SPECIAL REVIEW

Functional classifications and their application in phytoplankton ecology

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SUMMARY

1. Ecologists often group organisms based on similar biological traits or on taxonomic criteria. However, the use of taxonomy in ecology has many drawbacks because taxa may include species with very different ecological adaptations. Further, similar characters may evolve independently in different lineages.

2. In this review, we examine the main criteria that have been used in the identification of nine modes of classifying phytoplankton non-taxonomically. These approaches are based purely on morphological and/or structural traits, or on more complex combinations including physiological and ecological features.

3. Different functional approaches have proved able to explain some fraction of the variance observed in the spatial and temporal distribution patterns of algal assemblages, although their effectiveness varies greatly, depending on the number and characteristics of functional traits used. The attribution of functional traits to single species or broad groups of species has allowed a few classifications (e.g. *Functional Groups*, *FG*) to be used in the assessment of ecological status.

4. We stress that the misuse of functional classifications (by applying them under conditions other than those intended) can have serious consequences for interpreting ecological processes. Assigning functional traits or groups cannot be considered a surrogate for the knowledge of species or ecotypes, and the use of specific traits must always be justified and circumscribed within the limits of ecological questions and hypotheses.

5. An important future challenge will be to integrate advances in molecular genetics, metabolomics and physiology with more conventional traits; this will form the basis of the next generation of functional classifications.

Keywords: ecological indicators, ecological redundancy, functional classifications, phytoplankton, taxonomical classifications

Introduction

Species in any one community may have similar ecological roles, therefore revealing some 'redundancy' in ecological functions. This has led ecologists to group organisms with similar ecological features, with the aim of obtaining a framework that potentially simplifies the complexity of real ecosystems. Ecological groups defined in this way are called *adaptive syndromes* or *Functional Groups* (Solbrig, 1993). At the ecosystem level, a grouping based on feeding relationships was one of the first attempts to link species into functional groups, opening broad research fields including ecosystem energetics, physiological ecology and trophic interactions (Odum, 1959; Cummins & Klug, 1979; Azam *et al.*, 1983; Jørgensen & Kay, 2001). In plant ecology, functional

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classifications have been widely used (Körner, 1993) and progressively updated, leading to the development of new paradigms (Lavorel *et al.*, 2007; Grime & Pierce, 2012). In animal ecology, several studies have been performed where organisms were grouped into functionally coherent clusters, generally called 'guilds' (e.g. Fauth *et al.*, 1996; Barnett, Finlay & Beisner, 2007).

The phytoplankton is an extremely diverse, polyphyletic group of photosynthetic protists and cyanobacteria, which fuel food webs and drive biogeochemical cycling (Rousseaux & Gregg, 2014). Over the last few decades, various attempts have been made to categorise traits and functions in the phytoplankton (Lewis, 1976; Litchman & Klausmeier, 2008; Litchman *et al.*, 2010), most recently opening new research perspectives in 'chemotaxonomy' (Descy, Sarmento & Higgins, 2009) and 'ecometabolomics' (Peñuelas & Sardan, 2009). Much understanding of the role of the phytoplankton comes from studies in culture, which, for instance, determined the growth and nutrient uptake kinetics of a series of taxa (Morris, 1981; Reynolds, 2006). However, the clustering of species according to physiological features is difficult (because data are not always available), leading many authors to rely on classifications based on other biological traits (Kruk *et al.*, 2010).

'Ecology is evolution in action' (Krebs, 2009); thus, from an evolutionary perspective, functional criteria should comprise the biological processes and characters implicated in adaptation. The criteria used to define functional groups in phytoplankton include morphology, physiological features and, where appropriate, taxonomy. Besides biological and taxonomic traits, other criteria include ecological features, such as phenology, implicitly acknowledging that species showing similar seasonality respond similarly to a set of particular environmental conditions. In this respect, phytoplankton functional groups are arbitrary assemblages. Species could be classified taking into account their shape and the dimensions (Naselli-Flores, Padisák & Albay, 2007) or specific physiological requirements (e.g. nutrient demands). In these two cases, planktonic diatoms forming long filaments (e.g. *Aulacoseira*), and planktonic filamentous green algae (e.g. *Mougeotia*) would be merged or placed in two separate groups, depending on the trait chosen, that is shape type or silica in the cell walls, respectively. The number of functional groups that can be devised is potentially very large. The choice of criteria encompasses the whole gradient of levels of organisation or biocomplexity (Fig. 1). At one extreme, modern phylogenetic analyses are revolutionising our view of relationships between taxa (Krienitz & Bock, 2012; Komárek, 2013). Similarly, diverse groups of algae are clearly circumscribed in their ability to produce specific metabolites, for example toxins in cyanobacteria (Metcalf
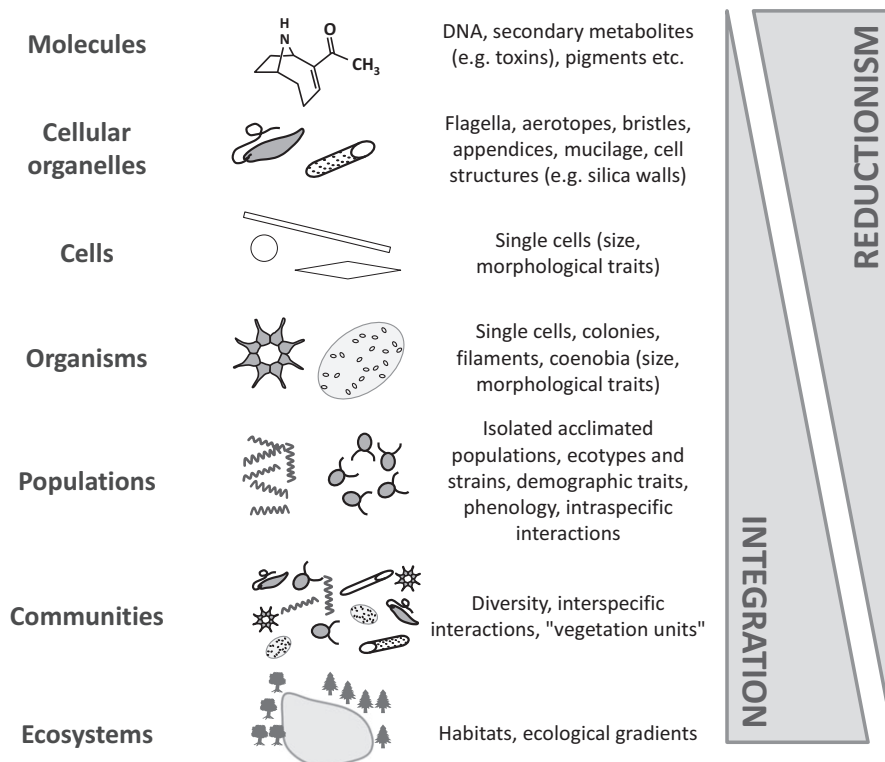

Molecules	DNA, secondary metabolites (e.g. toxins), pigments etc.
Cellular organelles	Flagella, aerotopes, bristles, appendices, mucilage, cell structures (e.g. silica walls)
Cells	Single cells (size, morphological traits)
Organisms	Single cells, colonies, filaments, coenobia (size, morphological traits)
Populations	Isolated acclimated populations, ecotypes and strains, demographic traits, phenology, intraspecific interactions
Communities	Diversity, interspecific interactions, "vegetation units"
Ecosystems	Habitats, ecological gradients

REDUCTIONISM

INTEGRATION

Fig. 1 The biocomplexity gradient in phytoplankton ecology. Physiological functions (e.g. mixotrophy, CO_2 concentrating mechanisms, N fixation) are carried out at the level of organisms, structures, cells and molecules.

& Codd, 2012; Newcombe *et al.*, 2012). At the other extreme, phenological features have traditionally played an important role in the identification of 'vegetation units' (Reynolds, 1980). Intermediate levels of biocomplexity, which include both morphological and physiological traits, are those that are most easily seen as useful in the identification of functional groups.

The aims of this review are as follows: (i) to examine the criteria used in identifying phytoplankton functional groups, summarising and evaluating critically the main classifications proposed so far. The use of functional classifications should always take into account the range of circumstances in which they are intended to apply Therefore, emphasis will be put on (ii) the limitations in the application of classifications, based on the particular choice of discriminant criteria involved. The article concludes (iii) with a discussion of the potential future development of functional groups in phytoplankton ecology.

We do not review every article that has made use of some sort of functional classification but have tried to include those that have proposed well-described, documented and widely applicable systems of classification (irrespective of the criteria used) and have contributed to the advance of functional classification in phytoplankton ecology. In Table 1, the different functional groups considered in this work have been roughly arranged based on the main criteria used for the classification. In particular, the work by Reynolds *et al.* (2002) set a milestone in the application of phytoplankton functional groups. This approach is considered here in detail, quantitatively testing the mutual relationships of the *Functional Groups* (FG) and their links with the main environmental constraints.

Taxonomic classifications

Species are the basic unit in ecosystem studies. Taxonomy at the species level brings the most complete level of information once the species niches are clearly defined. Higher taxonomic units were widely used to evaluate the distribution of phytoplankton (e.g. Wetzel,

Table 1 Phytoplankton functional classifications were analysed in this work

Functional group	Acronym	Principal criteria	Main discriminant features	Reference (relevant to phytoplankton ecology)
r and K selection	*r/K*	Functional	Functional (growth) and morphometric attributes (see Pianka, 1970)	Margalef (1978); Reynolds (1988b)
Competitive, Stress-tolerant and Ruderal strategists	*CSR*	Functional	Functional (growth) and morphological/morphometric attributes (see Reynolds, 2006)	Reynolds (1988a)
Biomass size spectrum; Normalised Biomass Size spectrum	*BSS, NBS*	Morphometrical	Size distribution	Platt & Denman (1978); Kamenir *et al.* (2004)
Traditional Taxonomic Size Spectrum	*TTSS*	Morphometrical	Size distribution	Kamenir *et al.* (2006)
Phytoplankton Geometric Shapes	*PGS*	Morphological	Shapes	Stanca *et al.* (2013)
Morphologically Based Functional Groups	*MBFG*	Morphometrical Structural	V, S, S/V, MLD, mucilage, flagella, aerotopes, heterocytes and siliceous exoskeletal structures	Kruk *et al.* (2010)
Functional Groups	*FG*	Phenological Ecological Functional	Phenology and ecological/functional attributes (tolerances to: low z_{mix}, light, temperature, SRP, DIN, Si, CO_2; high zooplankton grazing; see Table S1)	Reynolds (1980); Reynolds *et al.* (2002); Padisák *et al.* (2009)
Morpho-Functional Groups	*MFG*	Morphometrical Structural Functional Taxonomic	Structural, functional and taxonomic characters: flagella, mixotrophy, cellular organisation, aerotopes, dimensions, shapes, mucilage	Salmaso & Padisák (2007)

V, Volume; S, cell surface; MLD, maximum linear dimension; SRP, soluble reactive phosphorus; DIN, dissolved inorganic nitrogen; Si, reactive silica.

2001), particularly along trophic and physical gradients. However, only a few generalisations are possible, including, among the others, the increase of cyanobacteria in eutrophic (Downing, Watson & McCauley, 2001; Jeppesen *et al.*, 2005) and warmer lakes (Paerl & Huisman, 2008; Winder & Sommer, 2012), and the decrease of chrysophytes in eutrophic waterbodies (Kalff & Watson, 1986). Analyses based on finer taxonomic resolution (e.g. families; Salmaso *et al.*, 2006) are more difficult to apply and interpret due to the large number of taxonomic units.

Once originally based on pigment composition and cellular structure, modern phytoplankton taxonomy is being strengthened by molecular techniques (Wilmotte & Herdman, 2001; Rajaniemi *et al.*, 2005; Krienitz, 2009; De Clerck *et al.*, 2013). DNA sequencing allows obtaining quantitative data matrices that can be analysed numerically, providing lineage relationships between species (Ciccarelli *et al.*, 2006; Chakerian & Holmes, 2012). Nevertheless, the use of taxonomy in ecology has at least two severe drawbacks. On the one hand, many broader taxonomic groups include species with very different ecological properties (e.g. among diatoms, there are species forming large colonies and others with small single cells). On the other hand, distantly related species can share ecological attributes (e.g. mixotrophy) by convergent evolution, that is the independent evolution of analogous characters in different lineages (Wilson, 1992).

Classification of life history traits and the evolution of competitive abilities

The basics of competitive abilities: r and K selection (r/K)

The theory of r and K selection (Tables 1 & 2) was first proposed by animal ecologists (MacArthur & Wilson, 1967). In this classification, populations are characterised by the relative importance of the parameters r (rate of increase) and K (carrying capacity) of the logistic equation for population growth (Pianka, 1970; Begon, Townsend & Harper, 2006). Organisms selected for a high rate of increase (r) rarely reach the asymptotic density (K), but spend most of the time on the rising portion of the logistic curve, responding quickly to the availability of environmental resources but collapsing in response to disturbance or superior competitors, for instance. K-selected populations fluctuate near the asymptotic density for most of the time, have slower intrinsic rates of increase and use resources efficiently (thus being relatively tolerant of resource limitation).

Margalef (1978) interpreted the two r and K extremes as a continuum of life history strategies that could be represented along a gradient of decreasing concentrations of nutrients and turbulence. Species that are r-selected are small, with high surface area to volume ratios, while K-selected species have large dimensions, either consisting of large cells or large colonies, both resistant to grazing, and often motile. The concept of r and K selection has been widely applied in phytoplankton ecology (Sommer, 1981; Reynolds, 1988a,b; Steinberg & Geller, 1993). A few modifications were proposed to accommodate species sensitive to physical mixing, opening the way to the application of the *CSR* classification to phytoplankton (Reynolds, 1988a).

The CSR model

Taking into account the two extremes of 'stress' (physical and chemical limitations) and 'disturbance' (e.g. grazing, diseases, wind and frost), Grime (1977) identified for terrestrial vegetation four possible permutations. For one of these (high stress and high disturbance), no strategy was possible. The three remaining combinations included the Competitive (*C*), Stress-tolerant (*S*) and

Table 2 Criteria used to define the functional classifications (codes as in Table 1)

	Size	Shap	Stru	Func	Ecol	Habi	Taxo	TaxK	MisR
BSS/NBS	Y							None	None
TTSS	Y							None	None
PGS		Y						None	None
MBFG	Y	Y	Y					Basic	Low
rK	Y			Y	Y			None	Medium
CSR	Y	Y		Y	Y			Basic	Low
FG	Y	Y	Y	Y	Y	Y	Y	High	High
MFG	Y	Y	Y	Y			Y	Basic/High	Medium

Size, dimensions; *Shap*, shape; *Stru*, structural characters; *Func*, functions (explicit use of physiological properties); *Ecol*, ecological attributes, including trophic preferences; *Habi*, habitat; *Taxo*, use of taxonomical criteria. *TaxK* indicates the level of taxonomical knowledge required to include the species in a group, while *MisR* indicates the risk of misplacement.

Ruderal (*R*) strategies (Grime, Hodgson & Hunt, 2007). Reynolds (1988a) hypothesised that a similar classification of strategies could be applied to phytoplankton. Species must be adapted to exploit environments saturated by light and nutrients (*C*), to develop under low-nutrient conditions (*S*) or to endure turbulent transport through the light gradient (*R*). Species ascribed to a specific strategy were distinguishable by given morphometric physiological and metabolic features (growth rates, light harvesting, nutrient uptake, temperature optima and sinking).

Applications of the *CSR* classification are described in Reynolds (2006). For example, in several deep perialpine European lakes, it was possible to identify a vernal phase with *R* strategists (large diatoms: tolerant of vertical mixing and favoured by high nutrient availability), an early summer phase of *C* strategists (small flagellates; intolerant of mixing but good competitors for nutrients), a successive phase of *S* strategists (dinoflagellates and cyanobacteria; tolerant of low nutrients but not of vertical mixing), followed by a late-summer mixing phase favouring *R* strategists diatoms and conjugatophytes. This classification was further applied by, among the others, Lindenschmidt & Chorus (1998), Elliott, Reynolds & Irish (2001), Hart (2006), Naselli-Flores & Barone (2011), Barbosa, Barbosa & Bicudo (2013) and Naselli-Flores (2014).

Classifications based on morphology and structure

Three morphologically and structurally based classifications (one of them subsuming three kinds of size spectra) have been proposed in the recent years (Tables 1 & 2); the *Biomass Size Spectrum* (*BSS*), the *Normalised Biomass Size Spectrum* (*NBS*) and a *Traditional Taxonomic Size Spectrum* (*TTSS*); plus *Phytoplankton Geometric Shapes* (*PGS*) and *Morphologically Based Functional Groups* (*MBFG*).

Size spectra

Various size spectra (*SS*) have been studied both in marine and freshwater environments (Kamenir, Dubinsky & Zohary, 2004). To evaluate the *BSS*, phytoplankton cells were counted and measured and then distributed into geometric size classes according to their cell volume (V_i). The *BSS* was represented graphically, showing the distribution of biomass into classes of increasing cell volumes. Normalised *BSS* (*NBS*) were determined via normalisation of the total biomass in each V_i to the change in cell volume across the category (Platt & Denman,

1978). This way, *NBS* describes the mean cell density estimate (Kamenir *et al.*, 2004). In the *Traditional Taxonomic Size Spectrum* (*TTSS*) (Kamenir, Dubinsky & Zohary, 2006), size classes were defined as in *BSS*, and a *TTSS* was created as the frequency distribution of the cumulative number of taxa (recorded during one specific period of time) in size classes based on their cell volume.

Analyses of *BSS* and *TTSS* allowed an evaluation of differences in the distribution of cell size in waterbodies of contrasting trophic state and of any changes during pronounced ecosystem shifts (Kamenir *et al.*, 2004, 2008; Kamenir & Morabito, 2009).

Phytoplankton Geometric Shapes

Stanca, Cellamare & Basset (2013) used the geometric shapes of phytoplankton as the only criterion in studying the distribution of phytoplankton along the coast of the Salento peninsula (SE Italy). Phytoplankton species were allocated to the most similar geometric shape selected from those described by Hillebrand, Dürselen & Kirschtel (1999), Sun & Liu (2003) and Vadrucci, Cabrini & Basset (2007). At the same time, morphometric measurements (surface, volume and surface to volume ratios) were obtained from basic linear dimensions. Since no name was provided for this classification, we will refer to this approach as *Phytoplankton Geometric Shapes* (*PGS*) (E. Stanca, pers. comm.).

Stanca *et al.* (2013) argued that the high variability in *PGS* was related to morphological adaptations to the environment. Elongated shape maximises the cell surface exposed to light and was favoured (by mixing of the water column) during the winter. Rounded and combined shapes, mostly of mixotrophs, developed when the water column was thermally stable and nutrients depleted.

Morphologically Based Functional Groups

Kruk *et al.* (2010) classified phytoplankton into seven functional groups (*MBFG*) based on shape and structures. The classification was based on nine descriptors, namely volume, surface, surface to volume ratios, maximum linear dimension and the presence of mucilage, flagella, aerotopes, heterocytes and siliceous exoskeletal structures. Many functional and demographic features (which were excluded in the group definition) were differently distributed among the groups, suggesting a functional meaning in their separation and identification. The features tested included growth, sinking velocity,

silicon half saturation constant for growth- and abundance-related variables. The seven functional groups are characterised by a set of *a priori* features that allow the inclusion of new species. The *MBFG* classification has the advantage of simplicity and, not requiring specific knowledge about physiological traits and taxonomy, is also simple to apply in a variety of circumstances (Kruk & Segura, 2012).

Using data from 211 lakes, Kruk *et al.* (2011) showed that the occurrence of the various *MBFG* could be predicted from environmental conditions with an accuracy higher than for *Functional Groups* (*FG*, see below) and for the majority of species. Nevertheless, in a successive study of 83 lakes over a gradient from subpolar to tropical regions, Kruk *et al.* (2012) did not find systematic relationships between environmental gradients and phylogenetic affiliation or particular functional groups as defined by morphology.

Composite functional classifications

Besides biological traits, *Functional Groups* (*FG*) and *Morpho-Functional Groups* (*MFG*) (Tables 1 & 2) include also taxonomy and (for FG) ecology as discriminant attributes.

Functional Groups

The modern definition of *Functional Groups* (*FG*) by Reynolds *et al.* (2002) has its roots in the schemes, already available in the 1940s–1950s, where lakes were classified by the phytoplankton they supported (Reynolds, 1997). Using observations from a group of lakes in north-west England, and applying traditional phytosociological methods (Braun-Blanquet, 1964), Reynolds (1980) recognised 14 phytoplankton associations identified with alphanumeric labels (coda), each including species coexisting together and with similar seasonality. Successively, the use of 'association' was criticised, recognising that some species, although showing comparable adaptations and similar environmental optima, are not always found simultaneously. At present, the accepted term, *FG*, is intended to group together species with similar morphological and physiological traits, and with similar ecological features (Reynolds *et al.*, 2002). While originally the groups (coda) were allocated in blocks ordered alphabetically to reflect seasonal chronological shifts in a set of temperate lakes (Reynolds, 1984), with the successive incorporation of information from lakes located at different latitudes, the alphabetical order has lost its significance. The system was therefore expanded

to 31 coda accommodated based on expert judgment (Reynolds *et al.*, 2002; Table 3). A subsequent review by Padisák, Crossetti & Naselli-Flores (2009) recognised more than 40 coda, although not all of them yet sufficiently substantiated to be brought into the 'final' classification. Inclusion of new species in the *FG* coda requires a deep knowledge of the taxonomy and autecology of the species concerned. On the other hand, compared with the other classifications, the *FG* are well described in terms of habitat properties, environmental tolerance and trophic state (Reynolds, 2006; Padisák *et al.*, 2009).

Functional Groups represent the classical and the widest used system of classifying the phytoplankton. Nevertheless, relationships between the *FG* and their links with the main environmental constraints have never been tested quantitatively and confirmed. These points are briefly revisited in the next section.

Functional attributes of FG coda

A quantitative analysis of the relationships among the *FG* coda is presented here, based on their relative tolerance to different environmental conditions reported by Reynolds *et al.* (2002: their table III; for raw data and coding criteria see Table 3). Trophic classifications (coded from 1, ultra-oligotrophy, to 9, hypereutrophy) were obtained from Reynolds *et al.* (2002: their table I), integrating material from Reynolds (1984) and Padisák *et al.* (2009). *Functional Groups* were analysed by nonmetric multidimensional scaling (NMDS) and cluster analysis (Ward's method), both applied to Euclidean distance matrices (Oksanen *et al.*, 2013; Murtagh & Legendre, 2014). Environmental variables were related to the strongest gradients in *FG* composition by fitting environmental vectors to the NMDS configurations and by surface fitting (R Core Team, 2014; for R scripts see Table S1).

The cluster analysis and NMDS confirmed the close connection between some *FG* and their separation into five groups (Fig. 2a,b); for a description of coda see Table 3. The consistency of the data matrix used in the analysis was quite apparent in the relationships of groups 1–5 with the environmental variables (Fig. 2c). A high tolerance of low phosphorus (mostly oligotrophic environments) was contrasted with a high tolerance of low dissolved carbon dioxide and irradiance (mostly eutrophic environments). Tolerance of low water temperature (e.g. in winter) was contrasted with a tolerance of high filtration rates by zooplankton and low dissolved inorganic nitrogen (DIN, usually in early summer–autumn).

Table 3 For each functional groups (FG), the table reports the tolerances to different environmental conditions based on the data reported in Reynolds *et al.* (2002: their table III)

Codon (FG)	Representative species	z_m <3	I <1.5	Temp <8	SRP <10^{-7}	DIN <10^{-6}	Si <10^{-5}	CO$_2$ <10^{-5}	fZoo >0.4	Trophic Code	Trophic Code
A	*Urosolenia, Cyclotella comensis*	0	0.5	1	1	1	1	0	0	O	3
B	*Aulacoseira subarctica, A. islandica*	0	1	1	1	0	0	0	0	M	5
C	*Asterionella formosa, Aul. ambigua, Stephanodiscus rotula*	0	1	1	0	0	0	0.5	0	E	7
D	*Synedra acus, Nitzschia* spp., *Stephanodiscus hantzschii*	1	1	0	0	0	0	1	0	H	9
N	*Tabellaria, Cosmarium, Staurodesmus*	0	0	0	1	0	0.5	0	0.5	M	5
P	*Fragilaria crotonensis, Aulacoseira granulata, Closterium aciculare, Staurastrum pingue*	0	0	0	0	0	0.5	1	1	E	7
T	*Geminella, Mougeotia, Tribonema*	0	0.5	0	0.5	0	1	0.5	1	M	5
S1	*Planktothrix agardhii, Limnothrix redekei, Pseudanabaena*	1	1	1	0	0	1	1	1	H	9
S2	*Spirulina, Arthrospira, Raphidiopsis*	1	1	0	0	0	1	1	1	H	9
S$_N$	*Cylindrospermopsis, Anabaena minutissima*	1	1	0	0	1	1	1	1	E	7
Z	*Synechococcus*, prokaryote picoplankton	1	0	1	1	1	1	0.5	0	O	3
X3	*Koliella, Chrysococcus*, eukaryote picoplankton	1	0	1	1	0	1	0	0	O	3
X2	*Plagioselmis, Chrysochromulina*	1	0	0	0.5	0	1	0.5	0	ME	6
X1	*Chlorella, Ankyra, Monoraphidium*	1	0	0	0	0	1	1	0	EH	8
Y	*Cryptomonas*	1	1	1	0	0	1	0.5	0	E	7
E	*Dinobryon, Mallomonas (Synura)*	1	1	1	1	1	1	0	0	OM	4
F	Colonial Chlorophytes, e.g. *Chlorococcales*	1	0	0	1	0	1	0	0	M	5
G	*Eudorina, Volvox*	1	0	1	0	0	1	1	1	E	7
J*	*Pediastrum, Coelastrum, Scenedesmus, Golenkinia*	1	0.5	1	0	0	1	0.5	0.5	EH	8
K	*Aphanothece, Aphanocapsa*	1	0.5	0	0	0	1	1	0.5	E	7
H1	*Dolichospermum flos-aquae, Aphanizomenon/Chrysosporum*	1	0	0	0	1	1	1	1	E	7
H2	*Dolichospermum lemmermannii, Gloeotrichia echinulata*	1	0	0	0	1	1	1	1	M	5
U	*Uroglena*	1	0	0.5	1	0	1	0	1	OM	4
L$_O$	*Peridinium, Woronichinia, Merismopedia*	1	0	0	1	0	1	0	1	M	5
L$_M$	*Ceratium, Microcystis*	1	0	0	0	0	1	1	0	E	7
M	*Microcystis, Sphaerocavum*	1	0	0	0	0	1	1	0	E	7
R	*Planktothrix rubescens, P. mougeotii*	1	1	0	0	0	1	0.5	1	M	5
V	*Chromatium, Chlorobium*	1	1	0	0	0	1	0	0	E	7
W1	Euglenoids, *Synura, Gonium*	1	1	1	0	0	1	0.5	0	H	9
W2	Bottom-dwelling *Trachelomonas*	1	1	1	0	0	1	0.5	0.5	M	5
Q	*Gonyostomum*	–	–	–	–	–	–	–	–	–	–

FG are identified with alphanumeric labels (coda). The original table reports the tolerance (+/−) to a set of environmental conditions. In this work, for each FG, the table has been coded into a numerical matrix substituting 0, 0.5 and 1 to −, +/− and +, respectively. Where tolerance was suspected, but not proven, occasional question marks (?) in the original table have been replaced with 0.5. Owing to the incomplete description, group Q (Gonyostomum) was excluded from the analysis. z_m, depth of the surface mixed layer (m); I, mean daily irradiance (mol photons m^{-2} day^{-1}); Temp, water temperature (°C); SRP, soluble reactive phosphorus (mol L^{-1}); DIN, dissolved inorganic nitrogen (mol L^{-1}); Si, soluble reactive silicon (mol L^{-1}); CO$_2$, dissolved carbon dioxide (mol L^{-1}); fZoo, zooplankton grazing (proportion of the water processed daily by zooplankton). The trophic classes (ultraoligotrophy, oligotrophy, mesotrophy, eutrophy and hypertrophy and intermediate states) were coded numerically: U (1), UO (2), O (3), OM (4), M (5), ME (6), E (7), EH (8), H (9).
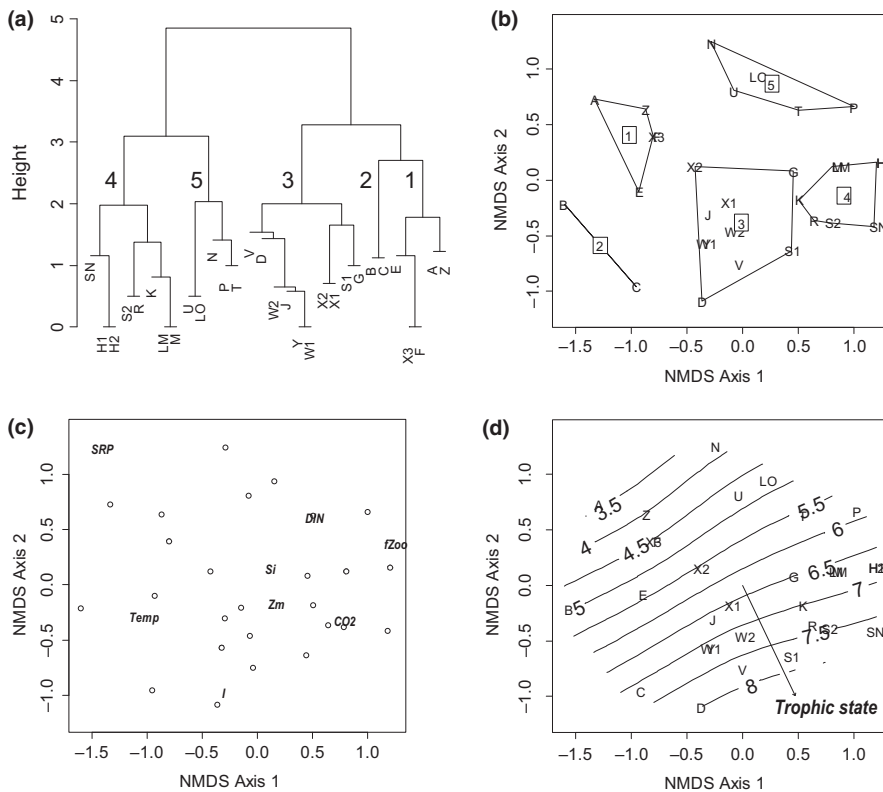**Codon J includes many Chlorococcales which are undergoing a wide taxonomical rearrangement (Krienitz & Bock, 2012).*

Fig. 2 Classification (a) and NMDS ordination (b) of the *Functional Groups* (*FG*) defined by Reynolds *et al.* (2002) (Table 3). The numbers 1–5 divide the main *FG*. The analyses were carried out taking into account the environmental tolerance, that is, depth of the surface mixed layer (z_m); mean daily irradiance (*I*); water temperature (*Temp*); soluble reactive phosphorus (*SRP*); dissolved inorganic nitrogen (*DIN*); soluble reactive silicon (*Si*); dissolved carbon dioxide (CO_2); zooplankton grazing (*fZoo*). (c) Ordination of tolerances as weighted averages of *FG* scores. (d) Vector and surface fitting of trophic state coded numerically from 1 (ultraoligotrophy) to 9 (hypereutrophy).

The position near the origin in the NMDS suggested that tolerances of euphotic depth and low silica availability were not consistently linked to the pattern of *FG* in the analysis. The gradient Temperature/grazing DIN allowed the clearest separation of two broad groups of *FG*, that is 1–2 and 4–5, respectively, whereas group 3 takes an intermediate position along this gradient (Fig. 2b,c). The first group (1–2) includes many *FG* that were originally defined to accommodate diatoms and other taxonomic assemblages developing in the spring and early summer (Reynolds, 1984). In the second group (4–5), *FG* are composed by species developing almost exclusively in warmer and stratified conditions. These differences were substantiated by a greater tolerance of *FG* 4–5 to zooplankton grazing (with many large species and colonies) and low nitrogen concentrations (with all the dinitrogen-fixing cyanobacteria in group 4). Orthogonal to this (i.e. 'upper left to bottom right'), the gradient phosphorus–carbon dioxide/irradiance further divided groups 1 and 5 from 2 to 4 (Fig. 2b,c). This can be interpreted as a trophic gradient. This was further supported by the results of the vector and surface fitting in Fig. 2d, which show a strong linear relationship between the trophic state (nutrient availability) and functional groups. At the eutrophic extreme, *FG* representatives (Table 3) were cyanobacteria developing in warm

epilimnia (S_N, S2) and turbid lakes (S1), purple and green sulphur bacteria (V), and small-celled and fast-growing diatoms (D). At the oligotrophic extreme, the diatoms were well represented with coda A and N, along with small and single-celled cyanobacteria (Z).

Morpho-Functional Groups

Morpho-Functional Groups were identified using *a priori* determined traits influencing functional processes and ecological characteristics (Salmaso & Padisák, 2007). Groups were classified based on the presence of flagella, the ability to obtain alternative sources of fixed carbon and nutrients, cellular organisation, dimensions, shapes, and, when ecologically relevant, taxonomy. Compared with the other classification systems, *MFG* do not make use exclusively of morphological/structural criteria in the definition of groups (as in *MBFG*), or even of phenological, habitat and trophic information (as in the *FG* classification). The criteria to define *MFG* were explicitly chosen as among the strongest drivers able to predict the best competitors under different environmental constraints (see Weithoff, 2003). Being based on an identification key, the inclusion of species in the system is quite straightforward. Conversely, the use of the classification requires, as a preliminary step, the ability to classify the

species from the genus to the order. The system is flexible enough to accommodate a greater number of groups, depending on the characteristics of the habitat analysed. An update of *MFG*, including some new groups (e.g. Tolotti *et al.*, 2012), is given in Table S2.

Following a similar approach, different morpho-functional classifications were subsequently conceived for benthic diatoms (*Morpho-Functional Diatom Groups*, *MFDG*; Centis, Tolotti & Salmaso, 2010) and river phytoplankton (Fraisse, Bormans & Lagadeuc, 2013).

Morpho-Functional Groups were used for the first time to compare the phytoplankton in lakes Garda and Stechlin (Salmaso & Padisák, 2007) and in two reservoirs with contrasting hydrological regimes (Tolotti, Boscaini & Salmaso, 2010). Other applications of *MFG* are discussed below.

Applicability of functional groups in the derivation of water quality indices

The development of phytoplankton functional group systems coincided with that of the European Union's Water Framework Directive (EC Parliament & Council, 2000). Of the functional approaches discussed above, the *FG*, *MFG* and the *TTSS* were included in studies aiming at assessing ecological status.

The assemblage (*Q*) index is based on the relative share of *FG* coda in the total biomass, multiplied by a numerical factor (*F*), defined for each functional group considering the phytoplankton assemblage likely to occur in a pristine lake of the corresponding type (Padisák *et al.*, 2006). A later version of this index was extended for the evaluation of river phytoplankton (*Q_R*, Borics *et al.*, 2007). Since the *F* numbers are (lake) type specific and can be adjusted for different kinds of human impacts, both *Q* and *Q_R* indices are conceptually different from most other metrics proposed for assessing the ecological quality of lakes, particularly in response to nutrient enrichment, the most widespread pressure affecting lakes (Thackeray *et al.*, 2013). The *Q* index provides results coherent with other phytoplankton-based quality indices (e.g. Becker, Huszar & Crossetti, 2009; Belkinova *et al.*, 2014; Molina-Navarro *et al.*, 2014) and, specifically, with the German *PSI* (Mischke *et al.*, 2008), the Polish *PMPL* index (Pasztaleniec & Poniewozik, 2010) and the Algal Group Index (*AGI*; Teneva *et al.*, 2014).

Morabito & Carvalho (2012), Lyche-Solheim *et al.* (2013) and Thackeray *et al.* (2013) evaluated different phytoplankton metrics to assess the ecological quality of lakes in response to eutrophication (expressed as total phosphorus, TP). The *Size Phytoplankton Index* (*SPI*) and the *Morpho-Functional Group Index* (*MFGI*) were derived from the *TTSS* and the *MFG*, respectively. Both indices showed a significant ($P < 0.01$) relationship with TP, but with different results and also non-significant relationships in different European regions. A combination of *SPI* and *MFGI* in a unique index (Functional Traits Index, *FTI*) improved the correlation with TP (Morabito & Carvalho, 2012).

Synoptic view and critical evaluations

Comparative analyses of functional classifications

Several authors have pointed out the strengths and weaknesses of *FG*, *MFG* and *MBFG* both in lakes and rivers (see references in Table 4). In general, these studies showed that both *FG* coda and *MFG* were suitable tools for explaining changes in phytoplankton assemblages in relation to major environmental drivers. These two approaches often produce similar (overlapping) results. However, since *FG* coda is associated with well-described environmental templates, they are generally acknowledged as being more helpful in explaining phytoplankton variability in relation to environmental factors. Classification of *MBFG*, based on seven groups, is closer to the diatom ecological guild approach (three groups; Passy, 2007) than to either *FG* or *MFG*. *Morphologically Based Functional Groups* can explain large-scale variations (Abonyi *et al.*, 2014) and therefore are suitable for analysing large, ecoregional data sets (Izaguirre *et al.*, 2012; Hu, Han & Naselli-Flores, 2013; Žutinić *et al.*, 2014). At finer regional and temporal scales, functional groupings (either *FG* or *MFG*) apparently perform better (Abonyi *et al.*, 2014).

A critical evaluation of functional approaches

Classifications founded on the concept of life history traits (*r/K* and *CSR*) have limited applicability in the study of phytoplankton. As stated by Roff (1992), 'attempts to transfer the concept to actual populations without regard to the realities of the complexities in life history have probably been detrimental rather than helpful' (see also Ricklefs and Miller, 2000). The *r/K* concept set the stage for the definition of successive approaches, such as the *CSR* classification. Nevertheless, as in the *r/K* approach, the *CSR* classification is more of conceptual value, highlighting the importance of the strong link between size and shape, and functional properties. The classification of phytoplankton into three *CSR* classes

Table 4 Summary of analyses comparing different phytoplankton functional classifications

Origin/site	Data set	Functional classifications compared	Statistical methods	Reference
Large floodplain rivers: (Mura, Drava, Danube and Sava) (Croatia)	Spatial and temporal, 24 samples	FG, MBFG	Canonical Correspondence Analysis (CCA), Self-organising Maps (SOM)	Stanković et al. (2012)
87 Andalusian lakes and ponds (S-Spain)	Spatial, 87 samples	FG, MBFG	Pearson correlations, Generalised Linear Models (GLM)	Gallego et al. (2012)
Three Pampa lakes, three sites per lake (Argentina)	Spatial and temporal, 72 samples	FG, MFG, MBFG	Redundancy Analysis (RDA), Detrended Correspondence Analysis (DCA)	Izaguirre et al. (2012)
Three small reservoirs (S-China)	Spatial and temporal, 18 samples	FG, MFG, MBFG	CCA	Hu et al. (2013)
River Loire (France)	Spatial and temporal, 170 samples	FG, MFG, MBFG	SOM	Abonyi et al. (2014)
A lateral channel of the Upper Paraná floodplain (Brazil)	Temporal, 49 samples	FG, MBFG	PCA, CCA, Indicator Value Analysis (IndVal)	Bortolini et al. (2014)
Two deep karstic lakes (Plitvice NP, Croatia)	Temporal, 384 samples	FG, MFG, MBFG	Principal Components Analysis (PCA), CCA	Žutinić et al. (2014)

does provide only a very limited set of attributes to study phytoplankton life history traits.

In the morphologically based classifications (*BSS/NBS*, *TTSS*, *PGS* and *MBFG*; Table 2), the presence of similar structures and/or sizes/shapes in phylogenetically distantly related species can be interpreted as a set of common analogous traits under strong natural selection. Although morphology and structure have implicitly functional roles, most (*MBFG*) or all (*BSS/NBS*, *TTSS*, *PGS*) physiological complexities are not taken into account. Characters such as (among others) pigment composition and photosynthetic efficiency are vital characteristics that cannot be predicted and modelled by size and shape. With these approaches, the common possession of silica walls in the large *Aulacoseira* and small (<5 μm) *Cyclotella* can identify the two genera as functionally equivalent, although they differ in their sinking rate in stable water columns (Winder, Reuter & Schladow, 2009). Similarly, large mucilaginous colonies share many related characters, such as the resistance to grazing and reduced susceptibility to sinking. However, no one can deny the differences between the large *Microcystis* colonies, which can move upwards by several metres per day, and the large colonial, non-motile Chlorococcales *s.l.* In a few occasions, attempts were made to investigate the reliability of size-based classifications in the trophic evaluation of waterbodies. However, in the case of trophic indices based on size classes (*SPI* and *MFGI*), better relationships with TP were obtained using other classical metrics, based on chlorophyll-a, the biovolume of cyanobacteria and species composition (Thackeray et al., 2013). More generally, the low discriminatory power of classifications based on a limited number of groups has been highlighted by Izaguirre et al. (2012), Stanković et al. (2012) and Žutinić et al. (2014).

Functional Groups and *MFG* make explicit use of functional properties in the delineation of groups of species. There are advantages in this, due to the recognition of specific ecological capabilities otherwise not distinguishable on a structural basis (e.g. mixotrophy, light optimum requirements); nevertheless, there is a high level of subjectivity in the approach.

An advantage of *FG* is that ecological features are linked with the trophic state or habitat preferences (Fig. 2; Table 2). Unlike any of the other classifications, species with very similar morphological characteristics but distinct environmental tolerances, such as *Planktothrix agardhii* and *P. rubescens*, are clearly separated into two functional groups, namely S1 and R, respectively. Similar considerations apply to other groups of species, for example small *Cyclotella* spp. or *Aulacoseira* spp.. On the other hand, the low number of representative species in each of the different *FG* forces investigators to 'guess' the inclusion of new species not yet assigned into a well-defined group. This issue was addressed by Padisák et al. (2009), where a number of misplacements were identified. A serious risk for *FG* is the blurring of differences in ecological tolerance between the groups,

due to the addition of further species to the existing coda. This classification must not be used when the ecological preferences of species are insufficiently known. The clear ecological delineation of *FG* coda was a prerequisite for the derivation of the assemblage Q and Q_R indices used in the evaluation of ecological status. However, the large degree of subjectivity in the choice of the factor number F poses serious limits to the possibility to generalising this approach, with applications limited to a case-by-case evaluation.

Contrary to the *FG* classification, *MFG* does not have a clear habitat characterisation, and investigations in this direction have begun only recently (e.g. Izaguirre *et al.*, 2012; Salmaso, Naselli-Flores & Padisák, 2012; Gallina *et al.*, 2013; Hu *et al.*, 2013; Mihaljević *et al.*, 2013; Thackeray *et al.*, 2013). Since species can be accommodated in several functional groups, *MFG* classifications can be efficiently used to overcome the problems related to differences in taxonomic accuracy and species identification in different ecosystems (e.g. Tolotti *et al.*, 2010).

The relationship between the functional groups is summarised in Fig. 3. The specificity of classifications purely based on size or shapes is apparent in the separation of *PGS* and *BSS/NBS/TTSS*. At a lower dissimilarity, *r/K* and *CSR* are closely connected, forming a separate group. The use of diversified structural attributes put the *MBFG* nearer to the *FG* and *MFG*.

Potentials and weaknesses of the functional approach: perspectives for the progress in phytoplankton ecology

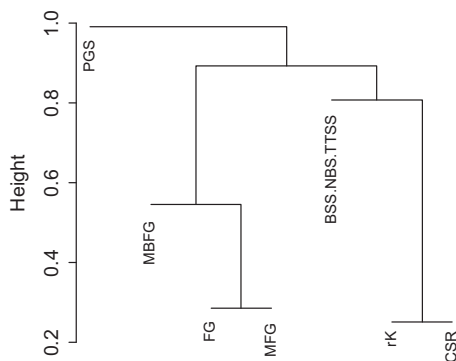Functional classifications allow comparisons between ecosystems around the world. Distant lakes appear

Fig. 3 Relationships between the functional groups based on the distinctive binary characteristics reported in Table 2, namely dimensions, shape, structural characters, functions, ecological attributes, habitat and taxonomy. Distances are based on the Jaccard dissimilarity, whereas the cluster analysis was performed with the Ward's method (R Core Team, 2014).

different based on species composition, but they could share a phytoplankton with similar functional characteristics. The identification of common traits under a range of environmental conditions (e.g. differing in the proportion of land use, nutrient inputs, grazing and climate) can improve our ability to generalise results, finding common patterns useful also in predicting shifts in the phytoplankton under climate- and environmental-change scenarios. Nevertheless, functional groups are not meant to be a substitute for the whole extent of information that can be gathered from species. The knowledge of which species dominate a functional group is of primary importance when information on conservation, trophic roles, toxicity or other characters pertaining to populations or strains are essential in addressing particular ecological questions or environmental issues.

The definition of functional groups requires categorising similarities in biological and ecological traits. At a broader scale (e.g. the ecosystem), such an approach has fostered important conceptualisations regarding the functioning of trophic webs and ecosystem energetics (Weisse *et al.*, 1990). At a finer scale, the numerous criteria and approaches that have been proposed to group adaptive phytoplankton traits may also reflect the lack of unifying concepts. Environmental drivers act at every level of the biological complexity, from single traits to communities. Therefore, the excessive reduction of traits can affect negatively the sensitivity and efficiency of classifications to explain the observed species distributions and changes.

The misuse of functional classifications outside a specific range of applications can have serious consequences in the interpretation of ecological processes. When using functional groups, we should take into account the limits and uncertainty implicit in the conceptualisation of ecological redundancy (Naeem, 1998). Isbell *et al.* (2011) argued that even more species would be needed to maintain ecosystem processes and services than suggested by previous studies. If only one or a few processes are considered, many species appear redundant within a specific set of environmental constrains.

Future progress will necessarily be founded on the delineation of functional traits defined with a broader and stronger theoretical framework. It is highly unlikely that the continuous application of functional classifications or selected traits would open the way to strong generalisations, that is in a 'let's apply and see what happens' approach. Adopting a deductive method (Ritchie, 2010), the robustness of functional traits and classifications should be tested experimentally, based on

clearly defined hypotheses addressing the power of traits and their mutual relationships. Simple examples include testing the increase of slow-sinking functional groups (or of selected traits, e.g. the fraction of gas-vacuolated cyanobacteria) in experimental setups and warming lakes (cf. Winder & Sommer, 2012), and testing whether physiological traits of phytoplankton can explain how species respond to environmental gradients (e.g. light and phosphorus; Edwards, Litchman & Klausmeier, 2013). Quantification of the links between species traits and environment should make use of robust statistical analyses, including methods specifically devised to test the species traits–environment relationships (e.g. the 'fourth-corner' method; Dray & Dufour, 2007; Dray & Legendre, 2008). On the other hand, following an inductive approach, refinement of functional classifications will require the study of the relationships between functional traits and environmental drivers. Important outcomes should include the identification of common patterns of change along environmental gradients.

Functional approaches have been based on discernible biological traits, integrated with phenology, ecology and taxonomy (Table 2). In this respect, more accurate phylogenetic analyses should be assessed for their potential to contribute synergistically to trait-based approaches (see Westoby, 2006; Kraft *et al.*, 2007; Cavender-Bares *et al.*, 2009; Litchman *et al.*, 2010; Vamosi, 2014). At the finer taxonomic levels, and considering the rapid progress in both molecular genetics and ecological metabolomics, future directions should also take into account 'cryptic adaptive traits'. Examples include the ability to produce a variety of toxins or to withstand hydrostatic pressure gradients through the synthesis of gas vesicles of different strengths in different strains of cyanobacteria (D'Alelio *et al.*, 2011; Kurmayer *et al.*, 2011; Salmaso *et al.*, 2013); the ability to exploit various light intensities and nitrogen compounds in different genotypes of *Prochlorococcus* (Moore *et al.*, 2002); the increasing presence of mycosporine-like amino acids (MAAs) and enhanced absorption of ultraviolet radiation of phytoplankton in high-altitude lakes (Ficek, Dera & Woźniak, 2013). As a cautionary note, the existence of ecotypes with different physiological adaptations (see also Rohrlack *et al.*, 2008; Zapomělová *et al.*, 2010; Üveges *et al.*, 2012; D'Alelio, Salmaso & Gandolfi, 2013) should be taken into account in the evaluation of the limits implicit in the use and interpretation of functional approaches based on easily measurable traits.

In conclusion, the various functional classifications available represent a first step towards the use of tools integrating phytoplankton 'functions'. Besides classical traits, an important future challenge will be to integrate, together with the advances in molecular genetics, metabolomics and physiology, our growing knowledge of phytoplankton taxa in the definition of functional classifications.

Acknowledgments

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. R code and input files used in the statistical analyses and statistical graphs.

Table S2. *Morpho-Functional Groups* (*MFG*; updated from Salmaso & Padisák, 2007).

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