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Proposal of a typology of Spanish mountain lakes and ponds using the composition of functional groups of macrophytes

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ABSTRACT

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Before establishing the ecological status of lakes, the Water Framework Directive requires their classification in types. Typically, the development of a typology has been based on abiotic variables. However, for the typology to have validity, the classification of lakes should be corroborated with the biological communities in the bodies of water. In this study, to develop a biologically relevant typology, the natural variability of the macrophyte communities in mountain lakes and ponds was evaluated. The use of functional groups of macrophytes as an alternative to the taxonomic approach was also evaluated. Thirty-one reference mountain lakes and ponds, located in the northwest quadrant of the Iberian Peninsula, were included in the study. The functional groups of macrophytes were based on the inorganic source of carbon used in photosynthesis. The typology developed from the functional groups was more conclusive than the classification derived from the taxonomic data. The primary determinants of the variability in the composition of the functional groups of macrophytes among the different types of lakes were the changes in the pH and in the orthophosphate concentration related to the decomposition of macrophytes. The submerged macrophytes dominated in the lakes with low concentrations of orthophosphate and the highest levels of alkalinity. In the lakes with lower pH values, the floating-leaved macrophytes were the dominant plants when the phosphorus concentration was higher, whereas at intermediate concentrations of phosphorus, the bryophytes and isoetids were more abundant; these two lake types were differentiated because of the dominance of the bryophytes in those lakes with higher acidity.

Key words: Typology, mountain lakes, ponds, macrophyte, functional group.

RESUMEN

Propuesta de una tipología de lagos y lagunas españoles de montaña utilizando la composición de grupos funcionales de macrófitos

Previamente al establecimiento del estado ecológico de los lagos, la DMA requiere su clasificación en tipos. Habitualmente, el desarrollo de una tipología se ha basado en variables abióticas. Sin embargo, su validez debería venir contrastada con las comunidades presentes en las masas de agua. En este estudio se evalúa la variabilidad natural de las comunidades de macrófitos en lagos y lagunas de montaña, con el fin de desarrollar una tipología que sea biológicamente relevante. Se evalúa además la validez del uso de grupos funcionales como una alternativa a la aproximación taxonómica. En el estudio se incluyeron treinta y un lagos y lagunas de montaña de referencia, localizados en el cuadrante noroccidental de la Península Ibérica. Los grupos funcionales de macrófitos se establecieron en base a la fuente de carbono inorgánica utilizada en la fotosíntesis. La tipología desarrollada a partir de grupos funcionales fue más concluyente que la derivada de los datos taxonómicos. La variabilidad en la composición de grupos funcionales entre los distintos tipos de lagos estuvo determinada fundamentalmente por los cambios de pH y de ortofosfato. Este último, relacionado con el proceso de descomposición de la biomasa macrofítica. En los lagos con bajas concentraciones de ortofosfato y los niveles más elevados de alcalinidad los macrófitos sumergidos fueron dominantes. Los macrófitos de hojas flotantes dominaron en los lagos con el pH más bajo y con las concentraciones de ortofosfato más elevadas, mientras que para valores intermedios de este nutriente, briófitos e isoétidos *fueron más abundantes. La diferencia entre los lagos definidos por estos dos grupos funcionales se debió a la mayor acidez de aquellos en los que dominaron los briófitos.*

Palabras clave: *Tipología, lagos de montaña, lagunas, macrófito, grupo funcional.*

INTRODUCTION

The Water Framework Directive (WFD; European Union, 2000) proposed an innovative model of water management by changing the concept of water quality to include a measure of the ecological status of the water body. The ecological status should be determined through the evaluation of a number of quality indicator elements, including the composition and the abundance of macrophyte communities. To assess the ecological status of a water body, reference conditions must first be established for the different types of aquatic systems, which have different flora and fauna in the natural state (Free *et al.*, 2006). Therefore, to establish the ecological status, the WFD requires a classification of the aquatic habitats into types or ecotypes. A type or ecotype is a group of aquatic habitats that, in reference conditions, has a specific composition or abundance of flora and fauna that is related to a particular combination of environmental factors for that group (van de Bund & Solimini, 2006). The purpose of the typology is to more easily detect the ecological changes caused by anthropogenic pressures, and therefore, a typology should ensure that the natural differences among aquatic ecosystems are clearly distinguished from those caused by human activity (CIS-WFD, 2003). The WFD allows member states to define the lake typology using either System A or B (European Union, 2000). Most states have opted to use the System B, which, in addition to several obligatory factors, allows more choice in the variables used and in the location of boundaries. In Spain, the water planning instruction classified the superficial water bodies into four categories (rivers, lakes, transitional waters and coastal waters), and types were assigned to each of the categories. For lakes, thirty types were established according to the variables of humidity index, altitude, lake origin, input regime, hydroperiod, and lake area, depth, conductivity and alkalinity (ORDEN ARM/2656/2008).

The assessment of the ecological status of lakes using macrophytes has gained importance because aquatic plants were recognized as one of the biological indicators for use in the biomonitoring programmes listed in the WFD (Free *et al.*, 2006; Nõges *et al.*, 2009). Therefore, the establishment of a typology based on the natural variability of macrophytes in aquatic ecosystems is required (Free *et al.*, 2006). Together with the biological elements, the value of supporting environmental variables (hydro-morphological and physico-chemical parameters) must be included when assessing the ecological status of a body of water. Thus, the typology should identify environmentally distinct types of water bodies with their biological communities in almost pristine reference conditions (Lyche Solheim, 2005). With this approach, the results derived from individual indicative parameters can be used to estimate the biological assemblage related to a particular type of water body (Anonymous, 2004). Different methods have been used to describe and to quantify the relationships among the environmental variables and the biological indicators, such as univariate, multivariate or probabilistic statistics (Moe & Ptacnik, 2007). For example, linear methods were used extensively to analyse the relationships between total phosphorus or total nitrogen and chlorophyll *a* as an ecological response and indicator of phytoplankton biomass (Vollenweider, 1976). Among the most adopted methods worldwide, the use of multivariate options clusters the groups based on the biological data and then group membership is predicted through environmental variables. Of the various applications of multivariate analyses, famous examples exist, including RIVPACS (Wright *et al*., 1997) and the BEAST (Reynoldson *et al*., 1995) methodologies, which both use benthic macroinvertebrate communities in reference rivers and lakes to define the reference community groups, respectively. Finally, probabilistic methods, such as Bayesian models, are increasingly used, and these models are based on and predict probability distributions, which incorporate uncertainties in a more explicit way than the other methods (Moe & Ptacnik, 2007).

A classification of aquatic systems based on the macrophyte community can be developed with two different approaches, i.e., taxonomic identifications or functional groups. Several factors affect the species of macrophyte in natural conditions that can be used to define the types of lake, and the examples include chemical variables such as alkalinity, pH or conductivity (Arts *et al*., 1990; Toivonen & Huttunen, 1995; Vestergaard & Sand-Jensen, 2000; Alahuhta *et al*., 2013). The physical properties, such as the littoral slope or the transparency of the water (Duarte & Kalff, 1986; Scheffer, 1998; Alahuhta *et al*., 2013), and the interaction of macrophytes with the other biotic elements of lakes such as fish, zooplankton, macroinvertebrates, phytoplankton and periphyton are also important (Sand-Jensen & Borum, 1991; Scheffer, 1998; Mulderij *et al*., 2005; Gross *et al*., 2007; Mulderij *et al*., 2007). The use of macrophyte functional groups has emerged as an alternative to the traditional taxonomic approach, and a classification based on this criterion assumes that the properties of a community are better understood and managed when the species are grouped into classes with similar characteristics or similar behaviours (Solbrig, 1993). Therefore, researchers focus on a small set of functional traits that are commonly shared by many plant species instead of a detailed study of each species in the community (Liao & Wang, 2010). Additionally, the functionally defined groups of plants tend to occupy discrete sections of environmental gradients. Thus, with the identification of the members of the group, we can predict the existence of predefined ranges in such gradients. Despite the advantages of the functional group approach, the biocoenotic typologies of lakes based on macrophytes have not used functional groups (Schaumburg *et al*., 2004; Kolada, 2009). The criteria selected to establish macrophyte functional groups are multiple, and include the morphological features of plants such as submerged leaf biomass or total length of roots (Ali, 2003), the growth form (McLaren, 2006) and the response of the seed bank (Araki & Washitani, 2000). However, the importance of the source of the inorganic carbon used in photosynthesis to establish functional groups has been used less frequently (Margalef, 1983).

The primary objective of this study was to develop a classification system of mountain lakes and ponds that was ecologically relevant, with the ability to explain the variation in macrophyte communities, as a previous step in the determination of the ecological status of this type of lakes.

Table 1. Types of human impact that typically affect mountain lacustrine systems in the study area, and the characteristics related to the two levels of impact: severe and low intensity. *Tipos de impactos que afectan habitualmente a los ecosistemas lacustres en el área de estudio y características asociadas a los dos niveles de impacto: severa y baja intensidad.*

Type of impact	Intensity	Characteristics
Livestock pressure	Low	No evidence (tracks, excrements) or very few around the pond or lake
	Severe	Abundant evidences (tracks, excrements) around the pond or lake
Tourism	Low	No significant erosion in the littoral area nor presence of solid waste
	Severe	Erosion of the littoral area or presence of solid waste
Water regulation	Low	No alterations or without severe alterations of hydrological regime of pond/lake
	Severe	Pond/lake with severe alterations of their hydrological regime
Fish introductions	Low	Small fish introductions
	Severe	Massive introductions of fishes with macrophyte bed alteration

We also assessed whether the existing Spanish typology is appropriate to stratify the variation in macrophyte assemblages in mountain lakes and ponds. Moreover, the use of functional groups as a valid alternative to the taxonomic approach in defining macrophyte communities was evaluated.

MATERIALS AND METHODS

Criteria for selecting reference ponds and lakes

The Water Framework Directive includes several possibilities for determining the reference conditions, and of these possibilities, a spatial network of reference sites was chosen. The most effective way to select such sites is to use ecological or environmental pressure criteria (CIS-WFD, 2003). Because understanding of the ecological functions of the studied mountain lakes was insufficient, we chose the environmental pressure criteria to select the sites. The WFD recognizes a reference locality as one that is minimally affected by human activity, and sites with low intensity pressures may be accepted as reference sites. Therefore, according to the information obtained from the "Catálogo de Zonas Húmedas de Castilla y León" (Decreto 194/1994; Decreto 125/2001) and our observations in the field, a list of potential impacts on the ponds and lakes was developed. The potentially four most influential effects of human activity, direct or indirect, on the macrophyte community were as follow: livestock pressure, tourism, water regulation and fish introductions. Because of the difficulty in establishing quantitative levels of the effects of human activities, we chose to identify the effects qualitatively. We established only two levels of effect (severe and low intensity; Table 1) because with a qualitative assessment, the probability of errors would most likely increase with more levels of the effects of human activity. Thus, all the lakes that were accepted as reference localities were those that did not experience any severe impact or did not accumulate more than two low intensity impacts from human activity.

Study area

In this study, we selected 31 mountain lakes and ponds of quaternary glacial origin that were located in the northwest quadrant of the Iberian Peninsula (Fig. 1). Twenty-nine of the lakes and

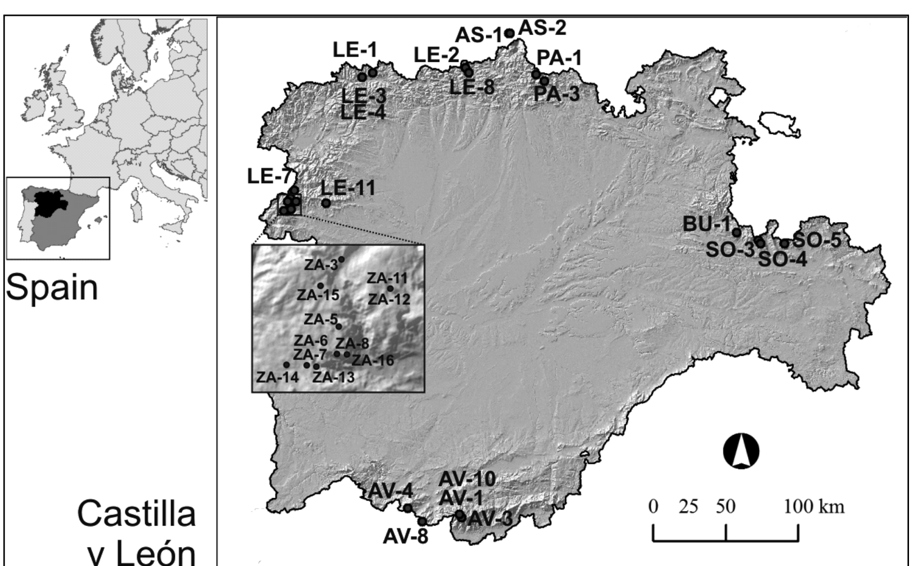


Figure 1. Locations of the 31 study lakes and ponds in northwest Spain. *Localización en el noroeste de España de los 31 lagos y lagunas estudiados.*

ponds are located on the Castilla and León region, which is a large area (94 223 km^2) that consists of a wide central plain from 600 to 800 m that is surrounded by mountains with elevations up to 2600 m.a.s.l. The other two lakes (AS1 and AS2) are located in the Cantabrian Mountains in the Asturias region. All these ponds and lakes are in protected areas and are reference sites or are minimally affected by human activity. Of these bodies of water, 26 are permanent systems and 5 are temporary systems at elevations that ranged from 1070 to 2140 m.a.s.l., with maximum depths between 0.3 and 25 m and lake areas between 0.15 and 12 ha. Tables S1 and S2 contain the morphometric and chemical characteristics and the geographic variables of the lakes, respectively (available at www.limnetica.net/internet).

Sampling of vegetation

The macrophyte vegetation was sampled in June and July in 2007 and 2008. In the ponds, the macrophyte vegetation was studied along profiles, which are defined as a line from one shore to the opposite shore at a right angle to the longest length. When the lake could not be crossed because of the depth, transects were used. The number of profiles varied according to the area of the lake and the development of the shore (Jensén, 1977); however, *in situ* corrections accounted for the heterogeneity of the macrophyte communities and the accessibility to the lake. Square sampling units were placed along the profiles at varying intervals of 0 to 5 m, depending on the homogeneity of the vegetation (the number of units varied according to the width of the lake), and the percentage cover of each species was quantified for each unit.

In the deep lakes, when the direct observation of macrophytes was not possible, the quantification of submerged vegetation was performed with a hook that was thrown from the boat. The sampling points were randomly located in zones of different depth, and four samples were collected in each of the zones. The number of sample points depended on the area of the lake (lakes < 1 ha: 5 points; lakes 1-5 ha: 10 points; lakes 5-10 ha: 15 points; and lakes > 10 ha: 20 points). The coverage values assigned to the species in the deep zones were 25 %, 50 %, 75 % and 100 %, depending on whether the species was collected in 1, 2, 3 or 4 of the samples, respectively.

The mean cover for each species was calculated as the sum of the coverage for that species from the different sample units divided by the total number of the sample units used for that lake. The sampling of the macrophytes was completed with a walk around each pond to register the species that were absent in the profiles. The nomenclature followed the Flora Ibérica (Castroviejo *et al.*, 1986-2010), the Flora Europaea (Tutin *et al.*, 1980) and Cirujano *et al.* (2008).

Functional types of macrophytes

In addition to the taxonomic approach, the composition of the functional groups of macrophytes was also determined. Because of the importan-

Table 2. Environmental variables included in the study, grouped into four categories. *Variables ambientales incluidas en el estudio, agrupadas en cuatro categorías.*

Local variables: Water chemistry variables	Local variables: Physical variables	Catchment variables	Geographical location variables
pH	Lake Surface Area	Catchment Area	Elevation
Conductivity	Maximum Depth	Catchment to Lake Area ratio	Latitude
Alkalinity	Littoral Slope	% Bare rock	Longitude
Nitrate	Persistence	% Shrubland	
Total Nitrogen	% Silt	% Forest	
Orthophosphate	% Sand	% Grassland	
Total Phosphorus	% Pebbels		
Chlorophyll *a*	Secchi Depth		
N:P			

ce of the inorganic carbon source used in photosynthesis (den Hartog & Van der Velde, 1988), we established the functional groups according to this criterion. The resulting groups were as follow: the helophytes, that use the atmospheric CO_2 as the inorganic carbon source; the floating-leaved hydrophytes, that use both the atmospheric CO_2 and the bicarbonate of the water; the submerged hydrophytes, which included the charophytes and the angiosperms, for which the bicarbonate of the water is the inorganic carbon source; the bryophytes, that assimilate the CO_2 from the water; and the isoetids, that primarily use the sediment CO_2 and to a lesser extent the CO_2 from the water.

Environmental variables

To determine the effects on the composition of the macrophyte, a total of 26 environmental variables were measured (Table 2).

The physicochemical variables were collected simultaneously with the macrophyte sampling. The conductivity, pH, temperature, oxygen concentration and percentage oxygen saturation were determined by direct measurement in the water of the pond or the lake with a multiparameter meter. The Secchi disk depth was also measured in the field. In each aquatic system, we established transects from the shore to the centre, and the water samples were collected randomly in areas without vegetation with a core that was 6 cm in diameter and one metre in length. These samples were integrated into a final sample of water for later laboratory determinations of the alkalinity, total nitrogen (TN), total phosphorus (TP), nitrate, orthophosphate and chlorophyll *a*. The samples that were used for the determination of total nutrients were fixed in the field with mercury, whereas those that were used for the analyses of the dissolved nutrients were filtered through a Whatman glass fibre filter (GF/C) and then were fixed with mercuric chloride in the laboratory. All samples were refrigerated at 4 °C until analysis. All analyses were conducted according to the standard methods (APHA, 1989).

The maximum depth was determined with several measurements along a series of transects. An approximate estimation of the littoral slope was performed using a scale of 1 to 4 (1 = very slight; 2 = slight; 3 = moderate; and 4 = steep). Additionally, the granulometric composition of

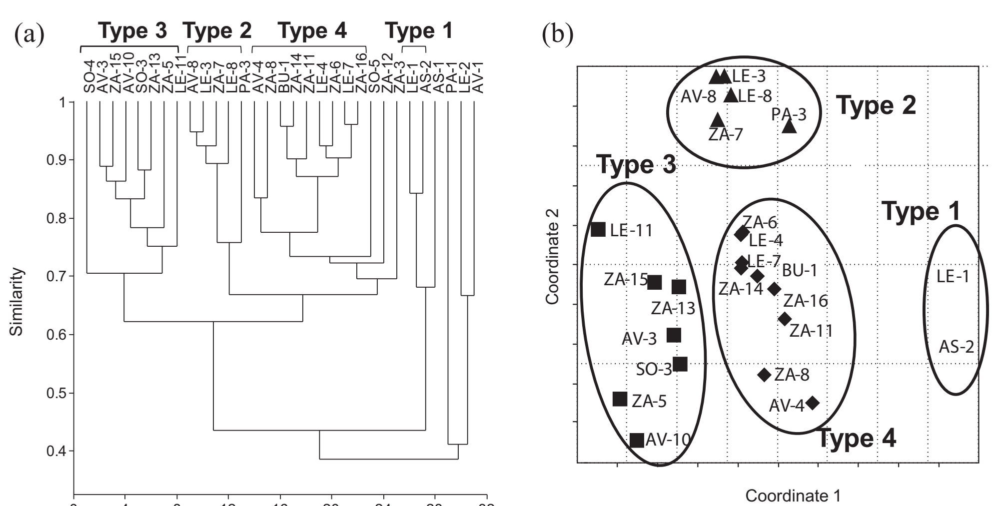


Figure 2. Cluster analysis (a) and NMDS ordination (b) of the 31 study lakes and ponds using the data of the five functional groups (isoetids, bryophytes, emergent, submerged and floating-leaved macrophytes). Type 1 = submerged hydrophytes; Type 2 = bryophytes; Type 3 = isoetids; Type 4 = floating-leaved hydrophytes. *Resultados de los análisis de agrupación (a) y de ordenación NMDS (b) basados en la cobertura de los cinco grupos funcionales (isoétidos, briófitos, helófitos, macrófitos sumergidos y de hojas flotantes) registrados en los 31 lagos y lagunas incluidos en el estudio. Tipo 1 = hidrófitos sumergidos; Tipo 2 = briófitos; Tipo 3 = isoétidos; Tipo 4 = hidrófitos de hojas flotantes.*

the substrate for the percentages of silt, sand and pebbles was estimated in the field. Using the application SIGPAC (www.sigpac.jcyl.es/visor/), we measured the area of the lake and the basin and estimated the land use in the basin (percentages of rocky land, shrubland, forest and grassland), and the geographical coordinates of each lake (latitude and longitude) were determined.

The persistence, temporary or permanent, was also used to separate the ponds and lakes, depending on whether the body of water dried up completely during the summer.

Data analyses

For the statistical analyses, the percentage of coverage of each taxon and of the different functional groups was transformed to a scale of 1 to 5 (1 ≤ 1 %; 2 = 1-2 %; 3 = 2-5 %; 4 = 5-30 %; and 5 ≥ 30 %).

The following steps were used to develop the typology:

- To determine if there were distinct groups of lakes using the macrophyte community in the reference lakes.
- To determine whether such biological groupings were significantly distinct for the macrophytes and the environmental variables.
- To attempt to assign environmental boundaries that were useful in the definitions of the distinct biological types.

To develop the typology, cluster analyses with the abundance data of the different functional groups or species in each reference lake or pond were performed. To assess the significance of the different types suggested by the dendrogram divisions, the differences in macrophyte composition among these types were evaluated using the ANalysis Of SIMilarities (ANOSIM). In this analysis, the statistic R is an absolute measure of the distance among the groups. The highest R-value within a given similarity threshold determined the types of reference lakes and ponds. To corroborate the groupings suggested by these analyses, we conducted a Non-Metric Multidimensional Scaling (NMDS) ordination. The Bray-Curtis index was the similarity measure used in all the analyses.

The second step was to determine whether the types of ponds and lakes previously suggested were different for the macrophyte community and the environmental variables. For this purpose, we conducted a SIMilarity of PERcentages (SIMPER) analysis with a cut-off level of 90 % to detect the species or functional groups that contributed the most to the differentiation of the types. Moreover, a one-way ANOVA was performed to determine whether significant differences were found for the environmental variables among the types. The homogeneity of the variance and the normal distribution were previously tested using the Levene's test and the Kolmogorov-Smirnov test, respectively. Any variables that did not have homogeneity of the variance and a normal distribution were transformed, and we used the arcsine $(x/100)^{-0.5}$ transformation for the variables expressed in percentages and the $\log(x)$ or $\log(x + 0.01)$ transformation for the rest of the data.

To visualize the differences among the types of water bodies, box-plot graphics of those environmental variables for which significant differences were detected in the ANOVA were constructed. We used the Scheffé test to identify the pairs of groups of samples among which significant differences ($p < 0.05$) existed. Because environmental factors may have complementary effects with one another, we evaluated the types for combinations of variables with a multiple discriminant analysis (CVA: Canonical Variate Analysis) (Ter Braak & Šmilauer, 2002). Those variables for which the types presented significant differences were sequentially introduced (forward selection) into the CVA. Before this analysis and to remove possible redundancies, we conducted a correlation analysis and eliminated the variables with a correlation value greater than 0.5.

The statistical packages used for these analyses were PAST v.2.14 for the clustering analyses and ANOSIM; PRIMER v.5 for the SIMPER and NMDS ordinations; STATISTICA v.8 for the ANOVAs, box-plot graphs, correlation analyses and the verification of normality and homogeneity of variances of the variables; and Canoco for Windows 4.5 for the CVA.

RESULTS

A total of 41 macrophyte taxa were identified, with 23 emergent macrophytes or helophytes, 6 floating-leaved hydrophytes, 5 submerged hydrophytes, 5 bryophytes and 2 isoetids (Table S3, available at www.limnetica.net/internet).

The helophytes, bryophytes and floating-leaved hydrophytes were the most frequent functional groups and were present in 97 %, 87 % and 77 % of the ponds and lakes, respectively. The isoetids and the submerged hydrophytes were present in 29 % and 26 % of them, respectively. Within the helophytes, the most frequent species was *Juncus squarrosus* L., which was present in 65 % of the aquatic systems. Other common helophytes were *Ranunculus flammula* L. and *Glyceria fluitans* (L.) R. Br., which were recorded in 48 % and 45 %, respectively, of the ponds and lakes, and *Carex rostrata* Stokes and *Viola palustris* L., which were found in the 32 % of them. The *Sphagnum* sp., *Warnstorfia exannulata* (Schimp.) Loeske and *Fontinalis antypiretica* Hedw. were the most common bryophytes, which were present in 74 %, 55 % and 32 % of the ponds and lakes, respectively. For the floating-leaved hydrophytes, *Ranunculus peltatus* Schrank, *Callitriche brutia* Petagna and *Potamogeton natans* L. were recorded in 55 %, 42 % and 23 % of the ponds and lakes, respectively. The most common isoetid was *Isoetes velatum* A. Braum in Bory & Durieu subsp. *asturicense* (Lainz), which was in 22 % of the aquatic systems, whereas among the submerged hydrophytes, the charophyte *Nitella flexilis* (L.) C. Agardh was the most common and was recorded in 13 % of the reference sites.

The dendrogram obtained from the taxonomic composition showed multiple groups that were poorly differentiated and were formed by a small number of lakes without a defined meaning. By contrast, the dendrogram obtained from the functional groups indicated better-defined groups of ponds and lakes than those obtained from the taxonomic composition (Fig. 2a). Thus, the functional group data were selected to elaborate the typology. The similarity analysis ($R = 0.871$;

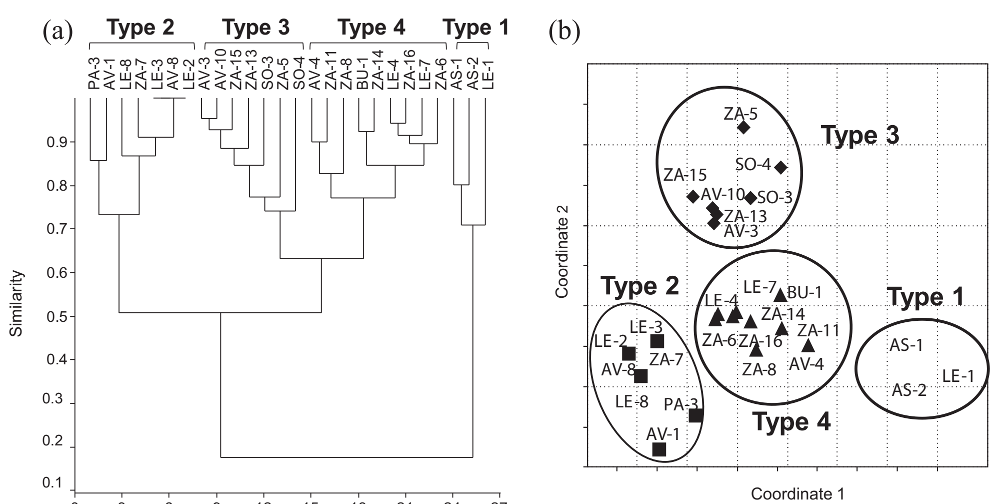


Figure 3. Cluster analysis (a) and NMDS ordination (b) of the 26 study lakes and ponds selected for the typology. The data for the four functional groups (isoetids, bryophytes, submerged and floating-leaved macrophytes) were used. Type 1 = submerged hydrophytes; Type 2 = bryophytes; Type 3 = isoetids; Type 4 = floating-leaved hydrophytes. *Resultados de los análisis de agrupación (a) y de ordenación NMDS (b) basados en la cobertura de cuatro grupos funcionales de macrófitos (isoétidos, briófitos, macrófitos sumergidos y de hojas flotantes) registrados en los 26 lagos y lagunas seleccionados para llevar a cabo la tipología. Tipo 1 = hidrófitos sumergidos; Tipo 2 = briófitos; Tipo 3 = isoétidos; Tipo 4 = hidrófitos de hojas flotantes.*

$p < 0.001$) identified four types of ponds and lakes, which were corroborated with the NMDS ordination (Fig. 2b) (Stress = 0.13). The SIMPER results showed the limited contribution of the helophytes in establishing the types of ponds and lakes because they were found in all the bodies of water. Therefore, the helophytes were omitted in the development of the typology.

In the dendrogram that used only the 4 functional groups (floating-leaved hydrophytes, submerged hydrophytes, bryophytes and isoetids), we selected the groups with the highest values of R ($R = 0.939$) that were statistically significant ($p < 0.001$) as the final types. The final number of ponds and lakes selected to establish the typology was 26, which were divided into the types

Table 3. The results of the SIMPER analysis showing the contribution of the functional groups to the dissimilarity among the types of lakes and ponds. *Se indica la contribución de los grupos funcionales a la disimilitud entre tipos de lagos y lagunas.*

Functional group	TYPE 1	TYPE 2	Mean dissimilarity: 96.89 %
	Mean abundance		Contribution %
Submerged hydrophytes	4.67	0	45.37
Bryophytes	0	4.29	41.66
Floating-leaved hydrophytes	1.67	0.29	12.96
	TYPE 1	TYPE 3	Mean dissimilarity: 83.23 %
	Mean abundance		Contribution %
Submerged hydrophytes	4.67	0.14	32.78
Isoetids	0	3.71	27.09
Bryophytes	0	3.29	23.39
Floating-leaved hydrophytes	1.67	3.29	16.74
	TYPE 1	TYPE 4	Mean dissimilarity: 70.60 %
	Mean abundance		Contribution %
Submerged hydrophytes	4.67	0.78	39.62
Bryophytes	0	3.44	34.49
Floating-leaved hydrophytes	1.67	3.89	25.89
	TYPE 2	TYPE 3	Mean dissimilarity: 56.28 %
	Mean abundance		Contribution %
Isoetids	0	3.71	44.33
Floating-leaved hydrophytes	0.29	3.29	35.66
Bryophytes	4.29	3.29	18.13
	TYPE 2	TYPE 4	Mean dissimilarity: 43.79 %
	Mean abundance		Contribution %
Floating-leaved hydrophytes	0.29	3.89	65.75
Bryophytes	4.29	3.44	21.75
Submerged hydrophytes	0	0.78	12.50
	TYPE 3	TYPE 4	Mean dissimilarity: 36.86 %
	Mean abundance		Contribution %
Isoetids	3.71	0	54.75
Bryophytes	3.29	3.44	18.31
Floating-leaved hydrophytes	3.29	3.89	15.58
Submerged hydrophytes	0.14	0.78	11.36

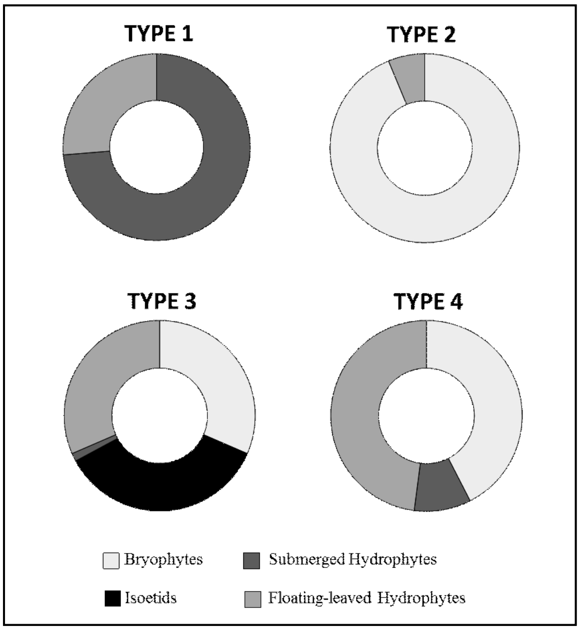


Figure 4. Mean relative abundance of the functional groups in the different types of lakes and ponds. *Valores medios de la abundancia relativa de los grupos funcionales en los tipos de lagos y lagunas.*

(Fig. 3a); the NMDS ordination corroborated the division of the lakes into the 4 types ($S = 0.08$) (Fig. 3b). The SIMPER results identified the functional groups that determined the differences among the types of ponds and lakes. The aquatic systems of type 1 were the most clearly differentiated because of the predominance of submerged hydrophytes. The differentiating characteristic of the type 3 bodies of water was the isoetids, whereas in type 2, the dominant functional group was the bryophytes. The bryophytes and the floating-leaved hydrophyte functional groups characterized type 4 ponds and lakes (Table 3). Figure 4 shows the relative abundance of each functional group in the different types of ponds and lakes, as determined by the cluster analysis.

The types of ponds and lakes were significantly differentiated by six environmental variables, which were conductivity ($F = 21.05$, $p < 0.001$), pH ($F = 15.91$, $p < 0.001$), alkalinity ($F = 13.79$, $p < 0.001$), orthophosphate ($F = 5.12$, $p < 0.01$), N:P ratio ($F = 5.06$, $p < 0.01$) and elevation ($F = 4.28$, $p < 0.05$). The type 1 lakes had pH, alkalinity and conductivity values that were significantly higher than the other three types of lakes. Moreover, in type 1 lakes, the orthophosphate concentration was significantly lower than that in the type 4 lakes in which the floating-leaved hydrophytes were the dominant functional group. The box-plot graphs (Fig. 5) suggested threshold values for the pH, alkalinity and conductivity, which allowed us to establish two groups: high alkaline lakes (> 1 mg/l) with high pH (> 7.5) and relatively high conductivity (> 150 μS/cm) and low alkaline lakes (< 1 mg/l) with low pH (< 7.5) and low conductivity (< 150 μS/cm). The high alkaline group was composed of the lakes of type 1, whereas the other types were in the second group.

The orthophosphate, N:P ratio, pH and elevation were included in discriminant analysis, whereas the alkalinity and the conductivity were excluded because of the high correlations with the pH ($r = 0.616$, $p < 0.001$; $r = 0.498$, $p < 0.05$, respectively). The variable pH was selected instead of conductivity or alkalinity based on the previous results of the ANOVAs and box-plot graphs, both of which indicated that the pH resulted in better differentiation among the types of lakes than the other variables. In this analysis, the combination of pH and orthophosphate was selected as the determinant for the ordination of the ponds and lakes (Fig. 6a). The axis 1 (eigenvalue = 0.713) summarized the distribution of the bodies of water along a pH gradient, and the lakes characterized by submerged hydrophytes (type 1) were located at the basic end of the gradient, whereas at the acidic end, the lakes were dominated by bryophytes (type 2). The ponds and lakes with isoetids (type 3) and those that were characterized by floating-leaved hydrophytes (type 4) occupied an intermediate position on the pH gradient (Fig. 6b). The axis 2 (eigenvalue = 0.399) represented an orthophosphate gradient (Fig. 6a), which thereby marked the differentiation of the lakes with floating-leaved hydrophytes; these hydrophytes had values of this nutrient that were slightly higher than those in the other lakes. By contrast, the lakes of type 1 had orthophosphate levels lower than the other types of bodies of water (Fig. 6b). Furthermore, an increase in the or-

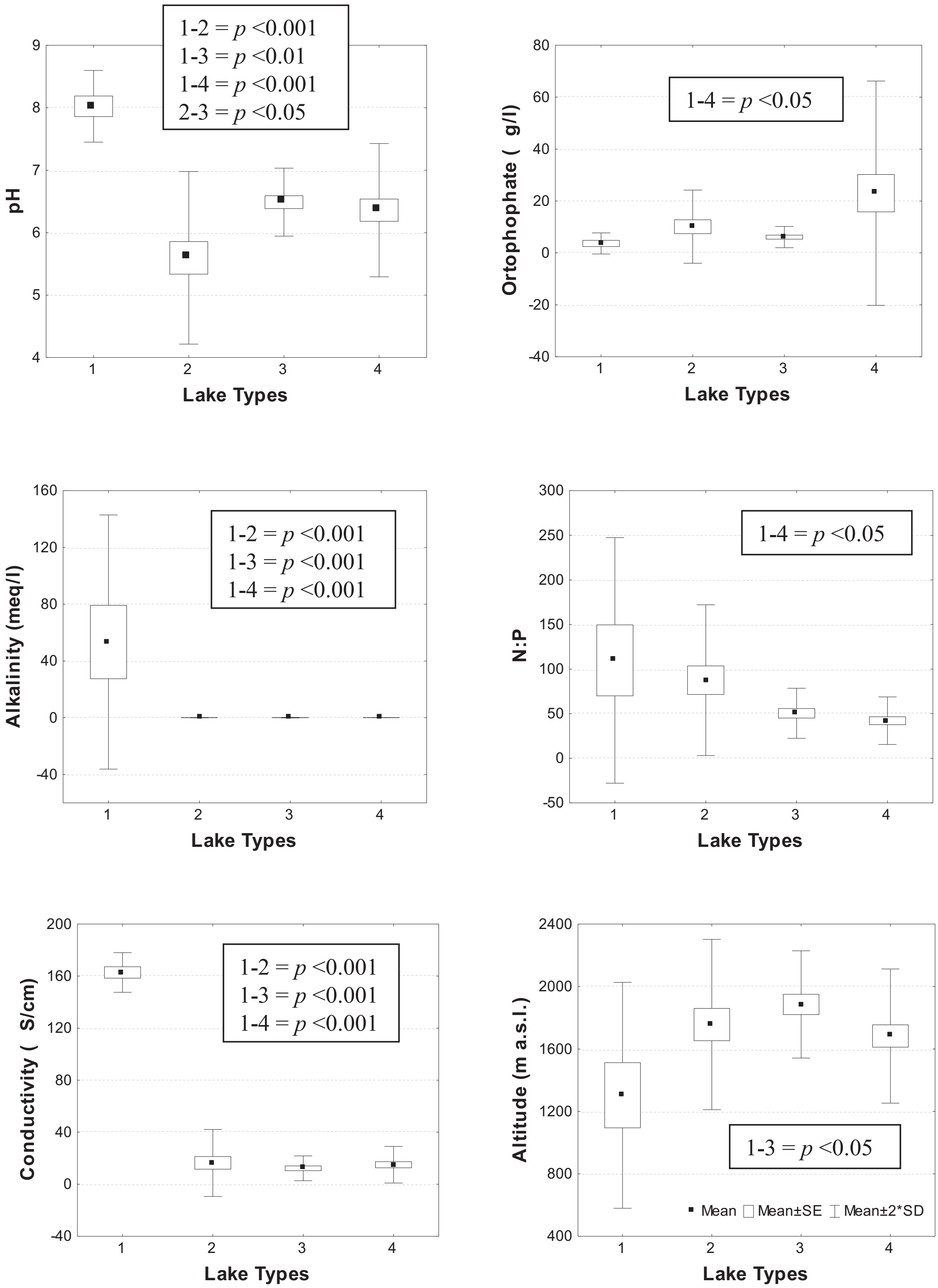


Figure 5. Box-plots of environmental variables that were significantly different among the different types of lacustrine ecosystems. ANOVA results are shown. *Gráficos box-plot de las variables ambientales que mostraron diferencias significativas entre los tipos de lagos y lagunas. Se muestran los resultados del ANOVA.*

thophosphate concentration resulted in an increase of chlorophyll *a* ($r = 0.637$, $p < 0.001$) and in a decrease of transparency ($r = -0.714$, $p < 0.001$).

DISCUSSION

The natural variability in the macrophyte community among the studied reference ponds and lakes was better understood when the species were grouped into functional groups that where based on the inorganic carbon sources used in photosynthesis. The study of this variability identified two local variables, pH and orthophosphate, as the optimum predictors of the differences among the aquatic systems. The pH was clearly related to the lithological characteristics of the basin. These two variables, which were not considered in the official lake and pond typology (ORDEN ARM/2656/2008), were used to classify the ponds and lakes into four types (acidic and oligotrophic; alkaline and oligotrophic; moderately acidic and oligotrophic; and moderately acidic with a greater availability of orthophosphate), which differed in the compositions of the macrophyte community.

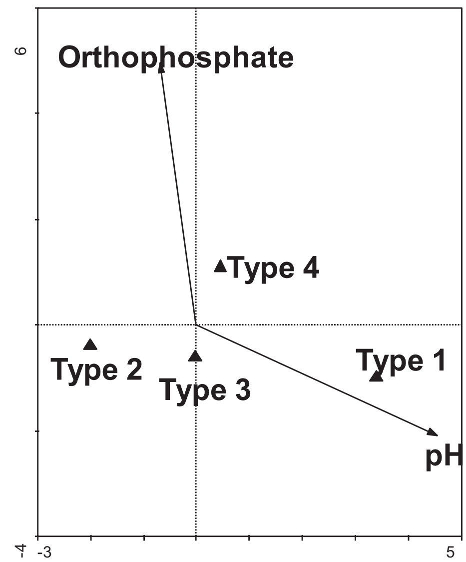
Functional groups of macrophytes versus taxonomy

One of the most important factors to affect the distributions of species and functional groups of macrophytes in lakes is the source of inorganic carbon used in photosynthesis (den Hartog & Van der Velde, 1988; Vestergaard & Sand-Jensen, 2000). Because different species show similar behaviours in relation to the carbon source used in photosynthesis, this criterion can be the basis to establish a series of functional groups of plants. A functional group includes species that have a similar response to the environment with similar effects on ecosystem function (Gitay & Noble, 1997). The species composition of the plant community in individual aquatic systems is often highly site-specific (Brock *et al.*, 2003; Alexander *et al.*, 2008; Barrett *et al.*, 2010). However, the use of functional groups reduces the noise caused by the spatial variability in the floristic data found in the taxonomic level identifications (Campbell *et al.*, 2014). Based on our study, the community characteristics were better understood when the species were grouped into functional groups because the results were more clear than those obtained with the taxonomic approach, which was consistent with the conclusion of previous works (Kumar & Narain, 2010). Moreover, the use of functional groups of macrophytes allowed us to focus on a smaller number of variables (5 functional groups compared with 41 macrophyte taxa) that were related to a unique functional trait instead of studying each species in detail, an advantage that clearly facilitated the development of a typology. The classification of species into functional groups enables to identify the relationships between the macrophytes and the water regime without the requirement for a detailed understanding of botany or a familiarity with scientific names (Campbell *et al.*, 2014). However, the usefulness of the functional group of helophytes for this type of studies seems to be limited because of the weak links with the water conditions. Moreover, variables such as water level fluctuations and wave exposure determine the distribution and composition of helophytes (Coops *et al.*, 1991, 2004); however, these variables were not significant or were not included in this study. Thus, we did not include the helophyte functional group in the analyses, which was consistent with other works related to the establishment of reference conditions and to the assessment of the ecological status of bodies of water (Schaumburg *et al.*, 2004; Free *et al.*, 2006; Søndergaard *et al.*, 2010).

The importance of pH

The clear relationship between the distribution of macrophytes and the pH was demonstrated in previous studies (Arts *et al.*, 1990; Vestergaard & Sand-Jensen, 2000; Chappuis *et al.*, 2014). The most extreme conditions of pH are a limitation to the development of certain functional groups; in such cases, a small number of functional groups are dominant in those lakes. This relationship was demonstrated for the submerged hydrophy-

(a)



(b)

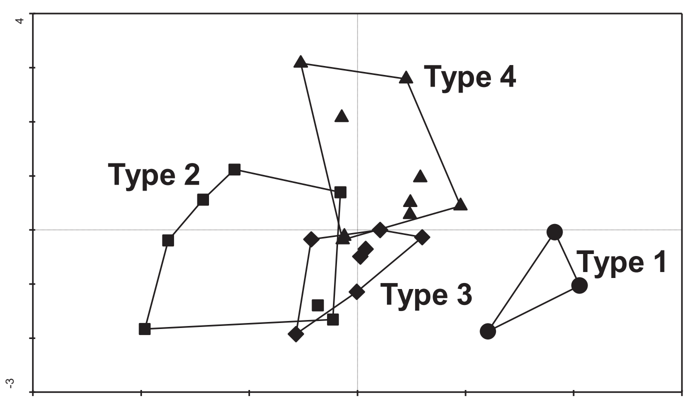


Figure 6. Canonical variate analysis scatter-plots based on five environmental variables and four macrophyte functional groups of 26 lakes and ponds. (a) Axes I and II of the CVA plot showing the centroids of the lacustrine ecosystem types. Environmental variables are indicated by arrows. (b) Ordination of the lakes and ponds of different types on the first two discriminant axes. *Resultados del Análisis Canónico Discriminante para los 26 lagos y lagunas seleccionados, basado en cinco variables ambientales y cuatro grupos funcionales de macrófitos. (a) Ordenación en el plano definido por los ejes I y II de los centroides correspondientes a los tipos de ecosistemas lacustres. Las variables ambientales se indican mediante flechas. (b) Ordenación de los lagos y lagunas pertenecientes a los diferentes tipos en el plano definido por los dos primeros ejes discriminantes.*

tes, which dominated the most mineralized of the lakes (type 1). Lacoul & Freedman (2006) noted that the species richness of this type of plant was greater in alkaline waters with a pH > 7. However, the ability to use and the affinity for bicarbonate varies among species in the same growth form. For example, the charophytes in the most mineralized aquatic systems were in the genus *Chara*, whereas *Nitella flexilis* was in the ponds and lakes with lower pH values in which the submerged hydrophytes were not dominant. The charophytes of the genus *Nitella* were in more acidic waters than those of *Chara*, which was previously noted (Hutchinson, 1975). Unlike the submerged hydrophytes, the growth of bryophytes is restricted or prevented in alkaline waters. The bryophytes cannot use bicarbonate, limiting the carbon source to free carbon dioxide, which is not available in water with even moderately high pH (Madsen & Sand-Jensen, 1991). In general, acidic lakes are poor in submerged species (Vestergaard & Sand-Jensen, 2000), and in this type of lakes the bryophytes are the dominant functional group because of the greater tolerance to acidity (Wetzel, 2001). In our study, this pattern was reflected in the composition of type 2 lakes and ponds in which the bryophytes dominated. Moreover, according to our results, the water acidity and the other factors that limit the development of a greater number of functional groups should be combined. Thereby, for the deepest systems at high elevation (> 2000 m.a.s.l.), the existence of a rocky littoral area and steep slope limited the development of aquatic plants and only the bryophytes had the conditions suitable for growth. Aquatic macrophytes are often limited by high elevations (Gacia *et al.*, 1994; Alahuhta *et al.*, 2011), whereas there is a positive relationship with aquatic bryophytes (Maristo, 1941). Similarly, in the more shallow lakes, the low heterogeneity of habitats favours the predominance of a small number of species or functional groups. Vestergaard & Sand-Jensen (2000) and Jones *et al.* (2003) both noted that with an increase in depth more habitats were available for macrophyte colonization.

The role of orthophosphate

Under the conditions of medium alkalinity, the orthophosphate concentration was the determining factor in establishing the typology. The extreme oligotrophic conditions and, therefore, the high water transparency favoured the development of the isoetids, and this functional group was identified as essential for the differentiation of type 3 bodies of water. The isoetids are dominant when the alkalinity or the pH values of ponds and lakes are not very high (Vestergaard & Sand-Jensen, 2000; Raun *et al.*, 2010); because the isoetids are unable to use the bicarbonate in the water (Madsen *et al.*, 2002), they use the carbon dioxide in sediments as the primary carbon source for photosynthesis (Sand-Jensen, 1987). Thus, the isoetids are photosynthetically independent of the bicarbonate concentration in water. At a similar position on the pH gradient, a greater availability of phosphorus resulted in an increase in chlorophyll *a* and in a decrease in transparency, which caused a shift in the composition of the functional groups. Thus, the isoetids and the submerged hydrophytes were replaced by the bryophytes and the floating-leaved hydrophytes. The transparency of the water, which is inversely related to the trophic status of the lake, is a conditioning factor in the composition of the aquatic plant community (Vestergaard & Sand-Jensen, 2000). As previously established, with a decrease in water transparency, the macrophyte community changes from one dominated with submerged species to a community of floating-leaved and emergent plants, which are groups of macrophytes that are not affected by the decrease in light availability with depth (Moss, 1988; Rodríguez *et al.*, 2003). Furthermore, bryophytes are characterized by the ability to grow under conditions of low light intensity (Riis & Sand-Jensen, 1997).

The identification of phosphorus as a determinant of the typology of ponds and lakes was surprising because these systems are not or are minimally affected by human activity. Typically, increased concentrations of phosphorus in a lake are related to an increase in the external phosphorus loading, which is characteristic of lakes located in landscapes that are heavily affected by human activities (Kagalou *et al.*, 2008). In our study, it would not be expected that the external loading of phosphorus could be the origin of the higher values recorded in type 4 compared with the rest of the types of ponds and lakes. Therefore, the role of macrophytes as a nutrient source should also be considered. Aquatic macrophytes play an important role in nutrient cycling because of the production of large quantities of biomass and the capacity to accumulate large concentrations of nutrients (Clarke & Wharton, 2001; Abdo & Da Silva, 2002). The intensity of nutrient uptake by roots and/or shoots and the site of this nutrient uptake are among the processes that determine the roles of the different macrophytes in nutrient dynamics (Pieczyńska, 1993). The concentrations of nutrients in the sediments are generally several orders of magnitude higher than those in the water (Barko & Smart, 1980; Morris & Lajtha, 1986), and therefore, sediments are the primary source of nutrients for aquatic macrophytes (Prentki, 1979; Barko *et al.*, 1991; Barko & James, 1998), with nutrients supplied by the water column as a secondary source (Thiebaut & Muller, 2000). The emergent and floating-leaved hydrophytes primarily obtain nutrients from the sediments, a process favoured by their typically large and well-developed root systems (Hutchinson, 1975; Granéli & Solander, 1988). However, for the submerged macrophytes, diverse studies showed that nutrient uptake occurred both from the water via the leaves and from the substrate via the roots (Bristow, 1975; Carignan, 1982). Moreover, this functional group generally has fine roots and are considered even pseudo-rooted macrophytes (Granéli & Solander, 1988). In these cases, the aquatic plants obtain most of the nutrients from the water column (Thiebaut & Muller, 2000; Shilla *et al.*, 2006). The type 4 bodies of water were characterized by the dominance of floating-leaved hydrophytes, and the aerial and underground biomass of this functional group, like that of the helophytes, are large compared with submerged vegetation (Granéli & Solander, 1988). The coverage of the helophytes and the floating-leaved hydrophytes was higher in type 4 lakes than in the other types. Because the sediment is the primary compartment for phosphorus storage (Da Silva *et al.*, 1994; Shilla *et al.*, 2006), these macrophytes accumulate large amounts of phosphorus during the growing season, and when these macrophytes die, the decomposition process begins and releases the nutrients back into the water column, which increases the concentration (Howard-Williams & Allanson, 1981; Godshalk & Barko, 1985; Wetzel, 1996).

Comparison with the previous Spanish typology (ORDEN/ARM/2656/2008)

The variables proposed for the classification of lakes in the nationwide typology (ORDEN ARM/2656/2008) differ from the variables that were used to determine the typology in our study. The lithology, which directly affects the pH of water, was the primary determinant of the differences in the composition of the macrophyte community in the study lakes. However, neither the pH nor the orthophosphate concentration are included in the national typology. Furthermore, lakes that were categorized as the same type based on the composition of macrophytes are identified as different types according to the abiotic criteria. Thus, the variables and limits selected for the nationwide typology are not useful to explain the natural distribution of the macrophytes in the mountain lakes of this study. For example, the three type 1 lakes, which were clearly dominated by submerged macrophytes and were identified as alkaline and oligotrophic lakes, are placed in three different types of lakes following the national typology. Notably, the Water Framework Directive indicates that the validity of a typology based on abiotic factors should be derived from the comparison with the biological communities of bodies of water (European Union, 2000).

CONCLUSIONS

The use of macrophyte functional groups, based on the criterion of the carbon source used in photosynthesis, to develop a typology for mountain ponds and lakes produced better results than the taxonomic approach. Four types of ponds and lakes were established as follow: acidic and oligotrophic, characterized by bryophyte dominance; alkaline and oligotrophic, dominated by submerged hydrophytes; moderately acidic and oligotrophic, distinguished by the presence of isoetids; and moderately acidic with a greater availability of phosphorus, characterized by floating-leaved macrophytes and bryophytes. The lakes at both ends of the pH gradient were characterized by a lower diversity of the macrophyte functional groups. Because the ponds and lakes in this study were not or were minimally affected by human activity, the phosphorus released by the breakdown of aquatic plants in lakes with a high coverage of floating-leaved and emergent macrophytes was the determinant of the variability of the macrophyte community in this type of mountain lakes. Our study presents a set of preliminary results for the classification of mountain lakes based on macrophyte functional groups in the Iberian Peninsula, and therefore, subsequent validation with other reference mountain lakes is required.

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