

1 **Headwaters fed by subterranean ice: potential climate refugia for mountain stream**
2 **communities?**

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23 **Running head:** Mountain stream climate refugia

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ABSTRACT

29 Near-term extirpations of macroinvertebrates are predicted for mountain streams worldwide as a
30 warming climate drives the recession of high-elevation ice and snow. However, hydrological
31 sources likely vary in their resistance to climate change and thus streams fed by more resistant
32 sources could persist as climate refugia for imperiled biota. In 2015-2016, we measured habitat
33 characteristics and quantified macroinvertebrate community structure along six alpine streams in
34 the Teton Range, Wyoming, USA. Strong differences in habitat characteristics (e.g., temperature,
35 bed stability, conductivity) confirmed three major stream sources: surface glaciers, perennial
36 snowfields, and subterranean ice. Subterranean ice-fed streams – termed “icy seeps” – appear
37 common in the Teton Range and elsewhere yet are globally understudied. Midges in the family
38 Chironomidae dominated our study sites, representing 78.6% of all specimens sampled, with
39 nematodes, caddisflies (*Neothremma*), and mayflies (*Epeorus*) also common. At the community-
40 scale, glacier- and snowmelt-fed streams differed significantly in multivariate space, with icy-
41 seep communities intermediate between them, incorporating components of both assemblages.
42 Because the thermal environment of subterranean ice, including rock glaciers, is decoupled
43 from large-scale climatic conditions, we predict that icy seeps will remain intact longer than
44 streams fed by surface ice and snow. Furthermore, our results suggest that icy seeps are suitable
45 habitat for many macroinvertebrates occupying streams fed by vulnerable hydrological sources.
46 Thus, icy seeps may act as key climate refugia for mountain stream biodiversity, an idea in need
47 of further investigation.

48

INTRODUCTION

49 The highest rates of climate change are occurring above the permanent treeline in alpine
50 and arctic ecosystems (Bradley et al. 2006). In the Rocky Mountains, warming is proceeding two
51 to three times more quickly than the global average (Hansen et al. 2005, Pederson et al. 2010),
52 resulting in extensive loss of glaciers and long-term snowpack (Hall & Fagre 2003). Streams fed
53 by permanent ice may exhibit short-term increases in flow as air temperatures rise and source ice
54 melting accelerates, but eventually they will shift to reduced flow and warm with the potential
55 for intermittency or drying permanently (Hotaling et al. 2017). As climate change proceeds,
56 invertebrate diversity at the mountain range scale is predicted to decrease due to both overall loss
57 of habitat and summit traps, where the highest-altitude species and communities have nowhere
58 left to disperse as warmer conditions and lower elevation communities shift upward. Biodiversity
59 loss will be compounded by the loss of specific aquatic habitat types, particularly the unique
60 conditions associated with meltwater from once-permanent hydrological sources like glaciers,
61 snowfields, or subterranean ice (Brown et al. 2007, Milner et al. 2009, Jacobsen et al. 2012, Finn
62 et al. 2013, 2014, Hotaling et al. 2017). Given that many alpine stream communities appear
63 uniquely adapted to cold thermal regimes (but see Hotaling et al. 2019c), they are likely to be
64 highly vulnerable to climate change as meltwater sources are lost (Giersch et al. 2017, Lencioni
65 2018). However, because alpine streams are heterogeneous with respect to hydrological source,
66 potential also exists to identify stream types that may be locally buffered from broad-scale
67 climate patterns and therefore could represent climate refugia for alpine stream biota (Morelli et
68 al. 2016).

69 A major, long-term focus in alpine stream biology has been understanding the links
70 between hydrological sources, the in-stream conditions they promote, and resident biotic

71 communities (Ward 1994, Hotaling et al. 2017). According to primary hydrological source
72 following Ward (1994), three types of alpine streams have historically been recognized: surface
73 glacier-fed, snowmelt-fed, and groundwater-fed streams. A fourth, understudied stream type also
74 exists – icy seeps – which are fed by subterranean ice (Hotaling et al. 2017, Hotaling et al.
75 2019a). In mountain ecosystems, the most common form of subterranean ice are rock glaciers,
76 masses of debris-covered ice that act as conveyor belts moving fallen rock and other debris
77 slowly downhill (Anderson et al. 2018; Jones et al. 2019). There may be more than 10,000 rock
78 glaciers in the western United States (Johnson 2018) and they are similarly common worldwide
79 (e.g., Lilleøren et al. 2011, Scotti et al. 2013, Charbonneau & Smith. 2018). In contrast, there are
80 ~1,250 surface glaciers and ~3,750 perennial snowfields in the western United States (Fountain
81 et al. 2017). Due to insulating debris cover, rock glaciers are largely decoupled from external
82 conditions (e.g., warm summer air temperatures; Clark et al. 1994, Anderson et al. 2018, Knight
83 et al. 2019), and should persist on the landscape longer than surface glaciers and snowfields. Icy
84 seeps, the outflow of rock glaciers and similar ice features, may therefore act as climate refugia
85 for cold-adapted stream biodiversity (Brighenti et al. 2019a,b; Hotaling et al. 2019a).

86 Like many mountain ranges worldwide, virtually nothing is known of alpine stream
87 ecology and biodiversity in the Teton Range, a granite-dominated subrange of the Rocky
88 Mountains. Previous studies of montane (2,000-3,000 m), but not alpine, streams revealed
89 considerable macroinvertebrate diversity in the region's higher elevation streams (Tronstad et al.
90 2016). Generally speaking, groundwater aquifers appear rare on the Teton Range massif, and
91 thus groundwater-fed streams are also rare (L.M.T., personal observation). Groundwater-fed
92 streams are considered the most resistant alpine stream type to warming because they are not
93 directly influenced by surface ice (e.g., Milner et al. 2009, Jacobsen et al. 2012). With a paucity

94 of groundwater-fed streams in the region, stream biodiversity in the Teton Range may be
95 especially vulnerable to climate change.

96 In this study, we addressed two major objectives. First, we made the first assessment of
97 alpine stream macroinvertebrate diversity in the Teton Range. Second, we explored associations
98 between primary hydrological sources (surface glaciers, snowfields, and subterranean ice) and
99 community structure. Specifically, we asked if benthic communities associated with icy seeps
100 have substantial taxonomic overlap with communities linked to sources more vulnerable to
101 climate change (e.g., glacier and snowfields). Our study provides an important first perspective
102 on an urgent need in freshwater ecology (see Brighenti et al., 2019a); testing whether an
103 underappreciated but globally common alpine stream type – icy seeps fed by subterranean ice –
104 may act as key refugia for mountain aquatic biodiversity threatened by global change. Our
105 results also provide new insight into the biodiversity of one of North America’s flagship
106 protected areas, Grand Teton National Park, and neighboring wilderness areas.

107

108 **MATERIALS AND METHODS**

109 **Study area**

110 During the summers of 2015 and 2016 (26 July-10 August), we sampled six streams in
111 the Teton Range of Grand Teton National Park and the adjacent Jedediah Smith Wilderness in
112 northwestern Wyoming, USA (Figure 1; Table 1). Study streams were selected to span the
113 breadth of alpine hydrological sources we have observed in the Teton Range and included two
114 streams fed by surface glaciers (‘glacier-fed’ hereafter), two fed by subterranean ice (‘icy seep’
115 hereafter) and two fed by permanent snowpack (‘snowmelt-fed’). In 2015, we sampled both
116 upstream (near the source) and downstream sites on each stream (Figure 1). On average, upper

117 sites were 111 m higher in elevation and 690 m in stream distance from lower sites (Table 1). In
118 2016, we re-sampled the upper sites with the same methods to assess inter-annual variability. We
119 focused on upper sites because they were as ‘true’ to primary hydrological source as possible
120 while lower sites inherently reflected various degrees of mixing among sources. In both years,
121 snow depth in the range was lower than average (152 cm in May, 1981-2010) with 2015 and
122 2016 at 63.3% and 80% of normal, respectively (Teton Pass, USDA SNOTEL).

123

124 Environmental data

125 At each site, we measured several environmental variables to characterize local habitat
126 and evaluate whether in-stream environmental conditions varied among stream types. We
127 measured water temperature for a full year (2015-2016) with *in situ* loggers (HOBO Pro v2,
128 Onset Computer Corporation) that recorded temperature hourly. We measured specific
129 conductivity (SPC), oxidation-reduction potential (ORP), pH, and dissolved oxygen (DO) with a
130 Yellow Springs Instrument (YSI) Professional Plus multiparameter sonde calibrated at the
131 trailhead (SPC, ORP, and pH) or at each site (DO). We estimated streambed stability with a
132 modified version of the Pfankuch Index (PI) following Peckarsky et al. (2014). Total suspended
133 sediments (TSS) were calculated by filtering known volumes of streamwater through pre-
134 weighed filters (PALL Type A/E glass fiber filters) and measuring dry mass to the 10^{-5} grams.
135 We used analysis of variance (ANOVA) statistical tests and the R package ‘plyr’ (R Core
136 Development Team 2017; Wickham 2011) to characterize differences in environmental variables
137 (Pfankuch Index, specific conductivity and total suspended solids) among stream types and
138 between study sites. When stream type was significant ($\alpha = 0.05$), we used Tukey’s HSD to

162 with ~80% ethanol. In the laboratory, invertebrate samples were divided into large (>2 mm
163 mesh) and small (between 250 μ m and 2 mm) fractions. For the large fraction, all invertebrates
164 were identified. The small fraction was subsampled using the record player method when
165 invertebrates were numerous (Waters 1969). Specimens were sorted, identified to the lowest
166 taxonomic level possible using keys in Merritt et al. (2008) and Thorp & Covich (2010), and
167 counted under a dissecting microscope. Insects were typically identified to genus when mature
168 specimens were present except Chironomidae which were classified as either Tanypodinae or
169 non-Tanypodinae. We estimated invertebrate density by summing the total number of individuals
170 for a given site and dividing by the area of streambed sampled. We calculated biomass by
171 measuring the length of the first 20 individuals of each taxon and then using length-mass
172 regressions to estimate individual biomass (Benke et al. 1999). We multiplied the mean
173 individual biomass for each taxon by the total number collected to estimate total biomass.

174

175 Biological data analysis

176 We used ANOVAs and Tukey's HSD tests performed on data summarized with 'plyr' (R
177 Core Development Team 2017; Wickham 2011) to characterize differences in invertebrate
178 density, biomass, and richness among stream types and study sites. To assess the relationship
179 between taxonomic richness or biomass with stream characteristics of interest, namely snow
180 cover ($S_{DURATION}$), temperature (T_{SUMMER}) and stability (Pfankuch Index), we performed both
181 Pearson and Spearman's rank-order correlations using the R package 'Hmisc' (Harrell Jr. 2013).
182 For correlation analyses, we focused exclusively on upper sites and averaged taxonomic richness
183 and biomass between 2015 and 2016.

184 We evaluated differences in community structure across streams, sites, and study years
185 using non-metric multidimensional scaling (NMS) with PC-ORD (McCune & Mefford 2006).
186 We $\log_{10}(n + 1)$ transformed density data for all taxa, removed rare taxa (either those private to a
187 single site in the matrix and/or representing $< 1\%$ of the total abundance), and used Sørensen's
188 dissimilarities to create distance matrices. We ran NMS analyses independently on two data
189 matrices: one including each of the upper and lower sites collected in 2015 only ($N = 12$ sites)
190 and the other including only the upper sites (sampled in both 2015 and 2016; $N = 12$ sites).
191 Dimensionality of the final solutions was chosen as the number of axes that produced the lowest
192 stress following 200 iterations. Following NMS we applied multi-response permutation
193 procedures (MRPP) in PC-ORD to assess whether there were differences in either community
194 structure and/or mean community distance within the following groups: upstream versus
195 downstream sites (2015 only) and among stream types for upper sites only (2015 and 2016). We
196 then used Indicator Species Analysis (ISA) in PC-ORD to assess whether any specific taxa in the
197 input matrices were indicative of (a) upper or lower sites or (b) a specific stream type. In ISA,
198 the indicator value (IV) for a taxon is the test statistic. The maximum IV is 100, which indicates
199 that a taxon is found in one group alone and is absent from other groups. We tested for
200 significance of all IVs using Monte Carlo randomizations with 15,000 permutations of the data
201 for each of the two tests (a and b above).

202

203

RESULTS

204

Environmental variation

205

Our upper sites clearly separated into three groups according to the glaciality index:

206

glacier-fed streams, snowmelt-fed streams, and icy seeps (Figure 2; Table 2). Glacier-fed streams

207 had less stable streambeds than both icy seeps and snowmelt-fed streams ($F = 19.9$, $df = 2$, $P =$
208 0.0001 ; Tukey's HSD, $P < 0.001$; Table 2). Annual temperature range (T_{RANGE}) was highest in
209 snowmelt-fed streams and lowest in icy seeps. Specific conductivity was highest in icy seeps
210 ($\text{SPC} > 100 \mu\text{S cm}^{-1}$ at upper sites; $F = 30.6$, $df = 2$, $P < 0.001$; Tukey's HSD, $P < 0.001$) and
211 lowest in glacier-fed streams (Table 2). More suspended solids (TSS) were present in glacier-fed
212 streams (mean = 0.157 g/L) compared to snowmelt-fed streams ($F = 3.6$, $df = 2$, $P = 0.44$;
213 Tukey's HSD, $P = 0.039$; Table 2). Summer temperatures (T_{SUMMER}) were lowest in glacier-fed
214 streams (mean = $1.6 \text{ }^\circ\text{C}$) and icy seeps (mean = $2.2 \text{ }^\circ\text{C}$), and higher in snowmelt-fed streams
215 (mean = $6.9 \text{ }^\circ\text{C}$; Table 2). Upper sites were on average $1.2 \text{ }^\circ\text{C}$ colder in the summer and had less
216 stable stream beds than lower sites (mean PI, upper = 27 ; mean PI, lower = 20.33 ; Table 1).
217 Other environmental variables (i.e., DO, pH, ORP, days under snow) did not vary among stream
218 types or between upper and lower sites (Table 2).

219

220 Biological variation

221 We collected 35 total invertebrate taxa of which 28 were insects (Supplementary
222 Appendix 1). Insects composed 95% of the total mean densities and 92% of the total biomass.
223 While invertebrate densities varied across stream types, biomass and richness were highest in
224 snowmelt-fed streams (Figure 3A). At upper sites, total macroinvertebrate density did not differ
225 among stream types ($F = 1.3$, $df = 2$, $P = 0.31$; Figure 3A), but biomass was $\sim 7\text{x}$ higher in
226 snowmelt-fed streams compared to glacier-fed streams and icy seeps ($F = 7.1$, $df = 2$, $P < 0.009$;
227 Tukey's HSD, $P \leq 0.02$; Figure 3B). Additionally, invertebrate richness was $\sim 2\text{x}$ higher in
228 snowmelt-fed streams than glacier-fed streams and icy seeps ($F = 10.4$, $df = 2$, $P < 0.002$,
229 Tukey's HSD, $P < 0.008$; Figure 3C). We observed generally higher and more variable

230 invertebrate densities ($F = 2.7$, $df = 1$, $P = 0.13$; Figure 3D) and higher biomass ($F = 2.6$, $df = 1$,
231 $P = 0.13$; Figure 3E) at lower sites. We also observed ~50% more taxa at lower sites ($F = 8.5$, df
232 $= 1$, $P = 0.012$; Figure 3F). Only T_{SUMMER} was significantly correlated with richness or biomass
233 (Richness: Pearson $r = 0.81$, $P = 0.002$; Biomass: Pearson $r = 0.63$, $P = 0.038$; Figure 4).
234 Spearman's rank-order correlations of the same relationships exhibited similar patterns, again
235 with only T_{SUMMER} exhibiting significant correlations with richness (Spearman's $r = 0.89$, $P =$
236 0.003) and biomass (Spearman's $r = 0.67$, $P = 0.023$).

237 The most common invertebrates in high-elevation Teton streams were non-Tanypodinae
238 midges (78.6% of all specimens sampled; >100 individuals/m² at all sites; Appendix 1) followed
239 by *Neothremma* (caddisflies), nematodes, and *Epeorus* (mayflies). Additionally, 45.7% of taxa
240 had densities >10 individual/m² among sites. Three taxa (Perlodidae, *Zapada* and Empididae)
241 were present at 67% of sites and twelve taxa (34.3%), including *L. tetonica*, were only found at a
242 single site (Appendix 1). Overall, we observed more stoneflies (6 taxa) than caddisflies (5 taxa)
243 and mayflies (4 taxa; Appendix 1). Only two taxa (midges and Collembola) were collected at
244 upper sites of both glacier-fed streams (Appendix 1). Two taxa were also present at both upper
245 icy seep sites (midges and the stonefly genus, *Zapada*) and four taxa were present in upper
246 snowmelt-fed sites [midges, stoneflies (Perlodidae), caddisflies (*Allomyia*), and flatworms
247 (Turbellaria)]. Of those, only Turbellaria were present at consistently high numbers (e.g., >20
248 individuals/m² per site; Appendix 1). No taxon exclusively occurred in one stream type and three
249 occurred in upper sites of all stream types (midges, *Allomyia* caddisflies, and *Helodon* black
250 flies; Appendix 1).

251 The most stable NMS solution comparing community structure between upper and lower
252 sites was three-dimensional (stress = 9.6) with the first two axes explaining 88% of the total

253 variation. Communities from the six lower sites overlapped substantially in ordination space with
254 those from the six upper sites (MRPP $A = 0.013$; $P = 0.27$). However, the mean pairwise
255 community distance was greater among upper versus lower sites (0.58 vs. 0.47), a trend that is
256 apparent in the NMS bi-plot (Figure 5A). Indicator species analyses revealed no taxon with IV
257 scores approaching the maximum of 100 (Tables S2-S3), an expected result when two
258 assemblages substantially overlap in ordination space. Mean IV across all taxa was 44.3, and
259 Empididae had the maximum IV (78.9; $P = 0.025$) to lower sites.

260 The NMS analysis that included upper sites only (2015 and 2016) converged on a two-
261 dimensional solution as the most stable result (stress = 8.9). The two axes explained 86% of the
262 variation, with axis-1 explaining 70% alone. In general, communities occupying glacier-fed
263 streams had the lowest axis-1 values, communities in snowmelt-fed streams had the highest axis-
264 1 values and icy seep communities had intermediate axis-1 values (Figure 5B). MRPP results
265 suggested that communities occupying these three stream types were significantly different from
266 one another ($A = 0.19$; $P = 0.006$). Pairwise differences were strong between glacier-fed and
267 snowmelt-fed communities ($A = 0.21$; $P = 0.005$) and were weaker but significant for the two
268 pairs that included icy seep communities (icy seep vs. glacier-fed: $A = 0.11$; $P = 0.05$; icy seep
269 vs. snowmelt: $A = 0.12$; $P = 0.03$). Indicator species analysis (Table S3) revealed IV scores >80
270 for three taxa, of which one was indicative of glacier-fed streams (Collembola; IV = 85.0; $P =$
271 0.02) and two were indicative of snowmelt streams (Tipulidae, IV = 82.1, $P = 0.004$; *Allomyia*,
272 IV = 81.5, $P = 0.004$). Turbellaria were also weakly indicative of snowmelt streams (IV = 72.5;
273 $P = 0.011$).

274

275

DISCUSSION

276 As climate change proceeds and mountain glaciers recede, there is a need to develop a
277 clearer understanding of how patterns of extant biodiversity and habitat heterogeneity are linked
278 in high-elevation ecosystems. In this study, we provide the first description of macroinvertebrate
279 diversity in the high Teton Range, Wyoming, where three major alpine stream types exist:
280 glacier-fed streams, snowmelt-fed streams, and icy seeps. To the best of our knowledge, no
281 streams fed by groundwater aquifers have been documented in the alpine zone of the granitic
282 Teton Range. From a global perspective, icy seeps, which are fed by subterranean ice (primarily
283 rock glaciers), rather than aquifers of liquid water, are of particular interest as they are likely to
284 persist on the landscape longer than surface ice features (Hotaling et al. 2019a). For the Teton
285 Range, a scarcity of high-elevation, groundwater-fed streams suggests that biodiversity in the
286 region may be even more reliant on meltwater than similar mountain regions (e.g., European
287 ranges, Brown et al. 2007; tropical Andes, Finn et al. 2016; Glacier National Park, Giersch et al.
288 2017). Thus, icy seep-associated climate refugia may be particularly important to Teton alpine
289 stream biodiversity as climate change proceeds. In the streams sampled for this study, glacier-
290 and snowmelt-fed streams exhibited significantly different invertebrate communities; however,
291 icy seeps were intermediate between the two in terms of community structure and invertebrate
292 density, biomass, and richness. These results suggest that icy seep communities share some
293 characteristics with both glacier- and snowmelt-fed streams and have the potential to act as
294 climate refugia for at least a subset of the unique communities present in each of the more
295 vulnerable stream types. Thus, the potential for icy seeps and ice-influenced terrestrial refugia
296 (Millar et al. 2015) to buffer climate-induced biodiversity loss has profound, global implications.

297 The recession of meltwater sources is predicted to strongly affect downstream
298 invertebrate communities (Jacobsen et al. 2012). In the near term, rising in-stream temperatures

299 are expected as ice melt comprises ever smaller proportions of stream flow. In alpine streams
300 worldwide, warmer conditions have been correlated with increased species richness for microbial
301 diversity (e.g., Wilhelm et al. 2013, Hotaling et al. 2019a), diatoms (Fell et al. 2018), and
302 macroinvertebrates (Finn & Poff 2005, Jacobsen et al. 2012). Our study adds another line of
303 evidence to this global pattern as we detected a positive correlation between species richness and
304 mean summer temperature (T_{SUMMER}). We also observed greater richness at lower (mean = 15
305 taxa) versus upper (mean = 10 taxa) sites which aligns with, and extends, the conclusions of
306 Tronstad et al. (2016), the only other study to investigate longitudinal patterns of
307 macroinvertebrate richness in montane streams of the Teton Range. Indeed, we observed far
308 fewer taxa at our highest elevation sites (10 taxa at ~3,150 m) versus the highest elevation sites
309 included in Tronstad et al. (2016): 26 taxa at ~2,700 m.

310 Although local (alpha) diversity will likely increase with warming water temperatures,
311 among-stream (beta) diversity may decrease as more diverse, generalist communities shift
312 upstream and specialized cold-adapted taxa are lost, effectively homogenizing biological
313 diversity at the regional scale (Jacobsen et al. 2012, Wilhelm et al. 2013, Hotaling et al. 2017). In
314 the Teton Range, we observed greater beta diversity at upper versus lower sites, and ISA
315 revealed indicator taxa for snowmelt and glacier-fed streams, but not for icy seeps. Collectively,
316 these patterns suggest that in the Teton Range, like elsewhere in the world, heterogeneous
317 hydrological sources bolster regional-scale alpine stream biodiversity. Icy seep communities also
318 appeared to bridge the taxonomic gap between glacier- and snowmelt-fed streams, and this
319 pattern remained stable between years (Figure 5B). While clear patterns of community
320 dissimilarity existed among our sites, ascribing a specific driver to these differences is difficult.
321 Many factors likely shape community structure in headwater streams (and our estimation of it),

322 including the primary source and its associated environmental regime, geographic location, and
323 both the method and timing of sampling (e.g., a site recently uncovered from seasonal snowpack
324 is likely to have a distinct community when compared to a site that melted out much earlier).

325 Our study represents the first perspective of macroinvertebrate biodiversity in the highest
326 elevation streams of the Teton Range. As such, there are many areas where future study will
327 expand, and refine, our conclusions. First, we did not have the resources to identify midges
328 (family Chironomidae) to lower taxonomic resolution. Midges are diverse in alpine streams
329 around the world (e.g., Montagna et al. 2016), and are typically the most common taxon (a
330 pattern we also observed). Thus, the limited taxonomic resolution of midges in our study may
331 have influenced our conclusions. For instance, Finn & Poff (2011) found 22 midge species in
332 snowmelt-fed alpine streams of the Colorado Rockies. Given our focus on three stream types, we
333 might expect even greater midge species diversity in the Teton Range. Second, our focus on a
334 limited number of sites, lack of within-year temporal sampling, and use of a single sampling
335 method/microhabitat (Surber samples from riffle habitats) rather than many approaches/habitats
336 (e.g., Ghani et al. 2016, Tronstad & Hotaling 2017), limits our ability to confidently describe the
337 full invertebrate community at a given site. We hope to fill these gaps in the future.

338 Ultimately, the degree to which alpine streams will be affected by climate change in
339 terms of flow magnitude and persistence remains largely unknown. In general, studies of alpine
340 stream ecology operate under the assumption that perennial flow will continue in the decades to
341 come (e.g., Jacobsen et al. 2012), but this may not be the case (e.g., Haldorsen & Heim 1999,
342 Herbst et al. 2019, Siebers et al. 2019). Thus, the biological ramifications of declining meltwater
343 sources in places like the Teton Range, where groundwater aquifer-fed streams are scarce, may
344 be even more profound than in ranges with a more equitable distribution of alpine stream sources

345 (e.g., Glacier National Park, Giersch et al. 2017). Indeed, if alpine streams supported by surface
346 glaciers and permanent snowfields transition to intermittency or dry completely, the future of
347 biodiversity in these ecosystems may depend almost exclusively on icy seeps. Our study paired
348 with the broader glaciological literature suggests there is room for optimism. Icy seeps have the
349 potential to span a wide beta diversity profile, perhaps bridging the taxonomic gap between
350 glacier- and snowmelt-fed communities (e.g., as observed for microbial diversity, Hotaling et al.
351 2019a). Moreover, rock glaciers and other subterranean ice forms (e.g., periglacial taluses, Millar
352 et al. 2013) are common in alpine regions worldwide and likely the most resistant ice form to
353 future warming. Clearly, research focused on subterranean ice sources and associated icy seeps,
354 and specifically the biological communities and hydrological flows they support, represent a
355 pressing need in alpine stream biology. We suggest that future studies incorporate temporal
356 monitoring of multiple alpine stream types, including icy seeps, to separate year-to-year variation
357 from true temporal signals. Long-term data will also allow for explicit tests of how biodiversity
358 and habitat characteristics may be altered among alpine stream types as climate change proceeds.
359

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368

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512

TABLES

513 Table 1. Key characteristics of our study streams and sites in the Teton Range, Wyoming, USA.

514 Distances to source (*D*) are cumulative. Both elevation and *D* are in meters.

Stream	Code	Site	Type	Lat., Long.	Elev.	<i>D</i>
Petersen Glacier	PG	Upper	Glacier-fed	43.782, -110.846	2,922	51
		Lower	Glacier-fed	43.785, -110.841	2,900	673
Middle Teton	MT	Upper	Glacier-fed	43.728, -110.795	2,955	178
		Lower	Glacier-fed	43.725, -110.790	2,802	780
South Cascade Creek	SCC	Upper	Icy seep	43.722, -110.838	3,152	165
		Lower	Icy seep	43.729, -110.837	2,943	1,051
Wind Cave	WC	Upper	Icy seep	43.666, -110.961	2,692	29
		Lower	Icy seep	43.667, -110.955	2,564	144
S. Fork Teton Creek	SFT	Upper	Snowmelt	43.691, -110.843	2,987	1,227
		Lower	Snowmelt	43.693, -110.859	2,881	2,757
N. Fork Teton Creek	NFT	Upper	Snowmelt	43.777, -110.860	2,955	9
		Lower	Snowmelt	43.775, -110.861	2,910	393

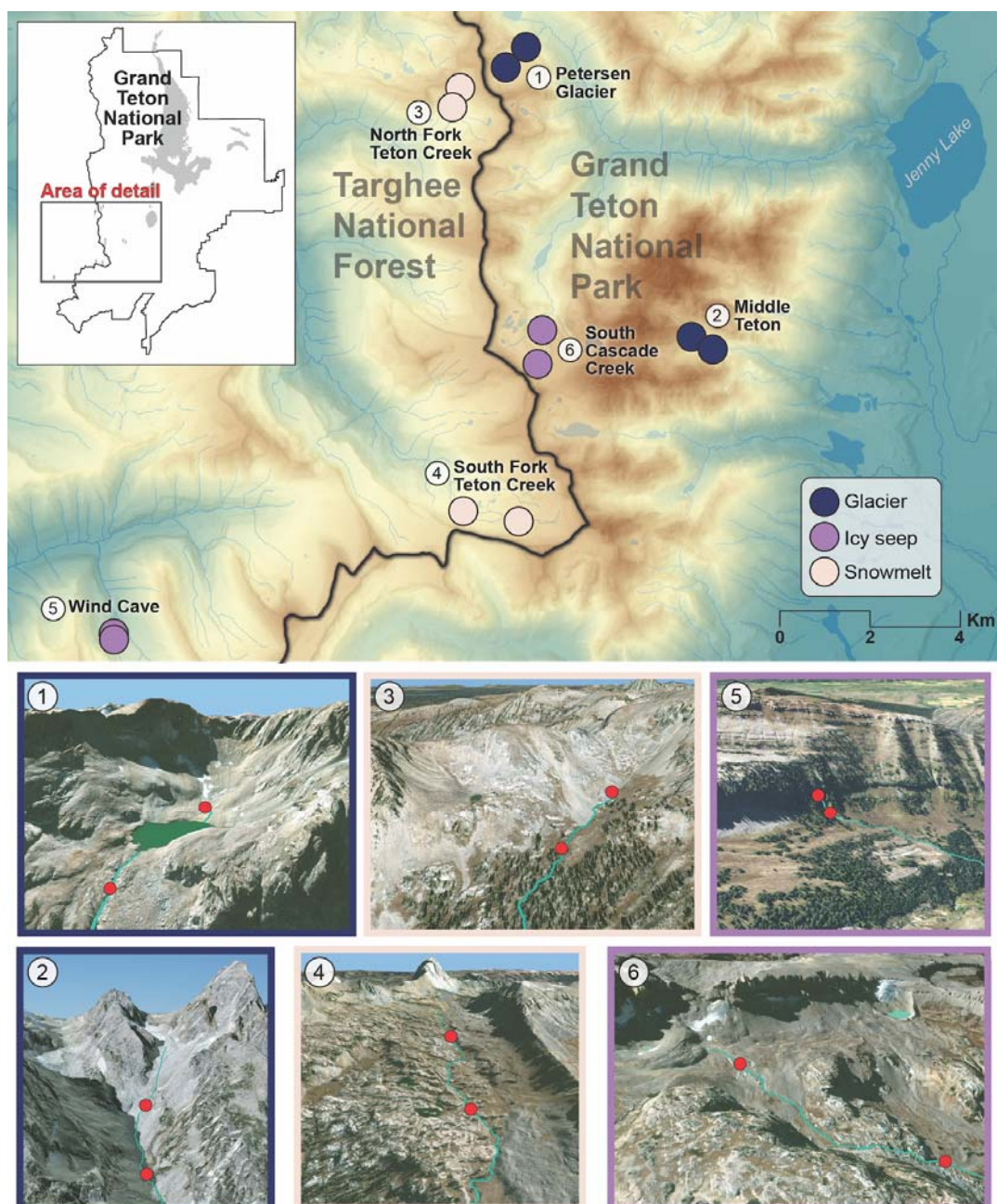
515

516 Table 2. Environmental characteristics of the streams and sites included in this study. T_{YEAR} :
 517 mean temperature for a calendar year. T_{SUM} : mean temperature between the summer solstice (21
 518 June) and autumn equinox (22 September). T_{RANGE} : the difference between the maximum and
 519 minimum temperatures recorded in the stream during the calendar year. S_{ON} and S_{OFF} indicate
 520 the day of the year when a site was covered (S_{ON}) or uncovered (S_{OFF}) by snow according to
 521 thermographs. S_{DURATION} (S_{DUR} below) is the approximate total number of days each site spent
 522 under snow in 2015-2016. Abbreviations and units: temperature (T; °C), dissolved oxygen (DO,
 523 percent saturation), specific conductivity (SPC; $\mu\text{S cm}^{-1}$), oxidation-reduction potential (ORP,
 524 mV), total suspended solids (TSS; g/L; upper sites only) and the Pfanckuch Index (PI; higher
 525 values correspond to a less stable streambed). All data (except for S_{ON} , S_{OFF} , and S_{DUR}) are for
 526 2015 only. Stream codes are provided in Table 1 and parentheses after the stream name indicate
 527 the stream type (GF: glacier-fed; SM: snowmelt-fed; IS: icy seep).

Stream	Site	T_{YEAR}	T_{SUM}	T_{RANGE}	S_{ON}	S_{OFF}	S_{DUR}	DO	SPC	pH	ORP	TSS	PI
PG (GF)	Upper	0.6	1.4	13.7	306	195	254	118	3.7	7.9	128.4	0.21	42
	Lower	1.9	6.0	16.2	303	128	190	103	9.9	8.6	24.8	--	23
MT (GF)	Upper	0.7	1.7	10.3	300	128	193	101	4.8	8.4	41.2	0.105	42
	Lower	1.5	4.1	13.6	296	150	219	100	5.7	8.9	62.9	--	30
SCC (IS)	Upper	0.5	1.5	10.0	298	204	271	104	113.2	8.5	-4.6	0.033	21
	Lower	1.5	4.6	13.0	297	155	223	104	110.3	8.4	58.4	--	16
WC (IS)	Upper	1.7	2.9	4.4	No cover			102	176.8	8.6	47	0.023	18
	Lower	1.9	4.2	7.3	309	98	154	99	175.2	8.4	77.2	--	18
SFT (SM)	Upper	1.9	7.1	17.7	300	174	239	101	74.3	8.6	-0.2	0.013	24
	Lower	Logger failed						101	114.7	8.5	23	--	17
NFT (SM)	Upper	2.5	6.6	15.2	300	125	190	101	11.1	8.5	64.9	0.024	15
	Lower	2.5	6.0	10.7	328	133	170	96	11.8	7.8	89.6	--	18

528

FIGURES



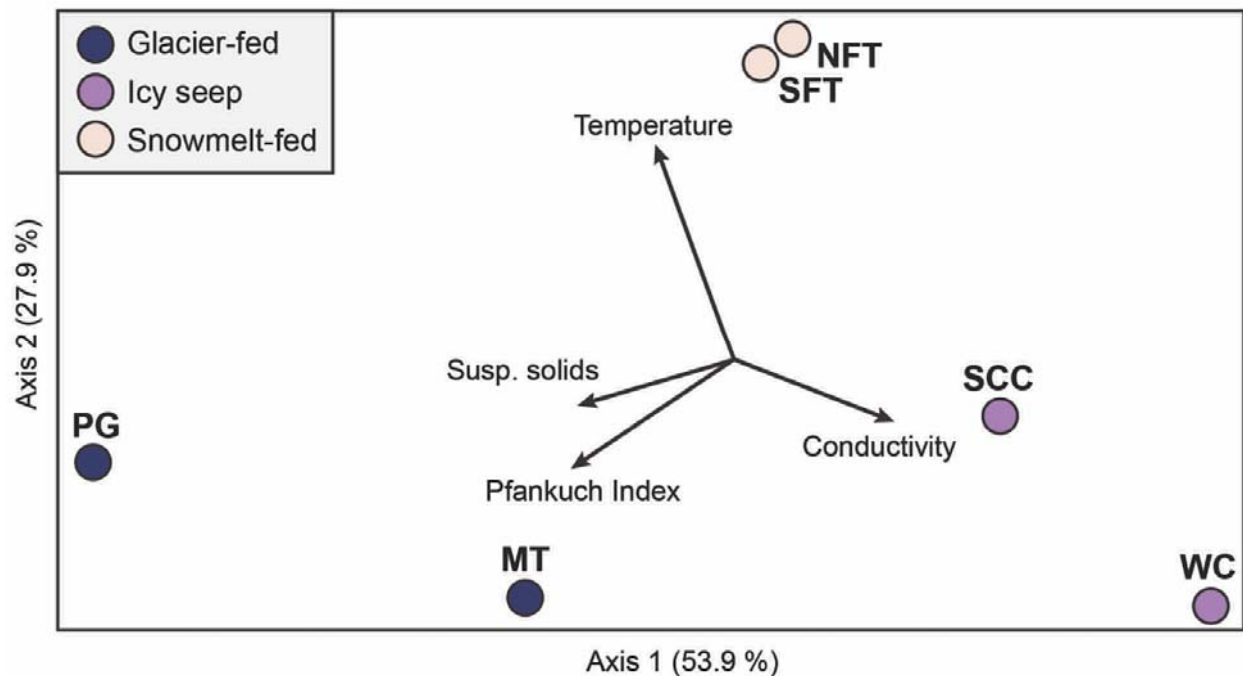
529

530 Figure 1. All streams and sites in the Teton Range, Wyoming, USA, included in this study.

531 Upper sites were selected to be as close to the primary hydrological source as possible. Numbers

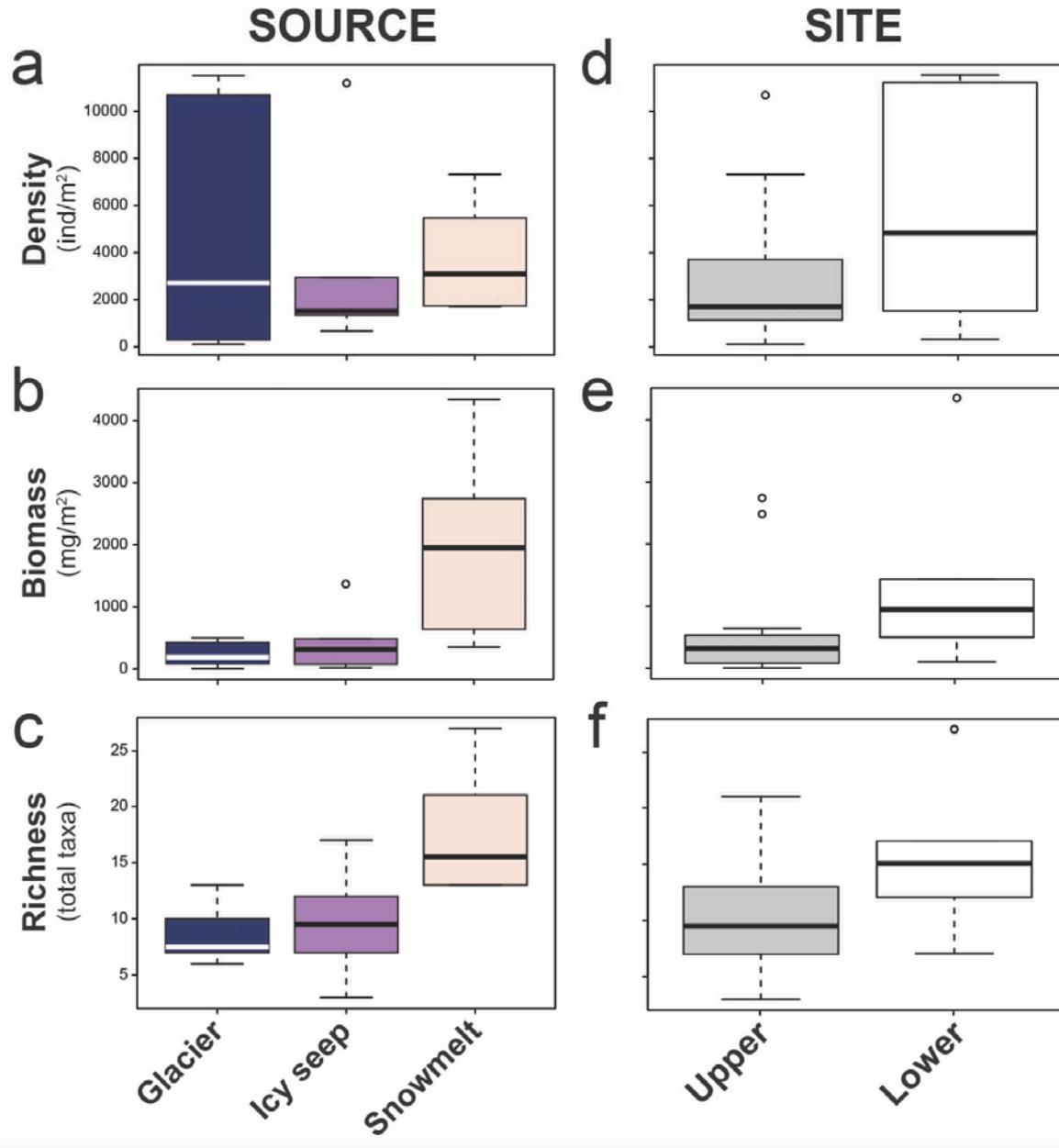
532 in the top panel correspond to satellite imagery (DigitalGlobe, 15 October 2015, bottom panel)

533 with stream channel (light blue) and sample sites (red dots) marked.



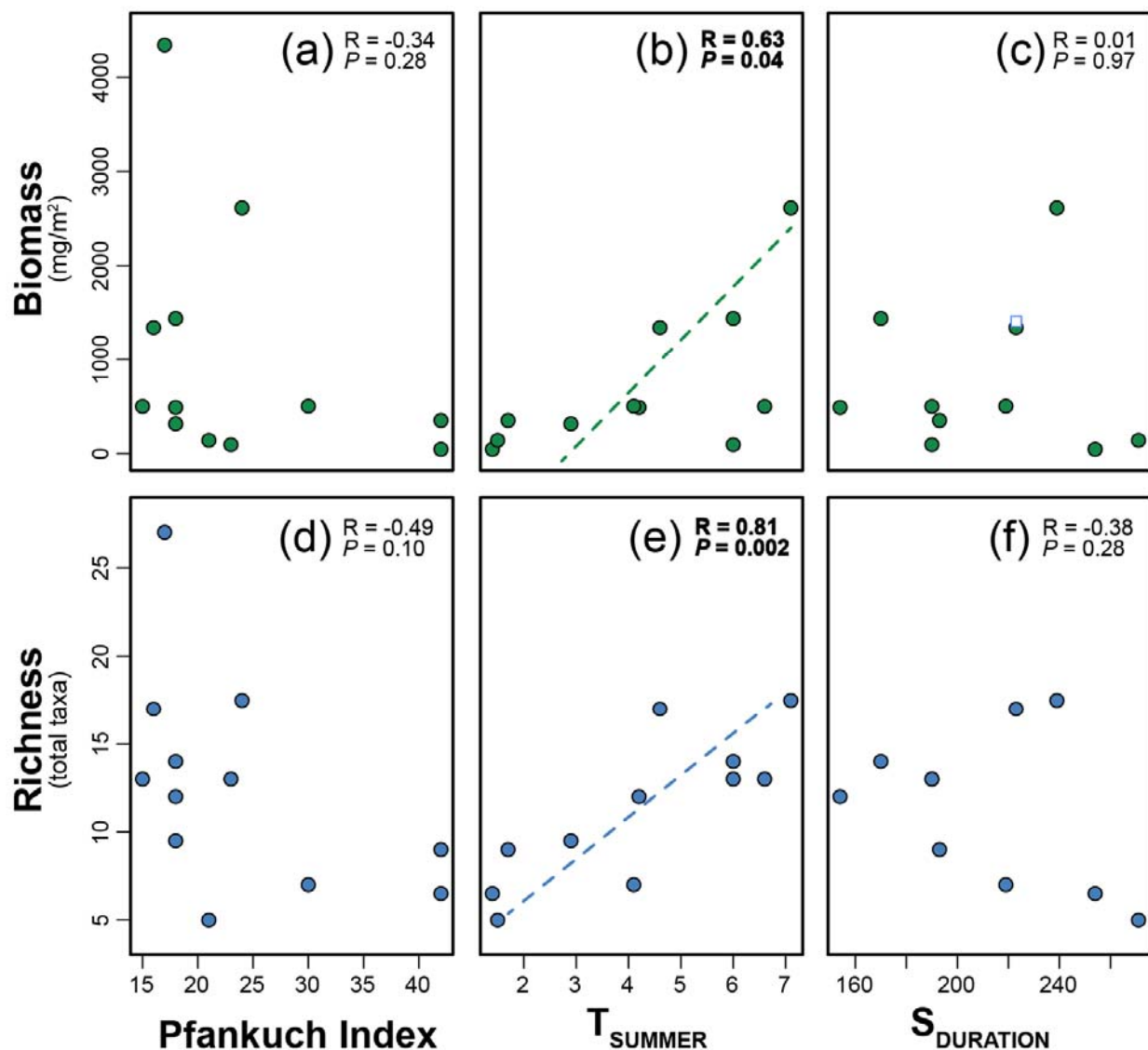
534

535 Figure 2. Principal components analysis (PCA) results showing environmental variation among
536 the upper sites according to the four variables of the glaciality index [specific conductivity,
537 streambed stability (Pfankuch Index), suspended solids, and temperature (T_{RANGE})].

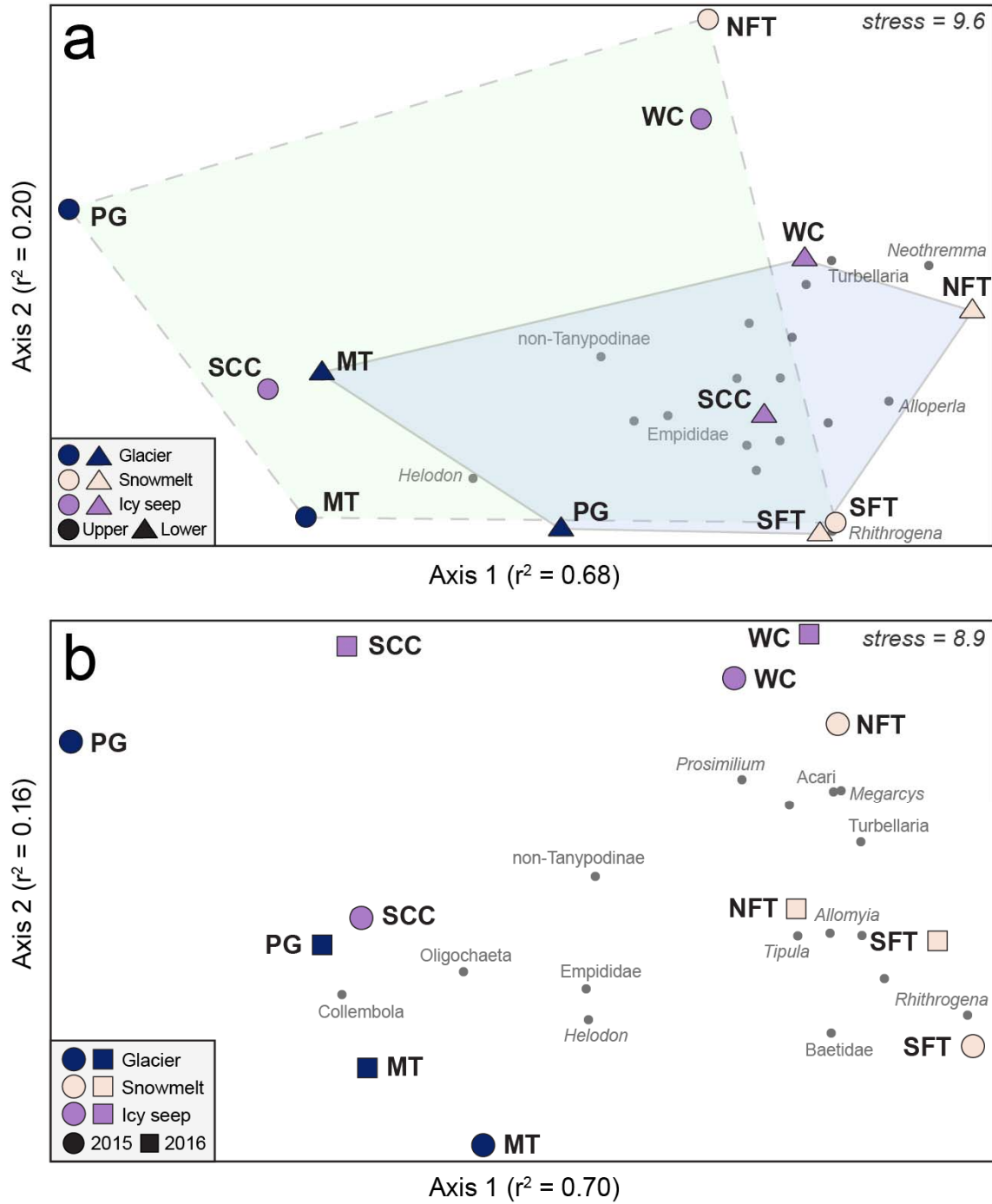


538

539 Figure 3. Macroinvertebrate density, biomass and richness among sites categorized by
540 hydrologic source (a-c; upper sites only in 2015 and 2016) or site (d-f; 2015 and 2106 data for
541 upper sites, 2015 data only for lower sites). Bold lines are median values, lower and upper box
542 limits indicate the 25th and 75th percentiles, respectively, and whiskers represent the lower and
543 upper limits of the data (excluding outliers which are shown as circles).



544
545 Figure 4. Bivariate plots of biomass (a-c) and richness (d-f) versus the Pfankuch Index, a
546 measure of stream bed stability (higher = less stable), S_{DURATION} (the approximate number of
547 days each stream was snow-covered in 2015-2016), and T_{SUMMER} (mean stream temperature
548 between the summer and autumn solstices). Significant Pearson correlations ($P < 0.05$) are in
549 bold with trendlines shown. The number of data points varies across plots due to a lack of snow
550 cover at upper Wind Cave during the study period and a failed temperature logger at lower South
551 Fork Teton Creek (see Table 2).



552

553 Figure 5. Non-metric multidimensional scaling (NMS) plots of macroinvertebrate communities

554 comparing (a) upper (circles) versus lower (triangles) sites in 2015 and (b) upper sites only

555 between 2015 (circles) and 2016 (squares). Colored polygons in (a) reflect the breadth of NMS

556 space occupied by upper (dashed lines, green fill) and lower (solid lines, blue fill) sites.