Environmental harshness mediates the quality of periphyton and chironomid body mass in alpine streams

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Abstract: Glacier retreat alters physical and chemical characteristics and biological communities of glacier-fed streams. Functional relationships, including effects of different food-source qualities on the density and biomass of primary consumers, are insufficiently known in alpine and subalpine headwaters. We measured: 1) the relative abundance of diatoms + chrysophytes, green algae, and cyanobacteria in the periphyton and assemblage patterns of the 2 dominant chironomid subfamilies, Diamesinae and Orthocladiinae, and 2) differences in chironomid body mass as an unexpected response to living conditions in harsh alpine stream ecosystems. Diatoms + chrysophytes dominated in harsh environments, and cyanobacteria were abundant in more benign streams. Patterns in algal dominance corresponded to differences in individual body mass of Diamesinae and Orthocladiinae among streams. Body mass of glacial-river specialists Diamesa steinboecki and Diamesa latitarsis gr. was higher in harsh than in benign streams, but other Diamesinae and Orthocladiinae biomass did not differ between harsh or less harsh conditions. Plausible explanations include higher nutritional quality of algal classes in periphyton and less competition for specialists in harsh than in benign streams. Our results indicate that harsh environmental conditions, such as low temperatures and high turbidity, lead to lower taxon numbers and food quality and that food quality influences invertebrate occurrence and body mass in alpine and subalpine headwater streams. Closely related species respond differently to specific environmental conditions and may perform differing functions. This result widens our general understanding of climate-change effects on alpine stream ecosystems and provides additional explanations for species abilities and performance.

Key words: Chironomidae, bottom-up effect, food quality, epilithon, alpine streams, BenthoTorch, glacier retreat, pigment analyses

Plasticity of body size and body mass with respect to environmental conditions has been observed in bacteria, protists, plants, and animals (Angilletta et al. 2004), and most species grow to larger adult size in colder environments. The intraspecific version of Bergmann’s rule, which states that body sizes of species tend to be larger in colder environments (Ashton 2004), also applies to ecototherms (Atkinson 1995), animals in which body temperatures closely follow ambient temperature. However, no single theory has been able to explain the general relationship of temperature and body size in ectothermal animals (Angilletta et al. 2004), so variables other than temperature also should be considered for disentangling this paradoxical relationship (Berrigan and Charnov 1994). Optimal study sites for such questions are environments with minimal human influences, except climate change, such as alpine streams.

Larvae of aquatic invertebrates colonize substrates of various water bodies and depend strongly on specific thermal conditions because they are ectotherms. The structure of invertebrate assemblages is stream-type specific (Hieber et al. 2001), according to the ecological tolerances of individual species (Niedrist and Füreder 2016), in different alpine-stream types, e.g., kryal and krenal streams with and without glacial influence (Ward 1994). These stream types are typically characterized by distinct environmental conditions, such as water temperature, discharge, and turbidity (e.g., Füreder et al. 2001). Living conditions in glacier-fed stream habitats are considered harsh (Clitherow et al. 2013) (e.g., cold water temperatures and highly variable turbidity, current, and discharge), whereas they become more benign when glaciers shrink (Milner et al. 2001). The timing of decreasing harshness depends on glacier size, elevation, and the rate of glacier
retreat. In the upper Rhône catchment in the central European Alps, for example, areal reduction of glaciers prevails during enhanced melt. Glacier thinning (Collins 2008) results in declining runoff in basins with intermediate-to-low glaciation (<60%), and living conditions are moderating in such catchments. In nonglacial fed streams, rising annual mean air temperature (Gobiet et al. 2014) will increase water temperatures (Kaulhal et al. 2010) with implications for the benthic communities.

Taxon richness and density are lower in glacier-fed than in less harsh nonglacial streams (Lods-Crozet et al. 2001a, Brown et al. 2010, Jacobsen and Dangles 2012). The few species able to persist in harsh sites possess physiological adaptations, as a study on heat shock proteins for freezing tolerance showed (Lencioni et al. 2015). Other investigators suggested that glacial stream specialists tend to be omnivorous or cannibalistic (Fürered et al. 2003, Clitherow et al. 2013, Niedrist and Fürered 2017a, 2018). Recent work in alpine streams has shown the effect of environmental stress gradients related to water-source dynamics on the structure of macroinvertebrate communities in terms of density, diversity, and species composition (Fürered 2007, Brown et al. 2010, Niedrist and Fürered 2016), but effects on the functions of communities and of single taxa are less known. Food availability and feeding relationships of invertebrates in these harsh environments are still poorly understood (Fürered et al. 2003, Clitherow et al. 2013, Niedrist and Fürered 2017b, 2018). Most benthic invertebrates in these headwater systems are primary consumers and an important feeding link between basal producers and higher trophic levels. In some cases, they are even important for fish species further downstream (Müller et al. 2009). Knowledge of such links is particularly important in terms of environmental change, which is likely to alter living conditions in alpine and subalpine streams considerably in the near future and, thus, will affect functional relationships of taxa (Niedrist and Fürered 2017b).

Despite the demonstrated qualitative relationships (who eats whom or what?) between grazing invertebrates and primary producers in alpine streams (e.g., Cummins and Klug 1979, Mihuc and Toetz 1996, Zah et al. 2001, Fürered et al. 2003, Niedrist and Fürered 2018), studies of potential bottom-up effects or quantifications of the relationship between compartments are scarce in such environments (Taylor et al. 2002). Many epilithic diatoms, several cyanobacteria, some green algae, and a few golden-brown (chrysophytes) and red algae (e.g., Saber et al. 2016) colonize benthic habitats in alpine (above the treeline) and subalpine streams (e.g., Hieber et al. 2001, Cantonati et al. 2006, Rott et al. 2006), and community composition and biomass vary between stream types (Rott et al. 2006) and seasons (Rott et al. 2000, Hieber et al. 2001).

According to the fatty-acid composition of microalgae in freshwater (Taipale et al. 2013) and their relative content of polyunsaturated fatty acids (Twining et al. 2016), the biochemical quality of epilithic groups in alpine streams might vary considerably (Guo et al. 2016a). Patterns of organismal groups within epilithic communities related to environmental change in alpine streams are important because shifts in the main groups (diatoms, green algae, and cyanobacteria) might result in changes of the nutritional quality of food available for grazers. This relationship is already known in lowland streams (Larson et al. 2013, Whorley and Wehr 2016), but information is missing for alpine streams. Biochemical food quality significantly influences growth and reproduction of lake zooplankton (Kilham et al. 1997, Koski et al. 1998, Burns et al. 2011, Pajk et al. 2012), benthic amphipods (Kainz et al. 2010), and invertebrates in lowland streams (Torres-Ruiz et al. 2007, Twining et al. 2016). For alpine streams, however, its role in invertebrate assemblage demography, body mass, and biomass availability has not been explored and the link between producers’ qualities and the performance of invertebrate consumers remains to be studied (Niedrist and Fürered 2017b).

Chironomidae are the dominant invertebrate group in alpine headwater streams (Fürered et al. 2001, Lods-Crozet et al. 2001b, Mülner et al. 2001, Niedrist and Fürered 2016). They are primary consumers of algae and process autochthonous (e.g., periphyton) and allochthonous (coarse particulate organic matter [CPOM] and detritus) products (Cummins and Klug 1979, Niedrist and Fürered 2017b, 2018). Thus, they are the first consumers along the alpine river continuum and an important food resource for other invertebrate species. Research from single watersheds of glacier-fed streams has illustrated patterns of longitudinal succession of chironomid subfamilies (Niedrist et al. 2017). Diamesinae usually dominate cold and dynamic headwater reaches, and are then accompanied and partially replaced by species of Orthocladiinae farther downstream (Fürered et al. 2001, Lods-Crozet et al. 2001b). Their biomass production will change as environmental conditions change in alpine environments. Longer growing seasons and increasing temperature will result in higher population densities and biomasses of invertebrates (Hogg and Williams 1996) with higher activity (Sweeney and Schnack 1977) and turnover ratio in production (Woodward et al. 2010).

The main ecological function of chironomids and other heterotrophs is to feed and, in turn, acquire biomass to produce offspring. However, biomass or body mass have barely been used as a community metric when assessing functional effects of ecosystem change on invertebrates in alpine streams (but see Hogg and Williams 1996 or Niedrist and Fürered 2018). Biomass as community metric is a promising and integrative indicator for an ecosystem service (Covich et al. 1999) because it is correlated with metabolism (Hirst et al. 2014) and depends on demography (Rudolf and Rasmussen 2013). Biomass varies considerably among invertebrate taxa (Nolte 1990, Benke et al. 1999) and cannot be inferred from density data. Thus, the ecological function of certain taxa to
provide biomass for invertebrate predators might be over- or underestimated when interpreting only density data and the question is whether it is beneficial to use biomass in addition to density for studies on alpine stream invertebrates.

Relationships of benthic invertebrates with the abiotic environment of glacier-fed stream systems have been studied along gradients of multiple environmental variables related to degree of glacial influence (Ilg and Castella 2006, Brown et al. 2010, Jacobsen and Dangles 2012, Khamis et al. 2016). Integrative proxies like glaciality index (Ilg and Castella 2006), glacial index (Jacobsen and Dangles 2012) or degree of meltwater contribution (Brown et al. 2010, Khamis et al. 2016) are used often to unite the most important abiotic variables influencing habitat and hence stream biota in glaciated alpine streams. We modeled a gradient of environmental harshness that combines the most important abiotic variables (water temperature, degree of glacial catchment, and sediment load) and separates sites based on differences in these variables. Thus, we can use a space-for-time substitution to estimate effects of expected environmental change (e.g., rising water temperature or reduced sediment load) on benthic producers and consumers in high-altitude glacial and nonglacial streams.

In the present study, we demonstrated potential bottom-up relationships and functional changes in consumer populations along a gradient of harshness. In stream reaches in 4 glaciated catchments in the Austrian Alps, we studied available food quantities and qualities of primary producers along a wide multifactorial gradient of environmental harshness, and we identified differences of invertebrate biomass and body mass of dominant chironomid subfamilies Diamesinae and Orthocladiinae. We hypothesized that (H1) the relative occurrence of algal and cyanobacterial classes that form the periphyton and the density and biomass of chironomids are affected by degree of environmental harshness. We further hypothesized that (H2) individual body mass of chironomids is related to the gradient of environmental harshness (Ilg and Castella 2006).

**METHODS**

**Study area**

Hohe Tauern National Park is in the central Austrian Alps in the Federal Provinces of Tyrol, Salzburg, and Carinthia. A freshwater monitoring program was implemented in 2009 in 4 glaciated catchments in the park (Füreder and Schöner 2013) to collect and analyze hydromorphic, physical, chemical, and biological data in glacier- and groundwater-fed stream reaches (Füreder et al. 2002). All stream reaches chosen for our study are remote, and direct anthropogenic influences are mostly absent. The monitoring design is a paired one, whereby a glacier- and a spring-fed reach above and below the treeline were chosen in each of the 4 catchments (Fig. 1A–C). The valleys are drained by single glacier-fed streams, so the kryal sites in each valley were longitudinally connected. In one of the catchments (Innergschlöß, Fig. 1C), the sites above the treeline are duplicated (i.e., 2 in Schlattenbach and 2 in Vriltenbach), resulting in 18 sampling reaches (9 glacial and 9 nonglacial) (Table 1). We selected sites from different stream types in terms of water source and degree of glacier cover (km²) so that the slightly different environmental conditions would form a gradient of environmental harshness (Füreder 2012), ranging from cold, dynamic, and turbid conditions in glacial streams to stable and clear habitats in nonglacial streams. We sampled benthic invertebrates, periphyton, and reach-scale environmental variables in a 10-d period in early July 2014, before peak summer glacial runoff.

**Chironomid community and organic matter**

At each site, we took 3 samples of aquatic invertebrates (54 total) with a Surber sampler (0.09 m², 100-μm mesh size). We randomly sampled different microhabitats focusing on 3 major substrates present in all of the riverbeds: mesolithal (cobbles, 6.33–20 cm), macrolithal (coarse cobbles, 20–40 cm), and megalithal (boulders, >40 cm grain size) (Blott and Pye 2012). We generally sampled before noon to avoid changing conditions resulting from alteration in discharge. We preserved all samples immediately in 75% ethanol. In the laboratory, we identified chironomids to species or species-group level with the aid of published and unpublished keys (e.g., Ferrarese and Rossaro 1981, Rossaro 1982, Schmid 1993) by mounting head capsules on slides in Eu- paral (CHROMA, Münster, Germany), to differentiate between main epilithic groups. BenthO-Torch™ (bbe Moldaenke, Germany), to differentiate between main epilithic groups. BenthO-Torch estimates community composition based on the in vivo fluorescence excitation spectra of cyanobacteria (pigment-group I), diatoms + chrysophytes (pigment-group II), and green algae (pigment-group III). These fluorescence spectra differ depending on the pigment.
composition of the primary producers (excitation at 470, 525, and 610 nm). Early versions of the BenthoTorch device had limitations (Kahlert and McKie 2014, Harris and Graham 2015) that arose from factors including thick and non-penetrable layers of primary producers or the presence of considerable amounts of red algae, which would be confused with cyanobacteria, in the system. In general, results of the BenthoTorch are not identical to those obtained by conventional microscopic analysis. For example, the BenthoTorch might also detect picocyanobacteria too small to be counted under the microscope or overestimate diatom contribution when chrysophytes are present in the system because both groups share the marker pigment fucoxanthin (Kahlert and McKie 2014, Harris and Graham 2015). However, this instrument still allows rapid, spatially resolved assessment of the total chlorophyll \( a \) (Chl \( a \)) concentration and a comparison of relative patterns of the main pigment groups within epilithic communities (e.g., Kamjunke et al. 2015, Mrowicki et al. 2016), especially in alpine streams, where red algae are rare (Rott et al. 2006) and algal layers are usually thin. Furthermore, the effect of reflection and periphyton thickness can be compensated with an additional light-emitting diode (700 nm). We analyzed 11 to 16 stones (diameter > 20 cm) in each stream (area = 0.78 cm\(^2\), 1 measurement/stone) without physically disturbing the periphyton.

Stream environmental characterization

We monitored water temperature continuously with digital loggers (Tidbit; Onset, Bourne, Massachusetts) throughout the year (every 30 min). We summarized temperature data over a 1-mo period before the sampling date and expressed it as the mean maximum daily water temperature (max\( T \)). Before collecting invertebrates, we collected and filtered a 1- to 2-L water sample through precombusted Whatman GF/C filters. In the laboratory, we dried, combusted, and weighed the filters to assess the concentration of suspended sediment. We estimated discharge through depth/velocity transects at each site with a vane wheel probe (ZS25; Höntzsch, Waiblingen, Germany). Subsequently, we modeled the inorganic sediment load (sediment load in mg/s; also called suspended sediment flux) as the combination of the concentration of suspended sediment (mg/L) and discharge (L/s). The degree of glaciation (glaciation) of the catchment was assessed using ArcVIEW (version 10.1; Environmental Systems Research Institute, Redlands, California) and ex-
pressed as the ratio of glaciated and total catchment area (% glaciated catchment).

Data analysis

Environmental harshness differed among all stream sites. To illustrate its potential effect on the biota, we calculated a multifactorial harshness gradient using maxT, sediment load, and glaciation, which provides a 1-dimensional gradient of environmental harshness across glacial and nonglacial alpine and subalpine streams, similar to the glaciality index (Brown et al. 2010). We selected maxT, glaciation, and sediment load as environmental factors because they have a strong influence on chironomids (Füreder et al. 2001, Milner et al. 2001, Niedrist and Füreder 2016). We log(x)-transformed the variables glaciation and sediment load because of skewed distributions before standardizing from 0 to 1. As the final gradient of environmental harshness, we used the component 1 scores from a noncentered principal components analysis (nPCA) of all studied streams divided by –1 (to provide a gradient with increasing values equal increasing harshness), similar to the approach of Ilg and Castella (2006).

We applied nonmetric multidimensional scaling (NMDS) of mean chironomid community structure at each stream to visualize the qualitative differences of the chironomid community structure. We used the enfit function and a permutation test (999 permutations) to obtain the p-value of the correlation between harshness and chironomid community structure. The analysis was based on log(x + 1)-transformed mean chironomid densities of replicate samples and the Bray–Curtis dissimilarity index.

To quantify trends of chironomid assemblages along this gradient, we used the following biotic indices: 1) taxonomic richness (number of chironomid taxa/sample), 2) density (number of chironomids/m²), 3) Shannon’s index of chironomid diversity, and 4) total biomass and mean individual body mass of Chironomidae, Diamesinae, and Orthocladiinae. Total biomass data were log(x)-transformed prior to statistical analysis. We used ranged major axis regressions (RMA, type II regression) with 99 permutations to describe the relationship between biomass and density.
environmental harshness to provide a demographic overview of the populations. We calculated the mean individual body mass of the chironomid larvae per site as the mean body mass of all individual larvae (Diamesinae or Orthocladiinae) per sample. We illustrated relative patterns of phytobenthic groups along the gradient of environmental harshness as the relative contribution of total Chl $a$ concentration (%) of each group. We used generalized additive models (GAMs) and simple linear models to describe and test significant relationships among biotic indices, algal groups, and the degree of environmental harshness based on mean data from each site to avoid spatial dependency of the replicate samples and measurements. For each analysis, we performed a regression diagnosis to avoid violations of model assumptions. Analyses were done in R (version 3.1.2; R Project for Statistical Computing, Vienna, Austria), with vegan (Oksanen et al. 2017), mgcv (Wood 2008), and lmodel2 packages (Legendre 2013).

**RESULTS**

**Stream habitats along the gradient of environmental harshness**

Principal component (PC) 1 of the nPCA (71% of cumulative variance) predominantly reflected the environmental variation among sites (Fig. 2). The explanatory power of PC 2 and 3 was considerably lower (21 and 8%, respectively; Fig. S1). Sediment load, glaciation, and maxT contributed primarily to PC 1 (Table 3). PC 1 was positively associated with maxT and negatively associated with sediment load and degree of glaciation (Table 3). All abiotic variables were significantly related to environmental harshness (linear models, $F > 20$, $p < 0.001$).

The gradient of environmental harshness (derived from the values of PC 1 × −1) ranged from −1.80 to 3.08. Higher gradient values (harsher environments) were based on lower water temperatures, higher sediment loads, and higher glaciation in the catchment (Fig. 2). The sites were normally distributed along this relatively wide gradient (Shapiro–Wilk normality test, $p = 0.12$). In general, glacial stream sites with high turbidity (sites 15, 16, 17, 18) showed the highest degree of environmental harshness. Streams fed by smaller glaciers (sites 8, 9, 10, 12, 14, and 15) and nonglacial streams (sites 2, 5, 7, and 11) had intermediate harshness regardless of elevation. The least harsh conditions were found at sites 1, 3, 4, 6, and 13, which were principally nonglacial, subalpine streams with very stable riverbeds (GN, personal observation) and clear nonglacial alpine streams. Among all sites, the mean maximum water temperature ranged from 1.42 to 13.08°C and transported sediment from 0.03 to 3559.01 mg/s.

**Patterns of epilithic pigment groups along environmental harshness**

Total periphyton abundance (Chl $a$) was not significantly related to environmental harshness. However, the relative contribution of the different pigment groups to total Chl $a$ concentration changed significantly along that gradient (Fig. 3A–C). The relative contribution of pigment-group I (cyanobacteria) increased as harshness increased ($F = 5.4$, $p < 0.05$; Fig. 3A), whereas the relative contribution of pigment-group II (mainly diatoms + chrysophytes) was highest in the harshest environments and decreased significantly when conditions improved (linear model: $F = 8.6$, $p < 0.01$; Fig. 3B). Relative contribution of pigment-group III (green algae) showed no significant response along the harshness gradient (Fig. 3C). Thus, pigment-group II dominated in harsh streams, whereas pigment-group I prevailed in more benign habitats.

**Chironomid assemblage and taxa distribution along environmental harshness**

A total of 18,795 chironomid larvae representing 64 different taxa were identified. The structure of chironomid
communities was mainly ordinated along 1 direction in the 2-dimensional solution of the NMDS (Fig. 4A, B). A smoothed fit for environmental harshness indicated that the variation of community structure followed the harshness gradient along the NMDS axis 1 (Fig. 4A). Assemblages from harsh sites (e.g., sites 17 and 18) generally had negative scores on NMDS axis 1. Those from intermediate sites had neutral scores, whereas communities from benign stream habitats assembled on the positive side of that axis. Taxa of the genus Diamesa (*D. steinboecki*, 2 morphotypes of *D. latitarsis* group (A and B), *D. cimerella* gr., *D. zernyi* gr.) dominated in harsh environments (bold in Fig. 4B), whereas more benign sites also were inhabited by other genera, such as *Eukiefferiella*, *Orthocladius*, *Paratriechocladius*, *Thienemanniella*, or *Tvetenia* (Fig. 4B). Taxon richness and harshness were strongly negatively related (*F* = 19.5, *p* < 0.001, *R*² = 0.55). Overall, taxon richness was lowest at site 18 (on average 11.3 ± 2.4 taxa) and highest at site 6 (26.3 ± 1.3 taxa).

**Density vs biomass and individual body mass along a harshness gradient**

Density of chironomids ranged from 89 (site 8) to 26,056 ind/m² (site 6) and was generally negatively related to harshness (*F* = 4.15, *p* < 0.05, *R*² = 0.21; Fig. 5A). However, the same relationship was not detected for chironomid biomass (Fig. 5B). Chironomid subfamilies, Diamesinae and Orthocladiinae, had opposite density and biomass patterns along the harshness gradient (Figs 5C, D, S2A, B).

The density of Diamesinae was not significantly related to harshness, but the density of Orthocladiinae clearly decreased with reduced harshness (*F* = 50.95, *p* < 0.001, *R*² = 0.76; Fig. S2A, B). Density and biomass of each subfamily (cf. Figs 5C, D and S2A, B) were similarly related to the harshness gradient, but they indicated different trends when Chironomidae was considered as a family (Fig. 5A, B). Density significantly decreased with increasing harshness (*F* = 4.15, *p* < 0.05, *R*² = 0.21; Fig. 5A), but we found no evidence for a decrease in biomass availability along this gradient.

When relating biomass to density, we identified a difference in total biomass between Diamesinae and Orthocladiinae at each site (Fig. S3). Generally, biomass stocks were higher for Diamesinae than Orthocladiinae. Assemblages of Diamesinae had, on average, 2.37 ± 1.16× the biomass (2.37 ± 95% CI), when density increased 10% (RMA regression, *p* < 0.01; Fig. S3, Table S2).

The relative abundance of older instars in populations of Diamesinae and Orthocladiinae was not significantly related to harshness (Fig. S4A, B). However, it increased slightly for Diamesinae and Orthocladiinae. Individual body mass of instars of Diamesinae increased significantly from 44.58 ± 10.77 µg/ind at benign sites to 102.92 ± 15.95 µg/ind at harsh sites (linear model, *F* = 6.5, *p* < 0.05, *R*² = 0.29; Fig. 6A). Hence, the mean individual body mass of Diamesinae larvae was 2.3× higher in harsh than in benign sites. In contrast, mean individual body mass of instars of Orthocladiinae larvae was not related to harshness and ranged from 15.36 to 30.97 µg/ind (Fig. 6B). Taxon-specific comparisons revealed that the mean body mass and harshness were significantly related for *D. steinboecki* (*F* = 13.9, *p* < 0.01, *R*² = 0.58).
DISCUSSION

Our study provides insights into the composition of periphyton in glacial and nonglacial high-altitude streams and into the community structure and biomass of chironomid subfamilies in these habitats. We analyzed the quality of periphyton (grazer food) along a wide gradient of environmental harshness and demonstrated a potential adaptive body mass accumulation of glacial-river specialists that might help them withstand harsh conditions in such habitats. Our work expands the understanding of the biotic/abiotic relationships provided in previous studies of alpine and subalpine streams in 3 key ways.

We showed that alpine and subalpine streams exist along a gradient of environmental harshness created by proximity to and influence of glaciers. Among the sites we studied, the relative contribution of algal and cyanobacterial groups in the epilithic community of the periphyton was significantly related to environmental harshness. Nutritional quality differs among algal and cyanobacterial groups (Twining et al. 2016). If we use a space-for-time substitution based on predicted loss of glaciers and the change in environmental harshness that would accompany this loss, then our results indicate that the nutritional quality of autochthonous food for invertebrate grazers might change as a consequence of glacier retreat.

We found that patterns of chironomid biomass differed significantly from patterns of chironomid density along the harshness gradient. Within the Chironomidae, the Diamesinae, which typically are dominant in harsh environments (Füreder et al. 2001, Milner et al. 2001, Lods-Crozet et al. 2001b), are able to acquire more biomass than assemblages of Orthocladiinae in the same streams. Applying the space-for-time substitution, decreasing environmental harshness will result in reduced biomass availability of chironomid populations in alpine streams as the relative abundance of Diamesinae decreases. This finding suggests that the biomass of populations should be included as a biotic metric when studying alpine stream invertebrates, especially when comparing different invertebrate families. Considering differences of biomass among invertebrate taxa, discussions about ecological consequences of species turnover or changes in densities (e.g., Milner et al. 2017) would incorporate the energetic state of the stream systems in the discussion about consequences of loss of glaciers.

We also found that individual larval body mass of Diamesinae was multiple times higher in harsh than in benign habitats. This pattern can be attributed to the plasticity of glacial river specialists (D. steinboecki and D. latitarsis gr. A) and might be facilitated by the nutritional quality of co-occurring algal and cyanobacterial groups in these habitats. The observed phenotypic plasticity of glacial-river specialist taxa might be the result of adaptations of Diamesinae to glacier-fed streams (e.g., Lencioni et al. 2015, Niedrist and Füreder 2018) and the low competition in these habitats (Milner et al. 2017).

Food availability and quality

Most studies with a focus on invertebrates in alpine or subalpine environments have used general proxies, such as
Chl a concentration, to estimate algal biomass (e.g., Füreder et al. 2001, Khamis et al. 2016). We found no significant relationship between algal biomass and environmental harshness, undermining the assumptions of previous investigators (Uehlinger et al. 2010) that additional local variables potentially influence general periphyton growth and density. Instead, we detected divergent patterns within the periphyton by comparing the 3 major pigment-groups: pigment-group I (mainly cyanobacteria), pigment-group II (diatoms + chrysophytes), and pigment-group III (mainly green algae) among sites. Pigment-group II dominated harsh habitats, whereas pigment-group I prevailed in streams that are more benign. In contrast, pigment group III showed no response to different degrees of environmental harshness, because green algae are usually scarcely represented in alpine streams (Rott et al. 2006). These patterns must be interpreted with a great deal of care, in particular concerning pigment-group II, which represents not only diatoms. The main marker pigment used to discriminate pigment-group II is fucoxanthin, which is shared by diatoms (Bacillariophyceae) and chrysophytes (Chrysophyceae). Therefore, the pattern we observed for pigment-group II is likely to have been generated by the summation of the occurrences of Hydrurus foetidus (chrysophytes) basal stages and diatoms. This conclusion is in agreement with the existing knowledge on the distribution and ecology of these 2 taxa in alpine streams. Hydrurus foetidus, although often found in stream stretches influenced by groundwater input, is widely distributed in different types of alpine streams with varying harshness, but with diverse seasonal patterns (Rott et al. 2000, Cantonati et al. 2016). Diatoms, on the contrary, are widely distributed in alpine streams but are markedly reduced in density and richness in harsh, turbid glacial streams (e.g., Cantonati et al. 2001).

We detected opposite patterns of diatoms (+ Hydrurus basal stages) and cyanobacteria within the periphyton assemblages when environmental conditions were moderate in alpine streams. Based on the composition and concentration of fatty acids, diatoms are characterized as potentially excellent food resources, whereas other groups, such as chrysophytes and green algae are characterized as intermediate- and cyanobacteria as low-quality food resources for herbivores in freshwater habitats (Taipale et al. 2013). Algal nutritional quality is generally of high importance for the fitness of consumers and for sustaining stream food webs (Guo et al. 2016a). Our results suggest a transition in grazers’ food from high nutritional quality in harsh environments (dominance of diatoms and chrysophytes) toward lower nutritional quality in benign habitats (dominance of cyanobacteria). However, the dietary choice of chironomid species in these streams is still insufficiently

Figure 5. Relationship between environmental harshness in alpine streams and density (A) and biomass (B) of Chironomidae spp., biomass of Diamesinae spp. (C) and Orthocladiinae spp. (D). Bold lines represent generalized additive model (GAM) best fit for significant (solid) and nonsignificant (dashed) models. Thin dashed lines indicate 95% confidence intervals. n.s. = not significant.
understood (but see Niedrist and Füreder 2018), and further research is warranted to assess feeding strategies of invertebrates in harsh environments. Furthermore, studies specifically testing the effect of ameliorating environmental conditions on the nutritional food quality (i.e., on the relative content of nutritious polyunsaturated fatty acids) of periphyton in alpine streams are needed.

Invertebrate patterns and underlying factors

In line with other studies investigating effects of gradients of environmental stressors on invertebrate communities (Füreder et al. 2001, Brown et al. 2015, Khamis et al. 2016), we also observed increasing taxon numbers, unimodal diversity response, and well-known turnover of species (Milner et al. 2001) within Chironomidae as harshness decreased. Such patterns, derived from only one part of the macro-invertebrate community, demonstrate the good indicator quality of Chironomidae for different degrees of environmental harshness. Different stream types in the Hohe Tauern National Park are characterized by different environmental conditions, which affect the structure of benthic assemblages (Niedrist and Füreder 2016). Congruent to previous work, our study underlines the effect of multifactorial stressors instead of single stressors for benthic invertebrates (Brown et al. 2015).

If the load of inorganic particles in the discharging water is low, algal food is easily accessible for grazing invertebrates at the lower end of the harshness gradient, which is represented by nonglacial stream sites below the treeline. High food availability in combination with benign abiotic conditions (e.g., high maximum water temperatures and low discharge) form very stable environments compared to the other end of the gradient (very low maximum water temperatures and high sediment loads).

We found 2 clearly separated groups of chironomid species along the harshness gradient. Harsh environments are dominated by Diamesa spp. (D. steinboeki, D. latitarsis gr. I and II, D. cinerella gr., D. zernyi gr.), Pseudokiefferiella parva, and a very few Orthocladiinae (Orthocladius luteipes and O. frigidus), whereas less harsh environments are dominated by the genera Eukiefferiella, Orthocladius, or Tvetenia. Cold-stenothermal taxa within the ‘harsh’ group are expected to be the first group to be affected by environmental shifts associated with climate change (Milner et al. 2017). Current trends of glacier retreat in temperate regions are predicted to continue and small glaciers in the Eastern Alps are expected to disappear in the coming decades (Zemp et al. 2006, Marzeion et al. 2018), which will generally decrease environmental harshness over time.

Secondary consumer biomass is a metric rarely used for studies on stream invertebrates in high-altitude streams (but see Buffagni and Comin 2000 or Niedrist and Füreder 2018), and as such the results of our study are an advance in knowledge on this topic. Our data clearly show that patterns of chironomid density and biomass differ along the gradient of environmental harshness. The relative abundance of chironomids usually increases when conditions get harsher (Brown et al. 2007). However, we found a decrease in density and an increase in biomass in harsher conditions. This discrepancy derives from the opposing shifts in relative abundances of chironomid subfamilies between which larvae differ strongly in size. We found no significant difference in density or demographic structure of the populations along the harshness gradient (i.e., the ratio of 3rd- and 4th-instar was similar among sites). Nevertheless, the summed dry mass of Diamesinae was, on average, 2.37 × the dry mass of Orthocladiinae because of interspecific differences in individuals at the same larval development stages. Thus, different levels of biological organization (e.g., subfamily vs family) responded differently to degrees of harsh-
ness, underlining the importance of species-level taxonomy (Knispel and Castella 2003).

**Food and invertebrates: mechanisms and constraints**

The capacity to accumulate body mass during the snow- and ice-free season is probably an essential requirement for survival and reproduction in habitats with a short growing season or a ‘windows of opportunity’ restricted to late spring (Downer and Matthews 1976, Sertić Perić et al. 2015). Unexpected patterns of larval size observed by Schütz and Füreder (2018) in a highly glaciated stream system over time led us to assume that pioneers in glacial headwaters are forced to fast growth in such habitats. Besides the difference in the total dry mass of chironomid subfamilies, we saw that individual larvae of the Diamesinae at similar stage of development possess an individual body mass that is $2.3 \times$ higher in streams with harsh conditions than in streams with less harsh conditions. Diamesinae have known adaptations to harsh physical conditions (Kohshima 1984, Bouchard et al. 2006, Danks 2007, Lencioni et al. 2015), and we assume that *D. steinboecki* and *D. latitarsis* gr. A are likely to be adapted to grow faster and acquire more biomass in harsh environments, as shown for *D. incallida* in colder waters (Nolte and Hoffmann 1992). Our study demonstrates phenotypic plasticity in body mass of glacial-river specialists in contrast to other co-occurring

[Figure 7. Mean ($\pm$SD) individual body mass of Diamesinae taxa *Diamesa steinboecki* (A), *Diamesa latitarsis* gr. A (B), *Diamesa latitarsis* gr. B (C), *Diamesa cinerella* gr. (D), and *Diamesa zernyi* gr. (E) along the gradient of environmental harshness. Bold lines represent significant linear relationships (*D. steinboecki* and *D. latitarsis* gr. A).]
nonspecialist species. However, our study is based on data from early summer only, so whether these co-occurring species also grow faster in harsh environments (as assumed by Schütz and Füreder 2018) remains unclear.

Previous investigators have emphasized that Diamesinae, although tolerant of more benign conditions (Ilg and Castella 2006), might be competitively excluded from benign sites. These taxa might benefit not only from the quantity of food available for them in glacial streams, but also from its higher nutritional quality because the relative abundance of diatoms and chrysophytes is higher in harsh than in more benign streams. A bottom-up effect of higher nutritious quality of food in harsh streams might also enhance invertebrate growth (Graça et al. 2001, Tank et al. 2003, Guo et al. 2016b, Twining et al. 2016) and reproduction (Twining et al. 2016), thus enabling Diamesinae to persist in these harsh environments. Algal biomass in glacial and nonglacial streams is significantly higher in autumn after the growing season (Bürgi et al. 2003). Thus, by sampling in early summer, we were able to illustrate the potential effect of nutritional quality on the body mass of invertebrate grazers, complementary to the amount of available food.

Conclusion

Our work demonstrated expected shifts in periphyton composition in response to amelioration of environmental conditions in alpine and subalpine streams. It also points to a complex functional relationship between available food sources and the phenotypic plasticity of invertebrate specialists, such as the Diamesinae, in harsh glacial streams. Size and body mass of chironomids, and invertebrates in general, have barely been considered in alpine stream studies, but present new perspectives on glacier-fed streams when studied (Schütz and Füreder 2018). Quantification of body mass and biomass of invertebrates in relation to food availability could provide a more accurate picture than density of the community in terms of the energetic state of high-altitude streams. Further, it would allow direct interpretations of the processes driving community structure and alterations in these changing environments. Differing assemblages of primary producers may alter bottom-up effects in streams and, thereby, affect the fitness of aquatic invertebrates differently in these ecosystems. By extension, differences in food quality might affect the productivity of fish in alpine headwater streams and downstream regions. Neglecting the dynamics of algae and cyanobacteria within the periphyton may lead to an underestimation of effects of differing food quality on aquatic grazers (Rott et al. 2006). Last, feeding strategies and associated adaptations, such as rapid biomass accumulation to ensure successful growth and reproduction, might be crucial for harsh-river specialists (Niedrist and Füreder 2018) and should be the focus of future research as already urged by Niedrist and Füreder (2017b).

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