A non-classical biomanipulation experiment in Gonghu Bay of Lake Taihu: control of *Microcystis* blooms using silver and bighead carp

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Summary

A non-classical biomanipulation experiment was carried out in Gonghu Bay of Lake Taihu in 2009. Silver and bighead carp were stocked in a large fish enclosure to control cyanobacterial blooms. Water quality, plankton abundance, and the intracellular and extracellular microcystins (MCs) in lake water were investigated monthly in 2009. The concentrations of nitrogen nutrients were significantly lower in the fish enclosure than in the surrounding lake, while phosphorus (especially total phosphorus) concentration was higher in fish enclosure. During the blooming period, Cyanophyta contributed to more than 90% of the phytoplankton in the surrounding lake, whereas it represented only 40–80% in the fish enclosure. The phytoplankton and crustacean zooplankton biomasses and the zooplankton/phytoplankton ratios were all significantly lower in the fish enclosure than in the lake. This result suggested that silver and bighead carp can suppress the phytoplankton and zooplankton biomass by grazing and increase the loading of nutrients through excretion, sediment resuspension and other simultaneous biological effects. During the blooming period, the intracellular and extracellular MCs in the fish enclosure were reduced by 93.8% and 69.8% compared with the surrounding lake. MCs content varied from 0.34 to 18.8 ng (mean 4.8 ng) MC-LR per g wet weight in the muscle sample of silver and bighead carp in the experimental enclosure, which suggested that these fish were safe to consume for human. However, the long-term effects of MCs on aquatic ecosystem and on public health cannot be overlooked.

Keywords: Lake Taihu, silver carp, bighead carp, *Microcystis* bloom, microcystin

Introduction

Lake eutrophication can lead to series of environmental problems such as high biomass of phytoplankton, the deterioration of water quality, the disappearance of submerged macrophytes and a decline in biological diversity (Chen, Xie, Zhang, Ke & Yang 2006). Cyanobacterial blooms occur in eutrophic lakes throughout the world. The manipulation of the food web structure is an effective method for controlling lake eutrophication. Studies have suggested that non-classical biomanipulation, mainly the stocking of silver carp (*Hypophthalmichthys molitrix*) and bighead carp (*Aristichthys nobilis*), is an appropriate tool for controlling algal blooms in tropical and subtropical lakes (Starling 1993; Xie & Liu 2001; Radke & Kahl 2002). This technique has been widely applied to control the outbreak of algal blooms in Chinese eutrophic lakes, including Lake Donghu, Lake Dianchi and Lake Taihu (Liu & Xie 1999; Xie 2003).

Silver and bighead carp can impose both top-down and bottom-up effects on the lake ecosystem; these carp can suppress the phytoplankton and zooplankton biomass by grazing and increase the loading of nutrients through excretion, sediment resuspension and other simultaneous biological effects.
There are many inconsistent results about the effects of stocking silver and bighead carp on water nutrient load. Tang, Xie, Lu, Xie and Wang (2002) reported that the concentrations of total nitrogen (TN) and total phosphorus (TP) were significantly higher in the silver and bighead carp enclosure in Donghu Lake. However, Chen, Bao and Zhou (2009) found the opposite result in Wuli Lake. Thus, it is necessary to conduct further research on the potential interactions between stocking fish and plankton biomass or nutrient loading.

Gonghu Bay and Meiliang Bay of Lake Taihu were two important sources of drinking water for Wuxi City in China. Unfortunately, toxic cyanobacterial blooms occurs in these regions each year since the 1980s (Xie 2008). From 2004 to 2005, we examined the possibility of bio-filtering toxic Microcystis blooms by stocking silver and bighead carp in large fish pens in Meiliang Bay of Lake Taihu (Ke, Xie, Guo, Liu & Yang 2007; Ke, Xie & Guo 2009). It was difficult to evaluate the effect of this practice on the water quality because of the free water exchange between the fish enclosure and the surrounding lake. Additionally, none of the physicochemical parameters was significantly different between the fish enclosure and the surrounding lake in these experiments (Ke et al. 2007). In 2007, a severe Microcystis bloom occurred in Gonghu Bay and created a drinking water crisis for Wuxi City (Xie 2008). Since then, several methods for improving water quality have been implemented in Gonghu Bay, including the restoration of aquatic macrophyte vegetation and the stocking of silver and bighead carp around the waterworks. In this study, we built a large enclosure in Gonghu Bay to study the effect of silver and bighead carp on water quality and plankton abundance with an emphasis on the effect of fish stocking on the accumulation of microcystins (MCs) in the water and in fish muscle. This study will contribute to the evaluation of non-classical biomanipulation as a restoration method for a subtropical eutrophic lake.

Materials and methods

Study area

Lake Taihu is located in Jiangsu Province of China and is characterised by its shallow water depth (average 1.9 m) and large surface area (2428 km²). It is an important aquaculture, industrial and drinking water resource in the Yangtze River Delta, and it is also a popular recreational and tourist attraction (Song, Chen, Peng, Wan, Gan & Zhang 2007). Gonghu Bay is in the northeast section of Taihu Lake and has a surface area of 150 km² and a depth of 1.8–2.5 m, which is the main source of drinking water for Wuxi City. To study the in situ effects of silver and bighead carp on water quality, a large fish enclosure was built in Gonghu Bay in late 2008. The total area of the enclosure was 0.08 km², with a length of 320 m and a width of 250 m, and the enclosure was separated from the surrounding lake with no water exchange. During March of 2009, 1200 kg of silver carp and 180 kg of bighead carp were stocked in this enclosure. The fingerling sizes of the silver and bighead carp were 120–170 g/ind. and 80–120 g/ind., respectively. The mass ratio of silver to bighead carp was ~4:1 and the initial stocking densities of silver and bighead carp were 7.5 and 1.1 g m⁻³ respectively.

Sampling and analyses

Four sampling stations were set up: Stations 1 and 2 were located in the centre of the fish enclosure, and Stations 3 and 4 were located in the lake surrounding the fish enclosure as a control (Fig. 1). Integrated water column samples were collected with a Patalas-Schindler trap monthly in 2009. Water temperature, pH, dissolved oxygen (DO), conductivity, turbidity, total dissolved solids and oxidation-reduction potential were measured in situ with a YSI Environmental Monitoring System 6600 (YSI Incorporated, Yellow Springs, OH, USA). Transparency was measured with a Secchi disk. Chemical oxygen demand, TN, total dissolved nitrogen (TDN), ammonia nitrogen (NH₄-N), nitrate nitrogen, nitrite nitrogen (NO₂⁻N), TP, total dissolved phosphorus and phosphate phosphorus were measured in a field research station laboratory, using the methods described by Zheng, Xie, Li, Yang, Wang and Guo (2004). Chlorophyll a (Chl-a) was determined spectrophotometrically (Lorenzen 1967) after filtration on a Whatman GF/C glass filter and extraction in 95% ethanol for 24 h.

For phytoplankton, a 1 L sample was preserved with 1% acidified Lugol’s iodine solution and was concentrated to 50 mL after sedimentation for
48 h. After complete mixing, 0.1 mL of the concentrated sample was counted under a microscope at 400× magnification. Phytoplankton species were identified according to Hu, Li, Wei, Zhu, Chen and Shi (1979) and John, Whitton and Brook (2002). Algae were counted on a cell-by-cell basis. Colonial *Microcystis* cells were separated using an ultrasonic crusher (JY88-II; Scientiz, Ningbo, China) and were counted. Zooplankton was sampled with a modified 5-L Patalas sampler and took 20 L of water from the bottom (0.5 m over sediment) to the surface (0.5 m, surface water) of the lake. The combined samples were filtered through a 69-μm plankton net and preserved with 5% formalin for further analysis. Crustacean zooplankton was examined at a magnification of 40× with an Olympus microscope (BX41; Olympus, Tokyo, Japan). Densities were determined by counting all individuals in the sample using a microscope at 40× magnification. Copepods and cladocerans were identified according to Shen (1979) and Chiang and Du (1979).

Fish samples were collected monthly from March to December. Ten silver carp and 10 bighead carp were captured randomly from the enclosure using multi-mesh gillnets in each month. These fish samples were measured, weighed and sacrificed immediately, and then dissected to collect liver and muscle samples. The collected organs were separately and carefully washed with distilled water to avoid cross-contamination, and then they were immediately frozen at −20°C at the field research station.

Based on our previous study in this lake (Guo, Ke, Xie & Ni 2009), we evaluated fish growth and feeding on plankton by sampling 10–15 individuals of silver and bighead carps, respectively, at 10:00–12:00 a.m. to measure their body length and body weight in April, July, October and December and then their guts were dissected. Gut contents weight of each fish was expressed as gut fullness, $F_i$ (g wet weight per 100 g wet body weight), which was estimated as $F_i = 100 \cdot G_i(W_i)^{-1}$ (where $G_i$ is the wet weight (g) of the gut content of fish $i$ and $W_i$ is the net wet body weight (g) of fish $i$ (total fish weight minus gut content weight; g).

### Extraction and determination of MCs

Lake water (1 L) was filtered with a glass fibre filter (Whatman GF/C; Cambridge, UK) to separate toxins dissolved in water (extracellular MCs) from toxins in particle form (intracellular MCs). The filter films were extracted three times with 30 mL of 75% methanol and the suspensions were centrifuged at 20 000 g (30 min at 4°C). The supernatant was diluted 1:5 with distilled water. The liquor and the distilled supernatant were directly concentrated on SPE cartridges (C18, 0.5 g), which had been preconditioned by washing with 10 mL of 100% methanol and 10 mL distilled water. The eluate from the cartridges was combined with 10 mL of 100% methanol and 10 mL distilled water. The eluate from the cartridges was combined with 10 mL of 100% methanol and was evaporated to dryness. The residue was dissolved with 100 μL distilled water and was used for the qualitative and quantitative analysis of the MCs by LC-MS (Thermo Electron Corporation, San Jose, CA, USA).

Extraction and analysis of the MCs in the liver and muscle tissue of the fish followed the method of Xie, Xie, Ozawa, Honma, Yokoyama and Park (2004): lyophilized samples (0.3 g dry weight for each organ) were homogenized and extracted three times with 25 mL BuOH:MeOH:H₂O (1:4:15) for 24 h while stirring. The suspensions were centrifuged at 29 000 g (1 h at 4°C). Each
supernatant liquor was diluted 1:1 with distilled water and was directly concentrated on an SPE cartridge (C_{18}, 5 g), which had been preconditioned by washing with 50 mL of 100% MeOH and 50 mL distilled water. The cartridge was washed with 50 mL distilled water, followed by 100 mL of 20% methanol. The eluate from the cartridge was combined with 100 mL of 90% methanol and was evaporated to dryness. The residue was dissolved with 5 mL of 100% MeOH and was applied to a silica gel cartridge (2 g), which had been preconditioned with 10 mL of 100% methanol. The cartridge was washed with 10 mL of 100% MeOH and then eluted with 20 mL of 70% MeOH. The elution fraction was evaporated to dryness. The residue was dissolved with 100 μL distilled water and was used for the qualitative and quantitative analysis of MCs by LC–MS according to the methods described by Chen and Xie (2005).

### Statistical analysis

A T-test was performed to test for significant differences between the fish enclosure and the surrounding lake. All statistical analyses were performed using SPSS for Windows version 13.0, SPSS, Chicago, IL, USA).

### Results

#### Physical and chemical parameters

The mean values of the physical and chemical variables measured in the fish enclosure and in the surrounding lake were generally similar during our study period (Table 1). The concentrations of the nitrogen nutrients (TN, TDN, NO₃-N, NH₄-N and NO₂-N) were significantly lower in the enclosure than in the surrounding lake (P < 0.05) while the TP concentration was significantly lower in the surrounding lake (P < 0.05). The mean value of transparency was slightly higher in the enclosure than in the lake and showed two peaks (March and August) during our study period.

#### Total nitrogen concentrations in both the enclosure and the lake increased from January to August, and then decreased from August to December (Fig. 2). Total nitrogen concentrations in the enclosure were significantly lower than those in the surrounding lake, although the difference showed no significance in the later stages of our study (from September to December). Total phosphorus concentrations showed a similar tendency as TN, which reached their peak values in July in enclosure and surrounding lake (Fig. 2). Total phosphorus concentrations were higher in the enclosure for all months except November. The TN: TP mass ratios were generally lower in the enclosure than in the lake and showed two peaks (March and August) during our study period.

### Biomass of phytoplankton

The seasonal succession of phytoplankton species was similar between the enclosure and the surrounding lake (Fig. 3); the phytoplankton were dominated by Cyanophyta (mainly *Microcystis*) and Bacillariophyta (mainly *Cylotella* sp.) from January to June, and followed by Cyanophyta (mostly *Microcystis*) from July to November. In December, the Cyanophyta biomass decreased significantly, and Cryptophyta and Bacillariophyta became the dominant types of phytoplankton. *Microcystis* predominated in the phytoplankton community from July to November in the surrounding lake (Fig. 3). Bacillariophyta contributed to a considerable proportion in the phytoplankton biomass both in the enclosure and in the surrounding lake throughout our study.

In general, the phytoplankton biomass was higher in the enclosure than in the surrounding

### Table 1 Physicochemical variables (mean ± SE) in the enclosure and in the surrounding lake

<table>
<thead>
<tr>
<th></th>
<th>Fish enclosure</th>
<th>Surprising lake</th>
</tr>
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<tbody>
<tr>
<td><strong>Water temperature (°C)</strong></td>
<td>19.52 ± 2.09</td>
<td>18.10 ± 2.13</td>
</tr>
<tr>
<td><strong>SD (cm)</strong></td>
<td>41.41 ± 1.96</td>
<td>46.09 ± 3.16</td>
</tr>
<tr>
<td><strong>DO (mg L⁻¹)</strong></td>
<td>9.65 ± 0.50</td>
<td>8.44 ± 0.57</td>
</tr>
<tr>
<td><strong>Conductivity (μS cm⁻¹)</strong></td>
<td>493.26 ± 12.03</td>
<td>494.75 ± 12.36</td>
</tr>
<tr>
<td><strong>pH</strong></td>
<td>8.01 ± 0.06</td>
<td>8.25 ± 0.20</td>
</tr>
<tr>
<td><strong>Turbidity (NTU)</strong></td>
<td>26.1 ± 1.59</td>
<td>28.47 ± 3.17</td>
</tr>
<tr>
<td><strong>TDS (mg L⁻¹)</strong></td>
<td>200.64 ± 5.02</td>
<td>199.41 ± 4.70</td>
</tr>
<tr>
<td><strong>TN (mg L⁻¹)</strong></td>
<td>1.491 ± 0.152*</td>
<td>1.651 ± 0.200*</td>
</tr>
<tr>
<td><strong>TDN (mg L⁻¹)</strong></td>
<td>0.799 ± 0.114*</td>
<td>1.225 ± 0.167*</td>
</tr>
<tr>
<td><strong>NO₂-N (mg L⁻¹)</strong></td>
<td>0.244 ± 0.029*</td>
<td>0.517 ± 0.101*</td>
</tr>
<tr>
<td><strong>NO₃-N (mg L⁻¹)</strong></td>
<td>0.012 ± 0.003*</td>
<td>0.015 ± 0.003*</td>
</tr>
<tr>
<td><strong>NH₄-N (mg L⁻¹)</strong></td>
<td>0.200 ± 0.028*</td>
<td>0.263 ± 0.059*</td>
</tr>
<tr>
<td><strong>TP (mg L⁻¹)</strong></td>
<td>0.082 ± 0.008*</td>
<td>0.065 ± 0.007*</td>
</tr>
<tr>
<td><strong>TDP (mg L⁻¹)</strong></td>
<td>0.026 ± 0.004</td>
<td>0.025 ± 0.004</td>
</tr>
<tr>
<td><strong>PO₄-P (mg L⁻¹)</strong></td>
<td>0.012 ± 0.002</td>
<td>0.012 ± 0.003</td>
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</tbody>
</table>

*Significant difference, P < 0.05.

TN, total dissolved nitrogen; TP, total phosphorus; TDP, total dissolved phosphorus; DO, dissolved oxygen; TDS, total dissolved solids; TN, total nitrogen; NO₂-N, nitrite nitrogen; NO₃-N, nitrate nitrogen; NH₄-N, ammonia nitrogen; PO₄-P, phosphate phosphorus.
lake from January to June. During the period of algal blooms (from July to September), the phytoplankton biomass was lower in the enclosure than in the surrounding lake, and Cyanophyta contributed to more than 90% of the phytoplankton in the surrounding lake, whereas it represented only 40–80% of the phytoplankton in the fish enclosure. The Microcystis biomass was low from January to June and suddenly increased to peak values in July (60.6 mg L$^{-1}$ in the surrounding lake and 3.6 mg L$^{-1}$ in the enclosure). During the bloom period, the Microcystis biomass decreased by ~94.1% in the enclosure compared with the surrounding lake water. The annual mean values of the Microcystis biomass were 0.61 mg L$^{-1}$ in the enclosure and 7.62 mg L$^{-1}$ in the surrounding lake respectively.

The Chl-a concentration was higher in the fish enclosure than in the surrounding lake from January to June (Fig. 4). The Chl-a concentrations in both the enclosure and the lake reached their peak values in July, with a significantly lower peak value in the enclosure than in the lake.

**Biomass of crustacean zooplankton**

A total of 15 crustacean taxa were identified and the crustacean community was mainly dominated by Bosmina coregoni, Ceriodaphnia cornuta, Limnothionina sinensis, Sinocalanus dorrii and Mesocyclops
The crustacean zooplankton biomass tended to be lower in the enclosure than in the surrounding lake, especially from August to November (Fig. 5). The annual average biomass for all crustacean zooplankton was 0.29 mg L\(^{-1}\) in the enclosure and 0.81 mg L\(^{-1}\) in the surrounding lake. The seasonal dynamics of the cladocerans and the copepods were different between the enclosure and the surrounding lake (Fig. 5). The biomasses of both the cladocerans and the copepods were significantly lower in the enclosure than in the surrounding lake during our study period (\(P < 0.05\)).

As showed in Fig. 6, the zooplankton to phytoplankton biomass ratios was different between in the surrounding lake and fish ecclosures. The results indicated that the higher grazing pressures of zooplankton on phytoplankton occurred in March, October and November in the surrounding lake, while in May in the fish enclosure.

**MCs content in water and fish muscle**

Three MC analogues (MC-LR, -RR and -YR) were identified, of which MC-LR and -RR were the main components. The average concentrations of intracellular MCs were 0.19 \(\mu\)g L\(^{-1}\) in the enclosure and 2.0 \(\mu\)g L\(^{-1}\) in the surrounding lake, and the average concentrations of extracellular MCs were
0.012 μg L⁻¹ in the enclosure and 0.023 μg L⁻¹ in the surrounding lake. Both intracellular and extracellular MCs concentrations were significantly lower in the enclosure than in the surrounding lake (Fig. 7). During the bloom period (from July to September), the intracellular and extracellular MCs in the fish enclosure were reduced by 93.8% and 69.8%, respectively, compared with the MCs content in the surrounding lake. In the enclosure, the peak values of the extra- and intracellular MCs were found in November, with values of 0.026 and 0.644 μg L⁻¹ respectively. In the surrounding lake, the peak values of the extra- and intracellular MCs were found in September, with values of 0.058 and 9.17 μg L⁻¹ respectively.

The monthly dynamics of the MCs concentrations in muscle of silver and bighead carp were shown in Fig. 8. Three MC analogues (MC-LR, -RR and -YR) were identified in the muscle of the silver and bighead carp, and MC-LR and -RR were the main components of the MCs. In the muscle, the maximum MCs concentration was found in July for the silver carp (0.094 μg g⁻¹ DW) and in September for the bighead carp (0.047 μg g⁻¹ DW). For most of the months, the MCs concentrations were higher in the muscle of the silver carp than in the muscle of the bighead carp. The annual average MCs concentrations in the muscle of the silver and bighead carp were 0.033 and 0.016 μg g⁻¹ DW respectively.

Figure 5 Seasonal variations of crustacean zooplankton biomass in the enclosure and in the surrounding lake.
The correlation coefficients between *Microcystis*, phytoplankton, zooplankton biomass, MCs concentrations in the water column and in fish muscle, and the different environmental factors (water temperature, SD, DO, pH, Chl-\( \alpha \), TN, TP and TN: TP) both in the fish enclosure and the surrounding lake were listed in Table 2. In the surrounding lake, both *Microcystis* and phytoplankton biomass were negatively correlated with DO and pH and positively correlated with Chl-\( \alpha \). In addition, phytoplankton biomass was positively correlated with temperature. A strong positive correlation was found between *Microcystis* and phytoplankton biomass (\( R = 0.955, P < 0.01 \)). The intracellular MC content was positively correlated with temperature, Chl-\( \alpha \) and *Microcystis* biomass and extracellular MC content was only positively correlated with Chl-\( \alpha \).
In the fish enclosure, the *Microcystis* biomass was positively correlated with Chl-a and TP. The intracellular MC content was positively correlated with TN and *Microcystis* biomass and negatively correlated with pH. The extracellular MC content was negatively correlated with temperature. The correlations between phytoplankton or zooplankton biomass and other factors were not significant ($P < 0.05$). The MC content in the muscle of the silver carp was positively correlated with Chl-a, *Microcystis* biomass and intracellular MCs, whereas the MC content in the muscle of the bighead carp showed no correlation with these factors.

### Growth and feeding intensity of the silver and bighead carp

Both the silver and bighead carp displayed rapid growth during the study period (Fig. 9). From April to October, the body weight increased approximately fivefold for silver carp and threefold for bighead carp. From October to the end of the year, the silver and bighead carp showed very slow growth rates, and their body weights in December were similar to those in October. In general, the feeding intensity of the silver and bighead carp gradually decreased from April to December as body weight increased (Fig. 9). The gut fullness of the silver carp (average 5.7%) was far higher than that of the bighead carp (average 2.7%) for each month.

### Discussion

In the present study, the annual mean values of transparency and nitrogen nutrient concentrations were slightly lower in the experimental enclosure than in the surrounding lake, suggesting that stocking silver and bighead carp improved the water quality to an extent. The ratio of TN: TP was generally lower in the enclosure than in the surrounding lake during our study period. Xie, Xie, Li, Tang and Liu (2003) found that a low N: P ratio is not a cause but a result of *Microcystis* blooms. Our study found an inconsistency between the cyanobacterial blooms and the low N: P ratio, which may be because the phytoplanktivorous fish (silver and bighead carp) accelerated the recycling of nutrients and the activation of phosphate
Table 2 Correlation analysis between phytoplankton biomass, MCs concentration and environmental factors in the enclosure and in the surrounding lake (E: enclosure S: surrounding lake)

<table>
<thead>
<tr>
<th></th>
<th>Microcystis</th>
<th>Phytoplankton</th>
<th>Zooplankton</th>
<th>Extra-MCs</th>
<th>Intra-MCs</th>
<th>MCs in fish muscle</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>E</td>
<td>S</td>
<td>E</td>
<td>S</td>
<td>E</td>
<td>S</td>
</tr>
<tr>
<td>Tem.</td>
<td>0.501</td>
<td>0.534</td>
<td>-0.222</td>
<td>-0.583*</td>
<td>0.215</td>
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<tr>
<td>pH</td>
<td>-0.561</td>
<td>-0.664*</td>
<td>0.088</td>
<td>-0.682*</td>
<td>0.016</td>
<td>0.184</td>
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<tr>
<td>SD</td>
<td>-0.03</td>
<td>-0.384</td>
<td>-0.157</td>
<td>-0.342</td>
<td>0.042</td>
<td>0.158</td>
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<tr>
<td>TN</td>
<td>0.577</td>
<td>0.051</td>
<td>-0.207</td>
<td>0.105</td>
<td>0.283</td>
<td>0.277</td>
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<tr>
<td>TP</td>
<td>0.672*</td>
<td>0.377</td>
<td>-0.426</td>
<td>0.353</td>
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<td>0.121</td>
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<tr>
<td>TN:TP</td>
<td>-0.027</td>
<td>-0.087</td>
<td>0.035</td>
<td>-0.016</td>
<td>0.133</td>
<td>0.29</td>
</tr>
<tr>
<td>DO</td>
<td>-0.479</td>
<td>-0.686*</td>
<td>0.049</td>
<td>-0.713**</td>
<td>-0.341</td>
<td>-0.197</td>
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<tr>
<td>Chl-a</td>
<td>0.761**</td>
<td>0.967**</td>
<td>-0.39</td>
<td>0.949**</td>
<td>0.273</td>
<td>-0.061</td>
</tr>
<tr>
<td>Microcystis</td>
<td>1</td>
<td>1</td>
<td>-0.197</td>
<td>0.995**</td>
<td>0.053</td>
<td>-0.184</td>
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<tr>
<td>Extra-MCs</td>
<td>-0.065</td>
<td>0.557</td>
<td>0.384</td>
<td>0.556</td>
<td>-0.34</td>
<td>0.3</td>
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<tr>
<td>Intra-MCs</td>
<td>0.704*</td>
<td>0.670*</td>
<td>-0.02</td>
<td>0.679*</td>
<td>-0.117</td>
<td>0.271</td>
</tr>
</tbody>
</table>

*Correlation is significant at the 0.05 level (two-tailed).
**Correlation is significant at the 0.01 level (two-tailed).
through their ingestion and excretion in the enclosure (Drenner, Hambright, Vinyard, Gophen & Pollingher 1987).

Silver carp mainly feed on phytoplankton while bighead carp mainly feed on zooplankton (Chen 1990; Ke et al. 2007). Top-down control from herbivorous zooplankton or fish was likely to be the key factor in regulating the phytoplankton biomass (Tang et al. 2002). The phytoplankton biomass was generally higher in the enclosure than in the surrounding lake from January to June, indicating a bottom-up effect of phytoplankton on silver and bighead carp. From July to September, the phytoplankton biomass and the proportion of Cyanophyta in the enclosure were substantially lower than in the surrounding lake, indicating that stocking the silver and bighead carp greatly inhibited the Cyanophyta biomass. During the bloom period, the Microcystis biomass was reduced by ~94.1% in the enclosure compared with the surrounding lake. Our results indicated that the cyanobacterial bloom was greatly suppressed by the silver and bighead carp. The initial stocking density of silver and bighead carp (7.5 and 1.1 g m\(^{-3}\), respectively) was effective for algae control in the present enclosure.

In our study, both cladoceran and copepod biomasses were significantly lower in the enclosure than in the surrounding lake (P < 0.05). It indicated that the presence of silver and bighead carp caused significant grazing pressure on the cladocerans and copepods. In the non-classical biomanipulation experiment of Meiliang Bay, there was no significant difference between copepod biomass in the fish enclosure and in the surrounding lake water (Ke et al. 2007). Many studies suggested that copepods may be more successful than cladocerans in evading the capture mechanisms of filter-feeding planktivorous fish (Drenner et al. 1987). While, in the present study, the evasive effect of copepods may have been weakened by the low proportion of cladoceran in fish enclosure.

In the surrounding lake, phytoplankton biomass was negatively correlated with DO and pH and positively correlated with Chl-a, temperature and Microcystis biomass. However, the correlation between phytoplankton or zooplankton biomass and other factors was not significant in the enclosure, which may be influenced by the top down effect of silver and bighead carp. Concretely speaking, the effect of silver and bighead carp on the phytoplankton biomass consists of direct (grazing) and indirect factors (changing the nutrient cycle by excretion and changing the grazing pressure of zooplankton on phytoplankton by grazing zooplankton; Vörös, Oldal, Présing & Balogh 1997; Xie 1999). It was complicated to evaluate whether the effect of the fish and nutrients on the phytoplankton or zooplankton biomass was positive or negative because the fish biomass and the nutrients largely overlapped within the measures of phytoplankton and zooplankton biomass (Jeppesen, Jensen, SØndergaard, Fenger-Grøn, Bramm, Sandby, Möller & Rasmussen 2004).

Zooplankton/phytoplankton biomass ratios may be a useful indicator of grazing pressure on phytoplankton (Jeppesen et al. 2004). In our study, the zooplankton to phytoplankton biomass ratio in the surrounding lake was significantly higher than the ratio in the enclosure, indicating that the presence of silver and bighead carp may relieve the grazing pressure on zooplankton on phytoplankton. The low zooplankton/phytoplankton ratio during the Microcystis blooms may be responsible for the decreased selectivity of the fish on the zooplankton (Dong & Li 1994).

Microcystins produced by some cyanobacteria are potent hepatotoxins and promote tumour growth by inhibiting protein phosphatase types 1 and 2A (Carmichael 1992; Dawson 1998). In our study, intracellular and extracellular MCs...
concentrations in the fish enclosure were significantly lower than in the surrounding lake, suggesting that stocking silver and bighhead carp could be an effective way to reduce MC concentrations in the lake by controlling the biomass of the toxic *Microcystis*. During the bloom period (from July to September), the intracellular and extracellular MCs in the enclosure were reduced by 93.8% and 69.8%, respectively, compared with the surrounding lake. Intracellular MCs were reduced by ~95.5% in an enclosure stocking silver carp at a high biomass compared with the lake water in Lake Shichahai (Beijing), while extracellular MCs showed no significant difference between each treatment group and the lake water (Zhang, Xie, Hao, Guo, Gong, Hu, Chen & Liang 2006). The *Microcystis* biomass and the intracellular MC concentration also decreased in the presence of fish in the study of Ke et al. (2007), but the difference was not statistically significant. The restoration effect of our study seemed to be stronger than those former experiments, likely because our fish enclosure was separated from the surrounding lake without water exchange.

Microcystins can accumulate in the various organs of fish through the ingestion of *Microcystis* (Chen, Xie, Zhang, Ke & Yang 2006; Chen, Xie, Zhang & Lei 2007), suggesting a potential risk to humans by consumption of the contaminated fish. In our study, the MC content in the silver and bighhead carp muscle was higher in summer and autumn: this tendency follows the seasonal variation of intracellular and extracellular MCs. The annual average MC content in the muscle of the silver carp (0.033 µg g⁻¹ DW) was higher than that of the bighhead carp (0.016 µg g⁻¹ DW), which may be the result of the different grazing rates and intestine lengths of the silver carp and the bighhead carp (Fig. 9). Chen et al. (2006, 2007) studied the bioaccumulation of MCs in silver and bighhead carp in Meiliang Bay of Lake Taihu, and found that the annual average MC concentrations in the muscle of the silver and bighhead carp were 0.197 and 0.124 µg g⁻¹ DW, respectively, which were six to eight times higher than the values found in our study. This result may be because of higher nutrient loading and intracellular MC concentrations in the water column in Meiliang Bay than in Gonghu Bay. Both this study and Chen et al. (2006, 2007) found that more MCs accumulated in the muscle of the silver carp than in the bighhead carp; this result may caused by ingestion more phytoplankton by silver carp than bighhead carp and different accumulation and metabolic mechanism for MCs between silver and bighhead carp.

In this study, the MC content in the silver and bighhead carp muscle varied from 0.34 to 18.8 ng (mean 4.8 ng) MC-LR eqg⁻¹ wet weight (a coefficient of 5 was used to convert dry weight to wet weight). Assuming an adult human ingests 300 g of fish muscle per day in Gonghu Bay, the daily uptake of MC-LR eq would be 0.102–5.64 μg (mean 1.44 μg), much lower than the TDI (2.4 μg per day for an adult human weighing 60 kg; WHO 2011). Thus, consumption of the fish in Gonghu Bay was safe to human health currently. However, the long-term impact of MCs on the aquatic ecosystem and on public health cannot be overlooked.

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