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Thermal Acclimation Ability Varies in Temperate and Tropical Aquatic Insects from Different Elevations

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Synopsis It has long been recognized that populations and species occupying different environments vary in their thermal tolerance traits. However, far less attention has been given to the impact of different environments on the capacity for plastic adjustments in thermal sensitivity, i.e., acclimation ability. One hypothesis is that environments characterized by greater thermal variability and seasonality should favor the evolution of increased acclimation ability compared with environments that are aseasonal or thermally stable. Additionally, organisms under selection for high heat tolerance may experience a trade-off and lose acclimation ability. Few studies have tested these non-mutually exclusive hypotheses at both broad latitudinal and local elevation scales in phylogenetically paired taxa. Here, we measure short-term acclimation ability of the critical thermal maximum (CT_{MAX}) in closely related temperate and tropical mayflies (Ephemeroptera) and stoneflies (Plecoptera) from mountain streams at different elevations. We found that stream temperature was a good predictor of acclimation ability in mayflies, but not in stoneflies. Specifically, tropical mayflies showed reduced acclimation ability compared with their temperate counterparts. High elevation tropical mayflies had greater acclimation ability than low elevation mayflies, which reflected the wider temperature variation experienced in high elevation streams. In contrast, temperate and tropical stoneflies exhibited similar acclimation responses. We found no evidence for a trade-off between heat tolerance and acclimation ability in either taxonomic order. The acclimation response in stoneflies may reflect their temperate origin or foraging mode. In combination with previous studies showing tropical taxa have narrower thermal breadths, these results demonstrate that many lower elevation tropical aquatic insects are more vulnerable to climate warming than their temperate relatives.

Introduction

Thermal acclimation is a form of plasticity that enables organisms to adjust their physiology following chronic or brief exposure to thermal stimuli (Bowler 2005; Angilletta 2009). Yet, the extent to which organisms exhibit thermal acclimation varies among species (e.g., Brattstrom 1968; Addo-Bediako et al. 2000; Deutsch et al. 2008; Beaman et al. 2016), types of traits (e.g., CT_{MAX}, CT_{MIN}, metabolic rate, performance; Johnson and Bennett 1995; Stillman 2003; Terblanche et al. 2005; Terblanche and Chown 2006; Calosi et al. 2008), and habitats (e.g., temperate, tropical, elevation; Feder 1978, 1982; Tsuji 1988). One fundamental challenge is therefore to understand what selection pressures

act on and shape acclimation ability in organisms (Beaman et al. 2016).

Theoretical models suggest that populations from more thermally variable environments should be under greater selection for the capacity to be plastic, because the presumed benefits of maintaining thermal plasticity exceed any potential costs (e.g., Levins 1968; Gavrillets and Scheiner 1993; Hoffmann 1995; Angilletta 2009). At macrophysiological scales, this theory has led to the general prediction that organisms living at low, tropical latitudes should have a reduced acclimation capacity compared with those from temperate environments, because temperate latitudes undergo greater seasonal changes in temperature (Janzen 1967; Ghalambor et al. 2006;

Tewksbury et al. 2008; Angilletta 2009; Gunderson and Stillman 2015). Indeed, the role of temperature or climate variability in shaping species acclimation ability between high and low latitudes is one of the primary predictions of Daniel Janzen's "climate variability hypothesis" (1967). Janzen proposed that organisms from temperate mountains should evolve broad thermal breadths and greater acclimation abilities in response to the large seasonal fluctuations and overlap in temperature across elevations. Selection for broader thermal breadths or increased acclimation responses should subsequently enable temperate organisms to disperse across elevations, unhindered by the changes in temperature they encounter. In contrast, the lack of seasonal variation and reduced overlap in temperature across elevations in the tropics should favor the evolution of narrower thermal breadths and reduced acclimation ability (reviewed in Ghalambor et al. 2006). However, the relationship between thermal breadths and acclimation capacity can be shaped by other factors. It has been proposed that the evolution of high thermal tolerance should come at the expense of acclimation ability (Cavicchi et al. 1995; Stillman 2003; Overgaard et al. 2011; Gunderson and Stillman 2015). The "trade-off hypothesis" (*sensu* Gunderson and Stillman 2015) therefore predicts a negative relationship between increased thermal breadths (particularly for tolerance to warmer temperatures) and acclimation ability, whereas the climate variability hypothesis predicts a positive relationship.

Macrophysiological comparisons of temperate and tropical organisms provide ideal conditions for testing how thermal limits and acclimation ability vary independently or jointly. To date, there is growing evidence that tropical organisms have narrower thermal breadths compared with temperate species (Ghalambor et al. 2006; Deutsch et al. 2008; Tewksbury et al. 2008; Gaston et al. 2009; Huey et al. 2009; Sunday et al. 2011). However, evidence for differences in acclimation ability due to climate variability (i.e., in support of the climate variability hypothesis) is mixed (Angilletta 2009). For example, while some studies have found reduced acclimation ability in tropical organisms (Feder 1978, 1982; Tsuji 1988; García-Robledo et al. 2016), other studies have found little or no difference in acclimation responses of temperate and tropical species (Hoffmann and Watson 1993; Gunderson and Stillman 2015; Seebacher et al. 2015; van Heerwaarden et al. 2016). Similarly, some studies have found evidence for the trade-off between thermal tolerance and acclimation ability (e.g., Cavicchi et al. 1995; Stillman 2003), but other studies do not find such evidence

(e.g., Calosi et al. 2008; Gunderson and Stillman 2015). These mixed results could, in part, reflect how differences in local temperature variation shape thermal acclimation. For example, tropical high elevation sites can exhibit temperature fluctuations on a daily basis that are similar to those experienced between summer and winter in temperate regions (Mani 1968; Ghalambor et al. 2006; Shah et al. 2017). Thus, latitudinal comparisons of critical thermal limits and acclimation ability could be complicated by the degree of local variation in temperature due to elevation.

Understanding what factors ultimately shape patterns of thermal plasticity requires comparisons across diverse regions and taxa (Seebacher et al. 2015). Here, we focus on temperate and tropical aquatic insects; a group that plays an important ecological role in freshwater habitats, but has been largely ignored compared with terrestrial insects (Chown et al. 2015). We test predictions from two non-mutually exclusive hypotheses: (1) the climate variability hypothesis, which predicts that increased local or regional temperature variability is positively correlated with increased acclimation ability (e.g., Brattstrom 1968; Patterson 1984; Rogowitz 1996), and (2) the trade-off hypothesis, which predicts that increased thermal tolerance is negatively correlated with acclimation ability (Stillman 2003). In addition to addressing the long-standing question of what factors shape patterns of thermal physiology in organisms, understanding geographic variation in acclimation ability is paramount to assessing vulnerability and species response to climate change (Sgrò et al. 2016).

We measured the short-term acclimation response (48 h; defined in Bowler 2005) of critical thermal maximum temperatures (CT_{MAX}) in two orders of freshwater aquatic insects, mayflies (Ephemeroptera) and stoneflies (Plecoptera). Previous work has shown that the thermal breadths (the difference between CT_{MAX} and CT_{MIN}) of these insects closely track environmental temperature variation (Shah et al. 2017). Here, we focused on short-term acclimation because both temperate and tropical aquatic insects experience short-term changes in temperature, whereas only temperate species experience long-term, seasonal changes in temperature. For instance, insects in high elevation temperate and tropical streams can experience rapid temperature changes because of the combined influences of reduced vegetation cover, cloud cover, and weather events that persist for several days (see the "Methods" section). The same taxonomic families can also be found in temperate and tropical regions facilitating

phylogenetically paired comparisons. Such conditions make freshwater streams an excellent model system to test how large-scale climate variability and local variation along an elevation gradient shapes patterns of thermal acclimation.

Methods

Study sites and species

We collected aquatic insects from shallow paired streams at ~2000 m (“low elevation”, hereafter) and ~2800 m (“mid elevation”, hereafter) in the Colorado Rocky Mountains and the Ecuadorian Andes between the months of June and December 2014 and 2015. We also collected tropical mayflies from a “high elevation” stream in Ecuador (~3683 m) but were unable to collect from an equivalent high elevation in the Colorado study site because of a lack of accessibility. To assess how stream temperature ranges differed across latitude and elevation, we recorded temperature in each stream using HOBO loggers (Onset Corporation). We calculated temperature range from the logger data collected over an 8–12-month period. We collected mayflies (Ephemeroptera) within the family Baetidae (*Baetis* spp. in Colorado and *Andesiops* spp. in Ecuador) and stoneflies (Plecoptera) within the families Perlidae (*Hesperoperla* sp. in Colorado and *Anacroneuria* spp. in Ecuador) and Perlodidae (*Megarcys* sp. and *Kogotus* sp. in Colorado). Most of these species occur at both latitudes, multiple elevations, and are numerically abundant in streams. Insects from each species were size-matched to reduce any effects of developmental stage on thermal physiology.

CT_{MAX} acclimation experiments

In the laboratory, we acclimated the field-caught insects for 48 h at one of two temperature treatments, “home” or “warming”, which were the same at both latitudes for a given elevation. We were specifically interested in determining whether CT_{MAX} increased in the “warming” treatment, suggesting an acclimation response. The “home” temperature treatments, calculated as the average stream temperature from the HOBO logger data, were 13 °C and 10 °C for low and mid elevation streams, respectively. The “warming” temperature treatments were 5 °C higher than the “home” temperatures. These were 18 °C and 15 °C for low and mid elevations, respectively. Thus, temperatures by elevation were paired across latitudes, because at these elevations the temperate and tropical sites had the same average temperatures. The use of different

temperature treatments between elevations within latitudes ensured insects, particularly the sensitive tropical populations, were unstressed in their “home” temperature treatments (Rezende et al. 2011). We also collected mayflies from an un-paired high elevation tropical site (3683 m, home temperature treatment = 7 °C; warming treatment = 12 °C). Because we did not have insects from a similar high elevation in Colorado, this population was analyzed separately (see the “Statistical analyses” section).

We chose a 5 °C increase from the “home” stream temperature as the “warming” treatment because it is within the range of natural variation experienced by most aquatic insects (Allan et al. 1991; Shah et al. 2017). The acclimation period of 48 h was used because it was the duration for which insects could be kept in the laboratory without exhibiting any visible signs of stress due to food deprivation (see Rezende et al. 2011; Shah et al. 2017). For example, when we attempted longer acclimation periods (>4 days), we found that insects experienced high mortality. All insects were starved during the “home” and “warming” acclimation period to ensure individuals were tested in a similar nutritional and post-absorptive state.

We measured CT_{MAX} following the 48-h acclimation period by placing up to 12 insects in separate mesh containers and immersing them in a water bath fitted with water and air pumps to provide flow and maximum oxygenation. The mesh allowed water to flow through the containers, but prevented insects from escaping during the experiment. We ramped temperature in the water bath at the rate of 0.3 °C min⁻¹ (Dallas and Rivers-Moore 2012) using a temperature controller (16C-2, Dwyer Instruments Inc.) connected to a 500 watt titanium heating rod. As temperature increased, we recorded changes in behavior (see Shah et al. 2017, for detailed documentation of behavioral changes) until insects displayed a loss of righting response (LRR, Lutterschmidt and Hutchinson 1997; Angilletta 2009). After exhibiting LRR, each insect was returned to cooler water for recovery. Only data from insects that recovered from the experiments were used in the analyses. After recovery, insects were euthanized in 95% EtOH, dried for 24 h at 56 °C, and weighed to obtain individual dried body mass measurements.

Statistical analyses

We used R version 3.1.3 (R Core Team 2013) for all statistical analyses. Between latitudes, the acclimation temperatures were the same at each elevation, facilitating direct statistical comparisons between

temperate and tropical insects under the same temperature treatments. For all analyses, an insect was considered to have acclimated if CT_{MAX} increased in the “warming” treatment compared with the “home” treatment. All model parameters were treated as fixed effects and effect sizes (partial η^2) were also calculated.

To test the influence of climate variability on acclimation ability, we first ran an ANOVA with CT_{MAX} as our response variable. Parameters for this analysis included the fixed factors latitude, treatment, elevation, taxonomic order, all interactions, and dry weight as a covariate to account for body size. Next, we assessed the effect of temperature variability on acclimation ability by directly comparing the reaction norms of the different insect populations. We did this by conducting separate analyses for each taxonomic order to control for phylogeny, and each elevation sampled across both latitudes. The models included latitude and treatment as fixed effects, an interaction between latitude and treatment, and dry weight as a covariate. We were particularly interested in finding whether there was a significant interaction indicating that acclimation ability (i.e., the slope of the reaction norm) differed between temperate and tropical populations of insects from a given elevation. Third, for the un-paired high elevation stream in Ecuador, we ran an analysis in which we only tested the treatment effect, because there was no Colorado counterpart. Fourth, we analyzed within-latitude differences in acclimation ability separately for each taxonomic order to assess the effect of elevation on acclimation ability. If thermal variability at a given elevation influences acclimation ability, we predicted a stronger acclimation response with increasing temperature variation. We included elevation and treatment as fixed effects with an interaction between elevation and treatment. As with the previous analysis with latitude, a significant elevation by treatment interaction would indicate that there are differences in acclimation ability among insects from different elevations. However, because different acclimation temperatures were used at different elevations, such comparisons must be interpreted cautiously. Finally, we tested the directional hypothesis that increased stream temperature variability is correlated with the percent change in CT_{MAX} (i.e., acclimation ability) for the two taxonomic orders.

To assess whether there is a trade-off between thermal breadth and acclimation ability, we tested the directional hypothesis that the percent change in CT_{MAX} decreases with increasing CT_{MAX} . For these analyses, we considered a decrease in CT_{MAX} after acclimation to be a non-acclimatory response.

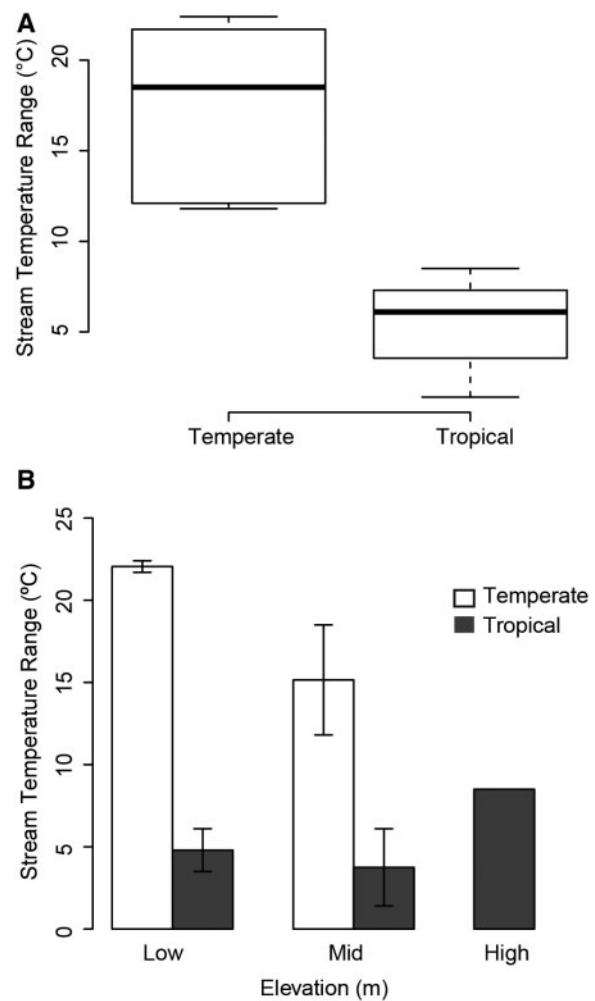


Fig. 1 Average stream temperature range (annual maximum – minimum) at temperate and tropical latitudes (**A**) and across low, mid, and high elevations (**B**).

Results

Temperature loggers placed in each stream at both latitudes revealed that stream temperature range was greater in temperate (Colorado) streams than in tropical (Ecuador) streams (Fig. 1A). Within the temperate latitude site, temperature ranges in low elevation streams were greater than mid elevation streams. However, the pattern was reversed in tropical streams where low and mid elevation streams had a narrower temperature range compared with the high elevation stream (Fig. 1B; also see Shah et al. 2017).

The full ANOVA model results showed that latitude, treatment, elevation, and taxonomic order had a significant effect on CT_{MAX} . Dry weight and all interactions were non-significant (Table 1). When we tested if acclimation varied among our paired temperate and tropical taxa at the same elevation,

Table 1 Output of the full model ANOVA in which latitude, treatment, and order are fixed effects, and dry weight is a covariate. The fixed effects are all significant but the interactions are not. Dry weight is not a significant covariate

Factor	F (df)	P-value	Partial η^2 (effect size)
Latitude	68.49 (1, 366)	<0.001	0.016
Treatment	56.74 (1, 366)	<0.001	0.133
Elevation	62.87 (2, 366)	<0.001	0.240
Order	78.54 (2, 366)	<0.001	0.305
Dry weight	0.26 (1, 366)	0.61	0.61
Latitude \times Treatment	1.24 (1, 366)	0.27	0.27
Latitude \times Elevation	0.002 (1, 366)	0.96	0.96
Treatment \times Elevation	0.20 (2, 366)	0.81	0.81
Latitude \times Treatment \times Elevation	0.36 (1, 366)	0.55	0.55

we found the latitude \times treatment interaction to be significant for all but the low elevation stoneflies (Table 2). Specifically, when examining the thermal reaction norms, we see that temperate, but not tropical mayflies increased CT_{MAX} in response to the warming treatment at the low and mid elevations (Fig. 2A, B; Table 3). Three of the stonefly populations exhibited a significant acclimation response across latitude (Fig. 3A, B), with no differences in the slope between latitudes at the low elevation site (Fig. 3A; Table 2). Interestingly, a significant latitude \times treatment interaction at the mid elevation revealed that the tropical species had a greater acclimation response compared with its temperate counterpart (Fig. 3B; Table 2). When we compared the acclimation response of the low and mid-elevation mayflies with the unpaired high elevation population, we found only the high elevation population exhibited a significant acclimation response (Table 2; Fig. 4).

Finally, we found a positive correlation between stream temperature variation and percent change in CT_{MAX} ($r = 0.85$; one-tailed P -value = 0.036) for mayflies, consistent with predictions from the climate variability hypothesis, but found no correlation in stoneflies ($r = -0.31$; one-tailed P -value = 0.347; Fig. 5A). We found no evidence of the trade-off hypothesis in mayflies ($r = -0.01$; one-tailed P -value = 0.492) or in stoneflies ($r = -0.79$; one-tailed P -value = 0.105; Fig. 5B).

Discussion

The capacity for thermal acclimation is thought to be an adaptive response that allows organisms to adjust their physiology and track variable environmental temperatures (Kingsolver and Huey 1998;

Table 2 Output of models assessing the latitude \times treatment interaction for mayflies and stoneflies from different elevations

Factor	F (df)	P-value	Partial η^2 (effect size)
Low elevation mayflies			
Latitude	14.41 (1, 26)	<0.001	0.35
Treatment	0.10 (1, 26)	0.75	0.00
Dry weight	1.00 (1, 26)	0.32	0.00
Latitude \times Treatment	4.62 (1, 26)	0.04	0.17
Mid elevation mayflies			
Latitude	2.52 (1, 119)	0.12	0.02
Treatment	17.34 (1, 119)	<0.001	0.13
Dry weight	0.05 (1, 119)	0.82	0.00
Latitude \times Treatment	5.97 (1, 119)	0.02	0.05
High elevation mayflies			
Treatment	8.23 (1, 21)	0.01	n/a
Dry weight	6.42 (1, 21)	0.02	n/a
Low elevation stoneflies			
Latitude	50.05 (1, 20)	<0.001	0.71
Treatment	12.57 (1, 20)	0.002	0.39
Dry weight	1.79 (1, 20)	1.20	0.08
Latitude \times Treatment	0.70 (1, 20)	0.41	0.03
Mid elevation stoneflies			
Latitude	200.36 (1, 93)	<0.001	0.68
Treatment	8.65 (1, 93)	0.004	0.09
Dry weight	3.55 (1, 93)	0.06	0.04
Latitude \times Treatment	5.17 (1, 93)	0.03	0.05

Gunderson and Stillman 2015; Seebacher et al. 2015). Nevertheless, the relationships between environmental thermal variability, thermal limits, and acclimation ability continue to be debated. Here, we tested two mutually non-exclusive hypotheses that have been proposed to explain variation in whole organism acclimation ability. The “climate variability hypothesis” posits that organisms from more variable climates should be under selection for greater thermal acclimation ability than those from stable climates (Janzen 1967; Ghalambor et al. 2006; Angilletta 2009). The “trade-off hypothesis” proposes that organisms that evolve high levels of thermal tolerance do so at the expense of their acclimation ability (Stillman 2003). We found support for the prediction that more variable thermal environments select for greater acclimation ability in mayflies. Temperate mayflies and high elevation tropical mayflies that experience more variable temperatures exhibit a significant acclimation response ($\sim 2^\circ\text{C}$ increase in CT_{MAX} over a 5°C warming treatment), whereas tropical mayflies from less variable lower

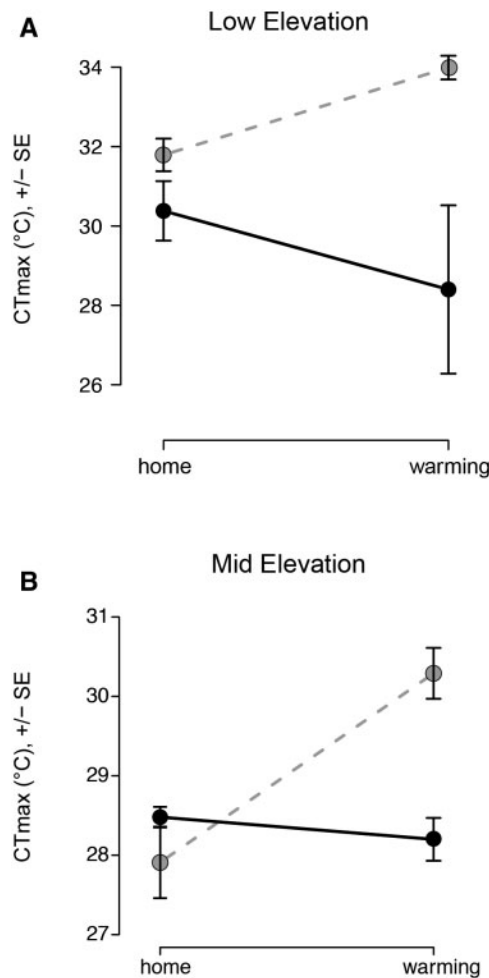


Fig. 2 Reaction norms showing acclimation response in mayflies from low elevation (**A**; home = 13 °C, warming = 18 °C) and mid elevation (**B**; home = 10 °C, warming = 15 °C). Temperate mayflies (gray circles, dashed line) exhibited a greater acclimation response than tropical mayflies (black circles, solid line).

and middle elevations do not exhibit an acclimation response ($\sim 0\text{--}0.5^\circ\text{C}$ increase in CT_{MAX} over a 5°C warming treatment; **Figs. 2A, B and 4**). In contrast, the stoneflies generally exhibit a similar acclimation response at all latitudes and elevations despite having relatively high upper critical temperatures (**Fig. 3A, B**). Thus, climate variability predicted acclimation ability in mayflies, but not in stoneflies (**Fig. 5A**). We did not find support for the trade-off hypothesis, as there was no general relationship between increasing thermal tolerance and reduced acclimation ability (**Fig. 5B**). The contrasting results between mayflies and stoneflies may reflect differences in evolutionary history, functional roles, challenges associated with aquatic life, or some combination of these factors. Below we discuss these results within the context of how temperature varies across latitude and elevation.

Table 3 Output of models assessing the elevation \times treatment interaction for mayflies and stoneflies from temperate and tropical latitudes

Factor	<i>F</i> (df)	<i>P</i> -value	Partial η^2 (effect size)
Tropical mayflies			
Elevation	5.47 (2, 58)	0.01	0.16
Treatment	0.06 (1, 58)	0.81	0.00
Dry weight	3.11 (1, 58)	0.08	0.05
Elevation \times Treatment	3.00 (2, 58)	0.05	0.09
Tropical stoneflies			
Elevation	230.77 (1, 44)	<0.001	0.84
Treatment	47.39 (1, 44)	<0.001	0.52
Dry weight	2.08 (1, 44)	0.12	0.05
Elevation \times Treatment	1.69 (1, 44)	0.20	0.04
Temperate mayflies			
Elevation	31.41 (1, 109)	<0.001	0.22
Treatment	24.32 (1, 109)	<0.001	0.18
Dry weight	0.02 (1, 109)	0.89	0.00
Elevation \times Treatment	0.02 (1, 109)	0.90	0.00
Temperate stoneflies			
Elevation	8.72 (1, 69)	0.004	0.11
Treatment	1.96 (1, 69)	0.17	0.03
Dry weight	12.43 (1, 69)	<0.001	0.15
Elevation \times Treatment	1.56 (1, 69)	0.22	0.02

Across latitude, tropical and temperate streams exhibit significant differences in temperature range (**Fig. 1A, B**). The warm summers and cold winters that characterize Rocky Mountain seasons result in wide ranges of annual stream temperatures. This range (i.e., from freezing to the warmest summer temperatures) is typically experienced from May, just before the spring-melt, through late August. Small ectotherms, such as insects, are highly responsive to their thermal environment because their body temperature closely matches that of their surroundings. Unlike terrestrial insects, aquatic insect larvae can remain active year-round and experience the full range of temperature variation at a given site. For example, stonefly larvae often live for 2–3 years, thus encountering repeated seasonal shifts in temperature. Similarly, while mayflies overwinter as eggs, they hatch in early June when snowmelt causes stream temperatures to be very low. These larvae rapidly develop and emerge as flying adults a few months later, when temperatures peak in the summer, thus experiencing the full range of seasonal variation during their development. In the aseasonal tropics, streams typically exhibit far less temperature variation (**Fig. 1A, B**; see also **Shah et al. 2017**) except

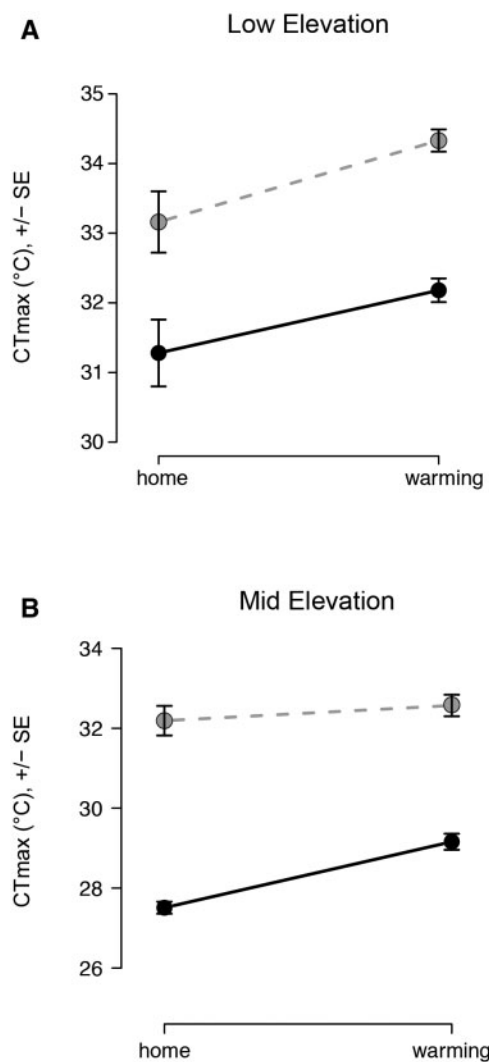


Fig. 3 Reaction norms showing acclimation response in stoneflies from low elevation (**A**; home = 13 °C, warming = 18 °C) and mid elevation (**B**; home = 10 °C, warming = 15 °C). There were no differences in acclimation responses between low elevation temperate (gray circles, dashed line) and tropical (black circles, solid line) stoneflies, but a borderline significant difference between the two groups at mid elevation, where tropical stoneflies acclimated better than their temperate relatives.

in the highest elevation tropical streams (Fig. 1B), where diel changes in temperature are much greater than those observed at lower tropical elevations. Thus, if temperature variation selects for acclimation ability in the tropics, it would be predicted to be more common only at high elevations, as we observed (Fig. 4).

We found greater acclimation ability in temperate mayflies compared with their tropical counterparts at similar elevations, whereas tropical stoneflies exhibited mostly similar acclimation ability compared with temperate stoneflies (Figs. 2A, B, 3A, B, and 4). In tropical streams, neither low nor mid elevation mayflies acclimated after the 48-h period (Fig. 2).

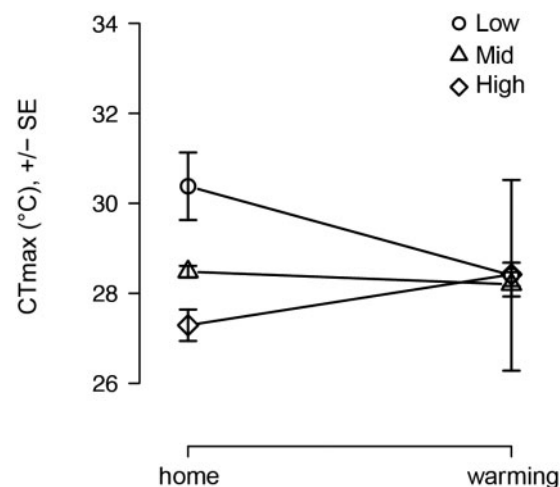


Fig. 4 Reaction norm showing differences in acclimation responses for mayflies from low (home = 13 °C, warming = 18 °C), mid (home = 10 °C, warming = 15 °C), and high (home = 7 °C, warming = 12 °C) elevations. Although no acclimation capacity was seen in mid and low elevation populations, high elevation mayflies exhibited an acclimation response.

In fact, not only was there a lack of an acclimation response in the low elevation tropical mayflies (genus *Andesiops*), but they also experienced ~55% mortality during the 48-h acclimation period prior to experimentation. We did not document such high rates of mortality in any of the other populations of insects in this study. These results suggest that even a seemingly moderate 5 °C increase in temperature can be stressful for low elevation populations. Thus, despite experiencing only slightly warmer year-round temperatures than their mid-elevation counterparts, low elevation tropical mayflies have the most reduced capacity for short-term thermal acclimation. Our results also suggest that differences in CT_{MAX} across elevations are unlikely to reflect plastic responses to different stream temperatures (Shah et al. 2017). For example, short-term acclimation to warmer temperatures does not result in mid-elevation mayflies increasing their CT_{MAX} temperatures to match that of the low elevation mayflies (Fig. 2), suggesting these populations have evolved different upper thermal limits.

Further evidence for the role of temperature variability in selecting for greater acclimation ability is observed in high elevation tropical mayflies that show a significant acclimation response (Fig. 4). High elevation tropical stream temperatures are significantly colder than lower elevation streams, but they can also vary by ~7 °C on a daily, weekly, or seasonal basis (A. A. Shah, personal observation) exposing insects to a remarkably large and rapid temperature change in an otherwise thermally stable

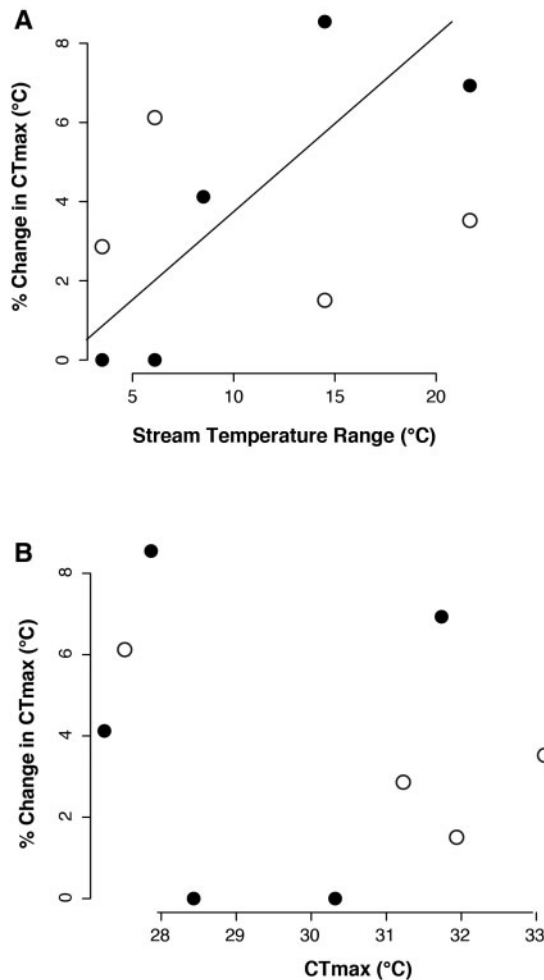


Fig. 5 Percent change in CT_{MAX} (showing acclimation ability) as a function of stream temperature variation to test the climate variability hypothesis (A). While the trend is positive for mayflies (filled circles), there is no relationship for stoneflies (open circles). B) shows acclimation capacity as a function of CT_{MAX} to test the trade-off hypothesis. We find no evidence for this hypothesis in mayflies (filled circles) or stoneflies (open circles).

environment. Shah et al. (2017) found that high elevation tropical aquatic insects had thermal breadths that were more like high elevation temperate species, rather than other tropical species. Thus, the observed plasticity in high elevation tropical mayflies is consistent with the view that it is the degree of local temperature variability that selects for thermal breadth (Shah et al. 2017) and plasticity, rather than simply latitudinal position. Indeed, there is a positive relationship between the amount of temperature variation at a given site and the magnitude of the acclimation response in mayflies (Fig. 5A). Collectively, such results suggest that mayfly acclimation responses are shaped by both large-scale seasonal changes in temperature and local temperature variation.

Tropical stoneflies acclimate as well as (Fig. 3A) or better than (Fig. 3B) their temperate relatives.

These results stand in sharp contrast to the mayfly results. While there was no general support for the trade-off hypothesis (Fig. 5B), as a group, temperate stoneflies are good candidates for tests of the trade-off hypothesis, as they have some of the highest CT_{MAX} values among all aquatic insects (Shah et al. 2017). A broader comparison of stoneflies throughout their geographic range and across a more diverse set of thermal environments is ultimately needed to test if thermal limits trade off against acclimation ability. In other aquatic organisms, studies have found evidence for acclimation capacity-thermal breadth trade-offs (e.g., Stillman 2003), but others have not (e.g., Calosi et al. 2008; Simon et al. 2015). Thus, the generality of this hypothesis remains to be explored.

The evolutionary history of stoneflies may also provide some insight into the similar acclimation capacities between temperate and tropical species. The order Plecoptera has a northern Pangean origin, and is thought to have split into two lineages, Arctoperlaria and Antarctoperlaria relatively recently compared with mayflies (McCulloch 2010). The Antarctoperlaria subsequently dispersed to South America (Zwick 2000). If tropical stoneflies retained their ancestral traits, then high CT_{MAX} values and acclimation abilities of tropical stoneflies may reflect the temperate origin of this lineage.

Differences in thermal physiology among orders could also result from alternative evolutionary responses and constraints in how respiratory physiology, life-history, foraging, and habitat use impact heat tolerance via oxygen limitation (Pörtner 2001) and oxygen availability (Verberk et al. 2011). For example, stoneflies are active predators that presumably have high oxygen demands when hunting, and maintaining acclimation ability could be necessary for their foraging success (Grigaltchik et al. 2012). In comparison, the herbivorous mayflies that “drift” along with the water flow may not require such precise thermoregulation. Stoneflies and mayflies also emerge at different times of the year, so some of the variation we observed could also be linked to differences associated with various stages of development, particularly those close to molting (Camp et al. 2014). Additional studies that include several species from multiple temperate and tropical drainages could be used to further explore the variation in acclimation of thermal limits between different taxonomic groups.

Summary and implications

We find evidence that climate variability plays an important role in determining acclimation ability

in phylogenetically related temperate and tropical mayflies but not stoneflies. More work comparing different groups of temperate and tropical insects are needed before broader generalizations can be made. Nevertheless, in combination with measures of thermal breadth (i.e., the difference between CT_{MAX} and CT_{MIN}) across a large number of temperate and tropical aquatic insect species (Shah et al. 2017), the mayfly results provide support for the role of climate variability in shaping thermal physiology across latitude and elevation gradients, as predicted by Janzen (1967).

We note that whole organism acclimation ability is only a first step in identifying how animals respond to changes in their thermal environment. Although beyond the scope of this study, a next step would include quantifying the responses to temperature at the molecular and biochemical level to better understand the physiological mechanisms driving shifts in whole organism thermal sensitivity (Hochachka and Somero 2002; Somero 2005, 2010).

These results also have important implications for how aquatic insect taxa, which are vital to healthy freshwater stream communities (Allan and Castillo 2007), will respond to warming global temperatures. Studies designed to estimate organismal responses to such changes often fail to incorporate acclimation capacity (see Gunderson et al. 2016). Freshwater ecosystems are predicted to respond to the warming of atmospheric temperatures (Daufresne and Boët 2007) and increase by a range of $+1^{\circ}\text{C}$ to $+3.7^{\circ}\text{C}$ (Bernstein et al. 2008; Stocker et al. 2013). Forecasts of climate change also suggest that thermal and natural disturbance regimes in small streams are likely to change in the near future, with warming being the most proximate effect (Bernstein et al. 2008; Stocker et al. 2013). Yet, thermal tolerance for freshwater aquatic invertebrates is not well understood (Chown et al. 2015).

Human exploits of stream environments—riparian clearing, dams, and urbanization to name a few—have also been shown to increase stream temperatures well above their natural range (e.g., Brown and Krygier 1970) over relatively short periods of time. In our study system, landslides due to heavy rains and clear-cutting at the mid-elevation tropical stream resulted in a temperature increase of $\sim 10^{\circ}\text{C}$ in just 24 h (personal observation). Our results indicate that while some acclimation ability exists in tropical aquatic insects, they are likely to be more vulnerable to warming than their temperate counterparts. This is especially true of low and mid elevation tropical mayflies. In fact, even the moderate increase in CT_{MAX} after acclimation in high

elevation tropical mayflies may not be enough to buffer those insects from warming streams. Although tropical stoneflies display some thermal plasticity, they too have lower CT_{MAX} temperatures compared with temperate species, which suggest greater sensitivity to warming. Future work should therefore address how temperature sensitivity determines species vulnerability, and alters species interactions, to better predict potential shifts in community composition of freshwater ecosystems.

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