Assessing thermal tolerance of vulnerable alpine stream insects as part of a long-term monitoring project in the Teton Range, Wyoming

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Abstract Alpine streams are predicted to decline as air temperatures warm and their water sources dry. Stream temperatures are expected to increase as glaciers and permanent snowfields decrease in size. For aquatic insects that are cold-adapted and restricted to small, high elevation streams fed by glaciers or snowfields, warmer water temperatures could be lethal. Conversely, less water in streams may increase the likelihood of insects freezing during winter months. We measured the critical thermal maximum (CT_{MAX}) – the highest non-lethal temperature an insect can survive, and supercooling temperature – the temperature at which an insect freezes, of three alpine stoneflies, *Zapada* sp., *Lednia tetonica* and *Lednia tumana*, collected in Grant Teton and Glacier National Parks. CT_{MAX} and supercooling point varied among species and with stream source (glacier-fed, snowmelt-fed and icy seep) and population (seven populations). Supercooling temperature was lowest in an alpine tarn and highest in glacier- and snowmelt-fed streams. *Zapada* sp. had the lowest CT_{MAX} of the three species. Stoneflies from icy seeps had lower CT_{MAX} than individuals from glacier- or snowmelt-fed streams. Individuals that likely experience the coldest winter temperatures had the lowest supercooling temperature. Similarly, stoneflies that experienced warmer water temperatures also had higher CT_{MAX} values. Investigating the thermal tolerances of alpine stoneflies allows us to predict how these insects may respond to future climate change scenarios.

Introduction

Alpine streams are geographically isolated and provide unique habitat for aquatic insects (Hotaling et al., 2017). Isolation, habitat specificity, and the rapid environmental shifts associated with climate change comprise a challenging combination for alpine specialists (Beever et al., 2003). Any directional change in environmental conditions means local extinctions of species with narrow limits of habitat tolerance, and isolation translates to a ‘no place to run’ scenario where local extirpations in alpine headwaters could result in regional or even global species extinctions (Giersch et al., 2015; Jordan et al., 2016). However, the physiology of alpine stream organisms is largely unknown, raising a difficult challenge for predicting their fate in a changing landscape. We typically assume that macroinvertebrates inhabiting alpine streams are cold-adapted, and therefore will be unable to persist as glaciers and perennial snowfields recede, and alpine streams warm.

In 2015, we established the Teton Alpine Stream Research (TASR) project to measure how stream biodiversity is currently structured and how it may change in the years to come. Instead of a space-for-time approach where conditions in one drainage (e.g., minimal glaciation) are used as a proxy for future conditions in another drainage (e.g., with extensive present-day glaciation), we are employing long-term monitoring of the same 10 sites which will provide a powerful means for disentangling how complex processes are affecting alpine streams of the Teton Range on decadal timescales. Our efforts have already yielded promising results. We have shown that population genetic differentiation varies across species (Hotaling et al., 2019*b*), microbial communities reflect their hydrological sources (Hotaling et al., 2019*a*), and macroinvertebrate communities also differ with hydrological source but not to the degree that microbes do (Tronstad et al., In prep.). However, our most striking finding has been the characterization of a new alpine stream type – icy seeps – which are fed by subterranean ice (Hotaling et al., 2019*a*). Historically, alpine streams have been classified into three main types which reflect the diversity of hydrological sources in mountain ecosystems: glacier-fed streams, snowmelt-fed streams, and groundwater-fed springs (Ward, 1994; Hotaling et al., 2017). Icy seeps are fed by subterranean ice in landscape features – primarily rock glaciers – which are predicted to persist on the landscape longer than glaciers and perennial snowfields. Across the American West, there are ∼5,000 glaciers and perennial snowfields of which around one-fourth are surface glaciers (Fountain et al., 2017). There are more than 10,000 rock glaciers across the same area (Johnson, 2018) and a similar story likely exists for other regions (e.g., Scotti et al., 2013; Lilleøren and Etzelmüller, 2011; Charbonneau and Smith, 2018). Thus, rock glaciers and icy seeps may dominate the alpine landscape yet comprise a minority of the alpine stream research focus. From the perspective of global change, perhaps the most intriguing aspect of rock glaciers is the expectation that they will persist on the landscape after surface glaciers and perennial snowfields are lost (Clark et al., 1994; Anderson et al., 2018; Knight et al., 2019). For aquatic ecosystems, this means there is clear potential for icy seeps to be the most persistent ice-fed habitat in a warming world, highlighting their potential to act as refugia for cold-adapted organisms (Hotaling et al., 2019*a*).

In 2018, our University of Wyoming-National Park Service (UW-NPS) funded research had two objectives: (1) continue our long-term research in the Teton Range by re-visiting our 10 sites and collecting another year of data, and (2) investigate the physiology of alpine stream stoneflies. For our second goal, we performed two experiments. First, we investigated the supercooling point (SCP) – the temperature at which the organism's body transitions from an undercooled to a frozen state (Renault et al., 2002) – for stoneflies representing multiple species, populations, and habitat types to shed light on the potential for these organisms to survive winter freezing stress. Second, we measured the critical thermal maximum (CT_{MAX}) – the highest non-lethal temperature that organisms can survive – for the same species and populations to gain insight into the degree to which putatively cold-adapted organisms are intolerant of warm temperatures.

Preliminary Results

In the summer of 2018 (July 29-August 6), we collected late-instar nemourid stoneflies (Plecoptera: Nemouridae) from seven populations in the Rocky Mountains (Figure 1). Study populations represented two Rocky Mountain sub-ranges centered around Glacier and Grand Teton National Parks in northwestern Montana and Wyoming, respectively (Figure 1C). We identified specimens belonging to three species – *Lednia tumana*, *Lednia tetonica*, and *Zapada* sp. – following previous studies (see Giersch et al., 2017; Hotaling et al., 2019*b*). Unlike *Lednia*, several *Zapada* species are not distinguishable as nymphs. Therefore, we could only identify *Zapada* to genus and cannot exclude the presence of more

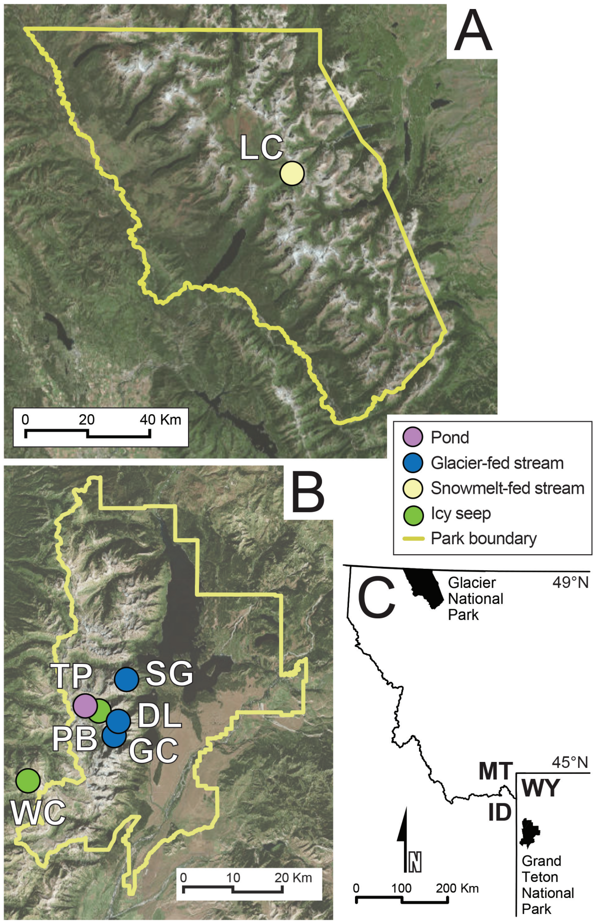

Figure 1. Populations and habitat types included in this study: A) Glacier National Park, B) Grand Teton National Park and surrounding mountains, C) and locations of the focal ranges in the Rocky Mountains. Acronyms: Lunch Creek (LC), Skillet Glacier (SG), Tetonica Pond (TP), Paintbrush Canyon (PB), Delta Lake (DL), Garnet Canyon (GC), and Wind Cave (WC).

than one species in our sampled Wind Cave populations. We classified populations into four habitat types – glacier-fed, snowmelt-fed, icy seep, or alpine tarn – based on environmental conditions and geomorphology.

Supercooling Point

Body size was a significant predictor of SCP (ANOVA, P=0.009), which warranted its inclusion as a covariate in our models. Across all samples, the mean SCP for late-instar alpine stoneflies was -6.5 ± 1.7°C. Population was a significant predictor of SCP (ANOVA, $P<0.001$) with lower SCPs for Tetonica Pond, Garnet Canyon, and Wind Cave versus other populations (Figure 2A). Habitat type also affected SCP (ANOVA, P=0.002). Stoneflies from the alpine tarn, Tetonica Pond, had lower SCPs than those from snowmelt-fed (ANOVA, P=0.05) and glacier-fed streams (ANOVA, P=0.001). Insects from icy seeps had lower SCPs than those from glacier-fed streams (ANOVA, P=0.03; Figure 2B). We also found differences in SCP among species (ANOVA, P=0.043), with *Zapada* sp. exhibiting a lower SCP than *L. tumana* (ANOVA, P=0.032; Figure 2C). Within *L. tetonica*, we found a strong signature of habitat influence on SCP (ANOVA, $P<0.001$). Individuals from the alpine tarn exhibited the lowest mean SCP overall (-7.5 ± 1.3°C) and those from glacier-fed streams the highest (Table 1). All group comparisons within *L. tetonica* were significant at $P\leq0.026$ (Figure 2D).

Critical Thermal Maxima

The interaction between stream type and average summer temperature had a significant effect on CT_{MAX} estimates for stoneflies (ANOVA, $P<0.001$). Specifically, streams identified as icy seeps had lower summer temperatures than other streams, and stoneflies inhabiting these streams had overall lower CT_{MAX} values (Figure 3; green box plots) whereas stoneflies from glacial- and snowmelt-fed streams had higher CT_{MAX} values; (Figure 3; blue and orange boxplots, respectively). Next, we identified significant differences in CT_{MAX} among species (ANOVA, $P<0.0001$; Figure 3). Among the three species tested, *Zapada* sp. appeared to have the lowest CT_{MAX} values, whereas the *Lednia* spp. exhibited greater heat tolerance.

Conclusions

The results of our 2018 UW-NPS funded research highlights the clear potential for physiology to deferentially affect alpine stream organisms in a changing climate. Below, we detail specific conclusions regarding our two primary lines of inquiry in 2018.

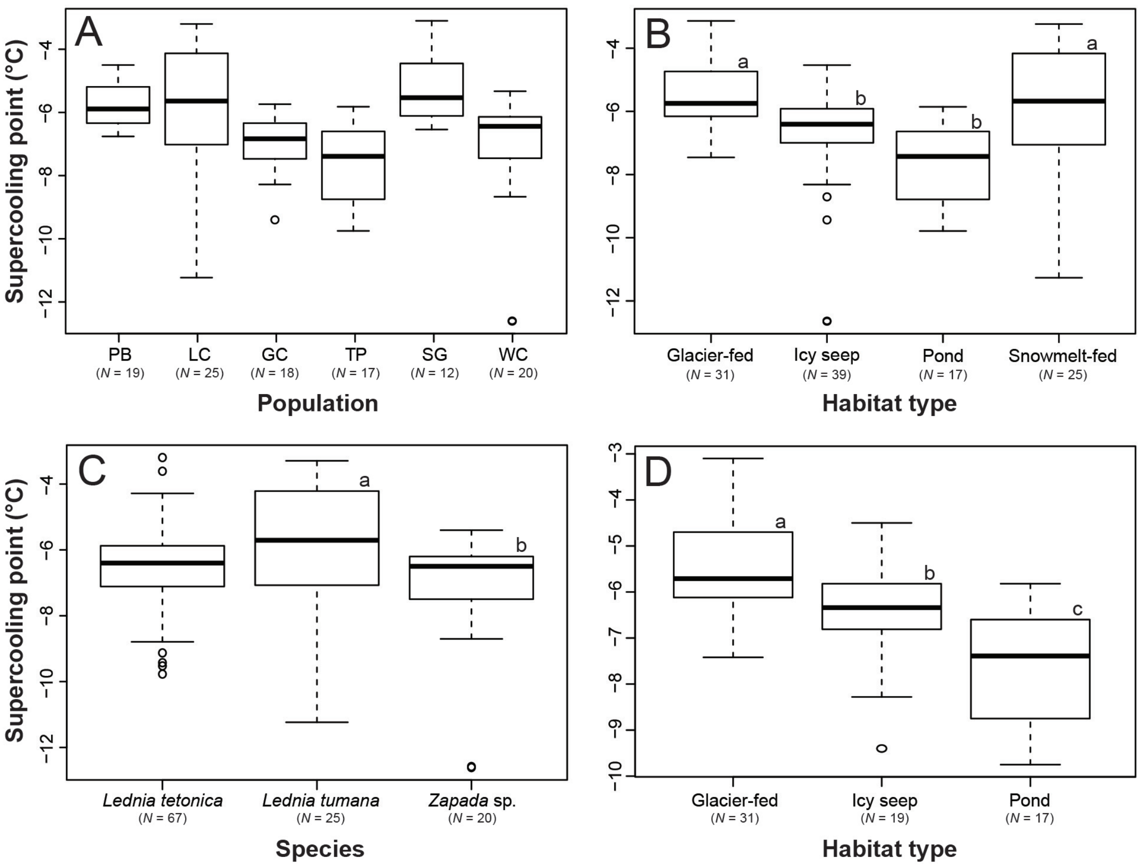

Figure 2. Box-and-whisker plots of the supercooling point (SCP) for alpine stream stoneflies in the Rocky Mountains grouped by (A) population, (B) habitat type, (C) species, and (D) habitat type for *Lednia tetonica* populations only. In each plot, groups of lower-case letters are significantly different at $P<0.05$ (statistical relationships are not shown for A).

Supercooling Point

We showed that SCP varies among and within nemourid stoneflies inhabiting alpine streams in the Rocky Mountains. The population most likely to experience winter freezing stress also exhibited the lowest SCP, suggesting a potential ecological role for SCP in freeze avoidance. As climate change proceeds, glaciers and perennial snowfields are declining worldwide which will result in reduced alpine streamflow (Hotaling et al., 2017). With reduced streamflow there will be a corresponding reduction in the thermal buffering capacity of streamwater (i.e., slower and/or reduced flows are more prone to freezing) and therefore an increased risk of freeze stress in alpine stream insects. This represents a potentially overlooked climate change risk. Only one stonefly, another nemourid, is known to exhibit freeze tolerance (Walters et al., 2009), but it is unclear if this is because freeze tolerance is particularly rare in cold-adapted stoneflies or if the lack of documentation is merely a product of few investigations. Future studies investigating supercooling and freeze tolerance in stoneflies, including those most at risk of climate change-induced extirpation, will yield important insight into this standing physiological question.

Thermal tolerance

Critical thermal maximum is a widely used assay of tolerance to heat stress in animals. In alpine ecosystems, stoneflies that routinely experience high summer temperatures exhibit higher CT_{MAX} values compared to populations that experience colder temper-

Population	Taxa	Type	T_{SPOT}	C	PI	Length	SCP
Lunch Creek	*L. tumana*	Snowmelt-fed	6.2	n/a	25	4.86 ± 0.5	-5.9 ± 2°
Wind Cave	*Zapada* sp.	Icy seep	3	101.1	18	4.41 ± 0.59	-7.2 ± 2°
Paintbrush Canyon	*L. tetonica*	Icy seep	1.3	25.0	34	5.56 ± 0.65	-5.8 ± 0.7°
Garnet Canyon	*L. tetonica*	Glacier-fed	2.4	4.1	32	4.50 ± 0.54	-7.0 ± 0.9°
Delta Lake	*L. tetonica*	Glacier-fed	2.6	4.3	18	6.50[a]	-7.4°[a]
Skillet Glacier	*L. tetonica*	Glacier-fed	8.5	3.1	34	5.55 ± 0.35	-5.2 ± 1.1°
Tetonica Pond	*L. tetonica*	Alpine tarn	4.1	29.3	n/a	4.64 ± 0.57	-7.5 ± 1.3°

[a] Single observation. Standard deviations could not be calculated.

Table 1. Environmental variation and habitat type classifications of streams included in this study. T_{SPOT}: spot estimate of stream temperatures (°C), C: specific conductivity (μS cm^{-1}), PI: Pfankuch Index, a standard deviation of the supercooling point (°C). Body lengths are in millimeters. All environmental data were collected in 2018 except Lunch Creek (data from 2014).

atures; however, caution must be exercised in interpreting these results. Because CT_{MAX} values are subject to variation due to methodology (Terblanche et al., 2007), we do not claim that stoneflies can live at temperatures that are equal to their CT_{MAX} values. Rather, our results suggest that stoneflies with high CT_{MAX} values are likely able to withstand greater heat stress than those with lower values. Thus, we have used CT_{MAX} in a comparative context, to serve as an index of thermal tolerance and sensitivity in alpine stoneflies. We found that stoneflies collected in icy seeps have low CT_{MAX} values likely because they do not experience high temperatures even in the summer. Although it is currently unclear whether these insects can take refuge in the cold hyporheic zone of the stream or in upwelling zones, prolonged increases in stream temperature in the future could negatively affect these populations. The differences measured among populations could be indicative of local thermal adaptation and further investigation into whether these populations are truly adapted to their habitat is warranted. Among the three stonefly species, *Zapada* sp. had the lowest CT_{MAX} values. In fact, when placed in the context of other studies that have measured critical thermal maxima in Rocky Mountain stoneflies (Shah et al., 2017), *Zapada* sp. still have some of the lowest values, suggesting greater sensitivity to warming than other stonefly species.

Future Work

We plan to submit a new UW-NPS funding proposal in 2019. The goals of this proposal will be two-fold. First, we will seek funding to continue our long-term monitoring of alpine streams and stoneflies in the Teton Range. After the 2019 field season, we will have collected five years of continuous data for our study which is already one of the longest running alpine stream ecological studies in the world. After this 5-year mark, we will carry out our first temporal analysis of the complete data set. We will be able to investigate year-to-year variability in environmental and biodiversity patterning, and test for the presence of any temporal trends in the data set. Second, we would like to expand our efforts to understand the thermal physiology of alpine stream insects as this represents a glaring gap in existing knowledge. We are interested in assessing thermal tolerance across the full summer growing season for *Lednia tetonica*, a rare, alpine stonefly which will contribute to our understanding of how alpine stream insect species may be affected by climate change. *Lednia tumana* in Glacier National Park, a sister species to *Lednia tetonica*, has been petitioned for listing under the U.S. Endangered Species Act due to climate-induced habitat loss (US Fish and Wildlife Service, 2016). We will seek to clarify how variation in temperature affects growth, reproductive capacity, and emergence of these potentially imperiled stoneflies.

In addition to 2019 research plans, we are currently

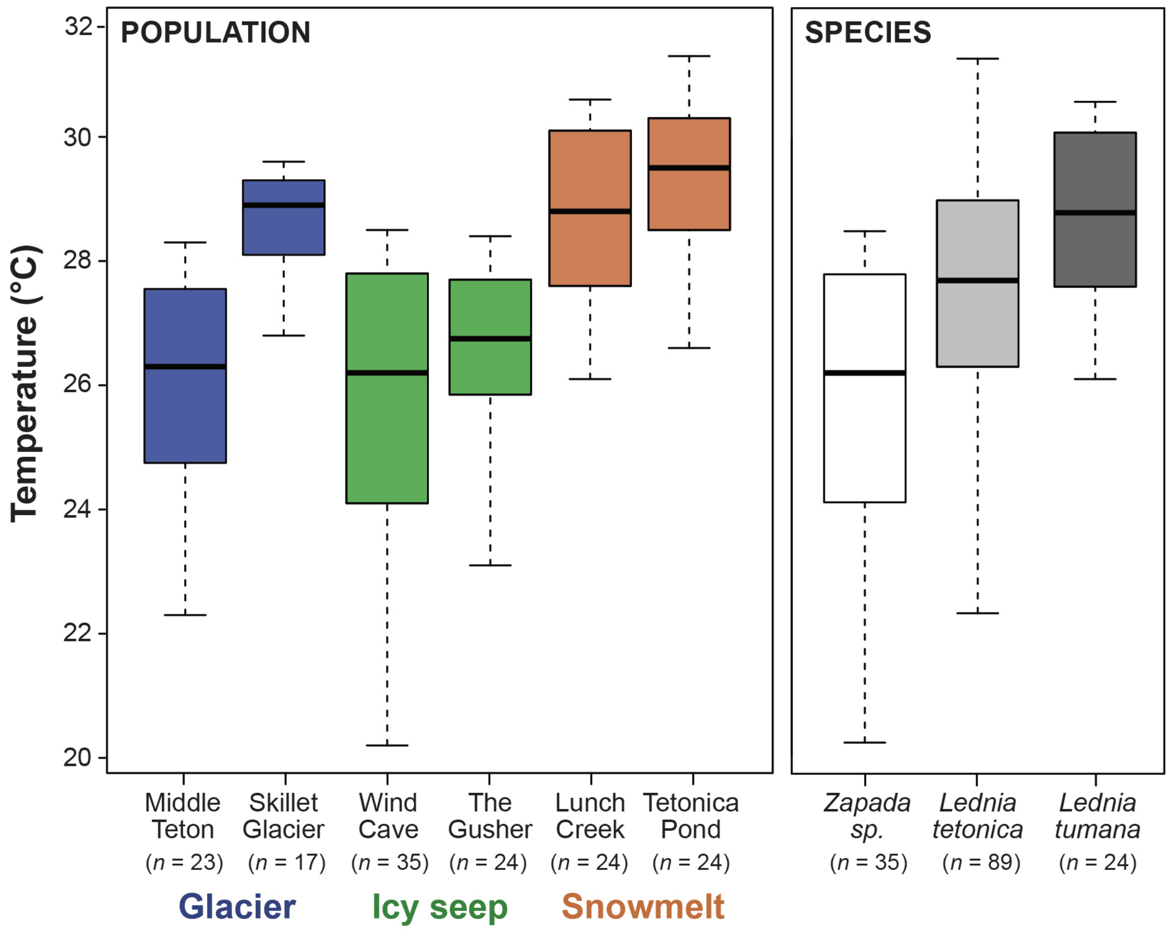

Figure 3. Box-and-whisker plots of the critical thermal maximum (CT_{MAX}) for alpine stream stoneflies in the Rocky Mountains grouped by populations and species.

preparing RNA sequencing libraries for specimens from our 2018 physiology data. Specifically, this will enable us to test whether or not there is a genomic signature of thermal stress in stoneflies at their CT_{MAX} that aligns with observed variation in CT_{MAX} in our physiological experiments. We plan to present the results of the full study linking thermal tolerance phenotype to the underlying genotype at the 2019 Society for Freshwater Science Annual Meeting. Publications for both 2018 studies will be submitted for publication in 2019.

Disclaimer

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