Can we improve our ability to interpret ectotherm thermal tolerance?

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Abstract

Thermal tolerances, such as critical temperatures, are important indices for understanding an organism's vulnerability to changing environmental temperature. Differences in thermal tolerance over ontogeny may generate a 'thermal bottleneck' that sets the climate vulnerability for organisms with complex life cycles. However, a species' microhabitat preference and methodological differences among studies can generate confounding variation in thermal tolerance that may mask trends in large-scale comparative studies and may hinder our ability to assess climate change vulnerability within and among species. Here, we evaluated two approaches to resolving ontogenetic and environmental drivers of thermal tolerance and methodological variation: mathematical standardisation of thermal tolerance and classifying microhabitat preferences. Using phylogenetically informed, multi-level models with a global dataset of upper critical temperatures from 438 Anuran species, we found ontogenetic trends in thermal tolerance were similar across microhabitat preferences and standardising critical temperatures against common methodological variation had little impact on our conclusions. Our results suggested thermal bottlenecks are not strongly present in Anurans but instead, implied strong developmental or genetic conservatism of thermal tolerance within families and ecotypes. We discussed

considerations for resolving confounding variation to interpret thermal tolerance at a macrophysiological scale.

Introduction

Thermal tolerance metrics, such as critical temperatures, have endured in ecological studies despite their criticisms because they are simple to conceptualise (e.g. impediment to movement), are generalisable across taxa, and can be readily assayed in the laboratory (Sinclair et al., 2016). These metrics facilitate our ability to link environmental temperature to organismal responses (Bates & Morley, 2020), synthesise general trends in physiological diversity from local to macro scales (Chown & Gaston, 2016), and index vulnerability or sensitivity to climate change via metrics such as thermal safety margins (Deutsch et al., 2008). For example, differences in upper critical temperature (CT_{max}) across life stages may indicate a thermal bottleneck at the most temperature-sensitive life stage (Dahlke, Wohlrab, Butzin, & Pörtner, 2020; Pandori & Sorte, 2019). For organisms with a complex life cycle such as frogs, juvenile or larval stages in aquatic environments are generally expected to have lower thermal tolerance and thus be more vulnerable to climate change than adult stages (Lenoir, Hattab, & Pierre, 2017).

Identifying a thermal bottleneck represents a significant amount of work that is limited to commonly studied species (e.g., Ruthsatz, Dausmann, Peck, and Glos (2022)). Thermal bottlenecks can also be identified using a macrophysiological approach which has described stage-specific differences in thermal sensitivities and tolerances in marine invertebrates (Collin, Rebolledo, Smith, & Chan, 2021; Pandori & Sorte, 2019; Truebano, Fenner, Tills, Rundle, & Rezende, 2018), fish (Dahlke et al., 2020; Moyano et al., 2017), insects (Kingsolver & Buckley, 2020; Klockmann, Günter, & Fischer, 2017) and reptiles (Levy et al., 2015). Among species comparisons in frogs are often limited to a single geographic region and are often limited to either adults or larva even if both are collected from the same region (Carilo Filho et al., 2021; Cheng et al., 2022; Pintanel et al., 2022; Pintanel, Tejedo, Ron, Llorente, & Merino-Viteri, 2019; Ruthsatz et al., 2022). Thus, whether thermal bottlenecks occur and how consistent they are across Anuran species is not well understood. A macrophysiological analyses of thermal tolerance throughout ontogeny will vastly improve our understanding of the presence and generality of thermal bottlenecks across an environmentally sensitive taxonomic group.

Comparative macrophysiological analyses present two key challenges towards assessing and interpreting thermal tolerances. Microhabitat preferences and among-study variation may generate confounding variation in thermal tolerances that may mask or accentuate the presence of a thermal bottleneck. Here, we analysed the CT_{max} of 438 Anuran species to 1) identify whether Anurans have a thermal bottleneck in their life cycle and their potential evolutionary drivers and ecological consequences at a macrophysiological scale, and 2) evaluate whether proposed solutions to two challenges of comparative analyses of ectotherm thermal tolerance improved our ability to identify thermal bottlenecks. We acknowledge there is a diversity of definitions and uses of thermal tolerance that would influence their interpretation. In this study, we only consider CT_{max} as the end point of a temperature ramping assay with a constant heating rate, or dynamic ramping protocol, and

we define heating tolerance as the temperature range between starting temperature and CT_{max} during the ramping assay.

Do microhabitat preferences improve identification of thermal bottlenecks?

The key challenge to interpret thermal tolerance over the life cycle is to disentangle confounding variation deriving from microhabitat selection from inherent developmental or physiological drivers. A diversity of microhabitats may mask broader adaptive covariation between thermal tolerance and microclimate selection to explain, in part, an observed lack of variation in CT_{max} at a global scale or drive strong associations with thermal tolerance (González-del-Pliego et al., 2020; Pincebourde & Woods, 2020). However, distinguishing between these patterns requires corresponding microclimate information for each ontogenetic stage that is not always collected for thermal tolerance studies or are available, but see Pintanel et al. (2022). If differences in thermal environments are consistent within broad microhabitat preferences in Anurans, then microhabitat preferences may be a reliable proxy for local environmental selection on thermal tolerance. Among species, frogs show strong microhabitat preferences that are associated with phenotypic syndromes encompassing behaviour, morphology and physiology and can broadly be categorised into ecological types ("ecotype") (Moen, Cabrera-Guzman, Caviedes-Solis, Gonzalez-Bernal, & Hanna, 2022; Moen & Wiens, 2017). In turn, the animal's ecotype may mediate the confounding interaction between life stages and their respective microenvironment (Carilo Filho et al., 2021; Pincebourde & Casas, 2015). We tested whether ecotypes are reliable proxies of environmental adaptation to resolve ontogeny by environment interaction of CT_{max} and identify thermal bottlenecks.

Does removing nuisance heterogeneity change thermal tolerance trends?

A large literature on ectotherm thermal tolerances has emerged because of the duality of critical temperatures as characteristics of a thermal performance curve or as physiological endpoints of a thermal regime (Bates & Morley, 2020; Sinclair et al., 2016). For comparative purposes, protocols to assay thermal tolerance are generally not standardised across taxa for either definition, and variation in the starting temperature and heating rate used will affect the measured CT_{max} among individuals in a ramping assay (Peck, Clark, Morley, Massey, & Rossetti, 2009; Terblanche, Deere, Clusella-Trullas, Janion, & Chown, 2007). Such methodological variation or "nuisance heterogeneity" generates uncertainty in thermal tolerances that may mask signals of interest (Noble et al., 2022). The significant nuisance heterogeneity of CT_{max} complicates how accurately this metric can be translated from the laboratory to the field regardless of any ecological relevance and undermines prediction accuracy and interpretation of climate vulnerability metrics with confidence (Pinsky, Eikeset, McCauley, Payne, & Sunday, 2019; Terblanche et al., 2011). One solution is to correct for variation induced by methodology to a standard protocol (Cupp, 1980; Lebenzon, Des Marteaux, & Sinclair, 2020). Resolving confounding variation will improve our ability to link environmental gradients to trait variation and test hypotheses of thermal tolerance evolution (Bates & Morley, 2020; Kovacevic, Latombe, & Chown, 2019). We applied a mathematical model that standardises thermal tolerances to the same heating rate and test

whether standardising methodological variation in thermal tolerance metrics improves our ability to resolve biological trends of interest while accounting for among-study variation in methodology (Kong et al., 2022).

Methods

Data compilation

We started with a published dataset of upper critical temperatures of Anurans complied from the literature (1962 – 2015) (Rohr et al., 2018). We supplemented this data with studies from 2016 to 2022 using Web of Science (accessed 25th February) and the keywords amphibian* AND ("critical thermal max*" OR CT_{max} OR "thermal toleran*" OR "critical temp*" OR "upper temp*")". The terms returned 90 studies that were screened for inclusion by one person (J.D.K.). Studies that measured CT_{max} using ramping assays were retained. Studies that estimated CT_{max} from fitted thermal performance curves or compiled values from literature were excluded. Only control animals were included from studies that used multiple stressors (e.g. dehydration or infection). The screening process yielded 36 papers that provided extractable data. Provided data were used over published values where possible. Populations were considered independent observations. Multiple endpoints of critical temperatures on an individual were recorded but only one CT_{max} was used per individual (loss of righting response where possible). We added studies that did not return from keywords from forward searching. We distinguished between temperatures animals were held at prior to experimentation (acclimation temperature) and the starting temperature of the ramping assay. We assume that observed CT_{max} reflected their acclimated CT_{max} as acclimation in frogs is reported on scales of hours (Brattstrom, 1970).

We obtained ecological types (hereby ecotype), from all Anuran species listed on the International Union for Conservation of Nature's Red List of Threatened Species (IUCN, 2022). Ecotypes were classified from ecological descriptions from IUCN based on adult behaviour and microhabitat preferences outside the breeding season (Moen & Wiens, 2017). We grouped species by ecotype even if the larval stage may have differing ecological types (e.g. lotic vs lentic systems). This is because the ecotype diversity of the larvae stage is broader than adults and is less described in the detail from the literature. The resulting dataset represented 32 families spanning the globe (Fig. S1). Thermal tolerance records were first matched to the ecotype dataset by binomial names used in the original source, then unmatched records were joined by IUCN names based on synonyms accepted by IUCN or Catalogue of Life. CT_{max} records that could not be matched with ecotype (56 observations) were excluded.

Standardisation of thermal tolerance metrics

The starting temperature and heating rate used affects the measured CT_{max} among individuals in a ramping assay (Peck et al., 2009; Terblanche et al., 2007). The temperature range between the start and end of a heating assay with a constant heating rate, the heating tolerance, describes an elapsed duration of time which scales with temperature similarly to heating tolerance. Heating rate has a consistent and predictable effect on the

effective duration of a heating assay, which accounts for a non-linear rate-temperature relationship, that in turn influences heating tolerance (Kong et al., 2022). This predictable relationship between heating rate, heating duration and consequently heating tolerance can be used to standardise heating tolerance to a given heating rate. To standardise CT_{max} to a heating rate of 1°C min^{-1}, we rescale heating tolerance into the effective duration of a heating assay, apply the correction for the effect of heating rate on effective heating duration, then rescale corrected heating duration back into a corrected heating tolerance (detailed in the Supplementary Information). A heating rate of 1°C min^{-1} was the most common heating rate and is considered ecologically relevant (e.g. (Leong, Tsang, & Guenard, 2022)). We did not standardise starting temperature because temperatures used may have reflected local or field temperatures and indicate local adaptation. There were 1318 observations from 438 species with CT_{max}, 1316 observations from 438 species with heating tolerance and 1238 observations from 432 species with corrected CT_{max} or corrected heating tolerance. In effect, we reduced potential methodological "nuisance heterogeneity" that may occur within and among ramping assays while retaining potential adaptive variation attributed with starting temperature that may reflect acclimation or developmental, physiological, genetic, or environmental constraints.

Relationship between CT_{max} and heating tolerance with ecotype and ontogeny

We constructed a phylogenetically corrected multi-level Bayesian model to investigate the relationship between CT_{max}, corrected CT_{max}, heating tolerance or corrected heating tolerance across ontogenetic stage and ecotype (see Supplementary Information for detail). We included starting temperature of the assay as a co-variate. Ontogenetic stages 1-5 correspond to larva, 6 to juveniles, and 7 to adults (Ruthsatz et al., 2022). We used an amphibian phylogeny dataset comprising of 7,238 species to account for phylogenetic relatedness (Jetz & Pyron, 2018). We pruned the phylogenetic tree to matched binomial names in the thermal tolerance data (438 species, Fig. 1). Species names based on Jetz and Pyron (2018) were used in analysis to account for duplicate names matching with the IUCN. Records that were not in the phylogenetic tree (n = 1) were excluded. We converted the pruned tree to a phylogenetic relatedness correlation matrix for subsequent analysis. For the CT_{max} model, we used a quadratic equation to model change in CT_{max} over ontogeny and the quadratic equation provided a better fit than the linear model (Table S1). All model outputs (Tables S2 – S5) and posterior predictive checks (Figs. S4 & S6) are presented in the Supplementary Information. All model parameters are reported as mean Bayesian posterior estimates ± 95% credible intervals (95% CI), which is equivalent to 95% confidence intervals for frequentist statistics. Summary statistics are presented as mean ± standard deviation.

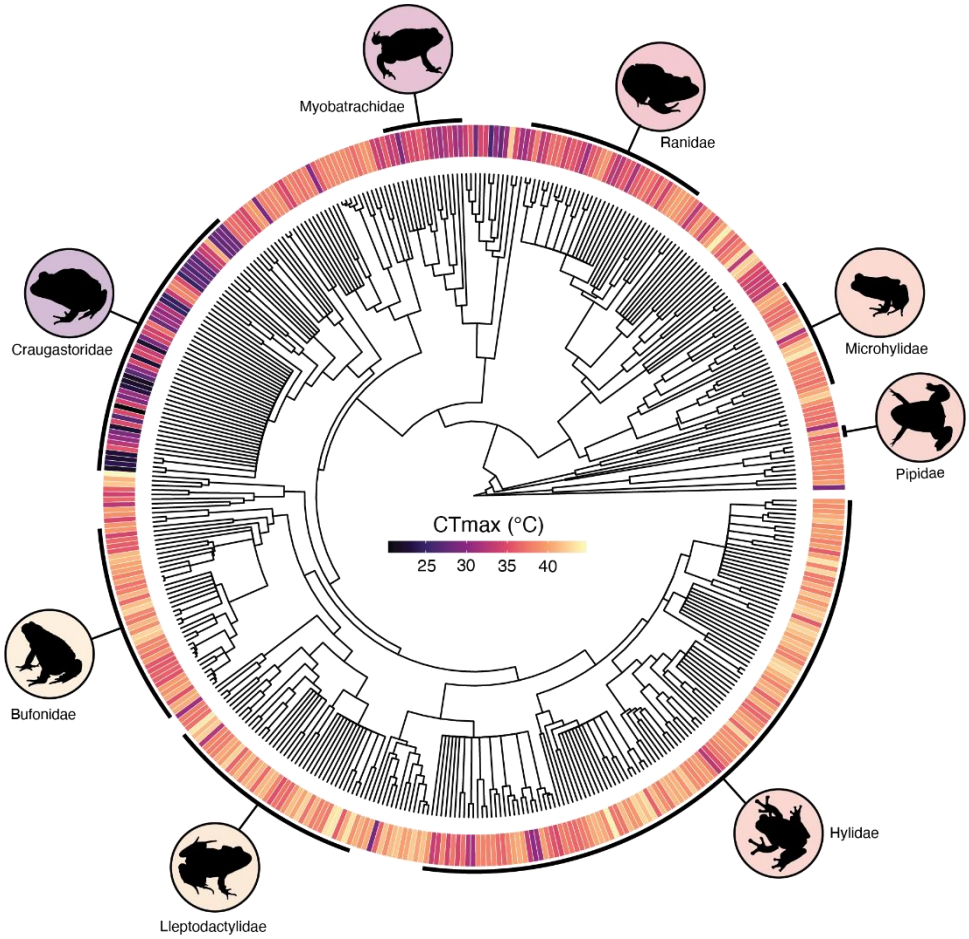

Figure 1. Phylogenetic patterns of CT_{max} across 438 Anurans based on Jetz & Pyron (2018). Colours represents species mean CT_{max}. Example families are indicated by outer black lines. Representative silhouettes obtained from phylopic.org and background colours within the circles represent mean CT_{max} at the Family level.

Results

CT_{max}

CT_{max} ranged between 20.4 °C and 46.1 °C (mean 37.1 ± 4.1 °C) across all ecotypes. On average, fossorial frogs have the highest mean CT_{max} (40.1 ± 3.1 °C) and semi-aquatic frogs have the lowest CT_{max} (35.4 ± 3.54 °C). Larval CT_{max} (Stage 1-5) was on average 3.5 °C higher than adult (Stage 7) and juvenile (Stage 6) CT_{max} combined; the mean difference ranged from 1.5 °C for semi-aquatic frogs and 5.2 °C for stream-dwelling frogs. However, data on CT_{max} for early larval (Stage 1) and juvenile (Stage 6) stages were low (n = 41 and n = 33, respectively) relative to later larval stages (Stage 3, n = 207) and adults (Stage 7, n = 733).

Variation in CT_{max} was strongly influenced by ontogeny where CT_{max} generally decreased across ontogeny (Table S2; Fig. 2). CT_{max} did not differ substantially between ecotypes, but on average, stream-dwelling frogs have lower CT_{max} (-0.74 [-2.63: 1.11]), while fossorial frogs generally had higher CT_{max} (1.96 [-0.06:4.05]). There was a strong phylogenetic effect on CT_{max} (λ = 0.85 [0.75:0.92]). Starting temperature also had a strong effect on CT_{max}, where the higher the starting temperature, the higher the CT_{max} (0.16 [0.14:0.18]).

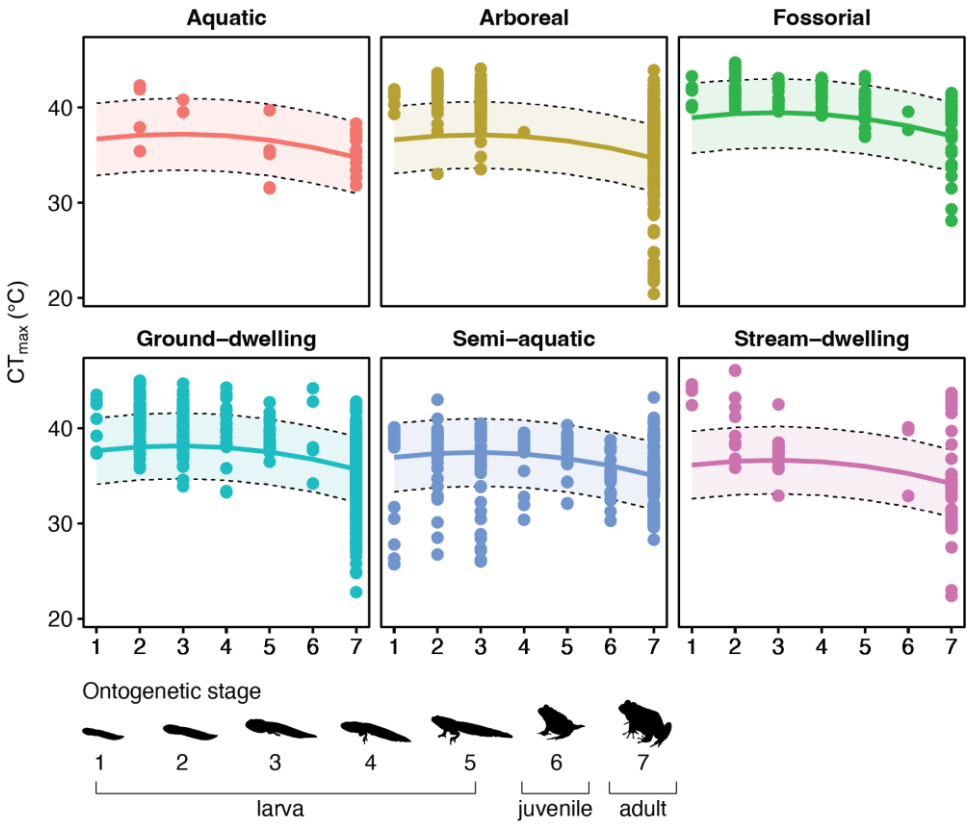

Figure 2. CT_{max} (°C) decreases over ontogeny (stage 1–7) across all Anuran ecotypes (438 species). Quadratic regression line and shaded area represents ecotype-specific model estimate ± 95% CI.

Corrected CT_{max}

CT_{max} corrected to 1°C min^{-1} was on average 1.1 °C higher (range difference between -1.6 – 6.8 °C) than observed CT_{max} (Fig. S3). Variation in corrected CT_{max} was strongly influenced by ontogeny where CT_{max} generally decreased across ontogeny (Table S3; Fig. 3). Corrected CT_{max} between ecotypes showed similar trends to the uncorrected CT_{max}, where stream-dwelling frogs had lower CT_{max} (-0.2 [-2.37: 1.95]), while fossorial frogs had higher CT_{max} (2.08 [-0.31:4.51]). There was a strong phylogenetic effect on corrected CT_{max} (λ = 0.85 [0.74:0.92), and starting temperature also influenced corrected CT_{max} (0.15 [0.13:0.17]).

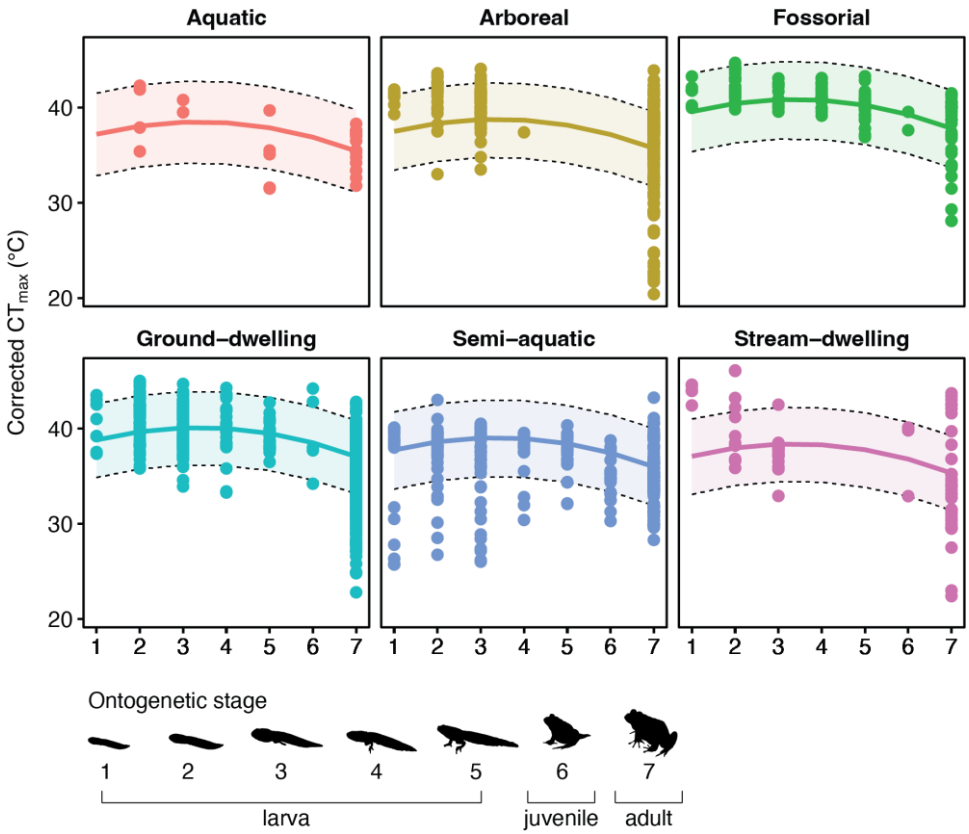

Figure 3. Corrected CT_{max} (°C) decreases over ontogeny (stage 1–7) across all ecotypes (432 species). Quadratic regression line and shaded area represent ecotype-specific model estimate ± 95% CI. CT_{max} was corrected to a heating rate of 1 °C min^{1}.

Heating tolerance

Heating tolerances ranged between 2 °C and 36.5 °C (16.7 ± 6.34 °C) across all ecotypes. Mean heating tolerance varied between ecotypes with the highest mean in fossorial frogs (18.6 ± 6.1 °C) and lowest in stream-dwelling frogs (16.0 ± 6.2 °C). Heating tolerance was also influenced by ontogenetic stage (-0.42 [-0.49:-0.35); Fig. 4), with marginal influence by ecotypes (Table S4). There was a strong phylogenetic effect on heating tolerance (λ = 0.86 [0.76:0.92]) and an effect of starting temperature where heating tolerance was lower at higher starting temperature (-0.83 [-0.85:-0.82]). Results were also consistent for the corrected heating tolerance (Table S5; Fig. S5).

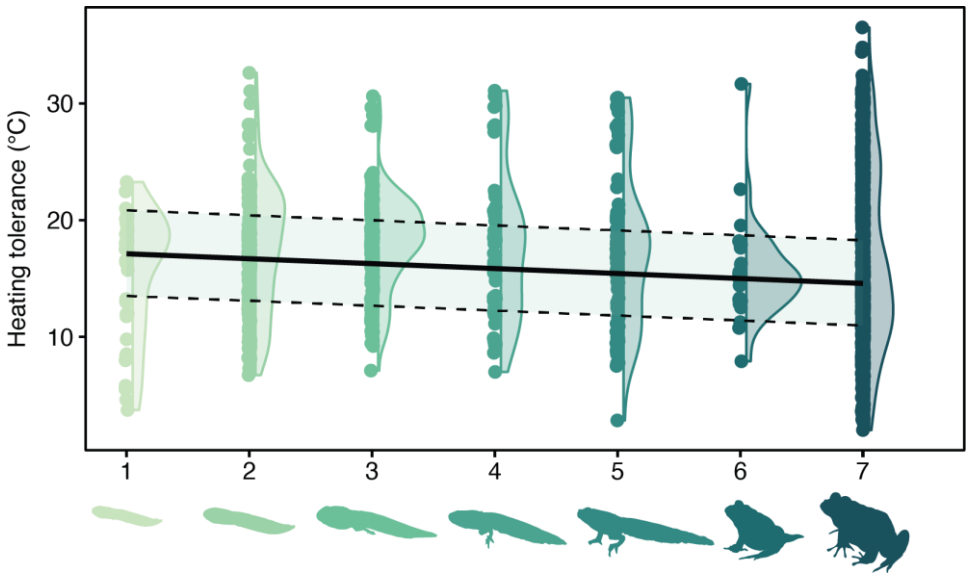

Figure 4. Heating tolerance (°C) decreases across ontogeny (stage 1–7, colours). Regression line and shaded area represents overall model estimate + 95% CI. Heating tolerance is CT_{max} – starting temperature of the ramping assay (438 species).

Discussion

No strong evidence for thermal bottlenecks in Anurans

Amphibians are an excellent system in which to investigate ontogenetic variation in thermal tolerance because they have a biphasic life cycle with life stages clearly delineated by metamorphosis. The delineation between life stages set by metamorphosis is key as metamorphosis may compartmentalise the life cycle and allow for a potential thermal bottleneck at one stage with minimal detrimental effects of temperature across a life cycle (Enriquez-Urzelai et al., 2019; Lowe, Martin, Skelly, & Woods, 2021).

We find striking differences between adults and larva across ecotypes, even after correcting for experimental variation, in the opposite direction than expected if larva represented a thermal bottleneck. A similar general negative relationship between thermal tolerance over ontogeny is widely reported for frogs (Cupp, 1980; Enriquez-Urzelai et al., 2019; Ruthsatz et al., 2022). Although lower thermal tolerances may be expected for aquatic larva, a higher thermal tolerance may be advantageous for small bodied larva that have reduced capacity to escape temperature extremes (Lenoir et al., 2017). Similarly, a wider selection of

microhabitats in terrestrial environments may allow adults to compensate for a lower thermal tolerance through behavioural site selection than aquatic larva which grow in a thermally homogeneous environment (Enriquez-Urzelai et al., 2020).

We find ecotype mediates the magnitude of ontogenetic differences in thermal tolerances but is not an accurate proxy for environmental selection or constraint. The discrete nature of complex life cycles with metamorphosis and the need to survive thermal stress at one stage until the transition to the next stage may place high importance on the interaction between traits and the environment where a thermal bottleneck is a selective disadvantage. Microhabitat preferences has been demonstrated to strongly correlate with thermal biology in reptiles (Greer, 1980; Michelangeli, Goulet, Kang, Wong, & Chapple, 2018), bees (Wilson, Murphy, Rinehart, Yocum, & Bowsher, 2020), frogs (Carilo Filho et al., 2021; Duarte et al., 2012), and ants (Bujan, Roeder, de Beurs, Weiser, & Kaspari, 2020; Leahy, Scheffers, Williams, & Andersen, 2022). We found strong phylogenetic conservatism in CT_{max} which likely reflects how ecotypes strongly correlate within Family (Moen & Wiens, 2017). For example, Hylidae have a high proportion of arboreal species (Vidal-García & Keogh, 2015). The use of ecotype as a proxy is complicated by variation in developmental characteristics that may not be consistent within Family or ecotypes to make generalisations. For example, direct-developing larva (not available in our dataset) may occupy more similar microhabitats to adults than species with an aquatic larva and fully terrestrial adults as discussed with the limitations of larvae ecotypes. Egg thermal tolerance is rare in the Amphibian literature, but eggs may also occupy vastly different microhabitats to larva and adults. For instance, arboreal frogs may create foam nests in the canopy but the larva inhabit puddles on the forest floor (Haddad & Prado, 2005). While it would be possible to classify larval ecotypes or egg microhabitats (Carilo Filho et al., 2021), this information is not readily available at the scale of this study, and we encourage further investigation into these modulators. Doing so may reveal whether diversity in developmental traits within ecotypes masks local scale signals of environmental adaption via interactions with ecotype. Nevertheless, we found fossorial species, on average, had higher thermal tolerance than other ecotypes.

The large overlap in thermal tolerance across ecotypes suggests developmental or genetic constraints have a greater influence on the evolution of thermal tolerances than environmental factors. Developmental or genetic constraints on climate niches or thermal traits are suggested for reptiles and amphibians (Bodensteiner et al., 2021; Carilo Filho et al., 2021; Pie, Campos, Meyer, & Duran, 2017). Several inferences support developmental or genetic constraints underlie consistent differences between larva and adult thermal tolerance. First, an absence for a bottleneck may indicate metamorphosis modulates thermal tolerances over ontogeny (Lowe et al., 2021). Minimal carry over effects of development temperature on CT_{max} are found between larva and adults (Enriquez-Urzelai et al., 2019). Physiological remodelling and morphological development during metamorphosis are hypothesised to trade-off with physiological processes and explain why CT_{max} decreases at metamorphic climax (Cupp, 1980; Ruthsatz et al., 2022).

Second, if juveniles and adults are not drastically different in their physiology (i.e., biochemical processes are not affected by metamorphosis), then differences in thermal

tolerance may indicate differences in energy allocation between life stages. An energetics interpretation depends on whether capacity to respond to a thermal challenge is considered part of maintenance metabolic costs and the flexibility of reallocating energy. Perhaps, the allocation of energy to reproduction at the adult stage trade-offs with the capacity to increase maintenance metabolic rates in response to heating, whereas larva without a reproductive metabolic cost have greater scope to rapidly respond to a thermal challenge at the cost of allocating energy to growth. This explanation is in line with how the temperature size rule emerges from differences between development and growth rates (Forster & Hirst, 2012; Gahm, Arietta, & Skelly, 2021; Rubalcaba & Olalla-Tárraga, 2020).

A higher physiological capacity to respond to change (i.e., plasticity) in larva is a parsimonious explanation from an energetics perspective. In contrast with studies that show decreasing acclimation capacity with increasing size (Burton, Lakka, & Einum, 2020; Rohr et al., 2018), *Rana temporaria* larva have lower acclimation capacity than adults (Ruthsatz et al., 2022). Assuming the cost of having a given thermal tolerance is the same between life stages, low plasticity in thermal tolerance suggests larva continuously express physiological mechanisms that permit higher CT_{max}, which is very costly (i.e., in maintenance metabolic cost) during a time when they should be allocating energy to growth. One explanation for low thermal plasticity is tadpoles have a narrow range of temperatures to select for behavioural thermoregulation and thus primarily rely on physiological thermoregulation via a higher CT_{max} to avoid heat stress (Casterlin & Reynolds, 1978). As larva would have lower absolute metabolic rate based on size allometry alone (in general), whether or not they can offset an increased energetic cost with increased food intake or by other means can be explored.

Third, if physiological mechanisms are different between life stages, then the differences may reflect developmental constraints. Perhaps the physiological machinery during larval growth covaries with greater capacity to respond to an environmental challenge, or that larva have lower energetic costs for responding to thermal challenges, which allows them to continuously express high CT_{max} at minimal cost to growth, in contrast with adults. This explanation would resolve the paradox of the high metabolic costs of physiological thermoregulation.

Standardisation reduces confounding interaction across life cycles but does not change our conclusions

Minimal difference between observed CT_{max} and corrected CT_{max} likely underpin the absence of significant changes to our inferences. There is a relative consistency in methods to assay CT_{max} in amphibians and the range of heating rates is narrow with 1 °C min^{-1} as the mode (Hutchison, 1961). Most studies in our literature search (2016-2022) assayed CT_{max} using a ramping protocol rather than static protocols (zero studies described) or from fitted thermal performance curves (three studies). Applying a correction to CT_{max} is likely more effective for other taxa or in broader scale interspecific comparisons. For example, heating rate varied between 0.0007 and 4 °C min^{-1} (mean 0.5 °C min^{-1}) in a global dataset of ectotherm CT_{max} that included tropical to polar organisms (Morley, Peck, Sunday, Heiser, &

Bates, 2018), and rescaling this data for non-linear rate-temperature dynamics produced high precision estimates of CT_{max} (Kong et al., 2022). In studies on frogs, ontogenetic stage is confounded with heating rates as lower heating rates are typically used for larva than adults or juveniles (Fig. S3). Thus, applying a correction to CT_{max} may be useful to infer true effects of ontogenetic stage and we suggest it a reasonable practice to account for nuisance heterogeneity in comparative study.

To our knowledge, our study is the first to apply a theoretic model incorporating heating rate and starting temperature to a comparative dataset of this scale (Pottier, Burke, Drobniak, & Nakagawa, 2022). Previous studies have used statistical regression to correct for variation in acclimation temperature on CT_{max} (e.g. Sunday et al. (2014)). In other words, rescaling of heating tolerances represents a null model of how CT_{max} may vary with heating rate and starting temperature, based on physiological and non-linear rate-time dynamics. However, missing covariates of CT_{max} reduced the effectiveness of applying a mathematical correction. We were not able to correct CT_{max} for studies that did not report acclimation temperature, starting temperature or heating rate (80 observations). As heating rate and starting temperature are important sources of variation in CT_{max}, we suggest these common covariates should be reported as best practice (Schwanz et al., 2022; Sunday et al., 2014).

Applying any correction relies on making generalisations about the nature of the relationship between CT_{max} and experimental covariates, and its consistency across taxa. The rescaling approach used here assumes that the underlying processes that generate what we observe as CT_{max} is modelled by a Boltzmann-Arrhenius function with an activation energy of 0.6eV, and this relationship is insensitive to change (e.g., acclimation or plasticity). This relationship may represent whole-organism metabolic rate encompassing the general rate-dependent physiology of animals, for example, and may be appropriate to describe thermal tolerances as emergent properties of several interacting processes at the sub-individual level, but this model may differ from other hypotheses about how thermal tolerance arises (MacMillan, 2019).

We suggest using a general model of thermal tolerance is more appropriate for large scale comparative studies where it is inappropriate to assume consistent or homogeneous mechanisms across taxa or large scales. Several other models of CT_{max} incorporating starting temperature and heating rate invoke more specific mechanisms, such as injury accumulation and damage repair (Jørgensen, Malte, Ørsted, Klahn, & Overgaard, 2021; Rezende, Bozinovic, Szilágyi, & Santos, 2020). In our dataset, CT_{max} endpoints are relatively consistent across studies and within ontogenetic stage, but ontogenetic stage is confounded by endpoint definition. For example, onset of spasms or heat rigour were more common for larva and loss of righting response is most common for adults. A model invoking a hypothesised mechanism could be relevant for investigating specific endpoints or definitions of upper critical temperatures. Overall, the appropriateness of a model for its use in comparative study depends on the underlying assumptions, and the utility of the model is a trade-off between the requirements for model parameterisation and its intended use.

Conclusions

Dealing with high among-study variation or problems of scale and resolution are resounding challenges of macrophysiology (Chown & Gaston, 2016). This necessity becomes critical when assessing vulnerability to future climate change because understanding trends in physiological diversity across taxa and environmental gradients forms a basis to predict or forecast climate change responses or vulnerability (Urban et al., 2016). For broad-scale comparisons, we suggest standardising against common experimental variation is a meaningful exercise regardless of any conclusion because potentially confounding variation may be tied with an organisms' life cycle, and uncertainty in CT_{max} is passed onto the derived climate vulnerability metrics (Garcia, Allen, & Clusella-Trullas, 2019; Pincebourde & Casas, 2015).

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