RESEARCH ARTICLE

Warming undermines emergence success in a threatened alpine stonefly: A multi-trait perspective on vulnerability to climate change

Alisha A. Shah[1,2] | Scott Hotaling[3,4] | Anthony B. Lapsansky[1,5] | Rachel L. Malison[6] | Jackson H. Birrell[1] | Tylor Keeley[1] | J. Joseph Giersch[6] | Lusha M. Tronstad[7] | H. Arthur Woods[1]

[1]Division of Biological Sciences, University of Montana, Missoula, Montana, USA; [2]W.K. Kellogg Biological Station, Department of Integrative Biology, Michigan State University, Hickory Corners, Michigan, USA; [3]School of Biological Sciences, Washington State University, Pullman, Washington, USA; [4]Department of Watershed Sciences, Utah State University, Logan, Utah, USA; [5]Department of Zoology, University of British Columbia, Vancouver, British Columbia, Canada; [6]Flathead Lake Biological Station, University of Montana, Missoula, Montana, USA and [7]Wyoming Natural Diversity Database, University of Wyoming, Laramie, Wyoming, USA

Correspondence
Alisha A. Shah
Email: aashah@msu.edu

Scott Hotaling
Email: scott.hotaling@usu.edu

Abstract

1. Vulnerability to warming is often assessed using short-term metrics such as the critical thermal maximum (CT_{MAX}), which represents an organism's ability to survive extreme heat. However, the long-term effects of sub-lethal warming are an essential link to fitness in the wild, and these effects are not adequately captured by metrics like CT_{MAX}.

2. The meltwater stonefly, *Lednia tumana*, is endemic to high-elevation streams of Glacier National Park, MT, USA, and has long been considered acutely vulnerable to climate-change-associated stream warming. As a result, in 2019, it was listed as Threatened under the U.S. Endangered Species Act. This presumed vulnerability to warming was challenged by a recent study showing that nymphs can withstand short-term exposure to temperatures as high as ~27°C. But whether they also tolerate exposure to chronic, long-term warming remained unclear.

3. By measuring fitness-related traits at several ecologically relevant temperatures over several weeks, we show that *L. tumana* cannot complete its life-cycle at temperatures only a few degrees above what some populations currently experience.

4. The temperature at which growth rate was maximized appears to have a detrimental impact on other key traits (survival, emergence success and wing development), thus extending our understanding of *L. tumana*'s vulnerability to climate change.

5. Our results call into question the use of CT_{MAX} as a sole metric of thermal sensitivity for a species, while highlighting the power and complexity of multi-trait approaches to assessing vulnerability.

KEYWORDS
aquatic insect, climate vulnerability, CT_{MAX}, development, flight performance, glaciers, growth rate, stonefly, thermal tolerance

1 | INTRODUCTION

Climate change is occurring more rapidly at high elevations than almost anywhere else on Earth (Pepin et al., 2015). Such change has led to massive recession of mountain glaciers and snowfields (Hugonnet et al., 2021) and contemporary warming of headwater streams (Niedrist & Füreder, 2020). In the future, rising stream temperatures will force mountain stream biota to either tolerate their new thermal regimes or be locally extirpated (Birrell et al., 2020; Hotaling, Finn, et al., 2017; Jacobsen, 2020), putting many species at risk of extinction (e.g. Giersch et al., 2017). In North America, two alpine stoneflies have been listed under the U.S. Endangered Species Act due to climate change-induced habitat loss (*Lednia tumana* and *Zapada glacier*, US Fish and Wildlife Service, 2019). However, the vulnerability of alpine stream insects to warming has rarely been measured, particularly beyond short-term tests of thermal tolerance.

Vulnerability to climate change is often assessed using short-term metrics such as the critical thermal limits—CT_{MAX} and CT_{MIN}—which represent, respectively, the high and low temperature limits of an organism and are marked by loss of locomotor capacity (Angilletta, 2009; Chown et al., 2009; Lutterschmidt & Hutchison, 1997; Sinclair et al., 2016). Although easy to measure, and powerful in a comparative framework (Addo-Bediako et al., 2000; Dallas & Rivers-Moore, 2012; Shah et al., 2017; Sunday et al., 2012), estimates of CT_{MAX} and CT_{MIN} are difficult to link to fitness in the wild (Huey & Berrigan, 2001). In general, single traits represent only components of fitness, and each trait may vary to different degrees across a thermal range (Kingsolver & Woods, 1997; Shah, Bacmeister, et al., 2020). For example, in a comparison of temperate and tropical mayflies, thermal breadth (i.e. the difference between CT_{MAX} and CT_{MIN}) was narrower for tropical mayflies indicating that they are more vulnerable to warming than temperate mayflies (Shah et al., 2017). However, for the same groups, swimming performance did not vary significantly suggesting that from a performance standpoint, they are equally sensitive to warming (Shah, Bacmeister, et al., 2020). These seemingly opposing conclusions about vulnerability can be addressed and clarified by measuring multiple fitness-related traits (Kellermann & van Heerwaarden, 2019; Pearson et al., 2014).

Critical thermal limits alone may provide only a partial understanding of vulnerability because they are typically measured over just a few hours and represent thermal "end points" (Hochachka & Somero, 2002). However, the absolute temperatures that animals can tolerate are not fixed, and they decrease with duration of exposure (Rezende et al., 2014). Critical thermal limits also do not reflect organismal responses to the slower, chronic nature of climate warming, in which performance is not dictated by survival alone but also by growth, development and reproduction (Verberk et al., 2016). For most wild organisms, little is known of the long-term effects of moderately higher temperatures on fitness, and whether warming will be a net cost or benefit. For instance, some ectotherms may grow and develop faster in warmer conditions (Deutsch et al., 2008), but fast growth itself may trade off with other important traits

(e.g. starvation resistance, development or immune function; Arendt, 1997) and may produce less robust or even abnormal adults (Arnott et al., 2006; Ficetola & De Bernardi, 2006). In fruit flies, for example, larvae grow faster when reared at warmer temperatures, but fly more slowly as adults due to under-developed wing muscles (Fraimout et al., 2018).

Assessing vulnerability to climate warming from single traits is particularly challenging for organisms with complex lifecycles, such as aquatic insects whose life histories bridge aquatic and terrestrial environments (Harvey et al., 2022). Aquatic juveniles typically experience less thermal variation in water, but they also have fewer opportunities to escape from suboptimal temperatures. By contrast, adults experience greater thermal variation as well as more micro-climatic options for thermoregulation (Shah, Dillon, et al., 2020; Woods et al., 2015). These environmental differences likely drive divergent physiological tolerances between immature and adult stages. Further, conditions that juveniles experience can carry over beyond the developmental environment and influence adult traits (Bonte et al., 2008; McCauley et al., 2018). Because most aquatic insects spend a large proportion of their lives in water before emerging as winged adults, changes in both aquatic and terrestrial environments can impact their responses to climate change.

Stoneflies in the genus *Lednia* (Family: Nemouridae) are endemic to alpine habitats of western North America (Green et al., 2022). *Lednia tumana* occur in alpine streams of Glacier National Park, USA, and the surrounding mountains including many glacier-fed streams. Given their close association with ice and snow, *Lednia* species are presumed to be highly vulnerable to climate warming (Green et al., 2022), but this prediction is based on correlations between abundances and stream temperatures (Giersch et al., 2017; Green et al., 2022) and lacks a mechanistic explanation. Still, in 2019, *L. tumana* was listed under the U.S. Endangered Species Act due to climate change-induced habitat loss (US Fish and Wildlife Service, 2019). Recent work has challenged the basic tenet of vulnerability for *L. tumana* by showing that two species in the genus—*L. tumana* and *L. tetonica*—can withstand short-term exposure to temperatures that are remarkably high compared to the in-stream temperatures they naturally experience (up to an average of 27.4°C; Hotaling, Shah, et al., 2019). Further, *L. tumana* and other members of its cold water community persist in alpine basins that have not been glaciated in ~170 years (Muhlfeld et al., 2020). Thus, there is a disconnect between where the species occurs, its presumed vulnerability, and the limited mechanistic data on its physiological tolerances.

Here, we attempted to clarify these apparent inconsistencies by experimentally assessing the effects of prolonged exposure to ecologically relevant temperatures on multiple development and performance-based traits for *L. tumana* nymphs and adults. Specifically, we addressed three questions: (1) What are the highest temperatures *L. tumana* nymphs can withstand over multi-week time-scales? (2) How do different traits vary in their responses to this prolonged warming? And, (3) how does long-term exposure to warmer temperatures during aquatic development affect the performance of terrestrial adults?

2 | MATERIALS AND METHODS

2.1 | Insect collection and rearing

We collected mid-to-late instar *Lednia tumana* nymphs (N = 419) from five streams in the alpine of Glacier National Park, MT, USA in mid-July 2019 (Figure 1; Table 1). Specimens were collected under USGS permit #GLAC-2016-SCI-0007. Sites were chosen because they contained high *L. tumana* abundances. In each stream, we deployed temperature loggers (HOBO Water Temperature Pro v2 data; Onset Computer Corporation) secured to rocks with coated steel cable. Loggers were checked 5 days after deployment, but later could not be retrieved for the next 2 years due to a rock slide at one site and fieldwork challenges as a result of the COVID-19 pandemic. However, based on longer-term datasets in other high Rocky Mountain streams, including in Glacier National Park (Hotaling, Foley, et al., 2019), we are confident that our short-term data are representative of summer temperatures in these streams. Nymphs were transferred from the stream in Whirlpak bags filled with stream water to the University of Montana in Missoula, MT. At the time of collection, we also scraped rocks to collect algae and biofilm, a typical food source for *L. tumana* (Karen Jorgensen, unpubl. data) and many other nemourids (Lieske & Zwick, 2007), in Lunch Creek, a representative stream with a very high density of *L. tumana*. The algae and biofilm (hereafter "biofilm slurry"), were used to feed nymphs during the experiment.

In the laboratory, nymphs were transferred to incubators (Percival Scientific, LT-36VLC8, I-66LLC8, I-36LLC8, I-66LLC8) that were initially held at 7°C to match the water temperature in the Whirlpak bags after transport. Over ~5 h, incubators gradually reached fixed test temperatures of 1, 4, 7, 13, 21°C. We also included a variable treatment (T_{MAX} = 10°C, T_{MIN} = 4°C, average = 7°C) to mimic the summer temperature regime of a thermally variable stream, Lunch Creek, included in our study. For the variable treatment, the incubator rose to the maximum temperature (10°C) over 3 h (07:00–10:00), remained stable for 9 h (10:00–19:00), then decreased to the minimum (4°C) over 3 h (19:00–22:00), which was then held overnight. For all treatments, light–dark cycles were set to 16:8 h L:D to approximate summertime in the northern Rockies. Each population × temperature treatment had two replicates of 7 individuals on average (max = 10, min = 3). Replicate groups of nymphs were placed in separate clear plastic containers with air stones for oxygenation and fed with 100 mL of biofilm slurry. We placed two ceramic tiles (2 cm diam.) in each plastic container to serve as substrate. Throughout the study, levels of food and water were checked once per day. The experiment lasted 31 days.

2.2 | Trait measurements and analyses

We measured growth rate, emergence success, survival and adult locomotor performance in *L. tumana* across 6 temperature
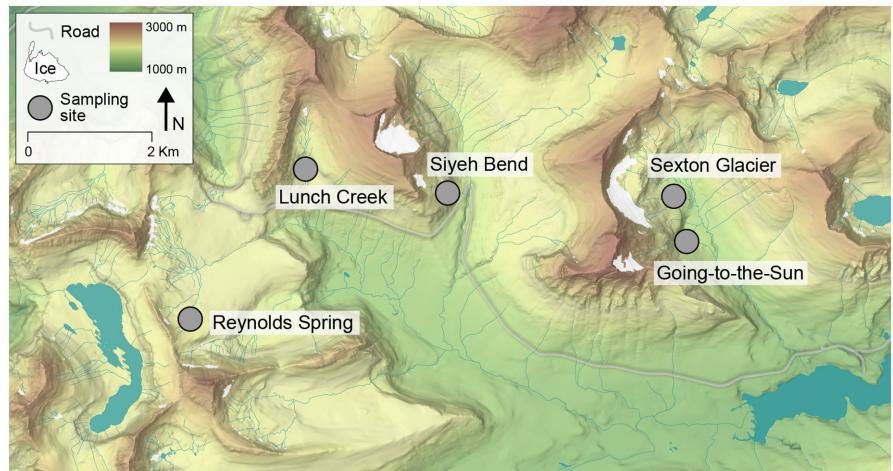
FIGURE 1 A map of our study sites in Glacier National Park, MT, USA where *Lednia tumana* nymphs were collected.

TABLE 1 Sampling sites and locations included in this study. Site temperature average (T_{AVG}), minima (T_{MIN}), and maxima (T_{MAX}) are in degrees Celsius. At each site, temperature was measured during a ~5-day period in the summer (13–17 August 2019).

Site	Elevation (m)	Latitude, Longitude	T_{AVG}	T_{MIN}	T_{MAX}	$N_{SAMPLED}$
Siyeh Bend	1830	48.7002°, −113.6701°	7.7	2.6	14.7	97
Sexton Glacier	1893	48.7003°, −113.6250°	3.0	1.6	5.3	110
Lunch Creek	2101	48.7049°, −113.7041°	6.5	4.1	10.8	105
Going-to-the-Sun	1746	48.6944°, −113.6201°	4.4	2.9	6.3	54
Reynolds Spring	2085	48.6818°, −113.7322°	7.1	2.8	14.5	53

treatments (5 fixed temperatures and 1 variable regime). To obtain growth rate data, nymphs were photographed from above (Olympus Tough TG-6) with a 2 cm scale placed in the field of view. To allow for assessment of emergence success, we placed plastic ladders, cut from embroidery mesh, in each container and stretched soft tule mesh over a wire frame to trap adults as they emerged. Each day the number of emergences and mortality were noted. Dead individuals were removed, and emerged adults were gently captured within 1 day of emergence and held at 7°C for up to 2 days for locomotor performance measurements. All analyses of data were conducted in R (R version 3.6.3).

2.3 | Growth rate

We measured body length (tip of head to tip of abdomen, not including cerci) from photos using the line tool in ImageJ (Abramoff et al., 2004). To obtain a more energetically-based response variable from body length, we converted length into dry mass using a length-mass regression:

$$Dry\,mass = aL^b,$$

where a and b are constants obtained from length-mass regressions of other nemourid species (Benke et al., 1999), and L is body length. Because it was not possible to tell individuals apart within containers, we calculated a mean dry mass for each replicate container. We found that growth rate had ceased in some of the warmer treatments after 2 weeks. This is likely because nymphs had completed growth and were very close to emergence. We therefore restricted our growth rate analyses to only the first 2 weeks of the experiment. The per-week growth rate was measured as the difference between mean mass between the first 2 weeks for each replicate divided by 2. Due to small sample size, we pooled replicates for each site×temperature for analyses. We initially tried to fit a number of functions to our data but due to high variance and small sample sizes, only the Gaussian function converged to a solution. We therefore used the Gaussian function (Angilletta, 2006):

$$Growth\,Rate = Gmax \times e^{-\left(\frac{T-Topt^2}{2c^2}\right)},$$

where $Gmax$ is the height of the peak, T is the sequence of temperature values over which growth rates were assessed, $Topt$ is the position of the centre of the peak, and c is the standard deviation of the curve. We chose starting parameter values that seemed likely by visually inspecting the plot: $Gmax = 0.07$, $Topt = 13$ and $c = 5$.

2.4 | Survival

We analysed survival using a logistic regression implemented as a generalized linear model (glm, family "binomial") in R. For each individual, survival was coded as a binomial variable, that is, as a 1

(survived) or a 0 (died). Emerged insects were also counted as having survived. We then assessed the fixed effects of temperature, site and their interaction on survival and used the random effect of container to account for the grouping of individuals into different containers.

2.5 | Emergence

We used the same logistic regression approach as survival for assessing the effect of temperature on emergence success. Emergence data were converted to binomial variables (1 = emerged, 0 = did not emerge) and we analysed the fixed effects of temperature, site and temperature×site on emergence success. To account for grouping effects, we included the random effect of container in the model.

2.6 | Adult locomotor performance and wing morphology

Flight is the primary mode by which adult aquatic insects find mates (swarming) and disperse. In addition to flying in air, adult stoneflies also skim across the water surface to move along a stream. When surface skimming, stoneflies use their wings to generate thrust without leaving the surface of the water (Marden & Kramer, 1994). This form of locomotion is widespread across Plecoptera (Marden et al., 2000; Thomas et al., 2000) and relatively well-studied because of its hypothesized role in the evolution of insect flight (Marden & Kramer, 1994; Will et al., 1995). We used the average sustained speed of surface skimming in *L. tumana* to test whether the temperature they experienced as juveniles affected their adult performance.

Collected adults were dropped from their plastic holding container onto water in a metal tray (30×40×2 cm deep) by inverting and tapping the container. Skimming was filmed from above using a levelled, tripod-mounted Fastcam Mini AX100 (Photron) recording at 2000 frames per second. Each adult was dropped 3 to 4 times consecutively and filmed for 120 s each time.

Stoneflies were auto-tracked from videos using DeepLabCut (Mathis et al., 2018). To account for digitization error, we smoothed these data using MATLAB R2021a (Mathworks) and the "smoothingspline" function (The Mathworks, 2019). We plotted speed versus time for each skim and used the average speed of the longest non-zero and approximately horizontal portion of the plot (exhibited after initial acceleration but before final deceleration) to measure the average sustained skimming speed. Because laboratory temperature was difficult to precisely control, we recorded the temperature and adjusted the skimming speed to an estimated value at 22.5°C by using the slope of a regression derived for the winter stonefly *Taeniopteryx burksi* (Marden et al., 2000; Marden & Kramer, 1994). This "adjusted skimming speed" was used in downstream analyses. After skimming tests, adults were immediately

frozen. We assessed the effect of rearing temperature on adjusted skimming speed using a linear model with each individual's mean adjusted skimming speed as a response variable and the interaction between temperature and site as a predictor variable in the model to test if temperature affected skimming performance differentially across sites.

We also measured wing length to use as a correlate of locomotor ability. We removed the fore- and hindwings from the right side of each frozen adult and photographed them (Nikon D7100) under a stereomicroscope (Nikon SMZ1500). Damaged wings were excluded from analysis. Wing length was measured as the distance between the upper corner of the wing base and the apex of the third radial (R3) wing vein near the wing tip using the line tool in ImageJ. We pooled measurements within sites and used a linear model in R to test whether developmental temperature affected fore- and hindwing length. We also measured wing area (see Appendix S1 in Supporting Information), but because this metric was highly correlated with wing length ($r_{(31)} = 0.93$, $p < 0.001$), we focused on wing length in our analyses. To confirm that wing morphology is correlated with adult locomotor performance given our study design, we analysed the relationship between each individual's forewing and hindwing lengths and their sustained speed during surface skimming.

2.7 | Variable versus static rearing treatment

To measure differences in trait sensitivity between individuals in the variable temperature treatment (min = 4°C, max = 10°C, mean = 7°C) versus the most similar fixed temperature treatment (7°C), we conducted Welch's two-sample *t*-tests for each trait, that is, growth rate, survival, emergence and skimming performance.

3 | RESULTS

3.1 | Growth rate

For all populations, growth rate was highest between weeks 1 and 2 of the experiment. Curve-fitting revealed that T_{OPT} lies at ~13°C (Figure 2a, Table 2).

3.2 | Survival

Survival declined with increasing temperature: individuals survived best at the coolest temperatures, 1°C (55%), 4°C (41.3%), 7°C (33.5%) and the worst at the warmest temperatures, 13°C (9.8%) and 21°C (3.2%; Figure 2b). Stoneflies from Sexton Glacier and Siyeh Bend had consistently low survival across all temperatures. The logistic regression with temperature, site, and their interaction as predictors indicated that the main effects of temperature and site were significant (temperature: chi sq = 34.44, $p < 0.001$; site: chi sq = 32.37, $p < 0.001$) but that their interaction was not (chi sq 1.86, $p = 0.76$).

Notably, survival for all sites was low in the 13°C treatment (mean across populations = 9.8%) where growth rate was highest.

3.3 | Emergence

The percentage of emerged individuals varied across populations but declined with increasing temperature. Specifically, more individuals emerged at the cooler temperatures: 1°C (30.8%), 4°C (24.5%), 7°C (31.8%) and the fewer at the warmest temperatures, 13°C (12.6%) and 21°C (0.1%) (Figure 2c). The logistic regression revealed a significant main effect of temperature (chi sq 10.76, $p < 0.01$), but non-significant effects of site and the temperature × site interaction (site: chi sq 6.94, $p = 0.14$; temperature × site: chi sq 1.65, $p = 0.80$). Only a few individuals emerged from the 13°C treatment and none from the 21°C treatment (Figure 2c). At 13°C, some individuals exhibited dark wing pads (indicating they were ready for emergence), but with hemolymph leaking from their bodies (Figure 2d).

3.4 | Adult locomotor performance and wing morphology

Consistent with previous studies (e.g. Marden & Kramer, 1994) there was a significant relationship between trial-averaged skimming speed and both forewing length ($F_{(1, 12)} = 8.20$, $p = 0.014$, $R^2 = 0.41$) and hindwing length ($F_{(1, 11)} = 9.15$, $p = 0.012$, $R^2 = 0.45$; see Appendix S2).

We then compared wing lengths among rearing treatments. Because few individuals with intact wings emerged from the 13 and 21°C treatments, we could only compare wing characteristics among insects incubated at 1, 4, and 7°C. Although fore- and hindwings were somewhat longer in the 4°C rearing treatment (Figure 3), the differences were not significant (forewing length: $F_{(1, 31)} = 0.64$, $p = 0.43$; Figure 3a; hindwing length: $F_{(1, 27)} = 0.16$, $p = 0.69$; Figure 3b). To assess how confident we should be in accepting the null hypothesis of no difference among groups, we evaluated the 95% confidence intervals of the *differences* between groups (Dziak et al., 2020). These confidence intervals were tightly grouped around zero, suggesting that the non-significant result did not arise simply from the lower power associated with small samples sizes. Skimming speed did not differ among adults emerging from different temperatures ($F_{(4, 12)} = 0.61$, $p = 0.66$; Figure 2e) because there was no variation among wing lengths and rearing treatments, as shown above. There was also no significant interaction between site and rearing temperature on wing length ($F_{(4, 12)} = 2.15$, $p = 0.18$).

3.5 | Variable vs. fixed rearing treatment

Mean trait values were not different between insects reared in the variable temperature treatment (average = 7°C) versus the fixed (7°C) treatment (Table 3).
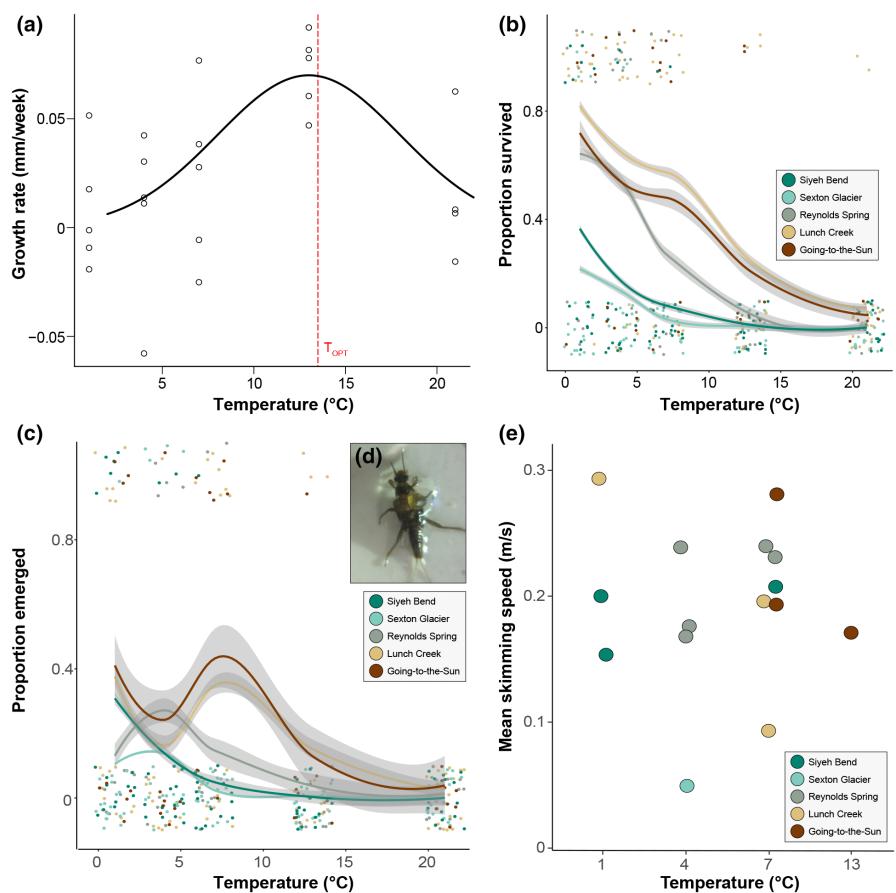
FIGURE 2 (a) A Gaussian function fitted to growth rates pooled across replicates for each site × rearing temperature treatment. The dashed vertical line indicates T_{OPT} at 13.5°C. (b) The proportion of individuals that survived in each temperature treatment. Survival declined with increasing temperature, and was notably low at 13°C, the temperature at which growth rates were highest. For better visualization, points are jittered on both the *x*- and *y*-axes and loess smooth lines with 95% CI are shown. (c) The proportion of individuals that emerged for all populations decreased with rearing temperature and was exceptionally low at 13 and 21°C. At 13°C, several individuals appeared to be injured during emergence. Points are jittered along the *x*-axis wherever they overlapped. (d) An example of a nymph that failed to emerge, with a partially split exoskeleton and a hemolymph bubble arising from its thorax. (e) Adult mean skimming speed across sites and temperature treatments. There was no variation in performance across sites or rearing temperatures.

TABLE 2 Parameter estimates of the thermal performance curve for growth rates in *Lednia tumana*.

Parameter	Estimate	Std. error	*t* value	Pr(>|t|)
G_{MAX}	0.07	0.01	5.03	<0.001
T_{OPT}	13.45	1.43	9.37	<0.001
c	4.36	0.90	4.84	<0.001

4 | DISCUSSION

Rising temperatures and shifting precipitation regimes are driving the recession of glaciers and permanent snowfields worldwide (Hugonnet et al., 2021, IPCC 2021), potentially endangering a multitude of species (Cauvy-Fraunié et al., 2015; Stibal et al., 2020). For aquatic biodiversity, rising water temperature presents the most pressing risk associated with declines of the cryosphere (Hotaling, Finn, et al., 2017;

Niedrist & Füreder, 2020). However, the presumed risk of warming to aquatic species is largely derived from current distribution patterns, that is, species restricted to meltwater are presumed to face greater risk of extirpation or extinction (e.g. Giersch et al., 2017). Although a useful starting point, assessing risk from distributions alone lacks an eco-physiological cause-and-effect context and may under- or overestimate the resilience of particular species to climate change. Gaining relevant eco-physiological insight through experimentation, however, can also be challenging, particularly for aquatic species endemic to remote regions, such as mountain tops, and whose life histories span aquatic and terrestrial habitats.

Short-term metrics of thermal tolerance (e.g. CT_{MAX}) are easy to implement, and have therefore been widely used to identify species' tolerance to warming (e.g. Bruno et al., 2018; Deutsch et al., 2008; Pinsky et al., 2019). However, short-term metrics are sensitive to methodology, including temporal scale (Leiva et al., 2019; Rezende et al., 2014) and do not address how species respond to chronically warm temperatures. Indeed, longer-term exposure to heat will

FIGURE 3 Adult wing length as a function of rearing temperature. There was no variation in wing length for different temperature treatments. However, for forewings (a) and hindwings (b), insects that emerged from the 4°C treatment showed a trend for longer wings compared to those emerged from 1°C and 7°C. Forewings and hindwings were measured according to the dashed red line shown on representative wings in (a) and (b), respectively.
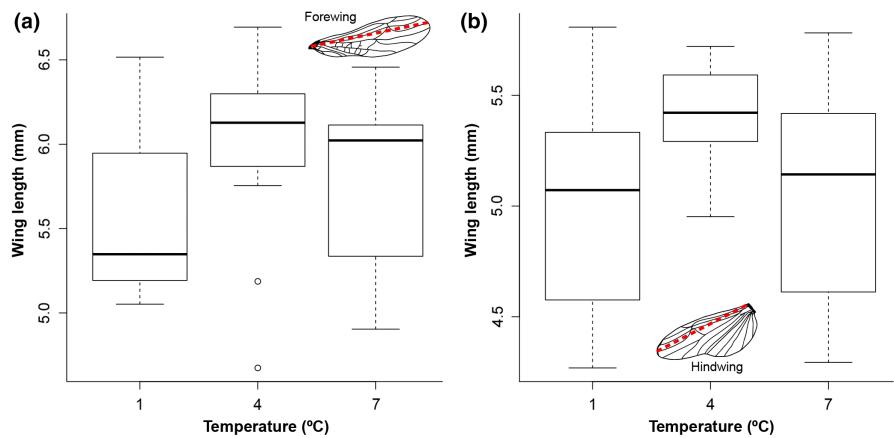

TABLE 3 Welch's two-sample test statistics for difference between means in the variable (mean temperature = 7°C) versus fixed (7°C) treatments for all traits.

Trait	df	t-statistic	p-value
Growth rate	12.59	2.01	0.07
Survival	4.85	−0.50	0.64
Emergence	6.34	1.06	0.33
Skimming speed	3.05	−0.68	0.55

undoubtedly lead to lower thermal tolerance trait values (Clusella-Trullas et al., 2021), but how much lower remains unknown for alpine aquatic insects facing rapid habitat warming. Thus, there is a pressing need to consider sub-lethal responses, which will be impacted at lower temperatures.

Here, using multiple fitness-related traits, we evaluated the consequences of warming over ecologically relevant time-scales for *Lednia tumana*, a threatened stonefly endemic to high-elevation streams in and around Glacier National Park, USA. The association between *L. tumana* and cold, often glacier-fed, meltwater has prompted investigations of its response to thermal stress (e.g. Hotaling et al., 2021; Hotaling, Shah, et al., 2019; Treanor et al., 2013), but to date, this work has consisted of only short-term measurements (i.e. 1–3 h) of tolerance to heat and cold stress. Overall, our results reveal that *L. tumana* is sensitive to even moderate warming. For example, we show that temperatures above 13°C yield high rates of mortality at longer time-scales. Moreover, thermal performance differed among traits; nymphs grew fastest at higher temperatures, whereas they transitioned into adulthood (emergence) best at much lower temperatures, with most individuals emerging between 1 and 7°C. Thus, there is clear value in determining which traits are impacted most by warming to more accurately predict climate change outcomes. We acknowledge that a variable temperature regime at the warmer temperatures (13°C and 21°C) may have supported accelerated growth overall as well as successful emergence during nighttime low temperatures. Such an experimental design, however, was not possible given our equipment and resources. Generally, our results indicate that mountain streams that exceed 7°C for prolonged periods in the summer may not support *L. tumana* populations. Beyond

new understanding of climate risks for a threatened species, we provide the first multi-trait perspective on climate vulnerability for any alpine stream insect. This is particularly relevant in light of global threats to alpine stream invertebrates (Birrell et al., 2020; Brown et al., 2007; Jacobsen et al., 2012; Leys et al., 2017), and our results have direct implications for their persistence under climate change.

4.1 | Temperature effects on growth rate, survival and emergence

Despite low emergence rates above 7°C, growth rate was maximized at higher temperatures (~13°C) although we note that the true T_{OPT} for growth in *Lednia* may have been obscured due to our small sample size and high variance in the growth rate data. Nevertheless, for small ectotherms living in temperate and high-elevation regions, growth is often limited by low temperatures (Roitberg & Mangel, 2016; Woods et al., 2022). At warmer temperatures, life processes such as metabolic rate, food intake, and rates of enzyme mediated reactions, tend to peak (Angilletta & Dunham, 2003). As we have shown for *L. tumana*, many temperate insects perform best at warm temperatures (Frazier et al., 2006), and some will choose temperatures that maximize growth (Miller et al., 2009). Assuming that stream productivity will increase with warming (Oleksy et al., 2020) and fuel faster growth, a high T_{OPT} for growth rate suggests that some *L. tumana* populations, much like many other types of insects (Deutsch et al., 2008), will initially benefit from warmer streams. However, additional research on important traits such as feeding and metabolic rates and egg count of *L. tumana* will be essential to understanding the full effects of temperature on their fitness.

In contrast to growth rate, other life-history traits in *L. tumana* showed greater sensitivity to temperature, which undermines conclusions about benefits and harm, and underscores the importance of investigating multiple traits. Survival declined steeply across populations with increasing temperature, and, emergence was low (~10%) at the temperature that yielded the highest growth rate (13°C). At this temperature, many emerging adults died before exiting the water, often with hemolymph leaking from their thoraxes. Adults from cooler treatments did not show similar injuries. Rapid

growth but low emergence at 13°C may reflect an underlying trade-off driven by a lack of adequate energy supply to fuel a complete emergence in warm water (Nash et al., 2021). This increased energy requirement for moulting may compound an already rising demand for oxygen at warmer temperatures (Dallas & Ross-Gillespie, 2015; Verberk et al., 2016).

4.2 | Temperature effects on adult traits

Temperature is a well-known determinant of ectotherm size at adulthood, and particularly, lower temperatures during development often result in larger adult size (French et al., 1998; Ray, 1960). For winged ectotherms, temperature can also affect morphology and, ultimately, flight performance (Frazier et al., 2008; Verberk et al., 2021). We examined the effects of developmental temperature on wing traits and skimming performance. Interestingly, adults emerging from the different temperature treatments (1, 4 and 7°C) did not show significant differences in the sizes of fore- or hindwings. However, similar to previous findings (Marden & Kramer, 1994), increased wing length translated to increased skimming velocity. Together, these results are consistent with our additional observation that skimming speed was not related to rearing temperature.

Lednia often fly just above streams (A. Shah & S. Hotaling, pers. obs.) and, like other small stoneflies (Macneale et al., 2005), they probably disperse both actively and passively with strong winds to nearby streams (Green et al., 2022). Evidence of dispersal activity can be inferred from *Lednia* population genetic structure, with populations exhibiting gene flow across different regions of the park (Hotaling, Muhlfeld, et al., 2017). However, fine-scale estimates of dispersal capacity for *L. tumana* do not currently exist, and this limits our ability to predict the degree to which local adaptation may be occurring. Still, if longer wings are correlated with better dispersal capacity in *L. tumana*, as they are in other species (Berwaerts et al., 2002; Malmqvist, 2000), long-winged individuals should contribute more to population connectivity. Given that high-alpine populations of many aquatic insects appear increasingly at risk from climate change, future work should focus on mechanistic links between in-stream conditions and dispersal capabilities.

4.3 | The challenge of integrating multiple traits to predict climate vulnerability

Assessing species' vulnerability to climate change is challenging, especially for species that reside in climatically harsh, remote habitats. Many studies implement a "*quick and dirty*" approach to vulnerability by measuring critical limits (Huey et al., 2012), which requires the least time and resources. This approach can of course be useful in a comparative framework, in instances where only a few individuals can be used, or in cases where the relationship between CTmax and sub-lethal responses are properly established. For most wild organisms, however, correlations between thermal limits

and sub-lethal thermal responses are unknown (but see Braschler et al., 2021) and render unreliable the sole use of thermal limit metrics in vulnerability assessments. Our study, like several others (Clusella-Trullas et al., 2021; Terblanche et al., 2007) serves as a cautionary tale for the use of single thermal tolerance indices. In addition to variation in response to high-temperature stress among key traits and life stages as has been shown in other species under thermal stress (Horne et al., 2019; Kingsolver & Buckley, 2020; Levy et al., 2015), we show that *L. tumana* fails to complete its life cycle at temperatures far below CT$_{MAX}$. Emergence—the moment of transition from an aquatic to a terrestrial phase—is also the most thermally sensitive component of its life history. Rising stream temperatures may therefore imperil populations of *L. tumana* by hindering emergence. This general conclusion is subject to obvious caveats—including lack of information regarding phenotypic and genotypic variation within and among *L. tumana* populations, the strength, timing, and frequency of selection exerted by high temperatures, and the roles of phenotypic plasticity in promoting persistence in the face of change. Of particular interest is the heritability of thermal tolerance traits. If the adults that managed to emerge at high temperatures also reproduced, and if their tolerance to heat stress is heritable, then strong selection could drive rapid adaptation to warmer conditions in these populations. Measuring heritability of thermal tolerance traits was beyond the scope of our study, but it should be incorporated into future investigations. Finally, existing or new biotic interactions may also further alter *Lednia*'s ability to cope with thermal change (Shah, Dillon, et al., 2020).

5 | CONCLUSIONS

As climate change proceeds and meltwater sources recede, flows in mountain streams will be reduced (Hotaling, Finn, et al., 2017). Smaller streams, which contain a majority of freshwater mountain biodiversity, will sustain higher mean temperatures and reach higher maximum temperatures (Birrell et al., 2020). Our results suggest that survival and emergence of *L. tumana* may decline significantly once stream temperatures consistently exceed 7°C. Over time, low emergence may reduce recruitment, drive population declines, and ultimately lead to population extirpation on local and regional scales. Continued monitoring and physiological testing of populations will help to refine our understanding of climate vulnerability for *L. tumana* and similar taxa worldwide.

AUTHOR CONTRIBUTIONS
Alisha A. Shah and Scott Hotaling designed the study with substantial input from J. Joseph Giersch and H. Arthur Woods; Alisha A. Shah, Scott Hotaling, J. Joseph Giersch, Jackson H. Birrell, Rachel L. Malison, Anthony Lapsansky and Tylor Keeley collected the data; Alisha A. Shah, Anthony Lapsansky and H. Arthur Woods performed statistical analyses; Alisha A. Shah and Scott Hotaling wrote the manuscript. All authors contributed substantially to revisions and gave final approval for submission.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflicts of interest pertaining to our manuscript.

DATA AVAILABILITY STATEMENT

All data and associated scripts used in this study are available on Zenodo: https://doi.org/10.5281/zenodo.7573829 (Shah et al., 2023).

ORCID

Alisha A. Shah https://orcid.org/0000-0002-8454-7905
Scott Hotaling https://orcid.org/0000-0002-5965-0986
Anthony B. Lapsansky https://orcid.org/0000-0001-7530-7830
Rachel L. Malison https://orcid.org/0000-0001-6803-8230
Jackson H. Birrell https://orcid.org/0000-0001-5339-0717
J. Joseph Giersch https://orcid.org/0000-0001-7818-3941
Lusha M. Tronstad https://orcid.org/0000-0002-1845-0267
H. Arthur Woods https://orcid.org/0000-0002-3147-516X

REFERENCES

Abramoff, M. D., Magalhaes, P. J., & Ram, S. J. (2004). Image processing with ImageJ. *Biophotonics International*, *11*(7), 36–42.

Addo-Bediako, A., Chown, S. L., & Gaston, K. J. (2000). Thermal tolerance, climatic variability and latitude. *Proceedings of the Royal Society B: Biological Sciences*, *267*(1445), 739–745.

Angilletta, M. J. (2006). Estimating and comparing thermal performance curves. *Journal of Thermal Biology*, *31*(7), 541–545.

Angilletta, M. J. (2009). *Thermal adaptation: A theoretical and empirical synthesis*. Oxford University Press.

Angilletta, M. J., & Dunham, A. E. (2003). The temperature-size rule in ectotherms: Simple evolutionary explanations may not Be general. *The American Naturalist*, *162*(3), 332–342.

Arendt, J. D. (1997). Adaptive intrinsic growth rates: An integration across taxa. *Quarterly Review of Biology*, *72*(2), 149–177.

Arnott, S. A., Chiba, S., & Conover, D. O. (2006). Evolution of intrinsic growth rate: Metabolic costs drive trade-offs between growth and swimming performance in *Menidia menidia*. *Evolution*, *60*(6), 1269–1278.

Benke, A. C., Huryn, A. D., Smock, L. A., & Wallace, J. B. (1999). Length-mass relationships for freshwater macroinvertebrates in North America with particular reference to the southeastern United States. *Journal of the North American Benthological Society*, *18*(3), 308–343.

Berwaerts, K., Van Dyck, H., & Aerts, P. (2002). Does flight morphology relate to flight performance? An experimental test with the butterfly Pararge aegeria. *Functional Ecology*, *16*, 484–491.

Birrell, J. H., Shah, A. A., Hotaling, S., Giersch, J. J., Williamson, C. E., Jacobsen, D., & Woods, H. A. (2020). Insects in high-elevation streams: Life in extreme environments imperiled by climate change. *Global Change Biology*, *26*(12), 6667–6684.

Bonte, D., Travis, J. M. J., De Clercq, N., Zwertvaegher, I., & Lens, L. (2008). Thermal conditions during juvenile development affect adult dispersal in a spider. *Proceedings of the National Academy of Sciences of the United States of America*, *105*(44), 17000–17005.

Braschler, B., Chown, S. L., & Duffy, G. A. (2021). Sub-critical limits are viable alternatives to critical thermal limits. *Journal of Thermal Biology*, *101*, 103106.

Brown, L. E., Hannah, D. M., & Milner, A. M. (2007). Vulnerability of alpine stream biodiversity to shrinking glaciers and snowpacks. *Global Change Biology*, *13*(5), 958–966.

Bruno, J. F., Bates, A. E., Cacciapaglia, C., Pike, E. P., Amstrup, S. C., Van Hooidonk, R., Henson, S. A., & Aronson, R. B. (2018). Climate change threatens the world's marine protected areas. *Nature Climate Change*, *8*(6), 499–503.

Cauvy-Fraunié, S., Espinosa, R., Andino, P., Jacobsen, D., & Dangles, O. (2015). Invertebrate metacommunity structure and dynamics in an Andean glacial stream network facing climate change. *PLoS ONE*, *10*(8), e0136793.

Chown, S. L., Jumbam, K. R., Sørensen, J. G., & Terblanche, J. S. (2009). Phenotypic variance, plasticity and heritability estimates of critical thermal limits depend on methodological context. *Functional Ecology*, *23*(1), 133–140.

Clusella-Trullas, S., Garcia, R. A., Terblanche, J. S., & Hoffmann, A. A. (2021). How useful are thermal vulnerability indices? *Trends in Ecology & Evolution*, *36*(11), 1000–1010.

Dallas, H., & Ross-Gillespie, V. (2015). Review: Sublethal effects of temperature on freshwater organisms, with special reference to aquatic insects. *Water SA*, *41*(5), 712–726.

Dallas, H. F., & Rivers-Moore, N. A. (2012). Critical thermal maxima of aquatic macroinvertebrates: Towards identifying bioindicators of thermal alteration. *Hydrobiologia*, *679*(1), 61–76.

Deutsch, C. A., Tewksbury, J. J., Huey, R. B., Sheldon, K. S., Ghalambor, C. K., Haak, D. C., & Martin, P. R. (2008). Impacts of climate warming on terrestrial ectotherms across latitude. *Proceedings of the National Academy of Sciences of the United States of America*, *105*(18), 6668–6672.

Dziak, J. J., Dierker, L. C., & Abar, B. (2020). The interpretation of statistical power after the data have been gathered. *Current Psychology*, *39*, 870–877.

Ficetola, G., & De Bernardi, F. (2006). Trade-off between larval development rate and post-metamorphic traits in the frog *Rana latastei*. *Evolutionary Ecology*, *20*(2), 143–158.

Fraimout, A., Jacquemart, P., Villarroel, B., Aponte, D. J., Decamps, T., Herrel, A., Cornette, R., & Debat, V. (2018). Phenotypic plasticity of Drosophila suzukii wing to developmental temperature: Implications for flight. *Journal of Experimental Biology*, *221*(13), jeb166868.

Frazier, M. R., Harrison, J. F., Kirkton, S. D., & Roberts, S. P. (2008). Cold rearing improves cold-flight performance in Drosophila viachanges in wing morphology. *Journal of Experimental Biology*, *211*(13), 2116–2122.

Frazier, M. R., Huey, R. B., & Berrigan, D. (2006). Thermodynamics constrains the evolution of insect population growth rates: "Warmer is better.". *The American Naturalist*, *168*(4), 512–520.

French, V., Feast, M., & Partridge, L. (1998). Body size and cell size in Drosophila: The developmental response to temperature. *Journal of Insect Physiology*, *44*(11), 1081–1089.

Giersch, J. J., Hotaling, S., Kovach, R. P., Jones, L. A., & Muhlfeld, C. C. (2017). Climate-induced glacier and snow loss imperils alpine stream insects. *Global Change Biology*, *23*(7), 2577–2589.

Green, M. D., Tronstad, L. M., Giersch, J. J., Shah, A. A., Fallon, C. E., Blevins, E., Kai, T. R., Muhlfeld, C. C., Finn, D. S., & Hotaling, S. (2022). Stoneflies in the genus Lednia (Plecoptera: Nemouridae): Sentinels of climate change impacts on mountain stream biodiversity. *Biodiversity and Conservation*, *31*, 1–25.

Harvey, J. A., Tougeron, K., Gols, R., Heinen, R., Abarca, M., Abram, P. K., Berg, M., Boggs, C., Brodeur, J., Cardoso, P., de Boer, J. G., De Snoo, G. R., Deacon, C., Dell, J. E., Desneux, N., Dillon, M. E., Duffy, G. A., Dyer, L. A., … Chown, S. L. (2022). Scientists' warning on climate change and insects. *Ecological monographs*, *93*(1), e1553.

Hochachka, P. W., & Somero, G. N. (2002). Mechanism and process in physiological evolution. In *Biochemical adaptation*. Oxford University Press.

Horne, C. R., Hirst, A. G., Atkinson, D., Almeda, R., & Kiørboe, T. (2019). Rapid shifts in the thermal sensitivity of growth but not development rate causes temperature–size response variability during ontogeny in arthropods. *Oikos*, *128*(6), 823–835.

Hotaling, S., Finn, D. S., Giersch, J. J., Weisrock, D. W., & Jacobsen, D. (2017). Climate change and alpine stream biology: Progress, challenges, and opportunities for the future. *Biological Reviews*, *92*(4), 2024–2045.

Hotaling, S., Foley, M. E., Zeglin, L. H., Finn, D. S., Tronstad, L. M., Giersch, J. J., Muhlfeld, C. C., & Weisrock, D. W. (2019). Microbial assemblages reflect environmental heterogeneity in alpine streams. *Global Change Biology*, *25*(8), 2576–2590.

Hotaling, S., Muhlfeld, C. C., Giersch, J. J., Ali, O. A., Jordan, S., Miller, M. R., Luikart, G., & Weisrock, D. W. (2018). Demographic modelling reveals a history of divergence with gene flow for a glacially tied stonefly in a changing post-Pleistocene landscape. *Journal of Biogeography*, *45*(2), 304–317.

Hotaling, S., Shah, A. A., Dillon, M. E., Giersch, J. J., Tronstad, L. M., Finn, D. S., Woods, H. A., & Kelley, J. L. (2021). Cold tolerance of mountain stoneflies (Plecoptera: Nemouridae) from the High Rocky Mountains. *Western North American Naturalist*, *81*(1), 54–62.

Hotaling, S., Shah, A. A., McGowan, K. L., Tronstad, L. M., Joseph Giersch, J., Finn, D. S., Woods, H. A., Dillon, M. E., & Kelley, J. L. (2019). Mountain stoneflies may tolerate warming streams: Evidence from organismal physiology and gene expression. *Global Change Biology*, *26*(10), 5524–5538.

Huey, R. B., & Berrigan, D. (2001). Temperature, demography, and ectotherm fitness. *The American Naturalist*, *158*(2), 204–210.

Huey, R. B., Kearney, M. R., Krockenberger, A., Holtum, J. A. M., Jess, M., & Williams, S. E. (2012). Predicting organismal vulnerability to climate warming: Roles of behaviour, physiology and adaptation. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *367*(1596), 1665–1679.

Hugonnet, R., McNabb, R., Berthier, E., Menounos, B., Nuth, C., Girod, L., Farinotti, D., Huss, M., Dussaillant, I., Brun, F., & Kääb, A. (2021). Accelerated global glacier mass loss in the early twenty-first century. *Nature*, *592*, 726–731.

Jacobsen, D. (2020). The dilemma of altitudinal shifts: caught between high temperature and low oxygen. *Frontiers in Ecology and the Environment*, *18*(4), 211–218.

Jacobsen, D., Milner, A. M., Brown, L. E., & Dangles, O. (2012). Biodiversity under threat in glacier-fed river systems. *Nature Climate Change*, *2*(5), 361–364.

Kellermann, V., & van Heerwaarden, B. (2019). Terrestrial insects and climate change: Adaptive responses in key traits. *Physiological Entomology*, *44*(2), 99–115.

Kingsolver, J. G., & Buckley, L. B. (2020). Ontogenetic variation in thermal sensitivity shapes insect ecological responses to climate change. *Current Opinion in Insect Science*, *41*, 17–24.

Kingsolver, J. G., & Woods, H. A. (1997). Thermal sensitivity of growth and feeding in Manduca sexta caterpillars. *Physiological Zoology*, *70*(6), 631–638.

Leiva, F. P., Calosi, P., & Verberk, W. C. E. P. (2019). Scaling of thermal tolerance with body mass and genome size in ectotherms: A comparison between water- and air-breathers. *Philosophical Transactions of the Royal Society B*, *374*(1778), 20190035.

Levy, O., Buckley, L. B., Keitt, T. H., Smith, C. D., Boateng, K. O., Kumar, D. S., & Angilletta, M. J. (2015). Resolving the life cycle alters expected impacts of climate change. *Proceedings of the Royal Society B: Biological Sciences*, *282*(1813), 20150837.

Leys, M., Keller, I., Robinson, C. T., & Räsänen, K. (2017). Cryptic lineages of a common alpine mayfly show strong life-history divergence. *Molecular Ecology*, *26*(6), 1670–1686.

Lieske, R., & Zwick, P. (2007). Food preference, growth and maturation of *Nemurella pictetii* (Plecoptera: Nemouridae). *Freshwater Biology*, *52*(7), 1187–1197.

Lutterschmidt, W. I., & Hutchison, V. H. (1997). The critical thermal maximum: History and critique. *Canadian Journal of Zoology*, *75*(10), 1561–1574.

Macneale, K. H., Peckarsky, B. L., & Likens, G. E. (2005). Stable isotopes identify dispersal patterns of stonefly populations living along stream corridors. *Freshwater Biology*, *50*(7), 1117–1130.

Malmqvist, B. (2000). How does wing length relate to distribution patterns of stoneflies (Plecoptera) and mayflies (Ephemeroptera)? *Biological Conservation*, *93*(2), 271–276.

Marden, J. H., & Kramer, M. G. (1994). Surface-skimming stoneflies: A possible intermediate stage in insect flight evolution. *Science*, *266*(5184), 427–430.

Marden, J. H., O'Donnell, B. C., Thomas, M. A., & Bye, J. Y. (2000). Surface-skimming stoneflies and mayflies: The taxonomic and mechanical diversity of two-dimensional aerodynamic locomotion*. *Physiological and Biochemical Zoology*, *73*(6), 751–764.

Mathis, A., Mamidanna, P., Cury, K. M., Abe, T., Murthy, V. N., Mathis, M. W., & Bethge, M. (2018). DeepLabCut: Markerless pose estimation of user-defined body parts with deep learning. *Nature Neuroscience*, *21*(9), 1281–1289.

McCauley, S. J., Hammond, J. I., & Mabry, K. E. (2018). Simulated climate change increases larval mortality, alters phenology, and affects flight morphology of a dragonfly. *Ecosphere*, *9*(3), e02151.

Miller, G. A., Clissold, F. J., Mayntz, D., & Simpson, S. J. (2009). Speed over efficiency: locusts select body temperatures that favour growth rate over efficient nutrient utilization. *Proceedings of the Royal Society B: Biological Sciences*, *276*(1673), 3581–3589.

Muhlfeld, C., Cline, T., Giersch, J., Peitzsch, E., Florentine, C., Jacobsen, D., & Hotaling, S. (2020). Specialized meltwater biodiversity persists despite widespread deglaciation. *Proceedings of the National Academy of Sciences of the United States of America*, In press, *117*, 12208–12214.

Nash, L. N., Antiqueira, P. A. P., Romero, G. Q., de Omena, P. M., & Kratina, P. (2021). Warming of aquatic ecosystems disrupts aquatic–terrestrial linkages in the tropics. *Journal of Animal Ecology*, *90*(7), 1623–1634.

Niedrist, G. H., & Füreder, L. (2021). Real-time warming of Alpine streams:(re) defining invertebrates' temperature preferences. *River Research and Applications*, *37*(2), 283–293.

Oleksy, I. A., Beck, W. S., Lammers, R. W., Steger, C. E., Wilson, C., Christianson, K., Vincent, K., Johnson, G., Johnson, P. T. J., & Baron, J. S. (2020). The role of warm, dry summers and variation in snowpack on phytoplankton dynamics in mountain lakes. *Ecology*, *101*(10), e03132.

Pearson, R. G., Stanton, J. C., Shoemaker, K. T., Aiello-Lammens, M. E., Ersts, P. J., Horning, N., Fordham, D. A., Raxworthy, C. J., Ryu, H. Y., McNees, J., & Akçakaya, H. R. (2014). Life history and spatial traits predict extinction risk due to climate change. *Nature Climate Change*, 4(3), 217–221.

Pepin, N., Bradley, R. S., Diaz, H. F., Baraer, M., Caceres, E. B., Forsythe, N., Fowler, H., Greenwood, G., Hashmi, M. Z., Liu, X. D., Miller, J. R., Ning, L., Ohmura, A., Palazzi, E., Rangwala, I., Schöner, W., Severskiy, I., Shahgedanova, M., Wang, M. B., … Yang, D. Q. (2015). Elevation-dependent warming in mountain regions of the world. *Nature Climate Change*, 5(5), 424–430.

Pinsky, M. L., Eikeset, A. M., McCauley, D. J., Payne, J. L., & Sunday, J. M. (2019). Greater vulnerability to warming of marine versus terrestrial ectotherms. *Nature*, 569(7754), 108–111.

Ray, C. (1960). The application of Bergmann's and Allen's rules to the poikilotherms. *Journal of Morphology*, 106(1), 85–108.

Rezende, E. L., Castañeda, L. E., & Santos, M. (2014). Tolerance landscapes in thermal ecology. *Functional Ecology*, 28(4), 799–809.

Roitberg, B. D., & Mangel, M. (2016). Cold snaps, heatwaves, and arthropod growth. *Ecological Entomology*, 41(6), 653–659.

Shah, A. A., Bacmeister, E. M. S., Rubalcaba, J. G., & Ghalambor, C. K. (2020). Divergence and constraint in the thermal sensitivity of aquatic insect swimming performance. *Current Zoology.*, 66(5), 555–564.

Shah, A. A., Dillon, M. E., Hotaling, S., & Woods, H. A. (2020). High elevation insect communities face shifting ecological and evolutionary landscapes. *Current Opinion in Insect Science.*, 41, 1–6.

Shah, A. A., Gill, B. A., Encalada, A. C., Flecker, A. S., Funk, W. C., Guayasamin, J. M., Kondratieff, B. C., Poff, N. L., Thomas, S. A., Zamudio, K. R., & Ghalambor, C. K. (2017). Climate variability predicts thermal limits of aquatic insects across elevation and latitude. *Functional Ecology*, 31(11), 2118–2127.

Shah, A. A., Hotaling, S., Lapsansky, A. B., Malison, R. L., Birrell, J. H., Keeley, T., Giersch, J. J., Tronstad, L. M., & Woods, H. A. (2023). Warming undermines emergence success in a threatened alpine stonefly: A multi-trait perspective on vulnerability to climate change. *Zenodo.* https://doi.org/10.5281/zenodo.7573829

Sinclair, B. J., Marshall, K. E., Sewell, M. A., Levesque, D. L., Willett, C. S., Slotsbo, S., Yunwei, D., Harley, C. D. G., Marshall, D. J., Helmuth, B. S., & Huey, R. B. (2016). Can we predict ectotherm responses to climate change using thermal performance curves and body temperatures? *Ecology Letters*, 19(11), 1372–1385.

Stibal, M., Bradley, J. A., Edwards, A., Hotaling, S., Zawierucha, K., Rosvold, J., Lutz, S., Cameron, K. A., Mikucki, J. A., Kohler, T. J., Šabacká, M., & Anesio, A. M. (2020). Glacial ecosystems are essential to understanding biodiversity responses to glacier retreat. *Nature Ecology & Evolution*, 4(5), 686–687.

Sunday, J. M., Bates, A. E., & Dulvy, N. K. (2012). Thermal tolerance and the global redistribution of animals. *Nature Climate Change*, 2(9), 686–690.

Terblanche, J. S., Deere, J. A., Clusella-Trullas, S., Janion, C., & Chown, S. L. (2007). Critical thermal limits depend on methodological context. *Proceedings of the Royal Society B: Biological Sciences*, 274(1628), 2935–2943.

The Mathworks I. (2019). Curve Fitting Toolbox.

Thomas, M. A., Walsh, K. A., Wolf, M. R., McPheron, B. A., & Marden, J. H. (2000). Molecular phylogenetic analysis of evolutionary trends in stonefly wing structure and locomotor behavior. *Proceedings of the National Academy of Sciences of the United States of America*, 97(24), 13178–13183.

Treanor, H. B., Giersch, J. J., Kappenman, K. M., Muhlfeld, C. C., & Webb, M. A. H. (2013). Thermal tolerance of meltwater stonefly Lednia tumana nymphs from an alpine stream in Waterton–Glacier international Peace Park, Montana, USA. *Freshwater Science*, 32(2), 597–605.

US Fish and Wildlife Service. (2019). Endangered and threatened wildlife and plants: Threatened species status for meltwater lednian stonefly and western glacier stonefly with a section 4(d) rule. *Federal Register*, 84, 64210–64227.

Verberk, W. C. E. P., Atkinson, D., Hoefnagel, K. N., Hirst, A. G., Horne, C. R., & Siepel, H. (2021). Shrinking body sizes in response to warming: Explanations for the temperature–size rule with special emphasis on the role of oxygen. *Biological Reviews*, 96(1), 247–268.

Verberk, W. C. E. P., Durance, I., Vaughan, I. P., & Ormerod, S. J. (2016). Field and laboratory studies reveal interacting effects of stream oxygenation and warming on aquatic ectotherms. *Global Change Biology*, 22(5), 1769–1778.

Will, K. W., Marden, J. H., & Kramer, M. G. (1995). Plecopteran surface-skimming and insect flight evolution. *Science*, 270(5242), 1684–1686.

Woods, H. A., Dillon, M. E., & Pincebourde, S. (2015). The roles of microclimatic diversity and of behavior in mediating the responses of ectotherms to climate change. *Journal of Thermal Biology*, 54, 86–97.

Woods, H. A., Legault, G., Kingsolver, J. G., Pincebourde, S., Shah, A. A., & Larkin, B. G. (2022). Climate-driven thermal opportunities and risks for leaf miners in aspen canopies. *Ecological Monographs*, 92(4), e1544.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Appendix S1. Figure S1. Wing area measurements.

Appendix S2. Figure S2. Relationship between wing length and skimming speed.

How to cite this article: Shah, A A., Hotaling, S., Lapsansky, A B., Malison, R L., Birrell, J H., Keeley, T., Giersch, J J., Tronstad, L M., & Woods, H A. (2023). Warming undermines emergence success in a threatened alpine stonefly: A multi-trait perspective on vulnerability to climate change. *Functional Ecology*, 37, 1033–1043. https://doi.org/10.1111/1365-2435.14284