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# Why scale matters? Life connections in the littoral of remote mountain lakes

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## Abstract

In low nutrient alpine lakes, littoral surfaces are the most productive part of the ecosystem, and they are biodiversity hotspots. It is not entirely known how the nature and properties of lake surrounding catchments, their ecological structure and larger landscape factors work together at different scales to shape the structure and functioning of littoral ecosystems.

A total of 114 high altitude lakes and ponds in the central Pyrenees were surveyed to assess the relative control of catchment properties at a variety of scales on littoral zoobenthic communities. At each location benthic invertebrate composition was recorded together with geolocation (altitude, latitude and longitude), composite factors representing hydrodynamics, geo-morphology and topography, riparian vegetation composition, presence of vertebrate predators (trout and frogs), and water pH and conductivity.

A two-step fuzzy set ordination (FSO)-multidimensional FSO (MFSO) model integrating benthic biota and environmental variables revealed that at geographic scale, longitude gradient surpassed altitude in its influence on littoral ecosystem, reflecting a transition between Atlantic and Mediteranean biogeographic regions. Within each catchment, topography (through its control of catchment type, shore and catchment snow coverage, and connectivity with other lakes) was the main driver of taxa composition, while hydrodynamics (waterbody size, type and inflow/outflow volumes) was secondary, and strongly covaried with the former. Locally, riparian plant composition was tightly connected with littoral invertebrate community structure, richness and morphotype diversity. These variables work directly and indirectly by creating habitats (for both, aquatic and terrestrial invertebrate stages), control the water renewal and nutrient input. They together sustained three ecologically tolerant associations adapted to different environments where the lakes are set.

In-lake predation, water conductivity and pH (broad measures of total dissolved ions/nutrients and their bioavailability) had no major influence on benthic communities, being potentially connected to their naturally high variability.

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The findings imply a strong dependence of altitude lake littoral ecosystems to surrounding landscape processes at scales beyond the local environment, which underpins their role as sensors of local and large-scale environmental change. The work also provides exhaustive data on processes characterizing relatively pristine sites, which can help evaluate how major climate/environmental changes can affect their environments.

**Keywords***:* altitude lakes, littoral zone, invertebrate, scale dependency ecotope, landscape factors, riparian vegetation, fish 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). High altitude 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 4000 (Castillo-Jurado, 1992). The littoral and riparian zones of these lakes are critical mediators between sediment and nutrient fluxes of 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 littoral processes take up a great portion of 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 high altitude lakes; this influences water and sediment chemistry, and ultimately their ecosystems (Vollenweider, 1968). Even though littoral zone is just a fraction of total lake area, it harbours the vast majority of species in a lake, and littoral nutrient productivity is vital for the aquatic food webs, contributing substantially to the whole lake ecosystem energy budget (Vander-Zanden et al., 2006, Vadeboncoeur et al., 2011).

High altitudes present numerous challenges to littoral biota, ranging from high solar radiation and water level fluctuations, to low food availability, a short growing season and strong seasonal temperature variation (Bretschko, 1995); most of them find here their distributional boundaries, and are highly sensitive to environmental changes (Bandyopadhyay et al., 1997). For example, winter mortality is a major factor shaping alpine lake insect population dynamics (Oswood et al., 1991). Lake food availability and duration of ice/snow cover during winter is another factor regulating littoral invertebrate communities (Bretschko, 1995), as there are also nitrate and ammonia levels, fish presence, lake morphology (Kernan et al., 2009) and type of shore coverage (Füreder et al., 2006).

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Extreme challenges of high altitudes allow for the formation of relatively simple ecosystems, characterized by a limited number of species and trophic levels, which are highly adapted to the ecotopes they inhabit. Research has shown that in mountain lakes, variability in external condition can affect species abundances, through their relative control on the proximal environment (Kernan et al., 2009). It has been shown that geographical location has a greater influence on macroinvertebrate communities than local environment (Kernan et al., 2009). It is therefore expected that large-scale geographical barriers and climate variability induce strong biogeographical variability, habitat fragmentation, and segregation of littoral biota into distinct ecosystems.

Despite the ecological and geochemical importance of alpine lakes and their littoral zone, the complexity of their ecosystem connectivity to surrounding landscape remains to be fully appreciated. To better anticipate the response of high altitude catchments to environmental changes, it is therefore imperative to integrate the littoral zone into our holistic understanding of catchment functioning, before major alterations occur. This study attempts to quantify the relative influence of catchment attributes on benthic ecosystem at scales from lake to large geographical gradients, and to identify natural littoral communities representing distinct ecosystems. We hypothesize that while local environment and nutrient resources directly support littoral community functioning, its composition is connected to landscape processes at scales beyond that of the lake. The study area has the advantages of being at the confluence of four major biogeographical regions of Europe: Atlantic, Continental, Mediterranean, and Alpine, which should facilitate capturing large-scale transitions over a relatively narrow region.

# 2 Methodology

## 2.1 The lakes under study

A total of 114 lakes and ponds 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). Their selection was largely dictated by their accessibility and size, reflecting a range of typically medium size ponds and lakes. The area is within the boundaries of Pyrenees National Park, France and comprises a series of postglacial catchments. 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 were surrounded by metasedimentary and sedimentary materials such as slate, limestone and sandstone (Zaharescu, 2011).

Most of the study lakes are above the tree line (alpine domain), and they are largely undisturbed by human activity. Pastoralism, leisure fishing and trekking are among the very few activities allowed in the park. Some of the waterbodies in the area are transformed into reservoirs and they are being used as freshwater reserves and for hydropower generation. The great majority of study lakes/ponds are oligotrophic. Their catchments have 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, 2011).

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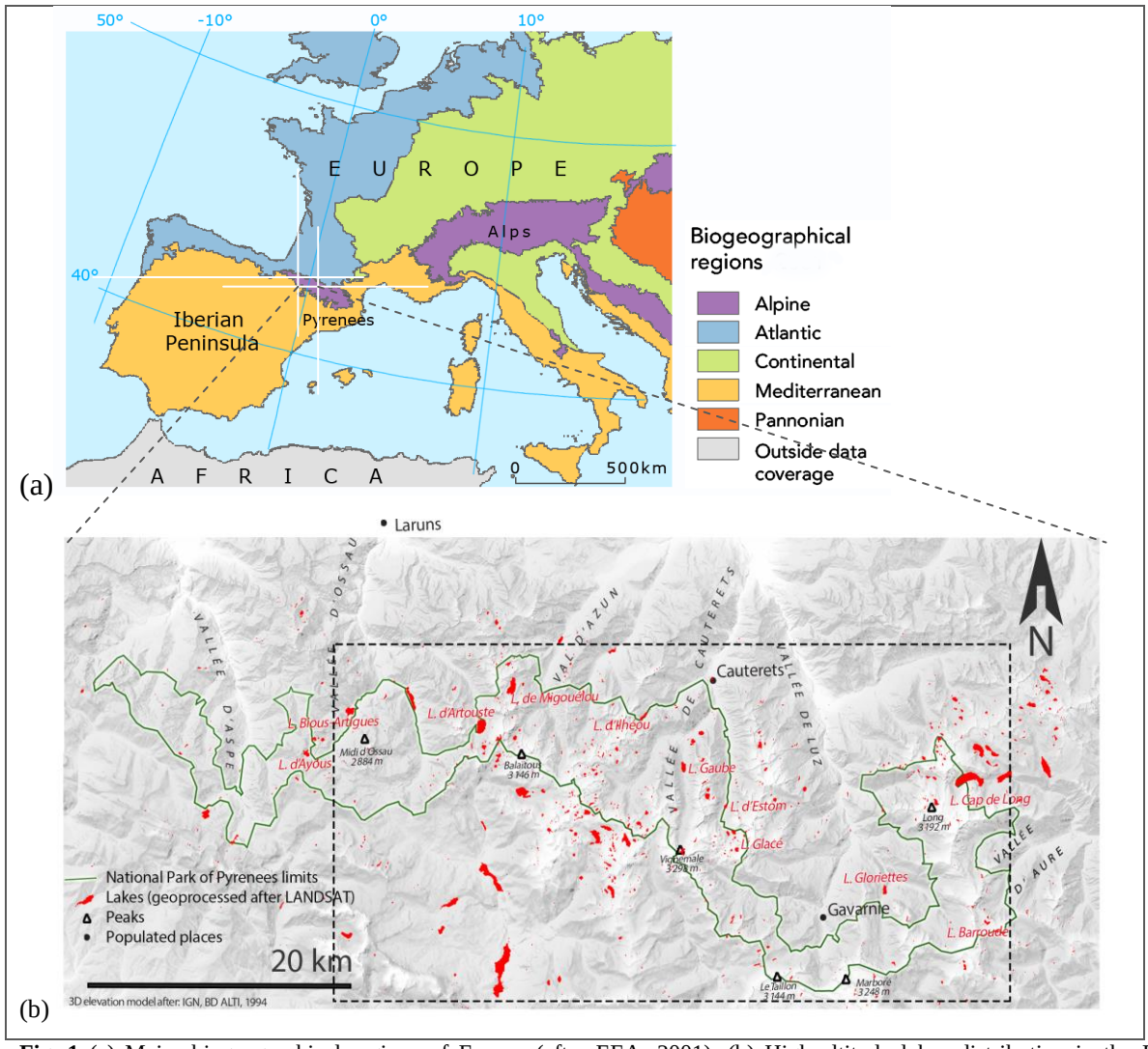


**Fig. 1** (a) Major biogeographical regions of Europe (after EEA, 2001). (b) High altitude lakes distribution in the Pyrenees National Park, France (green boundaries). Only lakes within park boundaries, which are enclosed in the dash line box were considered for this study.

The hydrological network, consisting of temporary and permanent lakes, ponds, pools and streams, is a natural legacy of the last glaciers retreat more than 5000 years ago. Water input in most lakes is by direct precipitation and permanent streams; glaciers and springs were present only in a few cases. Surface connectivity between lakes varied for the lakes investigated. Slope/bank snow coverage at the time of sampling was generally low, but had generally higher coverage at the head of catchments. Water pH was generally neutral (mean = 7.59) but varied between 5.2 and 8.8. Conductivity was also variable, ranging between 2 and 267 µS $cm^{-1}$ (mean= 40µS $cm^{-1}$).

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## 2.2 Sampling strategy and data collection

An exhaustive assessment was conducted for each visited water-body (Fig. 1). It included littoral macroinvertebrates, water pH and conductivity, the presence of vertebrate predators, i.e. frogs and trout, ecotope properties of near catchment, riparian vegetation assemblage and geolocation.

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-minute 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 to cover different micro-habitats in proportion to their occurrence. All substratum types (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. This taxonomic level will be regarded as morphotypes henceforth. For most statistical tests a family level resolution was used (see Supplementary Information).

Additionally, water pH and conductivity were recorded at the surface and the bottom (± 5m off the shore) at each site with portable pH/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. Furthermore, at each location a number of landscape factors were visually approximated according to dominant units. They were: nature of water input and output, tributary discharge, water-body size, % vegetation covering slopes and shore, slope, geology, presence of aquatic vegetation, shore development (fractal level), presence of snow deposits on the shore and in the catchment (%), catchment type and surface connectivity with other waterbodies (Zaharescu, 2011).

Riparian vegetation composition was recorded down to species level in the field at each site using multiple identification keys. A detail description of the procedure is described in Zaharescu (2011). Lakes geographical positions were recorded with a portable GPS device.

## 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, predation, water general chemistry and riparian vegetation.

### *2.3.1 Principal Component Analysis*

Detailed analysis of ecotope factors is exhaustively presented in Zaharescu et al. (2015). They will be summarized here briefly. 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

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groups (Varimax rotation). These composite factors were used as predictors of littoral zoobenthos in further analysis (Table 1). By default, the principal components of PCA with this rotation are uncorrelated.

**Table 1** Association between catchment variables characterising the Pyrenees lakes, and PCA components. Only highest variable correlation with any of the components is displayed. This allowed 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 others | | | 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).

### *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 these a distance (dissimilarity) matrix computed with Sørensen similarity index of invertebrate incidence 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), i.e. taxon (family) richness and sequential diversity comparison index- a simplified method for estimating relative differences in

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biological diversity (SCI; Barbour et al., 1999), where with *run* describing the morphotype and *taxon*, the family (Equation 1).

$$SCI = \frac{no.of\ runs\ x\ no.of\ taxa}{total\ no.of\ individuals}$$

(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/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 they 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 incidence) 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 incidence data. Plotting cluster solutions in discriminating space helped evaluate the reliability of

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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 for 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*

Biomes variability across large geographic areas are triggered by gradient changes in large-scale physical constrains, such as climate and topography. Understanding how lake ecosystems vary across such gradients can help predict their response to global environmental changes. Results of fuzzy set ordination (FSO) and multidimensional FSO (MFSO) of littoral families' composition against altitude, latitude and longitude are displayed in Fig. 2. The unidimensional FSO showed that individually, the three factors could reliably predict the variability in gross littoral taxa (Fig. 2). The relative contribution of these variables to a MFSO model 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, as independent factors).

Zoobenthos composition changes across large horizontal and vertical gradients in latitude, longitude and altitude have been reported before, and whole biome models have been used to evaluate changes in taxon distribution likely to occur with a changing climate (Johnson, 2000; Colwell et al., 2008, IPCC, 2014). At an estimated 60km longitudinal span, the study area is narrow. Its strong influence in the model can, nevertheless be explained by its position at the confluence of major biogeographic regions, i.e. Atlantic, Continental and Mediterranean (Fig. 1), with the W (Atlantic)-to-E (Mediterranean) gradient being the strongest. The results therefore provide evidence that high altitude littoral ecosystems can reflect transitional gradients between major biogeographical regions over relatively narrow spaces.

### *3.1.2 Catchment structure*

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). Detailed examination of catchment factors is presented in Zaharescu et al. (2015). Figures 2 and 3 illustrate the response of littoral organisms to these factors. Both, univariate and multivariate solutions of FSO show that topography was the

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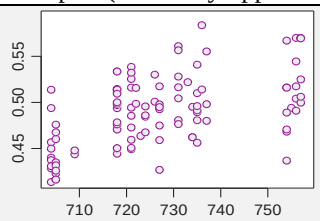
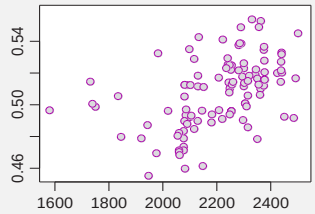
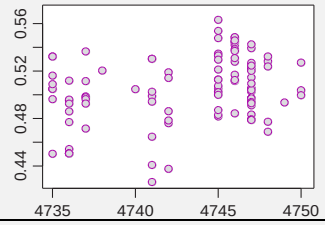
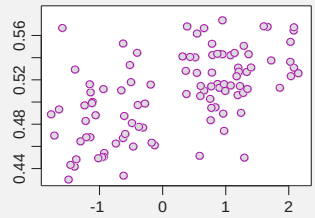
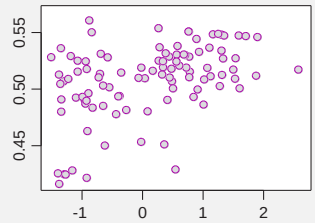
| Factor | *r* (Pearson) | *P* | FSO plot (x-factor/y-apparent factor as predicted by biota) |
|---|---|---|---|
| [a]Longitude (UTM) | 0.547 | 0.001 |  |
| [a]Altitude (m a.s.l.) | 0.470 | 0.001 |  |
| [a]Latitude (UTM) | 0.336 | 0.001 |  |
| PCA regression factor scores: | | | |
| [b]Topography formation | 0.566 | 0.001 |  |
| [b]Hydrodynamics | 0.439 | 0.001 |  |
| [b]Geo-morphology | -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 | |

**Fig. 2** One-dimensional fuzzy set ordination (FSO), showing the response of littoral invertebrate family structure to environmental variables in the Pyrenean 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 & Gallagher, 2001) previously to being used as constrain variables in the analysis.

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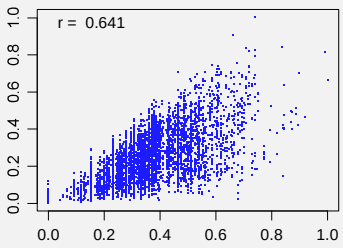
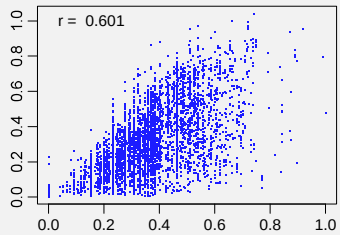
| Axis (log) | Cumulative *r* | Increment *r* | *P*-value | $\gamma$ | MFSO plot (x-matrix dissimilarity/y-ordination distance |
|---|---|---|---|---|---|
| *Geoposition* | | | | |   |
| Longitude | 0.499 | 0.499 | 0.044 | 1.000 | |
| Altitude | 0.623 | 0.124 | 0.167 | 0.360 | |
| Latitude | 0.641 |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

**Fig. 3** Multidimensional response of major littoral invertebrate composition to geoposition and composite catchment factors in 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. $\gamma$ (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.

most important predictor of major littoral biota communities with 99% degree of significance, followed by hydrodynamics. 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/ponds at the head of glacial valleys, with snow presence most of the year, would harbour functional taxa with adaptation for cryal 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. This ecosystem would also likely be more vulnerable to larger periods of snow presence.

The secondary effect of lake hydrodynamics suggests contributions from water source and lake area. For instance, large stream-fed lakes generally 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 smaller waterbodies, dominated by catchment runoff and/or snow melt (therefore not sourced by continuous streams), the extent of littoral surface can vary seasonally and warm faster. These different ecotopes will allow the

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persistence of functional groups adapted to particular conditions of each environment type. This is also supported by the results of studies conducted in altitude oligotrophic environments, which found clear differences in biotic assemblages in spring-fed streams under different flow regimes (Danehy and Bilby, 2009).

### *3.1.3 Effect of riparian vegetation*

With many of the benthic invertebrates also presenting terrestrial phases, relationships between littoral and riparian ecosystems may go beyond their simple proximity or nutrient exchange. (M)FSO model found a significant connection between the vegetation species composition and the invertebrate diversity and family richness (cumulative $r=0.48$, $p<0.05$; Fig. 4). Relatively low but significant relationship was also found between the compositions of vegetation and benthic invertebrates (Mantel test, Monte Carlo $r= 0.16$, $p<0.01$). It is therefore evident that in the nutrient-poor altitude lakes riparian plant species influence littoral ecosystem structure; e.g. by providing suitable microhabitats and food for emerging adults during short summer periods, protection against excessive solar radiation; and nutrient supply for benthic functional feeding groups (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). From our findings, it would appear that sparsely vegetated altitude catchments provide important functional links between vegetation composition around the lakes and the diversity, richness and functional composition of benthic invertebrates.

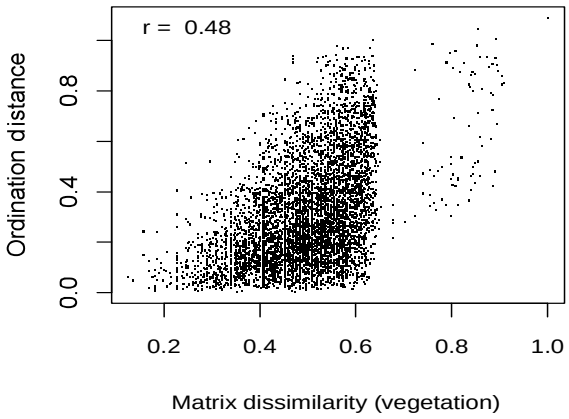


**Fig. 4** Relationship between riparian vegetation structure and littoral invertebrate morphotype diversity and family richness in a bidimensional FSO. A step-across function improved the ordination. Number of permutations = 1000.

### *3.1.4 In-lake predation and major 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

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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 means that vertebrate predators did not disrupt the major taxa composition of littoral fauna. It is possible that predators are size selective, affecting the abundance of certain groups, such as chironomids (Orthocladiinae and Chironominae) and planktonic crustaceans, as has previously been reported for trout (Kernan et al., 2009; Syväranta and Jones, 2009; Schilling et al., 2009). Or niche segregation between aquatic and terrestrial environments could have also played a role. It is known that frogs would largely prey on the more abundant terrestrial (adult) stages of insects (Vieites et al., 1997), which helps them maximise nutrient sequestration during the short period altitude lakes remain unfrozen. 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 support our results. Clearly, these interesting results merit further evaluation.

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

### 3.2 Major littoral communities

Harsh terrestrial environment and low nutrient content of headwater habitats present unique challenges to littoral biota, many of which have terrestrial life stages. These conditions may determine fragmentation of distributional ranges, and favour associations strongly dependent on local lake habitats. Flexible hierarchical clustering and indicator taxa analyses identified three large lake groups hosting characteristic biota (Fig. 5 and Table 2).

The first lake community (type A; Table 2) was the largest, and comprised a significant number of spring-dwellers, tolerant to wide ranges in temperature, altitude, flow regime, pH and micro-habitats (e.g. epi- and endobenthic, rock surfaces and epiphytic). They were mostly of gill and tegumentary respiration, and a feeding strategy largely detritivorous and microphytes, but a small proportion were predatory (e.g. Tanypodinae 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 of this group (eurytopic) means they can colonise a variety of headwaters. Association of Sphaeridae bivalves, Oligochaeta and Lumbriculidae worms with various members in this community has also been reported in headwaters of other regions, including the Oregon Coast Range and the Himalayas (Danehy and Bilby, 2009; Manca et al., 1998). The lakes group with this community are in Fig. 5.

Omnivorous beetles and predatory dragonflies represented the second community (type B). Both are strong flyers as adults, capable of active colonisation and maintaining connected populations not always at easy reach (Table 2). They have long life cycles (>1year) and tolerate a wide range of temperatures. They have affinity to low

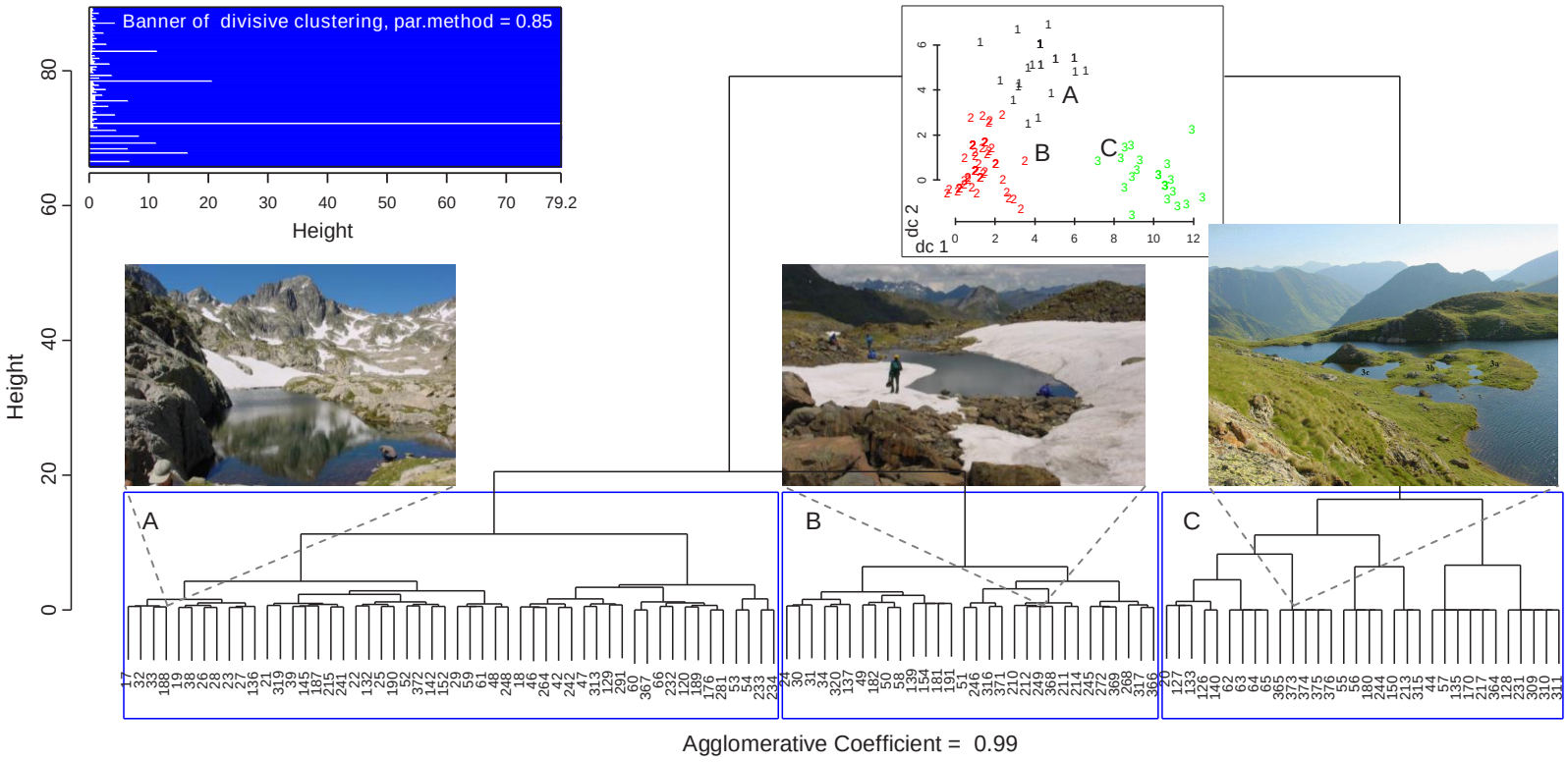


**Fig. 5** Major lake/ecosystem groups (A, B and C) as identified by hierarchical cluster analysis (flexible linkage, parameter = 0.85) based on shared littoral invertebrate families. A plot of cluster solutions in discriminating space (inset) demonstrate an effective clustering. Illustrated are: (A) Cambales Valley lake, (B) Mares de Montferrat, Ossoue Valley and (C), Barroude Petit, Aure Valley. The results are from an analysis of 114 lakes and 46 major invertebrate groups.

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**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.

| 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 Orthocladiinae | 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 & 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 114 central Pyrenean lakes, ponds and pools.

water flow regime and heterogeneous microhabitats (Tachet et al., 2002). Figure 5 displays lakes cluster sharing this littoral group.

The third littoral community (type C), was formed 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 was also diverse: shredders (Limoniidae), microphytes (Helophoridae) and, microinvertebrates and fine suspended matter (Culicidae) (Tachet et al., 2002). Adults of mosquitoes also feed on mammal blood.

Although the studied taxa formed associations, none of them showed preference for defined landscape characteristics along the assessed gradients (results of boxplot distributions not shown). This together with their wide ecological tolerance are evidence of distinct but ubiquitous lake communities, whose composition may result from natural evolution of lake ecosystems, or determined by lake/terrestrial factors beyond those analysed herein.

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# 4 Conclusions

The findings clarify the level of connectivity between the littoral ecosystem and the high altitude environment in the central Pyrenees, at a wide range of scales. Littoral invertebrates responded significantly to large-scale horizontal and vertical gradients, dominated by longitude. The short E-W span ecosystem changes in the study area is consistent with a sharp transition between two major European climates: Mediterranean and Atlantic, which overpowers the typical altitudinal gradient colonisation that followed Holocenic deglaciation. The central Pyrenees are therefore not a homogeneous ecoregion in terms of their lake ecosystems.

At catchment-scale, topography-related variables were the strongest predictors of littoral ecosystem composition, followed by hydro-dynamics. Topography controls habitats at relatively large scale, through traits such as lake basin morphology and riparian colonization. An secondary effect of hydro-dynamics suggests that larger and smaller lakes host distinct littoral communities, likely associated to differences in water balance, biogeochemical nutrient fluxes in the catchment, and the general lake metabolism.

Locally, riparian vegetation composition affected littoral invertebrate community structure. Although generally poorly developed, different vegetation assemblages could provide different microhabitats for the terrestrial phases of aquatic insects, shelter against harsh conditions of solar radiation and wind, and provide weathered nutrients and casing materials for benthic biota. In-lake vertebrate predation (trout and frogs), water pH and conductivity had no clear effect on littoral fauna composition, likely due to their usually high variabilities and control on abundance levels rather than taxonomic.

Community analysis revealed three, relatively simple, functional associations of wide ecological tolerances, characterizing distinct littoral ecosystems. They were of wide distribution across the studied region, a possible consequence of natural lake ecosystem evolution.

In the low nutrient and harsh climate high altitude catchments, riparian and littoral areas are the most bio-productive. The results demonstrate that the littoral ecosystem is connected in direct and indirect ways to a variety of physical, hydrological and ecological attributes from the terrestrial environment at scales extending from lake proximity, to its catchment and beyond. This is important as it helps appreciative the extent of terrestrial-aquatic interactions at high altitudes, and highlights their high vulnerability to external stress from environmental and climate changes. As climate-change continues to drive ecosystem shifts in the mountain biome, our results can serve as relatively pristine benchmark for further studies. Our work also suggests that using the regional scale to characterize ecosystem processes in high altitude lakes is sufficiently robust, and can be confidently used for other studies on high altitude environments.

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# Supplementary Information

## Supplementary List 1

Lakes and ponds in the central Pyrenees National Park surveyed for this study, together with their main hydrographical network, altitude and geographical location (decimal degrees).

| Lake no. | Name | Main valley | Altitude | Latitude | Longitude |
|---|---|---|---|---|---|
| 17 | Lake Berseau | Ossau | 2082 | 42.4959 | -0.3015 |
| 18 | Lake Berseau 1 | Ossau | 2080 | 42.4959 | -0.3015 |
| 19 | Lake Berseau 2 | Ossau | 2100 | 42.4959 | -0.3015 |
| 20 | Pond Berseau 1 | Ossau | 2085 | 42.4959 | -0.3015 |
| 21 | Pond Berseau 2 | Ossau | 2086 | 42.4959 | -0.3015 |
| 22 | Lake Larry 1 | Ossau | 2077 | 42.5018 | -0.3014 |
| 23 | Lake Larry 2 | Ossau | 2077 | 42.5018 | -0.3014 |
| 24 | Lake Larry 3 | Ossau | 2077 | 42.5018 | -0.3014 |
| 25 | Lake Larry 4 | Ossau | 2077 | 42.5018 | -0.3014 |
| 26 | Lake Ayous 1 | Ossau | 2060 | 42.5018 | -0.2929 |
| 27 | Lake Ayous 2 | Ossau | 2060 | 42.5018 | -0.2929 |
| 28 | Lake Ayous 3 | Ossau | 2060 | 42.5018 | -0.2929 |
| 29 | Lake Gentau 1 | Ossau | 1982 | 42.5018 | -0.2929 |
| 30 | Lake Gentau | Ossau | 1947 | 42.5018 | -0.2929 |
| 31 | Lake Miey | Ossau | 1920 | 42.5018 | -0.2929 |
| 32 | Lake Roumassot | Ossau | 1845 | 42.5018 | -0.2929 |
| 33 | Lake Castérau | Ossau | 1943 | 42.4945 | -0.2931 |
| 34 | Lake Paradis | Ossau | 1976 | 42.4945 | -0.2931 |
| 35 | Lake Peyreget | Ossau | 2074 | 42.4942 | -0.2719 |
| 36 | Lake Peyreget 3 | Ossau | 2159 | 42.4941 | -0.2635 |
| 37 | Pond Peyreget | Ossau | 2180 | 42.4941 | -0.2635 |
| 38 | Lake Col de Peyreget 1 | Ossau | 2220 | 42.4941 | -0.2635 |
| 39 | Lake Col de Peyreget 2 | Ossau | 2208 | 42.4941 | -0.2635 |
| 40 | Lake Pombie | Ossau | 2025 | 42.4941 | -0.2635 |
| 41 | Lake Artouste | Ossau | 1989 | 42.5110 | -0.2039 |
| 42 | Lake Arrémoulit Supérieur | Ossau | 2281 | 42.5005 | -0.1957 |
| 43 | Lake Arrémoulit | Ossau | 2285 | 42.5037 | -0.1956 |
| 44 | Lake Arrémoulit (bellow dam) | Ossau | 2255 | 42.5037 | -0.1956 |
| 45 | Lake Palas | Ossau | 2359 | 42.5037 | -0.1956 |
| 46 | Lake Palas 1 | Ossau | 2365 | 42.5037 | -0.1956 |
| 47 | Lake Palas 2 | Ossau | 2362 | 42.5037 | -0.1956 |
| 48 | Lake Arrémoulit Superior 1 | Ossau | 2300 | 42.5037 | -0.1956 |
| 49 | Lake Arrémoulit Superior 2 | Ossau | 2295 | 42.5037 | -0.1956 |
| 50 | Lake Arrémoulit Superior 3 | Ossau | 2297 | 42.5037 | -0.1956 |
| 51 | Lake Arrémoulit Superior 4 | Ossau | 2300 | 42.5037 | -0.1956 |
| 52 | Lake Arrémoulit Superior 5 | Ossau | 2300 | 42.5037 | -0.1956 |
| 53 | Lake Arrémoulit Superior 6 | Ossau | 2305 | 42.5037 | -0.1956 |
| 54 | Lake Arrémoulit Superior 6A | Ossau | 2305 | 42.5037 | -0.1956 |
| 55 | Lake Arrémoulit Superior 7 | Ossau | 2290 | 42.5037 | -0.1956 |
| 56 | Lake Arrémoulit Superior 8 | Ossau | 2285 | 42.5037 | -0.1956 |
| 57 | Lake Arrémoulit Inférieur | Ossau | 2241 | 42.5037 | -0.1956 |
| 58 | Lake Arrémoulit Inferior 1 | Ossau | 2248 | 42.5037 | -0.1956 |

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|---|---|---|---|---|---|
| 59 | Lake Arrémoulit Inferior 2 | Ossau | 2246 | 42.5037 | -0.1956 |
| 60 | Lake Arrémoulit Inferior 3 | Ossau | 2244 | 42.5037 | -0.1956 |
| 61 | Lake Arrémoulit Inferior 4 | Ossau | 2256 | 42.5037 | -0.1956 |
| 62 | Lake Arrémoulit Inferior 5A | Ossau | 2254 | 42.5037 | -0.1956 |
| 63 | Lake Arrémoulit Inferior 5B | Ossau | 2254 | 42.5037 | -0.1956 |
| 64 | Lake Arrémoulit Inferior 5C | Ossau | 2254 | 42.5037 | -0.1956 |
| 65 | Lake Arrémoulit Inferior 5D | Ossau | 2254 | 42.5037 | -0.1956 |
| 66 | Lake Arrémoulit Inferior 6 | Ossau | 2252 | 42.5037 | -0.1956 |
| 67 | Lake Arrémoulit Inferior 7 | Ossau | 2248 | 42.5037 | -0.1956 |
| 68 | Lake Arrémoulit Inferior 8 | Ossau | 2100 | 42.5037 | -0.1956 |
| 120 | Lake Micoulaou 1 | Azun | 2302 | 42.5034 | -0.1744 |
| 123 | Lake Micoulaou 3 | Azun | 2362 | 42.5001 | -0.1745 |
| 124 | Lake Micoulaou 4 | Azun | 2375 | 42.5001 | -0.1745 |
| 125 | Lake Batcrabère Supérieur | Azun | 2180 | 42.5034 | -0.1744 |
| 127 | Lake Batcrabère Supérieur 1 | Azun | 2182 | 42.5034 | -0.1744 |
| 128 | Lake Batcrabére Milieu | Azun | 2130 | 42.5034 | -0.1744 |
| 129 | Pond Batcrabére Milieu 1 | Azun | 2130 | 42.5106 | -0.1743 |
| 132 | Lake bellow Batcrabére Milieu | Azun | 2129 | 42.5034 | -0.1744 |
| 133 | Lake Batcrabère Inférieur | Azun | 2116 | 42.5106 | -0.1743 |
| 135 | Lake Batcrabère Inférieur 1 | Azun | 2116 | 42.5106 | -0.1743 |
| 136 | Pond next to Larribet Refuge | Azun | 2055 | 42.5106 | -0.1743 |
| 137 | Pond Pabat | Azun | 2062 | 42.5106 | -0.1743 |
| 139 | Lake La Claou Supérieur | Azun | 1750 | 42.5210 | -0.1656 |
| 140 | Lake La  Claou | Azun | 1739 | 42.5210 | -0.1656 |
| 142 | Lake Doumblas | Azun | 1580 | 42.5209 | -0.1612 |
| 145 | Pond Pluviometre | Azun | 1731 | 42.5135 | -0.1529 |
| 148 | Lake Remoulis Inférieur | Azun | 2017 | 42.5031 | -0.1532 |
| 150 | Lake  Remoulis Supérieur | Azun | 2019 | 42.5031 | -0.1532 |
| 152 | Pond Casteric | Azun | 2080 | 42.4958 | -0.1533 |
| 154 | Pond Toue | Azun | 2090 | 42.4958 | -0.1533 |
| 166 | Lake Col de Cambalés | Cauterets | 2582 | 42.4925 | -0.1451 |
| 167 | Lake Crete Du Cambalés | Cauterets | 2440 | 42.4925 | -0.1451 |
| 168 | Lake Peyregnets de Cambalés Grand | Cauterets | 2492 | 42.4925 | -0.1451 |
| 169 | Lake Peyregnets de Cambalés Petit | Cauterets | 2453 | 42.4925 | -0.1451 |
| 170 | Lake Cambalés 2 | Cauterets | 2424 | 42.4924 | -0.1407 |
| 171 | Pond Cambalés 2 | Cauterets | 2424 | 42.4924 | -0.1407 |
| 172 | Pond Cambalés Grand | Cauterets | 2380 | 42.4924 | -0.1407 |
| 173 | Pond Cambalés Grand 1 | Cauterets | 2386 | 42.4924 | -0.1407 |
| 174 | Pond Cambalés Grand 2 | Cauterets | 2390 | 42.4924 | -0.1407 |
| 175 | Pond Cambalés Grand 3 | Cauterets | 2441 | 42.4924 | -0.1407 |
| 176 | Lake Cambalés Grand | Cauterets | 2342 | 42.4924 | -0.1407 |
| 180 | Pond Opale | Cauterets | 2222 | 42.4923 | -0.1323 |
| 181 | Pond Opale 1 | Cauterets | 2248 | 42.4923 | -0.1323 |
| 182 | Pond Opale 2 | Cauterets | 2260 | 42.4923 | -0.1323 |
| 184 | Lake Opale Petit Inférieur | Cauterets | 2287 | 42.4923 | -0.1323 |
| 186 | Lake Opale Supérieur | Cauterets | 2320 | 42.4923 | -0.1323 |
| 187 | Pond Petit Laquet | Cauterets | 2360 | 42.4923 | -0.1323 |
| 188 | Lake Petit Laquet | Cauterets | 2350 | 42.4923 | -0.1323 |
| 189 | Lake Costalade Supérieur | Cauterets | 2320 | 42.4923 | -0.1323 |
| 190 | Pond Cambalés | Cauterets | 2315 | 42.4923 | -0.1323 |
| 191 | Lake Costalade Inférieur | Cauterets | 2310 | 42.4923 | -0.1323 |
| 209 | Lake Badéte | Cauterets | 2344 | 42.5024 | -0.1108 |
| 210 | Lake Col d'Arratille | Cauterets | 2501 | 42.4709 | -0.1033 |
| 211 | Pond Arratille 1 | Cauterets | 2363 | 42.4741 | -0.1031 |
| 212 | Pond Arratille 2 | Cauterets | 2330 | 42.4741 | -0.1031 |
| 213 | Pond Arratille 3 | Cauterets | 2315 | 42.4741 | -0.1031 |

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| | | | | | |
|---|---|---|---|---|---|
| 214 | Pond Arratille 4 | Cauterets | 2289 | 42.4741 | -0.1031 |
| 215 | Pond Arratille 5 | Cauterets | 2315 | 42.4741 | -0.1031 |
| 216 | Pond Arratille 6 | Cauterets | 2268 | 42.4741 | -0.1031 |
| 217 | Lake Arratille | Cauterets | 2247 | 42.4741 | -0.1031 |
| 231 | Oulettes. glacier runoff | Cauterets | 2151 | 42.4707 | -0.0905 |
| 232 | Pond Arraillé Inférieur | Cauterets | 2441 | 42.4706 | -0.0821 |
| 233 | Lake Arraillé Milieu | Cauterets | 2450 | 42.4706 | -0.0821 |
| 234 | Lake Arraillé Supérieur | Cauterets | 2485 | 42.4706 | -0.0821 |
| 238 | Pond Baysselance | Luz | 2555 | 42.4632 | -0.0739 |
| 239 | Pond Baysselance 2 | Luz | 2378 | 42.4632 | -0.0739 |
| 240 | Pond Baysselance 1 | Luz | 2236 | 42.4632 | -0.0739 |
| 241 | Pond Montferrat | Luz | 2207 | 42.4455 | -0.0743 |
| 242 | Lake Montferrat | Luz | 2374 | 42.4455 | -0.0743 |
| 243 | Pond Montferrat 1 | Luz | 2372 | 42.4455 | -0.0743 |
| 244 | Pond Montferrat 2 | Luz | 2440 | 42.4455 | -0.0743 |
| 245 | Lake Montferrat 1 | Luz | 2438 | 42.4455 | -0.0743 |
| 246 | Lake Montferrat 3 | Luz | 2438 | 42.4455 | -0.0743 |
| 247 | Lake Montferrat 4 | Luz | 2437 | 42.4455 | -0.0743 |
| 248 | Lake Montferrat 5 | Luz | 2437 | 42.4455 | -0.0743 |
| 249 | Lake Montferrat 6 | Luz | 2440 | 42.4455 | -0.0743 |
| 250 | Lake Montferrat 7 | Luz | 2440 | 42.4455 | -0.0743 |
| 251 | Lake Montferrat 8 | Luz | 2440 | 42.4455 | -0.0743 |
| 263 | Lake Estom | Cauterets | 1804 | 42.4808 | -0.0650 |
| 264 | Pond Sentier d’Estom 1 | Cauterets | 2235 | 42.4703 | -0.0653 |
| 266 | Pond Sentier d’Estom 2 | Cauterets | 2240 | 42.4703 | -0.0653 |
| 268 | Pond Sentier d’Estom 3 | Cauterets | 2240 | 42.4703 | -0.0653 |
| 270 | Pond Sentier d’Estom 4 | Cauterets | 2248 | 42.4703 | -0.0653 |
| 272 | Lake Labas | Cauterets | 2281 | 42.4702 | -0.0609 |
| 274 | Lake Oulettes d'Estom | Cauterets | 2360 | 42.4702 | -0.0609 |
| 276 | Lake Couy | Cauterets | 2445 | 42.4702 | -0.0609 |
| 278 | Lake Turon Couy | Cauterets | 2485 | 42.4630 | -0.0611 |
| 281 | Pond Turon Couy 2 | Cauterets | 2492 | 42.4630 | -0.0611 |
| 283 | Lake Couy Supérieur | Cauterets | 2500 | 42.4630 | -0.0611 |
| 285 | Pond Couy Supérieur | Cauterets | 2500 | 42.4630 | -0.0611 |
| 287 | Lake Glace | Cauterets | 2678 | 42.4630 | -0.0611 |
| 291 | Lake Ossue | Luz | 1834 | 42.4525 | -0.0614 |
| 295 | Lake Especiérès | Luz | 2195 | 42.4240 | -0.0409 |
| 296 | Lake Especiérès Infèrieur | Luz | 2186 | 42.4240 | -0.0409 |
| 297 | Pond Plaiteau de Saint André | Luz | 2075 | 42.4239 | -0.0326 |
| 298 | Ponds Labas Blanc | Luz | 2009 | 42.4239 | -0.0326 |
| 301 | Pond Bassia 1 | Luz | 2277 | 42.4613 | 0.0448 |
| 307 | Pond Serre Longue | Luz | 2190 | 42.4330 | 0.0523 |
| 308 | Pond Esbarris | Luz | 2139 | 42.4329 | 0.0607 |
| 309 | Lake Aires Supérieur | Luz | 2089 | 42.4329 | 0.0607 |
| 310 | Lake Aires Inférieur 1 | Luz | 2081 | 42.4329 | 0.0607 |
| 311 | Lake Aires Inférieur 2 | Luz | 2081 | 42.4329 | 0.0607 |
| 312 | Lake Comble 2 | Luz | 2099 | 42.4327 | 0.0651 |
| 313 | Lake Comble 1 | Luz | 2098 | 42.4327 | 0.0651 |
| 314 | Lake Troumouse 1 | Luz | 2098 | 42.4329 | 0.0607 |
| 315 | Pond Troumouse 1 | Luz | 2105 | 42.4329 | 0.0607 |
| 316 | Pond Troumouse 2 | Luz | 2102 | 42.4329 | 0.0607 |
| 317 | Pond Troumouse 3 | Luz | 2133 | 42.4329 | 0.0607 |
| 318 | Lake Troumouse 2 | Luz | 2135 | 42.4329 | 0.0607 |
| 319 | Lake Troumouse3 | Luz | 2145 | 42.4329 | 0.0607 |
| 320 | Lake Troumouse 4 | Luz | 2148 | 42.4329 | 0.0607 |
| 353 | Pond Aguilous | Luz | 2318 | 42.4506 | 0.0612 |

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|---|---|---|---|---|---|
| 354 | Pond Aguilous 1 | Luz | 2240 | 42.4506 | 0.0612 |
| 355 | Pond Agulious 2 | Luz | 2255 | 42.4506 | 0.0612 |
| 363 | Lake Badet | Aure | 2084 | 42.4536 | 0.0742 |
| 364 | Pond Barroude 6 | Aure | 2345 | 42.4326 | 0.0735 |
| 365 | Pond Barroude 5 | Aure | 2350 | 42.4326 | 0.0735 |
| 366 | Pond Barroude 4 | Aure | 2356 | 42.4326 | 0.0735 |
| 367 | Pond Barroude 3 | Aure | 2374 | 42.4326 | 0.0735 |
| 368 | Pond Barroude 2 | Aure | 2375 | 42.4326 | 0.0735 |
| 369 | Pond Barroude 1 | Aure | 2376 | 42.4325 | 0.0819 |
| 370 | Pond Barroude | Aure | 2385 | 42.4325 | 0.0819 |
| 371 | Pond Barraode refuge | Aure | 2377 | 42.4325 | 0.0819 |
| 372 | Lake Barroude Grand | Aure | 2355 | 42.4325 | 0.0819 |
| 373 | Lake Barroude Petit | Aure | 2377 | 42.4325 | 0.0819 |
| 374 | Pond Barroude Petit 1 | Aure | 2377 | 42.4325 | 0.0819 |
| 375 | Pond Barroude Petit 2 | Aure | 2377 | 42.4325 | 0.0819 |
| 376 | Pond Barroude Petit 3 | Aure | 2377 | 42.4325 | 0.0819 |
| 377 | Pond Barroude Grand 1 | Aure | 2458 | 42.4325 | 0.0819 |
| 378 | Pond Barroude Grand 2 | Aure | 2458 | 42.4325 | 0.0819 |
| 379 | Pond Barroude Grand 3 | Aure | 2458 | 42.4325 | 0.0819 |
| 380 | Pond Barroude Grand 4 | Aure | 2440 | 42.4325 | 0.0819 |

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# Supplementary List 2

Major zoobenthos taxa identified in the 114 lakes, ponds and pools of the central Pyrenees

O. Diptera, sO. Nematocera, SF. Psychodoidea, F. Psychodidae

O. Diptera, sO. Nematocera, SF. Culicoidea, F. Dixidae

O. Diptera, sO. Nematocera, SF. Culicoidea, F. Culicidae

O. Diptera, sO. Nematocera, SF. Chironomoidea, F. Ceratopogonidae & F. Thaumaleidae

O. Diptera, sO. Nematocera, SF. Chironomoidea, F. Chironomidae, sF. Tanypodinae

O. Diptera, sO. Nematocera, SF. Chironomoidea, F. Chironomidae, sF. Chironominae

O. Diptera, sO. Nematocera, SF. Chironomoidea, F. Chironomidae, sF. Orthocladiinae (lato sensu)= (stricto sensu) sF. Orthocladiinae+ sF. Diamesinae+ sF. Prodiamesinae

O. Diptera, sO. Nematocera, SF. Tipuloidea, F. Tipulidae

O. Diptera, sO. Nematocera, SF. Tipuloidea, F. Limoniidae

O. Diptera, sO. Brachycera, SF. Empidoidea

O. Trichoptera, GR. Spicipalpia, SF. Rhyacophiloidea, F. Rhyacophilidae

O. Trichoptera, GR. Spicipalpia, SF. Hydroptiloidea, F. Hydroptilidae

O. Trichoptera, GR. Integripalpia, SF. Limnephiloidea, F. Limnephilidae

O. Trichoptera, GR. Integripalpia, SF. Limnephiloidea, F. Uenoidae

O. Coleoptera, sO. Adephaga, F. Haliplidae

O. Coleoptera, sO. Adephaga, F. Dytiscidae

O. Coleoptera, sO. Polyphaga, GR. Haplogastra (=GR. Palpicornia), SF. Hydrophiloidea, F. Hydrophilidae

O. Coleoptera, sO. Polyphaga, GR. Haplogastra (=GR. Palpicornia), SF. Hydrophiloidea, F. Helophoridae

O. Coleoptera, sO. Polyphaga, GR. Heterogastra, SF. Byrrhoidea, F. Elmidae (=F. Helminthidae, =F. Elminthidae)

O. Megaloptera, F. Sialidae

O. Heteroptera, iO. Nepomorpha, F. Corixidae

O. Heteroptera, iO. Gerromorpha, F. Mesoveliidae

O. Heteroptera, iO. Gerromorpha, F. Veliidae

O. Heteroptera, iO. Gerromorpha, F. Gerridae

O. Odonata, sO. Anisoptera, F. Aeshnidae

O. Odonata, sO. Anisoptera, F. Gomphidae

O. Plecoptera, SF. Nemouroidea, F. Nemouridae

O. Plecoptera, SF. Nemouroidea, F. Capniidae

O. Plecoptera, SF. Perloidea, F. Chloroperlidae & F. Perlodidae

O. Ephemeroptera, F. Baetidae

O. Ephemeroptera, F. Siphlonuridae

O. Ephemeroptera, F.Heptageniidae

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| |
|---|
| Cl. Lamellibranchia, SF. Corbiculacea, F. Sphaeriidae |
| Cl. Gasteropoda, sCl. Prosobranchiata, F. Valvatidae |
| Cl. Gasteropoda, sCl. Prosobranchiata, F. Hydrobiidae |
| Cl. Gasteropoda, sCl. Pulmonata, F. Ancylidae |
| Cl. Gasteropoda, sCl. Pulmonata, F. Lymnaeidae |
| Phyl. Annelida, Cl. Hirudinea, O. Rhynchobdelliformes, F. Glossiphoniidae |
| Phyl. Annelida, Cl. Oligochaeta, F. Naididae |
| Phyl. Annelida, Cl. Oligochaeta, F. Tubificidae |
| Phyl. Annelida, Cl. Oligochaeta, F. Lumbriculidae |
| Phyl. Annelida, Cl. Oligochaeta, F. Enchytraeidae |
| Phyl. Annelida, Cl. Oligochaeta, F. Lumbricidae & F. Sparganophilidae |
| Phyl. Nemathelminthes, Cl. Nematoda |
| Phyl. Nemathelminthes, Cl. Gordiacea |
| Phyl. Plathelmintes, Cl. Turbelariata, O. Triclades, F. Planariidae |

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

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