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Divergence and constraint in the thermal sensitivity of aquatic insect swimming performance

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Abstract

Environmental temperature variation may play a significant role in the adaptive evolutionary divergence of ectotherm thermal performance curves (TPCs). However, divergence in TPCs may also be constrained due to various causes. Here, we measured TPCs for swimming velocity of temperate and tropical mayflies (Family: *Baetidae*) and their stonefly predators (Family: *Perlidae*) from different elevations. We predicted that differences in seasonal climatic regimes would drive divergence in TPCs between temperate and tropical species. Stable tropical temperatures should favor the evolution of “specialists” that perform well across a narrow range of temperatures. Seasonally, variable temperatures in temperate zones, however, should favor “generalists” that perform well across a broad range of temperatures. In phylogenetically paired comparisons of mayflies and stoneflies, swimming speed was generally unaffected by experimental temperature and did not differ among populations between latitudes, suggesting a maintenance of performance breadth across elevation and latitude. An exception was found between temperate and tropical mayflies at low elevation where climatic differences between latitudes are large. In addition, TPCs did not differ between mayflies and their stonefly predators, except at tropical low elevation. Our results indicate that divergence in TPCs may be constrained in aquatic insects except under the most different thermal regimes, perhaps because of trade-offs that reduce thermal sensitivity and increase performance breadth.

Key words: aquatic insects, climate variability, elevation, latitude, predator–prey, swimming performance, thermal performance curve

Ectotherm performance typically depends on body temperature. The highest performance (fastest rate) occurs at an optimum temperature (T_{OPT}) or within a range of optimum temperatures, and lower performance occurs outside this range (Huey and Kingsolver 1989;

Angilletta 2006). Change in performance with temperature is often visualized as a thermal performance curve (TPC). Like many other function-valued or plastic traits, TPCs may be subject to natural selection, which could alter both the location of T_{OPT} and the breadth

or shape of the curve (Angilletta 2009; Gomulkiewicz et al. 2018). A general expectation is that warm environments should select for higher T_{OPTS} and cooler environments should select for lower T_{OPTS} . Further, the difference between the end points of the curve (critical thermal limits) should be wider in variable than in stable environments (Janzen 1967; Ghalambor et al. 2006; Huey et al. 2009; Bozinovic et al. 2011). Consequently, the thermal environment is assumed to act as a source of natural selection on thermal physiological traits, including TPCs (Sinclair et al. 2012), such that T_{OPT} reflects a fitness optimum and is locally adapted (Angilletta 2009).

Alternatively, divergence in thermal sensitivity of ectotherm performance might be subject to evolutionary constraints. For example, behavioral thermoregulation allows ectotherms to stay within favorable temperatures across different thermal environments, effectively reducing the strength of divergent selection and inhibiting evolutionary shifts (Bogert 1949; Huey et al. 2003; Muñoz and Bodensteiner 2019). Divergence in TPCs may also be constrained if trade-offs exist between maximizing performance across a narrow range of temperatures and maintaining performance breadth across a wide range of temperatures (Huey and Kingsolver 1993; Gilchrist 1995; Tüzün et al. 2017). Thus, despite the long-held view that climate variability plays an important role in diversifying TPCs between tropical and temperate organisms that experience different thermal regimes (Van Berkum 1988; Gilchrist 1995; Wilson 2001), the matter still remains open to debate (Huey and Stevenson 1979; Angilletta 2009; Bozinovic et al. 2011).

Theory suggests that in the absence of constraints, temperate ectotherms should have wider thermal tolerance compared to their tropical relatives because they experience greater seasonal climate variability (i.e., the Climate Variability Hypothesis [CVH]; Huey and Stevenson 1979; Stevens 1989; Pither 2003). By contrast, species living in stable thermal environments should evolve narrow tolerances and perform best just in the restricted range of temperatures they usually experience (Huey and Hertz 1984; Gilchrist 1995; Angilletta 2009). Many studies have found support for the CVH when measuring thermal tolerance as the difference between the critical thermal limits (i.e., CT_{MAX} and CT_{MIN}) across marine (Sunday et al. 2011), freshwater (Gutiérrez-Pesquera et al. 2016; Shah et al. 2017b), and terrestrial (Huey et al. 2009) systems. However, because these studies focused on thermal limits (Lutterschmidt and Hutchison 1997; Angilletta 2009), they do not necessarily indicate how performance varies across ecologically realistic sublethal temperatures. Latitudinal comparisons of TPCs have found only mixed support for the CVH (Van Berkum 1988; Kingsolver and Gomulkiewicz 2003; Navas et al. 2008; Huey et al. 2009; Overgaard et al. 2014). Such findings call into question the generality of the CVH and indicate that there may be factors constraining the divergence of TPCs.

Elevation adds another dimension to the CVH (Janzen 1967; Ghalambor et al. 2006; Sunday et al. 2014). This insight was first articulated by Janzen (1967) to explain differences in thermal physiology, dispersal, and diversity of species along elevation gradients between temperate and tropical mountains. When elevation is considered, tropical temperatures can be either stable and cold at high elevation or stable and warm at low elevation, with little overlap across elevations. However, temperatures in temperate mountains fluctuate much more on several timescales (daily, seasonally), which imposes greater thermal overlap across elevations. These climatic differences should result in wider thermal tolerances and similar thermal sensitivity across elevations in temperate organisms and

narrower tolerance for tropical organisms with greater differences in thermal sensitivity across elevations (Janzen 1967; Heatwole et al. 1969; Huey and Webster 1976; Feder and Lynch 1982). In support, Navas et al. (2008) found that high-elevation tropical amphibians maintained high locomotor performance at cooler temperatures compared with either lowland tropical or temperate species, consistent with their stable cold environments. Lowland temperate amphibians also had higher performance at cooler temperatures compared with their lowland tropical counterparts, but the TPCs converged at moderate and high temperatures.

Temperate and tropical freshwater mountain streams contain diverse but related aquatic insects, providing an excellent natural laboratory for examining how climate variability shapes TPCs. Although all mountain streams are characterized by regular increases in temperature as water descends from high to low elevations, daily and yearly patterns of thermal variation in these streams depend strongly on latitude, and on interactions between latitude and elevation. In particular, the annual thermal fluctuations of temperate streams are greater than those of tropical streams, but the thermal range of these fluctuations depends on elevation. For example, high-elevation streams in the North American Rockies are less variable than lower elevations, but high-elevation streams in the Ecuadorian Andes are more variable than lower elevations (Table 1).

Although average winter temperatures in temperate streams are far colder than tropical streams, summer temperatures are more similar to the annual average temperatures of tropical streams at the same elevation. This similarity creates a control for comparative thermal physiological studies because the same temperature can be used to acclimate animals before testing. Previous studies of the CVH in this mountain aquatic system found that even when controlling for acclimation temperature, temperate species have larger differences between their critical minimum and maximum temperatures (Shah et al. 2017b; see Table 3 herein) and tropical mayflies have reduced plasticity of their critical thermal maxima (Shah et al. 2017a). These physiological differences are likely driven by the contrasting stream temperature regimes temperate and tropical aquatic insects experience throughout their life cycles. Specifically, temperate baetid mayflies hatch in early spring when water temperature is extremely low, but emerge as adults during warmer summer months. Temperate perlid stoneflies live for several years as nymphs experiencing multiple cold winters and hot summers (Brittain 1990). In contrast to the variable thermal regime experienced by their temperate relatives, tropical mayflies and stoneflies experience a stable and narrow range of temperatures from the time they hatch to the moment they emerge from the stream as adults.

Here, we examined thermal sensitivity of swimming performance in temperate and tropical latitudes across a range of elevations in 2 globally occurring families—*Baetidae* (mayflies) and *Perlidae* (stoneflies) (Fochetti and de Figueroa 2007; Brittain 2008). We conducted experiments during the temperate summer, when temperate and tropical insects at each elevation experience approximately the same mean stream temperature (Table 1). Because baetids and perlids from temperate streams are close relatives of and functionally similar to baetids and perlids in tropical streams, this presented a unique opportunity to assess the effect of environmental thermal variation on their physiology. Where these mayflies and stoneflies co-occur, they also interact with each other; herbivorous baetids are common prey of predatory perlids (Peckarsky 1980; Peckarsky and Dodson 1980; Gamboa et al. 2009). We expected that predatory stoneflies would have broader TPCs compared with their mayfly prey because predators and prey are thought to be under

Table 1. Elevation, location (latitude and longitude in decimal degrees), temperature, and taxa collected for each stream site in the temperate Rockies and tropical Andes

Elevation category	Elevation (m)	Latitude (DD)	Longitude (DD)	Avg. temperature at collection (°C)	Annual min. (°C)	Annual max. (°C)	Taxa
<i>Temperate Rockies</i>							
High	3,166	40.62348	-105.708004	6.16 ± 1.84	-0.1	11.9	Mayflies
Mid	2,798	40.81334	-105.708821	6.71 ± 1.12	-0.2	11.5	Stoneflies, Mayflies
Low	2,212	40.70346	-105.5847	11.35 ± 1.65	-0.3	21.4	Stoneflies
	1,992	40.69997	-105.441491	15.05 ± 1.92	-0.4	22	Mayflies
<i>Tropical Andes</i>							
High	3,683	-0.286931	-78.1153583	8.60 ± 0.98	4.6	13.1	Mayflies
Mid	2,694	-0.37639	-78.0747191	10.05 ± 0.93	7.2	13.3	Stoneflies, Mayflies
Low	1,845	-0.45034	-77.8907438	13.63 ± 0.87	10.8	16.9	Stoneflies
	2,003	-0.44924	-77.943	13.9 ± 0.61	12.9	16.4	Mayflies

Average temperatures and standard deviations are reported for only the months during which insects were collected and tested (late June–August in the Rockies and late October–December in the Andes). Because the Andean streams are thermally stable throughout the year, these temperatures represent their annual average temperatures. For Rocky Mountain streams, these temperatures represent summer means, when streams are warmest.

asymmetrical selection pressures (Dawkins and Krebs 1979; i.e., a thermal version of the “life-dinner principle,” Brodie and Brodie 1999). Thus, our findings also have important implications for the outcome of predator–prey interactions, especially as climate change occurs in these regions.

Materials and Methods

Study sites and insect collections

We tested baetid mayfly and perlid stonefly nymphs collected from streams occurring along an elevation gradient at each of two latitudes. We sampled insects from streams in the Andean Papallacta-Quijos river drainage, Napo, Ecuador (tropical), from late October to December 2016 and the Rocky Mountain Cache la Poudre river drainage, Colorado, USA (temperate), from late June to August 2016. During this time, average stream temperatures were very similar between temperate and tropical streams for a given elevation. Streams at both latitudes were roughly matched in elevation and assigned elevation categories relative to each other such that tropical streams were at 1,845 m and 2,003 m (low), 2,694 m (mid), and 3,683 m (high) and temperate streams were at 1,992 m and 2,212 m (low), 2,798 m (mid), and 3,166 m (high) (Table 1). We had two “low elevation” streams at each latitude because some taxa were difficult to find in the year of this study. Indeed, average temperatures of these low-elevation streams were very similar (Table 1). We recorded stream temperature by placing a temperature logger (HOBO pendant, Onset Corporation) in each stream. Loggers were attached using rebar and placed close to the streambed (~25 cm depth) to keep them inconspicuous and reduce the chances that they would be exposed to air at any point. In each stream, we placed the loggers in a random spot within the 10–15 m reach of stream where we collected insects. Temperature was logged once every 4 h. Here, we report temperatures recorded during the months our experiments were conducted (Table 1).

Because different genera and species occur at temperate and tropical locations, we controlled for phylogeny by comparing species in the same taxonomic family. We collected mayflies from the family *Baetidae* at low-, mid-, and high-elevation sites at both latitudes. The species *Baetis bicaudatus* was collected at mid and high elevations and species *B. tricaudatus* at low elevation in Colorado, USA. We collected individuals from the species complex *Andesiops*, which vary across the elevation gradient (Gill et al. 2016) in Napo,

Ecuador. Stoneflies from the family *Perlidae* were collected from low- and mid-elevation sites at both latitudes. In Colorado, we focused on the species *Hesperoperla pacifica*. In Napo, we collected *Anacronuria guambiana* at mid elevation, and *A. rawlinsi* at low elevation. Previous DNA-barcoding in combination with morphological descriptions allowed us to be confident about the taxonomic identity of the insects used in this study (see Gill et al. 2016; Shah et al. 2017b; Polato et al. 2018).

Incubation

Wild-caught insects were transported to the lab where they were placed in clean stream water and held for a total of 48 h. The insects were kept at the average temperature of their native streams, which were approximately equivalent between elevation pairs. At both latitudes, high-elevation insects were held at 7°C, mid-elevation insects at 10°C, and low-elevation insects at 14°C. Different incubation temperatures were necessary because, in previous studies, tropical high- and low-elevation mayflies appeared stressed when held for long periods at temperatures outside average stream temperature (Shah et al. 2017a). We did not feed insects during the incubation period to ensure the insects would not be digesting food during experiments.

After the 48 h incubation (acclimation) period, on the day of swimming performance trials, we gradually decreased holding temperatures for all insects to 5°C over a period of ~75 min. We then measured swimming performance at each of 5 temperature treatments (5°C, 10°C, 15°C, 20°C, and 25°C). We started at the lowest temperature (i.e., 5°C), then ramped to the next test temperature (e.g., Wilson and Franklin 1999), each time allowing insects to incubate at the new test temperature for ~1 h.

Swimming performance

We tested 5 individuals per elevation and latitude and performed repeated measurements of swimming velocity at each temperature in a water bath. We placed insects individually into swimming lanes made in a divided acrylic tray (each lane = 5 cm × 30 cm). The tray was immersed part way in a water bath which could be heated or cooled to achieve the desired test temperature. Once insects were tested at a given temperature, we used a 500 W titanium aquarium heater to gradually increase temperature (over a period of 1 h) to the next test temperature. We covered the open ends of the lanes with a fine mesh to confine insects while allowing water to pass through,

and used air stones and a pump to maintain oxygenation at ~80% saturation. We chose this value because none of the streams in our study system appear to drop below 80% oxygen saturation (Shah et al. 2017b). This method ensured that dissolved oxygen was as high as possible at any given temperature. Because oxygen saturation was maintained by the air stone and pump, we did not create additional flow in our experimental arena. The floor of the swimming lanes was patterned with a grid of 1 × 1 cm squares. All swimming trials were filmed at a frame rate of 30 fps from above with an iPad (iPad 2, Model A1395, Apple, Inc. USA). During swimming trials, we shut off the air pump to make the surface of the water still, allowing for easier viewing of the insects' movements in the video.

To induce swimming, we gently touched each insect on the posterior of the abdomen with a metal probe. We avoided prompting a response more than once per trial in order to reduce bias in the analysis; thus, if an insect did not respond after being touched, we moved on to the next individual. Insects underwent 3 trials per test temperature. Occasionally, the 25°C and 5°C test temperatures would induce a loss of righting response or other presumably thermally-induced stress response (Shah et al. 2017b), particularly in the tropical insects. In these cases, we were unable to perform more than 1 trial for that temperature.

Analysis of videos for performance measurements

We obtained maximum velocity using the digitizing program DLTdv5 in MATLAB (Hedrick 2008). We calibrated each video in the first frame by assigning an X, Y coordinate to the 1-cm grid. This allowed us to track an individual across a known distance over the length of the video clip. During analysis, the focal insect was assigned a point marker which automatically followed its movement across the grid until the last frame of the clip or until movement ceased. Maximum velocity was extracted from every video clip, ~3 values per individual, from which the highest value out of 3 was used in our analyses. After all trials were completed, we measured standard body lengths using the Image J software package (Abramoff et al. 2004).

Statistical analyses

We used polynomial mixed effect models to analyze the relationship between swimming performance and temperature in relation to latitude (temperate and tropical) and elevation (low, mid, and high) in mayflies and stoneflies. Individual identity was included as a random factor to account for repeated measurements. To explore whether temperature dependence of swimming performance varies between latitudes and elevations, we analyzed the interaction terms temperature × latitude and fit separate models for each latitude × elevation combination when the interaction terms were significant. We also explored possible differences in the response to temperature of mayflies and stoneflies by analyzing data from both orders and testing the interaction temperature × order at each latitude and elevation. In all cases, regardless of whether the interaction terms were significant, we represented the relationship between swimming performance and temperature for each latitude, elevation, and order using LOESS tendency lines. Finally, we estimated T_{OPT} and B_{95} (i.e., respectively, the temperature at which swimming velocity was maximum, and the 95% range around this maximum).

Because thermal performance functions are generally unimodal curves with a maximum in relation to temperature, we fitted second-order polynomial models and compared the goodness-of-fit of the linear and quadratic models using the Akaike information

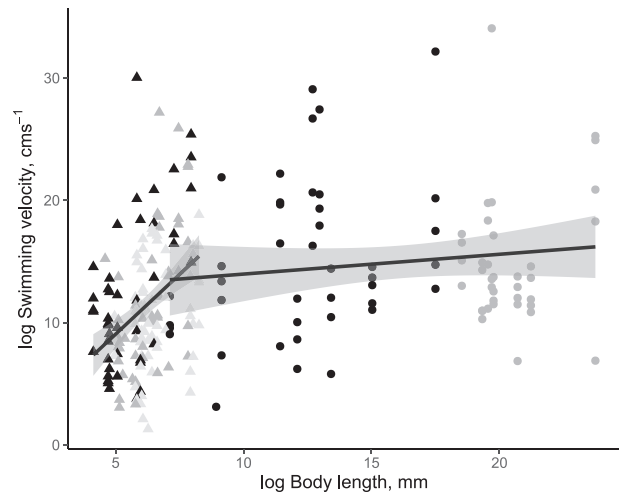


Figure 1. Relationship between swimming velocity and body size of mayflies (triangles) and stoneflies (circles) for each elevation (low: black, mid: gray, high: light gray). There is a strong positive relationship between length and velocity in mayflies, but there is no relationship in stoneflies.

criterion corrected for small sample sizes (AICc). The quadratic model was preferred over the linear model when the difference of AICc was >2 (Burnham and Anderson 2002). We analyzed the linear regression between log-swimming velocity and log-body length for each taxonomic order (mayflies or stoneflies). We used the residual values of these regressions as a response variable in all subsequent analyses to control for size effects on swimming velocity. Linear mixed effect models were fitted using the R package lme4 (Bates et al. 2015). To compute the denominator degrees of freedom and significance levels of the mixed effect models, we used Type III ANOVA with Satterthwaite's approximation using the package lmerTest (Kuznetsova et al. 2017) in R v3.5.3 (R Core Team 2019).

Data Availability

These files can be found on GitHub here: <https://github.com/Alisha-Shah/Swimming-performance-of-aquatic-insects>.

Results

We found a positive relationship between body length and swimming velocity in mayflies (0.98 ± 0.26 [$\beta \pm SE$]; $F_{1,32.4} = 13.86$; $P = 0.001$; mean body length $\pm SE$ 6.18 ± 1.06 mm) but not in stoneflies (0.41 ± 0.24 ; $F_{1,11.9} = 2.95$; $P = 0.12$; mean body length $\pm SE$: 16.19 ± 4.69 mm; Figure 1).

In mayflies, TPCs were different between temperate and tropical groups at low elevation (temperature × latitude: $F_{1,32.1} = 6.85$; $P = 0.013$; Table 2). Specifically, we found that the relationship with temperature was steeper and peaked at lower temperatures in low-elevation tropical mayflies ($T_{OPT} = 13.6^\circ\text{C}$, $B_{95} = 11.9^\circ\text{C} - 15.5^\circ\text{C}$; Figure 2 and Table 3), but flatter with a maximum at higher temperatures in temperate mayflies ($T_{OPT} = 18.9^\circ\text{C}$, $B_{95} = 16.7^\circ\text{C} - 21.2^\circ\text{C}$; Figure 2; Table 3). The differences between latitudes were nonsignificant at mid (temperature × latitude: $F_{1,36.6} = 1.86$; $P = 0.181$) and high elevations ($F_{1,51.8} = 2.26$; $P = 0.139$). At high elevation, TPCs for tropical and temperate mayflies peaked at similar temperatures (temperate: $T_{OPT} = 13.0^\circ\text{C}$, $B_{95} = 11.7^\circ\text{C} - 14.3^\circ\text{C}$; tropical: $T_{OPT} = 14.4^\circ\text{C}$, $B_{95} = 13.3^\circ\text{C} - 15.5^\circ\text{C}$; Figure 2). These values, however, need to be interpreted with caution because we

Table 2. Comparison of the linear and quadratic models fitted to swimming performance of mayflies and stoneflies in relation to temperature for each latitude and elevation

Elevation	Latitude	Intercept	Temperature	Temperature ²	R ² (%)	AICc linear	AICc quadratic
<i>Mayflies</i>							
Low	Temperate	0.60 (0.22)	0.56 (0.11)	-0.38 (0.12)	47.31	61.56	58.73
	Tropical	1.15 (0.35)	-0.05 (0.24)	-0.86 (0.29)	29.56	73.48	70.56
Mid	Temperate	-0.39 (0.23)	0.026 (0.15)	—	0.1	79.33	81.66
	Tropical	0.92 (0.29)	0.32 (0.18)	-0.59 (0.21)	33.01	63.92	62.19
High	Temperate	0.44 (0.20)	-0.16 (0.13)	-0.61 (0.14)	37.59	134.38	123.73
	Tropical	-0.19 (0.21)	0.01 (0.17)	—	0.03	67.78	66.86
<i>Stoneflies</i>							
Low	Temperate	-1.72 (0.53)	0.08 (0.02)	—	17	60	73.02
	Tropical	-0.89 (0.40)	0.09 (0.02)	—	36	56	66.78
Mid	Temperate	-0.14 (0.26)	-0.017 (0.015)	—	5	56	67.83
	Tropical	-0.38 (0.65)	0.055 (0.04)	—	12	53	64.94

Bolded parameter values—mean (SE)—indicate there was a significant effect of temperature.

could not statistically distinguish different models for temperate and tropical mayflies, and there was little statistical support to fit separate quadratic models at each latitude (Table 2). Finally, at mid elevation, we did not find significant differences between tropical and temperate mayflies and both families displayed monotonic relationships with temperature (Table 2).

TPCs did not differ between temperate and tropical stoneflies at low elevation (temperature \times latitude: $F_{1,28,4} = 0.27$; $P = 0.610$; Table 2). Latitudinal differences were also nonsignificant at mid elevation ($F_{1,33} = 3.67$; $P = 0.064$; Table 2), but with a tendency in temperate stoneflies to perform better at lower temperatures ($T_{OPT} = 12.1^{\circ}\text{C}$, $B_{95} = 8.4^{\circ}\text{C}$ – 15.5°C) than their tropical counterparts ($T_{OPT} = 16.3^{\circ}\text{C}$, $B_{95} = 14.1^{\circ}\text{C}$ – 18.9°C ; Figure 3 and Table 3).

When comparing TPCs of coexisting predatory stoneflies and their mayfly prey, we found differences at the low-elevation tropical site (temperature \times order: $F_{1,30,2} = 9.93$; $P = 0.004$; Figure 4). Mayflies displayed their maximum swimming velocity at a lower temperature than stoneflies (Figure 4). We found no significant differences at mid-elevation in the tropics ($F_{1,29} = 0.001$; $P = 0.98$), where both mayflies and stoneflies maximized swimming velocity at similar temperatures (Figure 4). At the temperate latitude, the difference between orders was not significant both at low ($F_{1,29,3} = 0.39$; $P = 0.53$) and mid elevations ($F_{1,38,5} = 0.53$; $P = 0.47$). In these cases, however, the TPCs did not follow the expected curvilinear relationship with a maximum at intermediate temperatures (Figure 4).

Discussion

Our understanding of variation in the thermal sensitivity of locomotor performance has largely been influenced by two opposing perspectives; 1) differing thermal regimes impose divergent selection and act as “motors” driving the adaptive evolution of TPCs, or 2) thermal physiology is conserved by various mechanisms acting as “brakes” to constrain the evolution of TPCs (Bogert 1949; Hertz et al. 1983; Huey and Kingsolver 1989; Muñoz and Bodensteiner 2019). Here, we shed light on these alternative views by comparing the thermal sensitivity of swimming performance in phylogenetically paired mayflies and stoneflies between latitudes and elevations. We found support for both divergence (motors) and constraints (brakes) acting on the shape of TPCs. Specifically, we found significant divergence in the shape of TPCs between low-elevation temperate and

tropical mayflies that were consistent with predictions from the CVH, but in all other cases, we found a lack of divergence between TPCs from temperate and tropical sites. Further, mayflies and their stonefly predators exhibited variation in TPCs at only the tropical low-elevation site. This difference in thermal sensitivity could impact the outcome of predator–prey encounters. To our knowledge, these are the first estimates of thermal swimming performance curves for any aquatic insect species. The lack of other such studies of aquatic insects prevents us from making broader generalizations of how temperature impacts performance in this ecologically important group of freshwater ectotherms.

Motors of divergence: climate variability as source of selection on TPCs

The difference in climate variability between temperate and tropical localities is greatest at low elevations (Table 1; see also Shah et al. 2017b) and should drive the evolution of locally adapted TPCs (Van Berkum 1988; Gilchrist 1995). In tropical lowlands, seasonally stable and warm temperatures should favor thermal specialists that perform best across the narrow range of temperatures they most commonly experience. By contrast, temperate lowlands that fluctuate between cold winter and warm summer temperatures should select for generalists that maintain performance across a wider range of temperatures. Consistent with these predictions, we found low-elevation tropical mayflies maintained high performance over a narrower range of temperatures compared with their temperate counterparts (Figure 2, see B_{95} values in Table 3). Temperate low-elevation mayflies displayed high performance at warmer temperatures (Figure 2), consistent with the warm summer temperatures they experience (Table 1). The TPC of low-elevation tropical mayflies was also initially steeper, peaking near 13.6°C , whereas low-elevation temperate mayflies had a shallower slope toward a peak of 18.9°C . The putative T_{OPT} s correspond with the average (tropical) and max (temperate) stream temperatures, respectively (Figure 2). These peaks also likely reflect the fact that animals were collected and tested during the temperate summer, when the streams were relatively warm. Performance of low-elevation tropical mayflies declined steeply beyond T_{OPT} and the B_{95} range (Figure 2). In fact, we were only able to induce swimming performance in 1 out of the 5 individuals tested at 25°C . The other 4 individuals were unresponsive at this test temperature and died shortly after experiments; similar patterns were not observed in the

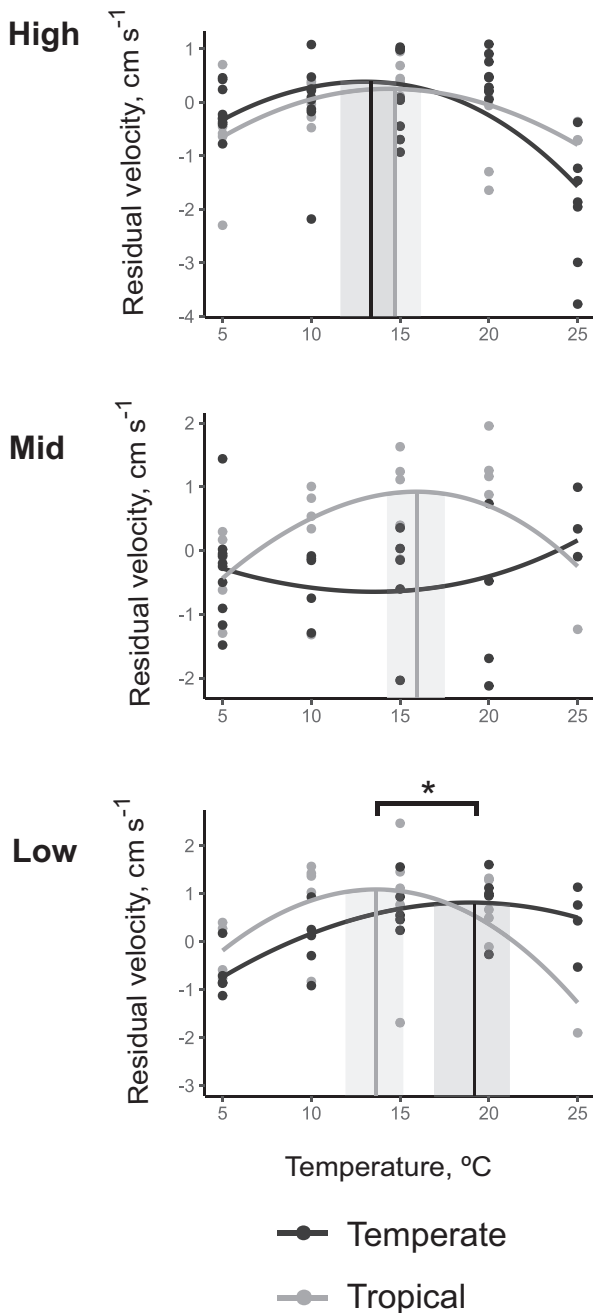


Figure 2. Thermal sensitivity of swimming performance shown as size-corrected velocity of mayflies for each elevation (low, mid, high) at the temperate and tropical latitudes. Points are repeated measurements per individual, and lines are LOESS tendencies (analyses were performed using quadratic mixed effect models including the individual ID as a random factor). Vertical lines and shadings represent the 95% range around the optimal temperature.

temperate mayflies. The divergent TPCs of low-elevation mayflies observed here are consistent with the different thermal regimes of temperate and tropical streams at this elevation, as has also been found for locomotor performance in anurans (Navas et al. 2008) and some lizards (Van Berkum 1988).

Brakes on divergence: conserved TPCs across latitude

Despite the expectation that TPCs should diverge in response to the differing temperate and tropical climatic regimes, most mayfly and

stonefly TPCs did not show significant differences, supporting the perspective that TPCs may be conserved by various constraints. Indeed, the lack of variation in TPCs across different thermal regimes (i.e., latitude) is more consistent with other comparative studies of terrestrial insect TPCs for development and growth (Sengupta et al. 2016; Ruybal et al. 2016; e.g., Kutcherov et al. 2015; Fragata et al. 2016; Wang et al. 2018). The mechanisms constraining TPC divergence between latitudes are, however, less clear. Several possible mutually nonexclusive hypotheses could explain the lack of divergence in TPCs. First, behavioral thermoregulation may reduce divergent selection between different thermal regimes by minimizing differences in body temperature (Bogert 1949; Hertz et al. 1983; Van Berkum 1988; Huey and Kingsolver 1989; Muñoz and Bodensteiner 2019). But given the small size of aquatic insects, their reduced capacity for movement, and the general homogeneity of water temperature, aquatic insects are less likely to exhibit active behavioral thermoregulation (Shah et al. 2020). Some aquatic invertebrates behaviorally select specific temperatures (Mundahl 1989; Chapperon and Seuront 2011; Piasečná et al. 2015), but there is no compelling evidence suggesting mayflies or stoneflies can actively track preferred temperatures or have body temperatures that differ from the water in which they occur.

A second explanation for the lack of divergence in TPCs could be that we measured the thermal sensitivity of burst swimming. Such short-term measures of performance are less sensitive to variation in both temperature and body size compared with longer-term measures of endurance (Videler and Wardle 1991; Austin and Shaffer 1992; Careau et al. 2014). Indeed, temperature was only a significant predictor of burst swimming performance at low elevations and for temperate mayflies at mid elevation, and body size was only a significant predictor for mayflies. However, unlike fish, aquatic insects do not actively engage in sustained swimming behavior but rather use short bursts of movement or simply drift in the water current (Waters 1972). Thus, it is unclear what other metrics of locomotor performance would capture how temperature and body size interact with lower-level effects on muscle kinetics, oxygen supply, diffusivity, and water viscosity (Verberk et al. 2011).

A third and potentially compelling explanation for the reduced sensitivity to temperature and the similarity in TPCs between temperate and tropical aquatic insects is that they are not constrained at all in their response to selection, but rather there is selection to maintain performance breadth across a broad range of temperatures (Huey and Hertz 1984; Van Berkum 1988; Gilchrist 1995). Previous studies have also considered a role for selection in the maintenance of a generalist strategy, particularly if there is a fitness trade-off with high performance over a restricted range of temperatures (Huey and Hertz 1984; Hertz et al. 1988; Gilchrist 1995; Tüzün and Stoks 2018). The challenge is to understand why a generalist strategy should be maintained in the tropics, which lack the seasonality of temperate regions. One possible explanation is the increasing diel variation in temperature that occurs with increasing elevation in the tropics (Shah et al. 2017a, 2017b). Tropical streams at higher elevations can exhibit considerable diel variation, such that with increasing elevation the difference between minimum and maximum water temperatures start to converge between temperate and tropical sites (Shah et al. 2017b). As a result, the lack of divergence in TPCs across latitude could reflect the somewhat similar thermal regimes of temperate and tropical streams at mid and high elevation. Indeed, we have found a concurrent decrease in thermal tolerance windows (measured as the difference between critical thermal limits) in temperate aquatic insects at these elevations (shown here in Table 3),

Table 3. Optimal temperatures (T_{OPT}) and tolerance breadths ($^{\circ}C$) of mayflies and stoneflies at each latitude and elevation

Elevation	Latitude	T_{OPT}	Breadth -95%	Breadth $+95\%$	CT_{MAX}	CT_{MIN}
Mayflies						
Low	Temperate	18.95	16.74	21.19	32.7	0.4
	Tropical	13.66	11.89	15.46	29.9	6.1
Mid	Temperate	—	—	—	28.8	0.8
	Tropical	15.90	14.02	17.83	25.70	2.8
High	Temperate	13.02	11.73	14.34	29.30	0.5
	Tropical	14.38	13.26	15.54	20.50	0
Stoneflies						
Low	Temperate	—	—	—	31.3	0.6
	Tropical	—	—	—	29.1	4.6
Mid	Temperate	12.07	8.43	15.51	33.20	0.90
	Tropical	16.31	14.09	18.94	27.40	1.10

Temperatures were calculated using LOESS tendencies fitted to data. Bold numbers indicate that the curvilinear relationship with temperature, required to calculate T_{OPT} , was supported by quadratic mixed effect models. Critical thermal maximum and minimum temperatures ($^{\circ}C$) summarized from Shah et al. (2017b) are also provided for ease of comparison.

suggesting selection on physiology could also be weaker (Shah et al. 2017b). This pattern of narrowing thermal tolerance with increasing elevation has also been detected in terrestrial ectotherms, such as lizards in temperate Tibet. High-elevation populations had narrower thermal performance compared with their low-elevation counterparts owing to decreased air temperature variation (Wu et al. 2018). Thus, elevation complicates the expectation of thermal stability in the tropics and these regions may be more variable than previously thought.

Comparing TPCs of stonefly predators and mayfly prey

The perlid stoneflies studied here are well-known predators of baetid mayflies (Peckarsky 1980; Gamboa et al. 2009). How each species responds to temperature could influence their interactions and ultimately community dynamics (Domenici et al. 2007). Our results showed that there were no strong differences in TPCs between mayflies and stoneflies except at low elevation in the tropics. This does not support the prediction that predator and prey experience asymmetrical selection (Dawkins and Krebs 1979) but instead suggests that both predator and prey may be under similar selection pressures to maintain swimming performance across a range of sublethal temperatures. We did, however, note qualitative differences in performance at the cold and hot extremes. Although stoneflies generally had lower performance compared with mayflies at $5^{\circ}C$, they had higher performance than mayflies at $25^{\circ}C$. This pattern is particularly prominent at the tropical low-elevation site (Figure 4). Thus, mayflies may have an advantage over their predators at cooler temperatures, but lose this advantage at higher temperatures. Indeed, predators from many taxonomic groups are more warm tolerant than their prey (Dell et al. 2011). In the stonefly–mayfly system, such differences in thermal sensitivity may have major consequences for predator–prey interactions and community structure (Rall et al. 2009; Sheldon et al. 2011; Shah et al. 2020) especially if mountain stream temperatures rise by several degrees as predicted (Pörtner et al. 2019).

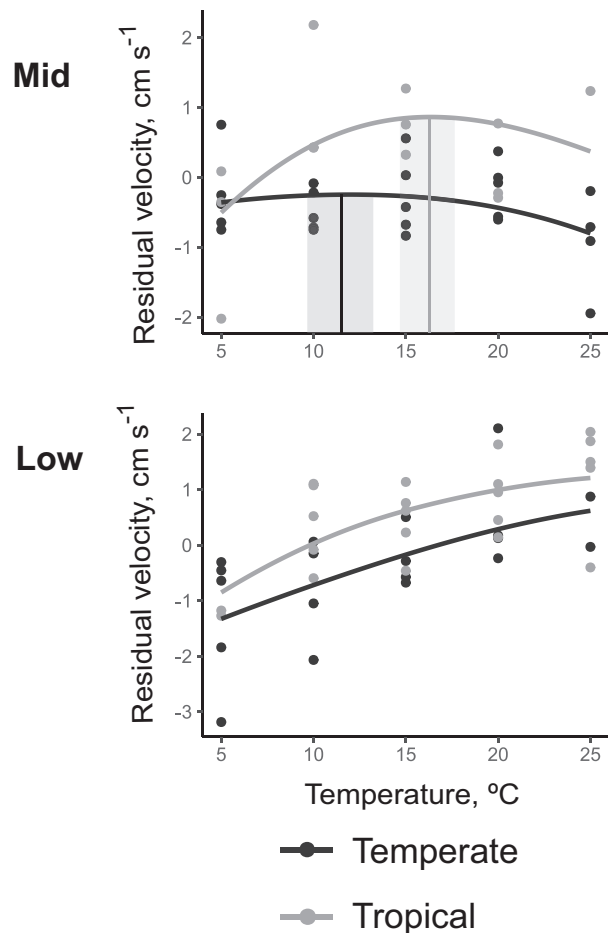


Figure 3. Thermal sensitivity of swimming performance of stoneflies for each elevation (low, mid) and latitude (temperate, tropical). Points are repeated measurements per individual and lines are LOESS tendencies (analyses were performed using quadratic mixed effect models including the individual ID as a random factor). Vertical lines and shadings represent the 95% range around the optimal temperature.

Conclusions

Despite the long-held assumption that variation in climatic regime is a strong driver of diversification in thermal physiology, we only found support for this view between temperate and tropical mayflies from low elevation, and not at any other elevations. This result contrasts sharply with the finding that thermal tolerance windows, defined by critical thermal limits, differ significantly between temperate and tropical species in this same study system (Shah et al. 2017b). Clearly, additional comparisons are needed to better understand which thermally sensitive traits are subject to evolutionary motors and brakes. Still, insights from the present study represent an important step in understanding what drives the evolution of thermal physiology in aquatic insects and how this translates to their vulnerability to climate change. Future avenues of research should assess the effects of temperature on not only additional species, but also on the outcome of interactions among species within communities (Barton and Schmitz 2009; Sheldon et al. 2011) under various climate change scenarios (Matassa and Trussell 2015; Tüzün and Stoks 2018; Wang et al. 2020).

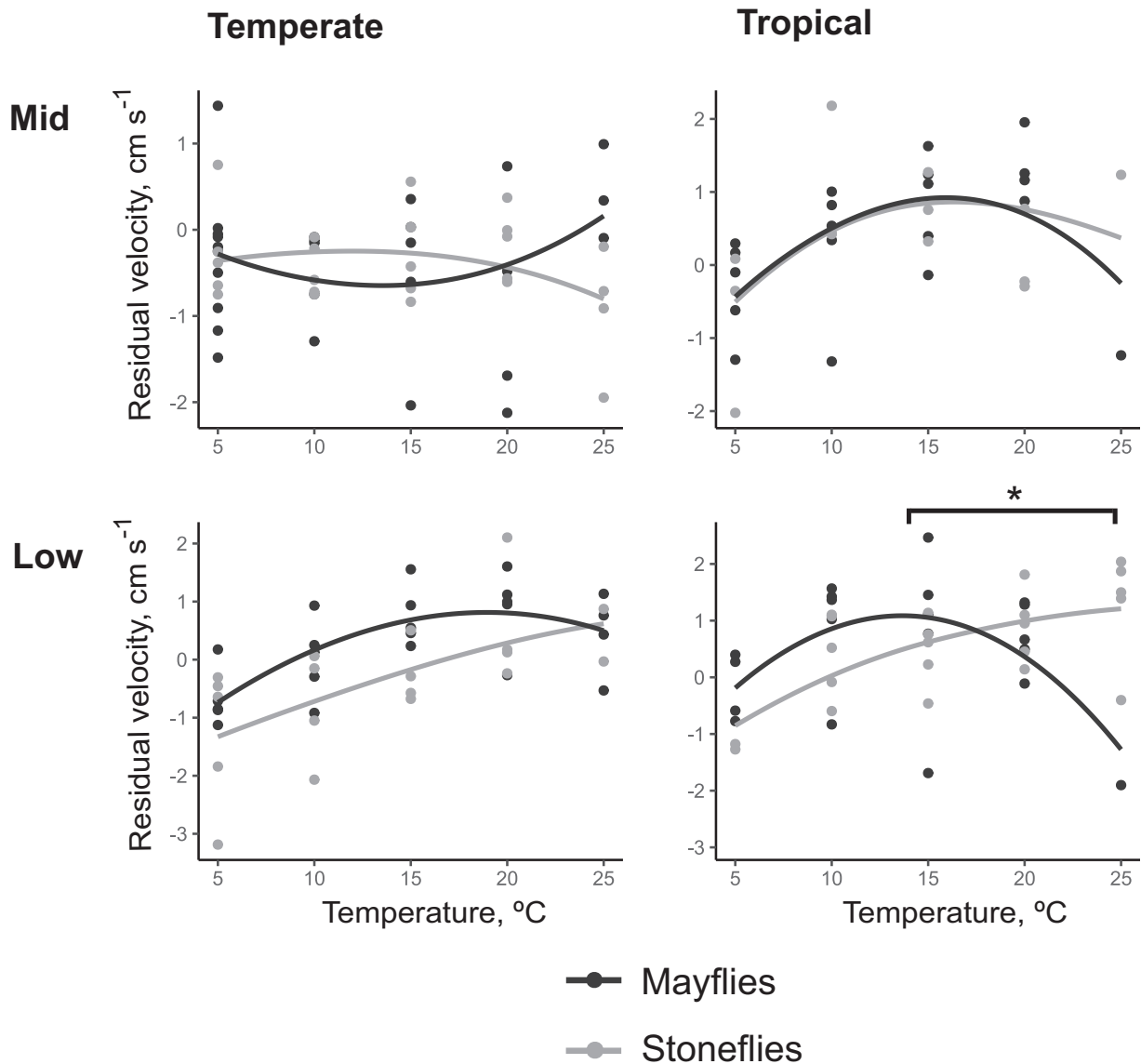


Figure 4. Comparison of TPCs of stonefly predators and their mayfly prey at each latitude and elevation. *Significant interaction between temperature and taxonomic order (i.e., stoneflies vs. mayflies).

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Authors' Contributions

A.A.S. and C.K.G. designed the research; A.A.S. and E.M.S.B. collected data; E.M.S.B. processed videos; J.G.R. performed analyses; A.A.S. wrote the manuscript with contributions from all authors.

Conflict of Interest statement

None declared.

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