


RESEARCH ARTICLE

Climate variability predicts thermal limits of aquatic insects across elevation and latitude

Alisha A. Shah¹  | Brian A. Gill^{1,2} | Andrea C. Encalada³ | Alexander S. Flecker⁴ | W. Chris Funk^{1,2} | Juan M. Guayasamin^{3,5} | Boris C. Kondratieff^{1,2,6} | N. LeRoy Poff^{1,2,7,8} | Steven A. Thomas⁹ | Kelly R. Zamudio⁴ | Cameron K. Ghalambor^{1,2}

¹Department of Biology, Colorado State University, Fort Collins, CO, USA

²Graduate Degree Program in Ecology, Colorado State University, Fort Collins, CO, USA

³Colegio de Ciencias Biológicas y Ambientales, Universidad San Francisco de Quito, Quito, Ecuador

⁴Department of Ecology and Evolutionary Biology, Cornell University, Ithaca, NY, USA

⁵Centro de Investigación de la Biodiversidad y Cambio Climático, Ingeniería en Biodiversidad y Recursos Genéticos, Facultad de Ciencias de Medio Ambiente, Universidad Tecnológica Indoamérica, Quito, Ecuador

⁶Department of Bioagricultural Sciences and Pest Management, Colorado State University, Fort Collins, CO, USA

⁷Institute of Applied Ecology, University of Canberra, Canberra, ACT, Australia

⁸Bioagricultural Sciences and Pest Management, Colorado State University, Fort Collins, CO, USA

⁹School of Natural Resources, University of Nebraska, Lincoln, NE, USA

Correspondence

Alisha A. Shah
Email: alisha.shah@colostate.edu

Funding information

U.S. National Science Foundation, Grant/Award Number: DEB-1046408, DEB-1045960 and DEB-1045991; Graduate Research Fellowship, Grant/Award Number: DGE-1321845; Ministerio del Ambiente of Ecuador, Grant/Award Number: #56-IC-FAU/FLO-DPN/MA and MAE-DNB-CM-2015-0017

Handling Editor: Sarah Diamond

Abstract

1. Janzen's extension of the climate variability hypothesis (CVH) posits that increased seasonal variation at high latitudes should result in greater temperature overlap across elevations, and favour wider thermal breadths in temperate organisms compared to their tropical counterparts.
2. We tested these predictions by measuring stream temperatures and thermal breadths (i.e. the difference between the critical thermal maximum and minimum) of 62 aquatic insect species from temperate (Colorado, USA) and tropical (Papallacta, Ecuador) streams spanning an elevation gradient of c. 2000 m.
3. Temperate streams exhibited greater seasonal temperature variation and overlap across elevations than tropical streams, and as predicted, temperate aquatic insects exhibited broader thermal breadths than tropical insects. However, elevation had contrasting effects on patterns of thermal breadth. In temperate species, thermal breadth decreased with increasing elevation because CT_{MAX} declined with elevation while CT_{MIN} was similar across elevations. In tropical insects, by contrast, CT_{MAX} declined less sharply than CT_{MIN} with elevation, causing thermal breadth to increase with elevation.
4. These macrophysiological patterns are consistent with the narrower elevation ranges found in other tropical organisms, and they extend Janzen's CVH to freshwater streams. Furthermore, because lowland tropical aquatic insects have the narrowest thermal breadths of any region, they may be particularly vulnerable to short-term extreme changes in stream temperature.

KEYWORDS

aquatic insects, climate change, CT_{MAX} , CT_{MIN} , Janzen's hypothesis, thermal breadth, vulnerability

1 | INTRODUCTION

A fundamental goal of integrative biology is to understand how variation in climate shapes the thermal physiology, behaviour, and geographic distribution of organisms (Angilletta, 2009; Bozinovic, Calosi, & Spicer, 2011; Spicer & Gaston, 2009). The “climate variability hypothesis” (CVH) predicts that more variable climates select for organisms with broader thermal tolerances, whereas less variable (stable) climates select for narrower thermal tolerances (Angilletta, 2009; Dobzhansky, 1950; Gaston & Chown, 1999; Janzen, 1967; Stevens, 1989). Thus, compared to the climatically stable tropics, mid-to-high latitude temperate environments should select for organisms with broader thermal breadths because of the seasonal variation between warm summer and cold winter temperatures (Angilletta, 2009). In an extension of the CVH, Janzen (1967) incorporated the influence of elevation on thermal breadth. In temperate mountains, seasonal temperature changes cause low and high elevation localities to have similar annual ranges, which should allow species to physiologically tolerate a wide range of temperatures. However, in tropical mountains at any given elevation, a narrow annual range of temperatures should lead to a narrow thermal tolerance. The narrower thermal breadth of tropical species should lead to reduced dispersal and a reduced elevation distribution. Thus, Janzen (1967) proposed that mountain passes should be “higher” for lowland tropical species because colder temperatures at higher elevations would be a greater physiological barrier to dispersal compared to temperate lowland species (Chan et al., 2016; Ghalambor, Huey, Martin, Tewksbury, & Wang, 2006; Huey, 1978; McCain, 2009).

Macrophysiological studies of terrestrial species have shown general support for the predicted relationship between thermal tolerance and latitude (Addo-Bediako, Chown, & Gaston, 2000; Brattstrom, 1968; Calosi, Bilton, & Spicer, 2008; Calosi, Bilton, Spicer, & Atfield, 2008; Deutsch et al., 2008; Feder, 1978; Sunday, Bates, & Dulvy, 2011; van Berkum, 1988) and elevation (Addo-Bediako et al., 2000; Bozinovic et al., 2011; Ghalambor et al., 2006; Sheldon & Tewksbury, 2014). For most organisms, this relationship is driven by maximum (CT_{MAX}) and minimum (CT_{MIN}) critical thermal limits that track maximum and minimum environmental temperatures respectively (Angilletta, 2009). In the warmer lowland tropics, organisms should exhibit higher CT_{MAX} and CT_{MIN} values compared to organisms from colder, temperate regions. High elevation tropical species may have low CT_{MIN} values similar to those found in temperate species. Consequently, we predict that temperature variation across elevation and latitude should shape macrophysiological patterns in thermal limits. The generality of such patterns remains undescribed for most taxa (Gaston et al., 2009), and studies of temperate and tropical freshwater ectotherms are particularly lacking (Chown, Duffy, & Sørensen, 2015; Gutiérrez-Pesquera et al., 2016).

Freshwater streams naturally occur along elevation gradients and exhibit global scale variation in thermal regimes that can be similar to those observed for air temperatures (Ward, 1985). As with air temperature, increasing elevation is accompanied by a decrease in stream temperatures as a function of the adiabatic lapse rate (Dillon, Frazier, & Dudley, 2006). However, partly due to the high heat capacity of

water, stream temperatures are more stable and do not vary linearly with air temperatures (Mohseni & Stefan, 1999). Across latitude, lowland tropical streams may be generally warmer and temporally more stable than temperate streams (Boulton et al., 2008). This variation in stream thermal regime likely drives several aspects of aquatic insect life-history, such as dormancy, growth rate, and timing of emergence (Ward & Stanford, 1982).

Other factors also interact with stream temperature to impose unique challenges on the thermal tolerance of aquatic ectotherms, most notably oxygen availability, which might vary with elevation and stream flow (Clark, Webb, & Ladle, 1999; Jacobsen, Rostgaard, & Vasconez, 2003; Pörtner & Knust, 2007; Verberk, Bilton, Calosi, & Spicer, 2011). The degree to which global scale climatic variability is mitigated by such local scale effects on thermal breadth in temperate and tropical stream organisms is not known for aquatic insects, but has important implications for our understanding of how aquatic and terrestrial organisms differentially cope with temperature variation, and for their vulnerability to climate change (Verberk, Durance, Vaughan, & Ormerod, 2016).

Here, we test two important components of Janzen’s (1967) CVH in temperate and tropical stream insects. We first test the assumption that tropical stream temperatures are less variable and exhibit reduced overlap across elevation compared to temperate zone streams. Second, we test the prediction that tropical aquatic insects have narrower thermal breadths relative to their temperate counterparts, by measuring the difference between the maximum (CT_{MAX}) and minimum (CT_{MIN}) critical thermal limits (Huey & Stevenson, 1979). We focus on species of aquatic insects that occur across a range of elevations in temperate Colorado, USA and tropical Ecuador.

2 | MATERIALS AND METHODS

2.1 | Study sites

Our study streams were distributed at a mid-latitude temperate location, the Rocky Mountains of northern Colorado, USA (40° 35’N, 105° 5’W), and a low latitude tropical location, the Andes Mountains of Ecuador (0.3° 10’S, 78° 28’W). We selected five streams from 1,992 to 3,166 m a.s.l. in the Cache la Poudre drainage in Colorado and seven streams from 1,845 to 3,898 m a.s.l. in the Papallacta-Quijos drainage (headwaters of the Amazon Basin) in Ecuador. To control for any confounding effects of stream flow, water depth, or width, we only sampled low-order (wadeable) streams that were similar in size.

2.2 | Quantifying stream temperature variability

At each stream, (1,992, 2,212, 2,950, 2,798 and 3,166 m in Colorado and 1,845, 2,694 and 3,683 m in Ecuador) we submerged temperature loggers (3001, Solinst Canada Ltd. Georgetown, Ontario) to record hourly fluctuations in water temperature over a period of several months (October–April). Loggers could not be submerged in streams at 2,957 and 3,387 m in Ecuador. Landslides and flooding of the Andean streams prevented loggers from collecting data for an entire year. We also measured stream temperature with a field meter (Pro

ODO, YSI Incorporated, Yellow Springs, Ohio) at every site during each insect collection.

2.3 | Aquatic insect collection, identification, transport and housing

We focused on species of aquatic insects from three ecologically important orders: Ephemeroptera (mayflies), Plecoptera (stoneflies) and Trichoptera (caddisflies). Because the majority of freshwater insect life cycles are spent in the water as juveniles, we tested this stage in our experiments and not the short-lived flying adult stage. While the climatic variation in water experienced by the juvenile stage is likely reduced compared to what the adults experience in air (Kingsolver et al., 2011), successful emergence for the adult stage is dependent on the growth and survival challenges of the juvenile larval stage, which for some species, can last several years. Thus, we focused our efforts on testing the effects of climate variability on thermal tolerance during the juvenile stage. We selected species from families that occur at multiple elevations at both latitudes, were members of various functional feeding groups (Table S1), and were numerically abundant to ensure adequate sample sizes. Insects were collected, acclimated, and tested from one elevation at a time. We collected aquatic insects between 08.00 and 11.00 hr using a D-frame kick net (mesh size: 500 μm). Insects were identified based on morphology to the lowest taxonomic level possible in the field without a microscope (i.e. morphological taxonomic units, MTUs), usually to genus and in some cases to species.

After collection from the stream, insects were transported to a laboratory (Fort Collins in the Colorado Rockies or the town of Baeza in the Ecuadorian Andes). In the lab, we maintained insects at the average temperature of stream from which they were collected. For a given elevation, these temperatures were the same between the two latitudes. A moderate water current was generated within the holding cooler using an aquarium pump. Insects remained in mesh-enclosed containers for 48 hr on a 12 : 12 hr light–dark cycle. This period allowed insects to acclimate to laboratory conditions and ensured that all individuals experienced a similar environment prior to experiments.

2.4 | Determining critical thermal limits

Our goal was to test if there is a relationship in the field between the amount of temperature variability at a given site and the observed patterns of thermal breadth (i.e. difference between CT_{MIN} and CT_{MAX}). We measured critical thermal limits using thermal ramping (Duarte et al., 2012; Lutterschmidt & Hutchison, 1997). While estimates of critical thermal limits can be sensitive to the measurement protocol (Rezende, Tejedo, & Santos, 2011; Terblanche, Deere, Clusella-Trullas, Janion, & Chown, 2007), the same methodology was used for all species to facilitate comparisons. One aspect of the experimental design that requires particular attention is the choice of acclimation and starting temperatures. Because comparisons were made across species from very different thermal environments, all species were acclimated to average stream temperature at the time of collection and that same temperature was used at the start of each experiment. Thus,

what we report are the natural “un-manipulated” patterns of CT_{MIN} and CT_{MAX} across elevation and latitude that likely reflect a combination of genetic and plastic influences. Using the stream temperature at the time of collection also allowed us to avoid the confounding effects of deteriorating body condition associated with acclimating all species to a single temperature outside their preferred temperature range (see Rezende et al., 2011). Indeed, during separate experiments designed to test the degree of plasticity in critical thermal maximum rates, we found the use of acclimation temperatures outside the range normally experienced resulted in obvious stress and mortality, particularly in tropical species, making comparisons less meaningful (A. A. Shah, W. Christopher Funk, C. K. Ghalambor, unpublished). See Appendix S1, Supporting Information, for more information regarding methods.

CT_{MAX} experiments were conducted during the months of January–March 2013 and 2014 in Baeza, Ecuador (1,795 m) and from June to August 2014 and 2015 in Fort Collins, Colorado (1,524 m) (Appendix S1, Methods). We ramped temperature at the rate of 0.3°C per min until a pre-determined, sub-lethal behaviour was observed (Table S2). In each experiment, we tested up to 12 individuals (mean = 8.34, min = 4) per MTU per stream site ($n = 847$ individuals, 23 MTUs). When individuals approached CT_{MAX} —determined by a loss of righting response—we transferred individuals to aerated water at normal stream temperature for recovery.

We conducted CT_{MIN} experiments on 299 individual insects (11 MTUs), during October–December 2014, in Ecuador, and June–August 2015 in Colorado (Appendix S1, Methods). In Ecuador, all insects could not be collected at two elevations (1,845 and 2,798 m a.s.l.), where CT_{MAX} data were collected, due to an oil pipeline construction project and a severe landslide. To measure CT_{MIN} , we ramped temperature down at the rate of ~0.3°C/min. Each CT_{MIN} trial consisted of testing a maximum of nine individuals per MTU (mean = 8.5, min = 5). We used data from only those insects that recovered from the experiments, i.e. resumed normal activity.

2.5 | Taxonomic identification and phylogeny reconstruction

To establish the taxonomic identity of every individual in our study, we used a hierarchical approach to species assignment. First, we DNA barcoded (Hebert, Stoeckle, Zemplak, & Francis, 2004) a subset of specimens ($n = 323$ out of 956) following standard protocols from the Canadian Center for DNA barcoding (Hajibabaei et al., 2005; Ivanova, DeWaard, Hajibabaei, & Hebert, 2006; Ivanova, DeWaard, & Hebert, 2006). Second, in cases where no specimens were DNA barcoded for a MTU and site, we used a larger dataset of DNA barcoded specimens to determine the known species composition at that site. These data were used to assign a species to every non-barcoded MTU. In some cases ($n = 21$), MTUs that were not barcoded could not be assigned to a single species because more than one cryptic species was present at a site. In these cases, we assumed proportional representation and distributed the MTUs evenly between the possible species present. This combination of morphological, genetic, and geographic information was then used to develop a phylogenetic hypothesis for all specimens in the study (see Appendix S1 for details).

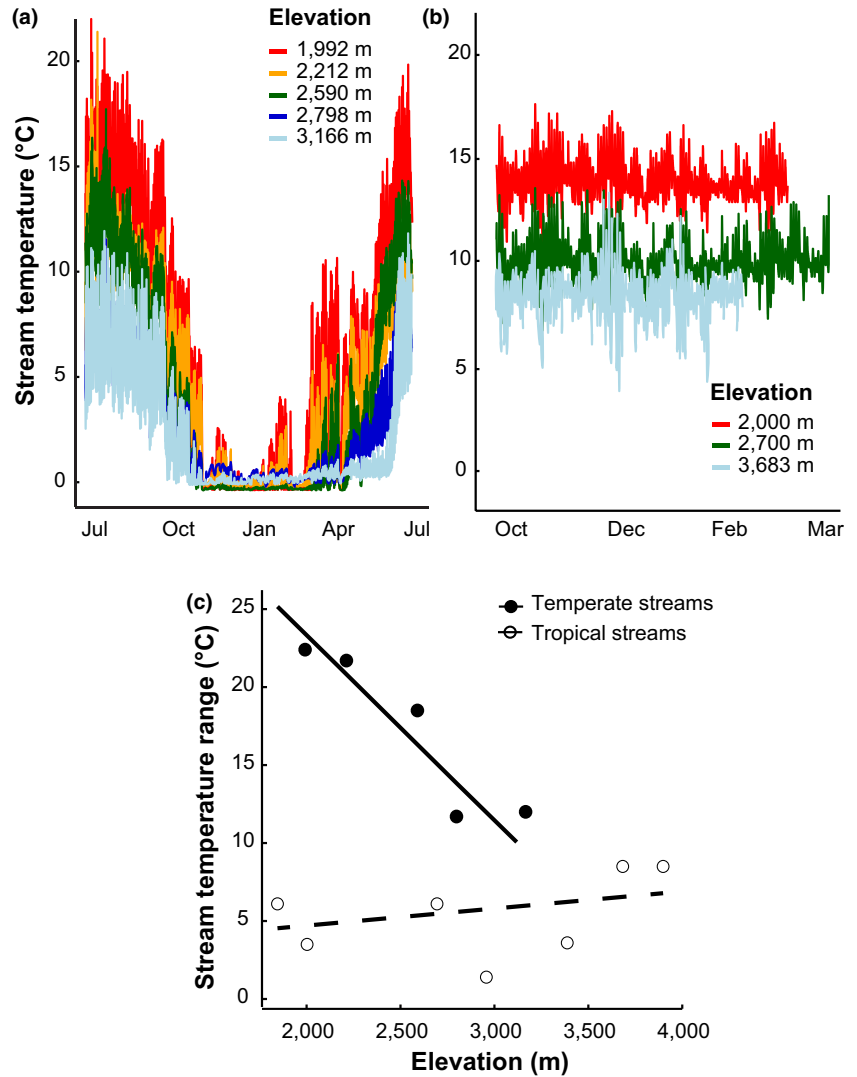


FIGURE 1 Stream temperature profiles for low-to-high elevation temperate streams, showing increased overlap in temperature, especially in the winter months (a), tropical streams with reduced overlap (b). (c) Shows the relationship between stream temperature range (annual maximum–minimum) and elevation, where it decreases with elevation in temperate streams, but has an increasing trend with elevation in tropical streams

2.6 | Statistical analyses

All statistical analyses were conducted in R version 3.1.3 (R Core Team 2013). To validate the assumption that temperature regimes are more variable in the temperate streams than in the tropical streams in our study, we calculated the coefficient of variation (CV) in temperature for each stream. Next, we used a general linear model (GLM) to test the effect of elevation, latitude, and their interaction on stream temperature range. Lastly, we conducted ANOVAs for each latitude, to explore how stream temperature varies with elevation.

2.7 | Effects of latitude and elevation on critical thermal limits

To test the effects of latitude and elevation on CT_{MAX} , CT_{MIN} , and thermal breadth we used phylogenetic generalized least squares regression (PGLS; Grafen, 1989) fit with an Ornstein–Uhlenbeck model of trait evolution (OU; Butler & King, 2004; Hansen, 1997). We chose the OU model based on its low AIC and higher log likelihood values in a model selection analysis (Table S4). We ran PGLS for CT_{MAX} , CT_{MIN} ,

and thermal breadth separately, using a model that included latitude, elevation, dry weight (Gaston & Spicer, 1998), and an interaction between latitude and elevation. We included elevation in the model because it was highly correlated with maximum and average stream temperature (Figure S1) and dry weight as a covariate to account for differences in thermal limits due to body size. Because thermal breadth was calculated from different individuals used in the CT_{MAX} and CT_{MIN} experiments, we used a mean dry weight of individuals. Finally, we used separate PGLS analyses for each latitude to test if there was a relationship between stream temperature range and thermal breadth while controlling for dry weight.

3 | RESULTS

3.1 | Stream temperature variability

Tropical streams were less thermally variable than temperate streams regardless of elevation (Figure 1a,b; Table 1). The major qualitative difference between the temperate and tropical regions is the clear influence of seasons in the temperate streams. Cold winters result in

TABLE 1 Elevations, annual maximum, minimum, range, and average temperatures of our study streams. Coefficients of variation (CV) were calculated for each stream

	Stream elevation (m)	Maximum (°C)	Minimum (°C)	Range (°C)	Average (°C)	CV (%)
Temperate (Colorado, USA)	1,992	22.0	-0.4	22.4	6.77	89.69
	2,212	21.4	-0.3	21.7	4.84	95.16
	2,590	18.1	-0.4	18.5	4.64	108.64
	2,798	11.5	-0.2	11.8	2.83	99.38
	3,166	11.9	-0.1	12.1	2.04	134.46
Tropical (Papallacta, Ecuador)	1,845	16.9	10.8	6.1	13.53	6.27
	2,003	16.4	12.9	3.5	13.95	4.4
	2,694	13.3	7.2	6.1	10.02	8.86
	2,957	10.3	8.9	1.4	9.62	n/a
	3,387	12.7	9.1	3.6	10.9	n/a
	3,683	13.1	4.6	8.5	8.57	11.0
	3,898	11.5	3.0	8.5	6.59	20.02

all streams converging on 0°C, at all elevations (Figure 1a). At lower elevations temperate streams warm more quickly and reach higher values in the transition from winter to spring and summer. But there is also a signal of spring snowmelt runoff that returns seasonally rising temperatures to near freezing for *c.* 3 weeks between February and March. In contrast, tropical streams exhibit no seasonal patterns in temperature, and their thermal coefficients of variation (CV) are largely driven by diel variation in stream temperature (Figure 1b). Annual thermal CV were consistently lower for tropical streams compared to temperate streams (Welch's two-sample *t* test corrected for unequal variance: $t_{4,9} = 11.435$, $p < .0001$). For any given elevation, temperate streams had higher maximum and lower minimum annual temperatures (Table 1). Yet, on average, temperate streams were colder (mean annual temperature $4.2 \pm 0.99^\circ\text{C}$) than tropical streams (mean annual temperature $10.5 \pm 0.83^\circ\text{C}$). We found a significant elevation by latitude effect on stream temperature range (GLM: $F_{1,16} = 73.14$, $p < .001$). Specifically, tropical stream temperature range remains stable across elevation (although it exhibits an increasing trend, ANOVA: $F_{1,5} = 1.00$, $p = .362$), but temperate stream temperature range decreases with elevation (ANOVA: $F_{1,3} = 19.57$, $p = .021$; Figure 1c).

3.2 | Effects of latitude and elevation on critical thermal limits and breadth

The PGLS analysis revealed that latitude was a significant predictor of CT_{MAX} estimates (PGLS: $t_{82} = -2.745$, $p = .007$). Specifically, CT_{MAX} was lower in tropical compared to temperate stream insects (Figure 2; Figure S2). CT_{MAX} also decreased with increasing elevation in temperate and tropical aquatic insects (Figure 2). Dry weight was not a significant covariate. For CT_{MIN} , we found a significant latitude by elevation interaction (PGLS: $t_{46} = -6.473$, $p < .001$) reflecting the fact that CT_{MIN} remains relatively constant and low across elevation in temperate aquatic insects, but decreases with elevation in tropical aquatic insects (Figure 3; Figure S2). Dry weight was not a significant predictor of CT_{MIN} .

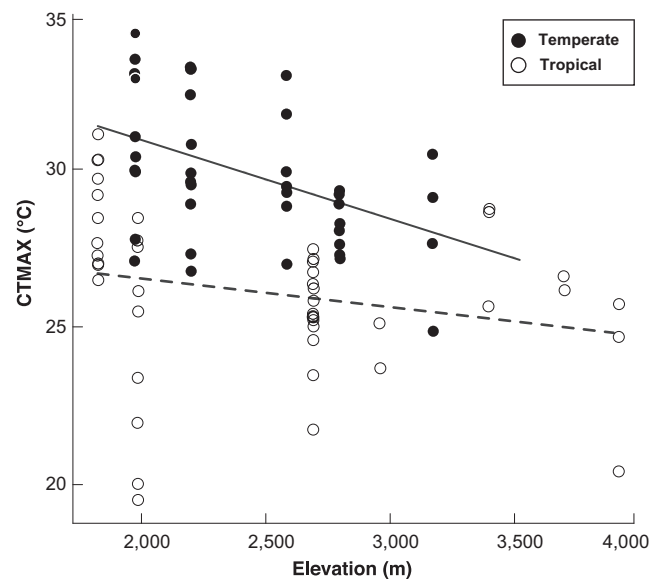


FIGURE 2 CT_{MAX} declines with increasing elevation in Ecuador and Colorado. The regression lines, generated from the phylogenetic generalized least square analysis do not show a significant interaction. In general, CT_{MAX} was higher in insects from Colorado than in those from Ecuador

The results of the PGLS analysis for thermal breadth revealed a significant interaction between latitude and elevation on thermal breadth (PGLS: $t_{46} = 3.937$, $p < .001$). This interaction reflects the large difference in thermal breadth between temperate and tropical species at low elevations and the convergence of thermal breadth at high elevations (Figure 4a). Lastly, we found that stream temperature range predicted aquatic insect thermal breadth (PGLS: $t_{48} = 7.550$, $p < .001$) (Figure 4b). This relationship was also significant *within* Colorado (PGLS: $t_{19} = 3.525$, $p = .003$) and Ecuador (PGLS: $t_{32} = 2.326$, $p = .027$), when controlling for phylogeny and dry weight. Thus, insects that experienced the widest

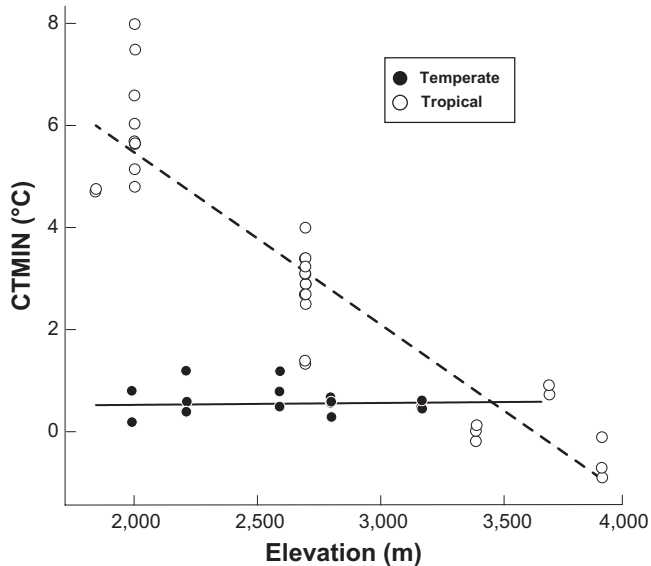


FIGURE 3 CT_{MIN} declines with increasing elevation only in Ecuador. In Colorado, there is no change in CT_{MIN} across elevation, resulting in a significant interaction between latitude and elevation. All regression lines were generated from the phylogenetic generalized least square analysis

stream temperature ranges had the broadest thermal breadths (Figure 4b).

4 | DISCUSSION

Janzen (1967) predicted that reduced climatic variability across an elevation gradient would result in tropical organisms with narrower thermal breadths compared to their temperate counterparts. Narrower thermal tolerance in the tropics has implications for patterns of dispersal, elevation range sizes, and rates of speciation (Ghalambor et al., 2006; Janzen, 1967). We tested the relationship between temperature variability on patterns of thermal breadth ($CT_{MAX}-CT_{MIN}$) and found support for Janzen's predictions; tropical aquatic insects on average have narrower thermal breadths compared to their temperate relatives. Indeed, these results complement recent findings that tropical aquatic insects have reduced dispersal and smaller elevation ranges (Gill et al., 2016). However, variation in thermal breadth is not simply a response to different latitudes, but instead reflects the magnitude of temperature variation experienced at a given site. For example, the difference in thermal breadths are greatest when comparing temperate and tropical species at lower elevations, and as elevation increases the relative differences in thermal breadth across latitude decline (Figure 4a). The close relationship between temperature variation and thermal breadth is thus influenced not only by large-scale latitudinal differences, but also by local scale effects such as elevation. Such relationships appear to be a general pattern in freshwater systems, as Gutiérrez-Pesquera et al. (2016) report a similar pattern in a comparison of temperate and tropical tadpoles. We explore these findings in greater detail below.

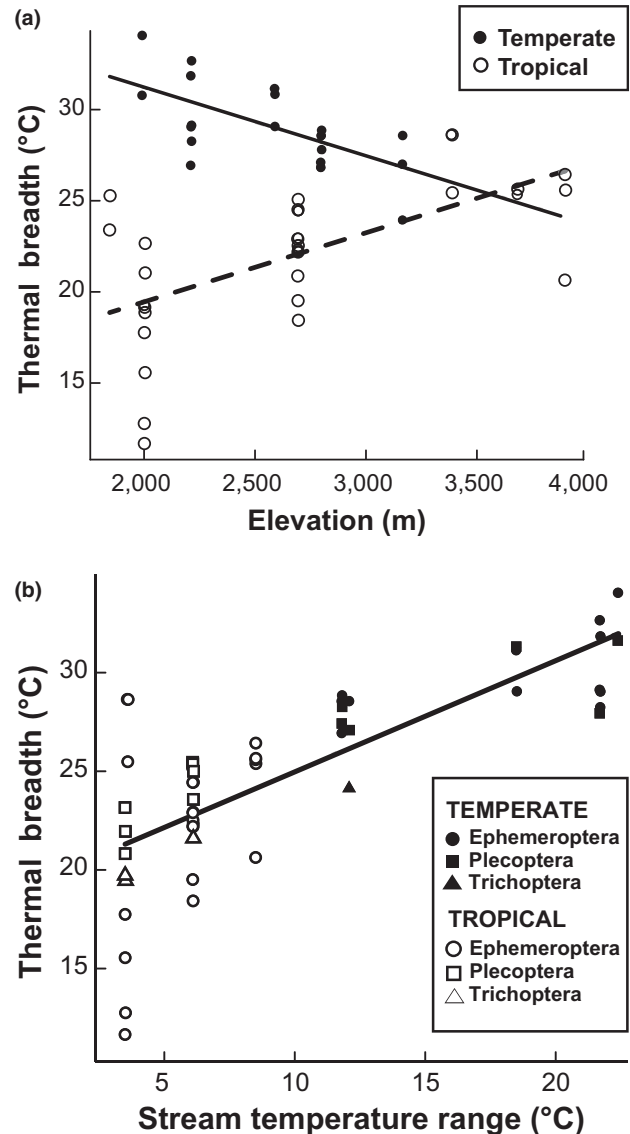


FIGURE 4 Thermal breadth shows a decreasing trend in Colorado, but an increasing trend in Ecuador (a). This matches the patterns of stream temperature range variation suggesting that within latitude stream temperature variation plays a role in shaping thermal breadth. (b) shows that between latitudes, stream temperature range predicts thermal breadth in aquatic insects. Colorado insects that experience on average wider temperature ranges also have wider thermal breadths compared to their relatives in Ecuador where temperature range is narrow

4.1 | Patterns of stream temperature variation across elevation and latitude

Temperate and tropical streams can exhibit substantial variation in characteristics such as width, depth, groundwater input, and insolation (Ward, 1985). We controlled for much of this variation by focusing on low-order mountain streams. While individual streams exhibit local variation, our comparative results indicate that tropical stream temperatures are (1) less seasonally variable than temperate streams, and (2) exhibit less overlap across elevation (Figure 1). Aquatic organisms

in these tropical streams experience considerably less annual variation in mean daily temperature (mean tropical range = 5.4°C; mean temperate range = 17.3°C, Table 1). Subsequently, there is reduced overlap in tropical stream temperature range across elevation, where the primary source of thermal variation appears to occur at the diel scale, particularly at higher elevations (Figure 1c; Table 1). Temperate streams, however, exhibit reduced annual temperature variability with increasing elevation because maximum stream temperatures decrease with elevation, while minimum temperatures are similar across elevations (converging on 0°C). Thus, organisms living in lower elevation temperate streams experience the widest variation in stream temperature, because these streams are warmer in summer and approach freezing temperatures in winter. In contrast, lower elevation tropical streams have the narrowest temperature ranges, as the variation in water temperature is low year-round. This variation in stream temperature explains patterns of thermal breadth across latitude and elevation (Figure 4).

4.2 | Effects of latitude and elevation on thermal breadth

We found strong support for the prediction that thermal breadth is narrower in tropical compared to temperate streams (Figure 4). However, across the elevation gradient, temperate and tropical aquatic insects exhibit contrasting patterns in their range of thermal tolerance. In tropical streams, insect thermal breadth increases with elevation as the difference between CT_{MAX} and CT_{MIN} increases, mostly driven by a sharper decrease in CT_{MIN} with elevation (Figures 2 and 3). In temperate streams, the unchanging CT_{MIN} and the decreasing CT_{MAX} with elevation result in a narrowing of thermal breadth as elevation increases (Figures 2 and 3). Thus, the low elevation temperate insects in this study system have the broadest thermal breadths, while low elevation tropical insects have the narrowest thermal breadths (Figure 4). Had we been able to include even lower elevation tropical streams, we suspect thermal breadths could become narrower still. For high elevation species at both latitudes, in contrast, thermal breadths converge (Figure 4a).

These patterns in thermal breadth reflect how stream temperatures change with elevation in each region. In the tropics, the average annual minimum and maximum temperatures declines with increasing elevation, but the difference between them remains constant (Table 1). The exception to this pattern occurs at the highest elevation above tree-line, where the grass-dominated tropical páramo receives high insolation, and stream temperatures show greater thermal variation than lower elevation forest-canopied streams (Table 1; Finn, Encalada, & Hampel, 2016; see also Huey et al., 2009). In temperate streams, thermal breadth decreases with increasing elevation as the difference between annual minimum and maximum temperatures also decreases with elevation (Figure 4a). In Colorado our highest sampling point was at 3,100 m a.s.l. and additional work is required to determine whether thermal breadth continues to decrease at even higher elevations. Nevertheless, such results contrast with studies of terrestrial insects that find thermal breadth decreases with elevation because cold

tolerance increases while heat tolerance remains unchanged (Gaston & Chown, 1999).

4.3 | Effects of latitude and elevation on critical thermal limits

We found that temperate species exhibited greater tolerance for high temperatures compared to their tropical counterparts (Figure 2). This result may appear counterintuitive at first because temperate streams are colder on average than tropical streams. However, at any given elevation, annual maximum temperatures are higher during the temperate summer (Table 1, Figure 1), and the greater CT_{MAX} values in the temperate species reflect these maximum temperatures. Indeed, previous studies have shown high maximum environmental temperatures to be correlated with higher CT_{MAX} values for ectotherms (e.g. Kaspari, Clay, Lucas, Yanoviak, & Kay, 2015; Turriago, Parra, & Bernal, 2015; Wu & Kam, 2005). Within latitude, CT_{MAX} is also sensitive to water temperatures, as it declines with elevation (Figure 2). These results are intriguing because studies of terrestrial ectotherms have generally found CT_{MAX} to be largely invariant across elevation and latitude (e.g., Addo-Bediako et al., 2000; Gaston & Chown, 1999; Ghalambor et al., 2006; Hoffmann, Chown, & Clusella-Trullas, 2013). In marine ectotherms, however, a meta-analysis by Sunday, Bates, and Dulvy (2012) showed CT_{MAX} to closely track changes in ocean temperature. An increase in CT_{MAX} was also detected across latitude in freshwater diving beetles (Calosi, Bilton, Spicer, Votier, & Atfield, 2010). Thus, aquatic ectotherms may differ from terrestrial ectotherms in maintaining more variation in their physiological sensitivity to maximum temperatures. While the causes of this difference have yet to be explored, they may be linked to differences in oxygen availability. Water has 33 times less oxygen than air, which severely reduces the amount available for respiration (Jones, 1972). Unlike air, warm water holds less oxygen than cold water and this property has been implicated in limiting thermal tolerance in aquatic organisms due to decreased oxygen supply (Pörtner, 2001; Verberk et al., 2011).

Ectotherm CT_{MIN} values typically track minimum temperatures in both terrestrial and aquatic environments (Addo-Bediako et al., 2000; Sunday et al., 2012), and we found similar patterns in aquatic insects. In tropical stream insects, CT_{MIN} values decreased with increasing elevation (Figure 3). Thus, high elevation tropical species are considerably more cold-tolerant than low elevation species. For instance, tropical Ephemeroptera at the highest elevation could withstand short periods of freezing during these experiments (A. Shah pers. obs.). This high cold tolerance reflects the persistent cold temperatures in the tropical highlands (Figure 1b). In temperate streams, by contrast, near freezing temperatures routinely occur at all elevations in the winter, and not surprisingly, CT_{MIN} values for the temperate taxa were near freezing and invariant across elevation (Figure 3). Indeed, our experiments with temperate insects often had to be terminated due to ice formation in the experimental water bath rather than the cessation of locomotor function. Such high levels of cold tolerance in temperate species call into question the ecological relevance of CT_{MIN} , and suggest that many

temperate species could be freeze-tolerant. Although the streams we sampled never freeze solid and water temperatures remain at or above 0°C, many aquatic and semi-aquatic insect species have been documented to be freeze tolerant (e.g. Danks, 2007; Sinclair, Vernon, Klok, & Chown, 2003).

The measurement of critical thermal limits can be affected by methodological factors like the acclimation temperature and ramping rate (Rezende et al., 2011; Terblanche et al., 2007). We avoided this problem using the same methodological approach for all species. The only source of environmental variation across species was acclimating species at the average stream temperature at the time of capture. While such an approach minimizes the effects of thermal stress on estimates of critical thermal limits, it does not allow us to partition variation across species due to genetically based evolved differences, vs. plastic responses to the acclimation temperatures. However, certain comparisons suggest plasticity alone cannot explain the patterns. For example, our temperate and tropical low elevation sites had similar stream temperatures at the time of the experiments, and all species were acclimated at ~15°C. Despite the same acclimation temperature, there are very large differences in CT_{MAX} among species between latitudes (Figure 2). The estimates for CT_{MIN} are also informative in this regard, because CT_{MIN} is thought to be very sensitive to acclimation temperatures (Chown, 2001; Chown & Terblanche, 2007; Terblanche et al., 2007). Despite different acclimation temperatures along the elevation gradient, almost all temperate species have approximately the same CT_{MIN} (Figure 3). Thus, even when we restrict ourselves to comparisons of species reared under the same temperatures, or compare similar responses of species held under different acclimation temperatures, we still find the influence of latitude and elevation on species differences.

4.4 | Implications for dispersal and vulnerability

Determining critical maximum and minimum limits lends insights into the physiological mechanisms driving species distributions and vulnerability of organisms to climate warming (Chown & Gaston, 2015). Janzen (1967) predicted that the narrow thermal tolerances of tropical organisms result in reduced dispersal and narrower elevation ranges. Linking physiological tolerance to elevation range size is challenging (Bozinovic et al., 2011), but our results suggest that the wider thermal breadths of temperate Ephemeroptera may permit increased dispersal ability and broader elevation distributions compared to their tropical counterparts (Gill et al., 2016). Other studies have documented similar differences in the elevation range sizes of temperate and tropical organisms (e.g. Chan et al., 2016; McCain, 2009), but the degree to which physiology alone determines geographic distributions remains a topic of debate (Bozinovic et al., 2011; Jankowski, Londono, Robinson, & Chappell, 2013).

Narrow thermal breadths are predicted to increase species vulnerability to rapid global climate change (e.g. Calosi, Bilton, & Spicer, 2008; Calosi, Bilton, Spicer, et al., 2008; Chan et al., 2016; Deutsch et al., 2008; García-Robledo, Kuprewicz, Staines, Erwin, & Kress,

2016). A key result from our study is that lower elevation tropical aquatic insect populations appear to be especially vulnerable to short-term extreme warming (or cooling) events. Consequently, even though the magnitude of projected warming is predicted to be relatively small in the tropics, such reduced thermal tolerance may increase vulnerability (see Deutsch et al., 2008). The degree to which dispersal to higher elevations could ameliorate such impacts in aquatic insects remains open to debate, because most long-distance dispersal is confined to the short-lived adult stage (Kovats, Ciborowski, & Corkum, 1996). Perhaps surprisingly, the least vulnerable group of aquatic insects may be low elevation temperate species that naturally experience wide seasonal fluctuations in stream temperature, possess relatively broad thermal breadths, and appear to have the potential to disperse to higher elevations (Finn et al., 2016).

In summary, the interaction between elevation and latitude provides a cautionary lesson when generalizing across temperate and tropical organisms, particularly with respect to vulnerability and sensitivity to changes in temperature. Physiologically, high elevation temperate and tropical aquatic insects may exhibit similarly narrow thermal breadths, whereas low elevation temperate and tropical species represent the ends of the thermal tolerance continuum with the broadest and narrowest thermal breadths, respectively. Acknowledging these differences and incorporating them into future climate scenarios will likely provide more accurate predictions of how different populations will be impacted by warming.

ACKNOWLEDGEMENTS

This paper was supported by the U.S. National Science Foundation through a collaborative Dimensions of Biodiversity grant DEB-1046408, DEB-1045960, and DEB-1045991; and a Graduate Research Fellowship DGE-1321845 awarded to A.A.S. We thank B. Choat, L. Nagle, M. Rojas, T. Waneka, and numerous other assistants for field and lab work, and K. Anderson for logistical support. We also thank J. Anderson, J. Bernardo, S. Brinkman, and A.R. Shah for their ideas and technical assistance in designing the CT_{MAX} and CT_{MIN} experiments. The Ministerio del Ambiente of Ecuador provided research permits (#56-IC-FAU/FLO-DPN/MA, MAE-DNB-CM-2015-0017).

AUTHORS' CONTRIBUTIONS

N.P., C.G., C.F., A.E., A.F., J.G., B.K., S.T., and K.Z. conceived the ideas. A.S. and C.G. designed methodology; A.S. collected and analysed the data; B.G. produced DNA barcodes and conducted the PGLS analysis; A.S. led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

DATA ACCESSIBILITY

Data are available from the Dryad Digital Repository <https://doi.org/10.5061/dryad.g1372> (Shah et al., 2017).

REFERENCES

- Addo-Bediako, A., Chown, S. L., & Gaston, K. J. (2000). Thermal tolerance, climatic variability and latitude. *Proceedings of the Royal Society of London B: Biological Sciences*, 174, 595–612.
- Angilletta, M. J. (2009). *Thermal adaptation: A theoretical and empirical synthesis*. Oxford, UK: Oxford University Press.
- Boulton, A. J., Boyero, L., Covich, A. P., Dobson, M., Lake, S., & Pearson, R. (2008). Are tropical streams ecologically different from temperate streams. In D. Dudgeon (Ed.), *Tropical stream ecology* (pp. 257–284). London, UK: Academic Press.
- Bozinovic, F., Calosi, P., & Spicer, J. I. (2011). Physiological correlates of geographic range in animals. *Annual Review of Ecology, Evolution, and Systematics*, 42, 155–179.
- Brattstrom, B. H. (1968). Thermal acclimation in anuran amphibians as a function of latitude and altitude. *Comparative Biochemistry and Physiology*, 24, 93–111.
- Butler, M. A. & King, A. A. (2004). Phylogenetic comparative analysis: A modeling approach for adaptive evolution. *The American Naturalist*, 164, 683–695.
- Calosi, P., Bilton, D. T., & Spicer, J. I. (2008). Thermal tolerance, acclimatory capacity and vulnerability to global climate change. *Biology Letters*, 4, 99–102.
- Calosi, P., Bilton, D. T., Spicer, J. I., & Atfield, A. (2008). Thermal tolerance and geographical range size in the *Agabus brunneus* group of European diving beetles (Coleoptera: Dytiscidae). *Journal of Biogeography*, 35, 295–305.
- Calosi, P., Bilton, D. T., Spicer, J. I., Votier, S. C., & Atfield, A. (2010). What determines a species' geographical range? Thermal biology and latitudinal range size relationships in European diving beetles (Coleoptera: Dytiscidae). *Journal of Animal Ecology*, 79, 194–204.
- Chan, W. P., Chen, I. C., Colwell, R. K., Liu, W. C., Huang, C. Y., & Shen, S. F. (2016). Seasonal and daily climate variation have opposite effects on species elevational range size. *Science*, 351, 1437–1439.
- Chown, S. L. (2001). Physiological variation in insects: Hierarchical levels and implications. *Journal of Insect Physiology*, 47, 649–660.
- Chown, S. L., Duffy, G. A., & Sørensen, J. G. (2015). Upper thermal tolerance in aquatic insects. *Current Opinion in Insect Science*, 11, 78–83.
- Chown, S. L. & Gaston, K. J. (2015). Macrophysiology—progress and prospects. *Functional Ecology*, 30, 330–344.
- Chown, S. L. & Terblanche, J. S. (2007). Physiological diversity in insects: Ecological and evolutionary contexts. *Advances in Insect Physiology*, 33, 50–152.
- Clark, E., Webb, B. W., & Ladle, M. (1999). Microthermal gradients and ecological implications in Dorset rivers. *Hydrological Processes*, 13, 423–438.
- Danks, H. V. (2007). The elements of seasonal adaptations in insects. *The Canadian Entomologist*, 139, 1–44.
- Deutsch, C. A., Tewksbury, J. J., Huey, R. B., Sheldon, K. S., Ghalambor, C. K., Haak, D. C., & Martin, P. R. (2008). Impacts of climate warming on terrestrial ectotherms across latitude. *Proceedings of the National Academy of Sciences USA*, 105, 6668–6672.
- Dillon, M. E., Frazier, M. R., & Dudley, R. (2006). Into thin air: Physiology and evolution of alpine insects. *Integrative and Comparative Biology*, 46, 49–61.
- Dobzhansky, T. (1950). Evolution in the tropics. *American Scientist*, 38, 209–221.
- Duarte, H., Tejedo, M., Katzenberger, M., Marangoni, F., Baldo, D., Beltrán, J. F., ... Gonzalez-Voyer, A. (2012). Can amphibians take the heat? Vulnerability to climate warming in subtropical and temperate larval amphibian communities. *Global Change Biology*, 18, 412–421.
- Feder, M. E. (1978). Environmental variability and thermal acclimation in neotropical and temperate zone salamanders. *Physiological Zoology*, 51, 7–16.
- Finn, D. S., Encalada, A. C., & Hampel, H. (2016). Genetic isolation among mountains but not between stream types in a tropical high-altitude mayfly. *Freshwater Biology*, 61, 702–714.
- García-Robledo, C., Kuprewicz, E. K., Staines, C. L., Erwin, T. L., & Kress, W. J. (2016). Limited tolerance by insects to high temperatures across tropical elevational gradients and the implications of global warming for extinction. *Proceedings of the National Academy of Sciences USA*, 113, 680–685.
- Gaston, K. J. & Chown, S. L. (1999). Why Rapoport's rule does not generalise. *Oikos*, 84, 309–312.
- Gaston, K. J., Chown, S. L., Calosi, P., Bernardo, J., Bilton, D. T., Clarke, A., ... van Kleunen, M. (2009). Macrophysiology: A conceptual reunification. *The American Naturalist*, 174, 595–612.
- Gaston, K. J. & Spicer, J. I. (1998). Do upper thermal tolerances differ in geographically separated populations of the beachflea *Orchestia gammarellus* (Crustacea: Amphipoda)? *Journal of Experimental Marine Biology and Ecology*, 229, 265–276.
- Ghalambor, C. K., Huey, R. B., Martin, P. R., Tewksbury, J. J., & Wang, G. (2006). Are mountain passes higher in the tropics? Janzen's hypothesis revisited. *Integrative and Comparative Biology*, 46, 5–17.
- Gill, B. A., Kondratieff, B. C., Casner, K. L., Encalada, A. C., Flecker, A. S., Gannon, D. G., ... Funk, W. C. (2016). Cryptic species diversity reveals biogeographic support for the 'Mountain Passes and Higher in the Tropics' Hypothesis. *Proceedings of the Royal Society of London B: Biological Sciences*, 283, 20160553.
- Grafen, A. (1989). The phylogenetic regression. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, 326, 119–157.
- Gutiérrez-Pesquera, L. M., Tejedo, M., Olalla-Tárraga, M. Á., Duarte, H., Nicieza, A., & Solé, M. (2016). Testing the climate variability hypothesis in thermal tolerance limits of tropical and temperate tadpoles. *Journal of Biogeography*, 43, 1166–1178.
- Hajibabaei, M., Ivanova, N. V., Ratnasingham, S., Dooh, R. T., Kirk, S. L., Mackie, P. M., & Hebert, P. D. (2005). Critical factors for assembling a high volume of DNA barcodes. *Philosophical Transactions of the Royal Society of London B: Biological Sciences*, 360, 1959–1967.
- Hansen, T. F. (1997). Stabilizing selection and the comparative analysis of adaptation. *Evolution*, 51, 1341.
- Hebert, P. D., Stoeckle, M. Y., Zemlak, T. S., & Francis, C. M. (2004). Identification of birds through DNA barcodes. *PLoS Biology*, 2, e312.
- Hoffmann, A. A., Chown, S. L., & Clusella-Trullas, S. (2013). Upper thermal limits in terrestrial ectotherms: How constrained are they? *Functional Ecology*, 27, 934–949.
- Huey, R. B. (1978). Latitudinal pattern of between-altitude faunal similarity: Mountains might be "higher" in the tropics. *American Naturalist*, 112, 225–254.
- Huey, R. B., Deutsch, C. A., Tewksbury, J. J., Vitt, L. J., Hertz, P. E., Pérez, H. J. Á., & Garland, T. (2009). Why tropical forest lizards are vulnerable to climate warming. *Proceedings of the Royal Society of London B: Biological Sciences*, 276, 1939–1948.
- Huey, R. B. & Stevenson, R. D. (1979). Integrating thermal physiology and ecology of ectotherms: A discussion of approaches. *American Zoologist*, 19, 357–366.
- Ivanova, N. V., DeWaard, J. R., Hajibabaei, M., & Hebert, P. D. N. (2006). Protocols for high-volume DNA barcode analysis.
- Ivanova, N. V., DeWaard, J. R., & Hebert, P. D. (2006). An inexpensive, automation friendly protocol for recovering high quality DNA. *Molecular Ecology Notes*, 6, 998–1002.
- Jacobsen, D., Rostgaard, S., & Vasconez, J. J. (2003). Are macroinvertebrates in high altitude streams affected by oxygen deficiency? *Freshwater Biology*, 48, 2025–2032.
- Jankowski, J. E., Londono, G. A., Robinson, S. K., & Chappell, M. A. (2013). Exploring the role of physiology and biotic interactions in determining elevational ranges of tropical animals. *Ecography*, 36, 1–12.
- Janzen, D. H. (1967). Why mountain passes are higher in the tropics. *American Naturalist*, 101, 233–249.
- Jones, J. D. (1972). *Comparative physiology of respiration*. London, UK: Edward Arnold.

- Kaspari, M., Clay, N. A., Lucas, J., Yanoviak, S. P., & Kay, A. (2015). Thermal adaptation generates a diversity of thermal limits in a rainforest ant community. *Global Change Biology*, 21, 1092–1102.
- Kingsolver, J. G., Woods, H. A., Buckley, L. B., Potter, K. A., MacLean, H. J., & Higgins, J. K. (2011). Complex life cycles and the responses of insects to climate change. *Integrative and Comparative Biology*, 51, 719–732.
- Kovats, Z., Ciborowski, J. A. N., & Corkum, L. (1996). Inland dispersal of adult aquatic insects. *Freshwater Biology*, 36, 265–276.
- Lutterschmidt, W. I. & Hutchison, V. H. (1997). The critical thermal maximum: History and critique. *Canadian Journal of Zoology*, 75, 1561–1574.
- McCain, C. M. (2009). Vertebrate range sizes indicate that mountains may be 'higher' in the tropics. *Ecology Letters*, 12, 550–560.
- Mohseni, O. & Stefan, H. G. (1999). Stream temperature/air temperature relationship: A physical interpretation. *Journal of Hydrology*, 218, 128–141.
- Pörtner, H. (2001). Climate change and temperature-dependent biogeography: Oxygen limitation of thermal tolerance in animals. *Naturwissenschaften*, 88, 137–146.
- Pörtner, H. O. & Knust, R. (2007). Climate change affects marine fishes through the oxygen limitation of thermal tolerance. *Science*, 315, 95–97.
- R Core Team. (2013). *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing. Retrieved from: <http://www.R-project.org/>
- Rezende, E. L., Tejedo, M., & Santos, M. (2011). Estimating the adaptive potential of critical thermal limits: Methodological problems and evolutionary implications. *Functional Ecology*, 25, 111–121.
- Shah, A. A., Gill, B. A., Encalada, A. C., Flecker, A. S., Funk, W. C., Guayasamin, J. M., ... Ghalambor, C. K. (2017). Data from: Climate variability predicts thermal limits of aquatic insects across elevation and latitude. *Dryad Digital Repository*, <https://doi.org/10.5061/dryad.g1372>
- Sheldon, K. S. & Tewksbury, J. J. (2014). The impact of seasonality in temperature on thermal tolerance and elevational range size. *Ecology*, 95, 2134–2143.
- Sinclair, B. J., Vernon, P., Klok, C. J., & Chown, S. L. (2003). Insects at low temperatures: An ecological perspective. *Trends in Ecology & Evolution*, 18, 257–262.
- Spicer, J. & Gaston, K. (2009). *Physiological diversity: Ecological implications*. London, UK: John Wiley & Sons.
- Stevens, G. C. (1989). The latitudinal gradient in geographical range: How so many species coexist in the tropics. *American Naturalist*, 133, 240–256.
- Sunday, J. M., Bates, A. E., & Dulvy, N. K. (2011). Global analysis of thermal tolerance and latitude in ectotherms. *Proceedings of the Royal Society of London B: Biological Sciences*, 278, 1823–1830.
- Sunday, J. M., Bates, A. E., & Dulvy, N. K. (2012). Thermal tolerance and the global redistribution of animals. *Nature Climate Change*, 2, 686–690.
- Terblanche, J. S., Deere, J. A., Clusella-Trullas, S., Janion, C., & Chown, S. L. (2007). Critical thermal limits depend on methodological context. *Proceedings of the Royal Society of London B: Biological Sciences*, 274, 2935–2943.
- Turriago, J. L., Parra, C. A., & Bernal, M. H. (2015). Upper thermal tolerance in anuran embryos and tadpoles at constant and variable peak temperatures. *Canadian Journal of Zoology*, 93, 267–272.
- van Berkum, F. H. (1988). Latitudinal patterns of the thermal sensitivity of sprint speed in lizards. *American Naturalist*, 132, 327–343.
- Verberk, W. C., Bilton, D. T., Calosi, P., & Spicer, J. I. (2011). Oxygen supply in aquatic ectotherms: Partial pressure and solubility together explain biodiversity and size patterns. *Ecology*, 92, 1565–1572.
- Verberk, W. C., Durance, I., Vaughan, I. P., & Ormerod, S. J. (2016). Field and laboratory studies reveal interacting effects of stream oxygenation and warming on aquatic ectotherms. *Global Change Biology*, 22, 1769–1778.
- Ward, J. V. (1985). Thermal characteristics of running waters. In B. R. Davies, & R. D. Walmsley (Eds.), *Perspectives in Southern Hemisphere limnology* (pp. 31–46). Dordrecht, The Netherlands: Springer.
- Ward, J. V. & Stanford, J. A. (1982). Thermal responses in the evolutionary ecology of aquatic insects. *Annual Review of Entomology*, 27, 97–117.
- Wu, C. S. & Kam, Y. C. (2005). Thermal tolerance and thermoregulation by Taiwanese rhacophorid tadpoles (*Buergeria japonica*) living in geothermal hot springs and streams. *Herpetologica*, 61, 35–46.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

How to cite this article: Shah AA, Gill BA, Encalada AC, et al. Climate variability predicts thermal limits of aquatic insects across elevation and latitude. *Funct Ecol*. 2017;31:2118–2127. <https://doi.org/10.1111/1365-2435.12906>