

Climate-induced glacier and snow loss imperils alpine stream insects

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Abstract

Climate warming is causing rapid loss of glaciers and snowpack in mountainous regions worldwide. These changes are predicted to negatively impact the habitats of many range-restricted species, particularly endemic, mountaintop species dependent on the unique thermal and hydrologic conditions found only in glacier-fed and snow melt-driven alpine streams. Although progress has been made, existing understanding of the status, distribution, and ecology of alpine aquatic species, particularly in North America, is lacking, thereby hindering conservation and management programs. Two aquatic insects – the meltwater stonefly (*Lednia tumana*) and the glacier stonefly (*Zapada glacier*) – were recently proposed for listing under the U.S. Endangered Species Act due to climate-change-induced habitat loss. Using a large dataset (272 streams, 482 total sites) with high-resolution climate and habitat information, we describe the distribution, status, and key environmental features that limit *L. tumana* and *Z. glacier* across the northern Rocky Mountains. *Lednia tumana* was detected in 113 streams (175 sites) within Glacier National Park (GNP) and surrounding areas. The probability of *L. tumana* occurrence increased with cold stream temperatures and close proximity to glaciers and permanent snowfields. Similarly, densities of *L. tumana* declined with increasing distance from stream source. *Zapada glacier* was only detected in 10 streams (24 sites), six in GNP and four in mountain ranges up to ~600 km southwest. Our results show that both *L. tumana* and *Z. glacier* inhabit an extremely narrow distribution, restricted to short sections of cold, alpine streams often below glaciers predicted to disappear over the next two decades. Climate warming-induced glacier and snow loss clearly imperils the persistence of *L. tumana* and *Z. glacier* throughout their ranges, highlighting the role of mountaintop aquatic invertebrates as sentinels of climate change in mid-latitude regions.

Keywords: alpine ecosystems, conservation biology, Endangered Species Act, global change, *Lednia*, macroinvertebrates, Plecoptera, stoneflies, *Zapada*

Received 28 August 2016; revised version received 12 October 2016 and accepted 25 October 2016

Introduction

Mountainous regions in the mid-latitudes are warming at a rate two to three times faster than the global average (Hansen *et al.*, 2005; Pederson *et al.*, 2010), causing rapid shrinkage of glaciers and snowpack (Hall & Fagre, 2003; Rauscher *et al.*, 2008). These changes are predicted to have significant effects on biodiversity in mountain ecosystems (Parmesan, 2006), particularly for aquatic invertebrates constrained to alpine streams (Brown *et al.*, 2007; Jacobsen *et al.*, 2012). Alpine aquatic invertebrates are particularly sensitive to climate change because their distributions and abundances are tightly linked to narrow thermal and hydrologic niches (Milner *et al.*, 2001; Jacobsen *et al.*, 2009). Increases in temperature with increasing distance from snow and ice sources is the primary factor determining patterns of biodiversity and

species occurrence (Brown *et al.*, 2007; Jacobsen *et al.*, 2012, 2014; Cauvy-Fraunié *et al.*, 2016). Therefore, habitat loss and fragmentation caused by shrinking glaciers and permanent snow masses may result in the near-term extinction for many alpine aquatic species, resulting in substantial declines in regional and global freshwater biodiversity (Hauer *et al.*, 1997; Monaghan *et al.*, 2005; Brown *et al.*, 2007; Muhlfeld *et al.*, 2011; Jacobsen *et al.*, 2012; Finn *et al.*, 2013a; Giersch *et al.*, 2015).

Macroinvertebrates inhabiting alpine stream environments in the Rocky Mountains of North America are especially vulnerable to anthropogenic climate change due to rapid regional warming and associated loss of glaciers and snowpack (Byrne *et al.*, 2014). Glacier National Park (GNP) in northwestern Montana is an iconic example of the potential impacts of global warming – over 80% of the park's glaciers have been lost since the mid-19th century (Carrara, 1987). Only 25 glaciers larger than 0.1 km² remain in GNP (US Geological

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Survey, 2014), and these are predicted to fully recede over the next two decades (Hall & Fagre, 2003). Tied to this ongoing deglaciation will be major hydrologic changes including, but not limited to, temperature increases, altered flow regimes, changes in turbidity and water chemistry, intermittency, and, in extreme cases, the complete loss of alpine stream reaches (Haldorsen & Heim, 1999; Brown *et al.*, 2007; Jacobsen *et al.*, 2012). These changes threaten the stability of critical habitat for alpine-restricted stream invertebrates and heighten risks of extinction and biodiversity loss.

Two species particularly susceptible to the effects of climate-induced glacier and snow loss are the meltwater stonefly (*Lednia tumana*) and western glacier stonefly (*Zapada glacier*). Both species were previously considered to be endemic to the GNP area (northern Rocky Mountains, USA) and have been recommended for listing under the U.S. Endangered Species Act (ESA) due to climate-change-induced habitat loss (U.S. Fish and Wildlife Service, 2016). The distribution of *L. tumana* is fragmented and restricted to short sections of cold, alpine streams directly below glaciers, permanent snowfields, and springs (Muhlfeld *et al.*, 2011; Jordan *et al.*, 2016). Bioclimatic models indicate *L. tumana* is predicted to lose over 80% of its current range under future warming scenarios (Muhlfeld *et al.*, 2011). Similarly, *Z. glacier* occupies an extremely restricted distribution in GNP (only three previously known locations), which has been truncated due to rising stream temperatures associated with glacial and snowfield decline over the last several decades (Giersch *et al.*, 2015). Although climate change threatens the persistence of many plant and animal species, just one species, the polar bear (*Ursus maritimus*), has been listed under the U.S. Endangered Species Act due to contemporary climate change and associated loss of permanent ice (U.S. Fish and Wildlife Service, 2008). However, climate change impacts likely imperil many more enigmatic organisms, particularly glacially tied aquatic macroinvertebrates (Bálint *et al.*, 2011; Jacobsen *et al.*, 2012). Given potential climate-induced threats to *L. tumana* and *Z. glacier*, a comprehensive evaluation of their current status, distribution, and habitat requirements is needed.

Here, we use a large alpine stream macroinvertebrate dataset and high-resolution climate and habitat information to describe the status, distribution, and habitat requirements of two ESA-recommended species, *L. tumana* and *Z. glacier*, throughout their range in the Rocky Mountains, USA. Through extensive field sampling and genetic analyses, we address three primary objectives: (1) to describe the range-wide distribution of *L. tumana* and *Z. glacier*; (2) to identify environmental factors controlling the distribution of both species; and (3) to identify factors influencing the density of

L. tumana in streams with systematic longitudinal sampling. Based upon previous studies (Muhlfeld *et al.*, 2011; Giersch *et al.*, 2015; Jordan *et al.*, 2016), we hypothesize that both species are restricted to high-elevation habitats in the Rocky Mountains and that permanent glacier or snow cover is a major environmental factor determining both distributions and *L. tumana* densities. Because *Z. glacier* nymphs are not diagnosable from morphology alone, we use genetic barcoding to identify specimens and gain an initial glimpse into *Z. glacier* population genetic patterns. Collectively, our results provide a rare example linking climate-change-induced habitat loss with pressing conservation implications for rare, understudied species.

Materials and methods

Study area and species

We extensively sampled the alpine stream network of GNP, Montana, from 1996 to 2015. Additionally, we sampled several sites outside the known distribution of *L. tumana* and *Z. glacier* across the Rocky Mountains of Montana and Wyoming. For GNP, the most recent glaciation event peaked at the end of the Pleistocene, approximately 20 000 BP, and was completed by 10 000 BP. A short period of glacial advance, the 'Little Ice Age', occurred between 1500 and 1900 AD, and the glaciers present in GNP today are remnants of ice formed during that time.

The genus *Lednia* was first described by Ricker (1952) as a subgenus of *Nemoura*, but later elevated to generic status by Illies (1966). *Lednia* species are endemic to alpine areas of western North America, and the current taxonomy includes four species: *Lednia tumana* (Ricker; Fig. 1a and b) of the northern Rocky Mountains, *L. borealis* Baumann and Kondratieff of the Cascades, *L. sierra* Baumann and Kondratieff of the Sierra Nevada, and *L. tetonica* Baumann and Call of the Teton Range.

Zapada is the most widely distributed stonefly genus of family Nemouridae in the Rocky Mountains (Baumann *et al.*, 1977). The western *Zapada* taxonomy recognizes seven species: *Z. cinctipes* (Banks), *Z. columbiana* (Claassen), *Z. cordillera* (Baumann and Gaufin), *Z. frigida* (Claassen), *Z. glacier* (Baumann and Gaufin), *Z. haysi* (Ricker), and *Z. oregonensis* (Claassen) (Baumann, 1975). *Zapada glacier* (Fig. 1c and d) is known by multiple common names, including the 'glacier forestfly' (Stark *et al.*, 1998) and 'western glacier stonefly' (Jordan *et al.*, 2010). While the adults of *Z. glacier* are easily distinguished from other *Zapada* species through characters described by Baumann & Gaufin (1971), nymphs are indistinguishable from *Z. haysi*. Therefore, we used previous barcoding data linking *Z. glacier* nymphs to adults described by Giersch *et al.* (2015) to identify *Z. glacier* specimens from all *Zapada* collected.

Characterizing species distributions

We sampled stream invertebrates at 482 sites (>1300 m; $N = 272$ streams) from 1996 to 2015 to determine the presence or absence of *L. tumana* and *Z. glacier* across the northern

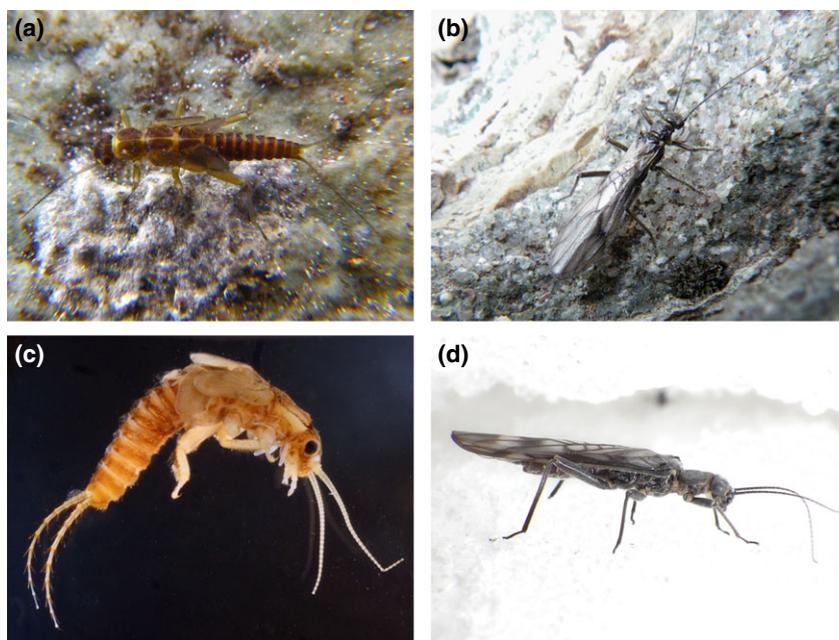


Fig. 1 Nymphs and adults of *Lednia tumana* and *Zapada glacier*. (a) *L. tumana* nymph; (b) *L. tumana* adult; (c) *Z. glacier* nymph; (d) *Z. glacier* adult. Identification of *Z. glacier* nymph was confirmed by molecular barcoding.

Rocky Mountains (Figs 2 and 3). Although glacial loss has occurred throughout the study area over the sampling interval, the vast majority (>95%) of sampling sites visited prior to 2010 have been revisited since. Therefore, we consider the ongoing effects of glacier loss on the results of this study to be minimal. We primarily focused on GNP because type specimens of both species were first described from there. Sampling methods followed those outlined in Muhlfeld *et al.* (2011) and Giersch *et al.* (2015). For most streams, nymphs and adults were qualitatively sampled by either hand-picking rocks in the stream (nymphs) or sweeping vegetation and aspirating individuals from substrate (adults). In a subset of sampling localities ($N = 56$ streams and 130 sites, Appendix S1), quantitative benthic samples were collected in triplicate using a Surber sampler (area 0.09 m², 250 μ m mesh size). Number of individuals per square meter (*L. tumana* only) was calculated based on the fractions subsampled, total area sampled, and averaged across triplicates. All *Zapada* nymphs were included in molecular barcoding (see below). Due to limited resources, it was not possible to collect quantitative samples at all sites included in this study. However, given the density of *L. tumana* observed in quantitative samples (e.g., Table S1, Appendix S1), observing at least one specimen through qualitative sampling alone was likely a near certainty. All specimens were preserved in 95% ethanol, and locations were recorded with a GPS. Both nymph and adult *L. tumana*, and adult *Z. glacier* were verified in the laboratory.

Zapada barcoding and genetic analyses

To identify *Z. glacier* specimens, we sequenced the 'DNA barcoding locus', cytochrome oxidase subunit I (COI), for all *Zapada* specimens collected ($N = 271$; nine adults, 262

nymphs). COI is widely used in DNA barcoding as it is variable across species yet retains conserved primer binding sites (Hebert *et al.*, 2003). Barcoding was performed by the Canadian Centre for DNA Barcoding at the Biodiversity Institute of Ontario, University of Guelph, following established protocols (see Appendix S5 for additional details). Sample information and photographs of barcoded specimens are available through the Barcode of Life Data System (BOLD; Ratnasingham & Hebert, 2007; project name = 'GNPZP').

After barcoding, we combined COI data from the new dataset ($N = 271$) with the dataset ($N = 154$) included in Giersch *et al.* (2015), to form the 425-sample *Zapada* dataset included in downstream analyses. COI sequences were visually inspected and aligned using MUSCLE (Edgar, 2004) as implemented in GENIOUS version 6.1.8 (Kearse *et al.*, 2012). For phylogenetic analysis, we 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 build a phylogeny using five chains for 10 million generations with a 1-million generation burn-in, inspected in TRACER (Rambaut & Drummond, 2007). To clarify genetic relatedness of specimens from other mountain ranges to those in GNP, we built a haplotype network of identified *Z. glacier* specimens in POPART (Leigh & Bryant, 2015) with the TCS network implementation (Clement *et al.*, 2000).

Stream temperature modeling

Stream temperatures were recorded at 43 sites (>1500 m; Appendix S2) across the study area from 1998 to 2013 using digital thermographs (Hobo and Tidbit models; Onset Computer Corporation, Pocasset, MA, USA). Thermograph

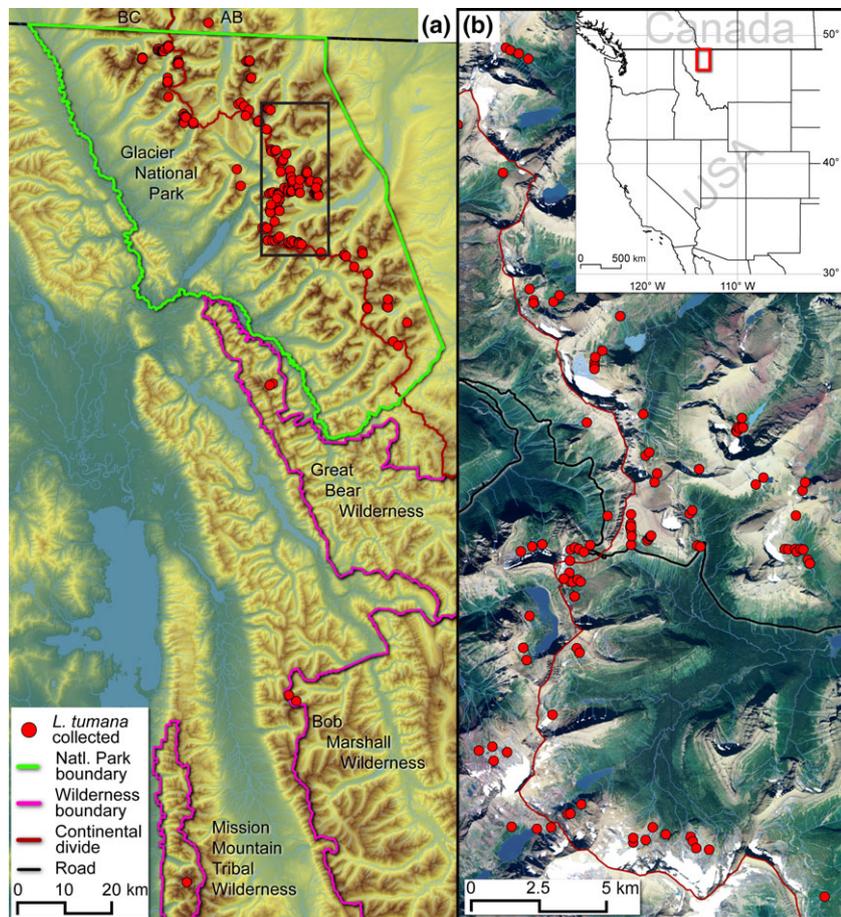


Fig. 2 Distribution of *Lednia tumana* based upon sampling conducted over the 20-year study period (1996–2015). (a) Study area from Glacier National Park (GNP) to the Mission Mountain Tribal Wilderness superimposed on an elevation gradient (black rectangle is the area shown in panel (b)). (b) Detail of *L. tumana* collection locations centered around Logan Pass, GNP, superimposed on NAIP satellite imagery from September 2005. The red rectangle in the map inset shows the size and placement of the study area in western North America.

locations were georeferenced at the time of installation, and data were summarized to mean monthly temperatures for each site and year of the study period. Temperature modeling methods followed those outlined in Jones *et al.* (2014). Briefly, we employed a generalized linear model framework to predict mean August temperatures for all sampling locations (Benyahya *et al.*, 2007). To parameterize the model, we used gridded Daymet air temperature surfaces (1-km resolution, Thornton *et al.*, 2012) and temporally joined values to each site's temperature record. Geomorphic predictor variables (elevation, slope, and aspect) were used to account for topographic and geomorphic effects across the landscape. We also included a categorical variable to account for the presence of glaciers at the stream reach level, which are known to influence downstream thermal regimes (Giersch *et al.*, 2015). To interpolate temperature predictions at sample sites across the study area, we considered stream reaches downstream of glaciers as glacier-affected and digitized them as such to the confluence of the next highest stream order. The resulting model was then used to predict mean August stream temperatures for

1986–2005 and provided baseline data for species occurrence modeling.

L. tumana occurrence modeling

For occurrence modeling, glacier and snow features, as well as alpine streams, lakes, and ponds, were digitized using satellite imagery in ARCGIS version 10.2 (Environmental Systems Research Institute, Redlands, CA, USA). To attain the minimum seasonal snow coverage possible, multiple satellite imagery sources were used including National Aerial Photography Program imagery (September 2003) and National Agriculture Imagery Program (NAIP; September 2005 and 2015). Specifically, true glacial features were coded as 'ice' based upon the publicly available georeferenced GNP glacier layer (NPS Geospatial Dataset #1019881) or if crevasses were visible in satellite imagery. All snow present at the minimum snow coverage time points as well as smaller snow features around ice masses were coded as 'permanent snow'. Streams were digitized to extend the National Hydrologic Dataset to the alpine zone using the

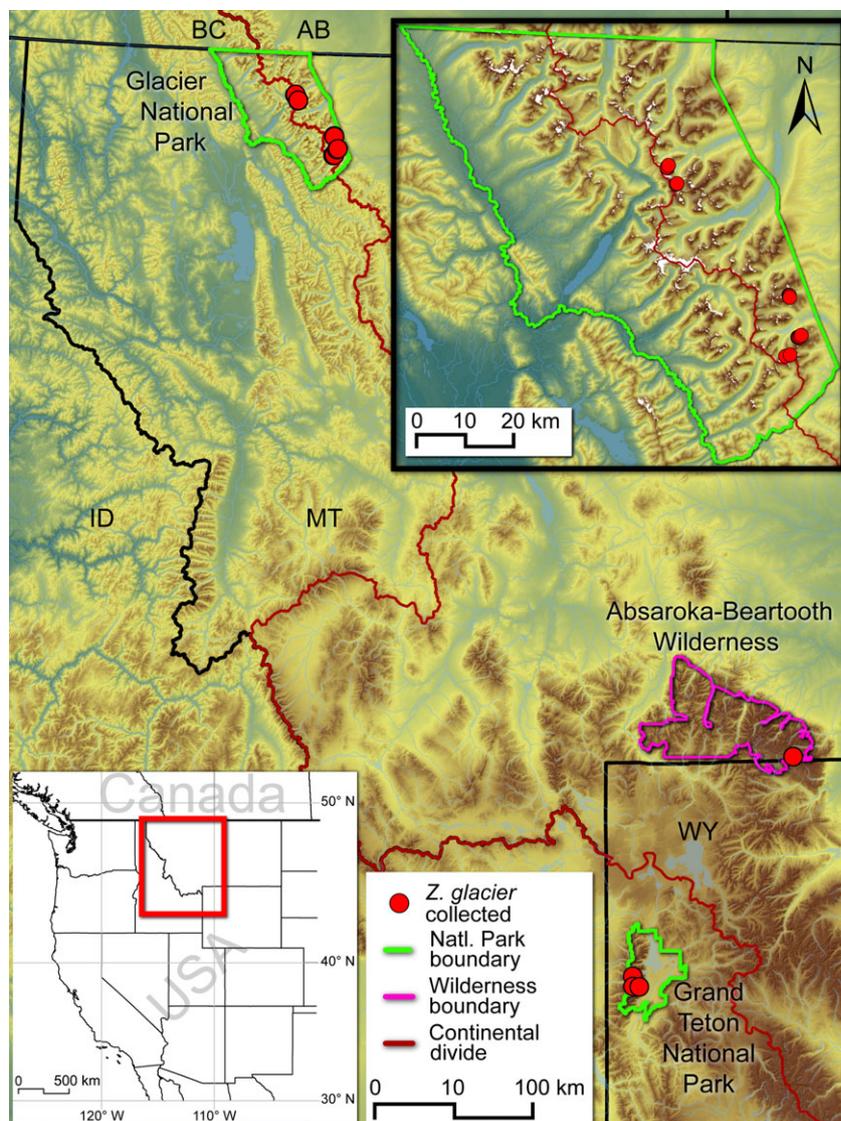


Fig. 3 Distribution of *Zapada glacier* based upon sampling conducted over the 20-year study period (1996–2015). Study area showing Glacier National Park (GNP), the Absaroka-Beartooth Wilderness, and Grand Teton National Park superimposed on an elevation gradient. The black outlined inset shows a detailed view of GNP, where most *Z. glacier* occurrences were documented. The red rectangle in the map inset shows the size and placement of the study area in western North America.

same 1-m resolution NAIP 2005 satellite imagery described above. This imagery was also used to digitize stream, ice, and snow features from the Absaroka-Beartooth Wilderness (ABW) and Grand Teton National Park (GRTE). Streams emanating from ice or permanent snow features were coded as sourced by ‘ice’ or ‘permanent snow’, respectively. Known groundwater-fed springs were coded as such, and all other streams were considered sourced by seasonal snow. Drainage area above collection locations was calculated using 10-m Digital Elevation Model (DEM) data. Watershed boundaries were refined based upon visible drainage channels using 0.6-m resolution satellite imagery (DigitalGlobe, Longmont, CO, USA) accessed through the ARCGIS World Imagery map. 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.

The extensive distribution data available for *L. tumana* were used to generate occurrence models and test hypotheses regarding the impacts of environmental variation on *L. tumana*'s spatial distribution. We used an information theoretic approach (Burnham & Anderson, 2002) and general linear mixed models to determine which environmental variables of *a priori* interest – source type, modeled stream temperature, distance to stream source, and basin proportion with perennial snow cover – were related to *L. tumana* occurrence. We used a binomial error distribution to account for the binary outcome of the response variable (presence vs. absence). Given that multiple sampling locations were nested within some alpine streams, we used a linear mixed modeling

approach to account for covariation in the presence or absence of *L. tumana* within drainages and inherent pseudoreplication (Zuur *et al.*, 2009). Stream name was used as a random intercept term for spatially grouped sampling locations. To identify a best-supported model explaining *L. tumana* occurrence, we compared AIC values for all possible subsets of the covariates. Covariates that did not decrease by $AIC \geq 2$ relative to a less parameterized model were not included in subsequent modeling steps (Arnold, 2010). The best-supported model was used for all parameter estimation. To compare effect sizes of the different environmental variables, all continuous covariates were standardized prior to analyses by subtracting the mean from each value and dividing by the standard deviation. The extremely limited number of detections for *Z. glacier* prohibited us from using a general linear mixed modeling framework to describe factors influencing occurrence of this species; instead, only descriptive statistics are provided.

L. tumana density and longitudinal variation

To better understand the environmental conditions that influence the density of *L. tumana*, we used quantitative samples for 18 streams ($N = 80$ sites, Appendix S1) with at least three sampling sites along the stream continuum (range 3–9, median = 4). We used *L. tumana* density (individuals per m^2) at each site as a response variable in Poisson general linear mixed models. As with the occurrence modeling, stream name was used as a random intercept term to account for covariation and the nested structure of replication (repeated measures; Zuur *et al.*, 2009). We also employed an individual random effect to deal with overdispersion in the count data (Elston *et al.*, 2001; Harrison, 2014). We used an identical model selection approach to determine which environmental variables – source type, modeled stream temperature, distance to stream source, and basin proportion with perennial snow cover – were related to the density of *L. tumana*. For all mixed models (occurrence and abundance), we used R (R Development Core Team, 2008) and the package LME4 (Bates *et al.*, 2015) to fit models.

Results

Characterizing species distributions

Lednia tumana were detected in 113 first-order alpine streams (175 total sites). Of these, 109 streams (170 sites) were in GNP and four were outside of the park (Fig. 2; Appendix S3). Elevations ranged from 1357 to 2544 m (mean = 2027 m; SD = 198 m). Adults were observed emerging from late July through early October. *Lednia tumana* were collected a mean distance of 592 m (SD = 455 m) from stream source and distance to source affected distributions depending upon the primary hydrologic influence; *L. tumana* were collected farther downstream in glacier-fed streams (max. = 2355 m) versus those fed by either permanent snow or springs (max. = 1289 and 910 m, respectively) (Table 1). Total

area of catchments with *L. tumana* ranged from 3948 m^2 to 5.4 km^2 (mean = 0.8 km^2 , SD = 1.1 km^2) and contained more total ice and snow (mean = 12.1%, SD = 15.2) than those without (5.3%, SD = 13.8), as well as a greater percentage of ice coverage (10.2%, SD = 15.5% vs. 4.2%, SD = 13.6%).

Of the 272 streams (482 sites) included in this study, *Z. glacier* was only collected in 10 streams (24 sites), including six in GNP and four outside of the park (Fig. 3; Appendix S4). Elevation of *Z. glacier* locations ranged from 1828 to 3205 m (mean = 2200 m, SD = 373 m), and catchment area ranged from 4127 m^2 to 2.9 km^2 (mean = 1.08 km^2 , SD = 0.9 km^2). *Zapada glacier* was collected a maximum of 1407 m (mean = 569 m, SD = 459 m) from stream source with 42% of locations sourced by ice, 54.2% below springs, and one location below a permanent snowfield (Table 2). *In situ* temperatures were recorded at two *Z. glacier* locations in GNP, below Grinnell Glacier Lake and near the springhead of the Upper Dry Fork tributary. Maximum temperature recorded below Grinnell Glacier was 6.3 °C (mean = 2.0 °C) with a range of 3.9 °C. At the springhead of the Upper Dry Fork tributary, the maximum temperature recorded was 13.3 °C (mean = 4.7 °C) with a range of 9.3 °C (Figs S1 and S2).

Zapada barcoding and genetic analyses

Our final COI alignment was 658 bp with 2.7% missing data across all *Zapada* specimens. Phylogenetic analyses strongly supported the seven previously recognized *Zapada* species with posterior probabilities (PPs) of 1.0 (Fig. 4a). In total, we identified 186 new *Z. glacier* specimens (Fig. 4a). A haplotype network constructed for all *Z. glacier* samples ($N = 238$) recovered 19 distinct haplotypes from three general areas (Fig. 4b): GNP and the surrounding mountains ($N = 195$ specimens; 11 haplotypes), ABW ($N = 9$ specimens; three haplotypes), and GRTE ($N = 34$ specimens; five haplotypes). All haplotypes from GNP differed by ≤ 2 substitutions (Fig. 4b). The ABW haplotypes, ~500 km to the southeast, differed from those in GNP by 1–4 substitutions. Another ~170 km to the southwest, the five GRTE haplotypes differed from GNP by 5–8 substitutions and ABW by 3–7 substitutions. The total number of haplotypes (19) included 10 new haplotypes which were not observed in Giersch *et al.* (2015). For additional results for *Zapada* systematics, see Appendix S5.

Stream temperature modeling

The stream temperature model was parameterized with air temperature, elevation, slope, aspect, and glacier covariates ($r = 0.84$ and RMSE = 1.35 °C; Table 3). All

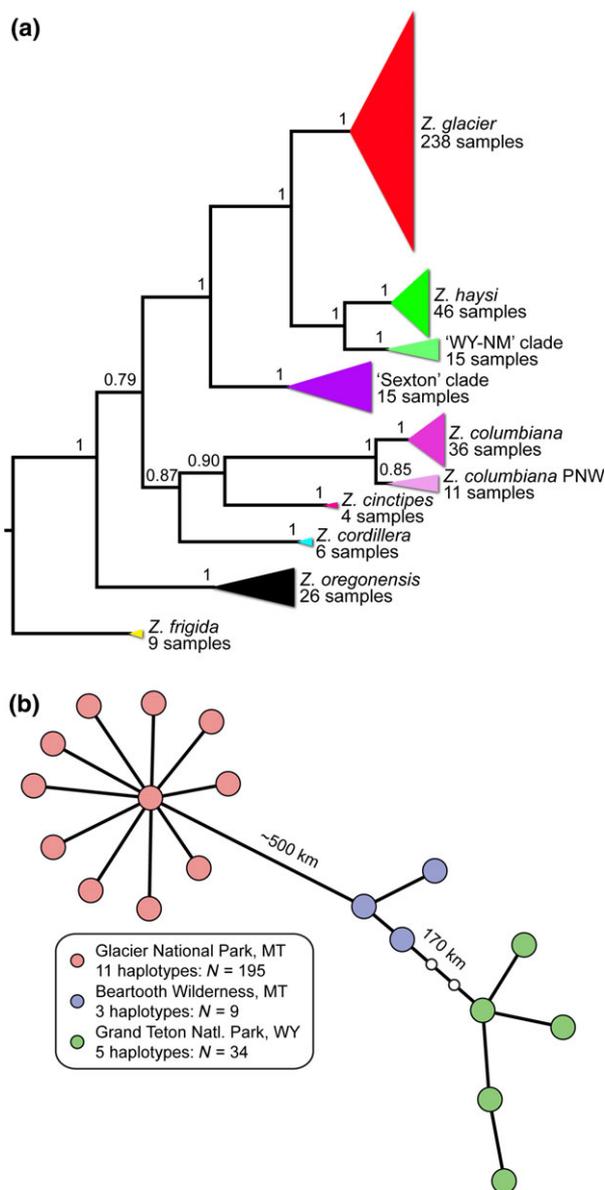


Fig. 4 (a) Phylogeny of 425 *Zapada* specimens barcoded for the cytochrome oxidase *c* subunit I (COI) gene. This dataset is a combination of 154 specimens from Giersch *et al.* (2015) and 271 new specimens. Terminal nodes were compressed into triangles and scaled according to number of specimens. Node numbers indicate posterior probability. (b) A COI haplotype network of all 238 *Z. glacier* specimens. Large circles represent compressed haplotypes with one mutational difference between them. Small white circles represent an additional mutational step each.

predictor variables were statistically significant ($P < 0.05$) and confirmed general expectations (i.e., stream temperatures were warmer where air temperatures were warmer). A significant cooling effect was observed for sites downstream of glaciers ($P < 0.05$), and this effect was estimated as an average of -3.32 °C

(SE = 0.29) during August–September (Table 3). Stream temperature modeling showed that mean temperature of glacier-fed streams is lower than locations fed by other sources (Table 3).

L. tumana occurrence modeling

Lednia tumana occurrence was strongly linked to permanent snow and ice within catchments. *Lednia tumana* occurred more frequently in streams derived from glaciers or permanent snow (Tables 4 and 5) than streams originating from seasonal snow or springs. The best-supported model explaining *L. tumana* occurrence also included stream temperature and distance from the stream source (Fig. 5; Tables 4 and 5). *Lednia tumana* were observed more frequently at sites that were close to the stream source and where temperatures were lower; they were rarely found in locations with the warmest temperatures and/or greatest distances to stream source (Tables 4 and 5). Over the range of observed variation, the effect of distance to source was nearly twice as strong as the effect of stream temperature (Table 5). The fixed and random effect structure of the best-supported model effectively accounted for spatial autocorrelation in the data (i.e., spatial autocorrelation was not present in residuals from the best-supported model; Fig. S3).

L. tumana density and longitudinal variation

Similar to patterns in occurrence, *L. tumana* density declined with increasing distance from stream source ($P < 0.001$; Table 6; Fig. 6). Other environmental variables (stream source type, stream temperature, and basin proportion with perennial snow cover) did not explain variation in *L. tumana* density. However, it is important to note that the lack of an effect for stream source type reflects the limited number of streams with longitudinal abundance data ($N = 18$) and the inclusion of a random effect structure that blocks observations by each stream (i.e., there are very few data points for each stream source type). If that random effect is removed, there are significant differences in abundance among the different stream sources (i.e., the best-supported model without a 'stream' random effect did include stream source; Table S3). Sites in streams derived from permanent snow had the highest average density ($P = 0.04$), while sampling locations in streams derived from spring sources had the lowest ($P = 0.04$). In either case (i.e., including or omitting a random intercept for stream), the model structure effectively addresses spatial autocorrelation (Figs S4 and S5).

Table 1 *Lednia tumana* occurrences in relation to stream source, distance to source (D), percentage of ice coverage (IC), permanent snow coverage (SC), and ice + permanent snow coverage (ISC) in a given catchment. Temperatures (T) are modeled August temperatures and reported in degrees Celsius (°C). Distances are in meters (m), and values in parenthesis are standard deviations. Modeled temperatures are based upon stream temperatures recorded *in situ* at 43 sites from 1998 to 2013

Source	Streams	D, max.	D, mean	IC, mean	SC, mean	ISC, mean	T, max.	T, mean
Ice	48	2355	769 (500)	21.4% (16.4)	1.2% (1.6)	22.6% (16)	10	4.7 (1.9)
Permanent snow	37	1289	523 (329)	0.008% (0.1)	4.1% (5.7)	4.1% (5.7)	9.3	6.4 (1.5)
Seasonal snow	22	1190	352 (350)	None	0.1% (0.5)	0.1% (0.5)	8.1	6.3 (1.1)
Spring	6	910	233 (254)	None	1.6% (1.8)	1.6% (1.8)	8.6	5.4 (3.6)
Total	113	2355	593 (455)	10.2% (15.5)	1.9% (3.7)	12.1% (15.2)	10	5.5 (2.0)

Table 2 *Zapada glacier* occurrences by stream source, distance to source (D), percentage of ice coverage (IC), snow coverage (SC), and ice + snow coverage (ISC) in a given catchment. Distances are in meters (m), and all values in parenthesis are standard deviations

Source	Streams	D, max.	D, mean	IC, mean	SC, mean	ISC, mean
Ice	5	1407	958.4 (319.2)	13.6 (13.6)	1.6 (1.5)	15.2 (12.6)
Permanent snow	1	1114	1114	0	7	7
Seasonal snow	0	n/a	n/a	n/a	n/a	n/a
Spring	4	643	227.2 (223.4)	0 (0)	0.6 (1.1)	0.6 (1.1)
Total	10	1407	568.8 (459.1)	5.7 (10.9)	1.3 (1.8)	7 (10.7)

Discussion

We identified a strong linkage between climate-driven environmental conditions and the fundamental niche of two alpine stoneflies, *L. tumana* and *Z. glacier*, in the northern Rocky Mountains. Both species have been recommended for listing under the ESA due to climate-change-induced loss of alpine glaciers and permanent snow (U.S. Fish and Wildlife Service, 2016), yet prior to this study, a comprehensive evaluation of their status, distribution, and habitat requirements was lacking. Over 20 years of research showed that both species are restricted to short sections of cold, alpine streams, primarily within GNP. This narrow band of habitat is under threat as glaciers, and permanent snow sources rapidly decline throughout the western United States, especially in the northern Rocky Mountains (Hall & Fagre, 2003; Edmunds *et al.*, 2012). However, the identification of both *L. tumana* and *Z. glacier* populations in alpine springs may bode well for the future of both species as groundwater-fed springs could act as refugia buffered against changes in the alpine cryosphere. Still, the decline of meltwater contributions will greatly alter temperature and hydrologic regimes for the majority of *L. tumana* and *Z. glacier* habitats and may lead to streams transitioning to ephemeral flows (Haldorsen & Heim, 1999). Moreover, *L. tumana* and *Z. glacier* are representative of a broader community of alpine, cold-adapted macroinvertebrates that face similar, although largely understudied, threats (Brown *et al.*, 2007;

Jacobsen *et al.*, 2012; Leys *et al.*, 2016). The results of this study, when combined with previous research (Muhlfeld *et al.*, 2011; Giersch *et al.*, 2015; Jordan *et al.*, 2016), clearly shows that continued decline of glaciers and permanent snowfields is the most significant threat to the long-term persistence of *L. tumana* and *Z. glacier*.

We also identified significant expansions and important refinements to the known ranges of both species. Prior to this study, the southern extent of *L. tumana*'s known range was the Grant Glacier basin just beyond GNP's southern boundary. Our discovery of *L. tumana* ~100 km south of GNP greatly extends this southern distributional limit over multiple mountain ranges. As there are few glaciers or permanent snowfields in the mountains surrounding GNP, these southern populations are geographically isolated and may be at even greater risk of extirpation. For *L. tumana*, additional undiscovered populations beyond the range described here are unlikely. For example, sampling in the Cabinet Mountains (~150 km west) did not identify any new localities. Furthermore, higher elevations further west are within the range of *L. borealis*, and ranges farther to the south may be within the range of *L. tetonica*. However, we cannot rule out the possibility of undiscovered *L. tumana* populations north of GNP in southwestern Canada. Indeed, one record of *L. tumana* has been reported from just north of the GNP border (Donald & Anderson, 1977). Future efforts to sample the southern Canadian Rockies will fill the only significant remaining gap in knowledge of *L. tumana*'s distribution.

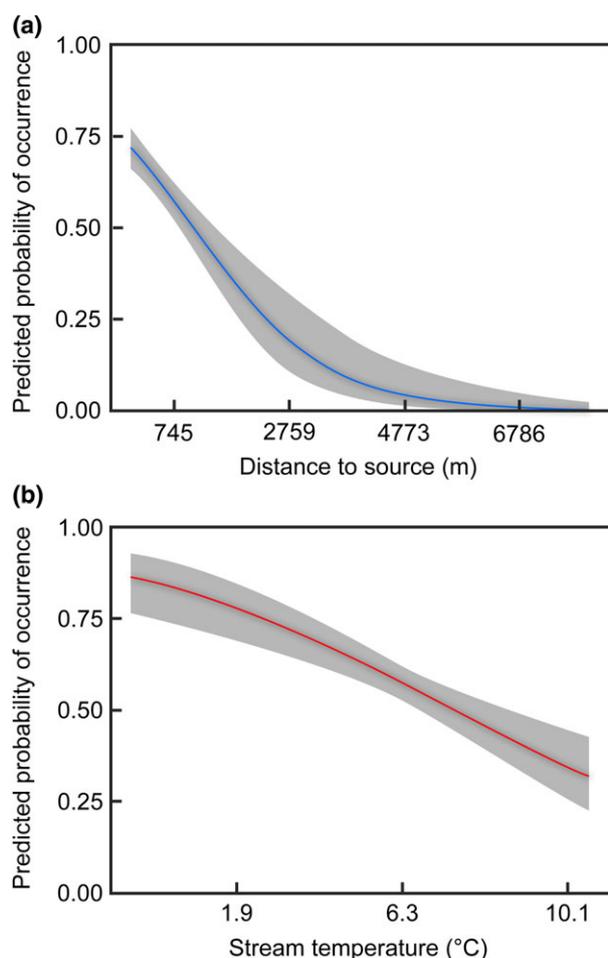


Fig. 5 Predicted probability of *Lednia tumana* occurrence as a function of (a) distance to stream source (m) and (b) stream temperature (°C). Probabilities are based upon streams fed by glaciers (i.e., probabilities are lower in streams with other sources).

Zapada glacier occurs in at least three mountain ranges and/or national parks in the Rocky Mountains (GNP, ABW, and GRTE). Despite this wide geographic distribution, *Z. glacier* is far more rare than *L. tumana* (observed in only 10 streams versus 113 streams for *L. tumana*). Given the ~500 km distance between the GNP and GRTE, it is conceivable that more *Z. glacier* populations (aside from the ABW populations) exist between the two areas. Also, the link between GNP *Z. glacier* and specimens from other ranges was solely based on mtDNA. A single marker, especially one with the unique evolutionary history of mtDNA, is not sufficient to delimit species nor provide robust population genetic information (e.g., Dupuis *et al.*, 2012; Carstens *et al.*, 2013). Additional genetic data incorporating multilocus, nuclear markers for *Z. glacier* paired with robust species delimitation approaches (e.g., Grummer *et al.*, 2014; Hime *et al.*, 2016; Hotaling *et al.*, 2016) are

Table 3 Parameter estimates for the stream temperature model. Root mean square error (RMSE) is in degrees Celsius (°C). Abbreviations include the regression coefficient (b) and associated standard error (SE)

Coefficients	b (SE)	P	r	RMSE
Air temperature	0.20 (0.05)	<0.001		
Elevation	-0.002 (0)	0.0076		
Slope	-0.097 (0.02)	<0.001		
Aspect	-0.007 (0.002)	<0.001		
Glacier effect	-3.32 (0.29)	<0.001		
Intercept	7.41 (1.50)		0.84	1.35

Table 4 Results of model selection for *Lednia tumana* occurrence. The best-supported model is highlighted in bold

Fixed	AIC	ΔAIC
Ice + Dist + Temp. + Snow	537.0	1.6
Ice + Dist. + Temp.	535.4	0
Ice + Dist. + Snow	544.6	9.2
Dist. + Temp. + Snow	561.5	26.1
Ice + Dist.	542.6	7.2
Ice + Temp.	552.4	17.0
Ice + Snow	574.3	38.9
Temp. + Dist.	564.9	29.5
Temp. + Snow	564.0	28.6
Dist. + Snow	572.9	37.5
Ice	576.0	40.6
Dist.	584.3	48.9
Temp.	567.3	31.9
Snow	581.7	46.3

Table 5 Parameter estimates for the best-supported model explaining occurrence of *Lednia tumana*

Parameter	Estimate	SE	P
Intercept (Ice)	0.3	0.298	0.314
Permanent snow	-0.819	0.377	0.03
Seasonal snow	-2.173	0.442	<0.001
Spring	-2.575	0.578	<0.001
Distance to source	-0.872	0.241	<0.001
Stream temperature	-0.474	0.159	<0.005

needed to clarify the presence or absence of cryptic species within *Z. glacier*.

Mountaintop species threatened by climate change present a conservation paradox. Given the limited probability of dispersal and the ongoing habitat reduction, conservation introductions, where organisms are moved to suitable habitat outside of their known range (Thomas, 2011; IUCN/SSC, 2013), may be the only viable management tool to avoid extinction. However, beyond obvious resource-related

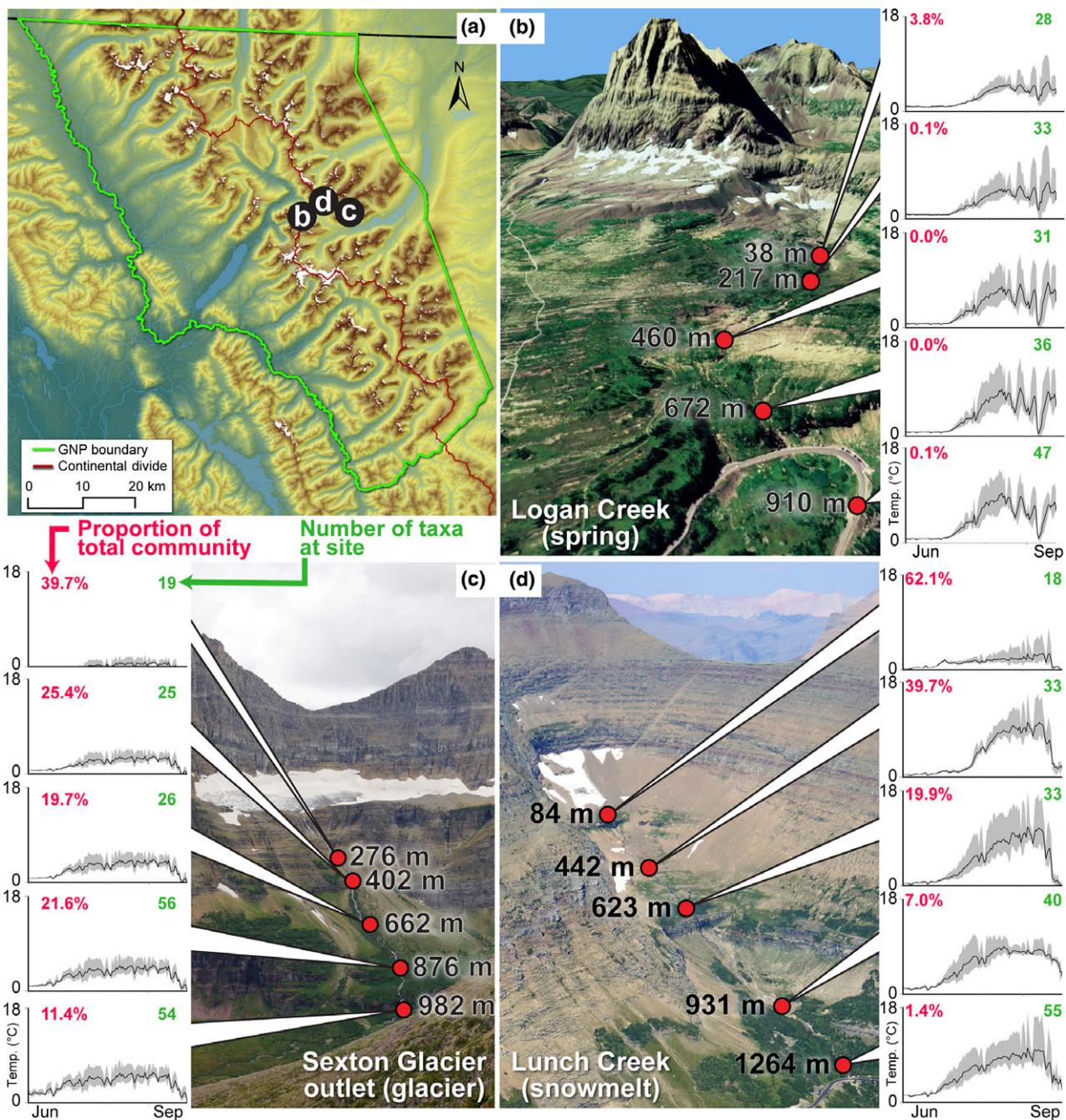


Fig. 6 Stream temperatures, percentage of *Lednia tumana* versus the total macroinvertebrate community, and number of taxa observed for longitudinal sampling of three representative hydrologic source types where *L. tumana* is known to occur. Distances (m) are to stream source. Dark lines in the thermographs indicate mean daily temperature with gray area indicating intraday range. (a) Glacier National Park, Montana, USA showing stream locations in panels b, c, and d. (b) Logan Creek, a groundwater-fed spring. (c) Sexton Glacier outlet, a glacier-fed stream. (d) Lunch Creek, a permanent snowfield-driven stream. Data and logger locations are included in Tables S1 and S2.

challenges, conservation introductions also come replete with biotic challenges including the need for available habitat, lack of threats to other taxa, minimized potential for hybridization, and the consideration of standing genetic diversity and how it may be

affected (IUCN/SSC, 2013). For low- and mid-latitude, glacially tied species, the former consideration is particularly difficult, with the only habitat option for longer-term success being higher latitude glacier-fed rivers far outside of traditional, mountaintop habitat, a likely

Table 6 Results of model selection for *Lednia tumana* density. The best-supported model is highlighted in bold. The effect of distance to source on *L. tumana* density was significant ($P < 0.001$)

Fixed	AIC	Δ AIC
Ice + Dist. + Temp. + Snow	808.8	2.0
Ice + Dist. + Temp.	807.3	0.5
Ice + Dist. + Snow	808.1	1.3
Ice + Temp. + Snow	822.8	16
Dist. + Temp. + Snow	808.2	1.4
Ice + Dist.	806.6	-0.2
Ice + Temp.	821.4	14.6
Ice + Snow	826.5	19.7
Temp. + Dist.	806.3	-0.5
Temp. + Snow	821.4	14.6
Dist. + Snow	808.8	2.0
Ice	825.3	18.5
Dist.	806.8	0
Temp.	819.5	12.7
Snow	825.8	19.0

unrealistic solution. Unfortunately, the most beneficial (and difficult to implement) tool for conserving alpine stream biodiversity may rest in reducing anthropogenic stressors, such as global carbon emissions, which contribute to global warming, glacier decline, and loss of alpine habitat (Khamis *et al.*, 2013).

The distributions of *Z. glacier* and *L. tumana* will likely contract dramatically as climate change effects are realized in the northern Rocky Mountains (Hall & Fagre, 2003; Muhlfeld *et al.*, 2011; Giersch *et al.*, 2015). Rapid habitat reduction and increased fragmentation portend greater probability of extinction, distributional shifts, and loss of genetic diversity. For both species, shifts to higher altitude are not viable options, and shifts across latitude are limited by sheer distance, lack of corridors, and poor adult stonefly dispersal ability (Brown *et al.*, 2009). Moreover, genetic diversity and population connectivity are critical for long-term resiliency of species in the face of environmental change. Temporal loss of habitat can increase population isolation and reduce gene flow, a pattern observed for some alpine-restricted species (Rubidge *et al.*, 2012; Bi *et al.*, 2013), including *L. tumana* (Jordan *et al.*, 2016). For *L. tumana*, temporal genetic comparisons highlighted a possible link between loss of glaciers and reduced genetic diversity (Jordan *et al.*, 2016). Similarly, molecular evidence from *Z. glacier* populations in GNP were consistent with these findings, revealing a dramatic reduction in its occupied range in recent years (Giersch *et al.*, 2015). Taken together, these data highlight an important but relatively unexplored concern when considering the conservation of *L. tumana*, *Z. glacier*, and other imperiled alpine, aquatic species – that is, assuming populations do persist into the future, what are the

ecological and conservation implications of climate-driven loss of genetic diversity (Bálint *et al.*, 2011; Pauls *et al.*, 2013)? A species that has lost much of its standing genetic diversity, and therefore its adaptive potential, will have greatly reduced capacity to mitigate future environmental changes (Hoffmann & Sgrö, 2011) and may suffer the near-term effects of inbreeding depression.

Through the loss of glaciers and snowfields, alpine stream networks will become more environmentally homogenous. While this homogeneity will lead to a net increase in within stream (alpha) diversity as a more diverse, warmer elevation community shifts upward, it will simultaneously cause declines in between stream (beta) and regional (gamma) diversity as climate-sensitive species and communities are lost (Hauer *et al.*, 1997; Brown *et al.*, 2007; Jacobsen *et al.*, 2012; Finn *et al.*, 2013b). Mountaintops act as isolated biological islands that restrict immigration and increase the likelihood of speciation and endemism, but also increase the risk of local extinction. The decline and loss of glaciers worldwide will likely lead to the local or regional extinction of aquatic alpine invertebrates, with up to 38% of species predicted to be lost with the complete recession of glaciers in regional catchments of Ecuador, the Alps, and Alaska (Jacobsen *et al.*, 2012).

Ultimately, the disappearance of glaciers from mid-latitude alpine ecosystems will dramatically alter landscapes, as well as hydrologic and thermal regimes, with clear implications for biodiversity on local to global scales and across taxonomic levels, from genes to communities. However, little is still known regarding the ecological roles of imperiled species inhabiting alpine streams (but see Füreder *et al.*, 2003; Clitherow *et al.*, 2013; Khamis *et al.*, 2015), particularly in North America. In a related example from an Alaskan glacier-fed stream, macroinvertebrates proved an important link between glacially derived organic carbon and higher trophic levels (Fellman *et al.*, 2015). *Lednia tumana* and *Z. glacier* likely represent an entire community of organisms increasingly imperiled by climate change with important, but unresolved ecological value. The results of this study emphasize near-term threats to the conservation of these enigmatic species and represent the need for more research and monitoring to inform management, understand evolutionary consequences, and predict the potential ramifications of their extirpation on ecosystem integrity.

Acknowledgements

Many institutions and individuals contributed to the existing knowledge of *Lednia tumana* and *Zapada glacier*. Funding was provided by the U.S. Fish and Wildlife Service, U.S.G.S. Ecosystems Mission Area and Status and Trends Program, and the

Glacier National Park Conservancy. We also acknowledge Rita Bennett, Vincent D'Angelo, Debra Finn, Cayley Faurot-Daniels, Andrew Lamont, Brady Miller, Jean Tabbert, and Lusha Tronstad for their assistance in the field. Debra Finn, Lusha Tronstad, and Bob Wisseman provided additional *Zapada* specimens for barcoding. James Boyd, Adam Sepulveda, and two anonymous reviewers provided helpful comments that improved the final version of this manuscript. Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Thermograph of the Grinnell Glacier Lake outlet stream where *Zapada glacier* is known to occur.

Figure S2. Thermograph of the Upper Dry Fork spring where *Zapada glacier* is known to occur.

Figure S3. Spatial correlogram of model residuals for *Lednia tumana* presence vs. distance between sampling sites.

Figure S4. Spatial correlogram of model residuals for *Lednia tumana* densities with distance between sampling sites.

Figure S5. Spatial correlogram of model residuals for *Lednia tumana* densities with distance between sampling sites for a model that did not include the random intercept term ‘stream’.

Appendix S1. All streams and sites where quantitative samples ($N = 130$) were taken and the sites where >3 observations were present along a single stream and included in *Lednia tumana* density modeling ($N = 80$).

Appendix S2. Complete site information for locations included in temperature modeling.

Appendix S3. *Lednia tumana* locations.

Appendix S4. *Zapada glacier* locations.

Appendix S5. Additional details DNA barcoding methods and *Zapada* systematics results.

Table S1. The data for five streams and 15 sites included in Figure 4.

Table S2. Locations of *in situ* temperature loggers included in Figure 4, S1–2.

Table S3. Model selection results for *Lednia tumana* density for a model that did not include a random intercept term ‘stream’.