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The use of invertebrates as indicators of environmental change in alpine rivers and lakes.

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Abstract

In alpine regions climatic change will alter the balance between water sources (rainfall, ice-melt, snowmelt, and groundwater) for aquatic systems, particularly modifying the relative contributions of meltwater, groundwater and rain to both rivers and lakes. Whilst these changes are expected to have implications for alpine aquatic ecosystems, little is known about potential ecological tipping points and associated indicator taxa. Within the EU-FP7 ACQWA project we examined changes in biotic communities along a gradient of glacier influence for two study systems; (1) a stream network in the French Pyrénées and (2) a network of lakes in the Italian Alps, with the aim of identifying potential indicator taxa (macroinvertebrates and zooplankton) of glacier retreat in these environments. To assess parallels in biotic responses across streams and lakes, both primary data and findings from other publications were synthesized. Using TITAN (Threshold Indicator Taxa ANalysis) threshold changes in community composition of river taxa were identified at <5.1% glacier cover and <66.6% meltwater contribution. Below this point the loss of cold stenothermic benthic invertebrate taxa, *Diamesa* spp. and the Pyrenean endemic *Rhyacophila angelieri* was apparent. Some generalist taxa including *Protonemura* sp., *Perla grandis, Baetis alpinus, Rhithrogena loyolaea* and *Microspectra* sp. increased when glacier cover was < 2.7 % and < 52 % meltwater. Patterns were not as distinct for the alpine lakes, due to fewer sampling sites; however, *Daphnia longispina* grp. and the benthic invertebrate groups Plectopera and Planaria were identified as potential indictors. While further work is required to assess potential indicator taxa for alpine lake systems findings from alpine river systems were consistent between methods for assessing glacier influence (meltwater contribution/glacier cover). Hence, it is clear that this approach (TITAN) could become a useful a management tool, enabling: (i) the identification of taxa particularly sensitive to glacier retreat; and (ii) conservation efforts/resources to be better directed in alpine aquatic systems.

Keywords:

Climate change, glacier retreat, macroinvertebrates, zooplankton, alpine aquatic systems, indicator species

1. Introduction

The cryosphere represents ~70% of all freshwater on the Earth's surface (Carpenter et al., 2011). The reliable flux from this frozen water store is a major control on abiotic and biotic patterns of high latitude and altitude lotic and lentic waters (Milner and Petts, 1994; Slemmons and Saros, 2012). However, many glaciers are shrinking worldwide and the rate at which they are changing has accelerated over the last 2-3 decades (Oerlemans 2005, Barry 2006, Haeberli et al., 2007). This is particularly the case in alpine regions where increases in air temperature will be higher than at lower altitudes (Beniston, 2003), primarily due to feedbacks between diabatic processes and the surface energy balance, which manifests in an amplification of regional climate signals (Ohmura, 2012). For larger glaciers that are receding, an initial increase in glacial meltwater generation may occur due to increased energy inputs, earlier disappearance of reflective snow cover and exposure of lower albedo ice (Hannah et al., 2000). However, initial flow increases will be followed by reduced glacial runoff in the long-term due to negative glacier mass-balance (Hannah and Gurnell 2001, Stahl et al., 2008), as observed in many European mountain regions (Zemp et al., 2006). Associated changes in the habitat and biota of alpine rivers and lakes are becoming evident (Adrian et al., 2009; Milner et al., 2009; Brown et al., 2006b; Finn et al., 2010; Brown and Milner, 2012).

Water source dynamics in glacially influenced rivers represent a major control on biotic communities at a range of spatial and temporal scales (Malard et al., 2006, Brown et al., 2007, Füreder 2007). Two major hydrological stores act as sources of runoff: (i) glaciers and snowpacks (meltwater); and (ii) alluvial or regional aquifers (groundwater) (Brown et al., 2006b). Different proportions of these water sources create spatial and temporal variability in sediment and thermal regimes and, linked to channel stability and habitat availability, determine benthic community assemblages (Malard et al., 2006). Where glacial meltwater dominates, low water temperature and unstable channel beds due to high discharge variability (Carrivick et al., 2013) creates deterministic patterns in benthic macroinvertebrate communities (Milner et al., 2001). These communities are typically dominated by chironomids well known to be tolerant of cold temperature and unstable channels, particularly *Diamesa* (Milner and Petts, 1994). However, with the projected reduction in glacial runoff, sediment loads will decrease and water temperature and channel stability increase, thereby driving potential significant shifts in the faunal composition of glacier-fed rivers (Ilg and Castella, 2006). Ephemeroptera, Plecoptera and Trichoptera will likely become increasingly abundant, along with other chironomid sub-families and Simuliidae (Milner et al., 2001). Many benthic organisms, sensitive to high water temperature, will be lost as they will be unable to migrate or respond physiologically to water temperature increase (Bellard et al., 2012). Because of this unique deterministic relationship between the physico-chemical habitat and the macroinvertebrate fauna, key taxa can potentially be used as biological indicators of climate driven changes in water source contributions within rivers in alpine environments (Brown et al., 2007). However, few studies have investigated this, despite findings from other environments highlighting the potential utility of indictor taxa for informing adaptive management plans (e.g. Bush et al., 2013).

Climate change effects on mountain lakes are mediated mainly through changes in air temperature, precipitation and wind regimes that determine snow and ice cover, catchment hydrology and lake temperature and mixing regimes (Thompson et al., 2005, Adrian et al., 2009). These factors, in turn, control many chemical and biological processes, which strongly influence habitats, ecological dynamics and species distributions (Battarbee et al., 2002, Catalan et al., 2006). Alpine lake ecosystems are characterized by simple food webs, particularly when compared to lowland lakes (Magnea et al., 2013). This lack of complexity enables web network/trophic properties to be characterised more completely and thus the responses of lake ecosystem to climatic change can be predicted (REF). Due to the presence of glaciers and the dynamics of snow cover, mountain lakes often respond to climate change according to indirect mechanisms (e.g. water temperature can decrease due to melting water from shrinking glaciers), which are less evident at low elevations (Thompson et al., 2005). Like rivers, the biological communities of alpine lakes can act as sensitive indicators of climate change, as even small perturbations of their natural equilibrium may cause detectable responses (Rogora et al., 2008). Hence, the identification of indicator taxa for monitoring glacier retreat in lake environments is feasible. Despite this no studies have linked contemporary glacier cover to pelagic or littoral faunal patterns, as to date most studies of high altitude lakes have focused on altitude or other environmental variables as key drivers of community composition (Mendoza and Catalan, 2010).

Climate change is expected to facilitate altitudinal range expansions of invertebrates and enhance the invasion potential of fish species, which will alter biotic interactions and community structure in alpine rivers and lakes (Bellard et al., 2012). In rivers, warmer water and more stable habitats can allow invertebrate predators to migrate upstream (Brown et al., 2007). Negative impacts of predator invasions or introductions on aquatic communities involve altering prey abundance and size distributions directly (Kratz, 1996; Dahl, 1998), or indirectly via prey behavioural responses (e.g. drift: Mcintosh and Peckarsky 1990, Lancaster 1990) or physiological responses (e.g. altered emergence size (Peckarsky et al., 2001)). In alpine lakes introduced fish are a major threat with significant effects on native communities (Knapp et al., 2001a, Schabetsberger et al., 2009). Less extreme conditions produced by climate change are projected to increase the invasion potential of introduced species, such as salmonids, into alpine lakes and assist the acclimatization of alien species (Holzapfel and Vinebrooke, 2005). However, synergistic and additive effects of these expansions/invasions have not been considered in the context of glacier retreat and climate change for alpine aquatic systems.

In this study, habitat and biotic records were used to assess the effects of glacier retreat on two contrasting alpine aquatic ecosystems: (i) a stream network in the French Pyrénées; and (ii) a network of lakes in the Italian Alps. Three specific objectives were identified: (i) to determine if distinct trends in physico-chemical habitat characteristics occur along a gradient of glacier influence for alpine streams and lakes; (ii) to identify potential indicator taxa of glacier retreat and associated ecological thresholds for both streams and lakes; and (iii) to determine if parallels exist between ecosystem responses to a shrinking cryosphere. It is rare that streams and lake responses are compared but in this way conservation strategies can be applied more holistically for climatically sensitive alpine aquatic ecosystems.

2.0 Study regions

Two key two study regions were identified, based on previous research conducted in the Alps and French Pyrénées. Prior knowledge of the habitat – biota relationships informed site selection and aided the development of a suitable sampling strategy given the logistical constraints of working at remote field sites. Furthermore, ACQWA related field campaigns contributed to existing historical data sets for these systems.

2.1 Study basins – French Pyrénées

Four glacier-fed streams from three river basins were studied in the Parc National des Pyrénées (PNP) during the summers of 2010 and 2011 (Fig. 1). The Taillon – Gabietous river basin covered an altitudinal range from 1800m at the lowest sample point to 3144m at the highest point in the catchment (Pic du Taillon). Two cirque small glaciers, the Glacier du

Taillon (0.09 km^2) and the Glacier des Gabiétous (0.08 km^2) are located on north facing slopes and feed the Taillon and Tourettes streams (Smith et al., 2001). The underlying geology is predominately sandstone (Marbore sandstone) and limestones (Santonien and Coniacien series). The Ossoue river basin, upstream of the lowest sampling point, spans an altitudinal ranging from 1850m to 3298m (Pic du Vignemale). The Glacier d'Ossoue (0.46 km^2) is located on the east facing slope and feeds the Oulettes d'Ossoue stream. The underlying geology is a mixture of metamorphic (Marble limestones) in the upper catchment (Del Río et al., 2012) with sedimentary rocks in the lower catchment. The Vignemale river basin, upstream of the lowest sampling point, covers an altitudinal range of 1820m to 3298m. The Glacier des Oulettes (0.13 km^2) and Glacier du petit Vignemale (0.03 km^2) feed the Gave des Oulettes. The catchment is underlain by igneous rocks (primarily Granodiorite). In total 17 sites were selected, with at least three along each stream, representing a gradient of glacier influence (Table 1).

2.2 Study sites - Gran Paradiso National Park

Seven high altitude alpine lakes were selected representing a gradient of glacier influence. All lake areas were > 10,000 m^2 and located within the Gran Paradiso National Park (PNGP), Western Italian Alps (45°25'- 45°45' N and 7°-7°30' W) (Fig. 1) above 2500m. Four of the lakes had glacier cover in the catchment; Lake Leynir 3.6 % Lake Lillet 20.8 %; Lake Motta 4.0% and Lake Leita 9.6% (Table 2). The underlying geology of the region is split between acidic gneiss in the east and calcareous schists in the west (Tiberti et al., 2010) and is a major control on vegetation development. Acidic grassland, consisting of *Festuca halleri, Festuca varia* and *Carex curvula* predominates in the alpine zone while basic grassland is less widespread. The nival zone is characterised by bare rock, moss, liverworts and lichens.

3.0 Material and methods

3.1 PNPstreams: physicochemical habitat and water source sampling

For PNP streams, field measurements were carried out at each study site on three occasions, during 2010 and 2011 (Early: 16 - 29 June, Mid: 15-30 July, late: 17-30 August). Spot measurements of electrical conductivity (EC) and pH were made using a Hannah HI 98129 handheld probe.Water samples (500ml) were collected at each reach to determine suspended sediment concentration (SSC). Samples were filtered through pre-weighed glass fibre filter papers (Whatman GF/C), dried at 95˚C for 2 h then re-weighed to the nearest mg (ATSM D3977 – 97). The bottom component of the Pfankuch index (PFAN) was also recorded. This involves a qualitative scoring assessment of five variables (rock angularity, substrate brightness, particle packing, percentage of stable materials, scouring and aquatic vegetation) with higher values representing unstable channels (Pfankuch, 1975). Water velocity (measured using a Sensa RC2 electromagnetic current meter), water depth and wetted width were recorded at five random locations along each 30m reach. Water temperature (T_w) was recorded continuously during both melt seasons, at each study reach, using either miniature digital temperature dataloggers (Gemini Tinytag Plus, instrument error ± 0.2°C) or CS547A temperature probes (Campbell Scientific, instrument error ± 0.2°C). To characterize the annual thermal regime, continuous water temperature monitoring was conducted between 09/10 and 09/12 at three sites; T3, G4 and Spr 1 (a small groundwater spring). Cross-calibration of all temperature sensors was conducted before and after the field season (Quilty and Moore, 2007). Correction factors were then applied to each logger based on a regression, which related the individual logger reading to the mean reading of all loggers

(Hannah et al., 2009). At each site, on one sampling occasion, 100 randomly selected clasts were measured (±0.1cm) and median b-axis (D_{50}) calculated.

Reach slope, altitude, catchment area and glacier cover were calculated for each site in ARC map 10 using aerial images and an ASTER DEM (30m resolution). Reach slope and average water depth were then used to calculate sheer stress (Castella et al., 2001):

$$SS = g\ s\ d\ \rho$$

Where g is the acceleration due to gravity, s the reach slope, d the mean depth and ρ the water density.

On each date, water samples were collected from potential hydrological source 'end-member's (i.e. snow, ice and groundwater) along with an additional sample from each study reach. Silica [Si] was selected as a tracer to characterize these end-members as it has been used in previous studies to split groundwater contributions to bulk streamflow from dilute meltwater (Anderson et al., 2000, Brown et al., 2006, Blaen et al., 2013). Silica concentrations were calculated in the laboratory using the molybdosilicic method (ASTM D859 – 10). Basin specific, two end-member mixing models were used for hydrograph separation approach to calculate meltwater and groundwater proportions for each reach using simple mass-balance equations (Sueker et al., 2000).

3.2 GPNP: physicochemical sampling

To characterize the annual thermal regime, temperature dataloggers (Maxim's iButton device DS1992L) collected hourly records at three of the lake sites (Lake Lillet, Lake Leynir and Lake Trebecchi Superiore). Loggers were located at 3.0m below the water surface, attached to a permanant buoy which marked the point of maximum depth and ran continously between 09/2009 and 09/2010. A light meter (LI-COR LI-250) with a submersible sensor was used to take vertical profiles of light intensity (Photosynthetically Active Radiation - PAR, 400–700 nm). Measurements were taken at 1 m depth intervals from the water surface to the lake bottom.

3.3 PNP streams: Biological sampling and processing

For PNP streams, five replicate macroinvertebrates samples were collected from random locations in suitable riffle habitat at each reach, using a Surber sampler (area 0.09m^2; mesh 250 µm). Macroinvertebrates were preserved in 70% ethanol in the field then, in the laboratory, all individuals were picked from the gravel and organic material under a magnifying bench lab. Ephemeroptera, Plecoptera and Trichoptera (EPT) and Chironomidae were identified to the lowest practical taxonomic level (species where possible) using a selection of identification keys (Müller-Liebenau, 1969; Tachet et al., 2000; Zwick, 2004). Taxa were then enumerated at the level of identification and also at the level of order.

3.4 Biological sampling and processing GPNP lakes

PNGP lakes were sampled during successive summers between 2006 and 2009. Zooplankton were collected at the deepest point of each lake by taking vertical tows with a conical plankton net (40 cm diameter, 48 µm mesh). Samples were preserved in 4% formaldehyde and transported back to the laboratory. Each sample was diluted to a fixed volume and three to five subsamples (0.1 to 1 ml volume) were obtained. Crustaceans and rotifers were identified to species level, where possible, according to Dussart (1969) Harding and Smith (1974), Braioni and Gelmini (1983) and Margaritora (1985). However, species groups were used for organisms with an uncertain taxonomy (e.g. *Daphnia longispina* and *Daphnia rosea* represent the *longispina* group) or when identification was not possible due to morphological deformations (e.g. *Synchaeta* gr. *stylata-pectinata*). Sample enumeration was conducted using a closed counting chamber, under a binocular dissecting microscope at 40x (Olympus CH-BI45-3). Subsequent counts were then converted to density (i.e. number of individuals per m^{-3}),

For each lake all the shore-accessible habitats were sampled for macroinvertebrates. Three littoral habitats were chosen according to clast diameter: (i) sand shores (clast diameter < 2mm); (ii) gravel shores (2mm < clast < 64mm); and (iii) stony shores (clast > 64mm). In each of these habitats, 1 m in sweeps (10 replicates), were conducted with a standard d-frame net (mouth, 25 x 20 cm; mesh 0.5 mm). Benthic macroinvertebrates were preserved in 70% ethanol, identified to the lowest practical level following Tachet (2000) and enumerated.

3.5 Data analyses

To test for differences in mean daily water temperature between (i) the stream sites and (ii) the lake sites, one-way ANOVA with Tukey's post-hoc test was used. Separate analyses were run for annual (all records), summer (June-August records) and winter (December – February) means.

For each site and sampling occasion stream Surber replicates were combined to avoid patch scale heterogeneity obscuring reach scale patterns, and taxonomic richness was calculated. Taxon densities were $\log_{10}$ (x+1) transformed to reduce the influence of extreme observations on the subsequent ordination procedure (Siddon et al., 2011). Hierarchical cluster analysis (Ward's method) was then applied to the taxa dataset and ANOSIM used to identify the number of clusters which maximised between-group dissimilarity while minimising within-group dissimilarity. Non-metric dimensional scaling (NMDS) was then carried out using Bray-Curtis dissimilarity with 1000 restarts. Reach-scale variables were related to the NMDS ordination using a vector fitting algorithm outlined by Faith & Norris (1989) to identify underlying habitat gradients. For lake sites, due to the relatively small sample size and as the measure of glacier influence (% glacier cover) was relatively static in time compared to meltwater contribution to flow, the mean across all sample dates was calculated and taxonomic richness recorded. The data were $\log_{10}$ (x+1) transformed and NMDS undertaken as described previously.

Prior to regression analysis we examined the PNP physical habitat/glacier influence data for co-linearity. Scatterplots were constructed and Pearson's correlation coefficients calculated for all pairwise relationships between: glacier cover, meltwater, altitude, distance from the glacier snout and stream width. Co-linearity between: (i) glacier influence and distance from the snout; and (ii) glacier influence and altitude, was apparent (Figure S1). In the case of distance from the snout we considered meltwater and glacier cover to be more representative of the hydrological sources, flow paths and processes that dictate physicochemical habitat characteristics (Malard et al., 2006). For altitude we considered the relationship an intrinsic characteristic of glaciation in alpine environments, particularly the Pyrénées where all glaciers are located at high altitude (Grunewald and Scheithauer, 2010). Furthermore, based on previous paired catchment studies of sites at similar elevations, both with and without glacier cover (see Füreder et al. 2005), it is clear that distinct taxa are associated with glacier-fed sites. For the stream data Generalized Linear Mixed Models (GLMMs, negative binomial) were used to investigate the relationship between the glacier influence predictor (i.e. meltwater/glacier cover) and: (i) taxonomic richness; and (ii) abundance of macroinvertebrate taxa (raw density data). Due to repeated measures, site was nested in sampling year and included as a random factor in the model. This induced a compound correlation structure, to account for temporal autocorrelation, which was preferable to an autoregressive model due to the uneven time periods between sample dates (Zuur et al., 2009). For the lake sites, due to the small sample size (n = 7), Spearman's rank correlation was used to identify relationships between glacier cover in the catchment and potential indicator taxa.

For the streams, Threshold Indicator Taxa ANalysis (TITAN) was used to identify taxa and community thresholds along a gradient of glacier influence as defined by (i) meltwater proportion and (ii) glacier cover in the catchment. TITAN is a non-parametric technique that orders and partitions observations along an environmental gradient, and, rather than using IndVal scores (indicator value method Dufrene and Legendre 1997) to interpret pre-existing typologies, it uses IndVal scores to define groupings. Following Baker & King (2010), multiple candidate change points are identified and indicator value scores calculated for each taxa (250 permutations). Standardized IndVals (based on SD and mean from permutation) were used for cross-taxon comparisons. Declining (z-) and increasing (z+) taxa are used to identify community-level change points. Bootstrapping (500 replicates) is used to identify reliable threshold indicator taxa and the uncertainty around the taxon and community change points. In this study community thresholds are presented as (i) TITAN thresholds, synchronous changes in the abundance of z- or z+ taxa within a narrow range of the glacier influence gradient and (ii) change point analysis (nCPA) where candidate change points are identified via deviance reduction of Bray-Curtis distance values. The latter approach (nCPA) is based on the assumption that the mean and variance of the Bray-Curtis distance will respond to compositional chances. The ‘changepoint’ separates the observations into two groups and deviance reduction is used to identify the most homogenous two groups (see Qian et al. (2003) for more detailed methods). All analysis was carried out in R.2.14.1 using the *Vegan* and *LME4* packages.

4.0 Results

4.1 Thermal regime and turbidity

A distinct seasonal dynamic was apparent for mean daily water column temperature (T_w) at all stream reaches, with minima observed during winter and maxima during late summer months (Fig. 2). T3 (5.7% glacier cover) was the only stream reach which displayed water temperature below zero, while winter temperature for both Spr 1 (0% glacier cover) and G4 (1.7 % glacier cover) were > 0˚C consistently and were less variable than T3. Maximum daily mean T_w was highest for Spr 1 (15.3 ˚C) and lowest for T3 (11.3 ˚C). However, the spring/summer temperature increase was latest for Spr 1, which started in May, and earliest for G4 which began in March (Fig. 2). Annual mean T_w for the entire sample period was significantly lower for T3 (ANOVA; $F = 42.0$, $P < 0.0001$) with no significant difference between T3 and Sp 1. For summer, all sites were significantly different (ANOVA; $F = 38.6$, $P < 0.0001$) in the order T3< G4 <Sp 1, while for Winter T3 was significantly lower than both G4 and Sp 1 (ANOVA; $F = 233.7$, $P < 0.0001$). A positive correlation between SSC and both measures of glacier influence, i.e. meltwater contribution ($r = 0.56$, $P < 0.0001$) and % glacier cover ($r = 0.51$, $P < 0.0001$), was evident for the PNP study sites.

Lake T_w displayed a distinct seasonal trend. During the winter, water temperature was similar in all three lakes, but with the advent of spring, Lake Trebecchi Superior maintained a similar temperature whereas in the other two lakes water temperature continued to decrease (Fig. 2). In June, lake temperature in the non-glacierized catchment (Lake Trebecchi Superior) increased rapidly following ice-off from 5 °C to >15 °C in less than 14 days. Lake Lillet (20.8 % glacier cover) increased slowly due to the input of glacial runoff and only reached 4.8 °C by the same period. However, T_w did rise during summer and reached a maximum in August (9.5 °C), significantly lower than Lake Trebecchi Superior. Lake Leynir

(3.6% glacier cover) showed a thermal regime between these two other lakes. For the entire sample period, mean water temperature was significantly different between all lakes (ANOVA; $F = 60.5$, $P < 0.0001$) in the order of Lilet< Leynir < T. Superior. Similar patterns were apparent for both summer mean temperature ANOVA; $F = 114.3$, $P < 0.0001$) and winter (ANOVA; $F = 167.7$, $P <0.0001$). Turbidity also appeared to be related to glacier cover. Lake Lillet showed markedly higher PAR attenuation (k = 0.37) than the lakes without glacier cover (all k < 0.23). On the contrary, Lake Leynir (k = 0.15) and Lake Leita (k = 0.18), displayed lower PAR attenuation, probably due to the longer distance from the glacier and the presence of sedimentation basins (e.g. alluvial plateaus, glacial stream meanders) along their tributaries.

4.2 Ordination and cluster analysis (PNP)

Cluster analysis revealed three distinct groups of sites based on taxonomic composition. These were mapped onto the NMDS ordination of macroinvertebrate abundance (stress = 0.16) for the 94 site-dates (Fig. 3). Taxa associated with Group 1 (i.e. negative scores on NMDS axis 1) were dominated by Diptera (*Diamesa* spp., *Cardiocladius capuncinus* and Empididae), although *Rhyacophila angelieri* and Oligochaeta were also abundant (Supplementary Table 1). Group 1 was related to low channel stability (high PFAN Index score), low water temperature and high shear stress, SSC and pH (Fig. 3). The variables driving Group 1 were linked to the high meltwater contribution to bulk stream flow(Fig. 4) corresponding to a mean glacier cover of 13%. Group 2 (Fig. 3) contained the fewest number of sites and was associated with *Baetis gemellus*, *Rhithrogena hercynia, Agapetus fuscipes,* elmids (*Esolus* spp. and *Elmis* spp.), *Amphinemura* sp., *Stempellinella* sp. and *Atherix* sp. (Supplementary Table 1). Variables associated with the grouping were EC and minimum and mean water temperature. Meltwater at these sites was intermediate between Group 1 and Group 3 (Fig. 4) and the underlying geology was calcareous. Glacier cover and meltwater contributions to flow were lowest in Group 3 (Fig. 4) associated with the taxa *Rhyacophila evoluta, Drusus discolour, Rhithrogena loyolaea*, *Arcynopteryx compacta* and Psychodidae. The physicochemical habitat of these sites was characterised by higher channel width and depth (i.e. sites further down valley from their sources) and low SSC and pH.

The ordination of lakes based on the zooplankton community (Fig. 5) displayed a negligible loss of information (Stress = 0.04). Axis 1 divided fishless lakes (positive scores) from lakes stocked with fish (negative scores). The cladoceran *Daphnia longispina* and rotifer *Lecane luna* were associated with positive scores on this axis, while the rotifers *Trichocerca* sp. and *Euchlanis* sp. were associated with negative scores. Axis 2 divided fishless lakes with no glacier influence (positive scores) from all other lakes (negative scores). *Lecane luna* and the cladoceran *Acropaerus harpae* were associated with positive scores, while the rotifer *Synchaeta* sp. and the copepod *Arctodiaptomus alpinus* were associated with negative scores.

4.3 Glacier influence – abundance and diversity (PNP & GPNP)

Plots of taxonomic richness against the two methods for quantifying glacier influence (i.e. meltwater contribution and glacier cover in the catchment) identified negative relationships (Fig. 6). For glacier cover (GLMM; AIC = 146.1, $P < 0.0001$) a stepped relationship was apparent with a sharp decrease in species richness at >5% glacier cover. For percent meltwater (GLMM; AIC = 155.1, $P = 0.007$), there was a marked decrease in richness between 60 and 100%.

These same trends were evident for a number of potential indicator taxa, which showed a marked decrease in density when meltwater contributions exceeded 60% until they were absent or nearly absent at 80% (Fig. 7). This was particularly the case for the orders, Ephemeroptera and Plecoptera. *Protonemura* sp., *Perla grandis, Baetis alpinus, Rhithrogena loyolaea* and *Microspectra* sp., which displayed significant negative relationships with meltwater (Fig. 7; Table 3). Similar (negative) trends were apparent between these taxa and % glacier cover, with a marked decrease in abundance at > 5%. Some taxa displayed no response across the meltwater gradient with only a negligible change in abundance, for example *Diamesa cinerella* (GLMM, $P = 0.64$), while other chironomids showed an increase in abundance with increased meltwater contributions. For example, *Diamesa latitarsis* gr. increased when percent meltwater exceeded 40% (GLMM, P = 0.023) and percent glacier cover 5% (GLMM, P < 0.001) (Fig. 7; Table 3).

Spearman's correlation coefficients indicated some significant relationships between abundance of lake taxa and percent glacier cover in the catchment (Table 4). Of the shoreline macroinvertebrates, Plecoptera (principally *Nemoura sp.*, *Nemurella* sp., *Leuctra* sp., *Perla* sp. and *Perlodes* sp.) showed a significant decrease in abundance with a reduction in glacier cover (Fig. 8). Planaria also displayed a similar response. Heteroptera showed an opposite response with abundance increasing with less glacial cover. Of the planktonic species, the most likely indicator of changing conditions in alpine lakes was *Daphnia longispina* gr. which showed a significant increase in abundance with a reduction in glacier cover (Fig. 8).

4.4 Change points and indicator taxa (PNP)

Results from the TITAN analysis broadly supported the GLMM results (Fig. 9). The only taxa that significantly increased with meltwater contributions (*z*+ taxa) were chironomids of the genus ***Diamesa*,** which displayed marked increases between 40 and 50% meltwater contribution (Fig. 9). A similar set of *z*+ taxa was observed for glacier cover with the addition of the caddisfly, *R. angelieri,* which increased above 2.5% glacier cover. Notably, the density of *Diamesa latitarsis* grp. displayed a significant increase at 7.5% glacier cover (Fig. 9). The other taxa for which significant change points could be identified all decreased across the glacier influence gradient (*z*- taxa). For meltwater contribution, 34 *z*-taxa were identified, a large proportion of which displayed change points between 55% and 75% (Fig. 9). For glacier cover, 35 *z*- taxa were identified, showed synchronous declines around two change points; 2.5% and 5% (Fig. 9). However, it is evident that there was a large amount of variation about the mean (wide confidence intervals), particularly for meltwater contribution, suggesting gradual increases in abundance and frequency along the gradient.

Change point analysis identified significant thresholds for both metrics used to quantify glacier influence (Fig. 9). When considered as the sum (*z*-) (i.e. the taxa decreasing across the glacial influence gradient) the change points were 5.1% for glacier cover and 67.5% for meltwater contribution (Table 4). For sum (z+) (i.e. the taxa increasing across the gradient) the change points identified were 2.7% and 51.7% for glacier cover and meltwater contribution respectively (Table 5). Similar change points were apparent for nCPA (i.e. Bray-Curtis distance) of 5.1% and 66.6% glacier cover and meltwater proportion, respectively (Fig. 9; Table 5).

5.0 Discussion

Drawing upon the primary data presented above and comparisons to published work, three main topics will be covered herein: (i) synergies between the physicochemical habitat of lakes and river ecosystems and implications for the biota; (ii) the identification of potential indicator taxa for these two biotypes; and (iii) change points and additive pressures.

5.1 Implications of physicochemical habitat change in alpine lakes and rivers

This study identified strong synergies between habitat characteristics of alpine rivers and lakes across a gradient of glacier influence. Water temperature, a key variable governing a range of physical and biological processes (Webb et al., 2008), was inversely related to catchment glacier cover for both lakes and streams. Similar patterns were observed by Brown and Milner (Brown and Milner, 2012) for a catchment with decreasing glacierization over time. When considering the GPNP sites, the warmer summer water temperature observed for Lake Trebecchi Superior (on glacial influence; Fig 2) is likely due to a combination of factors. First, reduced glacial meltwater input (advected coldwater) in the summer and increased residence times of lake water, both direct consequences of climate change, lead to greater potential for summer warming (Holm et al., 2011, Richards et al., 2012). Second, warming will typically lead to ice cover breakup occurring earlier in the spring and thus increased exposure to the atmosphere, thereby extending the summer warming period (Livingstone et al., 1999). For PNP streams, warmer summer water temperature recorded for sites with reduced glacial influence (Fig 2b) are likely due to lower stream discharge (decreased thermal capacity) and reduced meltwater input (Cadbury et al., 2008; Fellman et al., 2013). However, winter precipitation patterns will play an important role in future stream thermal regimes as snow cover dictates exposure time to the atmosphere and energy exchange processes (Dickson et al., 2012; Edwards et al., 2007). Once meltwater production decreases due to sufficient ice mass loss associated with climate change (Milner et al. 2009) reduced coldwater input and increased exposure to the atmosphere will increase water temperature and, in certain cases, decrease turbidity (Khamis et al. 2013). However, glacier size, catchment morphology and geology may alter response trajectories for water temperature and turbidity of both lakes and rivers (Leonard and Reasoner 1999, Brown and Hannah 2008, Richards et al., 2012).

Changes in water temperature and turbidity of alpine aquatic ecosystems will have implications for the distribution and behaviour of a range of taxa. Initially, as larger glaciers retreat (e.g. GPNP glaciers) and associated inputs of turbid meltwater increase, basal resources will be reduced in quantity and quality resulting in changes in zooplankton species composition and behaviour (e.g. vertical position in the water column). In this study water temperature and turbidity (i.e. glacier influence) appeared to be strongly linked to cladoceran community composition and abundance (Fig 8), particularly *Daphnia,* a relationship also identified by Fischer et al. (2011). For the stream sites, meltwater contribution is expected to decline (all glaciers are small in the French Pyrénées) and warmer water temperature and lower SSC will be more favourable for many taxa from the regional species pool (Brown et al., 2007b). The space for time approach adopted in this study suggests a shift from a species poor community dominated by *Diamesa* to a more diverse community with EPT taxa present (Fig 3 & Fig 7; c.f. Milner et al., 2001). Reach scale (alpha) diversity is likely to increase, while regional (gamma) diversity is likely to decrease as specialist taxa become extinct

(Jacobsen et al., 2012; Brown et al., 2007a) However, for stream systems in other regions (e.g. European Alps) fed by larger glaciers, meltwater is initially expected increase and alpha diversity to decrease (Khamis et al., 2013). Hence, clear parallels exist between lakes and streams regarding biodiversity responses to glacier retreat, and they depend on glacier volume and meltwater production.

5.2 Potential indicators of alpine aquatic ecosystem change.

Although a wide range of responses across the meltwater/glacier cover gradient exists, for most taxa, high glacial influence acts as an environmental filter to colonization (Brown and Milner, 2012). The most apparent biotic indicators of reduced meltwater contributions and % glacier cover was increased abundance of *Protonemura* sp., *P. grandis, B. alpinus, R. loyolaea* and *Microspectra* sp. (Table 3; Fig. 7), similar to findings from a single basin study in the French Pyrénées (Brown et al., 2007). Another clear indicator was reduced abundance of cold stenothermic taxa, in particular *Diamesa latitarsis* gr. and *R. angelieri* (Table 3; Fig. 7), which are both range restricted (the latter endemic to the Pyrénées) glacial stream specialists (Rossaro et al., 2006, Brown et al., 2007). A similar negative relationship between a cold stenotherm, endemic stonefly (*Lednia tumana*) and glacier cover in the catchment was found in the Waterton-Glacier International Peace Park, Canada (Muhlfeld et al., 2011). Hence, taxa sensitive to reductions in meltwater contribution share common attributes (traits) which enable them to colonise ‘harsh’ glacier fed streams (Ilg and Castella, 2006). Analysis at coarser taxonomic levels may not account for trait variability within families (Brown et al., 2007, Muhlfeld et al., 2011), particularly when considering speciose families such as Chironomidae, which are well represented in alpine aquatic systems (Rossaro et al., 2006). Therefore, a more complete taxonomy of alpine fauna is required to enable more in-depth indicator trait profiles to be developed (see Menezes et al., (2010) for review) which would facilitate larger scale studies, covering multiple biogeographical regions.

For the lake sites, patterns were not as distinct due to fewer sampling sites and the logistical constraints of sampling across the full gradient of glacier influence. Nevertheless, some potential indicator groups were identified, including *D. longispina,* a large bodied grazing crustacean*,* negatively related to % glacier cover. Whilst no studies to date have directly linked cladoceran taxa abundance to glacier cover, paleoecological studies have previously found strong relationships between lake water temperature (which shows a strong relationship with % glacier cover) and *D. longispina* abundance (Korponai et al., 2011). Stratographic analysis of sediments lake in western Norway found that *Daphnia* spp. were absent during a period of marked glacier recession, when increased glacial meltwater inputs would have reduced water temperature and transparency (Duigan and Birks, 2000).In this study potential indicator macroinvertebrate taxa were also identified for GPNP, although at the order level. Plecoptera and Planaria taxa decreased with glacier cover, likely due to higher water temperature as many represenatives are cold stenotherms (Tachet et al., 2000). Interestingly, this is in contrast to the response recorded for the streams sites where other factors such as resource availability and flow velocity may inhibit the colonisation of Plecoptera under high meltwater contributions (D L Snook and Milner, 2001). It is evident that numerous other variables (both biotic and abiotic) determine community composition in high altitude lake systems (see Kernan et al., 2009); hence, further work is needed to identify linkages between % glacier cover and community composition and the interaction with other variables such as fish presence (discussed below in sections 5.3 and 5.4) or organic enrichment.

5.3 Change points and additive effects

This study presents findings from the first change-point analysis for macroinvertebrate communities along a natural gradient of glacier influence. Clear community tipping points were identified for the gradient defined by meltwater contribution (~67%) and glacier cover (~5 %). Below this point the loss of specialist taxa, *Diamesa* spp. and the endemic *R. angelieri*, was apparent, similar to findings from other studies, highlighting their extinction risk as glaciers recede (Brown et al., 2007). Generalist taxa increased when glacier cover was < 3 % and meltwater < 52 %. Threshold analysis is still a relatively new field and most previous studies have focused on anthropogenic gradients, such as % impervious cover in the catchment or % of catchment mined (Baker and King 2010, Bernhardt et al., 2012), yet we suggest this approach could provide a useful monitoring tool in alpine environments. Further work is needed to identify if changes identified in this study represent alternative stable states and are consistent when considering multiple mountain ranges with a range of glacier sizes (Dodds et al., 2010).

For the lake system, while there were insufficient sites to run TITAN, although preliminary findings suggest that identification of a clear tipping point may not be possible as the additive effect of fish presence/absence alters community composition in a way broadly similar to glacier influence (see Fig 5). The presence of fish in high alpine lakes causes a shift from a community dominated by large bodied cladocerans to one dominated by small bodied rotifers and copepods (Knapp et al., 2001b, Magnea et al., 2013), similar to patterns observed in glacier fed lakes (Edmundson and Koenings 1986, Hylander et al., 2011). In streams some increases in taxa can have consequent effects with respect to biotic interactions and this is particularly the case with the colonization and increased abundance of the stonefly predator *P. grandis* into the benthic community (Khamis unpublished).

6.0 **Conclusions**

This research represents the first attempt to identify change points and indictors of alpine aquatic ecosystems change in the context of glacier retreat. A combination of glacial stream specialist taxa, sensitive to reductions in meltwater contributions, and generalist taxa sensitive to higher meltwater contribution have the potential to act as bio-indicators of ecosystem change. TITAN analysis identified a clear change point in physicochemical conditions for macroinvertebrate stream taxa, representing a shift from a specialist community to one that is more generalist based on percent meltwater or percent glacier cover (Fig. 9). This approach could provide a useful management tool for alpine aquatic systems under a changing climate. However, due to the relatively small spatial scale of our study, we suggest further work is needed to identify if these findings are consistent across larger spatial scales and a range of biogegraphical regions. For lakes, potential indicator taxa were identified in both the zooplankton and benthic macroinvertebrate community but community change points could not be clearly identified as the presence of predatory fish interacted with physicochemical habitat conditions to determine zooplankton community structure.

References

Adrian R, Reilly CMO, Zagarese H, Baines SB, Dag O. Lakes as sentinels of climate change. Limnology and Oceanography 2009;54:2283–97.

Anderson SP, Drever JI, Frost C, Holden P. Chemical weathering in the foreland of a retreating glacier. Geochimica et Cosmochimica Acta 2000;64:1173–89.

Baker ME, King RS. A new method for detecting and interpreting biodiversity and ecological community thresholds. Methods in Ecology and Evolution 2010;1:25–37. http://doi.wiley.com/10.1111/j.2041-210X.2009.00007.x.

Barry RG. The status of research on glaciers and global glacier recession: a review. Progress in Physical Geography 2006;30:285–306. http://ppg.sagepub.com/cgi/doi/10.1191/0309133306pp478ra.

Battarbee RW, Thompson Roy, Catalan J, Grytnes J. Climate variability and ecosystem dynamics of remote alpine and arctic lakes: the MOLAR project. Journal of Paleolimnology 2002;28:1–6.

Bellard C, Bertelsmeier C, Leadley P, Thuiller W, Courchamp F. Impacts of climate change on the future of biodiversity. Ecology Letters 2012;15:365–77.

Beniston M. Climate change in mountain regions: A review of possible impacts. Climatic Change 2003;59:5–31.

Bernhardt ES, Lutz BD, King RS, Fay JP, Carter CE, Helton AM, Campagna D, Amos J. How many mountains can we mine? Assessing the regional degradation of Central Appalachian rivers by surface coal mining. Environmental Science & Technology 2012;46:8115–22. http://www.ncbi.nlm.nih.gov/pubmed/22788537.

Braioni MG, Gelmini D. Rotiferi Monogodonti (Rotatoria: Monogodonta). Guide per il riconoscimento delle specie animali delle acque interne italiane, 23. CNR AQ/1/200: 180 pp; 1983.

Brown LE, Hannah DM. Spatial heterogeneity of water temperature across an alpine river basin. Hydrological Processes 2008;22:954–67.

Brown LE, Hannah DM, Milner AM. Vulnerability of alpine stream biodiversity to shrinking glaciers and snowpacks. Global Change Biology 2007a;13:958–66.

Brown LE, Hannah DM, Milner AM, Soulsby C, Hodson AJ, Brewer MJ. Water source dynamics in a glacierized alpine river basin (Taillon-Gabiétous, French Pyrénées). Water Resources Research 2006a;42:W08404. http://doi.wiley.com/10.1029/2005WR004268.

Brown LE, Milner AM. Rapid loss of glacial ice reveals stream community assembly processes. Global Change Biology 2012;18:2195–204. http://doi.wiley.com/10.1111/j.1365-2486.2012.02675.x.

Brown LE, Milner AM, Hannah DM. Stability and persistence of alpine stream macroinvertebrate communities and the role of physicochemical habitat variables. Hydrobiologia 2006b:159–73.

Brown LE, Milner AM, Hannah DM. Groundwater influence on alpine stream ecosystems. Freshwater Biology 2007b;52:878–90. http://doi.wiley.com/10.1111/j.1365-2427.2007.01739.x.

Bush A, Theischinger G, Nipperess D, Turak E, Hughes L. Dragonflies: climate canaries for river management. Ed. Ralph MacNally. Diversity and Distributions 2013;19:86–97. http://doi.wiley.com/10.1111/ddi.12007.

Cadbury SL, Hannah DM, Milner AM, Pearson CP, Brown LE. Stream temperature dynamics within a New Zealand glacierized river basin. River Research and Applications 2008;89:68–89.

Carpenter SR, Stanley EH, Vander Zanden MJ. State of the World's Freshwater Ecosystems: Physical, Chemical, and Biological Changes. Annual Review of Environment and Resources 2011;36:75–99. http://www.annualreviews.org/doi/abs/10.1146/annurev-environ-021810-094524.

Carrivick JL, Geilhausen M, Warburton J, Dickson NE, Carver SJ, Evans AJ, Brown LE. Contemporary geomorphological activity throughout the proglacial area of an alpine catchment. Geomorphology 2013;188:83–95. http://linkinghub.elsevier.com/retrieve/pii/S0169555X12001559.

Castella E, Adalsteinsson H, Brittain JE, Gislason GM, Lehmann A, Lencioni V, et al. Macrobenthic invertebrate richness and composition along a latitudinal gradient of European glacier-fed streams. Freshwater Biology 2001;46:1811–31.

Catalan J, Camarero L, Felip M, Pla S, Ventura M, Buchaca T, et al. High mountain lakes : extreme habitats and witnesses of environmental changes. Limnetica 2006;25:551–84.

Dahl J. The impact of vertebrate and invertebrate predators on a stream benthic community. Oecologia 1998;117:217–26.

Dickson NE, Carrivick JL, Brown LE. Flow regulation alters alpine river thermal regimes. Journal of Hydrology 2012;464-465:505–16. http://linkinghub.elsevier.com/retrieve/pii/S0022169412006610.

Dodds WK, Clements WH, Gido K, Hilderbrand RH, King RS. Thresholds, breakpoints, and nonlinearity in freshwaters as related to management. Journal of the North American Benthological Society 2010;29:988–97. http://www.bioone.org/doi/abs/10.1899/09-148.1.

Dufrene M, Legendre P. Species assemblages and indicator species : Theneed for a flexible asymmetrical approach. Ecological Monographs 1997;67:345–66.

Duigan CA, Birks HH. The late-glacial and early-Holocene palaeoecology of cladoceran microfossil assemblages at Kråkenes , western Norway , with a quantitative reconstruction of temperature changes. Journal of Paleolimnology 2000;23:67–76.

Dussart B. Les Copépodes des eaux continentales d'Europe occidentale...: Cyclopoïdes et biologie. vol. 2. N. Boubée et Cie; 1969.

Edmundson JM, Koenings JP. The influence of suspended glacial particles on the macro-zooplankton community structure within glacial lakes. Alaska Department of Fish and Game. FRED Division Repoort Series 67. 1986.

Edwards AC, Scalenghe R, Freppaz M. Changes in the seasonal snow cover of alpine regions and its effect on soil processes: A review. Quaternary International 2007;162-163:172–81. http://linkinghub.elsevier.com/retrieve/pii/S1040618206002552.

Faith DP, Norris RH. Correlation of environmental variables with patterns of distribution and abundance of common and rare freshwater macroinvertebrates. Biological Conservation 1989;50:77–98.

Fellman JB, Nagorski S, Pyare S, Vermilyea AW, Scott D, Hood E. Stream temperature response to variable glacier coverage in coastal watersheds of Southeast Alaska. Hydrological Processes 2013:n/a–n/a. http://doi.wiley.com/10.1002/hyp.9742.

Finn DS, Räsänen K, Robinson CT. Physical and biological changes to a lengthening stream gradient following a decade of rapid glacial recession. Global Change Biology 2010;16:3314–26. http://doi.wiley.com/10.1111/j.1365-2486.2009.02160.x.

Fischer JM, Olson MH, Williamson CE, Everhart JC, Hogan PJ, Mack JA, Rose KC, Saros JE, Stone JR, Vinebrooke RD. Implications of climate change for Daphnia in alpine lakes : predictions from long-term dynamics, spatial distribution, and a short-term experiment. Hydrobiologia 2011;676:263–77.

Füreder L. Life at the edge: habitat condition and bottom fauna of alpine running waters. International Review of Hydrobiology 2007;92:491–513.

Füreder L, Wallinger M, Burger R. Longitudinal and seasonal pattern of insect emergence in alpine streams. Aquatic Ecology 2005;39:67–78.

Grunewald K, Scheithauer J. Europe's southernmost glaciers: response and adaptation to climate change. Journal of Glaciology 2010;56:129–42.

Haeberli W, Hoelzle M, Paul F, Zemp M, Zu C-. Integrated monitoring of mountain glaciers as key indicators of global climate change : the European Alps. Annals Of Glaciology 2007;46:150–60.

Hannah DM, Gurnell AM. A conceptual, linear reservoir runoff model to investigate melt season changes in cirque glacier hydrology. Journal of Hydrology 2001;246:123–41. http://linkinghub.elsevier.com/retrieve/pii/S002216940100364X.

Hannah DM, Gurnell AM, Mcgregor GR. Spatio-temporal variation in microclimate, the surface energy balance and ablation over a cirque glacier. International Journal of Climatology 2000;20:733–58. http://doi.wiley.com/10.1002/1097-0088%2820000615%2920%3A7%3C733%3A%3AAID-JOC490%3E3.0.CO%3B2-F.

Hannah DM, Malcolm IA, Bradley C. Seasonal hyporheic temperature dynamics over riffle bedforms. Hydrological Processes 2009;23:2178– 2194.

Harding JP, Smith WA. A key to the British freshwater cyclopid and calanoid copepods: with ecological notes. vol. 18. Freshwater Biological Association; 1974.

Holm TM, Koinig K a., Andersen T, Donali E, Hormes A, Klaveness D, Psenner R. Rapid physicochemical changes in the high Arctic Lake Kongressvatn caused by recent climate change. Aquatic Sciences 2011;74:385–95. http://link.springer.com/10.1007/s00027-011-0229-0.

Holzapfel AM, Vinebrooke RD. Environmental warming increases invasion potential of alpine lake communities by imported species. Global Change Biology 2005;11:2009–15. http://dx.doi.org/10.1111/j.1365-2486.2005.001057.x.

Hylander S, Jephson T, Lebret K, Von Einem J, Fagerberg T, Balseiro E, et al. Climate-induced input of turbid glacial meltwater affects vertical distribution and community composition of phyto- and zooplankton. Journal of Plankton Research 2011;33 1239–48. http://plankt.oxfordjournals.org/content/33/8/1239.abstract.

Ilg C, Castella E. Patterns of macroinvertebrate traits along three glacial stream continuums. Freshwater Biology 2006;51:840–53.

Jacobsen D, Milner AM, Brown LE, Dangles O. Biodiversity under threat in glacier-fed river systems. Nature Climate Change 2012;2:361–4.

Kernan M, Ventura M, Bitusik P, Brancelj A, Clarke G, Velle G, Raddum GG, Stuchlik E, Catalan J. Regionalisation of remote European mountain lake ecosystems according to their biota: environmental versus geographical patterns. Freshwater Biology 2009;54:2470–93. http://dx.doi.org/10.1111/j.1365-2427.2009.02284.x.

Khamis K, Hannah DM, Hill Clarvis M, Brown LE, Castella E, Milner AM. Alpine aquatic ecosystem conservation policy in a changing climate. Environmental Science & Policy 2013. http://linkinghub.elsevier.com/retrieve/pii/S1462901113002128.

Knapp RA, Corn PS, Schindler DE. The introduction of nonnative fish into wilderness lakes: good intentions, conflicting mandates, and unintended consequences. Ecosystems 2001a;4:275–8. http://dx.doi.org/10.1007/s10021-001-0009-0.

Knapp RA, Matthews KR, Sarnelle O. Resistance and resilience of alpine lake fauna tofish introductions. Ecological Monographs 2001b;71:401–21.

Korponai J, Magyari EK, Buczkó K, Iepure S, Namiotko T, Czakó D, Kövér C, Braun M. Cladocera response to Late Glacial to Early Holocene climate change in a South

Carpathian mountain lake. Hydrobiologia 2011;676:223–35. http://link.springer.com/10.1007/s10750-011-0881-3.

Kratz KW. Effects of stoneflies on local prey populations : mechanisms of impact across prey density. Ecology 1996;77:1573–85.

Lancaster J. Predation and drift of lotic macroinvertebrates during colonization. Oecologia 1990;85:48–56. http://www.springerlink.com/index/10.1007/BF00317342.

Leonard EM, Reasoner MA. A Continuous Holocene Glacial Record Inferred from Proglacial Lake Sediments in Banff National Park, Alberta, Canada. Quaternary Research 1999;51:1–13. http://www.sciencedirect.com/science/article/pii/S0033589498920099.

Livingstone DM, Lotter AF, Walker IR. The decrease in summer surface water temperature with altitude in Swiss Alpine lakes: a comparison with air temperature lapse rates. Arctic, Antarctic, and Alpine Research 1999;31:341–52.

Magnea U, Sciascia R, Paparella F, Tiberti Rocco, Provenzale A. A model for high-altitude alpine lake ecosystems and the effect of introduced fish. Ecological Modelling 2013;251:211–20. http://www.sciencedirect.com/science/article/pii/S0304380012005947.

Malard F, Uehlinger U, Zah R, Tockner K. Flood-Pulse and riverscape dynamics in a braided glacial river. Ecology 2006;87:704–16.

Margaritora F V. Fauna d’Italia. Cladocera. Edizioni Calderoni Bologna; 1985.

Mcintosh AR, Peckarsky BL. Criteria determining behavioural respones to multiple predators by a stream mayfly. Oikos 1990;85:554–6.

Mcintosh AR, Taylor BW. Rapid size-specfic changes in the drift of Baetis bicaudatus (Ephemeroptera) caused by alterations in fish odour concentration. Oecologia 1999;118:256–64.

Mendoza G, Catalan J. Lake macroinvertebrates and the altitudinal environmental gradient in the Pyrenees. Hydrobiologia 2010;648:51–72. http://link.springer.com/10.1007/s10750-010-0261-4.

Menezes S, Baird DJ, Soares AMVM. Beyond taxonomy : a review of macroinvertebrate trait-based community descriptors as tools for freshwater biomonitoring. Journal of Applied Ecology 2010;47:711–9.

Milner AM, Brittain JE, Castella E, Petts GE. Trends of macroinvertebrate community structure in glacier-fed rivers in relation to environmental conditions: a synthesis. Freshwater Biology 2001;46:1833–47. http://doi.wiley.com/10.1046/j.1365-2427.2001.00861.x.

Milner AM, Brown LE, Hannah DM. Hydroecological response of river systems to shrinking glaciers. Hydrological Processes 2009;77:62–77.

Milner AM, Petts GE. Glacial rivers: physical habitat and ecology. Freshwater Biology 1994;32:295–307.

Muhlfeld CC, Giersch JJ, Hauer FR, Pederson GT, Luikart G, Peterson DP, Downs CC, Fagre DB. Climate change links fate of glaciers and an endemic alpine invertebrate. Climatic Change 2011;106:337–45.

Müller-Liebenau I. Revision der europaischen Arten der Gattung </i>Baetis</i> LEACH, 1815 (Insecta, Ephemeroptera). Gewasser und Abwasser 48/49. Göttingen. 1969.

Oerlemans J. Extracting a climate signal from 169 glacier records. Science 2005;308:675–7. http://www.ncbi.nlm.nih.gov/pubmed/15746388.

Ohmura A. Enhanced temperature variability in high-altitude climate change. Theoretical and Applied Climatology 2012;110:499–508. http://www.springerlink.com/index/10.1007/s00704-012-0687-x.

Peckarsky BL, Taylor BW, Mcintosh AR, Mcpeek MA, Lytle DA, Ecology S, Mar N. Variation in mayfly size at metamorphosis as a developmental response to risk of predation. Ecology 2001;82:740–57.

Pfankuch D. Stream Reach Inventory and Channel Stability Evaluation. United States Department of Agriculture Forest Service. United States Department of Agriculture Forest Service, Region 1, Missoula, MT, U.S.A; 1975.

Qian SS, King RS, Richardson CJ. Two statistical methods for the detection of environmental thresholds. Ecological Modelling 2003;166:87–97. http://linkinghub.elsevier.com/retrieve/pii/S0304380003000978.

Quilty E, Moore RD. Measuring stream temperature. Watershed Management Bulletin 2007;10:25–30.

Richards J, Moore RD, Forrest A. Late-summer thermal regime of a small proglacial lake. Hydrological Processes 2012;26:2687–95. http://doi.wiley.com/10.1002/hyp.8360.

Rogora M, Massaferro J, Marchetto A, Tartari G, Mosello R. The water chemistry of some shallow lakes in Northern Patagonia and their nitrogen status in comparison with remote lakes in different regions of the globe. Journal of Limnology 2008;67:75–86.

Rossaro B, Lencioni V, Boggero A, Marziali L. Chironomids from southern alpine running waters: ecology, biogeography. Hydrobiologia 2006;562:231–46. http://www.springerlink.com/index/10.1007/s10750-005-1813-x.

Del Río M, Rico I, Serrano E, Tejado JJ. GPR Prospection in the Ossoue Glacier (Pyrenees). In: . 14th International Conference on Ground Penetrating Radar (GPR). 2012. p. 684–8.

Schabetsberger R, Luger M, Drozdowski G, Jagsch A. Only the small survive: monitoring long-term changes in the zooplankton community of an Alpine lake after fish introduction. Biological Invasions 2009;11:1335–1345 LA – English. http://dx.doi.org/10.1007/s10530-008-9341-z.

Siddon E, Duffy-Anderson J, Mueter F. Community-level response of fish larvae to environmental variability in the southeastern Bering Sea. Marine Ecology Progress Series 2011;426:225–39. http://www.int-res.com/abstracts/meps/v426/p225-239/.

Slemmons KEH, Saros JE. Implications of nitrogen-rich glacial meltwater for phytoplankton diversity and productivity in alpine lakes. Limnol. Oceanogr. 2012;57:1651–63. http://www.aslo.org/lo/toc/vol_57/issue_6/1651.html.

Smith BPG, Hannah DM, Gurnell AM. A hydrogeomorphological context for ecological research on alpine glacial rivers. Freshwater Biology 2001;46:1579–96.

Snook D L, Milner AM. The influence of glacial runoff on stream macroinvertebrate communities in the Taillon catchment, French Pyrénées. Freshwater Biology 2001;46:1609–23.

Stahl K, Moore RD, Shea JM, Hutchinson D, Cannon AJ. Coupled modelling of glacier and streamflow response to future climate scenarios. Water Resources 2008;44:1–13.

Sueker JK, Ryan JN, Kendall C, Jarrett RD, Alpine A, National M. Determination of hydrologic pathways during snowmelt for alpine/subalpine basins, Rocky Mountain National Park, Colorado. Water Resources 2000;36:63–75.

Tachet H, Richoux P, Bournard M, Usseglio-Polatera P. Invertebres d’eau douce. Systematique, Biologie, Ecologie. CNRS Publishers: Paris; 2000.

Thompson R, Kamenik C, Schmidt R. Ultrasensitive alpine lakes and climate change. J. Limnol 2005;64:139–152.

Tiberti R, Holzapfel AM, Vinebrooke RD. Geomorphology and hydrochemistry of 12 Alpine lakes in the Gran Paradiso National Park, Italy. Journal of Limnology 2010;69:242–56.

Webb BW, Hannah DM, Moore RD, Brown LE, Nobilis F. Recent advances in stream and river temperature research. Hydrological Processes 2008;22:902–18.

Zemp M, Haeberli W, Hoelzle M, Paul F. Alpine glaciers to disappear within decades? Geophysical Research Letters 2006;33:6–9.

Zuur AF, Ieno EN, Walker N, Saveliev AA, Smith G. Mixed effects models and extensions in ecology with R. Genetics. Springer, New York; 2009.

Zwick P. Key to the west palaearctic genera of stoneflies (Plecoptera) in the larval stage. Limnologica 2004;34:315–48. http://linkinghub.elsevier.com/retrieve/pii/S0075951104800045.

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Table 1. Stream sampling reach physical habitat characteristics for the Pyrénées study.

Basin	**Stream**	**Site**	**Catchment area (km^2)**	**Glacier cover (%)**	**Distance from glacier (km)**	**Altitude (m)**	**Slope**
Taillon- Gabiétous (Calcareous)	*Tourettes*	G1	1.10	7.2	1.0	2150	0.10
		G2	1.60	5.0	1.4	2030	0.10
		G3	3.60	2.2	2.1	1900	0.04
		G4	4.65	1.7	2.8	1850	0.04
	Taillon	T1	0.28	32.1	0.3	2560	0.10
		T2	0.61	14.8	1.0	2150	0.15
		T3	1.72	5.2	1.4	1870	0.06
		T4	6.27	2.7	1.7	1850	0.05
		T5	6.70	2.5	2.2	1800	0.05
Ossoue (Metamorphic)	*Oulettes d'Ossoue*	O1	1.70	24.7	1.0	2250	0.20
		O2	5.01	8.4	1.9	2005	0.08
		O3	6.46	6.5	2.5	1870	0.03
Vignemale (Igneous)	*Gave des Oulettes*	V1	1.20	10.8	0.7	2250	0.11
		VHS	1.30	2.3	1.3	2150	0.06
		V2	5.77	2.3	2.1	2050	0.04
		V3	9.40	1.4	3.2	1980	0.04
		V4	11.60	1.1	4.5	1820	0.04

Table 2. Morphometric data of PNGP lakes and catchment characteristics Zm: maximum depth; A: area; L: perimeter; V: volume; Zr: relative depth; Z_{med}: average depth; DL: shore development; DV: volume development; B: catchment area; B/A: B/A ratio. Geology - AC: catchment entirely composed by Acidic Gneiss; geology - CS: catchment dominated by thick covering of Calcareous Schists. N.B. Nivolet Superiore – NIVSUP, Trebecchi Superiore – TRESUP, Losere – LOSERE, Lillet – LILLET, Motta – MOTTA, Leità – LEITA, Nero (in Leynir Valley) – NERO.

Lake	**Geology**	**Altitude (m)**	**A (m^2)**	**V (10^3 m^3)**	**Zm (m)**	**Zr (%)**	**Z_{med} (m)**	**DL**	**DV**	**B (ha)**	**B/A**	**Prairies (%)**	**Debris (%)**	**Rocks (%)**	**Glaciers (%)**	**Water (%)**
NIVSUP	*AG*	2538	34482	162.1	17.1	8.16	4.7	1.5	0.27	29.11	8.44	12.94	55.6	19.63	0	11.85
TRESUP	*CS*	2729	14172	48.9	7.5	5.58	3.45	1.34	0.46	23.66	16.69	77.59	6.37	11.58	0	4.46
LILLET	*AG*	2765	36249	233.1	13.2	6.14	6.43	1.25	0.49	91.86	25.34	0	41.37	33.85	20.84	3.95
MOTTA	*AG*	2656	101396	1257.4	51	14.19	12.4	1.97	0.24	289.87	28.59	0	43.47	48.8	4.03	3.7
LEITA	*CS*	2701	62171	244.6	11	3.91	3.93	2.25	0.36	315.59	50.76	8.55	53.02	26.7	9.36	2.37
LEYNIR	*CS*	2747	44691	466.4	22.1	9.26	10.44	1.28	0.47	156.47	35.01	22.34	50.38	20.82	3.63	2.84
NERO	*CS*	2671	17121	41.6	6	4.06	2.43	1.18	0.41	86.55	50.55	10.37	70.91	16.75	0	1.98

Table 3. GLMM model results for selected macroinvertebrate taxa.

Taxa	**Predictor**	**AIC**	**ΔAIC**	***t***	***P***
Baetis alpinus					
	Meltwater (^2)	999.5		1.4 (-2.1)	0.14 (0.03)
	Glacier cover	995.2		-3.2	0.0014
Perla grandis					
	Glacier cover	284.0		-4.0	***
	Meltwater	294.6		-4.0	***
Protonemura sp.					
	Meltwater	624.7	0.0	-5.6	***
	Glacier cover	616.1		-4.4	***
Rhithrogena loyolea					
	Glacier cover	607.7	0.0	-3.9	***
	Meltwater	616.3		-3.2	0.0014
Microspectra spp.					
	Glacier cover	380.9	0.0	-3.5	***
	Meltwater	377.3	0.6	-7.0	***
Diamesa latitarsis					
	Glacier cover	719.6	0.0	3.67	***
	Meltwater	728.1	1.8	2.2	*
Diamesa cinerella					
	Glacier cover	892.2		-	NS
	Meltwater	52.6		-	NS

Table 4. Spearman's correlation coefficients of mean taxa abundance and glacier cover in the catchment for lake samples from GPNP ($^{*}0.05 < P$, $^{**}0.01 < P$).

Taxa	***r***
Plecoptera	0.56^{**}
Coleoptera	-0.45
Heteroptera	-0.65
Planaria	0.38^{**}
Keratella quadrata	-0.04
Daphnia longispina	-0.52^{*}

Table 5. Threshold indicator taxa analysis community level results from runs using the two methods for quantifying glacier influence. Observed change points (Obs) and 5th and 95th quantiles of bootstrapped change points correspond to the value resulting in the largest sum of indicator value (IndVal) z scores for z- and z+ taxa. Tor nCPA thresholds correspond to the maximum deviance reduction (Bray-Curtis distance).

		Community change point		
Glacier gradient	**Method**	**Obs**	**0.05**	**0.95**
Glacier cover				
	sumz-	5.1	2.8	6.5
	sumz+	2.7	1.7	9.7
	nCPA	5.1	5.0	7.3
Meltwater				
	sumz-	67.5	54.7	78.5
	sumz+	51.7	37.8	75.5
	nCPA	66.6	60.8	79.8

Fig. 1. Map displaying: (a) the location of the study catchments in the French Pyrénées; and (b) the location of lake survey sites in the Italian Alps. In (a) glaciers are marked but are not to scale.

Fig. 2. Water temperature records from: (a) Gran Paradiso National Park (September 2009 - August 2010) for Lake Leynir 3.6 % glacier cover in catchment (GCC); Lake Tribecchi superiore no GCC; Lake Lillet 20.8 % GCC; and (b) stream sites in the Pyrénées between September 2011 and September 2012 (T3 5.7 % GCC, G4 1.7 % GCC and Spr 1 no GCC).

Fig. 3. NMDS ordination of: (a) $\log_{10}$ (x+1) transformed community data (stress = 0.16) shapes represent cluster group membership; and (b) post-hoc vector fit of physico-chemical habitat variables. Vectors lengths are scaled relative to their correlation coefficient.

Fig.4. Box plots of (a) meltwater contribution (%) and (b) glacier cover (%) by cluster group membership.

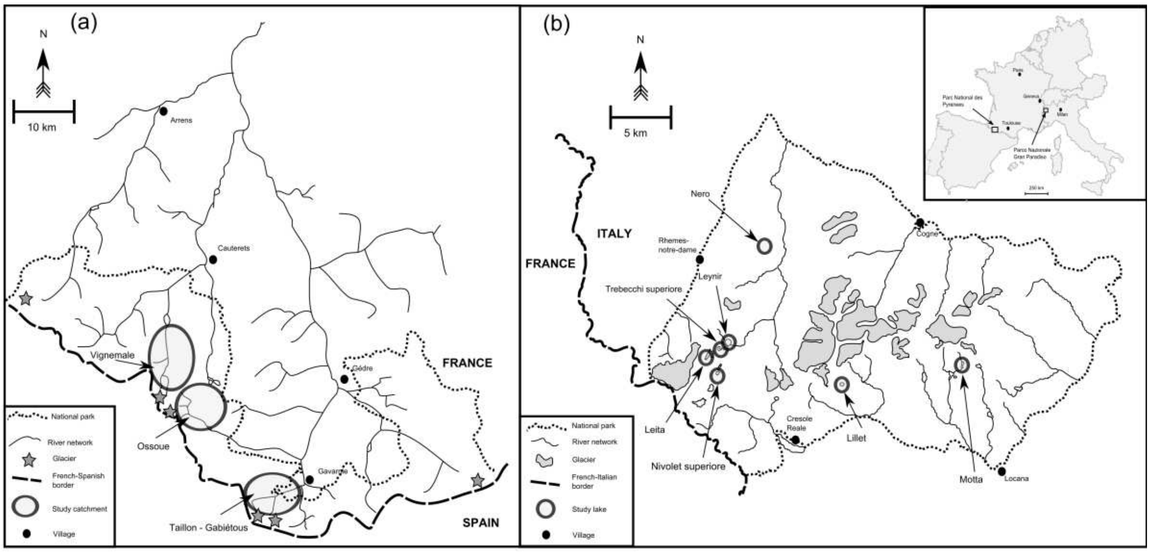
Fig. 5. NMDS ordination of $\log_{10}$ (x+1) transformed community data (stress = 0.04).Open squares represent lakes with fish present and filled squares lakes with fish absent. Glacier cover in the catchment (%) is displayed in parentheses.

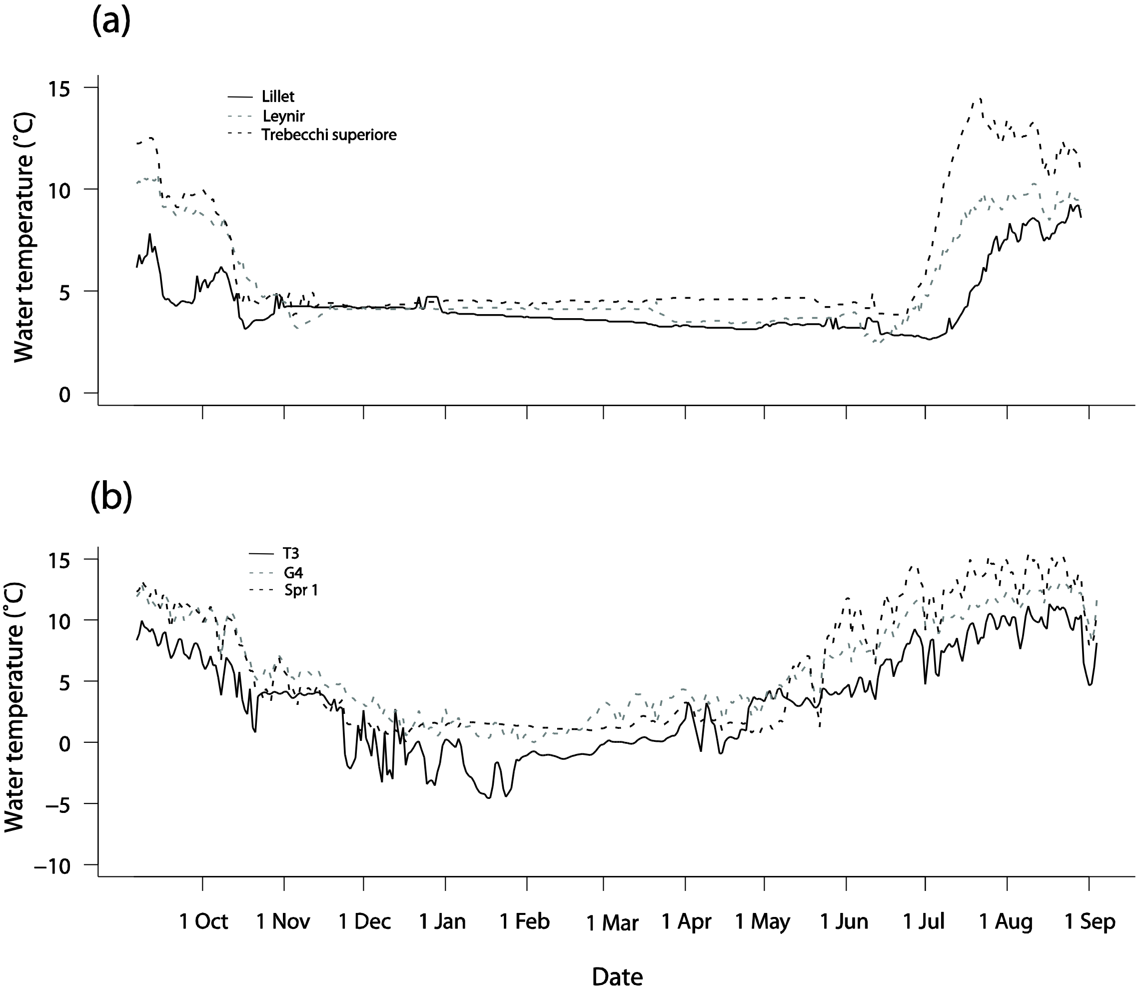
Fig. 6. Relationship between taxonomic richness (PNP stream sites) and: (a) meltwater contribution (%); and (b) glacier cover in the catchment (%). Line of best fit is a LOESS smoother (span = 0.5). Relationship between glacier cover (GPNP lake sites) and: (c) zooplankton taxonomic richness; and (d) Crustacean taxonomic richness. Line of best fit is a LOESS smoother (span = 0.8).

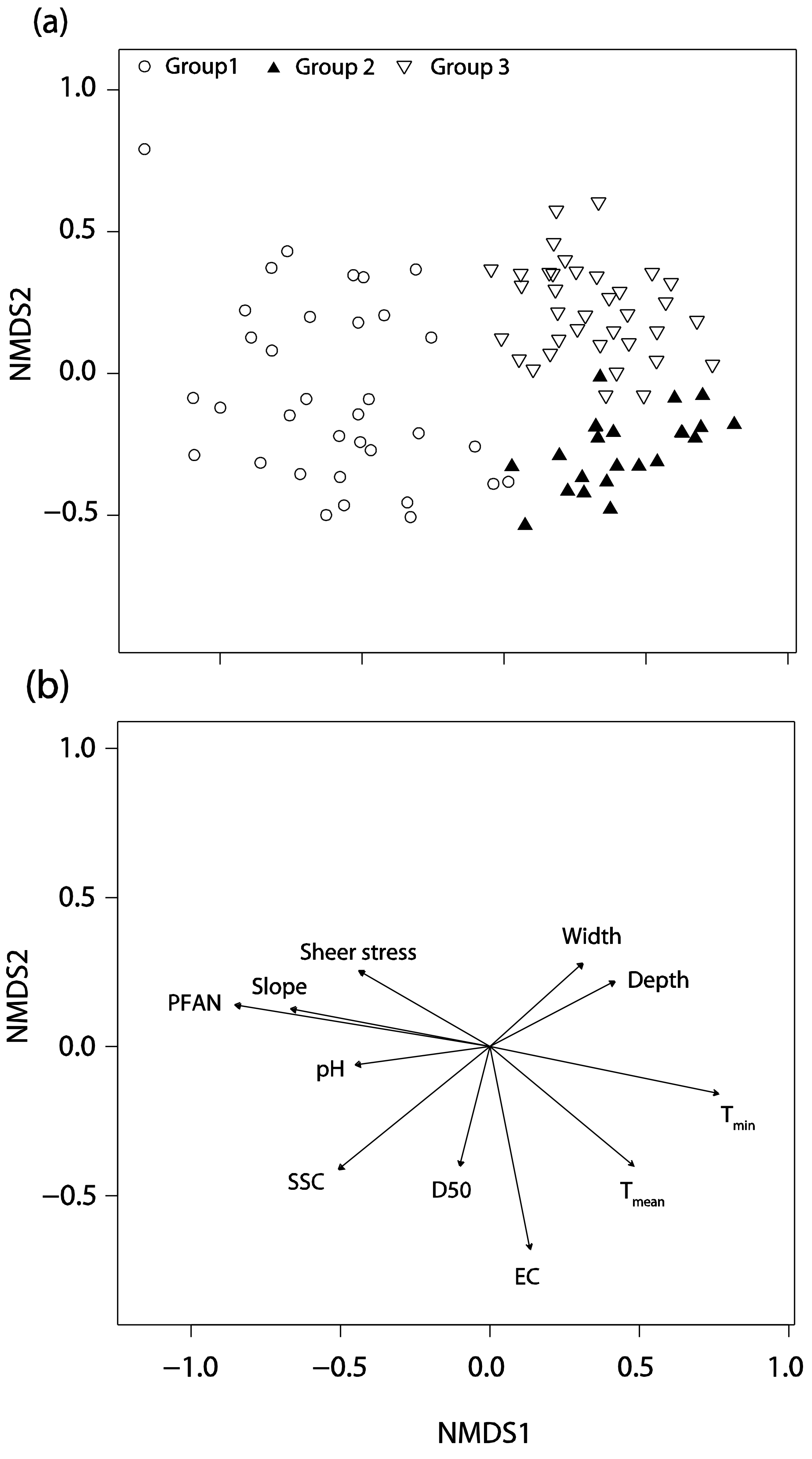
Fig. 7. Relationships between the most abundant indicator orders/taxa and (a) meltwater contribution (%) and (b) glacier cover (%). All panels are fitted with a LOESS smoother (span = 0.5)., *Leuctra fusca* gr. is a species complex outlined by Zwick (2004).

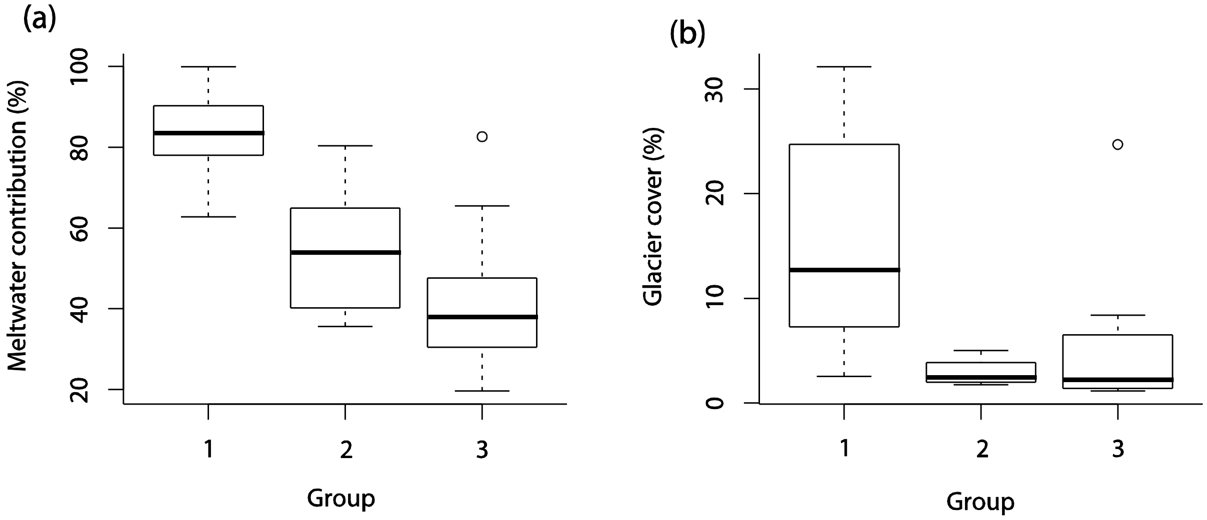
Fig. 8. Abundance (±CI 95) of (a) Plecoptera, (b) Heteroptera, (c) Planaria and (d) Daphnia longispina for the study lakes in GPNP. Lakes with fish present are displayed in italics.

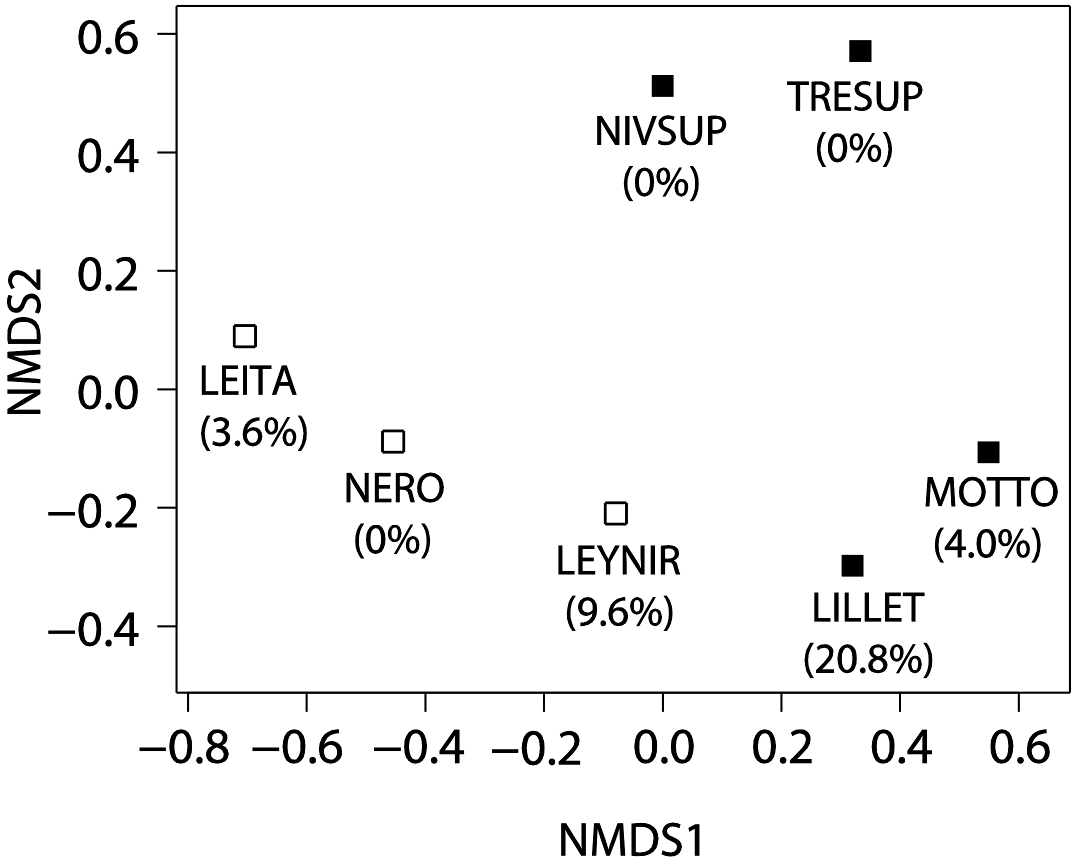
Fig. 9. Significant indicator taxa (TITAN, $P < 0.05$, purity > 0.95) identified across the habitat gradient defined by (a) meltwater contribution and (b) glacier cover in the catchment. Black symbols represent negative indicator taxa (i.e. abundance declines) and grey corresponds to positive taxa (i.e. abundance increases. Change point symbols are sized in proportion to response (z scores). Horizontal lines represent 5th and 95th percentiles from 500 bootstrap replicates. See Appendix 1 for full taxonomic names. Change-point analysis (nCPA, Bray-Curtis distance) of macroinvertebrate community response to (c) meltwater contribution (%) and (d) glacier cover (%).

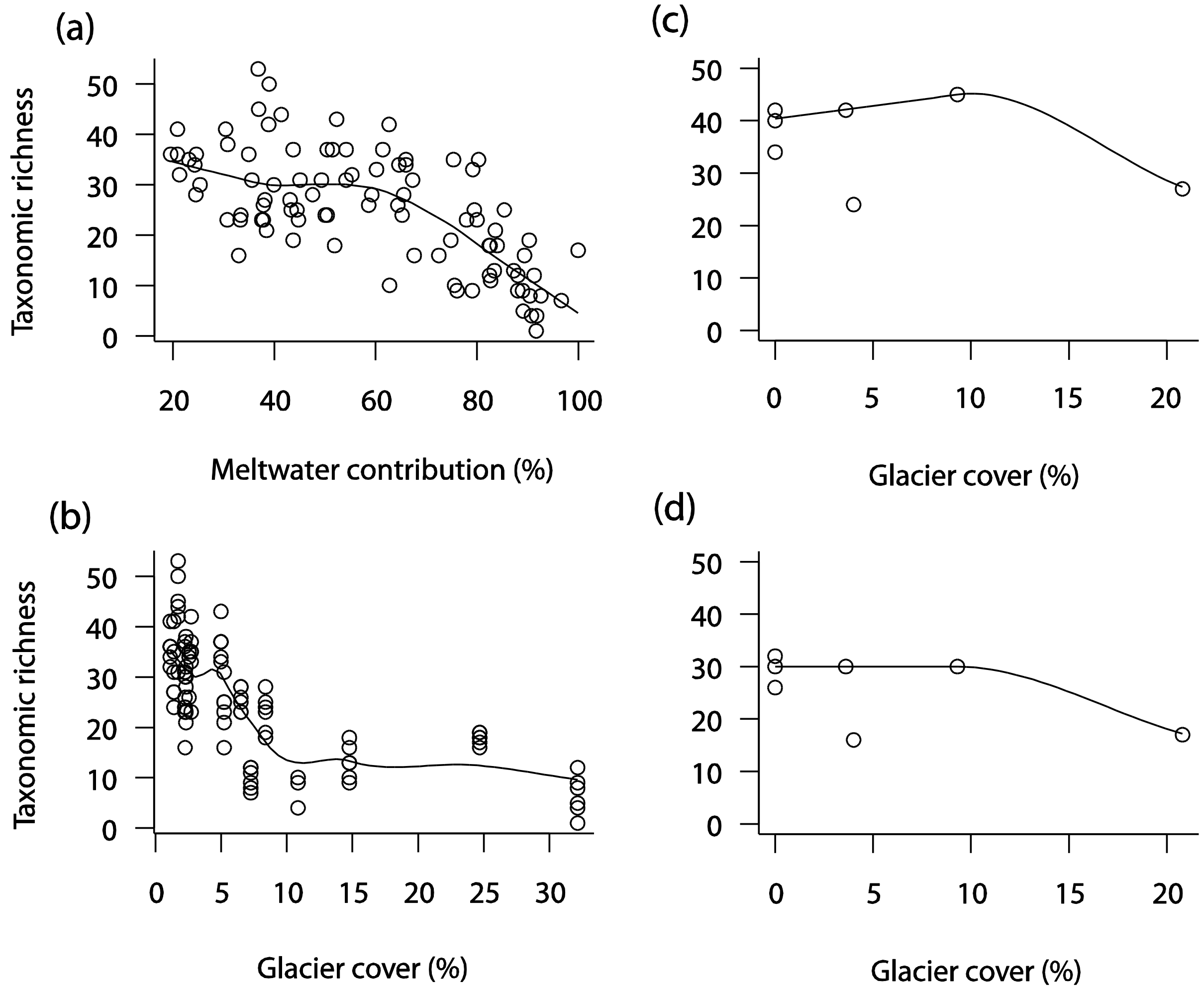

(a)
N
10 km
Arrens
Cauterets
Vignemale
Ossoue
Gèdre
FRANCE
Gavarnie
Taillon - Gabiétous
SPAIN
National park
River network
Glacier
French-Spanish border
Study catchment
Village
(b)
N
5 km
Nero
ITALY
Rhemes-notre-dame
FRANCE
Leynir
Trebecchi superiore
Leita
Nivolet superiore
Cresole Reale
Lillet
Cogne
Motta
Locana
National park
River network
Glacier
French-Italian border
Study lake
Village
Paris
Geneva
Parc National des Pyrenees
Toulouse
Milan
Parco Nazionale Gran Paradiso
250 km

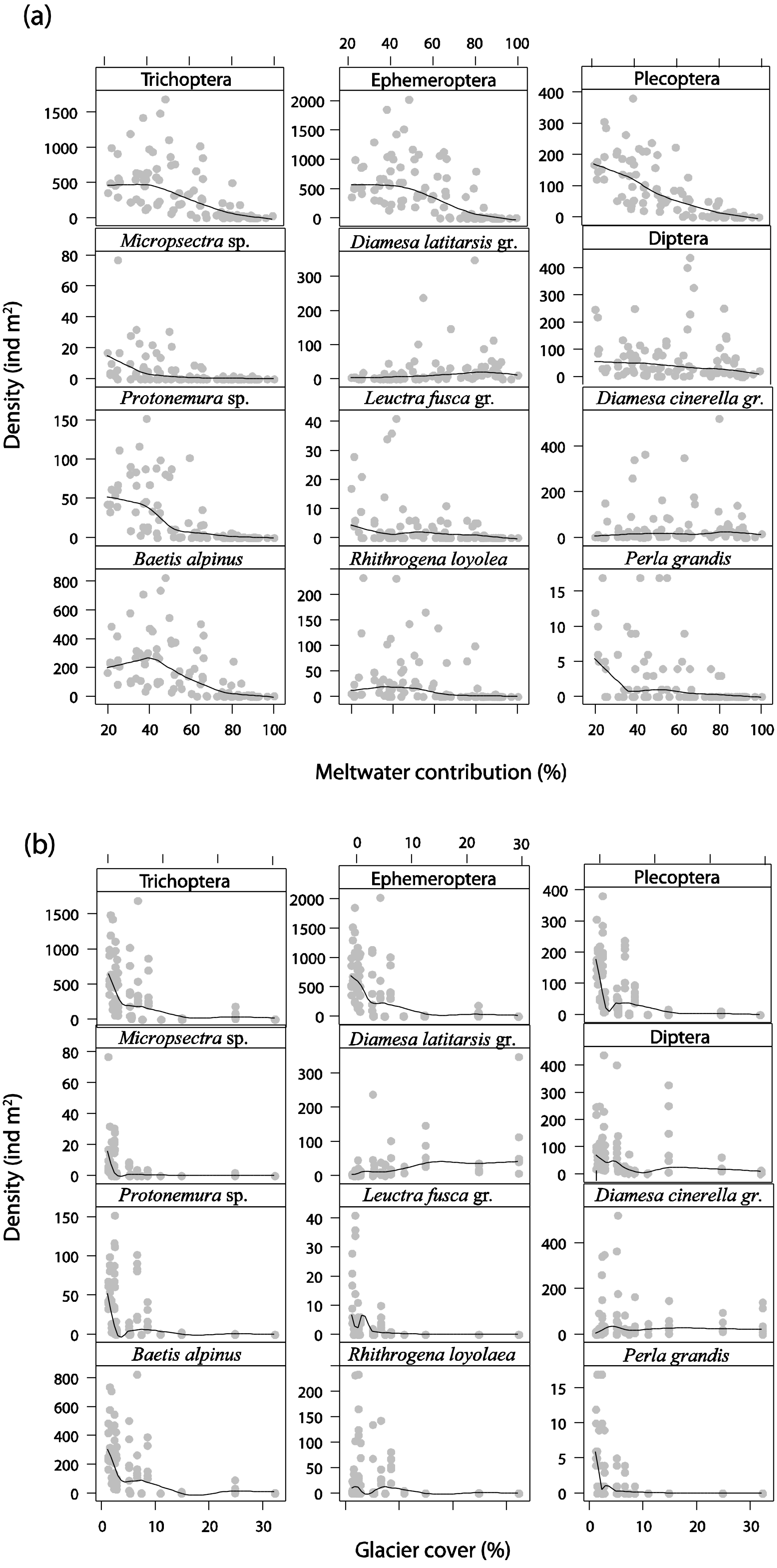

(a)
Lillet
Leynir
Trebecchi superiore
Water temperature (°C)
15
10
5
0
(b)
T3
G4
Spr 1
Water temperature (°C)
15
10
5
0
−5
−10
1 Oct
1 Nov
1 Dec
1 Jan
1 Feb
1 Mar
1 Apr
1 May
1 Jun
1 Jul
1 Aug
1 Sep
Date

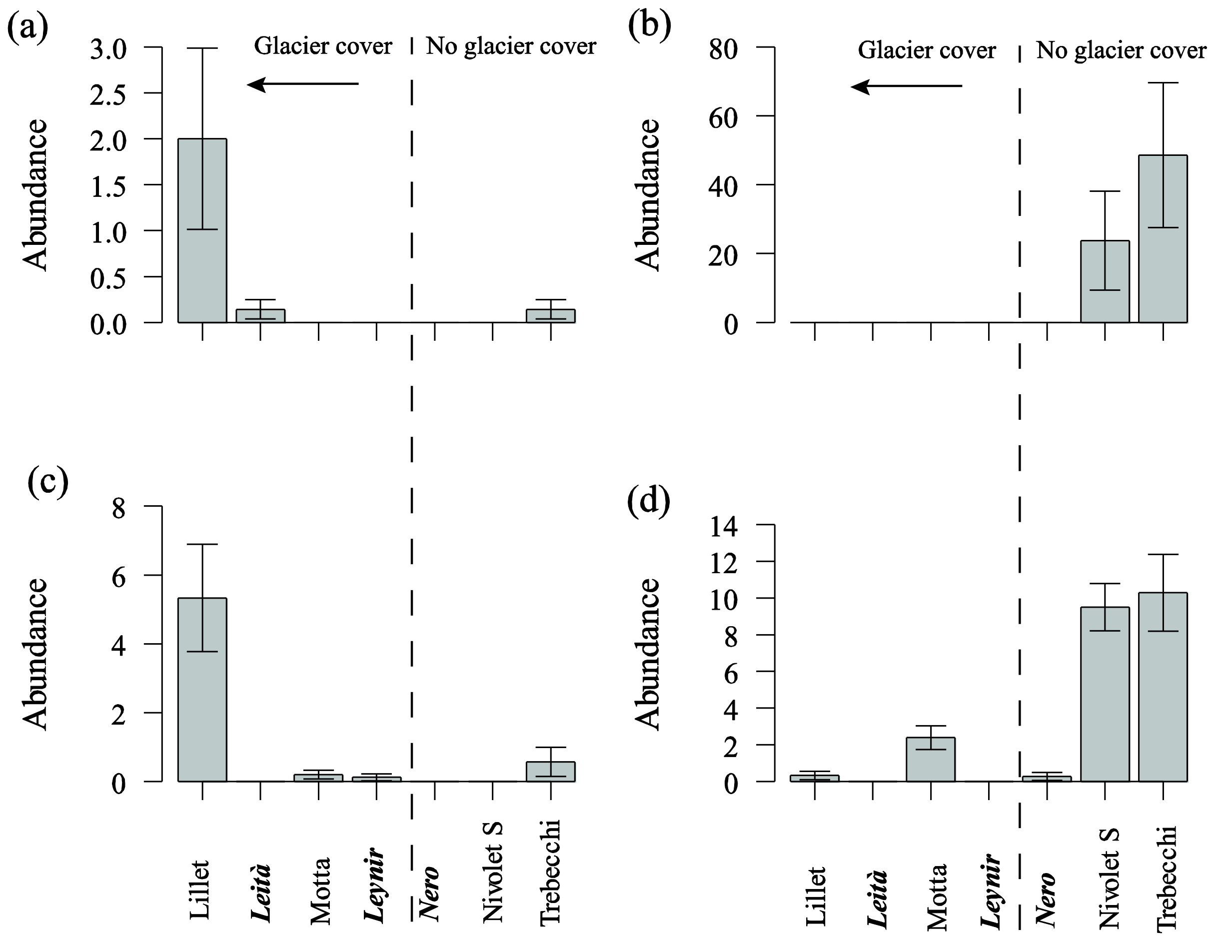

(a)
Group1
Group 2
Group 3
1.0
0.5
0.0
−0.5
NMDS2
(b)
1.0
0.5
0.0
−0.5
NMDS2
Sheer stress
Width
Depth
Slope
PFAN
pH
T_min
SSC
D50
T_mean
EC
−1.0
−0.5
0.0
0.5
1.0
NMDS1

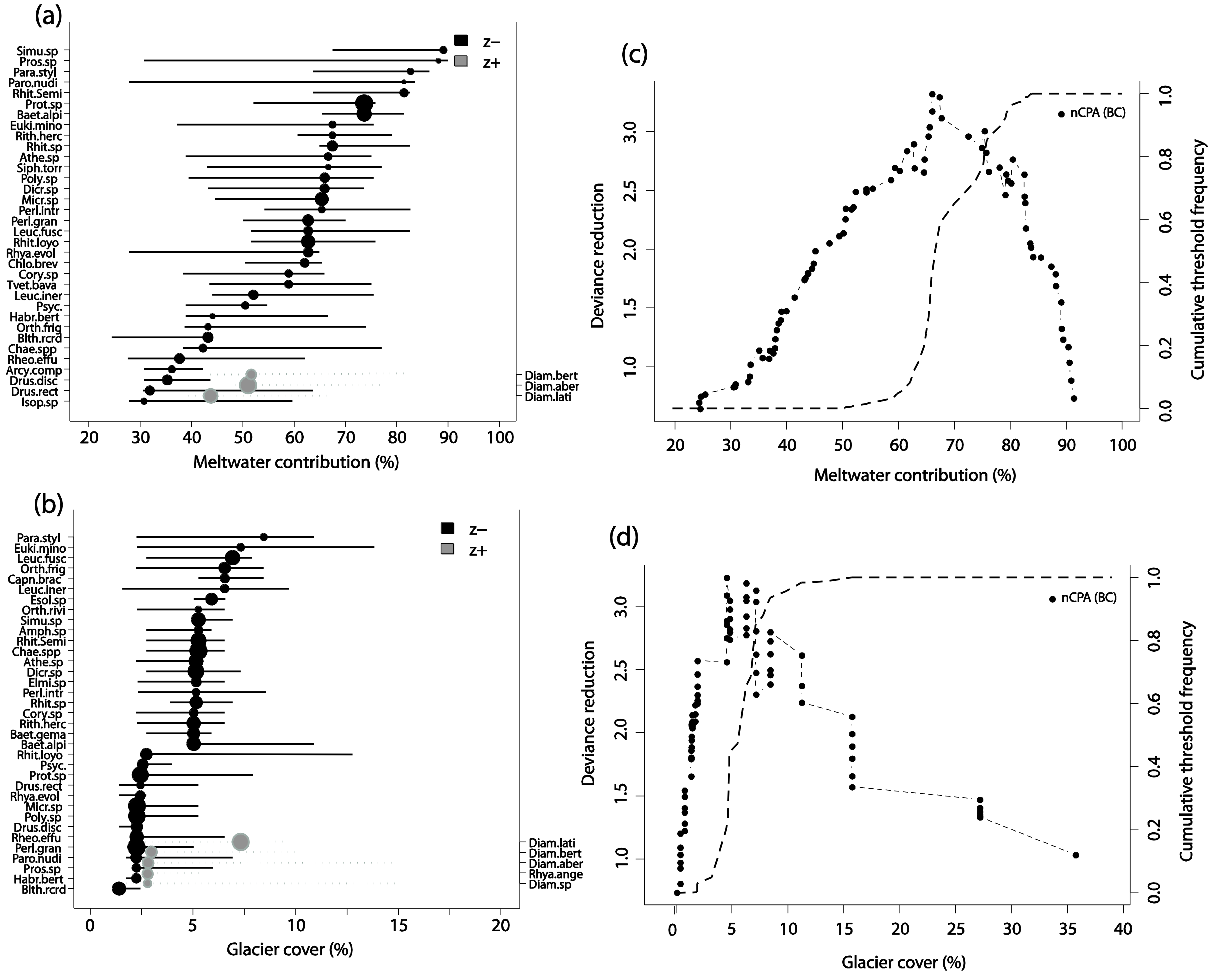

(a)
Meltwater contribution (%)
100
80
60
40
20
1
2
3
Group
(b)
Glacier cover (%)
30
20
10
0
1
2
3
Group

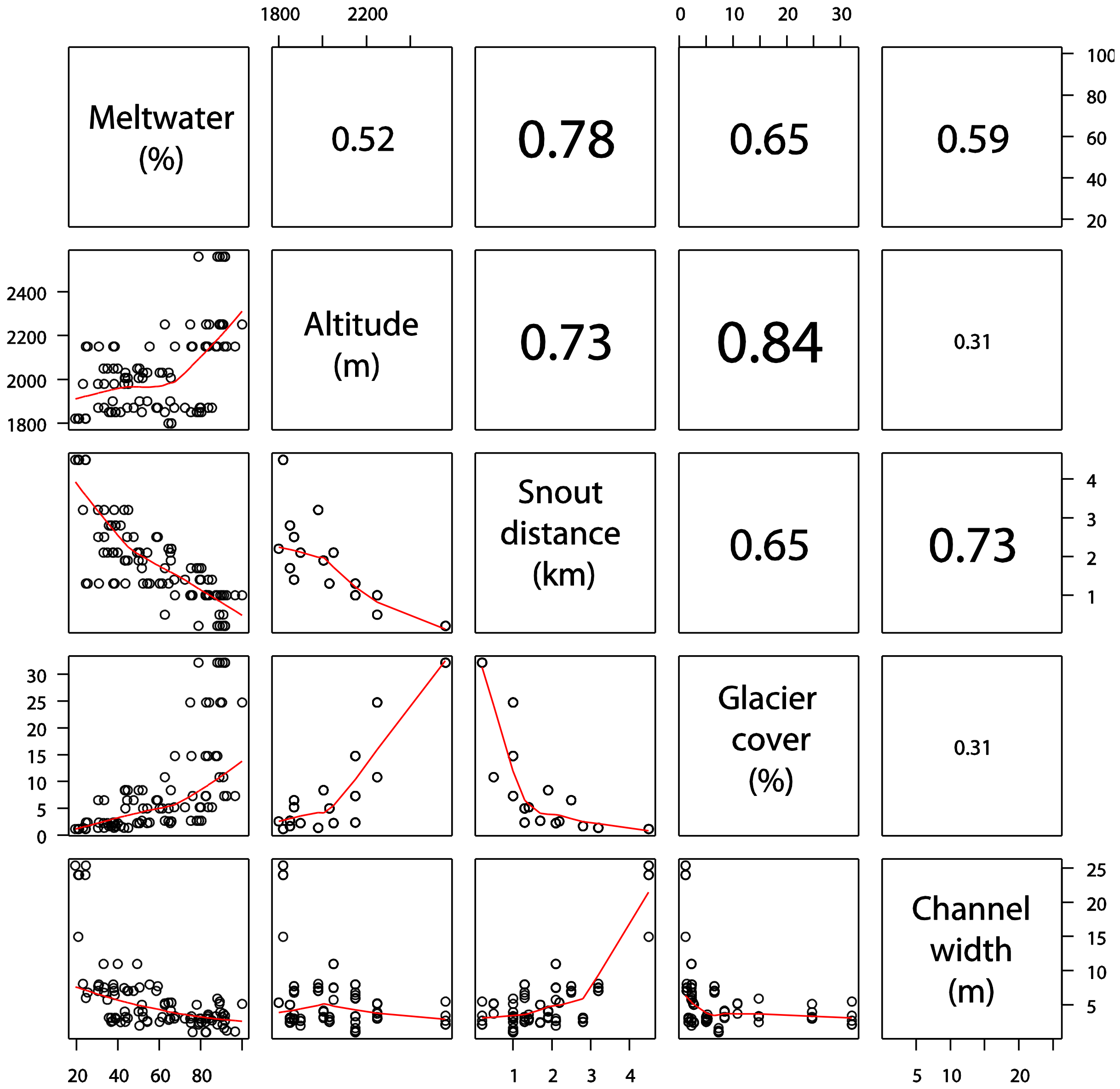

NIVSUP
(0%)
TRESUP
(0%)
LEITA
(3.6%)
NERO
(0%)
LEYNIR
(9.6%)
LILLET
(20.8%)
MOTTO
(4.0%)
NMDS2
0.6
0.4
0.2
0.0
−0.2
−0.4
−0.8
−0.6
−0.4
−0.2
0.0
0.2
0.4
0.6
NMDS1


(a)
Taxonomic richness
0
10
20
30
40
50
20
40
60
80
100
Meltwater contribution (%)
(b)
Taxonomic richness
0
10
20
30
40
50
0
5
10
15
20
25
30
Glacier cover (%)
(c)
0
10
20
30
40
50
0
5
10
15
20
Glacier cover (%)
(d)
0
10
20
30
40
50
0
5
10
15
20
Glacier cover (%)


(a)
Trichoptera
Ephemeroptera
Plecoptera
Micropsectra sp.
Diamesa latitarsis gr.
Diptera
Protonemura sp.
Leuctra fusca gr.
Diamesa cinerella gr.
Baetis alpinus
Rhithrogena loyolea
Perla grandis
Density (ind m^2)
Meltwater contribution (%)
20 40 60 80 100
(b)
Trichoptera
Ephemeroptera
Plecoptera
Micropsectra sp.
Diamesa latitarsis gr.
Diptera
Protonemura sp.
Leuctra fusca gr.
Diamesa cinerella gr.
Baetis alpinus
Rhithrogena loyolaea
Perla grandis
Density (ind m^2)
Glacier cover (%)
0 10 20 30


(a)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Abundance
Glacier cover
No glacier cover
(b)
80
60
40
20
0
Abundance
Glacier cover
No glacier cover
(c)
8
6
4
2
0
Abundance
(d)
14
12
10
8
6
4
2
0
Abundance
Lillet
Leità
Motta
Leynir
Nero
Nivolet S
Trebecchi
Lillet
Leità
Motta
Leynir
Nero
Nivolet S
Trebecchi


(a)
z−
z+
Simu.sp
Pros.sp
Para.styl
Paro.nudi
Rhit.Semi
Prot.sp
Baet.alpi
Euki.mino
Rith.herc
Rhit.sp
Athe.sp
Siph.torr
Poly.sp
Dicr.sp
Micr.sp
Perl.intr
Perl.gran
Leuc.fusc
Rhit.loyo
Rhya.evol
Chlo.brev
Cory.sp
Tvet.bava
Leuc.iner
Psyc.
Habr.bert
Orth.frig
Blth.rcrd
Chae.spp
Rheo.effu
Arcy.comp
Drus.disc
Drus.rect
Isop.sp
Diam.bert
Diam.aber
Diam.lati
20
30
40
50
60
70
80
90
100
Meltwater contribution (%)
(b)
z−
z+
Para.styl
Euki.mino
Leuc.fusc
Orth.frig
Capn.brac
Leuc.iner
Esol.sp
Orth.rivi
Simu.sp
Amph.sp
Rhit.Semi
Chae.spp
Athe.sp
Dicr.sp
Elmi.sp
Perl.intr
Rhit.sp
Cory.sp
Rith.herc
Baet.gema
Baet.alpi
Rhit.loyo
Psyc.
Prot.sp
Drus.rect
Rhya.evol
Micr.sp
Poly.sp
Drus.disc
Rheo.effu
Perl.gran
Paro.nudi
Pros.sp
Habr.bert
Blth.rcrd
Diam.lati
Diam.bert
Diam.aber
Rhya.ange
Diam.sp
0
5
10
15
20
Glacier cover (%)
(c)
nCPA (BC)
Deviance reduction
1.0
1.5
2.0
2.5
3.0
Cumulative threshold frequency
0.0
0.2
0.4
0.6
0.8
1.0
20
30
40
50
60
70
80
90
100
Meltwater contribution (%)
(d)
nCPA (BC)
Deviance reduction
1.0
1.5
2.0
2.5
3.0
Cumulative threshold frequency
0.0
0.2
0.4
0.6
0.8
1.0
0
5
10
15
20
25
30
35
40
Glacier cover (%)



Supplementary Figure 1. Scatterplot matrix displaying the relationship between glacier influence (i.e glacier cover and meltwater) and potentially collinear physical variables. The upper panels display the Pearson's correlation coefficient with the font scaled proportional to the strength of the correlation.