

## Research

### Elevational and local climate variability predicts thermal breadth of mountain tropical tadpoles

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The climate variability hypothesis posits that increased environmental thermal variation should select for thermal generalists, while stable environments should favor thermal specialists. This hypothesis has been tested on large spatial scales, such as latitude and elevation, but less so on smaller scales reflective of the experienced microclimate. Here, we estimated thermal tolerance limits of 75 species of amphibian tadpoles from an aseasonal tropical mountain range of the Ecuadorian Andes, distributed along a 3500 m elevational range, to test the climatic variability hypothesis at a large (elevation) and a small (microhabitat) scale. We show how species from less variable thermal habitats, such as lowlands and those restricted to streams, exhibit narrower thermal tolerance breadths than highland and pond-dwelling species respectively. Interestingly, while broader thermal tolerance breadths at large scales are driven by higher cold tolerance variation (heat-invariant hypothesis), at local scales they are driven by higher heat tolerance variation. This contrasting pattern may result from divergent selection on both thermal limits to face environmental thermal extremes at different scales. Specifically, within the same elevational window, exposure to extreme maximum temperatures could be avoided through habitat shifts from temporary ponds to permanent ponds or streams, while minimum peak temperatures remained invariable between habitats but steadily decreased with elevation. Therefore an understanding of the effects of habitat conversion is crucial for future research on resilience to climate change.

Keywords: climate variability hypothesis,  $CT_{max}$ ,  $CT_{min}$ , elevation, heat-invariant hypothesis, thermal tolerance limits, tropical mountains, warming tolerance

#### Introduction

In a rapidly changing world, understanding how climate shapes thermal physiological tolerances at different scales is becoming increasingly important. The climate variability hypothesis (CVH) predicts a positive relationship between thermal tolerance



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breadth and climate variability (Janzen 1967, Stevens 1989). In other words, ectothermic organisms should be physiologically adapted to the thermal regimes to which they are exposed. Thus, it is postulated that less variable climates in the tropics should select for species with narrower thermal breadths than in seasonal temperate areas (Janzen 1967). This prediction has been broadly described for both aquatic (Shah et al. 2017) and terrestrial ectotherms (Sheldon and Tewksbury 2014). Furthermore, low-elevation species should have narrower thermal breadths (thermal specialist) than high-elevation species (thermal generalists) (Muñoz and Bodensteiner 2019, Pintanel et al. 2019), since thinner air at higher elevations results in warmer temperatures during the day and colder temperatures at night (Blumthaler et al. 1997). Greater insolation at higher elevations results in warmer temperatures during the day, blurring the decline of  $CT_{max}$  with elevation, whereas  $CT_{min}$  decreases in response to adiabatic cooling of night temperatures. The prediction of narrower thermal breadth in tropical species has been employed as a mechanistic explanation of reduced dispersal capacity (Janzen 1967, Sheldon et al. 2018), increased rates of speciation (Ricklefs 2006, Martin et al. 2009, Polato et al. 2018), smaller elevational range sizes (Sheldon et al. 2011, Cadena et al. 2012, Gill et al. 2016) and predicted uplift dispersal of tropical lowland ectotherms to track thermal climatic niche due to increased heating (Colwell et al. 2008, Chen et al. 2011). However, the CVH should not be exclusive to large-scale (latitudinal and/or elevational) gradients as recent studies suggest that local temperature variation is also important (Mammola et al. 2019, Pintanel et al. 2019), with the existence of thermal specialist and generalist species at both extremes of the microenvironmental gradient (e.g. forest-restricted species and open-exploiter species, Pintanel et al. 2019; ground restricted species and above-ground dwelling ant species, Kaspari et al. 2015, Baudier et al. 2018).

A tenet in macrophysiological research (Chown and Gaston 2016) is that climatic gradients promote less geographic variation in upper than lower thermal limits ('heat-invariant hypothesis' or 'Brett's hypothesis'; Brett 1956, Araújo et al. 2013, Bozinovic et al. 2014, Chown and Gaston 2016). This rule is well documented in latitudinal gradients (Brett 1956, Addo-Bediako et al. 2000, Cruz et al. 2005, Sunday et al. 2011, Gutiérrez-Pesquera et al. 2016) and also, although less reported, in elevational gradients (Gaston and Chown 1999, Muñoz et al. 2014, Sunday et al. 2014, Pintanel et al. 2019).  $CT_{max}$  or heat tolerance has been considered a more static evolutionary trait than  $CT_{min}$  or cold tolerance in ectotherms (Araújo et al. 2013, Bozinovic et al. 2014, Muñoz et al. 2014), which may hamper the evolutionary potential of populations to respond to novel selective pressures through climate warming. Thermal tolerance limits are driven by natural selection through organismal exposure to extreme temperatures ('climate extremes hypothesis'; Bozinovic et al. 2011, Buckley and Huey 2016). Thus, given that minimum temperatures drop faster than maximum temperatures with elevation, we would expect

that cold tolerances will change faster than heat tolerances with elevation. At local scales, thermal variation is generally driven by maximum diurnal temperatures across microhabitats (Muñoz and Bodensteiner 2019) and thus we expect open habitat exploiters to present higher heat tolerances than forest restricted species (Pintanel et al. 2019) or subterranean species (Baudier et al. 2018), whereas their cold tolerance will not differ across habitats (Kaspari et al. 2015, Baudier et al. 2018, Pintanel et al. 2019). Accordingly, we should expect that thermal breadth will increase with elevation and in more thermally variable habitats, such as open temporary ponds, in contrast to thermally stable habitats, such as streams.

Amphibian tadpoles are an excellent group with which to examine the climate variability hypothesis at local and large geographic scales. They inhabit a wide variety of freshwater habitats, from streams to temporary ponds, across a broad elevational range in the Andes. Amphibians in Ecuador show an extraordinary species richness and diversity (629 species, 281 endemics, in a range of at 250 000 km<sup>2</sup>; Ron et al. 2020), which might have been promoted by the geological and climatic complexity of the region (Hoorn et al. 2010, Hazzi et al. 2018). Freshwater temperatures show similar variation to air temperatures. However, given the higher heat capacity and conductivity of water, freshwater temperatures are more stable than air temperatures (Spotila et al. 1992). This thermal homogeneity in freshwater habitats versus terrestrial habitats can limit tadpoles' thermoregulation and its potential to buffer from warming (Feder and Hofmann 1999). Therefore, tadpoles should be exposed to strong thermal stress which, in turn, may have prompted thermal evolution.

Here, we test the climate variability hypothesis in anuran tadpoles in an elevational gradient and across habitats. We measured critical thermal limits ( $CT_{max}$  and  $CT_{min}$ ) and in situ environmental temperatures of 75 species of amphibian tadpoles distributed along a ~3500 m elevational range. We first tested the assumption that minimum temperatures ( $t_{min}$ ) and cold tolerances ( $CT_{min}$ ) are more variable across elevation but less variable across habitats, compared to maximum temperatures ( $t_{max}$ ) and heat tolerance ( $CT_{max}$ ) respectively. Given that  $CT_{max}$  is considered to be evolutionary more static trait than  $CT_{min}$  (Hoffmann et al. 2013), we then tested if the evolutionary rates for  $CT_{min}$  are higher than for  $CT_{max}$ . Then, we tested the climate variability hypothesis, namely if there is a positive relationship between environmental temperature variation and thermal breadth ( $TB = CT_{max} - CT_{min}$ ). We expected that if temperature variation is broader at higher elevations and in open ponds in contrast to streams and lowlands, tadpoles' thermal breadth should also be broader in those places. Regarding spatial variation in thermal risk, given that  $CT_{max}$  did not vary with elevation, and environmental temperatures peak at open habitats, we expected that species from lowlands and ponds, in contrast to those from highlands and streams, will be exposed to higher vulnerability to heating risk.

## Material and methods

### Study sites

Tadpole sampling was carried out between June 2014 and April 2016 in Ecuador (between latitudes 1.2°N–4.8°S and elevations 23–3630 m a.s.l.). Larval amphibians occupy nearly all available aquatic environments, from open areas near the coast to streams and high elevation pasture-like páramos (Supporting information). We analysed thermal tolerance limits of 75 species of tadpoles (Fig. 1), which represents 22% of the total number of free-larvae amphibian species of Ecuador (Ron et al. 2020). Tadpoles or egg clutches were collected from their natural habitat and transported to the experimental facilities in the Pontificia Universidad Católica del Ecuador except for six species that were obtained through captive breeding from the ‘Balsa de los Sapos’ conservation initiative at the same university (Supporting information). In these cases, we assumed that the results did not differ from

those obtained in natural populations given the limited number of generations spent in captivity in the laboratory (F1 or F2) (Pintanel et al. 2020). Furthermore, the exclusion of those species or including captivity as a factor from the analyses, gave similar results (Supporting information). Finally, for each species, we also compiled a distributional range on elevation database of the Andean species studied here (Fig. 1 and Supporting information).

### Thermal data

We used Hobo Pendant temperature dataloggers (HOBO UA-002-64; Bourne, MA, USA) to obtain a continuous record of water temperature at each sampling site, placed at the bottom of each freshwater habitat. This is the coolest region of the habitat which could be selected by tadpoles to avoid peak temperatures (Duarte et al. 2012). Temperature was recorded every 15 min. We analysed maximum ( $t_{max}$ ), mean ( $t_{mean}$ ), minimum ( $t_{min}$ ) and mean daily range (dr)

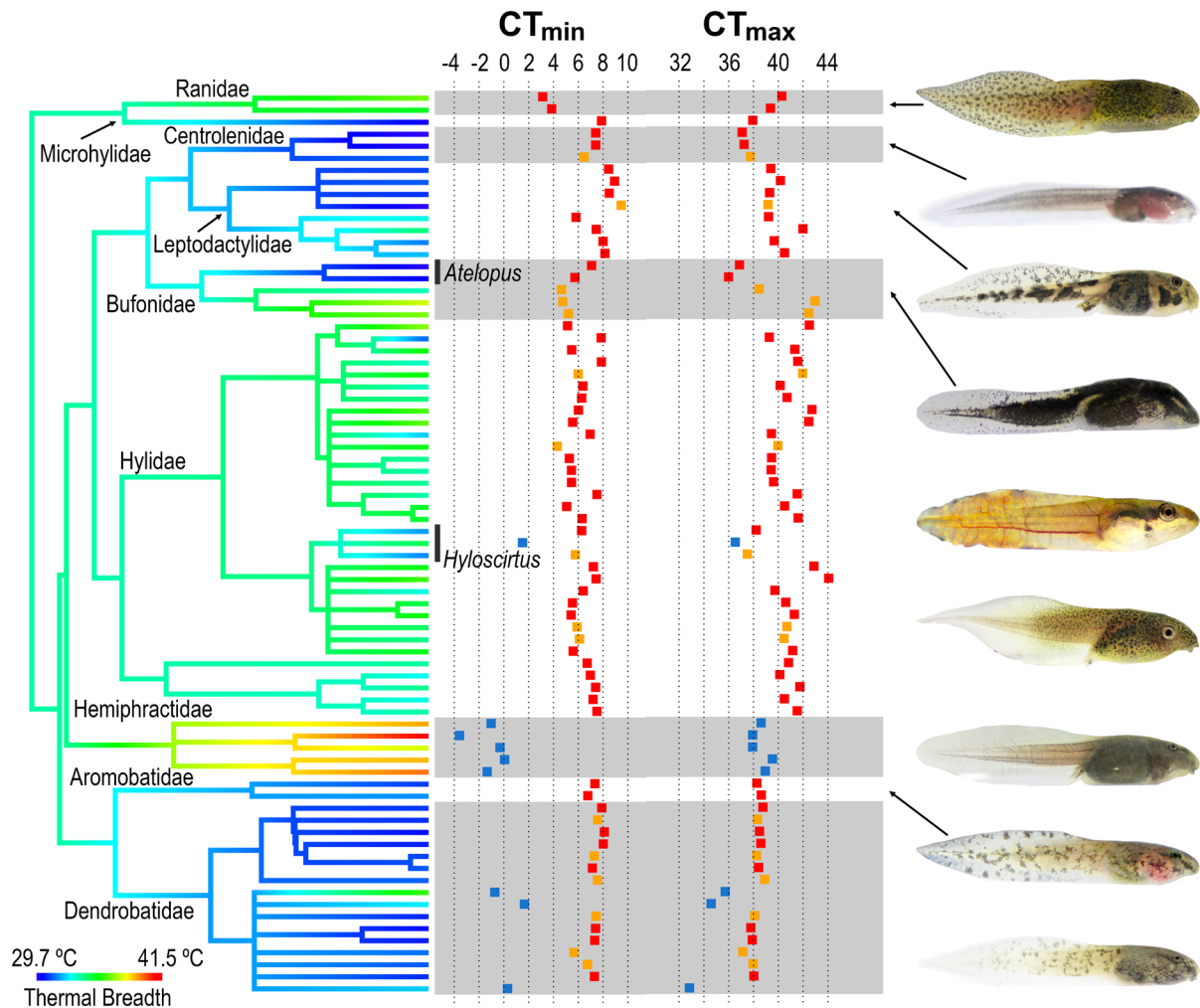


Figure 1. Evolution of thermal tolerance breadths and thermal limits in Ecuadorian tadpoles. Warmer colors in the phylogenetic tree indicate broader thermal breadths within each species. For visualization purposes, we further assigned a color (red squares < 1000 m a.s.l.; orange squares: 1000–2000 m a.s.l.; blue squares > 2000 m a.s.l.) according to their mid-elevational point of the distribution.

temperatures from each aquatic habitat. We also calculated the absolute thermal range (ar) as the difference between  $t_{\max}$  and  $t_{\min}$ .

We initially sorted each sampling site into four different habitat categories according to possible differences in their thermal characteristics (stream, permanent ponds, shaded temporal ponds and open ponds). Some species of tadpoles can be found in different aquatic environments making classification difficult and subjective. Thus, we decided to use the most parsimonious option, including only the two most restrictive levels of habitat type, which are stream-restricted species (thermal specialists) and the rest of species, referred to as pond-dwelling species (thermal generalists). We employed ANCOVA to determine the relationship of thermal data with habitat and elevation using the basic R package ver. 4.1.1 (<[www.r-project.org](http://www.r-project.org)>). The number of monitoring days ranged from 2 to 456 days (Supporting information) due to logistics and unexpected events. Therefore, we repeated the analyses using the mean value of daily maximum temperatures (mean  $t_{\max}$ ), daily minimum temperatures (mean  $t_{\min}$ ) and absolute range of temperatures (mean  $ar = \text{mean } t_{\max} - \text{mean } t_{\min}$ ) to exclude extreme peak temperature events, which gave similar results (Supporting information).

### Estimates of thermal tolerance

We estimated thermal limits for 1890 specimens from 75 species (Supporting information) using the same methodology as Pintanel et al. (2021). Before the experiments, larvae were acclimated at 20°C using a portable thermal bath (patent license ES1135983U; Madrid, Spain) with a photoperiod of 12L:12D for at least three days to stabilize thermal tolerance estimates of field sampled individuals living at diverse environmental temperatures (Hutchison 1961, Brattstrom 1968). During that time tadpoles were maintained in containers at low densities and fed with rabbit chow ad libitum. During the assays, each tadpole was placed individually in a separate container filled with 100 ml of dechlorinated tap water immersed in the thermal bath at a starting temperature of 20°C. We used Hutchison's dynamic method (Lutterschmidt and Hutchison 1997b) that consists of exposing each animal to a constant heating/cooling rate ( $\Delta T = 0.25^\circ\text{C min}^{-1}$ ) using a thermal bath (HUBER K15-cc-NR Kältemaschinenbau AG, Germany) until an endpoint is attained. The end-point was signalled for both thermal limits as the point at which the tadpole mobility ceased completely and failed to respond to external stimuli. Whenever possible, we used a minimum sample of 15 individual tadpole replicates per species and thermal tolerance limit. Once the endpoint was attained, we measured water temperature with a quick-recording thermometer (Miller and Weber; Ridgewood, NY, USA; 0.1°C accuracy) placed beside the tadpole (Lutterschmidt and Hutchison 1997a, Gutiérrez-Pesquera et al. 2016). Each tadpole was weighed and staged (Gosner 1960) immediately after the test. Only individuals between stages 25 and 38 were tested. Previous studies demonstrate that at later stages, when the metamorphic climax is approaching, tadpoles tend

to lower their thermal limits (Floyd 1983). Thermal breadth (TB) of each species was calculated as the difference between  $CT_{\max}$  and  $CT_{\min}$ .

Although we only included a single population for each species, we assumed that response variation among species is larger than variation within species (Klok and Chown 2003, Gutiérrez-Pesquera et al. 2016, but see Herrando-Pérez et al. 2019). Finally, to determine in what regions and specifically what species are exposed to extreme thermal conditions and, therefore, should be exposed to stronger thermal selection, we evaluated the risk of each species to suffer thermal stress. We estimated warming tolerance (wt) as the difference between  $CT_{\max}$  and  $t_{\max}$  (Duarte et al. 2012) and cooling tolerance (ct) as the difference between  $t_{\min}$  and  $CT_{\min}$  (Gutiérrez-Pesquera et al. 2016).

### Physiological and environmental variation with elevation and habitats

For the phylogenetic analyses, we used the package *ape* in R (Paradis et al. 2004) to reconstruct a consensus phylogenetic tree, using the most comprehensive and recent study on amphibians (Jetz and Pyron 2018). We used the position of a known sister taxon for four species not included in this phylogeny. Given that the phylogenetic tree contained polytomies, we used the function *multi2di* in *ape* that allows to make dichotomic a phylogenetic tree with multichotomies. We used a phylogenetic generalized least squares (PGLS) approach to determine the association between thermal physiology and vulnerability variables ( $CT_{\max}$ ,  $CT_{\min}$ , TB, wt and ct) with ecological variables, elevation (midpoint of species elevation and elevation of the population analysed) and habitat type (streams and ponds), accounting for phylogeny through maximum likelihood estimations of Pagel's  $\lambda$  using the R-package *caper* (Orme 2013). We employed a multiregression approach and selected the best models according to their Akaike information criterion ( $\Delta AIC < 7$ ). Also, we used simple PGLS regressions to examine the relationship between the physiological variables ( $CT_{\max}$ ,  $CT_{\min}$  and TB) and the environmental temperature ( $t_{\max}$ ,  $t_{\min}$ ,  $t_{\text{mean}}$  dr). Tadpole mass was not included in our analysis because, although it was significant for simple regression with  $CT_{\min}$ , it did not explain significant variation or change the results when included in the models (Supporting information).

### Evolutionary rates of thermal tolerance change

The heat-invariant hypothesis suggests that cold tolerance is more variable than heat tolerance (Araújo et al. 2013, Bozinovic et al. 2014), suggesting that  $CT_{\max}$  is static in comparison to  $CT_{\min}$  (Muñoz et al. 2014). Thus, we evaluated if it resulted from differential rates of evolution in thermal tolerance limits. We compared the evolutionary rates among  $CT_{\max}$  and  $CT_{\min}$  using the method and code of Adams (2013), incorporating the standard errors into our estimations to account for intraspecific variability.

## Results

### Thermal limits and habitat variation

We found that the temperature of Andean aquatic environments where amphibians breed (ponds and streams) decreases linearly with elevation. Furthermore, within an elevational band, ponds exhibited greater maximum temperature variation and overlap across elevations than streams but exhibited similar minimum temperatures (Fig. 2 and Supporting information). Both  $CT_{max}$  and  $CT_{min}$  correlated with maximum and minimum water temperatures respectively ( $\beta = 0.097 \pm 0.028$ ,  $t_{67} = 4.419$ ,  $p < 0.001$ ,  $\lambda = 1$  and  $\beta = 0.211 \pm 0.049$ ,

$t_{67} = 4.267$ ;  $p < 0.001$ ,  $\lambda = 1$ ). As predicted, both thermal limits decreased with elevation, but habitat was a significant predictor for  $CT_{max}$  only. Specifically, stream-restricted species exhibited lower  $CT_{max}$  than their pond-dwelling conspecifics (Fig. 3 and Supporting information). Accordingly, stream-restricted species exhibited narrower thermal breadths (TB) than pond-dwelling species ( $\beta = -2.148 \pm 0.671$ ,  $t_{72} = -3.202$ ,  $p = 0.002$ ,  $\lambda = 0.846$ ) and, furthermore, TB increased with elevation ( $\beta = 0.0015 \pm 0.0004$ ,  $t_{72} = 4.274$ ,  $p < 0.001$ ,  $\lambda = 0.846$ ) as expected by the climatic variability hypothesis (Table 4). Finally,  $CT_{min}$  showed a higher rate of evolutionary change than  $CT_{max}$  ( $\sigma^2_{CT_{max}} = 3.701$ ,  $\sigma^2_{CT_{min}} = 7.494$ ,  $LRT = 9.141$ ,  $p = 0.0016$ ).

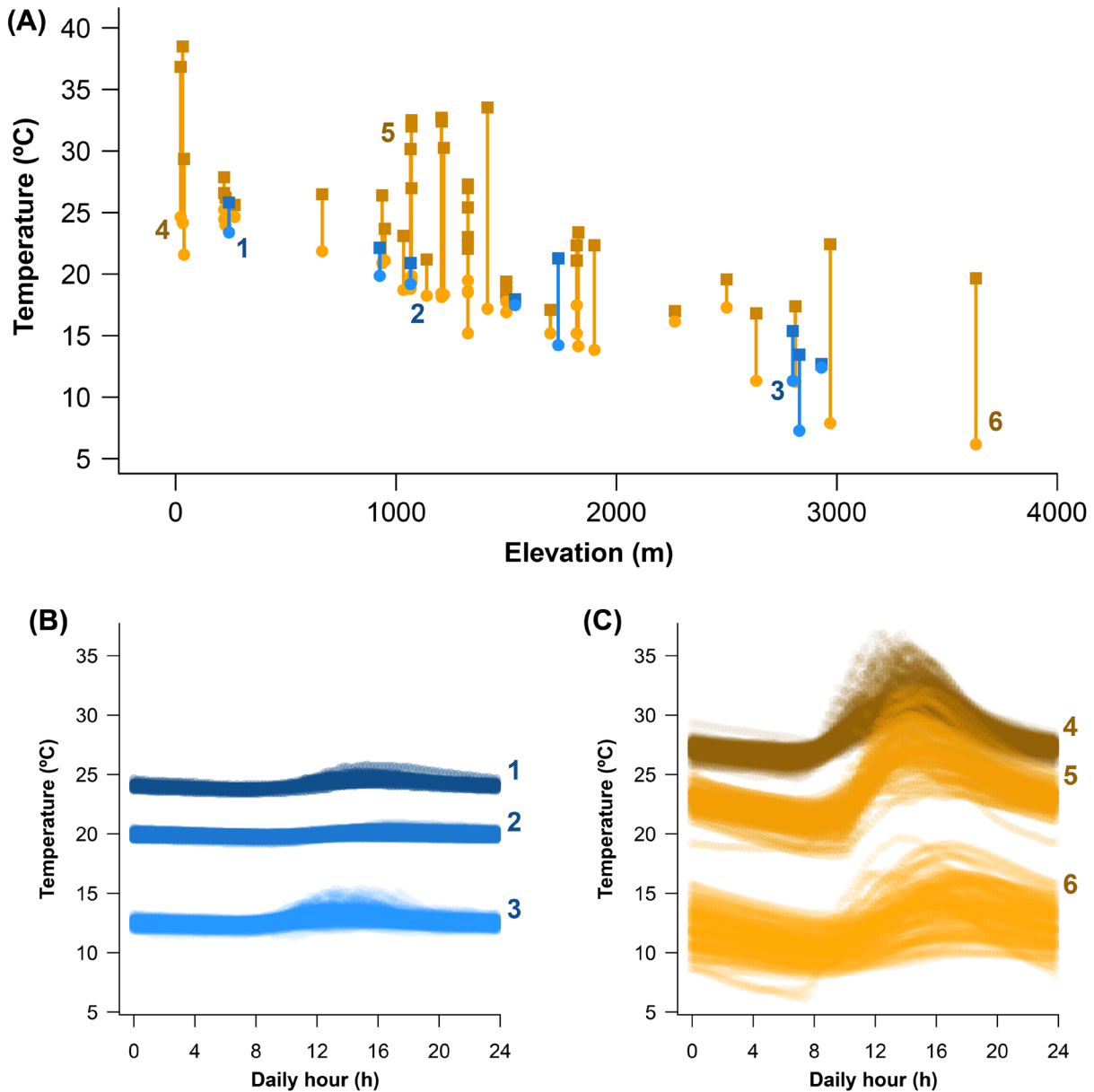


Figure 2. Environmental thermal variation throughout a tropical elevational range and across amphibian aquatic habitats in Ecuador. (A) Absolute maximum (squares) and minimum (circles) temperatures at ponds (orange) and streams (blue). (B and C) Daily temperature profiles at three different elevations in (B) streams and (C) ponds (see (A) for number assignment).

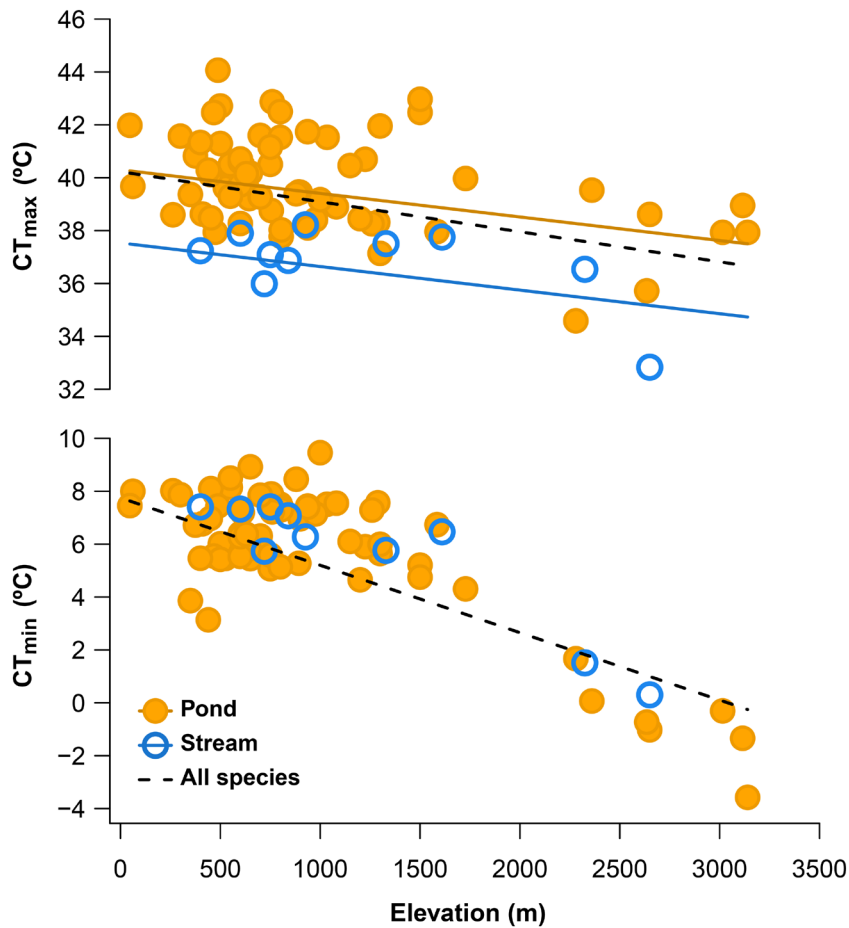


Figure 3. Upper and lower thermal limits ( $CT_{max}$  and  $CT_{min}$ ) variation in tadpoles from Ecuador with elevation midpoint of species distribution. 'Pond' (orange) corresponds to pond-dwelling species and 'Stream' (blue) to stream-restricted species. The dashed line corresponds to the analysis not accounting for habitat. All regression lines reflect the significant phylogenetic corrections without interaction (see Supporting information for the models).

The analyses of warming and cooling tolerance showed a positive and a negative correlation with elevation respectively ( $\beta = 0.0032 \pm 0.0008$ ,  $t_{67} = 4.328$ ,  $p < 0.001$ ,  $\lambda = 0.35$  and  $\beta = -0.0017 \pm 0.0005$ ,  $t_{67} = -3.593$ ,  $p < 0.001$ ,  $\lambda = 0$  respectively; Fig. 5, Supporting information). Thus, heat stress increases at lower elevations (i.e. lower wt) while cold stress increases at higher elevations (i.e. lower ct). Furthermore, pond-dwelling species show lower warming tolerances than stream-restricted species ( $\beta = 2.797 \pm 1.41$ ,  $t_{67} = 1.985$ ,  $p = 0.05$ ,  $\lambda = 0.323$ ; Fig. 5, Supporting information). Nonetheless, none of the highland species appears to be at risk of suffering from acute cold stress under current conditions (ct > 6.98°C), yet at least five low elevation species turned out to be highly vulnerable to suffer heat impacts (wt < 5°C).

## Discussion

Our results indicate that highland and pond-dwelling tadpole species have broader thermal breadths than lowland and stream-restricted species, supporting the climate

variability hypothesis at both large spatial scales (Stevens 1989, 1992, Sheldon and Tewksbury 2014, Shah et al. 2017, Pintanel et al. 2019) and local microenvironmental scales (Kaspari et al. 2015, Baudier et al. 2018, Pintanel et al. 2019, 2021). At a large spatial scale (i.e. elevation), broader thermal breadths with elevation are related to a greater decrease in  $CT_{min}$  in contrast to  $CT_{max}$ . Even though tadpole communities are exposed to maximum peak temperatures differing by 25°C (38.5°C at a 32 m a.s.l. lowland pond to 13.5°C in a stream at 2830 m a.s.l.),  $CT_{max}$  values were remarkably similar across elevations. For example, *Epipedobates machalilla*, which inhabits temporal shadowed ponds near sea level, presents roughly similar  $CT_{max}$  to *Gastrotheca pseustes*, the species registered at the highest elevation in this study (38.60 and 37.93°C; respectively, Fig. 1). In contrast, for these same species,  $CT_{min}$  values differed by 11.6°C. Similar geographical shifts in thermal limits have also been reported in large latitudinal gradients in different ectotherms (Addo-Bediako et al. 2000, Sunday et al. 2011). However, compared to latitudinal gradients, elevational clines integrate substantial climatic variation over much shorter geographical distances. The Andean uplifts that date from the Miocene

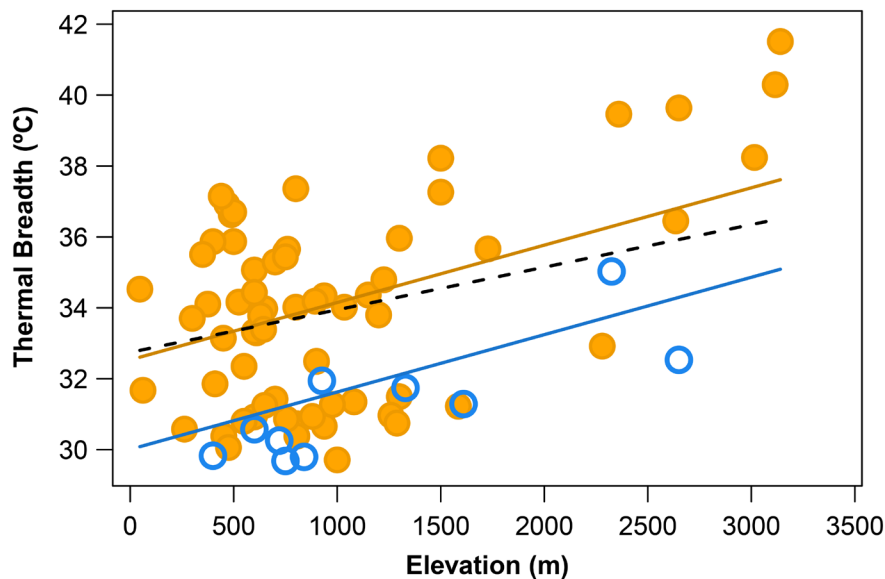


Figure 4. Thermal breadth ( $TB = CT_{max} - CT_{min}$ ) variation in tadpoles from Ecuador with elevation midpoint of species distribution. ‘Pond’ (orange) corresponds to pond-dwelling species and ‘Stream’ (blue) to stream-restricted species. The dashed line corresponds to the analysis not accounting for habitat. All regression lines reflect the significant phylogenetic corrections without interaction (Supporting information).

(Hoorn et al. 2010) and last glaciations of the Pleistocene (Torres et al. 2013, Flantua et al. 2014) could be responsible for the large decrease of  $CT_{min}$  in upland species (Fig. 3). In fact, vegetation currently found in high elevation Andes was not established until the last glaciations (van der Hammen 1974, Torres et al. 2013, Flantua et al. 2014). Specifically, the locations where high elevation ectotherms currently inhabit were proportionally much colder than lower elevations during the last glaciations ( $6-7^{\circ}\text{C}$  and  $3^{\circ}\text{C}$  respectively; van der Hammen 1974), explaining the current disparity found between environmental temperatures and cold tolerance. Therefore, the extreme cold tolerance found today may be a shadow of past adaptations.

Acknowledging the factors that promoted or constrained thermal evolution may improve our forecast to climate warming. Our analyses highlight that habitats impose local variability in maximum peak temperature and heat tolerance, not influencing minimum temperature or cold tolerance. This similar contrasting pattern appears in other terrestrial ectotherms (Frishkoff et al. 2015, Kaspari et al. 2015, Nowakowski et al. 2017, Pintanel et al. 2019, Anderson et al. 2022, Leahy et al. 2022). The divergence on the variation of extreme environmental temperatures (i.e.  $t_{max}$  and  $t_{min}$ ) should pose different selective pressures on critical thermal limits. If maximum temperatures of lotic habitats (i.e. streams) are lower and more constant than most lentic habitats (e.g. ponds, puddles), it is not surprising that species that are exclusive stream breeders (e.g. Centrolenidae family, *Atelopus* and *Hyloscirtus* genus; Fig. 1 and 3) show lower  $CT_{max}$  than species that can exploit temporal ponds (Duarte et al. 2012, Gutiérrez-Pesquera et al. 2016, Pintanel et al. 2021). Species might select suitable habitats within an elevational band

instead of being exposed to maximum extreme temperatures and, thus, habitat selection might have buffered  $CT_{max}$  evolution (Muñoz et al. 2014, Muñoz and Bodensteiner 2019). This, ultimately, may determine a pattern of physiological niche conservatism in upper thermal limits through elevational gradients. If habitat is responsible for heat tolerance stasis, addressing the possible effects of habitat conversion is crucial for future research on vulnerability to climate warming (Kearney 2013, Frishkoff et al. 2016). Contrary to maximum temperatures, minimum temperatures do not vary across habitats. In fact, most  $t_{min}$  variation is explained by elevation alone (89.4%; Supporting information). This implies that exposure to cold extremes at higher elevations cannot be ameliorated by thermoregulation (Muñoz et al. 2014, 2016, Gill et al. 2016). Therefore, if ectotherms cannot buffer extreme minimum temperatures, it leaves no other option for the population than to adapt physiologically, which might explain why  $CT_{min}$  is so highly variable through elevation and, also, has a faster rate of evolution than  $CT_{max}$  (Araújo et al. 2013, Muñoz and Bodensteiner 2019, but see Pintanel et al. 2019). Plasticity may extend thermal tolerance limits and our observed pattern could change if acclimation scope between species varied spatially. However, recent studies have revealed that thermal acclimation scope is generally of low magnitude (Clusella-Trullas and Chown 2014, van Heerwaarden et al. 2016) exhibiting low environmental signal (Schou et al. 2017).

Although none of the analysed species seem to be exposed to maximum temperatures higher than their  $CT_{max}$  (Fig. 4), our results suggest that some populations from low elevations will suffer short term thermal stress due to exposure to hotter environments (Deutsch et al. 2008, Tewksbury et al. 2008)

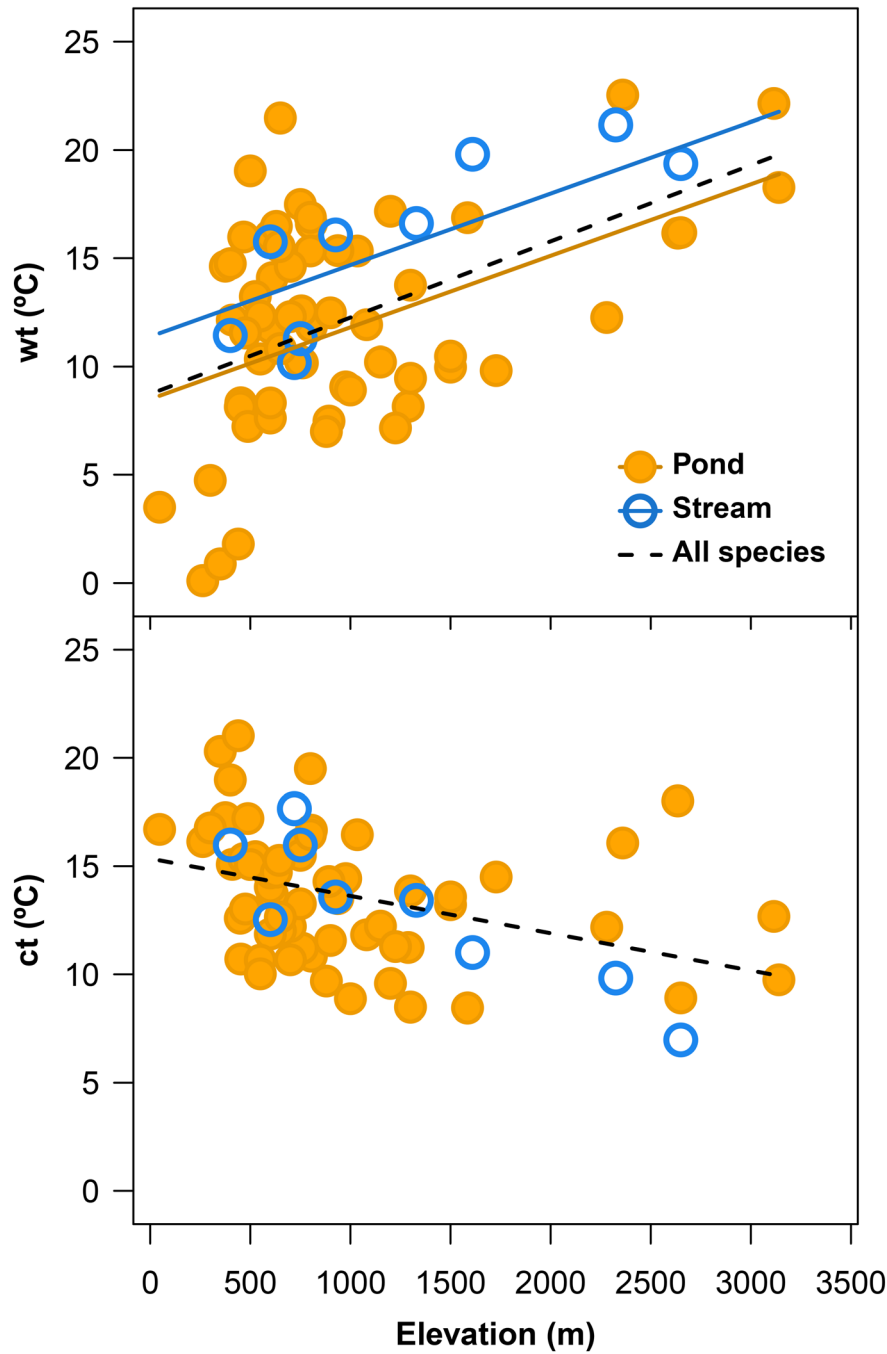


Figure 5. Warming (wt) and cooling (ct) tolerance variation in tadpoles with mid-elevation of the species' distribution using PGLS. 'Pond' (orange) corresponds to pond-dwelling species and 'Stream' (blue) to stream-restricted species. The dashed line corresponds to the analysis not accounting for habitat. All regression lines reflect the significant phylogenetic corrections without interaction.

which may force an uphill retreat (Colwell et al. 2008) or a move to colder habitats within the same elevational band. In contrast, upland species showed wide warming tolerances. Elevational species turnover has been argued to result from interactions between biotic and abiotic factors, which may play a primary role in distribution to lower and higher elevations respectively (Schemske et al. 2009, Jankowski et al. 2013, Wisz et al. 2013). For instance, some species may

have been excluded from particular elevations by competitively superior species, while the elevational range of the latter species may be limited by climatic factors (Paquette and Hargreaves 2021). Although none of the upland species seem to be exposed to maximum temperatures closer to their heat tolerance, the rise of temperatures may increase non-lethal chronic stress (Sinclair et al. 2016). In addition, the increase of temperatures can also alter the intensity of biotic



interactions (Pintanel et al. 2021), with the eventual upward migration of lowland species, threatening their survival in the long/medium term. Effects of global warming may manifest more strongly than expected in stream- and forest-restricted species. Both stream and forest habitats show lower and less variable temperatures than ponds and open habitats respectively. Also, the species found in the former habitats are usually less heat thermotolerant than the ones found in the latter (Gutiérrez-Pesquera et al. 2016, Pintanel et al. 2019). Thus, we might have overestimated warming tolerance in stream-restricted species, since the increase in stream intermittency, fragmentation and habitat destruction with global change may further exacerbate the effects of warming.

Here, we have provided a new perspective on the climatic variability hypothesis by testing it at both large (elevation) and small (microenvironmental) spatial scales, within a specific lineage of vertebrates and in the tropics, a region considered climatically stable. Overall, we show that thermal generalists usually occur in the most variable environments within each climatic gradient; i.e. open ponds and highlands. In contrast, stable thermal environments such as lowland streams promote more thermally specialized organisms. Finally, we have shown that microenvironments strongly shape heat tolerance, reiterating the importance of local spatial heterogeneity for the conservation of biodiversity.

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*Ethical statement* – Ministerio del Ambiente of Ecuador provided the permits to conduct this investigation (003-15/012-015/002-16 IC-FAU-DNB/MA). The CMAOT of Junta de Andalucía granted the permits for conducting the experiments to Estación Biológica de Doñana (Ref. 12\_44-Tejedo). Dirección de Investigación from Pontificia Universidad Católica del Ecuador conditions the project's acceptance and evaluates that the protocols follow bioethical guidelines.

*Conflicts of interest* – We declare no conflicts of interest.

## Author contributions

**Pol Pintanel:** Conceptualization (equal); Data curation (equal); Formal analysis (lead); Investigation (lead);

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## Data availability statement

Data are available from the Dryad Digital Repository: <<https://doi.org/10.5061/dryad.3j9kd51m3>> (Pintanel et al. 2022).

## Supporting information

The supporting information associated with this article is available from the online version.

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