Developmental plasticity in amphibian larvae across the world: investigating the roles of temperature and latitude

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Abstract

28 Temperature-induced developmental plasticity could allow amphibian larvae to complete
29 metamorphosis successfully despite new thermal challenges and increased desiccation risk due
30 to climate change. Here we investigated how the capacity for temperature-induced
31 developmental plasticity varies with latitude and whether population-specific biogeographic
32 background accounts for the different degree of plastic responses to temperature. We carried
33 out a combined analysis based on the data from 150 studies (93 articles) performed on 64
34 amphibian species. We collected empirical data for age and size at metamorphosis in amphibian
35 larvae acclimated to different temperatures during development and found that all larvae from
36 all populations in these studies revealed a change in metamorphic traits with a given change in
37 temperature and thus, were able to exhibit temperature-induced developmental plasticity. Age
38 at metamorphosis was more affected by temperature than size at metamorphosis. Age and size
39 at the onset of metamorphosis were generally lowest at warmest temperatures during
40 development. Furthermore, populations from tropical latitudes were less sensitive to a change
41 in developmental temperature compared to populations from higher latitudes. Accordingly, we
42 suggest tropical populations to be the most vulnerable to increasing temperatures during
43 metamorphosis. Our analyses reveal biases with respect to taxonomy, biogeographic
44 distribution of species, and study design. Data from tropical populations are underrepresented
45 and thus, the capacity for developmental plasticity of the most threatened species probably
46 remains poorly understood. Future studies should focus on under-represented regions, most
47 threatened species, and include a broader range of temperatures during development in order to
48 make robust projections on future sensitivity of populations to climate change.

49 **1.   Introduction**

50 Environmental temperature is by far the most important abiotic factor for animals, as it has the
51 largest impact on their distribution, ecology, and physiology (Angilletta, 2009; and references
52 therein). This is particularly true for ectotherms since environmental temperature determines
53 their body temperature, and therefore regulates the rates of all physiological and biochemical
54 processes impacting growth, development, and metabolism (Hochachka and Somero, 1973,
55 2002; Huey and Stevenson, 1979; Angilletta et al., 2002). Thus, the prospected rise in
56 environmental mean temperatures, as well as the increasing frequency of heatwaves associated
57 with global climate change (IPCC, 2021), pose a serious challenge to ectotherms (Daufresne et
58 al., 2009; Verberk et al., 2021). Potential compensatory strategies to face the effects of global
59 warming include behavioral thermoregulation (Kearney and Porter, 2009), evolutionary
60 thermal adaptation (Huey et al., 2012), and/or exhibiting phenotypic plasticity (Gienapp et al.,

61 2008). In relation to the rapid speed of ongoing climate change, behavioral responses are likely

62 the fastest and most flexible option, followed by temperature-induced phenotypic plasticity,

63 which could be favored if behavioral adjustments are hindered (Seebacher et al., 2015).

64 In ectotherms with complex life cycles such as holometabolous insects, marine invertebrates,

65 parasites, most teleost fish, and amphibians (Laudet, 2011), changing thermal conditions lead

66 to a plastic response both in size and time at which they metamorphosize (i.e., plastic growth

67 and developmental rate) (Wilbur, 1980; Denver and Middlemis-Maher, 2010; Kingsolver et al.,

68 2011). However, an increase in temperature influences development stronger than it influences

69 growth (Angilletta and Dunham, 2003; Gomez-Mestre et al., 2010) and this leads to a reduced

70 size at metamorphosis under higher temperatures. This temperature-induced developmental

71 plasticity can be explained by the intraspecific 'temperature-size rule' (Atkinson, 1994). This

72 rule predicts that ectothermic species reared at relatively higher temperatures display faster

73 growth rates, but a shortened larval period, when compared with conspecifics reared at lower

74 temperatures.

75 Amphibians are especially sensitive to changing thermal conditions due to their highly

76 permeable skin, their limited capacity for habitat selection (behavioral thermoregulation), and

77 their complex life history (rev. in Navas et al., 2008). This is even more so in larval stages

78 where they are likely to encounter higher variation in environmental temperature. Therefore,

79 the timing of metamorphosis is of key importance for reducing mortality risk (Rudolph and

80 Rödel, 2007) due to desiccation or temperature extremes. Several studies have demonstrated

81 that amphibian larvae exhibit plasticity in age and size at metamorphosis as a response to

82 variation in temperature (e.g., Merilä et al., 2000; Alvarez and Nicieza, 2002, Tejedo et al.,

83 2010; Yu et al., 2015; Ruthsatz et al., 2018b, 2020) following the temperature-size rule (rev. in

84 Ruthsatz et al., 2018a). The capacity for temperature-induced developmental plasticity may

85 provide a means for increasing fitness (Schlichting and Pigliucci, 1998; Boorse and Denver,

86 2004). For example, traits such as a short larval period and/or a smaller body size at

87 metamorphosis are hypothesized to confer greater fitness (Wilbur and Collins, 1973; Berven,

88 1990; Beck and Congdon, 2000; Ruthsatz et al., 2019).

89 In the light of declining amphibian populations worldwide (Stuart et al., 2004; Alroy, 2015;

90 Green et al., 2020) and an increase in erratic weather conditions as a result of global warming,

91 temperature-induced plasticity in physiological and morphological traits will be key for the

92 continued survival of many amphibians (Barria and Bacigalupe, 2017). However, the capacity

93 for temperature-induced plasticity might differ among species and populations (i.e.,

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94   phylogenetic contributions) and might be related to latitude and climate (i.e., thermal
95   adaptation; rev. in Edge et al., 2016). Not all amphibians might be able to use temperature-
96   induced plasticity as a mechanism to cope with climate change. For example, tropical
97   ectotherms are currently living very close to their optimal temperature and hence reveal reduced
98   thermal safety margins (Deutsch et al., 2008). As predicted by the 'climate variability
99   hypothesis' (Janzen 1967; Chown et al., 2004; Ghalambor et al., 2006), plasticity in thermal
100 tolerance exhibited by ectotherms such as amphibians is correlated with the magnitude of
101 variation in environmental temperature (Addo-Bediako et al., 2000; Ghalambor et al., 2006;
102 Bozinovic et al., 2011; but see Sørensen et al., 2016; Johansson et al., 2020) and increases as
103 we move from the equator towards the poles (Janzen et al., 1967). This expected relationship is
104 one of the several reasons (Gunderson and Stillman, 2015) that tropical species are suggested
105 to be at a higher risk from climate change (Tewksbury et al., 2008; Sunday et al., 2014; Pacifici
106 et al., 2015), but empirical evidence is still limited and controversial. While phylogenetic and
107 biogeographic patterns of plasticity in thermal tolerance have been well studied over the last 50
108 years (e.g., Brattstrom, 1968; Duarte et al., 2012; Gunderson and Stillman, 2015; Morley et al.,
109 2019), comprehensive studies investigating factors determining the capacity for temperature-
110 induced developmental plasticity are still rare (rev. in Ruthsatz et al., 2018a).

111 In this paper, we assessed the capacity for temperature-induced developmental plasticity in
112 amphibian larvae and tested for abiotic correlates of this trait. We performed an extensive
113 literature review and a combined analysis based on a total of 93 articles containing 1,154
114 estimates of metamorphic traits from 64 species in 150 studies. Specifically, we investigated
115 whether the latitude and/or the biogeographic background determines plasticity in metamorphic
116 traits of a given population. Based on the climate variability hypothesis, we hypothesized that
117 the capacity for temperature-induced developmental plasticity is the highest in species
118 distributed at higher latitudes, as higher latitudes have strong seasonality with a wider climatic
119 variation (Stevens, 1989; Calosi et al., 2010; Naya et al., 2011). Further, we quantitatively
120 evaluated published research on temperature-induced developmental plasticity in amphibians.
121 We hypothesized that the current knowledge on phenotypic plasticity in larval amphibians
122 might be biased towards temperate regions since tropical regions, despite their high species
123 richness and higher population declines, remain underrepresented in conservation research
124 (Schiesari et al., 2007; Hansen et al., 2018; da Silva et al., 2020).

125 **2.   Material and Methods**

126   *2.1 Systematic literature review*

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We conducted a systematic literature review using ISI Web of Science (ISI WOS, 2021) in April 2021 (searched for: "TOPIC"; search term: ((amphibian* OR newt OR frog OR toad OR salamander OR anuran* OR caudate*) AND (larv* OR tadpole* OR metamorph*) AND (temperature OR temperature during development OR climate change OR global warming OR climat* shift OR abiotic OR biotic OR acidi* OR pH OR oxygen OR hypox* OR salinity OR road salt OR O2 OR stress* OR pollut* OR heavy metal* OR fertilizer* OR predator OR pesticide* OR herbicide* OR hormone* OR desiccation OR disease* OR invasive species OR infection* OR environmental stress*) AND (effect* OR impact* OR cause* OR affect*) AND (age OR size OR growth OR weight OR mass OR growth rate OR develop* OR larval time OR larval duration OR time to metamorphosis OR mortality OR thermal window OR tolera* OR limit* OR critic* OR lethal OR aerobic scope OR metabolic scope OR sensitivity OR metabolism OR SMR OR metabolic rate OR CTMAX OR standard metabolic rate OR performance OR energy budgets OR heart rate OR body condition OR development* window OR plasticity OR acclimation OR development*plasticity OR growth plasticity). Timespan: All years.) (Pullin and Stewart, 2006). The reference lists of selected papers were also searched for further studies.

2.2 Data collection

The following selection criteria had to be fulfilled by the experimental design of the included studies: (1) experiments were conducted in the laboratory (i.e., no field studies), (2) at least at two different temperatures during development for the larvae, and (3) food was provided *ad libitum*.

We collected single data points (1154) for age (days after hatching to onset of metamorphosis), size, and growth rate (mg/day) at onset of metamorphosis from 93 published articles (Table S1). Size was measured by mass (mg), snout-vent length (SVL; mm), and/or total length (TL; mm) in respective studies. The onset of metamorphosis was defined as the emerging of at least one forelimb according to Gosner developmental stage 42 (Gosner, 1960). Furthermore, we obtained information on temperatures during development (i.e., tested temperature points) used in the respective studies as well as on variables representing sampling location as detailed as possible (i.e., GPS coordinates) and scientific classification according to the Linnean classification.

Some of these articles performed different studies on e.g., different populations of one species or on different species. Therefore, data collection resulted in 150 studies from 93 articles. *F*or

example, an article that tested the effect of two different temperatures during development on body mass of (a) *Rana temporaria* and (b) *Bufo bufo* was counted as one article, two studies, and four estimates/data points of body mass. All animals from one species sampled at the same location were considered as one population.

For studies that use figures instead of tabularization of their results, we used Engauge Digitizer 9.7 (Mitchell et al., 2021) to extract data from the graphs.

2.3 Biogeographic background

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°) or temperate (> 40-53.55°; Morley et al., 2019). Data were excluded if measurements were taken from specimens bred artificially such as university or hospital laboratory breeding or pet trade to reduce confounding issues associated with artificial selective history (Bennett et al., 2018). Consequently, analyses testing the effect of population-specific biogeographic background if measurements were carried out on a reduced dataset (i.e., reduced dataset).

For each sampling location, we extracted elevation (m) and 19 bioclimatic metrics related to temperature and precipitation (BioClim, WorldClim; Fick and Hijmans, 2017) for the time period of 1950 to 2000. The data were extracted, at a spatial resolution of 2.5 arc-min, using packages 'raster' and 'sp' in R (version 4.0.3; R Core Team, 2007).: 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) at a scale of 1 km^2 for each metric (Fick and Hijmans, 2017; Table S1).

We used these macroclimatic data (i.e., air temperature) as a proxy to estimate the thermal adaptation in amphibian larvae because we had no access to microclimatic data (e.g., actual water temperatures in the breeding pond) from the original articles. As most amphibians breed in small or shallow bodies of water, we assumed that the temperature of these breeding ponds

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190   might approximately equal average near-surface air temperature. Thus, the average near-surface

191   air temperature provided by WorldClim is an appropriate estimate of the temperature of

192   freshwater systems (Morley et al., 2019). Our study's inferences consider the limitation

193   imposed by using macroclimatic data because amphibian larvae inhabiting fluctuating

194   microhabitats are benefited from microenvironments that filter environmental fluctuations

195   (Woods et al., 2015; Oyamaguchi et al., 2018).

196   *2.4 Statistical analysis*

197   All statistical tests were performed in R (version 4.0.3; R Core Team, 2007). All plots were

198   constructed using ggplot2 (Wickham, 2009) unless mentioned otherwise, and Adobe Illustrator

199   2021. All independent variables were scaled before analyses with zero-mean standardization

200   using the *scale()* function in R.

201   *2.4.1 Temperature effects on metamorphic traits and plasticity index (PIX)*

202   To determine the thermal reaction norm (i.e., sensitivity) of metamorphic traits (as measured

203   by age, mass, SVL, TL, and growth rate) to temperature variation, we performed single linear

204   regressions of temperatures during development (independent variable) and metamorphic traits

205   (dependent variables) for each study included here (Fig. 1). Values for age, mass, SVL, TL, and

206   growth rate were log-transformed to account for high levels of regression residuals. The slope

207   of each regression describes the change in a metamorphic trait with a 1 °C change in

208   temperatures during development and was used as a plasticity index (PIX) according to e.g.,

209   Claussen (1977), Gunderson and Stillman (2015), and Ruthsatz et al. (2018a, 2020). Linear

210   regressions for each trait within the partial studies resulted in 399 data points for PIX (i.e., full

211   dataset), which were included as replicates in statistical analyses (Table S1). PIX values

212   indicate the sensitivity of growth and developmental rate to different temperatures during

213   development, and thus, the ability for a plastic response in metamorphic traits. Higher absolute

214   values of PIX correspond to higher plasticity (e.g., greater sensitivity of growth and/or

215   developmental rate to temperature).

216   *2.4.2 Effect of biogeographic background on the capacity for temperature-induced*

217   *developmental plasticity in amphibian larvae*

218   To examine the effect of population-specific biogeographic background (i.e., latitude,

219   elevation, and thermal adaptation) on the capacity for temperature-induced developmental

220   plasticity in amphibian larvae, data were analyzed using separate generalized linear mixed-

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effect models (GLMM). To account for possible phylogenetic effects of different species included in our dataset, we compared two methodological approaches for performing GLMMs: (1) the *glmer()* function (Bates et al., 2019) in the lme4 package (https ://cran.r-project.org/package=lme4) using the categorical predictor 'species' as a random factor and (2) the *MCMCglmm()* function (Hadfield, 2010) in the MCMCglmm package (https ://cran.r-project.org/package=MCMCglmm) using Markov Chain Monte Carlo sampling method for phylogenetic correction in R. Both GLMMs were performed with Gaussian distribution using the reduced dataset. The absolute plasticity indices for log-transformed age, mass, SVL, TL, and growth rate were used as dependent variables in separate models (Table 1).

2.4.2.1 Predictor selection procedure

Because many of the 19 bioclimatic variables are highly correlated, we first performed multiple Spearman's rank correlations between all possible predictor variables (i.e., latitude, elevation, and bioclimatic variables). Using the *caret* package in R, we then removed all variables with a correlation of $r > 0.55$ and used the five least-correlated predictor variables as fixed factors for our GLMMs, considering the random factor as specified above. Relationships between latitude and elevation on dependent variables as well as of latitude on retained bioclimatic variables were determined with multiple linear regressions (Fig. A1-A4).

Differences in acclimation capacity between latitudinal groups (i.e., temperate, sub-tropical, and tropical) were analyzed by Kruskal-Wallis test and applying Bonferroni correction using the reduced dataset (Fig. 3D; Fig. A1).

2.4.3 Phylogenetic effects on acclimation capacity in amphibian larvae

We obtained phylogenetic relationships among amphibians from a previously published supertree (Pyron and Wiens, 2011) using the package PhyloOrchard (https://rdrr.io/rforge/PhyloOrchard/), and trimmed the tree to reduce it to taxa of our interest for each PIX variable using the package Ape (Paradis and Schliep, 2019) and Geiger (Pennell et al., 2014). We tested for the effect of phylogenetic non-independence by using these trimmed phylogenetic trees as random factors in addition to species identity for all PIX variables separately. We used the same models and fixed effects as the aforementioned GLMM runs to compare results and deduce the effect of phylogenetic relatedness on our inferences. We re-ran GLMMs using the Bayesian Markov Chain Monte Carlo (MCMC) sampling method

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implemented in the package MCMCglmm (Hadfield, 2010) using the default priors given by the program and Gaussian distribution. MCMC estimation allows a better strategy to marginalize random effects such as phylogenetic non-independence and measurement errors (Hadfield, 2010) and effectively sample for posterior estimates from a large number of simulations (Gilks et al., 1995). Due to the low number of taxa for which PIX of Growth Rate (PIX GR) and PIX of Total Length (PIX TL) were available, we did not carry out GLMMs with MCMC sampling for these dependent variables as the number of posterior estimates in the model would exceed the number of available data points. After identifying key factors that affect various PIX variables, we plotted the PIX value and other key fixed factors alongside the phylogenetic tree utilizing the package ggplot2 (Wickham, 2016) and ggtree (Yu et al., 2017).

2.4.4 Biogeographic distribution of research in amphibian thermal biology

The distribution of the geographic locations at which experimental populations were sampled was illustrated in a world map using GeoMapApp (version 3.6.12; Ryan et al., 2009; Fig. 4). The proportion of studies carried out on each continent or in each latitudinal group were calculated in percentage.

3. Results

3.1 Systematic literature review

The systematic literature review returned 3,719 articles into an unfiltered reference library. After examining titles and abstracts, 717 articles were left as possibly relevant in the filtered reference library. Examining the full text of the filtered reference library led to 93 articles accepted in the reference library (Table S1). These articles were published between 1988 and 2020 (with 50.7 % of the studies published during the past 10 years) and comprised 1,154 data points from 150 studies representing 137 amphibian populations (Table S1). These 137 populations were from 64 species, 34 genera, 18 families, and 2 orders according to the Linnean classification. Temperatures during development ranged between 5.5 to 36 °C. The temperature range of the tests ranged between 1 and 18 °C. Sample sizes differ for independent variables age, mass, SVL, TL, and growth rate at metamorphosis because many studies do not include all of the five variables.

3.2 Effect of temperature during development on age and size at the onset of metamorphosis

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Age at metamorphosis decreased significantly in 99.1% of the studies (Table S1) and on average by 2.21 days with every 1 °C increase in temperature during development across all studies (P<0.001; Fig. 1A). The highest temperatures during development led to the youngest age and the lowest temperatures during development led to the oldest age at the onset of metamorphosis. SVL at the onset of metamorphosis decreased significantly in 66.6% of all studies with increasing temperature during development (P=0.002; Table S1; Fig. 1B). In contrast, TL at the onset of metamorphosis increased significantly in 51.7% of all studies with temperature during development (P<0.001; Table S1; Fig. 1C). SVL and TL at the onset of metamorphosis decreased and increased by 0.18 mm and 7.81 mm with every 1 °C increase in temperature during development across all studies, respectively. Neither log-transformed mass (P=0.761) nor growth rate (P=0.467) decreased or increased significantly with temperature during development across all studies (Fig. 1DE). On study level, slopes of mass at metamorphosis revealed a decrease with increasing temperature during development in 74.5% of the studies, whereas growth rate increased in 75.3% of the studies (Table S1).

Fig. 1 The effect of temperature during development on values of **A** age (in days after hatching), **B** mass (in mg), **C** snout-vent length (SVL, in mm), **D** total length (in mm), and **E** growth rate (mg/day after hatching) at the onset of metamorphosis. Dots and respective regression lines refer to the individual studies. Statistics for log-transformed values and linear regressions. Black regression line shows the general effect of temperature during development on dependent variables of all included studies if regression is significant; dotted line for non-significant regressions. The color code refers to the latitudinal group of spawn collection site of the respective populations (see text for further details). Yellow = temperate group (>40°). Orange = sub-tropical zone (25-40°). Red = tropical zone (0-25°). N=112.

The absolute PIX differed significantly between all metamorphic traits (Kruskal-Wallis test. H=158.033, df=4, N=400, P<0.001). The mean (± SD) absolute PIX was highest for growth rate (0.037 ± 0.03) and lowest in SVL (0.006 ± 0.00) across all partial studies (Fig 2).

Fig. 2 Absolute plasticity index (PIX) of log-transformed values of growth rate (mg/day after hatching), age (in days after hatching), mass (in mg), total length (in mm), and snout-vent length (SVL, in mm) at the onset of metamorphosis sorted by mean value. The plasticity index describes the change in metamorphic traits with a given change in temperature during development . Box = 1st and 3rd quartiles with median. Whiskers = 1.5-fold interquartile range. Dots = outliers. Numbers = sample size (number of studies) per trait. N=399.

314　　　　　　*3.3 Effects of the population-specific biogeographic background on the PIX of age and*
315　　　　　　*size at metamorphosis*

316　After excluding the data derived from specimens bred artificially such as university or hospital

317　laboratory breeding or pet trade to reduce confounding issues associated with artificial thermal

318　adaptation, 383 data points remained in the reduced dataset for latitudinal analyses. Absolute

319　latitude, elevation, Annual Temperature Range (Bio 7), Annual Precipitation (Bio 12), and

320　Precipitation Seasonality (Bio 15) were retained as factors representing the population-specific

321　biogeographic background from predictor selection procedure (Table A1; Fig. A1-A5).

322　GLMMs revealed a significant increase in the capacity for temperature-induced developmental

323　plasticity in age at the onset of metamorphosis (PIX age) with absolute latitude (Fig. 3AC;

324　Table 1), but PIX age did not significantly differ among latitudinal groups (Kruskal-Wallis test.

325　H= 4.895, df=2, N= 112, P=0.086; Fig. 3D). However, R² (0.073) from linear regression shows

326　that only a part of the variance was explained by latitude indicating that other factors might also

327　be important for the capacity to respond plastically to temperature variation (Fig. 3C). The

328　plasticity index of all other traits, however, was not correlated with latitude and did not

329　significantly differ among latitudinal groups (Table 1; Kruskal-Wallis test. Mass: H= 2.838,

330　df=2, N= 110, P=0.242; SVL: H= 0.145, df=2, N= 74, P=0.930; TL: H= 1.641, df=2, N= 25,

331　P=0.440; growth rate: H= 2.408, df=1, N= 62, P=121; Fig. A1). No study included in our dataset

332　measured growth rate in a tropical species at different temperatures during development .

333　We built separate GLMMs for the absolute PIX of all traits (i.e., age, mass, SVL, TL, and

334　growth rate at the onset of metamorphosis) from a combination of abiotic factors including

335　latitude, elevation, and the bioclimatic predictors from predictor selection procedure (Table

336　A1).

337　**Fig. 3** Absolute plasticity index (PIX) of age at metamorphosis (green and blue shades) and **A**

338　mean of absolute latitude of the source populations (°N/S) (latitude) of different species with

339　their phylogenetic relationships, **B** mean of Annual Temperature Range of different species

340　with their phylogenetic relationships, **C** as a function of absolute latitude of the source

341　population (°N/S), **D** for three latitudinal groups (i.e., tropical, sub-tropical, and temperate) in

342　amphibians, and **E** as a function of Annual Temperature Range (Bio 7). Black regression line

343　for significant linear regressions; dotted line for non-significant regressions. The color code

344　refers to the latitudinal group of spawn collection site of the respective populations (see text for

345　further details). Yellow = temperate group (>40°). Orange = sub-tropical zone (25-40°). Red =

346 tropical zone (0-25°).Box = 1st and 3rd quartiles with median. Tree only includes species

347 included in latitudinal analysis. Whiskers = 1.5-fold interquartile range. Dots = single data.

348 GLMMs revealed a significant decrease in the capacity for temperature-induced plasticity in

349 age at metamorphosis (PIX age) with increasing Annual Temperature Range (Bio 7) (Table 1;

350 Fig. 3B). However, R^2 (0.003) from linear regression shows that only a part of the variance

351 was explained by Annual Temperature Range (Bio 7) indicating that other factors might also

352 be important for the capacity to respond plastically to temperature variation (Fig. 3E). None of

353 the included bioclimatic variables explained PIX of SVL, mass, or growth rate (Table 1). The

354 capacity for temperature-induced developmental plasticity in TL at the onset of metamorphosis

355 was explained best by elevation and Annual Temperature Range (Bio 7)(Table 1; Fig. A4). The

356 higher the Annual Temperature Range (Bio 7)), the lower is the capacity for temperature-

357 induced plasticity in TL at metamorphosis. Plasticity in TL at metamorphosis increased with

358 elevation (Table 1; Fig. A4), whereas there was no effect of elevation on PIX of all other

359 metamorphic traits (Fig. A3).

360 **Table 1.** Generalized linear mixed models testing the effect of absolute latitude (°N/S).

361 elevation (m.a.s.l.), and three bioclimatic parameters on the capacity for temperature-induced

362 developmental plasticity in age and size at the onset of metamorphosis in amphibian larvae.

363 Species was included in the models as random factor. Bio 7 = Annual Temperature Range. Bio

364 12 = Annual Precipitation. Bio 15 = Precipitation Seasonality. N for datapoints, n for number

365 species. See text for further details.

Dependent variable [N(n)]	Fixed effects	Estimate	SE	df	t	P
PIX age [112(42)]	Intercept	0.036	0.003	33.03	11.13	**<0.001**
	Latitude	0.011	0.003	78.30	3.24	**0.001**
	Elevation	0.002	0.002	55.47	1.24	0.219
	Bio 7	-0.006	0.002	66.45	-2.87	**0.005**
	Bio 12	-0.001	0.002	62.16	-0.49	0.622
	Bio 15	0.003	0.002	62.31	1.62	0.110
PIX mass [110(39)]	Intercept	0.021	0.002	13.33	9.14	**<0.001**
	Latitude	-0.001	0.003	36.46	-0.47	0.641
	Elevation	0.001	0.002	49.21	0.56	0.578
	Bio 7	0.001	0.001	60.05	0.73	0.466
	Bio 12	-0.002	0.002	65.01	-0.98	0.330
	Bio 15	-0.003	0.002	71.72	-1.47	0.144
PIX SVL [74(32)]	Intercept	0.005	0.001	5.78	3.57	**0.012**
	Latitude	-0.001	0.001	2.31	-0.35	0.726
	Elevation	0.000	0.001	1.95	0.35	0.725
	Bio 7	0.000	0.001	1.93	0.06	0.946
	Bio 12	0.001	0.001	3.58	0.93	0.355
	Bio 15	-0.001	0.001	4.68	-0.49	0.625
PIX TL [25(6)]	Intercept	0.015	0.003	19.00	4.31	**<0.001**
	Latitude	-0.000	0.006	19.00	-0.05	0.958
	Elevation	0.020	0.009	19.00	2.26	**0.035**

	Bio 7	-0.011	0.004	19.00	-2.17	**0.042**
	Bio 12	-0.005	0.006	19.00	-0.77	0.446
	Bio 15	-0.007	0.007	19.00	-0.95	0.35
PIX growth rate [62(7)]	Intercept	0.045	0.021	33.06	2.17	**0.037**
	Latitude	-0.005	0.021	45.00	-0.26	0.796
	Elevation	0.019	0.029	55.11	0.64	0.522
	Bio 7	-0.016	0.011	53.95	-1.46	0.149
	Bio 12	-0.006	0.017	49.35	-0.40	0.691
	Bio 15	-0.001	0.021	36.57	-0.07	0.942

3.4 Phylogenetic effects on acclimation capacity in amphibian larvae

GLMMs carried out using the MCMC sampling approach where we account for phylogenetic non-independence produced similar results to the aforementioned GLMMs showing that phylogenetic relatedness among species included in this study does not have a clear effect on the plasticity indices of amphibian tadpoles. The PIX values for the age of metamorphosis (PIX age) increased with increase in elevation and decreased with increasing Annual Temperature Range (bio7) (see Fig. 3 and Supplementary tables S2-S4). No fixed effects had non-zero effects on other PIX variables (see Supplementary tables S2-S4). Visualization of posterior estimates of fixed effects and covariances estimated for random effects confirmed the convergence of MCMC chains in all the runs (see Supplementary tables S2-S4). In addition, histogram plots of posterior estimates of covariance for the two random factors – species identity and the phylogenetic tree - indicated that the phylogenetic relatedness does not have a significant effect on the value of PIX variables as the posterior estimates were close to 0 (see Supplementary Table S2-S4).

3.5 Biogeographic and taxonomic distribution of research in amphibian thermal biology

The dataset has a wide global spatial coverage (Fig. 4) including studies carried out on six continents and 25 countries (Table S1). All studies were carried out on the continent where the populations were sampled. Most studies were carried out in Europe (N=87 in 40 articles) and North America (N=29 in 26 articles), followed by Asia (N=16 in 16 articles), South America (N=12 in 7 articles), Australia (N=5 in 5 articles), and Africa (N=1 in 1 article). Thus, geographical data gaps exist, for example in Africa and large(r) parts of the Asian continent. Most studies were accordingly carried out in the temperate (58.6%) and sub-tropical zone (26%). Only 9.3% of the studies originated from the tropical zone highlighting the taxonomic mismatch and geographical bias since in the tropics species diversity is known to be greatest. Studies using amphibian populations from the temperate zone, investigated 20 different species, whereas only 14 different species were used in studies from the tropics. 30 different species

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from sub-tropical populations were included in the dataset. 54.1% (N=72) of the populations were sampled in the temperate zone, whereas 28.5% (N=38) and 9.7% (N=13) of the populations were sampled in the sub-tropical and tropical zone, respectively. Most studies were carried out in the Northern Hemisphere (86.6% from 77 articles). Only 7.3% (from 9 articles) of the studies were carried out in the Southern Hemisphere. 7.5% of the studies and thus, 7 articles were excluded from the analyses since these used animals from artificial breeding.

Most studies (N=20) used the European common frog (*Rana temporaria;* Table S1). European water frogs (*Pelophylax* spp.) were used as study species in 7 studies as well as the wood frog (*Lithobates sylvaticus).* Four studies used the striped marsh frog (*Limnodynastes peronii*). All other species were used in less than three studies. The three most studied species in our dataset are also among the ten most studied amphibian species for conservation as recently published by da Silva et al. (2020). Most studies were carried out using anurans (95.3%), whereas only 4.7% of the studies investigated caudates.

Fig. 4. A world map illustrating the geographic locations at which experimental populations were sampled. Points are colored according to the geographic zone in which the sampling locations belong. Yellow = temperate zone (>40°). Orange = sub-tropical zone (25-40°). Red = tropical zone (0-25°). Bar charts = Percentage of studies carried out on each continent. Frogs = Number of different species used in each latitudinal group/percentage of studies carried out in respective climate one. Figure made with GeoMapApp (www.geomapapp.org) / CC BY.

4. Discussion

Temperature-induced developmental plasticity could allow amphibian larvae to complete metamorphosis successfully despite new thermal challenges and increased desiccation risk due to climate change. Here, we collected empirical data for age and size of amphibian larvae at metamorphosis that are acclimated to different temperatures during development, in order to determine the population-specific capacity for temperature-induced plasticity. We found that larvae from 137 populations experience a change in metamorphic traits with change in temperatures during development and thus, were able to exhibit temperature-induced developmental plasticity. Our key findings suggest that age at metamorphosis seems to be much more affected by temperature than size at metamorphosis. Furthermore, we identified that tropical amphibian populations are the most vulnerable to increasing temperatures during metamorphosis because their metamorphic traits are less sensitive to changing temperatures during development compared to populations from higher latitudes. However, this study also

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425 detected that data on tropical populations are few and thus, the capacity for developmental

426 plasticity in most of the tropical amphibians remains to be thoroughly investigated.

427 *4.1 Thermal effects are consistent on age but not on size at metamorphosis across amphibian*

428 *populations*

429 For most ectothermic species with complex life cycles, the relationship between temperature,

430 development, and growth is well defined by the *temperature-size rule*. It states that the body

431 size of larvae that metamorphosize in warmer conditions is smaller, despite their initial fast

432 growth rate (Atkinson, 1994; Verberk et al., 2021). Our results confirm this pattern for the

433 majority of tested populations. We found that age and size at the onset of metamorphosis are

434 generally the lowest at the warmest temperatures during development and the highest at the

435 coldest temperatures during development. In our analysis, age at the onset of metamorphosis

436 was lower at warmer developmental temperatures in 99.1% of the studied populations. These

437 results indicate that developmental rate responds plastically to temperature variation

438 independent of respective thermal background Further, developmental rate turned out to be

439 more plastic than growth rate as age at metamorphosis was more affected by temperature than

440 size at metamorphosis. This is due to the accelerating effect of increasing temperature on

441 physiological and biochemical processes in general and on the endocrine mechanisms that

442 regulate amphibian metamorphosis (Smith-Gill and Berven, 1979; Angilletta, 2009; Little and

443 Seebacher, 2016). Thyroid hormones (TH), which are the major triggers of amphibian

444 metamorphosis (Shi 2000), are more effective at higher temperatures (Ruthsatz et al., 2018b;

445 2020). Further, environmental temperature variation can proximately cause stress which alters

446 the hormonal balance of amphibian larvae by activating the neuroendocrine stress axis (Navas

447 et al., 2017). Stress hormones are known to synergize with THs (Glennemeier and Denver,

448 2002a,b,c; Kulkarni and Buchholz, 2012) and thus, mediate temperature-induced

449 developmental plasticity (Denver, 2021). This impact of environmental temperature on the

450 endocrine pathways is independent of the genetic thermal adaptation of a population, which is

451 in line with the present results on age at metamorphosis.

452

453 In contrast to age, no consistent decrease at warmer temperatures was observed in mass, TL, or

454 SVL at the onset of metamorphosis, suggesting that size is not only explained by environmental

455 factors such as temperature. This is in line with findings of Lesbarreres et al. (2007), who

456 demonstrated that genetic variability rather than environmental factors predicted size at

457 metamorphosis in the European common frog (*Rana temporaria*). Nevertheless, growth rate is

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likewise accelerated at higher temperatures due to thermal effects on physiological and biochemical processes (Smith-Gill and Berven, 1979; Angilletta, 2009; Little and Seebacher, 2016). In our analysis, growth rate was higher at warmer developmental temperatures in 75.3% of the studied populations, indicating that growth rate responds plastically to temperature variation but is not significantly higher or lower than developmental rate. In general, growth rate is suggested to be less sensitive to temperature variation than developmental rate (Gomez-Mestre et al., 2010) since the thermal effect on THs favors developmental rate more than growth rate (Shi. 2000). This could result in the decoupling of both rates to some extent (Forster and Hirst. 2012), resulting in a larger impact on age than size at metamorphosis. The high degree of temperature-induced plasticity in growth rate in the present study could be associated and thus be biased to some extent, with the large number of studies included using *R. temporaria*, a species which is known to react more plastically to thermal variability than other amphibians (Laurila and Kujasalo, 1999; Ruthsatz et al., 2020). Accounting for phylogenetic relatedness yielded similar results showing that the elevation and Annual Temperature Range (bio7) had clear positive and negative effects on the PIX of age at metamorphosis (PIX age) (Supplementary Table S2). Despite the high intraspecific variation in PIX, we see that the species identity used as a random factor relatively predicts PIX age better than the phylogeny indicating that there is a lack of or a poor phylogenetic signal for PIX variables (see Supplementary Table S2). However, we did not measure phylogenetic signal per se because measures of phylogenetic signals are sensitive to non-random distribution of missing taxa on a phylogenetic tree, in addition to branch length information that is not often accurate when extracted from super trees. Together these factors can lead to misinterpretation of ecological and evolutionary processes (Münkemüller et al., 2012; Molina-Venegas and Rodríguez, 2017). In light of this, plasticity index data on more species sampled randomly across the amphibian tree could provide a better understanding of phylogenetic signal for plasticity in species' physiological traits.

The capacity for a temperature-induced plastic response in developmental and growth rate is adaptive in heterogeneous environments (Newman, 1992) but also results in different ages and sizes of larvae at metamorphosis. The age and size of larvae at metamorphosis are effective predictors of (future) fitness in amphibians (Smith, 1987; Beck and Congdon, 2000; Boone et al., 2001; Ruthsatz et al., 2019; but not: Semlitsch et al., 1988; Earl and Whiteman, 2015). Generally, being younger and larger at metamorphosis was found to be advantageous, because the individual will be more likely to survive to maturity (Berven, 1990). It has therefore been suggested that individuals that metamorphose at a smaller size (i.e., due to higher water

492 temperatures in ponds) may benefit from an earlier escape from desiccation risk in their larval
493 habitats but may also incur costs such as reduced juvenile survivorship (Smith, 1987; Semlitsch
494 et al., 1988; Berven, 1990; Ruthsatz et al. 2019). However, there is a growing body of literature
495 providing broad support that shrinking body sizes in ectotherms such as amphibians in response
496 to global warming might be beneficial at all life-stages. In small individuals, the risks of oxygen
497 (Verberk et al., 2021) as well as of food limitation are reduced and so is competition (Ohlberger,
498 2013; Van Buskirk et al., 2017; Rollins and Benard, 2020). Also, acclimation of metabolic rate
499 and thermal tolerance is faster in smaller individuals than in larger individuals (Rohr et al.,
500 2018). Thus, climate change poses a selection pressure that favors the capacity for temperature-
501 induced plasticity in amphibian larvae. Nevertheless, there are no studies so far demonstrating
502 that plasticity in general and in developmental rate actually increases fitness. Since little is
503 known generally about phenotypic plasticity in the wild (Loman, 2002), further studies are
504 required to investigate how a plastic response in developmental and growth rate affects fitness
505 in later life stages.

506 *4.2 Population-specific biogeographic background affects thermal sensitivity of metamorphic*
507 *traits*

508 In this study, we also investigated whether the population-specific biogeographic background
509 (e.g., latitude and associated thermal adaptation) accounts for the different degrees of plastic
510 responses in metamorphic traits to temperature. Along with a previous study on anuran larvae
511 (Ruthsatz et al., 2018a), the present study is the first to investigate temperature-induced
512 developmental plasticity patterns across latitude and associated thermal adaptation. We found
513 that the population-specific biogeographic background impacts the sensitivity of age and/or size
514 at the onset of metamorphosis to temperature variation and thus, requires the capacity for a
515 plastic response in developmental and growth rate.

516 In our analysis, the effects of the population-specific biogeographic background on the thermal
517 sensitivity of metamorphic traits were more consistent for age than for size at metamorphosis.
518 We found that populations from tropical latitudes show lower plasticity indices for age at
519 metamorphosis. Therefore, populations from colder climates with high precipitation, such as
520 temperate amphibians, are more likely to respond plastically in developmental rate to
521 temperature variation. In contrast, populations adapted to warmer climates with lower
522 precipitation revealed a lower sensitivity of age to temperature variation indicating lower
523 developmental plasticity (temperature-induced). Less plastic physiological traits are common

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in ectotherm populations from tropical climates due to the relatively stable thermal environments in the tropics (e.g., Janzen, 1967; Addo-Bediako et al., 2000; Somero, 2005; Ghalambor et al., 2006; Calosi et al., 2007; Bozinovic et al., 2011). In particular, the plasticity of thermal tolerance is well known to increase in populations found toward higher latitudes due to pronounced thermal seasonality (Calosi et al., 2010; Naya et al., 2011; Sunday et al., 2011; but not: Sorensen et al., 2016). As temperate populations experience more heterogenous thermal environments during their larval stage, selection favors a high sensitivity of developmental rate due to temperature variation resulting in a high capacity for a plastic response in both rates (Seebacher et al., 2015). We consequently identify populations from tropical latitudes as the most vulnerable to increasing temperatures during metamorphosis. However, temperature-induced developmental plasticity might have a greater potential to buffer the effects of global warming on amphibian larvae from all latitudes than plasticity in thermal tolerance (Sunday et al., 2014; Gunderson and Stillman, 2015; Barria and Bacigalupe, 2017).

4.3 Concluding remarks: Evidence of biogeographic, methodological, and taxonomic biases in thermal biology research and their consequences for predicting amphibian sensitivity to climate change

As the magnitude and speed of climate change accelerate (Mahlstein et al., 2013), there is a considerable interest in evaluating how different amphibian species and populations will cope with new thermal challenges. There is a growing body of research investigating mechanisms underlying responses of populations, and associated life history evolution as a result of changing climate (rev. in Lowe et al., 2021). This trend has greatly increased our knowledge on the potential of temperature-induced phenotypic plasticity and will contribute to identifying the world's most climate change vulnerable populations. Our effort considered populations from tropical latitudes as the most vulnerable to increasing temperatures during metamorphosis since metamorphic traits were less sensitive to changing temperatures during development compared to populations from higher latitudes. Nevertheless, our analyses reveal biases with respect to taxonomy, biogeographic distribution of species, and study design making global conclusions impossible. In our analysis, most studies were conducted in Europe and North America and used species that are common, widely distributed, or easily obtainable by researchers. This is a commonly found pattern in conservation research (Pawar, 2003; Schiesari et al., 2007; Winter et al., 2016; da Silva et al., 2020). The geographical bias is at least partially responsible for a taxonomic bias (Winter et al., 2016) since information gaps exist for Africa, Asia, and South America; these are the very regions where the vast majority of global

557 biodiversity hotspots are located (Mittermeier et al., 2011). Most threatened species occur in
558 these tropical regions, but also in countries with little investment in research (King, 2004).
559 Given the geographic differences in thermal sensitivity during metamorphosis that we have
560 detected despite low data from the tropics, we predict that an increase in studies on additional
561 tropical species could reveal greater risks. On the other hand, these additional studies will be
562 key in improving current predictions of biodiversity survival due to climate change and global
563 warming (Button and Borzée, 2021). Furthermore, most studies investigating the effect of
564 temperature on metamorphic traits use only 2–3 constant temperatures during development.
565 This methodical approach is insufficient to resolve the capacity for developmental plasticity
566 within the population-specific thermal tolerance (Kingsolver and Huey, 2008). Additional
567 research must focus on geographically under-represented regions, taxonomically under-
568 represented groups, species of higher conservation significance, and include a broader range of
569 temperatures during development in order to obtain robust interpretations on amphibian
570 survival in the light of accelerating global warming.

571 **5. Conflict of interest**

572 None declared.

573 **6. Author contributions**

574 KR conceived and designed the study. KR, NS, JG, MR, CZ, and ET conducted the data
575 extraction. KR, MLL, and AVM performed the statistical analysis. NS and KR led the writing
576 of the manuscript. NS, KR, and AVM designed the figures. All authors participated in
577 manuscript editing and final approval.

585 **9. Statement of Ethics**

586   The authors have no ethical conflicts to disclose.

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840 **11. Appendix**

841 **Table A1**. Correlation coefficients between absolute latitude (°N/S), elevation (m.a.s.l.), elevation, and bioclimatic predictor variables for all sampling

842 sites included in this study. Bold for correlation coefficients >0.55. Bio 1 = Annual Mean Temperature. Bio 2= Mean Diurnal Range. Bio 3 =

843 Isothermality. Bio 4 = Temperature Seasonality. Bio 5 = Maximum Temperature of Warmest Month. Bio 6 = Minimum Temperature of Coldest

844 Month. Bio 7 = Annual Temperature Range. Bio 8 = Mean Temperature of Wettest Quarter. Bio 9 = Mean Temperature of Driest Quarter. Bio 10 =

845 Mean Temperature of Warmest Quarter. Bio 11 = Mean Temperature of Coldest Quarter. Bio 12 = Annual Precipitation. Bio 13 = Precipitation of

846 Wettest Month. Bio 14 = Precipitation of Driest Month. Bio 15 = Precipitation Seasonality. Bio 16 = Precipitation of Wettest Quarter. Bio 17 =

847 Precipitation of Driest Quarter. Bio 18 = Precipitation of Warmest Quarter. Bio 19 = Precipitation of Coldest Quarter.

	Latitude	Elevation	Bio1	Bio2	Bio3	Bio4	Bio5	Bio6	Bio7	Bio8	Bio9	Bio10	Bio11	Bio12	Bio13	Bio14	Bio15	Bio16	Bio17	Bio18	Bio19
Latitude		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.011	<0.001	<0.001	<0.001	<0.001	<0.001
Elevation	-0.454		0.006	<0.001	<0.001	0.089	<0.001	0.279	<0.001	0.056	0.012	0.446	0.095	<0.001	<0.001	0.122	0.069	<0.001	0.001	<0.001	0.006
Bio1	**-0.833**	0.141		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.046	<0.001	<0.001	<0.001	<0.001	<0.001
Bio2	**-0.630**	**0.637**	0.393		<0.001	0.015	<0.001	0.025	<0.001	0.102	<0.001	<0.001	<0.001	<0.001	<0.001	0.858	<0.001	<0.001	0.193	<0.001	<0.001
Bio3	**-0.853**	0.478	**0.782**	**0.721**		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.003	<0.001	<0.001	<0.001	<0.001	<0.001
Bio4	0.550	-0.087	**-0.628**	-0.124	**-0.685**		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.003	0.215	<0.001	0.001	0.011	<0.001
Bio5	**-0.812**	0.190	**0.830**	**0.595**	**0.702**	-0.310		<0.001	0.434	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.127	0.000	<0.001	0.001	<0.001	<0.001
Bio6	**-0.648**	-0.056	**0.872**	0.115	**0.628**	-0.786	0.590		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.812	<0.001	<0.001	<0.001	<0.001	<0.001
Bio7	0.245	0.191	-0.453	0.306	-0.318	**0.832**	-0.040	**-0.725**		<0.001	<0.001	<0.001	<0.001	<0.001	0.150	<0.001	0.062	0.010	<0.001	0.620	<0.001
Bio8	-0.524	-0.098	**0.587**	0.084	0.319	-0.190	**0.573**	0.421	-0.203		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.001	0.000	<0.001	<0.001	0.309
Bio9	**-0.699**	0.129	**0.815**	0.340	**0.776**	-0.824	**0.616**	0.881	-0.586	0.203		<0.001	<0.001	<0.001	<0.001	0.038	0.020	<0.001	0.001	0.001	<0.001
Bio10	**-0.754**	0.039	**0.881**	0.356	**0.588**	-0.334	**0.900**	**0.680**	-0.213	**0.716**	**0.597**		<0.001	<0.001	<0.001	0.004	<0.001	<0.001	<0.001	<0.001	<0.001
Bio11	**-0.775**	0.086	**0.917**	0.291	**0.762**	-0.804	**0.677**	**0.955**	-0.622	0.431	**0.937**	**0.726**		<0.001	<0.001	<0.001	0.041	<0.001	<0.001	<0.001	<0.001
Bio12	-0.513	0.276	0.482	0.260	0.438	-0.301	0.402	0.464	-0.251	0.339	0.337	0.370	0.435		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

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Bio13	-0.699	0.511	0.523	0.569	0.626	-0.299	0.581	0.363	-0.074	0.323	0.403	0.447	0.439	0.779		<0.001	0.002	<0.001	<0.001	<0.001	<0.001
Bio14	-0.130	0.079	0.224	-0.009	0.154	-0.150	0.078	0.245	-0.232	0.188	0.106	0.147	0.194	0.749	0.397		<0.001	<0.001	<0.001	<0.001	<0.001
Bio15	-0.326	0.093	0.102	0.258	0.185	-0.064	0.288	0.012	0.096	0.171	0.119	0.206	0.105	-0.283	0.159	-0.673		0.035	<0.001	0.437	<0.001
Bio16	-0.721	0.460	0.557	0.523	0.627	-0.336	0.593	0.413	-0.132	0.365	0.429	0.482	0.478	0.827	0.977	0.445	0.108		<0.001	<0.001	<0.001
Bio17	-0.243	0.164	0.303	0.067	0.229	-0.165	0.173	0.309	-0.220	0.242	0.166	0.228	0.260	0.828	0.501	0.960	-0.618	0.549		<0.001	<0.001
Bio18	-0.558	0.395	0.436	0.329	0.396	-0.130	0.440	0.265	-0.025	0.518	0.165	0.398	0.308	0.782	0.781	0.545	-0.040	0.806	0.610		<0.001
Bio19	-0.338	0.140	0.373	0.213	0.427	-0.473	0.252	0.475	-0.368	-0.052	0.498	0.196	0.446	0.665	0.518	0.677	-0.385	0.537	0.675	0.286	

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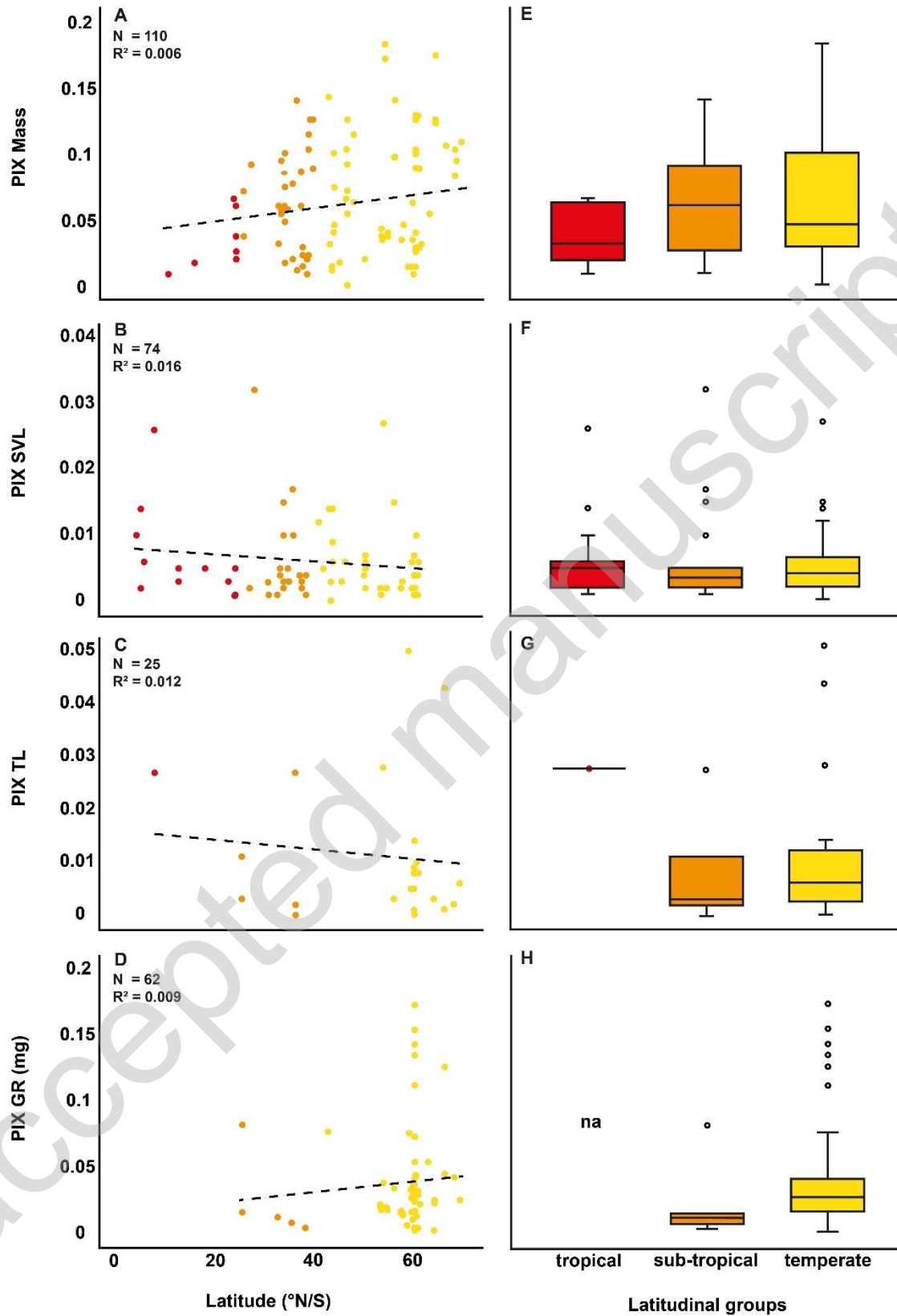
Fig. A1. Absolute plasticity index (PIX) for mass, SVL, TL, and growth rate at the onset of metamorphosis as **ABCD** a function of absolute latitude of the source population (°N/S) and **EFGH** for three latitudinal groups (i.e. temperate, sub-tropical, and tropical) in

amphibians. Regression line shows the general effect of absolute latitude on dependent variables if regression is significant; dotted line for non-significant regressions. Box = 1st and 3rd quartiles with median. Whiskers = 1.5-fold interquartile range. Dots = outliers. Color code for latitudinal groups; yellow = temperate, orange = sub-tropical, and red = tropical.
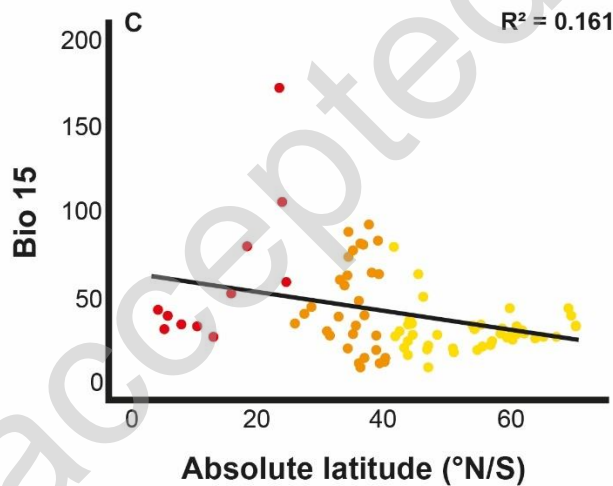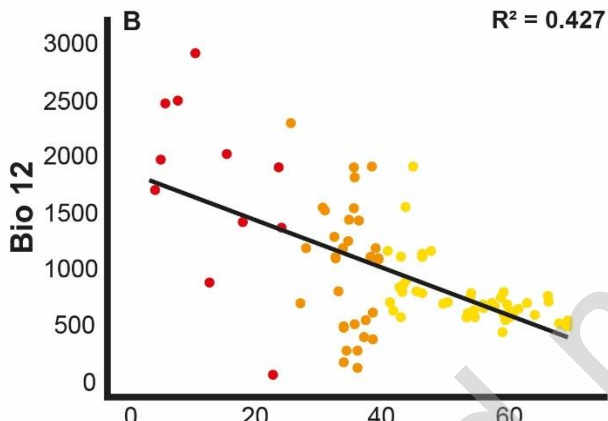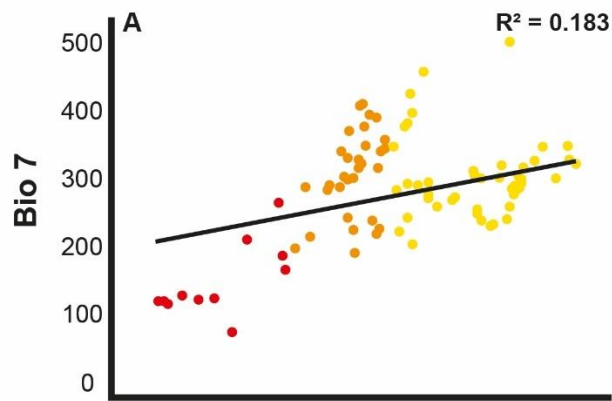
Fig. A2. Annual Temperature Range (Bio 7), Annual Precipitation (Bio 12), and Precipitation Seasonality (Bio 15) as a function of absolute latitude of the source population (°N/S). Black regression line shows the general effect of absolute latitude on dependent variables if regression is significant. Color code for latitudinal groups; yellow = temperate, orange = sub-tropical, and red = tropical.
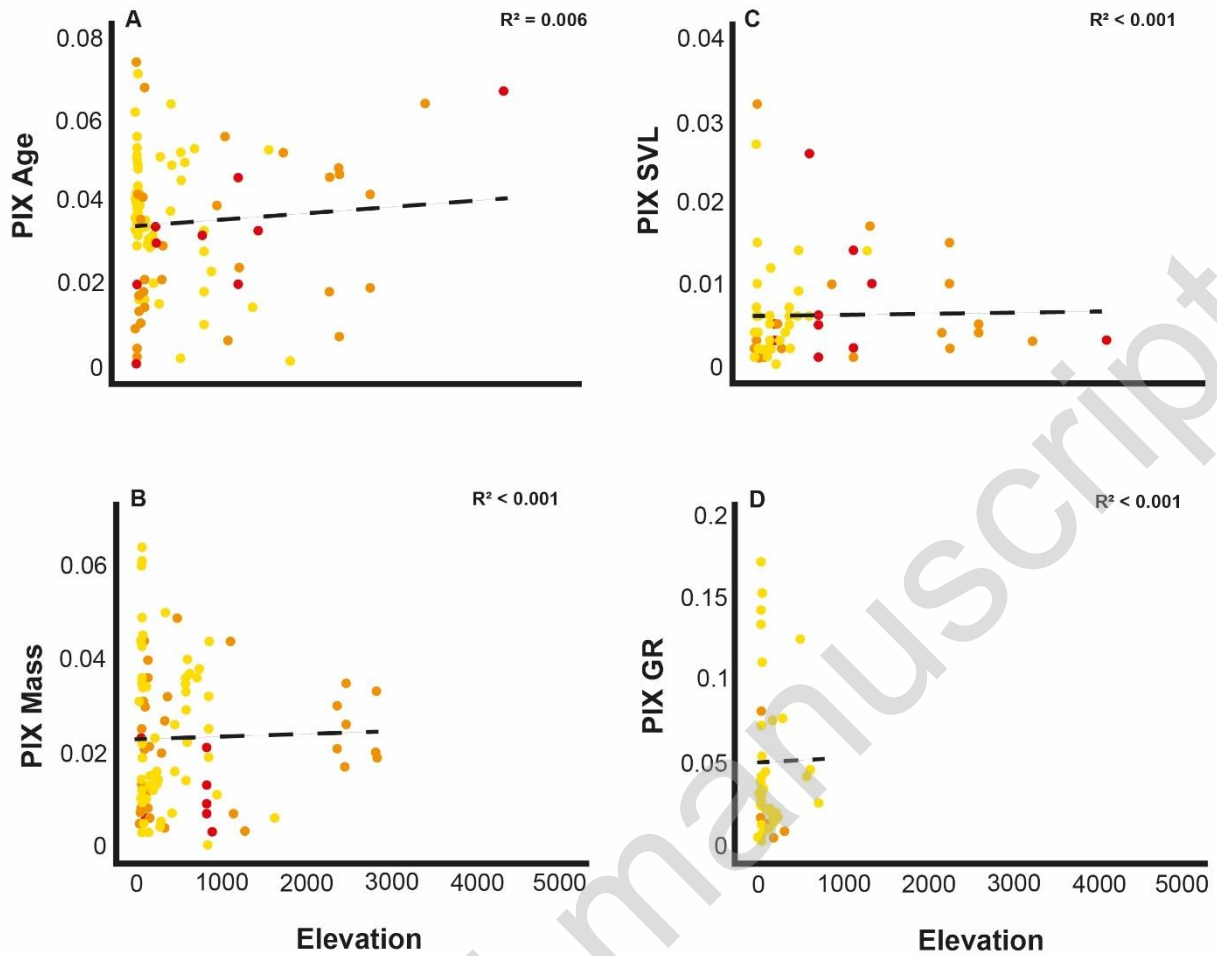
Fig. A3. Absolute plasticity index (PIX) for **A** age, **B** mass, **C** SVL, and **D** growth rate at the onset of metamorphosis as a function of absolute elevation of the source population (m.a.s.l.) in amphibians. Regression line shows the general effect of elevation on dependent variables if regression is significant; dotted line for non-significant regressions. Color code for latitudinal groups; yellow = temperate, orange = sub-tropical, and red = tropical.
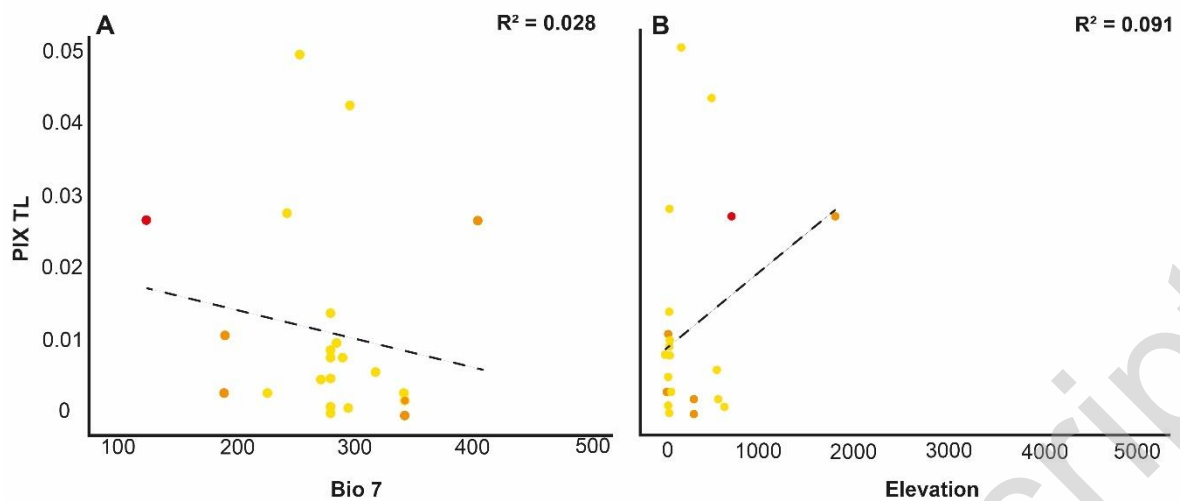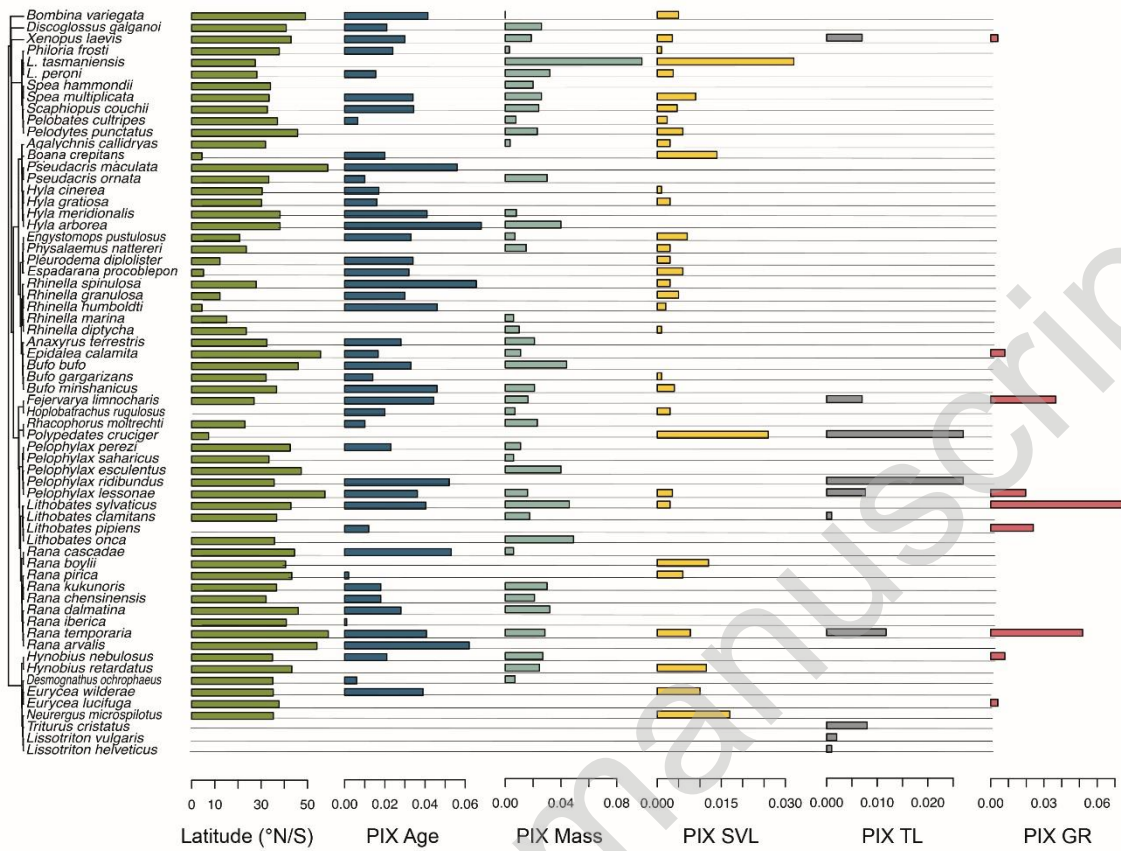
Fig. A4.Absolute plasticity index (PIX) for TL the onset of metamorphosis as a function of **A** Annual Temperature Range and **B** of absolute elevation of the source population (m.a.s.l.) in amphibians. Regression line shows the general effect of elevation on dependent variables if regression is significant; dotted line for non-significant regressions. Color code for latitudinal groups; yellow = temperate, orange = sub-tropical, and red = tropical.

Fig. A5 Absolute plasticity index (PIX) for age, mass, SVL, TL, and growth rate at the onset of metamorphosis in different species and absolute latitude of the source populations (°N/S). The phylogenetic tree shows the taxonomic relationships among species included in this study.

12. Vitae

12.1 Noa Sinai

Noa Sinai is a M. Ed. student for Biology and English at the Department of Education at Hamburg University.

12.2 Julian Glos

Dr. Julian Glos senior researcher at the Department of Animal Ecology and Conservation at Hamburg University. Research interests are community ecology, tropical biology, and amphibian ecology & conservation.

12.3 Ashwini V. Mohan

Dr. Ashwini Venkatanarayana Mohan is a Marie-Curie Postdoctoral fellow at the Natural History Museum London. Her recent work has focused on reptiles species distributed in island ecosystems, their bio-geographic origins, population genetics, and ecology.

12.4 Mariana L. Lyra

Dr. Mariana L. Lyra is a post-doc at the Instituto de Biociências and Centro de Aquicultura of the Universidade Estadual Paulista - UNESP, Rio Claro, São Paulo. She is interested in aspects related to biodiversity and evolutionary biology of neotropical amphibians.

12.5 Maya Riepe

Maya Riepe is a B. Sc. student for Biology and Chemistry at the Department of Education at Hamburg University.

12.6 Elena Thöle

Elena Thöle is a M. Ed. student for Biology and Art at the Department of Education at Hamburg University.

12.7 Cecile Zummach

Cecile Zummach is a M. Ed. student for Biology and History at the Department of Education at Hamburg University.

12.8 Katharina Ruthsatz

Dr. Katharina Ruthsatz is an Assistant Professor at the Technical University of Braunschweig. Her recent work has focused on amphibian conservation physiology and ecotoxicology, changes in energy budgets in the face of climate change in amphibians and ectotherms in general, developmental and physiological plasticity to environmental variation, and the influence on energetics and adaption to the environment. She is especially interested in the endocrine disruptive effects of environmental stress associated with global change on amphibian metamorphosis.

Fig. 1 The effect of temperatures during development on values of **A** age (in days after hatching), **B** mass (in mg), **C** snout-vent length (SVL, in mm), **D** total length (in mm), and **E** growth rate (mg/day after hatching) at the onset of metamorphosis. Dots and respective regression lines refer to the individual studies. Statistics for log-transformed values and linear regressions. Black regression line shows the general effect of temperatures during development on dependent variables of all included studies if regression is significant; dotted line for non-significant regressions. The color code refers to the latitudinal group of spawn collection site

of the respective populations (see text for further details). Yellow = temperate group (>40°). Orange = sub-tropical zone (25-40°). Red = tropical zone (0-25°).
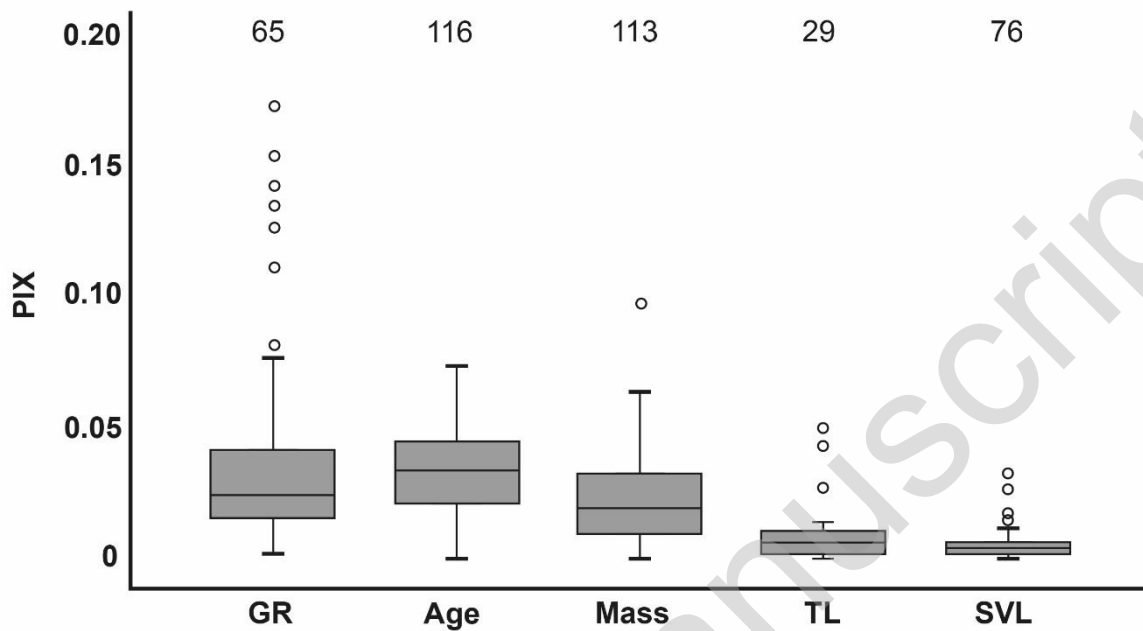

Fig. 2 Absolute plasticity index (PIX) of log-transformed values of growth rate (mg/day after hatching), age (in days after hatching), mass (in mg), total length (in mm), and snout-vent length (SVL, in mm) at the onset of metamorphosis sorted by mean value. The plasticity index describes the change in metamorphic traits with a given change in temperatures during development. Box = 1st and 3rd quartiles with median. Whiskers = 1.5-fold interquartile range. Dots = outliers. Numbers = sample size (number of studies) per trait. N=399.
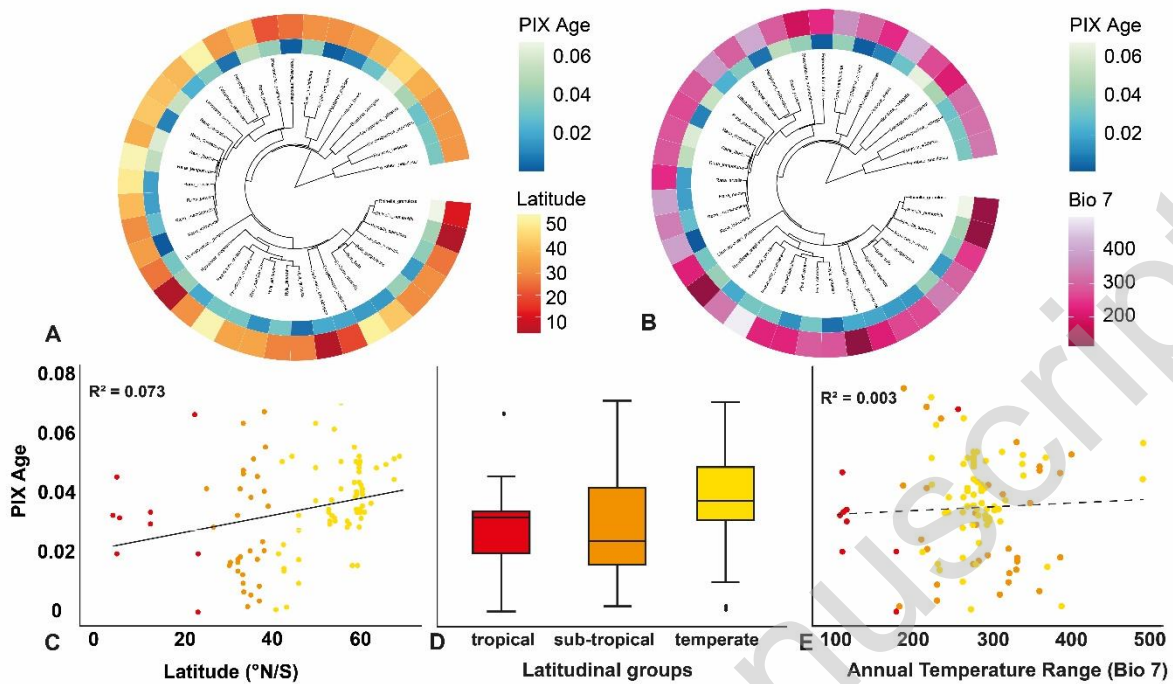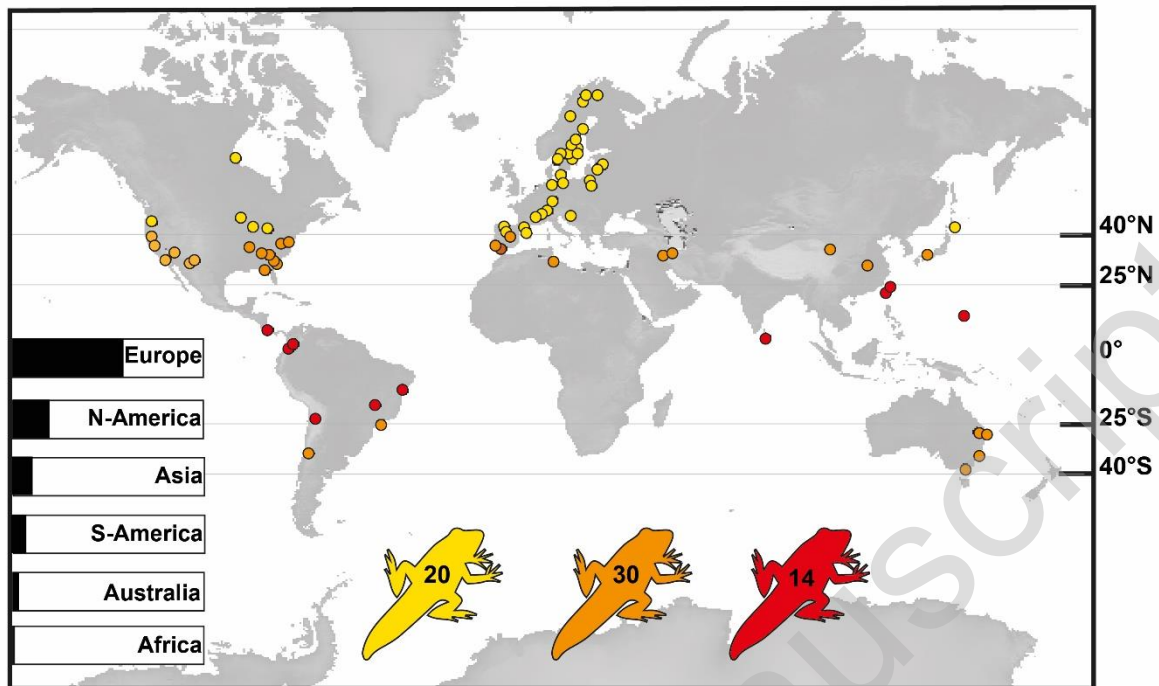
Fig. 3 Absolute plasticity index (PIX) of age at metamorphosis (green and blue shades) and **A** mean of absolute latitude of the source populations (°N/S) (latitude) of different species with their phylogenetic relationships, **B** mean of Annual Temperature Range of different species with their phylogenetic relationships, **C** as a function of absolute latitude of the source population (°N/S), **D** for three latitudinal groups (i.e., tropical, sub-tropical, and temperate) in amphibians, and **E** as a function of Annual Temperature Range (Bio 7). Black regression line for significant linear regressions; dotted line for non-significant regressions. The color code refers to the latitudinal group of spawn collection site of the respective populations (see text for further details). Yellow = temperate group (>40°). Orange = sub-tropical zone (25-40°). Red = tropical zone (0-25°).Box = 1st and 3rd quartiles with median. Tree only includes species included in latitudinal analysis. Whiskers = 1.5-fold interquartile range. Dots = single data.

Fig. 4. A world map illustrating the geographic locations at which experimental populations were sampled. Points are colored according to the geographic zone in which the sampling locations belong. Yellow = temperate zone (>40°). Orange = sub-tropical zone (25-40°). Red = tropical zone (0-25°). Bar charts = Percentage of studies carried out on each continent. Frogs = Number of different species used in each latitudinal group/percentage of studies carried out in respective climate one. Figure made with GeoMapApp (www.geomapapp.org) / CC BY.