

## Determinants of biodiversity in subtropical shallow lakes (Atlantic coast, Uruguay)

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### SUMMARY

1. Shallow lakes and ponds contribute disproportionately to species richness relative to other aquatic ecosystems. In-lake conditions (e.g. presence of submerged plants) seem to play a key role in determining diversity, as has been demonstrated for temperate lakes. When water quality deteriorates and turbidity increases, conditions in such lakes are affected drastically resulting in a loss of diversity. However, it is not clear whether subtropical lakes show the same pattern and whether the richness of all groups reacts similarly to environmental changes.
2. Our aim was to analyse the main factors explaining patterns of species richness in plankton, fish and submerged macrophyte assemblages in both turbid and clear subtropical shallow lakes. We analysed abiotic and biotic features of 18 subtropical, small- to medium-sized, shallow lakes along the Uruguayan coast. We compared both turbid and clear ecosystem states and evaluated the relative variance explained by the factors measured.
3. Variables describing lake and catchment morphology, as well as the percentage of the water column occupied by submerged macrophytes (%PVI) and water turbidity, had strong effects on taxon richness. Interestingly, individual biotic groups had dissimilar richness patterns. Macrophyte %PVI decreased with increasing lake area, while fish species richness showed the opposite pattern. Phytoplankton species richness increased with macrophyte %PVI, while the zooplankton richness pattern varied depending on the taxonomic group considered.
4. Overall, our results indicate that, as found for temperate lakes, a greater submerged plant cover promotes higher species richness in several groups, and that this may overwhelm the otherwise expected positive effect of lake size on species richness. On the other hand, small-bodied zooplankton predominated in lakes with high plant abundance. Our findings concur with recent studies, indicating that refuge capacity of aquatic plants might be weaker in (sub)tropical than in temperate shallow lakes.

5. The extremely high plant cover, frequently observed in warm lakes, could potentially lead to different richness patterns in some groups. This conclusion has important consequences for local managers and conservationists.

*Keywords:* fish, lake area, plankton, species richness, submerged macrophytes

## Introduction

Shallow lakes are crucial for the conservation of local and global biodiversity (Moss, 2000). These systems vary considerably in species richness, but at a regional level they may contribute more to diversity than other freshwater ecosystems, such as streams, ditches and temporary ponds (Williams *et al.*, 2003). Most shallow lakes occur in lowland areas, often with high human population densities, and as a consequence their environmental and socioeconomic value is being dramatically affected (De Meester & Declerck, 2005). Rather surprisingly, studies on species richness patterns in shallow lakes are underrepresented and largely ignored in monitoring and protection strategies (Waide *et al.*, 1999; Williams *et al.*, 2003). Furthermore, studies analysing the determinants of biodiversity in subtropical or tropical lakes are even more scarce (Havens *et al.*, 1996; Yuma *et al.*, 2006).

The application of the theory of island biogeography (MacArthur & Wilson, 1967; DeBinski & Holt, 2000) to aquatic systems, would predict higher species richness in larger, deeper and less isolated lakes than in smaller, more isolated systems. However, in temperate small shallow lakes and ponds without obvious surface connections to other waterbodies, different factors driving biodiversity (from those described in such theory) might explain their disproportionately high number of species (Williams *et al.*, 2003). Thus, the effects of in-lake processes may outweigh the effects of lake area and connectivity (Scheffer *et al.*, 2006). Local factors, such as productivity (often associated with a clear or turbid water state), are usually crucial in explaining patterns of species richness (Scheffer *et al.*, 1993). The turbid state, dominated by phytoplankton, has poorer water quality and fewer species. The clear water state, often accompanied by dominance of submerged macrophytes, is usually more species rich (Jeppesen *et al.*, 1997, 1998; Declerck *et al.*, 2005). Aquatic vegetation may play a key role in those systems, modulating (i.e. by modifying habitat heterogeneity) ecological inter-

actions and thus leading to a greater species richness than predicted by the theory of island biogeography (Scheffer *et al.*, 2006). In particular, submerged vegetation and the associated high spatial heterogeneity have been shown to promote relatively high richness of aquatic birds, amphibians and invertebrates (Søndergaard, Jeppesen & Jensen, 2005; Scheffer *et al.*, 2006). Recent research in subtropical ecosystems has shown that a much greater richness and density of fish, but not of invertebrates, is associated with beds of submerged plants in subtropical lakes than in similar temperate ones (Meerhoff *et al.*, 2007a,b; Teixeira-de Mello *et al.*, 2009).

Several studies have analysed the relationship between biodiversity and potentially explanatory variables in temperate shallow lakes. However, most studies of species richness have focussed only on one or two communities (e.g. zooplankton or macrophytes) and few works have addressed the simultaneous response of several groups to environmental change (Allen *et al.*, 1999; Jeppesen *et al.*, 2000; Declerck *et al.*, 2005; Beisner *et al.*, 2006). Therefore, it is not clear whether the species richness of all parts of the community changes in the same way in response to environmental drivers (Declerck *et al.*, 2005). Neither it is known how such patterns are affected by trophic interactions among the groups (Dyer & Letourneau, 2003).

Body size might affect the relative effect of the different potential factors. For example, lake area and connectivity are expected to be important for relatively large organisms, such as zooplankton and fish (Hillebrand & Azovsky, 2001). The effects of these factors are less clear for phytoplankton, with some studies showing contradictory results (e.g. Declerck *et al.*, 2005; Smith *et al.*, 2005). The fact that the richness of different species groups may respond differently to the environment could necessitate specific management or conservation programmes for such lakes.

Our objective was to analyse factors explaining richness patterns of several assemblages (phytoplankton,

zooplankton, fish and submerged macrophytes) in subtropical shallow lakes. Therefore, we studied several abiotic and biotic variables from 18 shallow lakes along the Uruguayan Atlantic coast that varied in environmental state (clear or turbid water). Based on the theoretical framework from the temperate zone, we expected that clear and plant-dominated shallow lakes would generally have more diverse species assemblages than turbid ones (e.g. Scheffer *et al.*, 1993), and that the in-lake attributes would be more important than area and connectivity (e.g. Scheffer *et al.*, 2006).

## Methods

### Study area

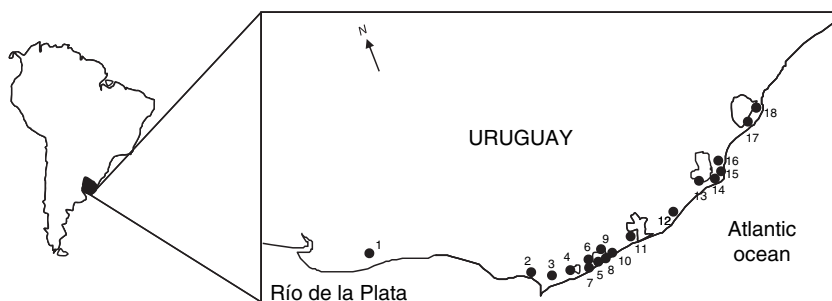
We studied 18 shallow lakes along the south-eastern coast of Uruguay during the 2003 Austral summer (Fig. 1). The lakes are classified as subtropical according to Salas & Martino (1990). Most of these lakes originated during marine transgressions in the Quaternary (García-Rodríguez *et al.*, 2004) and are located at sea level. All systems are freshwater, shallow and small to medium-sized (matching their catchments) and show a gradient in cover of submerged macrophytes. Most of them are surrounded by wetlands and may be considered in a semi-pristine state. Only Pajarera Lake is artificial, whereas Cisne and Diario lakes have been dammed, and Blanca, Escondida and García lakes are used as drinking water reservoirs.

### Data collection

All lakes were sampled once during summer (first half of January 2003) following the same procedure. We selected summer as the sampling season because it is the time of maximum biological activity for

most, if not all, organisms and was therefore best suited to give a representative picture of the ecosystems. We analysed an extensive list of variables (Table 1). Lake and catchment variables were calculated using aerial photographs and GIS methodology (Table 1). We measured in-lake variables *in situ* at the deepest point in each lake (e.g. dissolved oxygen, temperature, conductivity, light, etc.; Table 1). We took samples for water chemistry (e.g. alkalinity, nutrients, etc.) and plankton (Table 1) from three randomly selected shore to shore transects (thus reaching the limits of the surrounding wetland area). We divided each transect into five equidistant points at which we took whole water-column samples with a polyvinyl chloride (PVC) tube (20-cm diameter, length adjusted to lake depth). We mixed the water from the five points into a bulk sample, each transect being a sampling replicate. At the same points, we collected, by scuba diving, three 5-cm deep sediment cores for nutrient analysis. We sampled fish using two techniques (Table 1). The littoral zone was sampled during sunset, by point sample electrofishing, applying a short burst of electricity (hereafter referred to as a 'pulse'), to between 30 and 45 points in each lake, according to the lake perimeter. The fish abundance was estimated as CPUE (capture per unit effort), and calculated as the total number of individuals divided by the total number of electric 'pulses' applied (i.e. points sampled). The pelagic zone was sampled overnight (12 h) with three multi-mesh gill nets (Table 1). Pelagic abundance was estimated as CPUE, and calculated as the number of individuals divided by the net area and by the time of exposure.

We estimated the abundance of submerged macrophytes as the percentage of the water column inhabited by submerged plants (%PVI; *sensu* Canfield *et al.*, 1984) by measuring submerged macrophyte cover, plant height and water depth along equidistant transects



**Fig. 1** Geographical location of the lakes studied. 1, Cisne; 2, Diario; 3, Blanca; 4, Barro; 5, Escondida; 6, Chica; 7, Techera; 8, Nueva; 9, Mansa; 10, Nutrias; 11, Chaparral; 12, Ponderosa; 13, Clotilde; 14, García; 15, Aguada; 16, Moros; 17, Redonda; 18, Pajarera.

**Table 1** List of variables measured in the lakes studied, indicating the units, abbreviations and methods used for their estimation

Variables and units	Method	
<b>Lake and catchment variables</b>		
Catchment area (ha) and perimeter (km)	Aerial photographs and GIS methodology	
Lake area (ha) and perimeter (km)		
Surrounding wetland area (ha) and perimeter (km)		
Island area (ha) and perimeter (km)		
Ratio wetland area and lake area: W/L and lake area		
Number of tributaries		
Mean distance to the three closest freshwater systems (km): connectivity		
Lake mean and maximum depths (m): Zmean and Zmax		<i>In situ</i> measures
<b>In-lake <i>in-situ</i> variables</b>		
Temperature (°C): <i>T</i>		Photosynthetically available radiation profile
Dissolved oxygen (mg L <sup>-1</sup> ): DO		
Conductivity (µSm <sup>-1</sup> ): K		
Light attenuation coefficient (m <sup>-1</sup> ): K <sub>d</sub>		
Secchi disk depth (m): SD		
pH		
<b>In-lake chemical variables</b>		
Alkalinity (µeq L <sup>-1</sup> ): Alk	APHA (1985)	
Soluble reactive phosphorus (µg L <sup>-1</sup> ): SRP	Murphy & Riley (1962)	
Total phosphorus (µg L <sup>-1</sup> ): TP	Valderrama (1981)	
Total nitrogen (µg L <sup>-1</sup> ): TN	Valderrama (1981)	
Nitrate (µg L <sup>-1</sup> ): NO <sub>3</sub>	Müller & Widemann (1955)	
Ammonium (µg L <sup>-1</sup> ): NH <sub>4</sub>	Koroleff (1970)	
Dissolved inorganic nitrogen (µg L <sup>-1</sup> ): DIN	NO <sub>3</sub> + NH <sub>4</sub>	
Silicate (µg L <sup>-1</sup> ): SRSi	Müllin & Riley (1955)	
Total suspended solids (µg L <sup>-1</sup> ): TSS	APHA (1985)	
Coloured dissolved organic matter (nm <sup>-1</sup> ): CDOM	Fluorescence (Mcknight <i>et al.</i> , 2001)	
Sediment TP (µg DW g <sup>-1</sup> ): sed TP	Valderrama (1981)	
Sediment TN (µg DW g <sup>-1</sup> ): sed TN	Valderrama (1981)	
<b>Assemblages</b>		
Submerged macrophytes richness: Smac	Direct observation in the water body	
Percentage of plant volume inhabited (%): %PVI	Canfield <i>et al.</i> (1984)	
Submerged macrophytes spatial heterogeneity: heterogeneity	Nine classes (Semeniuk <i>et al.</i> , 1990)	
Phytoplankton abundance (org ml <sup>-1</sup> )	Lugol's iodine (Utermöhl, 1958) in random fields	
Zooplankton species richness: Szoo	69-µm-mesh size net	
Zooplankton abundance (org L <sup>-1</sup> ): Zoo	Formaldehyde 4% (Paggi & De Paggi, 1974)	
Fish abundance in the littoral zone (CPUE: ind. pulse <sup>-1</sup> )	Electrofishing (Perrow <i>et al.</i> , 1996). Elektrofishfanganlage Type FEG 1000. Range of electric pulses per lake: 30–45	
Fish abundance in the pelagic zone (CPUE: ind. net m <sup>-2</sup> h <sup>-1</sup> )	Multi-mesh gill nets (30 m × 1.5 m, mesh sizes: 15, 20, 25, 30, 35, 40 and 50 mm knot to knot)	
Fish species richness: Sfish	Electrofishing and multi-mesh gill nets	

covering the whole lake. The number of transects varied according to the lake area. With this information, we also classified the lakes in relation to the spatial heterogeneity of their submerged plant communities, according to Semeniuk *et al.* (1990). First, we assessed the spatial arrangement of the plant species in each lake, considering three classes: homogeneous (only one species), zoned (several species in different patches) and heterogeneous (several species co-occurring in patches). Secondly, we evaluated the spatial distribu-

tion pattern across the system, considering three classes: littoral, mosaic and complete. The combination of these two characteristics resulted in nine classes, with class 1 having the lowest heterogeneity (i.e. one submerged plant species only at the periphery of the lake) and class 9 the highest (i.e. several species in a heterogeneous arrangement, covering all the system). Lakes without submerged plants belonged to class 0.

We estimated the species richness of each assemblage (phytoplankton, zooplankton, fish and sub-

merged macrophytes) after identifying all individuals to the species level. We applied rarefaction to plankton counts by counting until the number of species reached an asymptote, when no more new species appeared after 2–3 U of counting effort. In the case of phytoplankton, we counted until we reached at least 100 individuals of the most frequent species, even if species saturation had been already reached. We did not include here those species strongly associated with periphytic communities. For the zooplankton community, we took 1 and 5 mL of subsamples and counted them fully, with the aim of counting at least 100 and 50 individuals of the commonest species of rotifers and microcrustaceans (copepods and cladocerans), respectively, and subsequently screened the rest of the sample for rare species.

We calculated the mean organism size for plankton communities as a weighted average of the mean species size. We measured fish in the field while, for plankton communities, we used unpublished data from studies in the same region (C. Kruk & G. Lacerot, Universidad de la República, unpubl. data). We considered only those phytoplankton species that made up more than 1% of the total, while we considered all zooplankton and fish species. For fish mean sizes, we used the data for the species collected by seine net and electrofishing separately, to take into account the different size selectivity of the two methods.

#### Data analysis

We used bivariate correlations (Pearson's coefficient,  $r$ ; or Spearman's coefficient,  $r_s$ ) to analyse the relationship between the abundance and richness of each assemblage, as well as that between richness and the biotic- and abiotic-measured variables. To analyse the effect of the biotic- and abiotic-measured variables on the species richness of each community, we carried a multiple regression model for each assemblage. We selected the best independent variables for each model by a forward selection procedure. The original variables included in all cases were:  $\log_{10}$  lake area, %PVI, fish CPUE, zooplankton abundance, phytoplankton abundance,  $K_d$  (light attenuation coefficient), TP (total phosphorus), TN (total nitrogen), SRP (soluble reactive phosphorus), DIN (dissolved inorganic N) and sediment TN (Table 1). We did not include the abundance of a particular assemblage as an explanatory factor in its corresponding model.

Furthermore, to analyse the effects of turbidity on taxon richness, we classified the lakes into clear or turbid and applied nonparametric correlations. We classified the lakes as clear when they had more than 1 m Secchi disk depth (SD) (following Scheffer *et al.*, 1993) and, alternatively, if lower than 1 m SD, had >50%PVI. The rest of the systems were classified as turbid and not dominated by submerged macrophytes, with high turbidity being either because of inorganic suspended solids or phytoplankton (as chlorophyll-a). We analysed the effects of turbidity by either including or removing the turbid systems from the data set. We tested for differences in the abiotic variables and the species richness per assemblage between the turbid and clear lakes with nonparametric Kruskal–Wallis tests ( $H$  statistic).

To assess the relative contribution of environmental variables to the richness of the assemblage studied, we performed a series of ordination analyses including lake and catchment morphology, in-lake variables, phytoplankton and zooplankton abundance, fish CPUE and submerged macrophytes %PVI as potential explanatory factors (Table 1). A preliminary detrended correspondence analysis with log-transformed biological data showed short gradient lengths (i.e. 0.87 SD). We therefore used redundancy analysis (RDA), a linear response model, for subsequent ordination analyses (ter Braak & Smilauer, 1998). A selection process was used to eliminate the variables lacking a unique contribution to the regression equation, in favour of a smaller number that explained a high proportion of the variance with low redundancy. Prior to the analysis, we assessed the importance of each variable using the forward selection procedure in a RDA and included only those with a significant ( $P < 0.05$ ) contribution in further analyses. Subsequently, to evaluate the severity of multicollinearity among the explaining variables, we inspected the variance inflation factors (VIF) and removed those variables with values higher than 20 (ter Braak, 1986). We applied the variance partitioning procedure (Borcard, Legendre & Drapeau, 1992) to quantify the effects and assess the relative contribution of the variables selected. We thus estimated the variation explained by all the variables selected and the partitioned variance explained by those finally selected, testing their significance using Monte Carlo simulations with 499 unrestricted permutations. All analyses were performed with CANOCO 4.52 software.



## Results

### Submerged macrophytes

We found 24 macrophyte species in total, most of which were submerged and rooted, whereas rooted floating-leaved species were infrequent. We did not exhaustively record the free-floating and emergent species. Free-floating plants were scarce and species poor (i.e. four taxa). Most systems were surrounded by wetlands with emergent macrophytes (of rather similar species composition in the different lakes), but emergents were scarce or absent from open water. The lakes varied widely in the richness (18 submerged macrophytes species in total), abundance (i.e. %PVI) and heterogeneity of submerged macrophytes (Table 2). Greater heterogeneity, macrophyte richness and %PVI were coincident (Table 3). *Cabomba carolineana* Gray, *Potamogeton illinoensis* Morong and *Ceratophyllum demersum* L. were the most widespread species.

We found no significant relationship between the species richness of submerged plants and lake area, but there was a negative relationship with the distance to other aquatic systems (connectivity), light attenuation coefficient ( $K_d$ ), total suspended solids (TSS) and chlorophyll-a (Chl-a; Table 3). From the set

of candidate factors in the multiple regression analysis, only  $K_d$  had a significant and negative effect on the richness of submerged macrophytes (Table 4).

Submerged plant abundance (%PVI), was negatively related to lake size (Fig. 2a) and turbidity (Fig. 2b). As turbid lakes consistently had almost no macrophytes, the pattern of an increase of %PVI with decreasing lake area was particularly notable when only clear lakes were considered (Fig. 2a).

### Phytoplankton

Phytoplankton consisted largely of small planktonic chroococcal cyanobacteria (e.g. *Aphanocapsa* Nägeli and *Aphanothece* Nägeli), nanoflagellates and picoplankton. There were 466 total species, but species richness per lake varied widely (from 26 to 134). We observed no blooms during the sampling period, and the cyanobacterium *Microcystis* Kützing ex Lemmermann was abundant only in Lake Chica. This lake also had the highest concentration of Chl-a (Table 2).

There was some tendency for total phytoplankton richness to decrease with increasing lake area (Fig. 2c), and a clear increase with increasing submerged vegetation %PVI (Fig. 2d). We observed a

**Table 2** Main limnological characteristics of the 18 lakes

Lake	Area Ha	TSS $\mu\text{g L}^{-1}$	TP $\mu\text{g L}^{-1}$	TN $\mu\text{g L}^{-1}$	SRSi $\mu\text{g L}^{-1}$	Sed TN $\mu\text{gDWg}^{-1}$	SD M	Chl-a $\mu\text{g L}^{-1}$	%PVI	Heterogeneity
Aguada	1.10	3.7	43.0	975	882	902	0.40*	3.9	100	9
<b>Barro</b>	13.5	28.0	32.8	884	855	887	0.26	4.9	0	0
<b>Blanca</b>	28.7	8.9	51.9	1017	3518	127	0.66	3.4	13	3
Chaparral	1.22	5.0	47.2	598	387	1279	0.73*	8.7	75	7
<b>Chica</b>	2.21	48.7	90.5	1164	76.6	1204	0.10	46.5	0	0
<b>Cisne</b>	157	30.1	413.0	1048	4848	1098	0.10	4.2	0	0
Clotilde	17.7	0.2	27.7	451	3219	1425	1.75	4.1	30	2
<b>Diario</b>	61.8	9.2	75.8	825	4640	674	0.55	1.3	34	8
Escondida	10.8	1.9	24.2	489	3865	1127	1.07	1.1	16	2
Garcia	5.22	1.0	29.8	332	2252	1410	1.62	0.0	5	1
Mansa	17.6	1.5	184.2	1534	2275	1251	1.02	4.5	68	8
Moros	0.96	1.5	28.7	437	3080	472	1.03	14.0	14	4
Nueva	0.53	6.3	60.9	1160	305	1822	0.63*	7.0	90	9
<b>Nutrias</b>	33.8	8.2	99.8	1136	203	1318	0.52	3.3	0	0
<b>Pajarera</b>	0.50	31.3	179.8	2691	4189	304	0.27	13.8	0	0
Ponderosa	0.61	6.3	86.5	888	1130	1422	0.88*	9.4	65	7
Redonda	4.70	0.9	23.9	514	2863	1051	2.02	1.5	58	9
Techera	1.01	15.7	37.9	1681	203	1574	0.70*	13.7	96	7
Mean	20.3	11.6	85.4	990	2155	1075	0.79	8.1	37	4.22
Max	157	48.7	413.0	2691	4848	1822	2.02	46.5	100	9.00
Min	0.52	0.2	23.9	332	76.6	127	0.10	0.0	0	0.00

Systems classified as turbid are bold type; the others were classified as clear. Abbreviations as in Table 1.

\*The systems where the Secchi disk depth (SD) was covered by very abundant submerged macrophytes.

Table 3 Bivariate Spearman's correlations showing the significant correlation coefficients

	Alk	SRSi	Lake area	Catchment area	Wetland area	Connectivity	K <sub>d</sub>	TSS	TP	TN	%PVI	S macrophytes	Heterogeneity	Chl-a	Zoo
%PVI															
SRSi	0.851*		-0.467**	0.735*	0.745*	-0.471**		-0.412**				0.738*	0.904*		
Lake area	0.458**	0.750*	0.750*	0.701*	0.735*	0.432**					-0.467**			-0.645*	
Catchment area	0.581*	0.735*	0.701*	0.703*	0.641*	0.555*								-0.581*	
Wetland area	0.439**	0.745*	0.973*	0.703*	0.467*	0.641*					-0.424**			-0.596*	
Connectivity	0.432*	0.555*	0.641*	0.467*	0.467*	0.467*					-0.471**				
Sediment TN	-0.517*	-0.552*	-0.458**	0.641*	0.467*	-0.484*					0.391**				
TSS							0.589*		0.628*	0.690*	-0.412**			0.422**	
S macrophytes							-0.454*		-0.459**		-0.542*			-0.438**	
S cyanobacteria	0.574*	0.679*	-0.761*	-0.516	-0.783*	-0.487*					0.738*			-0.427**	
Phytoplankton average size											0.533*			0.414**	-0.498*
Zooplankton average size															
Fish average size (electrofishing)			0.557*		0.486**										

Abbreviations as in Table 1. S = richness. We did not include the variables with nonsignificant relationships. Significant levels: \* $P < 0.05$ , \*\* $P < 0.10$  level (two-tailed).

similar but stronger pattern in case of cyanobacterial species richness (Table 3). Phytoplankton biomass (measured as Chl-a concentration) decreased with increasing size of the lake, catchment and surrounding wetland, as well as with higher TSS (Table 3). We also detected a negative relationship between species richness of cyanobacteria and chlorophyll-a (Table 3).

We then evaluated the relative effect of the main factors (lake area, %PVI, fish CPUE, zooplankton abundance, K<sub>d</sub>, TP, TN, sediment TN, SRP and DIN) on phytoplankton richness. Only sediment TN was a significant explanatory variable in the multiple linear regression (Table 4). We found no significant relationship between phytoplankton species richness and zooplankton or fish. Moreover, the mean size of phytoplankton taxa was correlated negatively with zooplankton abundance and positively with spatial heterogeneity (Table 3).

### Zooplankton

We found a total of 194 zooplankton species, with rotifers being the richest group (100 species). Species richness varied widely between lakes (from 9 to 41 species). Mean zooplankton abundance ranged between 43 and 1811 ind. L<sup>-1</sup>. Rotifers were most numerous, particularly *Keratella americana* Carlin and *Brachionus caudatus* Barrois & Daday. Cladocera constituted the second most abundant group, especially *Bosmina longirostris* Müller and *Diaphanosoma birgei* Korinek.

There was no statistically significant relationship between total zooplankton richness or total zooplankton abundance and %PVI or lake area. In the forward selection of the zooplankton richness regression model, no variable satisfied the entry criteria. In case of rotifers, no variable was significant in the forward selection model although backward selection yielded a model with a positive effect of K<sub>d</sub> and a negative effect of SRP. Cladoceran richness was related positively to K<sub>d</sub> and TN, and negatively to DIN, whereas copepod richness was positively related to log<sub>10</sub> lake area (Table 4). In all cases, the variables with the largest effect were K<sub>d</sub> and lake area, whereas nutrients, although significant, had less effect. Fish CPUE and phytoplankton abundance were not related to species richness or abundance of either the total abundance of zooplankton or that of any of the main zooplankton groups.

**Table 4** Regression models for taxon richness of submerged macrophytes, phytoplankton, rotifers, cladocerans, copepods and fish, showing the best independent variables for each model chosen by a forward selection procedure

Assemblage	Unstandardised coefficients	R <sup>2</sup>	ANOVA, F-value (P-value)
Submerged macrophyte richness	Constant = 3.733, K <sub>d</sub> = -0.363	0.375	9.003 (0.009)
Phytoplankton richness	Constant = 28.729, sediment TN = 0.033	0.293	6.220 (0.025)
Rotifer richness	Constant = 8.557, K <sub>d</sub> = 1.068, SRP = -0.041	0.309	3.124 (0.076)
Copepod richness	Constant = -0.210, Log <sub>10</sub> lake area = 1.695	0.257	5.197 (0.038)
Cladoceran richness	Constant = 1.986, K <sub>d</sub> = 0.786, DIN = -0.037, TN = 0.014	0.893	24.91 (0.001)
Fish richness	Constant = 9.469, Log <sub>10</sub> lake area = 3.538	0.310	6.745 (0.020)

The original variables included in all cases were: log<sub>10</sub> lake area, lake area, %PVI, fish abundance, zooplankton abundance, phytoplankton abundance, K<sub>d</sub>, TP, TN, sediment TN, SRP and DIN. Abbreviations as in Table 1.

Overall mean body size of the zooplankton increased with increasing distance to other aquatic systems (decreased connectivity) and TSS, and decreased with submerged macrophyte %PVI and richness, as well as with spatial heterogeneity (Table 3). Therefore, smaller bodied zooplankton tended to predominate in systems with abundant submerged plants.

### Fish

We caught a total of 32 fish species. Characiformes (10 species) was the richest and most dominant order. Fish species richness per lake varied from 0 to 18. The overall mean fish density in the littoral was 12.3, with a range from 0 to 39 CPUE (ind. electric pulse<sup>-1</sup>). The mean density obtained with the seine nets was 3.57, with a range from 0 to 15.8 CPUE [ind. (net m<sup>-2</sup>) h<sup>-1</sup>]. The small-bodied omnivorous fish *Jenynsia multidentata* Jenyns, *Cnesterodon decemmaculatus* Jenyns and *Cheirodon interruptus* Malabarba were the most common and dominant species. In most lakes, at least one carnivorous species was found, most frequently *Hoplias malabaricus* Bloch.

Fish species richness increased with log<sub>10</sub> lake area, showing a steeper regression line when only the clear lakes were considered (Fig. 2e), where it showed a significant and negative relationship with increasing %PVI (Fig. 2f). In the regression model for fish species richness (i.e. including lake area, %PVI, zooplankton abundance, phytoplankton abundance, K<sub>d</sub>, TP, TN, sediment TN, SRP and DIN) lake area was the only variable selected (Table 4). We found no significant relationship between fish richness and any aspect of the plankton community.

The mean body size of the fish species collected with electrofishing (i.e. in the littoral zone) increased with lake and wetland area (Table 3), while no

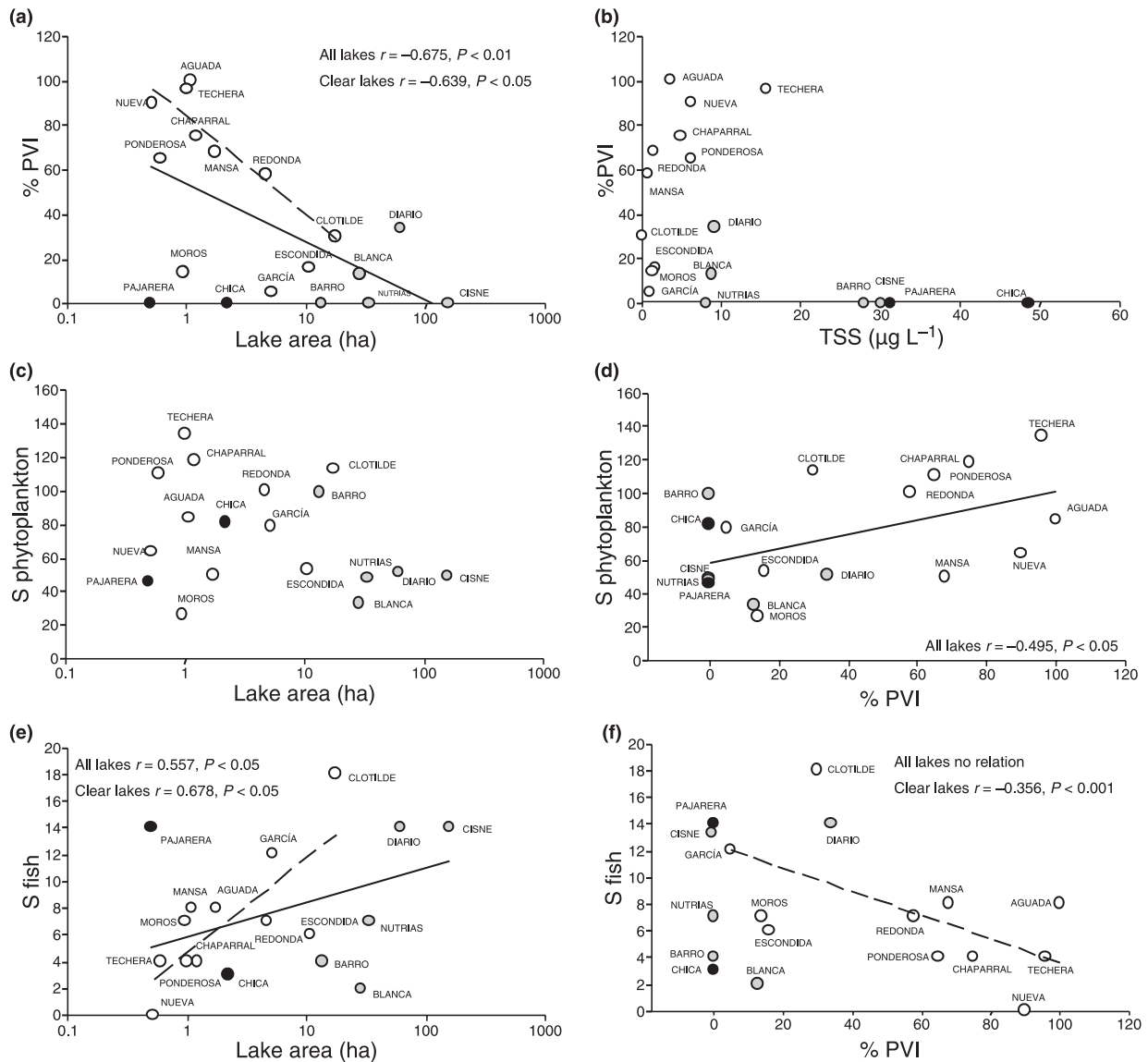
relation was observed in the size of fish captured by nets (i.e. in the pelagic zone).

### Correlational structure and the relative contribution of explanatory variables

We conducted RDA analyses to detect which variables best explained the species richness of all the assemblages studied. We began with all the measured variables (Table 1), but the forward selection procedure of RDA identified only four with significant contributions and low inflation factors (VIF): SRSi, Alk, %PVI and TSS (Fig. 3). The total variation in species richness explained by these four variables was 58.8% ( $P < 0.01$ ). Variables typically related to lake and catchment morphology, such as SRSi and Alk (with %PVI and TSS as co-variables), explained 20.0% ( $P < 0.05$ ) of overall species richness. Higher SRSi and Alk were observed in larger lakes that also had bigger catchments and wetlands, and were also more connected (Table 3). By contrast, %PVI and TSS (with SRSi and Alk as co-variables) explained 35.2% ( $P < 0.01$ ) of overall species richness. Plant cover (%PVI) and TSS were more related to in-lake factors, such as turbidity and nutrient concentrations, indicating the ecosystem state. Turbidity was most often related to TSS, except in the two lakes where phytoplankton was the main turbidity factor. TSS also increased with total nutrient concentrations (Table 3). Plant cover (%PVI) and TSS explained 1.8 times more variance than SRSi and Alk. The shared variance explained was 3.7%.

Lakes classified as turbid ( $n = 7$ ; Table 2) were related to higher Alk, SRSi, lake area and fish richness in the RDA triplot, while most lakes classified as clear ( $n = 11$ ; Table 2) were associated with higher %PVI (by definition), lower TSS, SRSi and Alk and phyto-





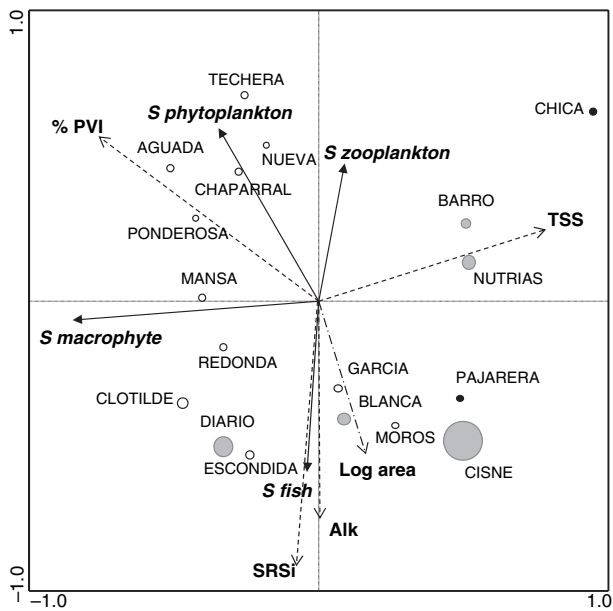
**Fig. 2** Relationship between: lake area and submerged macrophytes percentage of plant volume inhabited (%) (%PVI) (a), total suspended solids (TSS) and %PVI (b), richness of phytoplankton vs. lake area (c) and vs. %PVI (d), and fish richness vs. lake area (e) and vs. %PVI (f). White circles represent clear lakes, grey circles represent inorganic-turbid lakes (high TSS) and black circles represent phytoplankton-turbid lakes (high chlorophyll-a). Solid lines indicate the regression model with all the lakes while dashed lines indicate the models including only the clear lakes. Only those linear regressions that were significant ( $P < 0.05$ ) are included. Please note the log scale for lake area.

plankton richness (Fig. 3). Most of these relationships were confirmed by testing for differences in the mean values of these variables in each lake category (Kruskal–Wallis tests). Furthermore, turbid lakes had higher values of TP, while clear lakes also had larger wetland area/lake area ratios, higher sediment TN concentrations, and greater spatial heterogeneity and submerged macrophytes species richness. We found no significant differences in the richness of

zooplankton and fish communities between the turbid and clear lakes (Kruskal–Wallis tests).

## Discussion

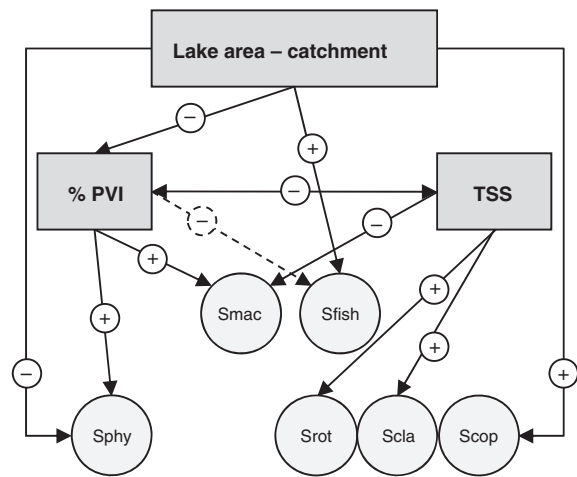
From our relatively large data set of water, sediment, morphology and biotic characteristics, only a few variables explained significant portions of the species richness of the assemblages studied (phytoplankton,



**Fig. 3** Redundancy analysis triplot showing phytoplankton, zooplankton, fish and submerged macrophyte richness (S, solid arrows) and the best explanatory variables (dashed arrows). Abbreviations as in Table 1. White circles represent clear lakes, grey and black circles represent turbid lakes, because of high total suspended solids and high chlorophyll-a respectively. The circle sizes are proportional to the respective lake area.  $\text{Log}_{10}$  lake area was included as a passive supplementary variable and is represented with a dot dashed arrow.

zooplankton, fish and submerged macrophytes). These variables were silicate concentration, alkalinity, %PVI and turbidity. These variables explained 60% of the total variance, a high percentage when compared with other studies that have focussed on the same assemblages (Beisner *et al.*, 2006,  $n = 18$  lakes; Declerck *et al.*, 2005,  $n = 98$  lakes). Silicate and alkalinity are generally related to catchment area (e.g. Conley, 2002). In contrast, %PVI and turbidity (TSS) mainly reflect the ecosystem state of the lake. Remarkably, these two groups of factors showed different, and even opposite, effects on the different assemblages. Plant cover (%PVI) and TSS alone explained more of the overall species richness than lake area and catchment-related variables.

In temperate lakes, some authors observed a considerable degree of agreement in the richness of the different assemblages (e.g. Allen *et al.*, 1999), while others have found no common patterns (e.g. Heino, Muotka & Paavola, 2003; Declerck *et al.*, 2005). Our results agree with the latter. Lake area and catchment-related variables affected submerged macrophytes



**Fig. 4** Theoretical diagram showing the potential main pathways among the key forcing factors (according to our study) and the richness of submerged macrophytes (Smac), fish (Sfish), rotifer (Srot), cladocerans (Scla), copepods (Scop) and phytoplankton (Sphy). The forcing variables are included in shaded boxes while the assemblage richness are in light boxes. Lake area - catchment refers to those variables related to lake and catchment morphology, thus including alkalinity and silicate concentration, while %PVI refers to submerged plant volume inhabited and total suspended solids (TSS) to water turbidity. The arrows represent the direction and sign of the influence. Please note that the negative effect of %PVI on fish richness corresponds to the clear plant-dominated lakes only (dashed arrow).

and fish, though in opposite directions (Fig. 4). Increasing lake area was related to decreased submerged macrophyte cover and increased fish richness, while phytoplankton richness increased with %PVI. Turbidity affected the relative importance of lake area and submerged plant cover.

Some studies conducted in temperate lakes, and along a similar TP gradient ( $50\text{--}400 \mu\text{g TP L}^{-1}$ ), have shown a significant decline in the species richness of zooplankton and submerged macrophytes with increasing TP, whereas fish, phytoplankton and floating-leaved macrophyte species richness were unimodally related to TP (Jeppesen *et al.*, 2000). However, despite a wide range in TP concentrations spanning mesotrophic to hypereutrophic conditions (from 24 to  $413 \mu\text{g TP L}^{-1}$ ), we did not find a clear relationship between nutrients and species richness.

#### The role of lake area

Larger lake and catchment areas are expected to lead to greater ecosystem richness, according to the theory

of island biogeography (MacArthur & Wilson, 1967; Allen *et al.*, 1999). However, in our study this was true only for fish and copepods. Phytoplankton total richness (and biomass) was lower in larger lakes, while the richness of submerged macrophytes and rotifers was not significantly related to area. Our results agree with other studies in temperate shallow lakes, showing a weak or no relationship between plankton, macroinvertebrates (not included in our study) or aquatic plant richness and lake size (e.g. Declerck *et al.*, 2005; Søndergaard *et al.*, 2005).

Plant species richness may increase with lake area because of the occurrence of more habitats in large lakes (Rørslett, 1991; Håkanson & Boulion, 2002). However, in our study, the larger lakes were also turbid, thus probably limiting a further increase in submerged macrophyte richness. This pattern partly coincides with previous studies (Weiher & Boylen, 1994; Vestergaard & Sand-Jensen, 2000) that showed no relationship between macrophyte richness and lake area in eutrophic turbid lakes.

Aquatic communities are characterised by organisms with different body sizes, physiologies and colonisation chances (Beisner *et al.*, 2006), leading to potentially different responses to the same environmental factors, such as lake area (Hillebrand & Azovsky, 2001; Williams *et al.*, 2003; Beisner *et al.*, 2006; Scheffer *et al.*, 2006). Similarly, we observed that the average body-size of fish in the littoral zone increased with increasing lake area.

#### *The role of submerged macrophytes*

Higher water transparency, %PVI and spatial heterogeneity coincided with greater richness of submerged macrophytes. Submerged macrophytes may offer spatially complex microhabitats and support highly diverse communities (Diehl, 1988; Jeppesen *et al.*, 1997). This is especially true in shallow meso-eutrophic lakes, where the area and volume occupied by submerged plants may be high (Jeppesen *et al.*, 1998), leading to an augmented impact on total richness (Declerck *et al.*, 2005). In this way, submerged plants may enhance the survival of several organisms, including pelagic large-bodied zooplankton, in temperate sites (Timms & Moss, 1984) and, to a lesser extent, in subtropical lakes (Iglesias *et al.*, 2007; Meerhoff *et al.*, 2007b). In our study, we did not observe a positive effect on large-bodied zooplankton.

Furthermore, we observed a negative relationship between %PVI and heterogeneity, and a positive relationship between turbidity and zooplankton average body size. Contrary to findings in temperate lakes (Declerck *et al.*, 2005; Vanormelingen *et al.*, 2008), phytoplankton showed the greatest richness in clear water, plant-dominated systems, even for the typically planktonic cyanobacteria.

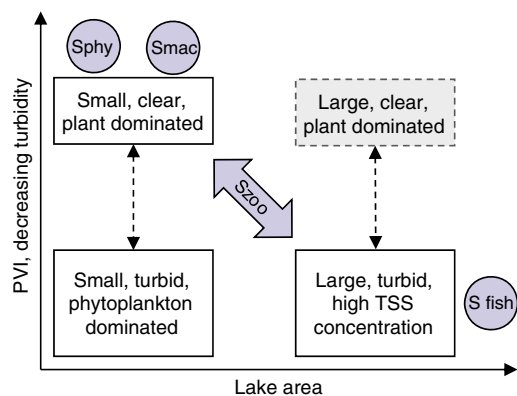
Aquatic vegetation is also one of the main structuring factors for fish (Jeppesen *et al.*, 1997). In subtropical lakes, higher fish richness and density occurs within submerged plant zones than in other habitats, regardless of lake area or trophic state (Meerhoff *et al.*, 2003, 2007a; Iglesias *et al.*, 2007; Teixeira-de Mello *et al.*, 2009). However, at the whole lake scale, fish richness was apparently more affected by lake area than by total %PVI. This may reflect the interacting effect of these two factors in our study, as we could not include large lakes with high %PVI, and the highest %PVI occurred in lakes <18 ha.

The apparent contradiction between our results and those from some temperate lakes (e.g. Declerck *et al.*, 2005), may be explained by the fact that very high submerged plant cover seems less common in cold temperate and temperate lakes than in warmer regions (S. Declerck, pers. comm.). In our study, seven out of the 18 lakes had a %PVI >50%. This extremely high plant cover could potentially lead to different richness patterns in some groups, after a certain (still unknown) threshold of plant cover or %PVI is reached.

Taxon richness in freshwater systems can also be critically impacted by the abundance and richness of predators (mostly fish; Dyer & Letourneau, 2003; Søndergaard *et al.*, 2005). Contrary to this expectation, we did not find clear correlations in taxon richness or abundance patterns among the components of the classic food web.

#### *The role of turbidity*

We placed our study lakes along two axes: increasing lake area vs. increasing %PVI (and simultaneous decrease in turbidity; Fig. 5). In this scheme, we identified three possible environmental scenarios based on our data, and a fourth speculative scenario. In the top left corner, we located the high richness lakes. These are small, clear systems with high %PVI and high richness of plankton and submerged mac-



**Fig. 5** Summary of potential lake alternative states in relation to %PVI, turbidity (either caused by phytoplankton or total suspended solids) and lake area, and the response of the richness of the main taxonomic groups. The richness of each assemblage (abbreviations as in Fig. 4) is shown near their observed maximum. Zooplankton richness is represented as an arrow, as the different taxonomic groups showed a range of optimum richness conditions. Dashed arrows indicate potential alternative state shifts. Large and clear, plant-dominated lake conditions are speculative and not based on our data as such systems did not exist in our data set.

rophytes. The bottom left group includes small turbid systems with high phytoplankton biomass and low overall richness. Finally, lakes located on the bottom right are turbid and relatively large. These lakes have high TSS concentrations but low phytoplankton biomass and are characterised by greater fish richness. Phytoplankton production may be light-limited there because of the high inorganic turbidity.

The richness of the zooplankton assemblage has an intermediate place between the small and large lakes depending on the taxonomic group considered (Fig. 5). Large-bodied zooplankton species were found in high TSS lakes, while smaller-bodied species dominated in highly transparent lakes. This pattern suggests that predator–prey interactions might be playing a role here, as predation by fish is usually dampened in turbid waters (e.g. Pekcan-Hekim & Lappalainen, 2006), and small fish aggregate in high numbers within submerged plants in subtropical lakes (e.g. Teixeira-de Mello *et al.*, 2009).

In conclusion, our results coincided with some of the predictions of the alternative states hypothesis for shallow temperate lakes (Scheffer *et al.*, 1993) and with some large sets of empirical data on the functioning and richness patterns in shallow temperate lakes (Declerck *et al.*, 2005, 2007; Søndergaard *et al.*, 2005). Some lakes had abundant submerged

plants, clear water and greater species richness of certain groups, whereas other lakes were turbid and with lower general richness. Lake area was also important and seemed to favour richness of large body-sized organisms, particularly of fish, and especially in clear, plant dominated lakes.

It has been suggested that the macrophyte-dominated state might be weaker under warm climates than in the temperate zone (Jeppesen *et al.*, 2007; Meerhoff *et al.*, 2007a), because some of the often described feedback positive effects on water transparency (such as a refuge for zooplankton and macroinvertebrate grazers) are weak or rare in the subtropics (Meerhoff *et al.*, 2007b). This conclusion has important consequences for local managers and conservationists.

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