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Small lakes in big landscape: Multi-scale drivers of littoral ecosystem in alpine lakes

Dragos G. Zaharescu^{1,2,3*}, Carmen I. Burghelea^{1,3}, Peter S. Hooda² Richard N. Lester⁴ and Antonio Palanca-Soler³

¹Biosphere 2, University of Arizona, Tucson, Arizona, USA

²School of Built and Natural Environments, Kingston University London, UK

³Faculty of Biological Sciences, University of Vigo, Vigo, Spain

⁴Formerly at Birmingham University Botanic Gardens, Birmingham, UK, who passed away in 2006

*Corresponding author: D.G. Zaharescu, zaharescu@email.arizona.edu, zaha_dragos@yahoo.com

C.I. Burghelea: bcarmen@email.arizona.edu

P.S. Hooda: p.hooda@kingston.ac.uk

A. Palanca-Soler: apalanca@uvigo.es

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Abstract

In low nutrient alpine lakes, the littoral zone is the most productive part of the ecosystem, and it is a biodiversity hotspot. It is not entirely clear how the scale and physical heterogeneity of surrounding catchment, its ecological composition, and larger landscape gradients work together to sustain littoral communities.

A total of 113 alpine lakes from the central Pyrenees were surveyed to evaluate the functional connectivity between littoral zoobenthos and landscape physical and ecological elements at geographical, catchment and local scales, and to ascertain how they affect the formation of littoral communities. At each lake, the zoobenthic composition was assessed together with geolocation, catchment hydrodynamics, geomorphology and topography, riparian vegetation composition, the presence of trout and frogs, water pH and conductivity.

Multidimensional fuzzy set models integrating benthic biota and environmental variables revealed that at geographical scale, longitude unexpectedly surpassed altitude and latitude in its effect on littoral ecosystem. This reflects a sharp transition between Atlantic and Mediterranean climates and suggests a potentially high horizontal vulnerability to climate change. Topography (controlling catchment type, snow coverage and lakes connectivity) was the most influential catchment-scale driver, followed by hydrodynamics (waterbody size, type and volume of inflow/outflow). Locally, riparian plant composition significantly related to littoral community structure, richness and diversity. These variables, directly and indirectly, create habitats for aquatic and terrestrial stages of invertebrates, and control nutrient and water cycles. Three benthic associations characterised distinct lakes. Vertebrate predation, water conductivity and pH had no major influence on littoral taxa.

This work provides exhaustive information from relatively pristine sites, and unveils a strong connection between littoral ecosystem and catchment heterogeneity at scales beyond the local environment. This underpins the role of alpine lakes as sensors of local and large-scale environmental changes, which can be used in monitoring networks to evaluate further impacts.

Keywords: Alpine lakes; Littoral zone; Benthic invertebrates; Scale dependency; Catchment heterogeneity; Riparian vegetation; Vertebrate predation; Environmental change.

1 Introduction

Integrative efforts linking landscape-scale biogeochemical, hydrological and ecological processes have been intensified in the last decade, and true whole-catchment perspectives are starting to crystalize (Richter and Billings 2015). Alpine catchments are of increased relevance, partly because they are younger than the average landscape, and they are major drivers of hydrological and biogeochemical cycles affecting the wider biosphere. Their high topography, remoteness and climate allow for the formation of waterbodies of unmatched water quality, which are ecological, biogeochemical and aesthetic hotspots.

Only across Europe, there are over 50,000 remote mountain lakes (Kernan et al., 2009), of which the Pyrenees, a relatively low-density lacustric region, accounts for an estimated 4,000 (Castillo-Jurado, 1992). The littoral and riparian zones of these lakes are critical mediators between sediment and nutrient fluxes from the surrounding terrestrial area and lake internal processes. Littoral surfaces also experience cross-ecosystem water and nutrient exchanges (both, autochthonous and allochthonous) with riparian zones, and provide habitat and resources for both aquatic and emerging stages of many aquatic taxa, such as most benthic insects (Gregory et al., 1991; Jonsson and Wardle; 2009; Kopacek et al., 2000). The Pyrenees are estimated to have >797 km of littoral zone in lakes above 1000m, which are of at least 0.5ha (Castillo-Jurado, 1992), meaning that littoral processes represent a great portion of the nutrient fluxes in the catchment.

The topography, the hydrology, the bedrock geology and the climate control the intensity of bedrock weathering and nutrient transport into alpine lakes; this influences water and sediment chemistry, and ultimately their ecosystems (Vollenweider, 1968). Even though the littoral zone is just a fraction of the total lake area, it harbours the vast majority of species in a lake, and the littoral nutrient productivity is vital for aquatic food webs, contributing substantially to the whole lake ecosystem energy budget (Vander-Zanden et al., 2006, Vadeboncoeur et al., 2011).

The challenges from inhabiting shallow lake areas at high elevation, range from high solar radiation and water level fluctuations, to low food availability, a short growing season, irregular freezing periods and strong seasonal temperature variation (Bretschko, 1995). Most of the aquatic invertebrates are at their distributional boundaries, and they are highly sensitive to environmental change (Bandyopadhyay et al., 1997). For example, winter mortality is a major factor regulating alpine lake macroinvertebrate populations (Oswood et al., 1991). Food availability and duration of ice/snow cover during winter are other factors affecting littoral macroinvertebrate communities (Bretschko, 1995), as

there are also nitrate concentrations (from acid deposition), fish presence, lake morphology (Kernan et al., 2009) and type of shore coverage (Füreder et al., 2006).

Elevated topography and low available nutrients generally support simple littoral ecosystems, which are characterized by a limited number of species and trophic levels (as compared with lowland lakes; Magnea et al., 2013), and are highly adapted to local environment. Research has shown that in mountain lakes, variability in terrestrial conditions can affect littoral macroinvertebrate abundances, through relative control on the proximal environment (Kernan et al., 2009). Moreover, geographical location can have a greater influence on macroinvertebrate communities than local environment (Kernan et al., 2009). It is expected that these topographical and climate restrictions introduce strong biogeographical variability and segregation of littoral macroinvertebrates into distinct communities. Climate/environmental change would further disrupt this natural heterogeneity, through mechanisms that alter the temperature, water and nutrient fluxes, significantly changing lake ecosystem balances. For example the functional diversity of alpine stream benthic invertebrate communities can be particularly affected by climate change-driven glacier retreat (Khamis et al., 2014).

Despite a great ecological and geochemical importance of the alpine lakes' littoral zone, the scale and complexity of its connectivity to surrounding landscape remains an open question. To better anticipate its response to environmental change it is, therefore, imperative to integrate the littoral surfaces into the mechanistic understanding of how physical and ecological heterogeneity of the catchment and littoral ecosystem interact across spatial scales before major alterations occur. This study attempts to evaluate the magnitude of the influence catchment attributes have on littoral macrozoobenthos community composition at scales from a lake to large geographical gradients. A second aim was to assess how these interactions determine the formation of littoral associations, which can potentially serve as sensors of environmental change. We hypothesize that while local littoral environment directly mediates the macroinvertebrate community, its composition is also sensitive to landscape processes at scales beyond that of the lake, through mechanisms that can affect both aquatic and terrestrial phases of its taxa. The study area has the advantages of being at the confluence of four major biogeographical regions: Atlantic, Continental, Mediterranean, and Alpine, which should facilitate capturing the large-scale heterogeneity in a relatively narrow region.

2 Methodology

2.1 The lakes under study

A total of 113 lakes were surveyed in July 2001 in the axial Pyrenees, between degrees: 42°51'34.76" - 42°43'8.19"N and 0°29'44.39"W - 0° 8'40.29"E (Fig. 1, Supplementary List 1). Their selection was largely dictated by their accessibility, and comprised a range of typical alpine ponds and lakes, with surface area varying between 9.4- 107,068 m². The area is within the boundaries of the Central Pyrenees National Park, France, and comprises a series of postglacial catchments on cirque and valley floors. Catchment geology varied between the various valleys and it was dominated by two large geologic units: in the central area and at the extreme east, lake catchments lie on acidic bedrock (granite batholith) while in between, granitic batholiths are surrounded by metasedimentary and sedimentary materials such as slate, limestone and sandstone (Zaharescu, 2011).

Most of the study lakes are above the tree line (altitudes ranged from 1580-2501 m a.s.l.; mean=2212m a.s.l.), and they are largely undisturbed by human activity. Low-level agro-pastoral activities, leisure fishing and trekking are among the very few activities allowed in the park. Two of the sampled lakes were transformed into reservoirs (lakes Artouste and Ossoue), and they are being used as freshwater reserve. The great majority of study lakes are oligotrophic. Their proximal catchment area (roughly 10-20 m around the lake) has generally low vegetation coverage (<20%), but this varies according to topography and location. Loose rocks dominate on most of the lake shores, though they were more abundant on the steeply slopes of granitic catchments (Zaharescu, 2016a).

The hydrological network, consisting of temporary and permanent lakes, ponds, pools and streams, is a natural legacy of the last glaciers retreat more than 5,000 years ago. Water input in most lakes is by direct precipitation and permanent streams; glaciers and springs were present only in a few cases. The water level in these lakes is a balance between precipitation input, seasonal water loss and lake basin capacity. Their geographical location on the north range of the Pyrenees, means they are at their full capacity most of the year. Surface connectivity between lakes varied for the lakes investigated. Slope and bank snow coverage at the time of sampling was generally low, but had higher coverage at the head of catchments. Water pH was mainly neutral (mean = 7.6), but varied between 5.2 (in granitic and *Sphagnum* moss vegetated waterbodies) and 8.8 (in lakes on schist and limestone) (Supplementary List 1). Conductivity was low, averaging 38 $\mu\text{S cm}^{-1}$ and positively related to pH (Pearson correlation, $r^2=0.18$, $p<0.01$). Neither parameter varied greatly between surface and bottom measurements.

2.2 Sampling strategy and data collection

An exhaustive assessment was conducted for each visited lake (Fig. 1). Because the response of benthic ecosystem to physical, chemical and ecological factors in the surrounding environment is expected to change with increasing landscape heterogeneity, composition and scale, we sampled major environmental parameters likely to influence biotic composition along a gradient of local, catchment and geographical scale influences. Local variables included littoral macroinvertebrates, water pH and conductivity, and the presence of vertebrate predators, i.e. frogs and trout. Catchment-scale parameters comprised ecotope properties of proximal part of lake catchment and riparian vegetation assemblages. We use the term “ecotope” to denote the integrated physical elements of a landscape that underlie an ecosystem, and that exchanges matter and energy with the surrounding environment (Zaharescu et al., 2016a). Geographical-scale influences were represented by horizontal and vertical gradients in, longitude, latitude and altitude.

Macroinvertebrate sampling deliberately targeted the littoral zone. This area generally supports far larger and more diverse populations of benthic invertebrates than the deeper zone (Vadeboncoeur et al., 2011). The littoral is also likely to relate more directly to the nearby riparian and catchment factors. Semi-quantitative 3 min kick-samples were collected in each lake using a standard pond net (Frost et al., 1971). Samples were collected at short distances while moving around the lake perimeter to cover different micro-habitats in proportion to their occurrence. Littoral substrate was highly variable and ranged from boulders to fine sands, vascular plants, mosses and algae. A composite sample (3-10 subsamples) was collected at each lake. In each visited lake about half of the perimeter was sampled. All substrates (rocks, cobbles, coarse and fine sand, epilithic moss, etc.) were sampled down to 60 cm water depth. Subsequently all samples were preserved in 96% alcohol for a comprehensive laboratory sorting and analysis. Benthic organisms were identified down to the lowest possible taxonomic level using Tachet et al. (2002) key, and counted under a stereomicroscope. The lowest taxonomic level identified (down to genus and species in some cases) of living and subfossil taxa will be regarded as morphotypes henceforth. For most statistical tests a family/subfamily level resolution was used. A list of identified taxons and their incidence is provided in Supplementary List 2.

Additionally, water pH and conductivity were recorded at the surface and the bottom (± 5 m off the shore) at each site with portable pH and conductivity probes. The water was collected with a standard bottom water sampler, following a clean protocol (Zaharescu et al., 2009). Presence of frogs (*Rana temporaria*) was visually inspected at each site. Trout presence data at each location was obtained from the stocking records maintained by the Pyrenees National Park.

Riparian vegetation composition (presence/absence data) was recorded down to species level in the field at each site (for 50-100 % of lake perimeter), or on plants collected in a vasculum and identified off site, using multiple identification keys (Grey-Wilson and Blamey, 1979; Fitter et al., 1984; and García-Rollán, 1985). A detail description of the procedure is described in Zaharescu (2011) and Zaharescu et al. (2016b).

Furthermore, at each location, a number of catchment-scale factors were visually approximated according to dominant units. They were: nature of water input (whether meteoric, spring or stream) and output (whether absent, temporary, surface small, medium and large, subterranean or dam), tributary discharge (from absent to high discharge), water-body surface area, % vegetation covering slopes and shore, slope (from flat to steep), main bedrock geology, presence of aquatic vegetation (from absent to abundant), shore development (1-4 fractal level), presence of snow deposits on the shore and in the catchment (%), catchment type (postglacial geomorphology: plain, U- and V-shape valleys, valley head slope and mountain pass) and surface connectivity with other waterbodies (whether absent, surrounded by a larger lake, connected with a second one, or in chain). They are detailed in Zaharescu (2011) and Zaharescu et al. (2016a).

Lake geolocation was recorded with a portable GPS and provided in Supplementary List 1.

2.3 Data analyses

Statistical data analyses included principal component analysis (PCA), fuzzy set ordination (FSO), multidimensional FSO (MFSO), cluster and indicator species analyses. For this, environmental factors were split into groups, i.e. geolocation, landscape/ecotope, invertebrate-vertebrate interaction, water chemistry and riparian vegetation.

2.3.1 *Principal Component Analysis*

First, the landscape variables were reduced to a limited number of meaningful composite factors (Principal Components) by using the PC regression scores from PCA, after maximizing their fit to variable groups (Varimax rotation). These composite factors were used as predictors of littoral zoobenthos in further fuzzy set analysis (Table 1). By default, the Varimax rotated principal components are uncorrelated.

2.3.2 *(Multidimensional) Fuzzy Set Ordination*

To analyse the relationship between littoral zoobenthos composition (presence-absence data) and environmental gradients we used fuzzy set ordination (FSO) followed by stepwise multidimensional

FSO (MFSO; Roberts, 2008). For this, a distance (dissimilarity) matrix computed with Sørensen similarity index of invertebrate presence-absence data was first calculated. This gave a measure of similarity between sites based solely on biotic composition (Boyce, 2008). Additionally, two more variables assumed to describe zoobenthos community structure were used in a (M)FSO with vegetation presence-absence data matrix (Sørensen similarity index). They were taxon (family) richness and sequential diversity comparison index, which is a simplified method for estimating relative differences in biological diversity (SCI; Barbour et al., 1999), and allowed considering morphotypes in the analysis (Equation 1), where *run* describes the morphotype and *taxon* refers to family classification:

$$SCI = \frac{\text{no. of runs} \times \text{no. of taxa}}{\text{total no. of individuals}} \quad (\text{Equation 1})$$

Fuzzy set ordination (FSO) concept (Roberts, 1986) is a generalised alternative to traditional ordination approaches, such as canonical correspondence analysis, in which cases are assigned gradual membership (fuzzy) values ranging from 0 to 1 (Roberts, 2008), instead of 0 or 1 (i.e. in-or-out of a given set) like in classical statistics. FSO is expected to perform better than other models on more complex data sets, and it is insensitive to noise in environmental factors and rare species (Roberts, 2009).

Variables were first screened in turn in FSO, and those with highest correlation with the zoobenthos distance matrix (at >95% efficiency) were retained for further MFSO. Technically, in MFSO, a FSO is performed on the variable that accounts for most of the variation first. Then, the residuals of the analysis are used with the next most important variable. The process is repeated until no more variables are left. Because only the fractions of variable membership that are uncorrelated are used by MFSO, each variable selected by the model is regarded as an independent process. This gives a high interpretability to the model (Roberts, 2008). Visually, the effect extent of each variable can be assessed by the increment in the correlation value attributable to that variable.

A total of 1000 random permutations were subsequently performed to test the significance of each variable in FSO/MFSO. Where the distance matrix was disconnected (sites and groups of sites with no shared species) or the dissimilarity was too high, a step-across function was applied to improve the MFSO. This finds the shortest paths to connect groups and removes rare observations/ groups of observations (Oksanen, 2008).

Because trout and frog variables were binary, and to achieve more accurate R^2 in the model, these variables were standardized by Hellinger transformation (Legendre and Gallagher, 2001) before using them in FSO.

2.3.3 Mantel test

To further assess the potential effect of riparian vegetation composition on major littoral invertebrate composition a Mantel test was performed on their distance matrixes. These matrixes were calculated with Baroni-Urbani & Buser similarity index. This index was preferred as it maximises the Pearson product-moment correlation coefficient between the two matrixes. A high significance of the correlation procedure was drawn after 9999 random permutations of Monte Carlo test. Mantel test was further used to test for the relationship between vegetation structure (computed using Sorensen similarity index) and zoobenthos family richness and morphotype diversity.

2.3.4 Community analysis

Finally, the littoral zoobenthos data (family presence-absence) was analysed for co-occurring taxa and their ecotope preferences. This was achieved by clustering the sites on the basis of shared species, and applying indicator species analysis for each resulting cluster. First, a flexible linkage Pair-Group Method using the Arithmetic Averages (PGMA; method parameter = 0.85) cluster analysis was run on a distance matrix computed from Sørensen similarity matrix of families presence-absence data. Plotting cluster solutions in discriminating space (by discriminant analysis) helped evaluate the reliability of cluster solution. Secondly, indicator species analysis was run at the nodes of the major clusters to identify invertebrate families that represent the resulting lake groups.

FSO and LabDSV packages were used to compute FSO and MFSO (Roberts, 2007a; Roberts, 2007b); ADE4, CLUSTER and FPC packages for Mantel test, clustering (Thioulouse et al., 1997; Kaufman and Rousseeuw, 1990; Hennig, 2005), and LabDSV for indicator species analysis (Dufrene and Legendre, 1997), all for the R statistical language and environment (R Development Core Team, 2005).

3 Results and discussion

3.1 Littoral diversity, landscape structure and scale

3.1.1 Large geographical gradients

Biome variability across geographic areas generally follows large-scale gradients in climate and topography. Results of FSO and MFSO of family composition against altitude, latitude and longitude showed that individually, these three factors could reliably predict littoral taxa composition (Fig. 2). The relative contribution of these variables to MFSO and their cumulative value are illustrated in Fig. 3. Longitude exerted by far the largest independent contribution, while altitude and latitude appeared to

incorporate a large covariant component with the former, as shown by their low significance (P value) as independent factors.

Compositional and functional changes in zoobenthos across large horizontal and vertical gradients have been reported before, and whole biome models have been used to evaluate changes in taxon distribution likely to occur with a changing climate (Colwell et al., 2008, IPCC, 2014). At an estimated 60km longitudinal span, the study area is relatively narrow. Nevertheless, longitude dominance in the model appears to be given by the area's unique position at the confluence of Atlantic and Mediterranean biogeographic regions (Fig. 1), which imprinted a sharp horizontal change in ecosystem composition. The two macro-regions are characterised by major climatic differences in water availability and temperature (López-Moreno et al, 2008), with Mediterranean climate being generally warmer, drier and comparatively of larger inter-seasonal variability than the Atlantic climate. This means potential tipping points in alpine lake ecosystems due to climate change effects (particularly through sharp changes in water temperature and dynamics; Khamis et al., 2014) is likely to happen faster across horizontal than vertical gradients in biogeographical boundary regions such as this one, with potentially unexpected effects. The changes could affect ecological processes such as niche retention in benthic biota, but they could also potentially affect longer-term biotic speciation in these regions (Doebeli and Dieckmann, 2003). This could be accentuated by the generally simpler composition of alpine benthic ecosystems as compared to lowlands (Magnea et al., 2013).

3.1.2 Catchment scale drivers

Principal component analysis (PCA) revealed three composite factors (Table 1). These factors were interpreted as: PC1, hydrodynamics (summarising input size, input and output nature, and lake size); PC2, geo-morphology (i.e. % vegetated shores and slopes, shore slope, geology, aquatic vegetation and shore development); and PC3, topography formation (catchment type, % shore and catchment snow coverage, connectivity with other lakes). They are exhaustively reported in Zaharescu et al. (2016a). The response of littoral invertebrates to these catchment factors is illustrated in Figures 2 (FSO) and 3 (MFSO). Both, univariate and multivariate solutions of the models show that topography was the most important predictor of littoral biota composition at a high degree of confidence ($p < 0.06$), followed by hydrodynamics (Figs. 2 and 3). Topography exerts its influence mainly through its structural variables: catchment type, shore and catchment snow coverage and connectivity with other lakes. These variables would sustain habitats at larger scale (e.g. lake's proximal catchment), and allow connectivity among populations of benthic communities, which need adequate habitats in both, aquatic and riparian areas

for survival. For instance, lakes at the head of glacial valleys, with snow presence most of the year, would harbour functional taxa with adaptation for near-freezing environment, very low nutrient input, and short reproductive time. On the other hand, valley floor lakes would harbour organisms with longer emergence periods, requiring additional nutrient and material inputs from the catchment, and allowing more diverse periphyton communities that serve as food and microhabitats for the zoobenthos. This ecosystem would also likely be more vulnerable to larger periods of snow presence.

While hydrodynamics was significant in FSO (Fig. 2), its small influence in MFSO can be explained by a high co-variability with topography (Fig. 3). The secondary effect of lake hydrodynamics suggests contributions from water source and lake area. For instance, large stream-fed lakes that maintain a continuous surface flow throughout the summer, would also maintain a generally low temperature and a heterogeneous structure of littoral habitats. Conversely, in relatively small waterbodies, dominantly fed by catchment runoff and/or snowmelt (therefore not sourced by continuous streams), the littoral surface can vary seasonally and warm faster. These different ecotopes will allow for the persistence of functional groups adapted to distinct lake environments, and they will vary with topography. This is supported by the results of studies conducted in other high altitude environments, which found clear differences in biotic assemblages in spring-fed streams under different flow regimes (Danehy and Bilby, 2009).

3.1.3 Local scale effect

(a) Riparian vegetation

Many of the benthic invertebrates, particularly insects, also have terrestrial phases. The relationship between littoral and riparian ecosystems may therefore go beyond their simple proximity or nutrient provision. (M)FSO model found a significant effect of plant species composition on the invertebrate diversity and family richness (cumulative $r=0.48$, $p<0.05$; Fig. 4). A relatively low but significant relationship was also found between the compositions of vegetation and benthic invertebrates (Mantel test, Monte Carlo $r=0.16$, $p<0.01$), which means commonly associated invertebrate groups are supported by commonly associated plant species. Although spatial covariability of flora and fauna along environmental gradients is not excluded, this relationship is meaningful in the sense that in the restricting alpine environment plant consortiums could provide niche separation for various competing invertebrates, including the terrestrial phases of most aquatic insects. This could include supplying nutrient for functional feeding groups, casing materials, microhabitats during short summer periods, and protection against excessive solar radiation (Gregory et al., 1991; Dudgeon, 2009).

Other studies have highlighted the importance of riparian plant coverage to macroinvertebrate communities along streams, especially in strong transitional gradients such as grassland-forest (Stone et al., 2005), but also the vegetation type (Cummins et al., 1989; Angradi et al., 2001). Our findings support the idea that sparsely vegetated alpine catchments provide important functional links between riparian vegetation composition and the diversity, richness and functional composition of benthic invertebrates.

(b) Vertebrate predation and water chemistry

Littoral productivity is vital for supporting higher trophic levels in lakes (Vadeboncoeur et al., 2011), and the presence of predators such as fish or amphibians, particularly in alpine lakes can result in a top-down driven ecosystem (Eriksson et al., 1980). Results of the relationship between the presence of fish and amphibians, and invertebrate groups surprisingly showed no effect (Fig. 2). This is evidence of the broad composition of littoral fauna being highly resilient to vertebrate predation. It is possible that predators were size selective, affecting the abundance of easily accessible groups, such as chironomids (Orthocladiinae and Chironominae) and planktonic crustaceans (Kernan et al., 2009; Syväranta and Jones, 2009; Schilling et al., 2009). Another explanation is that the generally coarse littoral substrate together with shielding mechanisms insects use in alpine lakes to protect against high solar radiation could also be effective against vertebrate predation. Niche segregation between aquatic and terrestrial environments could have also played a role. It is known that alpine lake frogs would largely prey on the more abundant terrestrial insect phases (Vieites et al., 1997), which helps them maximise nutrient intake during aestival season. Carlisle and Hawkins (1998) who observed that physical habitat might be more important than predation in structuring benthic communities in trout-stocked mountain lakes further supports our results.

Water pH and conductivity, measures of acidity, total ionic/nutrient content and their bioavailability, important lake parameters, could not explain diversity variation in major zoobenthic groups (Fig. 2). They are both indicators of bedrock geology and lake metabolism, and can change significantly during thaw periods in mountain lakes, influencing biotic composition (Olofsson et al., 1995). The very low relationship observed for either surface or lake bottom (pH and conductivity), suggests that their natural/seasonal variability in each lake may be strong enough to offset a direct response from biotic communities at a broader scale.

3.2 Major littoral communities

Low nutrient and strong environmental variability of alpine systems are expected to induce biogeographical fragmentation and formation of biotic communities that are strongly dependent to local conditions. Flexible hierarchical clustering and indicator taxa analyses identified three large lake groups hosting distinct biota (Fig. 5 and Table 2).

The first lake community (type A; Table 2 and Fig. 5) was the largest and the most widespread, consisting of a significant number of spring-dwellers, which were tolerant to wide ranges in temperature, altitude, water flow regime, pH and micro-habitats (e.g. epi- and endobenthic, epilithic and epiphytic). They were mostly sedentary invertebrates of gill and tegumentary respiration, feeding largely on detritus and microphytes. A small proportion were predators (e.g. Tanyptodinae larvae) and parasitic (nematodes). Their dispersion mode was mostly passive aquatic and aerial, which facilitates habitat connectivity (Tachet et al., 2002). The relatively wide ecological breadth (eurytopic distribution) of this group means they can colonise a variety of headwaters. Association of Sphaeriidae bivalves, Oligochaeta and Lumbriculidae worms with various members in this community has also been reported in headwaters of other alpine regions, including the Oregon Coast Range and the Himalayas (Danehy and Bilby, 2009; Manca et al., 1998).

The second community (type B, Table 2 and Fig. 5) was represented by omnivorous beetles and predatory dragonflies. Both are active groups, strong flyers as adults and capable of active colonisation and maintaining connected populations not always at easy reach. They also have long life cycles (>1year) and tolerate a wide range of temperatures. They have affinity to low water flow regime and heterogeneous microhabitats (Tachet et al., 2002), which most likely characterize the lakes cluster sharing this littoral group (Fig. 5).

The third littoral community (type C), had a low indicator value (Table 2). It was represented by craneflies, mosquitoes, water scavenger beetles and their parasitic worms. They share an aerial respiration (except gordiacea which are endoparasites in their larval stage) and a passive-to-active aerial dispersion mode in their adult stage. They tolerate a wide range of temperature and epibenthic microhabitats, with easy access to water surface where they breathe. Their feeding strategy is also diverse, from shredders (Limoniidae), to microphytes (Helophoridae), microinvertebrates and fine suspended matter (Culicidae) (Tachet et al., 2002). Females of most adult mosquitoes are ectoparasites. Further boxplot comparisons revealed that these communities did not display distinct preferences along the assessed catchment-scale variables (Supplementary Fig. 1). This, together with the wide ecological tolerance revealed by their taxon composition suggests ubiquitous distributions,

which may have resulted from natural evolution of lake ecosystems, or they were determined by lake or terrestrial factors beyond those analysed herein.

4 Conclusions

The findings simplify the complexity and highlight the level of connectivity between the littoral ecosystem of alpine lakes and the physical and ecological heterogeneity of their catchment at a wide range of spatial scales. Longitude dominance over other large-scale gradients in its influence on the littoral zoobenthic composition reflected the biogeographic boundary between Mediterranean and Atlantic climates. This suggests that climate change effects on alpine lake ecosystem are likely to be stronger across horizontal gradients than the expected altitudinal distribution in biogeographical boundary regions - an overlooked vulnerability of the alpine biome.

Catchment Topography and hydrodynamics (in this order of influence) were the leading catchment-scale drivers of littoral community composition. These factors control lake hydrological and biogeochemical processes including water balance, nutrient fluxes in the catchment, riparian vegetation colonization, lake temperature and metabolism, which ultimately influence littoral habitat and community formation and population connectivity.

Although generally poorly developed, riparian vegetation composition provided the main local scale effect on littoral invertebrate community structure, indicating that the proximal terrestrial habitat is critical to maintaining the structure and functioning of the littoral ecosystem. Different plant assemblages could provide distinct microhabitats for the terrestrial phases of aquatic insects, sheltering against harsh conditions of solar radiation and wind, and supplying weathered nutrients and casing materials for the aquatic phases of many invertebrates.

Community analysis revealed that the studied lakes were characterised by the presence of three simple functional zoobenthic associations, of which the sedentary group was the largest and the most widespread among the lakes. Overall the findings demonstrate that the littoral ecosystem is connected to a variety of topographical, hydrological and ecological attributes from the terrestrial environment at scales extending from lake proximity, to its catchment and beyond. Protecting the long-term natural status of these lakes as well as incorporating them into natural observatory networks should be a management priority, as they can serve as reference sites for the environmental stress affecting their ecosystems at a wide variety of scales.

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Authors contribution

Sampling campaign design, A Palanca-Soler; data collection, A Palanca-Soler, DG Zaharescu and RN Lester; study design, sample and data analyses, DG Zaharescu and CI Burghilea; manuscript preparation, DG Zaharescu, PS Hooda and CI Burghilea.

References

- Angradi TR, Hagan SM, Able KW. 2001. Vegetation type and the intertidal macroinvertebrate fauna of a brackish marsh: *Phragmites* vs. *Spartina*. *Wetlands* 21(1): 75–92.
- Bandyopadhyay J, Rodda JC, Kattelman R, Kundzewicz ZW, Kraemer D. 1997. Highland waters- a resource of global significance. In: Messerli and Ives (Eds), *Mountains of the world. A global perspective*. The Parthenon Publishing Group Ltd.
- Barbour MT, Gerritsen J, Snyder BD, Stribling JB. 1999. *Rapid bioassessment protocols for use in streams and wadeable rivers: periphyton, benthic macroinvertebrates and fish*. Second Edition. EPA 841-B-99-002. U.S. Environmental Protection Agency: Office of Water. Washington, D.C.
- Boyce R. 2008. Fuzzy set ordination web page. Northern Kentucky University. Accessed August 2009 from: <http://www.nku.edu/~boycer/fso/>.
- Bretschko G. 1995. Opportunities for high alpine research, the lake “Vorderer Finstertaler See” as an example (Kühtai, Tirol, 2237 m a.s.l.). *Limnologica* 25: 105–108.
- Carlisle DM, Hawkins CP. 1998. Relationships between invertebrate assemblage structure, 2 trout species, and habitat structure in Utah mountain lakes. *J N Am Benthol Soc* 17(3): 286–300.
- Castillo-Jurado M. 1992. *Morfometria de los lagos. Una aplicacion a los lagos del Pirineo*. PhD thesis, University of Barcelona (in Spanish).
- Colwell RK, Brehm G, Cardelús CL, Gilman AC, Longino JT. 2008. Global warming, elevational range shifts, and lowland biotic attrition in the wet tropics. *Science* 322: 258–261.

- Cummins KW, Wilzbach MA, Gates DM, Perry JB Taliaferro WB. 1989. Shredders and riparian vegetation. *BioScience* 39(1): 24–30.
- Danehy RJ, Bilby RE. 2009. Periphyton and macroinvertebrate assemblage responses to flow regime in spring-fed headwaters. *Verh Internat Verein Limnol* 30(8): 1210–1214.
- Doebeli M, Dieckmann U. 2013. Speciation along environmental gradients. *Nature* 421, 259–264.
- Dudgeon D. 2009. The influence of riparian vegetation on macroinvertebrate community structure in four Hong Kong streams. *J Zool* 216(4): 609–627.
- Dufrene M, Legendre P. 1997. Species assemblages and indicator species: the need for a flexible asymmetrical approach. *Ecol Monogr* 67(3): 345–366.
- EEA (European Environment Agency). 2001. Biogeographical regions, Europe 2001. Accessed February 22, 2010 at: <http://www.eea.europa.eu/data-and-maps/figures/biogeographical-regions-europe-2001>.
- Eriksson MOG, Henrikson L, Larsson P, Nilsson BI, Oscarson HG, Stenson JAE. 1980. Predator-prey relations, important for the biotic changes in acidified lakes. *Ambio* 9: 248–249.
- Fitter RSR, Fitter A, Farrer A. 1984. Grasses, sedges, rushes and ferns of Britain and Northern Europe. Collins, London, UK.
- Frost S, Huni A, Kershaw WE. 1971. Evaluation of a kicking technique for sampling stream bottom fauna. *Can J Zool* 49: 167–173.
- Füreder L, Ettinger R, Boggero A, Thaler B, Thies H. 2006. Macroinvertebrate diversity in Alpine lakes: effects of altitude and catchment properties. *Hydrobiologia* 562: 123–144.
- García-Rollán M. 1985. Claves de la flora de España (península y baleares). Vol.II: dicotiledoneas (l-z) / monocotiledoneas. Mundi-Prensa [in Spanish].
- Gregory SV, Swanson FJ, McKee WA, Cummins KW. 1991. An ecosystem perspective of riparian zones. *BioScience* 41(8): 540–551.
- Grey-Wilson C, Blamey M. 1979. The alpine flowers of Britain and Europe. Collins, London, UK.
- Hennig C. 2005. A method for visual cluster validation. In: Weihs, C. and Gaul, W. (eds), Classification - the ubiquitous challenge. Springer, Heidelberg 2005: 153–60.
- Intergovernmental Panel on Climate Change (IPCC). 2014. Climate Change 2014: Impacts, Adaptation, and Vulnerability. Contribution of Working Group II to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change [Field, C.B., V.R. Barros, D.J. Dokken, K.J. Mach, M.D. Mastrandrea, T.E. Bilir, M. Chatterjee, K.L. Ebi, Y.O. Estrada, R.C. Genova, B. Girma, E.S. Kissel, A.N.

- Levy, S. MacCracken, P.R. Mastrandrea, L.L. White (eds.)). Cambridge University Press, Cambridge, United Kingdom and New York, USA.
- Jonsson M, Wardle D. 2009. The influence of freshwater-lake subsidies on invertebrates occupying terrestrial vegetation. *Acta Oecol* 35: 698–704.
- Kaufman L, Rousseeuw PJ. 1990. Finding groups in data: an introduction to Cluster Analysis. Wiley, New York.
- Kernan M, Ventura M, Bitušík P, Brancelj A, Clarke G, Velle G, Raddum GG, Stuchlík E, Catalan J. 2009. Regionalisation of remote European mountain lake ecosystems according to their biota: environmental versus geographical patterns. *Freshwat Biol* 54: 2470–93.
- Khamis K, Hannah DM, Brown LE, Tiberti R, Milner a. M. 2014. The use of invertebrates as indicators of environmental change in alpine rivers and lakes. *Sci Total Environ* 493:1242–54.
- Kopacek J, Stuchlik E, Straskrabova V, Psenakova P. 2000. Factors governing nutrient status of mountain lakes in the Tatra Mountains. *Freshwat Biol* 43 (3): 369–83.
- Legendre P, Gallagher ED. 2001. Ecologically meaningful transformations for ordination of species data. *Oecologia* 129: 271–80.
- López-Moreno JI, Goyettea S, Benistona M. 2008. Climate change prediction over complex areas: spatial variability of uncertainties and predictions over the Pyrenees from a set of regional climate models. *Int J Climatol* 28: 1535–1550.
- Magnea U, Sciascia R, Paparella F, Tiberti R, Provenzale A. 2013. A model for high-altitude alpine lake ecosystems and the effect of introduced fish. *Ecol Mod*: 251: 211–20.
- Manca M, Ruggiu D, Panzani P, Asioli A, Mura G, Nocentini AM. 1998. Report on a collection of aquatic organisms from high mountain lakes in the Khumbu Valley (Nepalese Himalayas). In: Lami A and Giussani G (eds), Limnology of high altitude lakes in the Mt Everest Region (Nepal). *Mem Ist Ital Idrobiol* 57: 77–98.
- Oksanen J. 2008. Stepacross as flexible shortest paths or extended dissimilarities. R documentation for Vegan, available online at <http://vegan.r-forge.r-project.org/>.
- Olofsson E, Melin E, Degerman E. 1995. The decline of fauna in small streams in the Swedish mountain range. *Water Air Soil Pollut* 85(2): 419–24.
- Oswood MW, Miller LK, Irons III JG. 1991. Overwintering of freshwater benthic macroinvertebrates. In: Lee RE Jr, Denlinger DL (Eds), Insects at low temperature. Chapman and Hall, New York: 360–375.
- R Development Core Team. 2005. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <http://www.R-project.org>.

- Richter DD, Billings SA. 2015. One physical system: Tansley's ecosystem as Earth's critical zone. *New Phytologist* 206(3): 900–912.
- Roberts DW. 1986. Ordination on the basis of fuzzy set theory. *Vegetatio* 66: 123–31.
- Roberts DW. 2007a. FSO: fuzzy set ordination. R package version 1.0-1. <http://cran.R-project.org>.
- Roberts DW. 2007b. LabDSV: ordination and multivariate analysis for ecology. R package version 1.3-0 <http://cran.R-project.org>.
- Roberts DW. 2008. Statistical analysis of multidimensional fuzzy set ordinations. *Ecology* 89:1246–60.
- Roberts DW. 2009. Comparison of multidimensional fuzzy set ordination with CCA and DB-RDA. *Ecology* 90(9): 2622–2634.
- Schilling EG, Loftin CS, Huryn AD. 2009. Effects of introduced fish on macroinvertebrate communities in historically fishless headwater and kettle lakes. *Biol Conserv* 142: 3030–38.
- Stone ML, Whiles MR, Webber JA, Williard KWJ, Reeve JD. 2005. Macroinvertebrate communities in agriculturally impacted southern Illinois streams: patterns with riparian vegetation, water quality, and in-stream habitat quality. *J Environ Qual* 34: 907–17.
- Syväranta J, Jones RI. 2009. Isotopic variability in lake littoral organisms presents a challenge for food web studies. *Verh Internat Verein Limnol* 30(8): 1193–6.
- Tachet H, Richoux P, Bournaud M, Usseglio-Polatera P. 2002. Invertébrés d'eau douce. Systématique, biologie, écologie. CNRS Editions, Paris, France [in French].
- Thioulouse J, Chessel D, Doledée S, Olivier JM. 1997. ADE-4 A multivariate analysis and graphical display software. *Stat Comput* 7: 75–83.
- Vadeboncoeur Y, McIntyre PB, Vander Zanden MJ. 2011. Borders of biodiversity: Life at the edge of the world's large lakes. *BioScience* 61 (7): 526–537.
- Vander-Zanden MJ, Chandra S, Park S, Vade-Boncoeur Y, Goldman CR. 2006. Efficiencies of benthic and pelagic trophic pathways in a subalpine lake. *Can J Fish Aquat Sci* 63: 2608–20.
- Vieites DR, Nieto-Román S, Palanca A. 1997. Alimentación de las ranas pardas, *Rana* gr. temporaria, en el Circo de Piedrafita (Pirineos, España). *Pirineos* 149: 91–104 [in Spanish].
- Vollenweider RA. 1968. Scientific fundamentals of the eutrophication of lakes and flowing waters, with particular reference to nitrogen and phosphorus as factors in eutrophication. Organization for Economic Corporation and Development, Technical report, 250p. Paris, France.
- Zaharescu, DG. 2011. Landscape ecology and geochemistry of high altitude lakes. PhD thesis, University of Vigo, Spain.

Zaharescu DG, Hooda PS, Fernandez J, Soler AP, Burghilea CI. 2009. On the arsenic-source mobilisation and its natural enrichment in the sediments of a high mountain cirque in the Pyrenees. *Journal of Environmental Monitoring* 11: 1973-1981.

Zaharescu DG, Hooda PS, Burghilea CI, Palanca-Soler A. 2016a. A multiscale framework for deconstructing the ecosystem physical template of high altitudes lakes. *Ecosystems* (in press). bioRxiv doi: <http://dx.doi.org/10.1101/034405>.

Zaharescu DG, Palanca-Soler A., Hooda PS, Tanase C, Burghilea CI, Lester RN. 2016b. Sensors of change: riparian ecosystem sensitivity to local and large scale gradients in high elevation lakes. bioRxiv doi: <http://dx.doi.org/10.1101/035576>.

Tables

Table 1 Association between catchment variables characterising the Pyrenees lakes, and PCA components. Only highest variable correlation with any of the components is shown. This allowed to interpret PC1 as hydrodynamics, PC2 as geo-morphology and PC3 as topography formation.

	Principal component		
	1	2	3
Tributary discharge	0.92		
Nature of tributary	0.90		
Nature of water output	0.87		
Lake size	0.52		
% grass covered slopes		0.72	
% grass covered shore		0.68	
Slope of lake perimeter		-0.67	
Geology		0.60	
Aquatic vegetation		0.58	
Fractal order		0.50	
Catchment snow deposits			0.86
Catchment type			0.79
Shore snow coverage			0.75
Connectivity with other lakes			0.52
Total Eigenvalue (rotated)	3.07	2.69	2.46
% of variance explained	21.96	19.24	17.59
Cumulative %	21.96	41.20	58.79

Rotation method: Varimax with Kaiser normalization.

Kaiser-Meyer-Olkin measure of sampling adequacy= 0.73.

Bartlett's test of sphericity: approx. $\chi^2 = 1456.9$ ($P < 0.001$).

Table 2 Zoobenthic communities with significant association to lake groups (from prior cluster analysis), as given by indicator taxa analysis. A subject was classified into a group for which the indicator value was higher and significant (i.e. strong preference). Significance level is <0.05, unless stated otherwise.

Taxon	Common name	Biota and lake groups	Indicator value
Chironomidae Chironominae	Non-biting midges	A	0.67
Enchytraeidae	Microdrile oligochaetes	A	0.62
Chironomidae Tanypodinae	Non-biting midges	A	0.46
Chironomidae Orthoclaadiinae	Non-biting midges	A	0.46
Limnephilidae	Tube-case caddisflies	A	0.32
Sphaeriidae	Pea clams	A	0.23
Lumbriculidae	Microdrile oligochaetes	A	0.22
Naididae	Clitellate oligochaetes	A	0.22
Nematoda	Roundworms	A	0.21
Ceratopogonidae and Thaumaleidae	Biting & solitary midges	A	0.15
Baetidae	Mayflies	A	0.11
Haliplidae	Crawling water beetles	B	0.16
Aeshnidae	Dragonflies	B	0.31 (P=0.55)
Limoniidae	Craneflies	C	0.07
Culicidae	Mosquitoes	C	0.03
Gordiacea	Horsehair worms	C	0.03
Helophoridae	Water scavenger beetles	C	0.12 (P=0.16)

N (number of taxa used in the analysis) = 46 families from 113 central Pyrenean lakes, ponds and pools.

Figure Captions

Fig. 1 (a) Major biogeographical regions of Europe (after EEA, 2001). (b) Lakes distribution in the Pyrenees National Park, France (green boundaries). Only lakes within park boundaries, which are enclosed in the dash line box were considered for this study.

Fig. 2 One-dimensional fuzzy set ordination (FSO), showing the response of zoobenthic family structure to environmental variables in the Central Pyrenees lakes. Indices represent: (a) geolocation, (b) composite catchment (Table 1), (c) predation and (d) water physico-chemistry. Correlations are listed in descending order. Variables with highest influence in the model (correlations >0.3, in bold), also shown in plots, were retained for multidimensional FSO. *P* represents the probability. Predation variables were Hellinger transformed (Legendre and Gallagher, 2001) previously to being used as predictors in the analysis.

Fig. 3 Multidimensional response of littoral invertebrate composition to geolocation and composite catchment factors in a multidimensional FSO (MFSO) with step-across improvement. Variables are added to the model as log transformed, in the order of their decreasing fuzzy correlation (Pearson) with biota dissimilarity matrix. Permutation number = 1000. γ (gamma) represents a vector of the fraction of variance of a factor that is independent of all previous factors. Due to the high-dimensional variability of the dissimilarity matrix, the correlation probability for the one-dimensional solution sometimes has low significance, but it is still valid.

Fig. 4 Relationship between riparian vegetation structure and littoral invertebrate morphotype diversity and family richness in a bidimensional FSO. A step-across function improved the ordination. Number of permutations = 1000.

Fig. 5 Major lake/ecosystem groups (A, B and C) as identified by hierarchical cluster analysis (flexible linkage, parameter = 0.85) based on shared littoral invertebrate families. A plot of cluster solutions in discriminating space (inset) demonstrate an effective clustering. Illustrated are: (A) Cambales Valley lake, (B) Montferrat pond, Ossoue Valley and (C), Barroude Petit, Aure Valley. The results are from an analysis of 113 lakes and 46 major invertebrate groups.

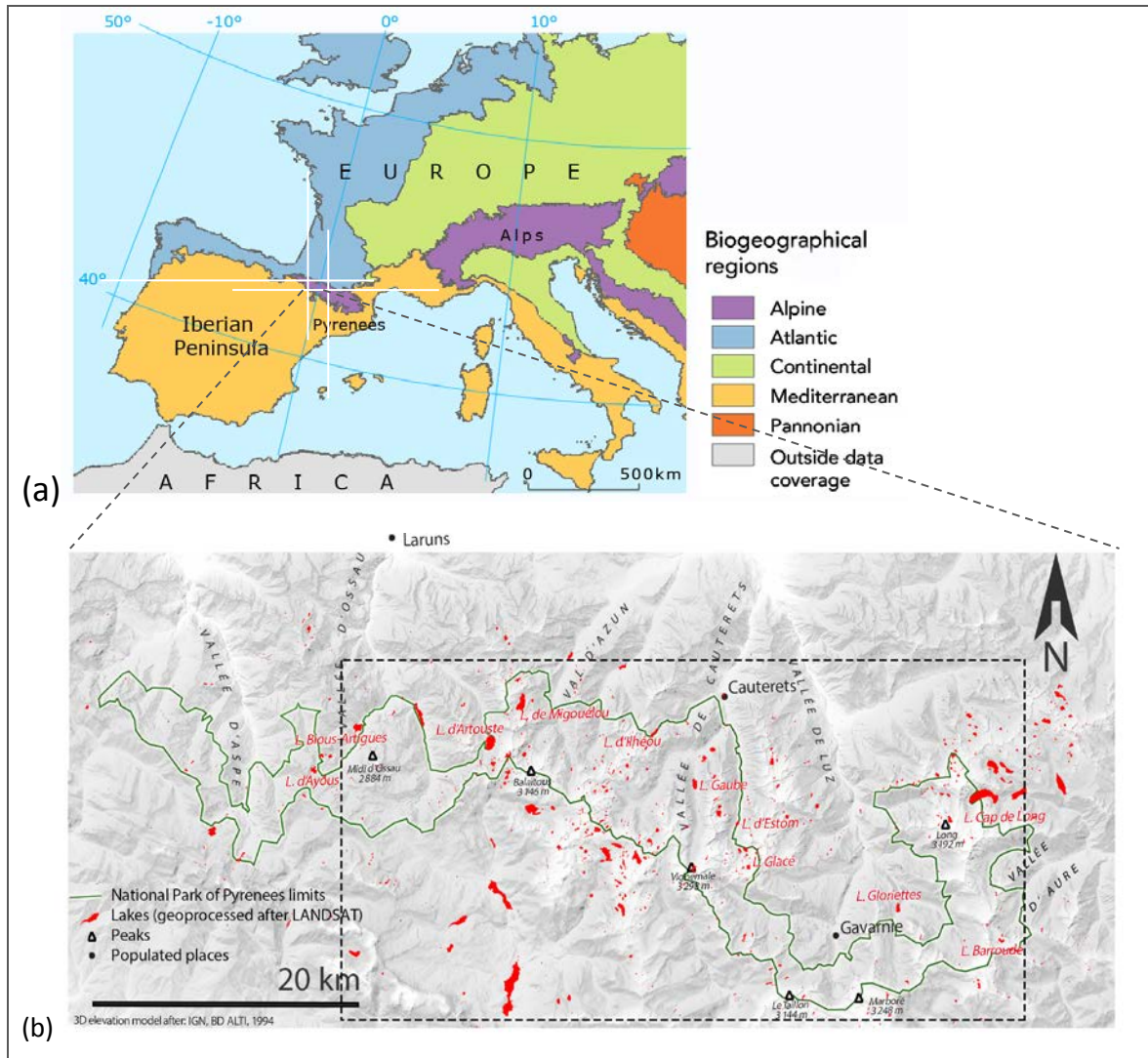


Figure 1

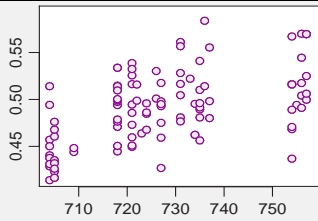
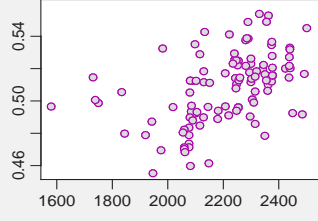
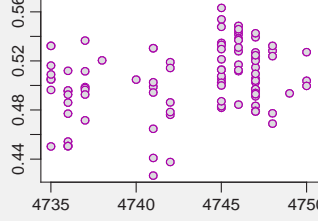
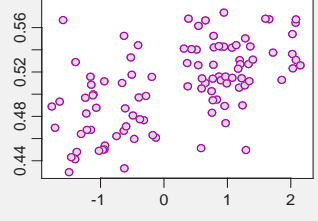
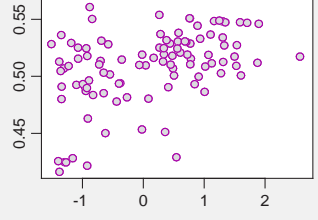
Factor	<i>r</i> (Pearson)	<i>P</i>	FSO plot (x-factor/y-apparent factor as predicted by biota)
^a Longitude (UTM)	0.547	0.001	
^a Altitude (m a.s.l.)	0.470	0.001	
^a Latitude (UTM)	0.336	0.001	
^b Topography formation (PCA regression factor scores)	0.566	0.001	
^b Hydrodynamics (PCA regression factor scores)	0.439	0.001	
^b Geo-morphology (PCA regression factor scores)	-0.061	0.627	
^c Trout (presence/absence)	0.068	0.277	
^c Frogs (presence/absence)	0.052	0.296	
^d pH(bottom)	0.235	0.047	
^d pH(surface)	0.074	0.278	
^d Conductivity (surface)	0.003	0.419	
^d Conductivity (bottom)	-0.009	0.457	

Figure 2

Axis (log)	Cumulative r	Increment r	P -value	γ	MFSO plot (x-matrix dissimilarity/y-ordination distance)
<i>Geoposition</i>					
Longitude	0.499	0.499	0.044	1.000	
Altitude	0.623	0.124	0.167	0.360	
Latitude	0.641	0.018	0.764	0.063	
<i>Landscape</i>					
Topography formation	0.491	0.491	0.064	1.000	
Hydrodynamics	0.601	0.110	0.373	0.797	

Figure 3

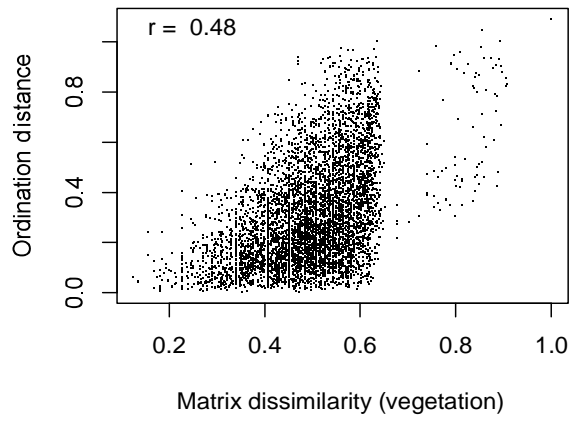


Figure 4

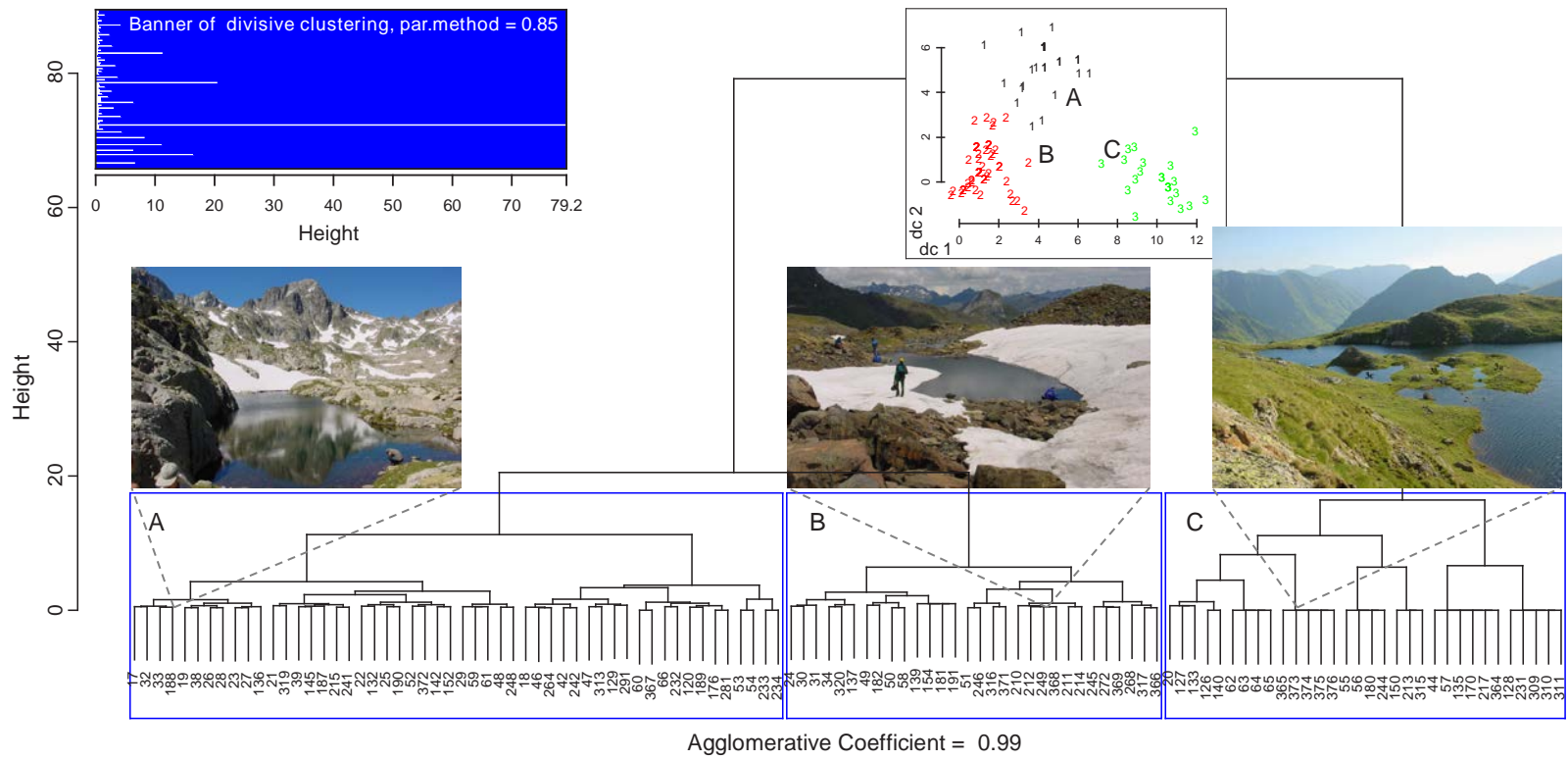


Figure 5

Supplementary Information

Small lakes in big landscape: Multi-scale drivers of littoral ecosystem in alpine lakes

Dragos G. Zaharescu, Carmen I. Burghilea, Peter S. Hooda, Richard N. Lester and Antonio Palanca-Soler

Supplementary List 1: Lakes and ponds from the central region of Pyrenees National Park surveyed in this study, together with their main hydrographical network, altitude (*below the tree line), geolocation (decimal degrees), surface area, pH and conductivity.

Index	Lake name	Main valley	Altitude m a.s.l.	Latitude	Longitude	Area m ²	pH (surface)	pH (bottom)	Conductivity (surface), μ S	Conductivity (bottom), μ S
17	Lake Berseau	Ossau	2082	42.4959	-0.3015	79223.11	7.94	8.01	23.00	24.00
18	Lake Berseau 1	Ossau	2080	42.4959	-0.3015	1484.40	7.75	7.70	25.00	26.00
19	Lake Berseau 2	Ossau	2100	42.4959	-0.3015	2419.81	7.63	7.63	14.00	14.00
20	Pond Berseau 1	Ossau	2085	42.4959	-0.3015	127.23	7.47	7.47	9.00	9.00
21	Pond Berseau 2	Ossau	2086	42.4959	-0.3015	180.64	7.69	7.69	25.00	25.00
22	Lake Larry 1	Ossau	2077	42.5018	-0.3014	1162.39	8.09	7.78	31.00	32.00
23	Lake Larry 2	Ossau	2077	42.5018	-0.3014	293.74	7.94	7.79	31.00	31.00
24	Lake Larry 3	Ossau	2077	42.5018	-0.3014	414.69	7.30	7.30	30.00	30.00
25	Lake Larry 4	Ossau	2077	42.5018	-0.3014	306.31	7.51	8.06	26.00	29.00
26	Lake Ayous 1	Ossau	2060	42.5018	-0.2929	722.57	7.94	8.28	31.00	34.00
27	Lake Ayous 2	Ossau	2060	42.5018	-0.2929	753.96	7.95	8.00	32.00	34.00
28	Lake Ayous 3	Ossau	2060	42.5018	-0.2929	769.69	7.91	7.69	31.00	31.00
29	Lake Gentau 1	Ossau	1982	42.5018	-0.2929	1850.40	8.57	8.68	62.00	62.00
30	Lake Gentau	Ossau	1947	42.5018	-0.2929	107068.62	8.03	8.33	40.00	45.00
31	Lake Miey	Ossau	1920	42.5018	-0.2929	9324.25	7.18	8.00	41.00	46.00
32	Lake Roumassot	Ossau	1845	42.5018	-0.2929	55694.15	8.52	8.55	43.00	45.00
33	Lake Castérau	Ossau	1943	42.4945	-0.2931	15013.67	8.60	8.68	121.00	127.00
34	Lake Paradis	Ossau	1976	42.4945	-0.2931	9495.97	8.20	8.11	53.00	54.00
38	Lake Col de Peyreget 1	Ossau	2220	42.4941	-0.2635	1473.41	7.85	8.14	11.00	19.00
39	Lake Col de Peyreget 2	Ossau	2208	42.4941	-0.2635	3758.13	7.74	7.71	6.00	6.00
42	Lake Arrémoulit Supérieur	Ossau	2281	42.5005	-0.1957	39654.75	6.46	6.71	20.00	22.00
44	Lake Arrémoulit (below dam)	Ossau	2255	42.5037	-0.1956	9680.03	7.79	7.98	13.00	15.00
46	Lake Palas 1	Ossau	2365	42.5037	-0.1956	2511.70	7.94	NA	8.00	NA
47	Lake Palas 2	Ossau	2362	42.5037	-0.1956	1226.79	8.06	7.60	5.00	9.00
48	Lake Arrémoulit Superior 1	Ossau	2300	42.5037	-0.1956	1272.35	7.90	7.98	5.00	7.00
49	Lake Arrémoulit Superior 2	Ossau	2295	42.5037	-0.1956	208.92	5.46	5.46	9.00	9.00
50	Lake Arrémoulit Superior 3	Ossau	2297	42.5037	-0.1956	23.56	5.28	5.19	7.00	9.00
51	Lake Arrémoulit Superior 4	Ossau	2300	42.5037	-0.1956	2104.08	7.34	6.92	3.00	5.00
52	Lake Arrémoulit Superior 5	Ossau	2300	42.5037	-0.1956	1503.25	5.51	5.60	3.00	5.00
53	Lake Arrémoulit Superior 6	Ossau	2305	42.5037	-0.1956	1237.00	8.04	7.38	5.00	6.00
54	Lake Arrémoulit Superior 6A	Ossau	2305	42.5037	-0.1956	1236.97	8.02	7.83	5.00	6.00
55	Lake Arrémoulit Superior 7	Ossau	2290	42.5037	-0.1956	384.85	7.37	6.90	2.00	4.00
56	Lake Arrémoulit Superior 8	Ossau	2285	42.5037	-0.1956	144.51	6.41	5.98	4.00	5.00
57	Lake Arrémoulit Inférieur	Ossau	2241	42.5037	-0.1956	9671.11	7.45	7.38	14.00	18.00
58	Lake Arrémoulit Inferior 1	Ossau	2248	42.5037	-0.1956	292.17	5.81	5.81	3.00	3.00
59	Lake Arrémoulit Inferior 2	Ossau	2246	42.5037	-0.1956	2831.36	6.47	NA	14.00	NA
60	Lake Arrémoulit Inferior 3	Ossau	2244	42.5037	-0.1956	4970.00	7.15	NA	14.00	NA
61	Lake Arrémoulit Inferior 4	Ossau	2256	42.5037	-0.1956	523.85	6.90	6.90	4.00	4.00
62	Lake Arrémoulit Inferior 5A	Ossau	2254	42.5037	-0.1956	282.74	6.38	6.38	5.00	5.00

63	Lake Arrémoulit Inferior 5B	Ossau	2254	42.5037	-0.1956	271.74	6.18	6.18	7.00	7.00
64	Lake Arrémoulit Inferior 5C	Ossau	2254	42.5037	-0.1956	278.02	6.77	6.77	8.00	8.00
65	Lake Arrémoulit Inferior 5D	Ossau	2254	42.5037	-0.1956	266.24	6.40	6.40	4.00	4.00
66	Lake Arrémoulit Inferior 6	Ossau	2252	42.5037	-0.1956	197.92	6.32	6.32	5.00	5.00
120	Lake Micoulaou 1	Azun	2302	42.5034	-0.1744	706.84	7.84	7.84	13.00	13.00
127	Lake Batcrabère Supérieur 1	Azun	2182	42.5034	-0.1744	285.88	7.75	7.92	14.00	15.00
128	Lake Batcrabère Milieu	Azun	2130	42.5034	-0.1744	1923.44	7.89	8.48	16.00	19.00
129	Pond Batcrabère Milieu 1	Azun	2130	42.5106	-0.1743	47.12	7.91	7.91	23.00	23.00
132	Lake below Batcrabère Milieu	Azun	2129	42.5034	-0.1744	1755.31	7.82	7.82	16.00	16.00
133	Lake Batcrabère Inférieur	Azun	2116	42.5106	-0.1743	18605.53	7.93	8.17	20	21
135	Lake Batcrabère Inférieur 1	Azun	2116	42.5106	-0.1743	3573.56	7.47	7.72	19.00	25.00
136	Pond next to Larribet Refuge	Azun	2055	42.5106	-0.1743	1979.20	5.98	5.98	10.00	10.00
137	Pond Pabat	Azun	2062	42.5106	-0.1743	518.35	6.01	7.32	4	6
139	Lake La Claou Supérieur	Azun	1750*	42.521	-0.1656	2964.09	8.32	8.24	19.00	22.00
140	Lake La Claou	Azun	1739*	42.521	-0.1656	2035.75	8.2	8.2	20	20
142	Lake Doumbblas	Azun	1580*	42.5209	-0.1612	1796.99	8.28	8.28	30.00	30.00
145	Pond Pluviometre	Azun	1731	42.5135	-0.1529	4546.54	8.22	8.22	23.00	23.00
150	Lake Remoulis Supérieur	Azun	2019	42.5031	-0.1532	12801.99	8.28	8.48	28.00	30.00
152	Pond Casteric	Azun	2080	42.4958	-0.1533	659.73	7.98	8.36	23.00	28.00
154	Pond Toue	Azun	2090	42.4958	-0.1533	639.31	7.24	7.60	33.00	27.00
170	Lake Cambalés 2	Cauterets	2424	42.4924	-0.1407	7297.92	7.87	8.72	7.00	13.00
176	Lake Cambalés Grand	Cauterets	2342	42.4924	-0.1407	13994.22	8.31	8.43	13.00	19.00
180	Pond Opale	Cauterets	2222	42.4923	-0.1323	175.93	8.04	8.04	21.00	21.00
181	Pond Opale 1	Cauterets	2248	42.4923	-0.1323	54.98	8.29	8.29	6.00	6.00
182	Pond Opale 2	Cauterets	2260	42.4923	-0.1323	1412.15	7.70	NA	32.00	NA
187	Pond Petit Laquet	Cauterets	2360	42.4923	-0.1323	169.65	7.83	7.83	8.00	8.00
188	Lake Petit Laquet	Cauterets	2350	42.4923	-0.1323	3765.98	7.94	8.16	5.00	9.00
189	Lake Costalade Supérieur	Cauterets	2320	42.4923	-0.1323	9519.03	7.93	8.31	12.00	5.00
190	Pond Cambalés	Cauterets	2315	42.4923	-0.1323	829.38	8.05	8.38	14.00	15.00
191	Lake Costalade Inférieur	Cauterets	2310	42.4923	-0.1323	10148.92	8.16	8.23	13.00	16.00
210	Lake Col d'Arratille	Cauterets	2501	42.4709	-0.1033	2670.28	NA	NA	NA	NA
211	Pond Arratille 1	Cauterets	2363	42.4741	-0.1031	141.37	7.64	7.72	59.00	43.00
212	Pond Arratille 2	Cauterets	2330	42.4741	-0.1031	63.62	7.50	7.50	45.00	45.00
213	Pond Arratille 3	Cauterets	2315	42.4741	-0.1031	3691.37	8.75	NA	85.00	NA
214	Pond Arratille 4	Cauterets	2289	42.4741	-0.1031	31.42	7.93	7.93	15.00	15.00
215	Pond Arratille 5	Cauterets	2315	42.4741	-0.1031	731.21	8.44	8.61	91.00	87.00
217	Lake Arratille	Cauterets	2247	42.4741	-0.1031	70038.67	8.32	8.31	77.00	74.00
231	Oulettes. glacier runoff	Cauterets	2151	42.4707	-0.0905	2434.66	7.00	7.00	90.00	90.00
232	Pond Arraillé Inférieur	Cauterets	2441	42.4706	-0.0821	714.71	7.72	7.01	34.00	29.00
233	Lake Arraillé Milieu	Cauterets	2450	42.4706	-0.0821	2544.69	6.92	7.08	25.00	26.00
234	Lake Arraillé Supérieur	Cauterets	2485	42.4706	-0.0821	2206.12	NA	NA	NA	NA
241	Pond Montferrat	Luz	2207	42.4455	-0.0743	109.96	7.40	6.83	19.00	9.00
242	Lake Montferrat	Luz	2374	42.4455	-0.0743	10445.80	6.81	7.28	56.00	42.00
244	Pond Montferrat 2	Luz	2440	42.4455	-0.0743	1011.59	7.78	7.47	38.00	37.00
245	Lake Montferrat 1	Luz	2438	42.4455	-0.0743	2111.15	7.42	7.58	84.00	73.00
246	Lake Montferrat 3	Luz	2438	42.4455	-0.0743	302.38	7.53	7.53	73.00	73.00
248	Lake Montferrat 5	Luz	2437	42.4455	-0.0743	314.15	NA	NA	NA	NA
249	Lake Montferrat 6	Luz	2440	42.4455	-0.0743	500.30	8.43	8.43	5.00	5.00
264	Pond Sentier d'Estom 1	Cauterets	2235	42.4703	-0.0653	320.44	7.25	7.60	69.00	54.00
268	Pond Sentier d'Estom 3	Cauterets	2240	42.4703	-0.0653	243.47	7.70	7.63	19.00	19.00
272	Lake Labas	Cauterets	2281	42.4702	-0.0609	49542.92	7.84	7.78	50.00	48.00
281	Pond Turon Couy 2	Cauterets	2492	42.463	-0.0611	471.24	7.56	7.88	10.00	10.00
291	Lake Ossue	Luz	1834	42.4525	-0.0614	38954.6	7.58	7.80	117.00	108.00
309	Lake Aires Supérieur	Luz	2089	42.4329	0.0607	8251.15	7.27	NA	199.00	NA
310	Lake Aires Inférieur 1	Luz	2081	42.4329	0.0607	1865.32	7.90	7.89	146.00	165.00
311	Lake Aires Inférieur 2	Luz	2081	42.4329	0.0607	7314.41	7.91	7.92	174.00	178.00
313	Lake Comble 1	Luz	2098	42.4327	0.0651	6660.18	7.66	7.60	175.00	166.00
315	Pond Troumouse 1	Luz	2105	42.4329	0.0607	11.78	8.15	8.15	60.00	60.00
316	Pond Troumouse 2	Luz	2102	42.4329	0.0607	9.42	7.78	7.78	61.00	61.00
317	Pond Troumouse 3	Luz	2133	42.4329	0.0607	25.13	7.60	7.60	38.00	38.00
319	Lake Troumouse3	Luz	2145	42.4329	0.0607	5006.91	6.97	6.97	88.00	88.00
320	Lake Troumouse 4	Luz	2148	42.4329	0.0607	1209.51	7.95	7.95	156.00	156.00
364	Pond Barroude 6	Aure	2345	42.4326	0.0735	400.55	8.62	8.30	12.00	11.00

365	Pond Barroude 5	Aure	2350	42.4326	0.0735	1157.68	8.10	8.16	43.00	42.00
366	Pond Barroude 4	Aure	2356	42.4326	0.0735	1762.43	7.28	7.14	52.00	54.00
367	Pond Barroude 3	Aure	2374	42.4326	0.0735	668.37	7.91	8.01	90.00	92.00
368	Pond Barroude 2	Aure	2375	42.4326	0.0735	186.92	7.53	7.96	20.00	23.00
369	Pond Barroude 1	Aure	2376	42.4325	0.0819	803.46	6.71	7.52	118.00	116.00
371	Pond Barraode refuge	Aure	2377	42.4325	0.0819	9.42	NA	NA	NA	NA
372	Lake Barroude Grand	Aure	2355	42.4325	0.0819	53603.42	7.80	8.38	151.00	267.00
373	Lake Barroude Petit	Aure	2377	42.4325	0.0819	62682.63	6.88	8.19	49.00	57.00
374	Pond Barroude Petit 1	Aure	2377	42.4325	0.0819	11.78	8.26	8.30	90.00	80.00
375	Pond Barroude Petit 2	Aure	2377	42.4325	0.0819	12.57	8.52	8.48	54.00	49.00
376	Pond Barroude Petit 3	Aure	2377	42.4325	0.0819	62.83	NA	NA	NA	NA
Mean			2212	42.481	-0.130892	7219.74	7.58	7.66	38.25	38.32
Maximum			2501	42.52	0.08	107068.62	8.75	8.72	199.00	267.00
Minimum			1580	42.43	-0.30	9.42	5.28	5.19	2.00	3.00

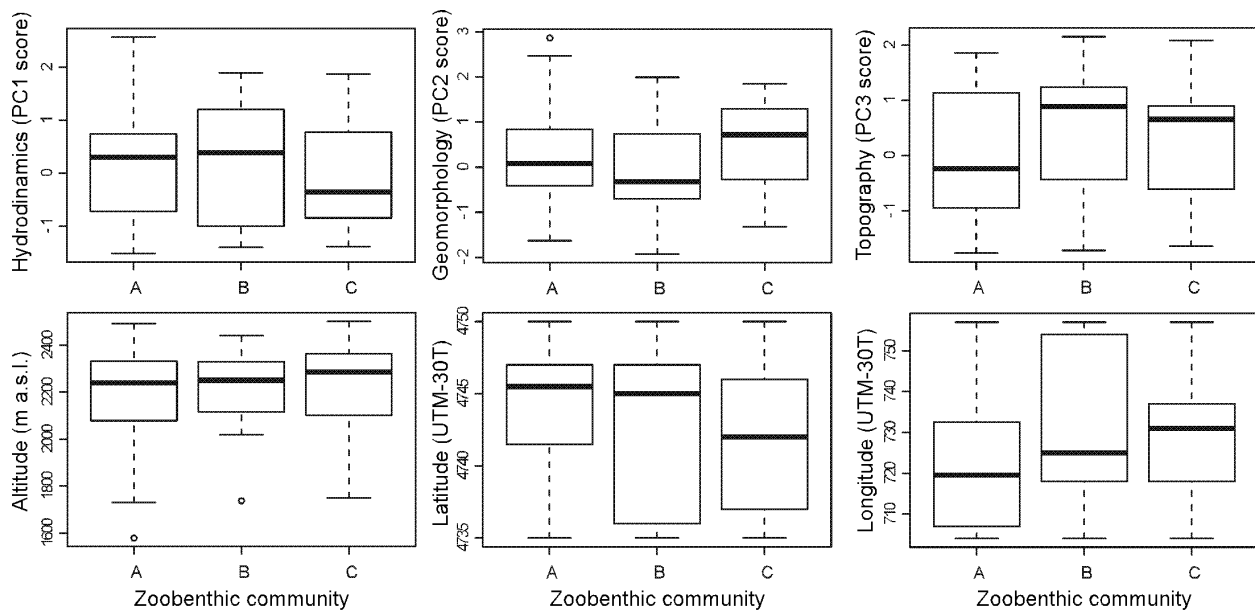
Supplementary List 2: Major zoobenthos taxa and their incidence in the 114 lakes, ponds and pools of this study.

Taxon	N. lakes
O. Diptera, sO. Nematocera, SF. Psychodoidea, F. Psychodidae	1
O. Diptera, sO. Nematocera, SF. Culicoidea, F. Dixidae	1
O. Diptera, sO. Nematocera, SF. Culicoidea, F. Culicidae	1
O. Diptera, sO. Nematocera, SF. Chironomoidea, F. Ceratopogonidae & F. Thaumaleidae	7
O. Diptera, sO. Nematocera, SF. Chironomoidea, F. Chironomidae, sF. Tanypodinae	25
O. Diptera, sO. Nematocera, SF. Chironomoidea, F. Chironomidae, sF. Chironominae	40
O. Diptera, sO. Nematocera, SF. Chironomoidea, F. Chironomidae, sF. Orthocladiinae (lato sensu)= (stricto sensu) sF. Orthocladiinae+ sF. Diamesinae+ sF. Prodiamesinae	59
O. Diptera, sO. Nematocera, SF. Tipuloidea, F. Tipulidae	1
O. Diptera, sO. Nematocera, SF. Tipuloidea, F. Limoniidae	2
O. Diptera, sO. Brachycera, SF. Empidoidea	1
O. Trichoptera, GR. Spicipalpia, SF. Rhyacophiloidea, F. Rhyacophilidae	1
O. Trichoptera, GR. Spicipalpia, SF. Hydroptiloidea, F. Hydroptilidae	1
O. Trichoptera, GR. Integripalpia, SF. Limnephiloidea, F. Limnephilidae	52
O. Trichoptera, GR. Integripalpia, SF. Limnephiloidea, F. Uenoidae	4
O. Coleoptera, sO. Adephaga, F. Haliplidae	2
O. Coleoptera, sO. Adephaga, F. Dytiscidae	28
O. Coleoptera, sO. Polyphaga, GR. Haplogastra (=GR. Palpicornia), SF. Hydrophiloidea, F. Hydrophilidae	2
O. Coleoptera, sO. Polyphaga, GR. Haplogastra (=GR. Palpicornia), SF. Hydrophiloidea, F. Helophoridae	13
O. Coleoptera, sO. Polyphaga, GR. Heterogastra, SF. Byrrhoidea, F. Elmidae (=F. Helminthidae, =F. Elminthidae)	7
O. Megaloptera, F. Sialidae	8

O. Heteroptera, iO. Nepomorpha, F. Corixidae	15
O. Heteroptera, iO. Gerromorpha, F. Mesoveliidae	2
O. Heteroptera, iO. Gerromorpha, F. Veliidae	1
O. Heteroptera, iO. Gerromorpha, F. Gerridae	6
O. Odonata, sO. Anisoptera, F. Aeshnidae	1
O. Odonata, sO. Anisoptera, F. Gomphidae	1
O. Plecoptera, SF. Nemouroidea, F. Nemouridae	1
O. Plecoptera, SF. Nemouroidea, F. Capniidae	2
O. Plecoptera, SF. Perloidea, F. Chloroperlidae & F. Perlodidae	3
O. Ephemeroptera, F. Baetidae	8
O. Ephemeroptera, F. Siphonuridae	1
O. Ephemeroptera, F. Heptageniidae	1
Cl. Lamellibranchia, SF. Corbiculacea, F. Sphaeriidae	22
Cl. Gasteropoda, sCl. Prosobranchiata, F. Valvatidae	1
Cl. Gasteropoda, sCl. Prosobranchiata, F. Hydrobiidae	1
Cl. Gasteropoda, sCl. Pulmonata, F. Ancyliidae	2
Cl. Gasteropoda, sCl. Pulmonata, F. Lymnaeidae	11
Phyl. Annelida, Cl. Hirudinea, O. Rhynchobdelliformes, F. Glossiphoniidae	1
Phyl. Annelida, Cl. Oligochaeta, F. Naididae	19
Phyl. Annelida, Cl. Oligochaeta, F. Tubificidae	2
Phyl. Annelida, Cl. Oligochaeta, F. Lumbriculidae	15
Phyl. Annelida, Cl. Oligochaeta, F. Enchytraeidae	39
Phyl. Annelida, Cl. Oligochaeta, F. Lumbricidae & F. Sparganophilidae	1
Phyl. Nematelminthes, Cl. Nematoda	24
Phyl. Nematelminthes, Cl. Gordiacea	1
Phyl. Plathelminthes, Cl. Turbellariata, O. Tricladetes, F. Planariidae	2

Abbreviations: Phyl.= Phylum; Cl.= Class; O.= Order; GR.= Group and F.= Family.

Prefixes: S= super-; s=sub- and i= infra-.



Supplementary Figure 1 Distribution of major zoobenthic communities (Table 2) along catchment scale (hydrodynamics, geo-morphology and topography formation, as summarized by principal component analysis) and geographical scale (altitude, latitude and longitude) gradients in the Central Pyrenees.