Temperature-dependence of metabolic rate in tropical and temperate aquatic insects: support for the Climate Variability Hypothesis in mayflies but not stoneflies.

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Running Head:

Climate vulnerability of aquatic insects

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Abstract:

A fundamental gap in climate change vulnerability research is an understanding of the relative thermal sensitivity of tropical and temperate organisms. Aquatic insects are vital to stream ecosystem function and biodiversity. With global temperatures rising at an unprecedented rate, it is imperative that we understand how sensitive aquatic insects are to increasing temperature and whether sensitivities vary among taxa across latitude and elevation. We measured thermal sensitivity of standard metabolic rate in stream-dwelling mayflies and stoneflies across a ~2,000 m elevation gradient in the temperate Rocky Mountains in Colorado, U.S.A., and the tropical Andes in Napo, Ecuador. Mayflies generally adhered to the predictions of the Climate Variability Hypothesis, such that tropical species exhibited greater thermal sensitivity than did temperate species. For stoneflies, by contrast, there was no evidence in support of our predictions; temperate stoneflies showed greater thermal sensitivity than tropical relatives. Differences among mayflies and stoneflies may reflect the evolutionary history of these insect clades or different ecological roles of mayflies (often herbivorous) and stoneflies (often predatory). Collectively our results suggest that there is physiological variation across elevations

and species and low elevation tropical mayflies may be the most imperiled by climate warming. However, broad generalizations about the vulnerability of tropical ectotherms should be made with caution.

Introduction:

Metabolism is crucial to all life. It integrates an organism's existing energy supplies and materials to fuel fitness-related activities like movement, growth, tissue maintenance, and development (Dillon, Wang, & Huey, 2010; Gillooly, Brown, West, Savage, & Charnov, 2001). In ectotherms, metabolic rates are sensitive to temperature, which can influence not only individual fitness, but distribution and abundance as well (Angilletta, 2009; Terblanche & Chown, 2007; Vannote & Sweeney, 1980). A major challenge in global change biology is to understand how temperature alters metabolism and energy budgets of different species, and in turn, impacts the ability of species to cope with climate change (Colwell, Brehm, Cardelús, Gilman, & Longino, 2008; Parmesan, Yohe, & Andrus, 2003; Walther et al., 2002). So far, most attempts to measure vulnerability to climate change have assessed species response to temperature challenges, such as lethal and critical thermal limits (Deutsch et al., 2008; Pinsky, Eikeset, McCauley, Payne, & Sunday, 2019). Despite their utility, these metrics address only the responses of organisms to extreme temperatures and do not capture the continuous or incremental effects of temperature on an organism's energy budget. Such effects are particularly important in ectotherms, because body temperature and metabolic rate increase with environmental temperature, resulting in higher energy expenditure. This response is likely to trade-off against other competing demands such as growth, reproduction, and locomotor performance (Pörtner et al., 2006). For example, in aquatic ectotherms, increased metabolic

demand can outpace supply of oxygen from the environment and result in decreased performance and lowered tolerance to heat stress (Pörtner, Bock, & Mark, 2017). Thus, understanding metabolic responses to temperature in species occupying different thermal environments can help us predict geographic variation in response to global warming (Dillon et al., 2010). In this study, we measured metabolic rate profiles in juveniles (nymphs) of two major groups of stream insects – mayflies and stoneflies – in temperate and tropical streams across a paired elevation gradient. At this aquatic stage, nymphs spend much of their time feeding, growing, and developing eggs and sperm packets (Brittain, 1990). Water temperature is therefore a major driver of life history traits in these taxa. We aimed to elucidate differences in patterns of thermal sensitivity and thus better evaluate potential vulnerabilities of mayflies and stoneflies to projected climate warming for which, currently, very little data exist (Chown, Duffy, & Sørensen, 2015).

Much like other types of thermal performance curves (TPCs), oxygen-consumption profiles (used to measure metabolic rate) vary with increasing temperature and display three identifiable regions (Pörtner, 2002; Schulte, 2015): an ascending phase; a peak, which indicates the onset of stress (Huey & Stevenson, 1979; Pörtner & Knust, 2007); and a descending phase of metabolic depression (Guppy & Withers, 1999). When coupled with other measures of performance and the critical limits, changes in oxygen consumption provide a more complete picture of how temperature shapes the ecology of an organism (Angilletta, 2009; Pörtner et al., 2017) (Fig. 1).

Although TPCs for traits like locomotion and metabolic rate have similar shapes, a metabolic rate curve is a special type of TPC (Pörtner, 2002; Schulte, Healy, & Fangue, 2011), with different implications for organismal fitness (Kellermann et al., 2019). The performance

traits used in many TPCs—running speed, feeding rates, growth, or reproduction—are often interpreted as components of fitness, such that *more* is *better*. For these traits, peaks indicate the location of thermal optima (T_{OPT}, Fig. 1) and suggest that performance is optimized at that temperature (Angilletta, Niewiarowski, & Navas, 2002; Huey & Kingsolver, 1989; Huey & Stevenson, 1979) or something close to it (Martin & Huey, 2008). For metabolism, in contrast, more is not necessarily better, and peak standard rates of oxygen consumption typically occur at warmer temperatures than do peak performance traits (Pörtner, 2001). For example, aerobic scope is maximized when the difference between standard metabolic rate (SMR) and maximum metabolic rate (MMR) is greatest (Fig. 1A; often the location of peak performance T_{OPT})—not when SMR itself is at its peak. At higher temperatures, when SMR meets MMR, a metabolic depression often follows as organismal functions decline (Fig. 1; see also Pörtner, 2002). Thus, the metabolic peak should lie to the right of T_{OPT} (i.e. at higher temperatures than T_{OPT}) and align with decreasing performance on the TPC (Fig. 1; Pörtner, 2001). We refer to this peak as T-MR_{PEAK}.

The shapes of metabolic rate curves found for different species, populations, and thermal experience (e.g., acclimitization; DeLong et al., 2018) could either reflect the acute effects of temperature on biological rates (Dillon et al., 2010; Payne & Smith, 2017) or the evolutionary response to past climatic conditions (Dell, Pawar, & Savage, 2011; Deutsch et al., 2008). If the shape of the metabolic rate TPCs simply reflects thermodynamics of biological reactions, we would predict that organisms of equivalent size experiencing a similar range of temperatures should exhibit similar metabolic rate curves (Payne & Smith, 2017).

Alternatively, the Climate Variability Hypothesis posits that if stable climatic regimes, such as those found in the tropics, expose organisms to only a narrow range of temperatures and

favor the evolution of thermal specialists (*sensu* Huey & Kingsolver, 1989), we predict that those organisms should have lower SMR within the range of temperatures they experience to maximize the allocation of energy to growth, reproduction, and aerobic performance (Fig.1B). These thermal specialists should also be more sensitive to temperature, i.e. have metabolic rates that change rapidly in response to temperature outside their normal range. Climates characterized by large seasonal temperature variation, such as those found at temperate latitudes, should select for thermal generalists capable of functioning effectively over a wide range of temperatures (Angilletta et al., 2002; Huey & Kingsolver, 1993). Thermal generalists are predicted to have relatively low SMRs across a wide range of temperatures (Fig. 1C) and be less sensitive to increasing temperature to keep their minimum energy demands low. The generality of these contrasting hypotheses remains open to debate because few comparative studies have measured metabolic rates in species from temperate and tropical regions under similar experimental conditions.

To test predictions generated by the alternative hypotheses detailed above, we examined metabolic rates of mayflies and stoneflies from temperate and tropical regions across different elevations. Because stream temperatures at a given elevation were similar at the time of the experiments, we predicted that the metabolic rate curves should look similar between temperate and tropical species if they were strictly determined by physicochemical effects of temperature on metabolic processes (Payne & Smith, 2017). But, if metabolic rates reflect adaptation to climate, we predicted greater sensitivity to temperature in the tropical species (i.e. larger Q_{10} values; Angilletta et al., 2002; Huey & Kingsolver, 1993); (Fig. 1B) compared to temperate ones (Fig. 1C). We also expected to find differences in SMR *within* each latitude, across elevations, such that insects experiencing greater thermal variation (e.g., in low elevation temperate streams)

would be less sensitive to temperature than insects experiencing reduced thermal variation (e.g., high elevation temperate streams).

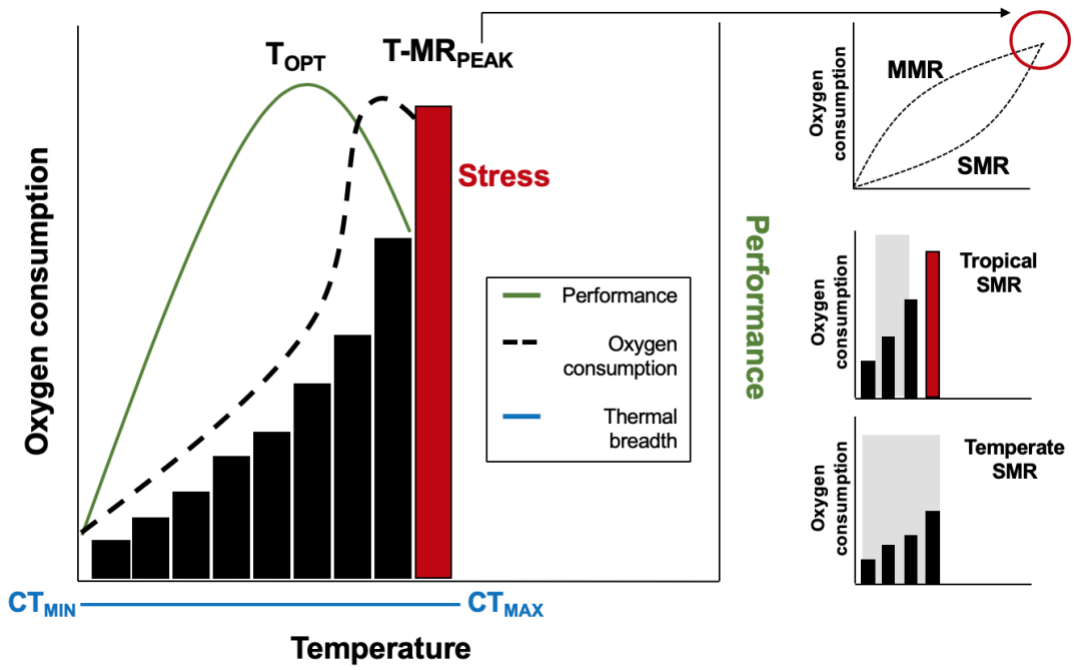

Figure 1. Expectations for change in standard metabolic rate (oxygen consumption) and performance in response to temperature in an aquatic ectotherm. The thermal performance curve (TPC, solid green line, left panel) could refer to any functional performance trait (e.g. swimming performance), where higher performance is better. T_{OPT}, where performance is highest, reflects the animal's preferred temperature. The oxygen consumption curve (dashed line) is a special case of TPC. The peak in this case ($T\text{-}MR_{PEAK}$) could represent the temperature at which standard metabolic rate (SMR) is so high that essentially equals the maximum metabolic rate (MMR) that the ectotherm can achieve (circle, right panel **A**). $T\text{-}MR_{PEAK}$ may therefore reflect stress (red color) and aligns with a decline in the functional performance trait. The general location of behaviorally determined critical thermal limits (CT_{MIN} and CT_{MAX}) are also shown (blue line, left panel). In this study we focus only on SMR. We predict that SMR will rise steeply in tropical species (right panel **B**) and be stressful when temperatures exceed the typical narrow range of temperatures they experience (gray rectangle). In comparison, temperate species should exhibit a gradual rise in SMR across the same temperatures (right panel **C**), reflecting the broad range of environmental temperatures they experience (grey rectangle).

Methods

Site description

Temperate sites were located in the Cache La Poudre river drainage of the southern Rocky Mountains in Colorado, USA, and tropical sites in the Papallacta-Quijos river drainage of the Andes Mountains in Napo Province, Ecuador. We chose small, relatively pristine streams where focal mayflies and stoneflies are typically found. Sites at both latitudes were matched by elevation, where high >3000 m, mid = 2000 – 3000 m, and low = 1000 – 2000 m. Each elevation pair was approximately the same temperature at the time of collection (Table 1). Thus, we were able to make latitudinal comparisons of insect metabolic rate for each elevation pair under the same thermal conditions. Ephemeroptera (mayflies) and Plecoptera (stoneflies) do not share genera between tropical and temperate sites. Therefore, we selected representative genera from the same family (mayfly family Baetidae and stonefly family Perlidae) at each location to contrast their metabolic rate. In the Rockies, we collected a total of 313 individual mayflies (genus *Baetis*) and 107 stoneflies (genus *Hesperoperla*). In the Andes, we collected 245 mayflies (genus *Andesiops*) and 76 stoneflies (genus *Anacroneuria*). All insects were identified to genus by morphology in the field. Although mayflies were easily collected across all elevations, stoneflies were much harder to find and were altogether absent at high elevation, so comparisons between stoneflies are at mid and low elevations only. We conducted experiments between January and April of 2013 in the Andes and June to August of 2013 in the Rockies.

Table 1. Average stream temperatures with standard errors and acclimation temperatures for each temperate (Rocky Mountain) and tropical (Andes) stream from which insects were collected.

Latitude	Site ID	Type	Elevation (m)	Stream temperature (°C)	Acclimation temperature (°C)
Temperate	COP2212	Low	2212	12.5 ± 3.73	14
	COP2590	Mid	2590	10.8 ± 4.68	10
	COP2798	Mid	2798	7.6 ± 1.41	10
	COP3166	High	3166	6.2 ± 3.56	6
Tropical	ECP1845	Low	1845	13.5 ± 0.72	14
	ECP2003	Low	2003	14.0 ± 0.38	14
	ECP2694	Mid	2798	10.0 ± 0.79	10
	ECP3683	High	3683	8.6 ± 0.89	6
	ECP3898	High	3898	6.6 ± 1.74	6

Lab Acclimation

Field-caught nymphs were brought back to the lab (~1600 m at both latitudes) and placed in containers of filtered stream water from their native streams without food for 48 h. During this time, high oxygen levels were maintained by bubbling air into the bath, and water was circulated by small pumps, with temperatures approximating the insects' home stream temperatures (Table 1). Temperate and tropical high elevation insects were acclimated to 6 °C, mid-elevation insects to 10 °C, and low elevation insects to 14 °C. We selected this paired design because tropical insects failed to acclimate to temperatures outside typical temperatures of their native streams (see also Shah, Funk, & Ghalambor, 2017a). For example, we often detected evidence of stress (e.g., jerking movements, unnatural swimming patterns) and even mortality, when tropical mayflies from high and low elevations were warmed outside native stream temperature (i.e., 12 °C and 10 °C, respectively). Therefore, the use of a single acclimation temperature across all elevations was not possible. Because acclimation temperatures differed among elevations but

were the same for each elevation pair, we restricted our comparisons to the same elevations between the two latitudes to control for the background acclimation temperature.

Metabolic Rate

Oxygen consumption rates (metabolic rates, hereafter) were measured using closed glass micro-respirometry chambers (Unisense A/S, Aarhus, Denmark). Mayflies were placed in 4 mL chambers, and stoneflies, which are larger, were placed in 50 mL chambers. After the 48 h acclimation period, we introduced insects randomly to one of nine test temperatures (5, 7.5, 10, 12.5, 15, 17.5, 20, 22.5, or 25 °C) by allowing temperature to rise naturally or by slowly cooling (0.5 $°C_{\text{-min}}$) to prevent thermal shock. On average, 16 individuals per population for mayflies and 6 per population for stoneflies were tested at each temperature (Tables S1, S2). Once at the test temperature, insects were moved individually into glass chambers filled with stream water that had been treated with UV light to reduce background microbial respiration, and immersed in a water bath held at test temperature (Fig. S1). Chambers were fitted with a glass-coated magnetic stir bar and a plastic mesh to which the insect could cling (Fig. S1). We allowed insects to acclimate to the chamber space for an additional hour before sealing each chamber with a glass stopper to prevent further introduction of atmospheric oxygen. Each stopper had a central capillary hole, allowing us to insert an oxygen microelectrode (500 μm diam.) connected to a picoammeter (Unisense OXY-Meter, Aarhus, Denmark) to measure change in oxygen concentration. To measure background respiration rates, a control chamber (without an insect) was included in each experiment. The microelectrode was first lowered into the control chamber at the start of the experiment. After reading oxygen concentration continuously for 2 minutes, it was moved to the first chamber that contained an animal, where data were recorded for another 2

minutes (Fig. S1), and so on. Experiments were terminated when oxygen levels reached 80% of the air-saturated values because natural dissolved oxygen levels in our study sites rarely fall below this value. Consequently, we made 3-6 readings for each chamber – fewer readings at the higher temperatures compared to the lower temperatures – which lasted around 128 minutes. Because there were typically 16 chambers running simultaneously, the average time interval between readings was 25 mins. While recording oxygen concentration, we also observed insects inside the chambers, noting any stress responses or mortality. Insects were then removed from chambers, euthanized in ethanol, dried, and weighed.

Statistical Analyses

We first calculated average rate of oxygen consumption for each insect or control (blank) chamber by measuring change in oxygen concentration across all measurements taken in a given chamber (see Supplemental Materials and Fig. S2 for an explanation of this calculation). We then averaged the control values for each temperature such that we calculated a control rate for each combination of test temperature, elevation, and latitude. Control rates were subtracted from insect consumption rates to correct for background microbial respiration. We conducted all statistical analyses on these 'control-corrected oxygen consumption rates' (hereafter, oxygen consumption) in R version 3.4.0 (R Core Team 2017).

Because we were interested in the thermal sensitivity of temperate and tropical metabolic rates between elevation pairs (i.e. the thermal reaction norm), we first compared metabolic rates during the ascending phase of the TPC by fitting a linear model in R with oxygen consumption as the response variable for each elevation type (i.e. high, mid, and low) separately. For this analysis, we used only the temperatures 5, 7.5, 10, 12.5, 15 and 17.5 °C because these

temperatures were within the ascending phase of the SMR curves for most populations in this study. The model included fixed effects of test temperature and latitude, and their interaction, with body mass as a covariate (Packard & Boardman, 1999). A significant interaction would denote a difference in thermal sensitivity (i.e. slope) between temperate and tropical species.

We conducted a similar analysis for the descending phase using data from the 17.5 and 20 °C treatments as these two temperatures encompassed the descending phase of the curve. At the highest temperatures (22.5 and 25 °C), we observed stress responses (e.g. unnatural swimming patterns, frenzied attempts to escape from the chamber and in some cases elevated metabolic rates) in tropical mayflies. We therefore did not include SMR at 22.5 and 25 °C in this analysis but instead calculated separate Q_{10} values for these temperatures (see below).

To compare the maximum SMR at each elevation between temperate and tropical taxa, we examined latitude x elevation combination individually to find the physiologically relevant peak (i.e., before CT_{MAX} and when the first signs of stress were apparent, if observed). This peak was typically at 17.5 °C in our study because in most cases, across both latitudes, SMR was highest at this temperature even if not always statistically different from 15 °C. We then compared these values using an ANOVA for each elevation pair using latitude as the fixed effect and body mass as a covariate.

Finally, we calculated Q_{10}, a measure of sensitivity to temperature, using the formula

$$Q_{10} = \left(\frac{R_2}{R_1}\right)^{\left(\frac{10}{T_2 - T_1}\right)}$$

where R_1 and R_2 are reaction (oxygen consumption) rates measured at two different temperatures, T_1 and T_2, respectively. Q_{10}s were calculated across cooler 'non-stressful'

temperatures (T_1 = 7.5 and T_2 = 15 °C) and warmer 'stressful' temperatures (T_1 = 15 and T_2 = 25 °C).

Results

Behavioral observations

Tropical mayflies showed visible signs of stress (jerking movements, loss of righting response) at several test temperatures outside the range they typically experience (e.g., 22.5 and 25 °C; Fig. 2 red bars, Tables S1; Fig. S3 A); many of the stressed individuals died at the end of the experiment (Table S1; Fig. S3 B). Low elevation tropical mayflies also experienced some mortality at 5 °C, which is much colder than the minimum temperatures they encounter in their home streams (Fig. 2F). Temperate mayflies, in contrast, experienced little-to-no visible signs of stress at any experimental temperature (Table S2). We also did not detect any overt signs of stress in any of the stoneflies in this study.

Thermal sensitivity based on standard metabolic rates and reaction norms

SMR profiles differed between temperate and tropical mayflies and stoneflies at all elevations (Figs. 2 and 3). In general, metabolic rates of mayflies from the two latitudes were lowest at temperatures approximating those in their native streams. Outside these temperatures, however, tropical mayflies exhibited a greater increase in metabolic rate (Fig. 2). In all mayfly populations across both latitudes, a secondary peak was observed at 22.5 and 25 °C that corresponded with visible stress during experiments. We denoted the initial peak in SMR as $T_{MR\ PEAK}$ (shown as yellow arrows on Figs. 2 and 3) or the onset of stress, and the secondary peak as an acute stress response because it was followed by death in some cases. As indicated in

the Methods, we excluded these stressful test temperatures from the analyses of the ascending and descending phases of metabolic rate profiles and dealt with them separately in an analysis of Q_{10}.

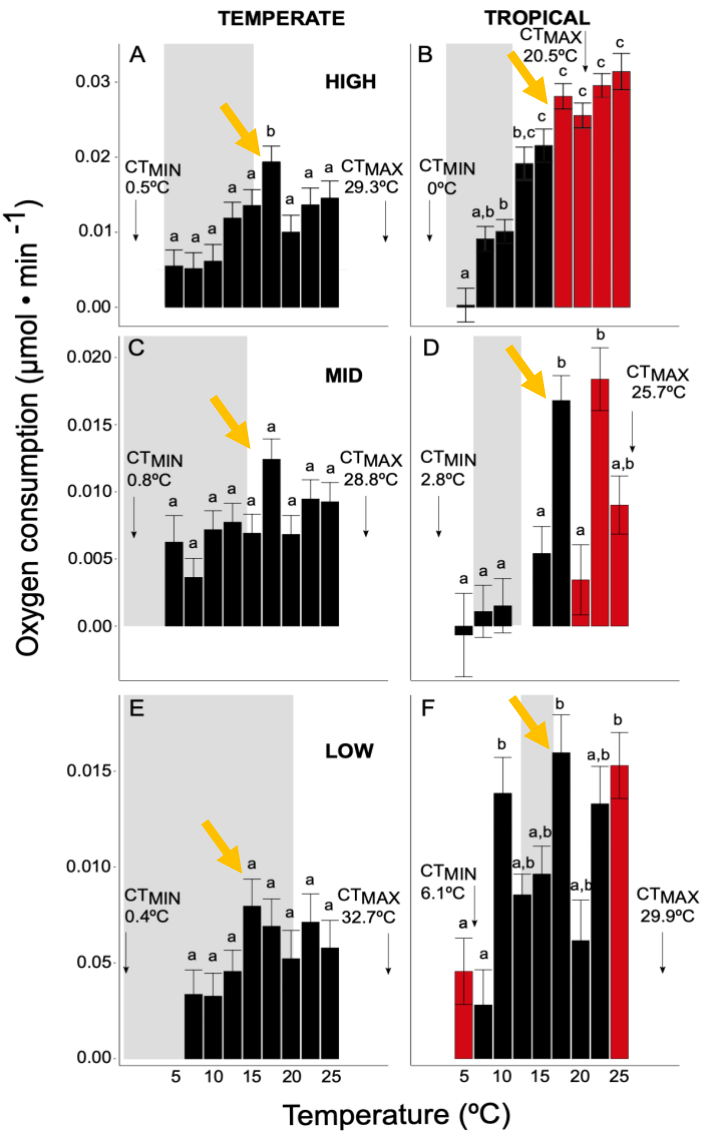

Figure 2. Plots of standard metabolic rate least square means in mayflies from high (A, B), mid (C,D), and low (E,F) elevations in temperate (left panel) and tropical (right panel) streams. Red bars indicate temperatures at which we observed stress. T-MR_{PEAK} is indicated by yellow arrows for each population. Gray rectangles represent the approximate stream temperature range measured in each stream. CT_{MIN} and CT_{MAX} determined in a previous study are shown.

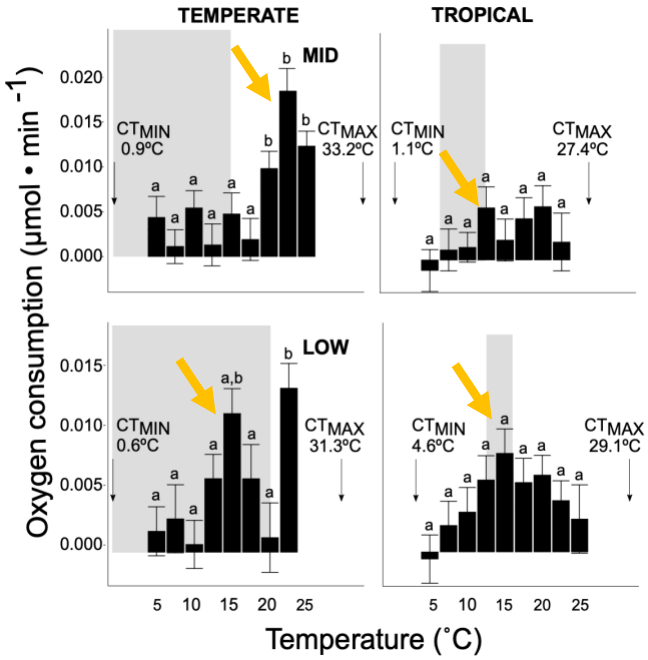

Figure 3. Plots of metabolic rate least square means in predatory stoneflies from mid (A, B) and low (C,D) elevations in temperate (left panel) and tropical (right panel) streams. Stoneflies were not found at high elevation. T-MRPEAK is indicated by yellow arrows for each population. Gray rectangles represent the approximate stream temperature range measured in each stream. We did not detect overt stress in stoneflies in this study. CTMIN and CTMAX determined in a previous study are shown.

In the ascending phase, metabolic rates in tropical mayflies across all elevations were more sensitive to temperature increases than were their temperate counterparts ($F_{(5,371)} = 8.57$, $p < 0.001$). Focusing on each elevation separately, we found tropical species to have greater increases in metabolic rate at high elevation ($F_{(5,97)} = 3.27$, $p = 0.009$, Fig. 4A), mid elevation ($F_{(4,98)} = 4.17$, $p = 0.003$, Fig. 4B), and at low elevation ($F_{(4,152)} = 6.77$, $p < 0.001$, Fig. 4C). In stoneflies, ascending phases did not differ between temperate and tropical populations (Figs. 5 A and B)

Temperate mayflies had a steeper decrease in metabolic rate at high elevation (Fig. 4A; $F_{(1,1)} = 4.04$, $p = 0.05$) and at low elevation (Fig. 4C; $F_{(1,1)} = 6.86$, $p = 0.01$). At mid elevation, there was no significant interaction (Fig. 4B). However, a Student's T-test revealed that oxygen

consumption values were significantly higher in tropical mayflies compared to temperate ones at 17.5°C (t = 3.946, p = 0.001), but rates were equally low between latitudes at 20°C (t = -1.006, p = 0.33). This suggests that the descending phase was steeper for tropical mayflies compared to temperate ones. Descending phases of stoneflies did not differ between tropical and temperate populations.

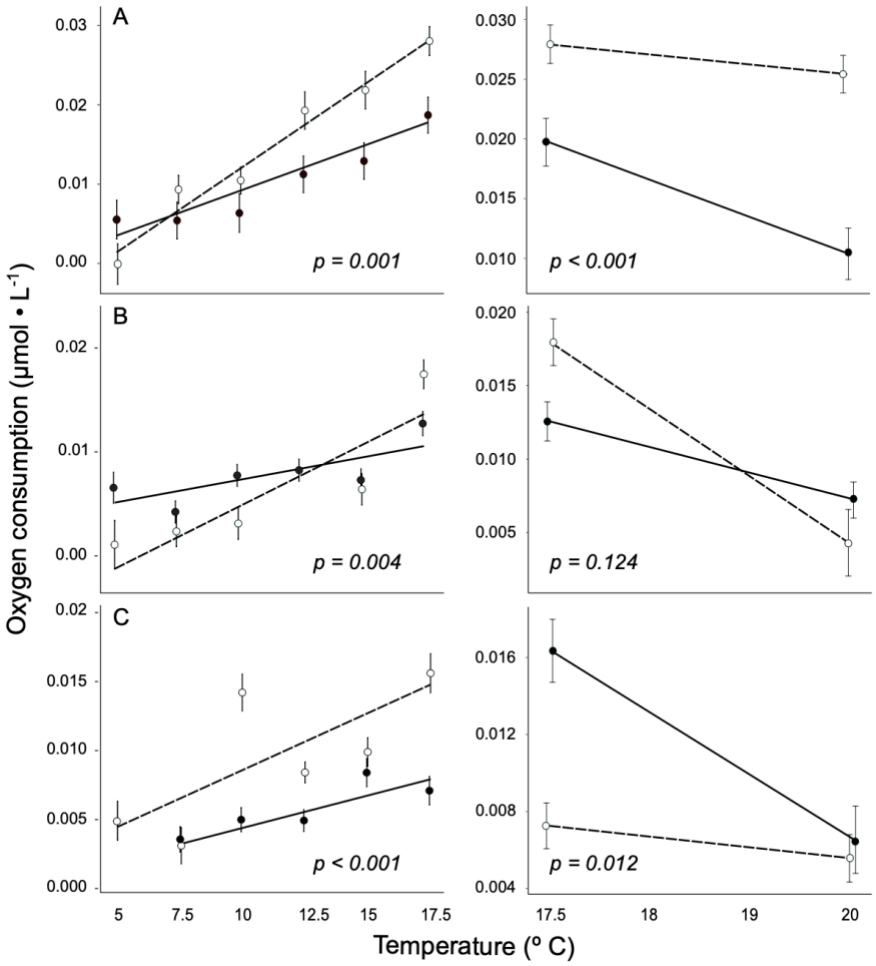

Figure 4. A comparison of the ascending (left panel) and descending (right panel) phases of metabolic profiles at high (A), mid (B) and low (C) elevations. Values are the least square means of metabolic rate in mayflies from tropical (white circles) and temperate (black circles) streams, after accounting for body mass in the model. Vertical bars are standard errors and p values are shown.

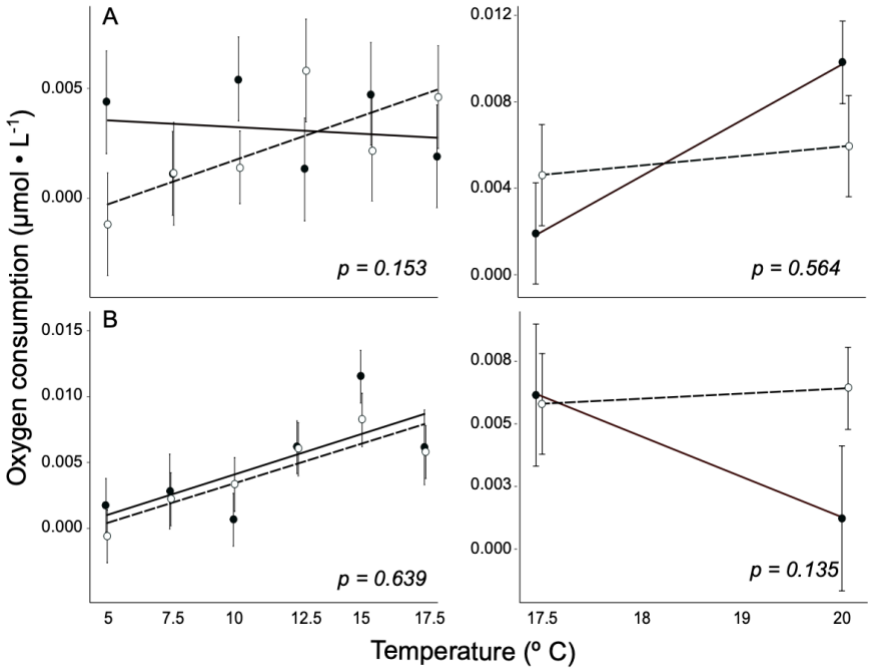

Figure 5. A comparison of the ascending (left panel) and descending (right panel) phases of metabolic profiles at mid (A), and low (B) elevations. Values are the least square means of metabolic rate in stoneflies from tropical (white circles) and temperate (black circles) streams, after accounting for body mass in the model. Vertical bars are standard errors and p values are shown.

Finally, the maximum SMR was higher in tropical compared to temperate mayflies at all elevations (Fig. 6). Stoneflies showed an opposite pattern where tropical species had lower peaks at both measured elevations, although this was significant only at mid elevation (Fig. 6).

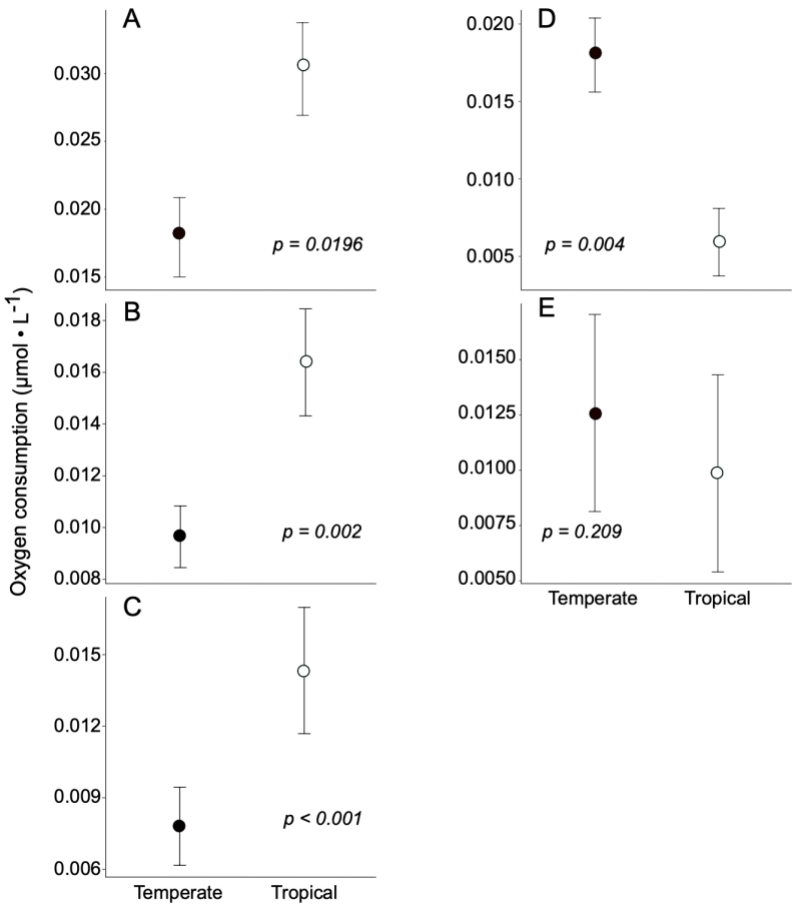

Figure 6. Peak SMRs (i.e. the highest metabolic rate) are compared between temperate and tropical mayflies from high (A), mid (B), and low (C) elevations and stoneflies from mid (D) and low (E) elevations. Vertical bars are standard errors and p values are shown.

Thermal Sensitivity based on Q_{10}

Our Q_{10} calculations revealed contrasting results for stoneflies, but not mayflies. When comparing Q_{10}s across 'stressful' and 'non-stressful' temperatures, we found Q_{10}s for both taxa to be higher at non-stressful temperatures than at stressful temperatures (Table 2). Across latitude, tropical mayflies showed greater sensitivity to temperature (higher Q_{10} values) at both non-stressful and stressful temperatures than their temperate counterparts (Table 2; Fig. 7). Notably, at mid and low elevations, tropical mayflies were highly sensitive to temperature even

at non-stressful temperatures (Q_{10} = 8.40 and 5.17, respectively). In contrast, tropical stoneflies were less sensitive than their temperate relatives for stressful and non-stressful temperatures (Fig. 7).

Table 2. A comparison of Q_{10} values calculated for temperate and tropical mayfly metabolic rates between typically non-stressful temperatures (7.5°C to 15°C) and typically stressful temperatures (15°C to 25°C).

		Mayflies		*Stoneflies*	
	Elevation	**Temperate**	**Tropical**	**Temperate**	**Tropical**
Non-stressful	High	3.63	3.15	n/a	n/a
	Mid	3.15	5.17	6.76	2.49
	Low	2.36	8.40	6.66	5.78
Stressful	High	1.07	1.46	n/a	n/a
	Mid	0.73	1.59	6.10	0.87
	Low	1.34	1.67	1.25	0.42

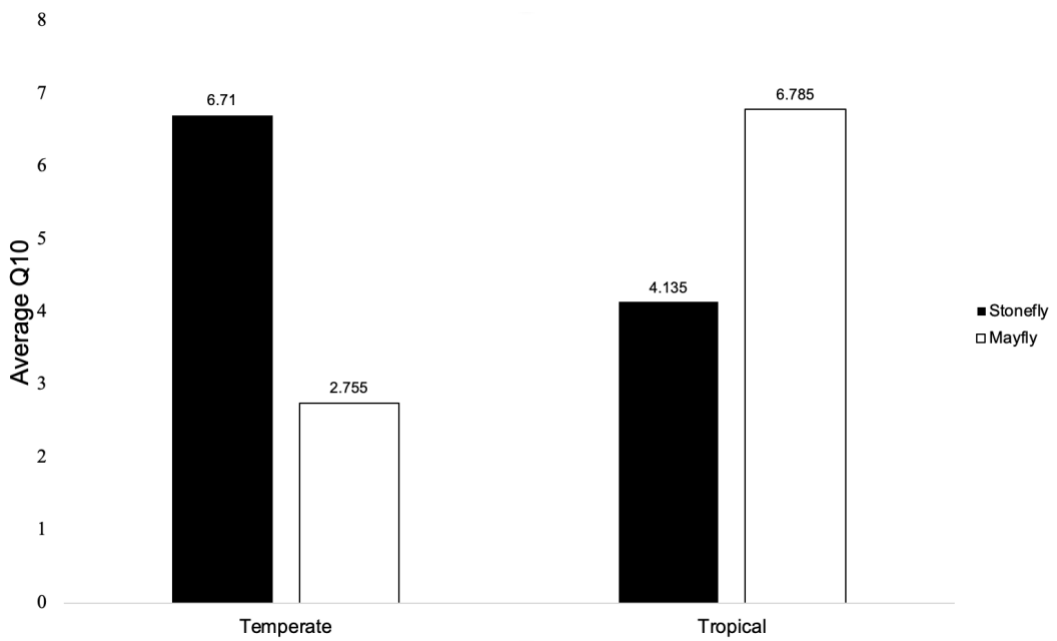

Figure 7. Comparison of average Q_{10} for mayflies and stoneflies during the ascending phase of the metabolic rate curve.

Discussion

Comparative studies of critical thermal limits have generally found tropical ectotherms to be adapted to narrower ranges of temperatures compared to their temperate counterparts, suggesting that tropical species are more vulnerable to global warming (Deutsch et al., 2008; Gutiérrez-Pesquera et al., 2016; Shah et al., 2017b; Sunday, Bates, & Dulvy, 2011, 2012). Payne and Smith (2017) recently challenged such conclusions by arguing that narrower thermal breadth may simply reflect the acute effects of temperature on biological rates of organisms living in warmer environment. Here, using a paired-elevation design, we compared the thermal sensitivity of SMR in closely related temperate and tropical aquatic insect nymphs. This study design enabled a comparison of temperate and tropical species under a common range of environmental, acclimation, and test temperatures. Despite originating in streams with similar environmental temperatures at the time of collection, tropical and temperate insects had different metabolic rate profiles (Figs. 2, 3). These results suggest that variation between temperate and tropical species does not reflect simple, direct effects of temperature on biological rates, but instead support the view that there are physiological differences between taxa from these regions. Tropical mayflies, in particular, exhibited greater sensitivity to temperature in terms of metabolic rates and the onset of stress compared to their temperate counterparts. By contrast, stoneflies showed little-to-no divergence in their thermal sensitivity. The ecological implications of greater thermal sensitivity remain unresolved for most ectotherms, but in the absence of compensatory changes in productivity, elevated metabolic rates could negatively impact the energy budget and capacity for aerobic performance in tropical mayflies (Dillon et al., 2010; Huey & Kingsolver, 2019).

Comparison of metabolic rate profiles

In most cases, metabolic rate generally rose with increasing temperature, peaked, and then fell. This pattern, however, varied considerably among taxa, populations, and elevations. As a group, tropical mayflies were more thermally sensitive than their temperate counterparts (Figs. 2, 4, 6 and 7). The metabolic rate curves of tropical mayflies had overall steeper ascending and descending phases, and higher Q_{10}s, across all three elevations. The particularly high Q_{10} for mid and low elevation tropical mayflies were similar to those of North Atlantic stenothermal fishes when experiencing temperatures away from an optimum (Johnston, Clarke, & Ward, 1991). These results support the prediction that tropical mayflies, which experience more stable stream temperatures (Shah et al., 2017b), are more sensitive to temperature.

Given that mayflies were paired by elevations that had similar environmental and acclimation temperatures, these differences cannot be attributed simply to the passive response of metabolic rate to higher temperatures. Instead, the greater thermal sensitivity of metabolism in tropical mayflies largely reflects a rapid increase and then decline outside the relatively narrow range of environmental temperatures that they experience (Figs. 2, 4, 6, 7). In contrast, temperate mayflies exhibited more modest increases in metabolism in response to temperature and had similar metabolic rate profiles across elevations (Fig. 2). For both temperate and tropical mayflies, metabolic rates tended to be lower within the range of environmental temperatures they experience (Fig. 2), suggesting there may be selection to reduce the minimum energetic costs associated with maintenance (Artacho & Nespolo, 2009; Bochdansky, Grønkjær, Herra, & Leggett, 2005; Brown, Marquet, & Taper, 1993). For example, even a moderate increase in stream temperature outside the normal range (Fig. 2, right edge of grey rectangles) is associated

with peaks in metabolism, which has potentially cascading effects on energy budgets and possible chronic stress (Fig. 2). If selection acts to minimize SMR under typical stream temperatures (Brown et al., 1993), these results would support the hypothesis that the narrower and more stable stream temperatures experienced by tropical mayflies underlie their greater thermal sensitivity. Such an interpretation is consistent with other aspects of temperate and tropical mayfly thermal physiology. First, thermal breadth (difference between critical thermal limits) measured in a parallel study were the narrowest for tropical mayfly populations (Shah et al., 2017b). Second, in this study, we observed visible signs of stress in the metabolic rate profiles of tropical but not temperate mayflies. Specifically, after the decline in metabolic rate, secondary peaks occurred at 22.5 °C and 25 °C for most groups, which corresponded to visible signs of stress and mortality in tropical but not temperate mayflies. Signs of stress at these high temperatures included leg spasms, loss of righting, release from substrate, and mortality (see Supplemental Information Table S1, Figs. S3 A, B). Other aquatic ectotherms, such as brown bullhead catfish (Heath, 1973) and harvester ants (Lighton & Turner, 2004), also show similar postmortem peaks in metabolism associated with heat stress. Collectively, these results are consistent with previous interpretations that temperate mayflies are thermal generalists, with broad thermal breadths and reduced metabolic sensitivity to temperature, while tropical mayflies are thermal specialists, with a narrower thermal breadth and increased metabolic sensitivity to temperature (c.f. Huey & Hertz, 1984).

The differences observed between temperate and tropical mayflies were generally unambiguous and supported by prior work in this system. In stoneflies, evidence in support of our predictions was less straightforward. However, the similarity between temperate and tropical stoneflies is consistent with previous research on other physiological traits (Shah et al., 2017a,

2017b). While there are some differences in metabolic rate profiles between temperate and tropical stoneflies (e.g. higher metabolic rates at warmer temperatures in temperate, but not tropical species), these differences are far less striking than those observed in mayflies (Fig. 2 vs. 3). Overall, the ascending and descending phases did not differ between temperate and tropical stoneflies (Figs. 3, 5), but absolute maximum metabolic rates and Q_{10}s across non-stressful and stressful temperatures were higher in the temperate species (Fig. 7). This lack of latitudinal differences in stoneflies was also found when comparing acclimation ability (Shah et al., 2017a). Another rare study of respiration rate in tropical stoneflies (Jacobsen & Brodersen, 2008) reports similar Q_{10} values to those reported here for non-stressful temperatures in mid-elevation stoneflies, confirming our conclusions of lower thermal sensitivity in at least this group of perlid stoneflies. Though many species in the tropics will be imperiled by warming (Deutsch et al., 2008; Dillon et al., 2010), the overall mixed support for this prediction in stoneflies a cautionary tale for generalizations about the vulnerability of tropical species.

Numerous hypotheses could explain the lack of divergence between temperate and tropical stoneflies. First, stoneflies as a group have a temperate origin and invaded tropical streams only recently (Baumann & Kondratieff, 1991; Stewart, Baumann, & Stark, 1974). Studies show that stoneflies are still adapting to tropical habitats (Polato et al., 2018), which may explain why thermal physiological traits in present-day temperate and tropical populations are similar. Second, perlid stoneflies and baetid mayflies have different life histories, which may contribute to differential sensitivity to temperature. As nymphs, semivoltine stoneflies live much longer than multivoltine mayflies, and may need to cope with multiple years of unpredictable variation in stream temperature (Clifford, 1982). In fact, many stoneflies enter diapause, unlike mayflies (Brittain, 1982; Hynes, 1976), giving them a greater ability to withstand adverse

thermal conditions (Brittain, 1990). Once emerged, adult stoneflies often remain close to natal streams, whereas adult mayflies in tropical (Finn, Encalada, & Hampel, 2016) and temperate (Polato et al., 2018) regions can disperse more widely. Therefore, stoneflies rely more heavily on the nymph stage for dispersal (Brittain, 1990) and as a consequence, may experience more thermal variation moving up-or- downstream than juvenile mayflies. Finally, stoneflies are important predators of mayflies and midges (Peckarsky, 1980; Tamaris-Turizo, Turizo-Correa, & del Carmen Zúñiga, 2007). Perlid stoneflies actively pursue prey in the wild (Peckarsky, 1980) as well as in laboratory settings (Sjöstrom, 1985; A.A.Shah *pers. obs*.). A reliance on aerobic metabolism and the need to maintain a high metabolic scope for active hunting (Kordas, Harley, & O'Connor, 2011) could constrain physiological divergence between temperate and tropical stoneflies, but too few comparative studies of metabolic profiles are available to test the generality of such hypotheses. Nevertheless, higher maximum metabolic rates (Fig. 6) and higher Q_{10}s (Fig. 6) do suggest some differences between temperate and tropical populations that could have ecological consequences when pursuing prey across a broad range of temperatures. Because perlid stoneflies and baetid mayflies interact as predator and prey in temperate and tropical streams (Gamboa, Chacón, & Segnini, 2009; Peckarsky, 1980), differential sensitivities to temperature may have community-level consequences (Gilman, Urban, Tewksbury, Gilchrist, & Holt, 2010; Grigaltchik, Ward, & Seebacher, 2012; Kordas et al., 2011; Pincebourde & Casas, 2019; Vucic-Pestic, Ehnes, Rall, & Brose, 2011). Our results underscore the importance of assessing community-level responses to global warming by explicitly testing interactions under different temperatures (Barton & Schmitz, 2009) and how these responses differ across latitude (Matassa & Trussell, 2015)

Comparison of metabolic rates and critical thermal maxima as indicators of vulnerability

Some of the results of the present study are surprising because we expected CT_{MAX}, a commonly used metric for thermal sensitivity (Diamond et al., 2012; Lutterschmidt & Hutchison, 1997; Piyaphongkul, Pritchard, & Bale, 2012; Ribeiro, Camacho, & Navas, 2012), to coincide with the descending limb of the metabolic rate profiles (Fig. 1). Yet, previously measured values of CT_{MAX} in temperate mayflies occur at much higher temperatures (Fig.1A, B, C). For example, CT_{MAX} was ~29 °C in high elevation temperate mayflies (Shah et al., 2017b) but they began to show elevated metabolic rates and behavioral signs of stress at lower temperatures (~17.5 °C) and an elevated metabolism. The large difference between CT_{MAX} and the onset of stress in this study is likely due to methodology, as rapid ramping or high starting temperatures can inflate estimated values of CT_{MAX} (Rezende, Tejedo, & Santos, 2011; Terblanche, Deere, Clusella-Trullas, Janion, & Chown, 2007). The discrepancy could also arise because CT_{MAX} and metabolic rates are governed by fundamentally different processes. CT_{MAX} is the result of changes in fluidity of cell membranes and whole-organism physiology in response to heat stress (Cossins, Friedlander, & Prosser, 1977; Hochachka & Somero, 2002). Standard metabolic rates arise from different activation energies of enzymes and sensitivity of biochemical processes to temperature (Rolfe & Brown, 1997). Thus, using CT_{MAX} in conjunction with other measures of thermal sensitivity such as metabolic rate provides a more complete assessment of thermal tolerance.

Climate change in Andean and Rocky Mountain Streams

Mountain streams are already increasing in temperature worldwide (Isaak & Rieman, 2013). Temperatures of small mountain streams, such as those in this study, are likely to show

concomitant increases with air temperature (Mohseni & Stefan, 1999; Pilgrim, Fang, & Stefan, 1998). Projections of mean air temperature increases of 2-3 °C in the Andes and 3-4 °C in the northern Rocky Mountains by the 2050s are therefore troubling (Nogués-Bravo, Araújo, Errea, & Martínez-Rica, 2007; Rangecroft, Suggitt, Anderson, & Harrison, 2016). However, evidence suggests that insects of the southern Rocky Mountains are relatively robust to warming because they may currently occur at suboptimal temperatures (Pyne & Poff, 2017). Moreover, mesocosm and field common garden experiments showed that temperate baetid mayflies grow faster when subjected to warmer water (N.L. Poff et al., *unpubl. data*). Given the wide elevation distribution and broad dispersal ability of these insects (Gill et al., 2016), they may be able to move across the landscape to find thermally suitable habitats as their environment warms. In contrast to mayflies of the Rockies, Andean mayfly growth declined with increasing temperature in mesocosms (N.L. Poff et al., *unpubl. data*) further supporting our conclusion that a warming environment will negatively affect tropical mayflies. Increased temperatures may also be locally exacerbated in some Andean streams where riparian forests are being cleared for human activities. The resulting exposure to increased solar radiation can alone raise stream temperature (Moore, Spittlehouse, & Story, 2005). The tropical mayflies in our study will experience the effects of these increasing temperatures leading to a possible mismatch of dissolved oxygen supply and demand (Pörtner, 2001; Verberk, Bilton, Calosi, & Spicer, 2011; Verberk et al., 2016b). The ability to maintain an adequate rate of oxygen uptake decreases at higher temperatures for many aquatic insects (Jacobsen & Brodersen, 2008; Rotvit & Jacobsen, 2013), thus reducing energy available for activities like movement, growth, and egg development. Thus, streams need not even reach temperatures as high as CT_{MAX} values to have a negative impact on insects. This is illustrated in a field experiment, where a moderate 2 °C temperature increase in

the stream resulted in declines in abundance of Eurasian mayflies, presumably as a result of decreased oxygen in the stream (Verberk, Durance, Vaughan, & Ormerod, 2016a). Whether tropical mayflies in our study will move upstream to track cooler temperatures is debatable given the low dispersal rates of aquatic insects in these streams (Gill et al., 2016). Future work should therefore also focus on whether aquatic insects can move upslope to counteract the effects of warming in their native streams (Sheldon, 2012).

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A.A.S. and C.K.G. designed the experiments. A.A.S. conducted fieldwork and experiments, analyzed results, and wrote the manuscript with contributions from all authors.

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