

Effects of nutrient availability and temperature on phytoplankton development: a case study from large lakes south of the Alps

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Abstract This work investigated the combined effects of nutrient availability and temperature on phytoplankton in large and deep lakes south of the Alps (lakes Garda, Iseo, Como, Lugano and Maggiore). The more eutrophic basins (Lugano and Iseo) showed a higher presence of cyanobacteria, green algae (Chlorophyta and Charophyta) and dinoflagellates (Dinophyta). Besides these two water bodies, high biomasses of cyanobacteria were recorded also in the oligo-mesotrophic Lake Garda. The development of these algal groups during the growing season showed a strong dependence on the surface spring availability of SRP, which, in turn, was related to winter climatic oscillations, deep mixing dynamics, and trophic status. A specific analysis carried out by applying additive mixed

modelling, generalized least squares and mixed modelling, allowed investigation of the direct, seasonal effects of water temperature variations and trophic status on different algal groups. The dominant cyanobacteria (Oscillatoriales) showed only a partial relationship with temperature, while Nostocales and Chroococcales, which did not appear to have a close relationship with the trophic status of the lakes, were characterised by abrupt increases during the warmer months. High positive relationships with temperature were found for a few other algal groups (e.g., Chlorophyta, Charophyta and Dinophyta). Overall, the results indicated a positive relationship between the seasonal development of the more abundant and eutrophic-sensitive algal groups and the concurrent effect of trophic status and water temperature. Nevertheless, it was stressed that specific differences could be interpreted taking into account the different autoecological characteristics and susceptibilities of different species and functional groups to other stressing factors favouring losses, including, e.g., vertical sinking and grazing.

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Introduction

Analyses of long-term datasets provide increasing evidence on the sensitivity of water bodies to nutrient concentrations and climatic fluctuations at different temporal scales. Particularly studied are the effects determined by increasing nutrient concentrations on primary production and on the composition, structure and dynamics of phytoplankton communities (Reynolds 2006; Schindler 2006; Staehr et al.

2011). In this regard, most investigations carried out worldwide in the last decades documented an increase in algal biomass and cyanobacteria in eutrophic environments (Padisák 2004; Istvánovics 2009).

Considering its role in multiple physical and biological processes in lakes, disentangling the direct and indirect effects of temperature on algal communities is difficult to address (Adrian et al. 2009). Nevertheless, the growing evidence confirming the existence of a gradual warming fostered an increasing number of studies about the effects of climate change on aquatic ecosystems and at different levels of organisation (Blenckner et al. 2002; Dokulil et al. 2006; Keller 2007; Hsieh et al. 2010). Water temperature directly controls the replication rates of planktonic organisms. Indirect effects are connected with changes in the timing of ice breakup, water turbulence intensity, viscosity and thermal stratification, with intense consequences on the vertical distribution of nutrients, the phenology of planktonic organisms and multiple biotic interactions (Winder and Schindler 2004; Nöges et al. 2010). In addition, the effects of water temperature fluctuations are strongly mediated by the physiographic characteristics of water bodies. In deep lacustrine ecosystems of the temperate regions, winter climatic fluctuations can strongly influence the development of algal biomass during the summer months by controlling the extent of lake overturn and the extent of surface nutrient enrichment during the late winter and early spring months (Salmaso 2010).

Due to their negative effects on water quality and exploitation, the effects of thermal fluctuations and trends were particularly studied in cyanobacteria (Paul 2008). Many recent investigations supported the view that an increase of nuisance cyanobacterial blooms could be linked—besides eutrophication—to recent warming trends (Weyhenmeyer 2001; Paerl and Huisman 2008; Wagner and Adrian 2009). In warmer environments, cyanobacteria can take advantage of their ability to grow faster at higher temperatures, developing massive populations in stratified, stable environments (De Senerpont Domis et al. 2007; Jöhnk et al. 2008). On the other hand, phytoplankton community models used to test the effects of elevated temperatures and increased nutrient loads on phytoplankton succession and productivity predicted a greater impact of nutrient loading over temperature, and a large dominance of cyanobacteria when high water temperatures were combined with high nutrient loads (Elliott et al. 2006). These results were consistent with the growing number of observations reporting an increase of algal blooms in nutrient-enriched environments. Climatic change may benefit various species of cyanobacteria by increasing their growth rates, dominance and geographic distributions (Paerl and Huisman 2009). In turn, this could suggest a considerable reduction in the effect of water temperature

change on phytoplankton communities at low nutrient levels. In deep lakes, the importance of considering nutrient conditions when analyzing the mechanisms underlying the effect of global warming on phytoplankton productivity was recently highlighted by Tadolnéké (2010).

The aim of this paper is to study the effects of water temperature fluctuations and trophic status on the development of phytoplankton in a group of large and deep lakes located at the southern margin of the Alps, namely, from east to west, lakes Garda, Iseo, Como, Lugano and Maggiore (hereafter, DSL, deep southern subalpine lakes). Data will be analysed taking explicitly into account the effects of both lakes (trophic status) and water temperature, by using different models, including additive models, generalised least squares, and mixed models. Specific objectives are (i) to identify the net effects of temperature fluctuations on the seasonal development of the principal groups of cyanobacteria and eukaryotic phytoplankton along a trophic state gradient of large lakes south of the Alps; (ii) to verify the type of temperature-biomass relationships characterizing the different algal groups; and (iii) to identify the most temperature- and eutrophic-sensitive algal groups. The results will be discussed critically in light of the role played by other abiotic and biotic explanatory variables.

Methods

Study sites

The largest lakes south of the Alps are narrow, elongated and delimited laterally by steep sides. Their bottom is generally flat. Lake Garda is the largest Italian lake, followed by lakes Maggiore, Como and Iseo (Table 1). Lake Lugano stands out for its subdivision into three basins. The northern basin is the largest of the three, accounting for around 80% of the total water volume. The maximum depths of the DSL range between 251 m (Lake Iseo) and 410 m (Lake Como). Theoretical renewal times are around 4–5 years (lakes Maggiore, Como and Iseo), 12 years (Lake Lugano, N-basin) and 27 years (Lake Garda). These water bodies contribute about 40% to the discharge of the River Po, the largest Italian river, and constitute more than 80% of the surface water resources in Italy. The altitude of the deep southern subalpine lakes ranges between 65 m (Garda) and 271 m (Lugano); lakes Iseo, Maggiore and Como are located at intermediate altitudes, between 186 and 198 m.

After the increase of nutrients observed in the 1960s, these lakes showed a complete recovery (Maggiore), a tendency to a decrease (Como, Lugano) or to a stabilization of nutrients (Garda, Iseo). Despite these signs of recovery, these lakes showed an increase in the appearance of new

Table 1 Morphometric and hydrological characteristics of deep southern subalpine lakes (DSL) in Northern Italy

	Garda	Iseo	Como	Lugano ^a	Maggiore
Latitude N	45°42'	45°44'	46°00'	45°58'	45°58'
Longitude E	10°43'	10°04'	9°15'	8°59'	8°39'
Altitude (m a.s.l.)	65	186	198	271	193
Area (km ²)	368	62	146	28	213
Maximum depth (m)	350	251	410	288	370
Mean depth (m)	133	123	154	171	178
Volume (km ³)	49.03	7.57	22.5	4.69	37.5
Catchment area (km ²)	2,290	1,842	4,508	297	6,599
Mean outflow discharge (m ³ s ⁻¹)	58.4	58.7	158.0	12.0	291.3
Renewal time (years)	26.6	4.1	4.5	12.4	4.1

^a Northern basin

cyanobacterial species and algal blooms (Salmaso 2010; Salmaso and Mosello 2010).

Deep lakes south of the Alps could be classified as warm monomictic lakes. In this category, lakes circulate completely once a year in the winter at or above 4°C, showing stable stratification for the rest of the year. Owing to their great depth, complete mixing occurs only during cold winters when the water column undergoes a complete cooling. This intermediate condition (oligomixis) is different from that of holomictic or meromictic lakes, which circulate freely every year or show a constant stratification, respectively. Therefore, in this typology of lakes, replenishment of phosphorus from the deeper to the surface layers during the period of maximum overturn is essential to assure a pool of nutrients that will be utilized by phytoplankton communities during the growing period. In this regard, a linked and ordered chain of causal factors including large-scale atmospheric patterns over the Atlantic and the Mediterranean regions, the winter air and spring water temperature, the extent of the spring lake overturn, and the extent of nutrient enrichment has important effects on the development of phytoplankton biomass and selected algal groups (Salmaso 2012). In contrast, the formation of a strong thermocline during the warmest period isolates large epilimnetic portions for many months, suppressing vertical mixing and inhibiting the transport of nutrients towards the trophogenic layers.

Sampling and laboratory analyses

Field measurements and water sampling were carried out between 2004 and 2006. The sampling frequency varied between 15 days and 1 month. To allow comparison of the data in the different lakes and years, analyses were carried out on a starting matrix of monthly averages (12 observations per year) of both environmental and biological data (see, e.g., Winder and Hunter 2008; Salmaso 2010). The ultimate matrices were complete (36 months) for lakes Garda, Lugano and Maggiore. Data for the months of

December 2005, and September and November 2006 were not available for Lake Como. As for Lake Iseo, data were not available in 2004 (Jan, Sep, Nov), 2005 (Jan, Feb, Oct) and 2006 (Jan, Jun, Sep). Therefore, annual or seasonal averages for Lake Iseo are approximate estimates. Nevertheless, the use of a more complete dataset, with missing data filled by values obtained by interpolation with linear regressions or by averaging data in adjacent months, provided practically coincident results in the computation and use of annual/seasonal averages reported in Figs. 1, 2, 3 and Table 2.

In the northern basin of Lake Lugano and in lakes Garda, Iseo and Maggiore, the sampling stations were located at the deepest point of the basins, whereas sampling in Lake Como was performed out of Dervio, where maximum depths are between 200 and 250 m. In the epilimnetic layers, sampling in lakes Lugano and Maggiore were carried out between 0 and 20 m with bottles designed to take integrated samples (Morabito et al. 2003; Simona 2003). In the same layer (0–20 m), average values of chemical variables and phytoplankton abundance in lakes Garda, Iseo and Como were estimated from samples collected at discrete depths (see details in Buzzi 2002; Salmaso 2000; Garibaldi et al. 2003). Laboratory analyses followed comparable and standardised protocols. The chemical determinations of total and soluble reactive phosphorus (TP and SRP), dissolved inorganic nitrogen (DIN, NO₃-N + NO₂-N + NH₄-N) and reactive silica (Si) were carried out following standard methods (APHA et al. 1995) and the results checked by regular intercalibration exercises among the laboratories.

As a rough indication of potential nutrient limitation, we considered the limiting levels discussed by Wehr and Descy (1998) and Reynolds (2006): SRP, 3–10 µg P L⁻¹; DIN, 15–30 µg N L⁻¹; reactive silica, 0.1–0.5 mg Si L⁻¹. In addition, potential limitation was assessed by computing atomic N:P ratios (based on DIN and SRP concentrations). Both ambient nutrient concentrations and deviations from the optimal Redfield ratio N:P = 16:1 (Stumm and Morgan

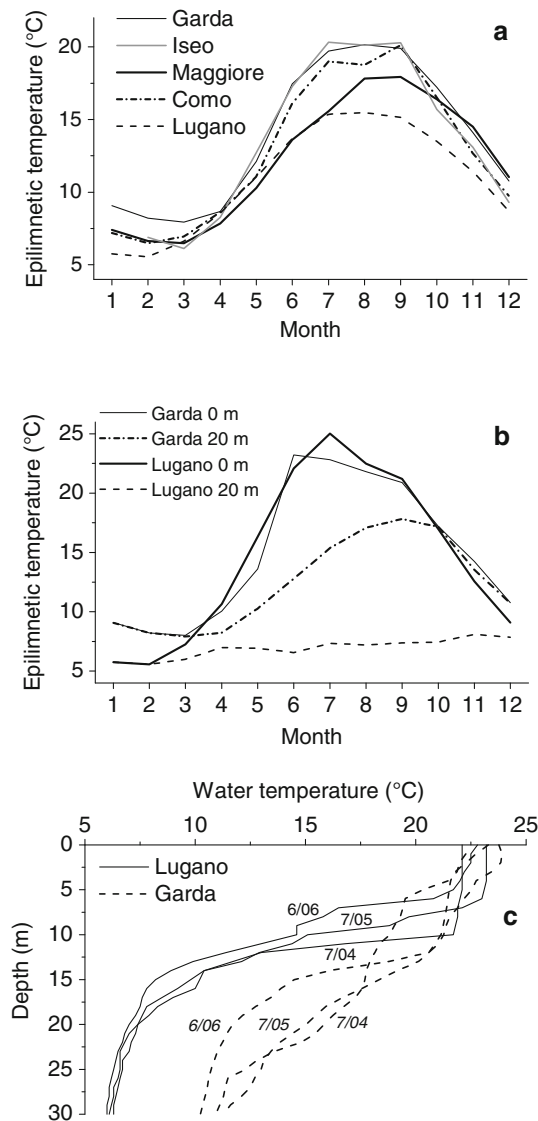


Fig. 1 **a** Monthly mean values (2004–2006) of epilimnetic (0–20 m) water temperature; in the legend, the five lakes have been ranked according to their altitudinal location (Table 1). **b** Monthly mean (2004–2006) water temperature values measured at the surface and at 20 m in the two lakes located at the opposite sides of the altitudinal gradient (Table 1: lakes Garda and Lugano). **c** Vertical profiles of water temperatures measured in single selected dates in July 2004–2005 and June 2006 in lakes Garda and Lugano

1996) were taken into consideration to assess potential phytoplankton growth limitation.

Chlorophyll-*a* concentrations were corrected by pheopigments. Phytoplankton counts were performed on samples fixed by acetic Lugol's solution following the Utermöhl method (Rott et al. 2007). Species identification and nomenclature followed the more recent monographs of the series Süßwasserflora von Mitteleuropa, established by A. Pascher (Gustav Fisher Verlag, and Elsevier, Spectrum Akademischer Verlag), specific manuals of the series Das

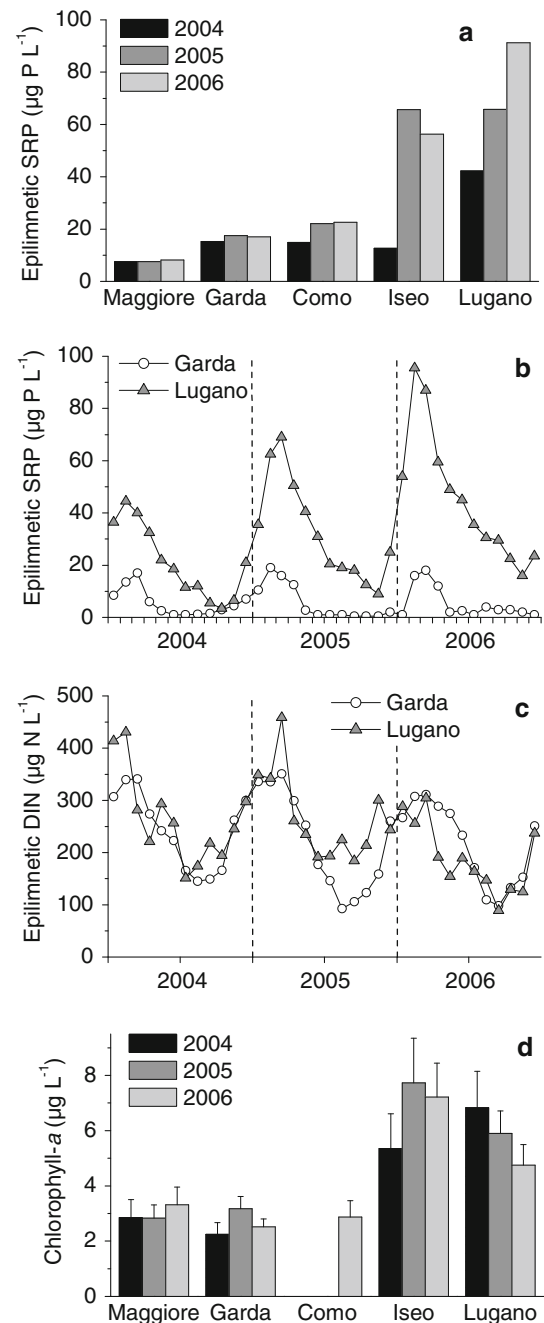


Fig. 2 **a** Mean concentrations of soluble reactive phosphorus at spring overturn (February–March) in the five lakes from 2004 to 2006. Monthly development of **(b)** SRP and **(c)** DIN from 2004 to 2006 in lakes Garda and Lugano. **(d)** Annual mean concentrations of chlorophyll-*a* from 2004 to 2006; the vertical bars indicate the standard errors of the means. Data refer to the layer 0–20 m

Phytoplankton des Süßwassers, established by G. Huber-Pestalozzi (E. Schweizerbart'sche Verlagsbuchhandlung), and specific papers (e.g., Wacklin et al. 2009). Before comparison, the phytoplankton data recorded in the study lakes were checked for taxonomical consistency. The definition of major groups of eukaryotic algae is coherent

Fig. 3 a–j Average values of the biovolumes ($\text{mm}^3 \text{m}^{-3}$) of the principal taxonomic algal groups in the deep southern subalpine lakes computed for the period 2004–2006. The vertical bars indicate the standard errors of the means. Note that graphs have different Y-scale ranges. *Black* and *gray* columns highlight the contribution of cyanobacteria and “green algae”, respectively. **k** Percentage contribution of the phytoplankton groups to biovolume

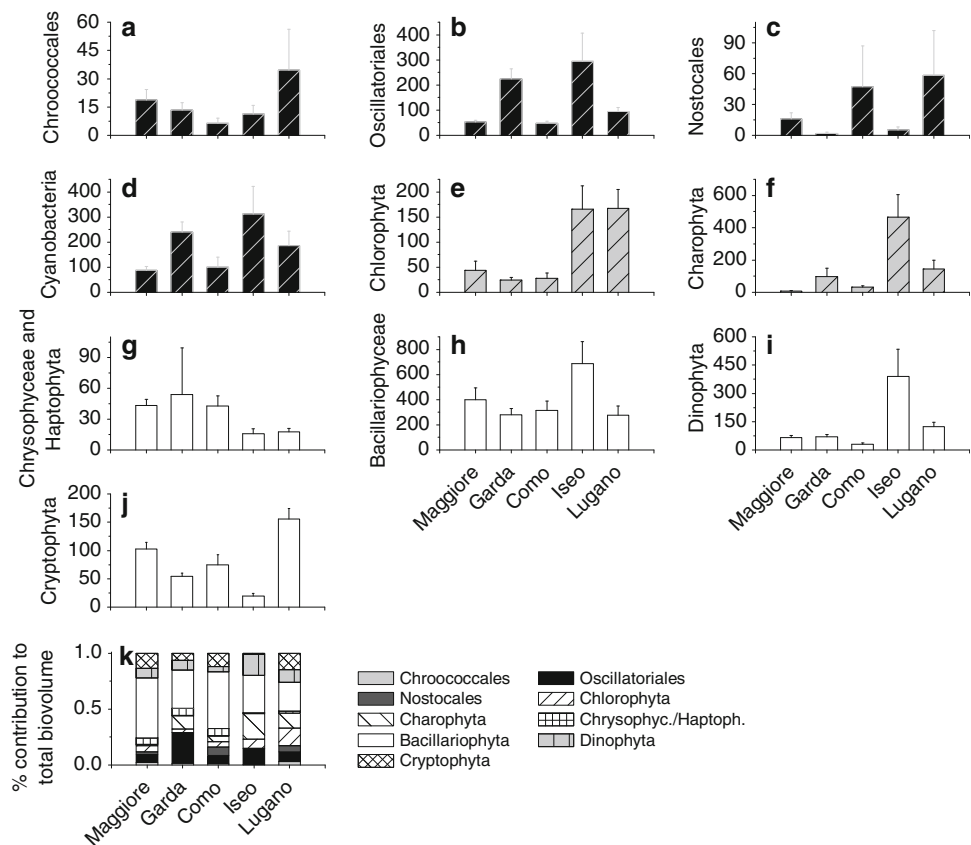


Table 2 Linear regressions between mean epilimnetic soluble reactive phosphorus concentrations at maximum spring overturn (February–March) and mean biovolumes of algal groups from late spring to autumn (June–December)

	Regression slopes	r^2
Cyanobacteria	1.22	0.27*
Chroococcales	−0.30	0.01
Oscillatoriales	1.30	0.24 ⁺
Nostocales	−0.04	0.00
Chlorophyta	2.27	0.53**
Charophyta	3.01	0.43**
Chrysophyceae and Haptophyta	−1.06	0.16
Dinophyta	0.89	0.13
Bacillariophyceae	−0.65	0.09
Cryptophyta	−0.59	0.06

Regressions were computed on $\log_e(\text{SRP})$ and $\log_e(\text{biovolume} + 1)$ transformed data. $n = 15$. Significance codes: ** $p < 0.01$, * $p < 0.05$, ⁺ $p < 0.1$

with the recent status in the systematic described by Krienitz (2009). In this paper, we considered separately the contribution of Chrysophyceae, Bacillariophyceae and Xanthophyceae (which are included in the Division Heterokontophyta). Due to their very low occurrences, Xanthophyceae (*Tribonema*) were not considered in the

analyses. Nomenclature and subdivisions in cyanobacteria followed the criteria defined by Komárek and Anagnostidis (2005).

A detailed account of the methods were reported by Salmaso (2000), Buzzi (2002), Garibaldi et al. (2003), Morabito et al. (2003), Simona (2003) and Salmaso et al. (2006).

Data analysis

In large and deep lakes, trying to correlate algal biomasses and nutrients over the whole temporal gradient is nonsense due to a changing role of the two variables in different seasonal periods. The variable “nutrients” at the beginning and during the seasonal growth period gradually changes its nature, from explanatory to a response variable, due to its consumption by the phytoplankton community. Therefore, in this work, the relationships between nutrients and phytoplankton were examined by relating the availability of nutrients at spring overturn and the average development of selected algal groups during the growing season. This approach agrees with the findings of Dillon and Rigler (1974), who found a significant relationship between TP concentrations from spring turnover and the mean summer values of Chl *a* in a large number of lakes. In this respect, after linearization of the data by $\log_e$ -transformation, the

single annual observations representing the mean biovolume values of phytoplankton groups in the growing season were regressed against the epilimnetic concentration of nutrients recorded at spring overturn of the same limnological year.

In the following models (1–3), the effects due to temperature were examined by relating the variations of water temperatures and the biovolumes of each taxonomic group in the trophogenic layers (0–20 m). Responses of phytoplankton groups were first modelled using generalised additive models (GAMs). This technique allows the identification of different types of relationships between variables, including nonlinear ones. Therefore, it can be used to identify the type of model required. GAMs allow non-linear regressions to be fitted to the data, based on specific smoothing splines. If GAM indicates that the smoother is a straight line, than the application of a linear regression model is correct (Zuur et al. 2009). To test the effects of trophic status on the above relationships, GAM models included also a categorical variable “lake”,

$$Phyto_{ij} = \alpha_j + f_j(Temperature_i) + \varepsilon_{ij} \quad (1)$$

where i and j are indices for the observations (monthly biovolumes of selected taxa and monthly water temperatures) and the five lakes, respectively, and f_j the smoothing function based on cubic regression spline. With this approach, a graph is needed to visualise the function f . In fact, contrary to usual regression models, in GAM we do not have an equation, but a smoother, which can be evaluated by plotting (Zuur et al. 2009). For each algal group, different smoothers for the five lakes (or different intercepts in the case of one smoother) were included only if significant.

Estimation of parameters in linear models was carried out by applying generalised least squares models (GLS; Pinheiro and Bates 2000). GLS allows estimation of unknown parameters in linear regression models even when assumptions are violated; i.e. when the variances of the observations are unequal or when the data have a certain degree of correlation (Zuur et al. 2009; see below). In this work, GLS models were applied using lake (a proxy for trophic status) as a categorical variable, water temperature as a continuous explanatory variable, and a lake-temperature interaction term:

$$Phyto_{ij} = \alpha_j + \beta_j \times Temperature_{ij} + \varepsilon_{ij} \quad (2)$$

where i and j have the same meaning as in equation (1). In the different models, different intercepts and slopes were included only if significant. The total number of parameters in this model adds up to 11 (a slope and intercept per lake, plus one noise variance term).

The inclusion of both years and lakes effects in model (2) would have implicated the estimation of up to 15

intercepts and 15 different slopes. If we disregard the exact effects of lakes and years, but want to take their variance into consideration within the models, then the use of mixed models can represent an effective solution (Zuur et al. 2009). Taking into consideration the effect due to different lakes and years, the 15 levels reflect the effects of the different spring SRP levels and other environmental and biotic differences (random effects) recorded in the five lakes and 3 years. Effects of water temperature and lakes were analysed by applying mixed effects modelling with random intercepts (Pinheiro and Bates 2000; Zuur et al. 2007),

$$Phyto_{ik} = \alpha + \beta \times Temperature_{ik} + a_k + \varepsilon_{ik} \quad (3)$$

In Eq. (3) the index i represents samples in a lake, while k (which takes values from 1 to 15) represents the five lakes in the three considered years. a_k represents the variation around the fixed intercept α at each lake/year level. With this technique, biovolumes of the single algal groups are modelled as a linear function of water temperature where the intercept is allowed to change per lake. With model (3) only four parameters are estimated (α , β , the variance of the random intercept a_k , and the variance of the noise). Temperature is used as a fixed effect (i.e. an informative factor level to be estimated from the data), whereas lakes in the 3 years (15 levels) are used as a random effect, assuming that the variation around the intercept, for each level, is normally distributed with a certain variance. A small variance (column “Random effects” in Table 4) means that differences per level (in terms of the intercept) are small, whereas a large variance allows for more variation (Zuur et al. 2009). We did not complicate further the model including separately “lake” and “year” effects due to the low number of levels (5 and 3, respectively), which were poorly suitable for a reliable estimate of variances.

Residuals were graphically examined for evidence of departures from normality, homogeneity and dependence. Due to their large variability, and before analysis, the biovolumes of the taxonomic groups were transformed by natural logarithm, $Y_i = \log_e(X_i + 1)$. If a temporal autocorrelation was identified between the residuals, an autoregressive process of order 1 was included in the models 1–3. Moreover, models were allowed to include different residual variations for the different lakes, therefore taking into account the existence of possible heterogeneity of variances among lakes. Selection of models based on Eqs. 1–3 and the inclusion of residual autocorrelation and variance structures was evaluated based on AIC (Akaike Information Criterion) values and ANOVA tests (Zuur et al. 2007, 2009).

Paired t-tests were performed to assess differences between the epilimnetic temperatures (Sokal and Rohlf 1995).

Statistical analyses were carried out with R version 2.11.0 (R Development Core Team 2010), and, in particular, with the “mgcv” (GAM procedures; Wood 2004) and “nlme” (regression analyses, GLS and mixed models; Pinheiro and Bates 2000) packages. As for GAM, “mgcv” package was used because it can perform cross-validation (allowing automatic determination of the optimal amount of cubic spline smoothing) and—among the others— inclusion of residual temporal correlations and variance structures.

Results

Water temperature

The lakes considered in this work showed strong differences in average epilimnetic temperatures (Fig. 1a). Differences were particularly marked during the warmest period when temperatures decreased, roughly following an altitudinal gradient from Lake Garda (65 m) to Lake Lugano (271 m). In the whole period, the main differences were between Lake Lugano and the other lakes (paired *t*-test, $0.000 < p < 0.015$), and between Lake Garda and lakes Maggiore and Como ($p < 0.001$). Though not significant, due to the low number of observations, averages of the summer (Jul–Sep) epilimnetic water temperatures of the 5 lakes over the three study years ($n = 5$) (range 17.1–19.9°C) showed a negative, linear dependence from lake altitude ($r^2 = 0.59$), with a mean decrease of 2°C every 100 m.

The low epilimnetic temperatures observed in Lake Lugano were due to the existence of very steep vertical thermal gradients in the summer period, up to 3.8°C m⁻¹ (2004), 3.7°C m⁻¹ (2005) and 1.6°C m⁻¹ (2006) in the layer between 10 and 15 m. In addition to lower values at the surface, water temperatures in Lake Lugano at 20 m were systematically lower than in the lower altitude Lake Garda, with maximum differences between 9 and 13°C in the summer months (Fig. 1b, c). The thermal structure of Lake Lugano in the warmest months was characterised by a distinct epilimnion (5–10 m in size) and by a metalimnion ranging from 5–10 m to 15–16 m. In contrast, at the opposite side of the altitudinal gradient, the thermal structure in Lake Garda was characterised by less marked discontinuities, and by the formation of a more extended metalimnion with maximum thermal gradients around 1°C m⁻¹ between 20 and 25 m (2004), and 10 and 20 m (2005–2006). Between July and September, average water temperatures in the layers 0–10 and 11–20 m in the three study years were 21.1–21.6 and 9.7–10.6 (Lugano), and 21.0–21.4 and 17.2–17.7°C (Garda), respectively. In addition, note (see “Discussion”) that in 2005 and 2006 the

mean epilimnetic (0–20) water temperatures in Lake Lugano during the period of maximum algal growth (summer and autumn) were considerably lower (9.7 and 9.4°C) than those measured in 2004 (14.3°C).

The average minimum epilimnetic water temperatures (February–March) in the five lakes over the three study years had a less ample range (6.1–8.1°C), showing a close negative, linear relationship with lake altitude ($r^2 = 0.89$, $n = 5$, $p < 0.05$), with a mean decrease of 1°C every 100 m. In the spring period, Lake Garda showed complete overturn in the three study years. In lakes Como, Iseo and Lugano complete spring circulation was observed in 2005 and 2006, whereas Lake Maggiore underwent complete overturn only in 2006.

Algal nutrients and biomass

In Fig. 2a, the five lakes were ranked according to their concentrations of SRP at maximum spring overturn. Lake Maggiore showed the lowest concentrations of phosphorus (<10 µg P L⁻¹), followed by lakes Garda and Como (<18 and <23 µg P L⁻¹, respectively). The higher concentrations of SRP (up to 66 and 91 µg P L⁻¹, respectively) in lakes Iseo and Lugano were associated with a higher year-to-year variability. In the biennium 2005–2006, SRP concentrations in these two lakes were around 4–5 and 1.5–2 times higher than in the preceding year (Fig. 2a). The increase of SRP in 2005 and 2006 was favoured by a greater replenishment of nutrients from the deep hypolimnion to the epilimnetic layers caused by complete overturn. Though characterised by minor concentrations, the effect was measurable also in Lake Como. Fertilisation of surface waters due to spring mixing in the DSL was the object of numerous studies. Among others, case studies (including 2005–2006) were discussed in Salmaso et al. (2006) and Salmaso and Mosello (2010), and references therein. Nevertheless, variations in the P-content in the epilimnetic layers also originated from the contribution of nutrients from rivers (not estimated), so that the fraction of phosphorus due to spring lake circulation cannot be clearly inferred. Overall, the temporal development of SRP followed a clear seasonal pattern every year. Two contrasting cases in Lake Garda (oligo-mesotrophic) and Lake Lugano (meso-eutrophic) are reported in Fig. 2b. Contrary to Lake Garda, the more enriched lake showed measurable concentrations of SRP also during the summer months, especially in years characterised by complete spring overturn and nutrient replenishment (2005–2006). In the remaining lakes, ranges of SRP (µg P L⁻¹) over the whole study period were 2–9 (Maggiore), 3–24 (Como), and 2–66 (Iseo).

The above results were confirmed taking into consideration total phosphorus. Annual averages, and ranges of TP concentrations (µg P L⁻¹) over the whole study period (in

brackets), were 8 (6–11) (Maggiore), 13 (7–25) (Garda), 16 (6–29) (Como), 24 (8–72) (Iseo), and 55 (14–110) (Lugano).

Annual average concentrations (2004–2006) of DIN were around 460–800 $\mu\text{g N L}^{-1}$ (Maggiore, Como and Iseo), and 190–270 $\mu\text{g N L}^{-1}$ (Garda and Lugano). During summer, epilimnetic DIN concentrations in lakes Garda and Lugano reached the lowest values (90–190 $\mu\text{g N L}^{-1}$) (Fig. 2c). In contrast, in the remaining lakes, minimum DIN concentrations were always greater than 200 $\mu\text{g N L}^{-1}$ (Iseo) and 600 $\mu\text{g N L}^{-1}$ (Maggiore, Como). Contrary to SRP (Fig. 2a), lakes Iseo and Lugano, after the complete spring overturn in 2005 and 2006, did not show a greater replenishment of DIN to the epilimnetic layers (cf. Fig. 2c).

N:P ratios in lakes Maggiore (>200), Como (60–750), Garda (38–700) and Iseo (21–400) were always >16. In contrast, in the spring months (2004–2005) and from winter to early autumn (2006), Lake Lugano showed N:P ratios <16 (namely, between 6 and 15); in the remaining period, N:P ratios were between 17 and 121. Overall, in decreasing order, average N:P ratios in the five lakes were 751 (Maggiore), 340 (Como), 224 (Garda), 216 (Iseo) and 26 (Lugano).

The lower concentrations of silica were measured in lakes Lugano and Garda. Annual average concentrations in these two lakes were 0.46 and 0.64 mg Si L^{-1} , respectively, while in the remaining lakes average values ranged between 0.72 and 0.98 mg Si L^{-1} . The lowest summer concentrations of silica were around 0.20 mg Si L^{-1} (Garda), 0.25 mg Si L^{-1} (Lugano) and 0.35 mg Si L^{-1} (Iseo, Como and Maggiore).

The higher trophic status of lakes Iseo and Lugano was confirmed by their higher annual average concentrations of chlorophyll-*a* (4.7–7.9 $\mu\text{g L}^{-1}$) compared with the remaining water bodies (2.2–3.3 $\mu\text{g L}^{-1}$) (Fig. 2d). In these two lakes, a positive effect of the greater replenishment of phosphorus on chlorophyll-*a* was observed in Lake Iseo.

Taxonomic groups and impact of nutrient availability

In Fig. 3a–j, the single lakes were ordered as in Fig. 2a, i.e. along a trophic gradient. Cyanobacteria showed a larger development in the two more eutrophic lakes (Lugano and Iseo) and in Lake Garda. This pattern was determined by the distribution of the more abundant cyanobacteria, i.e. Oscillatoriales (mainly *Planktothrix rubescens* followed by *Planktolyngbya limnetica* and *Limnothrix* spp.). The less abundant cyanobacteria—i.e. Chroococcales (mainly *Aphanocapsa/Aphanothece*, Gomphosphaerioidae) and Nostocales (*Dolichospermum lemmermannii* in lakes Garda, Iseo, Como and Maggiore, and *Aphanizomenon flos-aquae* in Lake Lugano)—showed no clear relationship with trophic status. The groups that showed a larger

development in the more eutrophic lakes were Chlorophyta (many colonial Chlorococcales, also with mucilaginous envelopes) and, to a lesser extent, Charophyta (conjugates: *Mougeotia* sp., *Closterium aciculare* and *Staurostrum* spp.) and Dinophyta (*Ceratium hirundinella*, *Peridinium* spp., and *Gymnodinium helveticum*) (see also Fig. 3k). At the other extreme, Chrysophyceae and Haptophyta (*Dinobryon* spp., *Mallomonas* spp., small Ochromonadaceae, *Chrys-ochromulina parva*) showed a clear tendency to decrease from the oligotrophic to the more eutrophic lakes. Bacillariophyceae (among others, *Asterionella formosa*, *Fragilaria crotonensis*, *Aulacoseira* spp. and *Cyclotella* spp.) decreased their relative importance (Fig. 3k) in the more eutrophic lakes, whereas Cryptophyta (mostly *Cryptomonas* spp. and *Plagioselmis nannoplantica*) showed a more scattered distribution.

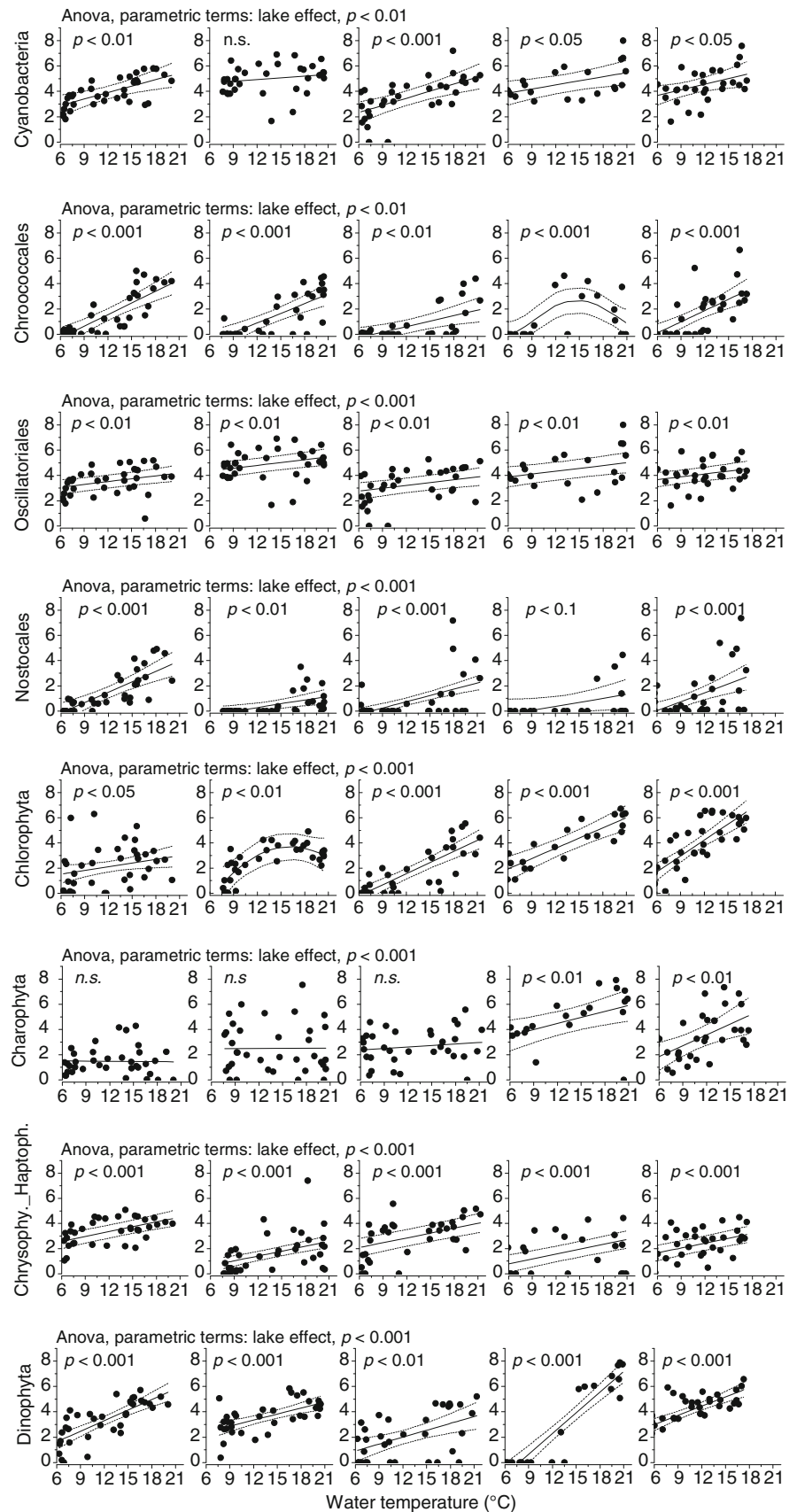
Identification of periods most affected by spring fertilization was performed by relating epilimnetic spring (February–March) nutrient concentrations and the biovolumes of the algal groups in different time intervals. Overall, the most informative results indicated a strong and positive impact of spring fertilisation of SRP on the average development of cyanobacteria and green algae (Chlorophyta and Charophyta) between late spring and autumn (Table 2). Among cyanobacteria, taxa that responded better to the spring replenishment of SRP were represented by the Oscillatoriales ($p = 0.06$; Table 2) and *Planktothrix rubescens* ($r^2 = 0.40$, $p = 0.01$). Among nutrients at spring overturn, DIN and Si showed no positive influence on the development of phytoplankton during the spring period and in the successive growing seasons.

Temperature effects on phytoplankton development along the trophic state gradient

With a few exceptions, the smoothers computed by GAM models relating water temperature and algal groups were straight lines (Fig. 4). Based on the AIC values, models included both different smoothers and intercepts in the different lakes, with the exclusion of Oscillatoriales and Chrysophyceae, where a model with a unique temperature smoother and different intercepts was identified. Positive relationships, significant ($p < 0.05$) in at least 4 lakes out of 5, were identified in the different groups of cyanobacteria, as well as in Chlorophyta, Dinophyta and Chrysophyceae. A significant effect by temperature in Charophyta was observed only in the two more eutrophic basins. Bacillariophyceae showed small and non-significant negative correlations with water temperature, whereas Cryptophyta showed positive relationships in lakes Garda and Como (data not shown).

Taking into consideration the transformation of the response variables, the linear, significant smoothers

Fig. 4 Smoothing curves obtained by applying the model (1) on $\log_e$ -transformed algal biovolumes ($\text{mm}^3 \text{m}^{-3}$). Dotted lines are 95% point-wise confidence bands. The graphs report the significance of differences between lakes, and the significance of smooth terms. For every algal group, from left to right, graphs refer to lakes Maggiore, Garda, Como, Iseo and Lugano, respectively ($n = 162$)



suggested the existence of exponential relationships between phytoplankton groups and water temperature:

$$(Phyto_i + 1) = a \cdot e^{b \cdot Temperature_i}$$

Estimation of the temperature slopes based on model (2) by GLS is reported in Table 3. Based on the AIC and ANOVA results, a number of algal groups showed, besides significant slopes, significant lake effects and lake $\times$ temperature effects (Nostocales, Chlorophyta, Charophyta, Dinophyta), whereas other groups included only different, significant intercepts (Cyanobacteria, Oscillatoriales, Chrysophyceae and Cryptophyta). As for Chroococcales and Bacillariophyceae, lake effects and lake $\times$ temperature effects were not significant. Confirming the results obtained by the application of GAM models, GLS highlighted positive and significant slopes between biomass and water temperature in all algal groups, with the exclusion of Bacillariophyceae, which showed a negative, though slight,

slope (-0.07 , Table 3). A few representative graphical solutions of the models obtained by GLS are reported in Fig. 5. In these models, the higher presence of Chlorophyta and Charophyta in the more eutrophic lakes (Iseo and Lugano) was well represented (see Fig. 3e, f). Similarly, the order of the regression lines in cyanobacteria and Dinophyta along the y-axes was consistent with the contribution of these algal groups in the different lakes (Fig. 3). In Table 3, a striking exception is due to the Nostocales, which, in line with Fig. 3c, showed temperature-biovolume slopes with values disconnected from the trophic status of the lakes. In this case, a lake effect cannot be interpreted in terms of differences due to nutrients.

The results of mixed models (fixed and random effects) are reported in Table 4. The standard deviations of the 15 levels determine the amount of variation around the population intercepts. Overall, the estimated standard deviation values showed a variation in intercepts a little lower or of

Table 3 Generalised Least Square modelling results for the DSL data (model 2)

	Temperature-phytoplankton relationships (F -values; phytoplankton-temperature slopes)	Anova—lake effect	Anova—lake: temperature interaction
Cyanobacteria	$F_{1,156} = 24.7***$; DSL, 0.12	$F_{4,156} = 2.7^*$	n.s.
Chroococcales	$F_{1,160} = 39.0***$; DSL, 0.18	n.s.	n.s.
Oscillatoriales	$F_{1,156} = 9.7**$; DSL, 0.07	$F_{4,156} = 4.1**$	n.s.
Nostocales	$F_{1,152} = 45.3***$; Mag, 0.27 Gar, 0.10** Com, 0.17 Ise, 0.11* Lug, 0.23	$F_{4,152} = 8.2***$	$F_{4,152} = 3^*$
Chlorophyta	$F_{1,152} = 94.6***$; Mag, 0.09 Gar, 0.16 Com, 0.30* Ise, 0.27 ⁺ Lug, 0.42**	$F_{4,152} = 11.5***$	$F_{4,152} = 3.3^*$
Charophyta	$F_{1,152} = 4.1^*$; Mag, -0.01 Gar, 0.01 Com, 0.04 Ise, 0.16 Lug, 0.28**	$F_{4,152} = 4.8**$	$F_{4,152} = 2.2^+$
Chrysophyceae and Haptophyta	$F_{1,156} = 20.5***$; DSL, 0.13	$F_{4,156} = 8.1***$	n.s.
Dinophyta	$F_{1,152} = 124.8***$ Mag, 0.27 Gar, 0.16 ⁺ Com, 0.19 Ise, 0.54*** Lug, 0.24	$F_{4,152} = 14.9***$	$F_{4,152} = 8.4***$
Bacillariophyceae	$F_{1,160} = 5.2^*$; DSL, -0.07	n.s.	n.s.
Cryptophyta	$F_{1,156} = 5.1^*$; DSL, 0.06	$F_{4,156} = 9.9***$	n.s.

Regressions were computed on $\log_e(\text{biovolume} + 1)$ transformed data. In the case of significant lake:temperature interaction effects, additional p -values in column 2 indicate whether the slopes of lakes Garda, Como, Iseo and Lugano are significantly different from the “baseline” level; i.e. the more oligotrophic Lake Maggiore. $n = 162$. Indices in F represent the usual degrees of freedom (Sokal and Rohlf 1995). Significance codes: *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, ⁺ $p < 0.1$

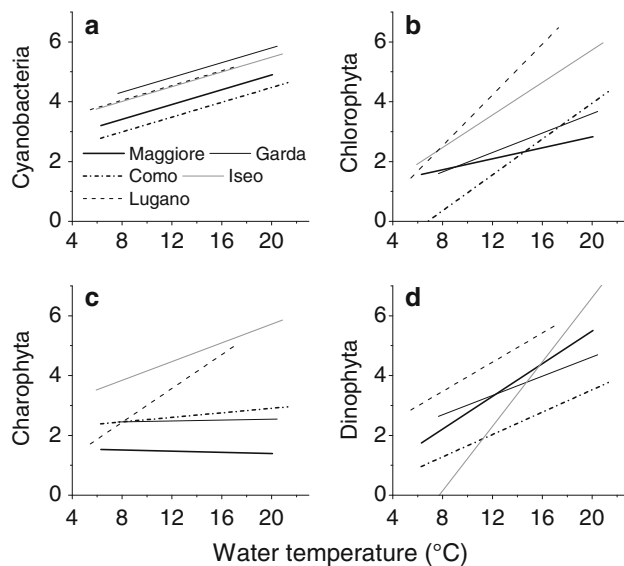


Fig. 5 Graphical solutions of the models obtained by GLS (Eq. 2 and Table 3) for the most abundant algal groups

the same order of magnitude as the residual variability, substantiating the effect of the different lakes and years on phytoplankton development. As for Chroococcales and Bacillariophyceae, final models coincided with GLS models (see Table 3; i.e. the inclusion of a random intercept did not improve the model). Overall, Dinophyta, Chlorophyta and Chroococcales exhibited the largest model slopes ($0.18\text{--}0.28\text{ mm}^3\text{ m}^{-3}\text{ }^{\circ}\text{C}^{-1}$, with biomasses on a $\log_e$ scale). The remaining groups had algal-temperature slopes ranging between 0.05 and $0.13\text{ mm}^3\text{ m}^{-3}\text{ }^{\circ}\text{C}^{-1}$, with the exclusion of diatoms, which showed a negative slope (Table 4).

The average combined effects of the availability of spring nutrient concentrations and water temperature on the

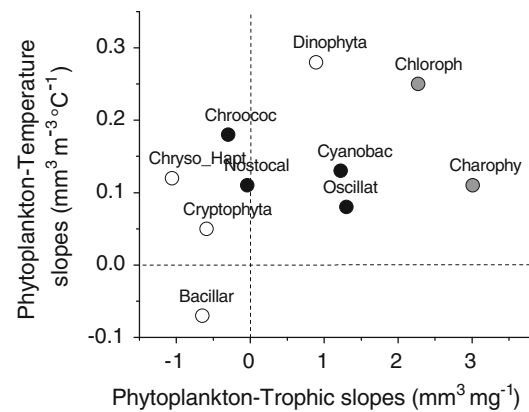


Fig. 6 Relationship between the trophic slopes and temperature slopes obtained from Tables 2 and 4, respectively. Black and gray circles highlight the contribution of cyanobacteria and “green algae”, respectively. The names of a few algal groups are abbreviated

development of phytoplankton groups were examined in Fig. 6. The group that showed the largest positive impact from P and temperature was represented by Chlorophyta. Dinophyta and Charophyta were positioned on the extreme side of the SRP and temperature gradient, respectively, while cyanobacteria (mainly with the Oscillatoriales) showed intermediate characteristics. Overall, the trophic and temperature slopes showed a positive ($r = 0.40$) though not significant relationship.

Discussion

The higher development of chlorophytes under higher phosphorus spring availability is consistent with the greater development of this group in nutrient enriched lakes (Jensen et al. 1994). The minor content of nutrients in large

Table 4 Mixed modelling results for the DSL data (model 3; mixed effects model with random intercept)

	Fixed effects Temperature-phytoplankton relationships (<i>F</i> -values; phytoplankton-temperature slopes)	Random effects Lakes in the 3 years; random intercept, Std. Dev.	Residual Std. Dev.
Cyanobacteria	$F_{1,146} = 31.2^{***}$; DSL, 0.13	0.60	1.06
Chroococcales	$F_{1,160} = 39.0^{***}$; DSL, 0.18	—	—
Oscillatoriales	$F_{1,146} = 9.7^{**}$; DSL, 0.08	0.72	0.78
Nostocales	$F_{1,146} = 16.5^{***}$; DSL, 0.11	0.23	0.22
Chlorophyta	$F_{1,146} = 62.9^{***}$; DSL, 0.25	0.82	1.39
Charophyta	$F_{1,146} = 7.2^{**}$; DSL, 0.11	0.48	0.88
Chrysophyceae and Haptophyta	$F_{1,146} = 29.2^{***}$; DSL, 0.13	0.90	1.18
Dinophyta	$F_{1,146} = 94.5^{***}$; DSL, 0.28	0.75	1.36
Bacillariophyceae	$F_{1,160} = 5.2^{*}$; DSL, -0.07	—	—
Cryptophyta	$F_{1,146} = 8.0^{**}$; DSL, 0.05	1.17	1.37

Regressions were computed on $\log_e(\text{biovolume} + 1)$ transformed data. Std. Dev., standard deviation. $n = 162$. Significance codes as in Table 3. As for the determination of the degrees of freedom in mixed modelling see Zuur et al. (2009). Explanations in the text

DSL contributed to maintain this broad group at low levels compared with smaller and more impacted systems. Dinoflagellates have an ample spectrum of tolerances. The tendency of this group to develop higher biomasses in lakes with higher SRP concentrations (Iseo and Lugano), appears consistent with the ability of taxa such as *Ceratium* and *Peridinium* to colonise summer epilimnia in mesotrophic and eutrophic lakes (Reynolds et al. 2002). The mixotrophic abilities of these phytoplankters allow them to utilize other food resources different from dissolved nutrients. In addition, these taxa are motile and so could also exploit higher nutrient concentrations in deeper waters via daily vertical migration.

Cyanobacteria, which are considered an algal proxy for eutrophication, exhibited a differentiated picture, with the more abundant group (Oscillatoriales) present, besides the two more eutrophic lakes, also in the oligo-mesotrophic Lake Garda. Considered separately, the three main cyanobacterial groups showed no clear relationship with trophic gradients. A few considerations about the autoecological characteristics of the dominant cyanobacterium in the DSL (*Planktothrix rubescens*) could help understanding these patterns. In many deep lakes at the northern and southern side of the Alps, *P. rubescens* is able to sustain massive summer production, forming thick metalimnetic layers as long as the light intensity is above the compensation point (Walsby et al. 1998; Salmaso 2000). This behaviour is favoured by the ability of this species to control vertical position in thermally stable environments and by its low light requirements (D'alelio et al. 2011). The decrease of this species with increasing eutrophication is due to an excessive darkening of metalimnia caused by algal shading (Lampert and Sommer 2007). The intensity of light saturation and light inhibition in *Planktothrix* was estimated around 10 and 130 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (Mur and Bejsdorf 1978). Net photosynthetic gain in this genus was estimated to be possible over about 3–4 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (Reynolds 2006). Considering that the 1% irradiance boundary is approximated no more closely than 2–20 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (Reynolds 2006), optimal depths for *Planktothrix* should be located around the lower limit of the euphotic zone. In the mid and late summer months, this limit in Lake Lugano extends up to around 7–12 m (Simona 2003), which corresponds to the depth of the steepest thermal gradient, immediately above the hypolimnion, therefore increasing the probability of this alga to experience low temperatures or to exit the metalimnion. In Lake Garda, euphotic depths in the summer months extends from 15 to 22 m (Salmaso 2000), corresponding to a zone with more relaxed temperature gradients and higher temperature than Lake Lugano.

In general, steep temperature gradients and lower mean water temperatures in the layers sampled in this work

(0–20 m) could contribute to explain the lower development of phytoplankton organisms (not only low-light tolerant taxa) in Lake Lugano compared to those expected under meso-eutrophic conditions. Despite the increase in the availability of spring SRP, the decrease in the annual mean concentrations of chlorophyll-*a* in 2005–2006 could have been favoured by low water temperatures (Figs. 1, 2). Another important factor is represented by the low N:P ratio, especially in 2005–2006. In Lake Lugano, DIN concentrations were always greater than 90–100 $\mu\text{g N L}^{-1}$ (Fig. 2c) i.e., according to Reynolds (2006), around 3–6 times greater than the limits below which phytoplankters may experience problems in obtaining sufficient N to half-saturate growth. Algal biomasses in lakes Maggiore, Como, Garda and Iseo were controlled by phosphorus, whereas the carrying capacity in Lake Lugano appeared to be controlled by both phosphorus and nitrogen. On the other side, the low N:P values in Lake Lugano were only partially matched by a higher development of cyanobacteria (Fig. 3). These considerations need to be interpreted with great care due to the high physiological variability in stoichiometric ratios in individual cells and taxa (Rhee and Gotham 1980).

Water temperature controls the replication rates of planktonic organisms (Eppley 1972). In culture conditions, and with saturating levels of nutrients and optimal light, growth rates (r , d^{-1}) increase exponentially from 0°C to just below the temperature of species-specific optima, which for most planktonic algae is between 20–25 and 35°C, but with temperature sensitivity of the division rate largely dissimilar among plankters (Padisák 2004). Many cyanobacteria develop massive populations at high temperatures, even in excess of 25°C (Coles and Jones 2000). This group is considered to perform better at elevated temperatures, competing most effectively with other algal groups. In fact, as the growth rates of eukaryotic taxa decline, cyanobacterial growth rates reach their optima (Elliott et al. 2006; Paerl and Huisman 2009). These specific characteristics are more efficiently exploited by those cyanobacteria that are not adapted to spend the summer months in colder and darkest metalimnetic layers, such as *Planktothrix* in Lake Lugano. Nevertheless, in field conditions, metabolic rates and primary production are under the control of both temperature and light. Contrary to light saturated photosynthesis, production rates of light-limited phytoplankton appear less responsive to temperature (Tilzer et al. 1986; Sommer and Lengfellner 2008). It was demonstrated how, under low water temperatures, both nutrients and radiant energy resources can only be utilised with low efficiencies (Tilzer et al. 1986).

The development of Nostocales and Chroococcales is ruled by different mechanisms. Both groups presented mean annual development 5–10 times lower than those

measured for the more abundant Oscillatoriales. In addition, these groups did not appear to have a close relationship with the trophic status of the lakes, nor with the variation in the spring availability of nutrients. As for Nostocales, *Dolichospermum lemmermannii* represents a case of very recent colonisation in the DSL. Apparently, irrespective of the trophic status, this species appeared for the first time in Lake Garda at the beginning of the 1990s in the form of extended blooms between July and September (Salmaso 2000). Since then, and following an altitudinal gradient, summer blooms of this species made their appearance in lakes Iseo (second half of the 1990s), Maggiore (2005), and Como (2006). Though characterised by high variability to temperature adaptation, being typical also of cold environments (e.g., Willén 2003), specific strains of *D. lemmermannii* show high temperature optima (i.e. between 19 and 26°C; Zapomělová et al. 2010) and have, in general, the capability to form huge water blooms in summer stratified conditions and during calm weather. In the epilimnetic layers of the DSL, this taxon generally showed high and abrupt relative increases in abundance at water temperatures higher than 16–17°C. Only in Lake Maggiore the increase appeared more regular. By contrast, it can be speculated that the absence of *Dolichospermum* in Lake Lugano could be due to the lower water temperatures and higher mixing that characterise its epilimnion (see a case study in Ojala et al. 2003). Instead, the dominance of *Aphanizomenon flos-aquae* in this lake is consistent with the ability of this genus to develop in mixed surface layers (Kangro et al. 2007) and to build up winter peaks detectable also under the ice (Padisák et al. 2010).

The relationships reported in Figs. 4 and 5 should be interpreted carefully. A first point to underline is that these empirical relationships imply different responses to water temperatures largely dependent on the level of nutrients. At the same temperature range, algae will replicate provided that nutrients are available. Differences in temperature-biovolume slopes do not imply differences in temperature sensitiveness of specific groups in the considered lakes. From a theoretical point of view, in the absence of limiting interfering factors, slopes relating the abundance of specific phytoplankton taxa and water temperature in a group of lakes should increase their values with the increase in nutrient concentrations. Statistically, this could be represented by the existence of significant interactions between temperature and lake trophic status. This property was shared by a few of the more eutrophic groups, i.e. Chlorophyta and Charophyta and, partly, Dinophyta (Table 3; Fig. 5). In the case of cyanobacteria, the parallel slopes indicated similar increases with water temperature, but based on different levels of average biomass partly linked to the trophic status of the different lakes. Similarly, Karentz and Smayda (1984) suggested an important role

played by the interactions between temperature and nutrients in the control of field phytoplankton abundance. The same authors underlined the possibility that species that require a fairly high level of nutrients might show a seasonal development similar to cold-water species, and that species tolerant of low nutrient levels might show warm-water occurrence patterns. More recently, related results were found by Tadonlécé (2010), who showed that the effects of global warming on chlorophyll-*a* normalized maximum rates of photosynthesis may be positive, non-significant, or negative, depending on nutrient availability. In our models, trophic status was represented as a “lake factor”. At least for the algal groups more affected by eutrophication (particularly Chlorophyta, Charophyta, Dinophyta and, partly, cyanobacteria; Fig. 3), the order of the regression lines along the y-axes and the magnitude of the slopes in the temperature-phytoplankton relationships were consistent with lake trophic status, and with the contribution of these algal groups in the different lakes. It is useful to stress that we did not compare the combined effects of temperature and nutrients collected at the same time. Rather, we examined the effects of temperature recorded over the whole study period, taking into account lake effects/trophic status of the five lakes. Nevertheless, the available results allow hypothesising the existence of ecological interactions between nutrient availability and water temperature, but a more detailed account could be obtained only with a larger dataset (see below).

A second point to take into account is the role assumed by explanatory variables other than temperature and trophic status. Interaction responses with other variables also dependent on temperature (e.g., water column stability, epilimnetic light regime, grazing rates and predator activities, competitive interactions) should be taken into consideration to correctly interpret the data. In our dataset, the negative temperature slope in diatoms is due to the high susceptibility of this group to the formation of stable and warm water columns that enhance the sinking and loss of heavier organisms (Padisák 2004). In Lake Tahoe, large planktonic diatoms were showed to decrease with increasing stratification over the last decades (Winder et al. 2009). In addition, water temperature indirectly controls the ratio between the euphotic to mixed depth, representing one of the most important factors influencing light regime (Hamilton et al. 2010); the strategies adopted by low light adapted species discussed above represent a distinctive example. Temperature is a key dominant variable driving interannual variability of large and small zooplankters (Schalau et al. 2008). In the DSL, the rapid increase of large Cladocerans and Copepods during and after the spring months is another factor acting on the phytoplankton development, being able to explain the low temperature slope in the smaller and edible cryptophytes (de Bernardi

and Canale 1995). Therefore, the interpretation of the biomass–temperature–nutrient relationships cannot be always clear. Besides nutrients, the role of temperature is mediated through interactions with other environmental factors that modify algal responses to thermal conditions (cf. Karentz and Smayda 1984). The temperature–phytoplankton relationships represent the effects of temperature on the different phytoplankton groups, and net of the effects of other competing variables. They should not be confused with the effects of temperature in cultures. Algae that are better able to contrast losses (e.g., due to sinking or grazing) will have a greater ability to exploit warm periods, which are more favourable for growth.

The existence of concurrent effects on phytoplankton due to both seasonal development of water temperature and trophic status was further highlighted by the positive relationship between trophic and temperature slopes (Fig. 6). In this typology of large and deep lakes, algal groups able to take advantage from high water temperature and spring nutrient availability were Chlorophyta and, partly, Dinophyta, Charophyta and dominant Cyanobacteria (Oscillatoriales). The size of individuals, colonies and coenobia, or the presence of mucilaginous envelopes, render these groups resistant against grazing and—excluding the large conjugatophytes—sinking. Ecological characteristics of these groups in deep lakes were discussed, among others, by Morabito et al. (2003), Reynolds (2006) and Salmaso (2010). As for the conjugates, the better performance in Lake Lugano could be favoured by enhanced turbulent conditions due to the formation of larger mixed epilimnia persisting during the summer months. The importance of autoecological characteristics minimizing loss processes (control of sinking and scarce susceptibility to grazing) becomes particularly apparent in dinophytes. In laboratory cultures and in natural populations, representatives of this group (e.g., *Ceratium*, *Peridinium*) have lower net growth rates compared to other taxa (Padisák 2004). Despite this, Dinophyta in this group of lakes had amongst the highest temperature slopes, implying a better exploitation of favourable temperature conditions compared to species with higher potential growth (including diatoms). Besides different nutrient availability, the patterns depicted above can be reversed because of different morphometric, hydrological and climatic characteristics in other typologies of lakes.

GAM and GLS methods are excellent tools to translate in statistical language ecological hypotheses. In many lakes, eutrophication and climate change represent two of the most important stressors (George 2010). In this work, GLS models showed effectively how the development of some algal groups could largely be explained by trophic status and temperature fluctuations. Nevertheless, with these models, the number of parameters to be computed

increases with the number of lakes and/or years (as in our specific case). A valuable solution to handle a larger number of cases in nominal variables is represented by mixed modelling. In this work, this technique proved to be extremely valid in summarising the relationships between development of algal groups and temperature, taking into account the variability in the single lakes/years.

It is worth noting that, for specific algal groups (Nostocales), observations were zero inflated, i.e. characterised by many zeroes due to the absence of the most widespread species (*Dolichospermum*) in the cold and mild months. Possibly, for these minor groups, other alternative modelling techniques could be adopted (Zuur et al. 2009).

In summary, this study demonstrated a significant effect of temperature fluctuations and trophic status on the development of the principal groups of cyanobacteria and eukaryotic phytoplankton. Nevertheless, only in a few algal groups (chlorophytes, charophytes, dinophytes and, partly, cyanobacteria) the temporal development was driven by both nutrient availability and temperature fluctuations. In these groups, the action of other important explanatory variables favouring losses, such as grazing and sinking velocity, appeared reduced compared to other groups (cryptophytes and diatoms), which, by contrast, exhibited low or negative temperature slopes. These results have implications in the evaluation of the impact of different climatic scenarios in lakes of differing trophic status, suggesting a net increase of only selected eutrophic- or eurytrophic-sensitive groups with increasing water temperature in more enriched systems.

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