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

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1 Abstract

2 In alpine regions climatic change will alter the balance between water sources (rainfall, ice-
3 melt, snowmelt, and groundwater) for aquatic systems, particularly modifying the relative
4 contributions of meltwater, groundwater and rain to both rivers and lakes. Whilst these
5 changes are expected to have implications for alpine aquatic ecosystems, little is known about
6 potential ecological tipping points and associated indicator taxa. Within the EU-FP7
7 ACQWA project we examined changes in biotic communities along a gradient of glacier
8 influence for two study systems; (1) a stream network in the French Pyrénées and (2) a
9 network of lakes in the Italian Alps, with the aim of identifying potential indicator taxa
10 (macroinvertebrates and zooplankton) of glacier retreat in these environments. To assess
11 parallels in biotic responses across streams and lakes, both primary data and findings from
12 other publications were synthesized. Using TITAN (Threshold Indicator Taxa ANalysis)
13 threshold changes in community composition of river taxa were identified at <5.1% glacier
14 cover and <66.6% meltwater contribution. Below this point the loss of cold stenothermic
15 benthic invertebrate taxa, *Diamesa* spp. and the Pyrenean endemic *Rhyacophila angelieri* was
16 apparent. Some generalist taxa including *Protonemura* sp., *Perla grandis*, *Baetis alpinus*,
17 *Rhithrogena loyolae* and *Microspectra* sp. increased when glacier cover was < 2.7 % and <
18 52 % meltwater. Patterns were not as distinct for the alpine lakes, due to fewer sampling
19 sites; however, *Daphnia longispina* grp. and the benthic invertebrate groups Plectopera and
20 Planaria were identified as potential indicators. While further work is required to assess
21 potential indicator taxa for alpine lake systems findings from alpine river systems were
22 consistent between methods for assessing glacier influence (meltwater contribution/glacier
23 cover). Hence, it is clear that this approach (TITAN) could become a useful a management
24 tool, enabling: (i) the identification of taxa particularly sensitive to glacier retreat; and (ii)
25 conservation efforts/resources to be better directed in alpine aquatic systems.

26 Keywords:

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

29

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 indicator 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

1 responses of lake ecosystem to climatic change can be predicted (REF). Due to the presence
2 of glaciers and the dynamics of snow cover, mountain lakes often respond to climate change
3 according to indirect mechanisms (e.g. water temperature can decrease due to melting water
4 from shrinking glaciers), which are less evident at low elevations (Thompson et al., 2005).
5 Like rivers, the biological communities of alpine lakes can act as sensitive indicators of
6 climate change, as even small perturbations of their natural equilibrium may cause detectable
7 responses (Rogora et al., 2008). Hence, the identification of indicator taxa for monitoring
8 glacier retreat in lake environments is feasible. Despite this no studies have linked
9 contemporary glacier cover to pelagic or littoral faunal patterns, as to date most studies of
10 high altitude lakes have focused on altitude or other environmental variables as key drivers of
11 community composition (Mendoza and Catalan, 2010).

12 Climate change is expected to facilitate altitudinal range expansions of invertebrates
13 and enhance the invasion potential of fish species, which will alter biotic interactions and
14 community structure in alpine rivers and lakes (Bellard et al., 2012). In rivers, warmer water
15 and more stable habitats can allow invertebrate predators to migrate upstream (Brown et al.,
16 2007). Negative impacts of predator invasions or introductions on aquatic communities
17 involve altering prey abundance and size distributions directly (Kratz, 1996; Dahl, 1998), or
18 indirectly via prey behavioural responses (e.g. drift: McIntosh and Peckarsky 1990, Lancaster
19 1990) or physiological responses (e.g. altered emergence size (Peckarsky et al., 2001)). In
20 alpine lakes introduced fish are a major threat with significant effects on native communities
21 (Knapp et al., 2001a, Schabetsberger et al., 2009). Less extreme conditions produced by
22 climate change are projected to increase the invasion potential of introduced species, such as
23 salmonids, into alpine lakes and assist the acclimatization of alien species (Holzapfel and
24 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 km²) and the Glacier des Gabiétous (0.08 km²) 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 km²) 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 km²) and Glacier du petit Vignemale (0.03 km²) 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 m² 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.

1

2 **3.0 Material and methods**

3 3.1 PNPstreams: physicochemical habitat and water source sampling

4 For PNP streams, field measurements were carried out at each study site on three
5 occasions, during 2010 and 2011 (Early: 16 - 29 June, Mid: 15-30 July, late: 17-30 August).
6 Spot measurements of electrical conductivity (EC) and pH were made using a Hannah HI
7 98129 handheld probe. Water samples (500ml) were collected at each reach to determine
8 suspended sediment concentration (SSC). Samples were filtered through pre-weighed glass
9 fibre filter papers (Whatman GF/C), dried at 95°C for 2 h then re-weighed to the nearest mg
10 (ATSM D3977 – 97). The bottom component of the Pfankuch index (PFAN) was also
11 recorded. This involves a qualitative scoring assessment of five variables (rock angularity,
12 substrate brightness, particle packing, percentage of stable materials, scouring and aquatic
13 vegetation) with higher values representing unstable channels (Pfankuch, 1975). Water
14 velocity (measured using a Sensa RC2 electromagnetic current meter), water depth and
15 wetted width were recorded at five random locations along each 30m reach. Water
16 temperature (T_w) was recorded continuously during both melt seasons, at each study reach,
17 using either miniature digital temperature dataloggers (Gemini Tinytag Plus, instrument error
18 $\pm 0.2^\circ\text{C}$) or CS547A temperature probes (Campbell Scientific, instrument error $\pm 0.2^\circ\text{C}$). To
19 characterize the annual thermal regime, continuous water temperature monitoring was
20 conducted between 09/10 and 09/12 at three sites; T3, G4 and Spr 1 (a small groundwater
21 spring). Cross-calibration of all temperature sensors was conducted before and after the field
22 season (Quilty and Moore, 2007). Correction factors were then applied to each logger based
23 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 (± 0.1 cm) 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 permanent buoy which marked the point of maximum depth and ran continuously 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 0.09m²; 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 log₁₀ (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

1 correlation was used to identify relationships between glacier cover in the catchment and
2 potential indicator taxa.

3 For the streams, Threshold Indicator Taxa ANalysis (TITAN) was used to identify
4 taxa and community thresholds along a gradient of glacier influence as defined by (i)
5 meltwater proportion and (ii) glacier cover in the catchment. TITAN is a non-parametric
6 technique that orders and partitions observations along an environmental gradient, and, rather
7 than using IndVal scores (indicator value method Dufrene and Legendre 1997) to interpret
8 pre-existing typologies, it uses IndVal scores to define groupings. Following Baker & King
9 (2010), multiple candidate change points are identified and indicator value scores calculated
10 for each taxa (250 permutations). Standardized IndVals (based on SD and mean from
11 permutation) were used for cross-taxon comparisons. Declining (z-) and increasing (z+) taxa
12 are used to identify community-level change points. Bootstrapping (500 replicates) is used to
13 identify reliable threshold indicator taxa and the uncertainty around the taxon and community
14 change points. In this study community thresholds are presented as (i) TITAN thresholds,
15 synchronous changes in the abundance of z- or z+ taxa within a narrow range of the glacier
16 influence gradient and (ii) change point analysis (nCPA) where candidate change points are
17 identified via deviance reduction of Bray-Curtis distance values. The latter approach (nCPA)
18 is based on the assumption that the mean and variance of the Bray-Curtis distance will
19 respond to compositional changes. The ‘changepoint’ separates the observations into two
20 groups and deviance reduction is used to identify the most homogenous two groups (see Qian
21 et al. (2003) for more detailed methods). All analysis was carried out in R.2.14.1 using the
22 Vegan and LME4 packages.

1 4.0 Results

2 4.1 Thermal regime and turbidity

3 A distinct seasonal dynamic was apparent for mean daily water column temperature
 4 (T_w) at all stream reaches, with minima observed during winter and maxima during late
 5 summer months (Fig. 2). T3 (5.7% glacier cover) was the only stream reach which displayed
 6 water temperature below zero, while winter temperature for both Spr 1 (0% glacier cover)
 7 and G4 (1.7 % glacier cover) were $> 0^{\circ}\text{C}$ consistently and were less variable than T3.
 8 Maximum daily mean T_w was highest for Spr 1 (15.3°C) and lowest for T3 (11.3°C).
 9 However, the spring/summer temperature increase was latest for Spr 1, which started in May,
 10 and earliest for G4 which began in March (Fig. 2). Annual mean T_w for the entire sample
 11 period was significantly lower for T3 (ANOVA; $F = 42.0$, $P < 0.0001$) with no significant
 12 difference between T3 and Sp 1. For summer, all sites were significantly different (ANOVA;
 13 $F = 38.6$, $P < 0.0001$) in the order $T3 < G4 < \text{Sp 1}$, while for Winter T3 was significantly
 14 lower than both G4 and Sp 1 (ANOVA; $F = 233.7$, $P < 0.0001$). A positive correlation
 15 between SSC and both measures of glacier influence, i.e. meltwater contribution ($r = 0.56$, P
 16 < 0.0001) and % glacier cover ($r = 0.51$, $P < 0.0001$), was evident for the PNP study sites.

17 Lake T_w displayed a distinct seasonal trend. During the winter, water temperature was
 18 similar in all three lakes, but with the advent of spring, Lake Trebecchi Superior maintained a
 19 similar temperature whereas in the other two lakes water temperature continued to decrease
 20 (Fig. 2). In June, lake temperature in the non-glacierized catchment (Lake Trebecchi
 21 Superior) increased rapidly following ice-off from 5°C to $>15^{\circ}\text{C}$ in less than 14 days. Lake
 22 Lillet (20.8 % glacier cover) increased slowly due to the input of glacial runoff and only
 23 reached 4.8°C by the same period. However, T_w did rise during summer and reached a
 24 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 Lilet 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

1 and minimum and mean water temperature. Meltwater at these sites was intermediate
2 between Group 1 and Group 3 (Fig. 4) and the underlying geology was calcareous. Glacier
3 cover and meltwater contributions to flow were lowest in Group 3 (Fig. 4) associated with the
4 taxa *Rhyacophila evoluta*, *Drusus discolour*, *Rhithrogena loyolaea*, *Arcynopteryx compacta*
5 and *Psychodidae*. The physicochemical habitat of these sites was characterised by higher
6 channel width and depth (i.e. sites further down valley from their sources) and low SSC and
7 pH.

8 The ordination of lakes based on the zooplankton community (Fig. 5) displayed a
9 negligible loss of information (Stress = 0.04). Axis 1 divided fishless lakes (positive scores)
10 from lakes stocked with fish (negative scores). The cladoceran *Daphnia longispina* and
11 rotifer *Lecane luna* were associated with positive scores on this axis, while the rotifers
12 *Trichocerca* sp. and *Euchlanis* sp. were associated with negative scores. Axis 2 divided
13 fishless lakes with no glacier influence (positive scores) from all other lakes (negative
14 scores). *Lecane luna* and the cladoceran *Acropaerus harpae* were associated with positive
15 scores, while the rotifer *Synchaeta* sp. and the copepod *Arctodiaptomus alpinus* were
16 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

1 sites with reduced glacial influence (Fig 2b) are likely due to lower stream discharge
2 (decreased thermal capacity) and reduced meltwater input (Cadbury et al., 2008; Fellman et
3 al., 2013). However, winter precipitation patterns will play an important role in future stream
4 thermal regimes as snow cover dictates exposure time to the atmosphere and energy
5 exchange processes (Dickson et al., 2012; Edwards et al., 2007). Once meltwater production
6 decreases due to sufficient ice mass loss associated with climate change (Milner et al. 2009)
7 reduced coldwater input and increased exposure to the atmosphere will increase water
8 temperature and, in certain cases, decrease turbidity (Khamis et al. 2013). However, glacier
9 size, catchment morphology and geology may alter response trajectories for water
10 temperature and turbidity of both lakes and rivers (Leonard and Reasoner 1999, Brown and
11 Hannah 2008, Richards et al., 2012).

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

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3 This study presents findings from the first change-point analysis for
4 macroinvertebrate communities along a natural gradient of glacier influence. Clear
5 community tipping points were identified for the gradient defined by meltwater contribution
6 (~67%) and glacier cover (~5 %). Below this point the loss of specialist taxa, *Diamesa* spp.
7 and the endemic *R. angelieri*, was apparent, similar to findings from other studies,
8 highlighting their extinction risk as glaciers recede (Brown et al., 2007). Generalist taxa
9 increased when glacier cover was < 3 % and meltwater < 52 %. Threshold analysis is still a
10 relatively new field and most previous studies have focused on anthropogenic gradients, such
11 as % impervious cover in the catchment or % of catchment mined (Baker and King 2010,
12 Bernhardt et al., 2012), yet we suggest this approach could provide a useful monitoring tool
13 in alpine environments. Further work is needed to identify if changes identified in this study
14 represent alternative stable states and are consistent when considering multiple mountain
15 ranges with a range of glacier sizes (Dodds et al., 2010).

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

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6.0 Conclusions

This research represents the first attempt to identify change points and indicators 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 biogeographical 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.

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

Basin	Stream	Site	Catchment area (km²)	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)	<i>Oulettes d'Ossoue</i>	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).

Glacier gradient	Method	Community change point		
		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 ($\pm$ 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)

(b)

(a)

(b)

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.