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Greater plasticity in CTmax with increased climate variability among populations of tailed frogs

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Temporally variable climates are expected to drive the evolution of thermal physiological traits that enable performance across a wider range of temperatures (i.e. climate variability hypothesis, CVH). Spatial thermal variability, however, may mediate this relationship by providing ectotherms with the opportunity to behaviourally select preferred temperatures (i.e. the Bogert effect). These antagonistic forces on thermal physiological traits may explain the mixed support for the CVH within species despite strong support among species at larger geographical scales. Here, we test the CVH as it relates to plasticity in physiological upper thermal limits (critical thermal maximum—CTmax) among populations of coastal tailed frogs (*Ascaphus truei*). We targeted populations that inhabit spatially homogeneous environments, reducing the potentially confounding effects of behavioural thermoregulation. We found that populations experiencing greater temporal thermal variability exhibited greater plasticity in CTmax, supporting the CVH. Interestingly, we identified only one site with spatial temperature variability and tadpoles from this site demonstrated greater plasticity than expected, suggesting the opportunity for behavioural thermoregulation can reduce support for the CVH. Overall, our results demonstrate one role of climate variability in shaping thermal plasticity among populations and provide a baseline understanding of the impact of the CVH in spatially homogeneous thermal landscapes.

1. Introduction

Climate change is increasing global temperatures as well as the frequency and severity of extreme climatic events, altering both the mean temperatures and temperature variability experienced by organisms [1]. The consequences of these changes may be magnified for ectotherms because external temperatures shape their physiology [2,3]. Organisms with the capacity for active, reversible physiological changes as a response to thermal cues [4], hereafter referred to as thermal plasticity, may be partly buffered from these consequences [5–9] as thermal plasticity can facilitate dispersal through different environments and/or allow organisms to persist *in situ* through thermal changes [10]. Consequently, thermal plasticity, or the lack of it, is an important component of species' adaptive capacity and vulnerability to climate change [10–12]. Understanding the evolution of thermal plasticity and how it varies across landscapes thus provides important insight into the coping capacity of organisms in the face of rapid climate change.

The climate variability hypothesis (CVH) is a well-established hypothesis explaining patterns in thermal physiological traits across landscapes [13,14]. The CVH postulates that ectothermic species inhabiting climatically variable environments evolve thermal physiological traits that increase or permit performance along a wider range of temperatures [13–18]. Under the CVH, exposure to greater seasonal temperature variability is predicted to favour the evolution of greater thermal plasticity as a mechanism to cope with thermal fluctuations in the environment [13,14,19,20].

Despite garnering much support across broad geographical ranges and taxonomic groups (e.g. [7,21–26]), investigations of the CVH within species are less common and provide mixed support (e.g. [24,27–31]). Of the intraspecific studies, few test the CVH as a mechanism to explain variation in thermal plasticity among populations (e.g. [27–29,31]) and most lack the sampling power required to identify spatial patterns in this trait. Uncovering trends in thermal plasticity among populations is important for understanding relative coping capacity to warming temperatures across species ranges. This information could also be used to infer broader patterns relevant to prediction in other, unsampled species, informing conservation efforts, especially since quantifying plasticity is challenging in many species. If the CVH is well-supported among populations, then populations experiencing climatic variability would be predicted to have greater thermal plasticity and thus higher coping capacity to warming temperatures than populations experiencing stable climates.

A major challenge when testing the CVH—and a possible explanation for the mixed support of the CVH within ectothermic species—is accounting for the effects of spatial temperature variation within sites [32]. Various components of microhabitats, such as the level of canopy cover and the diversity of abiotic structures, can lead to spatial thermal variability [33,34] and provide the potential for behavioural thermoregulation [34–36]. Thermoregulatory behaviour allows organisms to avoid unfavourable temperatures, potentially lessening the strength of selection for increased thermal tolerance and thermal plasticity (i.e. the Bogert effect; see [37–44]). For example, daytime behavioural thermoregulation by *Anolis* lizards has been found to shield them from environmental maximum temperatures and explains reduced rates of evolution in upper thermal limits among *Anolis* species [45,46]. Despite its potential in shaping thermal physiological traits, studies testing the CVH rarely account for spatial temperature variability as a proxy for behavioural thermoregulation, and implicitly assume its effects are negligible relative to the effects of temporal thermal variability across broad geographical scales (e.g. tropical versus temperate habitats). However, at finer geographical scales, variation in temporal thermal variability may not be as pronounced among populations [47]. Thus, the homogenizing effects of spatial thermal variability on experienced body temperatures at this scale may have an outsized impact on the evolution of thermal physiological traits.

The potential for spatial thermal variability to mediate selection from temporal thermal variability via behavioural thermoregulation is not ubiquitous among organisms or habitats. Effective behavioural thermoregulation requires both vagile organisms that can sample the thermal environment as well as a spatial configuration of temperatures that confers a benefit to behaviourally thermoregulating relative to the associated costs [34–36,44]. In systems lacking one or both of these components, the effects of temporal variability in temperature may be fully experienced and therefore provide unique opportunities to more directly test the CVH [32].

Here, we considered the role of the CVH in shaping plasticity in a thermal tolerance trait among populations of coastal tailed frogs (*Ascaphus truei*). This species inhabits cold, fast-flowing headwater streams along the mountainous regions of the Pacific Northwest, USA and western Canada. The larval tadpole life-stage, which is the focus of this study, is often observed attached to stream substrates via a modified sucker mouth and is limited in vagility, moving an average of 1.1 m day⁻¹ [48]. Although variation in structural microhabitat within these streams can cause spatial variability in temperature [49–54], their fast flows may alternatively homogenize temperatures across space at a scale relevant to coastal tailed frogs. Indeed, previous research suggests the tadpoles of many populations of this species appear to experience spatially homogeneous temperatures, limiting the potential for behavioural thermoregulation [55]. While coastal tailed frogs may experience relatively low diurnal temperature variation [56], high seasonal/annual temperature variability may drive selection for increased thermal plasticity and coping capacity.

We specifically investigated the CVH as it relates to thermal plasticity in critical thermal maximum (CT_{max}), a measurement of heat tolerance, among coastal tailed frog populations. Although many studies have demonstrated relatively low levels of variation in CT_{max} among populations/species compared with other metrics of thermal performance (e.g. critical thermal minimum—CT_{min}, [57,58]) and low magnitudes of plasticity in CT_{max} among ectotherms [19,59,60], coastal tailed frog populations vary in both CT_{max} and plasticity in this trait [55,61]. Furthermore, CT_{max} is strongly related to mortality from thermal stress among populations of the closely related Rocky Mountain tailed frog (*Ascaphus montanus*), such that small differences in CT_{max} can greatly impact the probability of mortality [62]. We used previously described estimates of population-level plasticity in CT_{max} [61] and quantified population-level variability in temporal and spatial temperatures using a combination of in-stream temperature loggers and fine-scale (<1 m) measurements. In the absence of spatial variability in temperature, we expected to find a strong, positive relationship between annual stream temporal temperature variability and the magnitude of thermal plasticity in CT_{max} among populations, such that populations from highly temporally variable habitats exhibit greater plasticity in CT_{max}. This result would support the CVH and provide insight into its role in shaping plasticity in CT_{max} among populations that do not have the capacity to behaviourally avoid stressful temperatures.

2. Methods

(a) Plasticity in CTmax

To capture a wide range of variation in environmental temperatures, we sampled coastal tailed frog populations along elevation gradients. We collected tadpoles (developmental stages 26.5–45 [63]) from 10 stream reaches in Oregon (electronic supplementary material, figure S1) using hand-nets (~48 tadpoles per population). Due to the distance between sampled stream reaches and limited vagility of tadpoles, we hereafter referred to these stream reaches as populations. Tadpoles from each population were evenly and randomly split into one of two holding temperature treatments: 8°C and 15°C, representing a typically experienced temperature (8°C) and a temperature near the warmest temperature experienced by these populations. After 3 days [64] of holding tadpoles without food (i.e. ensuring similar nutritional states among tadpoles—empty guts), CTmax experiments were performed via dynamic temperature ramping [65] at a rate of 0.3°C min⁻¹. As experimental ramping rate impacts estimates of CTmax [66–69], we used the same ramping rate for all experiments and populations. CTmax experiments were either performed from a starting temperature of 8°C or 15°C to account for acute temperature effects on CTmax (i.e. passive changes in CTmax due to changes in biochemical rate reactions) [70–72]. Oxygen saturation was maintained throughout holding and experiments using water pumps and aerators. CTmax was considered the point at which a tadpole no longer responded to tactile stimuli. After CTmax was reached, tadpoles were moved to a tank with ~8°C water to recover (i.e. defined as swimming and responding to tactile stimuli) to ensure that lethal temperatures were not reached. Following recovery, all tadpoles were euthanized using a 20% benzocaine solution, photographed for length measurements, fixed in 10% formalin and stored in 70% ethanol. We measured tadpole length (tip of nose to tip of tail) using IMAGEJ software [73].

(b) Characterizing stream temperatures

We deployed two stream temperature loggers (Hobo 64K Pendant Water Temperature Data Loggers, Onset Computer Corporation, Bourne, MA, USA) within the sampled stream area for each sampled population. Temperature loggers were housed in PVC pipes with drilled holes to allow for water flow and secured to a nearby tree using plastic-coated metal cable. Pipes were secured to in-stream metal poles to ensure loggers were held near the streambed, which is the microhabitat that tadpoles occupy. We logged temperatures every 4 h for approximately 1 year. Temperature data were reviewed for quality control [56], and used to calculate the following metrics (see electronic supplementary material, table S1 for more details): annual temperature range (the absolute maximum temperature – the absolute minimum temperature), range of mean daily temperatures (annual maximum of the average daily temperatures – the annual minimum of the average daily temperatures), absolute maximum temperature (the highest temperature experienced) and mean of the ten warmest days (average of the highest 10 daily maximum temperatures). As these metrics were highly correlated (Pearson's correlation coefficient ≥ 0.87 , $p < 0.01$; electronic supplementary material, table S2), we used annual temperature range for subsequent analyses.

We measured spatial variability in temperature for all populations but one (South Fork Steelhead Creek) due to logistical constraints. The full protocol for these measurements can be found in a previous paper [55]. Briefly, we measured streambed temperatures at ~100 points in space within the sampled stream area (~100 m, i.e. stream reach) in the late morning to afternoon. We measured streambed temperatures because tadpoles are primarily found there and because the streams sampled were relatively shallow (0.08–0.29 m), reducing the potential for vertical temperature stratification given their flow. Streambed temperatures were measured at fixed intervals with random offsets along horizontal transects of the stream (i.e. across stream width) at pre-identified transects along the length of the stream, targeting areas of *Ascaphus* habitat. We also opportunistically sampled stream seeps and confluences that may lead to thermal anomalies. Temperature measurements for each population were taken in 1 day (i.e. not over many days) during the summer months when temperatures are known to be highest (see [55]). We defined thermal refuges as temperatures with a 2°C difference from surrounding temperatures [74]. Using this definition as a guide, we identified spatially homogeneous populations as those having $<2^\circ\text{C}$ in the range of spatial temperatures measured.

(c) Statistical analyses

All statistical analyses were performed using R v. 4.2.2 [75] and use $\alpha = 0.05$ to denote significance. We used a mixed effects model to test the relationship between annual temperature range and plasticity in CTmax. In this model, CTmax was the response variable, the holding temperature and annual temperature range were both included as independent and interacting predictors. A significant interaction term would signify that the relationship between CTmax and holding temperature (i.e. plasticity) was dependent on annual temperature range. Based on previous results [61], we expected a significant positive effect of holding temperature, demonstrating beneficial acclimation (i.e. higher CTmax after being held in a warmer temperature). As such, a positive interaction term in our model would signify that plasticity increased with annual temperature range; a negative interaction term would demonstrate that plasticity was lower in populations that experience greater annual temperature ranges. We also included tadpole length and the experimental starting temperature as covariates to account for the effects of body size and acute temperature effects on CTmax [61], respectively. We included population as a random intercept to account for variation due to unaccounted population differences. We visually inspected the residuals and error variance of the model for assumptions of linearity and heteroscedasticity, respectively. We also checked for a lack of correlations among predictor variables before running the model and compared model performance against the null model (only a random population effect).

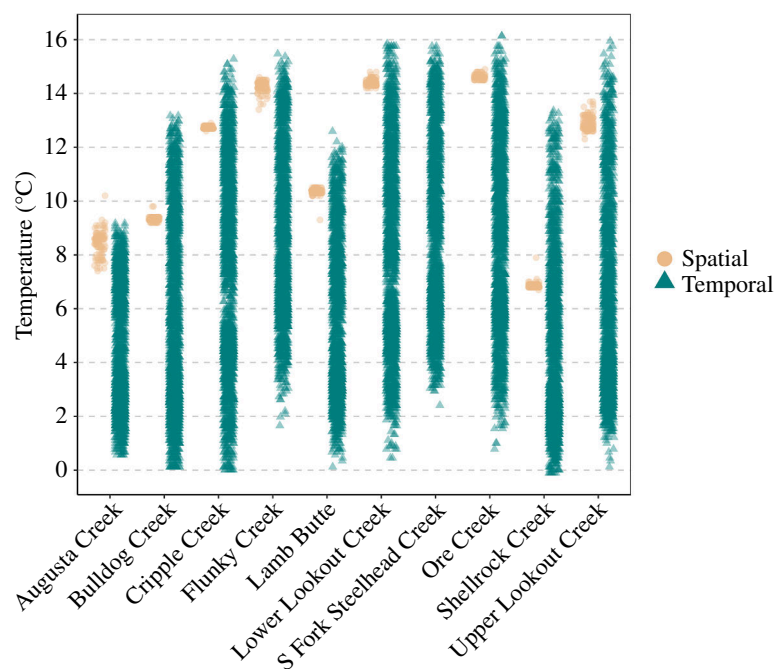


Figure 1. Temperature variation across time and space for each population. Temporal temperature points (green triangles) show all logged temperatures for ~1 year from our in-stream temperature loggers. Spatial temperature points (gold circles) show measured temperatures (~100 points) across space within the sampled stream area (<100 m) of each population except South Fork Steelhead Creek. Thus, temporal measurements represent temperatures over time at one spatial point in the stream, while spatial measurements represent temperatures over space at one point in time. Populations were considered spatially homogeneous in temperature if the range of temperatures was <2°C (grey dashed lines), thus Augusta Creek was the only population considered spatially thermally variable.

Table 1. Results from linear mixed effects model testing the effects of annual temperature range on plasticity in CTmax among populations with homogeneous spatial temperatures. Tadpole length and experimental starting temperature were included as covariates and population as a random effect. The significant positive interaction term between annual temperature range and holding temperature signifies that plasticity in CTmax increases with annual temperature range.

fixed effects	estimate	s.e.	d.f.	p-value
(intercept)	29.66	0.07	8.84	<0.0001
holding temperature	0.36	0.04	420.08	<0.0001
annual temperature range	−0.21	0.07	8.07	0.02
experimental starting temperature	0.07	0.04	420.12	0.05
tadpole length	−0.10	0.02	426.97	<0.0001
annual temperature range × holding temperature	0.12	0.04	420.08	0.0008
random effects	variance ± s.d.			
population	0.04 ± 0.20			
residual	0.14 ± 0.37			

3. Results

We sampled 485 tadpoles from 10 coastal tailed frog populations. Six tadpoles were removed from our analyses: five did not recover from CTmax experiments and one tadpole was missing a lateral photograph. Thus, the data presented represent 479 total tadpoles.

Stream temperature data revealed our sampled sites varied in annual temperature range (in-stream temperature loggers, min temperature range = 8.6°C, max temperature range = 15.8°C, var in temperature range = 4.07) but not in spatial temperature variability. All but one site (Augusta Creek) experienced less than a 2°C range in temperatures across space (figure 1). As we were interested in the effects of temporal variability on plasticity in CTmax in spatially homogeneous habitats, we excluded this site from our initial analysis.

Results from our mixed effects model showed that holding temperature did affect CTmax (i.e. there was a plastic response), as being held at 15°C resulted in significantly higher CTmax compared with being held at 8°C (table 1; figure 2). There was a slightly negative relationship between CTmax and annual temperature range, such that populations experiencing greater temperature variability had lower CTmax estimates. However, there was also a significant interaction between holding temperature and annual temperature range, such that plasticity in CTmax (i.e. the difference in CTmax in tadpoles held at different holding temperatures) increased with increasing stream annual temperature range (table 1; figure 2). The covariates

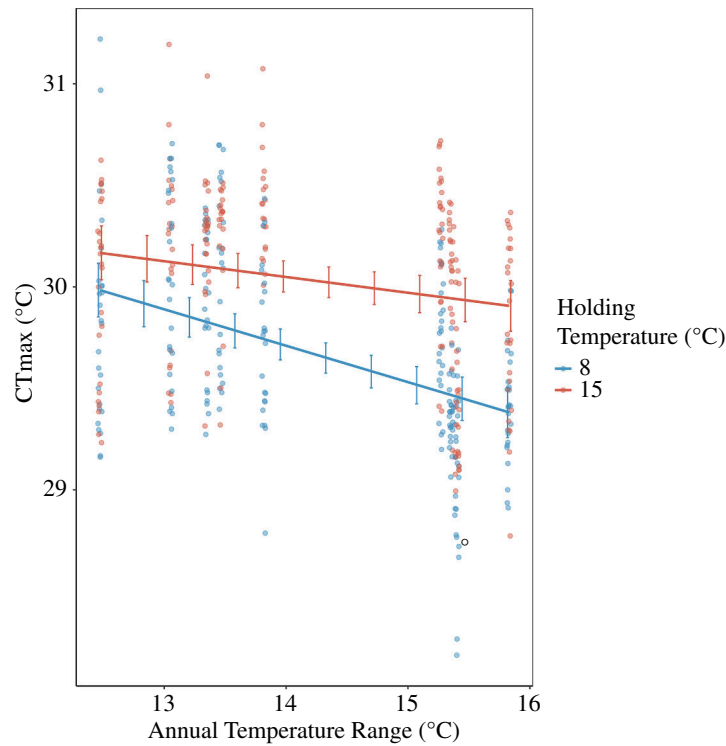


Figure 2. The effect of holding temperature (colours) on CTmax as a function of annual temperature range. Plasticity (i.e. the difference between holding temperatures) increases with annual temperature range ($p < 0.001$). Shown are raw CTmax data points with prediction slopes and confidence intervals estimated by the linear mixed effects model.

in our model also affected CTmax. Starting temperature had a weak positive effect on CTmax and total tadpole length had a significant negative relationship with CTmax, such that large tadpoles had lower CTmax estimates. When tadpoles close to or undergoing metamorphosis (Gosner stage ≥ 42) were excluded from the analyses, the results from the model were similar and the conclusions were not affected (electronic supplementary material, table S3). These model results were unaffected by excluding the population that was missing spatial temperature data (South Fork Steelhead Creek; electronic supplementary material, table S4).

To further explore how spatial variation might influence the relationship between the annual temperature range and the plasticity of CTmax, we included the data from Augusta Creek, and re-ran the model. The inclusion of this spatially variable population resulted in the loss of statistical significance for the interaction term and independent effect of annual temperature range (electronic supplementary material, table S5). The positive effect of holding temperature (i.e. demonstrating plasticity in CTmax), however, was still supported in this model.

4. Discussion

Moving towards a predictive understanding of how variation in organismal thermal tolerance could mediate responses to globally warming temperatures requires a strong understanding of the environmental conditions that ultimately shape these traits. The CVH has emerged as a leading hypothesis explaining variation in thermal physiological traits across variable environments, yet lacks consistent support among studies within species [27–29]. Here, we found support for the CVH among populations of coastal tailed frogs experiencing homogeneity in spatial temperatures. Specifically, we found that populations experiencing greater ranges in annual temperatures exhibited greater plasticity in CTmax. This pattern ultimately demonstrates the role of temporal temperature variability in shaping the evolution of plasticity in thermal physiological traits across populations.

We found opposing relationships between the independent effect of temporal temperature variability on CTmax (negative trend) and its interactive effect with holding temperature on CTmax (positive trend). This result shows that populations experiencing a wider range in annual stream temperatures exhibited both low CTmax estimates and high plasticity in CTmax, while those experiencing relatively stable stream temperatures exhibited high CTmax estimates and low plasticity in CTmax. Whether this relationship is driven by increased sensitivity in CTmax at 8°C or at 15°C requires further testing. Despite exhibiting greater plasticity in CTmax, populations inhabiting thermally variable streams did not achieve higher CTmax estimates than those from more thermally stable streams in the warm temperature treatment. Many studies have suggested a trade-off between these two traits such that populations/species with higher baseline CTmax have decreased plasticity in CTmax (tolerance–plasticity trade-off hypothesis [8,19,76,77]). Although we did not have the appropriate experimental design to directly test the tolerance–plasticity trade-off hypothesis [78], our results suggest support for it. Future studies investigating this potential trade-off and its underlying mechanisms (e.g. physiological constraints, plasticity threshold differences, reviewed in [78,79]) are necessary to fully understand the opposing trends between CTmax and plasticity in CTmax in this system.

Spatial variability is often considered a buffer against selection for increased thermal tolerance because it provides the potential for behavioural thermoregulation [42,44,45]. Most *A. truei* populations inhabit streams that lack spatial temperature variation, though we did find one site (Augusta Creek) which met the criteria for containing spatial thermal refuges. This site was an outlier both in terms of its relatively high spatial thermal variability and relatively low temporal thermal variability (figure 1). Following the CVH and the Bogert effect, we expected the Augusta Creek population to exhibit a low magnitude of plasticity in CT_{max}. However, this population exhibited a high magnitude of plasticity in CT_{max}, which weakened overall support for the CVH among populations of tailed frogs when included in our analyses (i.e. when not excluding spatially thermally variable populations). This result suggests that spatial thermal variability may reduce support for the CVH within species, though the evolutionary mechanisms underlying this prediction requires further investigation. Tailed frog tadpoles have been found to behaviourally thermoregulate [80], however, they also passively experience the spatial thermal environment when they lose suction on stream substrate and flow short distances downstream before reattaching [81]. Whether the role of spatial temperature variability on shaping plasticity in thermal tolerance is dependent on the type of movement tadpoles make in this system remains an open question.

Given the thermal landscape of these streams, our results make it challenging to predict the impact of increased plasticity in CT_{max} on vulnerability to warming temperatures for these populations. We quantified temporal thermal variability of temperatures experienced over the course of 1 year (i.e. annual scale), as this period of exposure reflects coastal tailed frog life-history and provides sufficient time to mount a plastic response. The metrics we used to describe thermal variability were strongly intercorrelated, suggesting that plasticity in CT_{max} may similarly be related to other aspects of the thermal landscape. For example, annual temperature variability of the streams in this study is largely dictated by maximum temperatures, as they all reach similar, near-freezing temperatures during the winter. As such, populations experiencing variable stream temperatures are also experiencing warmer maximum temperatures. Given the opposing relationships between CT_{max} and plasticity in CT_{max} described above, those populations have lower CT_{max} estimates (leading to a prediction of increased risk) but higher plasticity in CT_{max} (decreased risk). Overall, the relative physiological vulnerability of populations experiencing warm, variable stream temperatures will be dependent on whether their plastic response can be maintained over time and chronic warming and whether the magnitude of the plastic response scales with acclimation temperature.

5. Conclusions

Trends in organismal thermal physiological traits may be particularly useful for understanding sensitivity to warming temperatures [82–84], and broad geographical and taxonomic studies incorporating these traits have provided valuable insights into global trends of vulnerability to climate change [7,20,22–26,82,84–87]. Nonetheless, population-level inferences are critical for estimates of adaptive capacity—a major factor in assessing vulnerability to climate change [10–12]. By testing the CVH among populations of coastal tailed frogs, we uncovered a pattern of plasticity in CT_{max} across the landscape such that populations currently inhabiting temporally variable environments have greater plasticity in CT_{max}. Further intraspecific tests of the CVH present a research opportunity to test the generality of this result and confidently make predictions regarding relative vulnerability among populations within species for which physiological data are lacking. Our results contribute a baseline understanding of the potential impact of temporal thermal environments on the evolution of thermal physiological traits in the absence of spatial variability in temperatures.

Ethics. All experimental methods were approved by Colorado State University IACUC (16-6667AA) and University of Montana IACUC (024-17WLDDBS-042117). Collection was permitted by the Oregon Department of Fish and Wildlife (Permits 110-17, 114-18) and the Montana Fish, Wildlife and Parks (Permit 2017-060-W).

Data accessibility. Data and code are available on Dryad [88].

Supplementary material is available online [89].

Declaration of AI use. We have not used AI-assisted technologies in creating this article.

Authors' contributions. A.S.C.: conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, project administration, validation, visualization, writing—original draft, writing—review and editing; C.K.G.: conceptualization, funding acquisition, methodology, project administration, resources, supervision, writing—review and editing; B.R.F.: conceptualization, data curation, funding acquisition, investigation, methodology, validation, writing—review and editing; J.D.D.: conceptualization, funding acquisition, methodology, supervision, writing—review and editing; W.C.F.: conceptualization, funding acquisition, methodology, project administration, resources, supervision, writing—review and editing.

All authors gave final approval for publication and agreed to be held accountable for the work performed therein.

Conflict of interest declaration. We declare we have no competing interests.

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References

- Jackson ST, Betancourt JL, Booth RK, Gray ST. 2009 Ecology and the ratchet of events: climate variability, niche dimensions, and species distributions. *Proc. Natl Acad. Sci. USA* **106**, 19685–19692. (doi:10.1073/pnas.0901644106)
- Gillooly JF, Brown JH, West GB, Savage VM, Charnov EL. 2001 Effects of size and temperature on metabolic rate. *Science* **293**, 2248–2251. (doi:10.1126/science.1061967)
- Angilletta MJ. 2009 *Thermal adaptation: a theoretical and empirical synthesis*. Oxford, UK: Oxford University Press. (doi:10.1093/acprof:oso/9780198570875.001.1)
- Gabriel W, Luttbegg B, Sih A, Tollrian R. 2005 Environmental tolerance, heterogeneity, and the evolution of reversible plastic responses. *Am. Nat.* **166**, 339–353. (doi:10.1086/432558)
- Seebacher F, White CR, Franklin CE. 2015 Physiological plasticity increases resilience of ectothermic animals to climate change. *Nat. Clim. Chang.* **5**, 61–66. (doi:10.1038/nclimate2457)
- Kingsolver JG, Buckley LB. 2017 Evolution of plasticity and adaptive responses to climate change along climate gradients. *Proc. R. Soc. B* **284**, 20170386. (doi:10.1098/rspb.2017.0386)
- Morley SA, Peck LS, Sunday JM, Heiser S, Bates AE. 2019 Physiological acclimation and persistence of ectothermic species under extreme heat events. *Glob. Ecol. Biogeogr.* **28**, 1018–1037. (doi:10.1111/geb.12911)
- Stillman JH. 2003 Acclimation capacity underlies susceptibility to climate change. *Science* **301**, 65. (doi:10.1126/science.1083073)
- Fox RJ, Donelson JM, Schunter C, Ravasi T, Gaitán-Espitia JD. 2019 Beyond buying time: the role of plasticity in phenotypic adaptation to rapid environmental change. *Phil. Trans. R. Soc. B* **374**, 20180174. (doi:10.1098/rstb.2018.0174)
- Thurman LL *et al.* 2020 Persist in place or shift in space? Evaluating the adaptive capacity of species to climate change. *Front. Ecol. Environ.* **18**, 520–528. (doi:10.1002/fee.2253)
- Beever EA *et al.* 2016 Improving conservation outcomes with a new paradigm for understanding species' fundamental and realized adaptive capacity. *Conserv. Lett.* **9**, 131–137. (doi:10.1111/conl.12190)
- Nicotra AB, Beever EA, Robertson AL, Hofmann GE, O'Leary J. 2015 Assessing the components of adaptive capacity to improve conservation and management efforts under global change. *Conserv. Biol.* **29**, 1268–1278. (doi:10.1111/cobi.12522)
- Janzen DH. 1967 Why mountain passes are higher in the tropics. *Am. Nat.* **101**, 233–249. (doi:10.1086/282487)
- Ghalambor CK, Huey RB, Martin PR, Tewksbury JJ, Wang G. 2006 Are mountain passes higher in the tropics? Janzen's hypothesis revisited. *Integr. Comp. Biol.* **46**, 5–17. (doi:10.1093/icb/icj003)
- Calosi P, Bilton DT, Spicer JJ. 2008 Thermal tolerance, acclimatory capacity and vulnerability to global climate change. *Biol. Lett.* **4**, 99–102. (doi:10.1098/rsbl.2007.0408)
- Calosi P, Bilton DT, Spicer JJ, Votier SC, Atfield A. 2010 What determines a species' geographical range? Thermal biology and latitudinal range size relationships in European diving beetles (Coleoptera: Dytiscidae). *J. Anim. Ecol.* **79**, 194–204. (doi:10.1111/j.1365-2656.2009.01611.x)
- Sheldon KS, Huey RB, Kaspari M, Sanders NJ. 2018 Fifty years of mountain passes: a perspective on Dan Janzen's classic article. *Am. Nat.* **191**, 553–565. (doi:10.1086/697046)
- Pintanel P, Tejedo M, Merino-Viteri A, Almeida-Reinoso F, Salinas-Ivanenko S, López-Rosero AC, Llorente GA, Gutiérrez-Pesquera LM. 2022 Elevational and local climate variability predicts thermal breadth of mountain tropical tadpoles. *Eco. (Cop.)* 1–11. (doi:10.1111/ecog.05906)
- Gunderson AR, Stillman JH. 2015 Plasticity in thermal tolerance has limited potential to buffer ectotherms from global warming. *Proc. R. Soc. B* **282**, 20150401. (doi:10.1098/rspb.2015.0401)
- Shah AA, Funk WC, Ghalambor CK. 2017 Thermal acclimation ability varies in temperate and tropical aquatic insects from different elevations. *Integr. Comp. Biol.* **57**, 977–987. (doi:10.1093/icb/ix101)
- Shah AA *et al.* 2017 Climate variability predicts thermal limits of aquatic insects across elevation and latitude. *Funct. Ecol.* **31**, 2118–2127. (doi:10.1111/1365-2435.12906)
- Noer NK, Ørsted M, Schiffer M, Hoffmann AA, Bahrndorff S, Kristensen TN. 2022 Into the wild—a field study on the evolutionary and ecological importance of thermal plasticity in ectotherms across temperate and tropical regions. *Phil. Trans. R. Soc. B* **377**, 20210004. (doi:10.1098/rstb.2021.0004)
- Gutiérrez-Pesquera LM, Tejedo M, Olalla-Tárraga MÁ, Duarte H, Nicieza A, Solé M. 2016 Testing the climate variability hypothesis in thermal tolerance limits of tropical and temperate tadpoles. *J. Biogeogr.* **43**, 1166–1178. (doi:10.1111/jbi.12700)
- Bovo RP, Simon MN, Provete DB, Lyra M, Navas CA, Andrade DV. 2023 Beyond Janzen's hypothesis: how amphibians that climb tropical mountains respond to climate variation. *Integr. Org. Biol.* **5**, 1–18. (doi:10.1093/iob/obad009)
- Sunday JM, Bates AE, Dulvy NK. 2011 Global analysis of thermal tolerance and latitude in ectotherms. *Proc. R. Soc. B* **278**, 1823–1830. (doi:10.1098/rspb.2010.1295)
- Markle TM, Kozak KH. 2018 Low acclimation capacity of narrow-ranging thermal specialists exposes susceptibility to global climate change. *Ecol. Evol.* **8**, 4644–4656. (doi:10.1002/ece3.4006)
- Barria AM, Bacigalupe LD. 2017 Intraspecific geographic variation in thermal limits and acclimatory capacity in a wide distributed endemic frog. *J. Therm. Biol.* **69**, 254–260. (doi:10.1016/j.jtherbio.2017.08.010)
- Mottola G, López ME, Vasemägi A, Nikinmaa M, Anttila K. 2022 Are you ready for the heat? Phenotypic plasticity versus adaptation of heat tolerance in three-spined stickleback. *Ecosphere* **13**, 1–14. (doi:10.1002/ecs2.4015)
- Chiono A, Paul JR. 2023 The climatic variability hypothesis and trade-offs in thermal performance in coastal and inland populations of *Mimulus guttatus*. *Evolution* **77**, 870–880. (doi:10.1093/evolut/qpad005)
- Sasaki MC, Dam HG. 2019 Integrating patterns of thermal tolerance and phenotypic plasticity with population genetics to improve understanding of vulnerability to warming in a widespread copepod. *Glob. Chang. Biol.* **25**, 4147–4164. (doi:10.1111/gcb.14811)
- Molina-Montenegro MA, Naya DE. 2012 Latitudinal patterns in phenotypic plasticity and fitness-related traits: assessing the climatic variability hypothesis (CVH) with an invasive plant species. *PLoS One* **7**, e47620. (doi:10.1371/journal.pone.0047620)
- Kefford BJ, Ghalambor CK, Dewenter B, Poff NL, Hughes J, Reich J, Thompson R. 2022 Acute, diel, and annual temperature variability and the thermal biology of ectotherms. *Glob. Chang. Biol.* **28**, 6872–6888. (doi:10.1111/gcb.16453)
- Woods HA, Dillon ME, Pincebourde S. 2015 The roles of microclimatic diversity and of behavior in mediating the responses of ectotherms to climate change. *J. Therm. Biol.* **54**, 86–97. (doi:10.1016/j.jtherbio.2014.10.002)
- Pincebourde S, Murdock CC, Vickers M, Sears MW. 2016 Fine-scale microclimatic variation can shape the responses of organisms to global change in both natural and urban environments. *Integr. Comp. Biol.* **56**, 45–61. (doi:10.1093/icb/icw016)

35. Sears MW, Angilletta MJ. 2015 Costs and benefits of thermoregulation revisited: both the heterogeneity and spatial structure of temperature drive energetic costs. *Am. Nat.* **185**, E94–E102. (doi:10.1086/680008)
36. Sears MW, Angilletta MJ, Schuler MS, Borchert J, Dilliplane KF, Stegman M, Rusch TW, Mitchell WA. 2016 Configuration of the thermal landscape determines thermoregulatory performance of ectotherms. *Proc. Natl Acad. Sci. USA* **113**, 10595–10600. (doi:10.1073/pnas.1604824113)
37. Bogert CM. 1949 Thermoregulation in reptiles; a factor in evolution. *Evolution* **3**, 195–211. (doi:10.1111/j.1558-5646.1949.tb00021.x)
38. Huey RB, Hertz PE, Sinervo B. 2003 Behavioral drive versus behavioral inertia in evolution: a null model approach. *Am. Nat.* **161**, 357–366. (doi:10.1086/346135)
39. Huey RB, Kearney MR, Krockenberger A, Holtum JAM, Jess M, Williams SE. 2012 Predicting organismal vulnerability to climate warming: roles of behaviour, physiology and adaptation. *Phil. Trans. R. Soc. B* **367**, 1665–1679. (doi:10.1098/rstb.2012.0005)
40. Buckley LB, Ehrenberger JC, Angilletta MJ. 2015 Thermoregulatory behaviour limits local adaptation of thermal niches and confers sensitivity to climate change. *Funct. Ecol.* **29**, 1038–1047. (doi:10.1111/1365-2435.12406)
41. Farallo VR, Wier R, Miles DB. 2018 The Bogert effect revisited: salamander regulatory behaviors are differently constrained by time and space. *Ecol. Evol.* **8**, 11522–11532. (doi:10.1002/ece3.4590)
42. Muñoz MM, Bodensteiner BL. 2019 Janzen's hypothesis meets the Bogert effect: connecting climate variation, thermoregulatory behavior, and rates of physiological evolution. *Integr. Organ. Biol.* **1**, 1–12. (doi:10.1093/iob/oby002)
43. Enriquez-Urzelai U, Tingley R, Kearney MR, Sacco M, Palacio AS, Tejedo M, Nicieza AG. 2020 The roles of acclimation and behaviour in buffering climate change impacts along elevational gradients. *J. Anim. Ecol.* **89**, 1722–1734. (doi:10.1111/1365-2656.13222)
44. Bodensteiner BL, Agudelo-Cantero GA, Arietta AZA, Gunderson AR, Muñoz MM, Refsnider JM, Gangloff EJ. 2021 Thermal adaptation revisited: how conserved are thermal traits of reptiles and amphibians? *J. Exp. Zool. A Ecol. Integr. Physiol.* **335**, 173–194. (doi:10.1002/jez.2414)
45. Muñoz MM, Stimola MA, Algar AC, Conover A, Rodriguez AJ, Landestoy MA, Bakken GS, Losos JB. 2014 Evolutionary stasis and lability in thermal physiology in a group of tropical lizards. *Proc. R. Soc. B* **281**, 20132433. (doi:10.1098/rspb.2013.2433)
46. Muñoz MM, Losos JB. 2018 Thermoregulatory behavior simultaneously promotes and forestalls evolution in a tropical lizard. *Am. Nat.* **191**, E15–E26. (doi:10.1086/694779)
47. Klinges DH, Scheffers BR. 2021 Microgeography, not just latitude, drives climate overlap on mountains from tropical to polar ecosystems. *Am. Nat.* **197**, 75–92. (doi:10.1086/711873)
48. Wahbe TR, Bunnell FL. 2001 Preliminary observations on movements of tailed frog tadpoles (*Ascaphus truei*) in streams through harvested and natural forests. *Northwest Sci.* **75**, 77–83.
49. Caissie D. 2006 The thermal regime of rivers: a review. *Freshw. Biol.* **51**, 1389–1406. (doi:10.1111/j.1365-2427.2006.01597.x)
50. Dent L, Vick D, Abraham K, Schoenholtz S, Johnson S. 2008 Summer temperature patterns in headwater streams of the Oregon coast range. *J. Am. Water Resour. Assoc.* **44**, 803–813. (doi:10.1111/j.1752-1688.2008.00204.x)
51. Johnson SL. 2004 Factors influencing stream temperatures in small streams: substrate effects and a shading experiment. *Can. J. Fish. Aquat. Sci.* **61**, 913–923. (doi:10.1139/f04-040)
52. Torgersen CE, Price DM, Li HW, McIntosh BA. 1999 Multiscale thermal refugia and stream habitat associations of Chinook salmon in northeastern Oregon. *Ecol. Appl.* **9**, 301–319. (doi:10.1890/1051-0761(1999)009[0301:MTRASH]2.0.CO;2)
53. Vatland SJ, Gresswell RE, Poole GC. 2015 Quantifying stream thermal regimes at multiple scales: combining thermal infrared imagery and stationary stream temperature data in a novel modeling framework. *Water Resour. Res.* **51**, 31–46. (doi:10.1002/2014WR015588)
54. Schultz LD, Heck MP, Hockman-Wert D, Allai T, Wenger S, Cook NA, Dunham JB. 2017 Spatial and temporal variability in the effects of wildfire and drought on thermal habitat for a desert trout. *J. Arid Environ.* **145**, 60–68. (doi:10.1016/j.jaridenv.2017.05.008)
55. Cicchino AS, Shah AA, Forester BR, Dunham JB, Ghalambor CK, Funk WC. 2023 Multi-scale relationships in thermal limits within and between two cold-water frog species uncover different trends in physiological vulnerability. *Freshw. Biol.* **68**, 1267–1278. (doi:10.1111/fwb.14102)
56. Dunham JB, Chandler GL, Rieman B, Martin D. 2005 *Measuring stream temperature with digital data loggers: a user's guide*. Fort Collins, CO: US Department of Agriculture, Forest Service, Rocky Mountain Research Station. (Gen. Tech. Rep. RMRS-GTR-150WWW).
57. Araújo MB, Ferri-Yáñez F, Bozinovic F, Marquet PA, Valladares F, Chown SL. 2013 Heat freezes niche evolution. *Ecol. Lett.* **16**, 1206–1219. (doi:10.1111/ele.12155)
58. Hoffmann AA, Chown SL, Clusella-Trullas S. 2013 Upper thermal limits in terrestrial ectotherms: how constrained are they? *Funct. Ecol.* **27**, 934–949. (doi:10.1111/j.1365-2435.2012.02036.x)
59. Barley JM, Cheng BS, Sasaki M, Gignoux-Wolfsohn S, Hays CG, Putnam AB, Sheth S, Villeneuve AR, Kelly M. 2021 Limited plasticity in thermally tolerant ectotherm populations: evidence for a trade-off. *Proc. R. Soc. B* **288**, 20210765. (doi:10.1098/rspb.2021.0765)
60. Weaving H, Terblanche JS, Pottier P, English S. 2022 Meta-analysis reveals weak but pervasive plasticity in insect thermal limits. *Nat. Commun.* **13**, 1–11. (doi:10.1038/s41467-022-32953-2)
61. Cicchino A, Shah A, Forester B, Dunham J, Poff N, Ghalambor C, Funk W. Acute effects of temperature confound estimates of acclimation capacity of critical thermal maximum with consequences for vulnerability assessments. *Ecosphere*
62. Cicchino AS, Ghalambor CK, Funk WC. 2023 Linking critical thermal maximum to mortality from thermal stress in a cold-water frog. *Biol. Lett.* **19**, 20230106. (doi:10.1098/rsbl.2023.0106)
63. Gosner KL. 1960 A simplified table for staging anuran embryos and larvae with notes on identification. *Herpetologica* **16**, 183–190.
64. Turriago JL, Tejedo M, Hoyos JM, Camacho A, Bernal MH. 2023 The time course of acclimation of critical thermal maxima is modulated by the magnitude of temperature change and thermal daily fluctuations. *J. Therm. Biol.* **114**, 103545. (doi:10.1016/j.jtherbio.2023.103545)
65. Overgaard J, Kristensen TN, Sørensen JG. 2012 Validity of thermal ramping assays used to assess thermal tolerance in arthropods. *PLoS One* **7**, e32758. (doi:10.1371/journal.pone.0032758)
66. Kingsolver JG, Umbanhowar J. 2018 The analysis and interpretation of critical temperatures. *J. Exp. Biol.* **221**, jeb167858. (doi:10.1242/jeb.167858)
67. Terblanche JS, Hoffmann AA. 2020 Validating measurements of acclimation for climate change adaptation. *Curr. Opin. Insect Sci.* **41**, 7–16. (doi:10.1016/j.cois.2020.04.005)
68. Terblanche JS, Deere JA, Clusella-Trullas S, Janion C, Chown SL. 2007 Critical thermal limits depend on methodological context. *Proc. R. Soc. B* **274**, 2935–2943. (doi:10.1098/rspb.2007.0985)
69. Turriago JL, Tejedo M, Hoyos JM, Bernal MH. 2022 The effect of thermal microenvironment in upper thermal tolerance plasticity in tropical tadpoles. Implications for vulnerability to climate warming. *J. Exp. Zool. A Ecol. Integr. Physiol.* **337**, 746–759. (doi:10.1002/jez.2632)
70. Cicchino AS, Martinez CM, Funk WC, Forester BR. 2023 Temperature and development drive variation in oral morphology among tailed frog (*Ascaphus* spp.) populations. *Ichthyol. Herpetol.* **111**, 448–455. (doi:10.1643/h2022084)

71. Havird JC, Neuwald JL, Shah AA, Mauro A, Marshall CA, Ghalambor CK. 2020 Distinguishing between active plasticity due to thermal acclimation and passive plasticity due to Q10 effects: why methodology matters. *Funct. Ecol.* **34**, 1015–1028. (doi:10.1111/1365-2435.13534)
72. Einum S, Ratikainen I, Wright J, Pélabon C, Bech C, Jutfelt F, Stawski C, Burton T. 2019 How to quantify thermal acclimation capacity? *Glob. Chang. Biol.* **25**, 1893–1894. (doi:10.1111/gcb.14598)
73. Rasband W. 2018 *ImageJ v. 1.52a*. Bethesda, MD: US National Institutes of Health. See <https://imagej.nih.gov/ij>.
74. U.S.A Environmental Protection Agency. 2020 Columbia river cold water refuges. See <https://www.epa.gov/columbiariver/columbia-river-cold-water-refuges>.
75. R Core Team. 2021 *R: a language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing. See <https://www.R-project.org>.
76. van Heerwaarden B, Kellermann V, Sgrò CM. 2016 Limited scope for plasticity to increase upper thermal limits. *Funct. Ecol.* **30**, 1947–1956. (doi:10.1111/1365-2435.12687)
77. Armstrong EJ, Tanner RL, Stillman JH. 2019 High heat tolerance is negatively correlated with heat tolerance plasticity in Nudibranch Mollusks. *Physiol. Biochem. Zool.* **92**, 430–444. (doi:10.1086/704519)
78. Gunderson AR. 2023 Trade-offs between baseline thermal tolerance and thermal tolerance plasticity are much less common than it appears. *Glob. Chang. Biol.* **29**, 3519–3524. (doi:10.1111/gcb.16710)
79. van Heerwaarden B, Kellermann V. 2020 Does plasticity trade off with basal heat tolerance? *Trends Ecol. Evol.* **35**, 874–885. (doi:10.1016/j.tree.2020.05.006)
80. de Vlamming VL, Bury RB. 1970 Thermal selection in tadpoles of the tailed-frog, *Ascaphus truei*. *J. Herpetol.* **4**, 179. (doi:10.2307/1562892)
81. Gaige HT. 1920 Observations upon the habits of *Ascaphus truei* Stejneger. *Occ. Pap. Mus. Zool. Univ. Mich.* **84**, 1–11.
82. Deutsch CA, Tewksbury JJ, Huey RB, Sheldon KS, Ghalambor CK, Haak DC, Martin PR. 2008 Impacts of climate warming on terrestrial ectotherms across latitude. *Proc. Natl Acad. Sci. USA* **105**, 6668–6672. (doi:10.1073/pnas.0709472105)
83. Polato NR *et al.* 2018 Narrow thermal tolerance and low dispersal drive higher speciation in tropical mountains. *Proc. Natl Acad. Sci. USA* **115**, 12471–12476. (doi:10.1073/pnas.1809326115)
84. Sunday J *et al.* 2019 Thermal tolerance patterns across latitude and elevation. *Phil. Trans. R. Soc. B* **374**, 20190036. (doi:10.1098/rstb.2019.0036)
85. Gunderson AR, Dillon ME, Stillman JH. 2017 Estimating the benefits of plasticity in ectotherm heat tolerance under natural thermal variability. *Funct. Ecol.* **31**, 1529–1539. (doi:10.1111/1365-2435.12874)
86. Pinsky ML, Eikeset AM, McCauley DJ, Payne JL, Sunday JM. 2019 Greater vulnerability to warming of marine versus terrestrial ectotherms. *Nature* **569**, 108–111. (doi:10.1038/s41586-019-1132-4)
87. Wagner T, Schliep EM, North JS, Kundel H, Custer CA, Ruzich JK, Hansen GJA. 2023 Predicting climate change impacts on poikilotherms using physiologically guided species abundance models. *Proc. Natl Acad. Sci. USA* **120**, e2214199120. (doi:10.1073/pnas.2214199120)
88. Cicchino A, Ghalambor C, Forester B, Dunham J, Funk WC. 2024 Data from: Greater plasticity in CTmax with increased climate variability among populations of tailed frogs lacking opportunity for behavioral thermoregulation. Dryad Digital Repository (doi:10.5061/dryad.gmsbcc2xb)
89. Cicchino A, Ghalambor CK, Forester B, Dunham JD, Funk WC. 2024 Data from: Greater plasticity in CTmax with increased climate variability among populations of tailed frogs. Figshare. (doi:10.6084/m9.figshare.c.7494309)