Developments in Hydrobiology 221

Nico Salmaso · Luigi Naselli-Flores
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Phytoplankton responses to human impacts at different scales

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Phytoplankton Responses to Human Impacts at Different Scales

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PHYTOPLANKTON

Preface: phytoplankton responses to human impacts at different scales

16th Workshop of the International Association of Phytoplankton Taxonomy and Ecology (IAP)

Nico Salmaso · Luigi Naselli-Flores · Leonardo Cerasino · Giovanna Flaim · Monica Tolotti · Judit Padisák

Published online: 24 July 2012

The 16th workshop of the International Association for Phytoplankton Taxonomy and Ecology (IAP) on phytoplankton responses to human impacts was held at the Agricultural Institute of S. Michele all'Adige—Fondazione E. Mach from 21st to 28th August 2011. The stated IAP objectives are to get together pre-eminent as well as young scientists and students working on various aspects of phytoplankton taxonomy and ecology to discuss topics of current interest, to jointly examine water samples under the microscope and practice species identification under the guidance of leading experts, and to encourage young scientists to engage in taxonomy research—a field where expertise is vanishing.

No doubts that these objectives were achieved in the current workshop with 91 participants from 23 countries (Argentina, Australia, Austria, Brazil, Bulgaria, Canada, China, Croatia, Czech Republic, Denmark, France, Germany, Greece, Hungary, Israel, Italy, New Zealand, Spain, Sweden, Switzerland, Turkey, UK, Uruguay) attending the workshop, more or less equally divided between professional scientists and students. Grants were provided to encourage the participation of students. The hostel of the institute where the meeting was held provided a spectacular site located in the Adige Valley and surrounded by the Alps. The microscopy sessions in the afternoons were guided by established taxonomists and attended by keen-to-learn young scientists who jointly examined fresh and preserved samples brought by the participants.

The IAP was established in 1979, and since then held 16 workshops at different locations (Kristiansen, 1997). Since 1991, each workshop had a well-defined ecological theme, and taxonomic topics. Proceedings of all these workshops have been published as peer-reviewed articles. Since the early 1990s these appeared in dedicated volumes of Hydrobiologia:

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1991: Baja, Hungary (Padisák et al., 1993)
1993: Mont Rigi, Belgium (Descy et al., 1994)
1996: Granada, Spain (Álvarez-Cobelas et al., 1998)
1998: Shrewsbury, England (Reynolds et al., 2000)
1999: Delta Marsh, Canada (Hamilton et al., 2000)
2002: Castelbuono, Italy (Naselli-Flores et al., 2003)
2005: Sapanca, Turkey (Albay et al., 2007)
2008: Golan Heights, Israel (Zohary et al., 2010)

As tradition in all IAP workshops, the 2011 meeting had two main foci, one was ecological, the other taxonomic. The ecological theme was *Phytoplankton responses to human impacts at different scales*, the taxonomic topics were: *Chlorophyta*, *Dinophyta* and selected taxa of *Cyanobacteria*.

Apart of the foreword, this volume presents a selection of 26 reviews and original research papers. It is structured around the ecological theme of the workshop with many papers addressing global impacts like climate change and local influences like land-use on phytoplankton structure. Other papers addressed the trait concepts and, analyzed wax and wane, ecological responses, or physiological properties of individual species in the context of the central topics. Taxonomic papers review present state of the systematics of coccoid green algae (Krienitz & Bock, 2012), and provide an update to modern (2011) taxonomy of planktic heterocytous cyanobacteria (Komárek & Mareš, 2012). Zapomělová et al. (2012) while discussing biogeographically interesting nostocalean species revised taxonomic positions of two formerly *Anabaena* species and Zhu et al. (2012) provided a taxonomic and phylogenetic evaluation of *Limnothrix* strains.

The IAP participants are grateful to the Autonomous Province of Trento, which contributed to fund the workshop. We are also grateful to the Agrarian Institute—E. Mach Foundation for providing the access to the facilities of the campus. We thank the Regione Trentino Alto Adige, the APT—Azienda Promozione Turistica (Trento), Uwitech (Mondsee, (A), and Corr-Tech (S. Giovanni Lupatoto, VR) for their support. We are grateful to the Leica Microsystems BM Medical (Padova) for loaning us the Leica microscopes for the duration of the meeting. The assistance of the directorate, the staff members and the students of the IASMA Research and Innovation Centre was invaluable. Without their dedicated contribution the success of this meeting would not have been possible. Special thanks are due to Dr. Roberto Viola, the director of the IASMA Research and Innovation Centre, and to Dr. Ilaria Pertot, the coordinator of the Department of the Sustainable Agro-ecosystems and Bioresources Department. Dr. Domenico D'Alelio and Dr. Ukrike Obertegger provided great help and support in the general organisation of the meeting. Flavia Brescancin, Manuela Milan, Jayant Ranjan and Shiva Shams, members of the research centre, provided invaluable logistic help during the workshop. We greatly appreciated the assistance of the Administration of E. Mach Foundation, in particular Dr. Sabrina Tanzi, and of the director of the Hostel, Dr. Anna Maria Llupi. Special thanks are due to the CENTRAL EUROPE Programme (European Lakes Under Environmental Stressors, 2CE243P3) for help in financing the stay of some participants at the workshop.

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PHYTOPLANKTON

Review Paper

Phytoplankton response to a changing climate

Monika Winder · Ulrich Sommer

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Abstract Phytoplankton are at the base of aquatic food webs and of global importance for ecosystem functioning and services. The dynamics of these photosynthetic cells are linked to annual fluctuations of temperature, water column mixing, resource availability, and consumption. Climate can modify these environmental factors and alter phytoplankton structure, seasonal dynamics, and taxonomic composition. Here, we review mechanistic links between climate alterations and factors limiting primary production, and highlight studies where climate change has had a clear impact on phytoplankton processes. Climate affects phytoplankton both directly through physiology and indirectly by changing water column stratification and resource availability, mainly nutrients and light, or intensified grazing by heterotrophs. These modifications affect various phytoplankton processes, and a widespread advance in phytoplankton spring bloom timing and changing bloom magnitudes have both been observed. Climate warming also affects phytoplankton species composition and size structure, and favors species traits best adapted to changing conditions associated with climate change. Shifts in phytoplankton can have far-reaching consequences for ecosystem structure and functioning. An improved understanding of the mechanistic links between climate and phytoplankton dynamics is important for predicting climate change impacts on aquatic ecosystems.

Keywords Light · Water column stratification · Temperature · Phenology · Primary production · Cell size

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Introduction

Phytoplankton account for <1 % of the photosynthetic biomass on Earth, but are nevertheless responsible for nearly 50 % of global net primary production and are the primary energy source for aquatic ecosystems (Field et al., 1998), and are also of global significance for climate regulation and biogeochemical cycling. The fate of these processes is critically dependent on phytoplankton community composition. Understanding the factors that control species composition and dynamics of these microscopic organisms is a fundamentally important goal in order to predict the impact of environmental change on aquatic ecosystems. Alterations in physical conditions, nutrient input, and

grazing pressure strongly affects the diversity, community structure, and temporal dynamics of phytoplankton. Associated with increasing anthropogenic impacts on ecosystems, the Earth's climate has warmed by approximately 0.6 °C over the past 100 years, which is an unprecedented increase compared with the past 1,000 years (IPCC, 2007), and this rate is expected to accelerate in the current century. Complementing the analyses of long-term trends in global conditions has been the recognition of large coherent spatial and temporal climate variability through changes of the Earth's atmosphere–ocean system (Stenseth et al., 2003). Interannual and subdecadal fluctuations in large-scale climate oscillations can have a strong influence on local climate conditions (Mantua et al., 2002; Stenseth et al., 2003). Long-term climate change and large-scale climate fluctuations are a crucial attribute of global climate change, and a wide range of studies have shown links between fluctuations in climate and ecological processes that affect phytoplankton dynamics (Behrenfeld et al., 2006; Paerl & Huisman, 2008).

Climate-driven physical fluctuations exert strong impacts on aquatic ecosystems because climate is modifying the abiotic and biotic environments. A substantial body of research has demonstrated the sensitivity of phytoplankton to climate change. Consequently, any changes at the base of the aquatic food web can have repercussions for the entire ecosystem. In this review, we illustrate the chain of linked processes from alterations in climate and meteorological conditions to phytoplankton production and taxonomic species composition. This is not a comprehensive review of phytoplankton responses to climate change. Rather, we focus on better-known processes and highlight studies where climate change has had a clear impact on phytoplankton. First, we provide a theoretical framework on climate-driven environmental factors that limit primary production. Then, we review major phytoplankton responses to climate change and discuss how changes in phytoplankton might influence ecosystem functioning.

Mechanistic links between climate and factors limiting primary production

Phytoplankton dynamics are linked to annual fluctuations of temperature, water column stratification, light availability, and consumption (Sommer et al., 1986; Cloern, 1996). Changing climatic conditions can modify these environmental factors and alter phytoplankton structure and taxonomic composition. Phytoplankton response can be both directly through physiology and indirectly mediated through effects on environmental factors limiting primary production, most notably light and nutrients.

Temperature effects on phytoplankton

Temperature directly affects plant metabolism, which consists of both photosynthetic and respiratory activity, while metabolic rates of primary producers are primarily limited by photosynthesis (Dewar et al., 1999). Phytoplankton blooms on ice margin in polar and subpolar seas (Smith & Nelson, 1985) and under clear ice in lakes (Vehmaa & Salonen, 2009) indicate that low temperature does not prevent exponential growth of phytoplankton in liquid water. Light-limited rates of photosynthesis are insensitive to temperature, but light-saturated ones increase with temperature, which indirectly increases the light level where light saturation begins (Tilzer et al., 1986). Therefore, it is expected that global warming increases light-saturated rates of photoautotrohic production, but not light-limited ones. As a result, a temperature increase should lead to greater plant growth rates and biomass accumulation under adequate resource supply (Padilla-Gamino & Carpenter, 2007). However, compared to photosynthesis rates, the metabolism of heterotrophic organisms is more sensitive to temperature (Allen et al., 2005; Lopez-Urrutia et al., 2006). Consequently, warming should increase consumption by herbivores more strongly than primary production. This can strengthen top-down control over primary production by increasing grazing rates (O'Connor et al., 2009; Sommer & Lewandowska, 2011), and thus affect phytoplankton production and taxonomic composition.

The most significant climatic effects on phytoplankton species composition will very likely be mediated through changes in thermal stratification patterns such as the extent of the growing season and vertical mixing processes (Schindler et al., 1996; Rodriguez et al., 2001; Diehl et al., 2002; Smol et al., 2005) (Fig. 1). Vertical mixing is one of the key variables that conditions the growth performance of phytoplankton within the water column (Diehl et al., 2002; Salmaso, 2005), because mixing processes are

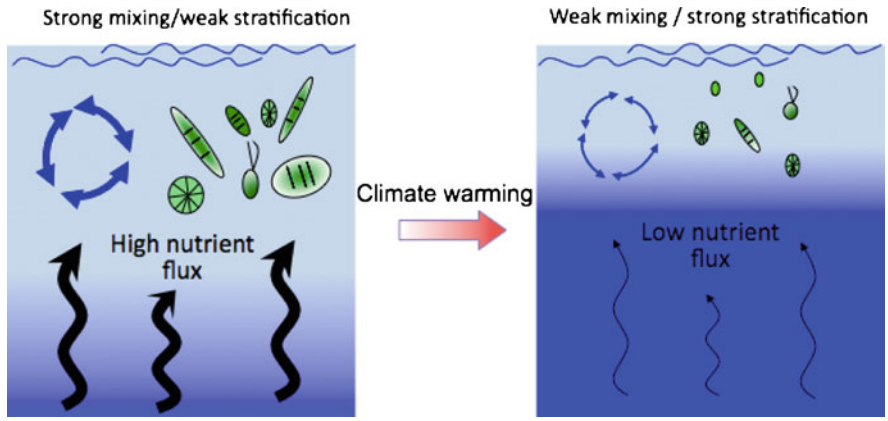

Fig. 1 The effect of increasing temperature on water column stratification (*blue arrows*), associated nutrient redistribution (*black arrows*), and phytoplankton production and cell size

usually accompanied by changes in resource availability of light and nutrients. Vertical mixing of natural waters is largely determined by meteorological variables. Heat exchange processes and wind action create two opposing tendencies—the tendency to stratify and suppress mixing, and the tendency for inputs of turbulent kinetic energy to promote mixing (Wetzel, 2001). The seasonal cycle of summer stratification and winter mixing is a product of the time-varying nature of these two tendencies. Changes in meteorological forcing result in a modification of the balance between stratification and mixing (King et al., 1997; Boyd & Doney, 2002; Livingstone, 2003).

A change in the intensity or duration of thermal stratification has a direct impact on turbulent diffusion and phytoplankton cell sedimentation, which are the major mechanisms causing vertical displacement for non-motile cells (Livingstone, 2003; Huisman et al., 2006). Thermal stratification and vertical mixing therefore has an immediate influence on phytoplankton sinking velocities, which increase non-linearly with size (Smayda, 1969), giving smaller and buoyant species an advantage in an environment where turbulence is not present to resuspend all planktonic species (Findlay et al., 2001; Huisman et al., 2004; Strecker et al., 2004).

Climate impacts on nutrient regimes

Water column mixing also affects nutrient availability for phytoplankton growth. Enhanced water column stratification suppresses the upward flux of nutrients from deep-water layers through vertical mixing, resulting in more nutrient-depleted conditions in surface waters (Livingstone, 2003; O'Reilly et al., 2003; Schmittner, 2005). As a consequence, altered mixing regimes affects the competitive advantage of specific algal cell types, that are better competitors for nutrients (Falkowski & Oliver, 2007) and that are able to maintain their vertical position in the surface water (Huisman et al., 2004).

On the other hand, for more eutrophic systems, mechanistic models predict that reduced vertical mixing will shift the competitive advantage between buoyant cyanobacteria and sinking phytoplankton species (Huisman et al., 2004). Intensified stratification can also lead to increased hypolimnetic oxygen depletion, which has widespread consequences for internal nutrient loading both for lakes and oceans (Jankowski et al., 2006; Shaffer et al., 2009). Thus, climate change might increase phosphorus concentration in concert with extended anoxic conditions (Wilhelm & Adrian, 2008), and effects of climate change may be similar to processes associated with eutrophication.

In addition, the frequency of extreme rainfall and severe drought has increased since the late 1970s (IPCC, 2007), affecting nutrient runoff from terrestrial sources (Briceño & Boyer, 2010). Increasing runoff can also modify the resource ratio in certain types of systems, depending on the nature of the geochemistry in the catchment and thus modifying the competitive advantage of phytoplankton species. For example, phytoplankton and bacterioplankton biomass is related to air temperature that controls the export of nitrogen and dissolved organic carbon from the catchment across subarctic Swedish lakes, indicating that climate may affect the balance between phytoplankton and bacterial production (Jansson et al., 2010). For coastal regions, enhanced upwelling due to an increasing

temperature gradient between land and sea are anticipated, which will increase nutrient availability and stimulate phytoplankton production (Rabalais et al., 2002). Similarly, melting ice at the poles can act as a nutrient source and can locally stimulate phytoplankton production (Smith et al., 2007). Overall, climate-associated changes in the nutrient environment vary strongly among ecosystems (Adrian et al., 2006).

Climate impacts on the underwater light environment

Light is essential for photosynthesis and therefore for food webs depending upon phototrophic production. The light experienced by phytoplankton is not independent of climatic conditions of temperature, wind, and precipitation. These climatic variables act on stratification, cloud cover, and—in coastal seas and lakes—runoff from land, which might transport suspended solids and dissolved humic substances that influence the underwater light climate. The impact of these variables can be demonstrated by calculating the mean light intensity of the mixed surface water layer I_{mix} ("epilimnion" in lakes) (Riley, 1957):

$$I_{\mathrm{mix}} = I_0(1 - \mathrm{e}^{-Kz})(Kz)^{-1}$$

where I_0 is the surface irradiance, K the vertical attenuation coefficient, and z the depth in meters. Under usual values of z and K, the term $(1 - \mathrm{e}^{-Kz})$ approaches 1, thus making I_{mix} inversely proportional to z and K. Each of the independent variables is under different climatic influences as discussed below.

The role of the mixing depth is the core of Sverdrup's (1953) classic "critical depth" concept for deep-water bodies. Vernal phytoplankton growth can only start when spring warming leads to a thermal stratification with a mixing less than a critical limit: at mixing depths (z_{m}) beyond that limit, water column respiration would be higher than water column photosynthesis, thus preventing an increase in biomass. In deep oceans and lakes, the onset of stratification easily leads to an order-of-magnitude decrease of z_{m} and thus an order of magnitude increase in mean mixed water column irradiance. The onset of stratification has thus been viewed as the big light switch that initiates the onset of the phytoplankton growth period. In accordance with this idea, Siegel et al. (2002) identified a minimal mixed water daily light dose of 1.3 mol photons m^{-2} days^{-1} (0.96–1.75) PAR for the onset of the spring bloom from remote sensing data of the North Atlantic. This threshold value was independent of latitude and, therefore, sea surface temperature. Siegel's threshold value was confirmed by mesocosms experiments with Baltic Sea plankton with temperature scenarios ranging from 2 to 8 °C warming (Sommer & Lengfellner, 2008).

Phytoplankton blooms have also been found before the onset of stratification when calm conditions permitted a sufficiently long residence of near surface phytoplankton in favorable light conditions (e.g., in Lake Constance; Tirok & Gaedke, 2007). Accordingly, the "critical depth" concept has been developed further into the "critical turbulence concept" (Huisman & Sommeijer, 2002). Such pre-stratification blooms in deep waters are, however, unstable, because any wind might induce deep mixing and distribute phytoplankton to greater depths and reduce the light supply. In shallow to medium deep-water bodies and non-stratifying systems, where either the bottom or a halocline is above Sverdrup's critical limit, phytoplankton blooms are strongly coupled to the external light regime, which is influenced by ice cover, cloud cover, or day length, and blooms can occur independently of temperature change (Sommer & Lengfellner, 2008).

The attenuation coefficient K depends on light attenuation by phytoplankton pigments through most of the growth season in waters where riverine input of turbidity or resuspension of sediments are unimportant, factors that are influenced by climate. The increase of K with chlorophyll acts as a negative feedback loop ("self shading") on phytoplankton growth, but at the start of the seasonal growth period, the background light attenuation is decisive for the light climate. In the open ocean, K is close to the value for clear water ($0.02\ \mathrm{m}^{-1}$), while it can reach values $>1\ \mathrm{m}^{-1}$ in brown-water lakes or turbid estuaries. In shallow waters, where wind can stir up the bottom sediment, sudden increases of K can happen any time of the year, creating an "optical winter". The same applies to suspended solids transported by floods of tributaries.

This means that the different aspects of the anticipated climate change will influence the light availability for phytoplankton differently and in partly counteracting ways: warming will lead to an earlier onset of thermal stratification in stratified water bodies, an earlier ice-melt, and a reduced mixing

depth in summer. All these tend to increase light availability. Increased windiness will partially counteract the effect of warming in deep-water bodies, i.e. delay the onset of stratification and increase mixing depth, and will increase the resuspension of sediments in shallow water bodies. Overall, the effect of wind on light availability is negative. Increased runoff from land will increase the transport of suspended particulate matter, and in certain geological settings (acidic bedrocks) increase the transport of humic matter to lakes and coastal seas. Due to their regional and episodic character, the changes in wind and runoff will increase the variance of light availability and thus phytoplankton production.

Climate effects on phytoplankton processes

Interactions between climate and phytoplankton are complex, because other factors such as resource availability, density dependence, and predation strongly control the abundance, distribution, and size structure of the community. Despite these complexity of interacting processes, some widespread climate-related responses have emerged, and the mechanisms involved in climate-related changes are becoming better understood (Richardson, 2008; Adrian et al., 2009). Impacts of climate change on plankton are mainly manifested as shifts in seasonal dynamics, species composition, and population size structure.

Phenology

Plankton blooms are important features in seasonal aquatic environments, where they drive many ecosystems and community processes, and are a major source of energy input for higher trophic levels (Smayda, 1997; Winder & Cloern, 2010). Seasonal phytoplankton succession is a community phenomenon that is controlled by processes that regulate population dynamics of various primary producers and consumers (Sommer, 1989). Blooms are triggered by individual species' life history and physiological responses to changing abiotic conditions. Timing and magnitude of blooms are controlled by population feedbacks and mediated through resource dynamics and predator–prey interactions (Sommer et al., 1986; Carpenter et al., 2001; Jäger et al., 2008). The onset of plankton spring blooms is usually initiated by changes in water temperature and light supply. In deep systems, spring phytoplankton blooms are coupled to the onset of thermal stratification, which increases the mean light exposure of phytoplankton cells in the mixed surface layer. Under these conditions, spring blooms are triggered by correlated increases in temperature and seasonal light availability (Edwards & Richardson, 2004; Winder & Schindler, 2004b; Peeters et al., 2007). In shallow, well-mixed systems, phytoplankton blooms are strongly coupled to the external light regime, that is influenced by ice cover, cloud cover, or day length, and can occur independently of temperature change (Sommer & Lengfellner, 2008).

A large number of studies have reported that the timing and magnitude of seasonal plankton blooms are shifting in response to climate change (Straile, 2002; Edwards & Richardson, 2004), which agrees with predictions from dynamical models of pelagic producer–grazer systems (De Senerpont Domis et al., 2007). Particularly, shifts in plankton spring phenology related to climate have been shown in several ecosystems, whereas later in the season other factors like biotic interactions often complicate the extraction of a clear climate signal. For example, vernal warming advanced the timing of stratification onset and the spring bloom in Lake Washington by 20 days over the last four decades (Winder & Schindler, 2004b). Shifts in bloom timing have also been observed in the Western Scheldt Estuary, where earlier onset of blooms have paralleled increasing temperature over the last 30 years (Kromkamp & Van Engeland, 2009). Similarly, a shift to the warm phase of the North Atlantic Oscillation (NAO) caused advancement of stratification onset and the spring bloom in the Baltic Sea (Smayda et al., 2004; Alheit et al., 2005), and shifted the timing of various phytoplankton taxa in the North Sea. Earlier timing of the spring bloom was also observed across central European lakes during the warm NAO phase as a result of accelerated early summer algal suppression due to faster growth of herbivores in warmer water (Straile, 2002). Similarly, new autumn phytoplankton blooms developed in San Francisco Bay through a trophic cascade induced by a shift of the east Pacific to its 'cool' phase in 1999 (Cloern et al., 2007). These studies are consistent with widespread observations in freshwater and marine systems (Blenckner et al., 2007; Thackeray et al., 2010).

Similar to field observations, increasing temperature advances the timing of phytoplankton spring peaks consistently in marine and freshwater systems and in taxonomic groups in mescosm experiments (McKee and Atkinson, 2000; Winder et al., 2012). These studies showed that, in well-mixed systems, earlier occurrence of phytoplankton peaks at high temperatures are independent of light intensity and are primarily driven by increased grazing pressure at higher temperatures that terminated the phytoplankton bloom earlier (Sommer & Lewandowska, 2011; Winder et al., 2012). Intensified grazing at increased temperature can also affect phytoplankton species composition and size structure, as has been shown in mesocosm experiments dominated by copepod grazers that prey preferentially on intermediate phytoplankton size classes (Lewandowska & Sommer, 2010).

The degree of advance varies, however, among taxonomic groups. For example, mesocosm experiments have demonstrated that cryptophytes and diatoms showed the strongest response to warming (Winder et al., 2012), which is in agreement with their physiological characteristics (Gervais, 1997; Litchman et al., 2007). In the North Sea, diatom taxa showed large phenologial variation, whereas the timing of total diatom biomass did not change (Edwards & Richardson, 2004), which is likely associated with the diatom community composition that is dominated by taxa forming resting stages that are triggered by the photoperiod and thus are not coupled to shifts in water column stratification. Differential responses to climate change can be expected and largely depend on the life strategies of the community.

In addition to peak timing, climate also affects phytoplankton peak magnitudes (Berger et al., 2010); the effects are, however, strongly sensitive to changes in algal carrying capacity as mediated by light supply (Jäger et al., 2008; Schalau et al., 2008). High light intensity typically increases phytoplankton bloom magnitude in mesocosm experiments (Berger et al., 2010; Winder et al., 2012). In contrast, intensified grazing at higher temperatures can create opposite patterns in phytoplankton bloom dynamics. This suggests that light limitation can have pronounced effects on plankton succession and that tight predator–prey coupling can suppress a response of phytoplankton to increased temperature.

Phytoplankton production

While climate-related phenological shifts towards earlier spring events are broadly observed, there has been a lack of consensus on how climate affects plankton production (Boyce et al., 2010; Taucher and Oschlies, 2011). Satellite and long-term field observations show that phytoplankton in the Pacific Ocean oscillate with large-scale climate patterns, and chlorophyll concentrations are typically lower during warm periods (Behrenfeld et al., 2006). Using a combination of water transparency and chlorophyll measurements, Boyce et al. (2010) extended the historical dataset and showed that ocean chlorophyll concentration decreased in large parts of the North Pacific and North Atlantic that parallelled increases in surface temperature. In contrast, long-term chlorophyll observations from continuous sampling programs showed that concentration increased in certain regions of the Pacific and Atlantic Oceans (McQuatters-Gollop et al., 2011). Simulation studies suggest that increased phytoplankton metabolism with increasing temperature may counteract the reduced nutrient redistribution into surface water, yielding a net increase in ocean productivity (Taucher & Oschlies, 2011). However, a temperature increase will also enhance the metabolism of heterotrophs that can intensify top-down control and may reduce phytoplankton production.

These contradictory results indicate the necessity for controlled observational programs. Phytoplankton production is a complex function of physical and physiological effects on predator–prey interactions, and effects of climate change on phytoplankton production will likely vary among sites, depending on resource limitation and species composition.

Species composition and size structure

The performance of individual phytoplankton species is strongly governed by the thermal stratification's impact on vertical mixing within the water column, which alters the position of phytoplankton relative to nutrients and light. Margalef (1978) proposed an empirical relationship between the interplay of turbulence, nutrient supply, and taxonomic composition. Based on this model, specific phylogenetic morphotypes (r versus K growth strategists) are selected along a continuum of habitat mixing and nutrient conditions.

The model predicts that, in marine systems, dinoflagellates are favored at weak mixing and diatoms at intensified mixing. In a further elaboration of his concept, Margalef separated the prediction under low turbulence: dinoflagellates will dominate under eutrophic and coccolithophore under oligotrophic conditions. For freshwater phytoplankton, Reynolds (1987) suggested a distinct association of phytoplankton key genera and morphological properties with the nutrient and turbulence environment. Similarly, experimental (Reynolds et al., 1983; Diehl et al., 2002; Ptacnik et al., 2003; Berger et al., 2007) and theoretical (Diehl et al., 2002; Huisman et al., 2004) work documented shifts in algal community structure and dynamics related to physical mixing processes.

Thermal stratification and vertical mixing has an immediate influence on phytoplankton sinking velocities that give smaller species an advantage in an environment where turbulence is not present to resuspend all planktonic species (Bopp et al., 2005). In contrast, buoyant species and flagellates have relatively low net sinking velocities (Findlay et al., 2001; Huisman et al., 2004; Strecker et al., 2004), and the latter are highly motile and are capable of selecting an appropriate light and nutrient environment in the water column (Fee, 1976). Similarly, bloom-forming phytoplankton species (e.g., cyanobacteria) may contain gas vesicles to decrease their density. These functional species traits have a distinct competitive advantage at reduced vertical mixing (Findlay et al., 2001; Huisman et al., 2004; Strecker et al., 2004).

In line with these predictions, several studies have shown that bloom-forming cyanobacteria have a competitive advantage over other phytoplankton taxa at higher temperatures in eutrophic systems (Jöhnk et al., 2008; Paerl & Huisman, 2008). Cyanobacteria have a higher maximum specific growth rate compared to diatoms and green algae at temperatures above 23 °C (Reynolds, 2006; Jöhnk et al., 2008), which makes cyanobacteria a strong competitor at high temperatures. Many cyanobacteria have the ability to form intracellular gas vesicles, which provide them with the ability to exploit habitats under intensified stratification and to outcompete other taxa (Paerl & Huisman, 2008). In addition, under eutrophic conditions, increasing temperatures will lead to steeper nutrient gradients in the thermocline, which gives an advantage to effective vertically migrating phytoplankton, such as cyanobacteria and dinoflagellates. The increase in cyanobacteria at higher temperatures has been reported in several lakes with different mixing regimes (Jöhnk et al., 2008), and along a latitudinal gradient in shallow lakes (Kosten et al., 2011).

Enhanced water column stratification and subsequently more nutrient-depleted conditions in surface waters is also expected to affect phytoplankton size structure (O'Reilly et al., 2003; Schmittner, 2005). Under a low nutrient concentration, small-sized algal taxa are expected to be favored because their high surface area to volume ratio enables rapid nutrient exchange through the cell surface (Litchman et al., 2007). In addition, small cells exhibit lower sinking rates and divide more rapidly, which is favorable under reduced mixing conditions.

In support of this hypothesis, shifts in size structure have been observed in planktonic organisms, including diatoms, dinoflagellates, and foraminifera, over geological and centennial time scales (Schmidt et al., 2004; Smol et al., 2005; Finkel et al., 2007; Rühland et al., 2008), and these shifts have been linked to changing water column stratification related to changing climate. Fossil records document that diatom community structure has been altered by environmental change over their evolutionary history (Finkel et al., 2005). On a geological time scale, macro-evolutionary changes coincide with changing hydrodynamic mixing processes, such as changes in sea-level and ocean thermal structure that are climate driven (summarized in Falkowski & Oliver, 2007). Marine diatom size structure and diversity shifted towards a smaller size in response to intensified thermal stratification linked to increasing ocean temperatures (Burckle et al., 1981; Finkel et al., 2005). Similarly, paleolimnological studies from high latitude and altitude systems indicate a widespread expansion of pelagic and small-sized diatoms in more recent decades (Sorvari et al., 2002; Saros et al., 2003; Rühland & Smol, 2005; Roberts et al., 2006), which is largely attributed to a longer ice-free season and increased stratification in deep lakes (Smol et al., 2005; Rühland et al., 2008).

Changes in temperature and physical mixing have also affected the competitive advantage of small-sized diatom cells in Lake Tahoe, as a response to contemporary climate warming (Winder et al., 2009). Small-sized cells within the *Cyclotella* genus increased with intensified stratification, whereas large-sized diatoms

dominated under stronger turbulent mixing conditions and decreased over the record of sampling. The selection for small-sized diatoms was accompanied with a shoaling trend in their vertical position in the water column (Winder & Hunter, 2008). In the North Atlantic, the dominance of pico-phytoplankton (<2 μm) increased with temperature and picophytoplankton cell size decreased with temperature (Moran et al., 2010). A trend towards smaller phytoplankton size under warming has also been reported from mesocosm experimental studies (Daufresne et al., 2009); however, its universal applicability is still controversial (Gardner et al., 2011; Rüger and Sommer 2012).

Conclusions

A floristic shift can have cascading ecosystem effects and, consequently, climate-driven changes likely alter important ecosystem functions, including primary production, biogeochemical cycling (Richardson & Jackson, 2007), energy transfer through the food web, and plankton community structure via size-dependent species interactions (Sommer et al., 2002). Shifting phytoplankton phenologies are critical for ecosystem production if phenological responses differ among primary producers and consumers (Edwards & Richardson, 2004; Winder & Schindler, 2004a; Seebens et al., 2007). The different degrees of change in phytoplankton peaks and zooplankton growth can lead to a decoupling of zooplankton food requirements and peak food availability. Asynchronization between peak food availability and requirements can result in predator–prey mismatches that can affect energy transfer to higher trophic levels (Cushing, 1974). Differences in the temporal match of a predator with its prey have been observed in algal–herbivore interactions (Edwards & Richardson, 2004; Winder & Schindler, 2004a; Adrian et al., 2006).

Reorganizations within the phytoplankton community with changing thermal structure of the water column will shift the dominance towards small-sized algal cells and species that are able to regulate their buoyancy (Findlay et al., 2001; Huisman et al., 2004; Strecker et al., 2004; Bopp et al., 2005; Smol et al., 2005). A shift towards small-sized cells will result in lower export production, which can have positive feedbacks on the climate system. These changes in primary producers will have associated effects on primary production and biogeochemical cycling of carbon and other elements. In addition, blooms of cyanobacteria or dinoflagellates have large ecosystem impacts on trophic transfer, water quality, and fish production.

Phytoplankton variability is a key driver of biogeochemical variability (Cloern, 1996; Behrenfeld et al., 2006) and fluctuations in annual fish recruitment (Platt et al., 2003). An improved understanding of the inherent natural variability of phytoplankton is therefore important for forecasting the extent of global change impact on aquatic ecosystem functioning. A current challenge is to predict how compositional shifts propagate up to higher trophic levels, and how synergistic effects of climate warming and other environmental changes will affect ecosystem functioning. The extent of physical changes and potential for species to adapt to changing environmental conditions will greatly influence food-web dynamics as the future climate warms and becomes more variable.

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PHYTOPLANKTON

Long-term trends and fine year-to-year tuning of phytoplankton in large lakes are ruled by eutrophication and atmospheric modes of variability

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Abstract This study demonstrated how the impact of eutrophication in a deep lake at the southern border of the Alps (Lake Garda) was regulated by specific modes of atmospheric circulation relevant for the Mediterranean area. At the decadal scale, nutrients and phytoplankton increased concurrently since the 1970s. At the annual scale, year-to-year fluctuations in nutrients and phytoplankton were controlled through a chain of causal factors centred on deeply penetrative mixing events determining an upward transport of phosphorus from the hypolimnion to the trophogenic layers. The extent of mixing was in turn controlled by lake and air winter temperature, which were ultimately regulated by the winter fluctuations of the East Atlantic pattern (EA). In its negative state, the EA shows an intense high pressure over the West Atlantic, causing a north-easterly air flow bringing cold air from continental Europe to Mediterranean, thus favouring greater lake mixing and nutrient fertilisation. Cyanobacteria (mostly *Planktothrix rubescens*) were the organisms which greatly benefitted from the long-term increase in phosphorus concentrations and the year-to-year fluctuations in surface phosphorus availability controlled by the EA. Given the same availability of phosphorus in the water column, positive winter EA phases weakened the eutrophication effects and phytoplankton development.

Keywords Deep lakes · Phytoplankton · Cyanobacteria · Eutrophication · East Atlantic pattern · North Atlantic Oscillation

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Introduction

Anthropogenic impacts on lakes include a wide range of physical, chemical and biotic stressors. The identification of the relative importance of these factors should take into account a whole spectrum of confounding processes (Adrian et al., 2009). Stressors may interact in additive or synergistic ways, resulting in cascade effects which are not always simple to disentangle. In addition, the impact of environmental and biotic stressors on the processes affecting planktonic organisms is mediated by latitudinal location, lake internal physical structures and hydrological settings. A paradigmatic example is represented by the effects of winter climate on lakes of different depth and geographic location. In northern temperate lakes,

reduced ice cover and increasing water temperatures are able to determine more intense and earlier blooms irrespective of phosphorous concentrations (e.g. Huber et al., 2008). Similar effects were found in shallow ice-free, temperate polymictic lakes (Meis et al., 2009). In contrast, a few investigations showed that warmer winters in the deep lakes have counter-intuitive effects, decreasing the extent of the spring mixed layer, the spring epilimnetic replenishment of nutrients and the growth of phytoplankton (Goldman & Jassby, 1990). These dynamics were outstandingly evident in the group of deep lakes south of the Alps, particularly in lakes Garda, Iseo and Lugano (Simona, 2003; Salmaso, 2005). Actually, the foremost anthropogenically linked stressors in this group of lakes are eutrophication and climate change.

Many investigations made use of teleconnection indices to study the effects of large-scale climate variability on different types of ecosystems. In Europe, a huge amount of studies related the temporal fluctuations of the North Atlantic Oscillation (NAO) with the temporal variability observed in different limnetic, marine and terrestrial ecosystems (e.g. Hurrell et al., 2003; Luoto & Helama, 2010; Lehmann et al., 2011). In lakes across Europe, fluctuations of NAO values were found to affect many limnological features, including physical (water temperature, lake ice cover and level changes), chemical (input and vertical distribution of nutrients) and biological responses (shift in the development of selected zooplankton and phytoplankton groups, and changes in species composition) (among the others, see Livingstone & Dokulil, 2001; Blenckner & Chen, 2003; George, 2007; Straile et al., 2003, and references therein). In general, these studies showed a strong connection between high positive NAO phases and mild winters, and between negative NAO phases and cold winters over Europe. By converse, the relationship between the winter climate and NAO in the lake district south of the Alps was uncertain, probably because of the location of the region, between different centres of action (the Mediterranean region and central and northern Europe). In Lake Garda, NAO was shown to have only a slight and non-significant influence on the winter climate and spring water temperatures (Salmaso, 2005). Similar uncertainties were found in Lake Maggiore, where Morabito (2007) suggested a possible coincidence in the temporal patterns of NAO and deep circulation episodes. However, the available data did not demonstrate the existence of statistically significant correlations between NAO on the one side, and mixing depth and biological variables on the other side. Again in Lake Maggiore, Manca & DeMott (2009) showed that between 1986 and 1993, *Bythotrephes* exhibited a greater than 10-fold increase in mean annual abundance, and that the increase was more intense between 1987 and 1990, during high NAO winter values and warm winter and spring conditions. However, no attempts were made to confirm statistically these relationships over longer periods.

In a recent study, Salmaso (2012) analysed the impact on the air winter temperatures of five teleconnection patterns potentially important for the interannual climate variability over the Mediterranean region and southern Europe. The indices included the NAO, the East Atlantic pattern (EA), the Scandinavia pattern (SCAND), the East Atlantic/West Russia pattern (EA/WR) and the Eastern Mediterranean Pattern (EMP). Among these indices, only the EA pattern and the EMP showed a clear relationship with the variables directly connected with the winter climate and limnological variables in the southern subalpine region. In this study we enlarge our focus, with the aim to understand, taking into account the relevant temporal scales, the effects of long-term eutrophication and atmospheric modes of variability on Lake Garda. Specific objectives are: (i) at the decadal scale, to identify the relationships linking long-term eutrophication and phytoplankton; (ii) at the annual scale, to examine the principal processes linked to climatic forcing and nutrients availability governing the selection and development of phytoplankton. In this work, our focus will be on the effects of the EA pattern during the winter months.

Materials and methods

Field measurements and water sampling were carried out at the deepest zone of Lake Garda, out of Brenzone (z_{max} = 350 m; 45°41′51″N, 10°43′15″E; mean surface level, 65 m a.s.l.). Samples were collected at least every 4 weeks, for a total of 13 samples per year. Temperature, pH, conductivity and dissolved oxygen in the water column were recorded since 1991 using underwater multiparametric probes (Idronaut and Seacat-Seabird probes). Overall, these data were used to

estimate the extent of maximum spring mixing, which was taken to be the maximum point at which an upper layer of almost-uniform values for the selected variables met a lower layer of rapidly changing values (Salmaso, 2005). As for water temperature, mixed conditions in the hypolimnetic layers were characterised by uniform values (practically equal to 0°C/m). Complete phytoplankton (0–20 m layer) and chemical data (8–9 depths from 0 to 350 m) are available since 1993 and 1995, respectively. Phytoplankton samples were fixed with acetic Lugol's solution and countings were performed on 10 ml sedimentation chambers observed with inverted microscopes. Algal biovolumes were calculated from abundances and specific biovolumes (Rott et al., 2007). Nutrients were analysed following standard methods (APHA, AWWA & WEF, 1995).

Mean daily air temperatures were measured since 1984 at the meteorological station of Arco (91 m a.s.l.), 5 km away from the northern border of the lake. The station is part of the Meteorological station network coordinated by the Agrarian Institute of S. Michele all'Adige.

The climatic conditions and limnological characteristics of Lake Garda were related to the mean values of the EA pattern computed for the winter period (December, January and February, EA_{DJF}). The mean monthly EA values were calculated by the NOAA-CPC (www.cpc.ncep.noaa.gov), using rotated principal components analysis (RPCA) applied to monthly mean standardised 500 hPa height anomalies measured since 1950 in the region 20°N–90°N. This work utilises the new indices, which have been standardised by the 1981–2010 climatology. EA is structurally similar to the NAO, and it is defined by a north–south dipole of anomaly centres spanning the North Atlantic from east to west. Contrary to the NAO, the anomaly centres of the EA pattern are displaced south-eastward. In its negative state, the EA_{DJF} mode shows an intense high pressure over the West Atlantic, causing a north-easterly airflow which brings cold air from continental Europe over the full Mediterranean basin (Josey et al., 2011; Fig. 1). This process is progressively attenuated and reversed with a progressive change of the EA_{DJF} towards positive values.

A detailed description of the methods used in the field and laboratory was reported by Salmaso (2011, 2012).

Relationships between variables were analysed by generalised least squares (GLS) models. Besides

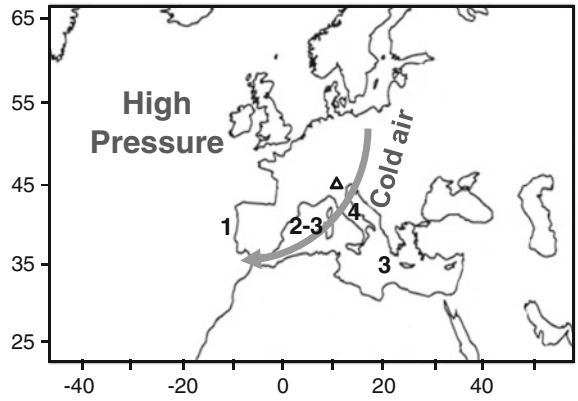

Fig. 1 The surface high pressure anomaly and airflow characterising the negative phase of the East Atlantic pattern. The *triangle* indicates the location of Lake Garda. The *numbers* indicate the locations where the impacts of the EA have been documented in the Mediterranean region. *1* deCastro et al. (2008); *2* Schroeder et al. (2010); *3* Josey et al. (2011); *4* Toreti et al. (2010). Modified from Josey et al. (2011)

computation of the Durbin–Watson statistic (5% level), assumption of independence of residuals in regression analyses was tested by including an autoregressive process of order 1 in the GLS models (Pinheiro & Bates, 2000). In this case, the inclusion of AR(1) models was verified based on the small sample Akaike information criterion (AICc), which includes a correction for finite sample sizes (Burnham & Anderson, 2004). However, autocorrelation in the residuals was found and verified only in one occasion (Fig. 4a). As for phytoplankton, computations were made on log-transformed biovolumes. Smoothing trends in the time series were calculated by applying a LOWESS smoother with a span equal to 2/3. To avoid spurious correlations originating from the presence of temporal trends, before regressions the variables in Figs. 3, 6 and 7 were linearly de-trended. Statistical analyses and statistical graphs were calculated in R 12.13.2 (R Development Core Team, 2011), specifically using the packages lmtest, nlme, mgcv and AICcmodavg.

Results

East Atlantic pattern and air temperature in the winter months

Winter EA pattern values increased significantly since 1951 (Fig. 2a). During the last 10–15 years, this index

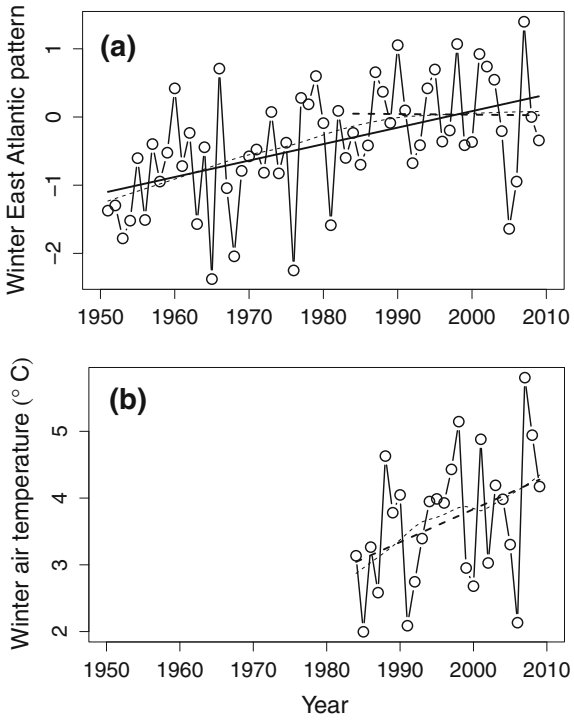

Fig. 2 Temporal variations of **a** the East Atlantic pattern (EA_{DJF}) and air temperature (T_{airDJF}) during the winter months (December–February). The years refer to the months of January and February, e.g. the 1951 value contains the average of December 1950, and January and February 1951. The linear regressions of EA_{DJF} (1951–2009) and T_{airDJF} (1984–2009) are significant at $P < 0.01$ and $P = 0.06$, respectively. The series are smoothed using the LOWESS procedure

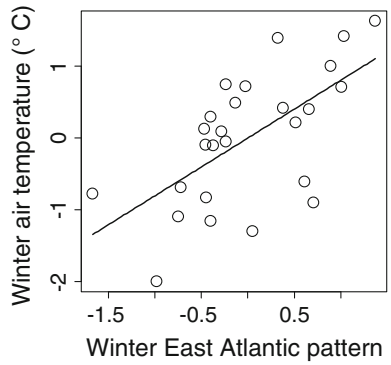

Fig. 3 Relationship ($P < 0.01$) between the air temperatures and the East Atlantic pattern during the winter months (December–February). Calculations were made on linearly detrended variables

showed large fluctuations, with values spanning from −1.5 to 1.4 and reaching, in 2005, negative values comparable to those measured in the 1970–1980s. For the sampling period (1993–2009) the index did not show any temporal pattern ($P \gg 0.05$). The interannual variations in the EA_{DJF} strictly controlled the corresponding variations in the winter air temperature values (T_{airDJF}; Figs. 2b, 3). However, contrary to EA_{DJF}, in the period for which the data were available (since 1984), T_{airDJF} showed a slight and only marginally significant tendency to increase (slope = 0.05°C year^{-1}, $P = 0.06$) (Fig. 2b). For the same period, EA_{DJF} did not show any particular trend (Fig. 2a). The decrease in the winter EA and air temperature values from 2004 to 2006 determined a greater cooling of spring water temperatures, which triggered the complete overturn of the lake. Other episodes of complete overturn associated with a complete replenishment of nutrients from the hypolimnetic to the epilimnetic waters (see over) were documented in 1991 and 1999–2000 (for a complete description see Salmaso (2005, 2011, 2012).

Long-term development of trophic variables and phytoplankton

The annual average values of total phosphorus (TP) in the whole water column (0–350 m, TP_{lakeYR}) of Lake Garda showed a tendency to increase, but with a stabilisation of values around 19 µg P l^{-1} during the last years (Fig. 4a). The vertical distribution of TP in the water column was characterised by marked inhomogeneities. In the whole study period, average concentrations of TP in the trophogenic layers (0–20 m) and in the deep hypolimnion (200 m bottom) were 10 and 31 µg P l^{-1}, respectively. The overall increase in the trophic status was followed by a significant decrease in water transparencies (Fig. 4b), and an increase in total phytoplankton biomass (Fig. 4c), especially due to an increase in cyanobacteria (Fig. 4d). On an annual basis, this group contributed with percent biovolumes on the total ranging from 5% to over 30% (Fig. 5). Cyanobacteria were almost all dominated by *Planktothrix rubescens*, which is considered the typical cyanobacterium in the deep southern subalpine lakes (D'Alelio & Salmaso, 2011), with minor contributions due to other species (*Snowella* cf. *aracnoidea*, *Planktolyngbya limnetica*, *Aphanothece* spp., *Limnotrix* sp.). In the considered period (1993–2009), *P. rubescens* represented the

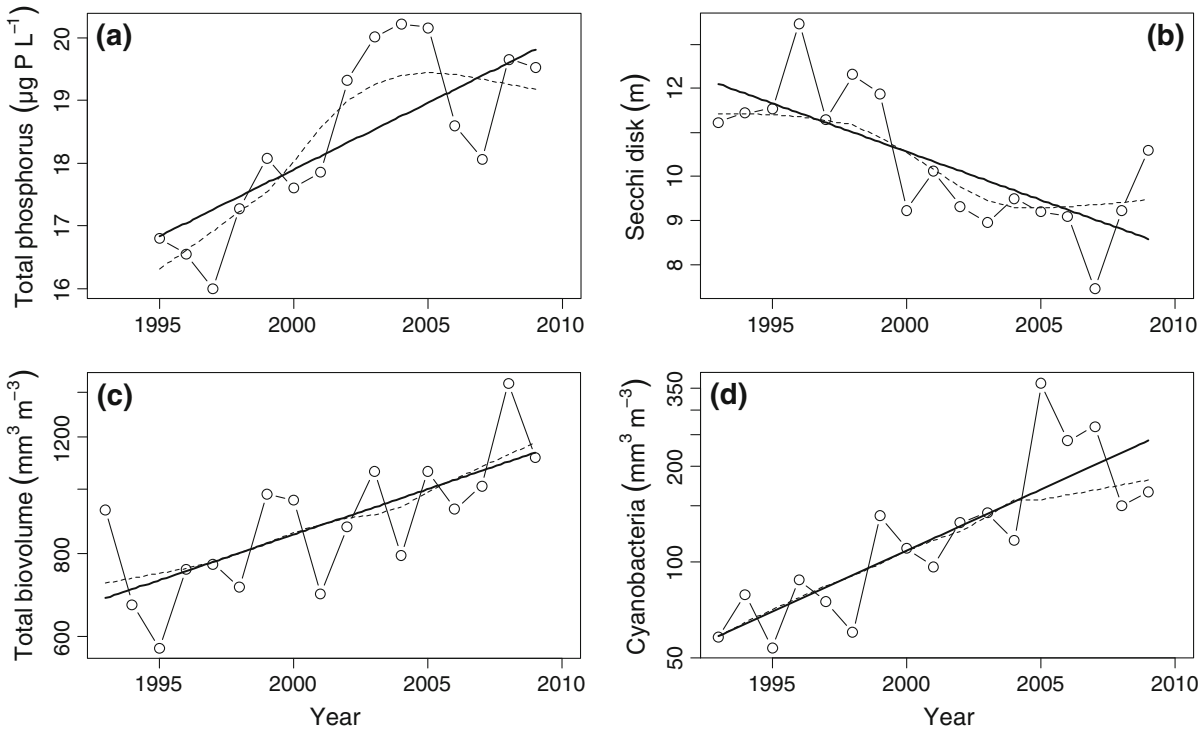

Fig. 4 Annual average values of **a** total phosphorus concentrations in the whole water column (0–350 m) from 1995 to 2009; the mean concentrations were obtained by computing the volume weighted average values measured in selected layers. Annual average values of **b** water transparency, **c** total phytoplankton biovolume and **d** biovolume of cyanobacteria in the layer 0–20 m from 1993 to 2009. The linear regressions are significant at $P < 0.1$ (**a**), and $P < 0.01$ (**b**–**d**), respectively. The series are smoothed using the LOWESS procedure

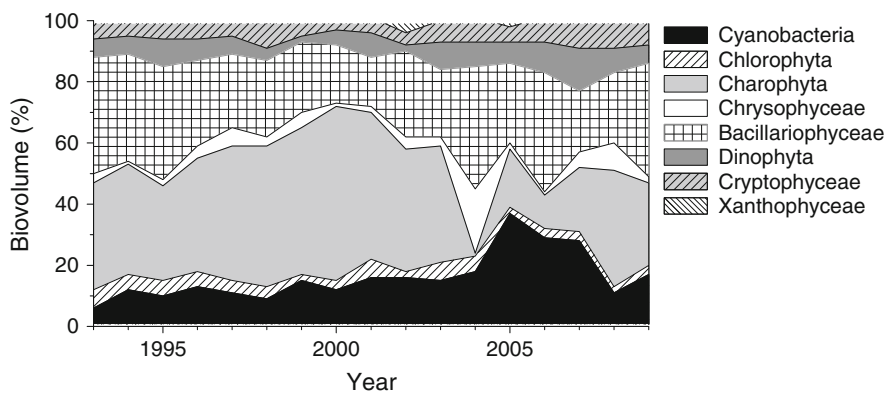

Fig. 5 Temporal changes in the percentage contribution of the phytoplankton groups to total biovolume in the layer 0–20 m

85% of the cyanobacterial biomass developing in the lake, constituting a serious concern due to its toxicity (Chorus et al., 2000). Another group which showed an increase in biovolume values (though at the border of significance, $P \sim 0.05$; figure not shown) was represented by the Dinophyta (mostly *Ceratium hirundinella*, *Gymnodinium* spp., *Peridinium* spp.). The remaining algal groups did not show any particular trend. After cyanobacteria, the most abundant groups were the large conjugatophytes (*Mougeotia* sp.), and the diatoms (*Fragilaria crotonensis*, followed by *Asterionella formosa*, *Tabellaria fenestrata* and *Diatoma tenuis*) (Fig. 5). Though based on previous periods, a detailed account on the long-term changes

in the phytoplankton community of Lake Garda was reported in Salmaso (2011, and references therein).

Overall, water transparency ($P = 0.02$), total phytoplankton biovolumes ($P = 0.01$) and the biovolume of cyanobacteria ($P < 0.01$) were significantly related to the concentrations of TP in the lake (figures not shown). It is worth noting that, as evidenced by the LOWESS smoothers, the tendency to the stabilisation of TP during the last 5 years seemed paralleled by a similar diminishing rate of change in the Secchi disk values (Fig. 4b) and biovolume of cyanobacteria (Fig. 4d). However, considering that these changes are very recent, a possible, consistent reversion of the long-term trends will need to be confirmed by future observations.

As we have seen, the increase in TP_{lakeYR} values was able to explain the long-term decadal changes in a few important trophic variables in Lake Garda. By converse, TP_{lakeYR} did not explain the large year-to-year fluctuations that characterised transparency and phytoplankton (see Fig. 4). In fact, after linear detrending, regression of water transparency and phytoplankton biovolumes on TP_{lakeYR} did not show any statistical significance ($P \gg 0.1$).

Multiple cascading effects in freshwater ecosystems: from atmospheric modes to phytoplankton fluctuations

As expected, and similarly to the relationship in Fig. 3, the winter EA pattern showed a clear, significant impact on the winter air temperatures (T_{airDJF}) measured in the period for which full environmental and biological data were available (1995–2009, Fig. 6a). In addition, EA_{DJF} was strictly related to the minimum water temperatures measured at maximum spring

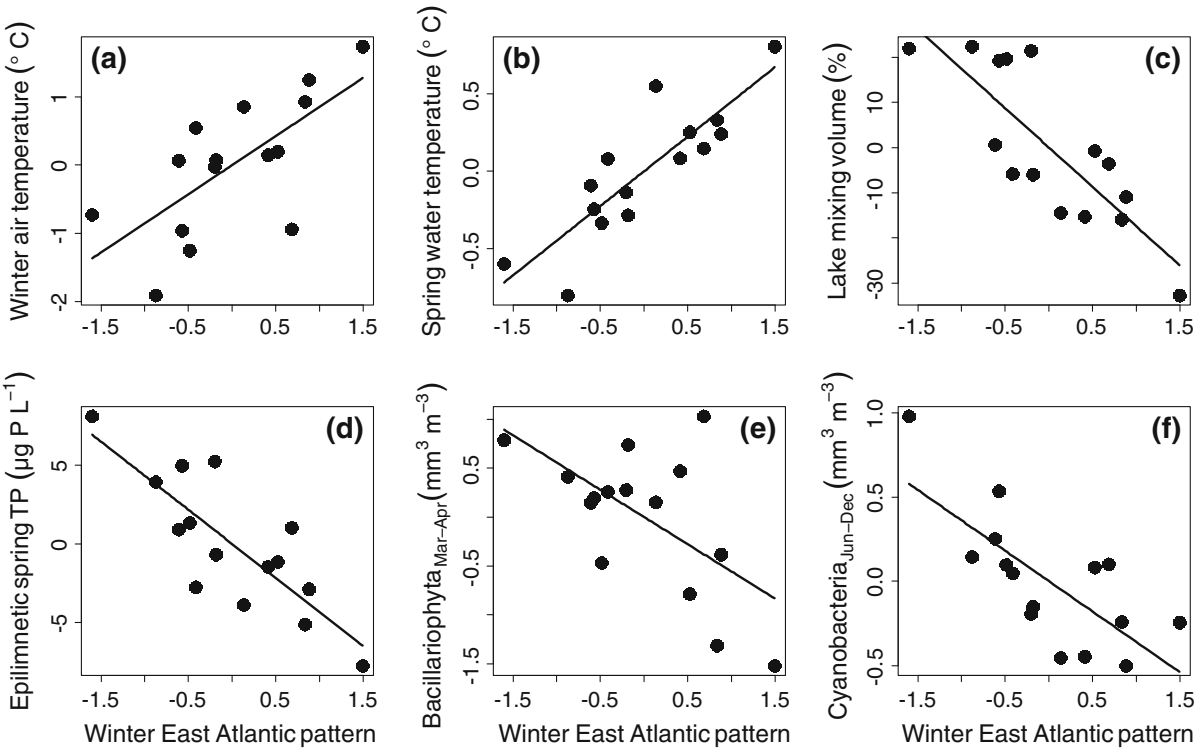

Fig. 6 Impact of the winter values of the East Atlantic (EA_{DJF}) pattern on **a** the winter air temperatures (T_{airDJF}), **b** the spring water temperatures in the upper hypolimnion and epilimnion (0–50 m; T_{0-50S}), **c** the extent of spring water mixing, as percent of the total lake volume ($MixVol_{\%}$), **d** the maximum concentrations of total phosphorus at spring overturn, between February and March (TP_{epiS}), and the average biovolumes of **e** Bacillariophyta in spring (March–April) and **e** cyanobacteria in summer and autumn (June–December). The data include only the period for which full environmental and biological data are available (1995–2009; $n = 15$). Before regressions, the variables were linearly de-trended. The relationships are significant at $P < 0.01$, with the exclusion of (**e**) ($P = 0.02$)

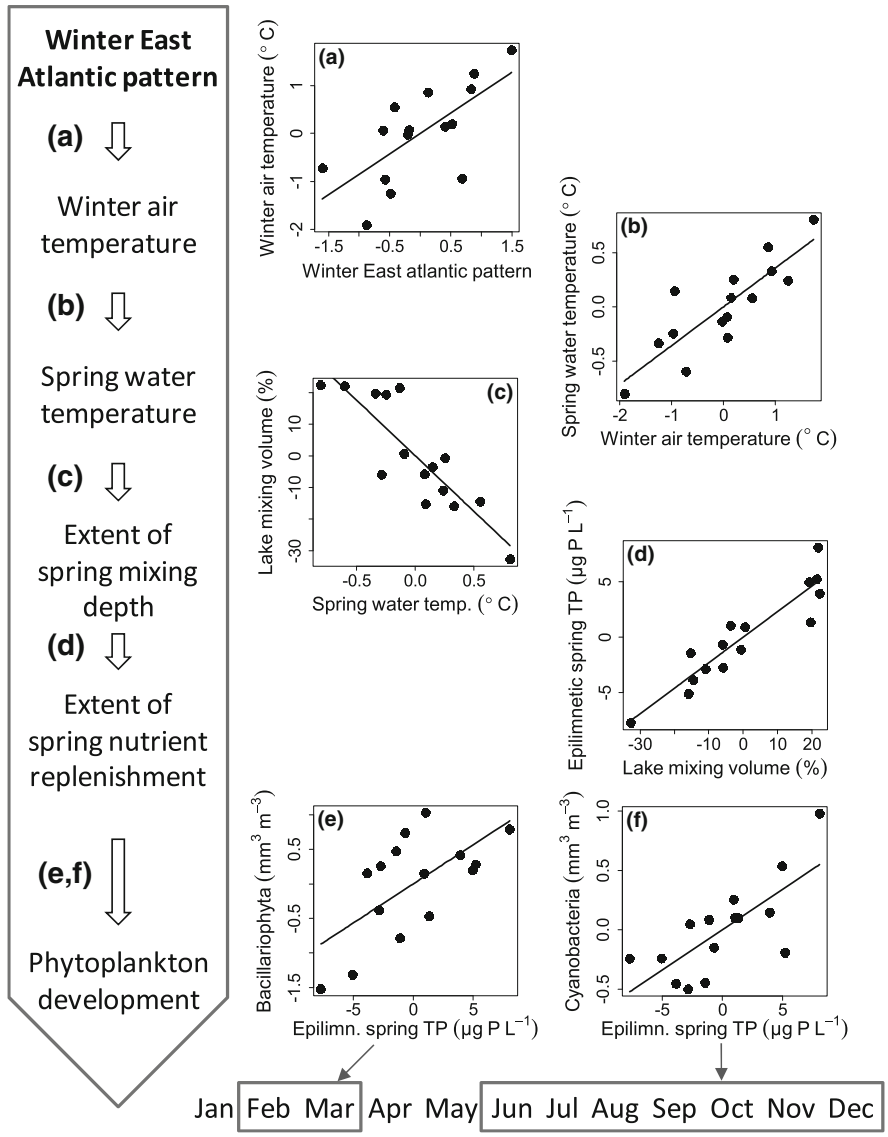

Fig. 7 Cascading effects on the limnological characteristics of Lake Garda originating from the year-to-year oscillations in the winter EA. The data include only the period for which full environmental and biological data are available (1995–2009; $n = 15$). Before regressions, the variables were linearly de-trended. The relationships are significant at $P < 0.01$. The average values of diatoms and cyanobacteria in the single years were computed for the periods indicated by the *two arrows*

overturn in the upper hypolimnetic and epilimnetic layer (between 0 and 50 m; $T_{0\text{–}50S}$, Fig. 6b), to the fraction of the spring lake volume undergoing complete mixing and homogenisation ($MixVol_{\%}$, Fig. 6c), and to the extent of replenishment of nutrients in the trophogenic layers during and at the end of lake mixing, in spring (TP_{epiS}, Fig. 6d). Complete lake circulation episodes determined a complete fertilisation of the trophogenic layers. During the years of complete overturn (1999–2000 and 2004–2006), average concentrations of TP in the layer 0–20 m were 21 µg P l^{-1}, compared with an average value of 14 µg P l^{-1} in the years of partial mixing. The concentrations of TP at maximum spring overturn strictly controlled the corresponding average concentrations of TP measured in summer and autumn (from June to December; results are similar for both not de-trended and de-trended data; $P = 0.01$, $n = 15$). Finally, EA_{DJF} did show a clear impact on the development of the two principal phytoplankton groups during their maximum seasonal development, namely the Bacillariophyta in early spring ($Bacil_{MA}$, March–April, Fig. 6e) and cyanobacteria in summer and autumn ($Cyan_{JD}$, June–December, Fig. 6f).

The above relationships are of little use if deprived of any functional meaning, that is an explanation able

to elucidate the functioning mechanisms they are supposed to describe. As stated by Stenseth et al. (2003), to fully understand how climate influences populations, we ought to understand the relationships between weather and climate, and ecological processes and patterns. Owing to the number of links, the risk of spurious relationships should be taken always into account.

Previous works showed the existence of a strict connection between winter climate, the limnological variables directly connected with winter temperature, and interannual phytoplankton fluctuations (e.g. Salmaso, 2005). Now, it is possible to update these relationships, including, at the top of the cascade, a specific atmospheric mode relevant for the Mediterranean and the southern subalpine region. These results are summarised in Fig. 7. Essentially, the interannual fluctuations of cyanobacteria and diatoms, in the periods of their maximum seasonal development, were under the strict control of the spring recycling of nutrients (e, f). In turn, interannual algal fluctuations were controlled by a chain of linked causal factors which included the fertilisation of epilimnetic waters (d), the cooling of hypolimnetic waters and deep vertical mixing dynamics (c), the winter air temperatures (b) and, finally, the large-scale atmospheric circulation patterns (a).

Discussion

The impact of the EA on the Mediterranean and southern subalpine regions

The EA pattern proved to be an important leading mode of atmospheric variability able to affect significantly the winter climate in the southern subalpine area and, in turn, the interannual fluctuations in the major limnological variables in Lake Garda. The existence of an increasing long-term trend in the winter EA values was recognised in a number of previous studies (Woolf et al., 2002; Conrad et al., 2003; Josey & Marsh, 2005). Nevertheless, our work demonstrated that, at least during the last 20 years, the EA winter values showed a tendency to a stabilisation, though characterised by ample interannual fluctuations. Research on the influence of atmospheric modes of variability relevant to the Mediterranean region and the southern subalpine area is still in its very early stages, and specific studies should be addressed to verify if the relationship between the EA pattern (and other analogous indices, i.e. the EMP; Hatzaki et al., 2007) and local weather varied over different periods of time. In this regard, non-stationarity in climatic indices was documented for example in the NAO values over North Europe (e.g. Solberg et al., 2002).

If compared with NAO, the effects of the EA pattern on the fluctuations of ecosystem variables were studied much less. A few recent papers demonstrated a strong influence of the EA on the air–sea heat exchange and temperature over the Mediterranean regions (Fig. 1). deCastro et al. (2008) showed that the main anomaly variability in the upwelling index calculated as the difference between coastal and oceanic sea surface temperature was explained by EA, with significant negative correlations along the entire western coast of the Iberian Peninsula; NAO was important only in a limited area (38–41°N), whereas other indices (including SCAND and EA/WR) did not show any impact. Schroeder et al. (2010) found that recent productions of anomalous warm and salty deep water in the north-western Mediterranean Sea were more closely related to the EA pattern than the NAO. Josey et al. (2011) found that winter anomalies dominated the annual mean heat budget of the Mediterranean Sea, and that the EA mode had the largest effect on both the eastern and western Mediterranean, of the order of 25 W m^{-2}. The EA/WR mode generated a dipole in the heat exchange, with an approximately equal and opposite signal of ca. 15 W m^{-2} on the western and eastern halves of the basin. NAO (<5 W m^{-2}) and SCAND had a smaller impact on the heat balance. Relationships between air temperature and large-scale atmospheric patterns in the Italian Peninsula were analysed by Toreti et al. (2010). The correlations with the EA pattern were significant in three seasons (winter, spring and summer) and in all the sub-areas considered in the analyses (North, Centre and South). A positive residual correlation was found also for NAO in Northern Italy in winter. From this brief examination, it appears that the EA pattern is emerging as an important climate index able to synthesise many weather variables relevant to the ecological phenomena occurring in aquatic and terrestrial ecosystems over the Mediterranean region. In addition, our work suggests a strong impact of the EA in fine-tuning the effects of human impact and eutrophication in the deep lakes south of the Alps.

Fine tuning of eutrophication impact in deep lakes

The ultimate effects of the winter EA fluctuations are strongly mediated by the physiographic characteristics of water bodies. In deep lakes, the focal mechanism is controlled by the spring deep mixing dynamics which act as a hinge between the winter EA fluctuations and the phytoplankton development during the growing period. This work updates and further confirms previous analyses, which showed a strong relationship between physical, chemical and biological factors in Lake Garda (e.g. Salmaso 2005, 2011). However, previous schemes did not include a link between large-scale climatic fluctuations, and environmental and biotic changes. The scheme reported in Fig. 7, therefore, represents a conceptual framework depicting an important ecological mechanism effective in deep lakes.

The increase of TP documented in Lake Garda during the last 15 years represents the last stage of an eutrophication process identified since the beginning of the 1970s, when the average concentrations in the whole water column were around 5–10 μg P l^{-1} (Mosello et al., 1997). In the group of deeper lakes south of the Alps, eutrophication still represents the main anthropogenic impact affecting the biotic structure and water quality (Salmaso et al., 2007). Lake Orta ($z_{max} = 143$ m) was the only one in this group of lakes to be heavily polluted by ammonium sulphate and copper discharged by a rayon factory. Other potential effects on the aquatic trophic webs in the deep subalpine lakes are due to the presence of persistent organic pollutants (POPs), even if these pollutants seem to represent a risk and an impact more for the higher trophic levels (fish, aquatic birds) and human health than for the lower trophic levels (Salmaso & Mosello, 2010).

In Lake Garda, cyanobacteria and diatoms represent the two principal dominant algal groups. Contrary to diatoms, cyanobacteria showed a quick response to TP at different temporal scales. At the decadal scale, they increased their biomass in reply to the long-term increase of TP (Fig. 4a, d), whereas, at the annual scale, they were positively and strongly affected by the fertilisation of the trophogenic layers due to the sequence of mechanisms a → b → c → d → f in Fig. 7. As for the last process (f), these results agree with previous findings (e.g. Dillon & Rigler, 1974), which reported significant relationships between TP concentrations at spring turnover and the mean summer values of chlorophyll-*a* in a large number of lakes. Not surprisingly, only a weak, non-significant relationship ($P = 0.16$, $n = 15$), was found between TP and cyanobacteria in the period between summer and autumn. In the growing months, and isolated epilimnia, TP is also dependent from phytoplankton activity and mineralisation processes, losing its nature of purely explanatory factor. As for cyanobacteria, we can envisage a development model made of two components, which include a long-term, decadal component (linked to TP_{lakeYR}), whose residuals, after linear de-trending, are strictly related to the year-to-year fluctuations in the winter EA values (and ultimately to TP_{epiS}). In this regard, cyanobacteria confirm their strict relationship with the availability of TP in this typology of lakes, and at different temporal scales. Anyway, it is not superfluous to underline that the relationship between TP_{lakeYR} and cyanobacteria can be flawed, because it takes into account the availability of P in the whole water column (including the deep hypolimnion), whereas complete fertilisation occurs only during complete mixing.

Diatoms appeared strictly related to the interannual availability of spring TP. The large and heavy organisms composing this group require high water turbulence to avoid sinking and losses (Padisák et al., 2003). For these reasons, although the presence of large diatoms is widespread in the cold months (from late autumn to spring), their largest net growth is observed only in a restricted period, coincident with the maximum nutrient pulses and before the maximum seasonal warming of the lake. Due to the increase in sinking losses their development in the stratified months (from May to November–December) is generally limited to the smaller and lighter forms (*Cyclotella* spp.), and to a few colonies of *F. crotonensis* (Salmaso, 2011).

Similar cascading effects, as those depicted for phytoplankton in Lake Garda (Fig. 7), were documented in many other lakes at the northern side of the Alps and all over central and northern Europe, but with the important difference that, in these environments, the ultimate factor was represented by the NAO (Straile et al., 2003; George, 2010). Brief exemplifications include effects on phytoplankton, zooplankton and fish. Examining a representative number of lakes in northern, western and central Europe, Blenckner et al. (2007) found a significant relationship between

the summer cyanobacterial biomass and the winter NAO. In Lake Constance the mass development of *Daphnia* and the subsequent timing of the clear-water phase in late spring and early summer was shown to be significantly related to the NAO in the winter months (Straile, 2000). In the same lake, Straile et al. (2007) showed a significant connection between climate variability associated with the NAO and the year-class strength of *Coregonus lavaretus*, through effects acting on the growth of larvae during spring and the egg development time. In the lakes south of the Alps, we can hypothesise the existence of similar effects triggered by the EA on the higher trophic levels. Overall, these selected examples and this work emphasise the key importance of different atmospheric modes of variability over the Atlantic and European regions in the regulation of ecological processes in aquatic ecosystems.

Conclusions

Eutrophication still represents the main source of human impact in the deep lakes south of the Alps. Nevertheless, this study showed how anthropogenic impacts can be finely regulated by climatic fluctuations which, in Lake Garda, have been adequately represented by the EA pattern, a teleconnection index relevant for the climate of the Mediterranean area and the region south of the Alps. The growth of cyanobacteria and diatoms was strongly enhanced by negative phases of the winter EA pattern through mechanistic links, which included higher fertilisation of trophogenic layers at spring overturn, higher vertical extension of circulation episodes, lower water temperatures and lower winter air temperatures associated to harsh winter conditions. In this regard, given the same availability of lake phosphorus, warmer temperatures in the winter period weaken the eutrophication effects and phytoplankton development. These effects are not entirely intuitive. The application of the EA could disclose important perspectives in the study of long-term climatic fluctuations and climate change on the phytoplankton development and, more in general, the ecology of freshwater ecosystems. This study regarded the impact of EA on the limnology of Lake Garda but, as shown by Salmaso (2012), similar considerations could have been obtained considering another index relevant for the climatology of the Mediterranean area, i.e. the EMP (Hatzaki et al., 2007). Many aspects regarding the connection between eutrophication of freshwater ecosystems and modes of atmospheric circulation (chiefly EA and EMP) in the southern subalpine region are still open to future research. (i) Effects are strictly mediated by lake size and depth, implying distinct responses due to different turnover times and mixing dynamics which, in turn, are dependent not only by winter air temperature but also other climatic features such as direction and velocity of wind. (ii) Relationships and functioning mechanisms change in the different seasons, whereas most seasons are not dominated by any particular climate regime in the extra tropics (Stenseth et al., 2003). (iii) Trends and shifts in the winter EA values could have important impacts on the frequency and intensity of deep mixing dynamics, with critical effects on environmental and biotic responses; the upward EA pattern was shown to have a significant role in freshwater flux variations to the eastern North Atlantic (Josey and Marsh, 2005). (iv) At the watershed level, other weather aspects (e.g. atmospheric precipitation) linked to climate indices may be connected with external input of nutrients and eutrophication (see George, 2010).

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PHYTOPLANKTON

Deep living *Planktothrix rubescens* modulated by environmental constraints and climate forcing

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Abstract The cyanoprokaryote *Planktothrix rubescens* has been studied for over four decades in an alpine lake, Mondsee, Austria. We hypothesise that impacts of climate change can be distinguished from other environmental constraints controlling its population dynamics. During thermal stratification, *P. rubescens* formed a deep chlorophyll maximum in the metalimnion. Seasonal and diurnal depth distributions indicated that *Planktothrix* lived well below the euphotic zone, at low light levels and moderate temperatures. Photosynthetic parameters indicated a shade adapted population. The eutrophication and rehabilitation periods of Mondsee were characterised by fluctuations controlled by phosphorus, with periods below the P-threshold associated with low biovolumes and relatively stable populations. Positive net changes of *Planktothrix* biovolume occurred during the spring–summer transition and in autumn. During summer, the population usually declined to an annual minimum. The standardised residuals of annual biovolumes responded positively to the climate signal of the Winter North Atlantic Oscillation and the timing of the onset of stratification. An inverse relationship existed between off-set and persistence of stratification. *P. rubescens* only benefits from climate warming early in the year, during late spring overturn and early summer. Longer periods of summer stratification did not favour biovolume development.

Keywords Deep chlorophyll maximum · Long-term · NAO · Eutrophication · Re-oligotrophication · Cyanobacteria · Oscillaxanthin

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Introduction

Most freshwater habitats in the world today are affected by eutrophication (Smith & Schindler, 2009), with the problem of nutrient enrichment now further exacerbated by the effects of climate change (Dokulil & Teubner, 2011). The underlying causes of eutrophication have long been recognised (Vollenweider, 1966) and even though there have

been great advances in understanding, it remains a particular problem because of algal blooms, scums and water quality issues (Schindler, 2006; Dodds & Cole, 2007). The deterioration in lake water quality is often associated with the excessive proliferation of cyanobacteria (Pearl & Ustach, 1982; Steinberg & Hartmann, 1988).

The most obvious nuisance is when there is a mass appearance of cyanobacteria in surface blooms. However, in deep lakes that stratify during summer, problems may be hidden in deeper water because some planktonic cyanobacteria thrive in the metalimnion where they build up deep chlorophyll maxima (DCM). This behaviour seems paradoxical because the epilimnion should be more attractive for autotrophic organisms (Davis et al., 2003). One of the best and most widespread examples of this behaviour is the cyanoprokaryote *Planktothrix rubescens* (de Candolle ex Gomont) Anagnostidis et Komárek (1988). This 'paradoxical' species is seen by others as an efficient 'ecosystem engineer' acting as a major organizing force (Padisák et al., 2010). Whatever the viewpoint, this species has a world-wide distribution in lakes of different sizes (Guiry& Guiry, 2011) but is of particular importance in deep, stratifying and eutrophic lakes. One adaptive advantage of *P. rubescens* is its ability to maintain populations even under declining or low phosphorus concentrations (Chorus et al., 2011, p. 84ff). Thriving mainly in the metalimnion, the species can become a nuisance because many strains can be toxic (Ostermaier & Kurmayer, 2010) and may transfer the toxin to higher trophic levels (Sotton et al., 2011). At higher nutrient levels, the species may even produce surface blooms (e.g. Almodóvar et al., 2004).

Planktothrix rubescens is one of the most studied organisms among the filamentous cyanoprokaryotes. This is because of its early seasonal appearance and the striking visual appearance of blooms. For example, it occurred frequently during the winter in Lac de Morat, Switzerland, during the nineteenth century. People at that time believed that the phenomenon was due to the reappearance of blood of Burgundy soldiers drowned during a battle in 1478. This myth triggered the German name 'Burgunderblutalge' (refer also to the red cock legend in Padisák et al., 2010). However, the cause of the red colouration was correctly identified by De Candolle (1825), who named the organism *Oscillatoria rubescens.*

The basionym of *P. rubescens* is, therefore, *Oscillatoria rubescens* D.C. ex Gomont 1892. (Skulberg & Skulberg, 1985; Anagnostidis & Komárek, 1988). It was first described as the purple or red-coloured form of planktonic *Oscillatoria* occurring in stratifying lakes, while the morphologically very similar taxon but blue-green form, *Oscillatoria agardhii*, was commonly described from polymictic, e.g. riverine and shallow lakes (shallow eutrophied 'H_2S-*Oscillatoria* lakes' described by Wundsch, 1940, see also *Oscillatoria agardhii/rubescens* Skulberg, 1977; Skulberg & Skulberg, 1985). Their different colouration relates to the presence of phycobilins, which are light harvesting pigments. The 'blue-green' phycocyanin is the only phycobilin occurring in *P. agardhii*, while *P. rubescens* also contains high concentrations of the 'purple' phycoerythrin, which gives it the characteristic colour. This different phycobilin pigment composition is seen as diacritical feature delineating strains of *P. agardhii* and *P. rubescens* according to phylogenetic analysis based on 16S rDNA sequences (Suda et al., 2002). Both *P. rubescens* and *P. agardhii*, contain oscillaxanthin. This carotenoid is not common in other taxa and was first isolated from *P. rubescens* (Karrer & Rutschmann, 1944; Goodwin, 1976, Rowan, 1989). The genus *Planktothrix* was described by Anagnostidis & Komárek (1988). The emended description of *P. rubescens* (D.C. ex Gomont) Anagnostidis et Komárek 1988 refers to the family Phormidiaceae. The main morphological features of *P. rubescens* include solitary trichomes or filaments that are able to move by gliding forwards and backwards (trembling), thylakoids arranged perpendicularly to the longitudinal side of cells, the even distribution of gas vesicles in the protoplast and the lack of false branching of trichomes (Fig. S1). Also, *P. rubescens* has cells where the length is slightly shorter than the width (Anagnostidis & Komárek, 1988; Komárek & Komárkova, 2004). Toxic and non-toxic strains of *Planktothrix* have been found in European lakes including Mondsee (Kurmayer et al., 2011) but the proportions of genes encoding the synthesis of toxic peptides differed between populations from different lakes but did not vary seasonally within a lake.

In Austria, *P. rubescens* appeared shortly after it was first found in several Swiss lakes in the late nineteenth century (e.g. Bachmann, 1897) in a lake north of the Alps. In 1909 it invaded the Wörthersee in

Carinthia south of the Alpine ridge and further spread through the lake districts thereafter (Findenegg, 1973). The taxon appeared in Mondsee in fall 1968, when phosphorus concentrations increased (Findenegg, 1969). *Planktothrix*, however, occurred throughout the re-oligotrophication phase and almost disappeared when the lake became oligo-mesotrophic. It still maintains a very small population at present.

The aim of the present review is to assess the population dynamics of *P. rubescens* in Mondsee over 40 years, from 1969 to 2010, and to identify the environmental constraints responsible for its rise and fall in abundance. We hypothesise that the metalimnetic populations of *P. rubescens* have been affected by climate change, which is now modifying ecological conditions within the lake.

The Study site

Mondsee is an alpine oligo-mesotrophic lake situated in central Austria (47°50′N, 13°23′E) at 480 m above sea level. It has an area of 13.8 km^2, a mean depth of 37 m and a maximum depth of 68 m. The lake regularly stratifies during summer while ice coverage in winter is sporadic. Therefore, Mondsee switches between dimictic and monomictic mixing regimes. For more details on the lake's location, morphometry and hydrology refer to Dokulil & Skolaut (1986).

Methods

Data sources

The quantitative data for *P. rubescens* for the years 1969–1972 were extracted from Findenegg (1969, 1972,1973), those for 1977–1980 from Schwarz (1979a, b, 1981) and Jagsch (1979), and for 2005–2010 from Schay & Wimmer (2010), Wolfram et al. (2010) and Mildner et al. (2010).

Annual average total phosphorus (TP) concentrations for 1969–1974 were inferred from *Planktothrix* biovolume (PB) using the regression equation PB = 1.54 TP + 12.8 ($r^2 = 0.42$, $n = 638$) and from palaeo-data as described in Dokulil & Teubner (2005). Average TP data for the years 1975–1981 and 2004–2006 were taken from Achleitner et al. (2007). Those for 2007–2010 come from Schay & Wimmer (2010), Wolfram et al. (2010) and Mildner et al. (2010). All other data between 1982 and 2004 are original. All methods used in these studies were standard techniques, mostly cross-checked to allow comparison of data from different laboratories.

Depth distribution of *P. rubescens* for the years 1982–84 are biomass estimates while the years 2003/04 were deduced from measurements using delayed fluorescence (DF) excitation spectroscopy (Gerhardt & Bodemer, 1998).

Euphotic depth (z_{eu}) was defined as the depth where photosynthetic available radiation (PAR) is 1% of surface light intensity. In the absence of direct measurements this depth was calculated from Secchi-disk readings by a factor of three empirically determined for Mondsee. Mixing depth (z_{mix}) was taken as the depth of maximum density difference calculated from water temperature.

Long-term sampling

Over the 40-year sampling period different sampling depths and strategies were used. Samples from discrete depths were usually taken with a Ruttner sampler (prior to 1982) or later with a Schindler–Patalas trap. Integrated samples for the top 20 m were obtained with a Schröder sampler (Schröder, 1969). Sampling intervals ranged from weekly to monthly. In a few cases, the interval was biweekly or daily as, e.g. when photosynthetic characteristics were measured. Similarly, sampling depths also varied over time. Details on the ^{14}C technique used for productivity estimates are given in Kaiblinger et al. (2007).

Diurnal sampling

High resolution measurements were performed during a stable thermal stratification period in mid-July 2002 for two diurnal cycles ('High moon' workshop). Samples for phytoplankton and lipophilic pigments were taken at 0.5, 1, 2, 3.5, 5.5, 7.5, 9.5, 12.5, 14.5, 18, 22 and 28 m at 3-h intervals. Methods of counting phytoplankton samples, analysis of chl-*a* and lipophilic pigments and related results are described in detail in Greisberger & Teubner (2007). Details of spectroscopic properties specifically used for analysing the concentrations of the carotenoid Oscillaxanthin are described in Goodwin (1976) and Rowan (1989). Hourly profiles of chlorophyll fluorescence

(Haardt-fluorimeter, Lincoln, USA), conductivity, oxygen and water temperature (YSI 6920 profiler) were measured at depth intervals of 0.25 m. Incoming PAR was continuously monitored over intervals of 10 min by using cosine corrected PAR sensor (LI 190). Under water light regime was measured with a scalar quantum sensor (LI 190 SB) connected to an integrating quantum meter (LI 188) made by LI-COR Inc, USA. The measurements were corrected for the immersion effect. Values of 1 and 0.1% surface light intensity were calculated from vertical attenuation coefficients. The maximum values of relative thermal resistance against mixing (RTRM; Vallentyne, 1957) were estimated from 0.25 m resolution profiles of water temperature and used to define the depth of thermocline (also defined here as z_{mix}).

Data treatment

The length of trichomes of *P. rubescens* at different depth was estimated by notched box-whisker plots using SYSTAT 10 (SPSS Inc.). Boxes were notched at the median; the length of the notches indicated 95% confidence intervals. Break points in time series were visualised by rescaled adjusted partial sums (RAPS) following Garbrecht & Fernandez (1994).

$$\sum_{t=1}^{k} \frac{Y_t - \overline{Y}}{S_Y} \quad (1)$$

with Y_t = value at time t, $\overline{Y}$ = mean of the series and S_Y = standard deviation of the mean. Schmidt stability was calculated according to Livingstone and Schanz (1994).

In situ net changes of PB were calculated from short time investigations ($\leq$1 week) according to

$$\mu' = \frac{(\ln B_2 - \ln B_1)}{t_2 - t_1} \quad (2)$$

with B_2 and B_1 biovolumes at time t_2 and t_1.

Long-term datasets of intra-annual biovolumes of *P. rubescens* were highly variable (coefficient of variation, CV = 0.96). Annual biovolumes of *P. rubescens* were hence standardised and linearly detrended to remove strong inter-annual variation prior to estimating climate response in the time series. These standardised residuals (SR) of *P. rubescens* were related to a climate signal, the Hurrel's station based North Atlantic Oscillation Index (NAO, Hurrel et al., 2001). Monthly indices were taken from http://www.cgd.ucar.edu/cas/jhurrell/indices.html, accessed 7 September 2011. Standardised residuals of annual biovolumes of *P. rubescens* were further correlated with original data of dates of lake stratification (low CV which ranged from 0.02 to 0.11) and its detrended time series data. Pearson correlations coefficients are shown for the detrended lake stratification data in Fig. 9 (r_{SR}) and also for the original lake stratification data (r) in the text.

RTRM values for the long-term data set were calculated in depth intervals of 1 m. The on-set of thermal stratification was defined as the date, the Julian day, when maximum RTRM-value exceeded the threshold value of 9.5. Similarly, the off-set of stratification was defined by the Julian day when the maximum RTRM-value fell below 9.5 leading to unstable thermal conditions in the water column and finally autumnal overturn. The duration of summer stratification was the time span in Julian days between dates of on- and off-set.

Results

Long-term dynamics

After its first appearance in autumn 1968 (Findenegg, 1969), *P. rubescens* rapidly increased in biovolume and reached a first peak in 1971 in Mondsee (Fig. 1). No data exist for the bloom years 1973–1976, only indirect evidence from visual observations of surface appearance of the species (Fig. S2) and palaeolimnological records of the marker pigment oscillaxanthin in the sediments (Schultze, 1985; Dokulil & Teubner, 2005). Maximum observed biovolumes occurred in the years 1977–1979 (4.4 $mm^3\ L^{-1}$ in 1979). As a consequence of restoration measures in the catchment, quantities of *Planktothrix* rapidly declined thereafter, reaching values below 0.002 $mm^3\ L^{-1}$ in 1998–2000. Beginning in 2001, biovolumes of *P. rubescens* increased again to values similar to the 1980s. The peak of 1.3 $mm^3\ L^{-1}$ in 2004 was followed by a rapid decline, leading to very low values from 2006 to 2010.

Annual average PB and TP concentrations strongly corresponded to each other during the period from 1969 to 2010 (Fig. 2), as is also indicated by the parallel progression of the RAPS in the lower panel. In the first phase, from 1969 to 1979, annual mean TP concentrations increased in the lake, peaking at 35 µg L^{-1} in 1978 (eutrophication phase), and *P. rubescens*

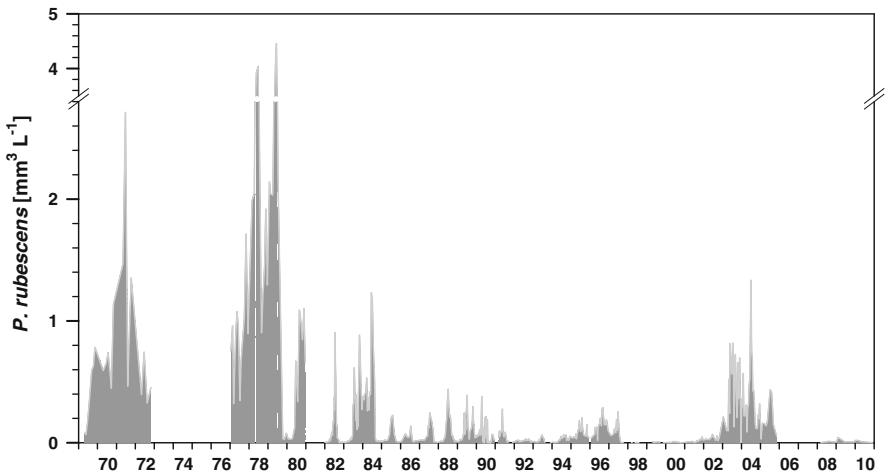


Fig. 1 Biovolume of *P. rubescens* as 10^6 µm^3 L^{-1} (equivalent to µg L^{-1} biomass) in Mondsee for the years 1969–2010. For data sources refer to relevant paragraph in "Methods"

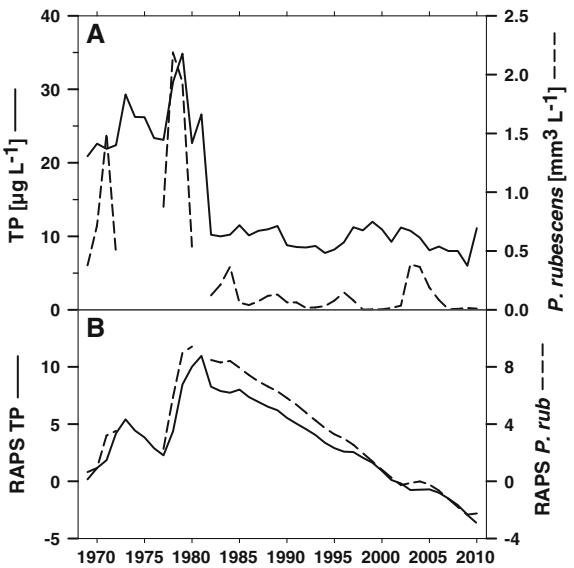


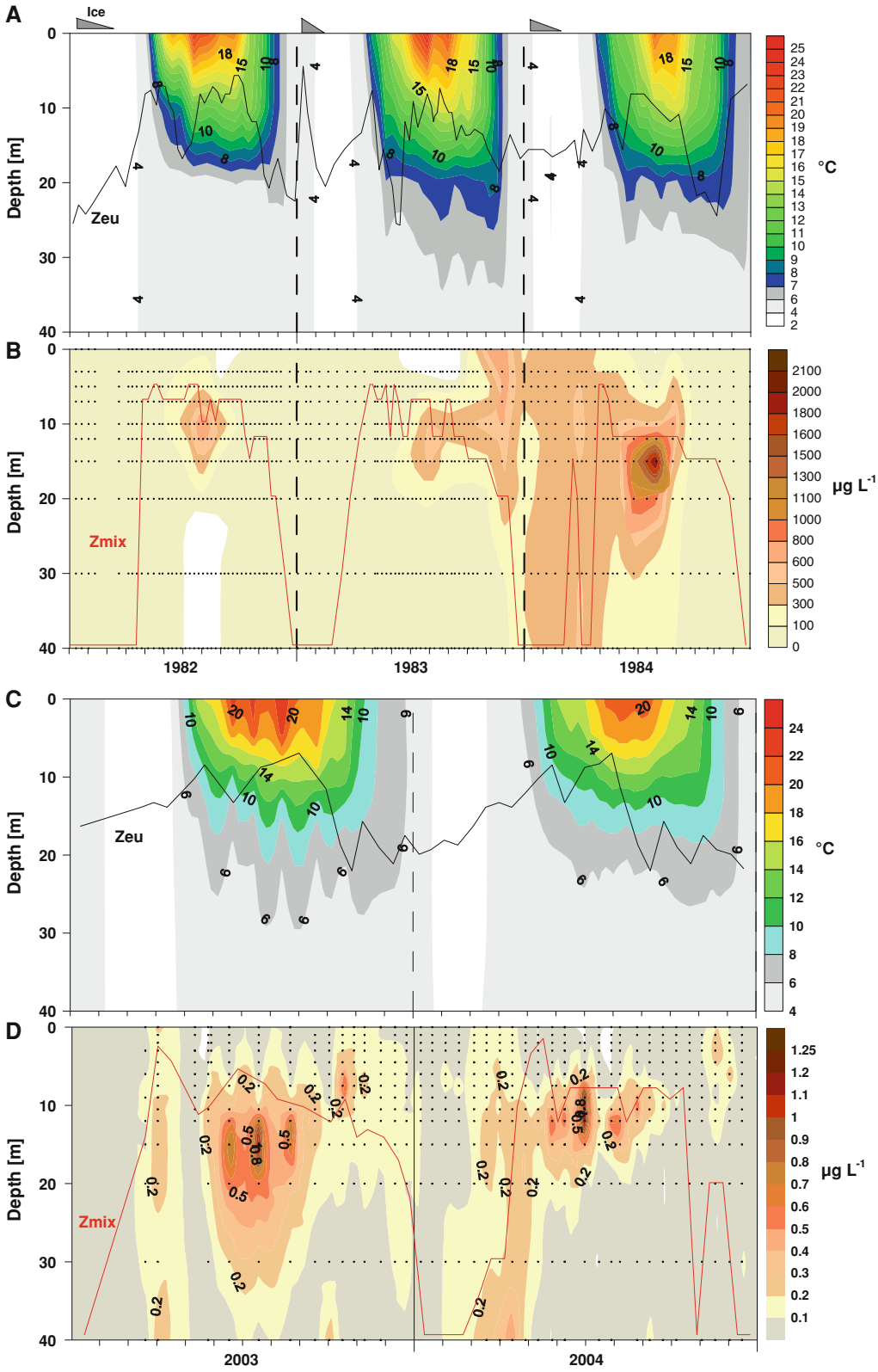
Fig. 2 Annual average total phosphorus (TP) and concentration of *P. rubescens* for 1969–2010 (**A**) and the readjusted partial sums (RAPS) of both variables (**B**)

proliferated. Both variables drastically declined then until TP concentrations dropped consistently below 10 µg L^{-1} and biovolumes below 0.1 mm^3 L^{-1} in 1990 (restoration phase). The next 10-year period, from 1990 to 2000, was characterised by low and further declining average biovolumes, reaching an annual minimum of 0.001 mm^3 L^{-1} in 1998 (stabilisation phase). Afterwards, *P. rubescens* mean biovolumes increased again, peaking at 0.4 mm^3 L^{-1} in 2003, followed by a drastic decline to values much below 0.01 mm^3 L^{-1} in very recent years.

Depth distribution

Depth distribution and formation of the metalimnetic maximum layer of *P. rubescens* is shown together with water temperature, z_{eu} and z_{mix} for the years 1982–1984 and 2003–2004 in Fig. 3. The formation of a *Planktothix*-layer between 10 and 15(20) m began when the lake started to thermally stratify. Maximum biovolumes were associated with water temperatures of 6–16°C and at depths of greatest density difference. *P. rubescens* grew well at depths below z_{eu}, i.e. at light levels of less than 1% and below the mixing zone during the stratified period. Positive net change of biovolume (μ') averaged 0.099 day^{-1} (0.002–0.446) were deduced from weekly observations. Positive net changes mainly occurred during late spring-early summer or in autumn. The layering was terminated usually when temperature stratification began to weaken and mixing depth increased. In the autumn of 1983, however, quite a large population remained in the epilimnion and managed to survive the winter, so it was able to proliferate throughout the water column at spring overturn.

The depth distribution of *P. rubescens* and related variables are further shown in detail for two diurnal cycles during summer stratification in Fig. 4. In addition, phytoplankton composition is shown for certain depths in Fig. 5 for these sampling dates. The density and the RTRM illustrate the usual separation with the epilimnion in the top 5–6 m, a thick metalimnetic layer from 6 to 13.5 m and the hypolimnion below during thermal stratification.


A
Ice
Depth [m]
Zeu
°C
B
Depth [m]
Zmix
µg L-1
1982
1983
1984
C
Depth [m]
Zeu
°C
D
Depth [m]
Zmix
µg L-1
2003
2004

◀**Fig. 3** Contour plot of water temperature (°C) with euphotic depth (z_{eu} in m) as *continuous line* (**A**) and *P. rubescens* biomass in µg L^{-1} with mixing depth (z_{mix} in m) for 1982–1984 (**B**). Contour plot of water temperature (°C) with euphotic depth (z_{eu} in m) as *continuous line* (**C**) and *P. rubescens* biomass as chl-*a* equivalents (µg L^{-1}) from DF-spectrometry with mixing depth (z_{mix} in m) for 2003 and 2004 (**D**)

The biovolume of *P. rubescens* was negligible in the top 9 m, because it contributed less than 1% to total biovolume of phytoplankton (Figs. 4, 5). At a depth of 9.5 m, coinciding with the depth of the thermocline at 9.4 m (depth variation from 8 to 11 m), the biovolume of *P. rubescens* increased and contributed 9% to total phytoplankton (Fig. 5, left panel). This depth also related to a light availability of 1%, because the euphotic zone was deepest at 10.7 and 10.3 m, respectively, during these 2 days in July. Biovolumes of *P. rubescens* further increased below the thermocline at 12.5 and 14.5 m reaching 0.97 and 0.91 mm^3 L^{-1}, respectively, which was about 85% of total biovolume. The *Planktothrix* layer occupied a zone at and below the margin of the thermocline where light was available between 1 and 0.1% (Fig. 4A). In the main layer at 12.5 m, 0.1% light intensity lasted for at least several hours per day (11 h on sunny 23.7.2002, 5 h on cloudy 24.7.2002). Based on the hourly fluorescence profiles (resolution 0.25 m), the depth of the population maximum of *P. rubescens* was even slightly deeper between 13 and 13.5 m and hence very close to the boundary between meta- and hypolimnion (Fig. 4B). The gravity point of the *P. rubescens* population moved marginally downwards in the afternoon and slightly dispersed during morning and night (Fig. 4A). Enhanced oxygen

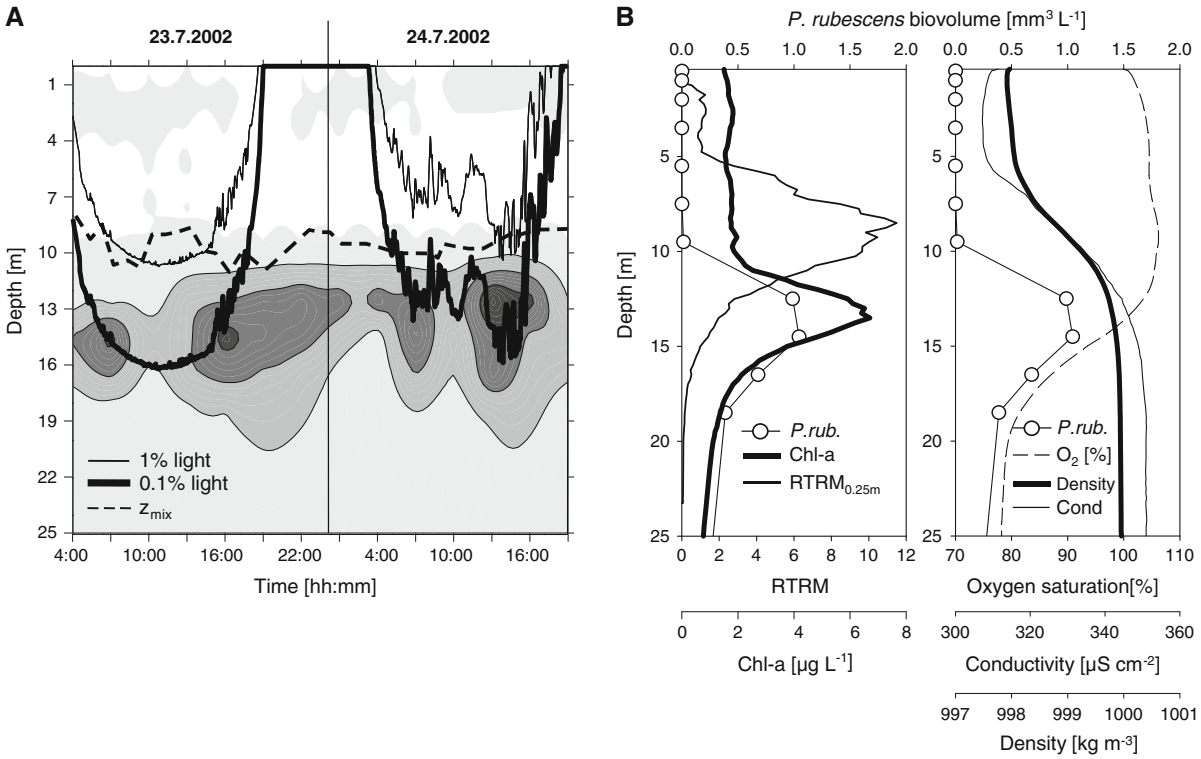


Fig. 4 Diurnal depth distribution of *P. rubescens* as contour plot (**A**) and mean verticals (**B**). All data are from Mondsee sampling at 23/24 July 2002. With exception of the biovolume of *P. rubescens* the depth resolution is 0.25 m. **A** Biovolume of *P. rubescens* is indicated by *shaded areas*: <0.4 (*white*), 0.5–1 (*light gray*), 1.1–1.5 (*mid-grey*), >1.6 (*dark grey*). *Lines* refer to the depth of 1% (euphotic depth) and 0.1% light availability, respectively, and mixing depth (thermocline as depth of maximum values of RTRM). **B** Profiles as averages of the two diurnal cycles: biovolume of *P. rubescens* (P. rub.), total chlorophyll-*a* of phytoplankton measured by fluorescence (chl-*a*), RTRM and density, conductivity (Cond) oxygen saturation (O_2%). The mean maximum value of RTRM is 11 and refers to a depth resolution of 0.25 m (maximum RTRM value of 1-m depth interval is 43 (not shown) and hence above the threshold of 9.5 used as threshold for the onset of stratification in Mondsee, see "Methods")

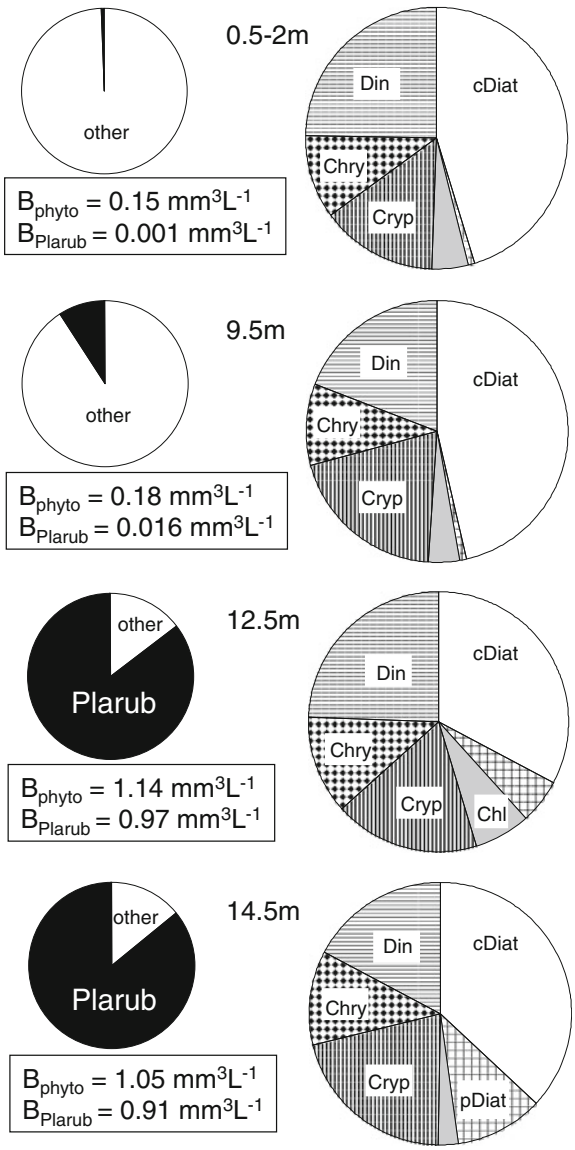


Fig. 5 Relative contribution of *P. rubescens* to total phytoplankton (*left panel*) and the composition of eukaryotic phytoplankton (*right panel*) at discrete depth of epilimnion (0.5, 1 and 2 m), metalimnion (9.5 m, 12.5 m) and hypolimnion (14.5 m). Data are averages from Mondsee sampling on 23/24 July 2002 (see method). *Chry* chrysophytes, *cDiat* centric diatoms, *pDiat* pennate diatoms, *Cryp* cryptophytes, *Din* dinoflagellates, *Plarub P. rubescens*, B_{phyto} biovolume of total phytoplankton, B_{Plarub} biovolume of *P. rubescens*

saturation was found from 7.5 to 11.5 m, which decreased slightly with depth and fell below 100% at 14 m (Fig. 4B).

The vertical distribution of phytoplankton other than *P. rubescens* was largely even (Fig. 5, right panel). The biovolume other than *P. rubescens* was dominated by diatoms (38–47 %), while dinoflagellates and cryptophytes contributed, on average, 16–24% and 14–20%, respectively. Chrysophytes and cyanobacteria other than *P. rubescens* were of minor importance. The only remarkable change with depth was found among the centric and pennate diatoms, e.g. centric diatoms made up 45–46% of phytoplankton in the surface layer (excluding *P. rubescens*), while pennate diatoms were almost absent (<1%). In the deeper layers below the thermocline at 12.5 and 14.5 m, the centric diatoms decreased to 33–35% while the pennate diatoms increased to 5–10%. The pennate diatoms were composed mainly of the genera *Asterionella*, *Fragilaria* (including former *Synedra*), *Nitzschia* and *Tabellaria*.

A distinct vertical pattern can be further seen by comparing the trichome length of *P. rubescens* at two metalimnetic strata (Fig. 6A). Trichomes at 9.5 m depth were significantly shorter than those at 12.5 m ($p < 0.05$, Fig. 6B). The trichome length varied from 28 to 1090 µm (median, see Table 2). In general, the frequency of shorter trichomes was much higher than of longer trichomes.

During the diurnal cycle survey in Mondsee, *P. rubescens* was the only abundant cyanobacterium containing the carotenoid pigment oscillaxanthin (Goodwin, 1976; Rowan, 1989). This marker pigment was significantly correlated to the abundant biovolume of *P. rubescens* below 9.5 m (Fig. 7). The median ratio of oscillaxanthin per biovolume of *P. rubescens* was 5.74×10^{-8} µg µm^{-3}, that of oscillaxanthin per chl-*a* of *P. rubescens* 0.081 µg µg^{-1} (Table 2). When biovolume was <0.025 mm^3 L^{-1} (samples from epilimnion) the spectroscopic signal of oscillaxanthin was under the detection limit applying a standard protocol aimed at analysing the many phytoplankton chlorophylls and carotenoids in a one analytical run.

Productivity

In July 1987 an in situ productivity ^{14}C-experiment was performed in which *Planktothrix* was taken from the DCM and then distributed over z_{eu}, exposing it to the entire light gradient. Comparison was made to the total phytoplankton assemblage, which was taken conventionally at each depth. The photosynthetic parameters obtained from chl-specific carbon uptake

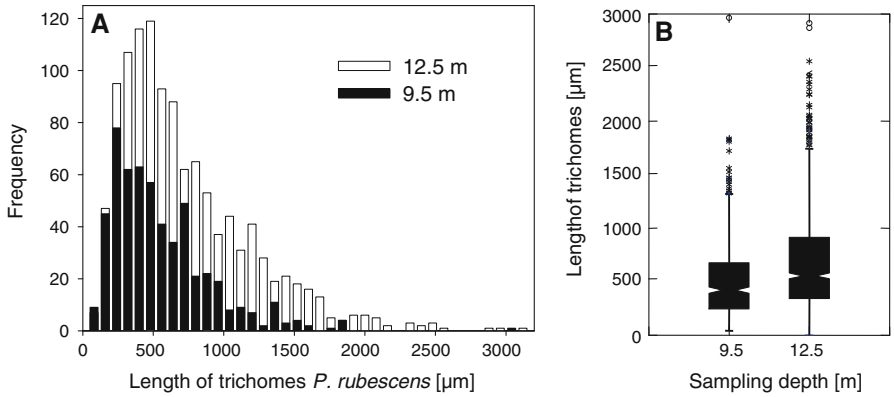


Fig. 6 Variation of length of trichomes of *P. rubescens* at sampling depth 9.5 (median length = 421 µm, $n = 552$) and 12.5 m (median length = 556 µm, $n = 1160$) in July 2002 in Mondsee. Variation is shown as frequency distribution (**A**) and as box-plot (**B**)

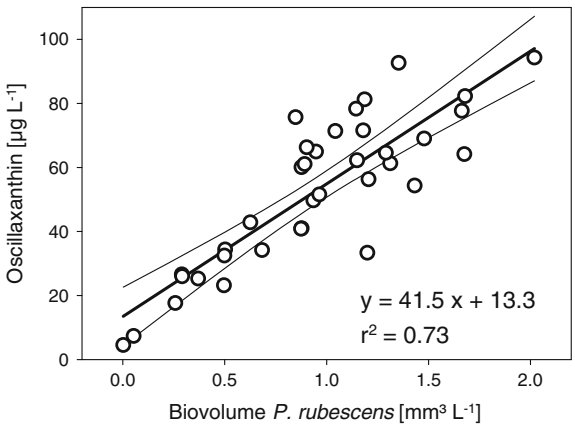


Fig. 7 Relationship between the biovolume of *P. rubescens* and its marker pigment oscillaxanthin at 9.5, 12.5 and 14.5 m depth. All data are from Mondsee sampling at 23/24 July 2002

versus light intensity (*P–I* curve) are summarised in Table 1.

Maximum rate of photosynthesis (P_{max}) of *Planktothrix* was lower and light harvesting efficiency at low light (α) was higher than that of total phytoplankton. As a result, light saturation (I_K) of photosynthetic rate was reached at lower light intensity for *P. rubescens*. Further, photoinhibition at light intensities above 640 µmol PAR was observed for *P. rubescens* while no inhibition was found for the total phytoplankton sample. All these parameters pointed to low light acclimation in *P. rubescens.*

Climate response

As the yield of *P. rubescens* was primarily linked to nutrient status (Fig. 8), annual average biovolumes of *P. rubescens* did not directly correspond to climate signals. To disentangle the phosphorus control and climate effects, all annual biovolumes of *P. rubescens* were converted to standardised residuals before testing correlations (SR biovolume of *P. rubescens*, see "Methods"). These residuals were significantly correlated with the large-scale climate phenomenon in February, the NAO_{Febr} ($r = 0.37$, $p < 0.05$, Fig. 9A). Statistical analysis with other months and periods of NAO did not reveal a significant response pattern of *P. rubescens* over the time-period from 1969 to 2004 and, therefore, is not shown. Physical lake mixing conditions, specifically the timing of on- and off-set of thermal stratification and the duration of summer

Table 1 Photosynthetic parameters deduced from ^{14}C-productivity versus light intensity (*P–I* curves) comparing *P. rubescens* and total phytoplankton

Photosynthetic parameter	*P. rubescens*	Total phytoplankton
P_{max} [mg C (mg chl-*a*)$^{-1}$ h^{-1}]	4.32	6.03
α [mg C (mg chl-*a*)$^{-1}$ h^{-1} (µmol photons m^{-2} s^{-1})$^{-1}$]	0.024	0.019
E_K (µmol photons m^{-2} s^{-1})	180	317
E_I (µmol photons m^{-2} s^{-1})	630	n.a.
Photoinhibition (% P_{max})	40	0

P_{max} maximum chl-*a* specific productivity, α light efficiency, E_K light intensity at onset of saturation, E_I light intensity at inhibition

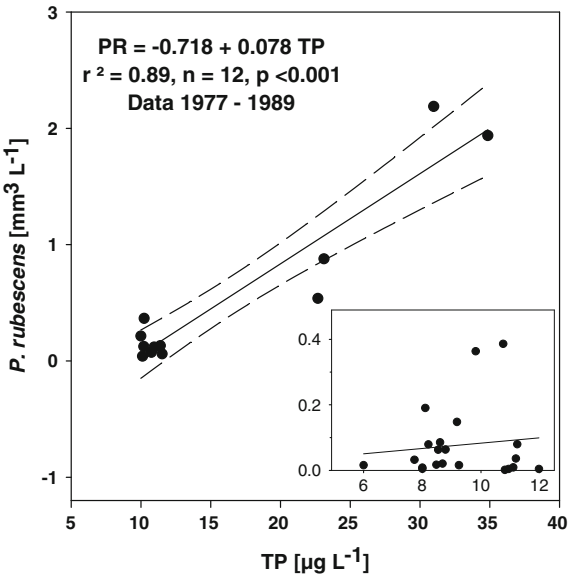


Fig. 8 Regression of *P. rubescens* (PR) annual average biomass versus TP concentration for 1969–1989 and for 1990–2010 (*inset*). The dashed lines are the 95% confident limits. The regression equation, explained variance and significance level is given in the main graph

stratification in Mondsee (Fig. 9B), were identified as factors which mediate the climate signal to biological response. This figure refers only to the years 1979 to 2003 because detailed depth profiles are lacking for the other years.

The on-set of stratification in Mondsee occurred on average in early May, on Julian day 122, and varied from day 85 to 143 in the year. The SR of biovolume of *P. rubescens* were positively correlated with the timing of this on-set, the original data (r) and the detrended (r_{SR}) Julian days, respectively ($r = 0.39$ and $r_{SR} = 0.41$, $p < 0.05$). The breakdown of summer stratification occurred on average in early November, on Julian day 307, and varied from day 290 to 323. In contrast to the on-set of stratification, we found an inverse relationship between the SR of *P. rubescens* and the timing of breakdown of thermal stratification ($r = -0.43$ and $r_{SR} = -0.44$, $p < 0.05$). An even stronger inverse response was found in the case of the time-span of thermal stratification ($r = -0.52$ and $r_{SR} = -0.55$, $p < 0.01$). The thermal stability of the water column can be expressed by Schmidt's stability. Maximum values for Schmidt's stability increased slightly with years and responded to warming in Mondsee (not shown). SR of biovolume of *P. rubescens* were negatively related with values of the annual maximum of Schmidt's stability ($r = -0.50$, $r_{SR} = -0.42$, $p < 0.01$, not shown in figures).

Annual average long-term seasonality

The annual patterns of *P. rubescens* biovolume, total chlorophyll-*a* concentration of phytoplankton, the mixing and euphotic depth are shown as long-term averages for the years 1979–2003 in Fig. 10. During spring overturn early in the year, biovolumes of *P. rubescens* only slightly increased (Phase I). With the transition into thermal stratification (Phase II), the biovolume of *P. rubescens* increased to an initial

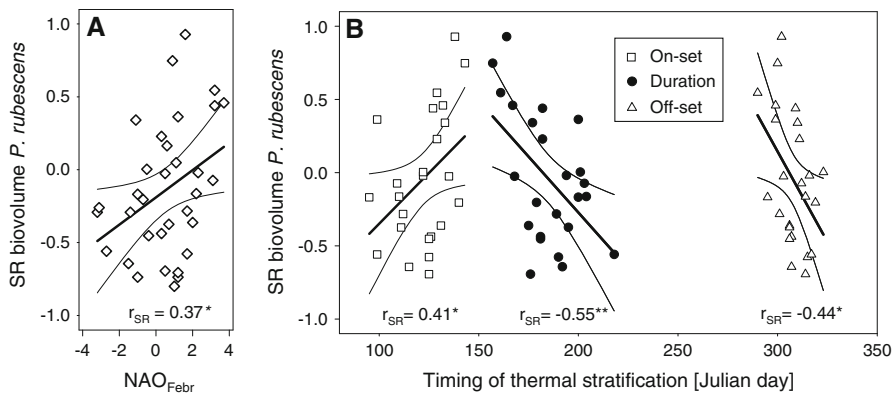


Fig. 9 Climate response of *P. rubescens*. The annual biovolumes of *P. rubescens* are displayed as standardised residuals (SR biovolume *P. rubescens*). **A** The response to the NAO-index in February (NAOFebr), and **B** the Julian days of the on-set, breakdown and duration of thermal stratification. **A** relates to the period 1969–1972, 1977–1980, 1982–2004 ($n = 30$), **B** for 1979–1980, 1982–1903 ($n = 25$). Pearson correlation coefficients for SR biovolume of *P. rubescens* versus residuals of the timing of stratification (r_{SR}) and 0.95 significance intervals are shown (*$p < 0.05$, **$p < 0.01$)

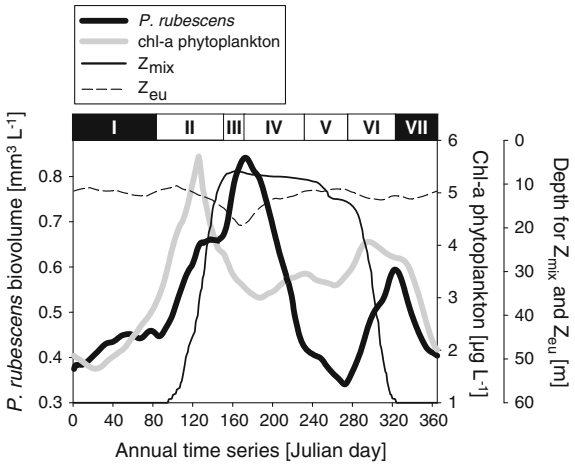


Fig. 10 Annual pattern for biovolume of *P. rubescens*, total chlorophyll-*a* concentration of phytoplankton and the depth of mixing and euphotic zone shown as long-term averages ($n = 25$, 1977–1980, 1982–2003)

peak, while the total phytoplankton already formed the spring maximum, which was usually the main peak of the year in Mondsee. Immediately after the on-set of thermal stratification, *P. rubescens* rapidly reached its maximum in the year, coinciding with the formation of the metalimnetic layer (Phase III). Total phytoplankton rapidly declined and water transparency increased during this clear-water phase. The annual average maximum of water transparency coincided, therefore, with the annual peak of *P. rubescens* biovolume, which occurred in the long-term average by mid to end of July (Julian day 173, median Julian day = 157, Table 2). In the following Phase IV, mainly characterised by a progressive deepening of the thermocline (see z_{mix} in Fig. 10), biovolume of *P. rubescens* rapidly decreased while total phytoplankton formed a summer high. By the end of summer, the decline of *P. rubescens* slowed down to an annual minimum by mid to end September (mean Julian day 272 in Phase V; median 248, Table 2). This was followed by a steep increase leading to a second, smaller peak by the end of mid-November, on Julian day 323 (transition Phase VI). Finally, with the start of the autumnal overturn (Phase VIII) both *P. rubescens* and total phytoplankton declined to winter values.

Table 2 Summary of main morphological and eco-physiological characteristics of *P. rubescens* in the alpine lake Mondsee for the thermal stratified period

Variable	Median (range)	*n*	Study period
Depth of *P. rubescens* layer (m)	12.5 (8–15)		1982–1984, 2000-2004
BV net change (μ', d^{-1})	0.099 (0.002–0.446)		1982–1984, 2000–2004
Trichome length (µm)	500 (28–1090)	1,712	July 2002
Trichome width (µm)	5.3 (5.1–6.4)	40	July 2002
Oscillaxanthin/Biovol. (µg μm^{-3})	5.74×10^{-8}	36	July 2002
Oscillaxanthin/chl-*a* (µg μg^{-1})	0.082 (0.042–0.18)	36	July 2002
Chl-*a* at DCM (µg L^{-1})	3.3 (0.4–32.1)	1,150	1977–2004
Light at DCM (% surface L.I.)	0.3 (0.1–1)		1982–1984, 2002
Water temperature at DCM [°C]	11.3 (4.9–24.0)	1,039	1982–2004
z_{eu}/z_{mix}	0.31 (0.18–4.83)	289	1982–2004
pH	8.20 (7.70–8.20	1,150	1982–2004
Conductivity (µS cm^{-1})	305 (268–320)	1,150	1982–2004
Alkalinity (mval)	3.0 (2.6–3.1)	1,150	1982–2004
PO_4-P (SRP, µg L^{-1})	0.7 (<0.3–2.6)	201	1982–2004
Total phosphorus (TP, µg L^{-1})	9.5 (4.0–28.1)	665	1982–2004
NH_4-N (µg L^{-1})	9.6 (0.0–61.9)	349	1982–2004
NO_3-N (mg L^{-1})	0.6 (0.2–1.1)	1,122	1982–2004
Total nitrogen (TN, mg L^{-1})	0.62 (0.12–1.20)	404	1982–2004
P_{max} [mg C (mg chl-*a*)$^{-1}$ h^{-1}]	1.85 (0.9–3.0)	15	2000
α [P_{max} (µmol photons m^{-2} s^{-1})$^{-1}$]	0.028 (0.003–0.031)	15	2000
E_K (µmol photons m^{-2} s^{-1})	79 (35–320)	15	2000
Timing annual peak (Julian day)	157 (91–195)	31	1969–1972, 1977–2004
Timing annual min (Julian day)	248 (198–301)	31	1969–1972, 1977–2004

Discussion

Long-term dynamics

Biovolume development of *P. rubescens* since 1969 can be characterised by a number of periods triggered by different events. The appearance and early success of *Planktothrix* must be attributed to high and increasing nutrient loading to the lake that originated from sewage effluents (Findenegg, 1969, 1973). Similar observations linking the appearance and expansion of *P. rubescens* to enhanced nutrient input have been made at several other sites (e.g. Edmondson et al., 1956). The beginning of sewage diversion in 1973 started a new era in which nutrient loads, particularly phosphorus, were reduced. Response of the lake was, however, delayed until 1979, when total phytoplankton biovolume began to decline until it reached a somewhat stable period of low values beginning in 1989 (Dokulil & Teubner, 2005). Such a reversal has been recognised in many other places (e.g. Schanz & Thomas, 1981).

The wax and wane of *P. rubescens* during these eutrophication and restoration periods was strongly controlled by phosphorus concentration ($r^2 = 0.89$, $p < 0.001$), as indicated in Fig. 8. The relation inserted in Fig. 8 shows that growth of *Planktothrix* was largely uncoupled from phosphorus for the last 20 years. Even the increase in biovolume in the years 2001–2006 (see Figs. 1, 2) was unrelated to TP and must have been triggered by factors other than P-load.

Analysis of biomass distribution versus a gradient of phosphorus concentration from several Austrian and Bavarian lakes revealed that *P. rubescens* proliferated under mesotrophic conditions (Teubner et al., 2004), opposing the general notion that the taxon is a 'eutrophic' species (see Table 1 in Ernst et al., 2009). Consequently, *P. rubescens* is one (among others) used to characterise mesotrophic to moderately eutrophic conditions when assessing the ecological status of lakes in the context of the Water Framework Directive (e.g. Wolfram et al., 2008).

Vertical niche separation

A number of plankton assemblages can form a DCM by aggregation or growth. Many types of deep living phytoplankton assemblages have been described from freshwater and marine systems (Cullen, 1982; Reynolds, 1992; Adler et al., 2000) that respond differently to environmental gradients.

The DCM formation by *P. rubescens* during summer thermal stratification is commonly described for oxygenic metalimnetic layers at or below the euphotic zone. This behaviour is well documented and is usually regulated by buoyancy changes responding to alterations in the light climate (e.g. Konopka, 1982; Micheletti et al., 1998; Teubner et al., 2003; Walsby et al., 2004). The strong density gradient in the metalimnion additionally creates a strong buoyancy force that prevents mixing and hence avoids entrainment. The composition of these phytoplankton assemblages in deep layers, mainly *P. rubescens*, can persist throughout a season, building a steady state assemblage that differs from assemblages in the epilimnion (e.g. Teubner et al., 2003; Lake Ammersee).

The occasional appearance at the lake surface, as was observed during the eutrophication period in Mondsee, also is a common feature (see "Introduction" and Fig. S2) and has been explained by changes in buoyancy regulation induced by mixing at the end of the stratified season (Walsby et al., 2006). Entrainment during mixing, therefore, also explains persistent biovolumes throughout the water column in autumn and, depending on meteorological conditions, during winter as was observed in 1983 to 1984 in Mondsee (see Fig. 3). Moreover, the species is acclimated or even adapted to low light intensities and low water temperatures allowing winter survival (Holland & Walsby, 2008).

High-resolution profile measurements have shown that the metalimnion is a zone almost free from turbulences in lakes (Ford & Johnson, 1983; Etemad-Shahidi & Imberger, 2001; Yamazaki et al., 2010). In Mondsee, we found a narrow stratum of the maximum population of *P. rubescens* at 13–13.5 m within the metalimnion extending over 7–8 m. It supports the theory that trichomes can easily move within this low turbulence layer by physiological buoyancy regulation in response to vertical light gradients (Walsby et al., 1983; Walsby, 2005). Effects of internal waves, as observed by Cuypers et al. (2011), can be safely ruled out because of permanent calm weather during the 2 days of observation.

Planktothrix rubescens modulated their buoyancy in Mondsee to aggregate in the dim light layer from 1 to 0.1% light intensity, which is sufficient light to support net growth but does not damage or inhibit the

photosynthetic apparatus. The population maximum layer at 13–13.5 m seemed to be optimal for *P. rubescens*, even if it was close to the boundary layer between the metalimnion and the hypolimnion (at 14 m in mid-July). These finding were substantiated by photosynthesis–irradiance response curves in Mondsee. Analysing the 11 µm size fraction of phytoplankton assemblage, which was almost exclusively composed of *P. rubescens* for the year 2000 (Kaiblinger et al., 2007), observed photosynthetic parameters were similar to those measured in an in situ experiment in 1987 (see Table 1). All photosynthetic parameters (Table 2) describe a strongly shade acclimated species, which utilises the small amounts of light reaching the metalimnion. The main light-harvesting pigments in *P. rubescens* are phycocyanin and phycoerythrin. These phycobilins enable this cyanobacterium to use low light intensities but also the narrow, almost monochromatic wave band of light quality in these deep layers.

Another aspect of physiological adjustment to deep layers is heterotrophic growth stimulated by dim light as reported from amino acid uptake experiments in Lake Zürich by Zotina et al. (2003) and Walsby & Jüttner (2006). The dim-light uptake rate of certain amino acids was two- to ninefold higher than the dark-uptake rate in their studies. Further, E_K values for amino acid uptake were much lower than for photosynthesis. Both studies from Lake Zürich found that heterotrophy, stimulated by low light intensity, supplemented autotrophic growth of *P. rubescens* in the deep metalimnetic strata. *P. rubescens* might be able, therefore, to grow even if there was insufficient light available for autotrophic growth. During the diurnal study in July 2002 in Mondsee, the maximum population layer at 13–13.5 m was 3–4 m below both the euphotic depth and the thermocline. Moreover, trichome length of *P. rubescens* at 9.5 m was significantly shorter than at 12.5 m. This observation supports findings by Walsby (2005) that longer trichomes tend to respond faster than shorter ones and are even able to migrate to deeper strata on sunny days. All these diurnal patterns confirm the physiological adjustment of *P. rubescens* to deep strata conditions discussed before (see vertical pattern 1983–1984 above).

The marker pigment oscillaxanthin

Oscillaxanthin is found in some phytoplankton species, mainly in filamentous cyanobacteria as *P. rubescens*, but also, e.g. in *P. agardhii* and *Aphanizomenon* spp. (Goodwin, 1976; Rowan, 1989; Rücker et al., 1995; Schlüter et al., 2004). This carotenoid has been commonly studied as a fossil pigment in sediments (e.g. Griffiths, 1978; Lami et al., 2000), while studies on plankton were more rare (Rücker et al., 1995; Schlüter et al., 2004; Greisberger & Teubner, 2007). Many pigments are ruled out as markers because they occur in numerous genera and even in several algal classes. In contrast, oscillaxanthin has an advantage because it is present in just a few species and of those, it is often possible to separate them by differences in their ecology. For example, *P. rubescens* and *P. agardhii* have different niches and, therefore, blooms in a lake usually consist of only one or the other. Our results confirm that this marker pigment can be used to infer biovolumes of *P. rubescens* when biomass is large. When biovolume contribution to total phytoplankton is low, the analytical techniques were at the detection limit, as has been described above for the epilimnetic samples with low biovolumes of *P. rubescens* in Mondsee. The median ratio of oscillaxanthin to chl-*a* calculated for *P. rubescens* from our field samples was twice as high as for cultures of *Aphanizomenon* and *Anabaena* grown under highlight conditions published in Schlüter et al. (2004).

Climate impact

How climate controls the lake's phenology and, therefore, affects the dynamics of *P. rubescens* was central to the analysis of the relationship between the NAO signal and ecosystem function. A positive NAO_{Febr} index relates to years of relative high insolation, high air temperature and low precipitation in the catchment of Mondsee. A negative NAO_{Febr} is associated with the opposite weather situation in the region (Dokulil et al., 2010; Nõges et al., 2010). We further show that an increase of hypolimnetic water temperatures by 0.1–0.2°C per decade was mediated by climate trends related to winter NAO signal (Dokulil et al., 2006). The response of *P. rubescens* to the winter climate signal is shown in Fig. 9. The annual biovolume development of *P. rubescens* increased during years of positive NAO_{Febr}, i.e. during years of warm winter–spring weather. The biovolumes were further dependent on the timing of the on- and offset of the stratification and the time span between these two dates. The response of *P. rubescens* to stratification, however, did not show a general stimulation or

inhibitory effect but changed within a year, as described by direct and inverse correlation patterns in Fig. 9. The annual average pattern shown in Fig. 10 indicates that the response of *P. rubescens* is in general different from the total phytoplankton during periods other than overturn. This independent, or indirectly related, development can be explained by vertical niche separation of epilimnetic and metalimnetic assemblages. Although *P. rubescens* was vertically separated, the species only benefited from thermal stratification in the beginning of the year, during Phases II and III. A prolonged period of summer stratification later in the year, as a consequence of climate warming, however, was not an advantage for biovolume development. These intra-annual shifts between early year forcing and mid-summer–autumn weakening of *P. rubescens*, explain the significant positive but weak correlation between the annual biovolume of *P. rubescens* and the climate signal NAO_{Febr}.

The temporal coincidence of the annual peak of water transparency and the annual metalimnetic peak of *P. rubescens* in Mondsee is a common pattern described for the transition from spring to summer in other studies (e.g. Salmaso et al., 2003; Teubner et al., 2003; Talling et al., 2005). During this growth period, *P. rubescens* might not only benefit from sufficient light and nutrient availability for autotrophic growth but also from low light-stimulated uptake of organic compounds released to the metalimnion after the breakdown of the phytoplankton spring peak. Adenylate energy charge measurements in Mondsee by Jewson & Dokulil (1982) support the organic breakdown in the thermocline. In this context, a zone of low turbulence to some extent seems crucial for the growth of *P. rubescens* at depth. A metalimnion that extends over several metres, as shown in this study for Mondsee, allows *P. rubescens* to move easily by physiological buoyancy regulation between strata with light intensities around E_K sufficient for photosynthesis or, at even deeper strata, to light intensities below E_K, beneficial for amino acid uptake. In some lakes (e.g. Anneville et al., 2004), the development of *P. rubescens* early in the year is discussed in view of overwintering biovolume. In Mondsee, winter biovolumes of *P. rubescens* were sometimes higher than summer minima and were even enhanced in certain years, as discussed for the transition from 1983 to 1984.

Climate warming, however, benefits the growth of *P. rubescens* only during the spring-early summer transition but not later in the year. The decline of *P. rubescens* to an annual minimum in summer lasts over several weeks during thermal stratification (indicated as Phases IV and V in our long-term seasonality Fig. 10). This decline to the summer minimum is confirmed by a statistically strong but inverse relationship between the annual biovolume of *P. rubescens* and the duration of thermal stratification. Therefore, the lengthening of the period of thermal stratification by warming is not beneficial for *P. rubescens*. Other phytoplankton species, which are mixotrophic and motile might benefit even more from a longer stratification period in summer, because they can exploit nutrient patches not only in the deep layer but also near the surface. Flagellates of crypto-, chryso- and dinophyceens play an important role in deep alpine lakes (Dokulil, 1988; Fott et al., 1999; Sonntag et al., 2006; Tolotti et al., 2012) and can account for more than 40% of the phytoplankton taxa as recorded for mesotrophic alpine Ammersee (Teubner et al., 2003). In the case of the diurnal distribution pattern in Mondsee 2002, many photosynthetic flagellates were evenly distributed over depth and, therefore, behaved differently from *P. rubescens*, living almost exclusively in deeper layers.

Summary and conclusions

The main characteristics of *P. rubescens* and associated environmental variables observed over four decades in the alpine lake, Mondsee are summarised for the stratified period in Table 2. The ranges for chlorophyll-*a* and water temperature are, at first glance, surprisingly wide. Considering the various phases of ecosystem changes during the four decades of observation, these ranges are clear because they cover periods of eutrophication and years of extremes in water temperature like 2003. The variation and median of the light climate substantiate that *P. rubescens* lives substantially below the 1% light level and prefers situations when the euphotic depth is equal or less than the mixing zone. The median and ranges of the light climate, the z_{eu}/z_{mix} ratio and the nutrients, particularly TP and TN fall well into values reported by Schreurs (1992) for Dutch lakes. The photosynthetic parameters obtained in 2000

(Kaiblinger et al., 2007) all indicate shade adaptation of *P. rubescens*. On average, the *Planktothrix* population peaks in May to June and reaches its minimum in August to September.

For deep living *Planktothrix rubescens* populations the following conclusions can be drawn:

- Preference for living under dim light (<1%) and cool water temperatures (5–12°C) at a depth of large density changes (metalimnion) and reduced turbulence.
- Life in or below the thermocline is aided by physiological acclimation of photosynthesis and buoyancy regulation.
- Survival during the stratified period is possible because of a potentially heterotrophic subsistence.
- Population dynamics during periods of lake eutrophication or rehabilitation are primarily controlled by phosphorus.
- Proliferation of *P. rubescens* is optimal at meso- to moderately eutrophic conditions.
- Benefits from climate warming are restricted to the winter signal transmitting weak climate forcing during late spring and early summer (transition period from vernal overturn to the on-set of thermal stratification). Longer periods of summer stratification showed no advantage for population development.
- The annual average long-term pattern indicates that population dynamics of *P. rubescens* are different from the development of total phytoplankton during the period of stratification.

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PHYTOPLANKTON

Resource ratio and human impact: how diatom assemblages in Lake Maggiore responded to oligotrophication and climatic variability

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Abstract Diatoms have been often used to track trophic changes from sedimentary records: recent studies demonstrated that these organisms can even be valuable indicators of climatic variability, although it is often difficult to discriminate the role of trophic and climatic drivers. Moving from the hypothesis that oligotrophication and climate affected the composition of the diatom assemblages by changing the resource ratio, we analysed the vernal diatoms succession in Lake Maggiore, between 1984 and 2007, using multivariate techniques (cluster analysis, canonical correspondence analysis, multivariate regression trees), in order to single out the oligotrophication effects from those attributable to climatic variability. Our results point out that Si, TP, temperature and wind emerged as key explanatory variables in species selection, with a stronger link between trophic and climatic drivers after the lake reached a stable oligotrophic status. Peculiar climate-driven events (deep mixing and floods) affected the in-lake Si:P ratio, giving an advantage to diatoms that are excellent P, but poor Si competitors. The classical role of *Fragilaria* and *Tabellaria* as early-warning indicators of eutrophication should be reconsidered, taking into account that both can be useful indicators of climate change, when links between their physiological resource needs and environmental data coming from robust limnological investigations can be established.

Keywords Diatoms · Seasonal succession · Oligotrophication · Meteorological variability · Nutrient ratio · Climate change

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Introduction

After eutrophication became evident in many lakes in the late 1960s, restoration plans were implemented to return some of the lakes to their original trophic condition. More recently, a major concern has been placed in analysing the effect of climate change on aquatic ecosystems (Dokulil & Teubner, 2011) and to what extent this could counteract the restoration measures directed at improving lake water quality. In this respect, the impact of climate and of meteo-

climatic events in relationship with lake trophy and trophic changes would deserve more attention, because the eutrophication drivers are expected to increase with climate change (Dokulil & Teubner, 2011).

Trophic as well as climatic changes may impact aquatic biota at various levels of the trophic web: we decided focusing this article on diatoms assemblage mainly because Lago Maggiore, due to its siliceous basin, is a 'diatom lake': considering biomass, diatoms are the dominant phytoplankton group for most of the year. Diatoms are known to be sensitive to environmental changes: shifts in diatom assemblage composition are frequently the first indication of ecological perturbations. As thermal stratification and physical mixing processes are controlled by climatic forcing, it is expected that climate change will particularly affect diatom abundance and community structure (Smol et al., 2005; Rühland et al., 2008). Many paleolimnological investigations (see Saros et al., 2003; Kirilova et al., 2008 and references therein) demonstrated that diatom profiles are useful in the reconstruction of limnological parameters such as lake-water pH and phosphorus concentrations, as well as climate-related variables. In the past decades, interest in the use of diatoms as indicators of water quality has grown (e.g. Birks et al., 2004; Bennion & Battarbee, 2007; Bigler et al., 2007). Studies on long-term changes of diatom assemblages mainly concern the sedimentary diatoms, although data describing the periodicity on monthly time scales are often needed to assess the response of diatoms to seasonal meteorological events. Moreover, from the analysis of the fossil records, it can be difficult to distinguish the role of direct and indirect effects of environmental and climatic changes on diatoms (Anderson, 2000; Köster & Pienitz, 2006).

In the case of Lake Maggiore, a valuable long record of the spring diatom succession is available, thanks to a lake's monitoring programme, started at the end of the 1970s. In the frame of this programme, complementary limnological data were collected, allowing to investigate the effects of both trophic changes and climatic variability. The key environmental constraints and their mutual relationships in the different periods of the lake's trophic evolution can be, therefore, analysed.

The change in phytoplankton species composition recorded during the last decades in Lake Maggiore can be mostly seen as the results of lake's oligotrophication (Ruggiu et al., 1998): some remarkable changes were observed in the basic structure of the phytoplankton assemblages, such as the constant increase in the number of taxonomic units, from about 50 *taxa* per year in 1984 to about 90 in 2003 and the decrease of average phytoplankton cell size (Kamenir & Morabito, 2009). On the other side, the variability recorded on shorter time scale, against an almost unchanged trophic status, can be regarded as a response to the variability of the local climate, sometimes interfering with the effects of the changing trophy. In the Lake Maggiore watershed, the strongest climatic events were recorded mainly across autumn and winter seasons, affecting also physical and chemical parameters, such as lake mixing and nutrient supply (Manca et al., 2000; Morabito, 2001). The effects of these climatic fluctuations are mainly experienced by populations growing in spring and can, therefore, determine changes in the diatom assemblage structure and abundance (see, Manca et al., 2000). We would like to remember here a dramatic *Tabellaria* bloom, occurred in Lake Maggiore in the early 1960s (1963–1964), at that time regarded as a sign of lake's eutrophication. However, in the same document, Tonolli claims for peculiar climatic winter conditions as a driver for the diatom's spring bloom. The reappearance of *Tabellaria* in significant numbers during the last decade apparently does not match with the present oligotrophic condition of the lake and could be related to climatic variability: this is, probably, the most intriguing change inside the planktonic diatom flora, stimulating us to investigate the long-term dynamics of this group in Lake Maggiore.

With our analysis, we would like to address three main questions:

(1) Are the changes recorded inside the diatom flora a sign of the trophic evolution? Or climatic variability can play an important role, confounding the response due to trophic changes?
(2) To what extent past and recent anomalies in diatom spring development are a clue for trophic variability?
(3) Which are the key climatic as well as trophic drivers controlling the long-term dynamics of the diatom assemblages?

Study site

Lake Maggiore, the second largest subalpine lake in Italy (lake area of 213 km^2, drainage area of

6,599 km^2), is situated at 193 m above mean sea level. It has a maximum depth of 370 m and an average depth of 178 m (see http://www.ise.cnr.it/lter/famemag.htm for a bathimetric map). It is oligotrophic by nature, as shown by early limnological studies (Baldi, 1949) and by analysis of the sedimentary pigments (Guilizzoni et al., 1983). The eutrophication process started in the 1960s: the nutrient concentration (phosphorus) in the lake water began to rise and was soon followed by an increase in phytoplankton abundance, biovolume, and primary production (Ravera & Vollenweider, 1968; Morabito & Pugnetti, 2000).

In the late 1970s, the lake reached a trophic state close to eutrophy, when the P loads peaked and the maximum in-lake TP concentration was recorded (around 30 μg l^{-1} at winter mixing; Ruggiu et al., 1998). Since that time, the P loads have been gradually reduced by various means, including the establishment of sewage treatment plants and the reduction of total phosphorus in detergents. As a result, the TP values have gradually decreased to some 10 μg l^{-1} (Ruggiu et al., 1998). The slow reversal of the trophic state of Lake Maggiore is documented in many papers: concerning phytoplankton, strong emphasis was put on the apparent resilience of the communities against falling levels of phosphorus (de Bernardi et al., 1988). However, starting from 1987 to 1988, major biological changes were at last manifested, especially in the phytoplankton (Manca et al., 1992).

Parameters analysed and methods

All the data used in the analysis presented here have been gathered in the frame of the research programmes devoted to the investigation of the trophic condition of Lake Maggiore and are published in the annual reports of the International Commission for the Protection of Swiss-Italian Waters (Commissione Internazionale per la Protezione delle Acque Italo-Svizzere; see also www.cipais.org). Samples for phytoplankton and chemical analysis were all collected at a central sampling site, corresponding to the point of lake's maximum depth (370 m). Samples were always collected with an integrating bottle in the 0–20-m water layer, corresponding to the euphotic layer in Lake Maggiore. Metalimnetic layer was always included in the 0–20-m water column during the stratification period. Meteorological data are published in the annual reports of CNR-ISE meteorological station (Arca & Barbanti, 1986–1991; Barbanti, 1992–1997; Ambrosetti et al., 2006a, b, 2007, 2008).

Phytoplankton

As explained before, only diatom taxa were considered in the analysis: in particular, we focused on the spring season, when diatoms have their growing phase peak. Data since 1984–2007 were used, including all the samples collected between January and June each year: sampling frequency was collected monthly in January and February, fortnightly in the period March–June. A total amount of 34 diatom *taxa* were recorded in the period 1984–2007: many of them reached a little importance and very low abundance and biovolume values, but some *taxa*, occasionally peaking to significant values in 1 or 2 year in the series, without a clear long-term trend: this is the case, for instance, of many small centric diatoms occasionally appearing after the year 2000. We decided to focus our analysis on the 12 dominant diatom *taxa*, contributing, each single year, to build up, on average, 80% of the total diatom biomass in the spring phase of succession and usually included among the *taxa* building up more than 80% of the total yearly phytoplankton biomass. In order to reduce the number of samples, making easier the interpretation of the multivariate statistical analysis, samples were grouped into three seasonal periods and averaged: Early Spring (ESP, including samples collected in January and February); Spring (SP, March and April samples) and Late Spring (LSP, May and June samples). To reduce the weight of the most dominant species in the statistical analysis, the original biovolume data were transformed in percentage values, then converted with a root–root transformation (see Salmaso, 1996).

Physical parameters

Physical parameters were averaged in each seasonal period. The following physical variables were considered in the analysis: mixing depth at maximum winter overturn (Zmix), wind velocity (Wind, total amount in km), epilimnetic water temperature (WaterT_e, 0–20 m) and air temperature (Air_T, °C). Monthly averages of wind, water and air temperature were used. Finally, the average values for the three seasonal

periods were calculated. Wind data were recorded by means of two different sensors across the time series: the old sensor from 1957 operated till 1997 and was replaced by a more sensitive one in that year. Therefore, the wind data for the period 1984–1997 are not comparable with those collected since 1997 onwards. For a limited period (1997–2003), both sensors were working. Because of this reason, two different sets of environmental parameters were used, the first covering the years 1984–1997 (in which Wind-OS indicates data from old sensor) and the second for the period 1997–2007 (with Wind-NS indicating new sensor's records).

Chemical parameters

Total phosphorus at mixing time (TP), monthly values of reactive phosphorus (SRP) and reactive silica (Si) were used. Si:P ratios were calculated as molar ratios. Monthly values, after averaging, were finally referred to the seasonal periods. Reactive silica and reactive and total phosphorus were determined by spectrophotometry (indophenol-blue and ammonium molybdate with ascorbic acid, respectively). Analytical methods were carefully evaluated across the years to assure the comparability of the data, as some changes were introduced during the period considered (Tartari & Mosello, 1997).

Statistical analysis

We calculated the dissimilarity matrix between pairs of samples using the Bray and Curtis index (Bray & Curtis, 1957) computed on biomass data, transformed as previously explained: the distance matrix was then analysed using the average linkage clustering method. Bray–Curtis similarity matrix calculation and cluster analysis were carried out using MASS and vegan packages in R v. 2.13.0 (R Development Core Team, 2011). The relationships among species and environmental variables were explored by means of canonical correspondence analysis (CCA; CANOCO 4.5), following the prescriptions reported in ter Braak & Smilauer (2002). The significance of the axes obtained by the CCA analysis was tested by means of Monte Carlo test with 499 permutations (ter Braak & Smilauer, 2002). Generalised linear modelling (GLM) was carried out to test the response of single species to the set of environmental variables and to identify the dominant pattern in the species–environment relationship: the change of species abundances has been analysed along the gradient of the environmental variables, using axes 1 and 2 as predictors and selecting the best fitting model from the value of the AIC statistics.

Multivariate regression trees (MRT; De'ath, 2002) were also used for modelling relationships between species and environmental variable. While the classical methods (e.g. generalised linear models and classification and regression tree; Breiman et al., 1984) place more emphasis to the explanation and description of distribution of species, this new approach allows making a prediction of biological community. MRT is a form of multivariate regression in that the response is explained, and can be predicted, by the explanatory variables. However, it is also a method of constrained clustering, because it determines clusters that are similar in a chosen measure of species dissimilarity, with each cluster defined by a set of environmental values. As with unconstrained clustering, each cluster can define an assemblage type, but additionally the environmental values define an associated habitat type. Trees are usually selected on the basis of their predictive accuracy. MRT analysis has been carried out using package mvpart in R v. 2.13.0 (R Development Core Team, 2011).

Results

Spring diatom succession

The succession of diatoms in Lake Maggiore has a typical time course, starting usually at the end of February and peaking between mid-March and late April, although the growing season usually lasts until June. However, since 1984, the species assemblages showed many changes (Fig. 1), with only few species remaining important during the entire period analysed (*Asterionella formosa* Hassal, *Fragilaria crotonensis* Kitton, *Aulacoseira islandica* var. *helvetica* O. Müller), although showing significant interannual fluctuations. Some *taxa* declined during the oligotrophication, such as *Aulacoseira islandica* v. *helvetica* and *Synedra acus* Kützing, while others (*Tabellaria flocculosa* (Roth) Kützing)) increased or became important only for a short time period (*Stephanodiscus parvus* Stoermer & Håkansson, *Aulacoseira ambigua* (Grunow) Simonsen)), without a clear trend. *Diatoma tenuis* C. Agardh showed

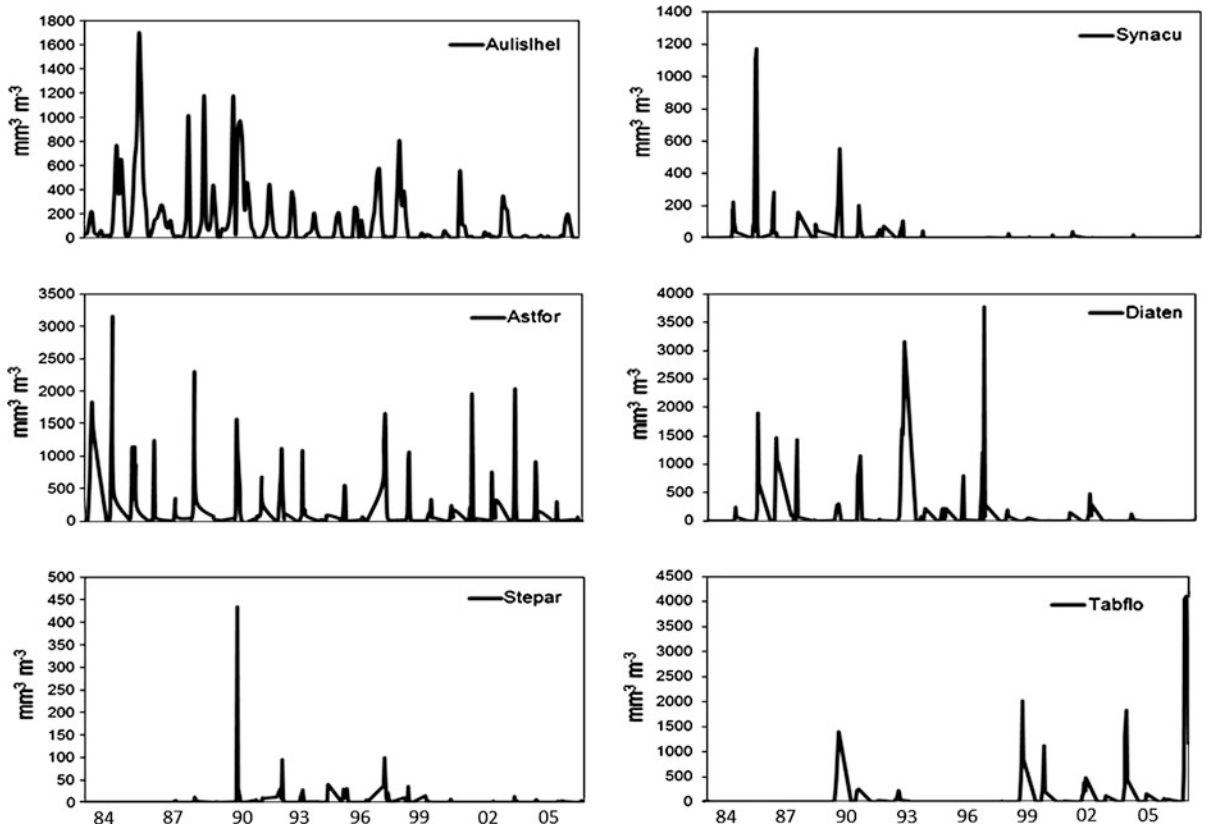

Fig. 1 Diatom *taxa* showing the clearer long-term trends in Lago Maggiore. Data from January to June of each year are shown. *Astfor*: *Asterionella formosa*; *Aulislhel*: *Aulacoseira islandica* var. *helvetica*; *Diaten*: *Diatoma tenuis*; *Stepar*: *Stephanodiscus parvus*; *Synacu*: *Synedra acus*; *Tabflo*: *Tabellaria flocculosa*

an interesting trend, characterised by a sharp decline, contemporary to the rise of *Tabellaria flocculosa*.

The cluster analysis (Fig. 2) aggregates the seasonal samples in five clusters at a 0.45 similarity level. The samples clustering mainly mirrors the seasonality, with clusters 1 and 2 including most of the Early Spring (January–February) samples, cluster 3 mostly Spring (March–April) samples, clusters 4 and 5 Late Spring (May–June) samples. The Spring assemblages are more homogeneous, whereas Early and Late Spring associations are both splitted in two clusters: the most recent samples are aggregated in clusters 1 and 5 and the samples collected before 2000 in clusters 2 and 4, indicating that changes in species composition affected mainly the initial and the final stage of the growing season. The fluctuations of some key *taxa* (Fig. 3) clearly explain the results of the cluster analysis: the Early Spring phase is characterised by the dominance of *Aulacoseira islandica* var. *helvetica* and *Synedra acus* in the period 1984–1995. Following their decline (1995–2007), *Cyclotella comensis* Grunow became more important. This species characterised also the Late Spring phase during the recent period, together with the dominance of *Tabellaria flocculosa*. The decrease of *Synedra acus* and *Diatoma tenuis* in the decade 1984–1994 is responsible for the splitting of the LSP samples in two clusters.

Species–environment relationships

As explained before, due to the changing of the wind sensors, two different set of environmental variables were tested (1984–2003 and 1997–2007). Considering the first period, the ordination along CCA axes 1 and 2 explains 76% of the variance for species–environment relationship. The most significant variables included in the model, identified through the Monte Carlo permutation test, were Si ($P = 0.002$), TP ($P = 0.002$), Zmix ($P = 0.026$) and the interaction between Zmix and TP (Zmix*TP, $P = 0.048$). Important, though

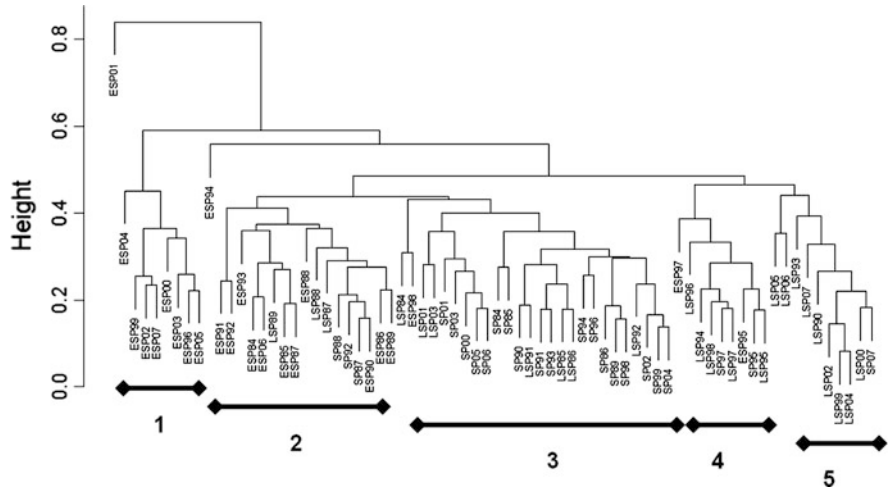

Fig. 2 *Dendrogram* resulting from the cluster analysis of the samples aggregated in the three seasonal groups. Seasonal codes are explained in the text

statistically not significant, was the air temperature (Air_T, $P = 0.07$). Concerning the second period, the proportion of variance explained by the first two ordination axes amounted to 67%, increasing to 81% when third axis is considered. The most significant variable was Air_T ($P = 0.002$), followed by the interaction Zmix*TP ($P = 0.074$) and by silica ($P = 0.078$). The reduced importance of the TP and Si in this second data set is due to the high stability of phosphorus concentration and to the limited fluctuations of Si during the decade 1997–2007.

The pattern of species response to environmental parameters in the period 1984–2003 results from the CCA biplot in Fig. 4. The species ordination is mainly driven by the first axis: the physical variables (wind, air and water temperature) determine the positive side of the axis, whereas the nutrients the negative side. Generalised linear modelling (see Supplementary Online Material 1) allows to analyse the response of single species to the environmental variables: in particular, the ordination axis is used as predictors for the growth response of a species. GLM outputs indicate that *Tabellaria flocculosa* and *Diatoma tenuis* are the species showing the strongest relationship with the physical variables: their position in the ordination biplot indicates that the two diatoms increase their abundance with increasing water and air temperature (LSP phase). The relationship of *T. flocculosa* with wind suggests that his growth is favoured by windy conditions, slowing down the sedimentation of this heavy silicified diatom.

In the left side of the CCA biplot, with a clear relationship with phosphorus, there are those species showing a decline with the improving trophic status (*Aulacoseira islandica* var. *helvetica*, *Synedra acus* and *Asterionella formosa*). According to GLM results, *A. islandica* var. *helvetica* and *C. comensis* both respond to the TP gradient, although in an opposite way, *Aulacoseira* increasing with TP and *Cyclotella* decreasing: the position of the species respect to Si probably reflects its consumption and cannot be taken as an indication of silica limitation.

In the second data set (1997–2007), the ordination plot along the first two axes is similar to that previously described for the 1984–2003 dataset (not shown). A first difference is due to the relatively high proportion of the variance explained by the third ordination axis, pointing out a clear seasonality effect: the *x*-axis (first axis) separates ESP and SP samples from the LSP ones, whereas the *y*-axis (third axis) separates SP and ESP samples. A further difference is that this ordination (see Supplementary Online Material 1) emphasises, along the *x*-axis, the separation of *D. tenuis* and *T. flocculosa*, the first species being mainly constrained by high temperature, whether the second one being more dependent on wind velocity. The species distribution along the *y*-axis seems to be mainly constrained by nutrient availability, in particular by reactive phosphorus: the decrease of SRP across seasons probably modifies the Si:P ratio, thus affecting the species composition, from a decrease of *Stephanodiscus parvus* to an increase of *Aulacoseira* spp.

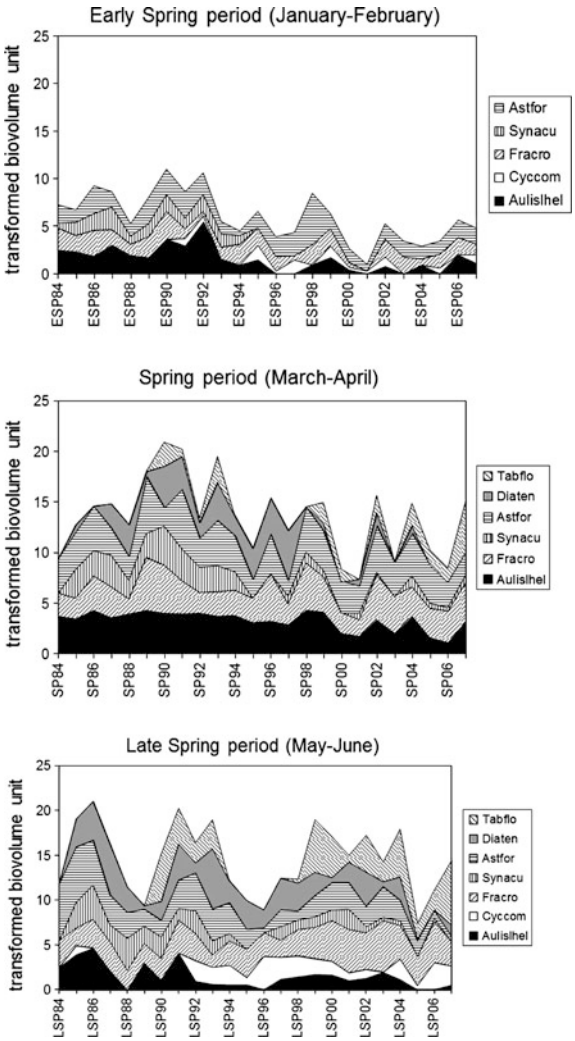

Fig. 3 Fluctuations of the most important diatoms in the period 1984–2007, in the three seasonal periods. The same seasonal codes are reported in the cluster dendrogram. Biovolume data on *y*-axis were obtained after transforming the raw data, as explained in the text. *Upper panel* early spring; *middle panel* spring; *lower panel* late spring. *Astfor*: *Asterionella formosa*; *Aulislhel*: *Aulacoseira islandica* var. *helvetica*; *Cyccom*: *Cyclotella comensis*; *Diaten*: *Diatoma tenuis*; *Fracro*: *Fragilaria crotonensis*; *Synacu*: *Synedra acus*; *Tabflo*: *Tabellaria flocculosa*

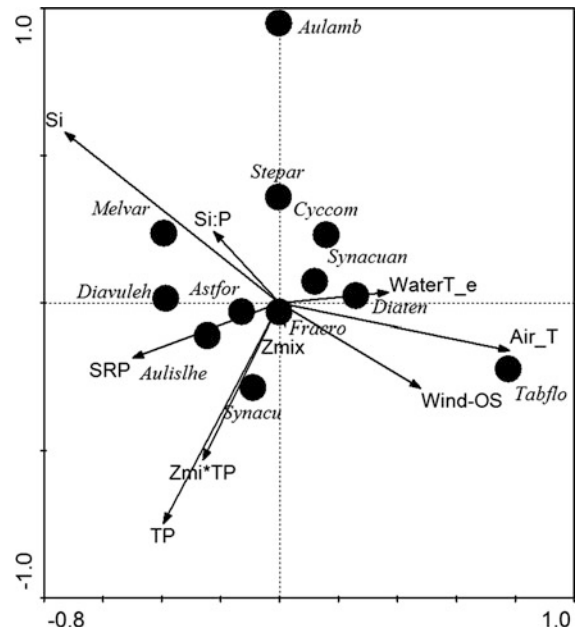

Fig. 4 Ordination of *taxa* and environmental variables resulting from the canonical correspondence analysis (1984–2003 period). Variable coding is explained in the text. Species coding as in Fig. 4, plus *Aulamb*: *Aulacoseira ambigua*; *Diavulhe*: *Diatoma vulgaris* var. *herembergi*; *Melvar*: *Melosira varians*; *Stepar*: *Stephanodiscus parvus*; *Synacuan*: *Synedra acus* var. *angustissima*

Multivariate regression trees analysis confirms silica, total phosphorus and air temperature as the environmental drivers in species selection. For both periods, the size of the tree was selected by cross-validation: the minimum value of the cross-validation error (CVRE = 0.483 for first data set and CVRE = 0.539 for the second one) was used to decide on the size of the tree (seven groups for both). The R^2 of the trees (1 − relative error) were 0.688 and 0.616, respectively.

The output is basically the same for the two data sets (1984–2003 and 1997–2007), therefore only the results of the longer series will be discussed (Fig. 5). The separation of species in the two groups resembles that already obtained in the CCA ordination plot. MRT placed the greater emphasis on the role played by Si concentration: the first separation level is among samples with a Si concentration above or below 1.06 mg l^{-1} (high-silica samples in Fig. 6, upper panel). Samples with higher silica are mainly those from ESP, whereas the other seasons have lower concentrations, because of the consumption due to diatom growth.

The finding that silica is the key driver in the selection of diatom assemblages seems to be trivial: however, looking at the samples included in the two groups, we find some SP or LSP samples even in the high-silica group (Fig. 6). Because silica concentration in Spring or Late Spring samples are expected to decrease below the 1.06 mg l^{-1} thresholds (see MRT results above), due to diatom consumption, the

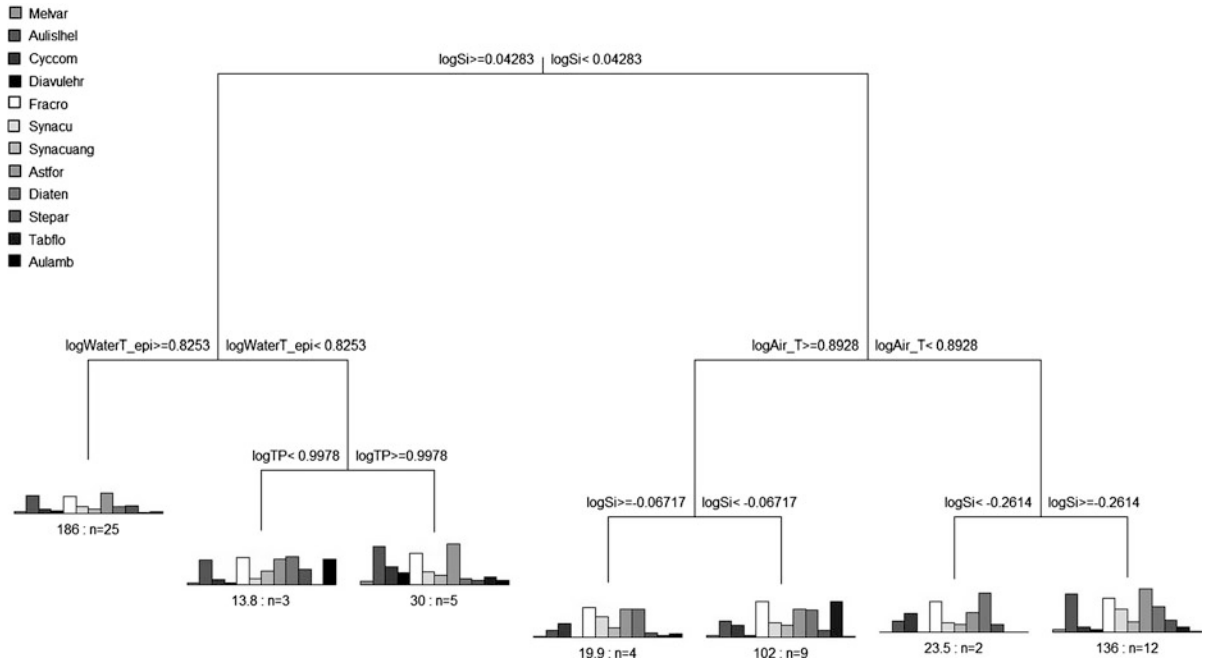

Fig. 5 Samples clustering obtained from the MRT analysis (1984–2003). Each node of the *dendrogram* shows the key discriminating environmental variable and its threshold value for samples separation. Species *bars* must be read from *right to left*, the *first bar* corresponding to the lower species in the legend

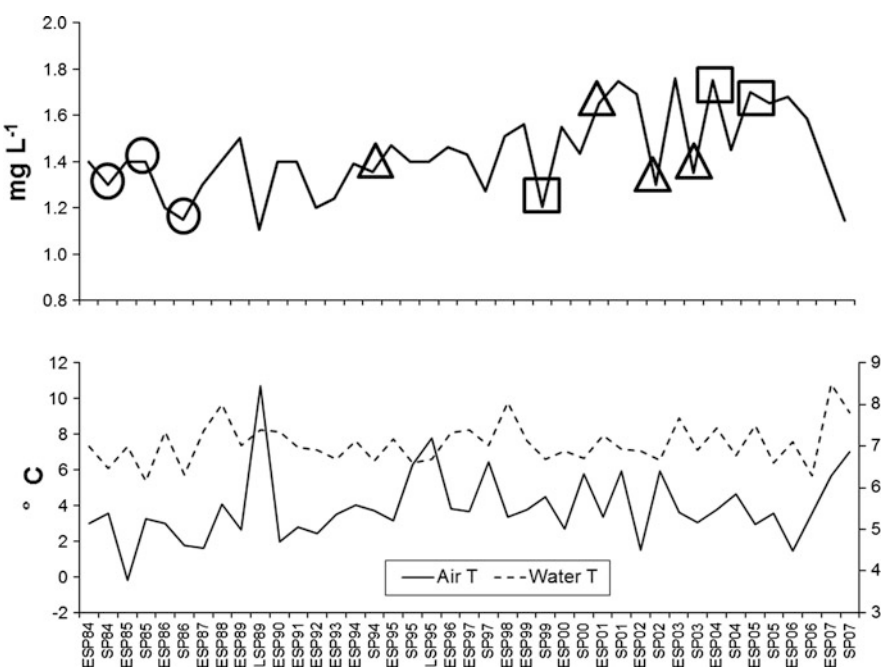

Fig. 6 *Upper panel* silica concentrations in the seasonal samples aggregated by the MRT analysis in high-silica group (silica concentration higher than 1.06 mg l^{-1}) Anomalous samples (SP and LSP seasons) and possible causes related to higher than usual silicates: *circles* cold periods; *triangles* floods effect; *squares* deep mixing episodes. *Lower panel* epilimnetic (0–20) water temperature and air temperature in the same 'high silica' samples

occurrence of such samples among those characterised by high silicates is anomalous. This can be due to a consumption lower than usual or to a Si availability higher than usual: therefore, a growth limitation of diatoms or an exceptional silica supply can explain such anomalies. Figure 6 shows the values of the silica

concentration in the samples belonging to high-silica group (upper panel): the anomalous SP and LSP samples are indicated by different symbols, according to the probable reason for their unusually high Si concentration. Among these reasons, there are very low water temperatures, such as those recorded in spring 1984–1986 and 1989 (see Fig. 6, lower panel), limiting diatom development and resulting in a lower than usual silica consumption. On the other side, deep mixing events (mixing depth exceeding 150 m), such those occurred in 1999, 2004 and 2005, can promote a silica enrichment of the upper water column, although the most important input of silica to the lake was due to the 1993, 2000 and 2002 flooding events.

Discussion

The most general pattern emerging from our analyses of the long-term data set is that seasonal environmental variability is the main driver for diatom species selection: this is not surprising, considering that diatoms usually show a marked and typical seasonal succession in lakes, where the species shift is constrained by the depletion of silica, the increase of water column stability as well as changes in the light regime (see Reynolds, 1984). However, comparable seasonal periods were also characterised by different diatom assemblages across years, as clearly shown by the cluster analysis. The oligotrophication of the lake has been certainly a strong driver for the species replacement: a recent paleolimnological study carried out on Lake Maggiore sediment cores (Marchetto et al., 2004) confirms the general trends pointed out by the analysis of the pelagic phytoplankton. Sedimentary records shown that the shift from oligotrophic to mesotrophic status during the 1960s and the recent oligotrophication process were accompanied by the same species shift recorded in the pelagic samples, where *Cyclotella* spp., *Stephanodiscus* spp., *Diatoma tenuis* and *Aulacoseira islandica* are good indicators of trophic changes, as pointed out by our analysis. *Asterionella formosa* and *Fragilaria crotonensis* show a less clear pattern in the sedimentary profiles: both species sharply increased during the eutrophication phase (Marchetto et al., 2004), but were still important under the Lake Maggiore oligotrophication. Our data confirm this trend, even though a smooth decline of *Asterionella* in the most recent years. The two pennate species seem to be able to thrive under a wide spectrum of trophic conditions, as pointed out by Wessels et al. (1999) who described a similar pattern during the trophic evolution of Lake Constance.

The interpretation of the pattern shown by *Tabellaria flocculosa* is, on the other side, less straightforward. Although this genus is commonly regarded as an indicator of early eutrophication warning (Wessels et al., 1999; Marchetto et al., 2004; Dokulil and Teubner, 2005), its dynamics in Lake Maggiore does not seem to be related to the ecological status of the lake. In fact, the frustules abundance of this species in the sediment's vertical profile shows quite a narrow peak at the beginning of the 1960s, followed by the disappearance of this diatom until the end of the 1990s, when a second, smaller peak was found (see Marchetto et al., 2004): this pattern is in good agreement with the data obtained from the analysis of the pelagic samples collected during the last decade (period not covered by the paleolimnological investigation), when the species increased significantly, in spite of the oligotrophic status of Lago Maggiore, and would support the idea that the long-term dynamics of *Tabellaria flocculosa* is not strongly related to the changing trophic condition.

The generalised linear models show that *Tabellaria flocculosa* is mainly controlled by physical factors (water, air temperature and wind), rather than by nutrient availability. The same factors play an important role in controlling the growth of *Fragilaria crotonensis* and *Diatoma tenuis*. The fluctuations of these species in the period analysed seems, therefore, mainly controlled by climate related rather than by nutrient-related factors, although the picture could be more complicated. For instance, Köster and Pienitz (2006) investigated the seasonal dynamics of diatoms comparing sediment traps and paleolimnological records: their conclusion was that the fluctuation of some species can be explained by their response to the cooling and mixing patterns of the water column, thus making difficult to understand to which factor (climate or nutrient availability) the diatom assemblages mainly respond, in particular when the species involved (such as *Tabellaria* and *Fragilaria*) respond to anthropogenic pressure as well. Moreover, it has been demonstrated that in deep lakes a strong link between climatic variability and nutrient supply exists (Manca et al., 2000; Salmaso, 2005), because temperature and wind are among the key drivers in determining the extent and the duration of lake

mixing. It is clear that climatic and trophic drivers are often acting together in regulating the vernal growth of diatoms. The key climatic constraint is not the direct temperature change, to which planktonic diatoms appear to be relatively insensitive (Anderson, 2000), but rather the factors on which they are dependent (turbulence, light and nutrients), ultimately controlled by climate (weather). The final outcome on the success of some *taxa* can be confounding: in this respect, the case of *Cyclotella* is paradigmatic. The increase of this centric diatom was usually taken as a clue for lakes' oligotrophication: however, recent investigations (Rühland et al., 2008; Winder & Hunter, 2008) in a wide number of temperate lakes in Europe and North America showed that increases in small-size *Cyclotella* species were a common occurrence, being the climatic warming over the last few decades a possible driver.

The possibility to investigate in detail the seasonal succession, such as in our case, would allow analysing the separate effects of climatic and trophic factors. The relationship with the wind vector in the CCA ordination indicates that high turbulence induced by a windy weather can be a crucial factor for sustaining the growth of *T. flocculosa*, *F. crotonensis* and *D. tenuis*. The influence of meteorological parameters on the dynamics of diatom assemblages has also been tracked by Kirilova et al. (2011), who analysed the seasonal varved sequences in the sediment of Sacrower See. Their analysis demonstrated the variable role played in different periods by climatic and trophic factors, pointing out that diatom assemblages can be even valuable indicators of climatic variability, when their seasonal succession is investigated in detail.

As stated by Anderson (2000), indirect meteorological effects on algal productivity (via control of nutrient inputs) are probably of greater importance than direct influence of temperature, usually taken as the most immediate proxy for climate changes. Other climatic-related factors, such as the amount and intensity of rainfall, affecting the basin runoff, can have major effects on the availability of nutrients for algae in lakes. Gibson (1981) demonstrated how the dry winters of 1975 in Northern Ireland resulted in lower than average inflows into Lough Neagh and caused a drop of the in-lake silica concentrations. As a result of these substantially lower than normal silica concentrations, the spring diatom crop failed in 1976. In the case of Lake Maggiore, the results of MRT analysis clearly point out the key role of silica in selecting different diatom assemblages: the timing of the anomalies in the silica concentration reveals that direct climatic factors (cold years) as well as climate driven events (deep mixing and floods) were involved in the increase of silica concentration. The runoff related to rainfall can be responsible for changing the nutrient ratio in lake water (Kilham et al., 1996) and, in particular, the increased availability of silica may favour a shift of the species composition towards high Si *taxa*. The hypothesis that diatoms are distributed along resource ratio gradients has been successfully tested in controlled experiments (Kilham et al., 1986), although often difficult to apply to field observations (Sommer, 1993). In our case, however, the change in the Si availability in Lake Maggiore seems to provide a reasonable explanation for the recent displacement of *Diatoma tenuis* by *Tabellaria flocculosa*. In Tilman et al. (1982), *D. tenuis* is reported as a good competitor for Si, but as a poor competitor for P, while the opposite is true for *T. flocculosa*. Since early 1990s, three of the major floods in the last two centuries took place in Lake Maggiore watershed, probably accelerating the decline of *Diatoma tenuis*, already limited by the decreased P availability, in favour of *T. flocculosa*, a better P competitor, taking an advantage from the increased Si supply, resulting from the higher runoff in the siliceous Lago Maggiore basin. The change in nutrient ratio could explain also the fluctuations of *A. formosa*, which have an optimal Si:P ratio similar to *Diatoma* (Makulla & Sommer, 1993): although its decline is less pronounced (Fig. 3), some low values of the LSP phase correspond to *T. flocculosa* peaks.

The succession shift towards the dominance of diatoms after an heavy rainstorm or a flood event has been documented in other lakes (Leitao et al., 2003; Anneville et al., 2004; Znachor et al., 2008), always resulting in the dominance of *Fragilaria crotonensis.* An increase of this species was also recorded in Lago Maggiore after 1997. We could hypothesise that, as both species are stimulated by an exceptional Si input, the heavy silicified *T. flocculosa* would outcompete *F. crotonensis* when the wind intensity is high enough to effectively contrast its sinking rate. The studies dealing with lakes' eutrophication attribute the appearance of these two diatoms to the worsening of the trophic status (see Wessels et al., 1999); however, their importance in Lago Maggiore is higher in the recent oligotrophic than in the past mesotrophic phase.

The resource ratio hypothesis indicates that both species can be limited by silica (although *Fragilaria* has a lower Si requirement than *Tabellaria*) rather than by phosphorus, so we can assume that their increase with increasing trophy is the result of a generally increased nutrient supply and is not necessarily driven by P increment. On the other side, the results presented in this article support the finding that climate variability and catchment hydrology affect the nutrient supply to the lake and alter seasonal nutrient ratios, which in turn structure the diatom phytoplankton (Kilham et al., 1996; Anderson, 2000), often favouring species usually regarded as meso- or eutrophic indicators. The changes in the diatom assemblage recorded in Lago Maggiore since the end of the 1990s seem, therefore, in agreement with the hypothesis that climate change should have effects on aquatic ecosystems comparable to those of eutrophication (Schindler, 2001). Among the consequences of climate warming, the frequency and severity of extreme events, such as heavy rainfalls, are expected to increase (Dokulil & Teubner, 2011), resulting in larger runoff, floods and increased erosion and wash out of nutrients. Increased erosion due to high winter runoff combined with higher water temperatures will, almost certainly, lead to widespread, climate-related eutrophication (Dokulil et al., 2009). Greater discharges are expected in winter and spring as more precipitation will come down as rain rather than snow (Dokulil & Teubner, 2011), so the effects could be particularly pronounced on the vernal growth phase of diatoms. Long-term lake monitoring in Sweden suggests that larger diatom crops are associated with increased nutrient flushing from the catchment (Anderson, 2000), similarly to what we recorded in Lago Maggiore. Although the global warming should determine an increase of water column stability in lakes (Nõges et al., 2009), making a deep mixing an exceptional events in deep and large lakes, on the other side, the nutrient supply from the catchment should increase, as a consequence of more frequent extreme meteorological events and higher runoff, giving diatoms an advantage, because of the mobilisation of silicates.

Diatom dynamics is strongly driven by abiotic variables that explained around 70% of the variance observed in the diatoms succession patterns. However, part of the unexplained variance could be attributed to biotic factors (competition, predation and parasitism): other papers investigated the changes of the food web structure in Lake Maggiore across its trophic evolution (Manca & Ruggiu, 1998; Manca et al., 2007), pointing out how modifications of the zooplankton assemblages, related to both climatic and trophic factors, could have affected the phytoplankton–zooplankton interactions through a decrease of the filter feeding cladocerans. Although large diatoms are less impacted than other phytoplankters by predation pressure from filter feeders (see Sommer, 1988, p. 249; Reynolds, 2006, p. 269), we cannot exclude that also biotic factors could have played a role in the dynamics previously described.

Conclusions

The aim of our study was to understand to what extent the long-term changes in the vernal diatom flora in Lake Maggiore were determined by trophic or by climatic constraints. The results of our analysis indicate:

1. The species fluctuations were probably mainly driven by trophic factors till the beginning of the 1990s, when the reduction of nutrient loads started affecting the in-lake concentrations. Under nutrient limitation, the supply was mainly regulated by autochthonous in-lake mechanisms, as testifies the role played by the interaction between mixing depth and phosphorus concentration, pointed out by the CCA.
2. In Lake Maggiore, the climatic variables started to play a significant role in controlling the spring phytoplankton growth when the nutrient limitation occurred: some deep mixing events were important in sustaining an abundant growth of diatoms, favoured by the turbulence of the water column as well as by the higher than usual silica supply.
3. The most recent evolution of the diatom assemblage, in particular as concerns the rise of *Tabellaria flocculosa*, was driven by an increase of silica concentration, as a consequence of a higher runoff and the mobilisation of silicates, in relation with some extreme meteorological events.
4. Diatoms can be useful indicators of climate change, when links between their physiological resource needs and environmental data coming

from robust limnological investigations can be established: the possibility to maintain long-term investigation programs could be crucial in this respect.

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PHYTOPLANKTON

Temperature modulated effects of nutrients on phytoplankton changes in a mountain lake

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Abstract Piburger See, a dimictic mountain lake in Austria, experienced moderate cultural eutrophication in the 1950s. Lake restoration led to a re-oligotrophication in the 1990s with a decrease in seasonal phytoplankton biovolume until the late 1990s, but a reversed trend from the early 2000s onwards. We hypothesize that recent changes in phytoplankton biomass and functional structure are triggered by changes in lake nitrogen and silica concentrations, and we expect climate-related factors to modulate the trophic status of Piburger See. Phytoplankton data were analyzed by non-metric multidimensional scaling (NMDS) applied on biovolume of morpho-functional groups, combined with correlation analyses of environmental variables. Since the 2000s, short-term changes in phytoplankton of Piburger See were explained by varying concentrations and ratios of nitrogen and silica, while the inter-annual variability in phytoplankton species composition was rather attributed to superimposed rising water temperature and lake thermal stability. Our results underline the co-dominant role of phosphorus and nitrogen as phytoplankton drivers in lakes that experience periods of nitrogen limitation. The combined impact of nutrients and climate on phytoplankton development can thus mimic short-term increases in the trophic level of less productive lakes.

Keywords Mountain lakes · Phytoplankton · Morpho-functional groups · Nutrients · Climate change · Multivariate analysis

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Introduction

The deterioration of the water quality in many temperate lakes in the mid twentieth century raised public concern about the consequences of cultural eutrophication (Dokulil & Teubner, 2011). Numerous fundamental studies and experiments (e.g. Vollenweider, 1968; Rohlich, 1969; Schindler, 1974) led to a

link between eutrophication effects and nutrient enrichment by identifying phosphorus as the key element in controlling freshwater eutrophication and as the primary target of restoration measures (OECD, 1982). The reduction of phosphorus load from point and diffuse sources successfully contributed to the reduction of primary production and to restoration of several lakes in industrialized countries (e.g. Sas, 1989), though cultural eutrophication still represents a major human impact on lakes at global level (Smol, 2008). Nevertheless, the recovery history of several temperate lakes contributed to the erosion of the phosphorus paradigm, as it became increasingly evident that lake recovery can be slowed down or even become counteracted by other factors, such as nitrogen load and climate change (Schindler, 2006; Blenckner et al., 2007; Lewis & Wurtsbaugh, 2008). Nitrogen often plays a co-dominant limiting role together with phosphorus (e.g. Scott & McCarthy, 2010), especially in oligotrophic mountain lakes (e.g. Dodds et al., 1989; Elser et al., 1990; Lanfrancois et al., 2003), while increasing atmospheric N deposition is often regarded as responsible for recent phytoplankton changes (Bergström & Jansson, 2006). Effects of climate change on lake ecological dynamics are multi-faceted (George, 2010). Several studies outlined a strong relation between climate change and phytoplankton abundance and species composition in boreal regions during the recent past (e.g. Huber et al., 2008; Rühland et al., 2008; Winder et al., 2009). Climate change can prevent recovering lakes to reach "good" status by shifting the ecological boundaries for "reference conditions" and thus requires a reconsideration of lake restoration targets (Bennion et al., 2011).

Piburger See has been oligo- to mesotrophic around the early twentieth century (Thies et al., 2011). The increase in recreational activities, tourism, and the application of fertilizers on nearby fields resulted in enhanced primary production and rising hypolimnetic oxygen depletion in the lake during the 1950s and 1960s (Pechlaner, 1968). Lake restoration started in 1970 by exporting anoxic and nutrient-rich hypolimnetic waters with an Olszewski tube (Pechlaner, 1979). External nutrient loading was reduced by altering fertilizer application and by diverting sewage from a public bath. Lake oxygenation rapidly improved after 1970 (Pechlaner, 1979), while the response of total phosphorus (TP) and phytoplankton biomass to lake restoration was delayed by two decades (Online Resources 1 and 2). Annual TP means and phytoplankton biomass increased up to maximum values in the early 1980s (Rott, 1983; Thies et al. 2011; Online Resources 1 and 2) and minimum values were reached in the late-1990 s (Pipp & Rott, 1995; Tolotti & Thies, 2002; Thies et al., loc. cit.). Since the early 2000s, phytoplankton biovolume has increased again and exceeded the highest values of the 1970s and 1980s, thus suggesting a reversing trend in lake trophic status (Thies et al., loc. cit.; Online resource 2). Simultaneously, only small changes in TP were recorded (Online Resource 1).

In this study, we investigate environmental and phytoplankton data of Piburger See for the period from 1998 to 2006 in order to disentangle the role of nutrients and climate as drivers for phytoplankton changes, and to discriminate climate-related factors in eutrophication processes of small mountain lakes.

Materials and methods

Study site

Piburger See is a soft-water mountain lake located in the Central Eastern Alps (47°11′42″N, 10°53′18″E, Tyrol, Austria). The catchment is mainly covered by coniferous forest and, apart from a single house, there are no settlements. Ice cover duration spans from December to April, and has recently shortened by about 2 weeks. The spring thermal overturn is often incomplete or very short and hypolimnetic anoxia develops during summer. Holomixis occurs in autumn, and can last for a few days only. Details on lake and catchment are given elsewhere (e.g. Pechlaner, 1979; Rott, 1986; Tolotti & Thies, 2002; Thies et al., 2011).

Sampling and analyses

During the study period (1998–2006), monthly water samples were collected with a Patalas-Schindler bottle (UWITEC, Mondsee, Austria) from the deepest point of Piburger See along a vertical profile at 3-m intervals from lake surface down to the bottom (i.e. 24 m). Water temperature was measured with a thermometer positioned inside the sampling device. Secchi transparency (Z_S) was measured during each sampling.

Concentrations of lake water nutrients were determined following standard methods (Tolotti & Thies, 2002). Chlorophyll *a* was determined spectrophotometrically according to Jeffrey & Humphrey (1975). Phytoplankton biovolume was determined for samples from 1998, 2002 (April–September), 2003 (April–December) and 2004–2006 by Utermöhl's method (1958), estimating cell volumes of the different taxa according to Rott (1981).

Data analysis

Air temperature and precipitation at Piburger See were derived from the gridded HISTALP data set (Auer et al., 2007). Data are based on monthly homogenized long-term series for the so-called "Greater Alpine Region" (4–19°E, 43–49°N, 0–3,500 m a.s.l.). Information on ice on and off were provided by local observations and by a web cam installed close to the lake since 2003. Monthly water temperature profiles were used to calculate the relative thermal resistance to mixing (RTR) based on water densities (Wetzel, 2001), the mixing depth (Z_{mix}), and the Schmidt stability of the water column (Idso, 1973). Z_{mix} was calculated from interpolated temperature profiles at an accuracy of ± 1.5 m. Monthly extension of the euphotic zone (Z_{eu}) was calculated from mean light attenuation coefficients (K_d), which were estimated from monthly Secchi transparency values (Z_S), according to the relation $K_d = 1.72\ Z_S^{-0.865}$ (Pipp & Rott, 1995). Secchi transparency was obtained from field measurements at Piburger See during the period 1973–1983 (Rott, 1986).

Analysis of environmental and phytoplankton data was based on volume weighted averages of the whole water column (0–24 m). In addition, volume weighted nutrient and RTR data of the mean lake epilimnion (0–6 m), metalimnion (9–12 m) and hypolimnion (15–24 m) were analyzed. Continuous temporal data series were smoothed using locally weighted polynomial regression (LOWESS), while time series showing gaps were smoothed by running averages ($N = 12$). The existence of significant monotonic trends was checked with the Mann–Kendall test (τ), while the slope of linear trends was estimated with the non-parametric Sen's method using MAKESENSE 1.0 (Salmi et al., 2002).

Phytoplankton data were analyzed at class and morpho-functional level, in order to reduce the variability of the data set and to focus on changes in phytoplankton functionality. Taxa biovolumes were allocated to different morpho-functional groups (MFGs) according to their morphological (i.e. size, shape, cell aggregation, presence of cell envelopes) and functional (i.e. motility, buoyancy, mixotrophic capability, specific nutrient requirements) traits (Salmaso & Padisák, 2007). In addition to the original definition of MFGs, a new group has been defined here (i.e. 6c-ColoPenn, including colony forming large pennate diatoms), considering that large pennates were represented almost exclusively by unicellular taxa till the late 1990s (Rott, 1983; Pipp & Rott, 1995; Tolotti & Thies, 2002) and by colony forming taxa during the last decade (see results).

Non-metric multidimensional scaling (NMDS, Kruskal & Wish, 1978) was applied to a Bray and Curtis' dissimilarity matrix on volume-weighted biovolume values of MFGs. NMDS was run by the SYSTAT 10.2 software package (Wilkinson, 1990) and consists in the simultaneous graphical visualization of Euclidean distances between the phytoplankton samples collected during the study period. MFG data were transformed by double square root to downweight the most abundant groups. MFGs found in <3 samples and with biovolume $<0.03\ mm^3\ l^{-1}$were excluded from the analysis. NMDS was started with several random configurations and the solution with the lowest 'stress' (Kruskal & Wish, 1978) was selected after 100 trials. This procedure avoids problems of local minima and allows to find repeatedly a consistent stable configuration (Legendre & Legendre, 1998).

The relation of MFGs with environmental variables was explored by correlation analysis of samples scores on the first two NMDS dimensions with air and water temperature, precipitation, lake nutrient concentrations and nutrient ratios. Chlorophyll *a* concentrations, total phytoplankton biovolume and biovolume of algal classes were also tested. The same approach was adopted to investigate the relation of MFGs biovolume with environmental variables. Phytoplankton and nutrient data were log transformed ($y = \log(x) + 1$) in order to stabilize the variance, and polynomially detrended nutrient data were also tested. A Kolmogorov–Smirnov distance test indicated Spearman's rank correlation as the most appropriate method for all data matrices.

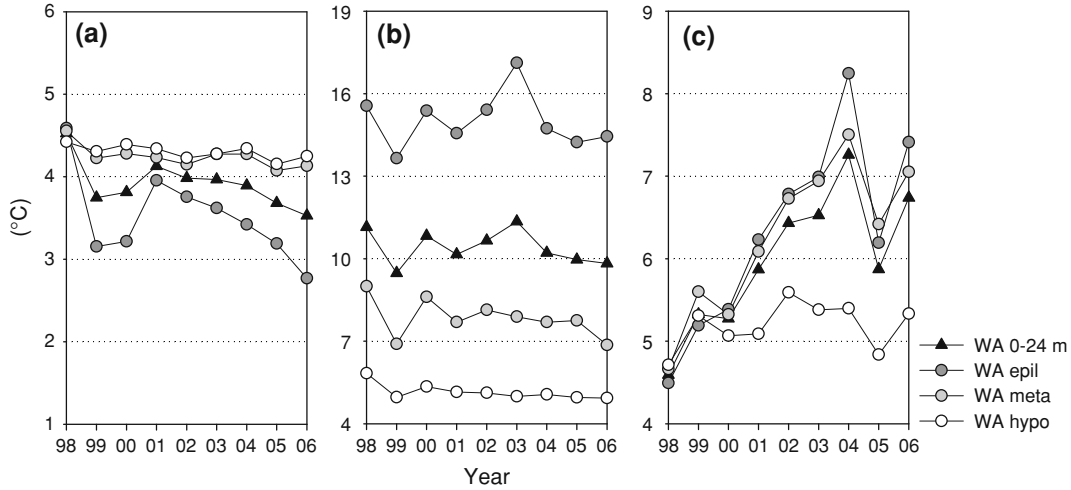

Fig. 1 Water temperature of Piburger See from 1998 to 2006 during ice cover from January to March (**a**), summer stratification from April to October (**b**) and fall circulation from November to December (**c**). WA 0–24 m = volume-weighted average water temperature, WA epil, WA meta and WA hypo = volume-weighted epi- (0–6 m), meta- (9–12 m) and hypolimnetic (15–24 m) temperature

Results

Water temperature and transparency

Average water temperature in winter (January–March) was determined by the timing of ice off (Fig. 1a). In particular, the high epilimnetic winter temperatures of 4.6 and 4.0 recorded in 1998 and 2001 relate to an exceptional early ice off at the beginning of March. Temperatures of the summer stratification (April–October) showed no major trend from 1998 to 2006 (Fig. 1b). The peak values of epilimnetic temperature in summer and fall 2003, reflect the high air temperatures recorded in Central Europe (Schär et al., 2004). Epi- and metalimnetic temperatures during fall circulation (November–December, Fig. 1c) increased from 1998 to 2004, and decreased in 2005 in relation to an earlier ice-on early in December. The increase in fall temperatures is as well reflected by the increase in November Schmidt stability from about zero in the late 1990 to values >50 J m^{-2} in the 2000s (Fig. 2). Annual stability remained almost unchanged.

Lake water transparency (Z_S) ranged from 4.5 m (e.g. April 2003 and autumn 2005) to 12 m (October 1999 and 2000), with average values of around 8 m (Fig. 3). In general, water transparency increased by the season, while a clear water phase was observed irregularly in spring (as in 2005 and 2006) or early summer (as in 1998 and 2003). Z_{eu} ranged from 8.7 to 18 m (average 13.4 m) and was $>Z_{mix}$ during the whole summer stratification each year.

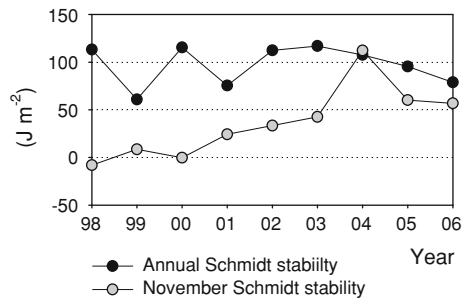

Fig. 2 Thermal Schmidt stability of Piburger See from 1998 to 2006

Nutrients

Annual average TP concentrations of Piburger See showed a significant and slight increasing trend (Mann–Kendall $\tau = 0.18$, $P < 0.05$) from 1998 to 2006, with values ranging from ca. 6 to 8 µg l^{-1} (Online Resource 1). Higher TP concentrations were recorded in the summer metalimnion, and in the hypolimnion in late summer to autumn (Fig. 4a), while dissolved inorganic nitrogen (DIN) showed a pronounced epi- and metalimnetic depletion during summer (Fig. 4b), with concentrations below the limiting values of 100 µg l^{-1} (Reynolds, 2006) in

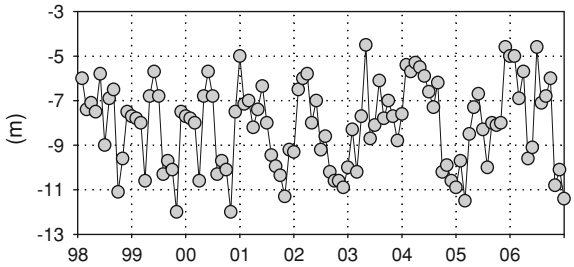

Fig. 3 Monthly water transparency (Secchi depth) of Piburger See from 1998 to 2006

1998 and from 2002 until 2006. Mean summer DIN increased in the lake hypolimnion till 2001. From autumn 1998 to spring 1999 DIN concentrations showed an increase of up to ca. 300 μg l^{-1}, but changes were less pronounced in the hypolimnion (Fig. 4b). Concentrations of dissolved reactive silica (DRSi) showed a gradual increase from winter 1998/1999 to early 2003 (up to ca. 2500 μg l^{-1}), followed by a rapid decrease down to values below those of 1998 (Fig. 4c). Epilimnetic DRSi depletion in summer was most pronounced in 1998 and from 2003 to 2006 (Fig. 4c), but concentrations were never limiting (i.e. <100 μg l^{-1}, Reynolds, 2006).

Phytoplankton

Mean chlorophyll *a* concentrations and phytoplankton biovolume in the growing season (Fig. 5) remained within the mesotrophic range (as defined in OECD (1982) and Rott (1984)) from 1998 to 2006, despite a pronounced inter-annual variability. Seasonal phytoplankton biovolume (showed in relation to seasonal

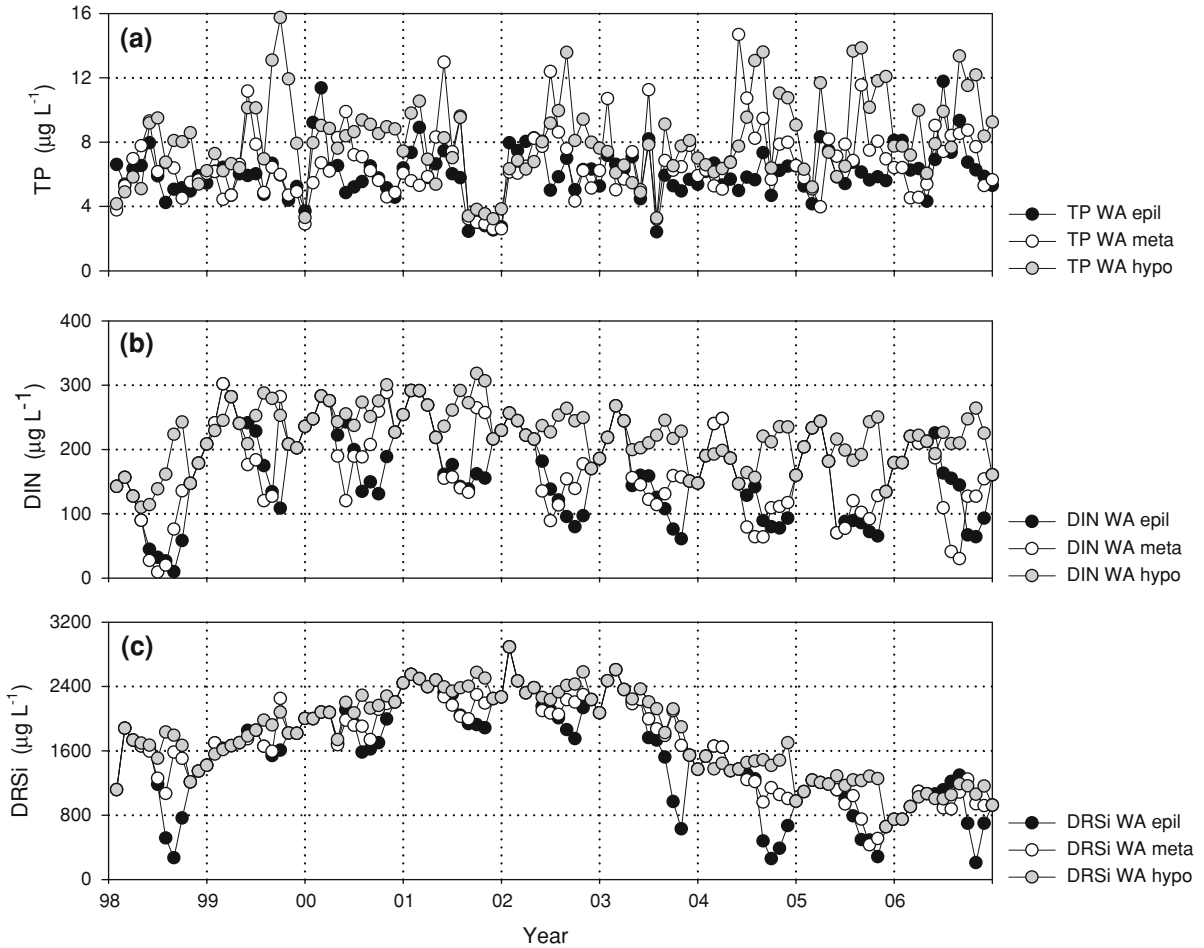

Fig. 4 Epi- (0–6 m), meta- (9–12 m) and hypolimnetic (18–24 m) volume-weighted average concentrations of TP (**a**), DIN (**b**) and DRSi (**c**) in Piburger See during the period 1998–2006

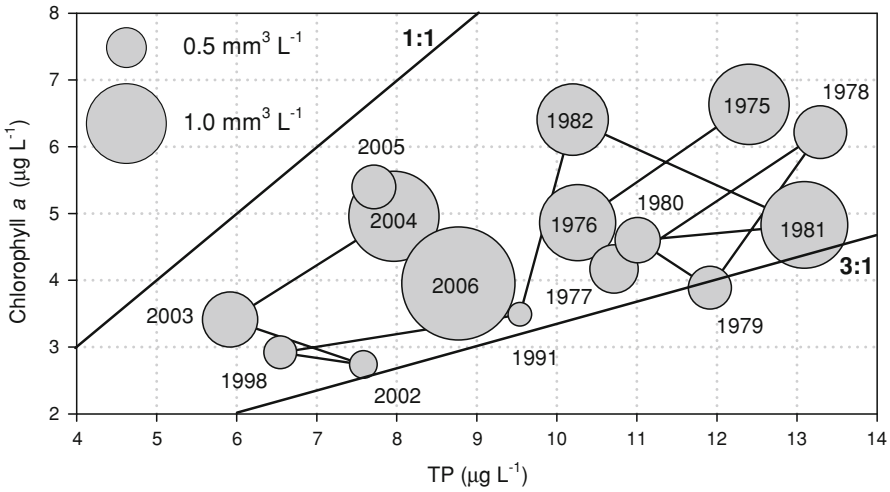

Fig. 5 Changes in total phytoplankton biovolume in relation to TP and chlorophyll *a* concentrations in Piburger See during the growing season (i.e. May–Oct, Apr–Sep for 2002) from 1975 to 2006. Area between the 1:1 and 3:1 ratios of TP to Chl *a* includes conditions where primary production of deep and/or oligotrophic lakes is dominated by phytoplankton (Dokulil & Teubner, 2003)

TP and chlorophyll concentrations in Fig. 5) decreased from the early 1980s to the late 1990s, but the trend was reversed from the early 2000s onwards, and the value of 2006 (1.3 mm^3 l^{-1}) was the highest ever recorded.

Monthly volume weighted biovolumes of the most variable MFGs (mean coefficient of variation from May to October, CV, ≥100%) are shown in Fig. 6, while representative taxa for each different MFG are reported in Online Resource 3. Unicellular and colonial flagellated groups (codes 1a, 2a, 2b; Fig. 6), which are among the most stable phytoplankton components of Piburger See (together with large dinoflagellates and cryptophytes, not shown), revealed a moderate seasonal variability (mean seasonal CV from 130% to 144%). Small dinoflagellates (code 2b, Fig. 6) became more abundant since the early 2000s. The occurrence of cyanobacteria has become increasingly irregular and short-lived since summer 2002. Vacuolated and non-vacuolated large colony forming groups (codes 5b and 5c, respectively, Fig. 6) and small non-vacuolated Chroococcales (code 5d, Fig. 6) were the most abundant and variable (seasonal CV up to 350%) cyanobacteria groups and reached a biovolume of up to 0.3 mm^3 l^{-1}.

Unicellular large pennates (code 6b, Fig. 6) represented the less abundant diatom MFG and their occurrence was mainly restricted to the first half of each year (Fig. 6). Large colony forming pennate (code 6c) and small centric taxa (code 7a, Fig. 6) showed the highest biomass among all phytoplankton MFGs and a pronounced variability in their occurrence (seasonal CV up to ca. 250%). Centric diatoms reached maximum biovolume in summer 1998, 2004 and 2006 (Fig. 6), while large colonial pennates were sporadic in 1998, but their biovolume markedly increased from 2002 onwards with peak values in 2003 and 2005.

Green algae sensu lato, like the unicellular taxa of MGF 9b and naked and gelatinous colonial taxa of MFGs 11a and 11b, showed an irregular seasonal variability and an enhanced occurrence in 2005 and 2006 (Fig. 6). Group 11b was the most frequent green algal group (biovolume <0.1 mm^3 l^{-1}, mean seasonal CV = 114%) and the phytoplankton group with the highest species richness.

The seasonal and vertical distribution of small centric and large colony forming diatoms (MFGs 6c and 7a) is showed in greater detail in Fig. 7, in relation to the high abundance and variability of these MFGs in Piburger See since the early 2000s. Small centric diatoms (mainly *Cyclotella comensis* Grunow and *C. radiosa* (Grunow) Lemmermann) were most abundant in the lake epilimnion (Fig. 7a) and reached a peak biovolume of 7 mm^3 l^{-1} and a relative abundance of 84% in September 2006 at the lake surface. In the metalimion of Piburger See, *Fragilaria crotonensis* Kitton (Fig. 7b) and *Asterionella formosa* Hass (Fig. 7c) were the two dominant taxa of MFG 6c, but their occurrence was characterized by opposing timing. *F. crotonensis* produced a metalimnetic bloom of about 5 mm^3 l^{-1} and a relative abundance of 83% in autumn 2003, and disappeared almost completely after summer 2004 (Fig. 7b), while *A. formosa* was

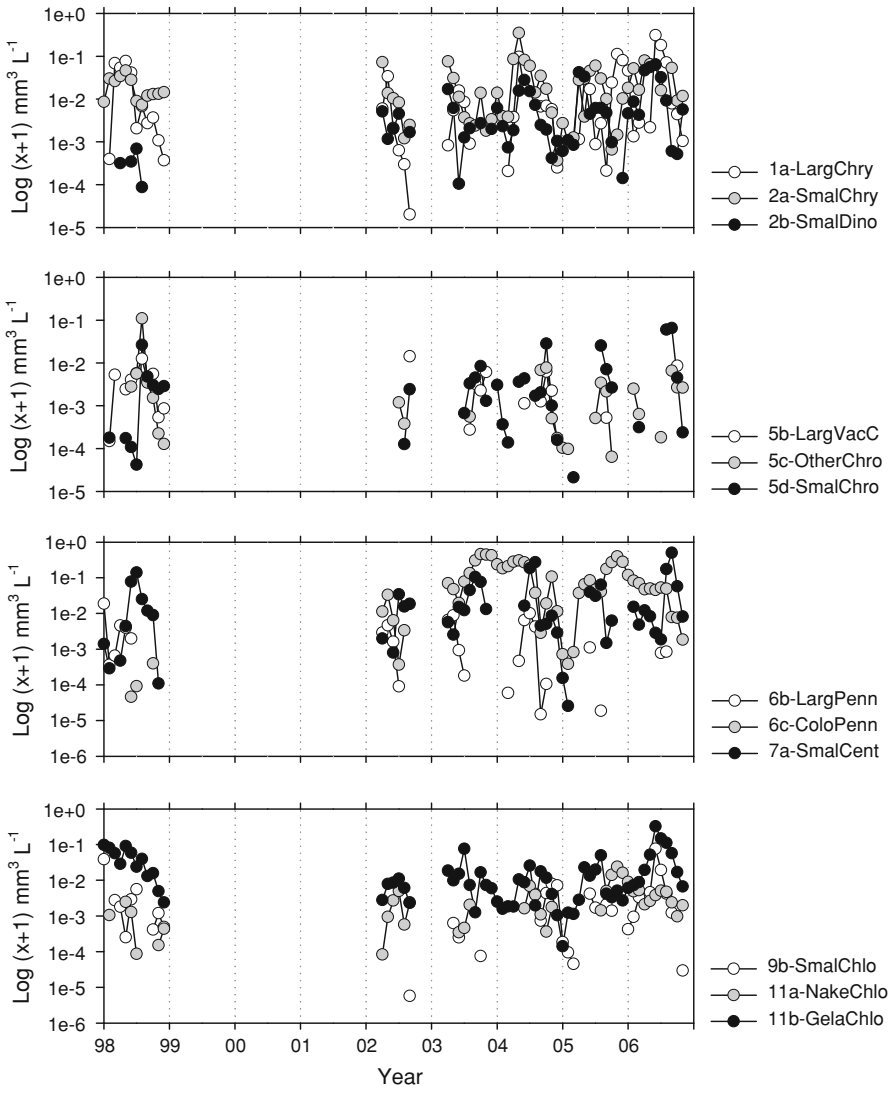

Fig. 6 Log-scaled biomass changes of the most important phytoplankton MFGs in Piburger See from 1998 to 2006. *1a-LargChry* large colony forming chrysophytes, *2a-SmalChry* small unicellular chrysophytes, *2b-SmalDino* small dinoflagellates (length ≤30 µm), *5b-LargVacC* large colony, gas vesicle forming cyanobacteria, *5c-OtheChro* non-vacuolated large colony forming coccal cyanobacteria, *5d-SmalChro* small colony forming coccal cyanobacteria, *6b-LargPenn* large unicellular pennate diatoms, *6c-ColoPenn* colony forming large pennate diatoms, *7a-SmalCent* small unicellular centric diatoms, *9b-SmalChlo* small unicellular chlorophytes, *11a-NakeChlo* cenobium forming naked Chlorococcales, *11b-GelaChlo* cenobium forming gelatinous Chlorococcales

completely absent before 2003 (i.e. in 1998 and 2002, Fig. 7c). Maximum biovolumes for *A. formosa* were fund in the summer metalimnion and were particularly high in July 2004 (i.e. 4 $mm^3\ l^{-1}$, corresponding to a relative abundance of 87%). In 2005, peak abundance of *Asterionella* only occurred in November (3 $mm^3\ l^{-1}$, corresponding to a relative abundance of 97%).

NMDS and correlation analysis

NMDS analyses indicated two dimensions as sufficiently consistent to explain the variability of phytoplankton MFGs. Adding further dimensions hardly improved stress values and variance explained, which was confirmed by the correlation of sample scores on the first two dimensions of the two- and three-dimensional NMDS analyses ($r \geq 0.97$ for both dimensions). The ordination of phytoplankton community based on monthly MFG biovolumes, as obtained by a unique NMDS analysis of the whole sample set ($n = 63$), explained 80% of the variance in phytoplankton MFG composition (Fig. 8). The patterns of the different years outline the high seasonal and inter-annual variability of phytoplankton in Piburger See. Samples of 1998 are concentrated in the left NMDS quadrants, with winter and autumn samples far from each other. From 2002 onwards, spring samples shifted toward the right quadrants, while summer and autumn

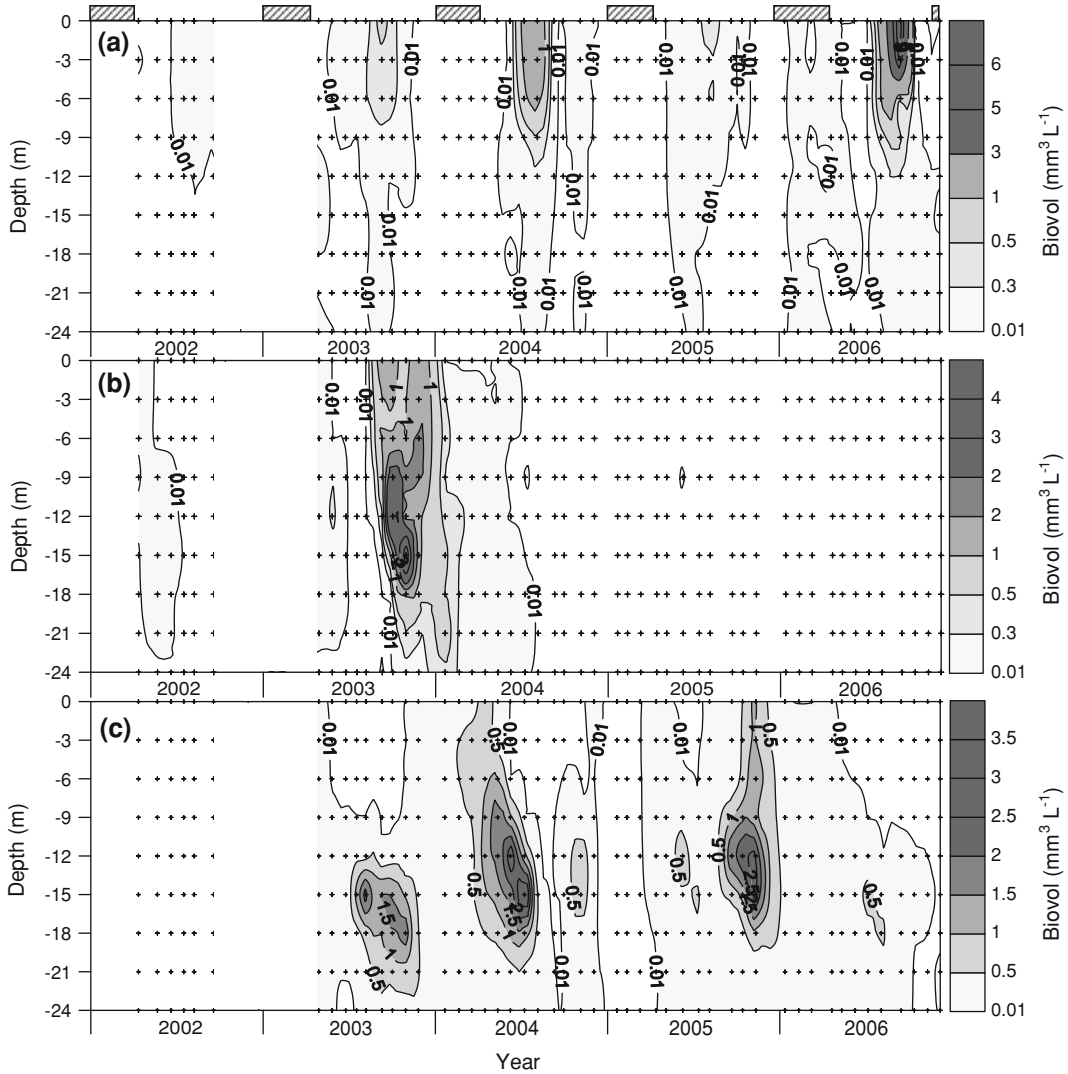

Fig. 7 Seasonal and vertical distribution of *Cyclotella* spp. (**a**), *Fragilaria crotonensis* (**b**) and *Asterionella formosa* (**c**) in Piburger See during the period 2002–2006. *Bars on top* = ice cover

samples shifted from the lower left toward the lower right quadrant in 2003. From 2003 onwards, summer and winter samples showed the highest inter-annual variability (Fig. 8). The NMDS configuration for the whole sample set (Fig. 9) reports the annual centroids (i.e. arithmetic mean of sample scores for each year), which underline the shift from 1998 to 2003 along the first NMDS dimension (D-1) and smaller changes along the second NMDS dimension (D-2) from 2003 to 2006. Correlation coefficients between samples scores on NMDS D-1 and D-2, and phytoplankton variables (Fig. 9) indicate a stronger increase in total phytoplankton biovolume and chlorophyll concentrations along D-2. Changes along D-1 were exclusively due to non-motile algal classes, i.e. to diatoms, cyanobacteria and coccal green algae. Flagellated algae were mainly responsible of the changes along NMDS D-2. According to these relationships, the 1998 centroid appears to be located in a NMDS region characterized by the highest cyanobacteria biovolume and by small contribution of flagellates and diatoms (Fig. 9). On the contrary, the year 2006 is characterized by a dominance of flagellates and diatoms. Spring and

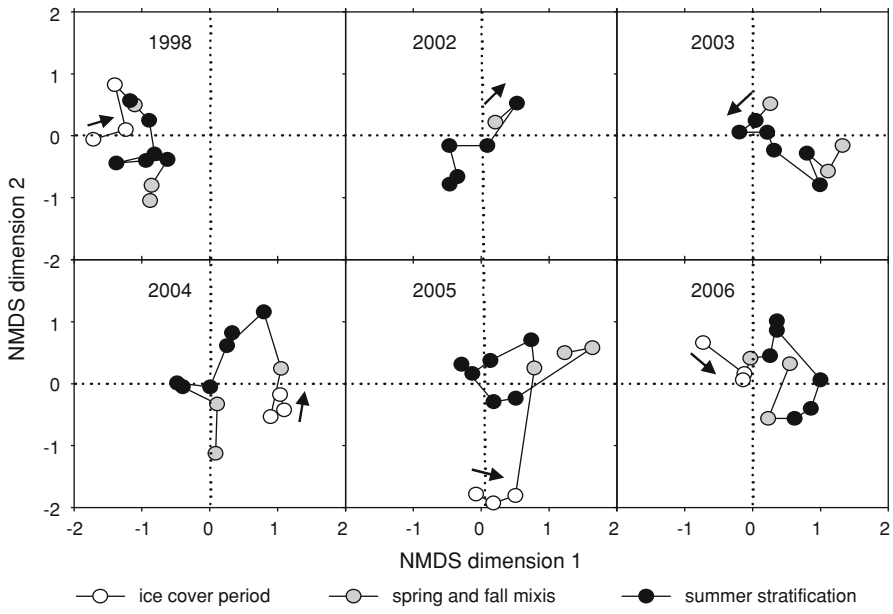

Fig. 8 NMDS ordinations of monthly phytoplankton samples. 1998 (Jan–Dec), 2002 (Apr–Sep), 2003 (Apr–Dec), 2004–2006 (Jan–Dec). *Arrows* indicate the beginning of the annual cycle

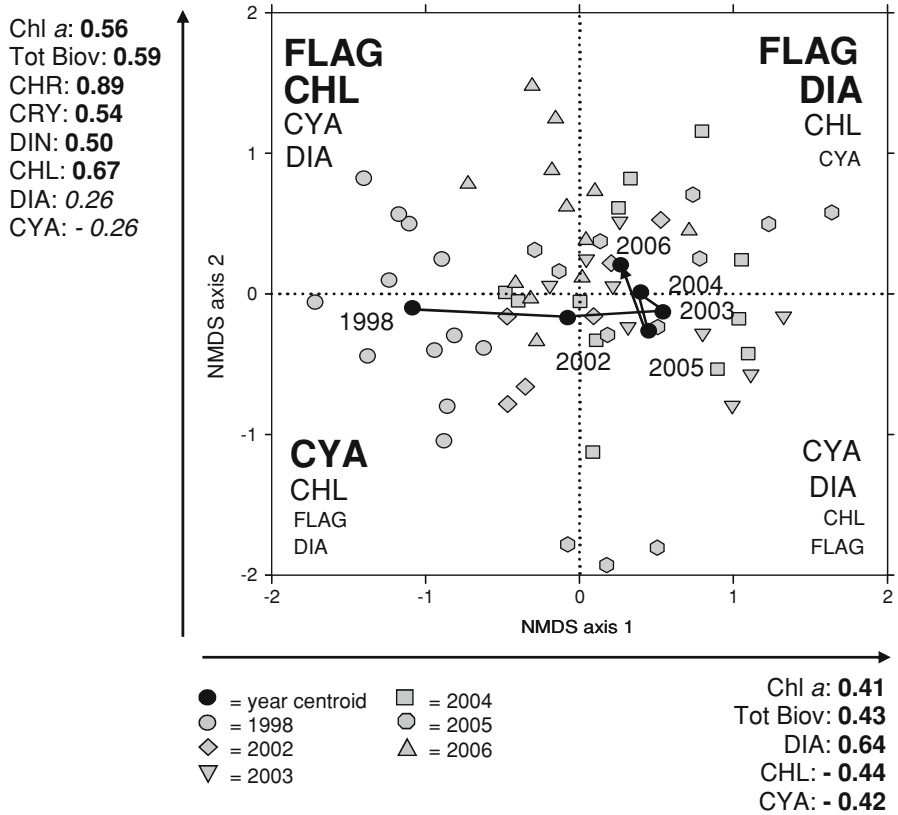

Fig. 9 NMDS ordinations of monthly phytoplankton samples in 1998, and 2002–2006. Correlation coefficients between sample scores on the first and second NMDS dimension, respectively, and chlorophyll (Chl *a*), total phytoplankton biovolume (Tot Bio), and the biovolume of the major algal classes are indicated. Significance levels as in Table 1. *CHR* Chrysophyceae, *DIN* Dinophyceae, *CRY* Cryptophyceae, *DIA* diatoms, *CYA* cyanobacteria, *CHL* non-motile chlorophytes. *Large*, *medium* and *small font* are used in the plot quadrants for dominant, frequent and less abundant algal groups, respectively

early summer samples are concentrated in the upper left NMDS quadrant characterized by high biovolume of flagellated and green algae (Fig. 9).

Correlation coefficients between sample scores on D-1 and environmental variables (Online Resource 4) show a significant positive relationship with DIN

Table 1 Correlation coefficients of MFGs with biomass ≥0.03 mg L^{-1} (used for NMDS analysis) and of the two key diatom taxa *Asterionella formosa* (*A. form*) and *Fragilaria crotonensis* (*F. crot*) and environmental variables

MFG	TP	DIN	DRSi	DIN:TP	DRSi:DIN	Z_{eu}	Air T-1	WT-1	Z_{mix}	Stab-1	RTR 06
1a-LargChry	0.36			**−0.42**		*0.26*					*0.27*
2a-SmalChry								−0.33		−0.33	
1b-LargDino			0.33		0.36		*0.26*				*0.31*
2b-SmalDino		*0.25*			*−0.25*			*−0.25*	*−0.25*		0.33
3a-UnicPhyt		−0.33			−0.37						
2d-Crypto								*−0.34*		−0.35	
5a-FilaCyan			*0.39*		**0.51**						
5b-LargVacC		**−0.47**			*0.30*	*−0.26*	*0.30*	**0.50**		**0.52**	−0.40
5c-OtheChro		*−0.27*	**−0.44**			**−0.44**	**0.42**	**0.50**		**0.58**	
5d-SmalChro		−0.34		*−0.27*			0.34	**0.60**		**0.48**	
6b-LargPenn					*−0.26*	−0.38			**−0.45**		
6c-ColoPenn	*0.27* d		**0.44**		**0.52**						**0.54**
A. form			*−0.30*		**−0.41**	*0.40*					
F. crot			**0.51**		0.35						
7a-SmalCent		**−0.42**		**−0.46**		−0.27	**0.70**	**0.65**	**−0.49**	**0.59**	*0.28*
9b-SmalChlo		−0.38									
11a-NakeChlo	**0.42**		−0.35	*−0.29*	−0.30		0.37		**−0.43**		*0.36*
11b-GelaChlo		**-0.42**		−0.39	*0.28*						

MFG abbreviations as in Fig. 6 and Online Resource 3. Nutrient concentrations and water temperature as volume weighted averages. *WT-1* water temperature of the previous month, *d* detrended data. Other variable abbreviation as in Online Resource 4. Bold, normal and italic figures indicate $P < 0.001$, $P < 0.01$ and $P < 0.05$, respectively

concentrations, DRSi:DIN ratios, Z_{eu} and the volume weighted average water temperature of the month previous to each sampling (WT-1). Scores on D-2 are correlated with TP concentration, DIN:TP and DRSi:TP ratios, Z_{eu}, and climate-related variables (Online Resource 4). The synoptic correlation matrix between MFGs biovolume and environmental variables is reported in Table 1. MFGs including flagellated taxa (i.e. groups 1a–3d) show some relation with nutrient concentrations and with nutrient ratios. However, only group 1a shows a significant correlation with TP concentrations and DIN:TP ratios. Groups 2a, 2b and 3d were negatively correlated with water temperature of the previous month. Groups 1a, 1b and 2b showed a positive relationship with RTR between 0 and 6 m water depth. Cyanobacteria groups (i.e. 5b–5d) showed only some negative correlation with nutrients, especially DIN, but strong positive correlation with water temperature and Schmidt stability. Unicellular pennate diatoms (group 6b) showed almost no significant correlation with nutrients, while large colonial taxa (group 6c) showed a positive relation with detrended TP and DRSi concentrations. The two dominant species of this group (i.e. *A. formosa* and *F. crotonensis*) showed opposite correlation with DRSi and DRSi:DIN. Small centric diatoms (group 7a) were negatively correlated with DIN and DIN:TP (Table 1). Z_{eu} was negatively correlated with unicellular pennate and small centric diatoms, but positively with *A. formosa*. Group 6c and 7a showed a positive relation with RTR between 0 and 6 m, and group 7a was the MFG with the strongest response to water temperature and stability. Among the green algae MFGs (group 9b–11b in Table 1), only naked taxa (11a) were correlated with TP concentration, air temperature and RTR 06.

Discussion

Major environmental changes at Piburger See in the period 1998–2006 comprise rising late summer and autumn lake water temperatures, and a general increase in lake nutrient concentrations since 1999.

Lake warming reflects the long term increase in air temperature recorded in the region (cf. Thies et al., 2011), which became particularly pronounced since the 1980s (Auer et al., 2007). As a major consequence, summer lake thermal stratification has intensified since the early 2000s. Increasing TP concentrations since the late 1990s suggest an ongoing, though slow, enrichment of this key nutrient, while nitrogen and silica concentrations have increased since the late 1990s with the highest silica concentrations occurring 3 years after DIN reached the maximum level.

Phytoplankton showed a strong response to environmental changes of the early 2000s. Total biovolume increased to peak levels of the 1980s (Rott, 1983), and a shift from stable species assemblages characterizing the 1990s (Pipp & Rott, 1995) towards a new assemblage for Piburger See occurred at phytoplankton functional level (Tolotti et al., 2005). The shift was due to non-motile algal groups (diatoms, cyanobacteria and coccal green algae), which first responded to the increase in lake DIN concentration, and then to rising DRSi:DIN ratios, water temperature and thermal stability. The increase in lake nitrogen concentrations during the late 1990s is regarded as a major trigger of recent phytoplankton changes in Piburger See, which persisted for about 4 years and were accentuated by an increase in silica and in thermal stability of the water column (cf. Thies et al., 2011). These latter variables played an important role in determining the inter-annual variability of the phytoplankton functional assemblage established in the early 2000s.

Multivariate analysis (NMDS) combined with correlation analysis showed that phytoplankton taxa categorized by their similar morphology and nutrient-light demand can be allocated to morpho-functional groups, which each respond differently to combined nutrient and climate changes that have recently occurred in Piburger See. Our results confirm findings of previous studies which showed algal groups to be described as multi-taxon functional groups responding in a coherent way to environmental drivers (e.g. Reynolds, 2006; Salmaso & Padisák, 2007; Padisák et al., 2009).

The capability of flagellates to cope with unfavourable light conditions and strongly segregated nutrients in thermally stable environments by mixotrophic nutrition and regulation of their position in the water column (e.g. Teubner et al., 2003; Reynolds, 2006), led to a moderate variability in both biomass and seasonal distribution of flagellates MFGs. Small chrysophytes and cryptophytes, which are among the most tolerant and opportunistic phytoplankton groups (Reynolds, 2006), hardly responded to nutrient changes in Piburger See.

Phenology of non-motile taxa (cyanobacteria, diatoms and coccal green algae) resulted to be much more susceptible to environmental changes, as these groups rely on water movements to remain within the euphotic zone and to access the optimum nutrient level (e.g. Reynolds, 2006; Tolotti et al., 2007; Winder et al., 2009; Wagner & Adrian, 2009). The lower abundance and the scattered distribution of large colony forming cyanobacteria and large unicellular pennate diatoms in comparison to the 1980s and 1990s (Rott, 1983; Pipp & Rott, 1995), agree with the nutrient depleted epilimnion at increased thermal stability (cf. Thies et al., 2011). The increase in gelatinous taxa, which include also large colony-forming taxa in Piburger See, agrees with their ability to overcome sinking losses by producing gelatinous mucilages (Reynolds, 2006). The increase in small coccal cyanobacteria, green algae and small centric diatoms, on the contrary, was related with increasing lake temperature and thermal stability. Generally, small phytoplankton forms are expected to be favoured in warm and thermally stable lakes, under pronounced nutrient segregation due to: (a) their higher cell surface to volume ratio and thus higher nutrient affinity (Litchman et al., 2006; Falkowski & Oliver, 2007); (b) their lower sinking velocity, which enables small phytoplankton forms to longer remain within the euphotic zone (Padisák et al., 2003; Huisman et al., 2004).

The biomass increase of small centric diatoms in the summer epilimnion of Piburger See, particularly since the enhanced lake thermal stability (2004–2006), agrees with observations from temperate lakes with rising water temperatures (e.g. Lotter & Bigler, 2000; Tolotti et al., 2007; Rühland et al., 2008; Winder et al., 2009). Small centrics are also highly competitive at low Si:P and N:P ratios, which are limiting for large pennate diatoms, as occurred in Piburger See in 2006, when *A. formosa* strongly declined and *Cyclotella* spp. bloomed in the summer epilimnion.

The rapid increase in the large colony forming pennate diatoms *A. formosa* and *F. crotonensis* was the major phytoplankton change during the study

period (1998–2006). Our results indicate nitrogen as trigger for the recent increase in large pennate diatoms in Piburger See. Both species are very strong competitors for P but have moderate to high N and Si requirements (e.g. Interlandi et al., 1999; Michel et al., 2006). Therefore, they can reach higher abundances under high N:P and Si:P ratios, as occurred in Piburger See from 1999 to 2005. Field studies and laboratory experiments indicate that *F. crotonensis* has higher N requirements than *A. formosa*, with its lower half saturation constant value for nitrogen (Rhee & Gotham, 1980; Interlandi, 2002). The different N requirements explain the timing of the two key species in Piburger See, with *F. crotonensis* developing in the period of highest DIN:TP ratios (i.e. 2002 and 2003) and *A. formosa* prevailing since 2004, under decreasing DIN:TP ratios. *A. formosa* appears to be also a better competitor for silica (e.g. Kilham et al., 1996), as it reached its highest biomass during the stage of decreasing DRSi concentrations and DRSi:TP ratios, when *F. crotonensis* was already outcompeted.

Large colony-forming diatoms are expected to decrease in warm and thermally stable water columns due to their high sinking rate from the euphotic zone (e.g. Winder et al., 2009). However, the increase of *F. crotonensis* and *A. formosa* in Piburger See since 2002 was sustained by the combination of favourable nutrient conditions with $Z_{eu} > Z_{mix}$. The development of meta- and hypolimnetic biomass of *A. formosa* lasting till late autumn is supported by its adaptation to low light intensity (Teubner et al., 2003; Reynolds, 2006).

Our study highlights the key role of nitrogen and silica as drivers of phytoplankton dynamics of Piburger See. Nevertheless, the causes of the increase in these two nutrients since the late 1990s still remain uncertain. The increase in lake N:P ratios could not be attributed to a decrease in TP concentrations, as commonly observed in recovering lakes (Jeppesen et al., 2005), and atmospheric nitrogen deposition in Tyrol is very low compared to other regions in the Alps (Rogora et al., 2006). Our data suggest that the abrupt increase in lake DIN between autumn 1998 and spring 1999 may be due to a reduced nitrogen uptake by phytoplankton, as indicated by the low biomass in the same period (Online Resource 2). Concomitantly, the increasing lake water temperature may have also promoted a greater nutrient release from the lake sediments (Jeppesen et al., 2010) in association with enhanced hypolimnetic oxygen depletion (data not shown here).

Lake DRSi data are only available since 1998. As DRSi concentrations in the lake inlet do not show significant changes, the observed DRSi increase in Piburger See between 1998 and 2002 is attributed to a reduced uptake by diatoms. From 2003 onwards, DRSi concentrations declined due to the first strong diatom bloom ever recorded in Piburger See, which underlines the key role of diatoms as factor controlling silica levels in temperate lakes (e.g. Reynolds, 2006).

Conclusions

Recent palaeolimnological studies on sediments of Piburger See outline that long-term phytoplankton changes are mainly attributed to increasing lake temperature, while nutrients are supposed to act as modulating factor. Short-term phytoplankton changes, however, seem to be mainly driven by changing nutrient (i.e. nitrogen and silica) concentrations and ratios, while rising water temperature and enhanced thermal stability rather regulate the inter-annual variability of Piburger See. Such a time-scale dependent role of nutrients and climate change as phytoplankton drivers is rather common in populated regions of the world where most lakes are suffering or recovering from the consequences of human activities, and where identifying the unique influence of climate change is more problematic.

Our results underline that phytoplankton development cannot be explained only by phosphorus dynamics because of a co-dominant role of nitrogen. This applies in particular to less productive, small mountain lakes and to restored lakes at a reduced phosphorus load, which can experience periods of nitrogen limitation. The combination of nutrient and climate drivers can sustain short term phytoplankton pulses, which may occur even in less productive lakes like Piburger See and may thus mimic increased trophic conditions.

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PHYTOPLANKTON

Coupling high-resolution measurements to a three-dimensional lake model to assess the spatial and temporal dynamics of the cyanobacterium *Planktothrix rubescens* in a medium-sized lake

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Abstract In a medium-sized pre-alpine lake (North Italy) the cyanobacterium *Planktothrix rubescens* has strongly dominated the phytoplankton assemblage since 2000, similar to many pre-alpine lakes, despite improvements in water quality. The objective of this study was to determine the factors governing the spatial distribution of *P. rubescens*, including the major hydrodynamic processes and the influence of long-term reduction in nutrient concentrations during a period of climate warming. We used an intensive field campaign conducted from February 2010 to January 2011, to evaluate distributions of phytoplankton phyla, as well as *P. rubescens*, using spectrally resolved fluorescence measurements. These data provided highly spatially and temporally resolved phytoplankton population data suitable to calibrate and validate a coupled three-dimensional hydrodynamic (ELCOM) and ecological model (CAEDYM) of the lake ecosystem. The simulations revealed the fundamental role of physiological features of *P. rubescens* that led to observed vertical patterns of distribution, notably a deep chlorophyll maximum, and a strong influence of lake hydrodynamic processes, particularly during high-discharge inflows in summer stratification. The simulations are used to examine growth-limiting factors that help to explain the increased prevalence of *P. rubescens* during re-oligotrophication.

Keywords Metalimnion · Hydrodynamics · Deep chlorophyll maximum · Phytoplankton · ELCOM–CAEDYM

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Introduction

Global changes in recent decades, including alterations in catchment nutrient yields and climate, as well as increasing translocation of species, appear to have induced changes in phytoplankton assemblages leading to increased rates of establishment of invasive or harmful species, such as cyanobacteria (Paerl & Huisman, 2008; Salmaso, 2010; Zhang et al., 2011). Many planktonic cyanobacteria can aggregate at the water surface and form dense blooms and scums, with

potential for production of potent toxins, including hepatotoxic microcystins (Carmichael, 2001). Gas-filled vesicles provide buoyancy and variable levels of photosynthetic carbohydrate reserves; this property can assist in buoyancy regulation in thermally stratified environments, to optimize nutrient and light acquisition and create greater propensity for bloom formation (Walsby et al., 1997). In thermally stratified lakes blooms may persist through the stratified period until the surface mixed layer begins to deepen preceding winter turnover (Huisman et al., 2005). Such blooms are certainly not new: from being well dispersed through the water column, they are 'telescoped' to the surface when the wind drops, concentrating along lee shores and giving a greatly exaggerated impression of abundance (Reynolds, 1971). The increasing incidence of blooms that impact negatively on water quality correlates well with increasingly enriched conditions, suggesting a powerful causal link between increased abundance of bloom with increased phosphorus availability (Gorham et al., 1974). Indeed reductions in nutrient concentrations before the growth season are still considered to be an important strategy to decrease the risk of cyanobacterial blooms, even under a scenario of warming temperatures (Brookes & Carey, 2011).

Some species of cyanobacteria can also form dense metalimnetic populations in oligotrophic lakes as other species compete less effectively at reduced irradiance levels and they are strongly nutrient-limited in the surface mixed layer (Dokulil & Teubner, 2000). Metalimnetic populations are also known to form dense surface blooms and scums when they become 'overbuoyant' at critical stages in the seasonal stratification cycle (Walsby & Booker, 1980).

Blooms of the cyanobacterium *Planktothrix rubescens* have been increasingly observed in lakes that have undergone recent re-oligotrophication, especially in lakes in pre-alpine European regions (Ernst et al., 2009). In many Swiss and Austrian lakes it has emerged as a 'keystone species' (often contributing >50% of total phytoplankton biomass) and forms a deep chlorophyll maximum (DCM). Luxury storage of internal phosphorus may increase its competitiveness during re-oligotrophication when phosphate can be strongly depleted from the water column (Feuillade et al., 1990). In general, the ecological success of *Planktothrix* species depends largely on gas vesicles, which provide cells with sufficient buoyancy to enable them to optimise resource capture at selected depths in thermally stratified lakes (Bürgi & Stadelmann, 2002; Walsby & Schanz, 2002; Walsby et al., 2006). *P. rubescens* can survive during complete water column mixing as it has a high light-capture efficiency (Reynolds, 2006). Warm winters may also lead to larger inocula of this species for the subsequent summer growth cycle. Hamilton et al. (2010) provide evidence that oligotrophic lakes are more likely to support DCMs as a result of a euphotic depth that extends at least as far as the depth range of the metalimnion but *Planktothrix* spp. are also known to alter gas vesicle strength as a response to lake morphometry, depth, hydrology and circulation patterns (D'Alelio et al., 2011). Plunging river inflows that insert into the metalimnion can additionally provide nutrients necessary to sustain or stimulate DCMs (Wurtsbaugh et al., 2001) whilst recurrent internal waves have been demonstrated to enhance metalimnetic cyanobacterial populations by vertical excursions of the metalimnion and exposure to strong gradients of light and nutrients (Pannard et al., 2011). A model was developed by Mellard et al. (2011) to explore how the phytoplankton forming DCMs respond through growth and movement, to opposing resource gradients (i.e. of light and nutrients) and different mixing conditions. They found how externally imposed heterogeneity in the form of resource gradients and mixing interact with internally generated heterogeneity in the form of competition and movement to determine variations in population density in the vertical dimension.

The use of complex deterministic models to reproduce aspects of the dynamics of aquatic systems may assist with capturing the spatial and/or heterogeneity within systems. Most such models have extensive data input requirements and the calibration and interpretation processes are made difficult by the requirement of intense spatial data, with often extended computer run times compared its simpler models (Grayson et al., 2002; Robson et al., 2008; Mooij et al., 2010). Mieleitner & Reichert (2008) tried to reduce this complexity by combining empirical observations of phytoplankton temporal variations with biological knowledge that enabled definitions of functional groups for particular lakes. Several complex models purport to simulate cyanobacteria species or populations with some accuracy (e.g. Serra et al.,

2007; Gal et al., 2009; Elliot, 2010) but most efforts to include spatial distributions and dynamics of different species have been focused on surface bloom-forming populations (Ibelings et al., 2003; Missaghi & Hondzo, 2010; Vilhena et al., 2010). Cuypers et al. (2011) used a simple physical model of cell transport to examine horizontal and vertical distributions of metalimnetic populations of *P. rubescens*, focusing on the role of seiches due to wind forcing during summer stratification. They found, however, that there were mismatches between simulated and observed data and attributed these to nonlinear effects on the biomass due to governing physical processes.

The objective of this study was to use a three-dimensional hydrodynamic-ecological model to reproduce discrete measurements of hydro-chemical variables, including inferred behaviour of metalimnetic populations of *P. rubescens*, in a pre-alpine lake. For the first time, we were able to obtain high-resolution spatial distributions of this keystone species to make direct comparisons with the corresponding state variable output from the model, using spectrally specific fluorometric responses and validation with traditional cell enumeration microscopy. We compared measured lake profiles to the high temporal resolution model output and investigated phytoplankton vertical distribution as a function of light, nutrient limitation, competition and governing hydrodynamics. We finally focused on the mechanism determining the metalimnetic growth and subsequent proliferation of *P. rubescens*.

Study site

Lake Pusiano (45°48′20 N, 9°16′33′E) is a medium-sized lake (surface area 5 km^2) located in North of Italy. It is a typical warm monomictic lake with maximum depth 25 m and residence time ca. 1 year, overturning between January and February at which time temperature is ca. 5°C. The watershed area is about 95 km^2 (mean slope = 39%, maximum altitude = 1,453 m a.s.l, median altitude = 683 m a.s.l.) which produces a rapid hydrological response (typically ca. 4 h) in the Lambro River (mean discharge is 1.4 $m^3\ s^{-1}$). Two other minor rivers, Gallarana and Emissario del Segrino, enter the lake on the west side (Fig. 1). The total mean inflow into the lake, calculated for the last 10 years (including rainfall) is 2.6 $m^3\ s^{-1}$. The outflow is a complex system of two channels,

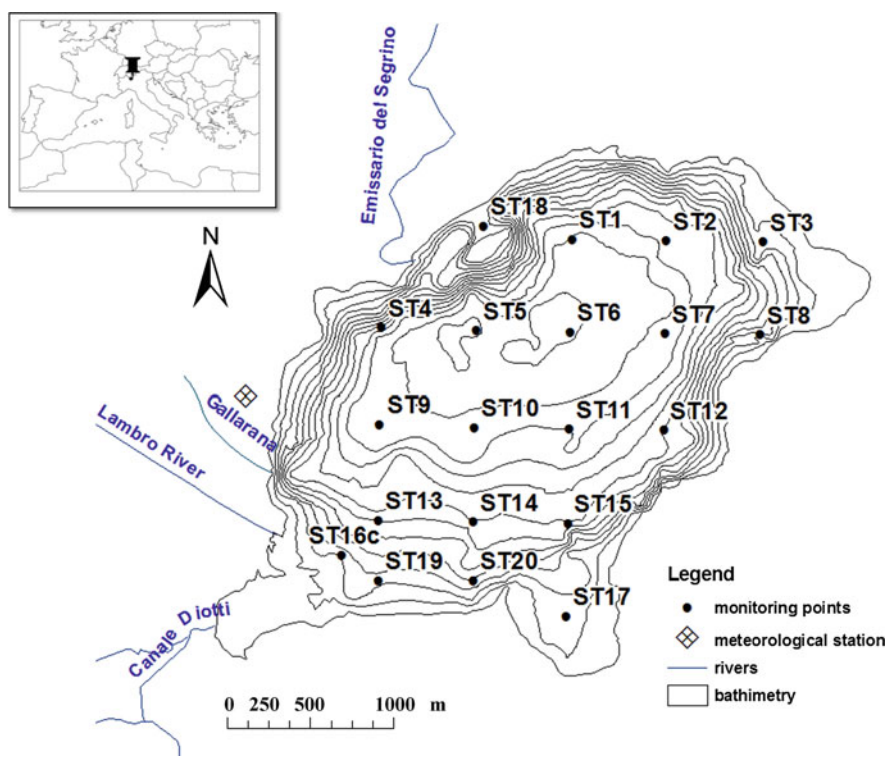

Fig. 1 Location and bathymetry of Lake Pusiano. The outermost *contour line* refers to the surface level (0 m) and other contours are at 2 m depths. In the *map* main inflows and outflows, the meteorological and the sampling stations are also indicated

mainly regulated by Canale Diotti located on the south-west side.

The temperature time series, measured in the lake at winter overturn, has increased in the last 40 years (ca. 0.015°C $year^{-1}$), in line with other studies on European alpine lakes (Ambrosetti & Barbanti, 1999; Livingstone, 2003). The lake is phosphorus-limited (Legnani et al., 2005). Total phosphorus (TP) concentrations increased until the mid-1980s (0.2 mg P l^{-1} at 1984 winter overturn) and progressively decreased towards the mesotrophic limit (0.04 mg P l^{-1}) by winter 2011, after the construction of a wastewater treatment plant in 1985. Total nitrogen (TN) concentrations have remained relatively constant (about 1.5 mg N l^{-1} at overturn) mainly due to substantial contributions of wet and dry atmospheric deposition to the nitrogen load (Balestrini et al., 2000).

Phosphorus loading to the lake has been described in Vuillermoz et al. (2006), who summarized several studies carried out in the Lake Pusiano watershed between 1972 and 2005. The main contribution to the lake comes from the Lambro River, which shows great variability, ranging from 20 to 7 t P $year^{-1}$ in 2000–2003, due mostly to the inter-annual rainfall variability (Salerno & Tartari, 2009).

The cyanobacterium *P. rubescens* became the dominant species in the phytoplankton assemblage in Lake Pusiano from 2001, blooming in the autumn, despite reduced nutrient concentrations, as simultaneously observed in many subalpine lakes (D'Alelio et al., 2011). However, after an intense flood event in November 2002 it completely disappeared for all of 2003 and a greater diversity was observed in the phytoplankton community in this year (Legnani et al., 2005).

Materials and methods

Field and laboratory methods

During the 2010 monitoring monthly program, water samples were collected for chemical and biological analysis at the deepest point in Lake Pusiano (ST6, Fig. 1) by a Van Dorn bottle at eight different depths (0.5, 2.5, 5, 7.5, 10, 15, 20 and 24 m). Dissolved inorganic nitrogen species (NH_4–N, NO_3–N), total dissolved nitrogen (TDN) and TN, phosphorus (PO_4–P), total dissolved phosphorus (TDP) and TP, silica (SiO_2–S) and other hydrochemical parameters such as sulphate, chloride, carbonate, calcium, magnesium, sodium and potassium were determined according to methods of APHA (1992).

Phytoplankton cell counts were conducted on Lugols-preserved water samples from ST6 at discrete depths within the lake epilimnion, metalimnion and hypolimnion. Identification to species level was carried out for each sample with an inverted microscope (Leica DMIRB) after sedimentation and the biovolume was estimated for each species by geometrical approximations (Legnani et al., 2005).

The zooplankton survey was conducted seasonally using a 74 μm plankton net (horizontal tow) sampling at five different depths (0.5, 5, 10, 15 and 23.5 m). Samples were pooled and preserved in 5% formalin and poured inside Imhoff sedimentation cones of 1,000 cm^3 volume. Organisms were then counted and identified under an Utermöhl inverted microscope to calculate densities on dilute representative subsamples of 1 cm^3 volume, recording for each taxon the developmental stages (nauplii, copepodites or juveniles, and adults).

From February 2010 vertical profiles of conductivity (at 20°C), pH, temperature, turbidity, redox, photosynthetically active radiation (PAR), dissolved oxygen (DO), percentage of oxygen saturation and chlorophyll-*a* were obtained on multiple stations in the lake (Fig. 1), using a multiparameter probe (Idronaut Ocean 7 Plus). Simultaneously vertical profiles of chlorophyll-*a* concentration were taken with a spectrofluorometric probe (BBE FluoroProbe, Moldaenke). The FluoroProbe (FP) differentiates up to four 'spectral groups' of phytoplankton in vivo and in situ. The original device is provided with spectral 'fingerprints' for Bacillariophyceae and Dinophyceae both recognized as Diatoms ('Diat'), Cyanobacteria ('Cyano'), Chlorophyceae ('Chlor') and Cryptophyceae ('Crypto'). The phylogenetic composition is determined from fluorescence stimulated by accessory pigments of the light-harvesting complex, and thus on the form which a characteristic phylum fingerprint is detected (Leboulanger et al., 2002). For a detailed description of FP, see Beutler et al. (2002). A dedicated software (FP 2.2.6, BBE-Moldaenke) was then used to calculate the relative amount of each algal class, expressed in terms of the equivalent amount of chlorophyll-*a* (as μg Chl-*a* l^{-1}). The FP algae fingerprints can be calibrated for a specific signal. A strain of

P. rubescens was isolated from Lake Pusiano at known chlorophyll concentration (between 50 and 100 μg Chl-*a* l^{-1}) and used to calibrate a specific fingerprint, after an offset determination for a filtered water sample (0.45 μm cellulose-acetate filter). Indeed this species has a higher phycoerythrin content and its fluorescence signal (dominated by red emission) is closer to the factory-calibrated Cryptophyceae signal than the Cyanobacteria one (rich in phycocyanin). The instrument reliability had been previously assessed by total chlorophyll analytical measurements.

The discharge of the main outflow from the lake was derived from hourly flow measurements whilst other minor tributaries and the outflow discharges were obtained daily from a hydrological model which was calibrated on Lake Pusiano watershed (Salerno & Tartari, 2009) and validated by lake measured levels and hydrological balance in 2010. Temperature, conductivity (and derived salinity), DO and turbidity were measured hourly in Lambro River with a fixed multiparameter probe (TROLL 9500, In Situ Inc.); water quality variables (TN and TP) were determined by high-frequency monitoring with an automatic water sampler (WATSAM portable and refrigerated type, ISOIL Ind.) which included periods of both high and low discharge. Monthly sampling and laboratory determinations were carried out for the water quality of other two tributaries.

Meteorological data of wind velocity and direction, solar radiation, air temperature, rainfall, relative humidity were recorded hourly at a meteorological station close to the lake (Fig. 1).

Model

The Computational Aquatic Ecosystem Dynamics Model (CAEDYM) was coupled with the 3D hydrodynamic Estuary, Lake and Coastal Ocean Model (ELCOM) to simulate the hydrodynamic, nutrient cycles and food web dynamics in Lake Pusiano in 3D. The model is maintained by Centre for Water Research (CWR) of University of Western Australia. ELCOM simulates velocity, temperature and salinity distributions in natural water bodies. It is based on the unsteady Reynolds-averaged, hydrostatic, Boussinesq, Navier–Stokes and scalar transport equations (Hodges et al., 2000; Laval et al., 2003a), and includes external environmental forcing (e.g. from meteorology, inflows and outflows). CAEDYM is a deterministic ecological model, based on the 'N–P–Z' (nutrient-phytoplankton-zooplankton) type, but also includes comprehensive descriptions of carbon, nitrogen, phosphorus, oxygen and silica (Hamilton & Schladow, 1997; Hipsey, 2008). A comprehensive presentation of all of the equations for phytoplankton and nutrient dynamics can be found in Robson & Hamilton (2004).

In the application to Lake Pusiano ELCOM–CAEDYM inflows characterization was driven by inputs from three tributaries. A multivariate model based on discharge, conductivity and turbidity (see e.g. Horsburgh et al., 2010) was calibrated ($R^2 = 0.8$ for TP; $R^2 = 0.9$ for TN) and validated ($R^2 = 0.9$ for TP; $R^2 = 0.6$ for TN) to obtain hourly estimation of water quality over a year for Lambro River. Dissolved and particulate forms of nitrogen and phosphorus were then derived from TN and TP (either from linear regressions or fixed ratios). Carbon (particulate and dissolved form) and silica were assumed to be constant in two ranges of discharge (0–10 and >10 m^3 s^{-1}), according to an approximation based on historical measurements. Constant daily values, derived from averages on monthly data, were assumed for the other minor rivers. Carbon, silica and all organic forms were assumed in the same proportion as for the main river.

Meteorological data were entered as input to the model at hourly frequency. The lake initial condition was provided through lake hydrochemical and biological profiles taken on 23 Feb. 2010 and the simulation duration was 322 days, ending on 12 Jan. 2011, at 1 min time step of computation. The applied grid size (100 × 100 × 1 m) was a compromise considering the spatial resolution for the monitoring multi-station grid, the thermal gradients, the computation time (CPU) and the numerical diffusion effects (Laval et al., 2003b).

A previous one-dimensional model calibration with DYRESM–CAEDYM for Lake Pusiano (Copetti et al., 2006), calibration by 'trial and error' (Robson et al., 2008) and literature information were used to define the biogeochemical parameters for CAEDYM.

Statistical analysis

Ordinary least squares (OLS) regression analysis was used to test the correlation between the biovolume and the chlorophyll concentration determined for each

phytoplankton group. Criterion for entry and acceptance was P value <0.05. Both dependent and independent axes were log-transformed to meet the statistical requirements for normal distribution; then the residuals of the regressions were tested for homoscedasticity (Howarth & Earle, 1979).

A qualitative (visual) approach was used to estimate the timing and the spatial dynamic of variables, particularly for each of the algal groups. The Nash–Sutcliffe index (E_{NS}) is a normalized index, used to evaluate the quantitative agreement between simulated and observed data, comparing the relative magnitude of residual variances (Nash & Sutcliffe, 1970). It is called 'modelling efficiency index' indicating the 1:1 line as reference for an optimal fit and ranges from $-\infty$ to 1 with $E_{NS} = 1$ being the optimal value. Values between 0.0 and 1.0 are generally viewed as acceptable levels of performance, whereas values <0 indicate that the mean observed value is a better predictor than the simulated value, or an unacceptable performance. The Mean Bias (MB) measures the average tendency of the simulated data to be larger or smaller than their observed counterparts. The optimal value of MB is 0.0, with low-magnitude values indicating accurate model simulation. Positive values indicate model underestimation and negative values indicate model overestimation (Gupta et al., 1999). The Root Mean Squared Error (RMSE) is one of the commonly used error index statistics. It represents the square root of the Mean Squared Error (MSE) and quantifies the model performance of each variable (e.g. Trolle et al., 2008). The RMSE and MB have the units of the variable tested, thus expressing the internal error.

Results

Phytoplankton assemblage and field patterns

Cyanobacteria prevailed over the other taxonomic groups, representing the 64% of the total annual biovolume across all samples (Table 1). Concentrations were highest in May due to an isolated bloom of *Aphanizomenon flos-aquae*, and from October to January due to a bloom of *P. rubescens*. Other significant contributions to the phytoplankton assemblage were from Cryptophyceae (15% of total annual biovolume), Dinophyceae (7%) and Bacillariophyceae (3%) which together with Cyanobacteria amounted to 90% of the total annual biovolume. Phytoplankton biomass was concentrated in the epilimnion in autumn and in the metalimnion in summer. Phytoplankton sampling identified 109 different species in total, with greatest diversity in late summer, as observed in Legnani et al. (2005). The total annual biovolume can be represented for most of the year by the sum of just five species (*Aphanizomenon flos-aquae* (Aph-f-a), *Asterionella formosa* (Ast-f), *Cryptomonas erosa* (Cry-er), *Cryptomonas rostratiformis* (Cry-rostr), *Cryptomonas* sp. (Cry-sp.), *P. rubescens* (P-rub)). Amongst these species P-rub exceeded the 90% of total biovolume in autumn–winter (Fig. 2a) and dominated the metalimnetic (>8 m depth) species composition by the end of the summer: it was 70% of the biovolume on 02/09/2010; 40% on 29/09/2010 (*Chrysochromulina parva* was 18%; *Cryptomonas ovata* was 15%; *Ceratium hirundinella* was 10%) and 95% on 28/10/2010.

The total biovolume was compared to the total Chl-*a* based on the sum of all phyla contributions (Fig. 2b). Taxonomic-biovolume data have been combined into spectral groups and related to the FP-determined Chl-*a*, after a log-transformation (see above in 'Statistical methods') for P-rub (Fig. 2c), Cyano (Fig. 2d), Diat (Fig. 2e) and Crypto (Fig. 2f).

Phytoplankton modelling

CAEDYM can simulate up to seven groups which can be parameterized according to the assigned specific species, genre, classes or groups. In this study, phytoplankton were characterized into three key algal groups (Crypto, Diat and P-rub) for the purpose of the model to represent the resultant dominant genera/species of *Cryptomonas* sp., *A. formosa* and *P. rubescens* observed during 2010 (Fig. 2a). Functionally (Reynolds et al., 2002; Padisák et al., 2009), the first species represents those commonly found in mixed, eutrophic small-medium lakes, with physiology characterized by tolerance to light and sensitivity to stratification and silica depletion; the second genre of species represents those found in mesotrophic small- and medium-sized lakes with sensitivity to stratification; the third represents a genre of phytoplankton common to the metalimnia of mesotrophic stratified lakes (usually deep lakes) with tolerance to low light and strong vertical segregation, and sensitivity to instability of water column.

Table 1 Number of phytoplankton species and percentage of total annual biovolume, assembled into taxonomic groups for each sampling date and water column location (according to the thermal profile) in Lake Pusiano

Date	No. of spp.	Bacillariophyceae	Chlorophyceae	Chrysophyceae	Cyanobacteria	Coniugate	Cryptophyceae	Dinophyceae	Ultraplancton	Total by date	Epilimnion	Metalimnion	Hypolimnion
27/01/2010	51	0.0	0.0	0.2	3.8	0.0	0.1	0.2	0.0	4.3	100.0		
23/02/2010	64	0.1	0.1	0.1	4.6	0.0	1.4	0.1	0.2	6.6	100.0		
17/03/2010	78	0.6	0.1	0.3	3.5	0.0	2.7	0.5	0.1	7.7	100.0		
20/04/2010	64	0.1	0.0	0.4	0.4	0.0	0.5	0.1	0.1	1.7	46.5	28.0	25.6
26/05/2010	56	0.7	0.1	0.4	10.9	0.3	4.2	0.8	0.1	17.5	3.7	63.6	32.6
29/06/2010	61	0.3	0.1	1.0	1.4	0.1	1.2	1.6	0.1	5.7	12.4	69.7	17.9
20/07/2010	87	0.3	0.5	0.3	1.4	0.1	1.6	0.6	0.3	5.2	18.2	55.9	25.9
02/09/2010	80	0.2	0.6	0.2	1.2	0.0	0.8	0.8	0.3	4.2	26.4	23.1	50.5
30/09/2010	83	0.1	0.0	1.1	0.9	0.0	0.6	1.2	0.1	4.0	24.3	47.2	28.5
28/10/2010	51	0.2	0.0	0.3	5.9	0.0	0.4	0.6	0.2	7.7	49.2	50.8	
17/11/2010	40	0.1	0.1	0.3	9.9	0.0	0.8	0.3	0.2	11.6	72.8	27.2	
15/12/2010	52	0.1	0.0	1.3	10.5	0.0	0.5	0.1	0.3	12.7	43.5	56.7	
12/01/2011	50	0.4	0.0	0.0	9.7	0.0	0.7	0.1	0.2	11.0	100.0		
Total by group	109 spp	3.2	1.7	5.8	64.2	0.7	15.3	6.9	2.2	100.0	53.6	32.5	13.9

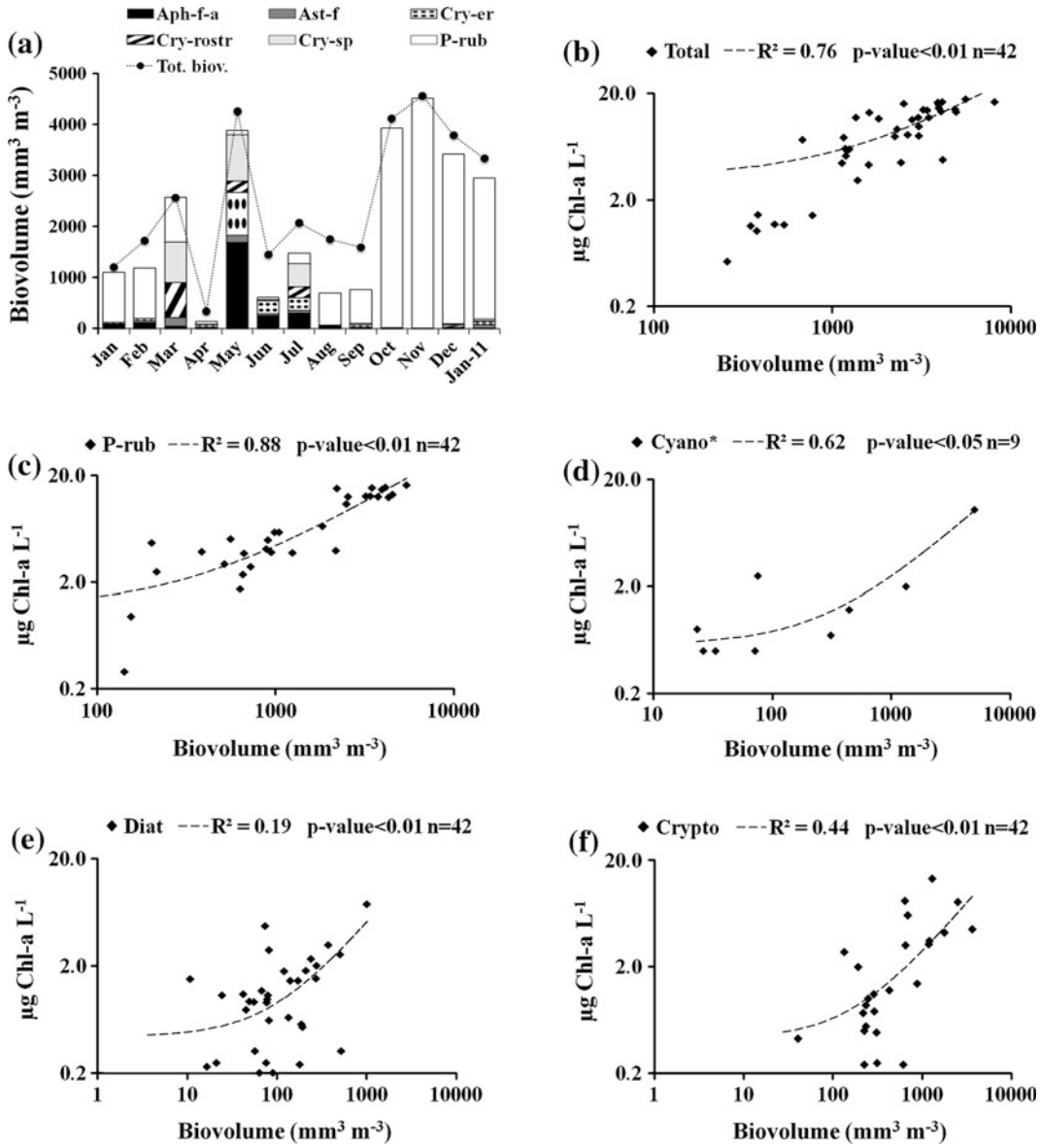

Fig. 2 **a** Dominant species of the phytoplankton assemblage as mean biovolume ($mm^3\ m^{-3}$) along water column by monthly identification. Mean total biovolume by all species is also reported as *black dots*. Log-regressions between biovolume ($mm^3\ m^{-3}$) and chlorophyll concentration (µg Chl-*a* l^{-1}) determined fluorometrically at every depth and date for: **b** total biovolume and chlorophyll concentration; **c** P-rub fingerprint; **d** cyanobacteria spectral group; **e** diatoms spectral group; **f** cryptophyta spectral group

The parameters for growth rate, temperature curve representation (as standard, optimum and maximum growth temperature), light limitation and vertical migration were obtained both from literature and previous modelling studies (Table 2). Carbon, nitrogen and silica (the last only for Diat) limitation were simulated with a simple Michaelis–Menten term whilst phosphorus limitation was simulated with the intracellular store option (for detailed explanation see Robson & Hamilton, 2004) since this is the limiting nutrient for phytoplankton growth in Lake Pusiano (Legnani et al., 2005; Copetti et al., 2006). The phosphorus limitation function was mathematically analysed and calibrated to make P-rub less frequently limited by P than Diat and Crypto (Feuillade et al., 1990; Dokulil & Teubner, 2000). Respiration rate and

Table 2 List of selected parameters differentiating the phytoplankton dynamic for the three algal groups set in ELCOM–CAEDYM

Phytoplankton parameters	Symbols	Units	Assigned values		
			Diat	Crypto	P-rub
Maximum potential growth rate of phytoplankton	Pmax	day^{-1}	1.0	1.0[c]	0.14[a]
Average ratio of C to chlorophyll-*a*	Ycc	mg C mg Chl-a^{-1}	40[d]	40[e]	90[e]
Light half saturation constant for algal limitation	IK[f]	µmol m^{-2} s^{-1}	60[d]		
Light saturation for maximum production	Ist[f]	µmol m^{-2} s^{-1}		80[d]	10[a]
Half saturation constant for phosphorus uptake	KP	mg l^{-1}	0.03	0.03[c]	0.002[c]
Half saturation constant for silica uptake	KSi	mg l^{-1}	0.24[c]		
Maximum internal phosphorus concentration	IPmax	mg P mg Chl-a^{-1}	0.085[b]	0.085	0.1
Minimum internal phosphorus concentration	IPmin	mg P mg Chl-a^{-1}	0.02[b]	0.02	0.0003
Maximum rate of phosphorus uptake	UPmax	mg P mg Chl-a^{-1} day^{-1}	0.11[b]	0.04	0.1
Standard growth temperature	Tsta	°C	8	15	10[a]
Phytoplankton optimum temperature	Topt	°C	12	18	13[a]
Phytoplankton maximum temperature	Tmax	°C	33[d]	25	20[a]
Temperature multiplier function for phytoplankton	vT	–	1.01	1.08	1.06[e]
Constant settling velocity	ws[g]	m day^{-1}	−0.086[d]	−0.035[d]	0.004
Phytoplankton mortality coefficient	kr	day^{-1}	0.3	0.18	0.005

[a] Bright & Walsby (2000)

[b] Bruce et al. (2006)

[c] Rigosi et al. (2011)

[d] Rinke et al. (2010)

[e] Copetti et al. (2006)

[f] Two options are available to model light limitation to growth (without photoinhibition *IK* or with photoinhibition *Ist*; refer to Robson & Hamilton (2004)

[g] Negative values indicates downward velocity; the positive value for P-rub indicates an upward velocity to simulate the buoyancy changes during autumnal waterblooms (Walsby et al., 2006)

mortality were calibrated for all the groups. The coefficient for phosphorus sediment release has been measured in Lake Pusiano (Vuillermoz et al., 2006) whilst those for nitrogen and silica were calibrated. A general herbivore zooplankton group was configured in the model to prey on Crypto (preference at 60%) and Diat (preference at 40%) since measured zooplankton consisted mostly of small herbivores (rotifers and copepod nauplii) whilst the predatory taxa (adult copepods, predatory rotifers) co-dominated only in summer and early winter.

Model assessment

Simulation profiles for temperature (T), DO, nutrients (SiO_2–Si, PO_4–P, NH_4–N and NO_3–N) and phytoplankton (Diat, Crypto and P-rub) were compared to the corresponding measured profiles at all the stations (note that nutrients were available only at ST6 station) for each sampling date, to estimate the model performance. The agreement was first tested computing E_{NS}, MB and RMSE over 6,092 observations for T, DO and algal groups and over 488 observations for nutrients. The comparison is represented in scatter plots (simulated data are on *Y* axes; measured data are on *X* axes) using smoothed densities computed with the algorithm of Eilers & Goeman (2004), to measure the spread of each data point from the 1:1 regression line (Fig. 3).

A good agreement resulted for T ($E_{NS} = 0.78$) and DO ($E_{NS} = 0.48$): ELCOM reproduced observed profiles by the boundary conditions and CAEDYM modulated them, mainly through the phytoplankton dynamics, with a mean underestimation (MB) of 1.66°C and 0.66 mg l^{-1}, respectively. Nutrient simulations depended on external loading input and the internal biogeochemical cycle (sediment–water column-biomass): a good performance was obtained for SiO_2–Si and NH_4–N ($E_{NS} = 0.35$ and $E_{NS} = 0.66$,

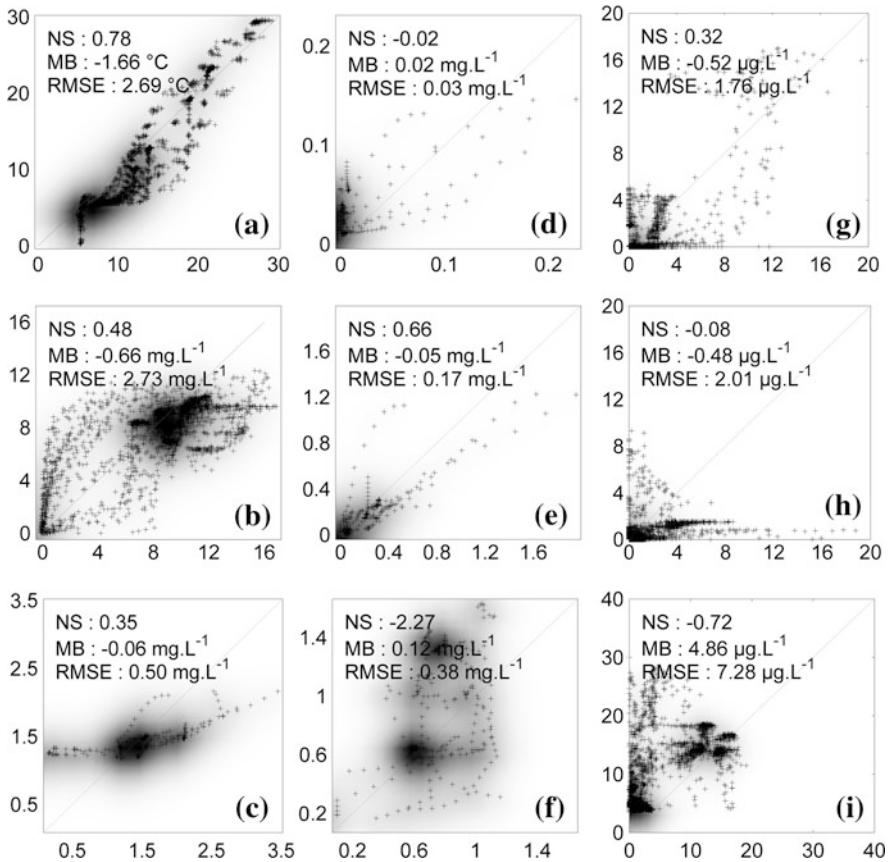

Fig. 3 *Scatterplots* with smoothed densities by simulated (on *Y* axes) and field (on *x* axes) data; E_{NS} value (NS), MB and RMSE are also reported as indices of model fit for **a** temperatures (°C), **b** dissolved oxygen (mg l^{-1}), **c** silica (mg l^{-1}), **d** orthophosphate (mg l^{-1}), **e** ammonium (mg l^{-1}), **f** nitrate (mg l^{-1}), **g** Diat (µg Chl-*a* l^{-1}), **h** Crypto (µg Chl-*a* l^{-1}) and **i** P-rub (µg Chl-*a* l^{-1}). See in the text for more information

with an underestimation of 0.06 and 0.05 mg l^{-1}, respectively) but deteriorated for PO_4–P and NO_3–N ($E_{NS} = -0.02$ and $E_{NS} = -2.27$, with an overestimation of 0.02 and 0.12 mg l^{-1}, respectively). For each algal group, the chlorophyll concentration (µg Chl-*a* l^{-1}) was dynamic on the basis of assigned parameters for nutrient uptake, growth, grazing, mortality, settling and sediment mineralization. Considering the global model performance (without space and time distinction) a good prediction level was gained for Diat ($E_{NS} = 0.32$, with a slight underestimation of 0.52 µg Chl-*a* l^{-1}) and deteriorated for Crypto and P-rub ($E_{NS} = -0.08$ and $E_{NS} = -0.72$, with an underestimation of 0.48 Chl-*a* l^{-1} and an overestimation of 4.86 µg Chl-*a* l^{-1}, respectively).

The vertical and seasonal patterns were analysed at the deepest station (ST6), where field data (at monthly scale) were overlapped with simulation data (at hourly scale) to describe the performance of the model to reproduce the seasonal evolution of each variable, in a qualitative and quantitative way. The RMSE and MB temporal evolution were thus computed by comparison between simulated and measured data at ST6 (Fig. 4). The evolution of T was matched during the year: the associated error (RMSE) increased only during stratification due to colder simulated temperatures at the bottom probably because the model underestimated the heat exchange between upper and lower layers during the summer. The evolution of DO was well matched especially in relation to the bottom depletion during the summer; the error values increased in this period because of the peak measured in the surface layers during the occasional bloom of the cyanobacterium Aph-f-a (not considered in the model) during late spring. Sediment release of SiO_2–Si, NH_4–N and PO_4–P were well simulated during the summer as well as NO_3–N bottom depletion. Some problems arose in the upper layers: SiO_2–Si was underestimated during early spring and overestimated during the summer probably due to the simplicity of the flow-based model for silica; NO_3–N was generally overestimated from spring, possibly due to inability to properly represent external loading and perhaps insufficient nitrogen uptake by phytoplankton;

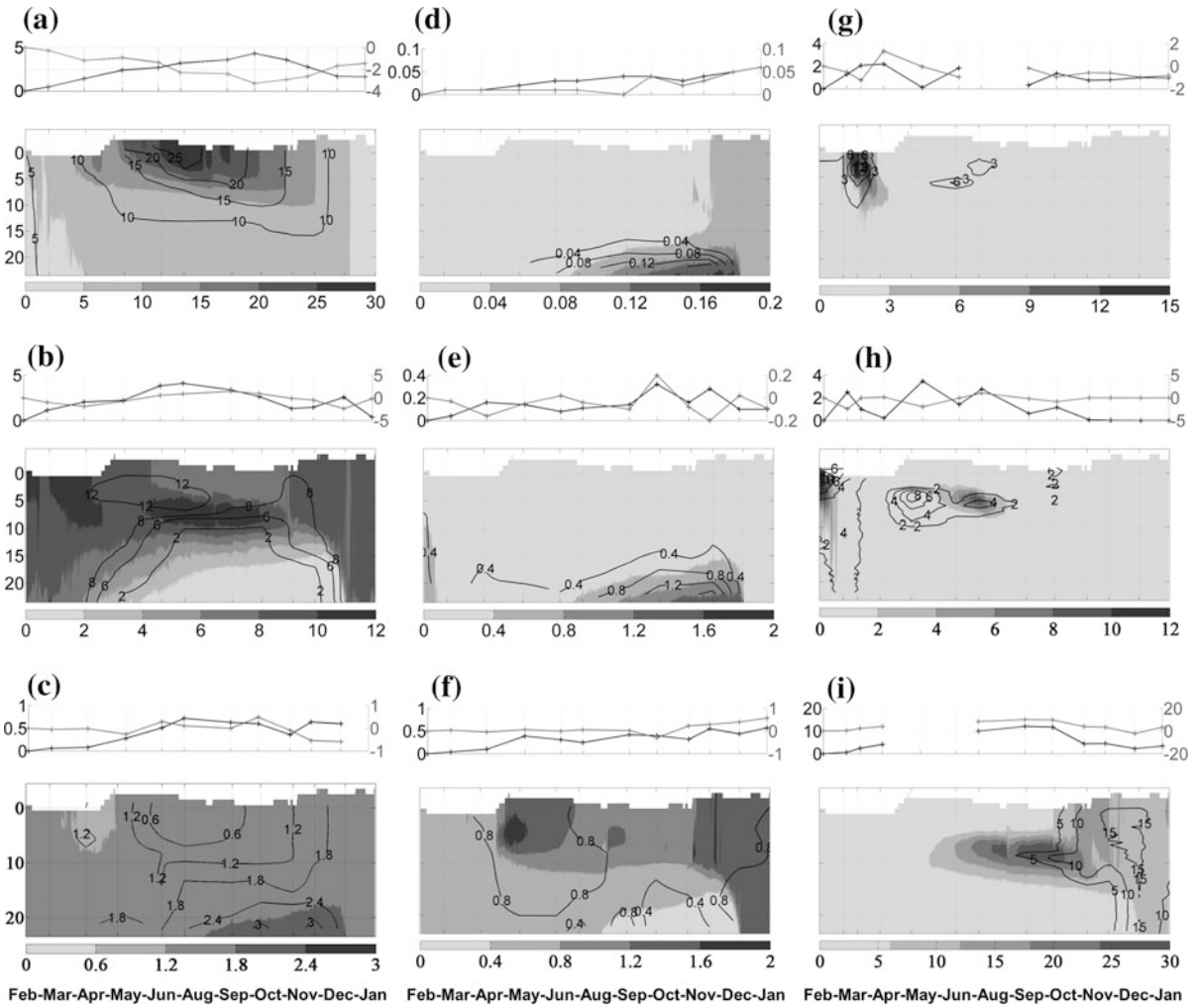

Fig. 4 On each *subplot*: RMSE (*black line*, referred to the main *Y* axes) and MB (*grey line*, referred to the secondary *Y* axes) at ST6 station computed by simulated and field for each sampling date (above); field (*black lines*) versus model (*contour plot* in scale of *grey*) profiles at ST6 station (below) as **a** function of depth (as metres on *Y* axes) and time (sampling date on *X* axes) for a temperatures in °C, **b** dissolved oxygen in mg DO l^{-1}, **c** silica mg Si–SiO_4 l^{-1}, **d** orthophosphate mg P–PO_4 l^{-1}, **e** ammonium mg N–NH_4 l^{-1}, **f** nitrate mg N–NO_3 l^{-1}, **g** Diat, µg Chl-*a* l^{-1} **h** Crypto µg Chl-*a* l^{-1} and **i** P-rub µg Chl-*a* l^{-1}. The missing values amongst the RMSE and MB of Diat and P-rub depended on the absence of measured profiles

PO_4–P was overestimated only during the lake overturn (Jan. 2011) probably due to an excess of sediment release in the model; the simulation of NH_4–N was the best matched amongst the nutrients. All the simulated algal groups resulted in correct reproduction of the timing and position in the water column based on qualitative comparisons with measured profiles of spectral groups: periods of rapid biomass increase of Diat were well matched with corresponding depletion of SiO_2–Si; Crypto growth was slightly underestimated during late-winter whilst early summer increases in biomass were simulated with the model but not so in spring. P-rub was well reproduced during the autumn bloom both in the spatial position and in the concentration values; the model correctly predicted the metalimnetic peak in summer but overestimated the productivity, probably because certain factors moderating the metalimnetic increase in the real world were not embraced.

The model performance in reproducing the spatial variability was shown through RMSE computed at each station, on the basis of the specific range of each variable (Fig. 5). The model had an optimal performance in reproducing temperatures along the south

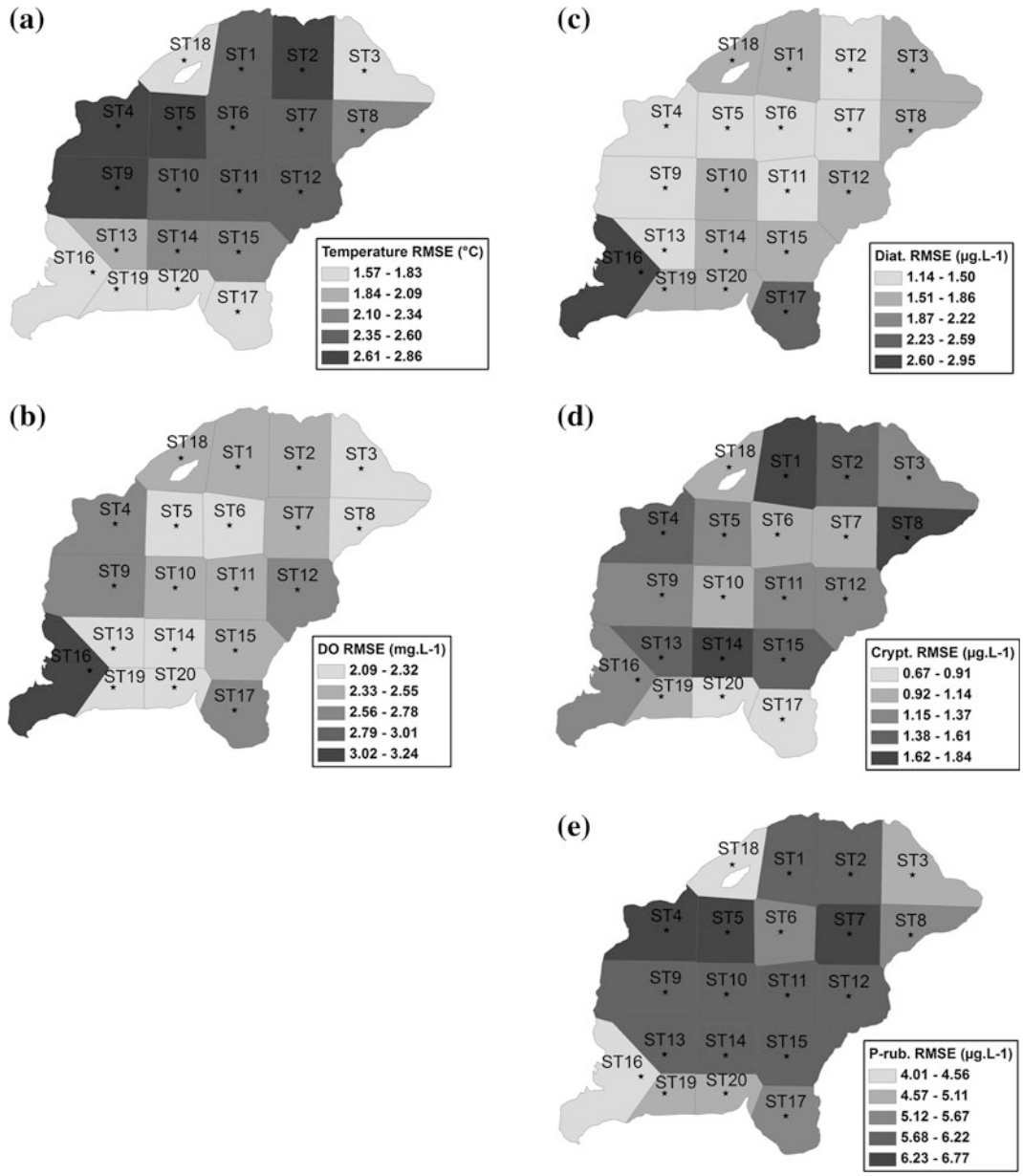

Fig. 5 Spatial model performance description through RMSE computed by simulated and measured data residuals at each monitoring station for **a** temperatures, **b** dissolved oxygen, **c** Diat, **d** Crypto and **e** P-rub. For each variable RMSE is relative to the full scale. *Light grey* expresses a lower error

side, near the entrance of Lambro River, as well as the north-east and the north-west shores near the entrance of a minor tributary at ST18 (see the map on Fig. 1). DO was generally well reproduced at all stations except ST16, where the main inflow enters. Phytoplankton simulations showed a good spatial performance for the Diat group, except ST16 where comparisons may have been affected by the major river inflow. Crypto relative error was generally small, deteriorating only in a few stations. For P-rub, the error was slightly larger due to metalimnetic overproduction in the model, and there was a better performance in the shallower stations close to the shore.

Model outputs for phytoplankton growth and losses, which included gross production, mortality, changes due to hydrodynamics and settling, were graphically examined. Each group was analysed in a representative surface layer (2 m) and in a representative deep layer (8 m) and their 'capacity to grow' was represented with growth-polygons. The vertex of each polygon represented individual growth limitation functions, spanning from 0 to 1 whilst the temperature effect and mortality (i.e. grazing and respiration) were also normalized to values between 0 and 1, allowing evaluation of how the limitation factors and loss terms affected the growth potential. Phytoplankton production is maximal when the value of limitation by nutrients and light and the temperature effect is close to 1 and when the value of grazing and respiration is close to zero. A bloom of Diat, from the end of winter to early spring, was supported by near-optimal temperatures for growth (ca. 12°C; Table 2), high nutrient availability and very low grazing and competition in the upper layer (depth 2 m). Smaller peaks in Diat concentrations in the deeper layer (8 m) were due mostly to sedimentation of populations in surface waters, as irradiance was strongly limiting at this depth. This group was highly sensitive to increases in water temperature and declined rapidly with increases in spring temperatures. A further influence was the occurrence of stratification in spring when there was a conspicuous loss of Diat from the upper layer. The combination of relatively high temperature and strong stratification meant that conditions were generally unsuitable to support their growth again until autumn (Fig. 6a).

Crypto at depth of 2 m attained highest concentrations in late-winter but quickly decreased in spring when there was strong limitation by temperature and light. In summer, they had large losses because of mixing through the surface mixed layer as well as high mortality, and thus their growth rate remained close to zero. At a depth of 8 m they grew rapidly during mid-summer, mainly due to concomitant occurrence of favourable temperature, adequate nutrients and stronger density stratification in this deeper layer (Fig. 6b).

Specific physiological features of P-rub were included in the model, such as a relatively low growth and mortality rate, low light saturation of growth rate and buoyant properties (simulated by a low negative value for the sedimentation rate). The combination of low values of growth rate and settling velocity made it particularly sensitive to high turbulence occurring either throughout the water column during winter mixing or in the surface mixed layer during summer. In the late-autumn period concentrations gradually declined due to increasing turbulence and impending water column mixing. In the deeper (8 m) layer, concentrations increased during summer and were almost static even under strong wind forcing, whilst they suffered a flood event in mid-summer (15 Aug.) and finally decreased in winter when low irradiance and cold temperature did not support net growth (Fig. 6c).

Discussion

Approach

A common problem in ecological modelling is the adequacy of representing changes in ecosystem dynamics without excessive complexity and over-parameterization of models (Van Nes & Scheffer, 2005). Interactions of abiotic and biotic components shape the spatial distributions of organisms, thus appropriate mathematical relationships need to be developed to provide for spatial heterogeneity. In this study, the physical variables (i.e. temperature) were first simulated satisfactorily at the deepest station (ST6) before calibration was undertaken of parameters relevant to the nutrient and algal dynamics.

Measurements of spectral signatures of phytoplankton based on functional groups proved to be a good strategy to parameterize different phytoplankton groups and allowed for direct calibration of the selected phytoplankton groups in the spatially resolved (3D) model. *P. rubescens* emerged as keystone species, requiring assignment of its own group in the model, whilst two more generic phytoplankton groups were chosen, representing Diatoms and Cryptophytes. The model algal-group parameters represented specific physiological features of each group, collectively representing the dynamics of the entire phytoplankton community in Lake Pusiano. As has been noted in a number of other studies (Arhonditsis & Brett, 2004; Trolle et al., 2008; Rinke et al., 2010) the model performance, evaluated statistically, showed that temperature and oxygen profiles were simulated well, but there was a lower performance for nutrient and biological variables (three phytoplankton groups), attributable to the complexity governing these higher

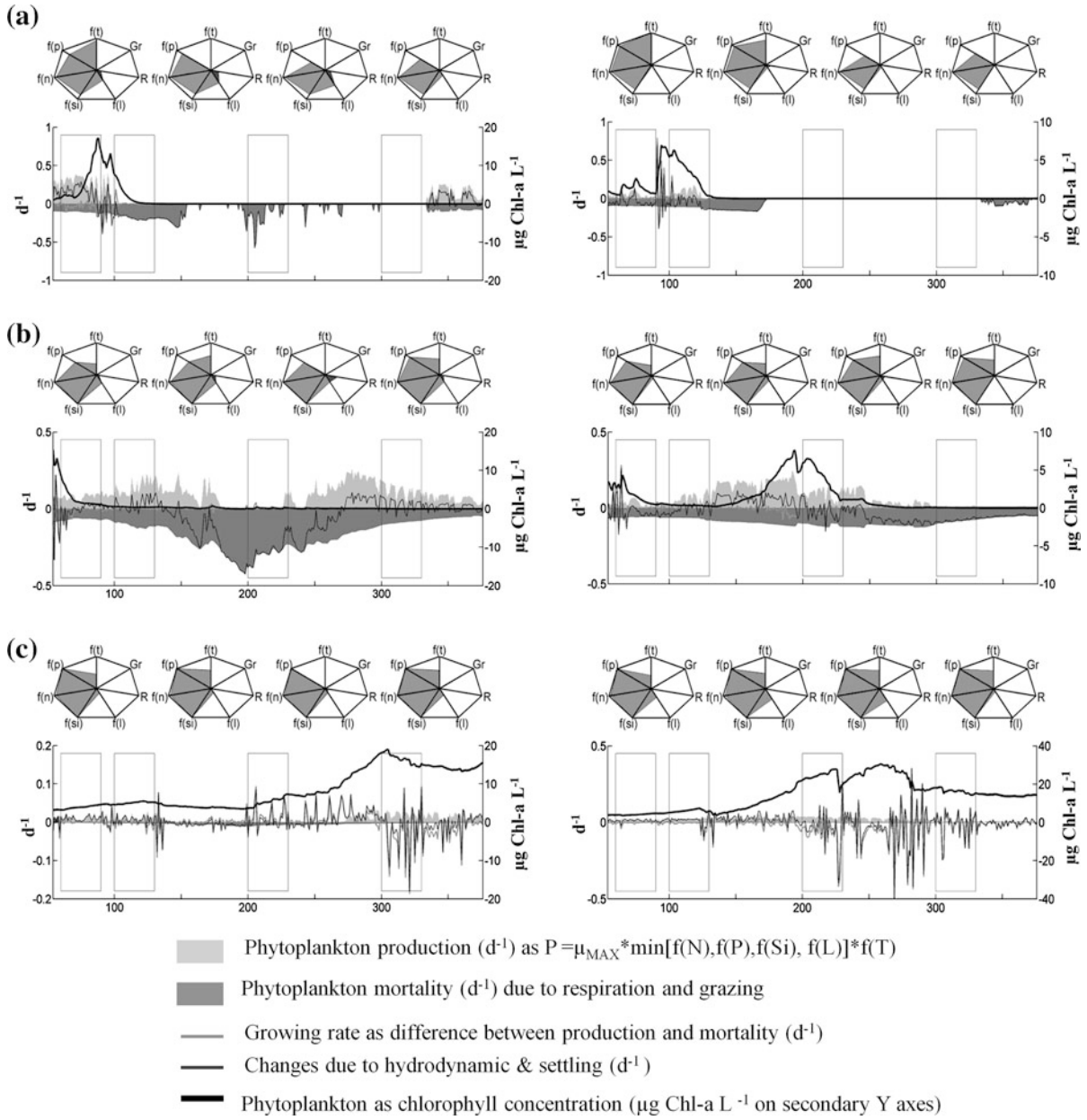

Fig. 6 Simulation output at the depth of 2 m (*left*) and of 8 m (*right*) at ST6 station for the limitation terms to phytoplankton growth. For each algal group the time evolution (322 days from 23 Feb. 2010 to 12 Jan. 2011, expressed as day of year). Each window of 30 days in the sub-plots corresponds to the area in the polygons (above each sub-plot) representing determinant periods for the phytoplankton phenology: (from *left to right*) late-winter from 53 to 80 day of year, early spring from 85 to 120 day of year, mid-summer from 200 to 230 day of year, late-autumn from 300 to 330 day of year. The algal growth is determined by the influence of temperature f(T), orthophosphate f(P), dissolved inorganic nitrogen f(N), silica f(Si), light f(L), respiration (R) and grazing (G) for: **a** Diat, **b** Crypto and **c** P-rub

ecological levels (Robson & Hamilton, 2004; Missaghi & Hondzo, 2010; Vilhena et al., 2010). In our study, the E_{NS} statistical index was positive for silica, ammonium and Diatoms and slightly negative for the other variables. A systematic further calibration (e.g. with a Bayesian approach) may be possible to parameterize mechanistic or process-based models (Arhonditsis et al., 2007) but it has been not yet

completely extended to coupled physical–biogeochemical models (Zhang & Arhonditsis, 2009), thus here it has not been undertaken with a 3D approach in which the spatial comparison has been explicitly evaluated. A common 'trial and error' approach was used, supported by an expert modeller with an understanding of both the biophysics of the system and the structure of the model. Field data were compared to model output with goodness of fit evaluated using a 'by eye' fit and with the aid of some measures of goodness of fit. In all cases parameter values were adjusted within literature or measured data ranges.

Vertical dynamics

The model provided good simulations of the seasonal patterns observed at the maximum depth for all of the variables. In particular, sediment nutrient releases (associated with increases in phosphate and ammonium concentrations) and depletion of nitrate (associated with denitrification) were inferred from the model simulations, whilst the timing and position of peaks in phytoplankton biomass in the water column were well matched. The model had some problems in reproducing the temporal dynamics of nutrients within the upper layers, likely due to the coarseness of certain measurements of external loading, though we could not discount other possible effects resulting from inaccuracies in the timing and magnitude of phytoplankton biomass.

Complex ecosystem models are often examined only in terms of their ability to reproduce observed data against outputs of state variables from the model. As a result important physiological processes embedded within the model structure are often not elucidated and yet can yield important information. The analysis of growth-polygons showed that *P. rubescens* is highly competitive in phosphorus-limiting conditions, especially compared with the other two phytoplankton groups. In spite of this, the physiological responses to nutrients were not the critical factor driving the algal growth in the model and other physiological properties (e.g. temperature range, growth rate, light and settling) determined ecological niches in which re-oligotrophication and increased duration and strength of stratification would favour such metalimnetic populations.

Planktothrix rubescens horizontal spatial patterns

Internal seiches derived from wind forcing are considered to drive the vertical and horizontal distributions of metalimnetic species such as *P. rubescens* (Cuypers et al., 2011; Pannard et al., 2011). In our study, wind (maximum speed: 5.7 m s^{-1}) had a strong effect on its distributions at short-time scales, mainly affecting vertical distributions. A spectral analysis of thermocline oscillations in Lake Pusiano has previously allowed identification of the rapid degeneration of basin-scale internal wave activity that occurs within a few hours during maximum stratification (Boegman et al., 2003).

The effects due to the Lambro River entrance were more pronounced and long-lasting during high-discharge events, both in terms of vertical and horizontal patterns. A description was attempted in order to compare salinity mean values to the maximum P-rub concentrations of vertical profiles at each station before (14 Aug.), during (15 Aug.) and after (16 Aug.) a flood event (maximum discharge: 263 m^3 s^{-1}). When the river entered the lake it was tracked by low-salinity water and was shown to have a circular anticlockwise distribution along the shores. In summer, maximum concentrations were in the metalimnion: before the flood, a stable condition was depicted by a horizontally uniform distribution; during the flood, water movements pushed the population towards the lake centre, then to the west shores and progressively to the south side. After 48 h, a horizontally uniform, but more diluted, distribution formed again in the metalimnion (Fig. 7).

Planktothrix rubescens is the primary constituent of the DCM in Lake Pusiano and its assigned physiological parameters led to simulations that replicated its presence in the metalimnion during the stratified period, as well as its horizontal variation. The other two algal groups that were simulated, representative of Diatoms and Cryptophytes, compete with it for resources over a typical annual phytoplankton succession sequence (Krivtsov et al., 2000). Vertical separation of resources, which is interrupted by storm events and inflow insertions, play a major role in alternating populations of these three groups through the annual cycle. Whilst the two competitors in the model had a higher potential growth rate, the growth-polygon for P-rub clearly showed how several of the key environmental variables (e.g. water temperature and light)

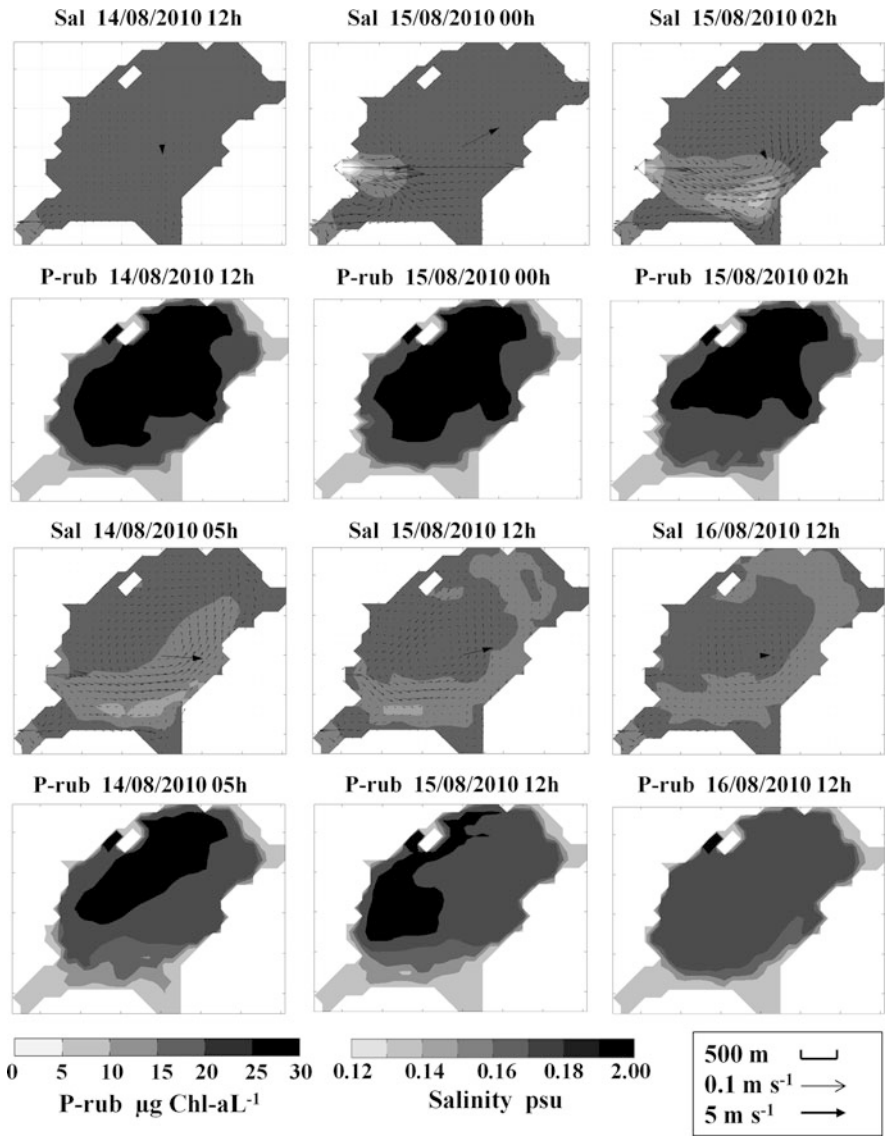

Fig. 7 Representation of spatial horizontal patterns before, during and after a flood event as mean values of salinity (Sal) and P-rub maximum Chl-*a* concentrations on surface plots. *X* and *Y* are spatial axes (m); *thin arrows* represent velocity and direction of the water currents in lake and the *thick arrow* on the lake centre represents velocity and direction of the wind

were closer to its optimal at 8 m than for the other two phytoplankton groups. The model results offer support to the hypothesis that physiological features (D'Alelio et al., 2011) explain the widespread success of that species. Stronger stratification during summer and warmer autumns associated with regional increases in air temperature (Livingstone, 2003) also need to be considered amongst factors leading to increasing dominance of *P. rubescens*. Variables relevant to a changing climate (e.g. air temperature) can be encompassed into model forcing terms and the length of simulation runs should be extended in future work in order to better understand, and potentially to isolate the effect of a changing climate on its distributions.

Conclusion

This study has evaluated the use a three-dimensional hydrodynamic-ecological model to reproduce discrete measurements of hydro-chemical variables, including inferred behaviour of metalimnetic populations of *P. rubescens* in a pre-alpine lake. An innovative methodological approach was used to allocate classic

taxonomic determinations into 'functional groups' in order to validate a hydrodynamic-ecological model with three 'key' groups including the metalimnetic species *P. rubescens*. Spectrally specific fluorometric responses and validation with traditional cell enumeration microscopy were used to provide highly temporally and spatially resolved distribution patterns of phytoplankton in Lake Pusiano, so that measured chlorophyll-*a* concentrations assigned to the three phytoplankton groups could be compared directly to the model output values of these groups.

Distributions of *P. rubescens* were strongly influenced by the lake hydrodynamics, particularly during high-discharge inflows in summer stratification. The river inflows shaped a circulation pattern in the lake, strongly altering spatial its distributions in the horizontal dimension. A vertically resolved assessment, based on analyses of model output at depths of 2 and 8 m, revealed strong separation of environmental drivers between the two depths, indicating niche separation according to the two depths. A difference was noted in near-shore and pelagic profiles in the model output and may be attributed to a depth-specific calibration strategy, inflow dilution of cells or phytoplankton nutrient uptake variations associated with the stream-lake transition zone (Mackay et al., 2011). A further parameterization of phytoplankton and zooplankton and an improvement in tributary input characterization are identified as areas where model performance could be improved. The model used in this study may help in the refinement of field programs, allowing for measurements to be targeted more specifically to locations or times in Lake Pusiano when there are likely to be rapid changes in phytoplankton populations. The model also revealed how the physiological features of *P. rubescens*, specifically its ability to grow well in the metalimnion, may be well suited to a re-oligotrophication phase occurring concurrently with the strengthening stratification of a warming climate.

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PHYTOPLANKTON

Mixotrophic phytoplankton is enhanced by UV radiation in a low altitude, P-limited Mediterranean lake

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Abstract UV radiation promotes harmful effects on phytoplankton populations, but it is influenced by the degree of sensitivity of different populations to the ultraviolet:photosynthetically active radiation ratio (UVR:PAR), part of which is P-dependent. Given the expected increase of UV radiation along with global change, one may ask if phytoplankton populations are able to adapt to the expectedly higher UVR:PAR ratio. If so, how would phytoplankton communities be affected? The main goal of this study is to answer these questions. Field and laboratory experiments were carried out with phytoplankton populations of an oligotrophic, low altitude lake in Central Spain. No changes were observed in abundance of phytoplankton fractions after UVR removal in the lake. However, autotrophic picoplankton underwent lower growth and contribution to total phytoplankton biomass when UVR increased. Phytoplankton biomass under enhanced UVR was one-third lower than the biomass reached under only PAR. UV-related growth changes were species-specific and linked to cell size and metabolism. An UVR increase would then promote phytoplankton assemblages who resulted from a trade-off between competitive advantages of picoplankton in a P-limited system and selected larger algae. Under these circumstances, the mixotrophic character of these larger species happened to be an evolutionary advantage.

Keywords UVR damage · Ecosystem vulnerability · Ruidera Lakes Natural Park · Autotrophic picophytoplankton · Mesocosm-experiments · Stoichiometry

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Introduction

Solar irradiances reaching the Earth fluctuate as result of atmospheric variables such as water vapour, atmospheric gases and processes related to human activity like the ozone hole, or global dimming and brightening due to aerosols and their interaction with clouds (Solomon, 1999; Wild et al., 2007). Thus, estimations of future UVR levels vary according to the different criteria considered; while some models

predict its increase (Madronich, 1994; Häder et al., 2007), others suggest its stabilization and even a decrease depending, for example, on the level and type of clouds and relating UVR with PAR changes (Helbling & Zagarese, 2003; Ravishankara et al., 2008). This, together with the fact that different light qualities have different vertical attenuation coefficients, means that a full range of UVR:PAR intensity ratios can be expected in waters of different optical properties and depth. Most studies focus on UVR incidence and its magnitude effects on organisms, but natural systems show a wide gradient of UVR and PAR irradiances; thus, not only are PAR and UVR changes important to aquatic populations, but also the UVR:PAR ratio trend (Hessen et al., 2008).

Due to aquatic-ecosystem sensitivity to UVR, many experiments have been done in marine and freshwater ecosystems across trophic, altitudinal and latitudinal gradients proving that different systems can be more or less protected from UVR depending on DOC levels and transparency (de Mora et al., 2000; Helbling & Zagarese, 2003). UVR has also been studied at organism level due to its harmful effect on different essential molecules such as lipids, nucleic acids or proteins, thus, affecting cellular metabolism. Therefore, the importance of variations in radiation on phytoplanktonic organisms is evident (see the literature reviews by Helbling & Zagarese, 2003; Harrison & Smith, 2009). Harmful UVR effects on the planktonic community are influenced by the degree of sensitivity or adaptation of different communities, and the response can be even species-specific (Quesada & Vincent, 1997; Kaczmarska et al., 2000; Helbling et al., 2001). These differences are partially due to specific size and metabolic properties taking into account that implicated process are photosynthetic inhibition, induction of DNA damage and repair (Banaszak, 2003; Buma et al., 2003; Villafañe et al., 2003). In this respect, evidence can be found in the literature indicating that cell size and UVR inhibition might be inversely related (Karentz et al., 1991; Callieri et al., 2001; Kasai et al., 2001; van Donk et al., 2001). Also, phylogeny is important because different taxonomic groups or even populations have different strategies to avoid UVR damage such as pigmentation, enzymes against reactive oxygen species, or different DNA or protein-repairing mechanisms (García-Pichel & Castenholz, 1993; Quesada & Vincent, 1997; Laurion & Vincent, 1998).

In high mountain lakes of the northern hemisphere (Carrillo et al., 2008), southern hemisphere (Souza et al., 2010) and boreal latitude (Xenopoulos et al., 2002), organisms are subjected to a higher incidence of UVR; however, inhibitory UVR effects were less pronounced of those that could be expected, indicating possible acclimation and/or adaptation to this radiation (Halac et al., 1997; Helbling et al., 2001; Xenopoulos & Frost, 2003). Moreover, many of the adaptive mechanisms are known to be phosphorous dependent (Medina-Sánchez et al., 2006; Carrillo et al., 2008), and this additional phosphorus in cells could be used for several UVR-damage repair mechanisms such as enhanced protein synthesis, nucleotide repair (Hessen et al., 2008) or sunscreen production (García-Pichel & Castenholz, 1993). It seems evident that in high UVR:PAR environments (in high altitude and/or latitude), planktonic organisms maintain a trade-off between the costs of growth and investment in protection and repair necessary to reduce UVR damage. In these environments, it has been demonstrated that, at least in the short term, when the incidence of UV radiation was removed by filters, phytoplankton reacts by increasing both growth and C:P ratio. This corroborates that survival under these high UVR:PAR conditions has a metabolic cost and systemic relevance in, for example, oligotrophic environments (Delgado-Molina et al., 2009).

But, the question that arises is whether these same populations can adapt to living in a higher UVR:PAR ratio than in their natural conditions, more specifically, under a UVR:PAR increase caused, for example, by the predicted higher incidence of UV radiation or a larger cloud cover (Gallardo et al., 2001) whose filter effect seems to be higher on PAR radiation than on UVR (Eilertsen & Holm-Hansen, 2000). The main goal of this study is to answer this question. To do so, first, changes in the density of phytoplankton populations (including autotrophic picophytoplankton) will be evaluated under field conditions by removing UV light in an oligotrophic mid latitude and altitude lake (Central Spain). Secondly, these populations will be placed under high UVR:PAR conditions in the laboratory to demonstrate whether they not only have a rapid response to change, growing less and increasing their phosphorus content, but also whether these changes are species-specific and related to algal sizes.

Methods

Study site

The field study was performed in La Conceja lake (Lagunas de Ruidera Natural Park, Spain) from 11 to 15 of July, 2009. The lake is sited at 37°N/2°W and 850 m.a.s.l., 14 m deep. It was considered a P-limited and N-enriched Mediterranean oligotrophic system (Álvarez-Cobelas et al., 2007). Sampling was carried out in the central area of the lake, where other limnological studies have been undertaken (Rodrigo et al., 2003; Álvarez-Cobelas et al., 2007). Limnological characteristics in the lake during this period of study are shown in Table 1; these data correspond to a composite sample obtained from the lake water taken with a Van-Dorn horizontal sampling bottle from three depths, spaced evenly within the epilimnion (0–10 m); in that, it is reflected that lake is still an oligotrophic system.

Irradiances through depth were measured using a multichannel submersible radiometer (Biospherical Instrument Compact Inc., BIC-2104 P). Diffuse attenuation coefficients for downward irradiance (K_d, λ) were determined from the slope of the linear regression of the natural logarithm of downwelling irradiance versus depth for each region of solar spectral irradiance. Light irradiances reaching algal communities (infield mesocosms and laboratory microcosms) were calculated, following Sterner et al. (1997), as the proportion of sub-superficial irradiance (I_0, Wm^{-2}) remaining in the water layer (I_m, Wm^{-2}). Then, $I_0 \cdot I_m$ is the mean light in a water layer determined by surface light, the diffuse attenuation coefficient (K_d) and depth. Thus, it is possible to test for different light qualities by modifying I_0 and depth span of experimental micro and mesocosms (enclosures), and to compare light availabilities for planktonic communities (Sterner et al., 1997) under natural and laboratory conditions (Table 2).

Experimental design

A field experiment was performed to study the effects of natural UVR irradiances on different size fractions of the autotrophic community. To do so, six UVR-transparent polyethylene enclosures (600 l volume; 0.7 m diameter and 1.6 m depth) were filled with 45 μm filtered water pumped from 0 to 1.7 m (photic layer affected by 1% of UVR_{305}), and were incubated in the lake for 4 days. Three of the enclosures received the full spectrum of solar radiation (UVR treatment), and the other three received only the PAR (PAR treatment). In the latter, UVR was excluded by covering the mesocosms with Ultraphan 395 filters (Digefra GmbH). The spectral characteristics of these filters were described by Villafañe et al. (2003). The two light treatments were therefore UVR plus PAR in natural doses (NPAB hereafter), and PAR alone in natural doses (NPAR).

The laboratory experiment aimed to shed light on the effects of increased UVR:PAR ratios on different

Table 1 Mean values of some chemical and biological variables under initial conditions and during experimental periods in La Conceja lake

	T-0 (11 July 2009)	T-1 (12 July 2009)	T-2 (14 July 2009)
DOC (mg C l^{-1})	1.88 ± 0.11	1.75 ± 0.14	3.05 ± 0.94
SRP (μg P l^{-1})	2.02 ± 0.04	1.34 ± 0.27	0.95 ± 0.09
TP (μg P l^{-1})	4.59 ± 0.40	8.49 ± 0.48	7.64 ± 0.12
TN (mg N l^{-1})	14.91 ± 0.04	15.44 ± 0.11	15.75 ± 0.14
NO_3^- (mg l^{-1})	17.94 ± 2.23	12.46 ± 2.86	14.29 ± 1.31
NH_4^+ (mg l^{-1})	0.024 ± 0.01	0.019 ± 0.00	0.089 ± 0.01
Chl *a* Nano (μg l^{-1})	0.93 ± 0.02	0.35 ± 0.01	0.38 ± 0.01
Chl *a* Pico (μg l^{-1})	0.37 ± 0.03	0.43 ± 0.03	0.41 ± 0.01
Chl *a* total (μg l^{-1})	1.30 ± 0.03	0.78 ± 0.02	0.79 ± 0.02

All values are the averages for the water column

DOC dissolved organic carbon, *SRP* soluble reactive phosphorus, *TP* total phosphorus, *TN* total nitrogen, *NO_3* nitrate, *NH_4* ammonia, *Chl a* chlorophyll a of total phytoplankton, picoplankton and nanoplankton fractions

Table 2 Laboratory and field enclosure sub-superficial irradiances (I_0, values for 305, 320, 340 and 380 nm, W m^{-2} nm^{-1}, values for PAR in μE m^{-2} s^{-1}), UVA, UVB and UVR:PAR ratio reaching the community (calculated from $I_0 \cdot I_m$ values in W m^{-2}, following Sterner et al., 1997)

	I_0 305 nm	I_0 320 nm	I_0 340 nm	I_0 380 nm	I_0 PAR	UVA	UVB	UVR:PAR
Field	0.008	0.078	0.157	0.268	1333.6	5.7	0.2	0.03
Laboratory	0.005	0.023	0.052	0.015	71.7	11.9	0.4	0.80

UVA and UVB field irradiances were calculated following Orce & Helbling (1997)

autotrophic size fractions. Sampled water was transported in 50-l containers and cold-preserved during the trip to the laboratory culture-room (3 h). Laboratory experiments started within 24 h from the water sampling. Twelve experimental microcosms (10 l of volume; 0.15 m × 0.30 m × 0.23 m dimensions) were set up under controlled conditions in a culture chamber with a day-night illumination cycle (13 h:11 h) at 20°C; these enclosures were filled to 7 l (0.20 m depth) to allow handling. Six microcosms were filled with lake water filtered through a 45 μm pore size mesh to remove zooplankton (phytoplanktonic community, hereafter). The other six microcosms were used to study the effects only on the autotrophic picoplanktonic community (APP fraction, hereafter) to avoid competitive interaction and potential predation by mixotrophic algae. These microcosms were filled with lake water filtered through a 3 μm pore size mesh at low pressure (<100 mmHg) to avoid cellular damage to filtered cells. Three enclosures of each planktonic size fraction (<45 and <3 μm) received UVR + PAR and three only PAR; they were used to study increased UVR:PAR in relation to lake UVR:PAR ratio. PAR was applied using three fluorescent tubes (PHILIPS L-D 58W/840 day light) that provided enough sub-superficial irradiation (Table 2) to allow gross photosynthesis (Carignan et al., 2000; Rodrigo et al., 2009). For UVR + PAR treatment, PAR irradiance was the same as in the PAR treatment, plus a single Q-Panel 340 fluorescent lamp (Q-Panel Lab, USA) which was mounted between the daylight tubes. Each set-up was coated with black plastic in order to provide a light field as homogenous as possible inside enclosures. The light period was from 8:00 am to 21:00 pm. UVR exposure lasted 5 h daily (13:00–18:00 pm), starting 5 h after the beginning of the first light period. To ensure equal exposure to all treatments, microcosms were exchanged randomly amongst 12 positions twice a day.

Laboratory and field enclosures were sampled at approximately the same intervals: immediately after filling (named T-0), after two UVR periods (T-1) and at the end of the experiment (T-2) after two more UVR periods (2 days later). Aliquots (100 ml) from each enclosure were used to quantify phytoplankton and APP abundances. Lugol-fixed phytoplankton were counted and their biovolume calculated, using an Olympus CK2 inverted microscope at 400× and 1,000× and Utermöhl counting chambers following the methods described in Rott (1981). At least 400 individuals of the more abundant species were counted in each sample with 10% probability of error (95% confidence limit, Lund et al., 1958). APP was immediately fixed with formalin at a final concentration of 1–2%. Samples for APP determination were filtered through 0.2 μm black polycarbonate membrane (Millipore) filters and counted by autofluorescence observation of picoplanktonic cells (Weisse, 1988) with a Nikon epifluorescence microscope at 1,250× magnification and with appropriate filters (Rodrigo et al., 2003). To count and measure the APP cells, we used photographs taken of the preparations; the scale in these pictures was 1 mm equivalent to 0.2 μm of observed object. To research if APP of different sizes responded to treatments equally, APP inhabiting both mesocosms and microcosms was counted in three size categories: up to 1.0 ± 0.2, 1.5 ± 0.2 and 2 ± 0.2 μm. When a pre-visualization was carried out, cells of 2 μm were less than 1% of the total organisms counted in each sample; therefore, we finally considered two size classes: APP < 1 μm (ranged between 0.8 and 1.2 μm) and APP > 1 μm (1.3–2.2 μm); larger cells were not found. APP cell biovolume was calculated by means of appropriated geometric form formula (mainly spherical). To obtain an estimation of phytoplankton's dry weight, the regression equation dry weight (DW) = 0.47 × $V^{0.99}$, where DW units are pg and algal volume (V) in μm^3, was used following Reynolds (1997). For the sake of simplicity, hereinafter, we refer to primary producers larger than 3 μm (observed under inverted microscopy) as 'phytoplankton' and the planktonic fraction smaller than 3 μm (counted by epifluorescence) as 'APP'.

To know inorganic, total and sestonic C, N, P concentrations, samples (2 l) were analysed for inorganic nutrients on the same day as their collection: the first day for the analysis of lake water and the last day of the laboratory experiment in the case of seston. UV spectrophotometric screening was used to determine nitrates (NO_3), phenol-hypochlorite techniques for ammonia and the acid molybdate technique to determine soluble reactive phosphorus (SRP). Samples for TP and TN were potassium persulfate-digested at 120°C for 30 min before their analyses as SRP or nitrate, respectively (American Public Health Association, 1998). DOC values were obtained by filtering through GF/F filters, acidifying them with HCl and oxygenating them to remove particulate organic carbon and inorganic carbon. Samples were then measured in a Shimadzu TOC-V CSH-CSN total organic carbon analyzer (TN P/TOC-V, TNM-1). For sestonic C, N and P determinations, samples were filtered through precombusted (1 h at 550°C) 1-Rm glass fiber filters (Whatman GF/B) at low pressure (<100 mmHg). Filters were then immediately analysed for P or were dried (24 h at 60°C) and kept desiccated until C and N analyses. Particulate C and N were determined using a Perkin-Elmer model 2400 elemental analyzer. For the analysis of particulate P, filters were introduced into acid-washed vials and determined as SRP by the acid molybdate technique. Blanks and standards were carried out for these procedures (American Public Health Association, 1998). All algal ratios were calculated on a molar basis. Nanoplanktonic and picoplanktonic Chl *a* were concentrated by filtration of 2 l of water through 3 μm (Whatman GF/D glass fiber filter, 25 mm diameter) and 0.7 μm (Whatman GF/F glass fiber filter, 25 mm diameter, serial filtration). Chl *a* was measured fluorimetrically by grinding of filters with pigments and keeping them in the dark in 90% acetone at 4°C for 24 h to extract the pigments. A Chl *a* standard (Sigma, Chl *a* from spinach) was used to transform the fluorescence data into Chl *a* concentrations.

Statistical analysis

A two-way ANOVA was made on biovolume of APP inhabiting microcosms at the beginning of the experiment to check initial conditions; the factors were (i) light quality, comparing data from microcosms to PAR and UVR + PAR treatments and (ii) microcosms containing seston smaller than 45 μm or smaller than 3 μm. After this, ANOVA Bonferroni post hoc tests were used to distinguish the mean differences that were statistically significant ($P < 0.05$).

As explained in previous paragraphs, two experiments were made on effect of light quality: once in the lake (NPAR vs. NPAB) and the other in the laboratory (UVR + PAR vs. PAR); results from these two independent experiments were analysed separately. Experiments had a similar design: light quality had two levels (treatments), three replicates (enclosures) were used for each treatment and three consecutive times were considered (T-0, T-1 and T-2) to measure variables. In these analyses, we tested the effect of incubation time, light quality and light by time interaction. Dependent variables were picoplankton density; biovolume of picoplankton, phytoplankton and some main populations; and stoichiometric composition. Because we know that it was insufficient residual degrees of freedom to produce multivariate test statistics including repeated measures, we analyse each variable (univariate) by means of a more simple and powerful repeated measures model treated as a mixed model (split-plot design) with enclosures considered as random effect. This random effect nested with light allows testing the light quality over time. Mauchly's (1940) sphericity test was used to validate the sphericity assumption of the repeated measures ANOVA. This assumption states the equality of the variances of the differences between levels of the repeated measures factor. Tukey's post hoc test was used to highlight the doses required to achieve some significant changes in the variable through multiple comparisons (times).

Normality of variable distribution was checked by the Kolmogorov–Smirnov test and homoscedasticity by Levene's test. When the data did not conform to these assumptions, they were log-transformed and then, indicated in the results tables. All analyses were performed by means of the SPSS 19.0 statistical package (www.spss.com).

Results

La Conceja lake water conditions for the experiments

Measurements and analyses of La Conceja lake water sampled from the surface to 10 m depth (water

column) showed that temperature ranged between 21.7 and 15.1°C. The K_d were 0.49 m^{-1} for PAR; and 0.63 m^{-1} for 380 nm, 1.71 m^{-1} for 380 nm, 2.63 m^{-1} for 320 nm and 4.74 m^{-1} for 305 nm; and sub-superficial UVR:PAR irradiance ratio was 0.03 (Table 2). It is a calcium bicarbonate-rich system; average DOC concentration was 1.88 mg C l^{-1} in the water column the first day of experiment (Table 1) and conductivity ranged between 890 and 840 μS/cm. Total nitrogen in natural sampled water filling the enclosures was 14.91 ± 0.04 (mg N l^{-1}) and total phosphorus was 4.59 ± 0.40 (μg P l^{-1}). Abundance distribution of plankton inhabiting the lake water column sampled to start experiences was: APP density 3.4×10^4 cells ml^{-1} (biovolume: 0.03 mm^3 l^{-1}); the rest of the phytoplankton sampled from the water column was 3.2×10^2 cells ml^{-1} (0.06 mm^3 l^{-1}) being *Cyclotella comensis* Grunow (7–9 μm diameter) the 8% of total biovolume (94% of density) and *Peridinium umbonatum* F. Stein (12–15 μm maxima long) the 58% of total biovolume (2% of density); longest primary producers were *Cryptomonas erosa* Ehrenberg (24 μm long) and the smallest *Scenedesmus ecornis* (Ehrenberg) Chodat or *Choricystis* sp. (3 μm long). Ciliates, which were small (<20 μm long), reached a maximum 0.01% of total density. In the water column, the smallest zooplankters were the mentioned ciliates, the rotifers *Ascomorpha ovalis* Bergendal (50 μm maximum length) and nauplii (76 μm maximum length). Therefore, almost all the observed zooplankters could be retained by the 45-μm mesh and all phytoplankton organisms went through the mesh; then, we considered this latter as the natural phytoplankton assemblage for the laboratory experiments.

APP and phytoplankton response to UVR in the field experiment

Phytoplankton grew in mesocosms during the experiment under the two light conditions or treatments (Fig. 1A) without significant differences between them ($P = 0.421$). The time factor (days) explains changes in both total phytoplankton biovolume ($F = 82.26$; df = 2,8; $P < 0.001$) and *Cyclotella meneghiniana* Kützing biovolume ($F = 166.298$; df = 2,8; $P < 0.001$), but not in *P. umbonatum* biovolume ($F = 2.88$; df = 2,8; $P = 0.114$). However, not a significant interactive effect, time × UVR was found. APP biovolume did not suffer the time × UVR interactive effect; however, analysing day to day, APP < 1 biovolume was significantly lower in NPAB than in NPAR conditions for first sampling time, after 2 days ($F = 12.46$; df = 1,4; $P = 0.024$), although no variation between light treatments was observed 2 days later (Fig. 1B).

APP response in laboratory to changes in UVR:PAR: abundance and structure

Differences in APP biovolume at T-0, T-1 and T-2 were analysed separately in both sets of microcosms: filled only with <3 μm-fraction and filled with <45 μm-fraction (Fig. 2A, B). Nevertheless, the same APP response was observed in both sets of microcosms: Differences between light treatments were significantly related to exposure time (Table 3). In PAR conditions, APP biovolume increased significantly between T-0 and T-2; in UVR + PAR conditions there were no time course changes in APP biovolume (Fig. 2A, B).

UVR + PAR effect was different for each size class of APP (Fig. 3; Table 3). Thus, interactive effect light quality × time only affected the density of the smallest APP (up to 1 μm), which decreased during the last UVR dose. However, for larger APP, interaction between factors was not significant; only the growth between T-0 and T-2 under PAR was statistically proven (Table 3), and there were no differences in density over time in UVR + PAR treatment.

Phytoplankton response to changes in UVR:PAR in the laboratory: abundance, structure and stoichiometry

Firstly, the slight difference in biovolume between treatments observed at time 0 (Fig. 4) is because of the distribution of frequency of biovolume measured in six microcosms filled with water from the same container: The lowest concentration occurred in one of the three microcosms for the PAR treatment and the higher value of the distribution was obtained in one of the three microcosms prepared for UVR + PAR treatment. Despite that, the initial concentration is homogeneous since no statistically significant differences between these averages were found.

Unlike APP, the phytoplankton grew over time even in UVR + PAR conditions. The average biovolume of phytoplankton increased over time, becoming

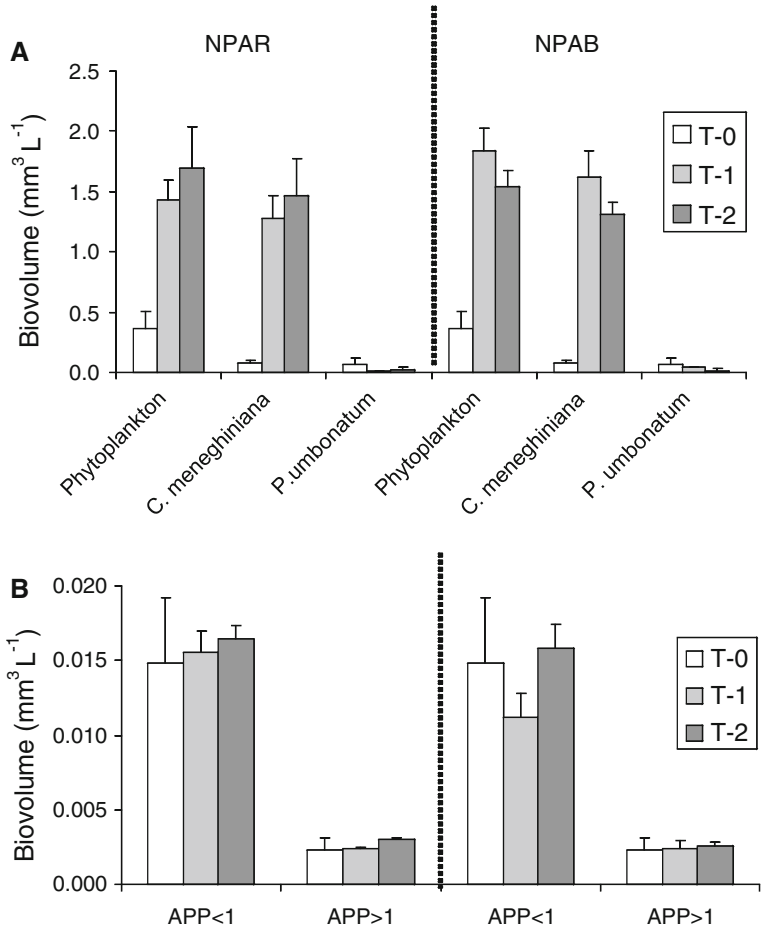

Fig. 1 Average and standard deviation of phytoplankton, *Cyclotella meneghiniana* and *Peridinium umbonatum* (**A**), APP equal to or lower than 1 µm and APP higher than 1 µm biovolume (**B**). Experiment in mesocosms placed in La Conceja lake under two ambient light quality conditions: photosynthetically active radiation (NPAR) and full sunlight (NPAB) doses (explained in text) observed at three consecutive times T-0: initial, T-1: after 2 days and T-2: 2 days later

higher in each sampled period (Fig. 4). Evidently, a synergistic effect existed between time and light quality (Table 3); time factor implies more UVR doses, and phytoplankton grew less only when subjected to more UVR episodes with the phytoplankton biovolume under UVR + PAR at T-2 being 67% which corresponds to PAR. This difference is partially due to APP fraction in phytoplankton: %APP biovolume in total phytoplankton varied from 50 ± 8% at the beginning to 37 ± 9 and 25 ± 2% at T-2 under PAR and UVR + PAR conditions, respectively. Analysing time course differences (Table 3) for each light treatment separately, it was observed that biovolume differed amongst sampling times under PAR conditions, and under UVR + PAR treatment the biovolume did not change from T-0 to T-1 and only T-2 differed.

Distribution of algal biovolume varied due to the different behaviour of dominant taxa (Fig. 4) throughout the experiment and under different light quality (Table 3). Thus, *P. umbonatum* grew significantly during the experiment reaching 81% of total phytoplankton biovolume under PAR and 65% under UVR + PAR, and differences occurred between light quality treatments only with the time course (significant interaction of factors; Table 3); in PAR condition time course changes were significant from the beginning; however, under UVR + PAR there were no differences in biovolume over time (Table 3). *C. meneghiniana* biovolume was statistically different for the interaction between time and light quality (Table 3); in PAR, biovolume growth from T-0 was statistically different amongst all three sampling times; in UVR + PAR, despite the first growth, no difference can be attributed to time. From the beginning to the end of the experiment, the abundance change rate of *C. comensis* was 200% in PAR treatment and −48% under UVR + PAR. This

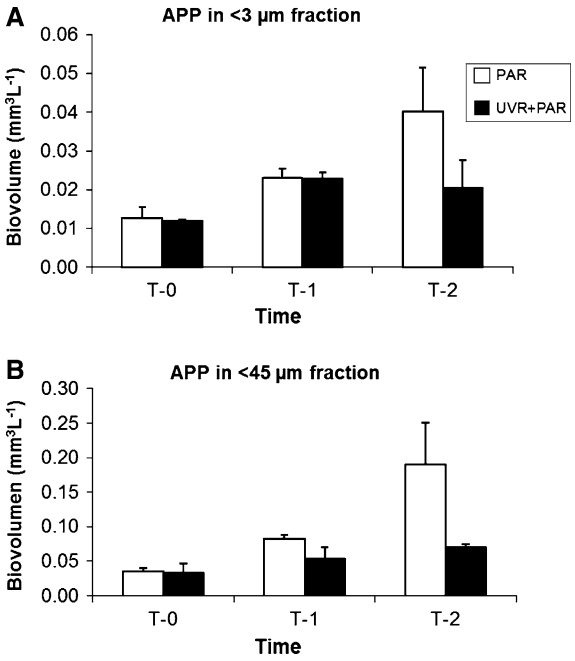

Fig. 2 Average and standard deviation of APP biovolume in microcosms filled with <3 µm (**A**) or <45 µm (**B**) size fraction plankton of under two light quality conditions in laboratory (PAR and UVR + PAR) at three consecutive times during the experiment (as in Fig. 1)

population was also affected by light quality over time, while its biovolume did not vary over time under UVR + PAR treatment (Table 3; Fig. 4).

Seston smaller than 45 µm showed a stoichiometric response to UVR + PAR (Fig. 5): at the end of the experiment, mol P/g DW of seston and percentage of phosphorus were almost double in seston grown under UVR + PAR conditions (df = 1,4; $F = 26.38$, $P = 0.007$). Consequently, molar ratios related to phosphorous were lower in phytoplankton cultivated in UVR + PAR (Fig. 5). C:P and N:P molar ratios were 167 ± 40 and 24 ± 8, respectively, in PAR treatment, and these molar ratios were significantly lower under UVR + PAR where C:P ratio was 92 ± 5 and N:P ratio was 13 ± 1 (df = 1,4; $F = 16.56$, $P = 0.015$ and $F = 8.15$, $P = 0.045$ respectively).

Discussion

The phytoplankton biomass in La Conceja lake was low and the size structure was dominated by pico and nanophytoplankton, as expected in nutrient-limited ecosystems (Marañón, 2009). APP represents half of the total phytoplankton biomass in La Conceja lake. This is in accordance with the model proposed by Stockner (1991) and later enriched with other data (Bell & Kalff, 2001; Callieri, 2008). Previous studies have described the APP in this lake, mainly represented by phycocyanin-rich chroococcoids (Rodrigo et al., 2003), likely the same as *Synechococcus* sp. now found in this study. In general, the greatest relative contribution of APP to the food web is caused by (i) the selective grazing of the crustacean-dominated zooplankton community on larger eukaryotic cells and (ii) the higher growth efficiency of APP in nutrient-depleted environments (Raven, 1998; Hudson et al., 2000). In this sense, populations of picocyanobacteria have a high affinity for orthophosphate and higher uptake rates than eukaryotic algae, which would explain their relative abundance in nutrient-depleted environments (Callieri et al., 2007; Winder, 2009). But, we could add another advantage for this community of small autotrophic organisms: in La Conceja P-limited lake, as we demonstrate, this small phytoplankton which inhabits in low UVR:PAR natural conditions does not improve its growth when UV radiation is artificially eliminated; thus, we can infer that the low UVR doses in that ecosystem do not damage these primary producers and, therefore, they do not have any cost in metabolic mechanisms related to that.

However, when UVR and UVR:PAR were increased substantially in the laboratory, phytoplankton responded, APP being significantly more affected than nanophytoplankton in the studied community. This difference was probably due to a higher sensitivity to UVR-induced damage on the smaller body size cells (cyanobacteria) rather than the larger ones (García-Pichel & Castenholz, 1993). The sensitivity of phytoplankton to UVR is known to vary interspecifically and with organism size (Leech & Williamson, 2000; Sommaruga & Buma, 2000; Callieri et al., 2001); even within a single population, differences in vulnerability due to the size could be found because very small cells have less volume available for accumulations of new biochemical components, as proposed by Marañón (2009) to explain their lower growth rate than expected in accordance to their size, and then could not dispose photoprotective pigments or remedial substances to be insensitive to UVR (Sereda et al., 2011).

Table 3 Results of univariate test (mixed model with enclosures as random effect nested with light) of APP biovolume in both sets of microcosms (with and without nanoplankton), and abundance of APP size fractions, total phytoplankton and main population biovolumes measured at time 0, 1 and 2 in microcosms in PAR and UVR + PAR treatments

	df	Biovolume				Abundance				Biovolume							
		APP in <3 μm fraction		APP in <45 μm fraction		APP < 1 μm		APP > 1 μm		Phytoplankton		*P. umbonatum*		*C. meneghiniana*		*C. comensis*	
		F	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Light quality	1,4	11.7	**0.027**	20.3	**0.011**	5.1	0.089	6.2	0.068	1.7	0.341	0.0	0.839	0.1	0.759	7.8	0.050
Time elapsed	2,8	12.2	**0.004**	18.4	**0.001**	2.4	0.153	8.7	**0.010**	123.7	**0.000**	97.0	**0.000**	14.2	**0.002**	9.2	**0.008**
Light × Time	2,8	4.6	**0.047**	7.5	**0.015**	9.4	**0.008**	2.8	0.121	18.5	**0.001**	0.0	**0.040**	4.5	**0.049**	25.7	**0.000**
Time (PAR)	2,4	9.1	**0.032**	13.5	**0.017**			13.5	**0.017**	90.3	**0.000**	2252.1	**0.000**	42.5	**0.002**	24.6	**0.006**
T-0/T-2			**0.030**		**0.016**				**0.014**		**0.000**		**0.000**		**0.003**		**0.006**
T-0/T-1											**0.037**		**0.001**		**0.003**		
T-1/T-2											**0.002**		**0.000**				**0.012**
Time (UVR + PAR)	2,4			7.8	**0.042**	7.8	**0.043**			34.1	**0.003**	16.1	**0.012**				
T-0/T-2					**0.037**		**0.037**				**0.003**		**0.019**				
T-1/T-2											**0.010**		**0.016**				

Univariate analysis of the same variables for each light treatment was separately made to test time effect under PAR and under UVR + PAR. Tukeys' post hoc test shows significant differences between incubation times. Calculated statistic *F*-value (*F*) and significance level or critical *P* value (*P*) and degrees of freedom (df) are reported; numbers in bold font mean statistical significant differences

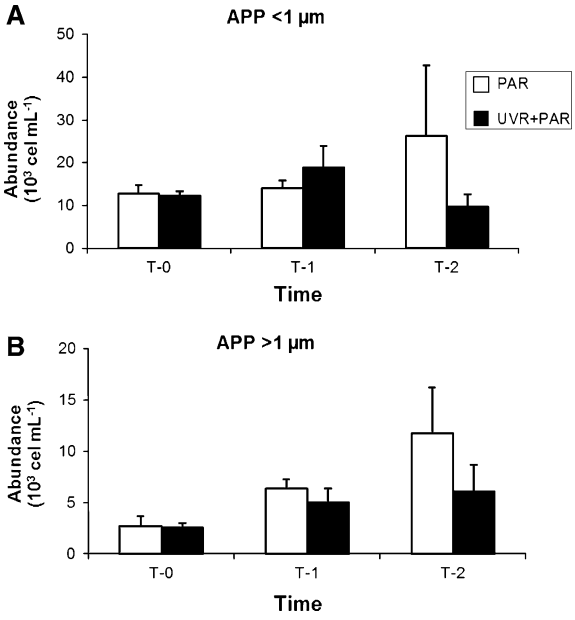

Fig. 3 Density average and standard deviation of two APP cell sizes in microcosms filled with <3 µm size fraction plankton under two light quality conditions in laboratory (PAR and UVR + PAR) at three consecutive times (as in Fig. 1)

Picocyanobacteria are considered more sensitive to UVR than other algal groups in nanoplankton (Quesada et al., 1995; Bertoni & Callieri, 1999), and their different response to UVR can depend on the geographical situation or environmental conditions (see examples in Bertoni & Callieri, 1999; Callieri et al., 2001; Xenopoulos & Frost, 2003). In this study of a mid altitude and low latitude lake, when the existing plankton were exposed to UVR addition, not only small cyanobacteria (picocyanobacteria) were more affected than nanoplankton but also, the three dominant populations of nanoplanktonic algae exhibited different behaviour: the smallest ones being the most affected by an increase in UVR (*C. comensis*) which reduce their population density; while *C. meneghiana*, that is larger in size, stopped growing after UVR dose; and *P. umbonatum*, although it grew under UVR, it did it less than without this radiation. The latter case could be explained by the ability of *Peridinium* to repair damage, demonstrated when it is under alternate UVR supply (Hayhome et al., 1981). Then, not only did the dominance of one or another microalgae population change due to the differential sensitivity of algal taxa to UVR (Xenopoulos & Frost, 2003), but the functional structure responded strongly too. In particular, both the

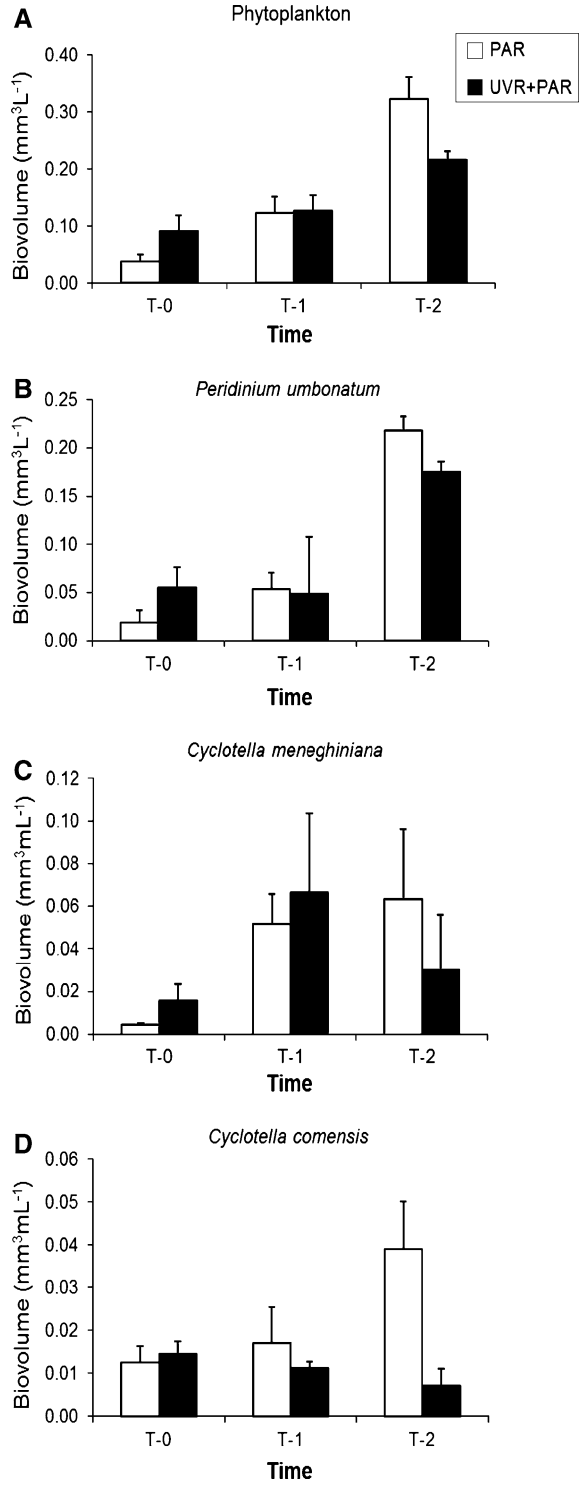

Fig. 4 Average and standard deviation of total phytoplankton and three dominant algal biovolume under two light quality conditions in the laboratory experiment (PAR and UVR + PAR) at three consecutive times (as in Fig. 1)

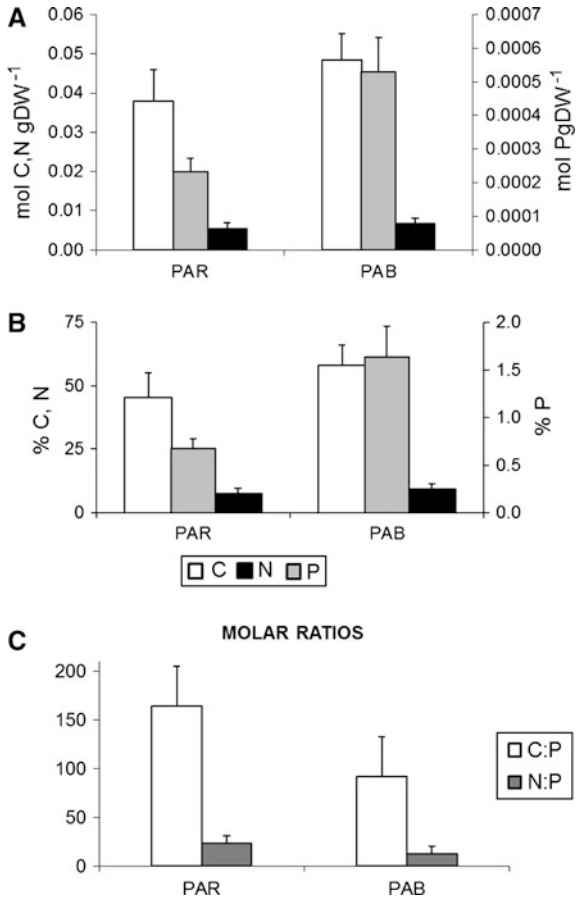

Fig. 5 **A** Average and standard deviation of molar C, N and P; **B** percentage of carbon (%C), nitrogen (%N) and phosphorus (%P) and **C** molar ratios in seston sampled in the microcosm after four doses of UVR at the end of experiment under two light quality conditions (PAR and UVR + PAR)

picoplankton and phytoplankton biomasses decreased and there was an increase of larger motile cells in the nanoplankton, in this case a prevalence of a mixotrophic dinoflagellate population.

According to currently available data, algal stoichiometry and its variability seem to depend on environmental conditions (Klausmeier et al., 2008); therefore, the deviation of stoichiometry phytoplankton assemblages from the Redfield ratio (Klausmeier et al., 2008) should reflect this (Litchman & Klausmeier, 2008) and, moreover, it should be more common in lakes than in oceans (Wetzel, 2001). It is in this context that the modification of the C:N:P ratio under an increase of UVR:PAR ratio is interesting. In the most similar to natural conditions (without a supply of UVR), the phytoplankton from La Conceja lake showed a C:N:P ratio higher than the reference molar ratio of 106C:16N:1P (Redfield, 1958, Reynolds, 1997), as was expected in water bodies of greater available light, P-limited (Sterner et al., 1997; Wetzel, 2001), rich in nitrogen and dominated by *Peridinium* with polysaccharides in theca (Popovsky & Préster, 1990; Menden-Deuer & Lessard, 2000). However, the resulting composition under the UVR + PAR acquired further P and the percentage of phosphorus in phytoplankton increased, reducing C:P and N:P ratios in a previously described trend (Xenopoulos et al., 2002; Carrillo et al., 2008; Klausmeier et al., 2008) and improving their quality as food for herbivores (Sterner et al., 1997; Villar-Argaiz et al., 2001, 2009).

Therefore, with an increased UVR:PAR ratio, the changes in phytoplankton assemblage involve a reduction of picophytoplankton, the most efficient P-uptaker in a P-limited ecosystem (Hudson et al., 2000), and concomitantly less carbon flows towards the microbial loop (Berman-Frank & Dubinsky, 1999). At the same time, in the nanoplanktonic fraction, a relative prevalence of *P. umbonatum*, a mixotrophic alga (Popovsky & Préster, 1990), occurred. This alga with mixotrophy as a nutritional advantageous strategy under low nutrient conditions contributes to the C-flux to the food web (Medina-Sánchez et al., 2004, 2006). However, with a higher relative UVR incidence, and, as suggested before, a decrease of picoplankton and then a weakening of microbial loop, *P. umbonatum* persistence and its phosphorous needs (Laybourn-Parry et al., 2005; Litchman & Klausmeier, 2008) would be only possible thanks to the photosynthetic metabolism and, at least in short term, to the P released from photolysis of DOM induced by UVR (Xenopoulos et al., 2002). Sereda et al. (2011) suggest that competition for acquisition of P in limnetic systems exposed to high levels of UVR may be a driving force structuring plankton assemblages to UVR tolerant, larger cells or a P-uptake system insensitive to UVR. In this sense, we might propose alternative states related to the algae that are more resistant to UVR:PAR changes, and in a P-limited system this alternative can be specific in a heterotrophic state based on microbial loop and mixotrophy and a phototrophic state based on phosphorus from released DOM. *Peridinium* organisms, mixotrophs that were also the best at resisting UVR amongst nanoplankton, would be the best adapted to alternating between these two situations.

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PHYTOPLANKTON

Sedimentation of phytoplankton: role of ambient conditions and life strategies of algae

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Abstract Pigment content in particles accumulated in sediment traps are often not directly correlated with phytoplankton abundance, but are rather indicative of transformations phytoplankton underwent on its downward move and following resuspension. We argue that the variability in temporal and spatial sedimentation patterns of different phytoplankton groups is not only an outcome of pigment persistence, but is also associated with dissimilarity in life strategies and dependent on the physical conditions of the water column. Pigment concentrations were measured on weekly–biweekly basis in the water column and in five sets of traps positioned in Lake Kinneret, Israel. Highly degradable peridinin and chlorophyll *c* reached the deep traps in minute quantities indicating that dinoflagellates mostly recycled in the epilimnion; these migrating algae dominated plankton community under low turbulence and high light. When fast-sinking diatoms persisted in the water column during holomixis they could reach the bottom intact, and fucoxanthin was found in equal proportions in water and traps, chlorophytes rarely dominated phytoplankton, but lutein and chlorophyll *b* harbored by this group were often the most abundant signature pigments in traps, reflecting the effect of high accumulation rates of these stable compounds in resuspensed particles from the bottom.

Keywords Sedimentation · Photosynthetic pigments · Phytoplankton · Decomposition · Resuspension · Material transport

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Introduction

Sinking phytoplankton is a major component in the downward flux of organic particles in aquatic ecosystems. The domination of algae in the sedimented particles is conspicuously manifested when algal blooms collapse (Graf et al., 1982). Organic material originating from different phytoplankton phyla is deposited to the bottom at various rates, which depend on the composition of the settling material: intact cells, cell debris, and fecal pellets produced by phytoplankton grazing. Despite the fairly uniform horizontal distributions of phytoplankton biomass in small and medium sized lakes, spatial variability of algal related organic matter in the topmost layer of bottom sediments can be high (Ostrovsky & Yacobi, 1999; Yacobi & Ostrovsky, 2000; Bloesch, 2004), indicating

that the deposited material is translocated laterally by resuspension-transportation processes. During holomixis, particles suspended in the water column are entrained by currents and large-scale water circulations (e.g., gyres) and deposited onto the bottom in inverse proportion to the near-bottom shear stress (Bloesh, 1995). When the water body is stratified and the water layers below the thermocline are largely detached from the direct impact of wind stress on the water surface, the internal seiching and water motions in the benthic boundary layer (BBL) produce highly heterogeneous fields of turbulence, near-bottom shear stress, and a complex system of currents in the deep layers. These contribute to the relocation of particles in the BBL and the uppermost sediment layer (Frechette et al., 1989). Although the dynamics of BBLs have been intensively studied during the last decade (e.g., Wüest & Lorke, 2003), the effect of boundary processes in basin-scale fluxes of particulate material is still not fully understood. The fate of deposited and resuspended phytoplankton may be traced using various cellular components, such as diatom frustules (Batterbee et al., 2003), chrysophyte scales (Zeeb & Smol, 2003) or biochemical markers, including sterols (Volkman, 1986) and pigments (Leavitt & Hodgson, 2003). It has been shown that the quantitative relationships between various photosynthetic pigments in the sedimented material are often different from the relationships found in the water column due to their different rate of decomposition/preservation (Louda et al., 2002; Yacobi & Ostrovsky, 2008); therefore, these pigments can be useful biomarkers for tracing diagenetic processing of algal detritus. In our previous study on Lake Kinneret (Ostrovsky & Yacobi, 2010), we showed that the dynamics of chlorophyll *a* (Chl *a*) and *β*-carotene (proxies of phytoplankton biomass) collected in hypolimnetic traps reflected the composition and abundance of phytoplankton in the upper mixed stratum and that traps deployed at the lake periphery and in the BBL notably overestimated the export flux of newly produced particulate organic material. This study is focused on the analysis of photopigments specific for different algal phyla. Here, we examine the dynamics and composition of various phyla-specific pigments in the water column and sedimentation traps to quantify the lateral changes of their sedimentation rates and to better understand the fate of different algal groups in a deep lake.

Methods

Spatial variability of sedimentation fluxes was studied from 2005 to 2009 using five sets of sedimentation traps, which were deployed at three stations (Stn.) positioned along an offshore transect stretched from the northwestern shore of Lake Kinneret (Israel) to its center (Fig. 1). This transect was positioned far away from the Jordan River inlet zone, where most of the particle load is deposited (Serruya, 1974; Markel et al., 1994). Trap locations were chosen to represent the littoral (Stn. *M*, 12 m depth), sublittoral (Stn. *F*, 22 m depth), and pelagic areas (Stn. *A*, 40 m depth) of the lake. At each station, a set of "lower" traps was positioned near the bottom ($\sim$2.5 m above the bottom at Stns. *A* and *F*, and $\sim$1.5 m above the bottom at Stn. *M*) and designated as A_{low}, F_{low}, and M_{low}, accordingly. At the two deeper stations, an additional set of traps was deployed above the BBL evaluated by Lemckert et al. (2004), based on turbulence profiles. In Stn. *F*, the "upper" traps were deployed 3 m above F_{low}, and in Stn. *A*, they were deployed 9 m above A_{low}, and were designated as F_{up} and A_{up}, respectively. Thus, the combination of "upper" and "lower" traps allowed assessing the trap performance under different turbulent conditions. Chemical and physical regimes at trap locations altered seasonally. At the deepest station, traps were in the anoxic hypolimnion from June to November (A_{up}) or December (A_{low}). Sedimentation traps at Stn. *F* were exposed to anoxic conditions from July to September–October, assuming an unvarying level of seasonal thermocline. However, in the summer strong internal seiching affected the peripheral parts of the lake, in such a way that large diurnal oscillations of the metalimnion could periodically expose these traps to the metalimnion and since early fall even to the epilimnion. The shallowest traps (M_{low}) were located in the permanently oxygenated epilimnion.

Each trap set consisted of four plastic cylinders (inner diameter 5 cm, height 50 cm). For a detailed description of the traps and their set up see Koren & Klein (2000). Traps were deployed usually for 1–2 weeks. The four sub-samples of collected material in each trap were pooled before further analysis. Particulate material accumulated in the traps was collected onto GF/C filters and stored in the dark at −18°C. Pigment extraction and analysis of the collected material from water column and traps

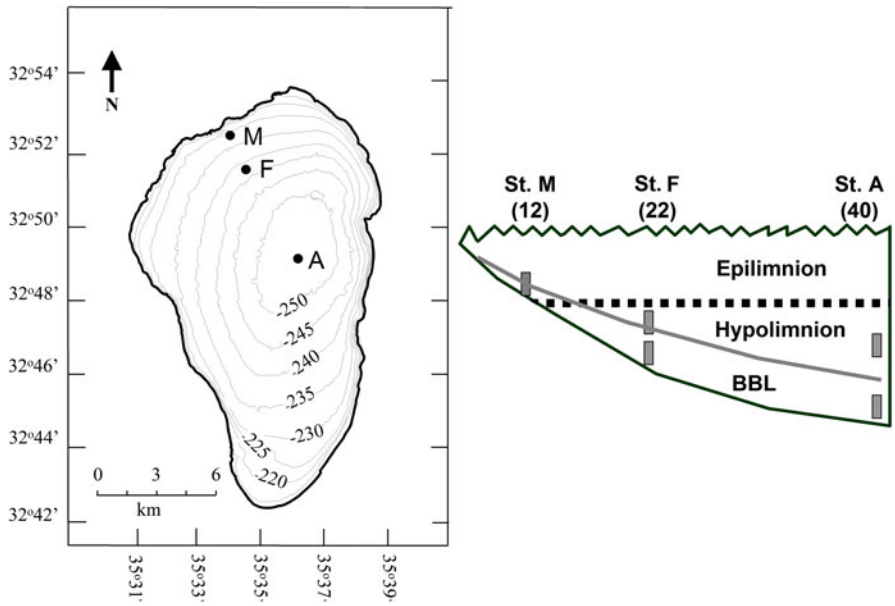

Fig. 1 Locations and scheme of trap deployment. *Left:* map of Lake Kinneret with locations of trap deployments and 5-m isobaths. *Right*: scheme of traps deployment relatively to the summer thermocline (*dashed line*). At Stns. *A* and *F* the "low" traps were deployed ~2.5 m above the bottom, and the "up" traps were positioned 9 and 3 m above the "low" traps, respectively. At Stn. *M* only a "low" traps were deployed ~1.5 m above the bottom. Average water depth at stations is shown in parentheses

followed the protocol described by Yacobi & Ostrovsky (2008, and citations therein). Particulate material collected on filters was processed within 1 week, following collection; frozen filters were ground in 3 ml of cold 90% acetone; an additional 3 ml of acetone were used to flush leftovers, and the pooled extract was left overnight in the dark at 4°C. Subsequently, the acetone extract was filtered through a GF/F filter and separated by a reverse-phase HPLC. Pigments were identified on the basis of the retention times and the absorption spectrum following isolation and spectrophotometric examination. The chlorophyll *c* (Chl *c*) isolated from lake samples showed absorption peaks at 448 and 634 nm (in HPLC eluant) suggesting a mixture of chlorophyll c_1 and chlorophyll c_2 (Jeffrey et al., 1997). For simplicity sake, we refer to that pigment Chl *c*. The quantification of the chromatograms was facilitated by injection of standards of known concentrations into the HPLC system, and calculating the response factor based on the area under the peak. In the system used, lutein and zeaxanthin were not separated, and we report this peak as if it was lutein (see "Discussion").

Water column samples were drawn from a depth of 1 m with a 5 l Aberg-Rodhe sampler at Stn. *A* (Fig. 1). A subsample of 1–2 l was stored in opaque plastic carboys until processing in the laboratory approximately 1 h after collection, where duplicate subsamples of several hundred milliliters were filtered onto GF/C filters and stored in the dark at −18°C. From 2006 to 2009 pigments in the water samples were examined with HPLC using the same protocol as described above for the analysis of the trap material. Only in 2005 we used the fluorometric method to measure Chl *a* concentrations in water.

The flux of organic particles measured with trap positioned in the middle quiescent part of the hypolimnion (A_{up}) provided the best possible estimate of particulate material export from the euphotic zone during stratification (Ostrovsky & Yacobi, 2010). The fate of various pigments in the water column during different periods of time was studied by computation of trap-to-water index (TWI), as follows:

$$\mathrm{TWI} = \frac{\mathrm{PI}_{\mathrm{trap}}}{\mathrm{PI}_{\mathrm{water}}} \tag{1}$$

$$\mathrm{PI}_{\mathrm{trap}} = \frac{F_i}{F_{\mathrm{Chl}}} \tag{2}$$

$$\mathrm{PI}_{\mathrm{water}} = \frac{C_i}{C_{\mathrm{Chl}}} \tag{3}$$

where F_i, F_{Chl} are the fluxes of the *i*th pigment and Chl *a* in A_{up}, respectively; C_i, C_{Chl} are the concentrations of the *i*th pigment and Chl *a* in the epilimnetic water, respectively $\mathrm{PI}_{\mathrm{trap}}$ and $\mathrm{PI}_{\mathrm{water}}$ are pigment indices (ratios) in the trap A_{up} and in the epilimnetic water, respectively. A TWI = 1 indicates that the proportion

of sedimenting pigments is identical to that in the euphotic layer.

Seasonal trends of pigment sedimentation rates were compared by averaging the rates during three periods (Fig. 2): (1) holomixis (January–March) characterized by well-mixed water column of low temperature; (2) a period of strong stratification (April–September), when the upper well-mixed productive stratum is separated from the nutrient-rich hypolimnion by strong thermocline, and (3) a period of fast reclining of the thermocline (October–December), characterizing by fast expending of the upper mixed layer. Further, we investigated the difference in sedimentation rates between various traps during the entire stratified period (April–December).

Sedimentation rates at A_{up} were used to elucidate the relative performances of other traps by calculating the relative difference (F) in sedimentation flux for each pigment comparatively to that measured in A_{up}, as follows:

$$F_{pig} = 100 \times \left(T_x - A_{up}\right)/T_x, \% \quad (4)$$

where F_{pig} is the relative flux difference, T_x the sedimentation flux of a given pigment measured with trap x, and A_{up} is the sedimentation flux of a given pigment measured with trap A_{up}.

Correlation analyses were used to reveal whether the sedimentation rates in different traps co-vary and to quantify the strength of these relationships. Since normal distribution could not be assumed, the correlation analyses were performed using Spearman rank correlation. For the same reason, we used the Mann–Whitney test to examine whether the calculated monthly averaged TWI were different from the hypothetical 1. Statistical analyses were performed using the statistics module of SigmaPlot 11 software.

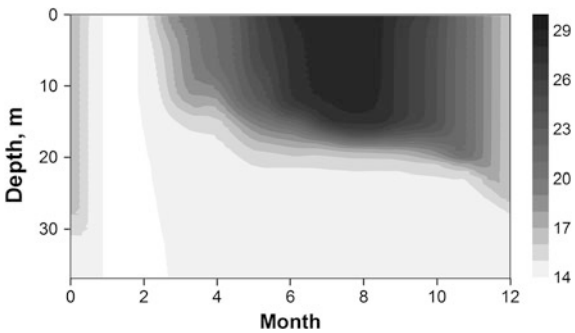

Fig. 2 Typical seasonal dynamic of temperature in the water column of Lake Kinneret, based on measurements on Stn. *A* in 2008

Results

The temporal dynamics of pigment sedimentation fluxes measured in traps deployed in the lower parts of the water column was often different from the standing stock dynamics of pigments in the upper, productive water layers. The comparison of phyla-specific (signature) pigments averages in water and traps (Fig. 3) indicated that the composition of pigments altered on the way from the upper productive stratum toward the bottom. As indicated by TWI, the ratio of peridinin and Chl *c* to Chl *a* in the traps were significantly lower than in those of the water column. In contrast, the ratio of chlorophyte signature pigments (lutein and Chl *b*) and diatom signature pigment (fucoxanthin) to Chl *a* were higher in the traps than in the water. Temporal partition showed a conspicuous difference in these relationships (Table 1). During holomixis and stratification, correlation coefficient between Chl *c* and peridinin (dinoflagellate signatures) and correlation coefficient between Chl *b* and lutein (chlorophyte signatures) were very high ($\sim$0.8–0.9), both in water and in A_{up}. The correlation coefficient of the pair Chl *c*–fucoxanthin was relatively low in water, and only in traps it was almost as high as for the other mentioned pigments. During the period of thermocline reclining, however, correlations between various pigments were usually notable lower than that for periods of holomixis and stratification, and particularly in the case of the pair of Chl *c*–peridinin.

Figure 4 shows the following regularities: (a) the sedimentation rate of pigments in A_{low} was 1.5–3 times higher than in A_{up} and the largest dissimilarity between A_{low} and A_{up} was observed for Chl *c* and peridinin during the period of holomixis; (b) the difference in pigment accumulation between the upper and lower traps at Stn. *F* was low and in all cases insignificant; (c) accumulation of the pigments, which can be used as proxy for total algal biomass (Chl *a* and β-carotene), and the pigments used as a proxy for chlorophytes (Chl *b* and lutein) were at F_{up} and F_{low} higher than at A_{up}; the largest difference was observed during period of thermocline reclining.

Pair-wise comparison of the sedimentation rates of all pigments in different traps showed a consistently

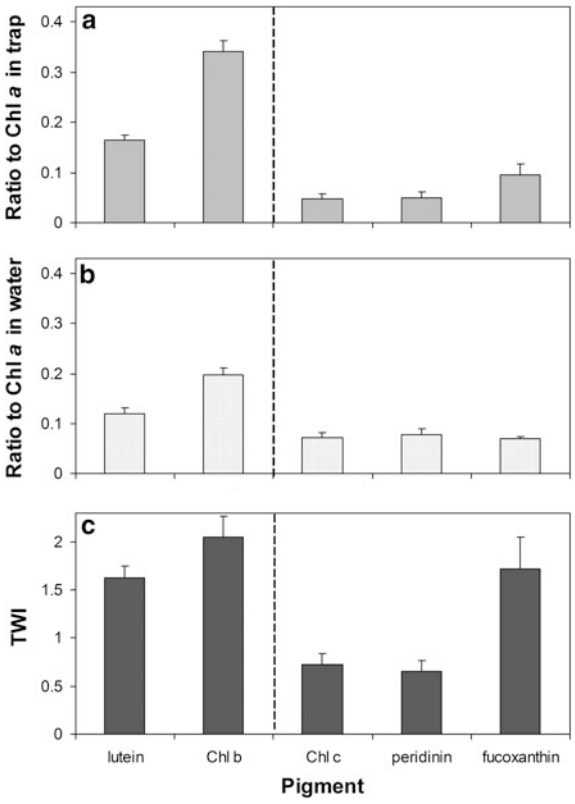

Fig. 3 Ratios of signature pigments of chlorophytes (Chl *b*, lutein), dinoflagellates (Chl *c*, peridinin), and diatoms (Chl *c*, fucoxanthin) to Chl *a* in: **a** water and **b** sedimentation traps A_{up} (in the middle of the lake). **c** Trap-to-water index (TWI). TWI was calculated using Eq. 1 (see "Methods"). Multiannual (2006–2009) averages are shown with standard errors

Table 1 Correlation coefficient (*r*) between concentrations of different pigments in water and in A_{up} during three periods: holomixis (January–March), stratification (April–September), and thermocline reclining (October–December)

Period	Peridinin vs. Chl *c*		Fucoxanthin vs. Chl *c*		Lutein vs. Chl *b*	
	Water	A_{up}	Water	A_{up}	Water	A_{up}
Holomixis	0.86	0.82	0.60	0.89	0.80	0.93
Stratification	0.90	0.80	0.43	0.78	0.89	0.82
Thermocline reclining	0.36	0.46	0.52	0.74	0.63	0.89

positive *r* coefficient, and in most cases with $P < 0.001$ (Table 2). These rather high correlations reflected coherent dynamics of pigments sedimentation rates in different locations. The correlation coefficients between pigment fluxes measured with A_{up} (used as a reference in this study) versus the fluxes measured in deep locations (A_{low}, F_{up}, F_{low}) were higher than the correlations between A_{low} versus F_{up} and F_{low}. The weakest correlations were found between traps in the deep Stns. *A* and *F* and traps in the littoral Stn. *M*.

The temporal pattern of sedimentation rates measured with the upper and the lower traps in Stns. *A* and *F* were similar in all pigments, as displayed by the sedimentation rates of Chl *a*, Chl *b*, and peridinin (Fig. 5). In Stn. *A* the lower traps collected more material almost consistently. The same was observed in most cases in Stn. *F*, but the difference between lower and upper traps was much lower than in Stn. *A*. Comparison of the averages at different stations, showed pigment-specific seasonal patterns. In the case of Chl *a* or Chl *b*, sedimentation rates were similar during holomixis and throughout the strong thermal stratification period, but conspicuously higher in Stn. *F* than in Stn. *A* from August or September throughout December, both in the upper and lower traps. Peridinin (Fig. 5), Chl *c* and fucoxanthin (data not shown) did not display defined differences in sedimentation rates between upper and lower traps and between rates at different locations compared with Chl *a*, Chl *b* (Fig. 5), and lutein (data not shown).

Differences in the average sedimentation rates measured with traps separated horizontally and vertically are shown on Fig. 6. The differences in the period when the lake was stratified were prominent for the pigments characterizing the total phytoplankton biomass (Chl *a* and *β*-carotene) and signature pigments of chlorophytes (Chl *b* and lutein). The differences were nearly pronounced equally in distant locations, and in upper and lower traps at the same station. In contrast, the signature pigments representing the most common chromophytes in Lake Kinneret—dinoflagellates (Chl *c* and peridinin) and diatoms (Chl *c* and fucoxanthin) showed very low discrepancy between traps, regardless of their locations.

Assuming that particles collected in A_{up} represent the best net vertical flux of pigments (see "Discussion"), one can quantify a "surplus" of deposited phytoplankton material in other traps, (Table 3). The "surplus" of Chl *a* and *β*-carotene (signature pigments of total phytoplankton) and lutein and Chl *b* (chlorophytes signature pigments) were conspicuously higher than those of Chl *c*, peridinin, fucoxanthin (chromophyte signature pigments). The signature pigments of

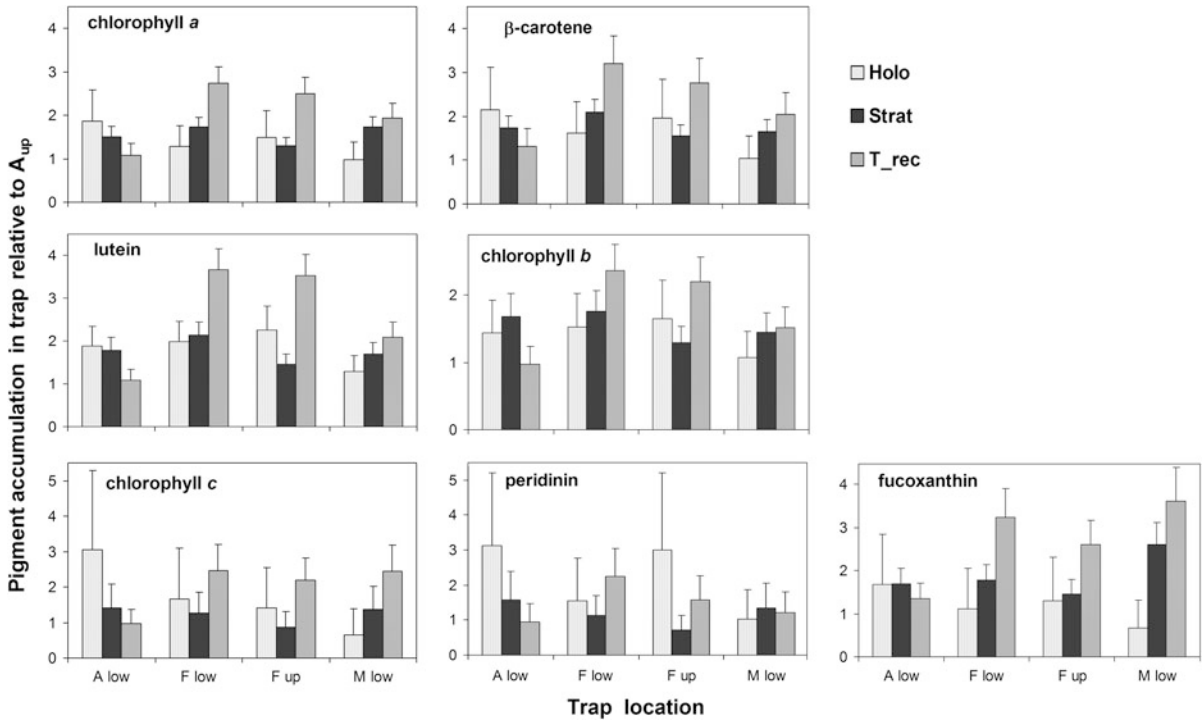

Fig. 4 Ratios of pigment accumulation rates in different traps relatively to A_{up}. Ratios are calculated for different periods (*Holo* holomixis, *Str* stable stratification, *T_rec* thermocline reclining). The multi-annual (2005–2009) averages are presented with standard errors. Trap locations are shown in Fig. 1

Table 2 Correlation coefficient (r) between pigment accumulation rates in pairs of various sedimentation traps[a]

Pigment	A_{low}–A_{up}	F_{low}–A_{up}	F_{up}–A_{up}	M_{low}–A_{up}	F_{low}–F_{up}	F_{low}–A_{low}	F_{up}–A_{low}	M_{low}–A_{low}	M_{low}–F_{up}	M_{low}–F_{low}
Chlorophyll *a*	0.76	0.71	0.80	0.46	0.71	0.65	0.59	0.44	0.41	0.55
β-Carotene	0.72	0.78	0.77	0.56	0.81	0.75	0.77	0.44	0.46	0.60
Lutein	0.65	0.61	0.57	0.55	0.64	0.69	0.55	0.71	0.49	0.73
Chlorophyll *b*	0.71	0.74	0.70	0.67	0.80	0.68	0.64	0.68	0.59	0.67
Chlorophyll *c*	0.96	0.89	0.74	0.23*	0.90	0.90	0.68	0.15**	0.49	0.50
Peridinin	0.86	0.89	0.84	0.64	0.91	0.86	0.84	0.53	0.36*	0.61
Fucoxanthin	0.47	0.98	0.85	0.67	0.89	0.28*	0.58	0.47	0.77	0.54

[a] The pair-wise correlations are calculated based on >190 pairs of measurements. In most comparisons $P < 0.001$, otherwise marked by * for $P < 0.05$ or ** where $P > 0.10$

total phytoplankton and chlorophytes showed an almost similar positive F_{pig} values, indicating "surplus" sedimentation rates, especially at the peripheral Stns. *F* and *M*. The highest values of F_{pig} were recorded in F_{low}, followed by F_{up}, and M_{low}, while the lowest "surplus" was found in A_{low}. The trends of chromophyte signature pigments were highly variable, and in several cases even small negative values were computed.

Discussion

The documentation of the distribution of pigments in the epilimnion of Lake Kinneret suggests that the spatial distribution of phytoplankton is rather homogenous, with an exception of the north tip of the lake near the Jordan River inflow (Berman & Elias, 1973; Yacobi & Schlichter, 2004; Ostrovsky & Yacobi, 2009). It is therefore, expected that if only the algal

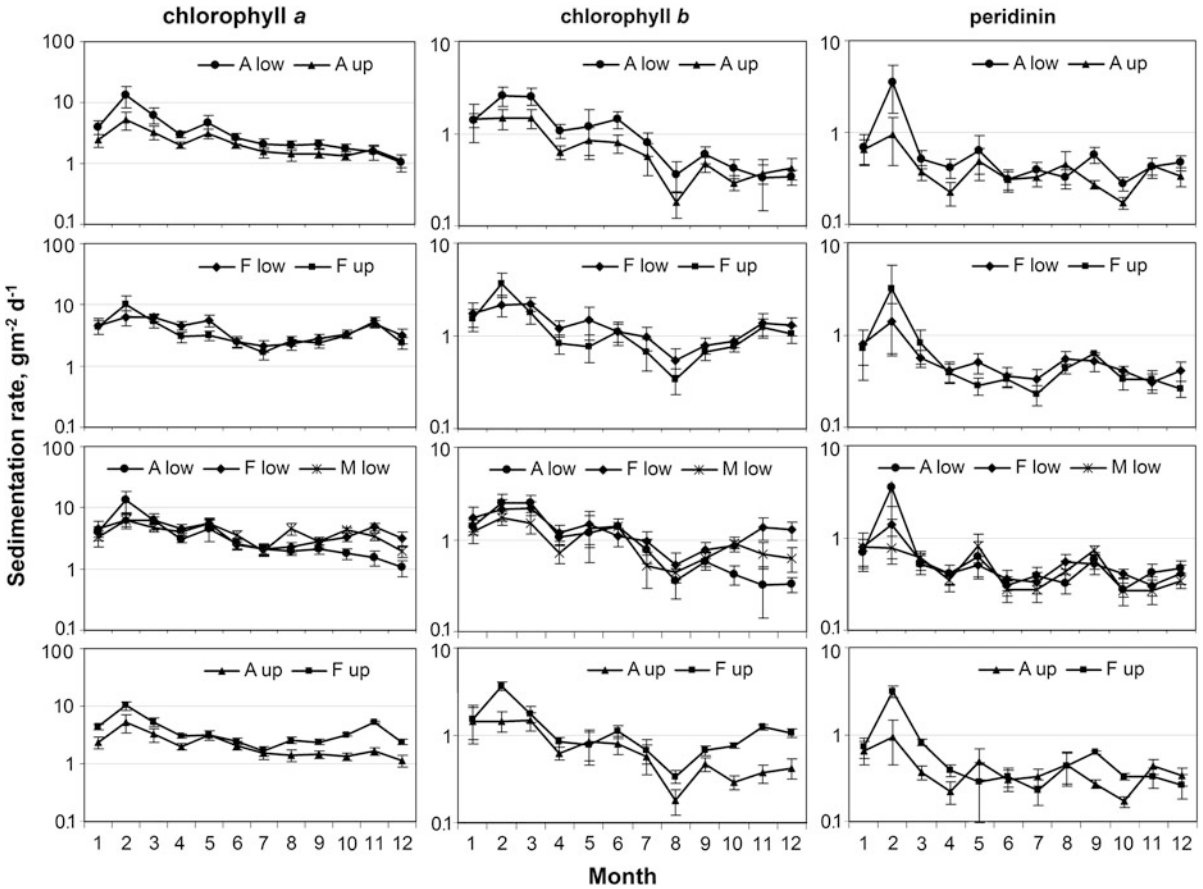

Fig. 5 Monthly averages of sedimentation rate of Chl *a*, Chl *b*, and peridinin in different traps. The averages were calculated for 2005–2009 and presented with standard errors. Trap locations are shown in Fig. 1

biomass is the main determinant of pigment downward flux, the flux measured by sedimentation traps located below the euphotic zone would be spatially homogeneous. However, that assertion does not hold in Lake Kinneret (Ostrovsky & Yacobi, 2010 and the current study) as well as in other lakes (Bloesch, 2004). In addition, if the vertical downward flux of pigments is only determined by sinking algal material, the measured sedimentation rates should decrease on the way of particle migration down to the bottom, due to cell lysis and material decomposition. Nevertheless, in most cases, the measured sedimentation rates of algal pigments were higher in the near-bottom traps, than in traps positioned shallower in the water column.

Phytoplankton cells and their debris have low-specific density (Reynolds, 2006) and are easily resuspended at the lake periphery, entrained by water motions (Schallenberg & Burns, 2004), and transported laterally via the BBL and metalimnion. The outcome of that fact is an increase in proportion of organic particles both in sedimentation traps and in the uppermost layer of bottom sediments from the lake littoral toward the lake center (Ostrovsky & Yacobi, 1999, 2010; Bloesch, 2004). In this study, we documented a large horizontal difference in sedimentation performance between chlorophyte signature pigments (Chl *b* and lutein) on the one hand, and signature pigments of the dinoflagellates (Chl *c* and peridinin), on the other hand (Fig. 6). Lutein, a chlorophyte signature pigment, is stored in sediments for hundreds of years (Züllig, 1981) or even more, as chlorophyte organic cellular residuals are known to be preserved for geological periods (Martín-Closas, 2003). This pigment, as well as Chl *b*, displayed large spatial heterogeneity in sedimentation fluxes because they contribute to the resuspended fraction, which dominates in peripheral areas of the lake. A similar tendency was displayed by diatoms, which are mostly fast-sinking algae (Reynolds, 2006) and

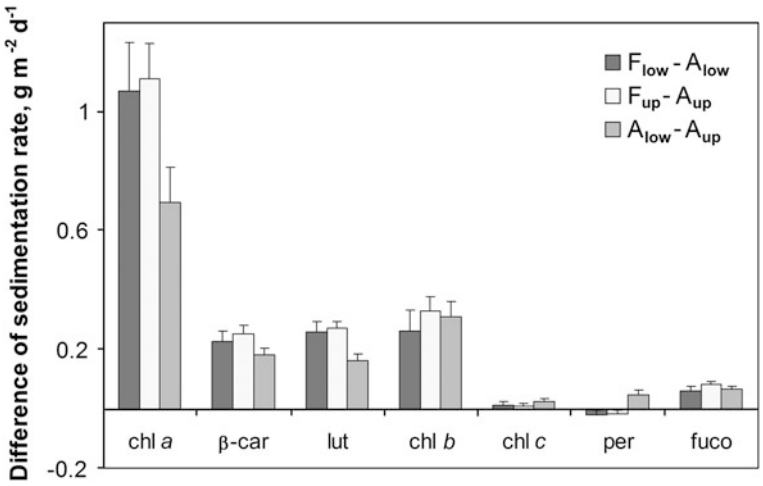

Fig. 6 Differences between the average sedimentation rates of signature pigments measured with various traps. The difference is calculated for traps separated horizontally ($F_{low} - A_{low}$, $F_{up} - A_{up}$) and vertically ($A_{low} - A_{up}$). The averages are calculated for stratified periods (April–December) of 2005–2009 sampling period and presented with standard errors. *Chl a* chlorophyll *a*, *β-car* β-carotene, *lut* lutein, *Chl b* chlorophyll *b*, *Chl c* chlorophyll *c*, *per* peridinin, *fuc* fucoxanthin. Trap locations are shown in Fig. 1

Table 3 The mean (±SE) of the flux difference, F_{pig} (%), in various traps relatively to A_{up}

Pigment	A_{low}	F_{low}	F_{up}	M_{low}
Chlorophyll *a*	28 (±6)	44 (±5)	41 (±5)	41 (±5)
β-Carotene	34 (±4)	43 (±7)	47 (±3)	33 (±6)
Lutein	27 (±5)	48 (±5)	36 (±8)	36 (±4)
Chlorophyll *b*	25 (±7)	47 (±5)	35 (±7)	27 (±8)
Chlorophyll *c*	13 (±10)	11(±16)	−1 (±16)	16 (±14)
Peridinin	23 (±9)	21 (±6)	9 (±7)	10 (±15)
Fucoxanthin	2 (±9)	8 (±6)	4 (±7)	−5 (±15)

F_{pig} was calculated using Eq. 4 based on pigment sedimentation rates averaged from January 2005 to December 2009

apparently decompose slowly in the water column (Yacobi & Ostrovsky, 2008).

We found that the horizontal changes in sedimentation rates of dinoflagellates were much lower than those of chlorophytes and diatoms (Fig. 5). This is apparently associated with the fact that signature pigments of the dinoflagellates vanish rather fast following their export from the epilimnion (Hurley & Armstrong, 1990; Leavitt, 1993; Steenbergen et al., 1994) and, thus, they are practically absent from the bottom sediments and cannot contribute to resuspended fraction in sedimentation traps. The evidence from Lake Kinneret shows explicitly that the sedimenting cells of the dinoflagellate *Peridinium gatunense* decompose rapidly (Viner-Mozzini et al., 2003). The later was a reason why the massive *Peridinium* bloom which lasted from February until May in 2007 did not leave a prominent pigment signature in trapped material (Yacobi, unpublished). It is, therefore, reasonable to conclude that only a slight proportion of the chromophytes reached the traps and if their signature pigments were detected, they originated in the meager left-over of cells that settled intact.

TWI (Fig. 3) indicates that dinoflagellates, which harbor peridinin and Chl *c*, are mostly recycled in the upper part of the stratified water column and reach the bottom only in minute quantities. The difference in TWI values for peridinin (dinoflagellates) versus fucoxanthin (diatoms) may be attributed to the fact that migrating dinoflagellates are able to compete with other algae under low-turbulence and high-light (strongly stratified water column) conditions, while

diatoms usually dominate during holomixis when turbulence is high and cells persist in the entire water column (Margalef, 1997). Dinoflagellates are dominant Chl *c*-containing algae in Lake Kinneret and that was the reason for the high correlation between Chl *c* and peridinin in water; whereas the relatively high degradability of peridinin (Hurley & Armstrong, 1990; Yacobi & Ostrovsky, 2008) explains its lower correlation with Chl *c* in traps (Table 1), where the contribution of diatoms was relatively higher. It is well documented that a number of species of diatoms can reach bottom sediments intact and be preserved there for a long time (e.g., Sicko-Goad, 1986). This mechanism allows the deposited cells to survive unfavorable conditions and regenerate new blooms from the resuspended cells. Such a life strategy was apparently the reason for the appearance of both sedimenting and resuspended particles in traps.

Although chlorophytes dominated phytoplankton biomass quite rarely (with the most prominent case of *Mougeotia* bloom in April–May 2005), their signature pigment, Chl *b*, was usually the most abundant pigment in sediment traps. The other chlorophytes' signature pigment, lutein, was closely correlated with Chl *b* ($r^2 = 0.78$, $n = 642$, $P < 0.001$). Although lutein could not be separated from zeaxanthin, which is harbored by cyanophytes (see above), zeaxanthin apparently had low contribution to lutein assessments. The latter is related to the fact that the dominant summer-blooming cyanophytes *Aphanizomenon ovalisporum* and/or *Cylindrospermopsis raciborskii* (Zohary & Shlichter, 2009; Alster et al., 2010) contain minute quantity of zeaxanthin (Yacobi, unpublished data). The scarcity of this pigment was also noted in other Nostocales (Hirschberg & Chamovitz, 1994). While both Chl *b* and lutein are relatively stable pigments, lutein has higher stability than Chl *b* (Leavitt & Hodgson, 2003; Yacobi & Ostrovsky, 2008).

The analysis of algal pigments in traps reveals that apparent sedimentation rates of phytoplankton varies notably between locations. The modification depends on several processes, such as the effect of turbulence on trap performance, decomposition of material in various parts of the water column, lateral transportation and focusing of newly produced material in deeper locations and contribution of resuspended particles in peripheral areas (Bloesch, 2004; Ostrovsky & Yacobi, 2010). In most comparisons, F_{pig} values (Table 3) indicate positive "surplus" of accumulated material in comparison to A_{up}, which evaluates the net sedimentation rate from the epilimnion. The evident reasons for the "surplus" can be (a) the trapping of the particles that are reintroduced into the water column due to resuspension at peripheral locations (Stns. *F* and *M*) and (b) oversampling of the sinking material under turbulent conditions (A_{low}). It is important to note that higher decomposition of dominant chromophytes before reaching the traps and during the period of material collection results in low "surplus" values, making the respective signature pigments to be non-indicative for the processes of over-trapping and resuspension. In contrast, the amount of less degradable pigments in various traps can well indicate these processes. In any case, the rate of material degradation should be taken into consideration for accurate assessment of the rates of material resuspension or over-trapping.

Using various photosynthetic pigments as biomarkers one can follow the general fate of different algal phyla in the lake and study the sedimentation trap performance under different ambient conditions. For further quantification of the fate of algal material in aquatic ecosystems it is important to specify the main reasons of the persistence (or vulnerability) of specific signature pigments, which vary with the degree of resilience of intact algal cells to breakdown (lysis) and/or the rate of pigment degradation in sinking debris. The quantification of these processes is a matter of further studies.

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PHYTOPLANKTON

Watershed land use types as drivers of freshwater phytoplankton structure

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Abstract The potential importance of watershed land use types, lake/watershed morphometry/topography and geographic distance as drivers of phytoplankton community composition was evaluated by using data collected from 18 freshwaters (lakes and reservoirs) distributed around Greece. In all freshwaters, phytoplankton species composition showed a strong correlation with the composition of land uses within their watersheds but no correlation with morphometry/topography and geographic distance. Cyanobacteria were found to be associated with artificial and agricultural land use types. Chrysophytes were closely associated to forested areas whereas euglenophytes to industrial, commercial, and transport units. Phytoplankton total biomass was significantly higher in freshwaters with a cover of agricultural and artificial land use >30% in their watersheds. This rather low threshold of agricultural and artificial land use cover might be indicative of the higher sensitivity of Mediterranean freshwaters to eutrophication process. Analysis performed separately for lakes and reservoirs revealed some diverse patterns with lake morphometric/topographic variables significantly affecting similarity in species occurrence. The results demonstrate that land use types reflecting anthropogenic pressures could act as critical drivers explaining phytoplankton structure. Our research suggests that Mediterranean freshwaters could be highly sensitive to land use types within their watersheds, thus landscape structure and configuration should be taken into account toward effective conservation and management plans.

Keywords Land use types · Drivers · Freshwater phytoplankton · Lakes and reservoirs · Mediterranean

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Introduction

Agriculture has been acknowledged as one of the most important drivers of land transformation in global scale (Lambin et al., 2006), whereas many global studies have shown that a big percentage of cultivated land has been created against forests and wetlands (Millennium Ecosystem Assessment, 2005). Freshwaters are important life resources. Whereas good water quality of these systems is a prerequisite for numerous ecosystem services (Meybeck & Helmer, 1996), in most parts of the world freshwater ecosystems have

suffered severe degradation (Carpenter et al., 2011). The causes of degradation of these ecosystems are strongly related to the surrounding land use types reflecting undergoing activities (Perry & Vanderklein, 1996). As a result, studies on the potential factors driving changes in water conditions and aquatic communities have become increasingly popular during the last decades, with our knowledge about individual drivers accumulating rapidly (Stomp et al., 2011).

Understanding the relationships between ecological lake functionality and watershed changes is an essential step for the selection and application of effective, long-term conservation and management strategies (Silva et al., 2011). Toward this direction, scientists have used biological indicators of water quality for detecting potential changes of biotic communities structure over spatial and temporal scales (Burns & Galbraith, 2007; Van Egeren et al., 2011). Phytoplankton is a primary biotic community indicating changes in ecological water quality due to its sensitivity and dynamic responses to the surrounding environment (Padisák et al., 2006). Shifts in the taxonomic composition of phytoplankton as a result of changes in nutrient conditions have been widely documented, with more clear evidence arising from studies on summer phytoplankton communities (Sommer, 1989; Watson et al., 1997). Phytoplankton groups are suggested to reflect trophic conditions (Rojo et al., 2000). For instance, in eutrophic freshwaters the relative importance of chrysophytes to community structure (biodiversity, biomass) decreases whereas that of cyanobacteria increases (Watson et al., 1997). Moreover, in stressed and heavily degraded ecosystems, a decrease in biodiversity is associated with an increase in biomass of few species (Perry & Vanderklein, 1996; Michaloudi et al., 2009).

Agricultural and urban land type uses are recognized as the main sources of nutrient input and various contaminants in lakes and reservoirs (Carpenter et al., 1998; Carney, 2009). When exceeding 40 and 15% of the watershed area, respectively, they are considered as significant morphological alterations and pressures in freshwaters (LAWA, 2003). The transformation of natural catchment into agricultural land, industry or urban areas and the associated eutrophication of the water are also considered a major threat to freshwater species diversity (Weijters et al., 2009). The role of land use and watershed development as descriptors of aquatic biodiversity is highlighted in a limited number of studies dealing with phytoplankton (Burns & Galbraith, 2007; Stomp et al., 2011) and other components of the aquatic biota such as bacteria, protozoa (Burns & Galbraith, 2007), and zooplankton (Van Egeren et al., 2011). Hoffmann & Dodson (2005) showed that watershed development was the best single descriptor of zooplankton richness for all lakes describing biodiversity patterns better than productivity or lake area alone. On the other hand, lake morphometry is considered as the best index of phytoplankton structure and productivity, reflecting climate and watershed conditions (Wetzel, 2001; Mazaris et al., 2010). For reservoirs it is well-known that hydraulic regime, size, and depth changes due to operational use have clear implications on their biotic communities (Kennedy, 1999). Still the limited studies that investigate phytoplankton composition and biomass changes in relation to freshwater/watershed morphology or land use (e.g., Maberly et al., 2003; Carney, 2009; Liu et al., 2011) provide information on a local rather than a broad spatial scale (Stomp et al., 2011). Furthermore, this information concerns mainly temperate areas whereas limnosystems of other regions such as the Mediterranean are quite distinct from the contemporary limnological paradigm; further raising the need for additional research (Alvarez Cobelas et al., 2005).

Clearly, there are a number of reasons why phytoplankton community structure can be used for assessing water quality. Notably, there is evidence to suggest that environmental heterogeneity could drive the observed compositional patterns. While occurrence of specific phytoplankton species could primarily be limited by abiotic conditions (lake depth, water retention time) and biotic interactions (Reynolds et al., 1993), recent evidence from biogeographical studies have raised the issue of distance decay patterns in microorganism community composition (Whitaker et al., 2003; Soininen et al., 2007). Considering these results, it is critical that before using phytoplankton community structure as an indicator of water quality and environmental conditions, we should improve our understanding on whether compositional structure and properties are driven by geographical distance limitations, by pure environmental factors or their combination. Only recently, studies on determinants of phytoplankton metacommunity structure across freshwaters have received great interest (Soininen et al., 2007).

This article aims to show the potential importance of pressures that human activities pose on phytoplankton structure of lakes and reservoirs contributing (i) to the limited number of papers linking land use types and phytoplankton and (ii) to the knowledge on phytoplankton metacommunity structure determinants. Therefore, phytoplankton structure of 11 lakes and 7 reservoirs in Greece was examined in relation to watershed land use types, morphometric/topographic variables, and geographical distance. Three were the basic objectives: (1) to assess the potential influence of watershed land use types and lake/watershed morphometry/topography on phytoplankton community descriptors (species, taxonomic groups, and total biomass) of a wide range of freshwaters, (2) to test whether compositional patterns of phytoplankton are driven also by geographical distance, and (3) to investigate if the relative importance of watershed land use types, lake/watershed morphometry/topography and/or geographical distance vary between the natural lakes and reservoirs.

Materials and methods

Study area

A total of 18 freshwaters (11 lakes and 7 reservoirs) of different trophic status and hydrological regimes were included in this study (Fig. 1). The freshwaters ranged in surface area from 1.3 to 65 km^2 and had a maximum depth from 1.1 to 120 m. They were located across an altitudinal gradient ranging from 25 to 850 m a.s.l. extending at a longitude from 20°53′E to 26°1′E and latitude from 38°10′N to 40°59′N. Their watersheds ranged in area from 76 to 11763 km^2 (Table 1).

Phytoplankton data

Phytoplankton data were obtained during the warm period (June–October) of the years 2007–2010. At least three samplings were performed during this period at each freshwater. Depth-integrated samples were collected from the euphotic zone near or at the deepest part of each freshwater. Live and preserved samples were examined in sedimentation chambers using an inverted microscope with phase contrast (Nikon SE 2000). Phytoplankton individuals were identified to species level using taxonomic keys. Cyanobacteria were evaluated by polyphasic approach (e.g., Moustaka-Gouni et al., 2010). Phytoplankton counts (cells, colonies, and filaments) were performed using Utermöhl's sedimentation method (Utermöhl, 1958). At least 400 individuals in total and 100 individuals of the most abundant taxa were counted per sample in sedimentation chambers of 50, 25, 10, 5, and 2 ml, depending on the density of the phytoplankters in the samples. For biomass estimation, the dimensions of 30 individuals (cells, colonies, or filaments) of each species were measured using tools of a digital microscope camera (Nikon DS-L1). Mean cell or filament volume estimates were calculated using appropriate geometric formulae (Hillebrand et al., 1999).

Freshwaters morphometric/topographic data and watersheds land use types

In the present analysis, we used a number of morphometric and topographic variables [surface area (km^2), mean and maximum depth (m), watershed area (km^2), and altitude (m)] as potential drivers of phytoplankton changes. ArcGis software (GIS, 1994) was employed to draw lake/reservoir and watershed areas based on physical barriers and topographical features. Mean and maximum depths were available from various sources (i.e., research programs, the Hellenic Ministry of Agriculture). Geographical distance between two freshwater systems, was estimated as the Euclidean distance between their centers.

The database of CORINE Land Cover 2000 for Greece (EEA, 1993) was used in order to identify land use types of watersheds. The CORINE Land Cover 2000 database represents the only land use type database validated at national level. Land use types are grouped into three levels, with increasing details on the spatial elements of the land use types presented; in this study we used the second and third levels of land use data for our analysis. Land-use cover proportions were calculated at the scale of the entire watershed using analytical tools of ArcInfo (ArcGis 10.x software). Basic land use types recognized within our study areas include: agricultural (arable land/permanent crops/pastures/heterogeneous areas), artificial (urban fabric/industrial, commercial and transport/mine, dump and construction/artificial non-agricultural vegetated areas), wetland (inland, maritime), forest and semi-natural (forests/scrubs and herbaceous vegetation/open spaces) land use categories.

Fig. 1 Map of Greece indicating the location of the studied freshwaters and their watersheds (*MP* Mikri Prespa, *KA* Kastoria, *CH* Chimaditis, *ZA* Zazari, *PE* Petron, *VE* Vegoritis, *PA* Pamvotis, *DO* Doirani, *KE* Kerkini, *VO* Volvi, *TH* Thisavros, *PL* Platanovrisi, *IS* Ismarida, *AI* Aisimis, *PO* Polyphytos, *TA* Tavropos, *YL* Yliki, *MA* Marathonas)

Data analysis

In order to examine any potential difference in phytoplankton biomass between freshwaters with a high and a low cover of artificial and agricultural land use we performed non-parametric Mann–Whitney test. To proceed with the pairwise comparisons in phytoplankton biomass, we grouped freshwaters by applying different thresholds of % cover in artificial and agricultural land use ranging from 15 to 45%.

To examine the influence of land use types, morphometry/topography and geographic distance on phytoplankton species composition we used standard Mantel and partial Mantel permutation tests (Green et al., 2004; Reche et al., 2005; Sommaruga & Casamayor, 2009). Mantel correlation coefficient (rs) ranges from −1 to 1, with the significance of the tests determined based on 999 permutations (Clarke & Warwick, 2001, Rosenberg, 2001).

The standard Mantel test is used in order to compare two independent similarity matrices that describe the same set of entities (Quinn & Keough, 2002). In our study, the entities were the different freshwaters and standard Mantel tests were used to test whether the associations between phytoplankton occurrence data (presence/absence per freshwater) and morphometric/topographic variables (per site) or percentage of land use types cover (per freshwater) or geographical distances

Table 1 Morphometric/topographic features of the studied freshwaters and percentage (%) of land use cover in their watersheds (*WA*:*LA* Watershed Area:Lake Area)

Code	Freshwater	Morphometric/topographic features					Land use cover (%)				
		Altitude	Area (km^2)	Watershed area (km^2)	WA:LA	Mean depth (m)	Artificial	Agricultural	Forests & Semi-natural	Wetlands	Freshwaters
MP	Mikri Prespa	850	39.15	197.83	5	4.1	0.26	13.89	4.85	4.85	19.84
KA	Kastoria	625	28.97	271.63	9	4.1	1.58	33.69	53.29	0.77	10.67
ZA	Zazari	600	2.04	75.73	37	1.7	1.55	40.17	55.58	0.00	2.69
CH	Chimaditis	590	10.56	112.58	11	1.2	1.16	35.54	52.11	0.00	11.19
PE	Petron	575	10.10	346.43	34	2.6	5.89	47.90	38.85	0.81	6.55
VE	Vegoritis	510	40.62	2108.53	52	20	6.96	40.08	49.82	0.13	3.02
DO	Doirani	150	34.83	299.42	9	3	1.94	34.79	49.16	2.48	11.63
IS	Ismarida	25	1.31	320.54	245	0.5	4.19	48.42	45.36	1.58	0.44
VO	Volvi	40	68.41	1281.67	19	13.8	1.08	44.14	49.07	0.37	5.34
PA	Pamvotis	500	20.07	479.94	24	4.3	8.17	44.85	42.07	0.73	4.18
YL	Yliki	100	20.65	2436.44	118	28	1.67	47.92	49.38	0.00	1.03
KE	Kerkini	50	65.16	11762.85	181	3.1	2.59	31.31	65.04	0.24	0.82
AI	Aisimis	200	8.73	93.99	11	15	0.00	10.88	79.84	0.00	9.29
PO	Polyphytos	300	64.37	5577.85	87	26.2	0.93	36.25	61.93	0.17	0.72
TA	Tavropos	800	21.62	163.077	8	47	0.00	14.74	72.00	0.00	13.26
MA	Marathonas	230	1.71	118.86	70	15	10.28	48.18	40.10	0.00	1.44
TH	Thisavros	400	6.93	4233.84	611	40	1.30	17.96	79.75	0.00	0.99
PL	Platanovrisi	250	2.03	4630.55	2281	15	1.22	16.42	81.38	0.00	0.98

between freshwater, were stronger than expected from chance. In addition, partial Mantel tests were used to examine the relationships between any two matrices while holding another one constant (Legendre, 2000). Phytoplankton composition refers either to species composition or to taxonomic groups composition. Species composition is based on the species occurrence data. The species occurrence data were used for the species presence/absence matrices of analysis.

We used Bray Curtis index to produce similarity matrices for every pair of freshwaters based on phytoplankton occurrence data. Similarity matrices were also produced separately for the morphometric/topographic variables and percentage of land use types cover by using Euclidian distance as a measure through multivariate space between the data points. Morphometric/topographic variables were standardized prior the production of the similarity matrix. An inter-lake/reservoir distance matrix was also produced based on Euclidian distances between the center of each freshwater.

Single matrices (morphometric/topographic variables, land use types, and geographic distance) were used to examine independently their potential influence upon phytoplankton similarity by mean of Mantel permutation tests. Partial Mantel tests were employed to examine for plausible correlation between phytoplankton species composition and each one of the above groups of variables by gradually keeping constant the remaining matrices. For example, we addressed the plausible correlation between geographic distance and phytoplankton species composition by initially holding the morphometric/topographic matrix constant; next we kept constant the land use matrix and finally both matrices were hold constant. This process was repeated for all three groups of parameters, resulting in a total of nine separated analyses.

To investigate whether patterns of phytoplankton community structure are influenced by the same groups of parameters we repeated the above analysis after grouping freshwaters as lakes or reservoirs.

Canonical Correspondence Analysis (CCA) (Quinn & Keough, 2002) was used to assess the relationship between land use types with (a) phytoplankton species composition and (b) phytoplankton taxonomic groups composition. In order to achieve a better interpretation of the results, land use types were grouped according the second scheme of Corine Land Cover data set which actually divides detailed land use types into 999 broader categories. Following this procedure, a total of 15 land use types were recognized in our study areas and maintained for the analysis.

Single Mantel tests and partial Mantel tests were run by Passage software (Rosenberg, 2001); CCA and the Mann–Whitney test were performed by Past software Version 2.09 (Hammer et al., 2001).

Results

The majority (72%) of the studied freshwaters belonged to watershed basins with artificial and agricultural land types covering more than 30% of their area (Table 1). According to the different land use cover types retrieved from Corine 2000, catchment modification is more intense around lowland lakes and reservoirs.

A total of 300 phytoplankton species were identified in the 18 freshwaters during the warm period of 2007–2010. These species belonged to nine taxonomic groups (Fig. 2). The most diverse taxonomic group was chlorophytes (138 species) followed by cyanobacteria (65) and euglenophytes (41). Mean phytoplankton biomass ranged from 0.6 ± 0.75 mg l^{-1} to 236.6 ± 2.7 mg l^{-1} (Fig. 3). Pairwise correlation analysis demonstrated that significantly higher values were recorded in those freshwaters with an agricultural and artificial land use cover >30% in their watersheds (Higher cover 167.76 ± 82.13 mg l^{-1}; lower 6.63 ± 4.02 mg l^{-1}) (Mann–Whitney $U = 7.01$; $P = 0.01$).

Phytoplankton composition (presence/absence matrix) was significantly related to the composition of land use types within their watershed (rs = 0.378, $P < 0.01$). In contrast, we found no significant relationship between phytoplankton composition and morphometry/topography similarity matrix or the geographic distance matrix of the freshwaters ($P > 0.05$). The significant relationship between phytoplankton species composition and land use type matrix was maintained even after we removed the effect of morphometry/

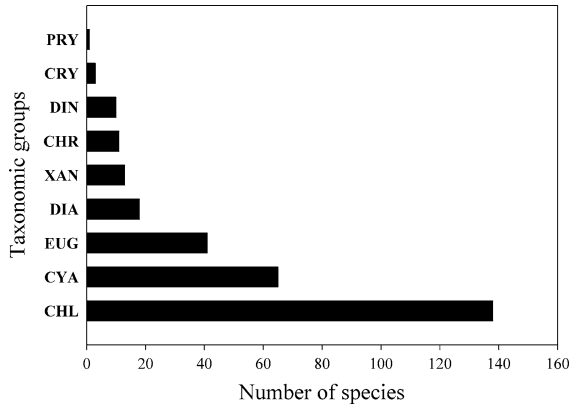

Fig. 2 Total number of species belonging to taxonomic groups (*DIN* Dinophytes, *CYA* Cyanobacteria, *CHR* Chrysophytes, *CRY* Cryptophytes, *DIA* Diatoms, *EUG* Euglenophytes, *CHL* Chlorophytes, *PRY* Prymnesiophytes, *XAN* Xanthophytes) in the studied freshwaters during the period 2007–2010

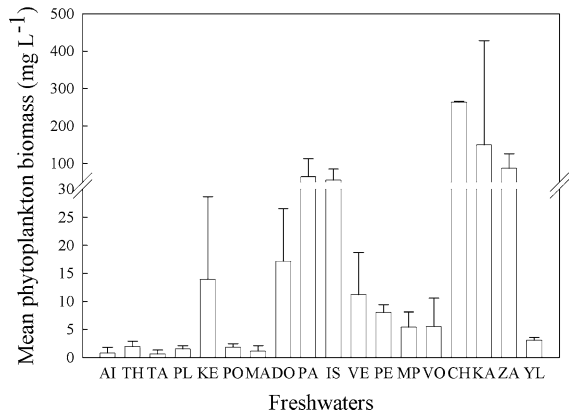

Fig. 3 Mean phytoplankton biomass values recorded in the studied freshwaters during the period 2007–2010. *Error bars* represent standard deviation

topography (rs = 0.32, $P < 0.01$) and geographic distance (rs = 0.367, $P < 0.01$) but also their combined effect (rs = 0.31, $P < 0.01$). Partial Mantel test revealed no other statistical significant relationship.

Different results were obtained when analyzing separately lakes and reservoirs. Phytoplankton species composition of lakes showed a significant correlation with morphometric/topographic variables (rs = 0.39, $P < 0.05$). Still, partial Mantel tests showed no significant correlation for any of the studied matrices. Standard Mantel tests revealed the lack of similarity between phytoplankton species composition of reservoirs and any of the explanatory matrices. However,

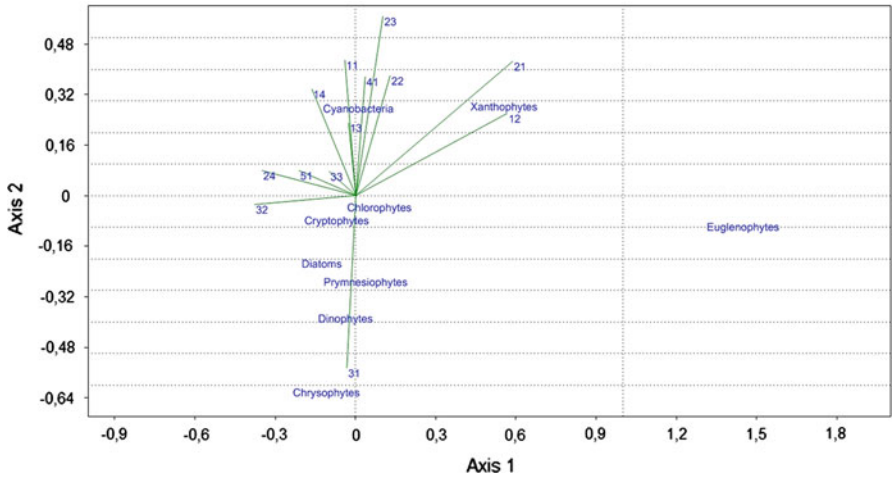

Fig. 4 Ordination diagram for the Canonical Correspondence Analysis of phytoplankton taxonomic groups present in the studied freshwaters and the land use types in the corresponding watersheds (*11–14* artificial: *11* urban fabric, *12* industrial commercial and transport units, *13* mine dump and construction sites, *14* artificial non-agricultural vegetated areas; *21–24* agricultural: *21* arable land, *22* permanent crops, *23* pastures, *24* heterogeneous agricultural areas; *31–33* forests: *31* forests, *32* scrub and/or herbaceous vegetation associations, *33* open spaces with little or no vegetation; *41* wetlands; *51* water bodies)

once the effect of land use types and geographic distance were removed, morphometry/topography was highly correlated with species composition matrix.

The results of the CCA for phytoplankton taxonomic groups (Fig. 4) showed that the first axis of the plot conditional model explained 58.4% of the variance in the species data. This axis represented a gradient from arable lands (Corine type 21) and industrial, commercial and transport units (Corine type 12) to natural shrub and/or herbaceous vegetation associations (Corine type 32). The second axis explained 22.04% of the variance in the species data and represented a gradient of pastures (23) to forests (31). The locations of the taxonomic groups in the biplot indicate the plot level conditions to which they were most closely associated. Cyanobacteria were more closely associated with artificial and agricultural land use types such as urban (Corine type 11) and artificial (Corine type 13, 14) sites and permanent crops (Corine type 22). Chrysophytes, dinophytes, prymnesiophytes, and diatoms were closely associated to forests (Corine type 31) and xanthophytes to arable land (Corine type 22) and industrial, commercial and transport units (Corine type 12). Chlorophytes were located very close to the axes cross point. In contrast, euglenophytes formed a separate group apart from the rest taxonomic groups with a location in the biplot more close to artificial and agricultural land types. CCA analysis did not reveal a clear pattern between phytoplankton species composition and land use types.

Discussion

The results of our analysis highlighted the importance of land use types as potential drivers shaping phytoplankton community structure. Despite the differences in hydrological regime and morphometric/topographic variables characterizing the 18 studied freshwaters, land use types were strongly correlated with phytoplankton community structure of all freshwaters even after controlling for geographical distance and morphometric/topographic heterogeneity. Among the most important land use types recognized to better distinguish phytoplankton community structure were agricultural and artificial areas.

According to the German Working Group on water issues (LAWA, 2003), agricultural and artificial (e.g., urban) land use types exceeding a 40 and 15% of the catchment area, respectively, are considered as important morphological alterations and pressures on freshwaters water quality. In our study, phytoplankton biomass was significantly higher in freshwaters with watersheds cover of agricultural and artificial land use >30%, thus revealing lower thresholds of agricultural

and artificial land use cover for changes in phytoplankton biomass. It is most likely that the lower values obtained in this study might show the critical transition threshold in eutrophication process from mesotrophic to highly eutrophic freshwaters under anthropogenic pressures in the Mediterranean landscape. In this region, due to the higher watershed area : lake area ratio of freshwaters compared to those of cold temperate ones, lakes and reservoirs should experience stronger watershed effects (Alvarez Cobelas et al., 2005) and might be more vulnerable to lower percentage of agriculture land use. In our study, most lakes and reservoirs had a quite higher watershed area:lake area ratio than the cold temperate ones, comparable to the ratio of other Mediterranean freshwaters (Alvarez Cobelas et al., 2005). Another plausible explanation could be the rather small number of sites used in our study in comparison to the sites used by LAWA.

Land use was also recognized as the most important remote driver of phytoplankton species composition for all freshwaters. Evidence from recent studies performed at the same region demonstrated that phytoplankton species occurrence and diversity is largely affected by environmental heterogeneity (Mazaris et al., 2010). At the long-term, land use types are likely to affect morphological and biochemical features of freshwaters; for example agricultural and urban land type uses are recognized as the main sources of nutrient input in lakes and reservoirs (Carpenter et al., 1998; Carney, 2009). Excessive input of nutrients, particularly phosphorus, regardless of the type of freshwater can result in implications for its phytoplankton community structure by increasing primary production, reducing water transparency and oxygen, reducing diversity and inducing the occurrence of phytoplankton algal blooms often dominated by toxin-producing species (Lampert & Sommer, 2007).

In this study, land use types were also found to be strongly correlated with phytoplankton composition at a higher taxonomic level. Cyanobacteria species were found to be associated with artificial and agricultural land use types such as urban and artificial sites and permanent crops. The high contribution of cyanobacteria species to phytoplankton diversity has been commonly observed in eutrophic lakes in Greece (e.g., Moustaka-Gouni, 1993; Mazaris et al., 2010) and worldwide (e.g., Reynolds, 1998). Chrysophytes, dinophytes, prymnesiophytes, and diatoms were closely associated to forests. These groups generally predominate at a lower nutrient status (Reynolds, 1984; Sommer, 1989; Padisák et al., 2009). The most species-diverse group of freshwater phytoplankton, chlorophytes, was located at the axes cross and, this may indicate their ubiquitous distribution in freshwaters, dominance in airborne phytoplankton and their role as pioneer colonists in aquatic systems (Chrisostomou et al., 2009; Genitsaris et al., 2011). In contrast, the location of euglenophytes in the biplot clearly separated this group apart from the rest taxonomic groups with them being closer to artificial (industrial, commercial, and transport units) and agricultural land types. A high number of euglenophytes species is reported in water bodies with a high content of organic substances (Borics et al., 2003). In our case, high species richness and similarity of euglenophytes were observed in four shallow lakes. The highest number of euglenophyte species along with the highest number of phytoplankton species was observed in the very shallow lake Ismarida (IS) which has a high watershed area: lake area ratio and a high percentage of water column occupied by macrophytes. An increase in phytoplankton species richness with percentage of the water column occupied with submerged macrophytes is also reported in subtropical shallow lakes (Kruk et al., 2009).

The distribution of phytoplankton species in the studied freshwaters was not related to geographical distance thus not exhibiting any spatially predictable occurrence pattern. The lack of distance decay phytoplankton pattern in our study does not clearly imply a random or cosmopolitan phytoplankton distribution. There is known evidence to support that both local environmental factors and geographical distance could drive microorganism community composition (Martiny et al., 2006). Soininen et al. (2007) found that plankton community composition may be jointly regulated by neutral and niche-based processes by examining determinants of plankton metacommunity structure across small spatial scale in boreal wetland ponds. Recently, large-scale biodiversity patterns in freshwater phytoplankton have been presented by Stomp et al. (2011) who showed that biodiversity gradients in phytoplankton were driven by local environmental factors, implying land use effects.

Our analysis performed separately for lakes and reservoirs revealed some different patterns. Lakes sharing similar morphometric/topographic variables (depth, surface, watershed area, and altitude) showed

significantly higher similarity in species composition (e.g., Mazaris et al., 2010). In reservoirs a strong effect of morphometry was only found after removing the direct effect of land use and geographic distance. It is well-known that reservoir hydraulic changes due to operational use have clear implications on the biotic communities of these highly dynamic systems (Kennedy, 1999). The lack of correlation between the community matrix and land use does not necessarily imply the lack of effect of the latter on the reservoir's phytoplankton composition. Actually, one could argue that the land use effect on phytoplankton community composition might be greater in reservoirs since nutrient loads to these systems are usually greater than for lakes located in drainage basins with similar land uses (Jørgensen et al., 2005). Reservoirs generally have higher watershed area:lake area ratios than lakes. In our study, the average watershed area:lake area ratio of the reservoirs (464.1) was considerably higher than that of lakes (51.2). A possible explanation for the lack of correlation between the community matrix and land use in reservoirs, considering also that the strong relationship between morphometry and community composition only appears when removing the effect of land use and geographic distance, is that drivers and responses interact (Carpenter et al., 2011). Consequently, a certain phytoplankton community composition is typically the result of multiple causes (drivers) which often interact and vary geographically. Therefore, it is not always feasible to disentangle these interactions so as to understand and moreover to explain separately the causes and consequences of a given state (Carpenter et al., 2011). Furthermore, the most striking feature of reservoirs, the shorter water retention time compared to lakes with profound effects on phytoplankton structure (Katsiapi et al., 2011) might suppress the correlation between the community matrix and land use types.

Overall, our study suggests that agricultural and urban land uses within a freshwater's watershed represent an important driver of phytoplankton community structure, (composition and diversity). Tracing the links between remote variables such as land use types and phytoplankton structure at different spatial and temporal scales provides a unifying approach toward understanding how biotic communities of lakes and reservoirs are shaped under anthropogenic pressures. This is rather important for water management in regions such as the Mediterranean, where freshwaters are insufficiently studied, different from the cold temperate ones and highly vulnerable to anthropogenic pressures and climate change.

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PHYTOPLANKTON

Catchment land use and trophic state impacts on phytoplankton composition: a case study from the Rotorua lakes' district, New Zealand

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Abstract Trophic state of lakes has been related to catchment land use, but direct links between phytoplankton taxa and land use are limited. Phytoplankton composition, represented by relative cell abundance of phyla, was measured over a period of 4 years in 11 lakes in the Rotorua region, New Zealand. The lakes differed in morphometry, trophic state and land use (as percentage catchment area). We tested whether relative proportion of land uses, indirectly representing relative nutrient loading, was the overarching driver of phytoplankton composition. Trophic state was correlated negatively with native forest and positively with pasture and urban area. Cyanoprokaryota were correlated negatively with native forest and positively with pasture and trophic state, Chlorophyta were correlated positively with native forest and urban land use and negatively with pasture and trophic state, and Bacillariophyta were positively correlated with dissolved reactive silica to dissolved inorganic nitrogen (Si:DIN) and Si to dissolved reactive phosphorus (Si:DRP) ratios. Lakes with higher nutrient loads had higher trophic state and Cyanoprokaryota dominance. Chlorophyta were negatively correlated with Cyanoprokaryota and Bacillariophyta, suggesting competition amongst these groups. Our results apply to lakes potentially subject to changes in catchment land use, which may have implications for trophic state, phytoplankton composition and Cyanoprokaryota blooms.

Keywords Phytoplankton · Cyanoprokaryota · Chlorophyta · Bacillariophyta · Nutrients · Land use · Trophic state · Lake morphology · Catchment

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Introduction

Increasing pressure on water resources through human activity has resulted in deterioration of water quality in many parts of the world (Vörösmarty et al., 2000). Poor water quality is often associated with increased trophic state and occurrence of cyanobacterial blooms (Paerl et al., 2001). The latter are of particular concern

due to their potential toxicity (Codd et al., 2005), unsightly appearance, musty odours and flavours in drinking water (Wood et al., 2011).

The trophic state of a lake can be determined from phytoplankton productivity or total biomass (Carlson, 1977). Chlorophyll-*a* (Chl-*a*) is frequently used as a proxy for phytoplankton biomass but phytoplankton composition can also be informative in relation to lake trophic state (Bellinger & Sigee, 2010). Indicators of trophic state have been formulated at the phytoplankton species level (e.g. Flint, 1977; Reynolds, 1990) and at the phyla and order levels (e.g. Nygaard, 1949; Stockner, 1972; Ptacnik et al., 2008), whilst species diversity and richness provide additional metrics that have been related to trophic state (e.g. Watson et al., 1997; Dodson et al., 2000; Interlandi & Kilham, 2001).

Phytoplankton composition is affected not only by trophic state of a water body, but also by other factors (Reynolds, 1998), such as mixing regime (e.g. Ryan et al., 2006), temperature (Paerl & Huisman, 2008; Brookes & Carey, 2011), water depth (Jeppesen et al., 2005), euphotic depth to mixing depth ratio, dissolved oxygen concentration (Vincent et al., 1984; Trimbee & Prepas, 1988; Dokulil & Teubner, 2000) and ratios of macro-nutrients (Smith, 1983; White et al., 1985; Hamilton et al., 2006). Some of these factors may be symptomatic rather than causal of the dominance of certain taxonomic groups. For example, cyanobacterial blooms are often associated with reduced water clarity but blooms also influence turbidity and deplete oxygen during the bloom collapse phase (Paerl & Huisman, 2008).

The availability of nutrients (e.g. phosphorus, nitrogen, carbon and silica) and light, as well as water temperature, most commonly regulate phytoplankton growth rates (Geider et al., 1997; Watson et al., 1997; Dignum et al., 2005). Nutrient availability is in turn related to catchment loading, which can have a strong anthropogenic component (Johnes et al., 1996; Smith et al., 1999; Abell et al., 2011). Lake restoration efforts have therefore focused on reduction of nutrient loads from catchments (e.g. Edmondson & Lehman, 1981; Dokulil, 1993; Jeppesen et al., 2005; Wagner & Adrian, 2009). It follows therefore that trophic state of lakes has been related to catchment land use (McColl, 1972; Harper & Stewart, 1987; Johnes et al., 1996; Soranno et al., 1996; Abell et al., 2010) but direct linkages of lake phytoplankton taxa to land use have been limited to a small number of studies (e.g. Jeppesen et al., 2005; Peterson et al., 2007).

The objective of this study was to test for relationships of phytoplankton composition to land use type in lake catchments. For this purpose, we selected 11 lakes within close geographic bounds in the Rotorua region, North Island, New Zealand, which had similar climatic forcing but differing morphometry, trophic state and catchment land use. We tested the hypothesis that, amongst an array of physical and chemical attributes of the lakes, relative proportion of different land uses, representing different rates of areal nutrient loading, was the overarching driver of changes in phytoplankton composition, represented by the relative cell abundance of each phytoplankton phyla for each lake over 4 years.

Materials and methods

Measurements of physical variables and collection of water samples for chemical and biological (phytoplankton) analysis were undertaken from mid-lake stations in the 11 Rotorua lakes four times per year (January, April, July and October) between October 2003 and October 2007 by Bay of Plenty Regional Council (BoPRC). Physical variables were derived from a water column profile of temperature and dissolved oxygen taken by means of a SBE 19 plus CTD profiler (Seacat, Seabird Electronics). A Secchi depth reading was also taken at the mid-lake station.

An integrated tube sampler was used to collect water samples at the mid-lake station from the surface to a depth 1 m above the thermocline in lakes that stratify (Okaro, Okareka, Okataina, Rotoiti, Rotoma, Rotomahana, Rotorua, Tarawera and Tikitapu) or down to 1 m above the bottom sediment in frequently mixed lakes Rerewhakaaitu and Rotoehu. This water was sub-sampled to provide samples for phytoplankton enumeration, which were preserved with Lugol's iodine and kept in the dark until examination. The analyses were carried out using Utermöhl settling chambers (Utermöhl, 1958) and an inverted microscope (Olympus, Ix71, Japan). Phytoplankton was identified to phylum and genus level, and relative abundance was determined for each phyla. Enumeration to find densities of phytoplankton (cells ml^{-1}) used methods adapted from Hötzel & Croome (1999) and US Environmental Protection Agency (2007). A

10-ml subsample was settled in a Utermöhl chamber. Phytoplankton were counted at 400× or 200× magnification in at least three transects, including at least 100 planktonic units (cells, colonies and filaments) of the dominant species. The whole chamber was counted for Cyanoprokaryota except when they were dominant. Measurements required for biovolume were not undertaken, and phytoplankton were identified to genus level. Since different species of the same genus can have quite different biovolumes, results were expressed as cell densities. Relative abundance was used as this displays the composition of the algal community, which may vary with productivity which determines total cell abundance. Relative abundance is a proportional measure and complementary to reporting of total cell counts and is simply a variable used to compare empirically with catchment indices.

The Shannon Diversity Index (SDI; Shannon, 1948) was calculated to characterise genera diversity in algal communities. The SDI was calculated as follows:

$$\mathrm{SDI} = -\sum_{i=1}^{S} p_i \ln p_i$$

where p_i is the relative density of the ith genera in the community that was calculated as the ratio of cell density of a given species to the total density of all species, and S is total number of species in the community.

Additional water sub-samples were placed on ice and returned to the laboratory where they were filtered through GC-50 filters (Advantec 0.5 μm). Nutrient analyses were performed using a flow injection analyser (FIA; Zellweger Analytics, 2000), according to APHA methods: 4500-NH_3 H for ammonium, 4500-Norg D for total Kjeldahl nitrogen (TKN), 4500-NO_3-I for nitrate-N + nitrite-N, 4500-NO_2-I for nitrite-N, 4500-P G for dissolved reactive phosphorus (DRP), and 4500-P H for total phosphorus (TP) (Clesceri et al., 1998). Total nitrogen (TN) was taken to be the sum of TKN and nitrate-N + nitrite-N, and dissolved inorganic nitrogen (DIN) was represented as the sum of nitrate-N + nitrite-N and ammonium-N. Silica was not measured during our study, but we used dissolved reactive silica (Si) concentrations from McColl (1975) as an approximation of concentrations in the lakes. These data were annual means of summer and winter Si concentrations, including both surface and bottom waters, from April 1970 to April 1971, in all of the lakes in our study. The mean dissolved reactive silica (Si) to DIN ratio and dissolved reactive silica to DRP ratio were based on data for Si for 1970–1971 from McColl (1975) and for DIN and DRP for 2003–2007; because of the different time periods, we use the ratio here only as an approximation for the most recent data. Chl-*a* concentrations were determined by filtering whole water samples through GC-50 filters (Advantec, 0.5 μm), freezing and then solvent extraction and fluorometric analysis with correction for phaoepigments (Arar & Collins, 1997). A trophic level index (TLI) score was calculated according to Burns et al. (1999) from seasonal samples collected at each lake and an average determined for the 2003–2007 sampling period. The TLI score was calculated from Secchi depth, TN, TP and Chl-*a* concentration (Burns et al., 1999), with higher values indicating an increase in trophic state. A mean TLI for 2004–2007 was also calculated from annual values (BoPRC, unpublished data; sample collection started in October 2003). Nutrient concentration data were used to indicate potential for nutrient limitation based on half-saturation values for growth limitation (Fisher et al., 1988, 1992) of total inorganic nitrogen (TIN) < 28 mg m^{-3} (N-limitation), DRP < 6.2 mg m^{-3} (P-limitation) and Si < 56 mg m^{-3} (Si limitation of Bacillariophyta only) together with atomic ratios of Si:DIN < 1 or Si:DRP < 10 (Bacillariophyta limitation) (Turner et al., 1998; Hamilton et al., 2006) and potential N or P limitation when TN:TP ratio deviated from 16 (Redfield et al., 1963).

Schmidt water column stability [S_t (J m^{-2}), sensu Idso, 1973] was calculated using LakeAnalyzer program version 3.4 (Read et al., 2011) as:

$$S_t = \frac{g}{A_S} \int_0^{Z_m} (Z - Z_v) A_Z \rho_Z \partial Z$$

where g is the acceleration due to gravity, A_S is the lake area at the surface, A_Z is the area at depth Z, ρ_Z is the density at depth Z, Z_m is the maximum water depth, and Z_V is the depth to the centre of volume, defined by:

$$h_g = \frac{\int_0^{Z_m} Z A_Z \partial Z}{\int_0^{Z_m} A_Z \partial Z}$$

Hypsographical curves for each of the lakes were used to define relevant areas and volumes corresponding to

specific elevations using data from Anon (2011) and BoPRC records. The percentages of pastoral, indigenous forest, exotic forest, urban and 'other' land covers in each catchment were taken from Anon (2011).

Statistical methods

Spearman's rank correlation analysis (Statistica v. 9, StatSoft, Inc.) was carried out to elucidate relationships (r_s) amongst the relative cell abundances of phytoplankton phyla (Cyanoprokaryota, Bacillariophyta, Chlorophyta, Pyrrophyta, Euglenophyta, Chrysophyta and Cryptophyta), total phytoplankton densities, Si:DIN ratio and proportions of land use, SDI, Chl-*a*, Secchi depth, mixed layer depth, mean lake depth, ratio of lake area to catchment area, euphotic depth to mixed layer depth ratio, anoxic to oxic volume ratio, Schmidt water column stability, nutrients, TN:TP ratio, and TLI. Relationships were considered to be significant when $P < 0.05$.

Principal Components Analysis (PCA) was applied to the variables of relative cell abundance of each phytoplankton phylum, and a suite of variables that we hypothesised could potentially influence the abundances. These variables included proportion of each catchment land use, SDI, Chl-*a*, Secchi depth, mixed layer depth, mean lake depth, ratio of lake area to catchment area, euphotic depth to mixed layer depth ratio, anoxic to oxic volume ratio, Schmidt water column stability, nutrients, TN:TP ratio, and TLI and compared with the lakes sampled. This technique finds orthogonal linear combinations of the variables. We sought to find linear combinations of variables that best explained the variation in our phytoplankton data using PCA. No significance tests were performed in the PCA and the identified principal components were uncorrelated by design, hence multi-co-linearity was not an issue. A bi-plot (Gabriel, 1971) was used to project variables from the PCA in two dimensions. The observations are depicted as points, and variables as arrows from the intersection of the axes. The direction of an arrow represents where samples with high positive values of the corresponding variable occur. Arrows which point in similar directions are positively associated with each other in the dimensions depicted. The relative lengths of the arrows represent how well the variables are represented in the reduced dimensions depicted. The variables used for the PCA were projected onto the plot as arrows. Each catchment land use was projected as a black arrow, the relative cell abundance of each phytoplankton phylum was projected as a red arrow and the remaining variables were projected onto the plot as blue arrows.

Results

The mean TLI values calculated from concentrations of TP, TN and Chl-*a*, and Secchi depth, ranged from 2.5 in Lake Rotoma to 5.5 in Lake Okaro for the study period (Table 1). Lakes Rotoma, Tarawera, Okataina had TLIs <3, which placed them in the oligotrophic category (cf. Burns et al., 1999) and lakes Rotoehu, Rotorua and Okaro had TLIs >4, which placed them in the eutrophic to hypertrophic category. Lakes Tikitapu, Okareka, Rotoiti, Rotomahana and Rerewhakaaitu were mesotrophic with TLIs between 3 and 4 (Table 1). Mean Chl-*a* concentrations (Fig. 1a) and phytoplankton cell densities (Fig. 1b) over the study period were strongly correlated ($r_s = 0.75$, $P < 0.01$).

There was generally a higher percentage of pasture in the catchments of lakes with high TLI scores, whilst native forest tended to dominate in the catchments of lakes where the TLI was lower (Fig. 2; Table 1). The TLI was negatively correlated with proportion of native forest in the catchment ($r_s = -0.7$, $P < 0.05$), and positively correlated with proportion of pasture ($r_s = 0.6$, $P < 0.05$), and urban land use ($r_s = 0.2$, $P < 0.05$).

Cyanoprokaryota, Chlorophyta and Bacillariophyta were the three main phytoplankton groups represented amongst the phytoplankton assemblage of the Rotorua lakes (Fig. 3). Cyanoprokaryota were found in all lakes except Tikitapu, although they represented only 2% of the population in Lake Okareka. The phytoplankton population in Lake Tikitapu was almost entirely represented by Chlorophyta and in Lake Okareka was strongly dominated by Bacillariophyta. Lakes with the highest mean TLI (2004–2007; Table 1) also had the greatest percentage of Cyanoprokaryota whilst Bacillariophyta constituted a greater percentage of abundance in lakes with low TLI. All lakes had at least five representatives of the seven phytoplankton phyla but Lake Rotoiti had representatives of all seven groups. Lakes Rerewhakaaitu and Tarawera had a higher proportion of Chrysophyta than the other lakes (Fig. 3). The numerically dominant genera of phytoplankton for each

Table 1 Characteristics of the Rotorua lakes

	Rotoma	Okataina	Tarawera	Tikitapu	Okareka	Rerewhakaaitu	Rotoiti	Rotomahana	Rotoehu	Rotorua	Okaro
Lake area (km^2)[a]	11.2	10.8	41.7	1.5	3.3	5.8	34.6	9	8.1	80.8	0.3
Catchment area (km^2)[a]	27.8	59.8	143.1	6.2	19.6	37	123.7	83.3	49.2	508	3.9
Mean depth (m)[b]	37	39	50	18	20	7	31	31	8	11	12.5
Max. depth (m)[a, d]	83	78.5	87.5	27.5	33.5	15.8	124	125	13.5	45	18
Mean Chl-*a* (mg m^{-3})[b]	1.3	2.1	1.5	1.8	3.9	2.9	9.6	4.2	10.6	22.8	33.5
Mean Secchi depth (m)[b]	13.2	11.1	8.8	6.8	7.3	6.7	5.1	5.2	2.8	2.5	2.3
Mean TP (mg m^{-3})[b]	5.0	8.9	11.6	6.7	8.5	9.2	33.0	38.2	40.0	40.0	89.6
Mean TN (mg m^{-3})[b]	156.2	143.0	118.3	235.5	216.8	393.7	353.8	215.6	415.2	482.6	1053.5
TLI[c]	2.5	2.8	2.9	3.1	3.3	3.4	3.8	3.9	4.6	4.9	5.5

Sources: [a] Anon (2011); [b] Scholes (2009); [c] BoPRC (unpublished data); [d] von Westernhagen et al. (2010). The TLI is a mean from results reported annually by BoPRC for the period from 2004 to 2007, and mean Chl-*a*, TP and TN concentrations and Secchi depths are for the period from July 2003 to June 2007

phylum found in the Rotorua lakes during this study were *Anabaena* and *Microcystis* for Cyanoprokaryota; *Cosmarium*, *Dictyosphaerium*, *Sphaerocystis* and *Staurastrum* for Chlorophyta; *Dinobryon* for Chrysophyta; *Cryptomonas* for Cryptophyta; *Asterionella*, *Aulacoseira* and *Fragilaria sensu lato* for Bacillariophyta and *Peridinium* for Pyrrophyta. The three most numerically dominant genera over all samples for each lake were *Anabaena*, *Microcystis* and *Fragilaria s.l.* for lakes Okaro and Rotoehu; *Anabaena*, *Microcystis* and *Dictyosphaerium* for Lake Rotorua; *Anabaena*, *Microcystis* and *Aulacoseira* for Lake Rotoiti; *Aulacoseira, Cyclotella* and *Mougeotia* for Lake Rotomahana; *Dinobryon*, *Coelosphaerium* and *Monoraphidium* for Lake Rerewhakaaitu; *Asterionella*, *Fragilaria s.l.* and *Dinobryon* for Lake Okareka; *Cosmarium*, *Staurastrum* and *Aulacoseira* for Lake Tikitapu; *Aulacoseira*, *Cryptomonas* and *Dinobryon* for Lake Rotoma; *Dinobryon*, *Aulacoseira* and *Anabaena* for Lake Tarawera; and *Fragilaria s.l.*, *Aulacoseira* and *Anabaena* for Lake Okataina.

The SDI was significantly, but weakly correlated with Chl-*a* ($r_s = 0.19$, $P < 0.05$) and with total phytoplankton cell concentration ($r_s = 0.25$, $P < 0.05$). There were more representatives at the genus level for Chlorophyta than for any other phyla and the SDI was significantly and positively correlated with Chlorophyta ($r_s = 0.26$, $P < 0.05$; Table 2).

Cyanoprokaryota cell abundance was negatively correlated with Secchi depth, depth of the upper mixed layer, mean lake depth, ratio of lake area to catchment area, native forest, and TN:TP ratio. It was positively correlated with Chl-*a*, percentage of pasture in the catchment, anoxic to oxic volume ratio, TP, TN, NH_4-N and DRP and TLI over the study period. Chlorophyta abundance relationships were almost diametrically opposed to those of Cyanoprokaryota, and there was a negative correlation between Cyanoprokaryota and Chlorophyta as well as between Chlorophyta and Bacillariophyta. Chlorophyta were positively correlated with native forest and urban land use, lake area:catchment area, Secchi depth, SDI, and TN:TP ratio, and negatively correlated with Chl-*a*, TP, DRP, TLI and proportion of pasture in the catchment. Bacillariophyta abundance was positively correlated with mean lake depth (Table 2).

In Fig. 4, the first two components of the PCA explained 27.1 and 12.7% of the variation in the data analysed (i.e. 39.8% of the total variation; see

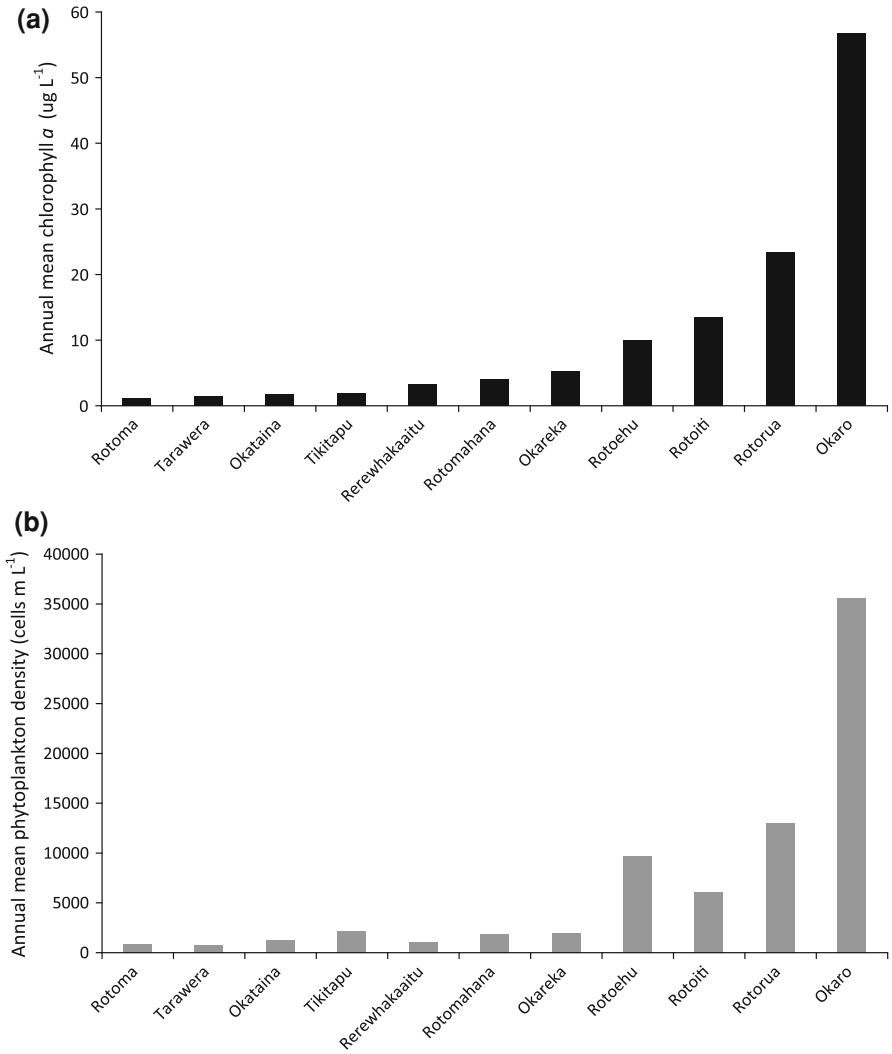

Fig. 1 **a** Chlorophyll-*a* concentrations and **b** phytoplankton densities as mean lake values of seasonal samples from October 2003 to December 2007

"Materials and methods"). The lakes were aligned strongly to the trophic state gradient, with those of higher trophic state (Okaro, Rotoehu and Rotorua) clustered to the right-hand side, oligotrophic lakes (Rotoma, Okataina and Tarawera) clustered to the left-hand side and mesotrophic lakes (Tikitapu, Rerewhakaaitu, Rotomahana, Okareka and Rotoiti) positioned close to the centre. Cyanoprokaryota were associated with the more eutrophic lakes, pastoral land use and nutrient (TN and TP) concentrations, Chlorophyta with native forest in the catchment, increasing Secchi depth and larger TN:TP ratios, whilst Bacillariophyta and less abundant phyla, such as Pyrrophyta, Cryptophyta, Chrysophyta and Euglenophyta, were positively aligned with SDI, mean lake depth, and depth of the upper mixed layer.

The Si:DIN and Si:DRP ratios were the lowest for lakes Tikitapu, Rotoiti, Okaro and Rerewhakaaitu, and the highest for Okareka and Okataina (Table 3). Relative abundance of Bacillariophyta was significantly and positively correlated with ratios of Si:DIN ($r_s = 0.72$, $P < 0.05$) and Si:DRP ($r_s = 0.79$, $P < 0.05$) (Table 3). Using the nutrient ratios for growth limitation given in Fisher et al. (1988, 1992), all lakes except Okaro, Rotoehu, Rotorua and Rotoiti (the lakes with the highest trophic state) showed potential for DIN or DRP limitation. None of these lakes had mean Si concentrations that were close to the value assigned for Si limitation (56 mg m^{-3}). In all lakes except Okaro, Rotoehu, Rotorua and Rotoiti, mean concentrations of DRP were below the value (6.2 mg m^{-3}) assigned for P limitation (Table 3),

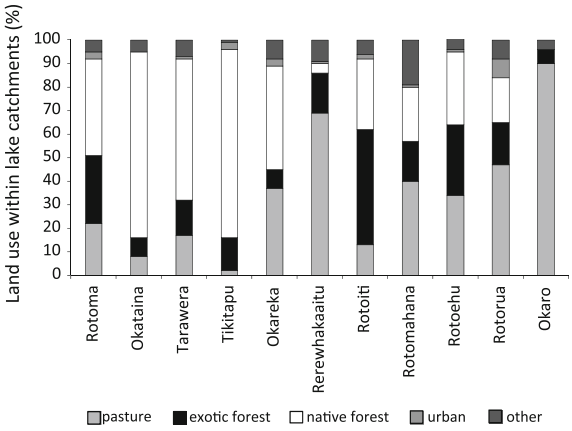

Fig. 2 Land use within the Rotorua lakes catchments. Lakes are ordered from (*left to right*) lowest to highest trophic level index (see Table 1)

although TIN concentrations in lakes Rotorua and Rotoehu were close to the value (28 mg m^{-3}) assigned for N limitation. The TN:TP molar ratio ranged from 5.56 for Lake Rotoehu to 21.4 for Lake Tikitapu. All lakes except Tikitapu and Rerewhakaaitu had TN:TP molar ratios <16 (Table 3). There was, however, large variability in both the mean concentrations of dissolved nutrients and in nutrient ratios in all lakes (Table 3).

Discussion

Our results show that phytoplankton composition is somewhat related to land use type in lake catchments where few other studies have made this link directly. In response to long-term reductions in external nutrient loading, Jeppesen et al. (2005) found that in a large proportion of their study lakes, there was decreased abundance of Cyanoprokaryota, some increases and decreases in Chlorophyta, and increased abundance of Bacillariophyta, Pyrrophyta, Cryptophyta and Chrysophyta. In a study of 40 lakes in North Island, New Zealand, Ryan et al. (2006) found that presence/absence of Cyanoprokaryota and Bacillariophyta genera was closely related to lake trophic state but also noted that mixing regime was a better predictor of phytoplankton composition. Dokulil (1993) demonstrated the complexity and specificity of such relationships; he found that the relative proportion of Cyanoprokaryota increased, and Bacillariophyta

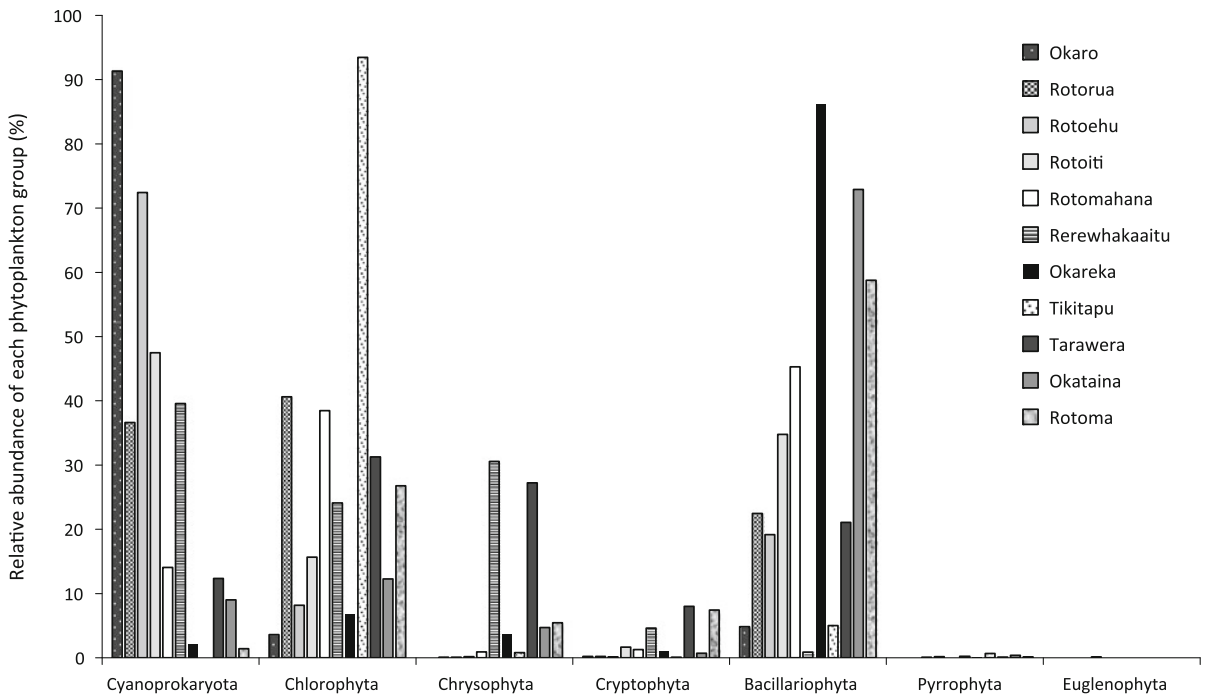

Fig. 3 Relative abundance of phytoplankton groups in the Rotorua lakes from October 2003 to December 2007. Lakes are ordered (*left to right*) from highest to lowest average trophic level index (see Table 1)

Table 2 Correlation coefficients between relative abundance of dominant phytoplankton groups and selected biological, physical and chemical variables in the Rotorua lakes in 2003–2007

	Cyanoprokaryota (%)	Chlorophyta (%)	Bacillariophyta (%)
Cyanoprokaryota (%)	1.00	−0.36*	−0.15
Chlorophyta (%)	−0.36*	1.00	−0.52*
Bacillariophyta (%)	−0.15	−0.52*	1.00
Shannon Diversity Index	0.07	0.26*	0.06
Chl-*a* (μg l^{-1})	0.47*	−0.36*	0.13
Secchi depth (m)	−0.48*	0.26*	−0.01
Mixed layer depth (m)	−0.23*	0.15	0.16
Mean lake depth (m)	−0.17*	0.12	0.23*
Lake area:catchment area	−0.25*	0.22*	0.07
Native forest (%)	−0.42*	0.23*	0.07
Exotic forest (%)	0.14	0.04	0.01
Pasture (%)	0.28*	−0.25*	0.00
Urban (%)	−0.16	0.28*	−0.03
Other (%)	−0.01	−0.12	0.15
Euphotic depth:mixed layer depth	−0.1	0.0	0.1
Anoxic:oxic volume	0.20*	−0.07	−0.12
Schmidt stability	0.05	0.14	−0.09
TP (mg m^{-3})	0.42*	−0.24*	0.13
TN (mg m^{-3})	0.41*	−0.16	−0.19*
NO_3-N (mg m^{-3})	0.15	−0.08	0.07
NH_4-N (mg m^{-3})	0.21*	−0.02	−0.10
DRP (mg m^{-3})	0.34*	−0.19*	0.01
TN:TP	−0.22*	0.17*	−0.32*
TLI	0.5*	−0.2*	0.0

Asterisks denote significant ($P < 0.05$) correlations. For all variables, $n = 141$ with the exception of the land use variables for which $n = 11$

TP total phosphorus, *TN* total nitrogen, *NO_3-N* nitrate, *NH_4-N* ammonium, *DRP* dissolved reactive phosphorus, *Chl-a* chlorophyll-*a*, *DIN* dissolved inorganic nitrogen, *TLI* trophic level index

decreased, over summer and spring during a period of re-oligotrophication of Lake Mondsee. In Lake Mondsee, the buoyancy-regulating cyanophyte, *Planktothrix rubescens*, was a major component of the proportional increase in Cyanoprokaryota, and increased water clarity and regional increases in temperature, together with changes in stratification, may have played a role in a long-term trend of increasing dominance of the metalimnetic populations of this species (Dokulil, 1993). Dominance of Cyanoprokaryota in metalimnetic phytoplankton populations has not been recorded in the Rotorua lakes despite the occurrence of distinct deep chlorophyll maxima in several of the oligotrophic lakes (Hamilton et al., 2010). Instead, we found that Cyanoprokaryota, represented primarily by surface-bloom forming populations were positively linked to land uses that increase external nutrient loading.

Our grouping at phylum level is a separation based primarily on morphological and physiological differences (e.g. pigmentation, cell wall composition) though molecular methods are increasingly being used for identification purposes. Komarek (2005) advocates a polyphasic approach, combining molecular and taxonomic information. Taxonomic grouping has been used as a means to implicitly include relevant environmental drivers that influence phytoplankton composition whilst also maintaining taxonomic separations that are important adaptive features (e.g. Dokulil, 1993; Watson et al., 1997). In our quest for simple causal mechanisms leading to changes in phytoplankton phyla, we did not cluster phytoplankton into functional groups which use traits such as volume; presence of flagella, siliceous exoskeleton structures and heterocysts, and length, which determine how phytoplankton respond to local environmental conditions that may include nutrient assimilation, growth rate and sinking rate (Margalef, 1978; Reynolds, 1980; Webb et al., 2002; Salmaso & Padisak, 2007; Kruk et al., 2010). A separation by functional groups to test

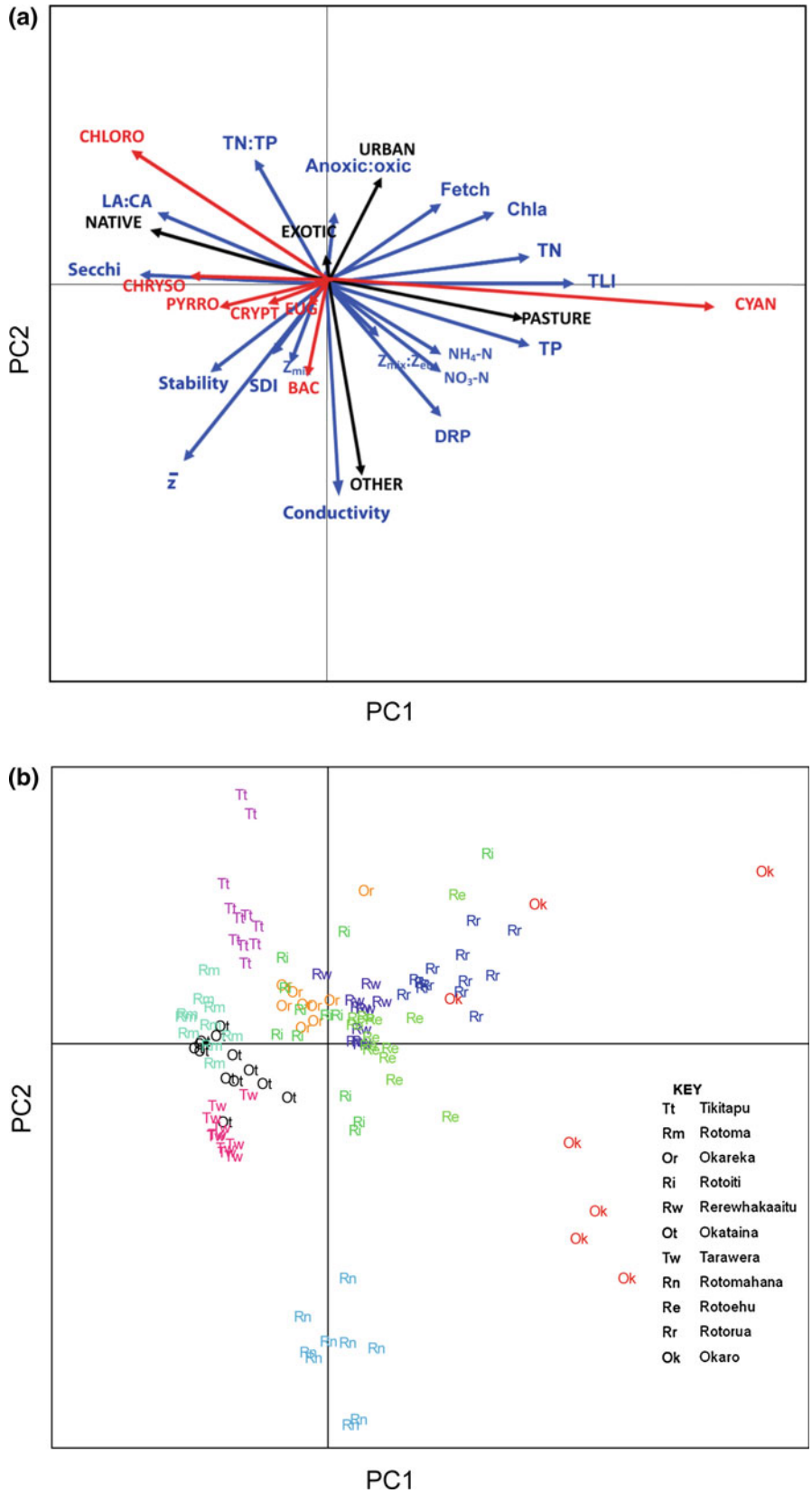

Fig. 4 Principal Components Analysis showing a bi-plot of the first two principal components, representing the two axes which account for the largest amount of variation in the data from Rotorua lakes sampled seasonally from October 2003 to December 2007. The *points* represent measurements for each lake. The variables have been scaled (see text). **a** Gradients for catchment land use shown by *black arrows*, other physical variables shown by the *blue arrows* and phytoplankton by the *red arrows*. **b** Lakes distribution. *TP* total phosphorus, *TN* total nitrogen, NO_3-*N* nitrate, NH_4-*N* ammonium, *DRP* dissolved reactive phosphorus, *Chl-a* chlorophyll-*a*, *DIN* dissolved inorganic nitrogen, *TLI* trophic level index, *SDI* Shannon diversity index, *LA* lake area, *CA* catchment area, *z* depth, z_{mix} mixing depth, z_{eu} euphotic depth, *CYAN* Cyanoprokaryota, *CHLORO* Chlorophyta, *BAC* Bacillariophyta, *CHRYSO* Chrysophyta, *CRYPT* Cryptophyta, *EUG* Euglenophyta, *PYRR* Pyrrophyta. (Color figure online)

Table 3 Annual mean values (from seasonal samples from October 2003 to January 2007) of concentrations of dissolved inorganic nitrogen (DIN) and dissolved reactive phosphorus (DRP), molar ratios of Si:DIN, Si:DRP and TN:TP, and percentage by cell density of Bacillariophyta in the Rotorua lakes

Lake	Annual mean Si (mg $l^{-1)}$	Annual mean DIN (mg m^{-3})	Annual mean DRP (mg m^{-3})	Annual mean Si:DIN ratio	Annual mean Si:DRP ratio	Annual mean TN:TP ratio	Bacillariophyta (%)
Tikitapu	0.2	7.3 (1.2)	1.9 (0.5)	101	170	21.4	5 (4)
Rotoiti	3.3	63.8 (20)	11.3 (3.4)	394	1095	7.5	35 (9.3)
Okaro	2.5	246.8 (96)	23.3 (7.4)	280	658	6.4	5 (8.1)
Rerewhakaaitu	1.4	9.7 (3)	2.4 (0.9)	536	1,289	20.2	1 (1.9)
Rotorua	4.9	30.9 (11)	3.3 (0.4)	814	1,651	6.1	22 (8.1)
Rotoehu	6.7	28.9 (7.7)	11.7 (3.5)	1,295	1,444	5.6	19 (10.9)
Rotoma	6.3	10.6 (2.2)	1 (0.3)	2,007	9,267	12.7	59 (8.4)
Rotomahana	20.1	19.3 (8.4)	14.3 (2.6)	3,910	1,965	5.6	45 (10.8)
Okataina	11.4	8 (1.7)	5.3 (1.4)	4,099	7,254	8.5	73 (8.6)
Tarawera	8.5	5.1 (1)	3.8 (0.7)	4,407	3,445	5.8	21 (8.4)
Okareka	6.6	3.6 (0.8)	1.9 (0.5)	5,598	5,932	11.8	86 (7.8)

Standard error for DIN, DRP and Bacillariophyta values is given in parentheses. Dissolved reactive silica (Si) concentrations are based on a historical annual mean value (McColl, 1975) ($n = 11$)

for variations against relevant morphological, physical and chemical attributes of the lakes represents a logical extension of the research for future studies.

Variables that were statistically related to Cyanoprokaryota abundance may be due to specific features of Cyanoprokaryota including, for example: nitrogen-fixation (e.g. in *Anabaena* species), buoyancy regulation and luxury storage of phosphorus (Pettersson et al., 1993; Zohary & Robarts, 1998). The positive relationship of Cyanoprokaryota to anoxic to oxic water volume ratio may be related to internal loading of phosphorus (Søndergaard et al., 2003) and reduced ratios of TN:TP (Smith, 1983) but some relationships may not necessarily be causal. For example, Cyanoprokaryota may also influence internal loading through the decay of blooms which act as a carbon source to enhance bacterial respiration, thereby leading to anoxia and increased internal nutrient loading (Paerl & Huisman, 2008). These types of complex positive feedbacks amongst Cyanoprokaryota, internal loading and TN:TP ratios (e.g. Hamilton & Mitchell, 1997) are often not easily resolved by a statistical analysis of data in which cause and effect are closely inter-related. The relationship of Cyanoprokaryota abundance to water column stability in this study reinforces the fact that has been well-established, namely that Cyanoprokaryota are more likely to proliferate during quiescent periods associated with low wind (Ryan et al., 2006).

Cyanoprokaryota dominated in three polymictic lakes (Rotorua, Rerewhakaaitu and Rotoehu), and two monomictic lakes (Okaro and Rotoiti) where ratio of mixing depth to euphotic depth tended to be higher than that in the other studied lakes. Prevalence of Cyanoprokaryota increases the turbidity of the water (Paerl & Huisman, 2008), thus reducing the euphotic depth and increasing the likelihood that the mixing depth will exceed the euphotic depth. One can expect that under such conditions, Bacillariophyta should outcompete buoyant Cyanoprokaryota since the former tend to have lower irradiance levels to saturate productivity (Reynolds, 1997; Huisman et al., 2004), but Bacillariophyta also require high levels of turbulence to offset negative buoyancy (O'Brien et al., 2003). Ratio of depth of the upper mixed layer to euphotic depth can also be complicated by varying tolerances to light of different Cyanoprokaryota species (Dokulil & Teubner, 2000).

Chlorophyta are often a sub-dominant group, which may be explained by a slower growth rate than other phyla in relation to TP concentrations and tolerance of lower TP concentrations than other phyla (Watson et al., 1997). Tolerance to low concentrations of phosphorus is the likely reason that Chlorophyta have a positive relationship with native forest and a

negative relationship with pastoral land use, as areal nutrient loads from native forest are low compared with those from urban and pastoral farming land use (Cooper & Thomsen, 1988; Elliot et al., 2005). Chlorophyta is, however, a large and heterogeneous group (Round, 1963) and can be dominant in lakes with high TP concentrations also (Jensen et al., 1994) and may increase and decrease in similar proportions in response to oligotrophication (Jeppesen et al., 2005). It is likely that competitive interactions between Chlorophyta, Cyanoprokaryota and Bacillariophyta occur along nutrient gradients (Reynolds, 1984; Watson et al., 1997). Lake Tikitapu, which was populated almost entirely with Chlorophyta, had the highest TN:TP ratio of all the lakes, lowest Si:DIN and Si:DRP ratios and very low reactive silica concentrations (McColl, 1972) indicating less favourable conditions for growth of Cyanoprokaryota and Bacillariophyta, respectively.

Bacillariophyta growth showed a significant positive relationship with Si:DIN and Si:DRP molar ratios. They tended to dominate in lakes of low trophic state but no significant relationship with trophic state or catchment land use was established. Chrysophyta may utilise silicate for scale formation (Bellinger & Sigee, 2010) and they clustered with Bacillariophyta.

Diversity of plankton communities has been explained by availability of exploitable niches (Reynolds, 1984), the intermediate disturbance hypothesis (Padisàk et al., 1993) and resource-competition (Interlandi & Kilham, 2001). From our data it is possible that a larger number of available niches occur in lakes of lower trophic state similar to observations by Interlandi & Kilman (2001) who found that the highest diversity occurred when resources were limiting. The influence of water column stability on diversity cannot be separated from that of trophic state, however, as lakes with the lowest trophic state tended to be large oligotrophic lakes with highest stability. Prevalence of Chrysophyta has also been found to be indicative of oligotrophic state (Ptacnik et al., 2008) as well as in our study SDI clustered with Chrysophyta and with the oligotrophic lakes.

Early studies of lakes in the Rotorua region had established relationships between land use type and trophic state (McColl, 1972) and independently, between trophic state and phytoplankton composition and abundance (Flint, 1977) but not directly between catchment land use type, trophic state and phytoplankton composition as shown here. Our findings allow us to make some generalisations about restoration efforts for the Rotorua lakes. The relationship between Cyanoprokaryota, trophic level and catchment land use implies that a reduction in blooms, particularly for eutrophic lakes such as Rotoehu and Lake Okaro, will likely require change of land use from one with high areal loading of nutrients (e.g. pastoral farming) to one with low levels (e.g. native forest). Lake restoration efforts for these may also have to address internal nutrient loading as has been undertaken for Lake Okaro through applications of alum (Paul et al., 2008) and a modified zeolite material for phosphorus adsorption (Hamilton & Landman, 2011). The Rotorua region has volcanically formed pumiceous soils that are permeable and free-draining (Hamilton, 2005), and the topsoils in pastoral areas have generally been fertilised to levels of TP > 500 mg g^{-1} (Ross et al., 2009).

In summary, we can infer that to some extent, phytoplankton composition was influenced by land use type and that nutrient loads associated with each land use type were responsible for the positive relationship between land use type and trophic state. Higher trophic states (i.e. poor water quality) were linked with dominance by Cyanoprokaryota, while lower trophic states appeared to be linked with increasing presence of Chlorophyta and possibly also Bacillariophyta. The latter group also showed an association with Si:DIN and Si:DRP molar ratios, indicating that silica may be depleted to limiting levels for Bacillariophyta growth, though the disconnect with the timing of Si measurements lends some subjectivity to these results and indicates the need for more regular monitoring of this variable. Similarly, other primary producers (e.g. macrophytes) and higher trophic levels (e.g. zooplankton, fish) are not monitored routinely in the Rotorua lakes but have previously been shown in other systems to influence phytoplankton production and to potentially structure the phytoplankton composition (e.g. Reynolds, 1987). Some of the residual variability may also be able to be teased apart by additional analysis at seasonal scales. We found that a number of factors were linked to the presence of Cyanoprokaryota, but the overarching factor was catchment land use type. The implications of this relationship are that rehabilitation of some lakes that have become eutrophic may necessitate changes in land use, reversing a trend that has existed for some decades of replacing indigenous forest with high-intensity pastoral farming in the Rotorua lakes area.

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PHYTOPLANKTON

Phytoplankton dynamics in permanent and temporary Mediterranean waters: is the game hard to play because of hydrological disturbance?

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Abstract Only few scientific investigations have been carried out, to our knowledge, on phytoplankton in Mediterranean temporary ponds. To test the hypothesis that climate forcing is the main factor affecting dynamics and structure of planktic algae in these peculiar ecosystems, and to assess the importance of human impacts on this basic component of the aquatic biota, phytoplankton structure and dynamics were analysed in two temporary, long lasting (9 months), ponds, and in a permanent one. The three studied water bodies can be classified as meso-eutrophic, which show extended macrophyte beds and are subjected to one or more human impacts, such as eutrophication, fish and plant introduction, and garbage pollution. Phytoplankton samples were collected monthly over two different periods in each pond. The identified phytoplankton taxa were grouped in functional coda and non-parametric ordination methods were used to analyse their annual patterns. Results showed a well-defined sequence of coda, which followed a common seasonal pattern in all the studied ponds, when the ordination techniques were applied to a singular water body. This pattern was overlapping in the three studied environments without apparent influence exerted either by the environmental typology (e.g. permanent or temporary) or by human impacts. However, when the analyses were carried out by means of a single matrix containing the coda shared by all the studied environments, they formed a cluster separating the single ponds rather than following common/overlapping seasonal patterns. The results suggest that local effects, particularly the specific composition and richness of phytoplankton assemblages, are as important as climate constraints.

Keywords Mediterranean temporary pond · Functional classification · Coda · Metaphyton · Rare species

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Introduction

Freshwater ponds are small ecosystems distributed worldwide, although they are often neglected. They disproportionately contribute to regional biodiversity because of the high compositional dissimilarity existing among sites (De Meester et al., 2005), which is

probably linked to their high productivity (Naselli-Flores & Barone, 1994). In addition, in the particular case of temporary ponds, the occurrence of organisms showing specific adaptations to bridge the dry periods results in a very peculiar biota, which is able to deal with the extreme variability of these ecosystems.

In recent years, the scientific community has been showing an increasing interest in these ecosystems, recognizing their complexity (Wilbur, 1997) and their role as model systems (Céréghino et al., 2008); symposia have been regularly organized on issues concerning the conservation and monitoring of temporary and permanent pond biodiversity (Oertli et al., 2005, 2010), and the *European Pond Conservation Network* (EPCN) was launched in 2004 to promote conservation of ponds and their biodiversity all around Europe. Unfortunately, this increased interest has not been counterbalanced yet by an equal and general interest from the public opinion and/or from local authorities.

Since then, several articles dealing with almost all the biological groups inhabiting temporary and permanents ponds have been published. However, to our knowledge, only a very small number of articles on phytoplankton in temporary ponds are available in the literature (e.g. Robarts et al., 1995; Moyá & Conforti, 2009; Cunha Pereira et al., 2010). Among these, only Naselli-Flores & Barone (2002) offered a view on both structural and dynamical aspects of phytoplankton in a Mediterranean temporary pond. This can be partly due to the inherent difficulties in clearly identifying the planktic compartment in these very shallow (maximum depth 1–2 m, but often less than 1 m) aquatic ecosystems. Actually, shallowness not only enhances competition among planktic, benthic and metaphytic primary producers, but, depending on hydrodynamic conditions, metaphytic microalgae may enter the plankton and spend there, eventually blooming, a large part of their life history. In the same way, truly planktic algae may settle on bottom sediments and pass a part of their life history out of the plankton. Moreover, the general low interest of the public for this kind of ecosystems makes difficult and not rewarding enough the setting up of the costly and time-consuming analyses aimed at clarifying phytoplankton dynamics and structure.

Among temporary ponds, those occurring in the Mediterranean part of Europe were recognized as being particularly important for their biodiversity, and they were included as a Priority Habitat for the EU under the auspices of the Habitats Directive 92/43/EEC. More in general, these ecosystems represent in the Mediterranean area the most diffuse typology of natural aquatic environments and, among these, they are presently disappearing at a very fast rate (Cancela da Fonseca et al., 2008). Actually, these ecosystems, which are generally small and shallow, can be recognized as aquatic environments only during the water phase, which, according to Mediterranean climatic features, occurs during the fall/winter rainy season. The extent of the hydroperiod varies from a few weeks to several months depending on the morphological characteristics of these environments, as well as their dry phase. In this last phase, they can hardly be recognized as aquatic ecosystems and appear as land depressions. They are thus severely subjected to human impacts since perfectly suitable to be filled up with litter or wastes and to be appointed for agricultural and urban development (Marrone et al., 2006). In addition, due to their small dimensions, they can greatly suffer pollution by fertilizers, pesticides or garbage, unsustainable water subtraction and/or deepening for conversion into permanent water bodies to fulfil irrigation needs or, paradoxically, to protect aquatic birds. However, the present climate change of Mediterranean basin and the associated overexploitation of underground and surface waters are the factors which mostly threaten temporary ponds, affecting the duration of their hydroperiod and its displacement.

Studies carried out on phytoplankton dynamics and structure in Sicilian temporary and permanent ponds (e.g. Naselli-Flores & Barone, 2002; Barone et al., 2010) have suggested the existence of a seasonal and compositional coherence in the dynamics and ecological structure of phytoplankton in these two typologies of aquatic ecosystems. This coherence is more pronounced in those temporary ponds characterized by long hydroperiods ($\geq$9 months), and it does not appear to be influenced by the breaking in the continuity of the aquatic phase caused by summer drying. This would suggest that temperature and precipitation patterns (and more in general climatic features) greatly contribute to determine the structure and dynamics of phytoplankton assemblages in Mediterranean ponds on a regional scale. Moreover, human impacts (e.g. eutrophication, water overexploitation, introduction of alien species and pollution) are supposed to be harsher on these small ecosystems,

and thus they should likely affect phytoplankton development causing a change in its seasonal patterns.

The aim of this article, in accordance with the main topic of the 16th Workshop of the International Association for Phytoplankton Ecology and Taxonomy (IAP), is thus to verify the influence of human impacts on the phytoplankton dynamics and structure of these fragile ecosystems, and to test the hypothesis that climate forcing is the main factor affecting the structure and the dynamics of planktic algae in Mediterranean natural ponds. Moreover, we also tried to evaluate how much and in which way it is possible to describe environmental changes by tracking the dynamics of phytoplankton functional groups. To fulfil this task, we compared phytoplankton structure and dynamics recorded, in different years, in one permanent and two temporary Sicilian ponds.

Materials and methods

Study sites

Three different ponds were selected in this study: two temporary ['Gorgo di Rebuttone' (GdR) and 'Stagno di Santa Rosalia' (SSR)] and one permanent ['Biviere di Gela' (BdG)]. All the water bodies can be classified as meso-eutrophic, show well-developed macrophyte beds and are subjected to one or more human impacts, such as eutrophication, fish/plants introduction, pollution, disturbance and other stresses.

The GdR is located close to the city of Palermo (38°01′42″N; 13°19′36″E) at 720 m a.s.l., just outside a natural reserve. It is a karstic depression with the bottom covered by clay. Its hydroperiod generally spans from October to July depending on precipitation intensity and duration. It has an elliptical shape with its main axis reaching about 80 m at maximum holding (z_{max}: 2 m). The bottom of the pond during the water phase is densely covered by macrophyte beds (*Nitella opaca* (C. Agardh ex Bruzelius) C. Agardh, *Potamogeton natans* L. and *Zanichellia palustris* L.). In spite of its temporary character, local people, from time to time, introduce fish, which are destined to die when the water body dries out. In 2008, *Cyprinus carpio* L. and *Perca fluviatilis* L. were observed in the pond.

The SSR is located on a calcareous promontory (Monte Pellegrino) which delimits the northern border of the Gulf of Palermo (38°10′12″N; 13°21′03″E), at 398 m a.s.l. This is also a karstic depression with the bottom covered by clay. In 1948, the Sicilian Forest Department built on its southern shore a concrete wall 2.5 m high, to increase the duration of the water phase and use the pond to drink cattle and to irrigate *Pinus halepensis* Miller and *Eucalyptus globulosus* Labillardière plantations, which in the present days is surrounding and sheltering the pond. G.E. Hutchinson was inspired to write his seminal article 'Homage to Santa Rosalia or why are there so many kind of animals?' after visiting the pond at the end of 1957 (see Naselli-Flores & Rossetti, 2010). In 1996, a natural reserve was created to protect the peculiar fauna and flora of the promontory on which lies the pond. As for the Gorgo di Rebuttone, its hydroperiod generally starts in October and lasts until mid-July. It has an elliptical shape with its main axis reaching about 50 m at maximum holding (z_{max}: 2 m). In both studied periods, the bottom of the pond during the water phase was densely covered by macrophyte beds (*Chara globularis* J.L. Thuiller, *Spirogyra ellipsospora* Transeau and *S.* cf. *lodziensis*). In 2010, the introduction of *Lemna minuta* Kunth, an invasive duckweed from North America, apparently caused the disappearing of most planktic organisms in the pond (Marrone & Naselli-Flores, 2011).

The BdG is the largest natural coastal lake in Sicily (≈ 1 km^2) and one of the few natural wetlands left on the island. It is a brackish water body, with conductivity values ranging between 2.2 and 2.7 mS cm^{-1}, located in SE Sicily (37°01′13″N, 14°20′30″E), 1.3 km from the Mediterranean coast and 8 m a.s.l. In 1987–1988, its maximum length was 2.5 km, maximum depth fluctuated between 6.5 and 7.5 m and mean depth was less than 3 m. The pond has no surface outflow and no important inflow, being fed during the rainy season by an ephemeral surface stream. In 1988, it has been designated for the List of Wetlands of International Importance and included in the 'Ramsar Act'. In 1997, a Nature Reserve has been created by the Sicilian Region to preserve the several rare and endangered vertebrate species (e.g., amphibians, turtles, and birds) that inhabit the pond. At the beginning of the millennium, it suffered an almost complete drying because of the decreased water availability from the catchment. To preserve this Ramsar site, at the end of 2004, it was suddenly filled up with water coming from a nearby reservoir and depth passed from 1.5 to 5.5 m in a few days. The

consequences of these events, which deeply influenced the composition and dynamics of phytoplankton, were analysed in details by Barone et al. (2010).

Phytoplankton and environmental data

Data collected in two different periods in each water body were selected for this study. Six different datasets were used: GdR was sampled in 2003–2004 and in 2007–2008, SSR in 1998–2000 and in 2008–2009 and BdG in 1987–1988 and 2005–2007. Samples were collected with different frequencies (weekly, biweekly or monthly) in the different water bodies and in the different periods. In the present study they were pooled in monthly data to uniform the six datasets.

Water samples for phytoplankton analysis were collected sub-superficially in all the three water bodies. At the same time, net-plankton samples were taken by towing vertically and/or horizontally a 25-μm mesh net. Half of the concentrated samples was fixed in 4% buffered formaldehyde. Species identification was carried out on living samples using the most up-to date phycological literature. Phytoplankton water samples for counting were immediately preserved in Lugol's iodine solution; cell counting was performed using a Zeiss-Axiovert 100 inverted microscope in accordance with the sedimentation method developed by Utermöhl. Wet weight biomass was calculated from recorded abundance and specific biovolume estimates, based on simple geometric shapes (Hillebrand et al., 1999) and assuming unit specific gravity. Species were pooled into functional groups following the recommendations given by Padisák et al. (2009). For those species not listed in this last quoted article (e.g. *Chaetoceros muelleri* Lemmerman, brackish water species), attribution to a specific codon was performed according to what specified in Reynolds et al. (2002).

Water samples for nutrients (SRP, N-NH_4, N-NO_2, and N-NO_3) were collected sub-superficially, refrigerated and taken to the laboratory where the analyses were performed according to Tartari & Mosello (1997). Temperature and conductivity values in the water were recorded using a mutiparametric probe.

Statistical analyses

The datasets used for statistical analyses consisted of species-specific biovolume estimates of phytoplankton. Phytoplankton taxa that were present with a relative biovolume greater than 1% in more than one sample, were included in the ordination analyses. The monthly biovolume values were transformed to reduce the weight of the most abundant taxa. Two different transformation procedures ($y_i = \log(x_i + 1)$; $y_i = \sqrt[4]{x_i}$) were used, according to data distribution. In each water body, three matrices were prepared and analysed: one phytoplankton matrix (taxa × sampling dates) considering the original data (the biomass of the single species), and two additional synthetic matrices after grouping the algae (i) into functional groups and (ii) into algal classes. For both the studied periods, the three matrices were transformed into dissimilarity matrices by computing the Bray–Curtis' dissimilarity index (Podani, 2000) and then ordinated separately in a two-dimension plane, by means of non Metric Multidimensional Scaling (nMDS). Two additional matrices were analysed in the same way. These were prepared using only (i) the functional groups and (ii) the species shared by all the studied environments. In addition, as regards the permanent water body (BdG) only the dataset collected in 1987–1988 was used, and only the 9-month period corresponding to the water phases of the two temporary environments were considered. All the ordinations were performed with the free software PAST (Hammer et al., 2001). To assess the reliability of NMDS graphs, stress value was computed according to Kruskal & Wish (1978).

Results

Environmental parameters and phytoplankton biomass

The annual variability of the main environmental parameters in the studied water bodies is shown in Figs. 1 and 2.

As regard the studied temporary ponds, the water phase generally begins in late September/early October and ends in July (Fig. 1). The figure represents a synthesis of the measures carried out in both the studied temporary ponds during all the sampling periods. In this case, the trends followed by the variables are considered much more informative than absolute numerical values (shown in Supplementary material), which may be highly variable depending on the variability of meteorological elements. Strictly

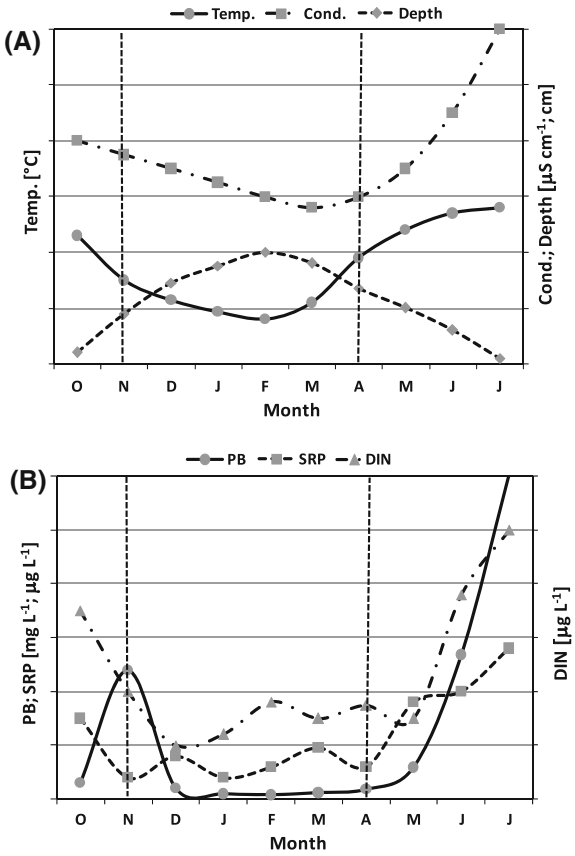

Fig. 1 Trends of selected environmental variables in the studied temporary ponds. **A** Temperature (Temp.), conductivity (Cond.). **B** Phytoplankton biomass (PB), soluble reactive phosphorus (SRP) and dissolved inorganic nitrogen (DIN). *Vertical dashed lines* delimit the most frequent period of occurrence of annual macrophytes

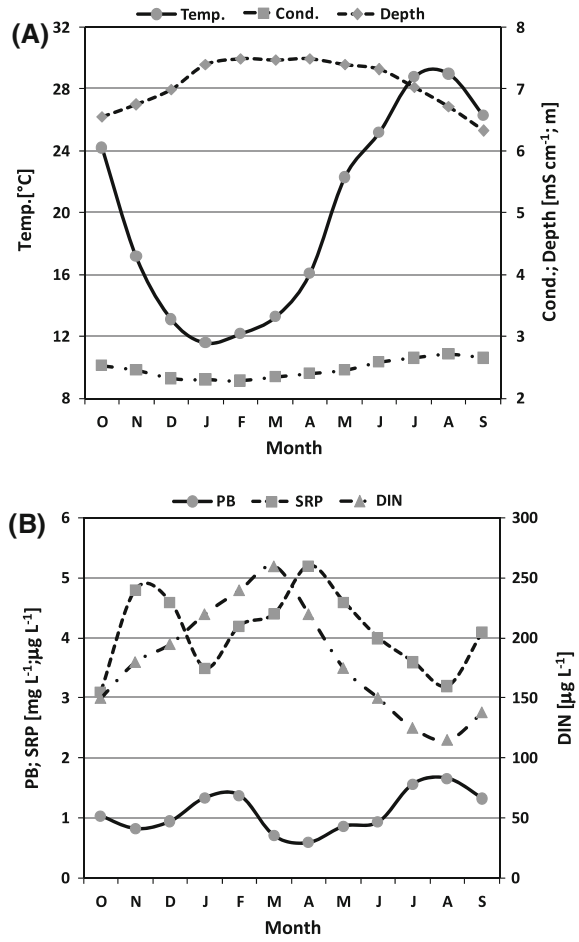

Fig. 2 Trends of selected environmental variables in the studied permanent pond during 1987–1988. **A** Temperature (Temp.), conductivity (Cond.). **B** Phytoplankton biomass (PB), soluble reactive phosphorus (SRP) and dissolved inorganic nitrogen (DIN)

linked to the precipitation regime under the Mediterranean climate, water temperatures and conductivity in the temporary ponds (Fig. 1A) show their minimum values at the end of winter/beginning of spring. The conductivity minimum appears to be delayed and follows that of temperature because of the occurrence of early spring precipitation events and their dilution effects. After this, the rapid spring increase of temperature and the absence of rain enhance evaporation effects and conductivity values again start increasing up to their maximum annual value, which generally precede the drying of the pond. The patterns of precipitation, water temperature and evaporation rates also influence the maximum depth, and thus the minimum temperature generally corresponds to the highest annual precipitation and maximum depth of these temporary water bodies. Deviations from the above described patterns largely depend on annual weather fluctuations, which are typical of Mediterranean climate. As an example, the water phase in GdR was much more prolonged in 2007–2008 because of the intense winter–spring precipitation in both the sampled years. This allowed the pond to maintain some water in the deepest depressions of its bottom throughout summer 2007 and 2008.

Phytoplankton total biomass (PB) in the temporary ponds showed a bimodal trend (Fig. 1B) with one peak (>20 mg l^{-1}) a few weeks after the beginning of the water phase and a second one which started in spring and reached its maximum (>60 mg l^{-1}) just before the complete drying of the water bodies. The period

between these two peaks is always characterized by very low values of phytoplankton biomass ($1 < PB < 2$ mg l^{-1}).

Soluble reactive phosphorus (SRP) and dissolved inorganic nitrogen (DIN) in the temporary ponds appear to be abundant and immediately available for phytoplankton growth since the beginning of the water phase (Fig. 1B). In both the studied environments and in all the studied periods, at the beginning of the water phase, SRP values are above 10 μg l^{-1} and DIN values are above 300 μg l^{-1}. Then, they rapidly decrease and fluctuate around lower values during winter and early spring. A second increase occurs in May, immediately followed by the second phytoplankton peak.

The trends of temperature, conductivity and depth in the permanent water body (Fig. 2A) are similar to those shown in temporary ponds, once the temporal sequence is ordered from October to September. Only the data recorded during 1987–1988 are shown in the figure because this period was considered more comparable than the period 2005–2007 when, to ward off the complete drying of the pond, management procedures were applied, which, being aimed at a sudden increase of the water level, deeply altered the phytoplankton composition and dynamics (for more details see Barone et al., 2010).

Phytoplankton biomass values are lower than in temporary ponds (<2 mg l^{-1}) with one peak in February and a second one in August (Fig. 2B). These lower values recorded in the permanent pond reflect the minor availability of dissolved nutrients for phytoplankton growth. As regards DIN, the values showed a unimodal trend with one peak in March (260 μg l^{-1}); a bimodal trend, out of phase with phytoplankton biomass, is followed by SRP, with one peak in November (4.8 μg l^{-1}) and a second one in May (5.2 μg l^{-1}).

Phytoplankton composition

A very high species richness characterized the microalgal flora found in the plankton of the studied ponds and more than 450 taxa were identified. Phytoplankton class composition in all the studied ponds showed a clear seasonal pattern with cryptophytes and/or diatoms dominating in winter and chlorophytes in early spring. These were followed by an assemblage rich in euglenophytes, often accompanied by dinoflagellates, which co-dominated the late-spring/summer assemblages. This pattern, although characterized by several different species, was quite regular in all the studied periods except in the permanent pond (BdG) in 2005–2007. In this period a quite different dynamics and structure of phytoplankton assemblages was detected: the haptophyte *Prymnesium parvum* Carter replaced cryptophytes and diatoms in winter and chlorophytes extended their period of dominance or subdominance throughout all the investigated period, with peaks of biomass in late summer. Moreover, cyanobacteria, which were virtually absent in 1987–1988, bloomed in early summer with relative biomass values ranging between 40 and 60% of the total.

In temporary ponds, among typical and common phytoplankton species (e.g. *Pediastrum* spp., *Coelastrum* spp., *Botryococcus braunii* Kützing, *Mucidosphaerium pulchellum* (H.C. Wood) C. Bock, Proschold & Krienitz, *Staurastrum* spp., *Ceratium hirundinella* (O.F. Müller) Dujardin, *Peridinium* spp., *Plagioselmis nannoplanctica* (Skuja) Novarino, Lucas et Morral, *Cryptomonas* spp.), the occasional presence of several metaphytic species was very often recorded. These species are not truly planktic but they can significantly contribute to the total biomass of phytoplankton. Their appearance in the plankton of the studied temporary ponds was generally punctual (e.g. occurring in a single sample) and linked to resuspension events. All these not-truly planktic species were grouped in the **MP** codon.

Among the most abundant metaphytic organisms exclusively found in the temporary ponds, *Glaucocystis nostochinearum* Itzigsohn, a member of Glaucocystophyta, reached, in April 2007, the 16% of total phytoplankton in GdR. In the same pond another member of this phylum, *Cyanophora paradoxa* Koršikov, was found in July 2004 with a much lower relative biomass value (2%). The volvocalean *Rusalka fusiformis* (Matv.) Nakada showed a relative contribution to total biomass higher than 20% in early spring 2008, whereas several other species (e.g. *Diacanthos belenophorus* Koršikov, *Vitreochlamys fluviatilis* (Pascher) Batko, *Treubaria triappendiculata* C. Bernard, *Chlorogibba allorgei* Bourrelly & Manguin, *Polyedriopsis spinulosa* (Schmidle) Schmidle) showed a contribution ranging between 2 and 3%. In SSR, the 'metaphytic contingent' was formed, among others, by *Keratococcus bicaudatus* (A. Braun ex Rabenhorst) J.B. Petersen, *Centritractus belenophorus* Lemmermann, *Spermatozopsis exsultans* Koršikov. Even in

this case, these species gave a small contribution to total biomass.

Phytoplankton ordination and occurrence of functional groups

In all the studied ponds and in all the periods, matrices formed by phytoplankton single species did not result in clear ordinations. Conversely, the configurations of the phytoplankton samples pooled into selected functional groups and obtained by NMDS resulted in a temporal arrangement in each pond for both the studied periods (Figs. 3, 4, 5). The stress values of these NMDS configurations are always lower than 0.20. Ordination obtained using the phylogenetic classes is superimposed to the one obtained using the functional groups. The latter contemporary allows a higher resolution than what was provided by classes and offer a higher readability than the single species. Functional groups clearly reveal the temporal ordination of the samples and are divided into two main groups, which may be identified with summer and winter seasons, in all the studied periods.

Phytoplankton species of the three ponds were allocated in 20 coda. However, only eight coda (**W1**, **W2**, **X1**, **X2**, **J**, **F**, **Y** and **MP**) were present in all the studied environments but in the permanent pond in the period 2005–2007, when no representatives of the coda **W1** (not bottom-dwelling euglenoids), **W2** (bottom-dwelling euglenoids) and **MP** (meroplankton) were found.

In GdR, 11 functional groups were present in 2003–2004 and 15 in 2007–2008 (Fig. 3). The higher number of functional groups in 2007–2008 was mainly due to the summer appearance of the coda **S1**, **H1** and $\mathbf{L_M}$. All these coda are formed by cyanobacteria: codon **S1** groups shade-adapted cyanobacteria

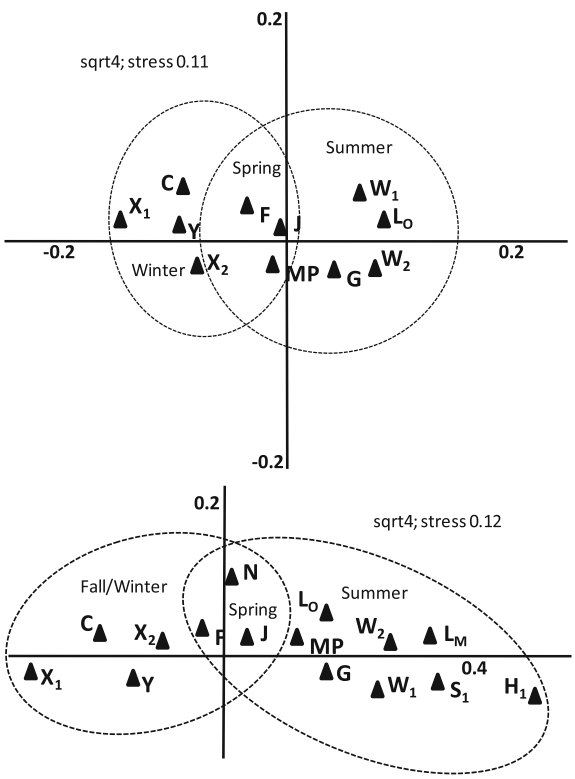

Fig. 3 Gorgo di Rebuttone (GdR). Non metric multidimensional scaling plots of the phytoplankton pooled into functional groups in the two studied periods. Above 2003–2004; below 2007–2008

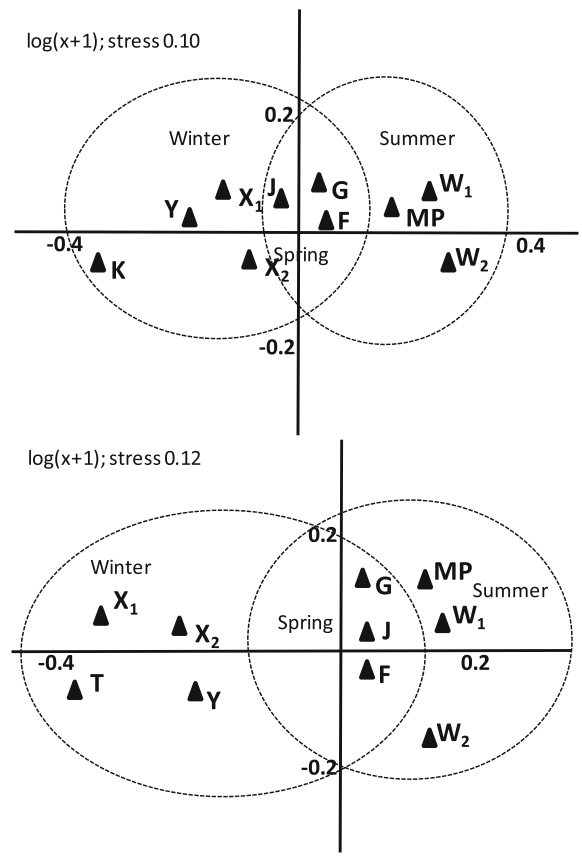

Fig. 4 Stagno di Santa Rosalia (SSR). Non metric multidimensional scaling plots of the phytoplankton pooled into functional groups in the two studied periods. Above 1998–2000; below 2008–2009

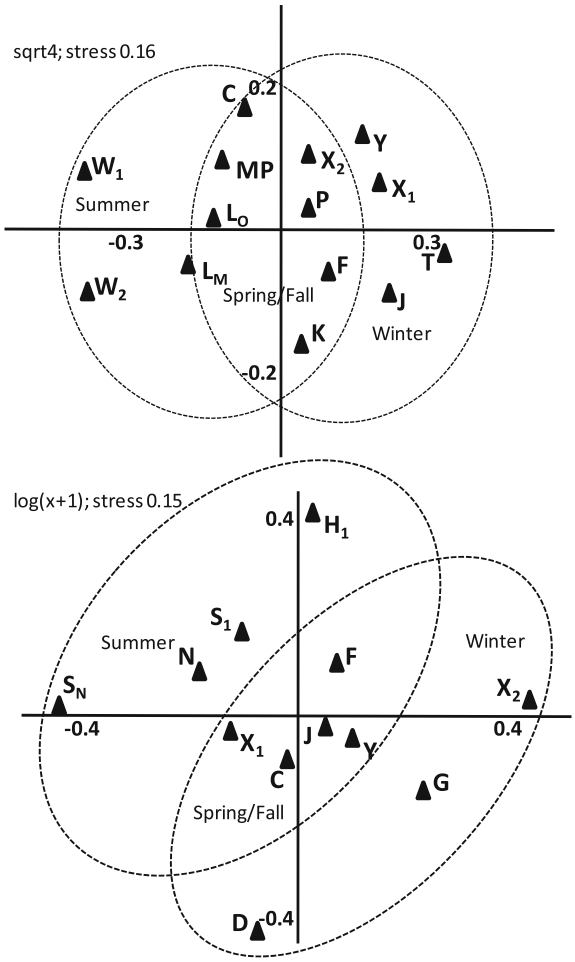

Fig. 5 Biviere di Gela (BdG). Non metric multidimensional scaling plots of the phytoplankton pooled into functional groups in the two studied periods. Above 1987–1988; below 2005–2007

(*Limnothrix* sp., *Planktothrix* sp.); codon **H1** collects gas-vacuolated nostocaleans [*Dolichospermum planctonicum* (Brunnth.) Wacklin, L.Hoffm. & Komárek and *Anabaenopsis elenkinii* V.V. Miller], and codon $\mathbf{L_M}$ is formed by *Microcystis* spp. co-occurring with *C. hirundinella* (O.F. Müller) Schrank. These coda were observed to co-occur with codon **W1** (*Euglena* spp., *Lepocinclis ovum* (Ehrenberg) Minkevich, *Phacus* spp.) and **W2** [*Trachelomonas* spp., *Strombomonas acuminata* (Schmarda) Deflandre]. In addition, codon **N** [*Staurastrum crenulatum* (Nägeli) Delponte], typical of continuous or semi-continuous mixed layer of 2–3 m in thickness, dominated late-winter/early-spring assemblage in 2008 accompanied by members of codon **J** (*Pediastrum* spp., *Coelastrum* spp., *Scenedesmus* spp., *Desmodesmus* spp.) and **F** (*Oocystis* spp., *Kirchneriella* spp., *B. braunii* Kützing). Coda **X1** (*Monoraphidium* spp., *Ankistrodesmus* spp.), **X2** (*Plagioselmis* sp., *Chlamydomonas* spp.), and **Y** (large cryptomonads, small dinoflagellates) characterized the fall/winter period in 2007–2008.

In SSR, 10 coda were identified in 1998–1999 as well as in 2008–2009 (Fig. 4). Codon **K** (*Aphanotece* sp., *Synechococcus* sp.) characterizing winter in 1998–1999 is replaced by codon **T** (*Planctonema lauterbornii* Schmidle, *Mougeotia* sp.) in 2008–2009. No other major changes can be noted by comparing the ordinations in the different studied periods but the appearance of the xanthophyceans *Goniochloris mutica* (A. Braun) Fott and *G. smithii* (Bourrelly) Fott (codon **J**) which bloomed at the beginning of March 2009, reaching in one single sample a relative value above 60% of total biomass.

In BdG, by comparing the two nMDS ordinations, it is possible to see that a lower number of coda characterized the second, more recent period, and only five of these coda (**C**, **F**, **J**, $\mathbf{X_1}$, **Y**) were present in both periods (Fig. 5). In some cases, the species included in the group were not the same: codon **C**, mainly formed by *C. muelleri* Lemmermann in 1987–1988, contained *Cyclotella meneghiniana* Kützing in 2005–2007, and codon **J** was much richer in species in 2005–2007 than in 1987–1988. Moreover, the seasonal occurrence of these shared groups showed a shift from winter, in 1987–1988, to summer in 2005–2007. New coda, not present in the pond in 1987–1988 appeared in 2005–2007 replacing euglenoids, which disappeared in the pond. These are formed by the cyanobacteria *Cylindrospermopsis raciborskii* (Wolosz.) Seena. & Subbar. ($\mathbf{S_N}$), *Aphanizomenon ovalisporum* Forti (**H1**) and *Pseudoanabaena limnetica* (Lemmermann) Komárek (**S1**).

The NMDS plot showing the ordination of the functional groups shared by all the studied ponds in all the investigated periods (with the exclusion of the permanent pond in the period 2005–2007) is shown in Fig. 6. Only eight coda (**W1**, **W2**, **J**, **F**, **X1**, **X2**, **Y** and **MP**) were used in this analysis. These may be regarded as the basic pool of ecological strategies exhibited by phytoplankton assemblages in Mediterranean ponds. No seasonal patterns resulted from the ordination. Conversely, the coda of each pond grouped together and formed three clearly separated clusters.

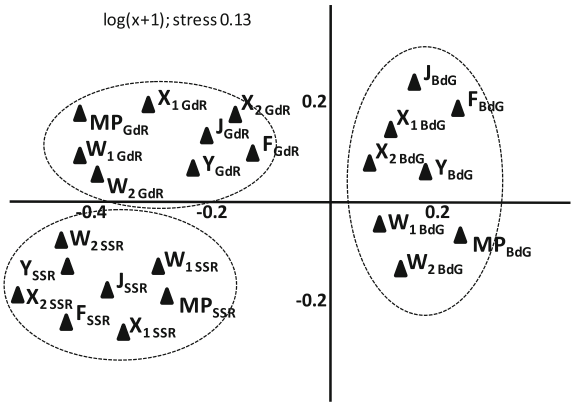

Fig. 6 Non metric multidimensional scaling plot of the eight phytoplankton functional groups shared by the studied ponds. BdG: Biviere di Gela; GdR: Gorgo di Rebuttone; SSR: Stagno di Santa Rosalia

Discussion

Environmental parameters and phytoplankton biomass

Mesotrophic and eutrophic shallow lakes and ponds exhibit alternative stable states either dominated by macrophytes or by phytoplankton (Scheffer, 1998). Macrophytes play a crucial role in creating and maintaining a clear water phase in these ecosystems by several mechanisms (e.g. Van Donk & Van de Bund, 2002). Among these, one of the most studied although not definitively demonstrated (Gross et al., 2007), is allelopathy. A major ecosystem stress is thus necessary to move an aquatic ecosystem from a clear water, macrophyte-dominated state to a turbid, phytoplankton-dominated alternative one. This was the case of the permanent pond BdG which, in the early years of this century, was almost disappearing because of a reduction of its water income (Barone et al., 2010). A disruption of the wide littoral zone occurred with the consequent loss of its perennial macrophytes and an increased nutrient availability for phytoplankton. Thus, the structure and dynamics of phytoplankton has shown deep changes and a shift from a clear state (1987–1988) to a turbid one (2005–2007) has taken place, as predicted by Sheffer's theory on the alternative stable states (Scheffer, 1998).

From the results as above achieved, it appears that, In contrast to what happen to permanent shallow lakes and ponds, temporary ponds pass physiologically from a turbid, phytoplankton dominated state to a clear, macrophyte dominated one and vice versa. The reasons for this peculiar behaviour lie in the intrinsic functioning features of these environments: temporary ponds are natural bodies of water which experience a recurrent dry phase; this is predictable both in its time of onset and duration (Williams, 2006). The cyclical nature of the dry phase and its predictability thus allows temporary water species to be adapted to water loss. Macrophytes inhabiting Mediterranean temporary ponds are not perennial, and are represented by annual plants (including Charophyta), which quickly germinate after the first rains in early autumn and grow throughout winter until reproduction in late winter or spring; other species germinate at the end of winter when water recedes and reproduce faster (Grillas et al., 2009). Macrophytes in the studied temporary ponds completed their life-cycles by mid-April and naturally disappeared afterwards. Before their germination and after the end of their annual cycle, phytoplankton have thus access to unlimited amounts of resources, which allow them to attain high biomass values.

In the studied temporary ponds amount of available nutrients (both DIN and SRP) higher than those occurring in the permanent one were recorded. This is also linked to the temporary nature of these environments. Mozley (1944), in one of the first articles dealing with temporary ponds, suggested that the seasonally alternating summer dry phase and winter water phase mutually feed each other. Terrestrial and aquatic communities are actually linked by a reciprocal legacy of organic matter which is seasonally left from one community to the following. The mineralization of this organic matter largely depends on the establishment of a well-structured and efficient microbial community, which could sustain a quick recycling of inorganic nutrients readily available to primary producers (Battle & Mihuc, 2000).

As shown by Fazi et al. (2008), in Mediterranean temporary waters the aquatic microbial community is fully functioning 9–28 h after a pond passes from the dry to the wet phase. Although the time needed to reactivate the aquatic microbial food web after the arrival of new water largely depends on the duration and intensity of the precedent dry phase (Amalfitano et al., 2008), this is generally quick enough to supply high amount of inorganic nitrogen and phosphorus to phytoplankton, allowing this primary producers to

peak in a few days, well before the establishment of the plant community. Later on, plants (and epiphytic algae) start their life-cycle and monopolize the resources keeping phytoplankton biomass at low values. A similar trend of phytoplankton biomass was described by Ribeiro Rodrigues et al. (2011) who studied the plankton dynamics of a subtropical temporary wetland and by Cunha Pereira et al. (2010) who investigated primary production in turloughs (Irish karstic temporary ponds).

When water starts receding in spring, plants have generally completed their cycle and their organic matter enters the microbial food chain. Available DIN and SRP tend to increase again in this period probably sustained by internal recycling and phytoplankton show its second biomass peak. This second, more intense, peak of biomass is also enhanced by the water reduction, which concentrates phytoplankton cells, and lasts up the complete desiccation of the water body.

Phytoplankton composition

The high number of species which characterizes the phytoplankton microflora of the studied ponds, and especially those of temporary ponds, has to be sought in the environmental features of these ecosystems. The temporary ponds selected in this study host a dense plant community during their water phase and the bottom was almost completely covered by macrophyte from mid-November to mid-April in the studied periods. During the macrophyte-dominated periods, planktic species were found to coexist with metaphytic microalgae and with microphytobenthos, since in these ecosystems, a typical pelagic zone do not exist during the plant growing period. This mixture of microalgae was more evident in the temporary ponds than in the permanent one and can be related to the higher macrophyte coverage of these environments. According to Borics et al. (2003), in those environments where aquatic macroflora is diverse and morphologically structured, many microhabitats may develop and a richer microflora generally occurs. In addition, as shown by Krasznai et al. (2010), the composition of phytoplankton assemblages in ponds is exclusively dependent on macrophyte coverage; these authors, studying 64 Hungarian ponds characterized by different macrophyte coverages, found that when the coverage was higher than 40%, the occurrence of cyanobacteria (coda **S1**, **H1**) was unlikely, whereas euglenoids and metaphytic volvocalean species developed frequently. Accordingly, in the present study cyanobacteria belonging to **S1**, **H1** appeared in the temporary GdR in summer 2007–2008, when an unusually prolonged water phase occurred after the disappearing of the annual plant community. Also in the permanent pond BdG, the suppression of the macrophyte coverage promoted the appearance of bloom-forming cyanobacteria belonging to the coda **S1**, **H1** and $\mathbf{S_N}$. Since the macrophytes of the permanent pond were perennial species, their removal caused a permanent shift from a macrophyte-dominated environment with clear waters to a phytoplankton-dominated, turbid environment.

More in general, a highly diversified microflora formed by metaphytic microalgae and euplanktic green algae was commonly found in winter and spring. Euglenoids were dominating the pond microflora since mid-April to the end of the water phase when they were generally found to attain high biomass values.

Phytoplankton ordination and occurrence of functional groups

Mediterranean area is characterized by periodical oscillations in precipitation and temperature which may deeply affect the functioning of the aquatic ecosystems subjected to its peculiar climate (Naselli-Flores, 2003; Naselli-Flores & Barone, 2005; Naselli-Flores et al., 2007). The interannual variability in the total amount and monthly distribution of precipitation causes significant variations not only at the length of the Mediterranean temporary ponds' hydroperiod but also at the start of their flooding period (Dimitriou et al., 2009). Naselli-Flores & Barone (2002) showed that these two variables may strongly condition the dynamics of phytoplankton assemblages inhabiting temporary ponds.

In spite of the strong influence exerted by meteorological elements, all the studied ponds exhibited a temporal gradient which was marked by a clear sequential appearance of functional groups. In particular, the seasonal sequence of coda **X1/Y** → **X2** → **F** → **J** → $(\mathbf{L_O})$**W1/W2**, which appeared proceeding from the flooding to the drying phase, strongly characterizes the seasonal succession of phytoplankton in Mediterranean temporary ponds. These coda,

along with the **MP** one, were commonly found in all the studied ponds. Since functional groups closely reflect the adaptive strategies of phytoplankton to environmental constraints (Reynolds et al., 2002), this recurrently observed sequence must have an ecological meaning. In fact, the codon **X1** collects small, fast-growing algae inhabiting turbid, nutrient-rich environments. They well cope with the initial flooding phase when a high nutrient availability and the absence of zooplankton may favour them in the formation of biomass bulks. Also members of the codon **Y**, which collects mixotrophic cryptomonads, are favoured by the absence of zooplankton and by the abundant organic matter typical of the flooding phase. Codon **X2** mainly develops in clearer meso-eutrophic water bodies. This is the condition which follows the flooding phase, when annual plants start growing, and water transparency increases. As the depth of the pond increases, codon **F** representatives become more frequent. This functional group is described as typically found in clear epilimnia. Later on, when the plants start disappearing, codon **J**, members of which are abundant in shallow, nutrient-rich environments, follows with high biomass values. The drying phase is characterized by the very diversified assemblage mainly formed by euglenoids (**W1** and **W2**) often accompanied by dinoflagellates ($\mathbf{L_O}$). They generally persist up to the complete desiccation of the pond, often forming a green thin layer on the wet soil. Moreover, due to their photoauxotrophic metabolic needs, they start becoming abundant when the life cycle of annual plants is completed and the availability of organic matter in the water is higher.

The sequence of these phytoplankton functional groups thus summarizes and represents the seasonal progression of ecological events, which characterizes the water phase of the Mediterranean temporary ponds. In addition, it may be argued that these groups collect species which are better adapted to survive the dry phase. Other groups of microalgae likely colonize these water bodies from surrounding permanent environments (Chrisostomou et al., 2009) and their success can be variable in different years. Moreover, several taxa belonging to the coda **X1**, **X2** and **J** are commonly found among airborne autotrophic eukaryotes (Genitsaris et al., 2011), thus suggesting that members of these coda are the most probable re-colonizers of ponds after the dry phase. A confirmation to this hypothesis comes from the minor number of coda, and their lower variability, recorded in the more sheltered SSR. This pond is actually surrounded by woodlands, conversely to GdR, which lays in a grassland landscape and which showed a higher variability in the number of coda recorded in the two studied periods.

It can be argued that the dry phase act as a 'resetting" phase of the temporary systems, which can re-open the competitive arena to new potential colonizers (Foissner, 2006), and eventually counteract some human impacts such as garbage pollution and the introduction of fish and plants. Actually, the survivorship of these alien elements is promptly stopped by the annual desiccation of the pond. This resetting phase largely smoothens the harshness of human impacts but those which interfere with the hydrological cycle and are depending on climate change of the Mediterranean area.

In permanent ponds, the resetting phase is absent and this absence likely decreases the resilience of the systems. Actually, the above quoted sequence of coda was also present in the permanent pond in 1987–1988, during the clear water, macrophyte-dominated period. Since the macrophyte species in permanent ponds are perennial species, they could not cope with the drying of the pond, which occurred at the beginning of the century (Barone et al., 2010). The permanent disappearing of the macrophyte in the pond allowed phytoplankton to become dominant and promoted a permanent shift towards an alternative stable state. The adaptive strategies to survive the dry phase performed by the inhabitants of temporary waters thus contribute to make these environments more resilient than permanent ones.

No temporal gradients appeared when the eight shared functional groups were pooled in a single matrix and ordinate by nMDS. This was in conflict with our initial hypothesis of a regional coherence in phytoplankton dynamics, which is driven by climatic features. If this hypothesis had been confirmed, the same coda should group together depicting a temporal sequence similar to that they showed in the ordinations obtained by analysing the water bodies one by one. Conversely, the coda did not show any successional trend but they were clearly separated and formed groups which identified the single water body. This result suggests that local conditions and the local pool of available species have thus a strong effect, which can be considered as important as that exerted by

seasonal/climatic constraints. To explain this result, concepts deriving from the game theory can be used. In mathematics, game theory models strategic situations, or games, in which an individual's success in making choices depends on the choices of others (Myerson, 1997). In this context, we can consider individual organisms as the players, the functional group which collects them as their strategies, and their relative biomass values (largely depending on their net growth rates or fitness) as their payoffs (see McGill & Brown, 2007). The game happens because the fitness of an individual is simultaneously influenced by its own strategy and by the strategies of others. A change in the composition of 'others" imposes a different strategy to all players. In this view, the rare species and the different annual colonizers, which may be regarded as occasional players, have the possibility to influence the strategies of the 'regular' players making each ecosystem unique and different from the others. In this way, rare species, as also shown by Padisák et al. (2010), assume a structuring effect on the dominant phytoplankton species, and besides constituting the ecological memory of aquatic ecosystems (Padisák, 1992), they can actively influence the dynamics of dominant phytoplankton groups.

In conclusion, the functional classification of phytoplankton allows to represents the ecological phases, which characterizes the seasonal succession of phytoplankton in macrophyte-dominated Mediterranean ponds. In particular, the specific and the recurrent sequence of coda, as resulted in the present study, seem to confirm the hypothesis of a regional coherence in the development of phytoplankton in Mediterranean ponds. However, the temporary character of a pond may allow for the possibility of a resetting phase, which enables these environments to counteract a large number of environmental impacts including the variability of the meteorological elements (temperature, duration and occurrence of precipitation) typical of Mediterranean climate. However, deep alteration of these climatic features as predicted by climate change models may enhance water overexploitation and severely endanger these ecosystems. The resetting phase eventually favours the possibility for new colonizers to develop, and potentially increases local biodiversity. This allows the existence of a large pool of rare species. The species richness is further increased by the coexistence of metaphytic, planktic and benthic species which constitute the element of compositional dissimilarity and dynamical singularity that makes each pond different and contributes to increase regional biodiversity.

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PHYTOPLANKTON

Present–absent: a chronicle of the dinoflagellate *Peridinium gatunense* from Lake Kinneret

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Abstract A long-term record dating back to the 1960s indicates that *Peridinium gatunense*, an armored dinoflagellate, dominated the phytoplankton of Lake Kinneret (Sea of Galilee, Israel) until the mid-1990s, with a relatively stable spring bloom. However, since 1996, these blooms became irregular, failing to develop in 10 out of the past 16 years. During the later period, a significant correlation ($R^2 = 0.605$, $P = 0.013$) was found between annual peak *P. gatunense* biomass and riverine inflow volume. In-lake surveys showed that patches of high *P. gatunense* densities were associated with water enriched with fresher inflowing Jordan River water. Supplementing laboratory cultures of *P. gatunense* with Hula Valley water stimulated its growth relative to un-enriched controls. A likely explanation to the recent irregular blooms of this dinoflagellate is a hydrological modification that was made in the catchment in the mid-1990s, preventing Hula Valley water from reaching Lake Kinneret in most years—except for exceptionally wet years. We propose that until the mid-1990s, the Jordan River water enriched Lake Kinneret with a growth factor (a microelement and/or organic compound) originating in the Hula Valley, which in recent years has arrived in sufficient quantities to support a bloom only in high-rainfall years.

Keywords Phytoplankton bloom · Biomass · Inflow volume · Selenium · Jordan River

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Introduction

Peridinium gatunense Nygaard 1925, a large (diameter: 44–60 μm; volume: ~66,000 μm^3) solitary motile protist, is a freshwater bloom-forming thecate dinoflagellate, famous for its blooms in Lake Kinneret (Sea of Galilee, Israel) (Pollingher, 1986, 1988). Initially, the Kinneret *Peridinium* was referred to by different species names (*P. cinctum*, *P. cinctum* fa. *westii*, etc.), until Boltovskoy (1983) and Hickel & Pollingher (1988) conclusively identified it as *P. gatunense*. This species has a cosmopolitan distribution, but usually it does not dominate the phytoplankton assemblage. The only other place known for blooms of this species is a small lake in northern Germany (Barbara Hickel, pers. Comm.).

For many years, and as documented since the mid-1960s, the most salient feature of the Kinneret phytoplankton has been the high-biomass winter–spring bloom of *P. gatunense* (hence: *Peridinium*)

(Pollingher & Kimor, 1970; Pollingher, 1986; Berman et al., 1992). Those intense blooms could be observed from the surrounding mountains as large patches of coffee-brown colored water, up to several square kilometers in area. At the peak of the bloom, *Peridinium* cell concentrations reached 500–2,000 cells ml^{-1}, comprising 150–250 g m^{-2} of wet weight biomass and >95% of total phytoplankton biomass (Pollingher, 1986, 1988). The bloom declined sharply in May–June, shortly after water temperature exceeded 25°C, thermal stratification was established, and nutrients became scarce, leaving behind resting cysts on the lake sediments. A low-biomass, high-diversity assemblage of mostly nanoplanktonic species characterized the summer and autumn (Pollingher & Kimor, 1970; Pollingher, 1981, 1986). A remarkable year-to-year stability of the annual phytoplankton succession pattern was reported: in Pollingher's (1981) words: "the annual succession at the species level has been an almost constant event in the lake for many years."

While a large, slow-growing cell like *P. gatunense* is a relatively poor competitor against the many co-existing nanoplanktonic species in rates of nutrient uptake, due to its relatively small surface area-to-volume ratio, it does possess several attributes that enabled it to compete successfully and dominate phytoplankton biomass year after year. Its motility and phototactic abilities (Liu et al., 1990) allow it to modify its position in the water column according to light and nutrient availabilities, and in this way, compensate for its low nutrient uptake rates. *Peridinium* performs daily vertical migrations in Lake Kinneret, traveling toward the surface in the early morning and sinking toward the nutrient-rich thermocline in the afternoon (Pollingher, 1988; Usvyatsov & Zohary, 2006). Its large cell size makes it inedible by most of the Kinneret zooplankton (Hambright et al., 2007), and so its grazing mortality is low. Its lifecycle, with resting cysts (Pollingher & Serruya, 1976; Pollingher, 1987, Alster et al., 2006), is aimed at maintaining perennial continuity. The cysts over summer in the sediments, germinating in November–December when nutrients from turnover and from the inflows are plentiful, to start a new bloom (Pollingher, 1986). With a cellular C:P molar ratio of 460:1 (Wynne et al., 1982; Zohary et al., 1998), it has an exceptionally low P requirement—an advantage in the mostly P-limited winter–spring situation (Berman, 1985). Furthermore, like other dinoflagellates (Rengefors et al., 1996), its resting cysts are capable of surplus P uptake, storing enough phosphorus for several cell divisions. As the blooms develop, CO_2 availability becomes growth limiting (Berman-Frank et al., 1994). To cope with this, *Peridinium* applies a C-concentrating mechanism (Berman-Frank et al., 1998). It was also shown to have allelopathic abilities at high cell densities, inhibiting the growth of *Microcystis* (Schatz et al., 2007).

After many years of recorded constancy, in the mid-1990s, the regular pattern of spring *Peridinium* blooms broke down. In 1996, for the first time, *Peridinium* failed to develop a spring bloom (Zohary, 2004; Roelke et al., 2007). Since then, *Peridinium* blooms in Lake Kinneret have become an irregular event (Zohary & Ostrovsky, 2011).

In this article, we present a long-term record (1969–2011) of *Peridinium gatunense* from Lake Kinneret and examine recent deviations from the previous highly regular annual pattern. Temporal changes in environmental and biological conditions were followed to identify environmental variables that may be associated with the disappearance of the earlier regular annual pattern and could possibly impose the lack of *Peridinium* blooms in recent years. Laboratory experiments in which *Peridinium* cultures were enriched with water from various inflows were conducted to examine the hypothesis that hydrological changes in the catchment were the ultimate reason for the current lack of *Peridinium* blooms.

Methods

Study site

Lake Kinneret is a meso-eutrophic warm monomictic lake, stratified from March–April till December–January. It is located in the Syrian-African Rift Valley, at 32°50′N 35°35′E, −210 m below sea level. At its full capacity, the lake covers 170 km^2, contains 4,300 × 10^6 m^3 water, has a maximum depth of 43 m and a mean depth of 25 m. Owing to the Mediterranean climate, it is subjected to cool wet winters and hot dry summers, with rainfall most of the external nutrient loading limited to 4–6 months of the year (usually from October–November to February–March). Water residence time in Lake Kinneret varies between 4 and 9 years, depending on winter

precipitation. Being a major source of Israel's drinking water, Lake Kinneret and its watershed area have been the focus for an intense monitoring program and accompanying research activities, initiated in 1969 and continuous till today.

The Jordan River is the main inflow into Lake Kinneret, supplying ca. 60% of its inflowing water and 70% of the nutrient loads. The sources of the Jordan River are the Dan, Banyas, and Hazbani Rivers, draining $\sim$2700 km^2 of mostly the mountainous watershed in Northern Israel and Southern Lebanon (Serruya, 1978). At the center of the drainage basin lies the Hula Valley ($\sim$180 km^2), which has been home to the shallow Lake Hula—until the 1950s, when this natural lake and its surrounding swamps were drained to increase arable land and eradicate malaria, exposing dry peats. The dried valley was subjected to new agricultural and ecological problems: dust storms, underground fires, infertile peat land, and release of large quantities of nitrates, sulfates, and organic matter, which drained into Lake Kinneret (Hambright & Zohary, 1998). To alleviate these problems, parts of the Hula Valley were re-flooded in 1994, the water table was elevated, and the hydrology was altered, such that the most of the water running through this valley no longer reaches Lake Kinneret. Only in above-average wet winters does overflow from the valley reach Lake Kinneret.

Long-term record

Long-term phytoplankton record (January 1969-June 2011): Phytoplankton species composition, abundance, and wet-weight biomass were followed routinely since January 1969 (and on-going). Water samples for phytoplankton determination were collected weekly or fortnightly from Station A, at the deepest part of the lake, from 10 to 14 discrete depths between the surface and the thermocline during stratification and along the entire $\sim$40-m water column (depending on water level) during holomixis. The record presented here is based on $\sim$1800 sampling dates (Jan 1969–Jun 2011) and a total of nearly 20,000 samples that were analyzed. Water samples were preserved in Lugol's solution, sedimented (10 ml) into sedimentation chambers, and phytoplankton was counted under an inverted microscope according to Lund et al. (1958). Concurrently, linear cell dimensions were measured for converting cell abundance to cell volume according to approximate geometric shapes and to wet-weight biomass assuming specific density of 1, as detailed by Hillebrand et al. (1999). The resulting phytoplankton biomass values from the discrete depths were depth-integrated to give a single value in g (w.w.) m^{-2} (hereafter g m^{-2}). Further details of sampling, counting, and biomass estimation procedures are given in Zohary (2004).

Long-term chemical record (January 1969–December 2010): Lake Kinneret water samples were collected weekly from 10 different depths at Station A using a Rhode sampler, stored in 1-l plastic bottles and brought back to the lab within 2–3 h. The analysis of Soluble Reactive Phosphorus (SRP) and NH_4 was performed within 5 h of sampling, while NO_3, organic nitrogen (total and dissolved), total nitrogen, and total phosphorus were determined on the following day, as described by Nishri (2011).

Long-term hydrological and nutrient loading record (October 1970 till September 2010): The Jordan River was sampled for flow rates and chemical composition at Huri Bridge, located downstream of the Hula Valley and 18 km upstream of Lake Kinneret. Water samples for chemical determinations were collected daily and stored in closed and cooled (4°C) 1-l plastic bottles until further analyzed, within 48 h of sampling, using the same analytic procedures as those used for the lake water samples. Jordan River discharge volumes were measured continuously, time-integrated to account for flood events, and reported as monthly inflow volumes. The discharge and nutrient concentration data were combined to derive monthly nutrient loads.

Spatial distribution of temperature, conductivity, and chlorophyll

An underwater towed undulating monitoring system, U-TUMS, was employed for collecting near-synoptic temperature, conductivity, chlorophyll fluorescence, and exact location data from Lake Kinneret. The U-TUMS comprises an underwater vehicle (carrier), a set of sensors, and navigation devices (geographic position system-GPS, SONAR, and speedometer), operated via an on-board computer. The U-TUMS is towed behind the boat along chosen trails. The computer-controlled vehicle steering mechanism forces the system to undulate from near surface to near bottom or to a maximal depth of 25 m if the bottom is at a deeper depth, while data from the sensors are presented on a screen in real time. The

underwater vehicle (MiniBAT, Guildline, Canada) was loaded with a multi-sensor probe CTD that measures water electric conductivity, temperature, and depth (Applied Microsystem Limited, Canada) and a fluorometer, set to measure chlorophyll (Minitracall, Chelsea Instrument UK). The U-TUMS was operated during the last *Peridinium* bloom event, during 26–28 March 2007, to study the patchy nature of this dinoflagellate population. It was towed along east–west transects in the northern part of the lake, ca. 3 km from the Jordan River inflow, at a speed of 4.5 knots while undulating (diving or climbing) at a rate of up to 90 cm s^{-1} (Ostrovsky & Sukenik, 2008). The collected data were filtered for errors (based on logic limits and expected values) and transformed from electronic values to physical qualities and quantities based on prior calibration. The data were then integrated into pseudo-three-dimensional presentation (Geographical latitude—X axis, Depth—Y axis, and the parameter quantity presented as a color map) using Surfer 7.0 (Golden Software Inc.) applying the near-neighbor interpolation procedure.

Enrichment experiments with *Peridinium* cultures

Cultures of *P. gatunense* Nygaard isolated from Lake Kinneret (Elgavish et al., 1982) were maintained continuously in a medium adjusted by Lindström (1991). A simple bioassay system was established to assess the effect of water originating from various geographic locations and reaching Lake Kinneret via the Jordan River or other streams, on the growth of *Peridinium*. An exponentially grown culture of *Peridinium* in freshly prepared Lindström medium was enriched (20% of the culture volume) with water from various sources. Flood waters were collected from the Jordan River near its inflow into Lake Kinneret (32°54′4.34″N 35°36′45.28″E) and from near the inflow of Meshushim River (32°54′14.45″N 35°38′23.85″E), a smaller river that drains part of the basaltic Golan Height, to the north-east of the lake. Additional water samples were collected from two sites in the Hula Valley: 1. Lake Agmon (33°6′10.35″N 35°36′29.30″E) and 2. Peat drainage site (33°6′51.75″N 35°36′3.01″E). Added water was filtered through a 0.22-μm nitrocellulose membrane to remove the suspended particles and microorganisms. The concentrations of P and N were measured in the assayed waters to allow the adjustment of experimental (with the added external water) and control (no addition of external water) cultures to the same initial concentrations of the main nutrients. Cultures, with initial concentrations of 1,250 cells ml^{-1}, 1.1 mM N, and 10 μM P, in triplicate, were incubated at 20°C under constant light of 35 μmol photon m^{-2} s^{-1} without agitation, for at least 40 days. Samples were withdrawn from each culture flask once a week, preserved in Lugol's solution, and sedimented (2 ml) into sedimentation chambers. *Peridinium* cells were counted under an inverted microscope according to Lund et al. (1958). Data were used to estimate specific growth rates, and maximal cell concentrations in response to the additions.

In an additional set of bioassays, the potential role of microelements in Hula Valley water in supporting and enhancing *Peridinium* growth was estimated using Lindström medium with reduced trace metal content and a *Peridinium* inoculum which was previously deprived of microelements for 4 weeks ("preconditioned" by incubation in Lindström medium containing only 10% of the trace metals mix). For the experiment, the preconditioned inoculum was transferred into Lindström medium (80% of total volume) with reduced (20% of standard) trace metal mix, supplemented with peat water from the Hula Valley (20% of total volume). Control cultures were transferred into Lindström medium (100% of the volume, no other addition) with reduced (20%) trace metal mix. Initial concentrations in all cultures were 750 cells ml^{-1}. The cultures were maintained and sampled as described above. Concentrations of microelements in floodwater used for the enrichment experiments were determined using ICP-MS Elan-6000 (Sciex, Perkin-Elmer).

Results

Peridinium gatunense—temporal dynamics in Lake Kinneret

The long-term record of *Peridinium* in Lake Kinneret demonstrates an extended stable period characterized by an annual spring bloom, followed by years in which bloom events were irregular. The time series of the yearly maximum monthly mean *Peridinium* biomass, or peak *Peridinium* biomass, for short, presented in Fig. 1 clearly shows higher variability since 1994.

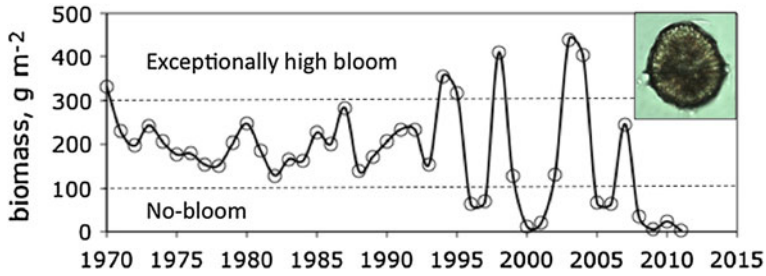

Fig. 1 Time series of the annual maximum monthly mean *Peridinium* biomass (= peak *Peridinium* biomass), 1970–2011. *Horizontal dashed lines* mark borderlines for the no bloom (below 100 g m^{-2}) and exceptionally high bloom (above 300 mg m^{-2}) situations. *Inset* A light microscope photograph of the thecate dinoflagellate, *P. gatunense* NYGAARD, photographed by Dr. Alla Alster

During the last 18 years (1994–2011), there were 10 "no-bloom" years in which monthly mean depth-integrated *Peridinium* biomass did not exceed 100 g m^{-2}. The *Peridinium* spring bloom failed to develop in 1996, 1997, 2000, 2001, 2005, 2006, 2008, 2009, 2010, and 2011.

During the said period (1994–2011),we also noted years of exceptionally high biomass blooms, defined as those in which peak *Peridinium* biomass exceeded 300 g m^{-2}. Until 1993, peak *Peridinium* biomass always fell within the 100 and 300 g m^{-2} range (except 1970), but since 1994, there were five exceptional bloom years: 1994, 1995, 1998, 2003, and 2004. Overall, the year-to-year fluctuations in peak *Peridinium* biomass have become much more extreme since the mid-1990s (Fig. 1). No-bloom years generally had lower or shorter peak phytoplankton biomass than the bloom years (Fig. 2), because other phytoplankton species temporarily dominating the lake ecosystem usually did not achieve the wet-weight biomass attained by *Peridinium*, or even if they did, it was for a shorter period.

The annual succession of phytoplankton species composition in bloom years tended to repeat year after year. As such, it is well represented by a single, averaged annual pattern for the years, 1969–1995 (Fig. 3, upper left panel). As previously reported by Pollingher (1986) and Zohary (2004), this pattern consisted of a spring bloom of *Peridinium* followed by a low-biomass, high-diversity assemblage in the summer and fall. A modified version of this pattern was observed in 1982, 1983, 1988, 1998, 1999, and 2003, when an early winter bloom of *Aulacoseira granulate* preceded the *Peridinium* bloom. In contrast, phytoplankton succession in no-bloom years differed from 1 year to another (Fig. 3); no single species or higher taxonomic group seemed to be able to consistently occupy the niche that opened in the absence of *Peridinium*. Furthermore, no single species was able to assume and maintain >90% dominance of total phytoplankton biomass as was typical for *Peridinium*. Instead, several alternative patterns were observed. In 1997 and 2001, an intense bloom of the filamentous diatom *Aulacoseira granulata* developed in January–February, declining in March. As an unusual event, *Peridinium* dominated phytoplankton biomass in the second half of 1997, but it remained well below the 100 g m^{-2} borderline. In 2001, no other species took over after *A. granulata* declined. In 2005, 2006, and 2010, the filamentous conjugating green alga, *Mougeotia* sp., a newcomer in Lake Kinneret first recorded in 1998, took over in spring. In 2010, *Mougeotia* remained abundant throughout the summer and fall, attaining its peak biomass in December and declining in early 2011. Another pattern observed was that of increased abundance of non-*Peridinium* dinoflagellates. *Ceratium hirundinella* dominated in March–April 2010, with peak monthly biomass of 75 g m^{-2}. In the springs of 1996, 2000, 2001, 2005, and 2006, *Peridiniopsis elpatiewskyi* and *Ps. cunningtonii*, occurring together, were the major biomass contributors, but the biomass of each never exceeded 35 g m^{-2}. In 2000, a diverse assemblage of chlorophytes dominated the winter–spring biomass, with *Coelastrum microporum*, *Pediastrum duplex*, *Pediastrum tetras,* and *Scenedesmus* spp. as main biomass contributors.

As opposed to the many different patterns in species composition in winter–spring, a noticeable summer–fall feature of the phytoplankton assemblage since the mid-1990s was the increased abundance and the relative contribution to total biomass of cyanobacteria,

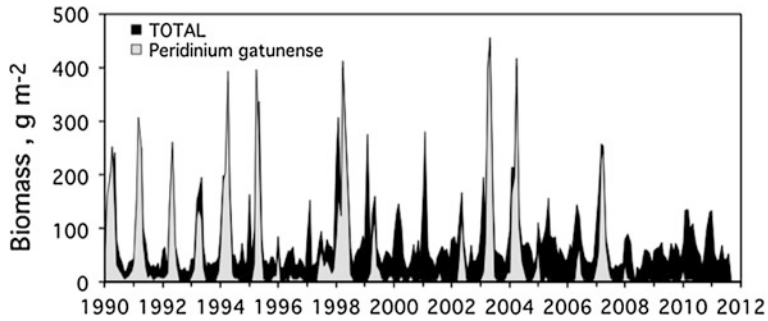

Fig. 2 Time series of monthly mean biomass of *P. gatunense* (*gray*) superimposed on total phytoplankton biomass (*black*) in Lake Kinneret, 1990–2011. Note the no-bloom years of 1996, 1997, 2000, 2001, 2005, 2006, 2008, 2009, 2010, and 2011

especially the N_2-fixing species, *Aphanizomenon ovalisporum*, and *Cylindrospermopsis raciborskii*. The summer–fall higher cyanobacterial abundance occurred in both bloom years and in no-bloom years. The summer–fall assemblages also changed in their dominant morphological forms, from the mostly single-celled often coccoid nanoplanktonic species to filamentous or elongated single-celled species.

Can the current bloom pattern be attributed to recent changes in hydrological and limnological variables?

Possible relationships between winter–spring water flow or seasonal nutrient loads (total N and total P) and the spring bloom of *Peridinium* are highlighted in Fig. 4. In this figure, we compare the seasonal development of a *Peridinium* bloom in drought years (low water inflow; low nutrient loads) with its development in flood years (high inflow and high nutrient loads). Before the mid-1990s, a spring bloom of *Peridinium* that exceeded areal biomass density of 200 g m^{-2} characterized nearly all the years, including drought years, such as 1990 and 1991. The years 2000 and 2001 had similarly low water inflows and loads of TN and TP as in 1990 and 1991, but no *Peridinium* bloom was observed in these latter years. In contrast, the years 2003 and 2004 were flood years with high winter–spring water inflow and nutrient loads. Both flood years supported exceptionally high blooms, with peak biomass exceeding 450 g m^{-2} (Fig. 4).

Of the many limnological variables monitored routinely in Lake Kinneret, only a few were highly and significantly correlated ($R^2 > 0.6$, $P < 0.05$) with the annual peak *Peridinium* biomass during the period, 1995–2010 (Table 1). The following variables showed high linear correlation with peak *Peridinium* biomass: (1) the annual Jordan River inflow volume (based on hydrological year: October of one year through September of the following year)—a proxy to total water inflow into the lake, $R^2 = 0.605$; (2) the annual Jordan River load of organic N—a proxy to the amount of organic matter that entered the lake, $R^2 = 0.512$; (3) epilimnion (0–10 m) mean spring (March–May) concentrations of dissolved organic nitrogen, DON, $R^2 = 0.607$. Interestingly, none of these variables, or any of many other variables examined, showed a significant correlation with peak *Peridinium* biomass during the period 1971–1994 (Table 1). In a subset of the data for 1995–2010, containing only the seven bloom years, the correlation coefficient increased for the annual river inflow ($R^2 = 0.768$), the river organic N load ($R^2 = 0.523$), and the in-lake concentrations of DON ($R^2 = 0.825$) (Table 1). These results indicate the potential impact of the riverine inflow and its associated load of organic and other materials on the *Peridinium* bloom during recent years, an impact that apparently was less important in the earlier period (1971–1994).

Another indication of the importance of riverine inflows to the blooming of *Peridinium* was obtained by the synoptic chlorophyll and conductivity data generated by the U-TUMS during the 2007 *Peridinium* bloom. On March 27, 2007 a distinct patch of *Peridinium*, indicated by elevated chlorophyll fluorescence (Fig. 5a), corresponded to a distinct patch of similar shape and dimensions of water with electric conductivity slightly lower than that of the surrounding waters (Fig. 5b). Microscopic examination confirmed that the indicated chlorophyll fluorescence signal corresponded to a patch of *Peridinium*, contributing >95% to total phytoplankton biomass. The conductivity of Jordan River water is ~0.500 mS cm^{-1}, whereas that of Kinneret epilimnion fluctuates between 0.850 and 1.650 mS cm^{-1}, with lower values recorded

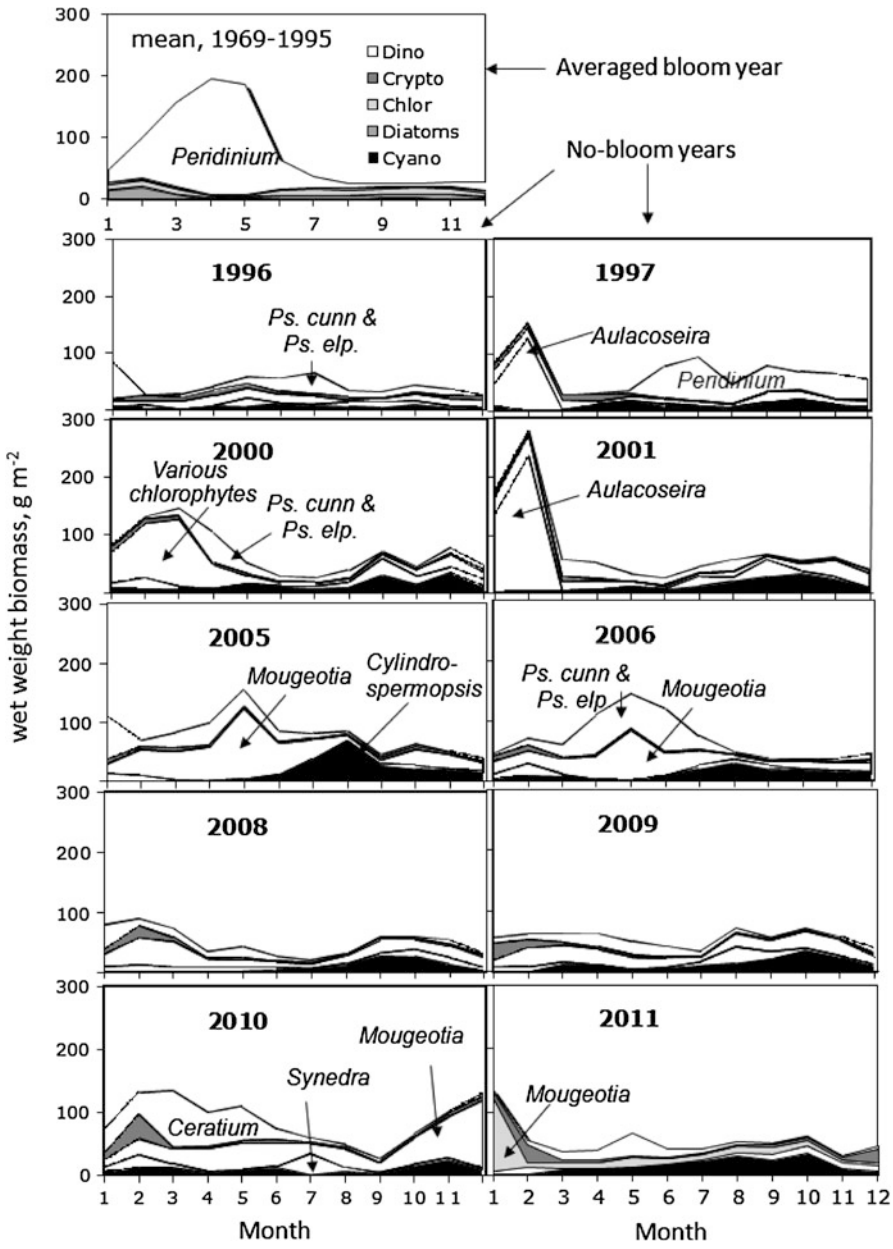

Fig. 3 The annual pattern of monthly mean biomass of the main taxonomic groups in Lake Kinneret in an averaged *Peridinium*-bloom year (*top left*) and in the 10 no-bloom years since 1996 (all other *panels*). Bloom-forming species are indicated. Ps. cunn.—*Peridiniopsis cunningtonii*, Ps. elp.—*Peridniopsis elpatiewskyi*

in association with river inflows. Relatively lower conductivity values recorded within the lake are considered as reliable tracers for riverine water mixed in the bulk of the Kinneret water (Ostrovsky & Sukenik, 2008). The data in Fig. 5 depict a plume of Jordan River water in the lake by a distinct patch of lower conductivity, of 0.886 mS cm^{-1}, surrounded by water with conductivity of 0.914 mS cm^{-1}. The *Peridinium* patch depicted by its chlorophyll signal in Fig. 5 apparently maintained its position within the patch of low-conductivity water, richer with nutrients and organic matter, brought in by the Jordan River. To demonstrate this difference in organic matter content, on March 27, DON concentration in Lake Kinneret at station A was 0.24 mg N l^{-1}, whereas, in the Jordan River a day later (the closest date with Jordan River data), it was 1.27 mg N l^{-1}.

These observations suggest that in addition to ample supply of nutrients, a "growth factor" such as an organic compound or a microelement (tentatively

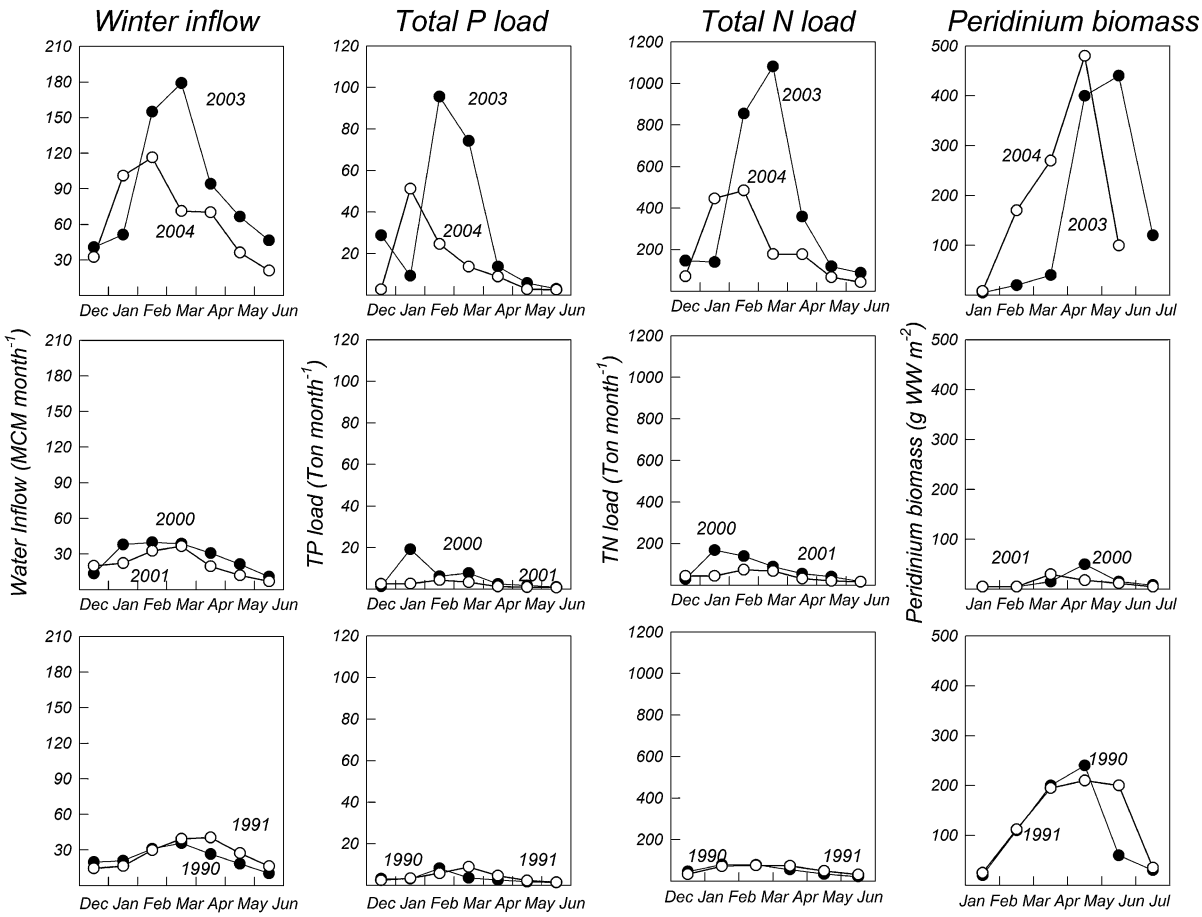

Fig. 4 The relationships between water inflow or nutrient loads and the seasonal bloom of *Peridinium* in Lake Kinneret in several representative years of high and low inflows. 2003 & 2004—high inflow, high loads, exceptionally highblooms; 2000 and 2001—low inflow, low loads, and no blooms; 1990 and 1991—low inflow, low loads, and regular blooms

determined as "*Peridinium* bloom-supporting factor") was needed to support the bloom. Probably, the supporting factor that previously reached the lake via the Jordan River during winter floods has not reached the lake in recent years in quantities and at the timing required for a sustainable bloom of *Peridinium*.

Identification of the "*Peridinium* bloom-supporting factor" using bioassays

The source and the gross chemical nature of the "*Peridinium* bloom-supporting factor" were assessed by bioassays conducted to examine the growth of *Peridinium* in a medium enriched with water sampled at different locations in the lake's catchment during winter floods. Water collected from the Jordan River during a 2008 winter flood enhanced the growth of *Peridinium* and yielded a 40% higher growth rate relative to control cultures that were maintained in full Lindström medium, 0.094 versus 0.067 day^{-1}, respectively ($P < 0.05$), (Fig. 6). The *Peridinium* cell yield in cultures fortified with Jordan River floodwaters was 16% higher than that of the control cultures, but this difference was statistically insignificant. Running the same experiment with water collected from the peat soil area of the Hula Valley showed a significant enhancement of growth rate and higher maximal cell yield relative to control cultures (Fig. 6): growth rate increased by 45% and cell yield by 30% ($P < 0.05$). Interestingly, the addition of water collected from floods of the Meshushim tributary, flowing through basaltic rock beds, had a minor effect on *Peridinium* growth (Fig. 6).

As all bioassay experiments were initiated with the same concentration of macronutrients (N and P) and

Table 1 R^2 values for linear correlation between a series of limnological parameters and annual peak *Peridinium* biomass for the periods 1970–1994 and 1995–2010

Parameter	1970–1994 (N = 23–24)	1995–2010 (N = 16)	1995–2010 bloom years (N = 7)
Jordan River, annual values (hydrological year)			
Inflow volume (10^6 m^3 $year^{-1}$)	0.005	**0.605***	**0.768***
Organic N load (tons $year^{-1}$)	0.027	0.512	0.523
Kinneret, 0–10 m, mean for March–May			
Alkalinity (mg $CaCO_3$ l^{-1})	0.013	0.322	0.176
pH	0.010	0.210	0.147
Dissolved oxygen (mg l^{-1})	0.012	0.047	0.058
Chloride (mg l^{-1})	0.163	0.218	0.254
Conductivity(mS cm^{-2})	0.108	0.336	0.256
NH_4 (mg l^{-1})	0.026	0.074	0.132
NO_3 (mg l^{-1})	0.023	0.075	0.263
Dissolved organic N (mg l^{-1})	0.058	**0.607***	**0.825****
Total dissolved P (mg l^{-1})	0.120	0.016	0.151

For the latter period only, correlations are also shown for a subset of the record, containing only the seven bloom years

* $P < 0.05$ and ** $P < 0.01$ for a 2-tailed probability test

Statistically significant R^2 values appear in bold letters

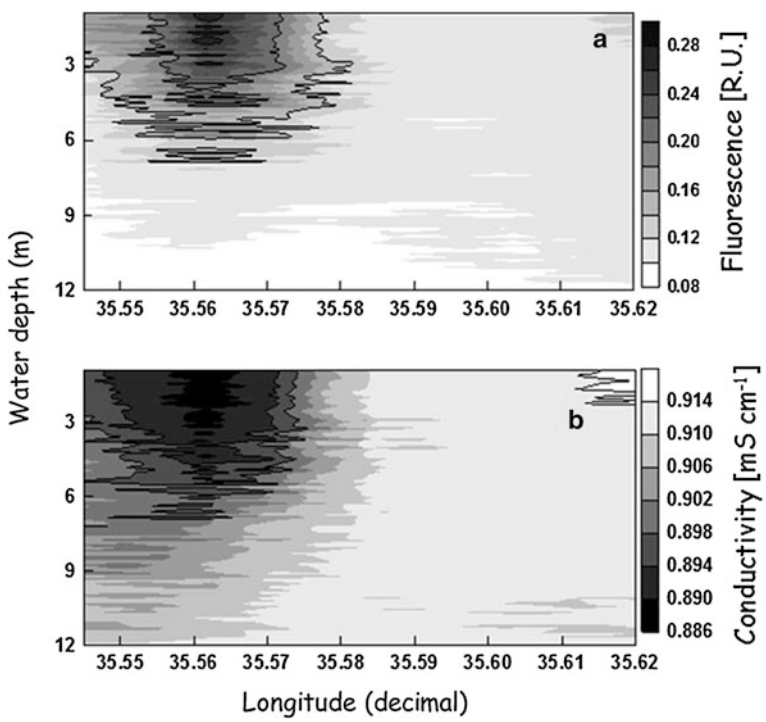

Fig. 5 Spatial distribution of chlorophyll fluorescence (**a**) and conductivity (**b**) during a *Peridinium* bloom in Lake Kinneret, based on U-TUMS measurements along an East–West transect (see "Methods" section), March 27, 2007; 22:15–23:50 hours, showing correspondence between high chlorophyll and low conductivity (Jordan River inflow). Note: reversed color scale for conductivity (*dark color* low concentration)

included the microelements provided with Lindström medium, the extra growth observed in cultures fortified with water samples from the Jordan River floods and from the Hula Valley could be attributed to the presence of "*Peridinium* bloom-supporting factor" in the form of trace elements and/or trace organic compounds in surplus. Therefore, the growth response of *Peridinium* inoculum, acclimated for 4 weeks in

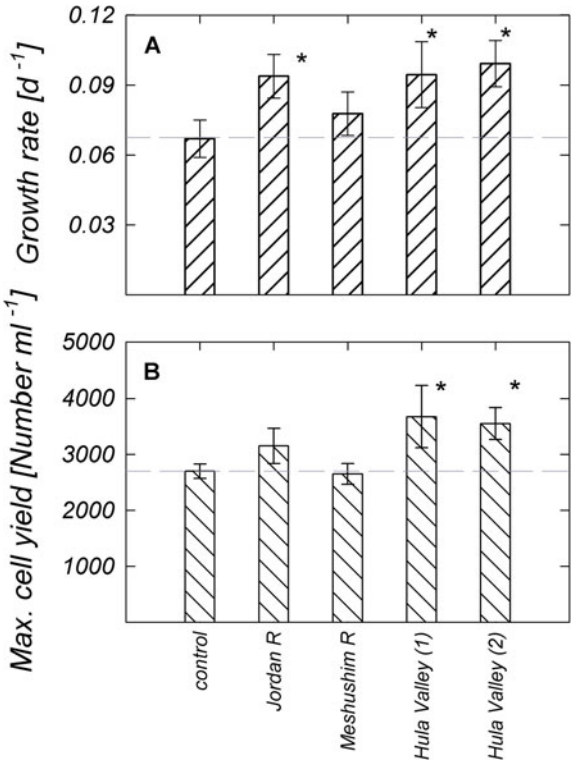

Fig. 6 Growth performance of *Peridinium* cultures grown in Lindstrom medium fortified with water collected from various sources in the catchment of Lake Kinneret. **A** Growth rate; **B** maximal cell yield. Added waters were flood water from the Jordan River; flood water from the Meshushim River; water from the reconstructed Lake Agmon in the Hula Valley [Hula Valley (1)], and that from Hula Valley peats [Hula Valley (2)]. Water was added to make up 20% of the total culture volume. *Bars* represent average and standard deviation of three culture replicates. An *asterisk* by the *bar* represents a value significantly higher than that of the control ($P < 0.05$)

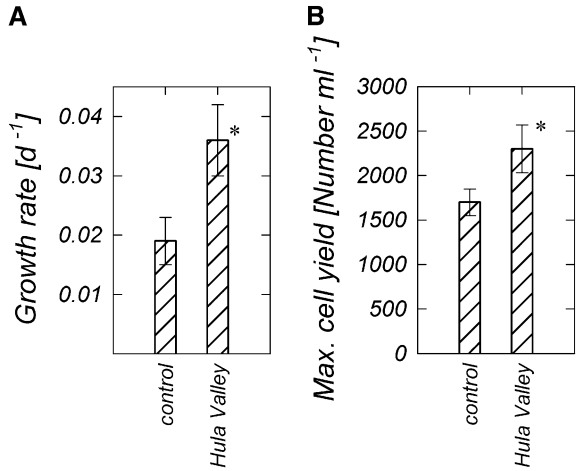

Fig. 7 The effect of peat water collected in the Hula Valley on the growth performance of *Peridinium* cultures starved of trace metals. Growth rate (**A**) and maximal cell yield (**B**) were measured in control cultures grown in medium with reduced amount of trace elements and fortified with 20% of their volume with peat water collected from the Hula Valley. *Bars* represent average and standard deviation of three culture replicates. An *asterisk* by the *bar* represents a value significantly higher than that of the control ($P < 0.05$)

Lindström medium that contained only 10% of the original amount of microelements (preconditioned), was evaluated in a growth bioassay with water from the Hula Valley. While the control cultures with reduced concentrations of microelements (20% of normal) demonstrated slow growth rates and low maximal cell yield, the cultures fortified with water from the Hula Valley grew at a faster pace and yielded significantly ($P < 0.05$) higher cell concentration (Fig. 7). However, the growth performance under these conditions was poorer relative to the growth with a normal dose of trace elements (Fig. 6). The potential role of the Hula Valley as well as Jordan River water as a source of trace elements was further evaluated by a direct chemical analysis (Table 2). This analysis indicated relatively high concentrations of B, Ba, and Sr in the Jordan River water, and of B, Ba, Mn, and Sr in the Hula water. The Co, Mo, and Zn concentrations in Lindström medium were similar or considerably higher than the concentrations measured in the various water sources. However, Se concentrations in Jordan flood and Hula Valley waters were 4 and 9 times, respectively, higher than that in the Lindström medium, suggesting the importance of this element to the growth of *Peridinium*.

Discussion

Possible reasons for the disappearance of *Peridinium*

The most obvious reason for the lack of blooms, shortage of N or P or both, was shown, from early on, not to be relevant to our case. Peak *Peridinium* biomass was not correlated with in-lake concentrations of ammonium, nitrate, or total dissolved P (Table 1). There was no evidence that inorganic nutrient loads have changed dramatically since the mid-1990s. Furthermore, no-bloom years such as 2000 and 2001 similarly had low loads of TN and TP as

Table 2 The concentrations of trace elements in floodwaters collected from the Jordan River and the Hula Valley peats as compared to the concentrations found in Lindstrom artificial medium

Element		Concentrations (nM)		
		Jordan River	Hula Valley	Lindstrom[a]
B	Boron	3,250	5,550	N.I.
Ba	Barium	685	290	N.I.
Cu	Copper	<32	32	N.I.
Co	Cobalt	<17	<8	40
Mo	Molybdenum	12.5	104	200
Mn	Manganese	<9	3,220	50
Ni	Nickel	<17	<17	N.I.
Rb	Rubidium	22.2	23.4	N.I.
Se	Selenium	2.8	6.2	0.7
Sr	Strontium	2,168	2,280	N.I.
V	Vanadium	78.5	<19	N.I.
Zn	Zinc	227	77	200

N.I. not included in Lindström medium

[a] Calculated from the medium recipe (Lindström, 1991)

those in 1990 and 1991, but the latter years were typical bloom years (Fig. 4).

Peridinium gatunense was never found in Jordan River water or in water bodies of the Hula Valley (Pollingher et al., 1998). The observation that *Peridinium* patches coincided with patches of water enriched with Jordan River inflow (Fig. 6) as well as the strong correlation between Jordan River inflow volume and *Peridinium* peak biomass since 1995, but not beforehand (Table 1), suggested to us that there is likely to be a "*Peridinium* bloom-supporting factor" (such as a microelement or organic compound) in Jordan River water that is essential for *Peridinium* growth. Furthermore, those findings suggested that since the mid-1990s this constituent is probably in short supply, enough of which arrives via the Jordan River only in the years of particularly high inflow. In the past, the Jordan River drained the Hula Valley with its extensive peat soils, unique to this part of the catchment. Starting in 1994, major man-made hydrological changes were made in the Hula Valley, and since 1998, the most of the water running through the Hula peat soils no longer reaches Lake Kinneret. Only in the high-rainfall years, when the Hula Valley gets flooded, excess water flows to Lake Kinneret via the Jordan River. We therefore hypothesized that the essential constituent originates from the peat soils. One possible "*Peridinium* bloom-supporting factor" is selenium, shown by Lindström (1985, 1991) to be essential for the growth of laboratory cultures of *P. gatunense*. In particular, Lindström & Rhode (1985) showed that optimum growth of *P. gatunense* isolated from Lake Kinneret was obtained with ca. 50 ng l^{-1} of Se[IV]. The Hula Valley is probably an important source of selenium (Table 2) for Lake Kinneret. Unfortunately, data on Se concentrations in Lake Kinneret and its catchment are mostly non-existent, due to the complexity of its determination and limited analytic sensitivity. As such, this parameter was not monitored routinely in Lake Kinneret and its catchment. An exceptional dataset from 1994, with monthly concentrations of soluble Se species in Lake Kinneret, showed that the 1994 crash of the *Peridinium* bloom coincided with depletion of Se[IV] to below its detection limit of 5 ng l^{-1} (Nishri et al., 1999). Our long-term experience of maintaining *Peridinium* in laboratory cultures, as well as our growth performance experiments (Fig. 7), confirmed that *Peridinium* would not grow without added microelements.

An alternative possible "*Peridinium* bloom-supporting factor" is an unknown organic compound. The loads of organic matter entering Lake Kinneret have declined consistently since the mid-1970s, resulting in significantly lower organic-N concentrations in Lake Kinneret (Nishri, 2011). These observations, as well as the correlations between peak *Peridinium* biomass and the loads of organic N, or ambient concentrations of dissolved organic N (as surrogate of dissolved organic matter) (Table 1), may suggest two possible roles for an organic compound as the "*Peridinium* bloom-supporting factor": (1) as a chelator, making micronutrients such as Se available to *Peridinium*; and (2) As a source of carbon—this last possibility asserts mixotrophic activity for *Peridinium*. Mixotrophy, the possession of both phototrophic and heterotrophic capabilities, has been emphasized as a mechanism for enhancing nutrient supplies in many dinoflagellate species (Burkholder et al., 2008). Possibly, *Peridinium* is capable of utilizing organic compounds and requires some component that is associated with DON. Toxic marine dinoflagellates such as *Gymnodinium breve* (renamed *Karenia breve*) and *Pfiesteria piscicida* are known to utilize urea (Steidinger et al., 1998; Lewitus et al., 2000). High levels of urea (>1.5 μM N) were found concomitantly with dinoflagellate blooms in commercial hybrid-striped bass aquaculture ponds

(Glibert & Terlizzi, 1999). Whether *Peridinium* is capable of supplementing its autotrophic activity by heterotrophic nutrition, thus providing another competitive advantage in the presence of organic matter, is yet to be proven. So far, we have no direct evidence for osmotrophic or phagotrophic nutrition in *Peridinium*. To the contrary, Pollingher and Berman (1976) demonstrated uptake of labeled glucose and amino acids by several planktonic species from Lake Kinneret but not by *Peridinium*. Also, we never observed microscopically other algal or bacterial cells within the protoplasm of *Peridinium* to infer phagotrophic nutrition. Nevertheless, we propose that this nutritional pathway was not pursued sufficiently and further studies in this direction are essential.

Years of exceptionally intense blooms

The long-term record of phytoplankton from Lake Kinneret had 6 years of particularly intense *Peridinium* blooms (Fig. 1), five of which occurred since the mid-1990s. All these years were high-rainfall years, with high Jordan River inflows ($>450 \times 10^6$ m^3) and consequently high nutrient loads. Before the mid-1990s, years with similarly high inflow of Jordan River and nutrient loads did not result in similar exceptional blooms. While we lack a clear explanation for this phenomenon, it could possibly be explained by more extreme water level fluctuations and additional supply of phosphorous during winters of rapid increase in water level (Zohary & Ostrovsky, 2011).

Ecosystem impacts of the lack of *Peridinium* blooms

In the past, mean winter–spring chlorophyll concentration and primary production in Lake Kinneret were positively correlated, respectively, with chlorophyll and primary production of the following summer–fall (Berman et al., 1995): springs of intensive *Peridinium* blooms were followed by summers of higher total phytoplankton biomass than that following springs with modest blooms. This correlation was interpreted as *Peridinium* biomass being the main source of nutrients, organic matter and energy fueling photosynthetic and heterotrophic activities in the lake in the summer, when external supplies are small. In particular, Hadas & Pinkas (1995) highlighted the importance of the *Peridinium* bloom biomass as the main source of organic matter for sulfate reduction processes, by which most of the organic matter in Lake Kinneret hypolimnion and sediments is being decomposed.

Dinoflagellates develop populations with low specific productivities and long generation times. Thus, they immobilize large amounts of nutrients and slow down overall ecosystem activity (Pollingher, 1988). In Lake Kinneret, these typical features were distinct, characterizing the blooms of *Peridinium* as well as overall ecosystem functioning. The above correlation between the summer–fall phytoplankton biomass and that of the preceding winter–spring continued to exist after the mid-1990s—but only for years in which *Peridinium* bloomed; no-bloom years did not show this correlation. This is indicative of fundamental differences in overall lake metabolism between years when *Peridinium* bloomed and no-bloom years. These differences may stem from various features of *Peridinium* and its blooms: (1) The different cellular C:N:P ratios of *Peridinium* (460:50:1, Wynne et al., 1982; Zohary et al., 1998) versus the Redfield ratio of 106:16:1 make it a food source of different nutritional values than other phytoplankton species. (2) Allelopathic capabilities of *Peridinium* (Schatz et al., 2007) made its blooms practically unialgal. No other species in Lake Kinneret could attain and maintain the >95% dominance typical of the *Peridinium* blooms. (3) No other species could attain the peak wet-weight biomass achieved by *Peridinium* (Fig. 3), and hence, the total amount of organic matter in spring was smaller. (4) The timing of peak biomass was altered: instead of a unimodal annual pattern with a spring peak, the no-bloom years showed various patterns, including unimodal with an early (February) peak (2001), bi-modal (2000, 2008), or a relatively flat pattern with only minor peaks (2009) (Fig. 3). (5) Higher abundance of toxin-producing cyanobacteria, both in winter–spring (*Microcystis*) and in the summer–fall (*Aphanizomenon ovalisporum*) since the mid-1990s are also likely to contribute to fundamental differences in ecosystem functioning, as well as poorer water quality.

A notable event in Lake Kinneret was a crash in 2008 of the commercial fishery, in particular that of *Sarotherodon galilaeus*, a native cichlid with a high commercial value. In the 1980s, it was shown that this species fed primarily on *P. gatunense* (Spataru, 1976). Using stable C isotopes Zohary et al. (1994) estimated that ~80% of its diet came from this single food source. The disappearance of *Peridinium* from the lake

water most likely contributed to its poor body condition in recent years (James Shapiro, pers. Comm.) and eventually to the crash of its fishery. Other factors thought to have contributed to the fishery collapse were over-fishing and loss of spawning grounds due to the increased water level fluctuations far beyond natural (Zohary & Ostrovsky, 2011).

In this context, we attribute the chronicle of *Peridinium* in Lake Kinneret to man-induced changes aimed at improvements (such as the hydrological changes made in the catchment of Lake Kinneret in the mid-1990s) but which led to unpredicted, undesirable changes. Lake Kinneret, previously a stable ecosystem—is no longer so. Its sensitive current status requires greater care and responsibility in its management as a major source of drinking water.

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PHYTOPLANKTON

Bloom forming cyanobacterial complexes co-occurring in a subtropical large reservoir: validation of dominant eco-strategies

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Abstract In this study, we analyse the spatial distribution of cyanobacterial summer blooms in a large subtropical reservoir located in the Uruguay River, from 2007 to 2011; these extraordinary algal growth events are mainly represented by scum-forming and nitrogen-fixing eco-strategists of the *Dolichospermum* and *Microcystis* genera. The use of the eco-strategists approach, based on ecophysiological work and field observations, allowed us to explain the differences in the distribution pattern and temporal dynamics of both cyanobacterial complexes. Spatial differences were produced due to much higher and fluctuating cyanobacterial abundances at the right margin of the reservoir and at the littoral areas closer to the dam. Satellite imagery (LANDSAT 5 TM) clearly depicted the stronger algal development in the reservoir arms and in the section closer to the dam. The *Microcystis* spp. complex achieved higher density than the *Dolichospermum* spp. complex. We hypothesise that the hydrological cycle explains the inter-annual fluctuations of the intensity and frequency of cyanobacterial blooms, and that spatial differences in cyanobacterial presence between the reservoir arms, its margins and the main channel is mainly a response to morphometrical and hydrological characteristics.

Keywords Cyanobacteria · Blooms · Eco-strategists · Subtropical reservoir

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Introduction

There is growing evidence that the spatial and temporal incidences of toxic algal blooms has increased steadily, thereby entailing potential risks to both human health and sustainability of ecosystems. Blooms of toxic cyanobacteria represent one of the most serious stressors in lakes, rivers, estuaries and marine environments (Ibelings & Havens, 2008). A wide range of impacts on the ecosystem generally occur when blooms with high cyanobacteria biomass persist or occur with great frequency: shading and growth inhibition of other primary producers (phytoplankton, benthic algae and vascular plants), pH raise and great diurnal fluctuations, interference of food collection for zooplankton filter feeders, increased organic loading and anoxic sediments,

accumulation of ammonia in water by senescence and subsequent bacterial decomposition of the blooms and mass mortality of birds and fish. The recurrence of this scenario produces a decline in biodiversity at all levels, from phytoplankton and zooplankton to birds, as well as changes in nutrient recycling and disruptions of carbon and energy flow in pelagic and benthic food webs (Ibelings & Havens, 2008). Although the relative importance of the effect of cyanotoxins over the above-mentioned stressors is not well defined in scenarios of massive fish kills, it is certain that toxins can cause death, cancer or birth defects in mammals (Kuiper-Goodman et al., 1999), increasing the costs of management and processing required for drinking water.

Eutrophication has been traditionally indicated as the main factor responsible for cyanobacterial blooms (Reynolds, 2006; Schindler et al., 2008), but there is recent evidence suggesting that global warming may also promote these events (Antoniades et al., 2007; Paerl & Huisman, 2008; Kosten et al., 2011). Nutrient enrichment stimulates cyanobacterial growth (Smith, 1983; Moisander et al., 2009) and its threshold value depends on the combination of several factors. For example, de Tezanos Pinto & Litchman (2010) described the interactive effects of nitrogen, phosphorus and light on the development of N-fixing cyanobacteria in a laboratory experiment, and Jiang et al. (2008) screened the interactions among significant factors (nutrients, iron, temperature and light) on the growth of *Microcystis aeruginosa*. This introduces the paramount influence of algal entrainment in nutrient-rich mixed layers and otherwise disentrainment from well-illuminated layers, which are reflected in natural ecosystems in terms of the ratio of euphotic depth to mixing depth of the water column (Reynolds, 2006).

The recognition of the environmental conditions that facilitate and promote the establishment of different species of cyanobacteria is mandatory to predict and manage the problems caused by these noxious blooms. Mur et al. (1999) developed a scheme that groups species according to their physiological traits in six eco-strategies, thus reducing the high diversity of this group in order to facilitate the predictability of blooms. The same species can be classified in more than one category: scum-forming, stratifying, homogeneously dispersed, nitrogen-fixing, benthic and small colony-forming eco-strategists. Oliver & Ganf (2000) proposed a synthetic way of classifying species forming blooms, and described three categories according to the mixing and the light in the water column, but did not consider the dynamics of nutrients. The approaches using ecological features allow the understanding and prediction of species distributions along environmental gradients (Litchman et al., 2010). Nevertheless, the monitoring of the spatial distribution is costly and therefore spatio-temporal patterns of bloom formation in most large inland waters are poorly known (Gons et al., 2005). The assessment of the spatial distribution of phytoplankton biomass may be greatly improved by remote sensing as satellite imagery complements in situ measurements and facilitates mapping large and heterogeneous systems; this technique provides synoptic and timely information on the abundance and distribution of cyanobacterial populations that, in turn, can facilitate public health risk assessment (Wheeler et al., 2011).

In subtropical South America, most studies related to problems caused by cyanobacterial blooms are performed to assess the toxicity risk of these events. There is little ecological research designed to fully understand the causality of blooms composed by different eco-strategists. Salto Grande Reservoir, located on the Uruguay River is one of the aquatic systems more affected in the region by recurrent cyanobacterial blooms composed of species with multiple morpho-physiological adaptations. The first studies performed just after the reservoir was filled in 1979, described a central zone of high flushing rate where phytoplankton was dominated by centric diatoms, and several arms where cyanobacteria rarely thrived, always providing that water level was low (Quirós & Cuch, 1982; Quirós & Luchini, 1982). Later on, De León & Chalar (2003) and Chalar et al. (2002 provided further information regarding the differences between the tributary arms located closer to the dam. Chalar (2009) suggested that decreases in either water discharge, wind-induced resuspension or water level caused *Microcystis aeruginosa* bloom formation. The toxicity of these blooms has been assessed at irregular intervals since 1999 yielding positive results (Chalar et al., 2002).

In this study, we perform an overall analysis of the spatial distribution of cyanobacteria in the Salto Grande Reservoir over the last five summer seasons (2007–2011). We aim to characterise the eco-strategies of the blooms by means of comparing the species composition, their physiological features and the prevailing environmental conditions at the different

sites of the reservoir. We hypothesise that the hydrological regime regulates the development and intensity of summer algal blooms and that the dominant eco-strategies depend on the environmental conditions prevailing at each location of the reservoir.

Methods

Study area

Salto Grande is a large river-like reservoir (750 km^2) with multiple arms located along 100 km of the main channel of the Uruguay River (29°43′ to 31°12′S and 57°06′ to 57°55′W) (Fig. 1). It is characterised by a high water period from April to November and a low water phase during summer time (December to March). Mean flow ranges from 2,800 to 5,563 $m^3\ s^{-1}$ with minimum and maximum records of 216 and 22,000 $m^3\ s^{-1}$ in dry and rainy periods, respectively. The reservoir is polymictic with short-lasting stratification under low flow conditions; it has a mean depth of 6.4 m, a maximum of depth of 35 m and a mean retention time of 11.3 days. This system has a single major entrance and five lateral arms; the Arapey and Mocoretá tributaries provide water inputs of minor importance (the mean flow of the Arapey represents <4% of the Uruguay River flow), whereas the supplies of the Mandisoví, Gualeguaycito and Itapebí are so small that they are not relevant (Quirós & Cuch, 1982). The reservoir is utilised for drinking water and recreational activities, including sports and fishing. Its waters are treated for drinking purposes using conventional water treatment technology that include activated carbon filters.

In the drainage area of the reservoir, the mean annual temperature is 19°C and the mean annual rainfall is 1,260 mm. Local winds have a NE direction during the entire year, with mean monthly velocities ranging between 10 and 12 km h^{-1}; in summer and spring the prevailing winds are N, NE, E and SE and in autumn and winter, without being dominant, the frequency of S and SW winds increases (Rojas & Saluso, 1987).

Sampling design

We here analyse the most recent cyanobacterial records produced in routine samplings by the Joint Technical Commission of Salto Grande (CTM) and the Uruguay River Management Commission (CARU). In Fig. 1, we indicate the 17 sampling points analysed over five warm periods from 2007 to 2011; for sites 15 to 17, data are only available since 2009. CTM provided the data of water level, measured as the depth at the deepest point closest to the dam, and the inflowing discharge estimated with a mass balance calculated as a function of water level.

Samples were collected each 7–10 days from January to April at 20 cm depth below the water surface. Subsurface temperature, pH and conductivity and dissolved oxygen were measured in situ using Orion Sa 720 and YSI 58 portable electronic metres. Temperature profiles were performed at sites 1, 9 and 10 during the 2010 and 2011 summer seasons at 1 m depth intervals between the surface and the bottom. Transparency was estimated with a Secchi disc; the euphotic depth (Z_{eu}) was estimated as 2.7 times the Secchi depth. Samples for soluble nutrients and chlorophyll *a* analyses were collected at the subsurface in plastic bottles pre-rinsed in lake water and preserved in dark and cold conditions until their filtration through fibreglass filters (Whatman GF/F). Nutrient determinations were only performed at sites 1, 9, 10, 14, 15, 16 and 17 from 2009 to 2011. Phosphate and nitrate were analysed following the stannous chloride method and cadmium reduction method, respectively, and ammonia was estimated by nesslerization. Total phosphorus (TP) and nitrogen (TN) were determined from unfiltered samples after digestion with persulfate (APHA, 2005). Inorganic suspended solids were evaluated by drying the non-filtrable residue at 550°C until constant weight (APHA, 2005). Concentrations of chlorophyll *a*, corrected for phaeopigments, were determined by spectrophotometry before and after acidification (HCl 0.1 N), using acetone at 90% solvent (Nusch, 1980). The equations published by Lorenzen (1967) were used for the calculations.

Water samples for quantitative phytoplankton analysis were preserved in PVC flasks with 1% Lugol's iodine solution. Counts were performed according to Utermöhl (1958). Replicate chambers were left to sediment for at least 24 h. Counting errors were estimated according to Venrick (1978), accepting a maximum of 20% for the most frequent species. When dense scums occurred, countings were performed with a Neubauer hemocytometer (0.1 mm

Fig. 1 Location of the Salto Grande Reservoir on the Uruguay River, indicating sampling sites:*1* Dam, *2* Playa Sol, *3* Las Palmeras, *4* Los Médicos, *5* Las Perdices, *6* La Toma, *7* Los Pinos, *8* Int. Munic. Salto (IMS), *9* Gualeguaycito, *10* Itapebi, *11* Playa Sur, *12* Playa Baly, *13* Playa Grande, *14* Santa Ana, *15* Belén, *16* Monte Caseros, *17* Bella Unión

deep) under light microscope, after hot digestion with sodium hydroxide (Reynolds & Jaworski, 1978). In this study, we follow Wacklin et al. (2009) who transferred all planktic *Anabaena* morphotypes into the new genus *Dolichospermum*.

Statistical analyses

In order to study the independence between the abundance of the *Microcystis* and *Dolichospermum* complexes at the different sites and locations (beaches,

tributaries, main channel, margins), we performed non parametrical analysis by Chi-Square tests. We then related the algal data of the resulting homogeneous sites to environmental variables by using the Spearman correlation index. The Canoco program was used to perform a redundant detrended analysis (RDA) using the matrix of mean annual physico-chemical and hydrological variables and the abundance of the two cyanobacterial complexes. This 'biological matrix' was tested separately against an environmental matrix obtained for the 17 sites without nutrient data and against a smaller matrix corresponding to the 7 sites with available nutrient information. The significance of canonical axes was analysed by a Monte Carlo permutation test (Ter Braak & Verdonschot, 1995).

Satellite imagery

We used two adjoining pairs of Landsat-5 TM images that represented contrasting scenarios in the reservoir: February 4, 2009 (extremely low waters), and March 14, 2011 (mid-waters). Both scenes were from the Landsat Path 225 and Rows 81–82. A water-only image was created using an unsupervised classification that was recoded and used to mask terrestrial areas. In order to locate the field sampling stations on satellite images and to extract the corresponding radiometric data, the images were geometrically corrected using well-distributed ground control points (GCP'S), with a positional accuracy of ±1 pixel. A radiometric correction was conducted with the Rayleigh dispersion model in order to reduce the atmospheric contribution (Stumpf, 1992). We extracted the radiometric data (reflectance) and analysed which bands or bands ratios were most suitable for creating a regression model for each image by performing Pearson correlations between satellite and in situ data. The spatial distribution of chlorophyll *a* was determined by applying the formulated regression model. Image processing was conducted using ERDAS Imagine 9.1 software.

Results

The fluctuation of the hydrological regime of the Salto Grande Reservoir from December 2006 to March 2011 is depicted in Fig. 2; the inflowing discharge registered a marked variation over the study period, from 434 in May 2009 to 29,730 $m^3\ s^{-1}$ in November 2009. Major inflows occurred periodically during late winter and early spring, except for summer 2010 when high river discharge persisted till late summer; contrarily, a prolonged drought period spanned from early summer to fall 2009. Water level of the dam only fluctuated between 29.9 and 36.8 m in July 2007 and December 2009, respectively. Inflowing discharge and water level were significantly correlated ($r = 0.44$, $P < 0.01$, $N = 903$). A rough estimation of mean water retention time showed strong differences among summer seasons, ranging from approximately 15 days in 2010 to 66 days in 2009.

The physico-chemical characteristics of the Salto Grande Reservoir during the five warm seasons here analysed are summarised in Table 1. Water temperature ranged from 13.8°C at site 1 (dam) to 35.9°C at the beach located at site 3; higher mean annual temperatures were always registered along the shoreline sites (2007, 2008, 2010 at site 3; 2009 at site 4; 2011 at site 15). The summer of 2007 was warmest (mean 28.4°C), whereas the lowest mean temperature (26.4°C) corresponded to 2009. Waters were generally well oxygenated, though at sites 9 and 10, concentrations as low as 3 $mg\ l^{-1}$ were measured. Highest oxygen concentrations were mostly registered at these same sites in the main channels of the inflowing tributaries, and at site 3 with a seasonal mean value of 15.7 $mg\ l^{-1}$. Waters were slightly alkaline with mean pH higher than 7.3 achieving a maximum value of 10.1 at site 9. Mean conductivity in the reservoir was low, 55.8 $\mu S\ cm^{-1}$, with higher values at the upstream sites and especially at those located at the left margin. Transparency (mean 0.66 m) was usually higher at the main channel of the river and its tributaries than in the beaches; it was inversely related to water discharge ($r = -0.3$, $P < 0.05$, $N = 831$). The mean concentration of inorganic suspended solids revealed a clear pattern with decreasing values from the upriver sites (20.3 $mg\ l^{-1}$) to the dam (5.3 $mg\ l^{-1}$).

The information regarding nutrients is scarce and available only for the sites located in the main channel of the river (1, 14) and tributaries (9, 10) and at the northern sites (15, 16, 17). Phosphorus and nitrogen inorganic dissolved fractions remained fairly constant along the reservoir, with mean concentrations of 20 and 680 $\mu g\ l^{-1}$, respectively. Contrarily, TP decreased downriver from 60 to 40 $\mu g\ l^{-1}$ and TN increased from 4,220 to 5,290 $\mu g\ l^{-1}$; interestingly,

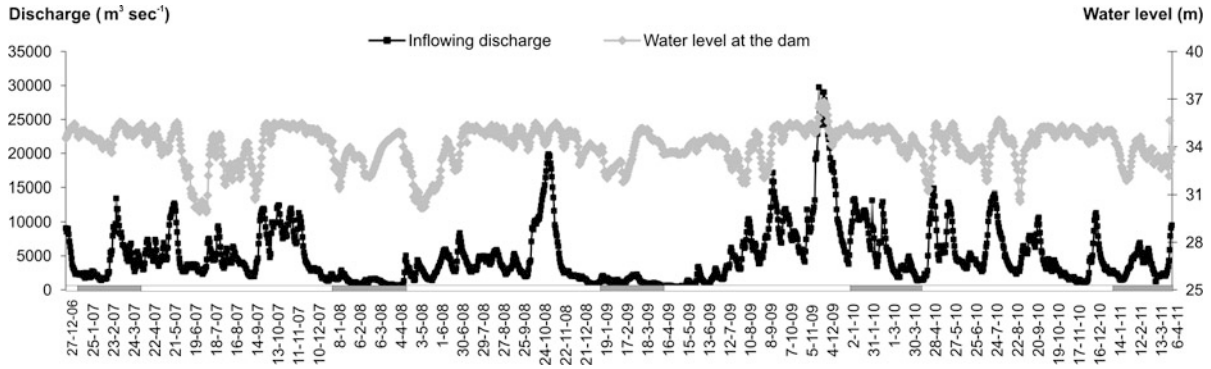

Fig. 2 Daily inflowing discharge of the River Uruguay to the Salto Grande Reservoir and water level at the dam over the study period (2007–2011). Sampling periods are indicated with a *grey shadow* on the *X* axis

discharge was directly correlated with TN concentration ($r = 0.62$, $P < 0.05$, $N = 155$) and inversely with SRP ($r = -0.35$, $P < 0.05$, $N = 155$). As for N:P ratios, TN:TP values were highly variable between years due to the extraordinary drought of 2009 when the enhancement of TN determined ratios as high as 331, whereas during 2010 and 2011 values ranged between 11 and 38.5. The DIN:SRP ratio ranged between 22 and 68.8, with a similar pattern at all sites decreasing from 2009 to 2011. TP (40 μg l^{-1}) and chlorophyll *a* (14.8 μg l^{-1}) mean concentrations correspond to eutrophic systems.

Phytoplanktonic chlorophyll *a* concentration in the Salto Grande Reservoir during the summer periods was significantly correlated with total cyanobacteria cell count ($r = 0.56$, $P = 0.01$, $N = 842$), which in turn was highly correlated with the abundance of species constituting the '*Microcystis* complex' ($r = 0.9$, $P = 0.01$, $N = 870$) and the '*Dolichospermum* complex' ($r = 0.67$, $P = 0.01$, $N = 862$). The most representative species were *Microcystis aeruginosa, M. wesenbergii, Dolichospermum circinale, D. spiroides* and *D. planctonicum*; only occasionally, *Raphidiopsis mediterranea* achieved relatively high abundances.

The abundance of both dominant complexes differed significantly among reservoir locations (Fig. 3), namely the beaches (2–8, 11–13 and 15–17), tributaries (9 and 10) and the middle of the main channel (1 and 14) ($P < 0.05$). There were significant differences between both tributaries (9 vs. 10), between the beaches located at opposite margins (2, 3, 4, 5, 11, 12, 13 vs. 6, 7, 8), and even among the beaches of the right margin located in the dam area (2, 3, 4, 5). Only two homogeneous sets of samples could be identified: the beaches at the left margin of the dam area (6, 7, 8) and at Federación (11, 12, 13). Site 3 presented the highest *Microcystis* mean cell density (113835.4 cells ml^{-1}) and interestingly, despite site 9 is located in a lotic environment, its abundances were also very high (105840.4 cell ml^{-1}). The lowest mean densities correspond to the northern site 17 at the tail of the reservoir where minimum abundances of *Microcystis*, *Dolichospermum* and total cyanobacteria were recorded (617.6, 2553.1 and 3170.7 cells ml^{-1}, respectively), even with quite high chlorophyll concentrations (Fig. 3) that corresponded to eukaryotic algae, mostly diatoms.

There were also annual differences at each site (Fig. 3). Higher cyanobacterial development was observed during 2011 at some sites, especially at the beaches located at the right margin of the dam area (sites 3, 4 and 5), and lower densities during 2010; the *Microcystis* complex was responsible for this general pattern. The distribution pattern of the *Dolichospermum* complex differed slightly, as it was fairly abundant in 2009 and at other sites, namely those located at the beaches in Federación (sites 11, 12 and 13).

The significant correlations with the environmental variables at different sites for both cyanobacterial complexes reveal different responses (Table 2). In general terms, *Microcystis* appears mainly negatively correlated to discharge, while *Dolichospermum* negatively to water level and positively to temperature. The increase of both discharge and water level had a negative effect on cyanobacterial development whereas temperature favoured their growth. Secchi depth showed controversial responses and no correlation was observed

Table 1 Mean values and standard deviations of physico-chemical variables for the sites analysed at the Salto Grande Reservoir over the five warm periods (2007–2011)

	Temp. (°C)	Oxygen (mg l^{-1})	pH	Conduct (µS cm^{-1})	Secchi (m)	SS550 (mg l^{-1})	SRP (µg l^{-1})	TP (µg l^{-1})	DIN (µg l^{-1})	TN (µg l^{-1})
1	27.0 ± 1.9	7.9 ± 1	7.5 ± 0.6	57 ± 7	0.7 ± 0.5	5.5 ± 3.5	20 ± 8	40 ± 20	660 ± 240	5290 ± 4890
2–5	27.7 ± 2.3	8.3 ± 1.6	7.6 ± 0.6	53 ± 5.3	0.5 ± 0.1	5.7 ± 5.2	nd	nd	nd	nd
6–8	27.5 ± 2	7.7 ± 0.8	7.4 ± 0.4	54.2 ± 5.6.	0.6 ± 1.6	4.6 ± 2.6	nd	nd	nd	nd
9	27 ± 2.2	8.8 ± 1.8	8.2 ± 0.9	56 ± 9.4	0.8 ± 0.4	5.1 ± 2.4	20 ± 9	40 ± 25	600 ± 230	2290 ± 4990
10	26.8 ± 2	8.4 ± 1.5	7.7 ± 0.5	60 ± 6.5	0.7 ± 0.5	8.9 ± 9.7	20 ± 9	30 ± 25	726 ± 226	4470 ± 4876
11–13	27.5 ± 2.2	7.8 ± 1.3	7.5 ± 0.7	55.8 ± 12.2	0.45 ± 1.3	9-5 ± 5-9	nd	nd	nd	nd
14	27 ± 1.8	7.5 ± 1.3	7.6 ± 0.5	60.7 ± 7.6	0.7 ± 0.5	9.4 ± 6.3	20 ± 9	40 ± 34	680 ± 159	2860 ± 4000
15–17	26.3 ± 3.3	7.9 ± 1.3	7.4 ± 0.6	61.4 ± 9.8	0.56 ± 0.2	15.8 ± 14.5	20 ± 13	50 ± 44	696 ± 241	4080 ± 4424

Mean values are shown for beaches located at the right (2–5) and left (6–8) margins of the dam area, at Federación (11–13) and at the northern area of the reservoir (15–17)

Nd no data

with nitrogen and phosphorus ratios. Few weak correlations were found with nutrient concentrations: between *Microcystis* and SRP at site 10 ($r = -0.43$, $P < 0.1$, $N = 23$) and *Dolichospermum* and N–NH_4 at sites 14 and 17 ($r = 0.49$, $P < 0.1$, $N = 17$ and $r = 0.47$, $P < 0.1$, $N = 22$, respectively).

The triplot resulting from the RDA using the abundances of the two cyanobacterial complexes and environmental variables from the 17 sites is shown in Fig. 4a. The environmental variables are significantly correlated with the first axis ($P = 0.002$), and the test of significance of all canonical axes is also significant ($P = 0.002$). The first axis explains a large proportion of variance in the cyanobacterial complex–environment relationship (75.6%) and is mainly defined by water level, oxygen, discharge and pH (intraset correlation coefficients: 0.45, −0.30, 0.29 and −0.25, respectively). The triplot represents 35% of the total variance of cyanobacterial data; temperature and oxygen were the variables most strongly correlated with the second axis (intraset correlation coefficients: 0.35 each). This analysis clearly shows that the hydrological variables were major factors controlling both complexes; *Dolichospermum* was benefited by the demise in water level (2009) and *Microcystis* was also positively influenced in warmer years (especially 2007) as depicted in the grouping of the samples corresponding to the different summer seasons. Oxygen and pH mostly respond to the metabolic activity of strong *Microcystis* blooms.

The influence of nutrients was analysed in a complementary RDA performed with the reduced dataset (7 sites, 3 years) (Fig. 4b). The environmental variables are significantly correlated with the first axis ($P = 0.034$), and the test of significance of all canonical axes is also significant ($P = 0.014$). The first axis explains 73.4% of variance in the cyanobacterial complex–environment relationship and is correlated with pH, TP, SRP, oxygen and water level (intraset correlation coefficients: 0.57, −0.48, −0.35, 0.34 and −0.26, respectively). The second axis is mainly associated with TP, DIN and pH level (intraset correlation coefficients: −0.36, 0.35 and −0.236, respectively). Thus, low values of water level and discharge once again evidenced the enhancement of cyanobacterial growth, but this additional analysis suggested that SRP and TP could have limited cyanobacterial growth.

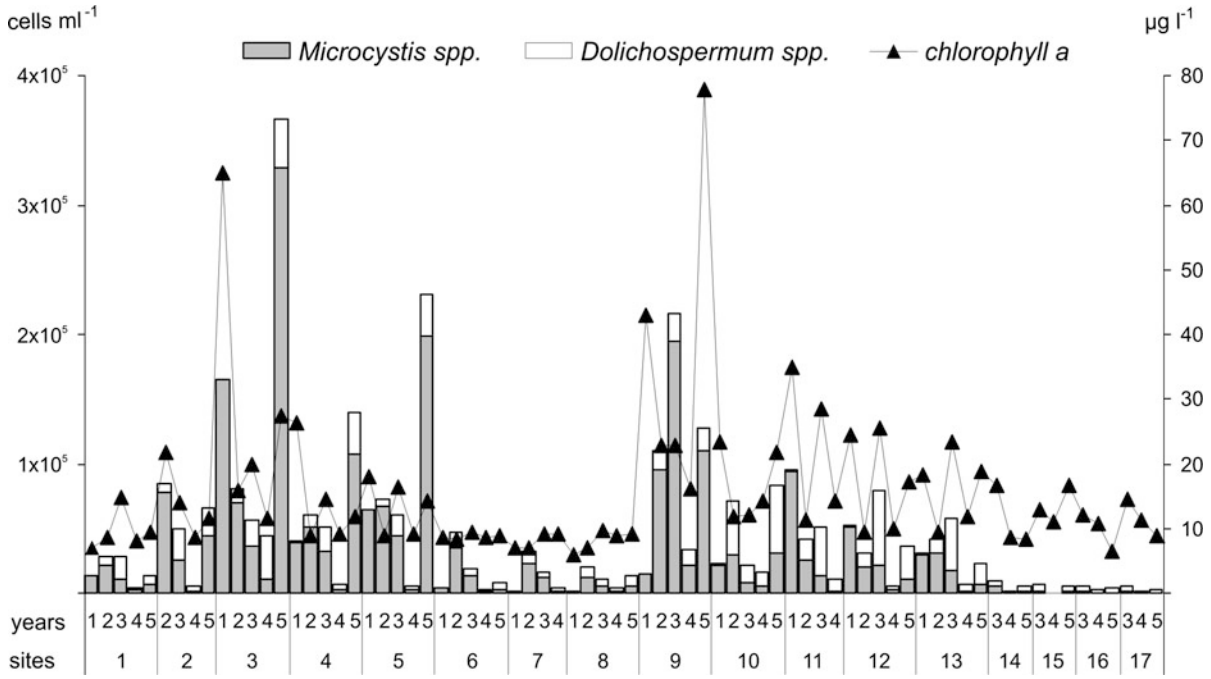

Fig. 3 Mean temporal variations at each site for the *Microcystis* spp. and *Dolichospermum* spp. complexes from 2007 to 2011 (*1* 2007, *2* 2008, *3* 2009, *4* 2010, *5* 2011)

The influence of temperature variation and stability of the water column on the abundance pattern of both eco-types in each summer season at deep sites (main channel and tributaries) is represented in Fig. 5 with data from site 9. *Dolichospermum* achieved highest densities (Fig. 5a) at elevated surface temperatures (>28.9°C), which coincided with stratification of the water column that was registered at the onset of summer (Fig. 5b). On the other hand, *Microcystis* increased its concentrations towards the end of summer or beginning of autumn, especially during 2011 (Fig. 5b). The pattern here illustrated for site 9, was also observed at sites 1 and 10 (data not shown).

Table 3 summarises the occurrence of blooms with more than 10^5 cyanobacterial cells ml^{-1} (WHO alert level 2 for drinking water supply) (Bartram et al., 1999) for each summer season at the different environments. It is evident that the worse scenarios (high cyanobacterial development) always corresponded to the beaches of the right margin located in the area close to the dam (RMDam); the higher frequency of events was observed in 2008 and 2011 when low water periods coincided with high temperatures. In the beaches along the shoreline, the light climate was good as estimated by the euphotic depth (Z_{eu}) that generally comprised the entire water column, whereas in the open channels the relation between the mixing and euphotic depth (Z_{mix}/Z_{eu}) ranged between 5 and 6 in the tributaries (sites 10 and 9, respectively) and 16 at the main flow near the dam.

Mapping chlorophyll *a* by satellite imagery

Figure 6 presents the chlorophyll *a* (chl *a*) thematic map predicted from the March 14, 2011 satellite imagery analysis. In situ chl *a* range was 0.9–62 µg l^{-1} and the higher correlation coefficient was found between ln chl *a* and band 2 (b2: 0.52–0.60 µm) ($r = -0.83$); the derived regression model was ln chl $a = 23.48–0.72$ b2 ($R^2 = 0.7$). The image shows great spatial variations between the reservoir areas, with remarkable higher chlorophyll *a* concentrations at the arms. Approximately 74% of the Gualeguaycito arm area shows values exceeding the threshold of alert level 2, 18% values exceeding alert level 1 and 8% values below the alert levels for drinking water supply (Bartram et al., 1999). Similarly, 69% of the Itapebi arm area presents chlorophyll *a* values exceeding alert level 2, 22% values exceeding alert level 1 and 10% show values below the alert levels. On the other hand,

Table 2 Significant Spearman correlation indexes between the abundance of the *Microcystis* and *Dolichospermum* complexes and environmental variables (* $P = 0.01$; ** $P = 0.05$) with the respective number of observations

	Temp.		pH		Conduct.		Secchi		Discharge		Water Level	
	Mic	*Dol*	*Mic*	*Dol*	*Mic*	*Dol*	*Mic*	*Dol*	*Mic*	*Dol*	*Mic*	*Dol*
1		0.28*	0.31*	0.36*	0.33*	−0.38**			−0.55*			−0.40**
		61	59	59	47	47			63			63
2		0.27**			0.41**				−0.67**		−0.34**	−0.32*
		53			53				57		57	57
3					0.57**		−0.27*					−0.29*
					62		67					67
4					0.34*				−0.55**		−0.33**	−0.32**
					59				65		65	65
5					0.39*				−0.59**		−0.34**	−0.34**
					63				67		67	66
6–8		0.24**	0.53**			−0.2**	0.33**		−0.61**		−0.26**	−0.29**
		170	157			159	177		179		179	172
9		0.51**	0.49**	0.42**					−0.32*			
		51	47	47					54			
10		0.47**						0.55**	−0.38**		−0.29*	−0.41**
		48						40	50		50	50
11–13		0.27*		0.37**	0.35*			−0.34**		−0.20*		−0.31**
		127		121	118			117		130		130
14					0.50*	0.53*			−0.45**	−0.64**		−0.55**
					21	21			23	23		23
15					0.45*							
					20							
16				0.39*				−0.32*				0.45**
				39				44				47
17		0.32*	0.41*	0.35*					−0.33*			−0.29*
		43	38	38					44			44

Sampling sites 6–8 and 11–13 are considered homogeneous (Chi-Square test)

near 100% of the area covering the main channel, the dam and beaches show lower concentrations (13–27 μg l^{-1}) that do not exceed alert levels.

Contrarily, the low waters scenario (2009, image not shown) presents a more homogeneous distribution and lower concentrations of chlorophyll *a*. Both Gualeguaycito and Itapebi arms present larger areas with values that did not exceed alert levels (79 and 72% of each arm area, respectively), while 22 and 28% show concentrations exceeding alert level 1. The in situ chl *a* range was 7–14 μg l^{-1} and the higher correlation with satellite data was found with ln band 3 (b3: 0.63–0.69 μm) ($r = -0.67$); the regression derived model was chl $a = 53.2–14.43$ ln b3 ($R^2 = 0.45$).

Discussion

Reservoirs are worldwide endangered by the proliferation of cyanobacterial blooms (Steel & Duncan, 1999; Bittencourt-Oliveira, 2003; Naselli-Flores & Barone, 2003; Bormans et al., 2005; Znachor et al., 2006; Gemelgo et al., 2009; Sotero-Santos et al., 2008; Moisander et al., 2009). The recognition of the environmental conditions that promote and facilitate the establishment of Cyanobacteria requires the identification of the prevailing eco-strategies in each system. In several tropical to subtropical reservoirs, the co-existence of the *Microcystis* and *Dolichospermum* complexes has been repeatedly registered

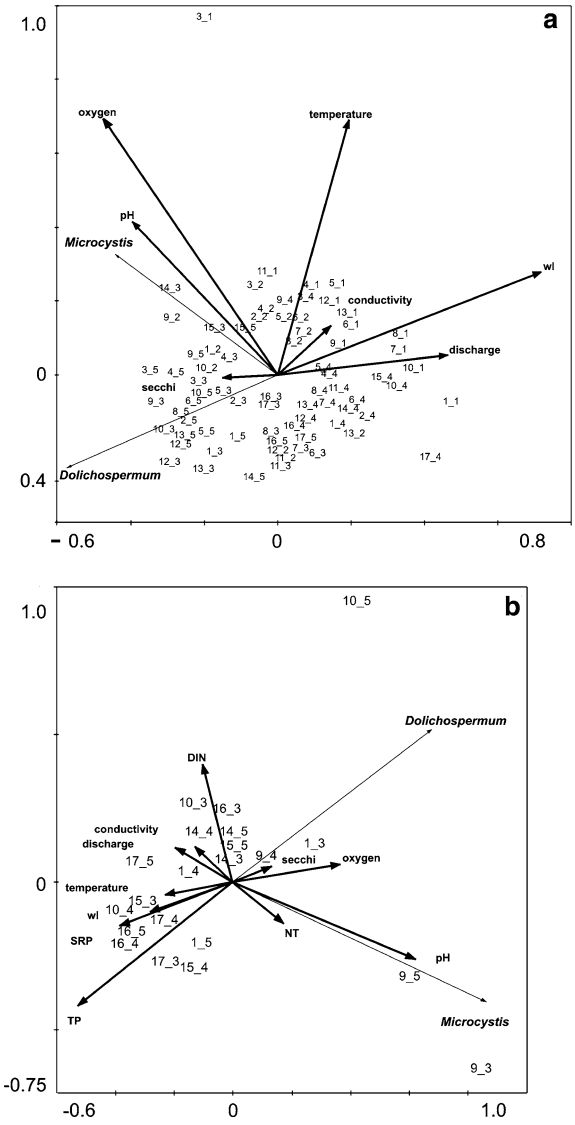

Fig. 4 **a** Triplot of the RDA of the 17 sites surveyed in the Salto Grande Reservoir; **b** triplot of the RDA of the 7 sites including nutrient information. *WL* water level

(Sotero-Santos et al., 2008; Li et al., 2010; Te & Gin, 2011). In the Salto Grande Reservoir, *Microcystis* dominated over *Dolichospermum* exceeding one order of magnitude its cell densities for the five last summer seasons recorded in this study. The same applies for the Kranji Reservoir (Te & Gin, 2011) and for eutrophic Czech reservoirs (Znachor et al., 2006), where *Microcystis* spp. (mainly *M. aeruginosa)* dominates over *Dolichospermum* spp. These species complexes were characterised by Mur et al. (1999) as scum-forming and, in the case of *Dolichospermum,* also as nitrogen-fixing. Both eco-strategies have the common feature to regulate buoyancy and thus, have an advantage in competing with other phytoplankton for light and nutrients. Under which conditions do these traits provide competitive advantages that can lead to extraordinary growth of the populations? Is N-fixation a beneficial trait in this enriched turbid system?

Our findings indicate that hydrological conditions were the driving factors in regulating cyanobacterial blooms in Salto Grande Reservoir: declining abundances of both eco-types are related to increasing discharge and water level as a result of flushing or dilution processes and by hindering upward flotation to the illuminated layers in deep and strongly mixed water columns through the suppression of persistent thermal stratification. Multivariate analyses showed that inter-annual differences of summer cyanobacterial development were strongly correlated with discharge and water level variations; satellite imagery supports these findings. These above-mentioned mechanisms appeared to have affected the algal abundance in Salto Grande Reservoir, triggering slightly different responses in the two blue-green complexes. *Dolichospermum* showed stronger proliferation during periods of lower water levels and more stratified water columns. This is reflected in the plot of the RDA (Fig. 4a) where tributary samples corresponding to low waters are associated to high cell counts of *Dolichospermum* (2009, 2011) and display the highest separation from those of high waters with scarce cell densities (2007, 2010). Interestingly, such high concentrations were not achieved during 2008 under comparatively low waters, probably because levels were still quite high at the beginning of summer (January). Permanently quiescent waters will more likely occur in the tributaries with little inflow (sites 9 and 10) and in sheltered beaches (site 3) at low waters, as these locations are more affected by flow decreases than the main channel. Likewise, in Australian nutrient-rich systems (Mitrovic et al., 2001; Thompson et al., 2003; Bormans et al., 2005), low flow conditions and great insolation by late spring or early summer, increased irradiance to cells in the upper mixed layer improving light conditions for *Dolichospermum* bloom development. *Microcystis* success in relatively better mixed and deeper water columns (e.g. Fig. 5) is explained by its higher ability for surface migration provided by gas vesicles with higher critical pressure

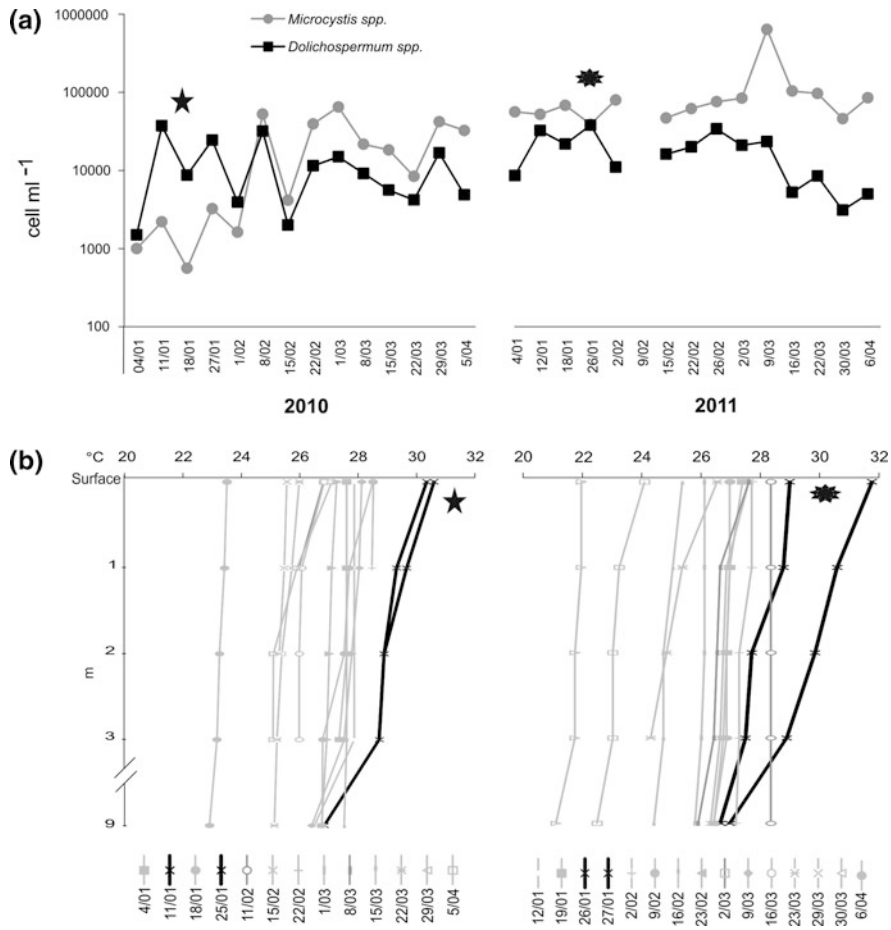

Fig. 5 **a** *Dolichospermum* and *Microcystis* temporal fluctuations at site 9 (Gualeguaycito arm) during the summers of 2010 and 2011. **b** Temperature profiles at site 9 for the same periods. *Note* the *bullets* in the *upper panel* (**a**) correspond to the sampling dates with highest temperatures indicated with *bold lines* in the *lower panel* (**b**)

Table 3 Cyanobacterial bloom frequency (number of weeks with more than 10^5 cells ml^{-1}) at the study sites of the Salto Grande Reservoir indicating the euphotic depth (Z_{eu}) and maximum depth (Z_{max})

	Main flow	Tributaries		Beaches			
	Dam (1)	Gualeg. (9)	Itapebí (10)	RMDam (2, 3, 4, 5)	LMDam (6, 7, 8)	Federación (11, 12, 13)	Northern (15, 16, 17)
Z_{eu}	1.89	2.16	1.89	1.89	1.89	1.17	1.71
Z_{max}	31	10	9.6	1.5	1.5	1.5	1.5
			Number of blooms				
2007	0	No data	0	10	0	6	No data
2008	0	2	3	14	4	5	No data
2009	1	4	1	11	0	4	0
2010	0	0	2	2	0	0	0
2011	0	5	0	21	0	1	0
	1	11	6	58	4	16	0

and buoyant density than those present in *Dolichospermum* species (Visser et al., 2005 and cites therein). Huisman et al. (2004) proved that *Microcystis* can escape from entrainment more easily in deep than in shallow waters, and coincidentally, Chalar (2009) described enhanced *Microcystis* growth at the deeper

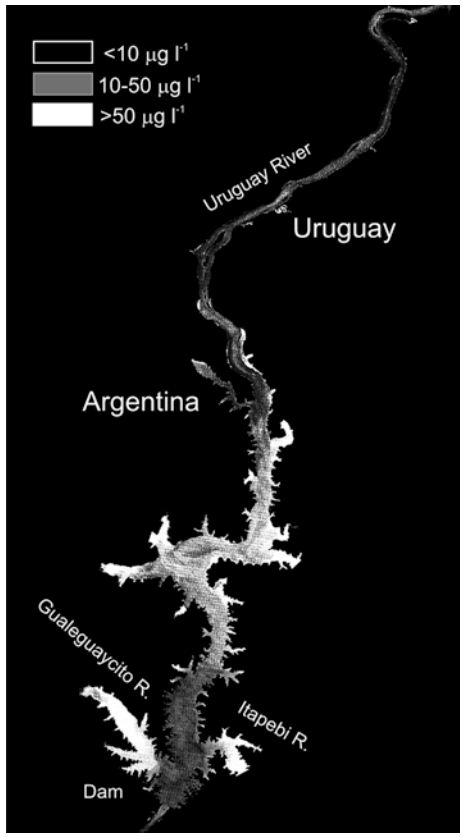

Fig. 6 Chlorophyll *a* thematic map predicted from March 14, 2011 satellite image of the Salto Grande Reservoir

sites in the Gualeguaycito and Itapebi arms of the Salto Grande Reservoir. *Microcystis* colony enlargement over the summer season may have favoured buoyancy regulation by late summer, due to the fact that floating velocity increases with colony size (Reynolds & Walsby, 1975). The analysis of physiological traits contributes to understand the causes of the heterogeneous spatial distribution of both complexes, as we suggest that thriving *Microcystis* superficial populations would be more prone to the drifting action of north-eastern winds that are dominant in the region; hence, cyanobacterial accumulation along the lee shore of the right margin is related to the scum-forming eco-strategy.

The influence of temperature also differed between both blue-green complexes and most interestingly, *Dolichospermum* showed different responses at different temporal scales. The inter-annual analysis performed with summer mean temperature values reveals that warmer years (e.g. 2007) boosted *Microcystis* blooms but did not equally promote *Dolichospermum* growth (Fig. 4a). The lowest mean temperature during the summer of 2009 probably caused the lower bloom frequency and impaired *Microcystis* growth registered in the reservoir, contrary to what would be expected during a drought period. Conversely, at a seasonal scale, *Dolichospermum* showed a positive relationship with temperature at most studied sites as reflected by the correlations performed using weekly values (Table 2). During each summer, maximum cell densities of *Dolichospermum* occurred early in the season and then dropped with early autumn decreasing temperatures (exemplified in Fig. 5a for site 9), just like in Australian systems (Thompson et al., 2003; Bormans et al., 2005). When enhanced temperatures were coupled to stratification in deeper water columns by mid-January (bold lines in Fig. 5b), *Dolichospermum* peaked even exceeding *Microcystis* cell densities.

Despite nutrient controls on phytoplankton production in reservoirs are mostly subordinated to physical factors (Forbes et al., 2008), the absence of abundant Cyanobacteria is better guaranteed at levels below 50 μg P l^{-1} (Dokulil & Teubner, 2000) and their presence is thought to be benefited at low TN:TP ratios (Smith, 1983). In Salto Grande, blue-greens dominated with P concentrations slightly exceeding this threshold and frequently relatively high ratios (DIN:SRP >30, TN:TP mostly exceeding 16). Our results indicate a weak correlation between SRP and discharge during summer seasons. Chalar (2006) asserted that P input occurs in winter months and thus, the lag with phytoplankton growth in warmer seasons favours P exportation from the system (low P retention: 3% of total load); the high nutrient loss probably accounts for low P inter-annual variation in spite of marked hydrological fluctuations. The RDA results show a negative effect of P on cyanobacterial growth: elevated cell density at the surface must have uptaken a great proportion of the SRP pool in the upper layers of the water column, although TP concentrations in Salto Grande were still sufficient to sustain large populations of Cyanobacteria. Although N:P ratios are imperfect predictors of cyanobacteria dominance (Reynolds, 1999; Downing et al., 2001), N-fixers dominance was found in many lakes under a boundary between the Redfield ratio (16) and ~32 (Smith et al., 1995; Nõges et al., 2008; Scott et al., 2008). During 2010,

when TN:TP <16, *Dolichospermum* cell density increased in relation to *Microcystis* at most surveyed sites and even though it did not dominate in terms of cell counts, its biomass may have been higher on some occasions. The mechanistic explanation that N-fixers have a competitive advantage when N is limiting would not be the main factor explaining their relative increase, because the low heterocyte frequency ($\sim$0.07%) here encountered suggests low reliance on fixation (most filaments with heterocytes corresponded to *D. planctonicum* and never to *D. spiroides*). As *Dolichospermum* was significantly correlated with ammonium only at two sites, the evidence is not sufficient to suggest a preference for reduced dissolved nitrogen (Ferber et al., 2004; Znachor et al., 2006), nor increased buoyancy control under ammonium availability (Spencer & King, 1989). The complete understanding of N and P influence on the growth of these two cyanobacterial complexes requires further measurements in the shallow areas (beaches) and at different depths of the water column in the deeper channels of the reservoir.

The positive relationships of both complexes with pH and dissolved oxygen are usually expected because both parameters are related to photosynthetic activities of the cyanobacterial bloom; it is difficult to establish whether high pH is the cause or the result of increased growth of cyanobacteria. Since the CO_2 fraction decreases as pH increases, this might be a limiting factor for photosynthesis. Cyanobacteria generally tend to be strong competitors under conditions of high pH and low CO_2, as they possess carbon concentration mechanisms which allow them to sequester carbon in a variety of forms (Badger & Price, 2003). Nevertheless, it is difficult to distinguish the effects of these parameters individually.

As indicated by Liu et al. (2011), the dominance of cyanobacteria is a stochastic result of a combination of multidimensional environmental factors. In the Salto Grande Reservoir, such interplay produced the above described temporal pattern, which was overlapped with a distinct spatial distribution that showed higher cyanobacterial development in the arms and beaches as compared to the main channel. The beaches at the right margin of the dam area, especially site 3, were characterised by the highest frequency of blooms (alert level 2 sensu Bartram et al. (1999)) on behalf of their shallowness that ensures cell entrainment in well illuminated and warmer water columns. Moreover, most beaches located in sheltered areas along the shore line (site 3 is almost surrounded by land) and the southern arms receiving little inflow from the Gualeguaycito and Itapebí rivers, act as dead zones ideal for phytoplankton growth and accumulation. During major inflow periods (2010), the small number of blooms registered corresponded to these sheltered sites with reduced flow influence from the Uruguay River. The lower Z_{mix}/Z_{eu} ratios in the tributaries as compared to the main channel supports that mixing of the water column was still low to enable Cyanobacteria buoyancy to overcome mixing so that cells could spend more time in well-illuminated layers. The chlorophyll *a* distribution depicted in the satellite images confirms strong algal biomass concentrations in the arms and shows how the right margin beaches are more exposed to receive the drift of cyanobacteria from the Gualeguaycito arm. The influence of the prevailing NE winds supports the occurrence of a higher concentration of surface scums at the right margin. Although LANDSAT imagery allowed analysis of a large area and thus detected high biomass concentrations in stretches that were not included in the original sampling programme, its use as a monitoring tool is not highly reliable during very low water periods when interference with sediments increases. The use of a satellite sensor such as MERIS, would improve the efficiency of remote sensing under all environmental scenarios due to its better spectral resolution and more suitable bands to detect chl *a* in turbid waters (Gons et al., 2005; Wheeler et al., 2011).

Studies that address the effects of several environmental factors on the growth and abundance of cyanobacterial populations under field conditions with a long-term monitoring record are scarce (Wiedner et al., 2007; Liu et al., 2011). Such investigations provide the necessary information for the application of adequate management measures. A long-term ecological study on the regulated Nakdong River supported the necessity of 'smart flow control' in order to destruct the bloom formation (Jeong et al., 2007); similar measures were proposed by Mitrovic et al. (2010) for Australian systems. Despite, the results of this 5-year study indicate that the way to reduce cyanobacterial biomass in Salto Grande would be related to increasing the mixing of the water column, the morphology of this multiple arm reservoir discourages the success of this measure as these inflow areas act as 'dead zones' mostly in summer time when

rivers have low discharges. In order to perform adequate plan managements, additional information is required namely nutrient data for the beaches area, dynamics of the inflowing river discharges and studies on viability and abundance of akinetes and dormancy stages in sediments.

Final remarks

Two cyanobacterial complexes dominated the Salto Grande Reservoir: *Microcystis* spp. and *Dolichospermum* spp. Their distribution was highly affected by fluctuating hydrological conditions and the morphology of the reservoir, differing along its 100 km length, between margins, over the 5-year survey and within each summer. The successful adaptation to the prevailing environment mostly by means of buoyancy control validates their classification as scum-forming eco-strategists. Nevertheless, as our results show a difference in the behaviour of both complexes that could not be clearly related to N-fixing, further studies should be performed to clarify whether this capacity constitutes a crucial strategy to develop *Dolichospermum* blooms under specific conditions in this eutrophic reservoir.

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PHYTOPLANKTON

The habitat template of phytoplankton morphology-based functional groups

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Abstract The identification of the main factors driving phytoplankton community structure is essential to understand and adequately manage freshwater ecosystems. We hypothesize that differences in morphological traits reflect phytoplankton functional properties that will be selected under particular environmental conditions, namely their *habitat template*. We apply a morphology-based functional groups (MBFG) approach to classify phytoplankton organisms and define each group template. We use machine learning techniques to classify a large number of phytoplankton communities and environmental variables from different climate zones and continents. Random forest analysis explained well the distribution of most groups' biovolume and the selected variables reflected ecological preferences according to morphology. By means of a classification tree it was also possible to identify thresholds of the environmental variables promoting groups dominance in different lakes. For example group III (filaments with aerotopes and high surface/volume including potentially toxic species) was dominant when light attenuation coefficient was >3.9 m^{-1} and total nitrogen was >2,800 $\mu g\ l^{-1}$. We demonstrate that morphology captures ecological preferences of phytoplankton groups and provides empirical values to describe their habitat template.

Keywords Morphological traits · Functional groups · Random forest · CART · Environmental change

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Introduction

Phytoplankton is essential for the functioning of our planet as it accounts for half of earth's primary production (Falkowski et al., 2003; Arrigo, 2005).

Also many problems of water quality are caused by phytoplankton with potentially serious implications for human and ecosystems health (Huisman et al., 2005). The identification of the main biotic and abiotic factors controlling phytoplankton in lakes is thus essential for the adequate management of freshwater ecosystems (Peretyatko et al., 2007).

Aggregated estimators of phytoplankton communities (e.g. total biomass) may work to describe overall community responses to varying environmental conditions (Vollenweider, 1976; Scheffer et al., 2003). However, phytoplankton species differ widely in their responses to environmental change, including their way of resources acquisition (light and nutrients) to grow, and the way of avoidance of mortality (washout, sedimentation and grazing) (Margalef, 1978; Reynolds, 1984a; Naselli-Flores et al., 2007). These features can be combined to describe the species habitat template (sensu Southwood, 1977). This concept views the habitat as a template on which evolution forges characteristic species traits (Southwood, 1988), and can be used to predict community organization (Keddy, 1992). Habitat templates have been built up for phytoplankton for different species, combining traits and environmental gradients (Margalef, 1978; Reynolds, 1988; Reynolds et al., 2002; Salmaso & Padisák, 2007).

In this vein, 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. Well-known examples are the Plankton Ecology Group (PEG) model (Sommer, 1989) that predicts the seasonal succession in temperate lakes and the Margalef mandala (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 (Le Quéré et al., 2005; Follows et al., 2007). These and other examples illustrate that clustering species based on their functional traits makes sense to summarise their response to environmental change.

Morphology-based functional groups

Morphological traits are relatively easy to measure and have clear relationships with the functional properties of phytoplankton (Lewis, 1976; Reynolds, 1984b; Naselli-Flores et al., 2007; Kruk et al., 2010). The morphology-based functional groups (MBFGs) approach clusters organisms in seven groups in terms of morphological traits (e.g. volume and the presence of flagella) independently from the organism's taxonomic affiliation (Kruk et al., 2010) (Fig. 1). In turn, significant differences in growth rate, sinking rates, demographic properties and competitive ability have been shown for these groups (Kruk et al., 2010; Segura et al., 2011, 2010). For example, group I represents small, high surface to volume ratio (S/V) organisms, with high growth rate and low sinking, and with better competitive ability at the beginning of temporal succession. The groups are also well predicted by environmental variables independently from geographical location (Kruk et al., 2011). Based on the morphological traits of each MBFG, potential ecological performance in terms of resources acquisition and avoidance of loss processes (consumption and sinking) have been derived (Table 4 in Kruk et al., 2010). However, the analysis of each MBFG environmental preferences has not been yet established. We hypothesize that differences in morphological traits among MBFG reflect phytoplankton functional properties which will be selected under particular environments. Therefore, MBFG anticipate phytoplankton habitat template.

In this article, we aim to link each MBFG to its habitat template using information from a very large number of phytoplankton communities and environmental variables from different climate zones and continents. We used random forest (RF) regression to evaluate which environmental variables explained best each MBFG biovolume distribution among lakes, and classification trees to detect the particular environmental threshold favouring the dominance of each MBFG. We also re-validate the classification in MBFG evaluating its power using classification trees.

Materials and methods

Fundamentals of the MBFG classification

Phytoplankton organisms are distinguished in seven MBFG groups based on eight morphological traits identified for each organism at the light microscopy (Fig. 1) (Kruk et al., 2010). Group I includes small

Table 1 Average and range for the analysed environmental variables and the seven MBFGs biovolume (I–VII)

Variables	Mean (range)
Temp (°C)	17.2 (0.4–33.0)
Z_{mix} (m)	2.2 (0.1–17.0)
K_D (m^{-1})	3.9 (0.4–43.6)
TN (μg l^{-1})	2424 (35–37,928)
TP (μg l^{-1})	191 (0.0–10,086)
RSi (μg l^{-1})	3492 (0.0–23,533)
TZ (org l^{-1})	1644 (0.5–26,319)
I (mm^3 l^{-1}), $N = 211$	4.8 (0.0–2453)
II (mm^3 l^{-1}), $N = 472$	0.6 (0.0–18)
III (mm^3 l^{-1}), $N = 446$	7.0 (0.0–1,798)
IV (mm^3 l^{-1}), $N = 675$	8.9 (0.0–3,173)
V (mm^3 l^{-1}), $N = 856$	3.3 (0.0–152)
VI (mm^3 l^{-1}), $N = 819$	10.4 (0.0–3,367)
VII (mm^3 l^{-1}), $N = 583$	6.3 (0.0–987)

N number of non zero values

organisms with high *S*/*V*. Group II clusters small flagellated organisms with siliceous exoskeletal structures. Group III represents large filaments with aerotopes. Organisms of medium size lacking specialized traits are included in group IV. Group V gathers unicellular or colonial flagellates of medium to large size. Non-flagellated organisms with siliceous exoskeletons are in group VI, and group VII includes large mucilaginous colonies.

The continuous traits included in the classification are: volume (*V*, μm^3), surface/volume (*S*/*V*, μm^{-1}) and maximum linear dimension (MLD, μm), and are calculated based on organisms geometrical approximations following Hillebrand et al. (1999). For all lakes, the organisms are considered as the unit (unicell, colony or filament). For colonial organisms with mucilage, *V* and *S* calculations are made for whole colonies including mucilage. The categorical traits incorporated are the presence or otherwise of flagella, mucilage, siliceous exoskeletal structures and aerotopes. To classify the organisms into the seven MBFG the estimation of continuous traits and the presence of the categorical traits has to be noted for each relevant organism for the original sample and not based on the species names. The absence of any of the traits after inspections at the larger magnifications should not be included even if expected based on the taxonomic classification of the species.

A code in the R software (R, 2011) to classify phytoplankton organisms into MBFG according to individual morphological traits is provided in the Supplementary material online. Using the provided code and R software, which can be free downloaded (http://www.r-project.org/) the user can upload a matrix with information about the organisms

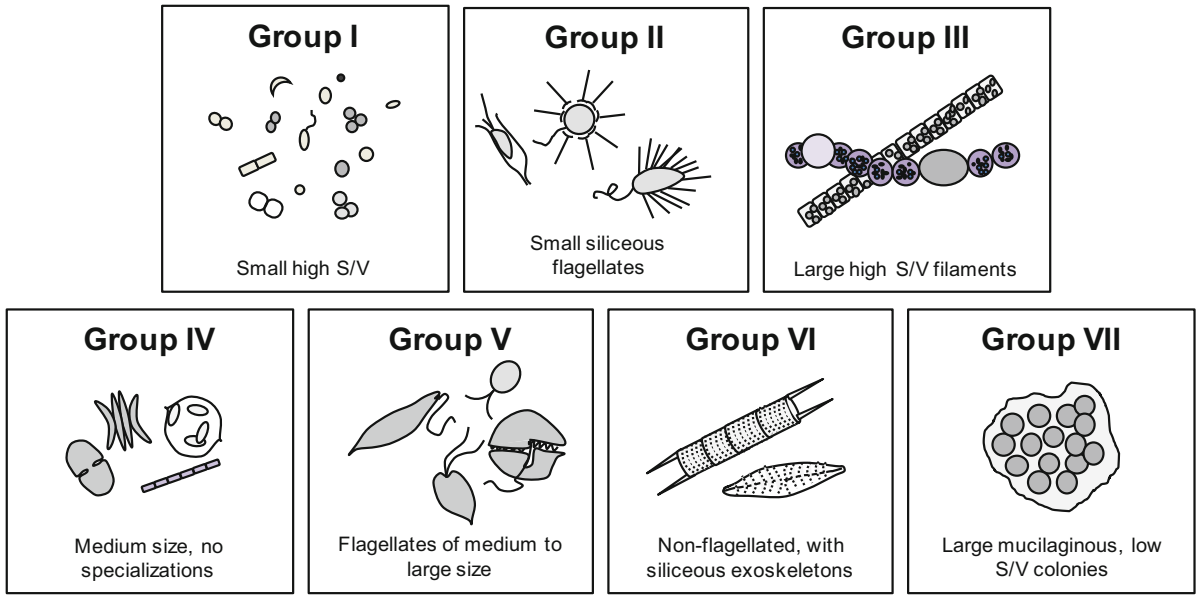

Fig. 1 Schematic representation of the seven MBFGs (Kruk et al., 2010) including a brief description of their morphology. *S* surface, *V* volume

morphological traits and will obtain a new matrix with the original organisms classified into MBFGs (Kruk et al., 2010).

Database

In order to evaluate the MBFG environmental preferences, we compiled a database of 711 species and lake environmental variables from 211 lakes with a total number of 925 samples located within four climate zones in South America, Europe and North America, and covering a wide range of environmental characteristics (Kosten et al., 2009; Kruk et al., 2009, 2011) (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, personal communication; 1999 Dutch multi-lake survey Gerben van Geest & Frank Roozen, personal communication). The remaining 104 lakes were sampled during 2005–2006 by standard procedures, described by Kosten et al. (2009) and Kruk 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. Light attenuation in the water column, temperature (Temp, °C) and oxygen profiles were measured in situ at noon. Light attenuation coefficient (K_D, m^{-1}) and water column mixing depth (Z_{mix}, m) were calculated from in situ measurements. Total nitrogen (TN, $\mu g\ l^{-1}$), total phosphorus (TP, $\mu g\ l^{-1}$) and soluble reactive silicate (RSi, $\mu g\ l^{-1}$) were estimated using standard procedures. For zooplankton abundance determination (TZ, org ml^{-1}), 2 l of lake water were filtered through a 50-μm sieve and preserved in a 4% formaldehyde solution (details on sample analysis in Kosten et al., 2009). Phytoplankton samples were fixed in Lugol's solution.

Phytoplankton traits and biovolume

Phytoplankton populations (individuals ml^{-1}) 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 (Lund et al., 1958). Organisms between 5 and 100 μm were counted at 400×, larger organisms were counted at 200×, and organisms between 5 and 2 μm were counted at 1,000×. We did not include species strongly associated with periphytic communities. Organism dimensions, including MLD were estimated for V and S calculations. The presence of aerotopes, flagella, mucilage and siliceous exoskeletal structures were noted for each relevant organism. Population biovolume ($mm^3\ l^{-1}$) was calculated as the individual volume of the species multiplied by the abundance of individuals. More than 80% of the samples were analyzed by the same group of scientists, using the same identification keys, a common protocol and fluid communication. The species were classified into the seven MBFG and their biovolumes were summed per sample (Table 1).

Statistical analyses

Machine learning

Natural systems generally do not meet statistical assumptions (e.g. normality, homoscedasticity). They are often of high order, non-linear and sometimes show abrupt shifts (Levin, 1992; McGill et al., 2006) which challenges the interpretation of classical statistical techniques (e.g. general lineal models). A number of highly computational statistical methods have recently emerged from the machine learning literature including classification trees and RF (De'ath & Fabricius, 2000; Cutler et al., 2007). These methods can cope with small sample size as compared to the number of variables (small n large p problems), complex interactions, and even with highly correlated predictor variables. See De'ath & Fabricius (2000) and Cutler et al. (2007) for a discussion of these statistical methods in an ecological framework. Despite, these problems are common in phytoplankton ecological studies, the application of the mentioned statistical methods is scarce (Zhao et al., 2008).

Classification and regression trees (CARTs)

In a standard regression situation, we aim to model the response variable based on one or several predictor variables. For example, classical multiple regressions

defines the linear combination of predictors that best explain the response variable in terms of explanatory power. In a different way, a tree is constructed by recursive binary partitioning of the response variable into regions that are increasingly homogeneous (i.e. nodes) until no improvement is possible. This final nodes are called leafs. In regression trees, at each node, the predictor variable that results in the most homogeneous partition of the response variable (measured by the sum of squared errors, SSE) is selected based on an optimization process (Breiman, 2001). This keep on going until no longer reduction of SSE is achieved. Similarly, the process can be performed for classification trees, with the aim of developing rules for assigning current and new observations into the classes using numerical and/or categorical predictors. These methods are easily interpretable and provide simple yes (>) or no (<) decision trees (De'ath & Fabricius, 2000).

Random forest

RFs are based on the combination of predictions made by many regression or classification trees to a specific data-set. The method selects many (e.g. 1,000) samples with replacement of the data (i.e. bootstrap samples) and fit a tree for a portion of the re-sampled data-sets. In each of the re-sampled data-sets, a small random number of predictor variables becomes available for the binary partitioning at each node until a full grown tree is constructed. For each bootstrap sample the best tree, as defined previously, is used to predict the data not used for the tree construction (i.e. out-of-bag data, OOB). Accuracy and error rates are computed for each observation using the OOB predictions and then averaged over all the observations. The importance of the predictor variables is assessed by randomly permuting the OOB observations, and then the modified OOB data is passed down the tree to obtain new predictions. The difference between the mean squared error (MSE) of the original and permuted OOB data, divided by its standard error, is a measure of the importance of the predictor variable (Cutler et al., 2007).

Validation of the MBFG classification using CART

We evaluated the validity and accuracy of Kruk et al. (2010) classification rules using an unconstrained classification tree. The nine organism traits defined by Kruk et al. (2010) were used as explanatory variables to classify the original 711 species into the seven MBFG. We then evaluated the accuracy of the classification based on the number of organisms well classified as compared to total number of organisms classified.

Habitat template of the MBFG

We used RF in the randomForest package (R, 2011) to evaluate the explained variance and the importance of environmental variables (K_D, RSi, Temp, TN, TP, TZ and Z_{mix}) in explaining each MBFG biovolume. The inorganic nutrient concentrations (soluble reactive phosphorus and dissolved nitrogen forms) were not considered in the analysis because they are highly variable, dependent on phytoplankton consumption and cause–effect relation are hard to disentangle. Therefore, using dissolved nutrients can lead to unclear relationships. The positive values of group's biovolume were $\log_{10}$ transformed. For each MBFG we constructed 1,000 regression random trees to compose the forest. Three environmental variables were randomly selected for each node of the 1,000 constructed trees. For each tree, the MSE on the OOB portion of the data was recorded. Then the same was done after permuting each predictor variable. Importance of a predictor variable was defined as the average over all trees of the difference between the two MSE normalized by the standard deviation of the differences (Cutler et al., 2007).

Environmental thresholds for MBFG dominance

We evaluated the environmental thresholds for dominance of the MBFG by means of a classification tree (CART). The lakes were classified as dominated by a particular group, when the groups accounted for at least 80% of the total phytoplankton biovolume in that lake. Then we used CART to classify lakes with a particular group dominating (I–VII; categorical variable) according to the environmental variables defined previously. After the full tree was constructed, we pruned it back to avoid overfitting. We did so by minimizing the cross-validated error (De'ath & Fabricius, 2000).

Results

Validation of the MBFG classification

CART classification of organisms based on the morphological traits was able to correctly separate ca. 97% of the organisms into the original MBFG. The number of miss-classified organisms was <3% (20 cases over the total 711 cases). The misclassified cases corresponded originally to groups I, III, IV and VII. Most of the misclassified species were related with group I, either classified as members of other groups or erroneously classified as group I.

Environmental template of the MBFG

Average and range for the environmental variables and the seven MBFGs biovolume (I–VII) are shown in Table 1. The average explained variance for the seven groups biovolume based on K_D, RSi, Temp, TN, TP, TZ and Z_{mix} was 42% (Fig. 2). Six of the seven groups showed an important explained variance reaching at least 34.3%. Group VI had the highest explained variance (57.7%) and group II the lowest (10.3%). All variables were selected as important explaining at least one group. Group I was mostly related to TN, followed by TP and K_D. Group II was not well explained by any of the variables, still RSi and Temp had the larger importance. The most important variables explaining group III were TP and K_D. TN and TZ explained group IV. Group V had a high explained variance by Temp, TZ and TN. Group VI had the highest explained variance including Temp, TN and TZ. Finally, the most important variables explaining group VII were RSi and Z_{mix}.

Environmental thresholds for MBFG dominance

From 925 lakes-cases, 147 presented one MBFG reaching >80% of total biovolume. Groups I and II were dominant only in four and three lake-samples, respectively. Group III dominated in 15 lake-samples, group IV in 11, group V in 37, VI in 58 and in 22 lake-samples group VII dominated. We then labelled each lake according to the dominant MBFG and classified them according to the environmental variables. The pruned tree had a complexity parameter of 0.01, a relative error of 0.18 and a cross-validated error of 0.85 (Fig. 3).

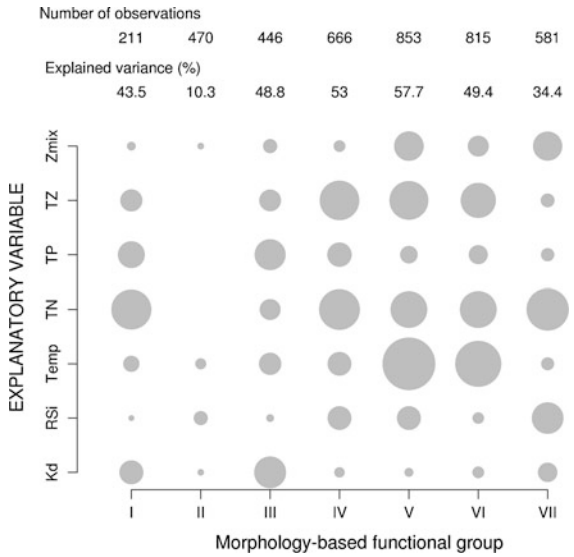

Fig. 2 Results of the RF analysis for the seven MBFGs of phytoplankton (MBFG, I–VII) based on seven environmental explanatory variables. Number of observations and average explained variance (%) for each MBFG are shown. The importance of each environmental variable in explaining each MBFG is proportional to circle diameter. K_D light attenuation coefficient, *RSi* reactive silicate, *Temp* temperature, *TN* total nitrogen, *TP* total phosphorus, *TZ* total zooplankton and Z_{mix} depth of the mixing zone

Lakes dominated by groups III–VII were adequately discriminated according to the environmental variables by the tree (Fig. 3). Light attenuation coefficient (K_D) was the first selected variable with a threshold value of 3.9 m^{-1} in the first node, the root node. The next two selected variables were TN to the left and Temp to the right, with threshold values of 2,800 μg l^{-1} and 24°C, respectively. The further selected variables included Z_{mix} (1.5 m), and reactive silicate (RSi, 1730 μg l^{-1}), and again K_D, Temp and RSi. TP and TZ were not important according to the analysis.

We now describe the environmental conditions driving groups dominance from the left to the right of the constructed tree (Fig. 3). Looking at the root node, to the left, lakes dominated by group III presented elevated K_D (>3.9 m^{-1}) and high TN (>2,800 μg l^{-1}). Lakes dominated by group V showed elevated K_D (>3.9 m^{-1}), TN lower than 2,800 μg l^{-1} and Temps <20°C. From the root node to the right, group VII clearly dominated at K_D <3.9 and Temp between 24 and 29°C. At K_D <3.9 m^{-1} and Temp <24°C, when RSi was below 1,730 μg l^{-1}, lakes were dominated by

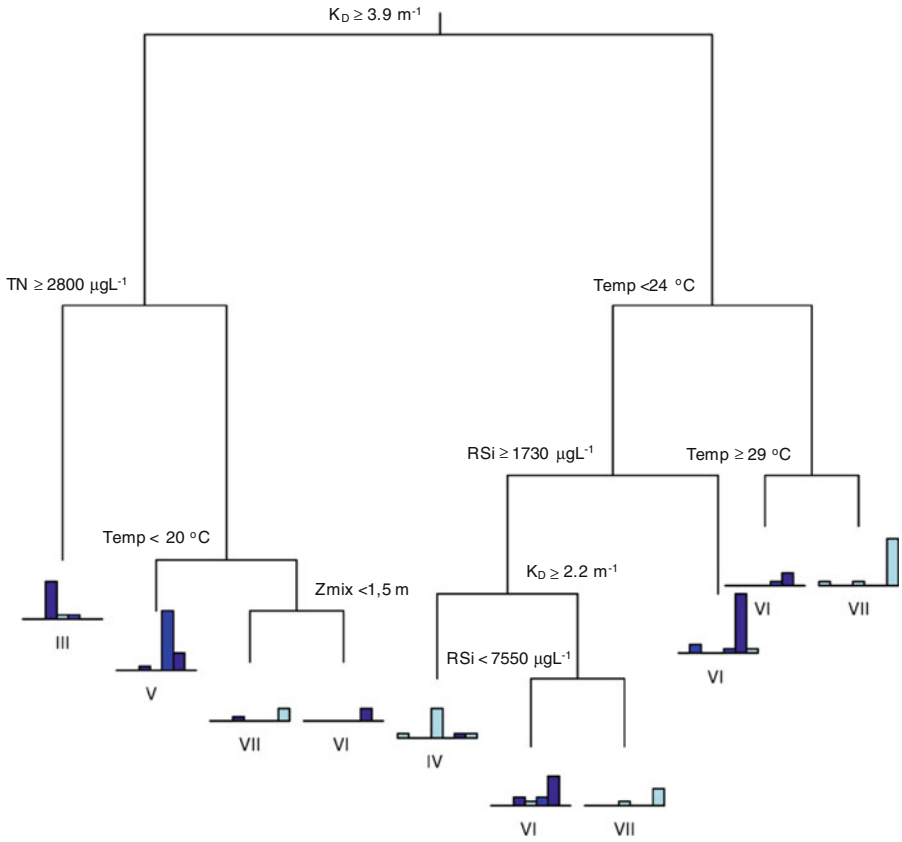

Fig. 3 Classification tree showing the environmental variables explaining the dominance (80% over total biovolume in $mm^3\ l^{-1}$) of MBFGs (MBFG: I–VII). In each node, the environmental variable and its threshold value are shown. K_D light attenuation coefficient, *RSi* reactive silicate, *Temp* temperature, *TN* total nitrogen and Z_{mix} depth of the mixing zone. The height of the branch is proportional to the variance explained by that split. At the end of each branch a histogram with bars representing the number of cases where a specific MBFG (I–VII: *left to right*) was found dominant is included. The MBFG with more cases as dominant is shown below each histogram

group VI. Similarly, lakes with dominance of group IV presented K_D between 2.2 and 3.9 m^{-1} and Temp <24 with RSi above 1,730 µg l^{-1}. In the rest of the leafs, there was a mix of lakes dominated by different groups that could not be separated according to the included environmental variables, which is the case for some subset of lakes dominated by group VI. On the contrary, lakes dominated by groups III–V where rather homogenous in their environmental settings.

Discussion

Differences in morphological traits among phytoplankton MBFG reflected well their different habitat templates. Main ecological processes including resources acquisition (light and nutrients), evasion of loss processes (mixing and zooplankton), as well as Temp, were represented in the results. The application of the MBFG approach was useful in reducing the diversity of species to a diversity of functions (sensu Dray & Legendre, 2008). This reduction of complexity provides an efficient tool to explore the effects of environmental changes on phytoplankton independently from geographical location and specific composition.

Though we used a huge database covering more than 900 lake-cases we obtained a relative high mean explained variance of MBFG biovolume (34%) in comparison with other ecological studies. Møller & Jennions (2002) found that biological studies, even experimental ones, often only explain a very small amount of variance (R^2: 0.3–29%). Therefore, ecological models with explained variances >30% might be considered good predictive tools.

RFs do not assume linear relationships among variables, thus allowing to correctly addressing the often nonlinear interactions occurring in phytoplankton communities (Zhao et al., 2008). However, RFs are black-box models, in which the fitted relationships cannot be written in the form of an equation. RF are data dependent and do not inform if the effect is positive or negative. This precludes an easy transference to managers or scientists (Cutler et al., 2007). To increase the applicability of the model, we constructed a classification tree, which allowed us to know the

specific environmental thresholds determining the dominance of a group in a lake. We successfully separated most of the group-dominated lakes according to the environmental variables. The identification of environmental thresholds is needed to understand ecosystems responses to environmental changes (Scheffer et al., 2001a; Bayley et al., 2007), including climate change and trophic interactions (Scheffer et al., 2001b) and forecast phytoplankton community changes (Roelke, 2000; Carpenter et al., 2009). Also the identification of thresholds is of paramount importance for the implementation of adequate management programs and water quality guidelines (i.e. Chorus & Bartram, 1999). TP and TZ were not selected in the CART to explain MBFG dominance which seems to be counterintuitive. This might have been the result of either a redundancy of the variables (i.e. TP and TN) or that the variable is particularly not important as a threshold for the 80% dominance (i.e. TZ). Below we describe in detail each group's habitat template and its relation with organism's morphological traits.

Group I: small organisms with high *S*/*V*

TP and TN were the most important variables explaining the distribution of group I biovolume. According to their morphology these organisms are *r*-selected (Pianka, 1970), have effective resources acquisition and high specific growth rate in resource-saturated and limited environments (Raven, 1998; Callieri & Stockner, 2002; Kruk et al., 2010). Group I biovolume might increase with total nutrients, while it's relative importance over total phytoplankton declines with increasing total nutrients (Raven, 1998; Bell & Kalff, 2001). Still, these organisms can be dominant in a wide variety of trophic conditions (Callieri & Stockner, 2002; Izaguirre et al., 2003) for example in flushed ecosystems or during transitional stages (Carrick et al., 1993; Reynolds, 2006; Kruk et al., 2010). Our data-set consisted on a low number of lakes dominated by group I which precludes further analysis of the lakes allocating their dominance.

Group II: small flagellated organisms with siliceous exoskeletal structures

Based on their morphology, we expected moderate resources gathering ability, moderate vulnerability to consumption and low to moderate sinking losses (Kruk et al., 2010). In general this group representatives have low optimum Temps (Kim et al., 2009; Jansson et al., 2010) and are more important in cold oligotrophic conditions (Kristlansen & Takahashi, 1982; Izaguirre et al., 2003), as well as in mesotrophic clear-water plant dominated lakes (Reynolds et al., 2002). Low explained variance can be a consequence of poor representation of lakes with this group. Also other environmental variables as is the case of pH and conductivity might have improved the explained variance (Siver & Hamer, 1989). In addition to the morphological traits originally considered, the production of resistant propagules and the facultative mixotrophy might improve their description (Sandgren, 1988).

Group III: large filaments with aerotopes

Light attenuation coefficient and TP were the main drivers of group III biovolume. Given their morphology, the species in this group may be mostly characterized as *K*-selected (Pianka, 1970) with high to moderate saturating nutrient concentration, and low losses due to consumption and sinking (Kruk et al., 2010). These features along with high *S*/*V* confer a greater tolerance to limiting light conditions (Naselli-Flores et al., 2007) and result in the success of these organisms in low-light high-trophic status environments (Padisák & Reynolds, 1998; Kruk et al., 2002; Reynolds et al., 2002; Bonilla et al., 2011). Furthermore, dominance of this group occurred mostly at very high K_D (>3.9 m^{-1}) and TN (>2,800 $\mu g\ l^{-1}$) values. This is in accordance with other studies implying that members of this group can succeed in turbid, eutrophic lakes as originally proposed for Oscillatoriales (Scheffer et al., 1997) and discussed also for Nostocales (Bonilla et al., 2011).

Group IV: organisms of medium size lacking specialized traits

The most important variables explaining group IV were TN and TZ. The small size and high quality as food (Sterner & Elser, 2002) of many of the species in this group (e.g. *Chlorella* sp.) make them liable to high grazing losses. The expected moderate tolerances to limiting resources (Kruk et al., 2010), including nutrients and light, might result in a positive relation

of biovolume with lower TN concentrations, and higher light in the water column (K_D in Fig. 3). Group IV dominated at the lowest values of light attenuation coefficient in the water column ($K_D < 2.2$ m^{-1}) and Temps <24°C. This combination of variables: low nutrient, high zooplankton abundance, low light attenuation might indicate the success of this group under good water quality conditions or during transitional ecosystem stages (Reynolds et al., 2002).

Group V: unicellular flagellates of medium to large size

Temp, TZ and TN explained the distribution of group V biovolume among lakes. According to their morphology, these organisms would have moderate aptitude to resources gathering and high to moderate vulnerability to consumption (Kruk et al., 2010). Motility that permits effective nutrient foraging in conjunction with the production of cysts might increase tolerance of lower nutrient conditions (Reynolds et al., 2002). 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 and limiting light conditions (Graham & Wilcox, 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). These organisms achieved dominance at low light ($K_D > 3.9$) and TN (<2,800 μg l^{-1}). Their moderate size and surface to volume ratio and the possession of flagella reduces high sinking losses therefore tolerates large mixings zones with lower light ($Z_{mix} > 1.5$ m) that can be characteristic of deeper meso to eutrophic lakes. These conditions are probably achieved not in the warmer seasons (Temp < 20°C) (Reynolds et al., 2002).

Group VI: non-flagellated organisms with siliceous exoskeletons

Temp, TZ and TN were the variables better explaining group VI biovolume. Their morphology indicates moderate resources gathering properties, with silicate requirements and moderate vulnerability to consumption (Kruk et al., 2010). Siliceous walls increase sinking but have advantages against certain types of grazers and viral infections (Smetacek, 2001). However, these organisms can suffer more than other groups from fungal infections, especially chytrids (Ibelings et al., 2004) and a wide variety of protists to crustacean zooplankton can successfully feed on diatoms (Hamm et al., 2003). Despite this group shows a wide range of responses to trophic status (Reynolds et al., 2002), its members are better competitors at lower temperatures (Tilman, 1982; Tilman et al., 1986). Owing to their high cell density and lack of motility, these organisms are rapidly excluded from illuminated waters, explaining their dominance in lakes with lower light attenuation than other groups ($K_D < 3.9$ m^{-1}). The obligate presence of a siliceous wall is probably the main constraining trait of these species (Kruk et al., 2010). Coincidently this group dominated in a close relationship with RSi values. High biovolume of group VI species might diminish RSi concentration in lakes. However, cause and effect cannot be disentangled from studies based on correlations.

Group VII: large mucilaginous colonies

Total RSi and mixing zone depth were the main environmental variables explaining the biovolume of group VII. RSi is highly correlated to the effect of the catchment area and nutrient access from it (Conley, 2002; Kruk et al., 2009). Large size and volume, and low surface to volume ratio, should tend to make species sensitive to low resource supply (Kruk et al., 2010). Therefore, we would expect an increase of this group in high-trophic status lakes, with larger catchment areas and probably deeper than shallow lakes. The presence of mucilage, along with lipids and aerotopes in the larger colonies, gives controllable buoyant properties (Walsby & Reynolds, 1980). However, water mixing can affect them negatively disrupting scums in the water surface (Chorus & Bartram, 1999). Dominance was attained with $K_D < 4.0$ m^{-1} and Temp between 24 and 28°C. The preference for high temperatures might indicate stratified and stable water column and is consistent with dominance this group in several tropical shallow lakes (Ganf & Viner, 1973; Huisman et al., 2005).

Here, we were able to describe the habitat template of most MBFG in terms of biovolume distribution among lakes. The conditions for 80% dominance of groups III, IV and V were also well discriminated by the environmental variables and constituted

homogeneous groups. However, the discriminatory power of the conditions for dominance of groups I and II was low, and groups VI and VII dominated in various environmental conditions. Deep, oligotrophic, high altitude lakes and lakes from extreme environments (i.e. polar and salty lakes) should be included to improve the results presented here. Also the validity of this approach to explain the temporal replacement of MBFG needs to be further analysed (but see Pacheco et al., 2010; Segura et al., 2010). Assuming community structure is shaped by similar processes, it would be a challenge to apply the MBFG classification to phytoplankton communities from coastal open waters and the ocean.

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 MBFGs appear to be relatively predictable (Kruk et al., 2011), it still makes a difference which species will dominate within 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). In this vein, the MBFG approach as we have presented it has some weaknesses and does not invalidate other approaches (Carpenter et al., 1993; Reynolds et al., 2002; Le Quéré et al., 2005; Salmaso & Padisák, 2007; Padisák et al., 2009), as does not rule out the classical taxonomical approach. However, morphological traits as a surrogate for species taxonomy allow researchers to produce more general models and generalize to ecosystems with very different taxonomic composition (Keddy, 1992; McGill et al., 2006; Dray & Legendre, 2008). This will likely bring us closer to the goal of predicting and managing nuisance algal blooms and to arrive to a verifiable quantitative method of describing community structure and change with other more "ecological" purposes.

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PHYTOPLANKTON

Comparison of morpho-functional phytoplankton classifications in human-impacted shallow lakes with different stable states

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Abstract The morpho-functional classifications of phytoplankton have been recently proposed as useful tools in the aquatic biomonitoring. In this study, we compared three different classifications in a range of different environmental conditions, a set of six shallow lakes with different stable states. The studied lakes are located in the Pampa Plain from Argentina, a region highly impacted as a consequence of the human activities. Among the selected lakes, three are in a turbid state, two of which have high phytoplankton abundances (phytoplankton-turbid), and one shows a high concentration of suspended inorganic matter (inorganic-turbid). Two lakes are clear and profusely colonized by submerged plants (clear-vegetated). Only one lake shows a typical alternative steady-state behavior, shifting turbid periods of high phytoplankton biomass with periods of more transparency and development of submerged macrophytes. We compared the three morpho-functional classifications applied by means of multivariate analyses in order to explore how much the variance of the biomass of the phytoplankton functional groups (for each functional classification) was explained by the environmental variables. The analyses performed showed a clear separation of the human-impacted turbid lakes from the clear-vegetated lakes. The advantages and disadvantages of the different morpho-functional classifications are discussed, concluding that the functional approach is adequate to analyze the phytoplankton communities in aquatic systems subjected to anthropogenic influence and for monitoring them.

Keywords Phytoplankton morpho-functional classifications · Alternative steady states · Shallow lakes · Human impact · Pampa Plain

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Introduction

Long time ago ecologists have tried to group organisms with similar structural and functional characteristics with the aim to obtain a better understanding of the functioning of the ecosystems (Salmaso & Padisák, 2007 and cites therein). In the phytosociological

approach, the associations are defined as groups of species that are typically found together (e.g., Margalef, 1978; Reynolds, 1980, 1984), and the species of a particular association share common ecological attributes. This approach was successively refined and expanded by different phytoplanktologists. Reynolds (1997) proposed different associations following functional criteria based upon both morphological and ecological properties. Later on, Reynolds et al. (2002) considered the structure of the phytoplankton assemblages and proposed a general scheme with 31 functional groups, defining their distinctive ecological features (functional classification). Groups are often polyphyletic, recognizing commonly shared adaptive features. This classification has been frequently used by phytoplankton ecologists in different aquatic systems (e.g., Kruk et al., 2002; Tolotti et al., 2005; Devercelli, 2006; Sarmento & Descy, 2008). Recently, this classification was revised and updated by Padisák et al. (2009).

The concept of functional diversity (FD) coined by Weithoff (2003) highlights another aspect of the functional characterization of species and communities and provides a new understanding of phytoplankton ecology; it is related to the functional multiplicity within the community rather than the multiplicity of species. In this respect, functional traits were defined as a property of an organism that can be measured and that influences one or more essential functional processes such as reproduction, growth, etc. (Weithoff, 2003). Using multivariate analyses Salmaso & Padisák (2007) evaluated classifications based on the morphological and functional characteristics. More recently, Kruk et al. (2010) proposed a new morphologically based functional classification, where simple morphological traits were found to capture much of the variability in functional properties of phytoplankton; the classification was tested using phytoplankton information obtained in more than 200 lakes along a climatic gradient (tropical to subpolar).

In this investigation, we applied different phytoplankton functional classifications to capture much of the differences among different shallow lakes located in the Pampean region of Argentina. This region contains thousands of shallow lakes which are subjected to a progressive eutrophication due to a combination of human activities in their catchment such as land-use change, increase of agriculture, livestock, non-regulated urbanization, fish introduction, drainage, canalization, and damming (Quirós et al., 2006). Limno-regional studies conducted in this area allowed to recognize three main types of shallow lakes: clear with abundant submerged and emergent macrophytes, turbid with high phytoplankton biomass ("green" shallow lakes), and turbid with great concentrations of inorganic suspended material (Quirós et al., 2002). Scheffer et al. (1993) have described the mechanisms involved in the transition of the shallow lakes from a clear state with submerged macrophytes to a turbid one dominated by phytoplankton: at low nutrient concentrations the clear-water equilibrium is the only possible stable state, in a hypertrophic situation just the turbid equilibrium exists, and between these two extremes there is a range of nutrient levels over which two alternative equilibria can exist. In the Pampa Plain, clear-vegetated lakes and turbid lakes with high phytoplankton biomass represent the two basic alternative states described in the model proposed by Scheffer et al. (1993). The third type of lake would be the result of a direct human impact on their drainage basin and includes systems where primary production is severely light limited by inorganic turbidity (Quirós et al., 2002). Some previous papers have reported information on the phytoplankton structure of these different types of shallow lakes (e.g., Izaguirre & Vinocur, 1994; Cano et al., 2008; Allende et al., 2009; Silvoso et al., 2011).

We seasonally analyzed the structure of the phytoplankton assemblages of six selected shallow lakes from the Pampa Plain that are representative of the different scenarios that can be recognized in this region, which in turn are mainly the result of the human impact in the area: two shallow lakes are clear-vegetated, two are phytoplankton-turbid, one is inorganic-turbid, and one can alternate periodically between clear and turbid states. The aim was to explore the adequateness of the morpho-functional approach in detecting changes in the phytoplankton assemblages in human-impacted systems by comparing three different classifications: (a) Reynolds et al. (2002) revised by Padisák et al. (2009); (b) Salmaso & Padisák (2007); (c) Kruk et al. (2010). Using multivariate analyses, we estimated how much the variance of the biomass of the phytoplankton functional groups (for each functional classification) was explained by the environmental variables.

Study area

The Pampa Plain (35°32′–36°48′S; 57°47′–58°07′W) from Argentina is encompassed in a warm temperate region, where mean annual temperature is about 15.3°C and winds have a mean annual speed of 10.1 km h^{-1} (Torremorell et al., 2007). This region is characterized by a marked interannual variability between wet and dry periods with a mean annual precipitation of about 935 mm (Sierra et al., 1994). Due to the very low regional topographic gradient (<0.1%) of this plain, the networks of surface water and salt evacuation toward the ocean are poor and water excesses often translate into flooding and salt redistribution (Jobbágy et al., 2008).

Pampean shallow lakes are typically permanent, relatively homogeneous in depth (mean depth ~2 m), and eutrophic or hypertrophic (Quirós & Drago, 1999). The study was conducted in six selected shallow lakes (Fig. 1), which represent the different states that the shallow lakes of this region can exhibit. Two lakes (Kakel Huincul and El Triunfo) are clear and are profusely colonized by submerged plants (mainly *Myriophyllum* sp. and *Ceratophyllum demersum*) and emergent macrophytes (*Schoenoplectus californicus*); these water bodies show a limited development of pelagic algae (Allende et al., 2009). Other two lakes are characterized by low Secchi depth values and high phytoplankton abundances (San Jorge and Chascomús). One lake (La Limpia) presents a high concentration of suspended inorganic matter and low development of both submerged macrophytes and phytoplankton. Finally, the sixth shallow lake (Lacombe) may alternate between turbid periods of high phytoplankton development and periods of more transparency and development of submerged macrophytes (Cano et al., 2008). The main characteristics of the six shallow lakes are summarized in Table 1.

Materials and methods

Three sampling sites were established in the six selected shallow lakes above described. Each site was sampled for physical, chemical, and biological parameters. Samples were collected in spring, summer, autumn, and winter (from November 2005 to September 2006).

In each shallow lake, temperature, pH, dissolved oxygen (DO), and conductivity were measured with HI 8314 and HI 8033 Hanna portable instruments. The water transparency was estimated with Secchi disk. The vertical diffuse photosynthetic active radiation (PAR) attenuation coefficients (Kd_{PAR}) were studied in order to characterize the underwater light climate in each of the six shallow lakes and seasons. We used an underwater turbidimeter (SCUFA, Turner™) and a spectrum-submersible radiometer (USB2000, Ocean Optics), during

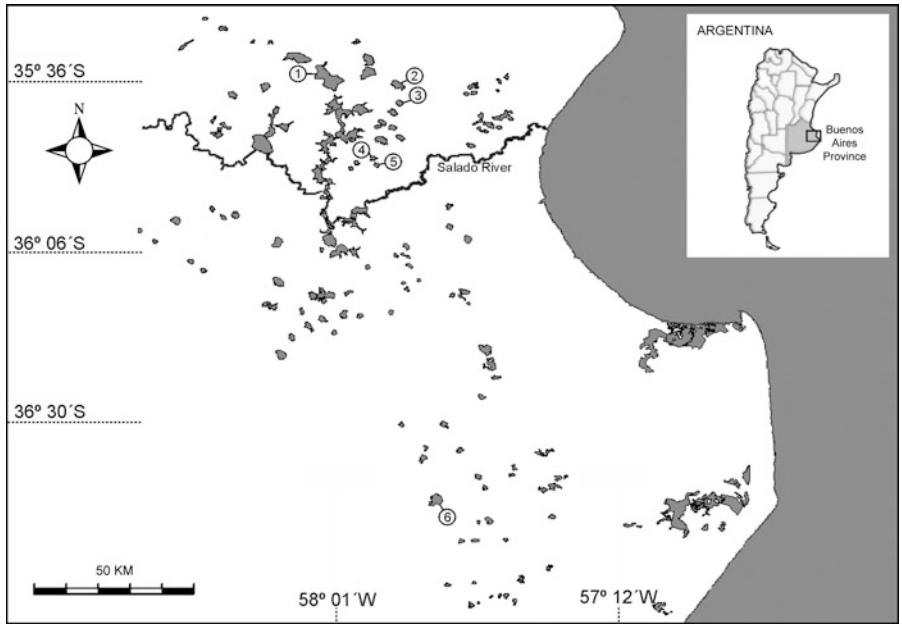

Fig. 1 Location of the studied shallow lakes in the Pampa Plain (Buenos Aires Province, Argentina). Chascomús (*1*); La Limpia (*2*); San Jorge (*3*); Lacombe (*4*); El Triunfo (*5*); Kakel Huincul (*6*)

Table 1 Geographic position, main morphometric features and states of the shallow lakes (data obtained from Silvoso et al. (2011)

	Kakel Huincul	El Triunfo	San Jorge	Chascomús	La Limpia	Lacombe
Geographic position	36°48′S; 57°47′W	35°51′S; 57°52′W	35°40′S; 57°47′W	35°36′S; 58°02′W	35°37′S; 57°48′W	35°49′S; 57°49′W
Surface area (km^2)	29.5	1.5	3.0	28.7	5.6	1.6
Max. depth (m)	4.0	nd	nd	1.9	2.3	2.0
Mean depth (m)	1.8	nd	nd	1.5	1.9	1.0
States	Clear-vegetated	Clear-vegetated	Phytoplankton-turbid	Phytoplankton-turbid	Inorganic-turbid	With alternative steady states

the annual period (2005–2006). The techniques used were described in detail in Pérez et al. (2010).

Samples for the determination of total suspended solids (TSS), percentage of organic matter in seston (%OM), total phosphorous (TP), and total nitrogen (TN) were collected subsuperficially (about 30 cm depth). All determinations were performed following the protocols given in APHA AWWA WEF (2005). Aliquots of filtered water (Whatman GF/F™) were acidified (pH 2) and stored at 4°C until analysis of dissolved organic carbon (DOC). DOC was determined using a high temperature Pt catalyst oxidation method (Shimadzu TOC-5000) following the recommendations of Sharp et al. (1993).

Phytoplankton samples were also collected subsuperficially at each of the three stations of the shallow lakes. Chlorophyll *a* (Chl *a*) concentration was estimated from triplicate samples filtered through glass-fiber filters (GF/F, Whatman™) by ion pairing reverse-phase HPLC, modified from Mantoura & Llewellyn (1983) and Hurley (1988), using an Äkta-basic chromatograph (Amersham™) controlled by the program Unicorn™ (C18 Phenomenex™; 5 µm particle size; 250 mm × 4.6 mm i.d.). The method applied has been described in detail by Laurion et al. (2002).

Quantitative phytoplankton samples were fixed with 1% acidified Lugol's iodine solution. Phytoplankton counts were performed using a Zeiss™ inverted microscope (Utermöhl, 1958) at ×400 magnification, and the counting error was estimated according to Venrick (1978). In all cases, we considered the individual algae as the unit (unicell, colony, coenobium, or filament), and we estimated cell numbers per colony or filament. Individual biovolumes were calculated using appropriate geometric formulae according to their shapes and the mean dimensions of the organisms in the samples (Hillebrand et al., 1999; Sun & Liu, 2003). For colonial organisms, calculations were made for the whole colony including mucilage. Biomass was estimated from biovolume, assuming unit specific gravity.

On the other hand, all the species recorded in the samples were classified into the functional groups proposed in the classifications of Reynolds et al. (2002)—reviewed by Padisák et al. (2009), Salmaso & Padisák (2007), and Kruk et al. (2010); the biomass corresponding to each functional group were also calculated for the numerical analyses.

Multivariate analyses

Redundancy analyses (RDA) were used to estimate how much variance of the biomass of the phytoplankton functional groups (Reynolds et al., 2002; Salmaso & Padisák, 2007; Kruk et al., 2010) was explained by the environmental variables. Previously, we preformed a detrended correspondence analyses (DCA), and as the data showed a linear response we applied RDA. Calculations were performed with the program CANOCO (ter Braak, 1988). The analysis was based on field data, on the biomass of the functional groups and on the environmental variables corresponding to each shallow lake and date. Species with a contribution of <5% of the total community biomass in any individual lake were excluded in this analysis. The statistical significance of the first axis and of all the axes was tested by a Monte Carlo permutation test. The importance of each variable was assessed using forward selection.

Results

Limnological variables

The contrasting features of the studied shallow lakes are indicated in Table 2. Clear-vegetated shallow

lakes (El Triunfo and Kakel Huincul) showed the lowest Kd_{PAR} values (mean 5.2 m^{-1}) and TSS (mean 4.1 mg l^{-1}). The highest Kd_{PAR} and suspended solids concentrations were observed in the inorganic-turbid shallow lake La Limpia, with mean values of 36.9 m^{-1} and 256.5 mg l^{-1}, respectively. The phytoplankton-turbid shallow lakes (Chascomús and San Jorge) were also characterized by high Kd_{PAR} values (mean 23.2 m^{-1}) and high TSS concentrations (mean 126.4 mg l^{-1}). The shallow lake Lacombe, which as it was mentioned exhibits periodic alternative steady states (clear and turbid), presented intermediate values of Kd_{PAR} (mean 9.2 m^{-1}) and TSS (mean 48.2 mg l^{-1}); in relation to this shallow lake it is important to point out that during the year of our study the system was in a turbid state with high phytoplankton abundance.

All the studied shallow lakes are eutrophic, as it is expressed from the relatively high concentrations of TP and TN registered. Nevertheless, the lowest values were measured in the clear-vegetated lakes, whereas the highest ones in the turbid systems. For all studied lakes TP ranged from 0.05 to 0.87 mg l^{-1} and TN from 0.26 to 1.82 mg l^{-1}.

Due to the characteristics of the catchments, which contain sedimentary rocks rich in sodium and carbonates, the waters of the lakes in this region are alkaline (mean pH varied from 8.6 to 8.9), and conductivities are also relatively high (mean values ranged from 0.95 to 2.27 mS cm^{-1}). The concentrations of DOC were rather high in all the lakes, registering the maximum values in the clear-vegetated water bodies (mean 49.7 mg l^{-1}) and the minimum ones in the inorganic-turbid lake (mean 21.3 mg l^{-1}). All the shallow lakes exhibited well-oxygenated waters, with mean concentrations ranging between 8.3 and 9.3 mg l^{-1}.

The highest values of phytoplankton Chl *a* were recorded in the phytoplankton-turbid lakes (mean value 205.8 μg l^{-1}), and the lowest one in the clear-vegetated lakes (mean value 5.1 μg l^{-1}). Intermediate values were registered in the inorganic-turbid shallow lakes La Limpia and Lacombe (mean values 28.5 and 41.2 μg l^{-1}, respectively).

Phytoplankton community structure

The six water bodies showed strong differences in total phytoplankton biomass (Fig. 2). As expected, the

Table 2 Ranges of the main limnological features for the six shallow lakes studied in the Pampa Plain

	Kakel Huincul	El Triunfo	San Jorge	Chascomús	La Limpia	Lacombe
State	Clear-vegetated	Clear-vegetated	Phytoplankton-turbid	Phytoplankton-turbid	Inorganic-turbid	With alternative steady states
Kd_{PAR} (m^{-1})	3.4–3.7 (3.6; SD 0.13)	3.4–11 (6.9; SD 3.1)	11.5–23.4 (17.2; SD 4.9)	16.6–35.8 (29.2; SD 8.6)	21.1–49.8 (36.9; SD 12.9)	5.2–13.5 (9.2; SD 2.9)
Suspended solids (mg l^{-1})	1.6–12 (5.4; SD 4.7)	1.0–4.8 (2.2; SD 1.8)	55–98 (73.7; SD 18.7)	70–265 (165; SD 94.2)	129–351 (256.5; SD 101.9)	25.5–69 (48.2; SD 20.8)
Dissolved oxygen (mg l^{-1})	8.0–10.6 (9.4; SD 1.1)	7.6–11.9 (9.2; SD 1.9)	8.4–9.4 (8.9; SD 0.5)	8.2–11.0 (9.5; SD 1.2)	7.8–8.6 (8.3; SD 0.32)	7.8–9.8 (8.9; SD 0.88
pH	7.3–9.2 (8.4; SD 0.79)	8.4–9.4 (9.1; SD 0.5)	8.3–9.1 (8.8; SD 0.34)	8.4–9.1 (8.8; SD 0.30)	7.8–9.1 (8.6; SD 0.55)	8.3–9.2 (8.9; SD 0.36)
Conductivity (mS cm^{-1})	1.54–2.19 (1.8; SD 0.28)	1.32–1.87 (1.62; SD 0.23)	1.34–1.51 (1.41; SD 0.08)	1.58–1.80 (1.72; SD 0.09)	0.82–1.13 (0.95; SD 0.13)	2.14–2.5 (2.27; SD 0.14)
TN (mg l^{-1})	0.30–1.14 (0.62; SD 0.4)	0.23–0.69 (0.39; SD 0.21)	0.95–1.72 (1.43; SD 0.35)	0.30–1.82 (1.11; SD 0.75)	0.72–0.97 (0.82; SD 0.11)	0.26–1.05 (0.74; SD 0.30)
TP (mg l^{-1})	0.05–0.28 (0.13; SD 0.11	0.07–0.25 (0.17; SD 0.08)	0.23–0.34 (0.30; SD 0.05)	0.41–0.70 (0.60; SD 0.13)	0.62–0.87 (0.75; SD 0.11)	0.11–0.29 (0.21; SD 0.07)
DOC (mg l^{-1})	38.7–82.4 (58.5; SD 18.1)	37.3–49.8 (40.9; SD 5.9)	42.1–60 (48.9; SD 8.6)	17.6–47.2 (28.2; SD 13.2)	13.7–40.3 (21.3; SD 12.7)	31.0–67.4 (41.6; SD 15.0)
Chlorophyll *a* (μg l^{-1})	1.6–7.7 (3.9; SD 2.8)	2.7–5.5 (3.8; SD 1.3)	36–334.6 (207.7; SD 125.9)	50.2–135.4 (101.1; SD 38.2)	18.3–35.4 (28.5; SD 7.3)	20.6–63.2 (41.6; SD 21.3)

Mean values and standard deviations (SD) are indicated between brackets

Kd_{PAR} vertical diffuse photosynthetic active radiation attenuation coefficient, *TN* total nitrogen, *TP* total phosphorus, *DOC* dissolved organic carbon, *nd* no data

highest biomasses were recorded in the two phytoplankton-turbid shallow lakes. Of these two lakes, San Jorge exhibited the highest values (217–1,730 mg l^{-1}), whereas in Chascomús they ranged between 146 and 232 mg l^{-1}. As the shallow lake Lacombe was in a turbid state during the year of this study, phytoplankton biomass was also very high, ranging from 56 to 1,620 mg l^{-1}.

In all four visits, the inorganic-turbid shallow lake (La Limpia) showed the lowest values of algal biomass, varying from 2.85 to 12.40 mg l^{-1}.

The two clear-vegetated lakes also presented low phytoplankton biomass. Values varied from 2.65 to 19.00 mg l^{-1} in the shallow lake El Triunfo. In Kakel Huincul, although the algal biomass was generally low, a peak was registered in summer; values in this lake varied from 0.59 to 273.00 mg l^{-1}.

The differences among the lakes were also reflected in the proportion of the algal groups (Fig. 3). In clear-vegetated lakes, total phytoplankton biomass was distributed in different groups, being the most representative Cryptophyceae, Chlorophyceae, Cyanobacteria, and Bacillariophyceae. In the phytoplankton-turbid shallow lakes, the dominant groups were Cyanobacteria, Chlorophyceae, and Bacillariophyceae, and the relative proportion of the algal groups was relatively stable in the different seasons. Cyanobacteria represented more than 90% of the total phytoplankton biomass in San Jorge. In the inorganic-turbid shallow lake, the dominant algal groups were Chlorophyceae and Bacillariophyceae. The shallow lake Lacombe was mainly dominated by Chlorophyceae and Cyanobacteria.

We identified a total of 184 phytoplankton species in the six shallow lakes. The main taxa recorded in each type of water body are listed in Table 3. In clear-vegetated lakes, different unicellular cryptophytes, chrysophytes, and chlorophytes were generally very abundant, together with small colonial cyanobacteria and colonial chlorophytes as accompanying species. In the turbid lake San Jorge, the phytoplankton community was almost completely dominated by the species cf. *Raphidiopsis mediterranea* in all sampling dates, which constituted a persistent algal bloom. In the other phytoplankton-turbid lake (Chascomús), the community was dominated by several unicellular and coenobial chlorophytes together with the diatom *Synedra berolinensis*; colonial cyanobacteria (mainly *Aphanocapsa delicatissima*) and colonial chlorophytes (mainly *Oocystis lacustris*) were less numerous but contributed considerably to the biomass. The inorganic-turbid lake La Limpia showed a higher contribution of filamentous diatoms (*Aulacoseira* spp.) and desmids (*Closterium aciculare*), and other taxa belonging to Chlorophyceae were also well represented in some seasons. In lake Lacombe, some small chlorophytes and filamentous cyanobacteria (mainly *Planktolyngbya limnetica*) were numerically abundant in certain seasons, whereas colonial cyanobacteria (*Cyanodictium imperfectum* and *A. delicatissima*) and several colonial chlorophytes were dominant in biomass in all sampling dates. The main morpho-functional groups according to the three classifications applied in this paper, are summarized in Table 4.

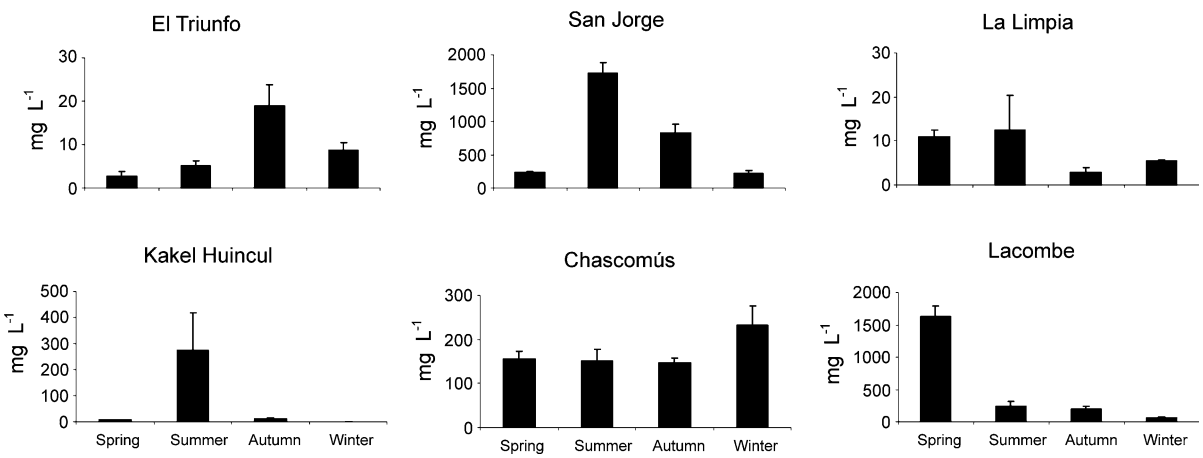

Fig. 2 Seasonal variation of total phytoplankton biomass in each shallow lake during the study period (*bars* standard deviation)

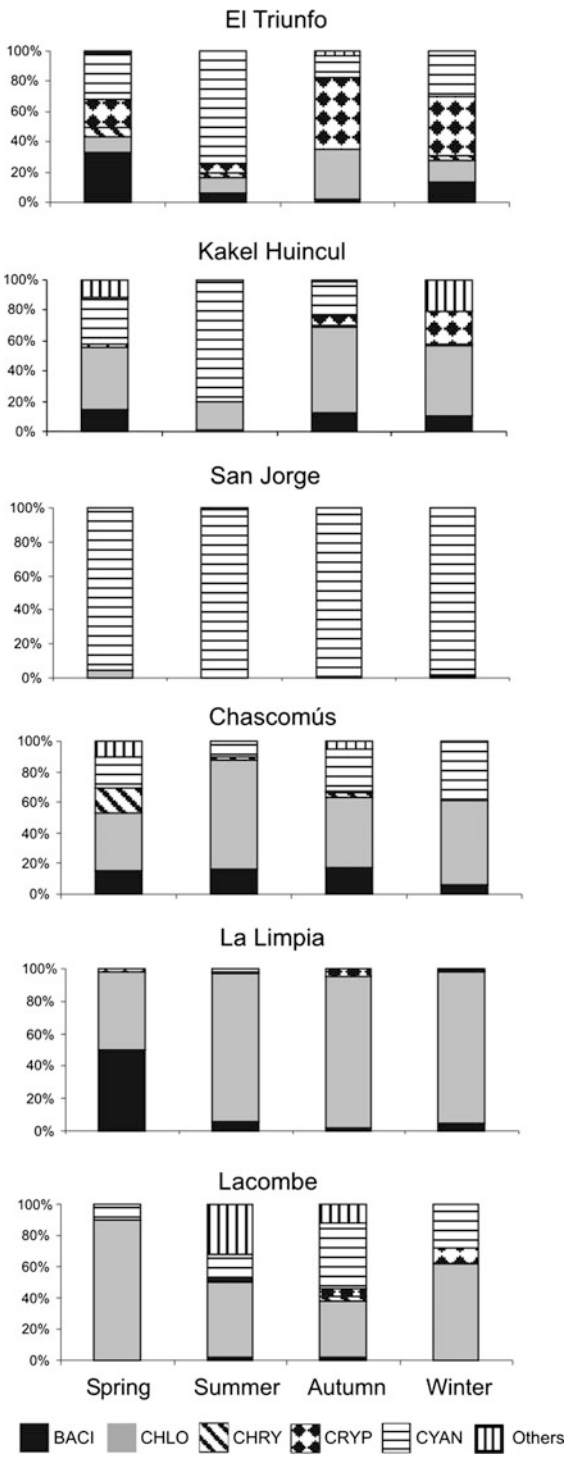

Fig. 3 Seasonal variation of the relative proportion of the different algal groups (in biomass) in the shallow lakes during the study period. Bacillariophyceae (*BACI*), Chlorophyceae (*CHLO*), Chrysophyceae (*CHRY*), Cryptophyceae (*CRYP*), Cyanobacteria (*CYAN*)

Multivariate analyses

In the redundancy analysis (RDA) based on the functional groups proposed by Reynolds et al. (2002), the first two axes accounted for 70.1% of the variance (axis 1: 39.9%; axis 2: 30.2%). The Monte Carlo test indicated that the environmental variables were significantly correlated with the first axis ($P = 0.012$) and the test of significance of all canonical axes was also significant ($P = 0.002$). The first axis was mainly correlated with Kd_{PAR}, TP, and DOC (intra-set correlation coefficients: 0.80, 0.71, and −0.62, respectively), and the second axis was mainly defined by conductivity (intra-set correlation coefficient: −0.61). Figure 4 shows the biplots (first two axes) of the shallow lakes (a) and the Reynold's groups (b) with respect to the environmental variables.

The results of the RDA based on the functional groups proposed by Salmaso & Padisák (2007) are shown in Fig. 5a, b. In this case, the total variance explained by the two first axes was 68.60% (axis 1: 40.90%; axis 2: 27.70%). The environmental variables were significantly correlated with the first axis ($P = 0.01$) and the test for all canonical axes was also significant ($P = 0.004$). In this analysis, the first axis was also mainly defined by a combination of TP, Kd_{PAR}, and DOC (intra-set correlation coefficients: 0.70, 0.67, and −0.57, respectively), and the second axis by conductivity (intra-set correlation coefficient: −0.66).

In the case of the RDA carried out with the morphologically based functional groups: MBFG (Kruk et al., 2010), the percentage of explained variance was 76% (axis 1: 46.60%; axis 2: 29.40%). The biplots corresponding to this analysis are shown in Fig. 6a, b. Monte Carlo test was significant for the first axis ($P = 0.012$) and for all canonical axes ($P = 0.006$). The first axis was correlated with conductivity and TN (intra-set correlation coefficients: −0.52 and 0.53, respectively), whereas the second axis was mainly defined by TP and Kd_{PAR} (intra-set correlation coefficients: 0.66 and 0.58, respectively).

The results of these analyses indicated that the biomass of the phytoplankton groups corresponding to the three classification approaches can be well predicted from the environmental variables. In all the ordinations, the turbid lakes are plotted separately from the clear-vegetated lakes. The samples of

Table 3 Dominant and frequent phytoplankton species in the studied shallow lakes from the Pampa Plain

Clear-vegetated shallow lakes	Phytoplankton-turbid shallow lakes	Inorganic-turbid shallow lakes	Shallow lake with alternative steady states
Cryptomonas marsonii	cf. *Raphidiopsis mediterranea*	*Aulacoseira granulata*	*Monoraphidium griffithii*
Cryptomonas erosa	*Aphanocapsa delicatissima*	*Aulacoseira granulata* var. *angustissima*	*Monoraphidium subclavatum*
Cryptomonas ovata	*Monoraphidium circinale*	*Closterium aciculare*	*Plagioselmis* sp.
Plagioselmis spp.	*Monoraphidium contortum*	*Coelastrum microporum*	*Aphanocapsa delicatissima*
Chlamydomonas spp.	*Monoraphidium griffithii*	*Pediastrum mustersii*	*Cyanodictium imperfectum*
Ochromonas spp.	*Monoraphidium minutum*	*Monoraphidium circinale*	*Bothryococcus braunii*
Monoraphidium circinale	*Scenedesmus quadricauda*	*Monoraphidium minutum*	*Oocystis marsonii*
Monoraphidium griffithii	*Tetrastrum staurogeniaeforme*	*Monoraphidium tortile*	*Oocystis nephrocystioides*
Monoraphidium komarkovae	*Oocystis lacustris*	*Monoraphidium obtusum*	*Planktolyngbya limnetica*
Aphanocapsa delicatissima	*Synedra berolinensis*	*Schroederia antillarum*	
Snowella lacustris		*Schroederia indica*	
Chroococcus minutus		*Schroederia setigera*	
Oocystis lacustris		*Cryptomonas marsonii*	
Bothryococcus braunii		*Plagioselmis* sp.	
Sphaerocystis schroeterii			

Table 4 Main morpho-functional groups according the three classifications used for to the different studied shallow lakes in the Pampa Plain

	Morpho-functional groups (Reynolds et al., 2002; Padisák et al., 2009)	Morphologically based functional groups (Kruk et al., 2010)	Morpho-functional groups (Salmaso & Padisák, 2007)
Clear-vegetated shallow lakes	X1, X2, Y, L0, K, F	V, IV, VII	2a, 2d, 5c, 5d, 11b
Phytoplankton-turbid shallow lakes	Sn, X1, K, F, D, J	III, IV, VI, VII	5c, 5d, 5e, 6b, 11a, 11b
Inorganic-turbid shallow lakes	P, X1, X2, Y, J	IV, V, VI	5c, 6b, 8a, 11a, 11b
Shallow lake with alternative steady states (in turbid state in this study)	X1, X2, S1, F, K	III, IV, V, VII	2a, 2d, 3a, 5c, 5d, 5e, 9b, 11b

Lacombe lake, that alternates between clear and turbid states, ordinated at a middle position, near the center of the graph or even closer to the samples of the clear-vegetated lakes. The optical conditions of the water bodies (expressed by the Kd_{PAR} values), together with conductivity and TP, seem to be the more important variables determining the algal groups. Nevertheless, the forward selection procedure showed that Kd_{PAR} was not significant in the analysis based on the MBFG of Kruk et al. (2010), whereas TP was not significant in the analysis based on the Reynold′s classification.

Discussion

As a consequence of the human activities, most shallow lakes located in the more impacted areas of the Pampa Plain have become turbid systems (Quirós et al., 2002, 2006). Once in a turbid state, the lake morphometry, the flatness of the terrain, and the persistence and strength of winds favor mixing and prevent stratification, and probably act as stabilizing factors of the turbid state (Torremorell et al., 2007). In turbid systems, the high light attenuation in the water

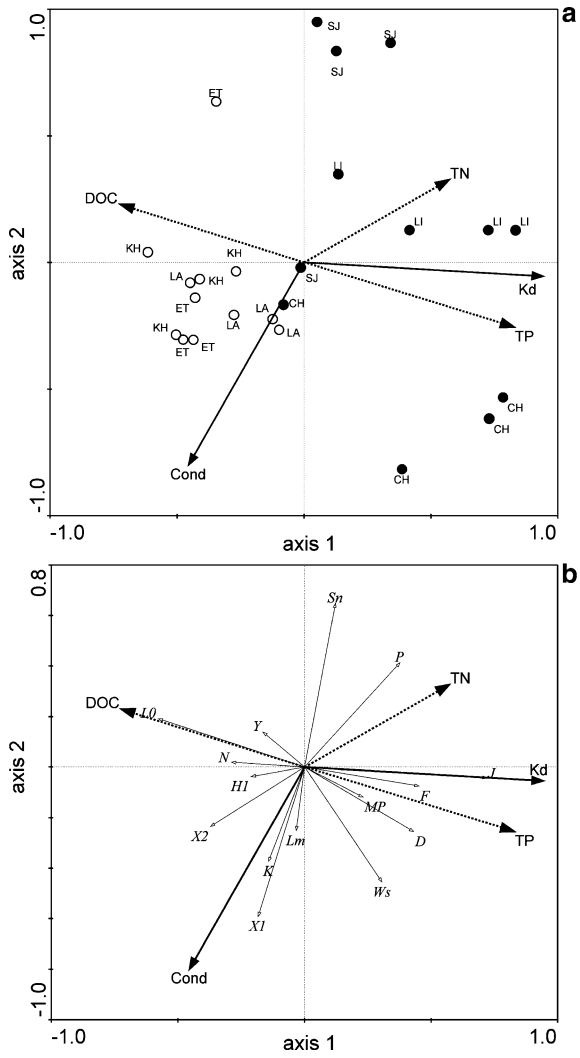

Fig. 4 Biplots of the RDA based on the biomass of the morpho-functional groups proposed by Reynolds et al. (2002). **a** Ordination of the samples and environmental variables (*CH* Chascomús, *SJ* San Jorge, *LI* La Limpia, *LA* Lacombe, *ET* El Triunfo, *KH* Kakel Huincul). **b** Functional groups and environmental variables. Turbid shallow lakes (*black circles*); clear-vegetated shallow lakes and lake with alternative steady states (*white circles*). Significant environmental variables ($P < 0.05$) are indicated with *solid arrows*, while *dotted arrows* are not significant. Conductivity (*Cond*), dissolved organic carbon (*DOC*), vertical attenuation coefficient (*Kd*), total nitrogen (*TN*), total phosphorus (*TP*)

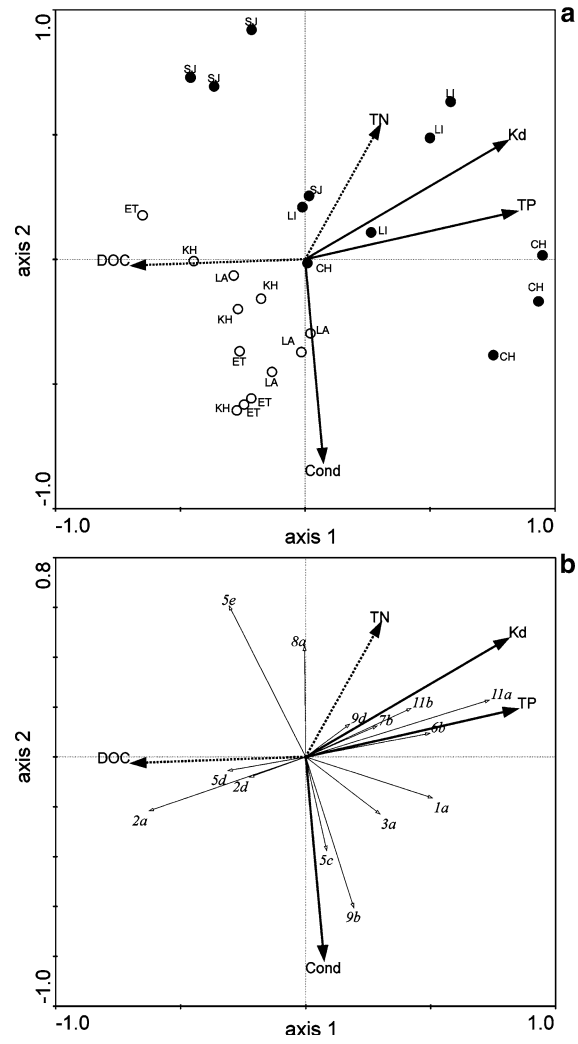

Fig. 5 Biplots of the RDA based on the biomass of the morpho-functional groups proposed by Salmaso & Padisák (2007). **a** Ordination of the samples and environmental variables (*CH* Chascomús, *SJ* San Jorge, *LI* La Limpia, *LA* Lacombe, *ET* El Triunfo, *KH* Kakel Huincul). **b** Functional groups and environmental variables. Turbid shallow lakes (*black circles*); clear-vegetated shallow lakes and lake with alternative steady states (*white circles*). Significant environmental variables ($P < 0.05$) are indicated with *solid arrows*, while *dotted arrows* are not significant. Conductivity (*Cond*), dissolved organic carbon (*DOC*), vertical attenuation coefficient (*Kd*), total nitrogen (*TN*), total phosphorus (*TP*)

column is one of the ecological key factors, and exerts profound effects on phytoplankton. Light availability affects algal competition (Huisman et al., 1999; Reynolds, 2006) and phytoplankton diversity (Reynolds, 1998; Stomp et al., 2004). Light-limiting conditions prevail in Pampean turbid lakes (Llames et al., 2009), and previous investigations have shown that the underwater light climate strongly affects the phytoplankton structure and the primary production in the lakes of this region (Allende et al., 2009). In the

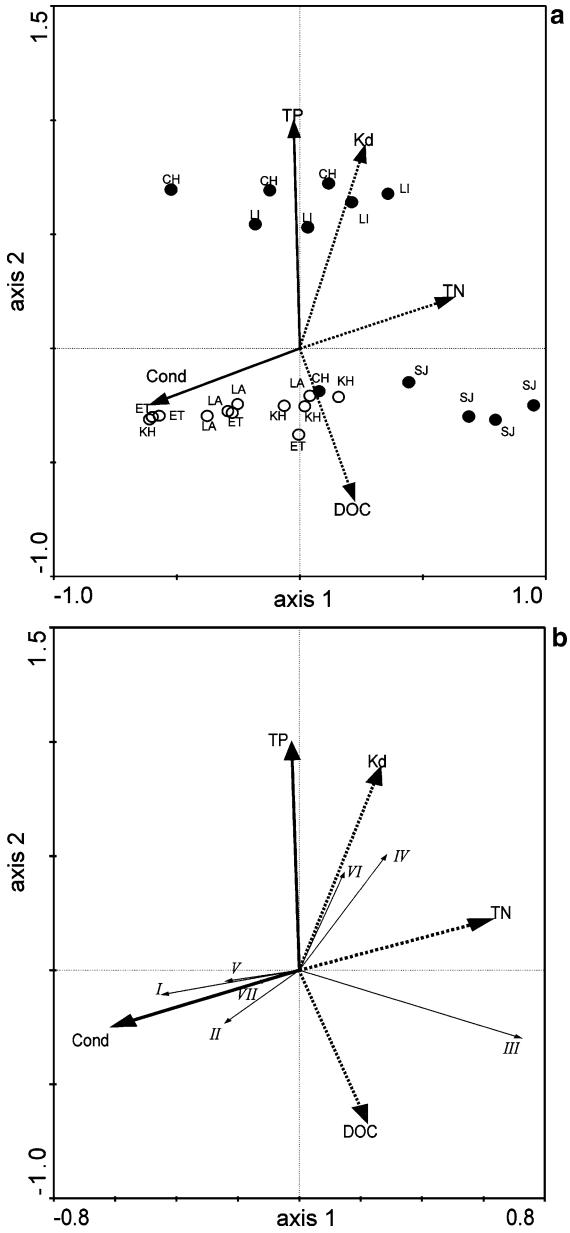

Fig. 6 Biplots of the RDA based on the biomass of the morphological-based functional groups proposed by Kruk et al. (2010). **a** Ordination of the samples and environmental variables (*CH* Chascomús, *SJ* San Jorge, *LI* La Limpia, *LA* Lacombe, *ET* El Triunfo, *KH* Kakel Huincul). **b** Functional groups and environmental variables. Turbid shallow lakes (*black circles*); clear-vegetated shallow lakes and lake with alternative steady states (*white circles*). Significant environmental variables ($P < 0.05$) are indicated with *solid arrows*, while *dotted arrows* are not significant. Conductivity (*Cond*), dissolved organic carbon (*DOC*), vertical attenuation coefficient (*Kd*), total nitrogen (*TN*), total phosphorus (*TP*)

passage of the shallow lakes from a clear-water to a turbid state, changes in phytoplankton community not only entail an increment in the pelagic algal biomass but also changes in algal composition. For example, a study conducted in the Pampa plain in the 1990s (Izaguirre & Vinocur, 1994) reported that almost 50% of the shallow lakes surveyed were in a clear state and these systems were characterized by low phytoplankton abundances, relatively high species richness, and a high proportion of nanoplanktonic species. Our current investigations in the region show that nowadays most lakes are characterized by high phytoplankton biomass, with dominance of one or few species, generally belonging to Cyanobacteria, Bacillariophyceae, and Chlorophyceae.

The Gleasonian line of reasoning assumes that individual species respond independently to the environment (Gleason, 1926), and the community composition reflects the response of individual species to the environmental conditions. As the environmental conditions select groups of species that share similar adaptive characteristics, there is a broad consensus in that communities are more reliable indicators of habitat conditions that are the presence or absence of component species (Naselli-Flores & Barone, 2011 and cites therein), and the morpho-functional phytoplankton approaches tends to simplify the taxonomical approach in environmental biomonitoring. In this sense, the aim of our investigation was to evaluate the strength of different classifications based on morphological and functional characteristics of phytoplankton to discriminate among the different types of shallow lakes.

Our results showed that the biomass of the phytoplankton groups corresponding to the three classification approaches used can be well predicted from the environmental variables. All the analyses showed the separation of the turbid lakes from the clear-vegetated ones.

The RDA performed using the Reynold′s functional classification showed a noticeable separation of the samples in relation to the turbidity of the lakes. The functional groups related with higher Kd_{PAR} values and nutrient concentrations were J, MP, D, and F. Codon J is commonly associated to shallow, mixed, highly enriched systems; codon MP includes meroplanktonic species (mostly diatoms) that are drifted to

the plankton living in turbid shallow lakes; codon D includes diatoms that live in shallow turbid waters (Padisák et al., 2009). Codon F is also typical of deeply mixed meso-eutrophic lakes, and although it was described living in clear epilimnia of the lakes, is tolerant to high turbidity (Reynolds et al., 2002). The codon Sn (dominant in the lake San Jorge) is typical of warm mixed environments; it comprises Cyanobacteria tolerant to low light conditions and as they usually have heterocysts, can also tolerate nitrogen deficiency. In the lake San Jorge, this functional group was almost exclusively represented by cf. *Raphidiopsis mediterranea*; it is important to mention that during the period of our studies we have never observed heterocysts in this species, but the system is not N-limited, and as it was discussed by Litchman et al. (2010), the development of heterocysts (the specialized cells where fixation occurs) indicates low nitrogen availability. Thus, we cannot discard that this population may belong to the genus *Cylindrospermopsis*. In the clear-vegetated lakes, the most representative functional groups were: Y mainly represented by cryptomonads; L0, which was described for mesotrophic systems and that seems to be sensitive to prolonged or deep mixing (Reynolds et al., 2002), and that in our lakes was represented by small colonial cyanobacteria; K that includes some small non-gas-vacuolated cyanobacteria typical of shallow, nutrient-rich water columns, and also sensitivity to deep mixing; X2 that comprises some unicellular flagellated species typical of shallow meso-eutrophic environments; X1, a codon described for shallow, eu-hypertrophic environments, mainly represented by small chlorococcaleans (Reynolds et al., 2002; Padisák et al., 2009). The functional groups proposed in the Reynold′s classification provided an appropriate characterization of the shallow lakes of the region, and the main groups observed in each type of lake reflect the main features of the phytoplankton assemblages previously described using complete lists of species (Allende et al., 2009). Particularly, the classification was able to detect the importance of mixotrophic flagellates (Chrysophyceae and Cryptophyceae) and small colonial cyanobacteria in the clear-vegetated lakes that can be explained by their lower sedimentation rates as compared with large non-buoyant cells (Søndergaard & Moss, 1998). One of the disadvantages of this classification is that the criteria for assigning species to groups are not formalized, and it is necessary a deeper knowledge of the autoecology of the species.

The morpho-functional classification proposed by Salmaso & Padisák (2007) constitutes another interesting approach where the criteria adopted to discriminate the groups include the traits proposed by Weithoff (2003) that are valuable for characterizing functional aspects of phytoplankton: motility, the potential capacity to obtain carbon and nutrients by mixotrophy, specific nutrient requirements, size and shape and presence of envelopes. The results of our multivariate analyses showed that three functional groups were prevalent in clear-vegetated lakes with higher DOC concentrations: 2a, 2d, and 5d; the two first include potentially mixotrophic nanoflagellates (Chrysophytes and Cryptophytes, respectively), and the third one comprises small colonial cyanobacteria belonging to Chroococcales. These results were similar to those obtained with the Reynold′s classification, but the dominant components in the assemblages of the clear-vegetated lakes were even more clearly reflected. In the turbid shallow lakes, the morpho-functional groups more related with high Kd_{PAR} values were 6b and 7b (large and small pennate diatoms, respectively); 11a and 11b (naked and gelatinous colonial Chlorococcales, respectively). Other groups, such as 1a (large Chrysophytes) and 3a (unicellular Phytomonadina) were also related with high turbidity. The groups 5e (Nostocales) and 8a (large Conjugatophytes), also abundant in phytoplankton-turbid lakes of the region, were associated to high TN levels.

An obvious requisite to use this classification, as well as that proposed by Reynolds et al. (2002), is that a deeper knowledge of the taxonomy and the functional aspects of phytoplankton are necessary.

The third classification used (Kruk et al., 2010) based on purely morphological traits, offers several advantages. Among them, 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. These features make this classification very useful for monitoring of ecosystems. The disadvantages with respect to the other two classifications may be the relatively low sensitivity to detect certain phytoplankton functional aspects that may be relevant in the system. For example, some mixotrophic taxa like

Cryptomonas spp., well represented in clear-vegetated lakes, were included in the same group (V) that other flagellate species that are strictly autotrophic, although these taxa are well represented in the opposite alternative steady state. Kruk et al. (2011) argued that the species in any particular group are basically interchangeable and ecologically equivalent, but this would not be the case in the mentioned example. Nevertheless, in our study, the multivariate analyses based on the MBFG proposed by Kruk et al. (2010) also showed a clear separation of the clear-lakes from the turbid ones. The percentage of explained variance obtained using this classification was higher than for the other two classifications, but this is because with a lower number of groups the total variation in the data set (total inertia) decreases. Contrarily to that observed with the other two classifications, in this analysis, the forward selection procedure indicated that Kd_{PAR} was not significant in the ordination obtained, whereas TP had more importance. The samples of the two turbid shallow lakes (Chascomús and La Limpia) were placed very close to each other, toward higher values of TP. The more representative MBFG in these turbid systems were IV (organisms of medium size lacking specialized traits) and VI (non-flagellated organisms with siliceous exoskeletons = diatoms). In the clear-vegetated lakes, the prevalent groups were I (small organisms with high S/V), II (small flagellates with siliceous exoeskeletal structures), V (represented in the studied systems by unicellular flagellates of medium size), and VII (mucilaginous colonies). The group III (large filamentous with aerotopes) was related with higher values of TN.

With all the classifications used, the results of the RDA showed that the samples of the phytoplankton-turbid lake Chascomús plotted closer to the samples of La Limpia (inorganic-turbid) than to the samples of the other phytoplankton-turbid lake (San Jorge). These results evidence the great importance of the light attenuation on the phytoplankton assemblages, as these two lakes are the most turbid among the studied systems. Investigations conducted in Chascomús, perhaps the most extensively studied Pampean shallow lake, indicate that the system has stabilized in a turbid state (Llames et al., 2009), and the results obtained by Torremorell et al. (2009) are consistent with a theoretical expectations based on a light limitation scenario.

Interestingly, in the two algal-turbid lakes, the dominant algal classes and the main functional groups were relatively constant throughout the study period. Other studies carried out in Chascomús have reported similar results. In particular, Torremorell et al. (2009) observed that the phytoplankton assemblage did not displayed significant variability in species composition during an annual period, and suggested that the seasonal trend in photosynthesis photoinhibition could have resulted from physiological adaptations of the cells. These observations seem to indicate that the phytoplankton assemblages of these turbid shallow lakes are in a steady state, and probably the stress due to a high light constraint would be the key factor in the selection of species well adapted to such conditions. Different investigations have shown that steady-state conditions occur regularly in shallow lakes and the stress factors may be one of the causes involved (Naselli-Flores et al., 2003 and cites therein).

Our results showed that in general the morpho-functional classifications constitute a good approach to analyze the differences among the shallow lakes of the Pampa plain. Although taxonomy cannot be replaced by a morpho-functional classification since the unequivocal link between any species and its traits is the basis for a correct inclusion of species into functional groups (Padisák et al., 2009), this approach can be very valuable in the environmental biomonitoring. In particular, the morphological-based classification proposed by Kruk et al. (2010) may be useful for long-term monitoring of aquatic systems or in the comparison of a great number of lakes, due to its relatively simplicity and objectivity. Nevertheless, in our study, the analysis based on this classification underestimated the importance of the light climate, one of the main regulator factors of the phytoplankton communities in the shallow lakes of this region, and it was not very sensitive to detect the importance of certain species in the clear-vegetated lakes (particularly some mixotrophic taxa). The functional classification proposed by Reynolds et al. (2002) provides more information, and allows a more detailed description of the algal assemblages. However, although the revision performed by Padisák et al. (2009) clarified many misplacements and modified some of the original habitat templates facilitating its application, this classification requires a deeper knowledge of the autoecology of single species or species groups.

The classification of Salmaso & Padisák (2007) reflected the differences in the algal assemblages among the systems, is also relatively simple, and in our study highlighted the importance of the variables that define the different steady states in the region. Thus, in this regional context, this classification seemed to be particularly sensitive and appropriate for monitoring the aquatic systems of the region.

All the classifications applied resulted adequate to separate the more deteriorated systems (turbid lakes) from the clear-vegetated ones, which are comparatively less impacted, concluding that the functional approach is an adequate tool to analyze the phytoplankton assemblages in human-impacted lakes.

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PHYTOPLANKTON

Phytoplankton functional and morpho-functional approach in large floodplain rivers

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Abstract Influence of hydrological characteristics and nutrient concentrations on phytoplankton was investigated in four large rivers (Mura, Drava, Danube and Sava) in the Pannonian ecoregion in Croatia to understand how phytoplankton of rivers can be explained by the "different functional group approach". To gain a clearer understanding of the factors that affect river phytoplankton, the present study examined phytoplankton biomass and composition in relationship with physical and chemical parameters assessed in detail by preparing self-organising maps using functional groups and morpho-functional groups. Total nitrogen along with water residence time showed to be the best predictor to determine phytoplankton biomass and chlorophyll *a*. Phytoplankton diversity increased with higher water discharge, but it had the consequence of diluting algae and decreasing biomass. Bacillariophyceae and Chlorophyceae species dominated the phytoplankton assemblages in all rivers. Diatoms predominated in rivers with shorter residence time. Dominant diatom codons of functional groups were C, D and TB while morpho-functional groups were represented by only diatom group VI. As residence time increased, the proportion of chlorococcalean green algae, represented by functional group codon T and morpho-functional group IV grew in summer. Since potamoplankton is dominated by diatoms, functional groups with its fine partition of diatom codons proved to be excellent descriptor of the potamoplankton. Application of morpho-functional groups originally developed from the lake data, showed to be limiting because of the predominating presence of only one diatom group.

Keywords Potamoplankton dynamics · Nutrients · River discharge · Water residence time · Large rivers

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Introduction

Lake eutrophication has become one of the most intensively investigated areas of hydrobiology in

recent decades. Several papers have been published on the relationship between nutrients and chlorophyll *a* content in lakes (reviewed in Phillips et al., 2008), and different trophic scales have been proposed for characterising the levels of lake eutrophication (Vollenweider & Kerekes, 1982). Running waters also suffer the negative consequences of anthropogenic eutrophication, such as high phytoplankton biomass and undesirable changes in species composition, but the literature focusing on the characteristics of phytoplankton in riverine ecosystems is much less extensive.

An explanation for the relative scarcity of studies on riverine phytoplankton may be that ecological assessment of rivers is usually based on investigation of the benthic elements of biota. Without a doubt, this approach has been successful over the 100-year history of river quality assessment. Rivers of lower order are naturally heterotrophic systems (Vannote et al., 1980; Reynolds et al., 1994; Dodds & Cole, 2007), and their benthic invertebrates are the biological elements most relevant for ecological assessment. The autotrophic component of these systems is the phytobenthos, which has been used successfully for monitoring (Van Dam et al., 1994; Kelly et al., 1998; Stevenson & Pan, 1999). Large floodplain rivers during medium and low-discharge periods can also be considered naturally autotrophic systems (Thorp et al., 1998; Wehr & Descy, 1998) because potamoplankton dominates the biota. Like lake phytoplankton, potamoplankton can be organised into functional groups (FGs) (Reynolds et al., 2002; Borics et al., 2007; Padisák et al., 2009) and morpho-functional groups (MFGs) (Kruk et al., 2010) on the basis of their morphological and physiological adaptive strategies for surviving in different environments. Such a classification of potamoplankton with FG approach has been described before (Devercelli, 2006; Várbíró et al., 2007; Abonyi et al., 2012) while MFG approach has never been used.

Several studies suggest that the biomass of river phytoplankton is determined by nutrients (Van Nieuwenhuyse & Jones, 1996) and by physical factors like catchment area, mean depth and flushing rate (Baker & Baker, 1979).

In light of previous studies on different FG and MFG approach on lake and river phytoplankton we hypothesised that they can also be applied in large floodplain Pannonian rivers. We tested FGs versus MFGs. In order to gain a clearer understanding of the factors that affect river phytoplankton, the present study examined phytoplankton biomass and composition of different FGs and investigated how those two are affected by nutrient composition and hydrological characteristics (water residence time and discharge).

Materials and methods

Study area

Croatia is located on the border of Western Europe and the Balkan Peninsula and it has two ecoregions: Pannonian and Mediterranean. The Pannonian ecoregion is influenced by Continental climate with average summer temperature between 20 and 22°C and annual precipitation between 700 and 120 mm, while the Mediterranean ecoregion is influenced by Mediterranean climate with average summer temperature between 20 and 26°C and annual precipitation between 700 and 1,000 mm. The Mediterranean ecoregion has short, fast-flowing, clearwater karstic rivers, while the Pannonian ecoregion has large, slow-flowing, lowland rivers.

Phytoplankton was investigated at nine sampling sites on the four largest Croatian floodplain rivers in the Pannonian ecoregion (Mura, Drava, Danube and Sava; Fig. 1). These rivers belong to the Black Sea watershed. Mura River is 493 km long and it flows through Croatian territory for only 53 km. It is the left tributary of the Drava River. Drava River flows through Croatian territory for 305 km, much of that along the Croatian-Hungarian Border and it is the right tributary of the Danube. Only 188 km flows through Croatian territory, mainly along the border with Serbia. Sava River is the longest Croatian river, with 562 km flowing through Croatian territory, mainly as a border river between Croatia and Bosnia and Herzegovina. Between our two sampling sites, Sava River receives two large tributaries from Bosnia and Herzegovina, the Bosna and Vrbas rivers.

All sites are Croatian National Monitoring Sites for tracking the ecological status of rivers according to the EU Water Framework Directive (WFD, 2000). Geological and hydrological properties of sampling sites are given in Table 1.

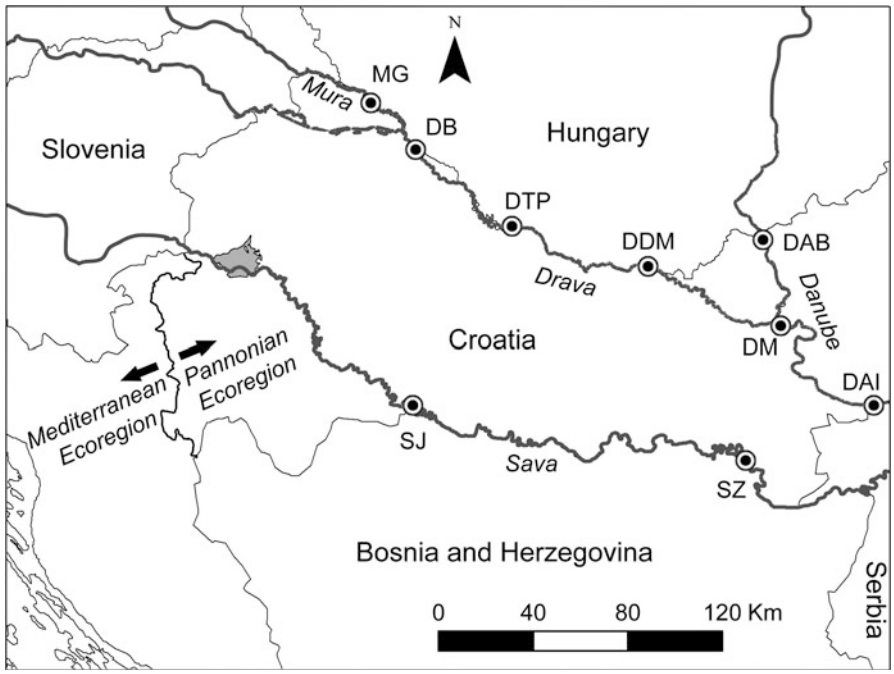

Fig. 1 Study area with the sampling sites indicated. Mura River, Goričan (MG); Drava River, Botovo (DB), Terezino Polje (DTP), Donji Miholjac (DDM) and river mouth (DM); Danube, Batina (DAB), Ilok (DAI); Sava River, Jasenovac (SJ) and Županja (SZ)

Table 1 Geological and hydrological properties of study sites

Sampling station	Code	Latitude	Longitude	Altitude (m)	Watershed (km^2)	Distance from mouth (km)	Width (m)	Depth (m)
Mura River								
Goričan	MG	46°24′43.40″	16°42′3.80″	140	13,148	33.5	94	4.7
Drava River								
Botovo	DB	46°14′29.76″	16°56′18.51″	123	31,038	226.8	127	3.1
Terezino Polje	DTP	45°56′44.15″	17°27′42.63″	98	33,916	152.3	210	5.5
Donji Miholjac	DDM	45°47′0.41″	18°12′3.79″	84	37,142	80.5	175	2.7
River mouth	DM	45°32′43.11″	18°54′45.49″	79	39,982	1.0	231	6.0
Danube								
Batina	DAB	45°53′21.90″	18°49′38.63″	82	210,250	1424.8	560	8.3
Ilok	DAI	45°13′57.16″	19°24′6.12″	73	253,737	1301.5	400	8.5
Sava River								
Jasenovac	SJ	45°16′7.85″	16°54′55.19″	89	38,953	500.5	210	8.1
Županja	SZ	45° 2′17.14″	18°42′10.63″	76	62,891	262.0	330	13.3

Sampling and sample analysis

Surface samples were collected monthly from the main-flow (thalweg) of the rivers between April and September 2010 (Kiss et al., 1996).

Phytoplankton samples were fixed with acidic Lugol's solution and stored in the dark at 4°C. Cells were counted with an inverted microscope following Utermöhl's (1958) method. A minimum of 400 settling units were counted in transect at 400× magnification, providing a counting error of <10% (Lund et al., 1958). Biovolumes were calculated by determining an average individual size from 30 randomly chosen cells of each species, and then multiplying by the observed species abundance (Rott, 1981). Biomass (freshweight) was derived from

biovolumes and used for further analyses, where $1\ mm^3\ l^{-1} = 1\ mg\ l^{-1}$ (Wetzel & Likens, 2000). Taxa were assigned to FGs based on Reynolds et al. (2002), Borics et al. (2007) and Padisák et al. (2009) and MFGs based on Kruk et al. (2010).

Samples for water chemistry were taken simultaneously with phytoplankton samples. They were stored in special bottles and analysed in the laboratory. Oxygen and total suspended particles were analysed following the APHA (2005). Conductivity and pH were measured with electrodes of the SevenMulti Modular Meter System (Mettler Toledo). Total phosphorus (TP) and dissolved silicates were detected using a UV–VIS spectrometer (Perkin Elmer Lambda 25). Total nitrogen (TN) and total organic carbon (TOC) were analysed using a Shimadzu TOC-VCPH equipped with an analyser for TN and TOC.

Chlorophyll *a* was filtered with Whatman GF/F glass filters, extracted in 96% ethanol and measured using a UV–VIS spectrophotometer (Perkin Elmer Lambda 25) according to standard guidelines (APHA, 2005).

Average monthly water discharge data were obtained from the Croatian National Hydrometeorological Institute. Theoretical water residence time (WRT) was calculated as a function of drainage area (A_d, km^2) and discharge (Q, $m^3\ s^{-1}$) using the equation $WRT = 0.08 \times A_d^{0.6} \times Q^{-0.1}$ (Soballe & Kimmel, 1987; Leopold et al., 1995).

Data analysis

Principal component analysis (PCA) was carried out using the program PRIMER 6 (Clarke & Gorley, 2006) in order to group sampling sites according to their environmental parameters. Data were normalised prior to PCA. Results of PCA were plotted together with similarity clusters after calculating Euclidean distances.

The Shannon–Wiener diversity index (Shannon & Weaver, 1963) was calculated for each sample using phytoplankton abundance values.

Self-organising map (SOM) was used to analyse and visualise the phytoplankton groups parallel to the environmental variables. SOM is a neural network analysis tool for visualisation of high-dimensional data by reducing its dimensionality, but still preserving the most important features of the data. In this case it is the topological and metric relationships of the original data that are preserved (Kohonen, 2001). SOMs have frequently been used in diatom ecology and in studies describing benthic algal assemblages in France, Luxembourg or to define (Rimet et al., 2004; Gosselain et al., 2005) riverine phytoplankton assemblages Hungary (Várbíró et al., 2007). The multidimensional data of environmental variables can be visualised using a SOM, initially training it with only biological variables by applying a mask function that assigns a weight of 1 to the biological variables and a weight of 0 to environmental variables (Céréghino & Park, 2009).

The SOM Toolbox (http://www.cis.hut.fi/projects/somtoolbox) was used to implement the SOM in a MATLAB™ environment. The data matrix consisted of 52 samples and 36 variables (24 codons, 5 physical–chemical–hydrological variables, 7 functional traits). In the selection phase of creating the SOM, the weights of the output layer were initially assigned randomly. Then a sample was chosen randomly and the best matching unit (BMU) was selected by calculating the Euclidean distance between the weights of the input layer and the output layer. Selection of the BMUs was based exclusively on the FGs coded biomass.

Physical–chemical–hydrological variables and functional traits were masked out during this selection phase of the neural network. When the learning phase was finished, a map with hexagons was obtained in which each hexagon contained a virtual unit containing the calculated weight/codon composition. The resulting hexagon map with its weights was visualised using the SOM Toolbox as component planes (CPs). Each CP represents the supplied variables that the SOM algorithm has learned.

Results

Physical, chemical and hydrological characteristics

Median values and ranges for all physical, chemical and hydrological data, as well as for the level of chlorophyll *a*, are presented for each sampling site in Table 2. Mura River had lower values for temperature, conductivity, alkalinity and oxygen than Drava River, but a higher concentration of nutrients. Most physi cal and chemical parameters tended to increase

downstream along the Drava River. The Danube had the highest parameter values among the four rivers investigated, but the values tended to decrease downstream. The same tendency was observed downstream the Sava River.

Many of the variables were correlated with each other, thus leaving only 11 distinct uncorrelated parameters for the consideration of multivariate statistical (Table 2). PCA grouped sites according to the rivers to which they belonged. The Mura River sampling site was statistically close to the Drava River sampling sites, while both Sava River sampling sites differed from those of the other rivers, and from each other. Danube sites were grouped separately from other investigated sites. The two axes in PCA analyses accounted for 56.3% of the cumulative variance in physicochemical data set with eigenvalues of 4.12 and 2.07, respectively. Four potamal rivers highly correlated with conductivity, TN, TP, oxygen and temperature. Axis 1 was presented by conductivity ($r = -0.41$), TN ($r = -0.40$) and TP ($r = -0.37$), explaining 37.4% of the variance. PCA axis 2 was positively influenced by temperature ($r = 0.56$) and oxygen ($r = 0.53$) and negatively by saturation ($r = -0.38$), explaining 18.8% of the variance.

Average monthly discharge (Q) and WRT significantly differed among the four tested rivers (ANOSIM analysis, $P = 0.001$). Both parameters tended to increase downstream in all cases (Table 2; Fig. 2). Mura River had the lowest average monthly discharge, with small fluctuations over time between 133 and 242 $m^3\ s^{-1}$. Drava River had the next-highest discharge, which ranged from 395 $m^3\ s^{-1}$ at the most upstream site (Botovo) to 878 $m^3\ s^{-1}$ at the mouth, with small fluctuations over time. The Danube had the highest average monthly discharge, between 2,286 $m^3\ s^{-1}$ at the upstream Batina site and 6,496 $m^3\ s^{-1}$ at the downstream Ilok site. The discharge peaked in June, but otherwise fluctuated little during the other months of the year. The Sava showed the greatest oscillations at both sampling sites and throughout the study period. The average monthly discharge ranged from 306 $m^3\ s^{-1}$ at the upstream Jasenovac site to 2,131 $m^3\ s^{-1}$ at the downstream Županja site.

Phytoplankton

A total of 222 algal taxa were identified in the samples. These belonged to nine major groups: Cyanobacteria (30), Euglenophyceae (13), Cryptophyceae (4), Dinophyceae (4), Chrysophyceae (9), Bacillariophyceae (65), Xanthophyceae (2), Chlorophyceae (91) and Zygnematophyceae (4).

Bacillariophyceae was the dominant taxonomic group in the majority of samples and contributed 6.4–93.7% to the total phytoplankton biomass. This group was the most dominant in all samples from Mura River, accounting for 73.1–93.7% of phytoplankton biomass. Chlorophyceae contributed 1.0–89.4% to the total biomass in Drava River, with its contribution to biomass peaking in August and September. This group contributed 4.1–49.1% to the total biomass of the Danube throughout the study period. On the contrary, it contributed only 0.4–11.1% to the total biomass in Sava River. Chryptophyceae were sometimes co-dominant with Bacillariophyceae and Chlorophyceae, contributing 0.1–46.8% to the total biomass, but the contribution varied widely and erratically over the study period. Euglenophyceae contributed significantly to the phytoplankton of the Sava River at the Jasenovac site, 16.9% in July and 26.8% in September. Other taxonomic groups made little or no contribution to the total phytoplankton biomass.

Taxa contributing more than 5% of the total biomass in individual samples were defined as descriptive species. A total of 41 descriptive species were identified (Table 3). The number of descriptive species was similar for the Mura (15), Danube (17) and Sava (18) rivers. Drava River had the greatest number (27). Three of these taxa were present in all four rivers: *Stephanodiscus hantzschii*, *Stephanodiscus* cf. *minutulus* and *Navicula lanceolata*. Nevertheless there were species that, despite accounting for a very small proportion of the total biomass, occurred in almost every sample: *Plagioselmis nannoplanctica*, *Cryptomonas* sp., small-celled *Stephanodiscus* sp., *Nitzschia acicularis* and *Scenedesmus* sp.

Total biomass was lowest in Sava River throughout the study period (0.03–0.71 mg l^{-1}), followed by the Mura (0.17–0.85 mg l^{-1}) and Drava River (0.14–3.92 mg l^{-1}). The Danube had the highest total biomass (0.21–20.94 mg l^{-1}) (Fig. 2).

Total phytoplankton biomass in relation to Shannon–Wiener diversity index and average monthly discharge is presented in Fig. 2. Two patterns of the total phytoplankton biomass were observed. At the Drava River sampling sites at Terezino Polje, Donji

Table 2 Median values and ranges of physical, chemical, and hydrological parameters and chlorophyll *a* concentration at all sampling sites from April to September 2010

	Mura		Drava							
	Goričan		Botovo		Terezino Polje		Donji Miholjac		Mouth	
	Median	Range	Median	Range	Median	Range	Median	Range	Median	Range
Water temperature (°C)	16.4	9.4–21.4	17.0	9.9–21.3	18.1	10.7–23.4	19.3	10.8–23.6	19.1	11.7–24.1
pH	8.01	7.85–8.15	8.03	7.95–8.18	8.11	7.95–8.21	8.14	7.93–8.17	8.16	7.92–8.38
Conductivity (μS cm^{-1})	318	262–509	299	279–379	302	283–377	318	301–386	319.5	288–393
Total suspended particles (mg l^{-1})	23	13.5–88.0	9.6	6.8–42.0	12.8	6.4–29.2	16.0	5.2–43.2	17.2	6.4–45.6
Dissolved oxygen (mg l^{-1})	8.9	7.8–10.2	8.8	8.0–10.6	8.7	8.3–10.5	8.85	8.4–10.6	8.5	6.9–10.8
Dissolved oxygen (%)	90	85.1–93.9	93.6	87.0–95.9	93.4	92.1–97.5	98.0	90.1–105.0	95.0	72.3–105.5
TN (μg N l^{-1})	1500	1360–2060	1170	960–1540	1235	920–1500	1280	930–1730	1310	920–1370
TP (μg P l^{-1})	81	58–215	51	28–109	67	30–90	69	40–114	79	42–130
Dissolved silicates (mg SiO_2 l^{-1})	6.3	5.1–7.6	5.1	4.2–6.2	5.06	4.7–6.5	4.8	3.9–6.7	5.4	2.8–6.2
TOC (mg l^{-1})	2.5	1.97–2.65	1.86	1.51–2.31	1.97	1.5–2.9	2.17	1.64–3.16	2.12	1.86–2.99
Q (m^3 s^{-1})	178	133–242	542	395–690	559	417–730	588.5	439–814	595.5	444–878
WRT (day)	14.1	13.9–14.5	21.2	20.6–21.8	22.2	21.6–22.9	23.4	22.6–24.0	24.4	23.4–25.1
Chlorophyll *a* (μg l^{-1})	2.7	0.5–7.9	3.8	1.7–5.1	4.9	1.4–8.8	8.51	2.2–20.3	8.4	2.7–28.4

	Danube				Sava			
	Batina		Ilok		Jasenovac		Županja	
	Median	Range	Median	Range	Median	Range	Median	Range
Water temperature (°C)	18.7	10.9–22.6	17.9	13.8–27.2	20.7	10.8–25.8	22.6	11.8–26.2
pH	8.32	7.98–8.63	8.01	7.91–8.23	7.81	7.63–8.09	7.95	7.87–8.06
Conductivity (μS cm^{-1})	464	387–565	392.5	367–450	394	365–434	413	381–468
Total suspended particles (mg l^{-1})	44.2	22.8–51.6	21.6	14.0–48.8	12.4	1.0–39.2	8.6	3.0–18.0
Dissolved oxygen (mg l^{-1})	9.2	7.7–12.0	8.1	6.5–10.1	6.8	5.1–9.0	6.8	6.1–9.3
Dissolved oxygen (%)	95.3	74.5–130.6	88.2	70.4–101.0	75.3	60.1–84.1	79.3	73.3–86.4
TN (μg N l^{-1})	1985	1760–2730	1755	1460–2440	1420	1270–2000	1380	1210–1910
TP (μg P l^{-1})	115	75–190	108	57–147	129	72–396	113	62–190
Dissolved silicates (mg SiO_2 l^{-1})	5.2	0.3–6.7	5.9	3.1–7.8	4.1	2.5–5.4	5.1	3.9–6.1
TOC (mg l^{-1})	3.11	2.52–3.51	3.30	2.74–4.16	3.82	1.82–4.91	3.10	1.63–4.79
Q (m^3 s^{-1})	3101.5	2286–5258	3740	2988–6246	905.5	306–1163	1171	441–2131
WRT (day)	9.1	8.7–9.4	24.2	23–24.8	23.0	22.4–25.6	29.9	28.1–32.9
Chlorophyll *a* (μg l^{-1})	6.2	3.6–80.5	14.2	3.3–22.6	3.8	0.2–9.2	0.9	0.2–5.6

TN total nitrogen, *TP* total phosphorus, *TOC* total organic carbon, *Q* mean monthly discharge, *WRT* water residence time

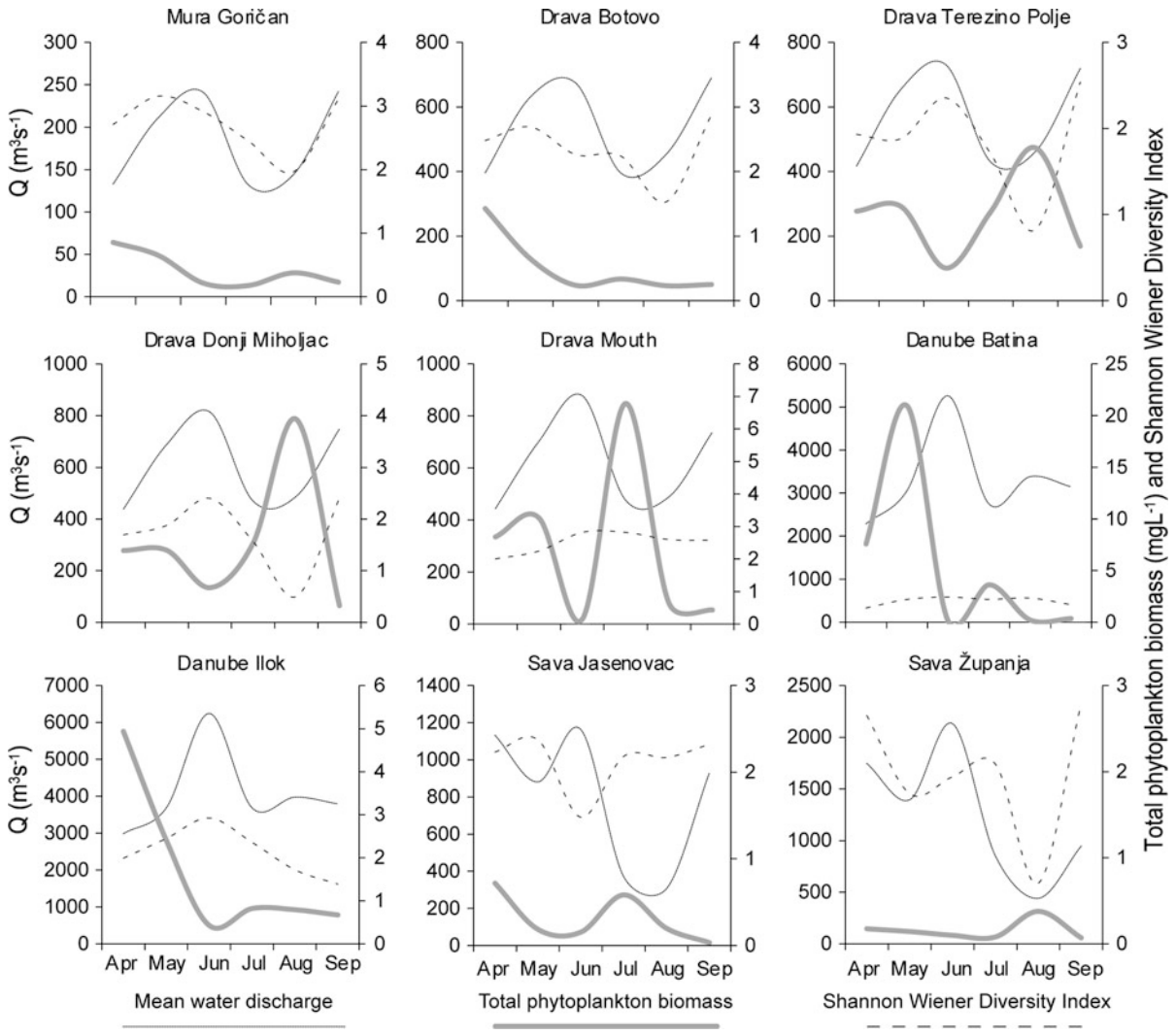

Fig. 2 Variations in average monthly discharge (Q), total biomass and Shannon–Wiener Diversity Index at the investigated sample sites

Miholjac and the river mouth, a maximum in biomass appeared in August. At all other sampling sites, the biomass reached a maximum in April or May.

Values of the Shannon–Wiener diversity index changed throughout the study period. It behaved similarly at all sampling sites at all four rivers (Fig. 2): it varied directly with average monthly discharge and inversely with total phytoplankton biomass, but without significance (linear regression, $P = 0.928$, $P = 0.250$).

A total of 24 FGs were detected. Those that contributed with low biomass (<10%) were grouped together as Others (Fig. 3). Throughout the study period, the dominant FGs were diatom groups with codons C, D and TB. Occasional dominance of codon T was also found in mid-summer, while other codons J, P, TD, W1, Y and X2 showed occasional co-dominance. Figure 3 also shows increasing tendency of codon T relative biomass in summer months with increase of WRT in Mura and Drava rivers. Downstream stations have higher relative biomass contribution with its maximum in Drava Donji Miholjac station, while in Drava Mouth station it decreases again, similar to Danube Batina. Sava River, as isolated hydrological system from other three investigated rivers had its specific FGs composition.

Table 3 List of taxa of dominant and subdominant species that contributed >5% to the total phytoplankton biomass recorded in the Mura, Drava, Danube and Sava Rivers during the study period

Cyanophyceae
Anabaena sp.—M, Dr, Da, S
Aphanizomenon flos-aquae—Dr, Da
Oscillatoria limosa—M, Dr, Da
Planktothrix agardhii—Dr, Da, S
Euglenophyceae
Euglena sp.—M, Dr, Da, S
Cryptophyceae
Cryptomonas sp.—M, Dr, Da, S
Plagioselmis nannoplanctica—M, Dr, Da, S
Bacillariophyceae
Acanthoceras zachariasii—M, S
Asterionella formosa—M, Dr, Da, S
Asterionella ralfsii—M, Dr, Da
Aulacoseira granulata—M, Dr, Da, S
Aulacoseira sp.—Dr
Cocconeis placentula—M, Dr, S
Cyclotella meneghiniana—M, Dr, Da, S
Diatoma ehrenbergii—M, Dr, S
Diatoma vulgaris—M, Dr, Da, S
Encyonema silesacum—M, Dr, S
Fragilaria crotonensis—M, Dr, Da, S
Gyrosigma acuminatum—Dr, Da, S
Gyrosigma scalproides—Dr, Da
Melosira varians—M, Dr, Da
Navicula lanceolata—M, Dr, Da, S
Navicula sp.—M, Dr, Da, S
Nitzschia acicularis—M, Dr, Da, S
Nitzschia sigmoidea—M, Dr, Da
Nitzschia sp.—M, Dr, Da, S
Pinnularia sp.—Dr
Stauroneis sp.—S
Stephanodiscus cf. *minutulus*—M, Dr, Da, S
Stephanodiscus hantzschii—M, Dr, Da, S
Stephanodiscus sp.—M, Dr, Da, S
Surirella brebissonii—M, Dr, Da, S
Ulnaria acus—M, Dr, Da, S
Ulnaria ulna—M, Dr, Da, S
Vibrio tripunctatus—M, Dr, S
Chlorophyceae
Actinastrum hantzschii—M, Dr, Da, S
Actinochloris sp.—Da
Chlamydomonas sp.—M, Dr, Da, S
Gleotilla sp.—M, Dr, Da, S
Pandorina morum—M, Dr, Da, S
Spermatozopsis exsultans—S

M Mura River, *Dr* Drava River, *Da* Danube, *S* Sava River

All seven MFGs were found (Fig. 4). The dominant MFG was group VI, corresponding to non-flagellated organisms with a siliceous skeleton. Group IV, corresponding to medium-sized organisms lacking special traits, showed mid-summer dominance. Group V, corresponding to unicellular flagellates of medium to large size, often showed subdominance.

The following MFGs did not contribute significantly to the total biomass of river phytoplankton: II, small flagellated organisms with siliceous exoskeletal structures; III, large filaments with aerotopes; and VII, large mucilaginous colonies. MFG I, corresponding to small organisms with high S/V, was present in only six samples and made an extremely low contribution to total biomass. Figure 4 shows that group IV has a tendency to increase in summer months following the increase of WRT in Mura and Drava rivers, where it is also observed that downstream stations have higher relative biomass contributions, with its maximum in Drava Donji Miholjac station. However, it was observed that Group IV decreases in Drava Mouth station similar to Danube Batina, while Sava river had different MFGs composition from other investigated rivers.

Different FGs and MFGs showed different relationships with nutrients and WRT, as described below.

SOM analysis

SOM analysis was performed based on relative codon biomass (Fig. 5). The rivers were clearly separated on the SOM map, which can be attributed to the different codon distribution in the four rivers. The darker colours of the CPs in Fig. 6 indicate higher biomass of the given codon.

Relationships were also investigated among the background variables TP, TN, WRT and biomass, expressed both in terms of chlorophyll *a* (μg l^{-1}) and biomass (mg l^{-1}) (Fig. 7). The SOM showed that the variables WRT and TN explain the variation in phytoplankton biomass. In contrast, TP showed an inverse relationship with algal biomass.

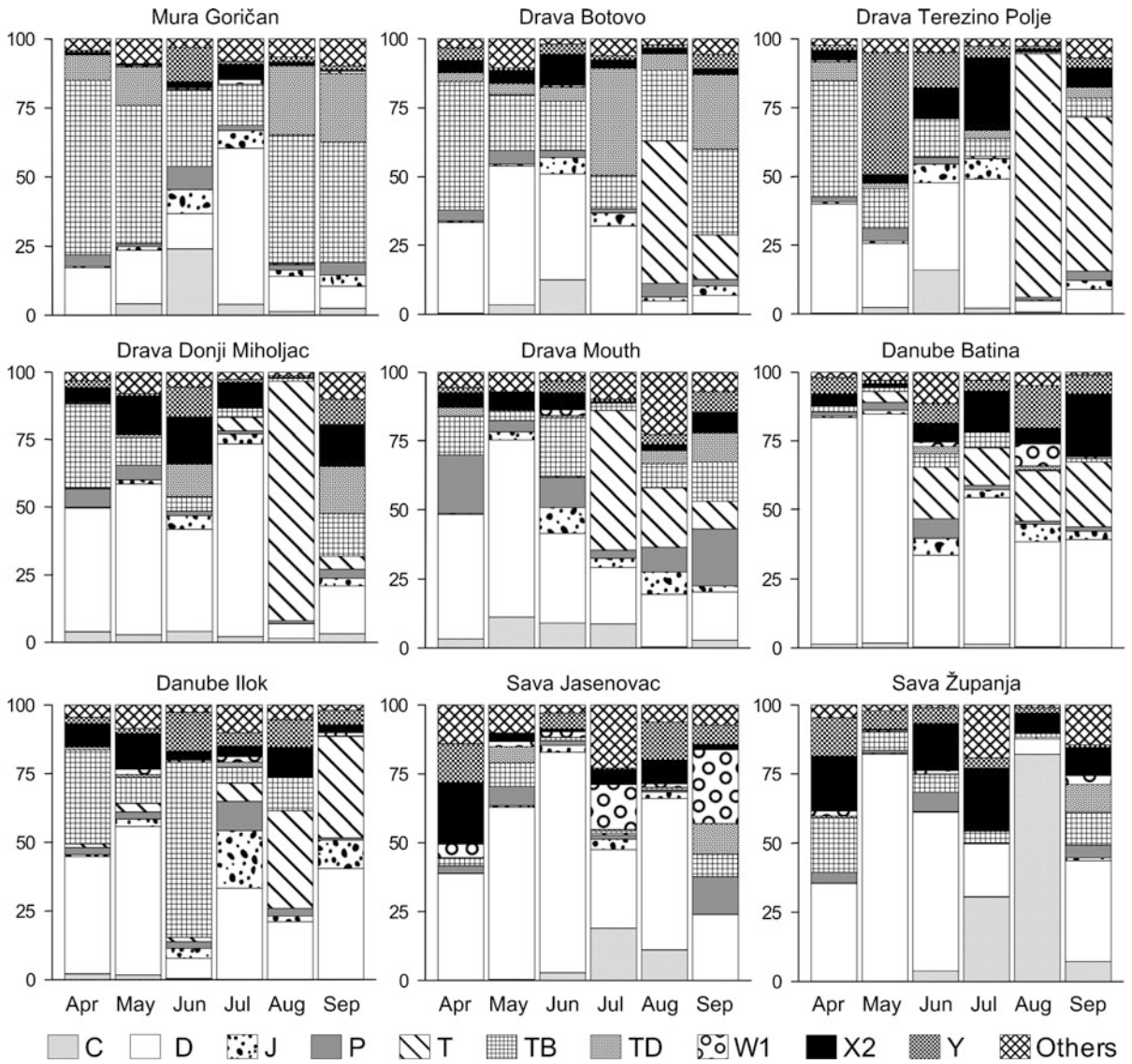

Fig. 3 Relative biomass of phytoplankton functional groups (Reynolds et al., 2002; Borics et al., 2007; Padisák et al., 2009) at all sampling sites. Functional groups that contributed with low biomass (<10%) presented as Others are: A, B, E, F, G, H1, K, L_M, L_O, N, TC, W2, X1 and X3

Comparison of the CPs of the physical and chemical variables with that of codons reveals that FGs have different preferences. The euplanktic C, D, J, L_O, Y, X1 and P codons prefer longer WRT. Conversely, the TB codon, corresponding to benthic diatoms that are the most characteristic of the upper river segments, preferred shorter WRT.

Analysis of the MFGs showed that groups V, VI and VII are not separated (Fig. 8). All of these MGFs were associated with higher WRT, while no MFG was associated with the shortest WRT values.

Discussion

Lotic systems have extremely dynamic hydrological regimes, leading to large fluctuations in abiotic and biotic factors along the river continuum (Vannote

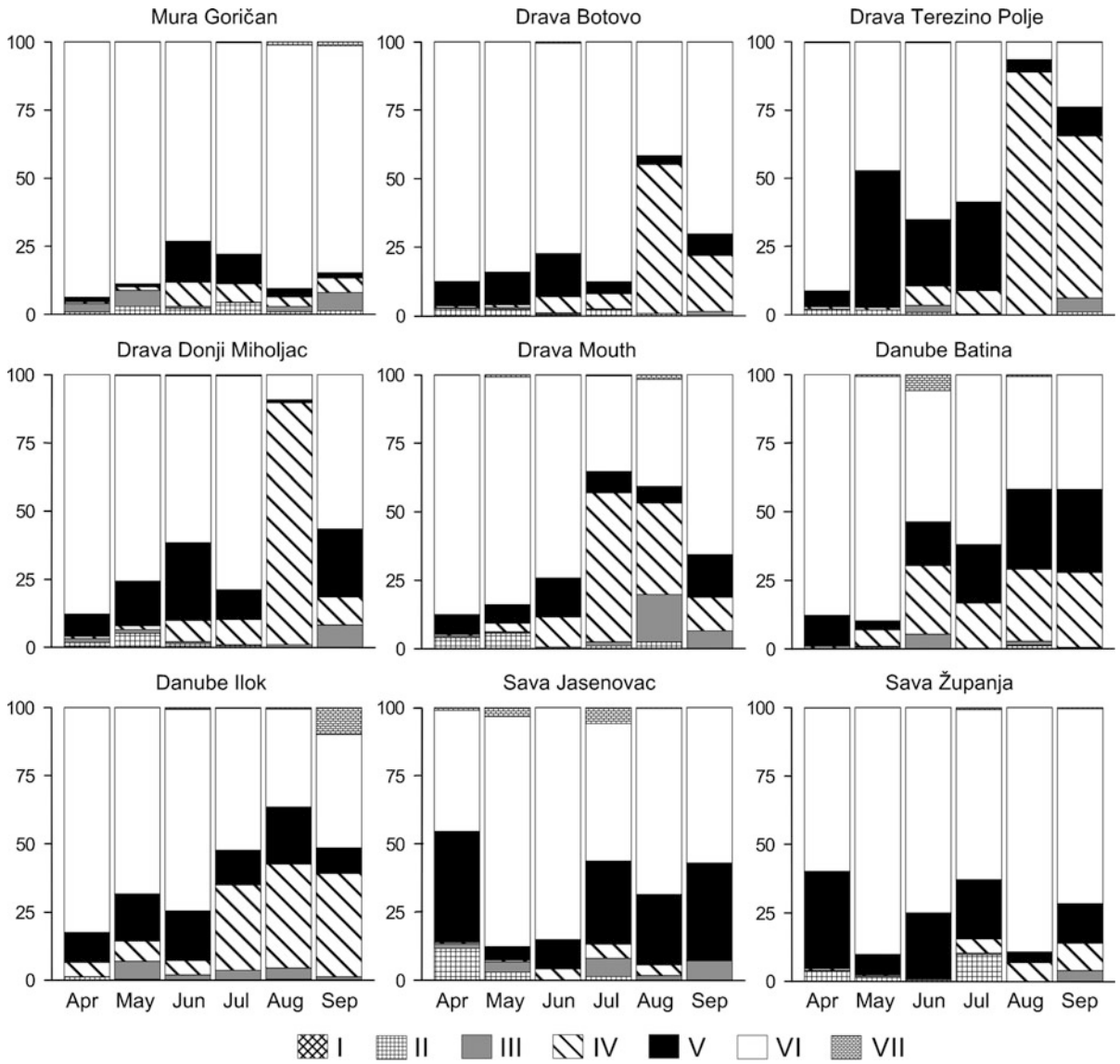

Fig. 4 Relative biomass of phytoplankton morpho-functional groups (Kruk et al., 2010) at all sampling sites

et al., 1980). Geology and geography, together with hydrology, are key factors of lotic environments that can make them more or less suitable biotopes for living organisms despite the turbulence in their characteristics (Wetzel, 2001). The rivers in the present study are large and flow in Pannonia lowlands; therefore they are predicted to be suitable habitats for the phytoplankton community known as potamoplankton (Borics et al., 2007).

The composition of the microflora of the four rivers in the present study is similar to that of other potamal rivers in the region (Várbíró et al., 2007). Similarly to the present study, a comprehensive, long-term evaluation of Danube microflora found that Chlorococcalean green algae and centric diatoms are the most relevant elements of the potamoplankton (Kiss, 1997). Indeed, the species identified in the present study as constant elements of the microflora are nearly identical to those identified in that dataset collected over more than 20 years. The tendency of the proportion of chlorophytes in the phytoplankton to increase with the size of the river, showed also in this study downstream in Mura and Drava rivers, can be considered a general feature of potamal river phytoplankton (Kiss & Genkal, 1996). In

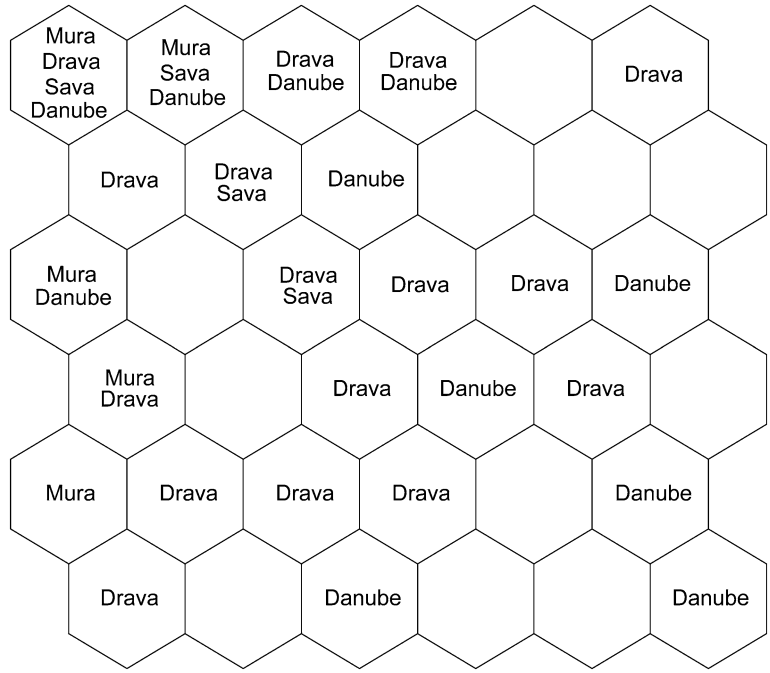

Fig. 5 The location of river sampling sites on the self-organising map (SOM). The map was produced as a result of the training and learning process based on functional group biomass

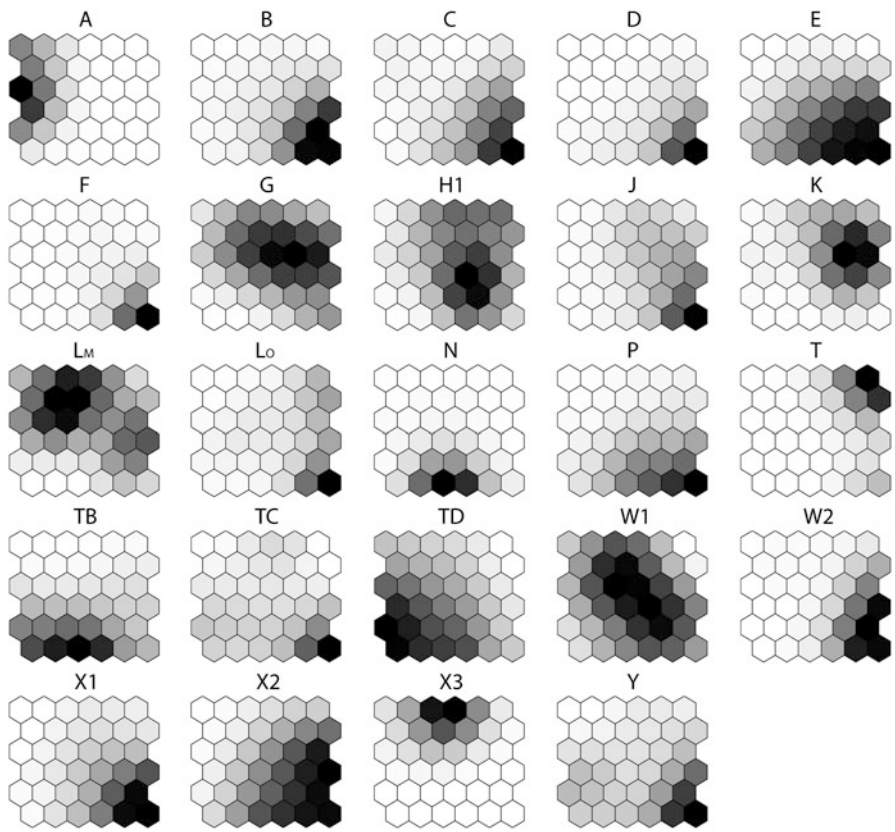

Fig. 6 Component planes of the self-organising map (SOM) showing gradient distribution of important functional codons. *Darker colours* indicate greater biomass

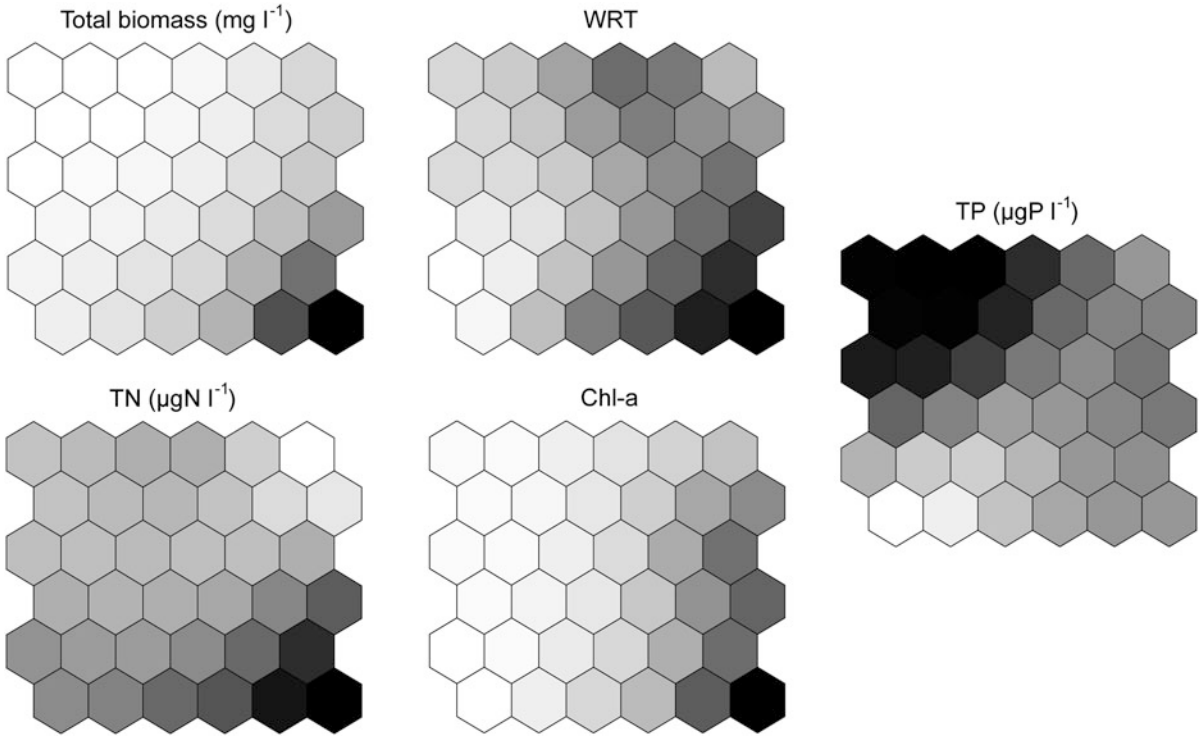

Fig. 7 Component planes of the SOM showing gradient distribution of the chemical and hydrological variables measured. *Darker colours* indicate greater values for the variable measured. *WRT* water residence time, *TN* total nitrogen, *TP* total phosphorus, *Chl-a* chlorophyll *a*

fact, chlorophytes can account for as much as 60% of the species diversity (Kiss & Schmidt, 1998). Euglenophytes occasionally appear as abundant members of microflora. For example, these taxa were observed to be more abundant in the Danube (Kiss, 1997) and in the Tisza River (Uherkovich, 1971). In both of those studies, the authors suggested that the organisms had arrived via the slow-flowing side arms of the rivers and/or via the oxbows of the floodplain. We found these taxa in the Sava River, which features a near-natural floodplain in the central, unregulated section.

Changes in phytoplankton diversity, not only in lakes but also in rivers, can be explained by the intermediate disturbance hypothesis (IDH) (Carvajal-Chitty, 1993; Padisák et al., 1993). The most frequent disturbances are river floods (Descy, 1993), which alter the entire river ecosystem. All discharge-driven variables like WRT, amount of suspended solids and concentration of nutrients are fundamental determinants of phytoplankton composition and biomass (Pieterse & van Zyl, 1988). During low-discharge periods, the phytoplankton of large rivers is similar to that of shallow lakes (Reynolds et al., 1994), dominated by euplanktic elements belonging to FGs J, X1, C and D (Várbíró et al., 2007). Floods restructure the phytoplankton, and the relative biomass of the tichoplanktic taxa increases. In the present study, the flood periods were associated with phytoplankton assemblages of high diversity and low biomass. Consistent with the predictions of the IDH, we found that the lengthy low-discharge periods in late summer allowed the development of high-biomass, low-diversity phytoplankton assemblages.

For both rivers and lakes, a clear non-linear relationship exists between TP and concentration of chlorophyll *a* (Van Nieuwenhuyse & Jones, 1996; Phillips et al., 2008). In rivers, this relationship is very strong when an extremely broad TP range is considered, but it is much less clear over narrower TP ranges. In the present study, the investigated rivers were neither phosphorus- nor nitrogen-limited because TP and TN values exceeded both mesotrophic (29 µg l^{-1} for TP and 285 µg l^{-1} for TN) and eutrophic boundary (71 µg l^{-1} for TP and 714 µg l^{-1} for TN) as suggested

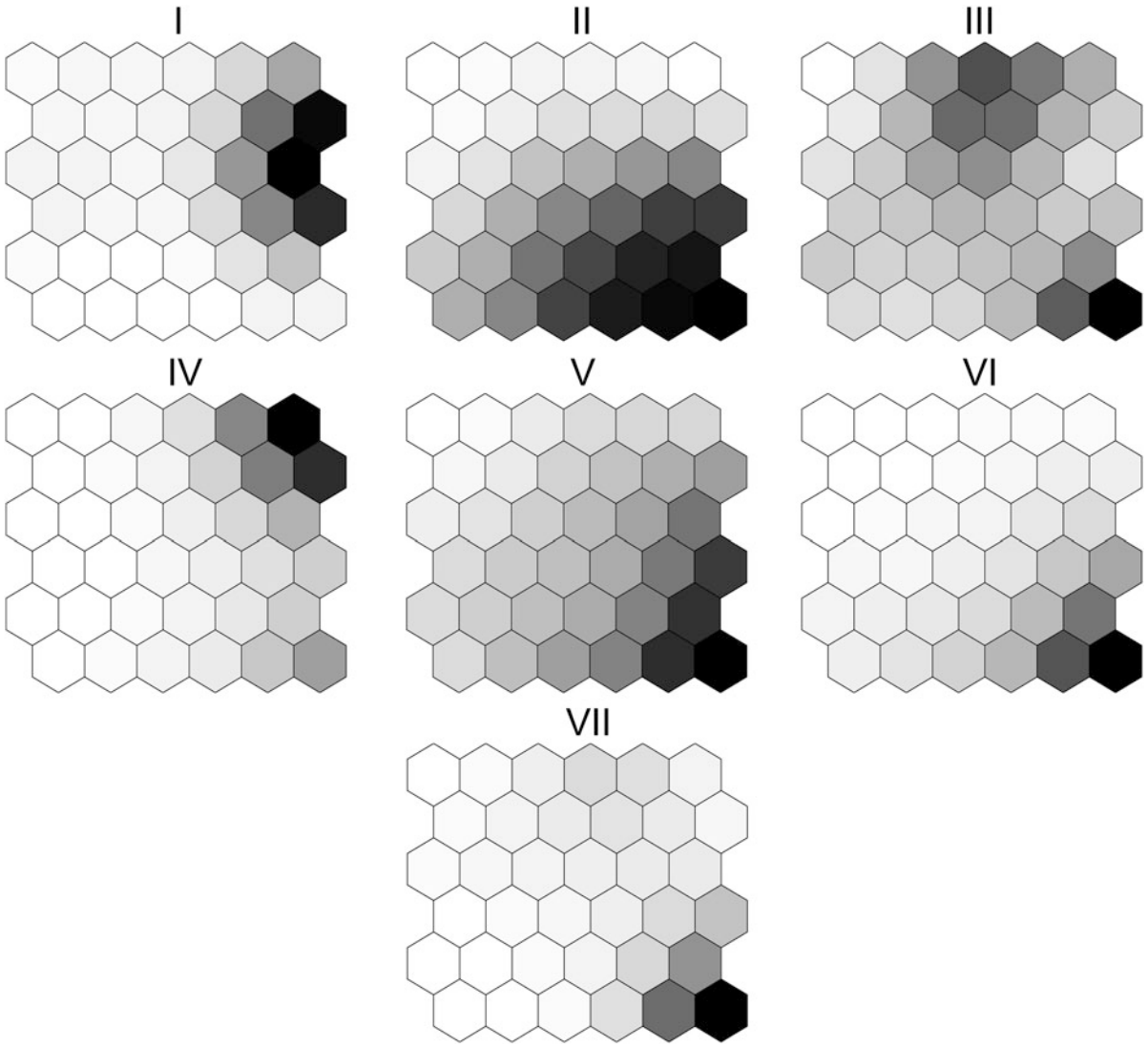

Fig. 8 Component planes of the SOM showing gradient distribution of the morpho-functional traits. *Darker colours* indicate greater biomass of the given functional trait

by Dodds (2006). The concentration of chlorophyll *a* highly varied from oligotrophic to hypertrophic situation with highest values (Dodds, 2006). In such nutrient-rich systems biotic interactions can be important, although phytoplankton biomass is controlled primarily by physical factors (Reynolds, 1988) which was confirmed by our SOM analysis that clearly indicated that in the four potamal rivers TP was not predictor of autotrophic biomass In this study, WRT and TN appeared as the best predictors of chlorophyll *a* and total biomass, in coincidence with Salmaso & Braioni (2008) and Salmaso & Zignin (2010) who asserted that discharge and variables directly linked to water fluxes significantly impacted phytoplankton biomass while nutrients showed occasional influence.

The analysis of the relationship between WRT and algal FGs revealed different behaviours. Not surprisingly, FGs D, J and X1 were strongly associated with high WRT. These groups frequently dominate the phytoplankton of shallow lakes and large, slow-flowing rivers (Reynolds et al., 1994; Schmidt, 1994). The tichoplanktic FGs TB, TD and TC (tichoplanktic Bacillariophyceae, desmids and cyanobacteria) show the opposite tendency (Borics et al.,

2007): these algae dominate rivers of lower order, but during high-discharge periods they can also dominate potamal river phytoplankton, since the habitat zones can shift downstream significantly during such periods. Indeed, this shift can be more than 100 km in the large lowland river Tisza (Uherkovich, 1971). Our data suggest that WRT did not influence the biomass of other FGs.

Abonyi et al. (2012) successfully used FGs for understanding seasonality and longitudinal changes of river phytoplankton and also like Borics et al. (2007) used FGs for water quality assessment. The results of our SOM analysis of MFGs in these four rivers that we obtained and discussed previously in detail were not as clear as those we found for FGs. In our rivers, MFG analysis did not show clear separation of euplanktic and tichoplanktic organisms because as already mentioned, MFGs that we tested are originally developed from lake data. The dominance of diatoms in potamoplankton also does not favour MFGs because they are represented by only one MFGs group and that is group VI, while FGs have fine partition of diatom codons. Thus, we found that the ability of MFGs analysis to reveal characteristics of river phytoplankton tested here for the first time is limited, while FGs were once more confirmed to be good predictor (Devercelli, 2006; Borics et al., 2007; Abonyi et al. 2012).

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PHYTOPLANKTON

Phytoplankton functional groups as indicators of human impacts along the River Loire (France)

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Abstract The phytoplankton functional group concept is successfully used to assess ecological status in lakes (Q index), and also provides a method for lotic ecosystems ($Q_{(r)}$ index). Here, we examine the $Q_{(r)}$ composition metric to demonstrate local to regional scale human effects on natural distribution of phytoplankton along the River Loire. Distribution of phytoplankton functional groups coupled with chemical and physical parameters are described at whole river scale (19 stations, between March and November 2009). Natural longitudinal changes were reflected by the switch from benthic Pennales ($\mathbf{T_B}$) towards meroplanktic greens (**J**) via unicellular centric diatoms (**D/C**). While upstream human pressure was mostly associated to species indicating eutrophic, stagnant environments (coda **P**, **M**, **H1**, **Y**), downstream attenuation of the $Q_{(r)}$ reflected enriched, shallow environments with prolonged residence time (coda **J**, **X2**, **X1**). Occurrence of minimum $Q_{(r)}$ index values were synchronized to late summer, but the longer was the distance from the source, the earlier was the seasonal decrease of $Q_{(r)}$. Increasing downstream co-dominance of codon **F** evidenced an ascending light availability in summer. The longitudinal distribution of functional groups allowed us to conclude that functional diversity might be able to sign human-affected richness, while simply species diversity does not.

Keywords River Loire · Phytoplankton assemblages · Water framework directive · Longitudinal changes · Functional groups · Ecological status index

Introduction

Water quality can be assessed in many ways according to the different metrics and human needs. One of the most relevant concepts of water quality assessment is the Water Framework Directive (Directive 2000/60/EC) of the European Parliament (WFD, 2000). The WFD has been stimulating a large number of researches to establish scientific basis to calibrate its assessment schemes and to define the so-called ecological quality ratios (EQR) (Schimming et al., 2010).

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In order to manage the ecological status of rivers according to the WFD, human effects on rivers must be defined to achieve the good ecological status. Despite the great quantity of available data, some results appear to contradict each other (Bragg et al., 2005). At present, WFD recommends the use of quality assessment based on qualitative and quantitative phytoplankton data in rivers, without specific details. The need for understanding general background mechanisms (Welch, 1952; Vannote et al., 1980; Elwood et al., 1983; Minshall et al., 1985), for understanding phytoplankton-related problems (Dokulil, 1996; Noppe & Prygiel, 1999) and to elaborate useful methods have been emphasised by many articles (Borics et al., 2007; Trifonova et al., 2007; Friedrich & Pohlmann, 2009).

Despite the need for holistic views to understand ecological processes in streams have been forced since the late 50's (Minshall et al., 1985), lotic environments remained less frequently studied. Apart from their stochastic behaviour, difficulties arise on whole river scale (many organisms, several countries, limited accessibility of background data, etc.). Different river concepts (Vannote et al., 1980; Elwood et al. 1983; Thorp & Delong, 1994; Thorp et al., 2006) represent milestones in viewing rivers on ecosystem level, and such approaches are highly required by the WFD.

One of the most recent phytoplankton research interests is the application of the so-called phytoplankton functional groups. In a functional group, ecologically (Reynolds et al., 2002; Padisák et al., 2009), morphologically (Kruk et al., 2010, 2011), or morpho-functionally (Salmaso & Padisák, 2007) similar species are assembled together and they are expected to represent a more or less well-defined functional trait. The usefulness of these concepts (Kruk et al., 2011; Stanković et al., this volume) is being tested.

Traditional phytoplankton monitoring is based on phytoplankton biomass or Chl-*a* (Mischke et al., 2011), in some cases on other accessory photosynthetic pigments or on these combinations (Friedrich & Pohlmann, 2009). As relationships between phytoplankton biomass and human impacts are often difficult to interpret, compositional changes seem to fulfil better the need for understanding these relationships (Walsh et al., 2005). In addition, traditionally monitored variables are not able to reflect species or functional trait level properties, and their quantities are highly conditioned by the age and growth conditions of the populations (Padisák, 2004).

The original idea of the phytoplankton functional group concept (Reynolds et al., 2002; Reynolds, 2006) was proposed as a new ecological status estimation method for lake phytoplankton (Q index—Padisák et al., 2006), then for river potamoplankton ($Q_{(r)}$ index—Borics et al., 2007). The use of this concept in this study relies on the fact, that phytoplankton composition is highly related to physical constraints (Reynolds, 1994; Naselli-Flores & Barone, 2011), and disturbances (Reynolds et al., 1993; Lindenschmidt & Chorus, 1998; Hambright & Zohary, 2000), both altering in time and space. Besides physical factors, trophic state also determines the relevant phytoplankton assemblages, altogether exhibiting quite similar dynamics in rivers and lakes (Reynolds et al., 1994).

The $Q_{(r)}$ index (Borics et al., 2007) is enabled to reflect human impacts at different scales by using specific F factor values for the different functional groups. These factor values were calculated using the following components: (i) nutrient status (from values 0-hypertrophic to 5-oligotrophic), (ii) turbulence (from values 0-standing waters to 5-highly lotic environment), (iii) sufficient time for the development of the given assemblage (from values 0-climax to 5-pioneer assemblages) and (iv) level of risk of functional traits (from values 0-high risk indicating pollution or being able to toxic to 5-low risk). The specified values of each component were summed, and then the F was calculated for each functional groups ranging between 0 and 5. The calculation of $Q_{(r)}$ is the following:

$$Q_{(r)} = \sum_{i=1}^{s} (p_i F),$$

where $p_i = n_i/N$, n_i is the biomass of the ith group, while N is the total biomass. F is the factor number allowing the quality index to range between 0 (the worst) and 5 (the best). The method has been already tested on large rivers of the Hungarian great plain (Duna, Tisza), and the Estonian part of the river Narva (Piirsoo et al., 2010).

In this research, we study the continuity of longitudinal changes of Loire phytoplankton at whole river scale, and are looking for human influences at local- and regional scales. Thus, we examine the phytoplankton composition with the following specific

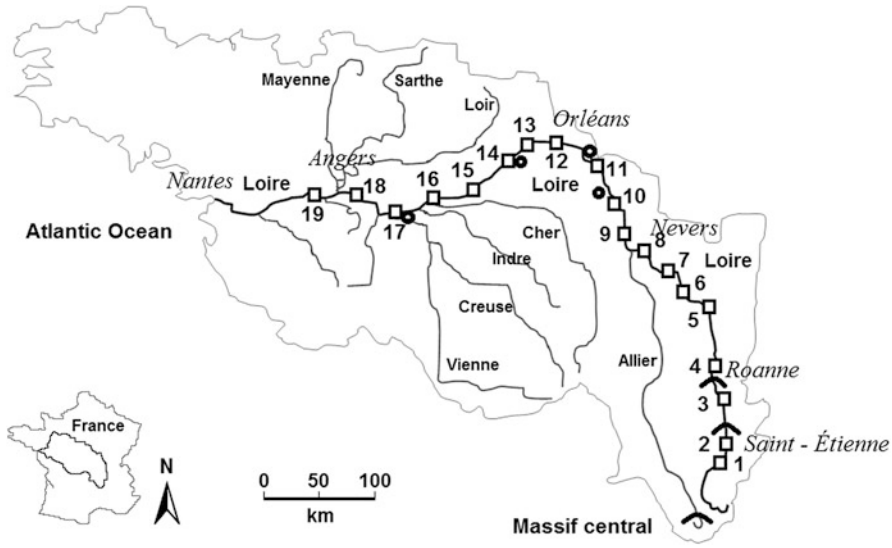

Fig. 1 Sampling stations of the Loire phytoplankton monitoring in 2009. *Semicircles* Dams, *thick circles* nuclear power plants along the River Loire

objectives: (i) which are the dominant functional groups along the River Loire? (ii) How natural is the seasonal succession along the Loire? (iii) Is it possible to define river sections by identifying corresponding phytoplankton patterns along the Loire? (iv) Which kinds of relationships are recognized between $Q_{(r)}$ and species composition, and $Q_{(r)}$ and Shannon–Weaver diversity?

This study is opened towards requirements of the Water Framework Directive (WFD, 2000) and it is also suit for the more and more advised whole river scale investigations of lotic ecosystems (Schimming et al., 2010).

Study area

The River Loire

Among the continental Atlantic rivers, the River Loire has the most extended catchment area (117,045 km^2) covering almost 20% of France. The Loire basin includes many protected areas by Natura 2000 or by the World Heritage of UNESCO, where exceptional landscapes and habitats have been still well preserved. The Loire crosses four ecoregions (two continental and two Atlantic), and covers six hydro-ecoregions, where its hydrological regime is mainly influenced by two main tributaries: the River Allier and the River Cher (Fig. 1). Between them, the Loire flows along a 300-km stretch without major inflow, then at downstream four tributaries increase the mean annual discharge more than twofold within 100 km. Here, anthropic pressure is imposed by towns such as Montluçon, Vierzon, and Bourges on the Cher, Chateauroux on the Indre, Limoges and Chinon on the Vienne. At the town of Angers, River Maine assembles discharge and human impacts of the Mayenne, Sarthe and Loir rivers.

Despite the existence of still natural areas, almost the whole catchment is influenced by humans especially by agriculture (70% of the catchment by grass—or cropland). Upstream of the basin, three dams were built: Grangent (1956) and Villerest (1988) on the River Loire and Naussac (1983) on the River Allier. There are two large cities (>100.000 inhabitants) in these regions: Saint-Etienne near the River Loire and Clermont-Ferrand over the River Allier. Dams mitigate flood peaks and sustain low flows for the functioning of downstream power plants (Oudin et al., 2009). While the middle section of the river is modified by dikes, its lower parts are mostly constrained by canalization constructed for small ship navigation. Besides cities, four nuclear power plants use Loire water (1) at Belleville-sur-Loire: 500 river kilometre (rkm), (2) at Dampierre-en-Burly: 550 rkm, (3) at Saint-Laurent-des-Eaux: 640 rkm and next to the town Avoine (4) Chinon: 793 rkm. Historically, this lower course has also been affected by several anthropogenic impacts such as industries, agriculture and wastewater discharges since centuries (Descy, 2009).

Materials and methods

Loire monitoring in 2009

This Loire monitoring was conducted by the Loire-Bretagne Water Authority. The number of the stations has been extended from the initial number 6 (in the 1990s) to 19 by 2008. The sampling stations were laid down between Malvalette and Montjean towns, without sampling the source and the lowermost river section. While sources normally lack phytoplankton (Reynolds & Descy, 1996), the lowermost section is excluded from the monitoring because of the tidal influence of the Atlantic Ocean. For simplifying this study, the station names are reduced to the corresponding station number along the river: (1) Malvalette: 145 river kilometre (rkm), (2) Saint Just Saint Rambert: 176 rkm, (3) Balbigny: 223 rkm, (4) Villerest: 258 rkm, (5) La Motte Saint Jean: 336 rkm, (6) Bourbon Lancy: 372 rkm, (7) Decize: 412 rkm, (8) Nevers: 448 rkm, (9) Fourchambault: 461 rkm, (10) Saint Satur: 506 rkm, (11) Gien: 555 rkm, (12) Jargeau: 609 rkm, (13) Meung sur Loire: 648 rkm, (14) Muides sur Loire: 672 rkm, (15) Chaumont: 707 rkm, (16) Villandry: 766 rkm, (17) Chouzé sur Loire: 794 rkm, (18) Saint Mathurin sur Loire: 840 rkm, (19) Montjean: 885 rkm.

Physical and chemical parameters

The main studied variables are discharge, water temperature, conductivity, pH, suspended solids, DO, DO(%), organic carbon, Kjeldahl-N and phytoplankton nutrients such as ammonium, nitrite, nitrate, soluble reactive phosphorus, total phosphorus and soluble reactive silica. Sampling frequency of these parameters in 2009 was once a month between January and December, only discharge data were based on daily measurements, here presented also as monthly averages. All of the physical and chemical data were available online at pages 'OSUR—http://osur.eau-loire-bretagne.fr/exportosur/Accueil' and 'Banque Hydro—http://www.hydro.eaufrance.fr/'.

Phytoplankton data and statistical analyses

Phytoplankton sampling frequency was once a month between March and November, 2009. Samples were taken from the middle of the water course using a bucket. Phytoplankton samples were fixed in situ with Lugol solution, and then overall 170 samples were sent to the Bi-Eau consultancy for phytoplankton analyses.

Phytoplankton samples were counted using the inverted microscope method (Utermöhl, 1958) counting 400 settling units per sample according to Lund et al. (1958). Phytoplankton biomass was estimated using specific biovolumes obtained by geometrical approximations based on Loire populations. In this standard monitoring system, biomass calculation includes only taxa representing >1% in the counts. Phytoplankton species were identified according to Ettl et al. (1978), Ettl et al. (1985), Fott (1968), Geitler (1930–1932), Huber-Pestalozzi (1955), Komárek & Anagnostidis (1999), Komárek & Anagnostidis (2005), Komárek & Fott (1983), Popovský & Pfiester (1990), Starmach (1985). In cases of diatom dominance, permanent slides were prepared, using the European standard method (Comité Européen de Normalisation (CEN) 2003). Diatoms were determined using manuals by Krammer (2002), Krammer & Lange-Bertalot (1986), Krammer & Lange-Bertalot (1988), Krammer & Lange-Bertalot (1991a, b) and Lange-Bertalot (2001).

For phytoplankton functional group classification, Reynolds et al. (2002), Padisák et al. (2009) and Borics et al. (2007) were used, and the ecological status estimations were calculated using F factor values proposed by Borics et al. (2007).

Hierarchical cluster analysis was performed with the Syntax 2000 software (Podani, 1988) with Bray–Curtis dissimilarity indices and the UPGMA fusion algorithm. Phytoplankton diversity was calculated following Shannon and Weaver (Shannon & Weaver, 1949 in Pielou, 1975), based on all counted individuals, using $\log_2$ and including benthic diatoms. Variables of phytoplankton and chemical data were visualized on 1-year scale using the Surfer 6 program.

Results

Physical and chemical variables

Discharge values showed nival drainage regime with discharge maxima in February (Fig. 2a, Image 2a in Supplementary material). While upstream stations showed sometimes uneven discharge fluctuations, between the two main confluents (River Allier and

River Cher) discharge remained more or less constant in all seasons. Among in situ measurements, water temperature showed similar seasonal patterns along the whole Loire length, with a slightly prolonged spring cold period upstream. Maximum temperature levels were observed in late summer, where temperature maxima (up to 26°C) occurred at stations 3 and between stations 11 and 16. The pH values increased from the middle section to downstream between April and October. Conductivity showed increasing values both downstream and seasonally, with maxima between 200 and 400 μS cm^{-1} (Fig. 2b, Images 2b, c in Supplementary material). In the middle section of the Loire (stations 11–16), dissolved oxygen reached supersaturated levels in summer (up to 170%).

Soluble reactive phosphorus and total-P (station 3), ammonium (between stations 2 and 4) and nitrite (stations 3–4) were higher at upstream stations. Values of organic carbon were low during spring and autumn at the downstream sections of the river, together with decreased suspended solid values. Nitrate showed significant increase downstream, with higher values in spring and autumn. Local increases in nitrate occurred at stations 10 and 17. Amount of soluble reactive silica was low between stations 5–8 and 10–18 during spring, but absolute minimum values occurred upstream between stations 2 and 8 (<2 mg l^{-1}) in late summer (Fig. 2c, Images 3–6 in Supplementary material).

Phytoplankton biomass and diversity

Most of the samples involved in this study were characterized by low biomass (Fig. 3a) and were mainly dominated by species belonging to diatoms, chlorococcalean green algae and Cyanobacteria. The total species number exceeded 300, of which the most abundant 161 taxa were converted to biomass (see Spreadsheet in Supplementary material), then classified into 23 different coda. The most frequent coda were **D** (37%), **J** (28%), $\mathbf{T_B}$ (11%), **C** (7%) and **B** (4%). The most species rich functional groups were $\mathbf{T_B}$ (39 spp.), **J** (25 spp.), **D** (15 spp.), **F** (15 spp.) and **X1** (14 spp.).

Shannon–Weaver diversity showed relatively high values in general (>80% between 2.8–5.0 bits ind^{-1}), but reflected considerable seasonal and longitudinal differences (Fig. 3b). Minimum values of Shannon–Weaver diversity occurred in spring with some exceptions like upstream stations 2 and 4 and downstream at station 17. Among patchiness of high diversity values, a late summer maxima between stations 5 and 8, and a midsummer maxima between stations 15 and 18 were observed. Species number (from 22 to 72) was highly related ($R^2 = 0.45$, $n = 170$) with Shannon–Weaver diversity (1.28–5.37).

Seasonality in phytoplankton coda distribution

Benthic diatoms ($\mathbf{T_B}$) were present during all seasons only at upstream stretches (*Nitzschia* spp., *Navicula* spp. and *Fragilaria construens* were the most frequent), while their distribution at other stations was mostly concentrated to spring and fall. At stations 9–10, they were highly represented even during summer. At station 4 (Villerest), a biomass peak was formed by *Melosira varians* ($\mathbf{T_B}$) in May with a biomass of 10,865 μg l^{-1}, then this species was replaced by coda **P** and **M**, in summer and autumn. The absolute biomass maximum (17,621 μg l^{-1}) was also found in May at the middle section of the Loire (station 12), with the significant contribution of *Cyclotella meneghiniana* (**C**), *Cyclostephanos dubius* (**B**) and *Skeletonema potamos* (**D**). Centric diatoms were dominant in spring, being well represented at all sampling stations (Fig. 4a–c), but exhibiting a mismatch at stations 2 and 4. Here, *Fragilaria crotonensis* (**P**) was dominant from May to August, where its contribution to the total biomass exceeded 50% around the year.

Chlorococcalean algae played a key role in summer from station 11 towards downstream (Fig. 4d–g). Most of them were belonged to coda **J** (*Scenedesmus* group *Armati* and group *Desmodesmus*), **F** (*Dichotomococcus curvatus*, *Dictyosphaerium* spp., *Crucigeniella* spp., *Kirchneriella* spp., *Oocystis* spp.) and **X1** (*Ankyra judayi*, *Didymocystis* spp., *Diplochloris* spp., *Monoraphidium* spp.). Members of codon **X2** (*Chlamydomonas* spp., *Plagioselmis* spp., *Spermatozopsis exsultans*) appeared only late spring and summer. At the downstream sections, coda **J** and **X1** showed a clear emergence between May and August (Fig. 4c–f), with the co-dominance of codon **F** (especially in June and July).

Cyanobacteria were only occasionally dominant in biomass, but well represented by species such as

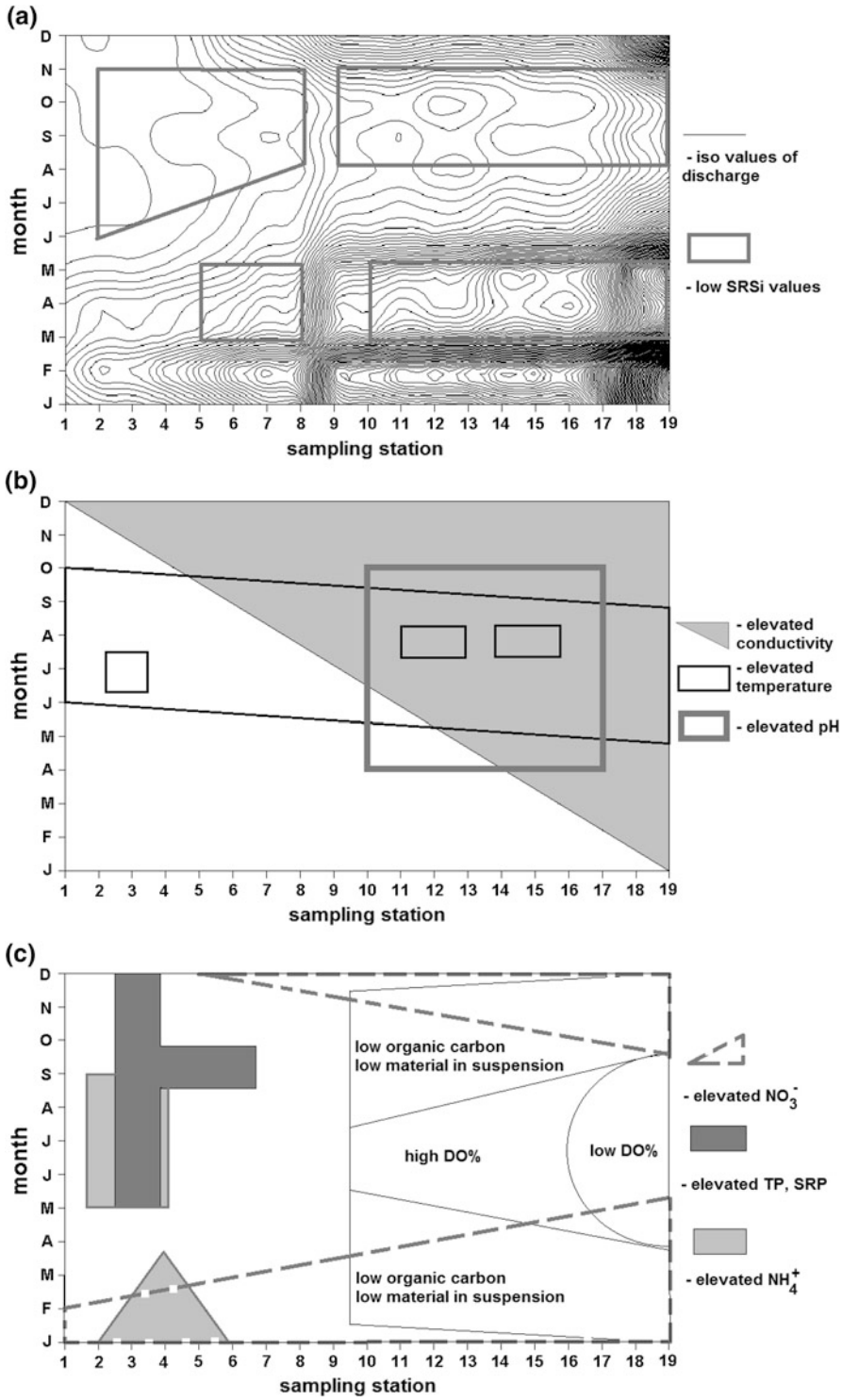

Fig. 2 Patchiness of **a** iso-values of discharge and low soluble reactive silica, **b** temperature, conductivity and pH, **c** nitrate, total-P, soluble reactive phosphorus, ammonium, organic carbon, dissolved oxygen saturation in the River Loire in 2009 (for more detailed information, see Images 3, 4, 5, 6 in Supplementary material)

(i) *Microcystis* spp. (**M**) between stations 2–4 and 7–8 late summer; (ii) a not yet identified Stigonematales sp., ($\mathbf{T_C}$) at station 2 in September and (iii) *Dolichospermum spiroides* (**H1**) and *Planktothrix agardhii* (**S1**) at station 7 in August (Fig. 4e–f).

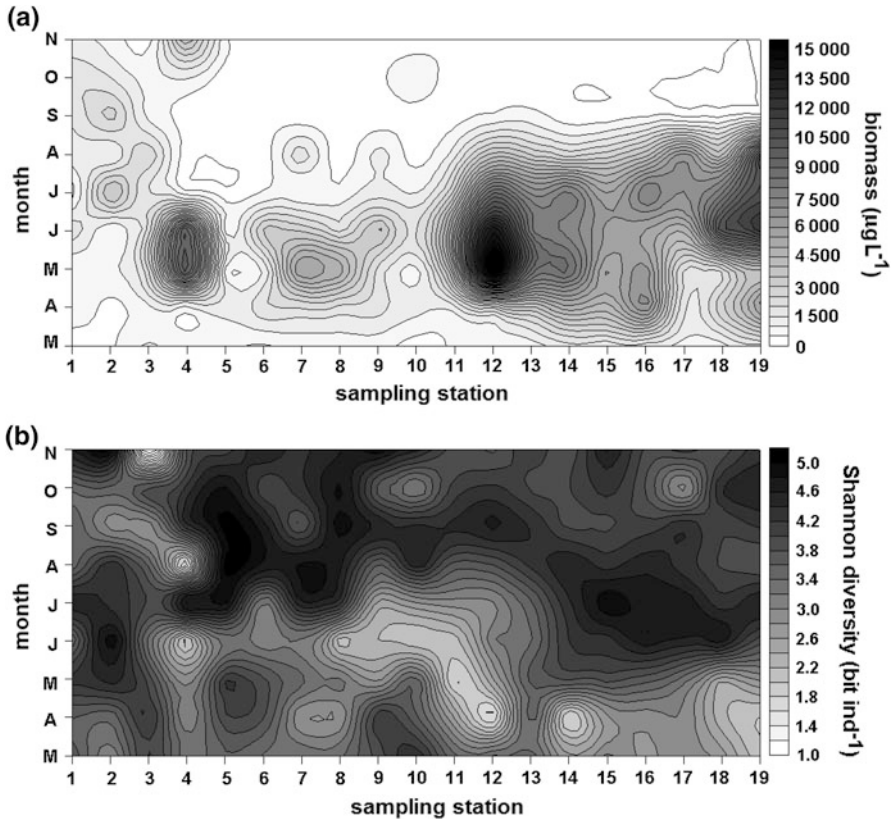

Fig. 3 Distribution of **a** total phytoplankton biomass, **b** Shannon–Weaver diversity in the River Loire in 2009

Longitudinal patterns by functional groups

While the distribution of coda $\mathbf{T_B}$, the sum of the centric diatoms (**B** + **C** + **D**) and **J** almost covered (around 90%) the whole the study period at almost all of the stations, these three groups showed different longitudinal patterns. Codon $\mathbf{T_B}$ decreased downstream with two peaks: stations 4 and 10. Centric diatoms were present at all stations, being dominant between stations 6 and 14. Codon **J** increased its contribution continuously towards downstream (Fig. 5a).

'Accessory' coda (around 10%) showed two markedly different distribution patterns upstream versus downstream. While they were represented by a few numbers of taxa belonging to many different functional groups upstream (Fig. 5b), from the station 10 downstream, their patchiness was designed by the fluctuations of only three functional groups: **X1**, **X2** and **F**. From the middle section of the River Loire, the contribution of coda **X1** and **F** increased on the account of codon **X2**. Though being characteristic at downstream stations, the above-mentioned coda had some sporadic occurrences at upstream stations as well. In the distribution profile of life forms along the river (Fig. 5c), planktonic algae were dominant with the exceptions presented at stations 4–5 and 9–10. Meroplanktic species increased their quantity towards downstream, especially below the inflow of the River Allier.

Cluster analysis of the main phytoplankton nutrients (Fig. 6a) resulted in four groups (and a singlet: station 10) at dissimilarity level of 0.15. Stations 1, 9 and 10 were separated, the group of stations 2–6, 7–18 were together, with the exception of stations 17 and 19.

Distribution of $Q_{(r)}$ values

Ecological status estimation based on Loire phytoplankton biomass for the year 2009 is shown in Fig. 7a. High $Q_{(r)}$ values were observed during spring and autumn, latter particularly in the middle section of the river. Low values characterized the late summer periods upstream (stations 2–4), and the summer periods downstream (station 12–19). Minimum values

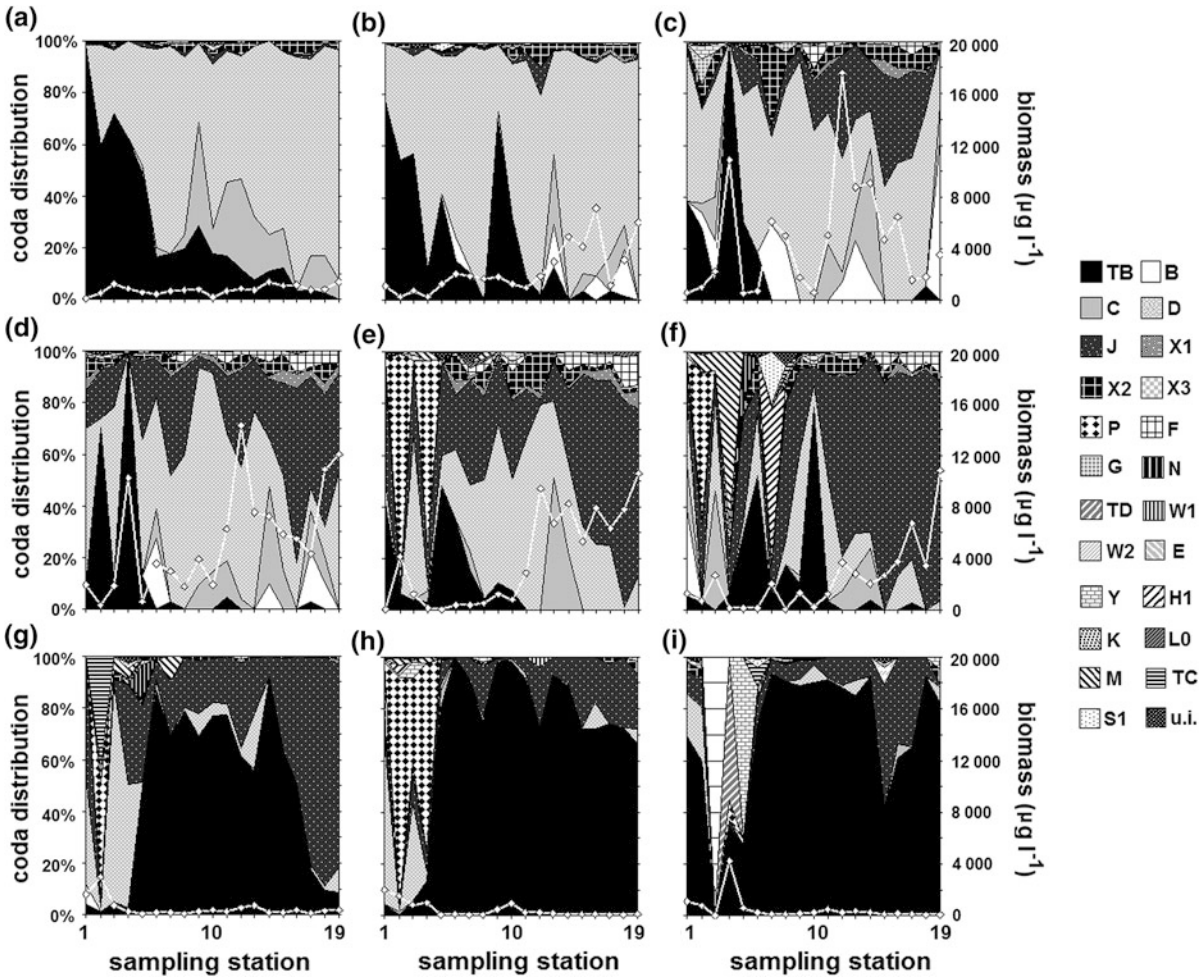

Fig. 4 Relative biomass (%) of phytoplankton functional groups (sensu Reynolds et al., 2002; Borics et al., 2007; Padisák et al., 2009) from **a** March to **i** November in 2009 in the River Loire. *Horizontal axes* Sampling stations along the river from stations 1 to 19. *White lines* Total biomass values using identical scale during the 9-month period

occurred in August at stations 4 ($Q_{(r)} = 0.82$) and 7 ($Q_{(r)} = 1.12$). At whole river scale, the longer is the distance of stations from the source, the earlier is the seasonal decrease of $Q_{(r)}$.

Average $Q_{(r)}$ index values varied around 4 at upstream and in the middle section of the River Loire, approximating value 3 downstreams. A clear decrease occurred at stations 2, 4 and 7, which was more markedly expressed in case of minimum index values (Fig. 7b). In both cases, index values showed progressive decrease from the station 10 downstreams. Based on the cluster analysis of $Q_{(r)}$ index values, four main groups were formed at dissimilarity level of 0.2 (Fig. 6b). Individual stations were 3, 4, 7; but stations 1–2, 5–14 and 15–19 were grouped together.

Discussion

Spatial gradients

Upstream sections of rivers have slight seasonality (Vannote et al., 1980), and the prevailing constant conditions select for algae belonging to benthic taxa (Leitão & Lepretre, 1998; Leland, 2003; Istvánovics et al., 2010). The more the sampling station is placed downstream, the more the seasonality overcomes. Changes in the river topography also require functional adaptations resulting zones versus continuums by the best suited biota (Huet, 1959; Vannote et al., 1980). In long rivers (like the River Loire), source area is relatively small, and the catchment area increases by

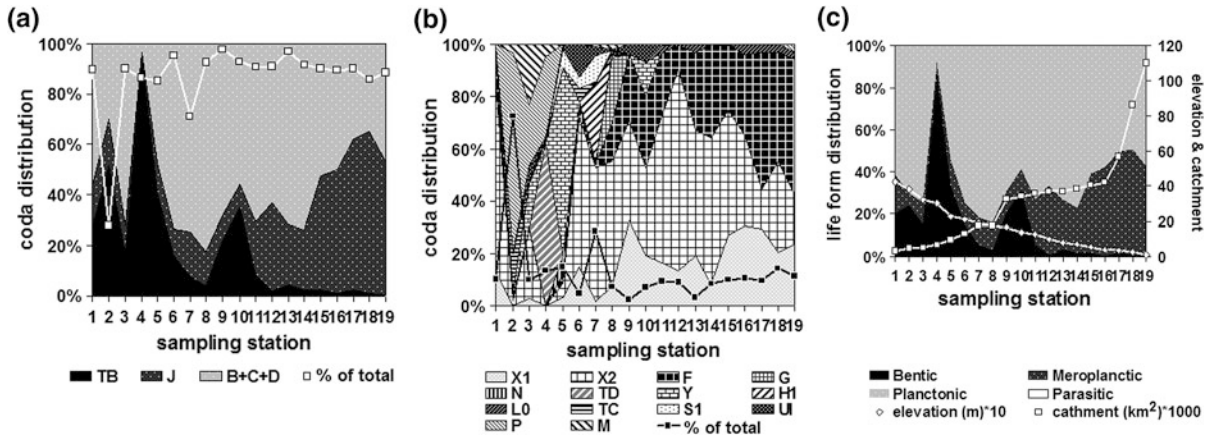

Fig. 5 Distribution of **a** coda $\mathbf{T_B}$, $\mathbf{B + C + D}$ and **J** along sampling station in 2009, and their relative contribution to the total accumulated biomass (*white line*), **b** distribution of 'accessory coda' (UI means unidentified) and their contribution to the total accumulated biomass (*black line*), **c** biomass distribution of algae belonged to different life forms, elevation level and water catchment area along the River Loire in 2009

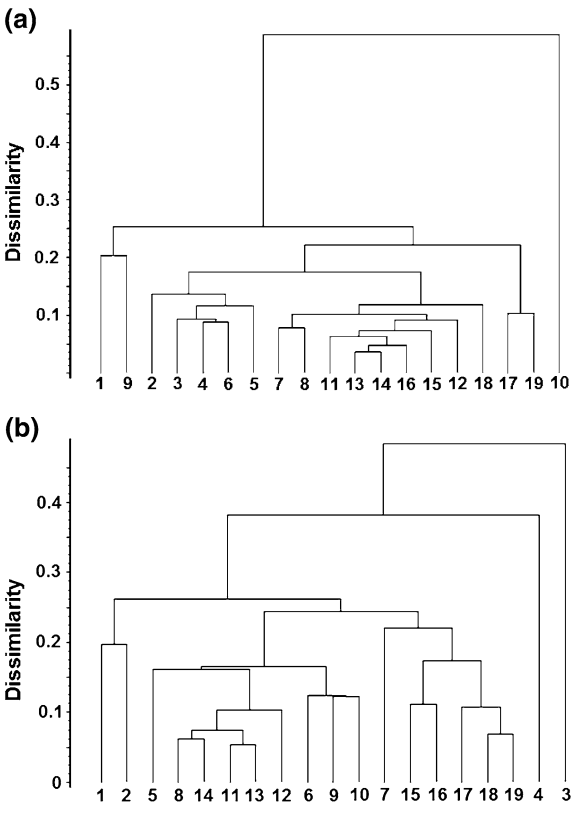

Fig. 6 Cluster analysis of the sampling stations **a** based on phytoplankton nutrients (N -, P-forms, organic carbon and soluble reactive silica), **b** based on $Q_{(r)}$ values

orders (Billen et al., 1994) providing evidence for longitudinal differences. Longitudinal succession of phytoplankton is redrawn by inflowing tributaries (Garnier et al., 1995; Istvánovics et al., 2010), by natural dead zones (Reynolds et al., 1991) or by human modifications on the river bed (dikes, reservoirs, flow modifications, stone disposal). Based on residence time, nutrient availability and light conditions, the maximum phytoplankton production occurs at middle sections of rivers (Reynolds & Descy, 1996) where phytoplankton is mainly dominated by centrics (Leitão & Lepretre, 1998; Bahnwart et al., 1999; Leland, 2003; Piirsoo et al. 2008; Istvánovics et al., 2010). Downstream sections of different rivers are variable in the potamoplankton, dominated by centric diatoms, chlorococcalean colonial greens (Leitão & Lepretre, 1998; Bahnwart et al., 1999; Friedrich & Pohlmann, 2009; Tavernini et al., 2011), euglenophytes, cryptophytes (Leland, 2003; Bahnwart et al., 1999), colonial Cyanobacteria (Ibelings et al., 1998), chrysophytes (Istvánovics et al., 2010), all depending on season and site location.

The above longitudinal considerations serve as background data to understand spatial phytoplankton distribution along the River Loire. As $Q_{(r)}$ index is based on biomass data, and most of the samples are dominated by only a few coda, it is interesting to show the coda distribution of the most frequent taxa. In Fig. 8, the dominance of coda $\mathbf{T_B}$, **D** and **J** is reflected, where other type of dominances (**P**, **M**, **H1**) occur occasionally, but pre-indicating decreases in $Q_{(r)}$ values.

Independently of sampling location, the lowest (worst) $Q_{(r)}$ values (circles in Fig. 8) occurred in late summer, but different river stretches are identifiable:

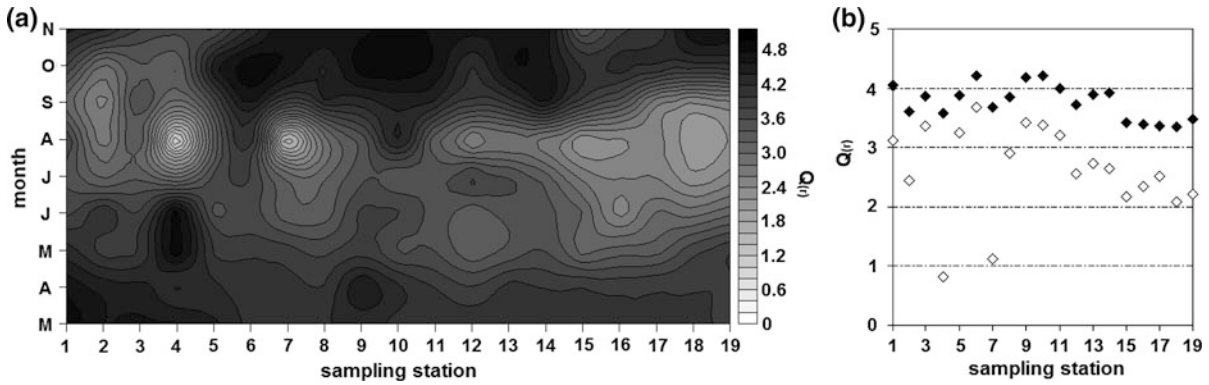

Fig. 7 **a** Seasonal and longitudinal differences of the $Q_{(r)}$ index in 2009, **b** average (*full diamond*) and minimum (*open diamond*) values of $Q_{(r)}$ index along the Loire in 2009

(i) upstream section of the Loire until Villerest dam (stations 1–4), which is dominated by benthic and centric unicellular diatoms which were replaced by lacustrine species (**P**, **M**) only in late summer;
(ii) after Villerest dam, where centric dominance was replaced by codon **J** only in late summer (stations 5–14, excepted station 7), and dominated by benthic taxa autumn;
(iii) from the station 15 downstream, where the dominance of codon **J** on centrics appeared earlier, and stayed longer before changing to codon $\mathbf{T_B}$.

All taxa, contributing to this patchiness are common (Rojo et al., 1994; Reynolds & Descy, 1996) and are in agreement with dominance patterns described in the aforementioned publications. Exceptions are *Fragilaria crotonensis* (**P**), *Microcystis* spp. (**M**) and *Dolichospermum spiroides* (**H1**) in the upper part of the River Loire.

Temporal gradients

Seasonally changing parameters such as discharge (Schmidt, 1994; Salmaso & Zignin, 2010; Centis et al., 2010; Tavernini et al., 2011), water temperature (Leland, 2003; Salmaso & Braioni, 2008; Tavernini et al., 2011), light (Vörös et al., 2000) and nutrient availability (Wu et al., 2011) have been still in the focus of recent scientific research. In rivers, seasonality basically determines which species are able to maintain their population, selecting the most capable to dominate. While weak selective physical conditions may explain highly variable planktonic vegetation (Reynolds & Descy, 1996), low species diversity may reflect severely selective environments.

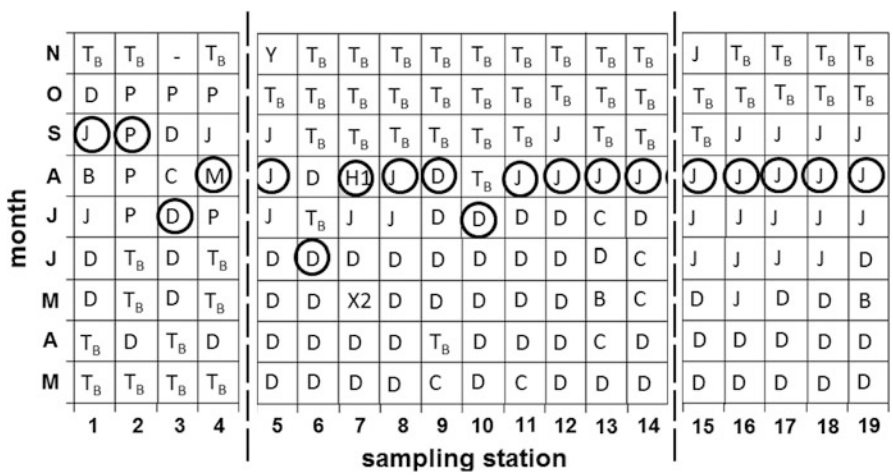

Fig. 8 Coda distribution of the first species in biomass, where *circles* indicate the minimum values of $Q_{(r)}$ index along the River Loire in 2009

Using the above-mentioned consideration, Fig. 9 visualizes the coda patchiness provided by the most frequent species in the samples. Similar to Fig. 8, coda **T_B**, **D** and **J** are frequent, but additional functional groups (coloured by grey background) occur: **X1** (*Monoraphidium*), UI (unidentified *Chlorella*-like small greens or isolated cells of *Dictyosphaerium* belonging to codon **F**) and **X2** (*Chlamydomonas* spp., *Spermatozopsis exsultans*).

Using this coda patchiness of the most abundant species, the three investigated seasons can be characterized by three different coda distributions:

(i) Spring is almost invariably dominated by centric diatoms along the whole river length;
(ii) In summer, the Loire is divided into three parts: an upstream section with mixed coda distribution, including both benthic (*Melosira varians*) and planktonic (*Microcystis* spp.) species dominance, followed by a middle section dominated by centric diatoms, and then a downstream section with the co-dominance of codon **X1** (*Monoraphidium*) and small greens (**X1/F**) accompanying codon **J**;
(iii) In late summer, centrics versus *Microcystis* dominance occur upstreams, benthic diatoms in the middle sections, and the dominance of codon **X2** (*Spermatozopsis exsultans*), and **T_B** downstreams.

The minimum diversity values (circles in Fig. 9) change continuously among seasons along the Loire: they appear in late summer in the upper parts, and at the beginning of spring at the lower parts. Among these dominant taxa, only the centrics are reported commonly (see Table 3 in Rojo et al., 1994).

Functional sections and human impacts

In the River Loire in 2009, benthic algae were more likely to dominate upstream, planktonic centrics in the middle part, and meroplanktic or metaphytic species downstream. Latter two life forms are described as strategies being fundamental for maintaining fluvial phytoplankton diversity (Stoyneva, 1994; Reynolds & Descy, 1996).

One of the most apparent human effect reflected by coda distribution is the influence of eutrophic reservoirs (Bonnet & Poulin, 2002; Latour et al., 2004; Briand et al., 2009) constructed in the upper part of the river. Dams modify the flowing regime, water residence time, nutrient distributions and light conditions (Hart et al., 2002; Palau, 2006), and therefore the seasonal succession of phytoplankton and species distribution. These can be identified on the Loire by the eutrophic, epilimnetic coda (**P**, **M**) resulting in lake type equilibrium assemblages (Naselli-Flores et al., 2003) in this area. The human controlled outlets of dams are reflected in the sporadic occurrence of lacustrine elements downstream: coda **M**, **P**, **Y**, **L_0**, and by the uneven quantitative dominance of benthic (flashed *Melosira varians* with single cells) species. Despite the presence of the planktonic elements, they cannot maintain persistent dominance downstreams, as they are not adapted to survive in lotic environments (Reynolds & Descy, 1996), but are able to enrich river phytoplankton with species in additional habitats. This

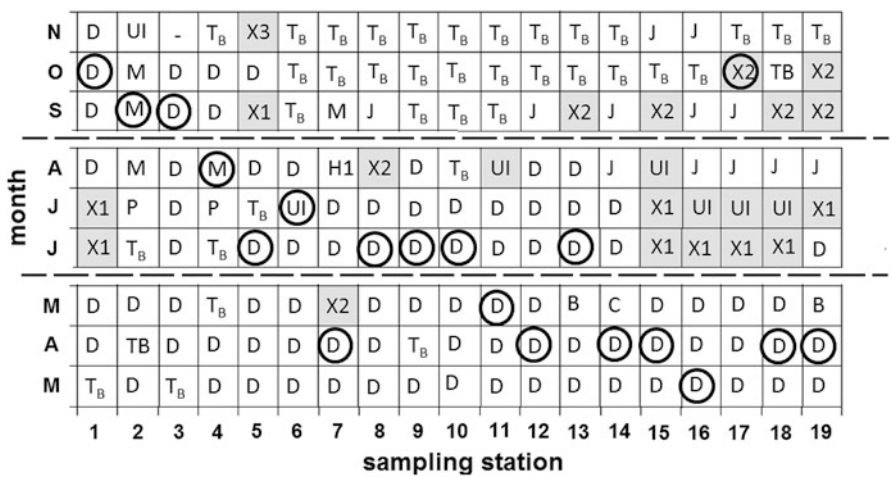

Fig. 9 Coda distribution of the most abundant species, where *circles* indicate the minimum values of Shannon–Weaver diversity (UI means unidentified single greens of 2–5 μm) in the River Loire in 2009

was also the case in the River Narva, sampled after the Narva Reservoir (Piirsoo et al., 2010).

This lake type succession is well reflected by the $Q_{(r)}$ index, emphasizing the lack of benthic diatoms dominance which is considered as natural in upstream sections of rivers. The upstream uneven distribution of physical and chemical components may also provide an example for effects of reservoirs in this part of the river. For example, very low soluble reactive silica concentrations (<2 mg l^{-1}) might be related to prolonged residence time, and to the dominance of epilimnetic, eutrophic diatom species like *Fragilaria crotonensis* (codon **P**). It is interesting to note that in spring at downstream sections, centric diatom maxima failed to result such a remarkable decrease (Image 6b in Supplementary material). Controlled outlets from the reservoirs are also apparent in the distribution pattern of the water temperature, soluble reactive phosphorus, nitrite and discharge values (Images 2, 4, 5 in Supplementary material). At Villerest dam (station 4) for example, two types of outflow work: an upper outflow between May and July, which then changed to the underneath one between August and April. This lower outflow is positioned at 8 m on the overall 24-m-high dam, which allows emission of hypolimnetic water in late summers.

The middle part of the river is characterized by high $Q_{(r)}$ index values, reflecting the presence of benthic diatoms during high flow and the dominance of centrics all around the year. The spring centric dominances emphasize their resistance against this highly selective environment (Margalef, 1978) that favours species with low-light tolerance and fast growth (Reynolds, 1994; Reynolds & Descy, 1996). This can be attributed to the natural elevating effect of the River Allier on discharge. This section of the Loire can be compared to other large rivers of Europe, as the 'Danube type' phytoplankton (Várbíró et al., 2007) dominated by coda **J**: *Scenedesmus* spp., **C**: *Cyclotella meneghiniana*, **D**: *Nitzschia acicularis*, *Skeletonema potamos*, *Stephanodiscus hantzschii*, that almost covers the main species occurring in the Loire.

The downstream increase of nitrate is a common human impact by agriculture (Strebel et al., 1989; Almasri & Kaluarachchi, 2004), and serves as a useful indicator of eutrophication in large rivers (Turner et al., 2003). The eutrophication in this middle part of the River Loire was demonstrated in the 1990s with elevated levels of Chl-*a* (Meybeck et al., 2003) up to 150 μg l^{-1} (the maximum in 2009 was around 90 μg l^{-1}). Besides the elevated level of nitrate, total phosphorus showed rather low values during spring at the whole river length, and also at the end of summer downstreams. In these cases, concentrations can be considered as background values (<0.05 mgP l^{-1}), as it was suggested for large German rivers (Mischke et al., 2011). The amount of total phosphorus is low enough for limiting algal growth (Descy et al., 2011), as it was recently concluded similarly in the downstream sections of the River Danube (Istvánovics & Honti, 2011).

The downstream decreasing $Q_{(r)}$ values reflected the increasing amount of codon **J**, indicating a switch in the primary energy source, as predicted by earlier studies (Borics et al., 2007). Besides the biomass dominance of codon **J** at the lower parts of the Loire, the change from codon **X2** to codon **X1** downstream also indicates an elevating trophic level. The uneven quantitative dominance of the volvocalean *Spermatozopsis exsultans* may require different assumptions (i) this species is able to reflect high organic content (Várbíró et al., 2007), (ii) may reflect uncommon environment, being dominant during downstream summer slow flow, tolerating very high light availability or (iii) as it was observed in all Shannon–Weaver diversity maxima (66 species at station 5: August, station 7: July, station 15: July), suggests evidence for some human-induced species addition independently of river stretch. These longitudinal changes can be explained by low discharge (Image 2a in Supplementary material) and the prolonged residence time, reflected also by higher Kjeldahl-N in some cases (Image 5a in Supplementary material). An increasing downstream light availability was reflected by the increasing dominance of codon **F**, showing underwater light changes, which may influence the longitudinal switch between centric diatoms and green algae as well.

Using these results, the River Loire can be characterized in 2009 by the following river stretches (Fig. 10):

(i) Upper section (stations 1–4) reflects natural features by the presence of benthic diatoms dominance in spring, but with strong human impacts by dams (station 2: Grangent and station 4: Villerest), resulting in lake type

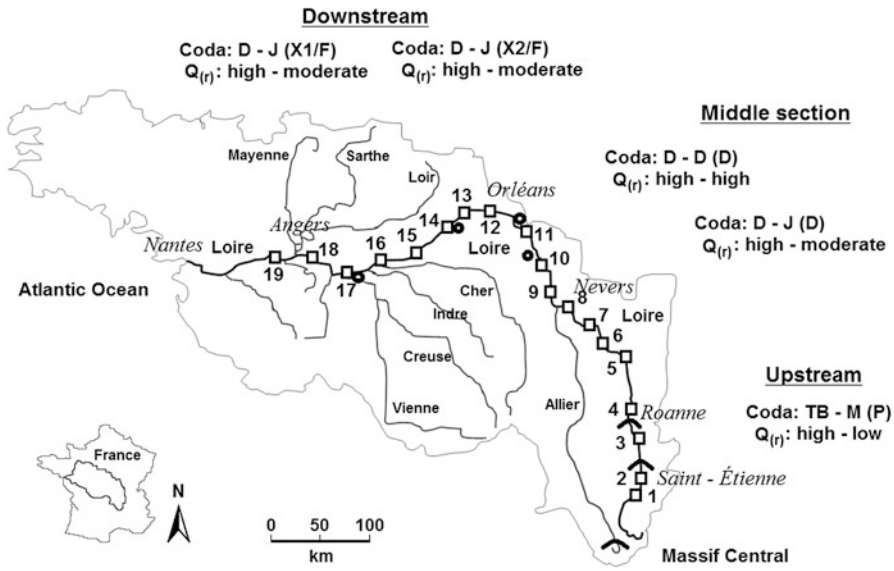

Fig. 10 Coda patchiness [spring–late summer (intermediate phase)] versus $Q_{(r)}$ index values along the River Loire in 2009

succession with eutrophic, epilimnetic cyanobacterial 'climax';

(ii) Between Villerest dam, and the River Allier inflow, stations 5–8 represent an intermediate and functionally diverse river stretch, influenced by both natural and human impacts;

(iii) After the confluence of the River Allier, the Loire shows a prolonged dominance of centrics (stations 9–11), reflecting more permanent physical conditions by discharge;

(iv) Further downstream, the plankton is more and more enriched by meroplanktic taxa (stations 12–16), including species thought to indicate elevated trophic levels and a prolonged residence time. Despite the high species diversity values observed in summer, phytoplankton functional classification does not allow us to identify a functionally diverse river section here;

(v) The downstream section (stations 17–19) of the River Loire does not separate from its upper part, but has an increased light availability during summer owing to slow flow velocity and low discharge effects. The increasing population density of invasive Asian clams (*Corbicula* spp.—Mollusca, Bivalvia, Corbiculidae) in the Loire (Brancotte & Vincent, 2002; Chovet & Lécureuil, 2008) is supposed to affect quantitatively the phytoplankton by grazing (Descy et al., 2011), but their presence may also influence the phytoplankton composition as well.

This Loire survey, based on the phytoplankton functional group concept, can be used to obtain comprehensive information on ecological status differences along this Atlantic river, providing an example for 'phytoplankton response to human impact at different scales'.

Final notes and methodological remarks

We need to call the reader's attention, that $Q_{(r)}$, as it is based on relative biomass data, provides quality values considering neither total biomass nor Shannon–Weaver diversity. As it was found in the Loire, species diversity can be affected by human impacts in some cases, providing additional species via additional habitat sources. For a better consideration of species richness, functional diversity might serve as a good tool.

As the method is based on the phytoplankton functional group concept, a possible worldwide application depends only on our knowledge about their usability in different climate regions, which could be a subject for further research for example in tropical zones, similarly to Q index (Crossetti & Bicudo, 2008; Becker et al., 2009).

An unfortunate feature of the method is that it does not separate between natural versus human-affected benthic diatoms dominance, and does not penalize invasive (*Achnanthidium catenata*, *Encyonema triangulum*—Coste & Ector, 2000) or brackish species

(*Actinocyclus normanii*, *Bacillaria paxillifera*) existing also in the River Loire.

A final observation is the difficulty of the classification between coda **B–C–D** in monitoring systems, where it is impossible to identify all the centric diatoms at species level. Even if size fractionating might be a tool (Mischke, 2007), coda classification can be biased by overlapping size dimensions of species.

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PHYTOPLANKTON

Functional groups of phytoplankton shaping diversity of shallow lake ecosystems

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Abstract Phytoplankton of eutrophic shallow lakes are frequently dominated by one species or species of the same functional group, resulting in species-pure algal assemblages. Knowledge of the structure of these assemblages is essential to understand their functioning; therefore, species and functional diversity were investigated in five sub-types of eutrophic shallow lake. Among the sub-types, astatic saline lakes and hypertrophic ponds had type-specific assemblages dominated by S_N and W0, W1 codons. The diversity of the phytoplankton in the sub-types was quite similar, except for the astatic saline lakes, which were characterised by lower values of both functional and species diversity. We found that both functional and species diversity were low when bloom-forming cyanobacteria (H1, S_N functional groups) became dominant. Dominance of other groups (J, Y, L_O and W1) did not coincide with decrease in species diversity. Analysis of the biovolume versus diversity relationships revealed that decrease in diversity might be expected at biovolume >20 $mm^3\ l^{-1}$ for shallow lakes.

Keywords Shallow lakes · Algal assemblages · Species diversity · Functional diversity

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Introduction

Due to geomorphology and climatic conditions, there are no oligotrophic deep lakes in the lowland area of the Carpathian Basin. In this region, eutrophic shallow lakes are typical. As a result of scientific achievements in recent years, much has been learned about the operation of these systems (Scheffer, 1998). A key result in shallow lake ecology is that such ecosystems have two alternative stable equilibria, e.g. macrophyte-dominated versus turbid state (Scheffer et al., 1993). This helps to understand the operation of the systems and provides theoretical background for lake restoration (Drenner & Hambright, 2002). It is now recognised that various alternative regimes might exist depending on lake depth and size, climate or nutrients (Scheffer & Carpenter, 2003; Scheffer & Van Nes, 2007). Krasznai et al. (2010) demonstrated that macrophyte dominance in shallow oxbow lakes does not necessarily result in clear water state, because dense

algal populations can develop in the small pools among the macrophytes.

The phytoplankton diversity of eutrophic lakes is usually lower than that of oligotrophic lakes (Moss, 1973). Nevertheless it was shown that those shallow lakes which have high habitat diversity have exceptionally rich algal flora (Borics et al., 2003). Experimental studies demonstrated that functional diversity may be a stronger determinant of ecosystem processes than species diversity (Hooper & Vitousek, 1997; Huston, 1997; Tilman et al., 1997; Wardle, 1999); therefore, investigation of the functional diversity of phytoplankton of shallow lakes is important to understand the operation of these systems. There are several ways of measuring functional diversity (Petchey & Gaston, 2006). One of these approaches is to gather species into functional groups, for which diversity metrics are calculated (Hadar et al., 1999). In phytoplankton ecology, use of functional groups can be traced back to the early 1980s. Reynolds (1980) identified 14 algal groups from analysis of the seasonal periodicity of lake phytoplankton. This system was upgraded and supplemented with other groups that share similar morphological and physiological features (Reynolds et al., 2002; Borics et al., 2007; Várbíró et al., 2007; Padisák et al., 2009); more than 30 functional groups were proposed in these studies, and their ecological traits were also outlined. The functional group concept became an increasingly popular approach in phytoplankton ecology, being used both in theoretical studies (Padisák et al., 2003, Salmaso & Padisák, 2007; Várbíró et al., 2007) and in applied hydrobiology, such as for water quality assessment (Padisák et al., 2006; Borics et al., 2007). Nevertheless, the diversity of functional groups has never been studied in shallow lake ecosystems.

Several papers have been published on the composition and species diversity of phytoplankton of eutrophic shallow lakes in the Carpathian Basin, but these studies usually report diversity in a single lake. Eutrophic shallow lakes include various types of water body that differ from each other in terms of alkalinity, macrophyte coverage, hydrology etc. These differences should also appear in the composition and diversity of phytoplankton.

In temperate eutrophic systems, transition of functional groups is expected during the phytoplankton succession C-G-M-P (Reynolds et al., 2002). Nevertheless, it is reasonable to suppose that other groups can also be dominant in the various sub-types of shallow lake. In Hungary, 17 sub-types of shallow lake exist, based on depth, size, macrophyte coverage, lakebed material and alkalinity (Szilágyi et al., 2008). This system can be considered as a mechanistic typology and not an operational one. Some types are represented by single lakes (Balaton, Neusiedler See, Lake Velence), whereas some of the other types are quite similar to each other. Validation of these sub-types based on biological elements demonstrated that several sub-types can be merged; therefore, the number of sub-types is <17, being 5–8 depending on the biological elements considered (Borics et al., 2009). According to these findings, five lake sub-types were defined based on hydrology, water depth, conductivity and macrophyte coverage (Table 1). These criteria have substantial influence on the composition of phytoplankton assemblages. Sub-type 1 includes the relatively deep oxbow lakes; these

Table 1 Sub-types of lakes with the hydrological, morphological, physical and biological criteria used for typological assignment

Code	1	2	3	4	5
Name of the types	Oxbows	Macrophyte-dominated lakes	Hypertrophic lakes	Open water lakes	Alkaline saline lakes
Hydrology	Perennial	Perennial	Perennial	Perennial	Astatic
Average depth (m)	<3	<3	<3	<3	<1
Max. depth (m)	10	3	3	3	1.5
Conductivity (μS cm^{-1})	400–800	400–800	400–900	400–900	>2,000
Macrophyte coverage (%)	5–20	>50	<5	5–20	0–10
Number of lakes	11	12	3	16	3
Number of samples	92	125	45	179	9

lakes can be stratified by growing season. Lakes in sub-types 2, 3 and 4 are identical in terms of hydromorphology, but differ in macrophyte coverage and fishing activity. In sub-type 5, very high-alkalinity astatic saline lakes, which are specific to the Carpathian Basin, are grouped (Felföldi et al., 2009). It seems reasonable to suppose that differences in the characteristics of the proposed sub-types will manifest themselves in the phytoplankton composition and diversity.

Comparative analysis of phytoplankton regarding composition and diversity has not been carried out for the possible sub-types of shallow lake in the Carpathian Basin. After compiling a large phytoplankton database for eutrophic shallow lakes, the dominance of the functional groups of algae in the various sub-types of lakes and the characteristics of the dominance–diversity relationships were studied.

To address these issues, we tested the following hypotheses:

- Despite their similar trophic state, sub-types of eutrophic lakes can be characterised by different dominant algal assemblages;
- Functional and species diversity of the phytoplankton depend on the lake sub-type;
- Besides the characteristic bloom-forming algae, other groups can also dominate the phytoplankton;
- Functional groups shape the diversity of the algal assemblages in a different way;
- Decreasing diversity is related to increasing algal biovolume.

Materials and methods

Database

Phytoplankton data were provided by the Hungarian National Monitoring System. Data for 26 lakes (294 samples, taken between 1993 and 2010) were inputted into a database. Monthly samples were taken by tube sampler from the trophic layer of the lakes in the growing season. In case of shallow lakes with maximum depth (D_{max}) less than 2 m, the whole water column was sampled. For algal counting, the Utermöhl (1958) technique was used. Phytoplankton biovolumes were calculated according to Hillebrand et al. (1999).

Diversity

Both species and functional diversity were calculated by the Shannon index of diversity (Shannon, 1948). Assignment of a species to a functional group was based on Reynolds et al. (2002), Borics et al. (2007) and Padisák et al. (2009). Functional diversity was defined as the biovolume-based diversity (H) of the functional groups in the sample. Functional groups making at least 80% contribution to total biovolume were considered as dominant.

Statistical analyses

One-way analysis of variance (ANOVA) was used to compare diversity among the five sub-types. During exploratory data analyses, line plots, scatterplots, LOWESS curves (Cleveland, 1979) and principal component biplot (PCA) were used to extract the functional groups associated with the lake sub-types.

Functional group–functional diversity relationships

The relationships between the diversity of the functional groups and the relative abundance of a given functional group were also investigated using a simple model (Fig. 1). This model can be applied to functional group/functional diversity and functional group/species diversity investigations. The grey field indicates the possible range within which diversity values may vary. The maximum diversity occurs in that state where all the elements are equal; i.e. H_{max} is at $p_a = p_b = \cdots = p_z = 1/Z$, where p_i is the relative abundance of the ith functional group (or species) and Z is the number of functional groups (or species). The upper boundary of the grey area indicates the actual maxima (H_{max}) of the diversity at a given abundance of the investigated functional group. The lower arch of the graph indicates the $Z = 2$ situation (H_{min}) at different relative abundance of the elements.

Functional group–species diversity relationships

Functional groups contain different numbers of species. There are only two species in the S_N functional

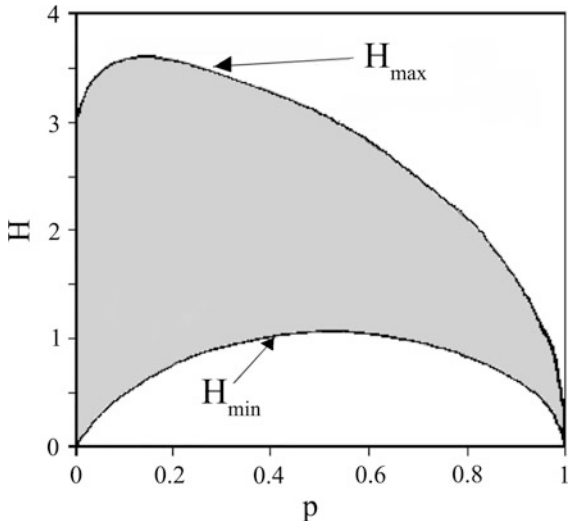

Fig. 1 Relationship between the relative abundance of a functional group and the diversity (H) of the assemblage. The *grey area* indicates the range in which diversity values may vary depending on the relative abundance of a functional group

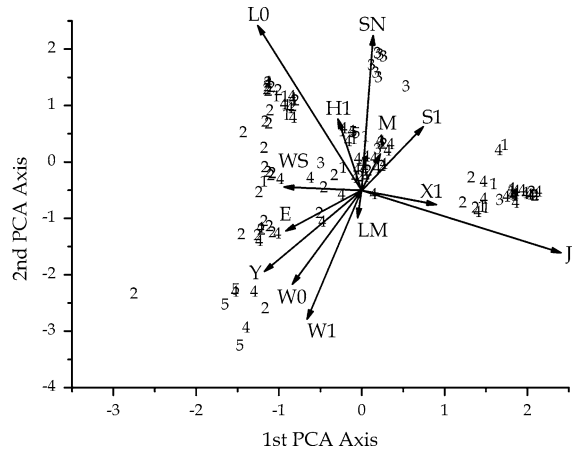

Fig. 2 PCA biplots of the lake sub-types and the functional groups of algae. Numbers encode the lake sub-types (Table 1)

group, while there are more than 100 species in the J functional group. Therefore, when the impact of a given functional group on species diversity was analysed, those species that belong to this functional group were deleted from the database and diversity was calculated for the rest of the species. This procedure was repeated as many times as there were functional groups. Species diversity calculated in this way is called "residual species diversity" (RSD) herein. When the relationships between functional groups and species diversity were analysed, the relative biomass abundance of the given functional group was considered as independent and the RSD values as dependent variables. The analyses were performed at sample level.

Results

Characteristic functional groups of the lake sub-types

The oxbows (1) and macrophyte-dominated lakes (2) were mostly characterised by the motile species groups (Lo, WS, Y and E) (Fig. 2). The hypertrophic lakes (3) were clearly separated by the principal component analysis. These lakes were frequently dominated by *Cylindrospermopsis raciborskii* (S_N). The open water lakes (4) could not be characterised by a certain group of algae. In this sub-type, phytoplankton was occasionally dominated by J, Lo, Y, S1 and E functional groups. The astatic saline lakes (5) were dominated by the elements of the W0 and W1 (euglenophytes) functional groups.

Phytoplankton diversity in the lake sub-types

One-way ANOVA indicated significant difference in both species ($F[4; 636] = 4.81$; $P < 0.005$) and functional diversity ($F[4; 635] = 3.07$; $P < 0.005$) among the lake sub-types (Fig. 3). In sub-types 1–4 both the median and range of the data were almost identical. The median values ranged from $H = 1.8$ to 2.1 for species and from 1.3 to 1.4 for functional diversity. The exception was the sub-type of astatic saline lakes, in which the lake phytoplankton was characterised by lower species and functional diversity.

Dominant functional groups

Based on the relative frequency of functional groups, 16 groups proved to be dominant (Fig. 4). In terms of absolute biovolume, there was five orders of magnitude difference among the dominant groups. Dominance of D (centric diatoms), U (*Uroglena* spp.), E (*Dinobryon* spp.), W1 (*Euglena* spp.) and X1, X2 (small-celled flagellates) was exceptionally rare. Nevertheless, in the higher biovolume range (biovolume $> \sim 20$ mm^3 l^{-1}) only bloom-forming cyanobacteria

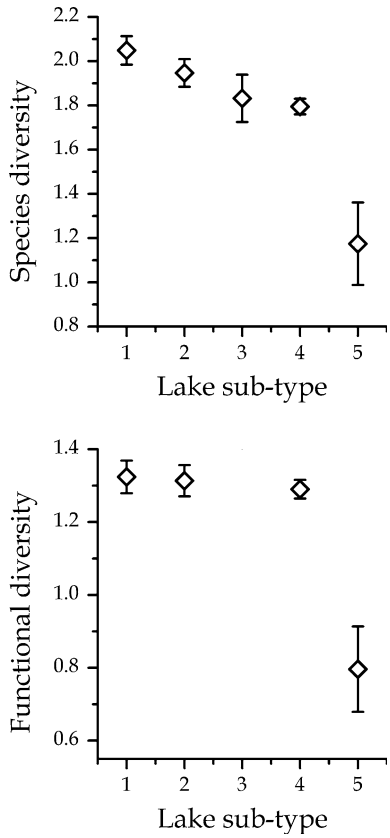

Fig. 3 Distribution of species and functional diversity values in the five investigated lake sub-types

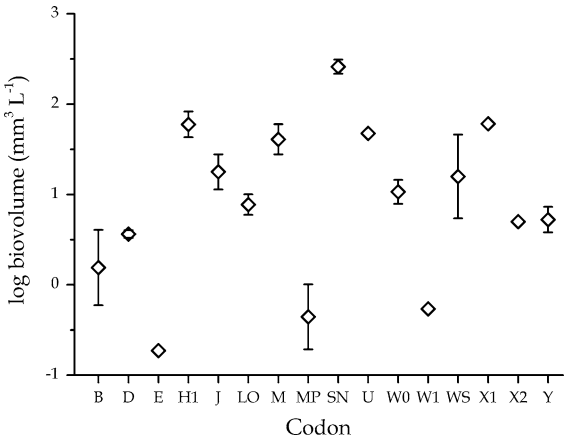

Fig. 4 Characteristic ranges of biovolume where the different functional groups occurred as dominant (relative biovolume abundance >80%)

(H1, S_N and M groups), *Synura* spp. (WS), chlorococcaleans (J) and dinoflagellates (Lo) were capable of developing dominant assemblages.

Functional group–functional diversity relationships

First, relationships between functional groups and functional diversity were investigated (Fig. 5). Scatterplots of the functional groups were similar. In most of the cases (D, J, Y, Lo, MP), the scatterplots of the data were identical to that of the theoretical model; that is, the maximum values of diversity occurred at about 0.1 relative abundance. Different distribution characterised the S_N, M and H1 functional groups, the most frequently occurring cyanobacteria. The maximum diversity values occurred when the abundance of these groups was zero. Minimal occurrence of these groups resulted in a steep decrease in the functional diversity. At higher abundance ranges, all the functional group–functional diversity relationships were characterised by a similar distribution pattern.

Functional group–species diversity relationships

In most cases, the impact of functional groups on the RSD was negligible (Fig. 6). High RSD values could be observed even in case of high relative abundance (>0.9) of the functional groups. A different pattern characterised the H1 and especially the S_N groups. At higher relative abundance of these groups, the RSD showed a decreasing tendency. The Lo and S_N functional groups showed the most characteristic type of functional group versus RSD relationship (e.g. no relationship and decreasing tendency). Fitting a LOWESS curve to the plots of these groups (Fig. 7), it seems clear that the RSD is independent of the relative abundance of the Lo group, but in case of the S_N group, a pronounced decline started from relative abundance of 0.5.

Biovolume–diversity relationships

Both functional and species diversity values showed hump-shaped, right-skewed curves on a logarithmic biovolume scale (plots not shown). Diversity values were highly scattered, even in the high (>50 $mm^3\ l^{-1}$) biovolume range. The LOWESS curves indicated that, for biovolume >20 $mm^3\ l^{-1}$, a sharp decline in diversity might be expected in terms of both functional and species diversity.

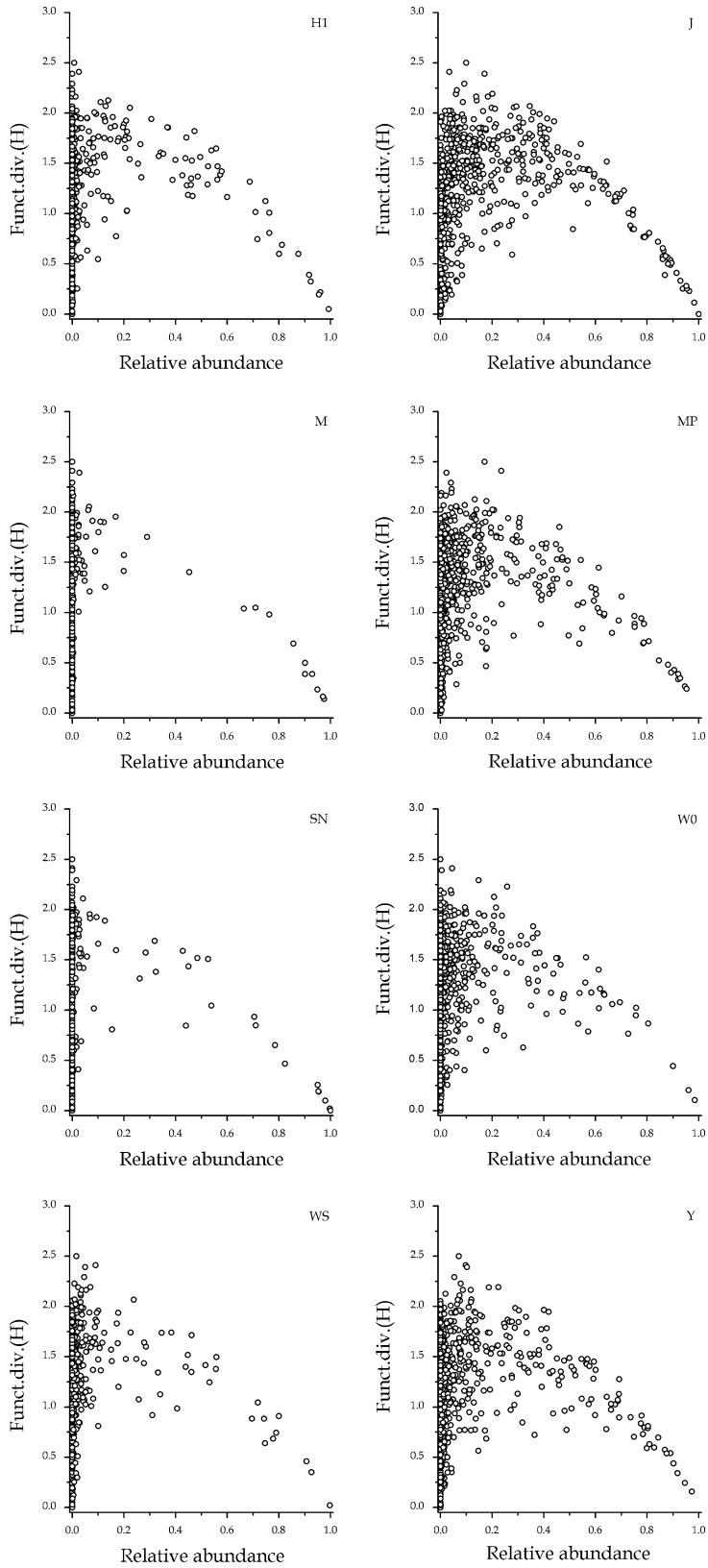

Fig. 5 Impact of the functional groups of algae on functional diversity

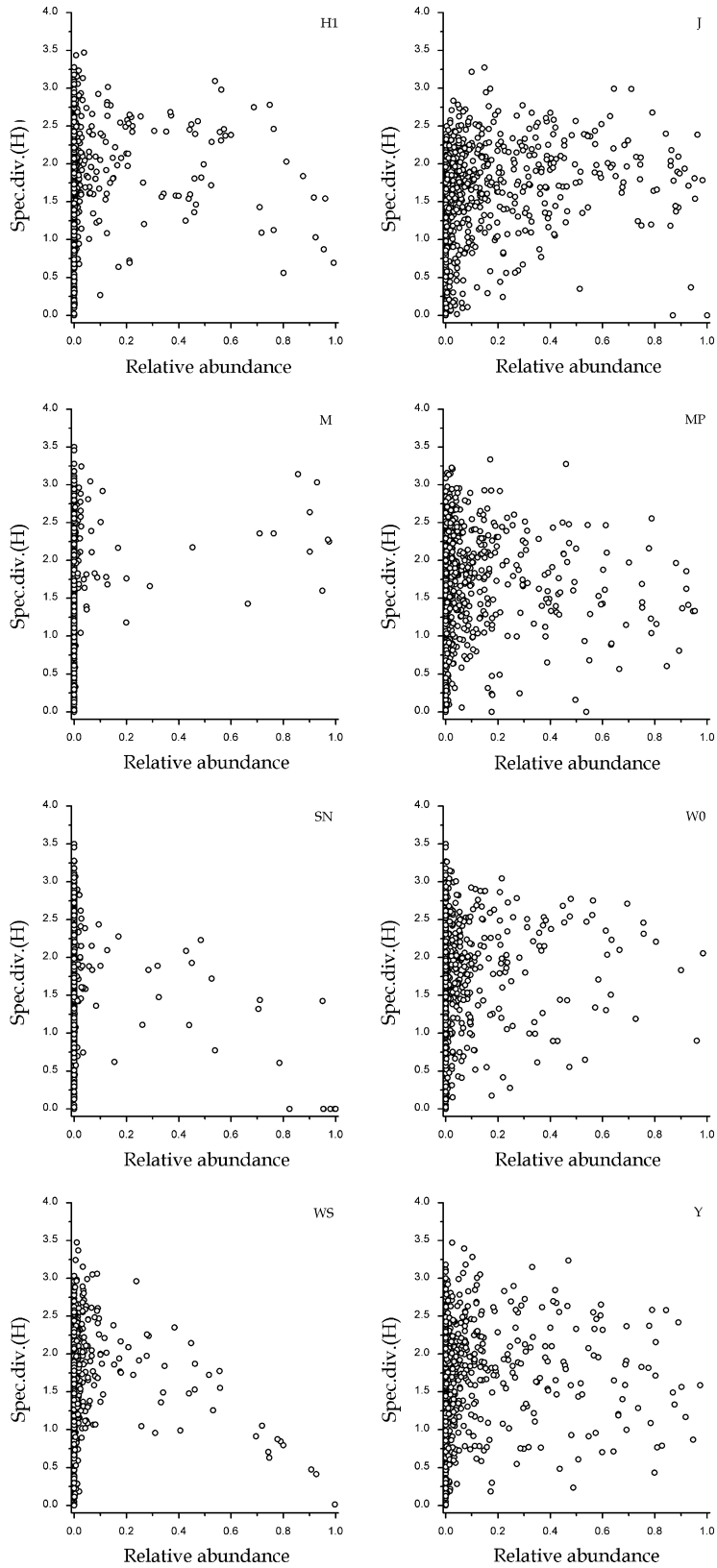

Fig. 6 Impact of the functional groups of algae on the residual species diversity (RSD) (where species belonging to the functional group used as the independent variable were not considered in the diversity calculation)

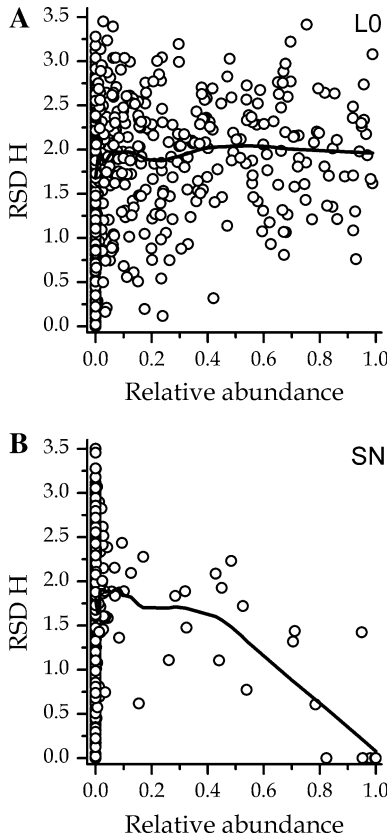

Fig. 7 LOWESS curves fitted to the plots of L_O—residual species diversity (**A**) and S_N—residual species diversity (**B**) relationships. These two functional groups showed the most characteristic type of functional group–RSD relationship (i.e. no impact and strong negative impact)

Discussion

We demonstrated that the composition of phytoplankton in the sub-types differ from each other. The taxonomic composition of the astatic saline lakes was remarkably different from the others. In these lakes, elements of the W0 (*Euglena* spp., which prefer polysaprobic conditions) and W1 (*Phacus* and *Lepocinclis* spp.) groups were dominant. Due to high concentration of organic compounds, these waters favour development of euglenophytes blooms. It is also known that these lakes are frequently dominated by photoautotrophic picoplankton as a result of serious light limitation (Felföldi et al., 2009). Phytoplankton of hypertrophic lakes was also remarkably different from the others. S_N dominance in this sub-type is not unusual (Borics et al., 2000) and causes serious blooms in other lakes in this region (Padisák & Reynolds, 1998). Success of this species is attributed to tolerance of self-shading and production of large numbers of akinetes. In the shallow hypertrophic lakes, the reactive phosphorus concentration is high and the temperature fluctuation in the early autumn period can be significant. These characteristics contribute to the development of the highest akinete concentration in case of *C. raciborskii* (Moore et al., 2005). The phytoplankton of the oxbows had much in common with that of the macrophyte-dominated lakes, partly because of different reasons, although motile taxa prevailed in both habitats. The oxbows are a specific type of water body which is wind sheltered, therefore being characterised by stable stratification. Investigating the temperature and oxygen profile of a sheltered oxbow, Borics et al. (2011) demonstrated that in summer period the mixing layer depth is only 2 m. In these lakes, non-motile organisms easily sink down from the photic layer; therefore, motility is one of the most important functional traits of species inhabiting oxbows (Krasznai et al., 2009). There are several mechanisms by which macrophytes exert impact on the planktonic food web. Macrophytes reduce light penetration, can produce allelopathic substances (Hasler and Jones, 1949; Körner and Nicklisch, 2002), increase the sedimentation rate (Van den Berg et al., 1997) and provide habitat for grazers (Jeppesen et al., 1997). Due to these mechanisms, macrophytes have a clear effect on the structure of phytoplankton communities (Jasser, 1995; Søndergaard & Moss, 1998; Van Donk & Van de Bund, 2002). In the presence of macrophytes, dominance of flagellated algae, e.g. *Chalmydomonas* spp., *Cryptomonas* spp. euglenophytes and dinoflagellates, is expected (Borics et al., 2003; Krasznai et al., 2010; Schriver et al., 1995; Van den Berg et al., 1997). In the well-mixed open water lakes, the chlorococcalean green algae (J) were the most characteristic phytoplankton elements. These algae frequently dominate the phytoplankton of shallow enriched ponds (Reynolds et al., 2002).

The phytoplankton diversity (H) of the lakes varies between 0 and 4.5 bits, but typically is in the range of 2.4–2.6 (Harris, 1986). The value of the biomass-based diversity (H) ranged between 1 and 2.5 bits in case of eutrophic Danish lakes (Jeppesen et al., 2000), but higher values characterise oligotrophic systems (Margalef, 1980). Weithoff (2003) showed that, in the oligotrophic Lake Constance, the average phytoplankton diversity is approximately 3 bits and occasionally

can be higher than 4. Besides the trophic state, diversity is influenced by other factors such as lake size, lake depth (Jeppesen et al., 2000), fish stock (Romo & Villena, 2005) or macrophytes (Declerck et al., 2007); therefore, we supposed that diversity is significantly different among the sub-types of shallow lakes. An unexpected result of this study is that, despite differences in the taxonomic composition and functioning of the lake sub-types, the diversity values were surprisingly similar. The lakes had high-diversity phytoplankton even in hypertrophic conditions (median values of *H* were within the range of 1.8–2.2). This can be explained by the high number of species with similar habitat template in naturally eutrophic water bodies (Reynolds, 1998). A common characteristic of these taxa is that they are evolutionarily adapted to elevated nutrient concentration. In case of the astatic saline lakes, low diversity can be explained by the astatic character and the extremely high salt concentration. These factors select the most tolerant euryhaline taxa such as euglenophytes (Caljon, 1987) and unique prokaryotic picocyanobacteria (Felföldi et al., 2009).

The fact that differences in diversity could not be found among the sub-types means that diversity is not a suitable metric for quality estimation in case of shallow eutrophic lakes.

The high number of functional groups that occurred as dominants was really surprising. It is known that lakes are frequently dominated by a few species or a certain functional group of algae in the late successional state, being called equilibrium (Sommer et al., 1993) or steady-state assemblages (Naselli-Flores et al., 2003). In shallow eutrophic systems, temperature and light availability are the most important factors driving development of phytoplankton assemblages. Usually, C, J, G, S1, H1, H2 and S_N assemblages are expected to be dominant in the growing season. Besides these, ten other groups proved to be dominant in the investigated lakes. There are several biotic and abiotic mechanisms that might result in steady-state assemblages (Rojo & Álvarez-Cobelas, 2003), among which competitive exclusion (Hardin, 1960) is the most important. Nevertheless, the overwhelming dominance of a few species does not necessarily mean that phytoplankton is in an equilibrium state. Short-term dominance can especially apply for those functional groups that dominate the first stage of phytoplankton succession (B, D, E) (Padisák et al., 2003), or for the mostly metaphytic W0, W1 groups, which can be protagonists in macrophyte-dominated lakes. In these habitats, benthic grazers could select small-celled taxa and help the dominance of large-sized species. In this case "the dominant species are not the best, but rather the remainder" (Rojo & Álvarez-Cobelas, 2003).

In parallel with the increasing dominance of any element of the assemblage, decreased diversity is expected. This tendency was quite obvious in case of the functional diversity, but was not observed in case of the RSD. In most functional groups, higher relative biovolume abundance did not coincide with lower RSD. This means that the dominant groups did not necessarily outcompete the other elements of the phytoplankton. In these cases, dominance of these groups (L_O, Y, W0, J, W_S) can be traced back to other biotic and abiotic reasons (Rojo & Álvarez-Cobelas, 2003). Dominance of motile taxa (L_O, Y, W0) is expected for stable stratification, or when nutrients are spatially segregated (Reynolds et al., 2002).

A characteristic decrease in RSD occurred exclusively in case of the dominance of S1 and especially S_N groups. Species in both groups are elongated, and this morphological adaptation makes them better photoadaptable light antennae (Reynolds, 1998); therefore, they are strong light competitors (Reynolds, 2006). It seems that only these strong light competitors exert impact on the diversity of the phytoplankton. When reasons other than light competition are responsible for the dominance of a certain functional group [species-specific abilities, e.g. mixotrophy or buoyancy regulation (Naselli-Flores et al., 2003)], the RSD of the other elements does not decrease. Although *Microcystis* spp. are also bloom-forming taxa and can dominate in late summer, these are not good light competitors, being instead rather sensitive to low light availability. Therefore, during the dominance of M functional group there was no reduction in RSD.

We found that minimal occurrence of the H1, M and S_N functional groups indicated loss of functional diversity. This means that, when functional diversity reaches its maximum, the system does not contain bloom-forming cyanobacteria at all. The presence of strong light competitors (H1 and S_N) indicates that light limitation drives the phytoplankton succession (Reynolds, 2006) and, if disturbances do not occur, results in low-diversity assemblages.

A unimodal relationship between productivity and diversity is quite common in both aquatic (Dodson et al., 2000) and terrestrial systems (Grime, 1973). The fact that increasing productivity coincides with an increase in diversity in the low and medium productivity range is well known (Abrams, 1995; Jeppesen et al., 2000). The descending arm of the curve in the higher productivity range has been explained by competition (Richman & Dodson, 1983), predation (Leibold, 1999) or abiotic factors (Jones et al., 1983). The hump-shaped distribution of the data in case of both species and functional diversity is in accordance with the previous findings. The unexpected result of this investigation is that the decreasing tendency revealed by the LOWESS curve appeared in a very high biovolume range (20 mm^3 l^{-1}). This concentration range corresponds to poor ecological quality based on the boundaries set for very shallow German hardwater lakes (Mischke et al., 2002), which are quite similar to the Hungarian lakes. Despite the decline of the curve, biovolume seems to be a poor predictor of diversity, meaning that the productivity–diversity relationship cannot be interpreted as indicating that productivity drives diversity (Gross & Cardinale, 2007). This kind of relationship can be observed exclusively in the extremely high biovolume range (Borics et al., 2000). These findings also support the view that diversity metrics do not work well in ecological state assessment.

Conclusions

1. Among shallow lakes, only hypertrophic lakes (sub-type 3) and astatic saline lakes (sub-type 5) had distinctive phytoplankton assemblage (Fig. 2).
2. Neither the functional nor species diversity showed differences among the lake sub-types (Fig. 3). Only alkaline saline lakes were less diverse than the others.
3. Besides the well-known bloom-forming groups (H1, S1, S_N, M, J), several other functional groups appeared as dominant (Fig. 4). The dominance of the functional groups developed in the extremely high range of biovolume (0.005–500 mm^3 l^{-1}).
4. The functional groups shaped the diversity in various ways (Fig. 5). Dominance of bloom-forming cyanobacteria reduced both functional and species diversity. When other groups dominated, the phytoplankton species diversity did not necessarily decrease (Fig. 6). The presence of bloom-forming cyanobacteria (even at low abundance) indicated loss in functional diversity.
5. Several groups can be dominant and produce high-biovolume assemblages, but they exert impact on the diversity only in the high biovolume range.

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PHYTOPLANKTON

Photosynthetic characteristics and physiological plasticity of an *Aphanizomenon flos-aquae* (Cyanobacteria, Nostocaceae) winter bloom in a deep oligo-mesotrophic lake (Lake Stechlin, Germany)

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Abstract In winter of 2009/2010, *Aphanizomenon flos-aquae* bloomed in the ice and snow covered oligo-mesotrophic Lake Stechlin, Germany. The photosynthesis of the natural population was measured at eight temperatures in the range of 2–35°C, at nine different irradiance levels in the range of 0–1,320 μmol m^{-2} s^{-1} PAR at each applied temperature. The photoadaptation parameter (I_k) and the maximum photosynthetic rate (P_{max}) correlated positively with the temperature between 2 and 30°C, and there was a remarkable drop in both parameters at 35°C. The low I_k at low temperatures enabled the active photosynthesis of overwintering populations at low irradiance levels under ice and snow cover. The optimum of the photosynthesis was above 20°C at irradiances above 150 μmol m^{-2} s^{-1}. At lower irradiance levels (7.5–30 μmol m^{-2} s^{-1}), the photosynthesis was the most intensive in the temperature range of 2–5°C. The interaction between light and temperature allowed the proliferation of *A. flos-aquae* in Lake Stechlin resulting in winter water bloom in this oligo-mesotrophic lake. The applied 2°C is the lowest experimental temperature ever in the photosynthesis/growth studies of *A. flos-aquae*, and the results of the P–I and P–T measurements provide novel information about the tolerance and physiological plasticity of this species.

Keywords *Aphanizomenon flos-aquae* · P–I and P–T characteristics · Oligo-mesotrophic Lake Stechlin · Cyanobacterial bloom · Winter · Ice cover · Physiological plasticity

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Introduction

The dynamics of phytoplankton populations are controlled by multiple factors including physiological and evolutionary adaptations, environmental and biological processes. The interactions between the different factors are important in understanding the response of organisms to these variables. The effects of temperature on phytoplankton cellular processes and growth are well known (e.g. Reynolds, 1984), being temperature an important, but not the only factor in determining the occurrence of a particular algal species. The physiological adaptations may contribute to the success of certain phytoplankton taxa to become efficient competitors, as demonstrated on several

species of cyanobacteria (e.g. Padisák, 1997; Price et al., 1998). Cyanobacterial dominance commonly occurs in eutrophic water bodies (e.g. Yamamoto & Nakahara, 2009a) at water temperatures above 20°C due to the high temperature optima of their growth (e.g. Reynolds, 1984; Wilhelm & Adrian, 2008), but the success of cyanobacteria is a result of their response to interactions between different environmental constraints (e.g. Dokulil & Teubner, 2000).

Aphanizomenon flos-aquae (L.) Ralfs is a filamentous and heterocytic cyanobacterium, which is capable of N_2 fixation (e.g. De Nobel et al., 1998; Reynolds et al., 2002). It produces akinetes that enable survival in unfavourable growth conditions (Yamamoto & Nakahara, 2007). The temperature optimum of its growth is above 20°C (e.g. Uehlinger, 1981; Dokulil & Teubner, 2000), but it depends on the light intensities at which the population was grown (Konopka & Brock, 1978). The species prefers shallow eutrophic freshwaters (e.g. Yamamoto & Nakahara, 2009a, b), and since external load reduction resulted in nitrogen scarcity in many lakes, relative biomass share of N-fixing genera, like *Aphanizomenon*, increased in many lakes during the last decades (Reynolds et al., 2002; Wagner & Adrian, 2009). *Aphanizomenon flos-aquae* cells contain gas vesicles, which have a significant role in regulating buoyancy (e.g. Walsby, 1994). It can accumulate in the illuminated layer near the water surface, developing a surface bloom (e.g. Dokulil & Teubner, 2000; Preußel et al., 2009). Temperature and light intensity are key factors in the regulation the growth of Nostocales (Mehnert et al., 2010). The competition between *A. flos-aquae* and other cyanobacterial species for light at different temperatures was investigated in several studies (e.g. De Nobel et al., 1998; Huisman et al., 1999; Yamamoto & Nakahara, 2006; Wagner & Adrian, 2009). The current knowledge about the temperature- and light-dependent growth of *A. flos-aquae* in temperature regions >15°C is well documented, but information is scarce about its winter occurrence. Wildman et al. (1975) found all developmental stages of *A. flos-aquae* in samples from an ice-covered lake in November. In another study, some filaments of the species were present in the phytoplankton throughout the winter months until the onset of exponential growth in mid-May in Kinnego Bay, Ireland (Jones, 1979). The possibility that these 'overwintering' filaments may have been the result of continuous germination of akinetes through the winter was discounted, since the observed filaments in winter and early spring were mostly long filaments consisted of many cells (Jones, 1979).

Aphanizomenon flos-aquae appeared in the oligo-mesotrophic, deep Lake Stechlin (Germany) first in 2001. Until 2006, the species was seasonal and sporadic between early summer and the autumnal overturn. Since 2006, it provided biomass peaks in late summers. In 2009, the biomass of the late summer peak was 400–500 µg wet weight L^{-1}. In autumn a drop of the biomass was observed, but after the autumnal overturn the population of *A. flos-aquae* began to grow, and provided another biomass peak in winter with 915–920 µg wet weight L^{-1}. In December, a macroscopically visible surface bloom of *A. flos-aquae* developed along the shorelines of the lake. Between December 2009 and January 2010, *A. flos-aquae* contributed 87–90% to total biomass therefore developed a sufficiently long-lasting winter equilibrium phase (Padisák et al., 2010) which continued in February 2010 when 17 cm thick ice developed on the lake and was covered by 20 cm snow. According to our knowledge about the species, appearance of *A. flos-aquae* in Lake Stechlin, and its winter bloom under ice cover were unexpected phenomena. They triggered the questions that the filaments of *A. flos-aquae* were physiologically active or not and that the bloom under winter conditions was a result of its low-temperature tolerance or low-temperature preference.

This study focused on the question of how temperature and irradiance level influence the photosynthetic activity of *A. flos-aquae*. We assumed that the interactions between temperature and the light availability would

(i) cause corresponding changes in the P–I (photosynthesis–irradiance) parameters of *A. flos-aquae*,
(ii) determine the occurrence of the species under extreme conditions, and
(iii) due to its physiologically distinctive features it could reach high biomass with active photosynthesis in an ice-covered lake.

To test these hypotheses, the photosynthetic activity of a natural population of *A. flos-aquae* collected on 26th February 2010 in Lake Stechlin was investigated at eight different temperatures (2–35°C) and at nine

different irradiance levels (0–1,320 μmol m^{-2} s^{-1}) in laboratory.

Materials and methods

Site description, sampling

Lake Stechlin is a medium-sized (4.2 km^2), deep (z_{mean}: 23.3 m; z_{max}: 69.5 m) lake without surface inflows at 53°10′N latitude, 13°02′E longitude and 84.5 m a.s.l. in Brandenburg, Germany. The lake has glacial origin, was originally oligotrophic, but during the last decade it turned oligo-mesotrophic. In February 2010, the following concentrations of nutrient were measured: SRP: 8.5 μg L^{-1}; TP: 27.7 μg L^{-1}; NO_3^--N: 58.7 μg L^{-1} (APHA, 1998).

Phytoplankton samples were taken on 26th February 2010 at a sampling site in front of the Leibniz-Institute of Freshwater Ecology and Inland Fisheries (Fig. 1), where the water depth was 26 m. At the sampling occasion, temperature and photosynthetically active radiation (PAR) (Li 192A Underwater Quantum Sensor, LI-COR Biosciences, Lincoln, Nebraska, USA) profiles were taken in the water column of the lake: the sensors were sank into different depth through a leak, while leak was recovered with ice and snow to get the real in situ temperature and light values during the measurements. The in situ temperature ranged between 0.3 and 2°C in the upper 10 m layer. The euphotic zone expanded to depth of 10–12 m. Samples were taken with a Van Dorn sampler in 2 m increments from the euphotic depths then were mixed (integrated sample). The sampling location and light field (under 17 cm thick ice and 20 cm snow cover) are shown on Fig. 1. Vertical light attenuation coefficient, K_d (m^{-1}) of different layers of the water column was calculated with the Lambert–Beer function (Kirk, 1994) from simultaneous measurements of irradiances per metre (0–20 m) in the field at time of the sampling. The K_d was in the upper 1 m layer the highest, 1.33 m^{-1}. The average K_d of the layers between 1 and 20 m was 0.42 ± 0.15 m^{-1}; the euphotic layer was lying at the depth of 11 m (Fig. 1).

The samples were kept in the dark and cool for 24–36 h, while they were transported from Germany to the location of the laboratory measurements at the University of Pannonia, Veszprém, Hungary. Subsamples of 300 ml were preserved in Lugol solution for microscopic counting (Utermöhl, 1958). The chlorophyll *a* concentrations of the samples were measured according to Wetzel & Likens (2000).

P–I and P–T measurements

The measurements were carried out in a laboratory incubation system (Üveges et al., 2011). The photosynthetic activity of the natural population was measured by the ^{14}C method (Steemann-Nielsen, 1952). The rate of photosynthesis was determined by adding $NaH^{14}CO_3$ with known activity (0.099181–0.114185 MBq) to 15 ml of samples in 20 ml scintillation vials (Econo Glass Vial, PerkinElmer, Waltham, MA, USA). The

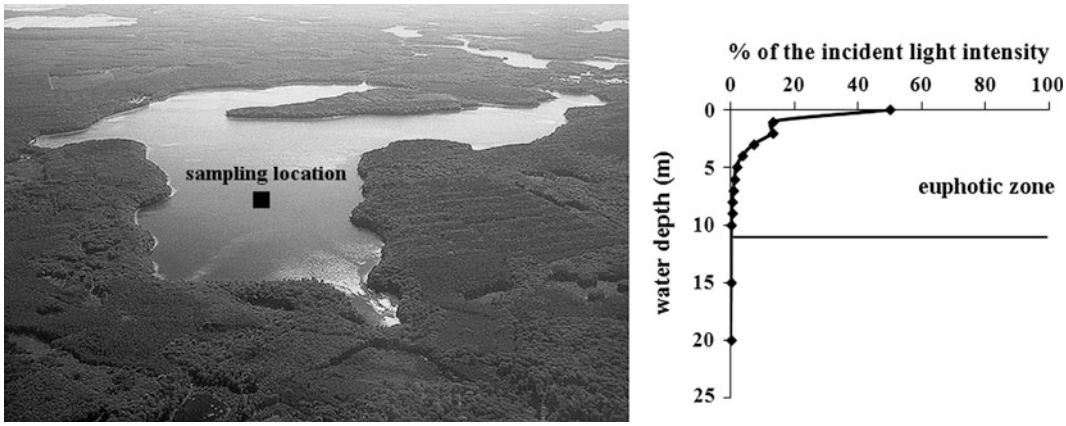

Fig. 1 Lake Stechlin and the sampling location in the lake. On the chart right the light field of the water column, covered by 17 cm thick ice and 20 cm snow, was shown at the sampling occasion. On 26th February 2010, the irradiance level was 134 μmol m^{-2} s^{-1} at 0 m (immediately below the ice), which corresponded to the 50% of the incident irradiance level

vials were pre-incubated at appropriate temperatures for 1 h before the addition of radioisotope to equilibrate the contents to the experimental condition. Three vials were incubated as replicates at each irradiance level. The photosynthesis of the natural population was measured for 2 h after the addition of radioisotope at nine irradiance levels (0; 7.5; 30; 75; 150; 260; 500; 920; 1,320 μmol m^{-2} s^{-1} PAR) at each applied temperature (2; 5; 10; 15; 20; 25; 30; 35°C). In all experiments, three vials were wrapped in aluminium foil to serve as dark control; they were incubated at 0 μmol m^{-2} s^{-1} irradiance. After the 2 h of incubation and removing of the externally attached radioisotope, the incorporation of ^{14}C into algal protein was measured in each vial with liquid scintillation analyzer (Packard Tri-Carb 3180TR/SL, GMI Inc., Ramsey, MN, USA). Non-photosynthetic C uptake, which was determined in control vials kept in the dark, was subtracted from photosynthetic C uptake.

To fit curves for photosynthesis vs. irradiance data and to determine the P–I parameters (Table 1), the GraFit software was applied (GraFit by R. Leatherbarrow, 1989–1992 Erithacus Software Ltd.) with the equation of Platt et al. (1980). The Platt et al. (1980) equation fits most photoinhibition data fairly well:

$$P = P_s\left(1 - \exp^{-(\alpha I/P_s)}\right)\left(\exp^{-(\beta I/P_s)}\right),$$

where P is the photosynthetic rate at irradiance I, P_s is the maximum photosynthesis obtained in the absence of photoinhibition, and α and β are parameters describing the initial slope and the photoinhibited section of the P–I curve, respectively. Photosynthetic parameters like maximal photosynthetic rate (P_{max}) and photoadaptation parameter ($I_k = P_{max}/\alpha$) were derived from the previous parameters (Table 1).

Q_{10}

The Q_{10} model (Ahlgren, 1987) was employed to describe the relationship between photosynthesis and temperature:

$$Q_{10} = \left(P_{I(T_2)}/P_{I(T_1)}\right)^{(10/(T_2-T_1))},$$

where $P_{I(T_2)}$ and $P_{I(T_1)}$ are photosynthetic activity at given irradiance level (I) at two temperatures, T_2 and T_1, respectively. In fact, algal growth rates increase up to the optimal temperature beyond which they decrease due to stressful conditions, so Q_{10} was calculated from the linear section of the photosynthesis–temperature (P–T) curves.

Results

Phytoplankton sample

On 26th February 2010, the chlorophyll *a* content of the integrated sample from 0 to 10 m upper layer of Lake Stechlin was 4.39 ± 0.40 μg chl *a* L^{-1}. According to the microscopic analysis, *A. flos-aquae* dominated in the samples; the aggregated filaments of *A. flos-aquae* provided more than 99% of the total biomass. The rest 1% was provided by the diatom *Stephanodiscus neoastraea*. Therefore, from ecophysiological point of

Table 1 Parameter values obtained by nonlinear regression of the photosynthesis–irradiance data for the natural population of winter blooming *Aphanizomenon flos-aquae* in Lake Stechlin (Germany)

T	α	P_s	β	P_{max}	I_k
2	0.195 (0.153)	4.31 (0.15)	0.0032 (0.0004)	3.96	20
5	0.143 (0.008)	6.15 (0.17)	0.0041 (0.0004)	5.39	38
10	0.160 (0.011)	12.98 (0.69)	0.0076 (0.0014)	10.70	67
15	0.132 (0.007)	13.96 (0.61)	0.0056 (0.0009)	11.69	89
20	0.138 (0.014)	15.34 (1.21)	0.0024 (0.0015)	14.04	102
25	0.121 (0.008)	20.98 (1.78)	0.0041 (0.0020)	18.07	149
30	0.113 (0.005)	22.46 (1.46)	0.0037 (0.0015)	19.42	172
35	0.109 (0.018)	10.45 (1.15)	0.0000 (0.0012)	10.57	97

SE is given in brackets for fitted parameters; SE was not calculated for the derived parameters, P_{max} and I_k. T, temperature (°C); α, initial slope (μg C L^{-1} h^{-1} (μmol m^{-2} s^{-1})$^{-1}$); P_s, maximum photosynthetic rate obtained at the lack of photoinhibition (μg C L^{-1} h^{-1}); β, photoinhibition parameter (μg C L^{-1} h^{-1} (μmol m^{-2} s^{-1})$^{-1}$); I_k, photoadaptation parameter (μmol m^{-2} s^{-1})

view the natural samples can be considered as a monoculture of *A. flos-aquae*.

P–I characteristics

The maximum photosynthetic rate (P_{max}) varied between 3.96 and 19.42 μg C L^{-1} h^{-1} (Fig. 2; Table 1). The population reached the highest P_{max} at 30°C, and the lowest at 2°C. At 2 and 5°C the fixed carbon was 20 and 27% of that fixed at 30°C. A substantial decrease in the P_{max} was measured between 10 and 5°C: *A. flos-aquae* photosynthesised at 50% of the maximum rate at 10°C. At 35°C, the P_{max} declined to 54% of that measured at 30°C. In the temperature range of 2–30°C, the P_{max} correlated positively with the temperature ($r = 0.975$; $P < 0.001$; $n = 7$).

The initial slope of the P–I curve (Fig. 2), α correlated negatively ($r = -0.876$; $P < 0.001$; $n = 7$) with the temperature; varied between 0.109 and 0.195 μg C L^{-1} h^{-1} (μmol m^{-2} s^{-1})$^{-1}$. Photoinhibition was observed at different irradiance levels in the experiments carried out at different temperatures (Fig. 2). At 2°C, the community was inhibited at the irradiance of 500 μmol m^{-2} s^{-1} by 20% and at 1,320 μmol m^{-2} s^{-1} by 55% relative to photosynthesis at 75 μmol m^{-2} s^{-1}. At 25°C, the photosynthesis was inhibited only at the highest irradiance levels, at 920 and 1,320 μmol m^{-2} s^{-1}. The strongest photoinhibition was observed in the experiment carried out at 10°C, the community was inhibited at 1,320 μmol m^{-2} s^{-1} by 54% relative to photosynthesis at irradiance of 260 μmol m^{-2} s^{-1}, in the region of P_{max}.

The photoadaptation parameter, I_k showed a strong positive correlation with the temperature in the range of 2–30°C ($r = 0.985$; $P < 0.001$; $n = 7$).

P–T characteristics

In the irradiance range of 260–1,320 μmol m^{-2} s^{-1}, the carbon uptake increased with the increasing temperature until the temperature reached the 30°C (Fig. 3A), strong positive correlation was observed in all cases ($P < 0.001$; $n = 21$): $r = 0.989$ at 1,320 μmol m^{-2} s^{-1}; $r = 0.986$ at 920 μmol m^{-2} s^{-1}; $r = 0.983$ at 500 μmol m^{-2} s^{-1}; and $r = 0.954$ at 260 μmol m^{-2} s^{-1}. The photosynthesis at these irradiance levels was inhibited by 41–48% at

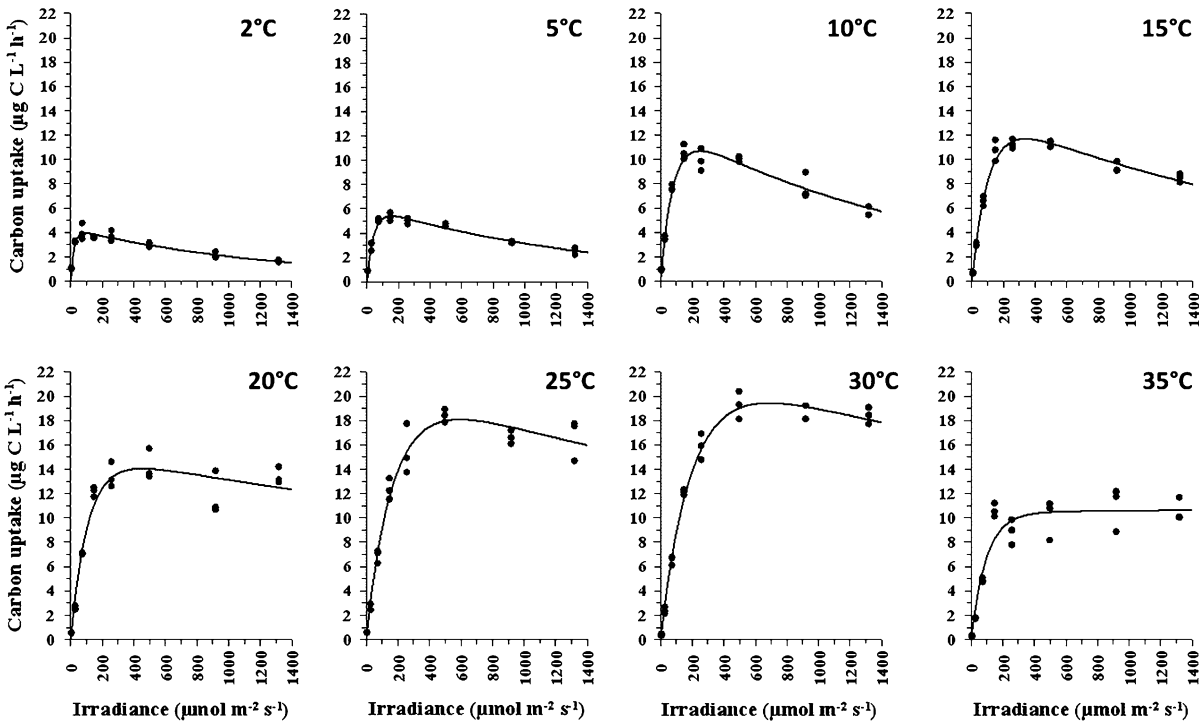

Fig. 2 Photosynthesis–irradiance curves of natural population of *Aphanizomenon flos-aquae* collected in Lake Stechlin, on 26th February 2010 at different experimental temperatures

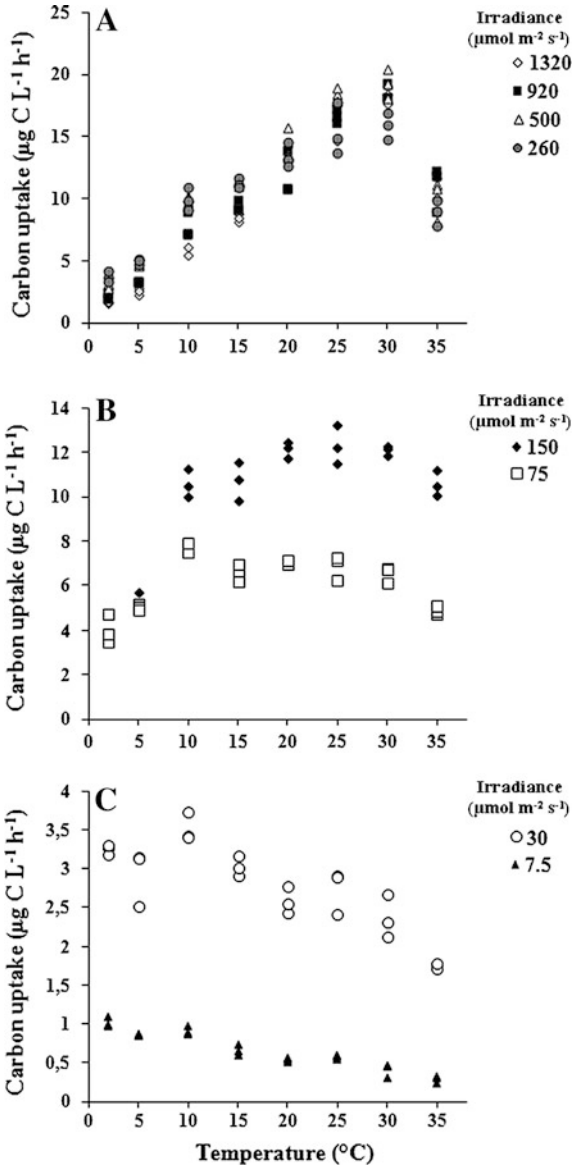

Fig. 3 Photosynthetic activity of natural winter population of *Aphanizomenon flos-aquae* as a function of temperature at different light intensities

35°C relative to that at 30°C. Strong positive correlation between carbon uptake and temperature was found only at the upper temperature region (2–10°C) at the irradiances of 150 and 75 μmol m^{-2} s^{-1} ($r = 0.984$ and $r = 0.971$; $P < 0.001$; $n = 9$) (Fig. 3B). At low irradiance levels, the photosynthetic activity correlated negatively with the temperature (Fig. 3C). At irradiance level of 30 μmol m^{-2} s^{-1}, the carbon uptake did not change remarkably with the increasing temperature, but above

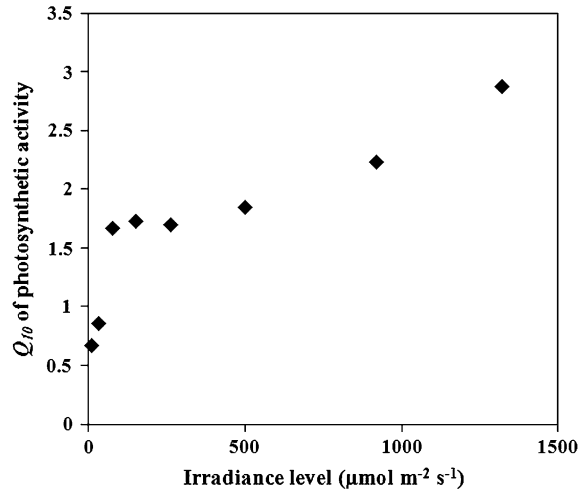

Fig. 4 Q_{10} of the photosynthetic activity of natural winter population of *Aphanizomenon flos-aquae* at different irradiance levels

15°C a negative correlation was observed ($r = -0.851$; $P < 0.001$; $n = 15$). At the lowest irradiance level (7.5 μmol m^{-2} s^{-1}), the photosynthesis of *A. flos-aquae* showed a strong negative correlation with the temperature in the whole temperature range ($r = -0.955$; $P < 0.001$; $n = 24$) (Fig. 3C).

Q_{10} of the photosynthetic activity

Q_{10} values computed for the linear section of the P–T curve (Fig. 3) varied from 0.67 to 2.88 (Fig. 4). At the lowest applied irradiances the photosynthesis did not show positive correlation to the temperature, the Q_{10} was less than 1. At the mid-irradiance levels (75–260 μmol m^{-2} s^{-1}) the average Q_{10} was 1.70 ± 0.03, which suggests lower variation of photosynthesis with temperature, than at the highest irradiance levels (500–1,320 μmol m^{-2} s^{-1}), where Q_{10} varied from 1.85 to 2.88.

Discussion

After the first appearance of *A. flos-aquae* in Lake Stechlin (Padisák et al., 2010), the life cycle of the species corresponded to the usual patterns described by Yamamoto & Nakahara (2009b) until 2009, when it overwintered with filaments near to the water surface. Overwintering filaments of the species were observed

in previous studies (e.g. Simona, 2003; Yamamoto, 2009), however, physiological activity of the observed winter populations has not been studied. Yamamoto & Nakahara (2007) explained the winter appearance with the high biomass in summer which did not disappear completely in winter. It was certainly not the case for the Lake Stechlin *A. flos-aquae* population in winter of 2009, since morphology of summer and autumn filaments were clearly different: in contrast to the summer filaments, those found in winter did not include heretocytes. In a study from 2009, Yamamoto & Nakahara (2009a) concluded that the increase of population density of *A. flos-aquae* in winter might be a result of the adaptation of the species to low water temperatures.

The influence of temperature on the cellular metabolism of the species was addressed in several studies. Konopka & Brock (1978) isolated *Aphanizomenon* from Lake Mendota, and studied the influence of temperature on rate of carbon uptake under low-light intensities. They found, that the optimal temperature of *A. flos-aquae* for photosynthesis was 20°C, but at 10°C *Aphanizomenon* was still photosynthesising at 8% of the maximum rate. In other studies, the optimum temperature of *Aphanizomenon*'s growth varied between 20 and 28°C similar to our results at saturating and sub-saturating irradiances, but in these studies the lower limit was found between 4 and 10°C (Uehlinger, 1981; Tsujimura et al., 2001). We assume that the pre-experiment growth temperature could affect the temperature responses of *A. flos-aquae*, as it has been shown in case of *Anabaena* (Scherer et al., 1981). This was confirmed by the results of Yamamoto & Nakahara (2005, 2006). They found, that the growth of *A. flos-aquae* maintained at 20°C, ceased at water temperature 11–12°C, and the lowest temperature at which *A. flos-aquae* could grow was 14°C. Konopka & Brock (1978) observed significant photosynthesis of natural samples at 4°C, but those samples were collected or maintained at higher temperatures than the natural samples in this study. At the highest temperature (35°C) applied in our experiment, there was a drop in the photosynthesis of the natural population of *A. flos-aquae*; the species showed moderate photosynthetic activity, similar to the results of Butterwick et al. (2005). Previously the photosynthetic activity of overwintering natural populations of *A. flos-aquae* was not studied at such a low temperature (2°C) and irradiance (7.5 μmol m^{-2} s^{-1}), therefore our findings in this temperature and irradiance levels cannot be compared to results of previous studies.

According to the P–T curves, the photosynthetic activity correlated negatively with the temperature under light-limited conditions. New and interesting result is that at the lowest irradiance level (7.5 μmol m^{-2} s^{-1}) the temperature optimum was at 2°C, and it was between 2 and 10°C at the irradiance of 30 μmol m^{-2} s^{-1}. For biological functions, the Q_{10} value is ranges generally between 1 and 3 (e.g. Bissinger et al., 2008). According to our results, the Q_{10} values at the applied lowest irradiances were <1 (0.67–0.87), and at irradiances >150 μmol m^{-2} s^{-1} it was between 1.67 and 2.88, similarly to other studies. These values suggest responsiveness to temperature changes only at mid and higher irradiance levels. These results can change our previous knowledge about the temperature dependence of the photosynthetic activity, and suggest that the responsiveness to temperature changes can be affected by other environmental parameters, like irradiance level. The interaction between temperature and the light availability affected the photosynthetic characteristics of *A. flos-aquae*, but the physiological distinctive features and survival strategies of the species contributed to the success at such extreme conditions.

The ability of *A. flos-aquae* to adapt to light-limited conditions was found higher than other cyanobacterial species (De Nobel et al., 1998). According to our study, the photoadaptation parameter was at each temperature low, the values were similar to those measured by De Nobel et al. (1998). The natural population of *A. flos-aquae* was more susceptible to photoinhibition due to the low-light conditions they grow in Lake Stechlin. At 2°C, the photoadaptation parameter was very low (20 μmol m^{-2} s^{-1}), but the photosynthetic efficiency was at this temperature the highest in the light-limited region, which suggests a competitive advantage of *A. flos-aquae* in case of light-limited conditions. The increase in the photosynthetic efficiency could have been caused by the changes in the ratio of phycobilin/chlorophyll *a* (Vincent, 2007). Photoinhibition was observed at each temperature (except 35°C), the poor ability to utilize high irradiances indicates the adaptation to low irradiance levels. Photoinhibition by PAR usually becomes increasingly severe at low temperatures (e.g. Krause, 1994), but in this study we found low

photoinhibition in the low-temperature range. The low photoinhibition at low temperatures can be attributed to the changed pigment composition and function of the photosystems (Lazarova et al., 2009); similar responses were observed in polar cyanobacteria to withstand the extremes of their environment (e.g. Vincent, 2007).

The deficiency of this study is that the pigment composition of the community was not examined; only the chlorophyll *a* was measured. According to previous studies cyanobacteria which contain phycoerythrin and phycoerythrocyanin, like *A. flos-aquae*, have competitive advantage in light-limited environments (Huisman et al., 1999). Phycoerythrin and phycoerytrocyanin synthesis did appear to be affected by light intensity (Gervais et al., 1997) and composition (Huisman et al., 1999). The light availability under the ice and snow cover was very low in Lake Stechlin in winter 2009/2010, the irradiance varied between 15 and 130 μmol m^{-2} s^{-1} in the upper 3 m layer during the day. Assuming that the spectral composition of light was similar in Lake Stechlin to that in Lake Pääjärvi (Lei et al., 2011), the green light dominated in the euphotic layer. At low-light conditions, *A. flos-aquae* could absorb green light (525–600 nm range) more efficiently because of its pigment composition (De Nobel et al., 1998; Huisman et al., 1999), therefore the spectral composition of the light under the ice and snow cover was favourable to its development. Despite our results show that the physiological plasticity of the species could enable them to develop deep chlorophyll maxima (DCM), in previous studies the appearance of the species in deeper water layers was not described nor in winter either in summer periods.

According to our results, the studied *A. flos-aquae* population had very similar ecophysiological characteristics to high-latitude cyanobacteria, which are dominant in cold ecosystems (Vincent, 2000): (i) *A. flos-aquae* could grow over a wide temperature range; (ii) at low temperatures it grows at slow rates; (iii) the low photoadaptation parameter enables the growth in dim light environment. Most of the previous studies about cyanobacteria supported the tenet that the optimum of photosynthesis and growth are above 20°C (e.g. Paerl & Huisman, 2008). This was also observed for cyanobacteria isolated from Antarctica: the species could grow over a wide temperature range (5–30°), but most of them were unable to grow at temperatures <5°C. Therefore, the polar cyanobacteria species are thought to be originated from warmer temperate regions (Seaburg et al., 1981). During long period of their evolution, they developed various adaptive mechanisms (e.g. Vincent, 2000), which contributed to their success and dominance in cold ecosystems. Microbial communities of cold environments are often unusual and intrinsically interesting because they have been subject to long periods of isolation with relatively low levels of disturbance (Vyverman et al., 2010). Not only the climate change (e.g. Vincent et al., 2009, 2011), but the species with high ecophysiological plasticity and success may have profound effects on the structure and efficiency of the food webs. *A. flos-aquae*, because of its ecophysiological plasticity and two temperature optima of its photosynthesis, can spread in habitats where its appearance was inconceivable according to our previous knowledge. The spread of different cyanobacterial species in temperate and polar regions at higher latitudes is often explained with the climate change, but an ongoing cyanobacterial adaptation cannot be ignored. The ecophysiological characteristics of species, like *A. flos-aquae*, may help us to understand the previous adaptation of cyanobacteria to cold environments, and their success in invasion and in extreme environments.

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PHYTOPLANKTON

The queer *Tetraëdron minimum* from Lake Kivu (Eastern Africa): is it a result of a human impact?

Maya P. Stoyneva · Jean-Pierre Descy · Vanessa Balagué · Pierre Compère · Maria Leitao · Hugo Sarmento

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Abstract The coccal unicellular green algal genus *Tetraëdron* Kütz. ex Korshikov, which can be easily identified by its typical polygonal shape, is a common member of freshwater plankton and metaphyton, frequently observed in lowland temperate and tropical waters. During the analysis of samples from tropical Lake Kivu (Eastern Africa), we found an interesting "lemon-shaped" alga, which, after observations in light microscope and scanning electron microscope, had been listed as *Tetraëdron* sp. Isolation in pure culture allowed a deeper study on morphology at different stages of the life cycle and the partial sequencing of the 18S rDNA. The results from the different combined approaches confirmed that it belongs to the species *Tetraëdron minimum* (A. Braun) Hansg. The unusual "lemon-shaped" forms predominant in Lake Kivu are young stages of the life cycle. This study contributes to the knowledge of the morphological variability, reproduction, and resting stages of *T. minimum* and discusses the reasons for the dominance of such unusual shape found in Lake Kivu, a lake strongly impacted by human activities as resulted by the large-scale biomanipulation following the introduction of the "Tanganyika sardine," *Limnothrissa miodon* (Boulenger, 1906), at the end of the 1950s.

Keywords Green algae · Phytoplankton · Zooplankton · Grazing · Tropical lake · Akinetes

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Introduction

"Although, from one point of view, *Homo sapiens* is just one more species among the millions, it is unique in its power to influence the environment of all the others" (Reynolds, 1997, p. 295). From this sentence, it is possible to turn to a special problem of a great concern—the human impact on the phytoplankton.

This peculiar community consists of organisms, and the distribution and success of each of them is a function of abiotic constraints and biotic processes with a hierarchical importance of different factors (Brönmark & Hansson, 2005). The abiotic environment of a water body often is altered by human-induced disturbances (Brönmark & Hansson, 2005), and the effects which some of them cause to the phytoplankton are generally well known. Among them are the consequences of eutrophication, acidification, and biomanipulation. However, in spite of the increasing number of reports on exotic species introductions and to the influence of invasive alien species on biological diversity and ecosystem integrity, it is possible to stand that the consequences of such introductions at the level of morphological variation of a given species, belonging to a different trophic level from that of the allochtonous species, are practically unknown.

This article shows the probability for the occurrence of unusual small-shaped cells of *Tetraëdron minimum* due to changes in the grazing pressure in tropical Lake Kivu, ca. 50 years after the introduction of the planktivorous endemic sardine *Limnothrissa miodon* from the lake Tanganyika in order to "improve" the food web and to become the basis of fisheries activities (Collart, 1960; Simberloff, 1995). With this article, based on microscopic observations on field and cultured material in combination with molecular methods, we would like to contribute also to the knowledge on the cytology, reproduction, and resting stages of *T. minimum.*

Materials and methods

Study site and sampling procedures

Lake Kivu, located between Rwanda and the Democratic Republic of the Congo (Kivu Province), is one of the Great Lakes of the East African Rift Valley and is formed by four main basins (Fig. 1). It is a deep (max. 489 m), meromictic lake, with an oxygenated epilimnion of about 70 m and a deep hypoliminion rich in dissolved gases (CO_2, methane). With an annual average of chlorophyll *a* in the mixed layer of 2.2 mg m^{-3} and primary production of 0.71 g C m^{-2} day^{-1} ($\sim$260 g C m^{-2} $year^{-1}$), the lake is clearly oligotrophic (Sarmento et al., 2006, 2009).

Phytoplankton samples were collected regularly from September 2002 till February 2004 twice a month in the southern basin, while northern, eastern, and western basins were visited twice a year (once in the dry season and once in the rainy season). The qualitative samples were collected by vertical plankton net (10 μm mesh size) in the 0–60-m layer, and the quantitative samples were collected with a Van Dorn bottle at different depths (surface, 5, 10, 20, 30, 40, 50, and 60 m). The samples were preserved immediately after collection with neutral formaldehyde (2–4% final concentration) and Lugol solution. Before observation, the samples were concentrated by settling. For scanning electron microscopy (SEM), samples were fixed with glutaraldehyde at 1–2% final concentration. More details on sampling sites and procedures can be found in Sarmento et al. (2007).

Strain isolation and cultivation

In September 2008, half a liter of subsurface water from Lake Kivu was shipped to the Institut de Ciències del Mar (CSIC) in Barcelona (Spain), within 48 h and then enriched with an equivalent volume of BG-11 culture medium. In sterile conditions, serial dilutions of the mixture were carried out in 12-well polystyrene plates. The plates were sealed with parafilm and incubated at 23°C under artificial photosynthetic active radiation (PAR) of 100 μmol photon m^{-2} s^{-1}, in a 16:8-h light:dark cycle for several weeks. Microbial growth was regularly checked directly on the plates without opening it, under an inverted microscope at 40× magnification. The wells in which the specimen of interest was found in large abundance were transferred to BG-11 culture medium agar plates for strain isolation. Individual dark green colonies were re-grown in fresh BG-11 liquid culture medium and filtered through a 0.2-μm filter (Durapore 47 mm), preserved in 750 μl of lysis buffer (40 mM EDTA, 50 mM Tris–HCl, 0.75 M sucrose) and stored at −80°C until nucleic acid extraction (Fig. 2).

In December 2008, a part of the material was transported to the Algal Collection of Sofia University (ACUS). There, the material was analyzed immediately by means of light microscopy (LM) and then transferred on new agar plates, enriched by Bold Basal medium (BBM). The transfer followed standard techniques (Ettl & Gärtner, 1995; Andersen, 2005). In an attempt to observe zoospore production, BBM

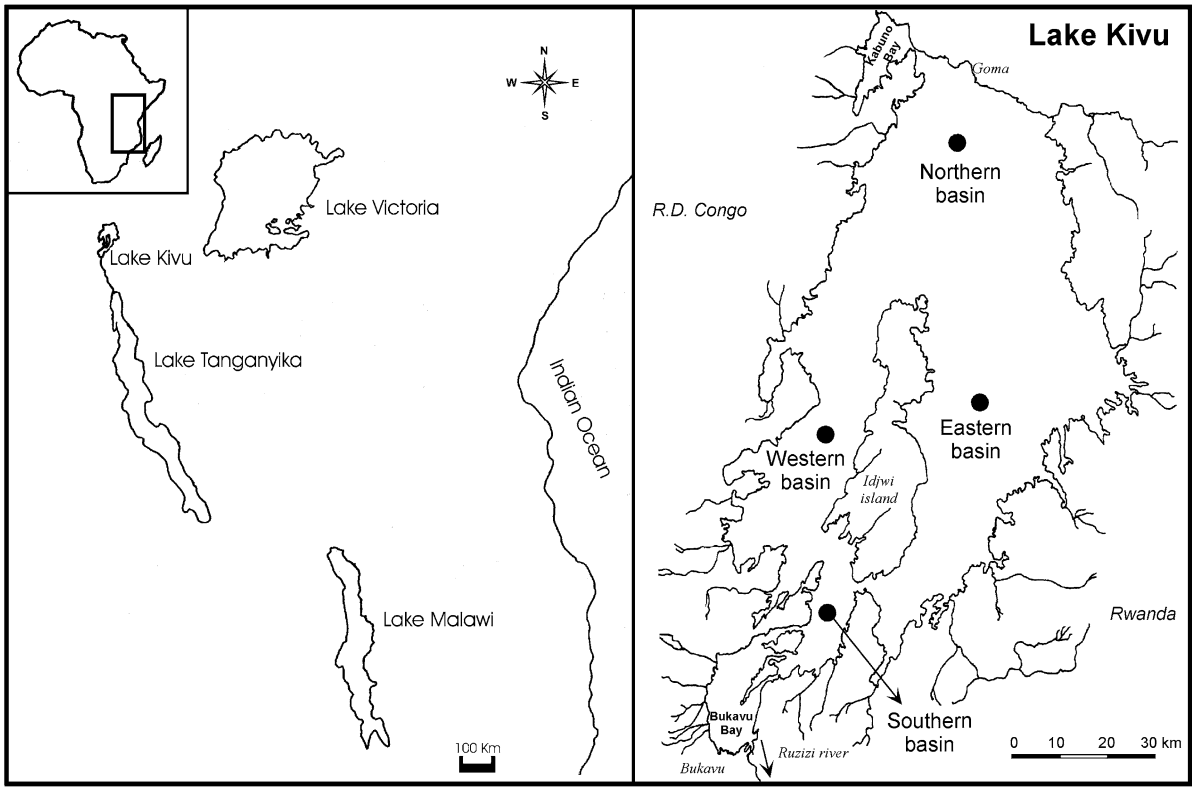


Fig. 1 Geographic situation of Lake Kivu with indication of its four major basins

liquid cultures were regrown several times, from the agar cultures. They were kept in darkness for ca. 16 h and then checked for zoospores.

LM and SEM processing with photo documentation of field and culture material

Light microscopic (LM) investigations were done on Olympus BX-50 (field material) and Motic BA 400 (culture material) microscopes with objectives 40× and 100× (oil immersion), both equipped with phase contrast. SEM study was done on Philips XL-microscope. Cell walls were stained with Gentian violet and Methylene Blue, and starch was colored with Lugol's solution (Ettl & Gärtner, 1995). Photomicrographs were taken with an Olympus Camedia digital camera (field material) and Moticam 2000 camera attached to the Motic BA 400 microscope with special adaptors (culture material). For processing of the photos, the computer software "Motic Images Plus 2.0" was used.

Nucleic acid extraction, amplification and sequencing

Nucleic acids were extracted by adding lysozyme (1 mg ml^{-1}) to the filter unit and incubating at 37°C for 45 min. Subsequently, proteinase K (0.2 mg ml^{-1}) and sodium dodecyl sulfate (SDS, 1%) were added, and the filter was incubated at 55°C for 1 h. The lysate was then extracted twice with an equal amount of phenol–chloroform–isoamyl alcohol (25:24:1, pH 8) and once with an equal amount of chloroform–isoamyl alcohol (24:1). The aqueous phase was spun down in a microconcentrator (Amicon-100, Millipore), washed with 2 ml of sterile MilliQ water three times, and reduced to a volume of 100 µl. The recovered DNA was quantified using Nanodrop (Thermo Scientific). Nucleic acid extract was stored at −80°C.

One nanogram of DNA was used as template for PCR amplification of eukaryotic 18S rDNA. The reaction (50-µl volume) contained 200 µM of each of the deoxynucleoside triphosphates, 0.5 µM of each of the primers, 1.5 mM $MgCl_2$, 1× PCR-buffer and 1.25

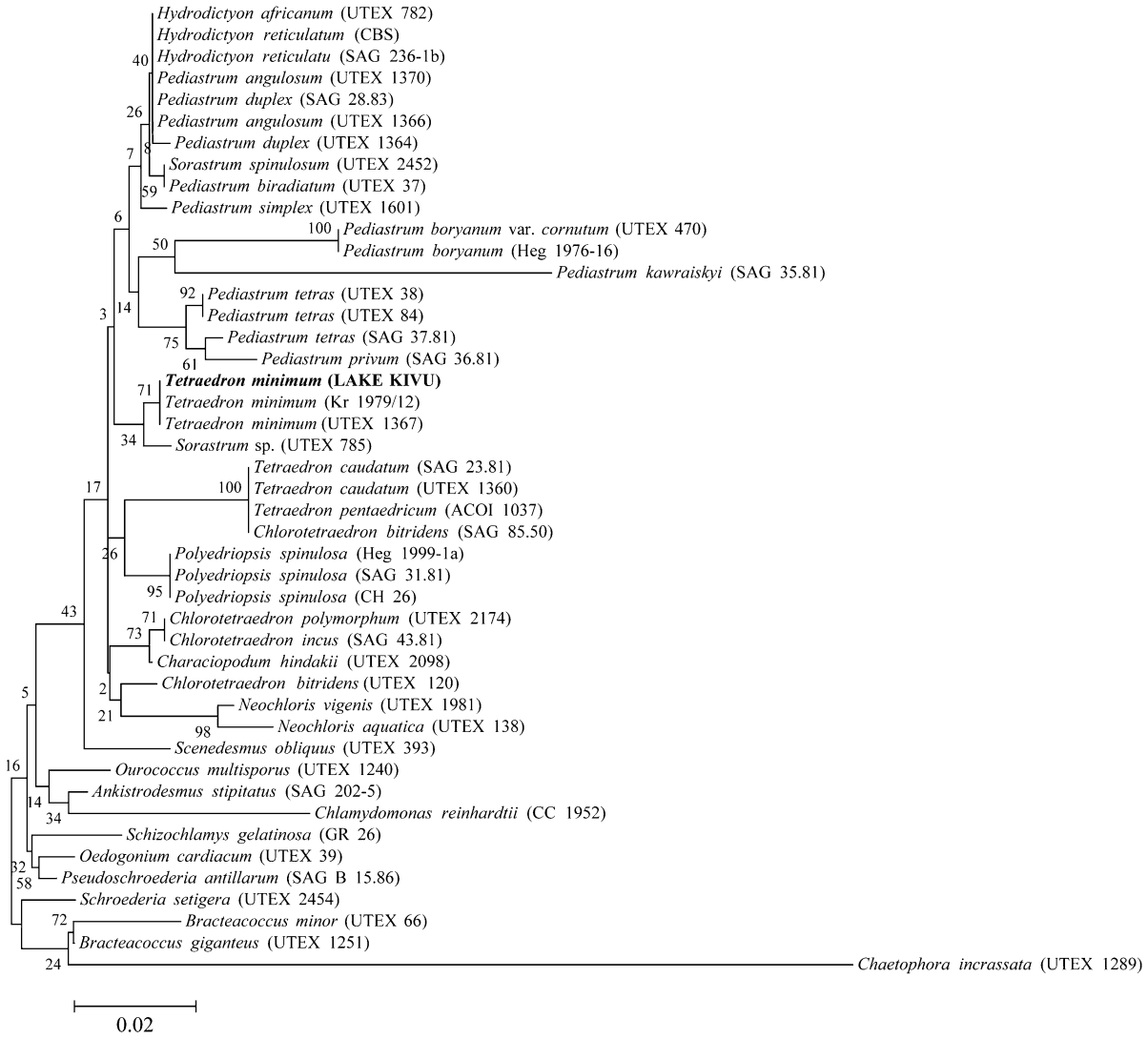


Fig. 2 Molecular phylogenetic analysis by maximum likelihood method. The evolutionary history was inferred using the maximum likelihood method based on the data specific model (Nei & Kumar, 2000). The bootstrap consensus tree inferred from 1,000 replicates is taken to represent the evolutionary history of the taxa analyzed (Felsenstein, 1985). Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1,000 replicates) is shown next to the branches (Felsenstein, 1985). The rate variation model allowed for some sites to be evolutionarily invariable ([+I], 64.2418% sites). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. All positions containing gaps and missing data were eliminated. There were a total of 345 positions in the final dataset. Evolutionary analyses were conducted in MEGA5 (Tamura et al., 2011)

Units of Taq DNA Polymerase (Invitrogen). We used the eukaryotic specific primers EUK1F (5′-AAC CTG GTT GAT CCT GCC AGT-3′) and 516r (5′-ACC AGA CTT GCC CTC C-3′). The PCR was performed with a thermal cycler (Bio-Rad) using the following program: initial denaturation at 94°C for 2 min 10 s; 30 cycles of denaturation (at 94°C for 30 s), annealing (at 56 for 45 s) and extension (at 72°C for 2 min 10 s); and a final extension at 72°C for 10 min. PCR products were verified and quantified by agarose gel electrophoresis with a standard in the gel (Low DNA Mass Ladder, Invitrogen).

Positive PCR products were purified and sequenced by Macrogen Sequencing Service (South Korea). The

nucleotide sequence was deposited in GenBank under accession number BankIt1523118 LKO1 JQ797441. The sequence obtained was aligned with the software MEGA 5.05 (Tamura et al., 2011) and compared with DNA sequences from algal culture collections used by Buchheim et al. (2005). Evolutionary analyses were conducted in MEGA5.05 (Tamura et al., 2011).

Results

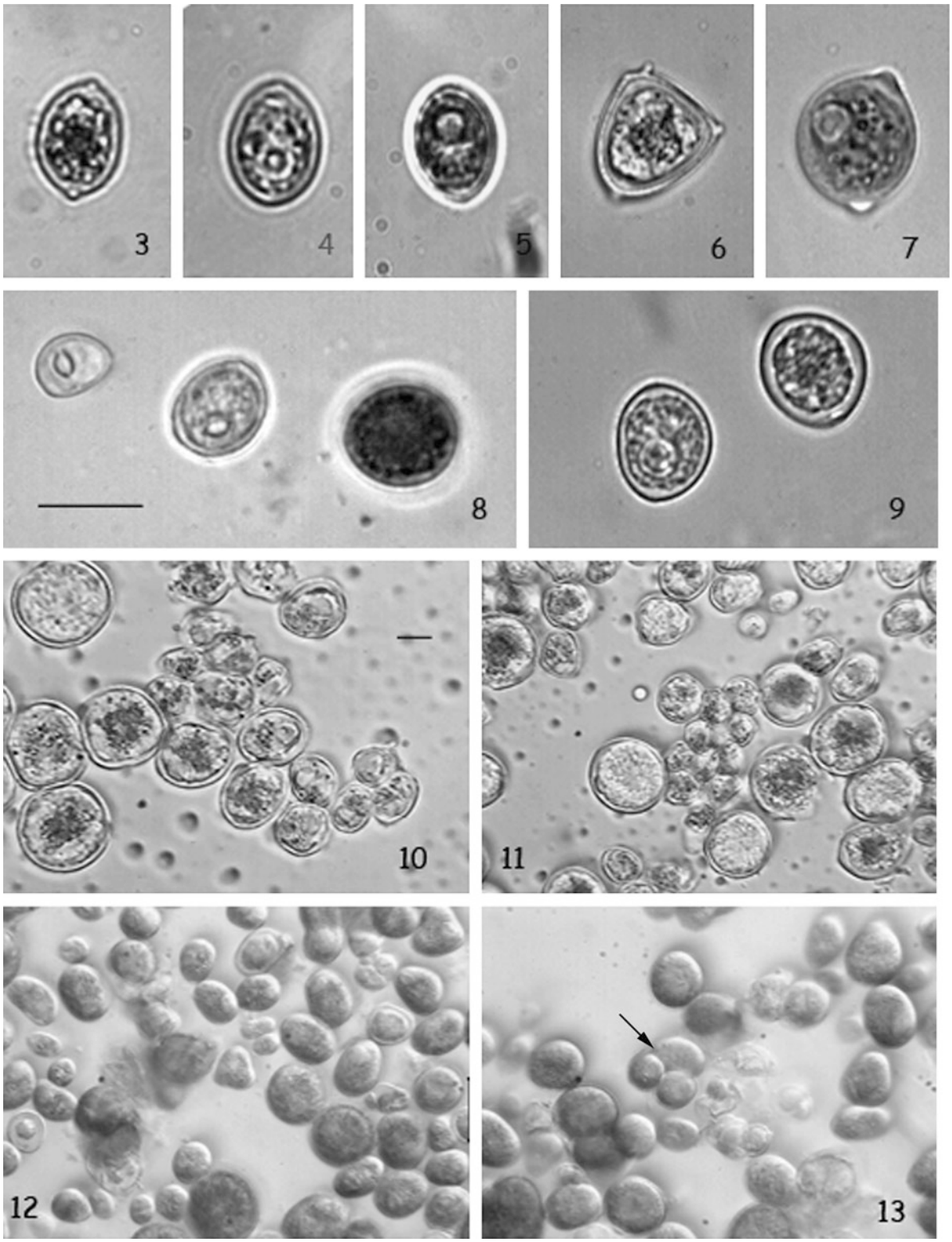
By means of LM in almost all phytoplankton samples from Lake Kivu, a free-floating alga with peculiar cell outline was found. The cells were solitary, ovoid, asymmetric to pyriform when seen in side view, and triangular (very rarely quadrangular) in front view, (5)–7–12–(14) µm in diameter. Each cell bears one or two, very rarely three or four, short-thickened polar protuberances, which were important for the "lemon-shaped" outline of the cell (Figs. 3–9, 20b). On higher magnifications, the rough character of the cell wall was visible, and by SEM, its scrobiculated character was confirmed (Figs. 14–17). Each cell contained a parietal, massive chloroplast, with a single pyrenoid (Figs. 5, 7–9) and oil droplets (Fig. 4). The pyrenoid bears a starch sheath, clearly visible after staining by iodine (Figs. 5, 8), thus confirming the disposition of the alga in the green lineage. The reproduction stages were rarely observed in the field material. They were represented by more or less developed autosporangia with 4 (8) autospores. Their release was preceded by cell wall rupture and its division in two parts. In the field material, the autospores have the peculiar "lemon-shape" of the free-floating cells, while some of the autosporangia showed a tetrahedral character. This was the first clear feature, which inspired the idea that the alga under investigation belonged to the genus *Tetraëdron.*

The next step—species identification—was more complicated. Finally, on the slides from the field material, we came to the conclusion that features observed overlap partially with the descriptions of two species, known for their polymorphism—*Tetraëdron regulare* Kütz. auct. post. (incl. var. *ornatum* Lemmerm.) and *T. minimum* (incl. var. *scrobiculatum* Lagerh.)—Sarmento et al. (2007). At the same time, we had to take into account that in less than 10% of the samples, in small amount, typical, and well-developed tetrahedral cells with pronounced protuberances of *T. regulare* were observed (Fig. 16 in Sarmento et al., 2007). In this case, the only possible correct solution was to postpone the final identification decision and to list the material as *Tetraëdron* sp. (Sarmento et al., 2007, Figs. 16, 47–50, 66) until we study it in pure cultures.

After the isolation of a clonal culture (in 2008), a part of the material was transferred for long-term cultivation on agar, and a small amount was immediately controlled for eventual zoospore production. However, zoospores were not observed. The first observations by LM of the material on agar plates did not reveal new features or significant morphological deviations compared with the data obtained from field material, except more abundant autosporangial and autospore production. In April 2009, on the original plate, sent to ACUS, a drying of the agar was detected, attended by the change in the color of algal stripes from green to yellowish-green. This was due to the abundant presence of akinetes—large (up to 25–30 µm in diameter) spherical cells with thick cell walls, which probably contained haematochrome (Figs. 10, 11). Some of them were in stage of division in two. The akinetes were immediately transferred to new agar plates.

In May 2010, PCR amplification and sequencing of the 18S rDNA of the material previously conserved from the first clonal cultures revealed the phylogenetic affiliation of the Kivu alga to *T. minimum* (Fig. 2). The partial sequence obtained was 100% similar to those of two *T. minimum* strains from other culture collections (Kr 1979/12 and UTEX 1367).

The culture material was studied again by LM from September 2010 to April 2011, after the algae in the new cultures, obtained from the akinetes, were developed more abundantly (Figs. 12, 13). Then an alteration in the abundance of the well-developed vegetative cells (some with typical for *T. regulare* shape) and autosporangia (Figs. 13, 18–28), and the smaller "lemon-shaped" cells was observed: the last ones dominated in February 2011 and then again in April 2011. All of the well-developed single vegetative cells were of bright green color and contained very large pyrenoids. Their starch sheath was clearly visible even without iodine staining (Fig. 7) and generally shows a bipartite character (Figs. 5, 8, 9). Again, a part of the material was transferred in BBM liquid and afterward checked by LM for zoospore formation. However, only autosporangia together with small "lemon-shaped" cells were recorded.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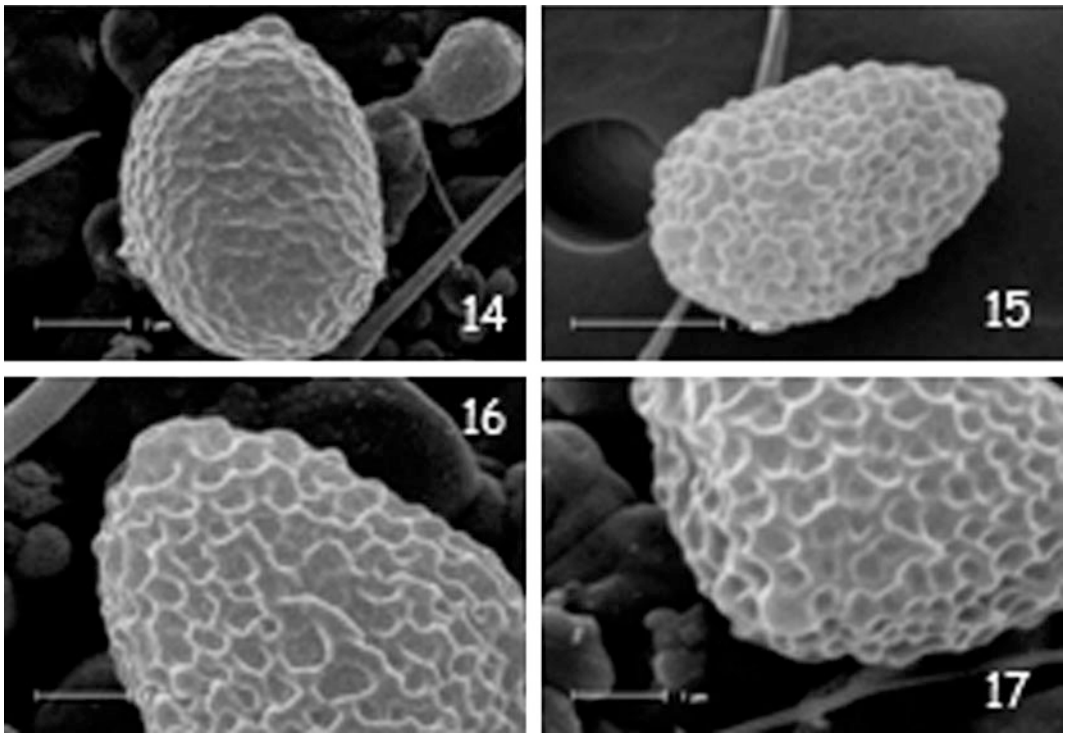
◀ **Figs. 3–13** *Tetraëdron minimum* in LM: 3–7, 9—single cells of the species; 8—single cells and initial autosporangium; 10, 11—akinetes with large *spots* or total content with resemblance to haematachromes and single cells in a drying culture; 12, 13—general view on a culture, developed from akinetes with new well developed vegetative single cells (some of them with typical triangular outfit like the cell in the centre of the photo, some more ovoid or spherical; among them smaller "lemon-shaped" cells could be seen), new young autosporangia and autosporangium with consecutive formation of autospores (arrow). Scale bar for Figs. 3–9—5 μm, for Figs. 10–13—10 μm

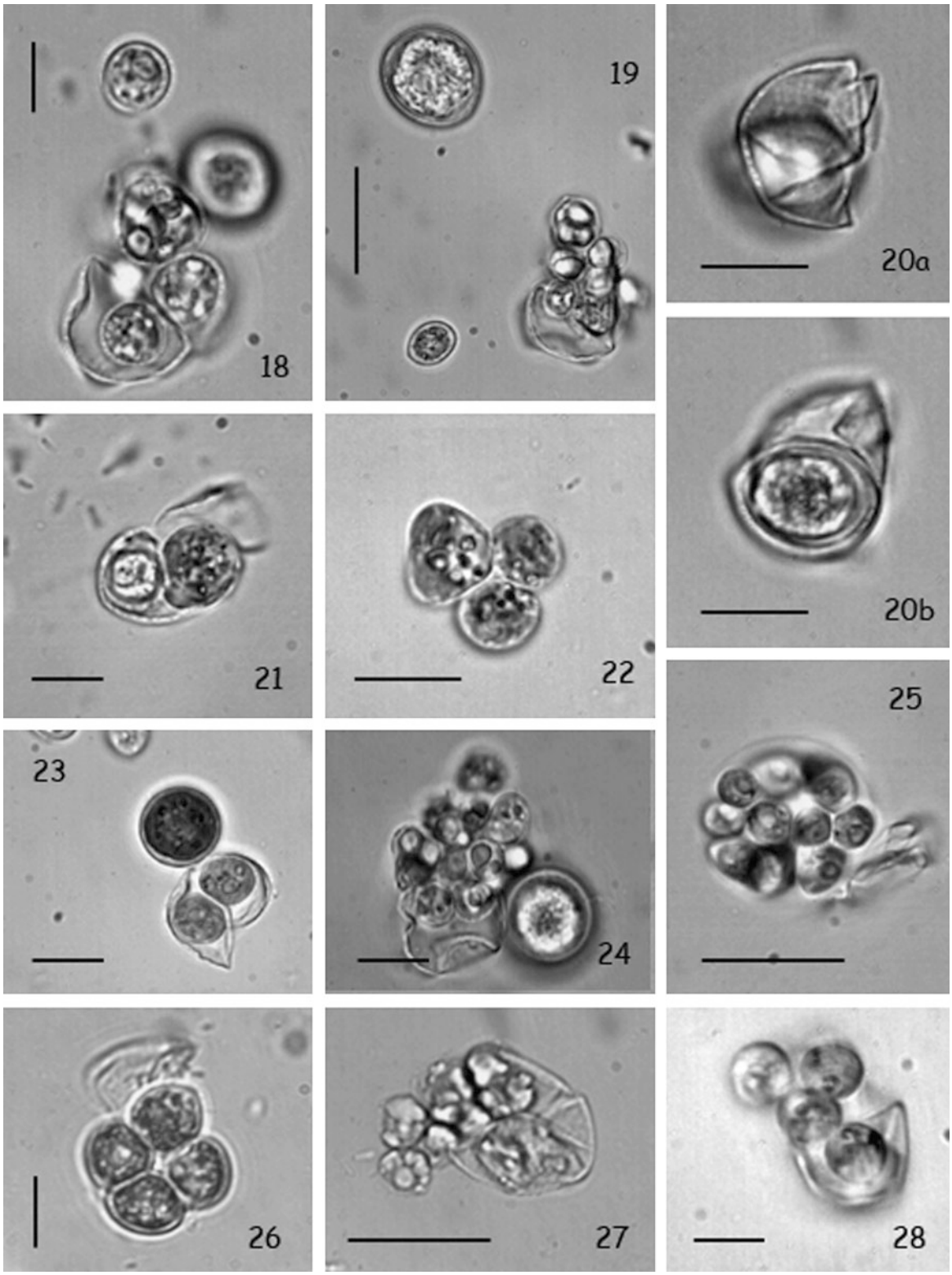
Discussion

The observations on the morphology and reproduction show that the peculiar alga, found in the Lake Kivu, belongs to the genus *Tetraëdron* and is able of asexual reproduction by autospores and, additionally, of akinete formation. Until recently, production of akinetes as a process of enlargement of cells under harsh conditions supplied by increase of dimensions and changes of the shape and coloration (from green to yellow or red) in *Tetraëdron* was described only by Troitzkaja (1933) for *T. minimum* and for *T. regulare*, and later by Korshikov (1953) for *T. incus* (Teiling) G. M. Sm. and by Davis (1966) for *T. bitridens* Beck-Managetta. All of the aforementioned authors noted their role as resting stages, but only Troitzkaja (1933) used the term "cysts" instead of "akinetes." Both terms could be applied to the "giant" cells, observed in our cultures, because they were formed in asexual way from vegetative cells through enlargement, supplied by cell wall thickening and change in cell protoplast toward production of reddish content (possibly haematochrome). Due to this asexual (vegetative) way of forming, which is not always clear when term "cyst" is used (Ettl, 1980) we prefer to refer the stages found as "akinetes." The vegetative division of akinetes, observed by us, is rarely reported in the physiological literature, but is a known process (Ettl, 1980).

The main cytological features observed in the vegetative cells (e.g., parietal chloroplast, single distinct pyrenoid with starch sheath, oil droplets) are on conformity with all former observations (see Kováčik, 1975 for details). Our records clarify the general bipartite character of the starch sheath around



Figs. 14–17 *Tetraëdron minimum* in SEM: 14, 15—general view on total cells; 16, 17—parts of cell surface with scrobiculated cell wall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◀ **Figs. 18–28** Autospores and autosporangia of *Tetraëdron minimum* in LM: 18, 22, 26, 28—autosporangia with four autospores (18 and 28—the release of the autospores after rupture of the autosporangium wall in two parts is seen; 22—autosporangium with one large and two smaller cells, showing the consecutive formation of autospores); 19, 24, and 27—autosporangia with more than four autospores; 20, 21, and 23—autosporangia with two autospores and ruptured autosporangium wall; 25—released autospores in a mucilage vesicle with remnants of the autosporangium wall beneath them. Scale bar for Figs. 18, 20–23, 26, and 28—5 μm, for Figs. 19, 24, 25, and 27—10 μm

the pyrenoid. The two parts of the sheath are distinct on the first photo from Plate 4 in the paper of Pickett-Heaps (1972) and on one drawing provided by Kovácik (1975, p. 365, plate 3c), but were not described or discussed by the authors. It could be supposed that the bipartite character of the pyrenoid sheath is typical for the genus. The cell wall surface in its outline by LM and its vision in SEM coincide with the data of Kováčik & Kalina (1975) on cell wall surface of *T. minimum*. The cell dimensions and mode of asexual reproduction by autospores only, as well as the data from molecular analyses, are on conformity with the same species.

The more frequent appearance of tetrahedral cells, resembling in outline *T. regulare*, than of more flat, quadrangular cells, widely known as typical of *T. minimum*, is on conformity with the data of Troitzkaja (1933), who underlined the simultaneous appearance of both types of cells in clonal cultures of *T. minimum*. However, the predominance of the peculiar "lemon-shaped" outline (with one or two small protuberances) of most of the cells found in the field and in some of the cultures provokes the question about the reasons which trigger the alga to appear in this form instead of its typical, widespread polygonal shape?

The change of the ratio of polyhedral and non-polyhedral cells in cultures of different age and observations of autosporangia and their development lead us to the idea that the "lemon-shaped" cells are just juvenile stages in the development of normal vegetative cells. This hypothesis finds support in the published details and illustrations on the development of different *Tetraëdron* species (e.g., Troitzkaja, 1933; Starr, 1954). After prolonged observations in cultures of *T. bitridens*, Starr (1954, p. 19) wrote that generally "each autospore is an exact replica of a mature vegetative cell, although, in some instances, where the spores are retained within a sporangial wall for a long time, the autospores may have less pronounced angular processes." Troitzkaja (1933) showed that in *T. minimum* the enlargement of the cell and increase in volume sometimes did not start from the central region (when a classical tetrahedral shape is formed), but from one of the sides, bringing to irregular, asymmetrical outline of the whole cell.

The data and conclusions of Kováčik & Kalina (1975) on cell wall surface, observed by SEM, are also on conformity with the idea that "lemon-shaped" cells found in Kivu waters and are young stages of *T. minimum*. The authors postulated that the network character of *Tetraëdron* cell wall surface, found by them in *T. caudatum* (Corda) Hansg. and *T. minimum*, is typical for the genus and the superficial undulation represents corrugation of two surface layers of the cell wall. Detailed analysis showed ontogenetical differences in the thickness and folding of the layers: in young cells the periphery of the middle layer is abundantly folded and the network is very dense, whereas in older cells the corrugated surface of outer layers evens out, the network thins out, and is composed of larger, often interlocked meshes. In old, large, rounded cells, the network is reduced or the cell surface is completely smooth (op. cit.). The photos of Kivu material, obtained by means of SEM, clearly show a well-developed mesh-network of the cell surface with deep folds (Figs. 14–17), which confirms that the "lemon-shaped" cells are young stages in development phase.

Alone, this result cannot explain the predominant occurrence of the immature stages of *T. minimum* in the oligotrophic Kivu waters. Lake Kivu phytoplankton composition is peculiar when compared to that of the other Rift Lakes (Sarmento et al., 2007): it is dominated by diatoms, cyanoprokaryotes (cyanobacteria/blue-green algae), and cryptophytes. Green algae, which, for example, in Lake Tanganyika are a dominant group, have here a secondary role in terms of abundance and biomass (Sarmento et al., 2006, 2008). Their diversity is also low, and the few taxa found are mainly colonial coccal green algae and desmids; unicellular forms are comparatively rare. This suggests that most green algae of Lake Kivu are grazing-resistant forms, as Stoyneva et al. (2007) supposed for a new *Eremosphaera* taxon in Lake Tanganyika.

Grazers in Lake Kivu are essentially three species: two cyclopoid copepods and one cladoceran (Isumbisho

et al., 2006). Although their grazing rates on algae are not known, their diet was studied using fatty acids (FA) as biomarkers: Masilya (2011) measured FA in several zooplankton size classes and found that small zooplankton (i.e., the 50–100 μm size class, comprising rotifers and copepod nauplii) fed essentially upon cryptophytes and diatoms. Copepods in the 100–300-μm size fractions also consumed chrysophytes, while the larger copepods (>300 μm) fed on cryptophytes, chrysophytes, and cyanoprokaryotes. FA from green algae were not found in zooplankton fractions, although they were present in the seston fractions. This indicates that green algae were either not ingested by zooplankters or that they were ingested but not assimilated.

Regarding *T. minimum* autospores, they are in the lower range of edible size for copepods (see, e.g., Sterner, 1989), and this could be a refuge strategy from grazing by the most abundant zooplankters in Lake Kivu. Small algae are more readily grazed by rotifers and by herbivorous protists. Rotifers are abundant in Lake Kivu, in contrast with the other oligotrophic Rift lakes (Isumbisho et al., 2006), and large ciliates are also present, although data on their abundance are lacking. Therefore, small algae, in order to survive in environments where grazing pressure is high and permanent, need to have traits that provide adequate refuge from grazers. In the case of *T. minimum*, fast reproduction rates with mass formation of autospores are a clear advantage for compensating grazing losses. Another trait which could be seen as a defense mechanism is the hard cell wall of *Tetraëdron* with high algaenans content, where the biopolymers are composed of long-chain even-carbon-numbered ω^9-unsaturated ω-hydroxy FA monomers varying in chain length from 30 to 34 carbon atoms (Blokker et al., 1998). This renders the cells and autospores resistant to digestive enzymes: several authors have reported that ingested phytoplankton cells may transit through zooplankton guts and be egested undamaged and able to grow (see a review of defense mechanisms in Van Donk et al., 2011).

Thus, grazing pressure in this tropical great lake may explain why these peculiar stages of *T. minimum* are so abundant and make the bulk of the population of this alga. In Lake Tanganyika, another green alga, *Eremosphaera tanganyikae* Stoyneva, Cocquyt, Gärtner, and Vyverman, efficiently escapes grazing thanks to large cell size (Stoyneva et al., 2007): this is a similar "strategy," at the other extreme of the size spectrum of the main grazers. A similar process was described for planktonic bacterial communities, who under high grazing pressure show a higher proportion of extremely small coccoid shapes or large filaments, out of the edible range for predators (Jürgens & Güde, 1994). The role of grazing in molding the size and shape of phytoplankters was recently summarized by Naselli-Flores & Barone (2011), who showed that inducible defenses in prey traits in response to predation risk are particularly common in natural systems and that these low energetic cost adaption reduces phytoplankton mortality due to herbivory.

As for the "human impact" issue, it may be indirectly involved in these morphological defenses of *T. minimum*. Indeed, Lake Kivu is an example of large-scale biomanipulation, which consisted in the introduction of the "Tanganyika sardine," *L. miodon*, at the end of the 1950s (Collart, 1960). This planktivorous fish induced important changes in the zooplankton structure, affecting both composition and abundance (Dumont, 1986). Thus, present zooplankton of Lake Kivu, different from that of the other Rift lakes in several respects, and the related grazing pressure, are the result of a major anthropogenic change. Due to the lack of detail in the knowledge of phytoplankton structure and composition before the sardine was introduced, the extent of the changes affecting the whole plankton is not easily evaluated. However, Sarmento et al. (2012) estimated that crustacean abundance may have declined by a factor of three as a result of the introduction; also, a major grazer, a large cladoceran has disappeared (Dumont, 1986). It is likely that a reduction in zooplankton body size distribution occurred from predation on large zooplankton (Brooks & Dodson, 1965), and that small zooplankton (small crustaceans, rotifers and protists) became more abundant, thereby resulting in an increased grazing pressure on small phytoplankton. In this context, phytoplankton taxa exhibiting traits providing efficient defense against grazing may have had an advantage, and this may explain the peculiar morphology of *T. minimum* in Lake Kivu as a device for reducing grazing losses, exploited consequently after the human impact on the food web of the lake.

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PHYTOPLANKTON

Changes in galactolipid composition of the cold freshwater dinoflagellate *Borghiella dodgei* in response to temperature

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Abstract The freshwater dinoflagellate *Borghiella dodgei* is adapted to cold temperatures. We investigated the effects of small temperature changes on its galactolipid composition, choosing 3 and 7°C as deviations from its optimal growth temperature (5°C). The galactolipid profile, important for maintenance of membrane fluidity, was determined by liquid chromatography–mass spectrometry and the influence of temperature on galactolipids was investigated by one-way ANOVA. We found 24 different galactolipid species, including novel tri-galactosyldiacylglycerols (TGDGs). The overall amount of mono- (MGDG), di- (DGDG) and tri- (TGDG) galactosyldiacylglycerols remained stable while single galactolipids varied with temperature. Few changes were found from 3 to 5°C, instead 11 galactolipid species changed from 5 to 7°C. Concomitantly with the unsaturation index of MGDGs, the more unsaturated galactolipids decreased at higher temperature, and the less abundant and less unsaturated galactolipids in each lipid class accumulated. Changes in the galactolipid profile of *Borghiella* underlined its cold-stenothermal nature: it can adapt to relatively 'higher' temperatures by reducing the synthesis of the more unsaturated MGDGs, DGDGs and TGDGs, but remains restricted by its lower growth rate. Based on our results, we predict that with climate change the galactolipid profile of cold-stenothermal algae will change with important repercussions on their consumers.

Keywords PUFA · MGDG · DGDG · TGDG · Climate change · Algae

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Introduction

In poikilotherms, response to temperature changes involves several important physiological processes including the modification of membrane lipid composition. While these aspects have been well studied in higher plants, the lipid structure, metabolism and other aspects of biochemistry have been rather poorly studied in eukaryotic algae (Harwood, 2004). Intra-

cellular membranes of algae are mainly constituted of thylakoid membranes whose lipid composition dominates the raw organic extracts of cells (Harwood, 2004). In eukaryotic chloroplasts, the thylakoid membrane is composed of (i) anionic non-phosphorous glycolipid sulfoquinovosyldiacylglycerols (SQDGs) and phospholipid phosphatidylglycerols (PGs), each accounting for 5–12% of the total lipids, providing the lipid matrix with a negatively charged lipid–water interface and (ii) neutral galactolipids mono- (MGDGs) and di-galactosyldiacylglycerols (DGDGs) accounting for about 50 and 30% of total lipids, respectively (Murata & Siegenthaler, 2004). The galactolipids are composed of a glycerol backbone with two acyl chains in position *sn*-1 and *sn*-2 and a galactose head group linked in β-configuration to the *sn*-3 position (in MGDG), while a second galactose moiety is bound to the first one in α-anomeric linkage (in DGDG). MGDGs have an inverted conical shape and form inverted micelles, while DGDGs have a cylindrical shape and form bilayers (Brentel et al., 1985).

The galactolipids MGDG and DGDG are essential for photosynthetic organisms (Awai et al., 2006) and are the most abundant membrane lipids in nature (Siegenthaler, 2004). Despite their ubiquity and abundance, our understanding of the relation between structure and function of these galactolipids in the photosynthetic processes is limited and controversial (Siegenthaler, 2004). Reasons for this might be that (i) in contrast to proteins, lipids themselves have no recognizable catalytic properties, and therefore it is not yet possible to determine directly the function of lipids in vitro, and (ii) lipids in thylakoid membranes are divided into 'bulk lipids' and 'functional lipids', and often these roles are not easily attributed (Murata & Siegenthaler, 2004). The galactolipids fill the spaces between membrane proteins and play an important role in maintenance of membrane packing (Gray et al., 2009). Saturated acyl chains are fairly linear and therefore can be packed more tightly in the membrane. This tight proximity increases van der Waals forces with consequently higher melting points. On the other hand, unsaturated fatty acids are bent in proximity of the double bonds and therefore do not pack neatly; the greater distance translates into weaker intermolecular interactions. Less energy is required to change their state, and therefore their melting point is much lower. Furthermore, the MDGDs are part of the photosystem I and are important for ATP synthases (Siegenthaler, 2004), and the DGDGs are part of photosystem II and the light-harvesting protein complex (Moellering & Benning, 2011).

The galactolipid tri-galactosyldiacylglycerol (TGDG) is also known from green algae (Benson et al., 1958), higher plants (e.g. Ongun & Mudd, 1968; Siebertz et al., 1979; Xu et al., 2010), diatoms (Vieler et al., 2007) and recently dinoflagellates (Gray et al., 2009; Leblond et al., 2010). The TGDGs play a role in the transport of lipids from the endoplasmic reticulum to the thylakoid membrane (Xu et al., 2010; Moellering & Benning, 2011) and probably also in membrane packing (Gray et al., 2009).

Generally, the interior of a membrane is highly fluid, and the hydrocarbon chain of lipids are disordered and in continuous motion, thus insuring membrane permeability. Lowering the temperature, however, leads to an ordered packing of lipids and a more rigid membrane, impairing its functioning. Lipid order is often incorrectly called membrane 'fluidity' (Guschina & Harwood, 2006); however, because this terminology is a figurative description of the membrane status and quite commonly used, we continue to use the term membrane fluidity.

Algal metabolism is influenced by different environmental factors; temperature is one of the most important (Raven & Geider, 1988). With more than 70% of the earth's ecosystems being cold with stable temperatures below or close to the freezing point of water, species adaptations to low temperature are wide spread and range from molecular to whole cell and ecosystem levels (Morgan-Kiss et al., 2006). At the molecular level, low temperature decreases reactivity of enzymes and membrane fluidity (Gurr et al., 2002) with important repercussions on cellular processes such as a general slowdown of repair mechanisms and metabolism (Davison, 1991), reduced carbon fixation (Huner et al., 1998) and high metabolic costs of growth (Vona et al., 2004; Flaim et al., 2010). Under cold conditions, reduced efficiency of enzymes can be compensated by a combination of different enzymes, by an increased abundance of enzymes, or by the production of isozymes especially suited to low-temperature conditions (Davison, 1991). According to the homeoviscosity principle (i.e. organisms tend to adjust the composition of their membrane lipids so that their fluidity or membrane order remains approximately constant at their growth temperature; Raison

et al., 1982), membrane fluidity can be altered by changes (i) in the type and quantity of unsaturated fatty acids, (ii) in the composition of molecular species comprising a given galactolipid class, (iii) in the size, hydrophobicity and charge of phospholipid head groups, (iv) in the balance between bilayer-stabilizing and -destabilizing lipids, (v) in the cholesterol:polar lipid and proteins:polar lipid ratios and (vi) in the activation of ion channels (Guschina & Harwood, 2006 and reference therein). Such fine tuning of membrane lipid composition is presumed to reflect a homeostatic mechanism of fundamental adaptive significance.

Cold-adaptation is an important trait in many species. Studies on cold-adapted cyanobacteria (Sakamoto et al., 1997), plants (Murata & Los, 2007), microalgae (Sato et al., 1996; Adlerstein et al., 1997; Fuschino et al., 2011), macroalgae (Becker et al., 2010) and dinoflagellates (Gray et al., 2009; Leblond et al., 2010) have clarified the general pattern of the effects of temperature on fatty acids. Besides temperature, ultraviolet radiation also alters galactolipid composition in algae (Obertegger et al., 2011). Algal fatty acid composition is an important factor in plankton ecology, affecting such aspects as algal buoyancy and food quality and consequently migration of zooplankton grazers (Müller-Navarra et al., 2000; Campbell & Dower, 2003). Considering the importance of algae as primary producers, information on temperature effects on fatty acid composition is pivotal in the face of climate change (Fuschino et al., 2011).

Unfortunately, the temperature range considered in most studies is very wide (range: 5–20°C; Sato et al., 1996; Sakamoto et al., 1997; Adlerstein et al., 1997; Becker et al., 2010; Leblond et al., 2010; Fuschino et al., 2011) and might not be compatible with the different scenarios of temperature increase according to the Intergovernmental Panel on Climate Change (IPCC, 2007). The IPCC future scenario B1, the most conservative, predicts a temperature increase of 2°C by 2100, considering improved amelioration efforts and a population decrease, while the worst case scenario (A1F1) predicts a temperature increase of 4°C (IPCC, 2007). However, climate change will not necessarily bring higher temperatures to all regions. With an eventual collapse of the Gulf Stream, we could perhaps see a cooling of some areas of the Northern Hemisphere (O'Hare, 2011).

We studied the effects of a slight increase and decrease in optimum temperature on lipid composition in a freshwater dinoflagellate. *Borghiella dodgei* (Moestrup et al., 2008) is a good model organism because (i) its base-line physiological properties are known and it is considered a strict cold-stenothermal species with a narrow temperature niche (Flaim et al., 2010; Obertegger et al., 2011) and (ii) studies on freshwater dinoflagellates are scarce (see Gray et al., 2009). We specifically focused on galactolipids because their content primarily determines membrane fluidity, and we provide a mechanistic understanding of how slight changes in temperature can change the relative amount of single galactolipid species.

Materials and methods

Culture conditions

Borghiella dodgei (hereafter called *Borghiella*) was cultivated in DY IV medium as described by Flaim et al. (2010) under a 14:10 light:dark cycle. Photosynthetic photon fluence rate was 100–125 μmol m^{-2} s^{-1} measured at the culture surface using a Quantum Photo Radiometer (Delta Ohm srl, Caselle di Selvazzano PD, Italy, mod HD 9021).

Experimental setup

Batch cultures of *Borghiella* were kept in 250 ml quartz glass flasks with 150 ml medium. Initial cell concentration was 10,000 cells ml^{-1}. The temperature experiment was conducted using a thermal gradient plate (2–10°C) (Filippi et al., 2006). Temperature was monitored by data loggers (Hobo U22-001 Water Temp Pro v2-Elkam SpA Italy). We selected three temperature treatments: ±2°C with respect to the optimum growth of *Borghiella* (5°C) (Flaim et al., 2010). Accuracy of temperature treatment was 0.2°C. Cell concentrations were determined by taking 1 ml subsamples fixed with Lugol's solution and by counting a minimum of 400 cells in a 1 ml gridded Sedgwick-Rafter chamber (Graticules Ltd., Tunbridge Wells, UK). If necessary samples were diluted before counting. The specific growth rate (μ) was calculated according to Guillard (1973). Algal growth was monitored every third day and when cultures reached the peak of exponential phase, they

were harvested for galactolipid analysis. Culture conditions and growth phase affect fatty acid content and composition (Alonso et al., 2000; Gurr et al., 2002). However, under stable laboratory conditions, cells are synchronised and therefore our results are not biased.

Galactolipid analysis

Fifty millilitres of each replicate were pooled, concentrated by 5 min centrifugation at 3,000*g* and 4°C, and washed twice in dH_2O. The final pellet was extracted with chloroform:methanol 2:1 (v/v) by cell homogenization with a glass/glass potter, reduced to dryness by rotary evaporation, re-dissolved in 2 ml of methanol and stored in dark glass vials under nitrogen until chromatographic analysis. Liquid chromatography/mass spectrometry (LC/MS) was performed using Hewlett-Packard Model 1100 Series liquid chromatography (Hewlett-Packard Development Company, L.P., Palo Alto CA, USA) coupled both to a photo diode-array detector (Agilent Technologies, Milan, Italy, Agilent 1100) and to a Bruker Esquire-LC quadrupole ion-trap mass spectrometer (Bruker Optik GmbH, Ettlingen, Germany) equipped with atmospheric pressure electrospray ion source (ESI). The analyses were carried out at room temperature on an Agilent ZORBAX Eclipse XDB-C8 150 × 4.6 mm, 3.5 μm using a methanol/water gradient elution. In our LC conditions, galactolipids eluted between 25 and 35 min and were detected both by UV absorption at λ 215 nm and by total ion current (TIC) generated by both positive and negative ion-mode electrospray ionization.

According to our HPLC setup, the rate of elution followed the order TGDGs > DGDGs > MGDGs, and within the single classes, the elution order depended on the length and unsaturation number of the fatty acyl groups. Galactolipids were identified by their ESI mass spectra producing both high $[M–H]^-$ ions by negative-ion and prominent $[M + Na]^+$ ions by positive-ion, and gave structural information concerning the fatty acid composition by the in-source collisional induced dissociation (CID) fragments. Moreover, the on-line fragmentation of $[M + Na]^+$ions by positive-ion ESI–MS–MS allowed us to establish the positional distribution of the *sn*-glycerol bound fatty acyl chains according to Guella et al. (2003).

For quantitative analysis, we integrated from the crude data (TIC) the corresponding extracted ion current (EIC) where only the ions of a particular molecular mass were taken into account. This methodology is justified because all the lipids belonging to a specific class (MGDG or DGDG or TGDG) can be reasonably assumed to ionize (and give fragments) equally. As an example, the EIC peak area of MGDG 18:5/18:5 (within the mass range $\Delta m/z = \pm 2$ Da) due to the ions at m/z 790.3 $[M + Na]^+$; 806.3 $[M + K]^+$ and their characteristic fragment ions (at m/z 606.3; 588.3, and 332.3) were integrated and compared with the EIC peak areas of all the lipids belonging to the MGDG class. This ratio represents a good estimate of the MGDG molar ratio of a particular species in the sample. If the ratio of the EIC area of the same component is calculated with respect to the sum of peak area of all galactolipids (MGDG + DGDG + TGDG), it can no longer be considered the true molar distribution ratio of this component among all the galactolipid components because the ESI efficiency ionization of different galactolipid classes can be significantly different. This bias cannot be easily overridden because suitable synthetic internal standards of galactolipids are not commercially available. However, if we can reasonably assume that the total galactolipids amount is not significantly affected by temperature changes, the temperature-induced variation of all the galactolipids components, expressed as a relative quantification through the ratio [peak area]/[sum of all peaks area], still represents a useful descriptor of temperature dependence of all the individual galactolipid species. From the analysis of our data, we calculated the individual unsaturation index (UI) for each galactolipid molecular species at different temperature treatments. This was obtained by multiplying the number of double bonds in each lipid by its mole percentage and summing the individual lipid unsaturation indices at the same temperature of all the lipids belonging to the same class.

Data treatment and statistical analysis

The effect of temperature (i.e. 3, 5 and 7°C) on single galactolipids was investigated by one-way ANOVA calculating the content of each lipid as the percent with respect to the total sum of peak area (i.e. 100%) of the respective galactolipid class (i.e. MGDG, DGDG and TGDG). The effect of temperature and galactolipid

class on the relative content of galactolipids and the UI was investigated by one-way ANOVA, respectively.

Homogeneity of variance was investigated by the Bartlett test. In the case of heterogeneity of variances, the non-parametric Kruskal–Wallis rank sum test was performed. All analyses were performed in R 2.13.1 (R Development Core Team, 2011).

Results

We found 24 galactolipids (five MGDGs, seven DGDGs, eight TGDGs and four SQDGs) and two PGs molecular species all of which are involved in the thylakoid membranes. Minor amounts of at least twenty phospholipids mainly belonging to the phosphatidylcholine (PC) class (following mass spectra and 1H and 31P-NMR spectra analysis) were also detected in the organic extract of *Borghiella*. Our analysis is here focused, however, only on neutral galactolipids which are the most abundant and are expected to be more sensitive to thermal changes than anionic SQDG and/or PG. In fact, the unsaturation index of the latter chloroplast classes (2.6 in SQDG and 3.7 in PG) was less than one half that of MGDG and DGDG. Worthy of note, several of the TGDG lipids found have never been reported for dinoflagellates. The length of acyl chains of galactolipids was determined for the most abundant galactolipid species (Table 1). While in TGDGs, C_{16} and C_{14} acyl chains were also present, in DGDGs and MGDGs, fatty acids were exclusively composed of C_{18} acyl chains. Only MGDGs showed a lower UI at 7°C than at 3 or 5°C with no difference between 3 and 5°C, while the UI of TGDGs and MGDGs did not show any difference between temperatures (Table 2). No significant changes in the distribution ratio of the three investigated lipid classes (MDGD, DGDG and TGDG) across temperature treatments were found.

Within MGDGs, the most abundant compound was 18:5/18:4 MGDG, followed by 18:5/18:5 MGDG and 18:4/18:4 MGDG (Table 1). The content of 18:5/18:5 MGDG was lower at 7°C than at 5 or 3°C, while content of 18:5/18:4 MGDG, 18:4/18:4 MGDG and 18:3/18:4 MGDG was higher at 7°C than at 5 or 3°C (Table 1). The content of the structural isomer of MGDG 36:8 (called MGDG 36:8 Iso in Table 1) did not change across temperatures.

Content of DGDGs showed patterns similar to MGDGs. The most abundant compound was 18:5/18:4 DGDG, followed by 18:5/18:5 DGDG and 18:4/18:4 DGDG (Table 1). The content of the most unsaturated DGDG (i.e. 18:5/18:5) was lower at 7°C than at 5 or 3°C (Table 1). The contents of 18:5/18:4 DGDG, 18:4/18:4 DGDG, 18:4/18:3 DGDG and 36:5 DGDG were higher at 7°C than at 5 or 3°C, while contents of 18:4/18:4 DGDG structural isomer and 36:6 DGDG did not change with temperature (Table 1).

Within TGDGs, the most abundant lipid species was 18:2/14:0 TGDG, followed by 18:1/14:0 (Table 1). While content of 18:2/14:0 TGDG was lower at 7°C than at 5 and 3°C, the content of 18:1/14:0 was higher at 7°C than at 5 or 3°C (Table 1). The contents of other TGDGs did not change with temperature (Table 1).

Growth rate (μ) of *Borghiella* was not different ($P > 0.05$) for cultures grown at 3 and 5°C (mean growth rate for cultures grown at 3 and 5°C = 0.23) but was higher ($P < 0.01$) than at 7°C ($\mu = 0.15$).

Discussion

A temperature change of only 2°C led to structural modifications of membrane lipids in *Borghiella*. The galactolipid profile of *Borghiella* was in agreement with that found by Gray et al. (2009) for cold-adapted dinoflagellates with both 18:5/18:5 and 18:5/18:4 DGDG present. Furthermore, the presence of only C_{18} acyl chains for MGDG and DGDG galactolipids in *Borghiella*, similar to four other cold-adopted dinoflagellates (Gray et al., 2009), could perhaps indicate another characteristic of psychrophilic dinoflagellates. *Borghiella* is already classified as strict cold-stenothermal species based on physiological characteristics (Flaim et al., 2010), and our study gave additional biochemical evidence for this conclusion.

Interestingly, the temperature differences used did not modify the overall proportions of the major galactolipid classes. Therefore, adaptation to higher or lower temperatures in *Borghiella* was achieved by increasing or decreasing specific galactolipid species.

Effects of decreasing temperature

When grown at a temperature slightly lower than its growth optimum, the overall composition of

Table 1 Profile of galactolipids in *Borghiella dodgei* with the positional distribution of their acyl chains

Galactolipids	Acyl chains	Temperature		Temperature		Temperature	
	sn-1/*sn*-2	3°C	3 versus 5°C	5°C	5 versus 7°C	7°C	Changes of % molar ration from 5 to 7°C
MGDG							
36:10	18:5/18:5	39.4 (2.3)	n.s.	36.5 (0.4)	>***	25.9 (1.3)	−29
36:9	18:5/18:4	43.6 (0.1)	n.s.	45.0 (0.5)	<***	48.7 (1.6)	+8
36:8	18:4/18:4	12.4 (1.8)	n.s.	13.0 (0.4)	<***	19.0 (0.7)	+46
36:8 Iso	18:5/18:3	1.5 (0.3)	n.s.	1.5 (0.1)	n.s.	1.6 (0.0)	–
36:7	18:3/18:4[a]	3.1 (0.3)	<*	3.9 (0.2)	<***	4.7 (0.4)	+21
DGDG							
36:10	18:5/18:5	8.2 (0.3)	n.s.	7.8 (0.7)	> **	6.2 (0.7)	−20
36:9	18:5/18:4	82.9 (2.6)	n.s.	82.6 (2.2)	<.	80.9 (2.1)	+2
36:8	18:4/18:4	4.5 (1.4)	n.s.	4.8 (0.5)	<*	7.2 (0.9)	+49
36:8 Iso	18:5/18:3	2.6 (0.5)	n.s.	2.5 (0.6)	n.s.	2.9 (0.5)	–
36:7	18:3/18:4	0.2 (0.0)	n.s.	0.2 (0.0)	< **	0.4 (0.1)	+83
36:6	18:3/18:3[a]	1.2 (0.2)	n.s.	1.6 (0.2)	n.s.	1.6 (0.2)	–
36:5	18:3/18:2[a]	0.4 (0.2)	n.s.	0.6 (0.2)	<**	0.8 (0.1)	+47
TGDG							
36:2	18:1/18:1[a]	7.2 (0.7)	n.s.	7.6 (1.5)	n.s.	8.8 (1.7)	–
34:4	18:3/16:1[a]	2.0 (0.3)	n.s.	2.4 (0.2)	n.s.	2.1 (0.4)	–
34:3	18:3/16:0[a]	3.1 (0.8)	n.s.	3.6 (0.7)	n.s.	3.2 (0.4)	–
34:2	18:2/16:0[a]	6.4 (1.4)	n.s.	7.2 (3.0)	n.s.	7.5 (1.3)	–
34:1	18:1/16:0[a]	0.0 (0.0)	n.s.	0.0 (0.0)	n.s.	0.4 (0.6)	–
32:3	18:3/14:0[a]	0.9 (0.8)	n.s.	1.2 (0.3)	n.s.	1.3 (1.1)	–
32:2	18:2/14:0	69.7 (1.5)	>*	64.3 (1.8)	>**	60.7 (3.4)	−6
32:1	18:1/14:0	10.7 (0.6)	n.s.	13.7 (2.5)	<**	16.1 (1.4)	+18

Intra-classes molar ratio of galactolipids are expressed as relative mean values (standard deviation in parenthesis) of the ratio between the EIC peak area of a given lipids with respect to the sum of the peak areas of all the lipids belonging to the same class. Each data point is the mean of three replicates. Results are compared by one-way ANOVA; the greater-than and lesser-than signs indicate at which temperature the relative proportion of galactolipids is greater or smaller, respectively; *n.d.* not determined, *n.s.* not significant, · $0.05 > P < 0.10$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, – not considered

[a] Tentative structural assignment

galactolipid species and the UI of galactolipid classes did not change for *Borghiella*; only two lipid species showed statistically significant changes from 5 to 3°C: TGDG 18:2/14:0 increased and MGDG 18:4/18:3 decreased. We suggest that adaptation of this psychrophilic dinoflagellate to lower temperatures was sufficiently maintained by its 'basal' composition of galactolipids and required minimum fine tuning for proper membrane functioning.

Effects of increasing temperature

A temperature 2°C higher than *Borghiella*'s growth optimum led to marked changes in the galactolipid profile with three species decreasing, eight increasing and a decrease in the UI of the MGDG lipid class. A decrease in unsaturated fatty acids with an increase in temperature is commonly seen as an adaptive mechanism and is valid across a wide range of temperatures encompassing cold, temperate and thermal algae (Sato et al., 1996; Gray et al., 2009; Becker et al., 2010). These studies and many others, however, occur over large temperature ranges (≥5°C within each study), forcing ecological significance. Our study instead, encompassed a smaller temperature fluctuation (2°C) compatible with conservative climate change scenarios.

For temperate marine dinoflagellates, MGDGs and TGDGs were relatively insensitive to temperature

Table 2 Unsaturation index (UI) for galactolipid classes in *Borghiella*

UI	3°C	3 versus 5°C	5°C	5 versus 7°C	7°C
MGDG	9.19 (0.10)	n.s.	9.14 (0.02)	>*	8.96 (0.03)
DGDG	8.95 (0.08)	n.s.	8.93 (0.04)	n.s.	8.87 (0.05)
TGDG	1.97 (0.03)	n.s.	1.96 (0.04)	n.s.	1.92 (0.02)

Mean value and standard deviation (in parenthesis) of the UI at different temperatures are reported. The effect of temperature on UI was investigated by one-way ANOVA; the greater-than sign indicates at which treatment the UI is greater; *n.s.* not significant, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

changes across the range considered (15–25°C), while DGDGs showed significant changes (Leblond et al., 2010). The inverted conical geometry of the MGDG molecules (Gurr et al., 2002), leading to an asymmetrical shape, is important to maintain membrane fluidity. We suggest that this role of MGDGs increases with low temperatures. Therefore for psychrophiles, MGDGs are more important in temperature adaptation than DGDGs and TGDGs.

The decrease of the most abundant and most unsaturated species (18:5/18:5 MGDG, 18:5/18:5 DGDG, 18:5/18:4 DGDG and 18:2/14:0 TGDG) concomitantly with an increase of the other less saturated species indicated the greater importance of the three more unsaturated galactolipids for membrane fluidity. On this basis and considering the highest percent increase observed for 36:8 DGDG/MGDG, we suggest that these species, and to a lesser extent their isomers, were precursors for 18:5/18:5 MGDG, 18:5/18:5 DGDG and 18:5/18:4 DGDG. Moreover, the less abundant and most saturated galactolipid species in each lipid class (18:4/18:3 MGDG, 36:5 DGDG and 18:1/14:0 TGDG) accumulated with increasing temperature, indicating that their conversion as potential precursors for the more unsaturated galactolipid species was not needed at higher temperatures. The insertion of double bonds in the acyl chains requires desaturase activity and is a costly process for an organism (Joyard et al., 2004). Therefore, we suggest that by reducing the synthesis of the more unsaturated MGDG, DGDG and TGDG species, *Borghiella* was more energy efficient.

TGDG has been observed in many photosynthetic taxa (Harwood, 2004; Xu et al., 2010; Gray et al., 2009; Leblond et al., 2010). Ours is the first report of TGDGs in a freshwater dinoflagellate. While it was not possible to fully compare TDGDs in *Borghiella* with Gray et al. (2009) and Leblond et al. (2010), we found additional, not previously reported species. In our study, the content of six of eight TGDGs remained stable with changes in temperature. A possible role of TGDGs related to membrane fluidity has been suggested by Gray et al. (2009): TGDGs could act as a 'viscosity-buffer', helping to keep the thylakoid membrane functioning irrespective of temperature. However, Xu et al. (2010) and Moellering & Benning, (2011) emphasise the trafficking functions of TGDGs. Based on our results, we suggest that the stability of the minor TGDG species with temperature would indicate a transportation role, while changes of the two more abundant species with temperature would indicate a role in maintenance of membrane fluidity.

Biological significance

There is little experimental evidence for truly psychrophilic taxa growing within narrow ranges of temperature (<10°C), even if algal species responsible for spring blooms must be capable of rapid growth below 5°C (Butterwick et al., 2005). Among the many dinoflagellates that form blooms (Hansen & Flaim, 2007) are some common psychrophilic species: *Peridinium aciculiferum* (Rengefors & Anderson, 1998), *Woloszynskia halophila* (Kremp et al., 2005) and *B. dodgei* (Flaim et al., 2010) all cease growth at temperatures >10°C. Like other psychrophiles, these species have carved a unique physiological niche that may reduce direct competition and grazing under similar environmental conditions (Richardson et al., 2000; Rose & Caron, 2007). *Borghiella* is a poor competitor in warmer waters and disappears from its natural habitat in early spring (Flaim et al., 2010). In fact, *Borghiella*'s growth rate was similar at 3 and 5°C reflecting the changes seen within the lipid classes considered. The lower growth rate at 7°C was in contrast to a presumed energy efficiency due to the

presence of less saturated galactolipids. This finding further underlines the strict cold-stenothermal character of this dinoflagellate: the algae can adapt to a relatively 'higher' temperature but remains restricted by its lower growth rate.

Maazouzi et al. (2008) show how in natural plankton assemblages rising water temperatures increase the degree of unsaturation in fatty acids and thus reduce food quality. Actually, nutritional quality of algae is determined by PUFAs (Müller-Navarra et al., 2000), and zooplankton selectively incorporate the most physiologically important PUFA species (Burns et al., 2011). Subsequently, zooplankton such as rotifers can elongate C_{18} to C_{20} to fulfil their nutritional needs (Wacker & Weithoff, 2009). The altered nutritional quality of *Borghiella* would have implications on its grazers and consequently on the aquatic food chain.

Specialists with sharp environmental niches are very competitive but require a stable environment. Among the various future climate scenarios possible, lower temperatures would lead to the conservation and possible expansion of *Borghiella*'s niche. However, with a more probable temperature increase, this algae would be further restricted, at least temporally. The fate of specialists such as *Borghiella* in a changing world will depend on the degree and direction of climate change and their ability to evolve new adaptive traits.

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PHYTOPLANKTON

Review Paper

Present state of the systematics of planktonic coccoid green algae of inland waters

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Abstract This review discusses the main developments in the systematics of coccoid green algae over the last three decades. The relationships of key groups of planktonic coccoid green algae are shown in the phylogenetic trees of Chlorophyceae and Trebouxiophyceae. The trees clearly show that the morphology of these algae do not adequately reflect their phylogenetic position. Different phylogenetic species can be hidden under one and the same morphotype. As most of the genera have a polyphyletic origin, they are in need of a systematic re-evaluation. Species classification using the phylogenetic species concept resulted in the establishment of new genera with smaller numbers of species and the description of new species that are not distinguishable by light microscopy. An overview of genera is given in tables and the revised designations of species as contained in the harmonized taxon list of the European Water Framework Directive lists is provided. In this transitional phase from an artificial to a more natural systematics of algae, field biologists and ecologists as well as molecular biologists must strengthen their interdisciplinary cooperation. The alignment of eco-functional groups of algae with true species identities using the barcoding conception will provide a better understanding of the interaction between organisms and their environment.

Keywords Coccoid green algae · Chlorophyceae · Trebouxiophyceae · Systematics · Genus and species concept · Barcodes · Functional groups

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Introduction

The classification of algae is presently going through an extremely interesting stage. The old, artificial classification system is being replaced by a new, more natural, phylogenetic system. This is especially the case for the coccoid green algae, mainly because numerous organisms in this morphological group do not propagate sexually. Hence, phenotypic criteria have to a large extent been used in the establishment of taxonomic clades. Initially, the introduction of molecular phylogenetic methods into the systematics of green algae led to a fundamental revision of the concepts of higher taxonomic lineages such as divisions, classes and orders (Melkonian & Surek, 1995; Friedl, 1997; Chapman et al., 1998; Leliaert et al.,

2012). Following these new phylogenetic conceptions the orders that contain coccoid green algae have been considerably changed. The systematics of lower taxonomic levels such as families and genera remain provisional because detailed taxon sampling for molecular sequence analyses have not been carried out for the taxa. Nevertheless, key groups of planktonic coccoid greens have in the recent past been studied by molecular methods, and as such a picture of their phylogenetic designation has started to take shape.

The central unit for biological classification is the species. The most established species concept is the biological species (Mayr, 1942), which refers to groups of interbreeding natural populations that are reproductively isolated. Because of its restriction to sexually reproducing organisms, this concept cannot be applied to most of the coccoid green algae. In an effort to deal with this limitation, limnologists adopted the morphological species concept using morphology-based diacritical characteristics. The climax of the morphological species concept for the coccoid green algae was reached following the publication of the famous handbook on "Chlorococcales" by Komárek & Fott (1983). Approximately 1,200 taxa from water, soil, and other habitats were compiled in this comprehensive work. The works of Hindák entitled "Studies on the Chlorococcal Algae (Chlorophyceae) I–V" (1977, 1980, 1984, 1988, 1990), which were published partly before and after the handbook of Komárek & Fott, have inspired many phytoplankton researchers. Combining a sharp eye for detail and experience, Hindák identified several new morpho-species. His observations triggered lively discussions on diacritic morphological characteristics such as presence or absence of pyrenoids, cell wall incrustations and mucilaginous envelopes.

Running parallel to these traditional approaches, an ultrastructural concept to classify green algae based on anatomy of flagellated cells and cytokinesis during mitosis was initiated by Melkonian (1982, 1984) and Mattox & Stewart (1984). Based on the orientation of the basal bodies of the flagellar apparatus, three main types have been suggested: *c*ounter *c*lock*w*ise orientation (CCW, 11–5 o'clock), *c*lock*w*ise orientation (CW, 1–7 o'clock), and *d*irectly *o*pposite orientation (DO, 12–6 o'clock). Later studies on molecular characteristics by Lewis et al. (1992) revealed that the relationships established on the basis of ultrastructural data were closely supported by molecular data.

As discussions on the relations between the various concepts of classifying green algae continued, the phylogenetic concept, which utilizes molecular markers to delineate taxa, steadily made its way into the daily routine of phycologists. The evidence that has so far accumulated clearly suggests that biological and morphological conceptions cannot solve the main questions on natural grouping of coccoid green algae. A polyphasic approach combining morphological, ecophysiological and molecular phylogenetic methods should contribute to modern systematics (Pröschold & Leliaert, 2007). In this review, we focus on the main developments in the systematics of coccoid green algae following the landmarks set by the works of Komárek & Fott, Hindák, and Mattox & Stewart in the middle of the 1980s.

Higher taxonomic lineages which contain coccoid green algae

The green algae evolved about 1,500 million years ago (Yoon et al., 2004) into two large lineages (divisions), the Chlorophyta and the Charophyta (Lewis & McCourt, 2004). In this review, we follow the work of Lewis & McCourt (2004) who suggested a "working classification of green algae and land plants" (Table 1).

The Charophyta has often been labeled the Streptophyta by Bremer et al. (1987). This division contains seven classes that include the Charophyceae (stoneworts), the Embryophyceae (higher land plants), the Zygnemophyceae (conjugates), and the Chlorokybophyceae (has only one species *Chlorokybus atmophyticus*, an aerophytic coccoid green alga). The Zygnemophyceae has within the order Desmidiales, a special type of coccoid green algae with a striking morphology characterized by two symmetrical halves (semicells). The majority of coccoid green algae considered here occur in several orders of Chlorophyceae, Trebouxiophyceae, and Prasinophyceae within the division Chlorophyta (Melkonian, 1990a; Fawley et al., 2000; Krienitz et al., 2003). Conventionally, these coccoid taxa were lumped in the order Chlorococcales *sensu lato* (*s.l.*) by Komárek & Fott (1983) and represented one of the most diverse groups of photoautotrophic cryptogams. However, this classical approach based on Pascher's (1918) idea of establishing orders according to life forms has not been

Table 1 Overview on main lineages of green algae

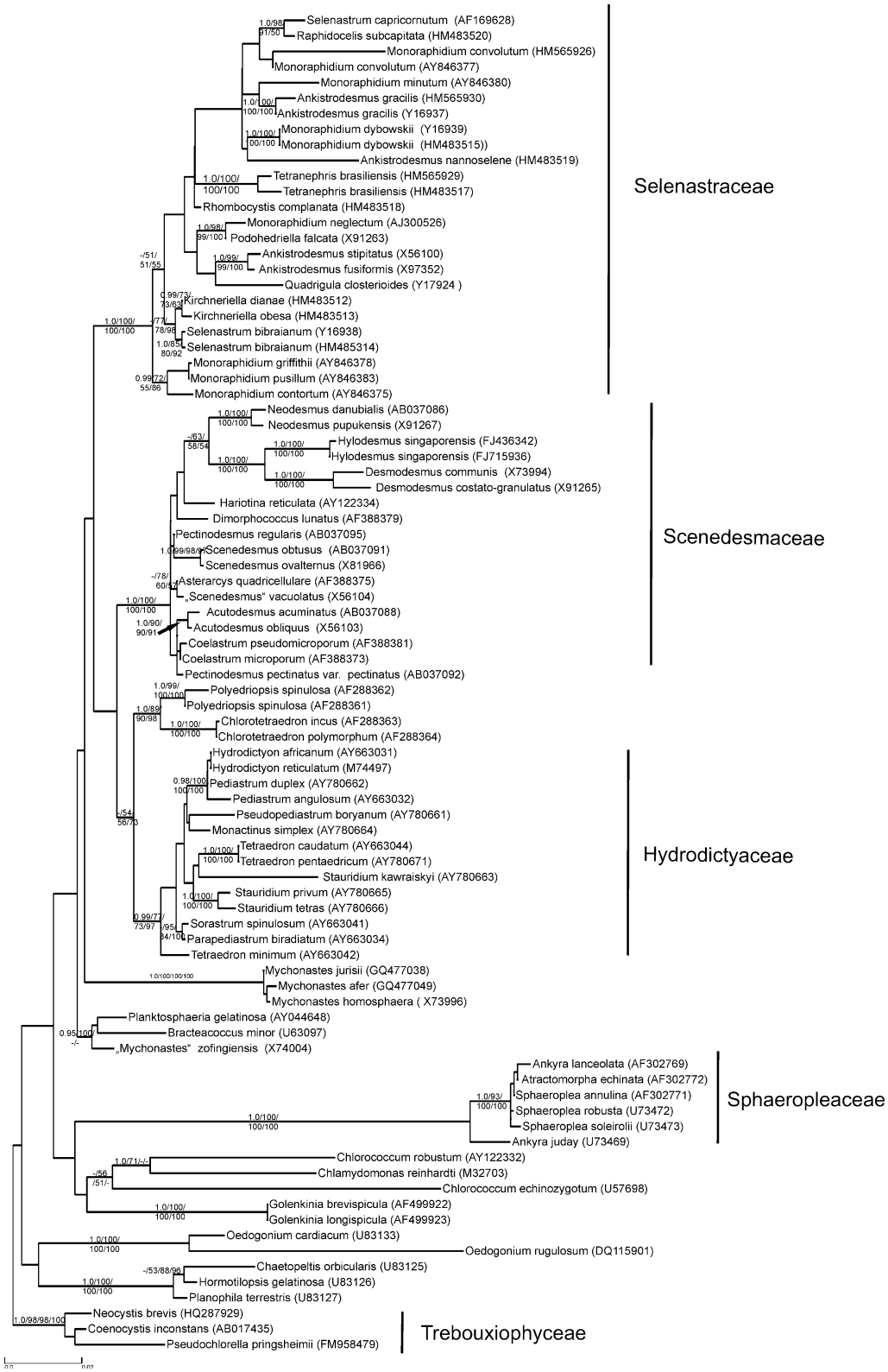
Kingdom Chlorobionta
Division Chlorophyta
Class Chlorophyceae
Order Chlamydomonadales
Order Chlorococcales* (*Chlorococcum*)
Order Sphaeropleales* (*Ankyra*, *Pediastrum*, *Scenedesmus*, *Selenastrum*)
Order Oedogoniales
Order Chaetopeltidales
Order Chaetophorales
Incertae sedis* (*Actinochloris*, *Nautococcus*)
Class Ulvophyceae
Order Ulotrichales
Class Trebouxiophyceae
Order Trebouxiales* (*Trebouxia*, *Chloroidium*, *Myrmecia*)
Order Microthamniales
Order Prasiolales
Order Chlorellales* (*Chlorella*, *Dictyosphaerium*, *Micractinium*)
Incertae sedis* (*Choricystis*, *Coccomyxa*, *Botryococcus*)
Class Prasinophyceae
Order Pyramimonadales* (*Halosphaera*, *Pachysphaera*)
Order Mamiellales* (*Bathycoccus*, *Ostreococcus*)
Order Pseudoscourfieldiales* (*Pycnococcus*)
Order Prasinococcales* (*Prasinococcus*)
Order Chlorodendrales
Incertae sedis* (*Picocystis*)
Division Charophyta
Class Mesostigmatophyceae
Class Chlorokybophyceae
Order Chlorokybales* (*Chlorokybus*)
Class Klebsormidiophyceae
Class Zygnemophyceae
Order Zygnematales
Order Desmidiales* (*Cosmarium*)
Class Coleochaetophyceae
Subdivision Streptophytina
Class Charophyceae
Class Embryophyceae

The orders containing coccoid phenotypes are indicated by an asterisk, and some examples of genera belonging into these orders are given

supported by phylogenetic methods and is therefore not applicable in modern systematic considerations of green algae. Consequently, most of the taxa that do not belong to the Chlorococcales *s.l.* have been transferred to other orders.

In the division Chlorophyta, coccoid taxa occur in three different classes; the Chlorophyceae, Trebouxiophyceae, and Prasinophyceae (Table 1). In the Chlorophyceae, the coccoid taxa occur in two orders; the Chlorococcales *sensu stricto* (*s.str.*) and Sphaeropleales. The only remaining taxa in the order Chlorococcales *s.str.* are those related to the polyphyletic genus *Chlorococcum* and some other genera that all are still under revision. It has also become evident that some taxa of Chlamydomonadales, Dunaliellales, and Volvocales occur in the order Chlorococcales *s.str.* (Nakayama et al., 1996; Booton et al., 1998; Chapman et al., 1998; Pröschold et al., 2001). The ultrastructures of all these taxa are characterized by a CW orientation of the basal apparatus of the flagella. Apart from the Chlorococcales *s.str.* and Sphaeropleales, several other lineages (incertae sedis) which do not correspond to the named clades have coccoid taxa. Future revisions of the CW-group of Chlorophyceae may probably lead to the establishment of several new orders. Furthermore, the content of ambiguous orders such as Actinochloridales, Chlorosarcinales, and Tetrasporales must be emendated.

A majority of chlorophycean members of Chlorophyta are placed in the order Sphaeropleales (Fig. 1). Some members of these taxa such as Sphaeropleaceae, Hydrodictyaceae, and *Bracteacoccus* produce zoospores with DO-orientation of the flagellar apparatus (Lewis, 1997; Buchheim et al., 2001; Wolf et al., 2002a; Shoup & Lewis, 2003). However, many non motile unicellular or colonial taxa such as Selenastraceae and *Mychonastes* are also included in this order (Krienitz et al., 2001, 2003, 2011a, b, 2012). The Scenedesmaceae also belong to this order. Members of this family are commonly non motile. However, presence of zoospores in cultures of *Acutodesmus* has been reported (Trainor, 1963). Keller et al. (2008) confirmed the monophyly of Sphaeropleales; however, from several other studies the relations between the clade containing the type genus *Sphaeroplea* (Sphaeropleales *s.str.*) and the other clades


Selenastrum capricornutum (AF169628)
Raphidocelis subcapitata (HM483520)
Monoraphidium convolutum (HM565926)
Monoraphidium convolutum (AY846377)
Monoraphidium minutum (AY846380)
Ankistrodesmus gracilis (HM565930)
Ankistrodesmus gracilis (Y16937)
Monoraphidium dybowskii (Y16939)
Monoraphidium dybowskii (HM483515))
Ankistrodesmus nannoselene (HM483519)
Tetranephris brasiliensis (HM565929)
Tetranephris brasiliensis (HM483517)
Rhombocystis complanata (HM483518)
Monoraphidium neglectum (AJ300526)
Podohedriella falcata (X91263)
Ankistrodesmus stipitatus (X56100)
Ankistrodesmus fusiformis (X97352)
Quadrigula closterioides (Y17924)
Kirchneriella dianae (HM483512)
Kirchneriella obesa (HM483513)
Selenastrum bibraianum (Y16938)
Selenastrum bibraianum (HM485314)
Monoraphidium griffithii (AY846378)
Monoraphidium pusillum (AY846383)
Monoraphidium contortum (AY846375)
Selenastraceae
Neodesmus danubialis (AB037086)
Neodesmus pupukensis (X91267)
Hylodesmus singaporensis (FJ436342)
Hylodesmus singaporensis (FJ715936)
Desmodesmus communis (X73994)
Desmodesmus costato-granulatus (X91265)
Hariotina reticulata (AY122334)
Dimorphococcus lunatus (AF388379)
Pectinodesmus regularis (AB037095)
Scenedesmus obtusus (AB037091)
Scenedesmus ovalternus (X81966)
Asterarcys quadricellulare (AF388375)
„Scenedesmus“ vacuolatus (X56104)
Acutodesmus acuminatus (AB037088)
Acutodesmus obliquus (X56103)
Coelastrum pseudomicroporum (AF388381)
Coelastrum microporum (AF388373)
Pectinodesmus pectinatus var. pectinatus (AB037092)
Scenedesmaceae
Polyedriopsis spinulosa (AF288362)
Polyedriopsis spinulosa (AF288361)
Chlorotetraedron incus (AF288363)
Chlorotetraedron polymorphum (AF288364)
Hydrodictyon africanum (AY663031)
Hydrodictyon reticulatum (M74497)
Pediastrum duplex (AY780662)
Pediastrum angulosum (AY663032)
Pseudopediastrum boryanum (AY780661)
Monactinus simplex (AY780664)
Tetraedron caudatum (AY663044)
Tetraedron pentaedricum (AY780671)
Stauridium kawraiskyi (AY780663)
Stauridium privum (AY780665)
Stauridium tetras (AY780666)
Sorastrum spinulosum (AY663041)
Parapediastrum biradiatum (AY663034)
Tetraedron minimum (AY663042)
Hydrodictyaceae
Mychonastes jurisii (GQ477038)
Mychonastes afer (GQ477049)
Mychonastes homosphaera (X73996)
Planktosphaeria gelatinosa (AY044648)
Bracteacoccus minor (U63097)
„Mychonastes“ zofingiensis (X74004)
Ankyra lanceolata (AF302769)
Atractomorpha echinata (AF302772)
Sphaeroplea annulina (AF302771)
Sphaeroplea robusta (U73472)
Sphaeroplea soleirolii (U73473)
Ankyra juday (U73469)
Sphaeropleaceae
Chlorococcum robustum (AY122332)
Chlamydomonas reinhardti (M32703)
Chlorococcum echinozygotum (U57698)
Golenkinia brevispicula (AF499922)
Golenkinia longispicula (AF499923)
Oedogonium cardiacum (U83133)
Oedogonium rugulosum (DQ115901)
Chaetopeltis orbicularis (U83125)
Hormotilopsis gelatinosa (U83126)
Planophila terrestris (U83127)
Neocystis brevis (HQ287929)
Coenocystis inconstans (AB017435)
Pseudochlorella pringsheimii (FM958479)
Trebouxiophyceae

◀ **Fig. 1** Molecular phylogeny of the Chlorophyceae based on SSU rRNA gene sequence comparisons. The phylogenetic tree shown was inferred using the maximum likelihood (ML) method (with substitution model: J1 [Optimum, Empirical]:G [Optimum]:5), based on 1558 aligned positions of 87 taxa using Treefinder (Jobb, 2008). Bayesian values (>0.95) (MB) were calculated by MrBayes 3.1 using GTR settings (Ronquist & Huelsenbeck, 2003; Posada & Buckley, 2004). The stationary distribution was assumed after 4 million generations when the average standard deviations of split frequencies between two runs was lower than 0.01. To test the tree confidence, bootstrap values (>50%) ML (1,000 replicates), neighbor-joining (NJ) (1,000 replicates; calculated using Paup 4.0), and maximum parsimony (MP) [1,000 replicates; calculated using Paup 4.0 (Swofford, 2002)] were determined. Support values are shown at the branches in the order: MB, ML, MP, NJ. *Scale bar* indicates substitutions per site. The sequences were obtained from Genbank [National Center for Biotechnology Information (NCBI)]. For each taxon, the NCBI accession number is given in brackets

(e.g., *Hydrodictyon*-clade, *Scenedesmus*-clade, *Ankistrodesmus*-clade, *Bracteacoccus*-clade) of the Sphaeropleales *s.l.* remain ambiguous (Fawley et al., 2005a; Pröschold & Leliaert, 2007; Krienitz et al., 2011a). Better-supported conclusions will require more data, based on sequences of a larger number of molecular markers.

Two major orders of coccoid taxa can be identified in the class Trebouxiophyceae; the Trebouxiales and Chlorellales (Fig. 2). Several other clades such as the *Choricystis*-clade and *Oocystis*-clade are distinctive and may probably be established as an independent order in future. Other clades (incertae sedis) hosting *Coccomyxa*, *Botryococcus*, *Coenocystis* and *Pseudochlorella* await delineation into higher taxonomic ranks. The Trebouxiales mainly comprises taxa from edaphic and aerophytic habitats as well as endosymbionts of lichens (Ettl & Gärtner, 1995; Friedl, 1995). Within Chlorellales (Fig. 3) are numerous new genera and species that were recently described from freshwaters (Fawley et al., 2005b; Bock et al., 2010, 2011a, b, c; Pröschold et al., 2010).

The class Prasinophyceae contains small flagellated and scaled green algae. A few of them have lost one or both the flagella and scales and now have a coccoid phenotype. Coccoid prasinophytes occur in different orders of the class Prasinophyceae (Table 1). It has been shown that this class is an artificial taxonomic lineage because these organisms have a paraphyletic origin; hence should better be named as prasinophytes (Steinkötter et al., 1994). The prasinophytes evolved in several independent lineages which probably represent independent classes. A report by Guillou et al. (2004) identifies seven different clades of the prasinophytes. Initial taxonomic revision of some of these clades has resulted in the description of the classes Nephroselmidophyceae (Cavalier-Smith, 1993), Chlorodendrophyceae (Masjuk, 2006), and Mamiellophyceae (Marin & Melkonian, 2010). The coccoid prasinophytes are restricted to marine habitats or saline inland waters (Guillou et al., 2004; Krienitz et al., 2012).

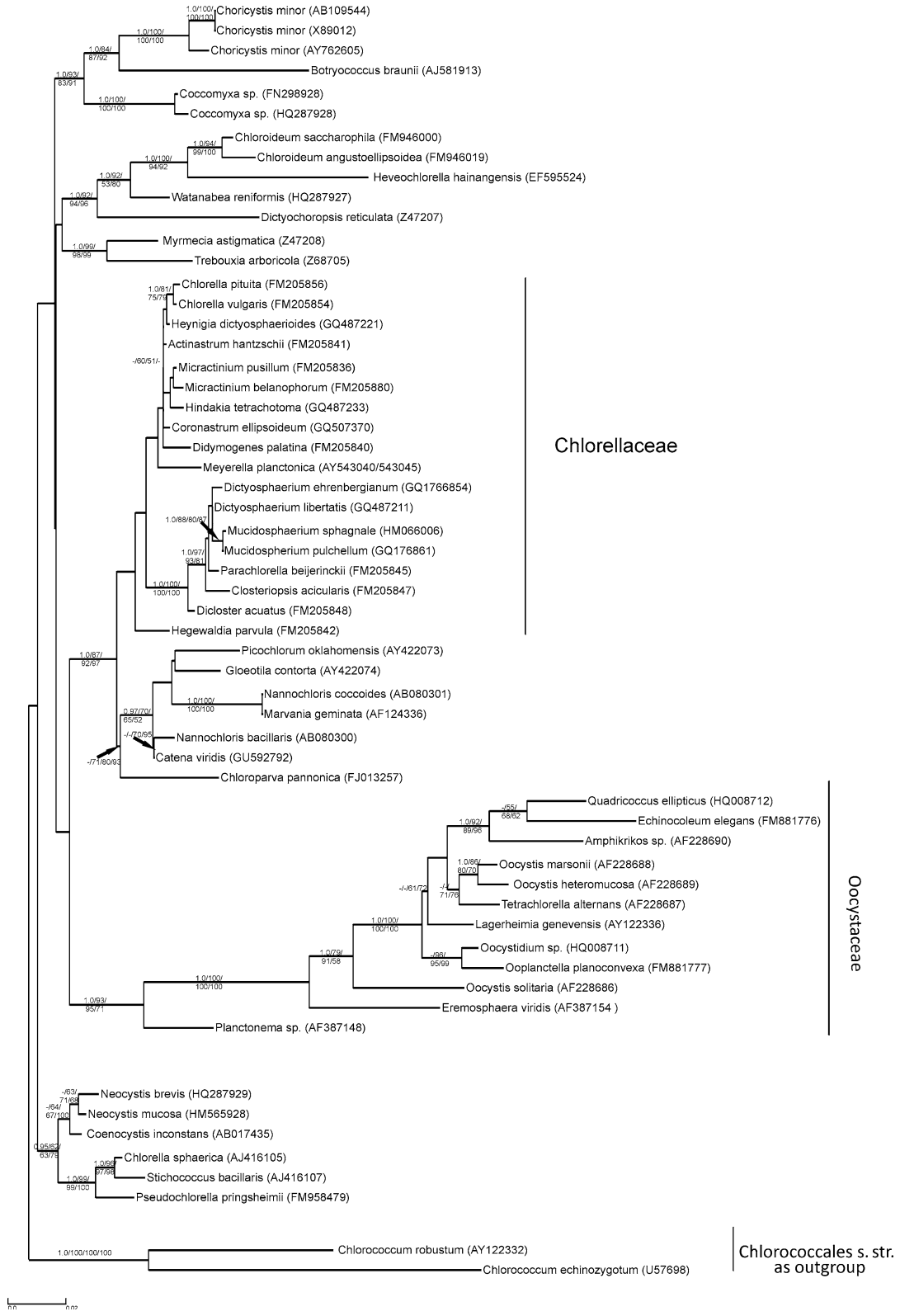
Recent systematics of key groups of coccoid green algae of inland waters

The ranking of the groups discussed in this section follow the topology in the phylogenetic trees (Figs. 1, 2, 3). These trees are based on a selection of sequences obtained from Genbank [National Center for Biotechnology Information (NCBI) http://www.ncbi.nlm.nih.gov/]. The accession numbers of these sequences are indicated in Figs. 1, 2, and 3).

Chlorophyceae

Selenastraceae

Morphologically, this group comprises of "needles and capricorns." Members of this family are very common in freshwaters and exhibit a high morphological diversity. They exclusively propagate by autospores. Discussions on the diacritical features of this group have been very intense. Marvan et al. (1984) investigated 18 genera of the Selenastraceae by means of numerical evaluation of morphological and ontogenetic characteristics. The shape of the cells or colonies, the arrangement of autospores inside the mother cell, the development of mucilage and incrustations on the cell wall, and the presence, number, and type of pyrenoids were used to differentiate the genera. According to this conception, several genera differ from each other only by one of the above characters. For example, *Selenastrum* differs from *Ankistrodesmus* by the curvature of the cells (Komárek & Comas, 1982), *Raphidocelis* differs from *Kirchneriella* by cell wall incrustations (Hindák, 1977), and *Chlorolobion* differs from *Monoraphidium* by the presence of a pyrenoid with starch envelope (Komárek, 1979; Heynig & Krienitz, 1982). Fawley et al. (2005a) correlated the morphological features of


Choricystis minor (AB109544)
Choricystis minor (X89012)
Choricystis minor (AY762605)
Botryococcus braunii (AJ581913)
Coccomyxa sp. (FN298928)
Coccomyxa sp. (HQ287928)
Chloroideum saccharophila (FM946000)
Chloroideum angustoellipsoidea (FM946019)
Heveochlorella hainangensis (EF595524)
Watanabea reniformis (HQ287927)
Dictyochoropsis reticulata (Z47207)
Myrmecia astigmatica (Z47208)
Trebouxia arboricola (Z68705)
Chlorella pituita (FM205856)
Chlorella vulgaris (FM205854)
Heynigia dictyosphaerioides (GQ487221)
Actinastrum hantzschii (FM205841)
Micractinium pusillum (FM205836)
Micractinium belanophorum (FM205880)
Hindakia tetrachotoma (GQ487233)
Coronastrum ellipsoideum (GQ507370)
Didymogenes palatina (FM205840)
Meyerella planctonica (AY543040/543045)
Dictyosphaerium ehrenbergianum (GQ1766854)
Dictyosphaerium libertatis (GQ487211)
Mucidosphaerium sphagnale (HM066006)
Mucidospherium pulchellum (GQ176861)
Parachlorella beijerinckii (FM205845)
Closteriopsis acicularis (FM205847)
Dicloster acuatus (FM205848)
Hegewaldia parvula (FM205842)
Chlorellaceae
Picochlorum oklahomensis (AY422073)
Gloeotila contorta (AY422074)
Nannochloris coccoides (AB080301)
Marvania geminata (AF124336)
Nannochloris bacillaris (AB080300)
Catena viridis (GU592792)
Chloroparva pannonica (FJ013257)
Quadricoccus ellipticus (HQ008712)
Echinocoleum elegans (FM881776)
Amphikrikos sp. (AF228690)
Oocystis marsonii (AF228688)
Oocystis heteromucosa (AF228689)
Tetrachlorella alternans (AF228687)
Lagerheimia genevensis (AY122336)
Oocystidium sp. (HQ008711)
Ooplanctella planoconvexa (FM881777)
Oocystis solitaria (AF228686)
Eremosphaera viridis (AF387154)
Planctonema sp. (AF387148)
Oocystaceae
Neocystis brevis (HQ287929)
Neocystis mucosa (HM565928)
Coenocystis inconstans (AB017435)
Chlorella sphaerica (AJ416105)
Stichococcus bacillaris (AJ416107)
Pseudochlorella pringsheimii (FM958479)
Chlorococcum robustum (AY122332)
Chlorococcum echinozygotum (U57698)
Chlorococcales s. str.
as outgroup

◀ **Fig. 2** Molecular phylogeny of the Trebouxiophyceae based on SSU rRNA gene sequence comparisons. The phylogenetic tree shown was inferred using the maximum likelihood (ML) method (with substitution model: J1 [Optimum, Empirical]:G [Optimum]:5), based on 1,429 aligned positions of 58 taxa using Treefinder (Jobb, 2008). Bayesian values (>0.95) (MB) were calculated by MrBayes 3.1 using GTR settings (Ronquist & Huelsenbeck, 2003; Posada & Buckley, 2004). The stationary distribution was assumed after 4 million generations when the average standard deviations of split frequencies between two runs was lower than 0.01. To test the tree confidence, bootstrap values (>50%) for ML (1,000 replicates), NJ (1,000 replicates; calculated using Paup 4.0), and MP [1,000 replicates; calculated using Paup 4.0 (Swofford, 2002)] were calculated. Support values are shown at the branches in the order: MB, ML, MP, and NJ. *Scale bar* indicates substitutions per site. The sequences were obtained from Genbank [National Center for Biotechnology Information (NCBI)]. For each taxon, the NCBI accession number is given in brackets

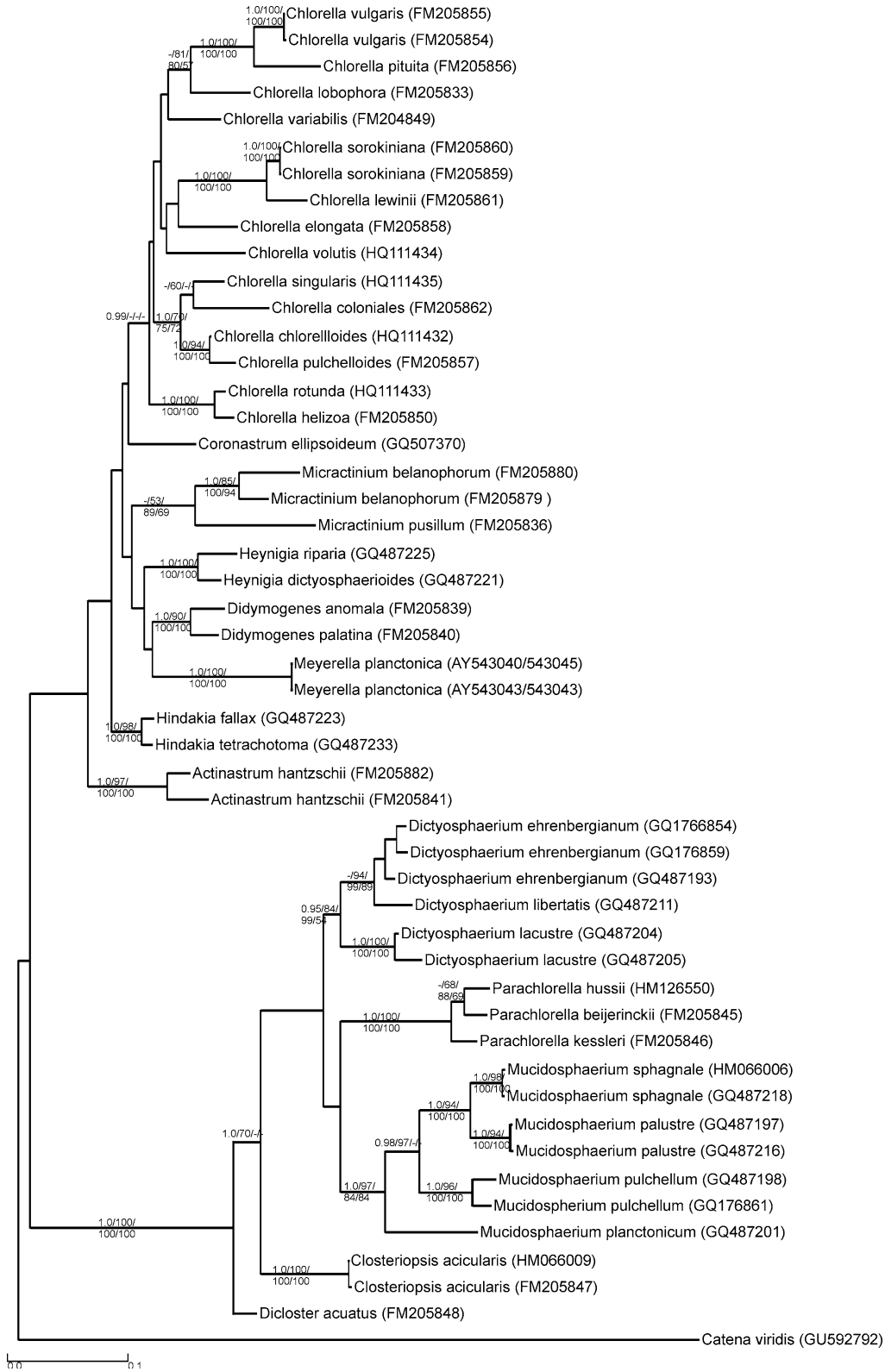
several morphospecies of Selenastraceae with their molecular characteristics and found that one morphotype can cover different phylotypes. The data suggested that a broad morphospecies concept would result in a substantial underestimation of species diversity. Subsequent molecular studies have confirmed the existence of "small" genera in Selenastraceae containing only a few species (Krienitz et al., 2001, 2011b). From the molecular studies, 10 different genera have been confirmed in this family (Table 2). Several other genera are yet to be subjected to detailed molecular investigation the outcome of which may be the description of new genera. This is especially the case for the relatives of the needle-shaped *Ankistrodesmus s.l.* and *Monoraphidium s.l.* which are found in nine different clades and probably represent different genera (Krienitz et al., 2011b). However, further taxon sampling is essential before conclusions on the taxonomic identities of these clades can be drawn.

The adjustment of the diacritical value of the main morphological characteristics in line with the molecular findings has revealed the following: the solitary versus colonial life form, the general shape of cells and colonies are relatively good criteria. Although changes in environmental conditions can result in some colonies disintegrating, the arrangement of autospores in the mother cells can be used for the differentiation of genera. The establishment of mucilage and incrustations are of limited taxonomic value because of the wide variability. Similarly, views on the value of starch formation and the presence of pyrenoids have changed over time. Traditionally, most of the genera were thought to be devoid of pyrenoids. However, this position was later, revised following the observation of pyrenoids in some genera. The first report on the presence of pyrenoids was made by Eloranta (1979), who used the TEM to observe pyrenoids in *Monoraphidium griffithii*. This finding was later confirmed for other species (Krienitz et al., 1985, 2001; Krienitz & Scheffler, 1994). In subsequent studies, pyrenoids were detected in all taxa studied; some exhibited a naked matrix, while others were equipped with starch grains covering the pyrenoid matrix (Krienitz et al., 2011b). In aerated cultures, it was observed that the concentration of CO_2 influenced pyrenoid formation (Miyachi et al., 1986). In *Monoraphidium terreste*, pyrenoids were well developed under normal air conditions. However, they were found to disappear under CO_2 enrichment (Krienitz & Klein, 1988). Hence the presence or absence of pyrenoids cannot be used as a basis for the differentiation of members of the Selenastraceae family.

Based on molecular phylogeny, several taxa traditionally included in the Selenastraceae were removed from this family. *Choricystis minor* is member of a picoplanktonic lineage in the Trebouxiophyceae (Krienitz et al., 1996a; Darienko et al., 2010). *Keratococcus bicaudatus* and *Pseudococcomyxa simplex* are closely related to *Choricystis* (Friedl, 1996). The authentic strain of *P. simplex* was recovered in the *Elliptochloris*-clade and found to be closely related to other strains of *Coccomyxa* (Pröschold et al., 2011). Consequently, the taxon *Coccomyxa simplex* has been re-established. Hence, other members of *Pseudococcomyxa* need a taxonomic revision. *Dicloster acuatus* is a needle-shaped coenobial member of Chlorellaceae (Hegewald & Hanagata, 2000). *Closteriopsis acicularis* is a solitary, needle-shaped member of the Chlorellaceae and is closely related to *Dicloster* (Ustinova et al., 2001). The ultrastructure of its pyrenoid is comparable to that of *Chlorella* and other Chlorellaceae (Hegewald & Schnepf, 1986). The apochloric *Hyaloraphidium curvatum* is a member of the lower fungi (Ustinova et al., 2000).

Scenedesmaceae

This is the largest group of coccoid green algae in freshwater ecosystems. Among the genera, the genus *Scenedesmus s.l.* with its extremely wide morphological variability is a nightmare for field ecologists who


Chlorella vulgaris (FM205855)
Chlorella vulgaris (FM205854)
Chlorella pituita (FM205856)
Chlorella lobophora (FM205833)
Chlorella variabilis (FM204849)
Chlorella sorokiniana (FM205860)
Chlorella sorokiniana (FM205859)
Chlorella lewinii (FM205861)
Chlorella elongata (FM205858)
Chlorella volutis (HQ111434)
Chlorella singularis (HQ111435)
Chlorella coloniales (FM205862)
Chlorella chlorellloides (HQ111432)
Chlorella pulchelloides (FM205857)
Chlorella rotunda (HQ111433)
Chlorella helizoa (FM205850)
Coronastrum ellipsoideum (GQ507370)
Micractinium belanophorum (FM205880)
Micractinium belanophorum (FM205879)
Micractinium pusillum (FM205836)
Heynigia riparia (GQ487225)
Heynigia dictyosphaerioides (GQ487221)
Didymogenes anomala (FM205839)
Didymogenes palatina (FM205840)
Meyerella planctonica (AY543040/543045)
Meyerella planctonica (AY543043/543043)
Hindakia fallax (GQ487223)
Hindakia tetrachotoma (GQ487233)
Actinastrum hantzschii (FM205882)
Actinastrum hantzschii (FM205841)
Dictyosphaerium ehrenbergianum (GQ1766854)
Dictyosphaerium ehrenbergianum (GQ176859)
Dictyosphaerium ehrenbergianum (GQ487193)
Dictyosphaerium libertatis (GQ487211)
Dictyosphaerium lacustre (GQ487204)
Dictyosphaerium lacustre (GQ487205)
Parachlorella hussii (HM126550)
Parachlorella beijerinckii (FM205845)
Parachlorella kessleri (FM205846)
Mucidosphaerium sphagnale (HM066006)
Mucidosphaerium sphagnale (GQ487218)
Mucidosphaerium palustre (GQ487197)
Mucidosphaerium palustre (GQ487216)
Mucidosphaerium pulchellum (GQ487198)
Mucidospherium pulchellum (GQ176861)
Mucidosphaerium planctonicum (GQ487201)
Closteriopsis acicularis (HM066009)
Closteriopsis acicularis (FM205847)
Dicloster acuatus (FM205848)
Catena viridis (GU592792)
0.0
0.1

◀ **Fig. 3** Molecular phylogeny of the Chlorellaceae based on a partitioned dataset of SSU, ITS1, 5.8S, and ITS2 gene sequences. The phylogenetic tree shown is based on 2,536 manually aligned base positions of 50 taxa, calculated by Treefinder (Jobb, 2008) using the maximum likelihood (ML) method under different substitutional models for each partition. The substitution models were as follows: 18S (1,686 bases) J2 [Optimum, Empirical]:GI [Optimum]:5; ITS1 (388 bases) J1 [Optimum, Empirical]:G [Optimum]:5; 5.8S (137 bases) HKY [{3,1,1,1,1,3}, Empirical]:G [Optimum]:5; and ITS2 (325 bases) GTR [Optimum, Empirical]:G [Optimum]:5. The Bayesian values (>0.95) (MB) were calculated by MrBayes 3.1. A general time reversible model with gamma shape parameter and proportion of invariable sites (GTR+I+G) was applied to each partition. The parameters were unlinked and allowed to vary across the partitions (Ronquist & Huelsenbeck, 2003; Posada & Buckley, 2004). The stationary distribution was assumed after 4 million generations when the average standard deviations of split frequencies between two runs was lower than 0.01. To test the tree confidence, bootstrap values (>50%) for ML (1,000 replicates), NJ (1,000 replicates; using Paup 4.0), and MP (1,000 replicates; using Paup 4.0 (Swofford, 2002) were calculated. Support values are shown at the branches in the order: MB, ML, MP, and NJ. *Scale bar* indicates substitutions per site. The sequences were obtained from Genbank [National Center for Biotechnology Information (NCBI)]. For each taxon, the NCBI accession number is given in brackets

wish to determine a taxon in a fixed sample under the inverted microscope. The "Annotated catalogue of *Scenedesmus* and nomenclaturally related genera," published by Hegewald & Silva (1988) is a milestone in the history of conventional systematics of green algae. More than 800 taxa were included in details that provide a clear testimony of how the game of nature creates a multitude of morphospecies. Although this catalogue is a good guide for microscopists, at the end of the day it is difficult to decide on the species delineation in Scenedesmaceae. Several publications, each justifying the different views of the authors, have been written.

According to Hegewald (1997), the great morphological variability of Scenedesmaceae can be attributed to the strictly non-sexual propagation. This argument may also be valid for many other coccoid green algae such as the Selenastraceae. The simple reproduction by means of autospores results in a situation whereby all mutations occurring, which do not significantly influence growth and ability of the mutants to compete, remain and are not lost by genetic processes. This discussion leads to the old question on the possible occurrence of flagellated stages in *Scenedesmus*, first reported by a Ukrainian phycologist J. J. Valz more than 130 years ago (according to Hegewald, 1997). Trainor (1963) recorded the presence of flagellates in cultures of *Scenedesmus*, while Lukavský (1991) and Cepák (1993) found them in outdoor mass cultures. Trainor (1996) has described the production and germination of zygospores. However, the majority of these algae propagate asexually by autospores, and the reasons given by Hegewald to explain the wide morphological variability remain valid.

Revisions of the class followed the introduction of molecular methods. First, it became evident that the morphologically differentiated subgenera should be upgraded to generic status. Consequently, the genus *Desmodesmus* was separated from *Scenedesmus* (An et al., 1999). Whereas *Desmodesmus* comprises species characterized by many substructures on the cell wall and are equipped with teeth, rosettes, warts and spines, the species of *Scenedesmus* have a smooth, non-ornamented cell wall (Hegewald, 2000). The genus *Acutodesmus*, which comprises of more or less ellipsoidal, spindle-shaped taxa that show longitudinal ridges under the TEM, was established by Tsarenko & Petlevanny (2001). Later, *Pectinodesmus* was erected for taxa with similar morphology as *Acutodesmus* but differing in molecular phylogeny (Hegewald et al., 2010). Several genera which are presently monotypic such as *Hylodesmus* and *Comasiella* have recently been established (Eliáš et al., 2010; Hegewald et al., 2010).

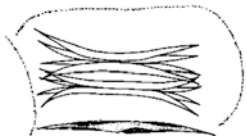
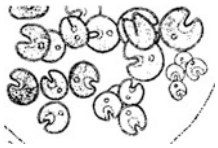
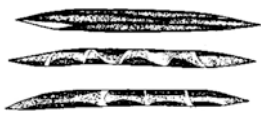
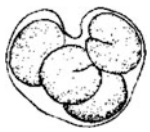
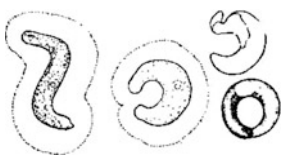
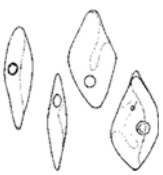
A major surprise resulting from the molecular studies is the clustering of *Coelastrum*-morphotypes with *Scenedesmus* suggesting that the flat coenobia with cells arranged in one or two rows of *Scenedesmus*-relatives are phylogenetically closely related to the spherical coenobia of *Coelastrum* and allied species (Fig. 1) (Hegewald et al., 2010). Following confirmation through molecular studies of the separate position of *Coelastrum reticulatum*, the old name *Hariotina* was reintroduced (Hegewald et al., 2002).

At present, 13 genera of Scenedesmaceae are phylogenetically and morphologically well defined (Table 3). Several other genera of this family, such as all the crucigenoid groups, are yet to be revised.

Hydrodictyaceae

The Hydrodictyaceae comprises microscopic colonies of *Pediastrum*, *Euastropsis*, and *Sorastrum* as well as macroscopic colonies of *Hydrodictyon*. The most

Table 2 Genera of Selenastraceae confirmed by 18S rRNA gene phylogeny and their main diacritic morphological characteristics

Genus	Drawing	Main diacritic morphology
Ankistrodesmus		Needle-shaped cells, in colonies, parallel arrangement of autospores
Kirchneriella		Semilunate- to crescent-shaped cells, in colonies, serial arrangement of autospores
Monoraphidium		Needle- to rod-shaped cells, solitary, serial arrangement of autospores
Nephrochlamys		Semilunate-shaped cells, in colonies, serial arrangement of autospores, widening mother cell wall
Podohedriella		Needle-shaped cells, solitary, heteropolar, serial arrangement of autospores
Quadrigula		Cylindrical cells with rounded ends, in quadricellular colonies, parallel arrangement of autospores
Raphidocelis		Capricorn-shaped cells, arcuated, solitary or in irregular colonies, serial arrangement of autospores
Rhombocystis		Cells rhomboidal with slightly thickened poles, solitary or colonial, parallel arrangement of autospores
Selenastrum		Semilunate-shaped cells in regular colonies, parallel arrangement of autospores
Tetranephris		Bean-shaped cells in quadricellular colonies, touched at the poles, serial arrangement of autospores

Drawings after Komárek & Fott (1983)

recent review reduced the number of *Pediastrum*-species to 24 (Komárek & Jankovská, 2001). Hydrodictyaceae exhibit distinct reproduction strategies: they produce asexually inside the parental cells through biflagellated zoospores that aggregate after swarming to daughter colonies which are released from the mother-sporangiae in a gelatinous bubble. Sexually, Hydrodictyaceae reproduce by isogametes. The ultrastructure of the flagellar apparatus is characterized by a directly opposite (DO) configuration in *Hydrodictyon* and *Pediastrum* (Wilcox & Floyd, 1988).

Phylogenetic analysis of *Hydrodictyon*, *Pediastrum*, and *Sorastrum* has revealed a pattern of colony-form evolution within the family from two-dimensionality to three-dimensionality (McManus & Lewis, 2005). Another molecular phylogenetic study of 28 hydrodictyacean strains revealed polyphyly in *Pediastrum* and resulted in taxonomic conclusions (Buchheim et al., 2005). Beside *Pediastrum*, the genera *Monactinus*, *Parapediastrum*, *Pseudopediastrum,* and *Stauridium* were delineated. It is interesting to note that *Pediastrum duplex* with a complex morphology of colonies evolved polyphyletically (McManus & Lewis, 2011). Consequently, a new genus *Lacunastrum* was erected (McManus et al., 2011). It has also been shown that members of the genus *Tetraedron* evolved as a sister clade to the Hydrodictyaceae. However, the evolutionary link between the tetraedric unicells of autosporic *Tetraedron*, zoosporic *Chlorotetraedron*, and the zoosporic colonial Hydrodictyaceae remains obscure (McEntee et al., 1977; Komárek & Kováčik, 1985; Hegewald et al., 2001; Buchheim et al., 2005). Presently, the Hydrodictyaceae has 10 genera as confirmed by morphological and molecular analyses (Table 4).

Sphaeropleaceae

This group provides a good example for demonstrating that life form sensu Pascher (1918) is not suitable for the natural grouping of algae. Two different life forms in this family, the filamentous (*Sphaeroplea*) and the coccoid (*Ankyra*) green algae cluster together (Fig. 1). *Sphaeroplea* is a filamentous green algal genus with multinucleate (coenocytic) cells (Buchheim et al., 2001). Its propagation is by asexual division of the cells in the unbranched filaments and oogamous sexual reproduction. *Actractomorpha* produces extremely long needle-shaped solitary cells that are coenocytic in character and can propagate asexually by zoospores and sexually by anisogamy or seldom by oogamy (Hoffman, 1983). Normally, this alga occurs mostly in soil. However, it has also been observed in freshwaters (Schmidt & Fehér, 1999–2000). Freshwater forms are not always correctly designated (e.g., as *Closteriopsis longissima* f. *gigantea* Heynig, 1980). Such planktonic cells can grow to lengths of 1,000–1,900 μm. The most frequently observed members of Sphaeropleaceae in the plankton are species of *Ankyra*, which produce spindle-shaped heteropolar cells with an anchor (Reymond & Hegewald, 1988). Species of *Ankyra* dominate in the clear water stages of stagnant waters (Barone & Naselli Flores, 1994). Although *Ankyra* mainly propagate asexually by zoospores, propagation in the genus needs more detailed investigation. Several types of unidentified aplanospores or resting stages have been observed in *Ankyra* cultures and field samples (Fott, 1971; Krienitz & Heynig, 1982).

Other clades

The *Mychonastes*-clade contains tiny, mostly spherical or oval cells of small size that occur as solitary cells or in colonies previously considered as two separate genera, *Mychonastes* and *Pseudodictyosphaerium*. Members of these genera belong to the most common pico- or small nanoplankton green algae in fresh or brackish waters. Molecular phylogenetic analyses have shown that both genera are mixed in the same clade (Krienitz et al., 1999, 2011a). Most species were previously described under the generic name of *Pseudodictyosphaerium* (Hindák, 1978a, b, 1988). However, since the genus *Mychonastes* was described four month earlier in 1978 (Simpson & Van Valkenburg, 1978), it therefore has nomenclatural priority. Consequently, the species of *Pseudodictyosphaerium* have been transferred to *Mychonastes*.

The *Bracteacoccus*-clade comprises mainly of soil algae. However, *Planktosphaeria gelatinosa*, which is a freshwater plankton commonly present, has been found to be a close relative of *Bracteacoccus* and *Radiococcus* (Wolf et al., 2003b). Unfortunately, this morphotype has been under-represented in recent taxon samplings for molecular considerations and needs further investigation. The edaphic "*Mychonastes*" *zofingiensis* does not belong to the true *Mychonastes* genus, and it is very likely that a new generic

Table 3 Genera of Scenedesmaceae confirmed by 18S rRNA or ITS gene phylogeny and their main diacritic morphological characteristics under LM and TEM

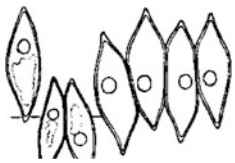
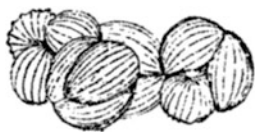
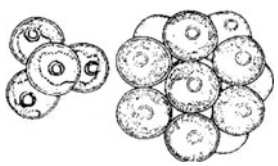
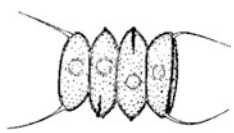
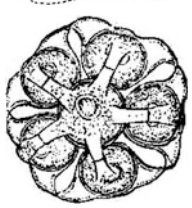
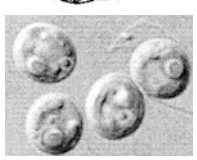
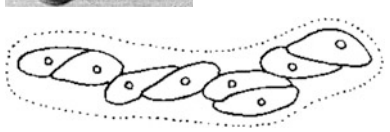
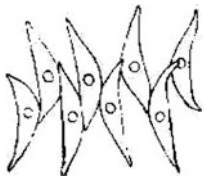
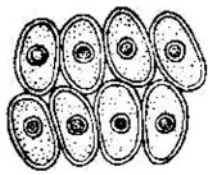
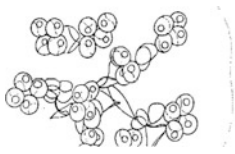
Genus	Drawing	Main diacritic morphology
Acutodesmus		Cells spindle-shaped with acute poles, without spines, in flat or curved coenobia or solitary, TEM: cell wall without or with few weak ridges
Asterarcys		Cells irregular ovoid, in 4- or 8-celled coenobia or solitary, chloroplast net-shaped
Coelastrella		Cells spherical or ellipsoid, in 2- or 4-celled groups, cell wall with meridional ribs
Coelastrum		Cells spherical or broad ellipsoidal, in spherical coenobia, cell wall smooth or rugose, no mucilage
Comasiella		Cells bean-shaped in flat, slightly curved coenobia, cell wall smooth
Desmodesmus		Cells cylindric, in flat coenobia, often with special cell wall ornamentations on an fourth outer layer, rosettes, tubes, warts, teeth, ribs, and spines
Dimorphococcus		Bean- or semilunate-shaped cells connected in syncoenobia by mucilaginous strands
Hariotina		Cells spherical, in large spherical coenobia connected by long cell wall extensions, mucilage
Hylodesmus		Cells spherical or oval, solitary, TEM: few delicate ribs
Neodesmus		Cells spindle- to drop-shaped, in 2-celled coenobia, which are connected in string-like of syncoenobia

Table 3 continued

Genus	Drawing	Main diacritic morphology
Pectinodesmus		Cells spindle-shaped, in flat or curved coenobia, TEM: cell wall with strong longitudinal ridges
Scenedesmus		Cells oval or cylindrical with obtuse or truncate poles, without spines, in flat or slightly curved coenobia, cell wall smooth
Westella		Cells spherical to ovoid, in 4-celled square-shaped coenobia, which are connected to syncoenobia

Drawings after Komárek & Fott (1983) and Krienitz (1990). Micrograph of *Hylodesmus* after Eliáš et al. (2010)

designation will be necessary for this taxon (Krienitz et al., 2011a).

The *Golenkinia*-clade is a member of the Chlorococcales *s.str.* (Fig. 1). The morphological and ontogenetic peculiarities, especially the CW basal body orientation shown by Hegewald & Schnepf (1984), have been supported by molecular analyses (Wolf et al., 2003a). Members of the polyphyletic genus *Chlorococcum* mostly occur in soils. However, after a heavy downpour, they can be washed out and transported into water bodies where they are able to propagate as observed in the case of *Chlorococcum robustum* (Krienitz et al., 1997).

Trebouxiophyceae

Chlorellaceae

This clade contains the classical "green balls." Following the description of the archetypical form of coccoid green algae, *Chlorella vulgaris* by Beijerinck (1890), more than 100 species of this genus have been described from freshwater, marine, and soil habitats. However, most of them need to be revised and transferred to other genera and other families. It has been shown that *Chlorella*-like green spheres evolved independently in different evolutionary lineages of Chlorophyceae, Trebouxiophyceae, and prasinophytes (Friedl, 1997; Chapman et al., 1998; Pröschold & Leliaert, 2007; Darienko et al., 2010). Based on the examination of biochemical and molecular data, Huss et al. (1999) have reduced the *Chlorella* genus to four species.

In the year 2000, a new development followed the work of Hegewald & Hanagata (2000) who found out that the coenobial ellipsoid *D. acuatus*, formerly classified in Scenedesmaceae, was closely related to *Chlorella kessleri*. The needle-shaped *C. acicularis*, traditionally considered as member of Selenastraceae, was also found to cluster in this lineage (Ustinova et al., 2001). *Actinastrum hantzschii*, a coenobial taxon formerly in the Coelastraceae, has also been transferred to Chlorellaceae (Wolf et al., 2002b). Krienitz et al. (2004) have identified two separate lineages within Chlorellaceae, designated as a *Chlorella*-clade and a *Parachlorella*-clade. Presently, these two clades have both old and new genera (Table 5; Figs. 2, 3).

The *Chlorella*-clade has eight different lineages that form clusters designated as genera. The scope of this genus has been extended to include 14 species. Among these species are solitary cells with or without mucilage and colonial forms that exhibit a morphology resembling *Dictyosphaerium* (Bock et al., 2011a). *Chlorella*-species occur in freshwater, soil and as endosymbionts (Pröschold et al., 2011). Fawley et al. (2005b) found *Meyerella*, a tiny sphere without pyrenoid, within the *Chlorella*-clade. According to

Table 4 Genera of Hydrodictyaceae confirmed by 18S or 26S rRNA gene phylogeny and their main diacritic morphological characteristics

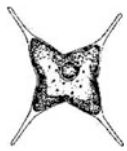
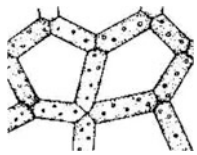
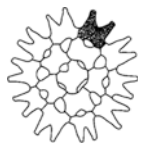
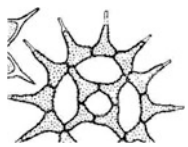
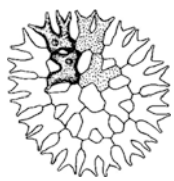
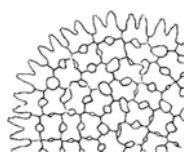
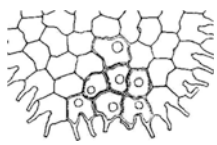
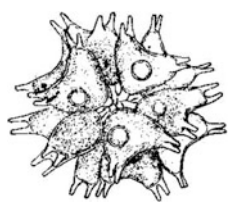
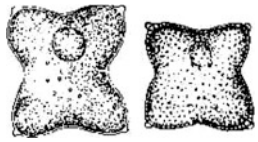
Genus	Drawing	Main diacritic morphology
Chlorotetraedron		Cells tetrahedral or polyhedral, with elongated cell wall protuberances at the corners, solitary
Hydrodictyon		Cells cylindrical in macroscopic net-like, one-layered coenobia
Lacunastrum		Flat coenobia with large intercellular spaces, marginal cells with two lobes
Monactinus		Flat coenobia with large intercellular spaces, marginal cells with one tapering lobe
Parapediastrum		Flat coenobia with intercellular spaces, marginal cells with 2 lobes each divided into 2 projections
Pediastrum		Flat coenobia with large intercellular spaces, marginal cells with 2 projections
Pseudopediastrum		Flat coenobia without intercellular spaces, marginal cells with two tapering lobes in one plane
Sorastrum		Three-dimensional coenobia, cells with 2 or 4 projections
Stauridium		Flat coenobia without intercellular spaces, marginal cells incised trapezoid or with projections in 2 planes

Table 4 continued

Genus	Drawing	Main diacritic morphology
Tetraedron		Cells flat or twisted, 3-, 4- or 5-sided, with rounded or elongated or spined corners, solitary

Drawings after Komárek & Fott (1983) and McManus et al. (2011)

Luo et al. (2010), the genera *Actinastrum*, *Didymogenes*, *Hegewaldia* and *Micractinium* also belong to this clade. *Hegewaldia* comprises taxa with facultative bristle production and oogamy (Pröschold et al., 2010). *Micractinium* usually occur in colonies and produce bristles. However, the colonies can disintegrate to form single cells without bristles. The formation of bristles can be triggered by substances produced by grazers such as the rotifer *Brachionus* (Luo et al., 2006). In addition to the several species of *Chlorella*, the *Chlorella*-clade has two other lineages with a colonial morphology similar to that of *Dictyosphaerium*. The two lineages belong to the genera *Heynigia* and *Hindakia* (Bock et al., 2010).

Six genera occur in the *Parachlorella*-clade. The genus *Parachlorella* has three species that are solitary or colonial, and covered by a mucilaginous envelope (Krienitz et al., 2004; Bock et al., 2011b). According to Krienitz et al. (2010), the *Dictyosphaerium*-morphotype evolved independently in different lineages of Chlorellaceae. The scope of the genus *Dictyosphaerium* has been reduced to three species, the type species *D. ehrenbergianum* (Bock et al., 2011c) and two new taxa. The genus *Mucidosphaerium* was established based on differences in its molecular phylogeny from that of *Dictyosphaerium*. *Mucidosphaerium* contains two former *Dictyosphaerium*-species, *D. pulchellum*, and *D. sphagnale*, as well as two new species. The taxa of the genera *Dictyosphaerium* and *Mucidosphaerium* are of great importance as they play an important role of establishing plankton communities. The spindle- to-needle-shaped genera *Closteriopsis* and *Dicloster*, as well as the marine, spherical *Marinichlorella* also belong to the *Parachlorella*-clade (Aslam et al., 2007).

Oocystaceae

The family of Oocystaceae is a natural lineage in Trebouxiophyceae confirmed by ultrastructural and molecular criteria. Many members of this group are very common in the plankton of stagnant and flowing waters. The extended cell wall is multilayered and constructed from crystalline cellulose fibers. Molecular phylogenetic data have revealed the monophyly of Oocystaceae. However, the species concept in this group has not been confirmed (Hepperle et al., 2000; Pažoutová et al., 2010; Krienitz & Bock, 2011). Because of the reduced taxon availability in strain collections and remarkable uncertainties on the concept of the type genus *Oocystis*, the circumscription of the genera of Oocystaceae has remained obscure. Hindák (1988) re-applied Lemmermanns (1903) criteria for distinguishing between *Oocystis* (without pyrenoids) and *Oocystella* (with pyrenoids). Hence, the taxonomic relevance of pyrenoids in Oocystaceae must be resolved. Several genera are characterized by incrustations (*Amphikrikos*, *Granulocystis*, *Granulocystopsis*, and *Siderocelis*); however, their origins and taxonomical values are still the subject of ongoing discussions.

Other clades

Choricystis and *Botryococcus*. *Choricystis*-species exhibit tiny bean-shaped, solitary cells and were traditionally assigned to the Chlorophyceae family. However molecular data have revealed that they have a close affiliation to the Trebouxiophyceae (Krienitz et al., 1996a, 1999). Surprisingly, the colonial and oil-producing alga *Botryococcus braunii* clusters close to *Choricystis* (Senousy et al., 2004). Komárek & Marvan (1992) established a multitude of *Botryococcus* species based on morphology. However, according to Plain et al. (1993) the morphological features of *B. braunii* vary depending on growth conditions hence it is not reliable to define more species. The figures provided by Plain et al. (1993) were exclusively those of *B. braunii*. This study did not include any of the new

Table 5 Genera of Chlorellaceae confirmed by combined 18S rRNA and ITS gene phylogeny and their main diacritic morphological characteristics

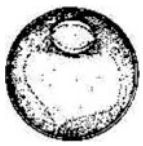
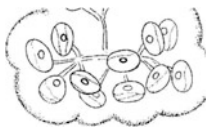
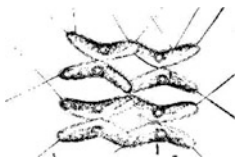
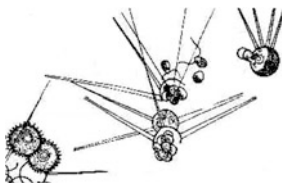
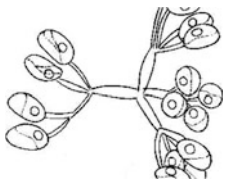
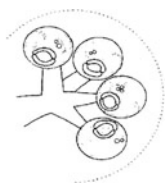
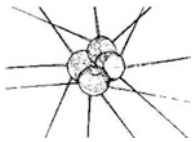
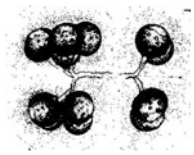
Genus	Drawing	Main diacritic morphology
Actinastrum		Cells rod-shaped, elongated, radially arranged in coenobia
Chlorella		Spherical or broad oval cells, with one pyrenoid, solitary or in mucilage covered colonies
Closteriopsis		Needle-shaped cells with several pyrenoids in the lateral chloroplast, solitary
Dictyosphaerium		Broad oval cells interconnected by strands attaching the elongated cell side, in mucilaginous colonies
Dicloster		Cells ellipsoidal arcuated with convex sides attached in 2- or 4-celled coenobia, 1 or 2 pyrenoids
Didymogenes		Cells cylindrical curved with convex side attached in 2-, 4- or 16-celled coenobia, one pyrenoid, spines
Hegewaldia		Spherical cells with or without bristles, solitary or in colonies, 1 pyrenoid, facultative oogamy
Hindakia		Broad oval cells interconnected by strands attaching the apical pole, in mucilaginous colonies, 1 pyrenoid
Heynigia		Spherical cells interconnected by mucilaginous strands in mucilaginous colonies, 1 pyrenoid

Table 5 continued

Genus	Drawing	Main diacritic morphology
Micractinium		Spherical cells with long bristles, in colonies, 1 pyrenoid
Meyerella		Cells short cylindrical, small, without mucilage, solitary, pyrenoid missing
Mucidosphaerium		Spherical cells interconnected by mucilaginous strands in mucilaginous colonies, 1 pyrenoid
Parachlorella		Spherical cells covered by mucilage, solitary or in groups, 1 pyrenoid

Drawings after Komárek & Fott (1983), Krienitz, et al. (2004), and Bock et al. (2010). Micrograph of *Meyerella* after Fawley et al. (2005b)

species described by Komárek & Marvan (1992) such as *B. terribilis* that has been shown to be very common in freshwater phytoplankton of diverse climatic regions (Krienitz et al., 1996b; Ballot et al., 2009; Fanés Treviño et al., 2009). The conversion of the *braunii*-morphotype into the *terribilis*-morphotype and vice versa was never demonstrated. It is therefore, important to as a matter of urgency include more *Botryococcus*-taxa into molecular studies. From an ecological point of view, the alliance of the picoplanktonic, invasive *Choricystis*, and the microplanktonic, attuning *Botryococcus* shown in Fig. 2, is not explainable. Padisák et al. (2009, 2010) grouped *Choricystis* into the functional group X1 and *Botryococcus* in group F. Later considerations will probably reveal the existence of some undiscovered lineages that have evolved between these two clades. Henley et al. (2004) documented a close relationship between *C. minor* and *Paradoxia multiseta* based on 18S rRNA phylogeny. However, according to the micrograph given (showing globular cells) in the catalogue of the UTEX strain collection, the identity of the *Paradoxia* strain investigated is doubtful.

Between the *Choricystis*-clade and the Chlorellaceae, several clades of green coccoid soil and aerophytic algae, such as *Chloroidium*, *Watanabea*, *Dictyochloris*, and the lichen symbionts of Trebouxiales have evolved (Friedl, 1995; Darienko et al., 2010; Rindi et al., 2010). These algae occasionally occur in the plankton of freshwaters. However, if a sufficient starting inoculum of these air- and soil-born algae is available and if the algae are able to propagate under the new conditions, then colonization of standing water bodies can occur rapidly (Happy-Wood, 1988).

Evolution of morphological peculiarities of coccoid green algae and their possible ecological functions

One of the most striking peculiarities of numerous coccoid green algae is the development of a uniform morphology hidden in the picoplankton size group which is nearly unidentifiable by LM. Convergent evolution resulted in a tiny, more or less spherical morphotype which covers an extremely high diversity with regard to phylogeny and physiology (Potter et al., 1997; Hepperle & Krienitz, 2001; Krienitz et al. 2011a). Based on its fast growth and high rates of reproduction and primary production, picophytoplankton can play a key role in food webs of

freshwater, marine, and saline habitats (Stockner & Antia, 1986; Raven, 1999). Picoplankton may establish large populations under nearly all levels of trophy (Stockner, 1991; Weisse, 1993; Padisák et al., 1997; Hehmann et al., 2001; Hepperle & Krienitz, 2001). Even under very saline conditions they can dominate all the succession stages of eukaryotic primary producers (Henley et al., 2004; Somogyi et al., 2011; Krienitz et al., 2012).

Picoplanktons evolved as adaptive strategies in all the classes considered here. In the Chlorophyceae, the *Mychonastes*-clade contains several species of picoplankton (Krienitz et al., 2011a). In the Trebouxiophyceae, a number of lineages, which include *Picochloron* and *Chloroparva* from salt pans (Henley et al., 2004; Somogyi et al., 2011), *Choricystis*, and *Nannochloris* from freshwaters (Krienitz et al., 1996a; Yamamoto et al., 2003), have picoplankton species. *Marvania* is a picoplankton species from different inland waters characterized by vegetative propagation through budding (Hindák, 1976; Reymond et al., 1986). Among the prasinophytes, there are many picoplankton lineages which are usually abundant in the oceans (Guillou et al., 2004; Leliaert et al. 2012). Extreme saline inland waters have one lineage with *Picocystis* (Lewin et al., 2000; Hollibaugh et al., 2001; Roesler et al., 2002). *Picocystis salinarum* from saline inland waters represents a link between picoplankton from marine and freshwater habitats and is therefore of great ecological and phylogenetic interest (Krienitz & Kotut, 2010; Krienitz et al., 2012).

Comparison of the fatty acid's contents of green (*Choricystis*, *Mychonastes*) and eustigmatophycean (*Nannochloropsis*) freshwater picoplankton revealed surprising differences in the composition of polyunsaturated fatty acids. The sums of n-6 and n-3 fatty acids are ten times higher in *Nannochloropsis limnetica* than in the green picoplankton (Krienitz et al., 2000; Krienitz & Wirth, 2006). Hence, the eustigmatophycean picoplankton has a higher nutritional value for grazers than green algal picoplankton. This raises the question on how the grazers can differentiate between the nutritious eustigmatophycean and the less nutritious green algae. As a follow-up to Hartmann & Kunkel's (1991) statement "The paradigm of invariate, nonselective feeding by zooplankton is rejected," limnologists have come up with possible mechanism of food selection that include: chemosensory, electrical charge, surface hydrophobicity, and chemical cues (Weisse, 2004). All these interactions between green algae and possible grazers need to be further elucidated.

Incrustations on the surface of coccoid green algae are common across the whole range of systematic groups of green algae. For example, they have been observed in Selenastraceae (*Raphidocelis*), Scenedesmaceae (*Scenedesmus*), Oocystaceae (*Amphikrikos*, *Siderocelis*), and Radiococcaceae (*Coenochloris*) (Hindák, 1977; Crawford, 1978; Krienitz, 1986; Vanormelingen et al., 2007). These incrustations are precipitates of ferric and manganic hydroxides and are of crystalline or amorphous nature (Crawford & Heap, 1978). The genesis and structure of this cell wall deposits varies with taxon suggesting a genetic influence and give the impression that this process is under the control of the cell (Crawford & Heap, 1978). So far, no vesicle driven expression through pores from inner to outer cell wall has been observed. The ecological function of these incrustations remains a subject of discussion. From our field observation, it was apparent that numerous algae tend to develop incrustations under highly disturbed conditions in rivers (Krienitz, 1990, 1998). It can therefore be hypothesized that the incrustations increase the weight of the cells, and this allows the cells to sink to the lower less disturbed habitats, which are conducive for propagation.

Spines and bristles of the plankton algae are adaptive features that promote buoyancy and reduce grazing pressure (Van den Hoek et al., 1995). A large number of publications have documented the interaction of kairomon-producing grazers and spine- and bristle-formations by coccoid green algae (summarized by Van Donk, 2005). Spines and bristles have different origins and compositions. Scenedesmaceae produce rigid, tube-like spines as elaborated parts of the outer sporopollenin-like cell wall layer (Schnepf et al., 1980). In contrast to spines, bristles develop after cell wall formation and lack cellulosic fibers and algaenan substances (Schnepf et al., 1980; Hegewald & Schnepf, 1984). *Didymogenes* and *Micractinium* produce bristles of the same type (Schnepf & Hegewald, 1993). The spines of *Golenkinia* contain cellulosic fibers and are produced after cell wall formation (Hegewald & Schnepf, 1984). The morphological and biochemical differentiation of spines and bristles in the various groups of coccoid green algae closely agree with their differences based on molecular data. The spine producing Scenedesmaceae belong to the

Chlorophyceae (Hegewald, 1997). The close relationship between *Didymogenes* and *Micractinium* as members of Trebouxiophyceae was demonstrated by Luo et al. (2006). The polyphyletic origin of bristles within the Trebouxiophyceae was confirmed by Pröschold et al. (2010). The separate position of *Golenkinia* as a member of Chlorococcales *s.l.* was revealed by Wolf et al. (2003a).

The production of mucilage by the cells is one of the most readily observed phenomena in coccoid green algae. It is commonly observed in all groups under discussion and has a polyphyletic origin. The production of mucilage largely depends on environmental influences and interaction with other species such as grazing pressure and resource competition (Reynolds, 2007). It may protect the algae from ingestion or, even if ingested, against digestion while passing through the intestinal tract of zooplankton (Porter, 1973). On the other side, the mucilaginous envelope can act as microhabitat for bacterial flora that produces substances with a nutritional value or have stimulatory effects (Cole, 1982). The mucilage can also act as a depository of nutrients (Decho, 1990). Furthermore, mucilage affects the buoyancy of the phytoplankton (Boney, 1981). The multitude of ecological functions makes it understandable that morphotypes characterized by mucilage evolved in different lineages and at different times as a response to the diverse interactions. This observation has clearly been demonstrated in the *Dictyosphaerium*-morphotype (Bock et al., 2010, 2011c; Krienitz et al., 2010). Even in one and the same genus such as *Chlorella* or *Mychonastes* mucilage possession has appeared and disappeared several times (Bock et al., 2011a; Krienitz et al., 2011a). Hence, the question on which of the contrasting features, "with mucilage" or "without mucilage," is more ancestral remains open.

A very complicated case is the elucidation of the origin of the radiococcacean morphotype. Kostikov et al. (2002) revised this "family" based on a detailed examination of morphology and ontogeny. However, a few molecular phylogenetic studies have indicated a polyphyletic evolution of members of this family (Wolf et al., 2003b; Bock et al., 2011b). The study of this group has been hampered by scarcity of cultures, because of their poor growth performance in culture. Future studies subjecting single colonies to PCR can help to shed some light on the phylogeny of this "slimy colonial green spheres."

Coccoid green algae represent the most diverse group among plankton algae. It is one of the groups well suitable for cultivation and therefore provides a wide range of experimental opportunities to study the genesis and ecological advantages of these organisms. Based on autecological features and functional characteristics, Reynolds et al. (2002) and Padisák et al. (2009) have placed most of the coccoid green algae in codon **X1** (shallow mixed layers in enriched conditions), and some of them (*Botryococcus*, *Dictyosphaerium*, and *Oocystis*) in codon **F** (clear epilimnia), and others (*Coelastrum*, *Pediastrum*, and *Scenedesmus*) in codon **J** (shallow enriched lakes, ponds, and rivers). Recent observations on species characterisics that include drastic changes in size through colony disintegration, and the periodic appearance and disappearance of features, such as mucilage, incrustations, and spines depending on interactions with abiotic and biotic environments suggest that a species can possibly deviate from this ecofunctional classification.

The phylogenetic species concept

The debate on the "right" species concept is certainly old and ongoing. In the post-Darwinian time, numerous concepts were proposed with different criteria for species delineation (e.g., Mayr, 1942; Henning, 1966; de Queiroz, 1998). As the biological (or reproductive) species concept of reproductive isolation (Mayr, 1942) is not applicable to asexually reproducing taxa (which is the case for many protists lineages, and especially the coccoid green algae), and the morphological species concept is very subjective, the phylogenetic species concept (or *diagnostic* concept sensu Mallet (2006)) has gained a lot of ground (Cracraft, 1989). This concept is based on genetic markers and recognizes the smallest monophyletic clusters of taxa worthy of taxonomic recognition as individual species. The populations/strains must share the same ancestor and all its descendants to be considered as individual species (Johansen & Casamatta, 2005; Mallet, 2006). This concept allows the delimitation of species by specific data like base changes in gene sequences. Hence, the number of recognized species largely depends on the chosen marker; the more conserved the analyzed region, the fewer the number of species recognized and vice versa (Hoef-Emden, 2007; Rindi

et al., 2009). The same applies to the chosen threshold of genetic divergence between sequences. A higher threshold for the congruence of sequences (e.g., 98% congruence) results in more species recognized than a lower threshold (e.g., 94%). The small subunit (SSU) of the ribosomal rRNA (18S) gene sequence is conventionally used as marker in phylogenetic studies (Chapman et al., 1998; Huss et al., 1999). The 18S rRNA is a universal gene and plays a major role in protein translation. It contains highly conserved regions which favors the development of universal primers and simplifies sequence alignment of distant taxa (Long & David, 1980; Sogin et al., 1986). As it is present in numerous copies within the genome, it is easy to amplify during a PCR. These criteria makes the 18S rDNA a useful tool for the phylogenetic resolution of higher algal ranks such as classes and orders (Friedl, 1995; Krienitz et al., 2003, 2011a). Exhaustive studies combining molecular and morphological data have shown that the 18S is too conserved to separate closely related species in coccoid green algal lineages that are clearly distinguishable by morphological and/or ecological factors (e.g., Krienitz et al., 2004; Bock et al., 2010; Darienko et al., 2010).

New insights and methodological opportunities in the molecular techniques have shifted the focus to the internal transcribed spacer 2 (ITS2). The ITS2 is a fast evolving marker region, situated between the 5.8S and the 28S rRNA on the ribosomal gene. To obtain a mature rRNA, the ITS2 needs to be excised during the maturing stage so that it folds up into a characteristic secondary structure. The primary sequence of the ITS2 is highly variable, even between closely related taxa. However, the ITS2 secondary structure motive seems to be extremely conserved in all eukaryotes (Mai & Coleman, 1997; Coleman, 2003, 2007; Schultz et al., 2005). Indeed the ITS2 folds normally in four helices, with helix III being the longest and the most conserved (Coleman, 2007). Due to this specific folding pattern, it is possible to align closely related sequences unambiguously.

The Compensatory Base pair Changes (CBC) concept refers to base changes within the secondary structure where a matching pair of bases in a double stranded section of the structure in one taxon is exchanged by a different matching pair in a second taxon (Gutell, 1994). Mating experiments in sexual protist lineages in combination with CBCs within the ITS2 have pushed the discussion toward concepts merging Mayr's biological species concept with molecular data (Coleman, 2000; Denboh et al., 2003; Behnke et al., 2004; Hoef-Emden, 2007). In protist lineages with at least occasional sexual reproduction, the occurrence of CBCs in conserved regions of the ITS coincides with the sexual incompatibility between species (Mai & Coleman, 1997; Müller et al., 2007; Coleman, 2009). As a consequence, the presence of CBCs or hemi-CBCs (only one-sided base changes) is often used for species delineation in morphological difficult groups or when only asexual reproduction is known (Krienitz et al., 2004; Hoef-Emden, 2007). Additionally, the CBC concept includes the step of calculating the secondary structure. Software for secondary structure prediction can help to facilitate the procedure but are not reliable if the entire ITS2 sequence is directly submitted to a RNA folding server. The sequences have to be submitted in small pieces which correspond to the different expected helices to get consistent results (Zuker, 2003; Hoef-Emden, 2007; Schultz & Wolf, 2009).

Apart from the nuclear regions (18S, ITS, 28S), a variety of markers have been used for species delineation. Among these markers is the chloroplast encoded large subunit of the ribulose-bisphosphate carboxylase (*rbcL*) gene. This protein coding region results in a straightforward alignment and tends to be more variable than the 18S but not as variable as the ITS regions. Another advantage of this marker over the nuclear markers is that since it is a plastid gene, the risk of amplifying contaminants like fungi is reduced. As a protein-coding gene, the *rbcL* sequence can be partitioned into codons with first, the second, and the third base positions. The third base (wobble) position has a higher evolution rate than the first or the second base positions because of its substitutional saturation (Rindi et al., 2009). However, the usually applied *rbcL* sequence has been criticized because it is not variable enough for the different substitution rates for the wobble position may cause problems within the phylogeny. Several other markers have been tested on closely related algae in an effort to separate species but none has proved to be exhaustive for a large number of groups. Examples are the trnG^{ucc} intron sequences for Desmidiales (Neustupa et al., 2010; Nemjova et al., 2011), actin I locus sequences for *Asterochloris* (Škaloud & Peksa, 2010); the *psbA*/*rbcL* spacer in combination with the *rbcL* gene for the *Tribonemataceae* (Rybalka et al., 2009) and many more.

The recent discussion proposing the use of a "barcode" to facilitate the identification of taxa has resulted in the recognition of the need to identify a specific DNA region for species delineation again (Hebert et al., 2003). The ideal barcode should be a short sequence, easy to amplify by universal primers and with the power to resolve organisms at species level (e.g., Hebert et al., 2003; Zimmermann et al., 2011). Under this context, various barcodes have been proposed for different organisms. For animals, part of the mitochondrial cytochrome oxidase c subunit I gene (COI, cox1) has been proposed and is now widely used (Hajibabaei et al., 2007). Evans et al. (2007) have proposed the cox1 as barcode for diatoms. The V4 subregion on the 18S rRNA gene was proposed by Zimmermann et al. (2011) for diatoms. Moniz & Kaczmarska (2010) have suggested use of the 5.8S and part of the ITS2 for diatoms, and the same was also successfully tested on *Chlorella*-related strains (Bock et al., 2011a). The ITS2 is favored by many scientists because of its variability combined with the conserved folding pattern (Buchheim et al., 2011). As the ribosomal operon may contain several versions of ITS2, indels of numerous nucleotides may be present in the less-conserved parts of the different ITS2 versions, which prevents direct sequencing and requires cloning or the use of specific primers (Pröschold et al., 2005). Hence, the establishment of a barcode conception for algae is of great practical importance. Unambiguous designation of species is essential for water quality studies. Barcodes can be used for the identification of standard organisms because morphological concepts often fail to provide accurate identification of algae (Zimmermann et al., 2011). It is especially important to identify barcodes for indicator species similar to the functional group conception established by Reynolds et al. (2002). There are many other indices in different parts of the world. However, it is important that whichever index is used, it should give a correct species identification.

The role of the field workers and experts of phenotypic and ecological characterization of algae

In the assessment of the quality of inland waters, phytoplankton community structure provides a useful indicator tool (Salmaso et al., 2006; Pearl et al., 2007). This is due to the following important attributes:

- being the main pelagic primary producers the phytoplankton play a key role in the functioning of standing water bodies,
- as a consequence of its high reproduction rates, phytoplankton gives a rapid response to changes in environmental conditions,
- phytoplankton communities are generally more diverse than other eukaryotic populations within aquatic food webs,
- species composition of the phytoplankton community, i.e., its biodiversity, has a critical influence on many kinds of water-utilization by man.

Hence phytoplanktologists worldwide contribute to the establishment of water quality assessment methods. In Europe, the Water Framework Directive is the key approach to the evaluation and protection of the inland surface waters (Padisák et al., 2006; Anneville et al., 2008). To support the practical field work, harmonized taxon lists have been established. For the phytoplankton, such a list was developed by Mischke (2006) and Mischke & Nixdorf (2008). About 300 taxa of coccoid green algae are included in this list. However, the list is under continuous improvement, and there are initiatives to assist the field-workers with information systems on taxonomy of algae such as AlgaTerra (Jahn & Kusber, 2006), and AlgaeBase (Guiry & Guiry, 2011). The value of ecological data is to a large extent dependent on the correct identification of organisms as any incorrectly identified samples cannot be improved by any statistical treatment or other sophisticated methods (Kotut & Krienitz, 2011). In this paper (Table 6), we have included 84 coccoid green algae from the harmonized taxon list, which have already been subjected to molecular phylogenetic examinations, and provided their old and new taxonomic designations. It has become evident that many taxa are still missing, and joint research activities are necessary to facilitate the acquisition of more cultures from field samples that can be included in the morphological, ecophysiological, and molecular analyses.

There is no doubt that there are difficulties in the microscopic identification of many species of coccoid green algae. Different phylogenetic species can be hidden under one and the same morphotype (Potter et al., 1997; Šlapeta et al., 2005). On the other hand,

Table 6 Coccoid green algae from the harmonized taxon list of the European Water Framework Directive, subjected to molecular phylogenetic examination, and their old, revised, or confirmed designations

Old designation	Revised or confirmed designation	Reference
*Actinastrum hantzschii**	*Actinastrum hantzschii** Lagerheim	Wolf et al. (2002b)
Amphikrikos sp.	*Amphikrikos* sp.	Hepperle et al. (2000)
Ankistrodesmus bibraianus	*Selenastrum bibraianum** Reinsch	Krienitz et al. (2011b)
Ankistrodesmus fusiformis	*Ankistrodesmus fusiformis* Corda	Krienitz et al. (2001)
Ankistrodesmus gracilis	To be included in a new genus not yet designated	Krienitz et al. (2011b)
Ankistrodesmus nannoselene	To be included in a new genus not yet designated	Krienitz et al. (2011b)
Ankistrodesmus stipitatus	*Ankistrodesmus stipitatus* (Chodat) Komárková-Legnerová	Krienitz et al. (2001)
Ankyra judayi	*Ankyra judayi* (G.M. Smith) Fott	Wolf et al. (2002a)
Ankyra lanceolata	*Ankyra lanceolata* (Korshikov) Fott	Wolf et al. (2002a)
*Botryococcus braunii**	*Botryococcus braunii** Kützing	Senousy et al. (2004)
Chlorella ellipsoidea	*Chloroidium ellipsoideum* (Gerneck) Darienko et al.	Darienko et al. (2010)
Chlorella minutissima	*Mychonastes homosphaera* (Skuja) Kalina & Punčochářová	Krienitz et al. (2011a)
Chlorella pyrenoidosa	*Pseudochlorella pyrenoidosa* (Zeitler) Lund	Darienko et al. (2010)
*Chlorella vulgaris**	*Chlorella vulgaris** Beijerinck	Huss et al. (1999)
Chlorotetraedron incus	*Chlorotetraedron incus* (Teiling) Komárek & Kováčik	Hegewald et al. (2001)
*Choricystis minor**	*Choricystis minor** (Skuja) Fott	Krienitz et al. (1996a, b)
Closteriopsis acicularis	*Closteriopsis acicularis* (G.M. Smith) Belcher & Swale	Ustinova et al. (2001)
Coelastrum astroideum	*Coelastrum astroideum* De Notaris	Hegewald et al. (2010)
Coelastrum microporum	*Coelastrum microporum* Nägeli	Hegewald et al. (2010)
Coelastrum morum	*Coelastrum morum* W. et G.S. West	Hegewald et al. (2010)
Coelastrum pseudomicroporum	*Coelastrum pseudomicroporum* Korshikov	Hegewald et al. (2010)
Coelastrum reticulatum	*Hariotina reticulata** Dangeard	Hegewald et al. (2010)
*Coelastrum sphaericum**	*Coelastrum sphaericum** Nägeli	Hegewald et al. (2010)
Coenochloris hindakii	*Parachlorella hussii* C. Bock, Pažoutová & Krienitz	Bock et al. (2011c)
*Coenochloris polycocca**	*Radiococcus polycoccus* (Korshikov) Kostikov et al.	Wolf et al. (2003b)
Coronastrum ellipsoideum	To be included in a new genus not yet designated	Bock et al. (2011c)
Crucigeniella rectangularis	To be included in a new genus not yet designated	Krienitz et al. (2003)
Dictyosphaerium chlorelloides	*Chlorella chlorelloides* (Naumann) C. Bock, Krienitz & Pröschold	Bock et al. (2011a)
Dictyosphaerium ehrenbergianum	*Dictyosphaerium ehrenbergianum* Nägeli	Krienitz et al. (2010)
Dictyosphaerium pulchellum	*Mucidosphaerium pulchellum* (Wood) C. Bock, Pröschold & Krienitz	Bock et al. (2011b)
Dictyosphaerium tetrachotomum	*Hindakia tetrachotoma* (Printz) C. Bock, Pröschold & Krienitz	Bock et al. (2010)
Didymocystis inermis	*Didymocystis inermis* (Fott) Fott	An et al. (1999)
*Didymogenes palatina**	*Didymogenes palatina** Schmidle	Luo et al. (2010)
*Golenkinia radiata**	*Golenkinia radiata** Chodat	Wolf et al. (2003a)
Kirchneriella aperta	*Kirchneriella aperta* Teiling	Krienitz et al. (2001)
*Kirchneriella obesa**	*Kirchneriella obesa** (W. West) Schmidle	Krienitz et al. (2011b)
Kirchneriella dianae	*Kirchneriella dianae* (Bohlin) Comas	Krienitz et al. (2011b)
Kirchneriella subcapitata	*Raphidocelis subcapitata* (Korshikov) Nygaard et al.	Krienitz et al. (2011b)
*Lagerheimia genevensis**	*Lagerheimia genevensis** (Chodat) Chodat	Krienitz et al. (2003)
*Micractinium pusillum**	*Micractinium pusillum** Fresenius	Luo et al. (2006)
Monoraphidium contortum	*Monoraphidium contortum* (Thuret) Komárková-Legnerová	Krienitz et al. (2011b)

Table 6 continued

Old designation	Revised or confirmed designation	Reference
Monoraphidium convolutum	To be included in a new genus not yet designated	Krienitz et al. (2011b)
Monoraphidium dybowskii	To be included in a new genus not yet designated	Krienitz et al. (2011b)
*Monoraphidium griffithii**	*Monoraphidium griffithii** (Berkeley) Komárková-Legnerová	Krienitz et al. (2001)
Monoraphidium minutum	*Nephrochlamys subsolitaria* (G.S. West) Korshikov	Krienitz et al. (2011b)
Monoraphidium pusillum	To be included in a new genus not yet designated	Krienitz et al. (2011b)
Neodesmus danubialis	*Neodesmus danubialis* Hindák	Hegewald & Hanagata (2000)
Nephrochlamys subsolitaria	*Nephrochlamys subsolitaria* G.S. West	Krienitz et al. (2011b)
Oocystis marssonii	*Oocystis marssonii* Lemmermann	Hepperle et al. (2000)
Oocystis solitaria	To be included in a new genus not yet designated	Hepperle et al. (2000)
*Paradoxia multiseta**	*Paradoxia multiseta** Svirenko	Henley et al. (2004)
Pediastrum biradiatum	*Parapediastrum biradiatum* (Meyen) E. Hegewald	Buchheim et al. (2005)
Pediastrum boryanum	*Pseudopediastrum boryanum* (Turpin) E. Hegewald	Buchheim et al. (2005)
*Pediastrum duplex**	*Pediastrum duplex** Meyen	Buchheim et al. (2005)
Pediastrum kawraiskyi	*Pseudopediastrum kawraiskyi* (Schmidle) E. Hegewald	Buchheim et al. (2005)
Pediastrum simplex	*Monactinus simplex* (Meyen) Corda	Buchheim et al. (2005)
Pediastrum tetras	*Stauridium tetras* (Ehrenberg) E. Hegewald	Buchheim et al. (2005)
*Planktosphaeria gelatinosa**	*Planktosphaeria gelatinosa** G.M. Smith	Wolf et al. (2003b)
*Polyedriopsis spinulosa**	*Polyedriopsis spinulosa** (Schmidle) Schmidle	Hegewald et al. (2001)
Pseudococcomyxa simplex	*Coccomyxa* sp.	Pröschold et al. (2011)
Pseudodictyosphaerium jurisii	*Mychonastes jurisii* (Hindák) Krienitz et al.	Krienitz et al. (2011a)
Quadricoccus ellipticus	*Quadricoccus ellipticus* Hortobágyi	Krienitz & Bock (2011)
*Quadrigula closterioides**	*Quadrigula closterioides** (Bohlin) Printz	Krienitz et al. (2001)
Scenedesmus arcuatus	*Comasiella arcuata** (Lemmermann) E. Hegewald et al.	Hegewald et al. (2010)
Scenedesmus acuminatus	*Acutodesmus acuminatus* (Lagerheim) Tsarenko	Hegewald & Wolf (2003)
Scenedesmus armatus	*Desmodesmus armatus* (Chodat) E. Hegewald	Hegewald et al. (2010)
Scenedesmus arthrodesmiformis	*Desmodesmus arthrodesmiformis* (Schröder) An, Friedl & E. Hegewald	An et al. (1999)
Scenedesmus costato-granulatus	*Desmodesmus costato-granulatus* (Skuja) E. Hegewald	Vanormelingen et al. (2007)
Scenedesmus denticulatus	*Desmodesmus denticulatus* (Lagerheim) An, Friedl & E. Hegewald	An et al. (1999)
Scenedesmus falcatus	*Pectinodesmus pectinatus** (Meyen) E. Hegewald et al.	Hegewald et al. (2010)
Scenedesmus obliquus	*Acutodesmus obliquus* (Turpin) Tsarenko	Hegewald & Wolf (2003)
Scenedesmus obtusus	*Scenedesmus obtusus* Meyen	Hegewald & Wolf (2003)
Scenedesmus opoliensis	*Desmodesmus opoliensis* (P. Richter) E. Hegewald	Hegewald et al. (2010)
Scenedesmus ovalternus	*Scenedesmus ovalternus* Chodat	Kessler et al. (1997)
Scenedesmus quadricauda	*Desmodesmus communis* (E. Hegewald) E. Hegewald	Kessler et al. (1997)
Scenedesmus serratus	*Desmodesmus serratus* (Corda) An, Friedl & E. Hegewald	An et al. (1999)
Scenedesmus subspicatus	*Desmodesmus subspicatus* (Chodat) E. Hegewald & Ant. Schmidt	Hegewald et al. (2005)
*Schroederia setigera**	*Schroederia setigera** (Schröder) Lemmermann	Buchheim et al. (2001)
*Tetrachlorella alternans**	*Tetrachlorella alternans** (G.M. Smith) Korshikov	Hepperle et al. (2000)
Tetraedron caudatum	*Tetraedron caudatum* (Corda) Hansgirg	Buchheim et al. (2005)
Tetraedron minimum	*Tetraedron minimum* (A. Braun) Hansgirg	Buchheim et al. (2005)

Table 6 continued

Old designation	Revised or confirmed designation	Reference
Treubaria setigera	*Treubaria setigera* (Archer) G.M. Smith	Buchheim et al. (2001)
Treubaria schmidlei	*Treubaria schmidlei* (Schröder) Fott & Kováčik	Buchheim et al. (2001)
*Westella botryoides**	*Westella botryoides** (W. West) De-Wildeman	Hegewald et al. (2010)

Species indicated by an asterisk are the type species of the genus. Species studied by molecular phylogeny however not designated to a genus are commented in column 2

one and the same genotype can exhibit different morphological peculiarities during its ontogeny or as result of interactions with the environment (Lürling & Beekman, 1999; Verschoor et al., 2004). Currently, molecular biologists are screening the contents of public strain collections (Day et al., 2004). However, these collections are not representative of the diversity of taxa in the field. Similarly, the designations of many taxa in culture collections are doubtful (Hegewald, 1989). Many algae are difficult to maintain in cultures and are not available for the sequencing work.

In the present situation, workers of both the types, the traditional phenotypic ones and those with a modern genotypic approach, should come together and forge interdisciplinary collaborations. Field limnologists have the privilege of working directly in ecosystems where evolution takes place. Organisms in their natural habitats are constantly interacting with each other and with their environment, while the natural selection process is acting on them. It is therefore so difficult to interpret the organismic structure of the ecosystem using the old phenotypic taxonomic units created by man using an artificial system. They do not adequately reflect how nature works. For ecologists, the genetic diversity within taxa is of major interest because of possible genetic shifts in populations as an adaptive response to a changing environment (Lynch et al., 1991; Wood & Leatham, 1992). Discussions on species concepts should in addition to the phenotype take into consideration intraspecific genetic variation. For species with well-studied phenotypic population characteristics from field observations, the description should be completed by genetic characteristics, especially for those cultured strains that conform to the original type description (Wood & Leatham, 1992). Limnologist should therefore support the efforts of molecular biologists to circumscribe phylogenetic species. Alongside this, large-scale ecophysiological experiments focusing on autecological features, physiological, and biochemical characteristics should be correlated to morphological variability of cultures of these phylospecies. Limnologists can also support an extensive taxon sampling. Through this exercise, they can advise on interesting algae, which need taxonomic revision and provide fresh samples, and isolate interesting strains for the public strain collections. On the other hand, molecular biologists have the basic tools for supporting the natural system of organisms. Their work should include developing methods for evaluating species composition in field (environmental) samples and comparing them with microscopic findings (Fawley et al., 2004; Richards et al., 2005; Medinger et al., 2010; Luo et al., 2011). A good example is the multiphased study of freshwater samples from México by Tavera & Diéz (2009).

In the near future, molecular tools may be used to identify individual filaments, colonies or cells picked from samples. It may also be possible to identify individuals directly within the samples. Promising results have already been published on cyanobacteria (Hayes et al., 2002), large sulfur bacteria (Salman et al., 2011), dinoflagellates (Ki & Han, 2005) and chrysophytes (Jost et al., 2010). Once the tools are fully developed, specialists with a good knowledge on morphospecies will have a good opportunity to align these individual phenotypes with the molecular findings (Auinger et al., 2008). However, presently we have to content with the use of two different and parallel systems. Although field biologists have no other option than to apply the traditional approach, they should be careful to provide very detailed documentation of their findings. The handbook by Komárek & Fott (1983) and the five volumes of Hindák's studies (1977, 1980, 1984, 1988, 1990) can be of great help in the determination of morphospecies of coccoid green algae. In the near future, the establishment of molecular signatures, the barcoding

conception, will allow an unambiguous designation of organisms. The alignment of eco-functional groups of algae with true species identities using the barcoding conception will provide a better understanding of the interaction between organisms and their environment. The reasons why and how an individual species adapts to different environmental conditions by producing spines, mucilage or incrustations, or losing them, establishing colonies or disintegrating them, exhibiting striking colonial appearances or returning to the simple green ball habit as a survival tactic will become evident.

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PHYTOPLANKTON

Review Paper

An update to modern taxonomy (2011) of freshwater planktic heterocytous cyanobacteria

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Abstract It is essential for the modern taxonomic classification of cyanobacteria to be continually updated in accordance with revisions based on molecular sequence comparisons and combined with morphological features, ecophysiological characters and other biochemical and molecular markers ("polyphasic approach"). Several genera, which are characterized by their planktic life form and contain indicator species important for the evaluation of aquatic biocenoses in majority of water bodies are recognized in the monophyletic group of heterocytous cyanobacteria. Current taxonomic revisions (and nomenclatoric consequences) of the specific contents of these heterocytous cyanobacterial generic units are covered by this article. Among these genera, 12 contain only planktic species, three remaining genera contain both planktic and non-planktic species. Comments and suggestions for future research are stressed especially in the ecologically distinct genera, which includes species dominating in the plankton of various reservoir types.

Keywords Cyanobacteria · Ecology · Heterocytous types · Plankton · Taxonomic revision

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Introduction

Modern taxonomy is a method for recognition and registration of organism diversity (both in natural populations and in cultures). Taxonomic classification of cyanobacteria is based on the so-called "polyphasic approach" (Johansen & Casamatta, 2005). In this approach, molecular phylogenetic analyses are the basic criterion for classification of genera and species, but the cytological and morphological markers (synapomorphic and autapomorphic characters) and the ecology (habitat preference, life strategy, ecophysiology) are an integral part of taxonomic definitions. All additional biochemical and molecular markers are also important. In order to evaluate the phenotypic plasticity within defined taxa, the variability observed in cultures has to be compared to the range in natural variation. In addition, the important nomenclatural corrections must be updated simultaneously with the respective taxonomic evolution. As a result, the taxonomic system must be continually corrected and modified according to the current findings.

As was confirmed by modern analyses, cyanobacteria with heterocytes (traditionally from the orders Nostocales and Stigonematales) form a highly supported monophyletic group (Giovannoni et al., 1988; Litvaitis, 2002; and many others). In the traditional system based on morphological criteria, species of the family Nostocaceae were classified in 8 (Geitler, 1932) up to 12 (Desikachary, 1959) or 10 (Starmach, 1966) genera. The Russian and Ukrainian authors divided the order Nostocales (without Stigonematales) in a different way—they established several additional families, Anabaenaceae, Aphanizomenonaceae, Aulosiraceae and Nostocaceae (cf. Kondrateva, 1968), but with the same lists of genera as in the previous monographs. According to the modern view, particularly the genus *Pseudanabaena* and evidently also *Isocystis*, which both have distinctly separate position in phylogenetic trees and have very different cytological and morphological characters (Guglielmi & Cohen-Bazire, 1984a, b; Turner, 1997; Komárek & Kaštovský, 2003; Hoffmann et al., 2005; our unpubl. results), obviously need to be transferred from the group of traditional nostocacean genera to other families and orders. On the other hand, the genera *Umezakia* (formerly classified to Stigonematales) and *Gloeotrichia natans* (formerly Rivulariaceae), have to be transferred to Nostocaceae according to the modern molecular sequences and revised cyto-morphological characteristics (Niiyama et al., 2011, this article). Traditional genera *Anabaena, Aphanizomenon* and *Anabaenopsis* were divided into several clusters on the generic level and several new genera have been defined following recent revisions combining molecular, phenotypic and ecological characters (Rajaniemi et al., 2005a, b; Willame et al., 2006; Wacklin et al., 2009; Zapomělová et al., 2009, 2010a, b; Komárek et al., 2010). A review of the nostocacean genera, known or studied up to the end of August 2011, is included in Table 1. The corresponding system according to the present knowledge (analysis of 16S rDNA data derived from GenBank and polyphasic revision of genera) is presented in Fig. 1 (see also "Results and discussion").

For modern delimitation of cyanobacterial genera it is obligatory that they occupy a separate position in the 16S rRNA phylogenetic tree. This must be complemented by the characteristic rRNA ITS region, and corrected showing clear morphological differences (autapomorphic features) and ecological and biogeographical markers (Komárek, 2011). For the classification in separate genera, sequence similarity of less than 95% in the 16S rRNA gene is usually accepted (Stackebrandt & Goebel, 1994), however, this limit is only approximate and must be connected with other, preferably qualitative markers. The cytological uniformity, biochemical markers (cyanotoxins, oligopeptides, etc.; Pearson et al., 2010) and sequencing of additional genomic loci (*rpoB*, *rbcLX*, *mcy*-genes, *gvp*-genes, *nif*-genes, etc.; Rajaniemi et al., 2005a; Tanabe et al., 2007) are also extremely beneficial as supporting data for the generic description. We therefore emphasize that not only the clear phylogenetic separation but also distinct (sharp) delimitation

Table 1 Genera from the family Nostocaceae (stage at August 2011), determined according to polyphasic evaluation

Planktic genera
Anabaena-like cluster I
Anabaena-like cluster II
Anabaena-like cluster III
Anabaenopsis (Wołoszyńska) Miller 1923
Aphanizomenon Morren ex Bornet et Flahault 1888
Cuspidothrix Rajaniemi et al. 2005
Cyanospira Florenzano et al. 1985
Cylindrospermopsis Seenaya et Suba Raju 1972
Dolichospermum (Ralfs ex Bornet et Flahault) Wacklin et al. 2009
Raphidiopsis Fritsch et Rich 1929
Sphaerospermopsis Zapomělová et al. 2010
Umezakia M. Watanabe 1987
Genera with planktic and benthic species
Cronbergia Komárek et al. 2010
Gloeotrichia J. Agardh ex Bornet et Flahault 1887
Nodularia (Mertens in Jürgens) ex Bornet et Flahault 1988
[*Isocystis* (Borzì) ex Bornet et Flahault 1988]
Benthic genera
Anabaena Bory ex Bornet et Flahault 1888
Aulosira Kirchner ex Bornet et Flahault 1988
Cylindrospermum Kützing ex Bornet et Flahault 1988
Hydrocoryne Schwabe ex Bornet et Flahault 1988
Macrospermum Komárek 2008
Mojavia Řeháková et Johansen 2007
Nostoc Vaucher ex Bornet et Flahault 1888
Trichormus (Ralfs ex Bornet et Flahault) Komárek et Anagnostidis 1989
Trichormus-like cluster A
Wollea Bornet et Flahault 1988
Endobiotic genera
Richelia J. Schmidt 1901

Fig. 1 Maximum likelihood (ML) phylogenetic tree based on 136 sequences (partial 16S rRNA gene) representing the current GenBank data on heterocytous cyanobacteria. Bootstrap values (512 replicates for ML, 1,000 replicates for MP) equal to 50 or higher are given at the nodes in this form: ML/MP. *Boldly printed branches* were present also in the strict consensus MP tree. The *dashed branch* leading to *Umezakia natans* AF516748 was shortened three times to make the figure more compact. The planktic heterocytous genera are generally clustered in an apical monophyletic lineage of nostocacean cyanobacteria in this tree, together with several non-planktic taxa such as *Wollea*, *Anabaena* and part of *Nodularia*. *Gloeotrichia echinulata* and *Cronbergia* appear to be more basal types within Nostocaceae. The *large numbers* correspond to the order of the genera in the text

by a complex of morphological and other suitable (i.e., cytological and ecophysiological) characters is highly desirable for generic separation. The nomenclatural rules as well as validation, identification of type species, etc., must be also respected in all cases.

A number of specific strategies for diversification can be found in cyanobacterial genera. Results of these different processes for diversification in the various genera include the occurrence of cryptospecies (Johansen & Casamatta, 2005), enormous morphological

variation within a single genotype, and a wide range of variation during the life cycles, etc. Therefore, there are no easily definable universal criteria for a uniform species delimitation. However, at least the population lineages and strains inside a single genus, which belong to the same genotype and morphotype with stable phenotypic features, and more or less stable and distinct ecological limits, should be considered as a species. Based on this definition, a single species must be genetically, ecologically, and morphologically uniform. All these premises should be applied in modern cyanobacterial classification.

Methods

For the purpose of our original phylogenetic analysis, all 16S rDNA sequences of heterocytous cyanobacteria longer than 800 bp available through GenBank by July 31, 2011 were downloaded into files according to the assigned generic names. The sequences were aligned within the genera by MAFFT v. 6 (Katoh et al., 2009) together with 2–3 suitable outgroup taxa, alignments were corrected manually, and a guide tree was constructed for each genus by the maximum likelihood (ML) method in SeaView v. 4.2.11 (Gouy et al., 2010). Phylogenetically relevant taxa were then chosen from these guide trees to construct the final data matrix of 136 nostocalean sequences and two outgroups (*Blennothrix* sp. EU586734 and *Chroococcidiopsis thermalis* AB039005). The sequences were aligned by MAFFT v. 6 and the resulting alignment was corrected manually to remove ambiguous gap regions. The final phylogenetic tree was constructed by the ML method via PhyML v. 3.0 (Guindon et al., 2010) using the generalized time-reversible (GTR) substitution model with discrete gamma distribution in six categories. The gamma shape parameter α as well as the proportion of invariable sites were estimated from the data set (GTR + I + G model), and 512 bootstrap replicates were executed to evaluate the relative support of branches. A maximum parsimony (MP) analysis was run using the same alignment as previously. One hundred replicate searches with starting tree obtained by random stepwise addition were performed using the tree bisection-reconnection (TBR) branch swapping algorithm in TNT v. 1.1. (Goloboff et al., 2008). One thousand nonparametric bootstrap replications were run with default settings to evaluate the relative branch support. All bases and base changes were weighted equally, and gaps were coded as missing data. Phylogenetic trees were drawn and edited using FigTree v. 1.3. (http://tree.bio.ed.ac.uk/software/figtree/).

For a better explanation of detailed genetic relations between the individual nostocacean genera, the positions of generic taxa are included in this review and often documented by several additional phylogenetic trees derived from various published studies (see Figs. 2, 3, 5, 6, 7, 8, 10, 11, 13, 14, 16).

Results and discussion

After deletion of gap regions, and including a major part of the 16S rRNA gene (positions 85–1421 relative to *Escherichia coli*), the final alignment sequence of heterocytous cyanobacteria derived from GenBank was 1325 bp long. Within the alignment, 724 sites (54.6%) sites were conservative, and 350 sites (26.4%) were parsimony informative. A single tree ($-\ln L = 18729.74$) was recovered in the ML analysis, while 24 most parsimonous trees ($l = 3{,}358$) were collapsed into a strict consensus tree. In all trees obtained, included sequences of the family Nostocaceae (except *Trichormus doliolum* AJ630455 and *Anabaena torulosa* GU396091) formed a monophyletic apical lineage, while the members of other traditional families (Scytonemataceae, Symphyonemataceae, Rivulariaceae, Stigonemataceae, Microchaetaceae) were in a basal position relative to this clade (Fig. 1). The nostocacean branch included all genera sequenced with exclusively planktic species (*Cuspidothrix*, *Dolichospermum*, *Aphanizomenon*, *Sphaerospermopsis*, *Cylindrospermopsis*, *Raphidiopsis*, *Anabaenopsis*, *Cyanospira* and *Umezakia*) as well as genera containing both planktic and benthic species (*Cronbergia*, *Nodularia, Gloeotrichia*). This lineage also included strictly non-planktic species or genera such as *Anabaena*, *Wollea*, *Trichormus* or *Nostoc*. The 16S rDNA data did not show a consistent phylogenetic signal at higher topological levels, however, some of the planktic genera clustered using both methods of cladogram construction (ML and MP), with relatively good bootstrap support. This included specifically the clusters of *Cylindrospermopsis*, *Raphidiopsis* and *Sphaerospermopsis* grouped together with *Wollea* and *Anabaena*, the cluster of *Anabaenopsis*, *Cyanospira*

and *Nodularia*, and the branch containing *Umezakia*, *Anabaena bergii* and *Aphanizomenon ovalisporum*. *Gloeotrichia echinulata* fell to different basal positions within Nostocaceae with low relative branch support, and *Cronbergia* clustered with *Aulosira* and part of *Cylindrospermum* at the basis of Nostocaceae.

Recently, the nostocacean genera containing primarily planktic species have been in the focus of modern study and taxonomic revisions (the genera are listed in our review according to their position in the phylogenetic tree in Fig. 1). The importance of genetic and taxonomic characters such as the ability to form gas vesicles (aerotopes) in vegetative cells during the life cycle is evident. About 10–12 generic units inside the Nostocaceae contain purely planktic species and another number (10 genera) do not contain any species with aerotopes. Only two genera (*Nodularia* and *Cronbergia*) contain both planktic (with aerotopes) and non-planktic species, but they need further study. It is also interesting that the planktic species *G. echinulata* probably belongs in the cluster of Nostocaceae, which contains species with gas vesicles, not in the Rivulariaceae (heteropolar types—*Calothrix, Rivularia*) where it was traditionally placed. However, other (non-planktic) species of *Gloeotrichia* have not yet been sequenced and the position of this whole genus can therefore not yet be solved. Planktic *Gloeotrichia* species are included in our review.

Umezakia M. Watanabe 1987 (Fig. 2)

This genus contains only one species, described and isolated from the Lake Mikata, Japan. It is characterized by straight or slightly flexuous, planktic, solitary filaments with slightly tapering ends, intercalary heterocytes and oval akinetes (distant from heterocytes). After isolation in culture, the distinct true T-type branching was observed in this species (Fig. 2b). Therefore, although the genus was originally classified into stigonematalean cyanobacteria, the recent molecular analyses (Niiyama et al., 2011) indicated its close relationship to *Aph. ovalisporum* (= *Anabaena*-like cluster I). *Umezakia* also displays the morphology of akinetes similar to the members of that cluster and produces the cyanotoxin "cylindrospermopsin", found up to now primarily in *Cylindrospermopsis* and "*Aph. ovalisporum*". Therefore, it must be classified into the family Nostocaceae.

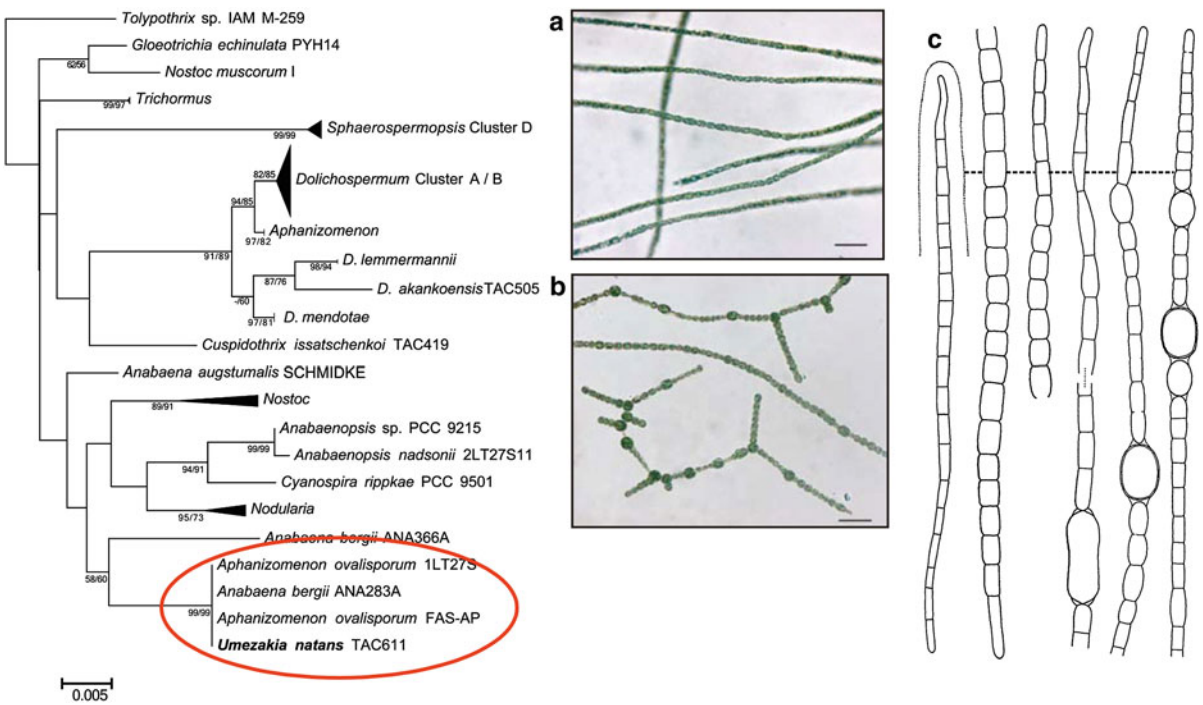

Fig. 2 *Umezakia* M. Watanabe; phylogenetic tree after Niiyama et al. (2011), documentation after Watanabe (1987); **a**, **c** vegetative filaments, **b** true-branched filaments with akinetes from cultures

Diacritical markers: Position in the phylogenetic tree; straight or flexuous filaments with tapering ends; metameric trichomes with intercalary heterocytes; paraheterocytic, oval akinetes; occasional true T-branching (in cultures).

List of species (monospecific genus): *U. natans* M. Watanabe 1987.

Selected references: Watanabe (1987), Niiyama et al. (2011).

Anabaena-like cluster I (Figs. 3, 4)

A portion of the traditional planktic *Anabaena/Aphanizomenon* group, which is clearly separated in its

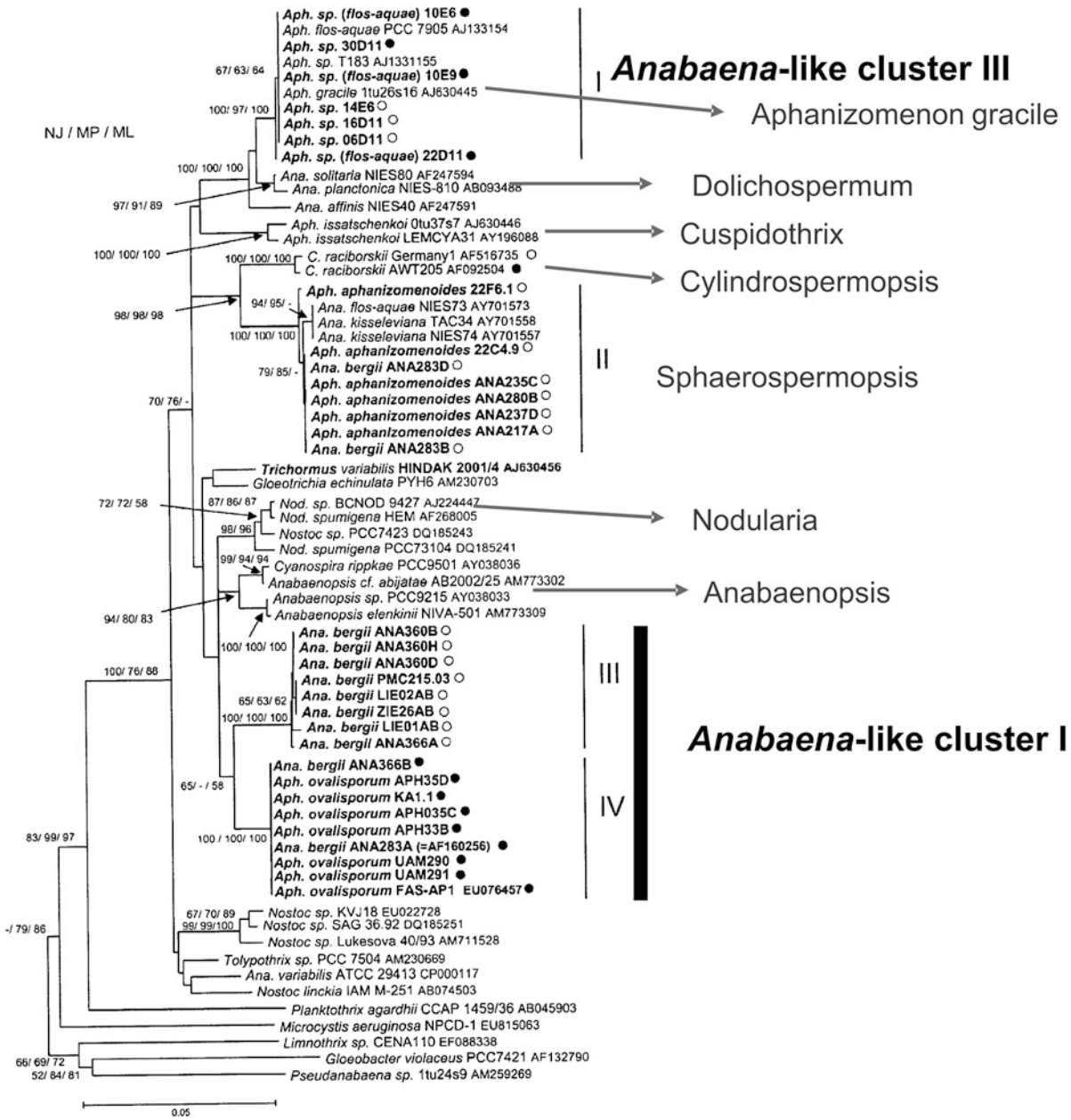

Fig. 3 Separation of *Anabaena*-like clusters I and III; phylogenetic tree after Stüken et al. (2009)

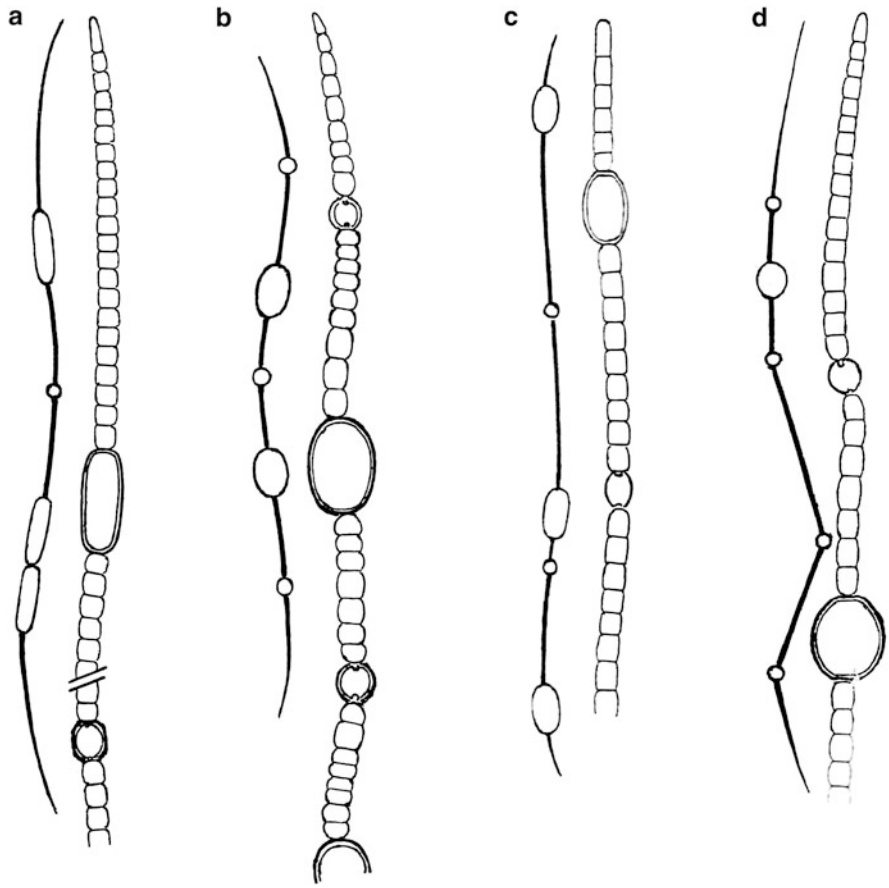

Fig. 4 Species from the *Anabaena*-like cluster I; **a** *Anabaena minderi*, **b** *Anabaena bergii*, **c** *Aphanizomenon ovalisporum,* **d** *Aphanizomenon manguinii* (from Komárek & Kováčik, 1989)

position in the phylogenetic trees (Stüken et al., 2006, 2009; Fig. 3). It contains only the planktic morphospecies with ±straight trichomes having a metameric structure, tapering ends, usually conical apical cells, cylindrical–oval intercalar akinetes distant from heterocytes and gas vesicles in cells (probably facultatively). *An. bergii*, *Aph. ovalisporum* and possibly also a few other species with similar phenotypic features belong into this cluster.

Diacritical markers: Position in the phylogenetic tree; planktic; solitary, free-floating, ±straight trichomes with ±tapering ends; metameric trichomes; solitary, intercalary heterocytes; paraheterocytic, elongated akinetes, distant from heterocytes.

List of species (three species evidently belong to this separated cluster): *Aph. ovalisporum* Forti 1911; *An. bergii* Ostenfeld 1908; *An. minderi* Huber-Pestalozzi 1938; however, other described species (*Anabaena austro-africana, An. carmichaelii, An. nodularioides, An. recta, An. salina, Aphanizomenon manguinii,* etc.) can be transferred into this proposed new genus after molecular analyses (e.g., Fig. 4d).

Selected references: Komárek & Kováčik (1989), Stüken et al. (2006, 2009).

Anabaenopsis (Wołoszyńska) Miller 1923 (Fig. 5)

A phylogenetically and phenotypically distinct and clearly definable genus. *Anabaenopsis* forms one clade together with *Cyanospira* and *Nodularia*, (Iteman et al., 2002), however, it differs especially from *Nodularia* strictly by its morphology. In habitat, it is similar to *Dolichospermum*, but characterized specifically by the formation of paired intercalar heterocytes, which develop metamerically, mirror-like, after the asymmetrical division of neighbouring intercalar vegetative cells (Fig. 5, scheme). Later the trichomes disintegrate between the ripe heterocytes into

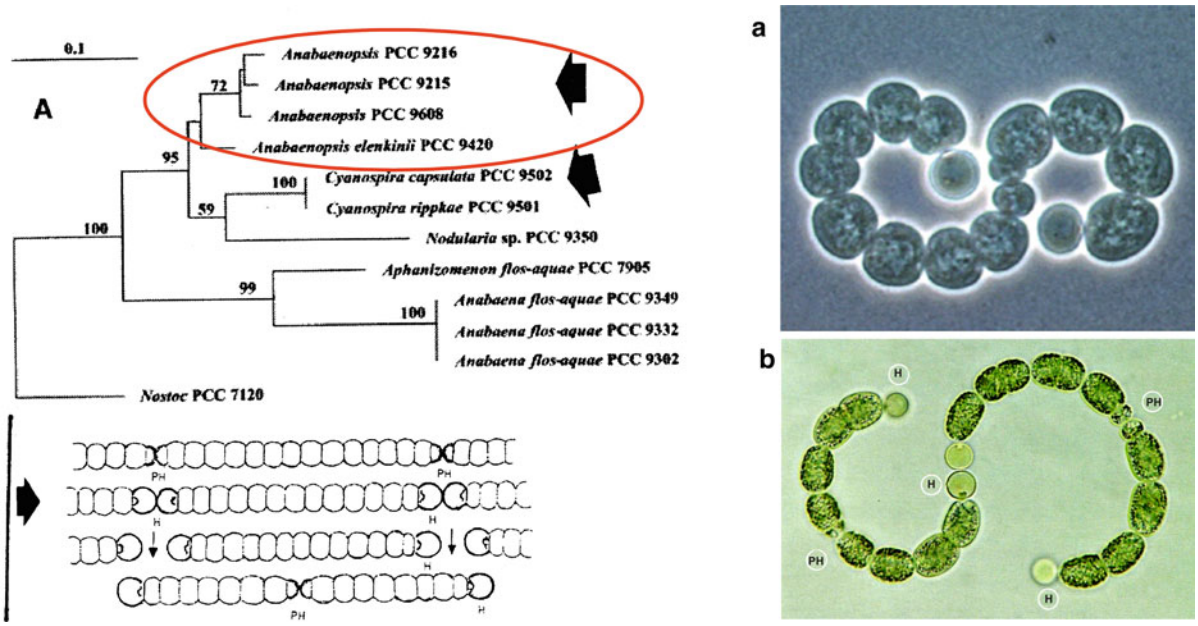

Fig. 5 *Anabaenopsis* (Wołoszyńska) Miller 1923 and *Cyanospira* Florenzano et al. 1985 (in phylogenetic tree); phylogenetic tree after Iteman et al. (2002) (A), photos from Hindák (2001), scheme of heterocyte formation from Komárek (2005); **a** *A. milleri*, **b** *A. elenkinii*

fragments (short filaments), which have relatively restricted length and possess secondary terminal heterocytes. Akinetes develop intercalarly. The genus is therefore clearly definable, but the infrageneric variation is wide and several species are often difficult to morphologically identify. The various species are known mainly from the plankton of inland or coastal halophilic or mineral water bodies with higher conductivity, usually from tropical and warm regions, less frequently from temperate zones. (Note: common in temperate central North American meso-eutrophic high conductivity or saline prairie pot holes.)

Diacritical markers: Position in the phylogenetic tree (Figs. 1, 5); solitary free-floating filaments; metameric structure of trichomes; specific formation of geminate heterocytes arising from neighbouring intercalary cells after asymmetric division; disintegrating of trichomes between ripe heterocytes; intercalary, paraheterocytic akinetes.

List of species (about 20 species were described): *A. abijatae* Kebede et Willén 1996; *A. ambigua* Pandey et Mitra 1962; *A. arnoldii* Aptekar' 1926; *A. circularis* (G.S. West) Wołoszyńska et Miller in Miller 1923; *A. cunningtonii* Taylor 1932; *A. doliiformis* Noda 1963; *A. elenkinii* Miller 1923; *A. hungarica* Halász 1939; *A. intermedia* Kogan 1967; *A. issatschenkoi* Voronichin 1934; *A. kelifii* Kogan 1962; *A. knipowitschii* (Usačev) Komárek 2005; *A. luzonensis* Taylor 1932; *A. magna* Evans 1962; *A. milleri* Voronichin 1929; *A. nadsonii* Voronichin 1929; *A. tanganyikae* (G.S. West) Miller 1923; *A. teodorescui* Moruzi 1960; *A. venkataramanii* Chandhyok 1966; *A. woltereckii* Behre 1956.

Selected references: Miller (1923), Komárek (1999, 2005), Hindák (2001), Iteman et al. (2002), Komárek et al. (2010), Rajaniemi et al. (2005a).

Cyanospira Florenzano et al. 1985 (Figs. 5, 6)

Another cluster, habitually similar to coiled *Dolichospermum* or *Anabaenopsis.* Phylogenetically closely related to *Anabaenopsis* and *Nodularia* (Fig. 5), with metameric trichomes, in which heterocytes develop intercalarly, solitary or in pairs. Obligate or facultative gas vesicles (the development of gas vesicles needs further studies) occur in cells. *Cyanospira* has been found in tropical regions of Africa and mountain areas of central Asia (one species). The main diacritical character is the apoheterocytic formation of akinetes in rows.

Diacritical markers: Position in the phylogenetic tree; solitary, coiled trichomes; metameric structure of trichomes; intercalary solitary or paired heterocytes;

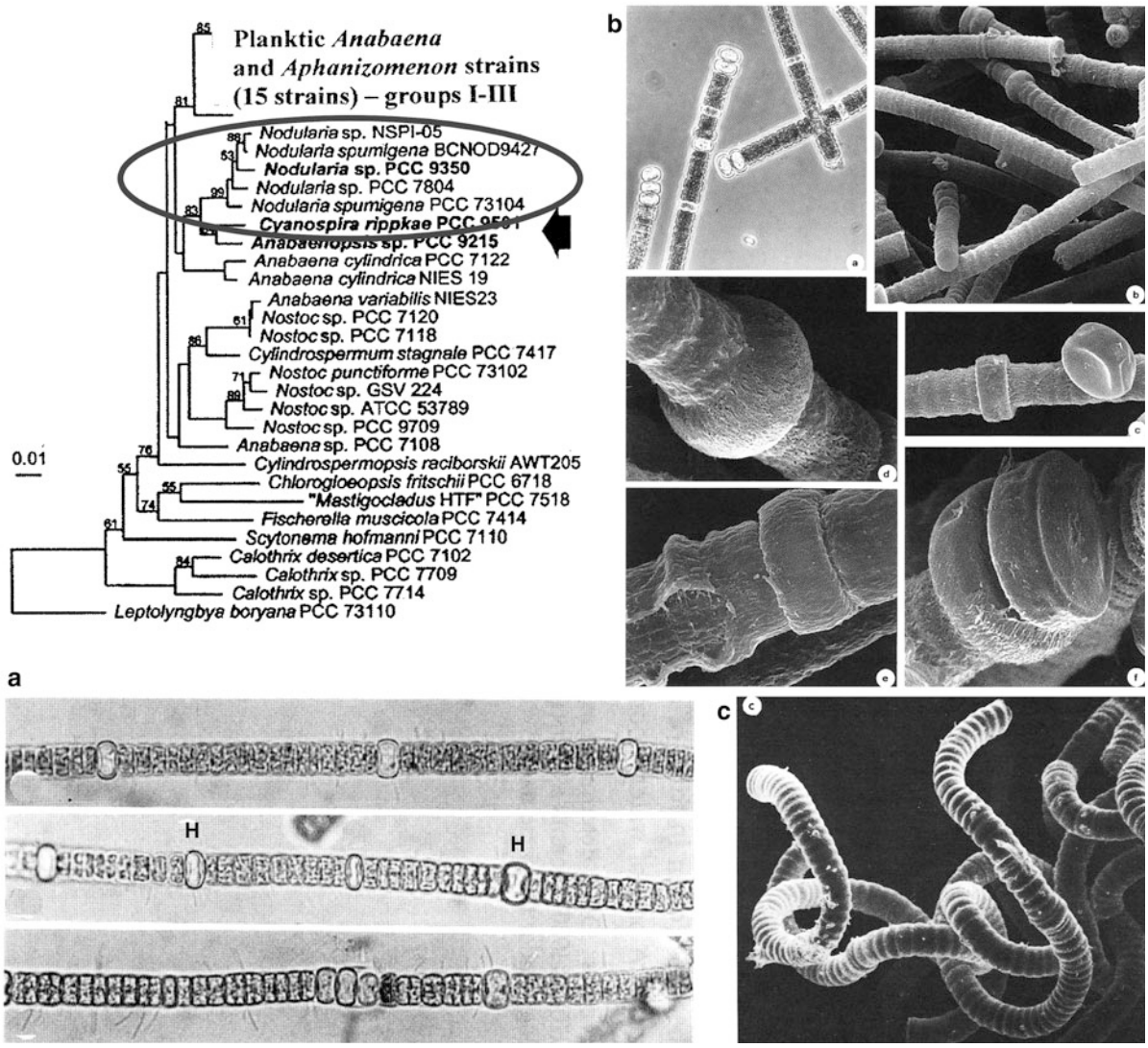

Fig. 6 *Nodularia* (Mertens in Jürgens) ex Bornet et Flahault; phylogenetic tree after Iteman et al. (2002); **a** *N. baltica*, **b** *N. spumigena*, **c** coiled forms of *N. spumigena* from Baltic Sea. Photos from Šmarda et al. (1988) and Komárek (2002)

facultative occurrence of gas vesicles; apoheterocytic formation of akinetes.

List of species (up to now there were only three planktic species described, but similar types occur perhaps also in tropical soils; Sili, pers. comm.): *C. rippkae* Florenzano et al. 1985; *C. capsulata* Florenzano et al. 1985; *C. globosa* (Hirano) Komárek 2012.

Selected references: Hirano (1963; sub *Anabaena*), Florenzano et al. (1985), Iteman et al. (2002), Komárek (2012).

Nodularia (Mertens in Jürgens) ex Bornet et Flahault 1888, nom. cons. (Fig. 6)

A genus containing both planktic and benthic species in a single phylogenetic cluster (Hašler et al., 2011). Planktic types contain gas vesicles in cells. Genera primarily related to *Nodularia* are *Anabaenopsis* and *Cyanospira*. Only *N. spumigena* is commonly cited from the planktic types which have a cosmopolitan distribution, but occur only in brackish inland and coastal localities very distant one from another. All

these populations (Baltic Sea, Caspian Sea, some central and western N American lakes, volcanic lakes in Mexico, coastal lakes in Uruguay, bays in SW Australia, etc.) have their own specific morphological characters and up to now the genetic identity or diversity of all geographically distinct populations is not known. The trichome and cell morphology, metameric heterocytes development, metamerically, irregular apoheteric akinete position are the most important diacritical characters.

Diacritical markers: Two groups (i) planktic, solitary trichomes, cells with gas vesicles, and (ii) benthic solitary filaments or in mats, without gas vesicles; phylogenetic position; metameric structure of trichomes; cells shorter than wide; irregular, apoheterocytic akinete formation.

List of species (Four planktic species with numerous morphotypes have been described but descriptions of further benthic or terrestrial species should be expected). Planktic species: *N. baltica* Komárek et al. 1993; *N. spumigena* Mertens ex Bornet et Flahault 1888 (about seven geographically distant morphotypes, probably genetically separated); *N. litorea* (Kützing) Thuret ex Komárek et al. 1993; *N. crassa* (Voronichin) Komárek et al. 1993. Benthic species: *N. harveyana* Thuret ex Bornet et Flahault 1888; *N. sphaerocarpa* Bornet et Flahault 1888; *N. moravica* Hindák et al. 2003; *N. major* (Kützing) ex Kirchner 1900; *N. rajkotii* Gupte 1964; *N. quadrata* Fritsch 1912; *N. turicensis* (Cramer) Hansgirg 1892; *N. willei* Gardner 1927.

Selected references: Nordin & Stein (1980), Šmarda et al. (1988), Komárek et al. (1993), Blackburn & Jones (1995), Hayes & Barker (1997), Bolch & Blackburn (1998), Barker et al. (1999, 2000), Bolch et al. (1999), Lehtimäki et al. (2000), Laamanen et al.

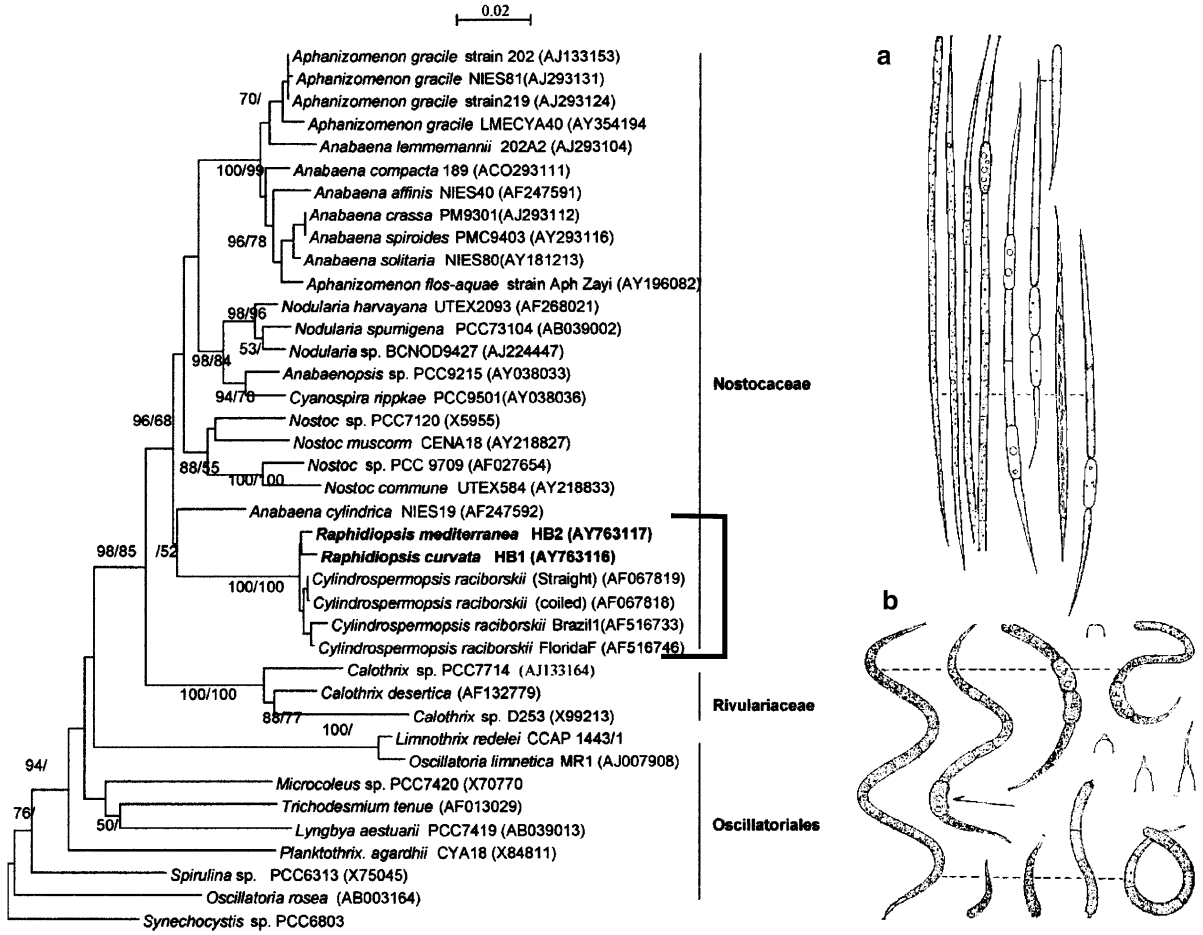

Fig. 7 *Raphidiopsis* Fritsch et Rich; phylogenetic tree after Li et al. (2008); **a** *R. mediterranea*, **b** *R. curvata* (from Komárek, 1999)

(2001), Iteman et al. (2002), Komárek (2002), Batley & Hayes (2003), Hašler et al. (2011).

Raphidiopsis Fritsch et Rich 1929 (Fig. 7)

Raphidiopsis is a validly described, but still very problematic genus. The main diagnostic feature is the obligatory absence of heterocytes; it is classified to the nostocacean cyanobacteria only on the basis of formation of intercalar akinetes of the same type which occurs in *Cuspidothrix* or *Cylindrospermopsis*. It is rarely cultured and up to now the heterocyte formation was never demonstrated experimentally. However, Li et al. (2008) and Moustaka-Gouni et al. (2009) showed that *Raphidiopsis mediterranea* (and *R. curvata*, respectively) are phylogenetically very close to the cluster of *Cylindrospermopsis raciborskii*, and that *R. mediterranea* should be conspecific with this species (Moustaka-Gouni et al., 2009). The mass development of *Raphidiopsis* has been recorded in last years from China, Greece and Argentine (Izaguirre, pers. comm.), and the taxonomic solution will be important in future. It is very probable that young filaments of *Cylindrospermopsis raciborskii* without heterocytes are often misinterpreted and identified as *Raphidiopsis mediterranea.* However, strains of *Raphidiopsis* exist, in which the heterocytes were not yet experimentally induced. Until the common absence of heterocytes in numerous *Raphidiopsis* populations is explained and more different species will be included in the analyses, the unification of both genera is premature. All these problems must be solved particularly in the type species *R. curvata* before the final solution of this genus. This revision concerns also the nomenclature (conservation of generic name *Cylindrospermopsis*?), because the unclear and little known *Raphidiopsis* (with the type species *R. curvata*) has a priority (1929) against *Cylindrospermopsis* (1972).

Diacritical markers: Solitary planktic trichomes; obligate absence of heterocytes; akinetes present, intercalary, solitary or in pairs; tapering terminal cells; trichomes subsymmetric.

List of species (seven species were described, from which all need taxonomic revision): *R. brookii* Hill 1972; *R. curvata* Fritsch et Rich 1929; *R. indica* Singh 1942; *R. longisetae* Eberly 1966; *R. mediterranea* Skuja 1937; *R. sinensis* Jao 1951; *R. turcomanica* Kogan 1967.

Selected references: Fritsch & Rich (1929), Geitler (1932), Hindák (1987), Gugger et al. (2005), Li et al. (2008), Moustaka-Gouni et al. (2009).

Cylindrospermopsis Seenayya et Suba Raju 1972 (Figs. 7, 8)

The type species of *Cylindrospermopsis* (*C. raciborskii*) originally described as *Anabaena*, was later transferred into the genus *Anabaenopsis* and in 1972 defined as a special genus with pantropical distribution and two species. This genus has a characteristic life cycle (changes of morphology during growth and development), trichome morphology and mode of development of apical heterocytes and subapical akinetes. The knowledge of changes in morphology during growth and development of these species is particularly urgent. The taxonomic position of *C. raciborskii* was many times supported by 16S rRNA gene sequencing (comp. Figs. 7, 8). The type species has originally a pantropical distribution, however, in the last 50 years it started to expand into the temperate zones. Now, it belongs to the most important planktic cyanobacteria and a lot of papers have already been published concerning its function in freshwater bodies over the world (cf. Padisák, 1997; Gugger et al., 2005; Stüken et al., 2006; Moustaka-Gouni et al., 2009). Its importance was further emphasized by the presence of the cyanotoxin cylindrospermopsin (Pearson et al., 2010). Expansion of this genus is indicated also by the fact that nine new tropical morphospecies were described in the last 20 years (from 1990 on), and that the genetic diversification of *Cylindrospermopsis* populations over different continents was reported in last years (Gugger et al., 2005; Fig. 9). However, the main studies were realized with the type species *C. raciborskii,* but ecology and variability of all other species are still almost unknown.

Diacritical markers: Solitary free-floating, planktic trichomes; facultative gas vesicles in cells; heterocytes develop from terminal cells, after asymmetrical division; akinetes subterminal; trichomes subsymmetric.

List of species (11 species described): *C. acuminato-crispa* Couté et Bouvy 2004; *C. africana* Komárek et Kling 1991; *C. catemaco* Komárková et Tavera 1996; *C. curvispora* M. Watanabe 1995; *C. cuspis* Komárek et Kling 1991; *C. gangetica* (Nair) Komárek 2012; *C. helicoidea* Cronberg et Komárek 2003; *C. philippinensis* (Taylor) Komárek 1984; *C.*

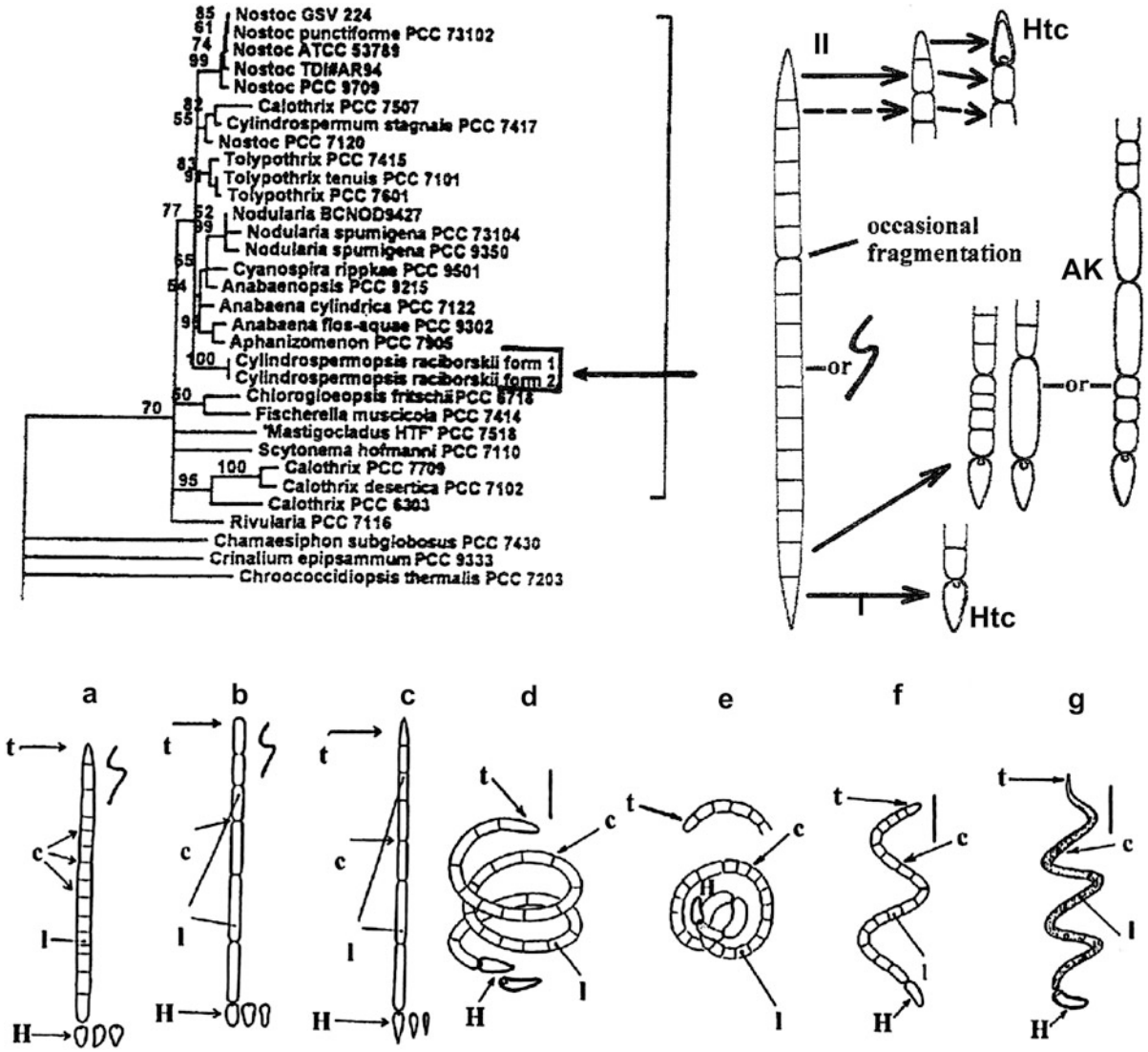

Fig. 8 *Cylindrospermopsis* Seenaya et Subba-Raju; phylogenetic tree from Castenholz (2001); selected species: **a** *C. raciborskii*; **b** *C. africana*; **c** *C. cuspis*; **d** *C. philippinensis*; **e** *C. curvispora*; **f** *C. taverae*; **g** *C. catemaco* (from Komárková, 1998)

raciborskii (Woloszynska) Seenaya et Subba-Raju 1972; *C. sinuosa* Couté et al. 2003; *C. taverae* Komárek et Komárková 2002.

Selected references: Seenayya & Subba Raju (1972), Horecká & Komárek (1979), Saitou & Nei (1987), Padisák (1991), Dokulil & Mayer (1996), Fabbro & Duivenvoorden (1996), Krienitz & Hegewald (1996), Padisák (1997, 2003), Couté et al. (1997, 2004), Komárková (1998), Castenholz (2001), Dyble et al. (2002), Komárek & Komárková (2003), Couté & Bouvy (2004), Gugger et al. (2005), Stüken et al. (2006), Moustaka-Gouni et al. (2009).

Sphaerospermopsis Zapomělová et al. 2010
(Figs. 3, 10)

Some of the of planktic species, which were earlier classified into the genus *Anabaena* (planktic) or *Aphanizomenon* belong in this cluster *Sphaerospermopsis*, which was first recognized after detailed

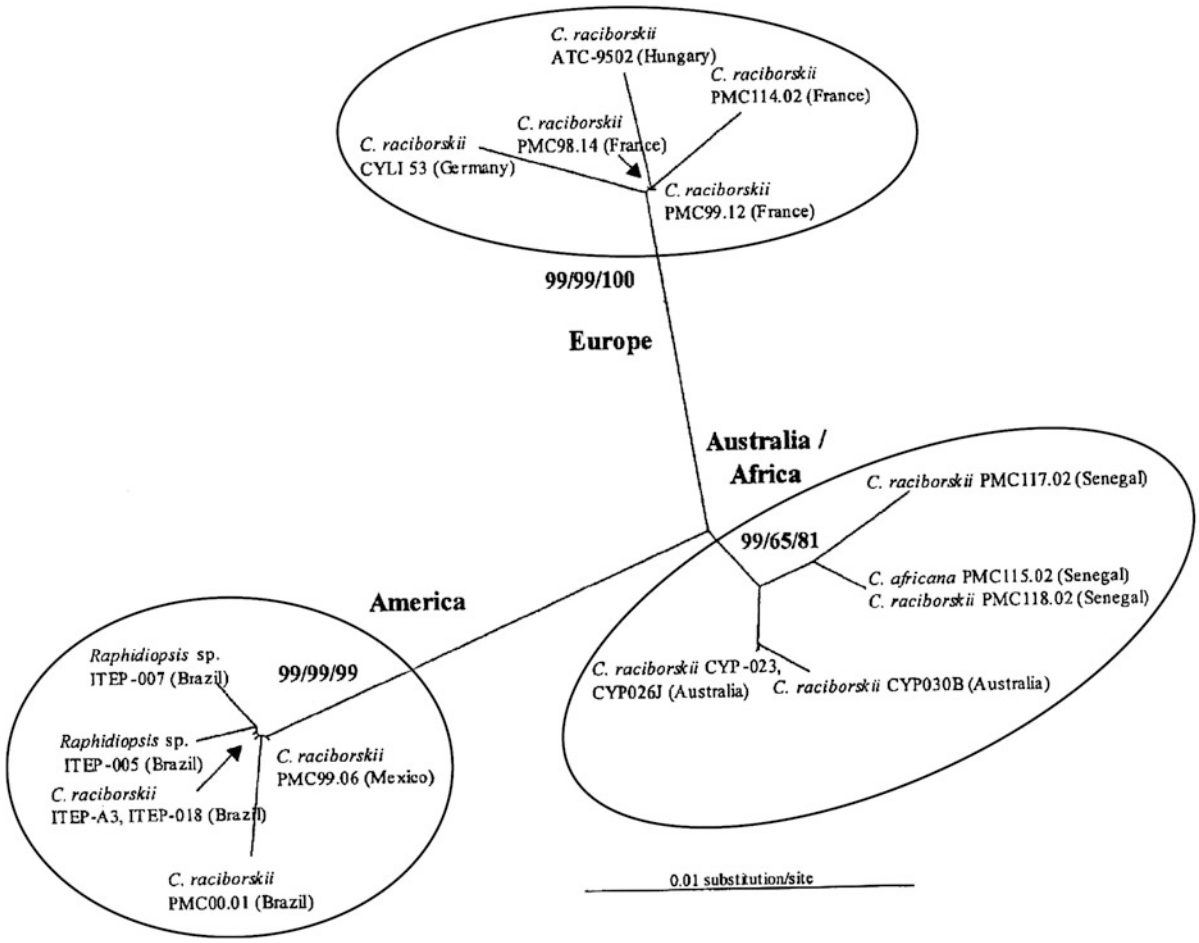

Fig. 9 Genetic diversity of *Cylindrospermopsis raciborskii* and *Raphidiopsis* sp. populations (strains) isolated from different continents (from Gugger et al., 2005)

molecular analyses (Zapomělová et al., 2009, 2010a). Almost spherical akinetes and their position localized on both sides of intercalary, solitary heterocytes are the main diagnostic morphological features in *Spaherospermopsis*. The terminal cells are often slightly tapered or morphologically modified. The genus comprises morphotypes (species) with both straight and coiled trichomes, which occur in temperate zone (mostly warmer areas) and in tropical regions.

Diacritical features: Unique position in the phylogenetic tree; solitary, free-floating coiled or straight metameric trichomes; obligatory presence of gas vesicles; position of spherical akinetes on both sides of heterocytes.

List of revised species (up to now six species were revised): *S. aphanizomenoides* (Forti) Zapomělová et al. 2010; *S. kisseleviana* (Elenkin) Zapomělová et al. 2010; *S. oumiana* (M. Watanabe) Tuji et Niiyama 2010; *S. reniformis* (Lemmermann) Zapomělová et al. 2010; *S. sphaericum* (Kiselev) Zapomělová et al. 2010; *S. torques-reginae* (Komárek) Werner et al. 2011.

Selected references: Stüken et al. (2006, 2009), Zapomělová et al. (2009, 2010a), Tuji & Niiyama (2010), Werner et al. (2011).

Dolichospermum (Ralfs ex Bornet et Flahault) Wacklin et al. 2009 (Fig. 11)

Traditionally part of the broad genus *Anabaena*. However, molecular analyses distinctly separated the planktic morpho- and ecotypes (planktic type of life,

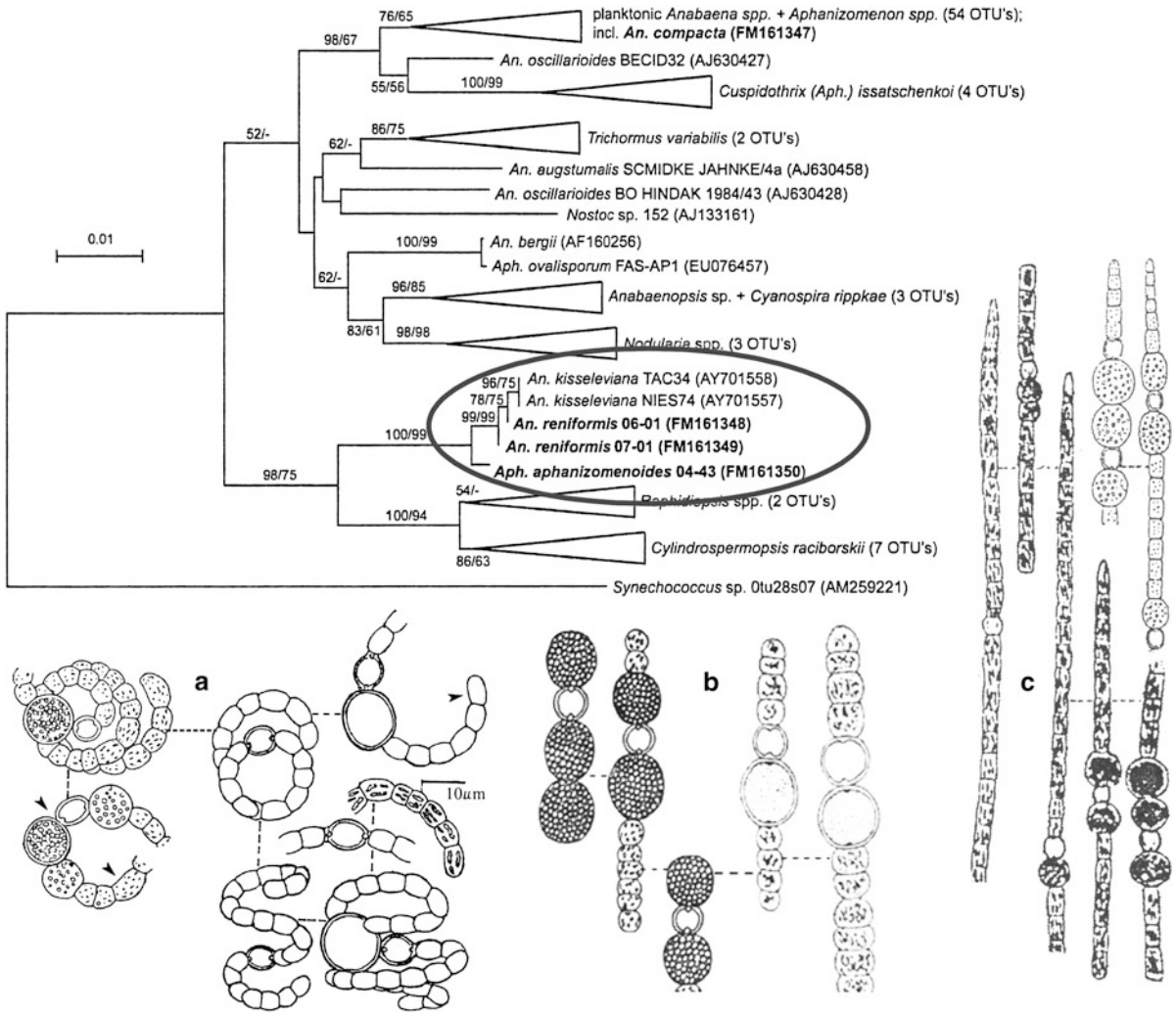

Fig. 10 *Sphaerospermopsis* Zapomělová et al.; phylogenetic tree according to Zapomělová et al. (2009), species from various authors: **a** *S. reniformis*, **b** *S. kisseleviana*, **c** *S. aphanizomenoides*

solitary trichomes or free-floating clustered filaments, gas vesicles in cells) from benthic species living in mats and biofilms, without gas vesicles (Rajaniemi et al., 2005a, b; Willame et al., 2006; Wacklin et al., 2009). The type species of *Anabaena, A. oscillarioides* belongs to the benthic group, for this reason the entire large cluster of typical planktic *Anabaena* had to be separated into a distinct genus and given another name. Therefore, the name *Dolichospermum* Ralfs, published as a section by Bornet & Flahault (1888), has been selected as the generic designation of this cluster, with the type species *D. flos-aquae* (syn.: *Anabaena flos-aquae*). The validation of this genus was published by Wacklin et al. (2009).

Diacritical markers: Position in the phylogenetic tree; planktic, free-floating, solitary, straight or variously coiled filaments (less frequently in colonies); trichomes metameric, ±cylindrical, with intercalary heterocytes; obligatory gas vesicles (aerotopes) in cells; akinetes paraheterocytic, ±elongated, solitary up to five in a row.

List of revised species (according to molecular evaluation and morphology, 42–45 morphotypes belong to this genus): *D. affine* (Lemmermann) Wacklin et al. 2009; *D. akankoense* (M. M. Watanabe) Wacklin et al. 2009; *D. arcticum* (Kiselev) Wacklin et al. 2009; *D. berezowskii* (Usačev) Wacklin et al. 2009; *D. bituri* (Cronberg et Komárek) Wacklin et al.

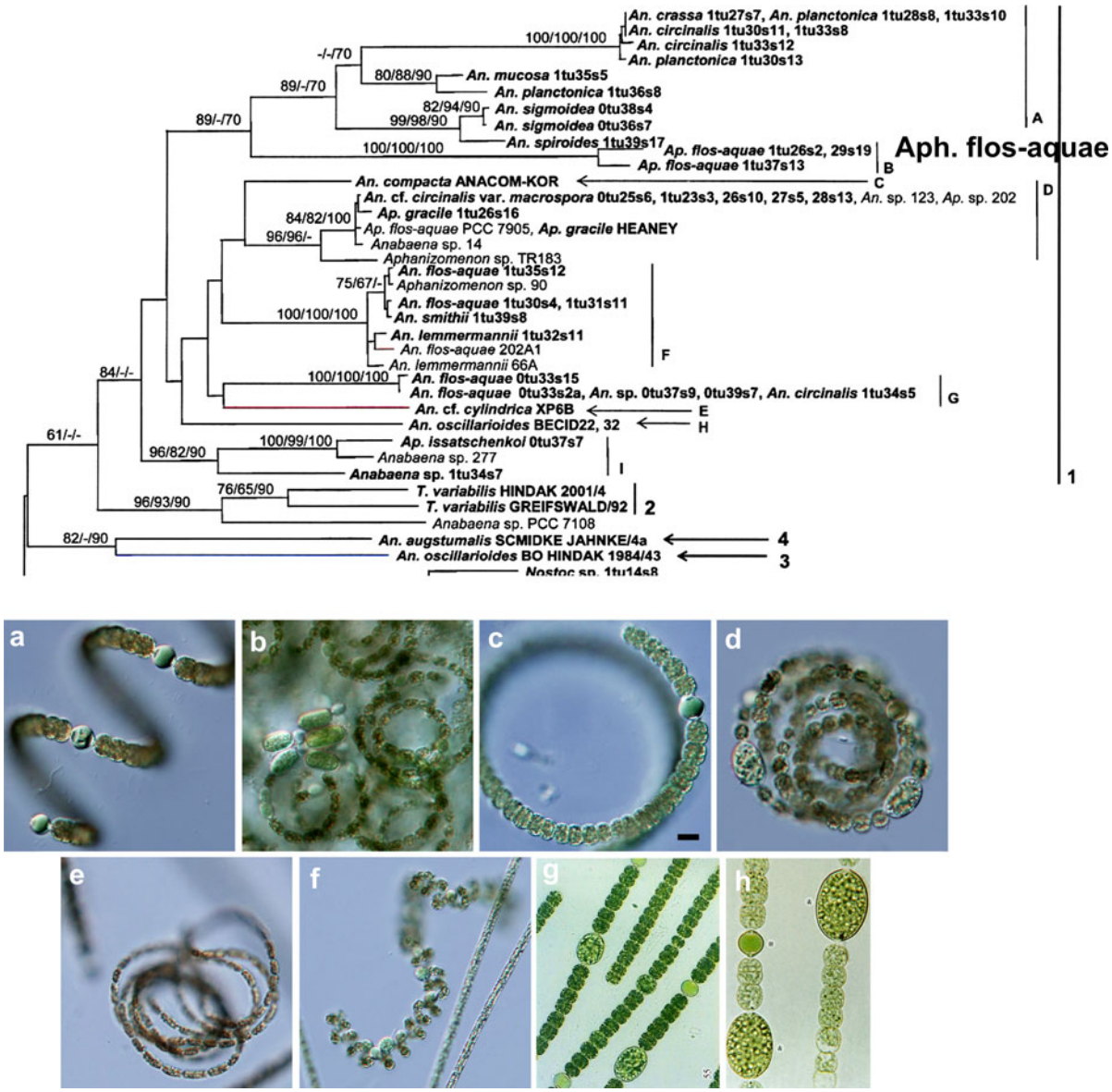

Fig. 11 *Dolichospermum* (Ralfs ex Bornet et Flahault) Wacklin et al.; phylogenetic tree according to Rajaniemi et al. (2005); **a** *D. crassum*; **b** *D. lemmermannii*; **c** *D. circinale*; **d** *D. flos-aquae*; **e** *D. mendotae*; **f** *D. compactum*; **g** *D. viguierii*; **h** *D. planctonicum*; **a**–**f** from Zapomělová (2008), **g** from Cronberg & Annadotter (2006), **h** from Hindák (2001)

2009; *D. bothai* (Welsh) Wacklin et al. 2009; *D. caspicum* (Ostenfeld) Wacklin et al. 2009; *D. circinale* (Rabenhorst ex Bornet et Flahault) Wacklin et al. 2009; *D. citrisporum* (M. Watanabe) Wacklin et al. 2009; *D. compactum* (Nygaard) Wacklin et al. 2009; *D. crassum* (Lemmermann) Wacklin et al. 2009; *D. curvum* (Hill) Wacklin et al. 2009; *D. danicum* (Nygaard) Wacklin et al. 2009; *D. delicatulum* (Lemmermann) Wacklin et al. 2009; *D. ellipsoides* (Bolochoncev) Wacklin et al. 2009; *D. fallax* (Komárek et Komárková-Legnerová) Wacklin et al. 2009; *D. farciminiforme* (Cronberg et Komárková-Legnerová) Wacklin et al. 2009; *D. flos-aquae* (Brébisson ex Bornet et Flahault) Wacklin et al. 2009; *D. fuscum* (Hill) Wacklin et al. 2009; *D. halbfassii* (Bachmann) Wacklin et al. 2009; *D. helicoideum* (Bernard) Wacklin et al. 2009; *D. heterosporum* (Nygaard) Wacklin et al. 2009; *D. jacuticum* (Kiselev) Wacklin et al. 2009; *D.*

lemmermannii (Richter) Wacklin et al. 2009; *D. longicellulare* (Pankow) Wacklin et al. 2009; *D. macrosporum* (Klebahn) Wacklin et al. 2009; *D. maximum* (Cronberg et Komárek) Wacklin et al. 2009; *D. mendotae* (Trelease) Wacklin et al. 2009; *D. mucosum* (Komárková-Legnerová et Eloranta) Wacklin et al. 2009; *D. nathii* (Vasishta) Wacklin et al. 2009; *D. nygaardii* (Cronberg et Komárek) Wacklin et al. 2009; *D. perturbatum* (Hill) Wacklin et al. 2009; *D. planctonicum* (Brunnthaler) Wacklin et al. 2009; *D. pseudocompactum* (M. Watanabe) Wacklin et al. 2009; *D. sigmoideum* (Nygaard) Wacklin et al. 2009; *D. skujaelaxum* (Komárek et Zapomělová) Wacklin et al. 2009; *D. smithii* (Komárek) Wacklin et al. 2009; *D. solitarium* (Klebahn) Wacklin et al. 2009; *D. spiroides* (Klebahn) Wacklin et al. 2009; *D. viguieri* (Denis et Frémy) Wacklin et al. 2009; *D. werneri* (Brunnthaler) Wacklin et al. 2009; *D. zinserlingii* (Kosinskaja) Wacklin et al. 2009.

Selected references: Bornet & Flahault (1888), Watanabe (1992, 1996), Komárková-Legnerová & Eloranta (1993), Komárek (1996, 1999), Li et al. (2000), Hindák (2001), Li & Watanabe (2001), Gugger et al. (2002a, b), Cronberg & Komárek (2004), Watanabe et al. (2004), Rajaniemi et al. (2005a), Willame et al. (2006), Komárek & Zapomělová (2007, 2008), Stüken et al. (2009), Wacklin et al. (2009).

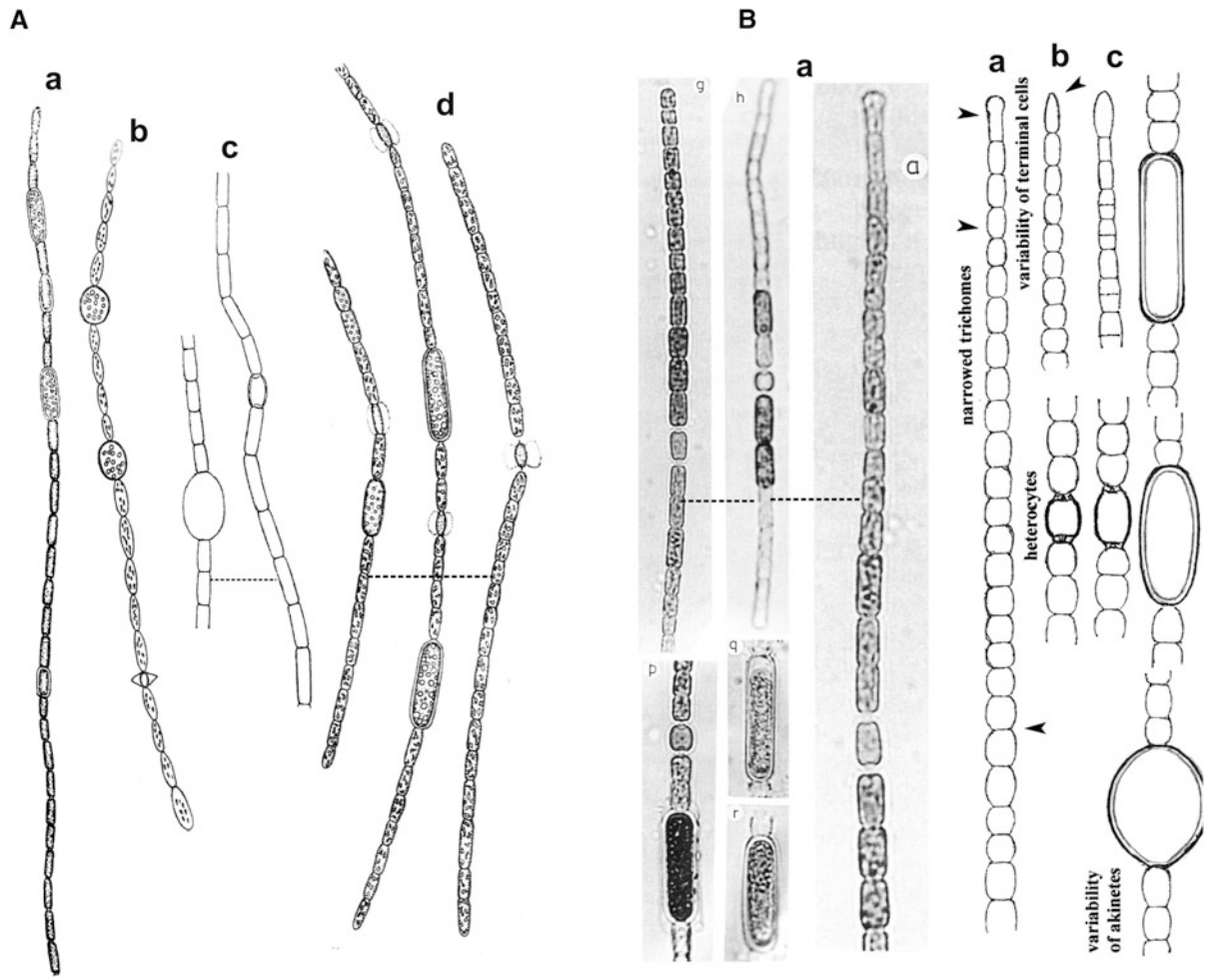

Fig. 12 **A** *Anabaena*-like cluster II: (*a*) *A. miniata*, (*b*) *A. elliptica*, (*c*) *A. levanderi*, (*d*) *A. tenericaulis*; **B** *Anabaena*-like cluster III: (*a*) *Aph. gracile*, (*b*) *Aph. skujae*, (*c*) *Aph. schindleri* (from various authors, cf. Komárek & Kováčik, 1989)

Anabaena-like cluster II (Fig. 12A)

This group contains four planktic "*Anabaena*" species with thin, ±cylindrical trichomes, which are composed from cells containing gas vesicles and which are always longer than wide and with no tapering end cells. Currently, only a few have been sequenced. A close relationship with the genus *Dolichospermum* was found during the first molecular analyses. This cluster was separated out before the final, more precise analyses.

Diacritical markers: Solitary floating, ±straight filaments; trichomes usually thin (−3, rarely up to 7 μm), with cells longer than wide; metameric trichomes; obligatory presence of gas vesicles; akinetes elongated, paraheterocytic, distant from heterocytes.

List of species (six morphospecies): *A. attenuata* Kiselev 1940; *A. abnormis* Proškina-Lavrenko 1968; *A. elliptica* Lemmermann 1898; *A. levanderi* Lemmermann 1906; *A. miniata* Skuja 1956; *A. tenericaulis* Nygaard 1949.

Selected references: Lemmermann (1898), Huber-Pestalozzi (1938), Nygaard (1949), Skuja (1956).

Anabaena-like cluster III (Figs. 3, 12B)

This cluster should be designated rather as "*Aphanizomenon*-like", because it contains only a few species, morphologically similar to *Aph. gracile* and traditionally identified as *Aphanizomenon* species. It differs from the typical *Aphanizomenon* (next genus) especially by the absence of formation of fascicles and different morphology of terminal cells (gradual narrowing of the trichome towards the end cells but never pointed). According to the molecular evaluation, it belongs to the genus *Dolichospermum*, in which it primarily resembles several thin species. Its taxonomy is not yet definitely resolved and the connection with *Dolichospermum* or definition of a separate genus must be assessed in future studies.

Diacritical markers: Free-floating, ±straight, solitary filaments; trichomes with slightly narrowed ends; terminal cells modified; trichomes metameric with intercalary, solitary heterocytes; paraheterocytic intercalary akinetes, cylindrical or elongated oval, solitary, slightly distinct from heterocytes.

List of species (in the following list only three species are included, however, other known "*Aphanizomenon*" species can also belong to the vicinity of this clade): *Aph. gracile* (Lemmermann) Lemmermann 1907; *Aph. schindleri* Kling et al. 1994; *Aph. skujae* Komárková-Legnerová et Cronberg 1992.

Selected references: Lemmermann (1907), Komárek & Kováčik (1989), Kling et al. (1994), Komárek (2005), Rajaniemi et al. (2005a, b), Komárek & Komárková (2006), Kaštovský & Johansen (2008).

Aphanizomenon Morren ex Bornet et Flahault 1888 (Figs. 11, 13, 14)

The traditional genus *Aphanizomenon* is heterogeneous according to molecular analyses. The heterogeneity is also supported by several phenotypic markers which are confirmed by the results from the molecular analyses (Figs. 13, 14). The genus most clearly separated from the original broad view of *Aphanizomenon* is *Cuspidothrix* (see the next genus). The typical part of the genus, based on the type species *Aphanizomenon flos-aquae*, is characterized by specific morphological characters and it is closely related to the genus *Dolichospermum* according to criteria from 16S rRNA sequences. It has a very distinct and specific morphology (with a tendency to form fascicles, and cylindrical, elongated and more or less hyaline terminal cells), and therefore it has been classified into a separate genus.

Diacritical markers: Cylindrical filaments (trichomes); solitary, but with tendency to form fasciculated colonies; trichomes subsymmetric, cylindrical ± up to the end, terminal cells elongate, hyaline; obligate gas vesicles in vegetative cells; heterocytes intercalary, solitary; akinetes elongated, intercalary, distant from heterocytes.

List of revised species (eight definable species after recent revisions, all known only from temperate (up to Mediterranean) zones of both hemispheres): *A. flexuosum* Komárek et Kováčik 1991; *A. flos-aquae* Ralfs ex Bornet et Flahault 1888; *A. hungaricum* Komárková et Mátyás 1995; *A. klebahnii* Elenkin ex Pechar 2008; A. *paraflexuosum* M. Watanabe 1991; *A. platense* Seckt 1922; *A. slovenicum* Rekar et Hindák 2002; *A. yezoense* M. Watanabe 1991.

Selected references: Elenkin (1909), Huber-Pestalozzi (1938), Cmiech et al. (1988), Komárek & Kováčik (1989), Komárek (1999), Li & Watanabe (2001), Li et al. (2003), Rajaniemi et al. (2005a), Komárek & Komárková (2006).

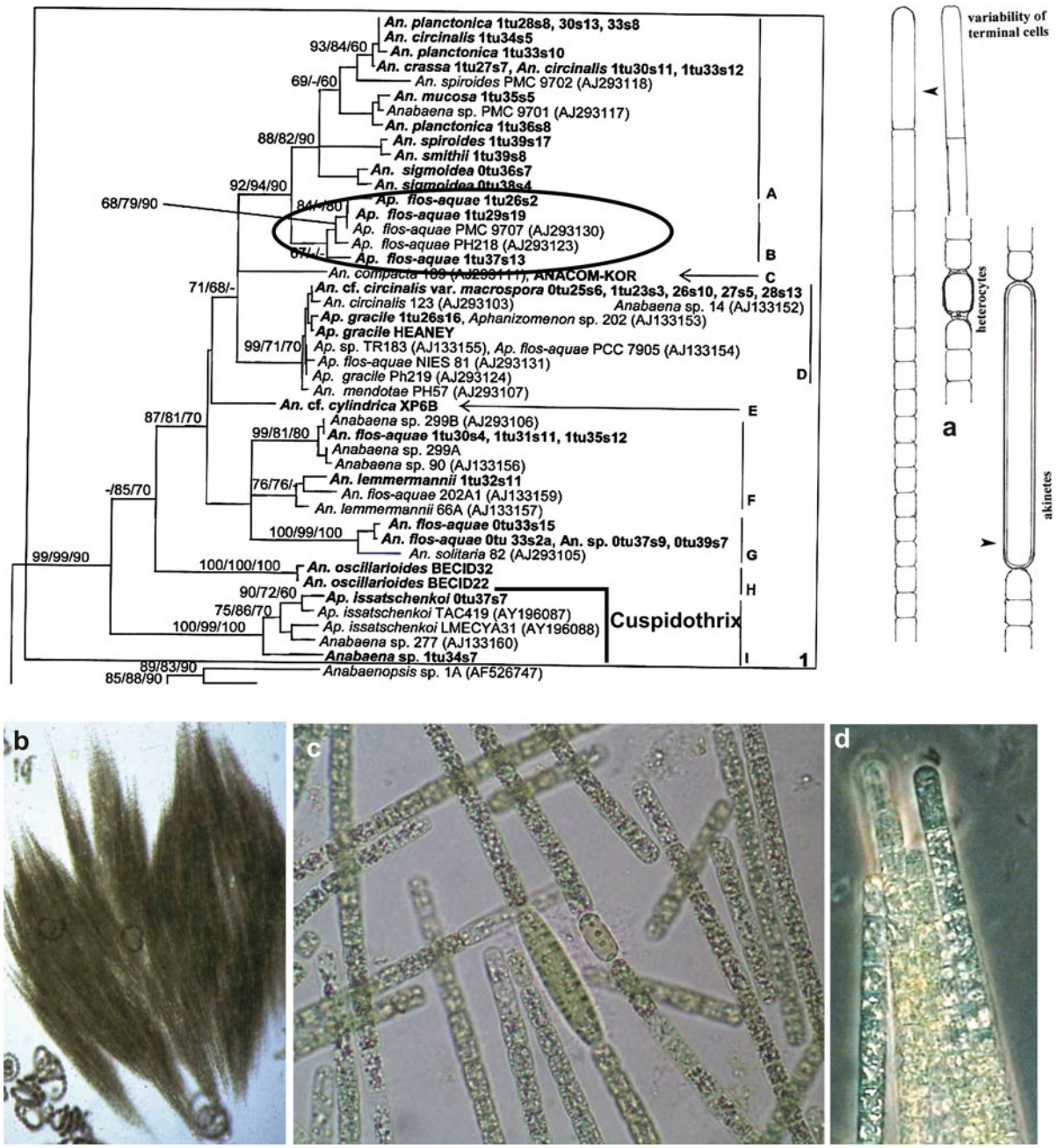

Fig. 13 *Aphanizomenon* Morren ex Bornet et Flahault, based on the type species *Aph. flos-aquae* (**a**–**c**); **d** terminal cells of trichomes; phylogenetic tree after Rajaniemi et al. (2005a, b), photos from Cronberg & Annadotter (2006)

Cuspidothrix Rajaniemi et al. 2005 (Figs. 13, 14)

Species of the genus *Cuspidothrix*, typically with tapering trichomes and pointed ends (based on "*Aphanizomenon issatschenkoi*"), were traditionally classified into the genus *Aphanizomenon*. However, molecular sequencing indicates that they represent a cluster separate from the typical *Aphanizomenon* (based on the type species *Aph. flos-aquae*) (Rajaniemi et al., 2005b). *Cuspidothrix* is clearly distinguishable using phenotypic characters (Li et al., 2003; Rajaniemi et al., 2005a, b; Willame et al., 2006; and others;

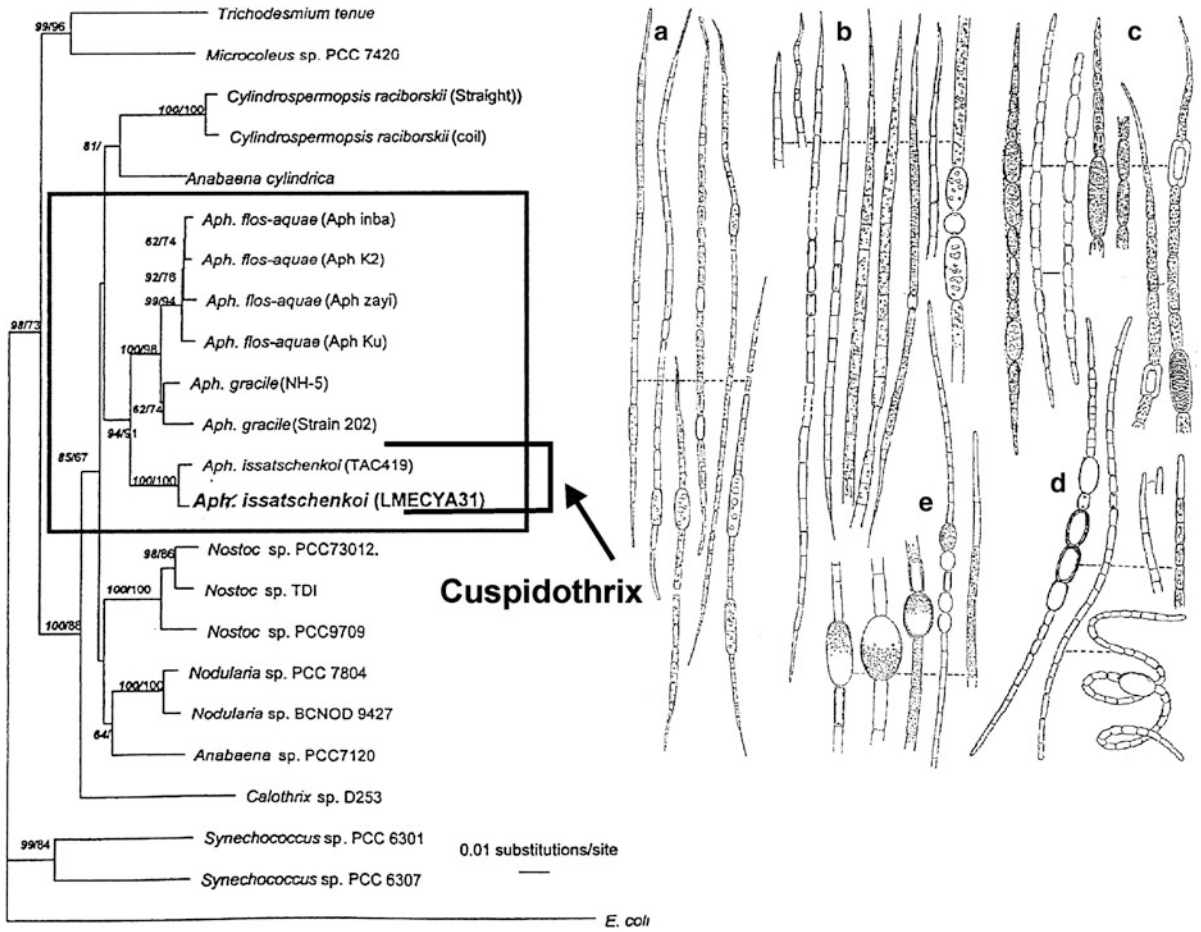

Fig. 14 *Cuspidothrix* Rajaniemi et al.; phylogenetic tree after Li et al. (2003); **a** *C. issatschenkoi*; **b** *C. tropicalis*; **c** *C. elenkinii*; **d** *C. capricorni*; **e** *C. ussatchevii* (from Komárek & Komárková, 2006)

Fig. 14), characteristic exclusively for this genus. Therefore, the genus *Cuspidothrix* was designated and validated by Rajaniemi et al. (2005b).

Diacritical markers: Solitary planktic, relatively thin trichomes; presence of intercalary heterocytes; intercalary akinetes, solitary or in pairs, distant or near to heterocytes; terminal cells narrowed and pointed; trichomes subsymmetric.

List of species (five described species, two known only from tropical regions, one is endemic in the Caspian Sea): *C. capricorni* (Cronberg et Komárek) Rajaniemi et al. 2005; *C. elenkinii* (Kiselev) Rajaniemi et al. 2005; *C. issatschenkoi* (Usačev) Rajaniemi et al. 2005; *C. tropicalis* (Horecká et Komárek) Rajaniemi et al. 2005; *C. ussatchevii* (Proškina-Lavrenko et Makarova) Rajaniemi et al. 2005.

Selected references: Li et al. (2003; under *Aphanizomenon*), Rajaniemi et al. (2005a), Komárek & Komárková (2006), Willame et al. (2006).

Gloeotrichia J. Agardh ex Bornet et Flahault 1886 (Figs. 1, 15)

Up to now, the genus *Gloeotrichia* has been always classified in Rivulariaceae based on to the heteropolar structure of trichomes, filaments with basal heterocytes and apical hairs, and spherical colonies with the development of akinetes above the basal heterocytes. It was the only genus outside the traditional family Nostocaceae (except for a specific group of several species of *Calothrix* not sequenced yet), which have the development of typical akinetes (not

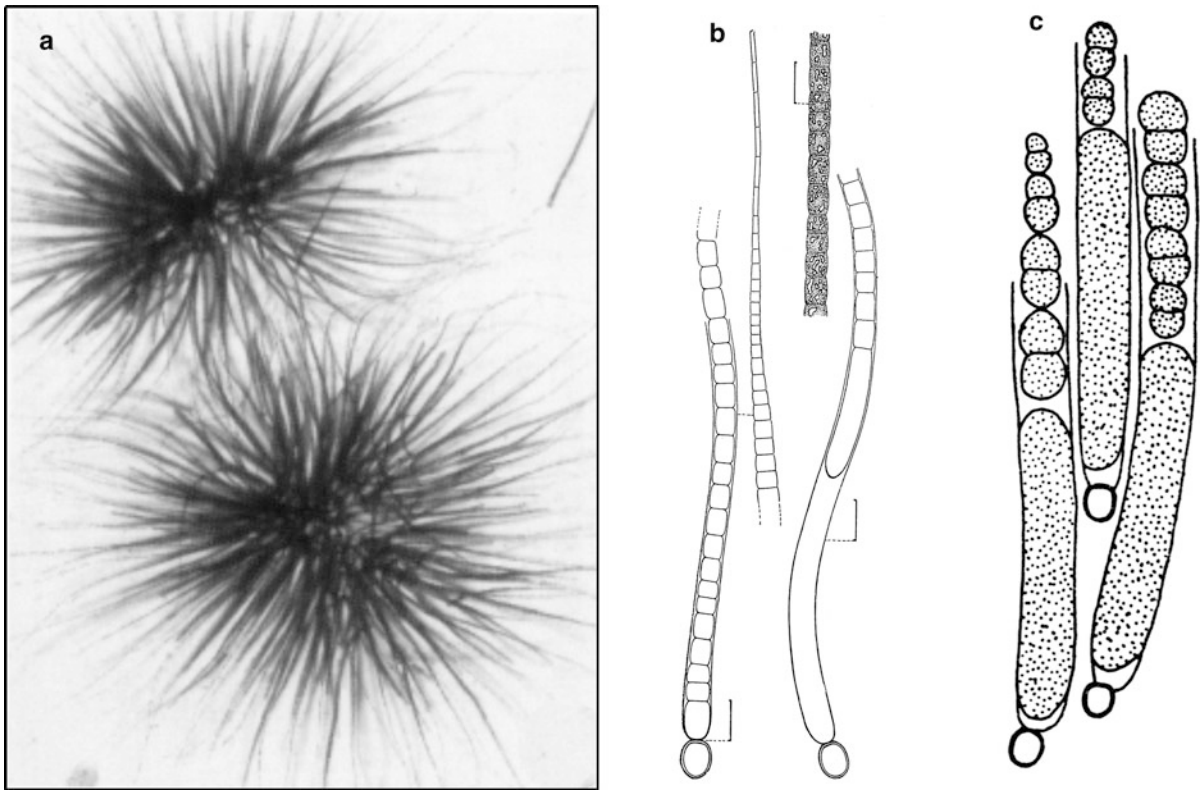

Fig. 15 *Gloeotrichia* J. Agardh ex Bornet et Flahault, the planktic species *G. echinulata*: **a** after Cronberg & Annadotter (2006), **b** solitary filaments after Komárek (1958), **c** akinetes after Richter from Starmach (1966)

arthrospores!). The molecular sequencing indicates that *Gloeotrichia* (at least planktic *G. echinulata* with aerotopes in cells) is more closely related to Nostocaceae than to Rivulariaceae and therefore it should be transferred into this family (Fig. 1). It is evident that the akinete type at least in this case, is a better indicator related to phylogenetic position than the heteropolar morphology of the trichomes.

Diacritical markers: Phylogenetic position; spherical, macroscopic colonies; heteropolar filaments and trichomes with basal heterocytes and terminal hairs; development of typical akinetes above the heterocytes.

List of species (only two planktic species; about 20 metaphytic or periphytic species are not included in the following list). Planktic species: *G. echinulata* [J. Smith et Sowerby] Richter 1894; *G. spiroides* Kondrateva 1954.

Selected references: Kondrateva (1954, 1968), Komárek (1958), Cmiech et al. (1984).

Cronbergia Komárek et al. 2010 (Fig. 16)

A group of *Anabaena*- or *Cylindrospermum*-like species with relatively short filaments and usually localized terminal heterocytes. The development of heterocytes is originally intercalary and in pairs (like in *Anabaenopsis*), but with a different method of formation: one intercalary, vegetative cell between two terminal heterocytes elongates and later divides using simple, perpendicular binary fission (Fig. 16a). The trichomes later separate between the two ripe heterocytes, similar to *Anabaenopsis.* Apoheterocytic akinetes develop solitary or in rows. The phylogenetic position of this cluster is separate from *Cylindrospermum* and *Nostoc*, its morphologically closest neighbours (Komárek et al., 2010).

Diacritical markers: Two groups, (i) planktic with solitary trichomes and facultative gas vesicles in cells, and (ii) benthic and metaphytic, in clusters, without

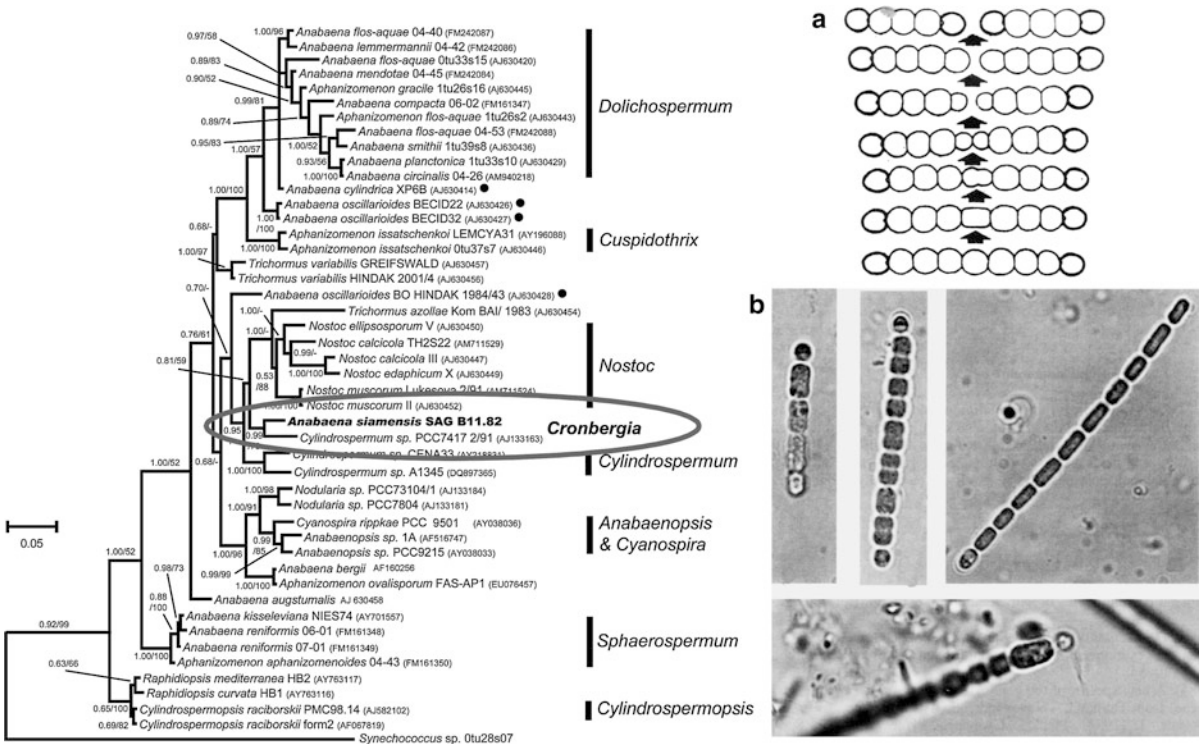

Fig. 16 *Cronbergia* Komárek et al. 2010; phylogenetic tree and the scheme (**a**) after Komárek et al. (2010); photos (**b**) of *C. planctonica* (after Cronberg 2003)

gas vesicles; position in the phylogenetic tree; trichomes originally metameric, but fragmentated; specific intercalary formation of paired heterocytes after binary fission of one elongated vegetative cell; akinetes apoheterocytic, solitary or in rows.

List of species (*Cronbergia* contains up to now only three species, from which one is planktic and occurs in Baltic Sea, but probably more species from this genus exits): *C. siamensis* (Antaricanonda) Komárek et al. 2010 (not planktic); *C. planctonica* (Cronberg) Komárek et al. 2010; *C. paucicellularis* Komárek et al. 2010 (not planktic).

Selected references: Hindák (2000, 2001), Cronberg (2003), Komárek et al. (2010).

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PHYTOPLANKTON

Biogeographically interesting planktonic Nostocales (Cyanobacteria) in the Czech Republic and their polyphasic evaluation resulting in taxonomic revisions of *Anabaena bergii* Ostenfeld 1908 (*Chrysosporum* gen. nov.) and *A. tenericaulis* Nygaard 1949 (*Dolichospermum tenericaule* comb. nova)

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Abstract Questions of biogeography of freshwater cyanobacteria and their ability to colonize new areas have been recently discussed in connection with increasing occurrence of some formerly rare morphospecies in temperate zones. Nevertheless, the general knowledge about the distribution of cyanobacterial species is still fragmentary, and any new findings on cyanobacterial biogeography and spread are valuable. In this study, we provide updated information on the occurrence of *Anabaena bergii*, *Raphidiopsis mediterranea*, and *Sphaerospermopsis aphanizomenoides* in the Czech Republic. In addition, more nostocacean morphospecies are newly reported from the Czech Republic (*A. fusca*, *A. tenericaulis*, *Dolichospermum curvum*, *D. mucosum*, and *S. reniformis*). All of these morphospecies were characterized from a morphological point of view, and their phylogenetic affiliations were assessed on the basis of their 16S rRNA gene sequences. Based on these results, *Anabaena bergii* was reclassified into *Chrysosporum* gen. nov., and *D. tenericaule* comb. nova was established.

Keywords *Anabaena* · *Dolichospermum* · *Sphaerospermopsis* · Taxonomy · Identification · Morphological variability · 16S rRNA gene · Biogeography · Alien species

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Introduction

Questions on the biogeography of freshwater cyanobacteria and their ability to colonize new areas have been recently discussed in connection with the increasing occurrence of some formerly rare

morphospecies in temperate zones. The most studied in this respect were *Cylindrospermopsis raciborskii* (Hindák, 1988; Padisák, 1991, 1997; Maršálek et al., 2000; Stefaniak & Kokociński, 2005; Stüken et al., 2006; Kaštovský et al., 2010) and *Sphaerospermopsis aphanizomenoides* (Padisák & Kovács, 1997; Hindák, 2000; Stefaniak & Kokociński, 2005; Stüken et al., 2006; Zapomělová et al., 2009, 2010; Kaštovský et al., 2010).

Nevertheless, in general, knowledge about the distribution of cyanobacterial species is still fragmentary and also its driving factors remain unclear. There are several reasons of this lack of information: floristic lists are missing or insufficient for many regions of the world, and the level of taxonomic knowledge is low in many groups (Hoffmann, 1996). In addition to this, the absence of akinetes in many nostocacean populations hampers the identification at species level as their size, shape, and position are important taxonomic criteria. Any piece of knowledge on cyanobacterial biogeography and spread is therefore valuable.

The only synoptic study summarizing the occurrence of alien cyanobacteria in the Czech Republic is by Kaštovský et al. (2010). Before it, several minor taxonomic studies mentioned potential non-native species but did not refer on their distribution (Gardavský, 1989; Marvan et al., 1997).

In this study, we provide updated information on the occurrence of *Anabaena bergii*, *Raphidiopsis mediterranea*, and *Sphaerospermopsis aphanizomenoides* in the Czech Republic. In addition, more nostocacean morphospecies are newly reported from the Czech Republic (*A. fusca*, *A. tenericaulis*, *Dolichospermum curvum*, *D. mucosum*, and *S. reniformis*). Further goals were to characterize these morphospecies from a morphological point of view and to assess their phylogenetic affiliations on the basis of their 16S rRNA gene sequences.

Materials and methods

Sampling, isolation, and cultivation of strains

Samples of phytoplankton were collected in 2000–2011 from various localities all over the Czech Republic using a 20-μm mesh plankton net. Occurrences of *Anabaena bergii*, *A. fusca*, *A. tenericaulis*, *Dolichospermum curvum*, *D. mucosum*, *Raphidiopsis mediterranea*, *Sphaerospermopsis aphanizomenoides*, and *S. reniformis* were recorded, and morphologies of these morphospecies were evaluated from the fresh material as described below. Single trichomes were isolated from the samples using a glass capillary and an inverted microscope (Olympus IX 71), and clonal strains were grown (Table 1). The strains were maintained in WC medium (Guillard & Lorenzen,

Table 1 Cyanobacterial morphospecies used in this study, corresponding strain codes, their origin, and year of isolation

Taxonomic assignment	Strain code	Geographical origin	Year of isolation
A. bergii	09-02	Očko sandpit lake, Czech Republic	2009
A. fusca	no[a]	Hubenov reservoir, Czech Republic	2008
A. tenericaulis	08-10	Máchovo fishpond, Czech Republic	2008
A. tenericaulis	08-11	Máchovo fishpond, Czech Republic	2008
D. curvum	04-19	Hejtman fishpond, Czech Republic	2004
D. mucosum	06-04	Horák fishpond, Czech Republic	2006
D. mucosum	06-05	Horák fishpond, Czech Republic	2006
D. mucosum	08-03	Mařka fishpond, Czech Republic	2008
D. mucosum	08-09	Mařka fishpond, Czech Republic	2008
R. mediterranea	07-04	Papež fishpond, Czech Republic	2007
S. aphanizomenoides	04-43	Svět fishpond, Czech Republic	2004
S. aphanizomenoides	09-03	Hostivař fishpond, Czech Republic	2009
S. reniformis	06-01	Pěšák fishpond, Czech Republic	2006
S. reniformis	07-01	Vyšehrad fishpond, Czech Republic	2007

[a] Only natural population was observed and described in this article

1972) under constant culture conditions (21°C, 70 μmol m^{-2} s^{-1} light intensity, 16 h/8 h light–dark cycle). The exception was *A. fusca* where no clonal strain was isolated, and only natural population was described in this article.

Evaluation of morphological characteristics

Field morphologies of all of the studied strains were evaluated. Microphotographs of at least 30 fresh trichomes per each population were taken with a digital camera (Olympus DP 70, magnification 400×). Dimensions of all cell types were measured. Five vegetative cells per trichome were measured in 30 trichomes and as many heterocytes and akinetes as it was possible to find in each sample. Akinetes were missing in the population of *A. tenericaulis*, and their dimensions were acquired from the cultivated strains during the first year of cultivation. Length:width ratios of vegetative cells, heterocytes, and akinetes were used as a rough approximation of the cell shapes. All size measurements were performed using image analysis (Olympus DP Soft).

Statistical analyses of morphological data

Basic statistical characteristics such as mean values, 25 and 75% percentiles and extreme values were computed for the morphological parameters of the field populations.

DNA extraction, PCR and sequencing

The biomass of the studied strains was harvested in the exponential phase of growth by repeated centrifugation, during which the trichomes were washed several times by NaCl solution (concentration 1 g l^{-1}) to remove mucilaginous substances. The biomass samples were stored at −20°C until DNA extractions. DNA was extracted using UltraClean™ Microbial DNA Isolation Kit (MO BIO Laboratories, Inc., Carlsbad, CA). The 16S rRNA gene and ITS region were amplified with primers 16S27F and 23S30R (Taton et al., 2003). Amplification was carried out as follows: one cycle of 5 min at 94°C; 10 cycles of 45 s at 94°C, 45 s at 57°C, and 2 min at 72°C; 25 cycles of 45 s at 94°C, 45 s at 54°C, and 2 min at 72°C; and a final elongation step of 7 min at 72°C. PCR product was used as a template for sequencing with primers 16S27F, 23S30R (Taton et al., 2003), primer CYA781F(a) (Nübel et al., 1997), and the reverse complement of Primer 14 (Wilmotte et al., 1993).

Phylogenetic analyses

Partial sequences of the 16S rRNA gene (1357 bp) were aligned using the program BioEdit version 7.0.9.0 (Hall, 1999), and the alignment was edited manually. Phylogenetic trees were constructed by maximum-likelihood (ML), maximum parsimony (MP), and neighbor-joining (NJ) (Saitou & Nei, 1987) algorithms in the program PAUP* version 4.0b10 (Swofford, 2003). The topology for the phylogenetic tree was derived from ML. The GTR + I + G evolutionary model of substitution was found for the best fit to the data using ModelTest 3.7 (Posada, 2008). The parameters (base frequencies, rate matrix of substitution types, and shape of gamma distribution) were estimated from the data. 100, 1000 and 1000 bootstrap replicates were performed for ML, MP and NJ analysis, respectively. Nucleotide sequences were deposited at GenBank under the accession numbers FM161348-1350, FN691914, FN691919, FN691920, and JQ237768-JQ237774.

Results

Occurrence of the studied nostocacean morphospecies in the Czech Republic

All of the studied nostocacean morphospecies are rather rare and sparsely distributed in the Czech Republic. Their occurrence is demonstrated in Fig. 1.

Anabaena bergii was only found at four localities in the Czech Republic (Máchovo jezero recreation pond, Dubice reservoir, Očko sandpit lake, and sandpit lake near Křenek; Fig. 1a).

Anabaena fusca was observed in Hubenov reservoir near Jihlava (Fig. 1b) in 2008, but no clonal strain was acquired.

Anabaena tenericaulis has been recently noticed at three localities (Máchovo jezero recreation pond, Dubice reservoir, and Očko sandpit lake). Besides that, it was reported from Mlýnský fishpond in South Moravia in the past (Fig. 1a).

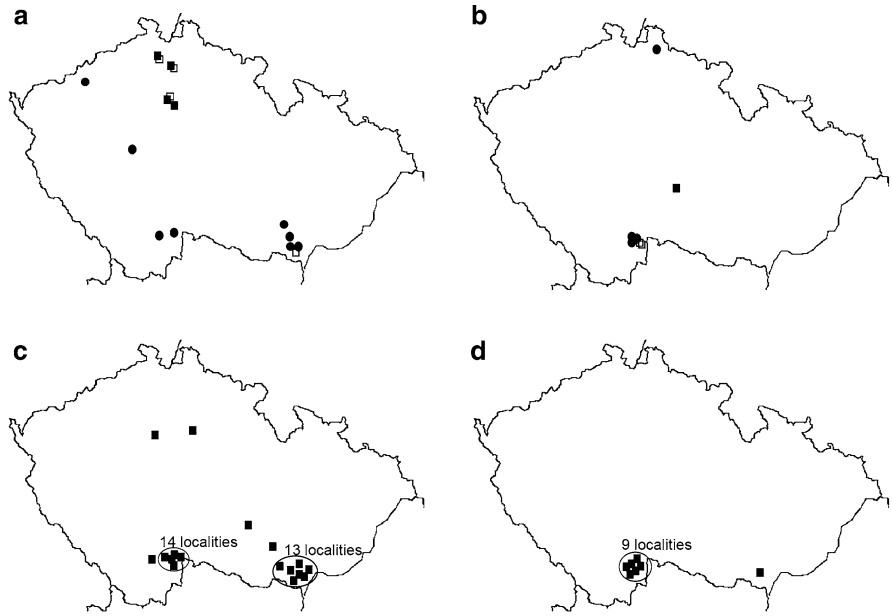

Fig. 1 Distribution of the studied cyanobacterial morphospecies in the Czech Republic: **a**—C. *bergii* (*filled square*), *D. tenericaule* (*open square*), and *R. mediterranea* (*filled circle*); **b**—*A. fusca* (*filled square*), *D. curvum* (*open square*), and *D. mucosum* (*filled circle*); **c**—*S. aphanizomenoides* (*filled square*); **d**—*S. reniformis* (*filled square*)

Occurrence of *Dolichospermum curvum* was noticed at two localities, Staňkovský and Hejtman fishponds, in South Bohemia (Fig. 1b).

Dolichospermum mucosum was found in three fishponds in South Bohemia (Horák, Mařka, Nový Vdovec) and at a locality in North Bohemia (Mšeno reservoir in Jablonec nad Nisou; Fig. 1b).

Sphaerospermopsis aphanizomenoides has been displaying an increasing occurrence frequency in the Czech Republic during the last decade. It has been found at 32 localities so far (Fig. 1c): 15 sites in South Bohemia (fishponds Baštýř, Dvořiště, Koclířov, Malý Tisý, Naděje, Nový Vdovec, Opatovický, Podsedek, Rožmberk, Stolec, Stříbřec, Svět, Velký Tisý, Víra, and Ženich), 2 sites in Central Bohemia (Poděbrady reservoir, Hostivař reservoir), and 15 localities in South Moravia (reservoirs Brno and Mostiště, fishponds Dolní Písečný, Dvorský, Hlohovecký, Horní Lužický, Horní Písečný, Mlýnský, Nad Sádkami, Novodvorský, Novoveský, Prostřední, Velký Martinec, Vrkoč, Výtopa, and Zámecký).

Sphaerospermopsis reniformis was found at nine localities in South Bohemia (fishponds Dobrá Vůle, Fišmistr, Horák, Klec, Láska, Naděje, Pěšák, Rožmberk, and Vyšehrad) and in Hlohovecký fishpond in South Moravia (Fig. 1d).

Raphidiopsis mediterranea was observed at seven localities: fishponds Bagr and Fišmistr in South Bohemia, fishpond Papež in Central Bohemia, coalpit lakes near Droužkovice in North-West Bohemia, and fishponds Františkův, Hlohovecký, and Zámecký in South Moravia (Fig. 1a).

Morphology of the studied nostocacean morphospecies

Morphometric characteristics of the studied morphospecies (Table 2) were measured directly from living water-bloom samples, except for akinetes of *A. tenericaulis* whose data were acquired from cultivated strains. General morphologies of these cyanobacteria are demonstrated in microphotographs (Fig. 2). Akinetes of *Anabaena bergii* from Očko sandpit were widely ovoid, with brownish epispore, vegetative cells were cylindrical, more-or-less isodiametric (length:width ratio around 0.9), and terminal cells were conical (Fig. 2a–d). Only proakinetes (immature stages of akinetes) were observed in the natural population of *A. fusca*. The missing data cannot be completed from culture conditions, as no clonal strain was isolated from this population. Nevertheless, the position of proakinetes was in accordance with the taxon description (near heterocytes, with 2–3 vegetative cells in between), as well as lemon-shaped vegetative cells and the pattern of trichome coiling (Fig. 2l–n). Trichomes of *A. tenericaulis* were very tiny, around 4 μm wide, and cross-walls between vegetative cells were almost invisible without

Table 2 Morphometric parameters of the studied nostocacean morphospecies measured from the original natural populations (living samples). The order of the strains in the table follows Table 1 where the strains are organized in alphabetical order. Values are means (minimum, maximum)

Strain code	Vegetative cells			Heterocytes			Akinetes		
	Length (μm)	Width (μm)	Length:width	Length (μm)	Width (μm)	Length:width	Length (μm)	Width (μm)	Length:width
09-02	4.2 (2.9, 6.0)	4.8 (3.8, 6.0)	0.9 (0.6, 1.4)	6.6 (5.6, 8.0)	6.2 (4.9, 7.5)	1.1 (0.9, 1.4)	17.1 (13.7, 22.7)	10.8 (8.5, 12.0)	1.6 (1.2, 2.5)
A. fusca	5.8 (3.8, 9.3)	5.3 (4.4, 6.0)	1.1 (0.8, 1.6)	7.5 (6.9, 8.1)	7.1 (6.6, 7.4)	1.1 (1.0, 1.2)	Only proakinetes present		
08-10, 08-11	9.6 (4.5, 18.0)	3.6 (2.6, 4.7)	2.8 (1.0, 5.1)	9.3 (4.2, 13.4)	3.9 (2.7, 5.1)	2.5 (1.1, 4.1)	28.6 (20.1, 39.7)	5.9 (5.0, 6.9)	4.9 (3.2, 6.8)
04-19	6.0 (4.2, 7.7)	7.0 (6.0, 8.2)	0.9 (0.7, 1.1)	7.2 (6.4, 8.9)	7.4 (6.3, 8.9)	1.0 (0.8, 1.1)	21.0 (20.6, 21.4)	10.7 (10.1, 11.3)	2.0 (1.8, 2.1)
06-04, 06-05	5.5 (3.3, 7.7)	6.2 (4.8, 7.4)	0.9 (0.6, 1.2)	8.9 (7.3, 11.3)	8.1 (6.5, 9.4)	1.1 (0.9, 1.3)	15.5 (11.7, 26.5)	13.7 (11.4, 16.9)	1.1 (0.9, 1.6)
08-03, 08-09	7.9 (4.2, 12.5)	8.7 (7.0, 11.2)	0.9 (0.5, 1.3)	10.1 (8.8, 11.6)	9.9 (8.8, 11.5)	1.0 (0.9, 1.2)	17.5 (14.7, 21.4)	15.7 (12.4, 18.8)	1.1 (1.0, 1.3)
07-04	9.5 (5.5, 16.7)	3.5 (2.8, 4.2)	2.7 (1.4, 4.8)	No	No	No	14.3 (9.5, 19.8)	4.0 (3.4, 4.6)	3.6 (2.3, 5.1)
04-43	5.5 (3.3, 11.2)	4.4 (3.1, 7.2)	1.3 (0.7, 2.3)	6.3 (5.2, 8.5)	5.9 (4.8, 7.1)	1.1 (0.9, 1.3)	10.0 (7.5, 11.4)	10.3 (8.9, 11.4)	1.0 (0.8, 1.2)
09-03	5.1 (2.1, 7.5)	3.8 (2.8, 6.1)	1.3 (0.6, 2.2)	6.2 (4.8, 8.7)	5.2 (3.3, 6.3)	1.2 (1.0, 2.1)	10.2 (7.8, 14.0)	9.9 (7.6, 12.7)	1.0 (0.9, 1.2)
06-01	5.3 (4.1, 7.7)	5.2 (4.3, 6.0)	1.0 (0.7, 1.4)	6.9 (5.8, 7.6)	7.1 (6.3, 8.2)	1.0 (0.9,1.1)	10.0 (8.5, 12.3)	9.5 (8.0,10.5)	1.1 (0.9, 1.2)
07-01	6.1 (3.9, 9.5)	4.8 (3.9, 5.6)	1.3 (0.8, 2.1)	7.0 (6.0, 7.9)	6.5 (5.4, 7.6)	1.1 (0.9, 1.3)	9.5 (8.7, 10.5)	9.4 (9.0, 9.8)	1.0 (0.9, 1.1)

constrictions. Akinetes in cultures were long cylindrical (Fig. 2e–h). Trichomes of *Dolichospermum curvum* were gathered in a massive mucilaginous mass both in nature and in cultured strains. Trichome coiling pattern was very specific: organization of trichomes into half-circles and trichome coiling direction is changed during the switch between the half-circles. Vegetative cells were spherical and akinetes kidney shaped (Fig. 2i–k). *D. mucosum* displayed irregular trichome coiling, spherical akinetes and thick mucilaginous envelope of trichomes (visible after staining with Indian ink; Fig. 2o–p). *Sphaerospermopsis aphanizomenoides* had spherical akinetes adjacent to heterocytes and narrowed but not pointed ends of trichomes (Fig. 2q–t). Trichomes of *S. reniformis* were regularly coiled but the compactness of coiling differed in the two populations studied (fishponds Pěšák and Vyšehrad). Nevertheless, both of these populations displayed an autapomorphic feature of the genus, i.e., spherical akinetes next to heterocytes (Fig. 2u–v). Trichomes of *Raphidiopsis mediterranea* were very thin (3–4 μm), with pointed terminal cells, invisible cross-walls, and relatively short cylindrical akinetes (Fig. 2w–z).

16S rRNA gene phylogeny of the studied nostocacean morphospecies

The studied nostocacean strains were spread in six different clusters A–F (Fig. 3) of ML, MP, and NJ phylogenetic trees based on partial 16S rRNA gene sequences (1357 bp). Cluster A contained two highly supported subclusters, *A. bergii* strains, including our strain 09-02, and *Aphanizomenon ovalisporum* strains. *Dolichospermum curvum*, *D. mucosum*, and *Anabaena tenericaulis* all appeared in the big clade of the genus *Dolichospermum*, but each of these morphospecies was situated in a different subclade. *D. curvum* 04-19 was in cluster B together with a Finnish strain of *D. flos-aquae* 0tu33s15, receiving a high-bootstrap support. *A. tenericaulis* 08-10 and 08-11 were both in cluster C, close to morphospecies *D. mendotae* and *Aphanizomenon gracile*. All these morphospecies display similar morphologies (vegetative cells longer than wide and elongated cylindrical akinetes). *D. mucosum* 06-04, 06-05, 08-03, and 08-09 appeared in cluster D together with *D. planctonicum*, *D. smithii*, *D. spiroides*, and *D. viguieri*. Common morphological features of the cyanobacteria of cluster D are the width

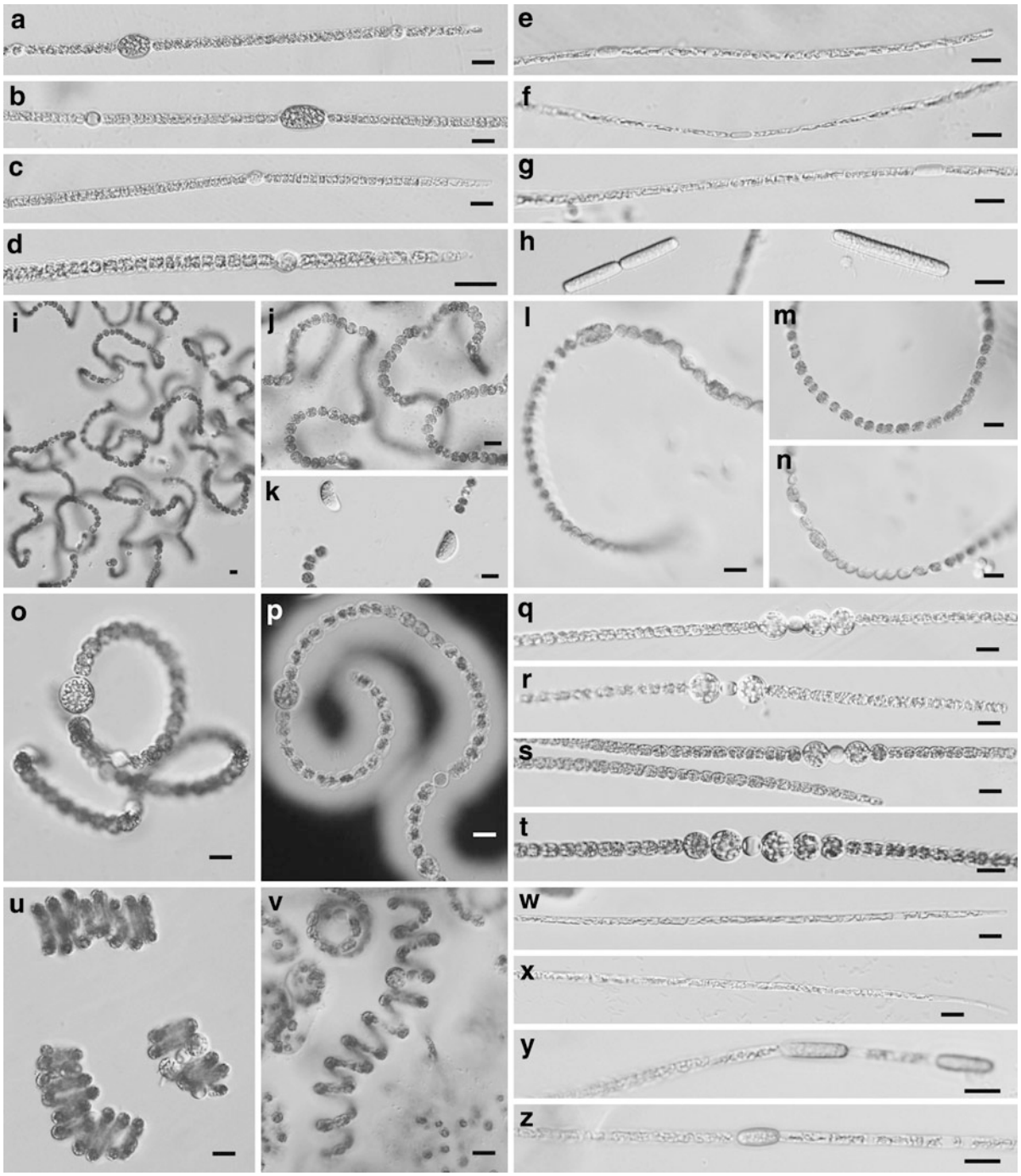

Fig. 2 Microphotographs of studied cyanobacterial morphospecies. *C. bergii* (**a**–**d**); *D. tenericaule* (**e**–**h**); *D. curvum* (**i**–**k**); *A. fusca* (**l**–**n**); *D. mucosum* (**o**–**p**); *S. aphanizomenoides* (**q**–**t**); *S. reniformis* (**u**–**v**); *R. mediterranea* (**w**–**z**). *Scale bars* represent 10 μm

of trichomes (≥8 μm) and length-to-width ratios of vegetative cells (usually <1, i.e., the cells are barrel shaped, shorter than wide). The strains of the genus *Sphaerospermopsis* (*S. aphanizomenoides* 04-43 and 09-03, and *S. reniformis* 06-01 and 07-01) clustered together in a highly supported cluster E. *Raphidiopsis mediterranea* 07-04 appeared in cluster F, together with *Cylindrospermopsis raciborskii*.

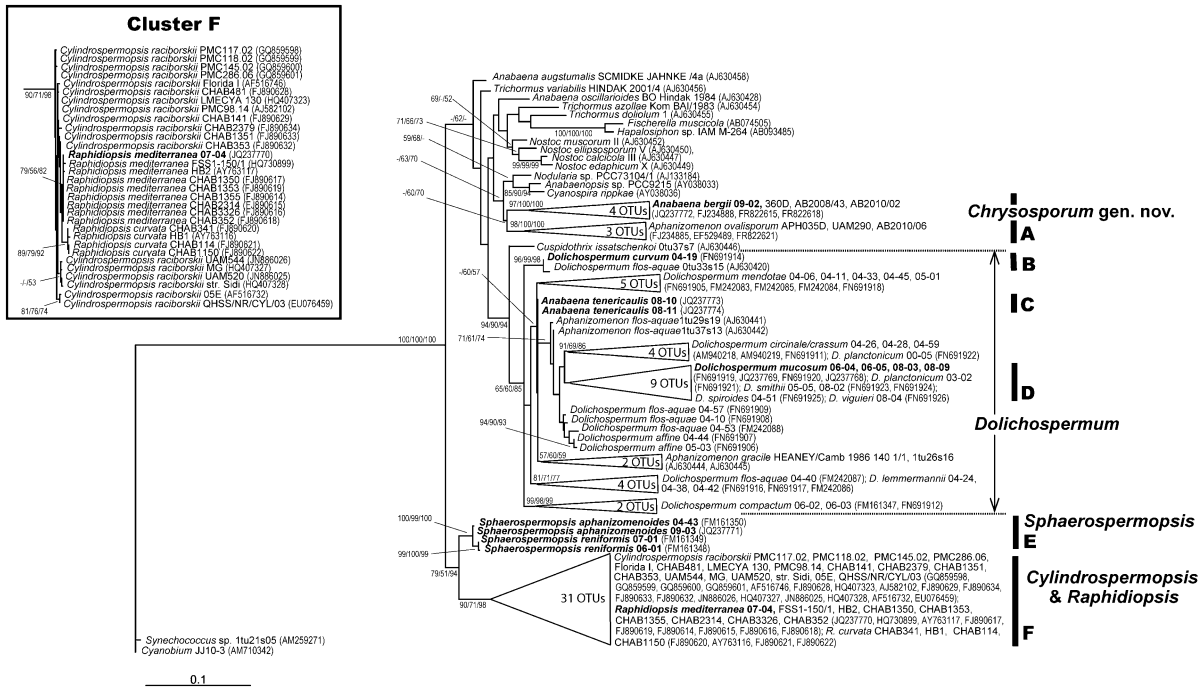

Fig. 3 Maximum-likelihood tree based on 16S rRNA gene sequences (1357 bp) showing the clustering of studied *Anabaena* strains. *Numbers* near the nodes indicate the bootstrap values over 50% for NJ, ML, and MP analyses (in format NJ/ML/MP). *OTUs*, operational taxonomic units; studied strains are in *bold*

Taxonomic revisions resulting from the herein presented phylogenetic analyses

Chrysosporum genus novum

Diagnosis Trichomes of varying length, solitary, free-floating, straight, more-or-less constricted at the cell-walls. Terminal cells conical. Vegetative cells with aerotopes, cylindrical, compressed during division. Heterocytes only intercalary, solitary, spherical, or slightly elongated. Akinetes widely ovoid (length:width ratio 1.2–2.5), with brownish or yellow–brown epispore, distant heterocytes. Planktonic in stagnant freshwater, usually sandpit lakes, halophilic lakes, reservoirs with higher conductivity, relict-lakes and estuaries of big rivers (Danube, Don).

Type species Chrysosporum bergii (Ostenfeld) comb. nova.

Basionym Anabaena bergii Ostenfeld, Izv. Turkestansk. Otd. Imp. Russk. Geogr. Obshch. 5 [Wiss. Ergebn. Aralsee-Exped. 8]: 142, pl. V: figs. 3, 4. 1908. (L)

Autapomorphic characteristics Brownish or yellow–brown epispore of akinetes, conical terminal cells.

Etymology The name of the genus is derived from a Greek word chrysos (gold) that reflects the characteristic color of the akinete epispore. The suffix sporum is derived from a Greek word spora (seed), which is a symbolic designation of akinetes commonly used in cyanobacterial nomenclature.

On the basis of its morphological and the 16S rRNA gene sequence similarities, the following taxon is also transferred to *Chrysosporum*:

Chrysosporum ovalisporum (Forti) comb. nova [basionym: Accad. Agr. Sc. Lett. Verona, Ser. 4, 12: 1911].

Affiliation of the following taxa to the genus *Chrysosporum* is to be confirmed using analysis of their 16S rRNA gene sequences:

Anabaena hatueyi Komárek [basionym: Preslia, Praha 77: 219, 2005]

Anabaena minderi Huber-Pestalozzi [basionym: Das Phytoplankton des Susswassers: Systematik

und Biologie, Schweizerbart'sche Verlagsbuchhandlung, Stuttgart: figs. 10, 11, 19, 20. 1938]
Anabaena recta Geitler et Ruttner [basionym: Arch. Hydrobiol. suppl. 14: 459, 1936]
Anabaena salina Liebetanz [basionym: Bull. Int. Acad. Polon. Sc. et Lett., Cl. Sc. Math. et Nat., Ser. B, 1925: 109, pl. 1: Fig. 5. 1925].

Dolichospermum tenericaule comb. nov

Both ML and NJ phylogenetic analyses of the 16S rRNA gene sequences proved the affiliation of *A. tenericaulis* strains (08-10, 08-11) within the genus *Dolichospermum*. We thus suggest reclassification of *Anabaena tenericaulis* Nygaard (1949) as *Dolichospermum tenericaule* comb. nov.

Discussion

This study refers on the occurrence of eight potentially non-indigenous nostocacean morphospecies in the Czech Republic: *Anabaena bergii*, *A. fusca*, *A. tenericaulis*, *Dolichospermum curvum*, *D. mucosum*, *Raphidiopsis mediterranea*, *Sphaerospermopsis aphanizomenoides*, and *S. reniformis.* For the species *D. curvum* and *A. fusca*, this is the first report on their occurrence in the Czech Republic. *A. tenericaulis* was previously found only once (Losos & Heteša, 1971) here. Three more nostocacean species were referred by Kaštovský et al. (2010) to be alien or potentially expansive in this region: *Cuspidothrix issatschenkoi*, *Cylindrospermopsis raciborski*, and *Dolichospermum compactum*.

Chrysosporum bergii comb. nova (former *Anabaena bergii*)

The herein presented localities of occurrence of *A. bergii* were all also reported by Kaštovský et al. (2010). The species was originally described from Lake Aral and still occurs there (Orlova & Rusakova, 1999). First records from the Czech Republic were by Heteša et al. (1997) from Košarská and Stulíková oxbow pool in Dyje river alluvium. Since 2001 to date, it has been repeatedly found in North Bohemia as was summarized in the "Results" section. This species has recently been reported also from other regions of Central Europe, such as Slovakia (Hindák, 1992, 2000) or northeast Germany (Stüken et al., 2006).

This morphospecies has been classified into the genus *Anabaena* so far but it displays several specific morphological features that are different both from this genus (currently containing species without gas vesicles) and from the genus *Dolichospermum* (planktonic representatives, separated from the traditional genus *Anabaena* by Wacklin et al. (2009)). These features are mainly the conical shape of terminal cells and brownish to yellow–brown epispore of akinetes. And also the cylindrical vegetative cells with little pronounced constriction at cross-walls is something different from typical *Anabaena* or *Dolichospermum*.

Based on the 16S rRNA gene sequence, a distinct and highly supported cluster was detected using maximum-likelihood, maximum parsimony, and neighbor joining phylogenetic analyses. This cluster (A) is markedly distant from the big *Dolichospermum* cluster (containing subclusters B, C, and D, Fig. 3). Also the type species of the genus *Anabaena*, *A. oscillarioides*, represented by the strain BO HINDAK 1984, appeared in a clearly different position in the phylogenetic tree, as well as another representative of benthic *Anabaena*, *A. augstumalis* SCMIDKE JAHNKE 4/a (Fig. 3). These findings are in agreement with results by Rajaniemi et al. (2005) and mainly by Stüken et al. (2009).

Regarding these specific morphological and molecular features of *Anabaena bergii*, we erected the new genus *Chrysosporum.* Besides *A. bergii*, we also proposed re-classification of *Aphanizomenon ovalisporum* into *Chrysosporum.* Stüken et al. (2009) demonstrated that *A. bergii* and *A. ovalisporum* form two sister phylogenetic clusters based on several genomic regions (16 s RNA, cpcA and cpcB genes). One of these clusters contained exclusively *A. bergii* strains, while two *A. bergii* were admixed to *A. ovalisporum* strains in the second cluster. Morphologies of these strains are not available, and reliability of species identification of these strains cannot be therefore assessed. Bootstrap supports of each of these clusters were higher (around 100) than clustering of these two clusters together (54–80, depending on genomic region and phylogenetic analysis), so the existence of a second genus containing *Aphanizomenon ovalisporum* might be discussed here. Nevertheless, we suggest an establishment of just one genus *Chrysosporum* for now because the species

identification of the strains by Stüken et al. (2009) cannot be verified. Furthermore, the original descriptions of *Anabaena bergii* and *Aphanizomenon ovalisporum* both encompass the important identification features (conical terminal cells, brownish epispore of akinetes), proposed autapomorphic for *Chrysosporum.* On the other hand, the only morphological difference between *A. bergii* and *A. ovalisporum* is the length:width ratio of vegetative cells (longer than wide in *A. ovalisporum*) that appears to be rather questionable (also depends on growth rate and division frequency of cells). A thorough revision of both these species is needed, and a special genus for *A. ovalisporum* might be separated from *Chrysosporum* if it will be proved well founded.

Anabaena fusca

Anabaena fusca has been found for the first time in the Czech Republic at the only locality of Hubenov reservoir near Jihlava. The species was described from a lake in Minnesota, USA (Hill, 1976b), and has not yet been reported from anywhere else than North America.

All morphological features observed in the Czech population fitted well to the original description of the taxon.

Nevertheless, the phylogenetic affiliation of this species remains unclear, because its 16S rRNA gene has not yet been sequenced. In this study, we only present the first observation of its occurrence, and the isolation of a strain is intended in the future. Wacklin et al. (2009) performed a substantial revision of the traditional genus *Anabaena* and reclassified the majority of planktonic representatives into the new genus *Dolichospermum*. *Anabaena fusca*, although morphologically similar to some *Dolichospermum* morphospecies, was excluded from this revision because of the lack of molecular data. Detailed polyphasic evaluation of this morphospecies is therefore highly desirable.

Dolichospermum tenericaule comb. nova (former *Anabaena tenericaulis*)

This is the first report on the occurrence of *D. tenericaule* in the Czech Republic since the finding by Losos & Heteša (1971). The species was described under the name *Anabaena tenericaulis* from Jægerbakke Dam, Selandia, Denmark (Nygaard, 1949) and was sparsely distributed in Nordic localities (Komárek, pers. comm.; Vuorio, pers. comm.; Willén, pers. comm.). Little is known about its distribution outside the Nordic countries. To our knowledge, it has never been reported from another country in Central Europe.

All morphological and morphometric parameters of the herein described strains (08-10, 08-11) are in agreement with the original description and drawings by Nygaard (1949).

The 16S rRNA gene of representatives of this species was sequenced for the first time. The herein presented phylogenetic analyses demonstrated that the strains 08-10 and 08-11 formed a tight cluster C (Fig. 3), close to *Dolichospermum mendotae* and *Aphanizomenon gracile* strains, whose sequences were acquired from Genbank. Cluster C is a part of a big clade of the genus *Dolichospermum*. It is possible to find morphological characteristics that are common for cluster C, *D. mendotae* and *A. gracile*: long cylindrical akinetes, elongated vegetative cells (length:width ratio > 1), and relatively thin trichomes (*ca.* 3–5 μm). Reclassification of *Anabaena tenericaulis* Nygaard 1949 as *Dolichospermum tenericaule* comb. nova was proposed based on these findings.

Dolichospermum curvum

This study reports on the occurrence of *D. curvum* at two sites in South Bohemia, fishponds Hejtman and Staňkovský. They are neighboring hypertrophic fishponds, interconnected by a channel, and therefore the two populations of *D. curvum* in these fishponds are presumably not isolated one from another. The species was described from a lake in Minnesota, USA, (Hill, 1976a) and later reported from Finland (Komárková-Legnerová & Eloranta, 1992) and Japan (Li et al., 2000).

From morphological point of view, our strain 04-19 is more similar to the Japanese (Li et al., 2000) population than to the population from USA (Hill, 1976a). According to the original description, the akinetes should be distinctly bent, while our population, as well as that one from Japan, displays kidney-shaped akinetes. Shape of akinetes of the Finnish population (Komárková-Legnerová & Eloranta, 1992) was somewhere in between the American and Japanese populations. Nevertheless, all of these populations displayed similar pattern of trichome coiling and a massive mucilaginous mass around trichomes.

Detailed polyphasic evaluation would be necessary to confirm that these populations from different parts of the world represent a tight phylogenetic cluster with the above-mentioned range of variability in the shape of akinetes.

For the present, our strain 04-19 represents the only *D. curvum* strain whose 16S rRNA gene has been sequenced. The herein published phylogenetic analyses have indicated that *D. curvum* belongs to *Dolichospermum* cluster where it represents a specific, highly supported subcluster (cluster B in Fig. 3). Besides our strain 04-19, a strain 0tu33s15 from Finland designated as *D. flos-aquae* also appeared in cluster B. Morphology of this strain was evaluated by Rajaniemi et al. (2005) but the information about trichome coiling pattern and presence of mucilage is missing in this publication and a microphotograph is not provided. As morphometric features of *D. curvum* are highly similar to those of *D. flos-aquae*, this Finnish population might have been misidentified and its affiliation to the species *D. curvum* cannot be excluded.

Dolichospermum mucosum

Occurrence of *D. mucosum* in the Czech Republic has only been noticed at four localities. This is the first finding from Central Europe as it has been reported from North Europe Komárková-Legnerová & Eloranta, 1992; Rajaniemi et al., 2005), Japan (Li et al., 2000), and Ukraine (Komárek & Zapomělová, 2007) so far.

Sequencing of the 16S rRNA gene of four Czech strains (06-04, 06-05, 08-03, and 08-09) confirmed that this species belongs to the genus *Dolichospermum* (revision performed by Wacklin et al., 2009). Its clustering together with *D. smithii* strains (straight trichomes of similar cell dimensions, spherical akinetes) is in accordance with results by Rajaniemi et al. (2005). These authors also presented a close phylogenetic affinity between coiled and straight morphospecies of *Dolichospermum* of similar cell dimensions and identical akinete shapes.

Sphaerospermopsis aphanizomenoides

The occurrence frequency of *S. aphanizomenoides* in the Czech Republic has been continuously increasing. For the first time, it was found by Horecká & Komárek (1979) from Central Moravia. Later findings were not until 1994 (Heteša et al., 1997) and then 2004 (Zapomělová et al., 2009). Kaštovský et al. (2010) referred on 20 sites where this species has recently occurred and since the publication of this study it has been newly found at more than 10 localities. Most of these localities are eutrophic to hypertrophic fishponds. An updated list of these localities has been provided above. This species was originally described from a lake in Anatolia (Geitler, 1932) and has predominantly been reported from the tropical and subtropical regions. Nevertheless, it appears to have expanded to the temperate zones of Central Europe within the last few years: Hungary (Padisák & Kovács, 1997), Slovakia (Hindák, 2000), Poland (Stefaniak & Kokociński, 2005), northeast Germany (Stüken et al., 2006), and the Czech Republic (Zapomělová et al., 2009). Reliable identification of *S. aphanizomenoides* is only possible when akinetes are present. Misidentification or oversight of populations without akinetes is therefore highly probable, and this species might be even more frequent than has been reported so far.

The first polyphasic evaluation of Czech strains of *S. aphanizomenoides* was provided by Zapomělová et al. (2009). A distinct highly supported molecular cluster was recognized based on the 16S rRNA gene, which resulted in erection of the new genus *Sphaerospermopsis* and reclassification of former *Aphanizomenon aphanizomenoides* (Zapomělová et al., 2009, 2010). Identical results were concurrently obtained from German and Portuguese strains, and were furthermore supported by analyses of some other genomic regions (Stüken et al., 2009; de Figueiredo et al., 2010).

Sphaerospermopsis reniformis

This article provides an information on nine localities of *S. reniformis* occurrence in the Czech Republic. For the first time, this species was noticed by Keršner (1997) in South Moravia and later Zapomělová et al. (2009) reported on occurrences in South Bohemia in 2006 and 2007. Little is known about the biogeography of this species. It appears to be sparsely distributed at very distant localities around the world: Germany, Ukraine, Japan, Cuba, and Africa (Lemmermann, 1898; Aptekar in Elenkin, 1938; Watanabe et al., 2004; Komárek, 2005; Cronberg & Annadotter, 2006; Komárek & Zapomělová, 2007). Similar to *S.*

aphanizomenoides, the absence of akinetes might hamper the recognition and identification of this species in natural populations and thus this taxon might well have been overlooked.

The herein described strains of *S. reniformis* (06-01, 07-01) have already been studied by Zapomělová et al. (2009). Based on this polyphasic evaluation, *S. reniformis* was established the type species of the newly erected genus *Sphaerospermopsis* (Zapomělová et al., 2009, 2010). Results of our present phylogenetic analyses are in accordance with that.

Raphidiopsis mediterranea

This study informs about the occurrence of *R. mediterranea* at seven localities in the Czech Republic. Kaštovský et al. (2010) mentioned four of them but alerted that this species is probably more widespread but previous findings might have confused it with a similar and better-known species *Cylindrospermopsis raciborskii. R. mediterranea* is considered a subtropical species but later has also spread to the temperate zone, as was summarized by Kaštovský et al. (2010). Regarding Central Europe, its occurrence has been recently reported from Slovakia (Maršálek et al. 2000).

Based on phylogenetic analyses of the 16S rRNA gene sequence, our *R. mediterranea* strain 07-04 clustered together with other *Raphidiopsis* and *Cylindrospermopsis raciborskii* strains from GenBank (cluster F, Fig. 3). The first evidence of the close phylogenetic relationship of these genera was published by Li et al. (2008). Later studies confirmed these findings and even declared that *R. mediterranea* represents a non-heterocytous life-cycle stage of *Cylindrospermopsis raciborskii* (Moustaka-Gouni et al., 2009, 2010).

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PHYTOPLANKTON

Taxonomic and phylogenetic evaluation of *Limnothrix* strains (Oscillatoriales, Cyanobacteria) by adding *Limnothrix planktonica* strains isolated from central China

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Abstract Six *Limnothrix* strains, isolated for the first time from a shallow eutrophic lake in central China, were taxonomically and phylogenetically evaluated by investigating their polyphasic characteristics, including morphological features, cellular ultrastructures, and 16S rRNA gene sequences. All the six strains were morphologically similar, and their trichomes were in average 1.7 μm wide and cells 4.0 μm long, and having small gas vesicles within cells, and therefore identified as *Limnothrix planctonica* (Woloszynska) Meffert. Cellular ultrastructures of them showed that peripheral thylakoids with 3–5 parallel layers were parietally distributed in the cells. The phylogenetic results based on the 16S rRNA gene sequences showed that all the *Limnothrix* strains, including the six in this study and those from the Genbank, formed two distinct clusters. The similarity in 16S rDNA sequences between these two clusters was lower than 90%, indicating that these *Limnothrix* strains belong to different genera. This is the first report on the morphology and phylogeny of *L. planctonica* strains, providing the new information on taxonomy of the genus *Limnothrix*.

Keywords Cyanobacteria · *Limnothrix* · Morphology · Phylogeny · Taxonomy

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Introduction

The cyanobacterial genus *Limnothrix* Meffert has been classified within the order Oscillatoriales, family Pseudanabaenaceae, subfamily Pseudanabaenoideae, under the current botanical taxonomic system (Anagnostidis & Komárek, 1988; Komárek, 2003; Komárek & Anagnostidis, 2005), and *L. redekei* (Van Goor) Meffert was established as the type species (Meffert, 1987). The *Limnothrix* species are characterized by solitary, unsheathed, and mostly unconstricted trichomes, mainly consisting of narrow cylindrical cells with polar and/or central aerotopes. Some species contain both phycocyanin (PC) and phycoerythrin (PE) as well as display complementary chromatic adaptation (CCA) (Kohl & Nicklisch, 1981). Such an adaptation allows these species to maximize the absorption of available light by regulating the ratio of PC to PE (Stomp et al., 2004, 2008).

With the difficulties in taxonomic delimitation, the genus *Limnothrix* has become a large group with 15 species and described forms, in which *L. redekei*, *L. planctonica*, and *L. rosea* are occasionally dominant in freshwater phytoplanktons (Meffert, 1987; Komárek & Anagnostidis, 2005). *Limnothrix* populations are also known to be present in marine environments in South Africa (Silva & Pienaar, 2000). *L. redekei* populations in Europe have been frequently reported to be massively present in shallow eutrophic lakes for decades (Bailey-Watts, 1972; Rojo & Cobelas, 1994; Vardaka et al., 2000; Moustaka-Gouni et al., 2007). Along with the development of molecular phylogenetics in Cyanobacteria, a few strains of *L. redekei* were shown to be polyphyletic. One mixed cluster is usually composed of *L. redekei* and *Pseudanabaena* strains (Gkelis et al., 2005; Willame et al., 2006; Acinas et al., 2009; Nishizawa et al., 2010). Therefore, the term *Pseudanabaena/Limnothrix* group, instead of two different genera, has been used in current researches (Zwart et al., 2005; Willame et al., 2006; Nishizawa et al., 2010). Another *Limnothrix* cluster typically represented by three Greek strains of *L. redekei* has been reported (Gkelis et al., 2005), and this cluster was shown to be complicated by adding strains morphologically identified as genus *Geitlerinema* (Perkerson et al., 2010; Bernard et al., 2011). Another difficulty related to the taxonomic assignment at the species level is that the conducted studies on the genus *Limnothrix* rarely documented other species than *L. redekei*. Hence, further evaluation of the phylogenetic position of the genus *Limnothrix* and further exploration of the defined taxonomy within this genus by applying more strains of *Limnothrix* and related genera, preferably from other geographic regions outside Europe, is necessary.

The occurrence of *Limnothrix* has never been reported in China since the establishment of this genus. In this study, six *Limnothrix* strains were isolated for the first time from a shallow eutrophic lake in central China. We aimed to perform the polyphasic examination of these six Chinese strains, to add further knowledge on the taxonomy and phylogeny of the *Limnothrix* genus.

Materials and methods

Isolation and cultivation of *Limnothrix* strains

The *Limnothrix* strains (Table 1) were isolated from the Donghu Lake in Wuhan City, Hubei Province in 2009. The micropipette method (Rippka, 1988) was used to isolate the cultivated strains. Six uni-algal strains initially identified at the *Limnothrix* genus level were obtained. All the six strains were cultured in liquid CT medium (Ichimura, 1979) under a constant white light intensity of 25 μmol photons m^{-2} s^{-1} on a 12:12 L:D cycle at 25 ± 1°C.

Morphological examination

The examined strains were morphologically identified according to the description of Meffert (1987) as well as that of Komárek & Anagnostidis (2005). Morphological and morphometrical studies were performed on specimens collected from cultures on the exponential growth phase. Trichome size was measured from ≥50 individuals per strain using a Nikon eclipse 80i light microscope with DS-Ri1 digital camera (Nikon, Japan). The image was analyzed using the NIS-Elements D 3.2.

Ultrastructure observation

The ultrastructural features of the isolated strains were studied using transmission electron microscopy

Table 1 Morphological characteristics of the *Limnothrix planktonica* isolates studied

Strain	Width (μm)	Length (μm)	L:W ratio	PE	Motility	Calyptra
CHAB709	1.3–1.7–2.0	2.3–4.1–6.2	1.9–2.4–3.1	+	+	−
CHAB751	1.2–1.7–2.0	2.0–3.8–6.0	1.7–2.4–3.1	+	+	−
CHAB753	1.3–1.8–2.0	1.8–4.1–6.7	1.8–2.4–3.2	+	+	−
CHAB756	1.3–1.9–2.2	2.2–3.9–5.7	1.6–2.2–3.2	+	+	−
CHAB759	0.8–1.6–2.0	2.0–4.2–6.0	2.0–2.7–3.1	+	+	−
CHAB763	0.9–1.6–2.2	2.0–4.0–6.8	2.0–2.5–3.2	+	+	−

(TEM). The strains during the exponential growth phase were fixed following a procedure similar to that described by Gkelis et al. (2005) and then observed under a TEM (FEI TECNAI G^2 20 TWIN, USA) at an accelerating voltage of 200 kV using the iTEM FEI software.

DNA extraction and PCR amplification

Total genomic DNA was extracted according to the method of Neilan et al. (1995) and the xanthogenate-SDS (XS) DNA extraction protocol (Tillett & Neilan, 2000). A 1 mL portion of the cultivated solution at the logarithmic growth phase was used, and 50 μL DNA solutions were finally obtained with 5–10 ng/μL DNA concentration.

The 16S rRNA gene sequences were amplified from the genomic DNA using the PCR primers 27F1 (5′-AGAGTTTTGATCCTGGCTCAG-3′) (Neilan et al., 1997) and B23S: 5′-CTTCGCCTCTGTGTGCC TAGGT-3′ (Taton et al., 2003). The polymerase chain reactions (PCR) were performed in a 50 μL reaction according to Lin et al. (2010). All PCR products were examined on 1% (w/v) agarose gels dyed with ethidium bromide and purified by the PCR purification kit (Omega, USA), and the purified PCR products were directly sequenced by Invitrogen Biotechnology Co. Ltd. (Shanghai, China).

Phylogenetic analysis

The 16S rDNA sequences, consisting of those examined in this study and those obtained from GenBank, were aligned using CLUSTALW integrated into the BioEdit package (Hall, 1999). Phylogenetic trees were constructed using neighbor-joining (NJ), maximum likelihood (ML), and Bayes algorithms. NJ with Kimura-2 and 1,000 bootstraps was used to construct the corresponding phylogenetic trees using the MEGA4 program package (Tamura & Dudley, 2007). DNA sequences were assessed for the best fit model to explain the sequence evolution using the test model (Posada & Crandall, 1998). The ML algorithms were constructed using PHYML version 3.5c (Guindon & Gascuel, 2003). One hundred bootstrap replicates were performed, and only bootstrap values above 50% were indicated at the nodes of the trees. Clade support was estimated using the general time-reversible (HKY) model. The parameters of the Ts/tv ratio and p-invar were set corresponding to the outputs from the test model (Posada & Crandall, 1998). The MrBayes program was used to execute the Bayes algorithms. The parameters in MrBayes were set to 5,000,000 generations, 50,000 trees, sampling at every 100th generation, use of the HKY model of DNA substitution, Nst = 6, and rates = gamma. The 16S rRNA sequence data were deposited in the GenBank under the following accession numbers: JQ004021–JQ004026.

Results

Morphological characteristics

The six *Limnothrix* isolates were morphologically examined,and they were all identified as *L. planctonica* (Woloszynska) Meffert based on the descriptions by Komárek & Anagostidis (2005). The trichomes of the six isolated strains were solitary, planktonic, or sometimes formed mats, settled to the bottom of the culture tubes, and they were unconstricted at the cross-wall (Fig. 1b, c). The color of the trichome was pale blue-green. The filaments contained PE because they were fluorescent through the PE-specific filter (490[Ex.]/580[Em.], Nikon, Japan) under a fluorescence light microscope. The trichomes were unsheathed, straight, or bent, consisting of cylindrical cells, and slowly glided with oscillation. The cells were 0.8–2.2 μm wide and 1.8–6.8 μm long, whereas the apical cells were rounded without calyptra. Gas vesicles were obviously observed in trichomes in the fresh samples from lake water (Fig. 1a). The ratios of the lengths to the widths of the cells were 2.5 to 3.2 on the average (Table 1). Based on the descriptions by Komárek & Anagostidis (2005), the six *Limnothrix* isolates were all identified as *L. planctonica* (Woloszynska) Meffert.

The six strains were examined for their ultrastructures. The cells have peripheral thylakoids annularly distributed at the cross section (Fig. 1d, e). However, the longitudinal thin sections of the trichomes showed that the thylakoids were only parietally distributed with 3–5 parallel layers. Gas vesicles were not found.

Phylogenetic analysis

The two strain pairs (CHAB751 and CHAB756; CHAB753 and CHAB759) of *L. planctonica* showed

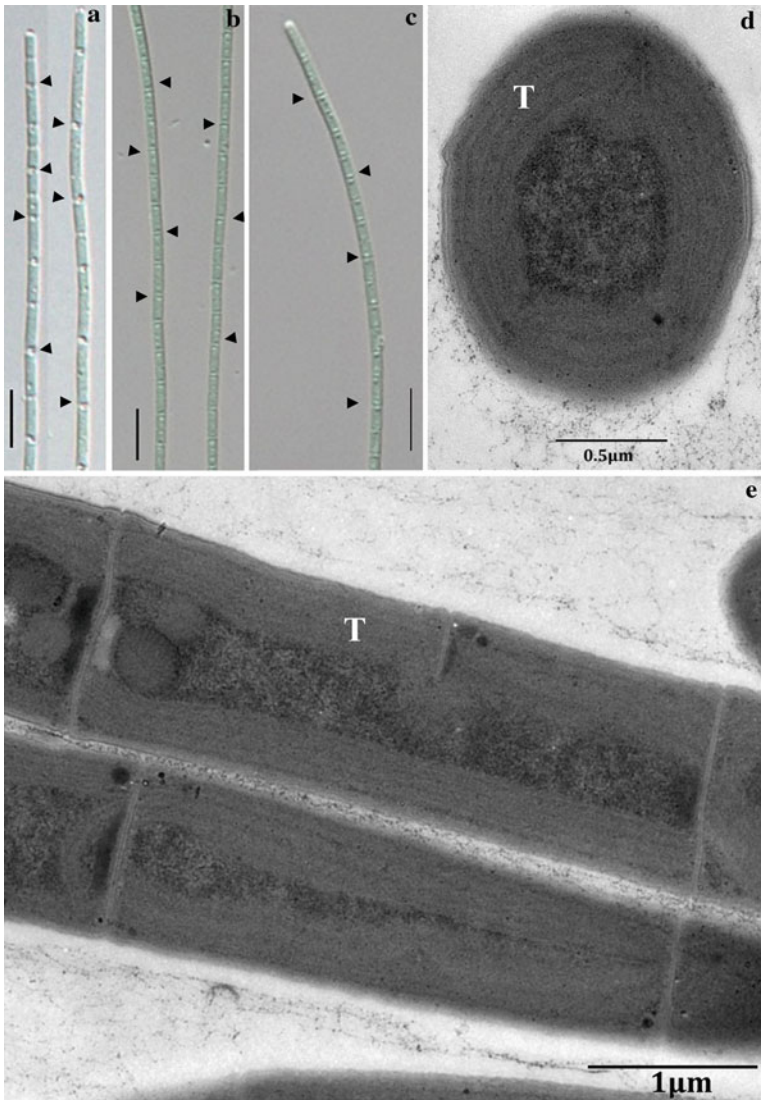

Fig. 1 Micrographs of *Limnothrix planctonica* strains in this study. **a** *L. planctonica* trichomes from a natural population. **b**, **c** Trichome of *L. planctonica* CHAB 709 strain; all *scale bars* = 10 μm, and the *arrows* indicate gas vacuoles. **d**, **e** Transmission electron micrographs of *L. planctonica* strain CHAB709, and thylakoids are marked with T

identical 16S rRNA gene sequences and had 99% similarity in 16S rRNA gene sequences with the other two strains. Six 16S rRNA gene sequences from this study and 52 previous 16S rRNA gene sequences from strains of *Limnothrix* and other Oscillatorean genera in Genbank were used to construct the phylogenetic trees using the NJ, ML, and Bayesian methods. All the *Limnothrix* strains form two distinct clusters, as shown in the Bayesian tree with the supporting values from the NJ, ML, and Bayesian methods (Fig. 2). The larger cluster II was formed mainly by *Limnothrix* strains from Asia, Europe, Africa, and America. However, the smaller cluster I was a mixture. The lowest similarity in 16S rRNA gene sequences among the *Limnothrix* strains within clusters I, II, and between Clusters I and II were 95.9%, 94.4%, and 86.2%, respectively, indicating large divergence in the *Limnothrix* strains reported so far.

Discussion

Taxonomic revisions have been continuously performed in Cyanobacteria during the last decades. In the order of Oscillatoriales, considerable treatments targeted on the genus *Oscillatoria* were performed, and the separation of several genera from *Oscillatoria*, such as *Planktothrix*, *Planktothricoides*, and *Limnothrix*,

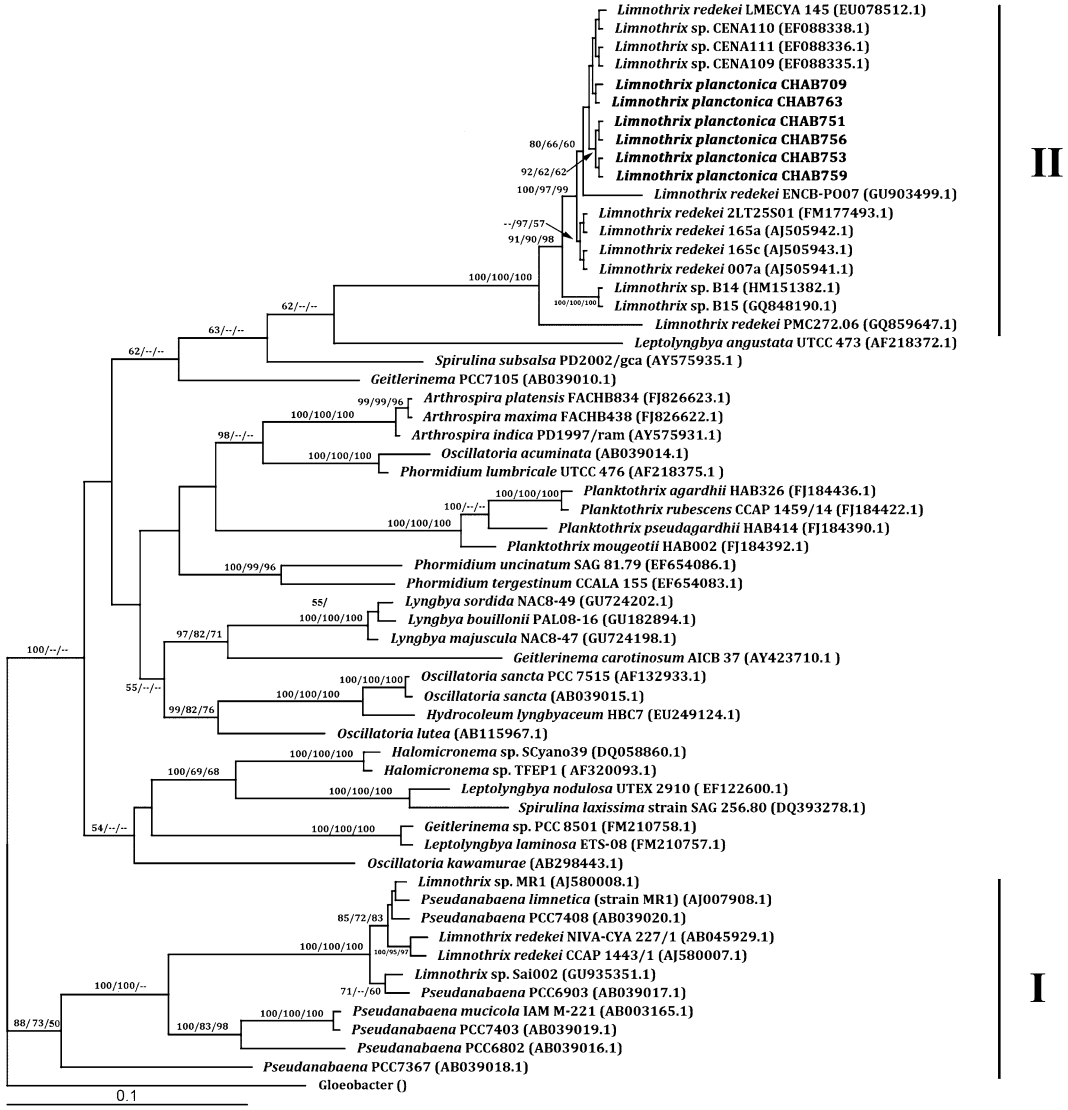

Fig. 2 Phylogenetic tree based on 16S rDNA region sequences (949 bp) of six 16S rRNA gene sequences from this study and 52 previous 16S rRNA gene sequences from strains of *Limnothrix* and other Oscillatorean genera in Genbank. Bootstrap values greater than 50% with Bayers/ML/NJ methods are indicated on the tree. Strains isolated from China are codes as "CHAB" strain number. *Gloeovacter violaceus* PCC7421 (BA000045) was used as the outgroup

successfully contributed to the taxonomic system of Oscillatorean organisms. The genus *Limnothrix* was established to distinguish from *Oscillatoria* based on several differences such as cellular shape and thylakoid arrangement. Suda et al. (2002) analyzed the 16S rDNA sequence of *L. redekei* NIVA-227 during the taxonomic studies on water-bloom-forming species of oscillatoroid Cyanobacteria and confirmed the molecular divergence among *Limnothrix*, *Oscillatoria*, and *Planktothrix*. Thus, they set *L. redekei* NIVA-227 as the strain type of the genus *Limnothrix*. However, three strains of *L. redekei* isolated from Lake Kastoria, Greece were shown to form a separate phylogenetic group from *L. redekei* NIVA-227, which clustered together with some *Pseudanabaena* strains (Gkelis et al., 2005), clearly exhibiting that *Limnothrix* is polyphyletic. Therefore, more strains isolated from more regions have to be examined to elucidate the detailed molecular divergence and phylogenetic relationship within the genus *Limnothrix*. This study is the first to examine

Table 2 Genetic distances of 16S rDNA sequences among the *Limnothrix* strains analyzed in this study (above 949 bp)

	CHAB 709	CHAB 751	CHAB 753	CHAB 756	CHAB 759	CHAB 763	B14	PMC 272.06	2LT 25S01	ENCB-PO07	LMECYA 145	CENA 110	CENA 111	CENA 109	B15	165c	165a	007a	NIVA 227	MR1	CCAP 1443
CHAB751	0.003																				
CHAB753	0.004	0.002																			
CHAB756	0.003	0	0.002																		
CHAB759	0.004	0.002	0	0.002																	
CHAB763	0.002	0.002	0.003	0.002	0.003																
B14	0.016	0.016	0.017	0.016	0.017	0.015															
PMC272.06	0.039	0.038	0.039	0.038	0.039	0.038	0.045														
2LT25S01	0.003	0.003	0.004	0.003	0.004	0.002	0.014	0.037													
ENCB-PO07	0.018	0.018	0.019	0.018	0.019	0.017	0.031	0.054	0.017												
LMECYA 145	0.003	0.003	0.004	0.003	0.004	0.002	0.016	0.039	0.003	0.018											
CENA110	0.002	0.002	0.003	0.002	0.003	0	0.015	0.038	0.002	0.017	0.002										
CENA111	0.002	0.002	0.003	0.002	0.003	0	0.015	0.038	0.002	0.017	0.002	0									
CENA109	0.002	0.002	0.003	0.002	0.003	0	0.015	0.038	0.002	0.017	0.002	0	0								
B15	0.016	0.016	0.017	0.016	0.017	0.015	0	0.045	0.014	0.031	0.016	0.015	0.015	0.015							
165c	0.004	0.004	0.005	0.004	0.005	0.003	0.015	0.036	0.002	0.018	0.004	0.003	0.003	0.003	0.015						
165a	0.003	0.003	0.004	0.003	0.004	0.002	0.014	0.037	0	0.017	0.003	0.002	0.002	0.002	0.014	0.002					
007a	0.004	0.004	0.005	0.004	0.005	0.003	0.015	0.036	0.002	0.018	0.004	0.003	0.003	0.003	0.015	0	0.002				
NIVA 227	*0.12*	*0.12*	*0.121*	*0.12*	*0.121*	*0.119*	*0.121*	*0.127*	*0.118*	*0.135*	*0.118*	*0.119*	*0.119*	*0.119*	*0.121*	*0.118*	*0.118*	*0.118*			
MR1	*0.122*	*0.122*	*0.123*	*0.122*	*0.123*	*0.121*	*0.123*	*0.129*	*0.12*	*0.137*	*0.12*	*0.121*	*0.121*	*0.121*	*0.123*	*0.12*	*0.12*	*0.12*	*0.02*		
CCAP 1443/1	*0.119*	*0.119*	*0.12*	*0.119*	*0.12*	*0.118*	*0.12*	*0.127*	*0.117*	*0.134*	*0.117*	*0.118*	*0.118*	*0.118*	*0.12*	*0.117*	*0.117*	*0.117*	*0.01*	*0.019*	
Sai002	*0.115*	*0.115*	*0.116*	*0.115*	*0.116*	*0.114*	*0.116*	*0.123*	*0.113*	*0.127*	*0.113*	*0.114*	*0.114*	*0.114*	*0.116*	*0.113*	*0.113*	*0.113*	*0.024*	*0.024*	*0.022*

Four strains in italics belong to Cluster II

Limnothrix based on cultivated strains from China, and the six strains were identified as *L. planctonica* by careful morphological examination. The *L. planctonica* populations from the Donghu Lake were shown to have small-sized gas vesicles, similar to the descriptions by Meffert (1988). However, the cultivated strains in this study tended to lose the gas vesicles, as shown in both the light microscope and TEM images (Fig. 1). Such loss of gas vesicles in the *Limnothrix* strains was also illustrated in the study by Gkelis et al. (2005). It was also shown that several strains encoded as *Limnothrix* sp. CENA isolated from Brazil had inconspicuous gas vesicles compared with *L. redekei* NIVA 227. The phylogenetic tree based on the 16S rDNA sequences, including most *Limnothrix* strains so far analyzed with longer lengths (above 949 bp) of the 16S rRNA gene, revealed that all *Limnothrix* strains form two distinct clusters, namely, cluster I (a mixture of *L. redekei* NIVA227 and *Pseudanabaena* strains) and cluster II (mostly *Limnothrix* strains). The 16S rDNA sequence similarity between clusters I and II was lower than 90% (Table 2). Hence, the reported *Limnothrix* strains should belong to at least two different genera given that 95% of 16S rRNA gene sequence is regarded as the cut-off for genus definition in bacteriological classification (Ludwig et al., 1998). The cluster considered as the relatively reasonable genus *Limnothrix* has to be determined. The *L. redekei* NIVA 227 was set as the strain type by Suda et al. (2002), and the *L. redekei* PCC9416 (SAG 3.89) was set as the reference strain for the *Limnothrix*-form genus (Castenholz, 2001). The *L. redekei* NIVA 227 was in cluster I based on the results of this study and that by Gkelis et al. (2005), whereas *L. redekei* SAG 3.89 was also shown to be in cluster I by Perkerson et al. (2010). By contrast, the *Limnothrix* strains in Cluster II, including three Greek strains of *L. redekei*, three Brazilian strains (*Limnothrix* sp. CENA 109-111), and the six strains of *L. planctonica* in this study, were all shown to lose gas vesicles or have small-sized gas vesicles under the cultivated conditions. Such difference in gas vesicles within *Limnothrix* corresponds well to the classified types of gas vesicles in the genus *Limnothrix* initially proposed by Meffert (1988) and it is very interesting to get that their morphological and molecular characteristics coincided. Hence, *L. redekei* NIVA 227 and the other *Limnothrix* strains in cluster I are proposed to represent the genus *Limnothrix*, but this proposal requires that the confusion among *Pseudanabaena* strains in this cluster be cleared. The *Limnothrix* strains in cluster II represent a monophyletic group, similar to the work by Perkerson et al. (2010) who taxonomically revised some *Geitlernema* strains, and they may be reorganized as a new cyanobacterial genus. All the findings in this study allowed us to reevaluate the taxonomic system related to *Limnothrix* at both genus and species levels. However, additional molecular evidence from *Limnothrix* strains, such as divergence in gas vesicles genes (*gvp*) in the strains between clusters I and II, are necessary, which will help fully understand the phylogenetic relationship among *Limnothrix*-related genera.

Conclusively, the morphological and phylogenetic characteristics of *Limnothrix* strains originating from China were studied for the first time in this study. This is also the first report to obtain *L. planctonica* strains and to elucidate their ultrastructure. Based on the results from this and previous studies showing *Limnothrix* as polyphyletic, taxonomy of *Limnothrix*, still needs further examination.

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PHYTOPLANKTON

Impairing the largest and most productive forest on our planet: how do human activities impact phytoplankton?

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Abstract This article summarizes the outcomes of the 16th Workshop of the International Association for Phytoplankton Taxonomy and Ecology. Four major issues dealing with the impact exerted by human activities on phytoplankton were addressed in the articles of this special volume: climate change and its impacts on phytoplankton, the role of land use in shaping composition and diversity of phytoplankton, the importance of autecological studies to fully understand how phytoplankton is impacted by stressors and the role of ecological classification to evaluate community changes due to the different impacts. Case studies from different types of aquatic environments (rivers, deep and shallow lakes, reservoirs, mountain lakes, and temporary ponds) and from diverse geographical locations (not only from the Mediterranean and temperate regions, but also from subtropical and tropical ones) have shown that a complex spectrum of human impacts, not exclusively linked to eutrophication, severely conditions structure and dynamics of phytoplankton assemblage both in the short and long terms. Moreover, the trade-offs between climate change and other human-induced stresses as eutrophication, agricultural and urban land use or water overexploitation contribute to make more severe the impact exerted by humans on phytoplankton and, in turn, on the functioning of aquatic ecosystems.

Keywords Climate change · Eutrophication · Land use · Functional classification · Autoecology · Modelling

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Introduction

Though it accounts for less than 1 % of the photosynthetic biomass on Earth—representing something poorly visible and tangible compared with the terrestrial vegetation—phytoplankton fuels trophic webs and biogeochemical cycling, playing a key role in global climate regulation, C-sequestration and oxygen production (Boyce et al., 2010; McQuatters-Gollop

et al., 2011; Winder & Sommer, this volume). Compared with current published estimates for land plants and for coastal vegetation, the production of phytoplankton accounts for nearly 50 % of the global net primary production (Longhurst et al., 1995; Field et al., 1998). Nevertheless, biomass development and phytoplankton composition have a huge impact on water quality, affecting not only the recreational and landscape value of water bodies, but also the availability of drinking water.

Short- and long-term environmental changes in lake ecosystems are closely paralleled by changes in the composition and biomass of phytoplankton communities. Changes most likely result from several overlapping stressors and are measured at the population and community level (at different taxonomical detail), including modifications in biodiversity patterns, phenology, dominance and frequency of algal blooms. Human alterations include the effects due to eutrophication and climate change, as well as other impacts connected with human activities and management, e.g. hydrological alterations (damming of rivers, water overexploitation), introduction of alien species, food–web manipulations, over-fishing, shoreline landscaping, aquatic plant management, dredging, introduction of pollutants among the others. Many examples about the effects of these alterations have been documented in this special issue. However, in the plethora of anthropogenically driven environmental stressors, changes connected with nutrients and climatic dynamics are considered having paramount importance.

At division or class level, a number of long-term ecological investigations and synoptic studies comparing a large number of lakes have emphasized the raising importance of cyanobacteria and, to a lesser extent, diatoms over other algal groups coupled with the increasing phosphorus concentration (Watson et al., 1997). In rivers or in reservoirs, conditions of high-nutrient concentrations associated with the stability of the water column can lead to high increase of algal biomass and huge populations of cyanobacteria (e.g. Naselli-Flores & Barone, 2003). At the other extreme of physical gradients, high flow in large rivers or in reservoirs is detrimental for the development of high-phytoplankton biomasses and cyanobacteria (Salmaso & Braioni, 2008), influencing the community structure and the presence of meroplanktic and metaphytic species (Abonyi et al., this volume).

At species level, the role of trophic state of water bodies as a selective mechanism was investigated during the 11th IAP meeting. Resuming assembly rules, Reynolds et al. (2000) highlighted that nutrient availability represented only one of the environmental dimensions defining algal niches. At the same time, they argued that the mechanisms of species selection remained controversial, expressing a positive perception about the relationship between defined species-clusters and particular physical conditions (Padisák et al., 2010). The utilization of association labels (coda) in the study of phytoplankton ecology underwent a remarkable progress in recent years (Reynolds et al., 2002; Padisák et al., 2009). Contemporarily, the definition of ecological indices based on the trophic preferences of single phytoplankton species or groups received an increasing interest, particularly in Europe, under the demands of EU Water Framework Directive (e.g. Kaiblinger et al., 2009; Ptacnik et al., 2009). Nevertheless, the inability of many indices to track closely the trophic state in different lakes reminds us the hypervolumetric nature of algal niches and the need of more reliable ecology in applied and management-oriented studies.

In the last decade, several investigations documented a significant increase of temperature in marine and inland waters (Dokulil et al., 2006). Knowledge on the effects of warming on phytoplankton is still at an early stage, but it is accelerating (Keller, 2007; Matzinger et al., 2007). Strong effects of warmer years on the development of cyanobacterial biomass have been demonstrated (Paerl & Huisman, 2009), and these results were confirmed by modelling studies, which showed a tradeoff between nutrient loading and temperature, with a dominance of cyanobacteria in water bodies characterized by comparable nutrient loadings but with higher water temperatures (Elliott et al., 2006; Jöhnk et al., 2008). In general, algae capable of buoyancy regulation are favoured by increased water stability and reduced mixing, with important effects on the structure of phytoplankton assemblages (Winder & Hunter, 2008; Tolotti et al., this volume). Further complexity is added by geographic position, lake morphometry and ecological interactions in modulating the climate effects. For example, in northern temperate lakes, an earlier timing of the spring phytoplankton growth is favoured by earlier ice melting, stratification and light availability (Weyhenmeyer et al., 2008). Conversely, it was widely demonstrated that the effects

of temperature fluctuations and thermal stability in large deep lakes may involve a cascade of causal factors which include the extent of mixing, the epilimnetic nutrient replenishment and the algal growth (Goldman et al., 1989; O'Reilly et al., 2003; Verburg et al., 2003; Salmaso, 2011). The disentangling of the interactions between the North Atlantic Oscillation and trophic state over Northern and Central Europe was recently resumed by George (2010). The study of the impact of large scale climatic fluctuations in the Mediterranean area, including the southern subalpine lake district, has begun only recently (Salmaso, 2012; Salmaso & Cerasino, this volume).

Climate change

Several contributions in this special issue highlighted the importance of climate fluctuations on phytoplankton structure and development. Climate fluctuations are understood in a broad sense, from high frequency, annual/seasonal changes (Dokulil & Teubner, this volume; Salmaso & Cerasino, this volume; Morabito et al., this volume) to long-term climate trends (Tolotti et al., this volume).

Both Dokulil & Teubner (this volume) and Salmaso & Cerasino (this volume) investigated the effects of large scale atmospheric patterns on the development of phytoplankton in two deep lakes at the northern (Lake Mondsee) and southern (Lake Garda) side of the Alps, respectively. Fluctuations in phytoplankton biovolumes in Lake Mondsee were mostly due to the development of *Planktothrix rubescens* (de Candolle ex Gomont) Anagnostidis and Komárek. During the stratification months, this species formed deep chlorophyll maxima well below the thermocline at low temperatures and dim light. Decadal changes in the biomass of *P. rubescens* were controlled by phosphorus availability and eutrophication, while the interannual fluctuations over the baseline long-term trend were, in turn, influenced by the North Atlantic Oscillation, through its effects on the timing of the onset of stratification.

Similarly, in Lake Garda, the interannual fluctuations of cyanobacteria and *P. rubescens* showed a strong link with the extent of vertical mixing and the upward transport of phosphorus from the hypolimnion to the trophogenic layers. In turn, interannual fluctuations in P-replenishment were strictly controlled by lake and air winter temperature and, ultimately, by the winter fluctuations of large scale atmospheric modes of circulation [East Atlantic (EA) and Eastern Mediterranean Patterns (EMP)] (Salmaso & Cerasino, this volume). In this regard, the EA and EMP could become two emerging and valuable 'climatic tools' helpful in explaining the effects of winter climate variability on terrestrial and aquatic ecosystems over the Mediterranean area.

Indirect effects exerted by climate in Lake Maggiore were investigated by Morabito et al. (this volume). Silica, phosphorus, temperature and wind were the key explanatory variables in species selection. Specific climate-linked events driven by deep mixing and floods were shown to increase the Si:P ratio, favouring good-P/poor-Si diatom competitors. Based on these findings, these authors argued that the long-term development of key species, such as *Tabellaria flocculosa* (Roth) Kützing was driven by climate fluctuations and physical factors rather than nutrient availability.

Long-term effects due to changes in nitrogen and silica availability, and to rising water temperature and thermal stability were documented in Lake Piburger (Tolotti et al., this volume). The increase in nitrogen during the late 1990s was regarded as a major trigger of recent phytoplankton changes, followed by an increase of taxa such as gelatinous species and small centric diatoms able to overcome sinking losses in the more stable environments as represented by the summer epilimnion.

Winder & Sommer (this volume) reviewed the direct and indirect effects of increasing lake temperatures. Direct effects act on physiology and phenology, while indirect effects influence phytoplankton through modifications in water column stratification, availability of nutrients, light and grazing intensity. These modifications favour shifts in phytoplankton composition and structure, with straight consequences for ecosystem functioning.

Based on field and laboratory experiments with phytoplankton populations from an oligotrophic, low-altitude lake in Central Spain, Rojo et al. (this volume) analysed specific effects of climate change affecting PAR and the light spectrum. Under enhanced UVR, phytoplankton biomass was one-third lower than the biomass reached under only PAR due to a lower growth and contribution of autotrophic picoplankton to total biomass.

Implications of environmental conditions, including thermal regime, on the sedimentation and horizontal distribution of phytoplankton were analysed by Yacobi & Ostrovsky (this volume).

Modelling approach

Climate fluctuations at different temporal and spatial scales were instrumental to interpret phytoplankton changes observed in the water bodies analysed so far. A careful selection of explanatory variables and parameters was compulsory in the development of ecological models aimed at determining the factors governing the spatial distribution of *P. rubescens* in a southern subalpine lake (Lake Pusiano; Carraro et al., this volume). The distribution of the species in this lake was strongly influenced by lake hydrodynamics. Thus, the application of coupled physical/biological models (ELCOM/CAEDYM) showed how the characteristics of this species could be well suited to a re-oligotrophication phase occurring concurrently with the strengthening stratification of a warming climate. However, the use of these models can be affected by the lower performances for nutrient and biological variables, therefore advocating for a more comprehensive, multidisciplinary approach in the definition and development of modelling tools in phytoplankton ecology. This is considered an urgent task, because the integration of simple mechanistic models and the use of complex modelling represent promising ways to improve predictability and a tool to formulate and test hypotheses.

Land use and phytoplankton diversity

Since the development of Vollenweider's models, it was clear that the use and management of watersheds were deeply connected to water quality. As a consequence, the importance of anthropogenic activities in determining lake and stream water chemistry was unanimously recognized, and several papers have been published since then showing the effects of land use on nutrient enrichment (e.g. Harper & Stewart, 1987). However, the recognition that biodiversity in lakes is influenced by land use in the watershed is relatively new (Hoffman & Dodson, 2005). By studying phytoplankton from an ecological point of view, i.e. as a complex system of mutually interacting populations, rather than as simple chlorophyll a concentration, the impact of land use on biodiversity clearly appears as shown by several papers included in this volume.

In particular, by analysing phytoplankton assemblages in 18 Mediterranean lakes and reservoirs, Katsiapi et al. (this volume) found that despite differences in hydrological regime and morphometric/topographic variables, land use type was strongly correlated with phytoplankton community structure. Moreover, they showed that phytoplankton biomass was significantly higher in water bodies having a watershed with agricultural and artificial land cover exceeding 30 %. This threshold was much lower than what had been set in temperate lakes (Alvarez Cobelas et al., 2005) and it is probably linked to the higher catchment area/lake area ratio experienced by Mediterranean freshwater ecosystems. In addition, the authors highlighted that in Mediterranean reservoirs the effects of land use, although masked by the operational use (e.g. Naselli-Flores, 2003; Naselli-Flores & Barone, 2005), can be even greater than that observed in natural lakes due to the higher catchment area/lake area ratios.

Analogous results were obtained by Paul et al. (this volume) who studied 11 lakes in the Rotorua region (New Zealand). In particular, these authors demonstrated that Cyanoprokaryota were negatively correlated with native forest and positively with pasture, whereas Chlorophyta were positively correlated with native forest and urban land use and negatively with pasture.

Other effects of land use on aquatic ecosystem integrity, and ultimately on phytoplankton structure, were shown by Naselli-Flores & Barone (this volume) who studied phytoplankton dynamics in Mediterranean temporary ponds. These ecosystems can be recognized as aquatic environments only during their water phase which can last from a few days to a few weeks. In the rest of the year, they appear as land depressions perfectly suitable to be filled up with garbage or to be appointed for agricultural and urban development. In addition, due to their small dimensions, they are highly subjected to pollution by fertilizers, pesticides or garbage, to water overexploitation and/or to deepening for conversion into permanent water bodies to fulfil irrigation needs.

Overexploitation of water resources and land use changes are also important in large lakes. As shown by Zohary et al. (this volume), the disappearing of the recurrent *Peridinium gatunense* Nygaard spring bloom in Lake Kinneret is due to a modification in the amount of water from its main inflow, the Jordan River. The seasonal patterns of phytoplankton in this lake and the very regular spring bloom of its flagship

dinoflagellate are famous among phytoplankton ecologists as a paradigm of the seasonal succession of phytoplankton in lakes. A significant reduction in the amount of fresher waters from Jordan River to fulfil agricultural needs resulted in the interruption of the regular appearance of *P. gatunense* in Lake Kinneret from 1996 onward.

Analogously, modifications in the hydrological cycle were shown to be effective in explaining the interannual fluctuation of cyanobacterial blooms and their different distribution in side-arms of a large, dendritic reservoir by O'Farrell et al. (this volume).

Water regulation of freshwater ecosystems can deeply interfere with phytoplankton structure and dynamics as shown by Abonyi et al. (this volume). These authors analysed data collected in the River Loire. The catchment of this river covers almost 20 % of France and is the most extended among the European rivers flowing into the Atlantic Ocean. Several reservoirs interrupt the water flowing and interfere with potamoplankton dynamics by releasing lacustrine species into the river waters. In addition, urban and industrial wastewaters reaching the river contribute to further alter the composition of the phytoplankton assemblages.

Autoecology

Identifying phytoplankton is a time consuming work. Moreover, the reliability of identification (at least for those organisms which can be recognized without genetic analyses) is supported by the experience of the operator which, in turn, is based on the amount of time spent observing phytoplankton at the microscope. In addition, methodological correctness is of paramount importance since observations carried out on fixed material not always allow to correctly identify microalgae. On the other hand, live samples cannot be easily managed and maintained when routine monitoring is performed. Apart the aforementioned skills, all those dealing professionally with phytoplankton cannot avoid to have a good knowledge on autoecology of species. Achieving this kind of knowledge is a fundamental support to the correct identification of microalgae. At the same time, a clear account of the environmental conditions at the time of species collection (and even during the weeks before collection) can provide a strong help in species identification.

Knowledge on autoecology is also important when species have to be attributed to functional groups (coda). This work cannot be done a priori and requires some caution. As an example, it cannot be easy for an inexperienced observer to identify *Aulacoseira* or *Cyclotella* at species level. However, knowledge on the autoecological requirements of the different species can help in attributing the organism to the correct codon and in reducing the possibilities of species misinterpretation. Thus, if *Aulacoseira* is dominating in poor light conditions, it will likely belong to the *granulata* species rather than to the *italica* or to the *ambigua* ones. In the same way, *Cyclotella comensis* is unlikely to occur in eutrophic waters where most probably *C. meneghiniana* will be present.

Autoecology of selected phytoplankton species was investigated in six papers of this volume. Even for very well-known species, autoecological studies may provide new insights that can be helpful to better understand the dynamics and distribution of planktic algae. This was demonstrated by Zohary et al. (this volume) who found that the occurrence of *P. gatunense* in Lake Kinneret depends on a yet unknown microelement or/and an organic compound which reach the lake from the Hula valley during flood periods.

Dokulil & Teubner (this volume) presented an almost exhaustive account on deep living *P. rubescens* which confirms the preference of this organism for low temperature and dim light. Moreover, these authors argue that survival of *P. rubescens* during stratified period is possible because of heterotrophic subsistence and that life below the thermocline is aided by physiological acclimation of photosynthesis and buoyancy regulation. Analogous findings on metalimnetic adaptations are shown in the paper by Carraro et al. (this volume).

Adaptation to dim light was also investigated by Üveges et al. (this volume), who studied the photosynthetic characteristics and physiological plasticity of *Aphanizomenon flos-aquae* (L.) Ralfs blooming in winter under the ice cover of Lake Stechlin. The study underlined the importance of trade-off among different traits in Cyanobacteria. In particular, the authors showed that temperature optimum for growth depended on light intensity. At low light (7.5 μmol m^{-2} s^{-1}), the temperature optimum was found at 2°C, allowing *A. flos-aquae* to bloom under the ice cover.

Adaptation to low temperature implies the existence of peculiar metabolic pathways and a well-defined composition of metabolites. Flaim et al. (this volume) studied the changes in galactolipid composition of the cold-adapted dinoflagellate *Borghiella dodgei* Moestrup, Hansen & Daugbjerg in response to temperature. Galactolipids are a class of molecules which constitute one of the structural components of the thylakoid membrane in eukaryotic chloroplasts. As shown by the authors, the relative abundance of the different galactolipids changes in response to temperature in order to ensure to optimal fluidity to thylakoid membrane and to allow the correct functioning of the photosynthetic systems. This allows *Borghiella* to outcompete other algae when water temperature is between 3 and 5°C.

Stoyneva et al. (this volume) investigated the causes of the phenotypic variability of *Tetraëdron minimum* Kützing ex Korshikov isolated from Lake Kivu. In this lake, *T. minimum* was characterized by an unusual 'lemon shape' instead of the typical polygonal one. The authors carried out a detailed investigation on the morphological and ecological features of this species and suggested that the peculiar lemon shape of *T. minimum* represented a defense against grazing by small zooplankton. Actually larger zooplankton disappeared from the lake because of the introduction of a planktivorous fish; this caused a disproportionate increase of small-bodied herbivores and an increase in the grazing pressure on *T. minimum* which stimulated the occurrence of the 'lemon shape'.

Functional group approach

Increasing need for substitute traditional taxonomic approach to understand phytoplankton patterns has recently resulted in the emergence of three approaches of morpho-functional classifications (Reynolds et al., 2002; Salmaso & Padisák, 2007; Kruk et al., 2010). All these methods are in the focus of the papers in this volume. Here we partition the discussion on functional grouping to the following sections: notes on nomenclature, modifications in and additions to the three classifications, comparisons, use for diversity analysis of community structure and ecological status assessment.

Notes on the nomenclature

There is no written consensus on abbreviation of these functional approaches. In order to avoid the future confusion, the authors of this article advise to remain consequent to the following form:

- functional group concept by Reynolds et al. (2002)—FG
- morpho-functional group concept by Salmaso & Padisák (2007)—MFG and
- the morphology-based functional group concept by Kruk et al. (2010)—MBFG

For example, the MFG abbreviation in Stanković et al. (this volume) should be read as MBFG.

Modifications in and additions to the three methods

The FG method underwent a number of modifications earlier [see summary by Padisák et al., (2009)].

Concerning the MFG, Tolotti et al. (this volume) suggested to include another category (6c-ColoPenn) into the original group 6 (large diatoms) for chain-forming pennatae species since nutrient and climate-related long-term changes in phytoplankton of the Piburger See, Austria, was possible to handle only with the separation of large Pennales into chain-forming and single-celled species. This addition seems very reasonable since shape, size and complexity (and even symmetry) highly determine competitive performance of species (Padisák et al., 2003; Naselli-Flores et al., 2007; Naselli-Flores & Barone, 2011).

With detailed statistical analyses on a database comprising data of 711 species from 925 samples taken in 211 lakes, Kruk & Segura (this volume) clarified some environmental variables as main driving forces leading to dominance of MBFGs. They found that for Group I (small organisms with high S/V), TP and TN were the most important variables. For Group II (small flagellated organisms with siliceous exoskeletal structures), no clear environmental factors could be selected because of low-explained variation. Probably the group is too complex, because, for example, the *Dinobryon* visualized on Fig. 1 in the paper by Kruk & Segura (this volume), though have loricae, is not a siliceous flagellate. For Group III (large filaments with aerotopes), light attenuation and TP proved to be the main drivers. According to these authors, TN and TZ (total

zooplankton) were equally important for Groups IV (organisms of medium size lacking specialized traits), V (unicellular flagellates) and VI (non-flagellated organisms with siliceous exoskeletons), additionally temperature proved to be an important driver for both Groups V and VI. SRSi as a main explanatory variable for group VII (large colonies with mucilage) was supposed to be, as pointed out by the authors clearly, a proxy of catchment properties since these species do not have direct silica demand.

Comparison of approaches

So far, only few papers addressed the comparison of the above three grouping methods or at least their pair wise comparisons. Izaguirre et al. (this volume) applied all the three methods to phytoplankton of three different types of pampa lakes (clear vegetated, phytoplankton turbid and inorganically turbid). As a major result, they demonstrated that all the three classification systems separated the clear and the turbid lakes. One of the disadvantages of the MFBG approach with respect to the other two methods was its incapability of discrimination of potentially mixotrophic and non-mixotrophic flagellates having different representation in the studied pampa lakes (both in Group V). Though Kruk et al. (2011) argued that species in any particular group are basically interchangeable, this was not the case in this example and Kruk and Segura (this volume) also commented on this particular point of the MBFG grouping. The other weakness of the MBFG was its low sensitivity to light conditions (Kd_{PAR}) which (together with mixing depth) are key drivers of phytoplankton species selection in lakes (Zohary et al., 2010).

All the three grouping systems were developed for lakes, and in their original descriptions, application for river phytoplankton was either considered (FG; Reynolds et al., 2002) or at least not excluded (Salmaso & Padisák, 2007; Kruk et al., 2010). Stanković et al. (this volume) compared the FG and MFBG groupings on large lowland rivers. Since diatoms typically dominate in these ecosystems, the MFBG approach with its single group for all siliceous non-flagellated species failed to discriminate between eu- and tychoplanktonic diatoms which are crucial in evaluation of riverine phytoplankton (Borics et al., 2007) unlike the FG concept that allows clear discrimination.

Diversity approach

Phytoplankton diversity patterns along with their environmental drivers have been a recurrent research subject (Reynolds et al., 1993; Sommer et al., 1993). Recently, diversity is understood at a much more complex way than the simple compositional diversity as it was treated earlier. The morpho-functional approaches (any of the three methods) have the potential to assess functional diversity of phytoplankton as challenged by Borics et al. (this volume). The achievement of this approach has been manifold. These authors in accordance with what was found for the development of phytoplankton equilibrium patterns (Naselli-Flores et al., 2003) explored that in biomass ranges > 20 mg L^{-1} only some FGs (H1, S_N, M, W_S, J, Lo) were able to develop dominance, and if so, a sharp decline in diversity can be predicted. The unusual behaviour of bloom-forming cyanobacteria (H1, S_N, M) was demonstrated by their 'deviant' behaviour from what can be theoretically expected: the maximum diversity occurs when relative abundance of these groups is zero. In other words, it means that even an occurrence of these species in the flora results in an immediate drop in diversity probably due to their invasive nature. They attributed this behaviour to the strong competitiveness of these species for light and to certain other features like (for W_S) mixotrophy. This study partly explains why just relationships to light and potential mixotrophy were identified as weakness of the MFBG approach (Izaguirre et al., this volume; Kruk & Segura, this volume).

Ecological status assessment

Of the three morpho/functional grouping methods, the FG was developed further to be used as a metrics in the Water Framework Directive (European Parliament and Council, 2000) by developing the Q (Padisák et al., 2006) and the Q_R (Borics et al., 2007) indices. Performance of the Q_R indices was tested annually and on whole river stretch scale on River Loire (Abonyi et al., this volume). Apart that the FG classification allowed following both seasonal and spatial changes in phytoplankton assemblages of River Loire, it proved to be suitable to detect interruptions of the river continuum (Vannote et al., 1980) by reservoirs and large inflows and, moreover, alterations at lower sections by non-point nutrient loads due to agricultural

land use. This analysis represents a significant addition to the River Continuum Concept.

Abonyi et al. (this volume) call the attention to some limitations of the Q_R index, namely that it does not discriminate between natural and human-affected benthic diatom dominance and the difficulty in sorting diatoms to B-C-D coda in monitoring practice. Implications for WFD are also mentioned in Borics et al. (this volume) emphasizing that the threshold value of 20 mg l^{-1} algal biomass indicated by diversity patterns corresponds to the poor ecological quality threshold for German shallow lakes (Mischke et al., 2002).

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