Embryos are largely understudied in conservation physiology

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Lay summary

Understanding how different life stages respond to environmental stressors is essential for predicting climate change impacts. Yet, embryos are largely overlooked, being focus of fewer than 10% of studies. We highlight this gap and call for greater inclusion of early life stages to complement knowledge across the entire life cycle.

Abstract

Understanding how animals respond to environmental stressors across their life cycle is essential for predicting species' vulnerability to climate change. Here, we systematically reviewed the literature to quantify the variation in research effort on different life stages in the field of conservation physiology. Specifically, we reviewed experimental studies measuring physiological and life-history responses to climatic stressors across three scientific journals: *Conservation Physiology*, *Journal of Thermal Biology*, and *Journal of Experimental Biology*. Our systematic map of 1,276 studies revealed a pronounced underrepresentation of studies on embryos, representing only 8-9% of studies. This pattern was remarkably consistent across all axes considered (i.e., journals, taxonomic groups, physiological traits, and environmental stressors). We also found that 80% of studies only investigated single life stages, and over 5% of studies did not clearly report the life stage(s) used. Despite the increasing recognition of the ecological importance and sensitivity of early life stages to environmental stressors, we found no evidence that research on embryos has gained traction over the past decade (2013-2024). We argue that these ontogenetic biases likely reflect a combination of historical precedents and enduring methodological and logistical constraints that continue to shape research agendas. To build a more holistic understanding across the life cycle, we: i) call for a paradigm shift placing embryos at the center of experimental agendas, ii) outline emerging methodological advances that increase the feasibility of research on early life stages, iii) demonstrate how studies on embryos can navigate ethical considerations for animal research, iv) highlight perspectives for future evidence syntheses and study reporting, and v) promote investigations of the mechanisms underlying physiological variation across ontogeny. Closing the ontogenetic gap will be key to improving our ability to predict population-level impacts of climate change and guiding more effective conservation and management interventions.

1. Introduction

How animals respond to environmental change is a central question in ecophysiology, particularly in the face of ongoing climate change. By investigating the physiological mechanisms underlying organismal responses to climatic stressors, conservation physiology provides essential insights for predicting species resilience and guiding conservation strategies (Wikelski and Cooke, 2006; Cooke and O'Connor, 2010; Cooke *et al.*, 2013). However, a key aspect that often remains overlooked is the need to understand these responses across all life stages of a species (Radchuk *et al.*, 2013; Kingsolver and Buckley, 2020). A growing body of evidence suggests that the effects of climate change, such as rising temperatures and increased thermal variability, can vary significantly across life stages (e.g., Kingsolver *et al.*, 2011; Levy *et al.*, 2015; Truebano *et al.*, 2018; Dahlke *et al.*, 2020; Kingsolver and Buckley, 2020; Sales *et al.*, 2021; Ma *et al.*, 2024). Differences in sensitivity to environmental stressors across life stages can have profound implications for population persistence and species survival, making it essential to understand the stage-specific effects of climatic stressors, as they collectively shape population demography, dynamics, and long-term viability under climate change (Radchuk *et al.*, 2013; Levy *et al.*, 2015; Kingsolver and Buckley, 2020).

Early life stages, such as embryos and larvae/juveniles, are often hypothesized to be the most vulnerable due to their limited capacity for thermoregulation and heightened sensitivity to environmental fluctuations (Byrne, 2012; Huey *et al.*, 2012; Pandori and Sorte, 2019; Bodensteiner *et al.*, 2021; Burggren, 2021; Cheng *et al.*, 2023). These assumptions are supported by empirical data encompassing both ecto- and endothermic taxa (e.g., mammals (Zhao *et al.*, 2020); birds (Mainwaring *et al.*, 2017); amphibians (Ruthsatz *et al.*, 2022); reptiles (Sun *et al.*, 2021); fishes (Dahlke *et al.*, 2020); insects (Bowler and Terblanche, 2008); and aquatic invertebrates (Pandori and Sorte, 2019); *but see* Hamdoun and Epel, 2007; De Bonville *et al.*, 2025). However, recent comparative analyses have provided evidence that early life stages may be understudied relative to adults in conservation physiology, potentially overlooking key windows of climate vulnerability (Pottier *et al.*, 2022b; Weaving *et al.*, 2022; Ruthsatz *et al.*, 2024). For example, Pottier *et al.* (2022b) concluded that very little research has been conducted on the plasticity of thermal tolerance in ectotherm embryos, while Weaving *et al.* (2022) found that data on adults were more abundant than those on early life stages in insects. In contrast, a meta-analysis by Pandori & Sorte (2018) found research on early life stages (embryos and larvae) of aquatic invertebrates were more prevalent than research conducted on adults in the context of climate warming. However, the extent to which these ontogenetic biases are generalizable remains unclear, as there is currently no comprehensive evidence on how research effort varies across different life stages in conservation physiology. To address this gap, we systematically assessed for potential biases in research effort on different life stages across three conservation physiology journals.

In this study, we aimed to quantify the research effort dedicated to different life stages in the experimental conservation physiology literature. To achieve this, we conducted a systematic review map of studies published in three key journals: *Conservation Physiology*, *Journal of Thermal Biology*, and *Journal of Experimental Biology*. We focused on animal physiology and life-history responses to climatic stressors and categorized studies by life stages, taxa, and climatic stressors to assess the heterogeneity of patterns in published research. We sought to identify gaps in the representation of specific life stages, predicting that research on embryos would be underrepresented compared to studies on larvae/juveniles and adults. By highlighting this potential imbalance, we aimed to encourage research that is more inclusive of all stages in conservation physiology. We conclude by providing recommendations and perspectives on integrating underrepresented life stages, aiming to expand our understanding of population sensitivity to global change – knowledge essential for informing more effective conservation and management strategies.

2. Materials and Methods

2.1. Registration and reporting

This study was pre-registered prior to data extraction (Pottier *et al.,* 2024b). While we mostly followed our plans, we acknowledge few minor deviations (see *Deviations from registration*). We followed MeRIT (Nakagawa *et al.*, 2023), CRediT (McNutt *et al.*, 2018), and Dragon Kill Points (Martinig *et al.*, 2025) guidelines to report authorship contributions (Tables S2-3). We also followed recommendations for reporting the title, abstract, and keywords of this study and maximize indexing in search engines and databases (Pottier *et al.*, 2024a). All data, code, and materials are freely available at https://github.com/p-pottier/Cons_phys_life_stages, and will be archived in Zenodo upon acceptance.

2.2 Systematic review

We aimed to obtain a representative sample of experimental studies in animal conservation physiology. Specifically, we focused on studies assessing animal responses to climate change.

We systematically reviewed the literature to find studies that experimentally manipulated climatic stressors and measured physiological and/or life-history traits of non-human animals. Therefore, we excluded reviews, meta-analyses, field observations, theoretical studies, editorials, and other study types without experimental manipulations. Note, however, that we included experimental studies performed in both laboratory and field settings, as long as climatic stressors were experimentally manipulated (e.g., altering operative body temperatures by adjusting nest depth of turtle eggs in the field). We focused on whole-organism non-human animals, hence excluded humans, plants, microorganisms, or isolated cells and organs. Relevant climatic stressors included temperature, oxygen/carbon dioxide, pH, salinity, and humidity/water availability. We also included studies manipulating other stressors with climatic relevance, such as diet restriction and UV radiation levels. We only focused on physiological or life-history traits (environmental tolerance or preference, energetics and metabolism, development, immunity and stress physiology, osmoregulation, reproduction, and cardiovascular physiology), to gain an overview of the field of conservation physiology broadly defined. Therefore, we excluded studies focusing on behavioural traits, morphology, ecological interactions, or biodiversity measures, for instance.

PP performed the literature searches on 16/10/2024 in Web of Science (core collection, UNSW subscription). We did not use other databases because our searches were targeted to specific journals, so there is little discrepancy in results between databases. Searches were designed to capture studies manipulating climatic stressors in the journals *Conservation Physiology*, the *Journal of Thermal Biology*, and the *Journal of Experimental Biology*. Note that a more general search (i.e., no key terms on climatic stressors) was used for the *Journal of Thermal Biology* because all studies in this journal are focused on temperature, one of the key climatic stressors of interest. We restricted our searches to articles published after 2013 (when the journal *Conservation Physiology* was founded) to maintain a comparable timespan between journals. Our searches were not intended to be comprehensive, and these three journals were used as a case in point to assess the validity of informal statements made about the under-prevalence of experimental research on embryonic stages. The sample of studies we obtained is intended to provide a representative sample of the literature in the field of climate conservation physiology. Note that all searches were filtered to article document types, to exclude reviews, notes, and editorials.

Searches were designed as follows:

Conservation Physiology (600 results): IS=("2051-1434") AND TS=("climat*" OR "global change" OR "global warming" OR "environmental change" OR "temperature*" OR "therm*" OR "hypotherm*" OR "hypertherm*" OR "warm*" OR "heat*" OR "cold*" OR "cool*" OR "hot" OR "solar" OR "UV" OR "oxygen*" OR "hypoxi*" OR "hyperoxi*" OR "normoxi*" OR "anox*" OR "CO2" OR "carbon dioxide" OR "hypercapn*" OR "pH" OR "acidifi*" OR "salinity" OR "salt*" OR "humid*" OR "water" OR "drought*" OR "dry*" OR "dessicat*" OR "dehydr*" OR "rainfall" OR "moisture" OR "arid*")

Journal of Thermal Biology (2,136 results): IS=("0306-4565") AND PY=(2013-2024)

Journal of Experimental Biology (2,145 results): IS=("1477-9145") AND TS=("climat*" OR "global change" OR "global warming" OR "environmental change" OR "temperature*" OR "therm*" OR "hypotherm*" OR "hypertherm*" OR "warm*" OR "heat*" OR "cold*" OR "cool*" OR "hot" OR "solar" OR "UV" OR "oxygen*" OR "hypoxi*" OR "hyperoxi*" OR "normoxi*" OR "anox*" OR "CO2" OR "carbon dioxide" OR "hypercapn*" OR "pH" OR "acidifi*" OR "salinity" OR "salt*" OR "humid*" OR "water" OR "drought*" OR "dry*" OR "dessicat*" OR "dehydr*" OR "rainfall" OR "moisture" OR "arid*") AND PY=(2013-2024)

We identified 4,881 bibliographic records from these journals. All bibliographic records were combined and deduplicated in R (v. 4.4.2) (R Core Team, 2019) by PP using the litsearchr (v. 1.0.0) (Grames *et al.*, 2019) and synthesisr (v. 0.3.0) (Westgate and Grames, 2020) packages. A total of 4,868 unique bibliographic records were identified.

KA, RA, AC, ZLC, MLE, SSK, JCSM, ECGM, MM, AKP, LP, DMR, BJS, RV, and NCW independently screened studies for eligibility based on their titles, abstracts, and keywords in Rayyan QCRI (Ouzzani *et al.*, 2016) using the decision trees in Fig. S1. When eligibility criteria could not be assessed solely based on the title, abstract, or keywords, we inspected the full article. In total, we identified 1,276 relevant studies.

2.3. Data extraction

KA, RA, AC, ZLC, MLE, SSK, JCSM, ECGM, MM, AKP, LP, DMR, BJS, RV, and NCW extracted descriptive information about the studies from their abstract and/or PDF using Google Forms (Table S1). Specifically, we extracted: i) the journal and year of publication, ii) the taxonomic group(s) studied, iii) the climatic stressor(s) manipulated during the experiment(s), iv) the life stage(s) exposed to the climatic stressor(s), v) the life stage(s) of the animals when physiological or life-history traits were measured, vi) the broad category of physiological or life history trait measured. Information on life stages were divided into three broad categories to allow cross-taxa comparisons: embryos, larvae or juveniles, and adults. We did not separate larval and juvenile stages because we covered a broad range of taxa, and separations between these life stages were often unclear or difficult to establish based on study descriptions. For continuous experimental exposure overlapping multiple life stages, we noted if the climatic exposure was imposed on animals before and after hatching, or strictly after hatching. Climatic stressors were divided into five broad categories: i) temperature, ii) oxygen/carbon dioxide, iii) pH, iv) salinity, v) humidity/water availability, and vi) other (diet, UV radiation). We also noted if authors investigated interactions between climatic- and non-climatic stressors, although this was not one of our aims. Taxonomic groups were separated into seven broad categories: i) birds, ii) mammals, iii) fish, iv) non-avian reptiles, v) amphibians, vi) insects, vii) other invertebrates. Trait categories were separated in seven broad classes: i) environmental tolerance and preference (e.g., survival or tolerance to the climatic stressor, habitat selection, thermoregulation, heat shock proteins, etc.), ii) energetics and metabolism (e.g., oxygen uptake, metabolic rate, aerobic scope, digestion efficiency, etc.), iii) osmoregulation (e.g., ion balance, water loss, acid-base regulation, excretion, etc.), iv) cardiovascular physiology (e.g., blood pressure, heart rate, stroke volume, etc.), v) immune function and stress physiology (e.g., stress hormones, immune competence, oxidative stress, etc.), vi) development (e.g., growth rate, body size, phenology, etc.), and vii) reproduction (e.g., fecundity, sex hormones, gametogenesis, sperm count, etc.). We also collected whether studies investigated interactions between climatic and non-climatic stressors (e.g., predation). A few studies also reported data in trait categories we assigned as "Other" (e.g., thyroid function, neurophysiology, symbiont density, sensory physiology). Note that we also collected trait details within each category. The exact questions and response options presented in the Google form are presented in Table S1.

2.3. Data curation visualization

Data were curated and visualized in R (R Core Team, 2019). PP curated the extracted data to amend typologies, and standardize trait, climatic stressor, and life stage categories. All steps involved in the data curation are available at https://github.com/p-pottier/Cons_phys_life_stages. PP used the *tidyverse* collection of packages (Wickham *et al.*, 2019), *ggstream* (Sjoberg, 2025), *circlize* (Gu *et al.*, 2014) and *patchwork* (Pedersen, 2025) packages to produce the figures. We did not perform statistical analyses because our objective was to provide a systematic map of the current state of evidence.

2.4. Deviations from registration

While we mostly followed our original plans, we acknowledge one main deviation from our original plans: we did not perform bibliometric analyses to identify author clusters based on life stages. This was because the *bibliometrix* package (Derviş, 2019) does not currently allow for mapping of author clusters based on external variables, which prevented us from performing the planned analyses. Nevertheless, this analysis was not critical to our conclusions.

3. Results

3.1. Data description

We found 1,276 studies measuring physiological and/or life-history responses of animals to climate change. All studies were published between 2013 and 2024 to maintain a consistent timespan across studies (Fig. 1a,b). Approximately 45% (n = 562) of studies were published in the *Journal of Experimental Biology*, 43% (n = 533) in the *Journal of Thermal Biology*, and 15% (n = 181) in *Conservation Physiology* (Fig. 1c,d). Note that the values below do not represent a percentage of studies *per se* because some studies have investigated multiple life stages, taxa, climatic stressors, and traits.

We found that the eligible studies predominantly investigated environmental tolerance and preference (30.8%; n = 727) followed by energetics and metabolism (24.3%; n = 574), development (16.1%; n = 380), immune function and stress physiology (10.4%; n = 246), osmoregulation (7.2%; n = 170), reproduction (5.8%; n = 138), and cardiovascular physiology (5.2%; n = 122) (Fig. 2a,b). An additional 6 studies (0.3%) also investigated other traits not captured by these broad categories (e.g., sensory physiology, neurophysiology).

Studies covered a broad range of taxa, although we note that most of the data originated from fishes (31.1%; n = 398), insects (18.6%; n = 238), and other invertebrates (17.6%; n = 226). Mammals (10.7%; n = 137), birds (8.7%; n = 112), reptiles (7.8%; n = 100), and amphibians (5.5%; n = 70) were the least studied taxa in our literature sample (Fig. 2c,d).

Most studies investigated responses to temperature (65.1%; n = 1,041), followed by O_2/CO_2 (12.1%; n = 194), pH (5.2%; n = 83), salinity (4.3%; n = 68), and humidity/water availability (3.8%; n = 60; Fig. 2e,f). Approximately 1% (n = 16) of studies investigated other stressors (i.e., diet, UV radiation), and 8.7% (n = 138) studied interactions between climatic and non-climatic stressors. The over-representation of studies on responses to temperature is, perhaps, not surprising because 45% of studies were published in the *Journal of Thermal Biology* (Fig. 1a,b). Nevertheless, temperature remained the most studied climate change stressor (54% of studies) after excluding studies published in this journal. Our results were also robust to the exclusion of environmental stressors other than temperature (Fig. S2-4).

3.2. Variation in research effort across life stages

We found a strong bias towards the study of adults, larvae, and juveniles relative to embryos. Specifically, only 9.4% (n = 131) of studies exposed embryos to climatic stressors, while 49.5% (n = 690) and 28.8% (n = 402) of studies exposed adults, or larvae/juveniles, respectively (Fig. 1a,c; Fig. 2a,c,e). We also found that a notable percentage of studies exposed animals across different life stages, with 4.2% (n = 59) of studies exposing embryos and later life stages, and 2.2% (n = 31) of studies exposing animals across different life stages strictly after hatching (Fig. 1a,c; Fig. 2a,c,e). We also found that approximately 5.9% (n = 82) of studies did not clearly report which life stage was exposed to the climatic stressor (Fig. 1a,c; Fig. 2a,c,e).

We observed similar patterns for the life stages assessed for life-history or physiological traits (Fig. 1b,d; Fig. 2b,d,f; Fig. 3). Most studies (52.4%; n = 788) measured the traits of adult animals, followed by larvae/juveniles (34.1%; n = 513), and embryos (8.0%; n = 120) (Fig. 3). About 5.5% (n = 83) also did not clearly report which life stage was assessed. Notably, we found that most studies reported the responses of single life stages, and few studies investigated the responses of multiple life stages (Fig. 3). Of studies that measured traits on adults (n = 788), only 18% (n = 142) and 4.6% (n = 36) also measured traits on larvae/juveniles and embryos, respectively (Fig. 3). Furthermore, 14.6% (n = 75) of studies measuring traits on larvae or juveniles (n = 513) also performed measurements on embryos (Fig 3). Among the studies that specifically exposed embryos to climatic stressors (n = 84), 40.9% (n = 47) assessed potential carry-over effects in larvae or juveniles, while only 11.3% (n = 13) did so in adults.

Interestingly, biases towards the study of post-embryonic stages were consistent across time, and we did not observe a notable temporal change in research effort among life stages (Fig. 1a,b).

We also found consistent under-representation of studies on embryos across journals (Fig. 1c,d), taxonomic groups (Fig. 2c,d), and climate change stressors (Fig. 2e,f), for both the life stages exposed to the climatic stressors (Fig. 1a,c; Fig. 2a,c,e), and those measured for physiological or life-history traits (Fig. 1b,d; Fig. 2b,d,f). Patterns were also consistent among trait categories, with the exception of development, for which we found a larger proportion of studies on embryos, as expected (Fig. 2a,b). It is also worth noting that there were a few notable differences in research effort among taxonomic groups, with very few studies measuring traits on mammal embryos (n =1), but significantly more in reptiles (n = 16; 13.7%; Fig. 2c,d).

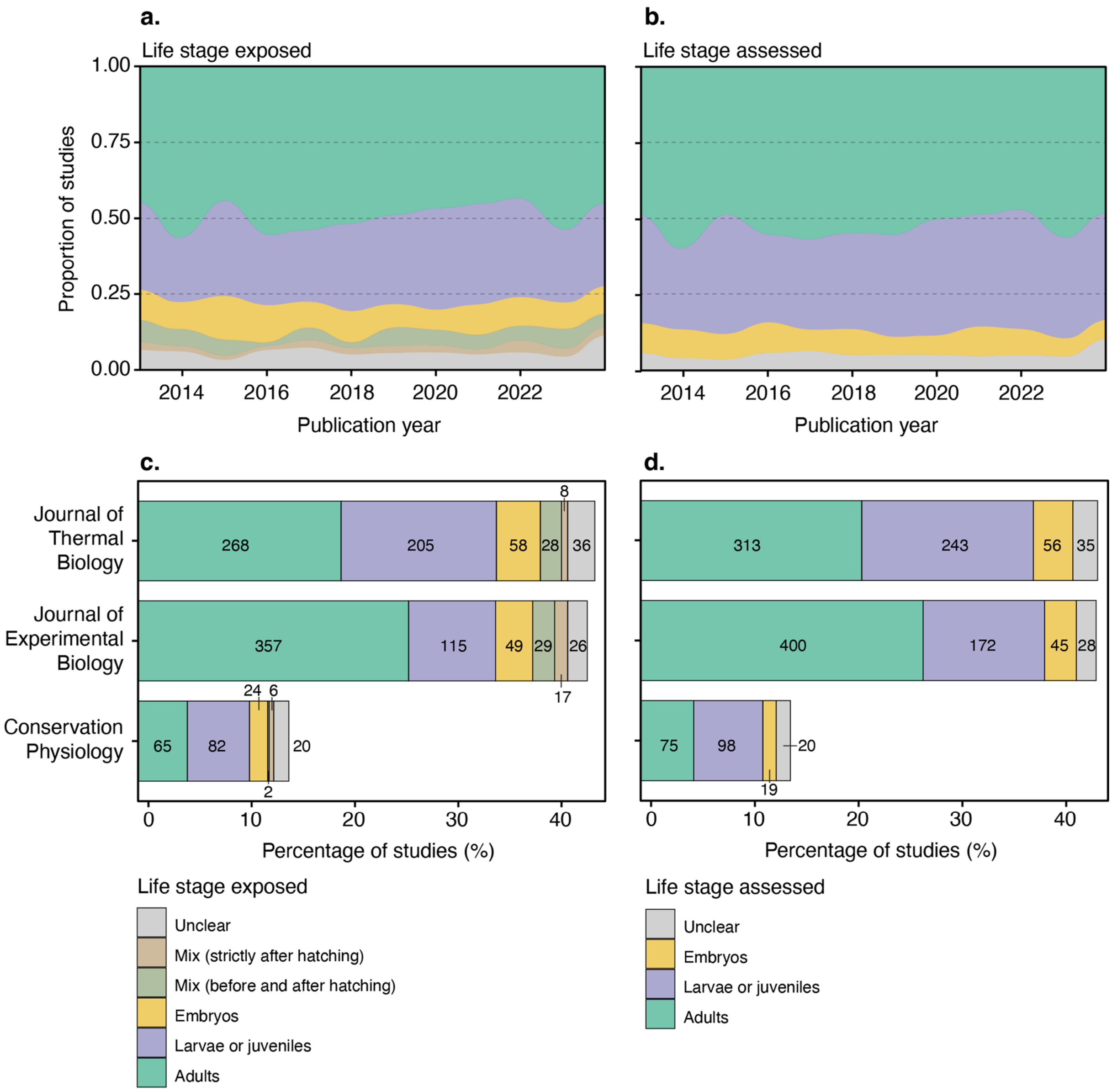

Fig. 1 | Differences in the relative proportion of life stages exposed to climatic stressor(s) (**a**) or assessed for physiological traits (**b**) over study publication years, and across the three representative journals surveyed (**c** exposed, and **d** assessed). Sample sizes (counts of studies) are presented for each category.

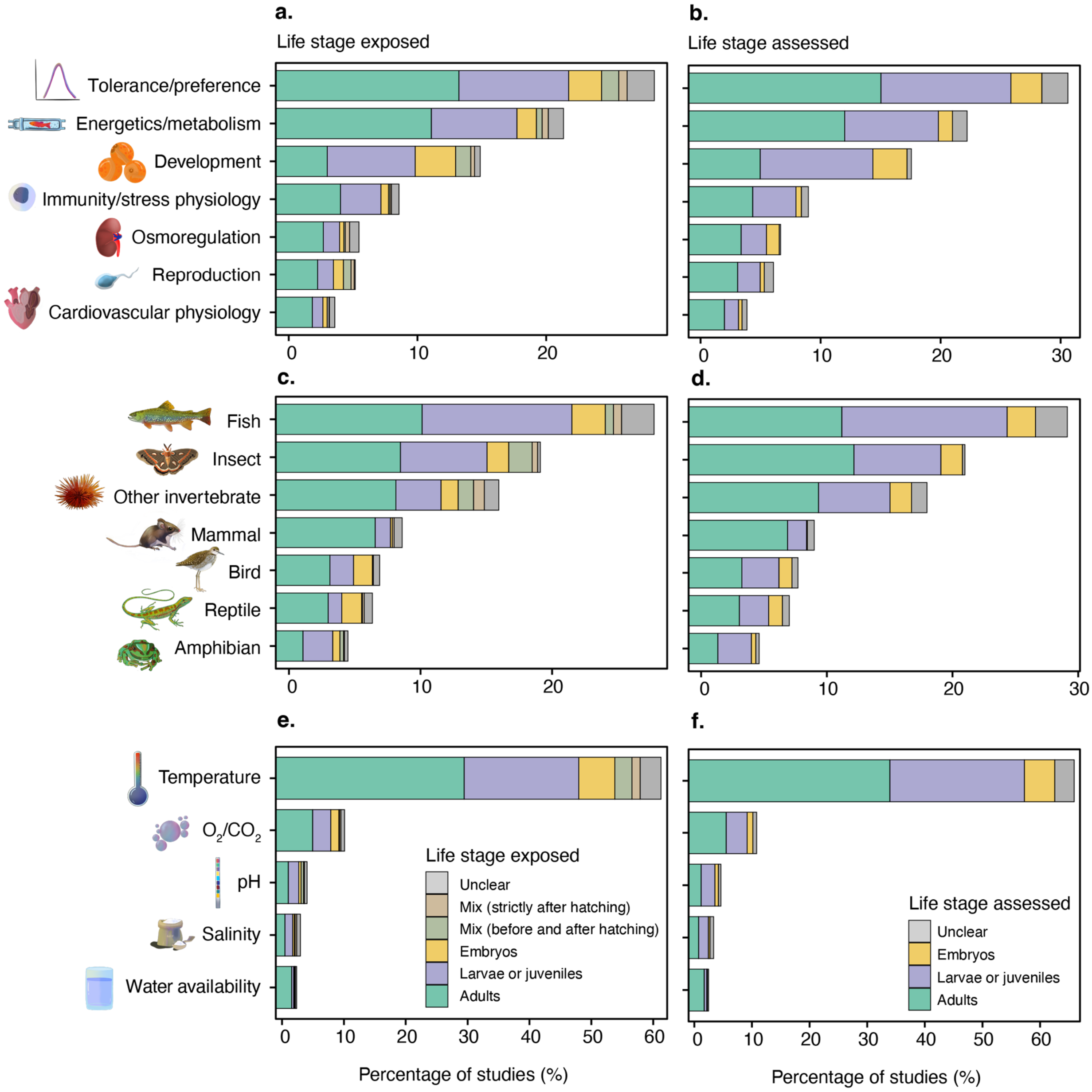

Fig. 2 | Differences in the relative proportion of life stages exposed to climatic stressor(s) (**a, c, e**) or assessed for physiological traits (**b, d, f**) across the climate change stressors (top row), taxa surveyed (middle row), and traits surveyed (bottom row). Studies categorized in "development" yet assessing adult animals are studies measuring whole animal size, growth, or measuring multiple life stages. Other stressors not captured by these broad categories (i.e., diet, UV radiation; n = 6) and studies investigating interactions between climatic and non-climatic stressors (n = 138) are not displayed for clarity. Representative diagrams were drawn by MLE.

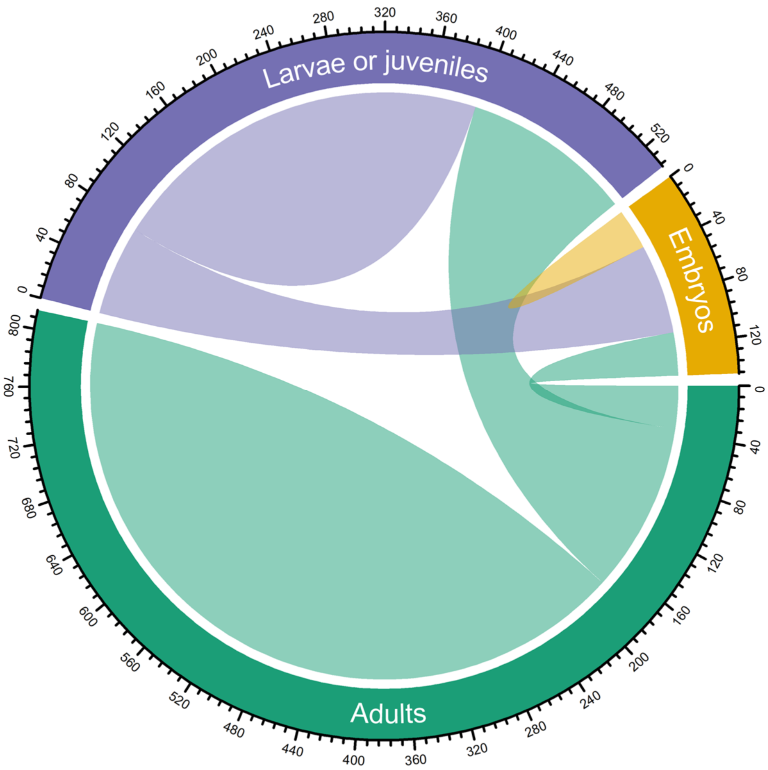

Fig. 3 | Chord diagram illustrating studies measuring traits on single or multiple life stages. Categories that are connected represent studies that investigated multiple life stages. Numbers in the outer circle represent the number of studies.

4. Discussion

Our systematic map revealed a pronounced and consistent bias in conservation physiology experimental research towards adult life stages, with embryos being largely underrepresented (8-9% of studies; Fig. 1-3). This pattern was remarkably consistent across journals, taxa, physiological traits, and climatic stressors, and has persisted over the past decade (Fig. 1-2). The underrepresentation of embryos is especially concerning given the increasing recognition of their ecological importance and sensitivity to environmental stressors (Dahlke *et al.*, 2020; Wu and Seebacher, 2020; Sales *et al.*, 2021; Sun *et al.*, 2021; Pottier *et al.*, 2022b; Vorsatz *et al.*, 2022). Neglecting this vulnerable life stage risks obscuring key demographic bottlenecks and may lead to inaccurate predictions of species' climate sensitivity, ultimately undermining the design of effective conservation strategies.

4.1 Ontogenetic biases in conservation physiology research: historical, methodological, and logistical constraints

Adult life stages were studied 1.5 to 1.7 times more than larvae and juveniles, and 5.3 to 6.6 times more than embryos in our literature sample (Fig. 1-3). Studies that focused on embryos also remained significantly underrepresented across journals, taxonomic groups, physiological traits, climatic stressors, and time, only representing between 1 and 15% of studies across all the axes we considered. The broad consistency of these patterns suggests that this study bias is not a product of editorial priorities or specific research areas, but instead reflects deep-rooted, structural limitations within scientific priorities and/or experimental design. Notably, this imbalance has persisted over the past decade, with no clear increase in embryo-focused studies, despite growing recognition in the ecological and developmental literature that early life stages often represent critical periods of heightened sensitivity to environmental change (Dahlke *et al.*, 2020; Wu and Seebacher, 2020; Sun *et al.*, 2021; Pottier *et al.*, 2022b; Vorsatz *et al.*, 2022; Vasudeva, 2023). The consistent ontogenetic bias in conservation physiology research likely reflects a combination of *historical*, *methodological*, and *logistical* constraints that have shaped the field over time.

Historical bias – Historically, ecological and conservation research has prioritized adult and juvenile life stages, grounded in a prevailing view of fitness and population viability that centers on survival, fecundity, and performance in post-embryonic stages. This adult- and juvenile-centric perspective shaped early physiological and ecological studies, which often focused on traits such as metabolic rate, stress tolerance, growth and reproductive performance in more developed life stages (e.g., McCay *et al.*, 1930; Gunn, 1942; Scholander *et al.*, 1950). These efforts likely reflect assumptions about the primary role of post-embryonic stages in driving population dynamics and adaptive potential. Conservation priorities were likely similarly influenced by this research focus, where population viability analyses and management strategies typically focused on juvenile and adult abundance, fecundity, and survival rates (Mills *et al.*, 1999; Salguero-Gómez *et al.*, 2016). While these metrics remain critically important, such emphasis has inadvertently marginalized physiological research on early developmental stages, despite their large influence on recruitment, lifetime fitness, and population persistence, as well as their potential to shape post-embryonic phenotypes and population trajectories through developmental plasticity and carry-over effects (Burggren and Mueller, 2015; Fawcett and Frankenhuis, 2015; Pettersen *et al.*, 2016; Noble *et al.*, 2018; Pottier *et al.*, 2022b). In turn, the under-appreciation of the importance of early life stages in the study of environmental stressors has likely constrained access to research funding, further reinforcing ontogenetic biases in the published literature.

Methodological constraints – Beyond historical biases, *methodological* challenges have likely strongly influenced the underrepresentation of embryos in conservation physiology research. Embryonic life stages often present practical difficulties due to their small size, fragility, and sensitivity to handling conditions, which complicate experimental design and physiological measurements, especially in small ectotherms. Many physiological traits require invasive or delicate techniques that are easier to implement in larger, more robust adults. For example, physiological assessments such as quantifying metabolic rates, hormone levels, or thermal tolerance in embryos demand specialized equipment or techniques which can be logistically challenging or have only recently become more accessible (Ellis-Hutchings and Carney, 2010; Pettersen *et al.*, 2018; Truebano *et al.*, 2018; Wu *et al.*, 2020; Cowan *et al.*, 2023). Moreover, certain assessments such as stress hormone measurements from blood or tissue samples require a minimum sample mass or volume that embryos often do not meet (Burraco *et al.*, 2015; Ruthsatz *et al.*, 2023). Additionally, some embryos develop within protective structures such as egg capsules or maternal tissues, further limiting direct manipulation and observation. The need for species-specific breeding knowledge and controlled developmental environments may present further methodological obstacles. Successful breeding protocols and rearing techniques must often be established or optimized for each species, requiring substantial time and resources, especially for rare or threatened species. Moreover, many embryos have narrow developmental windows during which environmental manipulations can be applied without causing mortality or developmental arrest (Limpus *et al.*, 1979; Du and Shine, 2015), necessitating precise timing and monitoring. These constraints increase experimental complexity and costs which may discourage researchers from prioritizing embryonic stages despite their ecological importance. Recent advances in non-invasive imaging (Ibbini *et al.*, 2022; McCoy *et al.*, 2023; Tills *et al.*, 2025), molecular techniques (El-Ghali *et al.*, 2010; Wu *et al.*, 2020), and miniaturized instrumentation (Lovegrove, 2009; Cowan *et al.*, 2023) are beginning to address these challenges, offering new opportunities to study early-life physiology more effectively. Continued development and dissemination of standardized protocols will be essential to overcome methodological barriers and improve representation of embryos in conservation physiology research.

Logistical constraints – In addition to methodological constraints, *logistical* factors may further limit the study of embryos from wild animals. Many early life stages occur in cryptic, transient, or inaccessible habitats, complicating their detection, collection, and experimental use. For instance, some aquatic species spawn in ephemeral pools or submerged substrates, while some terrestrial embryos may develop underground or within complex microhabitats, making field sampling labor-intensive and time-sensitive. Some taxa, such as anguillid eels, illustrate extreme logistical challenges where natural spawning grounds and embryonic stages remain poorly understood or unobservable (Tsukamoto, 2009), impeding ecologically relevant laboratory studies. Seasonality also constrains access to embryos, as reproductive events may be brief and highly synchronized (Lowerre-Barbieri *et al.*, 2011; Low, 2014), requiring precise timing for sample collection. These temporal limitations reduce flexibility in experimental design and can limit replication or longitudinal studies across developmental stages. Moreover, the need to maintain controlled environmental conditions during embryonic development adds logistical complexity, particularly for species with specific habitat requirements or sensitive life stages. In some species, thermal, moisture, or chemical conditions required by early life stages to thrive can be hard to replicate in the laboratory, requiring equipment and techniques that may differ from those routinely used for adult life stages (El-Ghali *et al.*, 2010, 2010; Ellis-Hutchings and Carney, 2010). Collectively, these logistical challenges compound methodological difficulties, contributing to the pervasive underrepresentation of embryos in conservation physiology research. Addressing these barriers will require coordinated efforts to improve field sampling techniques, develop flexible rearing protocols, and expand access to specialized laboratory equipment.

4.2 Future recommendations and concluding remarks

Our findings highlight the need for a more life-stage inclusive approach in conservation physiology research. The strong and consistent underrepresentation of embryonic stages across journals, taxa, traits, and climatic stressors suggests that this ontogenetic bias is pervasive and persistent, rooted in a combination of historical precedent and enduring methodological and logistical barriers. Addressing this gap is critical to improving the predictive power and ecological realism of conservation physiology research.

First, we recommend a deliberate shift toward understanding sensitivity to ecological stressors across multiple life stages, particularly embryos and larvae. Multi-stage studies can reveal hidden vulnerabilities that may be missed when focusing solely on adult stages. For example, early-life exposures to thermal, nutritional, or chemical stressors can induce physiological carry-over effects that influence later-life performance, survival, and reproductive success (Grandjean *et al.*, 2015; Noble *et al.*, 2018; Macartney *et al.*, 2019; Bodensteiner *et al.*, 2021; Wu *et al.*, 2025). However, among the few studies that specifically exposed embryos to climatic stressors, only 11% assessed potential carry-over effects in adulthood. Moreover, only 5% of studies on adults and 15% of studies on juveniles also measured traits on embryos (Fig. 3). Including underrepresented stages in physiological experiments would allow researchers to detect developmental trade-offs, identify critical windows of sensitivity, and better pinpoint demographic bottlenecks. Conservation physiology would benefit from increased integration of such longitudinal and life-stage inclusive designs, where trait expression and fitness are understood as dynamic, cumulative processes shaped across the life cycle (Fawcett and Frankenhuis, 2015; Hodgson *et al.*, 2016; Marshall *et al.*, 2016). These perspectives are key, particularly when aiming to forecast population responses to environmental change or design targeted conservation measures.

Second, improving the feasibility of embryo-focused studies will require coordinated investment in tools, protocols, and collaborations. Advances in non-invasive imaging (Keller *et al.*, 2008; Ibbini *et al.*, 2022; McCoy *et al.*, 2023; Tills *et al.*, 2025), molecular techniques (El-Ghali *et al.*, 2010; Wu *et al.*, 2020), and specialized instrumentation (Lovegrove, 2009; Kong *et al.*, 2016; Cowan *et al.*, 2023) have already begun to lower the technical barriers to studying small and fragile life stages. Recognition of the unique biology of embryos has also called for the standardisation of methods across ontogeny (Pottier *et al.*, 2022a), giving birth to interesting new experimental techniques (Cowan *et al.*, 2023). To accelerate progress, the field should prioritize the development and open sharing of standardized, scalable protocols tailored to embryonic and larval physiology, such as those developed in biomedical sciences and toxicology. Promoting embryo-based research has already gained substantial traction in these fields, often serving as a foundational step preceding investigations of later developmental stages. In fact, the Organisation for Economic Co-operation and Development (OECD) has already established standardized guidelines and protocols specifically tailored for embryo studies, ensuring consistency and reproducibility across research efforts. Adapting similar guidelines to the context of conservation physiology would be an effective way for research efforts on embryos to gain traction.

Third, we encourage the consideration of embryo-focused studies to align with ethical considerations for animal experimentation, particularly the 3Rs (*Replace, Reduce, Refine*). In many jurisdictions, experimentation on vertebrate embryos is exempt from ethical permit requirements until specific developmental milestones—often the onset of independent feeding (e.g., fishes) or a defined stage of embryonic development (e.g., reptiles, birds; experiments on viviparous species, however, typically require additional ethical consideration). This regulatory gap allows researchers to design and conduct studies on embryos more flexibly, without delays tied to permit approval. This is especially useful for short-term projects, such as those led by visiting students or early-career researchers with strict time constraints. Therefore, we see studies on embryos not only as an opportunity to tackle exciting research questions, but also as means to reduce reliance on more developed animals with stricter ethical regulations.

Fourth, while our synthesis focused on a subset of journals and physiological traits, the striking consistency of ontogenetic biases supports the generality of our findings. Nevertheless, future work could expand this mapping effort to include additional journals, trait categories (e.g., behavior, morphology), environmental stressors (e.g., toxicants) and life stage definitions (e.g., young larva vs. fully developed juvenile), as well as comparisons between aquatic and terrestrial systems. Fine-scale resolution of these patterns would help clarify which taxa or subfields have generated the most knowledge, and where the greatest opportunities remain for growth. We also encourage more detailed reporting of life stages in experimental studies. We found that over 5% of studies did not specify the exact life stage used (Fig. 1-2). While distinguishing life stages can be challenging in some species—especially when morphological changes are subtle and when ontogenetic variation is not a main study objective—providing clear descriptions of the likely life stage(s) used would improve interpretability and facilitate comparisons across studies.

Fifth, developing theoretical models for why differences in life stage-specific responses exist presents an extremely exciting avenue for research. Differences in life-stage specific response have been linked to size (Klockmann *et al.*, 2017; Medina-Báez *et al.*, 2023), mobility (e.g., pupal vs mobile adults, (Moghadam *et al.*, 2019), energy trade-offs between growth, maturation and reproduction (Makarieva *et al.*, 2004; Pörtner *et al.*, 2006; Sousa *et al.*, 2010), and the degree of phenotypic plasticity in response to a stressor (Pottier *et al.*, 2022b; Walasek *et al.*, 2022). Potential mechanisms have been proposed for differences in environmental sensitivity but not compressively assessed across the tree of life. For example, underdeveloped cellular repair mechanisms in developing insects (Bowler and Terblanche, 2008), tadpoles adjusting their energy investment under different stressors (Ruthsatz *et al.*, 2019), or mismatches between oxygen supply and demand in fishes (Dahlke *et al.*, 2020) are potential mechanisms. Across life stages, environmental cues also vary widely in their reliability and how they contribute to fitness, directly affecting the benefits and costs of changing phenotypes (Fawcett and Frankenhuis, 2015). A mixture of these mechanisms likely explains the variety of life-stage specific responses to ecological stressors, but elucidating shared responses among species can help conservation physiologists build better predictive models of species vulnerability to climate change.

In conclusion, the pervasive underrepresentation of early life stages in conservation physiology research likely reflects a legacy of structural constraints and adult-centric thinking that continues to shape research agendas. Yet, the biological rationale for studying embryos is compelling: they represent both a vulnerable life stage and a powerful window into the mechanisms of developmental plasticity, environmental sensitivity, and long-term fitness. Failing to address these biases risks overlooking key demographic bottlenecks, ultimately limiting our ability to predict species' responses to global change and design effective conservation strategies. More ontogenetically inclusive research in conservation physiology is not only increasingly feasible thanks to emerging methods for measuring environmental sensitivity across life stages, but also essential for meeting the challenges of biodiversity loss and climate adaptation in the decades to come.

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7. Author contributions

Conceptualization: PP, KR

Data curation: PP, KR

Formal analysis: PP

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Investigation: NCW, KA, AC, ZLC, MLE, SSK, ML, JCSM, ECGM, RA, MM, LP, AKP, DMR, BS, RV

Methodology: PP, ML, KR

Project Administration: PP, KR

Resources: PP

Software: PP

Supervision: PP, KR

Validation: PP, KR

Visualization: PP, NCW, MLE

Writing – Original Draft Preparation: PP, KR

Writing – Review & Editing: All authors.

Detailed contributions are listed in Tables S2-3, following Martinig *et al.* (2025)'s recommendations.

8. Conflicts of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

9. Statement of Ethics

Not applicable.

10. Funding

This project was not directly supported by a funding source. Nevertheless, this project was invited by the journal Conservation Physiology in response to a symposium organized at the Society for Experimental Biology Centenary conference (2023). The Society for Experimental Biology and The Company of Biologists (Scientific Meeting Grant EA735) provided funding to support expenses for this session, which made this project possible. PP is currently supported by a Wenner-Gren Stiftelserna postdoctoral fellowship (UPD2024-0239) and was supported by a Scientia PhD scholarship from the University of New South Wales during parts of this project. KR is currently supported by a Marie Sklodowska Curie Postdoc Fellowship AMPHISTRESS (no. 101151070) and was supported by German Research Foundation (Deutsche Forschungsgemeinschaft) project (459850971; A new perspective on amphibians and global change: Detecting sublethal effects of environmental stress as agents of silent population declines) during parts of this project. DMR is funded by Tamkeen under grant AD004. ZLC is supported by a Starting Grant in Natural and Engineering Sciences from the Swedish Research Council (Vetenskapsrådet) (registration number: 2023-03262). JCSM is supported by a Standard Grant from the Biotechnology and Biological Sciences Research Council (BBSRC) (BB/W004305/1). MLE is supported by a British Columbia Ministry of Land, Water, and Resource Management Grant. MM is supported by the Research Council of Norway (contract number 342628/L10). RA is supported by a Hellenic Foundation for Research and Innovation Grant (Project ID: 015424). ML is supported by Australian Research Council (ARC) Discovery Project Grants (DP210100812 and DP230101248). AKP is supported by by a European Commission Horizon 2020 Marie Sklodowska Curie Actions Global Research Fellowship (101022401). BJS is supported by the National Key Research Development Program of China (2022YFF0802300) and National Natural Science Foundation of China (32271572).

11. Data availability

All data and materials used in this study are available publicly (https://github.com/p-pottier/Cons_phys_life_stages) and will be archived permanently in Zenodo upon acceptance.

Supplementary Information

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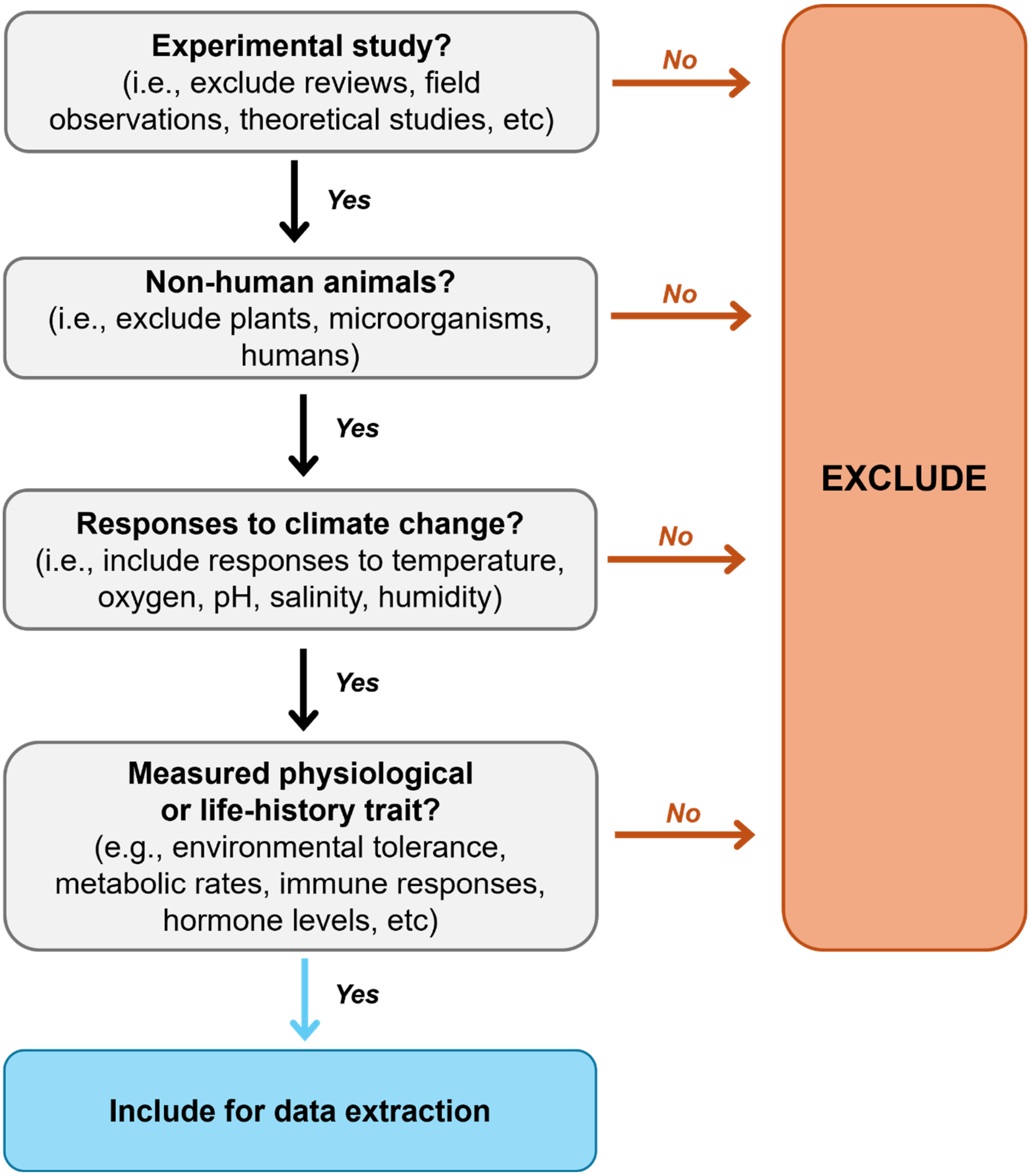

Figure S1: Decision tree used to screen studies for eligibility. When the title, abstract, or keywords provided insufficient detail to assess eligibility, the full article was examined.

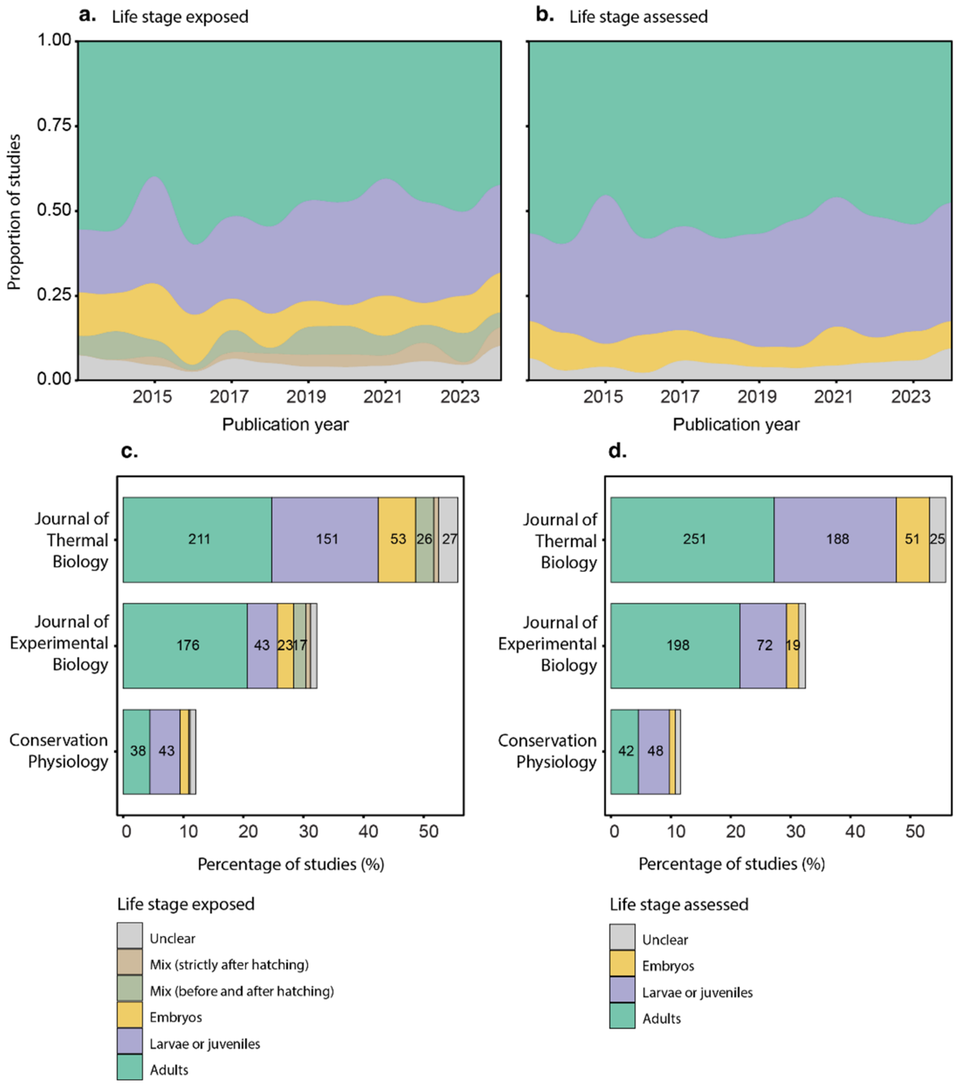

Figure S2: Differences in the relative proportion of life stages exposed to temperatures (**a**) or assessed for physiological traits after temperature exposures (**b**) over time, and across three representative journals surveyed (**c** exposed, and **d** assessed). Sample sizes (counts of studies) are presented for each category.

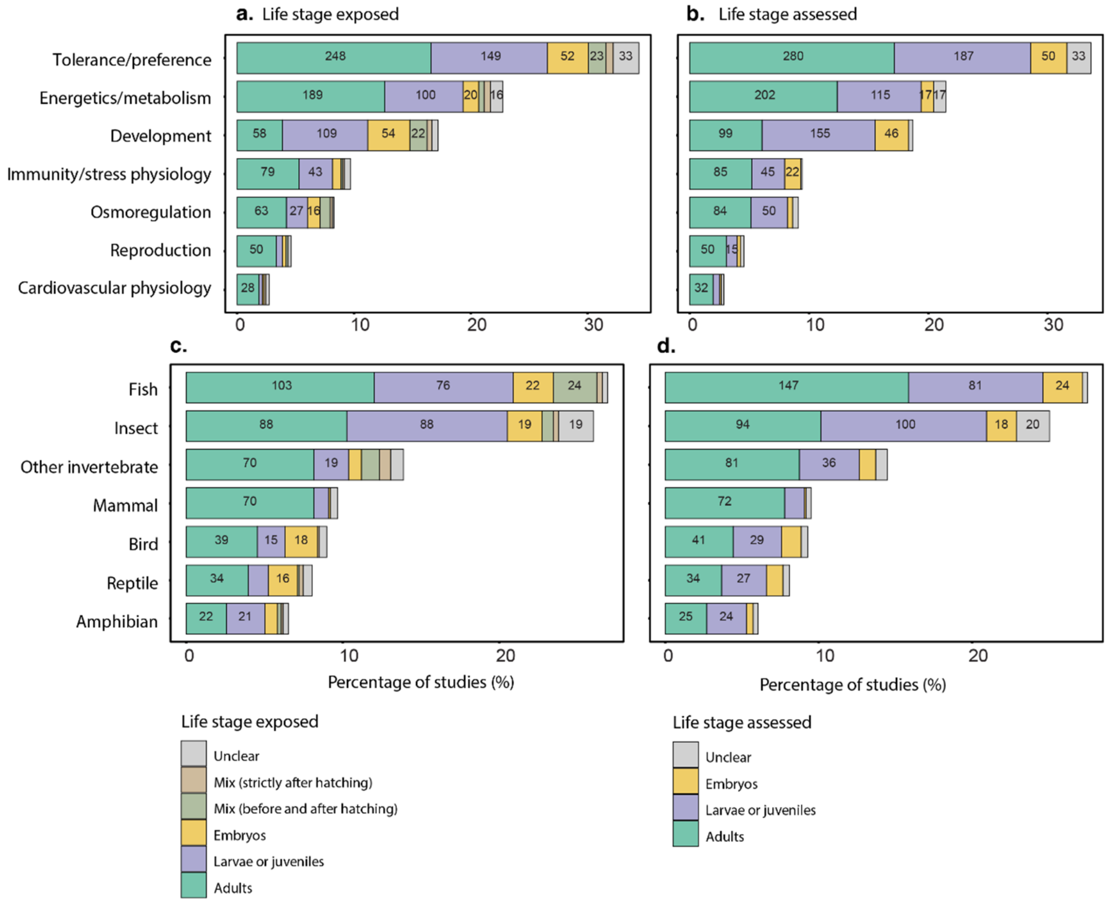

Figure S3: Differences in the relative proportion of life stages exposed to temperatures (**a, c, e**) or assessed for physiological traits after temperature exposures (**b, d, f**) across the traits (top row) and taxa (bottom row) surveyed.

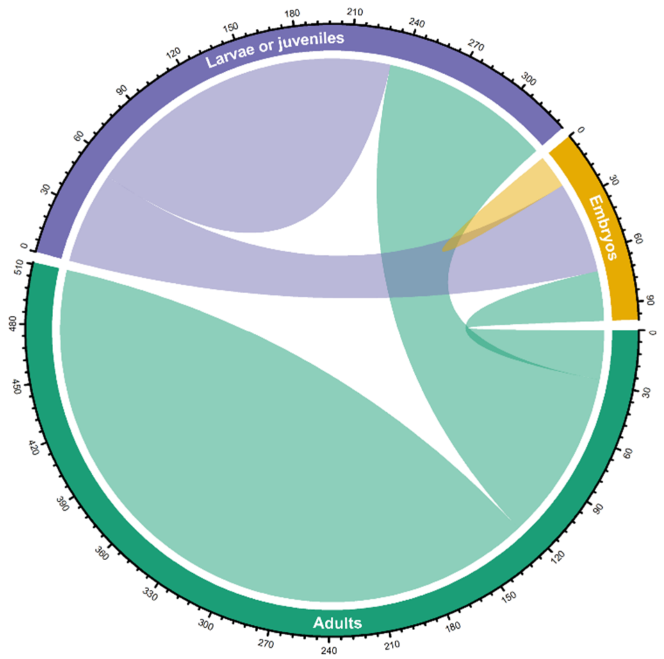

Figure S4: Chord diagram illustrating studies measuring traits on single or multiple life stages in a subset of studies on temperatures. Categories that are connected represent studies that investigated multiple life stages. Numbers in the outer circle represent the number of studies.

Table S1: Questions and instructions used in Google Forms to facilitate data extraction. This form was distributed to all the researchers responsible for extracting data from studies.

Question	**Description**	**Response options**
Your initials	E.g., PP	Short-answer text
Short reference	Use FirstAuthorName_et_al_YEAR **for studies with 3 or more authors** (e.g., Ruthsatz_et_al_2024) Use FirstAuthorName_and_SecondAuthorName_YEAR **for studies with 2 authors** (e.g., Ruthsatz_and_Pottier_2024) Use FirstAuthorName_YEAR **for studies with a single author** (e.g., Ruthsatz_2024)	Short-answer text
Title	Copy and paste title directly from Rayyan - **do not type**.	Short-answer text
DOI	Copy and paste DOI directly from Rayyan - **do not type**. 4 studies do not have their DOI displayed in Rayyan. If you encounter one of these, please google the article and find the DOI. Paste the DOI without the URL (e.g., "10.1242/jeb.138784")	Short-answer text
Journal	NA	Conservation Physiology; Journal of Thermal Biology; Journal of Experimental Biology
Taxonomic group	If the authors have used multiple taxonomic groups (e.g., one predatory fish and one invertebrate prey), tick multiple boxes.	Bird; Mammal; Fish; Reptile; Amphibian; Insect; Other invertebrate. Multiple responses allowed.
Climate change stressor	Tick the **climate change** stressor(s) the authors have **manipulated** during the experiments. Most studies manipulate a single stressor, but some may have used factorial designs with multiple stressors (e.g., temperature and acidification). Please note that you can use the "Other" category for additional climatic stressors that are not captured below, but these must be direct climate change stressors (e.g., not pesticides, light pollution, urbanisation, etc). If the authors used an experiment with an interaction between a climatic stressor **and** a non-climatic stressor (e.g., pollutant, disease, urbanisation), you can select "Interaction with non-climatic stressor". If they only used a non-climatic stressor, this study does **not** match our inclusion criteria.	Temperature; Oxygen; pH; Salinity; Humidity; Interaction with non-climatic stressor; Other (open text). Multiple responses allowed.
Life stage exposed to the stressor	Here, select the life stage **exposed** to the manipulated stressor (temperature, oxygen, humidity, pH, or salinity). If the authors performed separate experimental exposures on different life stages, select each life stage that applies. **However**, if the exposure **overlaps multiple life stages** (exposure from fertilisation to adulthood), select one of the "Mix" categories:	Embryos; Larvae or juveniles; Adults; Mix (before and after hatching); Mix (strictly after hatching); Unclear; Other (open text). Multiple responses allowed.

	• "*Before and after hatching*" refers to exposures that started before hatching (e.g., exposure from eggs to adults), • "*Strictly after hatching*" refers to exposures that started at the larval or juvenile stage. It is often easy to tell based on the abstract (e.g., look out for words such as "adults", "larvae", or "juveniles). However, there will be cases when you will need to dive into the PDF. Note also that there is a option "Unclear". Only use this option when the authors do not report what life stages were used, and these cannot be inferred. Please do not include cases where cells or organs were isolated from the animals before the exposure to the climatic stressor. We are interested in the responses of whole living organisms to climatic stressors.	
Life stage of the animals when traits were measured	Here, select the life stage of the animals **when the traits of interest were measured**. This can be different from the previous question. For instance, one study may incubate eggs at different temperatures and measure the oxygen consumption of embryos (in which case "embryos" should be selected in both questions). However, other studies may measure traits at a later point (e.g., at the juvenile stage) after incubating eggs to the climate change stressor (e.g., temperature). If authors measure traits in multiple life stages, select all that apply. Note also that there is a option "Unclear". Only use this option when the authors do not report what life stages were used, and these cannot be inferred.	Embryos; Larvae or juveniles; Adults; Unclear; Other (open text) Multiple responses allowed
Trait category	**Environmental tolerance and preference** --> survival or tolerance to different stressors (temperature, pH, hypoxia, salinity), habitat selection, thermoregulation, heat shock proteins, etc. **Energetics and metabolism** --> oxygen uptake, metabolic rate, aerobic scope, digestion efficiency, etc. **Osmoregulation** --> Ion balance, water loss, acid-base regulation, excretion, etc. **Cardiovascular physiology** --> Blood pressure, heart rate, stroke volume, etc. **Immune function and stress physiology** --> stress hormones, immune competence, oxidative stress, etc. **Reproduction** --> fecundity, sex hormones, gametogenesis, sperm count, etc. **Development** --> growth rate, body size, phenology, etc.	Environmental tolerance and preference; Energetics and metabolism; Osmoregulation; Cardiovascular physiology; Immune function and stress physiology; Reproduction; Development; Other (open text). Multiple responses allowed

	Use "Other" if none of these fit. <u>**However**</u>, remember that we are **only** interested in physiological and life-history traits, so most relevant traits are likely to fit into these categories. If authors measured physiological and/or life-history traits along with other traits (e.g., behaviour, morphology), do not use the "Other" category to add additional traits that are not relevant to our study. For instance, we are <u>**not**</u> interested in behavioural traits (e.g., dispersal, exploration, activity, learning, cognition, etc), morphological traits (e.g., body shape, abnormalities, pigmentation, coloration, etc), ecological interactions (e.g., predator-prey interactions, symbiosis, microbiome diversity), biodiversity parameters (e.g., abundance, species richness, heterozygosity), etc. Although we don't include morphological traits, note that we <u>include</u> measures of <u>whole animal size</u> (e.g., body size, body mass, snout-vent length, size at metamorphosis etc) in the "Development" category. Although we excluded cases where cells or organs were isolated from the animals <u>before</u> the exposure to the climatic stressor, we include physiological traits measured on cells/organs taken from living organisms <u>during/after</u> the exposure to the climatic stressor.	
Trait details	Indicate the specific traits that were measured (as described by the authors), **separated by semi colons.** For example, "development time; oxygen consumption".	Long-answer text
Additional comments	If you have important comments, please indicate them here. Otherwise, leave this question blank.	Long-answer text

Table S2: Authorship contributions according to the Dragon Kill Points guidelines (Martinig et al. 2025).

Contribution	**Author initials**
Conference session organisation	PP, NCW, KR
Communication with the journal Conservation Physiology	PP, KR
Study conceptualisation	PP, KR
Study design feedback	PP, ML, KR
Pre-registration (original draft)	PP
Pre-registration (review & editing)	PP, ML, KR
Literature searches	PP
Literature screening	NCW, MLE, ML, KA, AC, ZLC, SSK, JCSM, ECGM, RA, MM, LP, AKP, DMR, BS, RV
Data extraction	NCW, MLE, ML, KA, AC, ZLC, SSK, JCSM, ECGM, RA, MM, LP, AKP, DMR, BS, RV
Data extraction quality checks	PP, KR
Data cleaning and processing	PP
Figures (original draft)	PP
Figures (feedback)	NCW, MLE, KR
Figures (cosmetic adjustments)	PP, NCW, MLE
Manuscript introduction and discussion (original draft)	KR
Manuscript methods and results (original draft)	PP
Manuscript introduction and discussion (second draft)	PP, NCW, KR
Manuscript methods and results (second draft)	PP, NCW, KR
Manuscript (review and editing)	PP, NCW, MLE, ML, KA, AC, ZLC, SSK, JCSM, ECGM, RA, MM, LP, AKP, DMR, BS, RV, KR
Project administration	PP, KR

Table S3: Authorship contribution points, according to the Dragon Kill Points guidelines (Martinig et al. 2025)

Author initials	**Authorship points ("Dragon Kill Points")**
PP	16
NCW	8
MLE	5
ML	5
KA	3
AC	3
ZLC	3
SSK	3
JCSM	3
ECGM	3
RA	3
MM	3
LP	3
AKP	3
DMR	3
BS	3
RV	3
KR	12