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Long-term alpine stream monitoring in the Teton Range: Investigating multi-year patterns and thermal physiology

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Abstract Alpine streams and the biotic communities they contain are imperiled worldwide due to climate warming and the rapid decline of ice. The loss of glaciers and permanent snowpack may drive local populations to extinction, especially for organisms with narrow habitat tolerances. Since 2015, we have been monitoring alpine streams in the Teton Range that originate from three hydrological sources: surface glaciers, snowfields, or subterranean ice (e.g., rock glaciers). We call these stream types glacier-fed, snowmelt-fed, and icy seeps, respectively. We hypothesize that icy seeps may persist on the landscape longer than other hydrologic sources and that these features may act as a refuge for cold-adapted organisms such as the stoneflies *Zapada glacier* and *Lednia tetonica*. In November 2019, *Z. glacier* and a sister species of *L. tetonica*, *Lednia tumana*, were listed under the U.S. Endangered Species Act. This decision was based in part on work funded by the UW-NPS Research Station and highlights the pressing nature of our efforts. In 2019, we collected a 5th year of long-term data to begin investigating multi-year signals in the data. Our second 2019 objective was to further explore how thermal regimes affect tolerance of potentially imperiled insects. Because our annual data collection occurs in late summer with sample processing and analysis extending into the following year, this report will be a broad update on the project as whole, rather than solely 2019 activities. Through long-term monitoring of streams from different hydrological sources, we are building a dataset that will allow us to understand changes as air temperatures warm and permanent ice is lost in the alpine zone.

Introduction

Alpine streams are geographically isolated and provide unique habitat for aquatic organisms (Hotaling et al., 2017). Isolation, habitat specificity, and the rapid environmental shifts associated with climate change translate to a challenging combination for the persistence of alpine specialists (Beever et al., 2003). Any change in environmental conditions may mean local extinctions of species with narrow limits of habitat tolerance, especially those with 'no place to run' uphill (Giersch et al., 2015; Jordan et al., 2016). However, both the temporal dynamics of alpine streams through time and the physiology of resident organisms are largely unknown, raising a difficult challenge for predicting the fate of contemporary biodiversity in changing landscapes. We typically assume that macroinvertebrates inhabiting alpine streams are cold-adapted (e.g., Giersch et al., 2017), 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 structured now and how it may change in Grand Teton National Park and the surrounding mountains as climate change proceeds. 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, annual monitoring of 10 sites. This research framework provides a powerful means for disentangling how complex processes are affecting Teton Range alpine streams on decadal timescales while also providing an important space for student training. Our efforts have already yielded promising results. We have shown that population genetic differentiation varies across species (Hotaling et al., 2019*a*), microbial communities reflect their hydrological sources (Hotaling et al., 2019*b*), and macroinvertebrate communities also differ with hydrological source but not to the degree that microbes do (Tronstad et al., In review).

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*b*; Tronstad et al., In review). Historically, alpine streams have been classified into three main types that 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 even though they are rarely studied. 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 and Harrison, 2018). 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*b*; Tronstad et al., In review).

For 2019, our 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 thermal physiology of alpine stream stoneflies to clarify threats to their persistence. Here, we provide new results on both objectives, as well as updates on previously funded UW-NPS Research Station grants.

Methods

We collected aquatic invertebrates from 10 streams annually using a Surber sampler. Samples were processed and invertebrates were identified, counted and measured for length. For four sites, we have four consecutive years of data (2015-2018). The fifth year, 2019, is currently being processed. Because we were still refining our initial selection of sites (finalized in 2017), this number will grow in the years to come. For instance, we have three years of data for three additional sites.

In our 2018 UW-NPS report, we described our field and laboratory methods for thermal physiology as well as preliminary results. Briefly, we collected late-instar stoneflies representing three species (*L. tumana*, *L. tetonica*, and *Zapada* sp.) from seven populations. The *L. tumana* population was from Glacier National Park, Montana. All other populations were in the Teton Range. We showed that critical thermal maximum (CT_{max}), a measure of thermal tolerance, varies predictably with stream temperature.

That is, stoneflies from warmer streams exhibited correspondingly higher CT_{max}.

In 2019, we extended this work to include RNA sequencing (RNAseq). Specifically, we collected RNAseq data for three populations (two *L. tetonica*, one *L. tumana*) to link cellular stress to organismal physiology. Two groups of nymphs were compared: those flash frozen at their CT_{max} (treatment) and others that were held at 3°C (control). We expected to observe a clear signal of cellular stress with genes typical of heat stress responses [e.g., heat shock proteins (HSPs)] upregulated. We also expected nymphs that experience higher temperatures in nature to exhibit a correspondingly muted cellular stress response.

Preliminary results

Multi-year perspectives on alpine stream biodiversity

No clear patterns emerge from initial views of macroinvertebrate density and biomass across years (Figure 1A-B). Although invertebrate biomass in 2017 was generally quite low, which is interesting given that the preceding snow year was >10% below average (Figure 1C). The number of taxa observed each year has been largely stable through time and appears generally independent of snowpack (Figure 1D). However, North Fork Teton Creek, a snowmelt-fed site, has experienced year-over-year declines in the number of taxa present for four consecutive years. From 23 taxa present in 2015 to just 7 observed in 2018 (Figure 1D). We will be very curious what the 2019 data indicate for this trajectory.

Thermal physiology

We generated 368.8 million read pairs (mean = 20.6 million ± 1.9 million; min. = 2.6 million, max. = 39.2 million) for 18 libraries (3 treatment and 3 control for each population). After filtering and processing of the data set, our gene counts matrix contained 52,954 unique entries. We observed global differences in gene expression between *L. tumana* and *L. tetonica*. We identified 71 genes that were differentially expressed – 60 upregulated, 11 downregulated – including three HSPs (Figure 2).

To test if stoneflies from more thermally variable environments have muted cellular responses to stress, we identified all genes annotated as HSPs based on comparisons to protein databases. We calculated the mean difference in expression between treatment and control nymphs for each gene and population. Because the data were not normally distributed (P, Shapiro-Wilk < 0.001), we compared the distributions of mean differences for each population using a Kruskal-Wallis rank sum test followed by a Dunn test for multiple comparisons. Across all populations and species, 38 genes were annotated as HSPs. Of these, 12 unique genes were expressed at moderate to high levels. We found no support for our hypothesis that stoneflies naturally experiencing higher (and more variable) temperatures exhibit muted cellular stress responses versus those inhabiting colder (and more thermally stable) streams (Figure 3; P, Dunn's ≥ 0.66).

Conclusions

Species living in chronically cold habitats are generally assumed to be cold-loving stenotherms that are intolerant of warming. As glaciers disappear around the world (Huss and Hock, 2018), the demise of *Lednia*, *Z. glacier*, and similar imperiled taxa has been presumed to be directly linked to changes in thermal regime (Giersch et al., 2017). But typically there is mismatch between this simplified assumption and direct measurements of thermal tolerance. Alternative explanations for species ranges limited to coldest local habitats might include realized niche breadth associated with biotic interactions (e.g., food availability, Roughgarden, 1974, or competitive exclusion, Connell 1961), with thermal sensitivity potentially playing an interactive role (Gilchrist, 1995). While terrestrial habitats exhibit a wide array of thermal variation at the microhabitat scale (Woods et al., 2015), allowing greater thermal niche partitioning, the buffering capacity of flowing water reduces thermal diversity among microhabitats. Thus, with relatively high short-term thermal tolerance, far beyond anything experienced in nature, and cellular signatures of stress

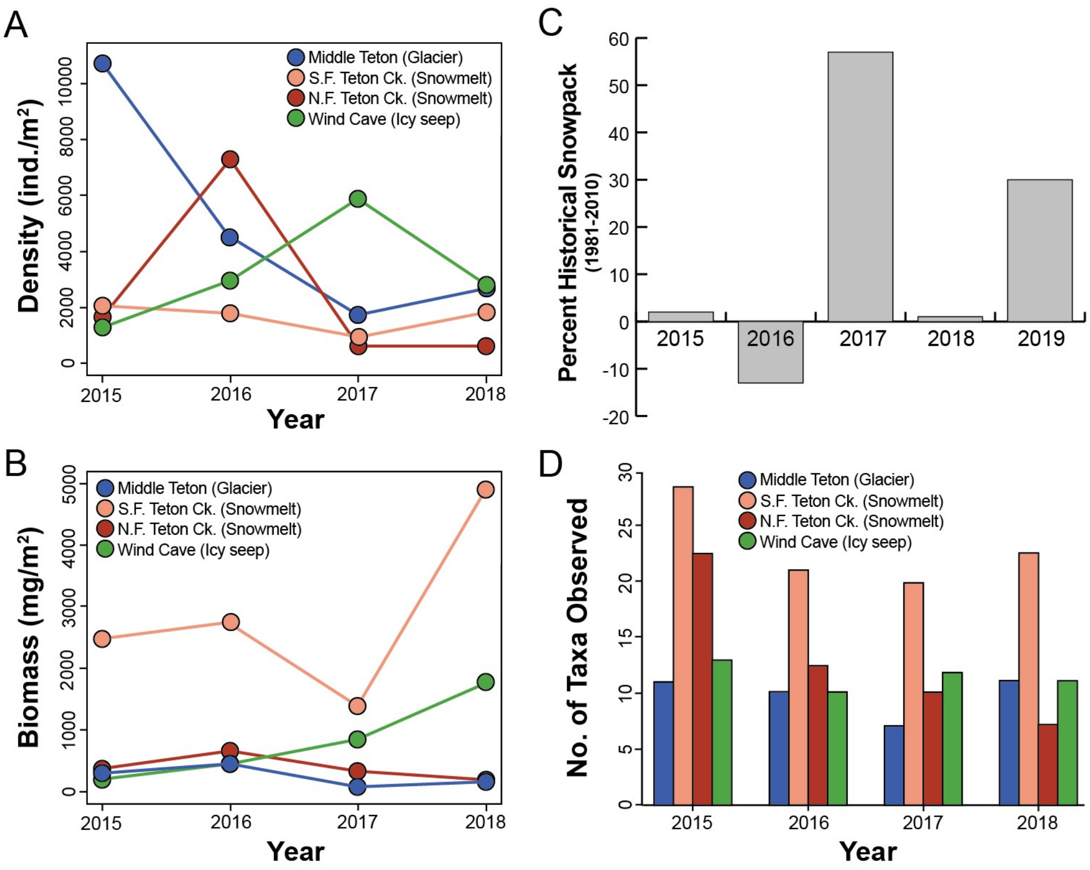

Figure 1. (A) Density of macroinvertebrates across the four sites with continuous data since 2015. (B) Invertebrate biomass through time for the same four sites. (C) Percentage of snowpack versus historical levels for each year at a representative SNOTEL site in the Teton Range. (D) Number of taxa recorded each year at each focal site.

even at low temperatures (e.g., constitutive expression of HSPs at 3°C), we hypothesize that present-day distributions of high-elevation stoneflies do not specifically reflect a preference for cold conditions but rather a tolerance for extreme cold that allows populations to persist in alpine streams even when they are excluded by biotic interactions from habitats closer to physiological optimum temperatures. Rather than being extreme cold stenotherms, *Lednia* and similar species may have evolved a wide thermal niche that allows them to colonize environments free of other factors limiting their distributions (e.g., competition, predation, or availability of specific food resources).

Future work

We plan to submit a UW-NPS funding proposal in 2020. Given the listing of *Zapada glacier* and *Lednia tumana* under the U.S. Endangered Species Act in November 2019, our monitoring efforts are more pressing than ever. Our proposal will be centered around two goals: (1) Continuing our long-term monitoring of alpine streams and potentially imperiled species (e.g., *Z. glacier*) in the Teton Range. (2) Clarifying foodweb dynamics in these enigmatic habitats. To the first goal, 2020 will mark our 6th year of continuous monitoring and any future research reports will include more detailed results of this long-term effort. To the second goal, resource availability and trophic dynamics are well-known influences on species distributions, yet they remain largely uncharacterized in alpine streams.

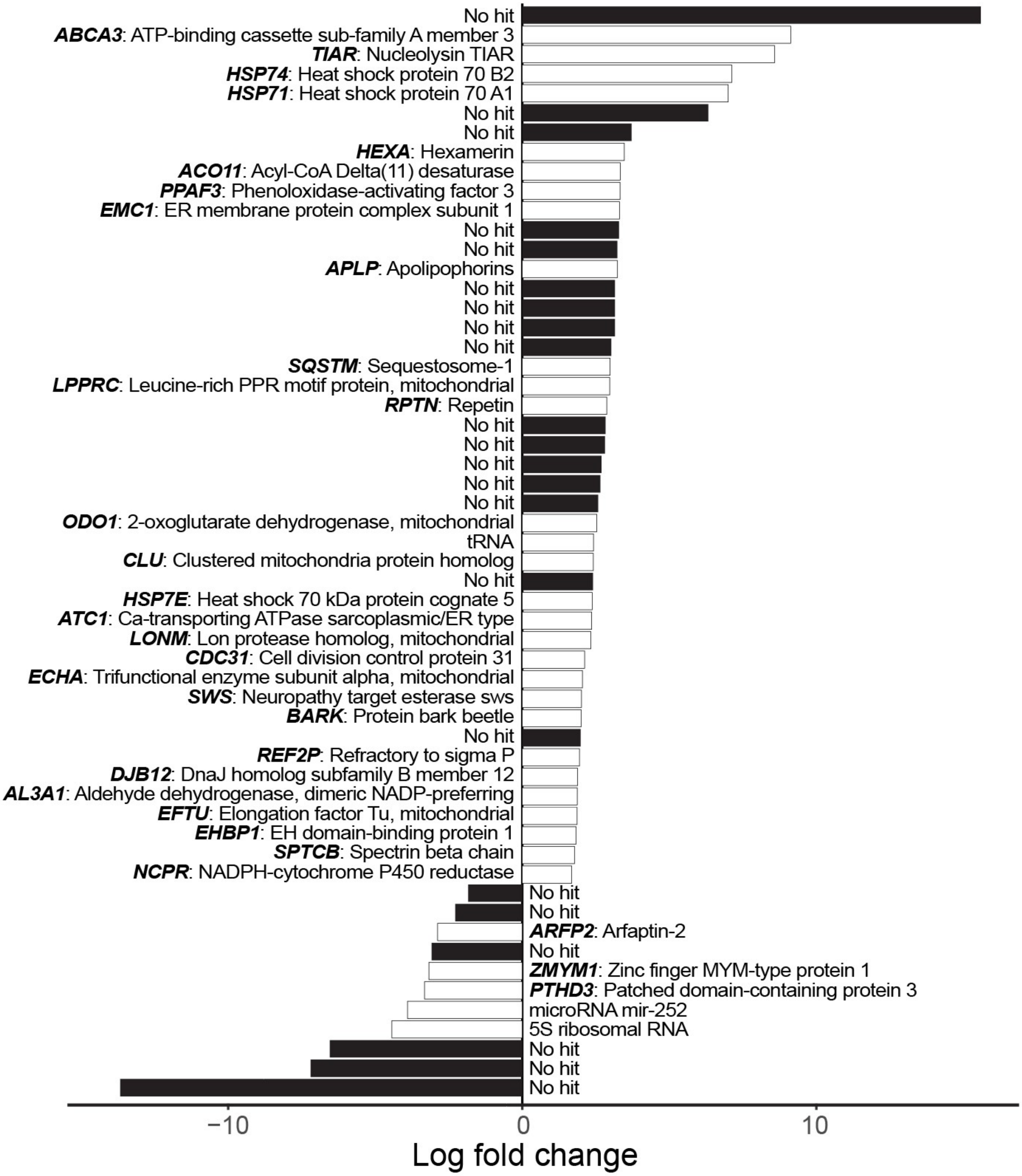

Figure 2. Log fold change with protein annotations (white = annotated; black = no hit) of *Lednia tetonica* DEGs (FDR ≤ 0.05). For annotated genes, only the hit with the lowest false discovery rate (FDR) is included.

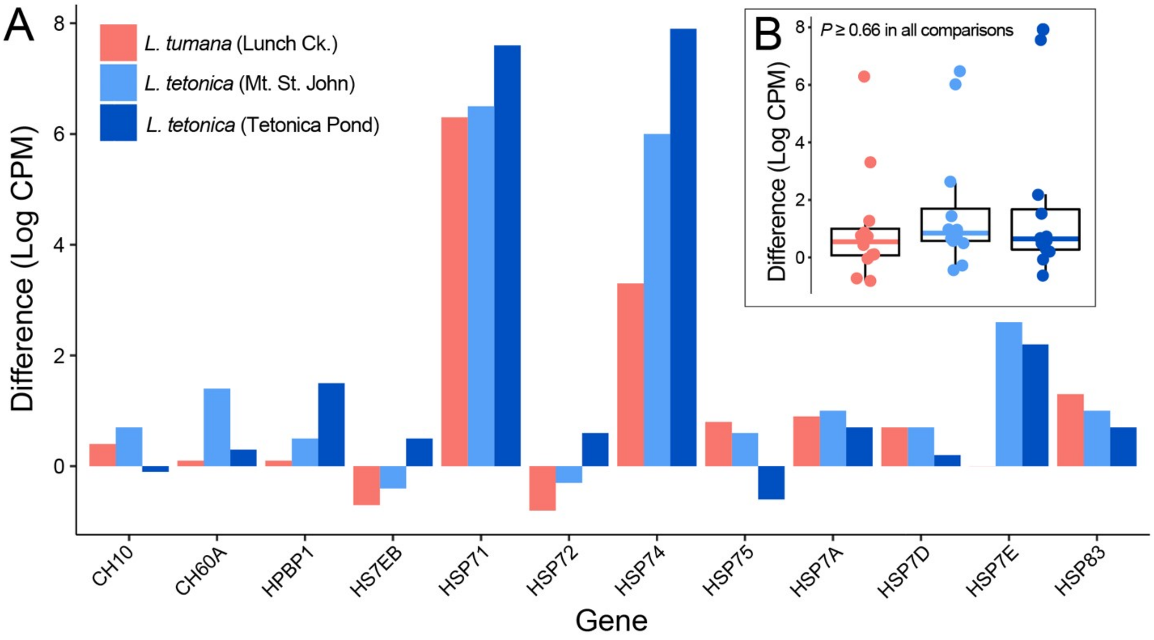

Figure 3. (A) Mean population-level differences in expression between treatment and control specimens of the 12 most highly expressed, unique HSPs annotated in this study. (B) Distributions of the values in (A) grouped by population. No significant differences (P, Dunn < 0.05) were present.

Manuscripts in preparation

In addition to 2020 research plans and manuscripts that we currently have in review detailing macroinvertebrate community structure in the high Teton Range (Tronstad et al., In review) and cold physiology of stonefly nymphs (Hotaling et al., in review), we are currently preparing three additional manuscripts. The first, led by Dr. Tronstad, is focused on the biological diversity of icy seeps specifically and stems from our summer 2016 fieldwork. The second, led by a doctoral student at UC Irvine, Matthew Green, will be a review of the *Lednia* genus in North America with a focus on distribution, ecology, and climate change threats. The third, led by doctoral student Taylor Price at the University of Minnesota, details the microbial diversity of our focal streams and stems from fieldwork conducted in 2018 and 2019. Taylor is being supervised from Dr. Trinity Hamilton, also at the University of Minnesota.

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Disclaimer: This draft report is distributed solely for purposes of scientific record keeping. Its content is deliberative and predecisional. Because the report has not yet been approved for publication by the U.S. Geological Survey (USGS), it does not represent any official USGS finding or policy.

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