

1 **Global Change Biology**

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

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

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32 *phenotypic plasticity, thermal bottleneck, metamorphosis, Bogert Effect, climate variability*  
33 *hypothesis*

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37 **Abstract**

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

61 **1. Introduction**

62 Global climate change is not only causing an increase in mean air and water temperatures, but  
63 also an increased magnitude and frequency of extreme climatic events (Lee et al. 2023). As a  
64 result, ectotherms are more likely to experience temperatures beyond their critical thermal  
65 maximum ( $CT_{max}$ ) in both terrestrial and aquatic habitats (Duarte et al. 2012; Sunday et al.  
66 2014). This is particularly true for populations already living close to their upper thermal limit.  
67 Consequently, the ability to mitigate thermal stress through either migration, evolutionary  
68 genetic adaptation or acclimation is crucial for the persistence of species in a changing climate  
69 (Franks & Hoffmann 2002; Huey et al. 2012; Seebacher et al. 2015). Given the limited dispersal  
70 ability of many species (e.g., freshwater species; Woodward et al. 2010) and rapid pace of  
71 global warming (Hoffmann & Sgró 2011), physiological acclimation is arguably the most  
72 important mechanism for coping with climate change (Gunderson & Leal 2015). Understanding  
73 differences in acclimation capacity of species and identifying global patterns can therefore help  
74 to identify climate change risks to biodiversity and develop effective conservation measures  
75 (Somero 2010).

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

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

97 In animals with complex life histories, thermal tolerance and acclimation capacity are thought  
98 to change during ontogeny according to physiological and morphological reorganizations and  
99 concomitant aerobic capacities in relation to oxygen demand (Pörtner 2002; Pörtner & Peck  
100 2010; Ruthsatz et al. 2020a, 2022a) as well as energetic costs associated with developmental  
101 processes (Ruthsatz et al. 2019). Furthermore, life stages might differ in their ability for  
102 behavioral thermoregulation (Navas et al. 2008; Little & Seebacher 2017) resulting in stage-  
103 specific adaptations in thermal traits (Huey et al. 1999). Therefore, determining taxon-specific  
104 acclimation capacity at different ontogenetic stages should be taken into account when studying  
105 climate adaptation of ectothermic species, as it will help identify life cycle bottlenecks and  
106 provide robust data on the vulnerability of populations or species to global warming  
107 (Bodensteiner et al. 2021; Pottier et al. 2022a; Dahlke et al. 2022). To date, most studies have  
108 focused on adult life stages when addressing the *climate variability hypothesis* (e.g., Gunderson  
109 & Stillman 2015; Sunday et al. 2011, 2014, 2019; Rohr et al. 2018) and the vulnerability of  
110 species to global change (e.g., Calosi et al. 2008; Comte & Olden 2017a; Morley et al. 2019;  
111 Molina et al. 2023), or have pooled several pre- and post-metamorphic life stages (Pottier et al.  
112 2022a; Weaving et al. 2022) thereby risking to overlook a critical thermal bottleneck in the life  
113 cycle (Dahlke et al. 2020). The extent to which thermal tolerance and acclimation capacity  
114 change during ontogeny is, therefore, not clear for many taxa.

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

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

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

## 146 2. Materials and Methods

### 147 2.1 Systematic literature review

148 We conducted a systematic literature review using ISI Web of Science (ISI WOS, 2021) on  
149 2022/06/30 and did not apply a timespan limit. The following Boolean search string was used  
150 to capture studies manipulating acclimation temperatures of amphibians and freshwater fishes  
151 at different life stages, and subsequently measured their  $CT_{max}$ : (“amphibian\*” OR “newt\*” OR  
152 “frog\*” OR “toad\*” OR “salamander\*” OR “freshwater” AND “fish\*”) AND (“early” OR  
153 “young” OR “life stage\*” OR “ontogen\*” OR “development\*” OR “hatchling\*” OR “alevin\*”  
154 OR “lary\*” OR “tadpole\*” OR “metamorph\*” OR “postmetamorph\*” OR “post-metamorph\*”  
155 OR “postlarva\*” OR “post-larva\*” OR “fry\*” OR “parr\*” OR “smolt\*” OR “subadult\*” OR  
156 “sub-adult” OR “juvenile” OR “fingerling\*” OR “adult\*”) AND (“thermal” OR “temperature”  
157 OR “acclimat\*” OR “heat” OR “warm\*”) AND (“tolerance\*” OR “thermal tolerance\*” OR  
158 “temperature tolerance\*” OR “warming tolerance\*” OR “heat tolerance\*” OR “thermal stress  
159 tolerance\*” OR “heat stress tolerance\*” OR “temperature stress tolerance\*” OR “limit\*” OR

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

164 Our search resulted in 11,057 documents (Fig. S1). After removing book chapters, conference  
165 contributions, reviews, meeting abstracts, editorial material, preprints, and proceedings articles,  
166 10,740 published peer-reviewed articles remained in our initial database. Additionally, we  
167 manually added articles included in the meta-analyses of Claussen (1977), Gunderson and  
168 Stillman (2015), Comte & Olden (2017b), Morley et al. (2019) and Dahlke et al. (2020) that  
169 met our inclusion criteria but were not obtained through the ISI Web of Science search. After  
170 an initial subjective evaluation of titles, 3,991 articles potentially containing results matching  
171 the objective of the present study were kept and further assessed for eligibility using the  
172 abstract. Thirty-four articles were not accessible. We contacted the authors of the original  
173 studies to request missing information and heard back from two authors. Finally, a total of 93  
174 articles (34 on amphibians; 59 on freshwater fishes) met our inclusion criteria. Search methods  
175 are summarized in a PRISMA flowchart (Fig. S1) and a list of included studies is available in  
176 the figshare data repository under [DOI:XXX](#) (to be included after manuscript acceptance).

177 *2.2 Inclusion criteria*

178 Studies were selected based on the following eight inclusion criteria:

179 (1) Studies were conducted on amphibians (anurans, caudates, or gymnophiones) or freshwater  
180 fishes (teleosts).

181 (2) Experiments were conducted under laboratory conditions (i.e., no field studies).

182 (3) Articles provided comprehensive information on methodology (acclimation temperatures  
183 and duration, ramping rate), phylogeny (species names), sampling location (GPS location),  
184 and life stage. If no GPS coordinates were provided but a concrete sampling location was  
185 stated (e.g., Central Park, New York City, NY, USA), we searched for the coordinates of  
186 the respective location on Google Maps.

187 (4) Animals were collected from their natural habitat. Data were excluded if measurements  
188 were taken from specimens bred artificially to reduce confounding issues associated with  
189 artificial selective history (Bennett et al. 2018). Studies were also included if adult animals  
190 were collected to immediately reproduce in the lab to obtain larvae.

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

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

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

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

### 213 2.3 Data extraction

214 When all inclusion criteria were met, data were collated in a spreadsheet. We extracted mean  
215  $CT_{max}$  for all acclimation temperatures resulting in 998 single data points (513 for amphibians;  
216 485 for freshwater fish). Some of these articles performed different studies on e.g., different  
217 populations of one species, different species, or different life stages. Therefore, all available  
218 datasets were included, resulting in 345 studies from 93 articles with 345 paired effect sizes  
219  $CT_{max}$  and acclimation capacity. Data presented in the text or tables were directly extracted  
220 from the article. When only raw data were available, mean values were calculated. For studies  
221 that presented results in figures instead of tables, Engauge Digitizer 12.1 was used (Mitchell et  
222 al. 2021) to extract data from the graphs. In addition to  $CT_{max}$  data, information on the

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

229 *2.4 Bioclimatic variables*

230 For each sampling location, 19 bioclimatic metrics, related to temperature and precipitation,  
231 and elevation were extracted using the WorldClim 2 database (<http://www.worldclim.org/>; Fick  
232 & Hijmans 2017) for the average of the years 1970–2000. The data were extracted at a spatial  
233 resolution of 30 seconds (~1 km<sup>2</sup>), using package ‘geodata’ (Hijmans 2021) in R (version 4.2.1;  
234 R Core Team, 2020). Bioclimatic variables are coded as follows: Annual Mean Temperature  
235 (bio1), Mean Diurnal Range (bio 2), Isothermality (bio 3), actual Temperature Seasonality (bio  
236 4), Maximum Temperature of Warmest Month (bio 5), Minimum Temperature of Coldest  
237 Month (bio 6), Annual Temperature Range (bio 7), Mean Temperature of Wettest Quarter (bio  
238 8), Mean Temperature of Driest Quarter (bio 9), Mean Temperature of Warmest Quarter (bio  
239 10), Mean Temperature of Coldest Quarter (bio 11), Annual Precipitation (bio 12), Precipitation  
240 of Wettest Month (bio 13), Precipitation of Driest Month (Bio 14), Precipitation Seasonality  
241 (bio 15), Precipitation of Wettest Quarter (bio 16), Precipitation of Driest Quarter (bio 17),  
242 Precipitation of Warmest Quarter (bio 18), and Precipitation of Coldest Quarter (bio 19)  
243 (<http://www.worldclim.org/data/bioclim.html>). These macroclimatic data were used as  
244 approximations to identify patterns of local adaptation in amphibians and freshwater fish, as  
245 microclimatic data (e.g. site-specific temperatures) were not available in original articles or in  
246 the WorldClim database. Following previous studies (Morley et al. 2019; Carilo Filho et al.  
247 2022), mean near-surface air temperature was assumed to reflect the temperature profile of  
248 freshwater systems and used to analyze thermal adaptation in both taxa. WorldClim  
249 (representing surface air temperatures) was selected for both realms. In contrast to marine  
250 habitats, the temperature of small or shallow bodies of water might fluctuate with the surface  
251 air temperature and animals at the surface of freshwater systems might be further exposed to  
252 high temperatures. Therefore, we assume that the average near-surface air temperature is a  
253 suitable estimate of the temperature of freshwater systems, thereby reflecting the thermal local  
254 adaptation of investigated amphibian and freshwater populations. This is in accordance with  
255 previous studies testing the effect of thermal adaptation on the thermal physiology of various

256 taxa (e.g., Gutiérrez-Pesquera et al. 2016; Morley et al. 2019; Carilo Filho et al. 2022; Sinai et  
257 al. 2022). Sampling locations were assigned to latitudinal groups based on the absolute latitude  
258 ( $^{\circ}$ N/S) and were categorized as either tropical ( $0$ – $25^{\circ}$ ), sub-tropical ( $>25$ – $40^{\circ}$ ), temperate ( $>40$ –  
259  $53.55^{\circ}$ ) or polar ( $>53.55^{\circ}$ ; Morley et al. 2019).

260 *2.5 Effect size calculation: acclimation response ratio (ARR)*

261 A well-established method to measure acclimation capacity in thermal tolerance in ectothermic  
262 animals is the calculation of the acclimation response ratio (ARR), i.e., the slope of the linear  
263 function describing the change in thermal tolerance with a given change in acclimation  
264 temperature (e.g., Hutchison 1961; Claussen 1977; Gunderson & Stillman 2015; Morley et al.  
265 2019). We separately calculated the ARR for  $CT_{max}$  within each study using the equation  
266 according to Claussen (1977):

$$267 ARR = \frac{CTmax_{[T2]} - CTmax_{[T1]}}{T2 - T1},$$

268 where T represents the acclimation temperature ( $^{\circ}$ C; with  $T2$  = highest acclimation temperature  
269 and  $T1$  = lowest acclimation temperature) and  $CT_{max}$  the heat tolerance estimates ( $^{\circ}$ C). When  
270 data on more than two acclimation temperatures were presented, we calculated the ARR for  
271 each stepwise comparison (e.g., 18–20 $^{\circ}$ C, 20–22 $^{\circ}$ C, 22–24 $^{\circ}$ C; Pottier et al. 2022a) and used the  
272 mean ARR of all comparisons in the statistical analysis. Higher absolute values of ARR  
273 correspond to higher plasticity in thermal tolerance limits (i.e., greater acclimation capacity;  
274 Claussen 1977; Kingsolver & Huey 1998; Gunderson & Stillman 2015; van Heerwarden et al.  
275 2016). An acclimation response ratio of 1.00 indicates a 100% acclimation in thermal tolerance  
276 to a temperature increase of 1 $^{\circ}$ C (Morley et al. 2019).

277 *2.6 Statistical analyses*

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

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

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

296 Phylogenetic trees for visualizations were created using the R package 'fishtree' (Chang et al.  
297 2019) for freshwater fishes (Teleostei). The amphibian ultrametric tree was obtained from the  
298 timetree.org website in June 2022 (Kumar et al. 2022). To ensure the validity, we developed a  
299 workflow to filter out taxonomically invalid taxa. Firstly, a time tree was generated using the  
300 "Build a Timetree" function on timetree.org. The taxa names were then extracted from the  
301 generated time trees using the R package 'ape' (Paradis & Schliep 2019), excluding non-  
302 binomial names. The extracted list was cross-checked with the GBIF Backbone Taxonomy  
303 (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  
304 extracted and matches with lower accuracy were manually verified. The resulting species list  
305 was uploaded back onto timetree.org using the "Load a List of Species" function. The newly  
306 generated time tree was then downloaded and used for visualizations.

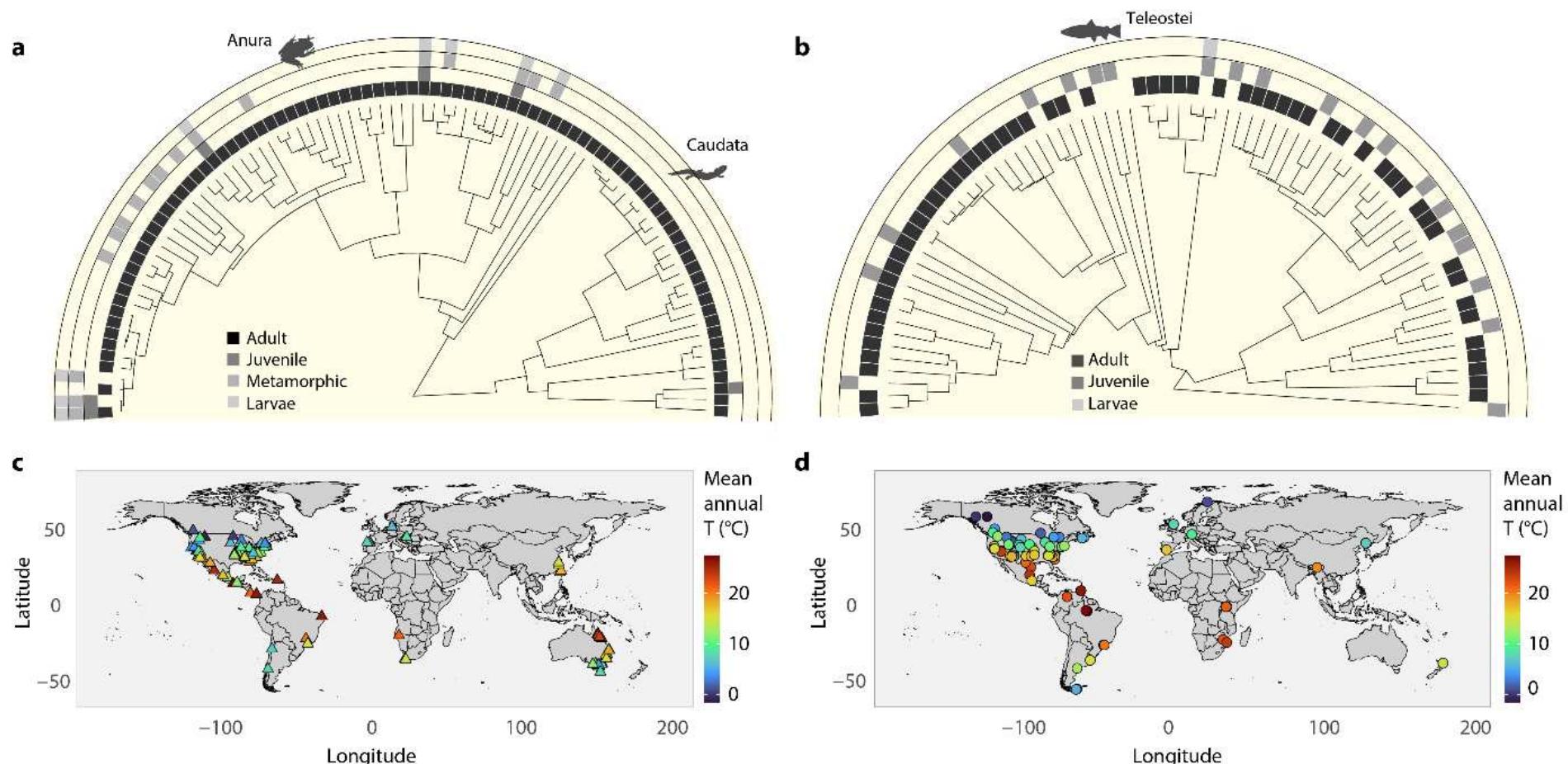
307 The relationship between absolute latitude ( $^{\circ}$ N/S) and the bioclimatic variables that revealed a  
308 significant effect on  $CT_{max}$  (Bio4, Bio7) and ARR (Bio3) in the CIT analysis was assessed using  
309 linear regressions. Differences in respective bioclimatic variables between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

### 313 3. Results

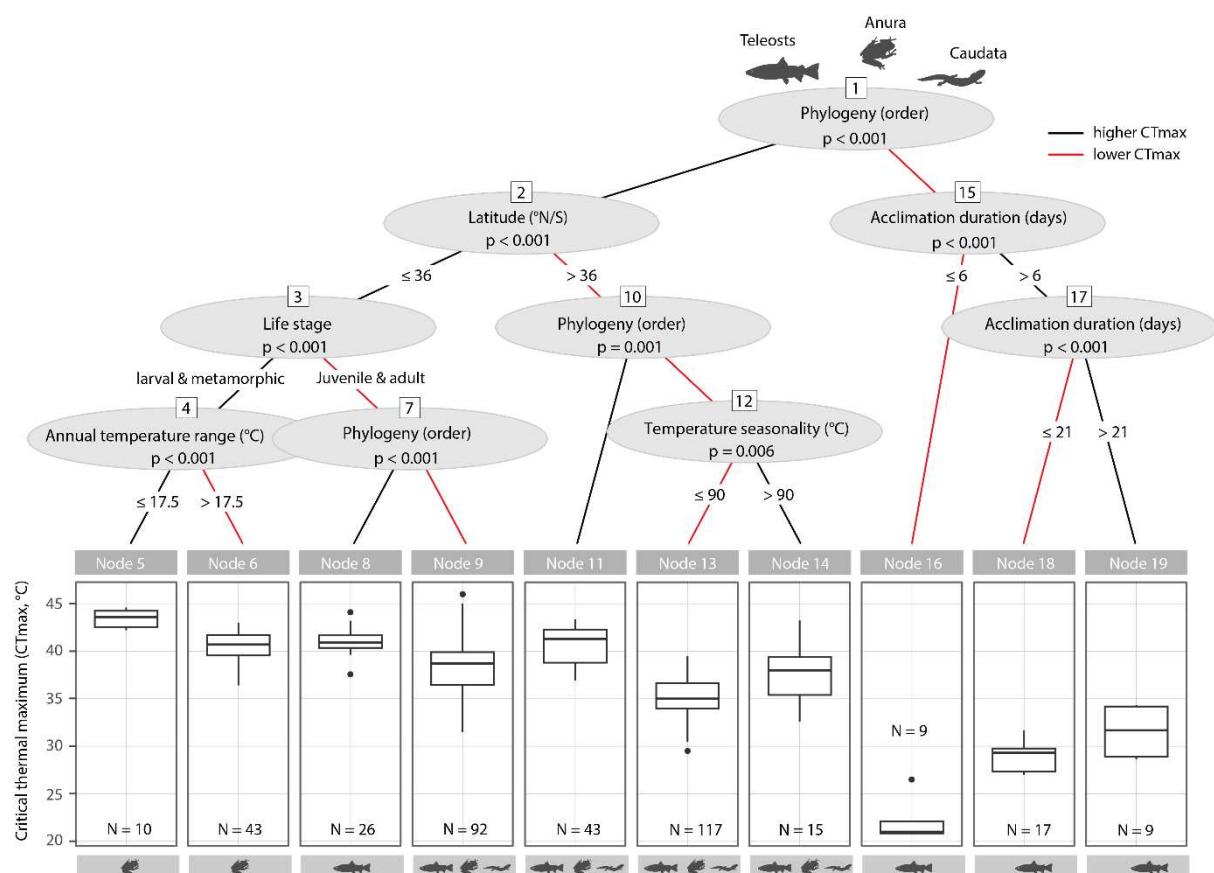
314 The analysis of paired  $CT_{max}$  and ARR values included 201 studies of 96 amphibian species (2  
315 orders) (Fig. 1a, b) and 144 studies of 93 teleost species (14 orders) (Fig. 1c, d) deriving from  
316 93 published articles, corresponding to a phylogenetic coverage of approximately 1% within  
317 each taxon. No data were available for Gymnophiona. Most studies determined  $CT_{max}$  and ARR  
318 in adult animals. For freshwater fishes, only one study determining  $CT_{max}$  in larvae was  
319 obtained, while 20% of the data covered juveniles (Fig. 1c). For amphibian species, 12% of the

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

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



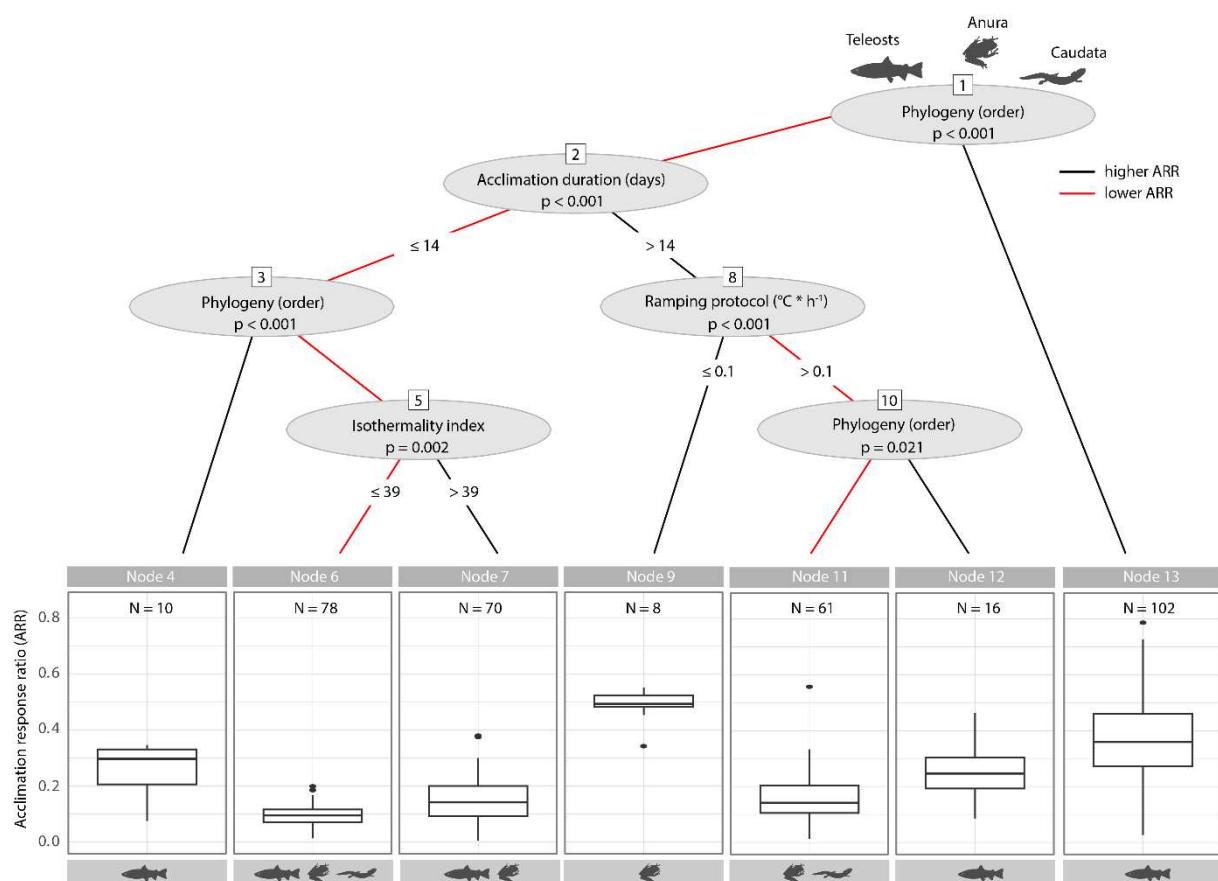
332 **Figure 1.** Phylogenetic, ontogenetic, and geographic background of critical thermal maximum ( $CT_{max}$ ) and acclimation response ratio (ARR)  
 333 measurements in (a, c) amphibians (Anura and Caudata) and (b, d) freshwater fishes (Teleostei). Phylogenetic trees contain (a) 96 amphibian and (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)).



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

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

355 in larval and metamorphic stages of anurans compared to juveniles and adults of anurans,  
 356 caudates and teleosts (Fig. S4). The influence of local bioclimatic variables was dependent on  
 357 phylogeny, latitude, and life stage. For low-latitude amphibians (early-life stages of anurans),  
 358  $CT_{max}$  was higher in regions with a lower annual temperature range (Bio 7,  $p < 0.001$ , node 4-  
 359 6; Fig. 2); and for high-latitude species,  $CT_{max}$  increased with temperature seasonality (Bio 4,  $p$   
 360 = 0.006, node 12-14; Fig. 2).



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

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

372 The first split of the classification tree separated a large group of fishes (8 orders, 68 species;  
373 Fig. 3) with a significantly higher mean ARR compared to the remaining fishes ( $P < 0.001$ ).  
374 The comparatively high ARR of this fish group was not related to experimental methodology  
375 nor any bioclimate variable. The second split was based on methodology, dividing the studies  
376 into groups with an acclimation duration longer or shorter than 14 d (node 3; Fig. 3). Longer  
377 acclimation generally resulted in higher ARR values ( $p < 0.001$ ), but the acclimation effect  
378 differed according to phylogeny, ramping rate and bioclimate (node 4-13). If the acclimation  
379 period was less than 14 days, a subsequent split occurred according to phylogenetic order ( $p <$   
380  $0.001$ ) and bioclimate (node 4 & 6), with a positive relationship between ARR and isothermality  
381 score (i.e., bio3;  $p = 0.002$ ; Fig. S4). When acclimation duration exceeded 14 d, studies were  
382 further subdivided according to ramping rate (node 9,  $P < 0.001$ ) and taxon (node 11,  $p = 0.021$ ).  
383 These terminal classifications imply higher ARR values at slow heating rates ( $<0.1^{\circ}\text{C min}^{-1}$ ),  
384 and higher ARR values in fish compared to amphibians at ramping rates  $>0.1^{\circ}\text{C min}^{-1}$  (Fig. 3).

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

#### 388 4. Discussion

##### 389 4.1 Context-Dependent Drivers and Broad-Scale Patterns of Physiological Limits

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

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

414 Unlike acclimation capacity, latitudinal origin was the primary determinant of heat tolerance  
415 in both taxa, with a higher heat tolerance in low-latitude species compared to high-latitude  
416 species, thereby supporting the *climate variability hypothesis* (Janzen 1967; Bozinovic et al.  
417 2011). Both taxa, therefore, exhibit physiologically adaptation to latitude-dependent thermal  
418 regimes to which they are exposed. Our results agree with the findings of a large body of  
419 research that has confirmed the link between physiological limits and large-scale geography  
420 based on a species' local adaptation to temperature and other associated climatic variables (e.g.,  
421 Gutiérrez-Pesquera et al. 2016; Sunday et al. 2011, 2019; Pintanel et al. 2022; but not: Addo-  
422 Bediako et al. 2000; Sørensen et al. 2016). Yet, in contrast to the broader literature, our synthesis  
423 indicated that  $CT_{max}$  was not *generally* higher in low-latitude populations and importantly it  
424 varied with ontogeny and local bioclimate. These findings emphasize the importance of  
425 assessing life stage-specific sensitivity to thermal stress as well as spatial climatic differences  
426 in conservation science. Therefore, focusing on large-scale geographical patterns for predicting  
427 how biodiversity will respond to future environmental change might bear the risk of  
428 overlooking context-dependent variation in thermal traits and thus, intraspecific differences in  
429 vulnerability to changing thermal conditions. For example, Bovo et al. (2023) demonstrated  
430 that responses of tropical amphibians to climate variation were heterogenous as a consequence  
431 of intraspecific variation in physiological traits and spatial variation in climate with elevation.  
432 Furthermore, Sunday et al. (2011) and Pinsky et al. (2019) reported that the physiological  
433 sensitivity of ectotherms across all latitudes depended on the realm, with terrestrial ectotherms  
434 being less sensitive to warming due to their higher capacity for behavioral thermoregulation.

435 4.2 *Life Stage-Specific Thermal Sensitivity as a Key Factor in Species Vulnerability to Climate*  
436 *Change*

437 In species with complex life-histories such as amphibians and teleost fish, life stages differ in  
438 size, morphology, physiology, and behavior (Wilbur 1980). Therefore, selection might promote  
439 stage-specific adaptations in thermal physiology (Enriquez-Urzelai et al. 2019; Ruthsatz et al.  
440 2022). Ignoring those life stage-specific differences in thermal physiology may drastically  
441 underestimate climate vulnerability of species with consequences for successful conservation  
442 actions. Here, we found  $CT_{max}$  but not acclimation capacity to differ between pre- and post-  
443 metamorphic life stages in amphibians, with a lower  $CT_{max}$  in juvenile and adult stages. Limnic  
444 larvae may have a reduced capacity for behavioral thermoregulation due to their limited body  
445 size impairing the movement between different microclimates (Kingsolver et al. 2011; Sinclair  
446 et al. 2016; Enriquez-Urzelai et al. 2019), making them more dependent on passive responses  
447 to temperature fluctuations. To cope with changes in temperatures, a high heat tolerance is  
448 therefore advantageous in early life stages (Ruthsatz et al. 2022). In contrast, post-metamorphic  
449 stages might rather be able to select favorable microclimates by behavioral thermoregulation  
450 (Navas et al. 2007; Haesemeyer 2020). This is particularly true for amphibians, as their post-  
451 metamorphic terrestrial habitats offer much spatial variability in operative thermal conditions  
452 (Gunderson & Stillman 2015), while juvenile and adult (freshwater) fish are able to  
453 behaviorally thermoregulate by performing vertical and horizontal movements (Amat-Trigo et  
454 al. 2023; but not: Clark et al. 2022). Moreover, juvenile and adult (freshwater) fish are known  
455 to show behavioral thermoregulation such as vertical and horizontal movements (Amat-Trigo  
456 et al. 2023; but not: Clark et al. 2022) with juveniles often displaying an ‘aggregation response’  
457 in cool water refuges (Breau et al. 2007). Our findings are in line with the pattern found for  
458 aquatic larvae by Cupp (1980), Enriquez-Urzelai et al. (2019), and Ruthsatz et al. (2022), who  
459 demonstrated a higher  $CT_{max}$  in amphibian larvae than in post-metamorphic stages. In contrast,  
460 Dahlke et al. (2020) found no difference in heat tolerance between larval and adult stages in  
461 marine and freshwater fish. Notably, our synthesis yielded only one estimate for larval  $CT_{max}$   
462 and acclimation capacity in freshwater fish and, thus, we lack the data for a definitive  
463 conclusion. Given that small body sizes of larvae restricts their capacity for behavioral  
464 thermoregulation, one would expect freshwater fish to exhibit the same life stage-specific  
465 differences in thermal sensitivity observed in amphibians. Finally, the lack of any life-stage  
466 specific pattern in acclimation capacity in the present study might be attributed to an inherent  
467 bias in life stage representation among the studied species. In a recent study on the European

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

476 *4.3 Understanding Context-Dependent Physiological Adaptation in Ectotherms*

477 Global syntheses on physiological studies can help us determine the winners and losers of  
478 climate change through assessment of broad-scale patterns of species' thermal limits and  
479 acclimation capacity for modifying their thermal tolerance (Somero 2010). This knowledge, in  
480 turn, enables us to develop suitable conservation strategies to mitigate the negative effects of  
481 climate change. However, our key findings emphasize that assessing species' vulnerability to  
482 changing thermal conditions based on large-scale geographic and/or phylogenetic patterns in  
483 thermal traits might cover up context-dependent physiological adaptations. In other words,  
484 tropical ectothermic species are considered particularly vulnerable to global warming as they  
485 live close to their physiological limits and have poor acclimation ability (Tewksbury et al. 2008;  
486 Huey et al. 2009; Sunday et al. 2014), but such generalizations might for instance ignore  
487 intraspecific variation in physiological limits across altitudinal variation in climate (Bovo et al.  
488 2023). Physiological adaptations are driven by the interplay between microclimate temperature  
489 heterogeneity and the behavioral thermoregulatory abilities of ectotherms (Huey et al. 2012;  
490 Pincebourde et al. 2016; present study) depending on their habitat characteristics (Pinsky et al.  
491 2019; Kulkarni et al. 2017), ontogeny (Enriquez-Urzelai et al. 2019; Ruthsatz et al. 2022a), life  
492 history traits such as body size (Rubalcaba et al. 2020; Peralta-Maraver & Rezende 2021) or  
493 activity patterns (Navas et al. 2007; Ruthsatz et al. 2022b), and/or energy balance (Pörtner et  
494 al. 2005; Muñoz et al. 2022). Physiological traits and limits are consequently rather  
495 evolutionary driven dynamic concepts than fixed values for a species (Bovo et al. 2018, 2023;  
496 Navas et al. 2022). In order to improve predictions of climate change impacts on biodiversity,  
497 it is imperative to deepen our understanding of context-dependent physiological adaptations  
498 (Meek et al. 2023), thereby advancing the development of suitable conservation  
499 measures/strategies that incorporate evolutionarily enlightened perspectives (Ashley et al.  
500 2003; Cook & Sgró 2018) beyond the species level (Fig. 4).

501 *4.4 Conclusion*

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

511 • **Biogeographic and taxonomic coverage**

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

524 • **Relevance of life stage and sex**

525 We urge future studies to measure a wider range of life stages and to measure both sexes within  
526 species to better identify potential life history thermal bottlenecks (Klockmann et al. 2017;  
527 Dahlke et al. 2020). All of the summaries to date likely overestimate physiological limits since  
528 most studies have been performed on adults and thermal tolerance increases (Klockmann et al.  
529 2017; Rubalcaba & Olalla-Tárraga 2020; but not di Santo & Lobel 2017) and acclimation  
530 capacity decreases (Pottier et al. 2021) with body size. Furthermore females and males differ in  
531 a wide range of morphological, physiological, and behavioral aspects as well as in their

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

537 • **Ecological relevance**

538 Future laboratory studies should adopt a more comprehensive and multifaceted approach for  
539 higher ecological relevance of thermal trait estimates (Desforges et al. 2023). Under natural  
540 conditions, organisms must often cope with multiple simultaneously occurring environmental  
541 stressors (Rohr & Palmer 2013; Gunderson et al. 2016) such as declining dissolved oxygen  
542 levels in freshwater habitats due to climate-induced temperature increases (Pörtner & Peck  
543 2010). As thermal limits are shaped by oxygen availability (Pörtner 2001, 2010), organisms  
544 might exhibit lower thermal limits under natural conditions. Moreover, exposure to pollutants  
545 might reduce thermal tolerance (Little & Seebacher 2015) or acclimation capacity (Ruthsatz et  
546 al. 2018b) due to increased metabolic demands of detoxification processes or disruption of  
547 endocrine pathways involved in physiological acclimation. Physiological responses to  
548 increased water temperature as performed in the studies summarized here (i.e., a single stressor)  
549 may not align with observed responses of individuals in the natural environments with multiple  
550 stressors (Potts et al. 2021).

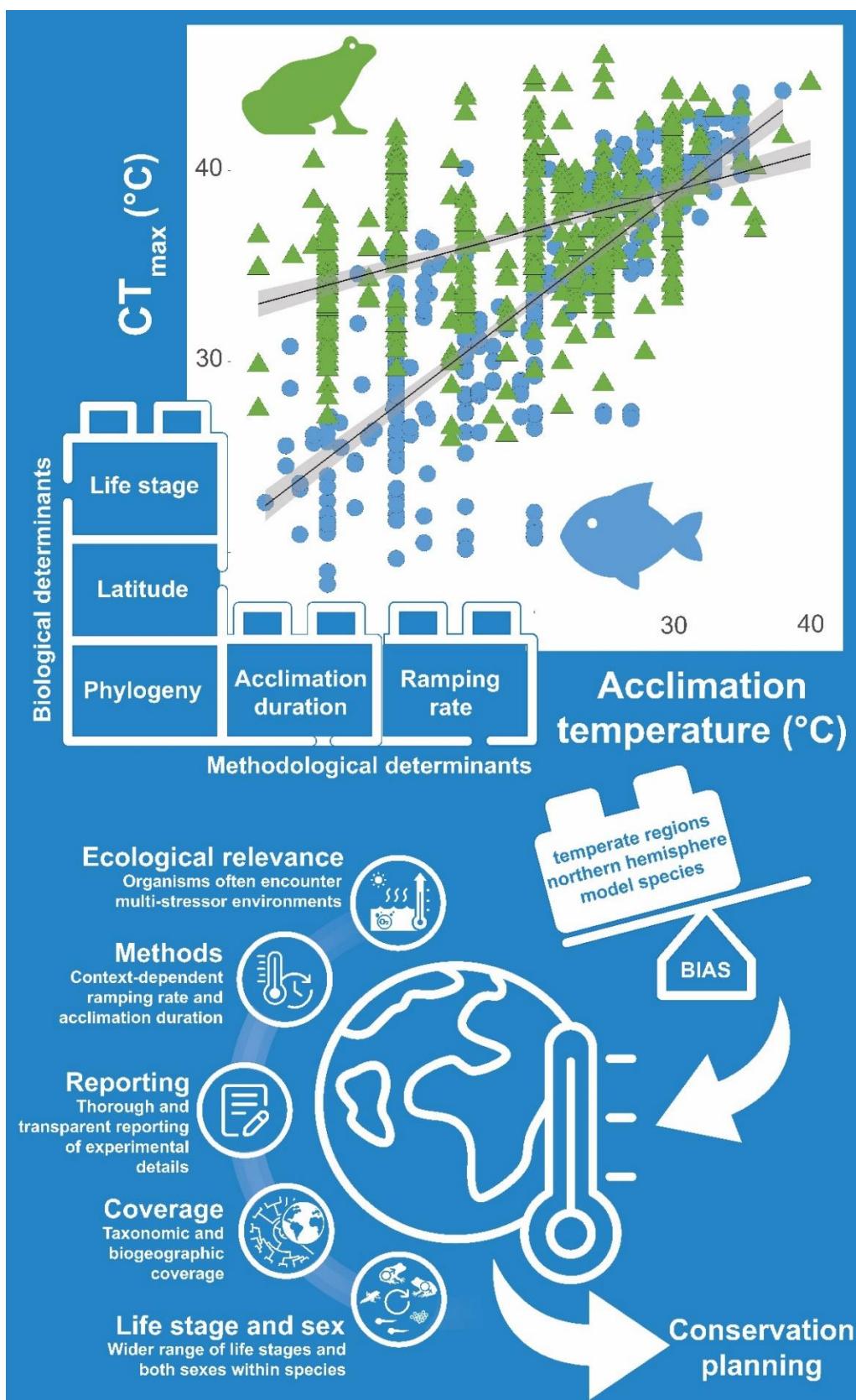
551 • **Methodological approach**

552 Customizing protocols to account for organismal and context-dependent variations in  
553 physiological limits (e.g., body size, life stage, sex, thermal history) will allow researchers to  
554 obtain more ecologically relevant estimates to inform conservation efforts. The application of  
555 an acute thermal ramping rate and a standardized endpoint such as the loss of equilibrium are  
556 used to measure critical thermal limits (Becker & Genoway 1979). The estimates are sensitive  
557 to differences in the methods. For example, faster ramping (heating) rates tend to yield higher  
558 thermal tolerance estimates compared to slower ramping rates (Moyano et al. 2017; Kovacevic  
559 et al. 2019; Penham et al. 2023). Using wild-collected animals is important as those reared in  
560 the laboratory may have physiology traits that differ from wild conspecifics (Pottier et al. 2021;  
561 Morgan et al. 2022). Methodological recommendations have been recently published and  
562 comparable methods are needed to compare thermal limits of different life stages (Cowan et al.  
563 2023; Desforges et al. 2023).

564 • **Through reporting of research details**

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

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



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

580 **5. Data availability**

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

583 **6. References**

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960 **8. Author contributions**

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

967 **9. Conflict of Interest**

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

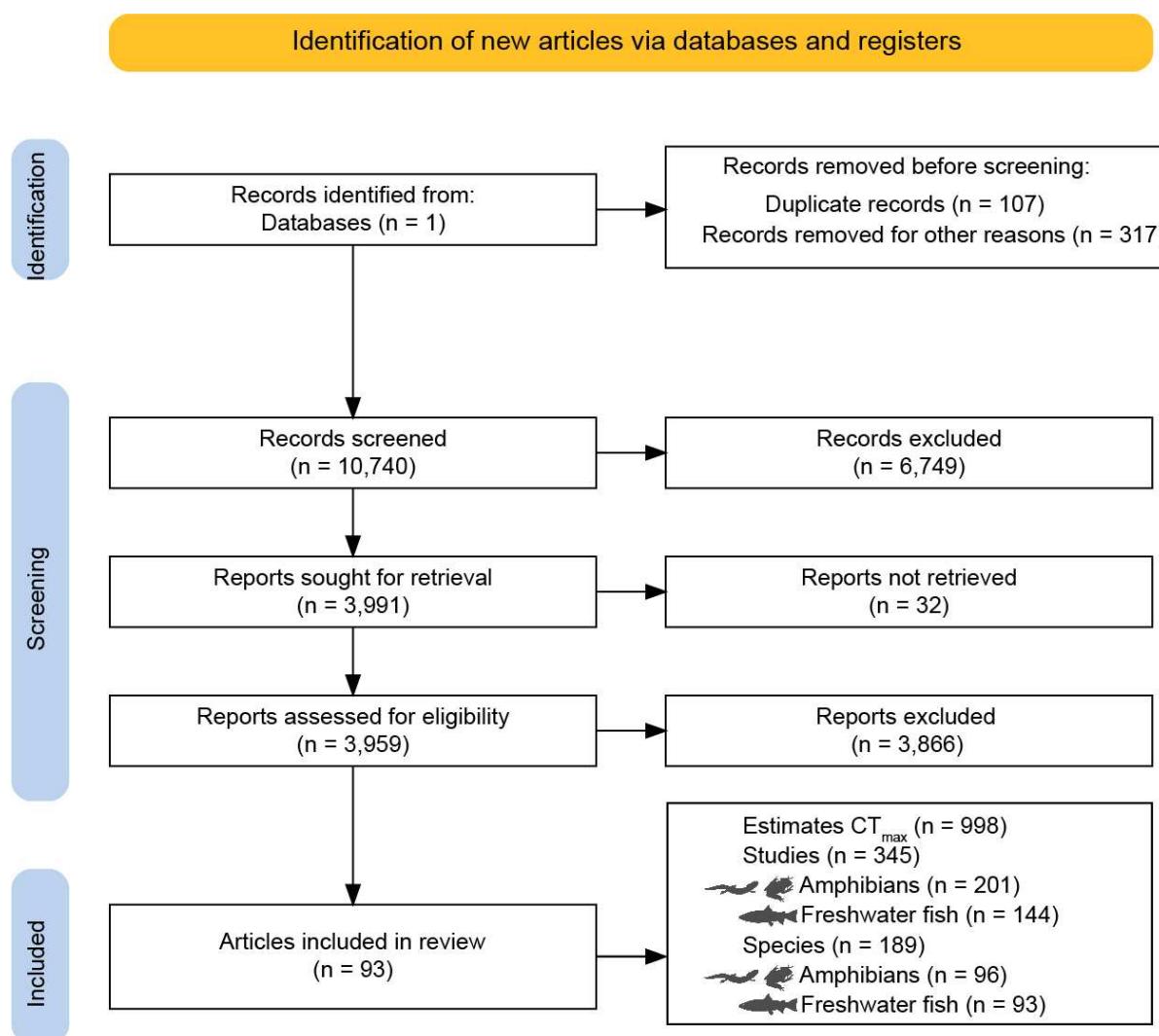
970 **10. Statement of Ethics**

971 The authors have no ethical conflicts to disclose.

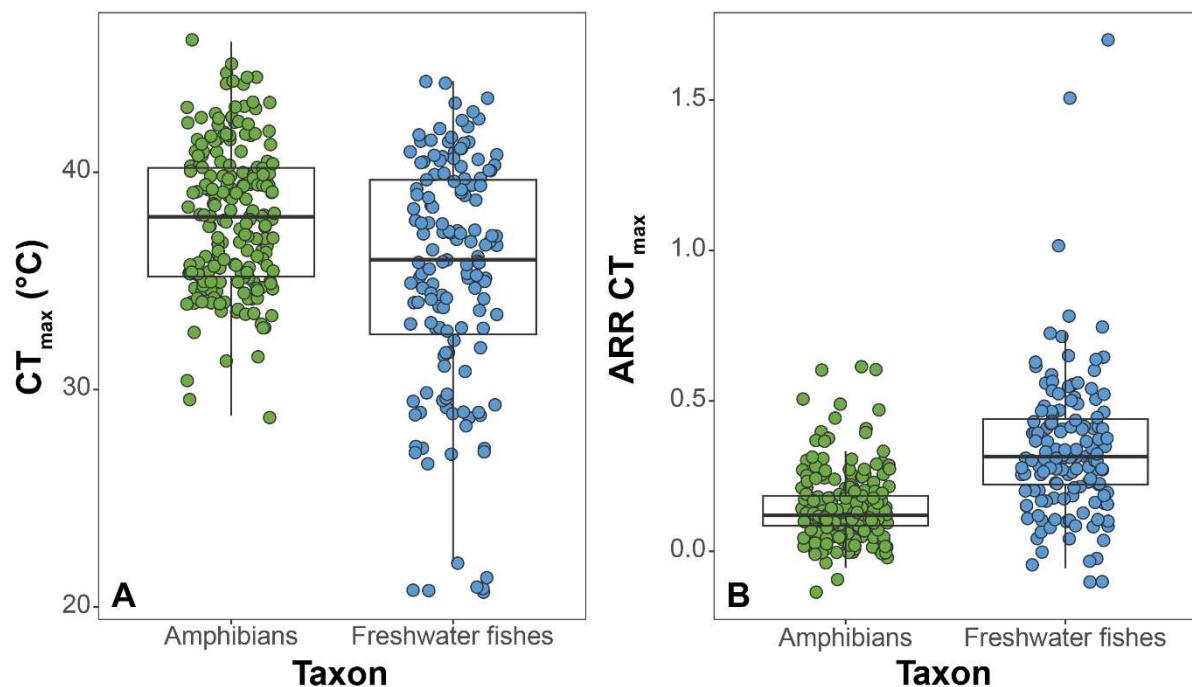
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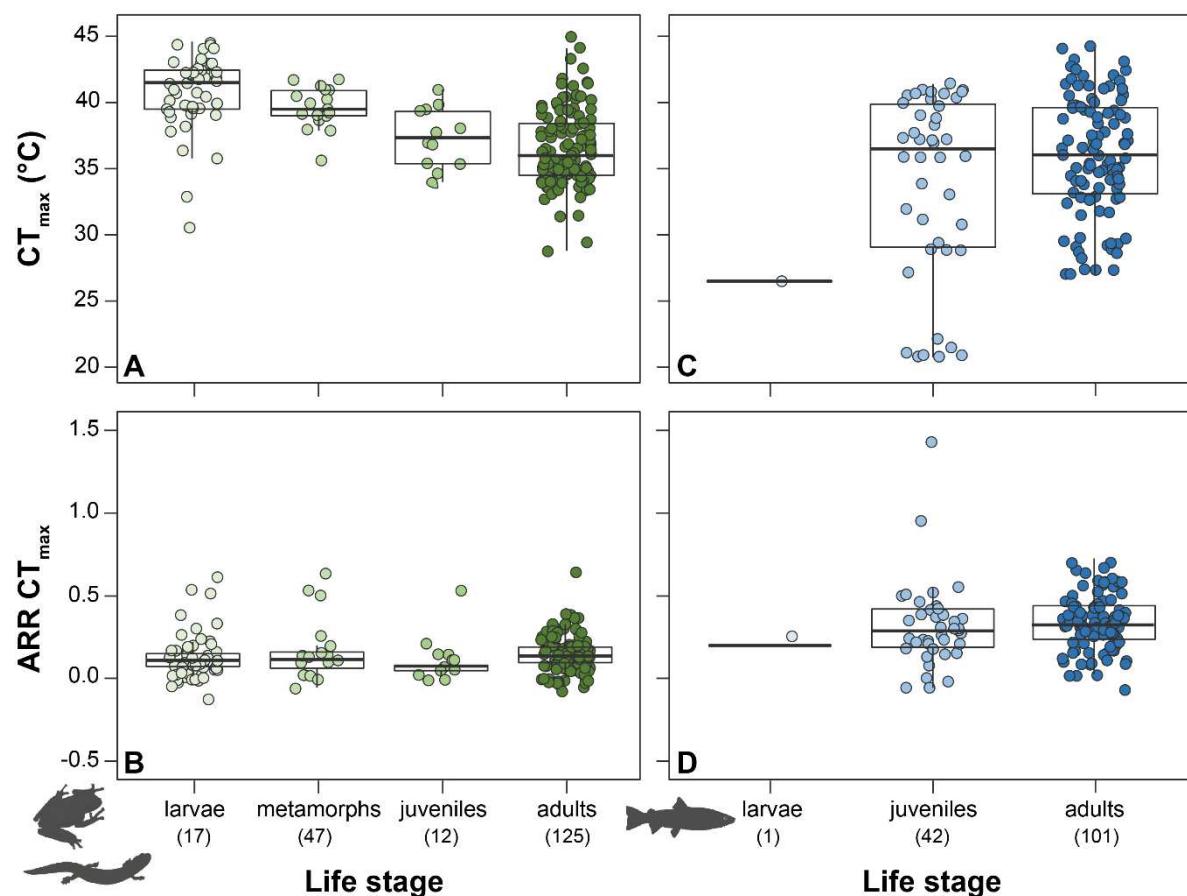
978 **Supplementary material**



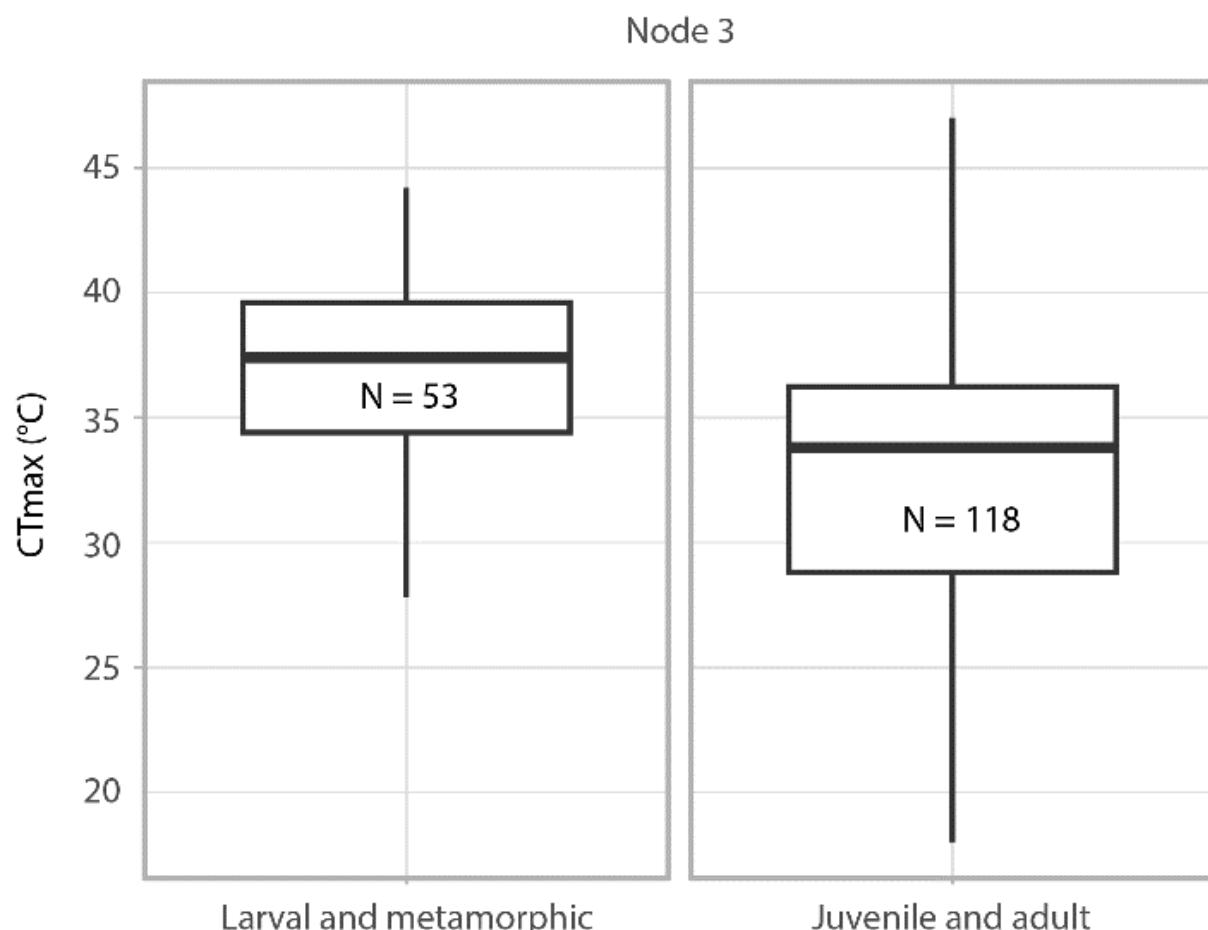
979 **Figure S1.** PRISMA flow diagram adapted from Page et al. (2021) showing literature search  
980 procedures and screening processes (created with Shiny app, Haddaway et al. 2022).

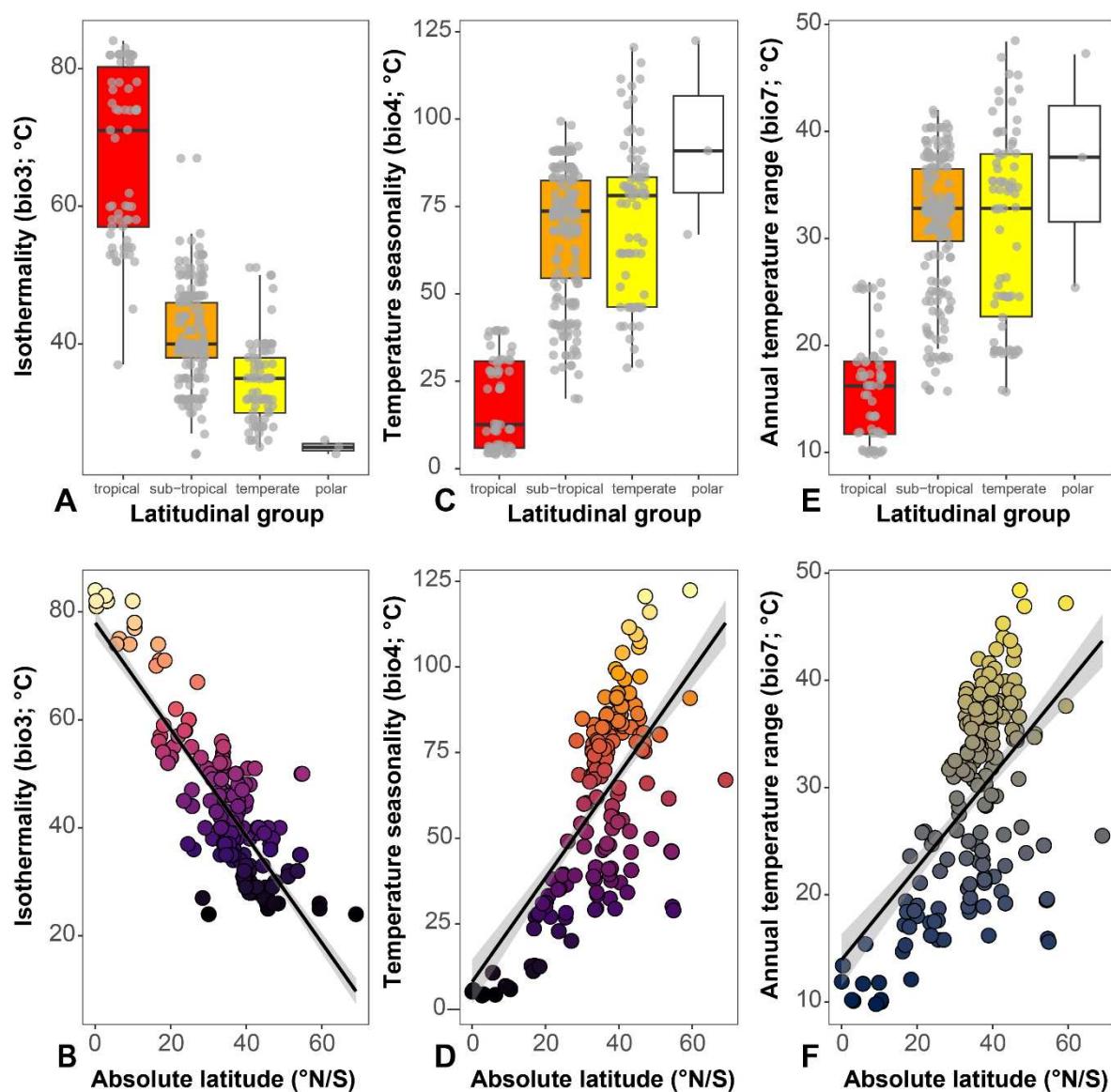


981 **Figure S2.** Taxon-specific **A** critical thermal maximum ( $CT_{max}$ ) and **B** Acclimation response  
982 ratio of  $CT_{max}$  (ARR  $CT_{max}$ ) for amphibians and freshwater fishes. Green: Amphibians. Blue:  
983 Freshwater fishes. Numbers in parentheses = sample size.



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





990 **Figure S5.** A. Isothermality (bio3), C. temperature seasonality (bio4), and E. annual  
991 temperature (bio7) range for BDF four latitudinal groups (i.e., tropical, sub-tropical, temperate,  
992 and polar) and as a function of absolute latitude ( $^{\circ}\text{N/S}$ ).