Morphology Captures Function in Phytoplankton
A Large-Scale Analysis of Phytoplankton Communities in Relation to their Environment

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Cover image: Schematic representation of the morphology-based functional groups of phytoplankton organisms (Kruk *et al.* 2010).

CHAPTER 1. General introduction

Phytoplankton is an assemblage of microscopic photosynthesizing algae and cyanobacteria. Although phytoplankton size varies widely (ca. 0.4 - 1000 μm) the organisms are simple and while colonies and filaments can be formed, unicells are the dominant form (Harris, 1986). Restrictions on their size are posed by their habitat and the need to cope with the acquisition of resources (light, nutrients) and with mortality caused by hydrological washout, sedimentation, and grazers (Reynolds, 1984a). It has been argued that unicells are the most adequate lifeform to deal with these restrictions because they allow high replications rates to compensate loss processes and a large surface to volume ratio, favouring resource acquisition (Lewis, 1976; Raven, 1998; Jiang *et al.*, 2005). Despite these restrictions, phytoplankton is an extremely diverse group. There are an estimated of 5000 extant species in the sea (Tett & Barton, 1995) and 5000 in freshwaters (Reynolds, 2006). Often more than 100 species co-exist (Hutchinson, 1961).

Phytoplankton is essential for the functioning of the earth system as it account for half of Earth's primary production (Falkowski *et al.*, 2003; Arrigo, 2005). Therefore, it has direct effects on global climate, for example as sink of greenhouse gases (Falkowski & Oliver, 2007; Litchman & Klausmeier, 2008; Kosten *et al.*, in press). On the other hand many problems of water quality are caused by phytoplankton (Huisman *et al.*, 2005) with potentially serious implications for human health (Paerl & Huisman, 2009).

Phytoplankton species differ widely in many functional aspects. Therefore, to understand these communities' roles in the functioning of ecosystems what is important is community composition rather than just biomass. For example, species differ in their potential for nitrogen fixing and carbon sequestration (Graham *et al.*, 2000; Verity *et al.*, 2002) and in their nutritional value for grazers (Sterner & Elser, 2002). Also, specific problems associated to phytoplankton blooms such as toxicity and odour, as well as the management strategies depend on the species that are present (Scheffer *et al.*, 1997; Reynolds *et al.*, 2002).

To predict the dynamics of biological communities we need to understand the mechanisms controlling community structure, assembly and functioning (Weiher & Keddy, 1995; Le Quéré *et al.*, 2005). However, modelling community dynamics in detail seems a daunting task as there are so many phytoplankton species, and a myriad of combinations of potential conditioning factors, including abiotic and biotic mechanisms as well as diverse spatial (local, regional and global) and temporal (hours to years) scales of action (Harris, 1986; Reynolds, 2006). Furthermore, even with full knowledge of all aspects of species

biology intrinsic chaos in communities may make detailed prediction fundamentally impossible (Hutchinson, 1961; Huisman & Weissing, 2001; Roy, 2007; Benincà *et al.*, 2008).

Aggregated estimators of phytoplankton communities (e.g. total biomass) may work to predict overall community responses to varying environmental conditions (Vollenweider, 1976; Scheffer *et al.*, 2003). However, as argued, it is important to know what kind of species will appear and consider the mechanisms behind the patterns. This thesis focuses on the question whether species might be clustered in groups that are reasonably homogeneous in a functional sense, and might be better predictable from environmental conditions than individual species.

Trait-based approaches have been increasingly applied to explain and predict the response of phytoplankton species to environmental conditions both in marine and continental aquatic systems (Weithoff, 2003; Reynolds, 2006; Follows *et al.*, 2007; Litchman & Klausmeier, 2008). Well-known examples are the Plankton Ecology Group (PEG) model (Sommer, 1989) that predicts the seasonal succession in temperate lakes and the Margalef mandala (Margalef, 1978) that explains the main strategies and mechanisms for marine plankton in terms of a trade-off between *r* and *K* selected traits. More recently, models based on functional traits have been shown to capture phytoplankton distribution in the world's oceans quite well (e.g. Dynamic Global Ocean Models, Le Quéré *et al.*, 2005). These and other examples illustrate that clustering species based on their functional traits makes sense to summarise their response to environmental change. Functional traits are morphological, phenological or physiological characteristics, measured at the level of the individual, that affect ecological performance, and ultimately fitness (Violle *et al.*, 2007). Functional traits can be continuous or categorical features that can be calculated as the average for a population in a particular environmental condition (Litchman & Klausmeier, 2008).

Most phytoplankton species are cosmopolitan and easily dispersed (Hillebrand & Azovsky, 2001; Finlay, 2002). Therefore, it is not unreasonable to assume that the local environmental conditions including the physical structure of the system, the availability of resources and predation by zooplankton will determine which species occur at any given moment and place (Reynolds, 1980). These local conditions will favour groups of species that share similar functional traits (Margalef, 1978; Tilman, 1982; Reynolds, 1984b, 1994, 2006). In my thesis I seek to define such groups of functionally similar species based on measurable morphological characteristics, and explore how well these groups can be predicted from environmental conditions.

Thesis outline

In the first 2 chapters of this thesis (2 and 3) I explore the factors that affect diversity and abundance at the species level. First, in Chapter 2 I evaluate which are the main factors that appear to determine the number of species in phytoplankton communities of a group of subtropical shallow lakes in relation to diversity of other groups of organisms found in these lakes. In Chapter 3 I analyze differences in predictability of individual species from environmental conditions in a wide group of lakes from different climate zones and continents. The subsequent three chapters focus on the question how well morphology-based groups capture ecology of the species and how well the distribution and abundance of such groups may be predicted from environmental conditions. In Chapter 4 I propose a functional classification of phytoplankton species based exclusively on organismic morphology and analyze whether the derived groups have coherent physiological traits and demographic parameters. In Chapter 5 I evaluate how well the abundance of such morphology-based groups is related to environmental conditions, comparing the results to those for other classifications and for individual species. Finally in Chapter 6 a mathematical model for simulating the dynamics of the morphology-based functional groups is presented.

CHAPTER 2. Determinants of Biodiversity in Subtropical Shallow Lakes (Atlantic Coast, Uruguay)

This chapter has been published previously in *Freshwater Biology*.

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Abstract

1. Shallow lakes and ponds contribute disproportionally 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.

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 disproportionally 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 water 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; Jeppesen *et al.*, 1998; Declerck *et al.*, 2005). Aquatic vegetation may play a key role in those systems, modulating (i.e. by modifying habitat heterogeneity) ecological interactions 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 *et al.*, 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; Meerhoff *et al.*, 2007b).

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 change in the same way in response to environmental drivers (Declerck *et al.*, 2005). Neither is it 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 (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 (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 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 '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. The pelagic zone was sampled overnight (12 hours) 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 them (%PVI, *sensu* Canfield *et al.*, 1984) by measuring submerged macrophyte cover, plant height and water depth along equidistant transects 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 distribution 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 submerged macrophytes) after identifying all individuals to the species level, when possible. We applied rarefaction to plankton counts by counting until the number of species reached an asymptote, when no more new species appeared after two to three units 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 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 (Kruk & Lacerot, Universidad de la República, unpublished 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.

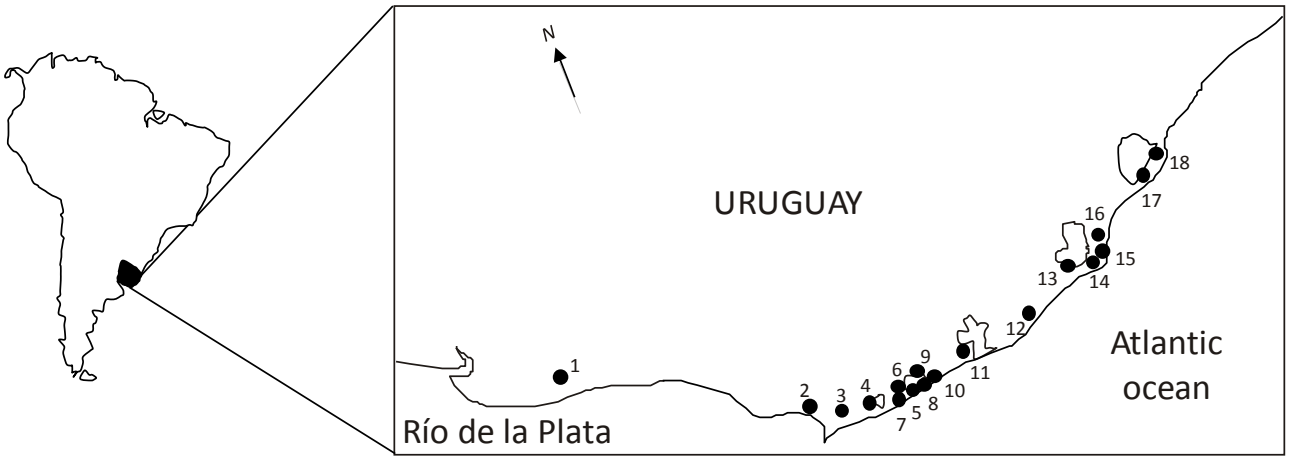


Figure 1. *Geographical location of the studied lakes. 1: Cisne; 2: Diario; 3: Blanca; 4: Barro; 5: Escondida; 6: Chica; 7: Techera; 8: Nueva; 9: Mansa; 10: Nutrias; 11: Chaparral; 12: Ponderosa; 13: Clotilde; 14: García; 15: Aguada; 16: Moros; 17: Redonda and 18: Pajarera.*

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

Variables and units	Method
Lake and catchment variables	
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	
Number of tributaries	
Mean distance to the three closest freshwater systems (km): connectivity	
Lake mean and maximum depths (m): Zmean and Zmax	*In situ* measures
In-lake *in-situ* variables	
Temperature (^{o}C): T	
Dissolved oxygen (mg L^{-1}): DO	
Conductivity (μSm^{-1}): K	
Light attenuation coefficient (m^{-1}): K_d	Photosynthetically available radiation profile
Secchi disk depth (m): SD	
pH	
In-lake chemical variables	
Alkalinity ($\mu eq\ L^{-1}$): Alk	Apha (1985)
Soluble reactive phosphorus ($\mu g\ L^{-1}$): SRP	Murphy & Riley (1962)
Total phosphorus ($\mu g\ L^{-1}$): TP	Valderrama (1981)
Total nitrogen ($\mu g\ L^{-1}$): TN	Valderrama (1981)
Nitrate ($\mu g\ L^{-1}$): NO_3	Müller & Widemann (1955)
Ammonium ($\mu g\ L^{-1}$): NH_4	Koroleff (1970)
Dissolved inorganic nitrogen ($\mu g\ L^{-1}$): DIN	$NO_3 + NH_4$
Silicate ($\mu g\ L^{-1}$): SRSi	Müllin & Riley (1955)
Total suspended solids ($\mu g\ L^{-1}$): TSS	Apha (1985)
Coloured dissolved organic matter (nm^{-1}): CDOM	Fluorescence (McKnight *et al.*, 2001)
Sediment TP ($\mu g\ DWg^{-1}$)	Valderrama (1981)
Sediment TN ($\mu g\ DWg^{-1}$)	Valderrama (1981)
Assemblages	
Submerged macrophytes richness: Smac	Direct observation in the water body
Percentage of plant volume inhabited (%): %PVI	Canfield (1984)
Submerged macrophytes spatial heterogeneity	9 classes (Semeniuk *et al.*, 1990)
Phytoplankton abundance (org ml^{-1})	Lugol's iodine (Utermöhl, 1958) in random fields
Zooplankton species richness: Szoo	69-µm-mesh size net
Zooplankton abundance (org L^{-1}): Zoo	Formaldehyde 4% (Paggi & De Paggi, 1974)
Fish abundance in the littoral zone (CPUE: ind $pulse^{-1}$)	Electrofishing (Perrow, 1996). Elektrofishfanganlage Type FEG 1000. Range of electric pulses per lake: 30-45.
Fish abundance in the pelagic zone (CPUE: ind net m^{-2} $hour^{-1}$)	Multi-mesh gill nets (30 m x 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

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 ran 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 non-parametric correlations. We classified the lakes as clear when they had more than 1 m Secchi disk depth (SD) (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 due to 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 non-parametric 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 (DCA) with log-transformed biological data showed short gradient lengths (i.e. 0.87 standard deviations). 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 *et al.*, 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. 4 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). Since 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 species in total, 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 similar but stronger pattern in the 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_d, 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).

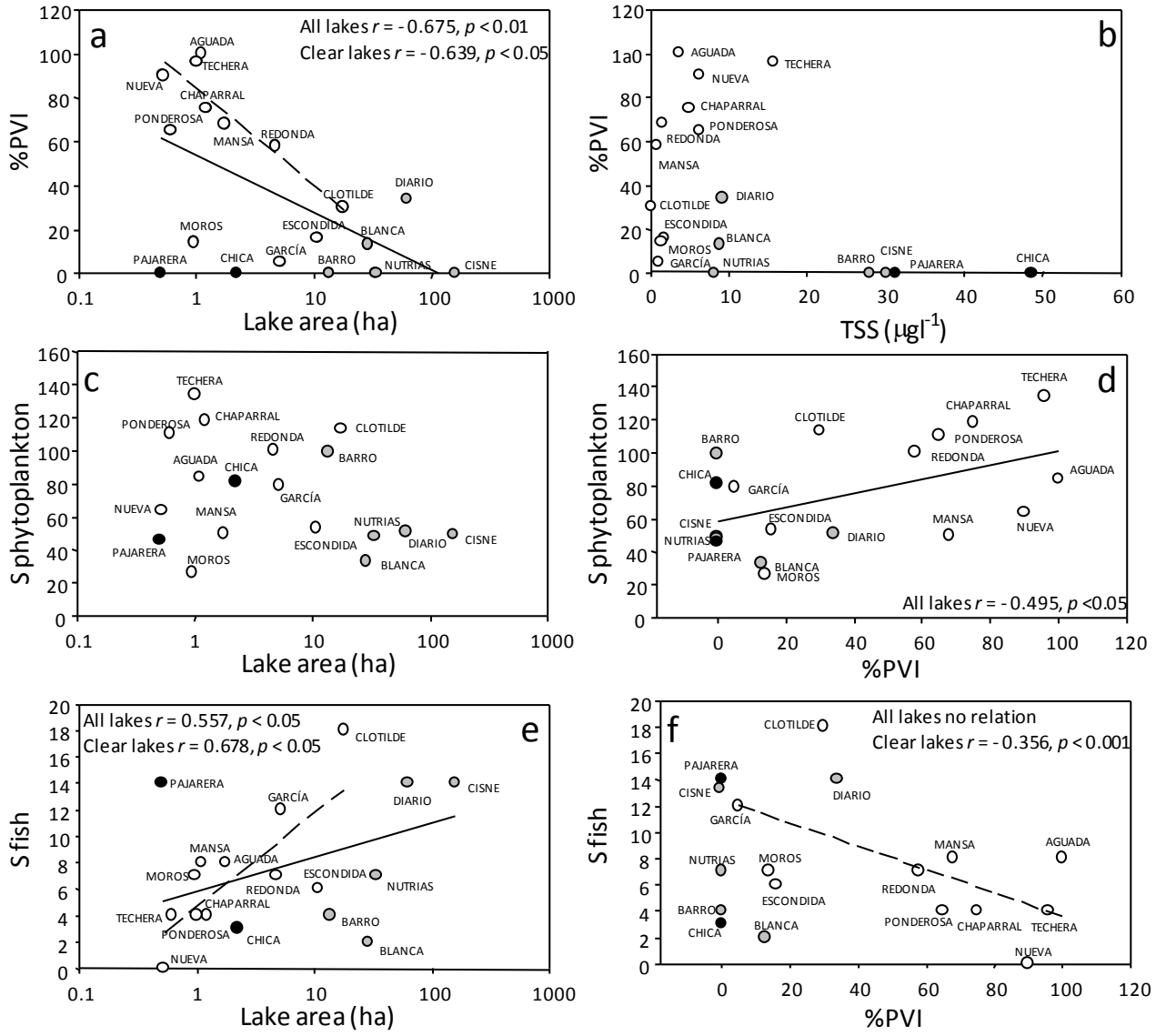
Zooplankton

We found a total of 194 zooplankton species, with rotifers being the richest group (100 species). Species richness varied widely between lakes (from nine to 41 species). Mean zooplankton abundance ranged between 43 and 1811 org L^{-1}. 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 the case of rotifers, no variable was significant in the forward selection model although backward selection yielded a model with a positive effect of K_d and a negative effect of SRP. Cladoceran richness was related positively to K_d and TN, and negatively to DIN, whereas copepod richness was positively related to Log_{10}lake area (Table 4). In all cases, the variables with the largest effect were K_d 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.

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). Smaller bodied zooplankton therefore tended to predominate in systems with abundant submerged plants.

***Figure 2**. Relationship between: lake area and submerged macrophytes %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.*



Fish

We caught a total of 32 fish species. Characiforms (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^{-1}). The mean density obtained with the seine nets was 3.57, with a range from 0 to 15.8 CPUE (ind net m^{-2} hour^{-1}). 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_{10}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_d, 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.

Table 2. *Main limnological characteristics of the 18 lakes. Systems classified as turbid are grey coloured; the others were classified as clear. Abbreviations as in Table 1. The systems where the Secchi disk (SD) was covered by very abundant submerged macrophytes are marked with an asterisk. Sed: sediment; Hetero: heterogeneity.*

Lake	Area ha	TSS μg L^{-1}	TP μg L^{-1}	TN μg L^{-1}	SRSi μg L^{-1}	sed TN μgDWg^{-1}	SD m	Chl-a μg L^{-1}	%PVI	Hetero
Aguada	1.10	3.7	43.0	975	882	902	0.40*	3.9	100	9
Barro	13.5	28.0	32.8	884	855	887	0.26	4.9	0	0
Blanca	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
Chica	2.21	48.7	90.5	1164	76.6	1204	0.10	46.5	0	0
Cisne	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
Diario	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
Nutrias	33.8	8.2	99.8	1136	203	1318	0.52	3.3	0	0
Pajarera	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

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 but that were more isolated (i.e. further from other aquatic systems) (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. Total suspended solids (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, Alk and phytoplankton 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).

Table 3. *Bivariate Spearman's correlations showing the significant correlation coefficients. Abbreviations as in Table 1. S: richness, Hetero: heterogeneity, macro: macrophytes. We did not include the variables with non-significant relationships. Significant levels: *p <0.05, [1]p <0.10 level (two-tailed).*

	Alk	SRSi	Lake area	Catchment area	Wetland area	Connectivity	K_d	TSS	TP	TN	%PVI	S macro	Hetero	Chl-a	Zoo
%PVI			-0.467[1]		-0.424[1]	-0.471[1]		-0.412[1]				0.738*	0.904*		
SRSi	0.851*		0.750*	0.735*	0.745*	0.432[1]									
Lake area	0.458[1]	0.750*		0.701*	0.973*	0.555*					-0.467[1]			-0.645*	
Catchment area	0.581*	0.735*	0.701*		0.703*	0.641*								-0.581*	
Wetland area	0.439[1]	0.745*	0.973*	0.703*		0.467*					-0.424[1]			-0.596*	
Connectivity		0.432*	0.555*	0.641*	0.467*						-0.471[1]	-0.487*			
Sediment TN	-0.517*	-0.552*	-0.458[1]			-0.484*					0.391[1]				
TSS							0.589*		0.628*	0.690*	-0.412[1]	-0.542*	-0.459[1]	0.422[1]	
S macrophytes						-0.487*	-0.454*	-0.542*	-0.459[1]		0.738*		0.742*	-0.438[1]	
S cyanobacteria	0.574*	0.679*	-0.761*	-0.516	-0.783*						0.533*			-0.427[1]	
Phytoplankton average size													0.414[1]		-0.498*
Zooplankton average size						0.539*		0.538*			-0.750*	-0.617*	-0.655*		
Fish average size (electrofishing)			0.557*		0.486[1]										

***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. The original variables included in all cases were: Log_{10} lake area, lake area, %PVI, fish abundance, zooplankton abundance, phytoplankton abundance, K_d, TP, TN, sediment TN, SRP and DIN. Abbreviations as in Table 1.*

Assemblage	Unstandardised coefficients	R^2	ANOVA
Submerged macrophyte richness	Constant = 3.733, K_d = - 0.363	0.375	F = 9.003 p = 0.009
Phytoplankton richness	Constant = 28.729, Sediment TN = 0.033	0.293	F = 6.220 P = 0.025
Rotifer richness	Constant = 8.557, K_d = 1.068, SRP = – 0.041	0.309	F = 3.124 p = 0.076
Copepod richness	Constant = -0.210, Log_{10} lake area = 1.695	0.257	F = 5.197 p = 0.038
Cladoceran richness	Constant =1.986, K_d = 0.786, DIN = -0.037, TN = 0.014	0.893	F = 24.91 p < 0.001
Fish richness	Constant = 9.469, Log_{10} lake area = 3.538	0.310	F = 6.745 p = 0.020

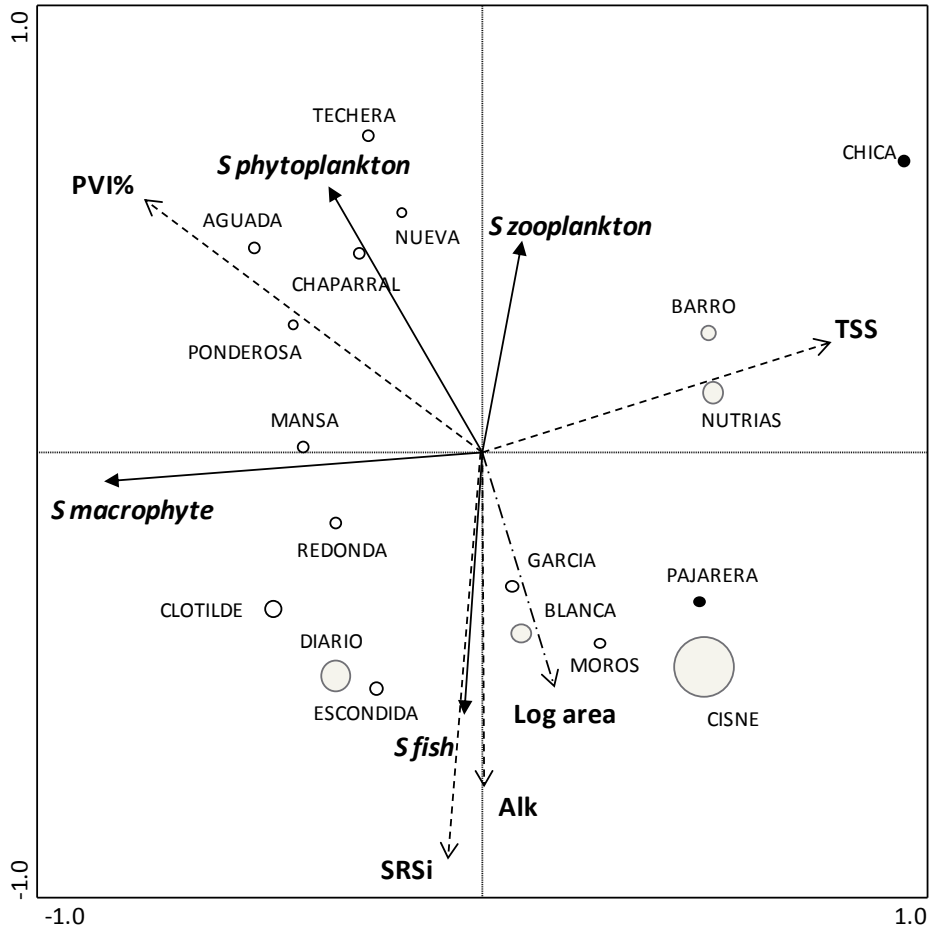


***Figure 3.** RDA triplot showing phytoplankton, zooplankton, fish and submerged macrophyte richness (S, solid arrows) and the best explanatory variables (dashed arrows). Abbreviations as in Table 1. White circles represent clear lakes, grey and black circles represent turbid lakes, due to high TSS and high chlorophyll-a, respectively. The circle sizes are proportional to the respective lake area. Log_{10} lake area was included as a passive supplementary variable and is represented with a dot dashed arrow.*

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, zooplankton, fish and submerged macrophytes). These variables were silicate concentration, alkalinity, %PVI and turbidity and explained 60% of the total variance, a high percentage when compared to other studies that have focussed on the same assemblages (Declerck *et al.*, 2005; Beisner *et al.*, 2006, 98 and 18 lakes respectively). 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 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 *et al.*, 2003; Declerck *et al.*, 2005). Our results agree with the latter. Lake area and catchment related variables affected submerged macrophytes 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 to 400 μg 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 μg 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 (Declerck *et al.*, 2005; Søndergaard *et al.*, 2005).

Plant species richness may increase with lake area due to the occurrence of more habitats in large lakes (Rørslett, 1991; Haakanson & 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 characterized by organisms with different body-sizes, physiologies and colonization chances (Beisner *et al.*, 2006), leading to potentially different responses to the same environmental factors, such as lake area (Williams *et al.*, 2003; 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). This is especially true in shallow meso-eutrophic lakes, where the area and volume occupied by submerged plants may be high (Jeppesen *et al.*, 2000), 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; Meerhoff *et al.*, 2007a). 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, since we could not include large lakes with high %PVI, and the highest %PVI occurred in lakes smaller than 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. com). 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 macrophytes. 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 characterized by greater fish richness. Phytoplankton production may be light-limited there because of the high inorganic turbidity.

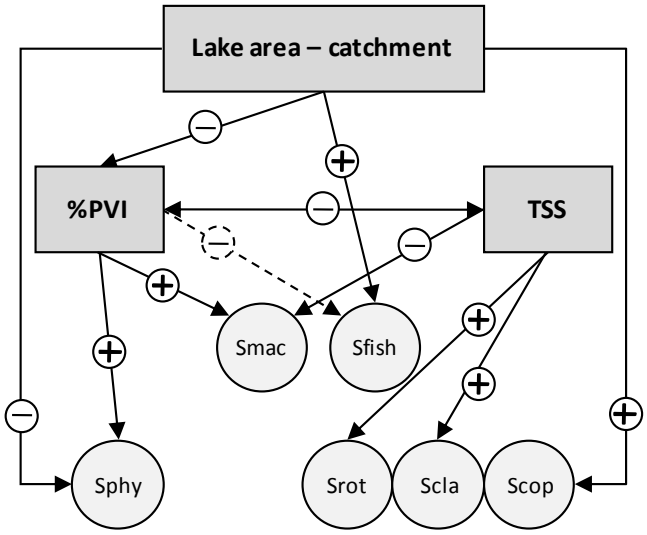


***Figure 4**. Theoretical diagram showing potential main pathways, key forcing factors (according to our study) and richness of submerged macrophytes (Smac), fish (Sfish), rotifer (Srot), cladocerans (Scla), copepods (Scop) and phytoplankton (Sphy). The forcing variables are included in shaded boxes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

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; Søndergaard *et al.*, 2005; Declerck *et al.*, 2007). 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.

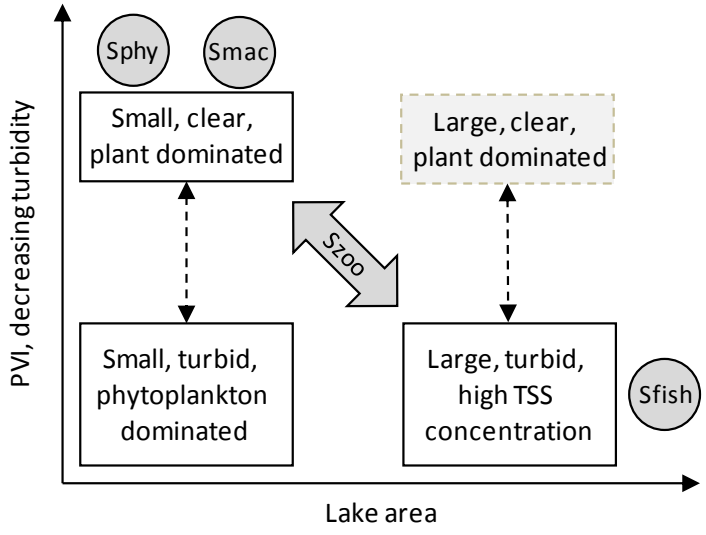


Figure 5. *Summary of potential lake alternative states in relation to %PVI, turbidity (either caused by phytoplankton or TSS) and lake area, and the response of the richness of the main taxonomic groups. The richness of each assemblage (abbreviations as in Fig. 4) is shown near their observed maximum. Zooplankton richness is represented as an arrow, since 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 since such systems did not exist in our data set.*

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CHAPTER 3. Species that tend to reach high biomass are relatively predictable in phytoplankton

Carla Kruk, Angel M. Segura, Edwin T.H.M. Peeters, Vera L. M. Huszar, Luciana S. Costa & Marten Scheffer

Abstract

1. Phytoplankton species differ strongly in their effect on ecosystem functioning and ecosystem services yet little is known about their predictability.
2. Here we explore systematic patterns in predictability of a large number of species using information from 203 lakes in South America, Europe and North America covering a wide range of environmental conditions.
3. We estimated the explained variance (proxy of predictability) in the presence and biomass of species using multiple regressions from commonly measured environmental variables such as nutrient levels, turbidity, depth, temperature and zooplankton abundance.
4. Predictability of presence or absence of species was approximately normally distributed. By contrast, species fell into two relatively distinct groups with respect to the predictability of their biomass. Species in the predictable group had a higher absolute (but not relative) biomass on average in lakes were they were present, but did not occur more frequently than other species. In fact, the average biomass was unrelated to the frequency of species presence. Biomass predictability was not systematically related to phylogenetic affiliation, neither were predictable species limited to particular functional groups as defined by morphology. However, predictable species occurred more in lakes with low chlorophyll-a levels but relatively high nutrient concentrations.
5. In conclusion, while the biomass of most species appears to have little relation at all with environmental variables, a small group of species from diverse phylogenetic and functional groups is rather predictable. Such predictable species tend to reach a high biomass, and occur relatively more in situations where competition for nutrients seems less severe.

Introduction

A priori, one might expect that phytoplankton community composition should be easier to predict than terrestrial vegetation. Due to their small size, phytoplankton species are much less dependent upon history and biogeography than terrestrial plants (Hillebrand & Azovsky, 2001; Cavender-Bares *et al.*, 2009). With the possible exception of some groups that may disperse less readily (Vanormelingen *et al.*, 2007; Vyverman *et al.*, 2007) most phytoplankton species are cosmopolitan, suggesting that they disperse easily and that the arrival to new habitats is hardly a limiting factor (e.g. Hillebrand & Azovsky, 2001; Fuhrman *et al.*, 2008).

Also, in contrast to terrestrial plants, phytoplankton grows very fast. Indeed, the time between two winters for phytoplankton may be comparable to the time between two glacial periods for forests, if one scales to generation times (Reynolds, 1993). In view of the fast growth and easy colonisation, it may seem reasonable to assume that all species might, in principle, develop in any spot at any time, and that therefore local conditions can almost be considered as an instantaneous 'filter' (*sensu* Weiher & Keddy, 1995) which allows only the sub-set of species that are sufficiently tolerant to these conditions (Reynolds *et al.*, 2002). This suggests that community composition may be predictable simply from physical conditions (hydrology, temperature), resources (light, nutrients) and predation by zooplankton at any given moment irrespective of the geographic region (Reynolds, 1980; Reynolds *et al.*, 2002).

On the other hand it has been argued that it is impossible to get accurate predictions of species abundances even with full knowledge of all aspects of environmental conditions and species biology (Huisman & Weissing, 2001). Such unpredictability may arise in part from the inability to track relatively fast environmental fluctuations but also from intrinsic complex dynamics (Scheffer *et al.*, 2003). Such chaotic dynamics may result from competition (Scheffer, 1991; Huisman & Weissing, 2000), or trophic interactions (Scheffer, 1991; Benincà *et al.*, 2008) and can be affected by seasonal forcing as well (Dakos *et al.*, 2009).

Importantly, the discussion about predictability is inextricably intertwined with the classical question how so many species can co-exist in nature. While, the role of intrinsic chaos has only be proposed more recently, Hutchinson already suggested that non-equilibrium conditions in general might explain how countless species of plankton can co-exist despite the fact that they are competing for just a limited number of resources in a

seemingly homogeneous environment; the famous 'paradox of the plankton' (Hutchinson, 1961).

An alternative explanation for the co-existence of many species is advocated by the neutral theory of biodiversity suggesting that species are functionally equivalent so that no species will outcompete other species (Hubbell, 2001). The related theory of self-organized similarity (also referred to as 'emergent neutrality') bridges the gap between neutral theory and niche-theory proposing that there may be a limited number of evolutionary self-organized functional groups of species (and corresponding niches), but that within each group an essentially unlimited number of ecologically equivalent species might co-exist neutrally (Scheffer & Van Nes, 2006). While the neutral theory implies a fundamental unpredictability at the species level, emergent neutrality implies predictability of functional groups, but unpredictability within such groups.

In the face of the theoretical interest in predictability it is remarkable that there are actually very few studies that evaluate the predictability of phytoplankton species from environmental conditions. Although numerous data-sets are available, studies typically analyse communities as a whole and do not address predictability of individual species (e.g. Beisner *et al.*, 2006; Vanormelingen *et al.*, 2008) except in few occasions and for particular groups (e.g. Chessman *et al.*, 1999).

Here we analyze data from more than 200 lakes ranging from tropical to subpolar regions, to explore how closely different species are related to environmental variables such as temperature, light, nutrients, physical mixing regime and zooplankton abundance. Subsequently we explore how such ecological predictability is related to phylogenetic affiliation, morphological traits and overall distribution.

Methods

We compiled a database of more than 700 species from 203 lakes located within four climate zones in South America, Europe and North America, and covering a wide range of environmental characteristics (Appendix I). For 107 of the lakes, information was obtained from published (De León, 2000; Mazzeo *et al.*, 2003) and unpublished sources (V.L.M. Huszar, pers. comm. 2006; and a 1999 Dutch multi-lake survey Gerben van Geest & Frank Roozen, pers. comm. 2004). The remaining 104 lakes were sampled at least once during summer in the period 2005-2006. We included only one summer sample per lake (203 cases). The sampling and sample-analysis protocols were comparable among the lakes with most of them sampled at random points integrating the water column and covering lake

area (detailed methodology elsewhere Kosten *et al.*, 2009; Kruk *et al.*, 2009). Environmental variables analysed were chosen to cover the main processes affecting phytoplankton species (e.g. mixing, temperature, resources availability, grazing) and included temperature (Temp), inorganic suspended solids (ISS), mixing depth (Z_{mix}), Secchi disk depth (Z_{SD}), light extinction coefficient (K_d), conductivity (Cond), alkalinity (Alk), dissolved inorganic nitrogen (DIN), total nitrogen (TN), soluble reactive phosphorus (SRP), total phosphorus (TP), soluble reactive silica (SRSi), total zooplankton abundance (TZ), Cladocera abundance (CLAD) and chlorophyll-a concentrations (Log_{10}Chlo-a).

Phytoplankton sample analysis and calculations

Phytoplankton populations (individuals mL^{-1}) were counted in random fields from fixed Lugol samples, using the settling technique (Utermöhl, 1958). We examined the samples at multiple magnifications and counted until we reached at least 100 individuals of the most frequent species. For all lakes, we considered the individual algae as the unit (unicell, colony or filament). Cell numbers per colony as well as organism dimensions, including maximum linear dimension (MLD, μm) were estimated. Individual volume (V, μm^3) and surface area (S, μm^2) were calculated according to geometric equations (Hillebrand *et al.*, 1999). For colonial organisms with mucilage, V and S calculations were made for whole colonies including mucilage.

Population biomass was estimated as biovolume ($mm^3\ L^{-1}$) and calculated as the individual volume of the species multiplied by the abundance of individuals.

The species were classified in major phylogenetic groups at the Class level according to Van Den Hoeck *et al.* (1997), except for Cyanobacteria and Bacillariophyceae for which we followed Komárek & Anagnostidis (1999; 2005) and Round *et al.* (1992) respectively. The included classes are described in Appendix II. Species were also classified in morphology-based functional groups (MBFG) (Kruk *et al.*, 2010).

The demographic parameters (sensu Violle *et al.*, 2007) considered were the frequency of occurrence of a species and its average biomass excluding observations with zero values (biomass). The percentage of biomass that each species contributes to the total community biomass was calculated (biomass%). A species was regarded to be dominant when it made up at least 30% of the community biomass.

Data analysis

From the total set of species we only considered those contributing >5% to the total biovolume at least in one lake and observed in five or more lakes. This left us with a group of 104 out of the original set of over 700 species.

To explore the explained variance in species occurrence we used binomial multiple logistic regression (BLR) with species occurrence expressed as presence-absence (McCullagh & Nelder, 1989). The independent variables included were Temp, ISS, Z_{mix}, K_d, Cond, alkalinity Alk, DIN, TN, SRP, TP, SRSi, TZ, CLAD and Log_{10}Chlo-a. We evaluated how well environmental variables explained the presence-absence of the species using the Nagelkerke R^2 coefficient of determination (Nagelkerke, 1991).

To analyze the explained variance in biomass of species across our lakes we used ordinary multiple linear regressions (OLR) with the same independent environmental variables as used for the binomial regressions. Species biovolume was transformed as $Log_{10}(x+a)$, with a equal to the minimum value of x across all species. The variance explained by the model was characterized as the R^2 coefficient of determination.

Hereafter, we shall refer to the Nagelkerke R^2 as the presence predictability and to the R^2 as the biomass predictability. These regressions were carried out with SPSS 15.0 for Windows.

Normality of the presence and biomass predictability was tested with the Kolmogorov-Smirnov test (*K-S d*) using SPSS 15.0 for Windows. Histograms were plotted to visualize the distribution of particular variables, and compared to a Kernel Density Estimation, computed with PAST version 1.81 (Hammer *et al.*, 2001).

We used non-parametric median Chi-Square (χ^2) tests and z *post-hoc* analysis to test for differences in demographic parameters and predictability of species presence and biomass (Nagelkerke R^2 and R^2) among phylogenetic groups and MBFG. We also divided the species in groups with different predictability (see the results section) and tested for significant differences between these groups in morphological traits, demographic parameters and environmental variables using Chi-Square (χ^2) tests. These analyses were carried out with SPSS 15.0 for Windows.

We used Spearman correlations (r_s) to investigate the relationships between presence predictability, biomass predictability, morphological traits (V, S, S/V, MLD), and demographic parameters. Non-linear curve and surface fitting were used to further

explore the relationships. These analyses were carried out with SPSS 15.0 for Windows and Tcurve software.

To check whether predictable species tend to occur together or rather have a complementary distributional pattern we compared their mutual correlation over lakes to that of other species, evaluating their significant differences in the median value of the Spearmean correlation coefficient with Chi-Square (χ^2) tests.

To analyse the effect of the environment on the number of predictable species, the number of predictable species corrected by the number of 5% dominant species and the percentage of the biomass of predictable species over the total community, we ran multiple regression models. We selected the best independent variables for each model by a forward selection procedure. The original variables included in all cases were: Temp, ISS, Z_{mix}, K_d, Cond, Alk, TN, TP, TZ, CLAD and Log_{10}Chlo-a.

Results

Biomass versus frequency and dominance

Species biomass in lakes where they were found was not correlated to their frequency of occurrence (Fig. 1). Overall, species with higher absolute biomass averaged over lakes where they were found, tended to have a higher relative contribution to the total community biomass (r_s = 0.698, p < 0.001). However, there are many exceptions in the sense that some dominant species had a quite low biomass on average (Appendix II). Also an overview of all species analyzed in detail (Appendix II) reveals that all phylogenetic groups had species that could become dominant in a particular lake (reach more than 30% of the total community biovolume). Examples are the cyanobacteria *Microcystis aeruginosa* (Kützing) Kützing, the chlorophyte *Microactinium pusillum* Fresenius and the diatom *Synedra acus* (Kützing). Also, species from different phylogenetic groups did not differ significantly in their average biomass (χ^2_8 = 10.84, p = 0.211) and only weakly in their frequency of occurrence (χ^2_8= 15.20, p = 0.0553).

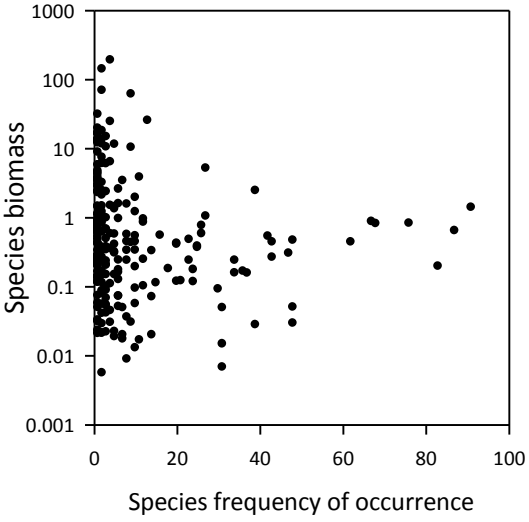

Figure 1. *Relationship between species frequency of occurrence and individual species average biomass in all the studied lakes.*

Predictability of presence

The predictability of the species presence (Nagelkerke R^2) was normally distributed (*K-S d* = 0.141, $p > 0.20$, Fig. 2a) and this was also true if we considered only cyanobacteria (*K-S d* = 0.124, $p > 0.20$). Presence of many species could be relatively well predicted from environmental variables. Almost 60% of the studied species had a Nagelkerke R^2 of at least 0.50 with a minimum value of 0.21 and a maximum of 0.99. (Fig. 2a). Species from different phylogenetic groups did not differ in predictability of their presence ($\chi^2_8 = 8.919$, $p = 0.349$). The same occurred with species from different MBFG ($\chi^2_6 = 10.50$, $p = 0.1052$). Also, morphological traits and demographic parameters were not related to the predictability of the species presence (Table 1).

Table 1. *Spearman correlation coefficients (r_s) of the predictability of species presence (Nagelkerke R^2) and species biomass (R^2) with each other and the demographic parameters and morphological traits. Relative biomass: % biomass; percentage of species with more than 30% of contribution to the total community biomass: 30% spp; species frequency of occurrence: frequency; species volume: V; surface: S; and maximum linear dimension: MLD. Significant levels: *p <0.05, **p <0.01 level.*

	Nagelkerke R^2	**R^2**
Nagelkerke R^2	-	-0.036
R^2	-0.036	-
Frequency	-0.197	0.102
Biomass	0.102	**0.478****
Biomass %	0.170	0.029
Max	0.042	0.185
30% spp	0.054	0.123
V	-0.109	-0.145
S/V	0.236	**0.224***
MLD	0.229	-0.033

Predictability of biomass

The overall pattern was different for predictability of the species biomass. The histograms as well as the kernel-smoothed distributions suggest two more-or-less distinct groups of species in terms of their biomass predictability (R^2): a large group of poorly predicted species and a small group of relatively well predicted species that we will refer to hereafter as 'predictable species' (Fig. 2b). The distribution of the predictability of the species biomass was significantly different from normal for the total set of species (*K-S d* =0.165, $p < 0.05$), for cyanobacteria (*K-S d* = 0.194, $p < 0.05$), and for the total set of species excluding cyanobacteria (*K-S d* = 0.165, $p < 0.05$). However, for cyanobacteria bimodality was most pronounced (Fig. 2c).

Which species are more predictable?

Species from different phylogenetic groups did not differ in predictability of their biomass (χ^2_8= 12.09, p = 0.147). Also, predictability differed only weakly between species from different morphology based functional groups (MBFG) (χ^2_6= 11.93, p = 0.064). Of the morphological traits we tested, only the surface to volume ration was related (positively) to the biomass predictability (Table 1). By contrast, predictability of species biomass (R^2) was strongly related the average biomass that species reached (Fig. 3). Higher biomass species tended to be better predicted by the environmental variables. The best fitted curve relating species biomass and predictability (R^2) was a sigmoid function (Fig. 3a, R^2 = 0.425, F_{81}=29.19, p <0.0001). For species with an average biomass of 0.3 mm^3L^{-1} (0.1 in log scale), predictability tended to be low (average R^2<0.2), whereas for species with a mean biomass of more than 3 mm^3L^{-1} (1 in log scale) R^2 was higher than 0.4 on average. A similar pattern was found if we considered only cyanobacteria (Fig. 3b, R^2 = 0.442, F_{31}=11.49, p <0.001). The predictability of species biomass was not correlated to a tendency to dominate (have a relative biomass larger than 30 %) (Table 1).

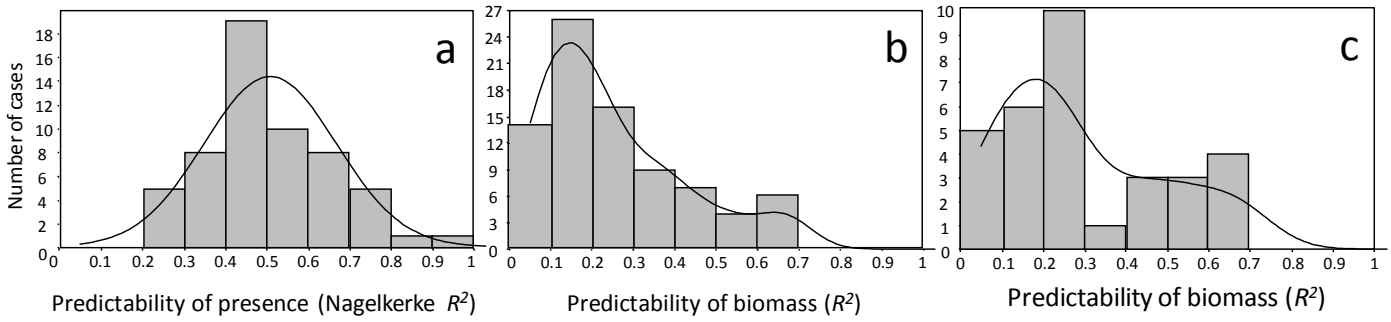

Figure 2. *Histograms showing the distribution of species predictability of species presence (Nagelkerke R^2 binomial logistic regressions with environmental conditions as explanatory variables) (a) and of species biomass (R^2 multiple linear ordinary regressions with environmental conditions as explanatory variables) for all species (b) and for cyanobacteria species (c). The normal fit (a) and the Kernel density functions are included (b and c).*

Contrasting predictable versus unpredictable species

In view of the apparent bimodality in predictability of biomass, we also explored if we could find systematic differences related to predictability by classifying species in 'predictable' (R^2> 0.4) versus 'unpredictable' (R^2< 0.4). In line with the other analyses, statistics on this dichotomy suggest that predictable species tend to reach a higher absolute biomass on average but are not systematically more dominant in relative terms, or more frequent in their occurrence (Table 2).

Predictable species were found in four (Bacillariophyceae, Chlorophyceae, Cryptophyceae, Cyanobacteria) out of the seven phylogenetic groups distinguished in our list of species. However, we did not observe significant differences in the number of predictable species among phylogenetic groups (χ^2_6 = 6.047, p = 0.418). Predictable species occurred in all except one MBFG (Group II) and there were no significant differences in the number of predictable species among MBFG (χ^2_6= 7.221, p = 0.301). Also, neither the volume, nor the maximum linear dimension of predictable species was markedly different from that of non-predictable species, although there was again a tendency for predictable species to have a higher S/V ratio (Table 2).

Although the previous analyses suggest that predictable species are by no means an ecologically homogeneous group, the number of predictable species in a community, as well as the relative number of predictable species to the total species and the percentage of the biomass represented by predictable species was related to particular environmental conditions (Figure 4). Specifically, multiple regressions indicated that the absolute number of predictable species is higher in communities in warmer lakes with relatively low chlorophyll a levels but high nutrient levels (Table 3). However, the temperature effect is not found for the relative number of predictable species, which just as the percentage of the total community biomass formed by predictable species, is only related to the chlorophyll-a level (negatively) and to nutrient levels (positively) (Figure 4).

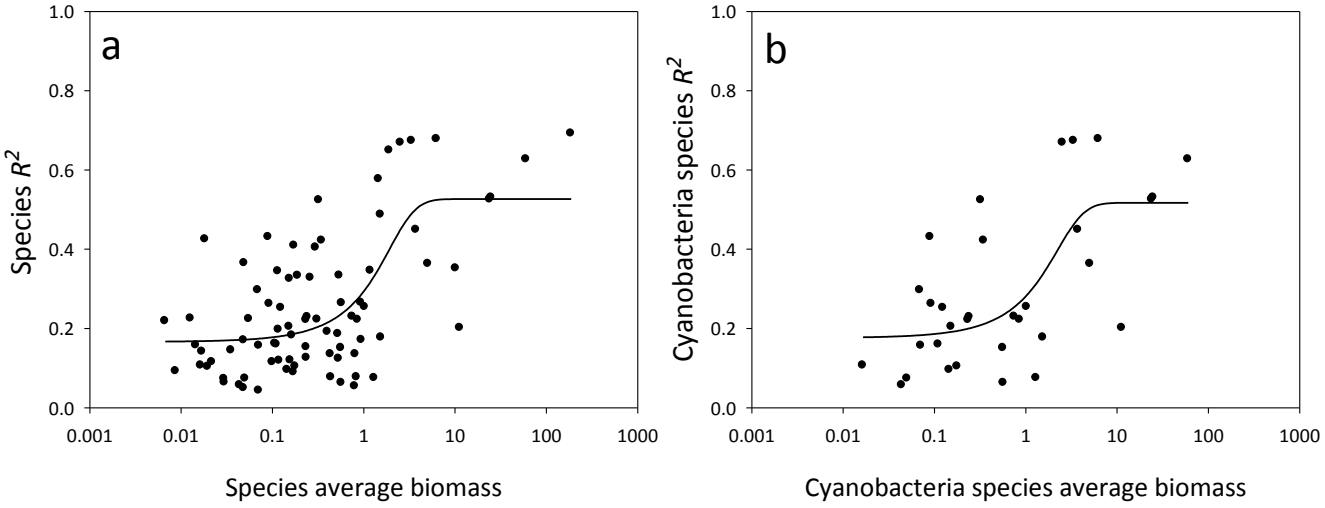

Figure 3. *Relationships between individual species average biomass and species biomass predictability (R^2 obtained by multiple linear ordinary regressions with environmental conditions as explanatory variables) for all the studied species (a) and for cyanobacteria species (b). Sigmoid functions were fitted in both cases: (a) R^2 = 0.425, F_{81}=29.19, p <0.0001, (b) R^2 = 0.442, F_{31}=11.49, p <0.001.*

Table 2. *Results the Chi square test (χ^2) for the differences in the median values between species classified in unpredictable (UP: R^2 <0.4) and predictable (P: R^2>0.4). The analyzed variables included the predictability of biomass (R^2) and presence (R^2 Nagelkerke), as well as other individual features: frequency of occurrence: Frequency; relative biomass; Biomass %; maximum biomass: Max; number of species with more than 30% of the total community biomass: 30% spp; volume: V; surface: volume ratio: S/V and maximum linear dimension: MLD. + and – are included when significant positive or negative values of the median were observed in the UP and P groups of species.*

Variable	χ^2_1	*p-level*	UP	P
R^2	28.23	<0.001	-	+
R^2 Nagelkerke	0.4909	0.484		
Frequency	0.7720	0.380		
Biomass	10.82	0.001	-	+
Biomass %	0.064	0.800		
Max	0.5761	0.448		
30% spp	1.854	0.173		
V	0.4269	0.514		
S/V	4.516	0.034	-	+
MLD	0.576	0.448		

To check whether predictable species tend to occur together or rather have a complementary distributional pattern we compared their mutual correlation over lakes to that of other species. Although the distributions of correlation coefficients overlapped, predictable species tended to be somewhat less correlated mutually than species in general (test for the median value $\chi^2_1 = 4.232$, $p = 0.040$).

Table 3. *Multiple regression models for the number of predictable species, the percentage of predictable species in the total community biomass and the number of predictable species relative to the total 5% dominant species, showing the best independent variables for each model chosen by a forward selection procedure.*

Variable	Standardised coefficients	R^2	ANOVA
Number of predictable species	Temp = 0.26 TN = 0.39 Log_{10}Chlo-a = -0.56	R^2 = 0.60	F_{160}=81.10 P<0.001
Percentage of community biomass formed by predictable species	TN = 0.27 Log_{10}Cloa = - 0.52 TP = 0.25	R^2 = 0.39	F_{159}=25.62 p<0.001
Number of predictable species/total number of species	Log_{10}Cloa = -0.54 TN = 0.40	R^2 = 0.34	F_{161}=47.23 p<0.001

Discussion

Our analysis shows that the presence or absence of phytoplankton species is quite predictable from local environmental conditions despite the fact that our data-set combined lakes across continents and climate zones. This is consistent with the idea that phytoplankton have no dispersal limits (Hillebrand & Azovsky, 2001; Fuhrman *et al.*, 2008) and therefore local conditions are sufficient to explain their occurrence (Vanormelingen *et al.*, 2008; Kruk *et al.*, 2009).

When it comes to predictability of biomass the results are more puzzling. We found that biomass of only a limited, relatively distinct group of phytoplankton species is predictable from environmental variables. Those predictable species were distributed quite evenly across morphology-based functional groups, although they had a slightly higher surface to volume ratio than unpredictable species. Also, predictable species occurred in phylogenetic groups ranging from Bacillariophyceae, Chlorophyceae and Cryptophyceae to Cyanobacteria. The only characteristic shared by most predictable species is that they tend to reach a higher absolute biomass, although they are not more dominant in relative terms or more common in terms of the number of lakes where they are found. The latter implies that predictability is not an artefact of the amount of data available for such species. The largest share of predictable species in a community (both in terms of species number and in terms of biomass) occurred in communities of lakes with relatively low chlorophyll-a concentrations, but high nutrient levels relative to the phytoplankton biomass.

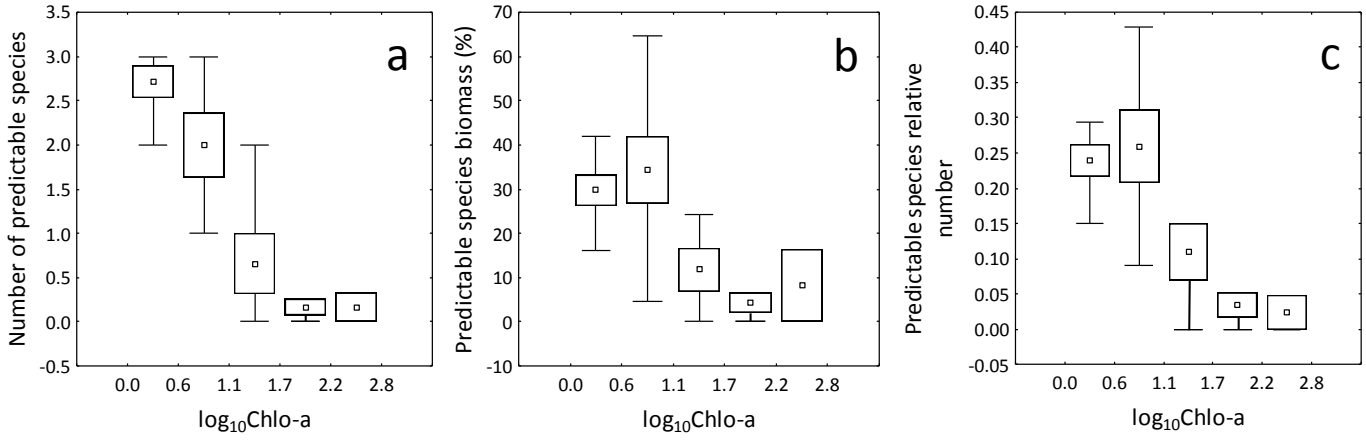

***Figure 4**. Relationship between the chlorophyll level of lakes and the importance of species for which the biomass is relatively predictable (R^2>0.4) in terms of predictable species number (a), the percentage of predictable species in the community (b) and the fraction of the total biomass of the community represented by predictable species (c).*

What could be the mechanism explaining these puzzling patterns in predictability of species biomass? As mentioned in the introduction unpredictability as well as species co-existence has been linked to four potential explanations: *Environmental stochasticity* (Hutchinson, 1961; Sommer, 1985); *Intrinsic chaos* (Huisman & Weissing, 2000; Scheffer, 2001; Benincà *et al.*, 2008); *Neutral coexistence* (Hubbell, 2001) and *Emergent Neutrality* (Scheffer & Van Nes, 2006). While empirical relations we unveiled do not put us in the position to infer much about the underlying processes, it is worth exploring whether our data suggest some of these possibilities to be more likely than others. We now turn to each of these potential explanations of unpredictability briefly.

Environmental stochasticity is certainly common, and it has been shown experimentally that it can allow co-existence of species that would otherwise loose species because of competition (Sommer, 1985). However, it is not easy to imagine why it would affect some species systematically more than others. Fast growing species could in principle follow changing conditions more closely. However, we see no correlation between predictability and individual volume, even though in phytoplankton, volume is a good proxy for growth rate (Reynolds, 1997; Kruk *et al.*, 2010).

Intrinsic chaotic dynamics have also been demonstrated experimentally in plankton (Benincà *et al.*, 2008), and models suggest that it might promote co-existence just as environmental fluctuation does (Huisman & Weissing, 2000). Predictable species are found more in environments where chlorophyll levels are low, but nutrient levels not. This suggests that environments with less competition (for nutrients and light) lead to higher predictability, which would be consistent with the idea that competition driven chaos could be a source of unpredictability. On the other hand, it is hard to see how chaos would affect part of the species much less than the others. If chaos is driven by competition for the same limited set of resources it should in principle affect all species. If some species would be driven out of chaotic communities, or would be specialized to resources in a way that would allow co-existence but set them apart from the chaotic remainder of the community, one would expect a correlation between predictability and particular functional traits. However we find no indication for such a relation, as neither phylogenetic affiliation, nor morphological traits that are believed to capture functional traits (Reynolds, 1988; Kruk *et al.*, 2010) are correlated to predictability. Predation by zooplankton can also drive chaotic dynamics in phytoplankton (Scheffer, 1991; Benincà *et al.*, 2008). Again one would expect a relationship to functional traits in this case. However, predictability is not related to aspects that are known grazing resistance such as the maximum linear dimension of organisms (Burns, 1968; Lampert, 1987). Also, grazing by zooplankton that might drive chaos is considered to be less in warmer lakes (e.g. Malthus & Mitchell, 2006), whereas we find no relationship to temperature.

Neutral coexistence in the original sense (Hubbell, 2001) can simply be excluded, as the functional equivalency implies that all species would be equally unpredictable. As we have seen, especially presence and absence of species is actually quite well predictable from environmental variables.

Emergent neutrality is subtly different in its predictions (Scheffer & Van Nes, 2006). It implies that self-organized functional groups take a 'niche', and should therefore be predictable as a group. However, within the groups, species should be interchangeable, and their dominance would therefore be unpredictable. This could lead to the observed relationship between biomass and predictability, if some species tend to be more dominant within a functional group (but not necessarily in the entire community) than others. This could either happen just by chance, or because those dominant species are for some biological reason systematically more successful in building up biomass than other species within the same functional group. This might explain the success of some invasive species such as *Cylindrospermopsis raciborskii* (Wolosz.) Seenayya & Subba Raju (Padisák, 1997). In any case, it would imply that the high-biomass species tend to occupy much of the available niche space for its functional group. Therefore, variation in environmental factors that determine the carrying capacity within that niche would translate relatively directly into the biomass of such species, leading to a large correlation between environmental conditions and their biomass. By contrast, species within a functional group that happen to take lower and more variable proportion of the space within a functional niche would be less correlated to the overall carrying capacity for that functional group, and therefore to the environmental conditions that affect this carrying capacity.

The idea that in this way consistently high biomass might necessarily lead to predictability of biomass from environmental conditions is in line with the fact that no such correlation exists for predictability of presence or absence. Indeed, presence of a species can on average be quite well predicted from environmental conditions, but this predictability is not correlated in any way to abundance or biomass (neither is it correlated to traits, phylogenetic affiliation or membership to particular morphology-based-functional groups). Overall, emergent neutrality is consistent with the observation that the environment allows one to predict which species can be present but that the actual biomass is quite unpredictable. The latter would arise because of the neutrality in competition within functional groups.

While some of the patterns we observed are thus consistent with the theory of emergent neutrality, this congruence can obviously not be considered as strong evidence. One more general view could be that causality might be reversed in the sense that phytoplankton

biomass causes much of the environmental conditions we measure. Indeed, 'environmental conditions' such as Secchi disk transparency and chlorophyll levels are obviously affected by algal biomass, and this is also true for total phosphorus and total nitrogen concentrations in the water (Scheffer, 1998). If the overall patterns would be ruled by this causality we should also expect that high biomass species tend to be more closely correlated to such environmental conditions, simply because they have a relatively stronger impact on their environment. An argument against this reasoning is that the predictability is actually not correlated to a tendency of the species to dominate the overall community in relative terms or to higher community biomass estimated as chlorophyll-a.

Another more general view could be that species that occur in low abundances have no real niche in the system, and are just present as a result of dispersion from neighbouring ecosystems where they thrive better, or remained in the system from earlier times when conditions favoured them more (Padisák, 1992; Rosenzweig Jr. & Buikema, 1994). For instance, estuarine fish communities have been shown to consist of two distinct groups of species: the abundant and persistent species and the rare occasional species. The first group is biologically associated with the particular environment while the second group consists of 'visiting' species that really belong to other habitats (Magurran & Henderson, 2003). In this view the poor relationship between environmental conditions and the biomass of scarce species could reflect the fact that they appear in the studied lakes just by chance. It should be remarked however that unpredictable species in our lakes are not less frequent than predictable ones.

In conclusion, while our data reveal clear patterns in predictability of species biomass, they do not really allow us to diagnose the mechanisms behind those patterns. Environmental fluctuations and intrinsic chaos seem likely drivers of unpredictable dynamics in phytoplankton. However, it is not obvious how they would explain the marked difference between predictability of species, especially as this difference appears to be unrelated to functional traits. Neutral coexistence can be excluded as an explanation in view of the predictability of the presence of species from environmental conditions. Emergent neutrality in self-organized communities appears to be consistent with the finding that predictability is correlated to the average biomass of a species and not to its frequency of occurrence. However, it remains puzzling why predictable species would occur more in lakes were low chlorophyll levels and relatively high nutrient concentrations and suggest that competition for resources in the phytoplankton community might be relatively less severe.

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Appendix I. *Range of environmental variables of lakes included in this study for the different regions: Subpolar (49°04'–55°06'S), Temperate (35°02'-39°08'S and 41°12'-52°36'N), Subtropical (29°09'-34°09'S) and Tropical (5°04'-23°05'S). Z_{max}: maximum depth; Z_{SD}: Secchi disk depth; T: water temperature; TN: total nitrogen; TP: total phosphorus; SRSi: soluble reactive silicate; CLAD: cladocera abundance; S: southern and N: northern hemispheres. N°: number.*

Latitude	N° of lakes	Z_{max} m	Z_{SD} m	T ºC	Ice cover	TN μgL^{-1}	TP μgL^{-1}	SRSi μgL^{-1}	Chlo-a μgL^{-1}	CLAD $orgL^{-1}$
Subpolar*		3.6	1.5	13.4	Yes	2106	438	1893	20.1	140
S	11	0.7-12	0.04-6.8	10.0-18.6		52-22993	15-9141	100-8900	<0.3-204	2-791
Temperate		8	1.0	16.0	Yes	1515	144	2545	31.9	142
S	11	0.2-31	0.01-4.0	0.4-33.0		63-25913	<10-5600	15.4-14200	0.3-595	0-9318
N	107									
Subtropical		3.1	0.8	19.6	No	4286	388	6660	76.7	113
S	40	0.3-7	0.2-3.9	10.0-30.1		68-37928	29-10087	<10-23533	<0.3-284	0-2791
Tropical		2.7	1.2	25.0	No	474	85.6	2492	12.2	20
S	42	0.5-18	0.2-4.5	14.3-33.0		35-1950	<10-394	<15.0-8955	<0.3-178	0-128

*Sampling in subpolar lakes only included summer season.

Appendix II. *List of species including their phylogenetic Class (Bacillariophyceae: Bac; Chlorophyceae: Chl; Chrysophyceae: Chr; Cryptophyceae: Cry; Cyanobacteria: Cya; Dinophyceae: Din; Euglenophyceae: Eug); their individual average biomass: Biomass; maximum contribution to the total community biomass: Max %; number of cases with more than 30% contribution to the total observations of the particular species: Dom %; R^2 from multiple linear regression; volume: V; surface/volume: S/V; and maximum linear dimension: MLD. Species are ordered from low to high R^2.*

Species	Class	Biomass	Max %	Dom %	R^2	V μm^3	S/V μm^{-1}	MLD μm
Urosolenia eriensis var. morsa	Bac	0.07	12.0	0	0.04	1254	0.8	55.1
Cryptomonas curvata	Cry	0.05	6.7	0	0.05	2526	0.4	27.3
Cyclotella sp.	Bac	0.80	94.2	16	0.05	2003	0.5	16.4
Chroococcus limneticus	Cya	0.04	6.9	0	0.06	47713	0.1	45.0
Pseudanabaena capillaris	Cya	0.57	35.6	25	0.06	19.9	5.9	25.8
Monoraphidium dybowskii	Chl	0.03	8.4	0	0.06	63.3	1.6	16.1
Hemidinium brasiliense	Din	0.03	14.4	0	0.07	1237	2.7	700
Rhabdoderma vermicularis	Cya	0.05	5.8	0	0.07	2444	1.0	14.9
Epigloeosphaera brasilica	Cya	1.31	20.9	0	0.07	2382	0.2	19.8
Urosolenia eriensis	Bac	0.44	27.8	0	0.08	1893	0.8	92.3
Peridinium umbonatum var. umbonatum	Din	0.84	15.4	0	0.08	691.8	0.7	16.2
Mallomonas tonsurata	Chr	0.17	52.4	17	0.09	492.8	0.7	19.7
Navicula sp.	Bac	0.01	5.9	0	0.09	221.2	1.0	23.9
Jaaginema geminatum	Cya	0.15	10.3	0	0.09	243.2	1.4	34.7
Aphanocapsa holsatica	Cya	0.18	20.7	0	0.10	15315	0.2	30.0
Cryptomonas marssonii	Cry	0.10	55.0	8	0.11	1200	0.5	31.9
Golenkinia sp.	Chl	0.02	7.6	0	0.11	443.6	0.6	20.1
Eutetramorus fotii	Chl	0.12	26.7	0	0.12	4513	0.5	17.4
Aulacoseira granulata var. angustissima	Bac	0.16	9.4	0	0.12	6264	0.6	101.0
Dinobryon divergens	Chr	0.53	70.5	10	0.12	254.5	1.1	150.0
Coelastrum indicum	Chl	0.24	25.9	0	0.13	5912	0.3	20.3
Cryptomonas sp.	Cry	0.82	76.6	18	0.13	343.2	0.7	31.9
Trachelomonas sp.	Eug	0.44	44.7	5	0.13	524.0		10.0
Chromulina ovaloides	Chr	0.02	7.7	0	0.14	280.7	0.8	8.0
Euglena sp.	Eug	0.04	18.4	0	0.14	688.7	1.7	20.0
Pseudanabaena catenata	Cya	0.57	25.0	0	0.15	110.0	3.4	41.5
Cryptomonas tetrapyrenoidosa	Cry	0.24	43.7	13	0.15	1829	0.4	19.2
Radiococcus planktonicus	Cya	0.07	7.9	0	0.16	3718	0.3	20.9
Chlorella homosphaera	Chl	0.01	5.1	0	0.16	61.6	1.3	4.8
Synechococcus aquatilis	Cya	0.11	41.0	7	0.16	17.2	1.9	3.2
Trachelomonas curta	Eug	0.11	29.8	0	0.16	2079	0.4	15.5
Cryptomonas marsonii	Cry	0.05	63.8	3	0.17	657.2	0.6	14.3
Coelastrum reticulatum	Chl	0.95	25.7	0	0.17	751.8	0.6	14.2
Microcystis ictyoblabe	Cya	1.55	62.5	17	0.18	32526	0.1	34.8
Cryptomonas brasiliensis	Cry	0.16	42.9	6	0.18	563.3	0.6	13.7
Aulacoseira granulata var. granulata	Bac	0.53	28.1	0	0.19	2014	1.3	244.0
Tetraedron minimum	Chl	0.40	24.2	0	0.19	244.8	1.0	8.8
Oocystis sp.	Chl	0.12	13.2	0	0.20	798.7	0.8	12.9
Coelomorom pusillum	Cya	11.32	71.1	40	0.20	6464	0.3	23.0

Appendix II cont.

Species	Class	Biomass	Max %	Dom %	R^2	V μm^3	S/V μm^{-1}	MLD μm
Cyanodictyon imperfectum	Cya	0.15	31.4	3	0.20	524.0	0.6	10.0
Aphanothece minutissima	Cya	0.24	26.5	0	0.22	113097	0.1	242.5
Aphanocapsa delicatissima	Cya	0.86	78.4	16	0.22	6876	0.6	15.0
Cryptomonas sp.2	Cry	0.31	14.9	0	0.22	658.5	0.6	12.3
Tetraeëdron caudatum	Chl	0.06	20.8	0	0.22	197.6	1.0	8.9
Gymnodinium cnecoides	Din	0.01	17.2	0	0.22	185.5	2.1	11.6
Cylindrospermopsis raciborskii	Cya	0.24	14.1	0	0.23	281.0	1.7	58.2
Aphanocapsa incerta	Cya	0.75	35.6	8	0.23	8420	0.1	24.1
Merismopedia sp.	Cya	0.13	11.8	0	0.25	839.5	0.6	15.3
Planktolyngbya limnetica	Cya	1.03	52.2	11	0.25	88.0	3.6	48.2
Pseudanabena recta	Cya	0.09	16.4	0	0.26	139.6	2.3	46.0
Oocystis lacustris	Chl	0.58	44.6	4	0.26	723.8	0.8	12.2
Dyctiosphaerium pulchellum	Chl	0.94	45.0	8	0.26	1122	0.7	93.5
Merismopedia duplex	Cya	0.07	5.0	0	0.30	350.3	0.9	12.1
Scenedesmus ellipticus	Chl	0.15	23.2	0	0.32	333.5	3.9	7.1
Chlorella vulgaris	Chl	0.26	14.1	0	0.33	126.3	1.1	5.8
Oocystis parva	Chl	0.19	22.5	0	0.33	1811	0.7	14.5
Oocystis marsonii	Chl	0.54	16.8	0	0.33	1072	0.9	15.1
Monoraphidium contornum	Chl	0.12	6.3	0	0.34	45.6	4.3	32.3
Peridinium sp.	Din	1.19	30.6	10	0.35	284567	0.1	67.5
Botryococcus braunii	Chl	10.21	90.8	56	0.35	47260	0.2	37.6
Merismopedia tenuissima	Cya	5.10	61.0	4	0.36	108.4	1.7	11.0
Chlorella minutissima	Chl	0.05	68.2	2	0.36	9.4	2.6	2.6
Rhodomonas minuta	Cry	0.30	27.3	0	0.40	43.0	1.4	5.1
Monoraphidium convolutum	Chl	0.17	14.4	0	0.41	15.8	4.2	8.8
Microcystis smithii	Cya	0.35	8.6	0	0.42	3773	1.0	12.1
Cyclotella meneghiniana	Bac	0.02	11.5	0	0.42	1077	0.6	15.1
Synechococcus nidulans	Cya	0.09	6.5	0	0.43	14.7	3.1	4.8
Lemmermmaniella pallida	Cya	3.77	74.5	9	0.45	6406	0.3	20.8
Nitzchia sp.	Bac	1.54	16.7	0	0.49	458.9	0.8	56.7
Jaaginemagracile	Cya	0.32	9.8	0	0.52	17.6	7.7	43.6
Microcystis aeruginosa	Cya	24.13	54.8	25	0.52	7941	0.3	30.4
Raphidiopsis mediterranea	Cya	25.00	89.5	23	0.53	952.1	1.7	146.9
Scenedesmus ovalternus	Chl	1.47	9.8	0	0.58	363.1	1.2	12.8
Planktothrix agardhii	Cya	60.22	98.8	67	0.63	1164	1.5	141.2
Synedra acus	Bac	1.92	28.1	0	0.65	413.0	1.9	86.0
Aphanocapsa nubilum	Cya	2.54	46.4	17	0.67	1303	0.6	12.1
Pseudanabaena acicularis	Cya	3.37	13.1	0	0.67	44.7	4.7	77.2
Oscillatoria sp.	Cya	6.31	15.4	0	0.68	3089	1.2	197.0
Microactinium pusillum	Chl	187.30	83.7	25	0.69	31.0	1.8	26.7

CHAPTER 4. A Morphological Classification Capturing Functional Variation in Phytoplankton

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Abstract

1. A logical way of distinguishing functional groups of phytoplankton is to cluster species according to their functional traits, such as growth rate and nutrient assimilation constants. However, data for such an approach are lacking for the vast majority of the species.
2. Here we show that a classification based on simple morphological traits may capture much of the variability in functional properties among the phytoplankton. We used information on more than 700 freshwater species, from more than 200 lakes situated in climate zones ranging from subpolar to tropical.
3. Morphological characteristics correlated well with functional properties, such as growth rate and sinking rate, and also with the population size and biomass attained in the field. This suggests that morphology is a good predictor of the functional characteristics of species.
4. Cluster analysis was used to define seven species groups based on morphology. Although some of the clusters are taxonomically homogeneous, others include species of several separate divisions. Functional traits (not used for the classification) differed significantly among the clusters, suggesting that the clusters may indeed represent meaningful functional groups.
5. Advantages of our morphological approach to classification include its objectivity, its independence from taxonomic affiliations, and the relative ease of its application to the majority of species for which physiological traits are unknown and are not readily determined.

Introduction

Phytoplankton communities are highly diverse and the occurrence of most species is difficult to predict (Benincà *et al.*, 2008). A long standing line of work aimed at predicting community composition of phytoplankton species is the phytosociological approach. The idea is to define groups of species that are typically found together (Margalef, 1978; Reynolds, 1980; Smayda & Reynolds, 2001). Statistical analyses have been used to recognise and label such clusters of species (Kruk *et al.*, 2002; Tolotti *et al.*, 2005). However, although the approach is pragmatic and the idea of communities as functional entities (*sensu* Clements, 1916) is appealing, there is no obvious basis for such a classification of species, nor is there a reliable way of constructing a general classification that is independent of local biological and environmental characteristics.

A more Gleasonian line of reasoning (Gleason, 1926) is to assume that individual species respond independently to the environment, and predict community composition on the basis of the response of individual species to conditions. Since phytoplankters disperse relatively well, assemblages are expected to be mainly shaped by local conditions (Hillebrand & Azovsky, 2001; Beisner *et al.*, 2006). The conditions should thus favour groups of species that share similar adaptive features (Webb *et al.*, 2002; Falkner *et al.*, 2006; Jäger *et al.*, 2008). Such species are not necessarily taxonomically related. It is often assumed that phylogenetically related species are also ecologically similar. However, phylogenetic 'overdispersion' is common (Webb *et al.*, 2002) in the sense that even closely related taxa can differ widely in habitat use and phenotypic characteristics (e.g. Lürling, 2003).

It would therefore be useful to be able to classify species according to their environmental requirements as a basis for a mechanistic prediction of community assembly (Lavorel *et al.*, 1997; McGill *et al.*, 2006). The most straightforward approach would be to group species according to their functional traits (Weiher & Keddy, 1995). Functional traits embrace physiological, morphological and phenological features that govern ecological performance (McGill *et al.*, 2006; Violle *et al.*, 2007). Such traits need to be measurable at the level of individuals, without reference to the environment (Naeem & Wright, 2003; Violle *et al.*, 2007). Relevant functional traits for phytoplankton should reflect the ability to acquire resources (light, nutrients), to grow and to avoid mortality, through such processes as hydrological washout, sedimentation and consumption by grazers (Margalef, 1978; Reynolds, 1984a; Naselli-Flores *et al.*, 2007). Unfortunately, the current paucity of information on species-specific physiological traits in the phytoplankton (Weithoff, 2003) limits our ability to arrive at *a priori* functional classifications (Reynolds *et al.*, 2002;

Salmaso & Padisák, 2007; Mieleitner *et al.*, 2008). Also, given the expense and practical limitations of systematically investigating physiological traits, this is not a situation that is likely to change in the near future.

By contrast, morphological traits are relatively simple to measure (Arnold, 1983; Hodgson, 1999) and, interestingly their relation to physiology is potentially well defined (Reynolds, 1997; Enquist *et al.*, 1999; Whitfield, 2001). Indeed, there are fundamental reasons to expect such relationships between morphology and the functional properties of phytoplankton (Lewis, 1976; Margalef, 1978; Reynolds, 1984a; Naselli-Flores *et al.*, 2007). Specific growth rate, resource-uptake and light-interception properties all depend on organismic dimensions, volumes and surface/volume ratios (Kirk, 1996). Furthermore, grazing efficiency by filter-feeding zooplankton is affected by phytoplankton morphology (Burns, 1968; Lampert, 1987; Reynolds, 2006).

A tight link between the morphology and autecology of species is also suggested by the fact that distribution in time and space, as well as demographic characteristics, are linked to phytoplankton morphometry (Lewis, 1977; Cermeño *et al.*, 2006). These relationships are so demonstrably robust that dynamic multispecies models, based on the morphology of the participating species, are able to simulate phytoplankton periodicity with considerable realism (Jones & Elliott, 2007).

While all this suggests that it should be possible to arrive at a functional classification of phytoplankton on the basis of their morphology that could be used for ecological prediction, we know of no systematic attempts to do this. In this paper, we propose and formalize a new functional classification of phytoplankton species based exclusively on organismic morphology. The system we present does not require knowledge of physiological traits, environmental conditioning and taxonomical affiliation. Instead it uses traits that are easily observable, such as volume, maximum linear dimension, surface area, and the presence of mucilage, flagella, gas vesicles (or aerotopes), heterocysts or siliceous exoskeletal structures. In order to validate the key assertion that these morphological traits are tightly linked to autecology of the organisms, we first analyse their relationships with measured physiological traits (e.g. maximum growth rate) and demographic data (e.g. mean population biomass).

We then construct a classification based on the mentioned morphological traits. Herein, we shall refer to this as a ‘morphology-based functional classification’, abbreviated to MBFC, and to the functional groups of species thus separated as ‘morphology-based functional groups’, or MBFG. Finally, we test whether the derived groups have coherent physiological traits and demographic characteristics.

Methods

Sample gathering and analysis

We compiled a database of 711 species from 211 lakes located within four climate zones in South America, Europe and North America, and covering a wide range of environmental characteristics (Table 1). For 107 of the lakes, information was obtained from published (De León, 2000; Mazzeo *et al.*, 2003) and unpublished sources (V.L.M. Huszar, pers. comm. 2006; a 1999 Dutch multi-lake survey Gerben van Geest & Frank Roozen, pers. comm. 2004). The remaining 104 lakes were sampled during 2005-2006 by standard procedures, described by Kosten *et al.* (2009). Of the total, 150 lakes were sampled only once, while 61 were sampled at least once every season. Both seasonal- and snapshot- sampling strategies were conducted in all the climatic regions and across the whole trophic spectrum. The sampling and sample-analysis protocols were comparable among the sampled lakes and in the published and unpublished sources from where we extracted the information. Most lakes were sampled at random points integrating the water column and covering the whole lake area. The phytoplankton samples were fixed in Lugol's solution.

Table 1. *Range of environmental variables of lakes included in this study for the different regions: Subpolar (49°04'–55°06'S), Temperate (35°02'-39°08'S and 41°12'-52°36'N), Subtropical (29°09'-34°09'S) and Tropical (5°04'-23°05'S). Z_{max}: maximum depth; Z_{SD}: Secchi disk depth; T: water temperature; TN: total nitrogen; TP: total phosphorus; SRSi: soluble reactive silicate; CLAD: cladocera abundance; S: southern and N: northern hemispheres. N°: number.*

Latitude	N° of lakes	Z_{max} m	Z_{SD} m	T ºC	Ice cover	TN μgL^{-1}	TP μgL^{-1}	SRSi μgL^{-1}	Chlo-a μgL^{-1}	CLAD $orgL^{-1}$
Subpolar*		3.6	1.5	13.4	Yes	2106	438	1893	20.1	140
S	11	0.7-12	0.04-6.8	10.0-18.6		52-22993	15-9141	100-8900	<0.3-204	2-791
Temperate		8	1.0	16.0	Yes	1515	144	2545	31.9	142
S	11	0.2-31	0.01-4.0	0.4-33.0		63-25913	<10-5600	15.4-14200	0.3-595	0-9318
N	107									
Subtropical		3.1	0.8	19.6	No	4286	388	6660	76.7	113
S	40	0.3-7	0.2-3.9	10.0-30.1		68-37928	29-10087	<10-23533	<0.3-284	0-2791
Tropical		2.7	1.2	25.0	No	474	85.6	2492	12.2	20
S	42	0.5-18	0.2-4.5	14.3-33.0		35-1950	<10-394	<15.0-8955	<0.3-178	0-128

*Sampling in subpolar lakes only included summer season.

Phytoplankton populations were counted in random fields using the settling technique (Utermöhl, 1958). We examined the samples at multiple magnifications and counted until we reached at least 100 individuals of the most frequent species. For all lakes, we considered the individual alga as the unit (unicell, colony, coenobium or filament). Cell numbers per colony, as well as organismic dimensions, including maximum linear dimension (MLD) were estimated. Individual volume (V) and surface area (S) were calculated according to the geometric equations of Hillebrand *et al.* (1999). Population biomass was estimated as biovolume and calculated as the individual volume of the species multiplied by the concentration of individuals. For colonial organisms with

mucilage, V and S calculations were made for whole colonies including mucilage. The presence of aerotopes (Aer), flagella (Fla), mucilage (Muc), heterocysts (Het) and siliceous exoskeletal structures (Si) was noted for each relevant organism.

We undertook an extensive literature search of physiological and ecological traits for the species included in our data set. All physiological data were obtained from laboratory experiments with similar experimental designs, including comparable temperature (18-25^{o}C) and saturating light conditions and not from size calculations. Sinking rates were obtained both from mesocosm and laboratory experiments. Traits lacking comparable experimental conditions or with fewer than 10 valid values (*n*) were not considered. We included maximum growth rate (*n* = 59), sinking velocity (*n* = 87), half-saturation constants for PO_4^{-3}-P uptake (*n* = 15), for P-limited growth (*n* = 19) and half-saturation constants for silicate-limited growth (*n* = 15).

Demographic parameters were deduced from the data and included for each species the population mean abundance in terms of numerical density, mean and maximum biomass, as well as its standard deviation (SD) and its coefficient of variation (CV).

Data analysis

Prediction using morphological traits

We used univariate general linear models (GLM) to evaluate the reliability of the nine morphological traits (V, S, MLD, S/V, Aer, Fla, Muc, Het and Si) in predicting the physiological and demographic features. GLM allow both continuous and categorical predictors to be included. Prior to the GLM, stepwise, least-square ordinary multiple linear regressions (MLR) were used to minimize co-linearity and to select the significant continuous morphological variables. We used GLM full factorial model with type III sums of squares. The intercept was set to zero in each case. When assumptions of normality were not satisfied, variables were Log_{10}-transformed. All analyses were performed using SPSS for Windows version 15.0.

Construction of MBFG with morphological traits

We used two-step cluster analysis to classify the species and construct the MBFG, based on the nine morphological traits. To determine the optimum number of clusters, each of the cluster solutions was compared using the Akaike Information Criterion (AIC). AIC reflects the model parsimony combining the goodness-of-fit and the number of estimated model parameters (Akaike, 1973). Outlier selection was done with noise handling of 25% and only

in one instance was a data point not assigned to one of the groups. Canonical variate analysis (CVA) was used to test whether the species clusters were more probable than random grouping; it was carried out using CANOCO for Windows version 4.5 with a focus on inter-species distances and Hill-type scaling, following ter Braak & Smilauer (1998). The significance was tested using Monte Carlo simulations with 499 unrestricted permutations. Finally, we tested for differences in the means of the continuous variables under the different morphology-based clusters using the non-parametric Kruskal-Wallis analysis. Post-hoc multiple comparison tests were performed in order to validate homogeneous sets of MBFG in relation to morphological, physiological and demographic traits. All analyses except CVA were carried out using SPSS for Windows version 15.0.

Results

Predictive value of morphological traits

Based on the nine morphological traits (V, S, MLD, S/V, Aer, Fla, Muc, Het, Si), we obtained significant models for predicting seven out of the 10 functional and demographic characteristics analysed. Three out of the five physiological characteristics (maximum growth rate, silicate half-saturation constant for growth and sinking velocity) and four of the five demographic analysed characteristics (maximum biomass, mean biomass, biomass SD and numerical abundance) were well predicted (Table 2). Only the half-saturation constants for PO_4^{-3}-P uptake (n = 15) and for P-limited growth (n = 19) were not significantly related to morphology.

Maximum growth rate decreased with volume (V) (Fig. 1a) and maximum linear dimension (MLD) (data not shown) and increased with the surface to volume ration (S/V) (Fig. 1b) (R^2 = 0.45, p <0.001). In the GLM, 88% of the variance in maximum growth rates was explained by V only (p < 0.001). The silicate half-saturation constant for growth was significantly related to S/V (R^2 = 0.25, p < 0.05). Sinking velocity increased significantly with MLD and decreased with S/V (R^2 = 0.37, p < 0.001). In the GLM, the variance explained was 84% (p < 0.001) and only the presence of siliceous exoskeletal structures showed a significant positive coefficient in the model (Table 2).

Numerical abundances were negatively related to V, S and MLD but increased with S/V (Fig. 1c, d; R^2 = 0.28, p < 0.001). Opposite patterns were found for population volume. Maximum and mean population biomass, as well as its SD, increased with organism-specific V, S and MLD but decreased with S/V (Figs 1e, f). The variances explained were 20%, 24% and 19%, respectively (p < 0.001). Compounding all dependent criteria in the

GLM, the continuous traits and the categorical traits accounted for 39 to 92% of the variance (Table 2). In all models, the number of morphological traits with significant coefficients was high. Overall, species with high population biomass and numerical abundance tended to be mucilaginous and to lack flagella. Although the presence of aerotopes contributed significantly to some models, no systematic effects on species biomass or abundance were identified.

Morphology-based functional groups

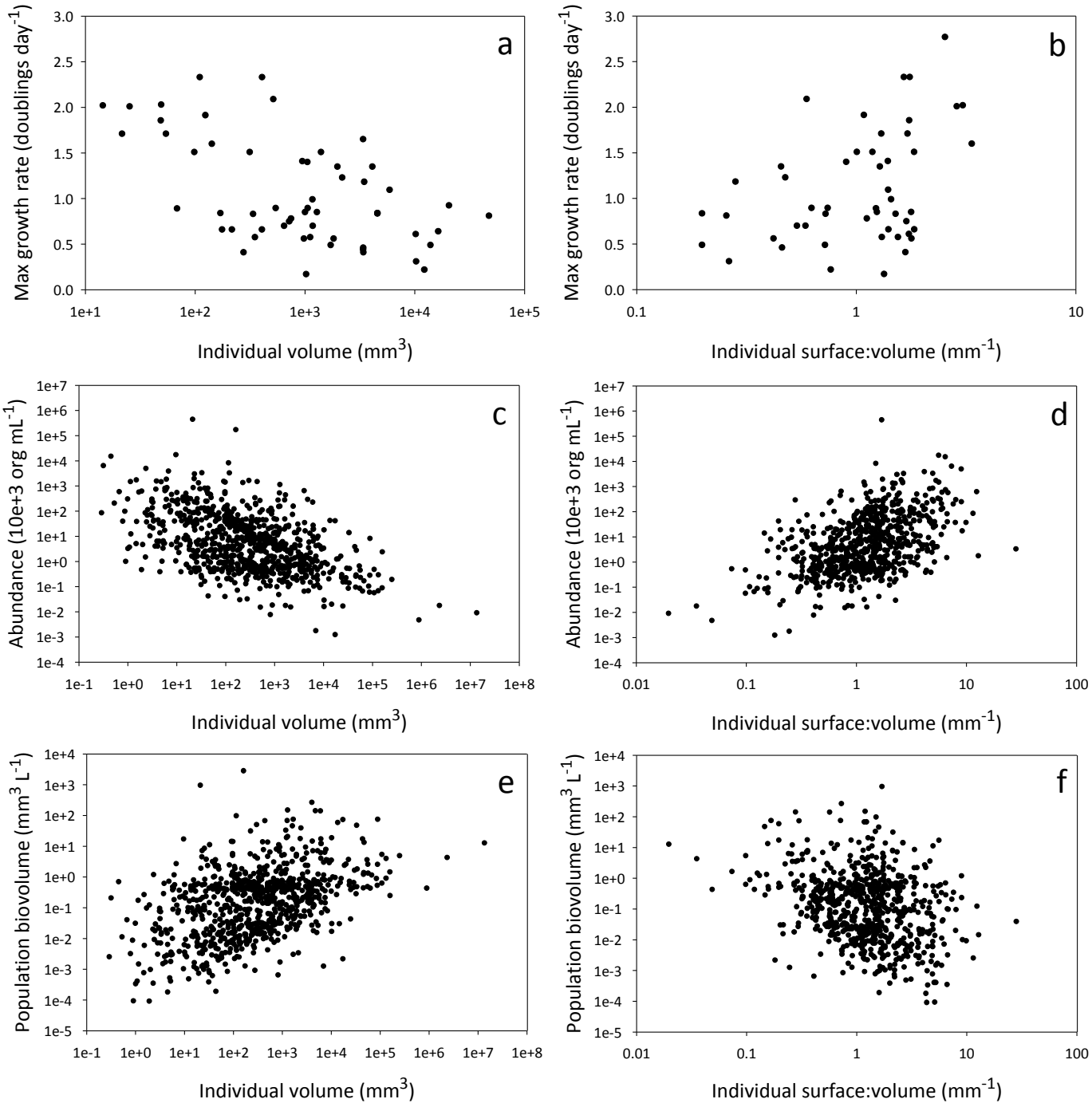
Two-step cluster analysis on the basis of the nine continuous and categorical morphological traits separated species into seven groups, with a similar proportion of cases in each. The seven groups are described in Table 3 and the basis for classifying new species is summarised in the form of a dichotomous key in Fig. 2. The CVA indicated that the groups constructed were significantly more probable than random groups ($F = 5.11$, $p < 0.01$).

***Table 2**. Results of the univariate general linear models for the physiological traits and demographic parameters. KSi growth: Si half saturation constant for growth, SD: standard deviation. Only the predictors with significant coefficients are shown (p <0.05). n: number of cases; V: individual volume; S: surface; MLD: maximum linear dimension all Log_{10} transformed; Aer: aerotopes; Fla: flagella; and Muc: mucilage. (0): absence, (1): presence of the trait.*

Dependent variables	*n*	Mean	Range	R^2 adj $p < 0.001$	Predictors with significant coefficients
Physiological traits					
Max growth (doubling day^{-1})	59	1.09	0.16-2.76	0.88	Log_{10} V = -0.27
Sink velocity (m day^{-1})**	87	0.22	-0.01-4.00	0.84	Silicate (0) = -0.24
KSi growth (μM)*	15	4.5	0.50-19.7	0.52	Non significant predictors
Demographic traits					
Abundance (org mL^{-1})*	711	4.0e+6	0.113-4.07e+7	0.92	Log_{10} MLD =-0.289, Log_{10}V = -0.602, Aer (0) = -2.27, Aer (1) =-1.23, Fla (0) = 0.48, Muc (0)=-1.14
Max biomass (mm^3L^1)*	711	11.5	8.56e-5-970	0.39	Log_{10} S = 0.548, Aer (0) = -2.34, Aer (1) = -1.21, Fla (0) = 0.460, Muc (0) = -1.05
Mean biomass (mm^3L^{-1})*	711	3.7	8.56e-5-896	0.59	Log_{10} S = 0.509, Aer (0)= -2.43, Aer (1) = -1.40, Fla (0) = 0.467, Muc (0)= -1.13
Biomass SD*	711	7.13	6.21e-5-1100	0.43	Log_{10} MLD = 0.391, Log_{10} V = 0.252, Aer (0) = - 1.65, Muc (0) = -1.13

*$Log_{10}(x)$ and ** Log_{10} (x+0.5) transformed

Figure 1. *Relationships of individual volume and surface: volume ratio to maximum (max) growth rate (a & b), numerical abundance (c & d) and population biomass (e & f).*



Not surprisingly, the nine morphological traits used to construct the groups differed significantly among them (Table 4). However, all the other traits (physiological and demographic) not used in the group construction, but significantly correlated with the nine morphological traits (Table 2), were also significantly different among the groups and showed large differences (Table 4; Fig. 3). In general, groups with larger V and lower S/V coincided with lower maximum population growth rate and lower numerical abundance; other variables, such as sinking velocity, showed no direct relation with size (Fig. 3). As individual volume thus seemed a major explanatory variable, we labelled the seven groups according to their individual volumes, **Group I** being the smallest organisms and **Group VII** the largest (Table 3).

Four out of the seven MBFG (**Groups I**, **IV**, **V** and **VII**) included organisms belonging to more than one major taxonomic group; four of the others consisted of only one taxonomic group (**Group-II** Chrysophytes, **Group-III** Cyanobacteria and **Group-VI** Bacillariophyceae). **Group I** was the most taxonomically rich group. Chlorophytes and Cyanobacteria fell into more than one of the MBFG, while the others phylogenetic groups were restricted to only one. Chlorophytes were included in four groups (**I**, **IV**, **V** and **VII**), as were cyanobacteria (**I**, **III**, **IV**, **VII**) with Oscillatoriales species in four groups.

Group I had the smallest MLD and V, and highest S/V. It included picoplankton (MLD < 2 μm) and small unicells (e.g. *Synechocystis aquatilis*), as well as a few representatives of small colonies (e.g. *Scenedesmus ellipticus*) and very thin filaments (e.g. *Jaaginema pallidum*) (Table 3; Fig. 3). This group exhibited the highest maximum population growth rates, low sinking velocities and greatest numerical abundance (Fig. 3). **Group I** differed from the rest in all variables except mean and maximum biomass. Although of smallest size, **Group I** showed the widest range of abundance and biomass (Fig. 3). **Group II** included all small flagellated species with siliceous exoskeletal structures and medium to low S/V (Chrysophytes) with a rather low maximum population growth rate (Table 3; Fig. 3). **Groups III** to **VI** showed similar individual volumes but differed significantly in their MLD, S/V and other variables (Fig. 3). **Group III** algae had the greatest MLD**,** high mean biomass, low maximum growth rate and low sinking velocities. It included filamentous cyanobacteria with aerotopes, both larger species of the Oscillatoriales (e.g. *Planktothrix agardhii*) and all the dinitrogen-fixing Nostocales (e.g. *Cylindrospermopsis raciborskii*). Despite their relatively high individual volume, the long narrow shape of the species of this group gave them the second highest S/V ratio. **Group IV** clustered large unicells, colonies and filaments lacking any of the categorical morphological traits (aerotopes, flagella, heterocysts, mucilage and siliceous exoskeletal structures) and was average in most traits (e.g. *Closterium acutum* Zygnematophyceae, *Coelastrum microporum* Chlorococcales and *Pseudanabaena limnetica* Oscillatoriales). **Group V** included most unicellular flagellated species (e.g. *Gymnodinium cnecoides* Dinophyceae, *Euglena proxima* Euglenophyceae),

which had intermediate values for most attributes, and low maximum growth rate, as well as low population biomass (Fig. 3). **Group VI** included non-flagellated organisms with siliceous exoskeleton (Bacillariophyceae), typically showed high sinking rates, and often maintained high biomass. Some of these species achieved relatively large size and individual volume, and moderate-to-high maximum growth rates. **Group VII** included all species forming large mucilaginous colonies. Component species achieved high mean V and S, and the lowest S/V; all had moderate or low maximum population growth rates (e.g. *Microcystis aeruginosa, Botryococcus braunii*).

Figure 2. *Key for classifying phytoplankton into morphology-based functional groups. V: volume (μm^3); S/V: surface: volume ratio (μm^{-1}); MLD: maximum linear dimension (μm).*

1 Presence of flagella	2 Presence of siliceous structures	**Group II**			
	2' Absence of siliceous structures	3 MLD < 2 μm	**Group I**		
		3' MLD > 2 μm	**Group V**		
1' Absence of flagella	2 Presence of siliceous structures	**Group VI**			
	2' Absence of siliceous structures	3 Presence of mucilage	4 Presence of aerotopes	5 S/V > 0.6 μm^{-1}	**Group III**
				5' S/V < 0.6 μm^{-1}	**Group VII**
			4' Absence of aerotopes	5 V < 10 μm^3	**Group I**
				5' V > 10 μm^3	**Group VII**
		3' Absence of mucilage	4 V < 30 μm^3	5 MLD < 20 μm^3	**Group I**
				5' MLD > 20 μm^3	**Group IV**
			4' V > 30 μm^3	5 Presence of aerotopes	**Group III**
				5' Absence of aerotopes	**Group IV**

***Figure 3**. Mean values and 1.00 and 1.96 standard errors for traits of each morphology-based functional group (MBFG from I to VII). Traits showed in panels' a-c were used to construct the groups; traits showed in panels' d-f were not. MLD: maximum linear dimension, max: maximum. All these traits were significantly different considering all MBFG; groups that belong to the same post-hoc subset are labelled with the same letter.*

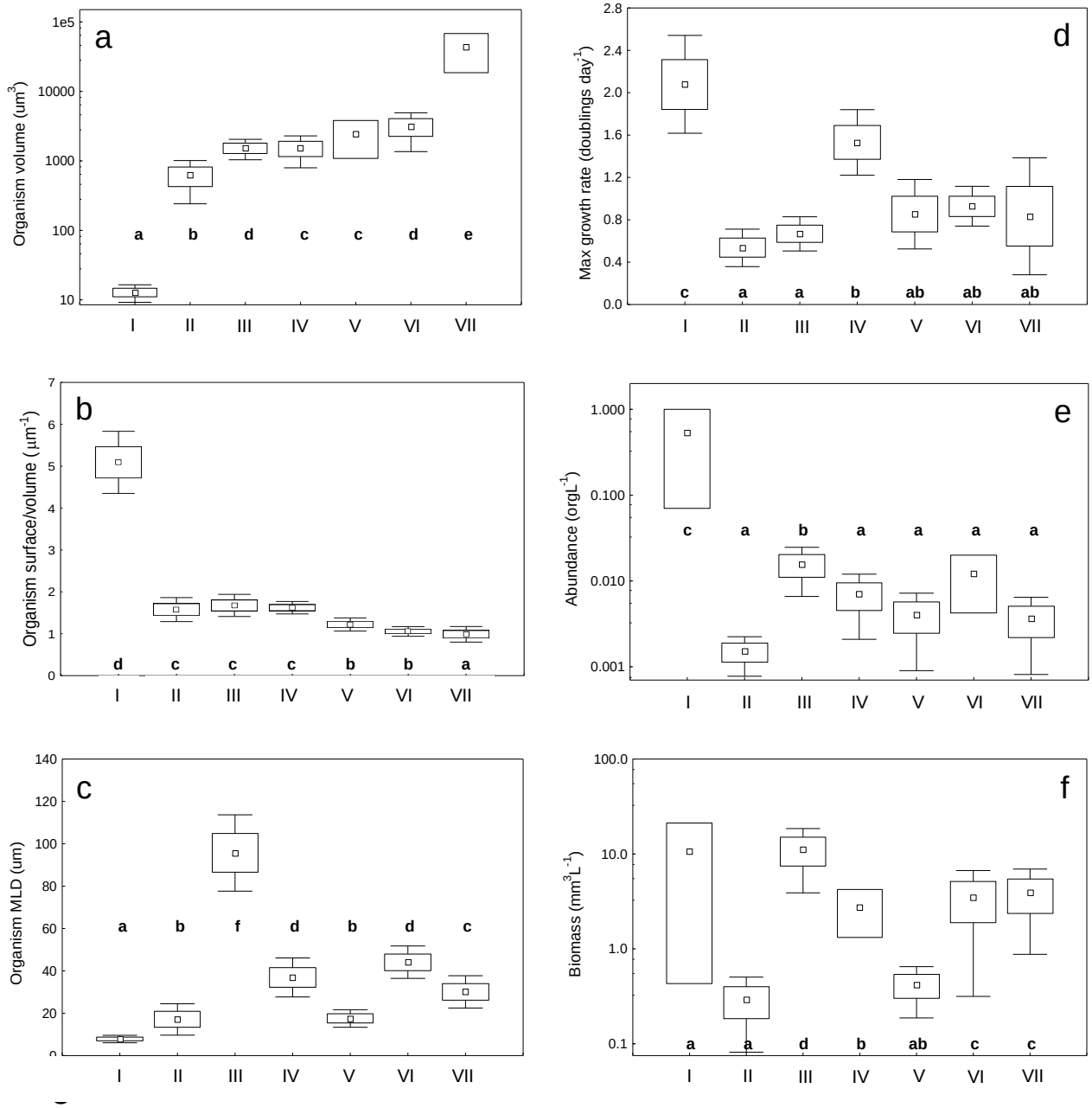


Table 3. *Description of the seven morphology-based functional groups (MBFG) of phytoplankton obtained using two-step cluster analysis of more than 700 species and 200 lakes. Continuous variables include: V: individual volume; S: surface area; MLD: maximum linear dimension, mean and range are shown. Categorical variables include: presence frequency of Aer: aerotopes; Fla: flagella; Muc: mucilage; Het: heterocysts; Si: siliceous exoskeletal structures; n: number of cases per cluster, mean frequency and range are shown in each case.*

MBFG	Description	Taxonomic group	Representative taxa	V μm^3	S μm^2	S/V μm^{-1}	MLD μm	Aer	Fla	Muc	Het	Si
I *n* = 87	Small organisms with high S/V	Chlorococcales, Chroococcales, Oscillatoriales, Xanthophyceae, Ulothricales	*Chlorella minutissima* Fott & Nováková, *Scenedesmus ellipticus* Corda, *Synechocystis aquatilis* Sauvageau, Chroococcales <1μm, *Jaaginema pallidum* (Böcher) Anagnostidis & Komárek, *Monodus* Chodat, *Stichococcus bacillaris* Naegli.	12.9	134	5.1	7.8	0.02	0.03	0.06	0.00	0.00
				0.3-120	2.3-8191	1.5-28.7	0.8-43.6	0-1	0-1	0-1	0	0
II *n* = 69	Small flagellated organisms with siliceous exoskeletal structures	Chrysophyceae	*Chromulina gyrans* Stein, *Dinobryon cylindricum* Imhof., *Mallomonas pulcherrima* (Stokes) Lemmermann, *Pseudopedinella* sp. Carter.	626	308	1.6	17.1	0.00	1.00	0.00	0.00	1.00
				1.0-10469	4.7-2783	0.3-6.8	2.0-164	0	1	0	0	1
III *n* = 45	Large filaments with aerotopes	Nostocales, Oscillatoriales	*Anabaena crassa* (Lemmermann) Komárková-Legnerová & Cronberg, *Aphanizomenon issatschenkoi* (Usacev) Proshkina-Lavrenko, *Cylindrospermopsis raciborskii* Wołoszyńska, *Limnothrix planctonica* Meffert, *Planktothrix agardhii* (Gomont) Anagnostidis & Komárek.	1541	1553	1.7	95.7	1.00	0.00	0.27	0.64	0.00
				8.1-8708	19.6-4598	0.4-4.3	2.5-259	1	0	0-1	0-1	0
IV *n* = 183	Organisms of medium size lacking specialized traits	Chlorococcales, Oscillatoriales, Xanthophyceae, Zygnematophyceae	*Coelastrum microporum* Nägeli, *Micractinium pusillum* Fresenius, *Monoraphidium griffithii* (Berkeley) Komárková-Legnerová, *Pediastrum duplex* Meyen, *Scenedesmus quadricauda* (Turpin) Brébisson, *Pseudanabaena limnetica* (Lemmermann) Komárek, *Arachnochloris minor* Pascher, *Closterium acutum* Brébisson, *Zygnema* sp. Agardh.	1543	791	1.6	37.0	0.00	0.00	0.00	0.00	0.00
				12.7-48255	29.1-18200	0.1-5.1	4.6-700	0	0	0	0	0
V *n* = 122	Unicellular flagellates of medium to large size	Cryptophyceae, Dinophyceae, Euglenophyceae, Volvocales, Chlorococcales	*Cryptomonas ovata* Ehrenberg, *Ceratium hirundinella* (Müller) Bergh, *Gymnodinium cnecoides* Harris, *Euglena proxima* Dangeard, *Trachelomonas curta* Cunha, *Pyramimonas longicauda* Van Meel, *Chlamydomonas globosa* Snow.	2444	764	1.2	17.5	0.00	1.00	0.00	0.00	0.00
				2.4-164779	8.9-20997	0.1-4.9	2.1-190	0	1	0	0	0
VI *n* = 98	Nonflagellated organisms with siliceous exoskeletons	Bacillariophyceae	*Acanthoceros zachariasii* (Brun) Simonsen, *Aulacoseira granulata* (Ehrenb.) Simonsen, *Cyclotella atomus* Hustedt, *Cyclotella meneghiniana* Kützing, *Urosolenia eriensis* (Smith) Round & Crawford, *Asterionella Formosa* Hassall, *Cylindrotheca closterium* (Ehrenb.) Lewin & Reimann, *Synedra acus* Kützing.	3143	1344	1.1	44.1	0.00	0.00	0.00	0.00	1.00
				7.8-57106	19.2-17473	0.2-3.6	2.8-244	0	0	0	0	1
VII *n* =106	Large mucilaginous colonies	Chlorococcales, Chroococcales, Oscillatoriales,	*Botryococcus braunii* Kützing, *Eutetramorus fotii* (Hindak) Komarek, *Oocystis lacustris* Chodat, *Aphanocapsa delicatissima* West & G.S. West, *Microcystis aeruginosa* (Kützing) Kützing, *Romeria okensis* (Meyer) Hindák.	43152	3062	1.0	30.1	0.04	0.00	1.00	0.00	0.00
				10.9 – 2.4e6	24.4-87616	0-6.0	3.5-244	0-1	0	1	0	0

Discussion

Our results indicate that phytoplankton can be classified into meaningful functional groups on the basis of simple morphological criteria. The correlations between physiological traits and morphological aspects and the fact that the MBFG are significantly different in functional traits suggest that morphology is a reasonable proxy for function. The only physiological features for which we found no significant relationship with morphology were phosphate uptake and growth half-saturation constants. This might be related simply to the few valid cases available for the analysis (n = 15 and 19) or to a wide range of physiological adaptation in these aspects. In fact, taxonomical relationships can be a poor predictor of ecological similarity in phytoplankton to the point that, even within a species, size and shape may vary substantially and functional adaptations such as heterocysts may be present or absent depending on conditions (Litchman *et al.*, 2003). Further, clumping (Jezberová & Komárková, 2007) and number of cells per colony (Lürling, 2003), are notoriously variable. In principle our approach allows the accommodation of such differences within species.

Although many earlier studies support the idea that there is a tight link between morphology and function in phytoplankton, attempts to create an ecologically meaningful classification had so-far taken a different approach. As argued in the Introduction, a functional classification has many advantages when it comes to ecological interpretation and prediction. However, direct experimental data on functional aspects such as the responses of growth to light and nutrients are rare. Therefore the use of easily determined morphological criteria as an indicator for functional properties is obviously a useful shortcut.

Clearly there are many more adaptive traits that could be important to ecological success than the ones we tested (e.g. differential cell-wall composition, ability to use alternative nutrient sources, perennation). Nonetheless, our results suggest that the MBFG might echo ecological functioning in a broader sense. Although interpretation of our groups in such terms is necessarily somewhat speculative, we now reflect on the ecological aspects that seem likely to be associated with the groups we distinguished (Table 4).

Group I: small organisms with high S/V

This group aligns small size with fast individual growth rate and high numerical abundance. These organisms are *r*-selected (Pianka, 1970); they are well adapted to rapid resource acquisition and diminished sinking losses (Reynolds, 1984a). Despite being palatable (Lehman, 1991), small species with a high rate of population growth might recover rapidly

after intense grazing (Sommer *et al.*, 2003). This group shows the highest maximum population biomass and numerical abundance. Few of its members have negative impacts on water quality, although some of the cyanobacteria can produce toxins (Dow & Swoboda, 2000).

Group II: small flagellated organisms with siliceous exoskeletal structures

This group contains only Chrysophyceae. These organisms may be classified as *r*-selected. Despite the presence of siliceous scales, small size and motility conferred by flagella allow algae of this group to avoid sinking and also to forage for nutrients (Reynolds, 1997). Flagella and siliceous spines might reduce losses due to grazing (Reynolds, 1997). In addition to the morphological traits we considered, the production of resistant propagules and the facultative mixotrophy shown for many of its constituting species (Sandgren, 1991) impart a tolerance to low nutrient conditions. Populations of these organisms typically exhibit low biomass, and pose no significant threats to water quality.

Group III: large filaments with aerotopes

The species in this group may be mostly characterized as *K*-selected (Pianka, 1970). They are large and grow slowly, but their high S/V confers a greater tolerance to limiting light conditions (Naselli-Flores & Barone, 2007). Moreover, the capacity of some members to fix nitrogen offers a tolerance of low-nitrogen conditions, and the potential production of resistant propagules (hormogonia and akinetes) may enhance their tolerance to low nutrients. Low sinking rates may result from the presence of aerotopes and from a high surface to volume ratio that also facilitates the access to resources (Ferber *et al.*, 2004). Large size (Lehman, 1991) and potential toxicity (Dow & Swoboda, 2000) give this group a higher resistance to grazing. Dominance by these organisms may have large impacts as a consequence of high attainable biomass (Scheffer *et al.*, 1997), and the potential to form toxic blooms (Dow & Swoboda, 2000; Vidal & Kruk, 2008).

Group IV: organisms of medium size lacking specialized traits

This group contains a spectrum from typically *r*-selected (e.g. *Monoraphidium griffithii*) to *K*- selected types (e.g. *Zygnema* spp.). The species have potentially moderate tolerances to limiting resources and low to moderate sinking rates. However, the small size and high quality as food (Sterner & Elser, 2002) of some of the species in this group (e.g. *Chlorella* sp. Beijerinck) make them liable to high grazing losses. This group contains no particularly nuisance species.

Table 4. *Description of the traits of the morphologically based functional groups (MBFG) and their potential ecological performance in terms of resources acquisition and avoidance of loss processes (consumption and sinking). Means and ranges are shown for the physiological traits. S/V: surface volume ratio; Max: maximum; P: phosphorus; N: nitrogen; Si: silicate; C: carbon. The actual sizes for the groups are given in Table 3.*

	Traits				**Potential ecological performance**		
MBFG	**Morphology**	**Max growth rate** doublings day^{-1}	**Sink velocity** m day^{-1}	**Population biomass** mm^3L^{-1}	**Resources gathering** (Light, P, N, Si, C)	**Vulnerability to consumption**	**Sinking losses**
I	Small organisms with high S/V	2.08 (1.7-2.76)	0.03 (-0.01-0.04)	10.7 (9e-5 -896)	Good gathering and low saturating concentration	High, good recovery	Low
II	Small flagellated organisms with siliceous exoskeletal structures	0.54 (0.30-0.74)	Potentially low	0.29 (3.1e-4 -6.61)	Moderate, silicate requirements	High to moderate	Low to moderate
III	Large filaments with aerotopes	0.67 (0.40-0.98)	-0.09 (-0.10-0.10)	11.2 (2.82e-3-139)	Moderate saturating concentration, some fix atmospheric nitrogen	Low	None
IV	Organisms of medium size lacking specialized traits	1.49 (0.45-2.32)	0.14 (-0.01-0.39)	2.76 (4.5e-4 -248)	Moderate	High loss	Low to moderate
V	Unicellular flagellates of medium to large size	0.89 (0.21-2.08)	0.192 (0.07-0.32)	0.42 (1.8e-4-12.7)	Moderate	High to moderate	Low to moderate
VI	Nonflagellated organisms with siliceous exoskeletons	0.93 (0.16-1.40)	0.68 (0.08-4.00)	3.50 (3.6e-4-130)	Moderate, silicate requirements	Moderate	High
VII	Large mucilaginous colonies	0.94 (0.48-1.39)	0.078 (0-0.52)	3.90 (1.2e-3-133)	High saturating concentration	Low	None or low

Group V: unicellular flagellates of medium to large size

Similarly to IV, this group embraces a variety of types ranging from *r*-selected species such as *Chlamydomonas globosa,* to more *K*-selected ones, such as *Ceratium hirundinella*. Their moderate size and surface to volume ratio, together with the possession of flagella, reduces high sinking losses. Motility also permits effective nutrient foraging that, in conjunction with the production of cysts, might increase tolerance of low nutrient conditions. In addition, the capacity of some species to benefit from mixotrophy and phagotrophy implies a means of tolerating conditions of reduced availability of dissolved nutrients (Graham *et al.*, 2000). Their relatively high maximum linear dimension and the presence of flagella may give substantial tolerance to grazing by all but the specialised zooplankton (Reynolds, 1997). Species in this group rarely pose serious threats to water quality.

Group VI: nonflagellated organisms with siliceous exoskeletons

This group contains only diatoms. It has species ranging from the small *r*-selected *Cyclotella atomus* to large, more *K*-selected *Aulacoseira granulata*. The obligate presence of a siliceous wall is probably the main constraining trait of these species. Owing to their high cell density and lack of motility, these organisms sink rapidly and are excluded from waters depleted in assimilable sources of silica. The formation of resistant cells, apparently confined to the centric species (Round *et al.*, 1992), potentially improves their survival in rivers (Reynolds, 2006). Furthermore, siliceous walls also have advantages against certain types of grazers (Hamm *et al.*, 2003) and viral infections (Smetacek, 2001). However, these organisms can suffer more than other groups from fungal infections, especially chytrids (e.g. Ibelings *et al.*, 2004). Diatoms rarely have substantial negative effects on the water quality but their dominance in reservoirs can cause serious filtration difficulties (Ridley, 1970).

Group VII: large mucilaginous colonies

Most organisms in this group are typically *K*-selected. The presence of mucilage, along with lipids and aerotopes in the larger colonies, gives controllable buoyant properties. Moreover, mucilage may help maintaining an adequate microenvironment for cells and avoidance of grazing (Reynolds, 2007). Also, survival may be prolonged by the facility of remaining as resting colonies in the sediment (Reynolds *et al*., 1981). Large size and volume, and low surface to volume ratio, should tend to make species in this group sensitive to low resource supply. On the other hand, these characteristics, together with the potential to produce toxins and allelopathic substances, may promote the fitness of these species. The large population biomass that some species can attain in surface water puts them in the category of notorious nuisance algae. Problems from the point of view of

water quality management can become especially serious if toxins are produced and surface scums are formed (Dow & Swoboda, 2000).

Our results do not invalidate other, more complex, ecological classifications of phytoplankton (Reynolds, 1988; Reynolds *et al.*, 2002; Salmaso & Padisák, 2007). While these classifications have the advantage of being more refined than ours, they do have the disadvantage of requiring information about traits that are typically not easy to obtain for all species (Salmaso & Padisák, 2007; Mieleitner *et al.*, 2008) and partially based on expert judgement (Reynolds *et al.*, 2002). Our MBFC is admittedly coarse. On the other hand, this easy-to-use, objective tool for determining functional groupings may be of value to phytoplankton ecologists, to modellers and to water-quality managers, attempting to understand and predict the composition of phytoplankton communities. Obviously, whether the MBFG may indeed be predicted better from in-lake conditions than species, taxonomic groups or classes of species proposed by other classification systems remains to be tested in practice.

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CHAPTER 5. Phytoplankton Community Composition can be Predicted Best in Terms of Morphological Groups

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Abstract

1. Although the relationship between total biomass of phytoplankton and environmental conditions is reasonably well understood, predicting the composition of phytoplankton communities is notoriously difficult.
2. Here we explore how well the aggregated biovolume of groups of species can be predicted from environmental variables using three different classification approaches: morphology-based functional groups (MBFG), phylogenetic groups, and functional groups proposed by Reynolds. We assessed the relationships between biovolume of each group and environmental conditions using canonical correlation analyses as well as multiple linear regressions, using data from 211 lakes worldwide ranging from subpolar to tropical regions.
3. We found that groups from all three classifications were more closely related to environmental conditions than individual species on average. However, both the multivariate and the regression analyses indicated that morphology-based groups can be predicted better from environmental conditions than groups based on the other classification methods.
4. This suggests that morphology captures ecological function of phytoplankton well, and that functional groups based on morphology may be most suitable to focus on if we aim at predicting the composition of communities.

Introduction

Aggregated estimators of phytoplankton abundance such as chlorophyll-a levels or total biovolume can be predicted relatively well from environmental conditions (McCauley & Murdoch, 1987; Scheffer *et al.*, 2003). However, community composition is less easily predicted. Nonetheless, species differ widely in aspects such as edibility or toxicity, making it important to understand what drives community composition, rather than total biomass. Unfortunately, there are probably thousands of phytoplankton species in lakes and oceans, and predicting species' individual responses is difficult if not impossible (Hutchinson, 1961; Roy, 2007; Benincà *et al.*, 2008). Therefore, it makes sense to concentrate prediction efforts on an intermediate level of species aggregation (Doney *et al.*, 2002; Falkowski *et al.*, 2003; Litchman *et al.*, 2006).

A simple and common way of aggregating species is the use of major phylogenetic groups. Such an approach assumes that different phylogenies somehow reflect important ecological differences among species (Webb *et al.*, 2002). An alternative approach is the use of explicit functional classifications (Padisák & Reynolds, 1998; McGill *et al.*, 2006; Litchman & Klausmeier, 2008). The idea is to cluster species with common traits and similar responses to environmental changes. Such functional classifications have been proposed for phytoplankton by several authors (Reynolds *et al.*, 2002; Salmaso & Padisák, 2007; Mieleitner *et al.*, 2008). Particularly influential is Reynolds et al. (2002) functional classification based not only on individual functional traits, but also on the range of environmental conditions over which the species are found to occur, as well as co-occurrence patterns. While this classification integrates a rich base of information, it also necessarily relies in part on expert judgment. Therefore an alternative, simpler classification has been proposed, based exclusively on objective morphological aspects such as individual volume, surface and maximum length (Kruk *et al.*, 2010).

So-far there is no consensus on which is the best approach to cluster species if it comes to predicting the effects of environmental change on community composition. Here we aim to resolve this issue by exploring how well the biomass of phytoplankton groups based on different classification systems can be statistically explained from environmental conditions in a lake, using information on a very large number of phytoplankton communities and environmental variables from different climate zones and continents.

Table 1. *Range of environmental variables of lakes included in this study for the different regions: Subpolar (49°04'–55°06'S), Temperate (35°02'-39°08'S and 41°12'-52°36'N), Subtropical (29°09'-34°09'S) and Tropical (5°04'-23°05'S). Z_{max}: maximum depth; Z_{SD}: Secchi disk depth; T: water temperature; TN: total nitrogen; TP: total phosphorus; SRSi: soluble reactive silicate; CLAD: cladocera abundance; S: southern and N: northern hemispheres. N°: number.*

Latitude	N° of lakes	Z_{max} m	Z_{SD} m	T ºC	Ice cover	TN μgL^{-1}	TP μgL^{-1}	SRSi μgL^{-1}	Chlo-a μgL^{-1}	CLAD $orgL^{-1}$
Subpolar*		3.6	1.5	13.4	Yes	2106	438	1893	20.1	140
S	11	0.7-12	0.04-6.8	10.0-18.6		52-22993	15-9141	100-8900	<0.3-204	2-791
Temperate		8	1.0	16.0	Yes	1515	144	2545	31.9	142
S	11	0.2-31	0.01-4.0	0.4-33.0		63-25913	<10-5600	15.4-14200	0.3-595	0-9318
N	107									
Subtropical		3.1	0.8	19.6	No	4286	388	6660	76.7	113
S	40	0.3-7	0.2-3.9	10.0-30.1		68-37928	29-10087	<10-23533	<0.3-284	0-2791
Tropical		2.7	1.2	25.0	No	474	85.6	2492	12.2	20
S	42	0.5-18	0.2-4.5	14.3-33.0		35-1950	<10-394	<15.0-8955	<0.3-178	0-128

*Sampling in subpolar lakes only included summer season.

Methods

Data-base

We compiled a database of 237 species from 211 lakes located within four climate zones in South America, Europe and North America, and covering a wide range of environmental characteristics (Table 1). For 107 lakes, information was obtained from published (De León, 2000; Mazzeo *et al.*, 2003) and unpublished sources (V.L.M. Huszar, *pers. comm*. 2006; a 1999 Dutch multi-lake survey Gerben van Geest & Frank Roozen, *pers. comm.* 2004). The remaining 104 lakes were sampled during 2005-2006 by standard procedures at least once during summer (Kosten *et al.*, 2009; Kruk *et al.*, 2009). For this study we included only one summer sample per lake (211 cases). The sampling and sample-analyses protocols were comparable among the lakes. Most lakes were sampled at random points integrating the water column and covering the whole lake area. Water samples for nutrients and plankton were taken integrating the water column with a PVC tube (30 cm diameter) and combining from 3 to 20 random replicates in each lake. Phytoplankton samples were fixed in Lugol's solution. Zooplankton samples were filtered on a 50µm sieve and preserved in a 4% formaldehyde solution. Environmental variables included temperature (Temp, ^{o}C), inorganic suspended solids (ISS, mg L^{-1}), water column mix depth (Z_{mix}, m), light attenuation coefficient (K_d, m^{-1}), conductivity (Cond, µS cm^{-1}), alkalinity (Alk, µeq L^{-1}), dissolved inorganic nitrogen (DIN, µg L^{-1}), total nitrogen (TN, µg L^{-1}), soluble reactive phosphorus (SRP, µg L^{-1}), total phosphorus (TP, µg L^{-1}), soluble reactive silicate (SRSi, µg L^{-1}), total zooplankton abundance (TZ, org L^{-1}), cladocera abundance (CLAD, org L^{-1}) and $Log_{10}(x+1)$ chlorophyll-a (LogChlo-a, µg L^{-1}) (details os sample analysis in Kosten *et al.*, 2009; Kruk *et al.*, 2009).

Phytoplankton abundance and biovolume calculation

Phytoplankton populations (individuals mL^{-1}) were counted in random fields from fixed Lugol samples, using the settling technique (Utermöhl, 1958). We examined the samples at multiple magnifications and counted until we reached at least 100 individuals of the most frequent species. For all lakes, we considered the individual algae as the unit (unicell, colony, or filament). Cell numbers per colony as well as organism dimensions, including maximum linear dimension (MLD, μm) were estimated. Individual volume (V, μm^3) and surface area (S, μm^2) were calculated according to geometric equations (Hillebrand *et al.*, 1999). For colonial organisms with mucilage, V and S calculations were made for whole colonies including mucilage. Presence of aerotopes, flagella, mucilage, heterocytes and siliceous exoskeletal structures were noted for each relevant organism. Population biomass was estimated as biovolume ($mm^3\ L^{-1}$) and calculated as the individual volume of the species multiplied by the concentration of individuals.

Data analysis

Species classification

We excluded species with a contribution of less than 5% to the total community biomass in any individual lake (leaving only 237 of the original total of 711 species). We classified the remaining species in 7 morphology-based functional groups (MBFG), in 8 major phylogenetic groups and in 29 Reynolds groups (Reynolds) (Table 2). For each group of each classification biovolumes were summed per sample.

Overall analysis and comparison of the three classifications and the species

Canonical correspondence analyses were used to make a global comparison of the three classifications. Four preliminary Detrended Correspondence Analyses (DCA) with log-transformed biological data were carried out for the three classifications and for the individual species. All showed long gradient lengths and therefore the unimodal response model was regarded as appropriate for our dataset (ter Braak & Smilauer, 1998). Therefore, we applied four Canonical Correspondence Analyses (CCA) to estimate how much variance of the biomass of the species and the groups in the three classifications was explained by the environmental variables. The importance of each variable was assessed using the forward selection procedure in a CCA, and only variables with a significant (p <0.05) contribution to the explained variance were included in further analyses. Subsequently, to evaluate the degree 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). The variance explained by the environment assessed through CCA was estimated as the variance explained by the sum of all canonical eigenvalues divided by total inertia. These analyses were carried out with the software

CANOCO for Windows version 4.5. All phytoplankton data were $Log_{10}(x+a)$ transformed, with *a* corresponding to the minimum value of the variable for the group of species of the particular classification. All environmental variables were standardized.

To evaluate the strength of each classification, five random versions of each classification were constructed by randomly assigning species in the same number of groups for each classification. The random versions were evaluated with the same CCA protocol. The difference in the explained variance from the environment between real and random groups was calculated for each classification.

***Table 2**. Phytoplankton species classifications. MBFG: morphology-based functional groups; N: number of groups per classification; V: volume; S: surface; MLD: maximum linear dimension.*

Classification	N	Reference	Groups	Classification method
MBFG	7	Kruk *et al.* (2010)	Small organisms with high S/V (I), small flagellated organisms with siliceous exoskeletal structures (II), large filaments with aerotopes (III), organisms of medium size lacking specialized traits (IV), flagellates unicells with medium to large size (V), nonflagellated organisms with siliceous exoskeletons (VI) and large mucilaginous colonies (VII).	Considering V, S/V, S, MLD, and the presence or otherwise of aerotopes, flagella, mucilage, heterocytes and siliceous structures.
Major phylogenetic groups	8	Van Den Hoeck *et al.* (1997) except Cyanobacteria (Komárek & Anagnostidis, 1999; Komárek & Anagnostidis, 2005)and Bacillariophyceae (Round *et al.*, 1992)	Bacillariophyceae (Bac), Chlorophyceae (Chl), Chrysophyceae (Chr), Cryptophyceae (Cry), Cyanobacteria (Cya), Dinophyceae (Din), Euglenophyceae (Eug), Zygnematophyceae (Zyg).	Phylogenetic classes
Reynolds' functional groups	29	Reynolds *et al.* (2002) and Reynolds (2006)	A, B, C, D, E, F, G, H_1, H_2, J, K, L_M, LO, M, MP, N, P, R, S_1, S_2, S_N, T, W_1, W_2, X_1, X_2, X_3, Y, Z	Considering phylogeny, morphological traits, co-occurrence and environment.

Regression models of individual species and of each group within each classification

In addition, we analyzed the predictability of each group within each classification and of all individual species from environmental conditions using ordinary multiple linear regressions (MLR). The same independent variables were used in all models (Temp, ISS, Z_{mix}, Kd, K, Cond, Alk, DIN, TN, SRP, TP, SRSi, TZ, CLAD and Log_{10} Chlo-a). We applied the non-parametric Kruskal-Wallis median test and non-parametric *z* post-hoc tests to analyze the differences in the R^2 values obtained for each classification method. All phytoplankton data were $Log_{10}(x+a)$ transformed, with *a* corresponding to the minimum value of the variable for the group of species of the particular classification. All analysis were performed using SPSS for Windows version 15.0.

***Table 3**. Results of the canonical correspondence analysis (CCA) relating the species and the different classification systems with the environmental variables. MBFG: morphology-based functional groups; N groups: number of groups per classification or number of species when corresponds; N env. var.: number of environmental variables included in the analysis; Total inertia: total variation obtained from the sum of all eigenvalues; Trace: explained variation obtained from the sum of canonical axes; Difference from random %: range of the difference of environmental explanation between the real classification and random classifications; F: F test values for all canonical eigenvalues with the finally selected environmental variables in all cases with $p < 0.01$.*

Classification	Environmental explanation %	N groups	N env. var.	Total inertia	Trace	Difference from random %
MBFG	37.1, *F* = 16.42	7	7	1.787	0.663	13.7 - 23.7
Phylogenetic groups	28.9, *F* = 16.05	8	5	2.192	0.634	-1.0 - 9.9
Reynolds' groups	26.8, *F* = 7.546	29	12	6.331	1.697	-0.4 - 2.2
Species	12.9, *F* = 2.568	237	10	24.199	3.118	-

Results

The CCA's indicate that the variance that can be explained from environmental conditions is much lower for individual species than for any of the three classifications (Table 3). The percentage of explained variance was highest for the MBFG reaching 37.1 %, followed by phylogenetic and Reynolds classifications (Table 3). Not surprisingly in view of the different degrees of aggregation, total variation in the dataset (total inertia) decreased from species via Reynolds and phylogenetic groups to MBFG. Also, the proportion of the variance explained by the environmental variables decreased with increasing number of groups in the classification. However, the difference in explained variance between MBFG and phylogenetic groups was rather large (8.3 %) despite the fact that the second classification has almost the same number of groups. Furthermore, the difference in explained variance between phylogenetic and Reynolds was rather small (2.1%) despite the fact that the number of groups differed widely (21). Also, the number of variables included in the multivariate analyses was approximately 2 times higher for the Reynolds groups and species than for MBFG and phylogenetic groups but this did not result in a higher percentage of explained variance.

To correct for the effect of differences in the number of groups in each classification we also compared results for each classification to those for random aggregations with the same number of groups. The MBFG classification had the largest difference from a random

classification, while Phylogenetic and Reynolds groups were only slightly better related to environmental conditions than random aggregations (Table 3).

Qualitatively comparable results were obtained from our analysis of multiple linear regression models, predicting the biovolume of groups or species from the same independent variables. Note that now we obtain a model with an R^2 for each species and for each group in each classification, and study the structure in this large set of results. The differences in average R^2 values between classifications were significant (Kruskal-Wallis $H_{120,3}$ = 14.82, p = 0.002). Post-hoc multiple comparisons showed that the average values of R^2 for models predicting MBFG and Reynolds' groups were significantly higher than those of the individual species (z = 2.95, p = 0.02 and z = 2.82, p = 0.03 respectively) while phylogenetic groups were not significantly different from individual species. In line with the findings from our multivariate analyses, models for groups in all classifications had on average higher R^2 than models for individual species (Fig. 1) and the MBFG classification resulted in the highest R^2 values on average.

The frequency distribution of the R^2 values for different classification reveals that while the maximum R^2 values do not differ much, the proportion of relatively low R^2 values varies markedly (Fig. 2). Most of the groups in the MBFG classification (n = 7) can be well predicted from environmental conditions whereas most individual species have models with a rather low R^2. A detailed description of the distribution of R^2 values among the different groups in Figure 2 is included in Appendix I.

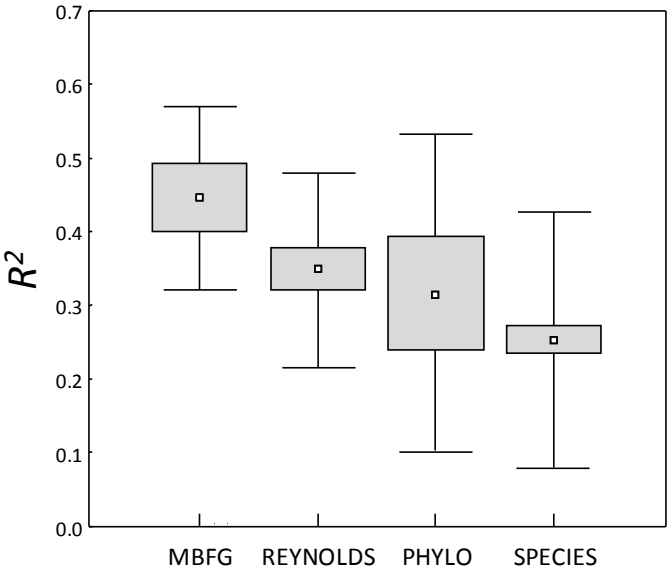
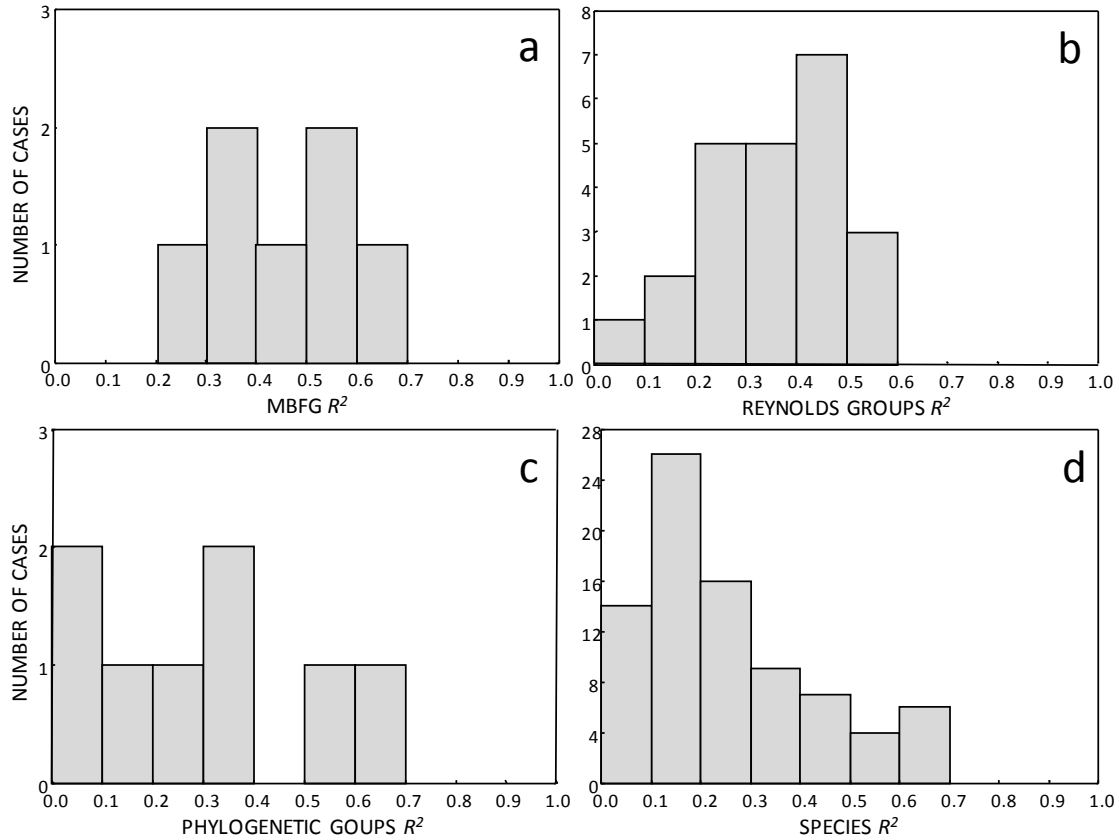


Figure 1. *The R^2 of multiple linear regression models for each of the studied phytoplankton classifications in relation to environmental conditions. Each classification is represented by a box-whisker plot. The dot is the mean of all the groups in the corresponding classification. Boxes correspond to the standard error and the whiskers to the standard deviation. MBFG: morphology-based functional groups; Reynolds: trait-based functional group following Reynolds; Phylo: phylogenetic groups and the species.*

***Figure 2**. Distribution of the R^2 of regression models for the groups in each phytoplankton classification against environmental conditions. a) morphology-based functional groups, b) Reynolds groups, c) phylogenetic groups, and d) species.*



Discussion

The emerging picture from the results of our regression analyses as well as the multivariate approach is that phytoplankton community composition may be predicted best in terms of morphological groups. The explained variance for individual species is much lower on average than that of any of the classifications (MBFG, phylogenetic and Reynolds groups). While, some species appear relatively predictable, a vast majority of the species shows no clear relationship to the environmental conditions we had measured.

The fact that morphological groups show a closer relationship to environmental conditions than phylogenetic groups suggests that morphology is a better proxy for ecological functioning than phylogeny. Put simply, our results suggest that two species that look alike but are phylogenetically unrelated appear more likely to be functionally similar than two species that are related but morphologically different. This is in line with a range of earlier findings (Huszar & Caraco, 1998; Kruk *et al.*, 2002; Salmaso & Padisák, 2007). Morphology of phytoplankton appears closely linked to ecological functioning (Weithoff, 2003; Naselli-Flores *et al.*, 2007), as shown in relation to physiological traits and demographic features,

as well as ecological performance (Margalef, 1978; Reynolds, 1984b; Marañón, 2008). By contrast, phylogenetic 'overdispersion' (Webb *et al.*, 2002) can lead closely related taxa to differ widely in functioning (e.g., Lürling, 2003).

Although in view of this phenomenon it may seem straightforward that local conditions favoring groups of species that share similar adaptive features can result in associations combining rather different phylogenetic origins (Reynolds *et al.*, 2002; Webb *et al.*, 2002; Salmaso & Padisák, 2007) this is not observed in all natural communities. In various terrestrial ecosystems the aggregation in phylogenetic groups rather coincides with natural species assemblages (Vamosi *et al.*, 2009). It has been suggested that in larger organisms, the occurrence of phylogenetic clustering may be caused in part by biogeographic history (Hillebrand, 2004; Cavender-Bares *et al.*, 2009). Indeed this seems less likely in phytoplankton which because of their small body-size have high rates of evolution and diversification (e.g., Nunn & Stanley, 1998; Schmidt *et al.*, 2006) and disperse easily, causing them to be largely cosmopolitan (Finlay & Clarke, 1999; Padisák, 2003; Fenchel & Finlay, 2004).

Obviously, some phylogenetic classes in phytoplankton are broader than others in their range of traits (Sandgren, 1988b; Graham *et al*., 2000). For instance, Cyanobacteria (Paerl, 1988) and Chlorophyceae (Happey-Wood, 1988) are found under a broad range of environmental conditions and display a wide range of growth forms ranging from small unicells to filaments with specialized cells. On the other hand, groups such as Chrysophytes have a more restricted environmental distribution and range of morphological variation (Sandgren, 1988a). This is reflected in our results in differences in predictability of groups from environmental factors. While the seven morphology-based groups are more or less equally predictable from environmental factors, the phylogenetic groups differ widely when it comes to the variance explained from environmental conditions. In fact even the 29 groups in Reynolds classification, are more homogeneous in terms of explained variance, than the eight phylogenetic groups.

In addition to being more closely related to environmental factors, the morphology-based classification has the advantage of being transparent and simple. Reynolds classification follows the traditional phytosociological approach (Tüxen, 1955; Braun-Blanquet, 1964) to describe aggregates of organisms that occur together and respond similarly to environmental changes. This approach has been well received and elaborated further by phytoplankton ecologists (Padisák & Reynolds, 1998; Padisák *et al.*, 2009). However, the criteria for assigning species to groups are not formalized, neither is the classification independent of phylogenetic affiliation. Also, the high number of groups in such refined classification systems (40 sensu Padisák *et al.*, 2009) may pose difficulties when it comes to

modelling their dynamics as it may be challenging to objectively assign parameter values for each group (Anderson, 2005; Le Quéré *et al.*, 2005).

On a more philosophical level the question of predictability of ecological community composition is related to some of the most profound issues in ecology. It has been argued that even with full knowledge of all species traits, it might be fundamentally impossible to predict phytoplankton development as dynamics may be ruled by chaos (Huisman & Weissing, 2001; Benincà *et al.*, 2008). Indeed, our results suggest that the majority of the phytoplankton species is rather unpredictable. Nevertheless, in line with what has been suggested by others (e.g., Elliott *et al.*, 2005; Litchman *et al.*, 2006; Mieleitner *et al.*, 2008) our results demonstrate that the occurrence of groups of species with similar traits is actually rather predictable from environmental conditions. One explanation might be that the morphology-based functional groups actually correspond to self-organized groups of similar species, in the sense of the theory of emergent neutrality (Scheffer & Van Nes, 2006). This would imply that while the biomass of such groups follows from environmental conditions, it will remain fundamentally unpredictable which of the species within such a group may dominate in any particular situation. This is because the species in any particular group are basically interchangeable and ecologically equivalent. While our results are consistent with that view, more refined analyses would be needed to test against predictions of other potential explanations (Vergnon *et al.*, 2009).

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Appendix I. *Description of the distribution of R^2 values among the different groups in each classification (Figure 2).*

To analyze the R^2 variance we compared the histograms of the R^2 values of the groups in each classification and among species (Fig. 2). The MBFG classification (n = 7) showed significant and well explained variances for most of its groups. The MBFGs were mostly well explained with six of the seven groups showing a R^2 higher than 0.3 **Group II** had a R^2 = 0.29 ($p < 0.001$, *df* =71) (Fig. 2A). The classification in major phylogenetic groups yielded significant models for all groups. Half of the phylogenetic groups showed R^2 values higher than 0.3 (Cyanobacteria, Chlorophyceae, Cryptophyceae and Bacillariophyceae) while the remaining half showed lower R^2. When the species were assembled with Reynolds classification, we obtained significant models for 23 out of the 29 groups, 15 reached R^2 values higher than 0.3 (Fig. 2D). The best explained groups corresponded to Cyanobacteria and included: filamentous non-N-fixing groups ($\mathbf{S_1}$: R^2 = 0.47, $p < 0.001$, *df* =71; and $\mathbf{S_2}$: R^2 = 0.53, p < 0.001, *df* =71), filamentous N-fixing groups ($\mathbf{S_N}$: R^2 = 0.33, p < 0.01, *df* =71; $\mathbf{H_1}$: R^2 = 0.55, p < 0.001, *df* =71), colonial large–celled groups (**M**: R^2 = 0.33, p < 0.01, *df*=71), and colonial small-celled groups (**K**: R^2 = 0.36, p < 0.001, *df* =71).

The extreme case was the species themselves; there were many low R^2 species and few high R^2 species (Fig. 2E). From the 237 species (5% dominant species in the 211 lakes) we obtained significant models for 82, 26 of them showed R^2 values higher than 0.3 (Fig. 2E), corresponding to the 11% of the total studied number.

CHAPTER 6. Trait-Based Approach Disentangles Core Features of Phytoplankton Succession

Angel M. Segura, Carla Kruk, Danilo Calliari & Hugo Fort

Abstract

1. Although trait-based approaches help to synthesize and to increase insight in community ecology, their use in describing how organisms are aligned within succession is scarce. Here we address this problem applying a recent morphology-based functional group classification (MBFG) of phytoplankton.

2. We construct a simple biogeochemical model and evaluate the competitive ability of each MBFG. Then we compare theoretical expectations with field data and model predictions.

3. We find a clear trade-off between maximum growth rate (μmax) and nutrient competitive ability for most MBFG. Then, we compare theoretical predictions of group performance to a well recorded phytoplankton succession and to records from 48 lakes. Groups with high surface/volume, no specialized cells and high μmax (opportunists) dominate at early stages. In contrast, large colonial and/or filamentous algae with lower μmax and specialized traits (gleaners), are the winners at late stages. Moreover, a biogeochemical model evidenced qualitative and quantitative agreement with empirical data**.**

4. This MBFG classification contributes to explain both ecological performance and phytoplankton community dynamics while reducing the complexity of phytoplankton communities in a mechanistic way. Despite incompleteness of the information (i.e. physiological rates for some species of each group) the groups have a common behaviour and show functional unity. Despite simplicity, MBFG classification allows to discriminate potentially noxious groups, configuring thus a powerful tool for managing lakes and ponds.

Introduction

Trait-based approaches have been increasingly applied in community ecology to predict anthropogenic effects in the environment and to evaluate general ecological questions (Lavorel *et al.*, 1997; McGill *et al.*, 2006; Litchman & Klausmeier, 2008). For example, the prediction of species timing of occurrence and biomass attained during succession is a problem that has puzzled ecologists and has a long history (Clements, 1916; Gleason, 1926). However, which is the best way to characterize and predict species presence or abundance remains unclear. A way through is to define functional groups and combine them in a mechanistic model to evaluate organisms performance (Litchman & Klausmeier 2008).

A recent proposal empirically combines two theories in the form of intrinsic growth and competitive ability to describe community succession and evaluate species performance (Litchman & Klausmeier, 2001; Litchman & Klausmeier, 2008). Competitive ability is based on the Resource-ratio theory (Tilman, 1982) and predicts winner species (gleaners) at late successional stages based on their capacity to survive at the lowest level of a limiting resource (R*). However, during early succession stages, fast growing species (opportunists) colonize first empty gaps (Tilman, 1994) or temporally out-compete late succession ones because of their high growth rates (Pacala & Rees, 1998). The combination of both theories predicts that distinct groups with contrasting functional traits dominate under different environmental conditions. However, empirical analyses of this approach are not common in literature (but see Litchman *et al.*, 2007).

The *morphology, performance and fitness* paradigm states that a set of morphological traits influences performance traits (*e.g.* growth rate), which in turn influence fitness (Arnold, 1983). Therefore it constitutes a useful framework to empirically evaluate ecological performance (sensu Violle *et al.*, 2007), and links organism individual traits with succession theory. Phytoplankton constitutes an ideal model to test these theoretical approaches. Small individual size makes community dynamics discernible for a human observer and allows performing whole community experiments (Reynolds, 2006; Litchman & Klausmeier, 2008). Moreover, well-defined morphological traits of phytoplankton individuals are directly related to their ecological performance (McGill *et al.*, 2006; Reynolds, 2006; Litchman & Klausmeier, 2008). However, phytoplankton high diversity and low predictability of its species (paradox of the plankton; Hutchinson, 1961; Huisman *et al.*, 2001; Benincà *et al.*, 2008; Dakos *et al.*, 2009) requires a reduction of the problem dimensionality before applying morphology-based approaches (Schaffer, 1981).

Within several modelling strategies (see Mieleitner *et al.*, 2008 for a review) reduction of dimensionality is generally achieved by clustering species according to their taxonomic affiliation, or by even less defined criteria (e.g. diatoms, greens and cyanobacteria; Litchman *et al.*, 2007). Such classifications are to some extent subjective and arbitrary and result in heterogeneous 'functional groups' in relation to their physiology and response to environmental forcing (Franks, 2009; Chapter 5). Another way to reduce dimensionality is to assume that some physiological characteristics are a power law of organism size (e.g. Follows *et al.*, 2007). However, certain traits (e.g. cells organization in colonies) alter the expected power law relationship (Nielsen, 2006; Reynolds, 2006) and may even disrupt the relationship between growth rate and size (Marañón, 2008).

In a novel approach Kruk *et al.* (2010) developed a morphological classification of freshwater phytoplankton based on nine morphological traits (e.g. cell volume, surface, presence of aerotopes). Traits selected are well defined, easy-to-measure, independent from environment and applicable without reference to taxonomy (Kruk *et al.*, 2010). The resulting classification consists on seven morphology-based functional groups (MBFG; Table 1) that are statistically different in their morphology, physiological rates (*i.e.* growth rate) and abundance patterns in the field (Kruk *et al.*, 2010). Furthermore they have been shown to be well predicted from environmental variables such as temperature and nutrients concentrations (Chapter 5).

In this article we link organisms' individual morphological traits with succession theory with two main objectives. The first one is to validate the functional unity of functional groups based only in morphology. The second is to construct a way of expressing ecological performance and analyse community succession based on morphology. To do so, we calculated the R* for each MBFG based on a simple biogeochemical model with only three mechanisms, namely growth, sinking and grazing. We applied the average values of the existing restricted information for each MBFG (i.e. physiological rates only for some species of the group) to the whole group. Then the resulting R* were compared in a gradient of losses and the trade-off in the growth-competitive ability space analysed. We then classified the species into MBFG, and contrasted theoretical and model predictions with the succession recorded in a subtropical shallow lake and a snapshot sampling from 48 temperate-subtropical shallow lakes. For the comparison we considered the temporal replacement and biomass levels attained by different MBFG, the ecological traits of the dominant MBFG and their richness at different stages of the succession. MBFG appeared as a simple, robust and useful tool to represent the temporal dynamics of functional diversity and to gain insight into phytoplankton community ecology.

Methods

Basics of the morphology-based functional groups (MBFG)

A comprehensive description of the MBFG is provided in Kruk *et al*. (2010). A summary of main characteristics and representative taxa for each MBFG is provided in Table 1. Maximum growth rate and sinking velocity were obtained independently from an extensive literature review (Kruk *et al.*, 2010). Maximum growth rate (μ_{max}; day^{-1}; N= 59) was obtained from laboratory experiments under similar protocols, including comparable temperature (18-25^{o}C) and saturating light conditions. Sinking velocities (*s;* m day^{-1}; N= 87) were obtained both from mesocosm and laboratory experiments. Species were then classified into MBFG, and rates were averaged for each group.

MBFG ecological performance

Nutrient competitive ability (R*) can be estimated measuring the concentration (R) of the resource in a steady state monoculture, or derived from the species growth kinetics (Tilman, 1982). We derived R* for each MBFG based on its growth kinetics. For simplicity, growth dependence upon external nutrient concentration (R; $mgPO_4L^{-1}$) was characterized using the classical Monod form with half-saturation concentration (*k;* $mgPO_4L^{-1}$):

$$netgrowth = \mu_{max}\frac{R}{(R+k)} \qquad (1)$$

where μ_{max} is an average value of empirically derived maximum growth rates for Group i (Table 1).

Considering such nutrient uptake model and adding phytoplankton losses and nutrient dynamics, we have a simple one-nutrient seven-phytoplankton model given by:

$$\begin{aligned}\frac{dP_i}{dt} &= P_i\left[n\mu_{maxi} - (m_i G + s_i z^{-1}) - f\right] \\ \frac{dR}{dt} &= (Ro - R)^* f - \left[\sum_{1-7} P_i\left[n\mu_{maxi} - (m_i G + s_i z^{-1})\right]\right] * Q\end{aligned} \qquad (2)$$

Where the index i, running from 1 to 7, denotes the seven MBFG, P_i =phytoplankton biovolume ($mm^3\ L^{-1}$) of MBFG i, μ_{maxi} , s_i are group's averages of empirically derived growth (day^{-1}) and sinking velocities (m day^{-1}), *z* is the depth of the lakes (*z*= 2 m), *f* is the flushing

rate (day^{-1}, fraction the whole lake water and phytoplankton flowing out per unit time), k (mg PO_4L^{-1}) is the half saturation constant for nutrient uptake; k was set equal among groups (k= 0.007 mg PO_4L^{-1}) as the average of empirically determined values, and similar to values previously used in algal competition models (*e.g.* Scheffer et al. 1997). The term m_iG represents the biomass removed by predation (*i.e.* zooplankton grazing). Grazing susceptibility in phytoplankton organisms is inversely related to maximum linear dimension (MLD; Reynolds 2006). We modelled predation as the grazing susceptibility (m_i=MLD_i^{-1}; Table 1) times an arbitrary group independent grazing pressure factor G representing the overall zooplankton grazing pressure of the system. In Group VII, m_7 was arbitrary set to the minimum of all groups since it posses the ability to generate mucilage and some of the component species potentially produce toxins that act as grazing deterrents (Reynolds, 2006), and is five orders of magnitude larger in volume than the other groups (Table 1). *Ro* is the nutrient concentration that enters into the system ($mgPO_4L^{-1}$). Instantaneous remineralisation of dead phytoplankton is assumed. Internal phytoplankton nutrient content was estimated assuming the carbon to phosphorus Redfield ratio (C:P = 106:1) and shields an empirically derived currency constant (Q = $5x10^{-3}$ mg PO_4 $L^{-1}mm^{-3}$) (Reynolds, 1984a pg. 31).

For the given biogeochemical model, R* can be expressed as:

$$R_i^* = \frac{(s_i z^{-1} + f + m_i G)k}{\mu_{\max i} - (s_i z^{-1} + f + m_i G)} \qquad (3)$$

where i goes from one to seven representing each MBFG, μ_{maxi}, s_i, z, f, m_iG and k are the same as in eqn 2. We compared R* among groups in a gradient from stable to highly perturbed (f from 1000^{-1} to 5^{-1} day^{-1}) and low to high grazing mortality environments (G from 0 to 10).

To explore possible trade-off in the growth-competitive ability space (μ_{max} vs. R*) grazing pressure (G in eqn 3) was set to three, in order to represent average groups mortality losses similar to experiments in chemostats (mortality = 0.01-0.015 h^{-1}; Huisman *et al.*, 2001; Litchman & Klausmeier, 2001; Passarge *et al.*, 2006). The overall relationship between μ_{max} and R* holds to moderate deviations in G (not shown).

Table 1. *Morphological description, representative taxa and physiological parameters of the seven morphology-based functional groups (MBFG). Average maximum growth rate (μ_{max}); sinking rate: s; volume: V and median maximum linear dimension: MLD used in eqns 2 and 3. The presence (1); absence (0) of: aerotopes (Aer); flagella (Fla); mucilage (M) and heterocysts (Het) are shown.*

MBFG	Description	Representative taxa	μ_{max} day^{-1}	*s* $mday^{-1}$	V μm^3	MLD μm	Aer	Fla	M	Het
I	Small organisms with high S/V	*Chlorella minutissima* Fott & Nováková, *Scenedesmus ellipticus* Corda	2.08	0.03	12.9	5	0	0	0	0
II	Small flagellated organisms with siliceous exoskeletal structures	*Chromulina gyrans* Stein, *Dinobryon cylindricum* Imhof.	0.54	0	626	7.3	0	1	0	0
III*	Large filaments with aerotopes	*Anabaena crassa* (Lemmermann), *Cylindrospermopsis raciborskii* Wołoszyńska,	0.67	0	1541	90	1	0	0	1
IV	Organisms of medium size lacking specialized traits	*Coelastrum microporum* Nägeli, *Scenedesmus quadricauda* (Turpin)	1.49	0.14	1543	21.8	0	0	0	0
V	Unicellular flagellates of medium to large size	*Cryptomonas ovata* Ehrenberg, *Ceratium hirundinella* (Müller) Bergh	0.89	0.192	2444	11.6	0	1	0	0
VI	Nonflagellated organisms with siliceous exoskeletons	*Acanthoceros zachariasii* (Brun) Simonsen, *Aulacoseira granulata* (Ehrenb.)	0.93	0.68	3143	34	0	0	0	0
VII*	Large mucilaginous colonies	*Aphanocapsa delicatissima* West & G.S. West, *Microcystis aeruginosa* (Kützing)	0.94	0.078	43152	19†	rare	0	1	0

*Groups with potential negative effects on water quality

† Grazing susceptibility (*m;* MLD^{-1}) of Group VII was set equal to the MLD of the Group III.

Characteristics of Lake Rodó and data gathering

Lake Rodó is an urban artificial shallow lake (mean depth= 1.7 m) which was subjected to a management programme (for details of the lake and management actions refer to Scasso *et al.* (2001). Water and sediments were removed in January 1997. After that, water was pumped to the lake at a constant rate giving a residence time of 43 days (f= 0.02 day^{-1}). Phosphate content of the pumped water was nearly 0.1 mgL^{-1}, which was the only input of phosphate to the system in the studied period. Water samples were collected from the deepest part of the lake at weekly intervals (January–April, 1997) and then biweekly until August 1997. Water samples were taken just beneath the surface of the lake and close to the bottom using a Ruttner bottle. For more details on sampling and sample analysis procedures see Kruk *et al.* (2002).

Phytoplankton sampling and analysis

Integrated samples for phytoplankton counts were collected and preserved in Lugol's iodine. Phytoplankton units (cells and colonies) were enumerated in random fields using the settling technique (Utermöhl, 1958). We examined the samples at multiple magnifications and counted until we reached at least 100 individuals of the most frequent species. Organism dimensions, including MLD were measured. Individual volume (V) and surface area (S) were calculated using geometric approximations (Hillebrand *et al.*, 1999). Population biomass was estimated as biovolume and calculated as the individual volume of the species multiplied by the concentration of individuals. For colonial organisms with mucilage, V and S calculations were made for whole colonies including mucilage. Presence of aerotopes (Aer), flagella (Fla), mucilage (Muc), heterocysts (Het) and siliceous exoskeletal structures (Si) were noted for each relevant organism. Species contributing >5% to the total biovolume were grouped in the morphology-based functional groups (MBFG) defined by Kruk *et al.* (2010).

In order to compare modelled temporal simulations with Lake Rodó observed dynamics, water column depth (z= 1.7 m), flushing rate (f= 0.02 day^{-1}) and allochtonous nutrient inputs (Ro= 0.1 $mgPO_4L^{-1}$) were set according to empirical values. Physiological parameters μ_{maxi}, s_i, m_i and k were set to physiological averaged MBFG values (Table 1), and grazing pressure factor (G) was set to three (see above). The model was run for 210 days (January-August) seeded with small inoculum of the seven MBFG ($P_{1\text{-}7}$= 0.01 mm^3L^{-1}), and an initial phosphate concentration R_{in}= 0.5 $mgPO_4L^{-1}$.

MBFG biomass and richness in middle and late succession

We analysed a total of 48 South American subtropical-temperate shallow lakes sampled once in the growing season between November and March (Austral Spring-Summer). Lakes were sampled at random points covering the whole lake area. Water samples for nutrients and plankton were taken integrating the water column with a PVC tube (30 cm diameter) and combining at least 3 random replicates in each lake. Phytoplankton samples were fixed in Lugol's solution and analysed with the same protocol as Lake Rodó. Zooplankton samples were filtered on a 50 µm sieve and preserved in a 4% formaldehyde solution. Temperature (T) was measured *in situ* using a conductivity-temperature meter. Reactive phosphorus (SRP) (Murphy & Riley, 1962) and total phosphorus (TP) (Valderrama, 1981) were determined. Zooplankton abundance was expressed in individuals per litre. For detailed description of sampling procedures and analyses see Kosten *et al.* (2009) and Kruk *et al.* (2009).

These lakes were grouped in middle or late succession according to the time spent since the beginning of the growing season (austral spring). That is to say, lakes sampled in December or January were classified as Middle succession while lakes sampled in February or March were termed Late succession lakes. From now onwards, we refer to them as Middle and Late respectively. Difference in days between Middle and Late sampling was on average 44. We calculated for each period the average groups biomass and its standard deviation as well as the number of dominant groups (*i.e.* groups accounting for more than 5% total biovolume), which we called group richness.

We compared lake snapshot-samplings with results from the biogeochemical model using a Monte Carlo approach. To compare Middle and Late succession data with model results we ran the biogeochemical model with random starting biomass and parameters 1000 times for 365 days. The model was seeded with the seven group's biovolume taken at random from an uniform distribution from zero to five (P_0= uniform [0, 5] mm^3L^{-1}). Average of model starting biovolume were not different from Middle empirical biomass values (*t* test; *df* =126; *p*= 0.25). Initial model nutrient concentration was chosen to match that of the Middle lakes from a uniform distribution between 0.1 and 0.2 (R_{in}= uniform [0.1, 0.2] $mgPO_4L^{-1}$). In order to account for the increase in total system biomass between Middle and Late lakes ($mean_{Middle}$= 0.315; $mean_{Late}$= 0.786 $mgPO_4L^{-1}$; *t* test, *df*=46; *p*=0.02) we fixed Ro= 0.05 $mgPO_4L^{-1}day^{-1}$. Depth was fixed to mean lakes depth (z= 2 m). Flushing rate of the model was variable among simulations but constant during each run and chosen at random from a uniform distribution to represent usually recorded flushing rates in lakes (*f*=uniform [20^{-1}, 1000^{-1}] $days^{-1}$; Reynolds, 2006). In each of the 1000 runs, we averaged the biovolume of MBFG in two time windows, from model days 1 to 50 (*i.e* model middle succession) and from days 95 to 145 (*i.e.* model late succession). Finally, we generated two (1000 x 7) output matrices that comprised the average Middle and Late modelled values for the seven MBFG biovolumes. Those values were used to compare with the set of biovolumes from the observed 48 lakes classified as Middle and Late. The 45 days difference between model averaging windows was chosen to account for the difference between mean sampling periods in the empirical data. The model was moderately robust to variations in the time windows used to average the results and to the number of days considered to average values (not shown).

Community structure

We described community structure in the empirical and modelled data in Middle and Latte succession. We estimated MBFG richness as the number of dominant groups (see above) and the Simpson diversity index D:

$$D = \frac{1}{\sum p_i^2} \quad (4)$$

where p_i denotes the proportion of MBFG i in the sample. We then compared richness and diversity between Middle and Late succession in empirical and model data by means of a t test.

Results

MBFG ecological performance

We first compared the R* among groups in a gradient of losses. **Group III** and **Group VII** out-competed other groups over a wide range of conditions (*i.e.* they presented lowest R*) in shallow lakes (Fig. 1) and can be characterized as gleaners. However, at low grazing pressures or relatively high flushing rates groups with highest growth rates (opportunists, **Group I** and **IV**) were able to dominate.

There was a clear indication of a compromise between competitive ability (R*) and maximum growth rate among groups **I**, **III**, **IV** and **VII** (Fig. 2). The other groups (**II**, **V** and **VI**) were not good competitors and had moderate to low growth rates.

Temporal succession in a shallow hypertrophic lake

Phytoplankton sampling began 25 days after refilling in Rodó Lake. Phytoplankton temporal sequence of species replacements responded to an autogenic succession from opportunist selected towards gleaner selected groups characteristic of a shallow eutrophic lake (Fig. 3a). Within the first days, phytoplankton biomass remained low (< 20 mm^3L^{-1}) and with presence of **Groups I**, **IV** and **VII**. Then the recorded temporal replacement was followed by the clear dominance of **Group IV** (*i.e.* organisms of medium size without specialized traits), followed by **Group VI** (mainly diatoms) and **Group VII** (large mucilaginous colonies) with the final dominance of **Group III** (large filaments with aerotopes). The simple biogeochemical model was able to reproduce well this succession (Fig. 3b). Main features of the phytoplankton dynamics were common to the observed and the modelled ecosystem. The modelled succession started with the initial dominance by **Group I**. **Group I** declined rapidly leading to a mixed dominance of **Groups IV** and **VII** after which **Group III** strongly excluded all other groups (Fig. 3b). The only important event that the model did not predict was one of the succession transitional groups (**Group VI**).

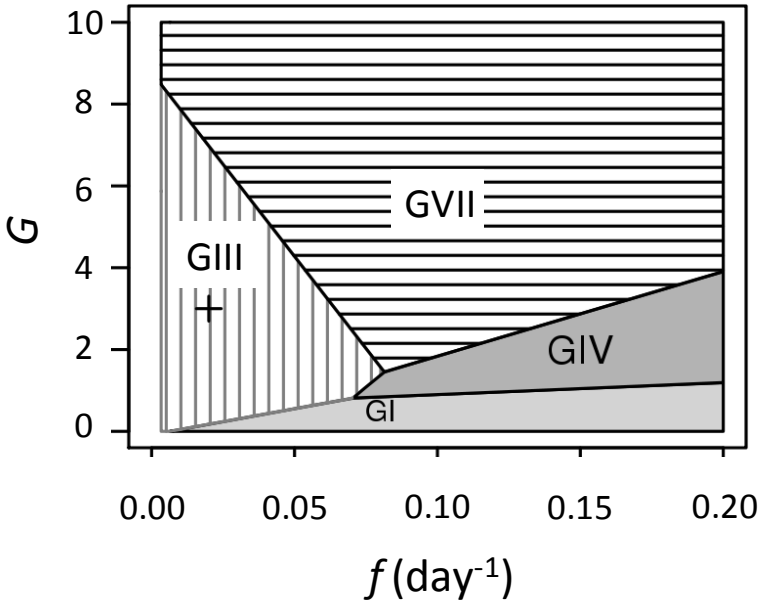

Figure 1. *Response surface of the morphology-based groups competitive ability (R*; calculated as in eqn. 3) in a gradient of grazing pressure (G) and flushing rate (f). The cross indicates the area with similar characteristics to Lake Rodó-Uruguay.*

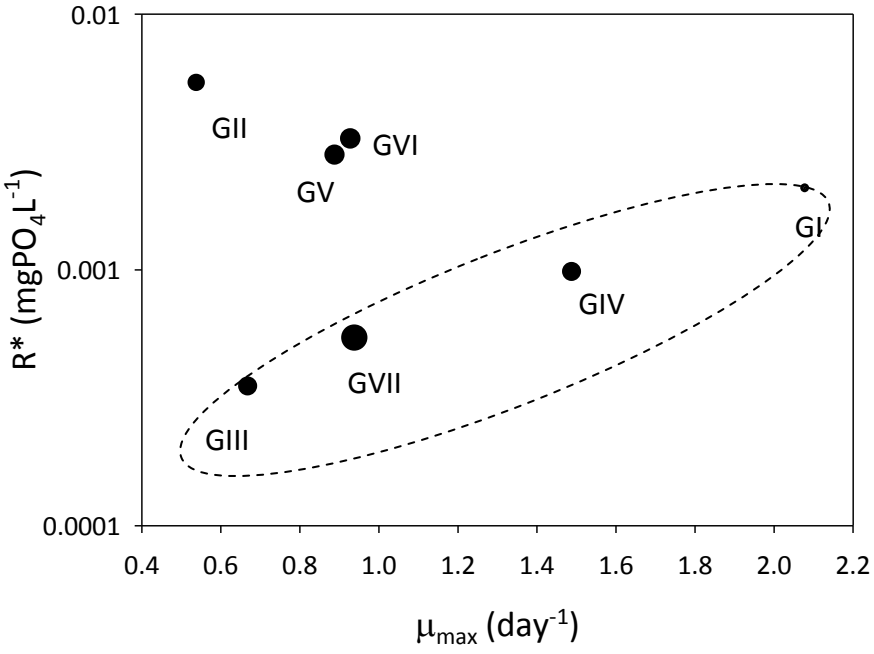

Figure 2. *Growth-Competitive ability space (R*; calculated as in eq. 3 vs. maximum growth rate (μ_{max}). Note that axes y is in logarithmic scale. Symbols are proportional to the logarithm of average MBFG volume. Dashed ellipse shows the groups aligned in the gleaner opportunist trade-off. Parameters of eq. 3 are: G= 3; k= $7x10^{-3}$ mg PO_4L^{-1}; f= 0 day^{-1}; z= 2 m; s and μ_{max} from Table 1.*

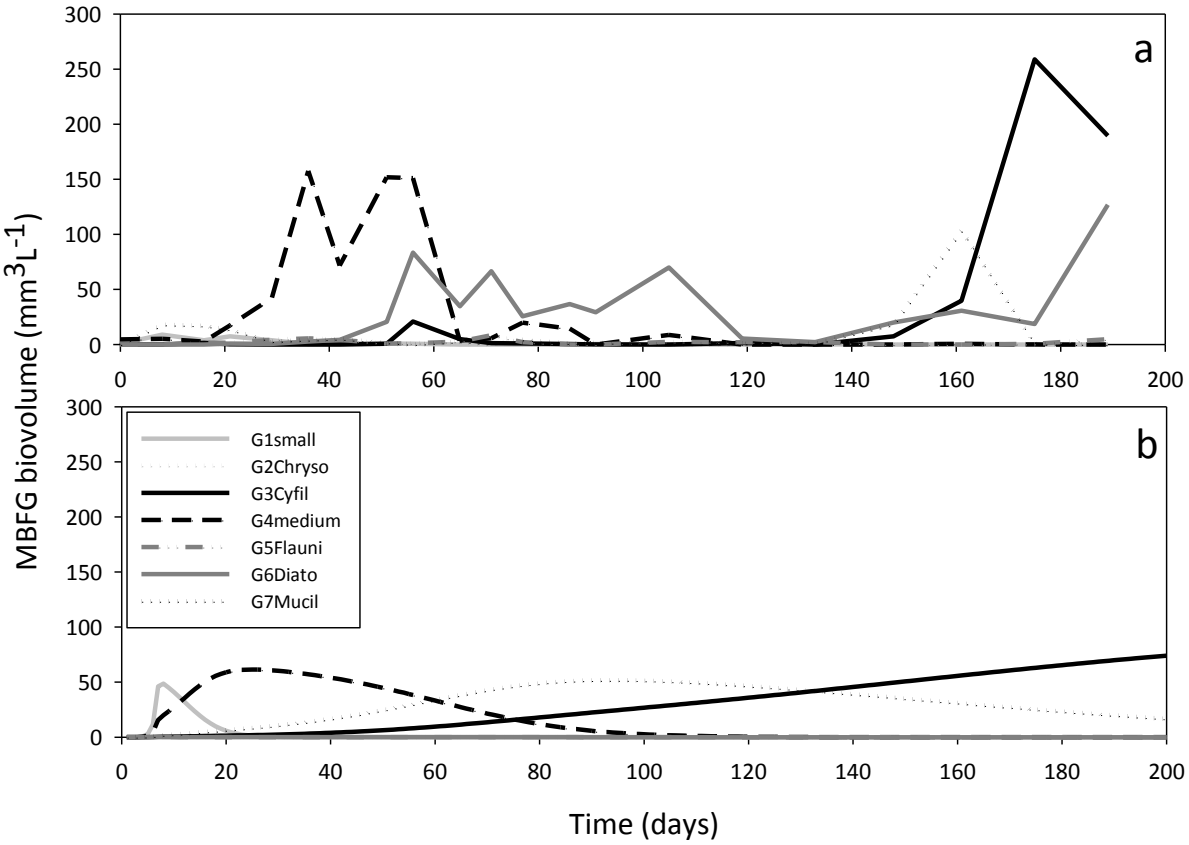

Figure 3. *(a) Biovolume of the morphology-based functional groups of the phytoplankton succession in Lake Rodó (Uruguay) after complete removal of water and sediments in January. (b) Modelled succession (as eq. 2); initial parameters: R=0.5 $mgPO_4L^{-1}$; $P_{1\text{-}7}$=0.01 mm^3L^{-1}; z= 1.7 m; f= 0.02 d^{-1}; G= 3; Ro= 0.35 mgL^{-1}.*

Table 2. *Average and standard deviation of main environmental variables, group richness (GR) and Simpson index (D) for Middle and Late succession in subtropical-temperate shallow lakes (empirical) and modelled data (modelled). Degrees of freedom (df) and p-level of a two sample t test between Middle and Late.*

Variable	Middle	Late	*df*	*p-level*
Water Temperature (°C)	23.0 (5.0)	21.0 (5.8)	42	0.21
Lake Area (Km^2)	428 (1219)	439 (1592)	44	0.97
Total Nutrients*	0.315 (0.44)	0.786 (1.10)	46	0.02
Total Zooplankton (ind L^{-1})	1252 (3026)	2449 (2188)	42	0.15
$GR_{Empirical}$	3.29 (1.30)	2.65 (1.31)	46	0.0513
$GR_{Modelled}$	3.63 (0.54)	2.10 (0.71)	1998	<< 0.001
$D_{Empirical}$	2.31 (0.87)	1.85 (0.86)	46	0.0398
$D_{Modelled}$	2.91 (0.54)	1.51 (0.51)	1998	<< 0.001

* It represents the sum of phosphate concentration plus total phytoplankton nutrient content. See text for calculations.

Biomass and richness in Middle and Late succession in subtropical-temperate lakes

In the field data of the 48 lakes, group richness and diversity were higher in middle than in late succession (Table 2). Model simulations captured well the decrease in group's richness

and diversity towards the late successional stage (Table 2). In the field data and during middle succession **Groups I** and **III** dominated, while the rest of the groups presented low biovolumes (Fig 4a). In Late succession **Group III** and **VII** were clearly dominants in biovolume (Fig 4c). Qualitative patterns of group dominance in Middle and Late succession were in accordance between modelled and empirical data for most MBFG (Fig. 4). Furthermore, the model captured the biomass pattern in Late succession correctly while not so in Middle succession (Fig. 4b and d).

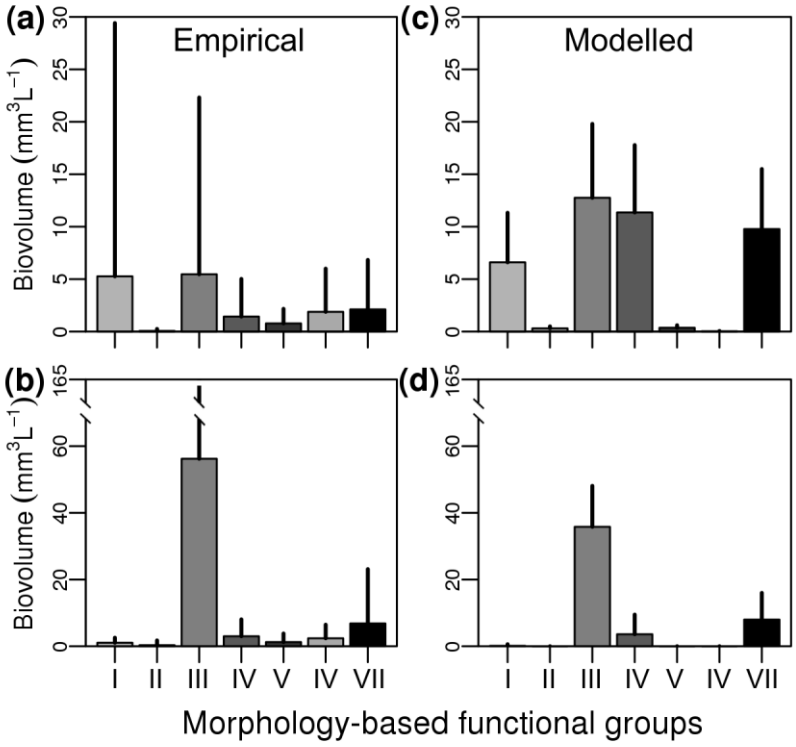

Figure 4. *Mean morphology-based functional groups biovolumes (bar=mean; whisker=SD) in the field (a, b) and modelled (c, d) in a middle (a, c) and late (b, d) succession. Succession stage for lakes was defined by the date lakes were sampled and in the model as the averaged biovolume between day 1 and 50 (Middle) and 95 to 145 (Late).*

Discussion

Here we demonstrated that the MBFG show concordance between theoretical expected successional patterns with model and field results that support a functional unity of this classification. Furthermore, the relevance of including a combination of morphological traits to classify organisms is reinforced coincidently with previous results (Kruk *et al.*, 2010). A clear trade-off was identified with ecological performance that leads to the dominance of organisms with particular traits (represented here by MBFG) during different stages of community succession. Thus the MBFG classification was instrumental to reduce

the dimensionality of this classical problem (*i.e.* unpredictability of phytoplankton species) and useful to gain insight into mechanisms driving phytoplankton community structure and dynamics.

Morphological traits summarize ecological performance

The extent of the winning surface (lower R*) of the groups in a gradient of losses (grazing and flushing) were well in accordance with expected patterns. Gleaners (Groups III and VII) succeeded under high grazing and low-medium losses according with the *resource-ratio theory* (Tilman, 1982).

The great extent of the 'winning' surfaces of Groups III and VII (Fig. 1) reflect the combination of morphological traits that define these groups: large filaments and colonies, respectively. Thus, their losses due to predation are diminished. Further, these groups have specialized cells and structures (*e.g.* aerotopes, heterocysts, mucilage) that make them successful competitors. For example, aerotopes and heterocytes allow controlling their position in the water column and fixation of N_2, respectively, which diminish their losses due to sinking and improve competitive ability for light and nutrients (Huisman *et al.*, 2005). However, these large-sized specialized organisms are constrained in their maximum growth rates. That excludes them from dominating at the beginning of the succession, or in highly perturbed (high flushing rates) environments where they are out-competed by Groups I and IV. Further, final dominance of Groups III and VII is in agreement with observations in several ecosystems. Group VII (e.g. *Microcystis spp.*) usually dominates at the end of succession in enclosures and lakes (Ganf & Viner, 1973; Huisman *et al.*, 2005; Reynolds, 2006). While other set of lakes are typical representatives of Group III dominance (Berger, 1975; Scasso *et al.*, 2001). Furthermore, the dominance of Group III is in accordance to the third stable state hypothesis for shallow lakes (Scheffer *et al.*, 1997).

Groups I and IV represent more opportunistic strategies. They invest energy in growth rather than in specialization. This is especially true for Group I constituted by very small-sized organism with high S/V ratio, which favours nutrient acquisition and high growth rate. However, their small size makes them very susceptible to grazing (Reynolds, 2006). Dominance of Group I in lakes with high flushing rates (close to the riverine flushing rates) supports the idea of the *successional niche* hypothesis (Pacala & Rees, 1998) that predicts out-competition of other groups that are not able to increase in biomass in highly perturbed environments. The conditions of high biomass losses and low grazing pressure under which Groups I and IV win are typical of early succession stages. In these early stages

grazing by zooplankton is generally not severe because zooplankton populations do not reach significant abundances (Sommer *et al.*, 2003; Reynolds, 2006).

The Growth-Competitive ability space: indicator of the gleaner-opportunist trade-off

Groups with high growth rates (Groups I and IV) were not the best competitors whereas good competitors (Groups III and VII) were not fast growing species. The trade-off between R* and maximum growth rate inhibits the existence of 'super species' (*sensu* Litchman *et al.*, 2007) that would lead to exclusion of other groups under any environmental condition.

The gleaner-opportunist trade-off gradient was accompanied by a decrease in the number of specialized traits. Better competitors presented a higher number of traits while opportunist did not. However, there was not a clear gradient associated to cell volume. For example, Group IV characterized here as opportunist and Group III, which is in the gleaner side of the spectrum have similar individual biovolume (Table 1). That supports the idea that using a single trait (*e.g.* cell volume alone) is not enough to predict the organism ecological strategy within a succession.

In the competitive ability-growth space we can separate the non-aligned groups into those that are not expected to dominate because they do not do so in nature in the modelled conditions (Group II and Group V), and those that are expected to dominate but did not in our model (Group VI). Within the formers, Group II and V include flagellates with potential heterotrophy and resistance cells that generally dominate in oligo to mesotrophic conditions or in clear-water plant dominated lakes (Reynolds *et al.*, 2002). Their traits would allow them to thrive in low nutrient-high light vertically heterogeneous habitats through their movement in the water column and use of organic compounds (Sandgren, 1988b). Our set of lakes and model represent shallow meso-hypertrophic turbid conditions in which nutrient related mechanisms dominate, whereas the structure of clear water systems is apparently controlled by other mechanisms (Karlsson *et al.*, 2009). So the dominance of these groups is not expected under the conditions here evaluated. Opportunists groups did so in highly perturbed low grazing conditions in agreement with the *successional niche* hypothesis (Pacala & Rees, 1998; Rees *et al.*, 2001).

Group VI, composed mainly by diatoms can actually dominate in natural systems. In our model formulation Group VI appeared as a poor competitor because of its enormous sinking rate caused by their heavy siliceous wall. If we emulate a well-mixed aquatic system (no sinking conditions), their competitive ability improves significantly: under vertically mixed conditions Group VI gets close to Group VII in the Growth-competitive ability space. Real lakes face alternation between periods of high stratification (strong

sinking conditions) and intense mixing (no sinking conditions) which may eventually allow dominance by this group (Reynolds, 2006). Water mixing should be considered (explicitly or implicit) in future model formulations to explore its effect on Group VI dynamics and overall functional diversity.

Succession dynamics in a hypertrophic shallow lake

The successional replacement of groups initially represented by Groups I and IV and the final dominance of groups VII and III is well in agreement with the *colonization-competition* trade off hypotheses (Tilman, 1994; Rees *et al.*, 2001) and the Tilman's *Resource-ratio* theory (Tilman, 1982). The final establishment and dominance by Group III agrees with observations in lake Rodó since 1992 (Sommaruga, 1995; Scasso *et al.*, 2001; Kruk *et al.*, 2002). Such patterns were adequately reproduced by the simple biogeochemical model despite it only included three mechanisms (growth, sinking and grazing).

Subtropical-temperate lakes during Middle and Late succession

The identity of dominant MBFG observed in our set of lakes in Middle and Late succession were in accordance to expected patterns (e.g. Sommer, 1989; Reynolds, 2006). Biomass levels, MBFG richness and diversity were also captured by our biogeochemical model. Those evidences suggest that the MBFG classification considers most important traits, and that the mechanisms included in the model are relevant drivers of succession in these environments. It is remarkable that the match in observed and modelled patterns with those theoretically expected occurred despite the set of observed lakes were highly heterogeneous (Table 2) and differed in their past histories.

Conclusions

The MBFG classification diminishes the high dimensionality of a typical phytoplankton community while keeping the system mathematically tractable. We recognize this is not the first attempt to understand phytoplankton community dynamics by grouping species based on individual traits (e.g. Margalef, 1978; Reynolds *et al.*, 2001). However, this is a step forward from classical schemes (e.g Mieleitner *et al.*, 2008) towards simple morphology-based functional groups (Kruk *et al.*, 2010) with a potential of future translation into performance currencies (McGill *et al.*, 2006). The MBFG classification is universal as all species can be objectively classified into MBFG according to a set off *a priori* defined morphological traits. Also, model flexibility could easily accommodate refinement, like the inclusion of temperature-dependence of growth or other physiological ratios in future model formulations. Despite pooling species in groups, MBFG allow to discriminate

potentially noxious groups, configuring thus a powerful tool for understanding and managing phytoplankton communities in a global changing world.

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CHAPTER 7. Synthesis

The work presented in this thesis all revolves around the question which aspects of phytoplankton communities are most predictable in the sense that they are related in a systematic way to environmental conditions. The ultimate goal is a practical one: predict when and where high densities may arise of particular species that are known to pose problems to water quality, and find ways to avoid such blooms. While prediction of the biomass of individual species is the most refined and notoriously difficult level of detail to aim for, prediction of the total biomass or chlorophyll-a concentration is at the other end of the range of level of detail (McCauley & Murdoch, 1987; Scheffer *et al.*, 2003). There is a long tradition of predicting total algal biomass from environmental conditions, and results are often quite good (e.g. Vollenweider, 1976). However, the character of the species that form the biomass, and hence the potential associated problems such as toxicity is more difficult to predict (Huisman & Weissing, 2001; Benincà *et al.*, 2008).

Phytoplankton communities as functionally filtered subsets

The thesis explores predictability of a range of phytoplankton communities' aspects: species richness (Chapter 2), the occurrence and biomass of individual species (Chapter 3), and the biomass of different groups of species (Chapters 4, 5 and 6). The results reveal that while species richness and the occurrence of individual species are more-or-less predictable, the predictability of the biomass of individual species varies widely. Only a small fraction of the species is predictable, in the sense that more than 40% of the variance in their biomass can be explained statistically from environmental conditions (Chapter 3). When species are grouped, the predictability of the biomass that different groups form obviously depends on the way they are grouped (Chapter 5).

The centre piece of this thesis is an approach to categorize species in functional groups on the basis of solely their morphological traits (Chapter 4). As such traits are known, this approach allows an objective categorization of all species. However, it is not *a priori* obvious whether the morphology would indeed capture functionality well enough for this approach to make sense. Three lines of evidence suggest that morphological groups may indeed be considered to represent functional groups. Firstly, for a subset of the species direct measurements of functional traits such as sinking rates, nutrient half-saturation constants and growth rates were available, and this allowed us to verify that the morphological groups did indeed tend to contain species that are functionally similar (Chapter 4). Secondly, the morphology-based functional groups (MBFG) turn out to be

more predictable from environmental conditions than random groups, taxonomic groups, or functional groups from a well known system based on expert judgement (Chapter 5). Finally, model predictions of a simple model based on the available physiological rates for each MBFG were consistent with patterns found in the field (Chapter 6).

These results suggest that the morphology-based functional groups do indeed contain functionally similar species, and also that the total biomass of such a functional group tends to be more predictable than the biomass of individual species. One interpretation of this pattern could be that species within these groups are functionally relatively equivalent and therefore interchangeable. This would be consistent with the view that phytoplankton has self-organized over evolutionary time-scales to form groups of similar species, and that within these groups competition is basically neutral (Scheffer & Van Nes, 2006).

In summary, we might think of phytoplankton communities as subsets of an omnipresent pool of cosmopolitan species selected by local environmental conditions (Fig. 1). The selection process would work on functional groups, whereas the relative importance of particular species within such groups could be essentially random.

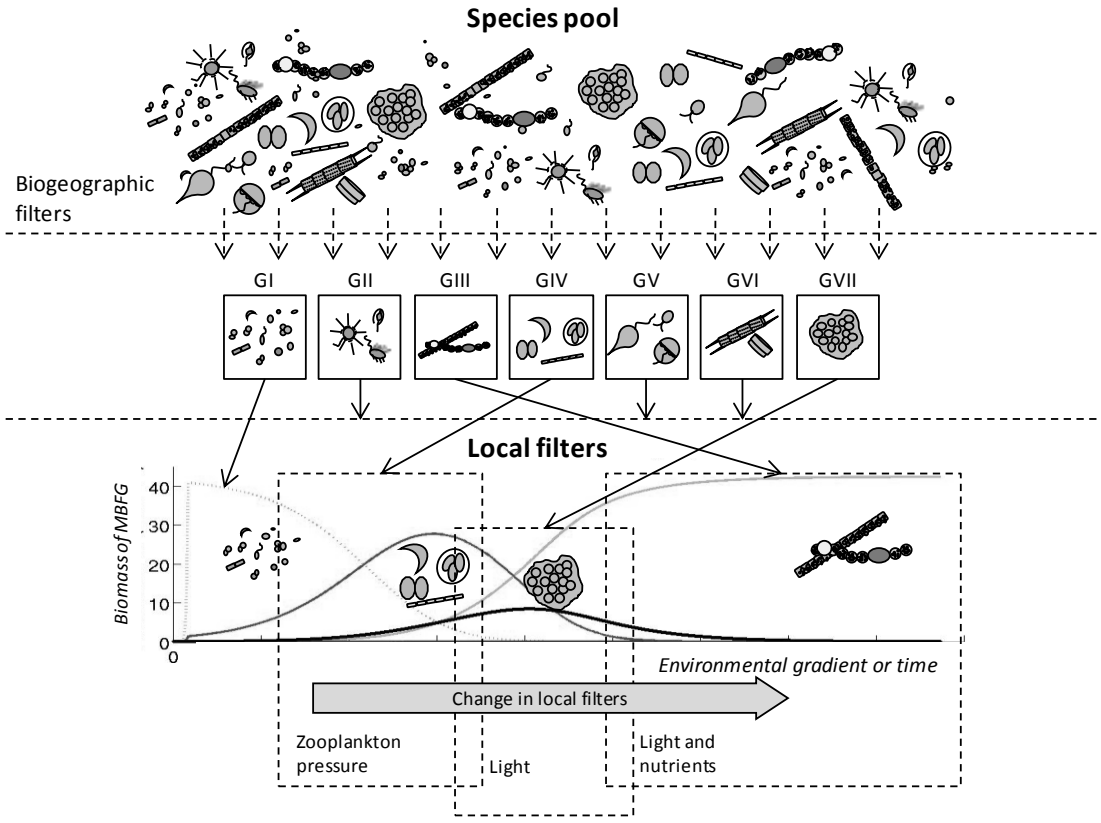

Figure 1. *Schematic representation of the hypothesis that phytoplankton communities at any given time or place represent a subset from an omnipresent global species pool that is determined by the environmental conditions that act as local 'filters'. The example of the autogenic succession in a eutrophic shallow lake is presented.*

Towards a deeper understanding

While the idea of phytoplankton communities as functionally filtered but internally neutral subsets is a fascinating idea, such abstract worldviews also necessarily capture only part of the complexity of real communities. Probably, if we aim at predicting specific nuisance species blooms, we will have to probe deeper into the mechanisms ruling the dynamics of algal communities. Indeed, while it is encouraging that morphology-based functional groups appear to be relatively predictable, it still makes a lot of difference which species will dominate in some of the groups, as features such as toxicity and edibility may still differ quite a bit within groups (Chorus & Bartram, 1999; Sterner & Elser, 2002; Huisman *et al.*, 2005).

Looking at the list of species in the different morphology-based functional groups it appears unlikely that they would really be functionally equivalent (Chapter 4). However, the actual classification is based on organisms' traits and not in the particular species as even categorical traits such as the ability to fix atmospheric nitrogen, which are generally considered as inherent to the species, may actually vary with environmental conditions (e.g. Ferber *et al.*, 2004). Also, the fact that within each group the biomass of some species is more predictable than that of others, suggests that there remain important differences within groups to be explored. Although one might be inclined to look at subtle differences between species that could separate them at a fine scale in niche space, we also know that many species are remarkably flexible, adapting rapidly to different conditions (Litchman *et al.*, 2003; Lürling, 2003; Jezberová & Komárková, 2007). This suggests that niches might in practise be quite broad and flexible. If this is true, modelling the dynamics of species in a more detailed way would have to address the mechanisms of adaptation explicitly (e.g. Jiang *et al.*, 2005).

Unfortunately, this is not the only aspect we may have to consider. Indeed, as we probe deeper into the mechanisms that could be involved in governing the dynamics at the species level we seem to be opening a Pandora's box. Experimental work has shown beautifully how phytoplankton species differ in their response to basic resources such as nutrients (Tilman, 1982, 1994; Huisman & Weissing, 2000) and light intensity (Huisman *et al.*, 1999; Litchman *et al.*, 2003) as well as colour (Stomp *et al.*, 2004; Stomp *et al.*, 2007). However, species can also be heterotrophic (Sandgren, 1988b) or move to forage actively using flagella (Sandgren, 1988b; Reynolds, 2006) or adjusting buoyancy (e.g. Walsby & Reynolds, 1980). Also, many more mechanisms than resource competition shape the overall competitive balance between species (Roy, 2007; Litchman & Klausmeier, 2008). Intricate webs of interaction with zooplankton may drive complex dynamics (Krivan & Schmitz, 2004; Benincà *et al.*, 2008) and pathogens such as viruses and fungal infections

can play havoc with populations in poorly understood ways (e.g. Ibelings *et al.*, 2004). Last but not least, it is becoming evident that phytoplankton species can be involved in complex induced allelopathic interactions (e.g. Legrand *et al.*, 2003; Hulot & Huisman, 2004; Solé *et al.*, 2005).

In addition to modelling as an indispensible tool to generate hypotheses and integrate knowledge, there are two obvious complementary approaches to probe deeper into the mechanisms that rule the assemblage of phytoplankton communities. Firstly, the results so-far suggest that an experimental approach, especially if it goes beyond testing the responses of single species to their environment could well be a gold mine when it comes to revealing surprising ways in which species interact. Secondly, the wealth of field data on phytoplankton communities may be mined much further. In fact, the analyses presented in this thesis just scratched the surface of what may be hidden in the particular data we explored. Although cluster analysis revealed no distinct groups that typically occur together, it may well be that hints for 'assembly rules' could be teased out of the data through more sophisticated approaches (Kraft *et al.*, 2007; Vergnon *et al.*, 2009). Obviously, as in other fields of ecology the combination of experimentation, modelling and field data analysis will be the most powerful way to obtain a deeper understanding of what drives dynamics in the field. This will likely bring us closer to the goal of predicting and managing nuisance algal blooms. Importantly, the spin off may also be formidable when it comes to obtaining more general fundamental insights into the functioning of ecological communities. The recent advances when it comes to evidence for important concepts such as chaos (Benincà *et al.*, 2008) and emergent neutrality (Vergnon *et al.*, 2009) illustrate the power of working with plankton communities in this sense.

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Summary

Predicting phytoplankton community dynamics in detail seems an overwhelming task as there are so many species, and a myriad of combinations of potential conditioning factors. Furthermore, even with full knowledge of all aspects of species biology intrinsic chaos in communities may make detailed prediction fundamentally impossible. Aggregated estimators of phytoplankton communities may work to predict overall community responses to varying environmental conditions. However, phytoplankton species differ strongly in their effect on ecosystem functioning and ecosystem services. Therefore, it is important to consider community composition rather than just biomass.

This thesis focuses on the question whether species might be clustered in groups that are reasonably homogeneous in a functional sense, and might be better predictable from environmental conditions than individual species. To answer this question we first explored the factors that affect richness and biomass at the species level and then evaluated how well trait-based groups of species capture function and may be predicted from environmental conditions. We used a large data base including more than 700 species from 200 lakes in different climate zones and continents.

In Chapter 2 we evaluate which are the main factors that appear to determine the number of species in phytoplankton communities of a group of subtropical shallow lakes in relation to diversity of other groups of organisms found in these lakes. Our results indicated that, as found for temperate systems, submerged plant cover and transparency promote higher species richness in several groups, including phytoplankton.

In Chapter 3 we analyze differences in predictability of individual species from commonly measured environmental variables such as nutrient levels and zooplankton abundance. The presence or absence of species could in general be relatively well predicted. By contrast, biomass of most species appeared to have little relation at all with environmental variables, with the exception of a small group of species from diverse phylogenetic and functional groups that appeared to be relatively predictable. Such predictable species tended to reach a high biomass, and occurred relatively more in situations where competition for resources seems less severe.

In Chapter 4 we propose a functional classification of phytoplankton species based exclusively on organismic morphology. We first showed that morphological characteristics are systematically correlated to functional properties, such as growth rate and sinking rate, and also to the population size and biomass attained in the field. Then we used cluster analysis to define seven morphology-based functional groups (MBFG) based on the

selected morphological traits. Functional traits and demographic parameters not used for the classification differed significantly among the clusters, suggesting that they may indeed represent meaningful functional groups.

In Chapter 5 we explored how well the aggregated biovolume of groups of species can be predicted from environmental variables using three different classification approaches: MBFG, phylogenetic groups, and functional groups proposed by Reynolds. Groups from all classifications were more closely related to environmental conditions than individual species on average. However, results indicated that MBFG can be predicted better from environmental conditions than groups based on the other classification methods. This suggests that morphology captures ecological function of phytoplankton well, and that functional groups based on morphology may be most suitable to focus on if we aim at predicting the composition of communities.

In Chapter 6 a simple model was constructed to simulate dynamics of morphology-based functional groups using information on physiological rates obtained from literature. A trade-off emerged between maximum growth rate and nutrient competitive ability for most MBFG groups. Furthermore, model predictions were in line with patterns in field data and with ecological characteristics typically associated to the morphological features of the different groups.

In the synthesis (Chapter 7) I argue that my results suggest that we might think of phytoplankton communities as subsets of an omnipresent pool of cosmopolitan species selected by local environmental conditions. I speculated that the selection process would basically work on functional groups, whereas the relative importance of particular species within such groups could be essential random, as those species are functionally equivalent and therefore interchangeable.

Samenvatting

Het modelleren van de dynamiek van fytoplanktongemeenschappen is een geweldige uitdagende taak want het betreft zo enorm veel verschillende soorten en een heel scala aan combinaties van potentieel conditionerende omgevingsfactoren. Daarnaast lijkt het geven van gedetailleerde voorspellingen fundamenteel onmogelijk, zelfs met volledige kennis van alle aspecten van de biologie van de soorten. Geaggregeerde schatters van fytoplanktongemeenschappen zoals totale biomassa zijn mogelijk wel bruikbaar om de reaktie van fytoplankton op veranderende omstandigheden te voorspellen. Echter, zulke geaggregeerde schatters gaan helemaal voorbij aan het feit dat de diverse fytoplanktonsoorten enorm kunnen verschillen in hun effect op het functioneren van het ecosysteem. Voor het in kaart brengen van de effecten van veranderingen in de omgevingsfactoren op het fytoplankton kan daarom kan niet volstaan worden met alleen biomassa, maar zullen kenmerken van de samenstelling van de gemeenschap betrokken moeten worden.

Dit proefschrift richt zich op de vraag of fytoplanktonsoorten geclusterd kunnen worden in homogene, functionele groepen die beter voorspelbaar zijn uit omgevingsfactoren dan individuele soorten. Om deze vraag te beantwoorden zijn eerst de factoren onderzocht die de rijkdom en biomassa op soortniveau bepalen. Vervolgens is geëvalueerd of bepaalde groepen van soorten beter voorspeld kunnen worden dan de individuele soorten. Een grote dataset van meer 700 soorten en 200 meren uit verschillende klimaatzones en continenten is daartoe geanalyseerd.

In hoofdstuk 2 wordt geanalyseerd welke hoofdfactoren bepalend zijn voor de diversiteit van het fytoplankton in een aantal subtropische, ondiepe meren. Daarbij is ook de diversiteit van andere biologische groepen betrokken De resultaten van het onderzoek laten over duidelijk zien dat de aanwezigheid van submerse planten in een meer en de helderheid van het water een sterke positieve invloed hebben op de soortenrijkdom van de verschillende groepen, inclusief het fytoplankton. De resultaten zijn in overeenstemming met bevindingen in ondiepe plassen in gematigde streken.

In hoofdstuk 3 wordt de voorspelbaarheid van individuele soorten onderzocht als functie van algemeen gemeten omgevingsfactoren zoals nutriëntengehalte en dichtheden van zooplankton. De aan- en afwezigheid van soorten kon over het algemeen aardig voorspeld worden. Anders was het met het voorspellen van de individuele biomassa. Voor de meeste soorten was de relatie tussen de biomassa en de omgevingsfactoren zwak, uitgezonderd voor een kleine groep soorten. De biomassa van deze groep van soorten, afkomstig uit verschillende phylogenetische en functionele groepen, liet zich aardig voorspellen. Deze

voorspelbare soorten bereikten doorgaans hoge biomassa's en kwamen relatief vaker voor in situaties waar de competitie om de bestaansmiddelen minder hard was.

In hoofdstuk 4 wordt een functionele classificatie van fytoplanktonsoorten voorgesteld, uitsluitend gebaseerd op morfologische kenmerken. In eerste instantie is nagegaan of deze morfologische karakteristieken bruikbaar zijn als functionele traits door de relatie te onderzoeken met bijvoorbeeld groeisnelheid, sedimentiesnelheid, populatiegrootte en bereikte biomassa in het veld. Vervolgens zijn met behulp van een clusteranalyse 7 groepen gedefinieerd. Functionele traits zoals fysiologische traits en demografische variabelen (die niet gebruikt zijn voor de classificatie in 7 groepen) vertoonden significante verschillen tussen de 7 morfologisch groepen, wat suggereert dat de clusters inderdaad zinvolle functionele groepen vertegenwoordigen. Voordelen van de morfologische classificatie zijn de objectiviteit, de taxonomische onafhankelijkheid en het relatieve gemak waarmee de kenmerken voor nieuwe soorten vastgesteld kunnen worden.

In hoofdstuk 5 wordt onderzocht hoe goed het totale biovolume van groepen van soorten voorspeld kan worden op basis van omgevingsfactoren voor 3 classificatie systemen. De gebruikte classificatie systemen zijn 1) de op morfologie gebaseerde functionele groepen, 2) phylogenetische groepen en 3) functionele groepen voorgesteld door Reynolds. Alle 3 de classificatie systemen resulteren in betere voorspellingen van de biovolumes dan individuele soorten. De resultaten laten tevens duidelijk zien dat de voorspelbaarheid van de biovolumes van het classificatiesysteem gebaseerd op morfologische kenmerken beduidend hoger was. Dit suggereert dat morfologie veel van het ecologisch functioneren van fytoplankton omvat en dat functionele groepen gebaseerd op morfologische kenmerken uitstekend bruikbaar zijn om voorspellingen te over de samenstelling van gemeenschappen.

Ten slotte wordt in hoofdstuk 6 een eenvoudig biogeochemisch model geconstrueerd om de ecologische waarde van de morlogische classificatie te evalueren. Daartoe wordt ook gebruik gemaakt van fysiologische informatie verkregen uit de literatuur. Een duidelijke samenhang komt naar voren tussen de maximale groeisnelheid en de competitie om nutriënten voor de verschillende op morfologie gebaseerde groepen. De voorspelde uitkomsten van het model waren zowel kwalitatief als kwantitatief in overeenstemming met de veldgegevens.

De resultaten van dit proefschrift suggeren dat het mogelijk is om functioneel vergelijkbare organismen te clusteren op grond van meetbare morfologische kenmerken. Daarnaast lijkt de totale biomassa van deze functionele groepen beter voorspelbaar dan de biomassa van individuele soorten ongeacht de geografische positie en tijd van het jaar. Eén interpretatie

van dit patroon is dat soorten binnen deze groepen functioneel gelijkwaardig zijn en dus onderling uitwisselbaar en dit is in overeenstemming met de 'Emergent Neutrality' theorie. Daarom zouden fytoplanktongemeenschappen opgevat kunnen worden als subsets van een overal aanwezige poel van cosmopolitische soorten geselecteerd door locale omgevingscondities. Het selectieproces werkt op het niveau van functionele groepen, terwijl het relatieve belang van de individuele soorten in essentie random is. Hoewel de resultaten in overeenstemming zijn met de idee van ' emergente neutrality' zullen experimentele benaderingen en verdergaande analyses nodig zijn om te toetsen tegen alternatieve voorspellingen en verklaringen.

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Finally, I want to thank my children Martina and Bruno, who were born during this thesis time, for the time we did not share but that will transform me in a better mother for them. A few months ago, during the last days before handing in the reading version of this thesis, Martina (aged 4) came to me and said: “Will you still want to live with us after you become a doctor? Or will you go take care of other children?” After I explained her that was not the case she said: “ok then I will make a thesis about phytoplankton as soon as I become an adult”. I might have sounded very convincing to her, but I truly wish them both many happy times an enjoyable-alternative-futures.

Curriculum Vitae

Carla Kruk was born in Montevideo Uruguay (1974). She holds a BSc. in Biochemistry from the Faculty of Sciences University of the Republic (2000, Uruguay). In 2002 she obtained her MSc. in Biological Sciences-Ecology at the same institution with Dr. Colin S. Reynolds (Centre for Ecology and Hydrology, UK) and Dr. Néstor Mazzeo (Faculty of Sciences) as supervisors. Her thesis was “Phytoplankton succession in a hypertrophic lake in restoration process”. From 1998 till 2003 she developed internships and participated in several courses, as well as regional and international congresses. She specialized in ecology and taxonomy of freshwater phytoplankton, with emphasis in functional ecology, trophic interactions, shallow lakes and biodiversity.

In 1996 she started working in the Limnology Section of the Faculty of Sciences (UdelaR) where she performed teaching, research and other activities. She participated in several projects related with water quality and biodiversity studies along with the development of shallow lakes restoration programs.

In 2003 she started her PhD studies with Prof. Marten Scheffer (Aquatic Ecology and Water Quality Management group, Wageningen University), obtained a WOTRO grant and begun her participation in the South America Lake Gradient Analsys group.

Currently, she has an effective position as teacher assistant in the Limnology Section where she participates in graduate and postgraduate courses about limnology and ecology and has graduate and postgraduate students. Her main actual interests are the analysis of phytoplankton species predictability and the further development of experiments and modelling approaches to evaluate the application of functional approaches to analyse more general ecological questions and practical problems of water quality.

List of Publications

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Sense certificate

Netherlands Research School for the
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CERTIFICATE

The Netherlands Research School for the
Socio-Economic and Natural Sciences of the Environment
(SENSE), declares that

Carla Kruk Gencarelli

Born on: *19 February 1974* at: *Montevideo, Uruguay*

has successfully fulfilled all requirements of the
Educational Programme of SENSE.

Place: *Wageningen* Date: *6 April 2010*

the Chairman of the
SENSE board

Prof. dr. R. Leemans

the SENSE Director
of Education

Dr. A. van Dommelen

The SENSE Research School declares that Ms. Carla Kruk Gencarelli has successfully fulfilled all requirements of the Educational PhD Programme of SENSE with a work load of 55 ECTS, including the following activities:

SENSE PhD courses:

- **Research Context Activity: " Organizing international workshop on 'Twenty Years of Limnology in Uruguay: History and Perspectives' (Montevideo, 20 November 2005"**

Other Phd and MSc courses:

- **Neural networks applications to environmental sciences**
- **Interactions among the UV solar radiation, the organic dissolved matter and lake organisms**
- **Diatoms from continental waters: identification, applications and ecological relevance**
- **Automatic learning applied to ecology problems**

Research and Management Skills:

- **Writing of PhD project proposal: "A new way of predicting and managing nuisance algae in Uruguayan lakes"**
- **Site-specific training in phytoplankton taxonomy, 1 - 15 October 2006, Uruguay**
- **Co-organisation of the First Workshop in Microalgae ecology in Uruguay. During the second Ecology Congress of Uruguay, 26 – 27 October 2005, Uruguay**
- **Integration of the board of my Research institute. Biology Institute, Faculty of Sciences, Universidad de la República Oriental del Uruguay.**
- **Organizing and lecturing in the Postgraduate Courses: Phytoplankton from continental waters (I and II)**
- **Organisation of the graduate course: Why green lakes? For the first year of the Bachelor in Biological Sciences (Faculty of Sciences-Universidad de la República)**
- **Co-organisation of the Workshop in taxonomic identification of phytoplankton from continental waters with special interest in Cyanobacteria. Faculty of Sciences, Universidad de la República, 5 – 7 October, 2009, Uruguay**
- **Co-organisation of the First Meeting of Cyanobacteria in Uruguay: Cyanobacteria from knowledge to management, 5 – 10 October 2009, Uruguay**
- **Organisation of the Workshop: Functional traits for the evaluation of bloom forming cyanobacteria. Faculty of Sciences, Universidad de la República, 10 October 2009, Uruguay**

Oral Presentations:

- **First Ecology Meeting of Uruguay, 13 – 17 October 2003, Montevideo, Uruguay**
- **Ninth Brazilian Congress Limnology, 20-25 July 2003, Juiz de Fora, Brazil**
- **Second Ecology Meeting of Uruguay, 24-28 October 2005, Montevideo, Uruguay**
- **Third Argentinean Limnology Congress, 31 October -2 November 2005, Chascomús, Argentina**
- **South America Lake Gradient Analysis Project (SALGA), Workshop I, 3-10 January 2007, Vitoria, Brazil**
- **International Association of Theoretical and Applied Limnology (SIL), Redefining Theoretical and Applied Limnology for the 21st Century, 12-18 August 2007, Montreal, Canada**
- **South America Lake Gradient Analysis Project (SALGA), Workshop II, 22 November 2007, Punta del Este, Uruguay**
- **Shallow Lakes Congress: Structure and Function of World Shallow Lakes, 23-28 November 2008, Punta del Este, Uruguay**
- **Ninth Zoology Meeting of Uruguay, 10-13 December 2008, Montevideo, Uruguay**
- **Seventh Latin America and Caribbean Phycology Congress, 25-29 August 2008, Lima, Perú**
- **ASLO Aquatic Sciences Meeting 2009, A cruise through Nice Waters, 25-30 January 2009, Nice, France**
- **First Uruguayan Meeting, Cyanobacteria: from knowledge to management, 7-9 October 2009, Montevideo, Uruguay**

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