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Establishing a long-term monitoring network for assessing potential climatic refugia in cold alpine streams

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Abstract Managing landscapes to maintain climate refugia is likely the best strategy to promote persistence of temperature-sensitive species with limited dispersal capacity. Rare, cold-stenothermic taxa occupy mosaic mountain stream networks due largely to hydrological source heterogeneity. We collected environmental and biological data from alpine streams in the Teton Range, Wyoming representing runoff from snowpack (N=3), glaciers (N=4) and subterranean ice (N=4), every summer from 2015-2017. We quantified differences in habitat among the streams according to a *glaciality* index that included bed stability, suspended solids, temperature and conductivity, and by comparing annual water temperature profiles for each stream. We measured to what degree macroinvertebrate and diatom assemblages varied by stream type. Abiotic and biotic characteristics appeared to differ among sources. Notably, streams fed by subterranean ice (icy seeps) maintained extremely low (mean <2°C) and stable water temperature. Rare, cold-stenothermic stonefly species (*Zapada glacier* and *Lednia tetonica*) were indicators for, although not exclusive to, icy seeps. Icy seeps and their sources may be refugia for temperature-sensitive taxa, as the subsurface ice is more insulated from warmer air temperatures.

Introduction

Long-term ecological data sets collected consistently and regularly are powerful tools for revealing gradual and complex responses to changing conditions (Lindenmayer et al., 2012). Long-term data sets are also recognized by the National Science Foundation (NSF) as an important funding priority (Long-Term Research in Environmental Biology, LTREB). Lindenmayer et al. (2012) outline five key benefits of long-term monitoring as: 1) understanding ecological responses to gradual change, 2) disentangling complex, interacting ecosystem processes that occur over decadal time scales, 3) providing core data that can be used to develop ecological models and test theory, 4) promoting multidisciplinary research, and 5) providing data for developing evidence-based management of natural resources. Our proposed program to study alpine streams in Grand Teton National Park (GRTE) will provide each of these benefits, and we intend to submit an NSF LTREB proposal for our long-term monitoring plan after collecting the six years of *core data* required for the LTREB

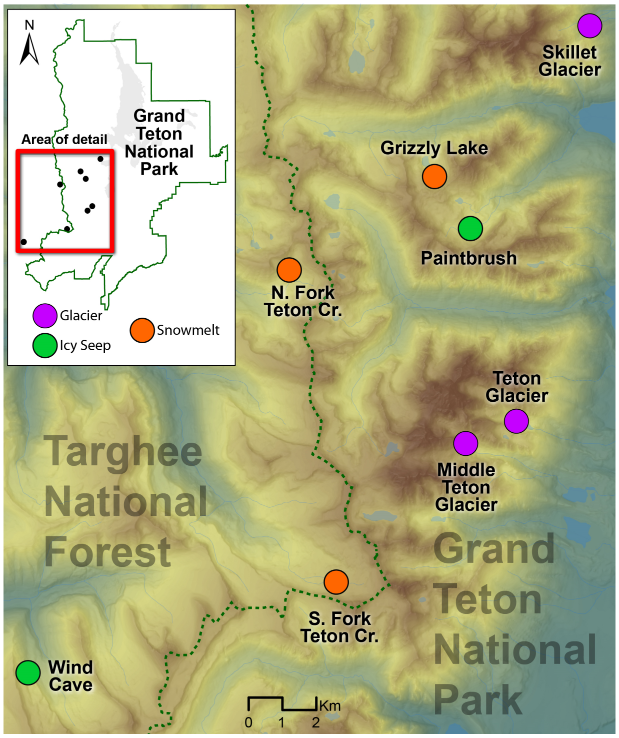

Figure 1. Sites for long-term monitoring in GRTE.

program. We also plan to partner with the NPS *Vital Signs* Monitoring Programs associated with GRTE, Glacier National Park (GLAC), and surrounding regions to bolster our data set and establish long-term partnerships with NPS scientists and resource managers.

We aim to include the GRTE sites (Figure 1) as a part of a broader network of alpine streams that includes GLAC and the Absaroka-Beartooth Wilderness (ABW). These three regions represent lingering strongholds of permanent alpine ice and associated stream ecosystems harboring rare invertebrate species. Our long-term monitoring will be essential to identifying stream types most resistant to climate change and will inform evidence-based conservation management. A consistently collected suite of response variables measured annually can reveal surprising results (Lindenmayer et al., 2012). Setting up a network to monitor both physicochemical habitat changes, and biological response variables representing the full range of ecosystem processes, will reveal long-term ecological effects that are not presently clear.

A secondary aim of our 2017 work was to gain some baseline population genetic inference for key indicator stonefly species in our focal streams. These included both *Lednia tetonica* and *Zapada glacier*. These molecular efforts allowed us to better understand the evolutionary history of these key species of conservation concern, to compare them to a similar (in terms of ecology, taxonomy and behavior) species in GLAC – *Lednia tumana* – and to set a monitoring baseline for future comparisons.

Methods

We collected a suite of data on the physical characteristics, water chemistry, and biodiversity of focal alpine streams (Fig. 1). These 12 indicators will form the core data for long-term monitoring at these sites:

Physical characteristics

1. Streambed stability through the Pfankuch Index, a component of the *glaciality* index.
2. Temperature: dataloggers record temperature continuously at an hourly rate.
3. Suspended solids (SS) measures the erosive power of the stream and is a component of the *glaciality* index (Hauer and Lamberti, 2006).

Water chemistry

4. and 5. Nitrate and ammonium concentrations are important indicators of source hydrology and atmospheric deposition (Saros et al., 2010; Elser et al., 2009), are essential nutrients that limit biological activity, and can therefore affect diversity across trophic levels. Moreover, higher nitrate concentrations have been associated with permanent ice at other sites in the middle-northern Rockies, including rock and surface glaciers (our own unpublished data; Saros et al., 2010).
6. Specific Conductivity (i.e., ionic concentration) is the third component of the *glaciality* index and can be used to estimate how long water has contacted sediment. We measured conductivity using a Yellow Springs Instrument (YSI) Professional Plus Multiprobe.
7. pH is an indicator of the flow path length and geological material that streamwater has interacted

with in the subsurface, and is a key physiological constraint on biological activity. We measured pH using a YSI Professional Plus Multiprobe.

8. Dissolved organic carbon (DOC) includes a mixture of simple and complex organic molecules, vital for heterotrophic biological activity. Through a simple colorimetric index of DOC, we can distinguish DOC that originated from algal production within the stream from DOC that originated from plant production outside of the stream. Moreover, long-term DOC data will help us evaluate whether nutrient chemistry is correlated with the proportion of stream water DOC derived from in-stream algal growth.
9. Chlorophyll a (chl a) is the pigment algae use to harvest light energy, and estimates biomass of primary producers.

Biodiversity

10. Invertebrate community structure. We collected three replicate Surber samples to characterize the invertebrate community in each stream.
11. Indicator species. In addition to quantitative sampling, we opportunistically sampled indicator species, particularly *Lednia tetonica* and *Zapada* sp. from a wider range of habitats. These samples will be stored for population genetic work to asses both spatial and temporal trends in genetic diversity and differentiation. *Zapada glacier* is a stonefly that received a warranted listing under the Endangered Species Act in 2016. Another stonefly, *Lednia tetonica*, is endemic to the Teton Range. A sister species, *Lednia tumana*, is only known from Glacier National Park and received a warranted listing under the Endangered Species Act in 2016 (US Fish and Wildlife Service, 2016).
12. Algal community structure. Algal assemblages differ among stream types in relation to nutrient and flow characteristics. For long-term monitoring, we collected three replicate samples from 4 cm^2 rock scrapes at each site annually.

Population genetics

We sampled *Zapada* and *Lednia* specimens from alpine streams in GLAC, ABW, and the Teton Range. To give broader genetic context to population genetic patterns observed for our focal species, we also obtained either existing mtDNA sequences or nymphs representing the full western *Zapada* taxonomy from mountain streams in California, Washington, New Mexico, and Oregon as well as specimens of *L. sierra* from Cold Water Creek in the Inyo National Forest of central California and *L. borealis* from the inlet to Snow Lake in Mount Rainier National Park. Despite efforts on multiple occasions, the genus *Lednia* has not been observed in ABW (J.J.G. and D.S.F., unpublished) nor in lower elevation streams or high-elevation lakes of the Teton Range (Hotaling et al., 2017; Tronstad et al., 2016). For all specimens, we sequenced the 'DNA barcoding' portion of the mitochondrial DNA (mtDNA) genome, a 658-bp region of the cytochrome c oxidase I (COI) subunit, for 79 newly collected specimens of *Zapada* sp. (n = 34), *L. tetonica* (n = 43), *L. sierra* (n = 1), and *L. borealis* (n = 1). COI is commonly used in DNA barcoding as it is variable both within and among species, yet retains conserved primer binding sites. Barcoding was performed by the Canadian Center for DNA Barcoding (CCDB) following established protocols.

For phylogenetic analyses, we analyzed the *Zapada* and *Lednia* data sets separately with the nemourid *Visoka cataractae* serving as the outgroup for all *Zapada* specimens and *Z. glacier* as the outgroup for *Lednia*. To construct trees, we first used an Akaike information criterion (AIC) test implemented in MrModeltest (Nylander, 2004) to select the best-fit model of DNA substitution (GTR+I+G). Next, we used MrBayes version 3.2.4 (Ronquist et al., 2012) to generate mtDNA gene trees for each data set using five chains analyzed for 10-million generations with a 1-million generation burn-in. Samples were taken every 10000 generations for two replicates. Retained posterior distributions for each replicate were combined to generate a majority-rule consensus tree. For *Zapada*, the placement of our 34 newly barcoded samples provided the basis for decisions regarding which species or lineage each specimen belonged

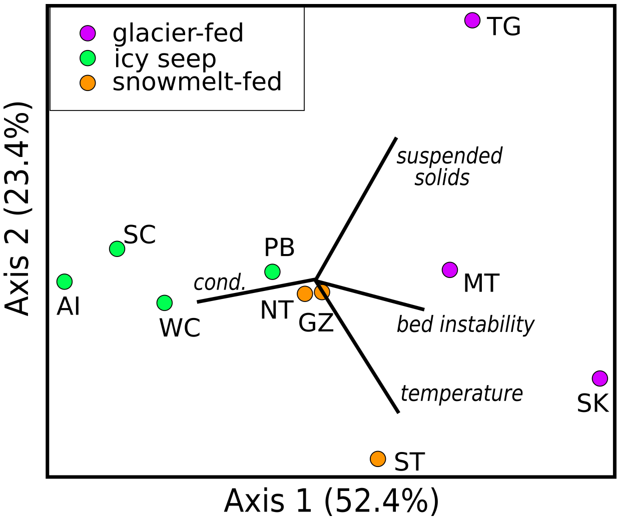

Figure 2. PCA performed on data collected in 2017 from 9 sites (plus data from 2016 on site South Cascade, because we were unable to sample that site in 2017 due to snow cover). Icy seeps are AI (Alaska Basin South), WC (Wind Cave stream), SC (South Cascade) and PB (Paintbrush). Glacier-fed streams are TG (Teton Glacier), MT (Middle Teton Glacier) and SK (Skillet Glacier). Snowmelt-fed streams are NT (NF Teton Creek), ST (SF Teton Creek), and GZ (Grizzly Lake inlet stream).

to. We constructed haplotype networks by compressing sequences into common haplotypes and generating networks in POPART (Leigh and Bryant, 2015). We performed a nested analysis of molecular variance (AMOVA) in Arlequin 3.5 (Excoffier and Lischer, 2010) to assess how genetic variation was partitioned across multiple sampling levels (i.e., among sub-ranges, among populations within sub-ranges, and within populations). The AMOVA was performed separately on the *Z. glacier* and *L. tumana*+*L. tetonica* data sets using mountain sub-ranges as the highest level of structure. We assessed significance and 95% confidence intervals using 5000 bootstrap replicates.

Preliminary Results

Physical characteristics

The suite of streams selected for long-term monitoring (Figure 1) classify readily into the three primary GRTE alpine stream types, according to the four key variables included in the *glaciality* index (Figure 2). For this report, note that we have only included in the principal component analysis (PCA) the site conditions at the time biological data were collected during our summer fieldwork campaign (annual stream temperatures not available yet). After we have retrieved the temperature dataloggers, the temporal data collected over a full year will further resolve the differences between icy seep and snowmelt-fed streams. Nonetheless, the PCA performed with the 2017 data collected in August on our 10 selected long-term monitoring sites reveal significant differences among the three stream types in ordination space (Figure 2).

Population genetics

Phylogenetic analyses supported the seven recognized *Zapada* species as monophyletic with posterior probabilities (PPs) of 1.0 (Figure 3a). Of our 34 newly barcoded *Zapada* specimens, 18 were identified as *Z. glacier*. These new specimens were from four streams where *Z. glacier* had not previously been recorded: three in ABW and one in the Teton Range, bringing the total number of streams known to contain *Z. glacier* to 13 (Giersch et al., 2017, 2015). A haplotype network connecting all *Z. glacier* specimens (n = 256) included 20 haplotypes from three sub-ranges: GNP (n = 198 specimens; 14 haplotypes), ABW (n = 23 specimens; 2 haplotypes), and the Teton Range (n = 35 specimens; 5 haplotypes). Each sub-range was generally characterized by a distinct set of haplotypes, however, haplotypes were shallowly diverged within sub-ranges (max. = 0.6% divergence within GNP) and only slightly more diverged among them with a maximum divergence between any two *Z. glacier* haplotypes of 1.22% (Figure 4a). Interestingly, one unique haplotype was found at both the Grinnell Glacier site in GNP (N = 1) and many sites in ABW (N = 22). For *Z. glacier*, among range differentiation explained 58.7% of the total observed variation and within population variation explained 41.1%, with little variance explained by populations within sub-ranges (0.2%). Overall ϕ_{ST} (0.59), variance among populations relative to total variance regardless of group structure, and ϕ_{CT} (0.59), were significant, revealing that the majority of population

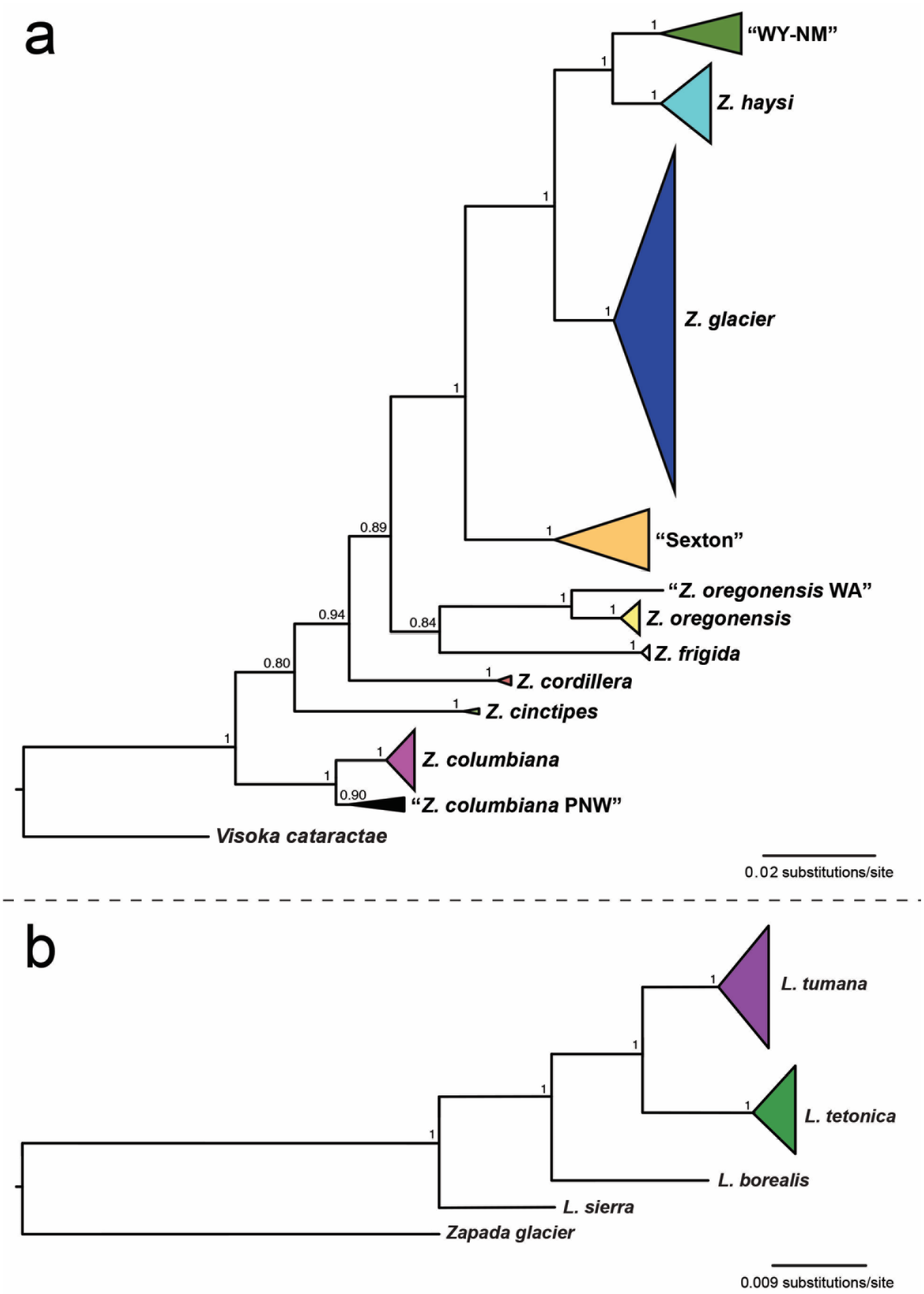

Figure 3. Cytochrome oxidase c subunit I (COI) gene trees of (a) western North American *Zapada*, and (b) the genus *Lednia* including 70 specimens from (Jordan et al., 2016) and 45 newly barcoded specimens. Terminal nodes were compressed into triangles and scaled according to number of specimens. Numbers above nodes indicate posterior probabilities.

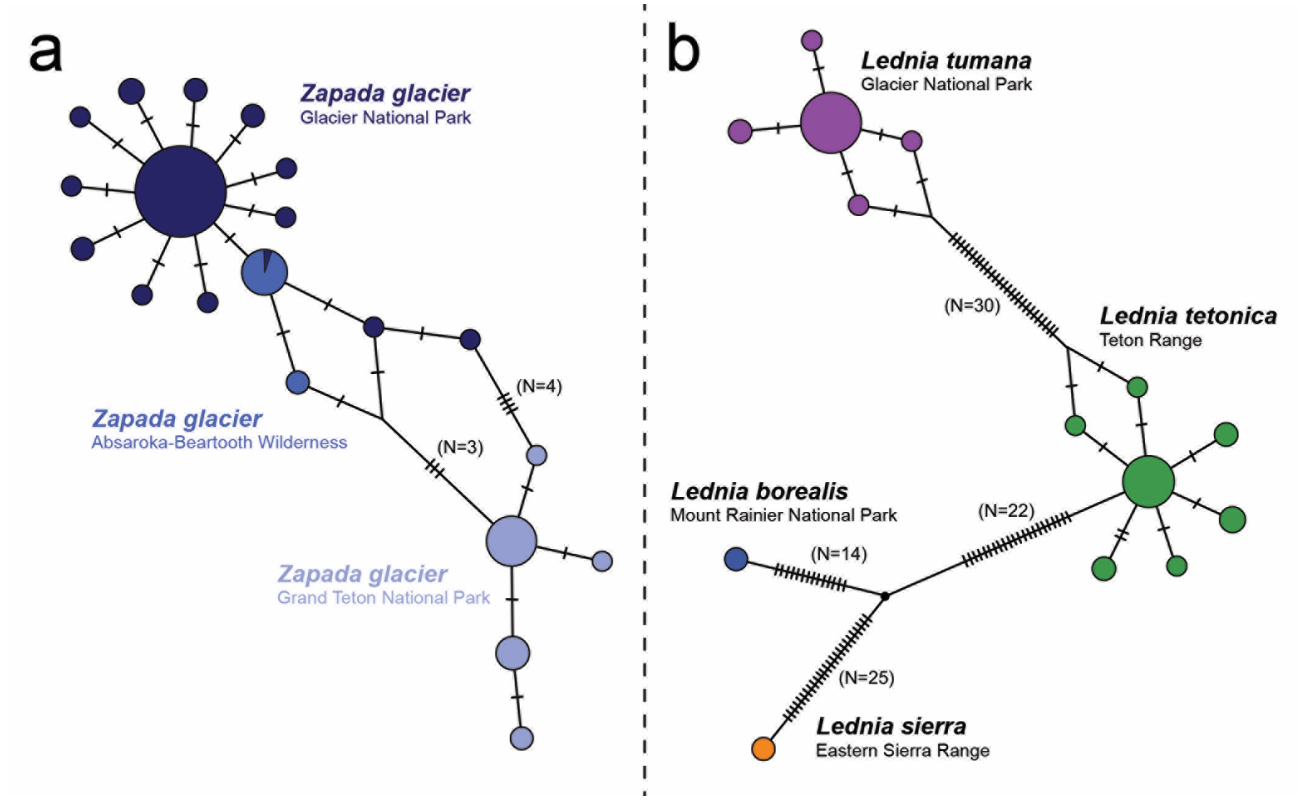

Figure 4. A COI haplotype network of (a) all *Zapada glacier* specimens and (b) all representatives of the current *Lednia* taxonomy. Colored circles represent haplotypes (with circle size scaled by frequency). Hashmarks between haplotypes represent one substitution step (i.e., one nucleotide difference).

structure in *Z. glacier* was explained by isolation among sub-ranges, rather than isolation among populations occupying the same sub-range.

Phylogenetic analyses strongly supported the existing, morphology-based *Lednia* taxonomy with PPs of 1.0 for all nodes and described species resolved as monophyletic (Figure 3b). The mtDNA gene tree placed *L. tetonica* and *L. tumana* as sister species, with *L. borealis* as the sister lineage to the *L. tetonica* + *L. tumana* clade, and *L. sierra* as the sister species to the other three (Figure 3b). Across all *Lednia* specimens, we identified five *L. tumana* haplotypes, seven *L. tetonica* haplotypes, and one haplotype each for the single specimens of *L. borealis* and *L. sierra*. A haplotype network connecting *Lednia* samples revealed strong divergence across described species (and by proxy, mountain sub-ranges; Figure 4b). These divergences ranged from a minimum of 4.6% between *L. tumana* and *L. tetonica* to a maximum of 11.9% between *L. tumana* and *L. sierra* (Figure 4b). For *L. tumana* and *L. tetonica*, among species differentiation explained 95.3% of the total variation observed and within population variation explained 4.4%, with little variance explained by populations within species (0.3%). Both ϕ_{ST} (0.95) and ϕ_{CT} (0.96) were significant. Like *Z. glacier*, the majority of genetic structure in *L. tumana* + *L. tetonica* was explained by isolation among sub-ranges, rather than isolation among populations within sub-ranges. This structure is further evidenced by deep sequence divergence between representative haplotypes for each species.

Invertebrate community structure

We sampled invertebrates at a number of alpine streams draining cold-water sources in the Teton Range. Densities ranged between 670 (South Cascade Stream) and 8740 ind/m^2 (Death Rock Glacier;

Stream	Density (ind/m^2)	Biomass (mg/mm^2)
Alaska Basin North Rock Glacier	1143	521
Alaska Basin South Rock Glacier	2489	721
Death Rock Glacier	8742	2687
Petersen Glacier outlet	934	83
Middle Teton Glacier outlet	4491	425
North Fork Teton Creek	7320	641
Paintbrush Rock Glacier	8130	747
South Cascade Stream	667	20
South Cascade Rock Glacier	2055	2253
South Fork Teton Creek	1738	2742
Wind Cave	2945	416

Table 1. Invertebrate density and biomass in 11 alpine streams in the Teton Range.

Table 1). Biomass ranged between 20 and 2090 mg/m^2 with the same sites having the minimum and maximum values. Non-Tanypodinae Chironomidae were the most abundant taxa collected (2635 ind/m^2 on average), followed by *Zapada* sp. (351 ind/m^2), Oligochaeta (141 ind/m^2) and Tubellaria (94 ind/m^2). *Zapada* sp. were in six streams that we sampled: Alaska Basin South Rock Glacier (208 ind/m^2), Death Rock Glacier (2488 ind/m^2), South Cascade stream (7 ind/m^2), South Cascade Rock Glacier (30 ind/m^2), South Fork Teton Creek (165 ind/m^2) and Wind Cave (966 ind/m^2). *Zapada* samples must be DNA barcoded to identify individuals to species as several species of *Zapada* co-exist in alpine streams of the Teton Range. We collected *Lednia tetonica* in two streams, Paintbrush Rock Glacier (5 ind/m^2) and South Cascade Rock Glacier (12 ind/m^2).

Algal community structure and biomass

Analysis of the 2017 diatom samples are in process. Chlorophyll a, an indicator of algal biomass, ranged between 0.6 and 28 g/m^2 (Figure 5). The two highest values were from icy seeps and the lowest value was from a stream draining a surface glacier. Snowmelt streams had intermediate chlorophyll a.

Conclusions

Population genetics

Our support for congruence between existing morphology-based taxonomic classifications and population genetic patterns also has important conservation implications. In an age when the diagnosis of cryptic diversity through genetic means is increasingly commonplace, it is important that we collectively remember that morphological differences can still be strong indicators of evolutionary divergence. Indeed, for *Lednia*, the morphological differences which species descriptions were based upon were roundly supported by our mtDNA sequence data. Based upon these results, we predict that for *Z. glacier*, a morphological comparison of adults would likely uncover relatively few, if any, morphological differences across mountain sub-ranges. However, given the difficulty of collecting *Z. glacier* adults, this prediction is difficult to confirm.

Perhaps most importantly, a conservation plan developed for any combination of population genetic patterns (*Lednia* or *Zapada*) or comparisons of *Lednia* adult morphology would capture the underlying population genetic pattern of isolation among mountaintops that exists for both groups. Given the recommendations for *L. tumana* and *Z. glacier* to be listed under the ESA due to climate-change-induced habitat loss (US Fish and Wildlife Service, 2016), and the multitude of less-studied alpine stream invertebrates worldwide, this theoretical point has clear policy implications as conserving one group can have an umbrella effect on co-occurring groups (Caro, 2010). In this case, this umbrella effect would extend beyond habitat conservation to also include the conservation of genetically distinct units.

Whether or not imperiled species actually contain additional cryptic species-level lineages is a topic of significant interest in conservation biology with important implications for management (Hime et al., 2016). Here, we find clear mtDNA support for both *L. tumana* and *L. tetonica* as separate, range-specific species with no contemporary gene flow between them. For *Z. glacier*, genetic diversity is clearly structured primarily by sub-range, but support for a limited degree of contemporary gene flow and the identification of a shared haplotype between GNP and ABW are evidence against cryptic species diversity within the *Z. glacier* complex. However, the possibility for discordance between mitochondrial and nuclear genomes (where evolutionary patterns do not align between the two) in population genetic and systematic studies is relatively common (Gompert et al., 2008). Therefore, multi-locus nuclear data paired with coalescent-based species delimitation methods are needed before any robust conclusions can be drawn (Grummer et al., 2014). However, additional genetic data collection and analyses should not occur in isolation; ideally, they would be complemented by a robust morphological study of *Z. glacier* adults across their known range paired with further ecological studies to better understand relevant dispersal and life-history traits for these rare stoneflies.

Invertebrate community structure

Invertebrate assemblages appear to differ among stream types. Snowmelt streams generally had consistently high densities, biomass and richness of invertebrates, while streams originating from surface glaciers had variable community attributes that appear linked to habitat conditions. For example, Petersen Glacier stream had low densities, biomass and richness of invertebrates, likely because the stream was highly unstable with small substrate. Conversely, the stream draining Middle Teton Glacier had the highest densities of invertebrates, likely because the stream had higher stability and large substrate size (cobbles and boulders). Streams draining icy seeps had intermediate density, biomass and richness of invertebrates. Both *Lednia tetonica* and *Zapada glacier* appear to be rare, as we collected them in a handful of alpine streams we sampled. These stoneflies tend to live in streams that are cold (e.g., 2°C) and stable draining icy seeps (e.g., rock glaciers).

Long-term monitoring

Analysis of samples and data collected in summer 2017 is still in progress, and one goal for the final assessment of 2017 results (and beyond) is to evaluate the level of intra-annual variability relative to among-stream variability. We expect icy-seep streams to be most stable in their habitat and biotic characteristics, due to their hydrological source stability. Conversely, we expect snowmelt streams to have much higher variability. Collecting long-term data is essential to identify landscape and habitat features, and associated indicator taxa and rare taxa that are more or less sensitive to temporal changes in climatic factors.

Future Work

We plan to submit a new proposal in 2018 to continue monitoring alpine streams originating from three sources (glaciers, rock glacier and snowmelt streams). We would also like to investigate the thermal tolerances of the rare stoneflies (*Zapada glacier* and *Lednia tectonica*) that live in very cold alpine streams.

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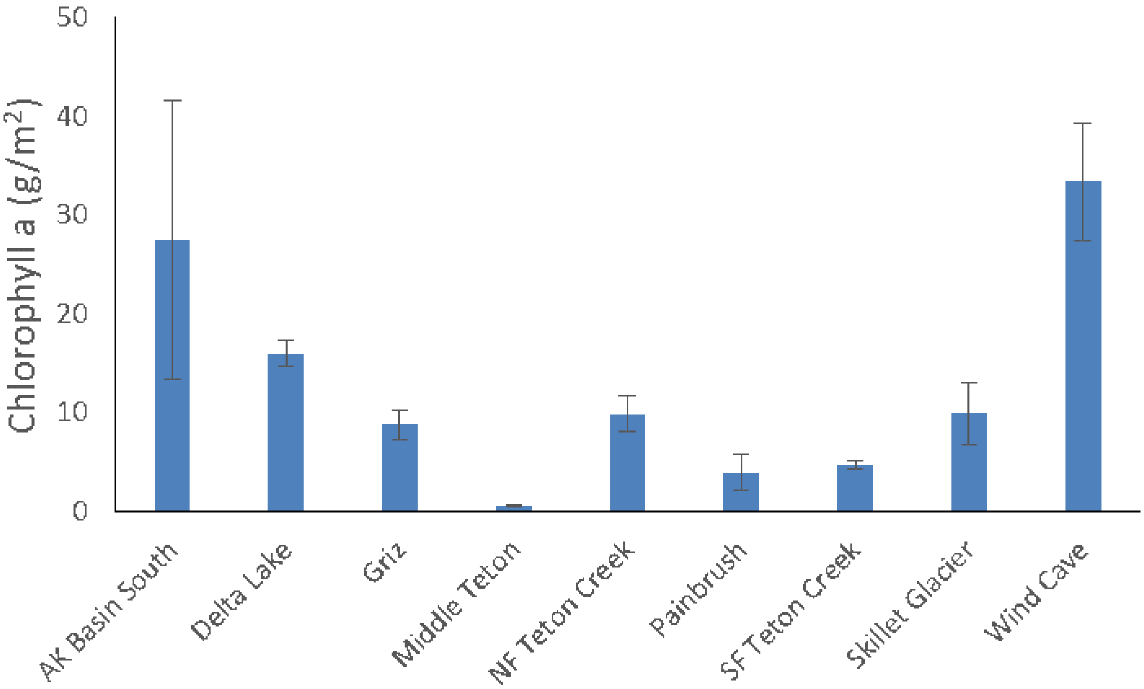

Figure 5. Chlorophyll a, an indicator of algal biomass, measured at 11 alpine streams in the Teton Range. Bars are standard errors.

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References

Caro, T. 2010. Conservation by proxy: indicator, umbrella, keystone, flagship, and other surrogate species. Island Press.

Elser, J. J., T. Andersen, J. S. Baron, A.-K. Bergström, M. Jansson, M. Kyle, K. R. Nydick, L. Steger, and D. O. Hessen. 2009. Shifts in lake N: P stoichiometry and nutrient limitation driven by atmospheric nitrogen deposition. Science **326**:835–837.

Excoffier, L., and H. E. Lischer. 2010. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Molecular Ecology Resources **10**:564–567.

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

Giersch, J. J., S. Jordan, G. Luikart, L. A. Jones, F. R. Hauer, and C. C. Muhlfeld. 2015. Climate-induced range contraction of a rare alpine aquatic invertebrate. Freshwater Science **34**:53–65.

Gompert, Z., M. L. Forister, J. A. Fordyce, and C. C. Nice. 2008. Widespread mito-nuclear discordance with evidence for introgressive hybridization and selective sweeps in Lycaeides. Molecular Ecology **17**:5231–5244.

Grummer, J. A., R. W. Bryson Jr, and T. W. Reeder. 2014. Species delimitation using Bayes factors: simulations and application to the *Sceloporus scalaris* species group (Squamata: Phrynosomatidae). Systematic biology **63**:119–133.

Hauer, F. R., and G. A. Lamberti. 2006. Methods in stream ecology. Elsevier Academic Press.

Hime, P. M., S. Hotaling, R. E. Grewelle, E. M. O'Neill, S. R. Voss, H. B. Shaffer, and D. W. Weisrock. 2016. The influence of locus number and information content on species delimitation: an empirical test case in an endangered Mexican salamander. Molecular Ecology **25**:5959–5974.

Hotaling, S., L. M. Tronstad, and J. C. Bish. 2017. Macroinvertebrate diversity is lower in high-elevation lakes vs nearby streams: evidence from Grand Teton National Park, Wyoming. Journal of Natural History **51**:1657–1669.

Jordan, S., J. J. Giersch, C. C. Muhlfeld, S. Hotaling, L. Fanning, T. H. Tappenbeck, and G. Luikart. 2016. Loss of genetic diversity and increased subdivision in an endemic alpine stonefly threatened by climate change. PLoS One **11**.

Leigh, J. W., and D. Bryant. 2015. POPART: full-feature software for haplotype network construction. Methods in Ecology and Evolution **6**:1110–1116.

Lindenmayer, D. B., G. E. Likens, A. Andersen, D. Bowman, C. M. Bull, E. Burns, C. R. Dickman, A. A. Hoffmann, D. A. Keith, and M. J. Liddell. 2012. Value of long-term ecological studies. Austral Ecology **37**:745–757.

Nylander, J. 2004. Evolutionary Biology Centre, Uppsala University; Sweden. MrModeltest v2. Program distributed by the author.

Ronquist, F., M. Teslenko, P. Van Der Mark, D. L. Ayres, A. Darling, S. Höhna, B. Larget, L. Liu, M. A. Suchard, and J. P. Huelsenbeck. 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology **61**:539–542.

Saros, J. E., K. C. Rose, D. W. Clow, V. C. Stephens, A. B. Nurse, H. A. Arnett, J. R. Stone, C. E. Williamson, and A. P. Wolfe. 2010. Melting alpine glaciers enrich high-elevation lakes with reactive nitrogen. Environmental Science & Technology **44**:4891–4896.

Tronstad, L. M., S. Hotaling, and J. C. Bish. 2016. Longitudinal changes in stream invertebrate assemblages of Grand Teton National Park, Wyoming. Insect Conservation and Diversity **9**:320–331.

US Fish and Wildlife Service. 2016. Endangered and threatened wildlife and plants; 12-month finding on a petition to list the western glacier stonefly as an endangered or threatened species; proposed threatened species status for Meltwater Lednian Stonefly and Western Glacier Stonefly. Federal Register **81**:68379–68397.