Phytoplankton productivity increased in Lake Geneva despite phosphorus loading reduction

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Long-term (1972–2005) data from Lake Geneva were analyzed to assess whether primary productivity (PP) has declined over years in response to phosphorus (P) reduction measures implemented in the 1970s, and to determine which factors were responsible for the response observed. The results revealed increases in PP and chlorophyll a, and a concomitant decrease in water transparency, which contrasts sharply with other large lakes undergoing nutrient loading controls. Our data revealed long-term increases in incident light, water temperature, water column stability (WCS), N:P ratios and Baccilariophyceae. Nitrogen, Daphnia and WCS, rather than P, were the major factors driving PP during the first part of the study (1972–1988). When P became the main nutrient driving PP (after 1988), the latter still increased, partly as a result of long-term increases in light and WCS, two factors not often considered when exploring climate influence on phytoplankton. Daphnia abundance decreased over time, and its coupling with phytoplankton changed from positive during the period of P abundance (1972–1988) to negative during the period of P scarcity (1989–2005), suggesting a change in the influence of Daphnia on phytoplankton and in food web structure. These data support the view that increases in climate-related factors may render the restoration of lakes more difficult, and showed that neglecting nitrogen, light and WCS, may hamper our understanding of lake responses to re-oligotrophication or lead to inappropriate management decisions, particularly in the context of global warming.

INTRODUCTION

Because of anthropogenic increases in the external nutrient loading, many lakes have suffered from increasing eutrophication over the past few centuries (Wetzel, 1990). This has led to a deterioration of their water quality. During the past decades, however, in several countries measures intended to improve the water quality of lakes have been implemented, including reducing external nutrient loading.

Lake Geneva is one of the largest lakes in Western Europe, and is socio-economically very important. As the largest freshwater reserve in this part of Europe, it is used for fishing, supplies drinking water to the surrounding towns, and attracts many tourists, especially during the summer. This lake underwent strong cultural eutrophication in the early 1960s, but following the implementation of phosphorus reduction measures in 1972, phosphorus (P) concentrations in the lake water decreased from ~90 μg P L⁻¹ in the mid-1970s to ~30 μg P L⁻¹ in 2005. Water quality has been monitored in Lake Geneva for more than 30 years, and this has generated a database that can be used to examine long-term changes in lake communities, and how they are influenced by anthropogenic activities and/or climate change. Previous studies of this lake have analyzed long-term changes in the succession of phytoplankton assemblages (1974–1998, Anneville et al., 2002), as well as the responses of rotifers (Molinero et al., 2006), copepods (Anneville et al., 2007) and fish communities (Gerdeaux, 2004; Gerdeaux et al., 2006; Gillet and Quetin, 2006; Dubois et al., 2008) to...
environmental changes. In the present study, we were interested in changes in the primary productivity (PP). A previous study of PP in this lake was undertaken by Anneville and Pelletier (Anneville and Pelletier, 2000); however, their study covered the period from 1974 to 1998, and did not investigate the mechanisms that may be driving the observed changes. In addition, these authors calculated the daily production from the rates of production measured during the incubation periods, using a conservative factor of 2.2 for the whole study (see our “Method” section for differences in the calculation method). PP is a rate process, and thus one of the best variables for elucidating how aquatic systems respond to environmental changes (Goldman, 1988).

Our understanding of how small, shallow lakes respond to nutrient reduction has advanced considerably (e.g. Jeppesen et al., 2005a, b), but we know less about the responses of large, deep lakes, especially with regard to inter-annual variability in phytoplankton productivity.

Reports from relatively large, deep lakes that have been subjected to nutrient reduction (surface area $>45 \text{ km}^2$ and mean depth $>30 \text{ m}$) have indicated that a decline in phytoplankton biomass and/or production, as well as an increase in water transparency, typically occurs within 6–10 years after initiating nutrient controls (e.g. Edmondson and Lehman, 1981; Dokulil, 1993, Häse et al., 1998). However, there is a considerable variability in the time lag of the response (e.g. Dokulil, 1993, Häse et al., 1998, Jeppesen et al., 2005b), and some of these lakes have shown a decline in phytoplankton biomass only in summer (Lake Constance, Häse et al., 1998). Moreover, a recent cross-system study has revealed that a substantial number of large deep lakes did not follow the general pattern found for smaller lakes (Jeppesen et al., 2005b), highlighting the complexity of the re-oligotrophication process.

Recovery from eutrophication is complex and depends on many factors, including internal nutrient regeneration, water temperature and lake morphometry (Genkai-Kato and Carpenter, 2005), as well as food web structure (Carpenter et al., 1992). Investigating individual lakes can help improve our understanding of the mechanism of oligotrophication, given that individual lakes may exhibit a specific trajectory as a result of their unique characteristics (Smith and Shapiro, 1981). In this study we examined in detail the responses of phytoplankton to marked changes in nutrient concentrations in Lake Geneva. We analyzed the long-term (1972–2005) records of PP, algal biomass and water chemistry, as well as the temporal shifts in zooplankton abundance and community composition; the latter is often not considered when long-term trends in phytoplankton biomass and productivity are analyzed. The aim of the study was to assess whether PP has declined in this lake over years in response to P control, and to find out which factors were responsible for the response observed.

**METHOD**

**Study site**

Lake Geneva is located at an altitude of 372 m on the border between Switzerland and France (46°27'N, 6°32'E). It is a semicircular lake with a surface area of 582 km$^2$, a maximum length of 72 km, and a maximum width of 14 km; the maximum and mean depths are 309 and 152 m, respectively. The lake contains $\sim$89 km$^3$ of water, has a residence time of about 11 years and does not freeze. Further details about the characteristics of the lake can be found elsewhere (Anneville et al., 2002).

**Sampling and sample analyses**

The data presented here cover the period 1972–2005, except for nutrients for which the data series starts in 1970. Incident solar radiation was recorded at a meteorological station located a few meters from the lake. Samples were collected at the deepest point in the lake using a common sampler for nutrients, chlorophyll $a$ (Chl $a$) and PP, and a 200-$\mu$m mesh net was towed vertically from 50 m to the surface to sample the zooplankton. Samples were collected once a month from 1970 to 1981, and two or three times a month thereafter. Samples for nutrients were collected at 12–20 depths ranging from the surface to the bottom of the lake (0–309 m), whereas samples for Chl $a$ and PP analyses were collected at 10 depths in the 0–30 m zone (0, 1, 2, 3.5, 5, 7.5, 10, 15, 20 and 30 m). Samples for phytoplankton species composition analyses were collected using a custom-made integrating bell-shape sampler; they were taken from the top 10 m until 2001, and from the top 20 m thereafter, because the maximum Chl $a$ value was observed at progressively deeper levels during the stratified periods (Anneville, 2001), and it was found that the annual mean concentration of Chl $a$ and rate of PP were increasing at depths below 10 m (see Results). Caution is thus called for in attempting to compare the phytoplankton composition observed prior to 2001 to that observed after 2001.

Water temperature was measured using a water sampler with a built-in thermometer in the early part of the study, and submersible probes thereafter. From the temperature values measured, the Brünt–Väisälä frequency (BVF) was calculated, and used as an index of the water column stability (WCS). BVF was calculated
using the equation \( N^2 = (g/\rho)/(d \rho/\text{d}z) \), where \( N^2 \) is the stability coefficient \( (s^{-2}) \), \( g \) the gravity \( (m/s^2) \), \( \rho \) the water density and \( z \) the depth. The water density \( (\rho) \) was estimated from the water temperature \( (T \text{ in } ^\circ \text{C}) \) according to the formula 
\[ \rho(T) = 1000 - 7 \times 10^{-3}(T-4)^2 \]
(Lerman, 1978).

The water transparency was measured using a 30-cm white disk. Although this is not the commonly used Secchi disk, this does not alter our conclusions as this white disk was used consistently throughout the entire study. The term “Secchi depth” is used hereafter to simplify.

Nutrients [ammonium, nitrates, nitrites, organic nitrogen, dissolved inorganic phosphorus (DIP) and total phosphorus (TP)] were analyzed by standard colorimetric methods (AFNOR, 1990). Total nitrogen (TN) was calculated as the sum of nitrate, nitrates, ammonium and organic nitrogen. The inorganic nitrogen concentration (DIN) was calculated as the sum of ammonia, nitrates and nitrate. The nutrient data presented here for the individual sampling dates are mean values for the 0–20 m zone of the water column, the zone where Chla and PP were determined (see below).

Phytoplankton composition and abundance were analyzed under an inverted microscope. The phytoplankton total biovolume was calculated from the biovolumes estimated from the cell dimensions and abundance of each species, assuming that \( 10^6 \mu m^3 = 1 \mu g \text{ fresh weight} \). Zooplankton abundance and composition were analyzed under a dissecting microscope.

Chla was analyzed by spectrophotometry (Strickland and Parsons, 1968) after extracting in 90% acetone. PP was measured in situ, generally during the local solar noon, using the \(^{14} \text{C} \) method (Steemann-Nielsen, 1952). We found that the yearly averaged Chla and PP levels increased over time below 10 m, and that PP at 30 m was usually very low or equal to zero. We therefore integrated Chla and PP values over the 0–20 m zone. The daily primary production was obtained by multiplying the PP per unit area (obtained for the incubation period) by the ratio of the daily global radiation to the irradiation measured during the whole period of primary production incubation. The rate of primary production per unit chl a (the light-saturated assimilation number=photosynthetic capacity, \( P_{\text{max}} \text{Chl} \)) is another sensitive physiological indicator of algal responses to ambient conditions (Eppley, 1972; Falkowski, 1981). Eutrophication is known to cause pronounced shifts in phytoplankton species composition, and \( P_{\text{max}} \text{Chl} \) ratios can respond to these compositional shifts due to alterations in the ratio of Photosystem II:Photosystem I components within the phytoplankton community (Smith, 2007). The \( P_{\text{max}} \text{Chl} \) ratio was calculated by dividing the maximum volumetric rate of PP recorded in the water column on any given date by the value of Chla recorded at the same depth on this date.

### Statistical analyses

This study deals with inter-annual variations. The long-term trends of variables investigated using Kendall’s Tau statistics and least-square regressions (Legendre and Legendre, 1998). For these regressions, linear models were used for simplification. To identify the factors that control phytoplankton production (PP and \( P_{\text{max}} \text{Chl} \)), two different methods were used to provide robust conclusions, because preliminary tests of correlations showed that the relationships were not always linear. One of the methods involved the use of regressions with explanatory purpose. Multiple regressions with backward elimination were used. This procedure eliminates redundant variables. Hence, only independent variables with significant partial regression coefficients remained in our regression models. Prior to these analyses, the data were log_{10}-transformed (except for the residuals) to stabilize the variance and attain homoscedasticity (the % DIP and % NO\textsubscript{3} were not used in analyses). The Durbin–Watson test and the first-order autocorrelation were used to check for autocorrelation. Tolerance was used to check for co-linearity. Partial \( T \) values in our statistical results indicate the size of the statistical effect of the independent variable on the dependent variable when all other independent variables are considered. The independent variables included Chla (for PP), light, temperature, nutrient (N, P) ratios and concentrations, the WCS index (BVF) and the abundances of zooplankton (total and different groups). For the second statistical method, the variables that showed long-term trends were first detrended (or differenced), using the variate difference method. This involved replacing each value \( Y_i \) by the difference \( (Y_{i+1} - Y_i) \). Dependent variables were then plotted against independent variables over time to check for evidence of serial dependence (Legendre and Legendre, 1998). This method is a simple but effective way to remove trends, especially those that are monotonic or cyclical (Legendre and Legendre, 1998), and has been used successfully in other long-term limnological studies (e.g. Berman et al., 1995). Systat and Statistica were used for the statistical analyses.

### RESULTS

#### Long-term trends of variables

Figure 1 shows inter-annual changes in the physical variables investigated. Table I summarizes the average
values of the variables studied, and the results of the statistical analyses performed to detect long-term trends. Global solar radiation and water temperature increased significantly over time. A similar trend was found for BVF from 1974 to 2001 (Fig. 1A–C); note that the very high and very low values found outside this period were not included in the analyses of trends. In contrast to these three variables, water transparency decreased significantly over the study period (Fig. 1D; Table I).

The mean annual concentrations of ammonium decreased significantly over time, and ranged from \(\sim 27\) to \(\sim 8\) \(\mu g\) L\(^{-1}\) (Fig. 2A; Table I). Ammonia always accounted for only a small fraction of TN. The highest concentration of ammonium was usually found in May–July (not shown), and coincided with the highest abundances of zooplankton (e.g. Gawler et al., 1988), suggesting that most of the ammonium was regenerated. In contrast, nitrate and its contribution to TN increased significantly over the study period, whereas the TN concentration increased from 1970 to about 1988, and subsequently decreased. TN displayed no significant long-term trend (Fig. 2B–D; Table I). Nitrate constituted a major proportion of the TN (52–80%, mean = 68%), and almost all the dissolved inorganic nitrogen pool (91–97%, mean = 95%).

TP, DIP and the contribution of DIP to TP all decreased significantly over the study period (Fig. 2E and F; Table I), which is consistent with the P loading control measures that had been implemented in Lake Geneva. DIP constituted 30–70% (mean = 48%) of TP.

As a consequence of the major contribution made by nitrate to TN, and of the contrasting long-term patterns for nitrate and phosphorus, the ratios of nitrogen to phosphorus (inorganic ratio = DIN:DIP) increased significantly over time (Table I; Fig. 2G and H). Interestingly, both the DIN:DIP and TN:TP ratios were low, and most of the time \(\sim 22:1\) in terms of moles (11:1 by mass) from the beginning of the study to about 1984. When TN:TP rose above this value, it remained \(< 31:1\) in terms of moles (<15:1 by mass). After 1984, the annual mean values of these ratios rose considerably, and after 1989, generally remained above 100:1 by moles for DIN:DIP or 60:1 by moles for TN:TP (Fig. 2G and H). The difference in the seasonal dynamics of inorganic nutrients between the period prior to 1989 and that after 1989 was consistent with the long-term shift in N:P ratios after 1989. Indeed the seasonal depletion of DIN was more marked prior to 1989 than after 1989, while the opposite was observed for DIP (Fig. 3A–D), indicating that N was more scarce than P prior to 1989, whereas P was more scarce than

![Fig. 1.](https://academic.oup.com/plankt/article-abstract/31/10/1179/1528073)

Long-term changes in global radiation (A), average temperature in the 0–5 m zone (B), water column stability index, i.e. the Brunt–Väisälä frequency (BVF) (C) and Secchi depth (D). Note that for BVF the extremely high or low values calculated for the years 1970 to 1973 and 2002, 2004 and 2005 were excluded when analyzing the long-term trends. The dotted lines indicated the years 1974 and 2001.
N after 1989. In addition, after 1989, the duration of strong DIP depletion was extended to early spring and fall (Fig. 3D).

The abundances of *Daphnia* and adult cyclopoids displayed long-term trends over the study period; *Daphnia* abundance decreased significantly, whereas adult cyclopoid abundance increased significantly. In contrast, neither *Bosmina* nor adult calanoid abundances displayed any discernable long-term trend (Fig. 4A–D; Table I).

Both primary production and phytoplankton biomass increased significantly over the study period (although biomass seemed to be relatively constant from 1972 to about 1990). The light-saturated photosynthetic capacity (*P*$_{\text{max}}$Chl) showed no discernable long-term trend (Fig. 5A–C; Table I). *P*$_{\text{max}}$Chl increased until about 1987 (whereas phytoplankton biomass showed no corresponding upward trend), and then became relatively constant over time. Year-on-year analysis revealed that most of the highest values of *P*$_{\text{max}}$Chl were found in spring during the clear water phases; at a time when *Daphnia* dominated the zooplankton community (Gawler et al., 1988; Gawler et al., unpublished results). Significant long-term increases were also found in PP and Chl $\alpha$ at discrete depths in the deeper waters (e.g. 15 m, Fig. 5D), despite the decrease in water transparency (Kendall’s Tau = 0.55, *P* < 0.0001 for PP; and Kendall’s Tau = 0.38, *P* = 0.0036 for Chl $\alpha$). The changes in the annual mean fresh weight biomass of phytoplankton groups indicated that only the *Bacillariophyceae* (Kendall’s Tau = 0.47; *P* = 0.0002) and the “other chrysophytes” (Kendall’s Tau = 0.57; *P* < 0.0001) exhibited significant long-term trends; they increased over time, even when the data set was restricted to the period 1974–2001 (Fig. 5E). As found for the Secchi depth, the long-term patterns for phytoplankton biomass and PP were not consistent with the results expected after implementation of nutrient loading control.

### Controlling factors

A statistical analysis that has allowed mapping of phytoplankton assemblages in Lake Geneva has also shown that major modifications in the timing and sequence of occurrences of phytoplankton groups have occurred since 1988 (Anneville et al., 2002). The long-term trends in many of the variables studied here showed a clear change between 1984 and 1989 (e.g. light, nitrate, TN and N:P ratios). For these reasons, in our multiple regression analyses we considered not only the whole data set, but also two different periods: 1972–1988 during which TP was $\geq 22$ µg L$^{-1}$ and DIP $> 10$ µg L$^{-1}$, and 1989–2005 when TP was <22 µg L$^{-1}$ except in 1995 and DIP $\leq 10$ µg L$^{-1}$ (Fig. 2E). The results revealed changes in the major factors driving phytoplankton between the two periods considered (Table II). For the period 1972–1988, the TN:TP ratio had highly significant partial effects on both PP and *P*$_{\text{max}}$Chl. The water column stability (BVF) also had
a significant partial effect on PP. These effects were all positive, and the proportions of variance explained by these independent variables were 68 and 70% for PP and $P_{\text{max}}$:Chl, respectively. We found, however, that our ability to explain inter-annual variations in $P_{\text{max}}$:Chl during this period increased considerably when we replaced the average of $Daphnia$ abundance for each year ($Daphnia$-AN) in the set of independent variables by the average $Daphnia$ abundances recorded during winter (W) of that year (January–March) and fall (F) of the preceding year (October–December) (i.e. $Daphnia$ October to December and January to March). This $Daphnia$ abundance (referred to below as $Daphnia$-FW) and BVF both had highly significant and positive partial effects on $P_{\text{max}}$:Chl, and accounted for 86% of the change (Table II). This result was probably due to the fact that $Daphnia$-FW was strongly positively correlated with the TN:TP ratio (and provided the same information), whereas $Daphnia$-AN was not (Fig. 6A and B). Similar results were found for the DIN:DIP
ratio: $R^2 = 0.72$, $P < 0.0001$ for *Daphnia*-FW and $R^2 = 0.13$, $P = 0.18$ for *Daphnia*-AN. Partial $T$ values for this period (1970–1988) indicated that the TN:TP ratio or *Daphnia*-FW had the strongest statistical effects on phytoplankton production and photosynthetic capacity (Table II).
In contrast to the results found for 1972–1988, the variables that had significant partial effects on phytoplankton productivity for the period 1989–2005 were DIN:DIP, light and calanoid and \textit{Bosmina} abundances for PP, and DIP, light, \textit{Bosmina} abundance and \textit{Daphnia}-AN for $P_{\text{max}}$:Chl. Among these variables, only DIN:DIP and \textit{Daphnia}-AN had negative statistical effects. The proportions of variance accounted for were 51 and 70\% for PP and $P_{\text{max}}$:Chl, respectively, and partial $T$ values indicated that DIP (for $P_{\text{max}}$:Chl) or DIN:DIP (for PP) had the strongest statistical effects, followed by light and zooplankton abundance (Table II).

For the whole data set (i.e. 1972–2005), the proportions of variance of PP and $P_{\text{max}}$:Chl explained were lower: 40 and 45\% respectively (Table II). TN:TP was the only variable that explained variations in PP (positive effects), while for $P_{\text{max}}$:Chl, nitrate, ammonia, BVF, \textit{Daphnia}-AN, cyclopoid and \textit{Bosmina} abundance had significant partial effects. Among the variables accounting for changes in $P_{\text{max}}$:Chl, \textit{Daphnia}-AN had the strongest statistical effect, and was the only variable with a negative effect.

Figure 6C clearly shows that despite the decrease in TP, the areal PP increased, and its values remained high even when TP dropped below 20 $\mu$g L$^{-1}$. Several authors have shown that the areal PP is less sensitive to nutrient variations in the water column than the light-saturated production ($A_{\text{opt}}=P_{\text{max}}$), and consequently may be a poor indicator of the response of the lake to nutrient changes compared with $A_{\text{opt}}$ (see Smith, 2007). According to these authors, $A_{\text{opt}}$ should decline significantly with a decrease in TP (Smith, 1979, 2007 and references therein); however, we found no consistent relationship between these two variables, using the whole data set in the present study (Fig 6C). $A_{\text{opt}}$ decreased significantly with TP ($R^2 = 0.47$, $P = 0.04$) only when TP was still above 40 $\mu$g L$^{-1}$ (i.e. prior to 1984). This contrasted with the pattern in the TP-$P_{\text{max}}$:Chl relationship over the same period (Figs 2D and 5C), indicating an influence of phytoplankton biomass.
Our findings also show that TN:TP, light, Daphnia-WF and temperature had significant partial effects on Chl \(a\), and explained 50% of its variance (Table II). The first two variables had positive effects, whereas the other two had negative effects. This negative effect of temperature could reflect the negative response of phytoplankton to rising temperature observed \((Q_{10})\) during the summer, when P was strongly depleted (Tadonléke, in preparation). Partial \(T\) values indicated that TN:TP and Daphnia-WF had the strongest statistical effects on Chl \(a\) (Table II).

Smith (Smith, 1982) developed a model to predict Chl \(a\) from TN and TP in temperate lakes \((\text{Log Chl} = 0.633 \text{ Log TP} + 0.548 \text{ Log TN} - 1.517)\). By applying this model to our data, we found that it predicted Chl \(a\) relatively well after 1990, particularly from 1997 to 2005 even though the model uses data from the growing season, but largely overestimated that measured prior to that period (Fig. 6D). Fitting our spring-summer (growing season) data to the model yielded similar results (not shown). It appeared that for the period 1972–1988, the difference between the measured and the predicted Chl \(a\) decreased as Daphnia-FW increased, while for the period 1989–2005 this difference increased as Daphnia-AN increased (Fig. 6E and F).

The results from the second method of analysis (based on differenced values) confirmed those obtained from regression analyses. They showed that fluctuations of annual means of PP and \(P_{\text{max}}:\text{Chl}\) were synchronized with changes in DIP concentrations after 1985 (Fig. 7A and B). Prior to 1985, inter-annual changes in phytoplankton production paralleled those of TN:TP (Fig. 7C and D). Note that all the potential controlling variables were tested.

### Table II: Results of multiple regressions testing the effects of independent variables on phytoplankton variables

<table>
<thead>
<tr>
<th>Data set</th>
<th>Dependent variable</th>
<th>Independent variable and constant</th>
<th>coefficient</th>
<th>Partial (T)</th>
<th>(P_{\text{T}})</th>
<th>(R^2)</th>
<th>(P)</th>
<th>DW</th>
<th>FOAC</th>
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<td>(P_{\text{max}}:\text{Chl})</td>
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Partial \(T\) values indicate the size of the statistical effect of the independent variable when all other independent variables are considered. \(P_{\text{T}}\) indicates the probability that a partial \(T\) value of equal or greater magnitude would be obtained by chance alone. DW and FOAC are the Durbin–Watson statistic and the first-order autocorrelation, respectively, used to check for autocorrelation (see detail in the text). \(P\) (probability), \(R^2\), DW and FOAC are for the regression model. PP is areal production, \(P_{\text{max}}:\text{Chl}\) is the light saturated photosynthetic capacity and Chl is areal chlorophyll \(a\). Daphnia-FW is mean abundance of Daphnia for fall of year \(n\) and winter of year \(n+1\). Daphnia-AN is mean annual abundance of Daphnia.
**DISCUSSION**

The data (≥30 years) from Lake Geneva presented here reveal a long-term increase in phytoplankton biomass and production, and a concomitant decline in the water transparency over the study period, despite phosphorus reduction. This contrasted sharply with previous studies of re-oligotrophication in large deep lakes, most of which concern periods generally <20 years, and have revealed a decline in these phytoplankton variables and an increase in water transparency within a maximum of 15 years after the beginning of P reduction (references in the Introduction). Previous studies of Lake Geneva (Anneville and Pelletier, 2000) did not reveal the patterns observed here, probably in part because of differences in the methods used to calculate the daily PP. Comparisons indicate that the method used by these authors (see Introduction and Method) does not adequately take into account the daily variations in light intensities (Tadonléke, unpublished results). So far attempts to understand...
phytoplankton responses in this lake have only considered phosphorus, even though the warming of water has been suggested as a potentially influential factor (Anneville and Pelletier, 2000). Our analyses indicate that the influence of nitrogen, the decline in the abundance and a change in the influence of Daphnia over time together with the long-term increase in light and WCS were the major factors responsible for the responses observed.

1970–1988: major influence of nitrogen and of Daphnia on phytoplankton productivity

Up to 13 years after the implementation of phosphorus reduction measures, nitrogen rather than phosphorus was the main factor driving phytoplankton productivity in Lake Geneva, and this helps explain why phytoplankton did not decline during this period. TN consisted mainly of nitrate, which increased in concentration and proportion over time (Fig. 2B–D). This has likely favored phytoplankton growth while P was decreasing, as suggested by the positive relationships between TN:TP and both Chl a and phytoplankton productivity (Table II). Several of our findings indicate that phytoplankton was N-limited during that period. First, the seasonal depletion of DIN was stronger prior to 1989 than after 1989 (Fig. 3A and B). Second, prior to 1985, TN:TP ratios were lower than or close to 22:1 by moles (Fig. 2G), the estimated boundary below which N limitation of lake phytoplankton occurs (Sakamoto, 1966; Guildford and Hecky, 2000). Third, cyanobacteria generally consisted mainly of the N2-fixing species Aphanizomenon flos aquae (Anneville et al., 2002). Fourth, ammonia and nitrate both had significant positive effects on \( P_{\text{max}} \text{-Chl} \), even though this involved the whole data set (Table II). An inter-annual increase in phytoplankton production with nitrate, and a concomitant decline in water transparency have also been reported in the large Lake Tahoe, but rather during its eutrophication (Goldman, 1988). In Lake Geneva, the phytoplankton is almost always dominated by large cells (Anneville et al., 2007). As the ammonium concentration was generally below 20 \( \mu \text{gN L}^{-1} \) and decreased over time (Fig. 2A), this dominance of large phytoplankton seems to be consistent with laboratory studies that have shown that...
biomass specific rates of uptake and accumulation of nitrate were lower for small compared with large phytoplankton cells, and have concluded that this difference may provide a competitive advantage to large cells in natural environments when nitrate is the main nitrogen source for phytoplankton (Stolte and Riegman, 1995). Moreover, several studies have indicated a preference for nitrate by phytoplankton >20 μm in size (e.g. Nalewajko and Gorside, 1983; Probyn et al., 1990). Other studies have demonstrated increases in the absolute uptake of nitrate by mixed communities (Lomas and Gilbert, 1999) or in the affinity for nitrate in individual marine algae (Reay et al., 1999), when the temperature increased below 25°C, an environmental circumstance found here (Fig. 1B).

N-reduction measures are not often implemented in parallel to P-reduction in attempts to restore lakes; this may alter nutrient stoichiometry and result in strong increases in the N:P ratios during re-oligotrophication, as observed here and in other aquatic ecosystems (e.g. Jeppesen et al., 2005b; Pearl et al., 2006). However, in most of these ecosystems, in contrast to Lake Geneva, a decline in phytoplankton variables was observed. Theory and both laboratory and field studies indicate that changes in nutrient ratios influence phytoplankton community structure (Tilman, 1977; Interlandi et al., 1999; Köhler et al., 2005). The influence of N:P ratios, which is not often considered in the study of re-oligotrophication [see however e.g. Köhler et al. (Köhler et al., 2005); Jeppesen et al. (Jeppesen et al., 2005b) for shallow lakes], might thus be a key factor causing changes in phytoplankton community structure in lakes undergoing this process. Our data contribute to the growing body of literature that is indicating that N-limitation of phytoplankton in lakes is common, and not exceptional as previously thought (Lewis and Wurtsbaugh, 2008).

Our data suggested that Daphnia was another major factor determining variations in phytoplankton productivity and has indirectly stimulated the latter during the period 1972–1988. Positive relationships between the ratio of phytoplankton production to biomass and the whole zooplankton or Daphnia alone, as found here, have been reported in other natural waters and in experimental mesocosms, and are considered to be indicative of Daphnia or zooplankton grazing on phytoplankton (e.g. Lampert et al., 1986; Elser and Goldman, 1991). The stock of Daphnia that developed in fall and winter (i.e. prior to phytoplankton growth) seemed to play a major role in these dynamics in Lake Geneva, as suggested by the significant relationships between Daphnia-FW and both the nutrient ratios (positive) and the difference between the measured and the predicted Chl a (Fig. 6A and E). Such results are consistent with mortality-related increases in per capita algal growth, and herbivore-enhanced nutrient regeneration rates (Glover, 1980; Callieri et al., 2004), and the coincidence between high values of production to biomass ratios and peaks of Daphnia during the clear-water phases (Lampert et al., 1986, this study). These findings are consistent with the only grazing study that we are aware of for Lake Geneva, which showed strong grazing of phytoplankton by herbivores, especially between May and July, when Daphnia was the dominant herbivore (Gawler et al., 1988). This strong grazing impact and the higher abundance of Daphnia during the period 1972–1988 might have been promoted by the negative impacts of piscivorous fish on planktivorous fish, as has been observed in shallow Danish lakes (Jeppesen et al., 2005a). The coregonids-cyprinids ratios were actually very low in Lake Geneva during the period 1970–1985 (Gerdeaux et al., 2006).

1989–2005: major influence of phosphorus and of Daphnia on phytoplankton productivity

Apparently there was a shift in the major factors driving phytoplankton from the period 1970–1988 to the period 1989–2005. This shift seems to have occurred between 1984 and 1989, and may be one reason why the proportions of variance of phytoplankton variables explained were lower for the whole data set than for each of the two study periods considered here (Table II). The observed shift from nitrogen to phosphorus as the main influential nutrient on phytoplankton is consistent with predictions (Sas, 1989). The synchronous variation of phytoplankton and P, together with the fact that this nutrient had the most marked statistical effects on phytoplankton productivity demonstrated that phytoplankton was P-limited (Fig. 7A and B; Table II), and indicated that P began to affect primary production only when annual mean DIP concentrations dropped below 10 μg P L−1. Consistent with this, the TN:TP ratios were >22:1 by moles (Fig. 2G), a threshold above which P limitation of phytoplankton generally occurs (Sakamoto, 1966; Guildford and Hecky, 2000). Moreover, the abundance of Bacillariophyceae and of “other chrysophytes” significantly increased over time (Fig. 5E). Previous studies have indicated that many diatoms are good competitors for P, and so increase at higher N:P ratios (e.g. Kilham, 1986). An increase in chrysophytes as P amounts decrease has been reported for other lakes during re-oligotrophication (e.g. Gammeter and Zimmermann, 2000). Our findings support the widely accepted, albeit seldom demonstrated view that during re-oligotrophication of lakes, phytoplankton becomes phosphorus-limited when SRP
Daphnia nutrient-poor conditions, their grazing pressure on phytoplankton. Under may sequestrate P (Sterner, 1990). A decrease in have a low body N:P ratio and high P requirements, this view is supported here by the increase in grazing from fish may lead to the reverse result. This et al (Anneville, 2004; Dubois et al, 2008). The increased abundance of planktivorous fish (e.g. 0+ perch and whitefish) has probably contributed to diminishing the abundances of Daphnia and other herbivorous cladocera (Anneville et al., 2007; Dubois et al., 2008), thus reducing their grazing pressure on phytoplankton. Under nutrient-poor conditions, Daphnia, which is known to have a low body N:P ratio and high P requirements, may sequesterate P (Sterner, 1990). A decrease in Daphnia abundance due to a high grazing pressure from fish may thus result in a higher availability of P and an increase in phytoplankton productivity, whereas an increase in Daphnia abundance resulting from a low grazing from fish may lead to the reverse result. This view is supported here by the increase in $P_{\text{max}}$ Chl with DIP and the negative coupling of $P_{\text{max}}$ Chl with Daphnia (Fig. 7; Table II). In P-poor waters, phytoplankton may also suffer from competition with organisms, such as bacteria, which have a lower half-saturation constant for P acquisition (Curie and Kalff, 1984). These processes can modify the structure of phytoplankton communities and change their coupling with zooplankton. In a whole-lake experiment where P was directly removed, causing the TP concentration to fall from ~74 to ~19 $\mu$g P L$^{-1}$ (a change similar to that found in the 0–20 m zone during our study, 56 to 16 $\mu$g P L$^{-1}$), the coupling between bacterio plankton and herbivorous zooplankton was enhanced (Teubner et al., 2003). Hence, based on P availability, our findings would seem to support the theory and the experimental results indicating that the coupling between zooplankton and phytoplankton under nutrient-poor conditions is different from that under conditions with moderate nutrient levels (e.g. Elser and Goldman, 1991).

Positive effects of climate-related factors on phytoplankton in Lake Geneva

Results from other large deep lakes have indicated that the phytoplankton variables declined when the TP concentration dropped below a threshold that varies from lake to lake. For example, in Lake Maggiore, phytoplankton biomass clearly declined when the decrease in TP reached 10 $\mu$g L$^{-1}$ (Manca and Ruggiu, 1998). In Lake Washington, the areal production declined sharply when the TP concentration fell below 20 $\mu$g L$^{-1}$ (Smith, 1979). In Lake Constance, the annual mean areal phytoplankton production consistently declined when the TP measured during the winter circulation dropped below 35 $\mu$g L$^{-1}$ (Häse et al., 1998). For deep lakes, it is generally thought that significant and sustained changes in phytoplankton variables and water transparency occur when TP in the pelagic zone of the lake falls below 30 $\mu$g L$^{-1}$ (Sas, 1989). One can speculate that the reduction of P in Lake Geneva, more than 30 years after the implementation of measures, is still not sufficient to cause such changes (note however that the mean annual TP in the whole lake dropped below 30 $\mu$g L$^{-1}$ after 2003, unpublished results). Our data indicate that the positive influence of climate-related factors, namely incident light and WCS, was the other reason (apart from the influences of nitrogen and zooplankton) why the responses of phytoplankton to P reduction deviated from those expected. The effect of light was supported by the rise of primary production during the study in relatively deep waters, despite the decline in the water transparency (Figs 1C and 5D). A positive effect of light on phytoplankton is not surprising, as light is necessary for photosynthesis. To our knowledge, this is the first study showing that an increase in WCS has a long-term positive impact on phytoplankton productivity. Other studies showing similar positive relationships have lasted only 1 or 2 years (Perissinoto et al., 1990, Tadonléké et al., 2000). Such results indicate that the photosynthetic apparatus of phytoplankton communities successfully adjusts to the physical variability of the water column. Previous studies in Lake Geneva have not explored the reasons for the overall dominance of large cells, particularly during periods of low phosphorus availability [e.g. the dominance of species such as Diatoma tenuis, Mougeotia gracillima and Oscillatoria limnetica since the 1990s (Anneville et al., 2002; Druart et al., 2006)]. Inconsistent results have been reported about the dominance of large versus small phytoplankton in lakes during re-oligotrophication (e.g. Willen, 2001; Dokulil and Teubner, 2005); however, in these studies, phytoplankton biomass declined within 5 years after P reduction. Our results suggest that WCS has favored large/filamentous phytoplankton in Lake Geneva, and together with the inedibility of these large prey to zooplankton, the ability of species to acquire nutrients and the above-mentioned nitrate effects, helps to explain the
continuously high phytoplankton biomass. Previous studies indicate that physically stable water columns favor large cells by reducing their sedimentation rates (Cushing, 1989). The WCS might thus be one of the factors that shape phytoplankton size-structure during re-oligotrophication in large deep lakes (especially those experiencing warming), as they are less affected by wind than small, shallow lakes. This factor and the incident light are not often considered when exploring the influence of climate on phytoplankton in lakes, particularly during re-oligotrophication. Our data indicate that they altered phytoplankton responses and should thus be taken into account in such studies.

To summarize, this study has shown that phytoplankton biomass and production increased and water transparency decreased in Lake Geneva, contrary to expectations following P reduction. Our analyses show that the responses observed were mainly due to the fact that the major factors driving PP during the first part of the study (1972–1988) were nitrogen, *Daphnia* and WCS, rather than P, and that when P became scarce and the main nutrient driving PP (after 1988), PP still increased, partly as a result of long-term increases in light and WCS. *Daphnia* coupling with phytoplankton switched from positive during the period of P abundance to negative during the period of P scarcity, suggesting a change in the influence of *Daphnia* on phytoplankton, which would be consistent with theory. These data support the view that increases in the major factors driving PP (after 1988), PP still increased, partly as a result of long-term increases in light and WCS. *Daphnia* coupling with phytoplankton switched from positive during the period of P abundance to negative during the period of P scarcity, suggesting a change in the influence of *Daphnia* on phytoplankton, which would be consistent with theory.

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