

Effects of the phytoplanktivorous silver carp (*Hypophthalmichthys molitrix*) on plankton and the hepatotoxic microcystins in an enclosure experiment in a eutrophic lake, Lake Shichahai in Beijing

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Received 14 November 2005; received in revised form 24 January 2006; accepted 7 March 2006

Abstract

The responses of nutrients, water transparency, zooplankton, phytoplankton and microcystins to a gradient of silver carp biomass (0, 18, 55, 110 g/m³) were assessed using enclosures in Lake Shichahai (Beijing). Picophytoplankton biomass increased with increasing fish stocking density ($r=0.64$, $p=0.09$). Silver carp significantly depressed zooplankton biomass, and thus, zooplankton grazing was too low to control phytoplankton. Intracellular microcystin (MC) content in the enclosures was correlated only to *Microcystis* biomass in the present study. *Microcystis* spp. biomass and intracellular microcystins content were much higher in lake water than those of enclosures with and without stocking fish. Stocking of silver carp could be an appropriate in highly productive Lake Shichahai, which naturally lacks of large cladoceran zooplankton. A fish stocking density of 55 g/m³ was most efficient at controlling *Microcystis* blooms and increasing water clarity. Mean extracellular MC concentration in the lake water was almost the same with that of the enclosures with fish.

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Keywords: Biomanipulation; Silver carp; Plankton; Algal biomass; Microcystins

1. Introduction

The effects of filter-feeding planktivorous fish on the plankton communities of aquatic ecosystem have been studied by Lazzaro (1987), Starling and Rocha (1990), Starling (1993), Fukushima et al. (1999) and others. Silver carp (*Hypophthalmichthys molitrix*) have been introduced worldwide for both aquaculture fish production and algal control (Gophen, 1990; Xie, 2003).

Stocking of silver carp as a biomanipulation tool to reduce phytoplankton biomass in lakes remains controversial (Costa-Pierce, 1992; Starling et al., 1998; Domaizon and Dévaux, 1999a) as responses vary

Table 1
Numbers and biomass of silver carp stocked in the enclosures

Treatment	Number of fish	Biomass of fish (g/m ³)
No fish (NF)	0	0
Low biomass (LF)	2	18
Median biomass (MF)	6	55
High biomass (HF)	12	110

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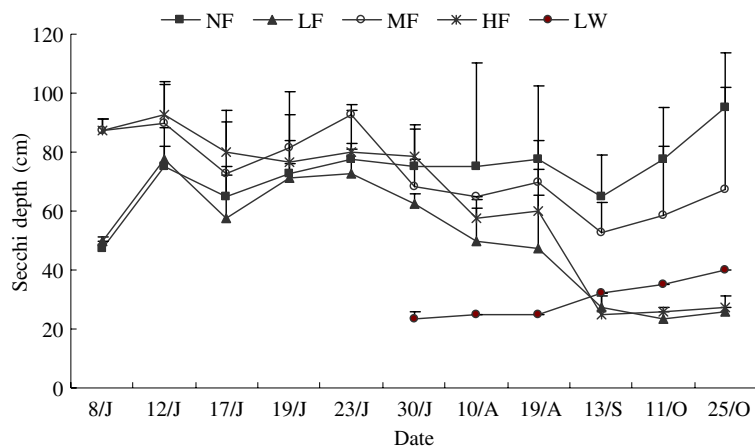


Fig. 1. The Secchi disc depth of the enclosures and in the lake during the experimental period. Error bars represent the standard deviation of the mean of the replicate enclosures, $n=2$. LF = low fish biomass; MF = median fish biomass; HF = high fish biomass; NF = fishless and LW = lake water.

according to body size, stocking density, food availability, and environmental conditions (Spataru and Gophen, 1985). Starling et al. (1998) list 14 studies that silver carp successfully control algal biomass, while others found no positive effects of silver carp, or even increased phytoplankton because of ichthyoeutrophication and reducing herbivore grazing from zooplankton (Miura, 1990; Wu et al., 1997; Domaizon and Dévaux, 1999b; Fukushima et al., 1999; Drenner et al., 1987). All of the successful experiments have in common the fact that they were performed under eutrophic or hypertrophic conditions where large or colonial algae (mainly cyanobacteria) were the predominant phytoplankton forms. In these studies nuisance algal blooms were suppressed. There have been many studies on the

impact of silver carp on phytoplankton and zooplankton communities; however, no information is available on the effects of silver carp stocking on the dynamics of intracellular and extracellular microcystins that are produced by some species of cyanobacteria. Microcystins are potent hepatotoxins and tumor promoters (Sivonen and Jones, 1999). Microcystins comprise a family of more than 60 closely related cyclic heptapeptides with the general structure of cyclo (D-Ala¹-X²-D-MeAsp³-Z⁴-Adda⁵-D-Glu⁶-Mdha⁷) in which X and Z are variable L amino acids, D-MeAsp is D-erythro-β-methylaspartic acid, Adda is (2S, 3S, 8S, 9S)-3-amino-9-methoxy-2, 6, 8-trimethyl-10-phenyl-deca-4, 6-dienoic acid, and Mdha is N-methyldehydroalanine (5). Three types (MC-LR, MC-RR and MC-YR) were identified

Table 2

Two-way ANOVA and mean values of physical, chemical, zooplankton biomass, phytoplankton biomass, microcystins in each treatment over the course of the experiment

Variable	Treatment mean				
	NF	LF	MF	HF	LW
TP (mg/l)	0.07 (a)	0.14 (ab)	0.77 (a)	0.16 (bc)	0.23 (d)
TN (mg/l)	1.10 (a)	1.70 (ab)	1.13 (a)	1.84 (ab)	2.13 (bc)
NH ₃ -N (mg/l)	0.07 (a)	0.08 (a)	0.07 (a)	0.07 (a)	0.12 (b)
NO ₃ -N (mg/l)	0.19 (a)	0.21 (a)	0.36 (a)	0.20 (a)	0.21 (a)
Secchi depth (cm)	77.50 (a)	39.50 (ab)	63.67 (ab)	45.75 (ab)	29.92 (b)
Crustacean biomass (μg/l)	224.02 (a)	13.58 (a)	2.24 (a)	8.78 (a)	1314.60 (b)
Chl <i>a</i> (μg/l)	21.14 (a)	48.14 (b)	23.74 (a)	66.84 (c)	123.86 (d)
Phytoplankton biomass (mg/l)	12.11 (ab)	23.95 (bc)	6.53 (a)	31.12 (c)	21.35 (bc)
Microcystis biomass (mg/l)	1.10 (a)	0.94 (a)	0.56 (a)	0.43 (a)	4.03 (b)
Picophytoplankton biomass (mg/l)	0.16 (a)	14.20 (b)	0.91 (a)	22.09 (b)	0.007 (a)
Intracellular microcystins (μg/l)	0.22 (a)	0.07 (a)	0.04 (a)	0.06 (a)	1.32 (b)
Extracellular microcystins (μg/l)	0.07 (a)	0.07 (a)	0.07 (a)	0.06 (a)	0.05 (a)

Means with different letter are significantly different (S–N–K Multiple Range Test: $\alpha=0.1$).

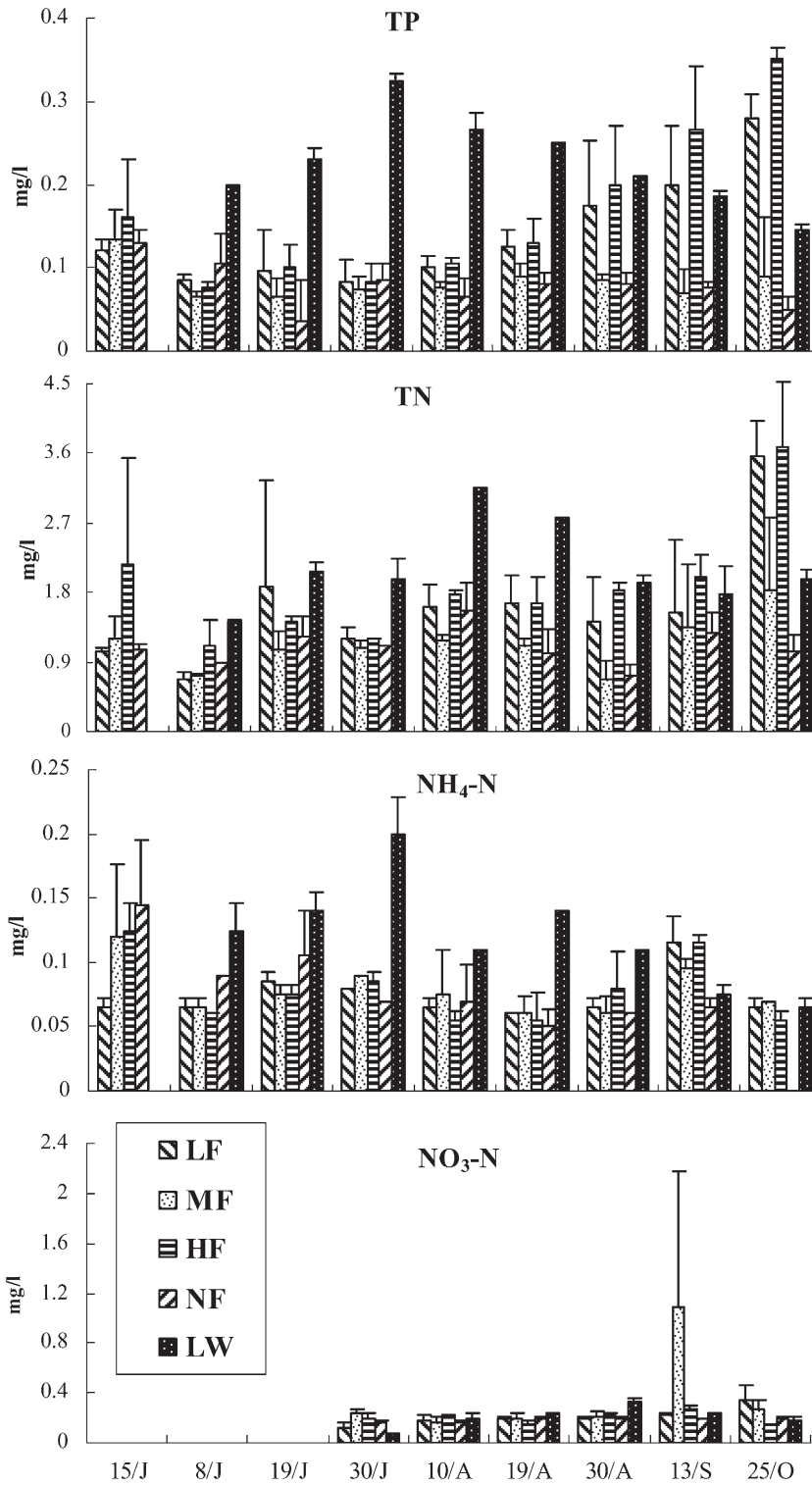


Fig. 2. TP, TN, NH₄-N and NO₃-N concentrations of the enclosures and the lake water during the experimental period (data are mean of the replicate enclosures).

and named by substitution of amino acids on two positions (X and Z) of the structure: MC-LR is named for leucine (L) and arganine (R), MC-RR is named for two arganine (R) and MC-YR is named for tyrosine (Y) and arganine (R). The objectives of the present study are to determine the responses of plankton and microcystins to a gradient of silver carp biomass using enclosure methods and to estimate an appropriate stocking density, which results in the efficient control of cyanobacterial blooms in a hypereutrophic temperate lake, Lake Shichahai in Beijing City.

2. Materials and methods

2.1. Study site

Lake Shichahai (39°58'N, 116°29'E) in Beijing City is a shallow hypereutrophic temperate lake with a surface area of 17.9 km², and a mean depth of 1.3 m. The concentrations of total nitrogen and total phosphorus in the most eutrophic part of the lake in 2003 were as high as 13.27 and 0.68 mg/l, respectively (unpublished data). Cyanobacterial

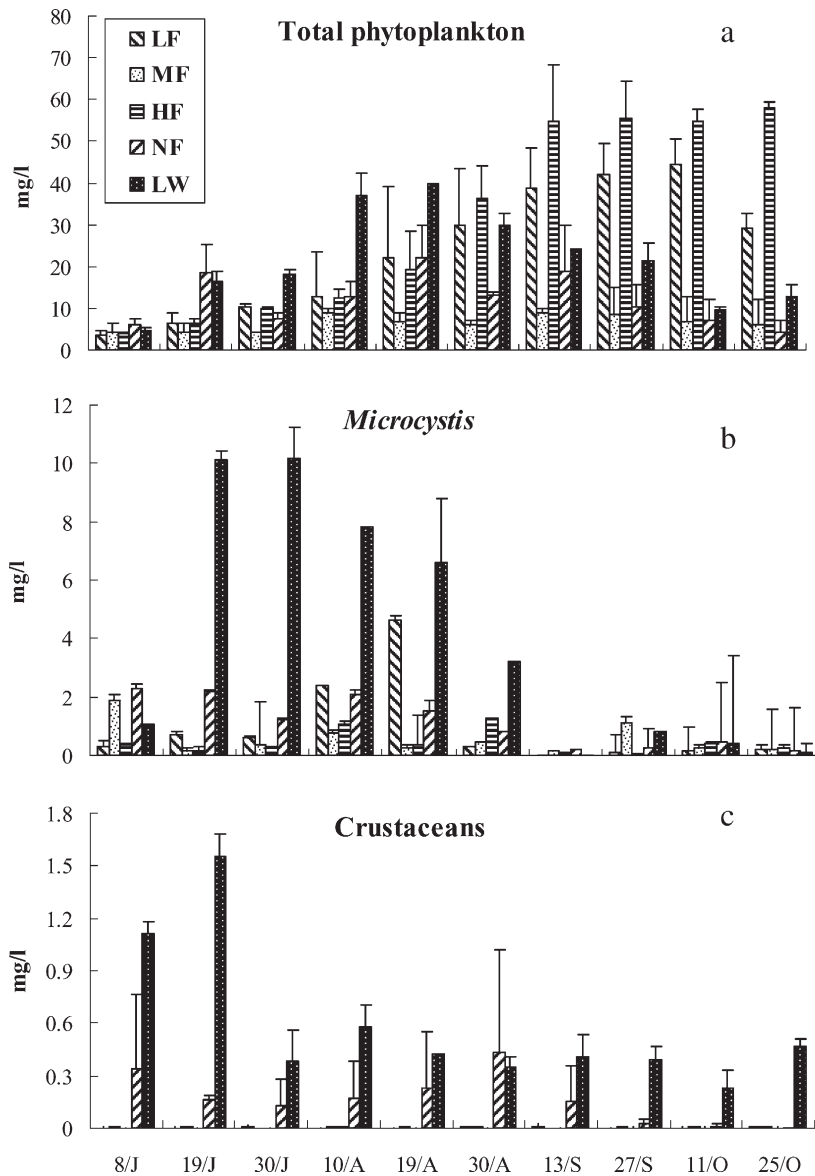


Fig. 3. Algal, *Microcystis* and crustaceans biomass of the enclosures and the lake water during the experimental period (data are mean of the replicate enclosures).

blooms dominated by *Microcystis* spp. occur regularly in the summer of each year.

2.2. Experimental design

We conducted experiments in eight enclosures located close to the lakeshore, in the most eutrophic part of Lake Shichahai. The experiment lasted from June 15 to October 25, 2004. Polyhexene enclosures (3×3×2.5 m) were sealed off from sediment at the bottom and filled with lake water to a depth of 1 m, which was approximately the depth of the surrounding lake water. Sediment from the lake was added into each enclosure at a depth of about 5 cm. Silver carp were collected from a nearby pond and acclimated in a net cage placed in the lake, and then put in the enclosures on June 15. Mean individual weight (\pm S.D.) of stocked fish were 100.0 g (\pm 11.5). Four fish biomass levels (no fish (NF), low fish biomass (LF), medium biomass (MF) and high biomass (HF)) were chosen with two replicates each (Table 1). Lake water near the enclosures was also sampled.

2.3. Sampling and analyses

Water temperature, Secchi depth (SD) and water chemistry were measured after fish stocking. Water samples for crustacean zooplankton and phytoplankton were collected from the surface and near the bottom from July 8 to October 25. Integrated water samples were from the surface and bottom layers taken using a 5-l modified Patalas's bottle sampler. Subsamples for phytoplankton including picophytoplankton were preserved with 1% acidified Lugol's iodine solution and concentrated to 30 ml after sedimentation for 48 h. After mixing, 0.1 ml concentrated samples were counted directly under 400× magnification. Picophytoplankton was identified as a group of algae in the size range 0.2–2 μ m (Sieburth et al., 1978). Colonial *Microcystis* spp. cell were separated using a high-speed blender (Ultra-Turrax) and counted as

above. Taxonomic identification was made according to Hu et al. (1979) and biomass was estimated from approximate geometric volumes of each taxon, assuming that 1 mm³ equals 10⁻⁶ μ g fresh weight (Shei et al., 1993). The geometric dimensions were measured on 10–30 individuals for each dominant species. Quantitative samples of crustaceans were collected by straining 10 l integrated water samples through a 60 μ m plankton net, and preserved with formalin. Copepods and cladocerans were identified according to Sheng (1979) and Chiang and Du (1979). Specimens were counted under microscope at a magnification of 10×6. Wet weight of cladocerans were estimated according to the formula of Huang and Hu (1986) and Chen (1981).

In the laboratory, total phosphorus (TP) concentration was measured by colorimetry after digestion of the unfiltered samples with K₂S₂O₈ + NaOH to orthophosphate (Ebina et al., 1983). TN was digested simultaneously with TP. After digestion, TN was measured as nitrate and absorbance was measured at 220 nm. Nitrate (NO₃-N) was analyzed using the automated Korolev/Cadmium reduction method. Chlorophyll *a* was determined by a spectrophotometer (Lorenzen, 1967) after filtration on Whatman GF-C glass filters and 24 h extraction in 90% acetone.

Microcystin (MC) was measured as intra- and extracellular MC according to the methods of Park and Lwami (1998) and Welker et al. (1999). The intracellular toxins were extracted from cyanobacterial cells that were filtered (500 to 1000 ml water) on the glass-fiber (GF/C, Whatman, UK) filters. The filtrate was used to measure the extracellular toxins. Both extra- and intracellular MC were determined by a reverse-phase high-performance liquid chromatography (HPLC) equipped with an ODS column (Cosmosil 5C18-AR, 4.6 × 150 mm, Nacalai, Japan) and a SPD-10A UV-vis spectrophotometer set at 238 nm. The sample was separated with a mobile phase consisting of 65% aqueous methanol with 0.05% trifluoroacetyl (TFA) at a flow rate of 1 ml/min. MC concentrations were determined by comparing the peak

Table 3
The means of biomass (μ g l⁻¹) of dominant crustacean zooplankton of five treatments over the course of the experiment

Species	Biomass (μ g/l) \pm SD				
	LF	MF	HF	NF	LW
<i>Bosmina coregoni</i>	1.30 \pm 1.41	0.26 \pm 0.26	0.18 \pm 0.16	34.08 \pm 31.87	11.06 \pm 4.52
<i>Diaphanosoma brachyurum</i>	6.32 \pm 7.43	0.18 \pm 0.25	3.03 \pm 4.03	37.91 \pm 49.09	526.35 \pm 185.78
<i>Moina micrura</i>	1.30 \pm 1.84	0	3.90 \pm 1.84	1.82 \pm 1.84	199.68 \pm 76.11
<i>Thermocyclops taihokuensis</i>	3.59 \pm 3.08	0.82 \pm 0.85	2.03 \pm 2.09	113.67 \pm 168.60	383.08 \pm 65.67
<i>Mesocyclops</i> sp.	0.44 \pm 0.44	2.43 \pm 2.19	0.38 \pm 0.34	17.46 \pm 10.25	63.80 \pm 5.47
<i>Neodiaptomus schmackeri</i>	1.80 \pm 0.93	0.74 \pm 0.58	1.56 \pm 1.97	19.08 \pm 18.88	130.6 \pm 184.16
Nauplii	32.75 \pm 19.00	10.36 \pm 5.18	23.91 \pm 21.81	246.21 \pm 303.15	518.00 \pm 172.20

areas of the test samples with those of the standards available (MC-LR, MC-RR and MC-YR, Wako Pure Chemical Industries-Japan).

2.4. Statistic analysis

The effects of fish biomass levels on TN, TP, NO₃-N, NH₄-N, Secchi depth, crustacean zooplankton biomass, phytoplankton biomass, Chl. *a* concentrations, intra- and extracellular MC contents were analyzed using two-way ANOVA, with treatment and time as the two factors simultaneously tested. Due to low replication and statistical power, we choose a probability level of $\alpha < 0.10$ to reduce the chance of making the type II error. If the inter-treatment effect reached significance level ($\alpha=0.1$), then the S–N–K multiple range was used to test significance of differences among the treatment means at $\alpha=0.1$ (Ma, 1985).

3. Results

3.1. Physical and chemical environments

Water temperature varied from 12.8 to 30 °C during the experiment. Fig. 1 shows the temporal changes in the Secchi depth in the enclosures and the surrounding lake water from July 8 to October 25. Initially, MF and HF enclosures had higher SD than NF and LF enclosures, but the situation was reversed at the end of the experiment for the HF treatment. SD was significantly higher in the fish-less enclosures than in the lake water, but SD did not differ between the LF, MF and HF treatments (Table 2). SD fluctuations of different enclosures were related to dynamics of algal biomass ($r=-0.77$).

Fig. 2 shows the temporal variation in concentrations of total nitrogen, total phosphorus, NH₄-N and NO₃-N in enclosures and the lake water from June 15 to October

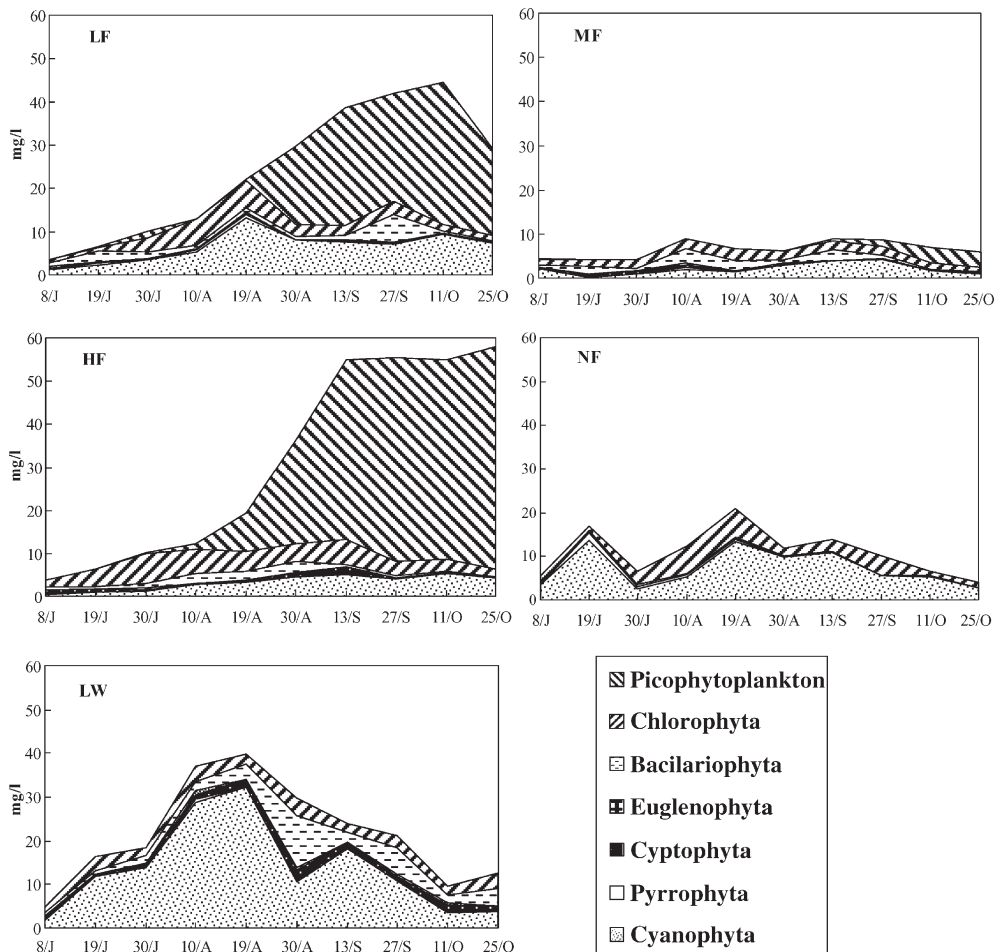


Fig. 4. Biomass distribution of the major algal groups in the enclosures and in the lake water (data are mean of the replicate enclosures).

25. Trends of $\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$ were similar to those of TP and TN, all of these nutrient concentrations were much higher at the end of the experiment. There were significant differences in the concentration of TP and TN among the treatments (Table 2). Concentrations of TP, TN and $\text{NH}_4\text{-N}$ were generally lower in the enclosures than in the lake water during July and August, but then increased remarkably in the HF and LF enclosures in September and October. Nutrient concentrations did not show consistent changes in NF and MF enclosures during the experiment.

3.2. Biomass of crustacean zooplankton and phytoplankton

Fig. 3(c) shows the biomass of total crustacean zooplankton over the course of the experiment in the

enclosures and the lake water. It was notable that biomass of crustacean zooplankton was much higher in the lake water than in the enclosures. There were no statistically significant differences of crustacean biomass among treatments (Table 2), although mean crustacean biomass was much higher in the NF treatment compared to the LF, MF and HF treatments. Table 3 shows biomass of dominant crustacean plankton among treatments. Except *Bosmina coregoni*, biomass of all zooplankton was much higher in the lake water than in the enclosures (Table 2).

Total phytoplankton biomass was significantly different among the treatments (Table 2). At the beginning of the experiment in July, silver carp exerted pressure on total phytoplankton biomass in the enclosures with fish. However, afterwards, both high and low densities of silver carp promoted algae

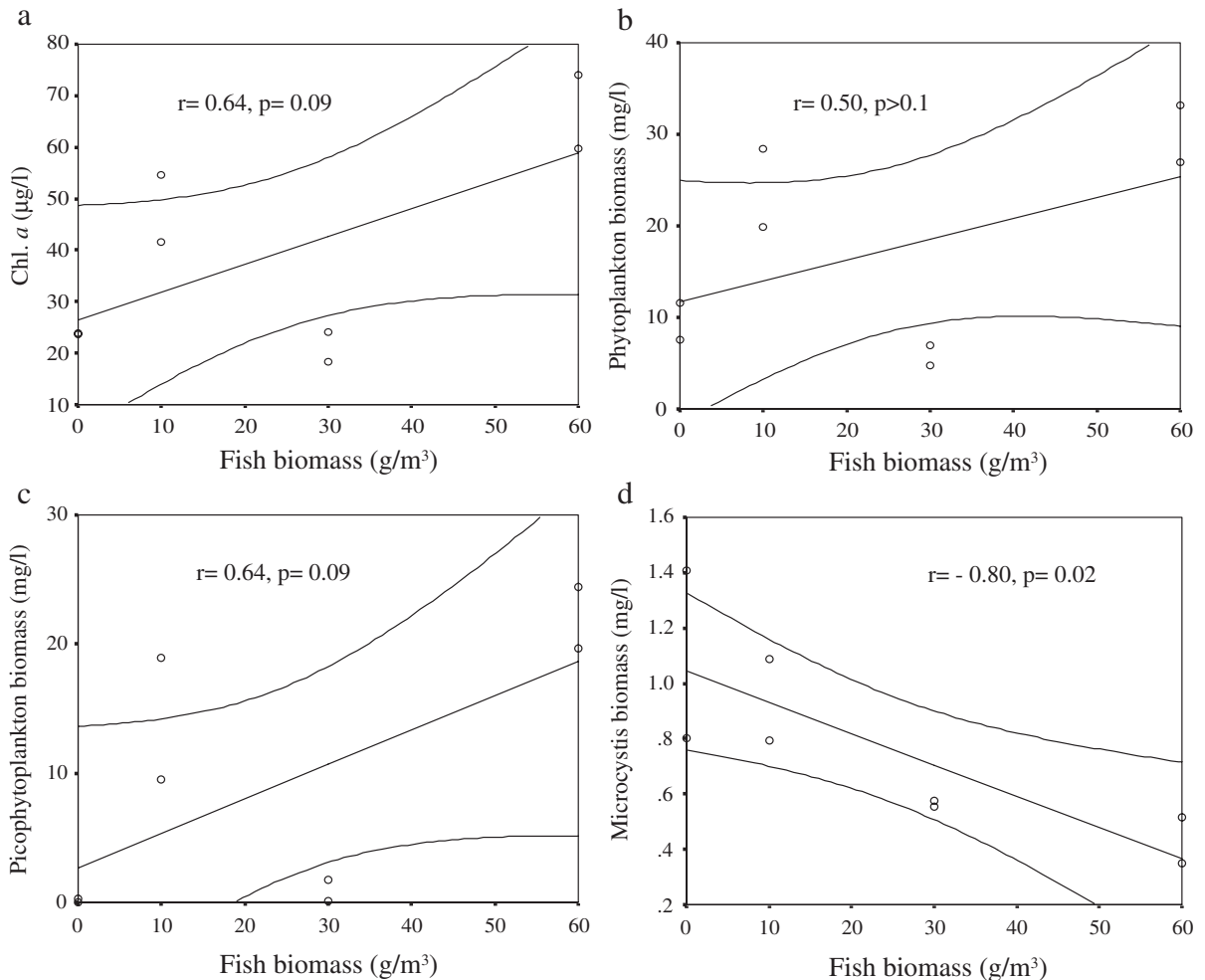


Fig. 5. Relationship between Chl *a* concentrations (a), phytoplankton biomass (b), picoalgae biomass (c), *Microcystis* biomass (d) and stocking fish density at 90% confidence level in eight enclosures, $n = 8$.

biomass (Fig. 3a). There was a positive relationship between the mean concentration of Chl *a* and stocking density of fish ($r=0.64$, $n=8$, $p<0.1$) (Fig. 5a), but no significant correlations were present between mean phytoplankton biomass and fish density ($r=0.50$, $n=8$, $p>0.1$) (Fig. 5b).

Small-sized picophytoplankton ($d<2\ \mu\text{m}$) contributed greatly to the phytoplankton assemblages in HF and LF enclosures and accounted for approximately 75% of the total algal biomass since August 30 (Fig. 4). There was a positive correlation between picophytoplankton abundance and stocking fish density ($r=0.64$, $n=8$, $p<0.1$) (Fig. 5c). Furthermore, transparency values and picophytoplankton biomass was negatively correlated ($r=-0.74$, $n=8$, $p=0.04$). Both TP and TN concentrations were positively related with abundance

of picophytoplankton ($r=0.97$, $n=8$, $p<0.01$), and picophytoplankton biomass was not significantly correlated with *Microcystis* biomass ($r=-0.43$, $n=8$, $p>0.1$) (Fig. 6).

There was a negative correlation between *Microcystis* biomass and fish biomass ($r=-0.80$, $n=8$, $p<0.05$) (Fig. 5d). However, *Microcystis* biomass was not correlated with TP, TN and SD (Fig. 7). *Microcystis* biomass was much higher in the lake water than in the enclosures from July 19 to August 30 (Fig. 3b).

3.3. Intracellular and extracellular microcystins

A pronounced bloom lasted from June 15 to August 30 in Lake Shichahai, while no visual surface blooms occurred since July 8 in all of the enclosures. Among

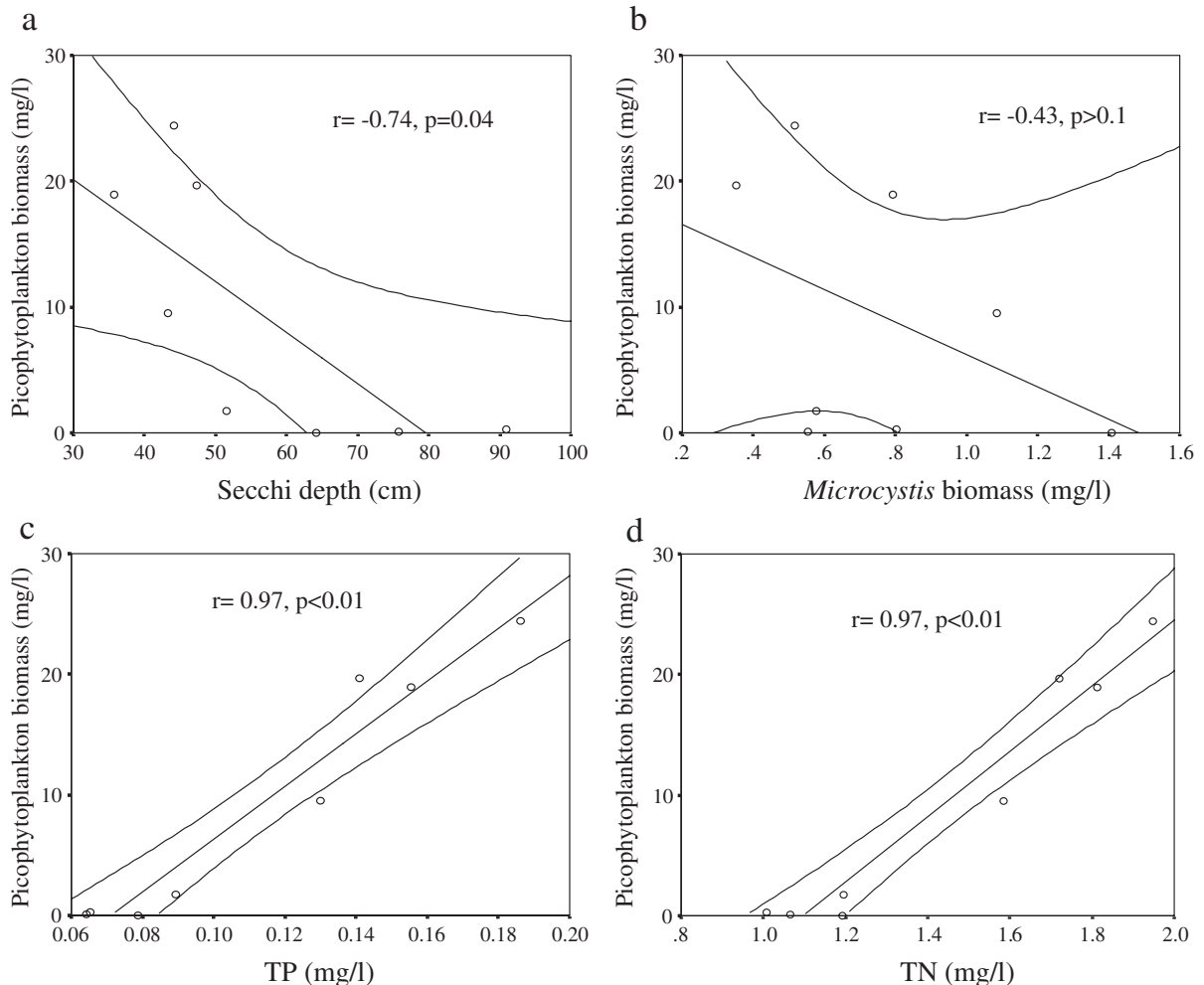


Fig. 6. Relationship between Secchi depth (a), *Microcystis* biomass (b), TP concentrations (c), TN concentrations (d) and picophytoplankton biomass at 90% confidence level in eight enclosures, $n=8$.

cyanobacteria, only one toxic genus was present (*Microcystis* spp.). The toxins contained in the phytoplankton samples were identified as MC-LR, MC-RR and MC-YR. MC-RR was the dominant form of microcystin quantified (Fig. 8). The maximum intra-MC concentration was 6.05 $\mu\text{g/l}$ in the lake water on August 10 and 0.94 $\mu\text{g/l}$ in NF enclosures on August 19. Intra-MC content was much higher in the lake water than in the enclosures (Table 2). Intra-MC showed positive correlations with *Microcystis* biomass and Secchi depth, but was not correlated with extracellular microcystins, TP and TN (Fig. 9).

The highest extracellular microcystin concentrations were detected on July 19 and October 25 in the enclosures and on October 25 in the lake water, but concentrations were low or under the limit of detection (<0.02 $\mu\text{g/l}$) on the other sampling dates. Mean concentrations of extracellular microcystins were higher in the LF, MF and NF enclosures than in the lake water.

4. Discussion

In the present study, mean picophytoplankton biomass in the enclosures was positively related to TN and TP (Fig. 6), indicating that small-sized algae were principally regulated by bottom-up force (nutrients); while the large size phytoplankton were regulated both by top-down forces (fish grazing) and by competition with small size algae. These observations were generally consistent with earlier experiments using silver carp by Domaizon and Dévaux (1999a). It is reported that picophytoplankton can out-compete larger phytoplankton for nutrients (Kerfoot et al., 1988), and that silver carp is not able to filter algae smaller than 10 μm (Cremer and Smitherman, 1980; Smith, 1989). Wang et al. (2004) found that picophytoplankton increased with increasing fish yield in three subtropical Chinese lakes and also in an enclosure experiment in the hypereutrophic Lake Donghu, which is consistent with our results. In our

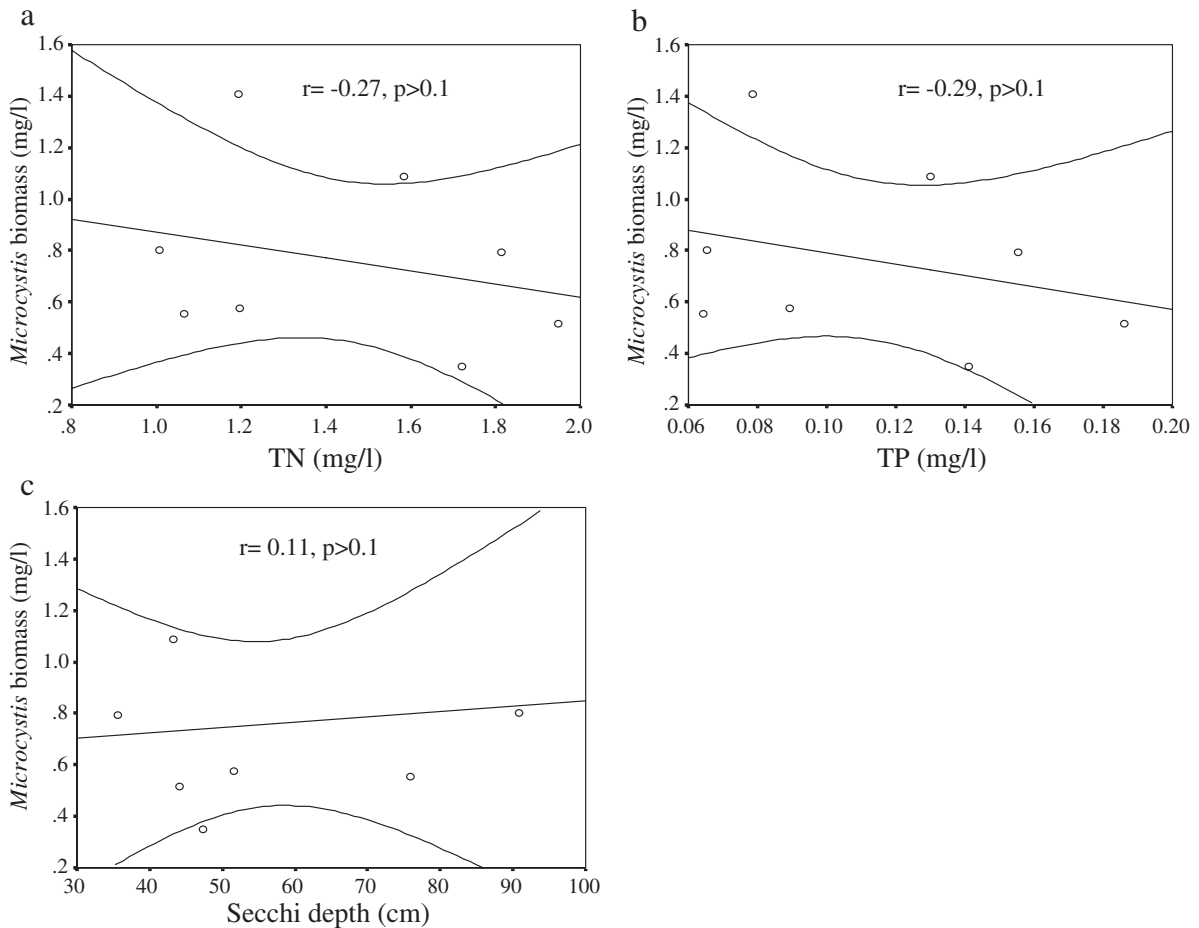


Fig. 7. Relationship between TN concentrations (a), TP concentrations (b), Secchi depth (c) and *Microcystis* biomass at 90% confidence level in eight enclosures, $n = 8$.

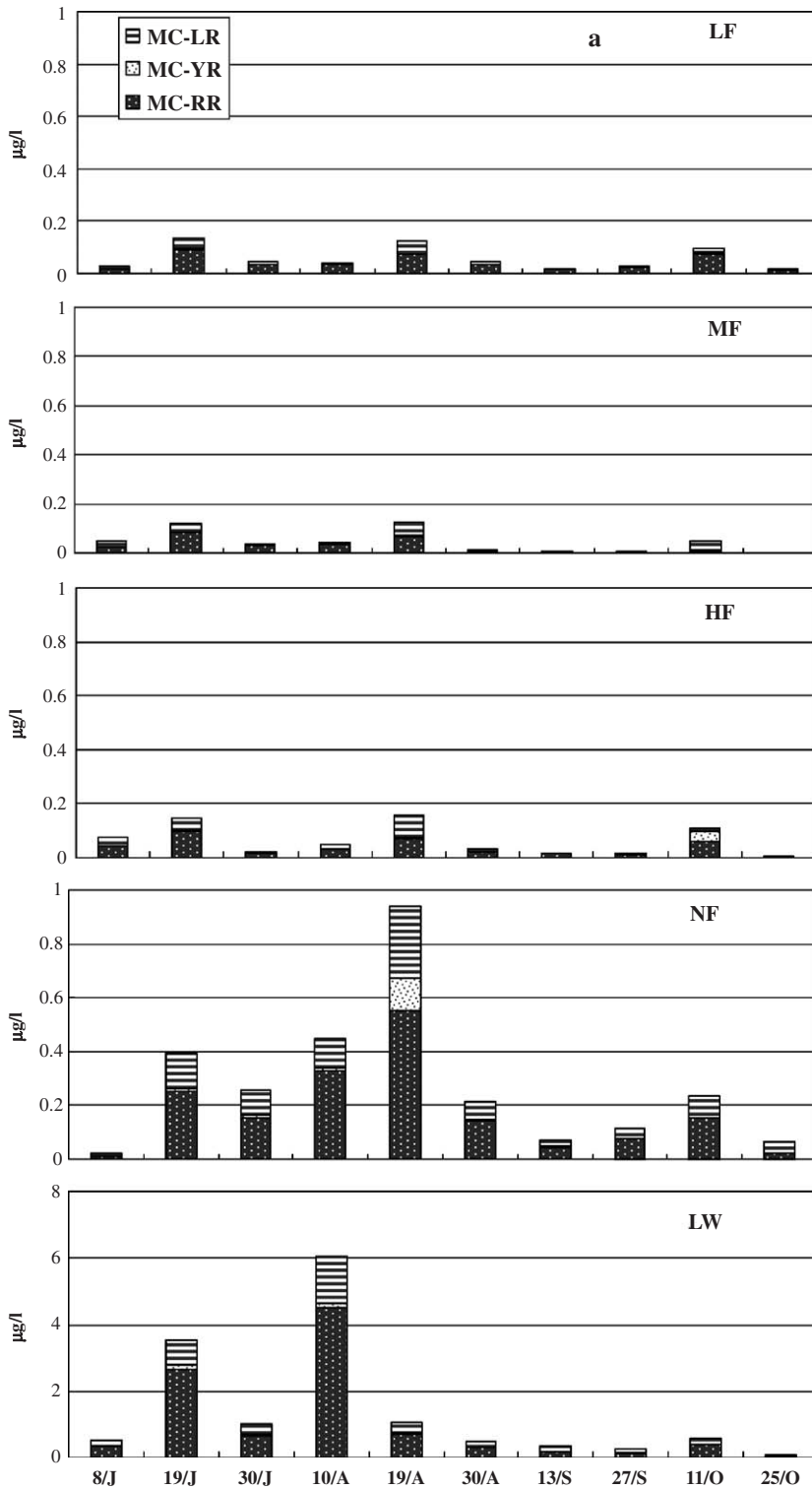


Fig. 8. Dynamics of the concentrations of intra- (a) and extracellular (b) microcystins in enclosures and lake (data are means of the replicate enclosures).

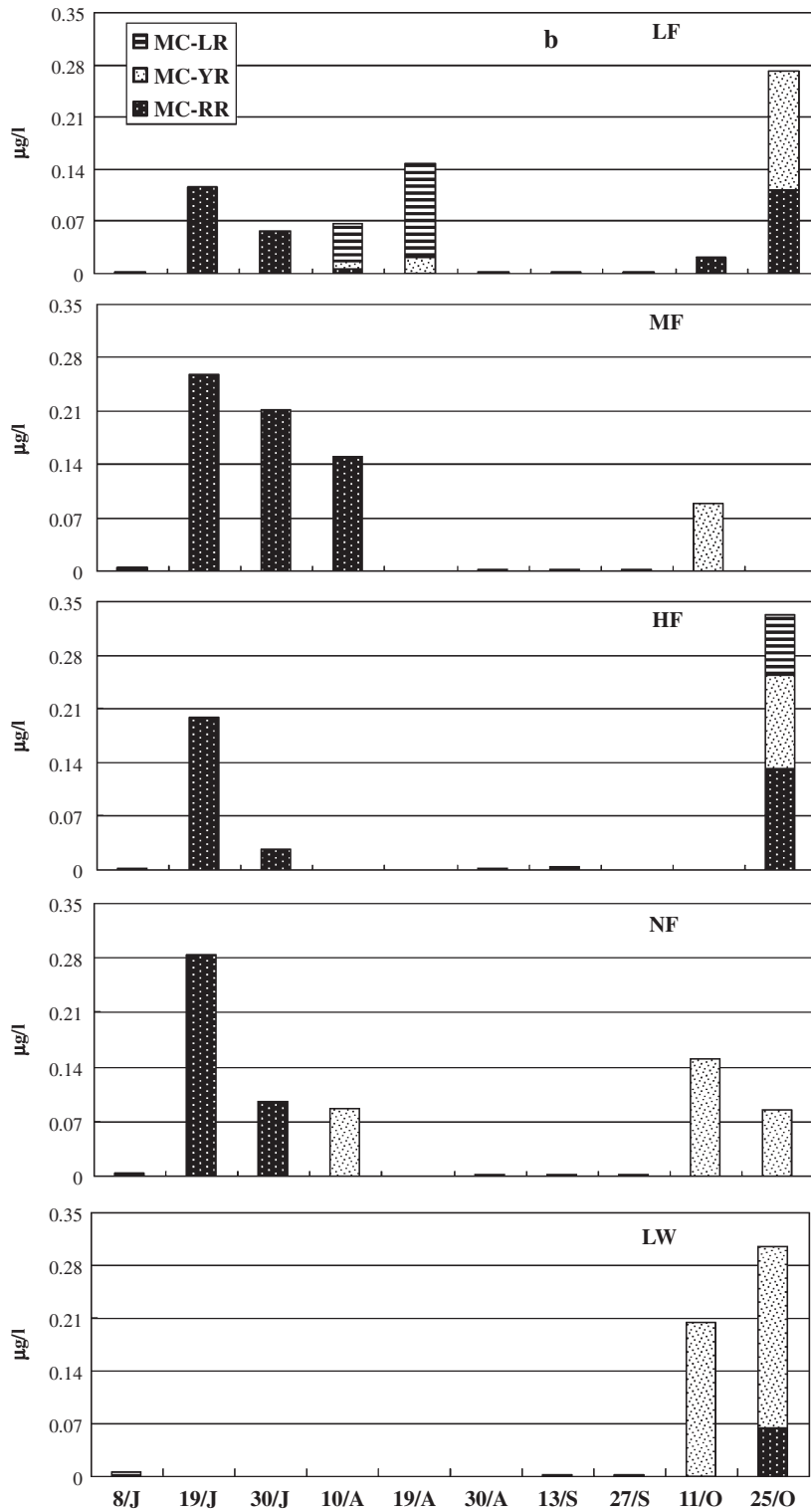


Fig. 8 (continued).

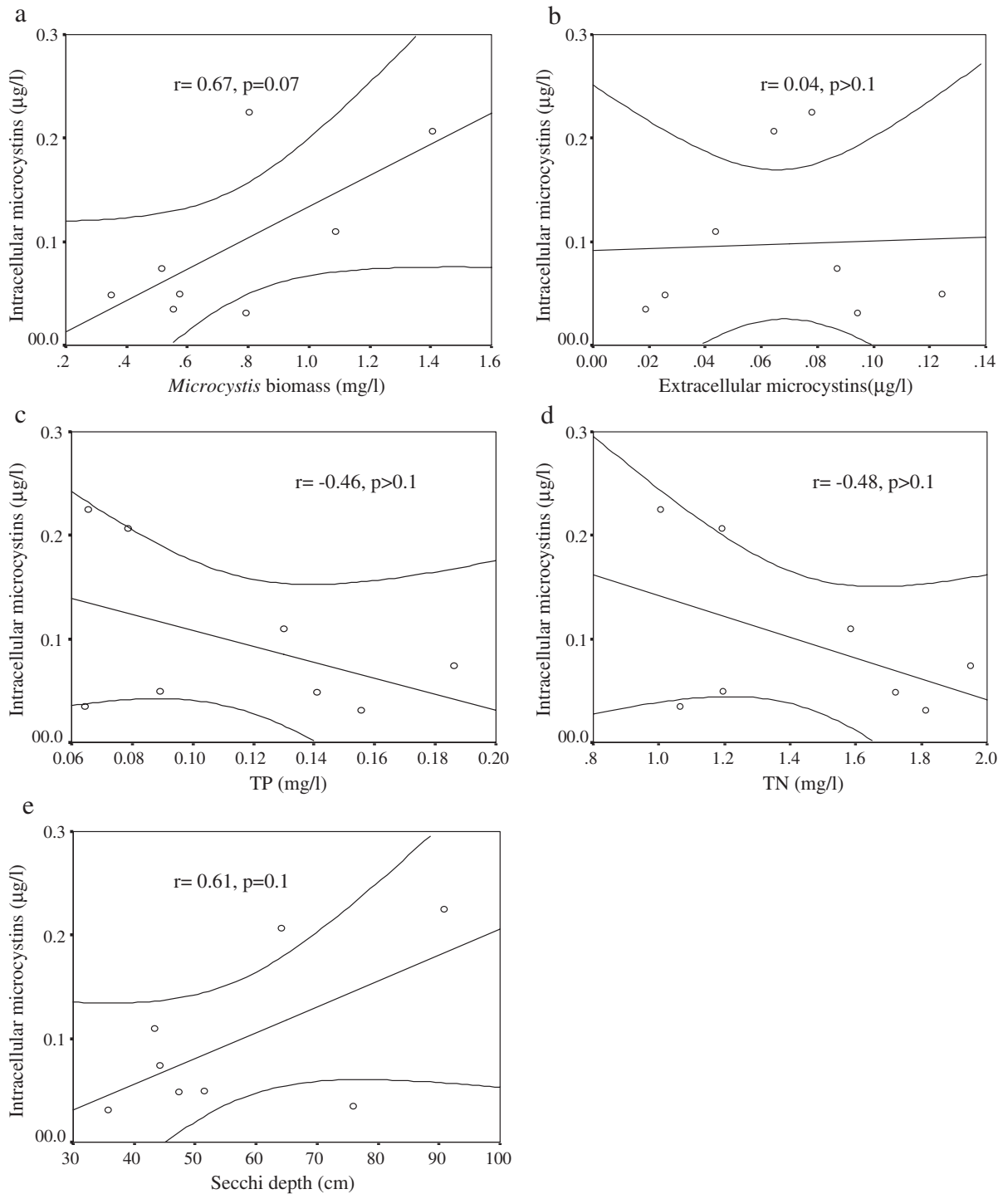


Fig. 9. Relationship between *Microcystis* biomass (a), extracellular microcystins content (b), TP concentrations (c), TN concentrations (d), Secchi depth (e) and intracellular microcystins concentrations at 90% confidence level in eight enclosures, $n=8$.

experiments, crustacean zooplankton were significantly suppressed by silver carp and thus had little ability to control phytoplankton biomass in the enclosures con-

taining fish. Picophytoplankton was rare in the fish-free enclosures probably because of high grazing pressure by zooplankton. These results indicate that silver carp at

high and low densities (110 and 18 g/m³) may strongly depress both competitors and predators of picophytoplankton, leading to massive growth of picophytoplankton and decrease of water clarity.

There have been previous experimental studies to examine the role of planktivorous fishes in the elimination of *Microcystis* spp. blooms using enclosure methods. Xie and Liu (2001) found that cyanobacterial blooms in the enclosures were completely eliminated within 10–20 days by introduction of silver carp. In the present experiment, although silver carp promoted total phytoplankton biomass at high and low stocking density, it efficiently suppressed cyanobacteria blooms in all the enclosures with fish, as well as the intracellular microcystin content. Furthermore, maybe the enclosures that reduced the disturbance of wind and decreased the penetrating light, which is called “wall effects” (Ramcharan et al., 1996). Physical disturbance is a very remarkable factor that promotes nutrients releasing to lake water from the sediment in shallow lakes (Douglas and Rippey, 2000). As a result, nutrient concentrations of the lake water were higher compared with that of the enclosures without fish. On account of the negative relationship between intracellular microcystins and SD, intracellular microcystin concentrations were higher in the lake water than that in the NF treatment with lower penetrating light into the water (Zhang, personal observations).

The mechanisms by which environmental factors affect toxin production by cyanobacteria are complex. Although nutrients have long been suspected as a contributing factor to both cyanobacterial abundance and toxin production (Kotak et al., 2000), in our study, there were no significant relationships between intracellular MC concentration and nutrient concentrations, and between the biomass of *Microcystis* spp. and nutrient concentrations. It is very possible that nutrient concentrations in our study were far in excess of growth requirements of phytoplankton and also *Microcystis*. In the present study, intracellular MC concentration was related positively with *Microcystis* spp. biomass but negatively with Secchi depth. Similarly, the investigations made by Kotak et al. (2000) showed that MC production was higher under lower light intensities. Many field studies indicate that the relationships between cyanobacteria, MC concentration, and environmental factors are invariably complex (Kotak et al., 1996). In the present study, intracellular MC content in the enclosures was mainly regulated by the biomass of *Microcystis*.

Intracellular MC content is strain dependant, varying by several orders of magnitude between strains (Chorus, 2001), and the ratio of MC-LR to MC-RR varies from strain to strain (Watanabe, 1996). In the present

experiment, this ratio varied greatly from 0.2 to 5.9, suggesting the possible coexistence of multiple toxic cyanobacterial strains in our study lake. Individual strains also have different environmental optima for growth and MC production, and respond differently to changing environmental conditions (Sivonen, 1990; Vézic et al., 2002). Therefore, observed MC dynamics in our study might be a result of interactions between environmental influences on MC production and dominance of individual cyanobacterial strains (Chorus, 2001).

The present study indicates that the phytoplanktivorous silver carp can be an efficient biomanipulation fish to reduce nuisance blooms cyanobacteria in eutrophic lakes where large herbivorous zooplankton are lacking. Although low and high stocking densities of planktivorous fish resulted in increased picophytoplankton and reduction of large cladocerans, a median stocking density (55 g/m³) significantly decreased biomass of *Microcystis*, picophytoplankton and microcystin content, and thus greatly improved water clarity compared with the high and low stocking densities.

Acknowledgements

Research was supported by “The technique and demonstration of improving Beijing urban water quality of water system in north ring road.” (Grant No. 2003AA661010) and by a Key Project of CAS (Grant No. KSCX2-SW-129).

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