

Effects of limiting nutrients and N:P ratios on the phytoplankton growth in a shallow hypertrophic reservoir

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Abstract The purpose of this study was to evaluate the effects of limiting nutrients and the N:P ratios on the growth of phytoplankton (mainly cyanobacteria) in a shallow hypertrophic reservoir between November 2002 and December 2003. Nutrient enrichment bioassays (NEBs) were conducted, along with analyses of seasonal ambient nutrients and phytoplankton taxa, in the reservoir. The average DIN:TDP and TN:TP mass ratios in the ambient water were 90 (range: 17–187) and 34

(13–60), respectively, during the study period. The dissolved inorganic phosphorus showed seasonal variation, but less than that of inorganic nitrogen. The TN:TP ratios ranged from 13 to 46 (mean: 27 ± 6) during June–December when the cyanobacteria, *Microcystis*, dominated the phytoplankton composition. The NEBs showed that phytoplankton growth was mainly stimulated by the phosphorus (all of total 17 cases), rather than the nitrogen concentration (8 of 17 cases). The rapid growth rate of cyanobacteria was evident with TN:TP ratios less than 30. According to the results of the NEBs with different N concentrations (0.07, 0.7 and 3.5 mg l⁻¹), but the same N:P ratios and when the nitrogen concentration was highest, the cyanobacterial growth reached a maximum at N:P ratios <1. Overall, the response of cyanobacterial growth was a direct function of added phosphorus in the NEBs, and was greater with increased N concentrations. Thus, cyanobacterial blooms favored relatively low N:P ratios in this hypertrophic reservoir system.

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Eutrophication of shallow lakes with special reference to Lake Taihu, China

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Introduction

The nitrogen-to-phosphorus ratio (N:P) in lentic systems has been frequently used as a key

indicator in predicting algal biomass and compositions, and its seasonal succession (Tilman, 1982; Kilham, 1990). It can also serve as an index that represents the nutrient limitation for the algal growth (Smith, 1983; Fugimoto & Sudo, 1997). The atomic ratio, 16N:1P, which is known as the Redfield ratio, has been used for generally describing the average elemental composition of phytoplankton. Many investigations; however, have shown this ratio to vary considerably, depending on the algal species and surrounding environmental conditions (Rhee, 1978; Forsberg & Ryding, 1980; Geider & La Roche, 2002; Ho et al., 2003), and that optimum nutrient ratios vary interspecifically within the diatoms due to growth competitions (Tilman, 1977, 1978).

A considerable variation in the nutrient stoichiometry of the natural phytoplankton community is a reflection of the type and extent of nutrient limitation and availability. In turn, this is likely related to the supply ratios of the elements loaded to the lake affecting the development of particular phytoplankton communities (Smith, 1983; Smith et al., 1987; Sommer, 1989; Fujimoto & Sudo, 1997; Xie et al., 2003). The hypothesis of “the dominance of cyanobacteria at low N:P ratios” is one of these examples. Smith (1983) pointed out that bloom-forming cyanobacteria had a tendency to dominate the lake when the N:P ratio was less than 29. The significance of the N:P ratio as a critical standard, however, is still controversial, due to variabilities in the other chemical characteristics and phytoplankton compositions within a geographic region.

Unlike Smith's ‘N:P ratio rule’, which stated that the relative proportion of cyanobacteria to the total algal biomass might decrease when the N:P ratio exceeded 29 (Smith et al., 1987), the bloom of cyanobacteria was affected by increases in the phosphorus concentration rather than simply by a decrease in the N:P ratio (Trimbee & Prepas, 1987; Sheffer et al., 1997; Xie et al., 2003). Moreover, the possibility that the growth response of algae to the limiting nutrient would be inconsistent with the N:P ratio rule in eutrophic systems that had greater amounts of nitrogen and phosphorus than are required for algal growths (Paerl et al., 2001). These results suggest

that the nutrient stoichiometry that algae require for growth would vary with the extent of nutrient deficiency and availability.

In this study, we evaluated the response of phytoplankton growth in relation to different ambient concentrations of N and P, and N:P ratios, in a hypertrophic reservoir where the nitrogen background concentrations were high. For these experiments, we conducted nutrient enrichment bioassays (NEBs) and compared the growth rates of phytoplankton among several treatments of the NEBs, along with seasonal analyses of the ambient nutrients and phytoplankton compositions.

Materials and methods

The study system

Singu Reservoir is a small agricultural reservoir, with surface area of 0.1 km² and maximum depth of 7.0 m, which has two inflowing streams with channel length of 1.3 and 3.4 km, respectively. The total watershed area is 0.255 km², with the water quality mainly influenced by the point source of livestock wastes (KARICO, 2001). The watershed of the reservoir is located in Monsoon climate region, and precipitation is concentrated during June–September. The use for irrigation affects the water level of the reservoir (usually between May and June). Thus, both irrigation and Monsoon rainfall affect both water quality and water level of the reservoir.

Limnological variables

Various limnological variables including temperature, dissolved oxygen (DO) concentration, pH, and electric conductivity (EC) were measured by Hydrolab data logger (RE-232/SDI-12) in situ. Temperature and DO were measured vertically at 20–50 cm intervals. Transparency was measured with a white disk with 20 cm diameter. Water level was measured as the maximum depth at each time. Rainfall data were obtained from the nearest meteorological observation center (Boryung Meteorological Observation Center, Chungnam Province, S. Korea).

Chemical analyses

The surface water was collected biweekly from the reservoir between November 2002 and December 2003. Stream water samples were also collected from an inflowing location prior to entering the reservoir. The discharge volume from the reservoir was estimated through the linear multiplication of the water velocity and the channel area.

Five liter water samples were collected for analyses of nutrients and phytoplankton taxa, using polyethylene bottles, which were kept in a dark ice-box during transportation. On arrival at the laboratory (within 5 h), the water samples were filtered through GF/F filters, and the filtrate used to measure the concentration of chlorophyll *a* (Chl *a*). Phosphorus fractions, such as dissolved inorganic phosphorus (DIP), total dissolved phosphorus (TDP) and total phosphorus (TP), were analyzed in triplicate using the ascorbic acid method from the standard method of the APHA (1995). The $\text{NH}_4^+\text{-N}$ concentrations were measured using an indophenol reagent, and those of nitrate–nitrogen ($\text{NO}_3^-\text{-N}$) and nitrite–nitrogen ($\text{NO}_2^-\text{-N}$) using the cadmium reduction method (APHA, 1995). The total nitrogen (TN) was measured using the cadmium reduction method, following persulfate digestion. Dissolve inorganic nitrogen (DIN) was obtained from the sum of $\text{NO}_3^-\text{-N}$, $\text{NO}_2^-\text{-N}$ and $\text{NH}_4^+\text{-N}$. The Chl *a* concentration was analyzed using the methanol extraction method of Maker et al. (1980).

Phytoplankton analysis

Water samples for the identification of phytoplankton species and the measurement of cell densities were collected from a 0.5–1 m depth from the reservoir, stored in Whirl-Pak bags and fixed with Lugol's solution (2%, final conc.). The cell density was measured using the Sedgwick-Rafter counting chamber under microscopic magnification of 200–400 \times . The phytoplankton composition was identified as containing three major and two minor taxa; Bacillariophyceae, Cyanophyceae and Chlorophyceae, and Dinophyceae and Cryptophyceae, respectively.

Nutrient enrichment bioassays (NEBs)

In addition to the regular sampling, nutrient enrichment bioassays (NEBs) were conducted for the evaluations of the limiting nutrients that regulate phytoplankton growth. The experiments for the evaluation of NEBs were conducted in a shaking incubator at a temperature of 20–25°C and light intensity of $100 \pm 5 \mu\text{E m}^{-2} \text{s}^{-1}$. For the experiments, the water was filtered using 100 μm mesh to minimize the effects associated with zooplankton grazing. Aliquots of filtrate (50 ml) were placed in 250 ml flasks, followed by the addition of KNO_3 and KH_2PO_4 as nutrient sources, to final concentrations of 1 mg l^{-1} for both nutrients. The final volume was made up to 100 ml with the addition of GF/F filtrate. A set of algal bioassay was conducted in triplicate sets for the control (no addition), +N (addition of nitrogen) and N + P (addition of nitrogen and phosphorus), and the responses measured as the difference between the final and initial Chl *a* at 0, 1, 3, 5 and 7 days after incubation. The growth rate (μ : day^{-1}) under each set of treatment conditions was calculated using the equation of the APHA (1995), as follows:

$$\mu (\text{day}^{-1}) = \ln (X_2/X_1)/(T_2 - T_1)$$

where, X_1 = the concentration of Chl *a* at the initial incubation stage (T_1 h);
 X_2 = the concentration T_2 h

Other triplicate experiments to measure the phytoplankton growth rate were conducted with five different P concentrations (+0.05, 0.1, 0.5, 1.0 and 1.5 mg l^{-1} , as KH_2PO_4 spiking), with nitrogen fixed at 1 mg l^{-1} . The medium into which different P concentrations were added was the same GF/F filtered lake water as above. The nitrogen concentrations of the medium used in the NEBs were similar between the treatments categorized with different N:P ratios. The spiking concentration of phosphorus, however, varied within the range 0.05– 1.5 mg l^{-1} .

The DIN:TDP mass ratio was calculated using the concentration measurements from the

subsamples taken from each treatment prior to the starting of the NEBs. The maximum growth rate (μ_{\max}) and half saturation constant (K_{μ}) were calculated as values to the phosphorus concentrations, with the corresponding growth rate (μ) obtained from the model of Monod (1950) employing the Sigma plot program (Version 7.0, SPSS Inc.).

Analysis of cyanobacterial growth under different N:P ratios

Using the same N:P ratio, the cyanobacterial growth rates were determined for different N and P concentrations. The incubation conditions were maintained at 20°C, with a light intensity of $53 \pm 2 \mu\text{E m}^{-2} \text{s}^{-1}$ (L:D cycle = 12:12). The light intensities used were values observed during the study period at the water depths from which the samples were taken. The cyanobacteria were collected by vertically pulling up a 30 μm pore size plankton net, with the collected sample then filtered through a 100 μm net to remove the large zooplankton. In order to isolate the cyanobacteria from composite phytoplankton, samples were placed in a large-mouth container for 24 h, and the cyanobacteria that rose to the surface due to their buoyancy were transferred by pipette to a GF/F pre-filtered lake water.

Cyanobacterial cells were starved in MA2 medium with phosphorus and nitrogen excluded. After 2-week incubation, a 2 ml aliquot of the cyanobacteria sample was placed in an acid-washed 250 ml flask, along with MA2 medium

that had been modified with different phosphorus and nitrogen concentrations. The N:P mass ratio was controlled by changing the amount of phosphorus with respect to fixed portions of nitrogen (0.07, 0.7 and 3.5 mg l^{-1}). Triplicate treatment sets were incubated for 5 days, and the cell growth determined as the difference between initial Chl *a*, prior to the incubation, and that 5 days later.

Statistical analysis

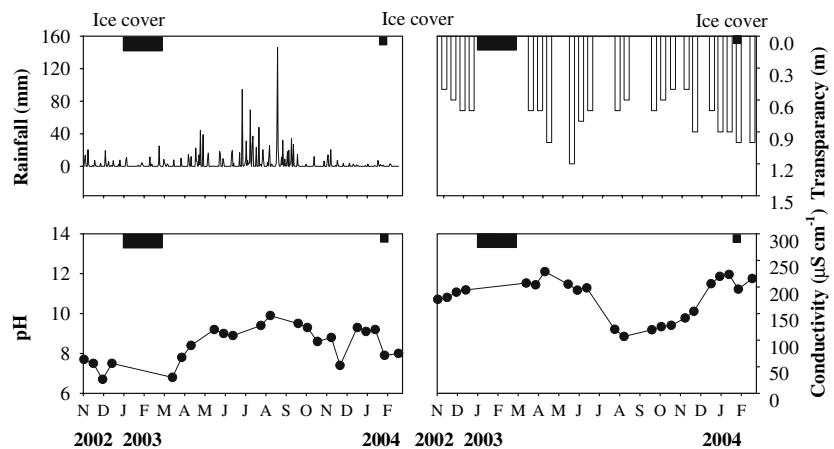
Statistical analyses were conducted between the variables using Pearson's correlation (SPSS 10.0). The differences in the growth response between the various NEB treatments were analyzed by an ANOVA, and the level of significance identified at $P < 0.05$.

Results

Variation of limnological variables

Rainfall during the study period was concentrated between June and September (41.5 mm in June, 391 mm in July, 79.5 mm in August, and 393 mm in September) (Fig. 1). The use for irrigation between the end of May and mid June decreased the reservoir water level from 7.1 to 5 m. The water level returned to 7 m due to seasonal monsoon rainfall during June–July. Transparency of the reservoir varied seasonally with the peak during May. The time of low transparency was

Fig. 1 Seasonal changes of precipitation, Secchi transparency, pH and electric conductivity in Shingu Reservoir during November 2002–February 2004



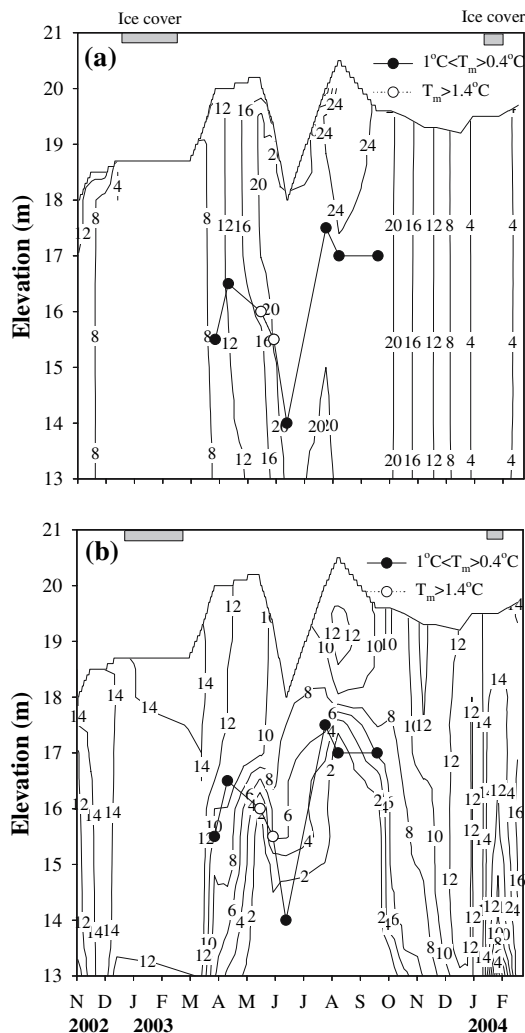


Fig. 2 Temporal and spatial changes of temperature (a) and dissolved oxygen concentration (b) in Shingu Reservoir during November 2002–February 2004. The line with black circles indicates the vertical temperature difference between 0.4 to 1.0°C, and the open-circled line indicates the vertical temperature difference greater than 1.0°C

coincided with the period of high pH, but EC variation was almost mirrored with pH. The reservoir had a weak thermal stratification near the hypolimnion, which persisted annually from mid-June to October (Fig. 2). Hypoxia $<2 \text{ mg O}_2 \text{ l}^{-1}$ in the hypolimnion was frequently observed from the end of May, and was most intense during mid-August. Hypolimnetic hypoxia was maintained until late October, the beginning of fall.

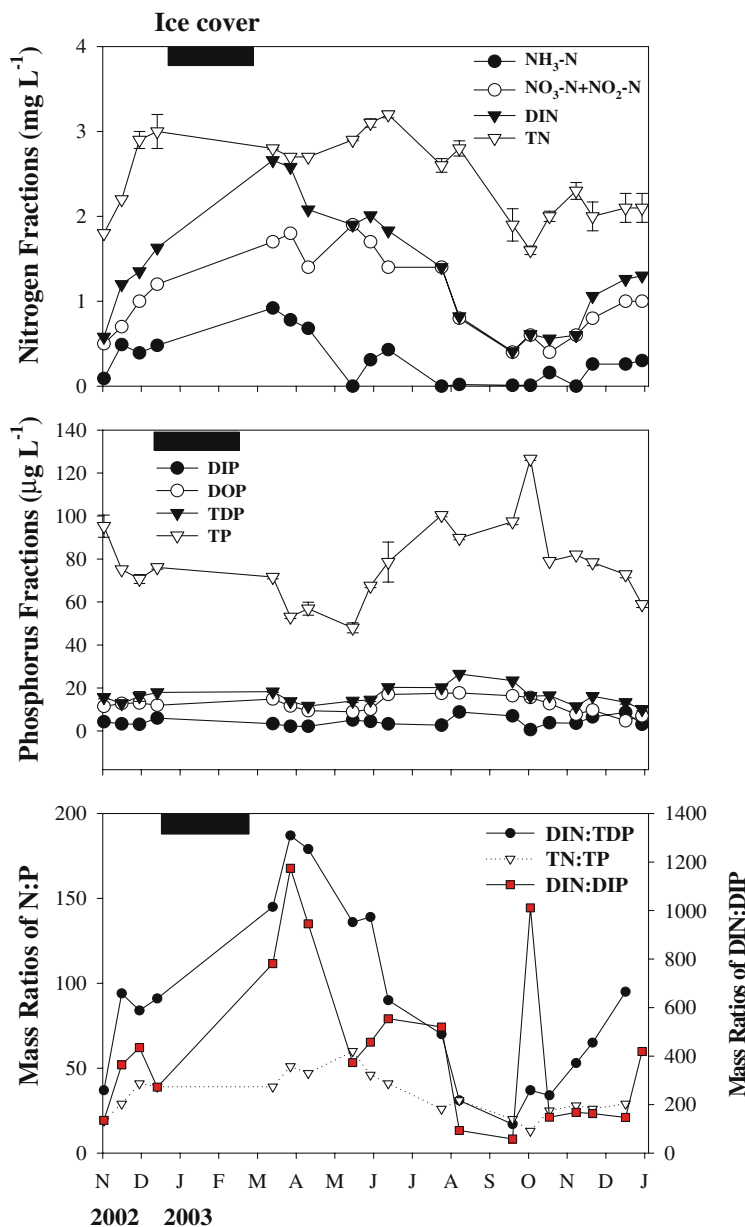
Variation of nutrients and phytoplankton

During the study period, the TN concentrations in the reservoir were $>1.2 \text{ mg l}^{-1}$ ($1.6\text{--}3.2 \text{ mg l}^{-1}$) (Fig. 3). The TN was mainly composed of $\text{NO}_3\text{-N}$, rather than $\text{NH}_4\text{-N}$, with $\text{NO}_2\text{-N} + \text{NO}_3\text{-N}$ and $\text{NH}_4\text{-N}$ ranging from 0.4 to 1.9 and 0.01 to 0.92 mg N l^{-1} , respectively (Fig. 3). Dissolved inorganic N peaked in early spring (March–April), then abruptly decreased during May–August. Thus, the variation in the dissolved inorganic N was greater than that of the TN. DIP concentration ranged from 0.6 to $8.8 \text{ } \mu\text{g P l}^{-1}$ and varied not much during the study period. DTP ranged from 10.1 to $25.6 \text{ } \mu\text{g P l}^{-1}$, with the peak concentration during August and minimum during December. The average TP was $76.4 \text{ } \mu\text{g P l}^{-1}$ and showed large seasonal fluctuations ($48.0\text{--}126.6 \text{ } \mu\text{g P l}^{-1}$). The TP increased from June, reaching a maximum in October. This reservoir was adjudged to be hypertrophic, based on the conventional criteria of nitrogen and phosphorus (Forsberg & Ryding, 1980). The TN:TP mass ratios in this hypertrophic reservoir were <60 , whereas those of DIN:TDP increased to >150 during March–June.

The concentration of Chl *a* varied from 28.8 to $109.7 \text{ } \mu\text{g l}^{-1}$ during the study period, and showed a similar seasonal pattern to that of the algal cell density (Fig. 4). The Chl *a* was at a minimum during early spring of 2003, but began to increase from May when cyanobacteria dominated the phytoplankton community. Thus, Chl *a* maxima $>90 \text{ } \mu\text{g l}^{-1}$ were observed between July and November, which coincided with the peaks in the cell density (Fig. 4). The Chl *a*:TP ratios, the Chl *a* yield for a given phosphorus concentration, were >1.0 , indicative of a massive algal bloom. Pearson's correlation analyses showed the Chl *a* concentrations to be positively correlated ($r = 0.66$, $P = 0.002$, $n = 19$) with the TP, but negatively ($r = -0.48$, $P = 0.042$, $n = 18$) with the TN. This result coincided with the inverse relations between Chl *a* and the TN:TP ratio ($r = -0.70$, $P < 0.001$, $n = 18$).

The cell densities (cell number ml^{-1}) in the reservoir showed high seasonal fluctuations. The community structure was characterized by the dominance of cyanobacteria during most of

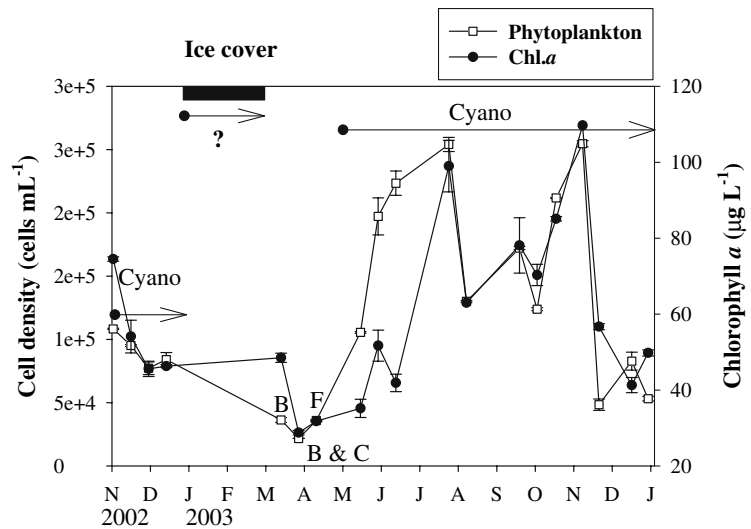
Fig. 3 Seasonal changes in the nitrogen and phosphorus fractions and N:P ratios in Shingu Reservoir during November 2002–December 2003



the year, with the exception of a short period during spring, which was dominated by a few other phytoplankton, such as a diatom (*Melosira varians*) and a green alga (*Dictyosphaerium puchellum*), and cryptomonads (*Rohdomonas* spp.) (Fig. 5). The dominant taxa of cyanobacteria were *Oscillatoria* spp., *Microcystis* spp. and *Aphanizomenon* sp., which dominated from May until the winter ice season. Seasonal succession of the

phytoplankton community showed that *Oscillatoria* spp. dominated at the beginning of the study period, which was then followed by *Aphanizomenon* sp. in late spring when the water level of the reservoir abruptly decreased. During the study, the major species causing the algal bloom was identified as *Microcystis* spp. (Fig. 5), and the high Chl *a* $>60 \mu\text{g l}^{-1}$, continued during July–December 2003 (Fig. 4).

Fig. 4 Seasonal changes in the phytoplankton cell density and chlorophyll-*a* in Shingu Reservoir during November 2002–January 2004. Cyano, B, C and F denote cyanophytes, bacillariophytes, chlorophytes and flagellated algae, respectively



Phytoplankton growth under limiting nutrients and various N:P ratios

Overall, the results of the NEBs experiments showed phosphorus as the key nutrient regulating the phytoplankton growth in the system (Fig. 6, lower panel), but nitrogen was also considered an important nutrient during a short period (Fig. 6, upper panel). Phosphorus enrichment caused a significantly ($P < 0.001$, $n = 17$, ANOVA) greater growth rate than that seen in the control and nitrogen treatments (Fig. 6), indicating P-limitation of the algal growth in the system. No difference was found in the NEBs between the P and N + P additions ($P = 0.229$, $n = 17$, ANOVA). Six of the 17 NEBs experiments (20 July, 8 August, 21 September, 2 October, 16 October, and 1 November) showed significantly ($P < 0.05$, $n = 6$, ANOVA) higher growth rates with the N + P treatments than the P, N treatments and control. This outcome suggests that simultaneous limitation of nitrogen and phosphorus occurred between July and early November 2003. In the mean time, only 3 cases (November 2002, March and April 2003) of the NEBs had greater growth rates in the +P than the N + P treatments (Fig. 6).

The phytoplankton growth was largely determined by the magnitude of P, although the ambient N and P concentrations were different for the same N:P ratio (Fig. 7). The growth rates over a wide range of DIN:TDP ratios decreased

with increasing ratio, but the most noticeable decrease was observed at ratios above 30 (Fig. 7). The growth rate consistently increased until the P concentration reached $50 \mu\text{g l}^{-1}$ (Fig. 8). The results plotted using the equation of Monod (1950) indicated a maximum growth rate of 0.54 day^{-1} and with a half saturation concentration of $33.1 \mu\text{g l}^{-1}$ for phosphorus. The maximum growth rate occurred in those treatments enriched with phosphorus alone or with both phosphorus and nitrogen. The treatments enriched with only nitrogen (+N) had lower growth rate, with the exception of one case during December 2002.

Cyanobacterial growth variation under different N:P ratios

The magnitude of the cyanobacterial growth responses showed differences with respect to the N:P ratios and N concentrations for the same N:P level. The N:P ratios under 20 displayed large differences in the growth rate with different nitrogen concentration (Fig. 9a), with the growth level tending to increase with increasing nitrogen concentration (Fig. 9b). The difference in the growth rates between 0.7 and 3.5 mg N l^{-1} were smaller ($P = 0.86$, ANOVA) than that of the lowest concentration, 0.07 mg N l^{-1} ($P = 0.001$, ANOVA).

At a constant N:P ratio, but with variable concentrations of N, the cyanobacteria showed

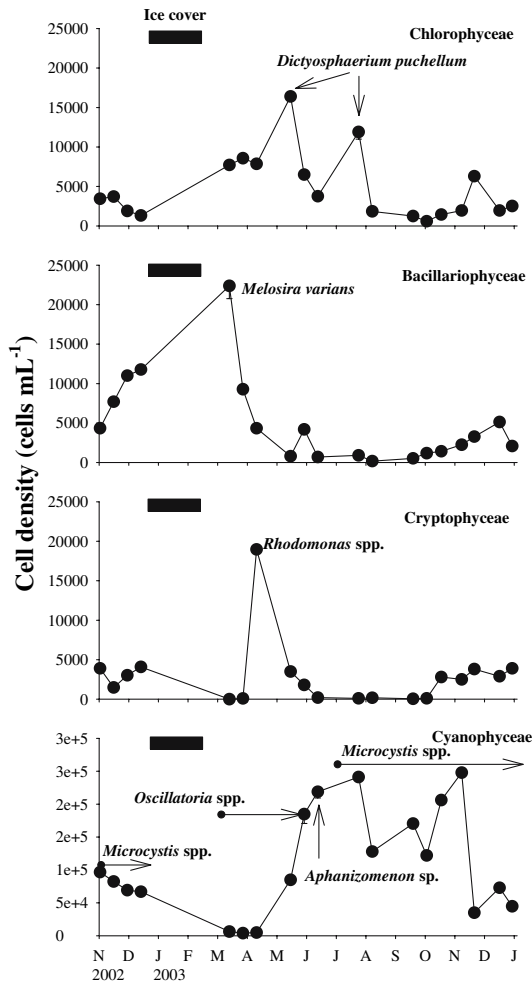


Fig. 5 The seasonal succession of dominant phytoplankton taxa in Shingu Reservoir during November 2002–December 2003

different growth patterns in the NEBs (Fig. 9c). The growth response reached a maximum at an N concentration of 3.5 mg l^{-1} when the N:P ratio in the NEBs was 1.0. The growth tended to decrease as the N:P ratio increased ($P < 0.01$, ANOVA) (Fig. 9c). A nitrogen concentration at 0.7 mg N l^{-1} caused the highest algal growth when the N:P ratio was 0.7 ($P < 0.05$, ANOVA). The growth rates were similar within the N:P ratio range 1–10 ($P > 0.5$, ANOVA), while the level of growth tended to decrease for N:P ratios above 10 ($P = 0.009$, ANOVA) (Fig. 9a). Conversely, the lowest nitrogen concentration, 0.07 mg N l^{-1} , showed a much lower level of growth, especially when the N:P ratio was

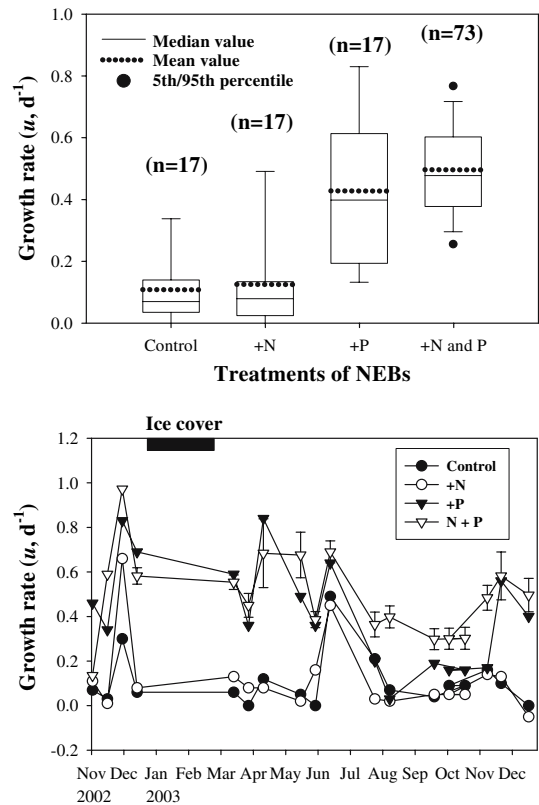


Fig. 6 The growth rates of phytoplankton toward various treatments (control, +N, +P, and N + P) in the NEBs, during November 2002–December 2003 (upper panel), and the seasonal growth response to nutrient additions in the NEBs (lower panel)

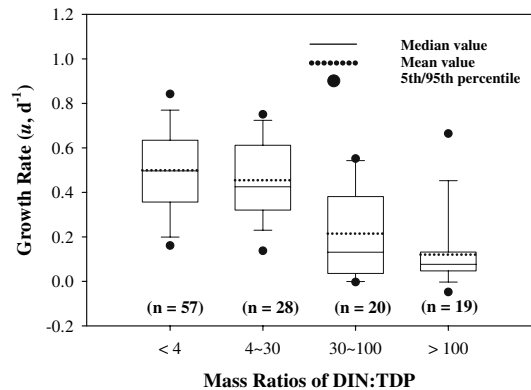


Fig. 7 The growth rates of phytoplankton at various DIN:TDP ratios

smaller than 1. Ratios >7 tended to give greater phytoplankton growth ($P < 0.004$, ANOVA) (Fig. 9c), compared to ratios <1 .

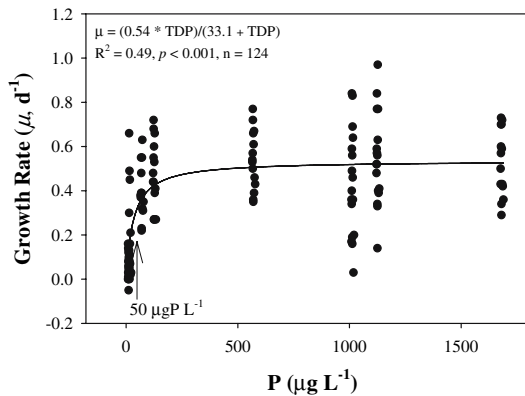


Fig. 8 Phytoplankton growth rate at various P (TDP = ambient + addition) concentrations using natural phytoplankton assemblages during November 2002–December 2003

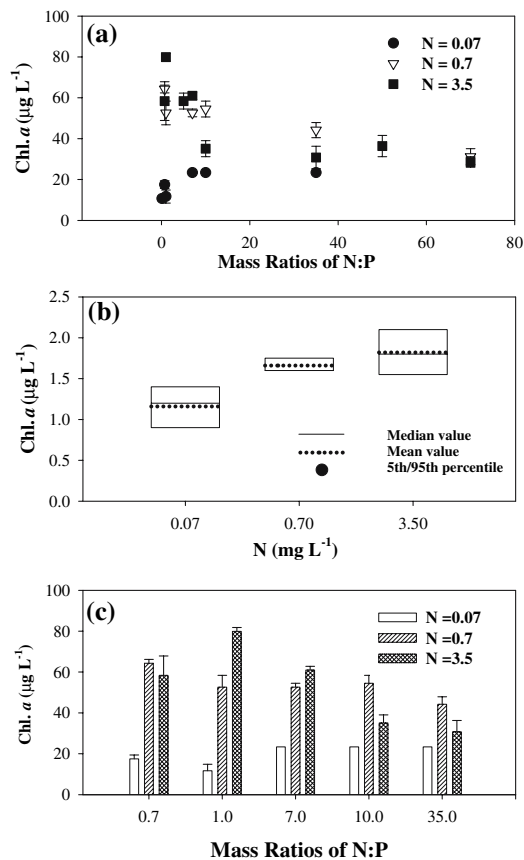


Fig. 9 Response of chlorophyll *a* to the N:P mass ratios (upper panel), and cyanobacterial responses to nitrogen (mid panel) and the N:P ratios (range: 0.7–35.0) at various nitrogen concentrations (lower panel)

Discussion

As important factors for determining the growth of phytoplankton, the ambient nutrient concentrations and nutrient ratios, known as stoichiometry (e.g., Sterner & Elser, 2002), are being widely used to estimate the physico-ecological consequences, such as the algal growth and succession of specific phytoplankton compositions (Goldman et al., 1979; Tilman, 1982; Sommer, 1989). An explanation for the mass conservation in aquatic organisms is that the external nutrient supply determines the cellular element composition of algae, which was supported by the earlier seminal work on stoichiometry (Redfield et al., 1963; Rhee, 1973) in an oligotrophic ocean and culture medium. The rules determining the constraints on stoichiometry; however, have been understood to vary depending on the organism, feeding mode and metabolic process (Geider & La Roche, 2002; Sterner & Elser, 2002; Ho et al., 2003).

A degree of nutrient deficiency and differential nutrients loading to a lake are also constraints on the stoichiometric variation and; thus, cause not only an imbalanced algal growth, but also lead the development of certain algal populations that have adapted to that particular situation (Smith et al., 1987). This implication calls attention to the need for eutrophic systems to be seen as having more variable and complicated limiting nutrients and critical nutrient ratio dynamics (Terry et al., 1985; Leonardos & Geider, 2004). Baring this in mind, our main concern in this study was to elucidate how algal growth (usually cyanobacteria) in a hypertrophic reservoir responded to both variations in ambient nutrient regimes and the N:P ratio in the NEBs in relation to the ambient N:P ratio.

Our major finding was that the phytoplankton (mostly cyanobacteria) in our hypertrophic study reservoir were P-limited and the degree of their growth in P addition increased with higher N concentrations added, indicating that at high nitrogen level, low N:P ratios were favorable for cyanobacterial development. These results suggest that phytoplankton in an N-rich environment appeared to demand a large amount of P to enable the use of a large supply of both N and P simultaneously to meet the great biomass

building. These results also suggest that higher nitrogen concentration is likely to induce stronger P-limitation on the phytoplankton growth, and are supported by the inverse relationship between the TN:TP ratio and Chl *a* concentration ($r = -0.7$, $P < 0.001$; Figs. 3, 4). An evident increase in the growth rate was observed with TN:TP ratios < 30 , which was sustained with increases in TDP up to $50 \mu\text{g l}^{-1}$, but reached a plateau thereafter (Figs. 7, 8).

With respect to the seasonal succession of phytoplankton, the variation in the N:P ratio appeared to be related with the cyanobacterial predominance in the reservoir. Due to the characteristic blooms in lentic systems with human-induced eutrophication, there have been numerous studies regarding the development of cyanobacteria and the interspecific changes of phytoplankton within eutrophic systems (Smith, 1983; Smith et al., 1987; Xie et al., 2003). The TN:TP ratios during the cyanobacterial blooms were 27 ± 6 , almost coinciding with the criteria of Smith (1983), who suggested a high possibility of cyanobacterial dominance at TN:TP ratios under 29. The phytoplankton growth rate to specific ambient nutrient concentrations and N:P ratios, via the nutrient enrichment experiments, suggests that the level of the nutrient limiting ratios was frequently found near 30 (Fig. 7), even if the criteria are still controversial. P limitation was observed with ratios exceeding this level, but no N-limitation was detected at ratios below 30. The possibility of N-limitation was only detectable under low N concentration conditions, even in the experiment subjected to the cyanobacteria (Fig. 9).

The causes of these results were suspected to be due to the special characteristics of our reservoir, which maintained much higher nitrogen than phosphorus levels, as well as DIN:TN ratios (53%) much higher than those for TDP:TP (23%). The N:P ratio standard for evaluating the possibility of potential P and N limitation would vary in cases where the absolute concentrations were different, even though the N:P ratio would be maintained at the same level. Also, the eutrophic system where the concentration of N is much higher than that of P might create the possibility of N limitation being relatively lower

than that of phosphorus, even with a decreasing N:P ratio. This result suggests that any standard for evaluating the limiting nutrient should consider the factors, not only the characteristics of phytoplankton species (Tilman 1977, 1978; Rhee, 1978), but also the absolute concentration of the nutrients existing within the aquatic system. A relative difference in the concentrations of the nutrients is necessary to endorse the growth of phytoplankton, rather than simply by their determination using the relative ratios of the nutrients in various aquatic systems with various nutrient stati (Leonardos & Geider, 2004).

Even though a number of studies have suggested the possibility of nitrogen-fixing cyanobacteria as the dominant species during periods of decreased N:P ratio (Horne, 1979; Tilman, 1982; Howarth et al., 1988; Paerl et al., 2001), our result indicated there was a dominant species shift, from *Aphanizomenon* to *Microcystis*, during periods when the N concentration decreased, and after completing the stratification, it has been suggested that the nitrogen concentration may not be the cause of the cyanobacterial species succession. The level of inorganic N tends to decrease at a higher rate than that of inorganic P during the algal growth period in both oligotrophic and eutrophic lakes and; thus, the growth limiting factor could change from P to N during the stratification period (Hendrey & Welch, 1974). Under this condition, dominant cyanobacteria species could succeed to taxa armed with the ability to fix nitrogen (Horne, 1979; Tilman, 1982; Howarth et al., 1988; Paerl et al., 2001). However, the nitrogen fixing *Aphanizomenon* which was observed before establishing the stratification had shifted to non-N fixing *Microcystis* during the period of N:P ratio decrease, which was caused by the drop in the N concentration and the rise in the P concentration after stratification had been established. Therefore, phosphorus seemed to be one of the most substantial factors influencing the dominance of *Microcystis* (Xie et al., 2003). Our result is also supported by Fujimoto & Sudo (1997), who showed that *Microcystis aeruginosa* was a superior competitor under low N:P supply ratio conditions than the filamentous cyanobacterium, *Phormidium tenue*.

A low phytoplankton growth rate was demonstrated, even in the experiments with P or N + P additions between July and October (Fig. 6), which suggests the physiological property of phytoplankton needs to be considered when predicting their growth response, rather than simply relying on the nutrient concentration and N:P ratio (Droop, 1968; Rhee & Gotham, 1981). Due to the luxury of P consumption, cyanobacteria could store enough P in the cell, so their growth would not necessarily rely on the ambient phosphate concentration (Goldman et al., 1987). The DIN:DTP ratio in our reservoir between July and October was in the range 17–37, with the exception of July (DIN:DTP = 70), and this range seemed to be comparable to the optimum DIN:DTP ratio (<30) (Fig. 7) for phytoplankton growth and; thus, both P and N could function as potential limiting nutrients. On the other hand, there could have been an excess amount of external phosphorus supplied to the phytoplankton with the inflowing discharge around this period (Table 1). Moreover, after the end of May, the phosphorus released from the sediment under hypo-oxic conditions (Kim, 2004) could have been supplied to the surface by wind disturbances. Therefore, these phosphorus additions could have resulted in the low growth rate. During August and September especially, when DIP concentration in the water column was at the maximum seasonal concentration, there had been a lot of standard precipitation prior to sampling. The precipitation from June to September accounted for 66% of the total annual precipitation (1,359 mm) and; thus, this concentrated precipi-

tation may have been responsible for increasing the discharge and P loading from the watershed. The increase in the TP concentration with both decreasing N and Chl *a* concentrations during this period may also be reflected by the increase of inorganic particulate matter.

Considering the mass conservation between an algal cell and the external nutrient supply, there should be no problem in using any type of nutrient for evaluating nutrient limitation (e.g., Sterner & Elser, 2002); for example, cellular or external and inorganic or organic forms. However, the factors causing variations in the cellular stoichiometry, such as the physiological status and the property of nutrient storage in phytoplankton species, need to be carefully considered to understand the ecological stoichiometry. The TN:TP ratios have been most frequently used as a measure, or criterion, to evaluate cyanobacterial blooms and limiting nutrients (Forsberg & Ryding, 1980; Smith, 1983; Trimbee & Prepas, 1987; Fujimoto & Sudo, 1997; Sheffer et al., 1997; Xie et al., 2003). Also, the DIN:DIP (Rhee, 1978; Patricia et al., 2000), DIN:OP or DIN:TP (Perkins & Underwood, 2000) ratios have been employed as surrogates of the TN:TP ratio for the assessment of lake conditions. In this study, the TN:TP ratios were closely related to the cyanobacterial density and nutrient ratios, which agreed with the growth response, as based on the soluble fractions of the DIN:TDP ratios. Even though organic N could be used for phytoplankton growth in the long run, the DIN content of our reservoir was 45 times higher than that of the DIP (less than 8.0 $\mu\text{g l}^{-1}$), and the average DIP

Table 1 Ambient nutrients, chlorophyll-*a* (Chl *a*) and discharge in two inflow streams and the outflow of Shingu Reservoir between May and October, 2003

Date	Inflow 1			Inflow 2			Outflow		
	Discharge $\text{m}^3 \text{ day}^{-1}$	TN mg l^{-1}	TP $\mu\text{g l}^{-1}$	Discharge $\text{m}^3 \text{ day}^{-1}$	TN mg l^{-1}	TP $\mu\text{g l}^{-1}$	TN mg l^{-1}	TP $\mu\text{g l}^{-1}$	Chl <i>a</i> $\mu\text{g l}^{-1}$
30 May	–	2.1 ± 0.03	197.7 ± 0.8	–	3.5 ± 0.10	315.8 ± 11.2	–	–	–
13 Jun.	–	6.2 ± 0.17	143.2 ± 2.3	–	7.6 ± 0.08	125.3 ± 9.3	–	–	–
25 Jul.	3,606	5.8 ± 0.04	94.4 ± 0.8	31,548	2.9 ± 0.17	89.8 ± 0.8	3.2 ± 0.10	134.4 ± 3.0	132.0 ± 4.6
8 Aug.	384	1.6 ± 0.04	74.9 ± 0.7	6,150	2.4 ± 0.12	91.2 ± 0.7	2.9 ± 0.13	86.8 ± 0.7	55.6 ± 2.7
19 Sep.	1,051	1.7 ± 0.25	96.7 ± 0.7	7,833	2.5 ± 0.17	105.3 ± 0.7	–	–	–
3 Oct.	303	1.4 ± 0.01	94.6 ± 2.8	2,378	2.5 ± 0.06	68.2 ± 2.1	–	–	–
18 Oct.	370	1.8 ± 0.10	29.1 ± 0.7	2,229	2.5 ± 0.00	84.2 ± 0.7	–	–	–

–: No inflow or outflow water

proportion of the total P was 5.8%. Therefore, we applied the soluble fraction of the TDP, which included the organic form of phosphorus, for phytoplankton to use under the environment with low DIP concentration levels (Islam & Whitton, 1992; Yelloy & Whitton, 1996). The phytoplankton growth in our system had a positive relationship with the dissolved P in the NEBs, which increased as both the TN:TP ($r = 0.596$, $P = 0.012$, $n = 17$) and DIN:DTP ($r = 0.575$, $P = 0.016$, $n = 17$) ratios increased. Thus, our experimental approach of NEBs, based on the algal growth response to the nutrient ratio and ambient nutrients, may be used as a useful tool for the diagnosis of lake eutrophication and efficient managements.

To conclude, cyanobacteria dominated in our hypertrophic study reservoir were P-limited in most times, and thus their growth rate increased with P addition. Also the degree of the cyanobacterial growth in P addition was magnified with more N supplied, reflecting that at high nitrogen level, low N:P ratios were favorable for cyanobacterial dominance. Our results suggest that limiting nutrients and N:P ratio are critical factors to evaluate the cyanobacterial bloom in eutrophic systems. However, cyanobacterial bloom events would be complicated in the ambient condition, so that some other factors may also be important to affect it such as temperature, light, and grazing (Hutchinson, 1957; Reynolds, 1984; Carpenter & Kitchell, 1993). In the companion study (Kim, 2004), we found that temperature had significant effect on the maximum growth rate (U_{max}) of cyanobacteria between 10 and 25°C ($Q_{10} = 2$). Light was also detrimental to their growth rate, but the degree of the growth rate by any single factor of either temperature or light varied with nutrients. As such, we do not deny the potentials of other factors to the cyanobacterial bloom in our reservoir, but, as we found in this study, limiting nutrients and their stoichiometry are important factors to understand cyanobacterial dominance in the eutrophic system.

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