

# Global Change Biology

## Acclimation Capacity to Global Warming of Amphibians and Freshwater Fishes: Drivers, Patterns, and Data Limitations

**Running title:** *Acclimation: Amphibians and Freshwater Fishes*

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## Abstract

Amphibians and fishes play a central role in shaping the structure and function of freshwater environments. These organisms have a limited capacity to disperse across different habitats and the thermal buffer offered by freshwater systems is small. Understanding determinants and patterns of their physiological sensitivity across life history is, therefore, imperative to predicting the impacts of climate change in freshwater systems. Based on a systematic literature review including 345 studies with 998 estimates on 96 amphibian and 93 freshwater fish species, we conducted a meta-analysis to explore phylogenetic, ontogenetic, and biogeographic (i.e. thermal adaptation) patterns in upper thermal tolerance (CT<sub>max</sub>) and thermal acclimation capacity (Acclimation Response Ratio, ARR) as well as the influence of the methodology used to assess these thermal traits using a conditional inference tree analysis. We found CT<sub>max</sub> and ARR differed between taxa, pre- and post-metamorphic life stages as well as with thermal adaptation. The ARR of freshwater fishes exceeded that of amphibians by more than twice across life stages. In amphibians, CT<sub>max</sub> decreased throughout early development, with juveniles exhibiting the lowest heat tolerance, potentially representing a life history bottleneck if other strategies to reach thermal refugia, e.g. through behavioral thermoregulation, would also be constrained. In contrast to the broader literature, CT<sub>max</sub> was not generally higher in low latitude populations but also varied with ontogeny, emphasizing the importance of assessing life stage-specific sensitivity to thermal stress. Importantly, the application of different methods (e.g. acclimation duration, ramping rates) changed life stage, phylogeny, and thermal adaptation patterns in CT<sub>max</sub> and ARR. Our analyses highlight biases and data limitations with respect to coverage in taxonomy, biogeographic distribution of species, life stage, and study design. We propose methods to improve robustness and comparability of thermal sensitivity knowledge needed to adopt interventions to safeguard freshwater biodiversity in a future climate.

## 1. Introduction

Global climate change is not only causing an increase in mean air and water temperatures, but also an increased magnitude and frequency of extreme climatic events (Lee et al. 2023). As a result, ectotherms are more likely to experience temperatures beyond their critical thermal maximum ( $CT_{max}$ ) in both terrestrial and aquatic habitats (Duarte et al. 2012; Sunday et al. 2014). This is particularly true for populations already living close to their upper thermal limit. Consequently, the ability to mitigate thermal stress through either migration, evolutionary genetic adaptation or acclimation is crucial for the persistence of species in a changing climate (Franks & Hoffmann 2002; Huey et al. 2012; Seebacher et al. 2015). Given the limited dispersal ability of many species (e.g., freshwater species; Woodward et al. 2010) and rapid pace of global warming (Hoffmann & Sgró 2011), physiological acclimation is arguably the most important mechanism for coping with climate change (Gunderson & Leal 2015). Understanding differences in acclimation capacity of species and identifying global patterns can therefore help to identify climate change risks to biodiversity and develop effective conservation measures (Somero 2010).

As an adaptive response to larger seasonal differences in temperature, thermal tolerance and acclimation capacity of ectothermic species or populations tend to increase with increasing latitude from tropical through temperate climate zones (e.g., Somero 2005; Deutsch et al. 2008; Sunday et al. 2011; Peck et al. 2014; Rohr et al. 2018; Cicchino et al. 2023; but see: Sørensen et al. 2016; Gunderson & Stillman 2015) and from higher to lower elevations (Enriquez-Urzelai et al. 2020; but not: Sunday et al. 2019). This biogeographical pattern is consistent with the *climate variability hypothesis* (Janzen 1967; Ghalambor et al. 2006), suggesting that climatic differences across latitudinal gradients lead to corresponding adaptations in thermal physiology. Low-latitude species adapted to relatively stable temperature conditions may have a lower acclimation capacity and, therefore, may be more vulnerable to climate change (Tewksbury et al. 2008; Sunday et al. 2014; but see: Bovo et al. 2023). However, there is still little empirical evidence supporting the climate variability hypothesis, possibly due to the limited geographical and phylogenetic coverage of observations, and because of the inconsistency of the methods applied to measure the acclimation capacity of different species and life stages (Gutiérrez-Pesquera et al. 2016; Shah et al. 2017). Moreover, the use of different methods or protocols might impact the estimation of thermal traits (Terblanche et al. 2007; Chown et al. 2009; Rohr et al. 2018; Pottier et al. 2022a; but not: Sunday et al. 2019). For example, acclimation duration (i.e., how long organisms were held at an acclimation temperature before exposing them to the

test temperature) and ramping protocol (i.e., heating or cooling rate in thermal tolerance trials) have been suggested to influence measurements of acclimation capacity, as the underlying physiological processes occur over certain time periods.

In animals with complex life histories, thermal tolerance and acclimation capacity are thought to change during ontogeny according to physiological and morphological reorganizations and concomitant aerobic capacities in relation to oxygen demand (Pörtner 2002; Pörtner & Peck 2010; Ruthsatz et al. 2020a, 2022a) as well as energetic costs associated with developmental processes (Ruthsatz et al. 2019). Furthermore, life stages might differ in their ability for behavioral thermoregulation (Navas et al. 2008; Little & Seebacher 2017) resulting in stage-specific adaptations in thermal traits (Huey et al. 1999). Therefore, determining taxon-specific acclimation capacity at different ontogenetic stages should be taken into account when studying climate adaptation of ectothermic species, as it will help identify life cycle bottlenecks and provide robust data on the vulnerability of populations or species to global warming (Bodensteiner et al. 2021; Pottier et al. 2022a; Dahlke et al. 2022). To date, most studies have focused on adult life stages when addressing the *climate variability hypothesis* (e.g., Gunderson & Stillman 2015; Sunday et al. 2011, 2014, 2019; Rohr et al. 2018) and the vulnerability of species to global change (e.g., Calosi et al. 2008; Comte & Olden 2017a; Morley et al. 2019; Molina et al. 2023), or have pooled several pre- and post-metamorphic life stages (Pottier et al. 2022a; Weaving et al. 2022) thereby risking to overlook a critical thermal bottleneck in the life cycle (Dahlke et al. 2020). The extent to which thermal tolerance and acclimation capacity change during ontogeny is, therefore, not clear for many taxa.

Amphibians and freshwater fishes tend to live in relatively shallow waters (e.g., wetlands, ponds, rivers, lakes) and may, therefore, experience strong seasonal and daily temperature fluctuations (Capon et al. 2021) and climate extremes such as heat waves (IPCC 2021). In addition, both taxa have a limited ability to disperse over larger distances and habitats to avoid unfavorable climatic conditions (Albert et al. 2011; Yu et al. 2013; Campos et al. 2021). Consequently, as a result of local adaptation (Meek et al. 2023), there should be a close correspondence between the capacity for thermal acclimation and the climatic conditions that amphibians and freshwater fishes experience during their life cycle. However, freshwater systems offer a wide range of thermal microhabitats that enable behavioral thermoregulation (Campos et al. 2021), especially for (post-metamorphic) amphibians that can switch between water and land. The potential of behavioral thermoregulation could reduce the need for physiological adaptations (also known as the *Bogert Effect*, (Bogert 1949)) and thus counteract

the emergence of geographical patterns in thermal acclimation capacity and/or thermal tolerance. Given the central role that amphibians and freshwater fishes play in shaping the structure and function of these ecosystems (Closs et al. 2016; Hocking & Babbitt 2014), understanding determinants and patterns of their physiological sensitivity is imperative to predicting the impacts of climate change on freshwater systems.

Here, we aimed to define the determinants and patterns of acclimation capacity in upper thermal tolerance in amphibians and freshwater fishes. To do so, we compiled literature on upper thermal tolerance and collected empirical data for  $CT_{max}$  in four amphibian (i.e., larvae, metamorphs, juveniles, and adults) and three freshwater fish (i.e., larvae, juveniles, and adults) life stages acclimated to different temperatures. Next, we calculated the population-specific acclimation capacity, i.e., mean acclimation response ratio (ARR) of upper thermal limits, and conducted a meta-analysis on the acclimation capacity of amphibians and freshwater fishes to test for differences among taxonomic groups, among life stages, and across thermal characteristics of populations, i.e., latitudinal and altitudinal differences based on local thermal adaptation. Further, we investigated how the methodological context, i.e., acclimation duration, ramping rate, affects estimates of thermal traits. Finally, we summarize methodological concerns, highlight key knowledge gaps and provide research recommendations for generating reliable and comparable data on the acclimation capacity of ectothermic species and life stages. This will improve our ability to predict future climate vulnerability of species and populations.

## 2. Materials and Methods

### 2.1 Systematic literature review

We conducted a systematic literature review using ISI Web of Science (ISI WOS, 2021) on 2022/06/30 and did not apply a timespan limit. The following Boolean search string was used to capture studies manipulating acclimation temperatures of amphibians and freshwater fishes at different life stages, and subsequently measured their  $CT_{max}$ : (“amphibian\*” OR “newt\*” OR “frog\*” OR “toad\*” OR “salamander\*” OR “freshwater” AND “fish\*”) AND (“early” OR “young” OR “life stage\*” OR “ontogen\*” OR “development\*” OR “hatchling\*” OR “alevin\*” OR “larv\*” OR “tadpole\*” OR “metamorph\*” OR “postmetamorph\*” OR “post-metamorph\*” OR “postlarva\*” OR “post-larva\*” OR “fry\*” OR “parr\*” OR “smolt\*” OR “subadult\*” OR “sub-adult” OR “juvenile” OR “fingerling\*” OR “adult\*”) AND (“thermal” OR “temperature” OR “acclimat\*” OR “heat” OR “warm\*”) AND (“tolerance\*” OR “thermal tolerance\*” OR “temperature tolerance\*” OR “warming tolerance\*” OR “heat tolerance\*” OR “thermal stress tolerance\*” OR “heat stress tolerance\*” OR “temperature stress tolerance\*” OR “limit\*” OR

“temperature stress\*” OR “thermal limit\*” OR “critical temperature\*” OR “CT max” OR “critical thermal m\*” OR “thermal performance breadth\*” OR “thermal breadth\*” OR “performance breadth\*” OR “thermal range\*” OR “thermal window\*” OR “thermal tolerance window\*” OR “tolerance window\*” OR “sensitivity\*” OR “thermal sensitivity”).

Our search resulted in 11,057 documents (Fig. S1). After removing book chapters, conference contributions, reviews, meeting abstracts, editorial material, preprints, and proceedings articles, 10,740 published peer-reviewed articles remained in our initial database. Additionally, we manually added articles included in the meta-analyses of Claussen (1977), Gunderson and Stillman (2015), Comte & Olden (2017b), Morley et al. (2019) and Dahlke et al. (2020) that met our inclusion criteria but were not obtained through the ISI Web of Science search. After an initial subjective evaluation of titles, 3,991 articles potentially containing results matching the objective of the present study were kept and further assessed for eligibility using the abstract. Thirty-four articles were not accessible. We contacted the authors of the original studies to request missing information and heard back from two authors. Finally, a total of 93 articles (34 on amphibians; 59 on freshwater fishes) met our inclusion criteria. Search methods are summarized in a PRISMA flowchart (Fig. S1) and a list of included studies is available in the figshare data repository under DOI:XXX (to be included after manuscript acceptance).

## 2.2 Inclusion criteria

Studies were selected based on the following eight inclusion criteria:

- (1) Studies were conducted on amphibians (anurans, caudates, or gymnophiones) or freshwater fishes (teleosts).
- (2) Experiments were conducted under laboratory conditions (i.e., no field studies).
- (3) Articles provided comprehensive information on methodology (acclimation temperatures and duration, ramping rate), phylogeny (species names), sampling location (GPS location), and life stage. If no GPS coordinates were provided but a concrete sampling location was stated (e.g., Central Park, New York City, NY, USA), we searched for the coordinates of the respective location on Google Maps.
- (4) Animals were collected from their natural habitat. Data were excluded if measurements were taken from specimens bred artificially to reduce confounding issues associated with artificial selective history (Bennett et al. 2018). Studies were also included if adult animals were collected to immediately reproduce in the lab to obtain larvae.

(5) The critical thermal maximum ( $CT_{max}$ ) was used as a standard measure of heat tolerance (Lutterschmidt & Hutchison 1997). Studies using other measures such as voluntary thermal maximum, time to death, heat knockdown, or lethal temperatures as well as extrapolations from thermal performance curves were not considered. In aquatic and terrestrial ectotherms such as amphibians and fish,  $CT_{max}$  is generally measured as loss of equilibrium (LOE) or loss of righting response (LRR) following a steady increase in water or air temperature [dynamic method according to Fry (1947)]. In comparison to other endpoints such as the onset of spasms (Lutterschmidt & Hutchison 1997), measuring  $CT_{max}$  as LOE or LRR is a non-lethal, robust method at various body sizes that is repeatable within individuals (Morgan et al. 2018).

(6) At  $CT_{max}$  measurements, the animals could be classified into one of four different categories representing the consecutive life stages of amphibians and freshwater fish: (a) larva (pre-metamorphic; amphibians: < Gosner stage 42), (b) metamorph (only for amphibians: Gosner stage 42-46), (c) juvenile (post-metamorphic), and (d) adult (after reaching sexual maturity). Embryos were not included in the present study because the assessment of acute heat tolerance in non-mobile life stages requires different endpoints that may not be directly comparable to the LOE/LRR-based  $CT_{max}$  of other life stages (Cowan et al. 2023; Lechner et al. 2023).

(7) Animals were acclimated to at least two constant acclimation temperatures prior to the  $CT_{max}$  measurements. Therefore, fluctuating treatments were not considered.

(8) Food was provided *ad libitum* during the acclimation time since food deprivation might decrease thermal tolerance and/or acclimation capacity (Lee et al. 2016).

### 2.3 Data extraction

When all inclusion criteria were met, data were collated in a spreadsheet. We extracted mean  $CT_{max}$  for all acclimation temperatures resulting in 998 single data points (513 for amphibians; 485 for freshwater fish). Some of these articles performed different studies on e.g., different populations of one species, different species, or different life stages. Therefore, all available datasets were included, resulting in 345 studies from 93 articles with 345 paired effect sizes  $CT_{max}$  and acclimation capacity. Data presented in the text or tables were directly extracted from the article. When only raw data were available, mean values were calculated. For studies that presented results in figures instead of tables, Engauge Digitizer 12.1 was used (Mitchell et al. 2021) to extract data from the graphs. In addition to  $CT_{max}$  data, information on the

methodology (i.e., acclimation temperatures and duration, ramping protocol), as well as on variables representing sampling location as detailed as possible (i.e., GPS coordinates), phylogeny (i.e., scientific classification according to the Linnean classification), and life stage at CT<sub>max</sub> assessment was extracted. The data extractions were performed by KR, KA and PCE, followed by an accuracy check of the data (KA: freshwater fish sub dataset; KR: amphibian sub dataset; PCE: both sub datasets).

#### 2.4 Bioclimatic variables

For each sampling location, 19 bioclimatic metrics, related to temperature and precipitation, and elevation were extracted using the WorldClim 2 database (<http://www.worldclim.org/>; Fick & Hijmans 2017) for the average of the years 1970–2000. The data were extracted at a spatial resolution of 30 seconds (~1 km<sup>2</sup>), using package ‘geodata’ (Hijmans 2021) in R (version 4.2.1; R Core Team, 2020). Bioclimatic variables are coded as follows: Annual Mean Temperature (bio1), Mean Diurnal Range (bio 2), Isothermality (bio 3), actual Temperature Seasonality (bio 4), Maximum Temperature of Warmest Month (bio 5), Minimum Temperature of Coldest Month (bio 6), Annual Temperature Range (bio 7), Mean Temperature of Wettest Quarter (bio 8), Mean Temperature of Driest Quarter (bio 9), Mean Temperature of Warmest Quarter (bio 10), Mean Temperature of Coldest Quarter (bio 11), Annual Precipitation (bio 12), Precipitation of Wettest Month (bio 13), Precipitation of Driest Month (Bio 14), Precipitation Seasonality (bio 15), Precipitation of Wettest Quarter (bio 16), Precipitation of Driest Quarter (bio 17), Precipitation of Warmest Quarter (bio 18), and Precipitation of Coldest Quarter (bio 19) (<http://www.worldclim.org/data/bioclim.html>). These macroclimatic data were used as approximations to identify patterns of local adaptation in amphibians and freshwater fish, as microclimatic data (e.g. site-specific temperatures) were not available in original articles or in the WorldClim database. Following previous studies (Morley et al. 2019; Carilo Filho et al. 2022), mean near-surface air temperature was assumed to reflect the temperature profile of freshwater systems and used to analyze thermal adaptation in both taxa. WorldClim (representing surface air temperatures) was selected for both realms. In contrast to marine habitats, the temperature of small or shallow bodies of water might fluctuate with the surface air temperature and animals at the surface of freshwater systems might be further exposed to high temperatures. Therefore, we assume that the average near-surface air temperature is a suitable estimate of the temperature of freshwater systems, thereby reflecting the thermal local adaptation of investigated amphibian and freshwater populations. This is in accordance with previous studies testing the effect of thermal adaptation on the thermal physiology of various

taxa (e.g., Gutiérrez-Pesquera et al. 2016; Morley et al. 2019; Carilo Filho et al. 2022; Sinai et al. 2022). Sampling locations were assigned to latitudinal groups based on the absolute latitude (°N/S) and were categorized as either tropical (0–25°), sub-tropical (>25–40°), temperate (>40–53.55°) or polar (>53.55°; Morley et al. 2019).

## 2.5 Effect size calculation: acclimation response ratio (ARR)

A well-established method to measure acclimation capacity in thermal tolerance in ectothermic animals is the calculation of the acclimation response ratio (ARR), i.e., the slope of the linear function describing the change in thermal tolerance with a given change in acclimation temperature (e.g., Hutchison 1961; Claussen 1977; Gunderson & Stillman 2015; Morley et al. 2019). We separately calculated the ARR for CT<sub>max</sub> within each study using the equation according to Claussen (1977):

$$ARR = \frac{CT_{max}[T_2] - CT_{max}[T_1]}{T_2 - T_1},$$

where T represents the acclimation temperature (°C; with T<sub>2</sub> = highest acclimation temperature and T<sub>1</sub> = lowest acclimation temperature) and CT<sub>max</sub> the heat tolerance estimates (°C). When data on more than two acclimation temperatures were presented, we calculated the ARR for each stepwise comparison (e.g., 18–20°C, 20–22°C, 22–24°C; Pottier et al. 2022a) and used the mean ARR of all comparisons in the statistical analysis. Higher absolute values of ARR correspond to higher plasticity in thermal tolerance limits (i.e., greater acclimation capacity; Claussen 1977; Kingsolver & Huey 1998; Gunderson & Stillman 2015; van Heerwarden et al. 2016). An acclimation response ratio of 1.00 indicates a 100% acclimation in thermal tolerance to a temperature increase of 1°C (Morley et al. 2019).

## 2.6 Statistical analyses

For all statistical tests R 4.0.2 (R Core Team, 2020) was used. All plots were constructed using R packages ‘ggplot2’ (Wickham & Wickham 2009), ‘ggtree’ (Yu et al. 2017) and Adobe Illustrator CS6.

Conditional inference tree (CIT) analysis (R package ‘partykit’, Hothorn & Zeileis 2015) was used to assess the influence of geographic origin (bioclimatic variables), phylogeny (taxon and order level), experimental methodology (ramping rate, acclimation duration), and ontogeny on CT<sub>max</sub> and ARR of amphibians and freshwater fishes. As an advantage over traditional (parametric) methods, CIT is a non-parametric method that handles complex non-linear relationships without making specific assumptions about data distribution or being sensitive to

outliers. CIT involves recursive partitioning to split data into subsets based on the relevance of predictor variables. At each node of the tree, a permutation-based test (Monte Carlo method with Bonferroni correction) determines whether the split is statistically significant ( $\alpha < 0.05$ ). The initial split in the tree indicates which predictor variable has the strongest correlation with the response variable ( $CT_{max}$  or ARR). The resulting tree provides a hierarchical structure and classification of significant predictor variables. A post-pruning strategy based on the Akaike Information Criterion (AIC, Akaike 1974) was used to avoid overfitting, i.e., removal of nodes that do not improve the overall fit of the model (Hothorn & Zeileis 2015). The raw data used for the analysis are provided in the electronic supplementary material (Table S1).

Phylogenetic trees for visualizations were created using the R package ‘fishtree’ (Chang et al. 2019) for freshwater fishes (Teleostei). The amphibian ultrametric tree was obtained from the timetree.org website in June 2022 (Kumar et al. 2022). To ensure the validity, we developed a workflow to filter out taxonomically invalid taxa. Firstly, a time tree was generated using the "Build a Timetree" function on timetree.org. The taxa names were then extracted from the generated time trees using the R package ‘ape’ (Paradis & Schliep 2019), excluding non-binomial names. The extracted list was cross-checked with the GBIF Backbone Taxonomy (GBIF Secretariat, 2021) using the species matching tool (<https://www.gbif.org/tools/species-lookup>, accessed June 2022). Matches at the species rank with an accuracy of 100% were extracted and matches with lower accuracy were manually verified. The resulting species list was uploaded back onto timetree.org using the "Load a List of Species" function. The newly generated time tree was then downloaded and used for visualizations.

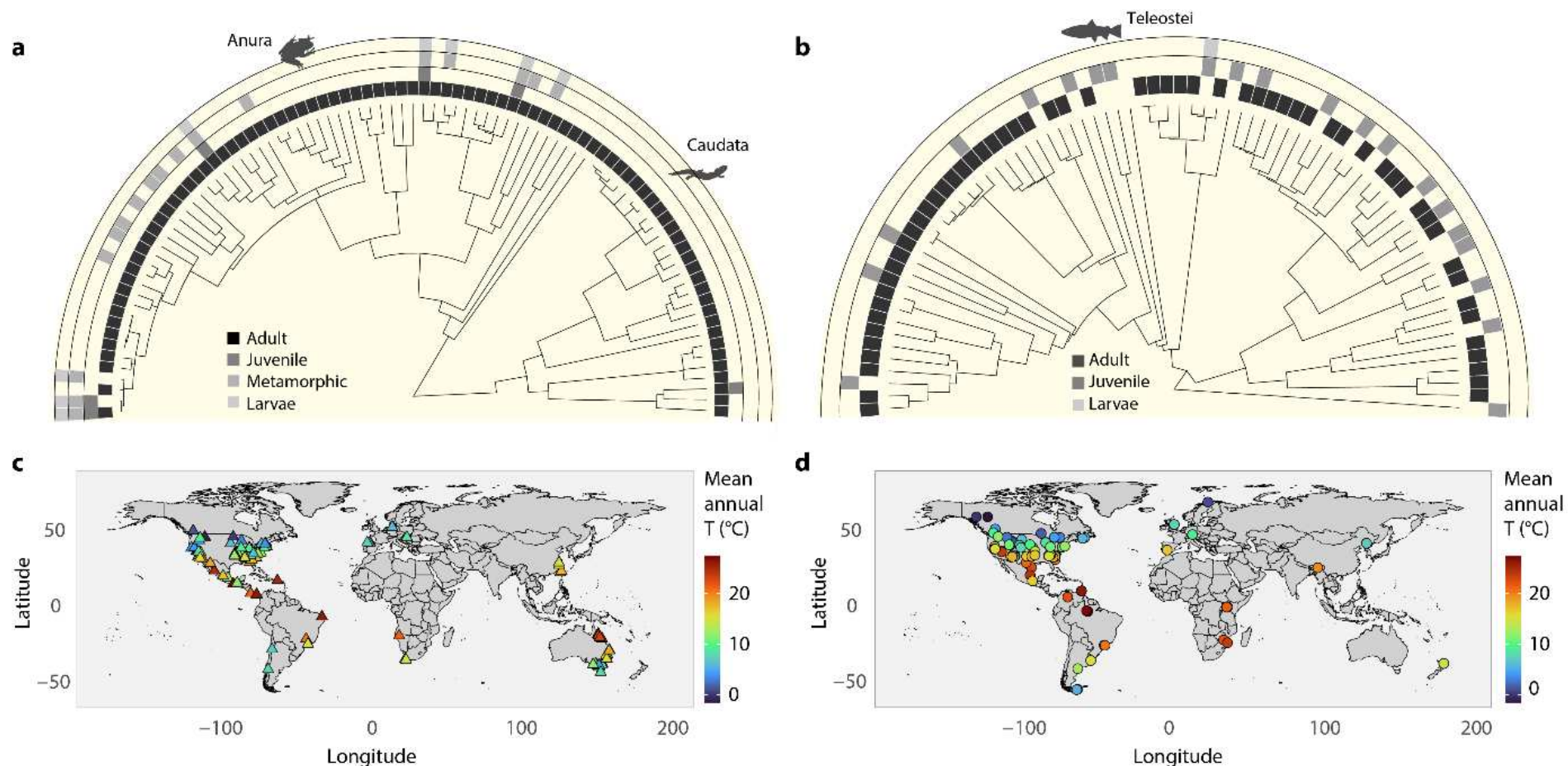
The relationship between absolute latitude ( $^{\circ}$ N/S) and the bioclimatic variables that revealed a significant effect on  $CT_{max}$  (Bio4, Bio7) and ARR (Bio3) in the CIT analysis was assessed using linear regressions. Differences in respective bioclimatic variables between latitudinal groups (tropical, sub-tropical, temperate, polar) were compared using Kruskal-Wallis tests followed by pairwise Mann-Whitney U- tests with false discovery rate (FDR)-correction.

### 3. Results

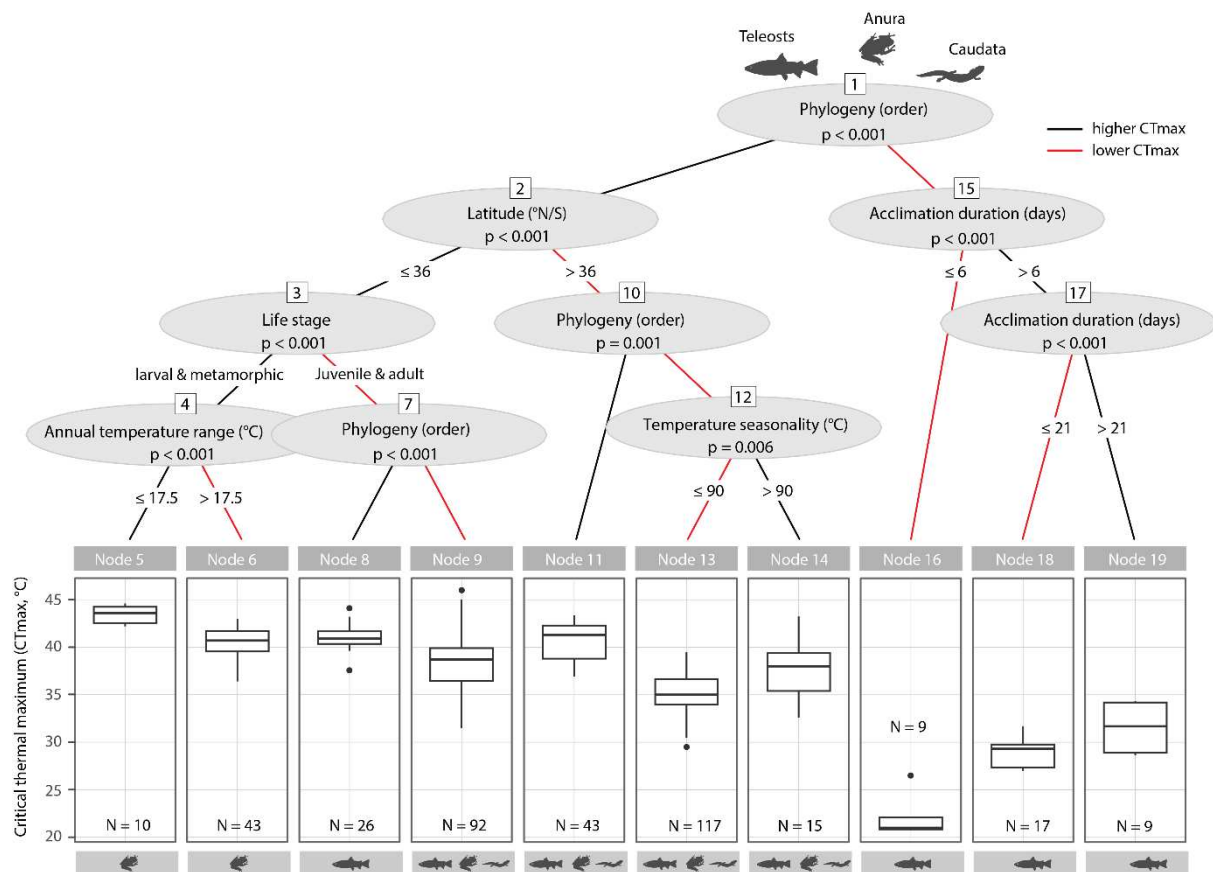
The analysis of paired  $CT_{max}$  and ARR values included 201 studies of 96 amphibian species (2 orders) (Fig. 1a, b) and 144 studies of 93 teleost species (14 orders) (Fig. 1c, d) deriving from 93 published articles, corresponding to a phylogenetic coverage of approximately 1% within each taxon. No data were available for Gymnophiona. Most studies determined  $CT_{max}$  and ARR in adult animals. For freshwater fishes, only one study determining  $CT_{max}$  in larvae was obtained, while 20% of the data covered juveniles (Fig. 1c). For amphibian species, 12% of the

data was available for one or several of the early-life stages, i.e. larvae, metamorphs, and/or juveniles (Fig. 1a). The geographic origin of amphibian and fish species displayed a bias towards temperate and subtropical regions, with 60% of all sampling sites located in North America, 10% in Australia and New Zealand, 8% in South America, 6% in Europe, and 4% in China (Fig. 1b, d). The  $CT_{max}$  protocols were variable in both taxa, with acclimation times ranging from 0.5 to 150 d (mean = 11.2 d) and ramping rates ranging from 0.02 to 1.0°C min<sup>-1</sup> (mean = 0.75 °C min<sup>-1</sup>).

Across all species studied,  $CT_{max}$  ranged from 20.75°C to 46.00°C (Table S1; Fig. S2a). Acclimation capacity in upper thermal tolerance was positive in 97.1 of the studies with an average of 0.22 °C with every 1 °C increase in temperature across all studies and life stages, whereas the acclimation response for  $CT_{max}$  was negative (i.e., negative ARR) in 2.0% of the studies. Across all species studied, ARR ranged from -0.08 to 1.68 (Table S1; Fig. S2b).



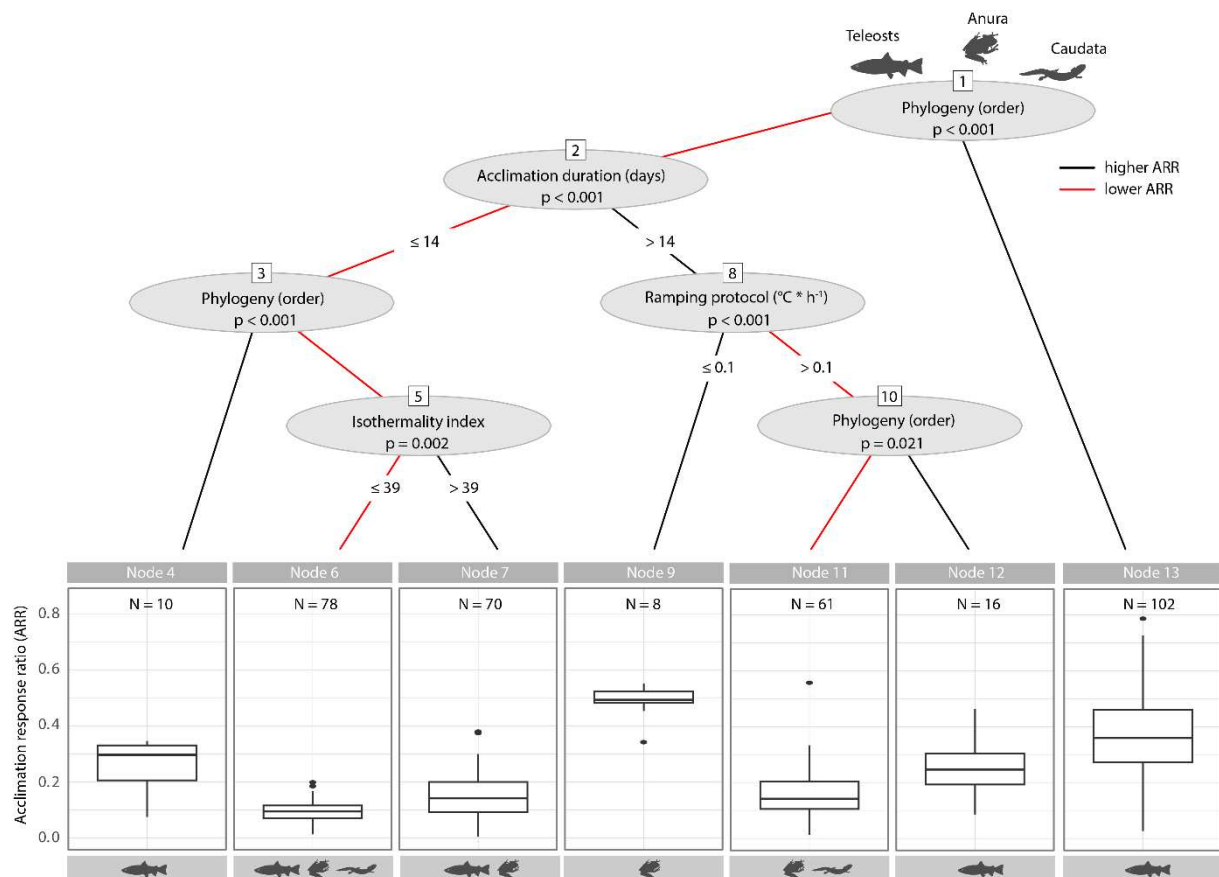
332 **Figure 1.** Phylogenetic, ontogenetic, and geographic background of critical thermal maximum (CT<sub>max</sub>) and acclimation response ratio (ARR)  
 333 measurements in (a, c) amphibians (Anura and Caudata) and (b, d) freshwater fishes (Teleostei). Phylogenetic trees contain (a) 96 amphibian and (b)  
 334 93 teleost species assigned to 2 and 14 orders, respectively. Grey shaded squares indicate which life stages of a species were studied. Maps show the  
 335 geographical origin and local mean temperature of (c) amphibian and (d) fish species. Phylogenetic coverage was approximately 1% within each  
 336 taxon. Silhouettes were taken from PhyloPic ([www.phylopic.org](http://www.phylopic.org)).



**Figure 2.** Conditional inference tree (CIT) for critical thermal maximum (CT<sub>max</sub>) of freshwater fish (Teleostei) and amphibians (Anura and Caudata). The sequence of internal nodes (splits) corresponds to a hierarchical structure of significant predictor variables. At each split, lines indicate the classification into groups with higher (black) and lower (red) CT<sub>max</sub> values. Boxplots show the distribution of CT<sub>max</sub> values for each terminal node (N = number of studies). Pictograms show the phylogenetic composition of terminal nodes. Silhouettes were taken from PhyloPic ([www.phylopic.org](http://www.phylopic.org)).

The CIT model explained 77% of the variability in the CT<sub>max</sub> studies. The model produced a classification tree with 9 internal nodes (splits) and 10 terminal nodes (Fig. 2). Phylogeny, latitudinal origin, and acclimation duration were the main discriminators of CT<sub>max</sub>, followed by life stage and local bioclimate. As indicated by the first split, acclimation duration was only a significant discriminator for a group of fish with relatively low CT<sub>max</sub> values. Within this group of cold-water fish (mainly salmonids), CT<sub>max</sub> increased with acclimation duration ( $p < 0.001$ , node 16-18). For most other fish and amphibian species, CT<sub>max</sub> depended primarily on latitude (node 2), with higher tolerance limits in low-latitude species ( $\leq 36^\circ$  N/S) compared to species at higher latitudes ( $> 36^\circ$  N/S). Differences in CT<sub>max</sub> between life stages were only significant within the group of low-latitude species ( $p < 0.001$ , nodes 3-9; Fig. 2, Fig. S3a). The classification in this group was phylogenetically unbalanced, suggesting higher CT<sub>max</sub> values

in larval and metamorphic stages of anurans compared to juveniles and adults of anurans, caudates and teleosts (Fig. S4). The influence of local bioclimatic variables was dependent on phylogeny, latitude, and life stage. For low-latitude amphibians (early-life stages of anurans),  $CT_{max}$  was higher in regions with a lower annual temperature range (Bio 7,  $p < 0.001$ , node 4-6; Fig. 2); and for high-latitude species,  $CT_{max}$  increased with temperature seasonality (Bio 4,  $p = 0.006$ , node 12-14; Fig. 2).



**Figure 3.** Conditional inference tree (CIT) for the acclimation response ratio (ARR) of freshwater fish (Teleostei) and amphibians (Anura and Caudata). The sequence of internal nodes (splits) corresponds to a hierarchical structure of significant predictor variables. At each internal node, lines indicate the classification into groups with higher (black) and lower (red) ARR values. Boxplots show the distribution of ARR values for each terminal node (N = number of studies). Pictograms show the phylogenetic composition of terminal nodes. Silhouettes were taken from PhyloPic ([www.phylopic.org](http://www.phylopic.org)).

The CIT model for ARR explained 47% of the variability in the study. The model produced a classification tree with 6 internal splits and 7 terminal nodes (Fig. 3). Phylogeny (order/taxon) was the main discriminator of ARR, followed by experimental methodology and a minor contribution of local bioclimate. Ontogenetic differences in ARR were not detected (Fig. S3b).

The first split of the classification tree separated a large group of fishes (8 orders, 68 species; Fig. 3) with a significantly higher mean ARR compared to the remaining fishes ( $P < 0.001$ ). The comparatively high ARR of this fish group was not related to experimental methodology nor any bioclimate variable. The second split was based on methodology, dividing the studies into groups with an acclimation duration longer or shorter than 14 d (node 3; Fig. 3). Longer acclimation generally resulted in higher ARR values ( $p < 0.001$ ), but the acclimation effect differed according to phylogeny, ramping rate and bioclimate (node 4-13). If the acclimation period was less than 14 days, a subsequent split occurred according to phylogenetic order ( $p < 0.001$ ) and bioclimate (node 4 & 6), with a positive relationship between ARR and isothermality score (i.e., bio3;  $p = 0.002$ ; Fig. S4). When acclimation duration exceeded 14 d, studies were further subdivided according to ramping rate (node 9,  $P < 0.001$ ) and taxon (node 11,  $p = 0.021$ ). These terminal classifications imply higher ARR values at slow heating rates ( $< 0.1^{\circ}\text{C min}^{-1}$ ), and higher ARR values in fish compared to amphibians at ramping rates  $> 0.1^{\circ}\text{C min}^{-1}$  (Fig. 3).

Isothermality (Bio3) decreased significantly with absolute latitude ( $R^2 = 0.725$ ,  $p = 0.001$ ). In contrast, both temperature seasonality (bio4;  $R^2 = 0.447$ ,  $p < 0.001$ ) and annual temperature range (bio7;  $R^2 = 0.322$ ,  $p < 0.001$ ) increased from the tropics to the polar latitudes (Fig. S5).

## 4. Discussion

### 4.1 Context-Dependent Drivers and Broad-Scale Patterns of Physiological Limits

The most striking result from our analyses is that the acclimation capacity of freshwater fish was more than twice that of amphibians, indicating a strong phylogenetic signal. Our findings align with those reported in previous syntheses (Gunderson & Stillman 2015; Rohr et al. 2018; Morley et al. 2019; Pottier et al. 2022a), demonstrating a higher thermal plasticity in organisms inhabiting aquatic habitats compared to their terrestrial counterparts, and with fish (marine and freshwater) exhibiting greater thermal plasticity compared to amphibians (Gunderson & Stillman 2015). Compared to most amphibians, freshwater fish are restricted to their aquatic habitat throughout their life cycle (Comte & Grenouillet 2013). As aquatic habitats tend to have less spatial variability in operative thermal conditions than terrestrial habitats (Gunderson & Stillman 2015; Sunday et al. 2014), behavioral thermoregulation is constrained, and freshwater fish are more likely to use thermal plasticity than amphibians to buffer against changing thermal conditions. Consequently, the capacity for thermal plasticity appears to be phylogenetically conserved between both taxa (Angilletta et al. 2002; Bodensteiner et al. 2021), depending on the ability for behavioral thermoregulation as explained by the *Bogert effect* (Bogert 1949; Cowles & Bogert 1944; Muñoz 2022), rather than on the level of thermal variation to which a

population is exposed to (Huey et al. 1999) as suggested by the *climate variability hypothesis* (Janzen 1967). Moreover, unlike freshwater fishes, most amphibians undergo a habitat shift from a larval aquatic to a post-metamorphic terrestrial habitat (Shi 2000). As an adaptive response to often shallow or temporary larval habitats (Newman 1992), amphibian larvae display a high degree of plasticity in growth and development (Kulkarni et al. 2017; Ruthsatz et al. 2018; Burraco et al. 2021; Sinai et al. 2022), providing a means for increasing fitness (Schlichting & Pigliucci 1998). Therefore, plasticity in timing of metamorphosis appears to be more important than that in thermal tolerance to reduce mortality risk (Rudolf & Rödel 2007) due to desiccation or temperature extremes (Burraco et al. 2022; Albecker et al. 2023).

Unlike acclimation capacity, latitudinal origin was the primary determinant of heat tolerance in both taxa, with a higher heat tolerance in low-latitude species compared to high-latitude species, thereby supporting the *climate variability hypothesis* (Janzen 1967; Bozinovic et al. 2011). Both taxa, therefore, exhibit physiologically adaptation to latitude-dependent thermal regimes to which they are exposed. Our results agree with the findings of a large body of research that has confirmed the link between physiological limits and large-scale geography based on a species' local adaptation to temperature and other associated climatic variables (e.g., Gutiérrez-Pesquera et al. 2016; Sunday et al. 2011, 2019; Pintanel et al. 2022; but not: Addo-Bediako et al. 2000; Sørensen et al. 2016). Yet, in contrast to the broader literature, our synthesis indicated that  $CT_{max}$  was not *generally* higher in low-latitude populations and importantly it varied with ontogeny and local bioclimate. These findings emphasize the importance of assessing life stage-specific sensitivity to thermal stress as well as spatial climatic differences in conservation science. Therefore, focusing on large-scale geographical patterns for predicting how biodiversity will respond to future environmental change might bear the risk of overlooking context-dependent variation in thermal traits and thus, intraspecific differences in vulnerability to changing thermal conditions. For example, Bovo et al. (2023) demonstrated that responses of tropical amphibians to climate variation were heterogenous as a consequence of intraspecific variation in physiological traits and spatial variation in climate with elevation. Furthermore, Sunday et al. (2011) and Pinsky et al. (2019) reported that the physiological sensitivity of ectotherms across all latitudes depended on the realm, with terrestrial ectotherms being less sensitive to warming due to their higher capacity for behavioral thermoregulation.

## 4.2 Life Stage-Specific Thermal Sensitivity as a Key Factor in Species Vulnerability to Climate Change

In species with complex life-histories such as amphibians and teleost fish, life stages differ in size, morphology, physiology, and behavior (Wilbur 1980). Therefore, selection might promote stage-specific adaptations in thermal physiology (Enriquez-Urzelai et al. 2019; Ruthsatz et al. 2022). Ignoring those life stage-specific differences in thermal physiology may drastically underestimate climate vulnerability of species with consequences for successful conservation actions. Here, we found  $CT_{max}$  but not acclimation capacity to differ between pre- and post-metamorphic life stages in amphibians, with a lower  $CT_{max}$  in juvenile and adult stages. Limnic larvae may have a reduced capacity for behavioral thermoregulation due to their limited body size impairing the movement between different microclimates (Kingsolver et al. 2011; Sinclair et al. 2016; Enriquez-Urzelai et al. 2019), making them more dependent on passive responses to temperature fluctuations. To cope with changes in temperatures, a high heat tolerance is therefore advantageous in early life stages (Ruthsatz et al. 2022). In contrast, post-metamorphic stages might rather be able to select favorable microclimates by behavioral thermoregulation (Navas et al. 2007; Haesemeyer 2020). This is particularly true for amphibians, as their post-metamorphic terrestrial habitats offer much spatial variability in operative thermal conditions (Gunderson & Stillman 2015), while juvenile and adult (freshwater) fish are able to behaviorally thermoregulate by performing vertical and horizontal movements (Amat-Trigo et al. 2023; but not: Clark et al. 2022). Moreover, juvenile and adult (freshwater) fish are known to show behavioral thermoregulation such as vertical and horizontal movements (Amat-Trigo et al. 2023; but not: Clark et al. 2022) with juveniles often displaying an ‘aggregation response’ in cool water refuges (Breau et al. 2007). Our findings are in line with the pattern found for aquatic larvae by Cupp (1980), Enriquez-Urzelai et al. (2019), and Ruthsatz et al. (2022), who demonstrated a higher  $CT_{max}$  in amphibian larvae than in post-metamorphic stages. In contrast, Dahlke et al. (2020) found no difference in heat tolerance between larval and adult stages in marine and freshwater fish. Notably, our synthesis yielded only one estimate for larval  $CT_{max}$  and acclimation capacity in freshwater fish and, thus, we lack the data for a definitive conclusion. Given that small body sizes of larvae restricts their capacity for behavioral thermoregulation, one would expect freshwater fish to exhibit the same life stage-specific differences in thermal sensitivity observed in amphibians. Finally, the lack of any life-stage specific pattern in acclimation capacity in the present study might be attributed to an inherent bias in life stage representation among the studied species. In a recent study on the European

common frog (*Rana temporaria*), young larvae may define the climate sensitivity of populations since that life stage exhibited the lowest acclimation capacity (Ruthsatz et al. 2022). Furthermore, it is worth noting that our synthesis did not encompass embryos, which have recently been reported to have the lowest heat tolerance in fish (Dahlke et al. 2020, 2022; Pottier et al. 2022b) and the lowest acclimation capacity across ectotherms (Pottier et al. 2022a). To better identify potential life history bottlenecks in thermal sensitivity in amphibian, fish and other taxa inhabiting freshwater, future studies should adopt a more comprehensive approach by considering a wider range of life stages within species.

#### 4.3 Understanding Context-Dependent Physiological Adaptation in Ectotherms

Global syntheses on physiological studies can help us determine the winners and losers of climate change through assessment of broad-scale patterns of species' thermal limits and acclimation capacity for modifying their thermal tolerance (Somero 2010). This knowledge, in turn, enables us to develop suitable conservation strategies to mitigate the negative effects of climate change. However, our key findings emphasize that assessing species' vulnerability to changing thermal conditions based on large-scale geographic and/or phylogenetic patterns in thermal traits might cover up context-dependent physiological adaptations. In other words, tropical ectothermic species are considered particularly vulnerable to global warming as they live close to their physiological limits and have poor acclimation ability (Tewksbury et al. 2008; Huey et al. 2009; Sunday et al. 2014), but such generalizations might for instance ignore intraspecific variation in physiological limits across altitudinal variation in climate (Bovo et al. 2023). Physiological adaptations are driven by the interplay between microclimate temperature heterogeneity and the behavioral thermoregulatory abilities of ectotherms (Huey et al. 2012; Pincebourde et al. 2016; present study) depending on their habitat characteristics (Pinsky et al. 2019; Kulkarni et al. 2017), ontogeny (Enriquez-Urzelai et al. 2019; Ruthsatz et al. 2022a), life history traits such as body size (Rubalcaba et al. 2020; Peralta-Maraver & Rezende 2021) or activity patterns (Navas et al. 2007; Ruthsatz et al. 2022b), and/or energy balance (Pörtner et al. 2005; Muñoz et al. 2022). Physiological traits and limits are consequently rather evolutionary driven dynamic concepts than fixed values for a species (Bovo et al. 2018, 2023; Navas et al. 2022). In order to improve predictions of climate change impacts on biodiversity, it is imperative to deepen our understanding of context-dependent physiological adaptations (Meek et al. 2023), thereby advancing the development of suitable conservation measures/strategies that incorporate evolutionarily enlightened perspectives (Ashley et al. 2003; Cook & Sgró 2018) beyond the species level (Fig. 4).

#### 4.4 Conclusion

There is a growing body of physiological studies assessing thermal limits and acclimation capacity of species, investigating physiological systems setting these limits to better predict shifts in the productivity and species distribution patterns in a warming world. Our synthesis points to representation biases in taxonomy, species' biogeographic distribution, life stage, and biases resulting from non-standardized study design. We found the influence of life stage, phylogeny, and thermal adaptation to depend on acclimation duration of the animals and the ramping rate used, underscoring the importance of a thoughtful selection of the methodological approach. Therefore, we conclude our synthesis by addressing those data inadequacies and proposing methods to enhance data collection presented in five themes (Fig. 4):

- **Biogeographic and taxonomic coverage**

We found strong latitudinal trends in physiological limits and additional research is needed in poorly represented (mostly tropical) regions and generally in low- and middle-income countries with little investment in research (King 2004) to yield additional, important insights. Our synthesis highlights that most studies have been conducted in North America, Europe, and Australia and information gaps exist for most parts of Africa, Asia, and South America (except Brazil). Such regional differences in research effort are common in conservation science (Schiesari et al. 2007; Winter et al. 2016; McLaughlin et al. 2022; Sinai et al. 2022) despite the fact that under-studied regions contain the vast majority of global biodiversity hotspots (Mittermeier et al. 2011). Furthermore, most of these studies used species that are common, widely distributed, and/or easily obtained by researchers. Studies on other species (particularly those already in decline) are needed to avoid taxonomic bias and reach stronger conclusions on whether specific taxa might be more sensitive to global warming (da Silva et al. 2020).

- **Relevance of life stage and sex**

We urge future studies to measure a wider range of life stages and to measure both sexes within species to better identify potential life history thermal bottlenecks (Klockmann et al. 2017; Dahlke et al. 2020). All of the summaries to date likely overestimate physiological limits since most studies have been performed on adults and thermal tolerance increases (Klockmann et al. 2017; Rubalcaba & Olalla-Tárraga 2020; but not di Santo & Lobel 2017) and acclimation capacity decreases (Pottier et al. 2021) with body size. Furthermore females and males differ in a wide range of morphological, physiological, and behavioral aspects as well as in their

energetic investment in gamete production (Hayward & Gillooly 2011). A recent meta-analysis across ectothermic taxa revealed that the acclimation capacity differed between males and females in wild-caught animals (Pottier et al. 2021). Furthermore, Dahlke et al. (2020) found narrower thermal tolerance ranges in spawning females and van Heerwaarden and Sgrò (2021) demonstrated that a low heat tolerance of male fertility is a critical bottleneck in insects.

#### • **Ecological relevance**

Future laboratory studies should adopt a more comprehensive and multifaceted approach for higher ecological relevance of thermal trait estimates (Desforges et al. 2023). Under natural conditions, organisms must often cope with multiple simultaneously occurring environmental stressors (Rohr & Palmer 2013; Gunderson et al. 2016) such as declining dissolved oxygen levels in freshwater habitats due to climate-induced temperature increases (Pörtner & Peck 2010). As thermal limits are shaped by oxygen availability (Pörtner 2001, 2010), organisms might exhibit lower thermal limits under natural conditions. Moreover, exposure to pollutants might reduce thermal tolerance (Little & Seebacher 2015) or acclimation capacity (Ruthsatz et al. 2018b) due to increased metabolic demands of detoxification processes or disruption of endocrine pathways involved in physiological acclimation. Physiological responses to increased water temperature as performed in the studies summarized here (i.e., a single stressor) may not align with observed responses of individuals in the natural environments with multiple stressors (Potts et al. 2021).

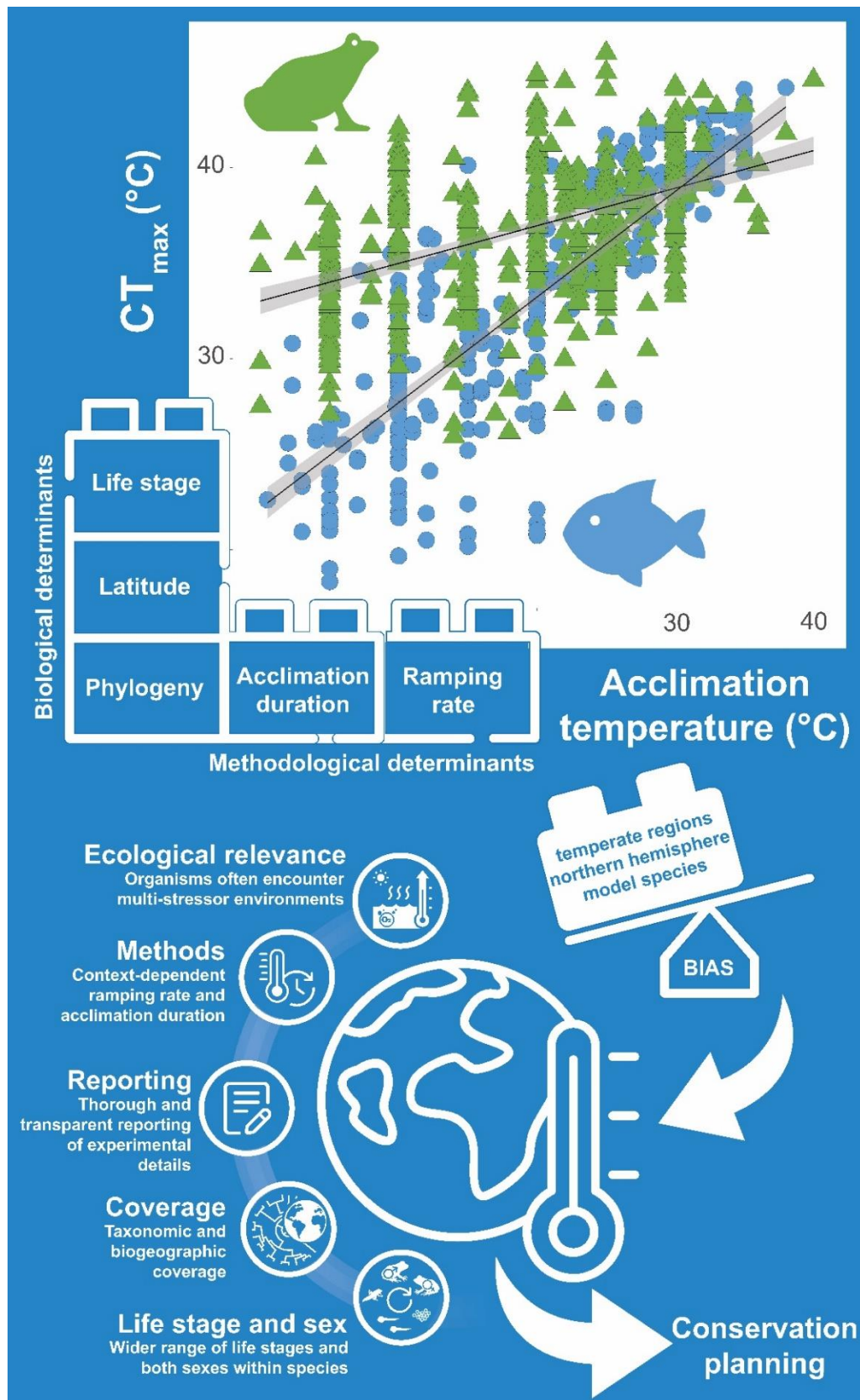
#### • **Methodological approach**

Customizing protocols to account for organismal and context-dependent variations in physiological limits (e.g., body size, life stage, sex, thermal history) will allow researchers to obtain more ecologically relevant estimates to inform conservation efforts. The application of an acute thermal ramping rate and a standardized endpoint such as the loss of equilibrium are used to measure critical thermal limits (Becker & Genoway 1979). The estimates are sensitive to differences in the methods. For example, faster ramping (heating) rates tend to yield higher thermal tolerance estimates compared to slower ramping rates (Moyano et al. 2017; Kovacevic et al. 2019; Penham et al. 2023). Using wild-collected animals is important as those reared in the laboratory may have physiology traits that differ from wild conspecifics (Pottier et al. 2021; Morgan et al. 2022). Methodological recommendations have been recently published and comparable methods are needed to compare thermal limits of different life stages (Cowan et al. 2023; Desforges et al. 2023).

• **Through reporting of research details**

Thorough and transparent reporting of experimental details in empirical studies such as sampling location and animal origin, among others, is required to enhance the comparability of studies on thermal traits. Furthermore, most studies working on adults did not report the sex of animals despite the potential for sex (or reproductive state) to be important factors in thermal sensitivity. The ability to make broad-scale comparisons of thermal tolerance across taxa, life stages and regions will be enhanced when studies report as much methodological detail as possible. Consequently, these future studies will contribute more robust estimates of climate vulnerability needed to guide climate change interventions.

By considering our recommendations, future studies will be more comparable, facilitating the utilization of respective findings in large-scale studies and models that assess species vulnerability and thus, population dynamics under global warming.



**Figure 4.** Synthesis of biological and methodological determinants as well as key directions for future research on acclimation capacity in amphibians and freshwater fish to advance the application of thermal traits in assessing species' and populations' vulnerability to climate change. See text for further details.

## 5. Data availability

Data associated with this study will be made available in the figshare data repository under DOI:XXX after manuscript acceptance.

## 6. References

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## **8. Author contributions**

Conceptualization: KR and MAP. Methodology: KR, KA, FD, SW, SG, MAP. Data Extraction and Quality Check: KR, KA, PCE, MLL, FD and SW. Formal Analysis: FD and SW. Investigation: KR, FD, and SW. Data Curation: KR. Visualization: KR, FD, and SW. Writing – Original Draft: KR, FD, and MAP. Writing – Review and Editing: all authors. Funding Acquisition: KR. Project Administration: KR. Supervision: KR and MAP. All authors gave their final approval for submission.

## **9. Conflict 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.

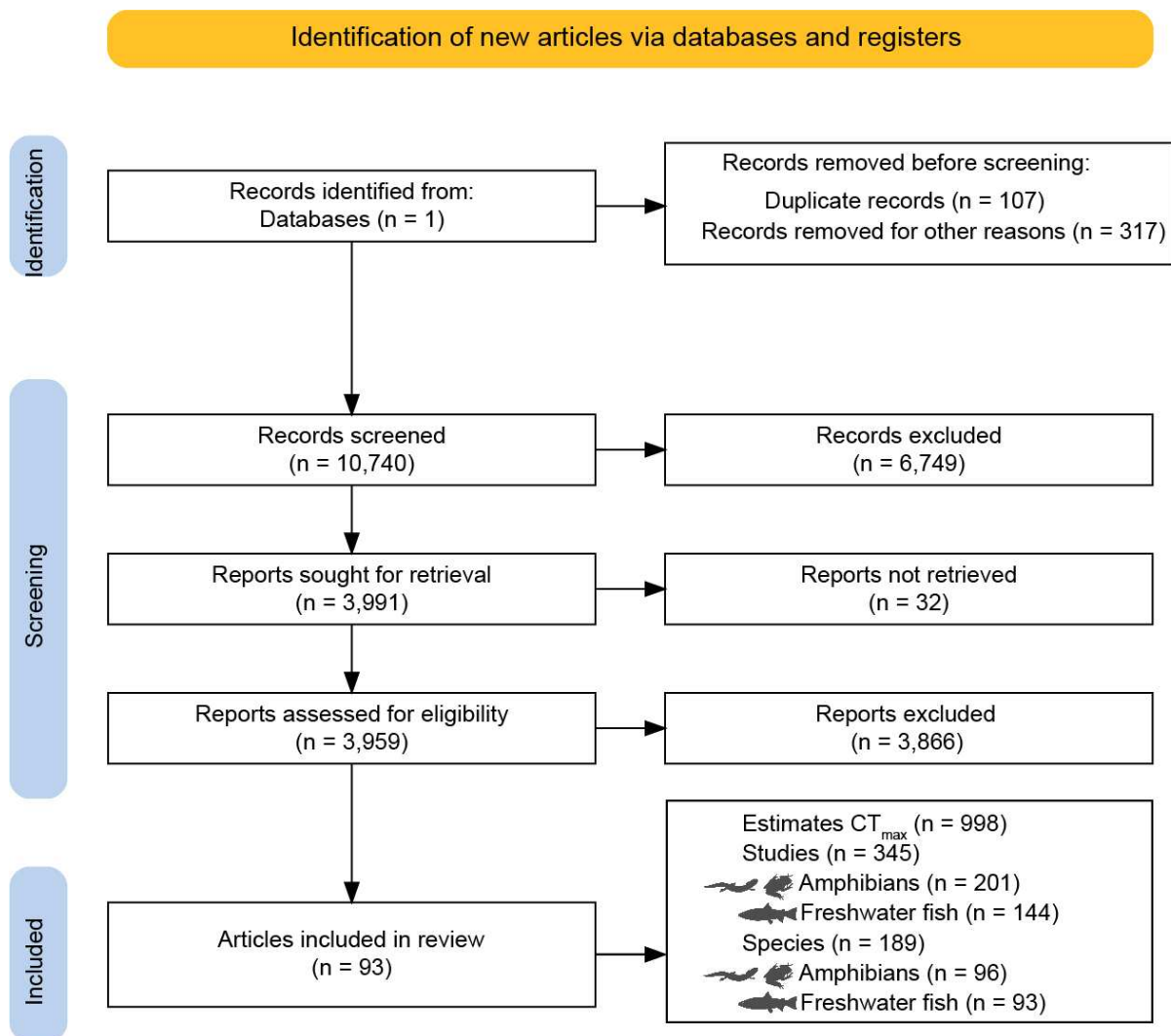
## **10. Statement of Ethics**

The authors have no ethical conflicts to disclose.

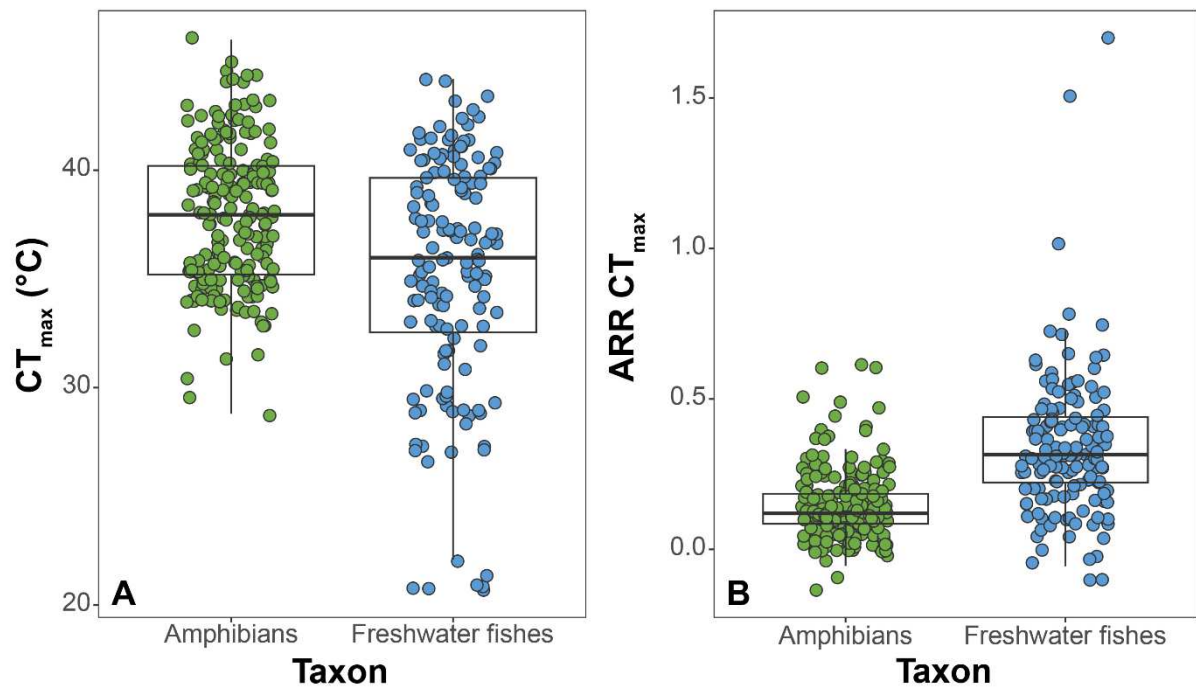
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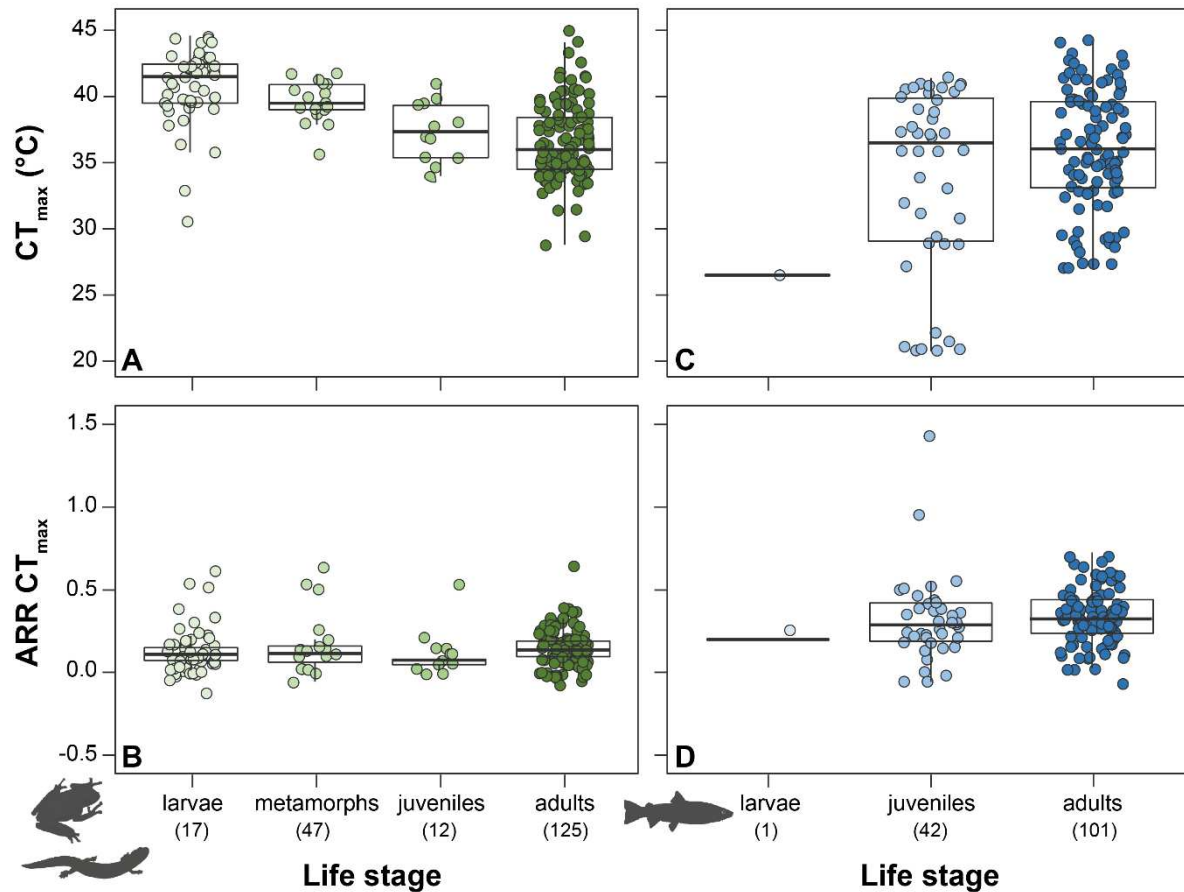
978 **Supplementary material**



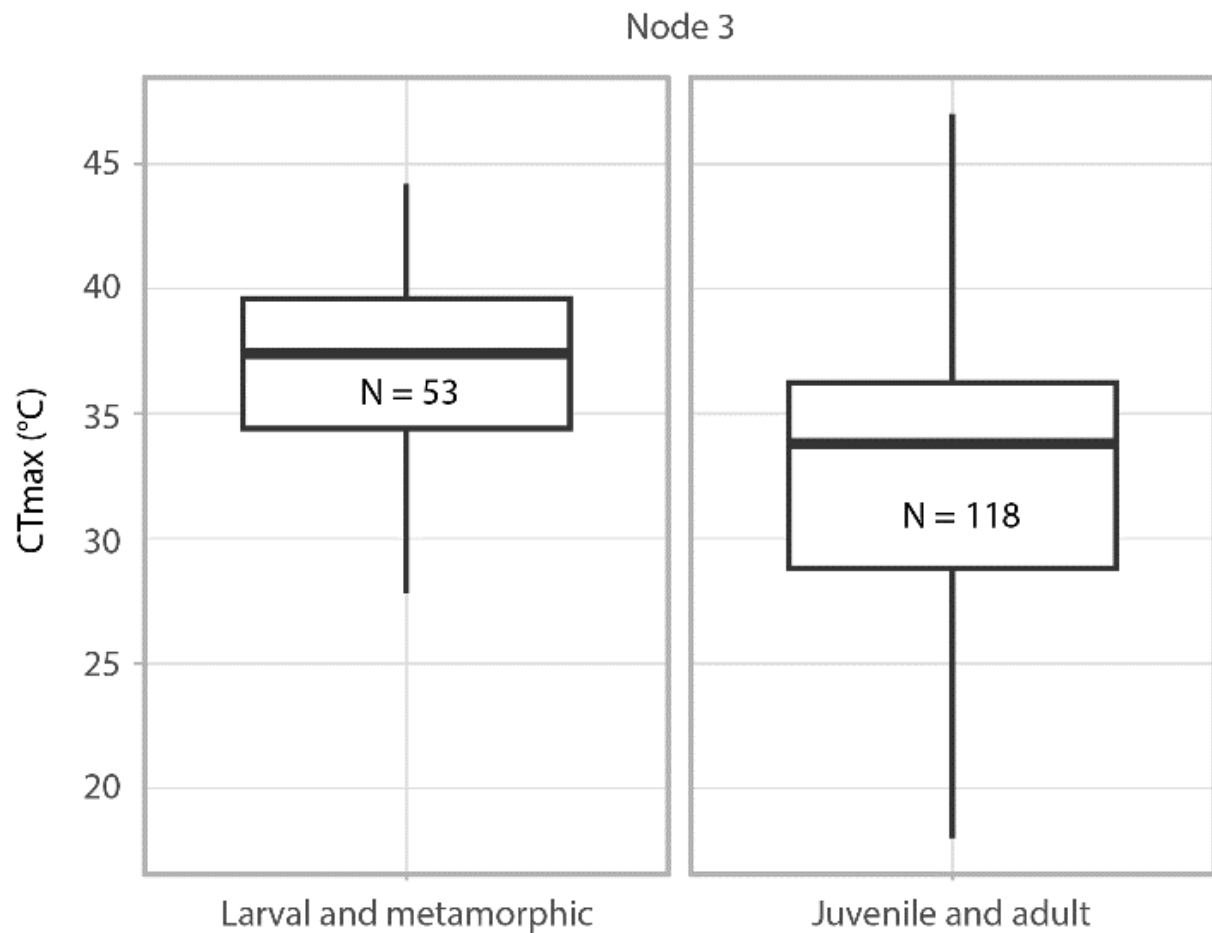
979 **Figure S1.** PRISMA flow diagram adapted from Page et al. (2021) showing literature search  
980 procedures and screening processes (created with Shiny app, Haddaway et al. 2022).



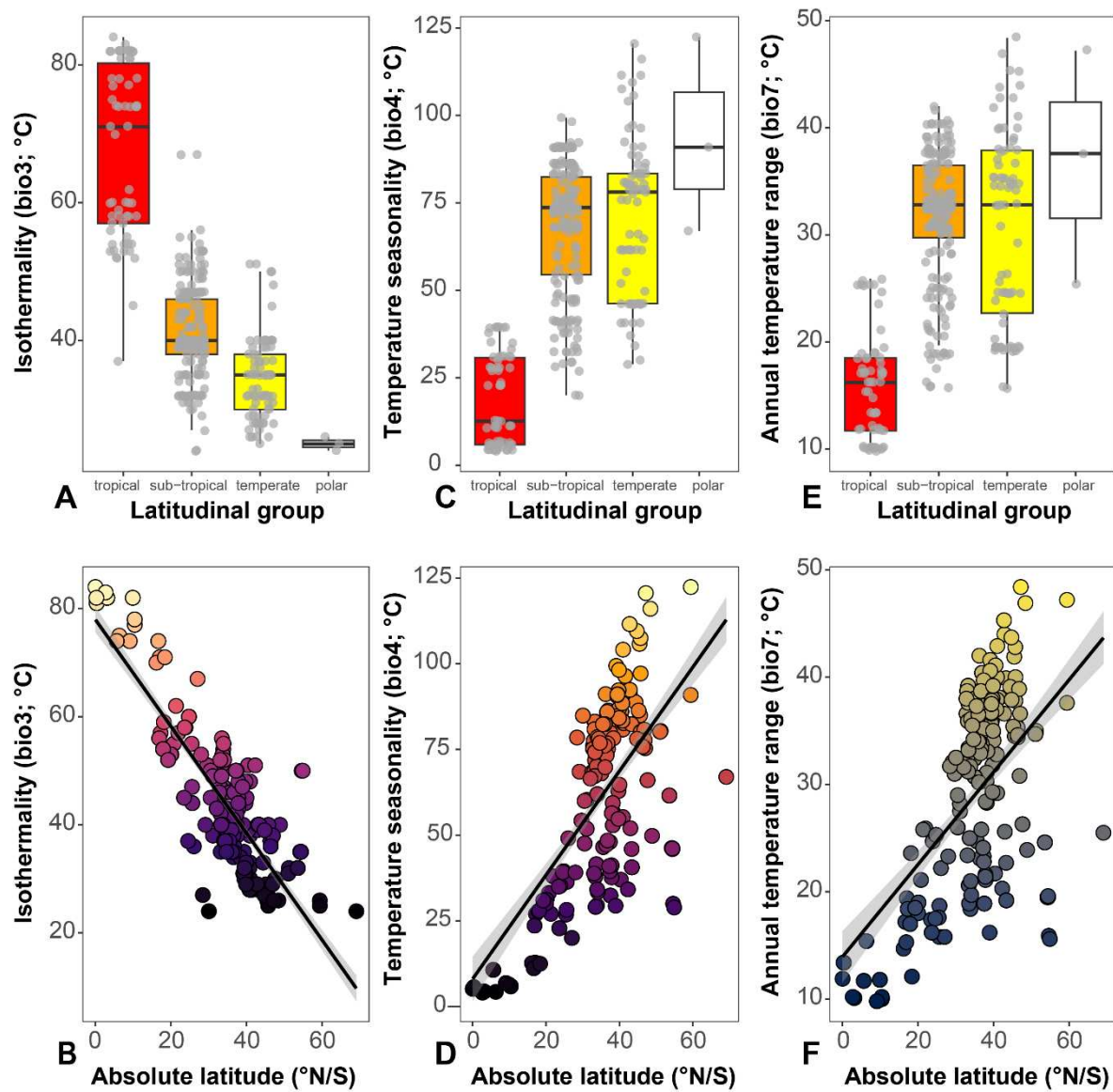
981 **Figure S2.** Taxon-specific **A** critical thermal maximum ( $CT_{max}$ ) and **B** Acclimation response  
 982 ratio of  $CT_{max}$  (ARR  $CT_{max}$ ) for amphibians and freshwater fishes. Green: Amphibians. Blue:  
 983 Freshwater fishes. Numbers in parentheses = sample size.



**Figure S3. AC** Life stage-specific critical thermal maximum (CT<sub>max</sub>) and **BD** Acclimation response ratio of CT<sub>max</sub> (ARR CT<sub>max</sub>) for amphibians and freshwater fishes. Green: Amphibians. Blue: Freshwater fishes. Numbers in parentheses = sample size.



987 **Figure S4.** Boxplots show the difference in CT<sub>max</sub> between larval/metamorphic stages and  
 988 juvenile/adult stages according to the classification in node 3 of the CIT model for both taxa (N  
 989 = number of studies).



**Figure S5.** A. Isothermality (bio3), C. temperature seasonality (bio4), and E. annual temperature (bio7) range for **BDF** four latitudinal groups (i.e., tropical, sub-tropical, temperate, and polar) and as a function of absolute latitude (°N/S).