# Sex reversal in vertebrates: drivers and consequences in a changing world

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## Abstract:

Sex reversal describes the process of external factors or genetic mutations overriding normal sex determination during sexual development. This phenomenon is widespread among vertebrates and occurs under a number of different developmental circumstances. Environmental factors such as temperature and stress, mutations in key sex determination genes, and exposure to exogenous hormones or endocrine-disrupting compounds may all cause sex reversal. Outcomes of the sex reversal process are incredibly varied. Some taxa are far more adept at switching sex than others, and different sex reversal triggers can lead to contrasting fertile, reproductively impaired, or intersex phenotypes, with subsequently divergent implications for both evolutionary and population-level dynamics. Sex reversal has received considerable scientific attention since early experiments with exogenous hormones revealed an inherent flexibility in the sex determination of vertebrates. However, this attention is spread over a variety of research disciplines, many of which use specialised terminology, and focus on specific aspects of sex reversal. This comes at a detriment to a rapidly-advancing field which would greatly benefit from increased cross-disciplinary study. Here, we conducted a systematic review of 589 studies to provide an overview of current research on vertebrate sex reversals. We summarise known taxonomic trends, common research applications, and contemporary theories of how and why sex reversal occurs. Drawing from the collected primary literature, we further examine specific triggers of sex reversal, affected taxa, and potential mechanisms. We then discuss the role of sex reversal as a natural phenomenon in wild populations, exploring current hypotheses of sex reversal as an adaptive trait. Finally, we conclude the review with a discussion of how projected future increases in anthropogenically-driven sex reversal triggers – namely extreme high temperatures and endocrine-disruptors – present a further challenge to vulnerable populations under global climate change.

## Keywords:

Sex inversion, Sex Control, Sex Differentiation, Endocrine-Disrupting-Chemicals, Climate Change, Environmental Sex Determination, Aquaculture, Toxicology, Charnov-Bull Model

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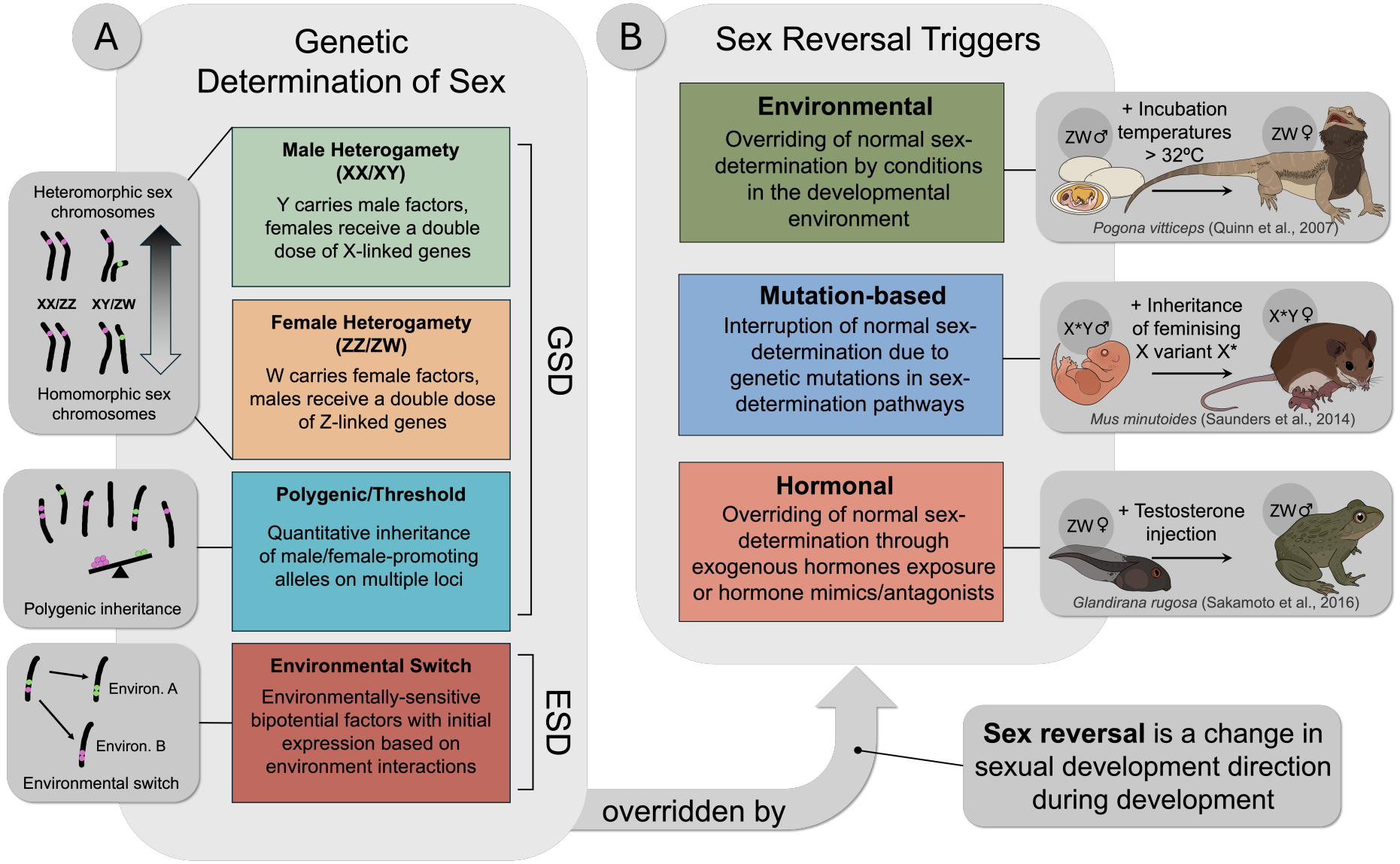
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## I. Introduction

Vertebrate sex determination is nuanced and incredibly varied. Broadly, most vertebrates possess some form of genetic sex determination (GSD), inheriting sex chromosomes (Bull, 1985) or, in polygenic systems, quantitative thresholds of male- or female-promoting alleles (Kocher et al., 2024). Conversely, some taxa show environmental sex determination (ESD). Similar genetic factors exist as in GSD species, but it is their bimodal expression under different environmental conditions, rather than their inheritance, that determines the direction of sexual development (see Fig. 1a; Deeming & Ferguson, 1988; Weber & Capel, 2018; Thépot, 2021). While diverse, these sex determination systems all operate using similar frameworks of gene-hormone interactions, and under the right conditions, all of these systems can be disrupted.

Sex reversal refers to external factors or genetic mutations overriding normal sex determination during the sexual development of an embryo or larvae (Bull, 1985; Weber and Capel, 2018). Individuals destined to be male become phenotypic females, and vice versa. Among GSD species, the sex-reversed individuals possess a phenotype at odds with their genetic sex, e.g. an XX or ZW male, or an XY or ZZ female. Such individuals are termed ‘discordant’ due to this mismatch (Holleley et al., 2015). Species with ESD can also be subject to sex reversal where factors cause them to develop as the sex opposite to that expected for their developmental conditions (see Fig. 1a; Warner and Shine, 2005; 2008; Rogerson et al., 2023). Sex reversal can occur in all vertebrates, but is most common in fish and amphibians, being comparatively rare in birds and mammals. However, these paradigms may shift into the future. Recent advances in genetic sex testing have markedly increased our ability to detect wild sex-reversed individuals, and lability in sex determination modes may be far more widespread than currently recognised (Holleley et al., 2016; Hall et al., 2025). Increases in the frequency and magnitude of the processes that drive sex reversal are also expected to drive an accompanying increase in the taxa and populations affected by this phenomenon (Schwanz et al., 2020).

Sex reversal can be instigated by a variety of triggers within three broad categories, across all sex determination systems (Fig. 1b). Environmental sex reversal involves the overriding of normal sex-determination by developmental conditions, such as temperature or resource availability (e.g. Radder et al., 2007; Dupoué et al., 2019). Environmental sex reversals specifically occur in ESD species where uncommon environmental variables override determining variables that are always present; e.g., masculinising oxidative stress at an otherwise female-producing temperature (Corona-Herrera et al., 2018). Second, mutation-based sex reversals involve genetic mutations (or incompatibilities in hybrids) that cause the failure of normal sex-determination gene pathways (e.g. Saunders et al., 2014; Parma et al., 2016). Finally, hormonal sex-reversals occur when developing individuals are exposed to hormones, hormone antagonists or endocrine-disrupting chemicals, which override normal sex-determination (e.g. Sakamoto et al., 2016; Piprek et al., 2012). Although these triggers appear varied at first glance, their proximate mechanisms tend to be similar. Sex reversal can occur due to mutations or external influences that alter the expression of sex-linked genes, adjust an individual’s balance or concentration of sex-steroid hormones, and/or interfere with primordial germ cell proliferation during early development (Capel, 2017; Weber and Capel, 2018).


A
Genetic Determination of Sex
Heteromorphic sex chromosomes
XX/ZZ
XY/ZW
Homomorphic sex chromosomes
Male Heterogamety (XX/XY)
Y carries male factors, females receive a double dose of X-linked genes
Female Heterogamety (ZZ/ZW)
W carries female factors, males receive a double dose of Z-linked genes
GSD
Polygenic inheritance
Polygenic/Threshold
Quantitative inheritance of male/female-promoting alleles on multiple loci
Environ. A
Environ. B
Environmental switch
Environmental Switch
Environmentally-sensitive bipotential factors with initial expression based on environment interactions
ESD
B
Sex Reversal Triggers
Environmental
Overriding of normal sex-determination by conditions in the developmental environment
ZW♂
+ Incubation temperatures > 32°C
ZW♀
Pogona vitticeps (Quinn et al., 2007)
Mutation-based
Interruption of normal sex-determination due to genetic mutations in sex-determination pathways
X*Y♂
+ Inheritance of feminising X variant X*
X*Y♀
Mus minutoides (Saunders et al., 2014)
Hormonal
Overriding of normal sex-determination through exogenous hormones exposure or hormone mimics/antagonists
ZW♀
+ Testosterone injection
ZW♂
Glandirana rugosa (Sakamoto et al., 2016)
overridden by
Sex reversal is a change in sexual development direction during development

109
110

Fig. 1. **Genetic determinants of sex and the sex reversal triggers that override them.** A). Vertebrate sex determination systems are generally either genetic (GSD; chromosomal/polygenic) or ESD (environmental cues overriding genes via interactions). While ESD is not considered a form of GSD, the genetic factors are still the same, but it is environment-moderated expression rather than inheritance of these genes that determines sex. B). Sex reversal overrides the normal paths in both ESD and GSD systems via environmental factors, genetic mutations, or modification of hormone levels. In the first example of environmental sex reversal, high incubation temperatures in the central bearded dragon (*Pogona vitticeps*) cause ZZ genetic males to develop as phenotypic females (Quinn et al., 2007). In the second example, of mutation-based sex reversal, an African pygmy mouse embryo (*Mus minutoides*) is genetically a XY male, but has inherited a variant form of the X chromosome, X*, which prevents masculinisation and lead to development of a female phenotype (Saunders et al., 2014). Finally, in the third example, of hormonal sex reversal, a ZW female tadpole of the Japanese wrinkled frog (*Glandirana rugosa*) is injected with exogenous testosterone, leading to the development of a male phenotype (Sakamoto et al., 2016).

In contrast to phenotypic sex-change, sex reversal occurs only before maturation of the gonads, and in conventionally gonochoristic species. The flexible hermaphroditic life-histories of many reef fishes, where fish reproduce as both males and females within their lifetime, are examples of sex-change (Kadota, 2022), as is the oft-reported spontaneous development of male gonadal tissue in adult female chickens following illness or trauma (Weber and Capel, 2018; Zhang et al., 2023a). While there is overlap in aspects of these two sex-inversions (see Table 1 for glossary) they can be quite different. Sequentially hermaphroditic fish are evolutionarily primed to switch between sexes, sometimes multiple times (Adolfi et al., 2023). In comparison, sex reversal in GSD species produces an individual with a novel combination of male and female factors (Tripathi et al., 2009; Capel, 2017). Partial or incomplete sex reversal is common, where individuals display weak to strongly intersex phenotypes, alongside reduced fertility or infertility. Reproductive impairment arises from the presence of intersex gonadal tissue, improperly connected reproductive systems, low numbers and poor quality of gametes, and/or irregular reproductive behaviour (Dorizzi et al., 1994; Kim et al., 1997; Bayley et al., 2002; Jobling et al., 2002). Furthermore, where sex reversal occurs in species with ESD, individuals may still be disadvantaged by an intersex phenotype (Dorizzi et al., 1994; Richard-Mercier et al., 1995), but also by the uncoupling of a presumably adaptive link between sex and developmental conditions (Charnov and Bull, 1977; Warner and Shine, 2005; 2008; Bock et al., 2023). Sex change is an evolutionarily stable state; sex reversal is a deviation from a stable state where, depending on the system, fitness may consequently increase, decrease, or remain unchanged (Weber and Capel, 2018).

Sex reversal is a popular area of research in a variety of disciplines. For example, experimental sex reversal is a core tool in the study of sexual development, helping to identify key factors in the processes of sexual differentiation and maintenance (often with an underlying aim to understand human variation; Ottolenghi et al., 2007; Rodríguez-Marí et al., 2011; Fujii et al., 2014). Discordant individuals specifically can teach us much about the relative contributions of hormones and genetics to the final sexual phenotype (Veyrunes et al., 2024). Sex reversal is frequently used to control sex-ratios in captive populations, particularly in aquaculture (Kavumpurath and Pandian, 1994; Singh and Srivastava, 2014; Zhang et al., 2021). Partial or incomplete reversals also act as a marker of endocrine-disrupting chemicals (Zillioux et al. 2001; Hayes et al., 2002). Yet an understudied facet of sex reversal is its natural occurrence. It is known that sex reversal occurs at constant low rates in

many wild populations (Shinomiya et al., 2004; Parma et al., 2016; Lambert et al., 2019; Castelli et al., 2021; Hill et al., 2022), but theoretical modelling predicts that slight changes in factors such as heritability or population size can increase reversal rates to the point they have a tangible influence over population demographics and dynamics (Cotton and Wedekind, 2009; Perrin, 2009; Holleley et al., 2016). Complete sex reversal may also be adaptive where it coincides with frequency dependent selection or environment/phenotype links (Schwanz et al., 2020). In this way, sex reversal may have facilitated historical transitions between sex determination systems, and in certain taxa, it may currently be shaping sex chromosome evolution (Holleley et al., 2015; Rodrigues et al., 2018). More concerningly, sex reversal could represent a developmental limitation that will hinder sensitive populations under the changing environment of the Anthropocene (Schwanz et al., 2020; Yu et al., 2022).

The study of sex reversal is a broad and rapidly advancing field; increases in our ability to detect and observe sex reversals are closely linked with technological advancements in modern genetic tools. This comes as sex reversal research itself wrestles with modern concerns: how will anthropogenic climate change and pollution alter the dynamics of sex reversal in wild populations? While numerous reviews explore sex reversal within specific contexts (e.g., a single taxon or mechanism), the field lacks general, broad-scale reviews that highlight shared mechanisms and theories, use standardised, defined terms, and foster connection between different research disciplines. Sex reversal occurring in natural populations is also underrepresented by the literature. Here, we conducted a systematic review to synthesise our understanding of the mechanisms and processes involved in sex reversal across non-human vertebrates. We summarise known triggers of sex reversal, highlighting both common and unique mechanisms, as well as affected taxa. We discuss where sex reversal is adaptive, or maladaptive, and the likely consequences of sex reversal in natural populations. Special consideration is given to how continuing anthropogenic change – climate change and exposure to endocrine-disrupting chemicals – will likely act to increase sex reversals and alter future population dynamics.

## II. Systematic review and literature compilation

We conducted a literature search through the *Scopus* and *Web of Science* databases, identifying papers that fit an intentionally broad initial inclusion criteria. *CitationChaser* (Haddaway et al., 2022) was then used to obtain both backwards and forwards citations from these initial articles. The resulting large pool of publications was refined to relevant articles using keyword searches. Remaining papers were then subject to a second stricter inclusion criteria to retain only relevant primary literature for the main review. Searches of the pre-print servers *EcoEvoRXiV* and *bioRXiV* were also used to identify additional upcoming papers. This review was structured following PRISMA guidelines for systematic reviews in ecology and evolution (O'Dea et al., 2021; Foo et al., 2021), particularly regarding execution and reporting of the literature search (Fig. 2). All searches and applications of the inclusion criteria were conducted by a single observer (NL).

Our initial database search was conducted from 4$^{th}$-26$^{th}$ April 2023. *Scopus* and *Web of Science* (WoS; core collection) were searched for publications containing the keywords '*sex reversal*'

and '*vertebrate*', excluding publications containing the keyword '*human*'. We retrieved 169 publications from both databases, with 51 unique records from SCOPUS and 84 unique records from WoS, for a total of 304 unique records. Abstracts were screened using an initial inclusion criteria, which only required mention of any sex-inversion in a non-human vertebrate. This search was purposefully broad (e.g. including sex change and all types of publications) to capture the literature as comprehensively as possible, including review articles or studies with inconsistent terminology. After this screening, which resulted in 192 relevant articles, we generated forwards (citing) and backwards (cited) citations using *CitationChaser* (Haddaway et al., 2022). *CitationChaser* allows a systematic search to capture large amounts of relevant literature that may be unable to be captured without either repeated refinement of database search strings, or screening large numbers of majority irrelevant articles obtained with broader search terms. This method is also particularly useful when terminology is inconsistent or differs across fields, as for sex reversal. Our pool of 192 initial papers included 51 review articles/book chapters, which are ideal for use as a base when 'citation chasing' (Foo et al., 2021). To generate backwards and forwards citations, publications were matched to the Lens.org database. Eight articles without DOIs were unable to be matched, and six were not present in the Lens.org database.

For the 178 papers present in the database, we generated 20,149 records, of which 8,577 were duplicates. Unique papers (n = 11,572) were processed by excluding articles that did not include at least one of several key words within the title or abstract. These keywords were '*sex-reversal'*, '*sex reversal'*, '*masculinization*', '*feminization*', '*sex inversion'*, '*sex change'*, '*sex-change'*, and '*override*' (including spelling variations). This resulted in a reduced dataset of 1262 papers which were subject to another title/abstract screen using the above broad inclusion criteria.

The full text of 1,049 remaining papers (including the 192 papers from the initial database searches) was then screened using a stricter criterion. This second inclusion criteria excluded publications reporting on sex-change, being inversions occurring in natural hermaphrodites or after the conclusion of sexual development in gonochorists. Publications were also excluded if they did not directly attempt or observe a sex reversal. A total of 100 reviews and book chapters relating to sex reversal were placed in a separate database. 580 articles were retained for this review. We did not include three papers that were not available in English, and a further 13 publications were excluded as their full text was not available, and the abstract did not contain key details. We added to the final included papers by conducting searches of two pre-print servers, *EcoEvoRxiv* and *bioRxiv*. Preprints were limited to those lodged after 1st January 2021, and up until the date of the search, 9th June 2023. The same keywords were used as in the initial searches of *Scopus* and *Web of Science*. Of 50 records, seven were already published and included in the database, and 34 did not meet the secondary inclusion criteria. Thus, nine additional papers were added to create a final sample of 589 publications.

We extracted data from 589 articles, aiming to synthesise current understanding of the mechanisms and processes involved in sex reversal of non-human vertebrates. Data extracted included examined taxa and their sex-determination systems, sex-reversal triggers, known mechanisms of those triggers, and the broader context of the research – including evidence of

occurrence in natural populations. These articles formed the basis of this review, but discussion was supported by previous reviews (including those captured by the initial database search), as well as papers encountered opportunistically after the time period of the systematic review (April-June 2023).

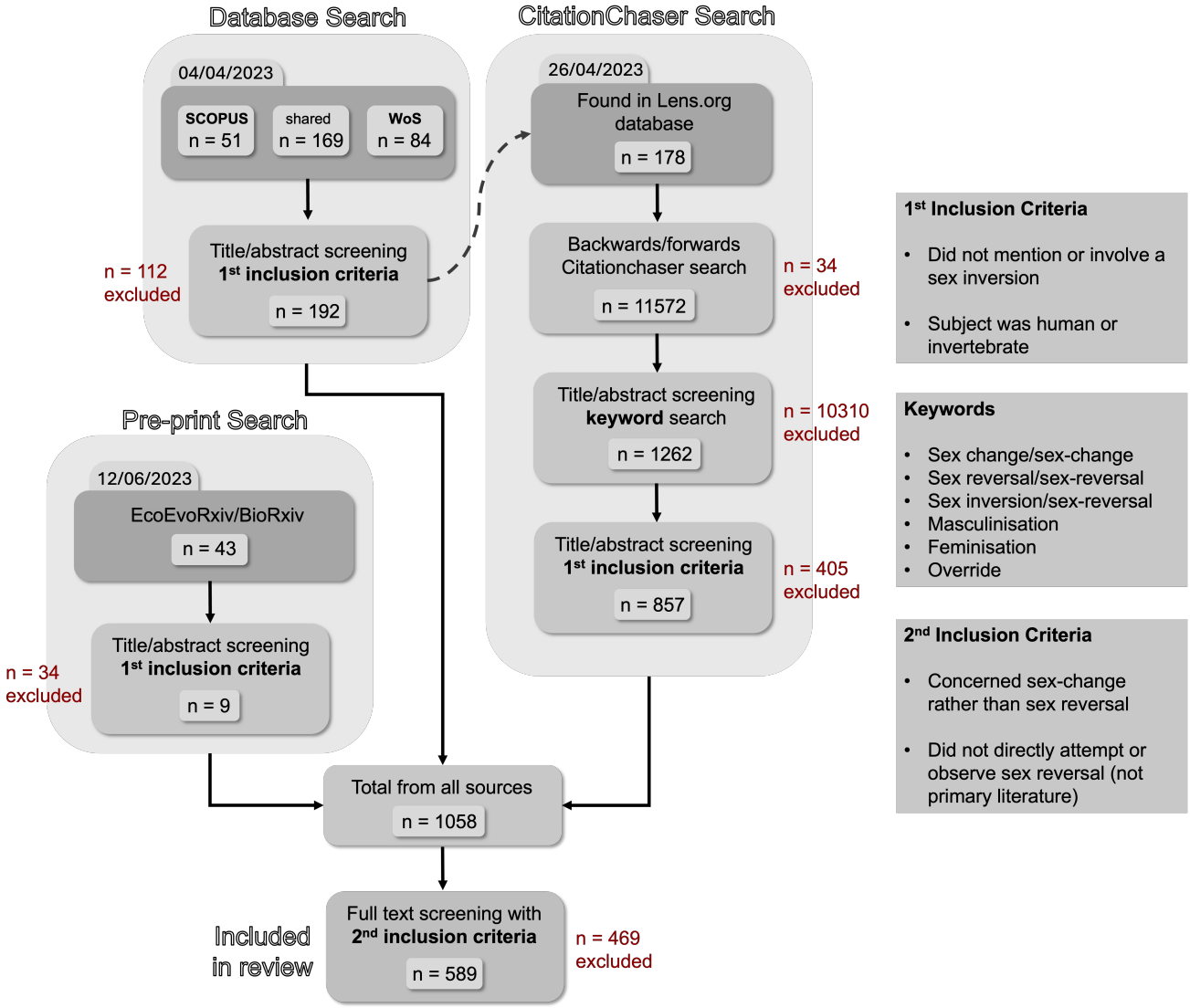



Fig. 2. **PRISMA flowchart summarising search methods.** Briefly, keyword searches were conducted in SCOPUS and Web of Science. Papers were screened with an initial broad inclusion criteria, with the citing/cited papers of applicable papers captured using CitationChaser. These articles were limited to those containing key terms within their abstract or title. All papers were then subject to a full-text screening with a stricter secondary inclusion criteria, to create the pool of papers used in this review. Duplicate removals at each stage are reported within the text.

## III. Overview of sex reversal research

### (1) Which vertebrates sex reverse?

Almost all vertebrate groups show some level of susceptibility to sex reversal (Fig 3, Fig 4a). However, the literature is presently dominated by studies on fish (61% of studies, n = 357/589), which are used in both the laboratory and aquaculture settings. Amphibians were the next most frequently studied group (14%, n = 80/589), being widely used in studies of endocrine-disrupting chemicals and sexual development. Non-avian reptiles (hereafter referred to as 'reptiles') made up a further 11% (n = 67/589) of studies, covering both experimental hormonal sex reversals, and evidence of naturally-occurring sex reversal. As a group, mammals were poorly represented in retrieved papers (total mammals; 9%, n = 50/589), with mammalian studies dominated by experimental genetic sex reversals in mice, *Mus musculus* (56%, n = 28/50). Similarly, domestic chickens (*Gallus gallus*) accounted for almost all bird studies captured by our search (total birds; 6%, n = 34/589, of which 31/34, 91%, were chickens). Only a single paper was retrieved on a vertebrate outside the Euteleostomi; an unsuccessful attempt to hormonally induce sex reversal in the lamprey *Lampetra planeri* (Hardisty and Taylor, 1965).

Despite the phenomenon of sex reversal being phylogenetically widespread (Stelkens and Wedekind, 2010), 50% of papers retrieved for this review focussed on just ten species (Fig 3). This included mice (28 studies) and chickens (31 studies), as well as three other common laboratory models; the medaka/ricefish (*Oryzias latipes*; 63 studies), the zebrafish (*Danio rerio*; 41 studies), and the African clawed frog (*Xenopus laevis*; 20 studies). Nile tilapia (*Oreochromis niloticus*; 59 studies) was the second-most frequently studied species, highlighting its role as a model aquaculture species. Emerging models of sex reversal include the central bearded dragon (*Pogona vitticeps*; 17 studies) and the olive flounder (*Paralichthys olivaceus*; 12 studies). Complete, reproductively functional sex reversals have been observed in all the above species, with the exception of chickens, which have been only recorded to display partial or transient sex reversals (Zhang et al., 2023a; 2023b).

All classic modes of sex determination were represented in the collected sex reversal studies (Fig. 3). Most species displayed GSD, commonly with male heterogamety (XX/XY; 362 mentions). Systems exhibiting female heterogamety (ZZ/ZW) were mentioned 126 times. Polygenic sex-determination systems were mentioned 49 times, while temperature-dependent sex determination systems (TSD) were mentioned 30 times. Several species displayed uncertain or unclassified sex determination systems (33 mentions).

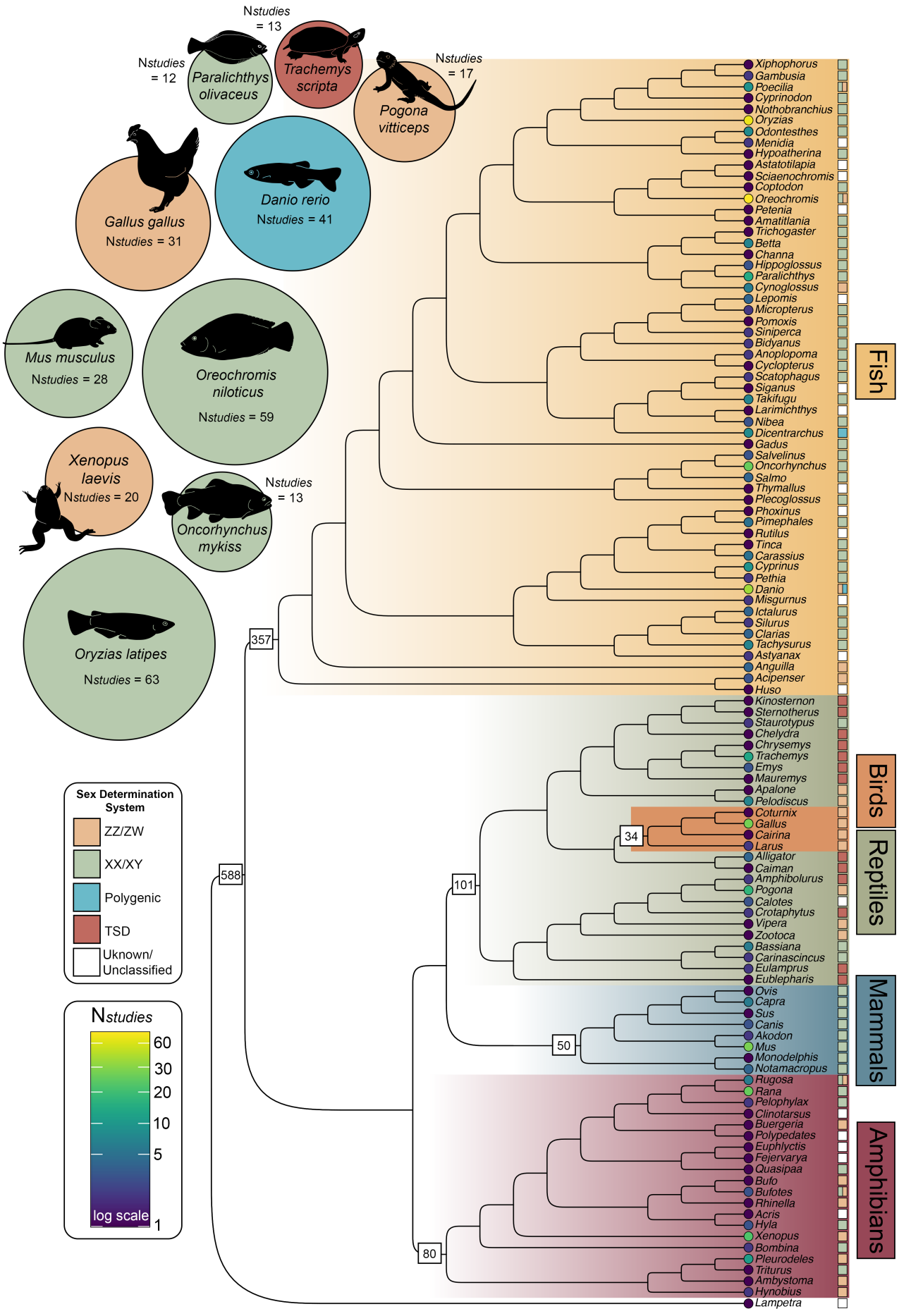

Nstudies = 13
Nstudies = 12
Paralichthys olivaceus
Trachemys scripta
Nstudies = 17
Pogona vitticeps
Gallus gallus
Nstudies = 31
Danio rerio
Nstudies = 41
Mus musculus
Nstudies = 28
Oreochromis niloticus
Nstudies = 59
Xenopus laevis
Nstudies = 20
Nstudies = 13
Oncorhynchus mykiss
Oryzias latipes
Nstudies = 63
Sex Determination System
ZZ/ZW
XX/XY
Polygenic
TSD
Uknown/ Unclassified
Nstudies
60
30
20
10
5
1
log scale
Xiphophorus
Gambusia
Poecilia
Cyprinodon
Nothobranchius
Oryzias
Odontesthes
Menidia
Hypoatherina
Astatotilapia
Sciaenochromis
Coptodon
Oreochromis
Petenia
Amatitlania
Trichogaster
Betta
Channa
Hippoglossus
Paralichthys
Cynoglossus
Lepomis
Micropterus
Pomoxis
Siniperca
Bidyanus
Anoplopoma
Cyclopterus
Scatophagus
Siganus
Takifugu
Larimichthys
Nibea
Dicentrarchus
Gadus
Salvelinus
Oncorhynchus
Salmo
Thymallus
Plecoglossus
Phoxinus
Pimephales
Rutilus
Tinca
Carassius
Cyprinus
Pethia
Danio
Misgurnus
Ictalurus
Silurus
Clarias
Tachysurus
Astyanax
Anguilla
Acipenser
Huso
Kinosternon
Sternotherus
Staurotypus
Chelydra
Chrysemys
Trachemys
Emys
Mauremys
Apalone
Pelodiscus
Coturnix
Gallus
Cairina
Larus
Alligator
Caiman
Amphibolurus
Pogona
Calotes
Crotaphytus
Vipera
Zootoca
Bassiana
Carinascincus
Eulamprus
Eublepharis
Ovis
Capra
Sus
Canis
Akodon
Mus
Monodelphis
Notamacropus
Rugosa
Rana
Pelophylax
Clinotarsus
Buergeria
Polypedates
Euphlyctis
Fejervarya
Quasipaa
Bufo
Bufotes
Rhinella
Acris
Hyla
Xenopus
Bombina
Pleurodeles
Triturus
Ambystoma
Hynobius
Lampetra
357
588
34
101
50
80
Fish
Birds
Reptiles
Mammals
Amphibians

Fig. 3. **Genus-level cladogram of vertebrate taxa which have been investigated for sex reversal.** 153 species from 112 genera were identified from the 589 papers included in this review. Taxonomic relationships were determined using open tree of life (OpenTreeOfLife et al., 2019). Numbers in white boxes denote the study numbers for each clade. Some studies included multiple species. Coloured circles at tree tips indicate number of studies retrieved per genus. Coloured boxes next to each genus name represent the known sex-determination system(s) of that genus. Bubbles on the left-hand side of the figure highlight the ten most frequent study species in the retrieved literature, which were the focus, or shared focus, of half of all publications (297/589 papers). Bubble colour denotes the species' sex-determination system. Note *Danio rerio* is labelled polygenic, as the majority of sex reversal experiments in this species use laboratory strains that have lost the ZZ/ZW sex chromosomes seen in wild populations.

## (2) Why is sex reversal studied?

Studies retrieved in this review broadly fit within five research areas: aquaculture methods, invasive species control, natural sex reversal, sexual development, and toxicology (Fig. 4b). Many papers touched on multiple topics (e.g., almost all papers mention sexual development), and thus assignation to a single main research area considered the authors' framing of the research, the chosen journal, and the intended audience. Aquaculture studies were defined as those intended to further knowledge in producing monosex fish populations for consumption, recreational fishing, or ornamental purposes. Invasive species control similarly focused on production of sex-skewed populations, but to limit reproduction in invasive populations. Natural sex reversal studies were defined as those reporting on instances, characteristics, or consequences of naturally occurring sex reversals. Studies testing or recording sex reversal effects from endocrine-disrupting-chemicals were classed under toxicology. Finally, the remainder of studies were classed under sexual development. This included experimental studies attempting to identify sex-determination or genes, classify sex determination pathways, or record the effects of hormones/hormone antagonists on sexual development.

Sexual development was the most prevalent topic among the retrieved studies (330/559, 59% of studies). All five vertebrate groups were represented among these studies, although 58% of studies were on fish, with the remaining studies split fairly evenly between birds, mammals, amphibians and reptiles (ranging from 10-12% of studies). Understanding the factors which govern sexual development has been a major goal since the beginning of sex reversal research (Fig. 4b inset). Retrieved papers included very early explorations of hormonal sex reversal in frogs and salamanders by Emil Witschi and colleagues (Witschi, 1929; Foote and Witschi, 1939; Bruner and Witschi,1954), alongside Toki-o Yamamoto's pioneering work on sex determination in the medaka (*Oryzias latipes*; Yamamoto, 1955; 1958; 1959).

Next most common were studies centred on methods in aquaculture (102/589, 17% of studies). Monosex aquaculture populations reduce energy allocated to reproduction rather than growth, and allow a focus on whichever sex has the most efficient growth - or the most popular appearance, for sexually dimorphic aquarium species (Kavumpurath & Pandian, 1993; Kipouros et al., 2011). Sex-specific aquaculture products, such as *Accipenser* sturgeon caviar, also benefit from sex-ratio control (Omoto et al., 2002; Grandi et al., 2007; Hassanzadeh et al., 2019). Furthermore, widespread use of sex reversal in aquaculture has provided the foundations for another small but emerging research area; invasive species control (5/589, 0.8% of studies; all in fish). Many discordant

individuals produce skewed sex ratios or inviable offspring due to mating with individuals of the same genotype (e.g., two XX individuals produce only XX offspring). When introduced into invasive populations, such individuals can reduce effective population size and reproductive output, limiting future invasion potential (Schill et al., 2016; Jiang et al., 2018*;* Norazmi-Lokman et al., 2021; Teal et al., 2023; Vorhees et al., 2023).

Studies on natural sex reversal comprised another 83 studies, or 14% of all studies. These included observations of naturally-occurring sex reversals (Shinomiya et al., 2004; Parma et al., 2016; Castelli, 2021), but also experimental laboratory-based studies used to understand the causes and consequences of natural sex reversals (e.g., Otake et al., 2006; Saunders et al., 2014; Li et al., 2016). While natural sex reversal research has risen sharply the last decade (Fig. 4b inset), the presence of natural sex reversal has long been recognised, with some of the earliest sex reversal research involving observations of mutation-based reversals in medaka with sex-linked colouration (Aida, 1936). Fish, amphibian, reptile and mammal systems were all represented in retrieved natural sex reversal studies, although reptiles have received the most attention (35% of studies). Recent advances in genetic testing have highlighted many new cases of natural sex reversals (Parma et al., 2016; Saunders et al., 2023). However, genetic tests do not reveal the mechanisms behind sex reversal, and the actual triggers of sex reversal for many of these systems remain unknown. For example, skewed sex-ratios in amphibians and fish may be due to high temperature masculinisation, exposure to endocrine-disrupting-chemicals, spontaneous or inherited sex reversal mutations, or a combination of these factors, highlighting the need for experimental studies to confirm sex reversal triggers in natural systems (Nagler et al., 2001; Lambert et al., 2019).

A further 69 or 12% of all studies focused on sex reversal as a consequence of exposure to environmental pollutants and endocrine-disrupting-chemicals. Toxicology research is overwhelmingly conducted on fish (54%) and amphibians (38%). These groups contain several key toxicology model organisms; they are sensitive to waterborne pollutants, easy to raise in the lab, and their biology is well-understood (Helbing, 2012, Gamse and Gorelick, 2016). A limited number of studies were retrieved concerning exposure to pollutants in mammals (one study), birds (two studies) or aquatic reptiles (three studies). Considering the immense literature on endocrine-disrupting-chemicals, toxicology studies may be an area in which this review is unable to capture the full scope of the literature. Specifically, if sex reversal or sexual disruption was not be the main focus, key search terms may not have been captured by our title/abstract searches as they were restricted to the main text of the study.

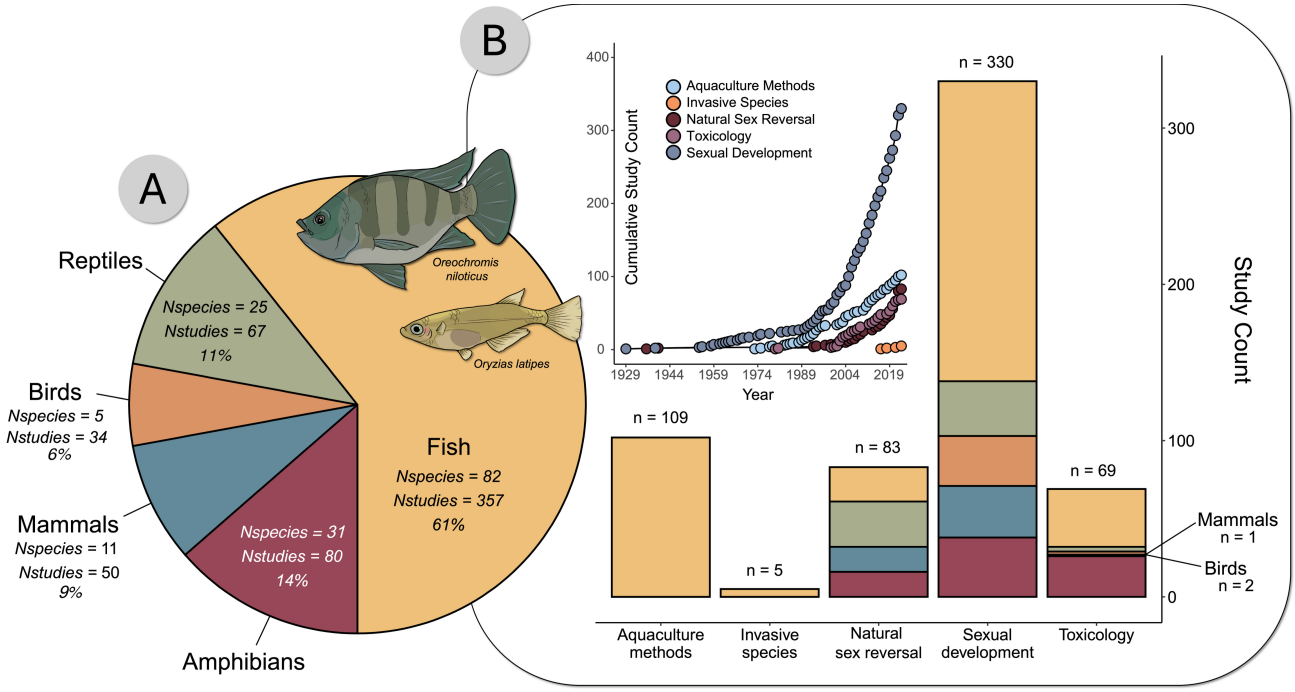



Fig. 4. **Broad-scale taxonomic distribution of sex reversal across the literature.** A). Pie chart showing the proportion (reported as %) of retrieved studies involving each major vertebrate clade. Also reported are the number of retrieved studies and species for each clade. Pictured are the two most frequently occurring taxa in the literature, the fish species *Oreochromis niloticus* and *Oryzias latipes*. B). Counts of studies retrieved in this review, coloured by vertebrate clade (as in A) and classed under five contexts of sex reversal research. Inset shows cumulative counts of studies per topic over time, visualising broad trends in context of retrieved literature from 1929 to 2023.

## (3) What causes sex reversal?

Sex-determination in gonochoristic vertebrates involves the initiation and subsequent maintenance of one development pathway – either male (testis) or female (ovaries) –in parallel with suppression of the alternate pathway. Initiation is primarily driven by genetic factors, often by 'master sex-determining genes' (e.g. *Sry* in mammals; Arnold, 2017). In GSD systems, expression of these genes is mediated by their inheritance (presence/absence or dosage dependence), which is often linked to inheritance of sex chromosomes. In purely ESD species, master sex determination genes are those that respond epigenetically to environmental conditions (Thépot, 2021). Master sex-determinants vary among vertebrates, but many genes within the sex determination pathway are conserved. DMRT1, SOX9, and AMH (anti-mullerian hormone) are key male pathway genes, driving development of the testes, suppression of ovaries, and supporting high androgen levels. Conversely, FOXL2 and CYP19A1 are integral elements of the female pathway, driving ovarian development, suppressing testes development, and supporting high estrogen levels. Expression of these genes forms positive feedback loops through upregulation of associated genes (concurrent with suppression of alternate pathway genes), guiding an individual's gonads to develop as either testes or an ovary. In turn, these developing gonads produce androgens and/or estrogens, which further reinforce the corresponding initiated pathway (Fig. 5; Arnold et al., 2017; Capel, 2017).

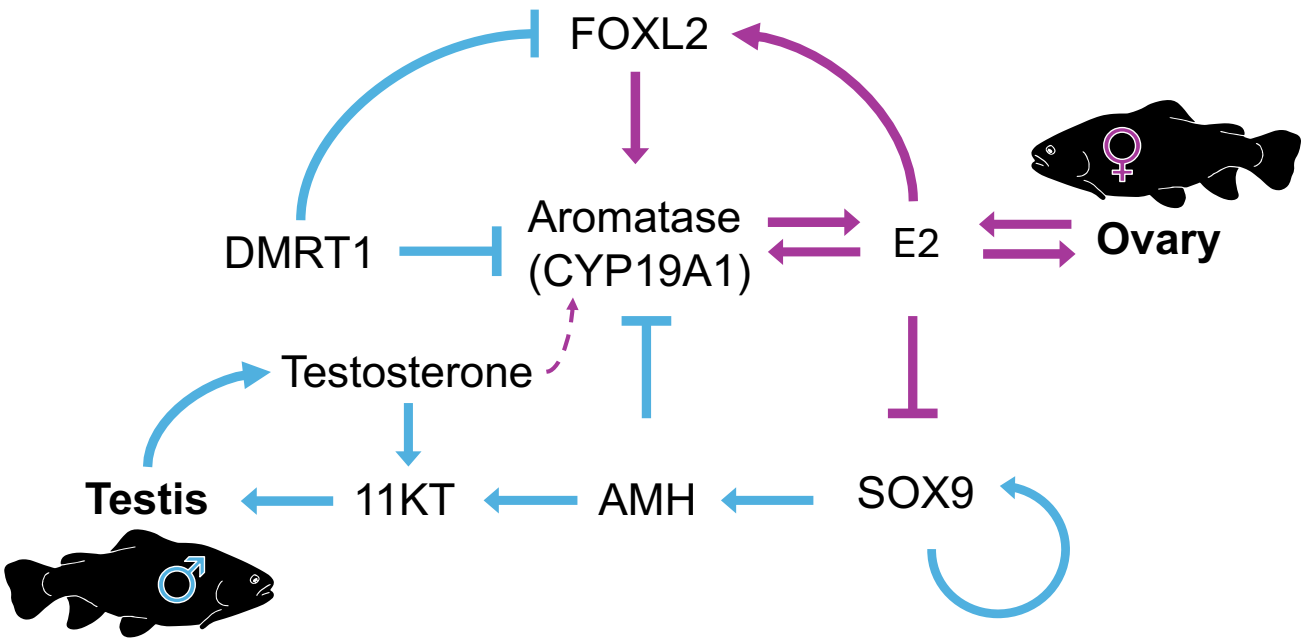



Fig. 5. **Example key genetic and hormonal pathways determining vertebrate gonadal fate.** Vertebrate sex determination is theorised as consisting of two antagonistic pathways, leading to either a testicular or ovarian fate. The initiated pathway is maintained by a series of positive feedback loops and by the inhibition of factors in the alternate pathway. Blunt arrows (⊥) indicate suppression relationships (between conflicting pathways), and sharp arrows (→) denote upregulation (within the same pathway). While fish are used here as an example, core sex-determination genes such as FOXL2, DMRT1, CYP19A1, and AMH are conserved across vertebrates, as is the action of the androgen testosterone and estrogen 17ß-estradiol (E2). The female-pathway hormone E2 is converted from testosterone via the enzyme aromatase (purple dashed line). Figure adapted from Capel (2017), Weber and Capel (2021), and Kocher et al. (2024).

At a basic level, sex reversal happens because the intended (e.g. genetic) pathway fails to suppress the alternate pathway, during either the initiation or maintenance phases of gonad development (Weber & Capel, 2017). Sex reversal triggers act either by causing failures in the intended pathway (e.g., *Sry* loss-of-function mutation; Warr et al., 2022) or by supporting the alternate pathway (e.g., exogenous estrogen; Díaz and Piferre, 2017). Although superficially varied, all sex reversal triggers appear to act by modulating developmental hormone levels, expression of sex-linked genes, and potentially through altering the numbers of primordial germ cells (Fig. 6). Identifying the exact proximate mechanisms of triggers can be complex. For example, sex reversal caused by temperature involves complex epigenetic responses that are known to vary between taxa (Castelli et al., 2021; Bókony et al., 2024). Furthermore, although many recent advances have been made in observing changes in sexual morphology and gene expression during sex reversal (Deveson et al., 2017; Liu et al., 2019; Zhang et al. 2023; Huang, 2023), identification of mechanisms still tends to be correlative. Distinguishing the initiating factors from those initiated downstream of sex reversal is particularly challenging (Yang et al., 2019; Wang W. et al., 2022).

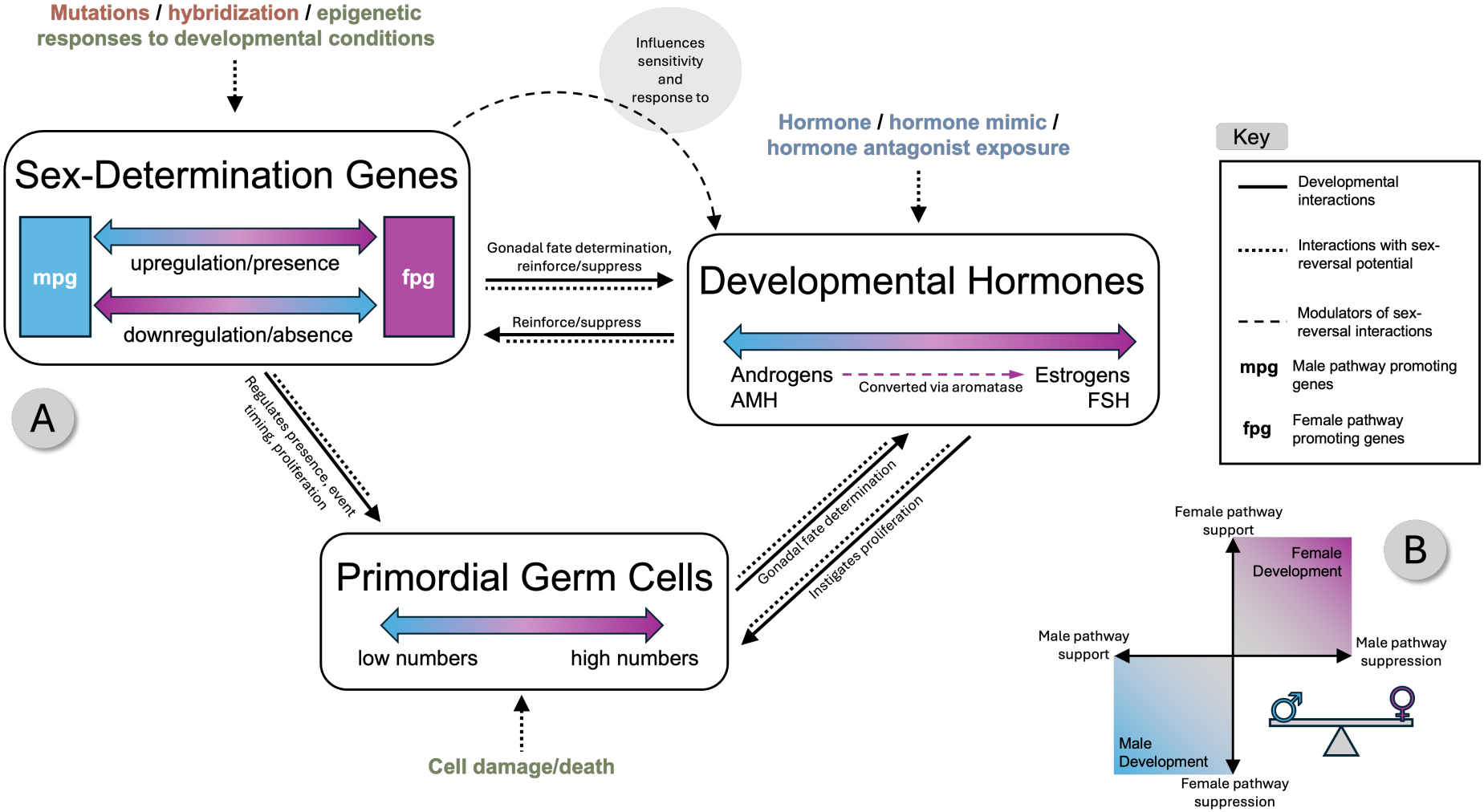

Mutations / hybridization / epigenetic responses to developmental conditions
Influences sensitivity and response to
Hormone / hormone mimic / hormone antagonist exposure
Sex-Determination Genes
mpg
upregulation/presence
downregulation/absence
fpg
Gonadal fate determination, reinforce/suppress
Reinforce/suppress
Developmental Hormones
Androgens
AMH
Converted via aromatase
Estrogens
FSH
A
Regulates presence, event timing, proliferation
Gonadal fate determination
Instigates proliferation
Primordial Germ Cells
low numbers
high numbers
Cell damage/death
Key
Developmental interactions
Interactions with sex-reversal potential
Modulators of sex-reversal interactions
mpg Male pathway promoting genes
fpg Female pathway promoting genes
B
Female pathway support
Female Development
Male pathway support
Male pathway suppression
Male Development
Female pathway suppression

Fig. 6. **Broad overview of potential sex determination mechanisms and sex reversal pathways.** A). Inherited sex-specific, or environmentally activated (in the case of purely ESD species) sex-determination genes initiate either male (testicular) or female (ovarian) gonad development. The developing gonad reinforces this initiated pathway by producing sex steroid hormones. However, this series of positive feedback loops may be interrupted by genetic mutations or external influences on *sex-determination genes*, *hormone levels*, or *numbers of primordial germ cells*. B). Current theory conceptualises sexual fate as being a balancing act between the upregulation and suppression of male and female factors. Sex reversal occurs if there is a failure to support the initiated pathway, or if enough factors support the alternate pathway. This can be visualised as a tipping scale of male and female pathway support/suppression (adapted from Capel, 2017).

From publications collected in this review, we recognised 13 ultimate triggers of sex reversal (Fig. 7; Table 2. Note some studies involved multiple triggers). The majority of studies were concerned with hormonal sex reversals (384 mentions, 58% of all studies), including both exogenous hormones (238 mentions), hormone antagonists (75 mentions), and EDCs (66 mentions). Three studies investigated sex reversal by gonadal transplant. Mutation-based sex reversals were mentioned in 137 studies (21% of all studies). Intentional experimental manipulations of genes were most common (99 studies), but there were also numerous observations of naturally occurring sex reversal mutations (32 studies) and a handful of sex reversals due to hybridisation (6 mentions). Environmental sex reversals were mentioned in 127 (19% of all studies) studies. Again, this group was dominated by a single trigger, temperature (108 mentions). The remaining studies investigated diverse triggers such as stress (12 mentions), oxidative stress (two mentions), irradiation (two mentions), food and water restriction (two mentions), and yolk mass (one mention). Finally, in 12 cases (2% of all studies), sex reversal occurred due to an unknown trigger.

Taxonomic group strongly influences the outcome of exposure (no effect / incomplete sex reversal / complete sex reversal) to triggers of sex reversal (Fig. 7; Pipreck et al., 2012). Fish, amphibians and reptiles show complete sex reversal with exposure to a variety of triggers. In contrast, mammals exhibit incomplete sex reversal, with only a handful of specific mutation-based triggers. Birds are the only group in which complete sex reversal has not yet been confirmed (but see Hall et al., 2025). However, while taxonomic groups do show broad trends, outcomes do vary across species within these groups, including due to genetic background (Bókony et al., 2021), reversal direction (Saidapur et al., 2001), and predictably, timing, magnitude and length of exposure to sex reversal triggers (Villalpando & Merchant-Larios, 1990; Iwamatsu et al., 2006; Van Nes & Andersen, 2006; Cek et al., 2007). We discuss each of the listed triggers and their mechanisms in detail below.

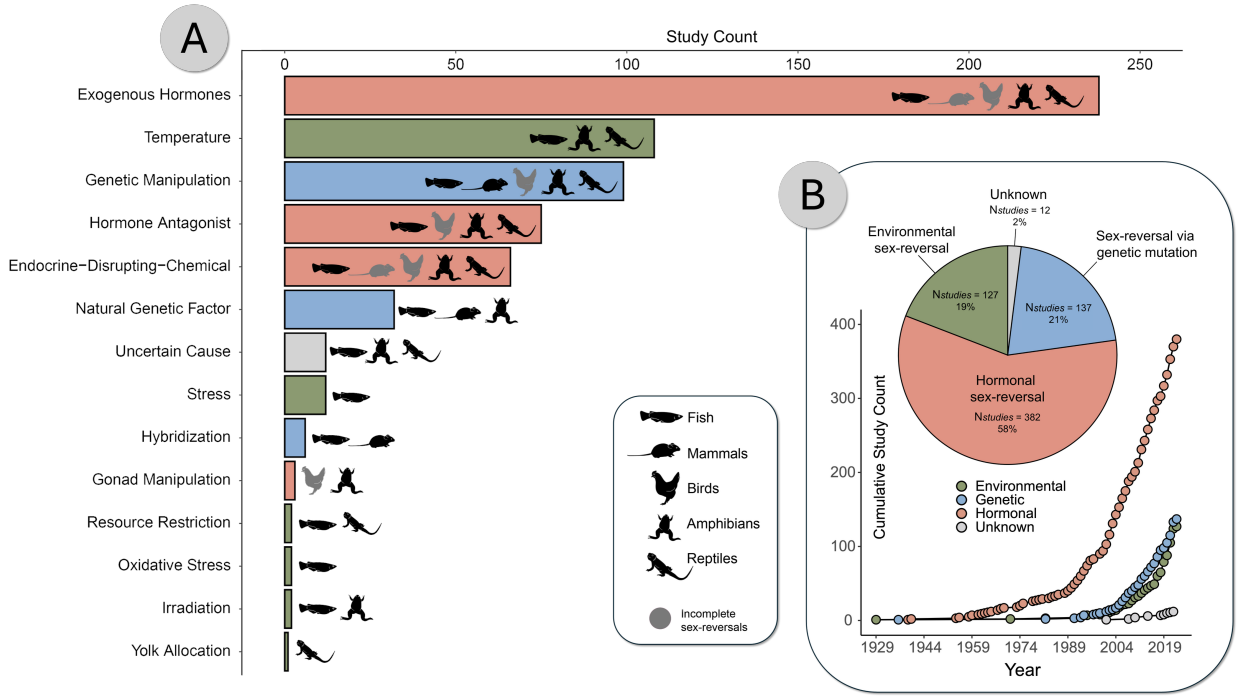



Fig. 7. **Known Triggers of Sex reversal**. Data collected from the 589 papers processed as part of this review. A). Known triggers are shown on the Y axis, with the number of retrieved studies involving that trigger shown on the X axis. Some studies involve more than one sex reversal trigger. Silhouettes mark which taxa are reported to experience sex reversal with each trigger. Grey silhouettes indicate incomplete sex reversal in all retrieved instances of a taxa/trigger combination. Black silhouettes indicate taxa/trigger combinations that can potentially result in complete sex reversal. B). Pie chart shows proportion of retrieved studies that fall within each main trigger category as described in figure 1b. The chart below shows the cumulative number of papers per each trigger category over time, visualising broad trends in the retrieved literature from 1929 to 2023.

## IV. Mechanisms of sex reversal: our contemporary understanding

### (1) Environmental sex reversals

#### *(a) Temperature*

Thermally-triggered sex reversal is widespread among fish (58%, n = 63/108 studies) and amphibians (16%, n = 17/108 studies), and well-studied, within a limited number of systems, in reptiles (26%, n = 28/108; Table 2). Systems with XX/XY, ZZ/ZW and polygenetic sex-determination are all represented among taxa known to sex reverse through temperature. Most thermally-triggered sex reversal occurs during a certain thermo-sensitive period during development, outside of which extreme temperatures have little effect on sex (Piferrer & Anastasiadi, 2021). Amongst the wide variety of species from different environments experiencing sex reversal through temperature, conditions required to instigate sex reversal tend to be relative to each species' natural ecology. For example, in the cold-water Atlantic halibut (*Hippoglossus hippoglossus*), 13°C constitutes a sufficiently high temperature to induce masculinisation (Van Nes & Andersen, 2006). Conversely, 13°C causes low-temperature masculinisation in the tiger pufferfish (*Takifugu rubripes*) of east Asia (Zhou et al., 2019).

*i.* *Fish*

High temperatures are overwhelmingly masculinising in fish (of 61 papers investigating high temp sex reversal in fish, 59 (97%) reported female to male sex reversal). A large body of work exists examining this phenomenon, partly due to the widespread use of high-temperature masculinisation in aquaculture, e.g., in the Nile tilapia (*Oreochromis niloticus,* 17/63 *, e.g.* Palaiokostas et al., 2015*;* Nivelle et al., 2019; Dussenne et al., 2022) which has subsequently become a model species in the study of teleost sex-determination. Comparatively fewer studies indicate low temperatures in fish can be feminising (e.g. *Odontesthes bonariensis*; Hattori et al., 2009, *Hypotherina tsurugae*; Miyoshi et al., 2020, *Paralichthys olivaceus*; Yamaguchi et al., 2010). Whether this is due to a lack of incentive to seek out these relationships, as high-temperatures are more relevant to ecology and aquaculture, or because low temperatures are less likely to cause sex reversal, is unclear. Exceptions to this high temperature masculinisation trend are not unheard of; either temperature extreme, low or high, is masculinising in *Paralichthys lethostigama* (Honeycutt et al., 2019) and *Dicentrarchus labrax* (Saillant, 2002). Low temperatures are known to masculinise the fry of *Takifugu rubripes* (Zhou et al., 2019), and there is evidence for high-temperature feminisation in *Trichogaster lalius* (Ramee et al., 2020) and in *O. niloticus* with a contrived YY genotype (Kwon et al., 2002).

Much of what we know about the mechanisms of thermally-triggered sex reversal is restricted to fish systems. While the mechanisms that drive temperature-triggered feminisation remain unclear, high temperature-triggered masculinisation appears to be epigenetic in nature, driven by interactions between high levels of the main fish glucocorticoid cortisol and expression of sex-linked genes (Yamaguchi et al., 2010; García-Cruz et al., 2020). Increased cortisol under high temperatures is, itself, instigated through upregulation of heat-shock proteins like *hsp70* (Uchimura et al., 2019; Wang et al., 2019; Wang C. et al., 2022). In *Paralichthys olivaceus*, high cortisol directly supresses expression of the aromatase gene *CYP19A1* (Yamaguchi et al., 2010), presumably increasing androgen levels and promoting the male sexual pathway. In both *P. olivaceus* and O*ryzias latipes*, high cortisol also suppresses expression of the follicle-stimulating hormone receptor gene, or *fshr* (Hayashi et al., 2010; Hara et al., 2021). This gene drives the proliferation and differentiation of germ cells, which tend to be higher in females than males in early development (Mustapha et al., 2021). Decreased germ cell numbers may prevent oogenesis, blocking advancement of the ovarian pathway and instead triggering testes development. Reduced, male-like germ cell numbers are seen in high-temperature stressed XX *O. latipes* (Hara et al., 2021), and XX *Tachysurus fulvidraco* (Yu et al., 2021). An alternate pathway of high cortisol masculinisation has been suggested for the pejerrey, *Odontesthes bonariensis.* High-temperature stressed individuals show upregulation of the gene *hsd11b2*, which codes for the dual-purpose enzyme 11β-hsd2 (Fernandino et al., 2012; García-Cruz et al., 2020). This enzyme functions to inactivate glucocorticoids and to synthesise the key fish androgen 11-ketotestosterone, which is also elevated in stressed *O. bonariensis* (Blasco et al., 2013; García-Cruz et al., 2020). Suppression of cyp19A1 is still supressed in XX *O. bonariensis* undergoing sex reversal, but as ever, it is difficult to untangle which factor is the direct instigator of the male pathway, and which is only the consequence of already instigated masculinisation (Fernandino et al., 2012).

DNA methylation is another epigenetic process that can alter the expression or function of genes, whose action may be crucial to temperature-linked sex reversal. Higher methylation levels generally result in reduced gene expression, or downregulation, and thus methylation of male or female-promoting genes can contribute to sex reversal (Furman et al., 2020). Low-temperature masculinised pufferfish (*Takifugu rubripes*) show an overall increase in DNA methylation compared to non sex-reversed fish (Zhou et al., 2019), while increased gonadal DNA methylation is seen in reversing high-temperature masculinised tilapia *Oreochromis niloticus* (Sun et al., 2016; Yao et al., 2022). Han et al. (2021) found that high temperature masculinisation of zebrafish (*Danio rerio*) increased levels of DNA methylation in promoters of the female-promoting genes *estrogen receptor 1* (*esr1*) and *sox9b*. Application of a DNA methylation inhibitor partly recovered expression of these genes, slightly reducing male sex-ratio bias in high-temperature treated groups. DNA methylation may be an important mechanism of *CYP19A* suppression; male *O. niloticus*, *Cyanoglossus laevis*, and *Dicentrarchus labrax* all have higher levels of DNA methylation in *CYP19A* promoter regions than females. High-temperature masculinisation in these three species is associated with increased methylation of this promoter to male-like or nearly male-like levels. Males raised at the same high temperatures also experience slight increases in DNA methylation, but not at the same magnitude seen in individuals who have undergone female-to-male sex reversal (Navarro-Martin et al., 2011; Wang et al., 2017; Liu et al., 2019). While there is a clear link between DNA methylation and teleost temperature-induced sex reversal, it is still unknown if methylation is a mechanism or consequence. Changes in methylation levels could be a mechanism of sex reversal itself, or simply the consequence of another factor (e.g. hormone levels) that has already initiated a switch to the opposite sexual pathway.

*ii.* *Amphibians & Reptiles*

Amphibians show similar patterns in temperature-triggered sex reversal to fish, with high temperatures known to masculinise several frogs: *Rana dalmatina* (Ujszegi et al., 2022), *R. dybowskii* (Xu et al., 2022), *R. catesbeiana* (Hsü et al., 2022), and *R. sylvatica* (Witschi, 1929). The salamanders *Pleurodeles watl* (Kuntz et al., 2003) and *Triturus cristatus* (Wallace & Wallace, 2000) similarly undergo female-to-male sex reversal at high temperatures. However, a close relative of *P. watl*, *P. nebulosus*, undergoes feminisation at high temperatures (Dorazi et al., 1995), as does another salamander, *Hynobius retardus* (Sakata et al., 2005). In reptiles, the bearded dragon *Pogona vitticeps* experiences feminisation through high temperatures (Quinn et al., 2007), while low temperatures masculinise embryos of two scincoid lizards, *Acritoscincus (Bassiana) duperreyi* (Radder et al., 2007) and the viviparous *Carinascincus (Niveoscincus) oscellatus* (Hill et al., 2022). Temperature-triggered sex reversal may be far more common in reptiles than currently recognised (Holleley et al., 2016, and see Cornejo-Páramo et al., 2020; Wiggins et al., 2020). Despite temperature-induced sex reversal in reptiles and amphibians occurring under presumably stressful conditions, there is as yet no compelling evidence for a relationship between stress, temperature, and sex reversal in these taxa (Castelli et. al., 2021; Bókony et al., 2023). Work on the *Pogona vitticeps* system instead implicates an obscure upregulation of female genes by temperature through a

pathway of cellular temperature sensing and calcium-redox signalling, temperature-sensitive proteins and DNA methylation. These are similar to the main sex determination pathways of TSD species such as the turtle *Trachemys scripta*, although in *P. vitticeps* these pathways also involve the overriding of sex chromosomes (Castelli et al., 2021; Whiteley et al., 2021; 2022a; 2022b; 2022c).

*(b) Stressful conditions*

As discussed above, high temperature masculinisation in many teleost fish appears to be driven by a stress response, with high cortisol levels suppressing genes coding for aromatase and follicle-stimulating-hormone reception, or initiating production of 11-ketotestosterone (Hattori et al., 2009; Hayashi et al., 2010; Yamaguchi et al., 2010; Yu et al., 2021). Thus, other stressful developmental conditions and direct application of cortisol can also induce sex reversal. Overcrowding – both real and simulated using mirrored enclosures – increases stress levels and subsequent masculinisation in *Odontesthes bonariensis*, likely through the same mechanism as high temperatures (*hsd11b2* upregulation and aromatase suppression; García-Cruz et al., 2020). In *Paralichthys lethostigma*, a bottom-dwelling fish which relies heavily on crypsis, larvae raised on blue backgrounds show increased cortisol and female-to-male sex reversals over those raised on grey or black backgrounds. Exogenous cortisol application was similarly masculinising, confirming stress as a sex reversal mechanism in this species (Mankiewicz et al., 2012). Overcrowding and application of exogenous cortisol similarly cause masculinization in *Danio rerio* (Ribas et al., 2017).

Oxidative stress is also a potential sex reversal trigger, occurring when harmful reactive oxygen species (ROS) outpace antioxidant and other biological defences within an individual's cells. Unchecked, ROS cause cellular damage, loss of cell reproductive ability, and eventually, cell death (Cohen-Jonathan et al., 2000; Dong et al., 2020). As number and proliferation of germ cells is linked to gonadal fate (discussed above; Mustapha et al., 2021), masculinisations induced by oxidative stress are likely driven by a loss of germ cells. Oxidative stress initiated by irradiation and hydrogen peroxide has been recorded to instigate female-to-male sex reversal and reduced germ cell numbers in *Pelophylax brevipoda* and *Oryzias latipes*, respectively (Terukiyo, 1982; Koki et al., 2022). Certain light wavelengths, or continuous light, can also induce irradiation/oxidative stress and subsequent female-to-male sex reversal. Constant-light masculinised *Chirostoma estor* showed upregulation of ROS-related genes but also high levels of cortisol. Thus, classic glucocorticoid stress may also contribute to certain sex reversals instigated by oxidative stress (Corona-Herrera et al., 2018; Hayasaka et al., 2019).

*(c) Resource restriction*

Resource restriction is implicated in a small number of fish and reptile sex reversals. While the mechanisms are not well-known and system specific, resource restriction could potentially represent a stressful condition, as above, or act by prevent developing individuals from synthesising key developmental hormones and metabolites. Water restriction during the pregnancy of two viviparous reptiles, *Vipera aspis* and *Zootica vivipara*, resulted in male-skewed offspring sex-ratios, despite the reported ZZ/ZW sex-chromosomes of these species. Dupoué et al. (2019) suggest that water

restriction constitutes a stressor like that of high temperatures in fish, with maternal stress hormones causing epigenetic modulation of genes like *cyp19a*, coding for aromatase. However, given the failure of recent attempts to induce sex reversal through stress in amphibians and reptiles (Castelli et. al., 2021; Bókony et al., 2023), other mechanisms may be involved. Starvation treatments of medaka fry, *Oryzias latipes*, resulted in a 20% rate of female-to-male sex reversal (Sakae et al., 2020). Fry showed breakdowns in their pantothenate (vitamin B5) pathway, which includes the enzymes that facilitate fatty acid production. Recreation of a suppressed pantothenate pathway – by treating fed fry with a pantothenate kinase inhibitor – produced the same sex reversal effect. Fatty acids may be required to suppress the male pathway gene *Dmrt*. Cortisol is not expected to be involved in this sex reversal, as starved fry likely experience an impaired ability to synthesise steroid hormones from cholesterol (Sakae et al., 2020). However, it is worth noting that cholesterol deficiencies might also affect production of other steroid hormones, including androgens and estrogens.

Yolk mass during development also constitutes another variable resource. Radder et al. (2009) reported that embryos within *Bassiana duperreyi* eggs incubated under cold conditions (16°C ± 7.5°C) responded to ablation or inflation of yolk storage with sex reversal. Yolk ablation had a masculinising effect, while yolk inflation was feminising. Again, it is unclear what the mechanism behind these sex reversals is, particularly considering interactions with temperature, but it may involve the chemical composition of the yolk influencing sex, or the maternal allocation of sex-steroid hormones to eggs in an offspring sex-dependent manner. Evidence for interactions, such as the above, between resource availability and environment highlight the potential for complex sex reversal responses under multiple stressors. Whether resource/environment interactions represent adaptive sex selection, or simply developmental limitations under extreme conditions is difficult to determine, and this is a research area that would strongly benefit from further exploration.

## (2) Mutation-Based Sex reversal

*(a) Hybridisation*

Divergence in sex-determination genes between lineages can lead to a disruption of sexual development in hybrids when they resume contact. Sex reversal in hybrid offspring from closely related species or distinct lineages has been confirmed in hybrids of *Oryzias latipes x O. curvinotus* (Shinomiya et al., 2006; Kato et al., 2010; 2011) and *Mus musculus* (Bullejos & Koopman, 2005). In both species, at least one parent was of an inbred laboratory strain, and specifically XY hybrids experienced low to high rates of male-to-female sex reversal. Differences in the timing of sex-determining gene expression appear to be involved in both cases. Derived forms of the Y-linked male factors *Dmy* or *Sry*, in inbred strains of *O.latipes* and *M. musculus* respectively, appear not to be expressed in time to prevent germ cell meiosis. This leads to higher female-like germ cell levels and initiation of the female pathway (Bullejos & Koopman, 2005; Martinez-Bengochea et al., 2022). Possible evidence of further sex reversals through hybridisation are reported by Mair et al. (1991), with highly variable sex-ratios in crosses of *Oreochromis aureus* with several other *Oreochromis* species.

While hybridisation as a trigger of sex reversal is yet to be observed in nature, it might be expected as one of many genetic incompatibilities that build between two lineages developing reproductive isolation (Schumer et al., 2015). That hybridisation of intra-specific lineages – such as strains of laboratory mice – can result in sex reversal effects may be an artifact of the strain creation process; laboratory strains undergo intense founder effects, possess little genetic diversity, and are subject to unusual selection pressures (Wade & Daly, 2005). However, the above results still flag the potential for incompatibilities between sex determination genes to contribute to outbreeding depression. Intraspecific lineages with small, inbred or fragmented populations may be at risk of developing such incompatibilities, which would be especially detrimental if hybrids showed only incomplete sex reversal.

*(b) Genetic mutations*

Broadly, the most common cause of natural sex reversal across vertebrates is the spontaneous mutation of genes involved in sexual development. Such mutations occur at low rates in all vertebrate groups. In a laboratory setting, gene editing tools such as CRISPR (Doudna & Charpentier, 2014) or TALENs (Joung & Sander, 2013) can be used to recreate known mutations or explore the effects of targeted or novel mutations. Such genetic manipulation studies are commonly conducted in laboratory models, namely mice (*Mus musculus)*, and the fish *Oryzias latipes* and *Oreochromis niloticus* (24%, 14%, and 11% of 99 retrieved papers, respectively). Hormonal treatments have historically been the main method of sex control research and in aquaculture, but genetic tools are becoming increasingly accessible, and viable in more taxa, not just model species (Fig. 7b; Malik et al., 2026). While the use of either method on animals for human consumption can be controversial, genetic editing does not carry the same risk of environmental pollution as hormone treatments.

Mutations that cause sex reversal often do so through a loss-of-function in key male or female-promoting factors. Usually, these key factors are ‘primary sex determination genes’ which act upstream of all other genes to initiate either the male or female pathway (e.g., *Sry*; Warr et al., 2012). Such genes can occasionally be translocated to the opposite sex chromosome, or to autosomes, resulting in reversed individuals with a discordant karyotypic and phenotypic sex but who test positive for certain genes associated with their phenotypic sex (e.g., *Sry* positive XX male *M. musculus* or *Sus scrofa*; Shapiro et al, 1982; Parma et al., 2016). Yet sex-determination involves cascades of numerous genes (Fig. 5), many of which can cause sex reversal effects when their expression is modified (Furman et al., 2020).

Sex reversal mutations are commonly deleterious, sporadic, or confer infertility because reversed individuals lack the genetic factors usually facilitating successful reproduction in their phenotypic sex (Parma et al., 2016). However, fish and amphibians often possess homomorphic sex chromosomes with limited sex-specific regions (see Fig 1a). Both the hetero- and homogametic sex share much of the gene content of their sex chromosomes. Reversed individuals are thus less likely to lack key genes for viability and fertility in their new phenotype. In turn, fertile reversed individuals can pass on reversal mutations to their offspring, leading to an increased population persistence of such mutations relative to taxa with heteromorphic sex chromosomes (Otake et al., 2008; Williamson

& May, 2011; Palaiokastas et al., 2015). Background mutation-based sex reversals in the ricefish (*Oryzias latipes*) are near 1% (Shinomiya et al., 2004), while in some anurans 2-16% of wild individuals and may be sex-reversed due to genetic factors (although contribution from environmental sex reversals cannot always be ruled out; Lambert et al., 2019; Nemesházi et al., 2020; Bòkony et al. 2021; Yang et al., 2022). Founder effects, genetic drift or unintentional anthropogenic selection can also concentrate heritable sex reversal mutations, regardless of their deleterious nature. For example, accidental proliferation of certain alleles in aquaculture stocks of *Cynoglossus semilaevis* resulted in many undesired female-to-male sex reversals (Jiang & Li, 2017; Cui et al., 2018), while breeding for the desired hornless trait in domestic goats (*Capra hircus)* also increased instances of a linked partial sex reversal mutation (Polled intersex syndrome*;* Boulanger et al., 2008; 2014). Prevalence of sex reversal conditions in stock and companion animals is likely driven by both low genetic diversity and increased levels of detection, as captive individuals are commonly screened for irregularities (e.g., Nagler et al., 2001; Fraslin et al., 2020; Albarella et al., 2020; Bertho et al., 2022).

*i. Primary Sex-Determination Genes*

Many major sex determination genes have been identified due to their capacity to induce sex reversal when their expression is altered. Primary sex determination genes differ across taxa, with perhaps the best-known being the mammalian Y-linked factor, *Sry*. Mutations in *Sry* and its associated downstream factors, like SOX9, are repeatedly shown to cause male-to-female sex reversal in mice (*Mus musculus*; Vidal et al., 2001; Barrionuevo et al., 2006; Warr et al., 2012). Similar Y-linked *Sry* analogues have been identified through sex reversal studies in fish, including *Sdy* and *Dmy* in salmonids and *Oryzias latipes* (Matsuda et al., 2007; Guiguen et al., 2018). More conserved genes, such as the male-linked *Dmrt,* can also act as major sex determination factors. Mutations in the *Dmrt* gene are responsible for sex reversal effects in a variety of taxa, from zebrafish to chickens and both GSD and TSD turtles. Although *Dmrt* may be Y-linked, Z-linked (where it is presumably dosage-dependent), or unlinked to any sex-chromosome, up or downregulation of this gene maintains a strong influence over sex determination (Smith et al., 2009; Webster et al., 2017; Ge et al., 2018). The ovarian-determination gene *FOXL2* is also conserved among vertebrates. While inheritance of FOXL2 is not sex-linked, it displays a dimorphic gonadal expression in accordance with the activated sexual pathway (Wang et al., 2007; Bertho et al., 2016). Upregulation of *FOXL2* disrupts testis development in XY *M. musculus* (Ottolenghi et al., 2007), while loss-of-function in *FOXL2* downstream causes high testosterone and partial sex reversal in XX females (Heikkilä et al., 2005). Similar, occasionally complete sex reversals are recorded from *FOXL2* mutations in fish and turtles (Wang et al., 2007; Li et al., 2013; Yang et al., 2017; Ma et al., 2022).

While genetic manipulation allows a direct assessment of the genetic factors behind sex reversals, it is more difficult to assess the factors behind natural sex reversal mutations. A naturally occurring variant of the X chromosome in the African pygmy mouse (*Mus minutoides*) prevents masculinisation of XY individuals, producing fertile XY females, but the mechanism by which is does so is currently unknown (Saunders, 2014; Veyrunes, 2024). The pygmy mouse is one of several rodent species which appear to have deviated from the normal strict XY/XX sex determination system

of most mammals, by evolving a variant sex chromosome (Parma et al., 2016). Similar, but independently evolved systems are seen in grass mice of the genus *Akodon* (Ortiz et al., 2009; Sánchez et al., 2010), the lemmings *Dicrostonyx torquatus* and *Myopus schisticolor* (Bull & Bulmer, 1981; Fredga, 1988), and the vole *Lasiopodomys mandarinus* (Roy, 2023; Saunders & Veyrunes, 2021).

*ii. Genes Encoding Hormone Production/Reception*

Genes encoding for hormone production or reception may also be exploited to reverse sex. Overexpression of the androgen receptor gene *AR* increases binding of androgens to cause partial female-to-male sex reversal in *Glandirana rugosa* (Fujii et al., 2014). Anti-müllerian hormone, coded for by the gene *amh*, is important for male development, supressing female reproductive tract development. Its influence is such that Y-specific *amh* alleles appear to be primary sex-determinants in some fish (Miyoshi et al., 2020; Nakamoto et al., 2021; Hattori et al., 2022). In other fish species, and in the turtle *Pelodiscus sinensis*, loss-of-function *amh* mutations have been shown to cause male-to-female sex reversal (Morinaga et al., 2007; Zhou et al., 2019; Liu et al., 2022). Equally important is the highly conserved vertebrate gene *Cyp19a1*. This gene codes for aromatase, the enzyme responsible for converting androgens to estrogens. Manipulation of this gene thus effects both androgen and estrogen concentrations, often resulting in sex reversal (e.g. Lambeth et al., 2013; Zhang et al., 2017; Yang et al., 2021; Shen et al., 2023). Control of *Cyp191a* expression is incredibly important in sexual development, and many masculinising edits to genetic factors upstream in the female developmental pathway seem to ultimately act by supressing *Cyp19a1* expression (Rodríguez-Marí et al., 2010; Liu et al., 2022).

*iii. Genes Regulating Germ Cell Proliferation*

Genetic mutations can also arise that directly affect germ cell numbers, a mechanism increasingly implicated in various sex reversals (Hara et al., 2021; Mukai et al., 2022). It is clear that, at least in fish, normal vertebrate sexual development results in higher numbers of germ cells in females compared to males (Nishimura et al., 2018; Mustapha et al., 2021; Pan et al., 2022). Female *Danio rerio fancl* gene mutants experience increased germ cell apoptosis (programmed cell death), and undergo sex reversal to become males, in line with their lowered germ cell number (Rodríguez-Marí et al., 2010). Another *D. rerio* mutant, with a homozygous knockout of the *lgf* gene, also shows female-to-male sex reversal associated with repression of germ cell proliferation (Xie et al., 2020). When engineered to lack germ cells entirely, females of both *D. rerio* and *Oryzias latipes* experienced a partial sex reversal to male (Kurokawa et al., 2007; Siegfried & Nüsslein-Volhard, 2008). In a similar vein, hotei-strain mutants of *O. latipes* with increased germ cell proliferation underwent sex reversal to female, although in this case *amh* signalling also appears to play a role (Morinaga et al., 2007; Nakamura et al., 2012).

## (3) Hormonal sex reversal

*(a) Exogenous Hormones*

Application of exogenous hormones has historically been the main method employed in aquaculture to maintain desired stock sex-ratios and is common in the study of sexual development. Of 589 retrieved articles in this review, 236 (40%) employed exogenous hormones. Sex-steroid hormone function is remarkably conserved, and at least partial feminisation or masculinisation has been reported to result from their application in all vertebrate groups. Sex reversal in this case is often achieved with synthetic versions of main vertebrate androgens and estrogens, which are often more effective at inducing sex reversal than their naturally occurring counterparts. However, this approach can carry extra risks in terms of environmental pollution and side effects on growth and mortality (Gorshkov et al., 2007; Ramírez et al., 2015; Asad et al., 2024). Developing embryos or larvae can be exposed to hormones through immersion in a solution (Kirankumar & Pandian, 2002), feeding of a hormone-supplemented diet (Baron et al., 2007), or via direct injections (Zhou et al 2021). Historically, gonadal transplants have also been used to induce sex reversal in developing individuals, although the effects are inconsistent and involve complex interactions with other signalling factors, besides sex steroids, that are produced by the donor gonads (Mikamo and Witsch 1963; 1964; Maraud et al., 1990).

Estrogens support a female developmental pathway, while androgen application tends to support a male pathway. Ambiguity arises where application of androgens can result in 'paradoxical feminisation', as the applied supplementary androgens are converted into estrogens by aromatase (Fig. 5). Paradoxical feminisation is seen in both fish and amphibians (Bruner & Witschi, 1954; Piferrer & Donaldson, 1991; Kawahara & Yamashita, 2000; Ramee et al., 2020). Occasionally, aromatisation may simply lead to reduced efficacy of a masculinisation treatment (Phelps & Okoko., 2011). Paradoxical feminisation can be avoided with the use of non-aromatisable androgens, or hormone antagonists rather than hormones themselves (e.g. Piferrer & Donaldson, 1991; Iwamatsu et al., 2006; Dumond et al., 2008). Overall, administration of additional hormones during development is very effective at influencing the gonads in either direction. However, as in other triggers, there is much taxonomic variation in the magnitude and functionality of the sex reversal response.

*i.* *Fish*

Sex-steroid hormone application is a tool used primarily and successfully in fish (comprising 171 (72%) of the 236 hormone manipulation studies retrieved in this review). As such, this specific manner of sex reversal has been extensively reviewed elsewhere (Pandian and Sheeler, 1995; Piferrer & Lim, 1997; Piferrer, 2001; Senior et al., 2012; 2016; Senior & Nakagawa, 2013; Wang & Shen, 2018; Li et al., 2022). Recently, concerns have been raised about the effect of these hormonal aquaculture treatments on both environmental and human health. Remnant hormones can potentially persist in waste runoff, and in fish used for human consumption, exposing natural populations and consumers to endocrine-disrupting factors. There is, therefore, a push for sex reversal methods that limit hormone use (Hoga et al., 2018; Malik et al., 2026). One strategy is to pair hormonally sex-reversed animals over several generations until genotype/phenotype pairings are reached that produce monosex offspring without further hormone treatments (e.g., an XY x YY pairing; Varadaraj & Pandian, 1989; Voorhees et al., 2023). This strategy requires the production of viable YY or WW

individuals, which is not possible for many species with degenerated Y or W sex-chromosomes (George et al., 1994; George & Pandian, 1996; Liu et al., 2013; Jiang et al., 2018). Sex-reversed individuals who produce sex-skewed or inviable offspring are of use, however, when attempting to control invasive species, as they reduce effective population size (Schill et al., 2016; Jiang et al., 2018; Norazmi-Lokman et al., 2021; Teal et al., 2023; Vorhees et al., 2023). Conversely, when WW individuals are viable, they can be used to boost reproduction in struggling species. Higher ratios of females to males in most species increases effective population size, as a larger proportion of individuals are available to reproduce (Cotton & Wedekind, 2007).

*ii. Amphibians*

Amphibians are model taxa for understanding the effects of hormones, hormone antagonists and other endocrine-disruptors, due to their accessible larval stage and sensitivity to waterborne disturbances. However, sensitivity to hormonal effects varies strongly across amphibians (Piprek et al., 2012a; 2012b; Xu et al., 2015). This is important; understanding a species' baseline response to known androgen/estrogen concentrations informs future tests of potential endocrine disruptors (Saidapur et al., 2001). Early experiments recorded masculinisation of *Rana dalmatina* tadpoles with testosterones (Vannini & Stagni, 1967; 1968), masculinisation and feminisation in hormone-treated larvae of *Pleurodeles watl* (Bruner & Witschi, 1954; Dumond et al., 2008), and successful masculinisation with testosterone, but not feminisation with estrogen, in *Rana clamitans* (Foote and Witschi; 1939). In more recent times, *Xenopus laevis* has become the model of choice for hormonal treatment tests (Oka et al., 2006; Lutz et al., 2008; Piprek et al., 2012a; 2012b; Li et al, 2020). As with temperature, hormonal sex reversal efficacy in amphibians is expected to vary with the developmental stage of the individual at the time of application (Villalpando & Mechant-Larios, 1990). Exposure method may also play a role in treatment success. Studies of *Glandirana rugosa* recorded successful masculinisation with testosterone injections (Nakamura et al., 2015; Sakamoto et al., 2016), whereas rearing female tadpoles in testosterone-spiked water led only to the development of ovotestes as adults (Oike et al., 2016).

*iii. Reptiles*

Reptile eggs may also be manipulated through hormone treatments. The most common use of hormone treatments in reptiles is to test hypotheses of the evolution and mechanisms of temperature-dependent sex-determination (TSD). Estradiol application overrides male-producing temperatures in TSD turtles (Bull et al., 1988; Rhen et al., 1996; Freedberg et al., 2006a; 2006b; Mork & Capel, 2013; Wu et al., 2022), crocodilians (Stoker et al., 2003; Rogerson et al., 2023), and geckos (Bull et al., 1988). Overriding TSD allows investigation of hypothesis of the adaptive link between sex and developmental temperature in TSD species, although it can be difficult to separate any effects of hormone treatments themselves on traits such as sex-biased survival and morphology (Bull et al., 1988). Reptiles with GSD also generally respond to estradiol treatment with complete male-to-female sex reversal (Radder & Shine, 2007; Ehl et al., 2017) although the lizard *Calotes versicolor* has both an uncertain sex determination system and an apparent resistance to estradiol treatments (Ganesh &

Raman, 1995). Beyond testing sex-environment interactions, estrogenic treatments in the softshell turtle *Pelodiscus sinensis* have recently been used in an attempt to create ZZ females, which can then be mated with ZZ males to maximise monosex stocks of fast-growing males for human consumption, just as has been done in fish (Zhou et al., 2021; Li et al., 2022; Wang Y. et al., 2022).

*iv. Birds and Mammals*

In contrast to other vertebrate groups, hormonal sex reversal in birds seems to always be partial, or in the case of feminised chickens (*Gallus gallus*), transient (Zhang et al., 2023a; 2023b). Although estradiol-treated chicks show strongly feminised phenotypes at hatching, male genetic factors persist and force a reversion back to a male phenotype after hatching (Shioda et al., 2021). In birds, hormone treatment resistance may be related to the notion of cell autonomous sex identity, where sex chromosomes appear to maintain a sexual identity in each individual cell regardless of hormone exposure (Zhang et al., 2023a, but see Hall et al., 2025). Mammals show similarly limited effects from hormonal treatments, although in-utero exposure to androgens can result in partially masculinised gonads and behaviour (Roberts et al., 2008). Resistance to estrogenic effects in mammals may have evolved to prevent feminisation of embryos in the womb by maternal estrogens (Capel, 2017). In marsupials, where uniquely among mammals gonad development can occur after birth, exogenous hormones are slightly more effective (but still do not cause complete sex reversal; Chew & Renfree, 2016). Nonetheless, important hormonal drivers of sexual phenotype in mammals have been identified through hormonal treatment of wallaby (*Notamacropus eugenii*) and opossum (*Monodelphis domestica*) joeys (Fadem & Tesoriero, 1986; Coveney et al., 2001; Leihy et al., 2002; Calatayud et al., 2010; Pask et al., 2010).

*(b) Hormone Antagonists*

Hormone antagonists either prevent hormone production, or hormone reception. Hormone antagonists can be more effective — causing fewer intersex individuals and higher rates of males — than androgen application in some taxa (e.g. *Tachysurus fulvidraco;* Shen et al, 2015), as they circumvent the issue of paradoxical feminisation (Piferrer & Donaldson, 1991). Masculinising anti-estrogens are most commonly used for intentional sex reversal. As endogenous androgens are precursors to endogenous estrogens, anti-androgens, such as flutamide, are often ineffective or less effective compared to estrogen treatments (Chadard et al, 2003; Filby et al., 2007; Navarro-Martín et al., 2009). However, they may be used to increase the efficacy of estrogen treatments (Jiang et al., 2018). Hormone antagonists may be administered to developing organisms through injections (Hu et al., 2019), diet supplementation (Joshi et al., 2017), topical application (Warner & Shine, 2005), or immersion in solution (Katare et al., 2015). In exposed individuals, high doses or certain compounds can cause detrimental effects on survival (Chadard & Dournan, 1999), or negatively effect morphological traits such as size or limb development (Katare et al., 2015).

By far the frequently employed hormone antagonists are aromatase inhibitors (AIs). Aromatase inhibitors block the aromatase enzyme's conversion of androgens into estrogens, increasing androgen levels and causing masculinisation. The non-steroidal AIs letrozole and

fadrozole reversibly bind to aromatase to block its function, producing at least partial masculinisation in fish (Kitano et. al, 2000; Bertolla et al., 2001; Gao et al., 2010), reptiles (Dorizzi et al., 1994; Rhen et al., 1996; Warner & Shine, 2005), amphibians (Chadard & Dournan, 1999), and birds (Hu et al., 2019). As with hormones, treatments are most effective in fish and amphibians (but see Zerulla et al., 2002), show mixed success in reptiles (Richard-Mercier et al., 1995; Jan et al., 2017), and only partial or non-functional sex reversal in birds (Mohammadrezaei et al., 2014; Hu et al., 2019). Unlike estrogen feminisation, non-functional sex reversal through AIs in chickens (*Gallus gallus*) will persist throughout the individual's life (Vaillant et al., 2001; Yang et al., 2008; 2011; Mohammadrezaei et al., 2014). Steroidal AIs, such as exemestane, which irreversibly bind to aromatase, are less commonly used but no less effective (Yu et al., 1993; Guiguen et al., 1999; Vizziano et al., 2008; Betancur López et al., 2014).

Tamoxifen is a modulator of estrogen receptors, commonly acting as a hormone antagonist by decreasing estrogen reception. Tamoxifen's masculinisation efficacy is moderate in comparison to AIs and comparatively higher doses may be required to see sex reversal effects (Guiguen et al.,1999; Singh & Srivastava, 2014). In teleost fish, it commonly causes masculinisation (Park et al., 2004; Kitano et al., 2007; Chakraborty, 2012; Singh & Srivastava, 2014), although it also been recorded to increase estrogen reception and cause feminisation in *Dicentrarchus labrax* (Navarro-Martín et al., 2009). In other taxa, high doses of tamoxifen have been recorded to masculinise embryonic gonads of female chickens (*Gallus gallus;* Trukhina et al., 2014) and mice (*Mus musculus;* Yu et al., 2014), as well as *Emys orbicularis* hatchlings incubated at otherwise female-producing temperatures (Dorizzi et al., 1994).

*(c) Endocrine-Disrupting-Chemicals*

The term 'endocrine-disrupting chemical' (EDC) covers a wide range of factors, grouped due to their potential to disrupt hormonal processes in exposed organisms (Diamanti-Kandarakis et al., 2009; Betram et al., 2022). There is considerable overlap between EDCs and compounds used to intentionally instigate sex reversal, including synthetic estrogens and androgens, and hormone antagonists like letrozole and tamoxifen, which have been discussed above. Disrupting chemicals are found in medications, manufacturing waste, stock effluent, and agricultural runoff, but are also present naturally in some plant material (Mukherjee et al., 2023). The study of endocrine disruptors is a broad multidisciplinary field, and providing a complete description of their effects is beyond the scope of this review. A brief overview of sex reversals through EDCs are presented below, but further reviews may be found elsewhere, providing specific information on EDC disrupting mechanisms (McLachlan, 2001), agrochemical EDCs (Hayes et al., 2011; Trudeau et al., 2020), exposure consequences in fish (e.g. Senior et al. 2012; Bhattacharya & Munshi, 2021; Dasmahapatra et al., 2023), and in aquatic environments in general (Matthiessen & Sumpter, 1998).

Natural populations are usually exposed to EDCs in relatively low concentrations, and those EDCs not specifically synthesised to be hormone substitutes or hormone antagonists also tend to induce only partial sex reversal effects. Despite this, EDCs cause significant negative effects in wild individuals. They promote the development of intersex phenotypes, reduce fertility, and result in

abnormal secondary sex-characteristics (Jobling et al., 2002). Many act as weakly androgenic or estrogenic compounds ('hormone mimics') after binding to an individual's hormone receptors. Famous estrogenic EDCs include those in the nonylphenol group, and the notorious bisphenol A, or BPA, which cause partial feminisation in fish (Barnhoorn et al., 2004; Horie et al., 2020; 2021), amphibians (Mackenzie et al., 2003; Levy et al., 2004; Man et al., 2020), crocodilians and testudines (Stoker et al., 2003; Manshack et al., 2017), and birds (Oshima et al., 2012). EDCs may alternatively bind to hormone receptors without causing estrogenic or androgenic effects, blocking the function of the receptor. The insecticide dichlorodiphenyltrichloroethane (DDT) causes feminisation when its metabolite DDE binds to and blocks androgen receptors (Bayley et al., 2002). As with non-phenols and BPA, it is known to produce partial sex reversal effects in most vertebrate taxa (Fry & Toone, 1981; Willingham & Crews, 1999; Edmunds et al., 2000; Bayley et al., 2002; Kuhl & Brouwer, 2006). A further group of EDCs act as hormone antagonists. Formerly use in boat paint to prevent biofouling, the compound tributyltin acts as an aromatase inhibitor. Exposure to tributyltin can be strongly masculinising in teleost fish (Shimasaki et al., 2003; Kuhl & Brouwer, 2006).

Actions of endocrine-disrupting chemicals in natural populations usually involve 'cocktails' of potential disruptors. These complex cases can involve the interaction of antagonistic estrogenic and androgenic effects (Kuhl & Brouwer, 2006), and/or increased overall exposure to disruptors of one kind (Howell et al., 1980; Jarque et al., 2015). Weakly disrupting compounds may cause significant sex reversal effects when applied in concert (e.g. Willingham & Crews, 1999). Conversely, combinations of EDCs with opposing effects rarely cancel each other out. Instead, sex reversal effects proceed in the direction of the stronger compound, or in the direction a taxon is more sensitive to. Feminisation appears to be the most common result of exposure to EDC cocktails (Kuhl & Brouwer, 2006; Soares et al., 2018). Accumulation of EDCs in prey species is also a concern. *Silurus meridionalis* fry experienced complete male-to-female sex reversal when fed annelid worms from a stream contaminated with various EDCs, including BPA and 4-nonylphenol (Dong et al., 2014), and a diet of artemia raised in tamoxifen solution was sufficient to induce a 90% male sex-ratio in *Tachysurus fulvidraco* fry (Park et al., 2004). Even with low contamination levels, bioaccumulation may lead to sex reversal effects in larger predator species (Dong et al., 2014; Ruhí et al., 2016; Lagesson et al., 2016). While there is experimental evidence of EDC effects reversing upon removal of the pollutant (Fenske et al., 2005; Paul-Prasanth et al., 2011; Baumann et al., 2014; Hu et al., 2019), the ability of wild individuals to recover normal development after exposure is likely extremely limited. Exposure lasting past the conclusion of sexual development, chronic exposure, or compounds with masculinising effects are highly unlikely to be reversible (Baumann et al., 2014).

As noted above, concerns are growing about the impact of aquaculture sex reversal treatments on consumers and the environment, and thus several studies have focused on finding 'natural alternatives' to conventional synthetic hormone treatments (Stadtlander et al., 2013; Malik et al., 2026). Weak endocrine-disrupting compounds can be found naturally in plant material, particularly plant saponins which may have aromatase-inhibiting effects. Saponins from *Trigonella foenum-graecum* and *Quillaja sp.* did not affect the sex-ratios or gonadal development of *Oreochromis niloticus* fry (Stadtlander et al., 2013), but those of *Tribulus terrestris* did induce male-skewed sex-

ratios in *O. niloticus, Cichlasoma nigrofasciatum,* and *Poecillia reticulata* (Çek et al., 2007a; 2007b; Ghosal et al., 2020; Mukherjee et al., 2023). Extracts from *Basella alba*, *Asparagus racemosus*, and *Mucuna prurien* were also successful at producing high levels of males in *O. niloticus* (Ghosal et al., 2020; Mukherjee et al., 2023). While masculinisation treatments are most highly sought-after, research has also highlighted the ability of phytoestrogens, such as those found in soybean meal (*Glycine max*), to reduce masculinisation from applied hormones, making it an unfavourable feed for stocks undergoing such treatments (El-Sayed et al., 2012).

## V. Is sex reversal adaptive?

### (1) Most sex reversals are not adaptive

Sex reversal typically reduces fitness because most cases are incomplete. Incomplete sex reversals create mechanical or physiological barriers to successful reproduction, including intersex morphology, improper development of primary or secondary sexual characteristics, and the production of poor quality or malformed gametes (Fig. 8, Endpoint 1; Dorizzi et al., 1994; Kim et al., 1997; Bayley et al., 2002; Jobling et al., 2002). Prior to reproduction, sex-reversed individuals with novel combinations of phenotypic, physiological, or behavioural traits may fail to recognise, or be recognised by, appropriate mates (Balasubramani & Pandian, 2008; Larsen et al., 2009; Weber & Leeds, 2022). Consequently, most spontaneous sex reversals represent a maladaptive deviation from typical gonochoristic function (Parma et al., 2016; Weber & Capel., 2018). Many such cases do not even arise under natural conditions; including all hormonally induced sex reversals, which have only recently become common due to anthropogenic influences.

Yet some sex reversals are complete and reproductively functional, or arise in response to commonly encountered natural conditions such as extreme temperatures, stress, resource availability, or genetic mutations. In certain systems, 1–16% of individuals within a population may be sex reversed (see Table 3., Shinomiya et al., 2004; Lambert et al., 2019). While sex reversal in these systems may be adaptive – as discussed in detail below – reversal rates can remain relatively high even when neutral or disadvantageous. This may simply reflect intrinsic constraints of vertebrate sexual development under suboptimal conditions. Even maladaptive sex reversals may persist when selection against them would require broad, and likely deleterious, changes to sexual development as a whole (Wright, 1932; Somjee et al., 2025).

### (2) Hypotheses for adaptive environmental sex reversal

Environmental sex reversal provides a mechanistic link between sex determination and environment. Selection can thus favour the production of whichever sex achieves higher fitness under current conditions, as predicted under the Charnov-Bull model of environmental sex determination (Charnov & Bull, 1977). While the Charnov-Bull model was developed to explain temperature–sex ratio relationships in TSD species, it can also be applied to environmental sex reversal – especially considering sex reversal may represent a transitional stage between GSD and pure ESD (Holleley et al., 2015). Sex-specific fitness differences may arise from direct mortality under extreme conditions (Eiby et al., 2008), or indirectly through environmental effects on size at hatch or birth, timing and

seasonality of emergence, resource availability, and subsequent growth rates (Noble et al., 2018). These factors impact male and female fitness differently where the sexes have different size, age, or body condition thresholds for reproduction. For example, whichever sex must reach a larger size in order to begin breeding will benefit from being produced at optimum conditions (often females; Schwanz et al., 2016). Alternatively, when reproductive success in one sex is more strongly linked to body condition, then that sex should be produced at the optimal development conditions (often males; Trivers and Willard, 1973; Lawson & Rollinson, 2021). Because sex reversal often occurs at environmental extremes, which presumably reflect suboptimal conditions, sex reversal may enable individuals to mitigate the fitness costs of developing under such conditions, by switching to whichever sex requires less investment for successful reproduction.

Exact sex-by-temperature interactions vary among taxa – both in which sex benefits most from optimum conditions and in which environmental variables are most important – but several broad patterns are apparent. In nature, fish and amphibians show a strong pattern of complete female-to-male reversal under extreme or stress-inducing conditions (Dorazi et al., 1995; Geffroy & Douhard, 2019; Ramee et al., 2020; Yu et al., 2022; Bókony et al., 2023). Comparable patterns occur in reptiles, where low-temperature masculinisation occurs at temperatures close to the lower thermal limit of embryos (Dissanayake et al., 2021a; Hill et al., 2022). Such stress-induced masculinisation may arise from conserved mechanisms acting on homologous sex-determination pathways (although see Bókony et al., 2023). Yet, if adaptive, this could indicate a general tendency for male advantage over females under suboptimal conditions (Geffroy & Douhard, 2019). Despite this trend, male-to-female reversals under extreme temperatures in the central bearded dragon (*Pogona vitticeps*), the dwarf gourami (*Trichogaster lalius),* and some salamander species demonstrate that the direction of sex reversal can vary across systems (Dorazi et al., 1995; Sakata et al., 2005; Quinn et al., 2007; Ramee et al., 2020).

Alternatively, sex reversal may be a mechanism that exploits frequency-dependent selection. Where sex ratios fluctuate from the evolutionarily stable 1:1, the relative fitness of each sex shifts in accordance with available reproductive opportunities, with the rarer sex generally gaining a fitness advantage through increased access to mates (Fisher, 1930). Sex reversal could allow individuals to capitalise on skewed sex ratios, provided reversal produces the rarer sex and is triggered by the same environmental conditions that generate skewed sex ratios. While complex, there is some precedent for this hypothesis. For example, Dupoué et al. (2019) found that water restriction during pregnancy in *Zootica vivipara* and *Vipera aspis* resulted in male-skewed offspring sex ratios that could not be explained by sex-specific mortality – indicating sex reversal. The authors also noted that, in the long term, male offspring condition under ‘drought conditions’ is poorer than that of female offspring. Thus, it is possible that mothers under drought conditions overproduce males that then benefit from frequency-dependent following male-biased juvenile mortality.

### (3) Sex reversal and frequency-dependent selection

While the above scenario focuses on environmentally mediated opportunities, frequency-dependent selection is also expected to shape the longer-term evolutionary consequences of sex reversal arising

from both environmental and mutational triggers (Schwanz et al., 2020). Sex-reversed individuals might gain a fitness benefit when populations temporarily exhibit uneven sex ratios, potentially maintaining low levels of sex reversal in some populations. However, as the rarer sex increases in frequency, its advantage diminishes, and populations move back towards the evolutionarily stable 1:1 sex ratio (Fisher, 1930). This feedback can constrain the spread of sex reversal, particularly when unidirectional sex reversal inflates one phenotypic sex at the expense of the other. Otherwise, discordant phenotypes that are selectively neutral can be rapidly eliminated by frequency-dependent selection – especially if there is fast generation turnover, where population sex ratios closely track those of the most recent generation (Fig. 8, Endpoint 2; Bull & Bulmer, 1989; Schwanz and Proulx, 2008; Saunders et al., 2014).

Nevertheless, frequency dependence can also amplify sex reversal. Very high reversal rates can occur when discordant phenotype have high fitness, when populations experience genetic bottlenecks or drift, or when exposure to environmental reversal triggers increases. Sudden increases in sex reversal can skew both phenotypic and genetic sex ratios because reversed individuals usually pair with partners of the same genetic sex (Schwanz et al., 2020; Hill, 2022). This can, in turn, foster selection favouring further sex reversal to balance sex ratios. For example, ZZ x ZZ pairings produce only ZZ offspring, which would all be male in the absence of further sex reversal. This scenario is not expected to be evolutionarily stable. The resulting loss of sex chromosomes (e.g. the female-promoting W) could theoretically lead to increased sex reversal until populations transition to non-chromosomal sex determination systems – such as TSD or polygenic systems – or the development of neo sex-chromosomes (including transitions from XY to ZW and vice versa; Holleley et al., 2015; Schwanz et al., 2020; Fig. 8, Endpoint 3). There is some empirical support for this, with laboratory-strain zebrafish having undergone a transition from ZW to polygenic sex-determination, potentially due to increased sex reversals driven by frequent pairings between related or discordant individuals (Wilson et al., 2014). Furthermore, sex reversal may contribute to the frequent historical transitions among sex-determination modes in various fish (Wang et al., 2024), amphibians (Ma & Veltsos, 2021), and reptiles (Gamble, 2010). Such transitions are much less common in mammals and birds, taxa in which successful sex reversal is itself rare (van Doorn, 2014).

## (4) Hypotheses for adaptive mutation-based sex reversal

Aside from frequency-dependent processes, mutation-based sex reversal can introduce flexibility into otherwise conventional GSD systems. The homomorphic sex chromosomes of fish, amphibians, and reptiles may be maintained by low but consistent background sex reversal rates that allow recombination among chromosomes in X/Y or Z/W chromosomes (‘fountain of youth’ hypothesis; Perrin, 2009; Rodrigues et al., 2018). Such recombination may prevent a potential ‘evolutionary trap’ facing mammals, in which progressive degeneration and eventual loss of the non-recombing Y chromosome necessitates the evolution of new male-determining mechanisms (Graves, 2006; Terao et al., 2022). Consequently, homomorphic sex chromosomes impose fewer barriers to the reproductive success of reversed individuals, allowing sex reversal – or a propensity for reversal, including environmentally induced reversal – to be passed on to offspring (van Doorn, 2014; Bókony

et al., 2023). As noted above, families that produce the rarer sex via sex reversal may initially benefit from frequency-dependent selection, although the same process is ultimately expected to constrain levels of sex reversal in the long-term.

The potential advantages of mutation-based sex reversal discussed above are either long-term and dependent on population trends (frequency-dependent selection) or else linked with adaptive environmental sex reversal. Can the discordant phenotype itself confer a direct advantage? There is evidence that the novel combination of genotypic and phenotypic sex in XY female African pygmy mice (*Mus minutoides*) confers higher fitness than in XX females. Female mice with XY genotypes show an increased ovulation rate, larger litters, earlier first litters, and a higher breeding chance compared to other female genotypes (Saunders et al., 2014). However, XY females build poor-quality nests and display heightened aggression, attacking male partners who would otherwise assist in parental care (Saunders et al., 2016; Heitzmann et al., 2023). How such laboratory-based observations of sex reversed fitness translate into population dynamics in the wild is still unclear (Wild et al., 2022; Veyrunes et al., 2024). Observing wild sex-reversed individuals is difficult, and many such individuals are recognised only after becoming specimens (Castelli et al., 2021; Wild et al., 2022; Hall et al., 2025).

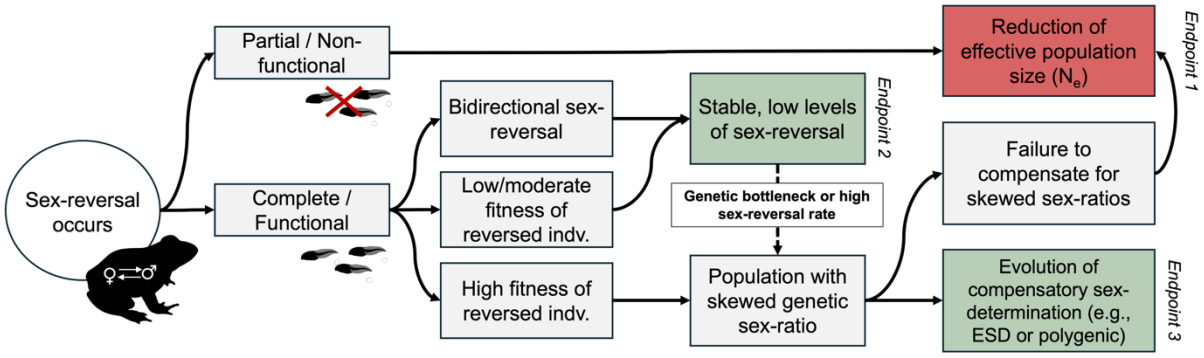



**Fig. 8. Potential effects of sex reversal occurring in a natural population with GSD.** The population-level consequences of sex reversal depend primarily on the reproductive ability of reversed individuals. Reversed individuals that cannot produce offspring reduce effective population size ($N_e$; *Endpoint 1*). When reversed individuals are reproductively functional, however, they produce offspring with skewed genetic sex ratios because their mates share their genetic sex. In most systems, such effects do not scale to the population level, either because sex reversal is rare or because reversed individuals have no fitness advantage (*Endpoint 2*). However, in populations where reversed individuals have elevated fitness or where sex reversal rates are very high (e.g., under widespread exposure to sex reversal triggers), populations can show skewed genetic sex-ratios. This is expected to reduce effective population size ($N_e$) as phenotypic sex ratios deviate from the evolutionarily stable 1:1 ratio – although, exceptions exist (see Cotton & Wedekind, 2007). Populations can nevertheless evolve to compensate for skewed genetic sex ratios by transitioning to ESD or by developing alternative genetic sex-determination systems, thereby restoring balanced sex-ratios (*Endpoint 3*).

## (5) Understanding fitness consequences of sex reversal

There is a clear need to evaluate the fitness of sex-reversed individuals to understand whether sex reversal is adaptive, neutral, or maladaptive, and under what circumstances each outcome arises. However, two major obstacles hinder our understanding.

First, fitness measured under laboratory conditions may not reflect outcomes in the wild. Sex reversal can influence mating receptivity, mate recognition, mate choice, mate competition, and parental care (Wild et al., 2022; Dussene et al., 2022; Heitzmann et al., 2023; Veyrunes et al., 2024). These effects can be obscured in captivity, where individuals are kept under ideal conditions, at unnaturally high densities, or – particularly for aquaculture species – bred through assisted reproductive technologies. While predictive population models exist for a few systems (Schwanz et al., 2016; Dissanayake et al., 2021b), these typically assume equal fitness between discordant and concordant individuals, an assumption that may often be violated (Cotton & Wedekind, 2009; Wedekind, 2017).

The second obstacle to understanding the fitness consequences of sex reversal is that sex reversal triggers themselves can strongly affect fitness. In *Rana dalmatina*, XX males resulting from mutation-based reversals exhibit unusual early-life traits, such as decoupling of development time and mass at metamorphosis, but none with clear negative or positive fitness effects (Bókony et al., 2023). By contrast, high-temperature masculinisation in this species is associated with low survival, reduced body condition, and presumably reduced fitness (Nemesházi et al., 2020; Mikó et al., 2021; Ujszegi et al., 2022; Bókony et al., 2023). Similar patterns occur in the yellow catfish (*Tachysurus fulvidraco*), where XX males reversed under naturally occurring high temperatures show poor fertility and increased mortality under multiple environmental stressors (Yu et al., 2022). In such cases, the discordant phenotype itself may not be inherently detrimental; rather, the developmental stress associated with the reversal trigger – such as extreme temperatures – may exaggerate or generate negative fitness effects (Bókony et al., 2023).

## VI. Sex Reversal in the Anthropocene

### (1) Anthropogenic Influences on sex reversal

As human populations grow and expand, so does the extent of anthropogenic influence on natural environments. In a sex reversal context, anthropogenic change has directly led to global increases in two reversal triggers; endocrine-disrupting-compounds (EDCs), and high developmental temperatures (Fig 9a; Wedekind, 2017). Natural systems have already seen severe consequences from exposure to EDCs; several notorious cases of sexual dysfunction in wild populations over the past few decades have been traced back to antibiological agents (pesticides, herbicides and antifouling agents; Fry and Toone, 1981; Shimasaki et al., 2003). Awareness of EDC risk has increased over time; certain compounds are now banned or tightly regulated (Godfray et al., 2019). Yet due to trade-offs with human health outcomes (e.g. use of DDT to control disease vector mosquitos; van den Berg, 2009), equally harmful alternatives and illegal usage (Godfray et al., 2019), the hazards remain. Waste material from manufacturing or sewage treatment containing endocrine disruptors (e.g. nonylphenols) has also repeatedly been implicated as the cause of skewed sex-ratios or intersex phenotypes in freshwater fish, and sexual disfunction in aquatic reptiles (Howell et al., 1980; Crain and Guillette, 1998; Dong et al., 2014; Pierron et al., 2021). Environmental contamination from synthetic hormones applied to livestock or present in pharmaceutical medications are also a concern (Hua et al., 2015; Leet et al., 2020). Synthetic hormones display increased sexual disruption efficacy in comparison with

other EDCs, as they are specifically intended to mimic natural vertebrate hormones (Aris et al., 2014). EDCs accumulate both in the environment (e.g. soil and water), and in prey items (Fry and Toone, 1981; Martin-Robichaud et al., 1994; Park et al., 2004; Dong et al., 2014). They may also be transferred across generations, with maternal exposure or contaminant loads passed on to offspring (Edmunds et al., 2000; Zhang et al. 2024).

Yet another challenge of the Anthropocene is anthropogenic climate change. Global temperatures are climbing, and extreme weather events are increasingly common (Dahlke et al., 2020; Jørgensen et al., 2022; Smith et al., 2023). Temperature increases have direct implications for environmental sex reversal systems. Fish and amphibians consistently show temperature-triggered sex reversal, mainly in the form of high-temperature masculinisation (Cristina et al., 2013; Flament, 2016). Thus, with global warming, sex reversal rates are expected to increase in these taxa (Honeycutt et al., 2019; Dussenne et al., 2020; Castañeda-Cortés et al., 2020; Yu et al., 2022). Conversely, for a handful of species with low-temperature sex reversal, reversal rates are predicted to decrease (Dissanayake et al., 2021a). While a number of both theoretical and experimental studies have begun to explore the outcomes of increased sex reversal through high temperatures (Schwanz et al. 2016; Lambert et al., 2018; Mikó et al 2021; Dussenne et al., 2022; Yu et al 2022), we know little about how reduction of sex reversal may affect natural population dynamics, if at all (Telemeco et al., 2009; Dissanyake et al., 2021; Hill et al., 2022).

### (2) Conflicting pathways and endocrine-disrupting chemicals

One pertinent question in the face of increasing EDC and high-temperature sex reversals is how these triggers will interact. Male and female developmental pathways are sustained through a complex balancing of factors – upregulation of factors associated with the initiated pathway, and suppression of those associated with the alternative pathway (Capel, 2017). The combined effects of high temperatures and EDCs likely depend on their directionality (e.g., masculinising or feminising), their disruptive ‘power’, and where in the developmental pathway they act (Fig. 9b). Empirical data is limited; estrogen treatments have been used to successfully prevent high temperature masculinisation in the fish fish *Paralichthys olivaceous*, *Dicentrarchus labrax* and *Oryzias latipes*, (Kitano et al., 2012; Yamaguchi et al., 2010; Días and Piferrer, 2017), but environmentally-relevant concentrations of estrogen treatments failed to prevent high temperature masculinisation of *Rana dalmatina* tadpoles (Mikó et al., 2021). There is likely both taxonomic variation in responses, and high threshold concentrations of EDCs needed to overcome high temperature masculinisation. The effects of exposure to multiple or contradictory EDCs are similarly variable and difficult to predict (Willingham & Crews, 1999; Kuhl & Brouwer, 2006). Additionally, while less well studied, we know that sex reversals can occur due to stressful environments beyond just high temperatures (García-Cruz et al., 2020), and that factors such as resource restriction and oxidative stress can also disrupt sexual development (Sakae et al., 2020; Mukai et al., 2022). This is an area that would greatly benefit from further experimental studies and direct comparisons between taxa.

### (3) Population consequences

Population consequences from sex reversal are initially driven by whether sex reversed individuals are able to reproduce (Fig 8). Anthropogenic sex reversals can cause both complete (reproductive) and incomplete (reproductively impaired or infertile) sex reversals. Intersex or reproductively impaired individuals reduce effective population size and may contribute to population declines (Fig 9c; Dissanayake et al., 2021b). Conversely, reproductive sex-reversed individuals can cause shifts in population sex-ratios, as they pass on sex reversal mutations to their offspring, or mate with individuals of the same genetic sex to produce offspring with skewed sex-ratios. These skewed sex-ratios can cause declines in effective population size, or drive selection for compensatory alternate sex-determination. While much discussion of sex reversal population effects has been theoretical (Schwanz et al 2016; Schwanz et al., 2020; Dissanayake et al., 2021b) concerns have already been raised for some species where sex reversal triggers result in poor quality individuals with impaired reproduction (Yu et al., 2022; Nemesházi et al., 2020). Small and fragmented populations are likely most at risk, as low genetic diversity can increase the prevalence of sex reversal mutations even if such mutations are deleterious or result in skewed sex ratios (Wilson et al., 2014; Ye et al., 2020).

### (4) Modulators of anthropogenic sex reversal

The above population effects from anthropogenic sex reversals are subject to several mitigating factors. Firstly, as detailed throughout this review, sex reversal outcomes vary widely across vertebrate groups. Amphibians and fish, and to a lesser extent, reptiles, demonstrate a high sensitivity to a broad range of sex reversal triggers including developmental temperature. Affected individuals can show complete sex reversal where they become fully functional members of the sex they were not genetically or environmentally predisposed to be. Conversely, birds and mammals show resistance to many sex reversal triggers, with thermally-triggered sex reversal absent from these two groups. When sex reversal triggers are sufficient to disrupt sexual development, sexual dysfunction and intersex phenotypes are the usual outcome; complete sex reversal is exceedingly rare (Parma et al., 2016; Hall et al., 2025). Resistance to environmental and hormonal sex reversal triggers in mammals and birds may be linked to their particularly strong genetic control of sex-determination (e.g., cell-autonomous sexual identity in birds; Clinton et al., 2012, and estrogen resistance in mammals; Capel, 2017). Mammals and birds show strongly heteromorphic sex-chromosomes where important genes governing fertility and sexual development may be positioned on the degraded Y or W. Reversing homogametic individuals (XX or ZZ) thus lack important genes for reproducing as the heterogametic sex (usually XY or ZW; Weber & Capel, 2018). Reversing heterogametic individuals also encounter strong barriers to reproductive success, although to a lesser extent than above. Heterogametic individuals have only one copy of usually paired factors, and meiotic recombination, which usually occurs in homogametic individuals, may be disrupted due to the Y or W not being homologous to the X or Z (Parma et al., 2016).

Where complete sex reversal does occur, reversal direction also has a role to play in population effects. Reversed individuals mate with partners of the same genetic sex, driving skewed genetic and phenotypic sex in their offspring (Schwanz et al., 2016; Wedekind, 2017). Two homogametic (XX/ZZ) individuals produce only heterogametic offspring which, barring further sex

reversal, are all one phenotypic sex (Fig. 9d). Alternatively, two heterogametic individuals produce 50% heterogametic offspring and 50% homogametic offspring. Theoretically, 25% of these offspring would be a novel homogametic combination – YY or WW – an inviable state in all but a handful of fish species (Parma et al., 2016; Jiang et al., 2018). While there is evidence some species can evolve around limitations of heterogametic pairings within naturally-occurring sex reversal systems (Saunders et al., 2022), populations faced with extreme anthropogenic heating or pollution events are very unlikely to develop such defences in the short-term.

Inherent genetic resistance is one avenue by which populations can mitigate increased exposure to sex reversal triggers. Recent studies have highlighted genetic factors driving both predisposition and resistance to temperature-triggered sex reversal. Theoretically, such factors provide opportunities for natural selection to reduce disadvantageous sex reversal (Holleley et al., 2015). Furthermore, genetic variation can be exploited to develop resistant populations for conservation, both through classic selective breeding (Hoelzel et al., 2019) and through ever-advancing modern genetic manipulation tools (Doudna & Charpentier, 2014). Family-level resistance to high temperature masculinisation has been reported in the teleost fish *Menidia menidia* (Lagomarsino and Conover, 1993), *Dicentrarchus labrax* (Saillant et al., 2002), *Oreochromis niloticus* (Lühmann et al., 2012), *Oncorhynchus mykiss* (Valdivia et al., 2014), *Danio rerio* (Ribas et al., 2017), *Cynoglossus semilaevis* (Wang et al., 2019), and *Cyprinus carpio* (Biswas et al., 2021). Such resistance appears to have a complex polygenic basis. They may reduce rates of overall sex reversal, or cause upward shifts in the range of temperatures that trigger reversals. Genetic resistance rarely prevents sex reversal outright, although the occurrence of high-temperature sex reversal in the dragon *Pogona vitticeps* is limited to the relatively cool southern part of the species' range, implying complete resistance in northern populations (Castelli et al., 2021).

There is also scope for some species to control levels of temperature-triggered sex reversal through their reproductive behaviour (Kearney et al., 2009). This strategy appears particularly relevant for reptiles. Flexible female thermoregulatory behaviour in the viviparous skink *Carinascincus oscellatus* has implications for rates of natural sex reversal through embryo exposure to low temperatures (Hill et al., 2022). Similarly, female *Bassiana duperreyi* and *Pogona vitticeps* show flexibility in both laying time, nest site selection and nest depth, traits which alter thermal regimes experienced by their eggs and thus instances of thermally-triggered sex reversal (Telemeco et al., 2009; Wild et al., 2022). Behavioural thermoregulation by embryos themselves potentially provides further opportunities for temperature buffering, although evidence for its efficacy is mixed (Liu et al., 2023). Behavioural mitigation of sex reversal is likely more limited in other taxa, such as oceanic fish species, who must travel great distances to find cooler habitats, or are subject to geographic and spatial limitations imposed by seasonal resource availability (Dahlke et al., 2020; Amat-Trigo et al., 2023). There is also evidence that some fish behaviour may elevate sex reversal rates; both *Oreochromis niloticus* and *Clarius gariepinus* fry have been recorded displaying a preference for supraoptimal high temperatures during the thermo-sensitive period. This behaviour may be genetically controlled, which raises the possibility of selection against, or genetic manipulation of, detrimental high temperature preferences (Santi et al., 2017; Nivelle et al., 2019).

The capacity for populations to prevent sex reversal triggered by endocrine-disrupting-chemicals (EDCs) is less certain. When pollutants enter a habitat, it is difficult for individuals to avoid exposure. Embryos and larvae developing in aquatic environments are most at risk, being almost permanently exposed to any dissolved EDCs (Gonsioroski et al., 2020). Amphibians and fish are thus most at risk, being both sensitive to sex reversal and inhabiting aquatic environments. Predators of these taxa are also vulnerable to exposure through bioaccumulation and contaminated prey (Fry & Toone, 1981). Again, birds and mammals show the least sensitivity to hormonal disruption, but EDC exposure can still cause harmful masculinisation/feminisation effects and reproductive impairment (e.g. Fry & Toone, 1981; Roberts et al., 2018). Proximity to human activity is a further predictor of sex reversal risk; sex reversal effects tend to be seen more frequently in anurans near urban or agricultural areas (Lambert et al., 2015; Zlotnik et al., 2019; Nemesházi et al., 2021; Bókony et al., 2023). Within groups, some species do show resistance to sex reversal through EDC exposure (Mackenzie et al., 2003; Piprek et al., 2012a; 2012b; Ramee et al., 2020), but few studies have yet investigated within-species variation in resistance which may harbour potential for selection against reversals (but see Myosho et al., 2019).

For both environmental and EDC-based sex reversals, the magnitude and timing of sex reversal triggers are expected to impact a species' resilience to sex reversal (Baker et al.,1988). Many triggers are only successful at instigating sex reversal inside a specific sensitive window of development (van Aerle et al., 2002; Phuge & Gramapurohit, 2015), and there is evidence for increased magnitude of both hormonal treatments and extreme temperatures resulting in more individuals reversing, or more 'complete' reversals (Mizukami-Murata et al., 2016; Lambert et al., 2018). However, increased exposure to high temperatures and high doses of EDCs also drive higher mortality and ongoing consequences such as supressed immune function, even in individuals that experience no sexual dysfunction (Sower et al., 1984; Ujszegi et al., 2022). For many EDCs outright mortality is actually the primary concern after exposure, although sexual disruption rates in survivors may have important implications for the ability of populations to persist through pollution events (Wedekind, 2017). Finally, in natural populations, repeated and frequent pollution/extreme weather events may have additive or synergistic effects (Folt et al., 1999; Orr et al., 2024). Such unpredictable and novel effects are expected to be particularly difficult for natural selection to respond to without further anthropogenic intervention (Catullo et al., 2019; Speakman et al., 2025).

## (5) Future research areas

Sex reversal has likely always been an uncommon but consistent process in fish, amphibians, and potentially, reptiles. Reproductive reversed individuals may play a role in historical transitions between sex determination systems, sex chromosome evolution, and allow individuals to exploit situations where fitness is sex-specific. Yet there is still much to learn, and confirm, about naturally occurring sex reversal – even as we expect increases in population exposure to anthropogenically-driven triggers. Specifically, to better understand the effect of sex reversal on population demography and dynamics, we need more direct comparative studies on the traits of discordant and concordant individuals. Different sex reversal triggers within a species may have differential effects on fitness,

while the intermediate and novel traits displayed by discordant individuals are complex, and difficult to place in their natural context. Trait-based research on natural sex reversals is moving in an encouraging comparative direction (see Li et al., 2016; Nemesházi et al., 2020; Mikó et al., 2021; Ujszegi et al., 2022; Yu et al., 2022; Dussenne et al., 2022; Bókony et al., 2023; Veyrunes et al., 2023; Wild et al., 2023; Laven et al., 2025). However, we still have few examples of how sex reversed animals act in the wild (Wild et al., 2022). Furthermore, there is a need to expand out from species where sex reversal is likely endemic, to those that are only now experiencing it due to recent anthropogenic change. Priority should be given to species that are at risk because of taxonomic predisposition, such as fish and amphibians, or because of ecological factors, e.g., aquatic life stages. Species with small population sizes and restricted habitats also face an increased risk from maladaptive sex reversals, as they are limited in their ability to buffer exposure to triggering conditions via natural selection and behavioural adjustments.

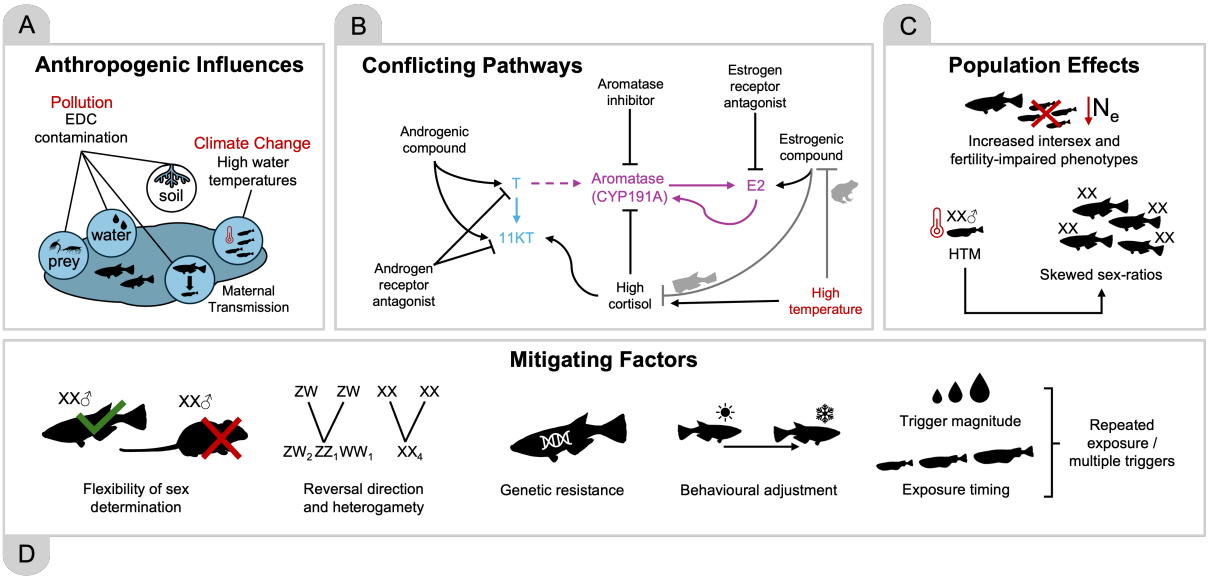



Fig. 9. **Visualisation of concepts discussed in section VI., "Sex reversal in the Anthropocene"**. A) Natural populations face combined risks from endocrine-disrupting-chemicals (EDCs) and increased average temperatures B) How different EDCs and high temperatures interact has not yet been well-studied, but predictions can be made by contrasting their mechanistic sex reversal pathways. Diagram shows a simplified sexual development pathway from Fig. 5. Black arrows indicate interactions, with blunt arrows (⊥) indicating suppression, and sharp arrows (→) denoting upregulation. Pale grey arrows display potential taxa-specific relationships as discussed in the text. C) Increased temperatures will likely increase high-temperature masculinisations (HTMs), while EDC exposure often creates reproductively impaired phenotypes, which both potentially reduce effective population size ($N_e$). D) Population effects from anthropogenic sex reversals are subject to several mitigating factors, as discussed in text.

## VII. Conclusions

1) Sex reversal occurs in all main vertebrate groups under a broad variety of triggering circumstances. However, these triggering circumstances share mechanisms; affecting either expression of sex-linked genes, hormones levels, or numbers of germ cells (or a combination of the three). Modification of these factors during development can push an individual towards

a male, female or intersex phenotype, despite the presence of usual inherited or environmental sex determination factors.

2) Fish and amphibians show an increased sensitivity and propensity to sex reverse. Sex reversal may represent an infrequent but important evolutionary process in these taxa. Conversely, mammals and birds rarely undergo complete sex reversal, and appear resistant to thermally-triggered sex reversal. This is potentially due to stronger genetic control over sex in birds and mammals compared to other vertebrate taxa.

3) Most sex reversal research concerns the inducement of reversals under laboratory or aquaculture conditions, often with triggers not present in nature (e.g., exogenous hormones). We lack empirical data on the consequences of sex reversal, both at the individual level (behaviour, physiology and reproduction), and the population level (genetic and phenotypic sex ratios, frequency dependent selection, and transitions between sex determination systems).

4) Thus, our understanding of sex reversal as a natural process is still fairly limited. Sex reversal commonly has negative effects on fitness, likely occuring only due to development constraints. Yet sex reversal may be adaptive in some rare cases. Reversing individuals could theoretically benefit from Charnov-Bull sex-by-temperature interactions, frequency dependent selection on skewed sex ratios, or possession of a novel phenotype with both male and female traits.

5) Current anthropogenically-driven changes in the environment involve more frequent extreme-temperatures and pollution events involving endocrine-disrupting-chemicals. Exposure to these conditions is expected to increase rates of sex reversal in wild populations, likely with negative effects on individual fitness and population viability. Research should be directed towards identifying at-risk taxa, and avenues by which sex reversal effects can be buffered, such as exploiting genetic variation in sex reversal sensitivity or propensity.

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