

## MULTI-GROUP BIODIVERSITY IN SHALLOW LAKES ALONG GRADIENTS OF PHOSPHORUS AND WATER PLANT COVER

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**Abstract.** This study aimed at unraveling the structure underlying the taxon-richness matrix of shallow lakes. We assessed taxon richness of a large variety of food-web components at different trophic levels (bacteria, ciliates, phytoplankton, zooplankton, fish, macro-invertebrates, and water plants) in 98 shallow lakes from three European geographic regions: Denmark (DK), Belgium/The Netherlands (BNL), and southern Spain (SP). Lakes were selected along four mutually independent gradients of total phosphorus (TP), vegetation cover (SUBMCOV), lake area (AREA), and connectedness (CONN). Principal-components analysis (PCA) indicated that taxon diversity at the ecosystem level is a multidimensional phenomenon. Different PCA axes showed associations with richness in different subsets of organism groups, and differences between eigenvalues were low. Redundancy analysis showed a unique significant contribution to total richness variation of SUBMCOV in all three regions, of TP in DK and SP, and of AREA in DK and BNL. In DK, several organism groups tended to show curvilinear responses to TP, but only one was significantly hump shaped. We postulate that the unimodal richness responses to TP that are frequently reported in the literature for many organism groups may be partly mediated by the unimodal response of macrophyte vegetation to lake productivity.

**Key words:** biodiversity; conservation management for species richness; phosphorus; productivity; richness; richness–productivity relationships; shallow lake; shallow-lake species richness structure; water plants.

### INTRODUCTION

Taxon richness is a major component of biological diversity and an important ecosystem characteristic (Chapin et al. 2000, Downing and Leibold 2002). A good knowledge of the extant patterns of taxon richness in natural ecosystems is essential for the formulation of relevant hypotheses on the drivers and ecosystem consequences of richness, and the development of efficient monitoring methods and conservation practices. On a regional scale, there are at least two mechanisms that may generate concordance in taxon-richness patterns of organism groups across trophic levels. First, maintenance of richness in different organism groups may be determined by the same driving variables (Hawkins and Porter 2003), such as productivity, structural diversity, climate, extreme environmental conditions,

or dispersal limitation. Second, associations in richness across trophic levels may be shaped by trophic interactions (Hunter and Price 1992, Dyer and Letourneau 2003). Richness at a lower trophic level may positively affect the richness at higher trophic levels, as high richness of food sources may allow the co-existence of high richness of consumers (Knops et al. 1999, Haddad et al. 2001). Conversely, high richness of predators may allow the co-existence of prey, because the different predators each selectively eliminate competitively superior prey species that otherwise tend to dominate the community (Sieman et al. 1998). Both bottom-up and top-down effects may cascade further along the food chain (e.g., “diversity cascades,” Dyer and Letourneau 2003). Most empirical or theoretical biodiversity studies focus on diversity of only one or a restricted number of organism groups, and information on diversity patterns across organism groups of different trophic levels is scarce (Allen et al. 1999, Heino et al. 2003).

Ecosystem productivity is generally considered to be a crucial factor determining the structure, dynamics,

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PLATE 1. Shallow lake with extensive vegetation of *Utricularia vulgaris* (nature reserve De Maten, Belgium). Photo credit: Frank Van de Meutter.

and diversity of food webs (Oksanen et al. 1981, Mittelbach et al. 2001). A large body of literature reports on significant associations between richness and ecosystem productivity for a wide array of biomes (Waide et al. 1999). The strength and form of these relationships vary widely, and differences have been attributed to the influence of disturbance (Kondoh 2001), niche specialization (Kassen et al. 2000), spatial scale (Chase and Leibold 2002), and history of community assembly (Fukami and Morin 2003).

Lakes provide good model systems for the study of taxon richness in relation to environmental gradients, because they form well-delineated ecological entities in the landscape (Dodson et al. 2000). Compared to terrestrial systems, however, studies on diversity patterns in lakes are underrepresented (Waide et al. 1999). As in terrestrial biomes, richness in lakes shows a wide variety of response functions to productivity gradients, depending on the taxon, scale, and study under consideration (Leibold 1999, Dodson et al. 2000, Chase and Leibold 2002). Nevertheless, recent and extensive reviews (Waide et al. 1999, Mittelbach et al. 2001) suggest hump-shaped patterns to predominate and to be more frequently found in freshwater systems than in other biomes.

Generally, the interpretation of diversity patterns in relation to environmental gradients in natural systems is complicated because different processes may be at play simultaneously. Various potentially important explanatory variables need to be considered, and interactions between these must be taken into account (Dodson 1992). A straightforward analysis is also often hampered by a high degree of association between explanatory variables (Graham 2003). One example of this problem in aquatic systems is the close association between water-plant cover and productivity in shallow lakes. Although aquatic vegetation is a key component of structural diversity and is a steering factor for in-

terspecific interactions in shallow lakes (Jeppesen et al. 1997, Scheffer 1998), no study has yet explicitly considered this variable in explaining observed productivity–richness patterns. Key features of aquatic vegetation (e.g., abundance, taxonomic composition, and physical structure) are strongly determined by the availability of nutrients (Rørslett 1991, Sand-Jensen and Borum 1991), which may seriously complicate the interpretation of richness–productivity relationships in freshwater ecosystems.

This paper seeks to unravel the structure underlying the taxon–richness matrix of shallow lakes. We studied taxon richness in a large variety of food-web components at different trophic levels (bacteria, ciliates, phytoplankton, zooplankton, fish, macro-invertebrates, and water plants) in 98 shallow lakes located in three geographic regions within Europe (Denmark, Belgium/The Netherlands and southern Spain). We focused on the association between richness and four key environmental variables that are generally considered to be important but whose independent association with diversity can often not be studied: (1) total phosphorus content, serving as a surrogate variable for potential lake productivity (Leibold 1999, Jeppesen et al. 2000), (2) vegetation cover (Scheffer 1998), (3) lake size (Dodson 1992), and (4) degree of connectedness (Cottenie and De Meester 2003).

A key feature of our approach is that our study lakes were selected in such a way as to allow estimation of the independent association of each of the investigated variables with the richness matrix. The design of our study thus allowed us to assess: (1) whether taxon richness in multiple trophic levels is concordant, or whether different groups of organisms (bacteria, algae, zooplankton, fish, etc.) react individually to the environmental gradients, and (2) the degree to which each of the key environmental gradients is independently associated with the taxon richness of the different organism groups and trophic levels.

## MATERIALS AND METHODS

### *Model systems*

For this study, we sampled 98 shallow lakes (see Plate 1). The study sites were located in three European regions at different latitudes: Denmark (32 lakes), Belgium/The Netherlands (34 lakes) and southern Spain (32 lakes). The lakes were not selected randomly. Instead we tried to select lakes along mutually independent gradients of four potentially important key variables (the “gradient variables”): submerged vegetation cover (SUBMCOV), total phosphorus (TP), lake surface area (AREA), and the degree of connectedness (CONN). We tried to create independent gradients for these variables to prevent interference from multicollinearity. We refer to Appendix A for a more detailed account on the aim and methodology of our lake selection procedure.

### Sampling

For the plankton component, the sampling design aimed at integrating seasonal and spatial variation. During either 2000 or 2001, plankton was sampled monthly in each lake during the growing season (May–October). Depending on the surface area of the lake, water samples of 6 L and 3 L were taken at 8 (<5 ha) and 16 (>5 ha) locations, respectively. The locations were randomly assigned on a grid covering both the littoral and the pelagic zones. Sampling was done with a tube sampler (6.7 cm in diameter). The tube sampler is efficient for taking integrated plankton samples in shallow lakes, as it also samples efficiently within water-plant beds. The samples of the different locations were pooled and the resulting sample (48 L) was then thoroughly stirred and quantitatively subsampled for bacteria, ciliates, flagellates, phytoplankton, and zooplankton. At the end of the six-month sampling period equal sub-volumes of the monthly samples of each planktonic group were merged to form one pooled sample integrating within-year variation.

Communities of fish, macro-invertebrates, and water plants were sampled once during the second part of the growing season. Coverage by submerged and floating-leaved water-plant vegetation was assessed on point locations along transects covering the entire lake. Macro-invertebrates were collected semi-quantitatively in the littoral zone using a sweep net (mesh size: 500  $\mu\text{m}$ ). The fish communities were sampled overnight (18 hours) with multiple gill nets in the littoral and pelagic zones.

For each lake, data were collected on physical (Secchi depth, conductivity, suspended matter, pH, temperature), chemical (total P, total N, nitrate, orthophosphate, and silicate), and morphometric (area, depth) variables on each sampling occasion. A detailed account on the sampling procedures is given in Appendix A.

### Sample analysis

Data on the taxon richness of 13 groups of aquatic organisms were obtained. The taxonomic resolution of the richness assessments differed among the studied groups depending on methodology (genetic vs. taxonomic methods) or feasibility of routine identification. Groups that were assessed with morphological methods were identified to the species (ciliates, adult copepods, cladocerans, fish, and water plants) or genus (rotifers, phytoplankton) level. Bacteria, heterotrophic flagellates, and ultraphytoplankton were enumerated with epifluorescence microscopy. Phytoplankton samples were counted using an inverted microscope. For the cladoceran *Daphnia*, richness within the *Hyalodaphnia* hybrid complex was assessed using genetic markers (restriction fragment length polymorphism [RFLP] analysis, Billiones et al. 2004). Identification of rotifers was restricted to an a priori defined set of genera. Ro-

tifer richness data thus only represent a fraction of the taxa that may be present in rotifer communities. We chose this approach because it allows standardizing the results and because many rotifer genera are difficult to identify in a reliable way within the context of routine ecological counts. Bacterial and protist communities in the two particle-size fractions were analyzed with denaturing gradient gel electrophoresis (DGGE, Muyzer 1999). Richness of macro-invertebrates was assessed as the number of a priori defined groups of taxa. For more details on the sample analysis, we refer to Appendix A. A detailed protocol for the DGGE analyses is given in Appendix B.

### Statistical analysis

We applied rarefaction to the count data using the statistical software package PRIMERv5 (Clarke and Gorley 2001). Cut-off values were chosen as a function of feasibility (time investment needed per investigated individual) and equaled 300 individuals for both rotifers and cladocerans, 125 individuals for ciliates and 20 individuals for cyclopoid copepods. Counts of phytoplankton were continued until species accumulation curves leveled off. We chose not to apply rarefaction to fish, or to submerged and floating water plants, because the lakes with no or few representatives of these groups were too numerous. Taxon richness in bacteria and protists was estimated by the number of bands discriminated by DGGE (van Hannen et al. 1999, Van der Gucht et al. 2001). Although the interpretation of these banding patterns in terms of taxon richness may be confounded by the fact that single bands may contain DNA from several species (Sekiguchi et al. 2001) and because several bands may be generated from a single species (Speksnijder et al. 2001), these interferences are rare. We therefore assume that the patterns reflect major gradients in the taxon richness of bacterial communities.

Patterns of concordance in the taxon richness of the studied organism groups were examined with standardized principal-components analysis (PCA) in the program CANOCO4.5 (ter Braak and Šmilauer 1997). PCA was chosen instead of unimodal methods like correspondence analysis because preliminary detrended correspondence analyses indicated that the response of taxon richness to major environmental gradients was predominantly linear (for more details on the analysis methods: see Appendix C). We also examined the degree of correspondence between geographic regions with respect to their multi-group richness patterns. This was done by constructing a Euclidean distance matrix for each region based on the standardized richness data and by correlating these distance matrices (Clarke and Ainsworth 1993; for more details: see Appendix C).

The association between the richness matrix and environmental variables was studied with redundancy analysis (RDA) and partial redundancy analysis (partial RDA). For each region, we first tested an RDA model

(the “Basic Model”) that was constructed with the gradient variables and their quadratic terms (except the nominal variable CONN). Statistical testing was done with the random permutation procedure of CANOCO (reduced model, 999 random permutations; ter Braak and Šmilauer 1998). With variables of the Basic Model, we then constructed a most parsimonious RDA model (the “Optimized Basic Model”) using the manual variable selection procedure of the CANOCO program (see Appendix C for more details). The explanatory contribution of each variable in the model was assessed with “variation partitioning” (Borcard et al. 1992).

Richness response curves to the gradient variables were further explored with univariate partial correlation analysis and multiple-regression analysis (for more details: see Appendix C). Richness curves to TP were deemed curvilinear if the quadratic term  $TP^2$  proved significant in the multiple-regression models. In the case of significant curvilinearity, we tested for unimodality with the test of Mitchell-Olds (MOS test; Mitchell-Olds and Shaw 1987). In case a maximum richness value was reached within the range of TP values obtained in the present study, curves were considered to be hump shaped. If a minimum was reached within the TP range, curves were considered U shaped. In order to allow a straightforward comparison with the results of Mittelbach et al. (2001) relationships were accepted to be significant at the 10% level.

Finally, we explored for the best set of explanatory variables available. A third RDA model (the “Extended Model”) was constructed upon running a forward-selection procedure on the total set of explanatory variables, including the gradient variables and the other environmental variables for which no gradients were enforced by our lake selection criteria (the physico-chemical variables conductivity, pH, Secchi depth, and concentration of suspended matter, total nitrogen, silicate, nitrite + nitrate, and orthophosphate; the morphometric variable “depth”; and the biological variables degree of infestation by submerged vegetation, phytoplankton biomass, wet mass and number of fish expressed as catch per unit of effort) (see Appendix C: Table C1).

To avoid spurious associations between community data and the investigated gradients, we also investigated the extent to which spatial structure contributed to the association between taxon richness patterns and explanatory variables, following the approach of Borcard et al. (1992). See Appendix C for more details.

Richness of fish and of submerged and floating vegetation were not included as dependent variables in the RDA models because they were not corrected for sample size effects by rarefaction (see above).

## RESULTS

### *Lake characteristics*

Despite the efforts to standardize the selection of model systems, not all a priori defined categories of

lakes were equally represented. Especially high-nutrient lakes with water plants and low-nutrient lakes devoid of water plants were hard to find. Nevertheless, we managed to find some lakes in each category, and in each geographic region we succeeded in making the chosen gradients mutually independent (with the exception of DK [Denmark] where total phosphorus [TP] and water-plant cover were negatively correlated; Appendix D: Table D1). Furthermore, the rigid lake-selection protocol avoided major systematic biases between geographic regions with respect to the chosen gradients. Although median values for TP tended to be higher in BNL (Belgium/The Netherlands) than in DK or SP (southern Spain) (Appendix D: Table D2), the differences were not significant (Mann-Whitney *U*-test, all *P*-values > 0.05). The range of the TP gradient was more pronounced in BNL than in DK and SP, with more lakes in the upper range: 25% of the lakes in BNL had TP values >400  $\mu\text{g/L}$ , while the upper quartile for lakes in DK and SP equaled  $\sim 150 \mu\text{g/L}$ . The degree to which lakes were covered by submerged water plants was highly comparable between regions, and lake-size median and range had the same order of magnitude (Appendix D: Table D2). The share of “isolated” lakes in the total set of selected lakes was about 50% in each geographic region (50% in DK and SP, 44% in BNL). More details on lake characteristics of the BIOMAN lakes (the EU project: Biodiversity and human impact in shallow lakes) will be described elsewhere.

### *Richness: patterns of association between organism groups*

From the principal-components analyses (PCAs) the structure in the richness matrices appeared to be complex (Fig. 1; Appendix E: Table E1). The total amount of variation explained by the first four PCA axes ranged between 60 (BNL) and 62.5% (DK). The amount of explained variation per PCA axis declined only gradually in each region: PCA4 still explained approximately half the amount of the variation explained by PCA1, suggesting a high degree of multidimensionality in the richness matrix. Each of the first four PCA axes, nevertheless, tended to show high associations with specific subsets of organism groups (Appendix E: Table E1). In DK, for example, PCA1 tended to be strongly associated with taxon richness in bacteria (BACLARGE), ciliates, cyclopoid copepods, rotifers, and phytoplankton; PCA2 with taxon richness in eukaryotes (EUKLARGE), ciliates, macro-invertebrates, fish, and floating vegetation; PCA3 with taxon richness in eukaryotes (EUKSMALL), cladocera, and submerged vegetation; and PCA4 with taxon richness in phytoplankton and cyclopoid copepods. Taxon richness of several organism groups also showed opposite responses along PCA axes (Fig. 1; Appendix E: Table E1). In DK, for example, the response of taxon richness in bacteria (BACLARGE) along PCA1 tended to be opposite to that of richness in ciliates, copepods, and



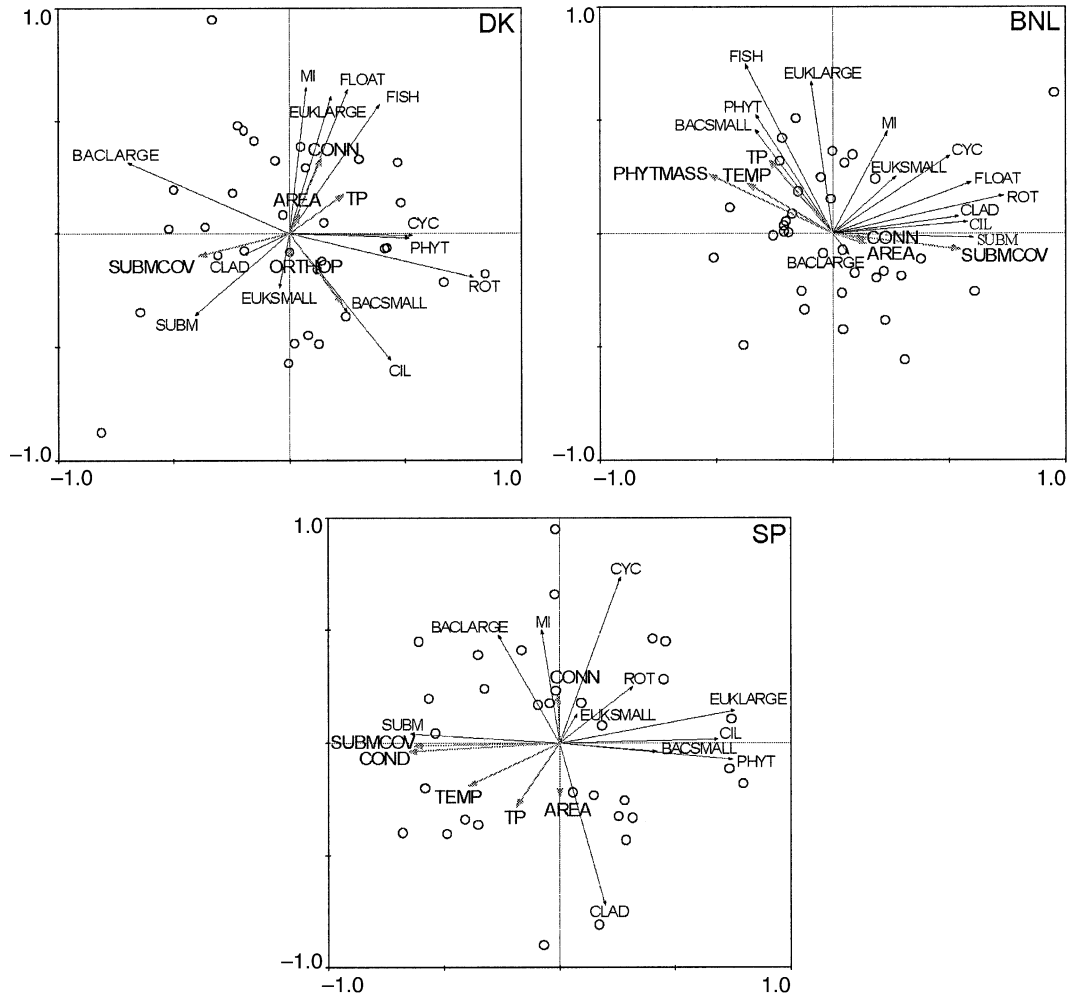


FIG. 1. Biplots of the two principal axes of PCAs performed on the centered and standardized richness data. The biplots represent the associations between the taxon richness of organism groups and the sampled lakes for each geographic region separately (DK, Denmark; BNL, Belgium/The Netherlands; and SP, southern Spain). Lakes are represented by circles. Important explanatory variables are projected as supplementary variables, and as such did not influence the ordinations. Key to abbreviations: SUBMCOV, degree of coverage by submerged vegetation; TP, concentration of total phosphorus; AREA, lake surface area; CONN, degree of connectedness; DEPTH, average lake depth; ORTHOP, orthophosphate concentration; PHYTMASS, total phytoplankton biomass; COND, conductivity; TEMP, temperature; BACSMALL, richness of bacterial DGGE strains in fraction of small seston; BACLARGE, richness of bacterial DGGE strains in fraction of large seston; EUKSMALL, richness of eukaryote DGGE strains in fraction of small seston; EUKLARGE, richness of eukaryote DGGE strains in fraction of large seston; PHYT, phytoplankton genus richness; CIL, ciliate species richness; ROT, rotifer genus richness; CYC, cyclopoid copepod species richness; CLAD, cladoceran species richness; MI, richness of macro-invertebrate groups; SUBM, submerged vegetation species richness; FLOAT, floating vegetation species richness.

rotifers. Richness patterns in BNL and SP were characterized by a comparable degree of complexity (see Appendix E for more detailed descriptions).

The degree of correspondence between geographic regions with respect to their multi-group richness patterns was generally low. Correlations between Euclidean distance matrices were marginally significant between DK and BNL ( $\rho = 0.189$ ,  $P = 0.1$ ) and between BNL and SP ( $\rho = 0.194$ ,  $P = 0.1$ ). Richness patterns tended to be most divergent between DK and SP ( $\rho = 0.050$ ,  $P = 0.36$ ).

#### *Richness: association with lake characteristics and environmental variables*

The Basic Model that related the richness matrix to the four gradient variables (SUBMCOV, TP, AREA, and CONN) and their quadratic terms was significant in SP and DK, but not in BNL (Table 1, Basic Model). The total amount of variation explained by the model equaled 32% in DK, 23% in BNL, and 30% in SP. Optimization of the Basic Model resulted in a substantial increase in the average amount of variation explained per explanatory variable (Table 1). The Op-

TABLE 1. Results of RDAs (redundancy analyses) applied on the richness matrices of the investigated European geographic regions following the three models.

Model, by region	Explanatory variables†	Trace‡	F	P§	AEV
Denmark (DK)					
Basic Model	SUBMCOV, TP, AREA, CONN, SUBMCOV <sup>2</sup> , TP <sup>2</sup> , AREA <sup>2</sup>	0.322	1.707	0.002	0.046
Optimized Basic Model	SUBMCOV, AREA, TP, TP <sup>2</sup>	0.254	2.294	0.001	0.064
Extended Model	SUBMCOV, TP, TP <sup>2</sup> , ORTHOP, DEPTH	0.334	2.603	0.001	0.069
Belgium/The Netherlands (BNL)					
Basic Model	SUBMCOV, TP, AREA, CONN, SUBMCOV <sup>2</sup> , TP <sup>2</sup> , AREA <sup>2</sup>	0.23	1.11	0.226	0.033
Optimized Basic Model	SUBMCOV, AREA	0.096	1.646	0.037	0.048
Extended Model	SUBMINF, PHYTMASS, TEMP	0.175	2.124	0.001	0.058
Southern Spain (SP)					
Basic Model	SUBMCOV, TP, AREA, CONN, SUBMCOV <sup>2</sup> , TP <sup>2</sup> , AREA <sup>2</sup>	0.302	1.484	0.014	0.043
Optimized Basic Model	SUBMCOV, TP	0.129	2.152	0.003	0.065
Extended Model	COND, TP, TEMP	0.243	3.004	0.001	0.081

Note: For more information on the models, see *Materials and Methods: Statistical analyses* and Appendix C.

† SUBMCOV is cover by submerged water plants; TP is total phosphorus; AREA is lake size; CONN is connectedness; DEPTH is lake depth; ORTHOP is concentration of ortho-phosphate; SUBMINF is infestation degree of submerged vegetation; PHYTMASS is phytoplankton biomass; COND is conductivity; TEMP is temperature.

‡ Fraction of total richness variation explained by the model.

§ P levels were assessed with random permutations.

|| Average amount of explained variation per independent variable included in the model.

timized Basic Models were significant in each of the three geographic regions.

Coverage by submerged vegetation contributed significantly to the Optimized Basic Models in each of the three geographic regions ( $P$  values < 0.1; Table 2). Submerged-vegetation cover was negatively associated with phytoplankton richness in all three geographic regions (Appendix F: Fig. F1). In DK and BNL, the taxon richness of several organism groups showed positive associations with submerged-vegetation cover (e.g., bacteria on large seston particles, cyclopoid copepods, and cladocerans in DK; rotifers and macro-invertebrate groups in BNL). In SP, the most pronounced associations between taxon richness and vegetation cover were negative (e.g., eukaryotes in large seston fraction and ciliates).

According to the Optimized Basic Models, TP explained a significant proportion of the variation in the richness matrix of DK and SP, but not of BNL (Table 2). In DK, TP<sup>2</sup> contributed significantly to the model (conditional effect: Trace [fraction of total richness variation explained by the model] = 0.084;  $P$  = 0.004) and the contribution of TP was only significant upon inclusion of TP<sup>2</sup> in the model (marginal effect of TP: Trace = 0.042,  $P$  = 0.21; conditional effect of TP: Trace = 0.086,  $P$  = 0.004). This indicates that curvilinearity was an important characteristic of the species responses to TP in DK.

A detailed examination of the response curve of taxon richness to TP (Appendix F: Fig. F2) revealed six linear associations, of which three were negative (phytoplankton in DK, rotifers and cyclopoid copepods in SP) and three were positive (bacteria on the small-

seston fraction and eukaryotes in the large-seston fraction in BNL, submerged vegetation in SP). Six response curves showed curvilinearity. Four of these curves belonged to organism groups of DK and were consistent with a humped shape (EUKLARGE, ROT, CLAD, MI), but only one curve was significantly hump shaped when tested with the Mitchell-Olds (MOS) test (MI).

AREA contributed to a limited extent to the richness variation in DK and BNL (Table 2, Appendix F: Fig. F3). No significant contribution for CONN was found in any geographic region.

In each region, forward selection on the entire set of environmental variables resulted in an Extended Model that explained a higher amount of variation than the Basic Model and the Optimized Basic Model (Table 1). For DK and BNL these models mainly involved an extension of the explanatory variable sets of the Optimized Basic Models. For DK, depth and orthophosphate were selected together with SVCOV, TP, and TP<sup>2</sup>, while the conditional effect of AREA became insignificant (Appendix G: Table G1). In BNL, phytoplankton biomass and temperature were selected. The conditional effects of SUBMCOV and AREA became insignificant in this region, but SUBMCOV was replaced in the model by the degree of infestation of submerged vegetation (SUBMINF; Appendix G: Table G1). In SP, TP, conductivity, and temperature were retained. Conductivity significantly explained a large proportion of the explained variation (Appendix G: Table G1 and Fig. G1) and tended to be negatively associated with taxon richness of at least six organism groups (phytoplankton, ciliates, fish, bacteria on small seston particles, and eukaryotes on both small and large seston parti-

cles). SUBMCOV became insignificant upon inclusion of conductivity in the model, mainly because it tended to be correlated with conductivity ( $r = 0.32$ ,  $P = 0.07$ ).

## DISCUSSION

### *Multidimensionality of taxon richness*

The results of our principal-components analyses (PCAs) provide little support for the existence of one major gradient of diversity variation across different organism groups of shallow lakes. The differences in the amount of variation explained by the first four axes of the richness PCAs were small, indicating that the richness hyper-volume is not dominated by one or a few single gradients, but tends to be truly multidimensional in each of the three European geographic regions. Although measurement error may potentially obscure richness associations to some extent (Allen et al. 1999), our observations clearly suggest that richness in different aquatic-organism groups responds to different (latent) variables, or even in an opposed way to the same variables. This observation is in agreement with other diversity studies in both aquatic (Allen et al. 1999, Heino et al. 2003) and terrestrial (Prendergast 1997) systems. Furthermore, the correspondence in the multi-group richness patterns between regions is low, and the patterns seem to deviate more with increasing distance along the latitudinal gradient.

The apparent absence of richness associations between adjacent trophic levels contrasts with the findings of some recent experimental studies performed in terrestrial systems (Knops et al. 1999, Haddad et al. 2001). A reason for this discrepancy may be that intertrophic diversity interactions are weaker in shallow lakes than in some terrestrial systems due to the circumstance that consumers in freshwater habitats tend to be less specific in their prey choice (e.g., filter-feeding zooplankton, fish) than is often the case in the highly specific plant–herbivore and herbivore–carnivore interactions of terrestrial habitats. Furthermore, the experiments that showed associations in diversity among trophic levels in terrestrial model systems (Knops et al. 1999, Haddad et al. 2001) were carried out within a very restricted locality, keeping other sources of variation constant, while our study was performed on a larger spatial scale. It may be that, on larger-than-experimental scales, richness patterns generated by trophic interactions are easily overruled by major gradients of environmental variables that differentially affect the richness at different trophic levels. Indeed, evidence for diversity concordance across trophic levels has generally been found to be weak in other large-scale surveys, too (Allen et al. 1999, Hawkins and Porter 2003).

The multidimensionality of taxon richness when considering the ecosystem as a whole has important implications with respect to both the assessment and management of taxon richness. Our results indicate that the

TABLE 2. Results of variation partitioning applied to the Optimized Basic Model of each geographic region using partial RDA (redundancy analysis).

Variable category by region <sup>†</sup>	Conditional effects			
	Trace <sup>‡</sup>	FEV (%) <sup>§</sup>	<i>F</i>	<i>P</i> <sup>  </sup>
Denmark (DK)				
SUBMCOV	0.080	32	2.912	0.005
AREA	0.045	17	1.613	0.092
TP + TP <sup>2</sup>	0.119	47	2.147	0.007
Explained in common	0.010	4		
Belgium/The Netherlands (BNL)				
SUBMCOV	0.050	52	1.719	0.074
AREA	0.046	48	1.576	0.094
Explained in common	0	0		
Southern Spain (SP)				
SUBMCOV	0.064	50	2.165	0.029
TP	0.065	50	2.189	0.018
Explained in common	0	0		

*Note:* For more information on the Optimized Basic Model and variation partitioning see *Materials and Methods* and Appendix C.

<sup>†</sup> SUBMCOV is cover by submerged water plants; TP is total phosphorus; AREA is lake size; “Explained in common” refers to the total amount of explained richness variation that cannot be uniquely attributed to one of the explanatory variables in the model.

<sup>‡</sup> Fraction of the total richness variation explained by the variable category.

<sup>§</sup> Fraction of explained variation contributed by the variable category.

<sup>||</sup> *P* levels were assessed with random permutations.

search for a single diversity index aimed at assessing taxon richness at the level of the entire system on a regional scale is probably of low relevance. Rather, useful indicators may only be found for one or a restricted subset of organism groups associated with a similar major gradient in the richness matrix. To assess richness at the ecosystem level, several of these indicators will have to be combined and weighed according to the final aim of the assessment (e.g., What is the target group? Is rarity to be considered?). The multidimensionality thus forces one to make choices when planning a biodiversity assessment, which will therefore always involve a degree of subjectivity. This issue is even more prominent when considering the management of the systems. Our observations imply that conservation practices designed to increase richness in one group of organisms may be inefficient or counterproductive for the management of richness in other organism groups. Thus, choices have to be made, which may be guided by functional importance, rarity, the historical condition of the lake, etc. Given that no single lake type or environmental condition can be associated with high taxon richness for all organism groups, an obvious statement of management advice is to maintain a variety of lake types at a regional scale.

### *Associations with gradient variables*

In many ecosystems including shallow lakes, explanatory variables that are potentially important in explaining diversity tend to be highly intercorrelated (multicollinearity, Graham 2003). The frequently observed association between water-plant abundance and total phosphorus (TP) in shallow lakes (Rørslett 1991, Sand-Jensen and Borum 1991) makes it very difficult to disentangle their separate effects on richness. Our solution to this important problem is that we applied criteria for the selection of study lakes aimed at creating independent gradients for these variables. This way of working allows an evaluation of the unique association of these variables with richness. It should nevertheless be acknowledged that the patterns of associations between the explanatory variables and richness observed in our data set do not necessarily reflect the patterns that would be found in a set of randomly selected lakes.

Although lake size has been found to be an important explanatory variable in several biodiversity studies on lakes (Dodson 1992, Allen et al. 1999), that variable contributed only marginally significantly to the explanation of taxon richness in DK (Denmark) and BNL (Belgium/The Netherlands). The limited importance of area in our study is probably due to the relatively small size range of the lakes compared to other studies (Dodson 1992, Allen et al. 1999). Because most of the shallow lakes in BNL and SP (southern Spain) are rather small, we chose the boundary between small and large lakes to be 5 ha. Søndergaard et al. (2005) did find a relation between taxon richness and lake size in a study involving a larger set of Danish lakes selected along a larger size gradient than in the present study.

Coverage by submerged vegetation was found to significantly contribute to a substantial fraction of the explained variation in the richness matrix, independently of phosphorus (Table 2, Optimized Basic Model). Vegetation may affect aquatic diversity because it mediates a multitude of interactions between organisms (Jeppesen et al. 1997). Water plants create a heterogeneous habitat (structural diversity). They form a physical structure that creates multiple environmental gradients supplying a unique substrate to many types of organisms (e.g., periphyton, macro-invertebrates) and serving as a refuge for predation (Jeppesen et al. 1997). Furthermore, water plants may provide additional food resources (periphyton, plant tissue, coarse particulate organic matter) and may compete with other autotrophic-organism groups for nutrients and light (phytoplankton, phytobenthos). The associations between water-plant cover and richness observed in our study tended to differ strongly among organism groups, varying from positive to negative, and were also different among geographic regions. With the exception of phytoplankton, the most pronounced associations between group richness and vegetation in DK and BNL were positive. In SP, richness of two organism groups

showed negative associations with vegetation cover, but these associations could not be disentangled from conductivity, as the vegetation effect was rendered insignificant upon inclusion of conductivity in the model.

TP was significantly associated with the richness matrices in Spain and Denmark, but not in BNL. TP contributed significantly in DK upon inclusion of TP<sup>2</sup> in the models only, which is indicative for the importance of curvilinearity in the response of richness to TP in this region. In their reviews, Mittelbach et al. (2001) stated that unimodal responses of richness to productivity do often occur in nature, but that other forms of relations are also common (e.g., positive, negative, hump shaped, and U shaped). Mainly based on the work of Leibold (1999) and Dodson et al. (2000), they stated that hump-shaped patterns were well represented in aquatic systems. For aquatic invertebrates, for example, they reported humped curves in 40% of the studies. Of the 36 taxon–region combinations in our study (excluding floating vegetation), we found evidence for six linear responses (16.6%) and four humped-like curvilinear responses (11.1%), of which only one (2.7%) was significantly hump shaped according to the Mitchell-Olds (MOS) test.

The large difference between the proportion of unimodal curves in our study and that of earlier studies on freshwater systems is striking. We hypothesize that this difference originates from the fact that none of the earlier studies considered or explicitly controlled for the potential impact of aquatic vegetation. The unimodal responses that are often observed for taxon richness along the productivity gradient in shallow lakes may indirectly reflect the unimodal response of water-plant vegetation to this gradient, rather than being directly steered by productivity itself.

Indeed, richness and abundance of vegetation have been reported to respond in a unimodal way to productivity (Rørslett 1991, Sand-Jensen and Borum 1991). Moreover, this unimodal response is easy to explain: in oligotrophic systems, submerged vegetation does not abound and is species poor because of nutrient limitation. Abundance and richness of water-plant vegetation tend to increase with increasing system productivity to reach a maximum at intermediate productivity levels (e.g., meso-eutrophic and eutrophic systems). In systems that are strongly eutrophied, vegetation becomes marginal or even absent, and is poor in species because water plants are inferior to phytoplankton and periphyton in the competition for light (Sand-Jensen and Borum 1991) and because they are negatively affected by secondary effects of increased productivity on ecosystem characteristics (e.g., unstable and anoxic sediments, predominance of large benthivorous fish that enhance predation on periphyton grazers, and higher abundance of zooplanktivorous fish that reduce the grazing capacity of the zooplankton community on phytoplankton). A unimodal response of vegetation abundance and richness to phosphorus



was not observed in our study, but this is likely to be the direct result of our deliberate choice to select our study lakes such that water-plant coverage and phosphorus gradients were independent from each other.

The number of samples involved in our studies was of the same order of magnitude as that of many other studies for which unimodal responses have been reported (Mittelbach et al. 2001). Consequently, it is improbable that the low proportion of hump-shaped responses in our study is due to a lack of statistical power. Even the most liberal approach of accepting significant negative quadratic terms as evidence for humped shapes results in a low proportion of humped curves. One might also argue that the TP gradients in our study were not large enough to allow the detection of unimodal responses. However, the gradients of TP in our study were comparable to the gradients of other studies reporting unimodal responses (Leibold 1999, Jeppesen et al. 2000). The fact that most evidence for curvilinearity was found in the region with the smallest TP range (DK) indicates that the TP range width is not likely to prevent us from detecting unimodal curves.

#### *Associations with other environmental variables*

Some of the variables for which no gradient was imposed by our lake-selection procedure explained a substantial amount of unique variation in the richness hypervolume, independently of the gradient variables. Depth and orthophosphate were important in DK, and phytoplankton biomass in BNL. The importance of temperature and conductivity in SP may reflect a gradient in hydrology and harshness in abiotic conditions. The lakes in southern Spain typically occur in closed, endorheic watersheds (those that have water inflow but no outflow). During summer, evaporation rates are high and conductivity levels often increase to very high levels as lake water levels drop (average of lakes in October: 5200  $\mu\text{S}/\text{cm}$ ; range: 54–16 300  $\mu\text{S}/\text{cm}$ ). A large number of lakes eventually dry out in summer. The strong increase in temperature and conductivity associated with this process undoubtedly limits the number of species and may account for the large number of organism groups showing negative associations with conductivity.

It is striking that all other associations with environmental factors, except lake size and temperature, are directly related to the theory of two alternative equilibrium states in shallow lakes (Irvine et al. 1989, Scheffer 1998): water-plant cover (all three regions), total phosphorus (DK and SP), depth and orthophosphate (DK), and biomass of phytoplankton (BNL).

#### CONCLUSIONS

In summary, two key messages emerge from our study. First, taxon diversity at the ecosystem level is a multidimensional phenomenon: richness in different taxonomic groups shows a low degree of concordance. Second, of the four a priori chosen gradients (total

phosphorus, submerged vegetation cover, lake area, and degree of connectedness), submerged vegetation and total phosphorus independently contributed most to the explained variation in taxon richness (except for TP in BNL). The associations between TP and richness in DK and SP suggest that TP indeed may affect aquatic diversity in direct ways, as proposed by several former studies (Leibold 1999). However, TP may also affect aquatic diversity in a more indirect way by influencing the aquatic vegetation. We postulate that such indirect mediation of richness by TP may provide an additional explanation for unimodal richness responses along broad TP gradients in aquatic ecosystems.

Together with shallow coastal zones (seagrass beds) and rivers, shallow freshwater lakes are fundamentally different from terrestrial biomes in that the abundance of their water-plant vegetation responds unimodally to system productivity. With increasing nutrient loading, species richness and structural diversity of terrestrial vegetations may decrease (Siemann 1998), but vegetations will not disappear. This contrasts with aquatic ecosystems where submerged water plants (and associated structural diversity) will strongly decline or disappear at the upper end of the productivity gradient (Scheffer 1998, Jeppesen et al. 2000). Given the potential importance of vegetation for diversity, this may be one of the reasons why unimodal richness–productivity responses are more frequently found in freshwater habitats than in other terrestrial biomes (Mittelbach et al. 2001).

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#### APPENDIX A

A detailed account of the aim and methodology of lake selection and of the sampling procedures and sample analyses is available in ESA's Electronic Data Archive: *Ecological Archives* E086-101-A1.

#### APPENDIX B

A detailed protocol on the application of DGGE for bacteria and eukaryotes is presented in ESA's Electronic Data Archive: *Ecological Archives* E086-101-A2.

#### APPENDIX C

A detailed description of the strategy followed for the statistical analysis of the data is given in ESA's Electronic Data Archive: *Ecological Archives* E086-101-A3.

#### APPENDIX D

Additional information on the gradients realized through the lake-selection procedure is provided in ESA's Electronic Data Archive: *Ecological Archives* E086-101-A4.

#### APPENDIX E

Detailed results of the principal-components analyses performed on the centered and standardized richness matrices of each geographic region are given in ESA's Electronic Data Archive: *Ecological Archives* E086-101-A5.

#### APPENDIX F

Scatter plots showing the associations between taxon richness values of the organism groups and gradients of submerged vegetation cover, total phosphorus content, and lake area are supplied in ESA's Electronic Data Archive: *Ecological Archives* E086-101-A6.

#### APPENDIX G

The Extended Model: results of variation partitioning and graphical representation of the association between taxon richness and conductivity in southern Spain are given in ESA's Electronic Data Archive: *Ecological Archives* E086-101-A7.