extinction risk from climate change

Amphibians are sensitive to climate change owing to their physiological vulnerability to temperature, humidity, and precipitation, high sensitivity to desiccation due to their highly vascularised skin (see vulnerabilities section), low dispersal (see movement ecology section), susceptibility to climate mediated factors such as disease outbreaks, and potential interactions with existing threats from habitat degradation, invasive species, and high levels of endemicity (Alford, Bradfield, & Richards, 2007; Blaustein et al., 2001; Corn, 2005; Gibbons et al., 2000; Gunderson & Stillman, 2015; McMenamin, Hadly, & Wright, 2008; Pounds et al., 2006; Reading, 2007; Wake, 2007). Although high relative vulnerability claims are frequently made, our ability to generalise is limited and uncertainty of how these trends are geographically and taxonomically distributed still remains high in the absence of validated model predictions.

Predicted risks

We aggregated data for amphibians and calculated the predicted relationship between warmer global temperatures and amphibian declines (Fig. 3.3). Based on multi-species models, amphibian extinction risk is expected to increase rapidly with temperature (slope = 0.69, N = 42, 95% Cis: 0.58, 0.73), and this rate is higher (~50% risk) than predictions for other taxonomic groups. Other syntheses based on expert opinion (Foden et al., 2013) and species-area approaches (Thomas et al., 2004) predict that climate change threatens 12–60% of amphibians with extinction.

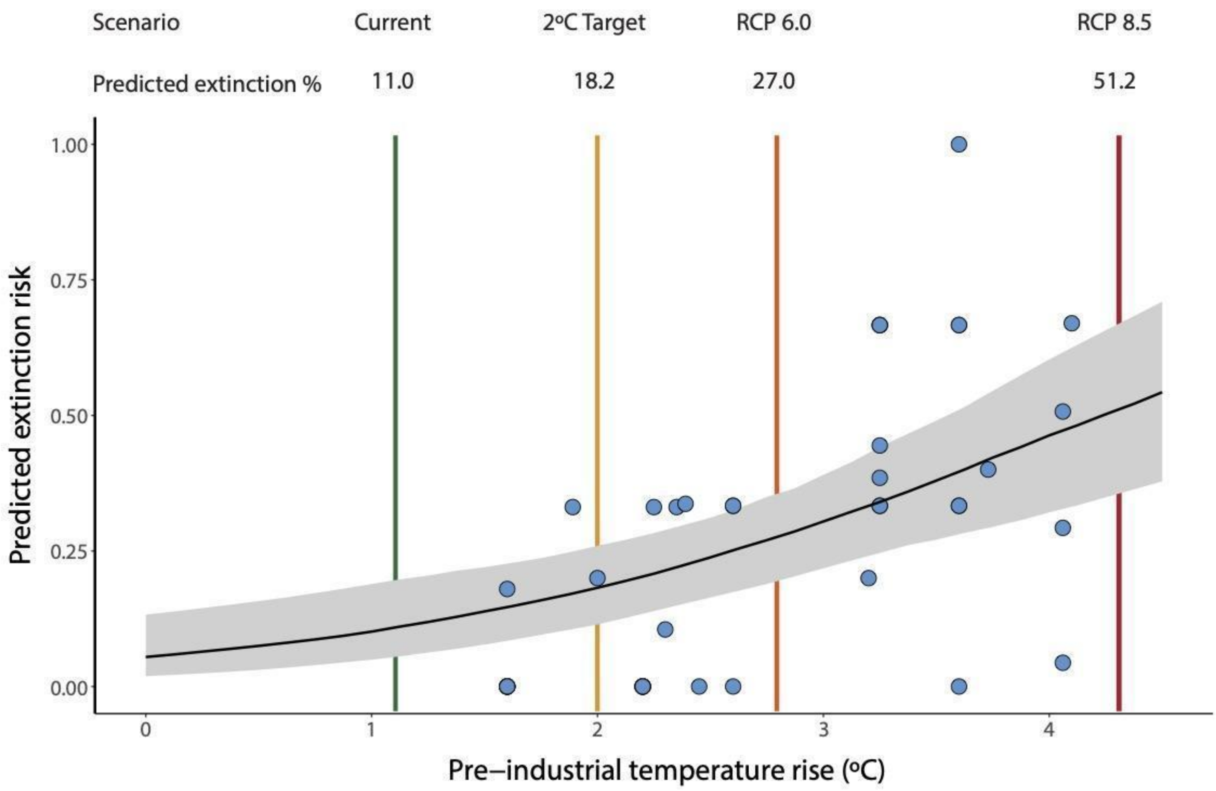


Fig. 3.3. Predicted global amphibian extinction risk based on pre-industrial temperature rise from 42 multi-species predictions of amphibian extinction risk from climate change. Estimated relationship transformed from logit and plotted with 95% confidence intervals (grey ribbon). Details of analysis in Urban (2015). Extinction risks are for four climate scenarios: current post-industrial temperature rise of 0.8 C, the policy target of 2 C, and Representative Concentration Pathways (RCPs) 6.0 and 8.5.

Genetic adaptation of amphibians to climate change

Although not unique to amphibians, we still know little about how amphibians might respond to climate change through genetic adaptation (Merilä & Hendry, 2014; Urban et al., 2016; Urban, Richardson, & Freidenfelds, 2014). Few predictions account for potential resilience through adaptation, with only 1 of 131 studies addressing potential adaptive change (Urban et al., 2016). Genetic variation can allow populations to adapt to climate change and thereby persist despite a changing climate (Carroll, Fredrickson, & Lacy, 2014; Hoffmann & Sgró, 2011). Local adaptation is especially important for poor dispersers, like most amphibians that cannot track shifting climates (Urban, De Meester, Vellend, Stoks, & Vanoverbeke, 2012).

It is also important to note that many species are comprised of hundreds or thousands of semi-independent populations (González-Suárez & Revilla, 2013; Hughes, Daily, & Ehrlich, 1997; Jetz, Ashton, & La Sorte, 2009), each potentially adapted to local conditions including climate (Rehfeldt et al., 2002). These divergent populations might respond differently to climate change in distinct ways depending on the match between their traits and changing local conditions (Pelini, Keppel, Kelley, & Hellmann, 2010). For poor dispersers, adaptive population differentiation can slow or preclude range shifts because all populations are perturbed from local optima (Pelini et al., 2010). For good dispersers, locally adapted populations can track changing climates across space through genotypic sorting (Urban et al., 2012) as opposed to evolution based on *in situ* standing genetic variation.

A review of genetic responses to climate variation uncovered 11 amphibian studies (Urban et al., 2014). Ten of the 11 studies documented genetic variation for at least one of the traits related to climate variation. Sixty-five percent of traits demonstrated significant genetic variation and 48% of the traits demonstrated significant genotype-by-environment interactions (Urban et al., 2014). For instance, frogs adapted to different thermal regimes in space (Freidenburg & Skelly, 2004; Orizaola, Quintela, & Laurila, 2010; Skelly & Freidenburg, 2000), and salamanders adapted different colour morphs over time (Gibbs & Karraker, 2006). Most studies focused on phenological changes in breeding and life history traits such as growth, development, and survival rates. However, most evidence comes from space-for-time substitutions, suggesting that genetic variation exists across heterogeneous landscapes that could contribute to climate resilience. However, we know much less about local genetic variation that allows responses *in situ*. Moreover, most studies are from North America and Europe so we know even less about the potential for adaptation in the tropics, where the greatest amphibians biodiversity occurs.

Genomics & evolvability

The large genomes of amphibians and limitations in funding have hindered efforts to generate genome assemblies for all but a handful of species. Advances in sequencing technologies have already started to remove this barrier, with completion of the first chromosome-level assemblies for salamanders (*Ambystoma mexicanum,* Nowoshilow et al., 2018) and caecilians (*Rhinatrema bivittatum*, Rhie et al., 2020). In addition to the early genomes for *Xenopus tropicalis* (Hellsten et al., 2010) and *X. laevis* (Session et al., 2016), chromosomal-level assemblies now also exist for several non-model frog species that encompass greater taxonomic and geographic variation (see Chapter 13). These genomic resources will be essential to identify genes that underlie critical climate-related traits. To date, few studies have pinpointed genes involved in amphibian climate change tolerance. As a rare example, Saito et al. (2019) demonstrated that neuronal heat sensor genes in *Xenopus* from warmer climates diverged from those species inhabiting cooler climates. A second example comes from a transcriptomics study of the montane salamander *Plethodon metcalfi* to identify genes involved in desiccation (Riddell, Roback, Wells, Zamudio, & Sears, 2019). A third study used time-series from a broadly distributed species to hone in on a set of candidate loci involved in thermal adaptation (Cayuela et al., 2021). Identification of a full suite of these genes would allow assessment of genetic variation within populations and across species ranges and could serve as a potential baseline estimate of adaptive capacity. Furthermore, transcriptomics (e.g. Riddell et al., 2019; Yang, Qi, & Fu, 2016) and epigenetics (Wogan, Yuan, Mahler, & Wang, 2020) may provide new insights into plasticity that could also be quantified within populations and across species' ranges in conjunction with adaptive capacity.

Landscape genetics

As a discipline, landscape genetics emerged quite recently, but there has been a proliferation of studies focused on amphibians due to their overall low vagility and their thermophysiological requirements that link them to the environments where they exist. With respect to climate change, these studies suggest environmental conditions that facilitate or impede dispersal (e.g. environmental resistance; Wang, Savage, & Shaffer, 2009), quantify fine-grained spatial genetic variation (Savage, Fremier, & Shaffer, 2010), and gene flow (Homola, Loftin, & Kinnison, 2019; Sánchez-Montes, Wang, Ariño, & Martínez-Solano, 2018). Furthermore, these same approaches could specifically be used to evaluate climate corridors by highlighting least-cost dispersal pathways (Epps, Wehausen, Bleich, Torres, & Brashares, 2007). Taken a step further, landscape genomics can be used to identify genotype-environmental associations and make predictions about the spatial distribution of adaptive alleles (Manel et al., 2018). There have recently been several amphibian landscape genomics studies that evaluate various genotype-environment associations, for example, local adaptation across a drying landscape in the Australian frog *Pseudophryne guentheri* (Cummins, Kennington, Rudin-Bitterli, & Mitchell, 2019), and lowland-highland adaptation across elevational transects in the Andes (*Boana platanura*, Medina et al., 2021), highland adaptation of genes coding for metabolism in the Tibetan plateau (*Bufo gargarizans*, Yang et al., 2016), as well as adaptations along latitudinal gradients (*Rana arvalis*; Rödin-Mörch et al., 2019).

Ecological interactions - species interactions

In addition to direct physiological effects, interspecific interactions frequently determine climate responses (e.g. Davis, Jenkinson, Lawton, Shorrocks, & Wood, 1998; Park, 1954). In cases where mechanisms of climate-induced declines and extinctions are understood, most involve indirect impacts via species interactions rather than direct physiological impacts (Cahill et al., 2013). Climate change can modulate the strength or even alter the direction of species interactions (Van Der Putten, Macel, & Visser, 2010; Visser, Van Noordwijk, Tinbergen, & Lessells, 1998), including elevational range expansions and contractions (Raxworthy et al., 2008). Divergent abilities to track climate changes through range expansion can create no-analogue communities composed of species with no history of co-occurrence or coevolution (Urban et al., 2012). Interactive effects often stem from stronger negative species interactions (e.g., novel or stronger predation risk or disease), fewer resources, interspecific competition, or loss of mutualists (Gilman et al., 2010).

For amphibians, climate change has likely contributed to declines by exacerbating disease, suggested by the case of the golden toad (*Incilius periglenes*). The golden toad declined in concert with climate-mediated changes to dry-season mist frequency and increased exposure to pathogens (Pounds et al., 2006; Pounds, Fogden, & Campbell, 1999; Pounds & Crump, 1994). Variation in breeding phenology (Beebee, 1995; Gibbs & Breisch, 2001) could also lead to changes in interaction strength, as responses can differ relative to photo- and hydro-period and weather cues. For instance, if *Hyla cinerea* tadpoles arrive late, they suffer greater predation from growing dragonfly nymphs (Rasmussen & Rudolf, 2016). In some cases, overwintering amphibians are expected to gain an advantage as winters become milder, supporting top amphibian predators (Herstoff & Urban, 2014). On the other hand, climate-mediated desiccation also increased behaviours that boost predation risk in red spotted newts (Rohr & Madison, 2003).

Differential changes in phenology can also alter competition among species, for example, by synchronising otherwise asynchronous competitors or causing one species to breed earlier and become a superior competitor. Interactions with other stressors -e.g., invasives and climate change - increased drying limits in *Rana sierrae* recruitment in small ponds while fish introductions limit their recruitment in larger lakes (Lacan, Matthews, & Feldman, 2008; M. Urban *pers. comm.*). Additionally, warming can cause outbreaks of pathogens and parasites such as outbreaks of parasitic copepods on *Rana boylii* and trematodes that cause malformations in developing frogs (Kupferberg, Catenazzi, Lunde, Lind, & Palen, 2009).

Evidence of management

Evidence of positive results for amphibian conservation with climate change

Conservation actions for climate change focus on habitat management and rescue measures for at-risk populations. Provision of breeding, foraging, or dispersal habitat conditions can be an effective adaptive management approach for climate change (Sutton et al., 2015). As mitigations are costly, Mims, Olson, Pilliod, & Dunham (2018) offered an approach for regional species prioritisation that merged species rarity with climate sensitivity.

At local scales, Shoo, Olson, & Hero (2011) provided examples of installation of microclimate and microhabitat refuges for amphibians, addressing: 1) riparian zones including their microclimate regimes (Olson, Anderson, Frissell, Welsh Jr., & Bradford, 2007; Olson, Coble, & Homyack, 2020; Olson, Leirness, Cunningham, & Steel, 2014; Olson & Burton, 2014); 2) microclimate refugia including downed wood (e.g. Kluber, Olson, & Puettmann, 2008, 2009; Rittenhouse, Harper, Rehard, & Semlitsch, 2008), leaf litter, and bromeliads (Donnelly, 1989; see also Stynoski, 2009); and 3) artificial wetting of terrestrial habitat by irrigation sprayers (Australia: Mitchell, 2001), an approach with additional applications (Central America: Pounds et al., 1999; Papua New Guinea: Bickford, 2005; Tanzania: Krajick, 2006). Smith, Meredith, & Sutherland (2018) reported 28 studies that created ponds and found that relative to amphibian use they were 80% effective (with 80% certainty) and 0% harm. Pond creation may be especially applicable to threatened species (Cushman & Pearl, 2007; Kupferberg, 1996; Shoo et al., 2011). Mathwin, Wassens, Young, Ye, & Bradshaw (2020) reviewed efficacy of manipulating water for amphibian conservation and found 17 examples of successful applications, with extension of hydroperiods to match larval requirements and off-season drying to control predators showing encouraging results. Biebighauser (2015) provided procedures for wetland creation and restoration (~6,000 designs created) that have been implemented across North America (~2,500 wetland projects supervised), with applications in urban and agricultural landscapes including schools (~250 wetlands), mined lands (~400 wetlands), and forests, including measures to forestall invasive predators (Biebighauser, 2007; Gamble & Mitsch, 2009; Hartel et al., 2007; Korfel, Mitsch, Hetherington, & Mack, 2010; Maret, Snyder, & Collins, 2006). Similarly, Petranka, Harp, Holbrook, & Hamel (2007) reported advantages of creating wetlands along a hydrologic continuum to reduce mortality from several risk factors including drought, pathogens, and predators on early life stages.

At landscape scales, the creation and retention of pond networks can address broader species sustainability functions by providing breeding, foraging, and dispersal habitats among populations (Fog, 1997; Piha, Luoto, Piha, & Merilä, 2007). For example, in managed forests, retention of tree islands and downed wood near riparian reserves may provide stepping-stone function that enhances habitat connectivity (Olson & Burnett, 2013; Olson & Kluber, 2014). Likewise, landscape genetic work supports frog dispersal along trajectories with downed wood and retained understory (Spear, Crisafulli, & Storfer, 2012). Furthermore, forest cover is a predictor of connectivity for headwater salamanders (Emel, Olson, Knowles, & Storfer, 2019). However, despite limited evidence for amphibian corridors retaining connectivity (Smith & Sutherland, 2014), one study found that corridors retained 8 of 13 frogs for as long as 20 years (Becker, Fonseca, Haddad, Batista, & Prado, 2007). At more regional to continental scales, creating corridors that align with the direction of climate changes might allow species to more easily track their climate niche.

Other approaches for species facing extinction from climate change include relocation, reintroduction, translocation, headstarting, captive rescue colonies, and bio-banking (Partners in Amphibian and Reptile Conservation (PARC), 2011; chapters in Walls & O’Donnell, 2021). Headstarting and relocations might be both easier and more cost-effective (Griffiths & Pavajeau, 2008). Three reviews found most (65%) amphibian translocations resulted in established breeding populations (Smith & Sutherland, 2014). Germano and Bishop (2009) identified guidelines to reduce failures.

Red List categories & climate change

An important research finding has been that areas with many species of high sensitivity and low adaptive capacity differ from areas where species are actually exposed to the brunt of climate change (Foden et al., 2013). Indeed, where exposure-based assessments of vulnerability to climate change are done exclusively, we can obtain misleading results that hamper conservation efforts. Some amphibian species (11-15%) are already threatened with extinction (on the IUCN Red List) and highly vulnerable to climate change. These species are a priority for conservation actions (Foden et al., 2013), no matter their climate change sensitivity. In other words, conservation efforts should not be competing unnecessarily, and when amphibians are already at high risk of extinction, those species deserve prioritised action, despite many uncertainties of their exposure to climate change.

Gaps: Research & knowledge

1. **Baseline data:** We have incomplete knowledge of the diversity and distribution of amphibians. As of May 27, 2021, there are 8,340 species of described amphibians, and in the past 10 years between 100-200 new species have been described each year (AmphibiaWeb, 2020). The ranges of many species are poorly known or known only from type localities and population trends and threats are unknown for 1,184 Data Deficient species out of 7,212 assessed species (IUCN Red List, see Howard & Bickford, 2014), which means over a quarter of amphibian species (2,312 species) are not assessed or lack sufficient basic data for assessment.
2. **Natural history:** Basic life history data are still lacking for many amphibian species. Efforts to compile life history traits for species into an accessible database for the scientific community is essential for addressing climate change vulnerabilities. Databases that address some of these aspects [AmphibiaWeb (amphibiaweb.org), ASW

(amphibiansoftheworld.amnh.org)] primarily address geography and taxonomy, but compiled species-specific trait databases are largely lacking, although the AmphiBIO database has started to fill this void (Oliveira, São-Pedro, Santos-Barrera, Penone, & Costa, 2017). We cannot stress enough the importance of renewed priority towards compiling these data. Fundamental research and its funding remain one of the most important, direct, and measurable ways to improve most things, including amphibians' conservation action plans.

3. **Amphibian physiology:** Although amphibian thermophysiology has been reasonably well-described, the group lags significantly behind other ectotherms, particularly lizards (Sinervo et al., 2010), in terms of large-scale applications of mechanistic modelling. Basic information on physiological responses to humidity shifts, demography, dispersal, and microhabitat use are lacking for most species, preventing more comprehensive mechanistic models from being built (Urban et al., 2016). How to reconcile the time and resources required for mechanistic models and parameter collection remains a challenge, especially if the scientific community aims to generate accurate global-level assessments of potential changes in species distribution. Given that mechanistic models are data-hungry, and that correlative models may lack biological realism or process-based insights, investments in hybrid or mechanistically-informed correlative species distribution models may be worth pursuing. Monitoring networks are needed to validate models and facilitate resurveys, and may be linked to Earth Observation efforts (GEO BON, 2015; Pereira et al., 2013).

4. **Models - scenario development:** As most species distribution forecasts developed nowadays focus on the impact of climate alone, the need exists for the inclusion of other change scenarios. The addition of land-use models, expected biological invasions, and synergies that may arise from future climate shifts may be particularly insightful. It

would also be extremely helpful to do some ground-truthing and validation of models generated from the early 2000s forecast to 2020 (e.g. Milanovich et al., 2010) to understand how well the predictions and real situations match, and to quantify error and bias.

5. **Phenotypic responses:** Phenotypic responses to climate change among amphibians are understudied, and additional studies measuring this are needed across taxonomic groups encompassing a wider range of geographic regions. This work either requires a space for time substitution (Wogan & Wang, 2018), or a time series from long term field sites and monitoring, or from dedicated resampling projects aligned with historical museum samples (Holmes et al., 2016; C. Moritz et al., 2008).
6. **Phenology:** Under climate change, shifting phenologies may alter interactions among species, for example Rollins & Benard (2020) demonstrated that different experimental combinations measuring body size and phenological shift in metamorphosis between two larval frogs led to divergent body mass outcomes at their terrestrial phase. There are, however, few empirical studies that have documented how shifting phenologies and novel interactions will affect individual species and restructure amphibian communities in the face of climate change.
7. **Dispersal:** We know little about amphibian dispersal. Dispersal syndromes and distances are known for only a handful of amphibians, yet these data are critical for understanding how well species will be able to track climate. We need these data from a wide taxonomic range of species encompassing lowlands, mountains, tropics, and temperate regions.
8. **Species interactions:** We still know little about how amphibians fit into local food webs and the strength of their interactions with other species. We often do not know what species they eat or a full list of their predators. Knowledge gaps also exist for

parasites and pathogens, which often interact with climate change in their impacts. Because many climate-induced declines in amphibians occur not through direct physiological impacts (Cahill et al., 2013), but rather indirectly through changes in species interactions, understanding biotic relationships could be important for accurately predicting climate change responses (Gilman et al., 2010).

9. **Adaptation:** Perhaps the largest gap is how much amphibian populations facing a new or even novel climates might be able to adapt and persist in place. To understand adaptability will require an understanding of what specific traits will be under selection in future climates (not just directly from climate, but indirect traits like dispersal or biotic interactions) and measuring genetic variation using experiments or tracking relatives. Ultimately, understanding the genes underlying these responses using genomic approaches could provide direct insights into the possibility and rate of adaptation.

Gaps: Conservation & management

1. There is a need for a proactive management framework to reduce risk of future catastrophic storm impacts on vulnerable populations of amphibians in hurricane-prone regions (Sterrett et al., 2019; Walls et al., 2019). Proactive (as opposed to reactive) conservation, in general, is geographically biased and needs to be strengthened in many parts of the world (Ryan, Palen, Adams, & Rochefort, 2014; Walls, 2018).
2. Strengthen and diversify stakeholder involvement in both conservation planning and action (Bickford, Posa, Qie, Campos-Arceiz, & Kudavidanage, 2012; Hartel, Scheele, Rozylowicz, Horcea-Milcu, & Cogălniceanu, 2020; Walls, 2018).
3. Encourage development and use of conservation tools (e.g. non-invasive stress hormone assays, genomic assessments) that may help natural resource managers and

conservation biologists identify at-risk populations relatively quickly, especially when potential threats are not readily apparent (Rollins-Smith, 2017; Walls, 2018).

4. Develop better models of species' reactions to climate change with defined and measurable biological mechanisms. Predictions from climate models, for example, need empirical tests to provide conservation managers with workable approaches to multiple impacts from climate change (Enriquez-Urzelai et al., 2019; Walls & Gabor, 2019).
5. Use more studies of behaviour, physiology, genetics and perhaps other disciplines that can have broad utility for understanding amphibian responses to climate change to inform strategies for amphibian conservation and management (Walls & Gabor, 2019).
6. Initiate and support long-term monitoring studies to understand how climate change-driven stress ultimately affects individual fitness, population resilience, relative abundances, and range shifts. Additionally, multiple measures of physiological health are needed to provide a more holistic assessment of how climate change-related factors impact individuals (Walls & Gabor, 2019; Winter et al., 2016).
7. Prioritise all amphibian species. Like species of conservation concern, non-threatened species, and especially those with data deficiency, also undergo localised population declines and losses due to climate change. Yet, common or obscure species typically are not the beneficiaries of conservation interventions. Proactively implementing conservation of common species could lead to early detection of climate change-driven issues before endangerment occurs (Walls & Gabor, 2019).
8. Develop and promote catastrophe response, rescue, and re-introduction work. In the face of increasingly extreme weather events predicted as a result of climate change, rescuing amphibians from the wild may be a necessary conservation management action for some species. These rescues may be short-term—holding individuals for weeks to months until the threat to the species or population in the wild is reduced—or may

involve the establishment of ex situ populations and reintroductions over longer time frames. Perhaps the first such instance of amphibian rescue in response to an extreme weather event related to climate change occurred in early 2020, when extreme drought conditions initiated the rescue of Booroolong Frogs (*Litoria booroolongensis*) from a population in northern New South Wales, Australia (NSW Department of Planning Industry and Environment, 2020).

9. Focus work on assisted migrations and managed relocation **for the most vulnerable species and geographic areas.** Most amphibians are dispersal-limited, making them one of the most climate change vulnerable groups of organisms (Foden et al., 2013). One management practice that has been suggested for dispersal-limited taxa is managed relocations, whereby populations, species, or genotypes are established in climatically suitable regions that exist outside of the natural/historical range of the species for the purpose of maintaining biological diversity or ecosystem functions (Hoegh-Guldberg et al., 2008; Richardson et al., 2009). Many ethical, legal, and ecological dilemmas arise from this practice (Schwartz et al., 2012), among them is the potential for unintended and unpredictable consequences (Ricciardi & Simberloff, 2009). Despite these caveats, there have been efforts to more generally establish frameworks for assessing which species possess traits that might make them candidates for managed relocation, and which ecosystems and communities might benefit most from managed relocations (Gallagher, Makinson, Hogbin, & Hancock, 2015). Sax, Early, & Bellemare (2013) further suggest approaches for estimating tolerance niches as a means to identify climatically suitable potential sites for the establishment of new populations, and for assessing which species require different management interventions (*in situ* conservation versus managed relocations versus *ex situ* rescue). With regard to amphibians, managed relocation has mostly been viewed as a potential management response to disease mitigation, which advocates translocating populations to climate refugia that are unfavourable for disease, preferably within or near their natural ranges (Scheele et al., 2014). To our knowledge, there are no instances where managed relocations have been implemented for amphibians to ameliorate the impacts of climate change, and large-scale implementation of assessment frameworks to identify which amphibian species, ecosystems, and communities might benefit from this intervention is lacking. Early and Sax (2011) estimated climate paths for 15 species of amphibians in the Western United States and found that a combination of dispersal and population persistence during short periods of unfavourable climate were needed for amphibians to successfully shift ranges in response to climate change; for those species for which climate paths could not be successfully identified, managed relocations were suggested as a possible intervention.

Discussion

Progress has been made on many aspects of how climate change is already changing and will continue to affect amphibian biodiversity. However, we still have a tremendous amount of work to better plan for and take actions against the negative effects of climate change. Advances in modelling and data mining, in particular, have enabled a new wave of research on theoretical trajectories and specification of taxa that are expected to be impacted negatively by changes in climate. Further work on gaps in mitigation and restoration research, response to extreme events (e.g., fires), and protected area design and management should also be prioritised. In addition, we clarify that conservation actions rely on thoughtful planning and, most critically, data from active and long-term fieldwork.

Challenges and prospects; we need more field data

Abundant metadata analyses and modelling studies are devoted to the topic of climate tracking, yet the number of carefully collected empirical datasets available for parameterisation is still small and not representative of sites where amphibian species richness or abundance is highest. This reflects a systemic undervaluation of boots-on-the-ground life history data and lengthy experimental assays by the scientific community (as reflected in estimates of scientific impact), and the science funding community. Moreover, while resurveys may be able to report changes in the altitudinal range of amphibians across multiple regions of the world (e.g. Bickford, Howard, Ng, & Sheridan, 2010; Bustamante, Ron, & Coloma, 2005), the lack of information on corresponding environmental shifts on the ground precludes statistical tests of associations. An increase in the number and quality of in situ observations can revolutionise our understanding of climate tracking in amphibians, and considerably change predictions in the face of future global change. However, there are several impediments to this, especially in countries that do not prioritise climate change and biodiversity conservation. This is often compounded by lack of training or infrastructure to conduct climate change research. Finally, in several countries, existing legal frameworks make field research increasingly difficult (e.g., India, Indonesia, Brazil) and newer legislations have unintentionally stifled international collaborations by making the collection and sharing of genetic material difficult (Prathapan et al., 2018; Rochmyaningsih, 2019). A long-term solution to these challenges lies in making policy changes that separate non-commercial and commercial research; having clear guidelines that enable research on amphibians and nurture international collaborations and skill transfer. The challenges could also be overcome especially in regions outside protected areas by engaging citizens via citizen science programmes and setting up long term monitoring databases (e.g. FrogID, available from https://www.frogid.net.au; iNaturalist, available from https://www.inaturalist.org; Frog watch India, available from https://indiabiodiversity.org; Herpmapper, available from https://www.herpmapper.org/), allowing comparative studies across time and space, and a rapid understanding of biodiversity across large scales after catastrophic events such as fire (e.g. Rowley, Callaghan, & Cornwell, 2020).

Box 3.1: Sea level rise and salinity

Freshwater systems are vital for amphibians with biphasic life cycles (i.e. those that occupy both aquatic and terrestrial habitats at different stages of their life cycle) and permanently aquatic species. Numerous species of amphibians may be found in coastal freshwater wetlands, which are becoming increasingly vulnerable to tropical cyclonic storms (hurricanes) and associated storm surge and coastal flooding (Walls et al., 2019). Globally, coastal wetlands are expected to be among the most severely impacted by climate change because of increased flooding and secondary salinisation from sea level rise along with increased frequency and intensity of coastal storms (Albecker & McCoy, 2017). Both the frequency and intensity of the strongest North Atlantic tropical cyclones have increased since the 1970s (Bhatia et al., 2019; Hartmann et al., 2013). Moreover, using the IPCC RCP8.5 baseline scenario of greenhouse gas emissions, Kirezci et al. (2020) projected that, by 2100, an increase of 48% (compared to present day) of global land area will be vulnerable to episodic coastal flooding from a 1 in 100-year return period event. Thus, any climate change-driven alterations to the frequency and intensity of storm events could amplify future coastal flooding due to sea level rise, posing an unprecedented challenge for conservation and management of amphibians in coastal ecosystems (Kirezci et al., 2020; Walls et al., 2019).

Box 3.2: Gaps in our knowledge on effects of climate-change on amphibians

In this box, we use data from a recent systematic review (literature from 2005-2015: Winter et al., 2016) on climate change in amphibians (and reptiles) to illustrate trends for the global, taxonomic, and distribution of research on climate change.

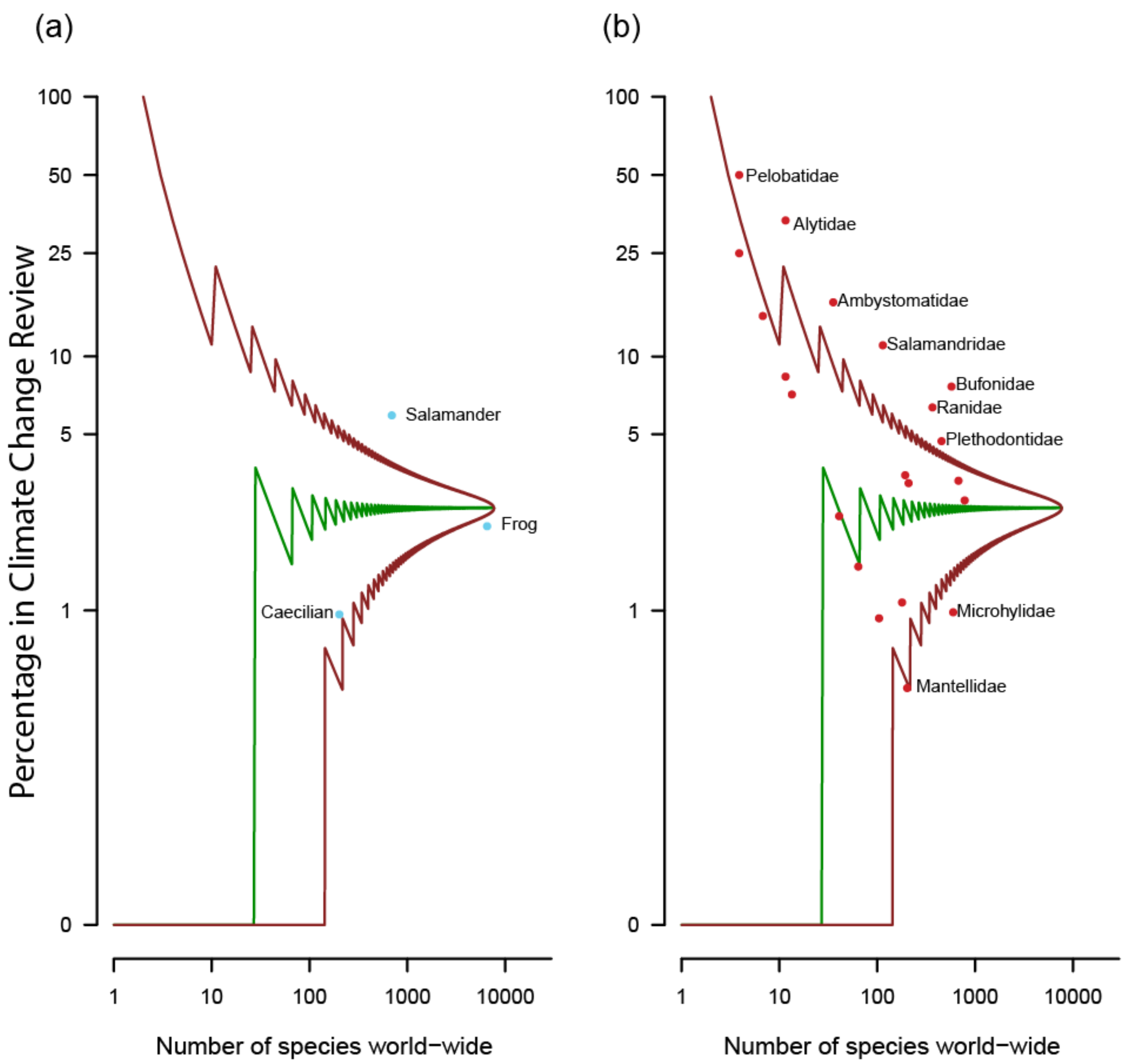
In this global dataset, there was a clear bias towards North American and European amphibians, a trend seen in amphibian studies more generally (da Silva et al., 2020), with a positive bias on studies on salamanders (Box Figure 3.1a). Studies are of only a single species or no studies at all came from Africa, Asia, and Australia despite their high amphibian biodiversity (Zellmer et al., 2020). South America was relatively well covered with studies covering many taxa in Argentina, Brazil, and Colombia (Box Figure 3.2). Efforts to model amphibian range shifts under future climates are geographically heterogeneous, with most studies in the United States, South America (primarily Brazil), and Europe. The taxa studied are indicative of regions where research was conducted.

Most studies reviewed by Winter et al (2016) use both temperature and precipitation (Box Figure 3.3.a), variables known to correlate with species richness in amphibians (Pyron & Wiens, 2013), and expected to alter under most climate change scenarios (Sodhi et al., 2008). However, studies that include extreme events such as storms, droughts and fires (see Box 3.3) are largely absent, despite the fact that these effects may be major drivers of extinction (Foden et al., 2019). Very few studies examine key environmental variables such as habitat requirements for amphibians, prey items, and soil and leaf litter characteristics (Box Figure 3.3b), and only a small subset examine human impact variables such as habitat fragmentation or presence of invasive species (Box Figure 3.3c). Taken together, this suggests that future studies of climate change and amphibians will need to rely on newer methods, more data, and better-quality datasets that include microhabitat data in order to be useful for providing needed insights for conserving amphibian biodiversity.

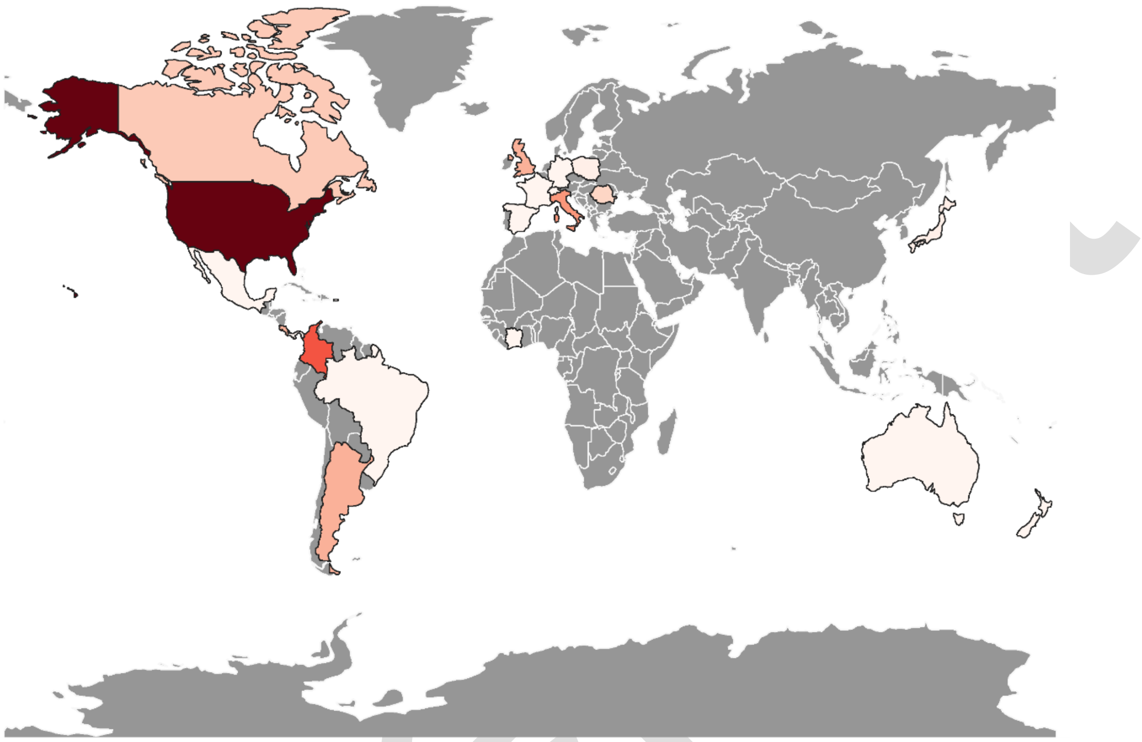
Why do we need data on tropical species?

The tropics hold the vast majority of extant amphibian species richness, yet data from these areas are most scant (Box Figure 3.2). It has been argued that these species are most vulnerable to the proximate effects of climate change (Foden et al., 2019). Tropical species often live close to their upper thermal tolerance limit and show narrow thermal performance breadths (Navas, Gomes, & Carvalho, 2008), which makes them particularly vulnerable to climate change. Underlying physiological mechanisms allowing some amphibians to cope with variation in temperature remain unclear, yet genetic studies suggest many mechanisms may be involved (Yang et al., 2016). To understand how animals cope with variation in temperature and hydric stress, we need a combination of physiological studies with those that investigate underlying genetic mechanisms.

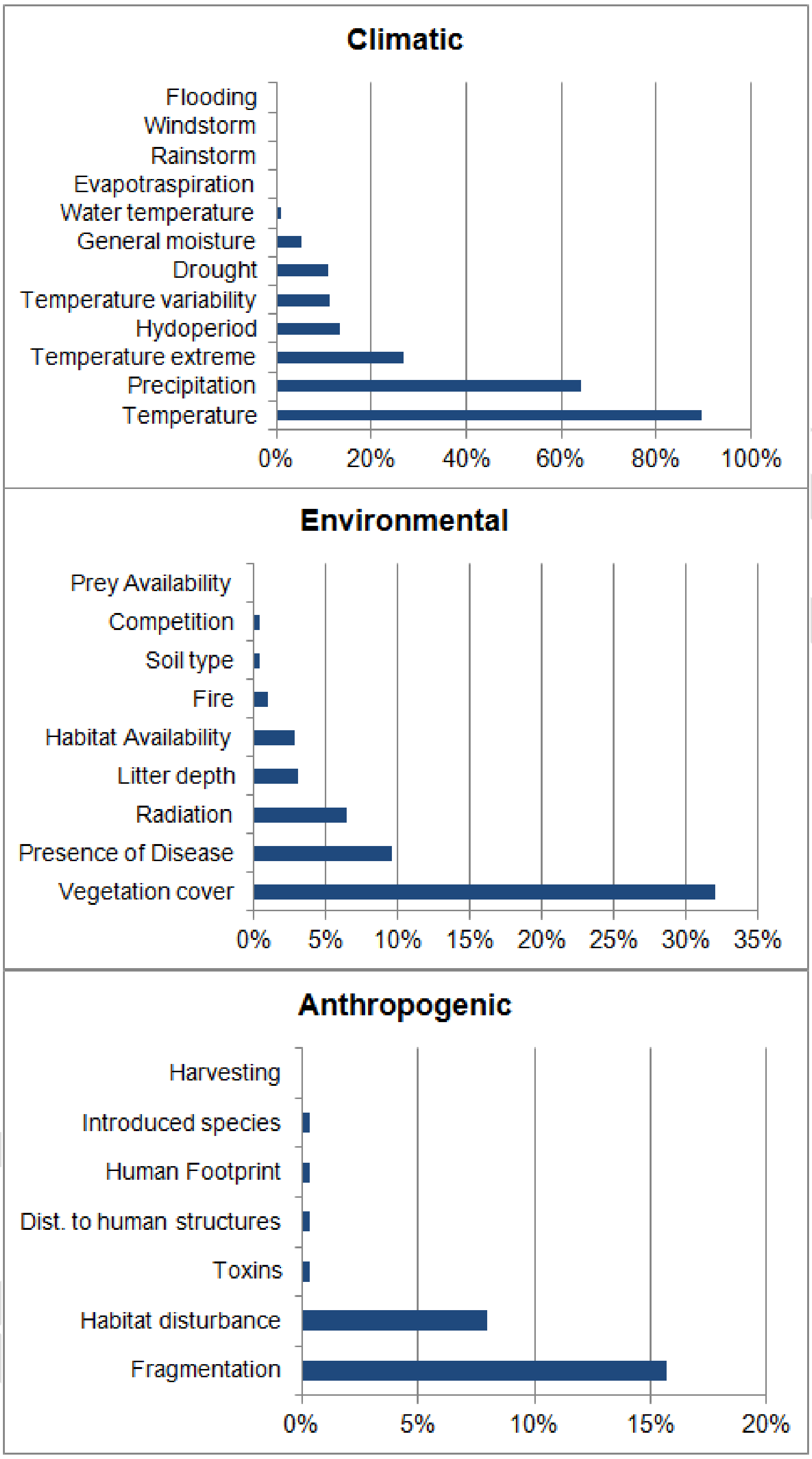
However, to predict future impacts of global change we also need species distribution models that use the biology of these understudied animals (Foden et al., 2019). Future models will require the use of physiological data to build mechanistic species distribution models, and transcriptomic data to provide a powerful tool to predict future impacts of climate change on all amphibians. This in turn relies on scientific capacity growing and being supported by these countries in order to facilitate data collection.



Box Figure 3.1. The taxonomic bias in data on amphibian climate change studies reflects the geographic distribution of species investigated. In this figure the taxonomic groups are placed with reference to their size (number of species on a log scale x-axis) and the proportion in the review (% in climate change review - y-axis). The brown and green lines represent parity and 95% confidence intervals, respectively, and are jagged due to the log scale of the x-axis. (a) Only two studies included caecilians (below the green line of parity but not outside the lower 95% confidence interval, brown line). Studies on salamanders were significantly overrepresented (above the brown 95% confidence line interval), while those on anurans were underrepresented (below the brown 95% confidence interval) in the dataset. Salamanders are particularly well represented in the dataset including ambystomatids, salamandrids, and plethodontids. (b) Of the frogs studied, boreal families such as ranids, bufonids, pelobatids and alytids were all overrepresented, while the more tropical mantellids and microhylids were underrepresented.



Box Figure 3.2. Data from Winter et al. (2016) on 193 species from 24 families of amphibians representing all three orders of amphibians that demonstrate a distinct geographic bias in the literature on amphibians and climate change. Darker colour indicates a greater number of studies. Note that the criteria for including literature in this review were relatively stringent (see Winter et al., 2016), and there may be more studies from other areas of the globe that were excluded or have been published since 2016.



Box Figure 3.3. a) Climatic, b) Environmental, and c) Anthropogenic variables used in studies reviewed by Winter et al (2016). Presence of the term was used to calculate percentage from 325 entries.

Box 3.3: Amphibians and fire

In many ways fire has set the most dramatic direct challenges to society that hinge on climate change and society's ability to become a part of natural systems and not apart from them. The size, frequency, and severity of fires are anticipated to increase under climate change (Dale et al., 2001). In particular, extreme fire-weather conditions including drought and hot-dry-windy air conditions coupled with human factors such as fire suppression activities of past decades (e.g. McDonald, Srock, & Charney, 2018; Moritz et al., 2014; Srock, Charney, Potter, & Goodrick, 2018; Turner & Romme, 1994) and increasing human-mediated fire starts are triggering widespread fires—a worldwide signature is evident (Box Figure 3.4). However, there is an overall lack of knowledge of the response of amphibians to fire (Driscoll et al., 2010), hindering our ability to assess fire risk and make informed management decisions. There is an urgent need to understand the impact of fires on amphibians, particularly in areas such as Australia, North America, and the Amazon basin, given the more frequent and more severe fires predicted as a consequence of global climate change (Moritz et al., 2012; Williams et al., 2008).

In North and South America, amphibian response to fire and fire effects to broader ecosystems are emerging. Pilliod, Bury, Hyde, Pearl, & Corn (2003) reviewed data from 15 studies of prescribed and wildfires in North America, finding: 1) spatial and temporal variability of fire effects on amphibians reflecting their life history, habitat associations, species range extent, and time-since-burning; 2) direct mortality effects as well as indirect effects on microclimate, aquatic habitat sedimentation and altered hydroperiod, nutrient pulses, and microhabitat changes to duff, litter, and down wood; and 3) a need for long-term data. Hossack & Pilliod (2011) reviewed seven studies with pre- and post-fire data and seven retrospective studies. They found that studies of plethodontid salamanders and southwestern-

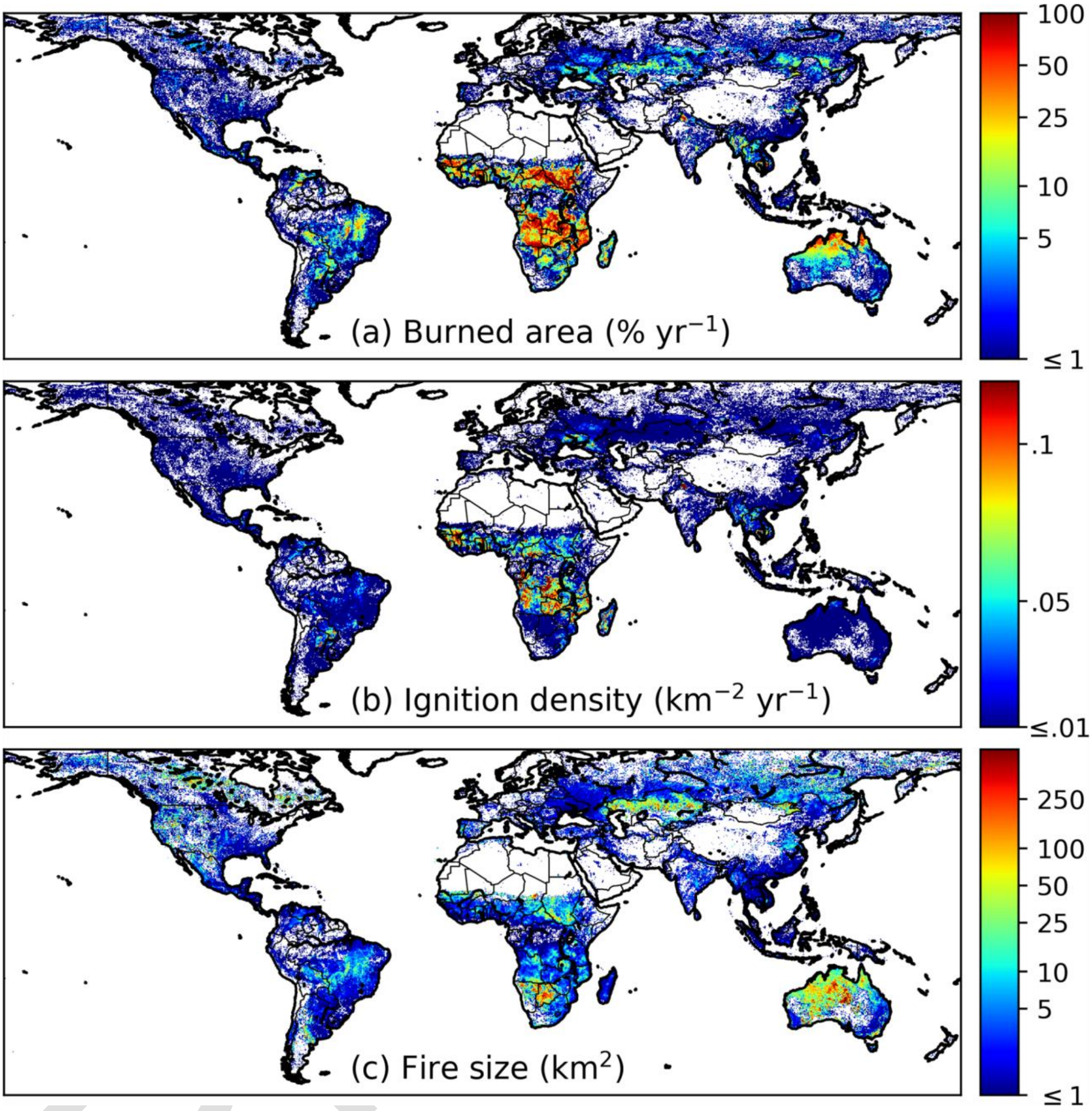
USA stream-breeding amphibian species reported negative fire effects on populations, individuals, or critical habitat attributes. Cousins, Leppin, Neill, Radin, & Olson (2019) reported high amphibian productivity in high-elevation pond-meadow complexes within areas with past wildfires in Oregon, supporting the apparent resilience of these amphibians to fire disturbance that may help bolster broader ecosystem recovery through food web interactions.

Although monitoring efforts in tropical regions of the Americas also give the impression that fires may be reasonably well tolerated by local amphibian communities (Drummond, Moura, & Pires, 2018; Warren-Thomas et al., 2013), this likely reflects the composition of the communities sampled to date, and the environmental filters that they have encountered. For instance, inventories in bamboo and *terra firme* forests in the Peruvian Amazon (Madre de Dios) detected generally lower (but not statistically significant changes in) amphibian diversity and abundance following anthropogenic fires associated with a severe drought in 2005 (Warren-Thomas et al., 2013). However, all species then recorded were known to be resistant to habitat alterations, and the inventory included no primary forest specialist species; as such, the sampling area may have been located in a transition zone already occupied by fire-resistant species. Similarly, in South American rupestrian grasslands known to be regularly subjected to wildfires, Drummond et al. (2018) found no statistically supported reduction in amphibian diversity following burning. The authors largely attributed these results to the timing of the fire (the dry season, when most riparian amphibians are hidden in rock outcrops, burrows or termite mounds), but noted that the single species known to be a direct developer and to utilise grasses as shelter was that with lower observation records and decreased abundance following burning. With increased attention recently turning to the burning of large tracks of rainforests in the Amazon region (Bullock, Woodcock, Souza, &

Olofsson, 2020), it remains to be seen whether more significant changes will be detected in the composition and abundance of the many direct developers and wet forest-dependent species known to occupy this domain.

Australia. Australia's 2019/2020 fire season brought the interaction of climate change and fires to the forefront in the country, with more than 17 million hectares of forest burnt in Australia (Boer, de Dios, & Bradstock, 2020; Noble, 2020). While a natural part of many ecosystems in Australia, fires of this extent are not typical (Boer et al., 2020), and a large proportion of wetter habitats, which historically burn infrequently, also burnt. The handful of studies on the impact of fire on Australian frogs have indicated overall resilience to fires (Bamford, 1992; Driscoll & Roberts, 1997; Lowe, Castley, & Hero, 2013; Potvin et al., 2017; Westgate, Driscoll, & Lindenmayer, 2012; Westgate, MacGregor, Scheele, Driscoll, & Lindenmayer, 2018), and short-term postfire persistence of many frog species across the fire was revealed via citizen science (Rowley et al., 2020), but the long-term impact of the summer 2019/2020 fires on Australian frogs remains unknown. Particular concern is held for species with small geographic ranges, especially rainforest-dependent species.

Mitigations to reduce fire effects. In the USA Southwest, society should reduce effects of human-mediated disturbances in fire-prone areas that could affect amphibian habitat conditions by: 1) reducing livestock grazing on native plants and near aquatic ecosystems that may result in altered fire-related processes and functions; 2) preventing human-transmission of invasive species, especially non-native plants that alter fire regimes; and 3) actively reducing fuel loads in areas subject to wildfire (Jones, Halama, & Lovich, 2016). These are all interacting factors as dry conditions and lightning strikes are predicted to increase with climate change in many areas. In the Northwest USA, management recommendations to address fuel treatments in forests to safeguard against wildfire risk were developed for known sites of the Siskiyou Mountains salamander, a species of concern (Clayton, Olson, Nauman, & Reilly, 2009). Due to elevated concerns for human communities-at-risk of wildfire within the salamander's range, alternative measures were developed to address salamander persistence to better inform management decisions when trade-offs between people and biota are used to inform decisions. In many ways, these actions mean a cultural reset of societal norms to integrate people into natural systems designed for mutual coexistence. Although a suite of approaches can be derived for multiple threats, a downscaled species-specific, geography-specific, and threat-combination specific approach is likely most effective to address the contexts of known local-to-regional issues, while simultaneously addressing human socioeconomics of the system.



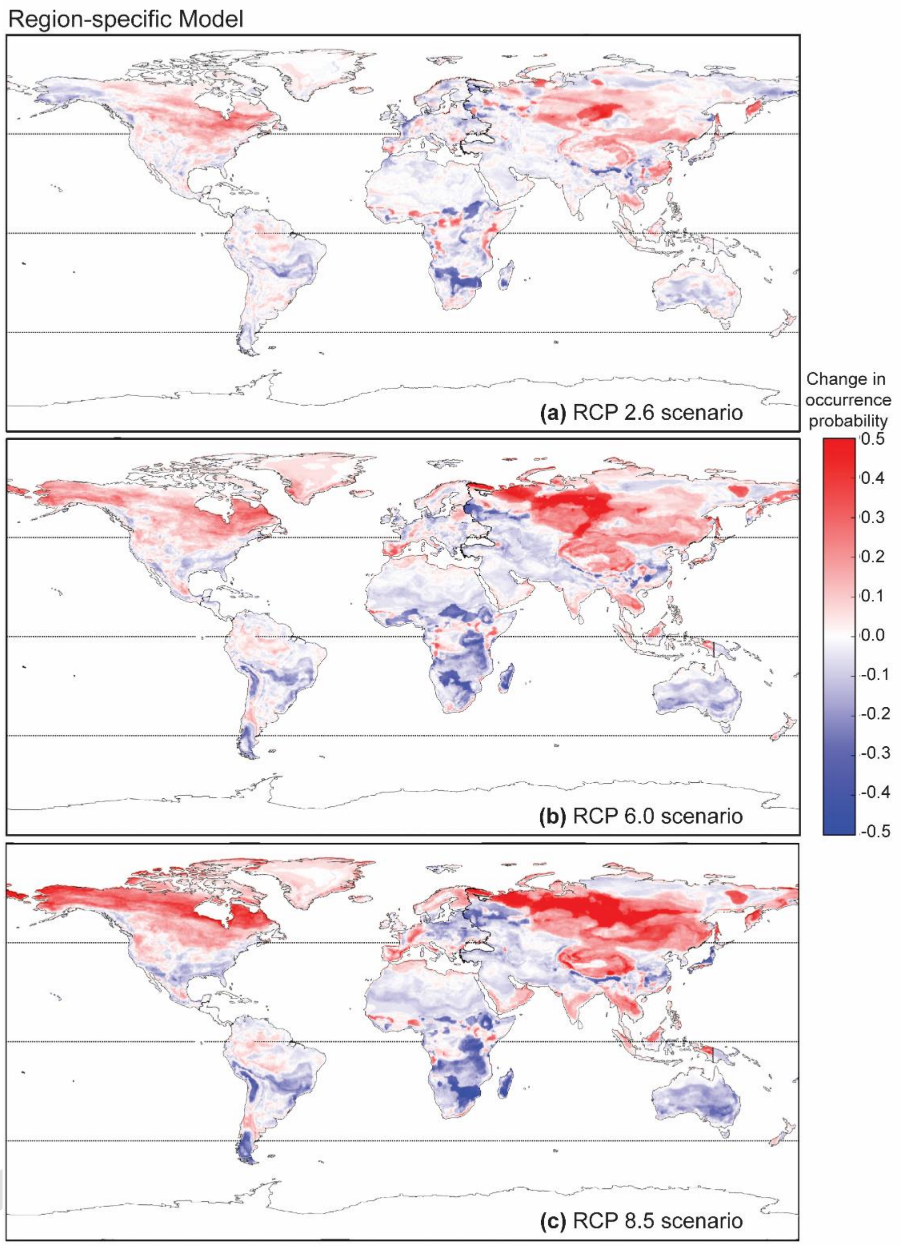
Box Figure 3.4. Average global burned area (from dataset MCD64A1: (Giglio, Boschetti, Roy, Humber, & Justice, 2018), ignition density and fire size over a 14-year study period, 2003-2016, representing 13,250 fires averaging 4.4 km^2 in average size. For any given location, burned area in panel (a) can be represented as the product of ignitions per year shown in (b) and fire size shown in (c). From Andela et al., (2019); globalfiredata.org, accessed 8 July 2021.

Box 3.4: Synergies: disease ecology

Synergies between climate change and infectious diseases have received a great deal of attention in recent years. In particular, several hypotheses have been proposed relating the emergence of the amphibian disease chytridiomycosis caused by fungi of the genus *Batrachochytrium* (primarily *B. dendrobatidis*, *Bd*), to climate change. Pounds et al. (2006) proposed the chytrid thermal optimum hypothesis, which posits that increased cloud cover led to a convergence between daytime and night-time temperatures leading to increased growth of *Bd* and amphibian declines in Monteverde, Costa Rica. They also proposed that climate change was increasing the number of dry days and decreasing mist frequency with detrimental consequences to amphibians. Subsequent analyses found no statistical support for the chytrid thermal optimum hypothesis (Rohr & Raffel, 2010; Rohr, Raffel, Romansic, McCallum, & Hudson, 2008). An isotopic tree ring study showed no long-term drying trend at Monteverde but did reveal that major declines in the 1980s corresponded to a particularly dry interval caused by a strong El Niño event. Analyses of temporally detrended data to account for epidemic *Bd* spread also support a role for extreme climatic conditions and increased climate variability caused by El Niño in amphibian declines in Latin America (Rohr & Raffel, 2010). Because climate change is predicted to increase climate variability Thornton et al. 2014 as well as the strength and frequency of extreme El Niño events (Wang et al., 2019), these results suggest the impact of chytridiomycosis outbreaks on amphibian populations could increase because of climate change (see Box Figure 3.5).

Climate change could increase the impact of *Bd* on amphibian populations through milder winter conditions in temperate montane regions. Decreased snowpack in Wyoming (Muths, Hossack, Grant, Pilliod, & Mosher, 2020) and earlier thaw date in the Pyrenees (Clare et al., 2016) have been associated with decreased survival with *Bd* and increased prevalence of *Bd*, respectively, and chytridiomycosis outbreaks in central Spain have been linked to milder winter conditions that allow for increased growth of *Bd* (Bosch, Carrascal, Durán, Walker, & Fisher, 2007). Beyond favouring conditions for growth of *Bd,* climate change may affect the interaction between host and parasites or pathogens. The thermal mismatch hypothesis (Cohen et al., 2018, 2017) proposes that while both parasites and hosts should have a performance optimum that matches local conditions, parasites typically have broader thermal tolerances than hosts and that cool-adapted hosts typically have a right-skewed performance curve while warm-adapted hosts typically have a left-skewed curve. Climate change could shift local conditions away from the host performance optimum and, because parasites have a broader performance curve, increase the performance advantage of parasite over host. Climate warming could thus promote increased *Bd* growth and outbreaks in cool-adapted species, a result that has been supported by both lab experiments and a meta-analysis of *Bd* outbreaks (Cohen et al., 2017). These results suggest that the effect of climate warming on *Bd* outbreaks may depend on host physiology, allowing predictions of which species may be most impacted by future outbreaks of *Bd* or other amphibian diseases.

992



993 Box Figure 3.5: Predicted change in the global occurrence probability of the amphibian
994 chytrid fungus *Batrachochytrium dendrobatidis* (Bd), a skin pathogen which can cause the
995 disease chytridiomycosis, with three climate change scenarios for the year 2100. These
996 projections were derived from region-specific models, likely providing a more accurate
997 perspective of the increasing occurrences of Bd in north-temperate zones and higher
998 elevations. From (Xie, Olson, & Blaustein (2016).

Box 3.5: Synergies: habitat alteration/degradation

Because amphibians are dependent on water or soil moisture, drought can have major negative effects on amphibian survival and reproduction (reviewed in Walls et al., 2013). Examples of drought effects on amphibians include extirpation of terrestrial species (e.g., from decreased soil moisture for lungless salamanders; Jaeger, 1980; reduction in number and water level of breeding pools for Australian frogs; Scheele et al., 2012), and changes in regional hydrology resulting in pond desiccation and population declines (e.g., frogs and salamanders in Yellowstone National Park; McMenamin et al., 2008). Increased evapotranspiration from wetlands and decreased hydrological input as a result of changes in precipitation could cause desiccation of amphibian breeding sites, causing reproductive failure of the species that use them.

Urbanisation, agricultural development, and intensive use of rangelands for livestock grazing are main drivers of habitat loss and degradation (Cameron, Marty, & Holland, 2014). The impact of habitat degradation on aquatic breeding amphibians can be exacerbated by climate change. The increase in frequency of droughts in some regions (e.g., California) has been linked to anthropogenic warming (Diffenbaugh, Swain, & Touma, 2015) and threatens species that rely on seasonal wetlands. For example, wetland habitat could be converted to grassland as a result of decreased hydroperiod resulting from climate change, eliminating both habitat and breeding sites for amphibians (Blaustein et al., 2010). Yet, the effects of wetland warming and drying on amphibians may be difficult to predict and not necessarily synergistic or even additive, in part because amphibians may be able to compensate by decreasing metamorphosis time or increasing growth rate in response to higher resource availability (O'Regan, Palen, & Anderson, 2014). Although with limited effectiveness, modified and created ponds have been shown to mitigate the impact of extreme drought and habitat loss on pond-breeding amphibians (Baumberger, Backlin, Gallegos, Hitchcock, & Fisher, 2020; Pechmann, Estes, Scott, & Gibbons, 2001).

Acknowledgements

We want to thank Ariadne Angulo, Amaël Borzée, Sally Wren for careful reading and thoughtful editorial suggestions that improved the manuscript.

References

Albecker, M. A., & McCoy, M. W. (2017). Adaptive responses to salinity stress across multiple life stages in anuran amphibians. *Frontiers in Zoology*, *14*, 40. doi: 10.1186/s12983-017-0222-0

Alford, R. A., Bradfield, K. S., & Richards, S. J. (2007). Global warming and amphibian losses. *Nature*, *447*(7144), E3–E4. doi: 10.1038/nature05940

Alroy, J. (2015). Current extinction rates of reptiles and amphibians. *Proceedings of the National Academy of Sciences of the United States of America*, *112*(42), 13003–13008. doi: 10.1073/pnas.1508681112

Alton, L. A., & Franklin, C. E. (2017). Drivers of amphibian declines: effects of ultraviolet radiation and interactions with other environmental factors. *Climate Change Responses*, *4*, 6. doi: 10.1186/s40665-017-0034-7

Amaro, R. C., Rodrigues, M. T., Yonenaga-Yassuda, Y., & Carnaval, A. C. (2012). Demographic processes in the montane Atlantic rainforest: Molecular and cytogenetic evidence from the endemic frog *Proceratophrys boiei*. *Molecular Phylogenetics and Evolution*, *62*(3), 880–888. doi: 10.1016/j.ympev.2011.11.004

AmphibiaWeb. (2020). AmphibiaWeb. Retrieved from https://amphibiaweb.org/

Andela, N., Morton, D. C., Giglio, L., Paugam, R., Chen, Y., Hantson, S., … Randerson, J. T. (2019). The Global Fire Atlas of individual fire size, duration, speed and direction.

Earth System Science Data, *11*, 529–552. doi: 10.5194/essd-11-529-2019

Bamford, M. J. (1992). The impact of fire and increasing time after fire upon *Heleioporus eyrei*, *Limnodynastes dorsalis* and *Myobatrachus gouldii* (Anura: Leptodactylidae) in Banksia woodland near Perth, Western Australia. *Wildlife Research*, *19*(2), 169–178.

Barnosky, A. D., Matzke, N., Tomiya, S., Wogan, G. O. U., Swartz, B., Quental, T. B., … Ferrer, E. A. (2011). Has the Earth's sixth mass extinction already arrived? *Nature*, *471*(7336), 51–57. doi: 10.1038/nature09678

Baumberger, K. L., Backlin, A. R., Gallegos, E. A., Hitchcock, C. J., & Fisher, R. N. (2020). Mitigation ponds offer drought resiliency for western spadefoot (*Spea hammondii*) populations. *Bulletin, Southern California Academy of Sciences*, *119*(1), 6–17. doi: 10.3160/0038-3872-119.1.6

Becker, C. G., Fonseca, C. R., Haddad, C. F. B., Batista, R. F., & Prado, P. I. (2007). Habitat split and the global decline of amphibians. *Science*, *318*(5857), 1775–1777. doi: https://doi.org/10.1126/science.1149374

Beebee, T. J. C. (1995). Amphibian breeding and climate. *Nature*, *374*(6519), 219–220. doi: 10.1038/374219a0

Bhatia, K. T., Vecchi, G. A., Knutson, T. R., Murakami, H., Kossin, J., Dixon, K. W., & Whitlock, C. E. (2019). Recent increases in tropical cyclone intensification rates. *Nature Communications*, *10*, 635. doi: 10.1038/s41467-019-08471-z

Bickford, D. (2005). Long-term frog monitoring with local people in Papua New Guinea and the 1997–98 El Nino Southern Oscillation. In M. A. Donnelly, B. Crother, C. Guyer, & M. H. Wake (Eds.), *Ecology and* *evolution in the tropics: a herpetological perspective* (pp. 260–283). Chicago, USA: University of Chicago Press.

Bickford, D., Howard, S. D., Ng, D. J. J., & Sheridan, J. A. (2010). Impacts of climate change on the amphibians and reptiles of Southeast Asia. *Biodiversity and Conservation, 19*(4), 1043–1062. doi: 10.1007/s10531-010-9782-4

Bickford, D., Posa, M. R. C., Qie, L., Campos-Arceiz, A., & Kudavidanage, E. P. (2012). Science communication for biodiversity conservation. *Biological Conservation*, *151*, 74–76. doi: 10.1016/j.biocon.2011.12.016

Biebighauser, T. R. (2007). *Wetland drainage, restoration, and repair*. Lexington, KY, USA: University Press of Kentucky.

Biebighauser, T. R. (2015). *Wetland restoration and construction: A technical guide. Second edition*. Morehead, Kentucky, USA: Center for Wetland and Stream Construction.

Blaustein, A. R., Belden, L. K., Olson, D. H., Green, D. M., Root, T. L., & Kiesecker, J. M. (2001). Amphibian breeding and climate change. *Conservation Biology*, *15*(6), 1804–1809. doi: 10.1046/j.1523-1739.2001.00307.x

Blaustein, A. R., Walls, S. C., Bancroft, B. A., Lawler, J. J., Searle, C. L., & Gervasi, S. S. (2010). Direct and indirect effects of climate change on amphibian populations. *Diversity*, *2*(2), 281–313. doi: 10.3390/d2020281

Boer, M. M., de Dios, V. R., & Bradstock, R. A. (2020). Unprecedented burn area of Australian mega forest fires. *Nature Climate Change*, *10*(3), 171–172. doi: 10.1038/s41558-020-0710-7

Bosch, J., Carrascal, L. M., Durán, L., Walker, S., & Fisher, M. C. (2007). Climate change and outbreaks of amphibian chytridiomycosis in a montane area of Central Spain; is there a link? *Proceedings of the Royal Society B: Biological Sciences*, *274*(1607), 253–260. doi: 10.1098/rspb.2006.3713

Brattstrom, B. H. (1968). Thermal acclimation in Anuran amphibians as a function of latitude and altitude. *Comparative Biochemistry And Physiology*, *24*(1), 93–111. doi: 10.1016/0010-406X(68)90961-4

Bucciarelli, G. M., Clark, M. A., Delaney, K. S., Riley, S. P. D., Shaffer, H. B., Fisher, R. N., … Kats, L. B. (2020). Amphibian responses in the aftermath of extreme climate events. *Scientific Reports*, *10*, 3409. doi: 10.1038/s41598-020-60122-2

Buckley, L. B., Hurlbert, A. H., & Jetz, W. (2012). Broad-scale ecological implications of ectothermy and endothermy in changing environments. *Global Ecology and Biogeography*, *21*(9), 873–885. doi: 10.1111/j.1466-8238.2011.00737.x

Bullock, E. L., Woodcock, C. E., Souza, C., & Olofsson, P. (2020). Satellite-based estimates reveal widespread forest degradation in the Amazon. *Global Change Biology*, *26*(5), 2956–2969. doi: 10.1111/gcb.15029

Burraco, P., Laurila, A., & Orizaola, G. (2021). Limits to compensatory responses to altered phenology in amphibian larvae. *Oikos*, *130*(2), 231–239. doi: 10.1111/oik.07919

Bustamante, M. R., Ron, S. R., & Coloma, L. A. (2005). Cambios en la diversidad en siete comunidades de anuros en los Andes de Ecuador. *Biotropica*, *37*(2), 180–189. doi: 10.1111/j.1744-7429.2005.00025.x

Cagnacci, F., Boitani, L., Powell, R. A., & Boyce, M. S. (2010). Animal ecology meets GPS-based radiotelemetry: A perfect storm of opportunities and challenges. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *365*(1550), 2157–2162. doi: 10.1098/rstb.2010.0107

Cahill, A. E., Aiello-Lammens, M. E., Fisher-Reid, M. C., Hua, X., Karanewsky, C. J., Ryu, H. Y., … Wiens, J. J. (2013). How does climate change cause extinction? *Proceedings of the Royal Society B: Biological Sciences*, *280*(1750), 20121890. doi: 10.1098/rspb.2012.1890

Cameron, D. R., Marty, J., & Holland, R. F. (2014). Whither the rangeland?: Protection and conversion in California's rangeland ecosystems. *PLoS ONE*, *9*(8), e103468. doi: 10.1371/journal.pone.0103468

Carnaval, A. C., Hickerson, M. J., Haddad, C. F. B., Rodrigues, M. T., & Moritz, C. (2009). Stability predicts genetic diversity in the Brazilian Atlantic forest hotspot. *Science*, *323*(5915), 785–789. doi: 10.1126/science.1166955

Carnaval, A. C., Waltari, E., Rodrigues, M. T., Rosauer, D., VanDerWal, J., Damasceno, R., … Moritz, C. (2014). Prediction of phylogeographic endemism in an environmentally complex biome. *Proceedings of the Royal Society B: Biological Sciences*, *281*(1792), 20141461. doi: 10.1098/rspb.2014.1461

Carroll, C., Fredrickson, R. J., & Lacy, R. C. (2014). Developing metapopulation connectivity criteria from genetic and habitat data to recover the endangered Mexican Wolf. *Conservation Biology*, *28*(1), 76–86. doi: 10.1111/cobi.12156

Caruso, N. M., Sears, M. W., Adams, D. C., & Lips, K. R. (2014). Widespread rapid reductions in body size of adult salamanders in response to climate change. *Global Change Biology*, *20*(6), 1751–1759. doi: 10.1111/gcb.12550

Catenazzi, A., Lehr, E., & Vredenburg, V. T. (2014). Thermal physiology, disease, and amphibian declines on the eastern slopes of the Andes. *Conservation Biology*, *28*(2), 509–517. doi: 10.1111/cobi.12194

Cayuela, H., Dorant, Y., Forester, B. R., Jeffries, D. L., Mccaffery, R. M., Eby, L. A., … Funk, W. C. (2021). Genomic signatures of thermal adaptation are associated with clinal shifts of life history in a broadly distributed frog. *Journal of Animal Ecology*, *Early View*, 1–17. doi: 10.1111/1365-2656.13545

Chen, I.-C., Hill, J. K., Ohlemüller, R., Roy, D. B., & Thomas, C. D. (2011). Rapid range shifts of species associated with high levels of climate warming. *Science*, *333*(6045), 1024–1026. doi: 10.1126/science.1206432

Christian, K. A., Nunez, F., Clos, L., & Diaz, L. (1988). Thermal relations of some tropical frogs along an altitudinal gradient. *Biotropica*, *20*(3), 236–239. doi: 10.2307/2388239

Clare, F. C., Halder, J. B., Daniel, O., Bielby, J., Semenov, M. A., Jombart, T., … Fisher, M. C. (2016). Climate forcing of an emerging pathogenic fungus across a montane multi-host community. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *371*(1709), 20150454. doi: 10.1098/rstb.2015.0454

Clayton, D., Olson, D. H., Nauman, R. S., & Reilly, E. C. (2009). Managing for the Siskiyou Mountains salamander (*Plethodon stormi*) in fuel treatment areas around at-risk communities. In D. H. Olson, D. Clayton, R. S. Nauman, & H. H. Welsh Jr. (Eds.), *Conservation of the Siskiyou Mountains salamander (Plethodon stormi). Northwest Fauna 6* (pp. 39–42). Society for Northwestern Vertebrate Biology.

Cohen, J. M., Lajeunesse, M. J., & Rohr, J. R. (2018). A global synthesis of animal phenological responses to climate change. *Nature Climate Change*, *8*(3), 224–228. doi: 10.1038/s41558-018-0067-3

Cohen, J. M., Venesky, M. D., Sauer, E. L., Civitello, D. J., McMahon, T. A., Roznik, E. A., & Rohr, J. R. (2017). The thermal mismatch hypothesis explains host susceptibility to an emerging infectious disease. *Ecology Letters*, *20*(2), 184–193. doi: 10.1111/ele.12720

Cordier, J. M., Lescano, J. N., Rios, N. E., Leynaud, G. C., & Nori, J. (2020). Climate change threatens micro-endemic amphibians of an important South American high-altitude center of endemism. *Amphibia-Reptilia, 41*(2), 233–243. doi: 10.1163/15685381-20191235

Corn, P. S. (2005). Climate change and amphibians. *Animal Biodiversity and Conservation*, *28*(1), 59–67.

Cousins, C., Leppin, M., Neill, A., Radin, M., & Olson, D. H. (2019). Wetland meadow habitats in the Cascade Range: Potential refugia for herpetofaunal communities accelerating post fire ecosystem recovery. *2019 Joint Annual Meeting of the Washington Chapter of The Wildlife Society, Society of Northwest Vertebrate Biology, and Northwest Partners in Amphibian and Reptile Conservation, Grand Mound, WA.*

Cruz-Piedrahita, C., Navas, C. A., & Crawford, A. J. (2018). Life on the edge: A comparative study of ecophysiological adaptations of frogs to tropical semiarid environments. *Physiological and Biochemical Zoology*, *91*(1), 740–756. doi: 10.1086/695705

Cucherousset, J., Marty, P., Pelozuelo, L., & Roussel, J. M. (2008). Portable PIT detector as a new tool for non-disruptively locating individually tagged amphibians in the field: A case study with Pyrenean brook salamanders (Calotriton asper). *Wildlife Research*, *35*(8), 780–787. doi: 10.1071/WR08074

Cummins, D., Kennington, W. J., Rudin-Bitterli, T., & Mitchell, N. J. (2019). A genome-wide search for local adaptation in a terrestrial-breeding frog reveals vulnerability to climate change. *Global Change Biology*, *25*(9), 3151–3162. doi: 10.1111/gcb.14703

Cushman, K. A., & Pearl, C. A. (2007). *A conservation assessment for the Oregon spotted frog (*Rana pretiosa*)*.

da Silva, A. F., Malhado, A. C. M., Correia, R. A., Ladle, R. J., Vital, M. V. C., & Mott, T. (2020). Taxonomic bias in amphibian research: Are researchers responding to conservation need? *Journal for Nature Conservation*, *56*, 125829. doi: 10.1016/j.jnc.2020.125829

Dale, V. H., Joyce, L. A., McNulty, S., Neilson, R. P., Ayres, M. P., Flannigan, M. D., … Wotton, B. M. (2001). Climate change and forest disturbances. *BioScience*, *51*(9), 723–734. doi: 10.1641/0006-3568(2001)051[0723:CCAFD]2.0.CO;2

Davis, A. J., Jenkinson, L. S., Lawton, J. H., Shorrocks, B., & Wood, S. (1998). Making mistakes when predicting shifts in species range in response to global warming. *Nature*, *391*, 783–786.

Delhey, K., Dale, J., Valcu, M., & Kempenaers, B. (2020). Why climate change should generally lead to lighter coloured animals. *Current Biology*, *30*(23), R1406–R1407. doi: 10.1016/j.cub.2020.10.070

Diffenbaugh, N. S., Swain, D. L., & Touma, D. (2015). Anthropogenic warming has increased drought risk in California. *Proceedings of the National Academy of Sciences of the United States of America*, *112*(13), 3931–3936. doi: 10.1073/pnas.1422385112

Donat, M. G., Alexander, L. V., Yang, H., Durre, I., Vose, R., & Caesar, J. (2013). Global land-based datasets for monitoring climatic extremes. *Bulletin of the American Meteorological Society*, *94*(7), 997–1006. doi: 10.1175/BAMS-D-12-

00109.1

Donnelly, M. A. (1989). Demographic effects of reproductive resource supplementation in a territorial frog, *Dendrobates pumilio*. *Ecological Monographs*, *59*(3), 207–221. doi: 10.2307/1942599

Driscoll, D. A., Lindenmayer, D. B., Bennett, A. F., Bode, M., Bradstock, R. A., Cary, G. J., … York, A. (2010). Fire management for biodiversity conservation: Key research questions and our capacity to answer them. *Biological Conservation*, *143*(9), 1928–1939. doi: 10.1016/j.biocon.2010.05.026

Driscoll, D. A., & Roberts, J. D. (1997). Impact of fuel-reduction burning on the frog *Geocrinia lutea* in southwest Western Australia. *Austral Ecology*, *22*(3), 334–339. doi: 10.1111/j.1442-9993.1997.tb00679.x

Drummond, L. de O., Moura, F. R., & Pires, M. R. S. (2018). Impact of fire on anurans of rupestrian grasslands (campos rupestres): A case study in the Serra do Espinhaço, Brazil. *Salamandra*, *54*(1), 1–10.

Early, R., & Sax, D. F. (2011). Analysis of climate paths reveals potential limitations on species range shifts. *Ecology Letters*, *14*(11), 1125–1133. doi: 10.1111/j.1461-0248.2011.01681.x

Emel, S. L., Olson, D. H., Knowles, L. L., & Storfer, A. (2019). Comparative landscape genetics of two endemic torrent salamander species, *Rhyacotriton kezeri* and *R. variegatus*: implications for forest management and species conservation. *Conservation Genetics*, *20*(4), 801–815. doi: 10.1007/s10592-019-01172-6

Enriquez-Urzelai, U., Sacco, M., Palacio, A. S., Pintanel, P., Tejedo, M., & Nicieza, A. G. (2019). Ontogenetic reduction in thermal tolerance is not alleviated by earlier developmental acclimation in *Rana temporaria*.

Oecologia, *189*(2), 385–394. doi: 10.1007/s00442-019-04342-y

Epps, C. W., Wehausen, J. D., Bleich, V. C., Torres, S. G., & Brashares, J. S. (2007). Optimizing dispersal and corridor models using landscape genetics. *Journal of Applied Ecology*, *44*(4), 714–724. doi: 10.1111/j.1365-2664.2007.01325.x

Evans, A. E., Forester, B. R., Jockusch, E. L., & Urban, M. C. (2018). Salamander morph frequencies do not evolve as predicted in response to 40 years of climate change. *Ecography*, *41*, 1687–1697.

Evans, A. E., Urban, M. C., & Jockusch, E. L. (2020). Developmental temperature influences color polymorphism but not hatchling size in a woodland salamander. *Oecologia*, *192*(4), 909–918. doi: 10.1007/s00442-020-04630-y

Fey, S. B., Siepielski, A. M., Nusslé, S., Cervantes-Yoshida, K., Hwan, J. L., Huber, E. R., … Carlson, S. M. (2015). Recent shifts in the occurrence, cause, and magnitude of animal mass mortality events. *Proceedings of the National Academy of Sciences of the United States of America*, *112*(4), 1083–1088. doi: 10.1073/pnas.1414894112

Fick, S. E., & Hijmans, R. J. (2017). WorldClim 2: New 1-km spatial resolution climate surfaces for global land areas. *International Journal of Climatology*, *37*(12), 4302–4315. doi: 10.1002/joc.5086

Foden, W. B., Butchart, S. H. M., Stuart, S. N., Vié, J. C., Akçakaya, H. R., Angulo, A., … Mace, G. M. (2013). Identifying the world's most climate change vulnerable species: A systematic trait-based assessment of all birds, amphibians and corals. *PLoS ONE*, *8*(6), e65427. doi: 10.1371/journal.pone.0065427

Foden, W. B., Young, B. E., Akçakaya, H. R., Garcia, R. A., Hoffmann, A. A., Stein, B. A., … Huntley, B. (2019).

Climate change vulnerability assessment of species. *WIREs Climate Change*, *10*, e551. doi: 10.1002/wcc.551

Fog, K. (1997). A survey of the results of pond projects for rare amphibians in Denmark. *Memoranda Societatis pro Fauna et Flora Fennica*, *73*, 91–100.

Freidenburg, L. K., & Skelly, D. K. (2004). Microgeographical variation in thermal preference by an amphibian. *Ecology Letters*, *7*(5), 369–373. doi: 10.1111/j.1461-0248.2004.00587.x

Gallagher, R. V., Makinson, R. O., Hogbin, P. M., & Hancock, N. (2015). Assisted colonization as a climate change adaptation tool. *Austral Ecology*, *40*, 12–20. doi: 10.1111/aec.12163

Gamble, D. L., & Mitsch, W. J. (2009). Hydroperiods of created and natural vernal pools in central Ohio: A comparison of depth and duration of inundation. *Wetlands Ecology and Management*, *17*(4), 385–395. doi: 10.1007/s11273-008-9115-5

Ge, Q., Wang, H., Rutishauser, T., & Dai, J. (2015). Phenological response to climate change in China: A meta-analysis. *Global Change Biology*, *21*(1), 265–274. doi: 10.1111/gcb.12648

GEO BON. (2015). *Global biodiversity change indicators. Version 1.2*. Leipzig.

Germano, J. M., & Bishop, P. J. (2009). Suitability of amphibians and reptiles for translocation. *Conservation Biology*, *23*(1), 7–15. doi: 10.1111/j.1523-1739.2008.01123.x

Gibbons, J. W., Scott, E., Ryan, T. J., Buhlmann, K. A., Tuberville, T. D., Metts, B. S., … Winne, C. T. (2000). The global decline of reptiles, déjà vu amphibians. *Bioscience*, *50*(8), 653–666.

Gibbs, J. P., & Breisch, A. R. (2001). Climate warming and calling phenology of frogs near Ithaca, New

York, 1900-1999. *Conservation Biology*, *15*(4), 1175–1178. doi: 10.1046/j.1523-1739.2001.0150041175.x

Gibbs, J. P., & Karraker, N. E. (2006). Effects of warming conditions in eastern North American forests on red-backed salamander morphology. *Conservation Biology*, *20*(3), 913–917. doi: 10.1111/j.1523-1739.2006.00375.x

Giglio, L., Boschetti, L., Roy, D. P., Humber, M. L., & Justice, C. O. (2018). The Collection 6 MODIS burned area mapping algorithm and product. *Remote Sensing of Environment*, *217*, 72–85. doi: 10.1016/j.rse.2018.08.005

Gilman, S. E., Urban, M. C., Tewksbury, J., Gilchrist, G. W., & Holt, R. D. (2010). A framework for community interactions under climate change. *Trends in Ecology and Evolution*, *25*(6), 325–331. doi: 10.1016/j.tree.2010.03.002

Godsoe, W., & Harmon, L. J. (2012). How do species interactions affect species distribution models? *Ecography*, *35*(9), 811–820. doi: 10.1111/j.1600-0587.2011.07103.x

González-Suárez, M., & Revilla, E. (2013). Variability in life-history and ecological traits is a buffer against extinction in mammals. *Ecology Letters*, *16*(2), 242–251. doi: 10.1111/ele.12035

Griffiths, R. A., & Pavajeau, L. (2008). Captive breeding, reintroduction, and the conservation of amphibians. *Conservation Biology*, *22*(4), 852–861. doi: 10.1111/j.1523-1739.2008.00967.x

Gunderson, A. R., & Stillman, J. H. (2015). Plasticity in thermal tolerance has limited potential to buffer ectotherms from global warming. *Proceedings of the Royal Society B: Biological Sciences*, *282*(1808), 20150401. doi: 10.1098/rspb.2015.0401

Hartel, T., Nemes, S., Cogălniceanu, D., Öllerer, K., Schweiger, O., Moga, C. I., & Demeter, L. (2007). The effect of fish and aquatic habitat complexity on amphibians. *Hydrobiologia*, *583*, 173–182. doi: 10.1007/s10750-006-0490-8

Hartel, T., Scheele, B. C., Rozylowicz, L., Horcea-Milcu, A., & Cogălniceanu, D. (2020). The social context for conservation: Amphibians in human shaped landscapes with high nature values. *Journal for Nature Conservation*, *53*, 125762. doi: 10.1016/j.jnc.2019.125762

Hartmann, D. L., Klein Tank, A. M. G., Rusticucci, M., Alexander, L. V., Brönnimann, S., Charabi, Y. A. R., … Zhai, P. (2013). Observations: Atmosphere and surface. In T. F. Stocker, D. Qin, G.-K. Plattner, M. Tignor, S. K. Allen, J. Boschung, … P. M. Midgley (Eds.), *Climate change 2013: The physical science basis. Contribution of Working Group I to the fifth assessment report of the Intergovernmental Panel on Climate Change* (pp. 159–254). Cambridge, UK and New York, NY, USA: Cambridge University Press. doi: 10.1017/CBO9781107415324.008

Hellsten, U., Harland, R. M., Gilchrist, M. J., Hendrix, D., Jurka, J., Kapitonov, V., … Rokhsar, D. S. (2010). The genome of the western clawed frog *Xenopus tropicalis*. *Science*, *328*, 633–636. doi: 10.1126/science.1183670

Henrique, R. S., & Grant, T. (2019). Influence of environmental factors on short-term movements of butter frogs (*Leptodactylus latrans*). *Herpetologica*, *75*(1), 38–46. doi: 10.1655/D-17-00012

Hernández-Pacheco, R., Plard, F., Grayson, K. L., & Steiner, U. K. (2021). Demographic consequences of changing body size in a terrestrial salamander. *Ecology and Evolution*, *11*, 174–185. doi: 10.1002/ece3.6988

Herstoff, E., & Urban, M. C. (2014). Will pre-adaptation buffer the impacts of climate change on novel species interactions? *Ecography*, *37*(2), 111–119. doi: 10.1111/j.1600-0587.2013.00116.x

Higgins, S. I., Larcombe, M. J., Beeton, N. J., Conradi, T., & Nottebrock, H. (2020). Predictive ability of a process-based versus a correlative species distribution model. *Ecology and Evolution*, *10*, 11043–11054. doi: 10.1002/ece3.6712

Hillman, S. S., Drewes, R. C., Hedrick, M. S., & Hancock, T. V. (2014). Physiological vagility: Correlations with dispersal and population genetic structure of amphibians. *Physiological and Biochemical Zoology*, *87*(1), 105–112. doi: 10.1086/671109

Hoegh-Guldberg, O., Hughes, L., McIntyre, S., Lindenmayer, D. B., Parmesan, C., Possingham, H. P., & Thomas, C. D. (2008). Assisted colonization and rapid climate change. *Science*, *321*, 345–346. doi: 10.1126/science.1157897

Hoffmann, A. A., & Sgró, C. M. (2011). Climate change and evolutionary adaptation. *Nature*, *470*, 479–485. doi: 10.1038/nature09670

Holmes, M. W., Hammond, T. T., Wogan, G. O. U., Walsh, R. E., Labarbera, K., Wommack, E. A., … Nachman, M. W. (2016). Natural history collections as windows on evolutionary processes. *Molecular Ecology*, *25*(4), 864–881. doi: 10.1111/mec.13529

Homola, J. J., Loftin, C. S., & Kinnison, M. T. (2019). Landscape genetics reveals unique and shared effects of urbanization for two sympatric pool-breeding amphibians. *Ecology and Evolution*, *9*(20), 11799–11823. doi: 10.1002/ece3.5685

Hossack, B. R., & Pilliod, D. S. (2011). Amphibian responses to wildfire in the western United States: Emerging patterns from short-term studies. *Fire Ecology*, *7*(2), 129–144. doi: 10.4996/fireecology.0702129

Howard, S. D., & Bickford, D. P. (2014). Amphibians over the edge: Silent extinction risk of Data Deficient species. *Diversity and Distributions*, *20*(7), 837–846. doi: 10.1111/ddi.12218

Hughes, J. B., Daily, G. C., & Ehrlich, P. R. (1997). Population diversity: Its extent and extinction. *Science*, *278*(5338), 689–692. doi: 10.1126/science.278.5338.689

IPCC. (2014). *Climate change 2014: Synthesis report. Contribution of Working Groups I, II and III to the fifth assessment report of the Intergovernmental Panel on Climate Change* (Core Writing Team, R. K. Pachauri, & L. A. Meyer, Eds.). Geneva, Switzerland.

IUCN. (2020). The IUCN Red List of Threatened Species. Version 2020-2. Retrieved from https://www.iucnredlist.org

Jaeger, R. G. (1980). Density-dependent and density-independent causes of extinction of a salamander population. *Evolution*, *34*(4), 617–621. doi: 10.2307/2408016

Jara, F. G., Thurman, L. L., Montiglio, P.-O., Sih, A., & Garcia, T. S. (2019). Warming-induced shifts in amphibian phenology and behavior lead to altered predator–prey dynamics. *Oecologia*, *189*(3), 803–813. doi: 10.1007/s00442-019-04360-w

Jetz, W., Ashton, K. G., & La Sorte, F. A. (2009). Phenotypic population divergence in terrestrial vertebrates at macro scales. *Ecology Letters*, *12*, 1137–1146. doi: 10.1111/j.1461-0248.2009.01369.x

Joly, P. (2019). Behavior in a changing landscape: Using movement ecology to inform the conservation of pond-breeding amphibians. *Frontiers in Ecology and Evolution*, *7*, 155. doi: 10.3389/fevo.2019.00155

Jones, L. L. C., Halama, K. J., & Lovich, R. E. (2016). *Habitat management guidelines for amphibians and reptiles of the southwestern United States*. Birmingham, AL, USA: Partners in Amphibian and Reptile Conservation (PARC).

Karger, D. N., Conrad, O., Böhner, J., Kawohl, T., Kreft, H., Soria-Auza, R. W., … Kessler, M. (2017). Climatologies at high resolution for the earth’s land surface areas. *Scientific Data*, *4*, 170122. doi: 10.1038/sdata.2017.122

Kearney, M. R., Gillingham, P. K., Bramer, I., Duffy, J. P., & Maclean, I. M. D. (2020). A method for computing hourly, historical, terrain-corrected microclimate anywhere on earth. *Methods in Ecology and Evolution*, *11*(1), 38–43. doi: 10.1111/2041-210X.13330

Kearney, M. R., & Porter, W. P. (2004). Mapping the fundamental niche: Physiology, climate, and the distribution of a nocturnal lizard. *Ecology*, *85*(11), 3119–3131.

Kirezci, E., Young, I. R., Ranasinghe, R., Muis, S., Nicholls, R. J., Lincke, D., & Hinkel, J. (2020). Projections of global-scale extreme sea levels and resulting episodic coastal flooding over the 21st Century. *Scientific Reports*, *10*, 11629. doi: 10.1038/s41598-020-67736-6

Kirk, M. A., Galatowitsch, M. L., & Wissinger, S. A. (2019). Seasonal differences in climate change explain a lack of multi-decadal shifts in population characteristics of a pond breeding salamander. *PLoS ONE*, *14*(9), e0222097. doi: 10.1371/journal.pone.0222097

Kluber, M. R., Olson, D. H., & Puettmann, K. J. (2008). Amphibian distributions in riparian and upslope areas and their habitat associations on managed forest landscapes in the Oregon Coast Range. *Forest Ecology and Management*, *256*, 529–535. doi:

10.1016/j.foreco.2008.04.043

Kluber, M. R., Olson, D. H., & Puettmann, K. J. (2009). Downed wood microclimates and their potential impact on plethodontid salamander habitat in the Oregon coast range. *Northwest Science*, *83*(1), 25–34. doi: 10.3955/046.083.0103

Korfel, C. A., Mitsch, W. J., Hetherington, T. E., & Mack, J. J. (2010). Hydrology, physiochemistry, and amphibians in natural and created vernal pool wetlands. *Restoration Ecology*, *18*(6), 843–854. doi: 10.1111/j.1526-100X.2008.00510.x

Krajick, K. (2006). The lost world of the Kihansi toad. *Science*, *311*(5765), 1230–1232. doi: 10.1126/science.311.5765.1230

Kroll, A. J., Runge, J. P., & MacCracken, J. G. (2009). Unreliable amphibian population metrics may obfuscate more than they reveal. *Biological Conservation*, *142*(11), 2802–2806. doi: 10.1016/j.biocon.2009.05.033

Kupferberg, S. J. (1996). Hydrolic and geomorphic factors affecting conservation of a river-breeding frog (*Rana boylii*). *Ecological Applications*, *6*(4), 1332–1344. doi: /10.2307/2269611

Kupferberg, S. J., Catenazzi, A., Lunde, K., Lind, A. J., & Palen, W. J. (2009). Parasitic copepod (*Lernaea cyprinacea*) outbreaks in foothill yellow-legged frogs (*Rana boylii*) linked to unusually warm summers and amphibian malformations in Northern California. *Copeia*, *2009*(3), 529–537. doi: 10.1643/CH-08-011

Lacan, I., Matthews, K. R., & Feldman, K. (2008). Interaction of an introduced predator with future effects of climate change in the recruitment dynamics of the imperiled Sierra Nevada yellow-legged frog (*Rana sierrae*). *Herpetological Conservation and Biology*, *3*(2), 211–223.

Lawler, J. J., Shafer, S. L., Bancroft, B. A., & Blaustein, A. R. (2010). Projected climate impacts for the amphibians of the western hemisphere. *Conservation Biology*, *24*, 38–50. doi: 10.1111/j.1523-1739.2009.01403.x

Li, Y., Cohen, J. M., & Rohr, J. R. (2013). Review and synthesis of the effects of climate change on amphibians. *Integrative Zoology*, *8*(2), 145–161. doi: 10.1111/1749-4877.12001

Loarie, S. R., Duffy, P. B., Hamilton, H., Asner, G. P., Field, C. B., & Ackerly, D. D. (2009). The velocity of climate change. *Nature*, *462*(7276), 1052–1055. doi: 10.1038/nature08649

Lowe, K., Castley, J. G., & Hero, J.-M. (2013). Acid frogs can stand the heat: Amphibian resilience to wildfire in coastal wetlands of eastern Australia. *International Journal of Wildland Fire*, *22*(7), 947–958. doi: 10.1071/WF12128

Lyons, M. P., & Kozak, K. H. (2020). Vanishing islands in the sky? A comparison of correlation- and mechanism-based forecasts of range dynamics for montane salamanders under climate change. *Ecography*, *43*, 481–493. doi: 10.1111/ecog.04282

Maclean, I. M. D. (2020). Predicting future climate at high spatial and temporal resolution. *Global Change Biology*, *26*(2), 1003–1011. doi: 10.1111/gcb.14876

Madelaire, C. B., Barsotti, A. M. G., Wagener, C., Vieira Sugano, Y. Y., Baxter-Gilbert, J., Gomes, F. R., & Measey, J. (2020). Challenges of dehydration result in a behavioral shift in invasive toads. *Behavioral Ecology and Sociobiology*, *74*, 83. doi: 10.1007/s00265-020-02866-5

Manel, S., Andrello, M., Henry, K., Verdelet, D., Darracq, A., Guerin, P. E., … Devaux, P. (2018). Predicting genotype environmental range from genome–environment associations. *Molecular Ecology*, *27*(13), 2823–2833. doi: 10.1111/mec.14723

Maret, T. J., Snyder, J. D., & Collins, J. P.

(2006). Altered drying regime controls distribution of endangered salamanders and introduced predators. *Biological Conservation*, *127*(2), 129–138. doi: 10.1016/j.biocon.2005.08.003

Mathwin, R., Wassens, S., Young, J., Ye, Q., & Bradshaw, C. J. A. (2020). Manipulating water for amphibian conservation. *Conservation Biology*, *35*(1), 24–34. doi: 10.1111/cobi.13501

McCallum, M. (2007). Amphibian decline or extinction ? Current losses dwarf background rates. *Journal of Herpetology*, *41*(3), 483–491.

McCartney-Melstad, E., Gidiş, M., & Shaffer, H. B. (2018). Population genomic data reveal extreme geographic subdivision and novel conservation actions for the declining foothill yellow-legged frog. *Heredity*, *121*(2), 112–125. doi: 10.1038/s41437-018-0097-7

McDonald, J. M., Srock, A. F., & Charney, J. J. (2018). Development and application of a Hot-Dry-Windy Index (HDW) climatology. *Atmosphere*, *9*(7), 285. doi: 10.3390/atmos9070285

McMenamin, S. K., Hadly, E. A., & Wright, C. K. (2008). Climatic change and wetland desiccation cause amphibian decline in Yellowstone National Park. *Proceedings of the National Academy of Sciences of the United States of America*, *105*(44), 16988–16993. doi: 10.1073/pnas.0809090105

Medina, R., Wogan, G. O. U., Bi, K., Termignoni-García, F., Bernal, M. H., Jaramillo-Correa, J. P., … Vázquez-Domínguez, E. (2021). Phenotypic and genomic diversification with isolation by environment along elevational gradients in a Neotropical treefrog. *Molecular Ecology*, *30*(16), 4062–4076. doi: 10.1111/mec.16035

Merilä, J., & Hendry, A. P. (2014). Climate change, adaptation, and phenotypic plasticity: The problem and the evidence. *Evolutionary Applications*, *7*(1), 1–14. doi: 10.1111/eva.12137

Milanovich, J. R., Peterman, W. E., Nibbelink, N. P., & Maerz, J. C. (2010). Projected loss of a salamander diversity hotspot as a consequence of projected global climate change. *PLoS ONE*, *5*(8), e12189. doi: 10.1371/journal.pone.0012189

Millennium Ecosystem Assessment. (2005). *Ecosystems and human well-being: Synthesis*. Washington, DC: Island Press.

Mims, M. C., Olson, D. H., Pilliod, D. S., & Dunham, J. B. (2018). Functional and geographic components of risk for climate sensitive vertebrates in the Pacific Northwest, USA. *Biological Conservation*, *228*, 183–194. doi: 10.1016/j.biocon.2018.10.012

Mitchell, N. J. (2001). Males call more from wetter nests: Effects of substrate water potential on reproductive behaviours of terrestrial toadlets. *Proceedings of the Royal Society B: Biological Sciences*, *268*(1462), 87–93. doi: 10.1098/rspb.2000.1334

Mittan, C. S., & Zamudio, K. R. (2019). Rapid adaptation to cold in the invasive cane toad *Rhinella marina*. *Conservation Physiology*, *7*(1), coy075. doi: 10.1093/conphys/coy075

Mokhatla, M., Measey, J., & Smit, B. (2019). The role of ambient temperature and body mass on body temperature, standard metabolic rate and evaporative water loss in southern African anurans of different habitat specialisation. *PeerJ*, *7*, :e7885. doi: 10.7717/peerj.7885

Moore, J. D., & Ouellet, M. (2015). Questioning the use of an amphibian colour morph as an indicator of climate change. *Global Change Biology*, *21*(2), 566–571. doi: 10.1111/gcb.12744

Moreno, G. (1989). Behavioral and physiological differentiation between the color morphs of the salamander, *Plethodon cinereus*. *Journal of Herpetology*, *23*(4), 335–341.

Moritz, C., Patton, J. L., Conroy, C. J., Parra, J. L., White, G. C., & Beissinger, S. R. (2008). Impact of a century of climate change on small-mammal communities in Yosemite National Park, USA. *Science*, *322*(5899), 261–264. doi: 10.1126/science.1163428

Moritz, M. A., Batllori, E., Bradstock, R. A., Gill, A. M., Handmer, J., Hessburg, P. F., … Syphard, A. D. (2014). Learning to coexist with wildfire. *Nature*, *515*(7525), 58–66. doi: 10.1038/nature13946

Moritz, M. A., Parisien, M.-A., Batllori, E., Krawchuk, M. A., Van Dorn, J., Ganz, D. J., & Hayhoe, K. (2012). Climate change and disruptions to global fire activity. *Ecosphere*, *3*(6), art49. doi: 10.1890/es11-00345.1

Moskwik, M. (2014). Recent elevational range expansions in plethodontid salamanders (Amphibia: Plethodontidae) in the southern Appalachian mountains. *Journal of Biogeography*, *41*(10), 1957–1966. doi: 10.1111/jbi.12352

Muths, E., Hossack, B. R., Grant, E. H. C., Pilliod, D. S., & Mosher, B. A. (2020). Effects of snowpack, temperature, and disease on demography in a wild population of amphibians. *Herpetologica*, *76*(2), 132–143. doi: 10.1655/0018-0831-76.2.132

Narins, P. M., & Meenderink, S. W. F. (2014). Climate change and frog calls: long-term correlations along a tropical altitudinal gradient. *Proceedings of the Royal Society B: Biological Sciences*, *281*, 20140401. doi: 10.1098/rspb.2014.0401

Nathan, R., Getz, W. M., Revilla, E., Holyoak, M., Kadmon, R., Saltz, D., & Smouse, P. E. (2008). A movement ecology paradigm for unifying organismal movement research. *Proceedings of the National Academy of Sciences of the United States of America*, *105*(49), 19052–19059. doi: 10.1073/pnas.0800375105

Navas, C. A. (1997). Thermal extremes at high elevations in the Andes: Physiological ecology of frogs. *Journal of Thermal Biology*, *22*(6), 467–477. doi: 10.1016/S0306-4565(97)00065-X

Navas, C. A. (2003). Herpetological diversity along Andean elevational gradients: Links with physiological ecology and evolutionary physiology. *Comparative Biochemistry and Physiology Part A*, *133*, 469–485. doi: 10.1016/S1095-6433(02)00207-6

Navas, C. A., Gomes, F. R., & Carvalho, J. E. (2008). Thermal relationships and exercise physiology in anuran amphibians: Integration and evolutionary implications. *Comparative Biochemistry and Physiology Part A*, *151*, 344–362. doi: 10.1016/j.cbpa.2007.07.003

Nix, H. (1986). A biogeographic analysis of Australian elapid snakes. In R. Longmore (Ed.), *Atlas of elapid snakes of Australia: Australian Flora and Fauna Series No. 7* (pp. 4–15). Canberra, Australia: Australian Government Publishing Service.

Noble, F. (2020). Government set to revise total number of hectares destroyed during bushfire season to 17 million. Retrieved from 9News website: https://www.9news.com.au/national/australian-bushfires-17-million-hectares-burnt-more-than-previously-thought/b8249781-5c86-4167-b191-b9f628bdd164

Nowakowski, A. J., Watling, J. I., Thompson, M. E., Brusch IV, G. A., Catenazzi, A., Whitfield, S. M., … Todd, B. D. (2018). Thermal biology mediates responses of amphibians and reptiles to habitat modification. *Ecology Letters*, *21*(3), 345–355. doi:

10.1111/ele.12901

Nowoshilow, S., Schloissnig, S., Fei, J., Dahl, A., Pang, A. W. C., Pippel, M., … Myers, E. W. (2018). The axolotl genome and the evolution of key tissue formation regulators. *Nature, 554*, 50–55. doi: 10.1038/nature25458

NSW Department of Planning Industry and Environment. (2020). Endangered frogs leap on a lifeline. Retrieved from https://www.environment.nsw.gov.au/news/endangered-frogs-leap-on-a-lifeline

O'Regan, S. M., Palen, W. J., & Anderson, S. C. (2014). Climate warming mediates negative impacts of rapid pond drying for three amphibian species. *Ecology*, *95*(4), 845–855.

Okamiya, H., Hayase, N., & Kusano, T. (2021). Increasing body size and fecundity in a salamander over four decades, possibly due to global warming. *Biological Journal of the Linnean Society*, *132*(3), 634–642. doi: 10.1093/biolinnean/blaa201

Oliveira, B. F., São-Pedro, V. A., Santos-Barrera, G., Penone, C., & Costa, G. C. (2017). AmphiBIO, a global database for amphibian ecological traits. *Scientific Data*, *4*, 170123. doi: 10.1038/sdata.2017.123

Olson, D. H., Anderson, P. D., Frissell, C. A., Welsh Jr., H. H., & Bradford, D. F. (2007). Biodiversity management approaches for stream-riparian areas: Perspectives for Pacific Northwest headwater forests, microclimates, and amphibians. *Forest Ecology and Management*, *246*(1), 81–107. doi: 10.1016/j.foreco.2007.03.053

Olson, D. H., & Burnett, K. M. (2013). Geometry of landscape connectivity for low mobility species: Thinking outside the box, diagonally. In P. D. Anderson & K. L. Ronnenberg (Eds.), *Density management in the 21st century: West side story. Gen. Tech. Rep. PNW-GTR-880* (pp. 220–238).

Portland, OR: U.S. Department of Agriculture, Forest Service, Pacific Northwest Research Station.

Olson, D. H., & Burton, J. I. (2014). Near-term effects of repeated-thinning with riparian buffers on headwater stream vertebrates and habitats in Oregon, USA. *Forests*, *5*(11), 2703–2729. doi: 10.3390/f5112703

Olson, D. H., Coble, A. S., & Homyack, J. A. (2020). Beyond best management practices. In C. A. Dolloff, R. J. Danehy, & G. H. Reeves (Eds.), *Reflections on Forest Management: Can Fish and Fiber Coexist? American Fisheries Society Symposium 92*.

Olson, D. H., & Kluber, M. R. (2014). Plethodontid salamander distributions in managed forest headwaters in western Oregon, USA. *Herpetological Conservation and Biology*, *9*(1), 76–96.

Olson, D. H., Leirness, J. B., Cunningham, P. G., & Steel, E. A. (2014). Riparian buffers and forest thinning: Effects on headwater vertebrates 10 years after thinning. *Forest Ecology and Management*, *321*, 81–93. doi: 10.1016/j.foreco.2013.06.013

Orizaola, G., Quintela, M., & Laurila, A. (2010). Climatic adaptation in an isolated and genetically impoverished amphibian population. *Ecography*, *33*(4), 730–737. doi: 10.1111/j.1600-0587.2009.06033.x

Ovaskainen, O., Skorokhodova, S., Yakovleva, M., Sukhov, A., Kutenkov, A., Kutenkova, N., … del Mar Delgado, M. (2013). Community-level phenological response to climate change. *Proceedings of the National Academy of Sciences of the United States of America*, *110*(33), 13434–13439. doi: 10.1073/pnas.1305533110

Park, T. (1954). Experimental studies of interspecies competition II. Temperature, humidity, and competition in two species of

Tribolium. *Physiological Zoology*, *27*(3), 177–238.

Parmesan, C., & Yohe, G. (2003). A globally coherent fingerprint of climate change impacts across natural systems. *Nature*, *421*(6918), 37–42. doi: 10.1038/nature01286

Partners in Amphibian and Reptile Conservation (PARC). (2011). Relocation, reintroduction, translocation, and headstarting. Spreadsheet of projects, April 15, 2011. Retrieved from https://parcplace.org/species/relocation-reintroduction/

Pašukonis, A., Warrington, I., Ringler, M., & Hödl, W. (2014). Poison frogs rely on experience to find the way home in the rainforest. *Biology Letters*, *10*(11), 20140642. doi: 10.1098/rsbl.2014.0642

Pechmann, J. H. K., Estes, R. A., Scott, D. E., & Gibbons, J. W. (2001). Amphibian colonization and use of ponds created for trial mitigation of wetland loss. *Wetlands*, *21*, 93–111. doi: 10.1672/0277-5212(2001)021[0093:ACAUOP]2.0.CO;2

Pekel, J.-F., Cottam, A., Gorelick, N., & Belward, A. S. (2016). High-resolution mapping of global surface water and its long-term changes. *Nature*, *540*(7633), 418–422. doi: 10.1038/nature20584

Pelini, S. L., Keppel, J. A., Kelley, A. E., & Hellmann, J. J. (2010). Adaptation to host plants may prevent rapid insect responses to climate change. *Global Change Biology*, *16*(11), 2923–2929. doi: 10.1111/j.1365-2486.2010.02177.x

Penner, J., & Rödel, M. O. (2019). Keep it simple? Dispersal abilities can explain why species range sizes differ, the case study of West African amphibians. *Acta Oecologica*, *94*, 41–46. doi: 10.1016/j.actao.2017.11.011

Pereira, H. M., Ferrier, S., Walters, M., Geller, G. N., Jongman, R. H. G., Scholes, R. J., … Wegmann, M. (2013). Essential biodiversity variables. *Science*, *339*(6117), 277–278. doi: 10.1126/science.1229931

Peterman, W. E., & Gade, M. (2017). The importance of assessing parameter sensitivity when using biophysical models: A case study using plethodontid salamanders. *Population Ecology*, *59*, 275–286. doi: 10.1007/s10144-017-0591-4

Petranka, J. W., Harp, E. M., Holbrook, C. T., & Hamel, J. A. (2007). Long-term persistence of amphibian populations in a restored wetland complex. *Biological Conservation*, *138*(3–4), 371–380. doi: 10.1016/j.biocon.2007.05.002

Phillips, S. B., Anderson, R. P., & Schapire, R. E. (2006). Maximum entropy modeling of species geographic distributions. *Ecological Modelling*, *190*, 231–259. doi: 10.1016/j.ecolmodel.2005.03.026

Piha, H., Luoto, M., Piha, M., & Merilä, J. (2007). Anuran abundance and persistence in agricultural landscapes during a climatic extreme. *Global Change Biology*, *13*, 300–311. doi: 10.1111/j.1365-2486.2006.01276.x

Pilliod, D. S., Bury, R. B., Hyde, E. J., Pearl, C. A., & Corn, P. S. (2003). Fire and amphibians in North America. *Forest Ecology and Management*, *178*, 163–181. doi: 10.1016/S0378-1127(03)00060-4

Pittman, S. E., Osbourn, M. S., & Semlitsch, R. D. (2014). Movement ecology of amphibians: A missing component for understanding population declines. *Biological Conservation*, *169*, 44–53. doi: 10.1016/j.biocon.2013.10.020

Potvin, D. A., Parris, K. M., Smith Date, K. L., Keely, C. C., Bray, R. D., Hale, J., … Melville, J. (2017). Genetic erosion and escalating extinction risk in frogs with increasing wildfire frequency. *Journal of Applied Ecology*, *54*, 945–954. doi:

10.1111/1365-2664.12809

Pounds, J. A., Bustamante, M. R., Coloma, L. A., Consuegra, J. A., Fogden, M. P. L., Foster, P. N., … Young, B. E. (2006). Widespread amphibian extinctions from epidemic disease driven by global warming. *Nature*, *439*(7073), 161–167. doi: 10.1038/nature04246

Pounds, J. A., & Crump, M. L. (1994). Amphibian declines and climate disturbance: The case of the golden toad and the harlequin frog. *Conservation Biology*, *8*(1), 72–85. doi: 10.1046/j.1523-1739.1994.08010072.x

Pounds, J. A., Fogden, M. P. L., & Campbell, J. H. (1999). Biological response to climate change on a tropical mountain. *Nature*, *398*(6728), 611–615. doi: 10.1038/19297

Prathapan, K. D., Pethiyagoda, R., Bawa, K. S., Raven, P. H., D., R. P., & 172 co-signatories from 35 countries. (2018). When the cure kills—CBD limits biodiversity research. *Science*, *360*(6396), 1405–1406. doi: 10.1126/science.aat9844 1406

Prodon, R., Geniez, P., Cheylan, M., Devers, F., Chuine, I., & Besnard, A. (2017). A reversal of the shift towards earlier spring phenology in several Mediterranean reptiles and amphibians during the 1998-2013 warming slowdown. *Global Change Biology*, *23*(12), 5481–5491. doi: 10.1111/ijlh.12426

Pyron, R. A., & Wiens, J. J. (2013). Large-scale phylogenetic analyses reveal the causes of high tropical amphibian diversity. *Proceedings of the Royal Society B: Biological Sciences*, *280*, 20131622. doi: 10.1098/rspb.2013.1622

Radchuk, V., Reed, T., Teplitsky, C., van de Pol, M., Charmantier, A., Hassall, C., … Kramer-Schadt, S. (2019). Adaptive responses of animals to climate change are most likely insufficient. *Nature Communications*, *10*, 3109. doi: 10.1038/s41467-019-10924-4

Rasmussen, N. L., & Rudolf, V. H. W. (2016). Individual and combined effects of two types of phenological shifts on predator-prey interactions. *Ecology*, *97*(12), 3414–3421. doi: 10.1002/ecy.1578

Raxworthy, C. J., Pearson, R. G., Rabibisoa, N., Rakotondrazafy, A. M., Ramanamanjato, J. B., Raselimanana, A. P., … Stone, D. A. (2008). Extinction vulnerability of tropical montane endemism from warming and upslope displacement: A preliminary appraisal for the highest massif in Madagascar. *Global Change Biology*, *14*(8), 1703–1720. doi: 10.1111/j.1365-2486.2008.01596.x

Reading, C. J. (2007). Linking global warming to amphibian declines through its effects on female body condition and survivorship. *Oecologia*, *151*, 125–131. doi: 10.1007/s00442-006-0558-1

Rehfeldt, G. E., Tchebakova, N. M., Parfenova, Y. I., Wykoff, W. R., Kuzmina, N. A., & Milyutin, L. I. (2002). Intraspecific responses to climate in *Pinus sylvestris*. *Global Change Biology*, *8*(9), 912–929. doi: 10.1046/j.1365-2486.2002.00516.x

Rhie, A., McCarthy, S. A., Fedrigo, O., Damas, J., Formenti, G., Koren, S., … Jarvis, E. D. (2020). Towards complete and error-free genome assemblies of all vertebrate species. *BioRxiv*, 2020.05.22.110833. doi: 10.1101/2020.05.22.110833v1

Ricciardi, A., & Simberloff, D. (2009). Assisted colonization is not a viable conservation strategy. *Trends in Ecology and Evolution*, *24*(5), 248–253. doi: 10.1016/j.tree.2008.12.006

Richardson, D. M., Hellmann, J. J., Mclachlan, J. S., Sax, D. F., Schwartz, M. W., Gonzalez, P., … Vellend, M. (2009). Multidimensional evaluation of managed relocation. *Proceedings of the National Academy of Sciences of the United States of America*, *106*(24), 9721–9724. doi: 10.1073/pnas.0902327106

Riddell, E. A., Odom, J. P., Damm, J. D., & Sears, M. W. (2018). Plasticity reveals hidden resistance to extinction under climate change in the global hotspot of salamander diversity. *Science Advances*, *4*(7), eaar5471. doi: 10.1126/sciadv.aar5471

Riddell, E. A., Roback, E. Y., Wells, C. E., Zamudio, K. R., & Sears, M. W. (2019). Thermal cues drive plasticity of desiccation resistance in montane salamanders with implications for climate change. *Nature Communications*, *10*, 4091. doi: 10.1038/s41467-019-11990-4

Riddell, E. A., & Sears, M. W. (2015). Geographic variation of resistance to water loss within two species of lungless salamanders: Implications for activity. *Ecosphere*, *6*(5), art86. doi: 10.1890/es14-00360.1

Rittenhouse, T. A. G., Harper, E. B., Rehard, L. R., & Semlitsch, R. D. (2008). The role of microhabitats in the desiccation and survival of anurans in recently harvested oak-hickory forest. *Copeia*, *2008*(4), 807–814. doi: 10.1643/CH-07-176

Rochmyaningsih, D. (2019). Indonesia gets tough on foreign scientists. *Science*, *365*(6451), 304–305. doi: 10.1126/science.365.6451.304

Rödin-Mörch, P., Luquet, E., Meyer-Lucht, Y., Richter-Boix, A., Höglund, J., & Laurila, A. (2019). Latitudinal divergence in a widespread amphibian: Contrasting patterns of neutral and adaptive genomic variation. *Molecular Ecology*, *28*(12), 2996–3011. doi: 10.1111/mec.15132

Rohr, J. R., & Madison, D. M. (2003). Dryness increases predation risk in efts: Support for an amphibian decline hypothesis. *Oecologia*, *135*(4), 657–664. doi:

10.1007/s00442-003-1206-7

Rohr, J. R., & Raffel, T. R. (2010). Linking global climate and temperature variability to widespread amphibian declines putatively caused by disease. *Proceedings of the National Academy of Sciences of the United States of America*, *107*(18), 8269–8274. doi: 10.1073/pnas.0912883107

Rohr, J. R., Raffel, T. R., Romansic, J. M., McCallum, H., & Hudson, P. J. (2008). Evaluating the links between climate, disease spread, and amphibian declines. *Proceedings of the National Academy of Sciences of the United States of America*, *105*(45), 17436–17441. doi: 10.1073/pnas.0806368105

Rollins-Smith, L. A. (2017). Amphibian immunity–stress, disease, and climate change. *Developmental and Comparative Immunology*, *66*, 111–119. doi: 10.1016/j.dci.2016.07.002

Rollins, H. B., & Benard, M. F. (2020). Challenges in predicting the outcome of competition based on climate change-induced phenological and body size shifts. *Oecologia*, *193*(3), 749–759. doi: 10.1007/s00442-020-04705-w

Rosenzweig, C., Karoly, D., Vicarelli, M., Neofotis, P., Wu, Q., Casassa, G., … Imeson, A. (2008). Attributing physical and biological impacts to anthropogenic climate change. *Nature*, *453*(7193), 353–357. doi: 10.1038/nature06937

Rowley, J. J. L., Callaghan, C. T., & Cornwell, W. K. (2020). Widespread short-term persistence of frog species after the 2019–2020 bushfires in eastern Australia revealed by citizen science. *Conservation Science and Practice*, *2*(11), e287. doi: 10.1111/csp2.287

Ryan, M. E., Palen, W. J., Adams, M. J., & Rochefort, R. M. (2014). Amphibians in the climate vise: Loss and restoration of resilience of montane wetland ecosystems in the western US. *Frontiers in Ecology and the Environment*, *12*(4), 232–240. doi: 10.1890/130145

Saito, S., Saito, C. T., Nozawa, M., & Tominaga, M. (2019). Elucidating the functional evolution of heat sensors among *Xenopus* species adapted to different thermal niches by ancestral sequence reconstruction. *Molecular Ecology*, *28*(15), 3561–3571. doi: 10.1111/mec.15170

Sánchez-Montes, G., Wang, J., Ariño, A. H., & Martínez-Solano, Í. (2018). Mountains as barriers to gene flow in amphibians: Quantifying the differential effect of a major mountain ridge on the genetic structure of four sympatric species with different life history traits. *Journal of Biogeography*, *45*(2), 318–331. doi: 10.1111/jbi.13132

Savage, W. K., Fremier, A. K., & Shaffer, H. B. (2010). Landscape genetics of alpine Sierra Nevada salamanders reveal extreme population subdivision in space and time. *Molecular Ecology*, *19*(16), 3301–3314. doi: 10.1111/j.1365-294X.2010.04718.x

Sax, D. F., Early, R., & Bellemare, J. (2013). Niche syndromes, species extinction risks, and management under climate change. *Trends in Ecology and Evolution*, *28*(9), 517–523. doi: 10.1016/j.tree.2013.05.010

Scheele, B. C., Driscoll, D. A., Fischer, J., & Hunter, D. A. (2012). Decline of an endangered amphibian during an extreme climatic event. *Ecosphere*, *3*(11), art101. doi: 10.1890/es12-00108.1

Scheele, B. C., Hunter, D. A., Grogan, L. F., Berger, L., Kolby, J. E., McFadden, M. S., … Driscoll, D. A. (2014). Interventions for reducing extinction risk in chytridiomycosis-threatened amphibians. *Conservation Biology*, *28*(5), 1195–1205. doi: 10.1111/cobi.12322

Scheffers, B. R., Brunner, R. M., Ramirez,

S. D., Shoo, L. P., Diesmos, A., & Williams, S. E. (2013). Thermal buffering of microhabitats is a critical factor mediating warming vulnerability of frogs in the Philippine biodiversity hotspot. *Biotropica*, *45*(5), 628–635. doi: 10.1111/btp.12042

Scheffers, B. R., De Meester, L., Bridge, T. C. L., Hoffmann, A. A., Pandolfi, J. M., Corlett, R. T., … Watson, J. E. M. (2016). The broad footprint of climate change from genes to biomes to people. *Science*, *354*(6313), aaf7671. doi: 10.1126/science.aaf7671

Schwartz, M. W., Hellmann, J. J., McLachlan, J. M., Sax, D. F., Borevitz, J. O., Brennan, J., … Zellmer, S. (2012). Managed relocation: Integrating the scientific, regulatory, and ethical challenges. *BioScience*, *62*(8), 732–743. doi: 10.1525/bio.2012.62.8.6

Seimon, T. A., Seimon, A., Yager, K., Reider, K., Delgado, A., Sowell, P., … Halloy, S. (2017). Long-term monitoring of tropical alpine habitat change, Andean anurans, and chytrid fungus in the Cordillera Vilcanota, Peru: Results from a decade of study. *Ecology and Evolution*, *7*(5), 1527–1540. doi: 10.1002/ece3.2779

Semlitsch, R. D. (2008). Differentiating migration and dispersal processes for pond-breeding amphibians. *Journal of Wildlife Management*, *72*(1), 260–267. doi: 10.2193/2007-082

Session, A. M., Uno, Y., Kwon, T., Chapman, J. A., Toyoda, A., Takahashi, S., … Matsuda, Y. (2016). Genome evolution in the allotetraploid frog *Xenopus laevis*. *Nature*, *538*(7625), 336–343. doi: 10.1038/nature19840

Sheridan, J. A., & Bickford, D. (2011). Shrinking body size as an ecological response to climate change. *Nature Climate Change*, *1*(8), 401–406. doi: 10.1038/nclimate1259

Sheridan, J. A., Caruso, N. M., Apodaca, J. J., & Rissler, L. J. (2018). Shifts in frog size and phenology: Testing predictions of climate change on a widespread anuran using data from prior to rapid climate warming. *Ecology and Evolution*, *8*(2), 1316–1327. doi: 10.1002/ece3.3636

Shoo, L. P., Olson, D. H., & Hero, J.-M. (2011). A call to amphibian biologists to share innovative ideas to minimize loss of species under climate change. *FrogLog*, *96*, 32–33.

Sinervo, B., Méndez-de-la-Cruz, F., Miles, D. B., Heulin, B., Bastiaans, E., Villagrán-Santa Cruz, M., … Sites Jnr., J. W. (2010). Erosion of lizard diversity by climate change and altered thermal niches. *Science*, *328*(5980), 894–899. doi: 10.1126/science.1184695

Sinsch, U. (2014). Movement ecology of amphibians: From individual migratory behaviour to spatially structured populations in heterogeneous landscapes. *Canadian Journal of Zoology*, *92*(6), 491–502. doi: 10.1139/cjz-2013-0028

Skelly, D. K., & Freidenburg, L. K. (2000). Effects of beaver on the thermal biology of an amphibian. *Ecology Letters*, *3*(6), 483–486. doi: 10.1046/j.1461-0248.2000.00186.x

Smith, R. K., Meredith, H., & Sutherland, W. J. (2018). Amphibian conservation. In W. J. Sutherland, L. V. Dicks, N. Ockendon, S. O. Petrovan, & R. K. Smith (Eds.), *What works in conservation 2018* (pp. 9–66). Cambridge, UK: Open Book Publishers.

Smith, R. K., & Sutherland, W. J. (2014). *Amphibian conservation: Global evidence for the effects of interventions*. Exeter: Pelagic Publishing.

Sodhi, N. S., Bickford, D., Diesmos, A. C., Lee, T. M., Koh, L. P., Brook, B. W., … Bradshaw, C. J. A. (2008). Measuring the meltdown: Drivers of global amphibian extinction and decline. *PLoS ONE*, *3*(2), e1636. doi: 10.1371/journal.pone.0001636

Spear, S. F., Crisafulli, C. M., & Storfer, A. (2012). Genetic structure among coastal tailed frog populations at Mount St. Helens is moderated by post-disturbance management. *Ecological Applications*, *22*(3), 856–869.

Srock, A. F., Charney, J. J., Potter, B. E., & Goodrick, S. L. (2018). The Hot-Dry-Windy Index: A new fire weather index. *Atmosphere*, *9*(7), 279. doi: 10.3390/atmos9070279

Sterrett, S. C., Katz, R. A., Brand, A. B., Fields, W. R., Dietrich, A. E., Hocking, D. J., … Grant, E. H. C. (2019). Proactive management of amphibians: Challenges and opportunities. *Biological Conservation*, *236*, 404–410. doi: 10.1016/j.biocon.2019.05.057

Storlie, C., Merino-Viteri, A., Phillips, B., VanDerWal, J., Welbergen, J., & Williams, S. (2014). Stepping inside the niche: Microclimate data are critical for accurate assessment of species’ vulnerability to climate change. *Biology Letters*, *10*(9), 20140576. doi: 10.1098/rsbl.2014.0576

Stynoski, J. L. (2009). Discrimination of offspring by indirect recognition in an egg-feeding dendrobatid frog, *Oophaga pumilio*. *Animal Behaviour*, *78*(6), 1351–1356. doi: 10.1016/j.anbehav.2009.09.002

Sunday, J. M., Bates, A. E., Kearney, M. R., Colwell, R. K., Dulvy, N. K., Longino, J. T., & Huey, R. B. (2014). Thermal-safety margins and the necessity of thermoregulatory behavior across latitude and elevation. *Proceedings of the National Academy of Sciences of the United States of America*, *111*(15), 5610–5615. doi: 10.1073/pnas.1316145111

Sutton, W. B., Barrett, K., Moody, A. T., Loftin, C. S., DeMaynadier, P. G., & Nanjappa, P. (2015). Predicted changes in climatic niche and climate refugia of conservation priority salamander species in the northeastern United States. *Forests*, *6*(1), 1–26. doi: 10.3390/f6010001

Tejedo, M., Marangoni, F., Pertoldi, C., Richter-Boix, A., Laurila, A., Orizaola, G., … Gomez-Mestre, I. (2010). Contrasting effects of environmental factors during larval stage on morphological plasticity in post-metamorphic frogs. *Climate Research*, *43*, 31–39. doi: 10.3354/cr00878

Thomas, C. D., Cameron, A., Green, R. E., Bakkenes, M., Beaumont, L. J., Collingham, Y. C., … Williams, S. E. (2004). Extinction risk from climate change. *Nature*, *427*, 145–148.

Tian, L., & Benton, M. J. (2020). Predicting biotic responses to future climate warming with classic ecogeographic rules. *Current Biology*, *30*(13), R744–R749. doi: 10.1016/j.cub.2020.06.003

Turner, M. G., & Romme, W. H. (1994). Landscape dynamics in crown fire ecosystems. *Landscape Ecology*, *9*, 59–77. doi: 10.1007/BF00135079

Urban, M. C. (2015). Accelerating extinction risk from climate change. *Science*, *348*(6234), 571–573. doi: 10.1126/science.aaa49

Urban, M. C., Bocedi, G., Hendry, A. P., Mihoub, J.-B., Pe'er, G., Singer, A., … Travis, J. M. J. (2016). Improving the forecast for biodiversity under climate change. *Science*, *353*(6304), 1113. doi: 10.1126/science.aad8466

Urban, M. C., De Meester, L., Vellend, M., Stoks, R., & Vanoverbeke, J. (2012). A crucial step toward realism: Responses to climate change from an evolving metacommunity perspective. *Evolutionary Applications*, *5*(2), 154–167. doi: 10.1111/j.1752-4571.2011.00208.x

Urban, M. C., Richardson, J. L., &

Freidenfelds, N. A. (2014). Plasticity and genetic adaptation mediate amphibian and reptile responses to climate change. *Evolutionary Applications*, *7*(1), 88–103. doi: 10.1111/eva.12114

Urban, M. C., Zarnetske, P. L., & Skelly, D. K. (2013). Moving forward: Dispersal and species interactions determine biotic responses to climate change. *Annals of the New York Academy of Sciences*, *1297*(1), 44–60. doi: 10.1111/nyas.12184

Valladares, F., Matesanz, S., Guilhaumon, F., Araújo, M. B., Balaguer, L., Benito-Garzón, M., … Zavala, M. A. (2014). The effects of phenotypic plasticity and local adaptation on forecasts of species range shifts under climate change. *Ecology Letters*, *17*(11), 1351–1364. doi: 10.1111/ele.12348

Van Der Putten, W. H., Macel, M., & Visser, M. E. (2010). Predicting species distribution and abundance responses to climate change: Why it is essential to include biotic interactions across trophic levels. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *365*(1549), 2025–2034. doi: 10.1098/rstb.2010.0037

Velasco, J. A., Estrada, F., Calderón-Bustamante, O., Swingedouw, D., Ureta, C., Gay, C., & Defrance, D. (2021). Synergistic impacts of global warming and thermohaline circulation collapse on amphibians. *Communications Biology*, *4*, 141. doi: 10.1038/s42003-021-01665-6

Venables, W. N., & Dichmont, C. M. (2004). GLMs, GAMs and GLMMs: An overview of theory for applications in fisheries research. *Fisheries Research*, *70*, 319–337. doi: 10.1016/j.fishres.2004.08.011

Visser, M. E., Van Noordwijk, A. J., Tinbergen, J. M., & Lessells, C. M. (1998). Warmer springs lead to mistimed reproduction in great tits

(*Parus major*). *Proceedings of the Royal Society B: Biological Sciences*, *265*(1408), 1867–1870. doi: 10.1098/rspb.1998.0514

von May, R., Catenazzi, A., Corl, A., Santa-Cruz, R., Carnaval, A. C., & Moritz, C. (2017). Divergence of thermal physiological traits in terrestrial breeding frogs along a tropical elevational gradient. *Ecology and Evolution*, *7*(9), 3257–3267. doi: 10.1002/ece3.2929

von May, R., Catenazzi, A., Santa-Cruz, R., Gutierrez, A. S., Moritz, C., & Rabosky, D. L. (2019). Thermal physiological traits in tropical lowland amphibians: Vulnerability to climate warming and cooling. *PLoS ONE*, *14*(8), e0219759. doi: 10.1371/journal.pone.0219759

Wake, D. B. (2007). Climate change implicated in amphibian and lizard declines. *Proceedings of the National Academy of Sciences of the United States of America*, *104*(20), 8201–8202. doi: 10.1073/pnas.0702506104

Walls, S. C. (2009). The role of climate in the dynamics of a hybrid zone in Appalachian salamanders. *Global Change Biology*, *15*(8), 1903–1910. doi: 10.1111/j.1365-2486.2009.01867.x

Walls, S. C. (2018). Coping with constraints: Achieving effective conservation with limited resources. *Frontiers in Ecology and Evolution*, *6*, 24. doi: 10.3389/fevo.2018.00024

Walls, S. C., Barichivich, W. J., & Brown, M. E. (2013). Drought, deluge and declines: The impact of precipitation extremes on amphibians in a changing climate. *Biology*, *2*, 399–418. doi: 10.3390/biology2010399

Walls, S. C., Barichivich, W. J., Chandler, J., Meade, A. M., Milinichik, M., O’Donnell, K. M., … Wetsch, O. E. (2019). Seeking shelter from the storm: Conservation and management of imperiled species in a changing climate. *Ecology and Evolution*, *9*(12), 7122–7133. doi: 10.1002/ece3.5277

Walls, S. C., & Gabor, C. R. (2019). Integrating behavior and physiology into strategies for amphibian conservation. *Frontiers in Ecology and Evolution*, *7*, 234. doi: 10.3389/fevo.2019.00234

Walls, S. C., & O'Donnell, K. M. (2021). *Strategies for conservation success in herpetology*. University Heights, OH, USA: Society for the Study of Amphibians and Reptiles.

Walpole, A. A., Bowman, J., Tozer, D. C., & Badzinski, D. S. (2012). Community-level response to climate change: shifts in anuran calling phenology. *Herpetological Conservation and Biology*, *7*(2), 249–257.

Walther, G.-R., Post, E., Convey, P., Menzel, A., Parmesan, C., Beebee, T. J. C., … Bairlein, F. (2002). Ecological responses to recent climate change. *Nature*, *416*, 389–395.

Wang, B., Luo, X., Yang, Y. M., Sun, W., Cane, M. A., Cai, W., … Liu, J. (2019). Historical change of El Niño properties sheds light on future changes of extreme El Niño. *Proceedings of the National Academy of Sciences of the United States of America*, *116*(45), 22512–22517. doi: 10.1073/pnas.1911130116

Wang, I. J., Savage, W. K., & Shaffer, H. B. (2009). Landscape genetics and least-cost path analysis reveal unexpected dispersal routes in the California tiger salamander (*Ambystoma californiense*). *Molecular Ecology*, *18*(7), 1365–1374. doi: 10.1111/j.1365-294X.2009.04122.x

Wang, I. J., & Shaffer, H. B. (2017). Population genetic and field-ecological analyses return similar estimates of dispersal over space and time in an endangered amphibian. *Evolutionary Applications*, *10*(6), 630–639. doi: 10.1111/eva.12479

Warren-Thomas, E., Menton, M., Huamán, J., Frisancho Vargas, R., Wadley, E., Price, N., & Axmacher, J. C. (2013). Frog communities in fire-disturbed forests of the Peruvian Amazon. *Herpetological Bulletin*, *126*, 14–24.

Westgate, M. J., Driscoll, D. A., & Lindenmayer, D. B. (2012). Can the intermediate disturbance hypothesis and information on species traits predict anuran responses to fire? *Oikos*, *121*(10), 1516–1524. doi: 10.1111/j.1600-0706.2011.19863.x

Westgate, M. J., MacGregor, C., Scheele, B. C., Driscoll, D. A., & Lindenmayer, D. B. (2018). Effects of time since fire on frog occurrence are altered by isolation, vegetation and fire frequency gradients. *Diversity and Distributions*, *24*, 82–91. doi: 10.1111/ddi.12659

While, G. M., & Uller, T. (2014). Quo vadis amphibia? Global warming and breeding phenology in frogs, toads and salamanders. *Ecography*, *37*(10), 921–929. doi: 10.1111/ecog.00521

Williams, J. W., Ordonez, A., & Svenning, J.-C. (2021). A unifying framework for studying and managing climate-driven rates of ecological change. *Nature Ecology and Evolution*, *5*, 17–26. doi: 10.1038/s41559-020-01344-5

Williams, R. J., Wahren, C. H., Tolsma, A. D., Sanecki, G. M., Papst, W. A., Myers, B. A., … Green, K. (2008). Large fires in Australian alpine landscapes: Their part in the historical fire regime and their impacts on alpine biodiversity. *International Journal of Wildland Fire*, *17*(6), 793–808. doi: 10.1071/WF07154

Winter, M., Fiedler, W., Hochachka, W. M., Koehncke, A., Meiri, S., & De la Riva, I. (2016). Patterns and biases in climate change research on amphibians and reptiles: A systematic review. *Royal Society Open Science*, *3*(9), 160158. doi: 10.1098/rsos.160158

Wogan, G. O. U., & Wang, I. J. (2018). The value of space-for-time substitution for studying fine-scale microevolutionary processes. *Ecography*, *41*(9), 1456–1468. doi: /10.1111/ecog.03235

Wogan, G. O. U., Yuan, M. L., Mahler, D. L., & Wang, I. J. (2020). Genome-wide epigenetic isolation by environment in a widespread *Anolis* lizard. *Molecular Ecology*, *29*(1), 40–55. doi: 10.1111/mec.15301

Xie, G. Y., Olson, D. H., & Blaustein, A. R. (2016). Projecting the global distribution of the emerging amphibian fungal pathogen, *Batrachochytrium dendrobatidis*, based on IPCC climate futures. *PLoS ONE*, *11*(8), e0160746. doi: 10.1371/journal.pone.0160746

Yang, W., Qi, Y., & Fu, J. (2016). Genetic signals of high-altitude adaptation in amphibians: A comparative transcriptome analysis. *BMC Genetics*, *17*, 134. doi: 10.1186/s12863-016-0440-z

Zellmer, A. J., Slezak, P., & Katz, T. S. (2020). Clearing up the crystal ball: Understanding uncertainty in future climate suitability projections for amphibians. *Herpetologica*, *76*(2), 108–120. doi: 10.1655/0018-0831-76.2.108

Zylstra, E. R., Swann, D. E., Hossack, B. R., Muths, E., & Steidl, R. J. (2019). Drought-mediated extinction of an arid-land amphibian: Insights from a spatially explicit dynamic occupancy model. *Ecological Applications*, *29*(3), e01859. doi: 10.1002/eap.1859

Chapter 4. Ecotoxicology: amphibian vulnerability to chemical contamination

Michelle D. Boone[1] & Jessica Hua[2], Caitlin R. Gabor[3], Ivan Gomez-Mestre[4], Marco Katzenberger[5], Taegan A. McMahon[6], and Samantha L. Rumschlag[7]

[1] Department of Biology, Miami University, Oxford, OH 45056, USA

[2] Department of Biological Sciences, Binghamton University, Binghamton, NY 13902, USA

[3] Department of Biology, Texas State University, San Marcos, TX 78666, USA

[4] Department of Wetland Ecology, Estación Biológica Doñana, CSIC, 41092, Sevilla, Spain

[5] Departamento de Genética, Universidade Federal de Pernambuco, Recife - PE, 50670-901, Brazil

[6] Department of Biological Sciences, Connecticut College, New London, CT 06320, USA

[7] Department of Biological Sciences, University of Notre Dame, Notre Dame, IN 46556, USA

Abstract

Amphibian populations are routinely exposed to chemical contaminants in their habitat because contamination is pervasive in industrial, residential, and agricultural areas; contamination moves to remoter regions through aerial drift, runoff, food webs via bioaccumulation and biomagnification, and the water cycle, resulting in contaminant exposure in all natural systems. Exposure to upwind agriculture has been one of the few causal factors linked to amphibian population declines across a large geographic area, yet expected environmental exposures are often below lethal thresholds, suggesting that interactions with other natural and anthropogenic factors may be the key avenue through which contaminants elicit impacts on individuals and populations. Recent data reveal that

direct exposure to contaminants can alter physiology or gene expression, causing long-lasting effects that go beyond the exposure period, in some cases even extending across generations. In their natural habitats, amphibians must cope with several biotic (competitors, predators, and pathogens) and abiotic stressors (temperature, precipitation and other environmental conditions). Anthropogenic stressors, such as habitat alteration/degradation, pollution and climate change, provide an additional challenge to these species. Evidence suggests that the presence of multiple stressors increases the likelihood that contaminants will cause effects on amphibians and their populations, potentially increasing their extinction risk. While some contamination is perhaps unavoidable in a human-dominated globe, there are ways to reduce amphibians' exposure to contaminants, such as managing their release and use, creating biological buffers from areas of exposure, and implementing better policies that protect natural systems. Managing the risk of contaminants to amphibians will require a concerted effort among scientists, policymakers, local communities, landowners, and other stakeholders around the world, to protect amphibians and the natural systems of which they are part.

Introduction

On a planet where over 6 billion pounds of active ingredient pesticides are sold each year (Atwood & Paisley-Jones, 2017) and where an estimated 90-100,000 chemicals are released into the environment from agricultural and industrial activities (Holt, 2000), chemical contaminants are widespread and found in every environment examined. Contamination from pesticide pollution alone is widespread with 64% of agricultural lands at risk to exposure to more than one contaminant (Tang, Lenzen, McBratney, & Maggi, 2021). Further, there is a high overlap between areas prone to pesticide exposure and high-biodiversity regions, particularly in South Africa, China, India, Australia, and Argentina, although the risk is global (Tang et al., 2021). These contaminants can be detected above and below ground, posing a threat to living organisms through direct exposure and indirect routes via water systems and food webs. Early reports of amphibian population declines (Wake, 1991) posited that contaminants could play an important role in declines and approximately 30% of globally threatened amphibians are affected by pollution (Baillie, Hilton-Taylor, & Stuart, 2004).

A recent assessment on our progress elucidating the causes of amphibian declines (Green, Lannoo, Lesbarrères, & Muths, 2020), however, did not explicitly include contaminants. Yet, of the many attempts to look for causal factors, contaminants have been one of the few statistically linked to declines: Upwind pesticide use has been associated with amphibian population declines in California, USA across numerous studies (Davidson, 2004; Davidson & Knapp, 2007; Davidson, Shaffer, & Jennings, 2001, 2002). Further, California is one of the places with the best records for pesticide use and application rates, making it one of the areas more likely to find associations if they existed. Yet, directly linking contaminants to declines is difficult (Bradford et al., 2011; Campbell Grant, Miller, & Muths, 2020; Campbell Grant et al., 2016; Davidson, Stanley, & Simonich, 2012) given that environmental concentrations are often below known effect thresholds, contaminant effects can appear years after exposure, the types of contaminants used change over time, testing often occurs long after a contaminant is used, peak concentrations that cause effects may occur well before testing, break-down products may have different toxicity, and demographic data on amphibians is scarce (Conde et al., 2019). Additionally, the sheer number of contaminants found in environments (Smalling, Orlando, Calhoun, Battaglin, & Kuivila, 2012) and the temporal and spatial variation in application make pinpointing contaminants as a driver of amphibian declines problematic. Indeed, despite chemical innovation that has led to a diversity of novel products (e.g., PFAS [perfluoroalkyl and polyfluoroalkyl substances], antimicrobials, microplastics;

Kumar, Borah, & Devi, 2020), our current understanding of the role of contaminants on amphibian declines stems from work on selected pollutants (Egea-Serrano, Relyea, Tejedo, & Torralva, 2012). However, population viability analysis by Willson et al. (2012) demonstrated how contaminants that impact larval and juvenile survival can increase the risk of local extirpation, suggesting that understanding the effects on key life stages can be important for predicting population consequences. For all of these reasons, determining cause-effect linkages is challenging even if contaminants were a central causative factor in declines.

Despite the risk of chemical contaminants to amphibians, the initial concern that amphibians may be more sensitive to contaminants than other vertebrates because of their permeable eggs, skin, and gills (Bishop & Pettit, 1992), has not been found to be the case (Bridges, Dwyer, Hardesty, & Whites, 2002; Kerby, Richards-Hrdlicka, Storfer, & Skelly, 2010). Larval amphibian susceptibility to contaminants is roughly similar to that of fish (Glaberman, Kiwiet, & Aubee, 2019; Ortiz-Santaliestra, Maia, Egea-Serrano, & Lopes, 2018), although variation exists within and between species and taxonomic groups (Bridges & Semlitsch, 2000), which can change with repeated exposure (Hua, Jones, & Relyea, 2014; Hua, Morehouse, & Relyea, 2013). Assessment of contaminant risks could also vary across biogeographical regions, but most research has focused on species in the northern hemisphere, which biases research toward certain types of contaminants, species with complex life cycles, and a narrow set of life history traits (Schiesari, Grillitsch, & Grillitsch, 2007). Nevertheless, amphibians are susceptible to environmental contaminants (Baker, Bancroft, & Garcia, 2013), and contaminants could pose an important threat to amphibian populations in the wild (Willson et al., 2012).

Collectively, while substantial progress has been made in past decades, the major goals of this chapter are to highlight research gaps, suggest key research directions towards the goal of continuing to understand amphibian vulnerability to chemical contamination, and identify actions to mitigate and reduce the effects of contamination on amphibian communities. In 2007, contaminant risks were assessed and reviewed by the IUCN working group (Boone, Semlitsch, Little, & Doyle, 2007; Gascon et al., 2007) and recommendations were updated in 2015 (Wren et al., 2015), which noted the potential for contaminant exposure risks to amphibians in ways that may be more obvious (mortality) to more subtle (endocrine disruption, impacts on fertility, reduced overwinter survival). These assessments and others have noted that the most serious threat to amphibians from contaminants is their potential to interact with other factors like habitat loss and degradation, novel diseases, climatic changes, exotic invasive species, and natural factors like predators/parasites and competitors (Campbell Grant et al., 2016; Carey et al., 2001; Hayes et al., 2006). The data have come to support this supposition in the last decade (e.g. Davis, Ferguson, Schwarz, & Kerby, 2020; Rohr et al., 2008; Rumschlag & Rohr, 2018). Contaminants can change community composition, which can alter critical life history traits and alter susceptibility to abiotic and biotic factors, and serve as a physiological stressor, which can influence the susceptibility to other environmental stressors and the likelihood for interactive effects.

Because current research suggests the important role of contaminants as both an additive (i.e., combined effects equal the sum of the effects of each factor alone) and interactive factor in natural systems, the potential for interactions between expected and observed environmental concentrations of contaminants and other factors is the focus of our review here. The objectives of this chapter are to (1) review key ecotoxicological research not addressed in previous IUCN assessments, (2) identify gaps in amphibian ecotoxicology knowledge, (3) evaluate the priorities for future amphibian ecotoxicology research, and (4) provide effective and strategic conservation recommendations to mitigate contaminant risks to amphibians.

Status update

Contaminant risks

Types of chemical risks to amphibians

Amphibians are vulnerable to toxicants and pollutants from several sources (Figure 4.1) and very different chemical natures, which have been reviewed extensively elsewhere (e.g. Sparling, Linder, Bishop, & Krest, 2010; Thambirajah, Koide, Imbery, & Helbing, 2019) and which are summarised here briefly. Industrial and agricultural chemicals likely constitute the most pervasive type of chemicals to which amphibians are exposed, as they contaminate soils and the water bodies that amphibians use as primary breeding habitats. These substances cause direct damage to larval and adult amphibians through poisoning, endocrine disruption, or other means of physiological impairment. Some of these substances are highly persistent in the natural environment and amenable to bioaccumulation, consequently remaining a grave concern even long after their use is stopped or legally banned. Insecticides (e.g., DDT, carbaryl, deltamethrin, parathion, rotenone, esfenvalerate, 3-trifluoromethyl-4-nitrophenol, endosulfan, endrin, toxaphene) and herbicides (glyphosate, atrazine, acetochlor, triclopyr, paraquat) pose a major threat to amphibians, given the frequent and extensive use of them worldwide. Phosphorus and nitrogenous compounds widely used as fertilisers in agricultural fields (e.g., nitrates, nitrites, ammonia, humic acid) often spill over to aquatic habitats, also decreasing survival and otherwise affecting larval development of amphibians. Similarly, secondary salinization of freshwater systems, which has increased over the past several decades due to human activities such as agricultural irrigation, coastal flooding, and the application of road salts (Cañedo-Argüelles et al., 2016; Saumure et al.,

2021) can result in direct mortality of freshwater species leading to deleterious outcomes for wildlife populations (Hintz & Relyea, 2019). Other contaminants derived from industrial activity are also a common concern for the well-being of amphibians, from flame retardants to chemicals used in the manufacture of plastics and resins. These include substances such as polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), bisphenol A (BPA), tetrabromobisphenol A (TBBPA), dioxins, genistein, furans, perfluorooctanesulfonate (PFOS), perchlorates or phthalates. Another group of toxicants derived from industrial and mining activities are metals, metalloids, and nanoparticles, including arsenic, boron, cadmium, chromium, copper, lead, mercury, nickel, selenium, silver, or zinc. Petroleum oil products can be often spilled to water bodies, and both their polycyclic aromatic hydrocarbons and the naphthenic acid represent direct threats to amphibians. Pharmaceutical and personal care products are additional sources of chemical pollution that raise concern, particularly considering that methimazole, ibuprofen, estrogen, propylthiouracil, ethylenethiourea, triclosan, and triclocarban, all can interfere with amphibians' endocrine pathways. In the end, chemical contaminants of diverse sources and types move through water in natural and human-made systems, making amphibians vulnerable to exposure to pollution during their life cycles.

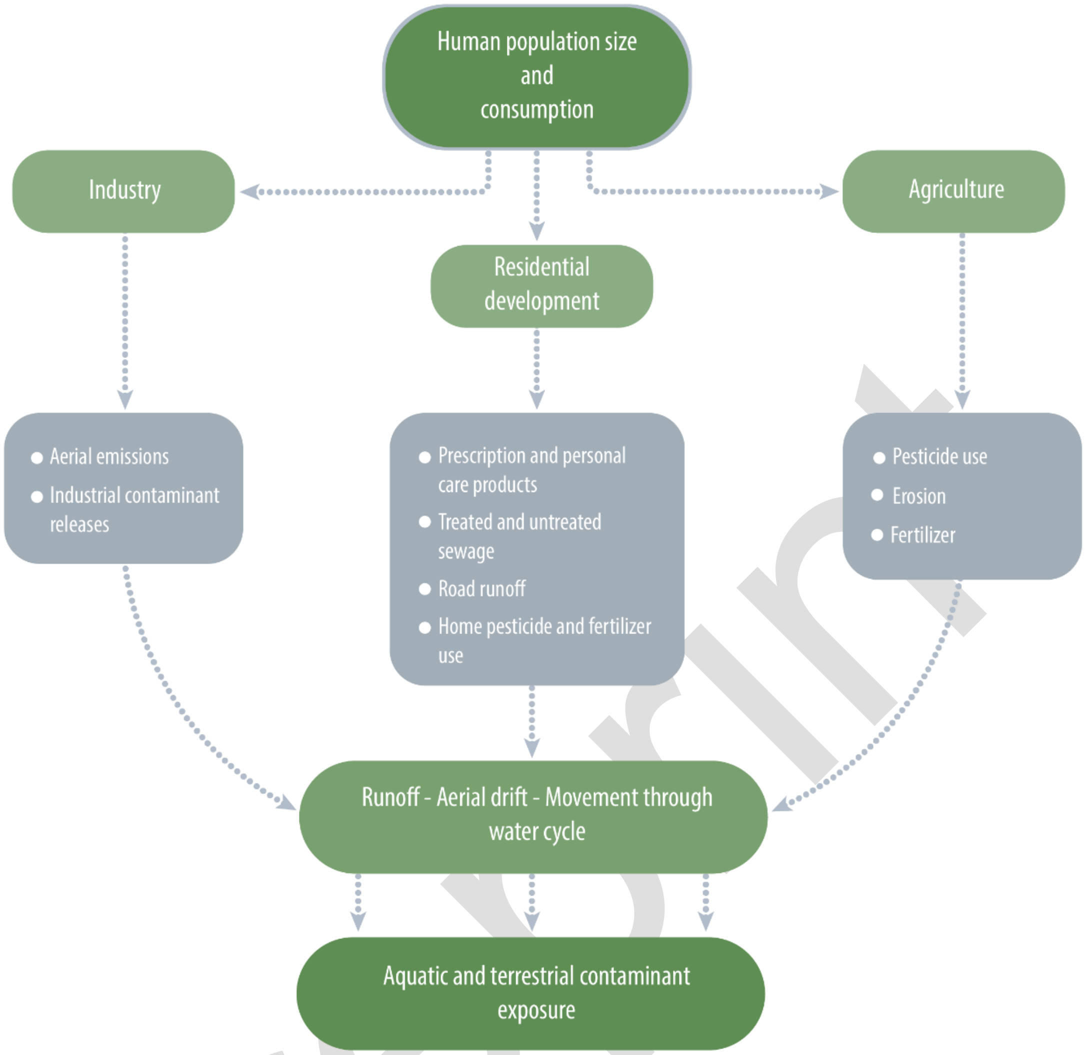


Figure 4.1. Human population size and consumption drives the industrial, residential, and agricultural footprints on the landscape that can contribute to chemical contamination of aquatic and terrestrial ecosystems.

Generalizable toxicity across classes, types, and modes of action of active ingredients

Predicting the effects of the thousands of environmental contaminants is enormously challenging because of the diverse array of contaminants to which ecosystems are exposed. Although basic toxicological data are available for a few model organisms, the ecological ramifications of exposure for most contaminants are not clear. Predicting responses in natural systems, however, is critical so that effects of exposure can be reasonably estimated for regulatory purposes—and such predictions are possible. An important means to anticipating community- and ecosystem-level effects can be coarsely achieved by using an active ingredient's chemical class, mode of action, and/or type (e.g., herbicide, insecticide, metal) to make predictions concerning the potential influence on natural systems.

By considering a contaminant through a categorical lens, some general principles can be reached. For example, Boone (2008) evaluated if combinations of insecticides with a different or the same mode of action were more or less likely to have additive or nonadditive effects on metamorphosis; in this study, aquatic environments containing two insecticides that were acetylcholinesterase inhibitors were more likely to have nonadditive effects than if the two insecticides had a different mode of action. Such approaches can improve our ability to anticipate effects of chemical mixtures, which are common in environments. Further, for contaminants that are well studied like the insecticide carbaryl (e.g. Boone, Semlitsch, Fairchild, & Rothermel, 2004; Boone et al., 2007; Zippel & Mendelson III, 2008), the herbicides atrazine (Rohr & McCoy, 2010) and glyphosate (e.g. Relyea, 2005), and the metal mercury (e.g. Bergeron, Hopkins, Todd, Hepner, & Unrine, 2011), the effects found in an array of studies from lab to field for these contaminants can offer insight for the ecological effects of contaminants with a similar mode of action or of a similar type/characteristic if we know that contaminants from similar classes and types have similar effects.

Data are beginning to suggest that chemical types and classes do have generalizable consequences. To evaluate chemical classes, Shuman-Goodier & Propper (2016) found effect sizes for swim speed and activity in fish and amphibians were similar for contaminants within the same chemical class. Using a meta-analysis, Egea-Serrano et al. (2012) determined that types of contaminants had different effect sizes across amphibian responses, suggesting that some contaminant types were more likely to have negative effects. Kerby et al. (2010) compared the sensitivity of amphibians via LC50s (lethal concentration of 50% of the population) with other taxonomic groups to contaminants based on chemical class and found amphibians had moderate to low sensitivity to pyrethroid, carbamate, organophosphate, and organochlorine pesticides; heavy metals; and inorganics relative to other groups; however, amphibians appeared to have higher sensitivity to phenols than other taxa. Evaluating sensitivity by chemical class or type is a useful way to infer contaminant categories that may be of more concern than others. Rumschlag et al. (2019) found that pesticides with the same chemical class or type (e.g., insecticide or herbicide) had similar impacts on amphibian host-trematode parasite communities, and Rumschlag et al. (2020) demonstrated that community structure and ecosystem function were impacted similarly based on a pesticide's effect through direct and indirect pathways. These studies suggest that based on class or type, we can expect some generality to contaminant effects, and we should be able to predict more complex ecological outcomes in systems based on direct effects at different trophic levels. These approaches offer a means of understanding contaminant impacts in natural systems so that we can minimise contaminant effects that can directly and indirectly impact species of concern, like amphibians, even without exhaustive studies for each particular contaminant.

Direct effects

Physiological

Extensive research has found that contaminant exposure at ecologically relevant concentrations can impact amphibian physiology in a myriad of important ways, from non-monotonic (a dose-response relationship characterised by a U-shaped or inverted U-shaped curve across increasing doses; Lagarde et al., 2015) modulation of stress hormones like corticosterone (Larson, McDonald, Fivizzani, Newton, & Hamilton, 1998; McMahon et al., 2011), to altered cardiac function (Jones-Costa et al., 2018; Palenske, Nallani, & Dzialowski, 2010), to the disruption of endocrine axes (including the feedback loops between hypothalamic-pituitary-adrenal axis or hypothalamic-pituitary-thyroid components of the endocrine system; Thambirajah et al., 2019; Trudeau et al., 2020), to immunomodulation (e.g. Forson & Storfer, 2006; McMahon et al., 2011), to impaired neuronal function (Sparling et al., 2010) or altered metabolism (Burraco & Gomez-Mestre, 2016). Moreover, contaminants have also been shown to be genotoxic (Maselli et al., 2010; Patar et al., 2016), and the damage caused to the DNA may potentially affect gene expression and lead to mutation-based diseases. One of the most commonly used pesticides in North America, the herbicide atrazine, has been shown to reduce size at metamorphosis, diminish immune function, and modulate gonadal morphology, impacting spermatogenesis and sex hormone production (Hayes et al., 2002; Rohr & McCoy, 2010; Vandenberg et al., 2012). Indeed, atrazine exposure can cause feminization in genetic male frogs (Hayes et al., 2002; Hayes, Khoury, et al., 2010; Rohr & McCoy, 2010), altering their overall fitness. Chlorothalonil, one of the most commonly used synthetic fungicides in North America, impacts immune response and degrades tadpole liver tissue in a non-monotonic fashion (McMahon et al., 2011). The severity of impact of contamination on amphibian physiology is also altered by timing of exposure (e.g. Rohr et al., 2013). Early life exposure is often, but not always, more detrimental than late life exposure. Additionally, there is evidence that the impact of contaminant exposure on physiology impacts the successive generations, as well. For example, male *Xenopus tropicalis* exposed to pesticides had reduced fertility due to endocrine disruption, were smaller in size, and their offspring had decreased plasma glucose levels (Karlsson et al., 2021). Many studies with amphibians do not examine physiological responses, but for those that do, effects appear to be commonplace (Thambirajah et al., 2019), suggesting biochemical changes that can have long-term effects are an important avenue for future research.

Carryover effects

Exposure to a contaminant has the potential to result in acute effects; understanding those effects and their ramifications can help managers minimise or mitigate the consequences. Yet even more pernicious are the effects that have consequences well after exposure, making short-term toxicity studies less useful in predicting effects in natural systems; further, effects that occur well after exposure make establishing cause-effect linkages challenging. Long-term effects stemming from conditions earlier in life are carryover effects. Carryover effects can occur when a contaminant has an obvious short-term effect with the consequences persisting or when a contaminant has no observed effect at exposure with impacts appearing later in life after exposure has ended (O'Connor, Norris, Crossin, & Cooke, 2014).

For instance, if contaminant exposure results in smaller size at metamorphosis in amphibians, then future fecundity, time to reproduction, and survival in the terrestrial environment (i.e., fitness) can be impacted (e.g. Altwegg & Reyer, 2003; Chelgren, Rosenberg, Heppell, & Gitelman, 2006; Earl & Whiteman, 2015; Scott, Casey, Donovan, & Lynch, 2007) even though contaminant effects may have been acute. Many contaminants affect endpoints correlated with fitness, through either direct chemical effects or indirect effects through changes in the food web (e.g. Relyea & Diecks, 2008). It follows that any contaminant that alters these critical endpoints have a higher probability of impacting future responses via carryover in ways that affect populations. Currently, studies that have followed amphibians after contaminant exposure early in development have found that carryover effects from acute exposures can have lasting effects on terrestrial growth and overwintering for some species and not for others (Boone, 2005; Distel & Boone, 2010).

Carryover effects from contaminant exposure in early life can also appear later in life despite no apparent effects immediately after exposure via altered physiology, behaviour, or gene expression (O'Connor et al., 2014). For instance, while negative chemical effects were not apparent in anurans reared in wastewater treatments relative to controls, terrestrial growth was reduced for those from wastewater, suggesting a metabolic cost of exposure was not apparent until later in development (Zeitler, Cecala, & McGrath, 2021). Similarly, Rohr & Palmer (2005) found that the herbicide atrazine unexpectedly increased terrestrial desiccation risk in salamanders through altered activity months after larval exposure. Delayed effects, like acute ones, are important because they can reduce survival, fertility, and growth; therefore, delayed carryover effects are critical to understand. Endocrine disruption caused by pesticide exposure may even affect subsequent unexposed generations, for at least two generations (Karlsson et al., 2021).

Contaminants that result in biochemical changes, such as changes in hormones (e.g., thyroid hormones, (Thambirajah et al., 2019); stress hormones, (Bókony et al., 2021; Davis et al., 2020); sex hormones, (Hayes, Khoury, et al., 2010)) or gene expression (e.g. Hinther, Bromba, Wulff, & Helbing, 2011; Zhang et al., 2019) may be more likely to have carryover effects. They appear to be a common, understudied consequence of contaminant exposure (Bergman et al., 2013; Edwards & Myers, 2007). Surprisingly, some carryover effects are positive: prior exposure to a contaminant can lead to greater tolerance to other stressors later in life, potentially through induction of a generalised stress response (Billet & Hoverman, 2020; Hua, Morehouse, & Relyea, 2013). However, general patterns have not yet been identified.

Carryover effects can also include those that cross generational boundaries--an area of research that offers many opportunities for discovery, given that the currently available data are quite limited. In particular, endocrine-disrupting chemicals (including phthalates, bisphenol A, microplastics pharmaceutical and personal care products, and persistent environmental contaminants like PCBs) are likely to have transgenerational impacts (Brehm & Flaws, 2019; Schwindt, 2015; Zhou et al., 2020). For instance, Karlsson et al. (2021) demonstrated that exposure of males to an anti-androgenic pesticide (linuron) resulted in effects across two generations in anurans. Additionally, maternal mercury exposure in anurans had negative effects on growth and survival in the next generation of tadpoles through maternal transfer of mercury (Bergeron et al., 2011), suggesting that contaminants that bioaccumulate in breeding females may have the potential to cross generational boundaries. Similarly, breeding pairs from agricultural and urban ponds with high concentrations of endocrine-disrupting pesticides (Bókony et al., 2018) produced tadpoles and juveniles with lower growth rates and development. Although there are few studies examining transgenerational impacts, current knowledge suggests that such effects may be common.

Carryover effects are understudied in amphibian ecotoxicology (as well as more broadly), and they have the potential to impact population health and persistence through time (O’Connor & Cooke, 2015). While we have a good understanding of the consequences that follow for some responses (e.g., effects on time and size at metamorphosis; early life stress hormones), species variation may still undermine broad generalisations, which could become predictable with more study (Earl & Whiteman, 2015). Making cause-effect linkages remains a major challenge for contaminants that have carryover effects and calls for studies across the life cycle and through multiple generations.

Indirect effects

Given that freshwater systems are among the most biodiverse in the world (Dudgeon et al., 2006), predicting the cumulative effects of contaminants on amphibians is hampered by the myriad possible indirect effects, mediated through and compounded by species interactions and food web structures. Despite the magnitude of the threat that contaminants impose on amphibians and freshwater systems (Bernhardt, Rosi, & Gessner, 2017; Burton, Di Giulio, Costello, & Rohr, 2017), indirect effects of contaminants are often overlooked by research communities and funding agencies. Classic toxicological lab-based experiments have documented scores of contaminants that can cause acute toxicity to organisms (Sparling et al., 2010), but they fail to predict complex suites of effects that can occur when contaminants enter freshwater systems (Bernhardt et al., 2017; Gessner & Tlili, 2016; Rohr, Salice, & Nisbet, 2016). Contaminant-induced changes in behaviour, competition, and predation/grazing rates can lead to changes in abundance, richness, and/or composition of community members (Fleeger, Carman, & Nisbet, 2003; Hillebrand & Matthiessen, 2009), which can impact amphibians via bottom-up and top-down trophic cascades (Fleeger et al., 2003; Hillebrand & Matthiessen, 2009). Advancements in replicated, field-based *in situ*, and mesocosm studies have offered a way to incorporate the complexity of multitrophic communities, so that the cumulative effects of contaminants on amphibians can be better evaluated.

Bottom-up indirect effects of contaminants alter food resources of amphibians. In the larval environment, alterations to algae can influence the survival and development of tadpoles. For instance, contaminants, including coal ash, fungicides, and herbicides, can decrease the abundance or alter the composition of phytoplankton and periphyton (Brock, Lahr, & Van den Brink, 2000; McMahon et al., 2012; Rowe, Hopkins, & Coffman, 2001; Rumschlag et al., 2020). Top-down effects of contaminants alter the community of amphibian predators. Insecticides can reduce survival of predators (Schäfer et al., 2011), which can benefit amphibian larval survival and growth through a predator release (Rumschlag et al., 2020). Amphibian behaviour can also be directly impacted by contaminants, which can indirectly lead to altered predator-prey interactions. Sublethal concentrations of contaminants, including copper and insecticides, can reduce tadpole activity, increase rates of abnormal swimming, reduce escape responses, or inhibit detection of predator cues by tadpoles, leading to increased predation risk (Hayden et al., 2015; Polo-Cavia, Burraco, & Gomez-Mestre, 2016; Sievers et al., 2019).

Contaminant-driven bottom-up and top-down effects can also alter transmission of parasites in amphibian populations by altering parasite exposure risk. For instance, in amphibian-trematode systems, triazine herbicides, organophosphate insecticides, and nutrients are linked with increases in snail abundance (first intermediate host) and thus trematode exposure, through increases in snail resources (periphytic algae, bottom-up effect) and changes to predator dynamics (top-down effect) (Johnson & Chase, 2004; Rumschlag et al., 2019). In an amphibian-chytrid system, effects of contaminants on parasite exposure and load can be non-monotonic (McMahon, Romansic, & Rohr, 2013), demonstrating complexity in predicting effects of contaminants on parasite transmission.

Indirect effects of contaminants on amphibians and other community members have even been linked to ecosystem-level consequences (Halstead et al., 2014). For instance, diverse arrays of insecticides can all lead to increases in primary productivity (through predation/grazing release) and ecosystem respiration through negative effects on larval salamanders and other zooplankton predators, which change zooplankton abundance and composition (Rumschlag et al., 2020).

The findings documenting the indirect effects on contaminants on amphibians highlight the need for a large-scale perspective in terms of ecology, community composition, and time. Amphibians do not experience chemical exposure in isolation, and therefore holistic research on the indirect effects of exposure is needed to understand the net ecological impact.

Evolutionary effects of contaminants

The call to incorporate evolutionary perspectives in our understanding of amphibian conservation and mitigation of amphibian declines was clearly articulated more than a decade ago (Blaustein & Bancroft, 2007). Indeed, since then, we have amassed ample evidence suggesting that amphibians can adapt in response to novel environmental conditions generated by pollutants (Brady, 2012; Cothran, Brown, & Relyea, 2013; Homola et al., 2019; Hua et al., 2015), although the ability to adapt depends upon the presence of resistant genotypes in the population.

Additionally, in the last 15 years, our understanding of the various adaptive mechanisms driving responses to pollutants has markedly improved. For example, endocrine flexibility is a crucial coping mechanism in response to anthropogenic environmental change. Generally, corticosterone, the main amphibian glucocorticoid associated with the hypothalamic-pituitary-interrenal axis (HPI axis), is predicted to be elevated with exposure to pollutants and other environmental stressors (Bókony et al., 2021; Forsburg, Guzman, & Gabor, 2021; Gabor, Davis, Kim, Zabierek, & Bendik, 2018; Gabor, Knutie, Roznik, & Rohr, 2018; Goff, Walls, Rodriguez, & Gabor, 2020; Hopkins, Mendonça, & Congdon, 1997; Tennessen et al., 2018). Yet not all populations (mostly endotherms) show elevated glucocorticoids in urbanised populations (Injaian et al., 2020; Murray et al., 2019). Further, Bókony et al. (2021) found that tadpoles of *Bufo* from anthropogenic and natural habitats that were reared in common garden experiments had higher baseline corticosterone-release rates in urban ponds; however, tadpoles from urban and agricultural ponds showed an adaptive response by responding to stressors with a greater stress-induced change than tadpoles from natural habitats, indicating that tadpoles from anthropogenic sites had a more efficient negative feedback (return to baseline). Collectively, these findings indicate the complexity of mitigating amphibian declines and suggest that more mechanistic studies may aid in exposing alternative methods for minimising the amphibian response to contaminants by decreasing application rate, changing the timing, or using different contaminants, even when the contaminants cannot be removed.

While the adaptive response to pollutants provides an optimistic perspective to amphibian populations facing contaminant exposure, recognition that these adaptations can lead to costs is growing (a reduction of fitness (Brady, 2012; Brady et al., 2019; Hua et al., 2015; Semlitsch, Bridges, & Welch, 2000); absence of protective co-tolerance effects to pollutants or natural stressors like predators and pathogens (Hua, Buss, Kim, Orlofske, & Hoverman, 2016; Hua, Cothran, Stoler, & Relyea, 2013; Hua, Morehouse, et al., 2013; Jones et al., 2021; Rumschlag et al., 2020). A number of advances in techniques to assess the evolutionary effects of contaminants on amphibians have been made, including traditional toxicity assays (e.g., time to death assays [TTD], LC50s) to compare functional traits like tolerance across groups, physiological coping capacity assays that measure stress physiology and capacity to cope with pollutants and environmental change (reviewed by Narayan, Forsburg, Davis, &

Gabor, 2019), and community metabarcoding to study diversity of amphibian microbiomes, which has applications in disease mitigation and captive breeding for reintroduction purposes (Ficetola, Manenti, & Taberlet, 2019).

Despite the growth in our understanding of evolutionary effects of contaminants on amphibians, few studies have directly implemented evolutionary principles and evaluated these efforts to inform and facilitate amphibian conservation. Future work should consider designing and testing conservation strategies based on our understanding of evolutionary effects of pollutants on amphibians. These may include selective breeding, introduction of adaptive variants through translocations, ecosystem interventions aimed at decreasing phenotype–environment mismatch, or genetic engineering (Pabijan et al., 2020). Some challenges to consider include: In captive breeding, should we expose amphibians to stressors that can help habituate the HPI axis and/or promote coping with unpredictable environments that they will experience if they are reintroduced to the wild? Similarly, can we engineer husbandry conditions that are similar to those in the wild (i.e., bioaugmentation techniques to initiate the establishment of healthy skin microbiotas in captive hellbenders prior to release; Kenison, Hernández-Gómez, & Williams, 2020).

While evolutionary responses may protect some amphibian populations from the effects of pollutants, other populations may not respond rapidly enough to cope with the pace of pollutant contamination even if genetic variation in resistance/tolerance exists in the population (Pabijan et al., 2020). Therefore, looking ahead, integrating evolutionary findings from the past 15 years to develop and directly test evidence-based evolutionary principles to protect the most vulnerable amphibian populations will be imperative to our amphibian conservation efforts.

Interactions of contaminants with other environmental factors

While contaminants alone and in mixtures have been put forward as a potential cause for amphibian population declines and while contaminants can theoretically cause local extinction (Willson et al., 2012) or serve as habitat sinks (e.g., coal ash, Rowe et al., 2001), the interactive effects of contaminants with other natural and anthropogenic factors has long-been anticipated to result in deleterious effects (Blaustein et al., 2011; Carey & Bryant, 1995; Hayes, Falso, Gallipeau, & Stice, 2010).

With habitat degradation and alteration

Land-use/Land-cover increases the risks of contamination:

Conversion of habitats to agriculture, residential, developed, and sub/urban lands can lead to increased contaminant exposures in the aquatic and terrestrial habitats used by amphibians (Sievers, Hale, Parris, & Swearer, 2018), which can directly affect amphibians and which can alter and degrade the quality of the habitat in ways that create the potential for multiple stressors. While contaminant exposure in the environment is pervasive in protected areas with low human impact to areas of agricultural and industrial activity (Battaglin et al., 2016; Bókony et al., 2018; Hageman, 2006; van Dijk & Guicherit, 1999), the likelihood of exposure is greater in some areas. Contaminants accumulate in water bodies, making these areas an important exposure pathway for amphibians with complex life cycles or living in areas near streams and wetlands (Battaglin et al., 2016; Bókony et al., 2018). Further, greater likelihood of contaminant exposure exists in aquatic habitats with concentration increasing dramatically for single contaminants and chemical mixtures (Anderson et al., 2013; Battaglin et al., 2016; Hayes et al., 2006) in both agricultural and protected areas (Sparling et al., 2015; Trudeau et al., 2020). Additionally, some types of agricultural techniques such as surface drainage ditches and subsurface tile drains contribute to habitat loss and transport pesticides, nutrients, and other contaminants into wetland habitats (Blann, Anderson, Sands, & Vondracek, 2009). Chemical mixtures increase the likelihood of effects (Hayes et al., 2006), which can ultimately reduce offspring fitness in amphibians (Bishop, Ashpole, Edwards, Van Aggelen, & Elliott, 2010; Bókony et al., 2018; Semlitsch et al., 2000), but which can also lead to pesticide tolerance or resistance (e.g. Cothran et al., 2013; Hua et al., 2015) in ways that alter populations.

Contaminants as habitat degradation:

Ponds are natural features on the landscape and are often added by people for recreational or aesthetic reasons, or for their ability to remove sediments moving across the landscape or water across impervious surfaces (Davis et al., 2021; Gallagher et al., 2011; Monaghan et al., 2016; Renwick, Smith, Bartley, & Buddemeier, 2005); both natural and human-made ponds are readily used by amphibians. Yet, environmental contaminants in these water bodies represent a form of habitat degradation. Ponds on human-dominated landscapes like golf courses, agricultural areas, parks, or multi-residential properties are more likely to be chemically managed to control algal or plant overgrowth, which can increase exposure risks to amphibians and influence population persistence (Sievers et al., 2018). For instance, golf courses manage water features for aesthetics impacted by fertiliser and pesticide runoff, occasionally applying chemicals like copper sulphate directly to ponds to reduce algal and plant growth, which can also be toxic to amphibians (Puglis & Boone, 2012). Use of pond dyes has become more common in residential and urban ponds as a means of reducing algal growth; effects have not been found to have direct impacts on amphibian metamorphosis, but such management practices change the food web, reducing algal and zooplankton food resources for amphibians (Bartson, Ogilvie, Petroff, Smith, & Rettig, 2018; Suski, Swan, Salice, & Wahl, 2018). Chemical exposure that reduces emergent vegetation can also impact the quality of a site for breeding and larval development via reduced cover and increased vulnerability to predators (Shulse, Semlitsch, Trauth, & Williams, 2010), although the direct and indirect consequences can make predicting outcomes difficult (Edge et al., 2020). The changes contaminants make to habitats can alter the quality of habitat, which can have population- and community-level repercussions, and which may not be obvious from traditional toxicological studies (e.g., LC50s in single species tests). Physiological and behavioural studies provide mechanisms for documenting systems in decline, especially in habitats that are experiencing conversion, before environmental stressors can be mitigated (Walls & Gabor, 2019).

While terrestrial buffers are mandated, for instance, in some areas near streams to reduce habitat degradation from nutrient runoff and soil erosion in waterways, they are generally not required around small temporary or permanent ponds often used by amphibians for breeding and larval development. Terrestrial buffers can promote contaminant and nutrient filtering from ponds (Cole, Stockan, & Helliwell, 2020; Mayer, Reynolds, Canfield, & McCutchen, 2005; Muscutt, Harris, Bailey, & Davies, 1993; Skagen, Melcher, & Haukos, 2008) and also serve as key upland habitats for terrestrial species or life stages (Semlitsch & Bodie, 2003). Physical habitat structure may also intercept aerial deposition of contaminants that may physically/directly impact amphibians in terrestrial habitats and can offer a solution to minimise contaminant impacts on water quality and on the species that live there.

Land-use/Land-cover influences environmental conditions and can interact with contaminant exposure

Land-use/land-cover changes alone have dramatic impacts on populations and communities, and amphibians can be affected by the interaction of habitat characteristics and contaminant exposure in ways that lead to the co-occurrence of environmental characteristics (e.g. Faulkner, 2004; Renick, Anderson, Morgan, & Cherr, 2015). For instance, loss of surrounding forest habitat can reduce leaf litter inputs and, thus, dissolved organic carbon that attenuates UV radiation; because some contaminants are more toxic in the presence of UV, changes in UV penetration can influence how toxic the same environmental concentration of a contaminant is and directly impact amphibian growth and survival (Puglis & Boone, 2011; Roberts, Alloy, & Oris, 2017).

Conversion of forest to rangeland can have impacts at a larger landscape scale and can interact with the resulting consequences, which may include reduction in emergent vegetation in ponds used for egg laying and predator protection of larvae, diminished quality of the terrestrial habitat for juvenile and adult growth and survival, changes in the hydroperiod of the wetland (which may be lengthened for cattle watering or shortened for planting), altered aquatic food webs resulting in changes in food availability and predators abundance, and reduced water quality (Moges et al., 2017) (Tilman, 1999). The addition of a contaminant that lengthens larval period in a habitat that has a shortened hydroperiod because of agricultural tiling or draining, for instance, can reduce recruitment of juveniles into the adult population, as Relyea & Diecks (2008) found for anurans reared in drying experimental ponds exposed to the insecticide malathion. Additionally, land use changes that impact water quality may result in algal blooms and higher water temperatures that spur management by land managers or residents. For instance, Goff et al. (2020) found that water quality and land cover type affected the physiological and bacterial diversity of ornate chorus frogs (*Pseudacris ornata*), thus affecting their overall population health. In this way, land-use and land-cover changes can alter a number of abiotic and biotic factors and interact with contaminant exposure to impact development and physiology of individuals, which can have acute and long-term consequences.

The potential for interactive effects of contaminants is illustrated in two field studies. The threatened Jollyville Plateau Salamander (*Eurycea tonkawae)* is a fully neotenic stream dwelling species found in central Austin, Texas, USA. This species is on the United States Endangered Species List because of threats from urbanization; indeed, counts of this species declined more in areas with the largest residential development than less developed areas throughout the species range (Bendik et al., 2014). In a follow-up study exploring the mechanisms associated with declines, Gabor et al. (2018) found that in two out of three years, salamanders from streams in more developed watersheds released higher corticosterone (an endocrine hormone associated with the stress axis) than salamanders from populations in preserves. Corticosterone levels were also higher in urban streams than in rural ones. Positive feedback between stream background corticosterone and baseline corticosterone may account for the higher corticosterone release rates found for *E. tonkawae* in urban streams, because amphibians can uptake exogenous corticosterone through their skin (Glennemeier & Denver, 2002). Because urban catchments are associated with septic systems and sewer lines, exogenous corticosterone from these systems plus runoff will continue to plague amphibians within these catchments. Further, Davis et al. (2020) found that salamanders located in agricultural wetlands compared to reference wetlands had higher ranavirus infection loads and higher corticosterone release rates. At the same time, corticosterone release rates were higher in ranavirus infected salamanders. Together, these results indicate that amphibians are being hit by multiple stressors, which likely increase the rates of amphibian declines. These studies show the usefulness of using water-borne corticosterone as one mechanism by which habitat impacts on amphibian population health can be measured in the field.

With disease

Given the important role disease has played in amphibian population declines (Scheele et al., 2019) — particularly ranaviruses and the amphibian chytrid fungi (*Batrachochytrium dendrobatidis* [Bd] and *B. salamandrivorans*)— and given that disease pathogens and contaminants are distributed across space while disease outbreaks appear more localised, the potential for disease by contaminant interactions is of critical importance (Blaustein et al., 2018). Because contaminants have a wide range of modes of actions, they have the potential to affect pathogens, hosts, or their interaction, which can alter disease dynamics and could explain the range of observed effects in experiments and natural systems (Blaustein et al., 2018). In experimental studies, the presence of contaminants may not alter the susceptibility of amphibians to a pathogen (as some studies have found, e.g., Buck et al., 2015; Gaietto, Rumschlag, & Boone, 2014; Kleinhenz, Boone, & Fellers, 2012) or it can increase susceptibility (e.g. Cusaac et al., 2021; Rohr et al., 2013; Wise, Rumschlag, & Boone, 2014), and these differences may be attributed to life stage exposure and species/population susceptibility. Field studies find associations between host-pathogen relationships and environmental contamination, although the type of contamination or effect may vary among study systems. For instance, King et al. (2010) found parasite infection risk was greater for anurans in polluted habitats, but risk varied with land cover in the landscape. Battaglin et al. (2016) found that frogs at field sites across the USA were more likely to be positive for Bd at sites with higher fungicide concentrations in water and sediments, and with more dissolved organic carbon, total nitrogen, and phosphorus in the water. Reeves et al. (2017) found Bd zoospore abundance was negatively associated with neonicotinoid concentration in wetlands in Iowa, USA. Rumschlag & Rohr (2018) found herbicide use was associated with low Bd infection prevalence in larval aquatic habitats and high infection prevalence in post-metamorphic terrestrial habitats. Further, populations exposed to salt runoff had slightly more frequent ranavirus-related mass mortality events, more lethal infections, and 117-times greater pathogen environmental-DNA (Hall, Brunner, Hutzenbiler, & Crespi, 2020). Generally, the presence of contamination in environments is associated with increased likelihood of pathogen/parasite infections in some systems in ways that are not currently predictable.

Anticipating how contaminants will impact pathogen-amphibian dynamics is difficult because underlying mechanisms determining these interactions are not well understood, because non-monotonic responses result with exposure to some contaminants (e.g., endocrine disruptors), and because amphibian populations/species (e.g. Hoskins & Boone, 2017; McMahon et al., 2011, 2013; Rohr & McCoy, 2010) and pathogens (e.g. Bd; McMahon et al., 2011) vary in response to contaminants. Yet, a promising research avenue for predicting pathogen-contaminant interactions is the examination of contaminant effects on immunomodulation (Hayes et al., 2006; McMahon et al., 2011) and on antimicrobial skin peptides or other defences that can prevent infections (McCoy & Peralta, 2018; Rollins-Smith et al., 2002). For instance, Davidson et al. (2007) found that an insecticide impacted the ability of anuran skin peptides to reduce Bd growth in vitro. Because pollution and other environmental conditions can influence the skin and gut microbiomes that can compromise an amphibian's ability to fight disease pathogens, contaminant effects on the amphibian host microbiome are likely an important mechanism influencing disease dynamics (McCoy & Peralta, 2018).

Contaminants can also alter the environment in ways that increase susceptibility to pathogens even if the contaminants themselves do not directly impact amphibians. For instance, Johnson et al. (2007) found that trematode infections were increased in amphibians through eutrophication of systems via nutrient runoff; in this way, contaminants can change the system to favour pathogens and increase infection rates. There are many ways that contaminants can alter the environment through changes in abiotic conditions or physical structure, or in the biotic community that could alter host-pathogen systems. For example, if contaminants can alter the abundance of microscopic aquatic predators that feed on infective stages of trematode parasites or Bd zoospores, they could influence infection prevalence and disease dynamics (Schmeller et al., 2014). Additionally, indirect effects of contaminant exposure can increase disease risk by increasing the abundances of intermediate hosts of pathogens in the environment or through slowing host development in stages especially vulnerable to infection (Halstead et al., 2014; Rumschlag et al., 2019). These interactions can be complex with outcomes mediated by host species, host and pathogen quality, and environmental properties.

Given that disease-causing parasites and pathogens are on the rise (Scheele et al., 2019), determining which factors can increase the likelihood of disease outbreaks is critical; current data suggest contaminants may be an important cofactor, yet there are thousands of chemicals that occur at different concentrations and that have divergent properties, creating a Russian roulette scenario in natural systems. Rumschlag et al. (2019) found that pesticide class predicted effects on trematode parasites and their hosts in aquatic communities, which offered some general conclusions that could be applicable to other areas. Such studies offer a powerful approach that provides predictive power to better shape both management and policy in ways that reduce the likelihood that contaminant exposure will lead to catastrophic disease outbreaks that negatively impact amphibian populations and species.

With climate change

The IPCC (2013) predicts changes in temperature and precipitation patterns across the globe, including shifts in average temperatures and increases in extreme climatic events (Diffenbaugh & Ashfaq, 2010; Schär et al., 2004). Understanding how contaminants will impact amphibians in a climate change scenario is a major challenge for amphibian conservation. Temperature can alter amphibian susceptibility to contaminants, but its effects are chemical dependent. Some studies find that higher temperatures can decrease sensitivity to pollutants (i.e., copper sulphate (Chiari, Glaberman, Serén, Carretero, & Capellini, 2015); atrazine (Rohr, Sesterhenn, & Stieha, 2011). In contrast, other studies report that increasing temperature results in greater toxicity (i.e., endosulphan, carbaryl, methomyl and pyrethroid insecticides (Boone & Bridges, 1999; Broomhall, 2002; Lau, Karraker, & Leung, 2015; Materna, Rabeni, & Lapoint, 1995)). It is clear that interactive effects between contaminants and temperature exist and understanding the mechanisms by which pollutants and temperature interact is important (similar to Burraco & Gomez-Mestre, 2016) to develop effective conservation strategies.

Further, climatic instability/unpredictability may also prompt amphibians to experience lower temperatures if reproduction events are prematurely cued (i.e., a false spring, Parmesan, 2007). Exposure to cold temperatures during embryonic stages can negatively affect amphibians by increasing tadpole susceptibility to pollutants (Wersebe et al., 2019). Similarly, phenological shifts that expose breeding amphibians to freezing conditions can have cascading consequences on offspring ability to tolerate pollutants (Buss, Swierk, & Hua, 2021).

Contaminants could also alter adaptive traits (morphological, physiological and behavioural) that are crucial for species to cope with climate change. In the past 15 years, our knowledge on amphibian thermal physiology traits has grown significantly (Duarte et al., 2012; Gutiérrez-Pesquera et al., 2016; Katzenberger, Duarte, Relyea, Beltrán, & Tejedo, 2021; Sunday et al., 2014). Contaminant effects on traits related to thermal physiology appear to be species- and chemical-dependent. Katzenberger et al. (2014), for instance, found that the herbicide Roundup® did not affect the critical thermal maximum (CTmax), but it changed the shape of the thermal performance curve; in contrast, Quiroga, Sanabria, Fornés, Bustos, & Tejedo (2019) found that tadpoles exposed to the insecticide chlorpyrifos showed a significant decline in CTmax but not in CTmin.

Currently, we have insight on how a few chemicals impact amphibians, but the vast majority remains untested and generalizations are difficult. An important and straightforward step would be to determine how toxicity of common contaminants changes with temperature for critical components of the food web (i.e., from reports like Aronson, Printup, Shuler, & Howard, 1998), which would improve our ability to mitigate deleterious effects in ecological systems.

Priorities in research

Amphibian ecotoxicological research has exploded in recent decades (Sparling et al., 2010) —assessing across scales from basic individual toxicity in the lab to ecologically relevant community-level questions in outdoor mesocosms and field enclosures, to landscape-level system questions. While research originally focused on mortality, it has now expanded to include responses across life stages (metamorphosis through to adult life stages), physiological responses such as endocrine and reproductive system modulation, and changes in behaviour, physiology, and genomic expression. Because amphibians are experimentally tractable across life stages they can serve as models for understanding the effects of contaminants in natural environments. The two key research areas for amphibian conservation related to pollution should focus on issues that will, first, protect populations in the wild that are impacted by contaminants and that will, second, improve regulatory data collection to better protect natural systems.

Population declines and amphibian conservation

We know amphibian populations are experiencing worldwide declines with no clear global explanation (Campbell Grant et al., 2020, 2016) and that contaminants are pervasive (e.g. Battaglin et al., 2016; Gibbs, MacKey, & Currie, 2009). To understand the role contaminants play in declines and in systems not experiencing declines, we need to focus on the ecological ramifications of contaminant exposure. We achieve this focus by identifying the important factors that interact with contaminant exposure to impact traits associated with amphibian fitness; these factors likely include habitat change, disease, and climate change, factors which are additional stressors in communities already experiencing naturally occurring competition, predation, and physiological stressors. We need to conduct experiments that examine exposure at multiple time points and that span life stages of diverse amphibian species because of the wide variety of life history strategies utilised by Amphibia. Biases in geography, ecosystems, life stages, and species of study creates a risk that we reach general conclusions that will not be reality-based, particularly given that some species and areas experiencing population declines are not those that have been the most extensively studied (Leaning, 2000; Trimble & van Aarde, 2012). Schiesari et al., (2007) found that while the majority of amphibian declines have taken place in the tropics, most studies were conducted on temperate systems using a small number of mainly temperate species. Hence, biogeographical and taxonomic biases can and should be addressed, at least partially, by including amphibians in routine federal toxicity testing, using native species from around the world.

Ecotoxicological studies for amphibian conservation

Traditional toxicological studies for regulatory purposes do not explicitly include amphibians, which is problematic given the role contaminants likely play in the amphibian biodiversity crisis, as outlined in this chapter. Yet, traditional toxicological approaches (e.g., LC50s) may not provide us with the information we need to protect this taxonomic group. Short-term studies often do not link exposure effects to critical traits correlated with fitness or to population dynamics, yet they are a good place to begin particularly in systems where there are little baseline data (e.g., many tropical systems). To determine long-term consequences of contaminant exposure, we need studies that examine consequences of exposure across life stages (i.e., carryover effects) and we need to use empirical data to parameterise population models to examine population viability in light of contaminant effects in complex communities (Willson et al., 2012). Linking responses that may happen with exposure (e.g., biomarkers like corticosterone; Gabor, Knutie, et al., 2018) to consequences later in life, offers promise to predict future consequences. Further, natural systems are more complicated and include contaminant mixtures and multiple potential stressors, so studies are needed that incorporate chemical as well as the natural complexity of ecological communities and can be powerful when paired with natural field studies (e.g. Hayes et al., 2003; Rohr, Raffel, Sessions, & Hudson, 2008; Rohr, Schotthoefer, et al., 2008). Such experiments can be logistically complicated, yet they are essential to establish cause-effect relationships and to evaluate the likelihood of additive or nonadditive effects. Many regulatory agencies in the US or Europe do not go beyond laboratory studies, but laboratories do not typically mimic systems--mesocosm or field studies are needed to do this (e.g. Halstead et al., 2014), and when experimental field conditions match natural systems, their results yield predictive power (e.g. Boone et al., 2004; Kidd et al., 2007). Complex ecotoxicology studies will be more easily achieved if chemical classes and types allow predictability, as the data currently suggest (Rumschlag et al., 2019, 2020); for then, a representative chemical can be used to explore interactions with other factors, across life stages, and general conclusions can be made for a suite of contaminants, which will help address the regulatory challenges associated with contaminant testing and regulatory delay.

Solutions for mitigating contaminant effects: Activities and opportunities

Considering that contaminant effects can be well-documented, are associated with amphibian population declines (Davidson et al., 2002), are predicted to interact with other stressors (above) and are predicted to cause declines when they affect survival (e.g. Willson et al., 2012), there are many reasons to reduce contaminant exposure in natural systems. Hence, stronger federal policies, improved and implemented conservation strategies, and individual actions can contribute to reducing the risk of amphibians being exposed to contaminants.

Policy

Environmental contaminants are pervasive largely because environmental policies (or lack thereof) support this outcome. As such, effective policies are the most important way through which exposure can be reduced. Given that contaminants move through food webs, atmospheric drift, and the water cycle, one or a few countries with poor policies can lead to global distribution of contaminants. However, contaminant release may at times be necessary for society or inevitable to meet national or global needs. The question of policy relates to societal decisions of assessing when benefits justify the environmental and health costs, which can be difficult to answer without adequate scientific evidence and transparent public discussions that are not obfuscated by misleading information from industry (e.g. Oreskes & Conway, 2010).

For instance, the herbicide atrazine increases crop yields by <6% at best and many reviews suggest average yields improve 1-3% (Ackerman, 2007). Atrazine is known to alter food webs by impacting the lowest trophic levels and, perhaps even more significantly, results in endocrine disruption across taxa (Hayes et al., 2011), although atrazine's manufacturer works to muddle these results from influencing public policy and regulation in the USA (Boone et al., 2014; Hayes, 2004; Rohr, 2021) by attacking scientists (e.g. Aviv, 2014) and funding/influencing research that disproportionately produces studies showing no effects of atrazine (Hanson, Solomon, Van Der Kraak, & Brian, 2019; Hayes, 2004). Is this an example of good policy where benefits disproportionately outweigh the costs or an example of the disproportionate influence of industry slowing regulatory processes (sensu Oreskes & Conway, 2010)? For amphibians, the weight of evidence suggests that there are significant costs to this policy that leads to widespread atrazine contamination of aquatic habitats (e.g. Rohr & McCoy, 2010), and the example of the regulatory process of atrazine is exceptional only in that the role of industry to slow the regulatory process has been well documented and publicised. Better policy that limits the role of industry in the experiments used to inform regulatory decisions could lead to better policy in the USA and other nations (Boone et al., 2014).

A policy of precaution, which is more pervasive in Europe, would also decrease the exposure risks to single chemicals and chemical mixtures, both of which increase the probability of biological effects and the interactive effects that result from interactions with other contaminants and environmental factors. However, for precaution to be an option, accurate predictions about how diverse contaminants will affect species and food webs are necessary. Towards this goal, while a wealth of data exist for amphibians and other taxa for a few contaminants, there are thousands of other regulated contaminants for which relatively little data exist. Looking ahead, expanding our understanding to include more contaminants and their potential interactions based on more general chemical properties or classes is an area of research that needs to be greatly expanded to allow informed decision-making or to adequately apply precaution. With more rigorous policy devoid of industrial influences, society and natural systems would reap more benefits from the trade-off than they currently do.

Conservation strategies

Even in the absence of policies that reduce contaminant release, strategies exist that can diminish the likelihood of exposure or the concentration to which systems are exposed (e.g. Smith & Sutherland, 2014) which influences the direct and indirect consequences experienced by organisms. Terrestrial buffers around aquatic habitats absorb nutrient and chemical contamination in runoff, and slow the rate of movement, which can reduce exposure risk (above). Policy that requires adequate habitat to surround aquatic environments could have a number of benefits including improved water quality and (potentially) flood control, which would benefit amphibians and a host of other taxa (including humans); however, buffer characteristics will vary across systems and are difficult to standardise (Kuglerová, Ågren, Jansson, & Laudon, 2014; Luke et al., 2019) with more known about riparian buffers than pond buffers. Terrestrial amphibians and terrestrial life stages are also vulnerable to contaminants (Brühl, Pieper, & Weber, 2011; Brühl, Schmidt, Pieper, & Alscher, 2013;

James & Semlitsch, 2011), and could benefit from terrestrial buffers around terrestrial habitats.

Societal calls for minimising environmental exposures to contaminants would benefit a host of species, including amphibians and humans. Reducing contaminant use by, for instance, accepting some agricultural losses to pests while using practices that benefit natural pest-predators provides effective and environmentally friendly approaches to achieve pest reduction without chemical pollution. In fact, some research suggests that organic techniques produce yields similar to conventional agriculture without the chemical footprint (Ponisio et al., 2015) and that enhancing the diversity of agricultural systems offers ecosystem services without a loss in yield (Tamburini et al., 2020). Further, reducing the use of contaminants to maintain public gardens and lawns in residential areas could also reduce contaminant inputs into natural systems given that homeowners use 10X more pesticides per acre than farmers (Meftaul, Venkateswarlu, Dharmarajan, Annamalai, & Megharaj, 2020). When the use of chemicals is unavoidable, such as when controlling the vectors of a zoonosis (e.g., *Aedes aegypti*, the mosquito responsible for spreading yellow fever, dengue fever, chikungunya, Zika fever, among others), their application should be accompanied by non-chemical actions (including population education) that add to the desired effect and help reduce the required number/dosage of applications. Prevention of pollution in the first place, particularly given that only a small amount of pesticides even reach pests (Pimentel & Burgess, 2012), is less economically and biologically costly than pollution clean-up.

Ultimately, cutbacks in consumption (as well as reduced human population size) would reduce pollution associated with industry and development and are steps that individuals can take to reduce their pollution footprint. If all stakeholders in industry, agriculture, government, and society members worked together to reduce the amount of pollution entering natural systems, amphibians and other species, including humans, are less likely to experience negative consequences of exposure--consequences that often do not reveal themselves for years.

Conclusions

In the last three decades, we have made substantial progress towards understanding how contaminants influence amphibians and the critical questions we need to address. Notably, we have addressed many priority points highlighted in the 2007 ACAP (Table 4.1). While we have made headway, there remain several research gaps. Of note, continued research is needed to understand the dynamics of how contaminants interact with other important stressors (i.e., habitat degradation, disease, climate change) to influence amphibians in potentially antagonistic, additive, or synergistic ways. Given the sheer number of different contaminants and the potential for diverse contaminant mixtures, an important need remains for predictive models that accurately assess the effects of individual and contaminant mixtures across ecological scales and organisations from molecular and physiological levels to systemic population and community levels. Importantly, this effort will require continued integration of multiple techniques (lab to field), as well as scientists with diverse expertise across biology (molecular to landscape levels). Researchers continue to study and understand the contribution of long-term and multi-generational effects of contaminants on amphibians. Lastly, a concerted effort should be made to address the geographical, ecosystem, and life stage biases that currently favour larval stages in temperate habitats. Addressing research priorities outlined here will allow us to better understand how contaminants influence amphibian declines. Current data indicate that amphibians are exposed to concentrations that elicit several effects (many of which are negative), that these effects are often (at a minimum) additive with other environmental stressors, and that they pose a threat to population viability worldwide. Collaborative work with scientists, policymakers, local human populations, landowners, and other stakeholders could lead to implementation of the best strategies to minimise the impacts on amphibians and the ecosystems at large.

Table 4.1: A summary of the research gaps highlighted in the 2007 ACAP update and current state of research on each of these gaps. The cool to warm colour scheme represents research gaps that have received relatively more attention to less attention in the past 30 years. In the last decades, we have made substantial progress on addressing the research gaps highlighted in the 2007 ACAP. For each of the gaps highlighted in the 2007 ACAP, we highlight areas in need of further investigation (**in bold)**.

Research gaps from ACAP 2007	**Current status**
Research is needed that goes beyond traditional toxicity testing by understanding complex chemical mixtures in complicated natural environments.	In the last 30 years, by integrating multiple toxicological techniques (lab to mesocosm to field), we have made substantial progress on understanding the complex direct and indirect effects of contaminants on amphibians. Studies have also worked to understand the interactive effects of complex contaminant mixtures. However, given the multitude of possible contaminant mixtures, we are still missing critical information that will allow us to make predictions about complex chemical mixtures in natural environments. Towards

	this goal, **future efforts that integrate experimental and predictive modelling efforts remain an important priority.**
Few studies have addressed physiological or genetic adaptation to chemical exposure, or how these adaptations to a chemical stressor may influence population persistence or make individuals vulnerable to other factors	In the last 30 years, research has worked to address our understanding of the physiological and evolutionary effects of contaminants as well as costs of responding to contaminants (See *Physiological effects* and *Evolutionary effects*). However, **we are still missing critical information to allow us to assess how these adaptations may influence population persistence or their relative contribution of mitigating contaminant-induced declines.**
We do not understand how contaminants may influence populations through time at multi-generational scales.	In the last 30 years, some efforts have been made to address multi-generational effects of contaminants though **this remains a research gap and this update includes two sections that address this point (See *Carryover effects* and *Evolutionary effects*).**
Examining the interactive effects of contaminants, disease, pathogens, global change, and habitat alteration will be	In the last 30 years, addressing interactive effects of contaminants appears to have been a research priority, **but this remains a central**

instrumental to planning mitigation measures to thwart declines.	**gap and major focus of this update (see *Interactive effects section*).**
Although much has been learned in recent years about the effects of a few contaminants (e.g., pesticides, coal combustion wastes), little is known about the effects of most other common pollutants on amphibians.	While we have made progress in expanding our understanding to more emerging contaminants (e.g., road salts, PFAS, microplastics, light pollution etc.), **there are many other contaminants that are not well studied. Understanding the impacts of chemical classes is a way to predict the effects of new chemicals that enter the market and is important baseline information that is needed. There is a need to consider not only the direct effects of these various contaminants but also their indirect effects.**
Experimental contaminant research has focused almost solely on the aquatic life stage for amphibians	This remains a significant weakness in our understanding of how contaminants influence amphibians. While aquatic exposure remains the most likely site of exposure for amphibians with complex life cycles, there are exposure risks to terrestrial life stages and species. **Research not only remains focused on aquatic life stages but there is**

	geographic bias that should be addressed in future efforts.

References

Ackerman, F. (2007). The economics of atrazine. *International Journal of Occupational and Environmental Health*, *13*(4), 437–445. doi: 10.1179/oeh.2007.13.4.437

Altwegg, R., & Reyer, H. U. (2003). Patterns of natural selection on size at metamorphosis in water frogs. *Evolution*, *57*(4), 872–882. doi: 10.1111/j.0014-3820.2003.tb00298.x

Anderson, T. A., Salice, C. J., Erickson, R. A., McMurry, S. T., Cox, S. B., & Smith, L. M. (2013). Effects of landuse and precipitation on pesticides and water quality in playa lakes of the southern high plains. *Chemosphere, 92*(1), 84–90. doi: 10.1016/j.chemosphere.2013.02.054

Aronson, D., Printup, H., Shuler, K., & Howard, P. (1998). *Chemical fate half-lives for toxics release inventory (TRI) chemicals*. Syracuse Research Corporation: SRC TR 98-008. Retrieved from https://www.epa.gov/sites/production/files/2015-09/documents/trichem.pdf)

Atwood, D., & Paisley-Jones, C. (2017). *Pesticides industry sales and usage 2008-2012 Market estimates*. USEPA Office of Pesticide Programs. Retrieved from https://www.epa.gov/sites/production/files/2017-01/documents/pesticides-industry-sales-usage-2016_0.pdf

Aviv, R. (2014). A valuable reputation. *The New Yorker*, 1–17. Retrieved from http://www.newyorker.com/reporting/2014/02/10/140210fa_fact_aviv?print able=true&currentPage=all

Baillie, J. E. M., Hilton-Taylor, C., & Stuart, S. N. (2004). *2004 IUCN Red List of Threatened Species: A Global Species Assessment*. Gland, Switzerland.

Baker, N. J., Bancroft, B. A., & Garcia, T. S. (2013). A meta-analysis of the effects of pesticides and fertilizers on survival and growth of amphibians. *Science of the Total Environment*, *449*, 150–156. doi: 10.1016/j.scitotenv.2013.01.056

Bartson, S., Ogilvie, J., Petroff, A. J., Smith, G. R., & Rettig, J. E. (2018). Effect of pond dye on the response of southern Leopard frog tadpoles (*Lithobates sphenocephalus*) to western Mosquitofish (*Gambusia affinis*) cues. *Basic and Applied Herpetology*, *32*, 71–76. doi: 10.11160/bah.93

Battaglin, W. A., Smalling, K. L., Anderson, C., Calhoun, D., Chestnut, T., & Muths, E. (2016). Potential interactions among disease , pesticides , water quality and adjacent land cover in amphibian habitats in the United States. *Science of the Total Environment*, *566–567*, 320–332. doi: 10.1016/j.scitotenv.2016.05.062

Bendik, N.F., Sissel, B.N., Fields, J.R., O'Donnell, L.J., Sanders, M.S. (2014). Effect of urbanization on abundance of Jollyville Plateau Salamanders (*Eurycea tonkawae*). *Herpetological Conservation and Biology*, *9*(1), 206-222.

Bergeron, C. M., Hopkins, W. A., Todd, B. D., Hepner, M. J., & Unrine, J. M. (2011). Interactive effects of maternal and dietary mercury exposure have latent and lethal consequences for amphibian larvae. *Environmental Science and Technology*, *45*(8), 3781–3787. doi: 10.1021/es104210a

Bergman, Å., Heindel, J. J., Kasten, T., Kidd, K. A., Jobling, S., Neira, M., … Woodruff, T. J. (2013). The impact of endocrine disruption: A consensus statement on the state of the science. *Environmental Health Perspectives*, *121*(4), 104–107. doi: 10.1289/ehp.1205448

Bernhardt, E. S., Rosi, E. J., & Gessner, M. O. (2017). Synthetic chemicals as agents of global change. *Frontiers in Ecology and the Environment*, *15*(2), 84–90. doi: 10.1002/fee.1450

Billet, L. S., & Hoverman, J. T. (2020). Pesticide tolerance induced by a generalized stress response in wood frogs (*Rana sylvatica*). *Ecotoxicology, 29*(9), 1476–1485. doi: 10.1007/s10646-020-02277-2

Bishop, C. A., & Pettit, K. E. (1992). Declines in Canadian amphibian populations: designing a national monitoring strategy. In *Occasional Paper Number 76,* Canadian Wildlife Service.

Bishop, Christine A., Ashpole, S. L., Edwards, A. M., Van Aggelen, G., & Elliott, J. E. (2010). Hatching success and pesticide exposures in amphibians living in agricultural habitats of the South Okanagan Valley, British Columbia, Canada (2004-2006). *Environmental Toxicology and Chemistry*, *29*(7), 1593–1603. doi: 10.1002/etc.202

Blann, K. L., Anderson, J. L., Sands, G. R., & Vondracek, B. (2009). Effects of agricultural drainage on aquatic ecosystems: A review. *Critical Reviews in Environmental Science and Technology*, *39*(11), 909–1001. doi: 10.1080/10643380801977966

Blaustein, A. R., & Bancroft, B. A. (2007). Amphibian population declines: Evolutionary considerations. *BioScience*, *57*(5), 437–444. doi: 10.1641/B570517

Blaustein, A. R., Han, B. A., Relyea, R. A., Johnson, P. T. J., Buck, J. C., Gervasi, S. S., & Kats, L. B. (2011). The complexity of amphibian population declines: Understanding the role of cofactors in driving amphibian losses. *Annals of the New*

York Academy of Sciences, *1223*(1), 108–119. doi: 10.1111/j.1749-6632.2010.05909.x

Blaustein, A. R., Urbina, J., Snyder, P. W., Reynolds, E., Dang, T., Hoverman, J. T., … Hambalek, N. M. (2018). Effects of emerging infectious diseases on amphibians: A review of experimental studies. *Diversity*, *10*(3). doi: 10.3390/d10030081

Bókony, V., Ujhegyi, N., Hamow, K., Bosch, J., Thumsová, B., Vörös, J., … Gabor, C. R. (2021). Stressed tadpoles mount more efficient glucocorticoid negative feedback in anthropogenic habitats due to phenotypic plasticity. *Science of the Total Environment*, *753*, 141896. doi: 10.1016/j.scitotenv.2020.141896

Bókony, V., Üveges, B., Ujhegyi, N., Verebélyi, V., Nemesházi, E., Csíkvári, O., & Hettyey, A. (2018). Endocrine disruptors in breeding ponds and reproductive health of toads in agricultural, urban and natural landscapes. *Science of the Total Environment*, *634*, 1335–1345. doi: 10.1016/j.scitotenv.2018.03.363

Boone, M. D. (2005). Juvenile frogs compensate for small metamorph size with terrestrial growth: Overcoming the effects of larval density and insecticide exposure. *Journal of Herpetology*, *39*(3), 416–423. doi: 10.1670/187-04A.1

Boone, M. D. (2008). Examining the single and interactive effects of three insecticides on amphibian metamorphosis. *Environmental Toxicology and Chemistry*, 1561–1568. doi: 10.1897/07-520

Boone, M. D., Bishop, C. A., Boswell, L. A., Brodman, R. D., Burger, J., Davidson, C., … Weir, S. (2014). Pesticide regulation amid the influence of industry. *BioScience*, *64*(10), 917–922. doi: 10.1093/biosci/biu138

Boone, M. D., & Bridges, C. M. (1999). The effect of temperature on the potency of carbaryl for survival of tadpoles of the green frog (*Rana clamitans*). *Environmental Toxicology and Chemistry*, *18*(7), 1482–1484. doi: 10.1897/1551-5028(1999)018<1482:TEOTOT>2.3.CO;2

Boone, M. D., Semlitsch, R. D., Fairchild, J. F., & Rothermel, B. B. (2004). Effects of an insecticide on amphibians in large-scale experimental ponds. *Ecological Applications*, *14*(3), 685–691.

Boone, M. D., Semlitsch, R. D., Little, E. E., & Doyle, M. C. (2007). Multiple stressors in amphibian communities : effects of chemical contamination , bullfrogs and fish. *Ecological Applications*, *17*(1), 291–301.

Bradford, D. F., Knapp, R. A., Sparling, D. W., Nash, M. S., Stanley, K. A., Tallent-Halsell, N. G., … Simonich, S. M. (2011). Pesticide distributions and population declines of California, USA, alpine frogs, *Rana muscosa* and *Rana sierrae*. *Environmental Toxicology and Chemistry*, *30*(3), 682–691. doi: 10.1002/etc.425

Brady, S. P. (2012). Road to evolution? Local adaptation to road adjacency in an amphibian (*Ambystoma maculatum*). *Scientific Reports*, *2*. doi: 10.1038/srep00235

Brady, S. P., Zamora-Camacho, F. J., Eriksson, F. A. A., Goedert, D., Comas, M., & Calsbeek, R. (2019). Fitter frogs from polluted ponds: The complex impacts of human-altered environments. *Evolutionary Applications*, *12*(7), 1360–1370. doi: 10.1111/eva.12751

Brehm, E., & Flaws, J. A. (2019). Transgenerational effects of endocrine-disrupting chemicals on male and female reproduction. *Endocrinology*, *160*(6), 1421–1435. doi: 10.1210/en.2019-00034

Bridges, C. M., Dwyer, F. J., Hardesty, D. K., & Whites, D. W. (2002). Comparative contaminant toxicity:

Are amphibian larvae more sensitive than fish? *Bulletin of Environmental Contamination and Toxicology*, *69*(4), 562–569. doi: 10.1007/s00128-002-0098-2

Bridges, C.M., & Semlitsch, R. D. (2000). Variation in pesticide tolerance of tadpoles among and within species of Ranidae and patterns of amphibian decline. *Conservation Biology*, *14*, 1490-1499.

Brock, T. C. M., Lahr, J., & Van den Brink, P. J. (2000). Ecological risks of pesticides in freshwater ecosystems Part 1 : Herbicides. *Alterra-Report 088*, (January), 128. Retrieved from http://en.scientificcommons.org/1136901

Broomhall, S. (2002). The effects of endosulfan and variable water temperature on survivorship and subsequent vulnerability to predation in *Litoria citropa* tadpoles. *Aquatic Toxicology*, *61*(3–4), 243–250. doi: 10.1016/S0166-445X(02)00061-9

Brühl, C. A., Pieper, S., & Weber, B. (2011). Amphibians at risk? Susceptibility of terrestrial amphibian life stages to pesticides. *Environmental Toxicology and Chemistry*, *30*(11), 2465–2472. doi: 10.1002/etc.650

Brühl, C. A., Schmidt, T., Pieper, S., & Alscher, A. (2013). Terrestrial pesticide exposure of amphibians: An underestimated cause of global decline? *Scientific Reports*, *3*, 1–4. doi: 10.1038/srep01135

Buck, J. C., Hua, J., Brogan, W. R., Dang, T. D., Urbina, J., Bendis, R. J., … Relyea, R. A. (2015). Effects of pesticide mixtures on host-pathogen dynamics of the amphibian chytrid fungus. *PLoS ONE*, *10*(7), 1–17. doi: 10.1371/journal.pone.0132832

Burraco, P., & Gomez-Mestre, I. (2016). Physiological stress responses in amphibian larvae to multiple stressors reveal marked anthropogenic effects even below lethal levels. *Physiological and Biochemical Zoology*, *89*(6), 462–472. doi: 10.1086/688737

Burton, G. A., Di Giulio, R., Costello, D., & Rohr, J. R. (2017). Slipping through the cracks: Why is the U.S. environmental protection agency not funding extramural research on chemicals in our environment? *Environmental Science and Technology*, *51*(2), 755–756. doi: 10.1021/acs.est.6b05877

Buss, N., Swierk, L., & Hua, J. (2021). Amphibian breeding phenology influences offspring size and response to a common wetland contaminant. *Frontiers in Zoology*, *18*(1), 1–11. doi: 10.1186/s12983-021-00413-0

Campbell Grant, E. H., Miller, D. A. W., & Muths, E. (2020). A synthesis of evidence of drivers of amphibian declines. *Herpetologica*, *76*(2).

Campbell Grant, E. H., Miller, D. A. W., Schmidt, B. R., Adams, M. J., Amburgey, S. M., Chambert, T., … Muths, E. (2016). Quantitative evidence for the effects of multiple drivers on continental-scale amphibian declines. *Scientific Reports*, *6*, 25625. doi: 10.1038/srep25625

Cañedo-Argüelles, M., Hawkins, C. P., Kefford, B. J., Schäfer, R. B., Dyack, B. J., Brucet, S., … Timpano, A. J. (2016). Saving freshwater from salts. *Science*, *351*(6276), 914–916.

Carey, C., & Bryant, C. J. (1995). Possible interrelations among environmental toxicants, amphibian development, and decline of amphibian populations. *Environmental Health Perspectives*, *103*, 13–17.

Carey, C., Heyer, W. R., Wilkinson, J., Alford, R. A., Halliday, T., Hungerford, L., … Lips, K. R. (2001). Amphibian declines and environmental change : use of remote-sensing data to identify environmental correlates.

Conservation Biology, *15*(4), 903–913.

Chelgren, N. D., Rosenberg, D. K., Heppell, S. S., & Gitelman, A. I. (2006). Carryover aquatic effects on survival of metamorphic frogs during pond emigration. *Ecological Applications*, *16*(1), 250–261. doi: 10.1890/04-0329

Chiari, Y., Glaberman, S., Serén, N., Carretero, M. A., & Capellini, I. (2015). Phylogenetic signal in amphibian sensitivity to copper sulfate relative to experimental temperature. *Ecological Applications*, *25*(3), 596–602.

Cole, L. J., Stockan, J., & Helliwell, R. (2020). Managing riparian buffer strips to optimise ecosystem services: A review. *Agriculture, Ecosystems and Environment*, *296*(February), 106891. doi: 10.1016/j.agee.2020.106891

Conde, D. A., Staerk, J., Colchero, F., da Silva, R., Schöley, J., Maria Baden, H., … Vaupel, J. W. (2019). Data gaps and opportunities for comparative and conservation biology. *Proceedings of the National Academy of Sciences of the United States of America*, *116*(19), 9658–9664. doi: 10.1073/pnas.1816367116

Cothran, R. D., Brown, J. M., & Relyea, R. A. (2013). Proximity to agriculture is correlated with pesticide tolerance: Evidence for the evolution of amphibian resistance to modern pesticides. *Evolutionary Applications*, *6*(5), 832–841. doi: 10.1111/eva.12069

Cusaac, J. P. W., Carter, E. D., Woodhams, D. C., Robert, J., Spatz, J. A., Howard, J. L., … Gray, M. J. (2021). Emerging pathogens and a current-use pesticide: potential impacts on Eastern Hellbenders. *Journal of Aquatic Animal Health*, *33*(1), 24–32. doi: 10.1002/aah.10117

Davidson, C. (2004). Declining downwind: Amphibian population declines in California and historical pesticide use. *Ecological Applications*, *14*(6), 1892–1902. doi: 10.1890/03-5224

Davidson, C., Benard, M. F., Shaffer, H. B., Parker, J. M., O'Leary, C., Conlon, J. M., & Rollins-Smith, L. A. (2007). Effects of chytrid and carbaryl exposure on survival, growth and skin peptide defenses in foothill yellow-legged frogs. *Environmental Science and Technology*, *41*(5), 1771–1776. doi: 10.1021/es0611947

Davidson, C., & Knapp, R. A. (2007). Multiple stressors and amphibian declines: Dual impacts of pesticides and fish on yellow-legged frogs. *Ecological Applications*, *17*(2), 587–597. doi: 10.1890/06-0181

Davidson, C., Shaffer, H. B., & Jennings, M. R. (2001). Declines of the California red-legged frog: Climate, UV-B, habitat, and pesticides hypotheses. *Ecological Applications*, *11*(2), 464–479. doi: 10.1890/1051-0761(2001)011[0464:DOTCRL]2.0.CO;2

Davidson, C., Shaffer, H. B., & Jennings, M. R. (2002). Spatial tests of the pesticide drift, habitat destruction, UV-B, and climate-change hypotheses for California amphibian declines. *Conservation Biology*, *16*(6), 1588–1601. doi: 10.1046/j.1523-1739.2002.01030.x

Davidson, C., Stanley, K., & Simonich, S. M. (2012). Contaminant residues and declines of the Cascades frog (*Rana cascadae*) in the California Cascades, USA. *Environmental Toxicology and Chemistry*, *31*(8), 1895–1902. doi: 10.1002/etc.1902

Davis, A. Y., Freund, A., Dumyahn, S. L., Mendoza, R., Muniz Torres, A., & Boone, M. D. (2021). Parcel management and perceived ecosystem services and disservices in the exurbs of a midwestern county in the United States. *Land*, *10*(5). doi: 10.3390/land10050448

Davis, D. R., Ferguson, K. J., Schwarz, M. S., & Kerby, J. L. (2020). Effects of agricultural pollutants on stress hormones and viral infection in larval salamanders. *Wetlands*, *40*(6), 2849. doi: 10.1007/s13157-020-01352-y

Diffenbaugh, N. S., & Ashfaq, M. (2010). Intensification of hot extremes in the United States. *Geophysical Research Letters*, *37*(15), 1–5. doi: 10.1029/2010GL043888

Distel, C. A., & Boone, M. D. (2010). Effects of aquatic exposure to the insecticide carbaryl are species-specific across life stages and mediated by heterospecific competitors in anurans. *Functional Ecology*, *24*(6), 1342–1352. doi: 10.1111/j.1365-2435.2010.01749.x

Duarte, H., Tejedo, M., Katzenberger, M., Marangoni, F., Baldo, D., Beltrán, J. F., … Gonzalez-Voyer, A. (2012). Can amphibians take the heat? Vulnerability to climate warming in subtropical and temperate larval amphibian communities. *Global Change Biology*, *18*(2), 412–421. doi: 10.1111/j.1365-2486.2011.02518.x

Dudgeon, D., Arthington, A. H., Gessner, M. O., Kawabata, Z. I., Knowler, D. J., Lévêque, C., … Sullivan, C. A. (2006). Freshwater biodiversity: Importance, threats, status and conservation challenges. *Biological Reviews of the Cambridge Philosophical Society*, *81*(2), 163–182. doi: 10.1017/S1464793105006950

Earl, J. E., & Whiteman, H. H. (2015). Are commonly used fitness predictors accurate? A meta-analysis of amphibian size and age at metamorphosis. *Copeia*, *103*(2), 297–309. doi: 10.1643/CH-14-128

Edge, C. B., Baker, L. F., Lanctôt, C. M., Melvin, S. D., Gahl, M. K., Kurban, M., … Houlahan, J. E. (2020). Compensatory indirect effects of an herbicide on wetland communities. *Science of the Total Environment*, *718*, 137254. doi: 10.1016/j.scitotenv.2020.137254

Edwards, T. M., & Myers, J. P. (2007). Environmental exposures and gene regulation in disease etiology. *Environmental Health Perspectives*, *115*(9), 1264–1270. doi: 10.1289/ehp.9951

Egea-Serrano, A., Relyea, R. A., Tejedo, M., & Torralva, M. (2012). Understanding of the impact of chemicals on amphibians: A meta-analytic review. *Ecology and Evolution*, *2*(7), 1382–1397. doi: 10.1002/ece3.249

Faulkner, S. (2004). Urbanization impacts on the structure and function of forested wetlands. *Urban Ecosystems*, *7*(2), 89–106. doi: 10.1023/b:ueco.0000036269.56249.66

Ficetola, G. F., Manenti, R., & Taberlet, P. (2019). Environmental DNA and metabarcoding for the study of amphibians and reptiles: species distribution , the microbiome , and much more. *Amphibia-Reptilia*, *40*, 129–148. doi: 10.1163/15685381-20191194

Fleeger, J. W., Carman, K. R., & Nisbet, R. M. (2003). Indirect effects of contaminants in aquatic ecosystems. *Science of the Total Environment*, *317*(1–3), 207–233. doi: 10.1016/S0048-9697(03)00141-4

Forsburg, Z. R., Guzman, A., & Gabor, C. R. (2021). Artificial light at night (ALAN) affects the stress physiology but not the behavior or growth of *Rana berlandieri* and *Bufo valliceps*. *Environmental Pollution*, *277*, 116775. doi: 10.1016/j.envpol.2021.116775

Forson, D. D., & Storfer, A. (2006). Atrazine increases ranavirus susceptibility in the tiger salamander, *Ambystoma tigrinum*. *Ecological Applications*, *16*(6), 2325–2332. doi: 10.1890/1051-0761(2006)016[2325:AIRSIT]2.0.CO

;2

Gabor, C. R., Davis, D. R., Kim, D. S., Zabierek, K. C., & Bendik, N. F. (2018). Urbanization is associated with elevated corticosterone in Jollyville Plateau salamanders. *Ecological Indicators*, *85*(March 2017), 229–235. doi: 10.1016/j.ecolind.2017.10.047

Gabor, C. R., Knutie, S. A., Roznik, E. A., & Rohr, J. R. (2018). Are the adverse effects of stressors on amphibians mediated by their effects on stress hormones? *Oecologia*, *186*(2), 393–404. doi: 10.1007/s00442-017-4020-3

Gaietto, K. M., Rumschlag, S. L., & Boone, M. D. (2014). Effects of pesticide exposure and the amphibian chytrid fungus on gray treefrog (*Hyla chrysoscelis*) metamorphosis. *Environmental Toxicology and Chemistry*, *33*(10), 2358–2362. doi: 10.1002/etc.2689

Gallagher, M. T., Snodgrass, J. W., Ownby, D. R., Brand, A. B., Casey, R. E., & Lev, S. (2011). Watershed-scale analysis of pollutant distributions in stormwater management ponds. *Urban Ecosystems*, *14*(3), 469–484. doi: 10.1007/s11252-011-0162-y

Gascon, C., Collins, J. P., Moore, R. D., Church, D. R., McKay, J. E., & Mendelson III, J. R. (2007). Amphibian conservation action plan. *Proceedings: IUCN/SSC Amphibian Conservation Summit 2005* (Vol. 22). Gland, Switzerland and Cambridge, UK.

Gessner, M. O., & Tlili, A. (2016). Fostering integration of freshwater ecology with ecotoxicology. *Freshwater Biology*, *61*(12), 1991–2001. doi: 10.1111/fwb.12852

Gibbs, K. E., MacKey, R. L., & Currie, D. J. (2009). Human land use, agriculture, pesticides and losses of imperiled species. *Diversity and Distributions*, *15*(2), 242–253. doi: 10.1111/j.1472-4642.2008.00543.x

Glaberman, S., Kiwiet, J., & Aubee, C. B. (2019). Evaluating the role of fish as surrogates for amphibians in pesticide ecological risk assessment. *Chemosphere*, *235*, 952–958. doi: 10.1016/j.chemosphere.2019.06.166

Glennemeier, K. A., & Denver, R. T. (2002). Developmental changes in interrenal responsiveness in anuran amphibians. *Integrative and Comparative Biology*, *42*(3), 565–573.

Goff, C. B., Walls, S. C., Rodriguez, D., & Gabor, C. R. (2020). Changes in physiology and microbial diversity in larval ornate chorus frogs are associated with habitat quality. *Conservation Physiology*, *8*(1), 1–19. doi: 10.1093/conphys/coaa047

Green, D. M., Lannoo, M. J., Lesbarrères, D., & Muths, E. (2020). Amphibian population declines: 30 years of progress in confronting a complex problem. *Herpetologica*, *76*(2), 97–100. doi: 10.1655/0018-0831-76.2.97

Gutiérrez-Pesquera, L. M., Tejedo, M., Olalla-Tárraga, M. A., Duarte, H., Nicieza, A., & Solé, M. (2016). Testing the climate variability hypothesis in thermal tolerance limits of tropical and temperate tadpoles. *Journal of Biogeography*, *43*(6), 1166–1178. doi: 10.1111/jbi.12700

Hageman, K. J., Simonich, S. L., Campbell, D. H., Wilson, G. R., & Landers, D. H. (2006). Atmospheric deposition of current-use and historic-use pesticides in snow at national parks in the western United States. *Environmental Science & Technology*, 40, 3174-3180.

Hall, E. M., Brunner, J. L., Hutzenbiler, B., & Crespi, E. J. (2020). Salinity stress increases the severity of ranavirus epidemics in amphibian populations. *Proceedings of the Royal Society B: Biological Sciences*, *287*(1926). doi: 10.1098/rspb.2020.0062

Halstead, N. T., McMahon, T. A.,

Johnson, S. A., Raffel, T. R., Romansic, J. M., Crumrine, P. W., & Rohr, J. R. (2014). Community ecology theory predicts the effects of agrochemical mixtures on aquatic biodiversity and ecosystem properties. *Ecology Letters*, *17*(8), 932–941. doi: 10.1111/ele.12295

Hanson, M. L., Solomon, K. R., Van Der Kraak, G. J., & Brian, R. A. (2019). Effects of atrazine on fish, amphibians, and reptiles: update of the analysis based on quantitative weight of evidence. *Critical Reviews in Toxicology*, *49*(8), 670–709. doi: 10.1080/10408444.2019.1701985

Hayden, M. T., Reeves, M. K., Holyoak, M., Perdue, M., King, A. L., & Tobin, S. C. (2015). Thrice as easy to catch! Copper and temperature modulate predator-prey interactions in larval dragonflies and anurans. *Ecosphere*, *6*(4), 1–17. doi: 10.1890/ES14-00461.1

Hayes, T. B. (2004). There is no denying this: Defusing the confusion about atrazine. *BioScience*, *54*(12), 1138–1149. doi: 10.1641/0006-3568(2004)054[1138:TINDTD]2.0.CO;2

Hayes, T. B., Anderson, L. L., Beasley, V. R., De Solla, S. R., Iguchi, T., Ingraham, H., … Willingham, E. (2011). Demasculinization and feminization of male gonads by atrazine: Consistent effects across vertebrate classes. *Journal of Steroid Biochemistry and Molecular Biology*, *127*(1–2), 64–73. doi: 10.1016/j.jsbmb.2011.03.015

Hayes, T. B., Case, P., Chui, S., Chung, D., Haeffele, C., Haston, K., … Tsui, M. (2006). Pesticide mixtures, endocrine disruption, and amphibian declines: Are we underestimating the impact? *Environmental Health Perspectives*, *114*(SUPPL.1), 40–50. doi: 10.1289/ehp.8051

Hayes, T. B., Collins, A., Lee, M., Mendoza, M., Noriega, N., Stuart, A.

A., & Vonk, A. (2002). Hermaphroditic, demasculinized frogs after exposure to the herbicide atrazine at low ecologically relevant doses. *Proceedings of the National Academy of Sciences of the United States of America*, *99*(8), 5476–5480. doi: 10.1073/pnas.082121499

Hayes, T. B., Falso, P., Gallipeau, S., & Stice, M. (2010). The cause of global amphibian declines: A developmental endocrinologist's perspective. *Journal of Experimental Biology*, *213*(6), 921–933. doi: 10.1242/jeb.040865

Hayes, T. B., Haston, K., Tsui, M., Hoang, A., Haeffele, C., & Vonk, A. (2003). Atrazine-induced hermaphroditism at 0.1 ppb in American leopard frogs (*Rana pipiens*): Laboratory and field evidence. *Environmental Health Perspectives*, *111*(4), 568–575. doi: 10.1289/ehp.5932

Hayes, T. B., Khoury, V., Narayan, A., Nazir, M., Parka, A., Brown, T., … Gallipeau, S. (2010). Atrazine induces complete feminization and chemical castration in male African clawed frogs (*Xenopus laevis*). *Proceedings of the National Academy of Sciences of the United States of America*, *107*(10), 4612–4617. doi: 10.1073/pnas.0909519107

Hillebrand, H., & Matthiessen, B. (2009). Biodiversity in a complex world: Consolidation and progress in functional biodiversity research. *Ecology Letters*, *12*(12), 1405–1419. doi: 10.1111/j.1461-0248.2009.01388.x

Hinther, A., Bromba, C. M., Wulff, J. E., & Helbing, C. C. (2011). Effects of triclocarban, triclosan, and methyl triclosan on thyroid hormone action and stress in frog and mammalian culture systems. *Environmental Science and Technology*, *45*(12), 5395–5402. doi: 10.1021/es1041942

Hintz, W. D., & Relyea, R. A. (2019). A review of the species, community, and ecosystem impacts of road salt salinisation in fresh waters. *Freshwater Biology*, *64*(6), 1081–1097. doi: 10.1111/fwb.13286

Holt, M. S. (2000). Sources of chemical contaminants and routes into the freshwater environment. *Food and Chemical Toxicology*, *38*(SUPPL.1). doi: 10.1016/s0278-6915(99)00136-2

Homola, J. J., Loftin, C. S., Cammen, K. M., Helbing, C. C., Birol, I., Schultz, T. F., & Kinnison, M. T. (2019). Replicated landscape genomics identifies evidence of local adaptation to urbanization in wood frogs. *The Journal of Heredity*, *110*(6), 707–719.

Hopkins, W. A., Mendonça, M. T., & Congdon, J. D. (1997). Increased circulating levels of testosterone and corticosterone in southern toads, *Bufo terrestris*, exposed to coal combustion waste. *General and Comparative Endocrinology*, *108*(2), 237–246. doi: 10.1006/gcen.1997.6969

Hoskins, T. D., & Boone, M. D. (2017). Variation in malathion sensitivity among populations of Blanchard’s cricket frogs (*Acris blanchardi*) and implications for risk assessment. *Environmental Toxicology and Chemistry*, *36*(7), 1917–1923. doi: 10.1002/etc.3715

Hua, J., Buss, N., Kim, J., Orlofske, S. A., & Hoverman, J. T. (2016). Population-specific toxicity of six insecticides to the trematode *Echinoparyphium sp. Parasitology*, *143*(5), 542–550. doi: 10.1017/S0031182015001894

Hua, J., Cothran, R., Stoler, A., & Relyea, R. (2013). Cross-tolerance in amphibians: Wood frog mortality when exposed to three insecticides with a common mode of action. *Environmental Toxicology and Chemistry*, *32*(4), 932–936. doi: 10.1002/etc.2121

Hua, J., Jones, D. K., Mattes, B. M., Cothran, R. D., Relyea, R. A., & Hoverman, J. T. (2015). The contribution of phenotypic plasticity to the evolution of insecticide tolerance in amphibian populations. *Evolutionary Applications*, *8*(6), 586–596. doi: 10.1111/eva.12267

Hua, J., Jones, D. K., & Relyea, R. A. (2014). Induced tolerance from a sublethal insecticide leads to cross-tolerance to other insecticides. *Environmental Science and Technology*, *48*(7), 4078–4085. doi: 10.1021/es500278f

Hua, J., Morehouse, N. I., & Relyea, R. (2013). Pesticide tolerance in amphibians: Induced tolerance in susceptible populations, constitutive tolerance in tolerant populations. *Evolutionary Applications*, *6*(7), 1028–1040. doi: 10.1111/eva.12083

Injaian, A. S., Francis, C. D., Ouyang, J. Q., Dominoni, D. M., Donald, J. W., Fuxjager, M. J., … Vitousek, M. N. (2020). Baseline and stress-induced corticosterone levels across birds and reptiles do not reflect urbanization levels. *Conservation Physiology*, *8*(1), 1–18. doi: 10.1093/conphys/coz110

IPCC. (2013). *Climate change 2013: the physical science basis. Contribution of Working Group I to the fifth assessment report of the Intergovernmental Panel on Climate Change*. Cambridge, UK and New York, NY: Cambridge University Press.

James, S. M., & Semlitsch, R. D. (2011). Terrestrial performance of juvenile frogs in two habitat types after chronic larval exposure to a contaminant. *Journal of Herpetology*, *45*(2), 186–194. doi: 10.1670/10-032.1

Johnson, P. T. J., & Chase, J. M. (2004). Parasites in the food web: Linking amphibian malformations and aquatic eutrophication. *Ecology Letters*, *7*(7), 521–526. doi: 10.1111/j.1461-0248.2004.00610.x

Johnson, P. T. J., Chase, J. M., Dosch, K.

L., Hartson, R. B., Gross, J. A., Larson, D. J., … Carpenter, S. R. (2007). Aquatic eutrophication promotes pathogenic infection in amphibians. *Proceedings of the National Academy of Sciences of the United States of America*, *104*(40), 15781–15786. doi: 10.1073/pnas.0707763104

Jones-Costa, M., Franco-Belussi, L., Vidal, F. A. P., Gongora, N. P., Castanho, L. M., dos Santos Carvalho, C., … Salla, R. F. (2018). Cardiac biomarkers as sensitive tools to evaluate the impact of xenobiotics on amphibians: the effects of anionic surfactant linear alkylbenzene sulfonate (LAS). *Ecotoxicology and Environmental Safety*, *151*(October 2017), 184–190. doi: 10.1016/j.ecoenv.2018.01.022

Jones, D. K., Hua, J., Mattes, B. M., Cothran, R. D., Hoverman, J. T., & Relyea, R. A. (2021). Predator- and competitor-induced responses in amphibian populations that evolved different levels of pesticide tolerance. *Ecological Applications*, *31*(4). doi: 10.1002/eap.2305

Karlsson, O., Svanholm, S., Eriksson, A., Chidiac, J., Eriksson, J., Jernerén, F., & Berg, C. (2021). Pesticide-induced multigenerational effects on amphibian reproduction and metabolism. *Science of the Total Environment*, *775*. doi: 10.1016/j.scitotenv.2021.145771

Katzenberger, M., Duarte, H., Relyea, R., Beltrán, J. F., & Tejedo, M. (2021). Variation in upper thermal tolerance among 19 species from temperate wetlands. *Journal of Thermal Biology*, *96*(January). doi: 10.1016/j.jtherbio.2021.102856

Katzenberger, M., Hammond, J., Duarte, H., Tejedo, M., Calabuig, C., & Relyea, R. A. (2014). Swimming with predators and pesticides: How environmental stressors affect the thermal physiology of tadpoles. *PLoS*

ONE, *9*(5), 1–11. doi: 10.1371/journal.pone.0098265

Kenison, E. K., Hernández-Gómez, O., & Williams, R. N. (2020). A novel bioaugmentation technique effectively increases the skin-associated microbial diversity of captive eastern hellbenders. *Conservation Physiology*, *8*(1), 1–12. doi: 10.1093/conphys/coaa040

Kerby, J. L., Richards-Hrdlicka, K. L., Storfer, A., & Skelly, D. K. (2010). An examination of amphibian sensitivity to environmental contaminants: Are amphibians poor canaries? *Ecology Letters*, *13*(1), 60–67. doi: 10.1111/j.1461-0248.2009.01399.x

Kidd, K. A., Blanchfield, P. J., Mills, K. H., Palace, V. P., Evans, R. E., Lazorchak, J. M., & Flick, R. W. (2007). Collapse of a fish population after exposure to a synthetic estrogen. *Proceedings of the National Academy of Sciences of the United States of America*, *104*(21), 8897–8901. doi: 10.1073/pnas.0609568104

King, K. C., Daniel Mclaughlin, J., Boily, M., & Marcogliese, D. J. (2010). Effects of agricultural landscape and pesticides on parasitism in native bullfrogs. *Biological Conservation*, *143*(2), 302–310. doi: 10.1016/j.biocon.2009.10.011

Kleinhenz, P., Boone, M. D., & Fellers, G. (2012). Effects of the amphibian chytrid fungus and four insecticides on Pacific Treefrogs (*Pseudacris regilla*)'. *Journal of Herpetology, 46*, 625-631.Kuglerová, L., Ågren, A., Jansson, R., & Laudon, H. (2014). Towards optimizing riparian buffer zones: Ecological and biogeochemical implications for forest management. *Forest Ecology and Management*, *334*, 74–84. doi: 10.1016/j.foreco.2014.08.033

Kumar, M., Borah, P., & Devi, P. (2020). Chapter 3—Priority and emerging pollutants in water. *Inorganic*

Pollutants in Water, 33–49. doi: 10.1016/b978-0-12-818965-8.00003-2 In P. Devi, P. Singh, & S. K. Kansal (Eds.), *Inorganic pollutants in water* (pp. 33–49). Elsevier. [DOI:10.1016/B978-0-12-818965-8.00003-2]

Lagarde, F., Beausoleil, C., Belcher, S. M., Belzunces, L. P., Emond, C., Guerbet, M., & Rousselle, C. (2015). Non-monotonic dose-response relationships and endocrine disruptors: A qualitative method of assessment. *Environmental Health*, *14*(1), 1–15. doi: 10.1186/1476-069X-14-13

Larson, D. L., McDonald, S., Fivizzani, A. J., Newton, W. E., & Hamilton, S. J. (1998). Effects of the herbicide atrazine on *Ambystoma tigrinum* metamorphosis: Duration, larval growth, and hormonal response. *Physiological Zoology*, *71*(6), 671–679. doi: 10.1086/515999

Lau, E. T. C., Karraker, N. E., & Leung, K. M. Y. (2015). Temperature-dependent acute toxicity of methomyl pesticide on larvae of 3 Asian amphibian species. *Environmental Toxicology and Chemistry*, *34*(10), 2322–2327. doi: 10.1002/etc.3061

Leaning, J. (2000). Environment and health: 5. Impact of war. *Canadian Medical Association Journal*, *163*(9), 1157–1161.

Luke, S. H., Slade, E. M., Gray, C. L., Annammala, K. V., Drewer, J., Williamson, J., … Struebig, M. J. (2019). Riparian buffers in tropical agriculture: Scientific support, effectiveness and directions for policy. *Journal of Applied Ecology*, *56*(1), 85–92. doi: 10.1111/1365-2664.13280

Maselli, V., Polese, G., Rippa, D., Ligrone, R., Kumar Rastogi, R., & Fulgione, D. (2010). Frogs, sentinels of DNA damage induced by pollution in Naples and the neighbouring Provinces. *Ecotoxicology and*

Environmental Safety, *73*(7), 1525–1529. doi: 10.1016/j.ecoenv.2010.05.011

Materna, E. J., Rabeni, C. F., & Lapoint, T. W. (1995). Effects of the synthetic pyrethroid insecticide, esfenvalerate, on larval leopard frogs (*Rana* spp.). *Environmental Toxicology and Chemistry*, *14*(4), 613–622. doi: 10.1002/etc.5620140409

Mayer, P. M., Reynolds, S. K., Canfield, T. J., & McCutchen, M. D. (2005). Riparian buffer width, vegetative cover, and nitrogen removal effectiveness: A review of current science and regulations. In *EPA/600/R-05/118*. Cincinnati, OH. Retrieved from http://nepis.epa.gov/Exe/ZyPDF.cgi/2000O182.PDF?Dockey=2000O182.PDF

McCoy, K. A., & Peralta, A. L. (2018). Pesticides could alter amphibian skin microbiomes and the effects of *Batrachochytrium dendrobatidis*. *Frontiers in Microbiology*, *9*(APR), 1–5. doi: 10.3389/fmicb.2018.00748

McMahon, T. A., Halstead, N. T., Johnson, S., Raffel, T. R., Romansic, J. M., Crumrine, P. W., & Rohr, J. R. (2012). Fungicide-induced declines of freshwater biodiversity modify ecosystem functions and services. *Ecology Letters*, *15*(7), 714–722. doi: 10.1111/j.1461-0248.2012.01790.x

McMahon, T. A., Halstead, N. T., Johnson, S., Raffel, T. R., Romansic, J. M., Crumrine, P. W., … Rohr, J. R. (2011). The fungicide chlorothalonil is nonlinearly associated with corticosterone levels, immunity, and mortality in amphibians. *Environmental Health Perspectives*, *119*(8), 1098–1103. doi: 10.1289/ehp.1002956

McMahon, T. A., Romansic, J. M., & Rohr, J. R. (2013). Nonmonotonic and Monotonic effects of pesticides on the pathogenic fungus *Batrachochytrium dendrobatidis* in culture and on tadpoles. *Environmental Science and Technology*, *47*, 7958–7964.

Meftaul, I. M., Venkateswarlu, K., Dharmarajan, R., Annamalai, P., & Megharaj, M. (2020). Pesticides in the urban environment: A potential threat that knocks at the door. *Science of the Total Environment*, *711*, 134612. doi: 10.1016/j.scitotenv.2019.134612

Moges, A., Beyene, A., Ambelu, A., Mereta, S. T., Triest, L., & Kelbessa, E. (2017). Plant species composition and diversity in wetlands under forest, agriculture and urban land uses. *Aquatic Botany*, *138*, 9–15. doi: 10.1016/j.aquabot.2016.12.001

Monaghan, P., Hu, S., Hansen, G., Ott, E., Nealis, C., & Morera, M. (2016). Balancing the ecological function of residential stormwater ponds with homeowner landscaping practices. *Environmental Management*, *58*(5), 843–856. doi: 10.1007/s00267-016-0752-9

Murray, M. H., Sánchez, C. A., Becker, D. J., Byers, K. A., Worsley-Tonks, K. E. L., & Craft, M. E. (2019). City sicker? A meta-analysis of wildlife health and urbanization. *Frontiers in Ecology and the Environment*, *17*(10), 575–583. doi: 10.1002/fee.2126

Muscutt, A. D., Harris, G. L., Bailey, S. W., & Davies, D. B. (1993). Buffer zones to improve water quality: a review of their potential use in UK agriculture. *Agriculture, Ecosystems and Environment*, *45*(1–2), 59–77. doi: 10.1016/0167-8809(93)90059-X

Narayan, E. J., Forsburg, Z. R., Davis, D. R., & Gabor, C. R. (2019). Non-invasive methods for measuring and monitoring stress physiology in imperiled amphibians. *Frontiers in Ecology and Evolution*, *7*(November), 1–10. doi: 10.3389/fevo.2019.00431

O'Connor, C. M., & Cooke, S. J. (2015). Ecological carryover effects complicate conservation. *Ambio*, *44*(6), 582–591. doi: 10.1007/s13280-015-0630-3

O'Connor, C. M., Norris, D. R., Crossin, G. T., & Cooke, S. J. (2014). Biological carryover effects: Linking common concepts and mechanisms in ecology and evolution. *Ecosphere*, *5*(3), 1–11. doi: 10.1890/ES13-00388.1

Oreskes, N., & Conway, E. M. (2010). *Merchants of doubt: how a handful of scientists obscured the truth on issues from tobacco smoke to global warming*. New York: Bloomsbury Press.

Ortiz-Santaliestra, M. E., Maia, J. P., Egea-Serrano, A., & Lopes, I. (2018). Validity of fish, birds and mammals as surrogates for amphibians and reptiles in pesticide toxicity assessment. *Ecotoxicology*, *27*(7), 819–833. doi: 10.1007/s10646-018-1911-y

Pabijan, M., Palomar, G., Antunes, B., Antoł, W., Zieliński, P., & Babik, W. (2020). Evolutionary principles guiding amphibian conservation. *Evolutionary Applications*, (February), 857–878. doi: 10.1111/eva.12940

Palenske, N. M., Nallani, G. C., & Dzialowski, E. M. (2010). Physiological effects and bioconcentration of triclosan on amphibian larvae. *Comparative Biochemistry and Physiology - C Toxicology and Pharmacology*, *152*(2), 232–240. doi: 10.1016/j.cbpc.2010.04.009

Parmesan, C. (2007). Influences of species, latitudes and methodologies on estimates of phenological response to global warming. *Global Change Biology*, *13*(9), 1860–1872. doi: 10.1111/j.1365-2486.2007.01404.x

Patar, A., Giri, A., Boro, F., Bhuyan, K., Singha, U., & Giri, S. (2016). Cadmium pollution and amphibians - Studies in tadpoles of *Rana* *limnocharis*. *Chemosphere*, *144*, 1043–1049. doi: 10.1016/j.chemosphere.2015.09.088

Pimentel, D., & Burgess, M. (2012). Small amounts of pesticides reaching target insects. *Environment, Development and Sustainability*, *14*(1), 1–2. doi: 10.1007/s10668-011-9325-5

Polo-Cavia, N., Burraco, P., & Gomez-Mestre, I. (2016). Low levels of chemical anthropogenic pollution may threaten amphibians by impairing predator recognition. *Aquatic Toxicology*, *172*, 30–35. doi: 10.1016/j.aquatox.2015.12.019

Ponisio, L. C., M'Gonigle, L. K., Mace, K. C., Palomino, J., Valpine, P. De, & Kremen, C. (2015). Diversification practices reduce organic to conventional yield gap. *Proceedings of the Royal Society B: Biological Sciences*, *282*(1799). doi: 10.1098/rspb.2014.1396

Puglis, H. J., & Boone, M. D. (2011). Effects of technical-grade active ingredient vs. commercial formulation of seven pesticides in the presence or absence of UV radiation on survival of green frog tadpoles. *Archives of Environmental Contamination and Toxicology*, *60*(1), 145–155. doi: 10.1007/s00244-010-9528-z

Puglis, H. J., & Boone, M. D. (2012). Effects of terrestrial buffer zones on amphibians on golf courses. *PLoS ONE*, *7*(6). doi: 10.1371/journal.pone.0039590

Quiroga, L. B., Sanabria, E. A., Fornés, M. W., Bustos, D. A., & Tejedo, M. (2019). Sublethal concentrations of chlorpyrifos induce changes in the thermal sensitivity and tolerance of anuran tadpoles in the toad *Rhinella arenarum*? *Chemosphere*, *219*, 671–677. doi: 10.1016/j.chemosphere.2018.12.059

Reeves, R. A., Pierce, C. L., Vandever, M. W., Muths, E., & Smalling, K. L. (2017). Amphibians, pesticides, and the amphibian chytrid fungus in restored wetlands in agricultural landscapes. *Herpetological Conservation and Biology*, *12*(1), 68–77.

Relyea, R. A. (2005). The lethal impact of roundup on aquatic and terrestrial amphibians. *Ecological Applications*, *15*(4), 1118–1124.

Relyea, R. A., & Diecks, N. (2008). An unforeseen chain of events: Lethal effects of pesticides on frogs at sublethal concentrations. *Ecological Applications*, *18*(7), 1728–1742. doi: 10.1890/08-0454.1

Renick, V. C., Anderson, T. W., Morgan, S. G., & Cherr, G. N. (2015). Interactive effects of pesticide exposure and habitat structure on behavior and predation of a marine larval fish. *Ecotoxicology*, *24*(2), 391–400. doi: 10.1007/s10646-014-1388-2

Renwick, W. H., Smith, S. V., Bartley, J. D., & Buddemeier, R. W. (2005). The role of impoundments in the sediment budget of the conterminous United States. *Geomorphology*, *71*(1–2), 99–111. doi: 10.1016/j.geomorph.2004.01.010

Roberts, A. P., Alloy, M. M., & Oris, J. T. (2017). Review of the photo-induced toxicity of environmental contaminants. *Comparative Biochemistry and Physiology Part - C: Toxicology and Pharmacology*, *191*, 160–167. doi: 10.1016/j.cbpc.2016.10.005

Rohr, J. R. (2021). The atrazine saga and its importance to the future of toxicology, science, and environmental and human health. *Environmental Toxicology and Chemistry*, *40*(6), 1544–1558. doi: 10.1002/etc.5037

Rohr, J. R., & McCoy, K. A. (2010). A qualitative meta-analysis reveals consistent effects of atrazine on freshwater fish and amphibians. *Environmental Health Perspectives*, *118*(1), 20–32. doi: 10.1289/ehp.0901164

Rohr, J. R., & Palmer, B. D. (2005). Aquatic herbicide exposure increases salamander desiccation risk eight months later in a terrestrial environment. *Environmental Toxicology and Chemistry*, *24*(5), 1253–1258. doi: 10.1897/04-448R.1

Rohr, J. R., Raffel, T. R., Halstead, N. T., McMahon, T. A., Johnson, S. A., Boughton, R. K., & Martin, L. B. (2013). Early-life exposure to a herbicide has enduring effects on pathogen-induced mortality. *Proceedings of the Royal Society B: Biological Sciences*, *280*(1772). doi: 10.1098/rspb.2013.1502

Rohr, J. R., Raffel, T. R., Sessions, S. K., & Hudson, P. J. (2008). Understanding the net effects of pesticides on amphibian trematode infections. *Ecological Applications*, *18*(7), 1743–1753. doi: 10.1890/07-1429.1

Rohr, J. R., Salice, C. J., & Nisbet, R. M. (2016). The pros and cons of ecological risk assessment based on data from different levels of biological organization. *Critical Reviews in Toxicology*, *46*(9), 756–784. doi: 10.1080/10408444.2016.1190685

Rohr, J. R., Schotthoefer, A. M., Raffel, T. R., Carrick, H. J., Halstead, N., Hoverman, J. T., … Beasley, V. R. (2008). Agrochemicals increase trematode infections in a declining amphibian species. *Nature*, *455*(7217), 1235–1239. doi: 10.1038/nature07281

Rohr, J. R., Sesterhenn, T. M., & Stieha, C. (2011). Will climate change reduce the effects of a pesticide on amphibians?: Partitioning effects on exposure and susceptibility to contaminants. *Global Change Biology*, *17*(2), 657–666. doi: 10.1111/j.1365-2486.2010.02301.x

Rollins-Smith, L. A., Doersam, J. K.,

Longcore, J. E., Taylor, S. K., Shamblin, J. C., Carey, C., & Zasloff, M. A. (2002). Antimicrobial peptide defenses against pathogens associated with global amphibian declines. *Developmental and Comparative Immunology*, *26*(1), 63–72. doi: 10.1016/S0145-305X(01)00041-6

Rowe, C. L., Hopkins, W. A., & Coffman, V. R. (2001). Failed recruitment of southern toads (*Bufo terrestris*) in a trace element-contaminated breeding habitat: Direct and indirect effects that may lead to a local population sink. *Archives of Environmental Contamination and Toxicology*, *40*(3), 399–405. doi: 10.1007/s002440010189

Rumschlag, S. L., Halstead, N. T., Hoverman, J. T., Raffel, T. R., Carrick, H. J., Hudson, P. J., & Rohr, J. R. (2019). Effects of pesticides on exposure and susceptibility to parasites can be generalised to pesticide class and type in aquatic communities. *Ecology Letters*, *22*(6), 962–972. doi: 10.1111/ele.13253

Rumschlag, S. L., Mahon, M. B., Hoverman, J. T., Raffel, T. R., Carrick, H. J., Hudson, P. J., & Rohr, J. R. (2020). Consistent effects of pesticides on community structure and ecosystem function in freshwater systems. *Nature Communications*, *11*(1), 1–9. doi: 10.1038/s41467-020-20192-2

Rumschlag, S. L., & Rohr, J. R. (2018). The influence of pesticide use on amphibian chytrid fungal infections varies with host life stage across broad spatial scales. *Global Ecology and Biogeography*, *27*(11), 1277–1287. doi: 10.1111/geb.12784

Saumure, R. A., Rivera, R., Jaeger, J. R., O'Toole, T., Ambos, A., Guadelupe, K., … Marshall, Z. (2021). Leaping from extinction: Rewilding the relict leopard frog in Las Vegas, Nevada, USA. In P. S. Soorae (Ed.), *IUCN Global conservation translocation* perspectives: 2021* (pp. 76–81). Gland, Switzerland and Abu Dhabi, UAE: IUCN SSC Conservation Translocation Specialist Group, Environment Agency - Abu Dhabi and Calgary Zoo, Canada. Retrieved from www.iucn-ctsg.org

Schäfer, R. B., Pettigrove, V., Rose, G., Allinson, G., Wightwick, A., Von Der Ohe, P. C., … Kefford, B. J. (2011). Effects of pesticides monitored with three sampling methods in 24 sites on macroinvertebrates and microorganisms. *Environmental Science and Technology*, *45*(4), 1665–1672. doi: 10.1021/es103227q

Schär, C., Vidale, P. L., Lüthi, D., Frei, C., Häberli, C., Liniger, M. A., & Appenzeller, C. (2004). The role of increasing temperature variability in European summer heatwaves. *Nature*, *427*, 332–336. doi: 10.1038/nature02230.1.

Scheele, B. C., Pasmans, F., Skerratt, L. F., Berger, L., Martel, A., Beukema, W., … Flechas, S. V. (2019). Amphibian fungal panzootic causes catastrophic and ongoing loss of biodiversity. *Science*, *363*, 1459–1463.

Schiesari, L., Grillitsch, B., & Grillitsch, H. (2007). Biogeographic biases in research and their consequences for linking amphibian declines to pollution. *Conservation Biology*, *21*(2), 465–471. doi: 10.1111/j.1523-1739.2006.00616.x

Schmeller, D. S., Blooi, M., Martel, A., Garner, T. W. J., Fisher, M. C., Azemar, F., … Pasmans, F. (2014). Microscopic aquatic predators strongly affect infection dynamics of a globally emerged pathogen. *Current Biology*, *24*(2), 176–180. doi: 10.1016/j.cub.2013.11.032

Schwindt, A. R. (2015). Parental effects of endocrine disrupting compounds in aquatic wildlife: Is there evidence of transgenerational inheritance? *General and Comparative*

Endocrinology, *219*, 152–164. doi: 10.1016/j.ygcen.2015.01.020

Scott, D. E., Casey, E. D., Donovan, M. F., & Lynch, T. K. (2007). Amphibian lipid levels at metamorphosis correlate to post-metamorphic terrestrial survival. *Oecologia*, *153*(3), 521–532. doi: 10.1007/s00442-007-0755-6

Semlitsch, R. D., & Bodie, J. R. (2003). Biological criteria for buffer zones around wetlands and riparian habitats for amphibians and reptiles. *Conservation Biology*, *17*(5), 1219–1228.

Semlitsch, R. D., Bridges, C. M., & Welch, A. M. (2000). Genetic variation and a fitness tradeoff in the tolerance of gray treefrog (*Hyla versicolor*) tadpoles to the insecticide carbaryl. *Oecologia*, *125*(2), 179–185. doi: 10.1007/s004420000443

Shulse, C. D., Semlitsch, R. D., Trauth, K. M., & Williams, A. D. (2010). Influences of design and landscape placement parameters on amphibian abundance in constructed wetlands. *Wetlands*, *30*(5), 915–928. doi: 10.1007/s13157-010-0069-z

Shuman-Goodier, M. E., & Propper, C. R. (2016). A meta-analysis synthesizing the effects of pesticides on swim speed and activity of aquatic vertebrates. *Science of the Total Environment*, *565*, 758–766. doi: 10.1016/j.scitotenv.2016.04.205

Sievers, M., Hale, R., Parris, K. M., Melvin, S. D., Lanctôt, C. M., & Swearer, S. E. (2019). Contaminant-induced behavioural changes in amphibians: A meta-analysis. *Science of the Total Environment*, *693*, 133570. doi: 10.1016/j.scitotenv.2019.07.376

Sievers, M., Hale, R., Parris, K. M., & Swearer, S. E. (2018). Impacts of human-induced environmental change in wetlands on aquatic animals. *Biological Reviews*, *93*(1), 529–554. doi: 10.1111/brv.12358

Skagen, S. K., Melcher, C. P., & Haukos, D. A. (2008). Reducing sedimentation of depressional wetlands in agricultural landscapes. *Wetlands, 28*(3), 594–604. doi: 10.1672/07-84.1

Smalling, K., Orlando, J., Calhoun, D., Battaglin, W., & Kuivila, K. (2012). *Occurrence of pesticides in water and sediment collected from amphibian habitats located throughout the United States , 2009 – 2010*. U. S. Geological Survey Data Series 707, 40

Smith, R. K., & Sutherland, W. J. (2014). *Amphibian conservation: Global evidence for the effects of interventions*. Exeter: Pelagic Publishing.

Sparling, D. W., Bickham, J., Cowman, D., Fellers, G. M., Lacher, T., Matson, C. W., & McConnell, L. (2015). In situ effects of pesticides on amphibians in the Sierra Nevada. *Ecotoxicology, 24*(2), 262–278. doi: 10.1007/s10646-014-1375-7

Sparling, D. W., Linder, G., Bishop, C. A., & Krest, S. K. (2010). *Ecotoxicology of amphibians and reptiles* (2nd edition). Boca Raton: CRC Press.

Sunday, J. M., Bates, A. E., Kearney, M. R., Colwell, R. K., Dulvy, N. K., Longino, J. T., & Huey, R. B. (2014). Thermal-safety margins and the necessity of thermoregulatory behavior across latitude and elevation. *Proceedings of the National Academy of Sciences of the United States of America, 111*(15), 5610–5615. doi: 10.1073/pnas.1316145111

Suski, J. G., Swan, C. M., Salice, C. J., & Wahl, C. F. (2018). Effects of pond management on biodiversity patterns and community structure of zooplankton in urban environments. *Science of the Total Environment, 619–620*, 1441–1450. doi: 10.1016/j.scitotenv.2017.11.153

Tamburini, G., Bommarco, R., Wanger, T. C., Kremen, C., van der Heijden, M.

G. A., Liebman, M., & Hallin, S. (2020). Agricultural diversification promotes multiple ecosystem services without compromising yield. *Science Advances, 6*(45). doi: 10.1126/SCIADV.ABA1715

Tang, F. H. M., Lenzen, M., McBratney, A., & Maggi, F. (2021). Risk of pesticide pollution at the global scale. *Nature Geoscience, 14*(4), 206–210. doi: 10.1038/s41561-021-00712-5

Tennessen, J. B., Parks, S. E., Swierk, L., Reinert, L. K., Holden, W. M., Rollins-Smith, L. A., … Langkilde, T. (2018). Frogs adapt to physiologically costly anthropogenic noise. *Proceedings of the Royal Society B: Biological Sciences, 285*(1891), 1–9. doi: 10.1098/rspb.2018.2194

Thambirajah, A. A., Koide, E. M., Imbery, J. J., & Helbing, C. C. (2019). Contaminant and environmental influences on thyroid hormone action in amphibian metamorphosis. *Frontiers in Endocrinology*, *10*, 276. doi: 10.3389/fendo.2019.00276

Tilman, D. (1999). Global environmental impacts of agricultural expansion: the need for sustainable and efficient practices. *Proceedings of the National Academy of Sciences of the United States of America* 96, 5995-6000.

Trimble, M. J., & van Aarde, R. J. (2012). Geographical and taxonomic biases in research on biodiversity in human-modified landscapes. *Ecosphere, 3*(12), art119. doi: 10.1890/es12-00299.1

Trudeau, V. L., Thomson, P., Zhang, W. S., Reynaud, S., Navarro-Martin, L., & Langlois, V. S. (2020). Agrochemicals disrupt multiple endocrine axes in amphibians. *Molecular and Cellular Endocrinology*, *513*, 110861. doi: 10.1016/j.mce.2020.110861

van Dijk, H. F. G., & Guicherit, R. (1999). Atmospheric dispersion of current-use pesticides: a review of the evidence from monitoring studies. *Water, Air, and Soil Pollution*, *115*, 21–70.

Vandenberg, L. N., Colborn, T., Hayes, T. B., Heindel, J. J., Jacobs, D. R., Lee, D. H., … Myers, J. P. (2012). Hormones and endocrine-disrupting chemicals: Low-dose effects and nonmonotonic dose responses. *Endocrine Reviews*, *33*(3), 378–455. doi: 10.1210/er.2011-1050

Wake, D. B. (1991). Declining amphibian populations. *Science*, *253*(5022), 860. doi: 10.1126/science.253.5022.860

Walls, S. C., & Gabor, C. R. (2019). Integrating behavior and physiology into strategies for amphibian conservation. *Frontiers in Ecology and Evolution*, *7*, 234. doi: 10.3389/fevo.2019.00234

Wersebe, M., Blackwood, P., Guo, Y. T., Jaeger, J., May, D., Meindl, G., … Hua, J. (2019). The effects of different cold-temperature regimes on development, growth, and susceptibility to an abiotic and biotic stressor. *Ecology and Evolution*, *9*(6), 3355–3366. doi: 10.1002/ece3.4957

Willson, J. D., Hopkins, W. A., Bergeron, C. M., & Todd, B. D. (2012). Making leaps in amphibian ecotoxicology: translating individual-level effects of contaminants to population viability. *Ecological Applications*, *22*(6), 1791–1802.

Wise, R. S., Rumschlag, S. L., & Boone, M. D. (2014). Effects of amphibian chytrid fungus exposure on American toads in the presence of an insecticide. *Environmental Toxicology and Chemistry*, *33*(11), 2541–2544. doi: 10.1002/etc.2709

Wren, S., Angulo, A., Meredith, H., Kielgast, J., Dos Santos, L., & Bishop, P. (2015). *Amphibian conservation action plan*. IUCN SSC Amphibian Specialist Group. Retrieved from https://www.iucn-amphibians.org/resources/acap/

Zeitler, E. F., Cecala, K. K., & McGrath,

D. A. (2021). Carryover effects minimized the positive effects of treated wastewater on anuran development. *Journal of Environmental Management*, *289*(March), 112571. doi: 10.1016/j.jenvman.2021.112571

Zhang, W., Chen, L., Xu, Y., Deng, Y., Zhang, L., Qin, Y., … Diao, J. (2019). Amphibian (*Rana nigromaculata*)exposed to cyproconazole: Changes in growth index, behavioral endpoints, antioxidant biomarkers, thyroid and gonad development. *Aquatic Toxicology*, *208*, 62–70. doi: 10.1016/j.aquatox.2018.12.015

Zhou, R., Lu, G., Yan, Z., Jiang, R., Bao, X., & Lu, P. (2020). A review of the influences of microplastics on toxicity and transgenerational effects of pharmaceutical and personal care products in aquatic environment. *Science of the Total Environment*, *732*, 139222. doi: 10.1016/j.scitotenv.2020.139222

Zippel, K. C., & Mendelson III, J. R. (2008). The amphibian extinction crisis: A call to action. *Herpetological Review*, *39*, 23–29

Chapter 5. Habitat loss: protection and management

Nicolás Urbina-Cardona[1], Ariadne Angulo[2], Andrew Turner[3,4], Dan Cogălniceanu[5], Deepthi Wickramasinghe[6], Ireri Suazo-Ortuño[7], James Watling[8], Luz Dary Acevedo[9], Omar Hernandez-Ordoñez[10], Thomas Doherty-Bone[11], Jeanne Tarrant[12]

[1]Department of Ecology and Territory, School of Rural and Environmental Studies, Pontificia Universidad Javeriana, Bogotá – Colombia

[2]IUCN SSC Amphibian Specialist Group, 3701 Lake Shore Blvd W, P.O. Box 48586, Toronto, Ontario M8W 1P5, Canada

[3]CapeNature Biodiversity Capabilities Directorate, Cape Town, South Africa

[4]Department of Biodiversity and Conservation Biology, University of the Western Cape, Cape Town, South Africa

[5]Ovidius University Constanta, Faculty of Natural Sciences and Agricultural Sciences, Al. Universitatii 1, 900470 Constanta Romania

[6]University of Colombo, Sri Lanka

[7]Universidad Michoacana de San Nicolás de Hidalgo, México

[8]Department of Biology, John Carroll University, University Heights, OH, USA

[9]World Conservation Society, Colombia

[10]Departamento de Zoología, Universidad Nacional Autónoma de México

[11]Conservation Programs, Royal Zoological Society of Scotland, Edinburgh, United Kingdom

[12]Threatened Amphibian Programme, Endangered Wildlife Trust, South Africa

Abstract

The protection and management of habitat are the most critical conservation actions needed for at least 60% of amphibians, with habitat loss accounting for population declines and extinctions at local and regional levels. Habitat loss is directly related to pollution, but it also exacerbates other major threats to amphibians, such as disease, illegal trade, and invasive species. Habitat loss also reduces the ability of amphibian species to disperse and alter their distribution within their ecophysiological tolerance ranges in order to adapt to climate change. Currently, less than 30% of amphibian species are represented in the global protected-area system. The restricted geographic distribution, high habitat-specificity, and dependence on narrow climatic envelopes of many amphibian species mean that amphibians are particularly prone to local extinctions. Of the 37 amphibian species reported as extinct, 48.6% were distributed in South and Southeast Asia, and 21% in Mesoamerica. These species mainly inhabited inland wetlands and forests around the world. Considerable research into understanding the effects of habitat loss on amphibians has been undertaken over the past 15 years, including a review on the effectiveness of amphibian-targeted conservation interventions.

Habitat protection and management priorities must include the urgent preservation of remnant native forest habitats, given that over 85% of amphibian species occur in these systems. Conservation actions must also include the protection and rehabilitation of other aquatic and terrestrial breeding habitats critical for supporting viable amphibian populations. The creation of new habitats, including in urban and agricultural landscapes, must not be excluded from the toolkit of key interventions needed to avoid declines of more generalist species. Beyond implementing direct habitat protection mechanisms, it is essential to ensure targeted management of newly created protected areas and improve that of existing protected

areas, inclusive of amphibians. For these actions to be sustainable, it is critical to facilitate the participation, communication, and involvement of a broad range of stakeholders, including government entities, productive-extractive sectors, NGOs, academia, local communities, and civil society.

Introduction

Through their 350-million-year presence on Earth amphibians have come to inhabit all continents, and have adapted to thrive in a vast array of habitats. From montane grasslands to coastal wetlands, tropical forests, and savannahs, amphibians make up a large proportion of the biomass in most temperate and tropical ecosystems (Burton & Likens, 1975; Duellman, 1999, see also Chapter 1) and provide important ecosystem services (Hocking, Babbitt, & Hocking, 2014; Valencia-Aguilar, Cortés-Gómez, & Ruiz-Agudelo, 2013). Only 5% of the earth's surface remains unmodified by anthropogenic transformations (Kennedy, Oakleaf, Theobald, Baruch-Mordo, & Kiesecker, 2019); with natural ecosystems currently under severe pressure from human presence and activity, amphibians are the animal Class most negatively impacted by the current extinction crisis (Catenazzi, 2015; Houlahan, Findlay, Schmidt, Meyer, & Kuzmin, 2000; IUCN, 2021; Kiesecker, Blaustein, & Belden, 2001), experiencing extinction rates as much as 200 times that of the background rate (Roelants et al., 2007). Habitat loss is the primary driver of amphibian declines (Green, Lannoo, Lesbarrères, & Muths, 2020; Nori et al., 2015; Stuart et al., 2004). Loss, transformation, modification and degradation of habitat affect the highest proportion of assessed amphibians, followed by the threat of invasive species and disease (IUCN, 2021; see also Chapters 1 and 4). The world's forests harbour 85% of amphibian diversity (IUCN, 2021); yet half of these habitats have been lost (Crowther et al., 2015). At an even larger scale, half of the world's habitable land has been converted for agricultural use (Ritchie & Roser, 2019) and freshwater systems are particularly impacted (WWF, 2020). Only a third of the world’s longest rivers remain free-flowing (Grill et al., 2019), with those that are dammed flooding important amphibian habitat (Dare, Murray, Courcelles, Malt, & Palen, 2020; Dayrell, Magnusson, Bobrowiec, & Lima, 2021; Jenkins, Van Houtan, Pimm, & Sexton, 2015). Alarmingly, 87% of all wetlands have been lost globally since 1700 (Ramsar Convention on Wetlands, 2018), with the rate of wetland destruction is three times faster than that of rainforests (Pearce & Madgwick, 2020). In addition to habitat destruction, degradation of remaining wetlands involves stressors such as pollution, loss of connectivity, biological invasions and emerging diseases (Buck, Scheessele, Relyea, & Blaustein, 2012; Lehtinen, Galatowitsch, & Tester, 1999).

Underpinning this loss of habitat is unsustainable human population growth, resource use, and consumption (Foley et al., 2005). To address this, conservation efforts must include addressing societal needs across local, regional, national and global scales. Conserving habitats critical to amphibians must bridge the spheres of policy, human wellbeing, governance, and education (Tarrant, Kruger, & du Preez, 2016; Vergara-Ríos et al., 2021). Perhaps more than ever, there is a growing awareness of environmental issues and willingness by the public to demand governments and corporations to drive necessary changes (Li, Hou, Cao, Ding, & Yuan, 2022; Pawaskar, Raut, & Gardas, 2018; Varumo et al., 2020). Without fundamental changes, further biodiversity loss will be inevitable and environmental sustainability undermined (Mace et al., 2018). The amphibian conservation community must play an active role in driving behaviour change at all levels to reduce, halt and ultimately reverse amphibian species loss.

The ASG Habitat Protection & Management Working Group was established to consolidate the habitat-related themes covered in the 2007 ACAP, namely the 'Key Biodiversity Areas' and 'Freshwater Resources and Terrestrial Landscapes' chapters. In this iteration of the ACAP, we provide a synopsis of knowledge, achievements, and challenges to addressing the threat of habitat loss over the last 15 years and identify a clear set of priority targets and actions towards realising these targets in the next ten years.

Status update

Drivers of land-use change: Habitat loss and fragmentation

The growth of the human population in the past two hundred years has led to an unprecedented increase in the demand for natural resources (Ellis, 2015). To meet the food, fibre, water, energy, and shelter needs of almost 8 billion people - as of 2020 (Kaneda, Greenbaum, & Kline, 2020) - natural ecosystems have been transformed into farmlands, pastures, plantations, urban areas, and infrastructure networks (Foley et al., 2005; Sutherland et al., 2021) (Figure 5.1). Habitat conversion for food production is a major driver of biodiversity loss (Newbold et al., 2016; Tscharntke, Klein, Kruess, Steffan-Dewenter, & Thies, 2005) and climate change (Godfray et al., 2018; Poore & Nemecek, 2018), reducing species richness in amphibian communities (Dudley & Alexander, 2017; Gardner, Barlow, & Peres, 2007) and decreasing the spatial and temporal distribution of species (Collins & Fahrig, 2017; Oliveira, Silva, Bastos, & Morais, 2015). On the other hand, urbanization reduces the number of amphibian species that can survive and disperse in urban and suburban landscapes due to the alteration of key processes related to habitat availability and quality (Hamer & McDonnell, 2008). While multiple drivers modify natural systems including urbanisation, energy production, and mining, we focus here on food production as the primary driver. Specifically, livestock production is the largest anthropogenic land-use type, accounting for 75% of agricultural land (Machovina, Feeley, & Ripple, 2015; Steinfeld et al., 2006). Meat production is directly responsible for 89% of rainforest conversion in South America (De Sy et al., 2015) and impacts freshwater availability and quality (Albert et al., 2020; Aritola, Walworth, Musil, & Crimmins, 2019). By 2050, agriculture is estimated to occupy one billion hectares of land (roughly the size of China), and will be coupled with increased use of fertilisers and pesticides (Tilman et al., 2001). The agricultural expansion will continue to transform biodiverse ecosystems in South America and sub-Saharan Africa, where large tracts of land still have unexploited agricultural potential (Laurance, Sayer, & Cassman, 2014). Although some agricultural practices such as rice paddies generate wetlands, they do not provide high quality habitat for all amphibians in the region (Borzée, Heo, & Jang, 2018; Fujioka & Lane, 1997; Holzer, Bayers, Nguyen, & Lawler, 2017; Naito, Sakai, Natuhara, Morimoto, & Shibata, 2013). Additionally, climate change may affect regional seasonality and increase extreme weather events (Cochrane & Barber, 2009), which in turn could affect land occupation, use, and intensity patterns (Laurance et al., 2014) (Figure 5.1, also see Chapter 3).

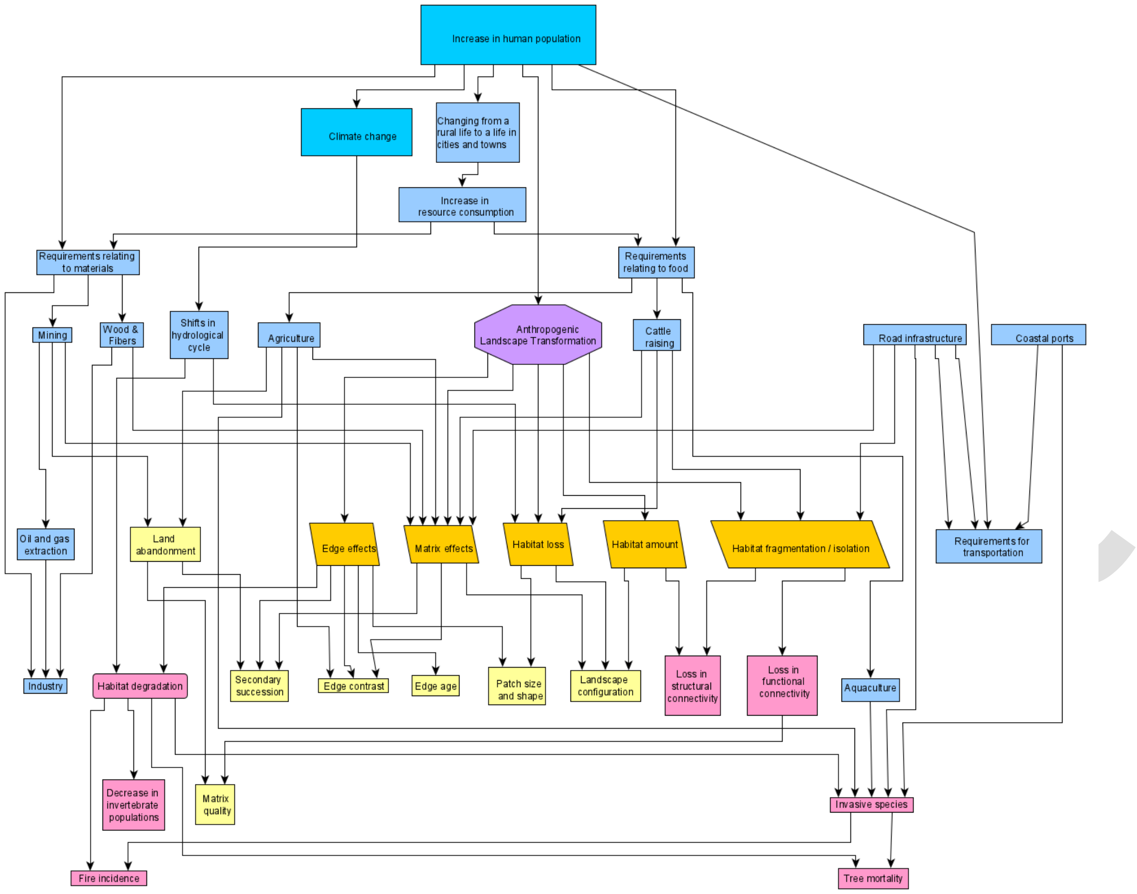


Figure 5.1. Causes and consequences of the anthropogenic transformation of the landscape. The causes are shown in blue; the main drivers of change are shown in orange; the consequences at landscape scale are shown in yellow; the ecological consequences for biodiversity are shown in pink.

A collateral driver of landscape transformation is the associated expansion of linear infrastructure, including road networks into previously inaccessible areas (Gallice, Larrea-Gallegos, & Vázquez-Rowe, 2019). Globally, the road network is expected to continue to expand, especially in megadiverse countries in Latin America and Africa (van der Ree, Jaeger, van der Grift, & Clevenger, 2011). Roads often decrease landscape connectivity (D’Amico, Périquet, Román, & Revilla, 2016) and increase animal-vehicle collisions with severe ecological, social, and economic consequences (Oddone Aquino & Nkomo, 2021).

Road infrastructure has both a direct impact on amphibians, and indirect impacts on biological processes (Andrews, Gibbons, Jochimsen, & Mitchell, 2008). Examples include habitat loss and increase in habitat damage and fragmentation, increase in edge effects, limited circulation of individuals, increasing genetic isolation of populations residing on each side of the road, higher mortality rate and consequent numerical impoverishment of the populations living on the side of the road, and increased human access to natural habitats (see Schmidt & Zumbach, 2008). Many amphibian species rely on different habitats for foraging, refuge, and reproduction, making landscape connectivity critical to the processes of dispersal and migration that maintain genetic and species diversity (Gilbert-Norton, Wilson, Stevens, & Beard, 2010; Resasco, 2019).

Another insidious form of habitat degradation that is often exacerbated by transportation networks is the introduction, intentional or accidental, of invasive alien species (Bucciarelli, Blaustein, Garcia, & Kats, 2014; Kats & Ferrer, 2003; Nunes et al., 2019). Introduction of invasive alien species to a habitat can threaten native amphibians through direct effects such as predation (Bosch, Rincón, Boyero, & Martínez-Solano, 2006; Ficetola et al., 2011; Maerz, Blossey, & Nuzzo, 2005; Martín-Torrijos et al., 2016; Vannini et al., 2018) and indirect effects such as altered water quality (Cotten, Kwiatkowski, Saenz, & Collyer, 2012; Maerz, Brown, Chapin, & Blossey, 2005; Pinero-Rodríguez, Fernández-Zamudio, Arribas, Gomez-Mestre, & Díaz-Paniagua, 2021), water availability (Cordero-Rivera, Velo-Antón, & Galán, 2007), and fire dynamics (Measey, 2011; van Wilgen, 2009). Likewise, some invasive and highly traded species such as the bullfrog *Lithobates catesbeianus* are vectors of emerging diseases such as ranavirus and chytrid fungus (Schloegel et al., 2009). Managing habitats and the invasion pathways that lead to them helps control existing invasions and minimise the risk of new invasions, and are thus essential for safeguarding amphibian populations (Falaschi, Melotto, Manenti, & Ficetola, 2020). Furthermore, it is critical to maintain continuity of invasive alien species control operations, particularly steady and reliable funding, to achieve success (Davies et al., 2020).

Effects of landscape transformation on amphibians

Landscape transformation resulting from habitat loss and fragmentation has led, directly and indirectly, to the decline of amphibian populations globally (Cushman, 2006; Gardner, Ribeiro-Júnior, et al., 2007; Hamer & McDonnell, 2008; Sutherland et al., 2021;Urbina-Cardona, 2008). The loss of natural areas limits habitat for species not able to adapt to anthropogenic landscapes (Ribeiro, Colli, & Soares, 2019) and leads to the homogenisation of biotic communities (Echeverría-Londoño et al., 2016; Ernst, Linsenmair, & Rödel, 2006). Generalist species can inhabit modified environments, depending on their habitat requirements, movement capacity, and reproductive mode (Crump, 2015; Dale, Pearson, Offerman, & O'Neill, 1994; Dixo & Metzger, 2010) (Figure 5.2). However, for many species, high habitat specificity and endemicity preclude them from surviving in altered habitats (Roach, Urbina-Cardona, & Lacher, 2020; Santos-Barrera & Urbina-Cardona, 2011). Most amphibian species occupy forest habitats (~85%), followed by wetlands (~ 66%), artificial terrestrial environments (~26%), grasslands (~17%), and to a lesser extent other habitat types (IUCN, 2021; numbers do not add up to 100% because a species may occupy more than one habitat) (Figure 5.3).

Figure 5.2. Effects of habitat transformation on amphibian species. Changes at the landscape level are shown in orange; aspects intrinsic to species are shown in green, and specific functional traits of amphibians are shown in red.

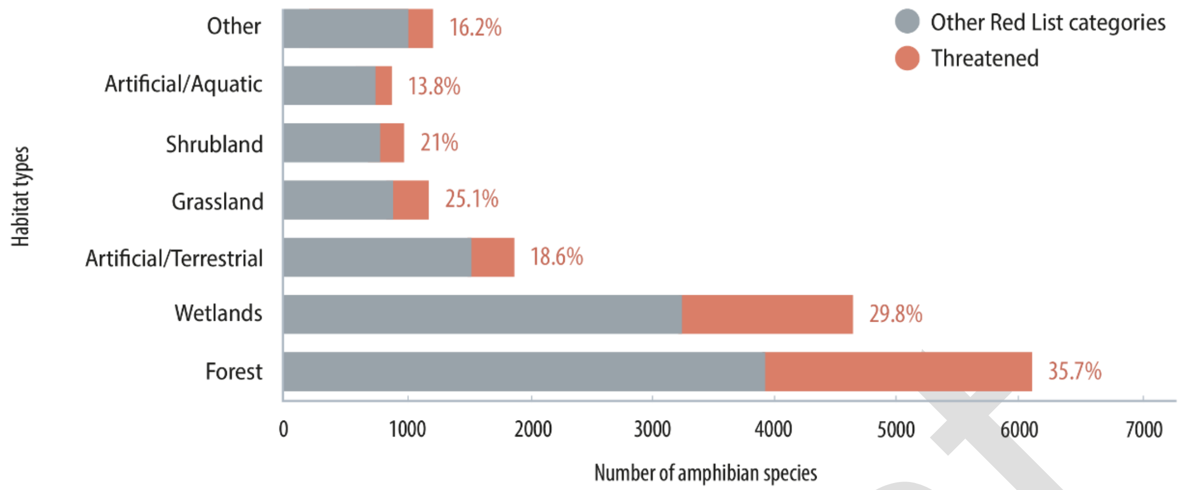


Figure 5.3. The top six habitat types for amphibians as reported on The IUCN Red List of Threatened Species (IUCN, 2021). The habitats are arranged according to the number of amphibian species occupying the habitat. The "Other" category in this figure includes marine intertidal, coastal, neritic, and supratidal, as well as introduced vegetation, savanna, desert, rocky areas, caves, and subterranean habitats. The percentage of threatened species that occupies each habitat is reported at the front of the bar; it should be noted that the total percentage does not correspond to 100% as a species may occupy more than one habitat.

Generalist species tend to have a wide geographic distribution in which they occur in a wide diversity of habitats with high abundance (Rabinowitz, Cairns, & Dillon, 1986). Many generalist species can adapt to modified habitats, so habitat management actions must address the creation and enhancement of such environments. Such actions can also encourage public involvement, for example, the creation of ponds, ditches, and rice fields (Hartel, Scheele, Rozylowicz, Horcea-Milcu, & Cogălniceanu, 2020; Magnus & Rannap, 2019; Mendenhall et al., 2014). This has the added advantage of giving people access to nature, instilling empathy and an appreciation of conservation efforts that can be leveraged to promote more effective policy (Balázsi, Riechers, Hartel, Leventon, & Fischer, 2019; Oscarson & Calhoun, 2007). In contrast, rare amphibian species tend to present a higher degree of threat given their high level of habitat specificity (Toledo, Becker, Haddad, & Zamudio, 2014). Creation and rehabilitation of habitats for specialist or threatened species is also being increasingly explored and being found to be effective (Fog, 1997; Ruhí et al., 2012; Valdez et al., 2019).

Forests contain diverse microhabitats that are used for shelter, foraging, and reproduction (Bowen, McAlpine, House, & Smith, 2007; Rios-López & Aide, 2007; Wells, 2007), making them home to more species of amphibians than any other habitat. Most rare species are particularly abundant in forest interiors (Schneider-Maunoury et al., 2016), where heterogeneous environments have greater stability in temperature and relative humidity (Brüning et al., 2018; Soto-Sandoval, Suazo-Ortuño, Urbina-Cardona, Marroquín-Páramo, & Alvarado-Díaz, 2017). Management and protection of primary forest cores are thus a priority for amphibian conservation (Pfeifer et al., 2017). Environmental changes affect the physiological and biological processes of amphibians, so their occurrence depends on factors such as temperature and humidity (McDiarmid & Altig, 1999). Life-history traits and habitat preferences can predict a species' ability to tolerate environmental change (Álvarez-Grzybowska, Urbina-Cardona, Córdova-Tapia, & García, 2020; Cortés-Gómez, Ramirez, & Urbina-Cardona, 2015) (Figure 5.2). For example, small-bodied species often avoid forest edges and the anthropogenic matrix where increased wind, light, heat (Pfeifer et al., 2017; Watling & Braga, 2015), and reduced canopy cover, leaf-litter and refugia (Demaynadier & Hunter, 1998) cause individuals to rapidly dehydrate (Figure 5.2). In contrast, large-bodied species with high dispersal capacity and aquatic larvae tend to inhabit pastures and food production systems (de Melo, Gonçalves-Souza, Garey, & de Cerqueira, 2017; Haddad et al., 2015; Mendenhall et al., 2014; Pineda, Moreno, Escobar, & Halffter, 2005; Queiroz, da Silva, & Rossa-Feres, 2015; Trimble & van Aarde, 2014; Vasconcelos, Santos, Rossa-Feres, &

Haddad, 2009). However, temporary water bodies created in pastures by anthropogenic activities (e.g. cattle or tractor tracks) harbour less than 15% of larval anuran species than natural temporary ponds (Camacho-Rozo & Urbina-Cardona, 2021).

Edge effects and habitat degradation

The effects of habitat loss and fragmentation often worsen due to edge effects (Fahrig et al., 2019; Fletcher et al., 2018). The edge effect is defined as the interaction that occurs between adjacent natural and anthropogenic vegetation covers creating an ecotone (Murcia, 1995). Globally, 70% of forest is less than 1km from an edge, so understanding edge effects is crucial for assessing the impact on biotic communities after deforestation (Alignier & Deconchat, 2011; Broadbent et al., 2008; Haddad et al., 2015). The diversity and structure of amphibian assemblages inhabiting forest fragments may be influenced by distance to disturbed areas (Pearman, 1997; Suazo-Ortuño, Alvarado-Díaz, & Martínez-Ramos, 2008). In the Neotropics, most amphibian species are sensitive to edge effects, even at distances of 400m, due to their responses to microclimatic changes in temperature, wind, and relative humidity (Schneider-Maunoury et al., 2016). Species most vulnerable to habitat loss and fragmentation are those inhabiting forest cores since they depend on high-quality habitat, and mostly avoid edges and the anthropogenic matrix (Lehtinen, Ramanamanjato, & Raveloarison, 2003; Urbina-Cardona, Olivares-Pérez, & Reynoso, 2006). Consequently, species adapted to mature forest interiors may disappear from small and irregularly shaped remaining patches in the absence of suitable breeding sites (Cabrera-Guzmán & Reynoso, 2012; Riemann, Ndriantsoa, Raminosoa, Rödel, & Glos, 2015; Tocher, Gascon, & Zimmerman, 1997) or structural connectivity (Gillespie et al., 2015). In West Africa, degradation on vegetation structure had a stronger deleterious effect on forest amphibian species richness than habitat fragmentation (Hillers, Veith, & Rödel, 2008). Likewise, it is important to consider that in highly fragmented landscapes, each forest patch may have a unique biotic community, so the loss of a single small fragment could lead to a regional loss of species (Fahrig, 2017; Fletcher et al., 2018). Linear remnants of native vegetation also constitute dispersal corridors for some amphibian species (De Lima & Gascon, 1999; Hansen, Scheele, Driscoll, & Lindenmayer, 2019).

Matrix effects and substitutable resources at a landscape level

In transformed landscapes, the dynamics between natural patches and other landscape elements are highly influenced by the anthropogenic matrix (Dixo & Metzger, 2010; Ferrante et al., 2017; Van Buskirk, 2012; Watling, Nowakowski, Donnelly, & Orrock, 2011). Matrix effects on population abundance and survival are associated with resource availability, the abiotic environment and the dispersal capacity of the study species (Driscoll, Banks, Barton, Lindenmayer, & Smith, 2013). In areas with intense agricultural practices (monocultures, burning, slashing, and logging, low temporal rotation, high use of pesticides-herbicides and soil mismanagement; Ellis, 2015; Kremen, Williams, & Thorp, 2002), amphibian assemblages show low species richness and high abundance of generalist species (Cáceres-Andrade & Urbina-Cardona, 2009; Gascon et al., 1999; Vasconcelos et al., 2009). In contrast, small-scale rural and family agricultural practices, with agro-ecological, multifunctional, or sustainable approaches, promote greater permeability of the matrix (Brüning et al., 2018). Permeable landscapes reduce the negative consequences of fragmentation (Foley et al., 2005; Oteros-Rozas, Ruiz-Almeida, Aguado, González, & Rivera-Ferre, 2019; Perfecto & Vandermeer, 2010) and facilitate the dispersal of amphibian species (Kehoe et al., 2015; Perfecto & Vandermeer, 2008, 2010), although this depends on the landscape elements that are used by species (Tarrant & Armstrong, 2013; Van Buskirk, 2012). Likewise, land cover type, structural complexity and the size of the matrix surrounding remaining natural patches play an important role in retaining connectivity and species richness (Cline & Hunter, 2016; Phillips, Halley, Urbina-Cardona, & Purvis, 2018; Watling et al., 2011).

In some tropical ecosystems, matrix effects may impact amphibians more than edge effects (De Lima & Gascon, 1999; Isaacs Cubides & Urbina Cardona, 2011; Mendenhall et al., 2014). For example, an intensively managed matrix with sparse, homogeneous vegetation such as a cornfield may increase edge effects on amphibian populations up to 150m into the forest (Santos-Barrera & Urbina-Cardona, 2011). In contrast, crops with a complex structure that maintain elements of the original native vegetation (e.g., shaded coffee or cocoa plantations) can buffer edge effects in native habitat by increasing amphibian species richness in the ecotone (Mendenhall et al., 2014; Rice & Greenberg, 2000; Roach et al., 2020; Santos-Barrera & Urbina-Cardona, 2011). These kinds of agroforestry systems could harbour an important percentage of amphibian species in montane cloud forests and tropical rainforests (Murrieta-Galindo, González-Romero, López-Barrera, & Parra-Olea, 2013; Murrieta-Galindo, López-Barrera, González-Romero, & Parra-Olea, 2013; Pineda & Halffter, 2004). Due to its use for biofuel, oil palm monocultures (of exotic invasive species *Elaeis guineensis*) have increased globally (Danielsen et al., 2009), reducing the richness of amphibian assemblages when compared to surrounding native forests (Faruk, Belabut, Ahmad, Knell, & Garner, 2013; Gallmetzer & Schulze, 2015; Gilroy et al., 2015; Konopik, Steffan-Dewenter, & Grafe, 2015; Scriven, Gillespie, Laimun, & Goossens, 2018). We recommend that the effects of forest edges and anthropogenic matrices be incorporated into systematic conservation planning protocols to identify corridors that may allow animal movement in response to global change (Baldwin, Calhoun, & deMaynadier, 2006; Muths et al., 2017; Nori et al., 2015; Pence, 2017).

In the larval or juvenile stage, amphibians are more vulnerable to dehydration, predation, and the effect of contaminants (Crump, 2015; also see Chapter 4). Anthropogenic systems thus affect the quality and quantity of habitat found at the edges of remaining fragments (Didham, Kapos, & Ewers, 2012; Harper et al., 2005; Murcia, 1995; Saunders, Hobbs, & Margules, 1991). It is important to consider that species use different habitats that allow them to maintain populations over time, and habitats within the matrix could be relevant to different life stages and activities of species (Pope, Fahrig, & Merriam, 2000; Van Buskirk, 2012). For example, some native forest-dwelling amphibian species may pass through anthropogenic matrices or use them for reproduction (Gascon et al., 1999). Neckel-Oliveira & Gascon (2006) found that the Tarsier tree frog (*Phyllomedusa tarsius*) was more abundant in the anthropogenic matrix due to the presence of large and permanent ponds, but also reported low reproductive success and survival of eggs and embryos due to predation and desiccation. In contrast, Van Dyke et al. (2017) found that amphibian species richness was positively linked to clustered pools in forests compared to isolated ones. Thorough knowledge of the life history, behaviour, and dispersal of target amphibian species is key to ecological restoration and species reintroductions (Tarrant & Armstrong, 2013; also see Chapter 14).

Heterogeneity in vegetation structure has a strong impact on amphibian assemblages (Cortés-Gómez, Castro-Herrera, & Urbina-Cardona, 2013; Gardner, Ribeiro-Júnior, et al., 2007) across spatial scales from microhabitats to landscape level (Duarte-Ballesteros, Urbina-Cardona, & Saboyá-Acosta, 2021). For instance, matrices with high structural complexity can reduce temperature extremes (Scheffers, Edwards, Diesmos, Williams, & Evans, 2014) and buffer edge effects on forest fragments (e.g., coffee plantations; Santos-Barrera & Urbina-Cardona, 2011). In heterogeneous agricultural landscapes, vegetation buffers environmental extremes by reducing exposure of amphibians to unfavourable conditions such as dehydration and elevated temperatures (Farallo & Miles, 2016; Watling & Braga, 2015; Whitfield & Pierce, 2005). The rate of temperature increase may be 60% lower in microhabitats located in forested areas compared to more exposed microhabitats (Scheffers et al., 2013, 2014). It is therefore important to maintain heterogeneity in vegetation cover and aquatic resources within the matrix, and to promote environmentally friendly management practices (e.g., low use of agrochemicals, fire management, maintenance of hedgerows and native vegetation, control of invasive species, and maintenance of leaf litter on the ground) (Arroyo-Rodríguez et al., 2020; Melo, Arroyo-Rodríguez, Fahrig, Martínez-Ramos, & Tabarelli, 2013; Urbina-Cardona, Bernal, Giraldo-Echeverry, & Echeverry-Alcendra, 2015; Zabala-Forero & Urbina-Cardona, 2021).

Colonisation and persistence of amphibian diversity in secondary forest

Secondary forests are forests regenerating largely through natural processes after significant human and/or natural disturbance of the original forest vegetation (floristic composition and structure have been modified) at a single point in time or over an extended period (Brown & Lugo, 1990; Chokkalingam & De Jong, 2001). Anthropic secondary forests can be classified based on the original type of disturbance: i) abandoned open areas with intense agricultural practices (monocultures); ii) burned forests; iii) abandoned selective logging sites; and iv) agroforestry. Those forests have become a frequent or even dominant vegetation type in human-modified landscapes (Arroyo-Rodríguez et al., 2017) and there is a continuous increase in this type of forest, mainly in tropical regions (Hansen et al., 2019). Despite increasing agricultural intensification globally, about 1.47 million km^2 of agricultural systems have been abandoned due to loss of soil productivity or socioeconomic and political factors (Bowen et al., 2007; Guariguata & Ostertag, 2001). Secondary forests are important biodiversity repositories and may provide complementary and supplementary resources to fauna (Arroyo-Rodríguez et al. 2017), and the abandonment and recovery through time of biodiversity can allow other species to colonise these forests (Laurance et al., 2011).

Secondary succession pathways depend on multiple factors and processes at different scales, driving direct or indirect changes at different levels:

- On previous land use and landscape composition (e.g., type, duration, intensity, and frequency of disturbance regime; Chazdon, 2003; Thompson & Donnelly, 2018; Walker, Wardle, Bardgett, & Clarkson, 2010).
- Landscape configuration (e.g., proximity to remaining forest patches and anthropogenic matrix structure; Brüning et al., 2018; Laurance et al., 2002; Tscharntke et al., 2012) and composition (Tscharntke et al., 2012).
- Patch characteristics (e.g. soil properties, size, shape, isolation, and microclimate; Chazdon, 2003; Guariguata & Ostertag, 2001).

With increasing time since agricultural abandonment and structural complexity of vegetation, some amphibian assemblages can increase their richness and number of individuals (Acevedo-Charry & Aide, 2019; Thompson & Donnelly, 2018). There is mainly an increase in the abundance of generalist forest species, given the colonisation of species from the matrix (Bowen et al., 2007). However, changes in the structure and composition of assemblages in secondary forests are dynamic given the increase in abundance of generalist forest species, colonisation of species from the matrix, and the possible arrival of specialists from the mature forest (Acevedo-Charry & Aide, 2019; Bowen et al., 2007). Vegetation succession interacts with species traits (e.g., tolerance to extremes in temperature and relative humidity, diet specialisation, preference for oviposition sites and breeding seasons; Gottsberger & Gruber, 2004; Suazo-Ortuño et al., 2018; Thompson & Donnelly, 2018) and natural disturbance regimes (e.g., hurricanes: Marroquín-Páramo, Suazo-Ortuño, Urbina-Cardona, & Benítez-Malvido, 2021; fires: Dunn, 2004; Mora et al., 2015), making the recovery process complex at the landscape, community, and population levels (Russildi, Arroyo-Rodríguez, Hernández-Ordóñez, Pineda, & Reynoso, 2016; Walker et al., 2010). For example, a study found that the increase in frequency and intensity of hurricanes created a homogenisation of amphibian assemblages inhabiting tropical dry mature forests, but amphibian assemblages inhabiting pastures were highly resilient to change (Marroquín-Páramo et al., 2021).

There is a trend towards increasing functional diversity (Ernst et al., 2006; Hernández-Ordóñez et al., 2019) and amphibian species richness in mature forests (Basham et al., 2016; Pawar, Rawat, & Choudhury, 2004) in late-successional stages (Herrera-Montes & Brokaw, 2010; Hilje & Aide, 2012) and in the interior of native forest fragments (Zabala-Forero & Urbina-Cardona, 2021). This is because small changes in plant structure, the number of available microhabitats, and the presence of water bodies generate drastic changes in species composition in forests with different successional stages (Cortés-Gómez et al., 2013; Hernández-Ordóñez, Urbina-Cardona, & Martínez-Ramos, 2015; Magnus & Rannap, 2019; Urbina-Cardona & Londoño-M, 2003). Once food-production systems were abandoned and rainforest regeneration began, amphibian species richness was the first parameter to recover (after 23 years), followed by species density (28 years for amphibians; Hernández-Ordóñez et al., 2015). In contrast, other parameters such as species composition are estimated to take between 80 and 150 years to recover (Bowen et al., 2007; Thompson & Donnelly, 2018). Management of secondary forests is thus crucial for biodiversity conservation because of their role in maintaining connectivity between older forest patches, facilitating dispersal of species with low matrix tolerance, as well as the mitigation of edge effects in remaining forest fragments (Goldspiel, Cohen, McGee, & Gibbs, 2019; Suazo-Ortuño et al., 2015; Thompson & Donnelly, 2018).

Amphibian representation in the protected area system

The IUCN defines protected areas (PAs) as “a clearly defined geographical space, recognised, dedicated and managed, through legal or other effective means, to achieve the long-term conservation of nature with associated ecosystem services and cultural values”. PAs are a fundamental cornerstone in the conservation of biodiversity, including amphibians (Le Saout et al., 2013; Venter et al., 2014). The Convention on Biological Diversity (CBD) Strategic Plan for Biodiversity 2011-2020 included the Aichi Biodiversity Targets and set five strategic goals and 20 targets to be achieved by 2020. As of October 2020, however, many of these had not been met (Convention on Biological Diversity, 2020). Strategic Goal C focused on improving the status of biodiversity by safeguarding ecosystems, species, and genetic diversity under Target 11, which sought to protect at least 17% of terrestrial and freshwater, and 10% of marine environments by 2020. Maintaining and improving habitats for amphibians and broader biodiversity addresses Goal 15 (Life on Land) of the United Nations Sustainable Development Goals (SDGs). By 2015, it was clear that while existing terrestrial PA proportions were relatively close to the proposed targets (14.6% of terrestrial and 2.8% of marine environments), >59% of ecoregions, >77% of important sites for biodiversity, and 57% of 25,380 species were not well represented in the PA network (Butchart et al., 2015). Within the existing PA system, 137 sites represent high irreplaceability for the conservation of amphibians, birds, and mammals, with the potential to conserve 385 amphibian species of which 179 species are threatened (Le Saout et al., 2013). Recently, Button and Borzée (2021) identified the geographic priorities for amphibian habitat protection globally.

The global PA network is fragile because many PAs do not guarantee the persistence of representative species and ecosystem processes (Kukkala & Moilanen, 2013; Margules & Sarkar, 2007). Globally, 25% of amphibian species have distributions totally outside PAs, and 18% have less than 5% of their distribution represented in Pas (Butchart et al., 2015; Nori et al., 2015). Regionally, for example, only 32% of the range of South Africa's threatened amphibians occurs within PAs (Skowno et al., 2019). We need to ensure that priority amphibian habitats are included within formally declared PAs as well as other types of conservation areas, and that management of these is improved with amphibians and their habitats as conservation targets (Nori et al., 2015). Historically, amphibians have often not been prioritised in conservation planning, both in establishing PAs and in the development of management plans (Rodrigues, Akçakaya, et al., 2004; Rodrigues, Andelman, et al., 2004; Urbina-Cardona & Loyola, 2008; Venter et al., 2014). For amphibians with restricted geographic distribution, it is necessary to protect all remaining habitats, as these are often irreplaceable (*sensu* Ochoa-Ochoa, Bezaury-Creel, Vázquez, & Flores-Villela, 2011; Ochoa-Ochoa, Urbina-Cardona & Flores-Villela, 2011). For example, South Asia is rich in amphibian species richness and endemism, representing four amphibian hotspots - Eastern Himalayas, Indo-Burma, Western Ghats, and Sri Lanka - that are underrepresented in Pas (Pratihar et al., 2014). Asia and Latin America are the regions that harbour the greatest number of species worldwide without any representation in the PA system (115 gap species; Nori et al., 2015). Yet, the declaration and establishment of Important Amphibian Areas (IAAs) and related regulations are lagging (Rowley et al., 2010).

However, amphibians are increasingly being recognised in PA planning (Ford et al., 2020). For example, the WWF Oasis network of Italy was specifically assessed for contributions to amphibian conservation (Bombi et al., 2012). Various NGOs have been actively working to facilitate the creation of PAs specifically to protect amphibians (Moore, 2011; Smith, Meredith, & Sutherland, 2019; see also Table 5.1). Although private and community-managed PAs are usually small in area, they play an important role in amphibian conservation. For example, in Mexico, 73% of endemic species are represented in private reserves (Ochoa-Ochoa, Urbina-Cardona, Vázquez, Flores-Villela, & Bezaury-Creel, 2009). However, achieving representation of amphibian species in a single PA is insufficient, because it can lead to small, isolated subpopulations. Rather, it is critical to ensure that species' core distributions are within PAs (Urbina-Cardona & Loyola, 2008). Some of the regions with the greatest amphibian species richness, including the tropical Andes in Peru, Ecuador and Colombia, southern Mexico, eastern Brazil, Papua New Guinea, and Indonesia, parts of Madagascar, Cameroon, and southwest India, are also areas with the highest rates of deforestation and least representation within the PA system (Nori et al., 2015); this underscores their great importance as priority areas for conservation (Button & Borzée, 2021). Thus, it is crucial to have clear spatial priorities that enable coordinated local planning of conservation area networks involving both government PAs and private initiatives (Ochoa-Ochoa et al., 2009).

Table 5.1. Examples of different types of protected areas established to protect amphibian species.

Site name	Date established	Target amphibian species	Site size (ha)	Country	Significance	Type of protection
Jorepokhri Wildlife Sancturary	1985	*Tylototriton himalayanus*	4	India	It has a small breeding population of the Himalayan newt. It is in danger because of the constructions made in the sanctuary.	Strict Protection, West Bengal State Forest Department
Natural Reserve “Monticchie”	1985	*Rana latastei*	230	Italy	One of the remaining large populations of this Italian endemic Ranidae	Special Area of Conservation – Europe Natura2000 site code IT2090001
“Paludi di Arsago” Area of Herpetological	1995	*Pelobates fuscus insubricus*	543	Italy	Last remaining large population of this very rare Italian Pelobatidae	Special Area of Conservation – Europe Natura2000

National Relevance						site code IT2010011
Guayacán Rainforest Reserve	2003	*Agalychnis lemur*	49	Costa Rica	Reserve is home to one of two known metapolulations of *A. lemur*, and has more species of amphibians (70+) than any other site in Costa Rica (https://cramphibian.com/guayacan-rainforest-reserve/)	Private Reserve
Ranita Dorada Reserve	2008	11 species	120	Colombia	Formerly an AZE site, trigger species *Andinobates dorisswansonae* and *A. tolimensis* now improved in status causing the site to be de-listed	Private Reserve

Ranita Terribilis Reserve	2012	*Phyllobates terribilis*	66.4	Colombia	KBA site. In 2020 the Eperãra Siaapidarã people incorporated their K´õk´õi Eujã Natural Reserve into the National Protected Area System, expanding the species' protection to 11,641 ha	Private Reserve
Sierra Caral Reserve	2012	10 threatened species; 7 endemic species	1901	Guatemala	The new reserve stimulated the declaration of the Sierra Caral National Protected Area in 2014	Private Reserve followed by National Protected Area
Yal Unin Yul Witz Reserve	2015	11 species	845	Guatemala	Within the larger Cuchumatanes KBA/AZE	Private Reserve
Elandsberg Nature Reserve	In progress	*Vandjikophrynus amatolicus*	4783	South Africa	First PA for this Critically Endangered species	Biodiversity Stewardship site

						(landowner agreements)
Sobonakhona Protected Environment Reserve	In progress	*Hyperolius pickersgilli Natalobatrachus bonebergi*	535	South Africa	First PA within a Traditional Authority area to be declared in the country with an amphibian as a target species	Biodiversity Stewardship site (landowner agreement)
Mount David Nature Reserve	In progress	*Capensibufo selenophos*	821	South Africa	Also the only remaining population of *Erica jasminiflora* occurs on the property	Biodiversity Stewardship site (landowner agreement)
Gingingdlovu Protected Environment Reserve	In progress	*Hyperolius pickersgilli*	125	South Africa	Linking coastal wetland across three private properties	Biodiversity Stewardship site (landowner agreement)
Hampton Nature Reserve	1998	*Triturus cristatus*	145.8	United Kingdom	Largest population of Great Crested Newt in Europe	Special Area of Conservation -

						Europe Natura 2000 UK0030053; Site of Special Scientific Interest (UK); owned by private company managed by conservation NGO (Froglife).
Hyla Park Nature Preserve	1995	*Hyla versicolor*	8	Canada	Protecting most northeasterly population of *Hyla versicolor*	Public land leased by conservation organisation

Site prioritisation and management effectiveness

The creation and designation of PAs does not, by itself, ensure adequate species protection. Disturbance, hunting, and forest-product exploitation threaten the integrity of reserves worldwide (Laurance et al., 2012; Pouzols et al., 2014). The effectiveness of PAs to resist anthropogenic pressures is influenced by multiple factors including a country's socio-economic and governance conditions (Barnes et al., 2016; Schleicher, Peres, Amano, Llactayo, & Leader-Williams, 2017). PAs are not just under the management jurisdiction of governments, but also local communities, private enterprises, and NGOs, as well as co-management between partners (Dudley, 2008; Roach et al., 2020). Examples of differing management structures include state protection, landowner agreements that provide formal protection of important biodiverse areas in the long term (Barendse, Roux, Currie, Wilson, & Fabricius, 2016), conservation agreements with local community zoning for land and resource use (e.g., areas for timber extraction), and indigenous conservation areas (Aguilar-López et al., 2020; Berkes, 2009; Ochoa-Ochoa et al., 2009). It is essential to align the objectives and goals of the PAs with the visions of the people living around them to ensure that human pressure is not increased due to cropland conversion and instead allows for increases in human development indices (Geldmann, Manica, Burgess, Coad, & Balmford, 2019; Laurance et al., 2012). Community-based conservation initiatives (Meine, Soulé, & Noss, 2006) allow for the integrated management of transformed landscapes that support biodiversity conservation (Arroyo-Rodríguez et al., 2020; Garibaldi et al., 2021; Melo et al., 2013; Palomo et al., 2014). Megadiverse countries often have a low socioeconomic status (i.e. those with the highest amphibian species richness are highly impacted by human activities; Nori et al., 2015). Effective habitat protection in these developing nations must therefore be supported by adequate management actions (Smith & Sutherland, 2014) and integrated with development activities that improves the socio-economic well-being of the local communities', who are often directly dependent on nature for their resources, in order to increase their resilience to future challenges and reduce negative environmental impacts (Adger, 2000; Bennett, Radford, & Haslem, 2006; Perfecto & Vandermeer, 2008, 2010).

An understanding of critical sites for the survival of amphibian species is essential, but the functional traits and degree of endemism of species should also be considered in PA designation (Cortés-Gomez, Ruiz-Agudelo, Valencia-Aguilar, & Ladle, 2015; Loyola et al., 2008; Menéndez-Guerrero, Davies, & Green, 2020; Tsianou & Kallimanis, 2016). It is essential to understand the distribution of amphibian species within each PA to inform management plans (Nori et al., 2015) and monitor not only their presence, but other aspects such as biomass, body condition, demography, trophic structure, and functional diversity (Álvarez-Grzybowska et al., 2020; Riemann, Ndriantsoa, Rödel, & Glos, 2017; Trimble & van Aarde, 2014; Urbina-Cardona et al., 2015). To fulfil these tasks, PA management requires strengthening through improving facilities, ranger training, reinforcing compliance, and supporting research. For PAs associated with low socio-economic communities, improving general land-use practices as well as including development activities to reduce the negative environmental impacts of nature-dependent local communities is critical.

Given their often-limited distributions and habitat specificity, amphibian protection needs to be more species-focused and allow for the creation of smaller PAs that might otherwise be lost. Several approaches allow for this: Key Biodiversity Areas (KBAs) are sites that contribute significantly to the global persistence of biodiversity and provide a standardised approach to identifying sites of particular importance for biodiversity under Aichi Target 11 and its successor(s) in the post-2020 global biodiversity framework (Smith, Bennun, et al., 2019). Sites qualify as global KBAs if they meet one or more of 11 criteria in "A Global

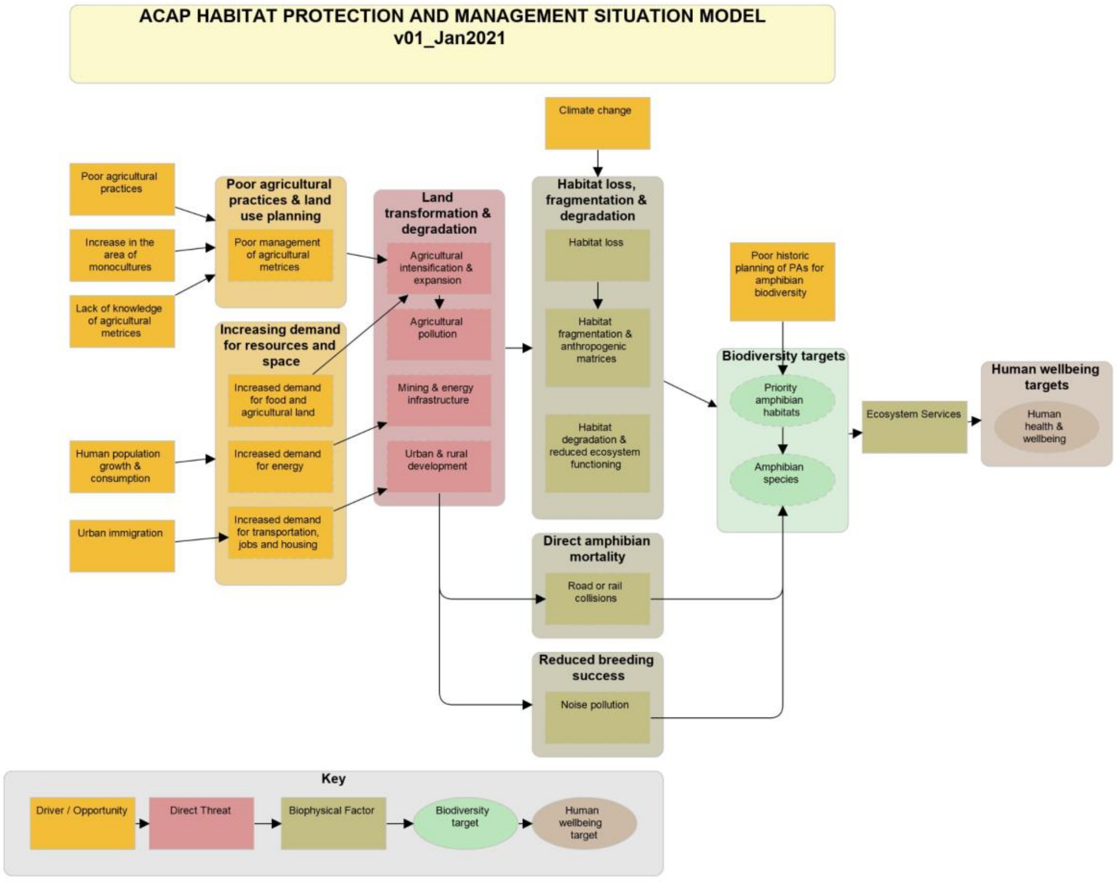
Standard for the Identification of Key Biodiversity Areas" (IUCN, 2016), which harmonises existing approaches to the identification of important sites for biodiversity and has received considerable support from the conservation community. The Key Biodiversity Area Partnership—a coalition of 13 international conservation organisations—was formed to address the rapid loss of biodiversity by supporting the identification, monitoring, and safeguard of sites that are critical for the survival of species and ecosystems.

Alliance for Zero Extinction (AZE) sites comprise the most irreplaceable subset of KBAs, holding Critically Endangered or Endangered species restricted to a single site globally. Unless AZEs are properly conserved, they are sites where species extinctions are imminent (Ricketts et al., 2005). Nearly 40% of current AZEs are triggered by amphibians (334 out of 865 sites), the largest of any taxonomic group; yet, fewer than half are currently protected. By identifying and mapping AZE sites and other KBAs, information about the global importance of these areas for the survival of range-restricted amphibians can be provided to key stakeholders to make the best decisions about how to manage that land (or water), where to avoid development, and how to best protect the biodiversity for which the sites are so important. Given limited resources for conservation, this information is vital for conservation efforts centred on habitat protection to prioritise sites of global significance for threatened amphibians.

If amphibian species are not considered within systematic conservation planning, the resulting network of conservation areas may not be congruent with the geographic distribution of this taxonomic group, even where "umbrella" species of groups such as mammals have been used (as demonstrated by Urbina-Cardona & Flores-Villela, 2010). Due to the high habitat specificity of some rare amphibian species, umbrella species are not a good tool for their conservation (Branton & Richardson, 2014; Caro, Engilis, Fitzherbert, & Gardner, 2004; Roni, 2003). Likewise, amphibians have rarely been used as umbrella, flagship or keystone species to understand the consequences of landscape change (Lindenmayer & Westgate, 2020). Additionally, these spatial priorities must be re-evaluated in the context of climate change scenarios and land use to ensure the persistence of species and assemblage populations (Agudelo-Hz, Urbina-Cardona, & Armenteras-Pascual, 2019; Grant, Miller, & Muths, 2020; Urbina-Cardona, 2008). For example, in Australia 10-15% of land cover has been determined to be the target for the national reserve system; however, the representation of amphibians is highly variable and this management approach ignores species` requirements for connectivity (Lemckert, Rosauer, & Slatyer, 2009). Protecting KBAs is critical, but so is promoting connectivity between different initiatives to ensure a network of conservation areas and not just isolated points that will not allow the dispersal of species under global change scenarios (Carvalho, Brito, Crespo, & Possingham, 2010).

Box 5.1: Situation model

This figure shows the Situation Model of the key issues relevant to integrating habitat protection and management for amphibians into strategic planning. The model is a visual map of the observed and presumed causal relationships in the context of habitat protection and management and the factors influencing direct and indirect threats and those affecting conservation targets. Such planning allows for identification of key points for interventions to address threats and develop well-informed strategies. It was developed using the Conservation Standards approach to guide strategic planning to address contributing factors influencing direct and indirect threats to amphibian conservation targets.



<END BOX>

Actions and opportunities for habitat protection and management

Actions and opportunities

Conservation actions should be informed by the best available evidence. However, evidence is often scarce and dispersed, and practitioners may not always use it to guide decisions (Fabian et al., 2019; Knight et al., 2008), instead relying on experience (Cook, Hockings, & Carter, 2010) or even anecdotes and myths (Sutherland, Pullin, Dolman, & Knight, 2004). Smith, Meredith & Sutherland (2021) compiled 129 actions for amphibian conservation based upon 430 studies worldwide (https://www.conservationevidence.com/), of which 42 have proven some conservation benefit, 8 demonstrate to be ineffective or harmful, 18 show a trade-off between benefit and harms, and in 61 the effectiveness is still unknown or there is no evidence found of assessed. Fifty-four actions focused on reducing the impact of anthropogenic landscape transformation, 20 focused on species management, and 35 focused on ecosystem protection and management. Three actions focused on education and awareness, while others focused on the legal protection of species, or livelihood and economic incentives such as engaging landowners and other volunteers to manage land for amphibian protection or pay farmers to cover costs of conservation measures (Smith et al., 2021). Interventions that have been reported in the literature are not always comparable for various reasons: lack of standardisation in the metrics, lack of robust experimental designs such as BACI (Before-After; Control-Impact), or a bias towards better-known biomes and regions (Christie et al., 2020).

This chapter presents suggestions for habitat management and research needed to maintain and improve habitat quality for amphibians. Below we highlight these recommendations (in no particular order), which will also inform a targeted implementer document:

1. Monitoring and evaluation: to determine the benefits and limitations of conservation interventions it is key to monitor and assess their impact (Darrah et al., 2019; Schmidt, Brenneisen, & Zumbach, 2020). Habitat interventions need to consider the requirements of each species (Urbina-Cardona et al., 2015), tolerance to environmental filters (Navas & Otani, 2007; Watling & Braga, 2015), historical landscape disturbance (Betts et al., 2019; Marroquín-Páramo et al., 2021), and spatial-temporal scale (Tscharntke et al., 2012).
2. Connectivity: amphibians benefit from matrices with remnant corridors, water sources (natural and artificial; Mendenhall et al., 2014), and reduced use of agrochemicals. Vegetated riparian areas, as well as agricultural wetlands, are key to facilitating the dispersal of amphibian species and increasing landscape connectivity (Borzée et al., 2018; Ficetola, Padoa-Schioppa, & De Bernardi, 2009; Holzer et al., 2017; Luke et al., 2019; Semlitsch & Bodie, 2003). Some countries (e.g. Colombia and Costa Rica) have considered the conservation of riparian vegetation in their public policy. Connectivity, however, is not limited to riparian corridors. There are interventions to mitigate the impact of infrastructure development on amphibians and their habitats that focus on habitat connectivity, such as the installation of wildlife underpasses and culverts (Beier, Majka, Newell, & Garding, 2008), rows of stumps or branches to reduce erosion and manage sediments (Goosem et al., 2010) and through the protection and restoration of sensitive habitats (Mitchell, Breisch, & Buhlmann, 2006).
3. Sustainable and regenerative agricultural practices: agroecology provides the ecological basis for biodiversity conservation from agriculture, promoting, from the self-sufficiency principle, natural resource renewal, natural biological control, provision of ecosystem services, and crop rotation (Altieri & Nicholls, 2000; Melo et

al., 2013). Embracing beneficial land-use practices, such as traditional farming, sacred forest sites, and incorporating indigenous knowledge into collaborative approaches is key to strengthening conservation effectiveness (Cocks, 2006; Oscarson & Calhoun, 2007).

4. Stakeholder agreements: habitat protection based on collaboration between landowners and communities, while still allowing productive land use with regular monitoring, is effective in both conserving habitat and restoring degraded ecosystems (Charles, 2021; South African National Biodiversity Institute (SANBI) and Wildlands Conservation Trust, 2015). Such approaches are cost-effective and rely on landowner engagement, often resulting in landscape-level protection and improved habitat management (South African National Biodiversity Institute (SANBI), 2015).

5. Voluntary biodiversity offsets: "Biodiversity offsets are measurable conservation outcomes resulting from actions designed to compensate for significant residual adverse biodiversity impacts arising from project development and persisting after appropriate avoidance, minimisation and restoration measures have been taken" (IFC, 2012). Biodiversity offsets are being adopted across international lending, corporate business, national policy, and voluntary programmes (Gelcich, Vargas, Carreras, Castilla, & Donlan, 2017). The IFC determines the need for critical habitat conservation through evaluating specific habitat attributes to conserve a prioritised restricted-range species, and then demonstrating a positive net gain from a monitoring system. Recently, offsets projects are prioritising amphibian species to assess, conserve and monitor their habitat (Sangermano et al., 2015; World Bank, 2019); so there are still no robust results on the effect of conservation actions on the populations of prioritised amphibian species. There are, however, important ethical considerations (Karlsson & Edvardsson Björnberg, 2021), risks (Carreras Gamarra, Lassoie, &

Milder, 2018), limitations, and evidence gaps (Gardner et al., 2013; zu Ermgassen et al., 2019) associated with biodiversity offsets, so thought needs to be given to these aspects in any proposed offset project.

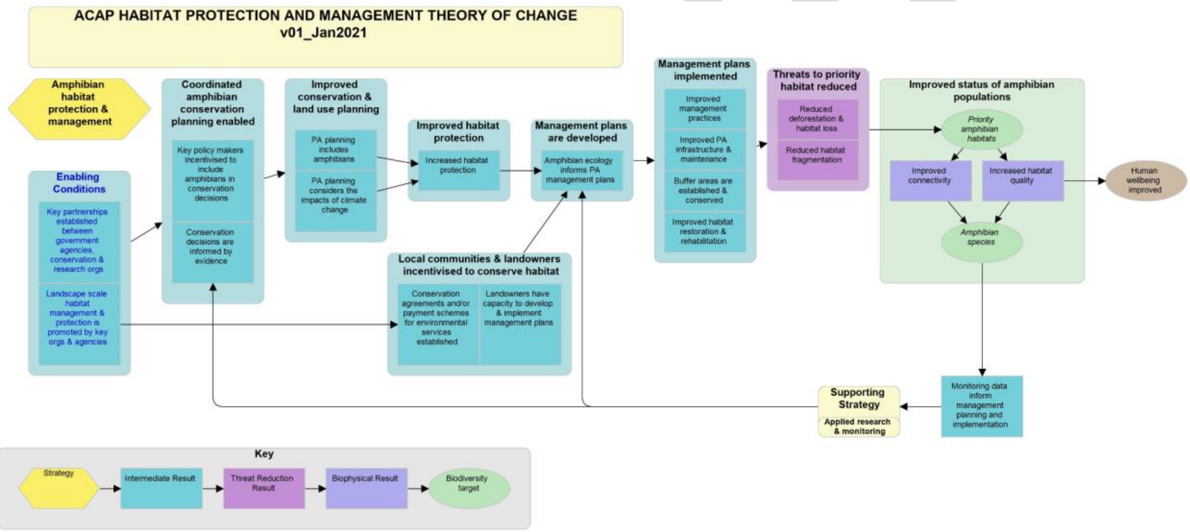
6. Higher-level interventions: certain interventions to support the protection of remaining natural habitats need to be at the policy level, although many can be integrated locally. These can include safeguarding KBAs and AZEs, ending subsidies for damaging agricultural practices, reducing monoculture expansion (e.g. soy, rice, oil palm, etc.), allocating resources to less environmentally damaging alternative land-uses, halting rainforest conversion (McAlpine, Etter, Fearnside, Seabrook, & Laurance, 2009), and demand-side mitigation measures (Bajželj et al., 2014), such as promoting dietary shifts, waste reduction (Foley et al., 2011) and ecological restoration of land illegally appropriated from fires (Driscoll et al., 2021).

   Reproductive health and empowering women is a cross-sectoral approach that can be both national policy-level and locally scaled, led by diverse agents, and linking reproductive health, education, sustainable development, community organisation, and habitat conservation. Although still relatively few in number, cross-sectoral initiatives are key in the context of the SDGs given their aim to improve both planetary and human well-being (Mayhew et al., 2020). A first step for the amphibian conservation community towards this could be to initiate conversations with reproductive health and sustainability organisations to explore aligned opportunities and generate funding. The Population and Sustainability Network (PSN), for example, brings together development, environment and reproductive health organisations to ensure that investment in rights-based family planning programmes are a core part of development initiatives and runs projects integrating family planning and conservation action.

7. Rehabilitation of degraded habitat and creation of artificial habitat: with over 3000 species, including a significant number of threatened species, benefiting from artificial habitats (Figure 5.3), the creation of habitats, such as ponds and seasonal wetlands, is an important tool for enhancing amphibian biodiversity (Ruhí et al., 2012; Scott, Metts, & Whitfield Gibbons, 2008; Simon, Snodgrass, Casey, & Sparling, 2009) as well as protecting threatened species (Beranek, Clulow, & Mahoney, 2020). Such interventions need to consider characteristics such as age, vegetation cover, water quality of the created habitats (Briggs, 2010; Stumpel & van der Voet, 1998), as well as the habitat requirements for target species, ecological connectivity and ideally be implemented at the landscape level to ensure viable populations (Petranka & Holbrook, 2006; Rannap, Lõhmus, & Briggs, 2009).

Box 5.2: Theory of Change

This figure shows a Theory of Change model (results chain) to illustrate how interventions linked to habitat protection and management can lead to improved status for amphibians and their habitats. This approach supports project planning and monitoring, mapping the pathways to achieving conservation goals, identification of activities and development of indicators to measure outcomes in response to interventions. This results chain was developed using the Conservation Standards approach illustrating the theory of change for habitat protection and management as a strategy for reducing threats in response to actions for achieving biodiversity targets (in this case, improved status of amphibian populations)



Box 5.3: Case study – KBAs and local human communities

Key Biodiversity Areas (KBAs) are often situated near impoverished communities that depend on the natural resources from within the site for their livelihoods. The Mount Nimba Strict Nature Reserve on the borders of Guinea, Liberia, and Côte d'Ivoire offer an important case study for conservation prioritisation. Covering 17,540 ha, the site is an AZE that contains the entire known populations of *Hyperolius nimbae* and *Nimbaphrynoides occidentalis*. In addition to a wealth of other biodiversity, the Mount Nimba range contains valuable minerals and dense forests. These resources have attracted mining and logging companies but are also vital to the livelihoods of local communities. Recognising the increased pressure on Mount Nimba from unsustainable resource extraction, the Critical Ecosystem Partnership Fund funded a project "Strengthening capacity of local communities to sustainably manage Mount Nimba's natural resources", which was completed in 2018. Local communities around Mount Nimba received training in improved gardening and livestock farming practices, sustainable resource use, as well as project and financial management, improving their farming yields and subsequently, their income. As a result, the local communities are less reliant on Mount Nimba's natural resources. Through community empowerment focused on sustainable conservation, this project has improved the likelihood that these forests will persist and improve into the future and support the long-term survival of these amphibians (Birdlife International, 2018; UNESCO, 2018).

Box 5.4: Case study – conservation agreements

The Wildlife Conservation Society has developed conservation agreements with private landowners and ethnic communities in areas surrounding four PAs (Farallones NP, Florencia Forest NP, Chingaza NP, and Tatama NP) with a high diversity of threatened species in Colombia. Under these conservation agreements, the owner of each property or community defines the area that will be left for preservation and implementation of management actions (exclusion of livestock or crop areas, maintenance of riparian vegetation, ecological restoration, trafficking reduction, participatory greenhouses, technical advice for the implementation of silvopastoral systems, the establishment of trails for ecotourism and eradication of illicit crops; World Conservation Society, 2020).

Successful agreements have been measured in habitat recovery through freeing up areas for active restoration and reducing intervention for agricultural or livestock uses. To date, 10 agreements are covering 630.96 hectares in conservation agreements in three protected areas and their surroundings: Five agreements in Farallones NP (237.26 hectares and 16 threatened species), three in Selva de Florencia NP (268, 6 hectares and 13 threatened species), and two in Chingaza NP (125.1 hectares and 4 threatened species). Conservation agreements are being developed with ethnic communities for species in a critical state of threat such as *Oophaga histrionica*, which is being worked with Embera chami reservation, area of influence of Tatama NP. Some of the threatened species benefiting from these agreements are *Oophaga histrionica*, *Oophaga anchicayensis*, *Atelopus lozanoi*, and *Andinobates daleswansoni*.

<End Box>

Identification of knowledge gaps and research

To improve habitat protection and management effectiveness for amphibians and provide cost-effective interventions in the field, we draw attention to the need to fill the following knowledge gaps (in no particular order of priority):

1. Based on the systematic conservation planning protocol (Margules & Sarkar, 2007), conservation area networks should be prioritised at a global level to connect different initiatives such as PAs, KBAs, and AZE, among others. These networks should be projected into the future under different scenarios of climate change and land use/land cover (LULCC).
2. To refine conservation networks at the local scale, functional connectivity models for amphibian target species should be conducted at an appropriate resolution. Target species can be habitat specialists, ensuring that essential core habitats are conserved, or threatened flagship species that act as an ‘umbrella’ for protecting multiple species and important habitats.
3. For these target species, physiological experiments should be carried out to understand their dehydration rates, locomotor performance curves, and critical temperatures, along different types of vegetation cover, to make inferences about their response to climate change and LULCC scenarios.
4. Where interventions are carried out (e.g. ecological restoration, implementation of agrosilvopastoral systems, planting of live fences, creation of ponds, among others), monitoring should be conducted at the demographic level for the target species and at the assemblage level for the facets (taxonomic, functional and phylogenetic) of diversity. It is crucial that the results of this monitoring are compiled in a global

database to be able to compare the effectiveness and success of interventions across regions, ecosystems and biotic groups.

5. Likewise, at the level of amphibian assemblages, it is necessary to know the scale of effect at which the landscape configuration operates and what is the amount of habitat required to maintain the values of the diversity facets within the ranges of a natural reference ecosystem (Watling et al., 2020).
6. Partnerships with social scientists and development agencies should be strengthened to improve the social development aspects that often underlie the success of amphibian conservation interventions and to ensure a holistic, integrated approach to achieving environmental objectives.

Acknowledgements

We thank Andrew Snyder, Diego Alejandro Gomez Hoyos, Frank Lemckert, Ian Little, James Lewis, Jeff Dawson, Luis Fernando Marin, Stefano Doglio and Vincenzo Ferri for giving freely of their time towards the development of this chapter and providing diverse and important insights, expertise and extremely helpful commentary. Claire Relton (Endangered Wildlife Trust, South Africa) created the Situation Model and Theory of Change diagram. We are grateful to Phil Bishop for showing us the way and inspiring us to never give up on amphibian conservation.

References

Acevedo-Charry, O., & Aide, T. M. (2019). Recovery of amphibian, reptile, bird and mammal diversity during secondary forest succession in the tropics. *Oikos, 128*, 1065–1078. doi: 10.1111/oik.06252

Adger, W. N. (2000). Social and ecological resilience: Are they related? *Progress in Human Geography, 24*(3), 347–364. doi: 10.1191/030913200701540465

Agudelo-Hz, W. J., Urbina-Cardona, N., & Armenteras-Pascual, D. (2019). Critical shifts on spatial traits and the risk of extinction of Andean anurans: An assessment of the combined effects of climate and land-use change in Colombia. *Perspectives in Ecology and Conservation*, *17*, 206–219. doi: 10.1016/j.pecon.2019.11.002

Aguilar-López, J. L., Ortiz-Lozada, L., Pelayo-Martínez, J., Mota-Vargas, C., Alarcón-Villegas, L. E., & Demeneghi-Calatayud, A. P. (2020). Amphibian and reptile conservation in a privately protected area from a highly transformed region in southern Veracruz, Mexico. *Acta Zoológica Mexicana*, *36*, 1–14. doi: 10.21829/azm.2020.3612164

Albert, J. S., Destouni, G., Duke-Sylvester, S. M., Magurran, A. E., Oberdorff, T., Reis, R. E., … Ripple, W. J. (2020). Scientists' warning to humanity on the freshwater biodiversity crisis. *Ambio, 50*, 85–94. doi: 10.1007/s13280-020-01318-8

Alignier, A., & Deconchat, M. (2011). Variability of forest edge effect on vegetation implies reconsideration of its assumed hypothetical pattern. *Applied Vegetation Science*, *14*, 67–74. doi: 10.1111/j.1654-109X.2010.01105.x

Altieri, M., & Nicholls, C. I. (2000). *Agroecología: Teoría y práctica para una agricultura sustentable*. México, DF. México: Programa de las

Naciones Unidas para el Medio Ambiente.

Álvarez-Grzybowska, E., Urbina-Cardona, N., Córdova-Tapia, F., & García, A. (2020). Amphibian communities in two contrasting ecosystems: functional diversity and environmental filters. *Biodiversity and Conservation*, *29*, 2457–2485. doi: 10.1007/s10531-020-01984-w

Andrews, K. M., Gibbons, J. W., Jochimsen, D. M., & Mitchell, J. (2008). Ecological effects of roads on amphibians and reptiles: a literature review. *Herpetological Conservation*, *3*, 121–143.

Aritola, J. F., Walworth, J. L., Musil, S. A., & Crimmins, M. A. (2019). Soil and land pollution. In M. L. Brusseau, I. L. Pepper, & C. P. Gerba (Eds.), *Environmental and pollution science* (pp. 219–235). Elsevier.

Arroyo-Rodríguez, V., Fahrig, L., Tabarelli, M., Watling, J. I., Tischendorf, L., Benchimol, M., … Tscharntke, T. (2020). Designing optimal human-modified landscapes for forest biodiversity conservation. *Ecology Letters*, *23*(9), 1404–1420. doi: 10.1111/ele.13535

Arroyo-Rodríguez, V., Melo, F. P. L., Martínez-Ramos, M., Bongers, F., Chazdon, R. L.. Meave, J. A., …, Tabarelli, M. (2017). Multiple successional pathways in human-modified tropical landscapes: New insights from forest succession, forest fragmentation and landscape ecology research. *Biological Reviews*, *92*(1), 326-340.

Bajželj, B., Richards, K. S., Allwood, J. M., Smith, P., Dennis, J. S., Curmi, E., & Gilligan, C. A. (2014). Importance of food-demand management for climate mitigation. *Nature Climate Change*, *4*, 924–929. doi: 10.1038/nclimate2353

Balázsi, Á., Riechers, M., Hartel, T., Leventon, J., & Fischer, J. (2019). The impacts of social-ecological system change on human-nature connectedness: A case study from Transylvania, Romania. *Land Use Policy*, *89*, 104232. doi: 10.1016/j.landusepol.2019.104232

Baldwin, R. F., Calhoun, A. J. K., & deMaynadier, P. G. (2006). Conservation planning for amphibian species with complex habitat requirements: A case study using movements and habitat selection of the wood frog *Rana sylvatica*. *Journal of Herpetology*, *40*(4), 442–453. doi: 10.1670/0022-1511(2006)40[442:CPFASW]2.0.CO;2

Barendse, J., Roux, D., Currie, B., Wilson, N., & Fabricius, C. (2016). A broader view of stewardship to achieve conservation and sustainability goals in South Africa. *South African Journal of Science*, *112*(5/6), #2015-0359. doi: 10.17159/sajs.2016/20150359

Barnes, M. D., Craigie, I. D., Harrison, L. B., Geldmann, J., Collen, B., Whitmee, S., … Woodley, S. (2016). Wildlife population trends in protected areas predicted by national socio-economic metrics and body size. *Nature Communications*, *7*, 1–9. doi: 10.1038/ncomms12747

Basham, E. W., González del Pliego, P., Acosta-Galvis, A. R., Woodcock, P., Medina Uribe, C. A., Haugaasen, T., … Edwards, D. P. (2016). Quantifying carbon and amphibian co-benefits from secondary forest regeneration in the Tropical Andes. *Animal Conservation*, *19*, 548–560. doi: 10.1111/acv.12276

Beier, P., Majka, D., Newell, S., & Garding, E. (2008). *Best management practices for wildlife corridors*. Retrieved from http://corridordesign.org/dl/docs/corridordesign.org_BMPs_for_Corridors.pdf

Bennett, A. F., Radford, J. Q., & Haslem, A. (2006). Properties of land mosaics:

Implications for nature conservation in agricultural environments. *Biological Conservation*, *133*(2), 250–264. doi: 10.1016/j.biocon.2006.06.008

Beranek, C. T., Clulow, J., & Mahoney, M. (2020). Wetland restoration for the threatened green and golden bell frog (*Litoria aurea*): development of a breeding habitat designed to passively manage chytrid-induced amphibian disease and exotic fish. *Natural Areas Journal*, *40*(4), 362–374.

Berkes, F. (2009). Community conserved areas: Policy issues in historic and contemporary context. *Conservation Letters*, *2*, 19–24. doi: 10.1111/j.1755-263x.2008.00040.x

Betts, M. G., Wolf, C., Pfeifer, M., Banks-Leite, C., Arroyo-Rodríguez, V., Ribeiro, D. B., … Ewers, R. M. (2019). Extinction filters mediate the global effects of habitat fragmentation on animals. *Science*, *366*(6470), 1236–1239. doi: 10.1126/science.aax9387

Birdlife International. (2018). Fighting rural poverty: Community empowered to conserve Guinean mountain reserve. Retrieved from https://www.birdlife.org/news/

Bombi, P., D’Amen, M., Salvi, D., Bologna, M. A., Marcone, F., Maggio, C., & Canu, A. (2012). Amphibians conservation in Italy: The contribution of the WWF Oases network. *Italian Journal of Zoology*, *79*(2), 287–295. doi: 10.1080/11250003.2011.623722

Borzée, A., Heo, K., & Jang, Y. (2018). Relationship between agro-environmental variables and breeding Hylids in rice paddies. *Scientific Reports*, *8*, 1–13. doi: 10.1038/s41598-018-26222-w

Bosch, J., Rincón, P. A., Boyero, L., & Martínez-Solano, I. (2006). Effects of introduced salmonids on a montane population of Iberian frogs.

Conservation Biology, *20*(1), 180–189. doi: 10.1111/j.1523-1739.2005.00296.x

Bowen, M. E., McAlpine, C. A., House, A. P. N., & Smith, G. C. (2007). Regrowth forests on abandoned agricultural land: A review of their habitat values for recovering forest fauna. *Biological Conservation*, *140*(3–4), 273–296. doi: 10.1016/j.biocon.2007.08.012

Branton, M. A., & Richardson, J. S. (2014). A test of the umbrella species approach in restored floodplain ponds. *Journal of Applied Ecology*, *51*, 776–785. doi: 10.1111/1365-2664.12248

Briggs, L. (2010). Creation of temporary ponds for amphibians in northern and central Europe. *Freshwater Forum*, *17*, 63–70.

Broadbent, E. N., Asner, G. P., Keller, M., Knapp, D. E., Oliveira, P. J. C., & Silva, J. N. (2008). Forest fragmentation and edge effects from deforestation and selective logging in the Brazilian Amazon. *Biological Conservation*, *141*(7), 1745–1757. doi: 10.1016/j.biocon.2008.04.024

Brown, S., & Lugo, A. E. (1990). Tropical secondary forests. *Journal of Tropical Ecology*, *6*, 1–32. doi: 10.1017/S0266467400003989

Brüning, L. Z., Krieger, M., Meneses-Pelayo, E., Eisenhauer, N., Ramirez Pinilla, M. P., Reu, B., & Ernst, R. (2018). Land-use heterogeneity by small-scale agriculture promotes amphibian diversity in montane agroforestry systems of northeast Colombia. *Agriculture, Ecosystems and Environment*, *264*, 15–23. doi: 10.1016/j.agee.2018.05.011

Bucciarelli, G. M., Blaustein, A. R., Garcia, T. S., & Kats, L. B. (2014). Invasion complexities: The diverse impacts of nonnative species on amphibians. *Copeia*, *2014*(4), 611–632. doi: 10.1643/OT-14-014

Buck, J. C., Scheessele, E. A., Relyea, R.

A., & Blaustein, A. R. (2012). The effects of multiple stressors on wetland communities: pesticides, pathogens and competing amphibians. *Freshwater Biology*, *57*, 61–73. doi: 10.1111/j.1365-2427.2011.02695.x

Burton, T. M., & Likens, G. E. (1975). Salamander populations and biomass in the Hubbard Brook Experimental Forest, New Hampshire. *Copeia*, *1975*(3), 541–546.

Butchart, S. H. M., Clarke, M., Smith, R. J., Sykes, R. E., Scharlemann, J. P. W., Harfoot, M., … Burgess, N. D. (2015). Shortfalls and solutions for meeting national and global conservation area targets. *Conservation Letters*, *8*(5), 329–337. doi: 10.1111/conl.12158

Button, S., & Borzée, A. (2021). An integrative synthesis to global amphibian conservation priorities. *Global Change Biology*, *27*(19), 4516–4529. doi: 10.1111/gcb.15734

Cabrera-Guzmán, E., & Reynoso, V. H. (2012). Amphibian and reptile communities of rainforest fragments: Minimum patch size to support high richness and abundance. *Biodiversity and Conservation*, *21*(12), 3243–3265. doi: 10.1007/s10531-012-0312-4

Cáceres-Andrade, S. P., & Urbina-Cardona, J. N. (2009). Ensamblajes de anuros de sistemas productivos y bosques en el piedemonte llanero, departamento del Meta, Colombia. *Caldasia*, *31*(1), 175–194.

Camacho-Rozo, C. P., & Urbina-Cardona, N. (2021). Tadpoles inhabiting natural and anthropogenic temporary water bodies: which are the environmental factors that affect the diversity of the assemblages? *Frontiers in Environmental Science*, *9*, 1–17. doi: 10.3389/fenvs.2021.667448

Caro, T., Engilis, A., Fitzherbert, E., & Gardner, T. (2004). Preliminary assessment of the flagship species concept at a small scale. *Animal Conservation*, *7*(1), 63–70. doi: 10.1017/S136794300300115X

Carreras Gamarra, M. J., Lassoie, J. P., & Milder, J. (2018). Accounting for no net loss: A critical assessment of biodiversity offsetting metrics and methods. *Journal of Environmental Management*, *220*(April), 36–43. doi: 10.1016/j.jenvman.2018.05.008

Carvalho, S. B., Brito, J. C., Crespo, E. J., & Possingham, H. P. (2010). From climate change predictions to actions - conserving vulnerable animal groups in hotspots at a regional scale. *Global Change Biology*, *16*(12), 3257–3270. doi: 10.1111/j.1365-2486.2010.02212.x

Catenazzi, A. (2015). State of the world's amphibians. *Annual Review of Environment and Resources*, *40*, 91–119. doi: 10.1146/annurev-environ-102014-021358

Charles, A. (2021). *Communities, conservation and livelihoods*. Gland, Switzerland: IUCN; Halifax, Canada: Community Conservation Research Network, 2021. doi: 10.2305/iucn.ch.2021.01.en

Chazdon, R. L. (2003). Tropical forest recovery: Legacies of human impact and natural disturbances. *Perspectives in Plant Ecology, Evolution and Systematics*, *6*(1–2), 51–71. doi: 10.1078/1433-8319-00042

Chokkalingam, U., & De Jong, W. (2001). Secondary forest: A working definition and typology. *International Forestry Review*, *3*(1), 19–26.

Christie, A. P., Amano, T., Martin, P. A., Petrovan, S. O., Shackelford, G. E., Simmons, B. I., … Sutherland, W. J. (2020). Poor availability of context-specific evidence hampers decision-making in conservation. *Biological Conservation*, *248*, 108666. doi: 10.1016/j.biocon.2020.108666

Cline, B. B., & Hunter, M. L. (2016). Movement in the matrix: Substrates and distance-to-forest edge affect postmetamorphic movements of a forest amphibian. *Ecosphere*, *7*(2), 1–23. doi: 10.1002/ecs2.1202

Cochrane, M. A., & Barber, C. P. (2009). Climate change, human land use and future fires in the Amazon. *Global Change Biology*, *15*, 601–612. doi: 10.1111/j.1365-2486.2008.01786.x

Cocks, M. (2006). Biocultural diversity: Moving beyond the realm of “indigenous” and “local” people. *Human Ecology*, *34*(2), 185–200. doi: 10.1007/s10745-006-9013-5

Collins, S. J., & Fahrig, L. (2017). Responses of anurans to composition and configuration of agricultural landscapes. *Agriculture, Ecosystems and Environment*, *239*, 399–409. doi: 10.1016/j.agee.2016.12.038

Convention on Biological Diversity. (2020). Aichi Biodiversity Targets. Retrieved October 1, 2020, from https://www.cbd.int/sp/targets/

Cook, C. N., Hockings, M., & Carter, R. W. (2010). Conservation in the dark? The information used to support management decisions. *Frontiers in Ecology and the Environment*, *8*(4), 181–188. doi: 10.1890/090020

Cordero-Rivera, A., Velo-Antón, G., & Galán, P. (2007). Ecology of amphibians in small coastal Holocene islands: local adaptations and the effect of exotic tree plantations. *Munibe*, *25*, 94–103.

Cortés-Gómez, A. M., Castro-Herrera, F., & Urbina-Cardona, J. N. (2013). Small changes in vegetation structure create great changes in amphibian ensembles in the Colombian Pacific rainforest. *Tropical Conservation Science*, *6*(6), 749–769. doi: 10.1177/194008291300600604

Cortés-Gómez, A. M., Ramirez, M. P., & Urbina-Cardona, N. (2015). Protocolo para medición de rasgos funcionales en anfibios. In B. Salgado Negret (Ed.), *La ecología funcional como aproximación al estudio, manejo y* *conservación de la biodiversidad: protocolos y aplicaciones* (pp. 126–179). Bogotá, Colombia: Instituto de Investigación de Recursos Biológicos Alexander von Humboldt.

Cortés-Gomez, A. M., Ruiz-Agudelo, C. A., Valencia-Aguilar, A., & Ladle, R. J. (2015). Ecological functions of Neotropical amphibians and reptiles: A review. *Universitas Scientiarum*, *20*(2), 229–245. doi: 10.11144/Javeriana.SC20-2.efna

Cotten, T. B., Kwiatkowski, M. A., Saenz, D., & Collyer, M. (2012). Effects of an invasive plant, Chinese tallow (*Triadica sebifera*), on development and survival of anuran larvae. *Journal of Herpetology*, *46*(2), 186–193. doi: 10.1670/10-311

Crowther, T. W., Glick, H. B., Covey, K. R., Bettigole, C., Maynard, D. S., Thomas, S. M., … Bradford, M. A. (2015). Mapping tree density at a global scale. *Nature*, *525*(7568), 201–205. doi: 10.1038/nature14967

Crump, M. L. (2015). Anuran reproductive modes: Evolving perspectives. *Journal of Herpetology*, *49*(1), 1–16. doi: 10.1670/14-097

Cushman, S. A. (2006). Effects of habitat loss and fragmentation on amphibians: A review and prospectus. *Biological Conservation*, *128*(2), 231–240. doi: 10.1016/j.biocon.2005.09.031

D'Amico, M., Périquet, S., Román, J., & Revilla, E. (2016). Road avoidance responses determine the impact of heterogeneous road networks at a regional scale. *Journal of Applied Ecology*, *53*(1), 181–190. doi: 10.1111/1365-2664.12572

Dale, V. H., Pearson, S. M., Offerman, H. L., & O'Neill, R. V. (1994). Relating patterns of land-use change to faunal biodiversity in the Central Amazon. *Conservation Biology*, *8*(4), 1027–1036. doi: 10.1046/j.1523-1739.1994.08041027.x

Danielsen, F., Beukema, H., Burgess, N., Parish, F., Brühl, C., Donald, P., … Fitzherbert, E. (2009). Biofuel plantations on forested lands: Double jeopardy for biodiversity and climate. *IOP Conference Series: Earth and Environmental Science*, *6*(24), 242014. doi: 10.1088/1755-1307/6/24/242014

Dare, G. C., Murray, R. G., Courcelles, D. M. M., Malt, J. M., & Palen, W. J. (2020). Run-of-river dams as a barrier to the movement of a stream-dwelling amphibian. *Ecosphere*, *11*(8). doi: 10.1002/ecs2.3207

Darrah, S. E., Shennan-Farpón, Y., Loh, J., Davidson, N. C., Finlayson, C. M., Gardner, R. C., & Walpole, M. J. (2019). Improvements to the Wetland Extent Trends (WET) index as a tool for monitoring natural and human-made wetlands. *Ecological Indicators*, *99*(December 2018), 294–298. doi: 10.1016/j.ecolind.2018.12.032

Davies, S. J., Jordaan, M. S., Karsten, M., Terblanche, J. S., Turner, A. A., van Wilgen, N. J., … Measey, J. (2020). Experience and lessons from alien and invasive animal control projects in South Africa. In B. van Wilgen, J. Measey, D. M. Richardson, J. R. Wilson, & T. A. Zengeya (Eds.), *Biological Invasions in South Africa* (pp. 629–663). Springer, Cham.

Dayrell, J. S., Magnusson, W. E., Bobrowiec, P. E. D., & Lima, A. P. (2021). Impacts of an Amazonian hydroelectric dam on frog assemblages. *PLoS ONE*, *16*(6 June), 1–18. doi: 10.1371/journal.pone.0244580

De Lima, M. G., & Gascon, C. (1999). The conservation value of linear forest remnants in central Amazonia. *Biological Conservation*, *91*, 241–247. doi: 10.1016/S0006-3207(99)00084-1

de Melo, L. S. O., Gonçalves-Souza, T., Garey, M. V., & de Cerqueira, D. (2017). Tadpole species richness within lentic and lotic microhabitats: An interactive influence of environmental and spatial factors. *Herpetological Journal*, *27*(4), 339–345.

De Sy, V., Herold, M., Achard, F., Beuchle, R., Clevers, J. G. P. W., Lindquist, E., & Verchot, L. (2015). Land use patterns and related carbon losses following deforestation in South America. *Environmental Research Letters*, *10*, 124004. doi: 10.1088/1748-9326/10/12/124004

Demaynadier, P. G., & Hunter, M. L. (1998). Effects of silvicultural edges on the distribution and abundance of amphibians in Maine. *Conservation Biology*, *12*(2), 340–352. doi: 10.1046/j.1523-1739.1998.96412.x

Didham, R. K., Kapos, V., & Ewers, R. M. (2012). Rethinking the conceptual foundations of habitat fragmentation research. *Oikos*, *121*(2), 161–170. doi: 10.1111/j.1600-0706.2011.20273.x

Dixo, M., & Metzger, J. P. (2010). The matrix-tolerance hypothesis: an empirical test with frogs in the Atlantic Forest. *Biodiversity and Conservation*, *19*(11), 3059–3071. doi: 10.1007/s10531-010-9878-x

Driscoll, D. A., Armenteras, D., Bennett, A. F., Brotons, L., Clarke, M. F., Doherty, T. S., … Wevill, T. (2021). How fire interacts with habitat loss and fragmentation. *Biological Reviews*, *96*(3), 976–998. doi: 10.1111/brv.12687

Driscoll, D. A., Banks, S. C., Barton, P. S., Lindenmayer, D. B., & Smith, A. L. (2013). Conceptual domain of the matrix in fragmented landscapes. *Trends in Ecology and Evolution*, *28*(10), 605–613. doi: 10.1016/j.tree.2013.06.010

Duarte-Ballesteros, L., Urbina-Cardona, J. N., & Saboyá-Acosta, L. P. (2021). Anuran assemblages and spatial heterogeneity in a paramo ecosystem of Colombia. *Caldasia*, *43*(1), 126–

137. doi: 10.15446/caldasia.v43n1.84860

Dudley, N. (2008). *Guidelines for applying protected area management categories*. Gland, Switzerland: IUCN. WITH Stolton, S., Shadie, P. & Dudley, N. (2013). IUCN WCPA Best practice guidance on recognising protected areas and assigning management categories and governance types. Best practice protected area guidelines series No. 21, Gland, Switzerland.

Dudley, N., & Alexander, S. (2017). Agriculture and biodiversity: a review. *Biodiversity*, *18*(2–3), 45–49. doi: 10.1080/14888386.2017.1351892

Duellman, W. E. (1999). *Patterns of distribution of amphibians: a global perspective*. Baltimore and London: The Johns Hopkins University Press.

Dunn, R. R. (2004). Recovery of faunal communities during tropical forest regeneration. *Conservation Biology*, *18*(2), 302–309.

Echeverría-Londoño, S., Newbold, T., Hudson, L. N., Contu, S., Hill, S. L. L., Lysenko, I., … Purvis, A. (2016). Modelling and projecting the response of local assemblage composition to land use change across Colombia. *Diversity and Distributions*, *22*, 1099–1111. doi: 10.1111/ddi.12478

Ellis, E. C. (2015). Ecology in an anthropogenic biosphere. *Ecological Monographs*, *85*(3), 287–331. doi: 10.1890/14-2274.1

Ernst, R., Linsenmair, K. E., & Rödel, M. O. (2006). Diversity erosion beyond the species level: Dramatic loss of functional diversity after selective logging in two tropical amphibian communities. *Biological Conservation*, *133*(2), 143–155. doi: 10.1016/j.biocon.2006.05.028

Fabian, Y., Bollmann, K., Brang, P., Heiri, C., Olschewski, R., Rigling, A., … Holderegger, R. (2019). How to close the science-practice gap in nature conservation? Information sources used by practitioners. *Biological Conservation*, *235*, 93–101. doi: 10.1016/j.biocon.2019.04.011

Fahrig, L. (2017). Ecological responses to habitat fragmentation per se. *Annual Review of Ecology, Evolution, and Systematics*, *48*(May), 1–23. doi: 10.1146/annurev-ecolsys-110316-022612

Fahrig, L., Arroyo-Rodríguez, V., Bennett, J. R., Boucher-Lalonde, V., Cazetta, E., Currie, D. J., … Watling, J. I. (2019). Is habitat fragmentation bad for biodiversity? *Biological Conservation*, *230*, 179–186. doi: 10.1016/j.biocon.2018.12.026

Falaschi, M., Melotto, A., Manenti, R., & Ficetola, G. F. (2020). Invasive species and amphibian conservation. *Herpetologica*, *76*(2), 216–227. doi: 10.1655/0018-0831-76.2.216

Farallo, V. R., & Miles, D. B. (2016). The importance of microhabitat: A comparison of two microendemic species of *Plethodon* to the widespread *P. cinereus*. *Copeia*, *104*(1), 67–77. doi: 10.1643/CE-14-219

Faruk, A., Belabut, D., Ahmad, N., Knell, R. J., & Garner, T. W. J. (2013). Effects of oil-palm plantations on diversity of tropical anurans. *Conservation Biology*, *27*(3), 615–624. doi: 10.1111/cobi.12062

Ferrante, L., Baccaro, F. B., Ferreira, E. B., Sampaio, M. F. de O., Santos, T., Justino, R. C., & Angulo, A. (2017). The matrix effect: How agricultural matrices shape forest fragment structure and amphibian composition. *Journal of Biogeography*, *44*(8), 1911–1922. doi: 10.1111/jbi.12951

Ficetola, G. F., Padoa-Schioppa, E., & De Bernardi, F. (2009). Influence of landscape elements in riparian buffers on the conservation of semiaquatic amphibians. *Conservation Biology*, *23*(1), 114–123. doi: 10.1111/j.1523-

1739.2008.01081.x

Ficetola, G. F., Siesa, M. E., Manenti, R., Bottoni, L., De Bernardi, F., & Padoa-Schioppa, E. (2011). Early assessment of the impact of alien species: Differential consequences of an invasive crayfish on adult and larval amphibians. *Diversity and Distributions*, 17(6), 1141–1151. doi: 10.1111/j.1472-4642.2011.00797.x

Fletcher, R. J., Didham, R. K., Banks-Leite, C., Barlow, J., Ewers, R. M., Rosindell, J., … Haddad, N. M. (2018). Is habitat fragmentation good for biodiversity? *Biological Conservation*, *226*, 9–15. doi: 10.1016/j.biocon.2018.07.022

Fog, K. (1997). A survey of the results of pond projects for rare amphibians in Denmark. *Memoranda Societatis pro Fauna et Flora Fennica*, *73*, 91–100.

Foley, J. A., DeFries, R., Asner, G. P., Barford, C., Bonan, G., Carpenter, S. R., … Snyder, P. K. (2005). Global consequences of land use. *Science*, *309*(5734), 570–574. doi: 10.1126/science.1111772

Foley, J. A., Ramankutty, N., Brauman, K. A., Cassidy, E. S., Gerber, J. S., Johnston, M., … Zaks, D. P. M. (2011). Solutions for a cultivated planet. *Nature*, *478*(7369), 337–342. doi: 10.1038/nature10452

Ford, J., Hunt, D. A. G. A., Haines, G. E., Lewis, M., Lewis, Y., & Green, D. M. (2020). Adrift on a sea of troubles: can amphibians survive in a human-dominated world? *Herpetologica*, *76*(2), 251–256. doi: 10.1655/0018-0831-76.2.251

Fujioka, M., & Lane, S. J. (1997). The impact of changing irrigation practices in rice fields on frog populations of the Kanto Plain, central Japan. *Ecological Research*, *12*(1), 101–108. doi: 10.1007/BF02523615

Gallice, G. R., Larrea-Gallegos, G., & Vázquez-Rowe, I. (2019). The threat of road expansion in the Peruvian

Amazon. *Oryx*, *53*(2), 284–292. doi: 10.1017/S0030605317000412

Gallmetzer, N., & Schulze, C. H. (2015). Impact of oil palm agriculture on understory amphibians and reptiles: a Mesoamerican perspective. *Global Ecology and Conservation*, *4*(June), 95–109. doi: 10.1016/j.gecco.2015.05.008

Gardner, T. A., Barlow, J., & Peres, C. A. (2007). Paradox, presumption and pitfalls in conservation biology: the importance of habitat change for amphibians and reptiles. *Biological Conservation*, *138*(1–2), 166–179. doi: 10.1016/j.biocon.2007.04.017

Gardner, T. A., Ribeiro-Júnior, M. A., Barlow, J., Ávila-Pires, T. C. S., Hoogmoed, M. S., & Peres, C. A. (2007). The value of primary, secondary, and plantation forests for a Neotropical herpetofauna. *Conservation Biology*, *21*(3), 775–787. doi: 10.1111/j.1523-1739.2007.00659.x

Gardner, T. A., Von Hase, A., Brownlie, S., Ekstrom, J. M. M., Pilgrim, J. D., Savy, C. E., … Ten Kate, K. (2013). Biodiversity offsets and the challenge of achieving no net loss. *Conservation Biology*, *27*(6), 1254–1264. doi: 10.1111/cobi.12118

Garibaldi, L. A., Oddi, F. J., Miguez, F. E., Bartomeus, I., Orr, M. C., Jobbágy, E. G., … Zhu, C. D. (2021). Working landscapes need at least 20% native habitat. *Conservation Letters*, *14*(2), e12773. doi: 10.1111/conl.12773

Gascon, C., Lovejoy, T. E., Bierregaard, R. O. J., Malcolm, J. R., Stouffer, P. C., Laurance, W. F., … Broges, S. (1999). Matrix habitat and species persistence in tropical forest remnants. *Biological Conservation*, *91*(2–3), 223–229. Retrieved from http://www.sciencedirect.com/science/article/pii/S0006320799000804

Gelcich, S., Vargas, C., Carreras, M. J., Castilla, J. C., & Donlan, C. J. (2017). Achieving biodiversity benefits with offsets: research gaps, challenges, and needs. *Ambio*, *46*(2), 184–189. doi: 10.1007/s13280-016-0810-9

Geldmann, J., Manica, A., Burgess, N. D., Coad, L., & Balmford, A. (2019). A global-level assessment of the effectiveness of protected areas at resisting anthropogenic pressures. *Proceedings of the National Academy of Sciences of the United States of America*, *116*(46), 23209–23215. doi: 10.1073/pnas.1908221116

Gilbert-Norton, L., Wilson, R., Stevens, J. R., & Beard, K. H. (2010). A meta-analytic review of corridor effectiveness.*Conservation Biology*, *24*(3), 660–668. doi: 10.1111/j.1523-1739.2010.01450.x

Gillespie, G. R., Howard, S., Stroud, J. T., Ul-Hassanah, A., Campling, M., Lardner, B., … Kusrini, M. (2015). Responses of tropical forest herpetofauna to moderate anthropogenic disturbance and effects of natural habitat variation in Sulawesi, Indonesia. *Biological Conservation*, *192*, 161–173. doi: 10.1016/j.biocon.2015.08.034

Gilroy, J. J., Prescott, G. W., Cardenas, J. S., Castañeda, P. G. D. P., Sánchez, A., Rojas-Murcia, L. E., … Edwards, D. P. (2015). Minimizing the biodiversity impact of Neotropical oil palm development. *Global Change Biology*, *21*(4), 1531–1540. doi: 10.1111/gcb.12696

Godfray, H. C. J., Aveyard, P., Garnett, T., Hall, J. W., Key, T. J., Lorimer, J., … Jebb, S. A. (2018). Meat consumption, health, and the environment. *Science (New York, N.Y.)*, *361*(6399). doi: 10.1126/science.aam5324

Goldspiel, H. B., Cohen, J. B., McGee, G. G., & Gibbs, J. P. (2019). Forest land-use history affects outcomes of habitat augmentation for amphibian conservation. *Global Ecology and Conservation*, *19*, e00686. doi:

10.1016/j.gecco.2019.e00686

Goosem, M. W., Harding, E. K., Chester, G., Tucker, N., Harriss, C., & Oakley, K. (2010). *Roads in rainforest: Best practice guidelines for planning, design and management*. Cairns, Australia: Reef and Rainforest Research Centre Limited.

Gottsberger, B., & Gruber, E. (2004). Temporal partitioning of reproductive activity in a Neotropical anuran community. *Journal of Tropical Ecology*, *20*(3), 271–280. doi: 10.1017/S0266467403001172

Grant, E. H. C., Miller, D. A. W., & Muths, E. (2020). A synthesis of evidence of drivers of amphibian declines. *Herpetologica*, *76*(2), 101-107. doi: 10.1655/0018-0831-76.2.101

Green, D. M., Lannoo, M. J., Lesbarrères, D., & Muths, E. (2020). Amphibian population declines: 30 years of progress in confronting a complex problem. *Herpetologica*, *76*(2), 97–100. doi: 10.1655/0018-0831-76.2.97

Grill, G., Lehner, B., Thieme, M., Geenen, B., Tickner, D., Antonelli, F., … Zarfl, C. (2019). Mapping the world's free-flowing rivers. *Nature*, *569*(7755), 215–221. doi: 10.1038/s41586-019-1111-9

Guariguata, M. R., & Ostertag, R. (2001). Neotropical secondary forest succession: changes in structural and functional characteristics. *Forest Ecology and Management*, *148*(1–3), 185–206. doi: 10.1016/S0378-1127(00)00535-1

Haddad, N. M., Brudvig, L. A., Clobert, J., Davies, K. F., Gonzalez, A., Holt, R. D., … Townshend, J. R. (2015). Habitat fragmentation and its lasting impact on Earth's ecosystems. *Science Advances*, *1*(2), 1–10. doi: 10.1126/sciadv.1500052

Hamer, A. J., & McDonnell, M. J. (2008). Amphibian ecology and conservation in the urbanising world: a review. *Biological Conservation*, *141*(10), 2432–2449. doi: 10.1016/j.biocon.2008.07.020

Hansen, N. A., Scheele, B. C., Driscoll, D. A., & Lindenmayer, D. B. (2019). Amphibians in agricultural landscapes: the habitat value of crop areas, linear plantings and remnant woodland patches. *Animal Conservation*, *22*(1), 72–82. doi: 10.1111/acv.12437

Harper, K. A., Macdonald, S. E., Burton, P. J., Chen, J., Brosofske, K. D., Saunders, S. C., … Esseen, P. A. (2005). Edge influence on forest structure and composition in fragmented landscapes. *Conservation Biology*, *19*(3), 768–782. doi: 10.1111/j.1523-1739.2005.00045.x

Hartel, T., Scheele, B. C., Rozylowicz, L., Horcea-Milcu, A., & Cogălniceanu, D. (2020). The social context for conservation: Amphibians in human shaped landscapes with high nature values. *Journal for Nature Conservation*, *53*, 125762. doi: 10.1016/j.jnc.2019.125762

Hernández-Ordóñez, O., Santos, B. A., Pyron, R. A., Arroyo-Rodríguez, V., Urbina-Cardona, J. N., Martínez-Ramos, M., … Reynoso, V. H. (2019). Species sorting and mass effect along forest succession: evidence from taxonomic, functional, and phylogenetic diversity of amphibian communities. *Ecology and Evolution*, *9*, 5206–5218. doi: 10.1002/ece3.5110

Hernández-Ordóñez, O., Urbina-Cardona, N., & Martínez-Ramos, M. (2015). Recovery of amphibian and reptile assemblages during old-field succession of tropical rain forests. *Biotropica*, *47*(3), 377–388. doi: 10.1111/btp.12207

Herrera-Montes, A., & Brokaw, N. (2010). Conservation value of tropical secondary forest: A herpetofaunal perspective. *Biological Conservation*, *143*(6), 1414–1422. doi: 10.1016/j.biocon.2010.03.016

Hilje, B., & Aide, T. M. (2012). Recovery of amphibian species richness and composition in a chronosequence of secondary forests, northeastern Costa Rica. *Biological Conservation*, *146*(1), 170–176. doi: 10.1016/j.biocon.2011.12.007

Hillers, A., Veith, M., & Rödel, M. O. (2008). Effects of forest fragmentation and habitat degradation on West African leaf-litter frogs. *Conservation Biology*, *22*(3), 762–772. doi: 10.1111/j.1523-1739.2008.00920.x

Hocking, D. J., Babbitt, K. J., & Hocking, D. J. (2014). Amphibian contributions to ecosystem services. *Herpetological Conservation and Biology*, *9*(1), 1–17.

Holzer, K. A., Bayers, R. P., Nguyen, T. T., & Lawler, S. P. (2017). Habitat value of cities and rice paddies for amphibians in rapidly urbanizing Vietnam. *Journal of Urban Ecology*, *3*(1), 1–12. doi: 10.1093/jue/juw007

Houlahan, J. E., Findlay, C. S., Schmidt, B. R., Meyer, A. H., & Kuzmin, S. L. (2000). Quantitative evidence for global amphibian population declines. *Nature*, *404*, 752–755.

IFC. (2012). *International Finance Corporation's Guidance Note 6: Biodiversity conservation and sustainable management of living natural resources*. Retrieved from https://www.ifc.org/wps/wcm/connect/topics_ext_content/ifc_external_corporate_site/sustainability-at-ifc/policies-standards/performance-standards/ps6

Isaacs Cubides, P. J., & Urbina Cardona, J. N. (2011). Anthropogenic disturbance and edge effects on anuran assemblages inhabiting cloud forest fragments in Colombia. *Natureza & Conservação*, *9*(1), 39–46. doi: 10.4322/natcon.2011.004

IUCN. (2016). *A global standard for the identification of Key Biodiversity Areas, Version 1.0.* Gland, Switzerland: IUCN.

IUCN. (2021). The IUCN Red List of Threatened Species. Retrieved March 30, 2021, from Version 2021-2 website: https://www.iucnredlist.org

Jenkins, C. N., Van Houtan, K. S., Pimm, S. L., & Sexton, J. O. (2015). Reply to Brown et al.: Species and places are the priorities for conservation, not economic efficiency. *Proceedings of the National Academy of Sciences of the United States of America*, *112*(32), E4343. doi: 10.1073/pnas.1511375112

Kaneda, T., Greenbaum, C., & Kline, K. (2020). *World population data sheet*. Washington, D.C, USA. Retrieved from https://www.prb.org/wp-content/uploads/2020/07/letter-booklet-2020-world-population.pdf

Karlsson, M., & Edvardsson Björnberg, K. (2021). Ethics and biodiversity offsetting. *Conservation Biology*, *35*(2), 578–586. doi: 10.1111/cobi.13603

Kats, L. B., & Ferrer, R. P. (2003). Alien predators and amphibian declines: review of two decades of science and the transition to conservation. *Diversity and Distributions*, *9*(2), 99–110.

Kehoe, L., Kuemmerle, T., Meyer, C., Levers, C., Václavík, T., & Kreft, H. (2015). Global patterns of agricultural land-use intensity and vertebrate diversity. *Diversity and Distributions*, *21*(11), 1308–1318. doi: 10.1111/ddi.12359

Kennedy, C. M., Oakleaf, J. R., Theobald, D. M., Baruch-Mordo, S., & Kiesecker, J. (2019). Managing the middle: a shift in conservation priorities based on the global human modification gradient. *Global Change Biology*, *25*(3), 811–826. doi: 10.1111/gcb.14549

Kiesecker, J. M., Blaustein, A. R., & Belden, L. K. (2001). Complex causes of amphibian population declines. *Nature*, *410*(6829), 681–

684. doi: 10.1038/35070552

Knight, A. T., Cowling, R. M., Rouget, M., Balmford, A., Lombard, A. T., & Campbell, B. M. (2008). Knowing but not doing: Selecting priority conservation areas and the research-implementation gap. *Conservation Biology*, *22*(3), 610–617. doi: 10.1111/j.1523-1739.2008.00914.x

Konopik, O., Steffan-Dewenter, I., & Grafe, T. U. (2015). Effects of logging and oil palm expansion on stream frog communities on Borneo, Southeast Asia. *Biotropica*, *47*(5), 636–643.

Kremen, C., Williams, N. M., & Thorp, R. W. (2002). Crop pollination from native bees at risk from agricultural intensification. *Proceedings of the National Academy of Sciences of the United States of America*, *99*(26), 16812–16816. doi: 10.1073/pnas.262413599

Kukkala, A. S., & Moilanen, A. (2013). Core concepts of spatial prioritisation in systematic conservation planning. *Biological Reviews*, *88*(2), 443–464. doi: 10.1111/brv.12008

Laurance, W. F., Camargo, J. L. C., Luizão, R. C. C., Laurance, S. G., Pimm, S. L., Bruna, E. M., … Lovejoy, T. E. (2011). The fate of Amazonian forest fragments: A 32-year investigation. *Biological Conservation*, *144*(1), 56–67. doi: 10.1016/j.biocon.2010.09.021

Laurance, W. F., Carolina Useche, D., Rendeiro, J., Kalka, M., Bradshaw, C. J. A., Sloan, S. P., … Zamzani, F. (2012). Averting biodiversity collapse in tropical forest protected areas. *Nature*, *489*(7415), 290–293. doi: 10.1038/nature11318

Laurance, W. F., Lovejoy, T. E., Vasconcelos, H. L., Bruna, E. M., Didham, R. K., Stouffer, P. C., … Sampaio, E. (2002). Ecosystem decay of Amazonian forest fragments: A 22-year investigation. *Conservation Biology*, *16*(3), 605–618. doi:

10.1046/j.1523-1739.2002.01025.x

Laurance, W. F., Sayer, J., & Cassman, K. G. (2014). Agricultural expansion and its impacts on tropical nature. *Trends in Ecology and Evolution*, *29*(2), 107–116. doi: 10.1016/j.tree.2013.12.001

Le Saout, S., Hoffmann, M., Shi, Y., Hughes, A., Bernard, C., Brooks, T. M., … Rodrigues, A. S. L. (2013). Protected areas and effective biodiversity conservation. *Science*, *342*(6160), 803–805. doi: 10.1126/science.1239268

Lehtinen, R. M., Galatowitsch, S. M., & Tester, J. R. (1999). Consequences of habitat loss and fragmentation for wetland amphibian assemblages. *Wetlands*, *19*(1), 1–12. doi: 10.1007/BF03161728

Lehtinen, R. M., Ramanamanjato, J. B., & Raveloarison, J. G. (2003). Edge effects and extinction proneness in a herpetofauna from Madagascar. *Biodiversity and Conservation*, *12*(7), 1357–1370. doi: 10.1023/A:1023673301850

Lemckert, F., Rosauer, D., & Slatyer, C. (2009). A comparison of Australia's anuran records against the reserve system. *Biodiversity and Conservation*, *18*(5), 1233–1246. doi: 10.1007/s10531-008-9542-x

Li, Z., Hou, Y., Cao, J., Ding, Y., & Yuan, X. (2022). What drives green development in China: public pressure or the willingness of local government? *Environmental Science and Pollution Research*, *29*(4), 5454–5468. doi: 10.1007/s11356-021-16059-8

Lindenmayer, D. B., & Westgate, M. J. (2020). Are flagship, umbrella and keystone species useful surrogates to understand the consequences of landscape change? *Current Landscape Ecology Reports*, *5*(3), 76–84. doi: 10.1007/s40823-020-00052-x

Loyola, R. D., Becker, C. G., Kubota, U., Haddad, C. F. B., Fonseca, C. R., & Lewinsohn, T. M. (2008). Hung out to dry: Choice of priority ecoregions for conserving threatened Neotropical anurans depends on life-history traits. *PLoS ONE*, *3*(5), 10–13. doi: 10.1371/journal.pone.0002120

Luke, S. H., Slade, E. M., Gray, C. L., Annammala, K. V., Drewer, J., Williamson, J., … Struebig, M. J. (2019). Riparian buffers in tropical agriculture: scientific support, effectiveness and directions for policy. *Journal of Applied Ecology*, *56*(1), 85–92. doi: 10.1111/1365-2664.13280

Mace, G. M., Barrett, M., Burgess, N. D., Cornell, S. E., Freeman, R., Grooten, M., & Purvis, A. (2018). Aiming higher to bend the curve of biodiversity loss. *Nature Sustainability*, *1*(9), 448–451. doi: 10.1038/s41893-018-0130-0

Machovina, B., Feeley, K. J., & Ripple, W. J. (2015). Biodiversity conservation: the key is reducing meat consumption. *Science of the Total Environment*, *536*, 419–431. doi: 10.1016/j.scitotenv.2015.07.022

Maerz, J. C., Brown, C. J., Chapin, C. T., & Blossey, B. (2005). Can secondary compounds of an invasive plant affect larval amphibians? *Functional Ecology*, *19*(6), 970–975. doi: 10.1111/j.1365-2435.2005.01054.x

Maerz, J. C., Blossey, B., & Nuzzo, V. (2005). Green frogs show reduced foraging success in habitats invaded by Japanese knotweed. *Biodiversity and Conservation*, *14*(12), 2901–2911. doi: 10.1007/s10531-004-0223-0

Magnus, R., & Rannap, R. (2019). Pond construction for threatened amphibians is an important conservation tool, even in landscapes with extant natural water bodies. *Wetlands Ecology and Management*, *27*(2–3), 323–341. doi: 10.1007/s11273-019-09662-7

Margules, C. R., & Sarkar, S. (2007). *Systematic conservation planning*. Cambridge, UK: Cambridge University Press.

Marroquín-Páramo, J. A., Suazo-Ortuño, I., Urbina-Cardona, N., & Benítez-Malvido, J. (2021). Cumulative effects of high intensity hurricanes on herpetofaunal assemblages along a tropical dry forest chronosequence. *Forest Ecology and Management*, *479*, 118505. doi: 10.1016/j.foreco.2020.118505

Martín-Torrijos, L., Sandoval-Sierra, J. V., Muñoz, J., Diéguez-Uribeondo, J., Bosch, J., & Guayasamin, J. M. (2016). Rainbow trout (*Oncorhynchus mykiss*) threaten Andean amphibians. *Neotropical Biodiversity*, *2*(1), 26–36. doi: 10.1080/23766808.2016.1151133

Mayhew, S. H., Newman, K., Johnson, D., Clark, E., Hammer, M., Mohan, V., & Ssali, S. (2020). New partnerships, new perspectives: The relevance of sexual and reproductive health and rights for sustainable development. *Health Policy*, *124*(6), 599–604. doi: 10.1016/j.healthpol.2019.03.010

McAlpine, C. A., Etter, A., Fearnside, P. M., Seabrook, L., & Laurance, W. F. (2009). Increasing world consumption of beef as a driver of regional and global change: a call for policy action based on evidence from Queensland (Australia), Colombia and Brazil. *Global Environmental Change*, *19*(1), 21–33. doi: 10.1016/j.gloenvcha.2008.10.008

McDiarmid, R. W., & Altig, R. (1999). *Tadpoles: The biology of Anuran larvae*. Chicago, USA: University of Chicago Press.

Measey, G. J. (2011). *Ensuring a future for South Africa's frogs: A strategy for conservation research*. Pretoria, South Africa: South African National Biodiversity Institute.

Meine, C., Soulé, M., & Noss, R. F. (2006). "A mission-driven discipline”: the growth of conservation biology. *Conservation Biology*, *20*(3), 631–651. doi: 10.1111/j.1523-1739.2006.00449.x

Melo, F. P. L., Arroyo-Rodríguez, V., Fahrig, L., Martínez-Ramos, M., & Tabarelli, M. (2013). On the hope for biodiversity-friendly tropical landscapes. *Trends in Ecology and Evolution*, *28*(8), 462–468. doi: 10.1016/j.tree.2013.01.001

Mendenhall, C. D., Frishkoff, L. O., Santos-Barrera, G., Pacheco, J., Mesfun, E., Mendoza Quijano, F., … Pringle, R. M. (2014). Countryside biogeography of Neotropical reptiles and amphibians. *Ecology*, *95*(4), 856–870.

Menéndez-Guerrero, P. A., Davies, T. J., & Green, D. M. (2020). Extinctions of threatened frogs may impact ecosystems in a global hotspot of anuran diversity. *Herpetologica*, *76*(2), 121–131. doi: 10.1655/0018-0831-76.2.121

Mitchell, J. C., Breisch, A. R., & Buhlmann, K. A. (2006). *Habitat management guidelines for amphibians and reptiles of the Northeastern United States*. Technical Publication HMG-3. Partners in Amphibian and Reptile Conservation (PARC). Retrieved from http://northeastparc.org/habitat-management-guidelines/

Moore, R. D. (2011). *Protecting the smaller majority: Amphibian conservation case studies*. Conservation International and the IUCN/SSC Amphibian Specialist Group.

Mora, F., Martínez-Ramos, M., Ibarra-Manríquez, G., Pérez-Jiménez, A., Trilleras, J., & Balvanera, P. (2015). Testing chronosequences through dynamic approaches: time and site effects on tropical dry forest succession. *Biotropica*, *47*(1), 38–48. doi: 10.1111/btp.12187

Murcia, C. (1995). Edge effects in

fragmented forests: implications for conservation. *Trends in Ecology & Evolution*, *10*(2), 58–62. doi: 10.1016/S0169-5347(00)88977-6

Murrieta-Galindo, R., González-Romero, A., López-Barrera, F., & Parra-Olea, G. (2013). Coffee agrosystems: An important refuge for amphibians in central Veracruz, Mexico. *Agroforestry Systems*, *87*(4), 767–779. doi: 10.1007/s10457-013-9595-z

Murrieta-Galindo, R., López-Barrera, F., González-Romero, A., & Parra-Olea, G. (2013). Matrix and habitat quality in a montane cloud-forest landscape: Amphibians in coffee plantations in central Veracruz, Mexico. *Wildlife Research*, *40*(1), 25–35. doi: 10.1071/WR12076

Muths, E., Chambert, T., Schmidt, B. R., Miller, D. A. W., Hossack, B. R., Joly, P., … Grant, E. H. C. (2017). Heterogeneous responses of temperate-zone amphibian populations to climate change complicates conservation planning. *Scientific Reports*, *7*(1), 1–10. doi: 10.1038/s41598-017-17105-7

Naito, R., Sakai, M., Natuhara, Y., Morimoto, Y., & Shibata, S. (2013). Microhabitat use by *Hyla japonica* and *Pelophylax porosa brevipoda* at levees in rice paddy areas of Japan. *Zoological Science*, *30*(5), 386–391. doi: 10.2108/zsj.30.386

Navas, C. A., & Otani, L. (2007). Physiology, environmental change, and anuran conservation. *Phyllomedusa*, *6*(2), 83–103. doi: 10.11606/issn.2316-9079.v6i2p83-103

Neckel-Oliveira, S., & Gascon, C. (2006). Abundance, body size and movement patterns of a tropical treefrog in continuous and fragmented forests in the Brazilian Amazon. *Biological Conservation*, *128*(3), 308–315. doi: 10.1016/j.biocon.2005.09.037

Newbold, T., Hudson, L. N., Arnell, A. P., Contu, S., Palma, A. De, Ferrier, S.,

… Zhang, H. (2016). Has land use pushed terrestrial biodiversity beyond the planetary boundary? A global assessment. *Science, 353*(6296), 288–291.

Nori, J., Lemes, P., Urbina-Cardona, N., Baldo, D., Lescano, J., & Loyola, R. (2015). Amphibian conservation, land-use changes and protected areas: a global overview. *Biological Conservation*, *191*, 367–374. doi: 10.1016/j.biocon.2015.07.028

Nunes, A. L., Fill, J. M., Davies, S. J., Louw, M., Rebelo, A. D., Thorp, C. J., … Measey, J. (2019). A global meta-analysis of the ecological impacts of alien species on native amphibians. *Proceedings of the Royal Society B, 286*, 20182528.

Ochoa-Ochoa, L. M., Bezaury-Creel, J. E., Vázquez, L.-B., & Flores-Villela, O. (2011). Choosing the survivors? A GIS-based triage support tool for micro-endemics: Application to data for Mexican amphibians. *Biological Conservation*, *144*(11), 2710–2718. doi: 10.1016/j.biocon.2011.07.032

Ochoa-Ochoa, L. M., Urbina-Cardona, J. N., Vázquez, L. B., Flores-Villela, O., & Bezaury-Creel, J. (2009). The effects of governmental protected areas and social initiatives for land protection on the conservation of Mexican amphibians. *PLoS ONE, 4*(9). doi: 10.1371/journal.pone.0006878

Ochoa-Ochoa, L. M., Urbina-Cardona, N., & Flores-Villela, O. (2011). Planning amphibian conservation in Mexico. *FrogLog*, *97*, 51–52.

Oddone Aquino, A. G. H. E., & Nkomo, S. L. (2021). Spatio-temporal patterns and consequences of road kills: a review. *Animals, 11*(3), 1–23. doi: 10.3390/ani11030799

Oliveira, S. L. De, Silva, D. de M., Bastos, R. P., & Morais, A. R. (2015). Anfíbios anuros nos covais do município de Jataí, Estado de Goiás. *Geoambiente On-line, 24. Retrieved* *from https://www.revistas.ufg.br/geoambiente/article/view/34139.* doi: 10.5216/revgeoamb.v0i24.34139

Oscarson, D. B., & Calhoun, A. J. K. (2007). Developing vernal pool conservation plans at the local level using citizen-scientists. *Wetlands*, *27*, 80–95. doi: 10.1672/0277-5212(2007)27[80:DVPCPA]2.0.CO;2

Oteros-Rozas, E., Ruiz-Almeida, A., Aguado, M., González, J. A., & Rivera-Ferre, M. G. (2019). A social-ecological analysis of the global agrifood system. *Proceedings of the National Academy of Sciences of the United States of America*, *116*(52), 26465–26473. doi: 10.1073/pnas.1912710116

Palomo, I., Montes, C., Martín-López, B., González, J. A., García-Llorente, M., Alcorlo, P., & Mora, M. R. G. (2014). Incorporating the social-ecological approach in protected areas in the Anthropocene. *BioScience*, *64*(3), 181–191. doi: 10.1093/biosci/bit033

Pawar, S. S., Rawat, G. S., & Choudhury, B. C. (2004). Recovery of frog and lizard communities following primary habitat alteration in Mizoram, Northeast India. *BMC Ecology*, *4*(10), 1–18. doi: 10.1186/1472-6785-4-10

Pawaskar, U. S., Raut, R. D., & Gardas, B. B. (2018). Assessment of consumer behavior towards environmental responsibility: a structural equations modeling approach. *Business Strategy and the Environment*, *27*(4), 560–571. doi: 10.1002/bse.2020

Pearce, F., & Madgwick, F. J. (2020). *Water lands: A vision for the world's wetlands and their people*. The Netherlands: Harper Collins.

Pearman, P. B. (1997). Correlates of amphibian diversity in an altered landscape of Amazonian Ecuador. *Conservation Biology*, *11*(5), 1211–1225. doi: 10.1046/j.1523-1739.1997.96202.x

Pence, G. K. Q. (2017). *The Western Cape*

Biodiversity spatial plan: Technical report. Cape Town, South Africa.

Perfecto, I., & Vandermeer, J. (2008). Biodiversity conservation in tropical agroecosystems: A new conservation paradigm. *Annals of the New York Academy of Sciences*, *1134*, 173–200. doi: 10.1196/annals.1439.011

Perfecto, I., & Vandermeer, J. (2010). The agroecological matrix as alternative to the land-sparing/agriculture intensification model. *Proceedings of the National Academy of Sciences of the United States of America*, *107*(13), 5786–5791. doi: 10.1073/pnas.0905455107

Petranka, J. W., & Holbrook, C. T. (2006). Wetland restoration for amphibians: Should local sites be designed to support metapopulations or patchy populations? *Restoration Ecology*, *14*(3), 404–411. doi: 10.1111/j.1526-100X.2006.00148.x

Pfeifer, M., Lefebvre, V., Peres, C. A., Banks-Leite, C., Wearn, O. R., Marsh, C. J., … Ewers, R. M. (2017). Creation of forest edges has a global impact on forest vertebrates. *Nature*, *551*(7679), 187–191. doi: 10.1038/nature24457

Phillips, H. R. P., Halley, J. M., Urbina-Cardona, J. N., & Purvis, A. (2018). The effect of fragment area on site-level biodiversity. *Ecography*, *41*(7), 1220–1231. doi: 10.1111/ecog.02956

Pineda, E., & Halffter, G. (2004). Species diversity and habitat fragmentation: frogs in a tropical montane landscape in Mexico. *Biological Conservation*, *117*(5), 499–508. doi: 10.1016/j.biocon.2003.08.009

Pineda, E., Moreno, C., Escobar, F., & Halffter, G. (2005). Frog, bat, and dung beetle diversity in the cloud forest and coffee agroecosystems of Veracruz, Mexico. *Conservation Biology*, *19*(2), 400–410.

Pinero-Rodríguez, M. J., Fernández-Zamudio, R., Arribas, R., Gomez-Mestre, I., & Díaz-Paniagua, C.

(2021). The invasive aquatic fern *Azolla filiculoides* negatively impacts water quality, aquatic vegetation and amphibian larvae in Mediterranean environments. *Biological Invasions*, *23*(3), 755–769. doi: 10.1007/s10530-020-02402-6

Poore, J., & Nemecek, T. (2018). Reducing food's environmental impacts through producers and consumers. *Science*, *360*, 987–992. doi: 10.1126/science.aaq0216

Pope, S. E., Fahrig, L., & Merriam, H. G. (2000). Landscape complementation and metapopulation effects on leopard frog populations. *Ecology*, *81*(9), 2498–2508.

Pouzols, F. M., Toivonen, T., Minin, E. Di, Kukkala, A. S., Kullberg, P., Kuustera, J., … Moilanen, A. (2014). Global protected area expansion is compromised by projected land-use and parochialism. *Nature*, *516*(7531), 383–386. doi: 10.1038/nature14032

Pratihar, S., Clark, H. O., Dutta, S., Khan, M. S., Patra, B. C., Ukuwela, K. D. B., … Das, S. (2014). Diversity and conservation of amphibians in South and Southeast Asia. *Sauria*, *36*, 9–59.

Queiroz, C. de S., da Silva, F. R., & Rossa-Feres, D. de C. (2015). The relationship between pond habitat depth and functional tadpole diversity in an agricultural landscape. *Royal Society Open Science*, *2*(7), 150165. doi: 10.1098/rsos.150165

Rabinowitz, D., Cairns, S., & Dillon, T. (1986). Seven forms of rarity and their frequency in the flora of the British Isles. In M. E. Soulé (Ed.), *Conservation biology: the science of scarcity and diversity*. Sunderland, USA: Sinauer Associates, Inc.

Ramsar Convention on Wetlands. (2018). *Global wetland outlook: State of the world's wetlands and their services to people 2018*. Gland, Switzerland. Retrieved from https://www.global-wetland-outlook.ramsar.org/outlook

Rannap, R., Lõhmus, A., & Briggs, L.

(2009). Restoring ponds for amphibians: A success story. *Hydrobiologia*, *634*, 87–95. doi: 10.1007/s10750-009-9884-8

Resasco, J. (2019). Meta-analysis on a decade of testing corridor efficacy: what new have we learned? *Current Landscape Ecology Reports*, *4*, 61–69. doi: 10.1007/s40823-019-00041-9

Ribeiro, J., Colli, G. R., & Soares, A. (2019). Landscape correlates of anuran functional connectivity in rice crops: a graph-theoretic approach. *Journal of Tropical Ecology*, *35*(3), 118–131. doi: 10.1017/S026646741900004X

Rice, R. A., & Greenberg, R. (2000). Cacao cultivation and the conservation of biological diversity. *Ambio*, *29*(3), 167–173. doi: 10.1579/0044-7447-29.3.167

Ricketts, T. H., Dinerstein, E., Boucher, T., Brooks, T. M., Butchart, S. H. M., Hoffmann, M., … Wikramanayake, E. (2005). Pinpointing and preventing imminent extinctions. *Proceedings of the National Academy of Sciences*, *102*(51), 18497–18501.

Riemann, J. C., Ndriantsoa, S. H., Raminosoa, N. R., Rödel, M. O., & Glos, J. (2015). The value of forest fragments for maintaining amphibian diversity in Madagascar. *Biological Conservation*, *191*, 707–715. doi: 10.1016/j.biocon.2015.08.020

Riemann, J. C., Ndriantsoa, S. H., Rödel, M. O., & Glos, J. (2017). Functional diversity in a fragmented landscape — Habitat alterations affect functional trait composition of frog assemblages in Madagascar. *Global Ecology and Conservation*, *10*, 173–183. doi: 10.1016/j.gecco.2017.03.005

Rios-López, N., & Aide, T. M. (2007). Herpetofaunal dynamics during secondary succession. *Herpetologica*, *63*(1), 35–50. doi: 10.1655/0018-0831(2007)63[35:HDDSS]2.0.CO;2

Ritchie, H., & Roser, M. (2019). Land

Use. Retrieved from OurWorldInData.org website: https://ourworldindata.org/land-use

Roach, N. S., Urbina-Cardona, N., & Lacher, T. E. (2020). Land cover drives amphibian diversity across steep elevational gradients in an isolated Neotropical mountain range: Implications for community conservation. *Global Ecology and Conservation*, *22*, e00968. doi: 10.1016/j.gecco.2020.e00968

Rodrigues, A. S. L., Akçakaya, H. R., Andelman, S. J., Bakarr, M. I., Boitani, L., Brooks, T. M., … Yan, X. (2004). Global gap analysis: Priority regions for expanding the global protected-area network. *BioScience*, *54*(12), 1092–1100. doi: 10.1641/0006-3568(2004)054[1092:GGAPRF]2.0.CO;2

Rodrigues, A. S. L., Andelman, S. J., Bakarr, M. I., Boitani, L., Brooks, T. M., Cowling, R. M., … Yan, X. (2004). Effectiveness of the global protected area network in representing species diversity. *Nature*, *428*, 640–643. doi: 10.1038/nature02459

Roelants, K., Gower, D. J., Wilkinson, M., Loader, S. P., Biju, S. D., Guillaume, K., … Bossuyt, F. (2007). Global patterns of diversification in the history of modern amphibians. *Proceedings of the National Academy of Sciences of the United States of America*, *104*(3), 887–892. doi: 10.1073/pnas.0608378104

Roni, P. (2003). Responses of benthic fishes and giant salamanders to placement of large woody debris in small Pacific Northwest streams. *North American Journal of Fisheries Management*, *23*(4), 1087–1097. doi: 10.1577/m02-048

Rowley, J., Brown, R., Bain, R., Kusrini, M., Inger, R., Stuart, B., … Phimmachak, S. (2010). Impending conservation crisis for Southeast

Asian amphibians. *Biology Letters*, *6*(3), 336–338. doi: 10.1098/rsbl.2009.0793

Ruhí, A., San Sebastian, O., Feo, C., Franch, M., Gascón, S., Richter-Boix, À., … Llorente, G. (2012). Man-made Mediterranean temporary ponds as a tool for amphibian conservation. *International Journal of Limnology*, *48*(1), 81–93. doi: 10.1051/limn/2011059

Russildi, G., Arroyo-Rodríguez, V., Hernández-Ordóñez, O., Pineda, E., & Reynoso, V. H. (2016). Species- and community-level responses to habitat spatial changes in fragmented rainforests: Assessing compensatory dynamics in amphibians and reptiles. *Biodiversity and Conservation*, *25*(2), 375–392. doi: 10.1007/s10531-016-1056-3

Sangermano, F., Bol, L., Galvis, P., Gullison, R. E., Hardner, J., & Ross, G. S. (2015). Habitat suitability and protection status of four species of amphibians in the Dominican Republic. *Applied Geography*, *63*, 55–65. doi: 10.1016/j.apgeog.2015.06.002

Santos-Barrera, G., & Urbina-Cardona, J. N. (2011). The role of the matrix-edge dynamics of amphibian conservation in tropical montane fragmented landscapes. *Revista Mexicana de Biodiversidad*, *82*, 679–687. doi: 10.22201/ib.20078706e.2011.2.463

Saunders, D. A., Hobbs, R. J., & Margules, C. R. (1991). Biological consequences of ecosystem fragmentation: A review. *Conservation Biology*, *5*(1), 18–32. doi: 10.1016/0006-3207(92)90725-3

Scheffers, B. R., Brunner, R. M., Ramirez, S. D., Shoo, L. P., Diesmos, A., & Williams, S. E. (2013). Thermal buffering of microhabitats is a critical factor mediating warming vulnerability of frogs in the Philippine Biodiversity Hotspot.

Biotropica, 45(5), 628–635. doi: 10.1111/btp.12042

Scheffers, B. R., Edwards, D. P., Diesmos, A., Williams, S. E., & Evans, T. A. (2014). Microhabitats reduce animal’s exposure to climate extremes. *Global Change Biology*, *20*(2), 495–503. doi: 10.1111/gcb.12439

Schleicher, J., Peres, C. A., Amano, T., Llactayo, W., & Leader-Williams, N. (2017). Conservation performance of different conservation governance regimes in the Peruvian Amazon. *Scientific Reports*, *7*, 11318. doi: 10.1038/s41598-017-10736-w

Schloegel, L. M., Picco, A. M., Kilpatrick, A. M., Davies, A. J., Hyatt, A. D., & Daszak, P. (2009). Magnitude of the US trade in amphibians and presence of *Batrachochytrium dendrobatidis* and ranavirus infection in imported North American bullfrogs (*Rana catesbeiana*). *Biological Conservation*, *142*, 1420–1426. doi: 10.1016/j.biocon.2009.02.007

Schmidt, B. R., Brenneisen, S., & Zumbach, S. (2020). Evidence-based amphibian conservation: A case study on toad tunnels. *Herpetologica*, *76*(2), 228–239. doi: 10.1655/0018-0831-76.2.228

Schmidt, B. R., & Zumbach, S. (2008). Amphibian road mortality and how to prevent it: a review. In J. C. Mitchell, R. E. Jung Brown, & B. Bartholomew (Eds.), *Urban Herpetology* (pp. 157–167). St. Louis, Missouri: Society for the Study of Amphibians and Reptiles. doi: 10.5167/uzh-10142

Schneider-Maunoury, L., Lefebvre, V., Ewers, R. M., Medina-Rangel, G. F., Peres, C. A., Somarriba, E., … Pfeifer, M. (2016). Abundance signals of amphibians and reptiles indicate strong edge effects in Neotropical fragmented forest landscapes. *Biological Conservation*, *200*, 207–215. doi: 10.1016/j.biocon.2016.06.011

Scott, D. E., Metts, B. S., & Whitfield Gibbons, J. (2008). Enhancing amphibian biodiversity on golf courses with seasonal wetlands. In J. C. Mitchell, R. E. Jung Brown, & B. Bartholomew (Eds.), *Urban herpetology* (pp. 285–292). Society for thc Study of Arnphibians and Reptiles.

Scriven, S. A., Gillespie, G. R., Laimun, S., & Goossens, B. (2018). Edge effects of oil palm plantations on tropical anuran communities in Borneo. *Biological Conservation*, *220*(February), 37–49. doi: 10.1016/j.biocon.2018.02.006

Semlitsch, R. D., & Bodie, J. R. (2003). Biological criteria for buffer zones around wetlands and riparian habitats for amphibians and reptiles. *Conservation Biology*, *17*(5), 1219–1228. doi: 10.1046/j.1523-1739.2003.02177.x

Simon, J. A., Snodgrass, J. W., Casey, R. E., & Sparling, D. W. (2009). Spatial correlates of amphibian use of constructed wetlands in an urban landscape. *Landscape Ecology*, *24*(3), 361–373. doi: 10.1007/s10980-008-9311-y

Skowno, A. L., Poole, C. J., Raimondo, D. C., Sink, K. J., Van Deventer, H., Van Niekerk, L., … Driver, A. (2019). National biodiversity assessment 2018: The status of South Africa’s ecosystems and biodiversity. Synthesis report. South African National Biodiversity Institute. In *South African National Biodiversity Institute*. Pretoria, South Africa: South African National Biodiversity Institute, an entity of the Department of Environment, Forestry and Fisheries. Retrieved from http://bgis.sanbi.org/NBA/NBA2011_metadata_formalprotectedareas.pdf%5Cnpapers2://publication/uuid/786A77C5-B11A-4F8D-B139-F3F626EBC802

Smith, R. J., Bennun, L., Brooks, T. M., Butchart, S. H. M., Cuttelod, A., Di Marco, M., … Scaramuzza, C. A. d. M. (2019). Synergies between the key biodiversity area and systematic conservation planning approaches. *Conservation Letters*, *12*, e12625. doi: 10.1111/conl.12625

Smith, R. K., Meredith, H. M. R., & Sutherland, W. (2021). Amphibian conservation. In W. J. Sutherland, L. V. Dicks, S. O. Petrovan, & R. K. Smith (Eds.), *What works in conservation 2021*. Cambridge, UK: Open Book Publishers. doi: /10.11647/OBP.0267 In

Smith, R. K., Meredith, H. M. R., & Sutherland, W. J. (2019). Amphibian conservation. In W. J. Sutherland, L. V. Dicks, N. Ockendon, S. O. Petrovan, & R. K. Smith (Eds.), *What works in conservation 2019* (pp. 9–65). Cambridge, UK: Open Book Publishers. doi: 10.11647/obp.0179

Smith, R. K., & Sutherland, W. J. (2014). *Amphibian conservation: Global evidence for the effects of interventions*. Exeter, UK: Pelagic Publishing.

Soto-Sandoval, Y., Suazo-Ortuño, I., Urbina-Cardona, N., Marroquín-Páramo, J., & Alvarado-Díaz, J. (2017). Efecto de los estadios sucesionales del bosque tropical seco sobre el microhábitat usado por *Agalychnis dacnicolor* (Anura: Phyllomedusidae) y *Smilisca fodiens* (Anura: Hylidae). *Revista de Biologia Tropical*, *65*(2), 777–798. doi: 10.15517/rbt.v65i2.24706

South African National Biodiversity Institute (SANBI). (2015). *The business case for biodiversity stewardship. A report produced for the Department of Environmental Affairs*. Pretoria, South Africa.

South African National Biodiversity Institute (SANBI) and Wildlands Conservation Trust. (2015). *Case study: Biodiversity partnership area. uMzimvubu Catchment Partnership*

Programme. Pretoria, South Africa.

Steinfeld, H., Gerber, P., Wassenaar, T., Castel, V., Rosales, M., & de Haan, C. (2006). *Livestock's long shadow: environmental issues and options*. Rome, Italy: Food and Agriculture Organisation (FAO).

Stuart, S. N., Chanson, J. S., Cox, N. A., Young, B. E., Rodrigues, A. S. L. L., … Waller, R. W. (2004). Status and trends of amphibian declines and extinctions worldwide. *Science*, *306*(5702), 1783–1786. doi: 10.1126/science.1103538

Stumpel, A. H. P., & van der Voet, H. (1998). Characterizing the suitability of new ponds for amphibians. *Amphibia-Reptilia*, *19*, 125–142.

Suazo-Ortuño, I., Alvarado-Díaz, J., & Martínez-Ramos, M. (2008). Effects of conversion of dry tropical forest to agricultural mosaic on herpetofaunal assemblages. *Conservation Biology*, *22*(2), 362–374. doi: 10.1111/j.1523-1739.2008.00883.x

Suazo-Ortuño, I., Alvarado-Díaz, J., Mendoza, E., López-Toledo, L., Lara-Uribe, N., Márquez-Camargo, C., … Rangel-Orozco, J. D. (2015). High resilience of herpetofaunal communities in a human-modified tropical dry forest landscape in western Mexico. *Tropical Conservation Science*, *8*(2), 396–423. doi: 10.1177/194008291500800208

Suazo-Ortuño, I., Urbina-Cardona, N., Lara-Uribe, N., Marroquín-Páramo, J., Soto-Sandoval, Y., Rangel-Orozco, J., … Alvarado-Díaz, J. (2018). Impact of a hurricane on the herpetofaunal assemblages of a successional chronosequence in a tropical dry forest. *Biotropica*, *50*(4), 649–663. doi: 10.1111/btp.12544

Sutherland, W. J., Dicks, L. V., Petrovan, S. O., & Smith, R. K. (2021). *What works in conservation 2021*. Cambridge, UK: Open Book Publishers. doi: https://doi.org/10.11647/OBP.0267

Sutherland, W. J., Pullin, A. S., Dolman, P. M., & Knight, T. M. (2004). The need for evidence-based conservation. *Trends in Ecology and Evolution*, *19*(6), 305–308. doi: 10.1016/j.tree.2004.03.018

Tarrant, J., & Armstrong, A. J. (2013). Using predictive modelling to guide the conservation of a critically endangered coastal wetland amphibian. *Journal for Nature Conservation*, *21*, 369–381.

Tarrant, J., Kruger, D., & du Preez, L. H. (2016). Do public attitudes affect conservation effort? Using a questionnaire-based survey to assess perceptions, beliefs and superstitions associated with frogs in South Africa. *African Zoology*, *51*(1), 13–20. doi: 10.1080/15627020.2015.1122554

Thompson, M. E., & Donnelly, M. A. (2018). Effects of secondary forest succession on amphibians and reptiles: A review and meta-analysis. *Copeia*, *106*(1), 10–19. doi: 10.1643/CH-17-654

Tilman, D., Fargione, J., Wolff, B., D’Antonio, C., Dobson, A., Howarth, R., … Swackhamer, D. (2001). Forecasting agriculturally driven global environmental change. *Science*, *292*(5515), 281–284. doi: /10.1126/science.1057544

Tocher, M. D., Gascon, C., & Zimmerman, B. L. (1997). Fragmentation effects on a central Amazonian frog community: A ten-year study. In W. F. Laurance & R. O. Bierregaard (Eds.), *Tropical forest remnants: Ecology, management and conservation of fragmented communities* (pp. 124–137). Chicago, USA: University of Chicago Press.

Toledo, L. F., Becker, C. G., Haddad, C. F. B., & Zamudio, K. R. (2014). Rarity as an indicator of endangerment in Neotropical frogs. *Biological Conservation*, *179*, 54–62. doi: 10.1016/j.biocon.2014.08.012

Trimble, M. J., & van Aarde, R. J. (2014).

Amphibian and reptile communities and functional groups over a land-use gradient in a coastal tropical forest landscape of high richness and endemicity. *Animal Conservation*, *17*(5), 441–453. doi: 10.1111/acv.12111

Tscharntke, T., Klein, A. M., Kruess, A., Steffan-Dewenter, I., & Thies, C. (2005). Landscape perspectives on agricultural intensification and biodiversity - ecosystem service management. *Ecology Letters*, *8*(8), 857–874. doi: 10.1111/j.1461-0248.2005.00782.x

Tscharntke, T., Tylianakis, J. M., Rand, T. A., Didham, R. K., Fahrig, L., Batáry, P., … Westphal, C. (2012). Landscape moderation of biodiversity patterns and processes - eight hypotheses. *Biological Reviews*, *87*(3), 661–685. doi: 10.1111/j.1469-185X.2011.00216.x

Tsianou, M. A., & Kallimanis, A. S. (2016). Different species traits produce diverse spatial functional diversity patterns of amphibians. *Biodiversity and Conservation*, *25*(1), 117–132. doi: 10.1007/s10531-015-1038-x

UNESCO. (2018). Mount Nimba Strict Nature Reserve. Retrieved from whc.unesco.org

Urbina-Cardona, N. (2008). Conservation of Neotropical herpetofauna: research trends and challenges. *Tropical Conservation Science*, *1*(4), 359–375. doi: 10.1177/194008290800100405

Urbina-Cardona, N., Bernal, E. A., Giraldo-Echeverry, N., & Echeverry-Alcendra, A. (2015). El monitoreo de herpetofauna en los procesos de restauración ecológica: indicadores y métodos. In M. Aguilar-Garavito & W. Ramirez (Eds.), *Monitoreo a procesos de restauración ecológica aplicado a ecosistemas terrestres* (pp. 134–147). DC, Colombia: Instituto de Investigación de Recursos Biológicos Alexander von Humboldt. doi:

10.13140/RG.2.1.4400.7129

Urbina-Cardona, N., & Flores-Villela, O. (2010). Ecological-niche modeling and prioritization of conservation-area networks for Mexican herpetofauna. *Conservation Biology*, *24*(4), 1031–1041. doi: 10.1111/j.1523-1739.2009.01432.x

Urbina-Cardona, N., & Londoño-M, M. C. (2003). Distribución de la comunidad de herpetofauna asociada a cuatro áreas con diferente grado de perturbación en la Isla Gorgona, Pacífico colombiano. *Revista de La Academia Colombiana de Ciencias*, *27*(102), 105–113.

Urbina-Cardona, N., & Loyola, R. D. (2008). Applying niche-based models to predict Endangered-Hylid potential distributions: are Neotropical protected areas effective enough? *Tropical Conservation Science*, *1*(4), 417–445. doi: 10.1177/194008290800100408

Urbina-Cardona, N., Olivares-Pérez, M., & Reynoso, V. H. (2006). Herpetofauna diversity and microenvironment correlates across a pasture-edge-interior ecotone in tropical rainforest fragments in the Los Tuxtlas Biosphere Reserve of Veracruz, Mexico. *Biological Conservation*, *132*, 61–75. doi: 10.1016/j.biocon.2006.03.014

Valdez, J. W., Klop-Toker, K., Stockwell, M. P., Fardell, L., Clulow, S., Clulow, J., & Mahony, M. J. (2019). Informing compensatory habitat creation with experimental trials: A 3-year study of a threatened amphibian. *Oryx*, *53*(2), 310–320. doi: 10.1017/S0030605317000394

Valencia-Aguilar, A., Cortés-Gómez, A. M., & Ruiz-Agudelo, C. A. (2013). Ecosystem services provided by amphibians and reptiles in Neotropical ecosystems. *International Journal of Biodiversity Science, Ecosystem Services and Management*, *9*(3), 257–272. doi:

10.1080/21513732.2013.821168

Van Buskirk, J. (2012). Permeability of the landscape matrix between amphibian breeding sites. *Ecology and Evolution*, *2*(12), 3160–3167. doi: 10.1002/ece3.424

van der Ree, R., Jaeger, J. A. G., van der Grift, E. A., & Clevenger, A. P. (2011). Effects of roads and traffic on wildlife populations and landscape function: road ecology is moving toward larger scales. *Ecology and Society*, *16*(1), 48. doi: 10.5751/ES-03982-160148

van Dyke, F., Berthel, A., Harju, S. M., Lamb, R. L., Thompson, D., Ryan, J., … Dreyer, G. (2017). Amphibians in forest pools: Does habitat clustering affect community diversity and dynamics? *Ecosphere*, *8*(2), e01671. doi: 10.1002/ecs2.1671

van Wilgen, B. W. (2009). The evolution of fire and invasive alien plant management practices in fynbos. *South African Journal of Science*, *105*, 335–342.

Vannini, A., Bruni, G., Ricciardi, G., Platania, L., Mori, E., & Tricarico, E. (2018). *Gambusia holbrooki*, the 'tadpolefish': The impact of its predatory behaviour on four protected species of European amphibians. *Aquatic Conservation: Marine and Freshwater Ecosystems*, *28*(2), 476–484. doi: 10.1002/aqc.2880

Varumo, L., Yaneva, R., Koppel, T., Koskela, I. M., Garcia, M. C., Sozzo, S., … Dictor, M. C. (2020). Perspectives on citizen engagement for the EU post-2020 biodiversity strategy: an empirical study. *Sustainability*, *12*(4), 1532. doi: 10.3390/su12041532

Vasconcelos, T. S., Santos, T. G., Rossa-Feres, D. C., & Haddad, C. F. B. (2009). Influence of the environmental heterogeneity of breeding ponds on anuran assemblages from southeastern Brazil. *Canadian Journal of Zoology*, *87*(8), 699–707. doi: 10.1139/Z09-058

Venter, O., Fuller, R. A., Segan, D. B., Carwardine, J., Brooks, T., Butchart, S. H. M., … Watson, J. E. M. (2014). Targeting global protected area expansion for imperiled biodiversity. *PLoS Biology*, *12*(6), e1001891. doi: 10.1371/journal.pbio.1001891

Vergara-Ríos, D., Montes-Correa, A. C., Urbina-Cardona, J. N., De Luque-Villa, M., Cattan, P. E., & Granda-Rodríguez, H. D. (2021). Local community knowledge and perceptions in the Colombian Caribbean towards amphibians in urban and rural settings: Tools for biological conservation. *Ethnobiology and Conservation*, *10*, 24. doi: 10.15451/ec2021-05-10.24-1-22

Walker, L. R., Wardle, D. A., Bardgett, R. D., & Clarkson, B. D. (2010). The use of chronosequences in studies of ecological succession and soil development. *Journal of Ecology*, *98*(4), 725–736. doi: 10.1111/j.1365-2745.2010.01664.x

Watling, J. I., Arroyo-Rodríguez, V., Pfeifer, M., Baeten, L., Banks-Leite, C., Cisneros, L. M., … Fahrig, L. (2020). Support for the habitat amount hypothesis from a global synthesis of species density studies. *Ecology Letters*, *23*(4), 674–681. doi: 10.1111/ele.13471

Watling, J. I., & Braga, L. (2015). Desiccation resistance explains amphibian distributions in a fragmented tropical forest landscape. *Landscape Ecology*, *30*(8), 1449–1459. doi: 10.1007/s10980-015-0198-0

Watling, J. I., Nowakowski, A. J., Donnelly, M. A., & Orrock, J. L. (2011). Meta-analysis reveals the importance of matrix composition for animals in fragmented habitat. *Global Ecology and Biogeography*, *20*(2), 209–217. doi: 10.1111/j.1466-8238.2010.00586.x

Wells, K. D. (2007). *The ecology and behavior of amphibians*. Chicago, USA: University of Chicago Press.

Whitfield, S. M., & Pierce, M. S. F. (2005). Tree buttress microhabitat use by a Neotropical leaf-litter herpetofauna. *Journal of Herpetology*, *39*(2), 192–198.

World Bank. (2019). *Forest-smart mining: Offset case studies*. 76. Retrieved from https://www.profor.info/sites/profor.info/files/FOREST-SMART MINING_Offset Case Studies.pdf

World Conservation Society. (2020). *Proyecto Estrategia de Conservación de Anfibios de Colombia*. Bogotá, Colombia.

WWF. (2020). *Living Planet Report 2020: Bending the curve of biodiversity loss*. Gland, Switzerland.

Zabala-Forero, F., & Urbina-Cardona, N. (2021). Respuestas de la diversidad taxonómica y funcional a la transformación del paisaje: relación de los ensamblajes de anfibios con cambios en el uso y cobertura del suelo. *Revista Mexicana de Biodiversidad*, *92*, e923443. doi: 10.22201/ib.20078706e.2021.92.3443

zu Ermgassen, S. O. S. E., Baker, J., Griffiths, R. A., Strange, N., Struebig, M. J., & Bull, J. W. (2019). The ecological outcomes of biodiversity offsets under “no net loss” policies: A global review. *Conservation Letters*, *12*(6), e12664. doi: 10.1111/conl.12664

Chapter 6. Infectious diseases

Molly C. Bletz[1,2], Brian Gratwicke[3,4], Anat Belasen[5], Alessandro Catenazzi[6], Amanda L. J. Duffus[7], Margarita Lampo[8,9], Deanna Olson[10] and Karthik Vasudevan[11]

[1]Amphibian Survival Alliance

[2]University of Massachusetts Amherst, Amherst, MA, USA

[3]Smithsonian's National Zoo, Washington DC, USA

[4]Conservation Biology Institute, Front Royal, VA, USA

[5]Department of Integrative Biology, College of Natural Sciences, University of Texas at Austin, Austin TX, USA

[6]Florida International University, Miami, FL, USA

[7]Department of Natural Sciences, Gordon State College, Barnesville, GA, USA

[8]Fundación para el Desarrollo de la Ciencias Físicas, Matemáticas y Naturales (FUDECI), Venezuela

[9] Instituto Venezolano de Investigaciones Científicas (IVIC), Venezuela

[10]USDA Forest Service, Pacific Northwest Research Station, Corvallis, OR, USA

[11]CSIR-Centre for Cellular and Molecular Biology, Laboratory for the Conservation of Endangered Species, Hyderabad, India

[12]IUCN Amphibian Specialist Group, Amphibian Disease Working Group

Abstract

Emerging infectious diseases are major threats to amphibian biodiversity. Significant advances in our understanding of these diseases have been made with respect to the pathogens themselves, the amphibian hosts and how they respond to and defend against pathogens, and the environment conditions that can influence the course of disease. Here, we review recent advances in our understanding of infectious diseases of amphibian related to these three components – pathogen, host, and environment -, and identify information gaps as research priorities. In particular, we highlight current diagnostic tools, we focus on ecological dimensions with relevance to development effective management strategies as well as provide a review of current proposed intervention strategies. We also discuss human dimensions of amphibian diseases with a focus on management and policy actions that can confront these threats and potentially minimise disease-driven declines at local and global scales.

Introduction

Pathogens and parasites including viruses, bacteria, protozoa, fungi, helminths and arthropods infect amphibians (Densmore & Green, 2007). Our knowledge of amphibian diseases and how to diagnose and treat them has improved dramatically in recent years, in part due to efforts of pathologists and veterinarians working with captive zoo collections (Wright & Whitaker, 2001), and work of molecular biologists and ecologists (Byrne et al., 2019; Rebollar et al., 2016). Infectious diseases are a natural part of any functioning ecosystem, and may fluctuate in natural cycles, leading to constraints between transmission and virulence fuelled by natural selection (Boots & Sasaki, 2003). Pathogens do not generally make their hosts go extinct, because that would also result in extinction of the pathogen, but exceptions may occur (De Castro & Bolker, 2005).

Emerging wildlife diseases are usually caused by invasive pathogens or parasites that spread to areas inhabited by naive hosts that do not have natural defences leading to population declines (Langwig et al., 2015). Amphibian populations have disappeared worldwide, primarily in places that have no evolutionary history with the disease, although the exact number of species affected remains controversial (Lambert et al., 2020; Scheele, Pasmans, et al., 2019). Indeed, proving disease-induced declines is a challenging task and simply surveying for a pathogen or disease in a declining population is not sufficient to infer causality (Pessier, 2017). The gold standard for demonstrating disease-related declines involves collecting population data prior to emergence, estimating disease prevalence, observing disease signs and population effects or mortality, isolating the pathogen and fulfilling Koch's postulates e.g. (Martel et al., 2013). These steps require substantial resources not normally devoted to wildlife taxa, and may partly explain why it took so long for amphibian diseases to be attributed to 'enigmatic amphibian declines' (Collins, Crump, & Lovejoy, 2009; Collins, 2010).

Many factors influence the course of disease including the pathogen, the host and the environment (Figure 6.1). We consider recent advances in our understanding of infectious amphibian diseases related to these three components as well as human dimensions (Figure 6.1). We identify information gaps as research priorities for the revised Amphibian Conservation Action Plan.

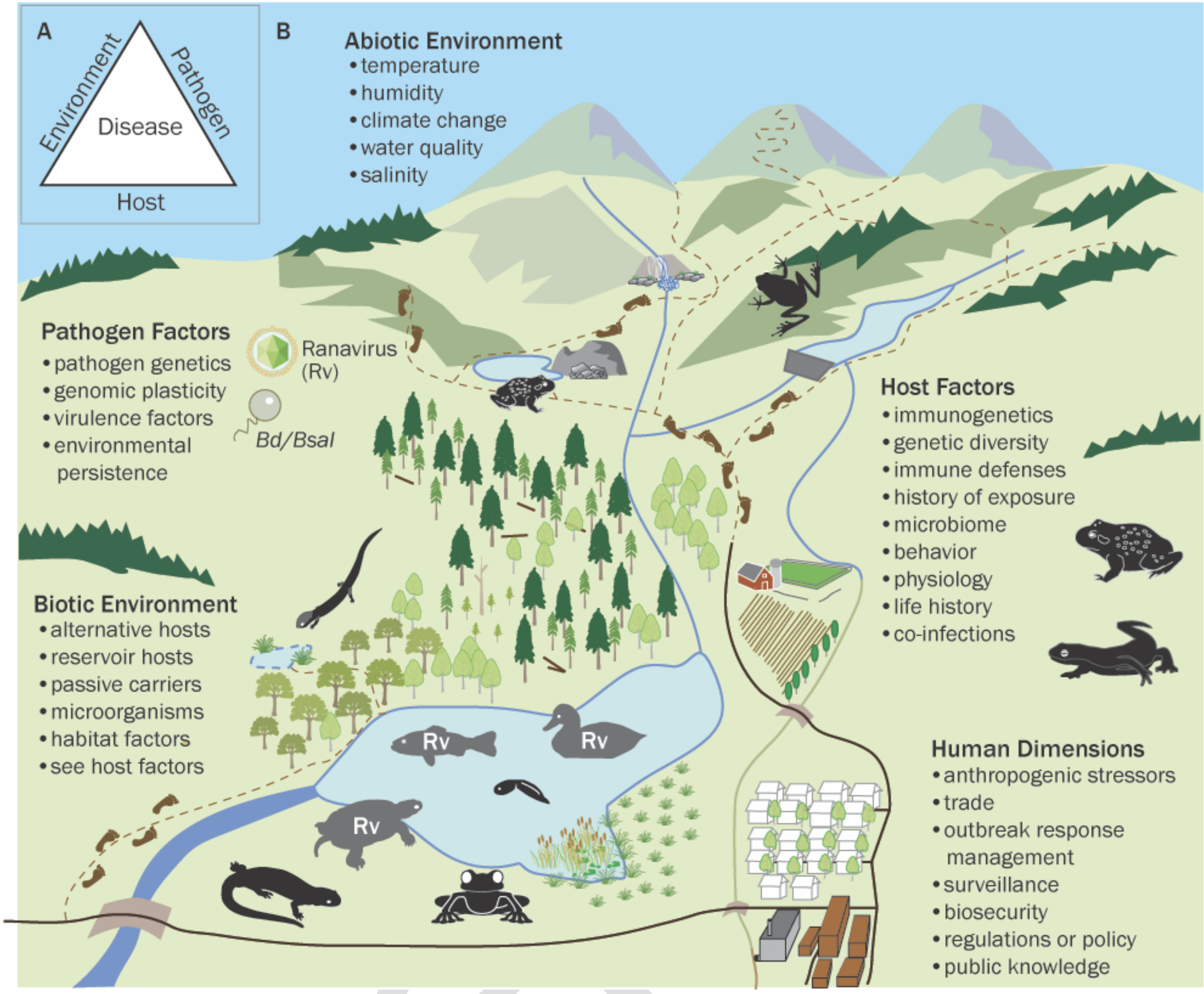


Figure 6.1: Schematic representation of specific elements of the disease triangle (A). Disease may develop where conducive factors of the environment (abiotic, biotic, human dimensions), pathogen, and host overlap (B). Inspired by Fisher & Garner (2020).

Status Update

Pathogen

A pathogen is a microscopic infectious viral, bacterial or mycotic agent that causes disease in a host, and various macroscopic parasites such as helminths, protozoa and arthropods also cause well-known diseases and illness in amphibians (Densmore & Green, 2007). General veterinary approaches have been developed for diagnosing and treating various amphibian diseases (Densmore & Green, 2007; Wright & Whitaker, 2001), but much attention has focused on context-dependent responses to emerging diseases (Langwig et al., 2015).

Emerging amphibian diseases

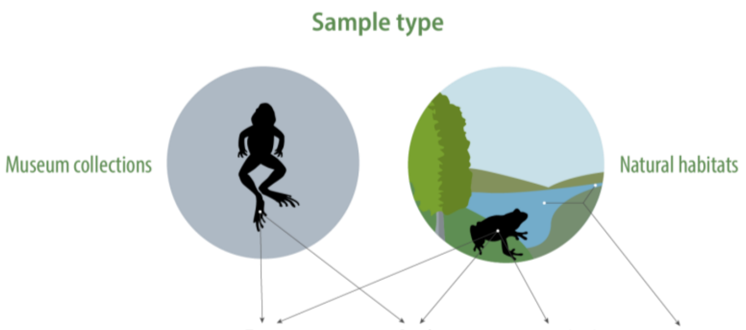
In the last 15 years, understanding of emerging amphibian pathogens has grown immensely (Table 6.1). Ranavirus emergence in naive amphibian populations has been associated with steep amphibian population declines of multiple species in Europe (Price et al., 2014; Teacher, Cunningham, & Garner, 2010)about:blank. Whereas ranaviruses have been documented globally, their population-level impacts in many places have not yet been adequately assessed (Brunner, Olson, Gray, Miller, & Duffus, 2021; Duffus et al., 2015). Three ranavirus species are known to affect amphibians, *Ambystoma tigrinum virus* (ATV), *Common midwife toad virus* (CMTV) and *Frog virus 3* (FV3) (Chinchar et al., 2017), but FV3 and CMTV are known to recombine as chimeric ranaviruses that have increased virulence and pose a large threat to wild populations (Peace et al., 2019; Vilaça et al., 2019). *Batrachochytrium dendrobatidis* (Bd) was described in 1999 (Longcore, Pessier, & Nicholes, 1999), but in 2013 a new *Batrachochytrium* species was reported, *B. salamandrivorans* (Bsal:(Martel et al., 2013)). Bd has a global distribution on every continent (James et al., 2015; Olson et al., 2013; Olson, Ronnenberg, Glidden, Christensen, & Blaustein, 2021), and Bsal has a restricted distribution in Asia, where it originates; it is also found in Europe where it is invasive and spreading in European salamanders (particularly fire salamanders) (Beukema et al., 2018; Lötters & Vences, 2020; Spitzen-van der Sluijs et al., 2016). In the US, a pathogenic protist causes severe Perkinsea infections resulting in mortality of tadpoles, a potential third emerging infectious disease of amphibians (Isidoro-Ayza et al., 2017). *Elizabethkingia miricola* is an example of a recently discovered emerging bacterial disease in amphibians. This zoonotic pathogen can also affect humans, and causes meningitis-like symptoms and mass die-offs in Chinese spiny frogs (*Quasipaa spinosa*) farmed for food (Hu, Dong, Kong, Mao, & Zheng, 2017; Lei et al., 2019).

Table 6.1: Known emerging amphibian infectious pathogens, and their characteristics.

Emerging Infectious Pathogens	**Type**	**Susceptible taxa**	**Known Distribution**
Ranavirus	Virus	Amphibians, Reptiles, Fish	Global
Batrachochytrium dendrobatidis	Fungus	Amphibians, susceptible species concentrated in the Americas and Oceania.	Global
Batrachochytrium salamandrivorans	Fungus	Primarily salamanders, with alternate amphibian hosts	Asia (Native range) Europe (Invasive)
Perkinsea	Protist	Amphibians, primarily tadpoles	US, Europe, MesoAmerica
Elizabethkingia miricola	Bacteria	Anurans, additional concern as it is a zoonotic pathogen. (mostly in captive and frog farm settings)	China, Europe, Madagascar, possibly global.

Diagnostics and monitoring

Amphibian pathologists have established a growing body of diagnostic knowledge that has improved our ability to evaluate disease signs and attribute them to causative agents that may have historically been dismissed as "Red Leg Disease" (Forzán, Heatley, Russell, & Horney, 2017; Pessier, 2017). The fact that severe Perkinsea infections were only recently discovered as a source of amphibian mortality in the US is a lesson to retain disciplined vigilance when examining new amphibian mortality events and declines. Multiple tools are available for detecting pathogens, confirming infection and diagnosing disease. Histology and microscopy remain the primary tool of pathologists forensically examining contemporary specimens, especially when preservation techniques limit use and consistency of DNA-based diagnostic tools, but advances in isolation techniques, molecular methods and DNA sequencing have expanded our understanding of amphibian pathogens, including lineage/strain distribution, genetic variation and virulence factors (Figure 6.2). However, documenting a pathogen is not necessarily indicative that it causes disease and decline (Russell et al., 2019). To better understand if a host is susceptible, tolerant or resistant, infection must be linked to longer-term clinical disease outcomes, such as death, persisting with infections, or clearing infections (Figure 6.3).



	Tissue	Swab	Water bath	eDNA
Histology	●			
Isolation	●[a]			
Molecular	●	●	●	●
Antigen-based	●[b,c]	●	●	

[a]Not from fixed tissues, [b]Virus only, [c]Blood sample

- Techniques
- Advantages
- Limitations
- * Test are not validated

Histological examination

- **Hematoxylin/Eosin Stain** (all pathogens).[1]
- **Immunohistochemistry** (all pathogens).[2]
- **In-situ hybridization assay** (Bd/Bsal).[3]

- Confirms true infection and disease.
- Can concurrently see histopathology.
- Detect co-infection.

- Low-moderate sensitivity.[4]
- Specialized personnel required.
- Can be expensive.
- Invasive or lethal sample.
- Time-consuming.

Isolation

- **Tail/toe clip** (Bd/Bsal).[5]
- **Internal organ, tail/toe clip***(Rv).[6]

- Diagnoses active infection.
- More detailed studies possible from obtained culture (genomics[7], experiments, etc.).

- Tissue required from living animal (Bd/Bsal).
- Time-consuming.
- High failure rate.
- Contamination prone.

Molecular examination

- **Traditional PCR.**[8]
- **Nested PCR.**[9]
- **Quantitative PCR.**[10]
- **Digital droplet PCR.**[11]
- **Fluidigm assay.**[12]
- **Metagenomic sequencing.**

- High sensitivity – detects low level infections.[5]
- Quantitative (selected techniques).
- High specificity (Bd/Bsal/Rv specific assays).
- Genotype specific detection possible.[13]
- Relatively quick and inexpensive.
- Can be done from non-invasive samples.
- Widely accessible (tPCR, nested qPCR).

- Only confirms presence of DNA, not infection or disease.
- Risk of DNA contamination or PCR inhibition possible.
- Can be expensive (Taqman, fluidigm).
- Fluidigm assay required high quality DNA and does work with low infection levels.
- eDNA detection cannot confirm which species in community are infected.

Antigen-based

- **Antigen-capture ELISA** (Rv only1[4]).

- Quick and cheaper than molecular methods.
- High sensitivity and specificity.

- Only works from blood samples.

- **Lateral flow assay** (*Batrachochytrium*[15]).

- Very quick.

- Poor sensitivity and specificity.
- Expensive.

[1]Berger et al. 1999, Forzan et al. 2017; [2]Olsen et al. 2004, Jerret et al. 2015; [3]Ossiboff et al. 2019, Forzan et al. 2019; [4]Kriger et al. 2006; [5]Cook et al. 2018; [6]Granoff et al.1965, Balseiro et al. 2009; [7]Rosemblum et al. 2008, O'Hanlon et al. 2018; [8]Annis et al. 2004; [9]Goka et al. 2009; [10]Boyle et al. 2004, Bloi et al. 2013, Standish et al. 2018; [11]Pinheiro et al. 2012; [12]Bryne et al 2019; [13]Ghosh et al. 2020; [14]Kim et al 2015; [15]Dillon et al. 2017.

Figure 6.2: Diagnostic tools for amphibian pathogens and disease. Diagnostic screening techniques can be applied to museum specimens, field-caught individuals as well as environmental substrates (e.g. water). Histological, isolation, molecular and antigen-based tools are available, each with their own set of advantages and disadvantages. Histological examination is still the only method capable of diagnosing clinical infection and disease, but has from low to moderate sensitivity and is costly. Isolation of pathogens can be difficult, but is essential for developing a deeper understanding of pathogens, their ecology, physiology and behaviour. Molecular tools offer non-invasive sampling methods and high sensitivity for detecting genetic material of pathogens, but quantitative PCR (qPCR) based methods don't come without important caveats. There can be wide variation in quantification according to laboratory methods making direct comparisons across studies difficult. This variation may be attributed to using different standard cultures/strains of Bd, different qPCR cycling parameters and molecular techniques, and different DNA extraction techniques (Bletz et al., 2015, Brannelly, Wetzel, West, & Richards-Zawacki, 2020). There has been an attempt to standardise across studies using Bd ITS copy number standards (Longo et al., 2013; Rebollar, Woodhams, LaBumbard, Kielgast & Harris., 2017); however, with variation in ITS within the fungal genomes the biological meaning can be skewed (e.g. 1000 ITS copies could be equivalent to 2 zoospores if copy number is 500, or 200 zoospores if the copy number is 5). It is additionally important to understand the detection limits of molecular techniques like qPCR. Low and inconsistent qPCR positives may be false positives, and mutations in the ITS region at the Taqman probe binding site in certain regions, such as Asia, can lead to false negatives (Mutnale et al., 2018). This caveat is also true for qPCR methods used to detect ranaviral DNA (Wynne, Puschendorf, Knight, & Price, 2020). Development of the lateral-flow assay by Dillon et al. (2017) shows some promise; however, this assay lacks specificity (it cross-detects related fungi) and sensitivity (it fails to detect low loads).

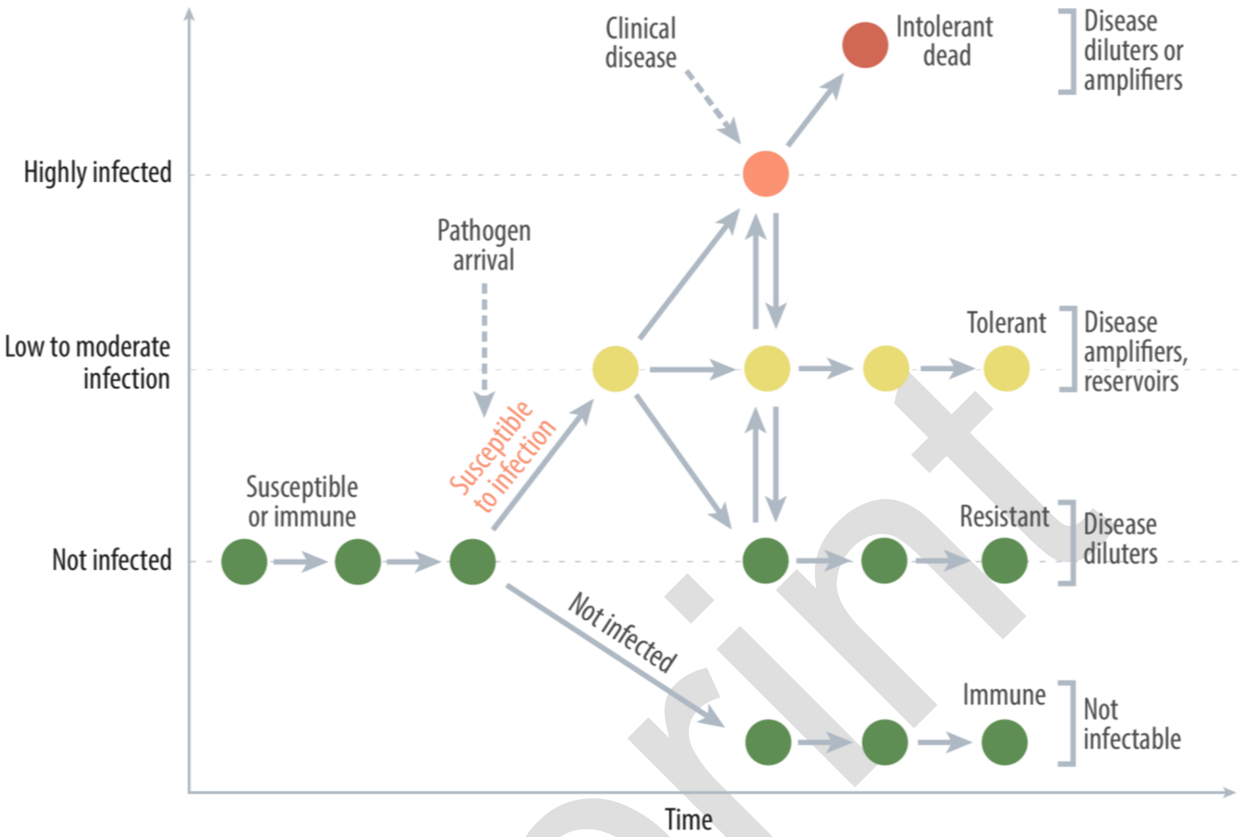


Figure 6.3: Diagrammatic representation of outcomes for amphibian hosts when exposed to a potential pathogen. **Immune** refers to individuals that cannot be infected. **Susceptible** refers to individuals that can become infected. **Resistant** refers to individuals that, once infected, exhibit resistance mechanisms that lower or eliminate the infection. **Tolerant** represents individuals that can survive infection and build up high infection loads with little negative impact. **Intolerant** refers to hosts that exhibit clinical disease and can ultimately succumb to infection (i.e., experience disease-induced mortality). These host states are not necessarily static across host species, populations, or individuals, and can vary with endogenous and exogenous factors.

Disease origins and virulence

The genomics revolution has advanced our understanding of the origins of amphibian pathogens, the multitude of pathogen genotypes, and virulence factors that make these pathogens deadly. Evidence suggests both Bd and Bsal originate in Asia - work that has been facilitated by improved isolation methods and genome sequencing (O'Hanlon et al., 2018). Our understanding of Bd has moved beyond seeing it as a singular pathogen to an understanding of a complex matrix of genotypes, some of which are endemic and others pandemic lineages that vary in virulence (Byrne et al., 2019; Jenkinson et al., 2016; Rosenblum et al., 2013). Bd genotypes have been cultured from hotspots, and whole genome sequences of globally distributed strains are identified: Bd GPL (Global Panzootic Lineage), Bd CAPE (Africa and Europe), Bd ASIA 1 (Asia), Bd Asia 2/ BRAZIL and Bd ASIA (O'Hanlon et al., 2018). Most cultured Bd isolates belong to Bd GPL lineage (Fisher, Hawkins, Sanglard, & Gurr, 2018), and that has led to strengthening of our knowledge about the GPL impacts on amphibians. In Bd infection 'coldspots' (e.g., Asia and Africa), where prevalence is low and Bd persists in amphibian populations (Mutnale et al., 2018), probability of obtaining pathogen cultures is poor, limiting our capacity to adequately understand the emergence and epidemiology of chytridiomycosis globally. Enzootic genotypes may be dominant in such regions and hybridization of enzootic hypovirulent and panzootic hypervirulent Bd strains can result in genotypes that show high virulence on native hosts (Greenspan et al., 2018). Similarly, recombination of ranaviruses can result in changes in virulence (Peace et al., 2019; Vilaça et al., 2019), while bacteria, particularly zoonotic pathogens like *Elizabethkingia miricola,* frequently evolve antibiotic resistance, reducing our ability to treat host infections (Lei et al., 2019). Lineage-specific diagnostics as well as genomic tools that don't require culturing can help fill this gap (Ghosh et al., 2021). The ability to genotype Bd from swab DNA has given the field an invaluable technique to understanding global Bd lineage distribution (Byrne et al., 2019).

Cultured isolates, experimental infection trials and -omics techniques have also expanded our understanding of virulence factors and mechanisms that may induce disease. Genomic and transcriptomic comparisons of Bd/Bsal as well as endemic and pandemic Bd genotypes show us signatures of virulence including metalloproteases, serine proteases and crinkle-like proteins (Ellison, DiRenzo, McDonald, Lips, & Zamudio, 2017; Farrer et al., 2017; McDonald, Longo, Lips, & Zamudio, 2020). Further understanding about these pathogens will emerge as culturing efforts and genomic techniques for Bd and Bsal are intensified globally (Fisher, Ghosh, et al., 2018).

Future steps & recommendations

Significant gaps in our knowledge of these pathogens remain. Greater understanding of hot and cold spots for pathogen presence and disease can give us a lens into what environmental conditions, host properties, and interactions between these allow amphibians to survive these diseases in nature and in-turn guide management for susceptible populations. Currently, there is no rapid, field-ready test for prominent amphibian pathogens. Such diagnostic tools could rapidly improve our understanding of pathogen distributions and fill rapid-detection needs, while genomic innovations like high-throughput sequences can continue to push the bounds of pathogen discovery globally.

Host

Once a pathogen infects a host, the host may survive through resistance mechanisms (e.g., the pathogen induces an effective immune response that reduces pathogen burden and clears infection), or through tolerance (i.e., negative impacts are minimised while the pathogen continues replicating and spreading (Figure 6.3)). However, if pathogen burdens increase to a level resulting in clinical disease, the burden to the host is magnified and may result in death if infection is not reduced or treated (Figure 6.3). Reviews are available for the patho-physiology of Bd (Baitchman & Pessier, 2013), Bsal (Martel et al., 2013), and ranaviruses (Miller, Pessier, Hick, & Whittington, 2015).

Host range & susceptibility

Host range differs between Bd, Bsal, and Ranavirus. Bd can infect all three amphibian orders (Olson et al., 2021); Bsal is known to infect anurans and caudates while disease primarily occurs in salamanders (Martel et al., 2014; Stegen et al., 2017); and ranaviruses infect amphibians, reptiles and fish (Brenes, Gray, Waltzek, Wilkes, & Miller, 2014; Duffus et al., 2015). Experimental infection of hosts can advance our understanding of host-pathogen-environment dynamics (Blaustein et al., 2018).

Host life stage also affects infection and disease progression. For Bd, larvae are typically tolerant, while recent metamorphs and juveniles experience higher mortality (Böll, Tobler, Geiger, Hansbauer, & Schmidt, 2012; Garner et al., 2009; Russell, Goldberg, Waits, & Rosenblum, 2010). Adults vary widely in susceptibility, tolerance, and resistance (Fig. 6.3). Bsal chytridiomycosis has only been documented in post-metamorphic amphibians to date. For ranavirus, larvae tend to be particularly vulnerable to disease and mortality (Duffus, Nichols, & Garner, 2014; Hoverman, Gray, Miller, & Haislip, 2012), but adults of some species show disease signs (Duffus, Nichols, & Garner, 2013).

There is wide variation in disease outcomes across host populations, space, and time (Bradley et al., 2015; Briggs, Knapp, & Vredenburg, 2010; Savage, Sredl, & Zamudio, 2011; Searle et al., 2011). Host susceptibility can shift over time as with Bd in South America (Becker, Rodriguez, Lambertini, Toledo, & Haddad, 2016; Carvalho, Becker, & Toledo, 2017; von

May, Catenazzi, Santa-Cruz, Kosch, & Vredenburg, 2020) and Panama (Voyles et al., 2018). Although Bd has been implicated in a number of species extinctions, several populations that were formerly believed extirpated have been "rediscovered" by scientists (Abarca, Chaves, Garcia-Roodriguez, & Vargas, 2010; Chaves et al., 2014; García-Rodríguez, Chaves, Benavides-Varela, & Puschendorf, 2012; González-Maya et al., 2013; Newell, Goldingay, & Brooks, 2013; Puschendorf, Hodgson, Alford, Skerratt, & VanDerWal, 2013; Rodríguez-Contreras, Señaris, Lampo, & Rivero, 2008; Whitfield et al., 2017). These cases present opportunities to understand what contributes to population recovery and mobilise this knowledge for conservation.

Differential disease outcomes over space and time may be related to host and ecological factors that mediate host susceptibility to infection and disease. Individual host factors include host defence mechanisms, e.g. innate and acquired immunity, and defence resulting from host-associated microbiomes. Ecological factors include biotic factors (*e.g.*, dilution effects, reservoir species, super-shedders), and abiotic conditions that impact host ecology and physiology. Understanding how these factors mediate host susceptibility is important for disease management and conservation.

Host defence mechanisms

Innate and acquired immunity play a role in amphibian pathogen infections, varying across host species and environments. Innate immune mechanisms comprise the first line of defence against infections and show general efficacy for a variety of pathogens (Conlon, 2011; Rollins-Smith, 2009; Smith et al., 2018). Acquired or adaptive immune mechanisms, such as the major histocompatibility complex pathway and T and B cells, provide a more specific pathogen response and are linked to both host genotype and exposure history. However, Bd can sometimes inhibit immune responses, limiting amphibians' ability to mount a robust adaptive response to Bd (Fites et al., 2014). MHC Class I molecules are hypothesised to mainly be associated with immune responses to ranaviruses (Teacher, Garner, & Nichols, 2009; Wang et al., 2017). MHC II immuno-genotype has been associated with susceptibility to Bd (Bataille et al., 2015; Kosch et al., 2016; Savage & Zamudio, 2011), ranavirus (Savage, Muletz-Wolz, Campbell Grant, Fleischer, & Mulder, 2019), and other potentially pathogenic microbes (Belasen, Bletz, Leite, Toledo, & James, 2019)about:blank. While immunity in amphibian larvae is less well-studied, tadpoles are known to have less functionally developed immune systems accompanied by immunosuppression through metamorphosis, while MHC expression expands greatly post-metamorphosis (Grogan et al., 2018).

Advances in molecular technologies, including high-throughput sequencing and transcriptomics, have deepened our understanding of cellular defence mechanisms and immune variation within and among host species (Zamudio, McDonald, & Belasen, 2020). Common Bd response mechanisms include skin repair (Ellison et al., 2014; Eskew et al., 2018; Poorten & Rosenblum, 2016) and innate and acquired immune activation (Ellison et al., 2017, 2014; McDonald et al., 2020). Recent studies have found that animals that down-regulated immune genes tolerated Bd infections better and highly susceptible species significantly upregulate immune responses (Savage, Gratwicke, Hope, Bronikowski, & Fleischer, 2020). These results suggest that immunopathology is a component of Bd susceptibility. Amphibian immune response reviews are available (chytridiomycosis: (Grogan et al., 2020; Grogan et al., 2018); ranavirus: (Grayfer, Edholm, De Jesús Andino, Chinchar, & Robert, 2015).

Improvements in our understanding of amphibian immunity have applications for disease management and mitigation, for example, selective breeding for genetically resistant or tolerant individuals, or development of vaccines that prime immune responses (Table 6.2). Vaccines against Bd have shown mixed success (e.g., (Stice & Briggs, 2010), perhaps because Bd-produced toxins inhibit amphibian adaptive immune response (Fites et al., 2013). Ranavirus vaccine trials, however, have shown promising results (Chen, Li, Gao, Wang, & Zhang, 2018; Zhou, Zhang, Han, Jia, & Gao, 2017).

Table 6.2 Overview of amphibian disease mitigation interventions targeting amphibian hosts.

Intervention	**Evidence**	***In situ* examples**
Treatment of the host directly with antifungals, antibiotics, deworming agents.	Treating hosts directly for the pathogen are widely used in veterinary medicine (Baitchman & Pessier, 2013; Wright & Whitaker, 2001), but they are mostly applicable in controlled settings and do not prevent reinfection.	Cascades frogs treated with itraconazole and released back into natural ponds showed reduced Bd pathogen burden and increased over-winter survival (Hardy, Pope, Piovia-Scott, RN, & Foley, 2015). Treatment of mountain chickens for Bd using itraconazole without environmental pathogen reduction had only short-term benefits (Hudson et al., 2016).
Treatment of host and translocation to disease-free refuge or disinfection of environment prior to reintroduction.	Disinfectants can be applied directly to the environment with varying environmental impacts (Lammens, Martel, & Pasmans, 2021; Rütte, Peyer, Schmidt, Keller, & Geiser, 2009).	Successful at controlling Bd on the island of Mallorca, with limited Mallorcan midwife toads and limited habitat (Garner et al., 2016). An attempt to create a Bd-free population of Archey's frogs through translocation in New Zealand was unsuccessful (Linhoff et al., 2021).

Translocation of individuals with resistant genotypes.	Recovering amphibian populations that have evolved resistance or tolerance to disease could serve as founders for low-cost reintroductions to historical sites (Mendelson, Whitfield, & Sredl, 2019). Genetic markers may be difficult to identify as resistance traits may be associated with reduced gene expression (Savage et al., 2020).	Not attempted yet, likely due to lack of knowledge of genotypes and/or concerns about negatively impacting recovering populations of threatened species.
Selective breeding for resilience traits.	The effectiveness of skin mucus secretions of frogs that survived a Bd epizootic became more inhibitory, providing evidence of natural selection that has the potential to be applied to captive populations (Scheele et al., 2014; Voyles et al., 2018).	Not attempted yet, due to high technical requirements, multigenerational timelines, and a need to better understand resistance mechanisms or genetic markers. Once clear resistance-associated genes are identified, genetic engineering for resistance may be a further possibility as has been used in American chestnuts (Newhouse et al., 2014).

Density reduction of hosts to reduce disease transmission.	Contact rates were reduced in low density groups of newts, suggesting reduced density may reduce Bsal transmission and spread (Malagon et al., 2020). However, a field experiment found that Bd was effectively transmitted between tadpoles regardless of density (Rachowicz & Briggs, 2007).	Translocation of limited numbers of mountain yellow-legged frog tadpoles to create new low-density populations were unsuccessful at preventing outbreaks (Woodhams et al., 2011). It seems unlikely that deliberately reducing healthy threatened amphibian populations to reduce disease risk would be justified by experimental evidence.
Increase population buffering capacity through head starting, captive-releases.	Demographically, increasing recruitment rates compensates for disease-related mortality (Lampo et al., 2017; Muths et al., 2011; Scheele et al., 2014)	Populations of wild Corroboree frogs declining due to Bd have been supplemented from captive populations and raised in predator-free enclosures to help sustain wild populations (Campbell, Australia, Environment Australia, Biodiversity Group, & Natural Heritage Trust (Australia), 1999; Linhoff et al., 2021). Head starting has helped to avoid extinctions and grow populations of Agile frogs in the UK and Northern Leopard Frogs in

		Canada (Linhoff et al., 2021), but has been unsuccessful at re-establishing breeding populations of Wyoming toads (Polasik, Murphy, Abbott, & Vincent, 2016). It is likely that success or failure of these efforts will be highly context-specific, and more studies are needed.
Augmenting protective skin microbes using probiotics.	Probiotics aim to boost host immunity in the mucosal environment through the addition of locally occurring, Bd-protective skin microbes to amphibians (Bletz et al., 2013). Experimental trials have given mixed results, some have been successful or partly successful (Bletz et al., 2018; Harris et al., 2009; Kueneman, Woodhams, Harris, et al., 2016; Muletz, Myers, Domangue, Herrick, & Harris, 2012), and others have been ineffective (Becker et al., 2011, 2015; Woodhams et al.,	One *in situ* field trial that augmented mountain yellow-legged frogs with *Janthinobacterium lividum* was associated with reduced Bd pathogen loads and improved survival after one season, but the population did not persist in the long term (Vredenburg, Briggs, & Harris, 2011). Our understanding of the role of skin microbiomes and immune function is not yet developed enough to reliably manipulate microbiomes to impart a desired function, and further research is needed to

	2012), and one study that genetically modified a core skin microbe to produce antifungal metabolites did not confer disease protection (Becker et al., 2021).	understand the relationship between host, pathogen and microbiome.
Vaccines	Effective ranavirus vaccines have been developed and used in Chinese giant salamanders (Chen et al., 2018; Zhou et al., 2017). Vaccinations for Bd however have been ineffective, or only weakly improve the ability to combat infection (Cashins et al., 2013; McMahon et al., 2014; Stice & Briggs, 2010), but recent attempts in Vegas valley leopard frogs have demonstrated improved effectiveness of previous exposure conferring improved survival (Waddle et al., 2021)	Not attempted yet, but has high potential, especially with long-lived species like giant salamanders. Whether highly effective Bd and Bsal vaccines can be developed remains unknown. Detailed studies of amphibian immune functional responses due to vaccination would be useful to improve Bd vaccine types, and delivery mechanisms, effectiveness and range of applicability.

Host-associated microbiomes

Host defences also include resident symbiotic bacteria, fungi and other micro-eukaryotes living on/in hosts, collectively called the host-associated microbiome. Mounting evidence suggests these communities play a role in disease dynamics (Jiménez & Sommer, 2016). High-throughput sequencing has enabled characterisations of microbiome communities of diverse amphibians, enhancing our ability to understand the protective role these communities play (Kueneman et al., 2019). Thousands of bacteria have been cultured from amphibian skin and tested for inhibition against *Batrachochytrium* pathogens (Bletz et al., 2017; Woodhams et al., 2015). Bd and Bsal may induce shifts in the microbiome (Bletz et al., 2018; Jani & Briggs, 2014), and microbiome composition may predict disease susceptibility (Becker et al., 2015). Populations with higher proportions of frogs with Bd-inhibiting skin bacteria may persist through Bd emergence (Lam, Walke, Vredenburg, & Harris, 2010; Woodhams et al., 2007), and cultured skin bacteria can inhibit Bd and Bsal in vitro (Bletz et al., 2017; Woodhams et al., 2015). Recent studies have explored the "mycobiome" (Kearns et al., 2017) as well as the full microeukaryotic community (Kueneman, Woodhams, Van Treuren, et al., 2016), and how these communities interact with bacteria (Belasen et al., 2021). Skin and gut bacterial microbiomes have also been associated with ranavirus susceptibility in laboratory and field studies (Harrison et al., 2019; Warne, Kirschman, & Zeglin, 2019). Modulating host immunity through probiotic bioaugmentation of hosts or their environments has been proposed as a disease mitigation strategy to capitalise on the role of these microbial communities (Table 6.2, reviewed in Bletz (2013) and Rebollar et al. (2016).

Impacts of pathogen co-occurrence and co-infections

In the wild, multi-pathogen-parasite landscapes occur, including coinfections of ranavirus, Bd, and Bsal (Lotters et al., 2018; Warne, LaBumbard, LaGrange, Vredenburg, & Catenazzi, 2016; Whitfield et al., 2013). Where pathogens co-occur they can affect different subsets of the amphibian community. For example, ranavirus may have greater impacts at lower elevations while Bd has impacts at higher elevations (Rosa et al., 2017). Mortality and sublethal effects can be exacerbated by coinfections in some cases (Longo, Fleischer, & Lips, 2019; McDonald et al., 2020); however, one recent study has suggested initial infection with low virulence Bd genotypes can shift Bsal infection dynamics (Greener et al., 2020).

Community-level factors

The biotic community can play a major role in determining disease outcomes, with important implications for disease management. As hosts vary in their susceptibility to the same pathogens, host community composition can determine whether a disease is enzootic or epizootic. For instance, with many immune or resistant hosts, the community may experience a *dilution effect,* whereby disease is kept to low, enzootic levels. Alternatively, if many reservoir (or highly tolerant) hosts or super-shedders are present, higher pathogen burdens may build up, resulting in negative impacts on susceptible hosts. Introduced African clawed frogs and American bullfrogs, and US-native Pacific chorus frogs are reservoir hosts for Bd (Reeder, Pessier, & Vredenburg, 2012), whereas various anuran and urodelan hosts, such as midwife toads and alpine newts, can be reservoir hosts for Bsal (Stegen et al., 2017). Non-amphibian hosts, such as crayfish or water fowl feet, have been suggested for Bd and Bsal; however, evidence remains controversial (Betancourt-Román, O’Neil, & James, 2016; McMahon et al., 2013; Van Rooij, Martel, Haesebrouck, & Pasmans, 2015). For ranavirus, non-amphibian hosts are well-documented, including fish and turtles (Duffus et al., 2015).

Future steps & recommendations

Over the last 15 years of research on amphibian disease hosts, there has been an increased understanding of the need to move beyond correlating pathogen presence with decline; rather, it is necessary to associate pathogen presence with disease, and in turn disease with decline.

Given high levels of intraspecific and interspecific variability in disease outcomes, broad predictive markers for susceptibility are needed. These may include genetic markers, mucosome activity against skin pathogens, proportion of the microbiome that is inhibitory against pathogens, or other measurable factors. Development of predictive assays will require additional comparative and validation studies. Further understanding of factors associated with populations experiencing recovery as well as “cold spots” for disease can advance development of targeted management methods.

Further, basic biological studies are lacking to provide context to correlational and experimental patterns. Studies of cellular responses to infection would enhance understanding of immune markers or responses most relevant to surviving pathogen infection. An improved understanding of the roles of non-bacterial microbes in amphibian microbiomes may clarify impacts of microbiome variation over species, space, and time, and of employing probiotic treatments in nature. Given that co-infections can exacerbate disease outcomes, understanding interactions of the widespread, well-studied pathogens featured in this review with more poorly studied pathogens and parasites will likely be important in effectively managing amphibian health broadly.

Environment

Environmental factors affect disease transmission and host-disease dynamics. Significant advances in understanding host-pathogen interactions with both abiotic and biotic environmental factors have been made in recent years.

Abiotic factors

Abiotic factors such as temperature, water, and altitude help explain spatiotemporal variability in amphibian pathogen occurrence (Brunner, Storfer, Gray, & Hoverman, 2015; Murray et al., 2011; Olson et al., 2013), Table 3. For example, reported localities of fatal chytridiomycosis are scarce, concentrated mainly in tropical regions of the Americas and Australia (Scheele, Pasmans, et al., 2019), and most ranaviral disease die-offs have been in temperate regions during warmer seasons (Price et al., 2019). Predicting disease impacts on amphibian populations, however, is challenging due to several interacting contexts (Blaustein et al., 2018). Furthermore, amphibian pathogens are a moving target, as amphibian trade (food, pets) spreads pathogens with panzootic potential (O'Hanlon et al., 2018) and climatic shifts may trigger new epizootic outbreaks (See Chapters 3 and 7).

Temperature also can affect pathogen life history traits. Optimal *in vitro* temperature ranges for Bd is 17- 25°C (Piotrowski, Annis, & Longcore, 2004), Bsal is 10-15°C (Martel et al., 2013), and Ranavirus is 20-28°C (Ariel et al., 2009). Experimental exposures of *Bd* strains to various thermal regimes *in vitro* showed that warmer temperatures may increase zoospore production within the host, but decrease zoospore viability in aquatic environments (Woodhams, Alford, & Briggs, 2008; Woodhams et al., 2012). Hence, *Bd* could have higher impact on populations under thermal conditions that are suboptimal for pathogen replication, if propagules remain viable outside their host for longer periods (Voyles et al., 2012; Voyles, Rosenblum, & Berger, 2011). Models have shown free *Bd* zoospore persistence in the environment is a major determinant of the fate of host populations (Doddington et al., 2013; Louca, Lampo, & Doebeli, 2014; Mitchell, Churcher, Garner, & Fisher, 2008). For ranavirus, a greater pathogenicity at warmer temperatures appears to be related to a faster viral replication (Brand et al., 2016).

Temperature effects on host immune systems are less clear. During host hibernation, the immune response involved in *Bd* clearance is impaired (Rollins-Smith, 2020), and hosts may be less effective at resisting disease after cold pulses (Greenspan, Bower, Webb, Berger, et al., 2017; Greenspan, Bower, Webb, Roznik, et al., 2017). Higher rates of *Bd* clearance in warmer environments have been attributed to increased amphibian skin sloughing, a mechanism that lowers infection burdens (Grogan et al., 2018), but repeated exposure to extreme heat also causes a corticosterone response characteristic of chronic stress that could suppress amphibian physiological endocrine sensitivity to pathogenic diseases (Narayan & Hero, 2014)about:blank. Temperature variability itself affects amphibian immune responses; further investigation is needed (Raffel, Rohr, Kiesecker, & Hudson, 2006).

Chytridiomycosis tends to have greater impact and higher infection prevalence on highland populations in cooler habitats (Catenazzi, Lehr, & Vredenburg, 2014; Scheele, Pasmans, et al., 2019; Woodhams & Alford, 2005). Warmer habitats have been proposed as thermal refuges where frogs are more likely to coexist with the fungus because Bd tends to grow sub-optimally (Puschendorf et al., 2009; Zumbado-Ulate, Bolaños, Gutiérrez-Espeleta, & Puschendorf, 2014)about:blank. Post-epidemic population recoveries have been more frequent in lowland than upland locations, supporting this hypothesis (Grogan et al., 2016; Lampo, Señaris, & García, 2017; Phillott et al., 2013). Hosts are not always constrained passively to ambient conditions; if hosts can raise their body temperature by spending more time in microhabitats where temperature exceeds the pathogen's optimum, amphibians can alter their infection risk (Richards-Zawacki, 2010; Rowley & Alford, 2013). However, recurring findings of conflicting correlations between prevalence, outbreaks, and climatic conditions (Ron, 2005) led to an examination of the effects of climatic conditions in terms of differential performance of the pathogen and its host relative to their thermal optima, an idea referred to as the thermal mismatch hypotheses (Cohen et al., 2017; Nowakowski et al., 2016). Consequently, "infection risk in ectotherms may change as the difference between host and pathogen environmental tolerances (i.e., tolerance mismatch) increases". Infection risk is expected to decrease, for example, if hosts can access thermal niche spaces suboptimal for *Bd* (Nowakowski et al., 2016). Conversely, infection risk could increase if available temperatures shift away from host optimums (Cohen, Civitello, Venesky, McMahon, & Rohr, 2019).

Humidity and water availability also play a role in amphibian disease dynamics. *Bd* has severely impacted populations associated with perennial waters (Scheele, Pasmans, et al., 2019), but hydrological regimes also can affect other pathogen-host dynamics. *Batrachochytrium* fungi do not tolerate desiccation and water availability or humidity is fundamental for effective transmission, but *Bd* transmission can increase during driest months when adults congregate near water sources (LaBumbard, Shepack, & Catenazzi, 2020; Piovia-Scott, Pope, Lawler, Cole, & Foley, 2011; Ruggeri et al., 2015). Also, *Batrachochytrium* fungi persist 1-7 months in sediment or lake water (Johnson & Speare, 2003; Martel et al., 2013; Stegen et al., 2017) and ranavirus can survive for >30 days in sediments (Munro, Bayley, McPherson, & Feist, 2016; Nazir, Spengler, & Marschang, 2012). Hence, pathogens can persist after their hosts have been removed from their habitats. Models suggest that one of the most important mechanisms promoting Bd establishment and driving host populations to extinctions is its capacity to survive outside its host in water or humid substrates (Doddington et al., 2013; Louca et al., 2014; Mitchell et al., 2008). Spatial distribution and zoospore life expectancy in the environment is becoming more apparent at some US amphibian breeding sites (Chestnut et al., 2014), but dynamics in tropical stream environments and the relationship to environmental factors remains a knowledge gap. Recent development of eDNA sampling techniques will hopefully expand zoospore detectability across microhabitats (Hauck, Weitemier, Penaluna, Garcia, & Cronn, 2019; Walker et al., 2007).

Extreme climatic events also can impact fecundity, recruitment and survival of uninfected amphibians, undermining the ability of populations to offset disease-induced mortality and possibly tipping infection outcome from coexistence to extinction. Extended droughts can lead to breeding failure, and reduce post-metamorphic survival and adult recruitment (Cayuela et al., 2016; Richter, Young, Johnson, & Seigel, 2003). Yet, post-epidemic recovery of remnant populations from several regions where *Bd* is highly pathogenic has been linked to a high recruitment of healthy adults (Lampo et al., 2017; Muths, Scherer, & Pilliod, 2011; Scheele, Hunter, Skerratt, Brannelly, & Driscoll, 2015). Similarly, in amphibian populations challenged by ranavirus, recruitment success was better explained by hydroperiod length than viral presence or other contaminants (Smalling, Eagles-Smith, Katz, & Grant, 2019). This suggests that population resilience to disease-associated impact is highly dependent on climatic conditions, and climate plays an important role in the probability of post-epidemic recovery.

Identifying conditions in which amphibian populations can coexist with infection opens a promising avenue for long-term conservation of wild populations threatened by chytridiomycosis (Hettyey et al., 2019). Although several interventions are proposed that modify temperature, hydrological regimes or water quality, manipulate host microbial communities, or use predators as biocontrol agents for reducing pathogen survival (Table 6.3), field tests have lagged.

Biotic factors

In addition to host-associated microbiome communities, complex host communities of reservoir and susceptible species, and human-mediated pathogen transmission, amphibian pathogens are part of complex aquatic communities, with natural predators and parasites. Some aquatic predators of chytrid zoospores are water fleas (Cladocera), copepods (Copepoda), and seed shrimp (Ostracoda) (Woodhams et al., 2011). Higher abundances of protozoans and microscopic metazoans reduced *Bd* zoospores amounts at amphibian breeding sites in the Pyrenees (Schmeller et al., 2014). Zoospore viability inversely correlated with *Bd* infection prevalence, suggesting that *Bd* predatory microfauna affected *Bd*-host dynamics (Schmeller et al., 2014). Mesocosm experiments using *Daphnia* further corroborated the idea that microfauna can reduce *Bd* zoospore counts in lentic habitats (Buck, Truong, & Blaustein, 2011; Hamilton, Richardson, & Anholt, 2012).

Ranaviruses have cross-taxonomic host boundaries (Brenes et al., 2014; Duffus, Pauli, Wozney, Brunetti, & Berrill, 2008; Schock, Bollinger, Gregory Chinchar, Jancovich, & Collins, 2008), and are further transmitted through scavenging, direct contact, and contact with contaminated water (Blaustein et al., 2018). Host predation can reduce ranavirus infection rates because predators tend to attack individuals who are weak or have altered avoidance behaviours; some pathogens including ranaviruses can alter tadpole behaviour and result in greater predation of infected individuals, leading to ‘healthier but smaller herds’ (DeBlieux & Hoverman, 2019).

Future steps & recommendations

While correlations between some environmental factors and mechanisms governing the infection dynamics are now well established, predicting and mitigating the impact of infections on amphibian populations continues to be a challenge. The relative contributions of mechanisms of transmission and disease tolerance in promoting pathogen-host coexistence appear to be context-dependent and field data are often scarce. Also, the role of biotic interactions in the infection outcome remains poorly understood. Future investigation and management of amphibian diseases will need to consider the context-dependence of interactions and address the complexities arising from multispecies and multiscale interactions. Context modelling can be useful for a rapid assessment of effective strategies, given the urge of mitigating amphibian diseases.

Human dimensions

Human dimensions in amphibian diseases are multifaceted including knowledge discovery through research and monitoring, inadvertent pathogen transmission, and direct conservation, management and policy actions (Olson & Pilliod, 2021).

Trade

International and national policies focus on reducing human-mediated transmission. For example, the recently proposed Asian origin of Bd and Bsal has raised concerns for risk of international transmission within trade markets (Carvalho et al., 2017; Nguyen, Nguyen, Ziegler, Pasmans, & Martel, 2017; O’Hanlon et al., 2018). In 2008, chytridiomycosis was added to the OIE's list of notifiable diseases due to increasing evidence of Bd spread through live amphibian trade. Both chytrid fungi and ranavirus are now OIE listed as notifiable diseases (Schloegel et al., 2009; OIE, 2020). In 2018, a motion was passed by the Convention on Biological Diversity (CBD) for member states to adopt measures to reduce risk of invasive alien species moving unintentionally in pathways associated with trade in live organisms (CBD, 2018).

Clean trade is a priority for immediate action across wildlife species due to rapidly increasing pathogen concerns for both wildlife and potential spillover to humans (Fisher, Ghosh, et al., 2018; Kolby, 2020). Research advances in rapid and cost-effective pathogen detection and procedures for biosecure captive-animal handling in trade markets are increasing the feasibility of taking measures to reduce risk of spreading diseases (e.g., Brunner et al., 2019; Gray et al., 2018). However, a web of regulatory authorities with overlapping regulations makes it challenging to make progress in effecting policy changes, and is compounded by a lack of funding, capacity and regulatory backing that has slowed progress in developing clean-trade markets (see Chapter 7 for more information on policy efforts).

Recognising the role of trade in spreading diseases is important but getting ahead of the problem and preventing spread is likely the most cost-effective action. Bsal is one example of a pathogen known only to occur in parts of Europe with a likely Asian origin (Martel et al., 2014). Scientists called for action to prevent its spread to North America which is home to exceptional salamander species richness that are naive to this pathogen (Gray et al., 2015). In June 2015, a US Geological Survey workshop in Colorado, USA convened to form a Bsal Task Force with 8 working groups to address response and control, surveillance and monitoring, diagnostics, communication and outreach, clean trade, research and decision science, and data management (North American Bsal Task Force, 2022). These emphasis areas each help to get ahead of disease impacts.

Surveillance and monitoring

In particular, pathogen surveillance in both captive and wild animals has been needed to understand geographic and taxonomic patterns of disease occurrence, the potential scope of trade effects, and the direction of biosecurity needs, however surveillance and monitoring to date has been primarily focused in North America, Europe and Australia, while many amphibian-rich regions lack capacity for widespread monitoring (although see National Monitoring Initiative in Madagascar - (Bletz et al., 2015; Weldon et al., 2013)). With severe documented Bd impacts, Australia was one of the first countries to establish survey protocols for national surveillance (Skerratt et al., 2008). Bsal detection in captive amphibians was reported in Europe (Fitzpatrick, Pasmans, Martel, & Cunningham, 2018; Sabino-Pinto, Veith, Vences, & Steinfartz, 2018), but no Bsal detections were reported in captive samples in North America (Klocke et al., 2017), which can greatly inform usefulness of biosecurity policies such as a trade moratorium. Bsal surveillance in North America and lack of detection to date (Waddle et al., 2020) further supports the role of trade restrictions. Surveillance of both Bd and ranavirus has accelerated rapidly in the last decade, supporting cross-jurisdiction concerns for amphibian disease threats. Global Bd and ranavirus community open-access databases are available with recent website updates. Worldwide, Bd has been detected in 1375 of 2525 (55%) species sampled, from 93 of 134 (69%) countries (Olson et al., 2021)(database: amphibiandisease.org). Metadata analyses using these data have aided understanding of disease threat and host-pathogen-environment associations. Ranavirus surveillance reports are dominated by amphibians (63 genera; vs. 27 fish and 34 reptile genera) in North America and Europe, with a history of detections related to mortality events, some of which were in production settings (Brunner et al., 2021); database: brunnerlab.shinyapps.io/GRRS_Interactive/).

Decision science & proactive planning

Decision science is a developing discipline to facilitate manager and policy maker decision-making processes. Importantly, decision science models can aid in predicting outcomes of alternative actions in preparing for and initiating responses to disease outbreaks (e.g. Canessa et al., 2018; DiRenzo & Campbell Grant, 2019; Hopkins, 2018). Proactive planning can be further aided by the development of Incident Command Systems (Box 6.1). An Incident Command System is a standardised approach to the command, control, and coordination of response providing a common hierarchy within which responders from all stakeholders can be effective.

Hopkins (2018) showcased the importance of development of a USA incident command system through scenario planning exercises. This work highlighted differing trajectories of amphibian die-off responses due to land ownership (US National Park System lands, US Forest Service lands, and neighbouring tribal lands), and stall points in responses due to national, state, and local permissions required for actions such as implementing ground-disturbing activities or chemical applications in field settings. The North American Bsal Task Force management and control working group has also outlined a Response Plan Template including an outlined of ICS (salamanderfungus.org). Importantly, these systems can and should be defined proactively at local, regional and national scales to expedite effective response and management actions.

Box 6.1. Incident Command System for rapid disease response

An Incident Command System (ICS) is a disaster management system that has been applied to emergency response situations such as for human hazards including wildfire, hurricanes, earthquakes, chemical spills, and search-and-rescue operations, invasive species and disease outbreaks. Development of An Incident Command System (ICS) for amphibian disease outbreaks can facilitate an effective response to through immediate and cascading follow-up actions, including assembly of a command team, biosecurity implementation, survey and diagnostics, development of an effective response actions, and active communication with stakeholders. (Hopkins, 2018) described an Incident Command System for responses to amphibian die-off scenarios from hypothetical outbreaks of chytridiomycosis due the chytrid fungus *Batrachochytrium salamandrivorans* (Bsal) in the salamander biodiversity hotspot of the Appalachian Mountains in the eastern United States.

END BOX

Disease control strategies

Biosecurity protocols outline basic steps to reduce amphibian pathogen transmission in both captive (Brunner, 2020; Gray et al., 2018; Pessier & Mendelson III, 2017) and field situations (Gray et al., 2017; Julian et al., 2020; Olson et al., 2021; Phillott et al., 2010). Biosecurity measures range from between-site hygiene measures to prevent pathogen transmission in field situations (Julian et al., 2020; More et al., 2018), to between-individual precautions (Cashins, Alford, & Skerratt, 2008; Gray et al., 2017; Greer et al., 2009), while stringent quarantine and disinfection measures can prevent disease outbreaks in both captive and field situations (Pessier & Mendelson III, 2017). Australia has developed national guidelines for intra- and inter-state implementation of hygiene protocols to prevent Bd spread (Commonwealth of Australia, 2016).

Disease-control strategies beyond biosecurity protocols have developed considerably in the last two decades. (Garner et al., 2016; Thomas et al., 2019) reviewed alternative strategies in the toolbox of approaches to mitigate pathogen outbreaks, many of which are in active research-and-development at this time, including: habitat modification, chemical treatments, vaccines, probiotics (Tables 6.2 and 6.3, see also (Smith & Sutherland, 2014) for evidence of effectiveness for disease control and biosecurity practices).

AmphibianArk (www.amphibianark.org) was created in 2006 to carry out ex situ components of the IUCN SSC Amphibian Specialist Group's Amphibian Conservation Action Plan (ACAP). Its vision was to leverage existing captive husbandry resources in zoos and aquaria around the world to meaningful ex-situ conservation efforts, and it has made great strides in training staff and building capacity, conducting prioritization and providing funding to support ex-situ amphibian conservation efforts globally (Reid & Zippel, 2008). It now spans more than 60 organisations in 28 countries working to conserve 115 anuran species (Gratwicke & Murphy, 2016; Harding, Griffiths, & Pavajeau, 2016). Whereas captive breeding efforts do not directly mitigate the threats, and have had mixed success (Harding et al., 2016), they have created numerous opportunities to conduct integrated research (Hudson et al., 2016; Lewis et al., 2019; Skerratt et al., 2016). Linhoff et al. (2021) provided guidelines for amphibian reintroductions and translocations, the final step in many ex-situ efforts (See Chapters 11 and 14 for more information on these topics).

Table 6.3: Potential disease interventions that manipulate environmental factors

Intervention	**Evidence**	***In situ* examples**
Prune overhanging vegetation to increase terrestrial or aquatic temperatures	Frogs that select habitats with higher temperatures reduce their Bd infections (Richards-Zawacki, 2010; Rowley & Alford, 2013). Canopy modification to create warmer microclimates is postulated as a tool to permit coexistence with the pathogen (Scheele, Foster, et al., 2019), Bd prevalence declines associated with cyclone-canopy disturbance in Australia supports this hypothesis (Roznik, Sapsford, Pike, Schwarzkopf, & Alford, 2015).	Riparian tree canopies in Australia were trimmed to reduce the suitability of the habitat for Bd at spotted tree frog release sites (Scheele et al., 2014), but the canopy pruning was discontinued (B.C. Scheele, pers. comm).
Translocations to environmental refugia	Release captive-bred animals in warmer parts of their range that may act as environmental refugia or disease-free refuges (Scheele et al., 2014). Timing of releases to coincide with low Bd prevalence may also influence post-release success.	A translocation of yellow-legged frogs to colder, higher elevations postulated to limit Bd in frogs, but did not work (Knapp, Briggs, Smith, & Maurer, 2011).

Artificial heating stations	Natural thermal springs act as Bd refugia for frogs (Savage et al., 2011), and provision of artificial heating stations in situ are postulated as a mitigation tool (Hettyey et al., 2019).	The Mountain Chicken Recovery Program is conducting release trials using artificially heated pools as one Bd-mitigation strategy (https://www.mountainchicken.org/blog/its-getting-hot-hot-hot-controlling-the-chytrid-fungus/).
Add fungicides or salts to ponds to reduce pathogen loads	Adding salt to experimental ponds reduced Bd transmission between infected and uninfected animals (Clulow et al., 2018). Addition of commercially available fungicides to mesocosms reduced Bd prevalence and load, but also affected tadpole growth rates (Geiger & Schmidt, 2013; Hanlon, Kerby, & Parris, 2012).	Addition of salt to ponds where captive-bred green and bell frog tadpoles were released improved survival and reduced Bd prevalence (Stockwell, Storrie, Pollard, Clulow, & Mahony, 2015). A multi-year study in Mallorca found that pond drying, environmental disinfection, and fungicidal treatment of resident midwife toads eliminated Bd for at least 2 yrs post mitigation efforts (Bosch et al., 2015)

Increase population buffering capacity through habitat improvements or predator removal.	This strategy aims to improve habitat, or optimise hydroperiods to increase recruitment in order to compensate for disease-related losses (Scheele et al., 2014). Ideally habitat improvement will occur proactively while populations are still resilient (Sterrett et al., 2019).	Construction of additional breeding ponds for Puerto Rican crested toads have been partly successful and increased the number of populations of this threatened species (Linhoff et al., 2021). Creation of habitats that excluded fish helped increase green and gold bell frogs even in the presence of Bd (Beranek, Maynard, McHenry, Clulow, & Mahony, 2021).
Microbial bioaugmentation of substrate	Experimental augmentation of soil with bacteria that produce antifungal metabolites prevented Bd colonization of amphibian skin (Muletz et al., 2012).	Not tested yet.
Micropredator augmentation	Zooplankton as a micro predators of Bd, and experimentally reduce Bd zoospores and transmission of Bd to tadpoles (Schmeller et al., 2014).	Not tested yet

Community engagement

Lastly, engaging people is a necessary component of mitigating disease spread. Although this takes many forms, important factors in this sociological component include: 1) accelerated scientist networking and collaborations to increase the global pace and scope of research and surveillance; 2) mobilising funding to build capacity for an effective response; 3) developing conservation partnerships to address common disease management goals; 4) developing a communication strategy to increase targeted communication with defined audiences including the public, environmental groups, and policy makers, natural resource managers and disease specialists. The Herp-Disease-Alert-System (HDAS; herp_disease_alert@parcplace.org) implemented by PARC (Partners in Amphibians and Reptile Conservation) in North America is an example of a public-management networking system gaining success for rapid disease responses that routes information to the correct authority for follow-up action. The Human Dimension may be the greatest challenge yet to mitigate amphibian disease threats, as the feral dynamics of the Anthropocene are all-encompassing, affecting multiple biodiversity threat factors (Tsing, Deger, Keleman, & Zhou, 2020).

Acknowledgements

We thank the following members of the ASG Infectious Diseases Working Group whose insightful survey responses built this chapter: Imadeddin Albaba, Ross Alford, Steven Allain, Claudio Azat, David Blackburn, Michelle D Boone, Jennifer Buchanan, James Collins, Angelica Crottini, Andrew Cunningham, Arvin C. Diesmos, Stefano Doglio, Vicky Flechas, Maria Forzan, Trent Garner, Richard Griffiths, Reid Harris, Jess Hua, Blake Klocke, Kaya Klop-Toker, Tiffany Kosch, Edgar Lehr, Roberta Lecis, Cynthia Paszkowski, Cori Richards-

Zawacki, Gonçalo M. Rosa, Bryan Stuart, Benjamin Tapley, Felipe Toledo, Jenny Urbina, Andrés Valenzuela, David B Wake and Mark Wilkinson.

References

Abarca, J., Chaves, G., Garcia-Roodriguez, A., & Vargas, R. (2010). Reconsidering extinction: Rediscovery of *Incilius holdridgei* (Anura: Bufonidae) in Costa Rica after 25 years. *Herpetological Review*, *41*(2).

Ariel, E., Kielgast, J., Svart, H. E., Larsen, K., Tapiovaara, H., Jensen, B. B., & Holopainen, R. (2009). Ranavirus in wild edible frogs *Pelophylax* kl. *esculentus* in Denmark. *Diseases of Aquatic Organisms*, *85*(1), 7–14. doi: 10.3354/dao02060

Baitchman, E. J., & Pessier, A. P. (2013). Pathogenesis, diagnosis, and treatment of amphibian chytridiomycosis. *The Veterinary Clinics of North America. Exotic Animal Practice*, *16*(3), 669–685. doi: 10.1016/j.cvex.2013.05.009

Bataille, A., Cashins, S. D., Grogan, L., Skerratt, L. F., Hunter, D., McFadden, M., … Waldman, B. (2015). Susceptibility of amphibians to chytridiomycosis is associated with MHC class II conformation. *Proceedings of the Royal Society B: Biological Sciences*, *282*(1805), 20143127. doi: 10.1098/rspb.2014.3127

Becker, M. H., Brophy, J. A., Barrett, K., Bronikowski, E., Evans, M., Glassey, E., Kaganer, A. W., Klocke, B., Lassiter, E., Meyer, A. J. & Muletz-Wolz, C.R. (2021). Genetically modifying skin microbe to produce violacein and augmenting microbiome did not defend Panamanian golden frogs from disease. *ISME Communications*, 1(1), pp.1-10.

Becker, C. G., Rodriguez, D., Lambertini, C., Toledo, L. F., & Haddad, C. F. B. (2016). Historical dynamics of *Batrachochytrium dendrobatidis* in Amazonia. *Ecography*, *39*(10), 954–960. doi: 10.1111/ecog.02055

Becker, M. H., Harris, R. N., Minbiole, K. P. C., Schwantes, C. R., Rollins-Smith, L. a, Reinert, L. K., … Gratwicke, B. (2011). Towards a better understanding of the use of probiotics for preventing chytridiomycosis in Panamanian golden frogs. *EcoHealth*, *8*(4), 501–506. doi: 10.1007/s10393-012-0743-0

Becker, M. H., Walke, J. B., Cikanek, S., Savage, A. E., Mattheus, N., Santiago, C. N., … Becker, M. H. (2015). Composition of symbiotic bacteria predicts survival in Panamanian golden frogs infected with a lethal fungus. *Proceedings of the Royal Society B*, *282*, 20142881–20142881.

Belasen, A. M., Bletz, M. C., Leite, D. da S., Toledo, L. F., & James, T. Y. (2019). Long-term habitat fragmentation is associated with reduced MHC IIB diversity and increased infections in amphibian hosts. *Frontiers in Ecology and Evolution*, *6*. doi: 10.3389/fevo.2018.00236

Belasen, A. M., Riolo, M. A., Bletz, M. C., Lyra, M. L., Toledo, L. F., & James, T. Y. (2021). Geography, host Genetics, and cross-domain microbial networks structure the skin microbiota of fragmented Brazilian Atlantic Forest frog populations. *Ecology and Evolution*, *n/a*(n/a). doi: 10.1002/ece3.7594

Beranek, C. T., Maynard, C., McHenry, C., Clulow, J., & Mahony, M. (2021). Rapid population increase of the threatened Australian amphibian *Litoria aurea* in response to wetlands constructed as a refuge from chytrid-induced disease and introduced fish. *Journal of Environmental Management*, *291*, 112638. doi: 10.1016/j.jenvman.2021.112638

Betancourt-Román, C. M., O'Neil, C. C., & James, T. Y. (2016). Rethinking the role of invertebrate hosts in the life cycle of the amphibian chytridiomycosis pathogen. *Parasitology*, *143*(13), 1723–1729. doi: 10.1017/S0031182016001360

Beukema, W., Martel, A., Nguyen, T. T., Goka, K., Schmeller, D. S., Yuan, Z., … Pasmans, F. (2018). Environmental context and differences between native and invasive observed niches of *Batrachochytrium salamandrivorans* affect invasion risk assessments in the Western Palaearctic. *Diversity and Distributions*, *24*(12), 1788–1801. doi: 10.1111/ddi.12795

Blaustein, A. R., Urbina, J., Snyder, P. W., Reynolds, E., Dang, T., Hoverman, J. T., … Hambalek, N. M. (2018). Effects of emerging infectious diseases on amphibians: A review of experimental studies. *Diversity*, *10*(3), 81. doi: 10.3390/d10030081

Bletz, M. C. (2013). *Probiotic bioaugmentation of an anti-Bd bacteria, Janthinobacterium lividum, on the amphibian, Notophthalmus viridescens: Transmission efficacy and persistence of the probiotic on the host and non-target effects of probiotic addition on ecosystem*. Master's theses, 2010-2019. 153. Retrieved from https://commons.lib.jmu.edu/master201019/153

Bletz, M. C., Kelly, M., Sabino-pinto, J., Bales, E., Praet, S. V., Bert, W., … Martel, A. (2018). Disruption of skin microbiota contributes to salamander disease. *Royal Society Proceedings B*, *285*, 1–10.

Bletz, M. C., Loudon, A. H., Becker, M. H., Bell, S. C., Woodhams, D. C., Minbiole, K. P. C., & Harris, R. N. (2013). Mitigating amphibian chytridiomycosis with bioaugmentation: Characteristics of effective probiotics and strategies for their selection and use. *Ecology Letters*, *16*(6), 807–820. doi: 10.1111/ele.12099

Bletz, M. C., Rosa, G. M. G. M. G. M., Andreone, F., Courtois, E. A. E. A., Schmeller, D. S. D. S., Rabibisoa, N. H. C. N. H. C., … Crottini, A. (2015). Widespread presence of the pathogenic fungus *Batrachochytrium dendrobatidis* in wild amphibian communities in Madagascar. *Scientific Reports*, *5*, 8633–8633. doi: 10.1038/srep08633

Bletz, M. C., Myers, J., Woodhams, D. C., Rabemananjara, F. C. E., Rakotonirina, A., Weldon, C., … Harris, R. N. (2017). Estimating herd immunity to amphibian chytridiomycosis in Madagascar based on the defensive function of amphibian skin bacteria. *Frontiers in Microbiology*, *8*, 1751–1751. doi: 10.3389/fmicb.2017.01751

Böll, S., Tobler, U., Geiger, C. C., Hansbauer, G., & Schmidt, B. R. (2012). The amphibian chytrid fungus in Bavarian populations of *Alytes obstetricans*: Past absence, current presence, and metamorph mortality. *Amphibia-Reptilia*, *33*(3–4), 319–326. doi: 10.1163/156853812X651856

Boots, M., & Sasaki, A. (2003). Parasite evolution and extinctions. *Ecology Letters*, *6*(3), 176–182. doi: 10.1046/j.1461-0248.2003.00426.x

Bosch, J., Sanchez-tome, E., Oliver, J. A., Fisher, M. C., Garner, T. W. J., Nacional, M., … Bosch, J. (2015). Successful elimination of a lethal wildlife infectious disease in nature.

Biology Letters, 11(11), 20150874. doi.org/10.1098/rsbl.2015.0874

Bradley, P. W., Gervasi, S. S., Hua, J., Cothran, R. D., Relyea, R. A., Olson, D. H., & Blaustein, A. R. (2015). Differences in sensitivity to the fungal pathogen *Batrachochytrium dendrobatidis* among amphibian populations. *Conservation Biology*, *29*(5), 1347–1356. doi: 10.1111/cobi.12566

Brand, M. D., Hill, R. D., Brenes, R., Chaney, J. C., Wilkes, R. P., Grayfer, L., … Gray, M. J. (2016). Water temperature affects susceptibility to ranavirus. *EcoHealth*, *13*(2), 350–359. doi: 10.1007/s10393-016-1120-1

Brannelly, L. A., Wetzel, D. P., West, M., & Richards-Zawacki, C. L. (2020). Optimized *Batrachochytrium dendrobatidis* DNA extraction of swab samples results in imperfect detection particularly when infection intensities are low. *Diseases of Aquatic Organisms*, 139, 233-243.

Brenes, R., Gray, M. J., Waltzek, T. B., Wilkes, R. P., & Miller, D. L. (2014). Transmission of ranavirus between ectothermic vertebrate hosts. *PLOS ONE*, *9*(3), e92476. doi: 10.1371/journal.pone.0092476

Briggs, C. J., Knapp, R. A., & Vredenburg, V. T. (2010). Enzootic and epizootic dynamics of the chytrid fungal pathogen of amphibians. *Proceedings of the National Academy of Sciences of the USA*, *107*(21), 9695–9700. doi: 10.1073/pnas.0912886107

Brunner, J. L. (2020). Pooled samples and eDNA-based detection can facilitate the “clean trade” of aquatic animals. *Scientific Reports*, *10*(1), 10280. doi: 10.1038/s41598-020-66280-7

Brunner, J. L., Olson, A. D., Rice, J. G., Meiners, S. E., Sage, M. J. L., Cundiff, J. A., … Pessier, A. P. (2019). Ranavirus infection dynamics and shedding in American bullfrogs: Consequences for spread and detection in trade. *Diseases of Aquatic Organisms*, *135*(2), 135–150. doi: 10.3354/dao03387

Brunner, J. L., Olson, D. H., Gray, M. J., Miller, D. L., & Duffus, A. L. J. (2021). Global patterns of ranavirus detections. *FACETS*, *6*, 912–924. doi: 10.1139/facets-2020-0013

Brunner, J. L., Storfer, A., Gray, M. J., & Hoverman, J. T. (2015). Ranavirus ecology and evolution: From epidemiology to extinction. In *Ranaviruses* (pp. 71–104). Springer, Cham.

Buck, J. C., Truong, L., & Blaustein, A. R. (2011). Predation by zooplankton on *Batrachochytrium dendrobatidis*: Biological control of the deadly amphibian chytrid fungus? *Biodiversity and Conservation*, *20*(14), 3549–3553. doi: 10.1007/s10531-011-0147-4

Byrne, A. Q., Vredenburg, V. T., Martel, A., Pasmans, F., Bell, R. C., Blackburn, D. C., … Brown, R. M. (2019). Cryptic diversity of a widespread global pathogen reveals expanded threats to amphibian conservation. *Proceedings of the National Academy of Sciences*, *116*(41), 20382–20387.

Campbell, A., Australia, Environment Australia, Biodiversity Group, & Natural Heritage Trust (Australia). (1999). *Declines and disappearances of Australian frogs*. Canberra, ACT: Biodiversity Group, Environment Australia.

Canessa, S., Bozzuto, C., Campbell Grant, E. H., Cruickshank, S. S., Fisher, M. C., Koella, J. C., … Schmidt, B. R. (2018). Decision-making for mitigating wildlife diseases: From theory to practice for an emerging fungal pathogen of amphibians. *Journal of Applied

Ecology, *55*(4), 1987–1996. doi: 10.1111/1365-2664.13089

Carvalho, T., Becker, C. G., & Toledo, L. F. (2017). Historical amphibian declines and extinctions in Brazil linked to chytridiomycosis. *Proceedings of the Royal Society B: Biological Sciences*, *284*(1848), 20162254–20162254. doi: 10.1098/rspb.2016.2254

Cashins, S., Alford, R., & Skerratt, L. (2008). Lethal effect of latex, nitrile, and vinyl gloves on tadpoles. *Herpetological Review*, *39*, 298.

Cashins, S. D., Grogan, L. F., McFadden, M., Hunter, D., Harlow, P. S., Berger, L., & Skerratt, L. F. (2013). Prior infection does not improve survival against the amphibian disease chytridiomycosis. *PLoS ONE*, *8*(2), 1–7. doi: 10.1371/journal.pone.0056747

Catenazzi, A., Lehr, E., & Vredenburg, V. T. (2014). Thermal physiology, disease, and amphibian declines on the eastern slopes of the Andes. *Conservation Biology:*, *28*(2), 509–517. doi: 10.1111/cobi.12194

Cayuela, H., Arsovski, D., Bonnaire, E., Duguet, R., Joly, P., & Besnard, A. (2016). The impact of severe drought on survival, fecundity, and population persistence in an endangered amphibian. *Ecosphere*, *7*(2), e01246. doi: 10.1002/ecs2.1246

CBD (2018). Invasive alien species. CBD/COP/DEC/14/11, Agenda item 26. 8 p. Retrieved from https://www.cbd.int/doc/decisions/cop-14/cop-14-dec-11-en.pdf

Chaves, G., Zumbado-Ulate, H., García-Rodríguez, A., Gómez, E., Vredenburg, V. T., & Ryan, M. J. (2014). Rediscovery of the critically endangered Streamside Frog, *Craugastor taurus* (Craugastoridae), in Costa Rica. *Tropical Conservation Science*, *7*(4), 628–

638. doi: 10.1177/194008291400700404

Chen, Z.-Y., Li, T., Gao, X.-C., Wang, C.-F., & Zhang, Q.-Y. (2018). Protective immunity induced by DNA vaccination against ranavirus infection in Chinese Giant Salamander *Andrias davidianus*. *Viruses, 10*(2). doi: 10.3390/v10020052

Chestnut, T., Anderson, C., Popa, R., Blaustein, A. R., Voytek, M., Olson, D. H., & Kirshtein, J. (2014). Heterogeneous occupancy and density estimates of the pathogenic fungus *Batrachochytrium dendrobatidis* in waters of North America. *PLOS ONE, 9*(9), e106790. doi: 10.1371/journal.pone.0106790

Chinchar, V. G., Hick, P., Ince, I. A., Jancovich, J. K., Marschang, R., Qin, Q., Subramaniam, K., Waltzek, T.B., Whittington, R., Williams, T., Zhang, Q., & ICTV Report Consortium. (2017). ICTV Virus taxonomy profile: Iridoviridae, *Journal of General Virology*, 98, 890–891. Retrieved from https://talk.ictvonline.org/ictv-reports/ictv_online_report/dsdna-viruses/w/iridoviridae

Clulow, S., Gould, J., James, H., Stockwell, M., Clulow, J., & Mahony, M. (2018). Elevated salinity blocks pathogen transmission and improves host survival from the global amphibian chytrid pandemic: Implications for translocations. *Journal of Applied Ecology*, *55*(2), 830–840. doi: 10.1111/1365-2664.13030

Cohen, J. M., Civitello, D. J., Venesky, M. D., McMahon, T. A., & Rohr, J. R. (2019). An interaction between climate change and infectious disease drove widespread amphibian declines. *Global Change Biology, 25*(3), 927–937. doi: 10.1111/gcb.14489

Cohen, J. M., Venesky, M. D., Sauer, E. L., Civitello, D. J., McMahon, T. A., Roznik, E. A., & Rohr, J. R. (2017). The thermal mismatch hypothesis explains host susceptibility to an emerging infectious disease. *Ecology Letters*, *20*(2), 184–193. doi: 10.1111/ele.12720

Collins, J. P., Crump, M. L., & Lovejoy, T. (2009). *Extinction in Our Times: Global Amphibian Decline*. New York, New York: Oxford University Press.

Collins, J. P. (2010). Amphibian decline and extinction: What we know and what we need to learn. *Diseases of Aquatic Organisms*, *92*(2–3), 93–99. doi: 10.3354/dao02307

Commonwealth of Australia. (2016). *Threat abatement plan: For infection of amphibians with chytrid fungus resulting in chytridiomycosis.* Department of the Environment and Energy, Commonwealth of Australia. Retrieved from https://www.deh.gov.au/biodiversity/threatened/publications/tap/chytrid/

Conlon, J. M. (2011). The contribution of skin antimicrobial peptides to the system of innate immunity in anurans. *Cell and Tissue Research, 343*(1), 201–212. doi: 10.1007/s00441-010-1014-4

De Castro, F., & Bolker, B. (2005). Mechanisms of disease-induced extinction. *Ecology Letters, 8*, 117–126.

DeBlieux, T. S., & Hoverman, J. T. (2019). Parasite-induced vulnerability to predation in larval anurans. *Diseases of Aquatic Organisms*, *135*(3), 241–250. doi: 10.3354/dao03396

Densmore, C. L., & Green, D. E. (2007). Diseases of Amphibians. *ILAR Journal*, *48*(3), 235–254. doi: 10.1093/ilar.48.3.235

Dillon, M. J., Bowkett, A. E., Bungard, M. J., Beckman, K. M., O’Brien, M.

F., Bates, K., … Thornton, C. R. (2017). Tracking the amphibian pathogens *Batrachochytrium dendrobatidis* and *Batrachochytrium salamandrivorans* using a highly specific monoclonal antibody and lateral-flow technology. *Microbial Biotechnology*, *10*(2), 381–394. doi: 10.1111/1751-7915.12464

DiRenzo, G. V., & Campbell Grant, E. H. (2019). Overview of emerging amphibian pathogens and modeling advances for conservation-related decisions. *Biological Conservation*, *236*, 474–483. doi: 10.1016/j.biocon.2019.05.034

Doddington, B. J., Bosch, J., Oliver, J. A., Grassly, N. C., Garcia, G., Schmidt, B. R., … Fisher, M. C. (2013). Context-dependent amphibian host population response to an invading pathogen. *Ecology*, *94*(8), 1795–1804. doi: 10.1890/12-1270.1

Duffus, A. L. J., Pauli, B. D., Wozney, K., Brunetti, C. R., & Berrill, M. (2008). Frog virus 3-like infections in aquatic amphibian communities. *Journal of Wildlife Diseases*, *44*(1), 109–120. doi: 10.7589/0090-3558-44.1.109

Duffus, A. L. J., Nichols, R. A., & Garner, T. W. J. (2013). Investigations into the life history stages of the common frog (*Rana temporaria*) affected by an amphibian ranavirus in the United Kingdom. *Herpetological Review*. Retrieved from https://qmro.qmul.ac.uk/xmlui/handle/123456789/10794

Duffus, A. L J, Nichols, R. A., & Garner, T. W. J. (2014). Experimental evidence in support of single host maintenance of a multihost pathogen. *Ecosphere*, *5*(11), art142. doi: 10.1890/ES14-00074.1

Duffus, A. L. J., Waltzek, T. B., Stöhr, A. C., Allender, M. C., Gotesman, M., Whittington, R. J., … Marschang, R. E. (2015). Distribution and host range of ranaviruses. In M. J. Gray & V. G. Chinchar (Eds.), *Ranaviruses: Lethal pathogens of ectothermic vertebrates* (pp. 9–57). Cham: Springer International Publishing. doi: 10.1007/978-3-319-13755-1_2

Ellison, A. R., DiRenzo, G. V., McDonald, C. A., Lips, K. R., & Zamudio, K. R. (2017). First in vivo *Batrachochytrium dendrobatidis* transcriptomes reveal mechanisms of host exploitation, host-specific gene expression, and expressed genotype shifts. *G3: Genes, Genomes, Genetics*, *7*(1), 269–278.

Ellison, A. R., Savage, A. E., DiRenzo, G. V., Langhammer, P., Lips, K. R., & Zamudio, K. R. (2014). Fighting a losing battle: Vigorous immune response countered by pathogen suppression of host defenses in the chytridiomycosis-susceptible frog *Atelopus zeteki*. *G3: Genes, Genomes, Genetics*, *4*(7), 1275–1289.

Eskew, E. A., Shock, B. C., LaDouceur, E. E. B., Keel, K., Miller, M. R., Foley, J. E., & Todd, B. D. (2018). Gene expression differs in susceptible and resistant amphibians exposed to *Batrachochytrium dendrobatidis*. *Royal Society Open Science*, *5*(2). doi: 10.1098/rsos.170910

Farrer, R. A., Martel, A., Verbrugghe, E., Abouelleil, A., Ducatelle, R., Longcore, J. E., … Cuomo, C. A. (2017). Genomic innovations linked to infection strategies across emerging pathogenic chytrid fungi. *Nature Communications*, *8*, 14742–14742.

Fisher, M. C., & Garner, T. W. J. (2020). Chytrid fungi and global amphibian declines. *Nature Reviews Microbiology*, *18*(6), 332–343. doi: 10.1038/s41579-020-0335-x

Fisher, M. C., Ghosh, P., Shelton, J. M. G., Bates, K., Brookes, L., Wierzbicki, C., … Garner, T. W. J. (2018). Development and worldwide use of non-lethal, and minimal population-level impact, protocols for the isolation of amphibian chytrid fungi. *Scientific Reports*, *8*(1), 7772. doi: 10.1038/s41598-018-24472-2

Fisher, M. C., Hawkins, N. J., Sanglard, D., & Gurr, S. J. (2018). Worldwide emergence of resistance to antifungal drugs challenges human health and food security. *Science*, *360*(6390), 739–742. doi: 10.1126/science.aap7999

Fites, J. S., Ramsey, J. P., Holden, W. M., Collier, S. P., Sutherland, D. M., Reinert, L. K., … Rollins-Smith, L. A. (2013). The invasive chytrid fungus of amphibians paralyzes lymphocyte responses. *Science, 342*(6156), 366–369. doi: 10.1126/science.1243316

Fitzpatrick, L. D., Pasmans, F., Martel, A., & Cunningham, A. A. (2018). Epidemiological tracing of *Batrachochytrium salamandrivorans* identifies widespread infection and associated mortalities in private amphibian collections. *Scientific Reports*, *8*(1). doi: 10.1038/s41598-018-31800-z

Forzán, M. J., Heatley, J., Russell, K. E., & Horney, B. (2017). Clinical pathology of amphibians: A review. *Veterinary Clinical Pathology, 46*(1), 11–33. doi: 10.1111/vcp.12452

García-Rodríguez, A., Chaves, G., Benavides-Varela, C., & Puschendorf, R. (2012). Where are the survivors? Tracking relictual populations of endangered frogs in Costa Rica. *Diversity and Distributions*, *18*(2), 204–212. doi: 10.1111/j.1472-4642.2011.00862.x

Garner, T. W. J., Schmidt, B. R., Martel, A., Pasmans, F., Muths, E., Cunningham, A. A., … Garner, T. W. J. (2016). Mitigating amphibian chytridiomycoses in nature. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*.

Garner, T. W. J., Walker, S., Bosch, J., Leech, S., Rowcliffe, J. M., Cunningham, A. A., & Fisher, M. C. (2009). Life history tradeoffs influence mortality associated with the amphibian pathogen *Batrachochytrium dendrobatidis*. *Oikos*, *118*(5), 783–791. doi: 10.1111/j.1600-0706.2008.17202.x

Geiger, C., & Schmidt, B. (2013). Laboratory tests of antifungal agents to treat tadpoles against the pathogen *Batrachochytrium dendrobatidis*. *Diseases of Aquatic Organisms*, *103*, 191–197. doi: 10.3354/dao02576

Ghosh, P. N., Verster, R., Sewell, T. R., O'Hanlon, S. J., Brookes, L. M., Rieux, A., … Fisher, M. C. (2021). Discriminating lineages of *Batrachochytrium dendrobatidis* using quantitative PCR. *Molecular Ecology Resources*, *21*(5), 1452–1459. doi: 10.1111/1755-0998.13299

González-Maya, J. F., Belant, J. L., Wyatt, S. A., Schipper, J., Cardenal, J., Corrales, D., … Fischer, A. (2013). Renewing hope: The rediscovery of *Atelopus varius* in Costa Rica. *Amphibia-Reptilia*, *34*(4), 573–578. doi: 10.1163/15685381-00002910

Gratwicke, B., & Murphy, J. B. (2016). Amphibian conservation efforts at the Smithsonian's National Zoological Park and Conservation Biology Institute. *Herpetological Review*. *47* (4), 711–718.

Gray, M., Duffus, A., Haman, K., Harris, R., Allender, M., Thompson, T., … Miller, D. (2017). Pathogen surveillance in herpetofaunal populations: Guidance on study design, sample collection, biosecurity, and intervention strategies. *Herpetological Review*, *48*, 334.

Gray, M. J., Lewis, J. P., Nanjappa, P., Klocke, B., Pasmans, F., Martel, A., … Olson, D. H. (2015). *Batrachochytrium salamandrivorans*: The North American Response and a Call for Action. *PLoS Pathogens, 11*(12). doi: 10.1371/journal.ppat.1005251

Gray, M. J., Spatz, J. A., Carter, E. D., Yarber, C. M., Wilkes, R. P., & Miller, D. L. (2018). Poor biosecurity could lead to disease outbreaks in animal populations. *PLOS ONE*, *13*(3), e0193243. doi: 10.1371/journal.pone.0193243

Grayfer, L., Edholm, E.-S., De Jesús Andino, F., Chinchar, V. G., & Robert, J. (2015). Ranavirus host immunity and immune evasion. In M. J. Gray & V. G. Chinchar (Eds.), *Ranaviruses: Lethal pathogens of ectothermic vertebrates* (pp. 141–170). Cham: Springer International Publishing. doi: 10.1007/978-3-319-13755-1_6

Greener, M. S., Verbrugghe, E., Kelly, M., Blooi, M., Beukema, W., Canessa, S., … Martel, A. (2020). Presence of low virulence chytrid fungi could protect European amphibians from more deadly strains. *Nature Communications, 11*(1), 5393. doi: 10.1038/s41467-020-19241-7

Greenspan, S. E., Lambertini, C., Carvalho, T., James, T. Y., Toledo, L. F., Haddad, C. F. B., & Becker, C. G. (2018). Hybrids of amphibian chytrid show high virulence in native hosts. *Scientific Reports, 8*(1), 9600. doi: 10.1038/s41598-018-27828-w

Greenspan, S. E., Bower, D. S., Webb, R. J., Berger, L., Rudd, D., Schwarzkopf, L., & Alford, R. A. (2017). White blood cell profiles in amphibians help to explain disease susceptibility following temperature shifts. *Developmental and*

Comparative Immunology, *77*, 280–286. doi: 10.1016/j.dci.2017.08.018

Greenspan, S. E., Bower, D. S., Webb, R. J., Roznik, E. A., Stevenson, L. A., Berger, L., … Alford, R. A. (2017). Realistic heat pulses protect frogs from disease under simulated rainforest frog thermal regimes. *Functional Ecology*, *31*(12), 2274–2286. doi: 10.1111/1365-2435.12944

Greer, A., Schock, D., Brunner, J., Johnson, R., Picco, A., Cashins, S., … Collins, J. (2009). Guidelines for the safe use of disposable gloves with amphibian larvae in light of pathogens and possible toxic effects. *Herpetological Review*, *40*, 145–147.

Grogan, L. F., Humphries, J. E., Robert, J., Lanctôt, C. M., Nock, C. J., Newell, D. A., & McCallum, H. I. (2020). Immunological aspects of chytridiomycosis. *Journal of Fungi*, *6*(4), E234. doi: 10.3390/jof6040234

Grogan, L. F., Phillott, A. D., Scheele, B. C., Berger, L., Cashins, S. D., Bell, S. C., … Skerratt, L. F. (2016). Endemicity of chytridiomycosis features pathogen overdispersion. *Journal of Animal Ecology*, *85*(3), 806–816. doi: 10.1111/1365-2656.12500

Grogan, L. F., Robert, J., Berger, L., Skerratt, L. F., Scheele, B. C., Castley, J. G., … McCallum, H. I. (2018). Review of the amphibian immune response to chytridiomycosis, and future directions. *Frontiers in Immunology*, *9*, 2536–2536.

Hamilton, P. T., Richardson, J. M. L., & Anholt, B. R. (2012). *Daphnia* in tadpole mesocosms: Trophic links and interactions with *Batrachochytrium dendrobatidis*. *Freshwater Biology*, *57*(4), 676–683. doi: 10.1111/j.1365-2427.2011.02731.x

Hanlon, S. M., Kerby, J. L., & Parris, M. J. (2012). Unlikely remedy: fungicide clears infection from pathogenic fungus in larval southern Leopard Frogs (*Lithobates sphenocephalus*). *PLOS ONE*, *7*(8), e43573. doi: 10.1371/journal.pone.0043573

Harding, G., Griffiths, R. A., & Pavajeau, L. (2016). Developments in amphibian captive breeding and reintroduction programs. *Conservation Biology*, *30*(2), 340–349. doi: 10.1111/cobi.12612

Hardy, B., Pope, K., Piovia-Scott, J., RN, B., & Foley, J. (2015). Itraconazole treatment reduces *Batrachochytrium dendrobatidis* prevalence and increases overwinter field survival in juvenile Cascades frogs. *Diseases of Aquatic Organisms*, *112*(3), 243–250.

Harris, R. N., Brucker, R. M., Walke, J. B., Becker, M. H., Schwantes, C. R., Flaherty, D. C., … Minbiole, K. P. C. (2009). Skin microbes on frogs prevent morbidity and mortality caused by a lethal skin fungus. *The ISME Journal*, *3*(7), 818–824. doi: 10.1038/ismej.2009.27

Harrison, X. A., Price, S. J., Hopkins, K., Leung, W. T. M., Sergeant, C., & Garner, T. W. J. (2019). Diversity-stability dynamics of the amphibian skin microbiome and susceptibility to a lethal viral pathogen. *Frontiers in Microbiology*, *10*. doi: 10.3389/fmicb.2019.02883

Hauck, L. L., Weitemier, K. A., Penaluna, B. E., Garcia, T. S., & Cronn, R. (2019). Casting a broader net: Using microfluidic metagenomics to capture aquatic biodiversity data from diverse taxonomic targets. *Environmental DNA*, *1*(3), 251–267. doi: 10.1002/edn3.26

Hettyey, A., Ujszegi, J., Herczeg, D., Holly, D., Vörös, J., Schmidt, B. R., & Bosch, J. (2019). Mitigating disease impacts in amphibian populations: capitalizing on the thermal optimum mismatch between a pathogen and its host. *Frontiers in*

Ecology and Evolution, *7*, 254–254. doi: 10.3389/fevo.2019.00254

Hopkins, M. C. (2018). *Batrachochytrium salamandrivorans (Bsal) in Appalachia: Using scenario building to proactively prepare for a wildlife disease outbreak caused by an invasive amphibian chytrid fungus*. Reston, Virginia: U.S. Department of the Interior, U.S. Geological Survey. Retrieved from https://search.library.wisc.edu/catalog/9913160356202121

Hoverman, J. T., Gray, M. J., Miller, D. L., & Haislip, N. A. (2012). Widespread occurrence of ranavirus in pond-breeding amphibian populations. *EcoHealth*, *9*(1), 36–48. doi: 10.1007/s10393-011-0731-9

Hu, W., Dong, B., Kong, S., Mao, Y., & Zheng, R. (2017). Pathogen resistance and gene frequency stability of major histocompatibility complex class IIB alleles in the giant spiny frog *Quasipaa spinosa*. *Aquaculture*, *468*, 410–416. doi: 10.1016/j.aquaculture.2016.11.001

Hudson, M. A., Young, R. P., D'Urban Jackson, J., Orozco-terWengel, P., Martin, L., James, A., … Cunningham, A. A. (2016). Dynamics and genetics of a disease-driven species decline to near extinction: Lessons for conservation. *Scientific Reports*, *6*(1), 30772. doi: 10.1038/srep30772

Isidoro-Ayza, M., Lorch, J. M., Grear, D. A., Winzeler, M., Calhoun, D. L., & Barichivich, W. J. (2017). Pathogenic lineage of Perkinsea associated with mass mortality of frogs across the United States. *Scientific Reports*, *7*(1), 1–10. doi: 10.1038/s41598-017-10456-1

James, T. Y., Toledo, L. F., Rödder, D., da Silva Leite, D., Belasen, A. M., Betancourt-Román, C. M., … Longcore, J. E. (2015). Disentangling host, pathogen, and environmental determinants of a recently emerged wildlife disease: Lessons from the first 15 years of amphibian chytridiomycosis research. *Ecology and Evolution*, *5*(18), 4079–4097. doi: 10.1002/ece3.1672

Jani, A. J., & Briggs, C. J. (2014). The pathogen *Batrachochytrium dendrobatidis* disturbs the frog skin microbiome during a natural epidemic and experimental infection. *Proceedings of the National Academy of Sciences*, *111*, E5049–E5058. doi: 10.1073/pnas.1412752111

Jenkinson, T. S., Román, C. M. B., Lambertini, C., Valencia-Aguilar, A., Rodriguez, D., Nunes-de-Almeida, C. H. L., … James, T. Y. (2016). Amphibian-killing chytrid in Brazil comprises both locally endemic and globally expanding populations. *Molecular Ecology*, *25*(13), 2978–2996. doi: 10.1111/mec.13599

Jiménez, R. R., & Sommer, S. (2016). The amphibian microbiome: Natural range of variation, pathogenic dysbiosis, and role in conservation. *Biodiversity and Conservation*, *26*(4), 763–786. doi: 10.1007/s10531-016-1272-x

Johnson, M. L., & Speare, R. (2003). Survival of *Batrachochytrium dendrobatidis* in water: quarantine and disease control implications. *Emerging Infectious Diseases*, *9*(8), 922–92. doi: 10.3201/eid0908.030145

Julian, J., Henry, P., Drasher, J., Jewell, S. D., Michell, K., Oxenrider, K., & Smith, S. (2020). Minimizing the spread of herpetofaunal pathogens in aquatic habitats by decontaminating construction equipment. *Herpetological Review*, *51*, 472–483.

Kearns, P. J., Fischer, S., Fernández-Beaskoetxea, S., Gabor, C. R., Zoological, N., & Si, P. (2017). Fight fungi with fungi: Antifungal properties of the amphibian mycobiome. *Frontiers in Microbiology*, *8*, 2494–2494. doi: 10.3389/fmicb.2017.02494

Klocke, B., Becker, M., Lewis, J., Fleischer, R. C., Muletz-Wolz, C. R., Rockwood, L., … Gratwicke, B. (2017). *Batrachochytrium salamandrivorans* not detected in U.S. survey of pet salamanders. *Scientific Reports*, *7*(1), 1–5. doi: 10.1038/s41598-017-13500-2

Knapp, R. A., Briggs, C. J., Smith, T. C., & Maurer, J. R. (2011). Nowhere to hide: Impact of a temperature-sensitive amphibian pathogen along an elevation gradient in the temperate zone. *Ecosphere*, *2*(8), art93. doi: 10.1890/ES11-00028.1

Kolby, J. E. (2020). To prevent the next pandemic, it's the legal wildlife trade we should worry about. Retrieved May 13, 2022, from National Geographic Website: https://www.nationalgeographic.com/animals/article/to-prevent-next-pandemic-focus-on-legal-wildlife-trade

Kosch, T. A., Bataille, A., Didinger, C., Eimes, J. A., Rodríguez-Brenes, S., Ryan, M. J., & Waldman, B. (2016). Major histocompatibility complex selection dynamics in pathogen-infected túngara frog (*Physalaemus pustulosus*) populations. *Biology Letters*, *12*(8), 20160345. doi: 10.1098/rsbl.2016.0345

Kueneman, J. G., Bletz, M. C., Mckenzie, V. J., Becker, C. G., Joseph, M. B., Abarca, J. G., … Vences, M. (2019). Community richness of amphibian skin bacteria correlates with bioclimate at the global scale. *Nature Ecology & Evolution*, *3*, 381–389. doi: 10.1038/s41559-019-0798-1

Kueneman, J. G., Woodhams, D. C., Harris, R., Archer, H. M., Knight, R., & McKenzie, V. J. (2016). Probiotic treatment restores protection against lethal fungal infection lost during amphibian captivity. *Proceedings of the Royal Society B: Biological Sciences*, *283*(1839), 20161553–20161553.

Kueneman, J. G., Woodhams, D. C., Van Treuren, W., Archer, H. M., Knight, R., & McKenzie, V. J. (2016). Inhibitory bacteria reduce fungi on early life stages of endangered Colorado boreal toads (*Anaxyrus boreas*). *The ISME Journal*, *10*(4), 934–934. doi: 10.1038/ismej.2015.168

LaBumbard, B. C., Shepack, A., & Catenazzi, A. (2020). After the epizootic: Host–pathogen dynamics in montane tropical amphibian communities with high prevalence of chytridiomycosis. *Biotropica*, *52*(6), 1194–1205. doi: 10.1111/btp.12824

Lam, B. A., Walke, J. B., Vredenburg, V. T., & Harris, R. N. (2010). Proportion of individuals with anti-*Batrachochytrium dendrobatidis* skin bacteria is associated with population persistence in the frog *Rana muscosa*. *Biological Conservation*, *143*(2), 529–531. doi: 10.1016/j.biocon.2009.11.015

Lambert, M. R., Womack, M. C., Byrne, A. Q., Hernández-Gómez, O., Noss, C. F., Rothstein, A. P., … Rosenblum, E. B. (2020). Comment on “Amphibian fungal panzootic causes catastrophic and ongoing loss of biodiversity.” *Science*, *367*(6484). doi: 10.1126/science.aay1838

Lammens, L., Martel, A., & Pasmans, F. (2021). Application of disinfectants for environmental control of a lethal amphibian pathogen. *Journal of Fungi*, *7*(6), 406. doi: 10.3390/jof7060406

Lampo, M., Señaris, C., & García, C. Z. (2017). Population dynamics of the critically endangered toad *Atelopus cruciger* and the fungal disease chytridiomycosis. *PLOS ONE*, *12*(6), e0179007. doi: 10.1371/journal.pone.0179007

Langwig, K. E., Frick, W. F., Reynolds, R., Parise, K. L., Drees, K. P., Hoyt, J. R., … Kilpatrick, A. M. (2015). Host and pathogen ecology drive the seasonal dynamics of a fungal disease , white-nose syndrome. *Proceedings of the Royal Society B*, *282*, 20142335–20142335. doi: 10.1098/rspb.2014.2335

Lei, X. P., Yi, G., Wang, K. Y., OuYang, P., Chen, D. F., Huang, X. L., … Yang, Z. X. (2019). *Elizabethkingia miricola* infection in Chinese spiny frog (*Quasipaa spinosa*). *Transboundary and Emerging Diseases*, *66*(2), 1049–1053. doi: 10.1111/tbed.13101

Lewis, C. H. R., Richards-Zawacki, C. L., Ibáñez, R., Luedtke, J., Voyles, J., Houser, P., & Gratwicke, B. (2019). Conserving Panamanian harlequin frogs by integrating captive-breeding and research programs. *Biological Conservation*, *236*, 180–187. doi: 10.1016/j.biocon.2019.05.029

Linhoff, L. J., Soorae, P. S., Harding, G., Donnelly, M. A., Germano, J. M., Hunter, D. A., … Eckstut, M. E. (2021). *IUCN guidelines for amphibian reintroductions and other conservation translocations: First edition*. IUCN. Retrieved from https://portals.iucn.org/library/node/49485

Longcore, J. E., Pessier, A. P., & Nicholes, D. K. (1999). *Batrachochytrium dendrobatidis* gen. et sp. nova chytrid pathogenic to amphibians. *Mycologia*, *91*, 219–227.

Longo, A. V., Fleischer, R. C., & Lips, K. R. (2019). Double trouble: Co-infections of chytrid fungi will severely impact widely distributed newts. *Biological Invasions*, *21*(6), 2233–2245.

Longo, A. V., Rodriguez, D., da Silva Leite, D., Toledo, L. F., Mendoza Almeralla, C., Burrowes, P. A., & Zamudio, K. R. (2013). ITS1 copy number varies among *Batrachochytrium dendrobatidis* strains: implications for qPCR estimates of infection intensity from field-collected amphibian skin swabs. *PLoS One*, *8*(3), e59499. doi: 10.1371/journal.pone.0059499.

Lotters, S., Norman Wagner, Kerres, A., Vences, M., Steinfartz, S., Sabino-Pinto, J., … Veith, M. (2018). First report of host co-infection of parasitic amphibian chytrid fungi. *Salamandra*, *54*(4).

Lötters, S., & Vences, M. (2020). The salamander plague in Europe – a German perspective. *Salamandra*, *56*(3), 2.

Louca, S., Lampo, M., & Doebeli, M. (2014). Assessing host extinction risk following exposure to *Batrachochytrium dendrobatidis*. *Proceedings. Biological Sciences / The Royal Society*, *281*(1785), 20132783–20132783. doi: 10.1098/rspb.2013.2783

Malagon, D. A., Melara, L. A., Prosper, O. F., Lenhart, S., Carter, E. D., Fordyce, J. A., … Gray, M. J. (2020). Host density and habitat structure influence host contact rates and *Batrachochytrium salamandrivorans* transmission. *Scientific Reports*, *10*(1), 5584. doi: 10.1038/s41598-020-62351-x

Martel, A., Blooi, M., Adriaensen, C., Van Rooij, P., Beukema, W., Fisher, M. C., … Pasmans, F. (2014). Recent introduction of a chytrid fungus endangers Western Palearctic salamanders. *Science*, *346*(6209), 630–631. doi: 10.1126/science.1258268

Martel, A, Spitzen-Van Der Sluijs, A., Blooi, M., Bert, W., Ducatelle, R., Fisher, M. C., … Pasmans, F. (2013). *Batrachochytrium salamandrivorans* sp. nov. causes lethal chytridiomycosis in amphibians. *Proceedings of the National Academy of Sciences of the*

United States of America, *110*(38), 15325–15329. doi: 10.1073/pnas.1307356110

McDonald, C. A., Longo, A. V., Lips, K. R., & Zamudio, K. R. (2020). Incapacitating effects of fungal coinfection in a novel pathogen system. *Molecular Ecology*, *29*(17), 3173–3186. doi: 10.1111/mec.15452

McMahon, T., Brannelly, L. A., Chatfield, M. W. H., Johnson, P. T. J., Joseph, M. B., McKenzie, V. J., … Rohr, J. R. (2013). Chytrid fungus *Batrachochytrium dendrobatidis* has nonamphibian hosts and releases chemicals that cause pathology in the absence of infection. *Proceedings of the National Academy of Sciences of the United States of America*. doi: 10.1073/pnas.1200592110

McMahon, T., Sears, B. F., Venesky, M. D., Bessler, S. M., Brown, J. M., Deutsch, K., … Rohr, J. R. (2014). Amphibians acquire resistance to live and dead fungus overcoming fungal immunosuppression. *Nature*, *551*, 224–227.

Mendelson, J. R., Whitfield, S. M., & Sredl, M. J. (2019). A recovery engine strategy for amphibian conservation in the context of disease. *Biological Conservation*, *236*, 188–191. doi: 10.1016/j.biocon.2019.05.025

Miller, D. L., Pessier, A. P., Hick, P., & Whittington, R. J. (2015). Comparative pathology of ranaviruses and diagnostic techniques. In M. J. Gray & V. G. Chinchar (Eds.), *Ranaviruses: Lethal pathogens of ectothermic vertebrates* (pp. 171–208). Cham: Springer International Publishing. doi: 10.1007/978-3-319-13755-1_7

Mitchell, K. M., Churcher, T. S., Garner, T. W. J., & Fisher, M. C. (2008). Persistence of the emerging pathogen *Batrachochytrium dendrobatidis* outside the amphibian host greatly increases the probability of host extinction. *Proceedings of the Royal Society B: Biological Sciences*, *275*(1632), 329–334. doi: 10.1098/rspb.2007.1356

More, S., Miranda, M. A., Bicout, D., Bøtner, A., Butterworth, A., Calistri, P., … Schmidt, C. G. (2018). Risk of survival, establishment and spread of *Batrachochytrium salamandrivorans* (Bsal) in the EU. *EFSA Journal*, *16*(4), e05259. doi: 10.2903/j.efsa.2018.5259

Muletz, C. R., Myers, J. M., Domangue, R. J., Herrick, J. B., & Harris, R. N. (2012). Soil bioaugmentation with amphibian cutaneous bacteria protects amphibian hosts from infection by *Batrachochytrium dendrobatidis*. *Biological Conservation*, *152*(August 2012), 119–126. doi: 10.1016/j.biocon.2012.03.022

Munro, J., Bayley, A. E., McPherson, N. J., & Feist, S. W. (2016). Survival of frog virus 3 in freshwater and sediment from an English lake. *Journal of Wildlife Diseases*, *52*(1), 138–142. doi: 10.7589/2015-02-033

Murray, K. A., Retallick, R. W. R., Puschendorf, R., Skerratt, L. F., Rosauer, D., McCallum, H. I., … VanDerWal, J. (2011). Assessing spatial patterns of disease risk to biodiversity: Implications for the management of the amphibian pathogen, *Batrachochytrium dendrobatidis*. *Journal of Applied Ecology*, *48*(1), 163–173. doi: 10.1111/j.1365-2664.2010.01890.x

Muths, E., Scherer, R. D., & Pilliod, D. S. (2011). Compensatory effects of recruitment and survival when amphibian populations are perturbed by disease. *Journal of Applied Ecology*, *48*(4), 873–879. doi: 10.1111/j.1365-2664.2011.02005.x

Mutnale, M. C., Anand, S., Eluvathingal, L. M., Roy, J. K., Reddy, G. S., & Vasudevan, K. (2018). Enzootic frog pathogen *Batrachochytrium dendrobatidis* in Asian tropics reveals high ITS haplotype diversity and low prevalence. *Scientific Reports*, *8*(1), 10125. doi: 10.1038/s41598-018-28304-1

Narayan, E. J., & Hero, J.-M. (2014). Repeated thermal stressor causes chronic elevation of baseline corticosterone and suppresses the physiological endocrine sensitivity to acute stressor in the cane toad (*Rhinella marina*). *Journal of Thermal Biology*, *41*, 72–76. doi: 10.1016/j.jtherbio.2014.02.011

Nazir, J., Spengler, M., & Marschang, R. E. (2012). Environmental persistence of amphibian and reptilian ranaviruses. *Diseases of Aquatic Organisms*, *98*(3), 177–184. doi: 10.3354/dao02443

Newell, D. A., Goldingay, R. L., & Brooks, L. O. (2013). Population recovery following decline in an endangered stream-breeding frog (*Mixophyes fleayi*) from subtropical Australia. *PLoS ONE*, *8*(3), e58559–e58559. doi: 10.1371/journal.pone.0058559

Newhouse, A. E., Polin-McGuigan, L. D., Baier, K. A., Valletta, K. E. R., Rottmann, W. H., Tschaplinski, T. J., … Powell, W. A. (2014). Transgenic American chestnuts show enhanced blight resistance and transmit the trait to T1 progeny. *Plant Science: An International Journal of Experimental Plant Biology*, *228*, 88–97. doi: 10.1016/j.plantsci.2014.04.004

Nguyen, T. T., Nguyen, T. V., Ziegler, T., Pasmans, F., & Martel, A. (2017). Trade in wild anurans vectors the urodelan pathogen *Batrachochytrium salamandrivorans* into Europe. *Amphibia-Reptilia*, *38*(4), 554–556. doi: 10.1038/srep44443

North American Bsal Task Force. (2022). A North American strategic plan to prevent and control invasions of the lethal salamander pathogen *Batrachochytrium salamandrivorans*. Retrieved from: https://www.salamanderfungus.org/

Nowakowski, A. J., Whitfield, S. M., Eskew, E. A., Thompson, M. E., Rose, J. P., Caraballo, B. L., … Todd, B. D. (2016). Infection risk decreases with increasing mismatch in host and pathogen environmental tolerances. *Ecology Letters*, 1051–1061. doi: 10.1111/ELE.12641

O'Hanlon, S. J., Rieux, A., Farrer, R. A., Rosa, G. M., Waldman, B., Bataille, A., … Fisher, M. C. (2018). Recent Asian origin of chytrid fungi causing global amphibian declines. *Science*, *360*(6389), 621–627. doi: 10.1126/science.aar1965

OIE (2020). Animal disease - List B. World Organization for Animal Health. Retrieved from https://www.oie.int/en/what-we-do/animal-health-and-welfare/animal-diseases/

Olson, D. H., & Pilliod, D. S. (2021). Amphibian and reptile conservation in the United States of America. In *Strategies for conservation success in herpetology*. University Heights, OH, USA.: Society for the Study of Amphibians and Reptiles.

Olson, D. H., Aanensen, D. M., Ronnenberg, K. L., Powell, C. I., Walker, S. F., Bielby, J., … Fisher, M. C. (2013). Mapping the global emergence of *Batrachochytrium dendrobatidis*, the amphibian chytrid fungus. *PLoS ONE*, *8*(2). doi: 10.1371/journal.pone.0056802

Olson, D. H., Haman, K. H., Gray, M., Harris, R., Thompson, T., Iredale, M., … Jennifer, B. (2021). Enhanced between-site biosecurity to minimize herpetofaunal disease-causing pathogen transmission. *Herpetological Review*, *52*(1), 29–39.

Olson, D.H., Ronnenberg, K. L., Glidden, C. K., Christensen, K. R., &

Blaustein, A. R. (2021). Global patterns of the fungal pathogen *Batrachochytrium dendrobatidis* support conservation urgency. *Frontiers in Veterinary Science and Zoological Medicine*. doi: 10.3389/fvets.2021.685877

Peace, A., O'Regan, S. M., Spatz, J. A., Reilly, P. N., Hill, R. D., Carter, E. D., … Gray, M. J. (2019). A highly invasive chimeric ranavirus can decimate tadpole populations rapidly through multiple transmission pathways. *Ecological Modelling*, *410*, 108777. doi: 10.1016/j.ecolmodel.2019.108777

Pessier, A., & Mendelson III, J. R. (2017). *A manual for control of infectious diseases in amphibian survival assurance colonies and reintroduction programs version 2.0: Updated 2017*. Apple Valley, MN: IUCN/SSC Conservation Breeding Specialist Group.

Pessier, A (2017). Hopping over red leg: the metamorphosis of amphibian pathology. *Veterinary Pathology*, *54*(3), 355–357. doi: 10.1177/0300985817699861

Phillott, A. D., Speare, R., Hines, H. B., Skerratt, L. F., Meyer, E., McDonald, K. R., … Berger, L. (2010). Minimising exposure of amphibians to pathogens during field studies. *Diseases of Aquatic Organisms*, *92*(2–3), 175–185. doi: 10.3354/dao02162

Phillott, A. D., Grogan, L. F., Cashins, S. D., McDonald, K. R., Berger, L., & Skerratt, L. F. (2013). Chytridiomycosis and seasonal mortality of tropical stream-associated frogs 15 years after introduction of *Batrachochytrium dendrobatidis*. *Conservation Biology*, *27*(5), 1058–1068. doi: 10.1111/cobi.12073

Piotrowski, J. S., Annis, S. L., & Longcore, J. E. (2004). Physiology of *Batrachochytrium dendrobatidis*, a chytrid pathogen of amphibians. *Mycologia*, *96*(1), 9–15.

Piovia-Scott, J., Pope, K. L., Lawler, S. P., Cole, E. M., & Foley, J. E. (2011). Factors related to the distribution and prevalence of the fungal pathogen *Batrachochytrium dendrobatidis* in *Rana cascadae* and other amphibians in the Klamath Mountains. *Biological Conservation*, *144*(12), 2913–2921. doi: 10.1016/j.biocon.2011.08.008

Polasik, J. S., Murphy, M. A., Abbott, T., & Vincent, K. (2016). Factors limiting early life stage survival and growth during endangered Wyoming toad reintroductions. *The Journal of Wildlife Management*, *80*(3), 540–552. doi: 10.1002/jwmg.1031

Poorten, T. J., & Rosenblum, E. B. (2016). Comparative study of host response to chytridiomycosis in a susceptible and a resistant toad species. *Molecular Ecology*, *25*(22), 5663–5679. doi: 10.1111/mec.13871

Price, S. J., Garner, T. W. J., Nichols, R. A., Balloux, F., Ayres, C., Mora-Cabello De Alba, A., & Bosch, J. (2014). Collapse of amphibian communities due to an introduced ranavirus. *Current Biology*, *24*(21), 2586–2591. doi: 10.1016/j.cub.2014.09.028

Price, S. J., Leung, W. T. M., Owen, C. J., Puschendorf, R., Sergeant, C., Cunningham, A. A., … Nichols, R. A. (2019). Effects of historic and projected climate change on the range and impacts of an emerging wildlife disease. *Global Change Biology*, *25*(8), 2648–2660. doi: 10.1111/gcb.14651

Puschendorf, R., Carnaval, A. C., VanDerWal, J., Zumbado-Ulate, H., Chaves, G., Bolaños, F., & Alford, R. A. (2009). Distribution models for the amphibian chytrid *Batrachochytrium dendrobatidis* in

Costa Rica: Proposing climatic refuges as a conservation tool. *Diversity and Distributions*, *15*(3), 401–408. doi: 10.1111/j.1472-4642.2008.00548.x

Puschendorf, R., Hodgson, L., Alford, R. A., Skerratt, L. F., & VanDerWal, J. (2013). Underestimated ranges and overlooked refuges from amphibian chytridiomycosis. *Diversity and Distributions*, *19*(10), 1313–1321. doi: 10.1111/ddi.12091

Rachowicz, L. J., & Briggs, C. J. (2007). Quantifying the disease transmission function: Effects of density on *Batrachochytrium dendrobatidis* transmission in the mountain yellow-legged frog *Rana muscosa*. *Journal of Animal Ecology*, *76*(4), 711–721.

Raffel, T. R., Rohr, J. R., Kiesecker, J. M., & Hudson, P. J. (2006). Negative effects of changing temperature on amphibian immunity under field conditions. *Functional Ecology*, *20*(5), 819–828. doi: 10.1111/j.1365-2435.2006.01159.x

Rebollar, E. A., Antwis, R. E., Becker, M. H., Belden, L. K., Bletz, M. C., Brucker, R. M., … Harris, R. N. (2016). Using “omics” and integrated multi-omics approaches to guide probiotic selection to mitigate chytridiomycosis and other emerging infectious diseases. *Frontiers in Microbiology*, *7*, 68–68. doi: 10.3389/fmicb.2016.00068

Rebollar E. A., Woodhams D. C., LaBumbard B., Kielgast J., Harris R. N. (2017). Prevalence and pathogen load estimates for the fungus *Batrachochytrium dendrobatidis* are impacted by ITS DNA copy number variation. *Diseases of Aquatic Organisms*, 123(3):213-226. doi: 10.3354/dao03097

Reeder, N. M. M., Pessier, A. P., & Vredenburg, V. T. (2012). A reservoir species for the emerging amphibian pathogen

Batrachochytrium dendrobatidis thrives in a landscape decimated by disease. *PLoS ONE*, *7*(3), 1–7. doi: 10.1371/journal.pone.0033567

Reid, G., & Zippel, K. (2008). Can zoos and aquariums ensure the survival of amphibians in the 21st century? *International Zoo Yearbook*, *42*, 1–6. doi: 10.1111/j.1748-1090.2007.00035.x

Richards-Zawacki, C. L. (2010). Thermoregulatory behaviour affects prevalence of chytrid fungal infection in a wild population of Panamanian golden frogs. *Proceedings of the Royal Society B: Biological Sciences*, *277*(1681), 519–528. doi: 10.1098/rspb.2009.1656

Richter, S. C., Young, J. E., Johnson, G. N., & Seigel, R. A. (2003). Stochastic variation in reproductive success of a rare frog, *Rana sevosa*: Implications for conservation and for monitoring amphibian populations. *Biological Conservation*, *111*(2), 171–177. doi: 10.1016/S0006-3207(02)00260-4

Rodríguez-Contreras, A., Señaris, J. C., Lampo, M., & Rivero, R. (2008). Rediscovery of *Atelopus cruciger* (Anura: Bufonidae): current status in the Cordillera de La Costa, Venezuela. *Oryx*, *42*(2), 301–304. doi: 10.1017/S0030605308000082

Rollins-Smith, L. A. (2009). The role of amphibian antimicrobial peptides in protection of amphibians from pathogens linked to global amphibian declines. *Biochimica et Biophysica Acta*, *1788*(8), 1593–1599. doi: 10.1016/j.bbamem.2009.03.008

Rollins-Smith, L. A. (2020). Global amphibian declines, disease, and the ongoing battle between *Batrachochytrium* fungi and the immune system. *Herpetologica*, *76*(2), 178–188. doi: 10.1655/0018-0831-76.2.178

Ron, S. R. (2005). Predicting the distribution of the amphibian pathogen *Batrachochytrium dendrobatidis* in the New World. *Biotropica*, *37*(2), 209–221.

Rosa, G. M., Sabino-Pinto, J., Laurentino, T. G., Martel, A., Pasmans, F., Rebelo, R., … Bosch, J. (2017). Impact of asynchronous emergence of two lethal pathogens on amphibian assemblages. *Scientific Reports*, *7*(1), 43260. doi: 10.1038/srep43260

Rosenblum, E. B., James, T. Y., Zamudio, K. R., Poorten, T. J., Ilut, D., Rodriguez, D., … Stajich, J. E. (2013). Complex history of the amphibian-killing chytrid fungus revealed with genome resequencing data. *Proceedings of the National Academy of Sciences of the United States of America*, *110*(23), 9385–9390. doi: 10.1073/pnas.1300130110

Rowley, J. J. L., & Alford, R. a. (2013). Hot bodies protect amphibians against chytrid infection in nature. *Scientific Reports, 3*, 1515–1515. doi: 10.1038/srep01515

Roznik, E. A., Sapsford, S. J., Pike, D. A., Schwarzkopf, L., & Alford, R. A. (2015). Natural disturbance reduces disease risk in endangered rainforest frog populations. *Scientific Reports*, *5*(1), 13472. doi: 10.1038/srep13472

Ruggeri, J., Longo, A. V., Gaiarsa, M. P., Alencar, L. R. V., Lambertini, C., Leite, D. S., … Martins, M. (2015). Seasonal variation in population abundance and chytrid infection in stream-dwelling frogs of the Brazilian Atlantic Forest. *PloS One*, *10*(7), e0130554. doi: 10.1371/journal.pone.0130554

Russell, D. M., Goldberg, C. S., Waits, L. P., & Rosenblum, E. B. (2010). *Batrachochytrium dendrobatidis* infection dynamics in the Columbia spotted frog *Rana luteiventris* in north Idaho, USA. *Diseases of*

Aquatic Organisms, *92*(2–3), 223–230. doi: 10.3354/dao02286

Russell, R. E., Halstead, B. J., Mosher, B. A., Muths, E., Adams, M. J., Grant, E. H. C., … Hossack, B. R. (2019). Effect of amphibian chytrid fungus (*Batrachochytrium dendrobatidis*) on apparent survival of frogs and toads in the western USA. *Biological Conservation*, *236*, 296–304. doi: 10.1016/j.biocon.2019.05.017

Rütte, M. von, Peyer, N., Schmidt, B., Keller, N., & Geiser, C. (2009). Assessing whether disinfectants against the fungus *Batrachochytrium dendrobatidis* have negative effects on tadpoles and zooplankton. *Amphibia-Reptilia*, *30*(3), 313–319. doi: 10.1163/156853809788795245

Sabino-Pinto, J., Veith, M., Vences, M., & Steinfartz, S. (2018). Asymptomatic infection of the fungal pathogen *Batrachochytrium salamandrivorans* in captivity. *Scientific Reports*, *8*(1), 11767–11767. doi: 10.1038/s41598-018-30240-z

Savage, A. E., Gratwicke, B., Hope, K., Bronikowski, E., & Fleischer, R. C. (2020). Sustained immune activation is associated with susceptibility to the amphibian chytrid fungus. *Molecular Ecology*, *29*(15), 2889–2903. doi: 10.1111/mec.15533

Savage, A. E., Muletz-Wolz, C. R., Campbell Grant, E. H., Fleischer, R. C., & Mulder, K. P. (2019). Functional variation at an expressed MHC class IIβ locus associates with ranavirus infection intensity in larval anuran populations. *Immunogenetics*, *71*(4), 335–346. doi: 10.1007/s00251-019-01104-1

Savage, A. E., Sredl, M. J., & Zamudio, K. R. (2011). Disease dynamics vary spatially and temporally in a North American amphibian. *Biological Conservation*, *144*(6), 1910–1915. doi: 10.1016/j.biocon.2011.03.018

Savage, A. E., & Zamudio, K. R. (2011). MHC genotypes associate with resistance to a frog-killing fungus. *Proceedings of the National Academy of Sciences of the United States of America*, *108*(40), 16705–16710. doi: 10.1073/pnas.1106893108

Scheele, B. C., Foster, C. N., Hunter, D. A., Lindenmayer, D. B., Schmidt, B. R., & Heard, G. W. (2019). Living with the enemy: Facilitating amphibian coexistence with disease. *Biological Conservation*, *236*, 52–59. doi: 10.1016/j.biocon.2019.05.032

Scheele, B. C., Guarino, F., Osborne, W., Hunter, D. A., Skerratt, L. F., & Driscoll, D. A. (2014). Decline and re-expansion of an amphibian with high prevalence of chytrid fungus. *Biological Conservation*, *170*, 86–91. doi: 10.1016/j.biocon.2013.12.034

Scheele, B. C., Hunter, D. A., Skerratt, L. F., Brannelly, L. A., & Driscoll, D. A. (2015). Low impact of chytridiomycosis on frog recruitment enables persistence in refuges despite high adult mortality. *Biological Conservation*, *182*, 36–43. doi: 10.1016/j.biocon.2014.11.032

Scheele, B. C., Pasmans, F., Skerratt, L. F., Berger, L., Martel, A., Beukema, W., … Canessa, S. (2019). Amphibian fungal panzootic causes catastrophic and ongoing loss of biodiversity. *Science*, *363*(6434), 1459–1463. doi: 10.1126/science.aav0379

Schloegel, L. M., Picco, A. M., Kilpatrick, A. M., Davies, A. J., Hyatt, A. D., & Daszak, P. (2009). Magnitude of the US trade in amphibians and presence of *Batrachochytrium dendrobatidis* and ranavirus infection in imported North American bullfrogs (*Rana* *catesbeiana*). *Biological Conservation*, *142*(7), 1420–1426. doi: 10.1016/j.biocon.2009.02.007

Schmeller, D. S., Blooi, M., Martel, A., Garner, T. W., Fisher, M. C., Azemar, F., … Pasmans, F. (2014). Microscopic aquatic predators strongly affect infection dynamics of a globally emerged pathogen. *Current Biology*, *24*(2), 176–180. doi: 10.1016/j.cub.2013.11.032

Schock, D. M., Bollinger, T. K., Gregory Chinchar, V., Jancovich, J. K., & Collins, J. P. (2008). Experimental evidence that amphibian ranaviruses are multi-host pathogens. *Copeia*, *2008*(1), 133–143. doi: 10.1643/CP-06-134

Searle, C. L., Gervasi, S. S., Hua, J., Hammond, J. I., Relyea, R. A., Olson, D. H., & Blaustein, A. R. (2011). Differential host susceptibility to *Batrachochytrium dendrobatidis*, an emerging amphibian pathogen. *Conservation Biology*, *25*(5), 965–974. doi: 10.1111/j.1523-1739.2011.01708.x

Skerratt, L. F., Berger, L., Clemann, N., Hunter, D. A., Marantelli, G., Newell, D. A., … West, M. (2016). Priorities for management of chytridiomycosis in Australia: Saving frogs from extinction. *Wildlife Research*, *43*(2), 105–120. doi: 10.1071/WR15071

Skerratt, L. F., Berger, L., Hines, H. B., McDonald, K. R., Mendez, D., & Speare, R. (2008). Survey protocol for detecting chytridiomycosis in all Australian frog populations. *Diseases of Aquatic Organisms*, *80*(2), 85–94. doi: 10.3354/dao01923

Smalling, K., Eagles-Smith, C., Katz, R. A., & Grant, E. (2019). Managing the trifecta of disease, climate, and contaminants: Searching for robust choices under multiple sources of uncertainty. *Biological*

Conservation, *236*(2), 153-161. doi: 10.1016/j.biocon.2019.05.026

Smith, H. K., Pasmans, F., Dhaenens, M., Deforce, D., Bonte, D., Verheyen, K., … Martel, A. (2018). Skin mucosome activity as an indicator of *Batrachochytrium salamandrivorans* susceptibility in salamanders. *PLOS ONE*, *13*(7), e0199295. doi: 10.1371/journal.pone.0199295

Smith, R., & Sutherland, W. (2014). *Amphibian conservation: Global evidence for the effects of interventions*. Exeter: Pelagica Publishing.

Spitzen-van der Sluijs, A., Martel, A., Asselberghs, J., Bales, E. K. E. K., Beukema, W., Bletz, M. C. M. C., … Lotters, S. (2016). Expanding distribution of lethal amphibian fungus *Batrachochytrium salamandrivorans* in Europe. *Emerging Infectious Diseases*, *22*(7), 1286–1286. doi: 10.3201/eid2207.160109

Stegen, G., Pasmans, F., Schmidt, B. R., Rouffaer, L. O., Van Praet, S., Schaub, M., … Martel, A. (2017). Drivers of salamander extirpation mediated by *Batrachochytrium salamandrivorans*. *Nature*, *544*(7650), 353–356. doi: 10.1038/nature22059

Sterrett, S. C., Katz, R. A., Brand, A. B., Fields, W. R., Dietrich, A. E., Hocking, D. J., … Campbell Grant, E. H. (2019). Proactive management of amphibians: Challenges and opportunities. *Biological Conservation*, *236*, 404–410. doi: 10.1016/j.biocon.2019.05.057

Stice, M. J., & Briggs, C. J. (2010). Immunization is ineffective at preventing infection and mortality due to the amphibian chytrid fungus Batrachochytrium dendrobatidis. *Journal of Wildlife Diseases*, *46*(1), 70–77. doi: 10.7589/0090-3558-46.1.70

Stockwell, M. P., Storrie, L. J., Pollard, C. J., Clulow, J., & Mahony, M. J. (2014). Effects of pond salinization on survival rate of amphibian hosts infected with the chytrid fungus. *29*(2), 391–399. *Conservation Biology*, *29*(2), 391-9. doi: 10.1111/cobi.12402

Teacher, A. G. F., Cunningham, A. A., & Garner, T. W. J. (2010). Assessing the long-term impact of ranavirus infection in wild common frog populations. *Animal Conservation*, *13*(5), 514–522. doi: 10.1111/j.1469-1795.2010.00373.x

Teacher, A. G. F., Garner, T. W. J., & Nichols, R. A. (2009). Evidence for directional selection at a novel Major Histocompatibility Class I marker in wild common frogs (*Rana temporaria*) exposed to a viral pathogen (ranavirus). *PLOS ONE*, *4*(2), e4616. doi: 10.1371/journal.pone.0004616

Thomas, V., Wang, Y., Van Rooij, P., Verbrugghe, E., Baláž, V., Bosch, J., … Pasmans, F. (2019). Mitigating *Batrachochytrium salamandrivorans* in Europe. *Amphibia Reptilia*, *40*(3), 265–290. doi: 10.1163/15685381-20191157

Tsing, A. L., Deger, J., Keleman, A. S., & Zhou, F. (2020). *Feral Atlas: The more-than-human Anthropocene*. Stanford: Stanford University Press.

Van Rooij, P., Martel, A., Haesebrouck, F., & Pasmans, F. (2015). Amphibian chytridiomycosis: A review with focus on fungus-host interactions. *Veterinary Research*, *46*(1), 1–22. doi: 10.1186/s13567-015-0266-0

Vilaça, S. T., Bienentreu, J.-F., Brunetti, C. R., Lesbarrères, D., Murray, D. L., & Kyle, C. J. (2019). Frog Virus 3 genomes reveal prevalent recombination between ranavirus lineages and their origins in Canada.

Journal of Virology, *93*(20), e00765-19. doi: 10.1128/JVI.00765-19

von May, R., Catenazzi, A., Santa-Cruz, R., Kosch, T. A., & Vredenburg, V. T. (2020). Microhabitat temperatures and prevalence of the pathogenic fungus *Batrachochytrium dendrobatidis* in lowland Amazonian frogs. *Tropical Conservation Science*, *11*(1). doi: https://doi.org/10.1177/1940082918797057

Voyles, J., Johnson, L. R., Briggs, C. J., Cashins, S. D., Alford, R. A., Berger, L., … Rosenblum, E. B. (2012). Temperature alters reproductive life history patterns in *Batrachochytrium dendrobatidis*, a lethal pathogen associated with the global loss of amphibians. *Ecology and Evolution*, *2*(9), 2241–2249. doi: 10.1002/ece3.334

Voyles, J., Rosenblum, E. B., & Berger, L. (2011). Interactions between *Batrachochytrium dendrobatidis* and its amphibian hosts: A review of pathogenesis and immunity. *Microbes and Infection*, *13*(1), 25–32.

Voyles, J., Woodhams, D. C., Saenz, V., Byrne, A. Q., Perez, R., Rios-Sotelo, G., … Richards-Zawacki, C. L. (2018). Shifts in disease dynamics in a tropical amphibian assemblage are not due to pathogen attenuation. *Science*, *359*(6383), 1517–1519. doi: 10.1126/science.aao4806

Vredenburg, V. T., Briggs, C. J., & Harris, R. N. (2011). Host-pathogen dynamics of amphibian chytridiomycosis: The role of the skin microbiome in health and disease. In L. Olson, E. Choffnes, D. Relman, & L. Pray (Eds.), *Fungal diseases: An emerging threat to human, animal, and plant health* (pp. 342–355). Washington DC: National Academy Press.

Waddle, A. W., Rivera, R., Rice, H., Keenan, E. C., Rezaei, G., Levy, J.

E., … Jaeger, J. R. (2021). Amphibian resistance to chytridiomycosis increases following low-virulence chytrid fungal infection or drug-mediated clearance. *Journal of Applied Ecology*, *n/a*(n/a). doi: 10.1111/1365-2664.13974

Waddle, J. H., Grear, D. A., Mosher, B. A., Grant, E. H. C., Adams, M. J., Backlin, A. R., … Winzeler, M. E. (2020). *Batrachochytrium salamandrivorans* (Bsal) not detected in an intensive survey of wild North American amphibians. *Scientific Reports*, *10*(1), 13012. doi: 10.1038/s41598-020-69486-x

Walker, S. F., Salas, M. B., Jenkins, D., Garner, T. W., Cunningham, A. A., Hyatt, A. D., … Fisher, M. C. (2007). Environmental detection of *Batrachochytrium dendrobatidis* in a temperate climate. *Diseases of Aquatic Organisms*, *77*, 105–112. doi: 10.3354/dao01850

Wang, S., Liu, C., Wilson, A., Zhao, N., Li, X., Zhu, W., … Li, Y. (2017). Pathogen richness and abundance predict patterns of adaptive major histocompatibility complex variation in insular amphibians. *Molecular Ecology*, *26*(18).

Warne, R. W., Kirschman, L., & Zeglin, L. (2019). Manipulation of gut microbiota during critical developmental windows affects host physiological performance and disease susceptibility across ontogeny. *Journal of Animal Ecology*, *88*(6), 845–856. doi: 10.1111/1365-2656.12973

Warne, R. W., LaBumbard, B., LaGrange, S., Vredenburg, V. T., & Catenazzi, A. (2016). Co-Infection by chytrid fungus and ranaviruses in wild and harvested frogs in the Tropical Andes. *PLOS ONE*, *11*(1), e0145864. doi: 10.1371/journal.pone.0145864

Weldon, C., Crottini, A., Bollen, A., Rabemananjara, F. C. E., Copsey, J., Garcia, G., & Andreone, F. (2013). Pre-emptive national monitoring plan for detecting the amphibian chytrid fungus in Madagascar. *EcoHealth*, *10*(3), 234–240. doi: 10.1007/s10393-013-0869-8

Whitfield, S. M., Alvarado, G., Abarca, J., Zumbado, H., Zuñiga, I., Wainwright, M., & Kerby, J. (2017). Differential patterns of *Batrachochytrium dendrobatidis* infection in relict amphibian populations following severe disease-associated declines. *Diseases of Aquatic Organisms*, *126*(1), 33–41. doi: 10.3354/dao03154

Whitfield, S. M., Geerdes, E., Chacon, I., Ballestero Rodriguez, E., Jimenez, R. R., Donnelly, M. A., & Kerby, J. L. (2013). Infection and co-infection by the amphibian chytrid fungus and ranavirus in wild Costa Rican frogs. *Diseases of Aquatic Organisms*, *104*(2), 173–178. doi: 10.3354/dao02598

Woodhams, D. C., & Alford, R. A. (2005). Ecology of chytridiomycosis in rainforest stream frog assemblages of Tropical Queensland. *Conservation Biology*, *19*(5), 1449–1459. doi: 10.1111/j.1523-1739.2005.004403.x

Woodhams, D. C., Alford, R. A., Antwis, R. E., Archer, H., Becker, M. H., Belden, L. K., … Davis, L. R. (2015). Antifungal isolates database of amphibian skin-associated bacteria and function against emerging fungal pathogens: Ecological Archives E096-059. *Ecology*, *96*(2), 595–595.

Woodhams, D. C., Alford, R. A., & Briggs, C. J. (2008). Life-history trade-offs influence disease in changing climates: Strategies of an amphibian pathogen. *Ecology*, *89*(6), 1627–1639.

Woodhams, D. C., Bosch, J., Briggs, C. J., Cashins, S., Davis, L. R., Lauer, A., … Voyles, J. (2011). Mitigating amphibian disease: Strategies to maintain wild populations and control chytridiomycosis. *Frontiers in Zoology*, *8*(1), 8. doi: 10.1186/1742-9994-8-8

Woodhams, D. C., Geiger, C. C., Reinert, L. K., Rollins-Smith, L. a, Lam, B., Harris, R. N., … Voyles, J. (2012). Treatment of amphibians infected with chytrid fungus: Learning from failed trials with itraconazole, antimicrobial peptides, bacteria, and heat therapy. *Diseases of Aquatic Organisms*, *98*(1), 11–25. doi: 10.3354/dao02429

Woodhams, D. C., Vredenburg, V. T., Simon, M.-A., Billheimer, D., Shakhtour, B., Shyr, Y., … Harris, R. N. (2007). Symbiotic bacteria contribute to innate immune defenses of the threatened mountain yellow-legged frog, *Rana muscosa*. *Biological Conservation*, *138*(3–4), 390–398. doi: 10.1016/j.biocon.2007.05.004

Wright, K. M., & Whitaker, B. R. (2001). *Amphibian medicine and captive husbandry*. Retrieved from https://www.cabdirect.org/cabdirect/abstract/20013162957

Wynne, F. J., Puschendorf, R., Knight, M. E., & Price, S. J. (2020). Choice of molecular assay determines ranavirus detection probability and inferences about prevalence and occurrence. *Diseases of Aquatic Organisms*, *141*, 139–147. doi: 10.3354/dao03518

Zamudio, K. R., McDonald, C. A., & Belasen, A. M. (2020). High variability in infection mechanisms and host responses: A review of functional genomic studies of amphibian chytridiomycosis. *Herpetologica*, *76*(2), 189–200. doi: 10.1655/0018-0831-76.2.189

Zhou, X., Zhang, X., Han, Y., Jia, Q., & Gao, H. (2017). Vaccination with recombinant baculovirus expressing ranavirus major capsid protein induces protective immunity in Chinese Giant Salamander, *Andrias davidianus*. *Viruses*, *9*(8). doi: 10.3390/v9080195

Zumbado-Ulate, H., Bolaños, F., Gutiérrez-Espeleta, G., & Puschendorf, R. (2014). Extremely low prevalence of *Batrachochytrium dendrobatidis* in frog populations from Neotropical dry forest of Costa Rica supports the existence of a climatic refuge from disease. *EcoHealth*, *11*(4), 593–602. doi: 10.1007/s10393-014-0967

Chapter 7. Trade and sustainable use

Jonathan E. Kolby[1,2], Amaël Borzée[3,2], Sinlan Poo[4,5], Nono LeGrand Gonwouo[6,2], N'Goran Germain Kouamé[7,2], Biraj Shrestha[2], Arturo Muñoz[8,2]

[1] College of Public Health, Medical and Veterinary Sciences, James Cook University, Townsville, Australia
[2] IUCN SSC Amphibian Specialist Group, Toronto, Canada
[3] Laboratory of Animal Behaviour and Conservation, College of Biology and the Environment, Nanjing Forestry University, Nanjing, People's Republic of China
[4] Department of Conservation and Research, Memphis Zoological Society, 2000 Prentiss Place, Memphis, TN 38112, United States
[5] Department of Biological Sciences, Arkansas State University, Jonesboro, AR 72401, United States
[6] Laboratory of Zoology, Faculty of Sciences, University of Yaoundé I, P.O. Box 812, Yaoundé, Cameroon
[7] Université Jean Lorougnon Guédé, UFR Environnement, Laboratoire de Biodiversité et Ecologie Tropicale, Daloa, BP 150, Côte d´Ivoire
[8] Department of Nutrition, Genetics and Ethology, Ghent University, Belgium

Abstract

The global trade in amphibians occurs at an extraordinary magnitude, involving the use of millions of animals locally and internationally every year. This activity is uniformly monitored and internationally regulated for less than 5% of described amphibian species, and the overall sustainability of present levels of trade are largely unknown. Amphibians are an

important source of protein in many regions of the world and are also frequently traded as pets and scientific research organisms. Thousands of amphibian species are either directly affected by this trade through their harvest or captive production, or indirectly affected by the deadly emerging infectious diseases this trade is spreading. This chapter highlights key points of concern that warrant additional investigation to ensure the long-term survival of amphibians is protected from the threat of trade, and concludes with a series of recommendations for constructive conservation actions.

Introduction

Millions of amphibians are traded globally every year for purposes ranging from use as a source of protein for human consumption (Warkentin et al., 2009; Gratwicke et al., 2010; Carpenter et al., 2014), to their use as exotic pets (Natusch & Lyons, 2012; Stringham & Lockwood, 2018; Altherr & Lameter, 2020), scientific research organisms, and for zoological conservation activities. Although a portion of these animals are produced in captivity, 42% are reported as wild caught (Hughes, Marshall & Strine, 2021), with 22% of the international amphibian trade comprised of species that are already threatened according to the IUCN Red List. It's important to note that the aforementioned trade characteristics refer only to the portion of international amphibian trade recorded by individual numbers of animals, whereas millions more are traded in units of mass, particularly those used as a source of food (Kolby, 2016). The impacts of these activities on global amphibian populations are largely unstudied.

A major challenge preventing deeper understanding of the impact of trade on amphibians is the scarcity of species-specific population estimates together with the absence of species-specific trade data recording by most countries. Currently, over 8,000 amphibian species have been scientifically described, but most readily available international trade data collected during official government inspections (i.e. the publicly accessible CITES trade database and the USFWS LEMIS trade database available through a Freedom of Information Act Request) only include information on several hundred species. At least 17% of amphibian species are internationally traded, with the majority originating from South America, China, and Central Africa, (Hughes, Marshall & Strine, 2021). Following capture or production in captivity, individuals are either consumed locally or exported (Warkentin et al., 2009; Auliya et al., 2016). Local consumption used for sustenance is more likely to demonstrate sustainable use (Kusrini, 2005) than international trade which is generally driven by market demands rather than necessity (Rowley et al., 2016; Hughes, Marshall & Strine, 2021; Morton et al., 2021). To consider whether present and future trade and use of amphibians is detrimental to the long-term survival of affected species, this chapter highlights key topics to explore, describes specific challenges in the measurement and evaluation of the impacts of trade (Box 1. Case study on amphibians in Ivory Coast), and recommends actions for the advancement of research and policy in this field of amphibian conservation science.

Amphibian trade records

Measurement of the trade in amphibians

Millions of amphibians are traded globally every year. Amphibians are harvested locally for trade, meat, and medicine (Onadeko, Egonmwan & Saliu, 2011; Van Vliet et al., 2017; Ribas & Poonlaphdecha, 2017) and exported internationally for meat, pets, and pharmaceutical research (Warkentin et al., 2009; Nijman & Shepherd, 2010; Auliya et al., 2016; Altherr & Lameter, 2020; see Text Boxes 7.1. 7.2 and 7.3). Although limited information about the international trade in amphibians is available, most countries either do not maintain or provide public access to records describing their domestic amphibian trade. This information gap represents a considerable hurdle preventing comprehensive assessments of the true impact of trade and consumption on amphibians globally.

Box 7.1. Domestic trade/biological use - Case study from Ivory Coast

Background

Vertebrate anatomy and physiology courses are the reason for a large volume of amphibian trade. In West and Central Africa, the species particularly affected by laboratory studies are the Northern Flat-backed Toad (*Sclerophrys maculata*), the Common Toad (*S. regularis*), the African Tiger Frog (*Hoplobatrachus occipitalis*), and the Grass Frogs (*Ptychadena* spp). These species have a wide distribution range and broad range of habitats across Africa (Kouamé et al., 2015; Channing & Rödel, 2019). Besides being collected for dissection, amphibians have always been used as food, medicine, or for cultural reasons by some particular West and Central African tribes (Gonwouo & Rödel, 2008; Mohneke & Rödel, 2009; Mohneke, Onadeko & Rödel, 2009; Mohneke, 2011) and a current increase in collection of these animals may be escalating beyond sustainability.

Origin of the trade

The increase in exploitation of amphibians is linked to the need for protein supplements due to rapid human population growth and a simultaneous decline in other protein resources, such as fishes. In some localities in southeastern Benin and Guinea, toads are used by villagers for treating diseases like Children's cough, appendicitis or skin injuries. Meanwhile, larger frog species like *Conraua* spp., *Hoplobatrachus occipitalis*, *Ptychadena* spp., *Pyxicephalus* sp. "edulis West", or *Trichobatrachus robustus* are collected for food from a wide range of West and Central African countries e.g. Benin, Burkina Faso, Cameroon, Ghana, Guinea, Ivory Coast, Nigeria, and Togo (Gonwouo & Rödel, 2008; Mohneke, Onadeko & Rödel, 2009; Mohneke et al., 2010; Mohneke, 2011; Kouamé et al., 2015). The known ethnic groups from West Africa, e.g. the Gourmanché and Mossi in Burkina Faso, the Hausa in Nigeria, and the Yacouba in Ivory Coast, and from Central Africa e.g. the Bakossi in Cameroon, traditionally use frogs as a source of protein or for medical and cultural reasons. On the Obudu plateau in Nigeria, tadpoles are intensively collected from small rivers (Mohneke, 2011). Likewise, amphibians are collected by university students for academic purposes. However, current rates of urbanisation and city development have greatly impacted local amphibian populations, which have become less abundant in recent years.

Amphibian harvest

Frog sellers generally collect the animals by hand at night using head lamps or hand torches around water ponds and microhabitats where the species are known to call. They collect any species they encounter and mostly target large adults for the ease of anatomical observations during practical sessions. Daily hunting rates range from about 40 to about 100 frogs per hunter and vary from one locality to another. Collected animals are kept in cartons and then sold on daily bases. Frog collection for food and trade is undertaken all year round with peaks in the dry season when the levels of the streams and ponds are low and collection is easier. More organised collection techniques include night searches along streams for large frogs using flashlights, machetes, spears, hooks, and nets (for detailed techniques used in hunting for trade see Gonwouo & Rödel, 2008; Mohneke, Onadeko & Rödel, 2009).

Growing harvest and trade

Since most attempts to commercially breed frogs under artificial, farm-like conditions have failed, the majority of amphibians are still taken directly from the wild. This trade provides a valuable source of revenue to local people. This practice is generally uncontrolled and likely to have an important negative impact on the natural populations of particular frog species. Similarly, every year, thousands of toads and frogs are collected in urban and suburban areas that host higher institutions of biological studies for use in laboratories. During such sessions, each student is entitled to one or two animals for practical sessions for anatomy and physiology studies. Each animal is sold for 200–250 FCFA (about 0.5 US dollars) depending on the size. As the number of students keeps on growing at universities there will be an equivalent increase in the demand of amphibians for practical work. Students enrolled in second year of biology in west and central Africa universities carry on three dissection sessions over the academic year. Assuming that all frogs and toads used during this practical work are collected from the wild, then this represents a considerable impact to the various populations where collection is done. Every year in higher institutions in Ivory Coast, for example, several hundred individuals are collected by students and subsequently killed and dissected in anatomy courses. Over collection seems to have negatively impacted local populations up to the point where the species are becoming rare to encounter in the city (Kouamé et al., 2015). The number is far higher if extrapolated across all higher institutions involved in biological studies across the continent. On the other hand, the trade of *H. occipitalis* at the different district markets of Daloa in Ivory Coast is still at a local scale with batches of five adult specimens sold for 500.00 FCFA (about 1 US dollars). The demand of amphibians for dissection in biology together with local markets for food increases the pressure on wild populations in urban areas.

Potential ecological consequences

Some amphibians species may not presently be categorised as threatened species by the IUCN Red List but may become so in the near future with the escalating combined threats. The unsustainable harvest of frogs in West Africa could likely have consequences including reduced control of arthropod pest species, especially species being vectors for human diseases such as *Anopheles* mosquitoes that transmit *Plasmodium* that cause malaria (Mohneke & Rödel, 2009). Given the targeting of large adult individuals during harvests, the reproduction of these animals is likely to be affected with consequences such as population declines (Gonwouo & Rödel, 2008; Mohneke, Onadeko & Rödel, 2009). The small-scale trade has just started to develop and it’s likely to continue and even increase given the growing populations. So far no actions have been taken to assess the rate of collection and its impact on wild populations. Consequently, population assessment and monitoring of *Sclerophrys maculata*, *S. regularis*, *Hoplobatrachus occipitalis* and *Ptychadena* spp. in regions where they are being collected are therefore highly recommended in addition to population-specific studies on recruitment and survival rates, to determine if populations can withstand the levels of harvest being experienced.

Box 7.2. Domestic trade/biological use - Case study from Nepal

Amphibians, and especially frogs, are the only group of multipurpose vertebrates in Nepal that are conjectured as permissible commodities for exploitation unaffected by the law. Their utilities expand much broader, as species particularly found in the hills and mountains across the country are highly regarded for their food value, therapeutic benefit, cultural belief, and customary ritual embedded in various ethnic groups (Rai, 2003; Shah & Tiwari, 2004). Some lowland frogs also fit in this category but a larger share in this region is captured and sold to high schools of Nepal offering science programmes (Suwal et al., 2011; Sah & Subba, 2012; Rai, 2014). The formalin-preserved specimens are eventually used in teaching concepts of vertebrates' anatomy to students through dissection curriculums in biology labs. Since the demand for such utility is entirely met from wild populations, this unregulated harvest poses serious threats to the survival of these frogs.

Amphibian harvest (Ethno-batrachology)

Nepal is a melting pot of various ethnic cultures and beliefs that are often shaped by human-environment interactions since bygone days. The majority of the ethnic communities in rural areas largely depend on natural resources and have championed ways to live in harmony with nature through the generation and transfer of rich traditional knowledge. They revere, protect and utilise all forms of natural resource (as food and medicine), including frogs vernacularly known as 'Paha'. It is, however an umbrella term that represents entire species used for subsistence living in different ecological belts of Nepal, particularly freshwater bodies; rivers, streams, waterfall, lake, pond, spring, irrigation canal, and wetland. The origin of paha terminology could be traced to the olden days of its use by Tamang people in Nepal to denote Liebig's paa frog and related species (Dubois, 1975; Dubois, 1992). Today, the use of paha has been documented by at least 12 ethnic groups both in the low and high land regions (Shah, 2001; Rai, 2003; Shah &

Tiwari, 2004; Lohani, 2010; Lohani, 2011; Lohani & Bharyang, 2011; Rai & Singh, 2015; Shrestha, Pandey & Gautam, 2019; Shrestha & Gurung, 2019). The harvest for sustenance, recreational eating, and presumed health benefits concentrates generally on fork-tongued frogs of the family Dicroglossidae, such as the genera *Paa*, *Ombrana*, and *Hoplobatrachus* (Shah & Tiwari, 2004; Kastle, Rai & Schleich, 2013). Among them, large-bodied species like Liebig's paa frog (*Paa liebigii*) are pervasively popular due to their wide distribution in the hills and high-mountains (below snowline) throughout Nepal, whereas bullfrogs (*Hoplobatrachus tigerinus* and *H. crassus*) are on the radar for lowland to small-hill communities. Because both these species take the lion's share in their multipurpose utility, they have massively been harvested across Nepal – a culture (practice) that is pervasive in villages. The rest of the frogs under Dicroglossidae can be quite specific to their purpose, for example, Sikkim Asian frog (*Ombrana sikimensis*) constitutes for food (Shrestha & Gurung, 2019). Some small-bodied species like Blanford's paa frog (*Paa blanfordii*) Polunin's paa frog (*Paa polunini*), Rostand's paa frog (*Paa rostandi*), qualifies for both food and curative uses, only in absence of *P. liebigii* (Rai, 2003). Another group of frogs from the family Ranidae, especially cascade frogs of the genus *Amolops*, such as *Assam* cascade frog (*Amolops formosus*), Marbled cascade frog (*Amolops* cf. *marmoratus*), and Mountain cascade frog (*Amolops monticola*) is also harvested for subsistence over the hills of Nepal (Rai, 2003; Shah & Tiwari, 2004). Species of the genus *Xenophrys* (eg. *Megophrys*) are used for their therapeutic properties as well (Shah & Tiwari, 2004).

Harvest for subsistence and collection strategy

Those used for traditional medicines, the meat is mixed with herbs to treat several minor ailments and diseases like dysentery, diarrhea, cough, cold, stomach ache, headache, urine problems, asthma, fever, measles, pneumonia, tuberculosis, typhoid, etc. (Shah, 2001; Rai, 2003; Shah & Tiwari, 2004; Shrestha & Gurung, 2019). Besides meat, eggs, skin secretion, and excreta are also used to heal open wounds, cuts, burns, typhoid, and rheumatism. Some communities believe that dried paha eggs cure impotency. Meat is an excellent source of nutrition for malnourished kids, people recovering from illnesses, pregnant women, and nursing mothers. For aforementioned meat-related usages, paha are skinned, eviscerated, and then used either raw for meat or preserved (as smoked) for the future. Hunting paha is rampant in villages, especially that of hills and mountains where different age-group people are involved. There is no harvest limit set or monitored and one may collect almost everything during their search effort. The collection is also year-round employing specific strategies except for the winter season. Such unchecked harvest spells grave danger to the population of paha frogs. Based on the local practice, paha is collected basically from streams in different ways; at night when frogs come out of hiding, the collectors keep bamboo flambeau – its light blinding frog's vision temporarily, later followed by handpicking. Some divert the river water into smaller channels and place bamboo traps on the end while some are involved in daytime hunting by flipping big rocks and handpicking. In recent days, paha collection is usually aimed for recreational purposes, especially recreational eating as their meat is relished and available free compared to poultry and livestock. Some forms of trade exist in villages with goods and money, somewhere in the range of USD 0.45-2.26 (Shrestha & Gurung, 2019).

Mass harvest for dissection

Four species from the Dicroglossidae family, Tiger frog (*H. tigerinus*), Jerdon's bullfrog (*H. crassus*), Terai cricket frog (*Minervarya teraiensis*), and Skittering frog (*Euphlyctis* cf. *cyanophlyctis*) make up most of the animals collected for the dissection classes. There is fragmentary evidence of quantification regarding mass harvest all across Nepal, some data primarily region-specific (Suwal et al., 2011; Sah & Subba, 2012; Rai, 2014). Each student requires an average of 2-6 frogs for dissection so the quantities technically exceed the total number of students studying biology every academic year. In 2001, around 47,000 frog specimens were used for dissection across educational institutes in the eastern region of Terai and some in Kathmandu, Nepal (Rai, 2014). For the academic year 2010/11, a range of 52,151 – 102,405 frogs was dissected across high schools, mostly from Kathmandu and lowland Terai regions (Suwal et al., 2011). Between 2010-2012, almost 14,000 bullfrogs (*H. tigerinus*) were dissected by Grade XI students across high schools in Biratnagar, eastern lowland Nepal (Sah & Subba, 2012). During the same period, harvesters also collected frogs for consumption which was estimated at a minimum of a thousand individuals per night. The authors posit that such haphazard collection may have pushed the local population on a declining trend as the capture quantities became less abundant within the same collection locality in just two years. It can be assumed that in absence of regulatory mechanisms, Nepal may face a similar fate in near future as of India and Bangladesh, where the population of overly harvested species saw a major decline, if the impact of such trade is kept overlooked. Since India banned exporting frogs to Nepal for dissection, all used specimens are wild-caught populations. The supply chain for dissection constitutes local collectors, based in Terai who supply the frogs either to biological enterprises (who then sell it to the colleges) or directly to high schools (colleges). An individual specimen may cost somewhere between NPR 20-100 (USD 0.18-0.90) based on the nature of the supply chain.

Probable ecological impacts of uncontrolled harvest

Many adult amphibians whose elevational range extends in the high-altitude region share several life-history traits such as body size, clutch size, and longevity (Zhang & Lu, 2012). Those living in high-altitude (> 2,500 m) compared to lowland relatives have a stunted developmental growth rate (low metabolism) throughout metamorphosis. They gain sexual maturity at older ages, thus have brief breeding seasons, rendering lesser spawning frequency with larger eggs (Morrison & Hero, 2003). The unchecked harvest for some species in line with their intraspecific differences may be detrimental to the overall population, including for example, *P. liebigii* (1,500-3,360 m), *P. polunini* (2,600-3,400 m), *P. rostandi* (2,400-3,500 m), *A. formosus* (1,190-2,896 m), *A.* cf. *marmoratus* (840-2,896 m), and *O. sikimensis* (1,210-2,500 m; Shah & Tiwari, 2004).

Because of the mass harvest for trade, frog populations in India collapsed for two species, *Euphlyctis hexadactylus* and *H. tigerinus* in 1985, compelling the authorities to list them in Appendix II of CITES (Altherr et al., 2011). Nepal is also a range country for *H. tigerinus* and despite the country not having international trade of frogs some forms of domestic trade largely exist, particularly for dissection purposes. Nepal doesn't have frog farming practices, thus all the frogs captured for human use are wild-caught. This, however, by no means advocates for introducing the concept of frog farms in the country. It is because such farms are prone to failures both ecologically and economically (Kusrini, 2005; Gratwicke et al., 2009; Schloegel et al., 2009).

Frogs are carnivorous and usually feed on insects, keeping their populations in balance. Some lowland frogs (genera *Hoplobatrachus, Limnonectes,* and *Euphlyctis*) have been found extremely helpful to the farmers by acting as pest control agents in the rice fields and controlling populations of harmful insects like houseflies and mosquitoes that affect human health (Khatiwada et al., 2016). In the hilly regions, *Amolops formosus* also consumes insects that are harmful to agriculturally important plants and human health. If frogs become less abundant, farmlands will see explosive growth in insect population and pesticides-use. Before they face rapid decline due to overharvesting, it is thus urgent to manage frog populations by gaining legal measures in a modality of participatory resource management. This may include but is not limited to banning destructive collection practice that harms the species and habitat, enacting open/closed harvest seasons, introducing catch limits, and imposing fines. Subsistence harvest should be monitored and allowed, without jeopardising the ability of the local population to continue their next generation. Dissecting real frogs has become obsolete in many countries, Nepal should also revamp the biology curriculum replacing real dissection with virtual programmes such as Froguts which is freely available and comprehensive (https://thesciencebank.org/pages/froguts). The existing information of species biology, niche, population ecology, and harvest rates must also be enhanced to investigate the dynamics of harvest, eventually to develop guidelines (policy) for sustainable harvesting, if needed.

Box 7.3. Domestic and international trade/medicinal and tourist use - Case study from Bolivia

Background

Bolivia holds more than 270 species of amphibians and in general, with the exception of a couple of species (*Telmatobius culeus* and *Rhinella spinulosa*), amphibians are not used for any purpose and are not seen as a protein source, although there are isolated reports of food source use in the lowlands. One of the two species used is the Titicaca water frog (*Telmatobius culeus*), consumed as a protein source in surrounding towns of Lake Titicaca and some Peruvian and Bolivian cities. Domestic pet trade is not officially reported in Bolivia, but there are informal reports of native species such as *Boana riojana, Boana geographica* and *Phyllomedusa camba,* offered together with exotic species such as albino Clawed frogs (*Xenopus spp.*) and Axolotl (*Ambystoma spp.*), being sold in pet markets in two main cities (La Paz and Cochabamba). There are no official reports of Bolivian species in the international pet trade, but there are Bolivian species in European pet shops. Local markets sell mainly high Andean amphibians such as *Rhinella spinulosa*, *Pleurodema cinereum* and *Telmatobius* spp. for traditional use, where different products and animals (including amphibians) are offered to Pachamama or Mother Earth. Previously, it was common to find hundreds of dissected frogs and toads with money in their mouths as a symbol of prosperity in local markets.

The Titicaca water frog and frog "juice"

The Titicaca water frog is an iconic amphibian species. Listed as Endangered on the IUCN Red List (IUCN, 2020), as Critically Endangered in the Bolivian red book of vertebrates (Ministerio de Medio Ambiente y Agua, 2009), and listed in Appendix I of CITES, it is endemic to Titicaca Lake and smaller surrounding lakes of Bolivia and Peru, where it is offered in different markets. Previously (early 1900s), *T. culeus* did not appear to be used for human consumption; at this time Allen (1922) reported that despite being a potential good source of protein, frogs were not used by local communities. Nowadays frogs are intensively harvested for human consumption, where in some cases between 2,000 and 4,500 individuals are reportedly illegally traded and confiscated, especially in Peru. In the 1970s and 1980s local communities were consuming the species, mainly in soup form. At the same time, they were actively harvesting large individuals to sell them as frog legs in local restaurants and restaurants in La Paz. In the last decade there has been an increasing demand for Peruvian and Bolivian markets, where the frog is used together with other ingredients for frog "juices", offered as a nutritional booster and presumed to have medicinal properties or potions presumed to improve the energy and sexual condition of consumers. Thousands of frogs are actively collected every month to be sold in markets; they are transported to Cuzco, Lima and other main cities in Peru, and La Paz, El Alto, Oruro and Cochabamba in Bolivia. These juices are even offered as part of tourist packages.

Other reports indicate that, in several towns on the Bolivian side of the lake, buyers come to buy hundreds of frogs per week from local fishermen, destined to go to Peru. Around 15,000 individuals were confiscated in 2006, and in 2011 visitors from Asia stopped in several towns around the lake seeking to buy large live individuals, possibly destined for international trade.

Legal instruments for the Titicaca water frog's conservation

There are different legal instruments in Bolivia to protect species like the Titicaca water frog, such as Environmental Law No. 1333, which establishes the obligation to carry out the sustainable use of authorised species; the General and Indefinite Ban No. 25458, that prohibits any use of Bolivian fauna; Resolution No. 309 of December 2006 issued by the National Competent Environmental Authority, which presents the technical standard with

Guidelines for Wildlife Management Plans; and finally resolution No. 024 of 2009 issued by the National Competent Environmental Authority, which regulates scientific research on biological diversity in Bolivia. In Peru, the Titicaca water frog is listed as Critically Endangered by Supreme Decree Nº 004-2014-MINAGRI, where all commercial activity is banned for this and other species listed in the decree. Internationally, the species has been added to Appendix I of CITES in 2017, which indicates that commercial international trade is prohibited.

Despite these legal instruments, they have been unable to curb the illegal use or domestic trade of this Endangered species. Also, the international trade between Peru and Bolivia in violation of CITES provisions is still very active, with insufficient law enforcement. Regarding trade to other countries, there are a couple of confiscations of individuals of this species in Ecuador and up until a couple of years ago it was still possible to find websites listing the species for sale in Europe. Due to the unique characteristics of this frog and interest in this species by the pet trade, stronger global monitoring is needed to better protect it from illegal trade.

Most of the publicly accessible amphibian trade data recorded within the English language originates from the United States Fish and Wildlife Service (USFWS) Law Enforcement Management Information System (LEMIS). The LEMIS data are made available through a Freedom of Information Act Request (FOIA) and represent the most comprehensive wildlife trade data for all amphibian species traded internationally by the USA. Although the USFWS LEMIS database provides detailed information about amphibians that were either imported or exported from the United States, it does not include data on domestic trade.

According to these LEMIS data, 769 individually recorded species of amphibians have been traded by the USA between 2000 and 2014, although the actual number might be lower since this includes an unknown quantity of taxonomic synonyms as well as taxonomic names that are no longer presently recognised as valid (Eskew et al., 2020). The information maintained in this database is unique compared to the trade records collected by most other countries where only the trade in CITES-listed species is uniformly maintained and all non-CITES species are excluded from recordkeeping. Therefore, patterns of international trade in hundreds of non-protected amphibian species from around the world are only available through government records of importation to the USA, maintained in the LEMIS database. It is however important to note that the inclusion of other languages results in a linear increase in cases of amphibian trade (Hughes, Marshall & Strine, 2021), and while Hughes, Marshall and Strine (2021) detected 1215 amphibian species in trade, including 575 species only found available online, additional hundreds can be found with the inclusion of two more languages in search queries: Korean and Portuguese (Koo et al., 2020; Máximo et al., 2021).

The amount of domestic harvest and use of amphibians, as well as the volume of international trade in non-CITES listed species, represent significant knowledge gaps in many parts of the world. The latter especially deserves greater effort to measure and record, because the level of international exploitation is a required piece of information for inclusion in proposals to list additional species in the CITES Appendices (https://cites.org/eng/disc/species.php). If such proposals become adopted, then standardised recordkeeping and reporting becomes a required component of international trade activity. At present (September 2021), only 201 of the more than 8,000 described amphibian species are CITES-listed, with a disproportionate number of species categorised as Data Deficient by the IUCN Red List of Threatened Species. Beyond the simple lack of information, Data Deficient species are of additional concern because they are likely to be under higher risk of extinction compared to species with sufficient information on the IUCN Red List (Howard & Bickford, 2014). The volumes of global trade in all CITES-listed amphibian species can be publicly accessed from the CITES Trade Database (https://trade.cites.org/). Unfortunately, due to the aforementioned limitations, it is presently largely unknown how many of the world's 8,000+ amphibian species have appeared in international trade, beyond the 201 reported to the CITES Secretariat, the few hundred non-CITES listed species traded and reported by the United States (Kolby, 2016), and those informally observed and reported from domestic markets (Altherr, Freyer & Lameter, 2020). Unlike the international trade records submitted to the CITES Secretariat, no centralised database exists to capture data that might be collected by governments describing domestic trade. A considerable research effort is therefore presently needed to integrate all sources of existing data to provide a comprehensive global snapshot of the trade in both CITES and non-CITES listed amphibians. This effort should not be restricted to the scientific research community, but should be a joint effort with regional and national governments, as well as other regional, national and international legislative agencies that can provide public access to databases of trade records.

Accuracy of species identification among trade records

The precision and accuracy of wildlife trade records varies considerably, both within and between different sources of information. In some circumstances, this is due to established institutional procedures whereby amphibian trade data are recorded at higher levels of classification, such as by genus or class, rather than by species. For example, customs border control officers often record shipments as "amphibians" or "frog legs" without any species information attached to these data. Amphibian trade records maintained by the USFWS LEMIS database contains potentially the most species-specific records accessible in English, and yet still includes many records described as “Non-CITES Amphibians” or with only the name of the genus. Therefore, the international trade in most amphibians that are not specifically protected or regulated is much less accurately and uniformly documented, and is consequently difficult to objectively characterise.

Another caveat to the interpretation and application of wildlife trade records for conservation purposes is the variable level of scientific accuracy expressed by law enforcement officers recording these data, both with respect to taxonomical precision and visual identification. For instance, in the United States, a Declaration for Importation or Exportation of Fish or Wildlife (Form 3-177) must be presented to a USFWS Wildlife Inspector in order for the shipment to be granted clearance and allowed to enter commerce. Sometimes, these decisions are made based on document inspections without physically inspecting the animals themselves, and the actual species traded might differ from those named on the documents provided by the traders. Thus, for shipments which are not physically inspected, these misidentifications can then become the accepted records of trade. Other times, wildlife trade enforcement officers might perform physical inspections but misidentify the species present. With 8,000+ described species of amphibians, and only 201 which presently require CITES permits for legal international trade to occur, there is little global incentive to train wildlife officers to identify the thousands of amphibian species which can potentially be traded without special permits. Therefore, law enforcement officers may sometimes misidentify unprotected species because their priority is instead to ensure permits are present, when required. Additional identification and monitoring challenges arise when amphibians are traded in the form of skinless frog legs and the species traded may not be those listed on the export documents. This has been demonstrated in Indonesia where shipments of frogs legs documented to included *Limnonectes macrodon*, *Fejervarya cancrivora, F. limnocharis,* and

Lithobates catesbeiana, were genetically sampled and proved only to contain *F. cancrivora* (Veith et al., 2000; Kusrini, 2005).

Without the ability to retrospectively spot-check the accuracy of amphibian trade records against what was physically traded, it is not currently possible to evaluate whether errors in species identification are commonplace or infrequent among these data. Irrespective of the frequency, any amount of species misidentification among official government wildlife trade records can have significant negative repercussions on the development of effective conservation policies aimed to reduce the threat of trade. For example, in 2019 a CITES listing proposal to include the genus *Paramesotriton* in CITES Appendix II (https://cites.org/eng/disc/species.php), stated that, "According to the LEMIS Database of the U.S. Fish & Wildlife Service, imports to the U.S.A. have involved a total of 38,273 individuals of *Paramesotriton* spp. between 2000 and 2016…" (CITES CoP18 Prop. 40). A closer examination of a subset of these same LEMIS records (trade from 2006-2010) showed that 233,924 individuals of *Paramesotriton* newts had been imported to the USA in just one third of the aforementioned time span (Kolby et al., 2014). It was discovered that this discrepancy occurred in part because USFWS had recorded 216,054 animals as *Triturus hongkongensis*, used as an invalid synonym for *Paramesotriton hongkongensis*, of which only 17,870 had been accurately recorded as the latter. Additionally, two shipments which were imported in 2012 and recorded in LEMIS as *Paramesotriton hongkongensis* had been incorrectly identified by the importers and accepted by USFWS, and were instead newts of the genus *Pachytriton* (J. Kolby, pers. comm.). The two aforementioned shipments each contained 1,600 individuals, and it is unknown how many more of the thousands of animals imported into the USA as *Paramesotriton hongkongensis* have similarly been recorded with incorrect species identifications. Although the CoP18 CITES listing proposal for inclusion of

Paramesotriton spp. in CITES Appendix II was successfully adopted despite the erroneously low trade data estimate (https://cites.org/eng/disc/species.php), it is plausible that similar misidentifications among wildlife trade records could have negative consequences for at-risk species in need of increased protection and regulation.

Amphibian trade data accessibility and biased communication of impacts

In addition to legal harvest and trade, a large portion of amphibians are harvested and traded illegally, both domestically and across international borders. The illegal international trade in wildlife is often considered sensitive information by law enforcement agencies, and even for CITES-listed species, these data are infrequently openly shared. Only recently, Parties to CITES have been requested by the CITES Secretariat to begin submitting reports of illegal wildlife trade, but unlike the reports of legal trade that are made publicly available, these illegal trade reports are not. Therefore, most of the publicly available government data describing the nature of global amphibian trade are restricted to records that describe primarily legal trade in CITES-listed species. Outside of the CITES framework, amphibian trade monitoring is equally deficient and the data available from organisations such as the World Customs Organization cannot be used adequately (Chan et al., 2015). Despite requests for improvements at the IUCN's 5th World Conservation Congress (WCC-2012-Res020) in 2012, the changes are so far not implemented.

As with most issues involving multiple countries and regions, identification of data collected on amphibian trade is sometimes limited by language barriers. Official documents from government and non-government agencies are recorded using the respective language of a given country. Consequently, most of the primary literature and secondary syntheses visible to the international scientific community are restricted by the data and information researchers are able to not only access but also comprehend. As such the apparent lack of data from certain regions may instead be an artifact of the presence of language barriers. For example, Altherr, Freyer and Lameter (2020) provided a report describing surveys of reptiles and amphibians offered for sale online and at exotic pet markets in Germany, published in German, which English-based data queries would fail to locate. It is also true that some countries don't gather this information or there is no system where all this data can be gathered.

When discussing harvest and consumption, there is a history and tendency to place the emphasis, and in essence the blame, on resource management within export countries. This prevalent but problematic view ignores the socioeconomic inequalities that are at least partially responsible for driving amphibian trade and harvesting. Aside from the biases it creates in the literature, failure to address the inequalities in trades can impede efforts to prevent further exploitation of amphibians. Major frogs' legs importing countries, for instance, are generally high-income countries, such as France, United States, Belgium, and Luxembourg (United Nations' Commodity Trade Statistics Database, United Nations Statistic Division, 2008; Warkentin et al., 2009). However, despite being one of the leading amphibian importers, policies and regulations in the EU are often insufficient to prevent overharvesting in export countries (Auliya et al., 2016). Even within regional markets, consumerism is largely driven by higher income countries such as Singapore and Hong Kong (Kusrini & Alford, 2006). While improving local and regional policies are fundamental to regulating amphibian trade, an acknowledgement of responsibility and an investment in addressing this issue by high-income, import countries is a key step that needs to be taken. A simple parallel can be seen in the shift in public consciousness from putting the burden of addressing deforestation on the export countries to acknowledging the role import countries play in driving the market and demand.

Sustainable amphibian trade

What is sustainable amphibian trade?

Efforts to assess sustainability of domestic and international use and trade in amphibians should be founded upon a common understanding of the term “sustainable”, to provide objective context for its use (Table 1). According to the Convention on Biological Diversity (CBD) from 1993, "Sustainable use" means “the use of components of biological diversity in a way and at a rate that does not lead to the long-term decline of biological diversity, thereby maintaining its potential to meet the needs and aspirations of present and future generations” (https://www.cbd.int/doc/legal/cbd-en.pdf. Accessed 10 May 2021). This CBD definition is also the working definition adopted by the Parties to CITES (CITES Resolution Conf. 13.2 Rev. CoP14; https://cites.org/sites/default/files/document/E-Res-13-02-R14.pdf). In this chapter, we similarly apply the term “sustainable” to describe use and trade activities that do not reduce wild populations of amphibians to levels likely to threaten their survival. Additionally, we define unsustainable amphibian trade to include any illegal trade activity, because the illegal trade in wildlife inherently undermines any nations’ rules and regulations enacted to protect affected species from overexploitation. Published examples of sustainable amphibian trade are rare (but see efforts by Kusrini (2005) to evaluate sustainability of the frog legs trade in Indonesia). Moreover, extinction risks associated with the trade of wild caught specimens is increasing (Hughes, Marshall & Strine, 2021), a trend that is likely to persist until additional regulations are implemented where appropriate (Borzée et al., 2021).

Table 1. Generalised types of use and primary sources of supply and demand of the global amphibian trade.

Type of use	Primary origin of supply	Primary market driving demand	Source (CITES)	Notes
Human consumption for food (subsistence, local consumption markets)	Africa, Asia, South America	Africa, Asia, South America	W,C	
Human consumption for food (exotic gastronomy, global consumption markets)	Asia	North America, Europe	W,C	Bullfrogs constitute a notable case as they are traded globally but also imported into the US (where they are native to)
Medicinal use	Africa, Asia, South America	Africa, Asia, South America	W,C, O	
Pet trade	Central and South America, Asia	Mostly North America, Europe	W,C,F, R, O	
Cultural use	Africa, Asia, Americas	Africa, Asia, Americas	W,C	
Educational use	Africa, Asia, Americas	Africa, Asia, Americas	W,C,F, O	

Zoological use	North America, Europe	North America, Europe	W,C,F, R	

Trade in wild-collected amphibians reported as bred in captivity

The trade in animals bred in captivity is often considered to exert reduced or negligible negative impacts on wildlife populations in their native environments compared to the trade in wild-collected animals. For this reason, the trade in wildlife produced in captivity is generally allowed to occur with fewer governmental restrictions in many countries. Particularly with respect to CITES-listed species, many countries that prohibit commercial exportation of wild-collected specimens allow for the regulated export of animals produced in captivity. Unfortunately, systems of relaxed provisions are sometimes exploited and there is growing evidence of illegal trade in wild-caught specimens of CITES-listed species traded with fraudulent documentation, particularly using incorrect source codes. The CITES source codes that are commonly used to describe the origin of a traded animal include W (wild: specimens taken from the wild), C (bred in captivity: Animals bred in captivity in accordance with CITES Resolution Conf. 10.16 (Rev.); https://cites.org/sites/default/files/document/E-Res-13-02-R14.pdf), F (born in captivity: animals born in captivity (F1 or subsequent generations) that do not fulfil the definition of 'bred in captivity' in Resolution Conf. 10.16 (Rev.)), and R (ranched: specimens of animals reared in a controlled environment, taken as eggs or juveniles from the wild, where they would otherwise have had a very low probability of surviving to adulthood; Table 2). To investigate and respond to this concern, in 2016 the Parties to CITES adopted Resolution Conf. 17.7 (Rev. CoP18) *Review of trade in animal specimens reported as produced in captivity* which stated that, "...the incorrect application of source codes and/or misuse or false declaration of source codes can reduce or negate such benefits where they exist, have negative implications for conservation and undermine the purpose and effective implementation of the Convention".

Table 2. Definitions of commonly used CITES source codes for traded amphibians.

Source Code	Code Name	Code Definition
W	Specimens taken from the wild	Specimens taken from the wild.
C	Animals bred in captivity	Animals bred in captivity in accordance with Resolution Conf. 10.16 (Rev.), as well as parts and derivatives thereof, exported under the provisions of Article VII, paragraph 5, of the Convention.
F	Animals bred in captivity that do not qualify for a “C” code	Animals born in captivity (F1 or subsequent generations) that do not fulfil the definition of 'bred in captivity' in Resolution Conf. 10.16 (Rev.), as well as parts and derivatives thereof.
R	Ranched specimens	Specimens of animals reared in a controlled environment, taken as eggs or juveniles from the wild, where they would otherwise have had a very low probability of surviving to adulthood.

(Source: CITES Trade Database – User guide, version 8. Available at https://trade.cites.org/cites_trade_guidelines/en-CITES_Trade_Database_Guide.pdf)

This Resolution established a process of review, dialogue, and evaluation to improve the capacity of CITES Parties to determine whether animals genuinely originated from the declared source or production system and to ascertain the legal origin of parental stock of captive bred specimens, especially those that may have been sourced outside their native ranges. This review process occurs in multiple stages and is meant to complete one full cycle every 2-3 years, bookended by the start of each CITES Convention of the Parties. At present (September 2021), this cycle has occurred only once, and the start of the second cycle, beginning with the selection of new species/country combinations for review, is now postponed until after CITES CoP19 due to delays caused by the COVID19 pandemic (CITES AC31 Doc. 19.1; https://cites.org/sites/default/files/eng/com/ac/31/Docs/E-AC31-19-01.pdf).

In the first iteration of this review process, two countries and two amphibian species were included for consideration: Panama for the strawberry poison frog (*Oophaga pumilio*) and Nicaragua for both the strawberry poison frog (*Oophaga pumilio*) and the red-eyed tree frog (*Agalychnis callidryas*). Both countries were sent a list of questions by the CITES Secretariat requesting information including the scientific basis by which these countries determined their exports were non-detrimental to these species, descriptions of the production methods by which they were producing frogs in captivity, wildlife trade and management methods, and additional details. The CITES Animals Committee then reviewed the responses received (see AC30 Doc. 13.1 A2 (Rev. 3); https://cites.org/sites/default/files/eng/com/ac/30/E-AC30-13-01-A2-R3.pdf) and determined that the trade in specimens of *A. callidryas* by Nicaragua reported as bred in captivity was in compliance with Article III and Article IV of the CITES Convention, as well as Article VII, paragraphs 4 and 5, meaning that their use of source code "C" was found to satisfy all requirements. In September 2018, in accordance with paragraph 2 g) of the Resolution, this species-country combination was excluded from further review (CITES AC31 Doc. 19.1).

Meanwhile, the trade in *O. pumilio* remained in review for both countries and the CITES Animals Committee recommended that by 1 February 2019, both Panama and Nicaragua should confirm that they would export specimens from facilities breeding this species only using the source code “W” or “F” and stop using the source code “C”, and will also make legal acquisition and non-detriment findings prior to authorising export (CITES SC70 Doc.31.3). At CITES Standing Committee 71 in August 2019, it was reported that Nicaragua confirmed it would implement this recommendation, but no response was received from Panama (CITES SC71 Doc. 13). The Standing Committee then requested that the CITES Secretariat publish an interim zero export quota for specimens of *O. pumilio* from Panama in the absence of their response (CITES AC31 Doc. 19.1). Panama did subsequently respond to the CITES Secretariat, but at present (September 2021), the content and evaluation of this response has not yet been made publicly available in either the CITES Animals Committee or Standing Committee documents posted on the CITES website and this issue does not yet appear to be resolved.

Spread of diseases by the amphibian trade

Highly pathogenic amphibian pathogens

The national and international trade in amphibians is the greatest contemporary source of global spread of amphibian pathogens (Kolby, 2016; Nguyen et al., 2017; O’Hanlon et al., 2018). The most devastating amphibian pathogens with respect to the number of species impacted and propensity to cause mass mortality are the two species of amphibian chytrid fungus (*Batrachochytrium dendrobatidis* and *B. salamandrivorans*) and ranaviruses. It has been estimated that approximately 500 species have already been negatively affected by chytridiomycosis, the disease caused by infection with chytrid fungus, and nearly 100 species may already be extinct due to this pathogen, in connection with other factors (Scheele et al., 2019).

Despite a growing body of scientific literature showing that the trade in amphibians is spreading deadly pathogens (Schloegel et al., 2009; Schloegel et al., 2012; Kolby et al., 2014; Kolby et al., 2015; Kolby, 2016; Nguyen et al., 2017; O'Hanlon et al., 2018), most governments have implemented relatively minimal biosecurity actions, if any at all. Novel regional strains of *B. dendrobatidis* with high virulence and the propensity to cause increased declines and extinctions if they spread continue to be identified (Schloegel et al., 2012), but there seems to be a general perception that since it's already been detected in dozens of countries, it's already too late for any meaningful efforts to reduce the continued global spread of this pathogen. Instead, most governmental attention, particularly in North America, has been directed towards controlling the spread of salamander chytrid fungus (*B. salamandrivorans*) as it has only recently emerged in Europe following introduction from Asia, and it has not yet been detected in the Western Hemisphere (Martel et al., 2014; Grear et al., 2021).

In 2016, the United States Fish and Wildlife Service banned the importation of 201 species of salamanders by listing them as injurious species under the Lacey Act. The intention was to prevent the introduction of species likely to carry this pathogen into the USA, based on results from laboratory exposure trials on a small number of tested species (Martel et al., 2014). If a species was found to be susceptible to infection, the entire genus was then listed as injurious. The USA is the global hotspot of salamander biodiversity and thus has good reason to take every reasonable measure to prevent a biodiversity catastrophe if native wild amphibians were to become exposed to this pathogen. Still, the US chose not to take a more precautionary approach, and does not prohibit the import and trade of species within genera for which susceptibility to infection is unknown. In 2017, it was discovered that frogs can also become infected with and vector *B. salamandrivorans* (Nguyen et al., 2017) but following this announcement, USFWS has continued allowing the importation of millions of frogs each year without any increased restrictions to control the possible presence of this pathogen among anurans.

In contrast to the approach adopted by the USA, where only one-third of described salamander species have been prohibited from importation, Canada has enacted legislation which prohibits the importation of all species of salamanders based on, "...the precautionary principle, and takes into consideration the limited and evolving understanding of the disease, as well as the enforcement challenges associated with identifying different salamander species at Canada's numerous ports of entry" (Government of Canada, 2017). Although initially enacted for one year pending further study, this import prohibition continues at present (September 2021).

In the European Union, "The Scientific Working Group of the European Union recently (June 2016) decided that an import prohibition for Asian salamanders should be implemented by placing those salamanders on Annex B of the EU regulation 338/97" (Auliya et al., 2016), and Switzerland has also banned their trade in amphibians (Schmidt, 2016). Although not specifically aimed to prevent the spread of amphibian diseases, shortly following the emergence of the COVID19 pandemic Vietnam enacted a ban on its wildlife trade, including amphibians, and the Republic of Korea now also prohibits the importation of non-native amphibians (Borzée et al., 2021).

Zoonotic pathogens carried by amphibians

In addition to pathogens that cause harm to amphibians, some pathogens transported through handling and consuming these animals can also cause disease in humans. For example, *Spirometra erinaceieuropaei*, a highly pathogenic tapeworm parasite responsible for the human disease sparganosis, was detected in 9.8% of frogs sampled from food markets in Guangdong, China (Wang et al., 2018). Research in Thailand found that 90% of amphibians sampled from frog farms were infected with *Salmonella*, demonstrating how the trade in frogs for food can serve as a pathway of *Salmonella* dispersal and exposure (Ribas & Poonlaphdecha, 2017). Additionally, frogs sampled from the pet trade in Japan have recently been discovered to carry *Veronaea botryosa*, a pathogenic fungus that caused lethal chromomycosis in many of the affected amphibians (Hosoya et al., 2015). Previously, humans were the only animal known to be susceptible to this pathogen. Sampling of confiscated frogs in Peru designated for human consumption showed a predominance of *Aeromonas* spp. and *Vibrio* spp. on Lake Titicaca frogs (Edery et al., 2021). As millions of farmed frogs are internationally traded as a source of protein for humans (Warkentin et al., 2009; Altherr, Goyenechea & Schubert, 2011), it is possible that the trade in amphibians for food may spread zoonotic pathogens more commonly than presently recognised. Major importing nations of live wildlife, such as the USA, do not sample amphibians for pathogens upon importation, and so there is little data to evaluate the frequency of zoonotic pathogen introduction through this dispersal pathway (Kolby, 2019).

Discussion

The global trade and use of amphibians are known to affect thousands of species (Hughes, Marshall & Strine, 2021), but records of amphibian trade are not often collected, maintained, or made publicly accessible for research purposes. Improved monitoring efforts are sorely needed to better understand whether additional species are threatened by local or international use and how these activities may be managed in a more sustainable fashion. The role of trade in the spread of batrachochytrids is particularly alarming because these pathogens are frequently detected among amphibians traded internationally (Kolby, 2016) and have caused more species declines and extinctions than any other disease in recorded history (Scheele et al., 2019). Despite the various uncertainties described in this chapter regarding regional and species-level amphibian population estimates, numbers of animals collected from the wild versus those bred in captivity, and how these factors relate to sustainable use, the overall trade in amphibians precautionarily appears currently unsustainable at the present time. This is particularly alarming due to the high frequency of disease vectors being transported without biosecurity measures to prevent pathogen transmission and the severely negative consequences of emerging infectious diseases on wild amphibians around the world today. Further research is needed to explore the feasibility of "pathogen-free" trade methods and governments should consider requiring animals to be free of chytrid, ranavirus, or other pathogens prior to allowing trade to occur. Although published case studies of species-specific sustainable amphibian trade are uncommon, this does not imply the absence of sustainable amphibian trade, as the annual legal trade in thousands of CITES Appendix-II listed amphibians occurs with governmental scientific evaluations that this trade is not detrimental to these species (https://cites.org/eng/disc/species.php). Still, it is likely that some of the 7,000+ non-CITES listed amphibian species may qualify for future listing actions as more information becomes available to evaluate. Taking into consideration the data challenges, uncertainties, and recommendations described in this chapter, efforts to better characterise the nature of amphibian trade and reduce known and potential negative impacts are urgently needed to help protect global amphibian biodiversity.

Recommended actions (in no order of priority):

1. Consider the development of a new Convention based upon principles similar to those of CITES, but specifically for monitoring and regulating the spread of wildlife diseases. Although the OIE functions in a similar manner, it only focuses on the spread of diseases among traded domesticated/farmed animals. An agreement was signed in 2015 between CITES and the OIE to cooperate in the control of diseases spread through wildlife trade, but no actions have yet been taken to reduce the spread of amphibian pathogens.
2. Support population assessments and monitoring of species that are collected and potentially overharvested for domestic use, including those used for food, pets, and biological purposes (e.g. dissection in university classes).
3. Encourage countries to establish stronger science-based policy actions to reduce the risk of *B. salamandrivorans* introduction through trade, based on recent publications showing that traded frogs to spread this pathogen, and not just salamanders.
4. Encourage all governments of countries that trade amphibians to develop and implement a disease surveillance program for amphibians being imported and exported. This should minimally include ranavirus and the two known amphibian chytrid fungi (*Bd* and *Bsal*).
5. Draft biosecurity policies to effectively control the spread of amphibian diseases through international trade. Particularly consider the unrestricted trade in species such as the American bullfrog (*Lithobates catesbeiana*) and African clawed frog (*Xenopus laevis*), which are known reservoir host species of amphibian chytrid fungus and ranavirus and traded in high quantities and densities.

6. Issue a request for countries to record their domestic and international trades in non-CITES listed amphibians, in any language (not restricted to English), and make these data available for scientific review.
7. Encourage governments, NGOs, and academics to report to the IUCN ASG Secretariat whether they have recorded in any language (not restricted to English), domestic and/or international amphibian trade data for non-CITES listed species. If available, these data should contribute towards future studies to better estimate threats to these species and help in the development of improved management plans to ensure amphibian trade sustainability, as appropriate.
8. Examine the socioeconomic inequalities that are driving amphibian exports and establish a dialogue on how policies can be improved on both the import and export sides of the trade.
9. Explore livelihood alternatives to frog consumption.
10. Identify species in trade in local markets and develop an identification guide for these species to help build awareness.
11. Build capacity to conduct surveys in local markets and support subsequent analysis of data.
12. Develop a local or regional database to track domestic amphibian harvesting and trade.

References

Allen, W. (1922). Notes on the Andean Frog, *Telmatobius coleus* (Garman). *Copeia* 108:52-54.

Altherr, S., Freyer, D., & Lameter, K. (2020). *Strategien zur Reduktion der Nachfrage nach als Heimtier gehaltenen Reptilien, Amphibien* *und kleinen*. Säugetieren, Germany: BfNSkripten 545.

Altherr, S., Goyenechea, A., & Schubert, D. J. (2011). *Canapés to extinction: the international trade in frogs' legs and its ecological impact*. Munich (Germany), Washington, D.C. (USA). A report by Pro Wildlife, Defenders of Wildlife and Animal Welfare Institute (eds.).

Altherr, S., & Lameter, K. (2020). The rush for the rare: reptiles and amphibians in the European pet trade. *Animals* 10:2085.

Auliya, M., García-Moreno, J., Schmidt, B. R., Schmeller, D. S., Hoogmoed, M. S., Fisher, M. C. ... Martel, A. (2016). The global amphibian trade flows through Europe: the need for enforcing and improving legislation. *Biodiversity and Conservation* 25(13):2581-2595.

Borzée, A., Kielgast, J., Wren, S., Angulo, A., Chen, S., Magellan, K. ... Bishop, P. J. (2021). Using the 2020 global pandemic as a springboard to highlight the need for amphibian conservation in eastern Asia. *Biological Conservation* 255:08973. doi:10.1016/j.biocon.2021.108973

Carpenter, A., Andreone, F., Moore, R., & Griffiths, R. (2014). A review of the international trade in amphibians: the types, levels and dynamics of trade in CITES-listed species. *Oryx* 48:565-574.

Chan, H. K., Zhang, H., Yang, F., & Fischer, G. (2015). Improve customs systems to monitor global wildlife trade. *Science* 348(6232):291-292. doi:10.1126/science.aaa3141

Channing, A., & Rödel, M.-O. (2019). *Field guide to the frogs and other amphibians of Africa*. Cape Town, South Africa: Struik Nature.

Dubois, A. (1975). Un nouveau sous–genre (*Paa*) et trois nouvelles espèces du genre *Rana.* Remarques sur la phylogénie des Ranidés (Amphibiens, Anoures). *Bulletin du Muséum National d'Histoire Naturelle* 324:1093–1115.

Dubois, A. (1992). Notes sur la classification des Ranidae (Amphibiens, Anoures). *Bulletin Mensuel de la Société Linnéenne de Lyon* 61:305–352. doi:10.3406/linly.1992.11011

Edery, S., Elias, R., Shiva, C., Weaver, T., & Reading, R. (2021). Cutaneous bacteria of confiscated *Telmatobius culeus* in Lima, Peru. *The Journal of Wildlife Diseases* 57(4):900-902. doi:10.7589/JWD-D-20-00076

Eskew, E. A., White, A. M., Ross, N., Smith, K. M., Smith, K. F., Rodríguez, J. P. ... Daszak, P. (2020). United States wildlife and wildlife product imports from 2000–2014. *Scientific Data* 7(22):6254. doi:10.1038/s41597-020-0354-5

Gonwouo, N. L., & Rödel, M.-O. (2008). The importance of frogs to the livelihood of the Bakossi people around Mount Manengouba, Cameroon, with special consideration of the Hairy Frog, *Trichobatrachus robustus*. *Salamandra* 44:23–34.

Government of Canada. (2017). Wild animal and plant protection and regulation of international and interprovincial trade act. In: *Regulations Amending the Wild Animal and Plant Trade Regulations*. Vol. 151, No. 11: May 31: https://canadagazette.gc.ca/rp-pr/p2/2017/2017-05-31/html/sor-dors86-eng.html#footnote.52700.

Gratwicke, B., Evans, M. J., Jenkins, P. T., Kusrini, M. D., Moore, R. D., Sevin, J., & Wildt, D. E. (2010). Is the international frog legs trade a potential vector for deadly amphibian pathogens? *Frontiers in Ecology and the Environment* 8(8):438-442.

Gratwicke, B., Evans, M. J., Jenkins, P. T., M.D., K., Moore, R. D., Sevin, J., & D.E., W. (2009). Is the international frog legs trade a potential vector for deadly amphibian pathogens? *Frontiers in Ecology and the Environment* 8:438–442.

Grear, D. A., Mosher, B. A., Richgels, K. L. D., & Grant, E. H. C. (2021). Evaluation of regulatory action and surveillance as preventive risk-mitigation to an emerging global amphibian pathogen *Batrachochytrium salamandrivorans* (Bsal). *Biological Conservation* 260:109222. doi:10.1016/j.biocon.2021.109222

Hosoya, T., Hanafusa, Y., Kudo, T., Tamukai, K., & Une, Y. (2015). First report of *Veronaea botryosa* as a causal agent of chromomycosis in frogs. *Medical Mycology* 53(4):369–377. doi:10.1093/mmy/myu094

Howard, S. D., & Bickford, D. P. (2014). Amphibians over the edge: silent extinction risk of Data Deficient species. *Diversity and distributions* 20(7):837-846.

Hughes, A., Marshall, B., & Strine, C. (2021). Gaps in global wildlife trade monitoring leave amphibians vulnerable. *eLife* 10:e70086. doi:10.7554/eLife.70086

IUCN. (2020). *The IUCN Red List of Threatened Species* (Vol. Version 2020-1). Gland, Switzerland: IUCN.

Kastle, W., Rai, K. R., & Schleich, H. (2013). *Field guide to amphibians and reptiles of Nepal*. Kathmandu, Nepal: ARCO-Nepal.

Khatiwada, J. R., Ghimire, S., Paudel Khatiwada, S., Paudel, B., Bischof, R., Jiang, J., & Haugaasen, T. (2016). Frogs as potential biological control agents in the rice fields of Chitwan, Nepal. *Agriculture, Ecosystems & Environment* 230:307–314. doi:10.1016/j.agee.2016.06.025

Kolby, J. E. (2016). *Pathways of amphibian chytrid fungus dispersal: global biosecurity and conservation implications.* (PhD). James Cook University, Townsville, Australia.

Kolby, J. E. (2019). *To prevent the next pandemic, it's the legal wildlife trade we should worry about.* Online. https://www.nationalgeographic.com/animals/article/to-prevent-next-pandemic-focus-on-legal-wildlife-trade Published 7 May 2020.: National Geographic: Wildlife Watch.

Kolby, J. E., Smith, K. M., Berger, L., Karesh, W. B., Preston, A., Pessier, A. P., & Skerratt, L. F. (2014). First evidence of amphibian chytrid fungus (*Batrachochytrium dendrobatidis*) and ranavirus in Hong Kong amphibian trade. *PLoS One* 9(3):e90750.

Kolby, J. E., Smith, K. M., Ramirez, S. D., Rabemananjara, F., Pessier, A. P., Brunner, J. L. ... Skerratt, L. F. (2015). Rapid response to evaluate the presence of amphibian chytrid fungus (*Batrachochytrium dendrobatidis*) and ranavirus in wild amphibian populations in Madagascar. *PLoS One* 10(6):e0125330.

Koo, K. S., Park, H. R., Choi, J. H., & Sung, H. C. (2020). Present status of non-native amphibians and reptiles traded in Korean online pet shops. *Korean Journal of Environment and Ecology* 3:106–114.

Kouamé, N. G., Ofori-Boateng, C., Adum, G. B., Gourène, G., & Rödel, M.-O. (2015). The anuran fauna of a West African urban area. *Amphibian and Reptile Conservation* 9(2):1–14 (e106).

Kusrini, M. D. (2005). *Edible frog harvesting in Indonesia: Evaluating its impacts and ecological context.* (PhD Thesis). James Cook University, Townsville, Australia.

Lohani, U. (2010). Man-animal relationships in Central Nepal. *Journal of Ethnobiology and Ethnomedicine* 6:31. doi:10.1186/1746-4269-6-31

Lohani, U. (2011). Traditional uses of animals among Jirels of Central Nepal. *Studies on Ethno-Medicine* 5:115–124. doi:10.1080/09735070.2011.11886398

Lohani, U., & Bharyang, S. (2011). Eroding ethnozoological knowledge among Magars in Cental Nepal. *Indian Journal of Traditional Knowledge* 10:466–473.

Martel, A., Blooi, M., Adriaensen, C., Van Rooij, P., Beukema, W., Fisher, M. C. ... Goka, K. (2014). Recent introduction of a chytrid fungus endangers Western Palearctic salamanders. *Science* 346(6209):630-631.

Máximo, I. M., Brandão, R. A., Ruggeri, J., & Toledo, L. F. (2021). Amphibian illegal pet trade and a possible new case of an invasive exotic species in Brazil. *Herpetological Conservation and Biology* 16(2):303-312.

Ministerio de Medio Ambiente y Agua. (2009). *Libro rojo de la fauna de vertebrados de Bolivia*. La Paz,Bolivia: Ministerio de Medio Ambiente y Agua.

Mohneke, M. (2011). *(Un) sustainable use of frogs in West Africa and* *resulting consequences for the ecosystem.* (PhD). Humboldt-Universität zu Berlin, Berlin, Germany.

Mohneke, M., Onadeko, A. B., Hirschfeld, M., & Rödel, M.-O. (2010). Dried or fried: amphibians in local and regional food markets in West Africa. *Traffic Bulletin* 22(117–128).

Mohneke, M., Onadeko, A. B., & Rödel, M.-O. (2009). Exploitation of frogs – a review with a focus on West Africa. *Salamandra* 45:193–202.

Mohneke, M., & Rödel, M.-O. (2009). Declining amphibian populations and possible ecological consequences – a review. *Salamandra* 45:203–210.

Morton, O., Scheffers, B. R., Haugaasen, T., & Edwards, D. P. (2021). Impacts of wildlife trade on terrestrial biodiversity. *Nature ecology & evolution* 5(4):540-548.

Natusch, D. J. D., & Lyons, J. A. (2012). Exploited for pets: the harvest and trade of amphibians and reptiles from Indonesian New Guinea. *Biodiversity Conservation* 21:2899-2911.

Nguyen, T. T., Van Nguyen, T., Ziegler, T., Pasmans, F., & Martel, A. (2017). Trade in wild anurans vectors the urodelan pathogen *Batrachochytrium salamandrivorans* into Europe. *Amphibia-Reptilia* 38(4):554-556. doi:10.1163/15685381-00003125

Nijman, V., & Shepherd, C. R. (2010). The role of Asia in the global trade in CITES II-listed poison arrow frogs: hopping from Kazakhstan to Lebanon to Thailand and beyond. *Biodiversity Conservation* 19:1963-1970.

O'Hanlon, S. J., Rieux, A., Farrer, R. A., Rosa, G. M., Waldman, B., Bataille, A. ... Fumagalli, M. (2018). Recent Asian origin of chytrid fungi causing global amphibian declines. *Science* 360(6389):621-627.

Onadeko, A. B., Egonmwan, R. I., & Saliu, J. K. (2011). Edible amphibian species: local knowledge of their consumption in Southwest Nigeria and their nutritional value. *West African Journal of Applied Ecology* 19(1):67-76. doi:10.4314/wajae.v19i1

Rai, K. R. (2003). *Environmental impacts, systematics and distribution of herpetofauna from East Nepal.* (PhD). Tribhuvan University, Nepal.

Rai, K. R. (2014). Anthropogenic impacts on herpetofauna; with special reference to the science education in Nepal. *Veröffentlichungen ARCO Newsletter*:12–17.

Rai, R., & Singh, N. B. (2015). Medico-ethnobiology in Rai community: a case study from Baikunthe village development committee, Bhojpur, eastern Nepal. *Journal of Institute of Science and Technology* 20:127–132. doi:10.3126/jist.v20i1.13935

Ribas, A., & Poonlaphdecha, S. (2017). Wild-caught and farm-reared amphibians are important reservoirs of salmonella, a study in north-east Thailand. *Zoonoses Public Health* 64(2):106-110. doi:10.1111/zph.12286

Rowley, J. J. L., Shepherd, C. R., Stuart, B. L., Nguyen, T. Q., Hoang, H. D., Cutajar, T. P. ... Phimmachak, S. (2016). Estimating the global trade in Southeast Asian newts. *Biological Conservation* 199:96-100.

Sah, M. K., & Subba, B. R. (2012). Anthropogenic impacts on Tiger frog *Hoplobatrachus tigerinus*, at Biratnagar and its surroundings, Nepal. *Nepalese Journal of*

Biosciences 2:156–157. doi:10.3126/njbs.v2i0.7505

Scheele, B. C., Pasmans, F., Skerratt, L. F., Berger, L., Martel, A., Beukema, W. ... Canessa, S. (2019). Amphibian fungal panzootic causes catastrophic and ongoing loss of biodiversity. *Science* 363(6434):1459-1463.

Schloegel, L. M., Picco, A. M., Kilpatrick, A. M., Davies, A. J., Hyatt, A. D., & Daszak, P. (2009). Magnitude of the US trade in amphibians and presence of *Batrachochytrium dendrobatidis* and ranavirus infection in imported North American bullfrogs (*Rana catesbeiana*). *Biological Conservation* 142(7):1420-1426.

Schloegel, L. M., Toledo, L. F., Longcore, J. E., Greenspan, S. E., Vieira, C. A., Lee, M. ... Hipolito, M. (2012). Novel, panzootic and hybrid genotypes of amphibian chytridiomycosis associated with the bullfrog trade. *Molecular Ecology* 21(21):5162-5177.

Schmidt, B. (2016). Import ban for salamanders and newts in Switzerland. Why? *Reptilien Un Amphibien in Gefahr* 57:8–9.

Shah, K. B. (2001). Herpetofauna and ethnoherpetology of the Southern Annapurna Region, Kaski District, Nepal. *Journal of Natural History Museum* 20:106–128.

Shah, K. B., & Tiwari, S. (2004). *Herpetofauna of Nepal: a conservation companion*. Kathmandu, Nepal: International Union for Conservation of Nature.

Shrestha, B., & Gurung, M. B. (2019). Ethnoherpetological notes regarding the paha frogs and conservation implication in Manaslu Conservation Area, Gorkha District, Nepal. *Journal of Ethnobiology and Ethnomedicine* 15:23. doi:10.1186/s13002-019-0304-5

Shrestha, B., Pandey, B., & Gautam, B. (2019). *Conservation guidelines for the Paha frogs from unchecked harvest in the Northern Regions of Bhojpur district, Nepal* (Vol. 17). Bhojpur district, Nepal: Save the Frogs.

Stringham, O. C., & Lockwood, J. L. (2018). Pet problems: biological and economic factors that influence the release of alien reptiles and amphibians by pet owners. *Journal of Applied Ecology* 55:2632-2640.

Suwal, M. K., Aryal, P. C., Bhurtel, B. P., & Rijal, B. (2011). *Exploitation of frogs in academic sector case study in +2 science stream in Nepal*. Kathmandu, Nepal: Companions for Amphibians and Reptiles of Nepal, .

Van Vliet, N., Moreno Calderón, J. L., Gómez, J., Zhou, W., Fa, J. E., Golden, C. ... Nasi, R. (2017). Bushmeat and human health: Assessing the evidence in tropical and subtropical forests. *Ethnobiology and Conservation* 112:123. doi:10.15451/ec2017-04-6.3-1-45

Veith, M., Kosuch, J., Feldmann, R., Martens, H., & Seitz, A. (2000). A test for correct species declaration of frog leg imports from Indonesia into the European Union. *Biodiversity and Conservation* 9:333-341.

Wang, F., Li, W., Gong, S., Wei, Y., Ge, Y., Yang, G., & Xiao, J. (2018). *Spirometra erinaceieuropaei* severely infect frogs and snakes from food markets in Guangdong, China: implications a highly risk for zoonotic sparganosis. *Tropicla Biomedicine* 35(2):408-412.

Warkentin, I. G., Bickford, D., Sodhi, N. S., & Bradshaw, C. J. (2009). Eating frogs to extinction. *Conservation Biology* 23(4):1056-1059.

Zhang, L., & Lu, X. (2012). Amphibians live longer at higher altitudes but not at higher latitudes. *Biological Journal of the Linnean Society* 106:623–632. doi:10.1111/j.1095-8312.2012.01876.x

INFORMING DECISION-MAKING

Chapter 8. Communications and education

Andrés Valenzuela-Sánchez[1,2], Gabriela Agostini[3,4], Lily Maynard[5,6], Javiera Cisternas[7], Candace M Hansen-Hendrikx[8] & Marcela Márquez-García[2,9]

[1] ONG Ranita de Darwin, Valdivia, Chile

[2] Instituto de Conservación, Biodiversidad y Territorio, Universidad Austral de Chile. Valdivia, Chile

[3] CONICET-Universidad de Buenos Aires, Instituto de Ecología, Genética y Evolución de Buenos Aires. Grupo de Estudios sobre Biodiversidad en Agroecosistemas. Ciudad Universitaria, Pabellón II. Güiraldes 2160, C1428EGA. CABA, Argentina

[4] COANA. Amphibian Conservation in Agroecosystems. La Plata, Argentina

[5] Disney Conservation - Disney's Animals, Science, and Environment. Orlando, Florida, USA

[6] Cincinnati Zoo & Botanical Garden, Ohio, USA

[7] ONG de Desarrollo Aumen o el eco de los montes, Coyhaique, Chile

[8] Amphibian Survival Alliance, Burlington, Ontario, Canada

[9] Centro de Humedales Río Cruces, Universidad Austral de Chile. Valdivia, Chile

Abstract

Most instances of detrimental environmental conditions are caused by human behaviour, and the amphibian decline crisis is not an exception. Although some species can be highly popular, amphibians are in general among the least preferred animals by people. This situation represents a source of direct and indirect threats to amphibians. In this chapter we

review key research on the human dimensions of amphibian conservation. The first section looks at human attitudes and behaviours that act as threats to amphibians. The second section offers a review about the factors that have been identified as drivers of amphibian-focused human cognition. In the third section we provide an overview of different conservation education and outreach techniques that can be used to change human behaviours and improve public support for amphibians, as well as about the role of communication in the co-production of usable science in amphibian conservation. We conclude this chapter by discussing some knowledge and methodological gaps that need to be addressed in order to better inform effective and strategic conservation education and communication actions to support amphibian conservation. Communications and education can increase stakeholder engagement and the success of amphibian conservation actions. Communicating the value of amphibian conservation using carefully designed messages, for instance by highlighting evidence about amphibians' relevance for ecosystem functioning and human well-being, or about the imperilled status of these animals, might provide a good starting point to increase the willingness to protect amphibians in decision makers and the public.

Introduction

Although some species can be highly popular, amphibians are in general among the least preferred animals by people (reviewed in Prokop & Randler, 2018). These animals can be associated with negative values, emotions, and wrong perceptions, usually resulting from the direct interpretation of folklore and superstition (Ceríaco, 2012; Deutsch, Grisolia, Bilenca, & Agostini, 2021; Tarrant, Kruger, & du Preez, 2016). This situation represents a source of direct and indirect threats to amphibians. Most instances of detrimental environmental conditions are also caused by human behaviour (Schultz, 2011), and the amphibian decline crisis is not an exception. Think of a challenging conservation problem you have encountered in relation to amphibians - protecting a rare species, cleaning up a river, implementing disinfection points to decrease pathogen dispersal in a protected area, or winning support for legislation. Inevitably, people are part of the problem and public education and outreach must be part of the solution (Jacobson, McDuff, & Monroe, 2015; Loyau & Schmeller, 2017). Good interpersonal relationships and communication among stakeholders is also necessary to produce usable science in amphibian conservation, to increase stakeholder engagement, and consequently, to boost the success of amphibian conservation actions (Wall, McNie, & Garfin, 2017; Wright et al., 2020). Therefore, although generally neglected, communications and education is a key topic to advance amphibian conservation science and practice.

Several authors have argued that efforts to promote biodiversity conservation must change human behaviours (Ehrlich & Kennedy, 2005; Schultz, 2011; Schultz & Kaiser, 2012). Education and communication strategies can play a central role in fostering conservation behaviours. Research has shown that appropriate education and outreach encourage sustainable behaviour, improve public support for conservation, reduce vandalism and poaching in protected areas, improve compliance with environmental regulations, increase recreation-carrying capacities, and influence policies and decisions that affect the environment and natural resources (e.g. Day & Monroe, 2000; Jacobson, 2009; Knudson, Cable, & Beck, 2003). For instance, amphibian-focused outreach at institutions such as zoos and aquaria can be a crucial intervention to support amphibian conservation worldwide (Dos Santos, Griffiths, Jowett, Rock, & Bishop, 2019).

In this chapter we review key research on the human dimensions of amphibian conservation. The first section looks at human attitudes and behaviours that act as direct or indirect threats to amphibians. The second section offers a review about the factors that have been identified as drivers of amphibian-focused human cognition. In the third section we provide a brief overview of different conservation education and outreach techniques that can be used to change human behaviours and improve public support for amphibians, as well as the role of communication in the co-production of usable science in amphibian conservation. We conclude this chapter by discussing some knowledge gaps that need to be addressed in order to better inform effective and strategic conservation education and communication actions to support amphibian conservation.

Direct and indirect threats

Human behaviours as a direct threat to amphibians

The presence of negative values and emotions towards amphibians can lead to anti-conservation behaviours, such as torturing and killing amphibians, illegal consumption, or removing these animals from gardens (Fig 8.1; Deutsch et al., 2021; Pagani, Robustelli, & Ascione, 2007; Tarrant et al., 2016). In general, little is known about the prevalence of these human behaviours and their consequences for amphibian populations. Persecution of amphibians based on negative values and emotions appears to be a non-significant threat in the Mediterranean basin (Cox, Chanson, & Stuart, 2006). A cross-cultural study on high school students' tolerance of frogs conducted in Chile, Slovakia, South Africa, and Turkey revealed that a low proportion of students reported negative behaviours toward amphibians such as active killing frogs (6% of respondents), although 30% of the students reported moving frogs away from their home gardens (Prokop et al., 2016). Contrastingly, a study conducted in Slovakia found that around 26% of pond owners killed adult amphibians (Prokop & Fančovičová, 2012). In South Africa, Xhosa people associate amphibians with witchery and perceive these animals as dangerous and poisonous (Brom, Anderson, Channing, & Underhill, 2020). The antidote to one of the many frog-related curses is to kill the animal, for instance, by sprinkling salt on its back (Brom et al., 2020). This salt sprinkling also occurs in Argentina, Brazil and Uruguay, with all toads (T. R. Kahn and G. Agostini, pers. obs.) and is a practice recommended in other countries to keep amphibians away from gardens (e.g. https://www.bobvila.com/articles/how-to-get-rid-of-frogs/). In a study in Argentina, Brazil, and Uruguay, Deutsch et al. (2021) found that 45% of respondents have a strong aversion to the frog *Ceratophrys ornata*, a situation that led to the death of more than 350 individuals. Keeping amphibians as pets can also represent a threat. For instance, Deutsch et al. (2021) revealed that 77% of the *C. ornata* individuals kept in captivity (=178 individuals in this study) were illegally caught from the wild. Due to overexploitation, spread of pathogens, and risk of invasions, the pet trade remains a main threat to amphibians worldwide (Mohanty & Measey, 2019).

Low conservation attention as an indirect threat to amphibians

Unfortunately, the comparatively low likeability of a species can translate into low conservation efforts, indicating that human predispositions and attitudes toward animals determine conservation agendas (Prokop & Randler, 2018). For instance, Bellon (2019) found that federal funding allocated under the Endangered Species Act to vertebrate species in the US during 2013 was significantly influenced by species' charisma and not by the federal priority assigned by the Fish and Wildlife Service. Although amphibians are among the most threatened vertebrates on Earth, they receive less conservation funding and research attention than mammals and birds (Dos Santos, 2018; Tapley, Michaels, Johnson, & Field, 2017; Tarrant et al., 2016). For example, Troudet Grandcolas, Blin, Vignes-Lebbe, & Legendre (2017) found that amphibian species have a small number of occurrence data in the GBIF database in comparison with other vertebrates, a situation that has not changed over time. Most of these data were specimen-based occurrences (e.g., from museum collections) rather than observation-based occurrences, which reflects a low number of records from enthusiasts (e.g., citizen scientists) compared to other vertebrate groups. Amphibians are also highly underrepresented among the flagship species featured on covers of US conservation and nature magazines (Clucas, McHugh, & Caro, 2008). Meredith, Van Buren, & Antwis (2016) argued that a poor representation of amphibians in education and outreach initiatives leads to little public engagement in the conservation of these animals.

Public acceptance and compliance of conservation measures

Amphibian-focused human cognition is also expected to affect the support and compliance of conservation measures, although this subject has been little explored. Prokop and Fančovičová (2012) found a high willingness to protect amphibians (similar to values received by birds and mammals) in participants attending five randomly selected primary and secondary schools in Slovakia. In the Pyrenees Mountains, Loyau and Schmeller (2017) found that all but one conservation measure (pay entrance fees) used to mitigate amphibian chytridiomycosis was well accepted by the public. Public willingness to support amphibian-focused conservation actions increased when people heard about the amphibian extinction crisis (Espinosa-Molina, Rodriguez-Jorquera, & Beckmann, 2021; Loyau & Schmeller, 2017) and become aware of the benefits that amphibians provide to human society (Tyler, Wassersug, & Smith, 2007).

Factors influencing attitudes and behaviours toward amphibians

Interpopulation variation in amphibian-focused human cognition

People of different cultural backgrounds perceive and relate to amphibians in very different ways. Local folklore associated with negative attitudes and behaviours towards amphibians has been found in several regions worldwide, e.g. Argentina (Deutsch et al., 2021), Ethiopia

(Kassie, 2020), Portugal (Ceríaco, 2012), and South Africa (Brom et al., 2020). For instance, in Argentina, Deutsch et al. (2021) reported that a third of the respondents that encountered the frog *Ceratophrys ornata* killed the animal. This behaviour was associated with myths and tales telling the danger and evil of this species (Deutsch et al., 2021). In contrast, in other places, amphibians are perceived as beneficial to humans (Jimenez & Lindemann-Matthies, 2015b). For example, in Southeast China, most people found toads and frogs beautiful and considered them important for pest control, medicinal purposes, and consumption (Jimenez & Lindemann-Matthies, 2015a). For some indigenous cultures, amphibians are sacred (Beebee, 1996; Valiente, Tovar, González, & Eslava-sandoval, 2010), thus, there is a cultural and spiritual connection that involves responsibility for the amphibians' welfare and their conservation for future generations (Cisternas et al., 2019). It is worth noting that local folklore and the related human behaviours toward amphibians can exhibit considerable differences even among groups of people inhabiting the same geographical area, as it is the case of South Africa between Xhosa-speaking and English-speaking people in their dislike towards amphibians (67% vs 6%, respectively) (Brom et al., 2020).

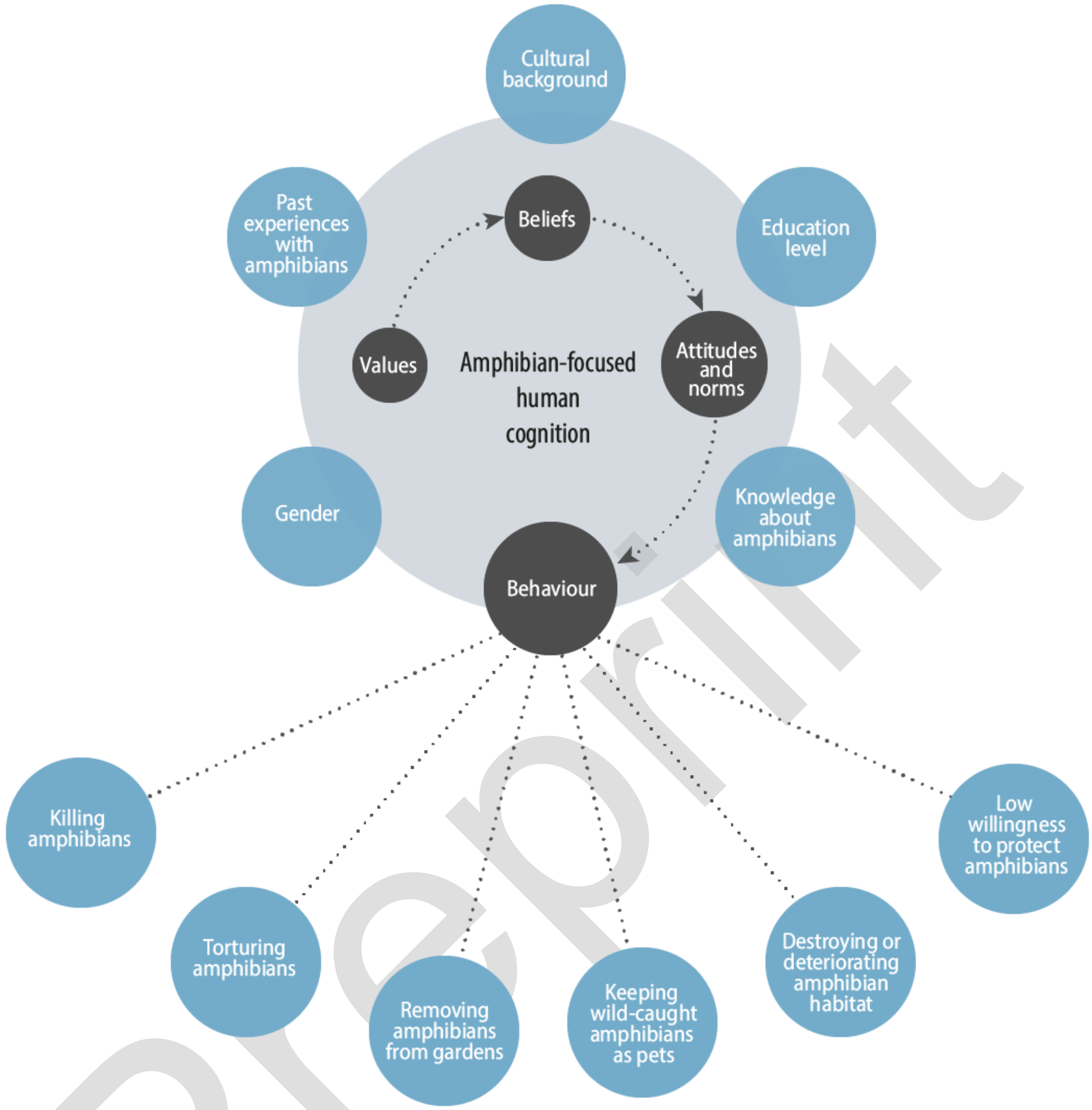


Figure 8.1. Factors that modulate amphibian-focused human cognition (black) and human behaviours that can represent a threat to amphibians (blue).

Intrapopulation variation in amphibian-focused human cognition

Research about the intrapopulation variation in attitudes and behaviours towards amphibians has highlighted that the interaction between intra- and interpopulation factors is common. For instance, gender is one of the main factors driving intrapopulation variation in attitudes and behaviours towards amphibians (Ceríaco, 2012; Deutsch et al., 2021; Prokop et al., 2016;

Tarrant et al., 2016), but whether women or men show more positive or negative attitudes or behaviours depends on the human population under scrutiny. For instance, in China, Jimenez and Lindemann-Matthies (2015a) found that women considered frogs more beautiful while the opposite was found in Colombia by the same authors (Jimenez & Lindemann-Matthies, 2015b). Some studies suggest that the effect of gender might depend on the level of the cognitive hierarchy model that is evaluated (Prokop et al., 2016). For example, Ceríaco (2012) reported that women have more dislike for amphibians than men, but men are more likely to persecute these animals. Some personality traits such as pathogen disgust (which in turn can be associated with gender and other personality traits such as neuroticism) are associated with amphibian-focused human cognition (Prokop et al., 2016). For instance, in Chile, Slovakia, South Africa and Turkey, Prokop et al. (2016) found that pathogen disgust negatively correlates with frog tolerance in respondents.

There is a positive correlation between direct past experiences with amphibians and positive attitudes and behaviours towards these animals (Schlegel & Rupf, 2010; Tomazic, 2008; Tomažič, 2011b, 2011a; Tomažic & Šorgo, 2017). For instance, in Indiana, Reimer et al. (2014) reported that respondents more familiar with hellbenders have more positive attitudes towards this salamander. Even human-wildlife interactions that can be considered as a threat (e.g., hunting for consumption) can be associated with positive attitudes towards amphibians (Jimenez & Lindemann-Matthies, 2015a; Nicholson et al., 2020). One important remark is the critical role that parental figures and other role models play in the experience that children might have with amphibians; children that were discouraged from playing with, observing, or going near amphibians in early childhood, retained their fear as adults, while those who were encouraged or facilitated by their parents showed affinity for these animals (Brom et al., 2020). In the cross-cultural study conducted by Prokop et al. (2016), the tolerance of frogs reported in parents or other family members positively influences the tolerance of frogs in high school students.

Finally, knowledge about amphibians (Brom et al., 2020; Jimenez & Lindemann-Matthies, 2015b, 2015a; Rommel, Crump, & Packard, 2016; Tarrant et al., 2016) and educational level in general (Deutsch et al., 2021; Kassie, 2020; Prokop & Fančovičová, 2012; Tarrant et al., 2016, but see Ceríaco 2012) can increase positive attitudes and behaviours in relation to these animals. For example, in Indiana, providing respondents with a small amount of information about the rarity and endemism of hellbenders increased their positive attitudes towards this species (Reimer et al., 2014). The perceived importance of amphibians also positively correlates with peoples' emotions, attitudes, and behaviours in relation to these animals (Jimenez & Lindemann-Matthies, 2015b, 2015a; Prokop & Fančovičová, 2012).

The importance of amphibian traits

Amphibian traits can influence how people perceive these animals. Some groups such as tree frogs (Schlegel & Rupf, 2010) or Darwin's frogs (Azat et al., 2021; A. Valenzuela-Sánchez, unpublished data) can be highly charismatic. Differences among amphibian species in their likeability can relate to aesthetic factors and anthropomorphic relatability (Brom et al., 2020; Prokop & Fančovičová, 2013). For instance, in the Czech Republic, Frynta, Peléšková, Rádlová, Janovcová, & Landová (2019) found that worm-like, legless, and small-eyed amphibians, such as caecilians, were less preferred by people. Morphological analyses also revealed that anuran species with a round body shape, short forelegs, small eyes, warts, pink and grey colouration, or dark and dull colouration were perceived as disgusting or ugly (Frynta et al., 2019).

Strategic education and communication actions

Education programmes

The need for improved education and outreach about amphibians is growing as these animals continue to decline. Careful planning and evaluation are critical for success. Thus, the development of education and outreach programmes should follow a systematic framework: planning-implementation-evaluation (PIE) process (Jacobson et al., 2015). Planning involves identifying goals and objectives, audiences, and educational strategies. Implementation concerns the operation of activities. Monitoring and evaluation of the results help identify successful activities as well as components in need of improvement (Table 8.1). This interactive process-PIE-leads to an education and outreach programmes that avoids common problems like targeting the wrong audience or using an inappropriate message or medium (Jacobson et al., 2015). In Figure 8.2 we propose some questions and best practices that amphibian conservationists can use to guide the planning, implementation, and evaluation of their education and outreach programmes.

The success of any education and communication strategy should be measurable. But what do we know about programme evaluation for amphibian conservation education? We found few studies that have evaluated the short and long-term impacts of amphibian-focused education activities. For instance, in a multi-partner educator workshop for the endangered Houston toad (*Anaxyrus houstonensis*), Rommel et al. (2016) reported significant increases in awareness/knowledge and values regarding general amphibian declines and the focal species. The workshop significantly increased participants' belief that they had necessary resources to teach about the Houston toad. Ninety-nine percent of participants agreed that they cared more about wild toads after meeting live "ambassador" toads. Post-workshop, the authors observed a 33% increase in use of amphibians or Houston toads in participant learning settings.

An evaluation of public understanding of the amphibian decline crisis carried out at 15 zoos in Brazil, New Zealand, and the United Kingdom, found that visitors in the three countries had relatively little understanding of amphibians and the global amphibian crisis (Dos Santos et al., 2019). They also found that zoo visitors in Brazil knew less about amphibian conservation than those in New Zealand or the United Kingdom. There was less amphibian-focused content in educational materials in zoos in Brazil than there was in the United Kingdom. An evaluation of an amphibian conservation education programme for middle schoolers in southern Chile showed increased knowledge but to a less extent, increased awareness (Soto Silva, 2015). This study used pre and post-test measures, as well as a control group.

Table 8.1. Data collection methods for programme evaluation proposed by Ernst, Monroe, and Simmons (2009).

Methods	**Overall purpose**
Interviews	To fully understand someone's impressions or experiences or learn more about their answers to questionnaires.
Focus groups	To explore a topic in depth through group discussion, e.g., reactions to an experience or suggestion, understanding common beliefs, etc.
Questionnaires and surveys	To quickly and/or easily get a lot of information from people in a non threatening way.
Observation	To gather accurate information about how a project actually operates, particularly about processes.
Literature review	To gather information on the audience and/or the issue. To identify what previous investigations have found about the state of the knowledge, skills, behaviours, or attitudes of the intended audience with relation to the issue.
Tests	To determine the audience's current state of knowledge or skill regarding the issue.
Concept or cognitive maps	To gather information about someone's understanding of and attitudes toward a complex subject or topic.
Document or product review	To gather information on how the project operates without interrupting the project.

Case studies or peer review	To fully understand or depict experiences of end-users in a project, and conduct comprehensive examination through cross-comparison of cases.

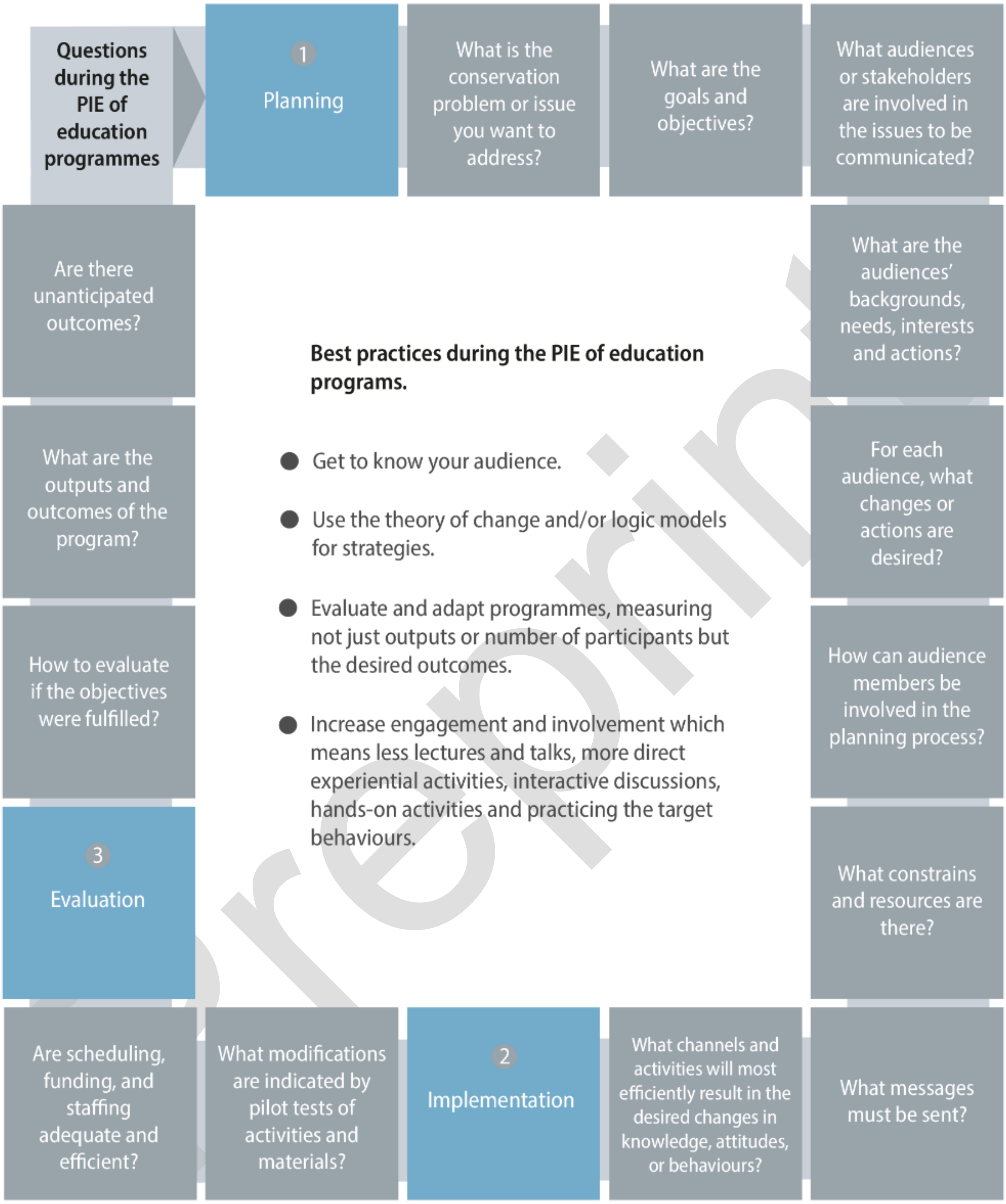


Figure 8.2: Best practices and questions during the planning-implementation-evaluation (PIE) of education programmes. Adapted from Jacobson et al. (2015).

Some authors have discussed the best type of learning experiences aimed at increasing knowledge and positive attitudes toward amphibians. In Slovenia, primary school students with prior direct experiences with amphibians were more willing to study animals and exhibited more positive attitudes towards them (Tomazic, 2008; Tomažič, 2011a). In Germany, Randler, Ilg, and Kern (2005) compared two types of learning experiences with 3rd and 4th graders (indoor-only vs. additional outdoor conservation action). They found that students who participated in the outdoor conservation action performed significantly better on achievement tests. Therefore, it seems that direct experiential activities (i.e. first-hand experiences) perform better than indirect experiences, such as classroom activities. There is a lack of information about the use and effectiveness of other education techniques in amphibian-focused contexts. In Figure 8.3 we show some examples of amphibian focused education and outreach interventions.

Communications and audience mobilisation

Strategic planning for amphibian conservation can use conservation psychology and behaviour change theories to connect actions to the threats amphibians face (Maynard, Monroe, Jacobson, & Savage, 2020). In Figure 8.4 we present a classification of conservation behaviours that can be used to guide strategic planning frameworks (Maynard et al., 2020). By promoting these behaviours, organisations can mobilise their audiences and enable the public to take action for amphibian conservation, increasing their reach and potential impact (Maynard et al., 2020; Salafsky et al., 2008)

Organisations and individuals interested in mobilising their audiences for amphibians should consider the range of communication strategies for their programmes (Fig. 8.3). Strategic communications tools include: 1) Mass media, such as social media, press kits, and advertisements; 2) Interpretive and educational media, such as exhibits, kiosks, publications, mail, social media, and clubs; 3) Events - such as presentations, workshops, tours, field trips, community running, meetings, and contests; and 4) community or citizen science, such as the iNaturalist "Global Amphibian BioBlitz" or the FrogWatch U.S.A. programme promoted across the Association of Zoos and Aquariums (AZA, 2021).

A powerful communication and outreach technique to consider for amphibians is community-based social marketing (Green, Crawford, Williamson, & DeWan, 2019; McKenzie-Mohr, 2011). By assessing the needs, motivations, and interests of the target audience, as well as any barriers hindering conservation actions, your communications programmes can inspire behaviour change. Other conservation psychology theories suggest additional communications techniques, such as the Elaboration Likelihood Model which highlights how reminders, cues, or celebrity spokespersons can spark interest in your audience (Petty & Cacioppo, 1986), or the Theory of Planned Behaviour that integrated social norms with behavioural intention to act (Ajzen, 1985). An example social marketing campaign for amphibians that used such strategies is the Amphibian Report Card, which created clear messages, a framework relatable to all people, and direct connections between the threats amphibian species face and the suggested actions to help them ("Amphibian Report Card," 2018).

Figure 8.3. Examples of amphibian-focused education and outreach interventions. (A, B) Education activities. (A) Classroom sessions about the mountain chicken frog to school children on Dominica (credits: Benjamin Tapley). (B) Children from Chilean Patagonia collaborate with ONG Ranita de Darwin members during the monitoring of Darwin's frog populations at the Reserva Elemental Melimoyu (credits: Daniel Casado). (C, D) Training workshops. (C) Training workshops for amphibian monitoring with tangata whenua (local indigenous communities in New Zealand) (credits: Phil Bishop). (D) A workshop in the Hoang Lien National Park, Viet Nam, encouraged porters and guides to adopt amphibian

friendly behaviours (credits: Benjamin Tapley). (E, F) Outreach activities. (E) Children paint frog watercolours at a zoo in central Chile. This outreach intervention also included a photo exhibition and infographics about amphibian ecology and conservation (credits: ONG Ranita de Darwin). (F) “Día de los anfibios” in the central square of Valdivia (southern Chile), a festival that gathered conservation organisations and the public to celebrate amphibians (credits: Felipe Rabanal).

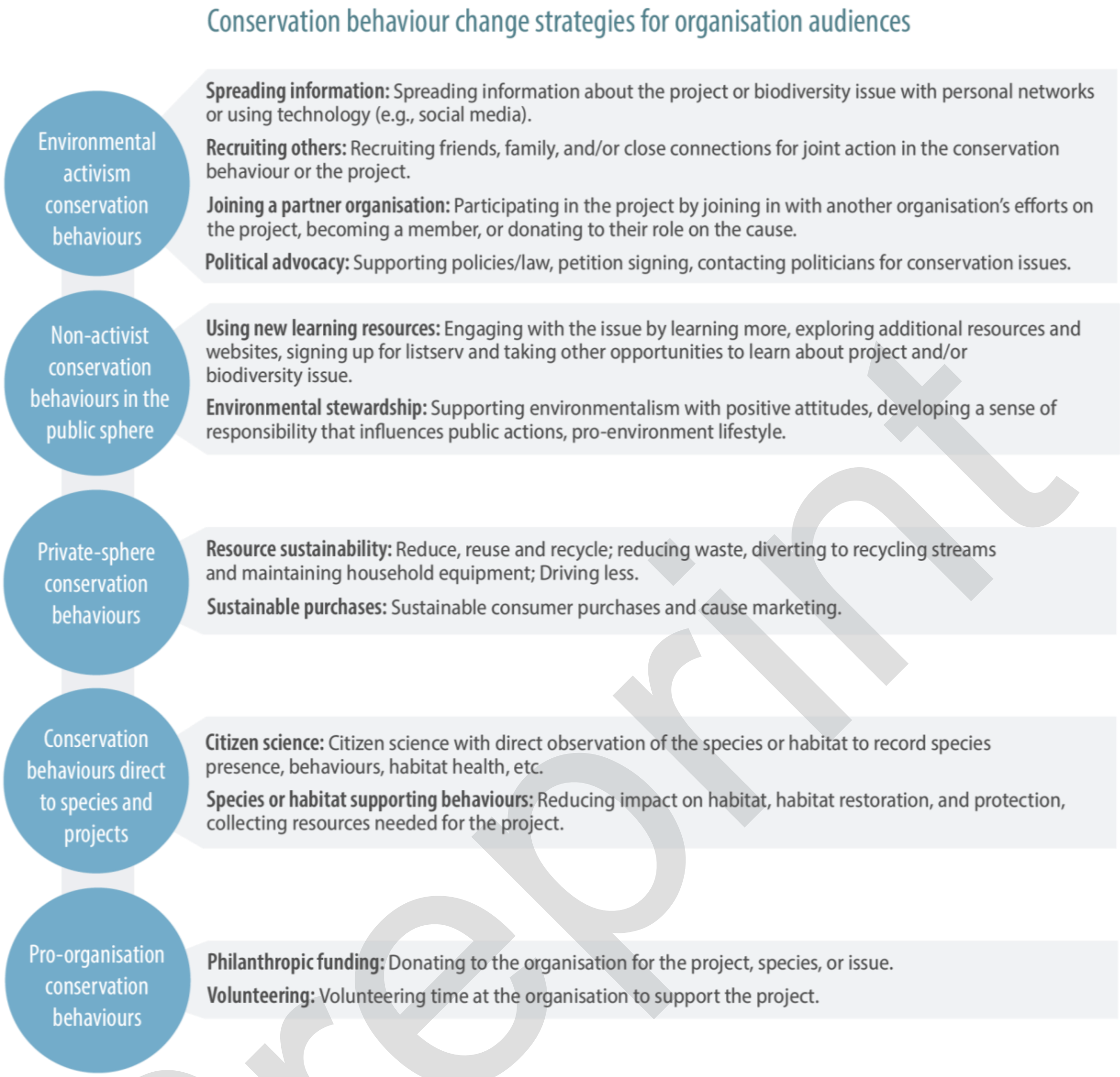


Figure 8.4. Classification of behaviours that can be promoted to mobilise organisation audiences for amphibian conservation. Adapted from Maynard et al. (2020)

The importance of stakeholder and community engagement

When creating impactful communication and education programmes, stakeholder engagement and community involvement are key factors to consider in order to align the local context with the proposed conservation actions (Bennett et al., 2017; Lin, Cheng, Chen, & Chang, 2008). For example, Kanagavel et al. (2020) found that to develop amphibian-based community conservation initiatives in the Western Ghats of India, frog conservation must be linked within a wider concept of forest protection since a significant proportion of community livelihoods depend on the presence of forests. Similarly, Cisternas et al. (2019) proposed that for achieving the feasibility of biocultural partnerships in New Zealand, building a relationship between participants would be the best way to optimise communication and validate the incorporation of different perspectives on frog conservation. A partnership between rural farmers and scientists in Mexico allowed the creation of a restoration programme focused on improving Axolotl (*Ambystoma mexicanum*) habitat while maintaining traditional agricultural practices (Valiente et al., 2010). Long-term partnerships between private landowners and conservationists have also allowed to protect amphibians' habitat in the USA (Kuyper, 2011; Milmoe, 2008; Symonds, 2008), United Kingdom (Pond Conservation: The Water Habitats Trust, 2012), and Chile (ONG Ranita de Darwin, 2021) (Fig. 8.5). In Romania, Hartel, Scheele, Rozylowicz, Horcea-Milcu, & Cogălniceanu (2020) concluded that lack of engagement from a broad range of local stakeholders was crucial for the failure of maintaining amphibian conservation initiatives within a protected area that changed its custodian. Therefore, amphibian conservation initiatives that focus on the broader cultural-socio-economic context would benefit from public support and long-term impact. Partnerships could also help to produce actionable science in amphibian conservation.

Figure 8.5. Examples of community engagement in amphibian conservation. (A) Citizen conservationists ("toad patrollers") set up a fence in canton Basel-Landschaft, Switzerland, to make sure that migrating amphibians are not killed on the road (credits: Benedikt Schmidt). (B) Landowners from southern Chile sign long-term voluntary or legal conservation agreements with a local amphibian conservation organisation to protect and monitor amphibians and related habitat in their land (credits: ONG Ranita de Darwin).

Communication and collaboration for actionable science in amphibian conservation

The need for actionable science in amphibian conservation is urgent, although generally there is a disconnection between research and practice (Grant, Muths, Schmidt, & Petrovan, 2019). This knowledge-implementation gap means that much of the amphibian scientific evidence available is not useful for end users, such as managers or decision makers (Schmidt et al. 2019). A way to address this problem is to communicate research in a way that can be directly used by end users (Schmidt, Brenneisen, & Zumbach, 2020). For instance, Indermaur and Schmidt (2011) quantified the requirements for wood deposits for populations of common toads (*Bufo bufo*) and European green toads (*Bufo viridis*). These authors reported their findings in a way that managers can easily determine the amount of woody deposits per hectare that are required to sustain a population of any size (Indermaur & Schmidt, 2011). The Conservation Evidence project is another good example of knowledge communication that can be directly used in conservation policy and management decisions. This project currently summarises evidence about the effectiveness of 129 amphibian conservation actions, mostly from North America, Europe, and Australia (Christie et al., 2021).

When thinking about communicating research to inform practice, one should ask what format(s) should be used to meet the needs of multiple end users (Wall et al., 2017). These formats can include websites, scientific and outreach articles, policy briefs, guidelines, smartphone apps, seminars, or hands-on workshops. It is likely that in most situations more than one format must be required. For instance, Schmidt et al. (2020) used a comparative effectiveness study to evaluate the effect of underpasses for amphibians (toad tunnels) and its physical characteristics on nearby amphibian populations in Switzerland. These authors decided to publish the key conclusions of this study in two outreach articles in two languages well before the scientific article was published (Schmidt et al., 2020).

Carefully thinking about how to communicate research findings does not guarantee that these findings will be relevant for solving amphibian conservation problems. If research is designed, implemented, and communicated only considering the scientist's perspective and knowledge of a conservation problem, there is the risk of failing to provide the information that is required by those who make policy and management decisions (Enquist et al., 2017; Wall et al., 2017). Most amphibian conservation problems require changing this unidirectional flow of information paradigm to a multidirectional one. Communication between scientists, managers, decision makers, and other stakeholders can improve the chances that research makes a true positive impact for amphibian conservation. There is a robust body of literature concerning collaborative production of knowledge in several scientific and medical fields, including conservation, which can be consulted by readers interested in the subject (e.g., Wall et al., 2017 and references therein). For example, translational ecology is “an approach that embodies intentional processes by which ecologists, stakeholders, and decision makers work collaboratively to develop and deliver ecological research that, ideally, results in improved environment-related decision making” (Enquist et al., 2017). A translational ecology approach, ideally guided by decision support frameworks (e.g. Wright et al., 2020), is an effective way to co-produce scientific evidence that informs conservation action (Wall et al., 2017).

It is important to consider that actionable science does not guarantee conservation success, as institutional barriers can play a significant role in the success of any conservation project (Wright et al., 2020). Institutional barriers can include conservation not being a political priority, amphibians not being preferred by the primary decision makers, and deficient engagement and communication between scientists and decision makers (Rose et al., 2019). For instance, Wright et al. (2020) evaluated 12 amphibian conservation case studies from Australia, Canada, Italy, and USA that used decision science to plan and implement conservation actions. Although all these case studies provided usable science by identifying optimal actions, less than 25% of the studies achieved conservation success. Most of the barriers for success were institutional barriers related to the complexity of the governance structures for a given decision problem, which led to over half of the studies failing, at least partially, at securing funding and implementing the actions (Wright et al., 2020). Therefore, communication among, and engagement of the different individuals and organisations involved in a project is critical. A conservation project that uses a translation approach should explicitly consider since its inception by what means, how frequently, and at what depth of engagement (e.g., presential or online workshops, emails, etc.) the researchers, end users, and other stakeholders are expected to communicate (Wall et al., 2017). Key leadership needs to be engaged to transcend organisational structures, which might require the involvement of multiple actors across time and space (Wright et al., 2020). This highly collaborative work can be an extenuating process, so careful consideration of “soft skills" such as listening, communicating, mediating, negotiating, and sharing, is very important for success (Enquist et al., 2017; Wall et al., 2017). It is also important for researchers to acknowledge that effective communication may require the participation of boundary-spanning organisations or professionals that can be better prepared to facilitate the collaboration across multiple disciplines and sectors (Wall et al., 2017; Wright et al., 2020).

Discussion

In this chapter we reviewed a representative body of literature to assist those researchers and practitioners who may undertake research and/or actions for amphibian conservation. We acknowledge a taxonomic and geographical bias in the evidence here reported. For instance, most studies about amphibian-centred human cognition were focused on anurans and conducted in Europe, South America, and South Africa. Additionally, there was an evident methodological bias towards an interpretivism research approach, and the application of questionnaires was the predominant data collection tool. Based on innovative examples of community and stakeholder empowerment with conservation (e.g. Charles, 2021; Lyver, Timoti, Davis, & Tylianakis, 2019), we encourage amphibian researchers to also incorporate innovative research methods that allow a bottom-up approach to knowledge construction, such as participatory action research, decolonising methodologies, and biocultural approaches.

Several factors have been identified as modulators of human attitudes and behaviours towards amphibians. These factors highlight different cultural and psychological sources of variation that need to be considered when designing conservation education and communication programmes. Two important remarks are worth discussing. First, most studies have focused on factors associated with intermediate levels in the cognitive hierarchy model of human behaviour (see Fulton, Manfredo, & Lipscomb, 1996; Fig 1), such as beliefs, attitudes and norms. How these intermediate levels translate into behavioural intentions and behaviours affecting amphibians remains poorly understood. Second, most studies on this topic have focused on the general public, while much less is known about factors influencing behaviour towards amphibians among private landowners, farmers, producers and entrepreneurs, conservation professionals, educators, natural resources managers, and policymakers (but see Pontes-Da-Silva, Pacheco, Pequeno, Franklin, & Kaefer, 2016; Prokop & Fančovičová, 2012; Rommel et al., 2016 for exceptions).

Conservation education and outreach techniques can be used to change human behaviours and improve public support for amphibian conservation. Although we found that some methods have produced positive results, programme evaluation in amphibian conservation education is still rare. Evaluation is critical to assess and improve the effectiveness of any conservation intervention, and therefore to ensure that limited funds go as far as possible in achieving conservation outcomes (Ferraro & Pattanayak, 2006). Most of the evaluation research that has been done focuses on classroom/experiential activities with pre- or middle-schoolers, and uses surveys or interviews to measure knowledge and attitudes. Thus, there is no evidence about the effectiveness of conservation education programmes on changing human behaviours and improving public support for amphibian conservation. We strongly suggest expanding the range of evaluation designs and methods traditionally used (Table 8.1) and assess other target audiences and conservation education techniques that could be used for amphibians (e.g., citizen science, storytelling, visual arts, interactive web sites, see

Jacobson et al., 2015 for more examples). This information is crucial to inform effective and strategic conservation education and communication actions. For instance, citizen science could be a useful tool to engage stakeholders and communities in amphibian conservation (Bonney et al., 2014; Lee et al., 2021). Participants of citizen science benefit from the experiential hands-on and field-based activities as well as gain confidence from the mastery of concepts and associated skills required for their participation (e.g. Cisternas, Germano, Longnecker, & Bishop, 2017; Lee et al., 2021). Citizen scientists or “citizen conservationists” can also directly benefit declining amphibian populations, for instance by reducing road mortality of pond-breeding amphibians (Fig 8.5; Sterrett, Katz, Fields, & Grant, 2019).

Communications and education can increase stakeholder engagement and the success of amphibian conservation actions. Increasing conservation attention towards amphibians could lead to a virtuous circle promoting career development of amphibian conservationists. For instance, media such as television, Internet, and magazines ranked as the most important career motivations for natural resources students in Florida (Haynes & Jacobson, 2015). Increasing the presence of amphibians in such media could increase students' interest in pursuing an amphibian-focused career. Improving positive attitudes toward amphibians in high-level decision makers (such as politicians, CEOs, board of directors, dean of colleges, or funders) should also be a high priority in the amphibian conservation community. Working with a species that is not preferred by the administration of your research institution or conservation organisation, or that receives less funding compared to other more charismatic species, can be a barrier difficult to sort in the career pipeline of an amphibian conservationist. Communicating the value of amphibian conservation using carefully designed messages, for instance by highlighting evidence about amphibians’ relevance for ecosystem functioning and human well-being, or about the imperilled status of these animals, might provide a good starting point to increase concern about amphibians in decision makers and the public.

Box 8.1. Glossary

Actionable science = "data, analyses, projections, or tools that can support decisions in natural resource management; it includes not only information but also guidance on the appropriate use of that information" (Enquist et al., 2017).

Biocultural partnerships = an association of persons joined as partners to develop conservation actions that sustain the biophysical and sociocultural components of dynamic, interacting, and interdependent social-ecological systems.

Citizen science = broadly, can be defined as the involvement of volunteers non-experts in scientific research.

Community involvement = the action of welcoming and integrating local people and communities into conservation decisions and implementation to effectively mobilise their action and reduce conflicts.

Folklore = traditional description of local beliefs and customs of a people often expressed in stories, myths, legends, and other artistic representations.

Stakeholders = include any community member, organisation, or individual with a stake in the conservation issue or location of a conservation project.

Acknowledgements

We are very thankful to Mauricio Akmentins, Ariadne Angulo, Stefano Doglio, Leida Dos Santos, Ted Kahn, Francis Lemckert, Ruth Marcec-Greaves, Rachel Rommel, Jeanne Tarrant, Jenny Urbina, and Sally Wren for their important contribution to an early draft of this chapter.

References

Ajzen, I. (1985). From intention to actions: a theory of planned behavior. In J. Kuhl & J. Beckmann (Eds.), *Action Control: From Cognition to Behavior* (pp. 11–39). New York: Springer.

Amphibian Report Card. (2018). Retrieved December 18, 2021, from https://www.amphibianreportcard.org

AZA. (2021). Association of Zoos and Aquariums: Frogwatch USA. Retrieved December 18, 2021, from https://www.aza.org/frogwatch?locale=en

Azat, C., Valenzuela-Sánchez, A., Delgado, S., Cunningham, A. A., Alvarado-Rybak, M., Bourke, J., … Angulo, A. (2021). A flagship for Austral temperate forest conservation: An action plan for Darwin’s frogs brings key stakeholders together. *Oryx*, *55*(3), 356–363. doi: 10.1017/S0030605319001236

Beebee, T. J. C. (1996). *Ecology and conservation of amphibians*. Chapman and Hall.

Bellon, A. M. (2019). Does animal charisma influence conservation funding for vertebrate species under the US Endangered Species Act? *Environmental Economics and Policy Studies*, *21*, 399–411. doi: 10.1007/s10018-018-00235-1

Bennett, N. J., Roth, R., Klain, S. C., Chan, K., Christie, P., Clark, D. A., … Wyborn, C. (2017). Conservation social science: Understanding and integrating human dimensions to improve conservation. *Biological*

Conservation, *205*, 93–108. doi: 10.1016/j.biocon.2016.10.006

Bonney, R., Shirk, J. L., Phillips, T. B., Wiggins, A., Ballard, H. L., Miller-Rushing, A. J., & Parrish, J. K. (2014). Next steps for citizen science: Strategic investments and coordination are needed for citizen science to reach its full potential. *Science*, *343*(6178), 1436–1437. Retrieved from http://www.sciencemag.org/content/343/6178/1436.short

Brom, P., Anderson, P., Channing, A., & Underhill, L. G. (2020). The role of cultural norms in shaping attitudes towards amphibians in Cape Town, South Africa. *PLoS ONE*, *15*(2), 1–18. doi: 10.1371/journal.pone.0219331

Ceríaco, L. M. P. (2012). Human attitudes towards herpetofauna: The influence of folklore and negative values on the conservation of amphibians and reptiles in Portugal. *Journal of Ethnobiology and Ethnomedicine*, *8*, 8. doi: 10.1186/1746-4269-8-8

Charles, A. (2021). Communities, conservation and livelihoods. In *Communities, conservation and livelihoods*. doi: 10.2305/iucn.ch.2021.01.en

Christie, A. P., Amano, T., Martin, P. A., Petrovan, S. O., Shackelford, G. E., Simmons, B. I., … Sutherland, W. J. (2021). The challenge of biased evidence in conservation. *Conservation Biology*, *35*(1), 249–262. doi: 10.1111/cobi.13577

Cisternas, J., Germano, J. M., Longnecker, N., & Bishop, P. J. (2017). Frog monitoring in New Zealand: Increased effort involving local communities. *FrogLog*, *118*, 83–85.

Cisternas, J., Wehi, P. M., Haupokia, N., Hughes, F., Hughes, M., Germano, J. M., … Bishop, P. J. (2019). Get together, work together, write together: A novel framework for conservation of New Zealand frogs. *New Zealand Journal of Ecology, 43*(3). doi: 10.20417/nzjecol.43.32

Clucas, B., McHugh, K., & Caro, T. (2008). Flagship species on covers of US conservation and nature magazines. *Biodiversity and Conservation*, *17*(6), 1517–1528. doi: 10.1007/s10531-008-9361-0

Cox, N., Chanson, J., & Stuart, S. (2006). *The status and distribution of reptiles and amphibians of the Mediterranean basin*. Gland, Switzerland and Cambridge, UK: IUCN.

Day, B. A., & Monroe, M. C. (2000). *Environmental education and communication for a sustainable world: Handbook for international practitioners*.

Deutsch, C., Grisolia, J., Bilenca, D., & Agostini, M. G. (2021). Human attitudes as threats in amphibians: the case of the Ornate Horned Frog (Ceratophrys ornata). *Human Dimensions of Wildlife*, *26*(3), 210–227. doi: 10.1080/10871209.2020.1808122

Dos Santos, M. M. (2018). *The role of education in amphibian conservation* (University of Otago). University of Otago. Retrieved from http://hdl.handle.net/10523/7891

Dos Santos, M. M., Griffiths, R. A., Jowett, T., Rock, J., & Bishop, P. J. (2019). A comparison of understanding of the amphibian crisis by zoo visitors across three countries. *Zoo Biology*, *38*(6), 471–480. doi: 10.1002/zoo.21516

Ehrlich, P. R., & Kennedy, D. (2005). Millennium assessment of human behavior. *Science, 309*, 562–563.

Enquist, C. A. F., Jackson, S. T., Garfin, G. M., Davis, F. W., Gerber, L. R., Littell, J. A., … Shaw, M. R. (2017). Foundations of translational ecology. *Frontiers in Ecology and the Environment*, *15*(10), 541–550. doi: 10.1002/fee.1733

Ernst, J., Monroe, M. C., & Simmons, B. I. (2009). *Evaluating your environmental education programs: A workbook for practitioners*. Washington D.C.: North American Association for Environmental Education.

Espinosa-Molina, M., Rodriguez-Jorquera, I. A., & Beckmann, V. (2021). Effect and difference between the threatened and endemic status on the general public support towards wildlife species in a biodiversity hotspot. *Biodiversity and Conservation*, *30*(11), 3219–3241. doi: 10.1007/s10531-021-02245-0

Ferraro, P. J., & Pattanayak, S. K. (2006). Money for nothing? A call for empirical evaluation of biodiversity conservation investments. *PLoS Biology*, *4*(4), 482–488. doi: 10.1371/journal.pbio.0040105

Frynta, D., Peléšková, Š., Rádlová, S., Janovcová, M., & Landová, E. (2019). Human evaluation of amphibian species: a comparison of disgust and beauty. *Science of Nature*, *106*(7–8). doi: 10.1007/s00114-019-1635-8

Fulton, D. C., Manfredo, M. J., & Lipscomb, J. (1996). Wildlife value orientations: A conceptual and measurement approach. *Human Dimensions of Wildlife*, *1*(2), 24–47. doi: 10.1080/10871209609359060

Grant, E. H. C., Muths, E., Schmidt, B. R., & Petrovan, S. O. (2019). Amphibian conservation in the Anthropocene. *Biological Conservation*, *236*(November 2018), 543–547. doi: 10.1016/j.biocon.2019.03.003

Green, K. M., Crawford, B. A., Williamson, K. A., & DeWan, A. A. (2019). A meta-analysis of social marketing campaigns to improve global conservation outcomes. *Social Marketing Quarterly*, *25*(1), 69–87. doi: 10.1177/1524500418824258

Hartel, T., Scheele, B. C., Rozylowicz, L., Horcea-Milcu, A., & Cogălniceanu, D. (2020). The social context for conservation: Amphibians in human shaped landscapes with high nature values. *Journal for Nature Conservation*, *53*. doi: 10.1016/j.jnc.2019.125762

Haynes, N. A., & Jacobson, S. (2015). Barriers and perceptions of natural resource careers by minority students. *Journal of Environmental Education*, *46*(3), 166–182. doi: 10.1080/00958964.2015.1011595

Indermaur, L., & Schmidt, B. R. (2011). Quantitative recommendations for amphibian terrestrial habitat conservation derived from habitat selection behavior. *Ecological Applications*, *21*(7), 2548–2554.

Jacobson, S. K. (2009). *Communication skills for conservation professionals*. Island Press.

Jacobson, S. K., McDuff, M. D., & Monroe, M. C. (2015). *Conservation education and outreach techniques* (2nd ed.). Oxford: Oxford University Press. doi: 10.1093/acprof:oso/9780198567714.001.0001

Jimenez, J. N., & Lindemann-Matthies, P. (2015a). Public knowledge and perception of toads and frogs in three areas of subtropical southeast China. *Society and Animals*, *23*(2), 166–192. doi: 10.1163/15685306-12341368

Jimenez, J. N., & Lindemann-Matthies, P. (2015b). Public knowledge of, and attitudes to, frogs in Colombia. *Anthrozoös*, *28*(2), 319–332.

Kanagavel, A., Parvathy, S., Tapley, B., Nirmal, N., Selvaraj, G., Raghavan, R., … Turvey, S. T. (2020). Are local and traditional ecological knowledge suitable tools for informing the conservation of threatened amphibians in biodiversity hotspots?

Herpetological Bulletin, *153*(153), 3–13. doi: 10.33256/HB153.313

Kassie, A. (2020). Attitude, beliefs and perception of people towards amphibian conservation around Chefa wetland, Oromo zone, Amhara National Regional State. *International Journal of Zoology Studies*, *5*(2), 1–4.

Knudson, D. M., Cable, T. T., & Beck, L. (2003). *Interpretation of cultural and natural resources*. Venture Publishing.

Kuyper, R. (2011). The role of safe harbor agreements in the recovery of listed species in California. *Endangered Species Bulletin*, *36*(2), 10–13.

Lee, T. S., Kahal, N. L., Kinas, H. L., Randall, L. A., Baker, T. M., Carney, V. A., … Duke, D. (2021). Advancing amphibian conservation through citizen science in urban municipalities. *Diversity*, *13*(211), 1–15.

Lin, H. C., Cheng, L. Y., Chen, P. C., & Chang, M. H. (2008). Involving local communities in amphibian conservation: Taipei frog Rana taipehensis as an example. *International Zoo Yearbook*, *42*(1), 90–98. doi: 10.1111/j.1748-1090.2008.00049.x

Loyau, A., & Schmeller, D. S. (2017). Positive sentiment and knowledge increase tolerance towards conservation actions. *Biodiversity and Conservation*, *26*, 461–478. doi: 10.1007/s10531-016-1253-0

Lyver, P. O. B., Timoti, P., Davis, T., & Tylianakis, J. M. (2019). Biocultural hysteresis inhibits adaptation to environmental change. *Trends in Ecology and Evolution*, *34*(9), 771–780. doi: 10.1016/j.tree.2019.04.002

Maynard, L., Monroe, M. C., Jacobson, S. K., & Savage, A. (2020). Maximizing biodiversity conservation through behavior change strategies. *Conservation*

Science and Practice, *2*(6), 1–11. doi: 10.1111/csp2.193

McKenzie-Mohr, D. (2011). Fostering sustainable behavior: an introduction to community-based social marketing. In *International Journal of Sustainability in Higher Education*. New Society Publishers. doi: 10.1108/ijshe.2003.24904bae.011

Meredith, H. M. R., Van Buren, C., & Antwis, R. E. (2016). Making amphibian conservation more effective. *Conservation Evidence*, *13*, 1–5. Retrieved from http://files/1146/Meredith et al. - 2016 - Making amphibian conservation more effective.pdf

Milmoe. (2008). Partnerships to conserve amphibian habitat. *Endangered Species Bulletin*, *33*, 36–37.

Mohanty, N. P., & Measey, J. (2019). The global pet trade in amphibians: species traits, taxonomic bias, and future directions. *Biodiversity and Conservation*, *28*(14), 3915–3923. doi: 10.1007/s10531-019-01857-x

Nicholson, D. J., Kanagavel, A., Baron, J., Durand, S., Murray, C., & Tapley, B. (2020). Cultural association and its role in garnering support for conservation: the case of the Mountain Chicken Frog on Dominica. *Amphibian and Reptile Conservation*, *14*(2), 133-144 (e241).

ONG Ranita de Darwin. (2021). Programa de Conservación de Tierras #YoProtejoMisRanitas. Retrieved July 7, 2021, from www.ranitadedarwin.org/conservaciondetierras

Pagani, C., Robustelli, F., & Ascione, F. R. (2007). Italian youths' attitudes toward, and concern for, animals. *Anthrozoös*, *20*(3), 275–293.

Petty, R. E., & Cacioppo, J. T. (1986). The elaboration likelihood model of persuasion. *Advances in Experimental Social Psychology*, *19*(C), 123–205. doi: 10.1016/S0065-2601(08)60214-2

Pond Conservation: The Water Habitats Trust. (2012). *Bringing back clean water to the countryside: Million Ponds Project Year 4 Report*. Retrieved from https://freshwaterhabitats.org.uk/wp-content/uploads/2013/08/MPP-Y4-REPORT-final_low-res.pdf

Pontes-da-Silva, E., Pacheco, M. L. T., Pequeno, P. A. C. L., Franklin, E., & Kaefer, I. L. (2016). Attitudes towards scorpions and frogs: a survey among teachers and students from schools in the vicinity of an Amazonian protected area. *Journal of Ethnobiology*, *36*(2), 395–411. doi: 10.2993/0278-0771-36.2.395

Prokop, P., & Fančovičová, J. (2012). Tolerance of amphibians in Slovakian people: A comparison of pond owners and non-owners. *Anthrozoos*, *25*(3), 277–288. doi: 10.2752/175303712X13403555186136

Prokop, P., & Fančovičová, J. (2013). Does colour matter? The influence of animal warning coloration on human emotions and willingness to protect them. *Animal Conservation*, *16*(4), 458–466. doi: 10.1111/acv.12014

Prokop, P., Medina-Jerez, W., Coleman, J., Fancovicová, J., Özel, M., & Fedor, P. (2016). Tolerance of frogs among high school students: Influences of disgust and culture. *Eurasia Journal of Mathematics, Science and Technology Education*, *12*(6), 1499–1505. doi: 10.12973/eurasia.2016.1241a

Prokop, P., & Randler, C. (2018). Biological predispositions and individual differences in human attitudes toward animals. In R. R. Nóbrega Alves & U. P. Albuquerque (Eds.), *Ethnozoology: Animals in our lives* (pp. 447–466). Elsevier Inc.

doi: 10.1016/B978-0-12-809913-1.00023-5

Randler, C., Ilg, A., & Kern, J. (2005). Cognitive and emotional evaluation of an amphibian conservation program for elementary school students. *Journal of Environmental Education*, *37*(1), 43–52. doi: 10.3200/JOEE.37.1.43-52

Reimer, A., Mase, A., Mulvaney, K., Mullendore, N., Perry-Hill, R., & Prokopy, L. (2014). The impact of information and familiarity on public attitudes toward the eastern hellbender. *Animal Conservation*, *17*(3), 235–243. doi: 10.1111/acv.12085

Rommel, R. E., Crump, P. S., & Packard, J. M. (2016). Leaping from awareness to action: impacts of an amphibian educator workshop. *Journal of Herpetology*, *50*(1), 12–16. doi: 10.1670/14-025

Rose, D. C., Amano, T., González-Varo, J. P., Mukherjee, N., Robertson, R. J., Simmons, B. I., … Sutherland, W. J. (2019). Calling for a new agenda for conservation science to create evidence-informed policy. *Biological Conservation*, *238*, 108222. doi: 10.1016/j.biocon.2019.108222

Salafsky, N., Salzer, D., Stattersfield, A. J., Hilton-Taylor, C., Neugarten, R., Butchart, S. H. M., … Wilkie, D. (2008). A standard lexicon for biodiversity conservation: Unified classifications of threats and actions. *Conservation Biology*, *22*(4), 897–911. doi: 10.1111/j.1523-1739.2008.00937.x

Schlegel, J., & Rupf, R. (2010). Attitudes towards potential animal flagship species in nature conservation: A survey among students of different educational institutions. *Journal for Nature Conservation*, *18*(4), 278–290. doi: 10.1016/j.jnc.2009.12.002

Schmidt, B. R., Brenneisen, S., & Zumbach, S. (2020). Evidence-based amphibian conservation: A case study on toad tunnels. *Herpetologica*, *76*(2), 228–239. doi: 10.1655/0018-0831-76.2.228

Schultz, P. W. (2011). Conservation means behavior. *Conservation Biology*, *25*(6), 1080–1083. doi: 10.1111/j.1523-1739.2011.01766.x

Schultz, P. W., & Kaiser, F. G. (2012). Promoting pro-environmental behavior. In S. Clayton (Ed.), *The Oxford handbook of environmental and conservation psychology*. Oxford. doi: 10.1093/oxfordhb/9780199733026.013.0029

Soto Silva, A. A. (2015). *Evaluación de la educación ambiental como herramienta de conservación: el caso de los anfibios endémicos de Mehuín, Chile*. Universidad Austral de Chile, Valdivia, Chile.

Sterrett, S. C., Katz, R. A., Fields, W. R., & Grant, E. H. C. (2019). The contribution of road-based citizen science to the conservation of pond-breeding amphibians. *Journal of Applied Ecology*, *56*(4), 988–995. doi: 10.1111/1365-2664.13330

Symonds, K. (2008). Ranchers restore amphibian-friendly ponds. *Endangered Species Bulletin*, *33*, 30–31.

Tapley, B., Michaels, C. J., Johnson, K., & Field, D. (2017). A global problem requires a global multifaceted solution. *Animal Conservation*, *20*(2), 122–123. doi: 10.1111/acv.12349

Tarrant, J., Kruger, D., & du Preez, L. H. (2016). Do public attitudes affect conservation effort? Using a questionnaire-based survey to assess perceptions, beliefs and superstitions associated with frogs in South Africa. *African Zoology*, *51*(1), 13–20. doi: 10.1080/15627020.2015.1122554

Tomazic, I. (2008). The influence of direct experience on students' attitudes to, and knowledge about amphibians. *Acta Biologica Slovenica, 51*, 39–49.

Tomažič, I. (2011a). Reported experiences enhance favourable attitudes toward toads. *Eurasia Journal of Mathematics, Science and Technology Education*, *7*(4), 253–262. doi: 10.12973/ejmste/75207

Tomažič, I. (2011b). Seventh graders' direct experience with, and feelings toward, amphibians and some other nonhuman animals. *Society and Animals, 19*(3), 225–247. doi: 10.1163/156853011X578901

Tomažic, I., & Šorgo, A. (2017). Factors affecting students' attitudes toward toads. *Eurasia Journal of Mathematics, Science and Technology Education*, *13*(6), 2505–2528. doi: 10.12973/EURASIA.2017.01237A

Troudet, J., Grandcolas, P., Blin, A., Vignes-Lebbe, R., & Legendre, F. (2017). Taxonomic bias in biodiversity data and societal preferences. *Scientific Reports, 7*(1), 1–14. doi: 10.1038/s41598-017-09084-6

Tyler, M. J., Wassersug, R., & Smith, B. (2007). How frogs and humans interact: Influences beyond habitat destruction, epidemics and global warming. *Applied Herpetology*, *4*(1), 1–18. doi: 10.1163/157075407779766741

Valiente, E., Tovar, A., González, H., & Eslava-Sandoval, D. (2010). Creating refuges for the axolotl (*Ambystoma mexicanum*). *Ecological Restoration*, *28*(3), 257–259.

Wall, T. U., McNie, E., & Garfin, G. M. (2017). Use-inspired science: making science usable by and useful to decision makers. *Frontiers in Ecology and the Environment, 15*(10), 551–559. doi: 10.1002/fee.1735

Wright, A. D., Bernard, R. F., Mosher, B. A., O'Donnell, K. M., Braunagel, T., DiRenzo, G. V., … Campbell Grant, E. H. (2020). Moving from decision to action in conservation science. *Biological Conservation, 249*, 108698. doi: 10.1016/j.biocon.2020.10869

Chapter 9. Conservation planning: the foundation for strategic action

Anne Baker[1], Sally Wren[2,3], Franco Andreone[4], Federico Kakoliris[5], Andolalao Rakotoarison[6], Deanna H. Olson[7], Dan Cogalniceanu[8], Jeff Dawson[9], Kevin Johnson[1], Ted R. Kahn[10], Esteban Lavilla[11], Cindy Paszkowski[12], Antonio W. Salas[13] and Nicolás Urbina-Cardona[14]

[1]Amphibian Ark, c/o CPSG, 12101 Johnny Cake Ridge Rd., Apple Valley, MN 55124, USA

[2]IUCN SSC Amphibian Specialist Group, 3701 Lake Shore Blvd W, P.O. Box 48586, Toronto, Ontario M8W 1P5, Canada

[3]Department of Zoology, University of Otago, 340 Great King Street, Dunedin 9016, New Zealand

[4]MRSN Museo Regionale di Scienze Naturali, Via G. Giolitti, 36, I-10123 TORINO – ITALY

[5] Seción Herpetología, Facultad de Ciencias Naturales y Museo, UNLP - CONICET. La Plata (1900), Buenos Aires

[6] Mention Zoologie et Biodiversité Animale,Faculté des Sciences, Universitéd'Antananarivo, Antananarivo, Madagascar

[7] Pacific Northwest Research Station, U.S.D.A. Forest Service, Corvallis, OR 97331, USA

[8] University Ovidius Constanta, Faculty of Natural Sciences and Agricultural Sciences, Al. Universitatii 1, corp B, Room P43, Constanta Romania

[9] Durrell Wildlife Conservation Trust, Les Augrès Manor La Profonde Rue, Trinity, Jersey JE3 5BP, British Channel Islands

[10] Research Fellow, Duke University, Nicholas School of the Environment

[11] UEL - Conicet / Fundación Miguel Lillo, Miguel Lillo 251 - 4000 Tucumán, Argentina

[12] Department of Biological Sciences, University of Alberta, Edmonton, Alberta T6G 2A7, Canada
[13] Biodiversidad, Medioambiente y Desarrollo, Jr. Moreno Alcalá 241, Lima 41, Perú
[14] Department of Ecology and Territory, School of Rural and Environmental Studies, Pontificia Universidad Javeriana, Bogotá – Colombia

Abstract

Comprehensive conservation planning is the starting point for effective conservation efforts. It clarifies the plan's goals and expected outcomes, evaluates threats to species, identifies missing scientific information, identifies and prioritises the actions that are needed to achieve objectives, establishes a timeline, identifies necessary resources including funding, personnel, and partnerships, and creates a monitoring plan to assess conservation impact and adaptive management needs. Because effective conservation is a long-term process, the short-term impact is often difficult to assess, but evidence is emerging that shows improved species status as a result of comprehensive conservation planning. In this chapter we identify the various levels at which planning occurs, discuss tools and processes available to assist with conservation planning, including some specific to amphibians, outline some of the major challenges to planning and plan implementation, and provide key recommendations to facilitate successful amphibian conservation planning.

Introduction

Conservation planning has important components that occur at global, national, and local levels. The IUCN Amphibian Conservation Action Plan (ACAP; Gascon et al., 2007; Wren et al., 2015) has identified cross-cutting needs across broad geographic and jurisdictional scales for amphibian conservation and has provided direction for addressing those needs relative to key risk factors. National and regional plans (e.g. Vaira, Akmentins, & Lavilla, 2018) often have established priorities regarding which species are most in need of conservation action at those spatial scales and what type(s) of actions are most urgent. In contrast, species *action* plans identify specific measures needed to implement the plans, as well as who would be responsible for which actions and over what timeframes, and the metrics of success. In addition to ensuring efficient use of resources, conservation action plans at all levels may be leveraged to increase funding opportunities and partnerships, and overall can improve the probability of success of grant applications as they ensure accountability with periodic reports and adaptive management, when needed.

Strategic species conservation planning increases the potential for effective conservation action that results in positive outcomes for the species. Such a positive outcome depends on several aspects: (i) the inclusion of all affected stakeholders in the planning process; (ii) consensus around well-defined and achievable goals, objectives, and actions; (iii) the best available scientific information to inform management and policy decisions; (iv) check points over time that enable adaptive management; (v) periodic reporting to stakeholders for transparency and accountability; and (vi) clear articulation of the measure(s) of success. In addition to these elements, clarification of the regulatory authority over species for conservation actions (including its legal enforcement capability), matching actions with available resources such as funding and personnel that may limit the capacity of the conservation program, and an understanding of how stakeholders consider risk and uncertainty relative to conservation planning, implementation of actions, and results are needed to maximise the success of programs (Olson, 2007). The importance of species conservation planning is recognised by the IUCN Species Survival Commission (SSC) as one of the essential elements of species conservation in the Assess, Plan, Act Cycle (Figure 9.1).

Figure 9.1: The IUCN SSC Assess-Plan-Act Cycle.

Conservation is a truly multi-disciplinary subject, requiring a wide range of expertise. Traditionally, biologists have moved into the conservation sphere as their research highlighted the decline of threatened species, but as the discipline of conservation planning has evolved, conservation biologists have recognised the need to engage diverse professions to improve the success of conservation initiatives. It might be beneficial, for example, to include experts in social marketing, human demographics, or resource economics in amphibian conservation decision-making. Undertaking a planning exercise is one of the best opportunities to bring that expertise together, strengthening stakeholder networks and increasing coordination and collaboration for, ultimately, better outcomes for the species, group of species, or site in question.

The history of amphibian conservation planning

The first conservation plans for amphibians (e.g. USFWS, 1983, 1984) were developed in the 1980s in response to the United States Endangered Species Act of 1973 (*The Endangered Species Act as Amended by Public Law 97-304 (the Endangered Species Act Amendments of*

1982), 1983). These and other early plans brought together important ecological information about a threatened species, highlighted knowledge gaps, and sometimes prioritised actions required for species recovery, but often failed to provide recovery criteria, thus making it difficult to know when a species had been recovered successfully. Whereas the first edition of the ACAP (Gascon et al., 2007) did not include a chapter on conservation planning, it was included in the 2015 ACAP revision (Wren et al., 2015). Despite this, the number of conservation plans that are known to be completed for previously un-planned amphibian species post-2015 has been substantially lower than in preceding years (Figure 9.2). During the 1982-2007 period an average of 1.24 plans were produced per year. In the subsequent 2007-2015 period, 4 plans per year were completed, while post-2015 only an average of 2.5 plans were produced annually. A full accounting of species conservation plans has been difficult to compile, hence inadequate reporting may contribute to some differences among timeframes.

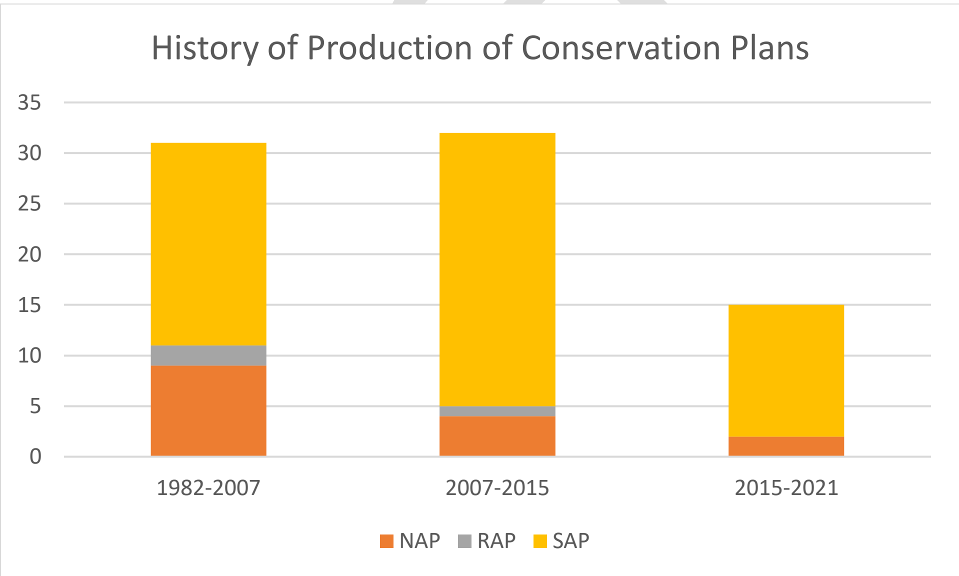


Figure 9.2. Number of amphibian conservation action plans produced globally since 1982, split by pre-ACAP (before 2007), First ACAP (2007-2015), and second ACAP (2015-2021). All plans for which references could be found, either on the ASG website, the CPSG website,

the USFWS website and through internet search engines were included. This probably underestimates the actual number of plans as some countries (i.e., Sweden) were reported to have plans for all nationally endangered species, which were not available. NAP refers to National Action Plans covering an entire country; RAP refers to Regional Plans covering a region within a country; SAP refers to Species Action Plans, usually for a single species, although a multi-species plan for *Atelopus* has recently been published and is included in the SAP count. Plans are recorded based on the year they were first produced. Some were updated in subsequent years, but these were not recorded as separate plans.

The number of plans produced between 1982 and 2021 also starkly differed with geographic region (Figure 9.3). The variation in number of plans among regions does not reflect species richness, relative number of threatened species within a region, or spatial extent of regions. Multiple complex interacting factors may explain variation in conservation plan initiation over time among geographic areas. Some of these are discussed further below. Many tie to low priority for amphibian conservation, resulting in limited resources and capacity to assess amphibian species status and to develop and implement conservation plans.

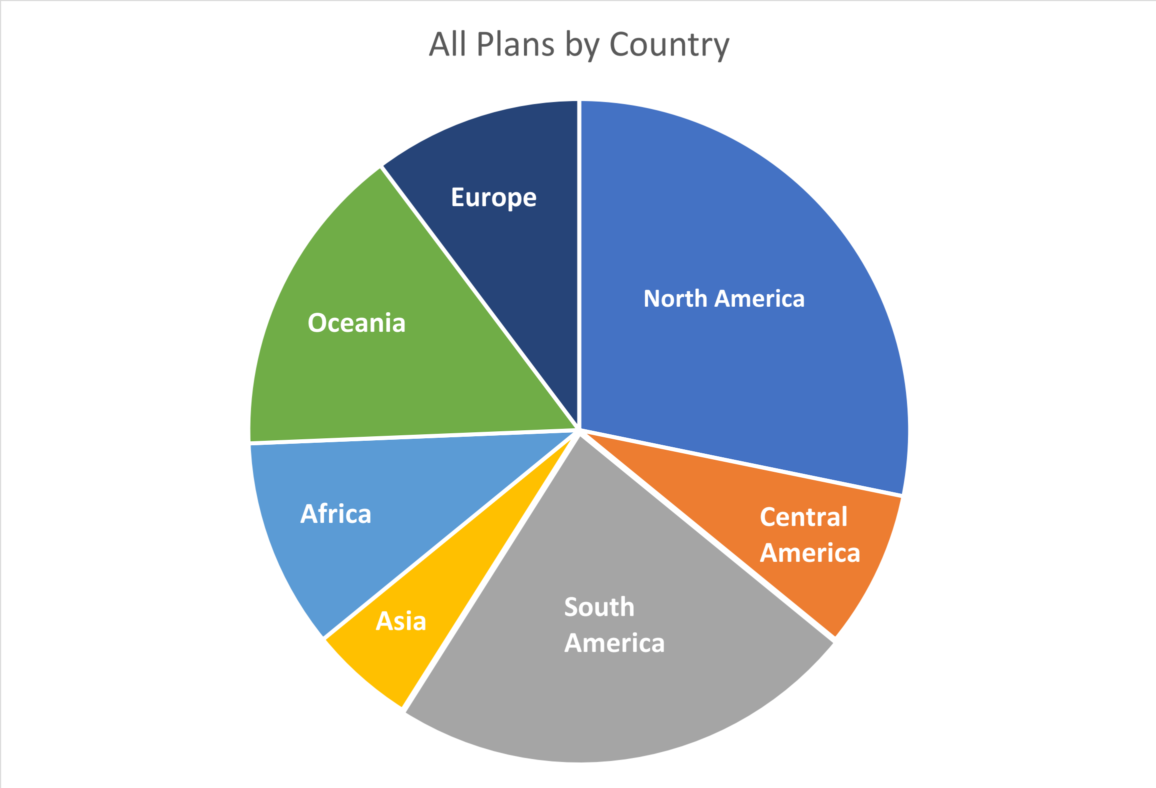


Figure 9.3. The total number of amphibian species conservation action plans (National, Regional and Species combined) by Geographic Region. There may be additional amphibian conservation plans that we did not find when assembling these data.

Assessing the effectiveness of conservation action plans is difficult for a number of reasons, not the least of which is identifying what measures will be used to evaluate success. At one end of the spectrum, success may be measured by activity, such as the number of prioritised actions completed, or by slowing declines in populations as is the case in a review of the Sahonagasy Action Plan (Andreone et al., 2012) published four years following the plan's completion. Alternatively, success may be measured by outcomes, such as the long-term viability of a species in the wild, for example, via changes in Red List status (Young et al., 2014). In general, it is difficult to quantify how many amphibian conservation plans have been implemented, and there is no standard review process of the effectiveness of amphibian conservation action plans in terms of achieving positive outcomes. This is not surprising, as the literature suggests that there is little evidence for the conservation outcomes of any conservation action planning (McIntosh et al., 2018), although individual actions are quite diverse and many have had support for positive effects (Smith, Meredith, & Sutherland, 2020). Assessing the impact of conservation planning for a species can take years as the effects of various efforts may not occur immediately. Lees et al. (2021), in an analysis of 35 species conservation plans completed in 23 countries over 13 years for a wide variety of species have documented positive outcomes (either increased or stable populations) for 26 species after periods of 15 years. Although the remaining species continued to decline over the same period, the decline slowed, and no species went extinct. As this analysis documented, measuring the impact of conservation planning is difficult and complex. It can take several decades for the effect of conservation actions to be seen, so it is unlikely that results will be seen immediately for more recently developed plans.

Assessment – a critical first step in planning

Good planning depends on good information about the current status of species. Several tools are available to assist in providing this information. The amphibian database assembled for The IUCN Red List of Threatened Species (IUCN Red List) provides collated information on species status across multiple standardised criteria, including some recommended conservation steps. The Conservation Needs Assessment (CNA) (Johnson et al., 2020) developed by the Amphibian Ark (AArk) is a transparent, logical and objective method which prioritises those species with the most pressing conservation needs. The CNA complements the IUCN Red List extinction risk assessments and together they provide a foundation for the development of holistic conservation action plans that combine *in situ* and *ex situ* actions as appropriate. Where they exist, National Red Lists or equivalent classification schemes also provide similar status information for species. Please see Chapters

2 and 10 for a deeper discussion on types of data required to make assessments, the issue of insufficient data, and methods that can be used for surveillance and monitoring to inform extinction risk assessments and planning. These assessment and prioritisation processes provide guidance for maximising the impact of limited conservation resources by identifying which measures could best serve those species requiring help.

Planning tools

Guidelines

As experience with conservation planning has increased, methods for species conservation planning have evolved, incorporating knowledge and decision-making tools from other disciplines. Published conservation planning guidelines reflect this improved knowledge.

Three fundamental approaches are described in the literature. The Open Standards for the Practice of Conservation (or 'Conservation Standards'; Conservation Measures Partnership, 2020) is an adaptive planning framework utilised to collaboratively and systematically conserve flora and fauna. It was created by the Conservation Measures Partnership (CMP). A full description of the Conservation Standards can be found at www.conservationmeasures.org. The IUCN SSC Conservation Planning Specialist Group (CPSG) publication *Species Conservation Planning Principles & Steps*, Ver. 1.0 (CPSG, 2020); www.CPSG.org) provides guiding principles for conservation planning and systematically describes the steps essential for effective conservation planning (http://www.cbsg.org/species-conservation-planning-cycle). A number of similarities (e.g., clear articulation of issues, identification of goals, objectives and actions, evaluation of impact) exist between the Conservation Standards and CPSG planning methods, although they also differ in some respects. One key difference between the Open Standards and the

CPSG process is that the latter focuses more heavily on identifying the key threats to the species as an initial step in the planning process. Less similar to these two methods is a process known as Structured Decision Making (Gregory et al., 2012), an approach for organised analysis of natural resource management decisions that can help address risk and uncertainty in the conservation planning process. In particular, Structured Decision Making is designed for use when there is substantial uncertainty regarding the effectiveness of possible conservation actions, whether because of inadequate understanding of factors such as fundamental ecological requirements of a species, or the probable impact of proposed actions. AArk has developed templates for formatting both national and species action plans which can be found in the AArk website's husbandry section (www.amphibianark.org).

Although there are guideline documents for the different approaches described above, they share some key points, which enable development of an effective conservation plan, and facilitate the implementation of that plan. All the methods help a group come together and work through complicated challenges, which may include conflicting stakeholder priorities and lack of data or evidence, to agree on a conservation solution. A skilled facilitator is key to ensuring an inclusive process. These methods also rely on making clear objectives (often following the SMART model: Specific, Measurable, Achievable, Realistic, and Time-bound). Furthermore, all these techniques are 'living methods' with a cyclical nature, which involve regularly re-evaluating decisions based on new information, and encourage assessment of past decisions to ensure the best possible outcomes.

Analytical tools

In cases where sufficient demographic information is known, Population Viability Analysis (PVA; Lacy, 2000) is an analytical tool that can project the future of threatened species' populations under various scenarios describing current and future conditions. This method is used in the management of threatened species to evaluate the relative impacts of threats, develop plans of action, judge outcomes of proposed management options, evaluate population recovery efforts and assess possible impacts of habitat modification or loss. It considers the interacting factors that could drive populations to extinction. PVA is used to estimate the likelihood of a population becoming extinct and to point out the need for conservation efforts, identifying key life stages or processes that should be the target of such conservation. One key value of a PVA is that it points out where data and expert opinion or intuition often lead to quite different results. While the predictive accuracies of PVAs have been criticised for lack of applied validation, they are objective and repeatable (Chaudhary & Oli, 2020; Doak et al., 2015) and the benefits of their use has been demonstrated in amphibians (Auffarth, Krug, Pröhl, & Jehle, 2017; Pickett, Stockwell, Clulow, & Mahony, 2016).

Unfortunately, these simulation models require solid data on population sizes and demographic parameters, information often not available for many threatened amphibian species. To date, only seven of the 60 amphibian species conservation action plans included PVA modelling. In all seven plans information on demographic parameters came mostly from captive populations or a single, small wild population.

Multi-species planning

With increasing recognition of the need to plan for threatened species across taxonomic groups, we are faced with the issue of limited capacity to plan for all the species that need these conservation efforts. Currently, 2,488 amphibian species are listed as Threatened on the IUCN Red List (classified as either Critically Endangered, Endangered, or Vulnerable), and from a global perspective it would not be feasible to undertake conservation planning for these species one-by-one. Therefore, efforts have been made to develop and carry out multi-species planning, to address the needs of several species in one process. This might be through the development of country-wide plans, e.g. the Action Plan for the Conservation of Amphibians of the Republic of Argentina (Vaira et al., 2018), which was developed following a nation-wide Conservation Needs Assessment; the Sahonagasy Action Plans developed by ASG Madagascar (Andreone, Dawson, Rabemananjara, Rabibisoa, & Rakotonanahary, 2016; Andreone & Randriamahazo, 2008; and see Box 9.2) and the China Herpetological Conservation Action Plan I: Amphibians (Pi-peng, 2010) Conservation plans may also cover a region within a country, e.g. the Action Plan for the Conservation of the Amphibians of the Valle del Cauca Region (Corredor Londoño et al., 2010).

Table 9.1: Software that may be useful in making objective decisions when conservation planning.

	VORTEX	RAMAS	HexSim	PMX	Outbreak
Author	Lacy & Pollak (2021); Lacy (2000b)	Akcakaya & Root (2005)	Schumaker (2016)	Lacy et al. (Lacy, Ballou, & Pollak, 2012)	Lacy et al. (Lacy, Pollak, Miller, Hungerford, & Bright, 2014)
Location	www.scti.tools/vortex/	www.ramas.com/software	www.hexsim.net	www.scti.tools	www.scti.tools
Cost	Free	$1K - $5K	Free	Free	Free

Description	Monte Carlo simulation, models population dynamics as discrete, sequential events (e.g., births, deaths, catastrophes, etc.) that occur according to defined probabilities. Probabilities of events are modelled as constants or as random variables that follow specified distributions.	Models population dynamics as discrete, sequential events (e.g., births, deaths, catastrophes, etc.) that occur according to defined probabilities. Probabilities of events are modelled as constants or as random variables that follow specified distributions, allows for species that live in multiple patches	Versatile, multi-species, life history simulator used for building spatially explicit and individual-based models of animal and plant population viability, interactions, and responses to disturbance.	Software for managing captive populations	Software for modelling dynamics of infectious diseases

Another option is taxon-based multi-species planning, suitable where there are taxonomic groups of amphibians with high numbers, or a high proportion, of threatened species and where the same actions are likely to have a positive impact on the whole group. For example, harlequin toads (Genus *Atelopus*) are among the most threatened amphibian genera; 82 of the 94 species that have been assessed by the IUCN Red List are categorised as Threatened or Extinct. In response, a partnership of organisations formed the Atelopus Survival Initiative, a collaborative network which aims to coordinate conservation responses for *Atelopus* species through a conservation action plan – HarleCAP - for the genus (Valencia & Fonte, 2021).

Multi-species plans don't need to be taxon-specific, covering only amphibians; it may be that we can increase the number of threatened amphibian species covered by conservation plans by explicitly including these species in site-based plans, for example plans for protected areas (e.g. Pulgar Vidal, Gamboa Moquillaza, Cabello Mejía, & Valdivia Pacheco, 2015), wetlands where waterfowl protections are implemented, or forests where stream-riparian protections are implemented to meet water quality standards or sensitive fish (e.g. Olson & Ares, 2022). These approaches may be especially effective for species where a significant proportion of their range falls within a protected area.

Another approach, which remains to be tested for amphibians, is the Assess to Plan (or A2P) approach, developed by the Conservation Planning Specialist Group (Gibson, Silva, Tognelli, & Karunarathna, 2020; C. Lees et al., 2020). A2P aims to move species more quickly through the Assess-Plan-Act Cycle (Figure 9.1) by using the IUCN Red List database to develop "bundles" of species that are sensible for multi-species conservation planning. Good bundles would comprise species anticipated to respond positively to the same set of conservation actions *and* whose conservation can be addressed by the same conservation actors or agencies. Typical planning categories expected from the A2P process might include: habitat-directed planning, for species dependent on the same habitat type which is subject to a common threat or set of threats; site-directed planning, for bundles of species inhabiting a defined area and subject to multiple localised threats linked to that site; threat-directed planning, for groups of species targeted by a common threat that is not anchored to a site, e.g. disease, overharvesting, or climate change; *ex situ* conservation feasibility assessment/planning, for species for which *in situ* conservation alone is considered unlikely to prevent extinction within the time available; and individual species recovery planning, for outlier species whose conservation needs do not overlap significantly with those of other species.

While single-species planning will remain key for some species, increasing efficiencies through multi-species planning approaches will be necessary; with such a large number of threatened amphibian species currently on the Red List, and a further 1193 listed as Data Deficient, as well as the continued discovery of new species (Tapley et al., 2018), planning and conservation efforts need to be scaled up significantly if we are to address the conservation needs of all amphibian species currently listed as threatened, and efficiencies can be gained with multi-species planning approaches.

Virtual planning

Traditionally, one of the key stages in a quality conservation planning process has been to bring together stakeholders in a multi-participatory planning workshop. There are several benefits to this method, including building stronger relationships and encouraging participants to focus on the task to hand. However, in 2020-21 in the face of the global pandemic, where international travel came to a halt, it was necessary to adapt and develop methods for continuing conservation planning work virtually.

There are significant challenges to effective virtual planning, not least ensuring that all participants have access to the relevant technology – both in terms of having reliable access to internet, as well an acceptable level of familiarity with the programmes used. It can be more challenging in a virtual process to ensure that there is equal engagement of all participants, and it may take additional capacity on the facilitation team to ensure that all avenues of communication – such as video, chat bar, and polls – are monitored sufficiently well, and that there is always somebody available to fix participants' technical issues.

Scheduling virtual meetings may present additional difficulties; first, timing meetings to be during working hours in all relevant time zones is not always possible, so some participants will be working at unusual hours. Furthermore, online sessions can often be more mentally draining for participants, so a virtual workshop may not be able to include day-long sessions, as is traditionally the practice for in-person workshops. Rather, it may be necessary to schedule workshops over a series of shorter sessions, which will extend the process, but allow participants to remain fully engaged within each session. However, sessions should not be scheduled too far apart, otherwise much time will be required to re-cap. Further guidance on setting up and facilitating a virtual workshop can be found in CPSG's document *A Guide to Facilitating Virtual Workshops* (IUCN SSC CPSG, 2020).

Despite these challenges to implementing effective workshops online, there are also benefits to this approach including significant reductions in cost and carbon emissions, and often the ability to invite a larger number of participants due to the lack of travel costs. As such, even when international travel increases again, it is likely that virtual workshops will remain a part of the future of conservation planning.

Challenges to planning

Key challenges to conservation planning in this section come from members of the ASG Conservation Planning Working Group who contributed their experiences in a brainstorm process. The factors listed below can be frequent and substantial challenges; some ways in which these challenges might be addressed are suggested.

Knowledge gaps. Although the ASG has tried to collate past and existing plans on the ASG's website (https://www.iucn-amphibians.org/resources/publications/action-plans/action-plans-by-regions/), this is not a comprehensive list, and it is difficult to track development and implementation of conservation plans. There may be species-specific plans that have been missed (e.g., those not appearing in an online literature search due to language differences), or species could be included in protected-area or habitat-management plans but are not specifically mentioned in the plan's title or keywords. It is important that efforts are made to better track and monitor the existence and implementation of plans for amphibian species to help decision-making for future planning efforts.

For individual conservation plans, actual or perceived **lack of data** is a further obstacle to undertaking planning for amphibians; decision-making can become more difficult where data is poorly available. Some evidence suggests that there may be a lower incentive for academic research on amphibians, due to the relatively low impact factor of herpetology compared with other biological sciences (Urbina-Cardona, 2008). The competitive academic system in many countries rewards research that can be completed and published quickly as opposed to the collection of data that, while not novel or cutting edge, would be useful to inform conservation decision-making, such as long-term monitoring of amphibian populations. Traditionally, much amphibian research has focused on taxonomy and systematics, with little or no attention paid to ecological research addressing life history parameters, population trends, or environmental threats, although this is gradually changing in a number of countries. Furthermore, specific impacts to amphibians may be overlooked even in research on relevant subjects; climate change, for example, is a threat to many amphibian species, but most studies modelling the impact of climate change focus on temperature rather than more difficult to model hydrological changes that are more likely to impact amphibians. It will be an ongoing challenge to ensure that sufficient data is available for decision-making in amphibian conservation planning. However, in cases where data is poor, an adaptive management approach may be used to test proposed actions (e.g. Canessa et al., 2019).

Amphibians aren't valued. Many participants felt that amphibians are often overlooked, not perceived as important as some other taxonomic groups (see more detailed discussion in Chapter 2), and therefore end up not being priorities for conservation planning. Addressing this may take education (see Chapter 8) to improve understanding of the importance of amphibians in the ecosystem. This reflects the importance of environmental education programmes to improve the direct experiences and interactions of people with amphibians beginning in childhood, that can develop more positive feelings and perceptions (Brom, Anderson, Channing, & Underhill, 2020). In this sense, education programmes at zoos are key for urban children while participatory sampling with rural people could be the most efficient strategy (Vergara-Rios et al., 2021). One strategy potentially useful with adults is to pinpoint the beneficial effects that amphibians have as controllers of pests, and to encourage the development of citizen science initiatives to bring understanding, interest, and care to the global public. Once such programme is the Global Amphibian BioBlitz organised by www.inaturalist.org and supported by the ASG (https://www.inaturalist.org/projects/global-amphibian-bioblitz).

Planning isn't valued. Another major challenge to undertaking conservation planning for amphibians is a lack of appreciation for the benefits of planning. It is true that it has been difficult to show the impact of developing a conservation plan empirically, partly due to the long time-period necessary to see impacts. However, evidence is now starting to show the positive impact of developing species-based conservation plans (IUCN SSC CBSG, 2017; C. M. Lees et al., 2021). Further, individuals that have participated in a conservation planning process often note the benefits of going through the steps of examining the evidence, developing a joint vision and goals, and critically thinking in a group setting with a variety of expertise present, about how best to achieve those objectives.

Conservation planning is perceived as difficult. Individuals may be daunted by the process of undertaking conservation planning, but as shown above, several guidelines are available to help support those undertaking planning for the first time (Conservation Measures Partnership, 2020; Copsey, Lees, & Miller, 2020; CPSG, 2020; Gregory et al., 2012; see Box 9.3 for a list of useful documents), as well as support offered from groups such as CPSG.

Lack of planning capacity can be another obstacle to developing conservation plans. Managing multi-stakeholder participation in the planning process requires facilitators with knowledge of planning processes and skill in facilitating both the interpersonal interactions within the stakeholder group and complex decision-making processes. A facilitator that can speak the major languages represented in the stakeholder group is also highly beneficial.

Limited funding. Funding for conservation planning is often limited and difficult to obtain. Bringing multiple stakeholders together, often including individuals from several different countries, requires significant financial resources; it is often perceived that such resources are better spent on action rather than planning. Some savings may be made with a virtual planning process, although virtual planning presents its own difficulties (see above). The use of virtual workshops for planning is a way to reduce the costs of planning, while allowing for even broader stakeholder participation.

Scientists and conservationists are disconnected. Finally, a lack of connection between research scientists and those implementing conservation actions was mentioned as a problem in undertaking planning. Scientists may follow a research cycle for knowledge discovery, focused on attainment of grants, research project implementation, and reporting in the scientific literature where information may not be freely available to conservation decision-makers and implementers. However, this highlights one of the specific benefits of bringing together diverse experts in a multi-participatory planning process – here information exchange is encouraged, and participants may benefit from networking with individuals who have both a different expertise and knowledge. It is this diversity of participants that helps build quality decision-making at a planning workshop, and ensures that proposed actions are based on the best possible evidence.

Challenges to implementing plans. Plans, once developed, must be implemented. Far too often plans are developed, made into a glossy document and then sit on shelves only to be referred to in funding proposals. The most successful conservation plans include an implementation component which identifies who is going to implement each action, by when, how that will be funded, etc. The same brainstorm of Working Group members identified a number of factors that may impede plan implementation.

Lack of resources. Implementing conservation plans requires resources – both human capacity and funding – over extended periods. This need for **sustained resources** may be a hurdle to implementing conservation plans, especially when funding for amphibians can be more difficult to obtain than for other taxa (see Chapter 2). The development of a conservation plan can assist with fundraising for the actions within the plan; some funders now request that applications are backed up by a conservation plan, and even for those that don't there are benefits to showing that a project is part of a larger, coordinated, and collaborative conservation strategy. This shift from funders may indicate that the benefits of planning are increasingly understood by funders, potentially increasing the availability of funds for planning itself.

Ineffective coordination or a breakdown in trust between partners can hinder implementation of a conservation plan; however, having a dedicated programme coordinator can help alleviate this issue. Someone who can review progress on specific actions, keep up communication with groups or individuals who had agreed to support or lead an action, identify new project partners, and report back to the wider stakeholder group on progress, helping to maintain the network that was instigated at the initial planning workshop and ensuring regular communication between relevant parties (Olson & Van Horne, 2017). Enhancing communication of conservation plan efficacy, such as through annual reports, can improve conservation plan accountability and engagement with complex stakeholder communities.

Lack of government support can be a major impediment to implementing a conservation plan, and this was also a common response in a more general survey of ASG members, when asked for impediments to conservation success (ASG Membership forms, 2013-2016 quadrennium and 2017-2020 quadrennium). There is often a disconnect between conservationists who identify problems and propose solutions, and the political actors necessary to ensure their execution. Conservation initiatives do not often transcend the scientific field and are rarely established as national policies that receive sustained state funding. Linked to a lack of government support, is the potential conflict (either real, or perceived) between economic development and species conservation. This problem may be alleviated when appropriate officials from relevant government agencies are afforded time-on-the-job to participate in or lead the development of a conservation action plan. As such, we recommend including relevant government departments in identified stakeholders when undertaking conservation planning.

Among local communities, **lack of public support** also can be a hurdle to conservation plan implementation, especially where there are negative public perceptions towards amphibians, or lower social values than other conservation priorities. These values may be related to negative experiences, oral traditions and superstitions, or negative media coverage of herpetofauna (Ceríaco, 2012; Iosif, Vlad, Stănescu, & Cogălniceanu, 2019; Prokop & Fančovičová, 2012; Tomažic & Šorgo, 2017). Urban dwellers may also show apathy towards amphibians, reducing support for implementation of conservation strategies.

Conclusions and approaches

Good conservation planning accrues a number of benefits. In addition to creating a roadmap for mitigating threats, it engages stakeholders in the conservation process, and increases funding opportunities. Evidence is beginning to emerge that conservation planning also results in positive outcomes for species. Implementation of the following steps will increase effective amphibian conservation planning.

1. Strive to include all Critically Endangered amphibians in a conservation plan that identifies threats and appropriate threat mitigation strategies, along with specific goals, objectives, actions, a timeline, monitoring, adaptive management, and expected positive outcomes.
2. Proceed with planning despite imperfect data; identify imperfect data, risks, and uncertainty in development of a plan.
3. Address all relevant areas identified in the ACAP (e.g., disease mitigation, education, genome banking) in plan development.
4. Identify trained facilitators and technical advisors to assist with conservation planning.
5. Include all relevant stakeholders in planning workshops.
6. Identify amphibian species of concern in all protected area (reserve) and habitat (e.g., forest, wetland) management plans that are not species conservation plans per se.
7. Establish a central database in which all amphibian conservation plans and plan updates are recorded, with capacity to include adaptive management, lessons learned, and implementation progress.
8. Ensure public access to plans and reports (e.g., see 7, above).
9. Promote planning as valuable to amphibian conservation efforts.

Box 9.1: Recovery of the El Rincon-stream frog - planning and execution by Federico Kacorilis

Plan development

In 2012 faculty and graduate students at La Plata Museum in Argentina started a planning project with a clear vision, ensuring the long-lasting viability of one of the most threatened amphibians in Argentina, the El Rincon-stream Frog, *Pleurodema somuncurense*. This frog was listed as Critically Endangered in the IUCN Red List and among the Top 100 EDGE amphibians worldwide due to its restricted range, declining population (including local extinctions), and the existence of several threats. However, as it happens with many threatened amphibians, there was a lack of information to clearly identify and set management actions. So, a stakeholder workshop was organised aimed at developing a Logical Framework for this species. Workshop participants first helped build a tree of threats and then, turned it into a tree of objectives to guide management activities (see Figure 9.4). However, because the real impact of threats was not fully known, it was decided to apply adaptive management to both measure the conservation impact of actions and, at the same time gather scientific information to allow assessment of the real effect of these threats on the frogs.

Plan implementation

Initially, the team focused on alleviating the main threats, invasive trout, which restricted frogs to a few remnants of habitat, and livestock, which promoted loss and fragmentation of these remnants through grazing and trampling. Removal of these threats was identified as crucial to enhance connectivity and natural movement of individuals to restored habitats, which would help the natural recovery of extinct sub-populations. However, there was a delay in obtaining permits to remove invasive trout, making natural recolonisation impossible. To address this, the team decided to add an ex-situ component and a translocation programme to help re-establishment of extinct sub-populations until permits to manage trout were approved.

While waiting for the permit to remove invasive trout, progress was made on the next step in the plan; working to exclude livestock from some sites, allowing rapid habitat regeneration of suitable frog habitat. Successful breeding in the ex-situ colony of this species, allowed for translocations from ex-situ facilities to the restored habitats, achieving the re-establishment of extinct sub-populations. Five years later, the permit to remove invasive trout was approved, which allowed the work of enhancing corridors to connect isolated sub-populations to begin, thus starting the recovery of the meta-population dynamics of the El Rincon-stream Frog.

Process evaluation

The Log Frame, or Logical Framework, represents a powerful tool for planning successful projects. This planning tool consists of a matrix which provides an overview of a project's goal, activities and anticipated results. It provides a structure to help specify the components of a project and its activities and for relating them to one another. It also identifies the measures by which the project's anticipated results will be monitored. Within this framework action plans resulting from a planning process should be flexible enough to address some uncertainty. In this case, the re-establishment of extinct sub-populations by natural recolonisation of frogs could have failed due to a delay in permits. This problem was solved by developing an ex-situ population and adding a translocation component to the original action plan. Additionally, adaptive management proves to be helpful to face both the lack of information about the real impact of some potential threats and the effectiveness of planned management actions.

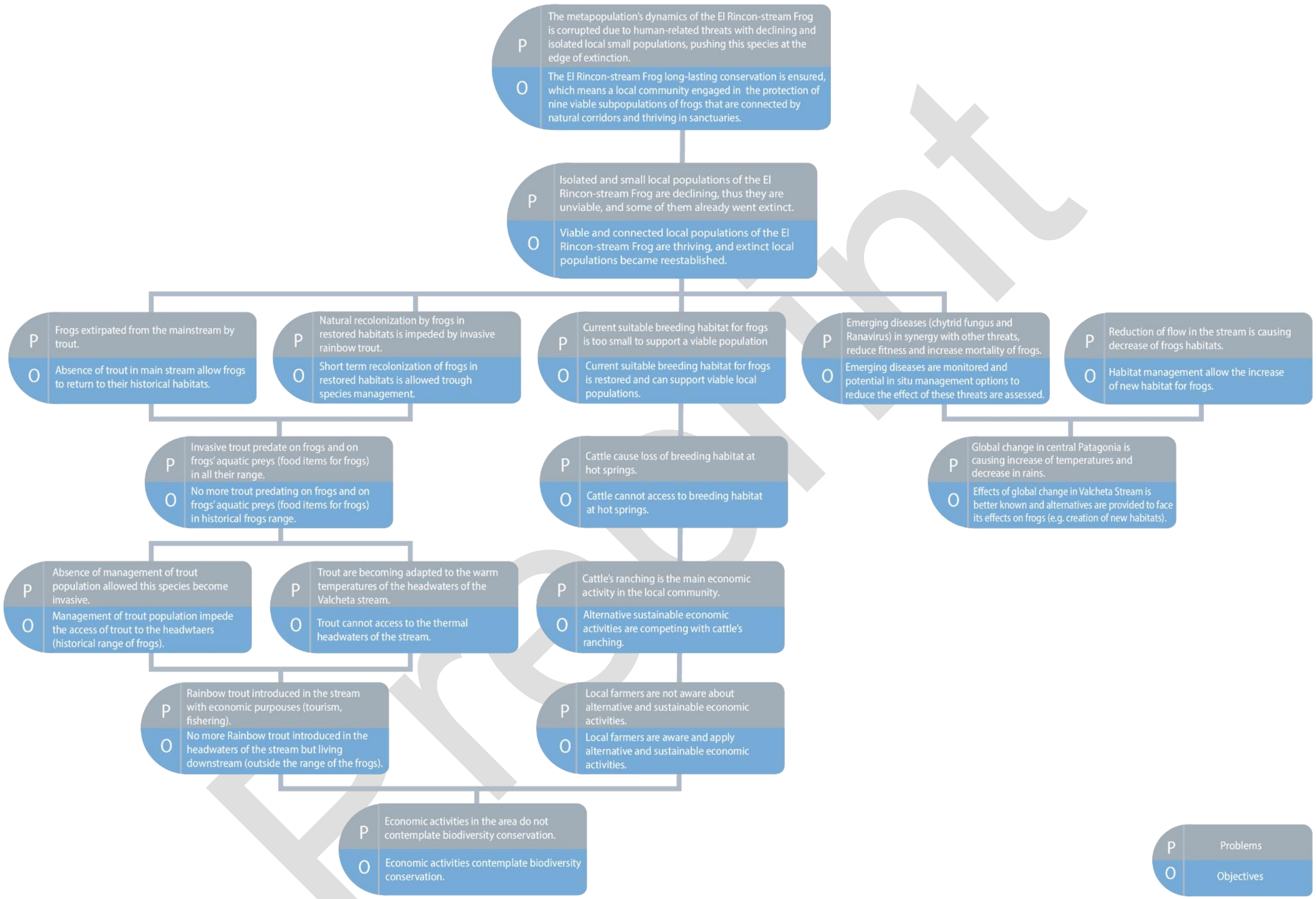


Figure 9.4: A tree of problems and threats that was developed during a conservation planning workshop for the El Rincon-stream Frog, *Pleurodema somuncurense* to guide management decisions.

Box 9.2: The endemic amphibians of Madagascar and the development of a country-wide conservation strategy

Background

Madagascar is well known for its astonishing biodiversity and endemicity rate. Amphibians are one of the most prominent vertebrate groups living there: current estimates indicate around 380 described species and many others still await formal description. The increasing deforestation rate of the natural habitats of Madagascar justifies priority attention be given to the conservation of this peculiar fauna. This was highlighted by the Global Amphibian Assessment and the first Amphibian Conservation Action Plan.

Plan development

A meeting was held in 2006 in Antananarivo to develop "A Conservation Strategy for the Amphibians of Madagascar" (ACSAM). During this meeting participants exchanged information, identified issues, and developed proposals for amphibian conservation in Madagascar. These discussions led to the formalization of the Sahonagasy Action Plan (SAP), "sahonagasy" being a Malagasy neologism, with "sahona" meaning "frog" and "gasy" an equivalent adjective to "Malagasy". The SAP was the first initiative to implement the ACAP at a national level and one of the first plans in a high endemism country. In the plan the meeting discussions were translated into eight themes addressing the major needs of Madagascan amphibians, including coordination of research and conservation activities, managing threats such as emerging disease, harvesting, and climate change, and monitoring species, accompanied by active safeguard and awareness initiatives.

Plan implementation and revision

The Sahonagasy Action Plan prompted research on iconic species and important amphibian communities. Workshops focussing on aspects of the plan were held, including one dedicated to chytrid fungus (*Bd*) and its prevention. This eventually led to the activation of a Chytrid

Emergency Cell and regular monitoring after screening found *Bd* positive individuals. Another workshop provided training on captive breeding and husbandry science for Malagasy amphibians. Conservation actions included a collaboration with Madagascar Fauna and Flora Group to organise a festival dedicated to the tomato frog (*Dyscophus antongilii*). At an ACSAM2 workshop held in Ranomafana National Park in 2012, participants assessed the results and process of the first SAP. A review of progress had been published prior to the workshop (Andreone et al., 2012), then at the meeting talks were followed by a brainstorm analysis and revision of the many tasks and objectives. Outcomes of the revised plan included a collaboration between ASG Madagascar, ASA and Durrell Wildlife Conservation Trust, who received funding from the Critical Ecosystem Partnership Fund to implement the new plan, including capacity building of local people, and the recruitment of two dedicated personnel. Further outcomes included scientific research training to support the understanding of the Ministry staff on how research is undertaken, with the goal of facilitating the delivery of scientific permits; a workshop sharing knowledge on the different amphibian-oriented protocols used in the field; a conference dedicated to the amphibians at Toamasina University; and an amphibian festival in the Ivoloina Park to increase public knowledge of amphibian conservation. Furthermore, a new species action plan, the McAP *Mantella cowanii* Action Plan, was finalised in 2021.

Process evaluation

The activity of ASG Madagascar and the workshops dedicated to amphibians highlighted these vertebrates as an important component of Madagascar's biodiversity; after being involved in the ACSAM the Malagasy Government is more aware of the importance of amphibians, which are now always considered in biodiversity strategies. Getting an amphibian action plan formally accepted by the Madagascar Government is a success in itself, and while there have been successful outcomes of the SAP, a lack of funding and insufficient coordination limited implementation of the original plan (see Andreone et al., 2012 for a full evaluation). While engaging the government has produced positive outcomes, implementation of long-term activities in a national strategy is possible only when there are stakeholders ready to support the actions with funds. For this it is compulsory that an NGO dedicated to amphibians is active in Madagascar to promote and sustain conservation actions. This is a great opportunity but also a great challenge for the Madagascar scientific community.

Franco Andreone & Andolalao Rakotoarison

IUCN SSC Amphibian Specialist Group - Madagascar

Box 9.3: Useful documents for undertaking conservation planning

Breitenmoser, U., Lanz, T., Vogt, K., & Breitenmoser-Würsten, C. (2015). How to save the cat - Cat Conservation Compendium, a practi- cal guideline for strategic and project planning in cat conservation. In *Cat News Special Issue* (Vol. 9). Retrieved from http://www.catsg.org/index.php?id=293

Conservation Measures Partnership. (2020). *Open standards for the practice of conservation. Version 4.0*. Retrieved from https://conservationstandards.org/download-cs/

Copsey, J., Lees, C., & Miller, P. (2020). *A Facilitator's Guide to Species Conservation Planning*. IUCN SSC Conservation Planning Specialist Group: Apple Valley, MN. Retrieved from https://www.cpsg.org/content/facilitators-guide-species-conservation-planning

CPSG. (2020). *Species Conservation Planning Principles & Steps, Ver. 1.0.* IUCN SSC Conservation Planning Specialist Group: Apple Valley, MN. Retrieved from https://www.cpsg.org/sites/cbsg.org/files/documents/CPSG Principles %26 Steps_English.pdf

Foden, E. W. B., & Young, B. E. (2016). *IUCN SSC Guidelines for assessing species' vulnerability to climate change. Version 1.0. Occasional Paper of the IUCN Species Survival Commission No. 59*. Cambridge, UK and Gland, Switzerland. doi: 10.2305/iucn.ch.2016.ssc-op.59.en

Gregory, R., Failing, L., Harstone, M., Long, G., McDaniels, T., & Ohlson, D. (2012). *Structured Decision Making: A Practical Guide to Environmental Management Choices*. Wiley-Blackwell.

IUCN/SSC. (2008). *Strategic Planning for Species Conservation: A Handbook. Version 1.0.* Gland, Switzerland. Retrieved from http://files/179/scshandbook_2_12_08_compressed.pdf

IUCN/SSC. (2013). *Guidelines for Reintroductions and Other Conservation Translocations. Version 1.0*. Gland, Switzerland: IUCN Species Survival Commission. Retrieved from IUCN Species Survival Commission website: https://www.iucn.org/content/guidelines-reintroductions-and-other-conservation-translocations

IUCN/SSC. (2014). *Guidelines on the Use of Ex Situ Management for Species Conservation. Version 2.0*. Gland, Switzerland. Retrieved from https://portals.iucn.org/library/sites/library/files/documents/2014-064.pdf

IUCN SSC CPSG. (2020). *A guide to Facilitating Virtual Workshops*. Apple Valley, MN, USA. Retrieved from https://www.cpsg.org/content/guide-facilitating-virtual-workshops

Linhoff, L. J., Soorae, P. S., Harding, G., Donnelly, M. A., Germano, J. M., Hunter, D. A., … Eckstut, M. E. (2021). *IUCN Guidelines for amphibian reintroductions and other conservation translocations*. Gland, Switzerland. Retrieved from https://www.iucn-amphibians.org/wp-content/uploads/2021/05/Ampb-Guidelines_170521_Final.pdf

World Organisation for Animal Health (OIE) & International Union for Conservation of Nature (IUCN). (2014). *Guidelines for wildlife disease risk analysis*. OIE, Paris. Retrieved from https://www.oie.int/en/document/guidelines-for-wildlife-disease-risk-analysis/

References

Akcakaya, H. R., & Root, W. T. (2005). *RAMAS GIS: linking landscape data with population viability analysis*. Setauket, NY: Applied Biomathematics. Retrieved from https://www.ramas.com/

Andreone, F., Carpenter, A. I., Copsey, J., Crottini, A., Garcia, G., Jenkins, R. K. B., … Raxworthy, C. J. (2012). Saving the diverse Malagasy amphibian fauna: Where are we four years after implementation of the Sahonagasy Action Plan? *Alytes*, *29*(1–4), 44–58.

Andreone, F., Dawson, J. S., Rabemananjara, F. C. E., Rabibisoa, N. H. C., & Rakotonanahary, T. F. (2016). *New Sahonagasy Action Plan 2016–2020 / Nouveau Plan d'Action Sahonagasy 2016–2020*. Museo Regionale di Scienze Naturali and Amphibian Survival Alliance, Turin.

Andreone, F., & Randriamahazo, H. (2008). *Sahonagasy Action Plan. Conservation Programs for the Amphibians of Madagascar / Programmes de conservation pour les amphibiens de Madagascar*. Museo Regionale di Scienze Naturali / Conservation International / IUCN Species Survival Commission / Amphibian Specialist Group.

Auffarth, J., Krug, A., Pröhl, H., & Jehle, R. (2017). A genetically-informed population viability analysis reveals conservation priorities for an isolated population of Hyla arborea. *Salamandra*, *53*(2), 171–182.

Brom, P., Anderson, P., Channing, A., & Underhill, L. G. (2020). The role of cultural norms in shaping attitudes towards amphibians in Cape Town, South Africa. *PLoS ONE*, *15*(2), 1–18. doi: 10.1371/journal.pone.0219331

Canessa, S., Ottonello, D., Rosa, G., Salvidio, S., Grasselli, E., & Oneto,

F. (2019). Adaptive management of species recovery programs: A real-world application for an endangered amphibian. *Biological Conservation*, *236*, 202–210. doi: 10.1016/j.biocon.2019.05.031

Ceríaco, L. M. P. (2012). Human attitudes towards herpetofauna: The influence of folklore and negative values on the conservation of amphibians and reptiles in Portugal. *Journal of Ethnobiology and Ethnomedicine*, *8*, 8. doi: 10.1186/1746-4269-8-8

Chaudhary, V., & Oli, M. K. (2020). A critical appraisal of population viability analysis. *Conservation Biology*, *34*(1), 26–40. doi: 10.1111/cobi.13414

Conservation Measures Partnership. (2020). *Open standards for the practice of conservation. Version 4.0*. Retrieved from https://conservationstandards.org/download-cs/

Copsey, J., Lees, C., & Miller, P. (2020). *A facilitator's guide to species conservation planning*. IUCN SSC Conservation Planning Specialist Group: Apple Valley, MN. Retrieved from https://www.cpsg.org/content/facilitators-guide-species-conservation-planning

Corredor Londoño, G., Velásquez Escobar, B., Velasco Vinasco, J. A., Castro Herrera, F., Bolívar García, W., & Salazar Valencia, M. L. (2010). *Plan de acción para la conservación de los anfibios del Departamento del Valle del Cauca*. Santiago de Cali. Retrieved from https://ecopedia.cvc.gov.co/sites/default/files/archivosAdjuntos/plan_de_accion_anfibios_valle.pdf

CPSG. (2020). *Species conservation planning principles & steps, Ver. 1.0.* IUCN SSC Conservation Planning Specialist Group: Apple Valley, MN. Retrieved from https://www.cpsg.org/sites/cbsg.org/fi les/documents/CPSG Principles %26 Steps_English.pdf

Doak, D. F., Boor, G. K. H., Bakker, V. J., Morris, W. F., Louthan, A., Morrison, S. A., … Crowder, L. B. (2015). Recommendations for improving recovery criteria under the US Endangered Species Act. *BioScience*, *65*(2), 189–199. doi: 10.1093/biosci/biu215

Gascon, C., Collins, J. P., Moore, R. D., Church, D. R., McKay, J. E., & Mendelson III, J. R. (2007). *Amphibian Conservation Action Plan: Proceedings IUCN/SSC Amphibian Conservation Summit 2005*. Gland, Switzerland: IUCN/SSC Amphibian Specialist Group.

Gibson, C., Silva, A. De, Tognelli, M. F., & Karunarathna, S. (2020). *Assess To Plan: Conservation action planning for the snakes and lizards of Sri Lanka*. Retrieved from https://www.cpsg.org/content/assess-plan-conservation-action-planning-snakes-and-lizards-sri-lanka

Gregory, R., Failing, L., Harstone, M., Long, G., McDaniels, T., & Ohlson, D. (2012). *Structured Decision Making: A practical guide to environmental management choices*. Wiley-Blackwell.

Iosif, R., Vlad, S. E., Stănescu, F., & Cogălniceanu, D. (2019). Perception of visitors regarding the wildlife inhabiting an archaeological site. *Human Dimensions of Wildlife*, *24*(4), 301–313. doi: 10.1080/10871209.2019.1603333

IUCN SSC CBSG. (2017). *Second Nature: Changing the future for endangered species*. St. Paul, Minnesota: IUCN SSC Conservation Breeding Specialist Group.

IUCN SSC CPSG. (2020). *A guide to facilitating virtual workshops*. Apple Valley, MN, USA. Retrieved from https://www.cpsg.org/content/guide-facilitating-virtual-workshops

Johnson, K., Baker, A., Buley, K.,

Carrillo, L., Gibson, R., Gillespie, G. R., … Zippel, K. (2020). A process for assessing and prioritizing species conservation needs: going beyond the Red List. *Oryx*, 1–8. doi: 10.1017/S0030605317001715

Lacy, R. C. (2000a). Considering threats to the viability of small populations using individual-based models. *Ecological Bulletins*, (48), 39–51.

Lacy, R. C. (2000b). Structure of the VORTEX simulation model for population viability analysis. *Ecological Bulletins*, (48), 191–203.

Lacy, R. C., Ballou, J. D., & Pollak, J. P. (2012). PMx: Software package for demographic and genetic analysis and management of pedigreed populations. *Methods in Ecology and Evolution*, *3*(2), 433–437. doi: 10.1111/j.2041-210X.2011.00148.x

Lacy, R. C., & Pollak, J. P. (2021). *VORTEX: A stochastic simulation of the extinction process. Version 10.5.5*. Brookfield, Illinois: Chicago Zoological Society. Retrieved from https://scti.tools/vortex/

Lacy, R. C., Pollak, J. P., Miller, P. S., Hungerford, L., & Bright, P. (2014). *Outbreak version 2.1*. IUCN SSC Conservation Breeding Specialist Group. Apple Valley, Minnesota, USA.

Lees, C., Gibson, C., Jaafar, Z., Ng, H. H., Tan, H. H., Chua, K. W. J., … Van Veen, F. J. F. (2020). *Assessing To Plan: Next steps towards conservation action for Threatened fishes of the Sunda Region*. Retrieved from https://www.cpsg.org/content/assess-plan-next-steps-conservation-action-threatened-freshwater-fishes-sunda-region

Lees, C. M., Rutschmann, A., Santure, A. W., & Beggs, J. R. (2021). Science-based , stakeholder-inclusive and participatory conservation planning helps reverse the decline of threatened species. *Biological*

Conservation, *260*(May), 109194. doi: 10.1016/j.biocon.2021.109194

McIntosh, E. J., Chapman, S., Kearney, S. G., Williams, B., Althor, G., Thorn, J. P. R., … Grenyer, R. (2018). Absence of evidence for the conservation outcomes of systematic conservation planning around the globe: A systematic map. *Environmental Evidence*, *7*(1), 1–23. doi: 10.1186/s13750-018-0134-2

Olson, D. H. (2007). Implementation considerations. In M. G. Raphael & R. Molina (Eds.), *Conservation of rare or little-known species: Biological, social, and economic considerations* (pp. 303–333). Island Press.

Olson, D. H., & Ares, A. (2022). Riparian buffer effects on headwater-stream vertebrates and habitats five years after a second upland-forest thinning in western Oregon, USA. *Forest Ecology and Management*, *509*, 120067. doi: 10.1016/j.foreco.2022.120067

Olson, D. H., & Van Horne, B. (2017). *People, forests and change - Lessons from the Pacific Northwest*. Washington, DC/Covelo, CA/London: Island Press.

Pi-peng, L. I. (2010). *WWF Project Technical Report China Herpetological Conservation Action Plan I : Amphibians*. Retrieved from https://www.amphibians.org/wp-content/uploads/2019/04/China-Amphibian-Action-Plan.pdf

Pickett, E. J., Stockwell, M. P., Clulow, J., & Mahony, M. J. (2016). Modelling the population viability of a threatened amphibian with a fast life-history. *Aquatic Conservation: Marine and Freshwater Ecosystems*, *26*(1), 9–19. doi: 10.1002/aqc.2564

Prokop, P., & Fančovičová, J. (2012). Tolerance of amphibians in Slovakian people: A comparison of pond owners and non-owners. *Anthrozoos*, *25*(3), 277–288. doi:

10.2752/175303712X13403555186136

Pulgar Vidal, M., Gamboa Moquillaza, P., Cabello Mejía, C., & Valdivia Pacheco, R. (2015). *Plan Maestro del Parque Nacional Yanachaga Chemillén: 2015-2019*. Retrieved from https://sinia.minam.gob.pe/documentos/plan-maestro-2015-2019-parque-nacional-yanachaga-chemillen

Schumaker, N. H. (2016). *HexSim (Version 3.1)*. Corvallis, Oregon: U.S. Environmental Protection Agency. Retrieved from www.hexsim.net

Smith, R., Meredith, H., & Sutherland, W. (2020). Amphibian conservation. In W. J. Sutherland, L. V. Dicks, S. O. Petrovan, & R. K. Smith (Eds.), *What works in conservation 2020* (pp. 9–64). Cambridge, UK: Open Book Publishers. doi: 10.1017/s0030605318000765

Tapley, B., Michaels, C. J., Gumbs, R., Böhm, M., Luedtke, J., Pearce-Kelly, P., & Rowley, J. J. L. (2018). The disparity between species description and conservation assessment: A case study in taxa with high rates of species discovery. *Biological Conservation*, *220*, 209–214. doi: 10.1016/j.biocon.2018.01.022

Tomažic, I., & Šorgo, A. (2017). Factors affecting students' attitudes toward toads. *Eurasia Journal of Mathematics, Science and Technology Education, 13*(6), 2505–2528. doi: 10.12973/EURASIA.2017.01237A

Urbina-Cardona, J. N. (2008). Conservation of Neotropical herpetofauna: Research trends and challenges. *Tropical Conservation Science*, *1*(4), 359–375. doi: 10.1177/194008290800100405

USFWS. (1983). *Recovery Plan for the Red Hills Salamander Phaeognathus hubrichti Highton*. Atlanta, Georgia. Retrieved from https://www.iucn-amphibians.org/wp-content/uploads/2018/11/Red-Hills-Salamander-Recovery-Plan.pdf

USFWS. (1984). *Recovery Plan for the Houston Toad (Bufo houstonensis)*. Albuquerque, New Mexico. Retrieved from https://www.iucn-amphibians.org/wp-content/uploads/2018/11/Huston-Toad-Recovery-Plan.pdf

Vaira, M., Akmentins, M. S., & Lavilla, E. O. (2018). Plan de Acción para la conservación de los anfibios de la República Argentina. *Cuadernos de Herpetología*, *32*(supl. 1), 56. doi: 10.31017/CdH.2018.(2018-s1)

Valencia, L. M., & Fonte, L. F. M. (2021). *Harlequin Toad (Atelopus) Conservation Action Plan (2021-2041)*. Retrieved from https://www.atelopus.org/

Vergara-Rios, D., Montes-Correa, A. C., Urbina-Cardona, J. N., De Luque-Villa, M., E. Cattan, P., & Dario Granda, H. (2021). Local community knowledge and perceptions in the Colombian Caribbean towards Amphibians in urban and rural settings: tools for biological conservation. *Ethnobiology and Conservation*, *24*(May). doi: 10.15451/ec2021-05-10.24-1-22

Wren, S., Angulo, A., Meredith, H., Kielgast, J., Dos Santos, L., & Bishop, P. (2015). *Amphibian Conservation Action Plan. April 2015*. Retrieved from https://www.iucn-amphibians.org/working-groups/thematic/

Young, R. P., Hudson, M. A., Terry, A. M. R., Jones, C. G., Lewis, R. E., Tatayah, V., … Butchart, S. H. M. (2014). Accounting for conservation: Using the IUCN Red List Index to evaluate the impact of a conservation organization. *Biological Conservation*, *180*(2014), 84–96. doi: 10.1016/j.biocon.2014.09.039

Chapter 10. Surveys and monitoring: challenges in an age of rapid declines and discoveries

A. Justin Nowakowski[1, 2, 3]*, Jessica L. Deichmann[1,2], Grant Connette[1,2], Izabela M. Barata[4], Edmund W. Basham[5], Deborah S. Bower[6], Michael W. N. Lau[7], Deanna H. Olson[8]

[1] Working Land and Seascapes, Conservation Commons, Smithsonian Institution, Washington, DC 20013, USA

[2] Smithsonian Conservation Biology Institute, Front Royal, Virginia, 22630, USA

[3] Moore Center for Science, Conservation International, Arlington, Virginia, USA

[4] Durrell Wildlife Conservation Trust, Jersey, Channel Islands

[5] School of Natural Resources and Environment, University of Florida, Gainesville, FL, USA

[6] School of Earth and Environmental Science, University of New England, Armidale, New South Wales, Australia

[7] Local Biodiversity and Regional Wetlands Programme, World Wildlife Fund, Hong Kong, China

[8] U.S. Department of Agriculture Forest Service, Pacific Northwest Research Station, Forestry Sciences Laboratory, Corvallis, OR, USA

Abstract

Surveys and monitoring are the core means of generating knowledge about the distributions, natural history, and conservation status of amphibians. In an age of rapid declines and discoveries across the globe, it is increasingly urgent that surveys and monitoring efforts are well-designed and linked to clear conservation goals. Here, we surveyed the amphibian conservation community and literature to review the state of the field and update recommendations for surveys and monitoring. Many of the advances of the past 15 years have been technological, including shrinking size and cost of hardware like data loggers and transmitters, which has enabled collection of vast amounts of data and required concomitant advances in analytical tools. Visual encounter surveys are still the most common field method for sampling amphibians, though, use of eDNA and automated recorders have increased in recent years. There are new opportunities to couple field techniques with rigorous sampling frameworks and recent advances in analytical methods. Myriad knowledge gaps persist, however, including basic understanding of amphibian biodiversity and natural history in under-sampled regions like the Congo basin and in understudied groups, such as caecilians. Because many knowledge gaps exist and surveys are resource intensive, there is heightened need to apply decision science to prioritise limited resources available for surveying and monitoring. The links between surveys and monitoring and conservation outcomes can ultimately be strengthened by: (1) defining clear conservation objectives for surveys and monitoring through a participatory process with stakeholders; (2) using decision support frameworks to prioritise survey efforts; (3) selecting the most appropriate combination of survey methods, monitoring framework, and analytical approach for the conservation objective; and (4) effectively communicating survey and monitoring results to decision-makers. Finally, (5) by leveraging new methods, technologies, and funding mechanisms, scientists and practitioners can enhance the surveys and monitoring efforts that are essential to achieving amphibian conservation goals.

Introduction

Surveys and monitoring are the means by which we not only detect changes in species distributions and populations but also discover and rediscover species. Across the globe, environmental changes are causing rapid amphibian declines, while at the same time more than 100 new species are described every year (Catenazzi, 2015). Rapid declines and discoveries together compound the urgency and challenges of linking surveys and monitoring to effective amphibian conservation. The threats causing amphibian declines – including land use, climate change, and disease – vary geographically in both degree of intensity and overlap with other threats (Hof, Araújo, Jetz, & Rahbek, 2011). Moreover, the diverse ecological traits of amphibians underlie considerable variation in species’ sensitivity to threats (Lips, Reeve, & Witters, 2003; Nowakowski et al., 2018). Resources for mitigating threats and monitoring populations are also unevenly distributed across the globe, with fewer resources available in hyper-diverse regions with the highest rates of species discovery and endangerment (Balmford & Whitten, 2003). These multidimensional challenges underscore the need to improve coordination of monitoring efforts, capitalise on effective new methods and technologies, prioritise limited resources, and strengthen the links among surveys, monitoring, and conservation action.

Decades of research and practice have led to a set of standards for integrating surveys and monitoring with conservation action through evidence-based adaptive management (Conservation Measures Partnership, 2020; Gillson, Biggs, Smit, Virah-Sawmy, & Rogers, 2019). Surveys and monitoring critically underpin several of the iterative stages of the adaptive management framework, including initial assessment of threats and population status, monitoring of changes in threats and populations, and evaluation of the effectiveness of interventions. Surveys and monitoring, therefore, provide the crucial evidence base for evaluating management options, decision making, and prioritising conservation actions. These actions can be most effective when designed and monitored with participation of local stakeholders and practitioners. Without adequate survey data and stakeholder participation, the adaptive management cycle breaks down.

The exact methods for surveying and monitoring amphibians are largely determined by the diverse life histories of species (Angulo, Rueda Almonacid, Rodríguez-Mahecha, & La Marca, 2006; Dodd, 2010; Heyer, Donnelly, McDiarmid, Hayek, & Foster, 1994). These characteristics frequently include a bi-phasic lifecycle, species-specific calling of male frogs, temporal variability in activity, and a common association with waterbodies. Anurans alone exhibit at least 39 known reproductive modes (Crump, 2015; Haddad & Prado, 2005), which determine how and where we survey for eggs, larvae, and adults. The habitat associations of species also have an outsized influence on our ability to detect and monitor amphibians. For example, fossorial species like most caecilians and canopy-dwelling species like some tree frogs are difficult to detect with conventional survey methods (Basham & Scheffers, 2020; Basham, Seidl, Andriamahohatra, Oliveira, & Scheffers, 2019; Gower & Wilkinson, 2005). Practitioners will need to carefully choose the most appropriate survey methods from a wide range of recent advancements and well-established techniques to effectively monitor focal species.

Confronted with these myriad challenges to amphibian conservation, how can scientists and practitioners more effectively survey and monitor amphibians? Recent advancements in technology in concert with continued population declines create a need to update our knowledge of current monitoring methods and identify existing knowledge gaps to better coordinate and prioritise future surveys. We solicited input from the amphibian conservation community to identify key developments and challenges in amphibian surveys and monitoring. Drawing on these responses, this chapter aims to highlight key knowledge gaps and recommendations for surveys and monitoring programmes (Table 10.1). In the sections below, we summarise (1) commonly used methods and recent methodological advancements;

(2) key knowledge gaps in amphibian conservation; (3) approaches to prioritising surveys and monitoring; (4) improving integration of survey and monitoring data into extinction risk assessments; (5) avenues for bridging the gap between surveys and conservation action; and (6) opportunities on the horizon for continued advancement of surveys and monitoring for amphibian conservation.

Table 1**.** Summary of key knowledge gaps and priorities for surveys and monitoring.

Key knowledge gaps	
1)	Knowledge of highly biodiverse and understudied landscapes - for example, the Congo rainforest
2)	Knowledge of understudied and difficult to detect groups, such as fossorial and arboreal species
3)	Resolution of cryptic species complexes
4)	Improved natural history and identification information, including calls and larval morphology
5)	Improved prediction of species responses to threats based on niches and adaptive capacity
6)	Understanding of interactive effects of threats on populations and assemblages
7)	Moving beyond presence-absence data to understand long-term population trends for many species
Priorities for better integration of survey data into IUCN Red List assessments	
1)	Increasing capacity for conducting species assessments through Red List training programmes
2)	Increased efficiency in integrating survey data into Red List assessments
3)	Encouraging species descriptions, which often represent the only information available for Red List assessments, to include information useful for assessments (e.g., survey effort, number of individuals, etc.)
4)	The development and maintenance of fewer but more permanent repositories for survey and monitoring data

5) A centralised platform for submitting relevant survey and monitoring data for species assessments

Priorities for survey and monitoring programmes

1) Designing surveys and monitoring to address clear questions
2) Identifying questions and design monitoring programmes in collaboration with local stakeholders
3) Addressing priority knowledge gaps that have clear outcomes for conservation
4) Using decision-support frameworks to prioritise limited resources for conservation projects
5) When possible, designing surveys and monitoring to evaluate effectiveness of interventions, as part of an adaptive management cycle
6) Facilitating use of standard database formats for survey and monitoring data by incorporating archival intent into study designs prior to survey implementation.

Potential advancements on the horizon

1) Improved machine learning methods to classify both visual (video and photos) and acoustic data for improved monitoring in remote locations
2) Continued development of new bioinformatic methods to increase the processing and analysis of increasingly large datasets

3) Increased portability of genetic analyses – such as portable sequencers and PCR machines – allow for molecular work in increasingly remote locations

4) Through open data repositories and other sharing platforms, improve the interoperability and accessibility of survey and monitoring data

5) Governments and institutions will need to better coordinate the collection and distribution of biodiversity monitoring data, adopting shared frameworks for information systems such as those promoted by the GEO Biodiversity Observation

6) Conservation financing and other creative funding mechanisms needed to address the large funding gap for surveys and monitoring

Advancements in amphibian surveys and monitoring in the last 15 years

Amphibian surveys and monitoring have a long history over which researchers have developed methods that are now commonly used across the globe (Figure 10.1). While many of these methods are established and well-tested, the last 15 years have brought technological advances in hardware, software and data analyses, as well as increases in knowledge and innovative techniques that have improved amphibian survey and monitoring efforts. For example, researchers have increasingly surveyed vertical transects using "persistent digging" to uncover fossorial species (Biju, Kamei, Gower, & Wilkinson, 2009) and climbing equipment to study the little-known ecology of canopy-dwelling amphibians (Basham et al., 2019). Hardware improvements have lowered the cost and enhanced performance of tools used for surveys and monitoring (Pimm et al., 2015) including autonomous recording units for passive acoustic monitoring (PAM) (Deichmann et al., 2018; Hill et al., 2018), tracking devices like passive integrated transponder (PIT) tags and miniaturised radio transmitters (Connette & Semlitsch, 2015; Forin-Wiart, Hubert, Sirguey, & Poulle, 2015; Lennox et al., 2017), eDNA samplers (Thomas, Howard, Nguyen, Seimon, & Goldberg, 2018), camera traps (M. T. Hobbs & Brehme, 2017), and drones (Koh & Wich, 2012). Growth in software development, machine learning, and bioinformatic tools has improved our ability to track species, analyse large scale spatial data (GIS), classify and detect species in images or audio recordings, and analyse big molecular datasets, such as those produced through metabarcoding and next-generation sequencing methods (e.g., whole genome sequencing). Novel molecular methods are allowing for species detection in samples of water, soil and faeces, identification of cryptic species complexes, and detection of pathogens and other microbiota through improved assays. Rapid accumulation of new species descriptions and natural history information has facilitated large-scale phylogenies and resolved taxonomies (Frost, 2021; Jetz & Pyron, 2018; Pyron & Wiens, 2011), improving the way we design surveys. Likewise, enhanced capacity at a local level has increased our ability to survey sites at broader spatial and temporal scales, for example, through national-level programmes for biodiversity monitoring (Schmeller et al., 2017) and coordinated citizen science programmes (Aceves-Bueno et al., 2015; O'Donnell & Durso, 2014). Advancements in statistical and conceptual approaches have resulted in new ways to design surveys (e.g., through participation of local communities as well as citizens across the globe; Table 10.2), integrate disparate datasets, and analyse survey data (e.g., recent advances in hierarchical population models) (DiRenzo, Che-Castaldo, Saunders, Campbell Grant, & Zipkin, 2019; Dorazio, 2014; Zipkin et al., 2014).

Although many survey and monitoring methods are currently widely used (Fig 10.1), each nevertheless has disadvantages to weigh alongside their benefits before implementation. For example, pitfall and funnel trapping can result in high mortality rates (Enge, 2001) and marking methods such as toe clipping and PIT tagging can also reduce survival in some species (Guimarães et al., 2014). Time- and area-constrained survey methods are often implemented in a way that precludes analysing the data with more rigorous statistical methods, such as those that account for imperfect detection. Methods that result in the accumulation of big data, like PAM, DNA sampled from an organism's environment (eDNA), camera trapping, or photographic mark-recapture, have the added challenge of immense data storage and management needs, as well as complex analytical methods that are still under development. Finally, it is important to consider sampling biases associated with different methods that can affect estimates of population abundances and demographic structure (Nowakowski & Maerz, 2009; Ribeiro-Júnior, Gardner, & Ávila-Pires, 2008). These challenges underlie the importance of carefully designing surveys around a question and selecting the most suitable method or combination of methods for answering that question. Fortunately, there is no end to the ingenuity of amphibian biologists and many of these methods, if combined with an effective monitoring framework (Table 10.2) and/or additional methodologies, can result in efficient data collection and high-quality data. For example, pairing on-the-ground methods (e.g., visual encounter surveys, quadrats, pitfalls) with remote sensing or molecular methods (PAM, eDNA) can provide complimentary data streams that, through modelling, can provide insights over much broader temporal and spatial scales than one method alone. These recent advances in surveys and monitoring can be used to address key knowledge gaps that currently hinder a concerted global conservation response to amphibian declines.

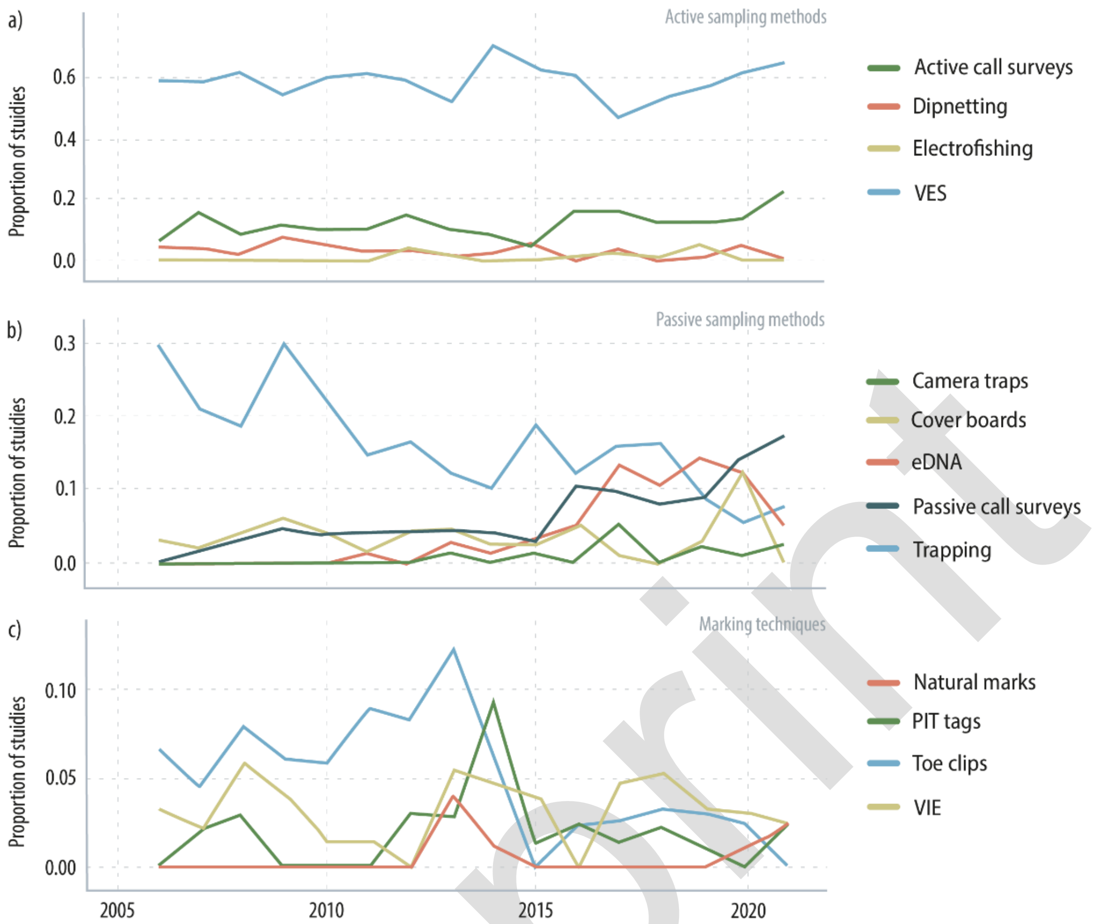


Figure 10.1 Trends in prevalence of active (A) and passive (B) sampling methods and marking techniques (C) in published literature. Active survey methods include those that require observers to actively search or listen for individual animals, including visual encounter searches (VES; inclusive of area and/or time constrained sampling such as transects and plots), dip netting, electrofishing, and active call surveys. Passive sampling methods include those where observed animals are detected in artificial structures (traps or coverboards), with sensors (passive acoustic monitoring and camera traps), or in environmental samples (eDNA). Common marking techniques include use of natural marking (e.g., dorsal patterns), toe clipping, passive integrated transponders (PIT tags), and visual implant elastomer (VIE). These trends are based on a Web of Science search of published literature from 2006-2021.

Table 10.2. A non-exhaustive list of frameworks for surveying and monitoring amphibians. Within each temporal category (static and dynamic) general sampling frameworks are listed in order of increasing rigour, complexity, and cost for a given number of locations. Opportunistic observations are playing an increasingly important role due to rapid increases in citizen science programmes and data platforms. However, these approaches come with limitations on analytical methods and inferences, stemming from lack of standardisation. Well-designed, planned surveys offer greater opportunity for standardisation and generate data that can be analysed with a wider array of modelling approaches, including those that account for imperfect detection. A 'robust design' generally refers to a class of standardised surveys wherein there are replicated temporal or spatial sub-samples within a defined spatial unit of aggregation (e.g., 1-ha plot) and that occur over a short enough time period to assume the populations are closed to immigration and emigration (MacKenzie & Royle, 2005; Pollock, 1982). Acronyms: SDM = species distribution model; GLM = generalised linear model; GLMM = generalised linear mixed model; VES = visual encounter surveys; MR = mark-recapture.

	Example activities	Example inferences	Example analyses
Static (single year)	***Opportunistic observations***		
	Citizen science (FrogWatch, eBird, iNaturalist, etc), rapid inventories, expert elicitation	Habitat suitability, projected range shifts, species lists, presence only, known range expansions	SDMs, integrated models
	Single visit, standardised surveys		
			GLM/GLMM, distance sampling,

	Surveys of occupancy and counts, distance sampling, molecular sampling	Drivers of spatial variation in occurrence, abundance, and genetic diversity; habitat associations; weaker inferences about interventions	ordination, single-season occupancy models
	Repeated surveys		
	Camera trapping, acoustic surveys, multiple VES	Drivers of spatial variation in occurrence or abundance while accounting for imperfect detection	Single-season occupancy, N-mixture models, MR abundance estimation
Dynamic (multiyear)	***Multiyear opportunistic observations***		
	Citizen science (FrogWatch, eBird, iNaturalist, etc), rapid inventories, expert elicitation	Phenology changes (e.g., timing of breeding), projected range shifts, and species lists	SDMs, integrated models
	Multiyear single visit (per year), standardised surveys		
	Mark-recapture, surveys of occupancy and counts, distance sampling	Population or community dynamics (survival, immigration), drivers of	GLM/GLMM, state-space models, integrated population model

	trends; demographic rates, stronger inferences about interventions	
Robust design		
Mark-recapture, Camera trapping, acoustic surveys, multiple VES, tracking studies	Population or community dynamics, drivers of trends, stronger inferences about interventions, accounting for imperfect detection	Dynamic occupancy and N-mixture models; multiyear MR abundance estimates

Key knowledge gaps that could be addressed with additional surveys

Considerable gaps remain in our knowledge of amphibians. At the most basic level, it is estimated that ~27% of amphibian species (~3,000 species) remain undescribed (Giam et al., 2012), and 25% of those that are described have too few range data to accurately predict threat status (González-del-Pliego et al., 2019). The primary causes of these data deficiencies are: 1) insufficient surveys in highly biodiverse and understudied landscapes, for example, the Congo rainforest, Papua New Guinea, and other habitats that are difficult to access in regions that are amphibian species-rich but resource-limited (Guerra, Jardim, Llusia, Márquez, & Bastos, 2020; Vieites, Wollenberg, & Andreone, 2009), and 2) difficulty in detecting some amphibian groups, including caecilians and canopy dwelling species. Thus, monitoring programmes that target understudied biodiversity hotspots combined with canopy and sub-surface survey methods, for example, would significantly improve our global understanding of amphibian distributions and status. Increased surveys and monitoring in these contexts would also lead to increased understanding of natural history, which would not only improve our overall ability to detect species, but also help us better understand how amphibians may be impacted by environmental change.

Undescribed species hidden within cryptic species complexes represent another important knowledge gap (McLeod, 2010). Such species make up a significant proportion of undescribed amphibian diversity (Funk, Caminer, & Ron, 2012) and resolution of these taxa could be addressed with increases in both the number of genetic studies and more widespread geographic sampling. These efforts can be accelerated by integrating genetic sampling (eDNA or tissue samples) and laboratory methods like gene sequencing into standard monitoring protocols. As they become increasingly affordable, genetic methods will uncover considerable hidden diversity and help overcome inaccuracies in field identifications, which can be an issue even for local experts (Deichmann et al., 2017). In addition to collecting tissues for molecular studies, it is also essential to collect additional data that can improve the efficacy of surveys and monitoring. Examples include tadpole morphology data that will allow for improved identification of larvae when adults are not present (Schulze, Jansen, & Köhler, 2015), and calls and photographs of voucher specimens that can be used as training data in machine learning methods for species classification (i.e. call and image recognition models) (Xie, Towsey, Zhang, & Roe, 2016).

With climate extremes increasing and habitat loss decimating tropical biodiversity hotspots, concerted survey efforts coupled with information on both species' exposure and sensitivity to threats –including traits, niche dimensions, and adaptive capacities – are needed to adequately forecast current and future threat impacts (Murray, Nowakowski, & Frichkoff, 2021; Urban et al., 2016). In particular, efforts to manage or conserve species may fall short of their goals if they fail to anticipate interactive effects of co-occurring threats, such as land use, climate change, and disease (Hof et al., 2011). As >70% of the Earth's land surface is modified by human activities (R. J. Hobbs, Higgs, & Harris, 2009), more work is needed to identify key habitats for amphibian persistence in working landscapes, such as riparian corridors and remnant trees (Mendenhall et al., 2014), while also identifying at-risk, intact habitats with high numbers of threatened species to prioritise for site protection (Nowakowski & Angulo, 2015; Venter et al., 2014). An important outcome of survey and monitoring can be the prioritization of areas of intact habitat that can serve as climate refugia and connected nodes in climate resilient protected areas networks (Marquet, Lessmann, & Shaw, 2019).

Although many datasets exist describing the presence of species in localities, there is very little information on population trends over time. Long-term data are needed to rigorously assess population and range dynamics, sensitivity to threats like land use and climate change, and the impacts of management interventions. Recent advances in statistical methods, such as dynamic occupancy and N-mixture models, and computing can be employed in conjunction with long-term monitoring of populations and communities, thereby enabling the detection of slow declines and species range shifts (Zipkin et al., 2014). Increases in open data repositories are facilitating comparative analyses and synthesis of amphibian population trends (Collen et al., 2009; Dornelas et al., 2018). Existing knowledge gaps are manifold and resolving each will likely have unequal returns on investment for conservation. In the face of such uncertainty, addressing the knowledge gaps identified here may serve as only one important criterion for prioritising limited resources for surveys and monitoring.

Prioritising limited resources for surveys and monitoring

Reliable, timely, and accessible information on the status of species and their threats is critical to achieving successful conservation interventions. However, despite considerable progress over recent decades in the standardisation of research methods and early detection of species declines, we have largely failed to halt ongoing declines in both common and rare amphibian species (Bishop et al., 2012; Campbell Grant, Muths, Schmidt, & Petrovan, 2019). Given the limited resources available for surveys and monitoring, a key goal should be to prioritise the collection of actionable information that provides the greatest chance to change conservation outcomes (Buxton et al., 2020; Lindenmayer, Piggott, & Wintle, 2013).

Even with this ‘value of information’ perspective, the challenge of how and where to prioritise research efforts remains daunting. Many rare and at-risk species are disproportionately under-studied by researchers (da Silva et al., 2020; Walls, 2014), while at the same time the proactive monitoring of widespread, common species can both decrease the cost of management interventions and increase the likelihood of success (Sterrett et al., 2019). In light of such trade-offs, decision science has produced an array of decision support frameworks that help practitioners and scientists structure potentially overwhelming complexity, including stakeholder interests and system uncertainty, to prioritise limited resources for conservation projects (See recent reviews of decision support frameworks) (Schwartz et al., 2018; Wright et al., 2020). Decision frameworks can help researchers identify cases where surveys and monitoring are needed and avoid cases where additional monitoring efforts would be unlikely to change management actions (McDonald-Madden et al., 2010). However, the evidence base for informing management decisions remains extremely limited for certain taxa and geographies, due to a lack of data on population status and effectiveness of management interventions (Canessa, Spitzen-van der Sluijs, Martel, & Pasmans, 2019; Christie et al., 2020). Although widely adopted, successful application of decision frameworks throughout a project, from initial planning to intervention and evaluation stages, remains relatively rare, including among amphibian projects (Redford, Hulvey, Williamson, & Schwartz, 2018; Wright et al., 2020). This clearly highlights the need for an objective-oriented approach to setting research priorities to provide baseline information on species with limited data, identify threats, monitor population status, and inform the implementation of specific management interventions (Table 10.3).

Addressing the magnitude of global amphibian declines requires considerable effort to expand the coverage of existing monitoring, particularly in under-studied geographies and for species lacking data. Filling these information gaps requires an increased commitment by funders and researchers to ensure that local researchers have the skills and resources to do effective monitoring, data reporting, and conservation planning. Establishing new monitoring networks in under-studied areas of high amphibian species richness would offer the potential for rapid, widespread deployment of standardised survey methods. Such monitoring networks would also ensure that data are accessible and comparable across time and space, while potentially affording opportunities for further expansion of surveillance capacity through the integration of volunteers and citizen scientists (Aceves-Bueno et al., 2015). As much of the tropics remain understudied, additional layers of prioritization of new monitoring networks could include (1) areas with many threatened or data deficient species, (2) highly threatened ecosystems, (3) areas with high endemism, (4) rediscovery of "lost species" that have not been observed for years or decades (González-Maya et al., 2013) and (5) using phylogenetic information to prioritise sensitive clades and evolutionarily distinct species (González-del-Pliego et al., 2019; Jetz & Pyron, 2018). Although this broadening of surveillance efforts would undoubtedly improve our ability to detect and respond to species declines, it is also imperative that researchers are equally committed to proactively proposing and evaluating potential conservation interventions in order to avoid simply monitoring species into extinction (Canessa, Spitzen-van der Sluijs, et al., 2019; Lindenmayer et al., 2013).

Table 10.3. Priorities for survey and monitoring in relation to perceived risk of species decline. (Adapted from Lindenmayer et al., 2013; Sterrett et al., 2019).

Perceived Risk of Decline	Survey & Monitoring Approaches
Unknown	1. Species discovery (prioritise poorly studied and species rich areas) 2. Basic assessment of genetic diversity (prioritise detection of cryptic species and evolutionarily-distinct lineages) 3. Collect distribution data to delineate species range, identify habitat associations, and identify potential threats
Low	4. Targeted surveillance with standardised methods to detect change 5. Targeted disease surveillance
Medium	1. Targeted monitoring of occurrence/abundance (ideally using methods capable of detecting gradual population trends) 2. Perform studies to evaluate management effectiveness (prioritise setting management triggers) 3. Test and adapt potential management strategies 4. Predict impacts of potential threats (e.g., habitat loss, climate change, etc.)
High	1. Intensive demographic monitoring of populations 2. Evaluate relative importance of threats 3. Intensive adaptive management and threat monitoring 4. Species rediscovery efforts

Improving integration of survey and monitoring data into red list assessments

IUCN Red List assessments are widely accepted standards for measuring species' risk of extinction on global and national scales and a powerful tool for conservation policy and planning (Brito, 2010; Hoffmann et al., 2010; Rodrigues, Pilgrim, Lamoreux, Hoffmann, & Brooks, 2006). Assessments are designed to be consistent, transparent, and structured by objective criteria and guidelines (Mace et al., 2008) to ensure repeatability over time. The effectiveness of the IUCN Red List depends on each assessment containing up-to-date information; however, data and the capacity needed to complete these assessments are unevenly distributed among geographic regions and across different taxonomic groups (Collen et al., 2009).

The high proportion of amphibian species that have not been assessed (13% of described species) or that are Data Deficient (16-17% of assessed species) illustrate the challenges posed by rapid species discovery and lack of meaningful data for many species, especially in the tropics (Collen, Ram, Zamin, & Mcrae, 2008; IUCN, 2021; Stuart et al., 2004). During the previous Global Amphibian Assessment for The IUCN Red List of Threatened Species (GAA), 5,743 amphibian species had been described, of which 22.5% were assessed as Data Deficient (Stuart et al., 2004). Since then, the number of known species has increased remarkably (currently 8,309 species; Frost, 2021). With so many new and little-known species, there is interest within the amphibian conservation community to increase the rate of species assessments. Addressing these challenges requires increased assessment capacity, new survey data, and more efficient integration of survey data into the assessment process.

Expanding the network of experts contributing to assessments and increasing Red List training and mentoring opportunities for the broader conservation community could help improve the speed, standardisation, and interpretation of Red List assessments. Training can enhance knowledge of the guidelines for applying the Red List Categories and Criteria as well as the data required to estimate trends in species abundance and distributions (Collen et al., 2016), assisting the design of future surveys and improving data integration into species assessments. For instance, assessment rates could be increased if authors of species descriptions, which often represent the only information available for species assessments, routinely included information such as descriptions of survey effort, abundance, habitats, and threats (Tapley et al., 2018). To this end, IUCN, in collaboration with The Nature Conservancy (TNC), developed a free online Red List training course available in IUCN's three official languages (see https://www.iucnredlist.org/resources/online). Expanded networks and increased capacity may also facilitate knowledge transfer and data sharing within and across regions, thereby synergising efforts across assessments and working groups and increasing rates of assessment.

As capacity to support Red List assessments improves and monitoring programmes continue to increase data availability, there is a need for more efficient dataflow to ensure that different types of survey and monitoring data effectively contribute to assessments. New approaches to data-sharing (e.g., online databases, repositories, data papers, data archiving) are required to improve dataflow and increase the availability of data across multiple regions. Current biodiversity data are spatially biased and are either scattered in many databases or reside on paper or behind pay walls, impeding access and collation for assessments (Beck, Böller, Erhardt, & Schwanghart, 2014; Chavan & Penev, 2011). A sustainable data management system requires the development and maintenance of fewer but more permanent data repositories (Bach et al., 2012) that are subject to data quality control (Costello, Michener, Gahegan, Zhang, & Bourne, 2013; Huang, Hawkins, & Qiao, 2013). Current standards and best practices for the management and publication of biodiversity data are already available (Costello & Wieczorek, 2014). Furthermore, the implementation of a process that awards professional recognition for contributors (e.g. citation and co-authorship) would likely increase contributions of scientists to open data repositories. However, to improve integration of available survey and monitoring data into Red List assessments, specific guidelines and a platform for submitting relevant data for species assessment could be implemented, strengthening links among experts and allowing a broader participation of trained professionals and citizen scientists alike. New survey and monitoring projects could facilitate data sharing and integration into Red List assessments by including data standards and plans for archival in the design phase of the project.

Bridging the gap between survey and monitoring data and conservation action

Frameworks for linking surveys and monitoring data to conservation actions

Adaptive management is a framework – widely used by non-governmental organisations (NGOs), government agencies and funders – that links survey and monitoring to conservation actions (Conservation Measures Partnership, 2020; Gillson et al., 2019; Schwartz et al., 2018). Following this framework, survey and monitoring data inform assessment of threats and population status, tracking of progress toward conservation goals, and evaluation of management interventions (Conservation Measures Partnership, 2020). Adaptive management is data and resource intensive, however, as it is tailored to system complexities and idiosyncrasies on the ground. In many understudied biodiversity hotspots, detailed population data are lacking and can take years or decades to accumulate; by then, actions may be too late (T. G. Martin et al., 2012). Other decision support frameworks exist – such as structured decision-making and evidence-based practice – and tools from each can be blended to achieve conservation objectives (Schwartz et al., 2018). For example, evidence-based conservation is a complementary framework that instead draws on the broader body of survey data and impact assessments to identify best practices, when at least some information exists on the state of the system; this approach mirrors the learning process widely used by medical practitioners (Gillson et al., 2019; Sutherland, Pullin, Dolman, & Knight, 2004). Adaptive management and evidence-based frameworks can be integrated to implement best practices as a starting point and then adapt interventions as monitoring data and impact assessments accumulate for a system. A complete cycle of adaptive management would (1) define clear conservation objectives that are part of a 'theory of change' results chain (Salafsky et al., 2008), with input from stakeholders; (2) plan and implement interventions alongside standardised, recurring surveys to monitor threats and focal taxa; and (3) use survey data to evaluate and adapt management interventions over time.

Linking surveys and monitoring to clear conservation objectives with stakeholder input

Critical to bridging the gap between data and effective conservation actions, is designing survey and monitoring efforts around clear conservation objectives, which are ideally defined with input from multi-stakeholder groups. These objectives may include: (1) protection of iconic places for a species or a location's natural heritage such as a national park; (2) assessing the status of rare or little-known species; (3) reversing suspected population declines; and (4) monitoring responses to specific threat factors. While long-term monitoring programmes are ideal for obtaining actionable data, such programmes often require significant human and financial resources and are less common outside of developed countries (Proença et al., 2017). With limited resources, it may only be possible to survey a site a single time. These one-off inventories are nevertheless essential for evidence-based conservation, as well-designed surveys may still allow researchers to discover new species, update species ranges, understand habitat associations, or identify potential threats (Tables 10.1 & 10.2).

Identifying the conservation objectives that guide a monitoring programme should ideally be a participatory process, involving input from multiple stakeholders and drawing on local knowledge. The importance of integrating stakeholder input into species monitoring and conservation programmes is increasingly recognised, especially for amphibians (Olson & Pilliod, 2021). This may include integration of local or regional communities in programme planning and implementation through conservation cooperatives, participatory panels, and citizen science involvement. Outreach and education can inspire appreciation for the awe, wonder, and importance of amphibians, which is needed to ensure their persistence for generations to come. Importantly, educating natural resource managers and policymakers about amphibians and their importance to ecosystems may be needed, especially if resources have been historically diverted to other priorities.

Development of Monitoring Programmes

The combination of standardised methodologies with recurrent surveys forms the foundation of a monitoring programme (example amphibian monitoring programmes: Boxes 10.1-10.4; Table S1). These programmes generate information on population status and dynamics that can be fed into decision support frameworks, such as adaptive management, and contribute to the planning and learning phases of a conservation project (Schwartz et al., 2018). A key aim of new monitoring programmes is often to conduct initial surveys that establish baseline information (Proença et al., 2017). This baseline can be used to assess current threats and the status of focal populations and may then contribute to conservation planning by prompting decisions about the need for additional monitoring and interventions. Other common aims of survey programmes are to understand species occurrence patterns and habitat associations, to quantify population trends and identify drivers of occurrence and trends, and to support planning and evaluation of management interventions. Some programmes may span multiple monitoring objectives. For example, the US northwest federal "Survey and Manage Program" is focused on five plethodontid salamanders (Text Box 10.4) and expanded over time to include surveys at additional sites, and of additional species, and using new survey methods to improve inferences about populations and their habitat associations across the landscape (Olson, Van Norman, & Huff, 2007).

Additionally, survey and monitoring programmes may have qualitative or quantitative goals, or a mix of each. For example, annual visits to breeding sites may generate qualitative information such as the date of breeding, lists of calling species, and anomalies noted – data-poor metrics that are potentially informative for detecting changing conditions that may warrant more rigorous follow-up surveys. At the other end of the spectrum, a mark-recapture programme could generate information about individuals across their life spans for more quantitative assessments of demographic status and trends. Data from long-term monitoring programmes can be used to develop reliable models that can inform conservation actions (e.g., determining extinction risk of development activities on focal species or identifying habitat critical for preservation to ensure survival of target metapopulations; Howell, R, Muths, Sigafus, & CHandler, 2020). Ancillary data collected during surveys for amphibian occurrence or population status may also have enormous long-term benefits to advance basic species knowledge, conservation, or research (Boxes 10.1-10.4). Lastly, tracking of multiple monitoring, conservation, or restoration programmes can facilitate synthesis of actions and outcomes across broad geographic areas. For example, the Canadian province of British

Columbia has developed an amphibian conservation and restoration database to help track these efforts across their province (Table S1).

Considerations of paramount importance for the long-term success of surveys and monitoring efforts include: 1) institutional support (e.g., can the programme become institutionalised, or are there multi-partner trust agreements to ensure longevity [researchers, local people, governments, decision makers, others]); 2) clear priorities and design; 3) capability (personnel infrastructure [e.g., biological, technical, administrative, policy]); 4) funding; 5) communication (stakeholder updates, reports, outreach and education, media and social media information releases); and 6) adaptive management (cyclic learning to improve execution of the programme). The last two considerations, communication and adaptive management, are opportunities to build stakeholder trust and leverage the results of surveys and monitoring into reactive conservation actions.

Impact assessments for adapting interventions

Evaluating the effectiveness of conservation interventions depends on the availability and design of survey and monitoring datasets. Often, interventions and monitoring programmes are designed independently, requiring retrospective impact evaluations that use existing monitoring data. In these cases, monitoring data from treated and untreated sites can be statistically matched after data collection, while accounting for confounding variables (Schleicher et al., 2020). In other cases, surveys and monitoring programmes are co-designed alongside interventions and ideally built on theories of change (Rice, Sowman, & Bavinck, 2020). Surveyed sites for planned impact assessments are either haphazardly assigned to treatments (sites receiving the intervention) and controls – as is most common – or are randomly assigned to each. Randomised controlled trials are the research gold standard but are rare in conservation impact assessments (Burivalova, Miteva, Salafsky, Butler, & Wilcove, 2019), perhaps owing to logistics or ethical concerns under certain contexts. Co-designing interventions and monitoring for impact evaluation requires a greater level of planning and coordination but, when well-implemented, can lead to stronger inferences about intervention effectiveness (Baylis et al., 2016; Burivalova et al., 2019), which in turn can reduce uncertainty and wasted resources in conservation (Buxton et al., 2021). Lessons learned from impact assessments then inform future implementation and adaptation of management interventions. For example, Canessa, Ottonello et al. (2019) monitored stage-specific survival rates of the threatened toad, *Bombina variegata*, to evaluate effectiveness of three methods of reintroduction, captive rearing, headstarting, and direct translocations. They then adapted to focus survey and implementation efforts solely on headstarting, based on the data generated during the first years of monitoring. The specific interventions implemented for an amphibian conservation initiative will invariably depend on species life history and system context; the evidence base for a number of interventions is reviewed in Smith, Meredith, & Sutherland (2018).

On the horizon: potential for advancing surveys and monitoring

As threats to amphibian populations increase, the future of surveying and monitoring will require increased capacity, efficiency, and funding if conservation is to succeed. Advances in technologies are enhancing efficiency of monitoring through remote detection and tracking of species with higher spatial and temporal resolution. A broad trend in greater accessibility of micro technologies for tracking small-bodied amphibians with corresponding analytical tools is likely to further increase the resolution of monitoring and the breadth of species that are appropriate to different methods. More passive monitoring through drones and remote technologies can help expand the geographic coverage of monitoring efforts by reducing time and resource requirements (Marvin et al., 2016; Wilson et al., 2016).

For amphibians in particular, technology has been an effective aid to surveys and monitoring. Many populations are now monitored through acoustic sensors at very high temporal resolution, thereby generating massive amounts of data. However, lags in development of analytical tools still constrain our ability to comprehensively process acoustic data (Brodie, Allen-Ankins, Towsey, Roe, & Schwarzkopf, 2020; Deichmann et al., 2018). In the future, we are likely to resolve these issues with improved machine learning methods that will classify both visual (video and photos) and acoustic data to enable the identification of cryptic species and allow improved monitoring in remote locations. This may lead to real-time monitoring at a large scale, for example, by employing automated detection of calls. Additionally, cutting-edge artificial intelligence, such as algorithms used in the gaming industry, may provide a means to test and predict scenarios as they unfold through monitoring and to guide management (Barbe, Mony, & Abbott, 2020). At the same time, continued development of new bioinformatic methods will enable the processing and analysis of increasingly large datasets (La Salle, Williams, & Moritz, 2016; Snaddon, Petrokofsky, Jepson, & Willis, 2013).

Accompanying advances in technology, the accessibility of genetic methods to inform monitoring has increased greatly. Genetic methods are an important piece of the conservation puzzle, informing our understanding of the underlying resilience of populations, resolving cryptic species, and guiding conservation strategy. The ongoing reduction in cost and increase in portability of genetic analyses – such as portable sequencers and PCR machines for molecular work in remote locations (Menegon et al., 2017) – coupled with the increased utility and complexity of laboratory and statistical analysis, will likely continue apace. For threatened amphibians, the continued rise in throughput and resolution of genetic methods will aid managers in prediction and decision-making around interventions for threatened species. Already we have seen the unit of focus change from species to sub-species management units in many cases, and with the advent of genomics we may soon be monitoring many populations at the individual or gene level.

Through open data repositories and other sharing platforms, there is a need to further improve the interoperability and accessibility of survey and monitoring data, including those generated by new technologies and molecular methods. However, these efforts will require a transformation in organisation and political will to ensure usefulness and equity of open data resources for conservation action (Stephenson et al., 2017). Governments and institutions will need to better coordinate the collection and distribution of biodiversity monitoring data, adopting shared frameworks for information systems such as those promoted by the GEO Biodiversity Observation Network (Navarro et al., 2017). The need for science to become more openly accessible, more robust and replicable is becoming increasingly crucial as resources are further restricted (Hampton et al., 2013). Digital platforms that manage data and enable sharing globally will need to become more coordinated and regulated over time, including adherence to meta-data standards. As developing countries gain better access to technology and communication, open data repositories and resources should be intentionally designed and maintained to improve equity of access and use of open data. Open data platforms can facilitate collaborations and knowledge exchanges between specialties and disciplines, from those collecting data on the ground to those analysing data in the cloud. Technology has the potential to reduce the resource disparity between different socioeconomic backgrounds and to provide access to open-source software and related training modules needed for planning and analysis of survey and monitoring data. This should increase the capacity of local stakeholders, which is an important goal in conservation (Brooks, Waylen, & Borgerhoff Mulder, 2012).

While there will always be a need for well-designed, on-the-ground monitoring programmes, surveys and monitoring efforts may increasingly take advantage of non-traditional sources of data to minimise the resources needed to gather data necessary for decision-making. With the proliferation of environmental impact assessments associated with infrastructure development projects, grey literature reports of species occurrences are becoming more accessible. Similarly, as the push to improve data formatting and data sharing bears fruit, mining biodiversity data portals may provide some of the information traditionally gathered in on-the-ground surveys. Consulting these portals will be an important initial step in designing strategic amphibian surveys and monitoring programs (Garcia Fontes, Stanzani, & Pizzigatti Correa, 2015). In addition, social media harbours a wealth of georeferenced biodiversity information that could be scraped and accessed through content analysis or other methods to inform amphibian surveys and monitoring (Toivonen et al., 2019).

Arguably, the greatest impediment to amphibian surveys and monitoring and to achieving amphibian conservation goals is lack of funding. This necessitates creativity to look beyond traditional sources of conservation research financing. Fortunately, there are opportunities on the horizon: it is increasingly feasible to engage the private sector to generate funding for biodiversity conservation. In some locations, the private sector’s stake in biodiversity is tied to its obligation to meet national and global development goals (Nationally Determined Contributions, post-2020 Biodiversity Targets, UN Sustainable Development Goals, etc.), to the will of activist shareholders and board members, and to the value of ecosystem services upon which corporations rely (Barbier, Burgess, & Dean, 2018). Multilateral development banks often fund projects initiated by corporations and they also play a role in financing conservation as part of the environmental responsibility standards tied to those projects. Development projects funded by the banks signed on to the Equator-Principles are required to implement the mitigation hierarchy to manage their impacts to biodiversity and to implement biodiversity offset mechanisms. Amphibian conservation activities can be strategically woven into these projects (Deichmann et al., 2013). Among private investors, there is growing interest in "impact projects", those that generate a measurable social or environmental benefit alongside a financial return (Rodewald et al., 2020). In amphibian-rich but resource-limited countries, these projects are often driven by an initial philanthropic contribution (blended financing), that catalyses investment from other entities. Ensuring survey objectives are clear and intentionally tied to national and global conservation goals will be essential in securing outside support for projects and conservation initiatives in resource-limited nations.

Amphibian surveys and monitoring vitally underpin much of our knowledge about the natural history, status, and population trends of amphibian species. As many populations have declined across the globe, ensuring that surveys and monitoring efforts are linked to conservation outcomes is increasingly urgent. These links can be strengthened by (1) defining clear, applied objectives for amphibian surveys and monitoring through a participatory process; (2) using decision support frameworks (such as adaptive management) to prioritise surveys; (3) selecting the most appropriate survey methods among traditional and recently advanced techniques; (4) and communicating survey and monitoring data in formats appropriate for informing decision-making. Finally, (5) by leveraging new methods, technologies, and funding mechanisms, we can ensure surveys and monitoring contribute to achieving amphibian conservation goals in an age of rapid amphibian declines and discoveries.

Box 10.1. Ancillary data

While in the field conducting surveys and/or monitoring, information that is important for conservation planning and research objectives can be collected with little additional effort. These data include: (1) habitat and microhabitat attributes (e.g., habitat types and sizes, vegetation, canopy cover, water depth and flow, stream gradient, substrates, water quality, calling site, hiding refugia); (2) species life history or behavioural observations (e.g., life stage occurrence, breeding/foraging/dispersal behaviours); (3) community composition (e.g., prey, predators, invasive species); (4) human activities (e.g., timber harvest, livestock grazing, agriculture, wildlife harvesting, nearby human community activities); (5) threats (e.g., algal bloom, fire, trees killed by pests/disease, chemical contamination, erosion, pathogens or poor animal health observations). For long-term monitoring, it could be useful to establish a monumented photo point (e.g., phenocams; Brown et al., 2016) to compare the habitat condition throughout the years, to show natural succession or effects of disturbances. As weather and microclimate conditions drive amphibian activity and distributions, it is important to obtain data from the nearest weather station or, preferably, to deploy weather data loggers at sampled sites. Additional sampling could include collection of a species voucher (adult, tadpole/larval and egg stages), genetic sample, carcass, vocalisations, eDNA samples for full analyses of the community and /or a photographic voucher - taken with species-specific characteristics shown, which may be of great value for later species confirmation or disease detection. Metadata from surveys should include disposition of samples and survey data in archived databases. Including these ancillary data and materials in standard survey and/or monitoring protocols will ensure they are collected. Although it may seem ambitious to record as many ancillary data as feasible and some data may require additional permitting (e.g., species vouchers and DNA samples), these ancillary data provide critical context to the species occurrence or abundance data and potentially the entire programme. Information ancillary to amphibian species occurrence or abundance is particularly useful for discerning environmental changes in long-term monitoring programmes and can shed light on the cause(s) of later-documented trends, information essential for conservation planning.

Box 10.2. The Mountain Chicken Recovery Programme

Once found on seven islands in the Caribbean, the Mountain Chicken (*Leptodactylus fallax*) is a Critically Endangered frog now restricted to the islands of Montserrat and Dominica. Chytridiomycosis caused by the fungal pathogen *Batrachochytrium dendrobatidis* (Bd) resulted in the near extinction of the species. In the early 2000s, a 3-year population monitoring and disease surveillance programme was established to determine the extent and impact of chytridiomycosis in Dominica (Cunningham, Lawson, Burton, & Thomas, 2008). Data from multiple years (2002-2014 on Dominica; 1998-2012 on Montserrat), showed a loss of over 85% of the population in fewer than 18 months on Dominica and near extinction on Montserrat, in one of the fastest observed vertebrate declines of all time (Hudson, Young, D'Urban Jackson, et al., 2016). This prompted Monserrat to develop the Mountain Chicken Species Action Plan, prioritising biosecurity measures (L. Martin et al., 2007). Despite this, Bd was detected in Montserrat in 2009 and subsequent surveys detected presence of the fungus in the last healthy Mountain Chicken population. In 2010, the Mountain Chicken Recovery Programme was formed (Adams et al., 2014), a collection of European Zoos and the governments of Dominica and Montserrat that coordinate conservation for this species based on robust long-term monitoring data. Between 2011-2014, the programme implemented experimental reintroductions of captive-bred animals (Hudson, Young, Lopez, et al., 2016), and in 2019 27 frogs were introduced to a semi-wild enclosure in Montserrat in an attempt to use environmental manipulation to enable frogs to survive in the face of endemic Bd in reservoir species. The first breeding pairs were recorded in 2020, culminating in what likely represents the first observed fertilised nest in Montserrat in 11 years, though this nest later failed. As part of the Long-Term Recovery Plan for the Mountain Chicken (Adams et al., 2014), monitoring of the species and pathogen continues on both islands, alongside research into mechanisms to ensure the survival of remnant populations and the reintroduction of others.

Box 10.3: *Atelopus* conservation

Survey efforts in Central America uncovered the first evidence of massive amphibian declines in the 1980s (Fig 10.2). Although the cause was unknown, continued monitoring in Costa Rica and Panama documented a south-east progression of population declines moving towards Colombia (Lips, 1999). By 1999, the emerging infectious disease chytridiomycosis, caused by the fungal skin pathogen *Batrachochytrium dendrobatidis* (Bd), had been identified as a major threat to the *Atelopus* genus of bufonid toads in particular. Survey data showed that Bd was an imminent threat to the continued existence of multiple threatened species, including the Panamanian Golden Frog (*Atelopus zeteki*), one of the world's most culturally significant, recognisable, and Critically Endangered amphibians (Gagliardo et al., 2008). Based on these alarming survey results, representatives from an international collaboration of universities, zoos, and conservation organisations established colonies of wild populations of multiple *Atelopus* species in ex-situ management centres (Zippel, 2002). In 2004, wild populations from Panamanian sites were decimated by *Bd* as predicted, rendering many *Atelopus* species Critically Endangered or possibly extinct in the wild (Zippel, 2002). Remnant captive populations have since been successfully bred in captivity as source populations for reintroductions, where surplus individuals are also providing a key role in understanding infection pathways and fungal resistance (Becker et al., 2011).

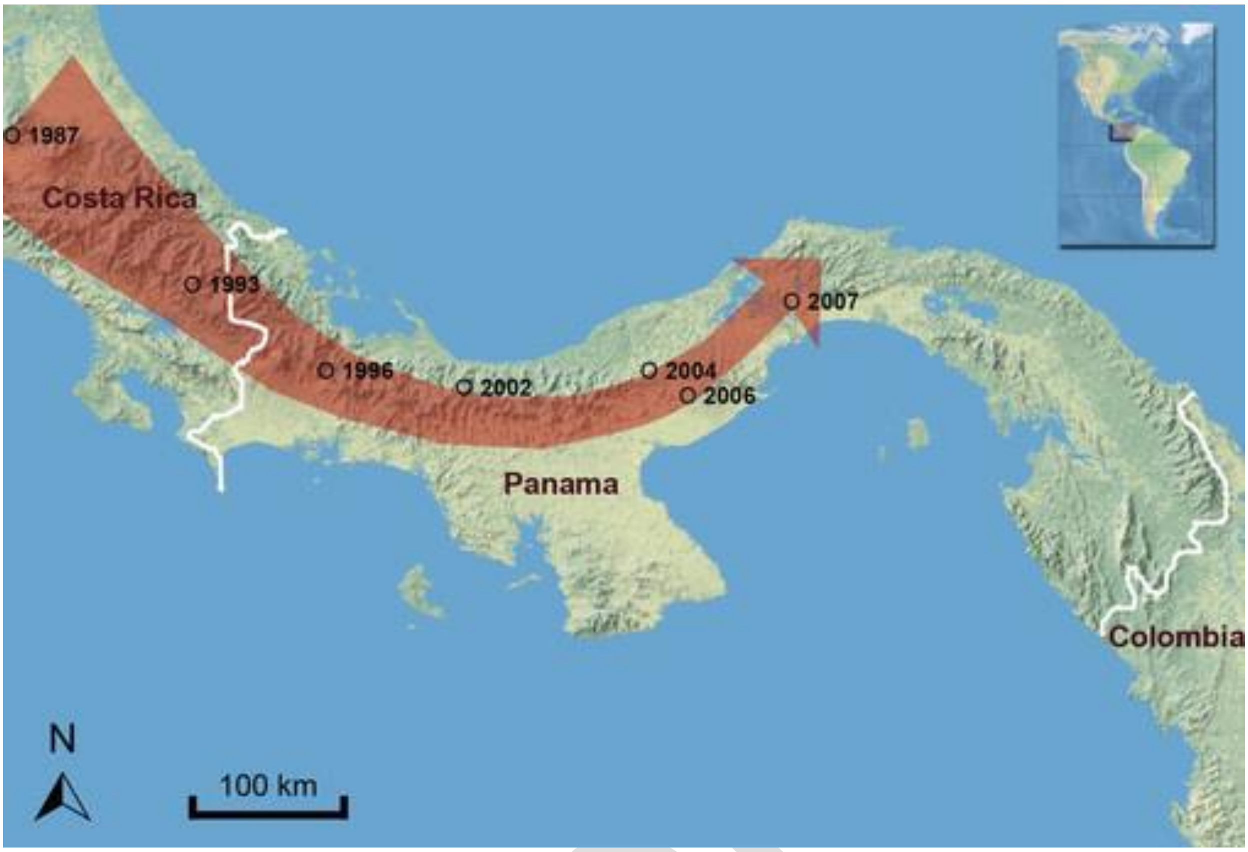


Figure 10.2. Amphibian surveys and monitoring in Central America documented a 20-year southeast progression of population declines that was eventually attributed to the skin disease chytridiomycosis caused by the amphibian chytrid fungus *Batrachochytrium dendrobatidis* (Box 10.3).

Box 10.4. The Survey and Manage Program: Siskiyou Mountains and Scott Bar salamanders

The US Pacific Northwest federal "Survey and Manage Program" was developed to address persistence of species associated with late-successional and old-growth forest conditions that were not protected by federal reserved lands (Molina, Marcot, & Lesher, 2006). One of the five amphibian species included in the programme was the Siskiyou Mountains salamander, (*Plethodon stormi*), a terrestrial woodland salamander with rocky substrate and shade habitat associations (Suzuki, Olson, & Reilly, 2008). Its range was not well delineated upon programme initiation in 1993, when 47 site localities were known for the species across a ~61-ha area. Hence, salamander occurrence surveys were mandated within 25 miles (40 km) of the outer-most known localities before any forest management proposals could be developed on federal lands within the species range. In addition, strategic surveys and independent research projects were conducted to collect additional data on occurrence, habitat associations, and genetic diversity. By 1999, there were 163 sites known for the species and the known range had doubled in size (~137 ha), extending 18 km to the south, 11 km to the east, and 16 km to the west (Nauman & Olson, 1999). To the south, a new morphologically and genetically distinct species was encountered, the Scott Bar salamander (*Plethodon asupak;* Mead, Clayton, Nauman, Olson, & Pfrender, 2005). The combined survey and research efforts for the Siskiyou Mountains salamander resulted in a tri-agency Conservation Agreement in Oregon where high-priority sites for conservation were identified as a pre-emptive effort to avoid its listing as federally Threatened or Endangered, while allowing for continued forest management within the species' range (Olson, Clayton, Nauman, & Welsh Jr, 2009). Additionally, species-management recommendations were developed to reduce fuel loading to reduce risk of wildfire at salamander sites (Clayton, Olson, Nauman, & Reilly, 2009). At this time, a multi-agency Conservation Agreement is in development for the Siskiyou Mountains and Scott Bar salamanders in California. The outcome of the Survey-and Manage Program for this originally little-known species has been significant knowledge discovery (reviewed in Olson et al., 2007) and a series of successful conservation measures with reconciliation of forest management disturbances and proactive measures to address the threat of wildfire.

Acknowledgements

We are grateful for the invaluable survey responses of ASG members and other amphibian experts whose feedback guided the topics and scope of the surveys and monitoring chapter. These contributors include: Antonio W. Salas, Andrés Merino-Viteri, Andrés Rymel Acosta Galvis, Segniagbeto H. Gabriel, Benedikt Schmidt, Ariel Rodríguez, Robert Ward, John Measey, Karthikeyan Vasudevan, Mark C Urban, David Bickford, Karen Lips, Rob Stuebing, Prof. Dr. Thomas Ziegler, Ángela M. Suárez Mayorga, Nicolas Urbina-Cardona, Luis Alberto Rueda Solano, John Wilkinson, Federico Kacoliris, Jean-Marc Hero, Francis Lemckert, Rayna Bell, Tahar Slimani, Ariadne Angulo, Nikki Roach, S.C. Bordoloi, Paul Crump, Víctor Jassmani Vargas García, Bill Peterman, Robert Puschendorf, Dede Olson, Vanessa K. Verdade, Dan Cogalniceanu, Maureen Donnelly, Daniel Rodríguez, Paul Doughty, Natalie Calatayud, PK Malonza, Karthikeyan Vasudevan, Francois Becker, N'Guessan Emmanuel Assemian, Diego Gómez-Hoyos, Aarón Josué Quiroz Rodríguez, Mark-Oliver Rödel, Luis Castillo Roque, Iñigo Martinez-Solano, Jeff Dawson, Valia Esther Herrera Alva, Amir Hamidy, Ninad Amol Gosavi, Amaël Borzée, Joseph Doumbia, Franco Andreone, Javiera Cisternas, Florina Stanescu, Dion Kelly, James Watling, Lily Rodriguez, Michelle Thompson, Cynthia Paszkowski, N`guessan Emmanuel Assemian, Luke Linhoff, Mauricio Akmentins, and Brett Scheffers. Thanks also to Ruth Marcec-Greaves and Sally Wren for their feedback on a draft of this chapter.

References

Aceves-Bueno, E., Adeleye, A. S., Bradley, D., Brandt, W. T., Callery, P., Feraud, M., … Tague, C. (2015). Citizen science as an approach for overcoming insufficient monitoring and inadequate stakeholder buy-in in adaptive management : Criteria and evidence. *Ecosystems*, *18*(3), 493–506. doi: 10.1007/s10021-015-9842-4

Adams, S. L., Morton, M. N., Terry, A., Young, R. P., Dawson, J., Martin, L., … Gray, G. (2014). *Long-Term recovery strategy for the Critically Endangered mountain chicken 2014-2034*. doi: 10.13140/RG.2.1.3836.8487

Angulo, A., Rueda Almonacid, J. V, Rodríguez-Mahecha, J. V, & La Marca, E. (2006). *Tecnicas de inventario y monitoreo para los anfibios de la region tropical andina*. Colombia.

Bach, K., Schäfer, D., Enke, N., Seeger, B., Gemeinholzer, B., & Bendix, J. (2012). A comparative evaluation of technical solutions for long-term data repositories in integrative biodiversity research. *Ecological Informatics*, *11*, 16–24. doi: 10.1016/j.ecoinf.2011.11.008

Balmford, A., & Whitten, T. (2003). Who should pay for tropical conservation, and how could the costs be met? *Oryx*, *37*(2), 238–250. doi: 10.1017/S0030605303000413

Barbe, L., Mony, C., & Abbott, B. W. (2020). Artificial intelligence accidentally learned ecology through video games. *Trends in Ecology & Evolution*, *35*(7), 557–560. doi: 10.1016/j.tree.2020.04.006

Barbier, B. E. B., Burgess, J. C., & Dean, T. J. (2018). How to pay for saving biodiversity. *Science*, *360*(6388), 486–488. doi: 10.1126/science.aar3454

Basham, E. W., & Scheffers, B. R. (2020). Vertical stratification collapses under seasonal shifts in climate. *Journal of Biogeography*, *47*(9), 1888–1898. doi: 10.1111/jbi.13857

Basham, E. W., Seidl, C. M., Andriamahohatra, L. R., Oliveira, B. F., & Scheffers, B. R. (2019). Distance-decay differs among vertical strata in a tropical rainforest. *Journal of Animal Ecology*, *88*(1), 114–124. doi: 10.1111/1365-2656.12902

Baylis, K., Honey-Rosés, J., Börner, J., Corbera, E., Ezzine-de-Blas, D., Ferraro, P. J., … Wunder, S. (2016). Mainstreaming impact evaluation in nature conservation. *Conservation Letters*, *9*(1), 58–64. doi: 10.1111/conl.12180

Beck, J., Böller, M., Erhardt, A., & Schwanghart, W. (2014). Spatial bias in the GBIF database and its effect on modeling species' geographic distributions. *Ecological Informatics*, *19*, 10–15. doi: 10.1016/j.ecoinf.2013.11.002

Becker, M. H., Harris, R. N., Minbiole, K. P. C., Schwantes, C. R., Rollins-Smith, L. A., Reinert, L. K., … Gratwicke, B. (2011). Towards a better understanding of the use of probiotics for preventing chytridiomycosis in Panamanian golden frogs. *EcoHealth*, *8*, 501–506. doi: 10.1007/s10393-012-0743-0

Biju, S. D., Kamei, R., Gower, D. J., & Wilkinson, M. (2009). *Conservation of Caecilians in the Eastern Himalayas Region*.

Bishop, P. J., Angulo, A., Lewis, J. P., Moore, R. D., Rabb, G. B., & Garcia Moreno, J. (2012). The amphibian extinction crisis - what will it take to put the action into the Amphibian Conservation Action Plan? *S.A.P.I.EN.S*, *5*(2), 97–111.

Brito, D. (2010). Overcoming the Linnean shortfall : Data deficiency and biological survey priorities. *Basic and Applied Ecology*, *11*(8), 709–713. doi: 10.1016/j.baae.2010.09.007

Brodie, S., Allen-Ankins, S., Towsey, M., Roe, P., & Schwarzkopf, L. (2020). Automated species identification of frog choruses in environmental recordings using acoustic indices. *Ecological Indicators*, *119*, 106852. doi: 10.1016/j.ecolind.2020.106852

Brooks, J. S., Waylen, K. A., & Borgerhoff Mulder, M. (2012). How national context, project design, and local community characteristics influence success in community-based conservation projects. *Proceedings of the National Academy of Sciences of the United States of America*, *109*(52), 21265–21270. doi: 10.1073/pnas.1207141110

Brown, T. B., Hultine, K. R., Steltzer, H., Denny, E. G., Denslow, M. W., Granados, J., … Richardson, A. D. (2016). Using phenocams to monitor our changing earth: toward a global phenocam network. *Frontiers in Ecology and the Environment*, *14*(2), 84–93. doi: 10.1002/fee.1222

Burivalova, Z., Miteva, D., Salafsky, N., Butler, R. A., & Wilcove, D. S. (2019). Evidence types and trends in tropical forest conservation literature. *Trends in Ecology & Evolution*, *34*(7), 669–679. doi: 10.1016/j.tree.2019.03.002

Buxton, R. T., Avery-Gomm, S., Hsein-Yung, L., Smith, P. A., Cooke, S. J., & Bennett, J. R. (2020). Half of resources in threatened species conservation plans are allocated to research and monitoring. *Nature Communications*, *11*(1), 4668. doi: 10.1038/s41467-020-18486-6

Buxton, R. T., Nyboer, E. A., Pigeon, K. E., Raby, G. D., Rytwinski, T., Gallagher, A. J., … Roche, D. G. (2021). Avoiding wasted research resources in conservation science. *Conservation Science and Practice*, *3*(2), e329. doi: 10.1111/csp2.329

Campbell Grant, E. H., Muths, E., Schmidt, B. R., & Petrovan, S. O. (2019). Amphibian conservation in the Anthropocene. *Biological*

Conservation, *236*(November 2018), 543–547. doi: 10.1016/j.biocon.2019.03.003

Canessa, S., Ottonello, D., Rosa, G., Salvidio, S., Grasselli, E., & Oneto, F. (2019). Adaptive management of species recovery programs: A real-world application for an endangered amphibian. *Biological Conservation*, *236*, 202–210. doi: 10.1016/j.biocon.2019.05.031

Canessa, S., Spitzen-van der Sluijs, A., Martel, A., & Pasmans, F. (2019). Mitigation of amphibian disease requires a stronger connection between research and management. *Biological Conservation*, *236*, 236–242. doi: 10.1016/j.biocon.2019.05.030

Catenazzi, A. (2015). State of the world's amphibians. *Annual Review of Environment and Resources*, *40*, 91–119. doi: 10.1146/annurev-environ-102014-021358

Chavan, V., & Penev, L. (2011). The data paper: a mechanism to incentivize data publishing in biodiversity science. *BMC Bioinformatics*, *12*(Suppl 15), S2. doi: 10.1186/1471-2105-12-S15-S2

Christie, A. P., Amano, T., Martin, P. A., Petrovan, S. O., Shackelford, G. E., Simmons, B. I., … Sutherland, W. J. (2020). Poor availability of context-specific evidence hampers decision-making in conservation. *Biological Conservation*, *248*, 108666. doi: 10.1016/j.biocon.2020.108666

Clayton, D., Olson, D. H., Nauman, R. S., & Reilly, E. C. (2009). Managing for the Siskiyou Mountains salamander (*Plethodon stormi*) in fuel treatment areas around at-risk communities. In Deanna H. Olson, D. Clayton, R. S. Nauman, & H. H. Welsh Jr. (Eds.), *Conservation of the Siskiyou Mountains salamander (Plethodon stormi). Northwest Fauna 6* (pp. 39–42). Society for Northwestern Vertebrate Biology.

Collen, B., Dulvy, N. K., Gaston, K. J., Gärdenfors, U., Keith, D. A., Punt, A. E., … Akçakaya, H. R. (2016). Clarifying misconceptions of extinction risk assessment with the IUCN Red List. *Biology Letters*, *12*, 20150843. doi: 10.1098/rsbl.2015.0843

Collen, B., Loh, J., Whitmee, S., McRae, L., Amin, R., & Baillie, J. E. M. (2009). Monitoring change in vertebrate abundance: the Living Planet Index. *Conservation Biology*, *23*(2), 317–327. doi: 10.1111/j.1523-1739.2008.01117.x

Collen, B., Ram, M., Zamin, T., & Mcrae, L. (2008). The tropical biodiversity data gap: addressing disparity in global monitoring. *Tropical Conservation Science*, *1*(2), 75–88.

Connette, G. M., & Semlitsch, R. D. (2015). A multistate mark-recapture approach to estimating survival of PIT-tagged salamanders following timber harvest. *Journal of Applied Ecology*, *52*(5), 1316–1324. doi: 10.1111/1365-2664.12472

Conservation Measures Partnership. (2020). *Open standards for the practice of conservation. Version 4.0*. Retrieved from https://conservationstandards.org/download-cs/

Costello, M. J., Michener, W. K., Gahegan, M., Zhang, Z., & Bourne, P. E. (2013). Biodiversity data should be published, cited, and peer reviewed. *Trends in Ecology & Evolution*, *28*(8), 454–461. doi: 10.1016/j.tree.2013.05.002

Costello, M. J., & Wieczorek, J. (2014). Best practice for biodiversity data management and publication. *Biological Conservation*, *173*, 68–73. doi: 10.1016/j.biocon.2013.10.018

Crump, M. L. (2015). Anuran reproductive modes: Evolving perspectives. *Journal of Herpetology*, *49*(1), 1–16. doi: 10.1670/14-097

Cunningham, A. A., Lawson, B., Burton,

M., & Thomas, R. (2008). *Addressing a threat to Caribbean amphibians: capacity building in Dominica. Darwin Initiative Final Report.*

da Silva, A. F., Malhado, A. C. M., Correia, R. A., Ladle, R. J., Vital, M. V. C., & Mott, T. (2020). Taxonomic bias in amphibian research: Are researchers responding to conservation need? *Journal for Nature Conservation*, *56*, 125829. doi: 10.1016/j.jnc.2020.125829

Deichmann, J. L., Acevedo-Charry, O., Barclay, L., Burivalova, Z., Campos-Cerqueira, M., D’Horta, F., … Aide, T. (2018). It’s time to listen: there is much to be learned from the sounds of tropical ecosystems. *Biotropica*, *50*(5), 713–718. doi: 10.1111/btp.12593

Deichmann, J. L., Mulcahy, D. G., Vanthomme, H., Tobi, E., Wynn, A. H., Zimkus, B. M., & McDiarmid, R. W. (2017). How many species and under what names? Using DNA barcoding and GenBank data for west Central African amphibian conservation. *PLoS ONE*, *12*(11), e0187283. doi: 10.1371/journal.pone.0187283 November

Deichmann, J. L., Sahley, C., Vargas, V., Chipana, O., Velasquez, W., Smith, E., … Catenazzi, A. (2013). Monitoring an endemic amphibian along a natural gas pipeline in the Peruvian Andes. *FrogLog*, *21*, 65–68.

DiRenzo, G. V, Che-Castaldo, C., Saunders, S. P., Campbell Grant, E. H., & Zipkin, E. F. (2019). Disease-structured N-mixture models: A practical guide to model disease dynamics using count data. *Ecology and Evolution*, *9*, 899–909. doi: 10.1002/ece3.4849

Dodd, C. K. (2010). Amphibian ecology and conservation. *Oxford University Press Inc., New York*, 556. doi: 10.1073/pnas.0703993104

Dorazio, R. M. (2014). Accounting for

imperfect detection and survey bias in statistical analysis of presence-only data. *Global Ecology and Biogeography*, *23*(12), 1472–1484. doi: 10.1111/geb.12216

Dornelas, M., Antão, L. H., Moyes, F., Bates, A. E., Magurran, A. E., Adam, D., … Zettler, M. L. (2018). BioTIME: A database of biodiversity time series for the Anthropocene. *Global Ecology and Biogeography*, *27*(7), 760–786. doi: 10.1111/geb.12729

Enge, K. M. (2001). The pitfalls of pitfall traps. *Journal of Herpetology*, *35*(3), 467–478.

Forin-Wiart, M. A., Hubert, P., Sirguey, P., & Poulle, M.-L. (2015). Performance and accuracy of lightweight and low-cost GPS data loggers according to antenna positions, fix intervals, habitats and animal movements. *PLoS ONE*, *10*(6), e0129271. doi: 10.1371/journal.pone.0129271

Frost, D. R. (2021). Amphibian species of the world: an online reference. Version 6.1. doi: doi.org/10.5531/db.vz.0001

Funk, W. C., Caminer, M., & Ron, S. R. (2012). High levels of cryptic species diversity uncovered in Amazonian frogs. *Proceedings of the Royal Society B*, *279*, 1806–1814. doi: 10.1098/rspb.2011.1653

Gagliardo, R., Crump, P., Griffith, E., Mendelson, J., Ross, H., & Zippel, K. (2008). The principles of rapid response for amphibian conservation, using the programmes in Panama as an example. *International Zoo Yearbook*, *42*, 125–135. doi: 10.1111/j.1748-1090.2008.00043.x

Garcia Fontes, S., Stanzani, S. L., & Pizzigatti Correa, P. L. (2015). A data mining framework for primary biodiversity data analysis. In A. Rocha, A. M. Correia, S. Costanzo, & L. P. Reis (Eds.), *New contributions in information systems and* *technologies* (pp. 813–821). Springer International Publishers. doi: 10.1007/978-3-319-16486-1

Giam, X., Scheffers, B. R., Sodhi, N. S., Wilcove, D. S., Ceballos, G., & Ehrlich, P. R. (2012). Reservoirs of richness : least disturbed tropical forests are centres of undescribed species diversity. *Proceedings of the Royal Society B*, *279*, 67–76. doi: 10.1098/rspb.2011.0433

Gillson, L., Biggs, H., Smit, I. P. J., Virah-Sawmy, M., & Rogers, K. (2019). Finding common ground between adaptive management and evidence-based approaches to biodiversity conservation. *Trends in Ecology & Evolution*, *34*(1), 31–44. doi: 10.1016/j.tree.2018.10.003

González-del-Pliego, P., Freckleton, R. P., Edwards, D. P., Koo, M. S., Scheffers, B. R., Pyron, R. A., & Jetz, W. (2019). Phylogenetic and trait-based prediction of extinction risk for data-deficient amphibians. *Current Biology*, *29*(9), 1557-1563.e3. doi: 10.1016/j.cub.2019.04.005

González-Maya, J. F., Belant, J. L., Wyatt, S. A., Schipper, J., Cardenal, J., Corrales, D., … Fischer, A. (2013). Renewing hope: The rediscovery of *Atelopus varius* in Costa Rica. *Amphibia Reptilia*, *34*(4), 573–578. doi: 10.1163/15685381-00002910

Gower, D. J., & Wilkinson, M. (2005). Conservation biology of caecilian amphibians. *Conservation Biology, 19*(1), 45–55.

Guerra, V., Jardim, L., Llusia, D., Márquez, R., & Bastos, R. P. (2020). Knowledge status and trends in description of amphibian species in Brazil. *Ecological Indicators, 118,* 106754. doi: 10.1016/j.ecolind.2020.106754

Guimarães, M., Corrêa, D. T., Filho, S. S., Oliveira, T. A. L., Doherty Jr, P. F., & Sawaya, R. J. (2014). One step forward: contrasting the effects of Toe clipping and PIT tagging on frog survival and recapture probability. *Ecology and Evolution*, *4*(8), 1480–1490. doi: 10.1002/ece3.1047

Haddad, C. F. B., & Prado, C. P. A. (2005). Reproductive modes in frogs and their unexpected diversity in the Atlantic Forest of Brazil. *BioScience*, *55*(3), 207–217.

Hampton, S. E., Strasser, C. A., Tewksbury, J. J., Gram, W. K., Budden, A. E., Batcheller, A. L., … Porter, J. H. (2013). Big data and the future of ecology. *Frontiers in Ecology and the Environment*, *11*(3), 156–162. doi: 10.1890/120103

Heyer, W. R., Donnelly, M. A., McDiarmid, R. W., Hayek, L. C., & Foster, M. S. (1994). *Measuring and monitoring biological diversity: Standard methods for amphibians*. Smithsonian Institution Press: London; Washington.

Hill, A. P., Prince, P., Piña Covarrubias, E., Doncaster, C. P., Snaddon, J. L., & Rogers, A. (2018). AudioMoth: Evaluation of a smart open acoustic device for monitoring biodiversity and the environment. *Methods in Ecology and Evolution*, *9*(5), 1199–1211. doi: 10.1111/2041-210X.12955

Hobbs, M. T., & Brehme, C. S. (2017). An improved camera trap for amphibians, reptiles, small mammals, and large invertebrates. *PLoS ONE*, *12*(10), e0185026.

Hobbs, R. J., Higgs, E., & Harris, J. A. (2009). Novel ecosystems: implications for conservation and restoration. *Trends in Ecology and Evolution*, *24*(11), 599–605. doi: 10.1016/j.tree.2009.05.012

Hof, C., Araújo, M. B., Jetz, W., & Rahbek, C. (2011). Additive threats from pathogens, climate and land-use change for global amphibian diversity. *Nature*, *480*(7378), 516–521. doi: 10.1038/nature10650

Hoffmann, M., Hilton-Taylor, C., Angulo, A., Böhm, M., Brooks, T. M., Butchart, S. H. M., … Stuart, S. N.

(2010). The impact of conservation on the status of the world's vertebrates. *Science*, *330*(6010), 1503–1509. doi: 10.1126/science.1194442

Howell, P. E., R, H. B., Muths, E., Sigafus, B. H., & CHandler, R. B. (2020). Informing amphibian conservation efforts with abundance-based metapopulation models. *Herpetologica*, *76*(2), 240–250. doi: 10.1655/0018-0831-76.2.240

Huang, X., Hawkins, B. A., & Qiao, G. (2013). Biodiversity data sharing: Will peer-reviewed data papers work? *BioScience*, *63*(1), 5–6.

Hudson, M. A., Young, R. P., D'Urban Jackson, J., Orozco-Terwengel, P., Martin, L., James, A., … Cunningham, A. A. (2016). Dynamics and genetics of a disease-driven species decline to near extinction: Lessons for conservation. *Scientific Reports*, *6*, 1–13. doi: 10.1038/srep30772

Hudson, M. A., Young, R. P., Lopez, J., Martin, L., Fenton, C., McCrea, R., … Cunningham, A. A. (2016). In-situ itraconazole treatment improves survival rate during an amphibian chytridiomycosis epidemic. *Biological Conservation*, *195*(February 2019), 37–45. doi: 10.1016/j.biocon.2015.12.041

IUCN. (2021). The IUCN Red List of Threatened Species. Retrieved March 30, 2021, from Version 2021-2 website: https://www.iucnredlist.org

Jetz, W., & Pyron, R. A. (2018). The interplay of past diversification and evolutionary isolation with present imperilment across the amphibian tree of life. *Nature Ecology & Evolution*, *2*, 850–858. doi: 10.1038/s41559-018-0515-5

Koh, L. P., & Wich, S. A. (2012). Dawn of drone ecology: low-cost autonomous aerial vehicles for conservation. *Tropical Conservation Science*, *5*(2), 121–132.

La Salle, J., Williams, K. J., & Moritz, C. (2016). Biodiversity analysis in the digital era. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *371*, 20150337. doi: 10.1098/rstb.2015.0337

Lennox, R. J., Aarestrup, K., Cooke, S. J., Cowley, P. D., Deng, Z. D., Fisk, A. T., … Young, N. (2017). Envisioning the Future of Aquatic Animal Tracking: Technology, Science, and Application. *BioScience*, *67*(10), 884–896. doi: 10.1093/biosci/bix098

Lindenmayer, D. B., Piggott, M. P., & Wintle, B. A. (2013). Counting the books while the library burns: why conservation monitoring programs need a plan for action. *Frontiers in Ecology and the Environment*, *11*(10), 549–555. doi: 10.1890/120220

Lips, K. R. (1999). Mass mortality and population declines of anurans at an upland site in Western Panama. *Conservation Biology*, *13*(1), 117–125. doi: 10.1046/j.1523-1739.1999.97185.x

Lips, K. R., Reeve, J. D., & Witters, L. R. (2003). Ecological traits predicting amphibian population declines in Central America. *Conservation Biology*, *17*(4), 1078–1088. doi: 10.1046/j.1523-1739.2003.01623.x

Mace, G. M., Collar, N. J., Gaston, K. J., Hilton-Taylor, C., Akçakaya, H. R., Leader-Williams, N., … Stuart, S. N. (2008). Quantification of extinction risk: IUCN's system for classifying Threatened species. *Conservation Biology*, *22*(6), 1424–1442. doi: 10.1111/j.1523-1739.2008.01044.x

MacKenzie, D. I., & Royle, J. A. (2005). Designing occupancy studies : general advice and allocating survey effort. *Journal of Applied Ecology*, *42*, 1105–1114. doi: 10.1111/j.1365-2664.2005.01098.x

Marquet, P. A., Lessmann, J., & Shaw, M. R. (2019). Protected-area management and climate change. In

T. E. Lovejoy & L. Hannah (Eds.), *Biodiversity and climate change: Transforming the biosphere* (p. 283). Yale University Press. doi: 10.12987/9780300241198-033

Martin, L., Morton, M. N., Hilton, G. M., Young, R. P., Garcia, G., Cunningham, A. A., … Mendes, S. (2007). *A Species Action Plan for the Montserrat mountain chicken Leptodactylus fallax*.

Martin, T. G., Nally, S., Burbidge, A. A., Arnall, S., Garnett, S. T., Hayward, M. W., … Possingham, H. P. (2012). Acting fast helps avoid extinction. *Conservation Letters*, *5*, 274–280. doi: 10.1111/j.1755-263X.2012.00239.x

Marvin, D. C., Pin, L., Lynam, A. J., Wich, S., Davies, A. B., Krishnamurthy, R., … Asner, G. P. (2016). Integrating technologies for scalable ecology and conservation. *Global Ecology and Conservation*, *7*, 262–275. doi: 10.1016/j.gecco.2016.07.002

McDonald-Madden, E., Baxter, P. W. J., Fuller, R. A., Martin, T. G., Game, E. T., Montambault, J., & Possingham, H. P. (2010). Monitoring does not always count. *Trends in Ecology and Evolution*, *25*(10), 547–550.

McLeod, D. S. (2010). Of Least Concern? Systematics of a cryptic species complex: *Limnonectes kuhlii* (Amphibia: Anura: Dicroglossidae). *Molecular Phylogenetics and Evolution*, *56*(3), 991–1000. doi: 10.1016/j.ympev.2010.04.004

Mead, L. S., Clayton, D. R., Nauman, R. S., Olson, D. H., & Pfrender, M. E. (2005). Newly discovered populations of salamanders from Siskiyou County California represent a species distinct from Plethodon stormi. *Herpetologica*, *61*(2), 158–177. doi: 10.1655/03-86

Mendenhall, C. D., Frishkoff, L. O., Santos-Barrera, G., Pacheco, J., Mesfun, E., Mendoza Quijano, F., …

Pringle, R. M. (2014). Countryside biogeography of Neotropical reptiles and amphibians. *Ecology*, *95*(4), 856–870.

Menegon, M., Cantaloni, C., Rodriguez-Prieto, A., Centomo, C., Abdelfattah, A., Rossato, M., … Delledonne, M. (2017). *On site DNA barcoding by nanopore sequencing*. *2*, 1–18.

Molina, R., Marcot, B. G., & Lesher, R. (2006). Protecting rare, old-growth, forest-associated species under the survey and manage program guidelines of the Northwest Forest Plan. *Conservation Biology*, *20*(2), 306–318. doi: 10.1111/j.1523-1739.2006.00386.x

Murray, A. H., Nowakowski, A. J., & Frichkoff, L. O. (2021). Climate and land-use change severity alter trait-based responses to habitat conversion. *Global Ecology and Biogeography*, *30*, 598–610. doi: 10.1111/geb.13237

Nauman, R. S., & Olson, D. H. (1999). Survey and Manage salamander known sites. In Deanna H. Olson (Ed.), *Survey protocols for amphibians under the Survey and Manage provision of the Northwest Forest Plan. Version 3.0.* (pp. 43–78). Interagency publication of the Regional Ecosystem Office, Portland, OR. Bureau of Land Management Publication BLM/OR/WA/PT-00/033+1792; U.S. G.

Navarro, L. M., Fernández, N., Guerra, C., Guralnick, R., Kissling, W. D., London, M. C., … Pereira, H. M. (2017). Monitoring biodiversity change through effective global coordination. *Current Opinion in Environmental Sustainability*, *29*, 158–169. doi: 10.1016/j.cosust.2018.02.005

Nowakowski, A. J., & Angulo, A. (2015). Targeted habitat protection and its effects on the extinction risk of threatened amphibians. *FrogLog*, *23 (4)*(116), 8–10.

Nowakowski, A. J., & Maerz, J. C. (2009).

Estimation of larval stream salamander densities in three proximate streams in the Georgia Piedmont. *Journal of Herpetology, 43*(3), 503–509.

Nowakowski, A. J., Watling, J. I., Thompson, M. E., Brusch IV, G. A., Catenazzi, A., Whitfield, S. M., … Todd, B. D. (2018). Thermal biology mediates responses of amphibians and reptiles to habitat modification. *Ecology Letters, 21*(3), 345–355. doi: 10.1111/ele.12901

O’Donnell, R. P., & Durso, A. M. (2014). Harnessing the power of a global network of citizen herpetologists by improving citizen science databases. *Herpetological Review*, *45*(1), 151–157.

Olson, D. H., Clayton, D., Nauman, R. S., & Welsh Jr, H. (2009). *Conservation of the Siskiyou Mountains Salamander (Plethodon stormi). Northwest Fauna 6*. The Society for Northwestern Vertebrate Biology.

Olson, D. H., & Pilliod, D. S. (2021). Philosophy, authority, and strategic pragmatism of amphibian and reptile conservation. In S. C. Walls & K. M. O’Donnell (Eds.), *Strategies for conservation success in herpetology*. Society for the Study of Amphibians and Reptiles.

Olson, D. H., Van Norman, K. J., & Huff, R. D. (2007). *The utility of strategic surveys for rare and little-known species under the Northwest forest plan. General technical report PNW-GTR-708*. Portland.

Pimm, S. L., Alibhai, S., Bergl, R., Dehgan, A., Giri, C., Jewell, Z., … Loarie, S. (2015). Emerging technologies to conserve biodiversity. *Trends in Ecology & Evolution, 30*(11), 685–696. doi: 10.1016/j.tree.2015.08.008

Pollock, K. H. (1982). A capture-recapture design robust to unequal probability of capture. *The Journal of Wildlife Management, 46*(3), 752–757.

Proença, V., Martin, L. J., Pereira, H. M., Fernandez, M., McRae, L., Belnap, J., … van Swaay, C. A. M. (2017). Global biodiversity monitoring: From data sources to Essential Biodiversity Variables. *Biological Conservation*, *213*, 256–263. doi: 10.1016/j.biocon.2016.07.014

Pyron, R. A., & Wiens, J. J. (2011). A large-scale phylogeny of Amphibia including over 2800 species, and a revised classification of extant frogs, salamanders, and caecilians. *Molecular Phylogenetics and Evolution*, *61*, 543–583. doi: 10.1016/j.ympev.2011.06.012

Redford, K. H., Hulvey, K. B., Williamson, M. A., & Schwartz, M. W. (2018). Assessment of the Conservation Measures Partnership's effort to improve conservation outcomes through adaptive management. *Conservation Biology*, *32*(4), 926–937. doi: 10.1111/cobi.13077

Ribeiro-Júnior, M. A., Gardner, T. A., & Ávila-Pires, T. C. S. (2008). Evaluating the efectiveness of herpetofaunal sampling techniques across a gradient of habitat change in a tropical forest landscape. *Journal of Herpetology*, *42*(4), 733–749.

Rice, W. S., Sowman, M. R., & Bavinck, M. (2020). Using Theory of Change to improve post-2020 conservation: A proposed framework and recommendations for use. *Conservation Science and Practice*, e301. doi: 10.1111/csp2.301

Rodewald, A. D., Arcese, P., Sarra, J., Tobin-de la Puente, J., Sayer, J., Hawkins, F., … Wachowicz, K. (2020). *Innovative finance for conservation: Roles for ecologists and practitioners. Issues in Ecology Report No. 22*.

Rodrigues, A. S. L., Pilgrim, J. D., Lamoreux, J. F., Hoffmann, M., & Brooks, T. M. (2006). The value of the IUCN Red List for conservation.

Trends in Ecology and Evolution, *21*(2), 71–76. doi: 10.1016/j.tree.2005.10.010

Salafsky, N., Salzer, D., Stattersfield, A. J., Hilton-Taylor, C., Neugarten, R., Butchart, S. H. M., … Wilkie, D. (2008). A standard lexicon for biodiversity conservation: Unified classifications of threats and actions. *Conservation Biology*, *22*(4), 897–911. doi: 10.1111/j.1523-1739.2008.00937.x

Schleicher, J., Eklund, J., Barnes, M., Geldmann, J., Oldekop, J. A., & Jones, J. P. G. (2020). Statistical matching for conservation science. *Conservation Biology*, *34*(3), 538–549. doi: 10.1111/cobi.13448

Schmeller, D. S., Arvanitidis, C., Böhm, M., Brummitt, N., Chatzinikolaou, E., Costello, M. J., … Belnap, J. (2017). Case studies of capacity building for biodiversity monitoring. In M. Walters & R. J. Scholes (Eds.), *The GEO handbook on biodiversity observation networks* (pp. 309–326). Springer Nature. doi: 10.1007/978-3-319-27288-7

Schulze, A., Jansen, M., & Köhler, G. (2015). Tadpole diversity of Bolivia's lowland anuran communities: molecular identification, morphological characterisation, and ecological assignment. *Zootaxa*, *4016*(1).

Schwartz, M. W., Cook, C. N., Pressey, R. L., Pullin, A. S., Runge, M. C., Salafsky, N., … Williamson, M. A. (2018). Decision support frameworks and tools for conservation. *Conservation Letters*, *11*(2), e12385. doi: 10.1111/conl.12385

Smith, R. K., Meredith, H., & Sutherland, W. J. (2018). Amphibian Conservation. In W. J. Sutherland, L. V. Dicks, N. Ockendon, S. O. Petrovan, & R. K. Smith (Eds.), *What Works in Conservation 2018* (pp. 9–66). Cambridge, UK: Open Book Publishers.

Snaddon, J., Petrokofsky, G., Jepson, P., & Willis, K. J. (2013). Biodiversity technologies: tools as change agents. *Biology Letters*, *9*, 20121029.

Stephenson, P. J., Bowles-Newark, N., Regan, E., Stanwell-Smith, D., Diagana, M., Höft, R., … Thiombiano, A. (2017). Unblocking the flow of biodiversity data for decision-making in Africa. *Biological Conservation*, *213*, 355–340. doi: 10.1016/j.biocon.2016.09.003

Sterrett, S. C., Katz, R. A., Brand, A. B., Fields, W. R., Dietrich, A. E., Hocking, D. J., … Campbell Grant, E. H. (2019). Proactive management of amphibians: Challenges and opportunities. *Biological Conservation*, *236*, 404–410. doi: 10.1016/j.biocon.2019.05.057

Stuart, S. N., Chanson, J. S., Cox, N. A., Young, B. E., Rodrigues, A. S. L., Fischman, D. L., & Waller, R. W. (2004). Status and trends of amphibian declines and extinctions worldwide. *Science*, *306*(5702), 1783–1786. doi: 10.1126/science.1103538

Sutherland, W. J., Pullin, A. S., Dolman, P. M., & Knight, T. M. (2004). The need for evidence-based conservation. *Trends in Ecology and Evolution*, *19*(6), 305–308. doi: 10.1016/j.tree.2004.03.018

Suzuki, N., Olson, D. H., & Reilly, E. C. (2008). Developing landscape habitat models for rare amphibians with small geographic ranges: A case study of Siskiyou Mountains salamanders in the western USA. *Biodiversity and Conservation*, *17*(9), 2197–2218. doi: 10.1007/s10531-007-9281-4

Tapley, B., Michaels, C. J., Gumbs, R., Böhm, M., Luedtke, J., Pearce-Kelly, P., & Rowley, J. J. L. (2018). The disparity between species description and conservation assessment: A case study in taxa with high rates of species discovery. *Biological*

Conservation, *220*, 209–214. doi: 10.1016/j.biocon.2018.01.022

Thomas, A. C., Howard, J., Nguyen, P. L., Seimon, T. A., & Goldberg, C. S. (2018). eDNA Sampler: A fully integrated environmental DNA sampling system. *Methods in Ecology and Evolution*, *9*(6), 1379–1385. doi: 10.1111/2041-210X.12994

Toivonen, T., Heikinheimo, V., Fink, C., Hausmann, A., Hiippala, T., Järv, O., … Di Minin, E. (2019). Social media data for conservation science : A methodological overview. *Biological Conservation*, *233*, 298–315. doi: 10.1016/j.biocon.2019.01.023

Urban, M. C., Bocedi, G., Hendry, A. P., Mihoub, J.-B., Pe’er, G., Singer, A., … Travis, J. M. J. (2016). Improving the forecast for biodiversity under climate change. *Science*, *353*(6304), 1113. doi: 10.1126/science.aad8466

Venter, O., Fuller, R. A., Segan, D. B., Carwardine, J., Brooks, T., Butchart, S. H. M., … Watson, J. E. M. (2014). Targeting global protected area expansion for imperiled biodiversity. *PLoS Biology*, *12*(6), e1001891. doi: 10.1371/journal.pbio.1001891

Vieites, D. R., Wollenberg, K. C., & Andreone, F. (2009). Vast underestimation of Madagascar’s biodiversity evidenced by an integrative amphibian inventory. *Proceedings of the National Academy of Sciences*, *106*(20), 8267–8272.

Walls, S. C. (2014). Identifying monitoring gaps for amphibian populations in a North American biodiversity hotspot, the southeastern USA. *Biodiversity and Conservation*, *23*, 3341–3357. doi: 10.1007/s10531-014-0782-7

Wilson, K. A., Auerbach, N. A., Sam, K., Magini, A. G., Moss, A. S. L., Langhans, S. D., … Meijaard, E. (2016). Conservation research is not happening where it is most needed. *PLoS ONE*, *14*(3), e1002413. doi: 10.1371/journal.pbio.1002413

Wright, A. D., Bernard, R. F., Mosher, B. A., O'Donnell, K. M., Braunagel, T., DiRenzo, G. V., … Campbell Grant, E. H. (2020). Moving from decision to action in conservation science. *Biological Conservation*, *249*, 108698. doi: 10.1016/j.biocon.2020.108698

Xie, J., Towsey, M., Zhang, J., & Roe, P. (2016). Acoustic classification of Australian frogs based on enhanced features and machine learning algorithms. *Applied Acoustics*, *113*, 193–201. doi: 10.1016/j.apacoust.2016.06.029

Zipkin, E. F., Thorson, J. T., See, K., Lynch, H. J., Grant, E. H. C., Kanno, Y., … Royle, J. A. (2014). Modeling structured population dynamics using data from unmarked individuals. *Ecology*, *95*(1), 22–29.

Zippel, K. (2002). Conserving the Panamanian Golden Frog: Proyecto Rana Dorada. *Herpetological Review*, Vol. 33, pp. 11–12.

Supplemental information

Table S1. Example amphibian survey and monitoring programs. Dept. = Department; Univ. = University; NGOs = non-governmental organizations [*Note: suggested examples are welcome, particularly beyond the USA*]

Program	Partners	Objectives	Methods	Geography/ Time	References
Agile Frog	NGOs, Jersey zoo, Jersey government	Prevent the extinction of the Agile Frog in Jersey	Pond survey of adult frogs; tadpole release and monitoring	Jersey/late 1980-present	-
Amphibian Research and Monitoring Initiative ARMI)	US Dept. Interior, US Geological Survey; other US Depts., academia, States	Monitor amphibians on public lands and determine factors affecting their status	Diverse survey and research methods used	US-wide with a focus on US federal and state lands/2000 to present	ARMI 2020 (see publication); Adams et al. 2013; Grant et al. 2016)
Atelopus Survival Initiative	National and international individuals, groups and institutions	Improve the conservation status of harlequin toads		Range-wide plan for the next 20 years (2021-2041)	https://www.atelopus.org/the-initiative
British Columbia, Canada amphibian conservation and restoration database	British Columbia Ministry of the Environment, Canada	Track amphibian conservation and restoration actions inclusive of inventory and monitoring programs	Any	Any	Leigh Anne Isaac, pers. Commun., BD Ministry of Environment, herpetofaunal expert

Corroboree Frog Recovery Program	AUS government, Zoos, NGOs	Secure the survival of the Northern and Southern Corroboree Frog in AUS, annually monitor wild populations	Survey number of calling males	Alpines of New South Wales and the Australian Capital Territory/2003 to present	https://www.corroboreefrog.org.au/
FrogID	Australian Museum	Understand the true species diversity, distribution and breeding habitats of Australian frogs	Anuran call surveys; citizen science	Australia/2017-2021	https://www.frogid.net.au/
Frogwatch USA	AZA		Citizen science; frog calls	US wide/1998-2014	www.aza.org/frogwatch
Golden Mantella	Malagasy NGOs, zoos	Address fundamental questions around species dispersion, migration and colonization	Capture-mark-recapture	Mangabe-Ranomena-Sahasarotra New Protected Area, Madagascar/2012-present	-
Greater Yellowstone Inventory and Monitory Network's Amphibian Monitoring Program	US Dept. Interior, US National Park Service, US Geological Survey; academia	Annually monitor native amphibian species and their habitats across 300 wetland sites in 30 watershed units.	Visual observations, Dip net surveys	Yellowstone National Park, Grant Teton National Park; Wyoming, USA/2005 to present	Ray et al. 2016, 2020; Hossack et al. 2015; Gould et al. 2019

Idaho Amphibian and Reptile iNaturalist Project	Idaho State Univ. Herpetology Laboratory; iNaturalist; citizen scientists	Improve species occurrence and distribution data in Idaho by collecting observations using iNaturalist, a mobile application	Crowdsourcing (iNaturalist) observations and purposive surveys	Idaho, USA/2016 to present	Peterson 2020
Long Term Ecological Research Program (LTER or PELD)	Brazilian government, National Institute for Research in the Amazon	Establish permanent research sites integrated in a network for the development and monitoring of long-term ecological research	Temporal dynamics of amphibians; visual and acoustic surveys	PELD Amazon/early 1990-present	https://ppbio.inpa.gov.br/
Mountain Chicken Recovery Programme	NGOs, zoo, academia, governments	To have healthy mountain chicken populations across their former year-2000 ranges on each of Montserrat and Dominica by 2034	Visual population surveys; screening the animals for disease	Montserrat and Dominica/2014-present	https://www.mountainchicken.org/

National Amphibian Survey	NGOs, UK government, academia	Determine trends in the occurrence and relative abundance of frogs, toads and newts in the UK	Trapping; capture-mark-recapture; citizen science	UK wide/2007-present	https://amphibian-survey.arc-trust.org/
North American Amphibian Monitoring Program (NAAMP)	US Dept. Interior, Geological Survey, Citizen science, academia, States, NGOs	Monitor calling amphibian populations	Anuran call surveys from roads	Eastern and central USA/1997 to 2015	NAAMP 2020 (see publications); Cosentino et al. 2014; Villena Carpio et al. 2016
Ranita de Darwin	NGOs, zoo, academia, governments	Long-term monitoring of Southern Darwin's frog (Rhinoderma darwinii) populations	Visual surveys; capture-recapture	4 sites across South Chile (Contulmo, Neltume, Chiloé, Melimoyu)/2014-present	https://www.ranitadedarwin.org/
Sierra Nevada Amphibian Monitoring Program	US Dept. Agriculture, US Forest Service	Long-term multi-scale monitoring of amphibians on national forest lands in the Sierra Nevada	Randomised, unequal probability, rotating panel design; Visual observations; Capture-mark-recapture; Egg mass surveys	Sierra Nevada Range, California: >2200 sites, 124 basins/2002 to 2009	Brown et al. 2012, 2013, 2014
US Dept. Defense Partners in Amphibian and Reptile	US Dept. Defense (Army, Air Force, Navy	Species inventory of 415 DoD properties (sites)	Literature, Database searches; Observations using variable methods	US-wide; 2013 to 2016	Petersen et al. 2018

Conservation (DoD PARC)	and Marine Corps)					

Table S1 References

Adams, M. J., Miller, D. A. W., Muths, E., Corn, P. S., Campbell Grant, E. H., Bailey, L. L., … Walls, S. C. (2013). Trends in amphibian occupancy in the United States. *PLoS ONE*, *8*(5), e64347. doi: 10.1371/journal.pone.0064347

ARMI. (2020). Amphibian Research and Monitoring Initiative. Retrieved from US Geological Survey website: https://armi.usgs.gov/

Brown, C., Kiehl, K., & Wilkinson, L. (2012). Advantages of long-term, multi-scale monitoring: assessing the current status of the Yosemite toad (*Anaxyrus (=Bufo) canorus*) in the Sierra Nevada, California, USA. *Herpetological Conservation and Biology*, *7*(2), 115–131.

Brown, C., & Olsen, A. R. (2013). Bioregional monitoring design and occupancy estimation for two Sierra Nevadan amphibian taxa. *Freshwater Science*, *32*(3), 675–691. doi: 10.1899/11-168.1

Brown, C., Wilkinson, L. R., & Kiehl, K. B. (2014). Comparing the status of two sympatric amphibians in the Sierra Nevada, California: Insights on ecological risk and monitoring common species. *Journal of Herpetology*, *48*(1), 74–83. doi: 10.1670/12-103

Campbell Grant, E. H., Miller, D. A. W., Schmidt, B. R., Adams, M. J., Amburgey, S. M., Chambert, T., … Muths, E. (2016). Quantitative evidence for the effects of multiple drivers on continental-scale amphibian declines. *Scientific Reports*, *6*, 25625. doi: 10.1038/srep25625

Chandler, R. B., Muths, E., Sigafus, B. H., Schwalbe, C. R., Jarchow, C. J., & Hossack, B. R. (2015). Spatial occupancy models for predicting metapopulation dynamics and viability following reintroduction. *Journal of Applied Ecology*, *52*(5), 1325–1333. doi: 10.1111/1365-2664.12481

Cosentino, B. J., Marsh, D. M., Jones, K. S., Apodaca, J. J., Bates, C., Beach, J., … Willey, A. (2014). Citizen science reveals widespread negative effects of roads on amphibian distributions. *Biological Conservation*, *180*, 31–38. doi: 10.1016/j.biocon.2014.09.027

Gould, W. R., Ray, A. M., Bailey, L. L., Thoma, D. P., Daley, R., & Legg, K. (2019). Multistate occupancy modeling improves understanding of amphibian breeding dynamics in the Greater Yellowstone Area. *Ecological Applications*, *29*(1), e01825. doi: 10.1002/eap.1825

NAAMP. (2020). North American Amphibian Monitoring Program. Retrieved November 18, 2020, from US Geological Survey website: https://www.usgs.gov/centers/eesc/science/north-american-amphibian-monitoring-program

Petersen, C. E., Lovich, R. E., & Stallings, S. (2018). Amphibians and reptiles of United States Department of Defense installations. *Herpetological Conservation and Biology*, *13*(3), 652–661.

Peterson, C. (2020). Using crowdsourced data for conservation: The Idaho Amphibian and Reptile iNaturalist Project. *Abstracts of the 9th World Congress of Herpetology, Dunedin, New Zealand*. Retrieved from https://web.archive.org/web/20200107102716/http://wchnz.com/wch2020/programme/programme

Ray, A. M., Gould, W. R., Hossack, B. R., Sepulveda, A. J., Thoma, D. P., Patla, D. A., … Al-Chokhachy, R. (2016). Influence of climate drivers on colonization and extinction dynamics of wetland- dependent species. *Ecosphere*, *7*(7), e01409. doi: /10.1002/ecs2.1409

Ray, A. M., Patla, D. A., & Peterson, C. (2019). Taking the pulse of wetlands: What are we learning from the amphibian vital sign? *Yellowstone Science*, *27*(1), 52–54. Retrieved from https://www.nps.gov/articles/short-taking-the-pulse-of-wetlands.htm

Villena, O. C., Royle, J. A., Weir, L. A., Foreman, T. M., Gazenski, K. D., & Campbell Grant, E. H. (2016). Southeast regional and state trends in anuran occupancy from calling survey data (2001–2013) from the North American amphibian monitoring program. *Herpetological Conservation and Biology*, *11*(2), 373–385.

SPECIES MANAGEMENT

Chapter 11. Conservation breeding

Benjamin Tapley[1], Kevin Johnson[2], Christopher Michaels[1], Kay Bradfield[3], Diane Barber[4], Lea Randall[5], Karthikeyan Vasudevan[6], Cynthia Paszkowski[7], Richard A. Griffiths[8], Natalie Calatayud[9,10], Edgardo Griffith[11], Federico Kacoliris[12]

[1] Zoological Society of London, Regent's Park, London, UK

[2] [1]Amphibian Ark, c/o CPSG, 12101 Johnny Cake Ridge Rd., Apple Valley, MN 55124, USA

[3] Perth Zoo, South Perth, WA, Australia

[4] Department of Ectotherms, Fort Worth Zoo, Fort Worth, Texas, USA

[5] Wilder Institute/Calgary Zoo Foundation, Calgary, Alberta, Canada

[6] Laboratory for Conservation of Endangered Species, CSIR-Centre for Cellular and Molecular Biology, Pillar 162, PVNR Expressway, Hyderguda, Hyderabad, 500048, Telangana, India

[7] Department of Biological Sciences, University of Alberta, Edmonton, Alberta T6G 2A7, Canada

[8] Durrell Institute of Conservation and Ecology, School of Anthropology and Conservation, University of Kent

[9] Conservation Science Network, 24 Thomas Street, Mayfield NSW 2304, Australia

[10] San Diego Zoo Wildlife Alliance, Beckman Center for Conservation Research, 15600 San Pasqual Valley Road, Escondido, CA 92025, USA

[11] El Valle Amphibian Conservation Center Foundation, El Hato, El Valle de Antón, Coclé, Panama

[12] Seción Herpetología, Facultad de Ciencias Naturales y Museo, UNLP - CONICET. La Plata (1900), Buenos Aires

Abstract

In the face of overwhelming and sometimes acute threats to many amphibians, such as disease or habitat destruction, the only hope in the short-term for populations and species at imminent risk of extinction is immediate rescue for the establishment and management of captive survival-assurance colonies (CSCs). Such programmes are not the final solution for conservation of any species, but in some circumstances may be the only chance to preserve the potential for eventual recovery of a species or population to threat-ameliorated habitat. A captive-assurance strategy should always be implemented as part of an integrated conservation plan that includes research on amphibian biology, advances in husbandry and veterinary care, training and capacity-building in range countries, mitigation of threats in the wild, and ongoing habitat and species protection and, where appropriate, disease risk analysis. The existence of captive assurance colonies also facilitates many of the goals of other ACAP branches, including research on amphibians and their diseases as well as the development and validation of methods that may be later used in the field. Captive programmes do not replace important programmes related to, *inter alia*, habitat preservation, control of harvesting, climate change, and ecotoxicology, but instead provide options and resources to enable survival of some species while these research programmes proceed, and to directly or indirectly support such programmes.

Introduction

The Amphibian Ark (AArk) was formed in 2007 to coordinate and support the ex situ component of the Amphibian Conservation Action Plan (ACAP), with the mission of ensuring the survival and diversity of amphibian species focusing on those that cannot currently be safe-guarded in their natural environments. AArk focuses its efforts on range-country programmes for those species which are otherwise likely to become extinct before the threats they face can be mitigated. In order for the Conservation Breeding Working Group (CBWG) priorities within the ACAP to be implemented, the global network of captive breeding programmes, which include CSCs, capacity-building programmes using analogue species, and applied ex situ research populations, must be explicitly linked to conservation and research programmes, both ex situ and in situ. To this end, AArk recommends that activities are implemented in five phases:

1. Assessment and prioritisation of species' conservation needs (Conservation Needs Assessment).
2. Establishment of captive operations in the range countries.
3. Research and long-term maintenance of captive operations.
4. Providing animals for research and reintroduction programmes.
5. Post-reintroduction monitoring and assessment (see translocation chapter).

The requirement and capacity for ex situ conservation varies regionally and is linked to spatial variation in amphibian species diversity, socioeconomic status of range states and the degree of threats posed to amphibian species in different parts of the world. The degree to which these variables are understood also varies considerably, and only ongoing field surveys, research and assessments will identify the actual numbers of species that will require a captive component to their overall conservation plan, and therefore determine the relative capacity of a region.

It should also be noted that, despite continuing advances in our understanding of the captive requirements of amphibians, captive husbandry capability is not sufficient to allow some species to thrive and breed ex situ. This is usually due to insufficient species-specific data, infrastructure and/or expertise.

Recognised challenges

A number of challenges can be faced by amphibian conservation breeding programmes, including a lack of funding, resources and expertise; inability to reverse some threats; insufficient understanding of species' life history and environmental parameters; limited capacity to establish the number of conservation programs required; and sometimes very low founder numbers. Table 1.1 includes a summary of recognised challenges that can be faced by ex situ amphibian conservation programs.

Table 11.1: Recognised challenges faced by amphibian conservation breeding programmes

Insufficient funding / resources	Relative to other taxa, amphibians remain grossly underfunded. Funding for CSCs comes from a diversity of sources but is often piecemeal, localised and short-term. CSCs require long term investment and take time to establish, this often results in project fatigue. Difficulties in sourcing specialist equipment in some range states has the potential to undermine programmes once they have been established.
Insufficient technical expertise and a lack of species champions	Amphibian captive husbandry expertise is sometimes lacking in the countries which support the greatest amphibian biodiversity and disproportionately threatened amphibian assemblages. Attempts have been made to address this balance, however the lack of technical expertise remains a problem. It can be difficult to train the appropriate people, there is high staff turnover and once training has occurred there are no mechanisms in place to ensure that the knowledge gained through training is put into practice and disseminated to others. This last issue is due, at least in part, to a lack of species champions to develop and formally manage programmes for target species. Some captive husbandry practitioners also have difficulty accessing scientific literature on amphibian husbandry. The expertise underpinning many programmes is based on short training experience and some programmes may lack the longer term experience required to adapt to the problems in husbandry.

Identifying suitable candidate species that require CSCs	Not all amphibians are suitable candidates for CSCs. The threats for some species are not currently reversible or may not ever be reversible. Deciding which species should be established as a CSC can be problematic and must to take into account the geo-political context and likelihood that the programme will succeed.
Failing to act and acting too late	CSCs are often seen as a measure of last resort and the establishment of a CSC is often postponed until numbers in the wild are dangerously low. This can greatly reduce the chances of establishing a viable CSC due to the issues inherent with small population sizes and the time potentially required to develop species-specific husbandry techniques. There is a choice to be made between prioritising small populations or larger, rapidly declining populations; in the one case extinction may be imminent, but programmes may fail, while in the other case there is still time for in situ only intervention.
Lack of field data on species biology and reliance on non–evidence based husbandry practices	Data on life history and environmental parameters are lacking for many species and life stages. This paucity of information has the potential to undermine CSCs for species which are established where little to nothing is known about the species biology, ecology and habitat / microhabitat requirements. There is a prevalence of anecdote-based husbandry over evidence-based approaches. There is a need to engage with field biologists, the scientific literature and the application of a methodical approach to changing

	husbandry. Engagement with industry / technical expertise may facilitate the design of better CSC facilities to provide appropriate conditions.
New threats and limited capacity	The captive breeding community must be able to respond to new threats as they emerge, in particular, emerging infectious disease. There is already limited captive breeding capacity and more species in need of CSCs than there are programmes established. As new threats emerge and more species become threatened there is a risk that the captive breeding community will be unable to respond. Working with field biologists to conduct health surveillance of wild populations is crucial.
Ex situ management can produce maladapted amphibians	Some amphibians fail to thrive and breed in captivity under the conditions currently provided to them. The husbandry requirements of amphibians are more complex than previously thought and for many species that require CSCs, the husbandry requirements are unknown. There is a danger of not producing any captive bred offspring or producing maladapted amphibians in CSCs which may not be suitable for reintroduction, especially if captive conditions differ greatly from field conditions.
Risk of novel pathogens in ex situ facilities	CSC facilities should be located within the indigenous range of a species to minimise the risk of individuals in such programmes becoming exposed to novel pathogens, or bringing pathogens into existing captive populations. Doing so may also simplify the provision of some environmental and climatic variables that may be important for successful husbandry. Capacity may be lacking in some regions, and as

	a result, facilities may need to be located outside of the range state and / or distributional range of the target species and there is a risk that such populations of amphibians will become exposed to novel pathogens. This is especially an issue if hosting organisations maintain cosmopolitan animal collections. Many pathogens of concern cannot currently be effectively screened for and this has the potential to undermine programmes and risk sympatric species at release sites at risk.
National, regional or local conservation authorities are / become unsupportive	Conservation priorities depend on the scale of operation. A regionally threatened species may not be a national or global priority, and vice versa. This can result in different priorities within organisations operating at different scales. Equally, the level of support provided will depend on the political motivations of the authorities concerned. State support is likely to improve with appropriate engagement with in-country parties.
Lack of sufficient numbers or genetic diversity for founding populations	Genetic analysis is expensive and the resources and expertise are not available to determine the genetic viability of many populations both in the field and in captivity that would benefit from it. Currently, some studbooks are not well implemented in existing CSCs.
Lack of post release monitoring	Inadequate post-release monitoring does not allow captive breeding practitioners to assess the success of their programmes. Poor survival and / or breeding of captive bred animals following their release to the

	wild needs to be identified as quickly as possible so that husbandry changes aimed at improving success can be identified and implemented.
Conflict of interests	Whilst conservation research has an important role in developing new husbandry techniques, disease mitigation and for developing reintroduction strategies, there is a risk that producing animals for research becomes the priority to the detriment of the captive population. The practical benefits of using captive bred offspring for research rather than release need to be critically assessed on a case-by-case basis.
Government and political constraints	If CSCs are implemented outside of range countries, the acquisition of permits to export wild-caught founder animals (and to subsequently import animals for eventual reintroduction) can be time-consuming and problematic. In-country collection permits can also be difficult to obtain in some countries, with long delays leading to further population declines in the wild.
Lack of stakeholder buy-in or involvement	Not all threatened amphibians are charismatic, and can be more difficult to attract resources, community and government buy-in to conservation actions. Within the ex situ community, these are also often ignored in favour of more charismatic species, not only other amphibians, but charismatic, larger species. There is an increasing trend in zoos and aquariums for merging departments together, which can lead to a loss of species-specific expertise.

Status update

Advances in species prioritisation and holistic programme planning

Given the inadequate global capacity to establish and maintain CSCs for all threatened species, and the necessary long-term nature of most CSCs, species prioritisation is a critical tool in a strategic approach to amphibian conservation, and a number of advances have been made in this area since the first ACAP (e.g. Gumbs, Gray, Wearn, & Owen, 2018; Isaac, Redding, Meredith, & Safi, 2012; Johnson et al., 2018). Additionally, the need for integration of ex- and in situ interventions (i.e. following the IUCN Conservation Planning Specialist Group’s One Plan Approach), which was not always the case for captive breeding programmes historically, was highlighted initially by the IUCN/SSC (2002), then subsequently by the first ACAP (Gascon et al., 2007) and continues to be the case. In 2006, a taxon selection and prioritisation working group developed a decision tree to help select and prioritise which species are most in need of ex situ assistance. In 2009 the AArk began expanding and refining this tool into the Conservation Needs Assessment (CNA) process (www.ConservationNeeds.org), as a method to promote needs-based species prioritisation, and holistic programme planning with defined exit strategies. A CNA assigns recommended actions to a species from a range of eleven conservation roles, from no current needs, through in situ conservation or research only, to full ex situ rescue or ark operations (Johnson et al., 2018), with national species priorities determined by scores allocated to responses within each CNA. Prioritisation of species is still constrained partly by incomplete knowledge of the total diversity of amphibians, and the current conservation status of the majority of described amphibian species (Tapley et al., 2018), and CNAs should be updated as additional or updated data are available, to ensure accurate priorities and recommendations for action. In order to inform conservation prioritisation the conservation needs of all threatened amphibians must be evaluated, and then re-evaluated on a ten-yearly basis, or when new data are available, to ensure the assessments remain current and valuable. CSCs should be established based on priorities at the time and reviewed as priorities change. Since 2018, a number of joint amphibian IUCN Red List and Conservation Needs Assessment workshops have taken place. This joint approach considerably reduces the financial and human resources required compared to conducting the two assessments separately and facilitates the necessary close link between the processes. It is envisioned that joint IUCN Red List and CNA assessments will continue into the future. However, completing assessments for all threatened amphibians and updating them on a cyclic basis to inform conservation action is costly. Moreover, the prioritisation process is only of value if it is followed by the establishment of captive programmes, as well as the other CNA recommendations, for those species that are identified as requiring them as part of integrated (or holistic) conservation recovery programmes. Therefore, the ex situ response must be strategically linked to the CNA process.

The conservation breeding community has responded positively to CNAs (Figure 11.1), as these assessments have been a pivotal consideration in the collection planning processes adopted by the ex situ conservation community (e.g. Barber & Poole, 2014; Garcia et al., 2020). However, mechanisms need to be developed to ensure that CNA recommendations are more-widely adopted at the national level when conservation strategies are developed for amphibians.

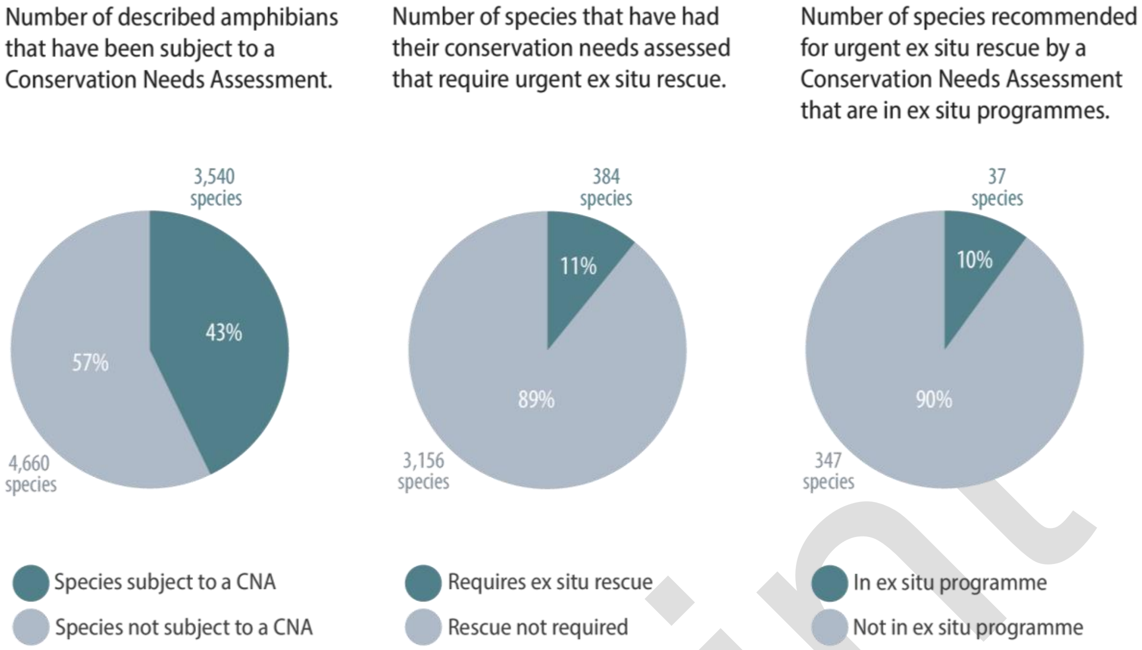


Figure 11.1. The number of amphibians that have had their conservation needs assessed, the proportion of assessed species that require urgent ex situ rescue and the proportion of the species requiring urgent ex situ rescue that are currently established as an CSC (data from AArk's programme progress database).

Paradigm shift and the development of regional capacity

The first ACAP recognised the importance of establishing CSCs within species range countries and using facilities dedicated to sympatric species with shared management histories (Gascon et al., 2007), and this recommendation is maintained by the CBWG and AArk (Zippel et al., 2011). If this is not the case, exposure to alien pathogens is possible (Pessier & Mendelson III, 2017; Zippel et al., 2011), which may create additional threats for the focal species and/or syntopic taxa following translocation of captive individuals (Cunningham, Daszak, & Rodríguez, 2003). Such pathogens have been detected in cosmopolitan amphibian collections (Cunningham et al., 2015; Miller et al., 2008; Walker et al., 2008), or may be unknown or unreliably detected and difficult to eradicate (e.g. Blooi et al., 2015; Rijks et al., 2018), and so pose a substantial and real threat (e.g. Walker et al., 2008). In addition to infectious disease, hosting CSCs outside of range countries potentially has cultural, political, legal and social implications for the long-term success of conservation programmes (Tapley, Bradfield, Michaels, & Bungard, 2015). An in-range approach requires capacity building, however, as regions most in need of CSCs are often those where few resources currently exist (Edmonds et al., 2012; Gagliardo et al., 2008). A number of high-profile CSCs have therefore been established outside of range countries because it was not possible to build capacity in time to rescue populations from acute declines, e.g. mountain chicken frogs (*Leptodactylus fallax*) and Kihansi spray toads (*Nectophrynoides asperginis*). Although these programmes are key to species survival and supported by AArk, they are acknowledged to be suboptimal in this respect. Both programmes work closely with in-range governments and communities, however, to partially address this issue.

In the years immediately following the publication of the ACAP in 2007, the number of amphibian captive programmes increased (Harding, Griffiths, & Pavajeau, 2016), and a number of well-equipped facilities dedicated to amphibian conservation breeding were established in regions where capacity was previously lacking (Bourke, 2010; Edmonds et al., 2012; Harding et al., 2016; Hernández Díaz, 2013; Tapley, Harding, et al., 2014; Ziegler, 2015). According to Harding et al. (2016), by 2014, approximately half of captive breeding programmes were undertaken by government or non-government agencies rather than zoos or aquariums. It is therefore important to avoid conflation of ex situ conservation as a concept with zoo and aquarium populations of animals.

Captive husbandry: advances and current limitations

There have been notable advances in a number of relevant areas since the publication of the first ACAP (Table 11.2).

Table 11.2. Advances in amphibian husbandry since the publication of the first ACAP.

Area of husbandry	Associated references
Nutrition	Antwis et al., 2014; Byrne & Silla, 2017; Dugas, Yeager, & Richards-Zawacki, 2013; Edwards, Byrne, Harlow, & Silla, 2017; Jayson et al., 2018; McInerney, Silla, & Byrne, 2019; Michaels et al., 2021; Ogilvy & Preziosi, 2012; Ogilvy, Preziosi, & Fidgett, 2012; Rodríguez & Pessier, 2014; Silla, McInerney, & Byrne, 2016; Venesky, Mendelson III, Sears, Stiling, & Rohr, 2012 Keogh et al., 2018
Provision of appropriate lighting	Baines et al., 2016; Michaels, Antwis, & Preziosi, 2015; Shaw et al., 2012; Tapley, Rendle, et al., 2014; Verschooren, Brown, Vercammen, & Pereboom, 2011; Whatley et al., 2020
Provision of enrichment	Michaels, Downie, & Campbell-Palmer, 2014
Behavioural syndromes	See review in Kelleher, Silla, & Byrne, 2018
Artificial manipulation of seasonally dependent adaptations	Calatayud et al., 2015 Calatayud et al., 2020

(brumation, aestivation, torpor)	
Larval rearing techniques	Behr & Rödder, 2018; Ciani et al., 2018; Fenolio et al., 2014; Gawor et al., 2012; Gower et al., 2012; Higgins et al., 2021; Lassiter et al., 2020; Michaels, Antwis, & Preziosi, 2014; Michaels & Preziosi, 2015; Michaels, Tapley, Harding, Bryant, & Grant, 2015; Pasmans, Janssens, Sparreboom, Jiang, & Nishikawa, 2012
Health assessment	Davis & Maerz, 2011; Jayson, Harding, et al., 2018; Narayan & Hero, 2011
Disease treatment protocols and pathogen management	Blooi, Martel, et al., 2015; Blooi, Pasmans, et al., 2015; Brannelly, Richards-Zawacki, & Pessier, 2012; Garner, Garcia, Carroll, & Fisher, 2009; Martel et al., 2011; Michaels et al., 2018; Rendle et al., 2015; Ujszegi et al., 2021.
Pre translocation training	Crane & Mathis, 2011
Assisted reproductive techniques and biobanking	See Chapter 12

Species-specific husbandry and management protocols have also been developed for a host of species via a range of channels including peer-reviewed articles and technical reports (Jameson et al., 2019; Poole & Grow, 2012; Tapley et al., 2016). Furthermore, techniques have been developed and validated that aid in situ actions such as population monitoring and disease mitigation (Hudson et al., 2016; Jayson, Ferguson, et al., 2018; Scheele et al., 2014;

Tapley, Michaels, Gower, & Wilkinson, 2020) and the establishment of facilities in range states (e.g. Nicolson et al., 2017).

While these advances have contributed to the ex situ community's ability to successfully maintain and breed an increasing number of species (e.g Ettling et al., 2013; McFadden et al., 2018; Michaels et al., 2015; Preininger, Weissenbacher, Wampula, & Hödl, 2012), it is still unable to meet the needs of many species due to husbandry limitations that ultimately derive from inadequate understanding of species biology and pathology, and insufficient resources and regional expertise (Flach et al., 2020; Pessier et al., 2014).

Adaptation to captivity

Over time, any captive population of amphibians will adapt genetically, phenotypically and behaviourally to captive environments, which inevitably differ from wild conditions in myriad ways. Potential adaptations to captivity include:

1. vocalisations / phonotaxis (Passos, Garcia, & Young, 2021, 2017);
2. antipredator behaviour (Crane & Mathis, 2011);
3. induced morphological antipredator responses (Kraaijeveld-Smit, Griffiths, Moore, & Beebee, 2006);
4. microbial communities (Antwis et al., 2014; Becker, Richards-Zawacki, Gratwicke, & Belden, 2014; Michaels & Preziosi, 2020; Passos, Garcia, & Young, 2018);
5. colouration (Dugas et al., 2013; Ogilvy et al., 2012);
6. size/morphology (Bennett & Murray, 2015);
7. nutritional state (Silla et al., 2016);
8. fecundity and offspring viability (Dugas et al., 2013);

Maximising genetic diversity is crucially important, particularly in instances where animals bred in captivity serve as a source population for reintroduction and translocation (Grueber, Hogg, Ivy, & Belov, 2015; Jameson et al., 2019). Understanding species-specific histories, early viability selection and local environmental adaptation is necessary as not all species will respond to inbreeding and artificial selection uniformly (Grueber et al., 2015). Suboptimal captive husbandry may also result in individuals with lower phenotypic fitness that are less likely to establish in wild habitats following translocation.

Adaptation to artificial captive environments could be reduced if every aspect of the natural environments could be replicated in captivity, although this is currently logistically and / or technologically impossible (Tapley et al., 2015) or ethically challenging in the case of predators, pathogens, parasites, and other stressors. Minimising the number of generations that a species is kept in captivity by reducing the length of time a species is held in captivity, increasing generation length or using cryopreservation are other methods that can be used to minimise adaptation to artificial captive environments (Frankham, 2008; Williams & Hoffman, 2009). Periodically importing individuals from the wild (Frankham & Loebel, 1992) and equalising families at each generation (Frankham, Manning, Margan, & Briscoe, 2000) are other strategies that can substantially reduce adaptation to captivity. Another strategy is to manage a population as several small reproductively isolated units where different components of diversity are lost randomly by drift, then crossing these units to rescue genetic diversity and produce animals for translocations (Margan et al., 1998).

Lessons learnt from reintroductions and translocations

Several high-profile amphibian species have been subject to captive breeding programmes which also include reintroduction efforts. Some of the reintroductions have been more successful than others, but for all of them, learning from any failings which are identified, as well as modifying processes, is vital to improving the success of future attempts. Reintroductions and other translocations are covered in far more detail in Chapter 14 of this publication; however, examples of lessons learned from a few programmes are included here.

Box 11.1: Puerto Rican crested toad (*Peltophryne lemur*)

As the Puerto Rican crested toad (*Peltophryne lemur*) reintroduction programme nears its fourth decade, the ebb and flow of failures and achievements is recognised as part of our functionality. Throughout this process, we have discovered that trust between partners is paramount and failures are not about proving someone wrong or assigning blame. Rather, failures are learning opportunities to build upon, adapt, and move forward as a group.

One of the biggest hurdles for this programme has been transitioning leadership from a few invested individuals overseeing a small reintroduction effort, to an island-wide programme instilling stewardship for a critically endangered species- connecting volunteers, universities, zoos, local and federal agencies, and nongovernmental organisations. The development of a Memorandum of Understanding (MOU) between primary agency partners responsible for the species protection and recovery, establishment of a Puerto Rican crested toad Working Group for all stakeholders, and increased communication and capacity building has been a slow process, but resulted in improved functionality and will prove paramount for this programme's long-term stability.

Wild crested toad populations were originally divided into two distinct northern (N) and southern (S) populations. Genetic analysis of N and S toads revealed that mitochondrial haplotypes within the lineages were moderately divergent, but they were determined to be no more divergent than other populations of amphibians (Beauclerc, Johnson, & White, 2010). By 1992 the N wild population was believed to be extirpated but N toads were still maintained as a distinct breeding group in the captive population. The N zoo population was established in 1980 from four inbred siblings and by 2008 was exhibiting signs of inbreeding depression. In 2011 it was decided to perform a genetic rescue of the N population by breeding them with wild caught S toads to establish a captive NxS population. Releases of NxS toads began at northern reintroduction sites in 2012 while S population releases continued at the southern sites. For the next several years, the Puerto Rican crested toad were managed as two populations (NxS and S), but limited space and resources created the need to manage the population as one species. However, not all partners agreed to a final merger despite the NxS toads' adaption to northern wet forests. There were concerns that there could be some deleterious alleles that might negatively impact survivorship for toads released in the dry scrub forest habitat in the south. After soliciting opinions from geneticists and biologists outside of the programme and thoughtful debate, it was agreed in 2017 to combine the captive populations and manage the Puerto Rican crested toad as one species. Resources were simply too limited to continue effective management of two captive populations and maximisation of genetic diversity and the potential for increasing overall fitness for the population outweighed concerns of low probability of negative outbreeding effects. Additionally, all reintroduction sites are isolated by geographic and man-made barriers, which helped alleviate concerns of causing any risk to the remaining wild population.

Lastly, there have been many challenges related to the creation and maintenance of reintroduction sites and monitoring in general. We still have limited knowledge of the toads' natural history and population sizes, and despite proven survivorship and recruitment at all but one reintroduction site, funding and staff shortages continue to hinder this programme. However, the establishment of reintroduction and field management standard operating procedures to assist partners at pond sites and increase monitoring efforts has become a useful tool and most importantly, we continue to move above the rising tide and use our mistakes to revaluate and adapt as we protect the remaining wild population and establish new ones for this species' recovery.

Box 11.2: White-bellied frog (*Geocrinia alba*)

Critically Endangered white-bellied frogs (*Geocrinia alba*) have a highly restricted and fragmented distribution in south-west Western Australia, with more than 50% of known populations disappearing over recent decades. Perth Zoo has been head-starting white-bellied frogs since 2008 to contribute to *in situ* recovery efforts. This is an effective strategy as more than 95% of fertile eggs / larvae brought into the Zoo survive to release, whereas survival to metamorphosis in the wild is only approximately 20% (McFadden et al., 2018). By late 2020, over 1,000 juvenile white-bellied frogs had been released to the wild to establish new populations and supplement existing small ones (K. Bradfield, pers. comm.). To minimise any adverse impacts of egg clutch collection on source populations, a proportion of each clutch is released at the site where it was collected (McFadden et al., 2018).

The results of post-release monitoring indicate that translocations of this species can be successful; a population established with head-started frogs is now one of the largest self-sustaining wild populations with others in a stable or increasing trend. However, one translocation attempt appears to have failed, and the distribution of frogs at one of the supplementation sites has changed (K. Williams, pers. comm.). Understanding the primary drivers of translocation success vs failure is therefore critical to on-going recovery efforts. Hoffmann, Williams, Hipsey, & Mitchell (2021) found that dry season soil moisture, which is likely to be decreasing in the area where *G. alba* occurs due to regional changes in climate and hydrology, predicts the outcomes of the translocations undertaken to date with a high degree of accuracy, and also explains the persistence/extinction of naturally-occurring populations. This clearly highlights the importance of understanding the fine-scale habitat associations of threatened amphibians when selecting release sites, particularly for species with limited dispersal ability.

Box 11.3: El Rincon Stream frog (*Pleurodema somuncurense*)

The El Rincon Stream frog, (*Pleurodema somuncurense*), is an endemic species conformed by a few isolated subpopulations, restricted to the hot springs of the headwaters of the Valcheta Stream in northern Patagonia, Argentina (Velasco, 2018). During the last four decades, this frog's range dramatically declined, and some subpopulations have gone extinct (Velasco, 2018).

In 2012 an ambitious recovery plan for this species and its habitat began, following guidelines highlighted in the Amphibian Conservation Action Plan (Gascon et al., 2007), the El Rincon Stream Frog Conservation Action Plan (Kacoliris et al., 2018), and the Conservation Action Plan for Amphibians of Argentina (Vaira, Akmentins, & Lavilla, 2018). As part of this plan, in 2015 we established an ex situ colony of this species aimed at producing individuals to conduct a reintroduction plan for this species. Between 2017 and 2021, we conducted a total of five translocations of individuals born in captivity to restored wild habitats where subpopulations of this species went extinct. Further monitoring allowed us to register the establishment of the reintroduced individuals (Martínez Aguirre et al., 2019). This news encouraged us to continue with the second step of the reintroduction plan, concluding in 2021 with three translocations of a total of 2,500 tadpoles born in captivity to three new restored habitats. Monitoring carried up a few months later showed that the tadpoles completed metamorphosis in the wild. If successful, these reintroductions will add a total of four new subpopulations of this Critically Endangered species, enhancing metapopulation dynamics and increasing long-lasting viability. Further observation will let us know which strategy is best in terms of effort and biological success (i.e. translocation of tadpoles vs translocation of froglets).

The reintroduction attempts carried on with the El Rincon Stream frog are the first rewilding experience made in Argentina with amphibians. Although results are even more positive than we expected, the road to get here was not easy and we had to face several barriers in terms of scepticism coming mainly from colleagues from the academic realm. This scepticism, based on the fact that no previous reintroductions were carried with amphibians in Argentina, made environmental authorities to be more cautious about giving permits, causing delays, and sometimes overcrowding in the ex situ facilities. This experience let us learn that future reintroduction programmes must be based on strong support coming from several stakeholders, thus in a more participative development of the conservation actions planned.

Box 11.4: El Valle Amphibian Conservation Center (EVACC)

Based on our experiences we highly recommend that before an amphibian ex situ programme with conservation purposes is launched, a complementary “Emergency Release Plan” (ERP) should be developed, which includes actions to be taken in the event of a serious situation within the ex situ facility (e.g. long-term lack of access during a pandemic, political instability, natural disaster or other emergency). This ERP does not replace a long-term release/reintroduction plan and would be implemented in cases involving releasing the animals back into the wild, when failing to do so could result in the imminent loss of the entire captive population. Based on the circumstances and magnitude of a given event, those releases could be evaluated as necessary and classified as soft or hard releases.

Under normal circumstances, releases or reintroductions should not be considered if life history or habitat requirements are not yet known. Basic population demographic data on the species should be gathered if these parameters are not already known, as these will be required for population viability analysis and for informing decisions about which stages of the life cycle should be used for the reintroductions. Similarly, habitat requirements need to be determined so that habitat management, restoration and creation can be carried out in a way that will maximise the chances of the reintroduction succeeding (Moore & Church, 2008).

Future directions

The amphibian ex situ community has made major advances in core areas since the launch of the first ACAP. However, further development is required in order to meet the global need for these programmes.

Whilst substantial advances have been made in the development of regional amphibian husbandry capacity, there are still gaps, particularly West, Central and East Africa, and southern and South-East Asia, which should be addressed by investment in these regions going forward. Furthermore, there must be continued effort to identify the conservation needs of individual species through the CNA process and to ensure that these CNAs remain up-to-date.

Our knowledge of the ex situ requirements of many amphibians has been enhanced by a substantial number of research projects over the past 15 years but gaps remain, particularly with regard to nutrition, diagnosis of disease and subsequent treatment, and methods for maintaining and breeding particular species in captivity, especially under biosecure conditions. These gaps can be addressed through further collaborative and co-ordinated research and partnership with, *inter alia*, nutritionists, wildlife health experts, field biologists and husbandry experts. The development of model systems based on existing knowledge from species that have been maintained successfully in captivity may be advantageous in understanding CSC requirements and implications for species that have not previously been kept in captivity, although this is not always the case.

Many amphibian conservation breeding programmes were established as a response to infectious diseases, especially fungal pathogens. Whilst there are promising advances in the mitigation of Bd and Bsal in the wild (e.g. Scheele et al., 2014; Woodhams et al., 2011), we are not at a stage where we can re-establish wild populations of amphibians that have been extirpated by Bd and Bsal, or other pathogens, while pathogens are still present in the environment (Mendelson III, 2018; See Chapter 6). Overcoming this challenge is critical to the success of many CSCs. As a result, the ex situ community must continue to fund costly breeding programmes for an indefinite period of time (Tapley et al., 2015). Exit strategies should be identified for all captive breeding programmes to ensure that limited resources are being used to the greatest effect.

The continued integration of ex situ interventions within well-defined holistic, prioritised conservation plans is critical to ensure that conservation efforts result in species recovery (e.g. Adams et al., 2014; Azat et al., 2021; Kissel, Palen, Govindarajulu, & Bishop, 2014; Lewis et al., 2019; Rosa et al., 2015; Scheele et al., 2014). Good communication and relationship-building skills, and thoughtful planning with appropriate participants who have the authority to implement actions and establish shared achievable goals are critical to achieving this. Holistic and inclusive planning processes such as those utilised by the IUCN Conservation Planning Specialist Group (CPSG, 2020) should be followed. Staff at captive institutions need to devote time to establishing relationships with those that work with threatened species in their region / focus area if they do not already exist and maintaining or strengthening existing relationships by engaging with all relevant stakeholders such as landowners, government, academia and local communities etc. Even with the above knowledge gaps addressed, there is not currently sufficient resourcing to meet the global need for CSCs. The pursuit and securing of additional funding streams and models to support long term, holistic conservation projects incorporating CSCs is needed.

It is encouraging to see the advances made over the past 15 years, and a number of successful programmes have been implemented despite the conservation breeding community falling short of the original aspiration due the constraints mentioned above. Many amphibian taxa will still become extinct without ongoing or new ex situ intervention, and it is more important than ever that new CSCs are established strategically, and as part of an integrated approach to recover highly threatened amphibian species.

References

Adams, S. L., Morton, M. N., Terry, A., Young, R. P., Dawson, J., Martin, L., … Gray, G. (2014). *Long-term recovery strategy for the critically endangered mountain chicken 2014-2034*. Mountain Chicken Recovery Program. doi: 10.13140/RG.2.1.3836.8487

Antwis, R. E., Haworth, R. L., Engelmoer, D. J. P., Ogilvy, V., Fidgett, A. L., & Preziosi, R. F. (2014). Ex situ diet influences the bacterial community associated with the skin of red-eyed tree frogs (*Agalychnis callidryas*). *PLoS ONE*, *9*(1), 1–8. doi: 10.1371/journal.pone.0085563

Azat, C., Valenzuela-Sánchez, A., Delgado, S., Cunningham, A. A., Alvarado-Rybak, M., Bourke, J., … Angulo, A. (2021). A flagship for Austral temperate forest conservation: An action plan for Darwin's frogs brings key stakeholders together. *Oryx*, *55*(3), 356–363. doi: 10.1017/S0030605319001236

Baines, F., Chattell, J., Dale, J., Garrick, D., Gill, I., Goetz, M., … Swatman, M. (2016). How much UV-B does my reptile need? The UV-Tool, a guide to the selection of UV lighting for reptiles and amphibians in captivity. Journal of Zoo and Aquarium Research, 4(1), 42–63. doi: 10.19227/jzar.v4i1.150

Barber, D., & Poole, V. A. (2014). *Association of Zoos and Aquariums Amphibian Taxon Advisory Group Regional collection plan, 3rd edition*. Maryland.

Beauclerc, K. B., Johnson, B., & White, B. N. (2010). Genetic rescue of an inbred captive population of the critically endangered Puerto Rican crested toad (*Peltophryne lemur*) by mixing lineages. *Conservation Genetics*, *11*(1), 21–32. doi: 10.1007/s10592-008-9782-z

Becker, M. H., Richards-Zawacki, C. L., Gratwicke, B., & Belden, L. K. (2014). The effect of captivity on the cutaneous bacterial community of the critically endangered Panamanian golden frog (*Atelopus zeteki*). *Biological Conservation*, *176*, 199–206. doi: 10.1016/j.biocon.2014.05.029

Behr, N., & Rödder, D. (2018). Captive management, reproduction, and comparative larval development of Klappenbach’s red-bellied frog, *Melanophryniscus klappenbachi* Prigioni and Langone, 2000. *Amphibian & Reptile Conservation*, *12*(1), 18–26.

Bennett, A. M., & Murray, D. L. (2015). Carryover effects of phenotypic plasticity: Embryonic environment and larval response to predation risk in wood frogs (*Lithobates sylvaticus*) and northern leopard frogs (*Lithobates pipiens*). *Canadian Journal of Zoology*, *93*(11), 867–877. doi: 10.1139/cjz-2015-0129

Blooi, M., Martel, A., Haesebrouck, F., Vercammen, F., Bonte, D., & Pasmans, F. (2015). Treatment of urodelans based on temperature dependent infection dynamics of *Batrachochytrium salamandrivorans*. *Scientific Reports*, *5*(January), 8037. doi: 10.1038/srep08037

Blooi, M., Pasmans, F., Rouffaer, L., Haesebrouck, F., Vercammen, F., & Martel, A. (2015). Successful treatment of *Batrachochytrium salamandrivorans* infections in salamanders requires synergy between voriconazole, polymyxin e and temperature. *Scientific Reports*, *5*(March 2015), 1–8. doi: 10.1038/srep11788

Bourke, J. (2010). Darwin’s frog captive rearing facility in Chile. *FrogLog*, *6*, 6–10.

Brannelly, L. A., Richards-Zawacki, C. L., & Pessier, A. P. (2012). Clinical trials with itraconazole as a treatment for chytrid fungal infections in amphibians. *Diseases of Aquatic Organisms*, *101*(2), 95–104. doi: 10.3354/dao02521

Byrne, P. G., & Silla, A. J. (2017). Testing the effect of dietary carotenoids on larval survival, growth and development in the critically endangered southern corroboree frog. *Zoo Biology*, *36*(2), 161–169. doi: 10.1002/zoo.21352

Calatayud, N. E., Hammond, T. T., Gardner, N. R., Curtis, M. J., Swaisgood, R. R. & Shier, D. M. (2020). Benefits of overwintering in the conservation breeding and translocation of a critically endangered amphibian. *Conservation Science and Practice*, 28, e341.

Calatayud, N. E., Langhorne, C. J., Mullen, A. C., Williams, C. L., Smith, T., Bullock, L., … Willard, S. T. (2015). A hormone priming regimen and hibernation affect oviposition in the boreal toad (*Anaxyrus boreas boreas*). *Theriogenology*, *84*(4), 600–607. doi: 10.1016/j.theriogenology.2015.04.017

Ciani, J. F. C., Guerrel, J., Baitchman, E., Diaz, R., Evans, M., Ibáñez, R., … Gratwicke, B. (2018). The relationship between spindly leg syndrome incidence and water composition, overfeeding, and diet in newly metamorphosed harlequin frogs (*Atelopus* spp.). *PLoS ONE*, *13*(10), 1–16. doi: 10.1371/journal.pone.0204314

CPSG. (2020). *Species conservation planning principles & steps, ver. 1.0.* IUCN SSC Conservation Planning Specialist Group: Apple Valley, MN. Retrieved from https://www.cpsg.org/sites/cbsg.org/files/documents/CPSG Principles %26 Steps_English.pdf

Crane, A. L., & Mathis, A. (2011). Predator-recognition training: A conservation strategy to increase postrelease survival of hellbenders in head-starting programs. *Zoo Biology*, *30*(6), 611–622. doi: 10.1002/zoo.20358

Cunningham, A. A., Beckmann, K., Perkins, M., Fitzpatrick, L., Cromie, R., Redbond, J., … Fisher, M. C. (2015). Surveillance: Emerging disease in UK amphibians. *Veterinary Record*, *176*(18), 468. doi: 10.1136/vr.h2264

Cunningham, A. A., Daszak, P., & Rodríguez, J. (2003). Pathogen pollution: Defining a parasitological threat to biodiversity conservation. *Journal of Parasitology*, *89*, S78–S83.

Davis, A. K., & Maerz, J. C. (2011). Assessing stress levels of captive-reared amphibians with hematological data: Implications for conservation initiatives. *Journal of Herpetology*, *45*(1), 40–44. doi: 10.1670/10-180.1

Dugas, M. B., Yeager, J., & Richards-Zawacki, C. L. (2013). Carotenoid supplementation enhances reproductive success in captive strawberry poison frogs (*Oophaga pumilio*). *Zoo Biology*, *32*(6), 655–658. doi: 10.1002/zoo.21102

Edmonds, D., Rakotoarisoa, J. C., Dolch, R., Pramuk, J., Gagliardo, R., Andreone, F., … Robsomanitrandrasana, E. (2012). Building capacity to implement conservation breeding programs for frogs in Madagascar: Results from year one of Mitsinjo’s amphibian husbandry research and captive breeding facility. *Amphibian and Reptile Conservation*, *5*(3), 57–69.

Edwards, C. L., Byrne, P. G., Harlow, P., & Silla, A. J. (2017). Dietary carotenoid supplementation enhances the cutaneous bacterial communities of the critically endangered southern Corroboree Frog (*Pseudophryne corroboree*). *Microbial Ecology*, *73*(2), 435–444. doi: 10.1007/s00248-016-0853-2

Ettling, J., Wanner, M. D., Schuette, C. D., Aldridge, R. D., Watkins-colwell, G., & Bell, C. (2013). Captive reproduction and husbandry of adult Ozark hellbenders , *Cryptobranchus alleganiensis bishopi*. H*erpetological Review*, 44, 605−610.

Fenolio, D. B., Gorman, T. A., Jones, K. C., Mandica, M., Phillips, L., Melde, L., … Haas, C. A. (2014). Rearing the federally endangered reticulated flatwoods salamander, *Ambystoma bishopi*, from eggs through metamorphosis. *Herpetological Review*, *45*(1), 62–65.

Flach, E. J., Feltrer, Y., Gower, D. J., Jayson, S., Michaels, C. J., Pocknell, A., … Masters, N. (2020). Postmortem findings in eight species of captive caecilian (Amphibia: Gymnophiona) over a ten-year period. *Journal of Zoo and Wildlife Medicine*, *50*(4), 879–890. doi: 10.1638/2019-0047

Frankham, R. (2008). Genetic adaptation to captivity in species conservation programs. *Molecular Ecology*, *17*, 325–333. doi: 10.1111/j.1365-294X.2007.03399.x

Frankham, R., & Loebel, D. A. (1992). Modeling problems in conservation genetics using captive *Drosophila* populations: Rapid genetic adaptation to captivity. *Zoo Biology*, *11*(5), 333–342. doi: 10.1002/zoo.1430110505

Frankham, R., Manning, H., Margan, S. H., & Briscoe, D. A. (2000). Does equalization of family sizes reduce genetic adaptation to captivity? *Animal Conservation*, *3*(4), 357–363. doi: 10.1017/S1367943000001074

Gagliardo, R., Crump, P., Griffith, E., Mendelson, J., Ross, H., & Zippel, K. (2008). The principles of rapid response for amphibian conservation, using the programmes in Panama as an example. *International Zoo Yearbook*, *42*, 125–135. doi: 10.1111/j.1748-1090.2008.00043.x

Garcia, G., Tapley, B., Marquis, O., Aparici Plaza, D., van der Meer, R., & Voorham, M. (2020). *Caudata regional collection plan for the EAZA Amphibian Taxon Advisory Group – Edition One*. Amsterdam.

Garner, T. W. J., Garcia, G., Carroll, B., & Fisher, M. C. (2009). Using itraconazole to clear *Batrachochytrium dendrobatidis* infection, and subsequent depigmentation of *Alytes muletensis* tadpoles. *Diseases of Aquatic Organisms*, *83*(3), 257–260. doi: 10.3354/dao02008

Gascon, C., Collins, J. P., Moore, R. D., Church, D. R., McKay, J. E., & Mendelson III, J. R. (2007). *Amphibian conservation action plan.* IUCN/SSC Amphibian Specialist Group. Gland, Switzerland and Cambridge, UK.

Gawor, A., Rauhaus, A., Karbe, D., Straeten, K. Van Der, Lötters, S., & Ziegler, T. (2012). Is there a chance for conservation breeding? Ex situ management, reproduction, and early life stages of the Harlequin toad Atelopus flavescens Duméril & Bibron, 1841 (Amphibia: Anura: Bufonidae). *Amphibian and Reptile Conservation*, *5*(3), 29–44.

Gower, R. A., Perl, A., Scheld, R. G. B., van der Straeten, S., Karbe, K., Pham, D., … Ziegler, T. (2012). Larval development, stages and an international comparison of husbandry parameters of the

Vietnamese mossy frog *Theloderma corticale* (Boulenger, 1903) (Anura: Rhacophoridae). *Asian Journal of Conservation Biology*, *1*(2), 51–66.

Grueber, C. E., Hogg, C. J., Ivy, J. A., & Belov, K. (2015). Impacts of early viability selection on management of inbreeding and genetic diversity in conservation. Molecular Ecology, 24(8), 1645–1653. doi: 10.1111/mec.13141

Gumbs, R., Gray, C. L., Wearn, O. R., & Owen, N. R. (2018). Tetrapods on the EDGE: Overcoming data limitations to identify phylogenetic conservation priorities. *PLoS ONE*, *13*(4), 1–19. doi: 10.1371/journal.pone.0194680

Harding, G., Griffiths, R. A., & Pavajeau, L. (2016). Developments in amphibian captive breeding and reintroduction programs. *Conservation Biology*, *30*(2), 340–349. doi: 10.1111/cobi.12612

Hernández Díaz, A. (2013). Crested toad reproduction at Africam Safari, Puebla, Mexico. *AArk Newsletter*, *23*, 17.

Higgins, K., Guerrel, J., Lassiter, E., Mooers, A., Palen, W. J., & Ibáñez, R. (2021). Observations on spindly leg syndrome in a captive population of *Andinobates geminisae*. *Zoo Biology*, *40*(4), 330–341. doi: 10.1002/zoo.21598

Hoffmann, E. P., Williams, K., Hipsey, M. R., & Mitchell, N. J. (2021). Drying microclimates threaten persistence of natural and translocated populations of threatened frogs. *Biodiversity and Conservation*, *30*, 15–34. doi: 10.1007/s10531-020-02064-9

Hudson, M. A., Young, R. P., Lopez, J., Martin, L., Fenton, C., McCrea, R., … Cunningham, A. A. (2016). In-situ itraconazole treatment improves survival rate during an amphibian chytridiomycosis epidemic. *Biological Conservation*, *195*(February 2019), 37–45. doi: 10.1016/j.biocon.2015.12.041

Isaac, N., Redding, D. W., Meredith, H. M., & Safi, K. (2012). Phylogenetically-informed priorities for amphibian conservation. *PLoS ONE*, *7*(8), e43912. doi: 10.1371/journal.pone.0043912

IUCN/SSC. (2002). *IUCN technical guidelines on the management of ex-situ populations for conservation*. Gland, Switzerland. Retrieved from https://portals.iucn.org/library/efiles/documents/Rep-2002-017.pdf

Jameson, T., Tapley, B., Barbon, A., Goetz, M., Harding, L., López, J., … Garcia, G. (2019). Best practice guidelines for the mountain chicken (*Leptodactylus fallax*). *European Association of Zoos and Aquaria,* (August), 1–119. Retrieved from https://www.eaza.net/assets/Uploads/CCC/BPG-2019/2019-Mountain-chicken-frog-EAZA-Best-Practice-Guidelines-Approved.pdf

Jayson, S., Ferguson, A., Goetz, M., Routh, A., Tapley, B., Harding, L., … Dawson, J. (2018). Comparison of the nutritional content of the captive and wild diets of the critically endangered mountain chicken frog (*Leptodactylus fallax*) to improve its captive husbandry. *Zoo Biology*, *37*(5), 332–346. doi: 10.1002/zoo.21442

Jayson, S., Harding, L., Michaels, C. J., Tapley, B., Hedley, J., Goetz, M., … Flach, E. (2018). Development of a body condition score for the mountain chicken frog (*Leptodactylus fallax*). *Zoo Biology*, *37*(3), 196–205. doi: 10.1002/zoo.21409

Johnson, K., Baker, A., Buley, K., Carrillo, L., Gibson, R., Gillespie, G. R., … Zippel, K. (2018). A process for assessing and prioritizing species conservation needs: going beyond the Red List. *Oryx*, 1–8. doi: 10.1017/S0030605317001715

Kacoliris, F., Velasco, M., Arellano, M., Martinez-Aguiree, T., Zarini, O., Calvo, R., … Williams, J. (2018). *Plan de acción para la conservación* *de la ranita del Valcheta (Pleurodema somuncurense), Meseta de Somuncura , Río Negro*. Facultad de Ciencias Naturales y Museo. La Plata, Argentina.

Kelleher, S. R., Silla, A. J., & Byrne, P. G. (2018). Animal personality and behavioral syndromes in amphibians: a review of the evidence, experimental approaches, and implications for conservation. *Behavioral Ecology and Sociobiology*, *72*(5). doi: 10.1007/s00265-018-2493-7

Keogh, L.M., Silla, A.J., McFadden, M.S. and Byrne, P.G. (2018). Dose and life stage-dependent effects of dietary beta-carotene supplementation on the growth and development of the Booroolong frog. *Conservation Physiology*, 6, coy052.

Kissel, A. M., Palen, W. J., Govindarajulu, P., & Bishop, C. A. (2014). Quantifying ecological life-support: the biological efficacy of alternative supplementation strategies for imperiled amphibian populations. *Conservation Letters*, *7*(5), 441–450. doi: 10.1111/conl.12093.

Kraaijeveld-Smit, F. J. L., Griffiths, R. A., Moore, R. D., & Beebee, T. J. C. (2006). Captive breeding and the fitness of reintroduced species: A test of the responses to predators in a threatened amphibian. *Journal of Applied Ecology*, *43*(2), 360–365. doi: 10.1111/j.1365-2664.2006.01137.x

Lassiter, E., Garces, O., Higgins, K., Baitchman, E., Evans, M., Guerrel, J., … Gratwicke, B. (2020). Spindly leg syndrome in *Atelopus varius* is linked to environmental calcium and phosphate availability. *PLoS ONE, 15*(6 June), 1–11. doi: 10.1371/journal.pone.0235285

Lewis, C. H. R., Richards-Zawacki, C. L., Ibáñez, R., Luedtke, J., Voyles, J., Houser, P., & Gratwicke, B. (2019). Conserving Panamanian harlequin frogs by integrating captive-breeding and research programs. *Biological Conservation*, *236*(May), 180–187. doi: 10.1016/j.biocon.2019.05.029

Margan, S. H., Nurthen, R. K., Montgomery, M. E., Woodwortn, L. M., Lowe, E. H., Briscoe, D. A., & Frankham, R. (1998). Single large or several small? Population fragmentation in the captive management of endangered species. *Zoo Biology*, *17*(6), 467–480. doi: 10.1002/(sici)1098-2361(1998)17:6<467::aid-zoo1>3.0.co;2-3

Martel, A., Van Rooij, P., Vercauteren, G., Baert, K., Van Waeyenberghe, L., Debacker, P., … Pasmans, F. (2011). Developing a safe antifungal treatment protocol to eliminate *Batrachochytrium dendrobatidis* from amphibians. *Medical Mycology*, *49*(2), 143–149. doi: 10.3109/13693786.2010.508185

Martínez Aguirre, T., Calvo, R., Velasco, M. A., Arellano, M. L., Zarini, O., & Kacoliris, F. P. (2019). Re-establishment of an extinct local population of the Valcheta frog, Pleurodema somuncurense, in a restored habitat in Patagonia, Argentina. Conservation Evidence, 16, 48–50.

McFadden, M., Gilbert, D. J., Bradfield, K. S., Evans, M., Marantelli, G., & Byrne, P. G. (2018). The role of ex situ amphibian conservation in Australia. In H. Heatwole & J. J. L. Rowley (Eds.), *Status of conservation and decline of amphibians; Australia, New Zealand, and Pacific Islands* (pp. 298–310). Melbourne: CSIRO Publishing.

McInerney, E. P., Silla, A. J., & Byrne, P. G. (2019). Effect of carotenoid class and dose on the larval growth and development of the critically endangered southern corroboree frog. *Conservation Physiology*, *7*(1), 1–11. doi: 10.1093/conphys/coz009

Mendelson III, J. R. (2018). Frogs in glass boxes: Responses of zoos to global amphibian extinctions. In: G. Rabb (Ed.), *The ark and beyond: The evolution of zoo and aquarium conservation* (pp. 298–310). Chicago, USA: University of Chicago Press.

Michaels, C. J., Antwis, R. E., & Preziosi, R. F. (2014). Impact of plant cover on fitness and behavioural traits of captive red-eyed tree frogs (*Agalychnis callidryas*). *PLoS ONE, 9*(4), 21–23. doi: 10.1371/journal.pone.0095207

Michaels, C. J., Antwis, R. E., & Preziosi, R. F. (2015). Impacts of UVB provision and dietary calcium content on serum vitamin D3, growth rates, skeletal structure and coloration in captive oriental fire-bellied toads (*Bombina orientalis*). *Journal of Animal Physiology and Animal Nutrition*, *99*(2), 391–403. doi: 10.1111/jpn.12203

Michaels, C. J., Downie, J. R., & Campbell-Palmer, R. (2014). The importance of enrichment for advancing amphibian welfare and conservation goals: A review of a neglected topic. *Amphibian and Reptile Conservation*, *8*(1), 7–23.

Michaels, C. J., & Preziosi, R. F. (2015). Fitness effects of shelter provision for captive amphibian tadpoles. *Herpetological Journal*, *25*, 7–12.

Michaels, C. J., & Preziosi, R. F. (2020). Clinical and naturalistic substrates differ in bacterial communities and in their effects on skin microbiota in captive fire salamanders (*Salamandra salamandra*). *Herpetological Bulletin*, *151*, 10–16. doi: 10.33256/hb151.1016

Michaels, C. J., Rendle, M., Gibault, C., Lopez, J., Garcia, G., Perkins, M. W., … Tapley, B. (2018). *Batrachochytrium dendrobatidis* infection and treatment in the salamanders *Ambystoma andersoni, A. dumerilii* and *A. mexicanum*.

Herpetological Journal, *28*(2), 87–92.

Michaels, C. J., Servini, C., Ferguson, A., Guthrie, A., Jayson, S., Newton-Youens, J., … Tapley, B. (2021). The effects of two calcium supplementation regimens on growth and health traits of juvenile mountain chicken frogs (*Leptodactylus fallax*). *Herpetological Journal*, *31*(1), 18–26. doi: 10.33256/31.1.1826

Michaels, C. J., Tapley, B., Harding, L., Bryant, Z., & Grant, S. (2015). Breeding and rearing the critically endangered Lake Oku clawed frog (*Xenopus longipes* Loumont and Kobel 1991). *Amphibian & Reptile Conservation*, *9*(2), 100–110.

Miller, D. L., Rajeev, S., Brookins, M., Cook, J., Whittington, L., & Baldwin, C. A. (2008). Concurrent infection with Ranavirus, *Batrachochytrium dendrobatidis*, and Aeromonas in a captive anuran colony. *Journal of Zoo and Wildlife Medicine*, *39*(3), 445–449. doi: 10.1638/2008-0012.1

Moore, R. D., & Church, D. R. (2008). Implementing the Amphibian conservation action plan. *International Zoo Yearbook*, *42*(1), 15–23. doi: 10.1111/j.1748-1090.2007.00041.x

Narayan, E., & Hero, J.-M. (2011). Urinary corticosterone responses and haematological stress indicators in the endangered Fijian ground frog (*Platymantis vitiana*) during transportation and captivity. *Australian Journal of Zoology*, *59*, 79–85.

Nicolson, D., Tapley, B., Jayson, S., Dale, J., Harding, L., Spencer, J., … Cunningham, A. (2017). Development of in-country live food production for amphibian conservation: the mountain chicken frog (*Leptodactylus fallax*) on Dominica, West Indies. *Amphibian and Reptile Conservation*, *11*(2), 59–68.

Ogilvy, V., & Preziosi, R. F. (2012). Can carotenoids mediate the potentially harmful effects of ultraviolet light in *Silurana (Xenopus) tropicalis* larvae? *Journal of Animal Physiology and Animal Nutrition*, *96*(4), 693–699. doi: 10.1111/j.1439-0396.2011.01197.x

Ogilvy, V., Preziosi, R. F., & Fidgett, A. L. (2012). A brighter future for frogs? The influence of carotenoids on the health, development and reproductive success of the red-eye tree frog. *Animal Conservation*, *15*(5), 480–488. doi: 10.1111/j.1469-1795.2012.00536.x

Pasmans, F., Janssens, G. P. J., Sparreboom, M., Jiang, J., & Nishikawa, K. (2012). Reproduction, development, and growth response to captive diets in the Shangcheng stout salamander, *Pachyhynobius shangchengensis* (Amphibia, Urodela, Hynobiidae). *Asian Herpetological Research*, *3*(3), 192–197. doi: 10.3724/SP.J.1245.2012.00192

Passos, L., Garcia, G., & Young, R. (2021). Do captive golden mantella frogs recognise wild conspecifics calls? Responses to the playback of captive and wild calls. *Journal of Zoo and Aquarium Research*, *9*(1), 49–54.

Passos, L. F., Garcia, G., & Young, R. J. (2018). Comparing the bacterial communities of wild and captive golden mantella frogs: Implications for amphibian conservation. *PLoS ONE*, *13*(10), 1–12. doi: 10.1371/journal.pone.0205652

Passos, L. F., Garcia, G., & Young, R. J. (2017). Neglecting the call of the wild: Captive frogs like the sound of their own voice. *PLoS ONE*, *12*(7), 1–11. doi: 10.1371/journal.pone.0181931

Pessier, A.P., & Mendelson III, J. R. (2017). *A manual for control of infectious diseases in amphibian survival assurance colonies and* *reintroduction programs. Ver. 2.0*. IUCN/SSC Conservation Breeding Specialist Group: Apple Valley, MN.

Pessier, A. P., Baitchman, E. J., Crump, P., Wilson, B., Griffith, E., & Ross, H. (2014). Causes of mortality in anuran amphibians from an ex situ survival assurance colony in Panama. *Zoo Biology*, *33*(6), 516–526. doi: 10.1002/zoo.21166

Poole, V. A., & Grow, S. (2012). Amphibian husbandry resource guide. *Association of Zoos and Aquariums,* (June), 1–118.

Preininger, D., Weissenbacher, A., Wampula, T., & Hödl, W. (2012). The conservation breeding of two foot-flagging frog species from Borneo, *Staurois parvus* and *Staurois guttatus*. *Amphibian and Reptile Conservation*, *5*(3), 45-56(e51).

Rendle, M. E., Tapley, B., Perkins, M., Bittencourt-Silva, G., Gower, D. J., & Wilkinson, M. (2015). Itraconazole treatment of Batrachochytrium dendrobatidis (Bd) infection in captive caecilians (Amphibia: Gymnophiona) and the first case of Bd in a wild neotropical caecilian. Journal of Zoo and Aquarium Research, 3(4), 137–140. doi: 10.19227/jzar.v3i4.112

Rijks, J. M., Saucedo, B., Brunner, J. L., Hick, P., Lesbarrères, D., Duffus, A., … Marschang, R. E. (2018). Report on the 4th International Symposium on Ranaviruses 2017. *Journal of Herpetological Medicine and Surgery*, *28*(1), 13. doi: 10.5818/17-10-131.1

Rodríguez, C. E., & Pessier, A. P. (2014). Pathologic changes associated with suspected hypovitaminosis a in amphibians under managed care. *Zoo Biology*, *33*(6), 508–515. doi: 10.1002/zoo.21161

Rosa, G. M., Cadle, J. E., Crottini, A., Dawson, J., Edmonds, D., Fisher, M.C., Garcia, G., Glaw, F., Glos, J., Harris, R. N., & Köhler, J. (2015).

ACSAM2, A conservation strategy for the Amphibians of Madagascar 2. Abstract book. Torino, Italy: Museo Regionale di Scienze Naturali, Regione Piemonte.

Scheele, B. C., Hunter, D. A., Grogan, L. F., Berger, L., Kolby, J. E., Mcfadden, M. S., … Driscoll, D. A. (2014). Interventions for Reducing extinction risk in chytridiomycosis-threatened amphibians. *Conservation Biology*, *28*(5), 1195–1205. doi: 10.1111/cobi.12322

Shaw, S. D., Bishop, P. J., Harvey, C., Berger, L., Skerratt, L. F., Callon, K., … Speare, R. (2012). Fluorosis as a probable factor in metabolic bone disease in captive New Zealand native frogs (*Leiopelma* species). *Journal of Zoo and Wildlife Medicine*, *43*(3), 549–565.

Silla, A. J., McInerney, E. P., & Byrne, P. G. (2016). Dietary carotenoid supplementation improves the escape performance of the southern corroboree frog. *Animal Behaviour*, *112*, 213–220. doi: 10.1016/j.anbehav.2015.12.012

Tapley, B., Bradfield, K. S., Michaels, C., & Bungard, M. (2015). Amphibians and conservation breeding programmes: do all threatened amphibians belong on the ark? *Biodiversity and Conservation*, *24*(11), 2625–2646. doi: 10.1007/s10531-015-0966-9

Tapley, B., Harding, L., Sulton, M., Durand, S., Burton, M., Spencer, J., … Cunningham, A. A. (2014). An overview of current efforts to conserve the critically endangered mountain chicken (*Leptodactylus fallax*) on Dominica. *Herpetological Bulletin*, (128), 9–11.

Tapley, B., Michaels, C., Harding, L., Bryant, Z., Gill, I., Chaney, N., … Bone, T. D. (2016). *Amphibian Taxon Advisory Group best practice guidelines for the Lake Oku frog* Xenopus longipes. 1–30.

Tapley, B., Michaels, C. J., Gower, D. J., & Wilkinson, M. (2020). The use of visible implant elastomer to permanently identify caecilians (Amphibia: Gymnophiona). *Herpetological Bulletin*, *150*, 18–22. doi: 10.33256/hb150.1822

Tapley, B., Michaels, C. J., Gumbs, R., Böhm, M., Luedtke, J., Pearce-Kelly, P., & Rowley, J. J. L. (2018). The disparity between species description and conservation assessment: A case study in taxa with high rates of species discovery. *Biological Conservation*, *220*, 209–214. doi: 10.1016/j.biocon.2018.01.022

Tapley, B., Rendle, M., Baines, F. M., Goetz, M., Bradfield, K. S., Rood, D., … Routh, A. (2014). Meeting ultraviolet B radiation requirements of amphibians in captivity: A case study with mountain chicken frogs (*Leptodactylus fallax*) and general recommendations for pre-release health screening. *Zoo Biology*, *34*(1), 46–52. doi: 10.1002/zoo.21170

Ujszegi, J., Molnár, K. & Hettyey, A. (2021). How to disinfect anuran eggs? Sensitivity of anuran embryos to chemicals widely used for the disinfection of larval and post-metamorphic amphibians. *Journal of Applied Toxicology*, 41, 387-398.

Vaira, M., Akmentins, M. S., & Lavilla, E. O. (2018). Plan de acción para la conservación de los anfibios de la República Argentina. *Cuadernos de Herpetología*, *32*(supl. 1), 56. doi: 10.31017/CdH.2018.(2018-s1)

Velasco, M. (2018). *Dinámica poblacional y conservación de la ranita del Valcheta (Pleurodema somuncurense) (Cei, 1969), Patagonia, Argentina*. PhD dissertation, Universidad Nacional de La Plata, La Plata, Buenos Aires.

Venesky, M. D., Mendelson III, J. R., Sears, B. F., Stiling, P., & Rohr, J. R. (2012). Selecting for tolerance against pathogens and herbivores to enhance success of reintroduction and translocation. *Conservation Biology*, *26*(4), 586–592. Retrieved from http://onlinelibrary.wiley.com/doi/10.1111/j.1523-1739.2012.01854.x/abstract

Verschooren, E., Brown, R. K., Vercammen, F., & Pereboom, J. (2011). Ultraviolet B radiation (UV-B) and the growth and skeletal development of the Amazonian milk frog (*Trachycephalus resinifictrix*) from metamorphosis. *Journal of Physiology and Pathophysiology*, 2, 34−42.

Walker, S. F., Bosch, J., James, T. Y., Litvintseva, A. P., Oliver Valls, J. A., Piña, S., … Fisher, M. C. (2008). Invasive pathogens threaten species recovery programs. *Current Biology*, *18*(18), 853–854. doi: 10.1016/j.cub.2008.07.033

Whatley, C., Tapley, B., Chang, Y.-M. R., Newton-Yowens, J., Mckendry, D., & Michaels, C. (2020). Impacts of UVB provision on serum vitamin D3, pigmentation, growth rates and total body mineral content in Mallorcan midwife toad larvae (*Alytes muletensis*). *Journal of Zoo and Aquarium Research*, *8*(1), 37–44.

Williams, S. E., & Hoffman, E. A. (2009). Minimizing genetic adaptation in captive breeding programs: A review. *Biological Conservation*, *142*(11), 2388–2400. doi: 10.1016/j.biocon.2009.05.034

Woodhams, D. C., Bosch, J., Briggs, C. J., Cashins, S., Davis, L. R., Lauer, A., … Voyles, J. (2011). Mitigating amphibian disease: strategies to maintain wild populations and control chytridiomycosis. *Frontiers in Zoology*, *8*, 8. doi: 10.1186/1742-9994-8-8

Ziegler, T. (2015). In situ and ex situ reptile projects of the Cologne Zoo: Implications for research and conservation of South East Asia’s herpetodiversity. *International Zoo*

Yearbook, *49*(1), 8–21. doi: 10.1111/izy.12084

Zippel, K., Johnson, K., Gagliardo, R., Gibson, R., McFadden, M., Browne, R., … Townsend, E. (2011). The Amphibian Ark: A global community for ex situ conservation of amphibians. *Herpetological Conservation and Biology*, *6*(3), 340–352.

Chapter 12. Amphibian assisted reproductive technologies and biobanking

Natalie E. Calatayud[1,2], Lachlan G. Howell[3,4,5], Rose Upton[3,5,], Benjamin Tapley[6], Kevin Johnson[7], Robert Browne[8], Ruth Marcec[9], Candace L. Williams[2], David O'Brien[10], Rebecca Hobbs[11], Vance L. Trudeau[12], Deborah S. Bower[13], Simon Clulow[14], John Clulow[3,5], Gina Della Togna[15,16]

[1]Conservation Science Network, 24 Thomas Street, Mayfield NSW 2304, Australia

[2]San Diego Zoo Wildlife Alliance, Beckman Center for Conservation Research, 15600 San Pasqual Valley Road, Escondido, CA 92025, USA

[3]Conservation Biology Research Group, School of Environmental and Life Sciences, The University of Newcastle, Callaghan, NSW 2308 Australia

[4]Centre for Integrative Ecology, School of Life and Environmental Sciences, Deakin University Geelong, Melbourne Burwood Campus, 221 Burwood Highway, Burwood, VIC, 3125, Australia

[5]FAUNA Research Alliance, Kahibah, NSW 2290, Australia

[6]Zoological Society of London, Regent's Park, London, UK

[7]Amphibian Ark

[8]Sustainability America, La Isla Road, Sarteneja, Corozal District, Belize

[9]Honduras Amphibian Rescue and Conservation Center, Honduras

[10]NatureScot (Scottish Natural Heritage), Great Glen House, Leachkin Road, Inverness, IV3 8NW, UK

[11]Taronga Institute of Science and Learning, Taronga Conservation Society Australia, Taronga Zoo, Bradleys Head Road, Mosman NSW 2088, Australia

[12]Department of Biology, University of Ottawa, Ontario Canada, K1S 1G9

[13]Centre for Conservation Ecology and Genomics, Institute for Applied Ecology, University of Canberra, Bruce ACT 2617

[14]School of Environmental and Rural Science, University of New England, Armidale, 2350 NSW

[15]Universidad Interamericana de Panama, Direccion de Investigación, Campus Central, Avenida Ricardo J. Alfaro, Panama

[16]Smithsonian Tropical Research Institute, Panama Amphibian Rescue and Conservation Project, Panama

Abstract

Continued amphibian species and population declines have led to the prioritisation of ex situ conservation breeding programme (CBPs) as one of the major strategies to safeguard and mitigate amphibian extinction. In the original version of the Amphibian Conservation Action Plan (ACAP), assisted reproductive technologies (ARTs) were incorporated as an appendix of the captive breeding programme's chapter, suggesting their application as an innovative and supplementary approach that could enhance the efficacy of CBPs. This updated version of the ACAP includes, for the first time, an entire chapter dedicated to ARTs and Biobanking exclusively. Created by a group of experts in the field, this chapter describes: 1) The current state of amphibian ARTs and biobanking, including hormonal stimulation for gamete release and collection, sperm and egg collection from live animals, sperm recovery form carcasses and wild-caught individuals, biobanking success in producing live animals and health and welfare considerations; 2) The acceptance of ARTs as viable tools for amphibian conservation, their evolution and general recommendations for expanding global amphibian ARTs and; 3) The incorporation of ARTs into a broader conservation action, describing their application in species conservation needs assessments and the incorporation of ARTs and

strategic gamete biobanking into CBP genetic management. The authors of this chapter are optimistic the information relayed here is a great contribution for amphibian conservation since ARTs could facilitate and aid in the preservation of genetic material to manage, augment or rescue populations and species from extinction. As with any ex-situ management strategy, ARTs including biobanking, should complement and support CBPs and habitat management programmes in conjunction with efforts to reduce or remove the pressures that initially led to a species' decline. This complementary conservation approach is recommended by the IUCN.

Introduction: statement and actions – the aim of the Working Group

With amphibians continuing to decline at an alarming rate, the establishment and management of ex situ conservation breeding programmes (CBPs[1]) to safeguard threatened species are of the utmost priority. In the original version of the Amphibian Conservation Action Plan (Gascon et al., 2007) the incorporation of assisted reproductive technologies (ARTs) was proposed as a necessary approach to enhance the efficacy of CBPs. Assisted reproductive technologies include procedures such as the use of hormones, biobanking, in vitro fertilisation to improve breeding success. In this updated version of the ACAP, and as proposed by Della Togna et al. (2020), the inclusion of a dedicated chapter on the role of ARTs will provide evidence of the legitimacy and practicality of their applications to amphibian conservation. Additionally, this chapter will provide up-to-date evidence of the ongoing use and value of ARTs and provide guidance to the broader amphibian conservation community on how these technologies can be incorporated into and complement existing conservation practices. In reviewing the progress of amphibian ARTs to date this document aims to provide the necessary information to establish a future framework for the incorporation of ARTs into existing conservation practises as well as promoting the growth of the ART and Biobanking Amphibian Working Group’s international community. Furthermore, it is acknowledged that outside of the immediate biobanking community, the information set out in this chapter should address the concerns and goals of a diverse set of stakeholders, governmental and non-governmental entities, and the conservation, academic and scientific communities. Incorporating ARTs into programmes and policies could help individuals and organisations to make accurate decisions, balancing the risks and costs of implementation.

As mentioned in the Conservation Breeding Working Group’s chapter and, in alignment with the IUCN’s World Conservation Strategy (Talbot, 1980) and the World Zoo Conservation Strategy (Wheater, 1995), ARTs should not act as the final solution for the management of declining amphibian biodiversity. Rather, ARTs should facilitate and aid the preservation of genetic material to manage, augment, or rescue populations and species from extinction. As with any ex-situ management strategy, ARTs including biobanking should complement and support CBPs and habitat management programmes in conjunction with efforts to reduce or remove the pressures that initially led to a species’ decline. This complementary approach is recommended by the IUCN (Gascon, 2007). Release of individuals following ARTs should conform to the IUCN’s reintroduction and translocation policies (Linhoff et al., 2021). Many approaches improve the management and success of amphibian CBPs. These include induction of spermiation and ovulation through the use of hormonal stimulation, gamete cryopreservation and refrigerated/cold, short-term storage and artificial fertilization (Browne, Wolfram, García, Bagaturov, & Pereboom, 2011; Clulow, Upton, Trudeau, & Clulow, 2019; Della Togna, 2015; Della Togna et al., 2020). However, successful genetic management using strategic biobanking can only be accomplished as part of a multidisciplinary approach in collaboration with all institutional, governmental, and private stakeholders. Therefore, the ASG Assisted Reproduction and Gamete Biobanking Working Group aims to coordinate international, regional, and local efforts for the development and implementation of ARTs for at-risk/threatened amphibian populations around the globe.

The current state of amphibian reproductive technologies and gamete banking

Gamete collection is the cornerstone of ARTs. Optimising protocols for gamete collection can improve the fertilization capability of individuals, artificial fertilization and artificial insemination (for internal fertilising species), and the quality of cryopreserved gametes in order to manage and maintain genetic diversity in CBPs.

In the 1800s, the concept of genome resource banks (GRBs) for cryopreserved gametes was established (Mantegazza, 1866). Commercial needs have driven major advances in ART protocols in fish aquaculture (Tiersch, Yang, Jenkins, & Dong, 2007; Walters, Benson, Woods, & Critser, 2009), agriculture, birds (Blesbois, 2007), mammals (Walters et al., 2009) 2009), and humans (Sherman, 1980; Walters et al., 2009). The uptake of GRBs in conservation has been slower, and despite catastrophic amphibian declines, the utility of biobanks for this class was not acknowledged until recently, where the importance of its development and application has become evident (Gascon, 2007).

In amphibians, protocols for cryopreserved gametes have been applied to in vitro fertilisation with free swimming sperm (reported for some species, both with fresh or cryopreserved sperm), cloning, and intracytoplasmic sperm injection (ICSI), but further refinements and improvements in protocols are needed to complement the conservation efforts. Here we review the current advances to date in amphibian ARTs.

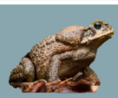
Hormonal stimulation for gamete release

Several amphibian studies have demonstrated the successful use of exogenous hormones for the collection of spermatozoa from Anura and Caudata. The most utilised hormones include peptides such as gonadotropin-releasing hormone agonist (GnRH-A [des-Gly10, D-Ala6, Pro-NHEt9]), human Chorionic Gonadotropin (hCG), and combinations of GnRH-A and dopamine antagonists such as metoclopramide, domperidone, or pimozide (Browne et al., 2019; Clulow et al., 2018; Della Togna et al., 2017; Silla & Langhorne, 2021; Silla, McFadden, & Byrne, 2019; Vu & Trudeau, 2016). Figure 12.1 shows some examples of successful hormonal treatments on amphibians (Della Togna et al., 2020).

The most commonly used methods of hormone administration are via injection either intraperitoneal, subcutaneous, or intramuscular. These techniques are minimally disruptive and provide the most rapid and effective delivery method reported to date. However, they do require basic training as they are considered ‘invasive’. In the USA, these procedures are categorised by the Institutional Animal Care and Use Committee (IACUC) [SW2] [GDT3] as a category “C” as they do not cause more than momentary or slight pain or distress and do not require the use of pain-relieving drugs (Federal Animal Welfare Regulations [CFR Ch.1, 2.36(b) (5-7), (Albus, 2012)]; however, the categorisation of these types of procedures will vary globally and even between institutions so it is up to researchers to inform themselves as to local procedural requirements.

		Species	Hormone induction	Cryopreservation	Stimulation method
EUROPE		*Pelohylax lessonae* (Uteshev et al., 2012)	GnRH 0.5 µg/µL bw	24% DMFA and 20% sucrose	Intraperitoneal injection
		*Rana temporaria* (Mansour et al., 2010; Uteshev et al., 2012; Kaurova etl al. 2021)	1.20 µg/g bw	15% DMSO ; glycerol and sucrose	Intraperitoneal injection
		*Rana sylvatica* (Constanzo et al., 1998; Beesley et al.,1998)	GnRH 50 µg/g	12% DMFA + 10% sucrose	Intraperitoneal injection
		*Epidalea calamita* (Arregui et al. 2020)	hCG 10 IU/g	10% DMFA and 10% sucrose	Intraperitoneal injection
		*Bufo bufo* (Uteshev et al., 2012)	1.20 µg/g bw	15% DMSO	Intraperitoneal injection
		*Pleurodeles waltl* (Uteshev et. al., 2015)	500 IU hCG, 0.025 + 0.1 µg/g	N/A	N/A
AFRICA		*Xenopus laevis and X. tropicalis* (Sargent and Mohun 2005; Mansour et al., 2009)	300 IU hCG	15% DMSO + 10% sucrose; 20% egg yolk and 0.8M sucrose + 20mM	N/A
ASIA		*Andrias davidianus* (Peng et al., 2011)	500 IU hCG & 0.025 + 0.1 µg/g bw	5 – 25% DMSO	N/A
		*Tylototriton kweichowensis* (Guy et. al., 2020)	Prime GnRH 0.025 µg/g bw & spermiation dose GnRH 0.1 µg/g bw (24 hr later)	10% DMSO + BSA 1% + w/wo 10% Trehalose	Intramuscular injection
		*Notophthalmus meridionalis* (Guy et. al., 2020)	Prime GnRH 0.025 µg/g bw & spermiation dose GnRH 0.1 µg/g bw (24 hr later)	10% DMSO + BSA 1% + w/wo 10% Trehalose	Intramuscular injection
NORTH AMERICA		*Rana muscosa* (Calatayud & Jacobs et al., manuscript in preparation)	0.3 – 3 µg/µL GnRH; 5, 10 IU/g hCG or combination 0.4, 0.6 µg/g GnRH with 5 & 10 IU/g hCG	10% trehalose + 10% DMFA	Intraperitoneal injection
		*Rana sylvatica* (Mugnano et al., 1998)	GnRH 2 µg / g bw	Testes macerates: 0.5M DMSO + 50% FBS v/v; 150 mmol Glycerol or (Constanzo et al., 1998)	Intraperitoneal injection
		*Rana pipiens* (Hopkins and Herr, 2008)	N/A	Testes macerates: 12% DMFA + 10% sucrose (Constanzo et al., 1998)	N/A
		*Rhaebo guttatus* (Hinkinson et al., 2019)	GnRH 0.4 ug/g GnRH + hCG 7.5, 10 IU/bw	DMFA (24%) + sucrose (20%)	Intraperitoneal injection
		*Anaxyrus boreas boreas* (Roth et al., 2010; Langhorne et al., 2021)	10 IU/g, 10 IU/g + 0.4 ug/g GnRH; GnRH 0.4 ug/g; GnRH 0.4 ug/g + 10 ug/g (Amphiplex)	0.5M trehalose + 10% DMFA	N/A
		*Anaryxus americanus* (Obringer et al., 2000; Kouba et al., 2012)	300 IU total; 4 ug total (intraperitoneal; subcutaneously; ventral & dorsal absorption)	Testes macerates: 0.5M DMSO + 50 % FBS v/v (Beesley et al. 1998)	Nasal & intraperitoneal injection
		*Anaryxus fowleri* (McDonough et al., 2016; Julien et al., 2019)	300 IU total; 4 ug total (injection); 20 ug total (nasal)	Testes macerates: 0.5M DMSO + 50 % FBS v/v (Beesley et al. 1998)	Nasal & intraperitoneal injection
		*Ambystoma tigrinum* (Marcec, 2016; Gonzalez, 2018)	hCG 500 IU; priming dose GnRH 0.025 µg/g bw + spermiation dose 0.1 µg/g bw	5 % DMSO + 0.5 % BSA	Intraperitoneal injection

148

		Species	Hormone induction	Cryopreservation	Stimulation method
NORTH AMERICA		*Ambystoma laterale* (Marcec pers. comm)	GnRH 0.5 μg/μL bw	N/A	N/A
NORTH AMERICA		*Ambystoma mexicanum* (Mansour et al., 2011; Rivera-Pacheco et al., 2021)	hCG 100 - 200 IU; Ovopel (GnRH 10 – 15 ug + 2.5 – 3 mg MET)	6% DMA	Intramuscular injection
NORTH AMERICA		*Cryptobranchus alleganiensis* (McGinnity et al., 2021)	GnRHa 0.4 μg/g bw + MET 10 μg/μL (Amphiplex)	10% DMSO	Intraperitoneal injection
NORTH AMERICA		*Necturus maculosus; Necturus beyeri* (Stoops et al., 2014; Calatayud et al., 2019)	GnRH 0.5 μg/μL bw	N/A	Intraperitoneal injection
CENTRAL AND SOUTH AMERICA		*Bufo marinus* (Browne et al., 1998)	hCG ; GnRHa 0.4 μg/g bw + MET 10 μg/μL (Amphiplex)	15% Me2SO + 10% sucrose; Testes macerates: 15% DMSO + 10% sucrose; 20% Glycerol + 10% sucrose	N/A
CENTRAL AND SOUTH AMERICA		*Atelopus zeteki* (Della Togna et al., 2015)	GnRH 4 μg/g bw; GnRHa 0.4 μg/g bw + MET 10 μg/μL (Amphiplex); 10 IU hCG	10% DMFA + 10% Trehalose	Intraperitoneal injection
CENTRAL AND SOUTH AMERICA		*Atelopus limosus* (Della Togna et al., 2020)	2.4 μg/g bw GnRH; GnRHa 0.4 μg/g bw + MET 10 μg/μL (Amphiplex); 10 IU hCG; GnRH 4 μg/g bw	10% DMFA + 10% Trehalose	Intraperitoneal injection
CENTRAL AND SOUTH AMERICA		*Atelopus certus* (Della Togna et al., 2020)	GnRH 4 μg/g bw	10% DMFA + 10% Trehalose	Intraperitoneal injection
CENTRAL AND SOUTH AMERICA		*Atelopus glyphus* (Della Togna et al., 2020)	GnRH 4 μg/g bw	10% DMFA + 10% Trehalose	Intraperitoneal injection
CENTRAL AND SOUTH AMERICA		*Atelopus varius* (Della Togna et al., 2020)	GnRH 4 μg/g bw	10% DMFA + 10% Trehalose	Intraperitoneal injection
CENTRAL AND SOUTH AMERICA		*Eleutherodactylus coqui* (Mann et al., 2010)	2M DMSO, 2M glycerol or 2M	2M DMSO, Glycerol with 2M sucrose	Intraperitoneal injection
CENTRAL AND SOUTH AMERICA		*Strabomantis bufoniformis* (Della Togna et al., 2020)	GnRH 4 μg/g bw	10% DMFA + 10% Trehalose	Intraperitoneal injection
CENTRAL AND SOUTH AMERICA		*Trypion spinosus* (Della Togna et al., unpublished)	2 μg/g bw GnRH; 5, 10 IU/g bw hCG	N/A	Intraperitoneal injection
CENTRAL AND SOUTH AMERICA		*Craugastor evanesco* (Della Togna et al., manuscript in preparation)	GnRH 4 μg/g bw	N/A	Intraperitoneal injection
CENTRAL AND SOUTH AMERICA		*Ceratophrys ornate, C. cranwelli* (Trudeau et al., 2010)	GnRHa 0.4 μg/g bw + MET 10 μg/μL (Amphiplex)	N/A	Intraperitoneal injection
CENTRAL AND SOUTH AMERICA		*Odontophrynus americanus* (Trudeau et al., 2010)	GnRHa 0.4 μg/g bw + MET 10 μg/μL (Amphiplex)	N/A	Intraperitoneal injection
AUSTRALIA		*Pseudophryne pengilleyi* (Silla et al., 2018)	hCG 40 IU/g	N/A	Intraperitoneal injection
AUSTRALIA		*Litoria fallax* (Upton et al., 2018)	hCG 20 IU/g	15% Me2SO + 1% sucrose	Intraperitoneal injection

149

	Species	Hormone induction	Cryopreservation	Stimulation method
AUSTRALIA	*Litoria raniformis* (Mann et al., 2010)	GnRH 1µg/µL bw	N/A	Intraperitoneal injection
AUSTRALIA	*Litoria aurea* (Upton et al., 2021)	hCG 20 IU/g	15% Me2SO + 1% sucrose	Intraperitoneal injection
AUSTRALIA	*Litoria castanea* (Hobbs et al. unpublished)	hCG 20 IU/g	0.5M trehalose + 10% DMFA	Intraperitoneal injection
AUSTRALIA	*Litoria caerula* (Clulow et al., 2018)	hCG 60, 100, 300 IU total	0.5M trehalose + 10% DMFA	Intraperitoneal injection
AUSTRALIA	*Litoria boorolongensis* (Silla et al., 2019)	hCG 20 IU/g	0.5M trehalose + 10% DMFA (Hobbs et al., unpublished)	Intraperitoneal injection
AUSTRALIA	*Mixophyes fasciolatus* (Clulow et al., 2018)	hCG 60, 100, 300 IU total	0.5M trehalose + 10%, 5% DMFA	Intraperitoneal injection
AUSTRALIA	*Litoria chloris* (Clulow et al., 2018)	hCG 60, 100, 300 IU total	0.5M trehalose + 10%, 5% DMFA	Intraperitoneal injection

Figure 12.1. A summary of exogenous hormone treatments reported in the literature used for the induction of spermiation in anurans and caudates. When available, cryopreservation treatments have also been identified. The most commonly reported mode of hormone administration is intraperitoneal injection; however, some species have also been successfully stimulated using topical application, subcutaneously and intra-muscular injections. Species were assigned to the continent of origin, not the location where the study took place. The figure does not show all the species reported in the literature.

In recent years, other forms of administration not requiring injection have been tested. These alternative methods include topical, oral ingestion, and nasal dripping and have been successfully tested in six anuran species (*Anaxyrus americanus, A. baxteri, A. valliceps, A. fowleri, Pseudophryne pengilleyi* and, *Xenopus laevis)* (Obringer et al., 2000; Ogawa, Dake, Iwashina, & Tokumoto, 2011; Rowson, Obringer, & Roth, 2001; Silla, Roberts, & Byrne, 2020). However, it is important to highlight that while hormonal administration through non-injectable methods requires less training and is less invasive, a basic knowledge of endocrinology is necessary to know how and when to apply these hormones. Furthermore, the success of all these studies have required the use of much higher concentrations of hormones compared to those used through injection, and had much lower rates of efficacy compared to injections, most likely due to partial absorbance. Therefore, the disadvantages of using non-injectable methods would imply that trading momentary discomfort for a far greater financial investment and the need to safely dispose of water containing hormones, does not offer viable alternative strategies to the traditional injection approach, unless they are used in instances where there is a restriction to the use of injections because of the size of the animals (Della Togna et al., 2020). Topical use of GnRH-A has been reported in only one species of caudate (*Eurycea rathbuni*) with successful increase in gamete production and breeding behaviour from both sexes post application (Glass Campbell, Anderson, & Marcec-Greaves, 2022). One study has successfully collected eggs from *Xenopus laevis* through non-invasive stimulation using progesterone and estradiol dissolved in water (Ogawa et al., 2011).

Gamete collection

Sperm and egg collection from live animals

Hormonal stimulation for gamete collection via injection has been successfully implemented in a number of amphibian species (Figure 12.1; Table 12.1). Sperm has been collected with different concentrations of hCG, GnRH, GnRH with hCG and, GnRH + dopamine antagonists (such as Amphiplex) in several species of caudates and anurans, some of which are shown in Figure 12.1. Non-invasive methods such as oral, dermal, or topical administration have also resulted in the successful collection of gametes for 5 anuran species using hCG and GnRH (Julien et al., 2019; Obringer et al., 2000; Rowson et al., 2001; Silla et al., 2019). Additionally, oocyte collection has been more challenging than sperm, but, nevertheless, successful collections have occurred with the use of different concentrations of hCG, GnRH, and GnRH with hCG, GnRHa with Metacloromide (Amphiplex), Follicle-stimulating hormone (FSH), pituitary extract (PE), pregnant mare serum gonadotropin (PMSG), Testosterone (T), corticosterone (C), Domperidone (D), Pimozide (P) and Lucrin to name a few (Table 12.1).

To date, most hormonally induced sperm and egg collections have been accomplished by the implementation of empirically developed protocols, or replicating those reported successful for other species, without further exploring if, in fact, those are the optimum protocols for new target species. Ideally, hormonal stimulation protocols should be standardised in a species-specific manner (for males and females), taking into consideration the identification of the best working hormones and concentrations, gamete concentration, quality and viability, sperm production peaks and oviposition timepoints (Della Togna, et al., 2020).

Table 12.1**.** A summary of amphibian species and corresponding exogenous hormones protocols used to induce ovulation.

					Administration				
Species	**Hormone**	**Procedure**	**Priming compound**	**Priming dose reported**	**Number of priming doses**	**Timing (hr prior to ovulatory dose)**	**Compound(s) administered for final ovulatory/oviposition**	**Doses**	**Reference**
Peltophryne lemur	GnRH & hCG	IP	hCG	1.5 IU/g	2	hCG - 48	GnRH; hCG; GnRHa + hCG	0.2 µg; 4 IU; 0.5 µg + 4 IU	Burger et al. 2021
Rana muscosa	Amphiplex, Lut	IP	GnRHa (des-Gly$_{10}$, D- Ala$_{6}$, Pro-NHEt$_{9}$-GnRH)	0.4 µg/g	1	24	GnRH + MET	1 x 0.4 µg/g + 10 µg/g	Calatayud et al., 2019
	PGF$_{2}\alpha$	IM	PGF$_{2}\alpha$	5 ng/g	1	48	PGF$_{2}\alpha$	5 ng/g	
Anaxyrus boreas boreas	hCG, GnRH	IP	hCG	3.7 IU/g	2	96, 24	hCG + GnRHa	13.5 IU/g + 0.4 µg/g	Calatayud et al., 2015
Acris crepitan	Amphiplex	added to water (10 mL)	None	None	0	na	GnRH + MET	0.17 µg + 0.42 µg / µl	Snyder et al., 2012
Lithobates pipiens	Amphiplex	IP	None	None	0	24	GnRH + MET	1 x 0.4 µg/g + 10 µg/g	Trudeau et al., 2010
Ceratophyrs ornata	Amphiplex	IP	None	None	0	24	GnRH + MET	1 x 0.4 µg/g + 10 µg/g	
Ceratophrys cranwelli	Amphiplex	IP	None	None	0	24	GnRH + MET	1 x 0.4 µg/g + 10 µg/g	
Odontophrynus americanus	Amphiplex	IP	None	None	0	24	GnRH + MET	1 x 0.4 µg/g + 10 µg/g	
Rana sevosa	hCG, GnRH	IP	hCG	3.7 IU/g	2	96 , 24	GnRH + hCG	1 x 0.4 µg/g + 13.5 IU/g	Graham et al., 2018
Eleutherodactylus coqui	Fish, avian, mammalian & GnRH (D-Ala, des-Gly, eth LHRH), hCG	SC	mLHRH; aLHR; fLHRH; GnRHa; hCG	None	0	na	mLHRH; aLHR; fLHRH; GnRHa; hCG	7µg, 33µg; 28µg; 7µg, 20µg; 5, 10, 15, 20 µg; 165 IU	Michael et al., 2004
Pseudophryne guentheri	GnRH		GnRHa	0.4 µg/g	1	26	GnRHa with or without prime	0.4 µg/g	Silla, 2010
Pseudophryne corroboree	Lucrin	SC	Lucrin	1 µg	1	26	Lucrin	5 µg	Byrne & Silla, 2010
Pseudophryne pengilleyi	GnRHa GnRH (D-Ala, des-Gly, eth LHRH)	TA	None	None	0	na	GnRHa	0.5 -2.0 µg/g	Silla et al., 2018
Pseudophryne bibronii	GnRHa GnRH (D-Ala, des-Gly, eth LHRH)	IP	GnRHa	0.4 µg/g	1	24	GnRHa	2 µg/g	Silla & Byrne, 2021
Pseudophryne caoriacea			GnRHa	0.4 µg/g	1	24	GnRHa	2 µg/g	
Helioporus eyrei			GnRHa	0.4 µg/ g	1	24	GnRHa	2 µg/g	
Necturus beyeri	[des-Gly$_{10}$, D- Ala$_{6}$]-LhRH-RH ethylamide acetate salt hydrate	IP	None	None	0	na	GnRHa	100 µg / 500 µL	Stoops et al., 2014
Litoria raniformis	des-Gly$_{10}$, D- Ala$_{6}$-[LHRH]	SC	None	None	0	na	des-Gly10, D- Ala6-[LHRH]	50 µg	Mann et al., 2010
Anaxyrus fowleri	GnRH, hCG, P$_4$	IP	hCG	3.7 IU/g					Browne et al., 2006
Ambystoma mexicanum	Follicle-stimulating hormones	IM	None	None	0	na	FSH	400IU	Trottier and Armstrong, 1974
Xenopus laevis	hCG & P$_4$	added water; IP	PMSG, hCG						Gillis et al. , 2021; Wlizla et al., 2016
Ambystoma tigrinum	hCG, LH								
Anaxyrus baxteri	hCG, GnRHa, P$_4$	IP	hCG + GnRHa	100 IU + 0.8 µg	1	72	hCG + GnRHa	100 IU + 0.8 µg	Browne et al., 2006
Lithobates pipiens	Pituitary extract (PE), P4, testosterone (T), corticosterone [C], Amphiplex, domperidone (D)	SC, IP	None	None	0	na	PE, PE+T, PE+P4, PE+C; Amphiplex, GnRH + D	~100 IU (LHRH) in 1 mL; PE+0.002µg/µL; PE+0.01mg/50mL; PE+0.1mg/50mL; 0.4 µg/g + 10 µg/g; 0.4 µg/g + D	Wright, 1961; Fort, 2000; Trudeau et al., 2013
Lymnodynastes tasmeniensis	Pituitary extracts, hCG, GnRHa, Pimozide	IP	GnRHa	0.9-1.2µg/g + Pimozide 10 µg/g	1	20	PE; PE + hCG; GnRH + PZ	PE vol; PE vol + 100 IU hCG; GnRH (0.9-1.2µg/g) + Pimozide (10µg/g)	Clulow et al., 2018
Litoia aurea	GnRH	IP	GnRHa	10 µg	1	72	GnRHa + hCG	20 µg + 300 IU	Clulow et al., 2018
Mixophyes fasciolatus	hCG & PMSG	SC	PMSG, hCG	50 IU & 25 IU; 1x100 IU	2; 2	PMSG-144 & 96; hCG-24	hCG	100IU	Clulow et al., 2012

Sperm recovery from carcasses

Testicular sperm sampling is usually achieved by euthanasia, followed by maceration of the testes, sperm analysis, and storage (either refrigerated or cryopreserved) for its immediate or later use. In cases where gamete recovery is part of a conservation strategy, euthanasia is not recommended; however, opportunistic sperm collection may be possible in instances where animals have died naturally or have had to be euthanised for medical reasons. Researchers must ensure dead animals are sufficiently intact and fresh, to ensure that an adequate quality sample can be obtained. Regardless of the environment and situation, this approach requires rapid detection and processing of the carcass to yield the highest quality gametes possible. Where samples may be collected opportunistically due to the death of a captive animal, a high degree of coordination between institutional departments (e.g. husbandry, reproductive biology, and pathology staff) is required to ensure timely processing and successful gamete recovery. We recommend establishing these communications before embarking on any collection, thereby ensuring all internal and external permitting and bureaucratic requirements are cleared, since any delay related to this process may result in the loss of valuable viable cells. In addition to opportunistic collection of testes from recently deceased animals, it is recommended that coordination with researchers for planned euthanasia also occurs. For example, euthanasia of type specimens or other common species used in approved research are sources of gametes that could be collected.

Sperm collection from wild-caught individuals

An important conservation strategy, particularly in the management of ex-situ populations, is preventing or mitigating the loss of genetic variability. The introduction of diverse genes into captive or inbred populations through in-situ gamete collection of wild individuals or populations increases the resilience of the rescued population without increasing the number of individuals in it. Additionally, these approaches can be complemented by using sperm cryopreserved in-situ as a potentially low-cost, spatially conservative, and long-term strategy to manage genetic diversity of CBPs. Equipment and resources that are cost-effective and are adaptable are optimal for use in the field and methods t should include some key considerations:

1. Knowledge of the best timing of when samples should be collected (e.g. peak concentrations and sperm quality parameters).
2. Use of effective and established cryopreservation protocols that have been pre-tested on the target species or a close relative).
3. Knowledge of field site accessibility to inform whether the operation requires a fully independent mobile laboratory facility, reduced capacity mobile laboratory facility or a field-kit only approach (see Della Togna et al., 2020 for specifics).
4. Implementation of established biosecurity protocols.

Biobanking success: producing viable offspring

Biobanking is a multi-decadal strategy that has been used to store biological samples for research and conservation of genetic information for a number of taxonomic groups by cryopreservation (Hewitt & Watson, 2013). To date, amphibian cryopreservation remains limited mostly to sperm and cell lines because of the large size, composition, and volume of oocytes, eggs, and embryos. Further technologies have been proposed to tackle the logistical challenges facing cryopreservation of the maternal lineage but will not be expanded upon in this chapter and we refer the reader to some of the following references for more detail (Browne et al., 2019; Clulow & Clulow, 2016; Clulow et al., 2019; Zimkus, Hassapakis, & Houk, 2018). Since the ACAP was published in 2007, papers citing sperm cryopreservation have been published for 41 species (35 Anurans and 6 Caudata) (Figure 12.1).

Six (and one sub-species) of the 41 biobanked species known to us represent salamanders, and include *Cryptobranchus alleganensis* (Peng, Xiao, & Liu, 2011; Unger, Mathis, & Wilkinson, 2013), *Ambystoma mexicanum* (Figiel, 2013) *Ambystoma tigrinum* (Gillis, Allen, & Marcec-Greaves, 2020; González, 2018; Marcec, 2016), *Notophthalmus meridionalis, Tylototriton kweichowensis* (Guy, Gillis, et al., 2020) and *Andrias davidianus* (Browne et al., 2019; McGinnity, Reinsch, Schwartz, Trudeau, & Browne, 2022). No caecilian species have been reported in biobanks to date (Figure 12.1).

Few publications report post-thaw artificial fertilization (Burger et al., 2021; Langhorne, 2016; Marcec, 2016; McGinnity et al., 2022; Upton et al., 2021; Upton, Clulow, Mahony, & Clulow, 2018) and truly demonstrate the biological competence of frozen amphibian sperm with the production of viable F1 individuals. Studies which reportedly produced offspring that successfully metamorphosed after artificial fertilisation include: *Anaxyrus boreas boreas, Lithobates sevosa* (Langhorne, 2016) and *Ambystoma tigrinum* (Marcec, 2016), *Litoria aurea* (Upton et al., 2021), *Litoria fallax* (Upton et al., 2018) and *Cryptobranchus alleganiensis* (Peng et al., 2011; Unger et al., 2013). Yet only two studies have demonstrated the reproductive fitness of those offspring, the *L. aurea* and *L. fallax* males produced by cryopreserved sperm reached sexual maturity and were capable of sperm production while ultrasounds showed that the two *L. aurea* females produced had reached sexual maturity and were gravid (Upton et al., 2021, 2018).

Health and welfare considerations

The health of an animal must be taken into consideration when preparing for ARTs. Certain species may be unable to withstand the stress of procedures such as sperm or egg collection.

Although at present, there is no evidence that the principal hormones used in ART directly cause toxicity or health complications in amphibians, the application of exogenous hormones should be done under careful consideration and consultation with trained personnel. Since hormonal control of amphibian reproduction is often species-specific (Norris, 2004; Ogielska & Bartmanska, 2009), caution is recommended when applying hormones to any species for the first time (Clulow et al., 2019; Silla, Calatayud, & Trudeau, 2021). To date, a few studies suggest that collection frequency can affect sperm quality in at least one anuran species (Guy, Martin, Kouba, Cole, & Kouba, 2020; McDonough, Martin, Vance, Cole, & Kouba, 2016) and overall animal health (Green, Parker, Davis, & Bouley, 2007; Wright & Whitaker, 2001). Contrasting studies suggest that while the effects of long-term or repeated exogenous hormone treatment may cause liver and kidney damage (Chai, 2016), with the appropriate attention, ARTs can provide benefits to animal health. In a captive setting, full or partial egg retention (dystocia) may occur in female amphibians when husbandry parameters are not ideal. Egg retention that does not resolve may follow in a multitude of secondary health complications that may result in death. However, in the event of egg retention, exogenous hormones can be administered to promote egg deposition (Calatayud, Chai, Gardner, Curtis, & Stoops, 2019; Chai, 2016; Wright & Whitaker, 2001). Furthermore, the use of cryopreservation in conjunction with hormone-induced gamete collection, allows for decreased transportation of animals from the wild, or between breeding colonies, which eliminates transport-induced stress and potentially life-threatening situations (Della Togna et al., 2020; Langhorne, 2016). ARTs also allow for improved long-term management of genetics and the prevention of inbreeding (Byrne, Gaitan-Espitia, & Silla, 2019; Howell, Mawson, et al., 2021; Silla et al., 2021) while offering greater potential of good health and high survivability in offspring.

Acceptance of ARTs as viable conservation tools

Evolution of ARTs as amphibian conservation tools

It is beyond the scope of this document to present information on the technical details of the emerging technologies that could be applied to amphibian conservation. A number of approaches have been reviewed by other authors and are referenced in this section. The future of ARTs relies on how these technologies will overcome the difficulties conservationists face with managing amphibian genome resources while preserving the highest genetic diversity (Clulow et al., 2019; Holt, Pickard, & Prather, 2004; Mastromonaco & Songsasen, 2020). Cloning (somatic cell nuclear transfer) is probably the most well-known technology resulting in the production of live young, but despite its success, it has not been incorporated into amphibian conservation. First described in an amphibian species, *Rana pipiens* and later *Xenopus laevis*, cloning was implemented to explore the fundamentals of developmental biology (reviewed by Gurdon & Byrne, 2002). Reproductive cloning followed shortly after when Gurdon (1968) reported the production of normal adult clones (individuals derived from nuclear transplantation that are identical to the parent). A suite of approaches is now available to support conservation across a number of taxonomic groups, particularly mammals (Mastromonaco & Songsasen, 2020). Cell transplantation (primordial and spermatogonial) may provide alternate sources of genetic material of a wild or threatened endangered species compared to sperm and oocytes alone. Through reprogramming and regeneration, cells can diversify into renewable and operational genetic material of infinite potential (Clulow & Clulow, 2016; Mastromonaco, González-Grajales, Filice, & Comizzoli, 2014; Mastromonaco & Songsasen, 2020). Somatic cell technologies also offer promise since their use precludes the need for viable gametes, thereby enabling genetic contribution of individuals that are reproductively dysfunctional or perish before reaching sexual maturity and fail to contribute to the gene pool (Mastromonaco et al., 2014).

General recommendations for expanding global amphibian ARTs

The 2020 global COVID-19 pandemic revolutionised work practises and this has once again transformed the manner in which training can be delivered to a wide and diverse group of users. Web-based communication will be instrumental in training but will still be limited by the number of people who have access to specific softwares and the internet. In many cases, increasing sustainable conservation will still require the considerations outlined by Della Togna et al. (2020) and at its core, fundamentally still needs the existence of human resources that can be trained and are backed by adequate resources with which to perform ARTs. The basic recommendations for increasing ARTs on-site, for sustainable conservation are:

1. Gamete collection and cryopreservation protocols tested for broad taxa.
2. Identification of biobanks in different regions of the globe that have secured long-term funding (Figure 12.2).
3. International (Nagoya Protocol (Kamau, Fedder, & Winter, 2010)) and national laws and policies that allow and facilitate the collection of gametes from existing CBPs or from the wild, transportation, and storage of biological materials.
4. Access to collection sites using local knowledge and expertise, taking into account that many species are located in or near indigenous communities and protected areas, each with particular restrictions.
5. Country policies on access to genetic resources allow such large-scale operations and have sustainable funding in place for long-term preservation of the collections.

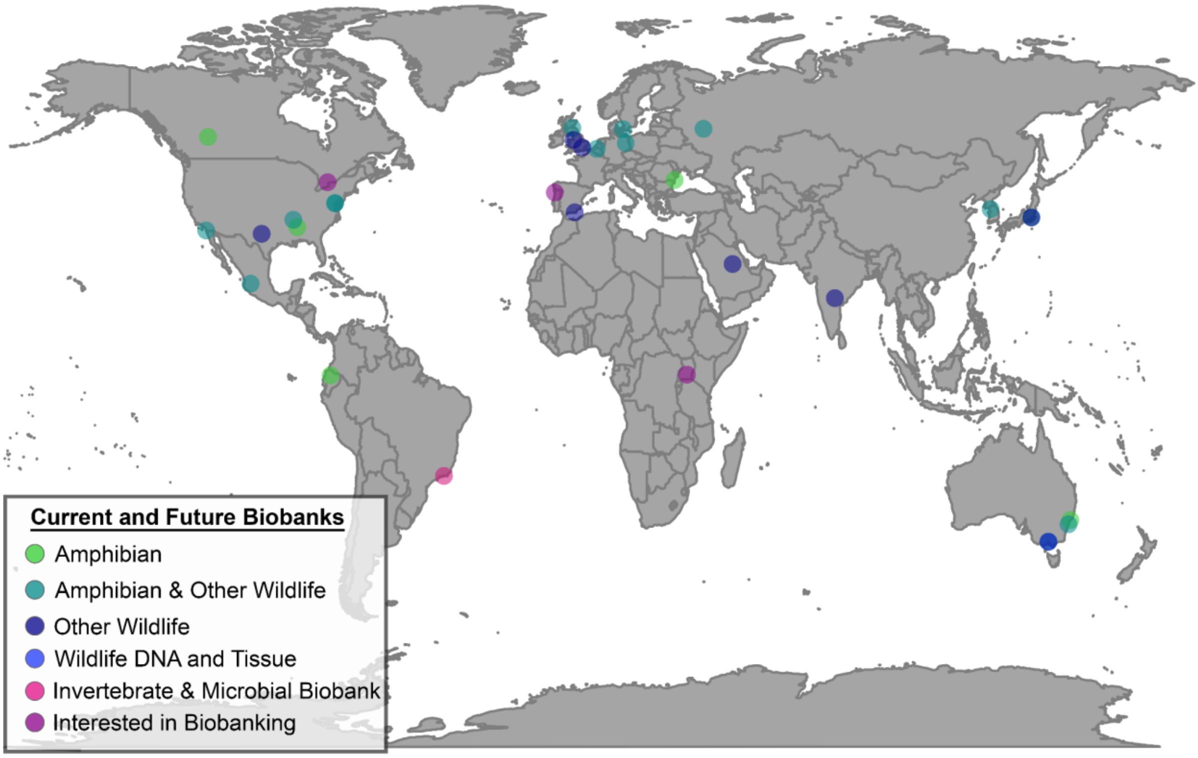


Figure 12.2. Location of known biobanks for wildlife species. Data collected by the ASG Amphibian ARTs and Biobanking Working Group from a survey conducted from 2018-2021.

Incorporating ARTs into broader conservation action: Informing effective management

Conservation Needs Assessments

The Amphibian Ark works with a range of amphibian field biologists and other experts to develop Conservation Needs Assessments (CNAs) for amphibian species, which in turn generate a range of recommended strategies, including ex-situ conservation actions (Johnson et al., 2020). This evaluation and prioritization help conservation managers to maximise the impact of limited conservation resources by identifying which amphibian species are most in need and are likely to receive the most benefit from particular types of conservation action. Biobanking is one of the conservation actions and is recommended for species which are under imminent danger of extinction (locally or globally) because the threats they face cannot or will not be reversed in time to prevent likely species extinction. They, therefore, require ex-situ management, or rescue, as part of an integrated program to ensure their survival. To date, CNAs have been completed for 3,461 amphibian species, out of which 372 have been recommended for biobanking (refer to Conservation Needs Assessments, 2021) (Figure 12.3). CNAs are one of the few conservation assessment tools which generate prioritised lists of species for biobanking, and as such, provide not only a logical and transparent procedure for guiding amphibian conservation activities within a country or region, but also a good reference for those involved with ARTs when considering species which should be targeted for biobanking (Figure 12.3). The Amphibian Ark recommends that detailed and collaborative species actions plans should be jointly developed by all relevant stakeholders for species which are considered for ex-situ rescue (Amphibian Ark, 2020), and the use of ARTs and gamete cryopreservation should be considered when appropriate in these action plans. Further detail on planning can be found within the Species Conservation Planning chapter.

Feasibility and design (strategy): Incorporation of ARTs and strategic gamete biobanking into CBP programmes

The integration of biobanking and ARTs into genetic management programmes has long been realised for agriculturally important animal and plant species yet continues to lag for wildlife species. This is likely due to a multitude of factors that differ between conservation management programs and these for-profit industries, including: access to sustainable financial resources and infrastructure, clear species prioritisation, need for taxa- or species-specific protocols, coordinated stakeholder engagement, and government support. Wildlife biobanking is a long-term genetic management strategy that requires all of these factors to work in concert and be dynamic and responsive to evolution in needs, technologies and management strategies over long-time scales; timescales that may reflect many generations of the target species.

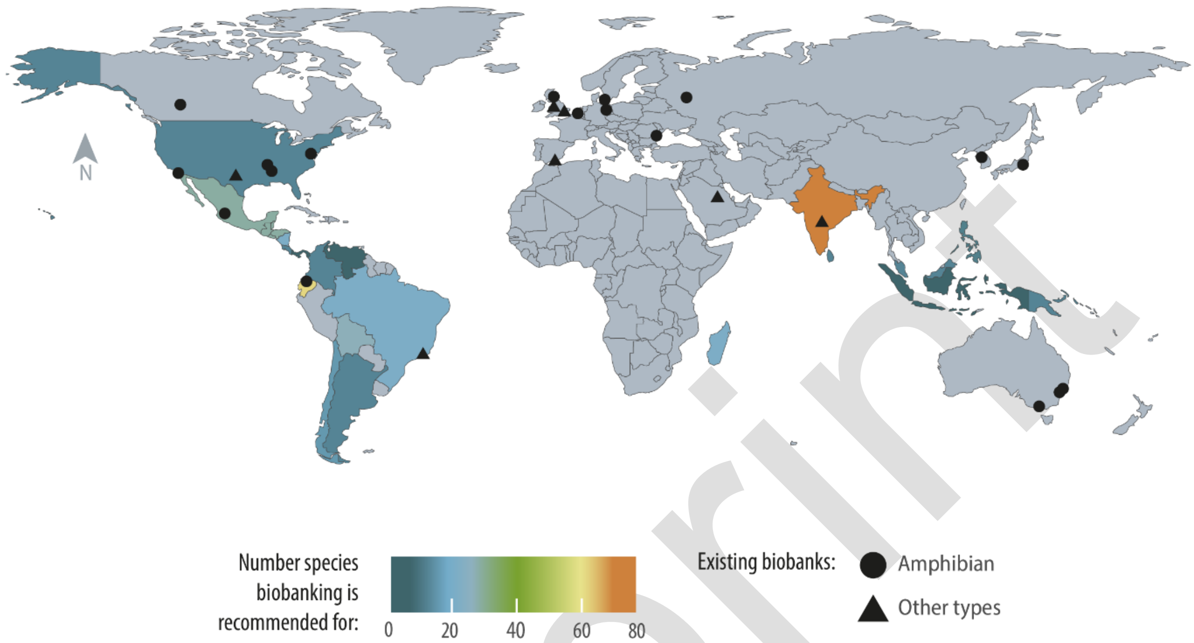


Figure 12.3. Existing biobanks containing general wildlife biomaterials or amphibian-specific samples superimposed over the regions where amphibian biobanking has been recommended by the Conservation Needs Assessments. Countries with biobanks marked with a triangle denote those with wildlife collections but that do not currently hold amphibian material or for which there has been no confirmation of banked amphibian samples. Circles denote areas where current amphibian-specific biobanks are located.

The current and emerging ARTs (Table 12.2) are primarily integrated within CBP programmes and, like many conservation actions, will be of greatest benefit when combined with a multifaceted conservation action plan. As such, biobanking strategic goals and decision frameworks are likely to reflect those for establishment of CBPs and may include additional considerations, i.e. do gamete collection protocols exist for this species? Is there a suitable model or subspecies should hybridisation be the only available sample end-use? Are there existing in-situ programmes that offer potential for opportunistic collection? Albeit collection of samples from species where data remains deficient or development of a CBP has not been forecast are not necessarily excluded, but this runs the risk of these resources becoming nothing more than a museum serving to support phylogenetic analysis and taxonomy, but little else.

Table 12.2. Summary of some of the institutions holding amphibian samples around the world which are linked to CBP programmes.

FACILITY	COUNTRY	NUMBER OF SPECIES	SOURCE	REFERENCE
San Diego Zoo Wildlife Alliance's Frozen Zoo	USA	26	Sperm / cell lines	Della Togna et al., 2020; Marlys Houck & Barbara Durrant, personal communication
Conservation Biology Group at the University of Newcastle	Australia	26	Sperm	Rose Upton, personal communication
Zoological Society of London	UK		Sperm / cell lines	Mike Bruford, personal communication
Taronga Conservation Society	Australia	12	Sperm	Rebecca Hobbs & Justine O´Brien, personal communication
Smithsonian Tropical Research Institute Panama Amphibian Rescue and Conservation Project	Panama	6	Sperm	Gina Della Togna, personal communication

One of the most significant driving factors of the poor representation of amphibians across CBP programs is high costs. The proposed budget for the development of CBP programmes in the original ACAP document was US$120,000 (2007) per year per species, with estimates of US$12,500,000 to captive-manage 100 species each for one year (Mendelson et al., 2006). Outdated by over a decade and lacking detail, these costs are likely highly conservative. More recent (2018) estimates in Australia suggest CBP programmes cost on average around A$200,000 per year per species, often for many years or even decades (Harley, Mawson, Olds, McFadden, & Hogg, 2018). Despite these high costs, there is emerging evidence of the cost-benefits and cost-reductions that are possible when integrating biobanking technology and ARTs into CBP programmes as practical support tools. Economic and genetic modelling using real-world data for the CBP program for Oregon spotted frogs (*Rana pretiosa*) suggests that biobanking technology could lower the size of the live colony required to be held in captivity, substantially lowering the costs of CBP programs, as well as reducing inbreeding of output amphibians from these programmes (Howell, Frankham, et al., 2021). This modelling provides an examination of programme costs and captive genetic diversity (heterozygosity H_t/H_o values derived from inbreeding rates) when a simple low-cost biobanking set-up (consisting of basic additional freezing infrastructure; e.g., freezers and liquid nitrogen dewars) is integrated into an established amphibian CBP programme. In hypothetical captive colonies designed to meet the same genetic retention target (90% of source population heterozygosity for a minimum of 100 years, in line with accepted global genetic benchmarks; Soulé, Gilpin, Conway, & Foose, 1986) there was a 26-fold cost reduction in populations with biobanking integrated compared to populations under conventional CBP programme conditions (Howell, Frankham, et al., 2021). This means that 26 species could be captive bred for the price of one in programs designed to meet globally accepted genetic retention targets under the with- and without-biobanking scenarios.

This research is further supported by recent modelling in Australian species, the Orange-bellied frog (*Geocrinia vitellina*) and White-bellied frog (*Geocrinia alba*), where similar proportionate cost and genetic benefits were exhibited (Howell, Mawson, et al., 2021). This study modelled the genetic and cost benefits of incorporating ARTs and biobanking into CBP programs of *G. vitellina* and *G. alba* at Perth Zoo, Australia. To meet the 90% heterozygosity retention target in conventional CBP program conditions would require 400 live *G. vitellina* and *G. alba*, costing A$1.1 million and A$718k in year one and A$466 million and A$284 million across 100 years respectively, compared to just 17 live individuals for each species, costing A$68k and A$48k in year one, and A$21 million and A$13 million across 100 years in CBP programs integrating ARTs and biobanking. The study also revealed that world-first ambitious targets of 95% and 99% *Ht/Ho* retention are also possible in amphibian CBP programs under realistic cost frameworks.

The integration of frozen founder spermatozoa would also provide significant genetic benefits. Conventional CBP programmes have various challenges with genetic diversity which can compromise the value of captive-bred animals for release to the wild, including inbreeding depression in unavoidably small captive colonies (Ralls, Ballou, & Templeton, 1988), reduced reproductive fitness (Farquharson, Hogg, & Grueber, 2018), and domestication and adaptation to captivity (Frankham, Briscoe, & Ballou, 2009). Biobanking and ARTs would reduce the rate of inbreeding in amphibian CBP programmes, and biobanked males would not be subject to domestication and adaptation to captivity, which would generally make animals produced using ARTs and biobanking better suited for release to the wild (Howell, Frankham, et al., 2021; Howell, Mawson, et al., 2021). Ultimately, these studies reveal a promising and potentially feasible model; the integration of ARTs and low-cost additional biobanking infrastructure into existing amphibian CBP programs globally to achieve cost and genetic outcomes for species, institutions and end-users. Given the generally poor understanding and transparency around the costs associated with amphibian biobanking, the slow progress towards a viable funding mechanism for amphibian biobanking, and the limited funding landscape for amphibian conservation efforts, this is likely the most feasible model for the integration of biobanking and ARTs into CBP programs (Della Togna et al., 2020). Amphibian conservationists and ART practitioners should focus on developing examples of this model in practice.

Howell, Mawson et al., (2021) provide a broad pathway of actions required to transition ARTs and biobanking into Australian CBP programmes under this model to produce practical examples. Since the model described above would be highly transferable, the pathway may also provide a feasible strategy to transition ARTs and biobanking into CBP programmes globally. The strategy is provided in more detail in Howell, Mawson et al. (2021), but would involve various key steps, including: 1) Continue building the case for amphibian biobanking using economic and genetic arguments; 2) Secure captive colonies of target species, through partnership with captive institutions or development of novel amphibian CBP programmes; 3) Financial planning and funding mechanism development (focussing on long-term biobank sustainability, understanding long-term required costs and the applicability of biobank funding mechanisms developed for biobanks in other sectors; 4) Leverage existing CBP program infrastructure through partnerships and secure additional biobanking and freezing infrastructure. This model of integrating additional biobanking infrastructure into established programs will be a low-cost option, e.g., around A$14,000 for basic freezing infrastructure as modelled in Howell, Mawson et al. (2021), which are supported by estimates of A$22,000 to incorporate basic biobanking capacity into CBP programs at Zoos Victoria (Della Togna et al. 2020) and the low-cost self-contained mobile laboratories for aquatic species cryopreservation presented in Childress, Caffey, & Tiersch (2018) and (Childress, Bosworth, Chesney, Walter, & Tiersch (2019); 5) Close species-specific knowledge gaps in target amphibian species in order to develop optimised species-specific biobanking protocols. This will require applied research effort, access to colony animals and skilled labour, and access to significant research funding (up to A$3.25 million in targeted applied research funds per species across 5-year research programs, as estimated in Howell, Mawson et al., (2021).

Conclusions and future directions

With more than 900 amphibian species requiring some form of ex-situ insurance population, (Zippel et al., 2011) predictions that global resources needed to sustain amphibian CBPs are extremely limited and are already around a decade old (Bishop et al., 2012). The reality is that the situation has worsened and continues to highlight the poor representation of amphibians in global CBP efforts. Therefore, maximising the global impact of amphibian gene banking is now at its most critical. Strategies for the best way to implement ARTs into broader amphibian conservation programs depend on biopolitical, biogeographical, and phylogenetic targeting. Biopolitical targeting should be designed and executed to target the obvious and publicly accessible benefits of safeguarding the target species. This will reciprocally garner greater public influence and political support leading to further resource allocation. The development of techniques for amphibian ARTs has almost exclusively been in moderate to high-income industrialised countries. Yet most amphibian species, except southeast Asia North America and eastern Australia, occur in the low to moderate-income countries within Central and South America, SE Asia, New Guinea, and Africa (Figure 12.3). Most threatened Anura come from Central and South America, Caudata from Asia and North America, and Gynophiona (caecilians) from India and Africa.

As we enter the new age of the sustainable management of biodiversity, increasingly technical options, such as the merging of CBPs and ARTs, are being offered to assist in achieving realistic goals. However, despite their application and reliability, financial constraints still pose a major obstacle. Generally, CBPs have been largely financed and supported by zoos as part of their conservation work; however, over the last two decades private groups, supported by seed grants or ongoing finance from various amphibian conservation organisations, have established private amphibian CBPs. When these are located in a priority region they provide the ideal opportunity to begin the merging of CBPs with gene banking of tissue, gamete or cell lines.

Biogeographically, emphasis should be on CBPs facilities that are located in the regions predicted to suffer the most loss of amphibian phylogenetic biodiversity. With the appropriate training, in-country CBPs can easily maintain fully genetically varied populations of species through broodstock management that incorporates sperm collected from individuals in their CBPs and in the field. However, this will require the adequate representation of experienced personnel on the ground willing to exchange, support and train in-country researchers, especially in instances where no technical expertise exists. For this, we propose the establishment of regional teams, led by one or more personnel specifically trained in ARTs procedures to be funded and willing to support any area where immediate intervention is required. The IUCN, the ASG and other large entities should help facilitate funding avenues to sustain this strategy if there is to be a long-term commitment to the preservation of amphibian species and the incorporation of ARTs into mainstream amphibian conservation strategies. Thus, biobanking can become incorporated into associated fieldwork for the species including maintaining or increasing suitable habitat. These works contribute not only to the perpetuation of amphibian species but also to global sustainability.

Prioritisation of regions for amphibian CBPs ARTs should address the urgency for conservation but should also take into account the practicality of conserving species based on their intrinsic value to the ecosystem and not on a singular species criterion. Second, determining what species to biobank should also consider the available, biogeographical patterns in genetic and phylogenetic diversity (Hu et al., 2021; Upton et al., 2021), predictions of future habitat loss through vegetation destruction or through changes in global temperatures (Zhang et al., 2021), and from recommendations generated by Conservation Needs Assessments (Johnson et al., 2020) and IUCN’s amphibian Red List. By prioritising resources to maximise conservation efficiency toward the protection of ecoregions closest to meeting targets, there can be a doubling benefit to cost, whilst excluding some areas of high biodiversity for species of particular taxon including amphibians (Chauvenet et al., 2020). Upton (2020) showed that up to 40% of amphibian phylogenetic diversity could be protected by increasing protection of 1.9% of global terrestrial area. Thus, the targeting of CBPs/ARTs should also be focused on these regions both in terms of their biodiversity but also in terms of increased risk to amphibian species.

References

Albus, U. (2012). *Guide for the care and use of laboratory animals (8th edition)*. London, England: SAGE Publications Sage UK.

Amphibian Ark. (2020). Taxon management. Retrieved from https://www.amphibianark.org/taxon-management/

Bishop, P. J., Angulo, A., Lewis, J. P., Moore, R. D., Rabb, G. B., & Garcia Moreno, J. (2012). The amphibian extinction crisis - what will it take to put the action into the Amphibian Conservation Action Plan? *S.A.P.I.EN.S*, *5*(2), 97-111.

Blesbois, E. (2007). Current status in avian semen cryopreservation. *World's Poultry Science Journal*, *63*(2), 213–222. doi: 10.1017/S0043933907001419

Browne, R. K., Silla, A. J., Upton, R., Della-Togna, G., Marcec-Greaves, R., Shishova, N. V., … Clulow, S. (2019). Sperm collection and storage for the sustainable management of amphibian biodiversity. *Theriogenology*, *133*, 187–200. doi: 10.1016/j.theriogenology.2019.03.035

Browne, R. K., Wolfram, K., García, G., Bagaturov, M. F., & Pereboom, Z. J. J. M. (2011). Zoo-based amphibian research and conservation breeding programs. *Amphibian and Reptile Conservation 5*(3), 1–14.

Burger, I., Julien, A. R., Kouba, A. J., Barber, D., Counsell, K. R., Pacheco, C., … Kouba, C. K. (2021). Linking in-situ and ex-situ populations of threatened amphibians through genome banking. *Conservation Science and Practice*, *3*(11). doi: 10.1111/csp2.525

Byrne, P. G., Gaitan-Espitia, J. D., & Silla, A. J. (2019). Genetic benefits of extreme sequential polyandry in a terrestrial-breeding frog. *Evolution*, *73*(9), 1972–1985. doi: 10.1111/evo.13823

Calatayud, N. E., Chai, N., Gardner, N. R., Curtis, M. J., & Stoops, M. A. (2019). Reproductive techniques for ovarian monitoring and control in amphibians. *Journal of Visualized Experiments*, *2019*(147). doi: 10.3791/58675

Chai, N. (2016). Surgery in amphibians. *Veterinary Clinics of North America - Exotic Animal Practice*, *19*(1), 77–95. doi: 10.1016/j.cvex.2015.08.004

Chauvenet, A. L. M., Watson, J. E. M., Adams, V. M., Di Marco, M., Venter, O., Davis, K. J., … Possingham, H. P. (2020). To achieve big wins for terrestrial conservation, prioritize protection of ecoregions closest to meeting targets. *One Earth*, *2*(5), 479–486. doi: 10.1016/j.oneear.2020.04.013

Childress, W. M., Bosworth, B., Chesney, E., Walter, R. B., & Tiersch, T. R. (2019). On-Site capabilities of a mobile laboratory for aquatic germplasm cryopreservation. *North American Journal of Aquaculture*, *81*(4), 349–363. doi: 10.1002/naaq.10099

Childress, W. M., Caffey, R. H., & Tiersch, T. R. (2018). Design and cost analysis of a self-contained mobile laboratory for commercial-scale aquatic species cryopreservation. *Journal of the World Aquaculture*

Society, *49*(5), 805–826. doi: 10.1111/jwas.12525

Clulow, J., & Clulow, S. (2016). Cryopreservation and other assisted reproductive technologies for the conservation of threatened amphibians and reptiles: Bringing the ARTs up to speed. *Reproduction, Fertility and Development*, *28*(8), 1116–1132. doi: 10.1071/RD15466

Clulow, J., Pomering, M., Herbert, D., Upton, R., Calatayud, N., Clulow, S., … Trudeau, V. L. (2018). Differential success in obtaining gametes between male and female Australian temperate frogs by hormonal induction: A review. *General and Comparative Endocrinology*, *265*, 141–148. doi: 10.1016/j.ygcen.2018.05.032

Clulow, J., Upton, R., Trudeau, V. L., & Clulow, S. (2019). Amphibian assisted reproductive technologies: moving from technology to application. In P. Comizzoli, J. L. Brown, & W. V Holt (Eds.), *Reproductive sciences in animal conservation* (pp. 413–463). Cham: Springer International Publishing. doi: 10.1007/978-3-030-23633-5_14

Conservation Needs Assessments. (2021). View assessments. Retrieved from https://conservationneeds.org/master/assessmentsearch

Della Togna, G. (2015). *Structural and functional characterization of the Panamanian Golden Frog (*Atelopus zeteki*) spermatozoa: impact of medium osmolality and cryopreservation on motility and cell viability*. PhD thesis. College Park: University of Maryland.

Della Togna, G., Howell, L. G., Clulow, J., Langhorne, C. J., Marcec-Greaves, R., & Calatayud, N. E. (2020). Evaluating amphibian biobanking and reproduction for captive breeding programs according to the Amphibian Conservation Action Plan objectives. *Theriogenology*, *150*, 412–431. doi: 10.1016/j.theriogenology.2020.02.024

Della Togna, G., Trudeau, V. L., Gratwicke, B., Evans, M., Augustine, L., Chia, H., … Comizzoli, P. (2017). Effects of hormonal stimulation on the concentration and quality of excreted spermatozoa in the critically endangered Panamanian golden frog (*Atelopus zeteki*). *Theriogenology*, *91*, 27–35. doi: 10.1016/j.theriogenology.2016.12.033

Farquharson, K. A., Hogg, C. J., & Grueber, C. E. (2018). A meta-analysis of birth-origin effects on reproduction in diverse captive environments. *Nature Communications*, *9*(1). doi: 10.1038/s41467-018-03500-9

Figiel, C. (2013). Cryopreservation of sperm from the axolotl *Ambystoma mexicanum*: implications for conservation. *Herpetological Conservation and Biology*, *8*(3), 748–855.

Frankham, R., Briscoe, D. A., & Ballou, J. D. (2009). *Introduction to conservation genetics*. Cambridge, UK: Cambridge University Press.

Gascon, C., Collins, J. P., Moore, R. D., Church, D. R., McKay, J. E., & Mendelson III, J. R. (2007). *Amphibian conservation action plan: proceedings IUCN/SSC Amphibian conservation summit 2005*. Gland, Switzerland and Cambridge, UK: IUCN/SSC Amphibian Specialist Group.

Gillis, A. B. (2020). *Assisted reproductive technologies in male Ambystoma tigrinum*. Master of Science thesis. Mississippi State University.

Glass Campbell, L., Anderson, K. A., & Marcec-Greaves, R. (2022). Topical application of hormone gonadotropin-releasing hormone (GnRH-A) stimulates reproduction in the endangered Texas blind salamander (*Eurycea rathbuni*). *Conservation Science and Practice*, *4*(3), 1–8. doi: 10.1111/csp2.609

González, M. A. (2018). *Desarrollo de técnicas de criopreservación para espermatóforos y embriones de Ambystoma mexicanum para su uso en reproducción asistida.* Master thesis. Universidad de Guadalajara.

Green, S. L., Parker, J., Davis, C., & Bouley, D. M. (2007). Ovarian hyperstimulation syndrome in gonadotropin-treated laboratory South African clawed frogs (*Xenopus laevis*). *Journal of the American Association for Laboratory Animal Science*, *46*(3), 64–67.

Gurdon, J. B. (1968). Changes in somatic cell nuclei inserted into growing and maturing amphibian oocytes. *Journal of Embryology and Experimental Morphology*, *20*(3), 401–414. doi: 10.1242/dev.20.3.401

Gurdon, J. B., & Byrne, J. A. (2002). Cloning of amphibians. In J. Cibelli, I. Wilmut, R. Jaenisch, J. Gurdon, R, Lanza, M. West & K. Campbell (Eds.), *Principles of cloning* (pp. 281–286). Elsevier.

Guy, E. L., Gillis, A. B., Kouba, A. J., Barber, D., Poole, V., Marcec-Greaves, R. M., & Kouba, C. K. (2020). Sperm collection and cryopreservation for threatened newt species. *Cryobiology*, *94*, 80–88. doi: 10.1016/j.cryobiol.2020.04.005

Guy, E. L., Martin, M. W., Kouba, A. J., Cole, J. A., & Kouba, C. K. (2020). Evaluation of different temporal periods between hormone-induced ovulation attempts in the female Fowler's toad *Anaxyrus fowleri*. *Conservation Physiology*, *8*(1), 1–8. doi: 10.1093/conphys/coz113

Harley, D., Mawson, P. R., Olds, L., McFadden, M. S., & Hogg, C. J. (2018). The contribution of captive breeding in zoos to the conservation of Australia's threatened fauna. In S. T. Garnett, P. Latch, D. B. Lindenmayer, & J. C. Z. Woinarski (Eds.), *Recovering Australian* *threatened species: a book of hope* (pp. 281–294). Melbourne, Australia: CSIRO Publishing.

Hewitt, R., & Watson, P. (2013). Defining biobank. *Biopreservation and Biobanking*, *11*(5), 309–315. doi: 10.1089/bio.2013.0042

Holt, W. V., Pickard, A. R., & Prather, R. S. (2004). Wildlife conservation and reproductive cloning. *Reproduction*, *127*(3), 317–324. doi: 10.1530/rep.1.00074

Howell, L. G., Frankham, R., Rodger, J. C., Witt, R. R., Clulow, S., Upton, R. M. O., & Clulow, J. (2021). Integrating biobanking minimises inbreeding and produces significant cost benefits for a threatened frog captive breeding programme. *Conservation Letters*, *14*(2), 1–9. doi: 10.1111/conl.12776

Howell, L. G., Mawson, P. R., Frankham, R., Rodger, J. C., Upton, R. M. O., Witt, R. R., … Clulow, J. (2021). Integrating biobanking could produce significant cost benefits and minimise inbreeding for Australian amphibian captive breeding programs. *Reproduction, Fertility and Development*, *33*(9), 573–587. doi: 10.1071/RD21058

Hu, Y., Fan, H., Chen, Y., Chang, J., Zhan, X., Wu, H., … Wei, F. (2021). Spatial patterns and conservation of genetic and phylogenetic diversity of wildlife in China. *Science Advances*, *7*(4), 1–10. doi: 10.1126/sciadv.abd5725

Johnson, K., Baker, A., Buley, K., Carrillo, L., Gibson, R., Gillespie, G. R., … Zippel, K. (2020). A process for assessing and prioritizing species conservation needs: going beyond the Red List. *Oryx*, 1–8. doi: 10.1017/S0030605317001715

Julien, A. R., Kouba, A. J., Kabelik, D., Feugang, J. M., Willard, S. T., & Kouba, C. K. (2019). Nasal administration of gonadotropin releasing hormone (Gnrh) elicits sperm production in fowler's toads (*Anaxyrus fowleri*). *BMC Zoology, 4*(1), 1–10. doi: 10.1186/s40850-019-0040-2

Kamau, E., Fedder, B., & Winter, G. (2010). The Nagoya Protocol on access to genetic resources and benefit sharing: what is new and what are the implications for provider and user countries and the scientific community? *Law & Development Journal (LEAD), 6*(3), 248–263.

Langhorne, C. J. (2016). *Developing assisted reproductive technologies for endangered North American amphibians*. PhD thesis. Mississippi State University.

Linhoff, L. J., Soorae, P. S., Harding, G., Donnelly, M. A., Germano, J. M., Hunter, D. A., … Eckstut, M. E. (2021). *IUCN Guidelines for amphibian reintroductions and other conservation translocations*. Gland, Switzerland.

Mantegazza, P. (1866). Fisiologia sullo sperma umano. *Rendic Reale Instit Lomb, 3,* 183–186.

Marcec, R. M. (2016). *Development of assisted reproductive technologies for endangered North American salamanders*. PhD thesis. Mississippi State University.

Mastromonaco, G. F., González-Grajales, L. A., Filice, M., & Comizzoli, P. (2014). Somatic cells, stem cells, and induced pluripotent stem cells: how do they now contribute to conservation? In W. V. Holt, J. L. Brown, & P. Comizzoli (Eds.), *Reproductive sciences in animal conservation* (pp. 385–427). Springer.

Mastromonaco, G. F., & Songsasen, N. (2020). Reproductive technologies for the conservation of wildlife and endangered species. In G. A. Presicce (Ed.), *Reproductive Technologies in Animals* (pp. 99–117). Elsevier.

McDonough, C. E., Martin, M. W., Vance, C. K., Cole, J. A., & Kouba, A. J.

(2016). Frequency of exogenous hormone therapy impacts spermiation in male Fowler’s toad (*Bufo fowleri*). *Reproduction, Fertility and Development*, *28*(7), 995–1003. doi: 10.1071/RD14214

McGinnity, D., Reinsch, S. D., Schwartz, H., Trudeau, V., & Browne, R. K. (2022). Semen and oocyte collection, sperm cryopreservation and IVF with the threatened North American giant salamander *Cryptobranchus alleganiensis*. *Reproduction, Fertility and Development*, *34*(5), 470–477. doi:10.1071/RD21035

Mendelson, J. R., Lips, K. R., Gagliardo, R. W., Rabb, G. B., Collins, J. P., Diffendorfer, J. E., … Brodie, E. D. (2006). Confronting amphibian declines and extinctions. *Science*, *313*(5783), 48. doi: 10.1126/science.1128396

Norris, K. (2004). Managing threatened species: The ecological toolbox, evolutionary theory and declining-population paradigm. *Journal of Applied Ecology*, *41*(3), 413–426. doi: 10.1111/j.0021-8901.2004.00910.x

Obringer, A. R., O’Brien, J. K., Saunders, R. L., Yamamoto, K., Kikuyama, S., & Roth, T. L. (2000). Characterization of the spermiation response, luteinizing hormone release and sperm quality in the American toad (*Bufo americanus*) and the endangered Wyoming toad (*Bufo baxteri*). *Reproduction, Fertility and Development*, *12*(1–2), 51–58. doi: 10.1071/rd00056

Ogawa, A., Dake, J., Iwashina, Y. K., & Tokumoto, T. (2011). Induction of ovulation in *Xenopus* without hCG injection: The effect of adding steroids into the aquatic environment. *Reproductive Biology and Endocrinology*, *9*(1), 11. doi: 10.1186/1477-7827-9-11

Ogielska, M., & Bartmanska, J. (2009). Oogenesis and female reproductive system in Amphibia—Anura. In M.

Ogielska (Ed.), *Reproduction of amphibians* (pp. 153–272). Enfield, New Hampshire: Science Publishers.

Peng, L., Xiao, Y., & Liu, Y. (2011). Effect of cryopreservation and short-term storage of Chinese giant salamander sperm. *Acta Hydrobiologica Sinica*, *35*, 325–332.

Ralls, K., Ballou, J. D., & Templeton, A. (1988). Estimates of lethal equivalents and the cost of inbreeding in mammals. *Conservation Biology*, *2*, 185–193. doi: 10.1111/j.1523-1739.1988.tb00169.x

Rowson, A. D., Obringer, A. R., & Roth, T. L. (2001). Non-invasive treatments of luteinizing hormone-releasing hormone for inducing spermiation in American (*Bufo americanus*) and Gulf Coast (*Bufo valliceps*) toads. *Zoo Biology*, *20*(2), 63–74. doi: 10.1002/zoo.1007

Sherman, J. K. (1980). Historical synopsis of human semen cryobanking. In G. David & W. S. Price (Eds.), *Human artificial insemination and semen preservation* (pp. 95–105). Springer.

Silla, A. J., Calatayud, N. E., & Trudeau, V. L. (2021). Amphibian reproductive technologies: Approaches and welfare considerations. *Conservation Physiology*, *9*(1), 1–23. doi: 10.1093/conphys/coab011

Silla, A. J., & Langhorne, C. J. (In press). Protocols for hormone-induced spermiation, and the cold storage, activation, and assessment of amphibian sperm. In A. J. Silla, A. J. Kouba, & H. Heatwole (Eds.), *Reproductive technologies and biobanking as tools for the conservation of amphibians*. Melbourne, Australia: CSIRO Publishing.

Silla, A. J., McFadden, M. S., & Byrne, P. G. (2019). Hormone-induced sperm-release in the critically endangered Booroolong frog (*Litoria booroolongensis*): effects of gonadotropin-releasing hormone and human chorionic gonadotropin. *Conservation Physiology*, *7*(1), 1–10. doi: 10.1093/conphys/coy080

Silla, A. J., Roberts, J. D., & Byrne, P. G. (2020). The effect of injection and topical application of hCG and GnRH agonist to induce sperm-release in the roseate frog, *Geocrinia rosea*. *Conservation Physiology*, *8*(1), 1–10. doi: 10.1093/conphys/coaa104

Soulé, M., Gilpin, M., Conway, W., & Foose, T. (1986). The millenium ark: How long a voyage, how many staterooms, how many passengers? *Zoo Biology*, *5*(2), 101–113. doi: 10.1002/zoo.1430050205

Talbot, L. M. (1980). The world's conservation strategy. *Environmental Conservation*, *7*(4), 259–268. doi: 10.1017/S0376892900007955

Tiersch, T. R., Yang, H., Jenkins, J. A., & Dong, Q. (2007). Sperm cryopreservation in fish and shellfish. *Society of Reproduction and Fertility Supplement*, *65*, 493–508.

Unger, S., Mathis, A., & Wilkinson, R. (2013). A comparison of sperm health in declining and stable populations of hellbenders (*Cryptobranchus alleganiensis alleganiensis* and *C.a. bishopi*). *American Midland Naturalist*, *170*(2), 382–392. doi: 10.1674/0003-0031-170.2.382

Upton, C. (2020). Conserving natures? Co-producing payments for ecosystem services in Mongolian rangelands. *Development and Change*, *51*(1), 224–252. doi: 10.1111/dech.12549

Upton, R., Clulow, S., Calatayud, N. E., Colyvas, K., Seeto, R. G. Y., Wong, L. A. M., … Clulow, J. (2021). Generation of reproductively mature offspring from the endangered green and golden bell frog *Litoria aurea* using cryopreserved spermatozoa. *Reproduction, Fertility and Development*, *33*(9), 562–572. doi:10.1071/RD20296

Upton, R., Clulow, S., Mahony, M. J., & Clulow, J. (2018). Generation of a sexually mature individual of the Eastern dwarf tree frog, *Litoria fallax*, from cryopreserved testicular macerates: Proof of capacity of cryopreserved sperm derived offspring to complete development. *Conservation Physiology*, *6*(1), 1–5. doi: 10.1093/conphys/coy043

Vu, M., & Trudeau, V. L. (2016). Neuroendocrine control of spawning in amphibians and its practical applications. *General and Comparative Endocrinology*, *234*, 28–39. doi: 10.1016/j.ygcen.2016.03.024

Walters, E. M., Benson, J. D., Woods, E. J., & Critser, J. K. (2009). The history of sperm cryopreservation. In A. A. Pacey & M. J. Tomlinson (Eds.), *Sperm banking: theory and practice* (pp. 1–17). Cambridge, UK: Cambridge University Press. doi: 10.1017/CBO9781139193771.002

Wheater, R. (1995). World zoo conservation strategy: a blueprint for zoo development. *Biodiversity & Conservation*, *4*(6), 544–552. doi: 10.1007/BF00222513

Wright, K. M., & Whitaker, B. R. (2001). *Amphibian medicine and captive husbandry*. Krieger Publishing Company.

Zhang, Z., Kass, J. M., Mammola, S., Koizumi, I., Li, X., Tanaka, K., … Usio, N. (2021). Lineage-level distribution models lead to more realistic climate change predictions for a threatened crayfish. *Diversity and Distributions*, *27*(4), 684–695. doi: 10.1111/ddi.13225

Zimkus, B. M., Hassapakis, C. L., & Houk, M. L. (2018). Integrating current methods for the preservation of amphibian genetic resources and viable tissues to achieve best practices for species conservation *Amphibian and Reptile Conservation*, *12*, 1–27.

Zippel, K., Johnson, K., Gagliardo, R., Gibson, R., McFadden, M., Browne, R., … Townsend, E. (2011). The Amphibian Ark: A global community for ex situ conservation of amphibians. *Herpetological Conservation and Biology*, *6*(3), 340–352

.

Chapter 13. Genomics

Lauren A. O’Connell[1], Ariel Rodríguez[2], Tiffany A. Kosch[3], Taejoon Kwon[4], Luciana Bolsoni Lourenço[5], H. Mauricio Ortega-Andrade[6], Daniel Selechnik[7], Katharina C. Wollenberg Valero[8], Andrew J. Crawford[9], John Measey[10]

[1]Department of Biology, Stanford University, Stanford, California, 94305, United States of America (loconnel@stanford.edu)

[2]Institut für Zoologie, Stiftung Tierärztliche Hochschule Hannover, Bünteweg 17, 30559 Hannover, Germany (Ariel.Rodriguez@tiho-hannover.de)

[3]AL Rae Centre for Genetics and Breeding, Massey University, 10 Bisley Drive, Hamilton 3240, New Zealand (tiffany.kosch@gmail.com)

[4]Department of Biomedical Engineering, Ulsan National Institute of Science and Technology, Ulsan, 44919, Republic of Korea (tkwon@unist.ac.kr)

[5]Department of Structural and Functional Biology, Institute of Biology, University of Campinas, 13083-863, Campinas, São Paulo, Brazil (lucianabolsoni@gmail.com)

[6]Biogeography and Spatial Ecology Research Group, Faculty of Life Sciences, Universidad Regional Amazónica Ikiam, 150101, 7km road to Muyuna, Tena, Napo, Ecuador (mauricio.ortega@ikiam.edu.ec)

[7]School of Life and Environmental Sciences (SOLES), University of Sydney, New South Wales, 2006, Australia (danselechnik@gmail.com)

[8]Department of Biological and Marine Sciences, University of Hull, Kingston-upon-Hull, HU67RX, United Kingdom (k.wollenberg-valero@hull.ac.uk)

[9]Depart of Biological Sciences, Universidad de los Andes, Bogotá, 111711, Colombia (andrew@dna.ac)

[10]Centre for Invasion Biology, Department of Botany and Zoology, Stellenbosch University, Stellenbosch, South Africa (johnmeasey@gmail.com)

Abstract

Amphibians are the most threatened major group of vertebrates worldwide and yet they are lagging behind other taxa in genomic resources that could aid in their conservation management. Here, we provide a status update on genomics technologies, how they have been used in amphibian research, and an outlook on how these approaches could inform future conservation planning and management strategies. Overall, amphibians lag far behind other vertebrates in the number of sequenced genomes, although both transcriptome and reduced representation sequencing have become popular tools for understanding amphibian physiology and population dynamics. Environmental DNA sequencing and epigenomics are also becoming useful tools for amphibian biology, although their adoption by the community has been slower. In addition to summarising technologies, their applications, and their challenges, we also provide case studies on how these approaches have been used for amphibian conservation projects. We focus on projects aimed at increasing pathogen resistance, informing captive breeding programs, and biocontrol of invasive species, although we acknowledge that many more unpublished projects are progressing our understanding of amphibian biology and conservation. Our future outlook includes pressing needs for increasing whole genome assemblies across the amphibian phylogeny, providing more bioinformatics training opportunities for conservation biologists, and increasing accessibility to genomics technologies and training to researchers in countries that hold most of the amphibian diversity on the planet.

Glossary

Chromatin conformation capture: a method to analyse the spatial organisation of chromatin in a cell.

Chromosome FISH: a method to identify the physical location of a piece of DNA on a chromosome by fluorescence *in situ* hybridisation.

Contigs: a DNA sequence reconstructed from a series of overlapping DNA fragments.

CRISPR gene editing: a method for engineering genetic elements of an organism derived from the prokaryotic antiviral system with clustered regularly interspaced short palindromic repeats (CRISPR).

DNA barcoding: a method of identifying species by sequencing a short segment of DNA that is conserved across distantly related species.

Environmental DNA (eDNA): DNA collected from environmental samples (e.g., water, faeces, soil) rather than directly from the organism.

Epigenetic sequencing: A method to analyse the gene activity changes caused by mechanisms other than DNA sequence changes, such as histone modification and DNA methylation.

Expressed transcripts: RNAs actively transcribed from DNA.

Genome annotations: A process to identify functional elements, such as genes, pseudogenes, promoters, repeats, on the genome.

Gene editing: techniques that modify DNA by inducing small changes such as single base pair edits.

Genetic rescue: method for increasing genetic diversity by facilitating immigration and gene flow into an isolated population.

Genome: an organism’s complete genetic sequence information.

Genome assembly: creation of a contiguous genome by piecing together smaller DNA sequence fragments decoded experimentally.

Genomic selection: a selective breeding method that predicts phenotypes of prospective breeding stock using impacts of genome-wide markers evaluated from a reference population.

Genetic markers: the physical location on a genome (and the DNA sequences associated with it) used to track genetic inheritance.

Genotype-by-sequencing (GBS): a method to analyse genotypes of samples by identifying genetic variants of a subset of genomic information.

Genome-wide association study (GWAS): A study to analyse associations between traits and genetic variations in distinct populations.

High-throughput sequencing: The technology that sequences millions of DNA and RNA simultaneously. Also known as next-generation sequencing (NGS).

Metagenomics: A collection of genetic material from a mixed community of organisms.

Optical mapping: a method to order the single molecule of DNA to construct a high-resolution map of restriction enzyme recognition sites.

Reduced representation sequencing: an umbrella term for many technological approaches that centre on obtaining genetic information for an organism by sequencing small portions of the genome.

Restriction-site associated DNA sequencing (RADseq): a method for obtaining genotype data throughout the genome of an organism by sequencing small fragments generated by restriction enzymes.

Transcriptome: A collection of RNAs transcribed from DNA, including messenger RNAs, long non-coding RNAs, microRNAs, transfer RNAs, ribosomal RNAs.

Whole Genome Sequencing (WGS): various methods for sequencing the entire genome of an organism by iterative sequencing of smaller fragments. Methods include Illumina short read, PacBio Hifi, and Oxford nanopore.

Introduction

Genetic diversity is critical for natural selection and the continued survival and fitness of species in a rapidly changing environment. The ability to generate genomic data for any species has progressed in technological approaches, accessibility through declining prices, and more widespread computational resources. However, the adoption of sequencing has been slow in amphibian research, including whole genome assembly, expressed transcripts, genomic markers and epigenetic modifications. This is surprising given how quickly amphibian species are declining and these technologies would be useful for rapid responses in establishing conservation strategies. Others have recently reviewed the state of amphibian genomes (Sun, Zhang, & Wang, 2020) and their application to understanding amphibian behaviour, physiology, and evolution (Funk, Zamudio, & Crawford, 2018; Shaffer et al., 2015; Walls & Gabor, 2019). Here we bring together the fields of genomics and conservation to provide a status update on sequencing technologies and their use for amphibian genomics and conservation projects. As genetic diversity is often used as a predictor of the long-term survival of populations, genomics is a toolkit that is broadly useful for every amphibian conservation project.

Many different genomics approaches have been used to study amphibian biology, although its application is not well distributed across species and geographic regions, which creates many challenges for amphibian conservation. Although genomics research in amphibians is more advanced than non-avian reptiles, it lags far behind birds and mammals (Figure 13.1a).

Most genomic research in amphibians has been conducted on IUCN Least Concern taxa, but among the threatened categories, the Critically Endangered species have received proportionately more attention (Figure 13.1b). Moreover, there is a geographic bias with respect to the percentage of species with genomics data in the Sequence Read Archive (SRA), where regions with more amphibian species have less genomic data (Figure 13.2). As we move forward with utilising genomics technologies for a greater understanding of amphibian biodiversity, we need to address the inequity in access to training and sequencing platforms in both instrumentation and the cost of data collection, especially in regions of the world that hold the greatest amphibian biodiversity. With equal access to training and technologies, amphibian conservation is poised to utilise genomics technologies in assessing species biodiversity and resilience to environmental stressors to inform conservation priorities, captive breeding programs, reintroduction surveillance, and management planning.

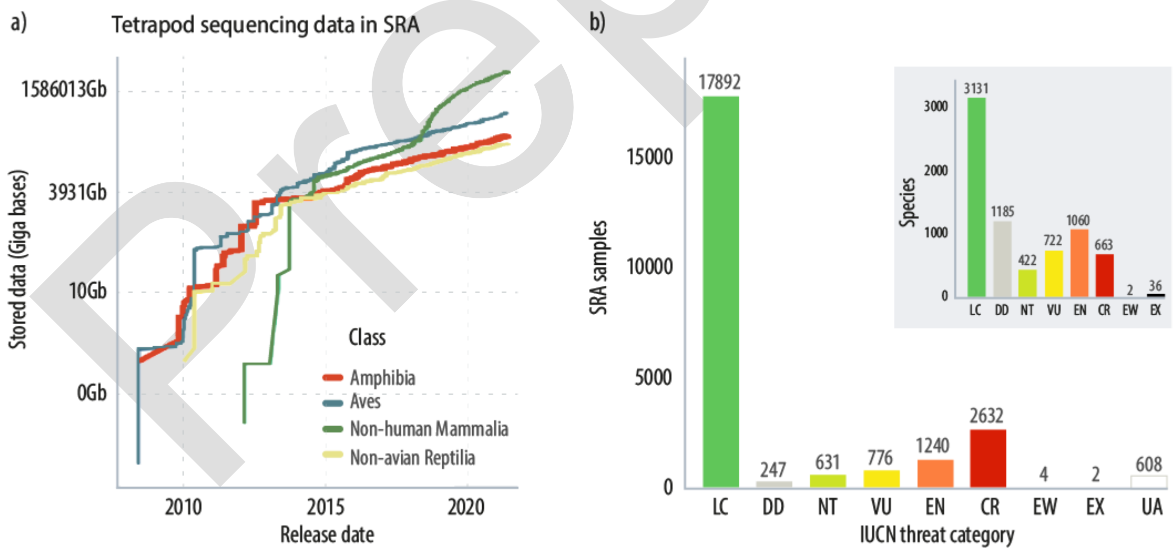


Figure 13.1: Genomic sequencing efforts in amphibians compared to other tetrapods. (a) Cumulative sum, in logarithmic scale, of high-throughput sequencing data stored in the Sequence Read Archive (SRA) for four main tetrapod groups. (b) Distribution of amphibian biosamples (equivalent to individuals) stored in SRA for each threatened category in the

IUCN Red List categories (UA: unassessed), the inset shows the number of species in each threat category. Data from SRA (www.ncbi.nlm.nih.gov/sra, accessed in January 2021) and IUCN Red List (www.iucnRed List.org/, accessed in January 2021).

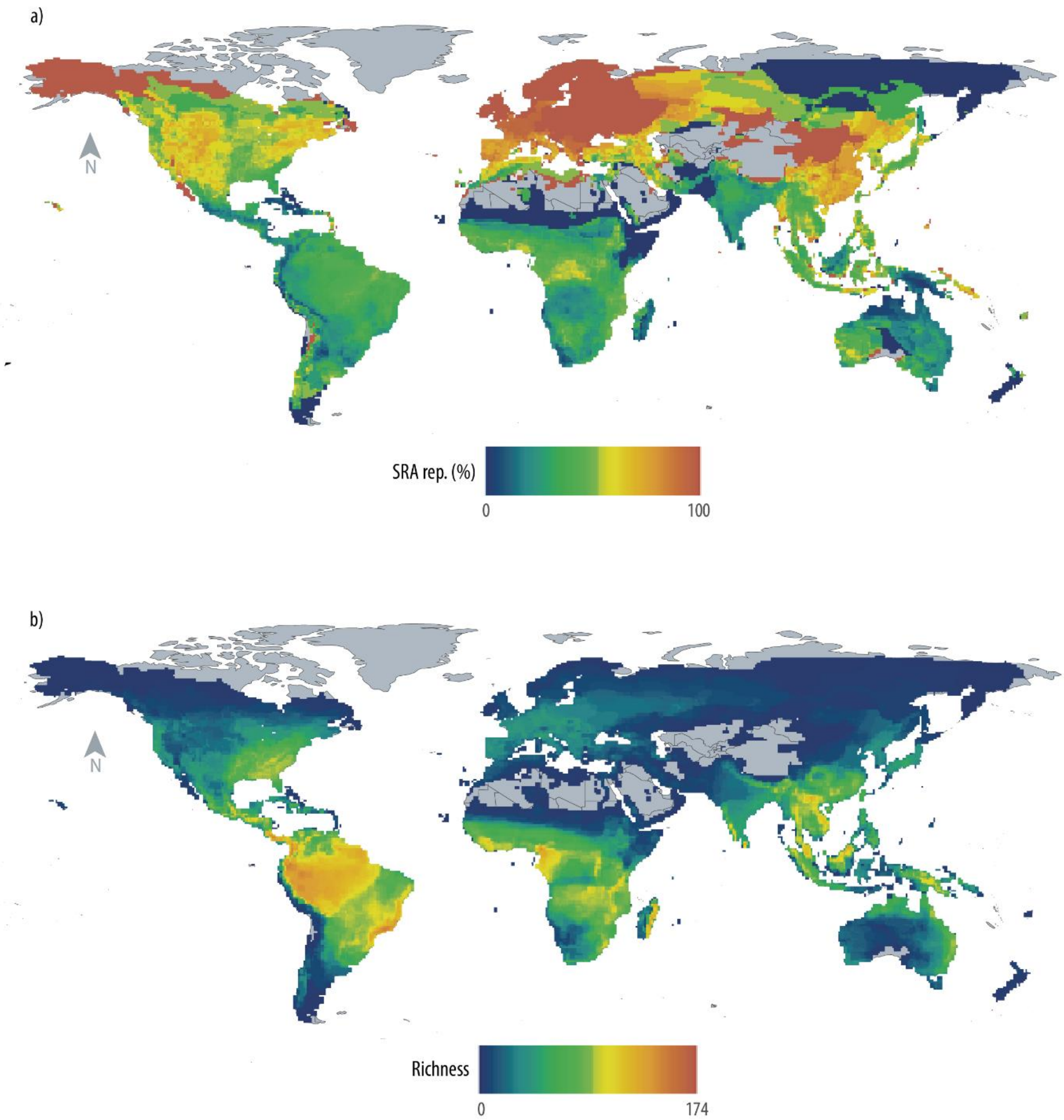


Figure 13.2: Biased geographic distribution of high-throughput sequencing effort. (A) Percentage of amphibian species sequenced and **(B)** amphibian species richness. Distribution polygons from the IUCN Red List and SRA records were spatially joined at ~10km resolution in ArcGIS® software (ESRI, Redlands, CA) to estimate the species richness and

the percentage of occurring species with high-throughput sequence information. Data from SRA (www.ncbi.nlm.nih.gov/sra, accessed in January 2021) and IUCN Red List (www.iucnRed List.org/, accessed in January 2021).

Status update

Genomic approaches to amphibian conservation

Genomics encompasses many approaches, including whole genome sequencing (WGS), RNA sequencing (RNASeq and IsoSeq), reduced representation sequencing (RRL), metagenomics, and epigenetic sequencing. Different approaches have been used depending on the scientific question and there are advantages and disadvantages of each approach (Table 13.1).

Table 13.1: Popular genomics approaches for amphibians**.** Advantages and disadvantages of each approach are summarised. Cost range estimates, in USD, refer to the direct sequencing cost (library preparation and sequencing). These cost estimates represent the authors' experience (in January 2021) and are provided as guidance, actual price quotes should be obtained from providers.

Advantages	**Disadvantages**	**Costs**
Whole Genome Sequencing		
1. Most comprehensive, genome-wide representation. 2. Broad taxonomic and biological applicability. 3. Provides detailed reference for the study of the target species and close relatives.	4. *Cost*: Medium to High depending on coverage and genome size. **5.** *Practicality*: Limited by the cost of sequencing (re-sequencing), assembly and annotation. **6.** *Particularities*: Repetitive regions in some amphibian genomes make assembly difficult.	$10K – 50K USD depending on genome size. Assembly and annotation are additional costs.
Transcriptomics		
7. Broad taxonomic and biological applicability.	11. *Cost*: Medium	$170 – 1,000 USD per sample (library prep. and sequencing).

8. Provides reference information for development of genomic markers for diverse applications. *9.* Provides information on coding and limited non-coding genomic regions. *10.* Functionally interpretable results that may provide genomic insights into the mechanisms underlying phenotypic variation and adaptation.	12. *Practicality*: Restricted (RNA instability prevents its application to museum samples). 13. *Particularities*: 1) Variability in gene expression at cell, tissue, organ, and individual levels; 2) Sub-optimal *de-novo* assemblies can affect downstream results; 3) Transcriptome annotation and construction of gene-to-transcript models can be challenging without a reference genome; 4) Misses most non-coding features of the genome	Price varies according to target exome size and desired depth.
Reduced Representation Libraries		
14. Reduced genome-wide representation at a relatively low cost.	17. *Cost*: Low	$8.5 – 100 USD per sample (price varies depending on the

15. Provides sufficient genotypic information for highly informative population genetic analyses. 16. Capture assays targeting conserved regions have broad applicability in terms of sampling and taxonomic scope.	**18.** *Practicality*: Restricted sampling and scalability (except for targeted capture protocols that can be applied to museum samples across many species). **19.** *Particularities*: 1) Design of the capture probes or selection of restriction enzyme is critical; 2) Strategies for loci selection can affect genotype calling in RADSeq assays; 3) Functional interpretation of results are limited without a reference genome.	amount of data, desired depth, and protocol)
Metagenomics		
1. A cost-effective approach that can target specific genome regions to assess a wide variety of fields, including systematics, ecology and conservation. 2. May be developed in the field or laboratory with portable devices.	1. *Cost:* Various techniques are available at relatively low costs. 2. *Practicality:* Restricted field availability of reagents, high variation in cost. 3. *Particularity:* 1) Studies on a single species need specific primers and the risk amplification of non-target sequences; 2) Bias from primers mismatches,	$10 – 100 USD per sample (price varies depending technology, target, desired depth, and protocol)

3. Accessible worldwide with standardised protocols that can improve the robustness of results.	bioinformatic issues, molecule and consensus accuracy, contamination, undersampling or incomplete databases.	
Epigenetics		
1. Can quickly provide genome-wide estimates of epigenetic modification patterns related to adverse. environmental changes for rapid screening purposes. 2. May be used as biomarkers for population stress vs. health.	1. *Cost:* Costs of different methods are reviewed extensively in (Eirin-Lopez and Putnam, 2019). 2. *Practicality:* More affordable methods give genome-wide resolution, more expensive ones specific modifications in specific loci or proteins. 3. *Particularity:* More research is needed as to which type of epigenetic modification and which genes modified are indicative of different stressors.	From $10 USD for mass spectrometry or gel-based assessment of global methylation to $1000 per sample for whole genome bisulfite sequencing.

A large taxonomic bias in sequencing effort exists in NCBI's Sequence Read Archive (SRA), where a limited number of amphibian families with few species are represented, including Caudata (Cryptobranchidae) and Archeobatrachian Anura (Ascaphidae, Pelobatidae, Pelodytidae, and Rhinophrynidae). Most amphibian families, however, are underrepresented with 23% of extant families having less than 5% of their species diversity represented in SRA.

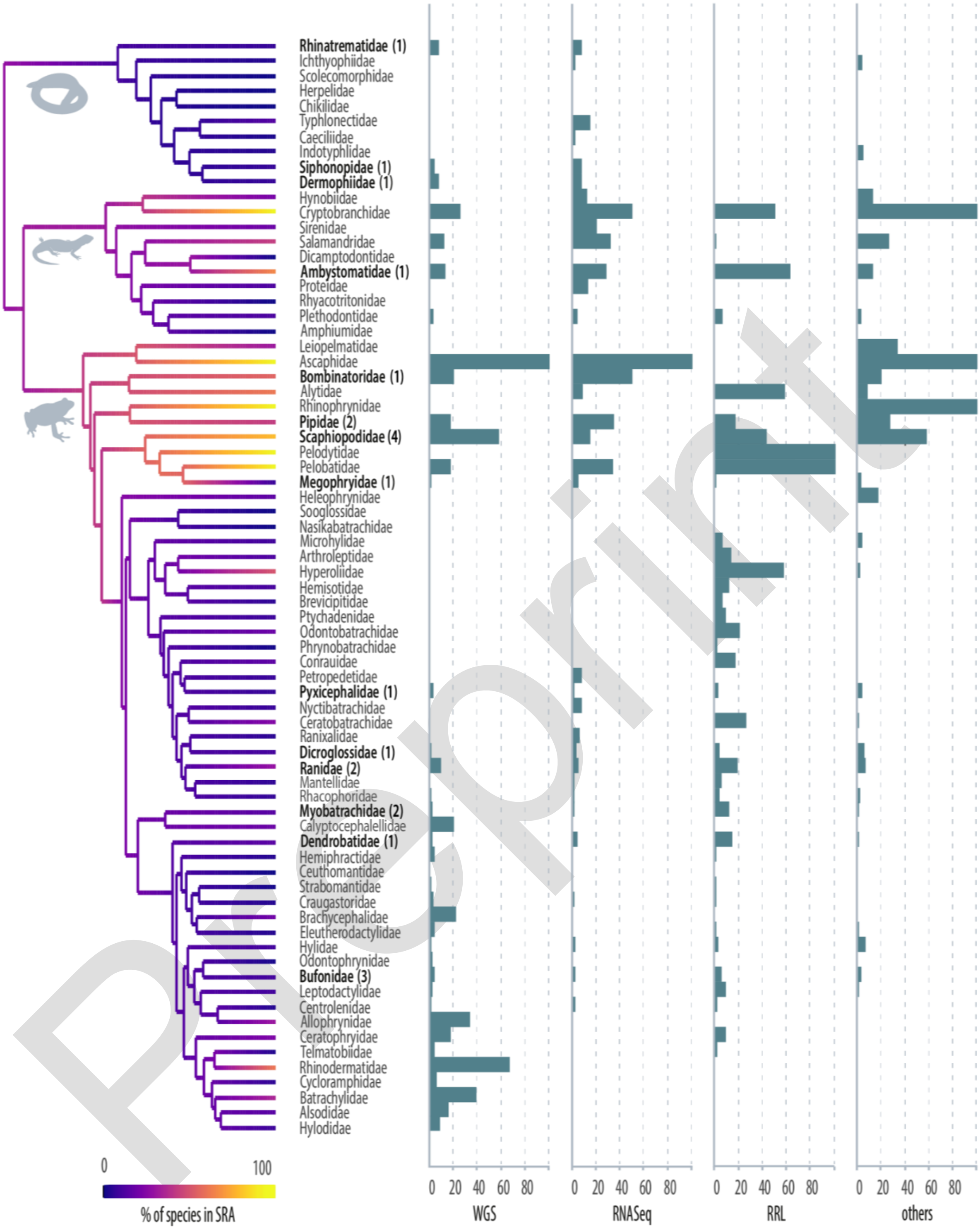


Figure 13.3. Taxonomic representation of amphibians in the Sequence Read Archive (SRA: www.ncbi.nlm.nih.gov/sra, accessed in January 2021). The percentage of species in each family is displayed on the amphibian phylogeny (*sensu* (Jetz & Pyron, 2018), pruned to family level), with bar plots on the right representing the percentage for each of the following

SRA assay categories: Whole genome sequencing (WGS), RNA sequencing (RNASeq), Reduced Representation Libraries (RRL), and all other assays (includes other approaches such as the sequencing of amplicons, transposase-accessible chromatin, bisulfite modifications, microRNA, and many others). Families with available reference genomes (as per the NCBI Genomes database, accessed in April, 2021) are highlighted in bold with the number of genomes in parentheses.

Amphibian genomes

Whole genome approaches

There are several amphibian genomes currently available and vary greatly in size and quality. The western clawed frog (*Xenopus tropicalis*) was the first amphibian species with a whole genome assembly (Hellsten et al., 2010). The African clawed frog (*Xenopus laevis*) was later sequenced at the chromosome level using high-throughput sequencing, chromatin conformation capture and chromosome FISH (Session et al., 2016). XenBase (https://www.xenbase.org) is the central resource for *Xenopus* genomic data and phenotyping information. Available genomes of 19 amphibian species are summarised in a recent review (Sun et al., 2020) and genomes of 22 species are currently deposited in the NCBI genome database (see Figure 13.3). Two additional species, the common toad (*Bufo bufo*) and the hourglass treefrog (*Dendropsophus ebraccatus*), are available through the GenomeArk of the Vertebrate Genome Project (https://vgp.github.io/genomeark/), and a third, the rufous grassfrog (*Leptodactylus fuscus*) was made available more recently (Mohammadi et al., 2021). Gene annotations are critical for these genomes to be widely useful to the community, and yet only eight amphibian genomes are fully annotated (*Xenopus laevis, X. tropicalis, Nanorana parkeri, Bufo bufo, Rana temporaria*, and three caecilians *Microcaecilia unicolor, Geotrypetes seraphini* and *Rhinatrema bivittatum*). UniProt (https://www.uniprot.org) is a broad resource for annotated genes and its current version (2021_01) contains five amphibian species (Anura: *X. laevis*, *X. tropicalis, L. catesbeianus*; Gymnophiona: *M. unicolor, G. seraphini*).

Genome assembly and annotation can be difficult due to the large size and repetitive elements of many amphibian genomes, especially in Caudata (Figure 13.4), For example, the 30 Gb haploid genome size of the axolotl *Ambystoma mexicanum* is about 10 times larger than the human genome (Nowoshilow et al., 2018; Smith et al., 2019). In Anura, some of the existing assemblies are also larger than the human genome: 5.8 Gb in *Lithobates catesbeianus* (Hammond et al., 2017), 6.76 Gb in *Oophaga pumilio* (Rogers et al., 2018), and 4.55 Gb in *Bufo gargarizans* (Lu *et al.*, 2021). Nevertheless, some anuran genomes are much smaller, like the 1.7 Gb genome of *X. tropicalis* and the 1.1 Gb genome of *Platyplectrum ornatum* (Lamichhaney, Catullo, Keogh, Clulow, & Edwards, 2021).

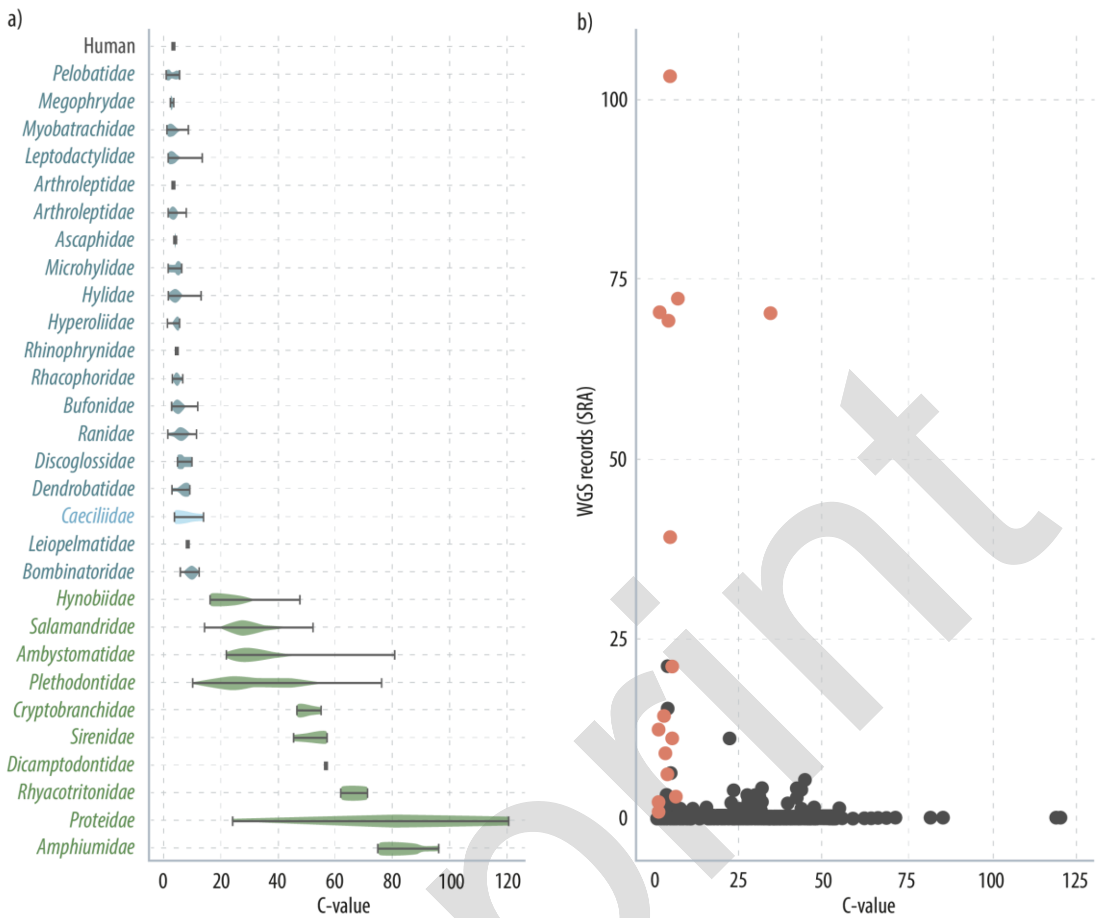


Figure 13.4: Genome size distribution across amphibian families and whole-genome sequencing (WGS) projects. (A) Genome size estimates (C-value, coloured by order with anurans in grey-blue, caecilians in light blue, and salamanders in green) vary widely by family. Human genome size is displayed at the top as a point of reference. (B) The relationship between genome sizes and submissions (WGS) per species is shown with assembled genomes marked by orange dots. C-values from Liedtke *et al.* (2018) and WGS records from NCBI SRA (www.ncbi.nlm.nih.gov/sra, accessed January 2021).

Whole genome challenges

The assembly of amphibian genomes remains challenging due to their large size and the vast amount of repeat elements (Rogers et al., 2018). The quality of available amphibian genomes ranges from near-complete chromosomal-scale genomes to fragmented contigs, and future efforts should focus on improving contiguity and completeness of these reference assemblies (Rhie et al., 2020). There are numerous threatened species with moderate genome sizes that we suggest be prioritised for sequencing (Table 13.2). Obtaining good estimates of genome sizes should be considered a top priority for threatened species, as this information is crucial for sequencing prioritisation. Data on genome size and chromosome numbers can be found at the phylogenetically aware database, GoaT (Genomes on a Tree; https://goat.genomehubs.org/). Even smaller genomes require sufficient computational resources, analytical expertise, and time to complete assembly and annotation. High repeat content necessitates that genome assemblers incorporate a variety of data types, including long reads (PacBio HiFi or Oxford Nanopore platforms), medium-range linked reads (Hi-C approaches by Dovetail or Arima Genomics), and optical mapping of genetic markers on whole chromosomes (e.g., BioNano platform) (Formenti et al., 2020; Nowoshilow et al., 2018; Rhie et al., 2020; Session et al., 2016). Dense genetic maps of F1 progenies can contribute to finalising chromosome-scale genome assembly (Mitros et al., 2019; Smith et al., 2019), and light-coverage sequencing of parental genomes can resolve a diploid genome assembly into its two component haploid genomes (Koren et al., 2018).

A central resource for amphibian genomic data (outside of *Xenopus*) with a standard procedure for annotation is critically needed. Amphibase (http://www.amphibase.org) was established to organise transcriptome resources with a unified gene annotation procedure, but more community effort is required for this to become a comprehensive resource. A database with diverse species is critically needed, as other sequence databases like UniProt are mostly derived from five amphibian species, which hinders our understanding of amphibian genome diversity. Overall, whole genome sequencing has not yet become a widespread tool for amphibian conservation. For example, a chromosome-scale reference genome is a valuable resource for understanding genetic diversity, although additional genomic samples are needed to estimate species genetic variation. We expect with decreased sequencing costs and more widely available annotation tools, whole genome sequencing will become a valuable conservation tool in the near future.

Table 13.2. Threatened species with moderate genome sizes that should receive priority in future genome sequencing projects. This list is not exhaustive and should be expanded as genome size estimates of more species become available. Estimates of genome size from (Liedtke, Gower, Wilkinson, & Gomez-Mestre, 2018) Red List categories from IUCN (2021).

Species	**Genome size (*C*-value)**	**Lineage**	**Red List category**
Leptopelis vermiculatus	3.1	Anura, Arthroleptidae	Endangered
Conraua goliath	3.1	Anura, Conrauidae	Endangered
Quasipaa boulengeri	3.1	Anura, Dicroglossidae	Endangered
Boulengerula taitana	2.9	Gymnophiona, Herpelidae	Endangered
Osteopilus vastus	2.5	Anura, Hylidae	Vulnerable
Phrynobatrachus krefftii	1.7	Anura, Phrynobatrachidae	Endangered
Buergeria oxycephala	1.6	Anura, Rhacophoridae	Vulnerable
Sooglossus sechellensis	1.8	Anura, Sooglossidae	Endangered
Telmatobius culeus	2.1	Anura, Telmatobiidae	Endangered

Transcriptomics

Transcriptomics approaches

Messenger RNA sequencing (RNASeq) is a method that sequences the expressed fraction of the genome. The assembled coding sequences of mRNAs can be compared with orthologous sequences in reference protein databases to infer and annotate their function. Transcript coding sequences could be used to design targeted enrichment probes and, along with the non-coding mRNA regions, can be used to develop microsatellite markers or genotyping panels for population genetic studies. The possibilities presented by the ability to quantify functional (presumptive amino acid sequence) variation without a reference genome makes this technique appealing for studying many molecular processes linked to conservation biology. Reference transcriptomes from 40 amphibian species are currently deposited in the NCBI Transcriptome Sequence Archive (TSA), a database of transcriptomes representing a fraction of the 222 species in SRA Database.

Best approaches for generating a transcriptome vary depending on the research question, and factors such as age, sex, and tissue type should be considered. For species with no reference genome assembly, transcriptomic data need to be assembled '*de novo*' into transcripts. Accurate annotation of the reference is also important for functional interpretation of downstream results (Hart et al., 2020; Musacchia, Basu, Petrosino, Salvemini, & Sanges, 2015) and several pipelines are now available for transcriptome assembly, annotation, and analyses (Cabau et al., 2017; Conesa et al., 2016; MacManes, 2018; McKenna et al., 2010; Smith-Unna, Boursnell, Patro, Hibberd, & Kelly, 2016; Van Den Berge et al., 2019). Although not currently widespread, transcriptomics studies are expected to benefit from long-read sequencing platforms (e.g., PacBio Iso-Seq, Oxford Nanopore Tech) for increased assembly contiguity and resolution of alternative splicing variants. However, the deep sequencing provided by short-read Illumina platforms may provide better depth, thus detecting rare transcripts useful for annotation.

Transcriptomics challenges

RNA sequencing is starting to be more widely applied to amphibian conservation projects and the current challenges are mostly associated with limited taxonomic diversity, as 76% of extant families have less than 5% of their species diversity represented by transcriptomic data (see Figure 13.3). In addition to identifying differentially expressed genes, RNA sequencing can also be used to study a range of important phenotypes linked to conservation planning. For example, these data can be used to identify a large set of SNPs to study signatures of selection in imperiled amphibian species to identify genotypes associated with adaptive polygenic traits like thermal tolerance, habitat preference, or disease resistance (Spurr et al., 2020). Finally, co-expression network analyses could be used to identify networks of genes with similar expression patterns across samples and how these vary under different conditions (Serin, Nijveen, Hilhorst, & Ligterink, 2016; van Dam, Võsa, van der Graaf, Franke, & de Magalhães, 2018). Combining gene co-expression networks with time series analyses in species experiencing drastic environmental challenges has the potential to uncover modules of co-expressed genes and changes in their interactions associated with a challenge of interest. This approach could pinpoint gene modules as markers for resilience or vulnerability, thus providing crucial information for implementing effective conservation measures.

Reduced representation library (RRL) sequencing

RRL approaches

Reduced Representation Libraries (RRL) are designed to focus sequencing on a subset of the genome. Restriction-site associated DNA sequencing (RADseq) and the targeted capture and sequencing of specific genomic regions are the two most common approaches currently used in amphibian genomics. RADseq was designed by (Miller et al., 2007) and further modified into genotyping-by-sequencing (GBS) (Elshire et al., 2011), double-digest RADseq (ddRAD, two restriction enzymes are used) (Peterson, Weber, Kay, Fisher, & Hoekstra, 2012), triple-digest RADseq (3RAD, three restriction enzymes are used) (Bayona-Vásquez et al., 2019), and Diversity Arrays Technology DArTseq (Lambert, Skelly, & Ezaz, 2016). There are also multiple methods of targeted capture such as Ultra Conserved Elements (UCE) (Faircloth et al., 2012; Mccormack et al., 2012), Anchored Hybrid Enriched (AHE) loci (Lemmon, Emme, & Lemmon, 2012). Restriction enzyme digestion and sequence capture probes can also be combined, as in the RADcap protocol (Hoffberg et al., 2016), and is exceptional at sequencing hundreds of specific loci across hundreds of individuals.

RRL methods provide hundreds to thousands of loci that allow for fine-scale analysis of population structure and genetic diversity, even with samples having low DNA quality like museum specimens, and thus have important implications in conservation recommendations. As a consequence, RRL techniques are useful for understanding reproductive isolation and gene flow as well as estimating hybridisation rates, species delimitation, and the identification of cryptic species (Dufresnes & Martínez-Solano, 2020; Dufresnes, Mazepa, et al., 2018; Guillory et al., 2019; Homola et al., 2019). Within species, population structure and demography are equally important, as gene flow and inbreeding depression influence adaptive potential and resilience to environmental change. For these questions, one of the most important parameters to quantify is effective population size, which can be used to study demographic history and extinction risk of populations. For example, RAD sequencing has been used with *Ambystoma* salamanders to determine effective population size, which could prove useful for population monitoring and management planning (Nunziata, Lance, Scott, Lemmon, & Weisrock, 2017; Nunziata & Weisrock, 2018).

RRL data has also been used for improving whole genome assembly methods by sequencing specific chromosomes (also known as ChromSeq, (Iannucci et al., 2021)). This approach resolved the assembly of the sex chromosomes of *Xenopus tropicalis* (Seifertova et al., 2013) and *Amolops mantzorum* (Luo, Xia, Yue, & Zeng, 2020), and helped to assemble the large genomes of *Ambystoma mexicanum* (Keinath et al., 2015; Smith et al., 2019) and *Notophthalmus viridescens* (Keinath, Voss, Tsonis, & Smith, 2017). In addition, RRL sequencing has enabled the identification of important genome features, such as sex-related markers (Cauret et al., 2020; Lambert et al., 2016) or candidate genes linked to conservation relevant traits (Guo, Lu, Liao, & Merilä, 2016).

RRL challenges

RRL approaches are likely to remain popular tools for informing amphibian conservation given their cost-effectiveness, especially for large amphibian genomes. However, a biased taxonomic distribution of RRL sequencing effort is noticeable (see Figure 13.3), as there is currently no data for Gymnophiona and multiple families of Anura (mostly Neobatrachians) and Caudata. Most families are underrepresented and only Pelobatidae and Pelodytidae have all of their species sequenced with RRL assays. Although public datasets may accelerate the improvement of specimen samplings, combining different RRL datasets may be very challenging, especially when they resulted from non-targeted genome-subsampling methods.

As the data produced by RADseq are randomly sampled across the genome, the sequences recovered in different experiments are not necessarily the same, even if the same restriction enzymes are used. Another challenge of RRL is that functional interpretations can be limited without a reference genome.

Metabarcoding and metagenomics

Metabarcoding and metagenomics approaches

Emerging from DNA barcoding (Hebert, Cywinska, Ball, & deWaard, 2003), metabarcoding focuses on the amplification and sequencing of specific genetic markers from multiple individuals while metagenomics corresponds to the study of genetic material from many individuals within an environment. Both approaches have broad applicability in taxonomy, ecology, population dynamics, evolution and biogeography, all of which are essential contributors to amphibian conservation biology (Ficetola, Manenti, & Taberlet, 2019). Metabarcoding and metagenomics, along with RNA sequencing, are also being used to profile microbial and parasitic communities of amphibians (Shakya, Lo and Chain, 2019). Successful examples include profiling parasites in the eastern dwarf tree frog (*Litoria fallax*) (Ortiz-Baez et al., 2020) and poison frogs (Dendrobatidae) (Santos, Tarvin, Connell, Blackburn, & Coloma, 2018).

The use of environmental DNA (eDNA) metabarcoding has been applied to survey amphibian communities in threatened ecosystems (Lopes et al., 2017; Sasso et al., 2017) rediscover “extinct” or “rare” species (Goldberg, Strickler, & Fremier, 2018; Lopes et al., 2021), detect invasive species (Bento, Egeter, Rebelo, Chaves, & Pinto, 2021; Dufresnes et al., 2019; Dufresnes, Denoël, Santo, & Dubey, 2017; Dufresnes, Leuenberger, et al., 2018; Secondi, Dejean, Valentini, Audebaud, & Miaud, 2016), identify emerging diseases

(Romero-Zambrano, Bermúdez-Puga, Sánchez-Yumbo, Yánez-Galarza, & Ortega-Andrade, 2021), and develop strategies in accordance with the Amphibian Conservation Action Plan (Wren et al., 2015). For example, this approach has successfully been used to monitor the distribution of the threatened great crested newt (*Triturus cristatus*) and detect invasive species associated with population declines (Harper et al., 2019).

Metabarcoding and metagenomics challenges

The success of metabarcoding studies for amphibian conservation is dependent on representative reference sequences within these databases. Metabarcoding and metagenomics facilitate the identification of relevant taxa from high-throughput sequencing data (Wilson, Sing, & Jaturas, 2019; Xu, Dimitrov, Rahbek, & Wang, 2015) and rely on reference sequences in public databases like BOLD (www.boldsystems.org), ENA (www.ebi.ac.uk/ena), GenBank (www.ncbi.nlm.nih.gov/genbank), and Silva (www.arb-silva.de), among others. BOLD, for example, contains reference sequences for only 3,247 species of amphibians (39% of described species) with Anura (2,728 spp., 37% of total species diversity) and Gymnophiona (84 spp., 39%) taxa being less well represented than those in Caudata (435 spp, 57%). Therefore, efforts toward reducing taxonomic gaps in reference databases are encouraged.

Epigenetics

Epigenetics approaches

Epigenetics describes genome-wide patterns of DNA modifications and structures that impact gene regulation. These can be inherited somatically or through the germline without altering the DNA sequence (Rando & Verstrepen, 2007). Such modifications can serve as stress biomarkers predicting population persistence in unstable environments (Rey et al., 2020). In this context, whole genome bisulfite-sequencing (WGBS) can be used, which relies on the conversion of cysteines into thymines by sodium bisulfite. Activity levels of methylation-inducing genes can then be measured using qPCR (Hudson, Lonhienne, Franklin, Harper, & Lehnert, 2008) or DNA methylation-specific antibodies (Hawkins & Storey, 2018; Zhang, Hawkins, & Storey, 2020). For example, temperature-related research in amphibians includes studies of expression of genes whose products have gene silencing functions in striped burrowing frogs (*Cyclorana alboguttata*) (Hudson et al., 2008), changes in methylation patterns linked to the freeze-thaw cycle in Wood Frogs (*Rana sylvatica*) (Hawkins & Storey, 2018; Hudson et al., 2008), and histone modifications linked to the onset of metamorphosis in *L. catesbeianus* (Mochizuki, Ishihara, Goda, & Yamauchi, 2012).

Epigenetic modifications can change under other environmental stressors such as endocrine disrupting chemicals (Jacobs, Marczylo, & Guerrero-Bosagna, 2017) or radiation. For example, exposure of *Xenopus laevis* to the pesticide atrazine causes disturbances in steroidogenesis via epigenetic modifications (Hayes et al., 2002). Japanese tree frogs (*Dryophytes japonicus*) sampled two years after the Fukushima nuclear accident show genome-wide increases in methylation patterns (Gombeau et al., 2020). These connections highlight the importance of epigenetic modifications as stress biomarkers and the untapped potential of this tool for amphibian conservation.

Epigenetics challenges

This approach requires a high quality reference genome and extensive sequencing depth, which is expensive at present but likely to decrease in cost in the future. Once epigenome markers are identified (Thorson et al., 2020), other more cost-effective methods may be used to assess their modification (reviewed in (Eirin-Lopez & Putnam, 2019)). To reliably relate epigenetic changes with environmental stressors, baseline research is needed to identify which external variables influence gene methylation (Mochizuki et al., 2012; Rey et al., 2020). Additionally, considering the longevity of epigenetic modifications across generations, there is a need for understanding the role of long-term acclimatisation in reintroduction efforts (van Oppen, Oliver, Putnam, & Gates, 2015). Including epigenetics in conservation planning (conservation epigenetics *sensu* (Rey et al., 2020)) would ensure that recent ecological history and phenotypic plasticity are taken into account.

Case studies on applying genomics approaches to amphibian conservation

The recent revolution in genomics technologies means that many projects are underway for which the successes and failures are not yet known. Here, we look at specific conservation projects that have successfully used genomics technologies to inform conservation approaches to disease resistance, captive breeding, and biocontrol of invasive species.

Understanding and increasing chytridiomycosis resistance

Understanding the genetic contribution to chytridiomycosis susceptibility caused by *Batrachochytrium dendrobatidis* (Bd) infection is critical for prioritising species for conservation efforts and producing species capable of surviving the disease through captive breeding programs. Most efforts to identify genetic regions associated with Bd immunity have involved targeted studies of immune genes or gene expression comparisons between infected and uninfected frogs (Table 3). The majority of Bd genetic association studies have targeted the major histocompatibility complex (MHC), which have detected correlations between MHC variation and Bd resistance (Table 3). One of the best examples comes from lowland leopard frogs (*Lithobates yavapaiensis*), where an MHC allele (the Q-allele) predicts increased survival (Savage & Zamudio, 2011; Sommer, 2005). RNA sequencing approaches have identified many immune genes that are differentially expressed in response to Bd infection including the MHC, B-cells, complement, and chitinase (Table 3). These studies also found that Bd suppresses lymphocyte expression (Ellison, Savage, et al., 2014), more resistant populations exhibit robust early immune response (Grogan et al., 2018), and dysregulation of immune genes is associated with susceptibility (Grogan et al., 2018; Savage, Gratwicke, Hope, Bronikowski, & Fleischer, 2020). Although these approaches have identified many candidate resistance genes for future study, their design does not permit testing the link between gene expression differences and Bd survival given study animals were euthanised for tissue sampling.

A thorough understanding of the genes underlying chytrid immunity and their effect size is critical for managing amphibians threatened by Bd. To date, only two studies have used genome approaches to investigate Bd resistance: a genome-wide association study in Southern Corroboree frogs (see Box 1) (Kosch et al., 2019) and targeted exome sequencing in harlequin frogs (Byrne et al., 2021). Although pioneering in their approaches, these studies lack the robust statistical power recommended before use in management. With the rapid development of genomics technologies in recent years, and the ever increasing availability of amphibian reference genomes, such investigations are now possible in many species. Future efforts should apply genomics approaches discussed in this Status Update to better understand genetic contributions to Bd resistance.

Table 13.3. Bd immunity studies using genetic/genomic approaches.

Species	**Experimental Design**	**Gene Region**	**Reference**
Bufo calamita	Field study	MHCII	(May, Zeisset, & Beebee, 2011)
Lithobates yavapaiensis	Laboratory challenge	MHCII	(Savage & Zamudio, 2011)
Multiple sp.	Field study and laboratory challenge	MHCII	(Bataille et al., 2015)
Lithobates yavapaiensis	Field study	MHCII	(Savage & Zamudio, 2016)
Physalaemus pustulosus	Field study	MHCII	(Kosch et al., 2016)
Lithobates chiricahuensis	Field study	MHCII	(Savage, Mulder, Torres, & Wells, 2018)
Thoropa taophora	Field study	MHCII	(Belasen, Bletz, Leite, Toledo, & James, 2019)
Lithobates pipiens	Field study	MHCII	(Trujillo *et al*., 2021)
Japanese *Rana* spp.	Field study	TLRs	(Lau, Igawa, Kosch, & Satta, 2018)

Xenopus tropicalis	Laboratory challenge	Transcriptome	(Rosenblum et al., 2009)
Lithobates muscosa, L. sierrae	Laboratory challenge	Transcriptome	(Rosenblum, Poorten, Settles, & Murdoch, 2012)
Atelopus zeteki	Laboratory challenge	Transcriptome	(Ellison, Savage, et al., 2014)
Agalychnis callidryas, Atelopus glyphus, Atelopus zeteki, Craugastor fitzingeri	Laboratory challenge	Transcriptome	(Ellison, Tunstall, et al., 2014)
Rana temporaria	Laboratory challenge	Transcriptome	(Price et al., 2015)
Rhinella marinus, Anaxyrus boreas	Laboratory challenge	Transcriptome	(Poorten & Rosenblum, 2016)
Lithobates sylvatica, L. catesbeianus	Laboratory challenge	Transcriptome	(Eskew et al., 2018)
Litoria verreauxii alpina	Laboratory challenge	Transcriptome	(Grogan et al., 2018; Savage et al., 2020)
Lithobates yavapaiensis	Laboratory challenge	Transcriptome	(Savage et al., 2020)

Pseudophryne corroboree	Laboratory challenge	Genome-wide SNPs, MHCI	(Kosch et al., 2019)
Atelopus varius, A. zeteki	Field study	Exome	(Byrne et al., 2021)

<begin Box 1>

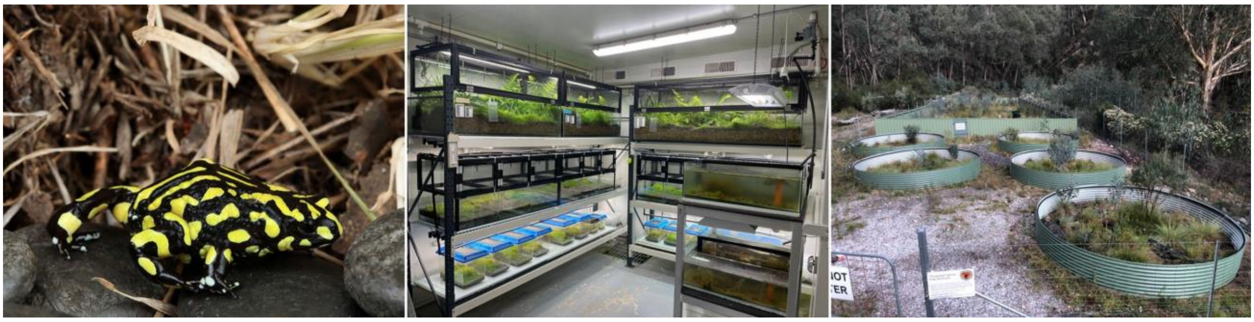
Box 13.1. Developing methods to increase Bd-resistance in Southern Corroboree Frogs

Southern Corroboree frogs (*Pseudophryne corroboree*) – an Australian alpine endemic species – have been driven to functional extinction in the wild by chytridiomycosis (Hunter et al., 2010) and their continued survival is dependent on captive breeding and reintroduction. Although a successful breeding program has been in place for over a decade, self-sustaining populations have yet to be established in the wild (Kosch et al., 2019). One of the challenges of re-establishing this species is that it co-occurs with Bd-tolerant reservoir species *Crinia signifera* (Scheele, Hunter, Brannelly, Skerratt, & Driscoll, 2017). As culling the reservoir host is not a desirable option, Bd-resistance will have to be increased to allow this species to survive along with the Bd pathogen.

Research is underway to understand the genetic basis of Bd-resistance and develop methods to enhance it in currently susceptible species (Kosch et al., 2019; Skerratt, 2019). The Southern Corroboree Frog Restoration Project consists of a multi-institutional group of academics, threatened species managers, and zoo practitioners dedicated to restoring this species in the wild (Lee Berger; Deon Gilbert; David Hunter; Tiffany Kosch; Michael McFadden; Jacques Robert; Kyall Zenger; James Cook University; NSW Department of Planning, Industry and Environment; Taronga Conservation Society; University of

Melbourne; University of Rochester; and Zoos Victoria). As genetic intervention is a long-term endeavour requiring decades before animals are fit for release, participants have committed to proceeding cautiously, involving all stakeholders, and vetting the safety and efficacy of each step before proceeding. The program consists of multiple stages: 1) understanding the genetic basis of immunity to Bd, 2) developing genetic tools to increase resistance, 3) testing effectiveness of genetic intervention by Bd-challenge in the lab and the field (by release into exclosures), 4) testing for off-target effects in the lab and the field, 5) release into the wild, and 6) long-term monitoring to evaluate success. Such methods, if successful, can be used as a proof of concept for other threatened amphibians worldwide.

One of the biggest challenges for this project has been developing genetic resources for *P. corroboree*. However, current efforts to sequence a reference genome and develop gene editing and transgenesis tools should help alleviate this problem. Pilot studies have been conducted to sequence immune genes, develop genome-wide DArT-seq markers, and begin to understand the genetic architecture of resistance (Kosch et al., 2017, 2019). Future plans involve testing other genotyping technologies such as targeted sequence capture and low-pass sequencing to increase genotyping coverage and performing well-powered genome-wide association studies with increased sample size. There are also plans to expand the standard phenotypes used to measure Bd-resistance by including molecular phenotypes and longitudinal gene expression data to better understand genetic architecture and identify putative Bd-resistance variants.



Box Figure 13.1. Southern corroboree frogs (*Pseudophryne corroboree*) are conservation-reliant due to their susceptibility to Bd. A captive-bred *P. corroboree* frog (left, photograph by Corey Doughty), *P. corroboree* breeding facility at the Melbourne Zoo (middle, photograph by Mikaeylah Davidson), and outdoor enclosures maintained by the Australian National Parks and Wildlife Service (right, photograph by Michael McFadden).

<end of Box 1>

Genomic approaches for invasive amphibian biocontrol

Invasive species are linked to approximately one-third of amphibian extinctions and threaten 16% of extant amphibian species (Blackburn, Bellard, & Ricciardi, 2019). These effects occur primarily through habitat alteration, predation, competition, hybridisation, and disease spread (Falaschi, Melotto, Manenti, & Ficetola, 2020; Nunes et al., 2019). The use of genomic approaches for understanding and managing invasions has rapidly increased in recent years (McCartney, Mallez, & Gohl, 2019), but is only beginning to be applied to amphibian systems (see Box 13.2).

Genomic tools offer powerful methods to study invasive-native hybridisation. For example, hybridisation with invasive salamanders (*Ambystoma tigrinum mavortium*) threatens endemic native salamanders (*Ambystoma californiense*) in California (Mccartney-Melstad & Shaffer, 2015), where hybrids outcompete and cannibalise pure natives and prey upon other amphibians in the community (Ryan, Johnson, & Fitzpatrick, 2009). Preservation of the native species requires introgression prevention, and genomic scans have been used to track the movement of non-native alleles (Shaffer et al., 2015). Moreover, genome regions associated with traits critical to population viability are candidates that may indicate to managers which populations have the strongest potential to further spread non-native alleles (Shaffer et al., 2015). For example, genomic regions associated with metamorphosis were identified using RRL sequencing (Voss, Kump, Walker, Shaffer, & Voss, 2012) and genes promoting thermal tolerance have been identified using RNA sequencing (Cooper & Shaffer, 2021). Thus, genomics approaches are critical tools for understanding invasive-native population dynamics and can inform conservation management practices (Dufresnes & Dubey, 2020).

Genomic tools also offer new perspectives into invader-mediated population declines. Invasive cane toads (*Rhinella marina*) in Australia increase parasitic infections in native amphibians (Kelehear, Brown, & Shine, 2013) that can be fatal (Pizzatto & Shine, 2011). RNA sequencing of invasive Australian cane toad livers revealed a novel virus at high prevalence (Russo et al., 2018), while follow up studies showed that native range cane toads contained a diversity of viruses (Russo et al., 2021). This suggests an “enemy release”, where viruses left behind in the native range may serve as effective control agents due to evolutionary distance (Russo et al., 2021). Although biocontrol through pathogenic agents has been suggested, selection of a suitable agent would require careful investigation due to the risk of infecting native frog species.

Cane toads also carry lethal toxins that lead to population-level declines in Australian predators (Shine, 2010), as well as shifts in behavioural traits of some predator populations (Pettit, Ward-Fear, & Shine, 2021). Gene editing in cane toads using CRISPR has been used to knock-out a toxin hydrolase that converts toad toxin from its storage form to a lethal active form (Cooper et al., 2020). Other genes that may enhance the toad's invasion success may also serve as future knockout candidates using these protocols. However, this approach requires caution due to the potential risk of gene-edited toads being inadvertently introduced back to the native South America range through human translocation.

<begin Box 2>

Box 13.2. Genomics of the cane toad invasion

Box 2 Figure. The invasive Australian cane toad (*Rhinella marina*). Photograph taken by Dr. Matt Greenlees.

Originally sourced from native South American populations, cane toads (*Rhinella marina*) were introduced to Puerto Rico in 1920, then to Hawaii in 1932, and finally to northeastern Australia in 1935 (Turvey, 2013). The cane toad invasion has since garnered much attention in Australia due to its ecological effects on a diversity of native taxa (Shine, 2010).

The collection of genomic data on invasive cane toads is relatively recent, enabled by the development of a multi-tissue reference transcriptome (Richardson et al., 2018) and draft genome assembly (Edwards et al., 2018). These tools have been critical for elucidating genetic changes that occur as the toads disperse across northern Australia to the arid western regions. Population genetics studies using RNA-Seq (Selechnik, Richardson, Shine, DeVore, et al., 2019) and RADSeq (Trumbo et al., 2016) have characterised population structure and identified two genetic clusters separated at a continental divide marked by an abrupt change in rainfall and temperature. Candidate genes involved in heat and dehydration resistance (Selechnik, Richardson, Shine, DeVore, et al., 2019) and those involved in metabolism and stress responses (Rollins, Richardson, & Shine, 2015) have been identified that may underlie the successful range expansion. Differential expression analyses on the RNA-Seq dataset suggest that environment-driven gene expression follows a similar pattern across the continental divide (Selechnik, Richardson, Shine, Brown, & Rollins, 2019).

The application of genomic techniques to the cane toad system has allowed for the investigation of invasion from novel perspectives. Analyses using 16S rRNA sequencing data to characterise colon microbiota in toads from each side of the continental divide revealed differences in both microbial compositional and functional variation (Zhou et al., 2020). Furthermore, behavioural traits were linked to microbial functional variation while infection prevalence of lungworm parasites was linked to both compositional and functional variation (Zhou et al., 2020). Further exploration of the relationships between gut microbiota, endoparasites, and invasive behaviours may cultivate new management strategies.

The role of epigenetics in shaping the cane toad invasion has also been investigated. Reduced representation bisulfite sequencing on common garden-bred cane toad tadpoles exposed to conspecific alarm cues revealed differential changes to DNA methylation in lineages from each side of the continental divide (Sarma et al., 2020). Further, these alarm cue-exposed individuals exhibited an induced defence mechanism and this defence was shown to be transferred to the next generation (Sarma et al., 2021). These are among the first studies to demonstrate a potential role for epigenetics in rapid evolution during invasion and suggest that such effects should be considered in future biocontrol studies.

<end of Box 2>

Discussion

Amphibians are less intensively researched than mammals or birds (Figure 13.1) and most genomic sequencing efforts in amphibians have concentrated on Least Concern taxa. Being the tetrapod group with the most threatened species, a boost on genome sequencing projects in threatened amphibian species is urgent. Although the lack of high-quality reference genomes may preclude some genomic applications, the use of reduced genome representation techniques (e.g. RNA-Seq, RAD-Seq, and Targeted Capture assays) are a viable alternative to genome-based approaches and should be more extensively applied to imperilled amphibian species. We strongly suggest that Red List assessments incorporate genomics approaches for estimating genetic diversity and species delimitation in biodiverse regions. We can now envision a future where genomic-informed interventions in translocations, genomic rescue, and disease prevention and mitigation are part of our arsenal for ensuring the long-term preservation of amphibian biodiversity.

Many approaches have been successfully used to conserve threatened amphibians including habitat conservation, restoration, and supplementation (Cook, 2010; Woodhams et al., 2011). Unfortunately, these approaches are not always effective for threats that are hard to mitigate such as disease, climate change, and invasive species, thus requiring the development of novel approaches to increase survival. If the goal of a conservation program is to establish self-sustaining populations in the wild, then genomic methods that promote survival alongside the threat should be considered. Measurement of genetic diversity is critical for assessing inbreeding and outbreeding depression prior to population augmentation or captive breeding strategies and genomics is currently the simplest way of tackling this problem (Byrne & Silla, 2020; Frankham et al., 2011). Although more complex and drastic, genetic intervention is also a promising approach for establishing self-sustaining populations of amphibians that can survive alongside key threats. Genetic intervention methods can include genetic rescue, CRISPR gene editing, and genomic selection, all of which rely on genomics technologies and reference genomes. Of these, only genetic rescue has been used for conservation purposes (but see (Newhouse & Powell, 2021; van Oppen & Oakeshott, 2020); Box 13.1). However, the widespread success of gene editing and/or genomic selection methods in medicine and agriculture (Meuwissen, Hayes, & Goddard, 2016; Piaggio et al., 2017) suggests these methods should be considered. Genetic intervention in wildlife is controversial (Kardos & Shafer, 2018; Redford, Brooks, Nicholas, & Adams, 2019) and should be performed with utmost caution along with careful testing to ensure that manipulated animals pose no environmental risk and are fit for release. Another challenge of applying genetic intervention methods in amphibians is the lack of fundamental genomic understanding of key survival traits, but this should increase as more genomic resources become available in the near future.

This Genomics Status Update has highlighted several critical needs for the amphibian conservation community, including equity in training and technology access, data resource management and transparency, and the involvement of stakeholders and conservation practitioners in genomics analyses. There is a clear geographic bias in the origins of genomics data compared to amphibian biodiversity hotspots (Figure 31.2). We call for more equity in training opportunities and access to genomics technologies for researchers from Central and South America, Africa, and Southeast Asia. Cheap and portable sequencing platforms are one promising avenue, coupled with bioinformatics training and decolonisation of field-based genomic studies. Data transparency and accessibility is another community challenge, as annotation and genomic resource management often lack funding but are critical for rapid progress. Additionally, transparency in data and sequencing should be a requirement of any funded project, including rapid public release of sequence data prior to publications that may take years to appear. Finally, there is a clear need to involve stakeholders and conservation practitioners in genomics research, which could include community driven annotation or metadata necessary for genome usability as well as “plug and play” platforms coupled with free online bioinformatics training opportunities that make these approaches more accessible in concept and in practice. Portable high-throughput nanopore MinION sequencers are now being used directly in the field to generate genomic data for rapid biodiversity assessments, thus strengthening local capacities for monitoring and conservation (Pomerantz et al., 2018). The ability to conduct massively parallel DNA sequencing studies *in situ* can also alleviate the need to export genetic material or digital sequence information on genetic resources (DSI), two key components of the Convention on Biological Diversity (CBD) and the Nagoya Protocol (https://www.cbd.int/dsi-gr/). Portable devices with quick high-throughput sequencing and analysis capabilities can boost data accessibility for decision-makers, researchers, and local government officials to improve amphibian management decisions. Genomics can make an important contribution to global amphibian conservation, but only if access to its power is equitable for all people involved.

Acknowledgements

We thank Dr. Lee Skerratt and Dr. David Hunter for their comments on Box 1 and Dr. Lee Ann Rollins for feedback on the invasive species section. TK is supported by a grant from the National Institute of Biological Resources (NIBR), funded by the Ministry of Environment (MOE) of the Republic of Korea (NIBR201803101, NIBR201905103, NIBR202005102). LAO is supported by the National Science Foundation [IOS-1845651, IOS-1827333, IOS-1822025], the National Institutes of Health [DP2HD102042], the Rita Allen Foundation, the

McKnight Foundation, Pew Charitable Trusts, and the New York Stem Cell Foundation. HMOA is supported by the Secretaría de Educación Superior, Ciencia, Tecnología e Innovación of the Republic of Ecuador [SENESCYT (ENSAMBLE-Grant-PIC-17-BENS-001)], the World Academy of Sciences (TWAS-Grant-16-095) and VLIR-UOS (SI2020SEL031-77258) as local co-promoter in Ecuador.

References

Bataille, A., Cashins, S. D., Grogan, L., Skerratt, L. F., Hunter, D., Mcfadden, M., … Waldman, B. (2015). Susceptibility of amphibians to chytridiomycosis is associated with MHC class II conformation. *Proceedings of the Royal Society B*, *282*(20143127).

Bayona-Vásquez, N. J., Glenn, T. C., Kieran, T. J., Finger, J. W., Louha, S., & Troendle, N. (2019). *Adapterama III : Quadruple-indexed , double / triple-enzyme RADseq libraries*. *PeerJ*, 7:e7724. doi: 10.7717/peerj.7724.

Belasen, A. M., Bletz, M. C., Leite, D. da S., Toledo, L. F., & James, T. Y. (2019). Long-term habitat fragmentation is associated with reduced MHC IIB diversity and increased infections in amphibian hosts. *Frontiers in Ecology and Evolution*, *6*, 1–12. doi: 10.3389/fevo.2018.00236

Bento, S., Egeter, B., Rebelo, R., Chaves, C., & Pinto, J. (2021). eDNA is a useful tool to evaluate the success of the eradication program of *Xenopus laevis* in Portugal. *ARPHA Conference Abstracts*, 4, e65055. doi: 10.3897/aca.4.e65055

Blackburn, T. M., Bellard, C., & Ricciardi, A. (2019). Alien versus native species as drivers of recent extinctions. *Frontiers in Ecology and the*

Environment, *17*(4), 203–207. doi: 10.1002/fee.2020

Byrne, A. Q., Richards-Zawacki, C. L., Voyles, J., Bi, K., Ibáñez, R., & Rosenblum, E. B. (2021). Whole exome sequencing identifies the potential for genetic rescue in iconic and critically endangered Panamanian harlequin frogs. *Global Change Biology*, *27*(1), 50–70. doi: 10.1111/gcb.15405

Byrne, P. G., & Silla, A. J. (2020). An experimental test of the genetic consequences of population augmentation in an amphibian. *Conservation Science and Practice*, (July 2019), 1–11. doi: 10.1111/csp2.194

Cabau, C., Escudié, F., Djari, A., Guiguen, Y., Bobe, J., & Klopp, C. (2017). Compacting and correcting Trinity and Oases RNA-Seq de novo assemblies. *PeerJ*, *5:e2988*. doi: 10.7717/peerj.2988

Cauret, C. M. S., Gansauge, M.-T., Tupper, A. S., Furman, B. L. S., Knytl, M., Song, X., … Evans, B. J. (2020). Developmental systems drift and the drivers of sex chromosome evolution. *Molecular Biology Evolution*, *37*(3), 799–810. doi: 10.1093/molbev/mst

Conesa, A., Madrigal, P., Tarazona, S., Gomez-Cabrero, D., Cervera, A., Mcpherson, A., … Mortazavi, A. (2016). A survey of best practices for RNA-seq data analysis. *Genome Biology*, *17*, 13. doi: 10.1186/s13059-016-0881-8

Cook, F. R. (2010). "Amphibian ecology and conservation: A handbook of techniques". edited by K. Dodd. 2010. [book review]. *The Canadian Field Naturalist*, *124*(1), 69. doi: 10.22621/cfn.v124i1.1043

Cooper, C. A., Woodcock, M. E., Kamalakkannan, V., Capon, R. J., Strive, T., & Tizard, M. L. (2020). Successful gene editing in the cane toad. *Abstracts from the UC Davis Transgenic Animal Research Conference XII August 11–15, 2019*, 467–485. doi: 10.1007/s11248-020-00210-1

Cooper, R. D., & Shaffer, H. B. (2021). Allele-specific expression and gene regulation help explain transgressive thermal tolerance in non-native hybrids of the endangered California tiger salamander (*Ambystoma californiense*). *Molecular Ecology*, *30*(4), 987–1004. doi: 10.1111/mec.15779

Dufresnes, C., Déjean, T., Zumbach, S., Schmidt, B. R., Fumagalli, L., Ramseier, P., & Dubey, S. (2019). Early detection and spatial monitoring of an emerging biological invasion by population genetics and environmental DNA metabarcoding. *Conservation Science and Practice*, *1*(9), e86. doi: 10.1111/csp2.86

Dufresnes, C., Denoël, M., Santo, L., & Dubey, S. (2017). Multiple uprising invasions of *Pelophylax* water frogs , potentially inducing a new hybridogenetic complex. *Scientific Reports*, *7*(1), 6506. doi: 10.1038/s41598-017-06655-5

Dufresnes, C., & Dubey, S. (2020). Invasion genomics supports an old hybrid swarm of pool frogs in Western Europe. *Biological Invasions*, *22*, 205–210. doi: 10.1007/s10530-019-02112-8

Dufresnes, C., Leuenberger, J., Amrhein, V., Bühler, C., Thiébaud, J., Bohnenstengel, T., & Dubey, S. (2018). Invasion genetics of marsh frogs (*Pelophylax ridibundus* sensu lato) in Switzerland. *Biological Journal of the Linnean Society*, *123*(2), 402–410. doi: 10.1093/biolinnean/blx140

Dufresnes, C., & Martínez-Solano, Í. (2020). Hybrid zone genomics supports candidate species in Iberian *Alytes obstetricans*. *Amphibia-*

Reptilia, *41*, 105–112. doi: 10.1163/15685381-20191312

Dufresnes, C., Mazepa, G., Rodrigues, N., Brelsford, A., Litvinchuk, S. N., Sermier, R., … Jeffries, D. L. (2018). Genomic evidence for cryptic speciation in tree frogs from the Apennine Peninsula, with description of *Hyla perrini* sp. nov. *Frontiers in Ecology and Evolution*, *6*, 144. doi: 10.3389/fevo.2018.00144

Edwards, R. J., Tuipulotu, D. E., Amos, T. G., O'Meally, D., Richardson, M. F., Russell, T. L., … White, P. A. (2018). Draft genome assembly of the invasive cane toad, *Rhinella marina*. *GigaScience*, *7*(9), giy095. doi: 10.1093/gigascience/giy095/5067871

Eirin-Lopez, J. M., & Putnam, H. M. (2019). Marine environmental epigenetics. *Annual Review of Marine Science*, 11, 335-368. doi: 10.1146/annurev-marine-010318-095114

Ellison, A. R., Savage, A. E., DiRenzo, G. V., Langhammer, P., Lips, K. R., & Zamudio, K. R. (2014). Fighting a losing battle: Vigorous immune response countered by pathogen suppression of host defenses in the chytridiomycosis-susceptible frog *Atelopus zeteki*. *G3: Genes, Genomes, Genetics*, *4*(7), 1275–1289. doi: 10.1534/g3.114.010744

Ellison, A. R., Tunstall, T., Direnzo, G. V., Hughey, M. C., Rebollar, E. A., Belden, L. K., … Zamudio, K. R. (2014). More than skin deep: Functional genomic basis for resistance to amphibian chytridiomycosis. *Genome Biology and Evolution*, *7*(1), 286–298. doi: 10.1093/gbe/evu285

Elshire, R. J., Glaubitz, J. C., Sun, Q., Poland, J. A., Kawamoto, K., Buckler, E. S., & Mitchell, S. E. (2011). A robust , simple genotyping-by-sequencing (GBS) approach for high diversity species. *PLoS ONE*, *6*(5), 1–

10. doi: 10.1371/journal.pone.0019379

Eskew, E. A., Shock, B. C., Ladouceur, E. E. B., Keel, K., Miller, M. R., Foley, J. E., & Todd, B. D. (2018). Gene expression differs in susceptible and resistant amphibians exposed to *Batrachochytrium dendrobatidis*. *Royal Society Open Science*, *5*(2). doi: 10.1098/rsos.170910

Faircloth, B. C., McCormack, J. E., Crawford, N. G., Harvey, M. G., Brumfield, R. T., & Glenn, T. C. (2012). Ultraconserved elements anchor thousands of genetic markers spanning multiple evolutionary timescales. *Systematic Biology*, *61*(5), 717–726. doi: 10.1093/sysbio/sys004

Falaschi, M., Melotto, A., Manenti, R., & Ficetola, G. F. (2020). Invasive species and amphibian conservation. *Herpetologica*, *76*(2), 216–227. doi: 10.1655/0018-0831-76.2.216

Ficetola, G. F., Manenti, R., & Taberlet, P. (2019). Environmental DNA and metabarcoding for the study of amphibians and reptiles : species distribution , the microbiome , and much more. *Amphibia-Reptilia*, *40*, 129–148. doi: 10.1163/15685381-20191194

Formenti, G., Rhie, A., Balacco, J., Haase, B., Mountcastle, J., Houde, P., … Skelton, J. (2020). Complete vertebrate mitogenomes reveal widespread gene duplications and repeats. *BioRxiv*. doi: 10.1101/2020.06.30.177956

Frankham, R., Ballou, J. D., Eldridge, M. D. B., Lacy, R. C., Ralls, K., Dudash, M. R., & Fenster, C. B. (2011). Predicting the probability of outbreeding depression. *Conservation Biology*, *25*(3), 465–475. doi: 10.1111/j.1523-1739.2011.01662.x

Funk, W. C., Zamudio, K. R., & Crawford, A. J. (2018). Advancing understanding of amphibian evolution, ecology, behavior, and conservation with massively parallel sequencing. In

P. A. Hohenlohe & O. P. Rajora (Eds.), *Population Genomics: Wildlife* (pp. 211–254). Springer, Cham. doi: 10.1007/13836_2018_61

Goldberg, C. S., Strickler, K. M., & Fremier, A. K. (2018). Degradation and dispersion limit environmental DNA detection of rare amphibians in wetlands : Increasing efficacy of sampling designs. *Science of the Total Environment*, *633*, 695–703. doi: 10.1016/j.scitotenv.2018.02.295

Gombeau, K., Bonzom, J., Cavali, I., Camilleri, V., Orjollet, D., Dubourg, N., … Adam-Guillermin, C. (2020). Dose-dependent genomic DNA hypermethylation and mitochondrial DNA damage in Japanese tree frogs sampled in the Fukushima Daiichi area. *Journal of Environmental Radioactivity*, *225*:106429. doi: 10.1016/j.jenvrad.2020.106429.

Grogan, L. F., Robert, J., Berger, L., Skerratt, L. F., Scheele, B. C., Castley, J. G., … McCallum, H. I. (2018). Review of the amphibian immune response to chytridiomycosis, and future directions. *Frontiers in Immunology*, *9*(NOV), 1–20. doi: 10.3389/fimmu.2018.02536

Guillory, W. X., French, C. M., Twomey, E. M., Chávez, G., Prates, I., May, R. Von, … Brown, J. L. (2019). Phylogenetic relationships and systematics of the Amazonian poison frog genus *Ameerega* using ultraconserved genomic elements. *Molecular Phylogenetics and Evolution*, *142*, 106638. doi: 10.1016/j.ympev.2019.106638

Guo, B., Lu, D., Liao, W. B., & Merilä, J. (2016). Genome-wide scan for adaptive differentiation along altitudinal gradient in the Andrew’s toad *Bufo andrewsi*. *Molecular Ecology*, *25*(16):3884-900. doi: 10.1111/mec.13722.

Hammond, S. A., Warren, R. L., Vandervalk, B. P., Kucuk, E., Khan, H., Gibb, E. A., … Birol, I. (2017).

The North American bullfrog draft genome provides insight into hormonal regulation of long noncoding RNA. *Nature Communications*, *8*(1), 1433. doi: 10.1038/s41467-017-01316-7

Harper, L. R., Lawson, L., Christoph, H., Boonham, N., Rees, H. C., Lewis, E., … Hänfling, B. (2019). Generating and testing ecological hypotheses at the pondscape with environmental DNA metabarcoding : A case study on a threatened amphibian. *Environmental DNA, 2*,184–199. doi: 10.1002/edn3.57

Hart, A. J., Ginzburg, S., Xu, M. (Sam), Fisher, C. R., Rahmatpour, N., Mitton, J. B., … Wegrzyn, J. L. (2020). EnTAP: Bringing faster and smarter functional annotation to non-model eukaryotic transcriptomes. *Molecular Ecology Resources*, *20*(2), 591–604. doi: 10.1111/1755-0998.13106

Hawkins, L. J., & Storey, K. B. (2018). Histone methylation in the freeze-tolerant wood frog (*Rana sylvatica*). *Journal of Comparative Physiology B, 188*, 113–125. doi: 10.1007/s00360-017-1112-7

Hayes, T. B., Collins, A., Lee, M., Mendoza, M., Noriega, N., Stuart, A. A., & Vonk, A. (2002). Hermaphroditic, demasculinized frogs after exposure to the herbicide atrazine at low ecologically relevant doses. *Proceedings of the National Academy of Sciences of the United States of America*, *99*(8), 5476–5480. doi: 10.1073/pnas.082121499

Hebert, P. D. N., Cywinska, A., Ball, S. L., & deWaard. (2003). Biological identifications through DNA barcodes. *Proceedings of the Royal Society B: Biological Sciences*, *270*, 313–321. doi: 10.1098/rspb.2002.2218

Hellsten, U., Harland, R. M., Gilchrist, M. J., Hendrix, D., Jurka, J., Kapitonov, V., … Rokhsar, D. S. (2010). The genome of the western clawed frog

Xenopus tropicalis. *Science*, *328*, 633–636. doi: 10.1126/science.1183670

Hoffberg, S. L., Kieran, T. J., Catchen, J. M., Devault, A., Faircloth, B. C., Mauricio, R., & Glenn, T. C. (2016). RADcap: Sequence capture of dual-digest RADseq libraries with identifiable duplicates and reduced missing data. *Molecular Ecology Resources*, *16*(5), 1264–1278. doi: 10.1111/1755-0998.12566

Homola, J. J., Loftin, C. S., Cammen, K. M., Helbing, C. C., Birol, I., Schultz, T. F., & Kinnison, M. T. (2019). Replicated landscape genomics identifies evidence of local adaptation to urbanization in wood frogs. *The Journal of Heredity*, *110*(6), 707–719.

Hudson, N. J., Lonhienne, T. G. A., Franklin, C. E., Harper, G. S., & Lehnert, S. A. (2008). Epigenetic silencers are enriched in dormant desert frog muscle. *Journal of Comparative Physiology B*, *178*, 729–734. doi: 10.1007/s00360-008-0261-0

Hunter, D. A., Speare, R., Marantelli, G., Mendez, D., Pietsch, R., & Osborne, W. (2010). Presence of the amphibian chytrid fungus *Batrachochytrium dendrobatidis* in threatened corroboree frog populations in the Australian Alps. *Diseases of Aquatic Organisms*, *92*(2-3), 209-16. doi: 10.3354/dao02118

Iannucci, A., Makunin, A. I., Lisachov, A. P., Ciofi, C., Stanyon, R., Svartman, M., & Trifonov, V. A. (2021). Bridging the gap between vertebrate cytogenetics and genomics with single-chromosome sequencing (ChromSeq). *Genes*, *12*(1), 124. doi: 10.3390/genes12010124

Jacobs, M. N., Marczylo, E. L., & Guerrero-Bosagna, C. (2017). Marked for life : Epigenetic effects of endocrine disrupting chemicals. *Annual Review of Environment and Resources*, 42, 105-160. doi:

10.1146/annurev-environ-102016-061111

Kardos, M., & Shafer, A. B. A. (2018). The peril of gene-targeted conservation. *Trends in Ecology & Evolution*, *33*(11), 827–839. doi: 10.1016/j.tree.2018.08.011

Keinath, M. C., Timoshevskiy, V. A., Timoshevskaya, N. Y., Tsonis, P. A., Voss, S. R., & Smith, J. J. (2015). Initial characterization of the large genome of the salamander *Ambystoma mexicanum* using shotgun and laser capture chromosome sequencing. *Scientific Reports*, *5*, 16413. doi: 10.1038/srep16413

Keinath, M. C., Voss, S. R., Tsonis, P. A., & Smith, J. J. (2017). A linkage map for the Newt *Notophthalmus viridescens*: Insights in vertebrate genome and chromosome evolution. *Developmental Biology*, *426*, 211–218. doi: 10.1016/j.ydbio.2016.05.027

Kelehear, C., Brown, G. P., & Shine, R. (2013). Invasive parasites in multiple invasive hosts : the arrival of a new host revives a stalled prior parasite invasion. *Oiko*s (January), 1317–1324. doi: 10.1111/j.1600-0706.2013.00292.x

Koren, S., Rhie, A., Walenz, B. P., Dilthey, A. T., Bickhart, D. M., Kingan, S. B., … Phillippy, A. M. (2018). De novo assembly of haplotype-resolved genomes with trio binning. *Nature Biotechnology*, *36*(1174–1182). doi: 10.1038/nbt.4277

Kosch, T A, Eimes, J. A., Didinger, C., Brannelly, L. A., Waldman, B., Berger, L., & Skerratt, L. F. (2017). Characterization of MHC class IA in the endangered southern corroboree frog. *Immunogenetics*, *69*, 165–174. doi: 10.1007/s00251-016-0965-3

Kosch, T A, Silva, C. N. S., Brannelly, L. A., Roberts, A. A., Lau, Q., Marantelli, G., … Skerratt, L. F. (2019). Genetic potential for disease resistance in critically endangered amphibians decimated by chytridiomycosis. *Animal Conservation*, *22*(3), 238–250. doi: 10.1111/acv.12459

Kosch, T. A., Bataille, A., Didinger, C., Eimes, J. A., Rodríguez-Brenes, S., Ryan, M. J., & Waldman, B. (2016). Major histocompatibility complex selection dynamics in pathogen-infected túngara frog (*Physalaemus pustulosus*) populations. *Biology Letters*, *12*(8). doi: 10.1098/rsbl.2016.0345

Lambert, M. R., Skelly, D. K., & Ezaz, T. (2016). Sex-linked markers in the North American green frog (*Rana clamitans*) developed using DArTseq provide early insight into sex chromosome evolution. *BMC Genomics*. doi: 10.1186/s12864-016-3209-x

Lamichhaney, S., Catullo, R., Keogh, J. S., Clulow, S., & Edwards, S. V. (2021). A bird-like genome from a frog : Mechanisms of genome size reduction in the ornate burrowing frog , *Platyplectrum ornatum*. *Proceedings of the National Academy of Sciences of the United States of America*, 118(11), e2011649118. doi: 10.1073/pnas.2011649118/-/DCSupplemental.Published

Lau, Q., Igawa, T., Kosch, T. A., & Satta, Y. (2018). Selective constraint acting on TLR2 and TLR4 genes of Japanese *Rana* frogs. *PeerJ*, *6*, e4842. doi: 10.7717/peerj.4842

Lemmon, A. R., Emme, S. A., & Lemmon, E. M. (2012). Anchored hybrid enrichment for massively high-throughput phylogenomics. *Systematic Biology*, *61*(5), 727–744.

Liedtke, H. C., Gower, D. J., Wilkinson, M., & Gomez-Mestre, I. (2018). Macroevolutionary shift in the size of amphibian genomes and the role of life history and climate. *Nature Ecology & Evolution*, *2*(11), 1792–1799. doi: 10.1038/s41559-018-0674-4

Lopes, Carla M, Sasso, T., Valentini, A., Dejean, T., Martins, M., Zamudio, K. R., & Haddad, C. F. B. (2017). eDNA metabarcoding: a promising method for anuran surveys in highly diverse tropical forests. *Molecular Ecology Resources*, *17*(5), 904–914. doi: 10.1111/ijlh.12426

Lopes, C. M., Baêta, D., Valentini, A., Lyra, M. L., Sabbag, A. F., Gasparini, J. L., … Zamudio, K. R. (2021). Lost and found: frogs in a biodiversity hotspot rediscovered with environmental DNA. *Molecular Ecology*, *30*(13), 3289–3298. doi: 10.1111/mec.15594

Luo, W., Xia, Y., Yue, B., & Zeng, X. (2020). Assigning the sex-specific markers via genotyping-by-sequencing onto the y chromosome for a torrent frog *Amolops mantzorum*. *Genes*, *11*(7), 727. doi: 10.3390/genes11070727

MacManes, M. D. (2018). The Oyster River Protocol: a multi-assembler and kmer approach for de novo transcriptome assembly. *PeerJ*, *6:e5428*. doi: 10.7717/peerj.5428

May, S., Zeisset, I., & Beebee, T. J. C. (2011). Larval fitness and immunogenetic diversity in chytrid-infected and uninfected natterjack toad (*Bufo calamita*) populations. *Conservation Genetics*, *12*, 805–811. doi: 10.1007/s10592-011-0187-z

McCartney-Melstad, E., & Shaffer, H. B. (2015). Amphibian molecular ecology and how it has informed conservation. *Molecular Ecology*, *24*, 5084–5109. doi: 10.1111/mec.13391

McCartney, M. A., Mallez, S., & Gohl, D. M. (2019). Genome projects in invasion biology. *Conservation Genetics*, *20*, 1201–1222. doi: 10.1007/s10592-019-01224-x

McCormack, J. E., Faircloth, B. C., Crawford, N. G., Gowaty, P. A., Brumfield, R. T., & Glenn, T. C. (2012). Ultraconserved elements are novel phylogenomic markers that resolve placental mammal phylogeny when combined with species-tree analysis. *Genome Research, 22*(4),746-54. doi: 10.1101/gr.125864.111.746

McKenna, A., Hanna, M., Banks, E., Sivachenko, A., Cibulskis, K., Kernytsky, A., … DePristo, M. A. (2010). The genome analysis toolkit: A MapReduce framework for analyzing next-generation DNA sequencing data. *Genome Research, 20*, 1297–1303. doi: 10.1101/gr.107524.110

Meuwissen, T., Hayes, B., & Goddard, M. (2016). Genomic selection: A paradigm shift in animal breeding. *Animal Frontiers*, *6*(1), 6–14. doi: 10.2527/af.2016-0002

Miller, M. R., Dunham, J. P., Amores, A., Miller, M. R., Dunham, J. P., Amores, A., … Johnson, E. A. (2007). Rapid and cost-effective polymorphism identification and genotyping using restriction site associated DNA (RAD) markers. 240–248. *Genome Research, 17*(2), 240–248. doi: 10.1101/gr.5681207

Mitros, T., Lyons, J. B., Session, A. M., Jenkins, J., Kwon, T., Lane, M., … Rokhsar, D. S. (2019). A chromosome-scale genome assembly and dense genetic map for *Xenopus tropicalis*. *Developmental Biology*, *452*(1), 8–20. doi: 10.1016/j.ydbio.2019.03.015

Mochizuki, K., Ishihara, A., Goda, T., & Yamauchi, K. (2012). RNA polymerase II phosphorylation at serine 2 and histone H3 tri-methylation at lysine 36 are key steps for thyroid hormone receptor b gene activation by thyroid hormone in *Rana catesbeiana* tadpole liver. *Biochemical and Biophysical Research Communications*, *417*(3), 1069–1073. doi: 10.1016/j.bbrc.2011.12.097

Mohammadi, S., Yang, L., Harpak, A., Dobler, S., Crawford, A. J., &

Andolfatto, P. (2021). Concerted evolution reveals co-adapted amino acid substitutions in frogs that prey on toxic toads. *Current Biology. doi:10.1016/j.cub.2021.03.089*. 2530–2538

Musacchia, F., Basu, S., Petrosino, G., Salvemini, M., & Sanges, R. (2015). Annocript: a flexible pipeline for the annotation of transcriptomes able to identify putative long noncoding RNAs. *Bioinformatics*, *31*(13), 2199–2201. doi: 10.1093/bioinformatics/btv106

Newhouse, A. E., & Powell, W. A. (2021). Intentional introgression of a blight tolerance transgene to rescue the remnant population of American chestnut. *Conservation Science and Practice*, *3*(4), 3:e348. doi: 10.1111/csp2.348

Nowoshilow, S., Schloissnig, S., Fei, J., Dahl, A., Andy, W., Pang, C., … Roscito, J. G. (2018). The axolotl genome and the evolution of key tissue formation regulators. *Nature, 554, 50–55.* doi: 10.1038/nature25458

Nunes, A. L., Fill, J. M., Davies, S. J., Louw, M., Rebelo, A. D., Thorp, C. J., … Measey, J. (2019). A global meta-analysis of the ecological impacts of alien species on native amphibians. *Proceedings of the Royal Society B*, *286*, 20182528. doi: 10.1098/rspb.2018.2528

Nunziata, S. O., Lance, S. L., Scott, D. E., Lemmon, E. M., & Weisrock, D. W. (2017). Genomic data detect corresponding signatures of population size change on an ecological time scale in two salamander species. *Molecular Ecology*, *26*(4), 1060–1074. doi: 10.1111/mec.13988

Nunziata, S. O., & Weisrock, D. W. (2018). Estimation of contemporary effective population size and population declines using RAD sequence data. *Heredity*, *120*, 196–207. doi: 10.1038/s41437-017-0037-y

Ortiz-Baez, A. S., Cousins, K., Eden, J.-S., Chang, W.-S., Harvey, E., Pettersson, J. H.-O., … Holmes, E. C. (2020). Meta-transcriptomic identification of *Trypanosoma* spp. in native wildlife species from Australia. *Parasites & Vectors*, *13*, 447. doi: 10.1186/s13071-020-04325-6

Peterson, B. K., Weber, J. N., Kay, E. H., Fisher, H. S., & Hoekstra, H. E. (2012). Double Digest Radseq : An inexpensive method for de novo snp discovery and genotyping in model and non-model species. *PLoS ONE, 7*(5), e37135. doi:10.1371/journal.pone.0037135

Pettit, L., Ward-Fear, G., & Shine, R. (2021). Invasion of cane toads (*Rhinella marina*) affects the problem-solving performance of vulnerable predators (monitor lizards, *Varanus varius*). *Behavioral Ecology AndSociobiology*, *75*, 39. doi: 10.1007/s00265-021-02978-6

Piaggio, A. J., Segelbacher, G., Seddon, P. J., Alphey, L., Bennett, E. L., Carlson, R. H., … Wheeler, K. (2017). Is it time for synthetic biodiversity conservation? *Trends in Ecology & Evolution*, *32*(2), 97–107. doi: 10.1016/j.tree.2016.10.016

Pizzatto, L., & Shine, R. (2011). The effects of experimentally infecting Australian tree frogs with lungworms (*Rhabdias pseudosphaerocephala*) from invasive cane toads. *International Journal for Parasitology*, *41*(9), 943–949. doi: 10.1016/j.ijpara.2011.03.013

Pomerantz, A., Peñafiel, N., Arteaga, A., Bustamante, L., Pichardo, F., Coloma, L. A., … Prost, S. (2018). Real-time DNA barcoding in a rainforest using nanopore sequencing: opportunities for rapid biodiversity assessments and local capacity building. *GogaScience*, *7*(4), giy033. doi: 10.1093/gigascience/giy033/4958980

Poorten, T. J., & Rosenblum, E. B. (2016). Comparative study of host response to chytridiomycosis in a susceptible and a resistant toad species. *Molecular Ecology*, *25*(22), 5663–5679. doi: 10.1111/mec.13871

Price, S. J., Garner, T. W. J., Balloux, F., Ruis, C., Paszkiewicz, K. H., Moore, K., & Griffiths, A. G. F. (2015). A de novo assembly of the common frog (*Rana temporaria*) transcriptome and comparison of transcription following exposure to ranavirus and *Batrachochytrium dendrobatidis*. *PLoS ONE*, *10*(6), e0130500. doi: 10.1371/journal.pone.0130500

Rando, O. J., & Verstrepen, K. J. (2007). Timescales of genetic and epigenetic inheritance. *Cell*, *128*, 655–668. doi: 10.1016/j.cell.2007.01.023

Redford, K. H., Brooks, T. M., Nicholas, B. W., & Adams, J. S. (2019). *Genetic frontiers for conservation: An assessment of synthetic biology and biodiversity conservation*. Gland, Switzerland: IUCN.

Rey, O., Eizaguirre, C., Angers, B., Baltazar-Soares, M., Sagonas, K., Prunier, J. G., & Blanchet, S. (2020). Linking epigenetics and biological conservation: Towards a conservation epigenetics perspective. *Functional Ecology*, *34*, 414–427. doi: 10.1111/1365-2435.13429

Rhie, A., McCarthy, S. A., Fedrigo, O., Damas, J., Formenti, G., London, S. E., … Friedrich, S. R. (2020). Towards complete and error-free genome assemblies of all vertebrate species. *Nature*, *592*, 737–746.

Richardson, M. F., Sequeira, F., Selechnik, D., Carneiro, M., Vallinoto, M., Reid, J. G., … Rollins, L. A. (2018). Improving amphibian genomic resources: a multitissue reference transcriptome of an iconic invader. *GigaScience*, *7*(1), gix114. doi: 10.1093/gigascience/gix114

Rogers, R. L., Zhou, L., Chu, C., Marquez, R., Corl, A., Linderoth, T., … Nielsen, R. (2018). Genomic takeover by transposable elements in the strawberry poison frog. *Molecular Biology and Evolution*, *35*(12), 2913–2927. doi: 10.1093/molbev/msy185

Rollins, L. A., Richardson, M. F., & Shine, R. (2015). A genetic perspective on rapid evolution in cane toads (*Rhinella marina*). *Molecular Ecology*, *24*, 2264–2276. doi: 10.1111/mec.13184

Romero-Zambrano, G. L., Bermúdez-Puga, S. A., Sánchez-Yumbo, A. F., Yánez-Galarza, J. K., & Ortega-Andrade, H. M. (2021). Amphibian chytridiomycosis, a lethal pandemic disease caused by the killer fungus *Batrachochytrium dendrobatidis:* New approaches to host defense mechanisms and techniques for detection and monitoring. *Bionatura*, doi: 10.21931/RB/2021.06.01.28

Rosenblum, E. B., Poorten, T. J., Settles, M., & Murdoch, G. K. (2012). Only skin deep : shared genetic response to the deadly chytrid fungus in susceptible frog species. *Molecular Ecology*, *21*(13), 3110–3120. doi: 10.1111/j.1365-294X.2012.05481.x

Rosenblum, E. B., Poorten, T. J., Settles, M., Murdoch, G. K., Robert, J., Maddox, N., & Eisen, M. B. (2009). Genome-wide transcriptional response of *Silurana (Xenopus) tropicalis* to infection with the deadly chytrid fungus. *PLoS ONE*, *4*(8), e6494. doi: 10.1371/journal.pone.0006494

Russo, A. G., Eden, J.-S., Tuipulotu, D. E., Shi, M., Selechnik, D., Shine, R., … White, P. A. (2018). Viral discovery in the invasive Australian cane toad (*Rhinella marina*) using metatranscriptomic and genomic approaches. *Journal of Virology*, *92*(17). doi: 10.1128/JVI.00768-18

Russo, A. G., Harding, E. F., Yan, G. J. H., Selechnik, D., Ducatez, S., DeVore, J. L., … White, P. A. (2021). Discovery of novel viruses associated with the invasive cane toad (*Rhinella marina*) in its native and introduced ranges. *Frontiers in Microbiology*, *12*, 733631. doi: 10.3389/fmicb.2021.733631

Ryan, M. E., Johnson, J. R., & Fitzpatrick, B. M. (2009). Invasive hybrid tiger salamander genotypes impact native amphibians. *Proceedings of the National Academy of Sciences of the United States of America*, *106*(27). 11166-11171.

Santos, J. C., Tarvin, R. D., Connell, L. A. O., Blackburn, D. C., & Coloma, L. A. (2018). Diversity within diversity: Parasite species richness in poison frogs assessed by transcriptomics. *Molecular Phylogenetics and Evolution*. doi: 10.1016/j.ympev.2018.03.015

Sarma, R. R., Crossland, M. R., Eyck, H. J. F., Devore, J. L., Edwards, R. J., Cocomazzo, M., … Rollins, L. A. (2021). Intergenerational effects of manipulating DNA methylation in the early life of an iconic invader. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *376*(1826), 20200125. doi: /10.1098/rstb.2020.0125

Sarma, R. R., Edwards, R. J., Crino, O. L., Eyck, H. J. F., Waters, P. D., Crossland, M. R., … Rollins, L. A. (2020). Do epigenetic changes drive corticosterone responses to alarm cues in larvae of an invasive amphibian? *Integrative and Comparative Biology*, *60*(6), 1481–1494.

Sasso, T., Lopes, C. M., Valentini, A., Dejean, T., Zamudio, K. R., Haddad, C. F. B., & Martins, M. (2017). Environmental DNA characterization of amphibian communities in the Brazilian Atlantic forest : Potential application for conservation of a rich and threatened fauna. *Biological Conservation*, *215*(321), 225–232. doi: 10.1016/j.biocon.2017.09.015

Savage, A. E., Gratwicke, B., Hope, K., Bronikowski, E., & Fleischer, R. C.

(2020). Sustained immune activation is associated with susceptibility to the amphibian chytrid fungus. *Molecular Ecology*, *29*(15), 2889–2903. doi: 10.1111/mec.15533

Savage, A. E., Mulder, K. P., Torres, T., & Wells, S. (2018). Lost but not forgotten: MHC genotypes predict overwinter survival despite depauperate MHC diversity in a declining frog. *Conservation Genetics*, *19*, 309–322. doi: 10.1007/s10592-017-1001-3

Savage, A. E., & Zamudio, K. R. (2011). MHC genotypes associate with resistance to a frog-killing fungus. *Proceedings of the National Academy of Sciences of the United States of America*, *108*(40), 16705–16710. doi: 10.1073/pnas.1106893108

Savage, A. E., & Zamudio, K. R. (2016). Adaptive tolerance to a pathogenic fungus drives major histocompatibility complex evolution in natural amphibian populations. *Proceedings of the Royal Society B: Biological Sciences*, *283*(1827), 20153115. doi: 10.1098/rspb.2015.3115

Scheele, B. C., Hunter, D. A., Brannelly, L. A., Skerratt, L. F., & Driscoll, D. A. (2017). Reservoir-host amplification of disease impact in an endangered amphibian. *Conservation Biology*, *31*(3), 592–600. doi: 10.1111/cobi.12830

Secondi, J., Dejean, T., Valentini, A., Audebaud, B., & Miaud, C. (2016). Detection of a global aquatic invasive amphibian, *Xenopus laevis*, using environmental DNA. *Amphibia-Reptilia*, *37*(1), 131–136. doi: 10.1163/15685381-00003036

Seifertova, E., Zimmerman, L. B., Gilchrist, M. J., Macha, J., Kubickova, S., Cernohorska, H., … Krylov, V. (2013). Efficient high-throughput sequencing of a laser microdissected chromosome arm. *BMC Genomics,* 14, *357* (2013). doi: 10.1186/1471-2164-14-357

Selechnik, D., Richardson, M. F., Shine, R., Brown, G. P., & Rollins, L. A. (2019). Immune and environment-driven gene expression during invasion: An eco-immunological application of RNA-Seq. *Ecology and Evolution*, *9*(11), 6708–6721. doi: 10.1101/583617

Selechnik, D., Richardson, M. F., Shine, R., DeVore, J. L., Ducatez, S., & Rollins, L. A. (2019). Increased adaptive variation despite reduced overall genetic diversity in a rapidly adapting invader. *Frontiers in Genetics*, *10*(November), 1–14. doi: 10.3389/fgene.2019.01221

Serin, E. A. R., Nijveen, H., Hilhorst, H. W. M., & Ligterink, W. (2016). Learning from co-expression networks: possibilities and challenges. *Frontiers in Plant Science*, *7*, 444. doi: 10.3389/fpls.2016.00444

Session, A. M., Uno, Y., Kwon, T., Chapman, J. A., Toyoda, A., Takahashi, S., … Matsuda, Y. (2016). Genome evolution in the allotetraploid frog *Xenopus laevis*. *Nature*, *538*(7625), 336–343. doi: 10.1038/nature19840

Shaffer, H. B., Gidis, Ã. M., McCartney-Melstad, E., Neal, K. M., Oyamaguchi, H. M., Tellez, M., & Toffelmier, E. M. (2015). Conservation genetics and genomics of amphibians and reptiles. *Annual Review of Animal Biosciences*, *3*,113-138. doi: 10.1146/annurev-animal-022114-110920

Shine, R. (2010). The ecological impact of invasive cane toads (*Bufo marinus*) in Australia. *The Quarterly Review of Biology*, *85*(3), 253–291. doi: 10.1086/655116

Skerratt, L. F. (2019). I*mproving disease resilience in ecosystems using synthetic biology*. The University of Melbourne, Grant number: FT190100462.

Smith-Unna, R., Boursnell, C., Patro, R., Hibberd, J. M., & Kelly, S. (2016).

TransRate: reference free quality assessment of de-novo transcriptome assemblies. *Genome Research*, *26*(8), 1134–1144.

Smith, J. J., Timoshevskaya, N., Timoshevskiy, V. A., Keinath, M. C., Hardy, D., & Voss, S. R. (2019). A chromosome-scale assembly of the axolotl genome. *Genome Research*, *29*, 1–8. doi: 10.1101/gr.241901.118.29

Sommer, S. (2005). The importance of immune gene variability (MHC) in evolutionary ecology and conservation. *Frontiers in Zoology*, *2*(16). doi: 10.1186/1742-9994-2-16

Spurr, L., Alomran, N., Bousounis, P., Reece-Stremtan, D., N M, P., Liu, H., … Horvath, A. (2020). ReQTL: Identifying correlations between expressed SNVs and gene expression using RNA-sequencing data. *Bioinformatics*, *36*(5), 1351–1359.

Sun, Y., Zhang, Y., & Wang, K. (2020). Perspectives on studying molecular adaptations of amphibians in the genomic era. *Zoological Research, 41*(4), 351–364. doi: 10.24272/j.issn.2095-8137.2020.046

Thorson, J. L. M., Beck, D., Maamar, M. Ben, Id, E. E. N., Mcbirney, M., & Id, M. K. S. (2020). Epigenome-wide association study for atrazine induced transgenerational DNA methylation and histone retention sperm epigenetic biomarkers for disease. *PLoS ONE, 15*(12):e02393801–29. doi: 10.1371/journal.pone.0239380

Trumbo, D. R., Epstein, B., Hohenlohe, P. A., Alford, R. A., Schwarzkopf, L., & Storfer, A. (2016). Mixed population genomics support for the central marginal hypothesis across the invasive range of the cane toad (*Rhinella marina*) in Australia. *Molecular Ecology*, *25*(17), 4161–4176. doi: 10.1111/mec.13754

Turvey, N. (2013). *Cane toads: A tale of sugar, politics and flawed science*. Sydney University Press.

van Dam, S., Võsa, U., van der Graaf, A., Franke, L., & de Magalhães, J. P. (2018). Gene co-expression analysis for functional classification and gene-disease predictions. *Briefings in Bioinformatics*, *19*(4), 575–592. doi: 10.1093/bib/bbw139

Van Den Berge, K., Hembach, K. M., Soneson, C., Tiberi, S., Clement, L., Love, M. I., … Robinson, M. D. (2019). RNA sequencing data: hitchhiker's guide to expression analysis. *The Annual Review of Biomedical Data Science*, *2*(1), 139–173. doi: https://doi.org/10.1146/annurev-biodatasci-072018-021255

van Oppen, M. J. H., & Oakeshott, J. G. (2020). A breakthrough in understanding the molecular basis of coral heat tolerance. *Proceedings of the National Academy of Sciences of the United States of America*, *117*(46), 1–3. doi: 10.1073/pnas.2020201117

van Oppen, M. J. H., Oliver, J. K., Putnam, H. M., & Gates, R. D. (2015). Building coral reef resilience through assisted evolution. *Proceedings of the National Academy of Sciences of the United States of America*, *112*(8), 2307–2313. doi: 10.1073/pnas.1422301112

Voss, S. R., Kump, D. K., Walker, J. A., Shaffer, H. B., & Voss, G. J. (2012). Thyroid hormone responsive QTL and the evolution of paedomorphic salamanders. *Heredity, 109*, 293–298. doi: 10.1038/hdy.2012.41

Walls, S. C., & Gabor, C. R. (2019). Integrating behavior and physiology into strategies for amphibian conservation. *Frontiers in Ecology and Evolution*, *7*(June), 1–13. doi: 10.3389/fevo.2019.00234

Wilson, J.-J., Sing, K.-W., & Jaturas, N. (2019). DNA Barcoding: bioinformatics workflows for beginners. In *Encyclopedia of bioinformatics and computational* *biology: Volume 3* (pp. 985–995). doi: 10.1016/b978-0-12-809633-8.20468-8

Woodhams, D. C., Bosch, J., Briggs, C. J., Cashins, S., Davis, L. R., Lauer, A., … Voyles, J. (2011). Mitigating amphibian disease: strategies to maintain wild populations and control chytridiomycosis. *Frontiers in Zoology*, *8*, 8. doi: 10.1186/1742-9994-8-8

Wren, S., Angulo, A., Meredith, H., Kielgast, J., Dos Santos, L., & Bishop, P. (2015). *Amphibian conservation action plan*. April 2015. IUCN SSC Amphibian Specialist Group. Retrieved from https://www.iucn-amphibians.org/working-groups/thematic/

Xu, X., Dimitrov, D., Rahbek, C., & Wang, Z. (2015). NCBIminer: sequences harvest from Genbank. *Ecography*, *38*(4), 426–430. doi: 10.1111/ecog.01055

Zhang, J., Hawkins, L. J., & Storey, K. B. (2020). DNA methylation and regulation of DNA methyltransferases in a freeze tolerant vertebrate. *Biochemistry and Cell Biology*, *98*(2), 1–31. doi: 10.1139/bcb-2019-0091

Zhou, J., Nelson, T. M., Rodriguez Lopez, C., Zhou, S. J., Ward-Fear, G., Stuart, K. C., & Rollins, L. A. (2020). Microbial function is related to behavior of an invasive anuran. *BioRxiv*.

Chapter 14. Translocations

Jen Germano[1], Gemma Harding[2], Jeff Dawson[3], Luke Linhoff[4], Lea Randall[5] and Richard Griffiths[2]

[1] Terrestrial Science Unit, Department of Conservation, Aotearoa New Zealand

[2] Durrell Institute of Conservation and Ecology, School of Anthropology and Conservation, University of Kent

[3] Durrell Wildlife Conservation Trust, La Profonde Rue, Trinity, Jersey, JE3 5BP, British Channel Islands

[4] Smithsonian Conservation Biology Institute, Center for Species Survival & Florida International University, Department of Biology

[5] Calgary Zoo Foundation, Calgary, Alberta, Canada

Glossary

Translocation: the movement of an organism by human agency that is then released in a different area; the most general and highest order term referring to human mediated movement of a species/subspecies/taxon.

Conservation translocation: intentional movement and release of living organisms where the primary objective is for conservation purposes.

Assisted colonisation: is the intentional movement and release of an organism outside its indigenous range to avoid extirpation of populations or extinction of the focal species. Assisted colonisation is primarily carried out where protection from current or likely future threats in the current range is deemed less feasible than at alternative sites outside the current range.

Reintroduction: is the intentional movement and release of an organism(s) inside the species' indigenous range from which the species has disappeared.

Reinforcement/Supplementation: is the intentional movement and release of an organism(s) into an existing population of conspecifics, and is synonymous with the terms augmentation, supplementation, and restocking. Reinforcement may be done for several reasons, including to enhance population viability, increase genetic diversity, or increase the representation of specific demographic groups or stages.

Mitigation translocation: the intentional removal of organisms from habitat that will be lost through anthropogenic land-use change or threat, and release at an alternative site.

Definitions are based on the 2013 IUCN Guidelines for Reintroductions and Other Conservation Translocations.

Abstract

Species translocations are highly complex and challenging and those involving amphibians are no exception to this. While outcomes have improved over the decades, the last review of published herpetofauna translocations found a success rate of 41%. This is likely due to the interplay of numerous factors that need to be addressed to give releases the greatest opportunity to thrive. Some of these factors include source population, animal behaviour, habitat quality, disease risks, genetics, welfare, and ensuring that the root cause of decline has been addressed. Where questions exist around key factors, trial releases and experimental research can help to address uncertainties. Additionally, it is critical that sufficient time and resources are put into planning and monitoring, with a contingency or exit strategy in place if the project does not go as planned. Future challenges that need to be addressed by the amphibian reintroduction community include the use of translocations in the mitigation space to deal with habitat destruction and human development as well as the application of assisted colonisation in the face of the global climate change crisis.

Introduction

Amphibian translocations, and in fact translocations of any taxonomic group, are complex undertakings. Success is not guaranteed, as project-specific uncertainties are inevitable and translocations require consideration of animal behaviour, disease, genetics, population ecology, political, socioeconomic, and stakeholder contexts (Ewen, Armstrong, Parker, & Seddon, 2012; IUCN/SSC, 2013; Linhoff et al., 2021). They are long-term commitments that do not end when animals are released. Often, they require years of adaptive management and years, if not decades, of monitoring to establish the level of success. Furthermore, if the initial threats to the species are not mitigated and if long-term security of the release site is not ensured, then these newly translocated populations will fail.

Historically, translocations have been used for a range of reasons. For amphibians, most past releases have been for conservation. Additionally, many releases have been carried out for pest control (e.g., cane toads) or inadvertently (e.g., American bullfrog) and while there are many lessons that can be learned from the study of invasive species, these are outside the scope of this chapter. In the past two decades, as the science of reintroduction biology has developed and gained international recognition, there has been a substantial increase in the use of translocations for the mitigation of habitat destruction for human development (Bradley, Tomlinson, Craig, Cross, & Bateman, 2021; Germano & Bishop, 2009; Germano et al., 2015; Miller, Bell, & Germano, 2014; Romijn & Hartley, 2016; Sullivan, Nowak, & Kwiatkowski, 2015). These mitigation translocations have lower success rates than conservation translocations (Germano & Bishop, 2009) and may not meet the animal welfare or species goals that they set out to achieve (Bradley et al., 2021; Germano & Bishop, 2009). The motivations driving future amphibian translocations are likely to continue to evolve. Perhaps one of the most probable developments over the coming years will be the use of assisted colonisation in an attempt to guarantee the survival of species facing dire circumstances in the face of climate change.

Status update

Progress in reintroductions and conservation translocations

The use of translocations for the conservation of amphibians and wildlife in general has been growing worldwide (Bubac & Johnson, 2019; Dodd & Seigel, 1991; Germano & Bishop, 2009). A comparison of data collected from 1966 to 2006 (Griffiths & Pavajeau, 2008) to data collected between the first ACAPs release in 2007 and 2014 showed the number of amphibian species involved in both captive breeding and translocation projects to have increased by 57% (Harding, Griffiths, & Pavajeau, 2016). Alongside this growth, a comparison of reviews of published herpetofauna releases have shown an increase in positive outcomes from a 19% success rate of reviewed cases in 1991 (Dodd & Seigel, 1991) to 41% in 2008 (Germano & Bishop, 2009). These successes are likely due to the development of reintroduction biology as a whole and a push towards adaptive management and the use of scientific approaches to address *a priori* goals. The trend after the 2007 ACAP also showed a shift towards research and a focus on captive assurance populations with very few new reintroductions (Harding et al., 2016). With more many releases targeting specific research questions this continues to add to our knowledge, refine our management practices and increase the chances of future successes. Detailed information and best practice can be found in the *IUCN guidelines for amphibian reintroductions and other conservation translocations* (Linhoff et al., 2021).

Planning and feasibility

Planning and feasibility studies are vital steps before a reintroduction is undertaken. Each programme will require consideration of different elements depending on the threats to the species and potential impacts to habitat, ecosystems and communities. There are numerous factors to consider and a wealth of tools available to assist with the process (Canessa et al., 2016). Although the focus of each programme will be different there are a few key considerations which apply (see Box 14.1).

Box 14.1: Key considerations for planning

1. Is the species a suitable candidate for reintroduction
2. Have other interventions such as habitat enhancement or threat management been considered
3. Are there ways to protect the species *in situ*
4. Is there sufficient knowledge on the species biology, ecology, and reasons for decline
5. Are there support and resources for a reintroduction (e.g., long-term funding, expertise, partnerships, political and community support)
6. Have threats been considered/removed/mitigated
7. Have release sites been identified
8. Is there a contingency plan or exit strategy for the reintroduction if needed

Detailed information on the considerations is listed in the IUCN Guidelines (IUCN/SSC 2013) and the IUCN guidelines for amphibian reintroductions (Linhoff et al., 2021).

Useful tools and procedures to assist feasibility and knowledge gathering

1. Species Action Planning Workshops (IUCN/SSC, 2014; IUCN – SSC Species Conservation Planning Sub-Committee, 2017)
2. Population modelling (see Linhoff et al., 2021)
3. Habitat Suitability Analysis (Jarchow, Hossack, Sigafus, Schwalbe, & Muths, 2016; Romero, Olivero, & Real, 2013)
4. Genetic studies (Wilson et al., 2008) and analysis (Weiser, Grueber, & Jamieson, 2012)
5. Strategic planning tools - Using decision analysis framework (Ewen, Soorae, & Canessa, 2014)
6. Collaborations with zoos, government, researchers, non-profit, traditional owners/indigenous people (Cisternas et al., 2019; Miller et al., 1994)

108

Experimental research such as trial translocations with a small number of individuals or using a similar species can provide useful data and test neutralization of threats and broaden feasibility. When undertaking trials, it is important to impose the same stringent protocols and procedures as the same risks are present. There are published trial releases that can provide examples of how to test translocation feasibility (Bodinof et al., 2012; Kemp, Norbury, Groenewegen, & Comer, 2015; McCallen, Kraus, Burgmeier, Fei, & Williams, 2018; Mortelliti, Santulli Sanzo, & Boitani, 2009; Valdez et al., 2019).

There are very few published examples of the process and decision-making elements involved in planning, particularly by programmes where translocations did not go ahead based on the outcomes of feasibility studies or research. It would therefore be useful to have examples of potential reintroductions that were not undertaken as a result of low feasibility or alternative management options. Similarly, it would be useful to have more examples of translocations that did not go to plan (see Case Study, Borzée, Kim, Kim, & Jang, 2018), and adaptive management that resulted in alternative interventions. Examples of amphibian reintroductions along with lessons learned can be found within the IUCN Reintroduction Perspective Publications (Soorae, 2008; 2010; 2011; 2013; 2016; 2018; 2021) and via Conservation Evidence, particularly the Amphibian Synopses (Smith & Sutherland, 2014; Sutherland, Dicks, Petrovan, & Smith, 2021).

Box 14.2. Case study: The Suweon treefrog

Background

The Suweon treefrog *Dryophytes suweonensis* was described in the eponymous city of Suwon in 1980, before becoming functionally extinct in the early 2010s. The local government decided to bring the frogs back a few years later and terraformed an island in a reservoir with all the habitat and vegetation types known to be needed for by the species at the time of the project. Researchers from local universities were tasked with the translocation part of the project, and selected a few localities based on genetic information and population dynamics at the site to be the origin of the translocated individuals.

Methods

To ensure a higher chance of success, amplexed pairs were caught and kept in clear plastic tanks filled with water from the rice paddy where they had been caught. Eggs were collected in the morning, and transferred to a laboratory to head start the froglets before release. The tadpoles, and metamorphs, were kept isolated by clutch, and as only 150 froglets were released at the translocation site, all others were released at the point of capture to reinforce the population at the site of capture (data non-published), after screening for pathogens.

Results and outcomes

The frogs at the translocation site were surveyed until the beginning of hibernation, and a few young males were found calling the following spring (showing a shorter generation time than expected). No amplexus or female were observed, a commonality in the species, but tadpoles were found, and their identity confirmed through molecular tools. More males were found calling the subsequent spring, highlighting the adequacy of protocols used. This was however the last year of the project, and management changed the following fall, with all hibernation sites removed and the vegetation cut as they did not look clean for the public. No observation of the Suweon treefrogs at the site could be confirmed at a later date, and the site was transformed into a water purification plan and car park shortly afterwards.

Current status and threats

The Suweon treefrog is listed as Endangered, it is present at other locations, but the probability of extinction through a PVA for the Republic of Korea is 1 within 50 years.

(Please refer to Borzée et al., 2018 for further details).

Source populations for translocations

Captive populations

Amphibians exhibit a variety of characteristics that make them suitable for captive breeding and head-starting for translocation such as their high fecundity, applicability of reproductive technologies, short generation time, small body size, lack of parental care, hard-wired behaviour, and low maintenance requirements (Balmford, Mace, & Leader-Williams, 1996; Bloxam & Tonge, 1995). However, not all amphibians are suitable for such programmes, and many species have husbandry requirements that are poorly understood or difficult to implement (Tapley, Bradfield, Michaels, & Bungard, 2015). Captive breeding over many generations can have unintended genetic consequences, possibly leading to inbreeding or loss of genetic diversity; additionally, populations may undergo selection to captive conditions unless they are carefully managed (Gilligan & Frankham, 2003; Groombridge, Raisin, Bristol, & Richardson, 2012; McDougall, Réale, Sol, & Reader, 2006; Witzenberger & Hochkirch, 2011). For an overview of conservation breeding, see Chapter 11.

Head-starting, the process by which early life stages (eggs, larvae, or juveniles) are temporarily raised in captivity and released at a later stage to avoid the heavy mortality associated with younger age classes in the wild, has also been used extensively in translocation efforts (Smith, Meredith, & Sutherland, 2020). Both captive bred and head-started animals may become behaviourally adapted to captive conditions and may not be suitable for release to the wild if they do not demonstrate appropriate anti-predator responses or foraging behaviour (Griffin, Blumstein, & Evans, 2000; McDougall et al., 2006). Behavioural adaptation may be partially mitigated by maintaining animals in conditions that closely resemble the wild; individuals may also benefit from pre-release and anti-predator training (Crane & Mathis, 2011; Griffiths & Pavajeau, 2008; Mendelson & Altig, 2016; Tapley et al., 2015; Teixeira & Young, 2014; Teixeira, de Azevedo, Mendl, Cipreste, & Young, 2007).

Captive breeding and reintroduction programmes have increased for threatened amphibian species. In the seven years following the first ACAP an estimated 83% of releases involved a captive breeding component (Harding et al., 2016). Although the number of captive breeding and reintroduction programmes are on the rise, this is primarily occurring in countries in South America, the Caribbean and Central America as programmes are shifted to within-country efforts where amphibian diversity and declines are greatest, meanwhile, the number of programmes in more developed, industrialised countries have decreased over the same time frame (Harding et al., 2016).

There is a lack of understanding about genetics, animal husbandry, and basic life history traits such as breeding cues for many species, and this has hampered the success of captive breeding programmes. Furthermore, captive bred animals often suffer from poor nutrition and health that can impact breeding behaviour and physiology, leading to poor production of offspring for translocation efforts. However, recent advancements have been made in the field of amphibian reproductive technologies (see Chapter 12) such as hormone therapies, artificial fertilization and cryobanking of sperm and eggs, all of which can improve reproductive capacity (e.g., Kouba, Vance, & Willis, 2009; Silla & Byrne, 2019). However, further research on amphibian reproductive biology, as well as on-going development and application of these tools, is needed (Della Togna et al., 2020).

Wild source populations

Wild-wild translocations avoid the costs and logistics involved with establishing and maintaining a captive facility. Equally, it circumvents the risk of adaptation to captivity through multiple generations of captive breeding. However, genetic management needs consideration, and it may be important to ensure individuals are from multiple clutches to avoid a founder effect at the release site. Likewise, although biosecurity may be less of an issue than in a zoo setting, care needs to be taken to avoid the transfer of invasive plants or pathogens between sites during the action. Wild-wild translocations are best carried out using eggs or tadpoles, as these can develop and disperse naturally at the release site (Denton, Hitchings, Beebee, & Gent, 1997; Ward, Liddiard, Goetz, & Griffiths, 2016). Translocation of post-metamorphic stages needs careful consideration given that such stages can have a strong homing ability (Pašukonis et al., 2013), and may become disoriented if moved to a new site. Consideration also needs to be given to the potential impact of removing stock from the donor site. Given the relatively high natural mortality of eggs and larval stages, combining head-starting of larvae – either in-situ in protective enclosures or ex-situ at a nearby facility – may be the optimal solution for amphibians whose life cycle suits such an approach.

Habitat

Habitat loss and degradation is the greatest single driver of amphibian population declines and species loss (Bishop et al., 2012). As such reintroductions and conservation translocations can be a valuable action to address these threats and safeguard amphibian populations (*Pelophylax lessonae*; (Foster, Buckley, Martin, Baker, & Griffiths, 2018); *Rana fisheri* (Saumure et al., 2021). Additionally, habitat quality is a key predictor of translocation outcomes (Bubac, Johnson, Fox, & Cullingham, 2019; Germano & Bishop, 2009; Griffith, Scott, J, Carpenter, & Reed, 1989).

Amphibians depend on the quality and quantity of microhabitats that provide adequate conditions for shelter, feeding, reproduction, stimulation, and escape from predators. Many amphibian species, particularly those that are threatened, have narrow or specific habitat requirements making them less adaptable to modified environments. It is therefore important to assess the habitat at a proposed translocation site to ensure it is suitable for the focal species. Whilst broad habitat requirements are generally known (e.g., if a species is forest dependent), specific habitat needs and therefore sensitivity to habitat modification is lacking (Nowakowski, Thompson, Donnelly, & Todd, 2017). Equally, as many poorly-known threatened species may be hanging on in degraded habitats that are far from optimal, caution is needed in trying to use such habitats as a template for restoration elsewhere in order to expand the species range. Further research into this area is required, both to understand the reasons for population declines and to help inform conservation translocations.

Obtaining this information prior to a translocation may be difficult, but we suggest some options. First, understanding the broad macro and micro-habitat features at a known species site and proposed translocation site will help inform site suitability. Second, when there is no or very little information about the species of conservation concern, evaluate available information on natural history known for a closely-related species – or a species thought to occupy a similar niche - to help inform the translocation.

Receptor site and habitat protection is crucial to ensure long-term success of the translocation. Protected areas are a cornerstone of global conservation of biodiversity, including amphibians, and operate under a diverse range of management models (Dudley, 2008). Effectiveness of protected areas is dependent on various factors including socio-economic and governance conditions (Barnes et al., 2016; Schleicher, Peres, Amano, Llactayo, & Leader-Williams, 2017) as well as management and resource capacity (Geldmann et al., 2018). Successful translocations will therefore need to ensure appropriate measures are in place to safeguard receptor site integrity.

Some final considerations:

1. Assess the impact of climate change on habitat suitability when selecting a site, as what is suitable now may not be in 20- or 50-years' time. Assisted colonisation is likely to become a more frequently used conservation tool in the future in light of climate change, increasing habitat loss, invasive species and the additional challenges this poses (Brodie et al., 2021).
2. Habitat restoration at the site may be required in order to provide the range of microhabitats or to connect habitat fragments within and between sites. Habitat restoration and/or creation should be part of any mitigation translocations undertaken.

3. When undertaking translocations for mitigation or reinforcement, the quantity and quality of habitat needs to be assessed to ensure long-term-viability and to ensure conservation gains are made.

Disease

All translocations must assess the risk of infectious diseases. For example, diseases present at the release site may imperil translocated animals, or translocated animals may become a vector to spread a pathogen to new localities, which may impact existing populations or other species already present at the release site (Walker et al., 2008). The spread of novel infectious diseases, including fungal, bacterial, and viral pathogens, has recently caused declines and even extinctions of numerous amphibian species (Bienentreu & Lesbarrères, 2020; Scheele et al., 2019). A more complete discussion of specific diseases and their impacts on amphibian conservation is discussed in Chapter 6. While it is virtually impossible to eliminate all risk associated with disease in a translocation, by implementing a variety of best practice measures and performing a thorough disease risk assessment it is possible to greatly reduce any negative impacts that may occur (Hartley & Sainsbury, 2017). Refining and adapting protocols via adaptive management experiments can also have the potential to assist translocations where disease threats are present (Scheele et al., 2021).

Best practice guidelines for reducing disease risks relating to amphibian translocation are available (e.g., Linhoff et al., 2021; Murray et al., 2011; Pessier & Mendelson, 2017), but several core principles should be followed. First, animals that are kept in captivity that will be reintroduced should be isolated from other species outside their native range that may be vectors for novel pathogens. Basic biosecurity measures when working with captive amphibians such as using dedicated footwear, hand washing, and sterilising equipment can help prevent the spread of diseases in captivity and the field (Pessier & Mendelson III, 2017). Second, a formal disease risk assessment should be performed (Hartley & Sainsbury, 2017; Sainsbury, Armstrong, & Ewen, 2012). Deciding on a translocation programme's goals and the acceptable risk thresholds are critical and can help make informed and calculated decisions. Disease risk analysis has been done for many amphibian translocations and some helpful herpetofauna examples exist (e.g Bobadilla Suarez et al., 2017; Sainsbury et al., 2017). Third, prior to any translocation a pre-release disease screening should be performed. Animals can be screened for general health and specific pathogens using methods such as faecal parasite examinations or using polymerase chain reaction (PCR)-based screening for the common fungal pathogens Bd and Bsal (Pessier & Mendelson 2017). Translocations of sick and unhealthy animals should also be avoided.

Genetics

Our understanding of conservation genetics and their application to reintroductions has developed considerably since the original ACAP (Jamieson & Lacy, 2012). Even though rigorous habitat assessment of the release site may maximise the chances of animals establishing a viable population, there is a risk that the released stock may be maladapted to some degree. This is particularly the case when the animals for release stem from multiple generations of captive breeding (see above), particularly if the habitat in the receptor site may have changed in subtle ways (see Chapter 11). Likewise, animals that have been rescued from a small, remnant population that is threatened or non-viable, may represent a bottle-necked founder population with low genetic diversity and low capacity to survive at the release site. In deciding the optimal genetic constitution of a founder population for a reintroduction, a balance may need to be struck between ensuring sufficient genetic diversity to allow the establishment of a viable population and adaptation to the new conditions and minimising the risk of outbreeding depression.

Rigorous pre-release and post-release genetic screening of a population may be desirable, but may be costly in terms of the overall reintroduction budget. Equally, as many rare and cryptic amphibian species have unresolved taxonomy and phylogeography, establishing genetic baselines for informing the reintroduction may involve timescales and funds that are difficult. Nevertheless, informed decisions based on existing knowledge of distribution and habitat requirements can be made concerning the number of individuals, stage structure and sources of donor populations. Integration of genetic and demographic modelling may be important in reintroduction decision models (Converse, Moore, & Armstrong, 2013), but in practice reliable data may be difficult to obtain for many amphibian species requiring conservation interventions.

Monitoring

Determining whether reintroduction goals have been met requires post-release monitoring at an appropriate scale, appropriate level, and appropriate timeframe. 'Scale' will vary geographically from a single site to a whole geographical region. 'Level' ranges from basic presence/absence, through simple population counts and population densities, through to estimates of population size. There may well be a trade-off between 'scale' and 'level', in that obtaining population estimates at a large geographical scale may be logistically difficult (as well as unnecessary); whereas establishing just presence or absence at a single release site may be convenient but uninformative. An appropriate timeframe for monitoring will reflect both 'scale' and 'level', as well as the milestones that have been set by the project to measure 'success'. Different success milestones can be established at different places within the timeframe. These are usually related to (1) establishing that released animals survive; (2) establishing that released animals are breeding; and (3) establishing that released animals have founded a self-sustaining, viable population or metapopulation (Griffiths & Pavajeau, 2008; Miller et al., 2014; Seddon, 1999). Milestone (3) will clearly take much longer to establish than either (1) or (2). Regardless, the timeframe set needs to be measured in terms of generation times rather than months or years, as different amphibians have different life histories that run at different speeds (Linhoff et al., 2021). Although there is no set timeframe for monitoring, a study of amphibian translocations found that on average, programmes showed higher levels of success after 15 years (Harding, 2014).

Whatever scale, level and timeframe are used, amphibians present some challenges for population assessment because many species are cryptic, with highly seasonal reproductive cycles. This means that any monitoring programme must account for issues associated with imperfect detection of populations or individuals (Schmidt, 2003). Fortunately, statistical models are now available that can account for such imperfect detection, and are recommended to be incorporated into the design of monitoring programmes at an early stage (Griffiths, Foster, Wilkinson, & Sewell, 2015). Monitoring may comprise direct observations of all stages of amphibians or the calls that they produce. Additionally, indirect observations may be informative. Environmental DNA (or eDNA) is proving to be an increasingly powerful tool for detecting species that are otherwise difficult to observe directly. Although extrapolating eDNA concentrations in the field to levels of abundance is currently difficult, metabarcoding approaches have the advantage of assessing a range of other taxa that may be relevant to conservation status (e.g., presence of disease, competitors or predators). eDNA methods are advancing rapidly and are likely to become a valuable part of the toolkit for assessing the status of cryptic species at large geographical scales (Harper et al., 2019).

Release methodology

The incredible diversity of amphibian species means that a programme's release methodology will likely be highly species-specific. Without previous experience with a species, a period of experimentation or adaptive management may occur during releases. It is important to continually re-assess translocation release methodologies, learn from prior mistakes, maintain flexibility, and not be afraid to apply creative solutions to solve difficult problems. There are a variety of release techniques that are worth testing, which have successfully been used for amphibians or other taxa groups (Tetzlaff, Sperry, & DeGregorio, 2019). There are generally two types of releases: hard-releases are where the animals are simply released, and soft-releases are where animals are released at the release site with some type of support. For example, soft-released animals may receive supplemental feeding, become acclimated to the release site in predator-proof enclosures (known as a delayed-release), or receive a combination of multiple supports (Parker, Dickens, Clarke, & Lovegrove, 2012).

Integrating experimental research into a translocation's release method can also be used to test explicit hypotheses (Kemp et al., 2015). For example, splitting release animals into separate treatments and releasing them under different conditions can provide direct comparisons of protocols if combined with post-release monitoring. Variations in release treatment location, season, life-stage, age, or tests of hard- and soft-release methods can be done. For example, in a study of Wyoming toads, a treatment of soft-released toads held in enclosures designed to acclimate animals to the release site reduced dispersal movements away from the release site compared to a treatment of hard-released toads. Soft-released animal's behaviour was also more similar to wild-conspecifics (Linhoff & Donnelly, In press). Experimental releases may help inform management decisions and answer foundational questions for any translocation. While some of these release methods have been trialled in amphibians, techniques to improve release success have been implemented in other taxonomic groups and may be useful for amphibians. Techniques such as delayed-releases (Linhoff & Donnelly, In press; Salehi, Akmali, & Sharifi, 2019), acoustic anchoring (Bradley, Ninnes, Valderrama, & Waas, 2011), supplemental feeding (Chauvenet et al., 2012), release with familiar individuals (Goldenberg et al., 2019), and predator control at the release site (Calvete & Estrada, 2004) may all be useful for some amphibian species.

Animal welfare

Every effort should be made to reduce stress or suffering during conservation translocations and programmes should adhere to internationally accepted standards for animal welfare (IUCN/SSC, 2013), such as the OIE Terrestrial and Aquatic Animal Health Codes. However, Harrington et al., (2013) determined that despite efforts to reduce stress and suffering, 67% of reintroduction projects reported animal welfare concerns for a variety of taxa. To address these concerns, they developed a useful decision tree for all stages of release (Harrington et al., 2013). There are many aspects of translocations that can negatively affect animal welfare (e.g., improper capture and handling, lengthy travel to release sites, and exposure to disease). Animal welfare can also be compromised if a release site lacks suitable quality, quantity, or connectivity of habitat to meet the needs of all life stages (Germano & Bishop, 2009).

Stress experienced during translocation or captivity can reduce the fitness of translocated individuals by interfering with reproduction and increasing disease susceptibility, predation risk, and likelihood of dispersing from the release site to unsuitable habitat (Dickens, Delehanty, & Romero, 2010; Griffin et al., 2000; Teixeira et al., 2007). Non-invasive methods of detecting stress have been developed by quantifying levels of corticosterone from skin or buccal swabs, urine, or water-borne hormone monitoring methods (reviewed in Narayan, Forsburg, Davis, & Gabor, 2019). However, stressors may not be equal for captive and wild translocated animals. Soft-releases may be beneficial for captive bred animals but may actually increase stress for wild-caught animals by prolonging their captivity (IUCN/SSC, 2013). Furthermore, because many amphibian translocations include a captive breeding component, animal welfare should be an important consideration for these programmes. Recent advancements in husbandry techniques have the potential to improve the welfare of captive individuals (See Chapter 11). Additionally, a better understanding of the sensory ecology of the species as it pertains to animal welfare can help improve management strategies for reintroduction (Swaisgood, 2010).

Discussion

Challenges for reintroductions

Translocations are not a risk-free management tool. It is often more cost-effective and biologically productive to protect a species *in situ*. In some circumstances, however, translocations have become a useful and/or necessary tool for the conservation management of amphibian species. There have been increases in success rates of herpetofaunal translocations in the past (Dodd & Seigel, 1991; Germano & Bishop, 2009), but success rates of roughly 40% leave significant room for the reintroduction community to strive for further improvements. One of the greatest challenges therefore is to ensure that translocations are done well and in a way that knowledge is gained and improvements, both species-specific and generally, can continue to be made and shared.

Perhaps one of the greatest threats to the use of translocations for amphibian conservation comes in the development space where they are being used as a tool to mitigate the impact of habitat destruction and human development. Thorough guidance on translocations has been provided by the IUCN (IUCN/SSC, 2013; Linhoff et al., 2021) but this guidance is rarely followed in these types of releases. Additionally, the initial threat to a species must be mitigated for a translocation to succeed and to have a net gain for conservation (e.g., destruction of habitat and translocation of animals to a small portion of remaining habitat equates to a net loss overall). For releases that cannot meet these standards, government agencies that regulate such releases, and the practitioners and managers who perform them, need to assess and use other tools that may deliver the desired conservation outcomes. The dilemma of reconciling the needs of burgeoning human populations with habitat destruction worldwide is one of the greatest threats facing amphibians. This is also an area where compensation and management dollars spent on translocations may not be delivering intended benefits to the species or mitigating damage to species and their habitat.

In addition, another challenge on the translocation horizon is how the reintroduction biology community can use this tool in the face of climate change. Whilst translocations linked to assisted colonisation are rare there is little doubt that they can play a role in this work, but it comes with other complexities that will need to be dealt with (Butt et al., 2021; Chauvenet, Ewen, Armstrong, Blackburn, & Pettorelli, 2013).

Recommendations

Both the amphibian conservation and reintroduction biology communities need to continue to build the capacity for practitioners and managers to work successfully in the translocation space. This includes education around the complexities and planning for translocations as outlined in some of the main detailed guideline documents (see Box 14.2). Government agencies and consultants also need to be educated about the success rates and dangers of using this tool for mitigation.

To continue to improve techniques, the results and challenges of releases must be shared amongst the amphibian and translocation communities. While scientific publications may be the gold standard of analysis and communication, publications such as the Global Re-introduction Perspective Series and databases of translocations are also key. Translocation databases are maintained by some government agencies and for some species (e.g., Lincoln Park Zoo maintains an avian translocation database), there is great potential for this to be developed on a wider scale as an accessible and evolving resource for practitioners worldwide.

Conclusions

Translocations are a tool that has grown in use throughout the world and across numerous taxonomic groups. Amphibian translocations have been a part of this growth. With a concerted effort for practitioners and managers to follow best practice guidelines provided by organizations such as the IUCN and others and the continued research into improving methodology, it is hoped that the success rates of these releases will continue to improve.

Box 14.3: Useful guidelines and reference documents for amphibian reintroductions

1. IUCN Amphibian reintroduction guidelines (https://www.iucn-amphibians.org/wp-content/uploads/2021/05/Ampb-Guidelines_170521_Final.pdf)
2. IUCN Guidelines for reintroductions and other conservation translocations (https://www.iucn.org/content/guidelines-reintroductions-and-other-conservation-translocations)
3. Department for Environment Food & Rural Affairs, 2021. Reintroductions and other conservation translocations: code and guidance for England https://www.gov.uk/government/publications/reintroductions-and-conservation-translocations-in-england-code-guidance-and-forms
4. Guidelines for conservation-related translocations of New Zealand lizards https://www.doc.govt.nz/globalassets/documents/getting-involved/translocation/translocation-best-practice-lizards-1.pdf
5. Great crested newt mitigation guidelines http://mokrady.wbs.cz/literatura_ke_stazeni/great_crested_newt_mitigation_guidelines.pdf -
6. Best management practices for amphibian and reptile salvages in British Columbia http://a100.gov.bc.ca/pub/eirs/finishDownloadDocument.do?subdocumentId=10351
7. Guidelines for mitigation translocations of amphibians: Applications for Canada's prairie provinces https://www.researchgate.net/publication/323783710_Guidelines_for_Mitigation_Translocations_of_Amphibians_Applications_for_Canada's_Prairie_Provinces
8. Kihansi spray toad re-introduction guidelines. http://www.amphibians.org/wp-content/uploads/2013/07/kihansi-spray-toad-re-introductionguidelines.pdf

9. The Scottish code for conservation translocations. Scottish Natural Heritage. http://www.snh.gov.uk/docs/A1327922.pdf
10. Kleiman, D. G., Stanley Price, M. R. & Beck, B. B. (1994). Criteria for reintroductions. In P. Olney, G. Mace, & A. Feistner (eds.), *Creative conservation: Interactive management of wild and captive animals* (pp.287–303). London, UK: Chapman & Hall.
11. Amphibian population management guidelines. http://www.amphibianark.org/pdf/Aark%20material/AArk%20Amphibian%20Population%20Management%20Guidelines.pdf

Acknowledgements

We are very grateful to Amaël Borzée, Javiera Cisternas Tirapegui, Kevin Johnson, Kaya Klop-Toker, Michael Lau, Cynthia Paszkowski and Benjamin Tapley, for contributing their time and expertise to the development of this chapter.

References

Balmford, A., Mace, G. M., & Leader-Williams, N. (1996). Designing the Ark: Setting priorities for captive breeding. *Conservation Biology*, *10*(3), 719–727. doi: 10.1046/j.1523-1739.1996.10030719.x

Barnes, M. D., Craigie, I. D., Harrison, L. B., Geldmann, J., Collen, B., Whitmee, S., … Woodley, S. (2016). Wildlife population trends in protected areas predicted by national socio-economic metrics and body size. *Nature Communications*, *7*, 1–9. doi: 10.1038/ncomms12747

Bienentreu, J. F., & Lesbarrères, D. (2020). Amphibian disease ecology: Are we just scratching the surface? *Herpetologica*, *76*(2), 153–166. doi: 10.1655/0018-0831-76.2.153

Bishop, P. J., Angulo, A., Lewis, J. P., Moore, R. D., Rabb, G. B., & Garcia Moreno, J. (2012). The amphibian extinction crisis - what will it take to put the action into the Amphibian Conservation Action Plan? *S.A.P.I.EN.S*, 5(2), 97-111.

Bloxam, Q. M. C., & Tonge, S. J. (1995). Amphibians: suitable candidates for breeding-release programmes. *Biodiversity and Conservation*, *4*, 636–644. doi: 10.1007/BF00222519

Bobadilla Suarez, M., Ewen, J. G., Groombridge, J. J., Beckmann, K., Shotton, J., Masters, N., … Sainsbury, A. W. (2017). Using qualitative disease risk analysis for herpetofauna conservation translocations Transgressing ecological and geographical barriers. *EcoHealth*, *14*, 47–60. doi: 10.1007/s10393-015-1086-4

Bodinof, C. M., Briggler, J. T., Junge, R. E., Beringer, J., Wanner, M. D., Schuette, C. D., … Millspaugh, J. J. (2012). Postrelease movements of captive-reared Ozark hellbenders (*Cryptobranchus alleganiensis bishopi*). *Herpetologica*, *68*(2), 160–

173. doi: 10.1655/HERPETOLOGICA-D-11-00033.1

Borzée, A., Kim, Y. I., Kim, Y. E., & Jang, Y. (2018). Translocation of an endangered endemic Korean treefrog *Dryophytes suweonensis*. *Conservation Evidence*, *15*, 6–11.

Bradley, D. W., Ninnes, C. E., Valderrama, S. V., & Waas, J. R. (2011). Does “acoustic anchoring” reduce post-translocation dispersal of North Island robins? *Wildlife Research*, *38*(1), 69–76. doi: 10.1071/WR10173

Bradley, H. S., Tomlinson, S., Craig, M. D., Cross, A. T., & Bateman, P. W. (2021). Mitigation translocation as a management tool. *Conservation Biology, 36*(1), e13667. doi: 10.1111/cobi.13667

Brodie, J. F., Lieberman, S., Moehrenschlager, A., Redford, K. H., Rodríguez, J. P., Schwartz, M., … Watson, J. E. M. (2021). Global policy for assisted colonization of species. *Science*, *372*(6541), 456–458. doi: 10.1126/science.abg0532

Bubac, C. M., & Johnson, A. C. (2019). Conservation translocations and post-release monitoring : Identifying trends in failures , biases , and challenges from around the world. *Biological Conservation*, *238*(August), 108239. doi: 10.1016/j.biocon.2019.108239

Bubac, C. M., Johnson, A. C., Fox, J. A., & Cullingham, C. I. (2019). Conservation translocations and post-release monitoring: Identifying trends in failures, biases, and challenges from around the world. *Biological Conservation*, *238*(May), 108239. doi: 10.1016/j.biocon.2019.108239

Butt, N., Chauvenet, A. L. M., Adams, V. M., Beger, M., Gallagher, R. V., Shanahan, D. F., … Possingham, H. P. (2021). Importance of species translocations under rapid climate change. *Conservation Biology*, *35*(3), 775–783. doi: 10.1111/cobi.13643

Calvete, C., & Estrada, R. (2004). Short-term survival and dispersal of translocated European wild rabbits. Improving the release protocol. *Biological Conservation*, *120*(4), 507–516. doi: 10.1016/j.biocon.2004.03.023

Canessa, S., Converse, S. J., West, M., Clemann, N., Gillespie, G., McFadden, M., … McCarthy, M. A. (2016). Planning for ex situ conservation in the face of uncertainty. *Conservation Biology*, *30*(3), 599–609. doi: 10.1111/cobi.12613

Chauvenet, A. L. M., Ewen, J. G., Armstrong, D. P., Blackburn, T. M., & Pettorelli, N. (2013). Maximizing the success of assisted colonizations. *Animal Conservation*, *16*(2), 161–169. doi: 10.1111/j.1469-1795.2012.00589.x

Chauvenet, A. L. M., Ewen, J. G., Armstrong, D. P., Coulson, T., Blackburn, T. M., Adams, L., … Pettorelli, N. (2012). Does supplemental feeding affect the viability of translocated populations? The example of the hihi. *Animal Conservation*, *15*(4), 337–350. doi: 10.1111/j.1469-1795.2012.00522.x

Cisternas, J., Wehi, P. M., Haupokia, N., Hughes, F., Hughes, M., Germano, J. M., … Bishop, P. J. (2019). Get together, work together, write together: A novel framework for conservation of New Zealand frogs. *New Zealand Journal of Ecology*, *43*(3). doi: 10.20417/nzjecol.43.32

Converse, S. J., Moore, C. T., & Armstrong, D. P. (2013). Demographics of reintroduced populations: Estimation, modeling, and decision analysis. *Journal of Wildlife Management*, *77*(6), 1081–1093. doi: 10.1002/jwmg.590

Crane, A. L., & Mathis, A. (2011). Predator-recognition training: A conservation strategy to increase postrelease survival of hellbenders in head-starting programs. *Zoo Biology*, *30*(6), 611–622. doi: 10.1002/zoo.20358

Della Togna, G., Howell, L. G., Clulow, J., Langhorne, C. J., Marcec-Greaves, R., & Calatayud, N. E. (2020). Evaluating amphibian biobanking and reproduction for captive breeding programs according to the Amphibian Conservation Action Plan objectives. *Theriogenology*, *150*, 412–431. doi: 10.1016/j.theriogenology.2020.02.024

Denton, J. S., Hitchings, S. P., Beebee, T. J. C., & Gent, A. (1997). A Recovery Program for the Natterjack Toad (Bufo calamita) in Britain. *Conservation Biology*, *11*(6), 1329–1338.

Dickens, M. J., Delehanty, D. J., & Michael Romero, L. (2010). Stress: An inevitable component of animal translocation. *Biological Conservation*, *143*(6), 1329–1341. doi: 10.1016/j.biocon.2010.02.032

Dodd, C. K., & Seigel, R. A. (1991). Relocation, repatriation, and translocation of amphibians and reptiles: are they strategies that work? *Herpetologica*, *47*(3), 336–350.

Dudley, N. (2008). *Guidelines for applying protected area management categories*. Gland, Switzerland: IUCN.

Ewen, J. G., Soorae, P. S., & Canessa, S. (2014). Reintroduction objectives, decisions and outcomes: global perspectives from the herpetofauna. *Animal Conservation*, *17*(S1), 74–81. doi: 10.1111/acv.12146

Ewen, John G., Armstrong, D. P., Parker, K. A., & Seddon, P. J. (2012). *Reintroduction biology: Integrating science and management*. Oxford, UK: Wiley-Blackwell.

Foster, J., Buckley, J., Martin, Y., Baker, J., & Griffiths, R. A. (2018). Reintroduction of the pool frog to the United Kingdom. In *Global reintroduction perspectives: 2018.*

Case studies from around the globe. (pp. 64–68). Gland, Switzerland and Abu Dhabi, UAE: IUCN Reintroduction Specialist Group / Environment Agency Abu Dhabi.

Geldmann, J., Coad, L., Barnes, M. D., Craigie, I. D., Woodley, S., Balmford, A., … Burgess, N. D. (2018). A global analysis of management capacity and ecological outcomes in terrestrial protected areas. *Conservation Letters*, *11*(3), 1–10. doi: 10.1111/conl.12434

Germano, J. M., & Bishop, P. J. (2009). Suitability of amphibians and reptiles for translocation. *Conservation Biology*, *23*(1), 7–15. doi: 10.1111/j.1523-1739.2008.01123.x

Germano, J. M., Field, K. J., Griffiths, R. A., Clulow, S., Foster, J., Harding, G., & Swaisgood, R. R. (2015). Mitigation-driven translocations: Are we moving wildlife in the right direction? *Frontiers in Ecology and the Environment*, *13*(2), 100–105. doi: 10.1890/140137

Gilligan, D. M., & Frankham, R. (2003). Dynamics of genetic adaptation to captivity. *Conservation Genetics*, *4*(2), 189–197. doi: 10.1023/A:1023391905158

Goldenberg, S. Z., Owen, M. A., Brown, J. L., Wittemyer, G., Oo, Z. M., & Leimgruber, P. (2019). Increasing conservation translocation success by building social functionality in released populations. *Global Ecology and Conservation*, *18*, e00604. doi: 10.1016/j.gecco.2019.e00604

Griffin, A. S., Blumstein, D. T., & Evans, C. S. (2000). Training captive-bred or translocated animals to avoid predators. *Conservation Biology*, *14*(5), 1317–1326. doi: 10.1046/j.1523-1739.2000.99326.x

Griffith, B., Scott, J, M., Carpenter, J. W., & Reed, C. (1989). Translocation as a species conservation tool: Status and strategy. *Science*, *245*(4917), 447–480. doi: 10.1126/science.245.4917.477

Griffiths, R. A., Foster, J., Wilkinson, J. W., & Sewell, D. (2015). Science, statistics and surveys: A herpetological perspective. *Journal of Applied Ecology*, *52*(6), 1413–1417. doi: 10.1111/1365-2664.12463

Griffiths, R. A., & Pavajeau, L. (2008). Captive breeding, reintroduction, and the conservation of amphibians. *Conservation Biology*, *22*(4), 852–861. doi: 10.1111/j.1523-1739.2008.00967.x

Groombridge, J. J., Raisin, C., Bristol, R., & Richardson, D. S. (2012). Genetic consequences of reintroductions and insights from population history. In J. G. Ewen, D. P. Armstrong, K. A. Parker & P. J. Seddon (Eds.), *Reintroduction biology: Integrating science and management* (pp. 395–440). Oxford, UK: Wiley-Blackwell. doi: 10.1002/9781444355833.ch12

Harding, G, Griffiths, R. A., & Pavajeau, L. (2016). Developments in amphibian captive breeding and reintroduction programs. *Conservation Biology*, *30*(2), 340–349. doi: 10.1111/cobi.12612

Harding, G. (2014). *Captive breeding and reintroduction of amphibians as a conservation tool* (University of Kent at Canterbury). Master of Science thesis. University of Kent at Canterbury.

Harding, G., Griffiths, R. A., & Pavajeau, L. (2016). Developments in amphibian captive breeding and reintroduction programs. *Conservation Biology*, *30*(2), 340–349. doi: 10.1111/cobi.12612

Harper, L. R., Buxton, A. S., Rees, H. C., Bruce, K., Brys, R., Halfmaerten, D., … Hänfling, B. (2019). Prospects and challenges of environmental DNA (eDNA) monitoring in freshwater ponds. *Hydrobiologia*, *826*(1), 25–41. doi: 10.1007/s10750-018-3750-5

Harrington, L. A., Moehrenschlager, A., Gelling, M., Atkinson, R. P. D., Hughes, J., & MacDonald, D. W.

(2013). Conflicting and complementary ethics of animal welfare considerations in reintroductions. *Conservation Biology*, *27*(3), 486–500. doi: 10.1111/cobi.12021

Hartley, M., & Sainsbury, A. (2017). Methods of disease risk analysis in wildlife translocations for conservation purposes. *EcoHealth*, *14*(s1), 16–29. doi: 10.1007/s10393-016-1134-8

IUCN/SSC. (2013). *Guidelines for reintroductions and other conservation translocations. Version 1.0*. Gland, Switzerland: IUCN Species Survival Commission.

IUCN/SSC. (2014). *IUCN Species Survival Commission guidelines on the use of ex situ management for species conservation. Version 2.* 1–7.

IUCN – SSC Species Conservation Planning Sub-Committee. (2017). *Guidelines for species conservation planning. Version 1.0*. Gland, Switzerland. doi: 10.2305/IUCN.CH.2017.18.en

Jamieson, I. G., & Lacy, R. C. (2012). Managing genetic issues in reintroduction biology. In J. G. Ewen, D. P. Armstrong, K. A. Parker & P. J. Seddon (Eds.), *Reintroduction biology: Integrating science and management.* (pp. 441–475). Oxford, UK: Wiley-Blackwell. doi: 10.1002/9781444355833.ch13

Jarchow, C. J., Hossack, B. R., Sigafus, B. H., Schwalbe, C. R., & Muths, E. (2016). Modeling habitat connectivity to inform reintroductions: A case Study with the Chiricahua leopard frog. *Journal of Herpetology*, *50*(1), 63–69. doi: 10.1670/14-172

Kemp, L., Norbury, G., Groenewegen, R., & Comer, S. (2015). The roles of trials and experiments in fauna reintroduction programs. *Advances in Reintroduction Biology of Australian and New Zealand Fauna*, (May 2015), 73–89.

Kleiman, D. G., Stanley Price, M. R. & Beck, B. B. (1994). Criteria for reintroductions. In P. Olney, G. Mace, & A. Feistner (Eds.), *Creative conservation: Interactive management of wild and captive animals* (pp. 287–303). London, UK: Chapman & Hall.

Kouba, A. J., Vance, C. K., & Willis, E. L. (2009). Artificial fertilization for amphibian conservation: Current knowledge and future considerations. *Theriogenology*, *71*(1), 214–227. doi: 10.1016/j.theriogenology.2008.09.055

Linhoff, L. J., & Donnelly, M. (In Press). Assessing release strategies for reintroductions of endangered Wyoming toads. *Wildlife Society Bulletin*.

Linhoff, L. J., Soorae, P. S., Harding, G., Donnelly, M. A., Germano, J. M., Hunter, D. A., … Eckstut, M. E. (2021). *IUCN Guidelines for amphibian reintroductions and other conservation translocations*. Gland, Switzerland.

McCallen, E. B., Kraus, B. T., Burgmeier, N. G., Fei, S., & Williams, R. N. (2018). Movement and habitat use of Eastern Hellbenders (*Cryptobranchus alleganiensis alleganiensis*) following population augmentation. *Herpetologica*, *74*(4), 283–293. doi: 10.1655/0018-0831.283

McDougall, P. T., Réale, D., Sol, D., & Reader, S. M. (2006). Wildlife conservation and animal temperament: Causes and consequences of evolutionary change for captive, reintroduced, and wild populations. *Animal Conservation*, *9*(1), 39–48. doi: 10.1111/j.1469-1795.2005.00004.x

Mendelson, J. R., & Altig, R. (2016). Tadpoles, froglets, and conservation: A discussion of basic principles of rearing and release procedures. *Amphibian and Reptile Conservation*, *10*(1), 20–27.

Miller, B., Reading, R., Conway, C., Jackson, J. A., Hutchins, M., Snyder, N., … Derrickson, S. (1994). A model for improving endangered species recovery programs. *Environmental Management*, *18*(5), 637–645. doi: 10.1007/BF02394630

Miller, K. A., Bell, T. P., & Germano, J. M. (2014). Understanding publication bias in reintroduction biology by assessing translocations of New Zealand's herpetofauna. *Conservation Biology*, *28*(4), 1045–1056. doi: 10.1111/cobi.12254

Mortelliti, A., Santulli Sanzo, G., & Boitani, L. (2009). Species' surrogacy for conservation planning: Caveats from comparing the response of three arboreal rodents to habitat loss and fragmentation. *Biodiversity and Conservation*, *18*(5), 1131–1145. doi: 10.1007/s10531-008-9477-2

Murray, K. A., Retallick, R. W. R., Puschendorf, R., Skerratt, L. F., Rosauer, D., McCallum, H. I., … VanDerWal, J. (2011). Assessing spatial patterns of disease risk to biodiversity: Implications for the management of the amphibian pathogen, *Batrachochytrium dendrobatidis*. *Journal of Applied Ecology*, *48*(1), 163–173. doi: 10.1111/j.1365-2664.2010.01890.x

Narayan, E. J., Forsburg, Z. R., Davis, D. R., & Gabor, C. R. (2019). Non-invasive methods for measuring and monitoring stress physiology in imperiled amphibians. *Frontiers in Ecology and Evolution*, *7*(November), 1–10. doi: 10.3389/fevo.2019.00431

Nowakowski, A. J., Thompson, M. E., Donnelly, M. A., & Todd, B. D. (2017). Amphibian sensitivity to habitat modification is associated with population trends and species traits. *Global Ecology and Biogeography*, *26*(6), 700–712. doi: 10.1111/geb.12571

Parker, K. A., Dickens, M. J., Clarke, R. H., & Lovegrove, T. G. (2012). The theory and practice of catching, holding, moving and releasing animals. In J. G. Ewen, D. P. Armstrong, K. A. Parker & P. J. Seddon (Eds.), *Reintroduction Biology: Integrating Science and Management* (pp. 105–137). Oxford, UK: Wiley-Blackwell. doi: 10.1002/9781444355833.ch4

Pašukonis, A., Ringler, M., Brandl, H. B., Mangione, R., Ringler, E., & Hödl, W. (2013). The homing frog: High homing performance in a territorial dendrobatid frog *Allobates femoralis* (Dendrobatidae). *Ethology*, *119*(9), 762–768. doi: 10.1111/eth.12116

Pessier, A. P., & Mendelson III, J. R. (2017). A manual for control of infectious diseases in amphibian survival assurance colonies and reintroduction programs. Ver. 2.0. In *IUCN/SSC CBSG*. IUCN/SSC Conservation Breeding Specialist Group: Apple Valley, MN.

Romero, D., Olivero, J., & Real, R. (2013). Comparative assessment of different methods for using land-cover variables for distribution modelling of *Salamandra salamandra longirotris*. *Environmental Conservation*, *40*(1), 48–59. doi: 10.1017/S0376892912000227

Romijn, R. L., & Hartley, S. (2016). Trends in lizard translocations in New Zealand between 1988 and 2013. *New Zealand Journal of Zoology*, *43*(2), 191–210. doi: 10.1080/03014223.2016.1146311

Sainsbury, A. W., Yu-Mei, R., Ågren, E., Vaughan-Higgins, R. J., Mcgill, I. S., Molenaar, F., … Foster, J. (2017). Disease risk analysis and post-release health surveillance for a reintroduction programme: the pool frog *Pelophylax lessonae*. *Transboundary and Emerging Diseases*, *64*(5), 1530–1548. doi: 10.1111/tbed.12545

Sainsbury, Anthony W., Armstrong, D. P., & Ewen, J. G. (2012). Methods of disease risk analysis for reintroduction programmes. In J. G. Ewen, D. P.

Armstrong, K. A. Parker & P. J. Seddon (Eds.), *Reintroduction biology: Integrating science and management*, (i), 336–359. Oxford, UK: Wiley-Blackwell. doi: 10.1002/9781444355833.ch10

Salehi, T., Akmali, V., & Sharifi, M. (2019). The soft-release of captive-born Kaiser's Mountain Newt *Neurergus kaiseri* (Amphibia: Caudata) into a highland stream, western Iran. *Journal of Threatened Taxa*, *11*(10), 14259–14267. doi: 10.11609/jott.4981.11.10.14259-14267

Saumure, R. A., Rivera, R., Jaeger, J. R., O'Toole, T., Ambos, A., Guadelupe, K., … Marshall, Z. (2021). Leaping from extinction: Rewilding the relict leopard frog in Las Vegas, Nevada, USA. In P. S. Soorae (Ed.), *IUCN Global conservation translocation perspectives: 2021* (pp. 76–81). Gland, Switzerland and Abu Dhabi, UAE: IUCN SSC Conservation Translocation Specialist Group, Environment Agency - Abu Dhabi and Calgary Zoo, Canada.

Scheele, B. C., Hollanders, M., Hoffmann, E. P., Newell, D. A., Lindenmayer, D. B., McFadden, M., … Grogan, L. F. (2021). Conservation translocations for amphibian species threatened by chytrid fungus: A review, conceptual framework, and recommendations. *Conservation Science and Practice*, *3*(11), 1–15. doi: 10.1111/csp2.524

Scheele, B. C., Pasmans, F., Skerratt, L. F., Berger, L., Martel, A., Beukema, W., … Flechas, S. V. (2019). Amphibian fungal panzootic causes catastrophic and ongoing loss of biodiversity. *Science*, *363*, 1459–1463.

Schleicher, J., Peres, C. A., Amano, T., Llactayo, W., & Leader-Williams, N. (2017). Conservation performance of different conservation governance regimes in the Peruvian Amazon.

Scientific Reports, *7*(1), 1–10. doi: 10.1038/s41598-017-10736-w

Schmidt, B. R. (2003). Count data, detection probabilities, and the demography, dynamics, distribution, and decline of amphibians. *Comptes Rendus - Biologies*, *326*, 119–124. doi: 10.1016/s1631-0691(03)00048-9

Seddon, P. J. (1999). Persistence without intervention: assessing success in wildlife. *Science*, *14*(12), 5347.

Silla, A. J., & Byrne, P. G. (2019). The role of reproductive technologies in amphibian conservation breeding programs. *Annual Review of Animal Biosciences*, *7*(October 2018), 499–519. doi: 10.1146/annurev-animal-020518-115056

Smith, R. K., & Sutherland, W. J. (2014). *Amphibian conservation: Global evidence for the effects of interventions*. Exeter, UK: Pelagic Publishing.

Smith, R., Meredith, H., & Sutherland, W. (2020). Amphibian conservation. In W. J. Sutherland, L. V. Dicks, S. O. Petrovan, & R. K. Smith (Eds.), *What works in conservation 2020* (pp. 9–64). Cambridge, UK: Open Book Publishers. doi: 10.1017/s0030605318000765

Soorae, P. S. (2008). *Global Re-introduction perspectives: Re-introduction case-studies from around the globe*. Gland, Switzerland and Abu Dhabi, UAE: IUCN Reintroduction Specialist Group / Environment Agency Abu Dhabi.

Soorae, P. S. (2010). *Global reintroduction perspectives: 2010. Additional case-studies from around the globe*. Abu Dhabi, UAE: IUCN/SSC Re-introduction Specialist Group.

Soorae, P. S. (2011). *Global re-introduction Perspectives: 2011. More case-studies from around the globe*. Gland, Switzerland and Abu Dhabi, UAE: IUCN/SSC Re-introduction

Specialist Group and Environment Agency - Abu Dhabi.

Soorae, P. S. (2013). *Global re-introduction perspectives: 2013. Further case studies from around the globe*. Gland, Switzerland and Abu Dhabi, UAE: IUCN Reintroduction Specialist Group / Environment Agency Abu Dhabi.

Soorae, P. S. (2016). *IUCN Global Re-introduction Perspectives: 2016. Additional case-studies from around the globe*. Gland, Switzerland and Abu Dhabi, AE : IUCN Reintroduction Specialist Group / Environment Agency Abu Dhabi.

Soorae, P. S. (2018). *Global reintroduction perspectives: 2018. Case studies from around the globe*. Gland, Switzerland and Abu Dhabi, UAE: IUCN Reintroduction Specialist Group / Environment Agency Abu Dhabi.

Soorae, P. S. (2021). *Global conservation translocation perspectives: 2021. Case studies from around the globe.* Gland, Switzerland: IUCN SSC Conservation Translocation Specialist Group, Environment Agency - Abu Dhabi and Calgary Zoo, Canada.

Sullivan, B. K., Nowak, E. M., & Kwiatkowski, M. A. (2015). Problems with mitigation translocation of herpetofauna. *Conservation Biology*, *29*(1), 12–18. doi: 10.1111/cobi.12336

Sutherland, W. J., Dicks, L. V., Petrovan, S. O., & Smith, R. K. (2021). *What works in conservation 2021*. Cambridge, UK: Open Book Publishers. doi: https://doi.org/10.11647/OBP.0267

Swaisgood, R. R. (2010). The conservation-welfare nexus in reintroduction programmes: A role for sensory ecology. *Animal Welfare*, *19*(2), 125–137.

Tapley, B., Bradfield, K. S., Michaels, C., & Bungard, M. (2015). Amphibians and conservation breeding programmes: do all threatened amphibians belong on the ark? *Biodiversity and Conservation*, *24*(11), 2625–2646. doi: 10.1007/s10531-015-0966-9

Teixeira, B., & Young, R. J. (2014). Can captive-bred American bullfrogs learn to avoid a model avian predator? *Acta Ethologica*, *17*(1), 15–22. doi: 10.1007/s10211-013-0150-8

Teixeira, C. P., de Azevedo, C. S., Mendl, M., Cipreste, C. F., & Young, R. J. (2007). Revisiting translocation and reintroduction programmes: the importance of considering stress. *Animal Behaviour*, *73*(1), 1–13. doi: 10.1016/j.anbehav.2006.06.002

Tetzlaff, S. J., Sperry, J. H., & DeGregorio, B. A. (2019). Effects of antipredator training, environmental enrichment, and soft release on wildlife translocations: A review and meta-analysis. *Biological Conservation*, *236*(February), 324–331. doi: 10.1016/j.biocon.2019.05.054

Valdez, J. W., Klop-Toker, K., Stockwell, M. P., Fardell, L., Clulow, S., Clulow, J., & Mahony, M. J. (2019). Informing compensatory habitat creation with experimental trials: A 3-year study of a threatened amphibian. *Oryx*, *53*(2), 310–320. doi: 10.1017/S0030605317000394

Walker, S. F., Bosch, J., James, T. Y., Litvintseva, A. P., Oliver Valls, J. A., Piña, S., … Fisher, M. C. (2008). Invasive pathogens threaten species recovery programs. *Current Biology*, *18*(18), 853–854. doi: 10.1016/j.cub.2008.07.033

Ward, R., Liddiard, T., Goetz, M., & Griffiths, R. A. (2016). Head-starting, re-introduction and conservation management of the agile frog on Jersey. In P. S. Soorae (Ed.), *Global re-introduction perspectives: 2016. Case-studies from around the globe.* (pp. 40–44). Gland, Switzerland and Abu Dhabi, UAE: IUCN/SSC Re-introduction Specialist Group and

Environment Agency - Abu Dhabi. doi: 10.13140/RG.2.1.3619.4969

Weiser, E. L., Grueber, C. E., & Jamieson, I. G. (2012). Allele Retain: A program to assess management options for conserving allelic diversity in small, isolated populations. *Molecular Ecology Resources*, *12*(6), 1161–1167. doi: 10.1111/j.1755-0998.2012.03176.x

Wilson, G. A., Fulton, T. L., Kendell, K., Scrimgeour, G., Paszkowski, C. A., & Coltman, D. W. (2008). Genetic diversity and structure in Canadian northern leopard frog (*Rana pipiens*) populations: implications for reintroduction programs. *Canadian Journal of Zoology*, *86*(8), 863–874.

Witzenberger, K. A., & Hochkirch, A. (2011). Ex situ conservation genetics: A review of molecular studies on the genetic consequences of captive breeding programmes for endangered animal species. *Biodiversity and Conservation*, *20*(9), 1843–1861. doi: 10.1007/s10531-011-0074-4