Annual Report Vol. 39 (2016)

A unique "icy seep" aquatic habitat in the high Teton Range: Potential refuge for biological assemblages imperiled by climate change

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Abstract Alpine streams are threatened as meltwater sources diminish. We established baseline monitoring efforts of alpine streams in the Teton Range during 2015 by describing biotic and abiotic conditions of surface glacier and snowmelt streams. Our results indicated a third alpine stream type, icy seeps. Icy seeps are fed by subterranean ice melt, extremely cold but stable water temperature (summer mean <2°C), moderately high streambed stability (Pfankuch Index $\approx$ 18-25), and relatively high specific conductivity ($>$50 μS cm^{-1}). In 2016, we documented several icy seeps in the Teton massif, and our data suggest that they have the potential to serve as climate refugia for organisms and processes associated with extremely cold meltwater, due to the subterranean ice sources being more insulated from atmospheric conditions than surface glaciers and snowpack. Our 2016 work focused on locating icy seeps and documenting physical conditions, invertebrates, diatoms, and microbes in these alpine streams. We are processing the biological data, but microbial assemblages in icy seeps are similar to those found in streams fed by dwindling surface glacier ice. We are preparing to submit a paper about the microbial patterns, including how they compare to alpine streams representing comparable hydrological sources in Glacier National Park.

Introduction

Alpine streams occur at elevations higher than the regional treeline. As the uppermost segments of extensive river systems (e.g., Snake/Columbia River basin), alpine streams tend to be insular in nature, with clusters of habitat occurring in high mountain ranges separated by great distances (Hotaling et al., 2017*a*). Isolation of alpine streams at the highest reaches of river networks has two major ecological effects: 1) physical isolation among streams and mountain ranges tends to amplify biodiversity by driving local adaptation of alpine-specialist species; and 2) alpine streams can act as "summit traps" in which species moving upstream in response to a warming climate can become trapped and driven to local extinction due to little to no opportunity for dispersal into suitable habitat on other isolated mountain ranges. Alpine streams therefore harbor both diverse and threatened assemblages of aquatic species (Brown et al., 2007; Jacobsen et al., 2012; Khamis et al., 2014).

In 2015, we received UW-NPS funding to undertake a preliminary survey of alpine streams in the Tetons (proposal "Assessing alpine aquatic invertebrate assemblages in Grand Teton National Park, Wyoming"). Our premise was that alpine stream research in North America was significantly lagging behind work done in Europe and elsewhere, and furthermore, that within North America virtually nothing was known about alpine stream ecology in the middle section of the Rocky Mountain range (i.e., between Colorado and Glacier National Park, Montana; Finn and Poff, 2005; Giersch et al., 2017). The unique geology of the Tetons combined with the rapid reduction of glaciers and permanent snowfields in the region made it imperative that research begin immediately to document key environmental types of alpine streams that exist in Grand Teton National Park (GRTE), and what unique aquatic biodiversity these streams harbor. We proposed to focus our efforts on two contrasting stream types that we hypothesized would dominate the Teton's alpine landscape: those fed by dwindling surface glaciers, and those fed by snowpack.

Physical and chemical characterization of our 2015 data (based on 6 focal alpine streams), revealed the presence of a third, unique alpine stream type in GRTE: streams fed by meltwater runoff from subterranean ice. The stream emerging from Wind Cave, on the west side of the Teton Range, is representative of this third key stream type, henceforth termed "icy seeps". A review of the geology literature and geologic maps suggested that icy seeps could be common in transition zones where loosely organized materials abruptly meet an impermeable bedrock layer – as occurs commonly in the high Tetons. In 2016, we emphasized fieldwork on exploring and documenting icy seeps, pursuant to the exciting possibility that the subterranean nature of the source ice to these streams could render them relatively resistant (compared to surface ice and snow) to climate change. If so, icy seeps could act as refugia for the unique biological assemblages and ecosystem processes associated with extremely cold meltwater streams of the middle Rockies. We added a microbial ecologist (LZ) and a diatom specialist (RJB) to our team for 2016, with the goal to document each of three key components of alpine stream food webs: microbes (which perform key ecosystem processes including decomposition and transformations of nitrogen), diatoms (the dominant primary producers in alpine stream ecosystems), and macroinvertebrates (the dominant consumers, including two indicator stonefly species of conservation concern).

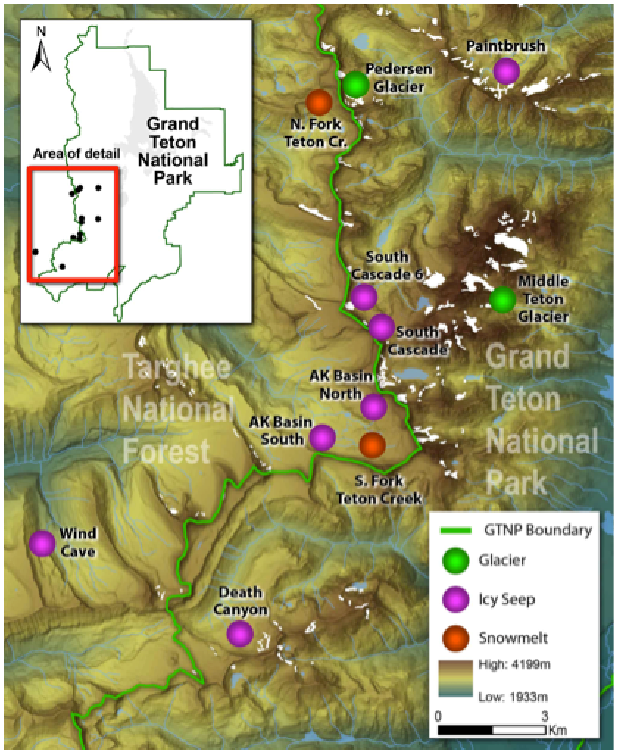

Figure 1. Map of the Teton Mountain Range showing the streams we sampled and their hydrological source.

We report here on the results and conclusions that we have accumulated thus far from the 2016 season in which we focused on the ecological characterization of GRTE icy seeps. We emphasize the microbial component, as that portion is near completion (including a manuscript that we will submit to *Global Change Biology* within the month). We also provide a comparison of the microbial results from GRTE to results from an analogous and concurrent study in Glacier National Park (GLAC), in order to provide regional context. Finally, we discuss some preliminary results from the macroinvertebrate component, and we are currently still processing the diatom samples.

Methods

Categorizing stream types and identifying icy seeps

Environmental data were collected for alpine streams in GRTE and the surrounding areas (Figure 1), along with a similar set of streams in GLAC (Lewis and Livingston Mountain Ranges) in the summer of 2016. All field sampling in both regions was performed within a six-week interval from early August to mid-September, when the influence of seasonal snowpack is minimized, improving site access and ensuring that hydrologic conditions most closely reflected their primary source. We completed the GRTE fieldwork during the first two weeks of that period. We included 13 total streams for the microbe-focused study: six in GRTE and seven in GLAC. We also located and sampled five additional GRTE icy seeps for the first time in 2016.

Primary hydrologic source for streams was identified by comparing a suite of environmental variables, satellite imagery, and field observation (more below). We categorized streams into one of four categories – glacier-fed, snowmelt-fed, groundwater-fed (in GLAC only), or an icy seep – according to the Pfankuch Index (PI; a measure of streambed/channel stability; Peckarsky et al., 2014), mean summer temperature (summer solstice to autumn solstice, T_{SUMMER}), specific conductivity (SPC, measured with a Professional Plus Multiprobe, Yellow Springs Incorporated, Yellow Springs, OH), and suspended solids in the water column, using standard filtration methods (note: the final two variables were collecting only in GRTE streams). Streams from both GRTE and GLAC were categorized as glacier-fed if satellite imagery (National Agricultural Imagery Program, 2015) revealed crevasses in the surface ice feeding a stream, $PI > 30$ indicating high streambed instability, and $T_{SUMMER} < 2°C$. Any other streams fed by meltwater from a permanent snow feature were categorized as snowmelt-fed. Groundwater-fed springs were categorized as such when the source could be identified, $T_{SUMMER} > 3°C$, and the streambed had high stability ($PI < 15$). Icy seeps were categorized by an obvious subterranean source, very cold summer temperatures ($T_{SUMMER} < 3°C$), and relatively stable streambed (PI = 15-24). Stream channels (from the source or location of emergence from talus), and surface snow and ice features were digitized in ArcGIS version 10.3 (Environmental Systems Research Institute, Redlands, CA, USA) using color satellite imagery captured on October 15, 2015. Imagery from this date was used to document the minimal snow/ice coverage. Distances of collection locations to stream source, glacier and snow feature area, lake area, and watershed area were calculated in ARCGIS. Percentage of ice or permanent snow per drainage was calculated by comparing total catchment area with area coded as ice or permanent snow. We also georeferenced putative glacier-fed streams and icy seeps from glacier boundaries digitized for GLAC from 1-meter NAIP 2005 satellite imagery and a rock glacier inventory database for the western United States (Johnson and Fountain, 2016), respectively.

Site name (see Figure 1)	**Stream type**	**% basin area containing *surface* ice/snow**	**Downstream distance of sample site from source (m)**
Wind Cave	icy seep	0	52
Death Canyon	icy seep	0.07	4
AK Basin - S	icy seep	0	1
AK Basin - N	icy seep	0	4
S Cascade (upper)	icy seep	7.1	8.5
S Cascade (Schoolroom)	icy seep	5.4	2.5
Paintbrush	icy seep	4.5	22
Middle Teton	glacier-fed	15.8	178
Petersen Glacier	glacier-fed	20.8	50
S Fork Teton Cr	snowmelt	0	1044
N Fork Teton Cr	snowmelt	0	9

Table 1. Stream type, percent surface area of basin covered in permanent snow/ice, and distance downstream of source water for all GRTE alpine stream sampled in 2015 and 2016

Summary physical data for all GRTE study sites are included in Table 1, and we ran a Principle Components Analysis (PCA) on the four key "glaciality" variables (temperature patterns, streambed stability, SPC, and suspended solids) collected in all GRTE streams, to confirm the existence in the Tetons of the three common alpine stream types: glacier-fed,

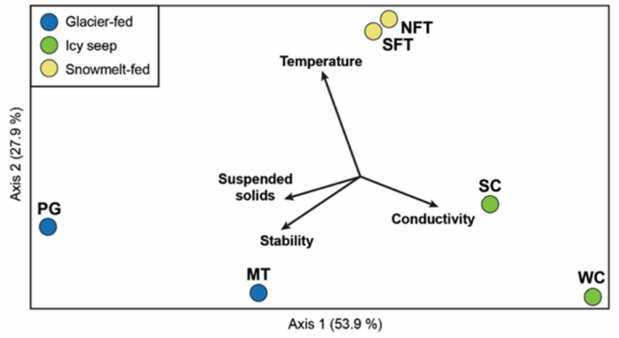

Figure 2. Principal components analysis of six alpine streams in GRTE according to four key environmental variables commonly used to assess the influence of glacier meltwater on stream ecosystems. Abbreviations: North Fork Teton Creek (NFT), South Fork Teton Creek (SFT), South Cascade Creek (SC), Wind Cave (WC), Middle Teton (MT), and Petersen Glacier (PG).

snowmelt-fed, and icy seep (Figure 2).

Fieldwork in 2016 verified the presence of a number of previously undocumented icy seeps in Death Canyon, Alaska Basin, South Cascade Canyon, and Paintbrush Canyon. Among those located, we collected environmental and biological data, including placing temperature dataloggers for a full year, in six newly located icy seeps (Figure 1, Table 1).

Assessing macroinvertebrate diversity

We collected aquatic invertebrates with a Surber sampler (243 μm mesh size; 0.0929 m^2) to measure the density of invertebrate taxa (ind/m^2) in streams. We collected six Surber samples near the source of each stream and composited the samples into Whirl-pak bags containing 95% ethanol as a preservative for transport to the laboratory at the University of Wyoming. In the laboratory, we separated samples into large (>2mm) and small (<2 mm) size fractions, and sorted invertebrates from the debris. We subsampled the small fraction using a record player (Waters, 1969) if invertebrates were abundant (e.g., >200 individuals). Invertebrates were identified, counted and measured under a dissecting microscope using available keys (Merritt et al., 2008; Thorp and Covich, 2010). We are calculating the density and biomass of all taxa (mg/m^2) using length-mass regressions (Benke et al., 1999).

Assessing diatom diversity

We collected diatoms by selecting three flat, upward-facing rocks fully exposed to streamflow and sunlight at each sample site, scraping a 9-cm^2 area from each of the three rocks, and immediately preserving each sample in 10% formalin. Samples were transported to the laboratory at the University of New Mexico for identification to species and estimation of relative abundance. Diatom sample processing is currently in-progress.

Assessing microbial diversity

We collected microbial samples from three microhabitats for each stream: streamwater, biofilm, and source ice (when possible). Streamwater was sampled by collecting three replicates per stream of one liter of flowing water into sterile Whirl-Pak bags (Nasco, Salida, CA). Biofilm samples were collected by scrubbing a 103 cm^2 section of three representative rocks from the stream bottom into an ethanol-sterilized plastic dish containing ~25 mL of sterile PCR water. A wire scrubbing brush was flame-sterilized before and after each sampling. When source ice was present and safely accessible, we sampled subsurface ice (~30 cm depth) from three locations that appeared typical of the glacier or snowfield surface. At each ice collection location, we first removed the upper ca. 30 cm of ice using a flame-sterilized ice axe adze. Next, we re-sterilized the adze and collected ca. 3 liters of ice into sterile Whirl-Pak bags. These samples were transported to the basecamp and allowed to melt before filtering. After collection, samples were filtered using BD Luer-Lok sterile syringes and filter holders (Becton, Dickinson and Company, Franklin Lakes, NJ) and 0.2-μm filters (Millipore, Billerica, MA). For streamwater and ice, ca. 0.5-1 L was typically filtered. For biofilm supernatants, ca. 2-20 mL were filtered. Filters were immediately placed into sterilized sucrose lysis buffer (SLB; 20 mM EDTA, 400 mM NaCl, 0.7 M sucrose, 50 mM Tris, pH 9.0, Mitchell and Takacs-Vesbach, 2008). At each site, three replicate samples were collected for all microhabitats. In addition, a "field negative" was collected for every site by using forceps to briefly expose a sterile filter to atmospheric conditions before preserving it in SLB.

DNA was extracted from filters and sequencing libraries were prepared following standard protocols. The 515f/806r PCR primer sets developed for the Earth Microbiome project (Gilbert et al., 2014) and described in Bates et al. (2011) were used to target the highly variable V4 region of the 16S rRNA gene. In total, we generated 13,093,344 sequences for 48 libraries. After quality filtering, we retained an average of 56,328 reads per library. For non-negative libraries only, the minimum reads retained was 20,725 and maximum was 312,514. Sequencing depth was determined to be sufficient for resolving the amount of diversity present based upon rarefaction curves with relatively little additional diversity being discovered after 5,387 sequences per sample.

To assess biodiversity differences within and among groups, including stream type, microhabitat, or region (GRTE and GLAC), we calculated within-site (α) diversity using the Shannon diversity index (H). We compared α diversity among groups with a two-sample, nonparametric t-test using H as our metric and 1,000 permutations. We assessed correlations between α diversity and temperature (including T_{RANGE}, T_{YEAR}, and T_{SUMMER}) and streambed stability (PI) using Pearson correlations in the R package 'Hmisc' (Harrell Jr, 2013). We also estimated among-site differences in microbial assemblages (β diversity) using Bray-Curtis dissimilarity distances. To test whether microbial assemblages varied among defined groups (GLAC vs GRTE, stream type, microhabitat), we performed adonis analyses (analogous to PERMANOVA) using the R package 'vegan' (Oksanen et al., 2007).

Preliminary Results

Distribution of stream types including icy seeps

For the microbial analyses, we included four glacier-fed streams (two each in GRTE and GLAC), four snowmelt-fed streams (two each in GRTE and GLAC), three icy seeps (two in GRTE, one in GLAC), and two groundwater-fed springs that were both in GLAC. In this analysis, stream beds were significantly less stable in glacier-fed streams than all other stream types (Tukey's HSD, $P < 0.001$). Icy seeps and glacier-fed streams were colder than both groundwater-fed springs and snowmelt-fed streams (Tukey's HSD, $P < 0.05$). The PCA run on the GRTE streams alone (Figure 2) also strongly suggests that elevated SPC can help to characterize streams as icy seeps and/or groundwater-fed, as a key driver increasing SPC is close contact of water with soil and/or rock. Precipitation, conversely, has SPC near zero, which explains why meltwater from snow or surface glaciers have extremely low SPC.

Watershed areas for the GRTE sampling locations varied from 0.05 km^2 (Alaska Basin South Rock Glacier) to 1.8 km^2 (Upper Paintbrush Rock Glacier). The percent surface ice and snow cover varied greatly among sites (Table 1). Surface snow or ice covered 20.8% of the watershed above the Petersen Glacier stream, and four sites did not have any late-season ice or snow coverage (Alaska Basin South icy seep, Alaska Basin North icy seep, the Wind Cave icy seep, and the North Fork Teton Creek snowmelt-fed stream). Distance to source varied from 1 meter (Alaska Basin South icy seep) to 1044 meters (South Fork Teton Creek snowmelt-fed stream).

Macroinvertebrate diversity

All samples are sorted, and individuals are currently being identified, counted and measured to calculate density and biomass. The sample from Alaska Basin North icy seep is processed, and we identified seven invertebrate taxa from that site. Total density was 228 ind/m^2 and biomass was 301 mg/m^2. Non-Tanypodinae midges and worms were the most abundant taxa, and *Tipula* (cranefly larvae) had the highest biomass. We anticipate finishing analysis on the remainder of the 2016 sites in spring 2018, and one of us will present the data at an international conference in May 2018.

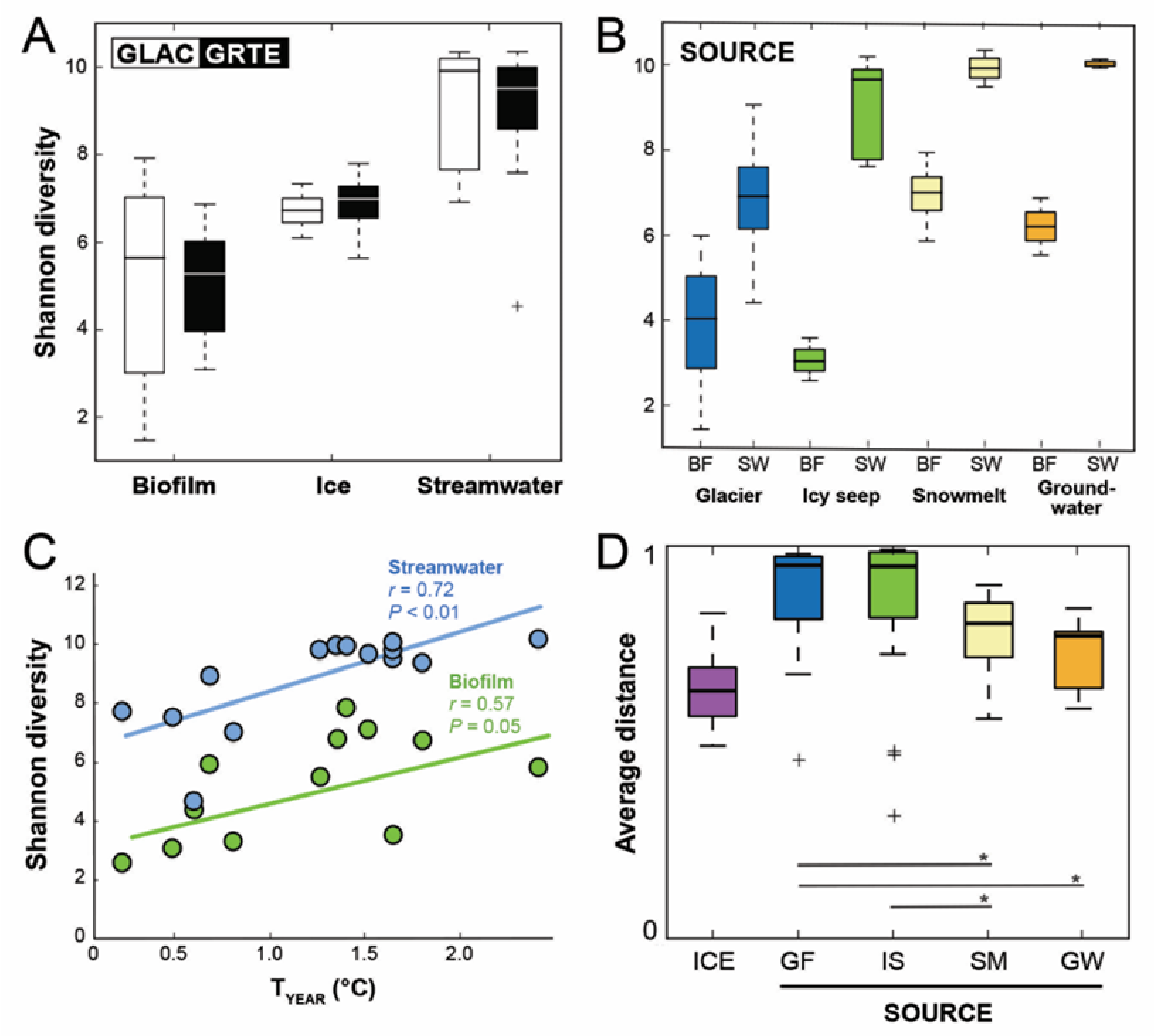

Figure 3. Alpha diversity by a) region + microhabitat and b) stream type ('source') + microhabitat. Upper and lower lines for each group indicate the highest and lowest, non-outlier values observed. Dark lines indicate median values. Outliers are denoted with crosses. c) Alpha diversity is positively correlated (Pearsons' r) with mean annual stream temperature for both streamwater and biofilm. P-values were calculated from Pearson's correlation coefficients. Circles represent one sample. d) Average Bray-Curtis distance between samples when grouped by either ice or source. Higher values indicate greater dissimilarity. Asterisks indicate significance at $P < 0.05$. All comparisons of source distances versus ice samples were significant and are not shown. Abbreviations: BF = biofilm, SW = streamwater, GF = glacier-fed, IS = icy seep, SM = snowmelt-fed, GW= groundwater-fed spring.

Microbial diversity

Our microbial analysis revealed a number of strong patterns in diversity among regions, stream types, and microhabitats within streams (Figure 3). We observed no difference in regional (γ) diversity between GLAC and GRTE (H_{GLAC} = 7.07 vs. H_{GRTE} = 6.87, P = 0.81) or stream types (range of pair-wise P = 0.1-1). For microhabitats, streamwater (mean H = 8.82) was more diverse than both biofilms ($P < 0.01$) and ice ($P < 0.01$). When stream type, microhabitat, and region were all taken into account, the sites with lowest microbial diversity were biofilms from glacier-fed streams and icy seeps in GLAC (mean H = 2.71 and

2.88, respectively). Conversely, streamwater from groundwater-fed springs and snowmelt-fed streams were the most diverse overall (mean H = 10.04 and 9.93). Generally, diversity of biofilms and streamwater scaled with source. That is, in higher diversity habitats like snowmelt-fed streams, both biofilms and streamwater were correspondingly more diverse than in lower diversity habitats like glacier-fed streams. Icy seeps had the largest variability in α diversity among microhabitats for a single stream type, with streamwater an average of 5.97 Shannon diversity units more diverse than biofilms – a much higher difference than the average of the same comparison for all other sources (mean H difference, BF to SW = 3.25). Across all sites, α diversity of streamwater and biofilm were both positively correlated with T_{YEAR} (Pearson's $r = 0.72$, $P < 0.01$ and $r = 0.57$, $P = 0.05$, respectively; Figure 3A) and T_{SUMMER} (Pearson's $r = 0.62$, $P = 0.02$ and $r = 0.70$, 0.01, respectively; Figure 3B). Only α diversity of biofilms was correlated with T_{RANGE} (Pearson's $r = 0.70$, $P = 0.01$; Figure 3C). Channel stability was negatively correlated with streamwater diversity (Pearson's $r = -0.70$, $P < 0.01$) but showed no significant relationship with biofilm diversity (Pearson's $r = -0.21$, $P = 0.52$). When among site (β) diversity was compared, no sub-range-specific signatures of microbial community assemblages were observed ($P = 0.072$). However, both source ($P = 0.013$, 22.59% of variance) and microhabitat ($P = 0.038$, 19.14% of variance) were significant drivers of community assemblage patterns.

Conclusions

Alpine stream types

The contributions of perennial snow and ice vs. seasonal snow inputs are primary factors determining thermal regimes and ultimately patterns of alpine stream biodiversity and species occurrence (Brown et al., 2007). Therefore, watershed size, the percent of surface snow and ice coverage in the basin, and natural downstream increases in temperature with distance from stream source are typically key drivers of the distributions of cold-water dependent biological communities and associated ecosystem processes in alpine streams. For instance, Giersch et al. (2017) reported that the extent of watershed ice and perennial snow coverage was a key indicator of the presence of the alpine stonefly *Lednia tumana* in GLAC.

Three primary stream types are formally recognized in the alpine stream literature: glacier-fed streams, snowmelt-fed streams, and groundwater-fed springs. Our results clearly support the existence of a fourth stream type that is especially prevalent in GRTE: icy seeps. However, icy seeps are not restricted to GRTE; these streams also occur in lower abundance in GLAC, and they likely occur in many other mountainous areas of North America and beyond. The paucity, or even absence, of late-season surficial snow and ice in sampled watersheds in which very cold, stable thermal regimes and stenothermic communities were recorded indicate the presence of subterranean ice feeding these sites. Icy seeps emanate from lobes of rock glaciers, talus debris fields, and moraines of Holocene glaciers, and contain unique biological assemblages and may be particularly resistant to climate change due to their naturally insulated geomorphology. Close temporal monitoring of icy seeps along with the other major alpine stream types will lend clarity to the rate of environmental and biotic change in alpine stream ecosystems. Characterizing stream types that are most resistant to environmental change is an important avenue for future research as these streams may represent a stronghold for cold-adapted, meltwater-associated biota and ecosystem processes in a post-glacial landscape in alpine regions.

Macroinvertebrate diversity

The low density of invertebrates from the Alaska Basin North icy seep stream was similar to the stream draining Petersen Glacier that we sampled in 2015 (Tronstad et al., in prep). Invertebrate biomass in the icy seep stream was lower than groundwater-fed streams previously sampled and similar to assemblages in other glacier-fed or icy seeps streams (e.g., Middle Teton Glacier and Wind Cave streams). We expect that invertebrates living in these streams are strongly influence by hydrologic source as well as stream size and substrate size. During spring 2018, we will finish analyzing the invertebrates in these streams to estimate the biodiversity, density and biomass of invertebrates living in these unique ecosystems.

Microbial diversity

Stream temperature and to a lesser degree, channel stability, significantly influences microbial diversity in alpine streams. For stream water and biofilm communities, an increase in temperature translates to an increase in α diversity. However, streams with more unstable channels (e.g., glacier-fed streams) have correspondingly reduced α diversity in stream water but not biofilms. Among sites, β diversity is highest for glacier-fed stream and icy seep communities, intermediate for snowmelt-fed streams, and lowest in groundwater-fed springs. Aside from taxa living on or within permanent glaciers and snowfields, stream microbial taxa most at risk under future warming scenarios may include members of cold-associated families, and particularly those that appear in the coldest (and most ice-associated) streams with little to no representation elsewhere (e.g., Exiguobacteraceae).

Future Work

Before we began working in GRTE alpine streams in 2015, we recognized that considerable site exploration and fundamental natural history research would be essential to establish a strong foundation on which to build further research. Through the first two years of this project, we have established this foundation through careful classification of hydrological and biological diversity in GRTE. These efforts are beginning to bear fruit with three manuscripts either in review (Hotaling et al., 2019*b*) or nearing submission (Hotaling et al., 2019*a*; Tronstad et al., in prep). These new studies and results complement previous efforts by our group to characterize macroinvertebrate diversity of subalpine streams (Tronstad et al., 2016) and high-elevation lakes (Hotaling et al., 2017*b*). Finishing this first round of publication efforts, while also completing analysis of our macroinvertebrate and diatom data collected in 2016, are two near-term goals. We intend to present macroinvertebrate results from 2015 and 2016 combined at the Society for Freshwater Science meeting in May 2018. Going forward, we have two primary research goals. In 2017, we laid the groundwork for long-term monitoring of alpine streams in GRTE. We established 10 long-term study sites that represent the three major stream types in GRTE. We identified key abiotic and biotic variables to monitor annually, and we achieved the greatest bang for the buck in evaluating responses associated with changing climate. We also began monitoring similar sets of study streams in both the Absaroka-Beartooth Wilderness (south-central Montana) and GLAC. We intend to continue collecting the same suite of response variables annually at each of the focal streams across these three regions. These annually collected data will allow us to describe in detail how different Rocky Mountain alpine stream types are responding to climate change and to test the specific hypothesis that icy seeps can serve as refugia for cold-water taxa and ecological processes.

We will also continue to hone techniques for characterizing alpine stream types in the Rocky Mountain using LIDAR elevation data, which are straightforward and useful for describing high-resolution stream watershed morphology but suffer some difficulties when applying the method to streams with subterranean sources such as icy seeps and groundwater springs. We will continue to research and adapt specialized methods for investigating these habitats, including the use of multispectral, high resolution satellite imagery for detecting icy seeps throughout GRTE. Ultimately, we will pursue NSF funding from their Long-Term Research in Environmental Biology (LTREB) after 3-4 more years of data collection. We plan to continue exploring alpine areas of GRTE and the surrounding mountains with a specific focus on characterizing the distributions of macroinvertebrates of conservation concern including two stoneflies, *Lednia tetonica* and *Zapada glacier*.

Acknowledgements

We thank the UW-NPS research grant for support and park permitting staff for their help attaining research and backcountry permits. Thanks also to Gunnar Johnson (Portland State University) for sharing his spatially georeferenced data on the distribu-

tion of rock glaciers in the western United States with us.

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