Microhabitat and ontogenetic variation of thermal tolerance in complex ectotherm life cycles

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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 life history can hinder our ability to assess climate change vulnerability in

large-scale comparative studies as these reflect local adaptation. Here, we used phylogenetically informed, multi-level models with a global dataset of upper critical temperatures from 438 Anuran species that explicitly included microhabitat preferences and ontogenetic stage to examine variation in upper critical temperatures. We found ontogenetic trends in thermal tolerance were similar across microhabitat preferences. We then used microclimate-driven models of the heat and water budget of a thermoregulating frog to show the contribution of behavioural thermoregulation towards mitigating exposure to thermal stress. 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, and that behavioural thermoregulation has a strong potential for buffering frogs against extreme temperatures.

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 and Morley 2020), synthesise general trends in physiological diversity from local to macro scales (Chown and 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 (Pandori and Sorte 2019, Dahlke et al. 2020). 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 et al. 2017).

Identifying a thermal bottleneck represents a significant amount of work that is limited to commonly studied species (e.g., Ruthsatz et al. (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 (Truebano et al.

2018, Pandori and Sorte 2019, Collin et al. 2021), fish (Moyano et al. 2017, Dahlke et al. 2020), insects (Klockmann et al. 2017, Kingsolver and Buckley 2020) 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 (Pintanel et al. 2019, Carilo Filho et al. 2021, Cheng et al. 2022, Pintanel et al. 2022, 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. 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 and 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 and Wiens 2017, Moen et al. 2022). In turn, the animal’s ecotype may mediate the confounding interaction between life stages and their respective microenvironment (Pincebourde and Casas 2015, Carilo Filho et al. 2021).

Thermal safety margins (TSM) link upper critical temperatures to environmental temperature to index the vulnerability of ectotherms to temperature extremes (Deutsch et

87 al. 2008). Most macrophysiological assessments of thermal safety margins use global
88 datasets of climate and mean critical temperatures for species but the resolution of climatic
89 data can have large impact on estimated TSM that leads to an underestimation of TSM
90 (Garcia et al. 2019, Pinsky et al. 2019). Behavioural thermoregulation is important in this
91 context as ectotherms that are able to behaviourally thermoregulate may be able to
92 mitigate the effects of warming (Sunday et al. 2014). Thus, assessments of climate
93 vulnerability using TSM should consider both the resolution of climate data and the innate
94 capacity of species to respond to temperature variation.

95 Here, we tested whether ecotypes are reliable proxies of environmental adaptation to
96 resolve ontogeny by environment interaction of CT_{max} and identify thermal bottlenecks. We
97 analysed the CT_{max} of 438 Anuran species to 1) identify whether Anurans have a thermal
98 bottleneck in their life cycle and their potential evolutionary drivers and ecological
99 consequences at a macrophysiological scale, and 2) evaluate whether proposed solutions to
100 two challenges of comparative analyses of ectotherm thermal tolerance improved our
101 ability to identify thermal bottlenecks. We acknowledge there is a diversity of definitions
102 and uses of thermal tolerance that would influence their interpretation. In this study, we
103 only consider CT_{max} as the end point of a temperature ramping assay with a constant
104 heating rate, or dynamic ramping protocol, and we define heating tolerance as the
105 temperature range between starting temperature and CT_{max} during the ramping assay.

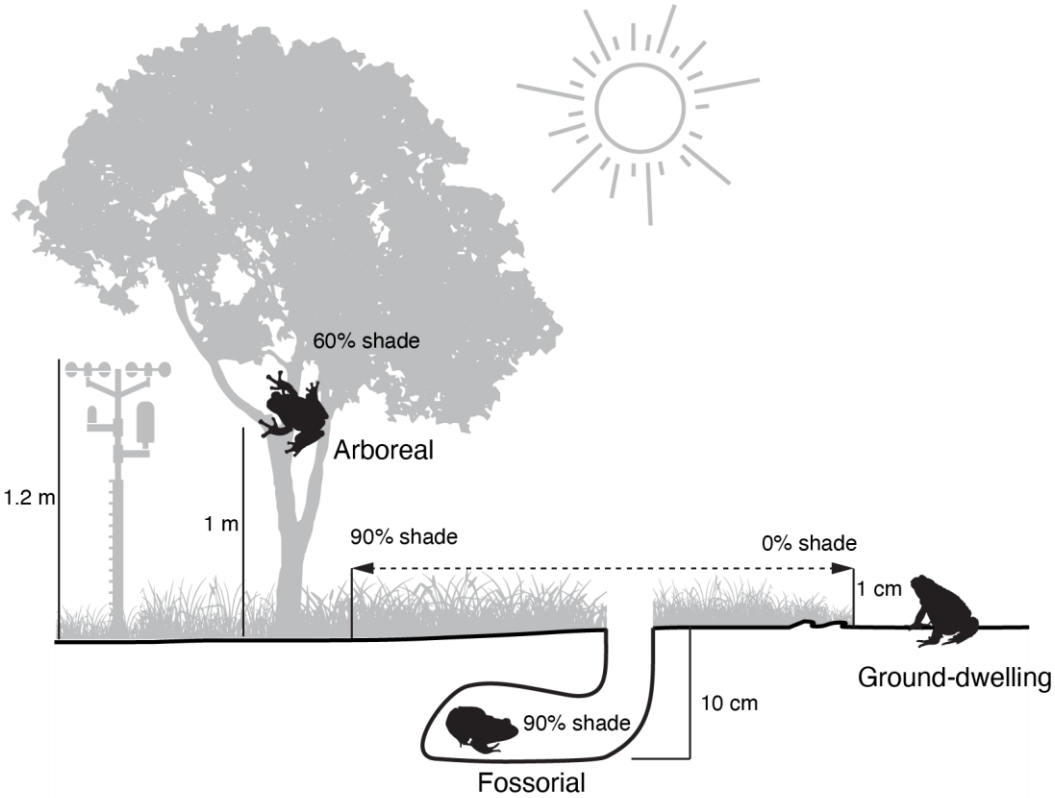

Figure 1 Schematic diagram of ecotype microclimate and key variables used to calculate representative microclimate. Arboreal frogs were modelled at 1m 60% shaded air temperature, fossorial frogs at 10cm soil depth in 90% shaded soil, and ground-dwelling frogs were modelled at 1cm 0% shaded (open) air temperature. Thermoregulating ground-dwelling frogs could move to 90% shaded 1cm air temperatures, indicated by the arrows.

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). The data were reported in (Wu et al. 2024). Ecotypes were classified from ecological descriptions from IUCN based on adult behaviour and microhabitat preferences outside the breeding season (Moen and Wiens 2017). We grouped species by ecotype even if the larval stage may have differing ecological types (e.g. lotic vs lentic systems) 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.

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} or 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 and Pyron 2018). We pruned the phylogenetic tree to matched binomial names in the thermal tolerance data (438 species, Fig. 2). 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.

Thermal safety margins at macro- and micro-climate scales

To calculate thermal safety margins we extracted mean annual temperature and maximum temperature of the warmest month from WorldClim 2.1 (resolution: 0.5 min of a degree) for each observation (Fick and Hijmans 2017). TSM was calculated as upper critical temperature (CTmax) - maximum temperature of the warmest month. Observations that could not be queried on WorldClim were removed from analysis. To illustrate the effect of spatial resolution on estimates of TSM for representative ecotypes, we chose frogs representing three ecotypes (Fig. 1): fossorial (*Neobatrachus pictus*), arboreal (*Litoria peronii*) and ground-dwelling (*Crinia signifera*). These species are sympatric in north-eastern New South Wales, Australia and were measured in the same study using the same conditions (5°C acclimation temperature and 1°C min^{-1} heating rate) (Brattstrom 1970). Because of the additional challenges of modelling microclimates involving water and the potentially large temperature difference between air and water, we were not able to conduct simulations for aquatic, stream-dwelling and semi-aquatic ecotypes We used NicheMapR to simulate representative microclimates for a fossorial (90% shaded 0.1 m soil temperature), arboreal (60% shaded 1m air temperature) and ground-dwelling species (0% shaded 0.01m air temperature) (Kearney and Porter 2017). These microclimates informed a biophysical energy and mass balance model of a hypothetical non-thermoregulating 5g frog (approximating a leopard frog) that we used to estimate operative temperature (maximum body temperature) for each species (Kearney and Porter 2019, Enriquez-Urzelai et al. 2020, Wu et al. 2024). To demonstrate the role of behavioural thermoregulation for estimating TSM, we ran the biophysical model twice for the ground-dwelling frog allowing for or restricting microhabitat selection by shuttling between shaded and unshaded microhabitats.

For each ecotype, we extracted maximum temperature at the locality of the individual and maximum body temperature, representing operative temperature, from the year-worth of output (Fig. 1).

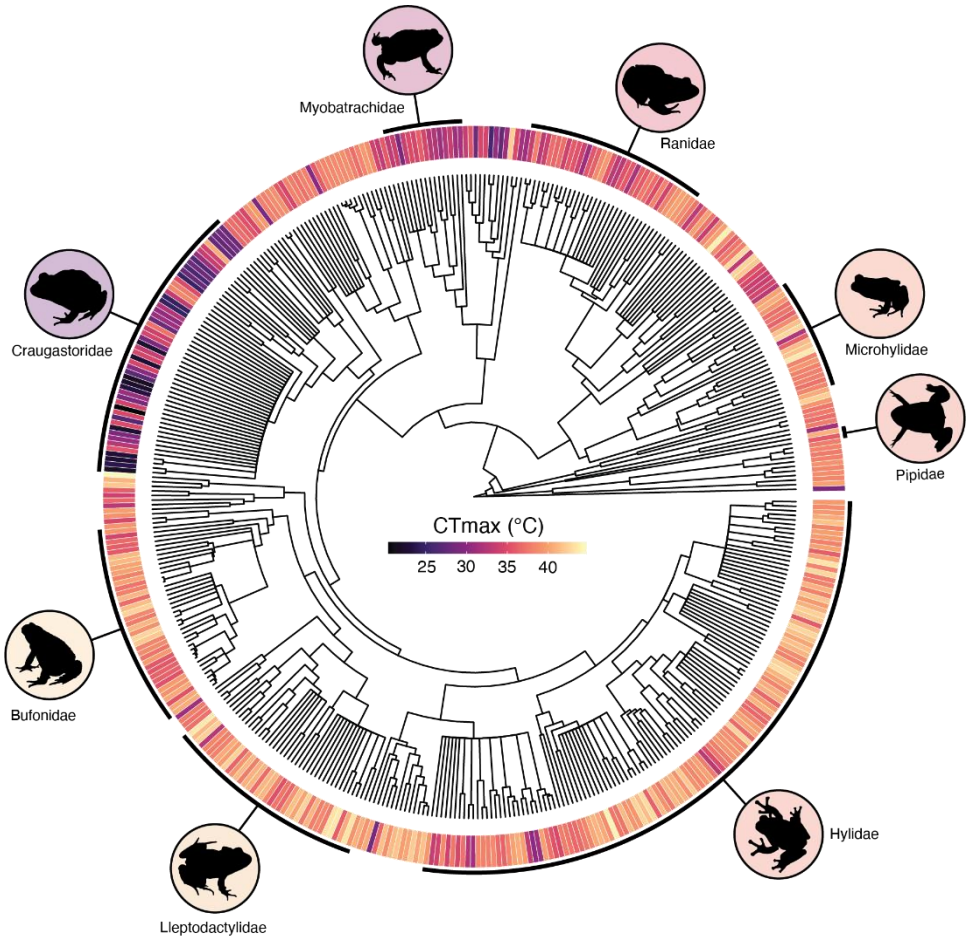

Figure 2. Phylogenetic patterns of CT_{max} across 438 Anurans based on Jetz & Pyron (2018). Colours represents species mean CT_{max}. Example families are indicated by outer black lines. Representative silhouettes obtained from phylopic.org and background colours within the circles represent mean CT_{max} 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, there are fewer data on CT_{max} for early larval (Stage 1, n = 41) and juvenile (Stage 6, n = 33) stages than 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. 3). 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]).

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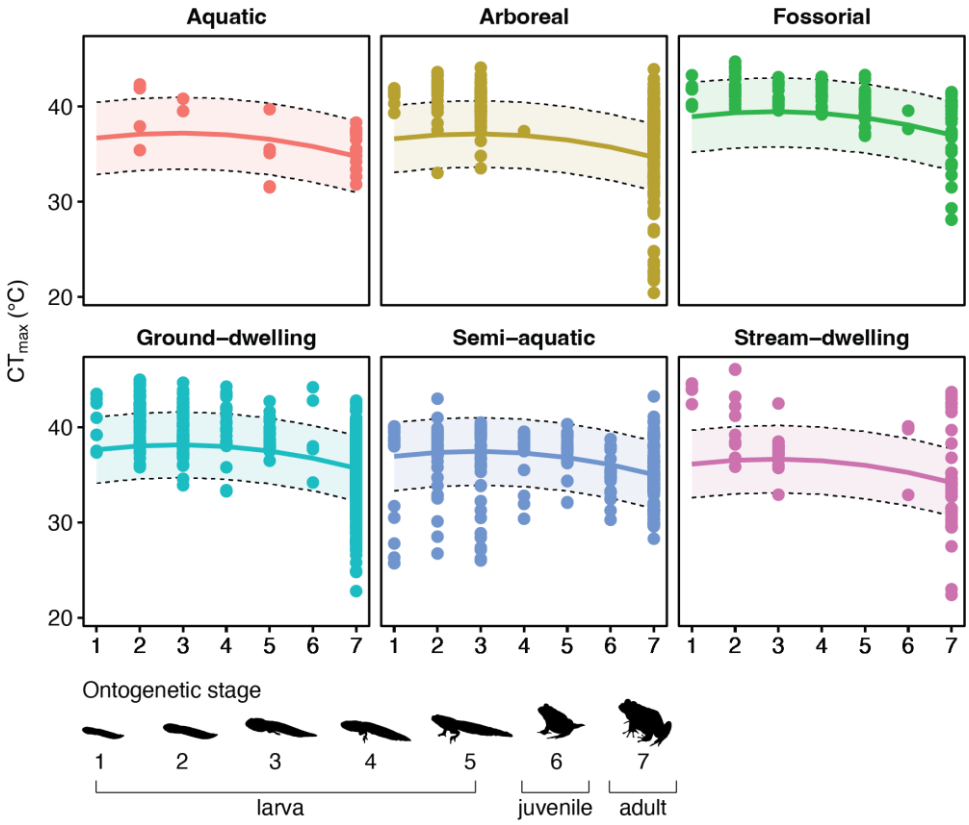

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211 *Figure 3. CT_{max} (℃) decreases over ontogeny (stage 1–7) across all Anuran ecotypes (438 species). Quadratic regression line*

212 *and shaded area represents ecotype-specific model estimate ± 95% CI.*

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]).

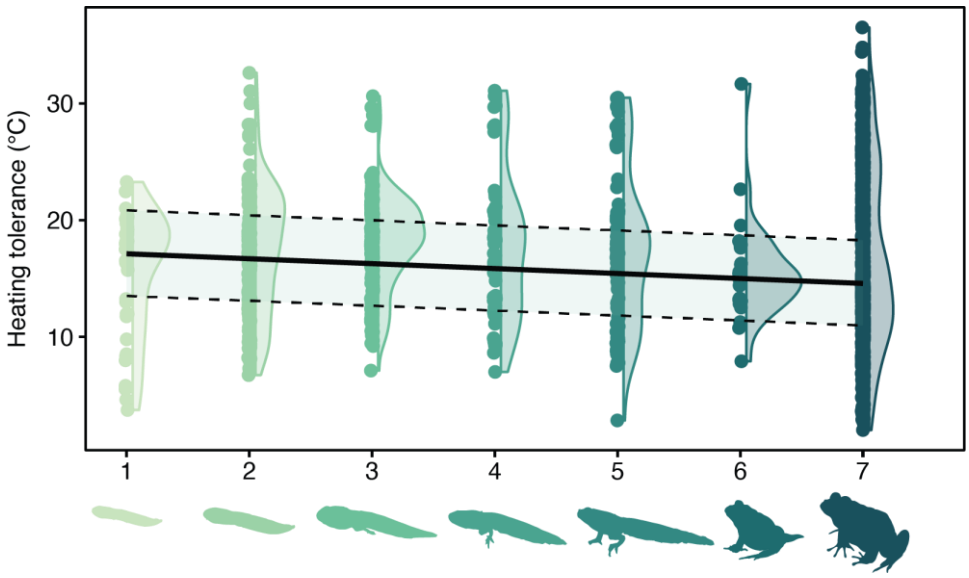

Figure 4. Heating tolerance (°C) decreases across ontogeny (stage 1–7, colours). Regression line and shaded area represents overall model estimate + 95% CI. Heating tolerance is CT_{max} – starting temperature of the ramping assay (438 species).

Thermal safety margin

Estimates of thermal safety margin vary with the spatial resolution of the environmental temperature used in calculations, microhabitat characteristics, and the presence of behavioural thermoregulation (Table 1, Fig. 5). Using macroclimate underestimates TSM for fossorial and ground-dwelling frogs because the microclimate of these ecotypes are not well captured by weather stations. Higher TSM using microclimate than macroclimate for a fossorial species are generated by a lower soil temperature than air temperature. There is little difference between using operative temperatures and microclimate because fossorial frogs do not leave their burrow. TSM are similar using all spatial resolutions for arboreal frogs because arboreal microhabitats represented by 1m shaded environments (60% canopy

cover) are similar to weather stations (e.g. 1.2m 90% canopy cover). A ground-dwelling frog in an open, sunny habitat will encounter maximum air temperatures and exhibit maximum body temperatures exceeding their upper critical temperature (negative TSM) at the relevant microclimatic scale of the individual (1cm above ground in our simulations). When allowed to select cooler microhabitats, predicted body temperatures of simulated ground-dwelling frogs never exceed CTmax (positive TSM).

Table 1 Thermal Safety Margin (TSM; CTmax – maximum temperature; °C) calculated using maximum temperatures of varying spatial resolution for sympatric species representing three ecotypes: fossorial (Neobatrachus pictus), arboreal (Litoria peronii) and ground-dwelling (Crinia signifera) species. Macroclimate: Maximum temperature of the warmest month (WorldClim, BIO5). Microclimate: Maximum temperature from downscaled climate to representative microhabitat conditions of the ecotype (NicheMapR). Operative temperature: maximum body temperature for a simulated 5g frog in their preferred microhabitat.

Temperature	Fossorial	Arboreal	Ground-dwelling
Current macroclimate	-0.88	7.40	6.70
Future macroclimate	-4.58	3.60	2.90
Microclimate	5.62	6.14	-7.90
Operative temperature (no thermoregulation)	5.56	4.78	-12.36
Operative temperature (thermoregulating)	NA	NA	2.32

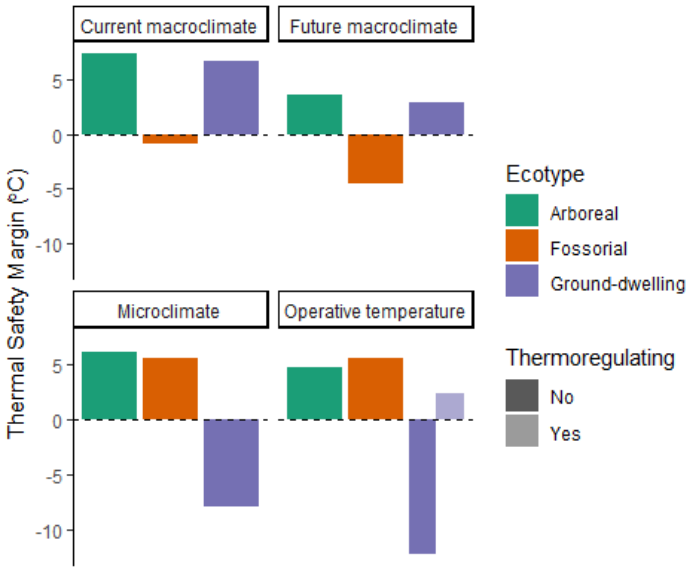

Figure 5 Thermal Safety Margin (TSM; CTmax – maximum temperature; °C) calculated using maximum temperatures of varying spatial resolution for sympatric species representing three ecotypes: fossorial (Neobatrachus pictus), arboreal (Litoria peronii) and ground-dwelling (Crinia signifera) species. Macroclimate: Maximum temperature of the warmest month (WorldClim, BIO5). Microclimate: Maximum temperature from downscaled climate to representative microhabitat conditions of the ecotype (NicheMapR). Operative temperature: maximum body temperature for a simulated 5g frog in their preferred microhabitat.

Discussion

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 et al. 2021).

We found striking differences between adults and larva across ecotypes 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 found 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 (Lowe et al. 2021). Microhabitat preferences has been demonstrated to strongly correlate with thermal biology in reptiles (Greer 1980, Michelangeli et al. 2018), bees (Wilson et al. 2020), frogs (Duarte et al. 2012, Carilo Filho et al. 2021), and ants (Bujan et al. 2020, Leahy et al. 2022). We found strong phylogenetic conservatism in CT_{max} which likely reflects how ecotypes strongly correlate within Family (Moen and Wiens 2017). For example, Hylidae have a high proportion of arboreal species (Vidal-García and 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-

285 developing larva (not available in our dataset) may occupy more similar microhabitats to
286 adults than species with an aquatic larva and fully terrestrial adults as discussed with the
287 limitations of larvae ecotypes. Egg thermal tolerance is rare in the Amphibian literature, but
288 eggs may also occupy vastly different microhabitats to larva and adults. For instance,
289 arboreal frogs may create foam nests in the canopy but the larva inhabit puddles on the
290 forest floor (Haddad and Prado 2005). While it would be possible to classify larval ecotypes
291 or egg microhabitats (Carilo Filho et al. 2021), this information is not readily available at the
292 scale of this study, and we encourage further investigation into these modulators. Doing so
293 may reveal whether diversity in developmental traits within ecotypes masks local scale
294 signals of environmental adaption via interactions with ecotype. Nevertheless, we found
295 fossorial species, on average, had higher thermal tolerance than other ecotypes.

296 The large overlap in thermal tolerance across ecotypes suggests developmental or genetic
297 constraints have a greater influence on the evolution of thermal tolerances than
298 environmental factors. Developmental or genetic constraints on climate niches or thermal
299 traits are suggested for reptiles and amphibians (Pie et al. 2017, Bodensteiner et al. 2021,
300 Carilo Filho et al. 2021). Several inferences support developmental or genetic constraints
301 underlie consistent differences between larva and adult thermal tolerance. First, an absence
302 for a bottleneck may indicate metamorphosis modulates thermal tolerances over ontogeny
303 (Lowe et al. 2021). Minimal carry over effects of development temperature on CT_{max} are
304 found between larva and adults (Enriquez-Urzelai et al. 2019). Physiological remodelling and
305 morphological development during metamorphosis are hypothesised to trade-off with
306 physiological processes and explain why CT_{max} decreases at metamorphic climax (Cupp
307 1980, Ruthsatz et al. 2022).

308 Second, if juveniles and adults are not drastically different in their physiology (i.e.,
309 biochemical processes are not affected by metamorphosis), then differences in thermal
310 tolerance may indicate differences in energy allocation between life stages. An energetics
311 interpretation depends on whether capacity to respond to a thermal challenge is considered
312 part of maintenance metabolic costs and the flexibility of reallocating energy. Perhaps, the
313 allocation of energy to reproduction at the adult stage trade-offs with the capacity to
314 increase maintenance metabolic rates in response to heating, whereas larva without a
315 reproductive metabolic cost have greater scope to rapidly respond to a thermal challenge at

316 the cost of allocating energy to growth. This explanation is in line with how the temperature
317 size rule emerges from differences between development and growth rates (Forster and
318 Hirst 2012, Rubalcaba and Olalla-Tárraga 2020, Gahm et al. 2021).

319 A higher physiological capacity to respond to change (i.e., plasticity) in larva is a
320 parsimonious explanation from an energetics perspective. In contrast with studies that
321 show decreasing acclimation capacity with increasing size (Rohr et al. 2018, Burton et al.
322 2020), *Rana temporaria* larva have lower acclimation capacity than adults (Ruthsatz et al.
323 2022). Assuming the cost of having a given thermal tolerance is the same between life
324 stages, low plasticity in thermal tolerance suggests larva continuously express physiological
325 mechanisms that permit higher CT_{max}, which is very costly (i.e., in maintenance metabolic
326 cost) during a time when they should be allocating energy to growth. One explanation for
327 low thermal plasticity is tadpoles have a narrow range of temperatures to select for
328 behavioural thermoregulation and thus primarily rely on physiological thermoregulation via
329 a higher CT_{max} to avoid heat stress (Casterlin and Reynolds 1978). As larva would have lower
330 absolute metabolic rate based on size allometry alone (in general), whether or not they can
331 offset an increased energetic cost with increased food intake or by other means can be
332 explored.

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

340 A notable characteristic of the amphibian CT_{max} literature is that there is a relative
341 consistency in methods to assay CT_{max} and the range of heating rates is narrow with a mode
342 of 1 °C min^{-1} (Hutchison 1961). Most studies in our literature search (2016-2022) assayed
343 CT_{max} using a ramping protocol rather than static protocols (zero studies described) or from
344 fitted thermal performance curves (three studies). In contrast, there is a much larger
345 diversity in heating rates reported for global ectotherm CT_{max} varying between 0.0007 and 4

346 °C min^{-1} (mean 0.5 °C min^{-1}) that reflects the tropical to polar origin of species (Morley et al.
347 2018). In studies on frogs, ontogenetic stage is confounded with heating rates as lower
348 heating rates are typically used for larva than adults or juveniles (Fig. S3). This different may
349 contribute towards nuisance heterogeneity in comparative study but the effects may be
350 minimal compared to other taxa (Noble et al. 2022). In our dataset, CT_{max} endpoints are
351 relatively consistent across studies and within ontogenetic stage, but ontogenetic stage was
352 confounded by endpoint definition. For example, onset of spasms or heat rigour were more
353 common for larva and loss of righting response is most common for adults. A model
354 invoking a hypothesised mechanism could be relevant for investigating specific endpoints or
355 definitions of upper critical temperatures.

356 Simulating body temperatures trades-off with making significant assumptions about the
357 microclimate characteristics of each ecotype and their thermal biology. For example,
358 arboreal frogs bask but we simulated they did not (Tracy et al. 2010). Using operative
359 temperature is best suited to species with well characterised thermal biology to derive
360 accurate TSM estimates and activity patterns at a local scale, for species whose body
361 temperatures are expected to be significantly different to environmental temperature, or
362 for large-scale studies that trade-off accuracy for scale. If the thermal biology of species is
363 known, then a microclimate-driven biophysical models of ectotherm body temperatures can
364 provide a fine-scale analysis of estimated activity times under warming scenarios and
365 hypothetical capacities to mitigate potential heat stress (Anderson et al. 2022).

366 A size-dependency of CTmax may explain why heating tolerances are relatively conserved
367 over ontogeny. Larger sizes may buffer adult frogs against higher heating rates via inertia
368 effects, thus a lower CTmax for an adult is equivalent to higher CTmax in smaller larva when
369 rate-time dynamics are considered. A lower size limit for thermal inertia in dry-skinned
370 ectotherms is approximately 1kg, which is larger than most frogs. A similar threshold for
371 frogs is unclear. While tadpoles are reported to be at thermal equilibrium with water
372 temperature, a small basking arboreal frog (<10g) can elevate their body temperature above
373 ambient temperature. Rates of heat exchange that influence body temperature changes in
374 frogs (a wet-skinned ectotherm) depends on resistance to water loss, size, and the
375 difference in thermal properties between water and air.

Microclimate and microhabitat preference strongly modulate estimates of climate vulnerability. We demonstrate that spatial resolution of temperature strongly influences estimated metrics of climate vulnerability. The magnitude of under- or over-prediction was highly dependent on the microhabitat preferences of species (Table 1). Specifically, canopy cover, which intercepts direct solar radiation, in the microclimate model set the ecological accuracy of the simulated microhabitat and may differ from the selected microhabitats in the field. In general, we suggest using simulated microclimates to estimate TSM than macroclimate, particularly for large scale studies for which field measurements of temperature are not feasible. Doing so relies on fewer assumptions than using a biophysical model of the heat balance of a generalised frog to estimate operative temperatures, requires one model instead of two for deriving environmental temperature to calculate TSM, and may be a reasonable proxy for body temperature for an ecotype. It may be reasonable to use soil temperature as a proxy for body temperature for fossorial frogs that do not frequently leave their burrow.

Our simplified simulations clearly illustrate the importance of behavioural thermoregulation (Bogert effect) and the availability of thermal refugia in the context of assessing thermal safety margins (Kearney 2013, Sunday et al. 2014). At face value, our results suggest for a given location, ground dwelling frogs are highly vulnerable to climate change, but the frog was not simulated to avoid temperatures above their activity thresholds. Several studies demonstrated microclimate is a better predictor of frog CTmax than microclimate (Gutiérrez-Pesquera et al. 2016, Katzenberger et al. 2018, Pintanel et al. 2019), and that variation in tadpole CTmax is explained by local scale water temperatures measured using data loggers (Bonino et al. 2020, Pintanel et al. 2022), or simulated microclimate (Oyamaguchi et al. 2018). Understanding environmental tolerance across all life stages is crucial to identify vulnerability in for organisms with complex life cycles (Lowe et al. 2021). The differences in thermal tolerance between aquatic larval stages and mobile adult stages suggested that there is an evolutionary capacity to maximise performance to the environment for a given life stage. However, the potential to fully decouple detrimental environmental effects on fitness at one life stage to the next (i.e. carry-over effects) in amphibians needs to be explored at a comparative global scale.

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