Are Western Glacier Stoneflies (*Zapada glacier*) more widely distributed in Wyoming alpine streams?

Lusha Tronstad[1], Bryan Tronstad[1], Audrey Lindsteadt[1] and Scott Hotaling[2]
[1]Wyoming Natural Diversity Database, University of Wyoming, Laramie, WY 82071
[2]Department of Watershed Sciences, Utah State University, Logan, UT

Dinwoody Creek with Dinwoody Glacier in the background.

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Abstract
Rare invertebrates are difficult to study because of their small size and infrequent occurrences, and information is usually lacking to make informed management decisions. Little is often known about rare species and they are more likely to have status to protect them (e.g., Sensitive species, Endangered Species Act). The Threatened Western Glacier Stonefly (*Zapada glacier*) is known from two areas in Montana (Glacier National Park and Beartooth Mountains) and one area in Wyoming (Teton Range), but survey effort has been limited leading to a lack of distributional knowledge for the species. We surveyed alpine streams in Wyoming to see if the *Z. glacier* was in other mountain ranges. We sampled streams from a variety of sources (i.e., surface glacier, rock glacier, springs and snowmelt) for invertebrates and measured basic stream characteristics. We collected the genus *Zapada* in 60% of the streams we sampled and we discovered *Z. glacier* in 8 streams (Wind River Range, Absaroka Mountains and Beartooth Mountains). Invertebrate communities in the Wind River Range, Bighorn Mountains, Absaroka Mountains, Beartooth Mountains and Snowy Mountains largely overlapped sharing many similar taxa. Streams fed by rock glaciers had the widest range of invertebrate biomass and richness. Rock and surface glacier streams had the coldest water temperatures and rock glacier-fed streams had the highest specific conductivity. *Zapada* is a genus that is widespread in western North America, but the *Z. glacier* is a rare taxa that appears to be restricted to cold streams draining surface glaciers and rock glaciers, and is more widely distributed than previously known.

Introduction

Rare plants and animals are challenging to study because of their low densities and the infrequency at which they are observed (Lyons et al. 2005), yet we often lack crucial information about these species that is needed for management. Basic information about species, such as how long they live, how they reproduce, needs of the species, habitat requirements and threats, is needed to estimate their scarcity, range and population trends over time. Invertebrates are especially prone to a lack of basic knowledge likely because of their small size and often cryptic nature making casual observations less likely than vertebrates and plants. Additionally, the distribution of rare species can be underestimated because of their scarce nature. Surveys and studies dedicated to such species are needed to learn more about them. Rare species are critical to ecosystems and processes occurring within them by acting as prey, predators, decomposers, nutrient cyclers and much more (Lyons et al. 2005). Rare species have a higher risk of extinction because of their low densities (Flather and Sieg 2007) making them more likely to be petitioned for Endangered Species Act (ESA) listing; however, managing rare species and the ecosystems where they live requires basic knowledge of the species.

A changing climate affects most species on Earth, but temperatures are increasing faster at higher elevations and latitudes (Hansen et al. 2005, Pederson et al. 2010). Therefore, species living in mountain ecosystems and closer to the poles are experiencing more drastic temperature shifts. Whether species are aquatic or terrestrial, rising air temperatures can alter conditions. Warmer air temperatures are melting glaciers and permanent snowfields that are critical water supplies (Milner et al. 2017). Surface glaciers are predicted to disappear from several mountain ranges in the next century (Hall and Fagre 2003, Milner et al. 2017) which could have profound effects on many plants, animals and humans (Hotaling et al. 2017, Milner et al. 2017, McKernan et al. 2018, Frederikse et al. 2020). Aquatic species that live downstream of glaciers are predicted to lose habitat (e.g., Giersch et al. 2015) as streams become intermittent and cease flowing as the mass of ice is exhausted (Jacobsen et al. 2014a, Jacobsen et al. 2014b). Species that depend on the resources and conditions supplied by surface glaciers may be extirpated, but some appear to persist (Muhlfeld et al. 2020). In contrast, rock glaciers and the streams originating from them (icy seeps; Tronstad et al. 2020) are predicted to persist longer on the landscape compared to surface glaciers potential providing habitat for cold-adapted species (Anderson et al. 2018, Brighenti et al. 2021). Rock glaciers are buried ice covered with rock debris that insulates them from atmospheric temperatures.

The Western Glacier Stonefly (*Zapada glacier*) is a rare, cold-adapted insect known only from alpine streams in Glacier National Park and the Beartooth Mountains of Montana and the Teton Range in Wyoming. The species was described in Glacier National Park in 1971 (Baumann and Gaufin 1971), and was more recently discovered in the Beartooth Mountains, Montana and the Teton Range, Wyoming (Figure 1; Giersch et al. 2017, Hotaling et al. 2019b). *Zapada glacier* was listed as Threatened under the Endangered Species Act in 2019. The genus *Zapada* is widely distributed primarily in western North America with 10 valid species. Adults can be differentiated morphologically, but adults are difficult to collect due to the short, moving window when they emerge each year. Stoneflies spend most of their life as nymphs living in streams making this life stage the easiest to collect; however, nymphs cannot be identified to species using morphology, but DNA barcoding can be used to identify them. No surveys have been done to see if the *Z. glacier* is more widely distributed in other mountain ranges.

We surveyed alpine streams in four mountain ranges of Wyoming to see if the *Z. glacier* is more widely distributed. These insects are ≤6 mm in length and emerge as adults during a short window in late summer. We collected aquatic insects using Surber samples and hand-picking before their expected emergence period to identify new populations and estimate the effectiveness of each method for detection. We also measured stream conditions to estimate the types of habitats that *Z. glacier* inhabit. Results will be used to inform the recovery plan for the US Fish and Wildlife Service and to inform other management decisions.

Methods

We sampled sites that drained surface glaciers, permanent snowfields, springs and rock glaciers (Johnson 2018, Johnson et al. 2021) to detect *Z. glacier* outside of their known range mid-July through August in 2019 and 2021. *Zapada glacier* are known from cold (<7°C) waters sourced from surface glaciers, rock glaciers and springs in Glacier National Park, Montana, four streams in the Beartooth Mountains, Montana and the Teton Range, Wyoming, USA (Giersch et al. 2017, Tronstad et al. 2020). We visited alpine streams in the Absaroka Mountains, Beartooth Mountains, Wind River Range, Bighorn Mountains and Snowy Mountains in Wyoming (Figure 1). We categorized streams based on the source of water for each stream based on rock glacier (Johnson 2018, Johnson et al. 2021) and surface glacier locations, professional opinion, and observations while sampling.

We collected aquatic invertebrates at all sites to detect the presence of *Zapada* nymphs. We recorded location at each site using a GPS (Garmin Oregon 700). We collected benthic invertebrates, suspended sediments, and physical habitat stability. We collected three separate Surber samples (243 µm mesh) to quantify benthic invertebrates. Samples were elutriated in the field and preserved in ~80% ethanol. We separated samples into large (>2 mm) and small (250 µm to 2 mm) fractions in the laboratory. All invertebrates were picked and identified in the large fractions. If invertebrates were abundant (>~1000 individuals) in the small fraction, we subsampled using a record player (Waters 1969). Invertebrates were identified, counted and measured under a dissecting microscope using available keys (Thorp and Covich 2010, Merritt et al. 2019). We measured the first 20 individuals of each taxon in each sample and calculated biomass (mg/m^2) using length-mass regressions (Benke et al. 1999). Additionally, we hand sampled 18 streams in addition to Surber samples to estimate the best way to detect the genus *Zapada* and we compared which method most often detected their presence. We prepared up to 8 specimens of the genus *Zapada* from each stream they were collected following protocols from the Canadian Center for DNA Barcoding (CCDB). The cytochrome oxidase I gene was sequenced at CCDB for identification. Species identifications of *Zapada* were made by comparing sequences from our specimens to sequences from known specimens in the Barcode of Life Datasystem.

We collected a variety of chemical, physical and biological data at each stream to measure stream characteristics. We measured basic water quality including water temperature using a Yellow Springs Instrument Professional Plus, but some values are missing due to instrument failure. Dissolved oxygen was calibrated daily and specific conductivity and pH were calibrated at the trailhead. We estimated total suspended sediment (TSS) by filter ≤800 mL of water through an ashed type A/E glassfiber filter (PALL; 1 µm pore size). Filters were dried at 30°C for 2 days and measured on a balance (0.01 mg accuracy). We measured physical habitat stability using the modified Pfankuch Index (Ilg and Castella 2006, Peckarsky et al. 2014). We categorized streams into one of four categories – surface glacier-fed, snowmelt-fed, spring-fed or icy seep using the Pfankuch Index (PI), water temperature, specific conductivity (SPC) and TSS. Icy seeps were categorized by an obvious subterranean source, very cold summer temperatures (<4°C), and relatively stable streambed (PI = 15-24; Tronstad et al. 2020).

We investigated how stream characteristics varied among ranges and stream sources using mixed-effect models where stream was a random effect. We summarized data using the plyr package (Wickham 2011) and we used emmeans (Lenth 2021) to compared levels within a factor. No statistics are reported when models did not converge. We evaluated differences in community structure across streams and stream types using non-metric multidimensional scaling (NMDS) with the Vegan package (Oksanen et al. 2013). We removed rare taxa (either those private to a single site in the matrix or representing <0.1% of the total abundance without midges). Dimensionality of the final solutions was chosen as the number of axes that produced the lowest stress following 200 iterations.

Results

We sampled 44 streams in the Wind River Range (n = 13), Absaroka Mountains (n = 5) Beartooth Mountains (n = 7), Bighorn Mountains (n = 5) and Snowy Mountains (n = 14) in Wyoming. Insects composed 90% of the individuals we collected, and annelids, mites, crustaceans, mollusks and nematodes were much less dense in decreasing order. Of the insects, Diptera composed 83% of individuals followed by Plecoptera (5%), Ephemeroptera (4%), Trichoptera (2%), and Coleoptera (<1%). On average, we collected 3581 ind/m^2 (±368 SE;

Figure 2a, b). Similarly, 99% of the assemblage biomass belonged to insects. Diptera composed 48% of the insect biomass followed by Ephemeroptera (15%), Trichoptera (14%), Plecoptera (12%) and Coleoptera composed <1% of the biomass. On average, we collected 664 mg/m^2 (±69) of invertebrates across ranges (Figure 2c, d). Invertebrate density was most variable in springs, but densities did not differ among stream water sources (glmer, t = 0.07-1.6, p = 0.12-0.95; emmeans, p = 0.39-1.0; Figure 2b). In total, we identified 55 invertebrate taxa, we collected 10 taxa (±0.7) in each sample on average (Figure 2e, f) and we collected fewer taxa in streams originating from rock glaciers (glmer, t = 2.2-3.7, p = 0.0009-0.03; emmeans, p = 0.001-0.11; Figure 2f). The invertebrate assemblages in alpine streams in the four mountain ranges largely overlapped (Figure 3a). Dissimilarity ranks among mountain ranges overlapped with values within a range (R = 0.005, p = 0.43; Figure 3b). The invertebrate assemblages of the streams sourced from rock glaciers and springs largely overlapped, while the assemblages from surface glaciers and snowmelt streams also overlapped but had less diversity (Figure 3c). Dissimilarity ranks among stream sources also overlap considerably, but the ANOSIM R value was negative (R = -0.029) suggesting that dissimilarities are greater within the groups than between the groups (p = 0.67; Figure 3d).

We identified the genus *Zapada* (Nemouridae) in 59% of the streams we sampled and their densities ranged between 11 and 569 ind/m^2. We found *Zapada* in the Wind River Range (n = 6), Absaroka Mountains (n = 5), Beartooth Mountains (n = 8), Bighorn Mountains (n = 2) and Snowy Mountains (n = 10). We collected *Zapada* in streams originating from springs (n =10), rock glaciers (n = 8), snowmelt streams (n = 5) and surface glaciers (n = 3). When Surber samples and hand-picked samples were collected in the same streams, Surber samples detected *Zapada* in 100% of samples and hand-picking discovered *Zapada* in 50% of samples; therefore, Surber samples were superior at detecting *Zapada*. We collected six species of *Zapada* including *Z. glacier* plus potentially undescribed species (Table 1). We collected *Zapada glacier* in two streams in the Wind River Range (surface glacier-fed streams), 1 stream in the Beartooth Mountains and 5 streams in the Absaroka Mountains (rock glacier-fed streams; Figure 7). Their densities ranged between 11 and 215 ind/m^2.

Conditions in streams where we discovered *Z. glacier* supported evidence that they prefer cold and sometimes harsh environments. Algal biomass measured as chlorophyll a did not differ in streams where we detected *Z. glacier* compared to other streams sampled (glmer, t = -0.92, p = 0.36; Figure 4a). Seston concentrations also did not differ between streams where *Z. glacier* were present or not detected (glmer, t = -0.8, p = 0.40; Figure 4 c). *Zapada glacier* tended to be in streams with higher specific conductivity (glmer, t = 2.0, p = 0.05; Figure 5a) and in colder streams (<11°C; glmer, t = 0.3, p = 0.76). Our surveys targeted cold, alpine streams so temperatures in streams where they were discovered them did not differ from other streams we sampled (Figure 5c). pH (7.5-8.5) and dissolved oxygen concentrations (2.5-12.5 mg/L) in streams where we discovered *Z. glacier* did not differ from others we sampled. *Zapada glacier* lived in streams with low (very stable; rock glacier-fed streams) to highly (unstable; surface glacier-fed streams) Pfankuck Index values (Figure 6a). These stoneflies were detected in small streams (cross-sectional areas <50 cm^2) to very large streams (~300 cm^2; Figure 6c). Streams that *Z. glacier* inhabited had smaller (Figure 6e) and more uniform particle sizes (Figure 6g).

Stream conditions often varied by stream source. Periphyton biomass, estimated from chlorophyll a concentrations, did not differ among stream sources (glmer, t = 0.28-1.3, p = 0.18-

0.32; emmeans, p = 0.54-0.99; Figure 4b). Seston concentrations were higher in surface glacier streams compared to snowmelt (glmer, t = 2.0-4.8, p < 0.001-0.05; emmeans, p = 0.08; Figure 4d). Specific conductivity (glmer, t = 0.1-0.9, p = 0.37-0.87; emmeans, p = 0.64-1.0; Figure 5b) and water temperature did not differ among stream sources (glmer, t = 2.3-3.5, p = 0.38-1.7; emmeans, p = 0.38-0.98); however, surface and rock glaciers tended to be colder and snowmelt streams were warmer (Figure 5d). pH (glmer, t = 0.3-1.7, p = 0.09-0.73; emmeans, p = 0.31-0.99) and dissolved oxygen concentrations did not differ among stream sources (glmer, t = 0.27-1.9; emmeans, p = 0.28-0.99). Surface glaciers tended to have higher Pkankuck Index values (less stable), and rock glaciers and springs had lower values (more stable; glmer, t = 1.0-2.3, p = 0.02-0.3; emmeans, p = 0.09-0.1; Figure 6b). The stream cross-sectional area of surface glaciers ranged between small and large, (glmer, t = 0.4-2.1, p = 0.03-0.72; emmeans, p = 0.13-0.26; Figure 6d), but most other streams we sampled were smaller. Mean particle size was largest in streams fed by surface glaciers and the variance of mean particle size was smallest in rock glaciers.

Discussion

We found the genus *Zapada* in all four mountain ranges sampled, in 71% of the basins surveyed and they occur in all stream types. *Zapada* is a common genus in western North America. A study in Glacier National Park and the Teton Range discovered 7 species of *Zapada* (*Z. cinctipes, Z. columbiana**, Z, cordillera, Z. frigida, Z. glacier*, Z. haysi** *and Z. oregonensis**; asterisk indicates they occurred in Wyoming) in alpine streams and two potentially undescribed species (both occurred in Wyoming; Hotaling et al. 2019a). We discovered six species plus potentially undescribed species in Wyoming including *Z. glacier*. We collected *Z. glacier* in eight streams two of which were large, cold surface glacier-fed streams in the Wind River Range and six streams were rock glacier-fed streams in the Absaroka and Beartooth Mountains (Figure 8). *Zapada glacier* are known from the Beartooth Mountains of Montana, and we discovered them in streams across the border in Wyoming as well as farther south in the Absaroka Range and even farther south in the Wind River Range. *Zapada glacier* in the Wind River Range are the most southerly occurrences of this species (Figure 1). *Zapada glacier* appear to be distributed more widely than we previous knew and more surveys of alpine streams would likely reveal more locations; however, these stoneflies are rare and only occupy a portion of cold, alpine streams. Our surveys targeted streams where they may occur based on previous knowledge, but we detected them in <20% of the streams we sampled. Interestingly, we did not detect the genus *Lednia*, another neumourid stonefly tied to cold-water habitats, in any of the streams we sampled. More information is needed to understand what habitats *Z. glacier* select for beyond cold streams below surface and rock glaciers

Surber samples reliably detected *Zapada* in our streams. When we compared Surber samples and hand-picked samples from the same streams, we always detected *Zapada* in Surber samples, but we only detected *Zapada* in 50% of hand-picked samples. Hand-picked samples detected *Zapada* when individuals were more numerous and had larger individual body sizes; however, Surber samples were superior when *Zapada* had lower densities and smaller body sizes. We suggest using a quantitative sampling method when surveying small, cryptic invertebrates.

The invertebrate communities we sampled in each stream source largely overlapped. We noticed many of the same taxa in alpine streams across ranges and they were similar to the invertebrates found in the Teton Range (Tronstad et al. 2020). The invertebrate community in each stream source overlapped, but the polygons were different sizes. For example, the polygon representing the invertebrate assemblage from surface glacier-fed streams was much smaller compared to the other stream sources. We interpret the smaller polygon as a smaller, specific invertebrate community that lives in such streams. Streams originating from surface glaciers had low taxa richness and were often dominated Chironomidae. The analysis of similarity also showed that this stream source had much lower dissimilarity ranks compared to the other water sources. We recognize that a limitation of our study was estimating water sources for streams, which we did using observations at the site (e.g., visual, water quality, water temperature, etc.), expert opinion and mapping (Johnson 2018, Johnson et al. 2021), but we realize that streams can have multiple sources which can alter our results. For example, Heap Steap Creek was fed by a surface glacier and rock glacier, but we catagorized it as a surface glacier-fed stream. Streams in the Snowy Mountains were likely fed by springs and snowmelt, but we usually categorized them as spring-fed streams because they flowed all summer (Figure 7). We recognize that stream water sources occur along a gradient instead of being categorized as only one type.

Streams originating from different sources can be differentiated using specific conductivity, seston (total suspended solids), water temperature and the Pfankuck Index (Tronstad et al. 2020). These differences are critical because *Z. glacier* was found in surface and rock glacier-fed streams in our study and in the Teton Range (Tronstad et al. 2020), but also in spring-fed streams in Glacier National Park (Giersch et al. 2015, Giersch et al. 2017). Rock glaciers tend to release waters with higher specific conductivity likely because rock glaciers are subterranean and intermixed with rock debris (Brighenti et al. 2021). Streams originating from rock glaciers generally had the highest specific conductivity in our study. Rock glacier and surface glacier-fed streams have cold water. These streams had cold instantaneous water temperature in our study, but a better estimate of water temperature is to measure the temperature range over the summer (Tronstad et al. 2020). Streams draining from surface glaciers had the highest seston concentrations dominated by inorganic substrate. Median seston concentration was highest from surface glaciers in our study, but we measured higher concentrations in other stream types. Seston concentrations from surface glaciers in our study were lower than those measured in the Tetons (Tronstad et al. 2020), perhaps because the slopes of the streams were more gentle in our study. Finally, surface glacier-fed streams have lower stream stability (higher Pfankuck Index values) and rock glacier-fed and spring-fed stream are much more stable (lower Pfankuck Index values). Streams draining rock glaciers and springs had the lowest values in our study. *Zapada glacier* can live in streams originating from three different sources which can have contrasting conditions. Despite appearing to tolerate diverse conditions, these stoneflies only live in a handful of streams.

Surveys dedicated to an invertebrate of interest can collect valuable information about their distribution, life history and other basic information. Here, we surveyed for the *Z. glacier* and found the genus in over half of the streams we sampled and the target species in 18% of streams sampled. Our surveys that were tailored to detect *Z. glacier* were lucrative by discovering them in the Wind River Range and the Absaroka Mountains which were >108 km from known locations. We recommend further surveys to discover the distribution of this stonefly in Montana and Wyoming, especially in rock glacier-fed streams of western Montana, Yellowstone National Park and many other un-sampled streams in the Beartooth, Absaroka, and Wind River Mountains. We developed methods that can be used in high elevation terrain that are only accessible by backpacking. These methods could be used to sample for other alpine aquatic insects of interest. Information we gathered will inform the US Fish and Wildlife Service that this species is more widely distributed and add to the knowledge of what types of habitats these stoneflies live in; however, the *Z. glacier* is rare on the landscape only occupying a portion of what appeared to be suitable habitat.

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Table 1. Species of *Zapada* collected in the Absaroka-Beartooth, Wind River, Bighorn and Snowy Mountains of Wyoming.

Zapada species	Absaroka-Beartooth	Wind River	Bighorns	Snowy
Z. cinctipes		X		X
Z. oregonensis		X		X
Z. columbiana	X	X		
Z. glacier	X	X		
Z. haysi	X		X	
Z. oregonensis group B BG	X	X		X
Undescribed		X	X	X

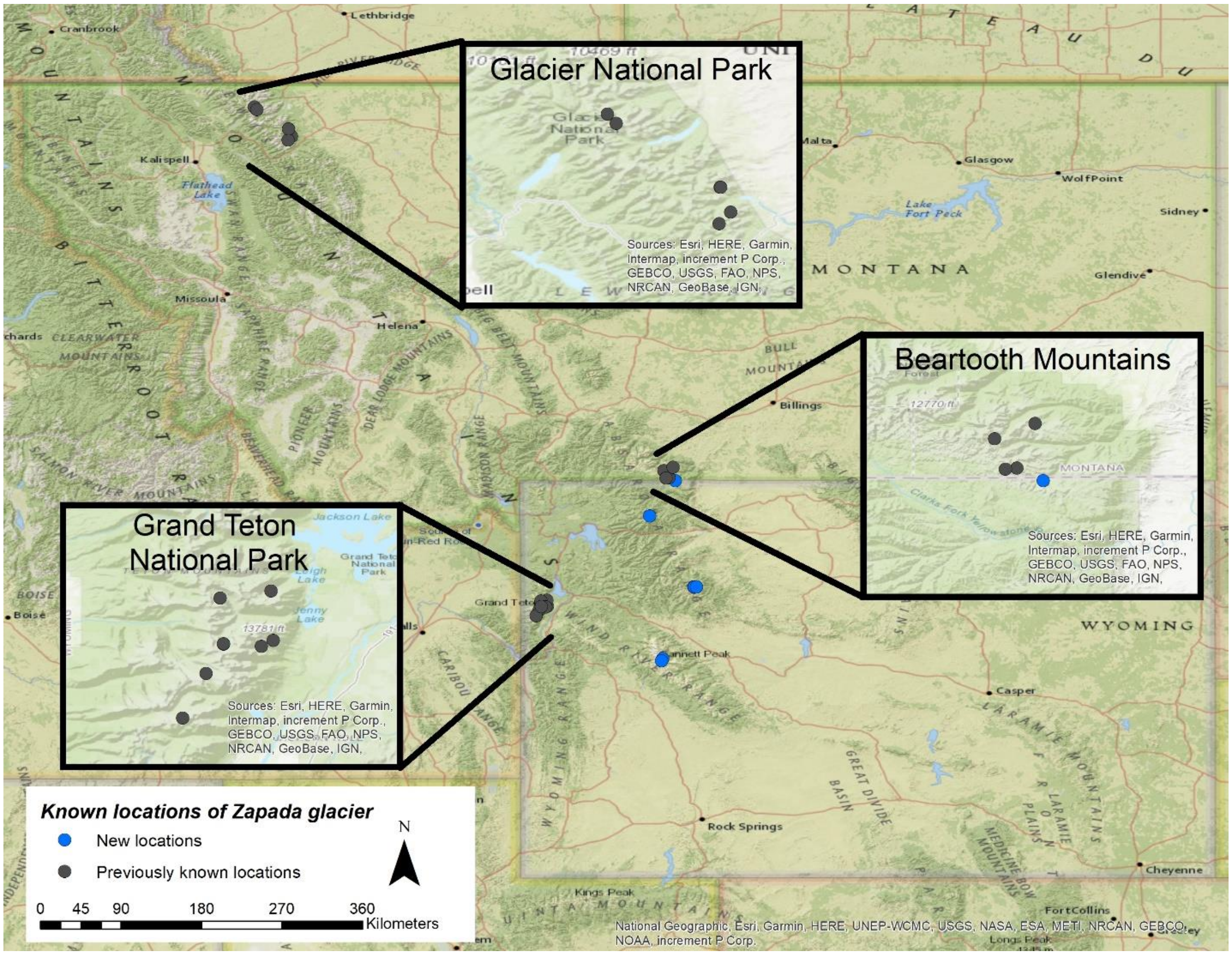

Figure 1. Previously known sites for Western Glacier Stonefly (*Zapada glacier*) in Montana and Wyoming and newly discovered streams in the current study. Scale bar is for the main map.

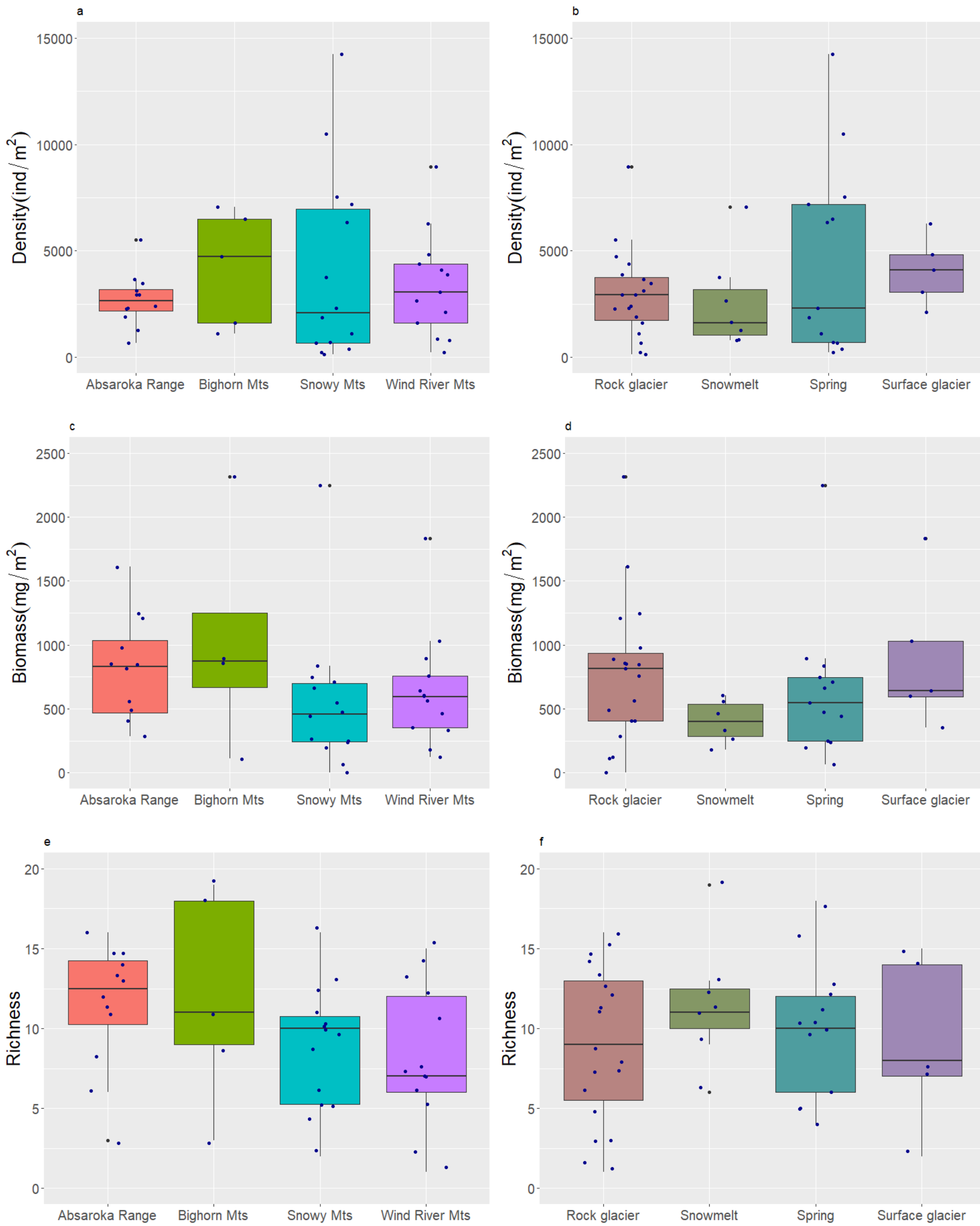

Figure 2. Density (a, b), biomass (c, d) and richness (e, f) of invertebrates in alpine streams by mountain range (a, c, e) and by stream source (b, d, f). The line is the median, the lower and upper edges of the box are the 25th and 75th percentiles, whiskers represent the minimum and

maximum values excluding outliers and points represent mean values from each stream (n = 3 Surber samples per stream).

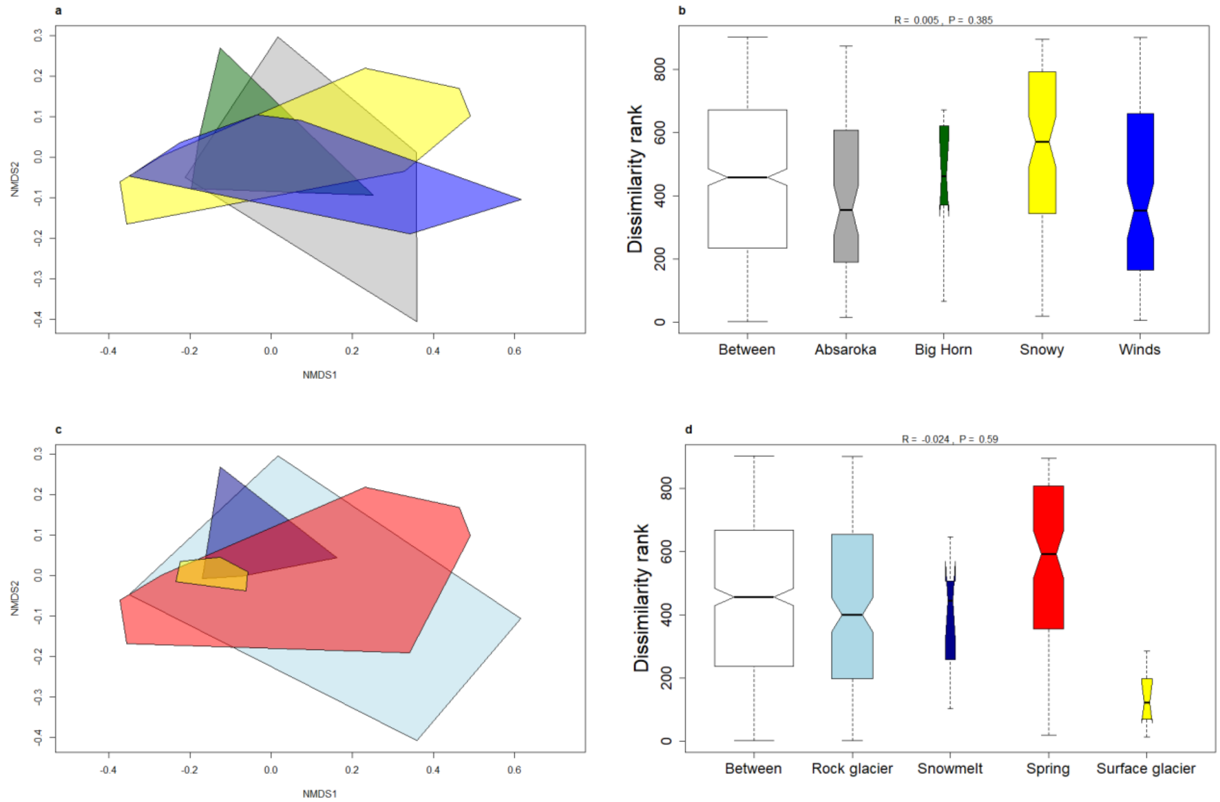

Figure 3. Non-metric multidimensional scaling (NMDS; a, c) and dissimilarity ranks (b, d) of invertebrate assemblages among mountain ranges (a, b) and stream sources (c, d). Refer to dissimilarity rank plots for NMDS color coding.

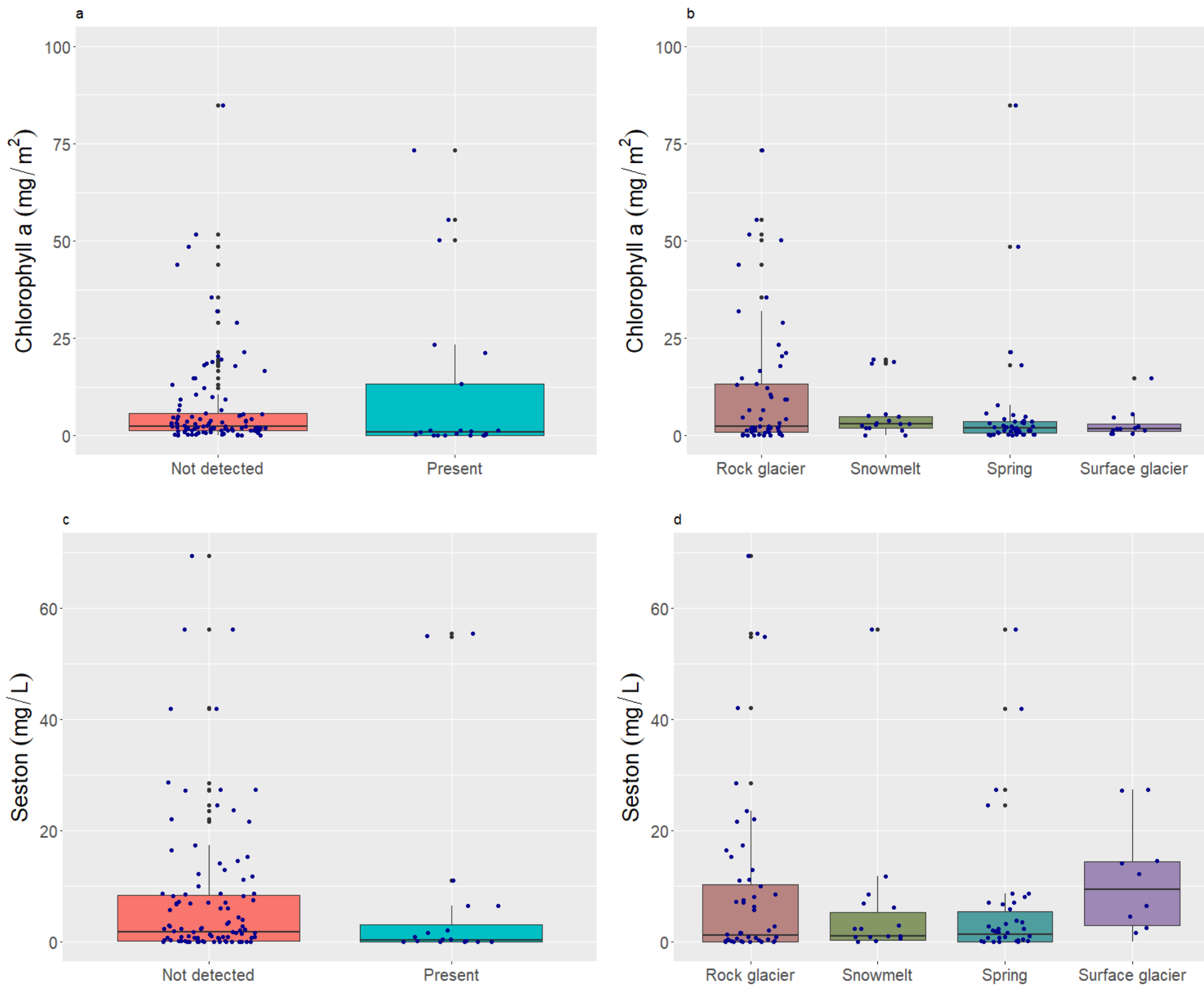

Figure 4. Periphyton biomass (a, b) and seston concentrations (c, d) in alpine streams where Western Glacier Stonefly (*Zapada glacier*) were and were not detected (a, c) and among stream sources (b, d). Lines are the median, lower and upper edges of the boxes are the 25th and 75th percentiles, whiskers are the minimum and maximum values excluding outliers, and points represent mean values from each stream (n = 3 samples per stream).

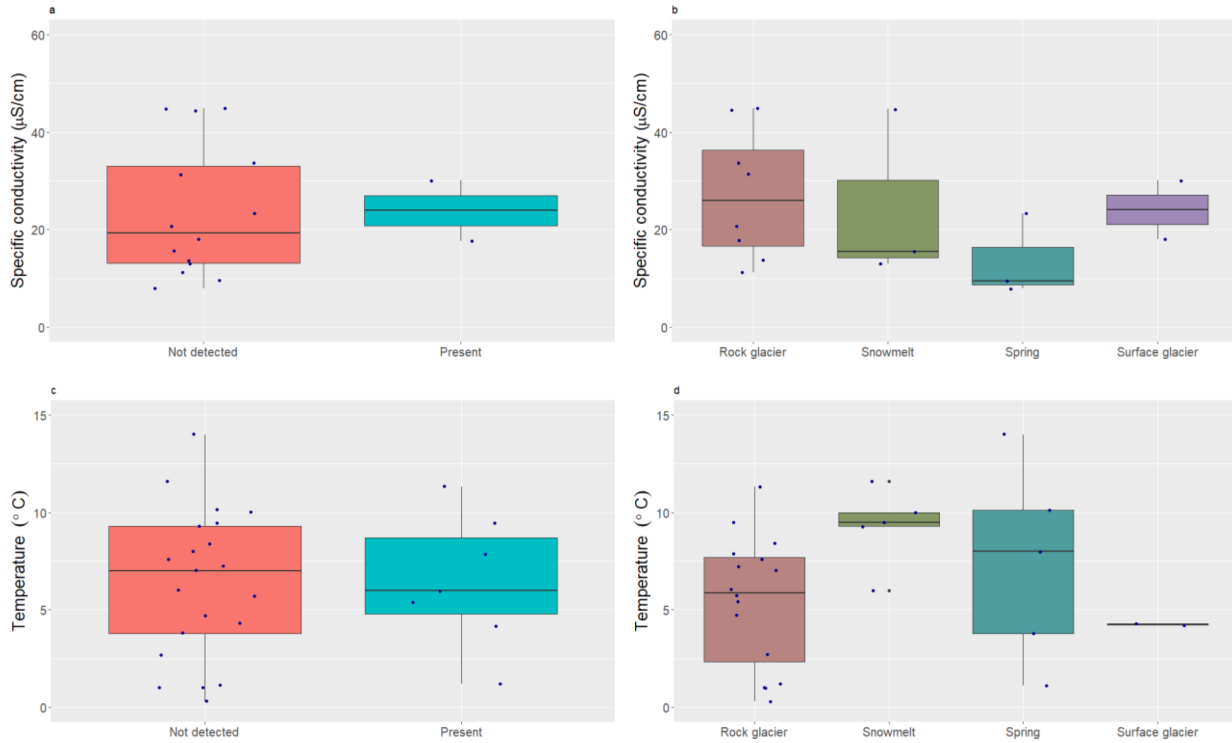

Figure 5. Specific conductivity (a, b) and water temperature (c, d) of alpine streams where Western Glacier Stonefly (*Zapada glacier*) was and was not detected (a, c) and by stream source (b, d). The line is the median, the lower and upper edges of the box are the 25th and 75th percentiles, whiskers represent the minimum and maximum values excluding outliers and points represent values from each stream. Our meter failed so we do not have data from all streams.

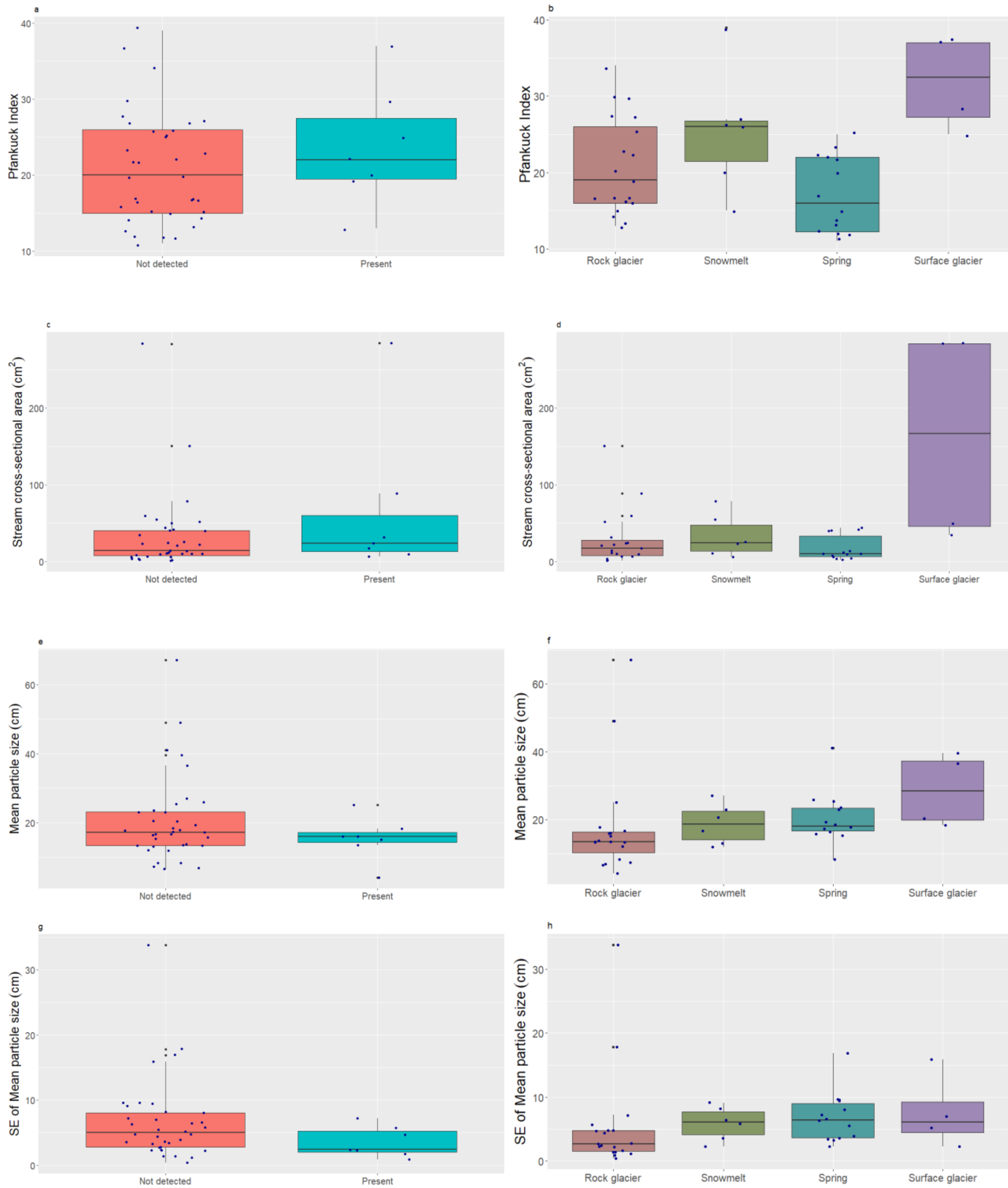

Figure 6. Pfankuck index (a, b), stream cross-sectional area (c, d), mean particle size (e, f) and the standard error (SE) of mean particle size (g, h) in alpine streams by mountain range (a, c, e, g) and by stream source (b, d, f, h). The line is the median, the lower and upper edges of the box are the 25th and 75th percentiles, whiskers represent the minimum and maximum values excluding outliers and points represent mean values from each stream.

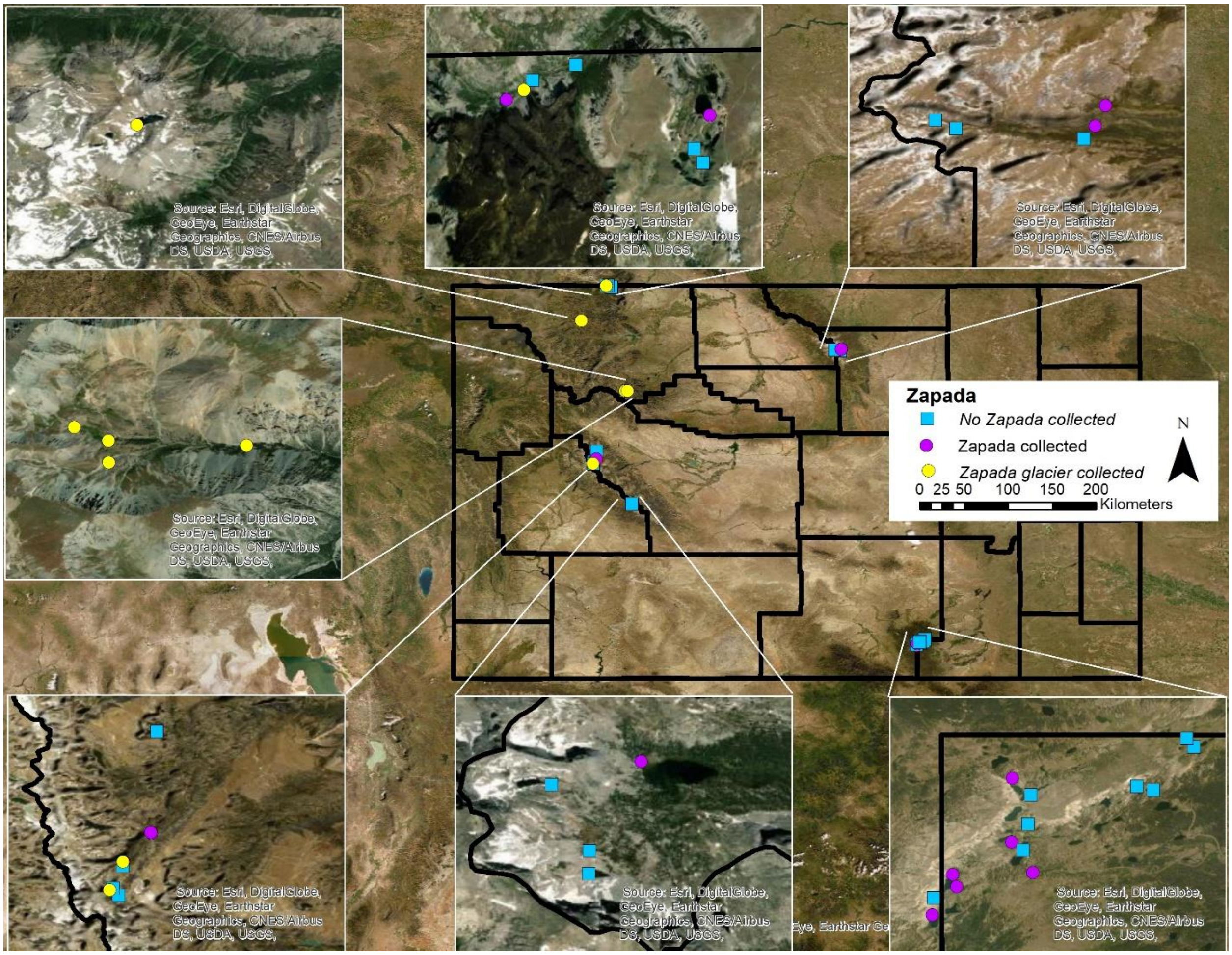

Figure 7. All alpine streams sampled in Wyoming (counties shown) including those where we did not detect stoneflies of interest, streams where we collected the genus *Zapada* and streams where we found *Zapada glacier*. Inset maps show details of sampling locations. Scale bar is for the main map.


a.
b.
c.
d.
e.
f.

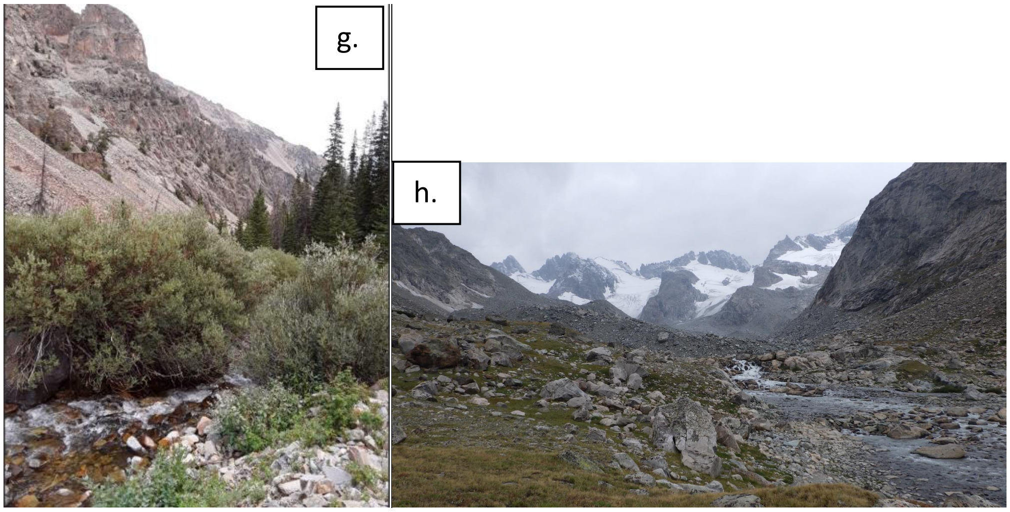

Figure 8. Photos of streams we collected *Zapada glacier*. Gannett Creek downstream from the surface glacier (a), Hiccup Creek (b), Dead Cabin Creek (c), Last Hour Creek (d), Cooper Lake Inlet (e), Tributary of Rock Creek (f), Meadow Creek (g) and Dinwoody Creek (h).