

# Classification schemes for phytoplankton: a local validation of a functional approach to the analysis of species temporal replacement

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*During ecosystem succession, phytoplankton species composition is hard to predict and although it is tempting to use taxonomic groups for predictive purposes, the conditions favouring their development are often cross-phyletic and, frequently, overlap. Another alternative is to consider functional groups. Reynolds (1997) proposed phytoplankton associations according to functional criteria, based upon identified coherent morphological and ecological properties. Here we apply data from the phytoplankton community of Lake Rodó in Montevideo, Uruguay (small, shallow, polymictic and hypertrophic lake under restoration) to test and quantify the effectiveness of the approach. The phytoplankton species were sorted into their main taxonomic groups and into the associations proposed by Reynolds. A canonical variate analysis was used to test the non-random occurrence of these classification schemes and to determine their discriminatory power. Both classification schemes, taxonomic and functional, showed a significant result, but classification into functional associations had a higher discriminatory power. The eigenvalue for the canonical correspondence analysis first axis for the functional associations was 0.708 and the cumulative explained variance for the species–environmental relationship was 78.6%. The environmental factors showed similar patterns between associations and individual species. Our data indicate that the scheme using functional associations does indeed capture much of the ecology of the phytoplankton.*

## INTRODUCTION

With the aim of predicting the effects of anthropogenic changes in the environment, the search for classification schemes of plant species according to their response to specified environmental conditions has increased (Lavorel *et al.*, 1997). Reynolds (Reynolds, 1980) applied the traditional phytosociological approach (Tüxen, 1955; Braun-Blanquet, 1964) to discern patterns in a long series of phytoplankton data from a group of lakes in Northwest England. He separated 14 species associations. These associations comprised hyperspace ‘aggregates’ of organisms, i.e. those that happened to be in the water at the same time and increased or decreased simultaneously. However, the behaviour was reproducible in the sense that associations were consistently represented under similar conditions at other times and in other lakes and, it was

claimed, were indicative of robust ecological affinities (Reynolds, 2000). The associations themselves are based on the physiological, morphological and ecological attributes of the species that potentially and alternatively may dominate or co-dominate the system. Subsequently, the scheme has been expanded to accommodate associations represented among a wider number of lakes (Reynolds, 1997; Padisák and Reynolds, 1998).

Although the selection of dominant phytoplankton species in lakes depends upon a complex and mainly unpredictable combination of factors, the annual variation of dominant species can be predicted with a high probability (Sommer, 1989). A reasonable starting assumption is that all species can potentially grow, but their establishment and development are conditioned by the variety of opportunities provided, the starting inoculum and the growth rates achieved, as well as the mechanisms for

perennation and the capacity to sustain biomass loss processes. This means that species-specific strategic adaptations can be selectively favoured in certain environments, the physical structure of the system and the availability of nutrients being among the most important variables (Reynolds, 1980).

The analysis of phytoplankton dynamics invokes both population and community ecology (Rojo and Alvarez-Cobelas, 2000). Tracing the trajectory of broad compositional responses to environmental changes (e.g. eutrophication) is one of the best ways to understand change in system function (Powell, 1995). Analysing how changes generated by imposed environmental variation, either natural or by deliberate management, reveals important aspects of the tolerances and susceptibilities of the 'normal' community. The impacts of major management measures, designed to mitigate the effects of eutrophication, on the phytoplankton community of a small urban lake provided a remarkable opportunity to examine the selection and resilience of the assemblages represented. In the specific case of Lake Rodó, a small, shallow and hypertrophic urban lake in Montevideo, Uruguay, management of the source and retention of the inflow waters, in order to alter the nutrient loads and dynamics, produced a series of clear phases of alternate plankton dominance. A continuous record of the phytoplankton of Lake Rodó throughout these changes presented a unique opportunity to evaluate the functional authenticity of species associations and their importance for the structure of the phytoplankton community, and to test the sensitivity of the responses to sharply imposed driving conditions.

We hypothesized that the associations would be of greater significance and more useful in reconstructing the compositional responses to environmental changes than either the species or their taxonomic affiliations. In order to test and consolidate this idea, we set out to demonstrate that structured groups of species exist in Lake Rodó with greater than random probability and that their occurrences respond to the anticipated controlling factors. Our findings also bear on the applicability of this method of classifying phytoplankton on functional lines. We first present data on the dominant species, their main taxonomic affiliations and their ascriptions to functional associations. The applicability and discriminatory power of the traditional and functional classifications are estimated and compared, particularly in relation to the factors believed to govern Lake Rodó.

## STUDY AREA

Lake Rodó is a small (1.3 ha), shallow (maximum depth 2.5 m) and continuously polymictic subtropical system,

built in 1917 in a park in Montevideo city (34°55'S, 56°10'W). The principal uses of the lake are recreation and cultural activities. The system has been subject to enrichment by nutrients derived from the surrounding urban area (Mazzeo *et al.*, 1999). Until December 1997, stormwater was received from an ill-defined urban catchment.

From the spring of 1996, the lake has been subject to a programme of supposedly restorative measures, aimed at improving water quality and regaining high water clarity. This has been the first such attempt in Uruguay (Scasso *et al.*, 2001). However, as the initial measures achieved limited success, other therapies were subsequently applied, so that a series of alternative management phases was achieved (Table I). The lake was emptied, its sediments removed and the inflows of water closed off. Nutrient-rich groundwater was used to re-fill and, subsequently (between January 1997 and January 1998), maintain the lake (360 m<sup>3</sup> day<sup>-1</sup>). Then, ground water pumping was stopped and, from July 1998, a water recirculation system between the lake and two adjacent pools, each covered with free-floating plants, was implemented. Aquatic plants in the main lake (especially *Eichhornia crassipes* and *Spirodela intermedia*) were cultivated and mechanically harvested before autumn (Rodríguez *et al.*, 2002). The recirculation broke down in July 1999 and was reinstalled in February 2000. In November, a floodgate was constructed to control the water transfer from the lake through the pools. On rainy days, the floodgate is closed to isolate the lake and, after a day of sunshine, it is opened to reconnect the system with the pools.

Because of the dominant presence of small omnivorous fish (*Cnesterodon decemmaculatus* and *Jenynsia lineata*), a form of biomanipulation was attempted by removing them with a net, especially in winter in 1997 and 1998.

## METHOD

Water samples were collected from the deepest part of the lake, initially at weekly intervals (January–April, 1997), then biweekly until January 1999, and monthly until December 1999. Water samples were taken just beneath the surface of the lake and close to its bottom, using a Ruttner bottle. Temperature (T) and dissolved oxygen (DO) profiles (every 20 cm), conductivity (K), pH, transparency (Secchi disk depth; SD) and light penetration [profile of photosynthetically active radiation (PAR) at 20 cm intervals, using a Li-Cor LI-192SA 4π-quantum sensor and LI-250 datalogger] were each measured *in situ*. The attenuation coefficient ( $k_d$ ) was estimated from the measured gradient of light readings. Alkalinity (Alk), soluble reactive phosphorus (SRP) (Murphy and Riley, 1962), total phosphorus (TP) and total nitrogen (TN)

*Table I: Restoration programme and hydrological regime applied in Lake Rodó during 3 years of management*

Measures	Date
Sediment removal	September 1996
Refilling with underground water	January 1997
Underground water pumping	January 1997–January 1998
Hydrological isolation I	January 1998–July 1998
Recirculation regime	July 1998–July 1999
Hydrological isolation II	July 1999–December 1999
Floodgate management	November 1999–April 2001, ongoing
Recirculation regime and underground water pumping	January 2000–April 2001, ongoing

Hydrological isolation corresponds to periods with no underground water or recirculation regime.

(Valderrama, 1981), nitrate (N-NO<sub>3</sub>) (Müller and Widemann, 1955), nitrite (N-NO<sub>2</sub>) (Strickland and Parsons, 1972), ammonium (N-NH<sub>4</sub>) (Koroleff, 1970), silicate (SRSi) (Mullin and Riley, 1955), suspended solids (TSS) and their organic matter content (OM) (American Public Health Association, 1985) and chlorophyll (Chl) *a* (Nusch, 1980) were determined following the methods cited. DIN (dissolved inorganic nitrogen) was the sum of N-NO<sub>3</sub>, N-NO<sub>2</sub> and N-NH<sub>4</sub>. The mixed depth was identified from the temperature profiles. Trophic state was estimated according to Salas and Martino (Salas and Martino, 1990).

Phytoplankton samples for qualitative analysis were taken with a net (2 µm mesh size) and with a bottle, and preserved in 4% neutralized formalin. Integrated samples for phytoplankton counts (three replicates) were collected with a 5 l Schindler trap, preserved in Lugol's iodine and counted in a 1 ml Sedgewick–Rafter chamber using a photonic microscope at ×400 magnification (Guillard, 1978). Phytoplankton units (cells and colonies) were enumerated in random fields. Phytoplankton biovolume was approximated according to Hillebrand *et al.* (Hillebrand *et al.*, 1999). Relative abundance, in terms of the biovolume of principal taxonomic groups, was considered following the system of Van der Hoek *et al.* (Van der Hoek *et al.*, 1997). Species contributing >5% to the total biovolume were grouped in the associations (AS) defined by Reynolds (Reynolds, 1997) and Padisák and Reynolds (Padisák and Reynolds, 1998), in concordance with their morphological and ecological characteristics. Shannon (Shannon, 1948) diversity and equitability as well as richness (Margalef, 1958) indices were calculated.

Zooplankton samples for qualitative analysis were collected with a 69-µm-mesh net and replicated samples were taken with a 5 l Schindler trap for counting in 2- to 5-ml Sedgewick–Rafter chambers. Zooplankton abundance

was expressed in individuals (ind.) per litre and organized in major groups as well as in small and medium-sized herbivores. The proportion of water processed by rotiferan and crustacean zooplankton was calculated from equations relating to species abundance, size and water temperature (Reynolds, 1984).

### Data analysis

Data were organized in a 'biological' matrix, to include phytoplankton species sorted in either principal taxonomic groups, associations or individual species; and an 'explanatory' matrix including the measured environmental variables, Chl *a*, rotifers, cladocerans, copepods, small and medium herbivores, zooplankton filtration rate and the effect of the past composition of the community as total phytoplankton biovolume 1 month previously [ $\text{Phy}_{(t-1)}$ ].

Following ter Braak and Smilauer (ter Braak and Smilauer, 1998), unimodal response models for the ordination analyses of long gradients data were selected. Two canonical variate analyses (CVA) were carried out to test the null hypothesis that the *a priori* classifications of phytoplankton species in principal taxonomic groups and in associations and its relationship with the temporal clustering of species are a random result. Correspondingly, the discriminatory power for each classification scheme was estimated through the total variance explained. Two CVA were performed, including the species belonging to a certain group according to each classification in the form of nominal variables as the matrix of explanatory variables according to ter Braak (ter Braak, 1995). This special case of canonical correlation analysis was carried out with the CANOCO 4.0 software (ter Braak and Smilauer, 1998) with focus on interspecies distances and Hill-type scaling.

The classification method with the highest discriminatory power was analysed in relation to its governing

factors. A series of canonical correspondence analyses (CCA) was performed using the same software, to elucidate the relationships between the groups of species classified with the selected scheme and their explanatory variables. The analysis of the partial multiple regression coefficients among all 24 explanatory variables standardized was carried out to eliminate the variables lacking a unique contribution to the regression equation in favour of a smaller number with high explained variance and low redundancy. The final CCA with the classified species was compared with one carried out with the individual species and the selected explanatory variables.

The significance of environmental variables to explain the variance of species data in the CVA and CCA was tested using Monte Carlo simulations with 199 unrestricted permutations. Variables were considered to be significant when  $P < 0.05$ .

## RESULTS

### Environmental characteristics

From its various site characteristics (Table II), Lake Rodó is classifiable as a hypertrophic subtropical system. Temperature varied seasonally, with a minimum value of 10°C. The system supported an overall mean Chl *a* concentration of 110 µg l<sup>-1</sup>, closely associated with low SD and high TSS values. Two clear-water phases were observed (SD depth higher than 1.00 m) during the spring of 1997 and 1998. SD, TSS and total phytoplankton abundance were significantly correlated with Chl *a* ( $r = -0.772$ ,  $P < 0.001$ ;  $r = 0.541$ ,  $P < 0.05$ ; and  $r = 0.773$ ,  $P < 0.001$ , respectively), while no significant linear relationship was found between Chl *a* and total phytoplankton biovolume. Irradiance at midday was always high.  $k_d$  and SD were significantly related ( $k_d = 0.822 + 0.677SD^{-1.30}$ ;  $r = 0.792$ ,  $P < 0.001$ ). By day, the water was usually saturated or supersaturated with respect to DO (42.3–175.1%), and neutral or alkaline in reaction (Table II). At most times, the mixing depth extended to the bottom. However, during summer, anoxic conditions were registered close to the bottom, probably as a result of diel stratification. Dissolved inorganic carbon was always high and showed a mean value of 60.1 mg C l<sup>-1</sup> (27.6–91.2). High concentrations of TN, TP and SRSi were found (Table II; Figure 1). Dissolved inorganic nitrogen was on average 2069 µg l<sup>-1</sup> (60.0–9956), nitrate being the dominant form, showing maximum values during ground water supply. TP was always high. SRP was below detection level most of the time (<10 µg l<sup>-1</sup>), but during the clear-water phases and second hydrological isolation (Figure 1). With respect to SRSi, important variations were observed mainly during the ground water supply and recirculation periods.

The zooplankton were dominated most of the time by small herbivores, principally rotifers (*Keratella tropica*), showing a mean value of 3076 ind. l<sup>-1</sup> (47–14 417) (Table II). Copepods showed a seasonal pattern, with higher abundance at the beginning of the spring (*Notodiaptomus incompositus*, *Metacyclops mendocinus* and *Tropocyclops prasinus meridionalis*) in the first 2 years. Cladocerans were the less abundant group (*Moina micrura* in spring and *Diaphanosoma birgei* in summer). From data on the abundance of the zooplankton, we calculated that the proportion of water processed by rotiferan and crustacean zooplankton never exceeded  $7.5 \times 10^{-4}$  l per litre of lake volume day<sup>-1</sup>, with a mean of  $2.4 \times 10^{-4}$  day<sup>-1</sup>.

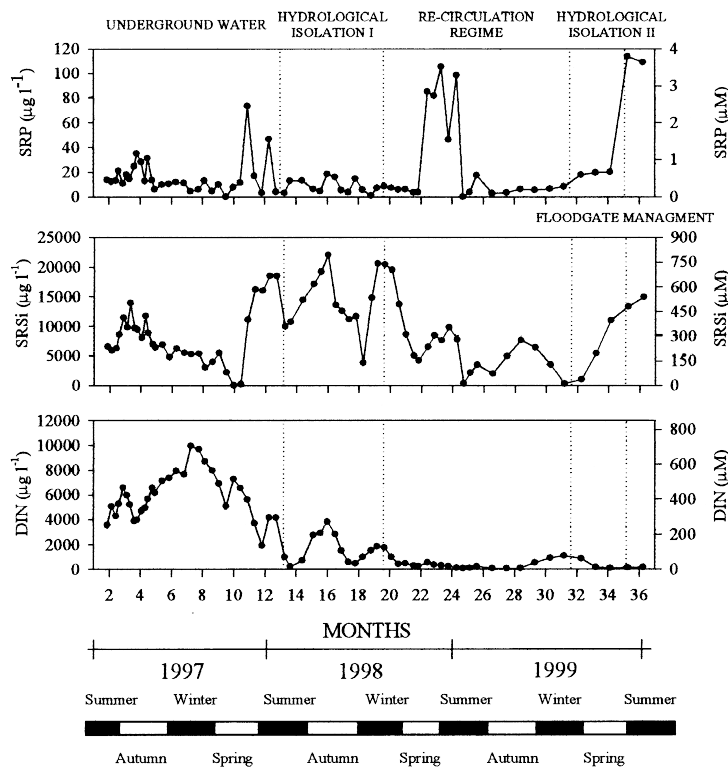
### Phytoplankton identification and characterization

Phytoplankton sampling began 50 days after refilling the lake. A total of 256 taxa, including many species not previously recorded in Uruguay, were identified and their

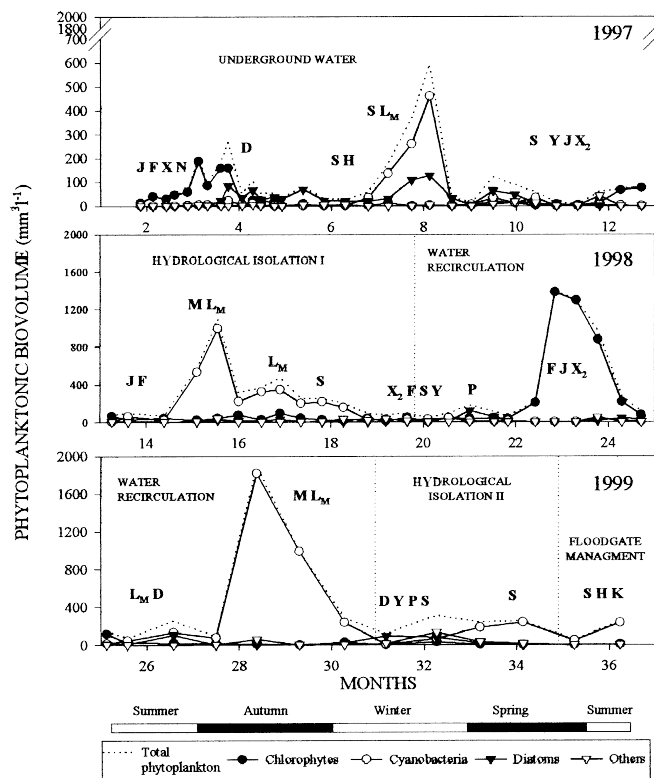
Table II: Mean and range of the physicochemical parameters and zooplankton during the study of Lake Rodó

Parameter	Mean and range of variation
Maximum depth (m)	1.95 (1.25–2.00)
PAR (µmol photons m <sup>-2</sup> s <sup>-1</sup> )	1269 (329–2396)
Secchi disk depth (m)	0.51 (0.25–1.40)
$k_d$ (m <sup>-1</sup> )	2.82 (1.06–6.11)
Temperature (°C)	19.1 (10.0–31.2)
Dissolved oxygen (mg l <sup>-1</sup> )	8.84 (3.73–19.55)
pH	8.13 (7.46–8.95)
Conductivity (µS cm <sup>-1</sup> )	836 (428–1103)
Alkalinity (mg CaCO <sub>3</sub> l <sup>-1</sup> )	267 (117–267)
TSS (mg l <sup>-1</sup> )	29.6 (6.5–79.8)
TSS organic matter (%)	57.4 (5.6–108)
Total nitrogen (µg l <sup>-1</sup> )	6524 (682–27 470)
N-NO <sub>3</sub> (µg l <sup>-1</sup> )	3014 (57–9917)
N-NO <sub>2</sub> (µg l <sup>-1</sup> )	70.48 (undet.–192.7)
N-NH <sub>4</sub> (µg l <sup>-1</sup> )	22.7 (undet.–269)
Total phosphorus (µg l <sup>-1</sup> )	179 (66–579)
SRP (µg l <sup>-1</sup> )	20.1 (<10–114)
SRSi (mg l <sup>-1</sup> )	9013 (undet.–22016)
Chlorophyll <i>a</i> (µg l <sup>-1</sup> )	110 (1.86–284)
TN/TP (by weight)	47.0 (2.1–253)
Rotifers (ind. l <sup>-1</sup> )	2441 (9–13 727)
Copepods (ind. l <sup>-1</sup> )	499 (26–2051)
Cladoceran (ind. l <sup>-1</sup> )	112 (0–1662)

PAR, photosynthetically active radiation at midday;  $k_d$ , light attenuation coefficient; TSS, total suspended solids; SRP, soluble reactive phosphorus; SRSi, soluble reactive silicate; undet., undetectable by standard methods.



**Fig. 1.** Temporal variation of soluble reactive phosphorous (SRP), soluble reactive silicate (SRSi) and dissolved inorganic nitrogen (DIN) in the water column at Lake Rodó. The hydrological periods are shown.



**Fig. 2.** Biovolume temporal variation of phytoplankton principal groups in Lake Rodó. The associations constructed with the dominant species are shown (Table III). The hydrological periods are also shown.

Table III: List of the phytoplankton associations (AS) and their representative species with >5% of relative abundance and in order of appearance observed in Lake Rodó

AS	Representative genera	ST
<b>B</b>	<i>Cyclotella meneghiniana</i>	CR
<b>D</b>	<i>Synedra acus</i>	R
<b>F</b>	<i>Sphaerocystis schroeteri</i> , <i>Sph. planctonicus</i> , <i>Bothriococcus neglectus</i> , <i>B. terribilis</i> , <i>Eutetramorus fotii</i> <sup>a</sup> , <i>E. planctonicus</i> <sup>a</sup> , <i>Planktosphaera gelatinosa</i> <sup>a</sup>	CS
<b>H</b>	<i>Anabaena planctonica</i> , <i>Anabaena spiroides</i> , <i>Aphanizomenon flos-aquae</i> , <i>A. gracile</i> , <i>Anabaenopsis</i> sp.	CS
<b>J</b>	<i>Pediastrum duplex</i> , <i>Coelastrum reticulatum</i> , <i>C. microporum</i> , <i>Oocystis lacustris</i> <sup>a</sup> , <i>Treubaria triappendiculata</i> <sup>a</sup> , <i>Scenedesmus</i> spp. <sup>b</sup>	CR
<b>K</b>	<i>Coelosphaerium</i> sp., <i>Aphanocapsa nubilum</i> , <i>A. delicatissima</i> , <i>A. elachista</i> , <i>Aphanothece minutissima</i>	CS
<b>L<sub>M</sub></b>	<i>Peridinium</i> cf. <i>cinctum</i> , <i>Gomphosphaeria</i> sp., <i>Microcystis flos-aquae</i> , <i>Microcystis wesenbergii</i> , <i>Coelomorom tropicalis</i> <sup>a</sup>	S
<b>L<sub>o</sub></b>	<i>Gymnodinium</i> sp., <i>Merismopedia tenuissima</i> , <i>M. punctata</i>	S
<b>M</b>	<i>Microcystis aeruginosa</i>	S
<b>N</b>	<i>Cosmarium</i> spp.	R
<b>P</b>	<i>Aulacoseira granulata</i> , <i>Closterium gracile</i>	R
<b>S</b>	<i>Planktothrix agardhii</i> , <i>Limnothrix planktonica</i> , <i>Pseudoanabaena galeata</i> , <i>Oscillatoria</i> spp., <i>Raphidiopsis mediterranea</i> <sup>a</sup>	R
<b>W</b>	<i>Lepocinclis salina</i>	R/CR/CRS
<b>X<sub>1</sub></b>	<i>Chlorella vulgaris</i> , <i>Monoraphidium</i> spp. <sup>b</sup> , <i>Schroedriella setigera</i> <sup>a</sup>	C
<b>X<sub>2</sub></b>	<i>Chlamydomonas</i> spp. <sup>b</sup> , nanoplankton, Haptophyta	C
<b>Y</b>	<i>Cryptomonas</i> sp., <i>Chroomonas</i> sp.	CRS
<b>Z</b>	<i>Chlorella minutissima</i>	C

<sup>a</sup>Species that were not mentioned before in the literature. The dominant strategies according to Reynolds (Reynolds, 1988) are also shown (ST).

<sup>b</sup>In some cases, only the genus is mentioned.

biovolume determined. The majority of the observed species were typical of nutrient-rich environments. Some of them were rare and went undetected during counting. Sixty-eight species were registered as having achieved a biomass >5% on any sampling occasion. Diversity and equitability indices showed a mean value of 2.17 (0.05–3.34) and 0.49 (0.80–0.001), respectively. The richness index was 1.76 (0.587–2.85), corresponding to a mean value of 22 different species. The highest diversity was observed at the beginning of the study and during the second year (1998), and decreased towards the last summer (1999).

Species were classified taxonomically in four major groups: chlorophytes, diatoms, cyanobacteria and others. Dinoflagellates, euglenophytes, cryptophytes, chrysophytes and haptophytes were joined as a group in ‘others’ having a low abundance (as a group, 3.8% of the total phytoplankton biovolume). Phytoplankton abundance was high throughout the study period with a mean of

$153.5 \times 10^5$  units ml<sup>-1</sup> ( $1.233 \times 10^4$ – $6.685 \times 10^5$ ), with chlorophytes, cyanobacteria and diatoms numerically dominant. Phytoplankton biovolume was also high with a mean value of 233 mm<sup>3</sup> l<sup>-1</sup> (5.76–1891). However, in this case, cyanobacteria and chlorophytes were the groups with greatest total biovolume (Figure 2).

Ascribing the dominant species to the associations outlined by Reynolds (Reynolds, 1997) and Padisák and Reynolds (Padisák and Reynolds, 1998), resolved by morphological, physiological and ecological thresholds nominated by Reynolds (Reynolds, 2000), we found that 17 of the proposed functional groups were represented in the full set (Table III). Seven genera and species that had not been assigned to the scheme previously were fitted to one or other of the groups by their morphological and ecological characteristics and by the conditions obtaining at the times they became dominant. *Oocystis lacustris* was included in the **F** association because it is a medium-sized member of the Chlorococcales, with mucilage, and

Table IV: Summary of the Monte Carlo permutation test (199 permutations under reduced model) using CVA to test the significance of species clusters in the temporal replacement sequence of the phytoplankton community in Lake Rodó

	Classification in taxonomic groups	Classification in associations
Test of significance of first canonical axis	Eigenvalue = 0.727 F ratio = 3.604 P value = 0.0150	Eigenvalue = 0.923 F ratio = 3.703 P value = 0.005
Test of significance of all canonical axes	Eigenvalue = 2.462 F ratio = 2.828 P value = 0.005	Eigenvalue = 5.882 F ratio = 2.338 P value = 0.005

CVA was performed considering main taxonomic groups (chlorophytes, diatoms, cyanobacteria and others: dinoflagelates, Cryptophyta and Haptophyta) defined by Van der Hoek *et al.* (Van der Hoek *et al.*, 1997) and species associations according to Reynolds (Reynolds, 1997) and Pádisak and Reynolds (Pádisak and Reynolds, 1998).

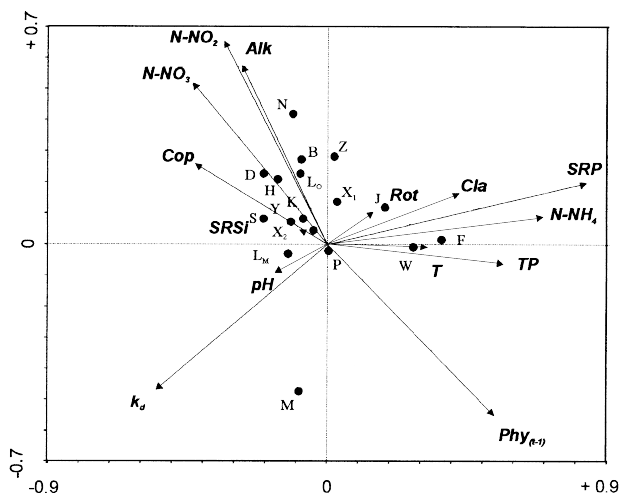


Fig. 3. Species–environment biplot CCA. The points represent 17 associations with one or more individual species (Table III) and the arrows represent each environmental variable pointing in the direction of its maximum change across the diagram during the Lake Rodó study. Cla, cladoceran; Cop, copepods; Rot, rotifers.

observed in a nutrient-rich system but following a clear-water phase. *Eutetramorus fotti* and *Planktosphaeria gelatinosa* were also assigned to the **F** association of large mucilaginous Chlorococcales. Like *O. lacustris*, they occurred after clear-water phases, in combination with *Sphaerocystis*. *Coelomorom tropicalis* was placed in the **L<sub>M</sub>** association with *Gomphosphaeria* because of the similarity in their morphology (colony type) and occurrence. Some of the recorded species were not completely in concordance with all the association definition requirements. *Treubaria triappendiculata* was assigned to the association **J** since it is a chlorophyte lacking mucilage, found in a shallow, nutrient-rich lake. However, it did not show the high abundance

expected for the species of this association. Finally, *Raphid-iopsis mediterranea* was assigned to the group **S** because it is a non-nitrogen-fixing member of the Nostocales that does not form aggregates.

The associations that showed relative biovolumes >40% were within the chlorophytes (groups **J**, **F** and **X<sub>2</sub>**), within diatoms (**D** and **P**), in the cyanobacteria (**S**, **H**, **L<sub>M</sub>** and **M**) and in others (**Y** and **N**). The associations replacement sequence is shown in Figure 2 along with the biovolume of principal groups.

### Validation of classification schemes

The classifications based on taxonomic affiliation and on Reynolds' functional criteria both showed greater probability of occurrence than random aggregates of species. Although both classifications were statistically significant and showed a high explained variance, the association scheme had a higher discriminatory power (Table IV). Diatoms and chlorophytes were not useful in the discrimination of species clustering through CVA, because they were always present.

Initial submission of the 17 functional associations to CCA involved the entire set of environmental factors. The Monte Carlo tests of the first canonical axis (0.371) and of all canonical axes were highly significant. The cumulative percentage of variance explained 72% of the species–environmental variation and the correlation was high, indicating significant correlations among the 17 associations and the 24 environmental variables. Reducing the environmental variables from 24 to 14 allowed more of the total variance, reaching 78.6% (Table V). Axis 1 (0.708), which showed the strongest relationship between species and environmental variables (0.932), was principally correlated with SRP, N-NH<sub>4</sub><sup>+</sup>, TP, *k<sub>d</sub>* and Phy<sub>(t-1)</sub>. Axis 2 with N-NO<sub>2</sub>, Alk, Phy<sub>(t-1)</sub> and N-NO<sub>3</sub>,

*Table V: Summary statistics for the first four axes of CCA for the species organized in the 17 associations performed in Lake Rodó*

	Axis				Total inertia
	1	2	3	4	
Eigenvalues	0.708				
	$F = 8.231$	0.637	0.444	0.396	5.357 <sup>a</sup>
	$P < 0.010$				
Species–environmental correlation	0.932	0.870	0.760	0.775	
Cumulative % variance of species data	13.2	25.1	33.4	40.8	
Cumulative % variance of species–environmental relationship	25.5	48.5	64.4	78.6	
Sum of all canonical eigenvalues					2.781
					$F = 4.158$
					$P < 0.005$

<sup>a</sup>Sum of all unconstrained eigenvalues. The results of the Monte Carlo test of significance of the first canonical axis and all canonical axes are shown.

*Table VI: Summary statistics for the first four axes of CCA for the 68 dominant species performed in Lake Rodó*

	Axis				Total inertia
	1	2	3	4	
Eigenvalues	0.815				
	$F = 3.486$	0.726	0.700	0.645	13.437 <sup>a</sup>
	$P < 0.05$				
Species–environmental correlation	0.974	0.934	0.882	0.906	
Cumulative % variance of species data	6.1	11.5	16.7	21.5	
Cumulative % variance of species–environmental relationship	15.5	29.3	42.5	54.8	
Sum of all canonical eigenvalues					5.272
					$F = 2.488$
					$P < 0.005$

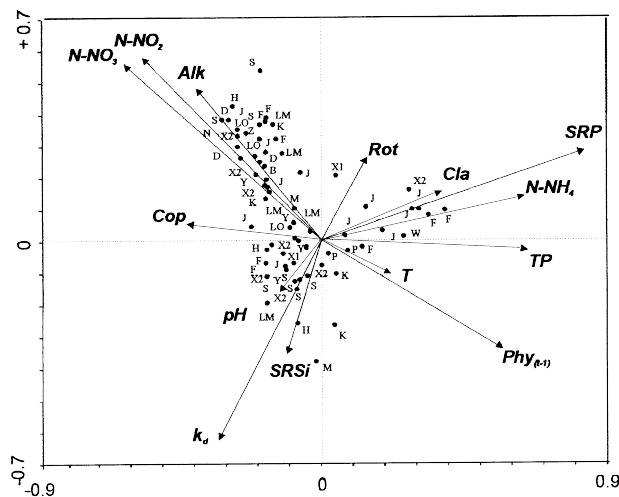
<sup>a</sup>Sum of all unconstrained eigenvalues. The results of the Monte Carlo test of significance of the first canonical axis and all canonical axes are shown.

axis 3 with SRSi and rotifers, and finally axis 4, included principally T besides other variables (Figure 3).

The associations were distributed in time (and in the diagrammatic representation) among these groups of environmental variables and in most cases with respect to their characteristics and preferences. The attenuation coefficient was positively related with the associations showing adaptive behaviours to light-deficient environments (**M**, **S**, **L<sub>M</sub>** and **Y**), and negatively correlated with

associations of clearer waters (**F**, **Z**, **W**, **J** and **X<sub>1</sub>**). Associations **H**, **D**, **L<sub>O</sub>**, **B** and **N** were related to the period of ground water supply, probably by SRSi availability and other still unknown mechanisms. The zooplankton community did not show an effect as a whole, but separated among the groups of controlling factors, copepods were positively related to the period of ground water supply, cladocera with the clear-water phases and rotifers in relation to the fourth axis and silicate.





**Fig. 4.** Species–environment biplot CCA. The points represent the individual phytoplankton species and the arrows represent each environmental variable pointing in the direction of its maximum change across the diagram during the Lake Rodó study. The species is denoted with a letter in relation to its association belonging. For association description, see Table III.

To determine how the individual species were organized in relation to the same environmental variables, a second CCA was carried out including the 68 dominant taxa against the 14 variables (Figure 4). In this case, the species were labelled according to the association ascribed to them. By comparing this result with that from the final CCA using the 17 functional associations, three main findings emerged. First, the total inertia (total variance in the species data), the importance of each axis and the species–environment correlation increased in the analysis of the 68 taxa and the 14 environmental variables. In addition, a decrease in the cumulative explained variance of the species and the species–environment relationship was also observed when the data set for individual species was used (Table VI). Second, the axes structure and their relative importance, as well as the spatial distribution in the diagrammatic representation, were similar to those observed for the associations. Only SRSi changed, showing a higher influence in the variation of individual species. Third, not all the species corresponding to the same association were distributed equally and identically to the associations.

## DISCUSSION

### Recognition of phytoplankton environments

After the sediment removal and refilling of Lake Rodó with water, the minimum growth requirements of most

phytoplankton were satisfied. However, some environmental factors soon became more critical to selection. Being a subtropical system, critical limiting temperatures and inadequate incident daily irradiances were not experienced. The low transparency became a limiting factor as a consequence of light attenuation through phytoplankton development. A continuous polymictic state and a mixing layer coinciding most of the time with maximum depth conditioned the column structure such that losses by sedimentation were kept low and ensured all entrained microalgae had frequent access to light. In addition, the low or moderate flushing rates (Scasso *et al.*, 2001) were not exclusive of species with low cell growth rates. According to Reynolds' (Reynolds, 2000) criteria, carbon and nitrogen supply did not fall below levels that would separate functional requirements. Silicate concentrations were generally high, but the important variations seemed to be related to diatom growth prior to either clear-water phase. SRP was below detection limits most of the time, confirming its importance as a growth- and yield-limiting factor in this lake. Simultaneous experiments carried out by Aubriot *et al.* have confirmed this assumption (Aubriot *et al.*, 2000). Zooplankton abundance was low and species composition was characteristic of nutrient-rich systems.

### Phytoplankton associations

The mathematical significance of our statistical analysis supported well the value and the basis for separation of Reynolds' functional groupings. Considering that the grouping scheme of associations is still under development and the associations themselves were formulated originally to describe algae encountered in temperate systems, the outcome is very encouraging. To be able also to assimilate easily several new species, on the basis of their morphological and functional attributes, also promises some flexibility of use. Some exceptions or possible improvements to this scheme need to be noted, however. As has been found by others, the properties of species ascribable to the **S** association may justify some further subdivision (Padisák and Reynolds, 1998; Reynolds, 2000). The transfer of *Chlorella minutissima* from the **Z** association to the **X<sub>1</sub>**, proposed by Huszar *et al.* (Huszar *et al.*, 2000), would facilitate the understanding of the successional events in nutrient-rich systems. This was the only species registered for the **Z** association, which appeared in abundance only during the colonization phase of the system. Only two associations not characteristic of nutrient-rich environments were observed in the hypertrophic Lake Rodó. *Gymnodinium* and *Merismopedia* (from group **L<sub>0</sub>**) and *Cyclotella meneghiniana* (**B**) are more often associated with more mesotrophic environments.

## The value of floristic classification

It seems obvious that when the number of dominant species is small, and their dominance persists throughout the year, phytoplankton might be most usefully characterized by the named individual species. Nevertheless, it is widely appreciated that phytoplankton in natural lakes are often rather more diverse and their dominance often more variable. Diverse and dynamic communities could perhaps be more appropriately described using coarser classification schemes based on the assemblage of species, as recognized by some statistical sorting methods. The result is not necessarily transportable, or at least, not until many such classifications reveal consistent patterns. These are also influenced by phytoplankton species richness, for which, apart from a supposed low diversity in hypertrophic waters (Reynolds, 1984), there is no reliable basis for interpretation of the differences between lakes or at different times in the same lake (Padisák, 1992). Besides, high frequencies of unpredictable microfloral oscillation are difficult to contend with and are poorly explained by quantitative indices of change. Rare species provide another potential identifier of particular and specific internal conditions, but the possibility that these merely represent some ecological memory of past events (Padisák, 1992) makes their reliability uncertain.

In the scheme we have tested, the sorting has taken place beforehand and, principally, on intuitive grounds. We have not sought to compare its validity against other schemes, but, rather, whether the separations have any demonstrable basis for discrimination. We have been able to show that the predetermined groups of species describe the observed assemblages with rather greater than random probability and, moreover, with greater probability than do the principal phylogenetic affinities. We emphasize that there is no prior assumption that the environment ‘recognizes’ particular groups of species, the scheme allows for many species to grow and increase, but anticipates that intensifying adversity will filter out the more tolerant or better adapted species. However, the supposition that is made is that there may well be common morphological or physiological properties that affect the relative advantage, and that these constitute the basis of the species clustering. The groupings originally proposed by Reynolds are also sensitive to filtration by chronic nutrient deficiencies (P, N, Si) and are thus sensitive to assembly formation in lakes where P or N is not (or not often) generally limiting.

Some of these are morphological (size, shape, motility) and they conform well to the patterns established by previous users of the scheme (Huszar *et al.*, 2000). Padisák and Reynolds were able to characterize the phytoplankton responses to a decreasing external load of phosphate to

Balaton Lake entirely in terms of floristic associations (Padisák and Reynolds, 1998). Huszar and Caraco (Huszar and Caraco, 1998) showed a predictive superiority over taxonomic groups, but other authors have continued to identify assembly patterns and rules using wholly taxonomic categories (Naselli-Flores, 2000; Rojo *et al.*, 2000). The discrepancies might be overcome by taking into account more dimensions of environmental variation, which are not usually considered specially in the definition of the trophic state. The use of mathematical sorting methods to identify clustering in real data and the current tendency to match morphological or physiological traits to ecological responses offer a real prospect of achieving probabilistic prediction of representation by recognized species associations (Reynolds *et al.*, 2000, 2002). Statistical analyses are useful in the recognition and validation of important hypotheses, even though the non-biological, mathematically imposed nature of the relationships should be recognized.

## Factors influencing the phytoplankton associations in Lake Rodó

The associations proved useful in reconstructing the community structure and sequence of replacement of the dominant and subdominant species observed in Lake Rodó. This is not surprising in the sense that applying labels to the plankton is likely to resurrect the original data on backtranslation. What is satisfying is the fact that the functional identities of the groups devised originally to apply in temperate Europe were, with minimal adjustment, applicable in Lake Rodó. In this extremely dynamic, nutrient-rich system, the observed associations were well matched to the principal environmental changes. The shallowness, polymixis and high nutrient concentration seem likely to have favoured the dominance of C-strategist, *r*-selected functional groups (**Z**, **X** and **J**) tending towards *r*-selected (**D**) and finally *K*-selected R-strategists (**S**). Four main controlling factors were observed during the course of the study. The attenuation coefficient ( $k_d$ ) was often high, representing high water turbidity (reciprocal to the clear-water conditions), the development of which ‘filters’ in favour of light deficiency-tolerant species of the **S** association. The relevance of the system memory and history in the temporal variation of associations and species was reflected. A group of variables appeared to be responding to the periods of low turbidity (SRP, N-NH<sub>4</sub>, TP, cladoceran). A further group of variables was associated with the period of ground water supply (nitrogen compounds, Alk) and copepods (Figure 3). SRP and sometimes SRSi represented the nutrient constraint, while light availability represented the energy limitation. The important change in the hydrological conditions and alteration of the

residence time also seems to have been important, though it could not be measured directly. Grazing pressure was hardly ever a significant determinant itself, because of the scarcity of zooplankton and the high abundance of small omnivorous–planktivorous fish (*C. decemmaculatus*) (Scasso *et al.*, 2001). The role of residence time and grazing pressure, as possible key factors, should be studied further.

### Perspectives for the application of associations in the selection of restoration measures

Once the non-random existence of associations and its relationship with environmental variables are established, they can be studied, particularly in relation to restoration measures. The question is how and to what extent the position of a lake in a trophic spectrum influences the aggregation of species in a particular way, which might allow us to predict distinctive associations. There is no single axis of variation and no unique variable that will allow us to predict or even explain the variation in the composition of the phytoplankton (Reynolds *et al.*, 2000). ‘Rules of assembly’ that have been proposed consider the inoculum, the growth rates, the memory, the traits, the water body characteristics, the food web and the environmental variability. In this sense, the combination of this scheme with a dynamic decision tree based on dominant associations should be developed to achieve better results during the implemented restoration measures. This final task of establishing the particular assembly rules to predict phytoplankton composition and select the best restoration measures will be the subject of a future paper.

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