Pre-treating algae-laden raw water by silver carp during *Microcystis*-dominated and non-*Microcystis*-dominated periods

Hua Ma, Fuyi Cui, Zhiquan Liu and Zhiwei Zhao

ABSTRACT

Performance of pre-treating algae-laden raw water by silver carp during a non-*Microcystis*-dominated period (period I) and a *Microcystis*-dominated period (period II) was investigated in terms of algae cell concentration, total phosphorus content, chlorophyll *a* and phytoplankton species structure. During period I the ineffective filter-feeding for small green algae resulted in the increase of small single algae, which led to the negative removal of chlorophyll *a*, and when the biomass was higher, the negative was more significant. However, due to the effective filter-feeding of silver carp for *Microcystis flos-aquae*, the average removal efficiency exceeded 50% at all stocking biomass levels (20–120 g/m^3) used in experiments during period II. Total phosphorus removal efficiencies could exceed 50% at silver carp biomass stocking levels of 60–80 g/m^3 during both period I and period II. The experimental results indicated that silver carp stocking contributed to the removal of colony-forming cyanobacteria, but led to the increase of single-cell algae (mainly green algae and diatoms) during both period I and period II. The initial phytoplankton community structure and the control of nutrient level were important factors in the choice of silver carp stocking biomass when used to purify algae-loaded water.

Key words | filter-feeding fish, *Microcystis flos-aquae*, phytoplankton community structure, silver carp, single cell algae, water purification

Hua Ma
Fuyi Cui (corresponding author)
Zhiquan Liu
Zhiwei Zhao
State Key Laboratory of Urban Water Resource and Environment,
Harbin Institute of Technology (SKLUWRE, HIT),
PO Box 2650,
Harbin 150090,
China
E-mail: *hit_cuifuyi@hotmail.com*

INTRODUCTION

Due to its ability to filter-feed and digest phytoplankton (Smith 1989; Chen *et al.* 1990), stocking of silver carp has been considered to be a valuable tool for phytoplankton control in eutrophic water bodies (Radke & Kahl 2002). In addition to the worldwide application of silver carp to improve water quality in large bodies such as lakes or reservoirs (Domaizon & Dévaux 1999; Lu *et al.* 2002), silver carp has also been used in relatively smaller water bodies such as water works, as a pre-treatment method to decrease the phytoplankton concentration in algae-laden raw water (Ma *et al.* 2010).

Although silver carp is able to collect particles as small as 4.5–10 μm, using highly modified gill rakers and secretion of mucus (Cremer & Smitherman 1980; Xie 1999), the filtration efficiency for algae with size below 10 μm is very low (Smith 1989; Dong *et al.* 1992). Moreover, the performance of silver carp stocking differed with different initial phytoplankton community structure or under different water quality conditions. In addition, the excretion from silver carp is likely to increase nutrient concentrations and thereby stimulate the growth of some phytoplankton. The stocking biomass has a close relationship with volume of excretion; therefore, the stocking biomass also affects the performance of silver carp.

In the present study, the effect of silver carp on phytoplankton in terms of total phosphorus content, chlorophyll *a* and phytoplankton species structure during *Microcystis*-dominated and non-*Microcystis*-dominated periods were compared. Silver carp stocking biomass is a very important parameter when applied in natural water bodies, so all experiments were carried out at known biomass gradients. The objectives of this study were: (i) to clarify the effects of silver carp on phytoplankton community at the above two typical periods; and (ii) to provide an

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approach as to how to choose an ideal silver carp stocking biomass for the treatment or purification of actual algae-laden water.

MATERIALS AND METHODS

Source of raw water

Raw water used in this study was from Yu-Qiao Reservoir, which is primarily used as a drinking water supply for northern China. In the last few years, *Microcystis* blooms have occurred every summer and have seriously affected the water treatment processes. Blooms occur mainly in July and August and the dominant algae species has been *Microcystis flos-aquae*. The experiments have been carried out from June to August so that both bloom and non-bloom periods were covered.

Experimental facility and design

The experiments were performed in four semi-underground tanks. Each tank was of length 4.0 mm × width 2.0 mm × depth 2.5 m, with an effective volume of 20 m^3. Raw water was pumped into the tank bottoms through 40 mm diameter PVC pipes and overflowed the tanks. The hydraulic retention time (HRT) in each tank was 72 h. Tanks were randomly selected and stocked with different biomass levels of silver carp. Mean individual biomass was 398.5 ± 16.0 g (mean ±1 SD). Initial biomasses were 20.2, 40.7, 56.4, 82.3, 97.9 and 122.8 g/m^3 (the corresponding numbers of fish were 1, 2, 3, 4, 5 and 6) during the non-*Microcystis*-dominated period and 18.0, 41.5, 59.7, 81.7, 99.9 and 120.5 g/m^3 (the corresponding numbers of fish were 1, 2, 3, 4, 5 and 6) during the *Microcystis*-dominated period. Each trial lasted for 15 days, and 5 days after the start of each trial phosphorus (TP), phytoplankton numbers and chlorophyll *a* (Chl *a*) concentrations were determined each day. All the values in the following figures were the average of at least seven data, and the error bars indicate standard deviation. One-factor ANOVA was performed to test for significant differences between treatments (fish presence versus absence) of all measured variables, and the least significant difference (LSD) method was performed to test for the difference among average values of cell concentrations, chlorophyll *a* and TP under different biomass levels ($\alpha = 0.05$).

Analysis methods

Phytoplankton species were identified and measured using a microscope (Olympus BX41, Tokyo, Japan) at 400× magnification following the method described by Ma *et al.* (2009). TP was measured in unfiltered water samples using a spectrophotometer (T6, Beijing, China). Chlorophyll *a* was extracted from the cell residue on a GF/C filter using methanol, and was quantified spectrometrically. All analyses were performed in accordance with Chinese Standard methods (SEPA of China 2002).

RESULTS

Water quality in the non-*Microcystis*-dominated period and the *Microcystis*-dominated period

The experiments were carried out during the non-*Microcystis*-dominated period (period I) and the *Microcystis*-dominated period (period II). Raw water characteristics for the two periods are given in Table 1. Before early July, phytoplankton cell concentrations were always below 2.8×10^7 cells/L. In this period the dominant species were mainly green algae (mainly *Chlamydomonas*, *Platymonas* and *Scenedesmus*) and diatoms (mainly *Cyclotella*). But after 10 July, *Microcystis flos-aquae* appeared in the raw water and became the absolute dominant species. Although *Microcystis flos-aquae* concentrations fluctuated dramatically in the following month, they were always the predominant phytoplankton species with a mean proportion of 83.0%.

Table 1 | Characteristic data of the raw water used for the experiments

	Period I		Period II	
Parameter	Range	Mean value	Range	Mean value
T (°C)	23.5–27.1	25.5	27.0–28.0	27.6
Turbidity (NTU)	4.00–7.97	5.80	5.51–21.70	12.18
pH	7.40–8.35	7.73	7.51–8.44	7.89
COD_{Mn} (mg/L)	2.35–4.09	3.10	3.23–4.62	3.81
Total phosphorus ($\times 10^{-2}$ mg/L)	2.2–4.9	3.9	4.1–5.7	4.9
Chl *a* (mg/m^3)	2.76–9.19	5.13	9.31–32.55	20.31
Phytoplankton ($\times 10^7$ cells/L)	0.37–2.73	0.98	2.99–6.43	4.33

Phytoplankton cell concentrations at different silver carp biomass

The changes of dominant species and total phytoplankton cell concentrations at different biomasses of silver carp are shown in Figure 1. During period I, Chlorophyta concentrations significantly increased in the presence of silver carp ($F = 9.89$, P-value $= 0.00023$), and total phytoplankton densities increased with the increase of green algae. When biomass of silver carp was above 60 g/m^3, Chlorophyta concentrations increased with the increase of silver carp biomass, but did not show significant differences among the biomass levels (different values between the averages were below LSD value). This observation indicates that the stocking of silver carp cannot effectively filter-feed single cell green algae, and the stocking of silver carp contributes to the increase of green algae. During period II, the stocking of silver carp resulted in a sharp decrease of cyanophyta concentrations in effluent, and the removal of total phytoplankton increased with the increase of silver carp biomass until the biomass was above 60 g/m^3. The average removal efficiency exceeded 50% at all stocking biomass levels used in experiments during period II. While the biomass was above 100 g/m^3, cyanophyta concentration showed a small increase.

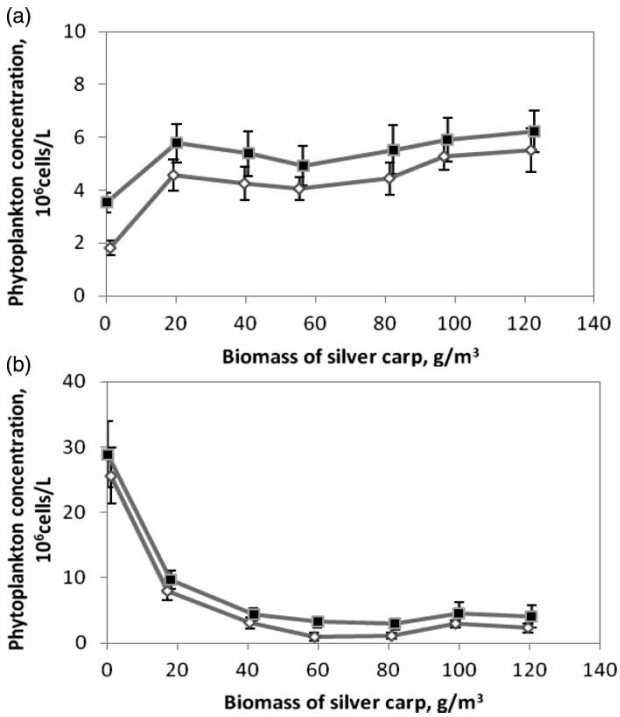

Figure 1 | Changes of phytoplankton concentrations at different biomass of silver carp in period I (a) and period II (b). Values are means ± SD.

Phytoplankton species structure and chlorophyll *a* at different silver carp biomasses

Phytoplankton species structures for different biomass levels of silver carp were different for the different periods. Figure 2 shows the changes of the main phytoplankton cell proportions at different biomasses of silver carp on the day of stable water quality in two periods. In period I, green algae and diatoms were the dominant groups, and the percentage of Chlorophyta showed a sharp increase in the presence of silver carp. This figure increased with increasing biomass of silver carp up to 82.3 g/m^3; further increase was not obvious at higher stocking levels. In period II the rapid increase of *Microcystis flos-aquae* concentration in raw water resulted in the dominance of cyanophyta among the phytoplankton groups. In the presence of silver carp the proportion of cyanophyta in water tended to decrease with increasing biomass of silver carp up to stocking levels of 59.7 g/m^3. The proportions of cyanophyta rose with further increase of stocking biomass. Overall, silver carp significantly inhibited the growth of cyanobacteria in raw water and enhanced the proportions of Chlorophyta.

Chlorophyll *a*, a major photosynthetic pigment in phytoplankton, is usually used to evaluate the primary productivity in water, so the value is indicative of phytoplankton biomass. Changes to phytoplankton species structures caused by silver carp also affected Chl *a*

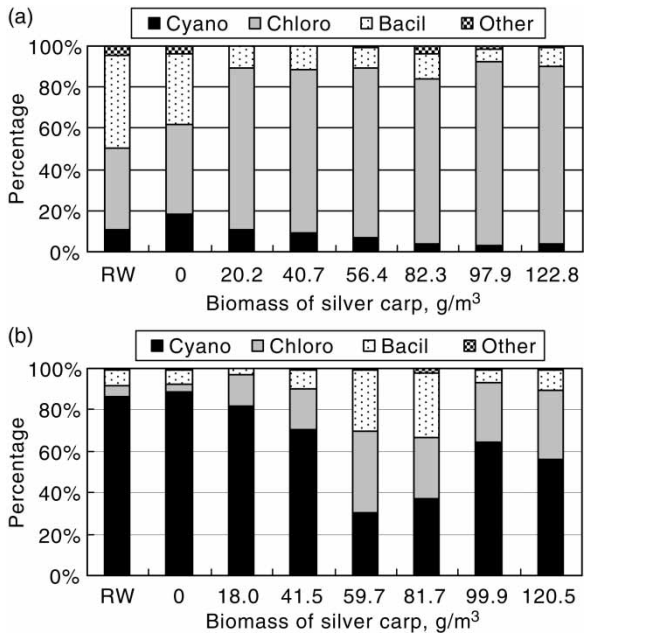

Figure 2 | Changes of main phytoplankton cell proportions at different biomass of silver carp in period I (a) and period II (b). RW = raw water; Cyano = cyanophyta; Chloro = Chlorophyta; Bacil = bacillariphyta; Other = Other species.

concentrations, and there was an obvious difference of this effect during the two periods (Figure 3). In period I, Chl *a* concentrations in the effluent were significantly higher than in the raw water, and this phenomenon was more evident when the biomass of silver carp was over 82.3 g/m^3 (different values between the averages were above LSD value 3.09). Chl *a* concentrations in effluent showed significant difference ($F = 7.81$, P-value $= 4.66 \times 10^{-5}$). Further tests using the LSD method indicated that Chl *a* did not show significant difference (different values between the averages were below the LSD value) when the biomasses of fish were 0 g/m^3, 20.20 g/m^3, 40.70 g/m^3 and 56.40 g/m^3, while at high biomass level there was also no significant difference. On the contrary, in period II, Chl *a* concentrations in effluent were lower than those in raw water at all silver carp stocking biomass levels. One-way ANOVA revealed significant difference between fish absence and fish presence ($F = 8.97$, P-value $= 1.32 \times 10^{-5}$), but the biomass had no significant effect on the efficiency of Chl *a* removal (different values between the averages were below the LSD value).

Changes of total phosphorus at different silver carp biomasses

The effects of silver carp on TP content in water mainly depend on two factors: the removal by filter-feeding of particulate phosphorus (in the form of suspended substances such as phytoplankton and organic or inorganic particulate matter) and the release of dissolved phosphorus from faecal pellets and directly from fish excretion. Figure 4 shows TP removal at different biomass levels of silver carp in the two periods. When the biomass of silver carp was 0, total phosphorus decreased by about 20%, which might result from the sedimentation of inorganic particulate matter. Because the rate of *Microcystis* sedimentation is very slow (Visser *et al.* 1995; Bormans *et al.* 1999), the phosphorus removal was mainly attributed to fish filter-feeding of algae particles. One-way ANOVA indicated that removals of TP showed significant differences both in period I ($F = 18.668$, P-value $= 1.34 \times 10^{-9}$, F critical $= 2.372$) and in period II ($F = 14.225$, P-value $= 3.89 \times 10^{-8}$, F critical $= 2.372$). When stocking biomass in raw water was at a low level, the removal of TP tended to increase with increasing biomass of silver carp. Removals of TP reached a maximum value when the biomasses were 56.4 g/m^3 and 81.7 g/m^3 in period I and period II, respectively. When the stocking biomass was increased to 100 g/m^3 and 120 g/m^3, the removal of TP decreased. A further test using the LSD method also indicated that when the biomass level was 56.4 g/m^3 in period I, TP removal was significantly higher than those in other biomass levels. When the biomass levels were 59.7 g/m^3 and 81.7 g/m^3, TP removals were significantly higher than those in other biomass levels, but removal of TP did not show significant differences between these two biomass levels (different values between the averages were below the LSD value of 8.70).

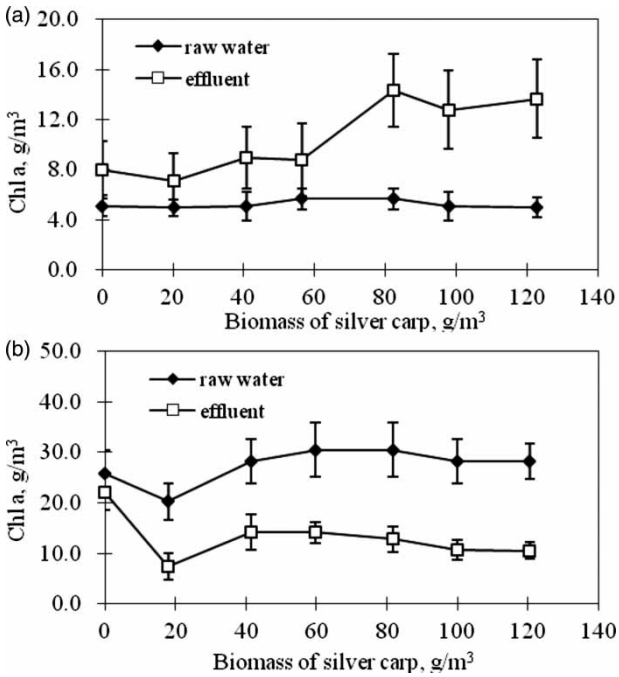

Figure 3 | Changes of Chl *a* concentrations at different biomass levels of silver carp in period I (a) and period II (b). Values are means ± SD.

DISCUSSION

The changes of different phytoplankton species concentrations in the water mainly depended on the grazing characteristics of silver carp, and the proliferation and

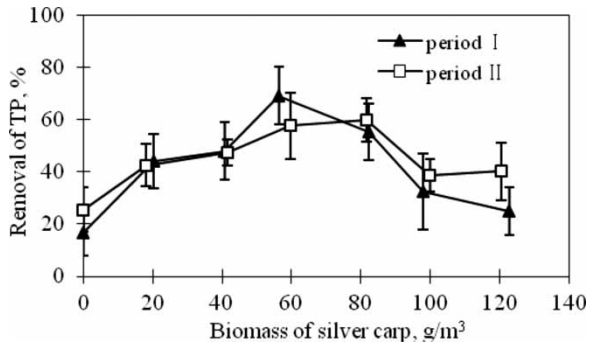

Figure 4 | Removal of total phosphorus (TP) at different biomass levels of silver carp in period I and period II. Values are means ± SD.

sedimentation of phytoplankton. In natural water phytoplankton mainly consisted of single-cell algae (such as green algae and diatom) and colony-forming cyanobacteria. A nutrition transfer to smaller size phytoplankton was bound to occur in the presence of silver carp in such water due to higher filtration rates of silver carp for large phytoplankton than the smaller phytoplankton (Dong *et al.* 1992; Turker *et al.* 2003). This phenomenon occurs not only in the *Microcystis* bloom period but also in the non-*Microcystis* bloom period. However, previous studies (Starling & Rocha 1990; Domaizon & Dévaux 1999) do not involve this factor although they were aware of the possible negative effect such as the increase of micro algae. In the present study the two periods were considered. Domaizon & Dévaux found that stocking of silver carp led to an increase in the proportion of *Microcystis* in total phytoplankton when the stocking biomass was 0–32 g/m^3 (Domaizon & Dévaux 1999). Obviously this conclusion is valid when the phytoplankton is composed of microalgae, such as green algae or diatom, which cannot be grazed effectively by silver carp. Our study clarified the difference between phytoplankton removal during the *Microcystis* bloom period and the non-*Microcystis* bloom period, which provided a guide for pre-treating algae-load raw water when silver carp was used to purify raw water for drinking water supply.

The increase in TP concentration in water also directly stimulated the growth of single-cell algae. This result was consistent with studies undertaken in Tianjin by Ma *et al.* (2009, 2010). However, this effect was not the same at different silver carp stocking biomass levels. At low silver carp stocking levels, the fish filter-feeding activities for phytoplankton was reduced and the nutrient transfer rate from water to fish body was also limited, so good removal efficiencies of phytoplankton and phosphorus could not be reached. At moderate stocking levels of silver carp the role of phytoplankton control by silver carp could function effectively, not being restricted by nutrient (i.e. phytoplankton food) limitation. At this point, the nutrient utilization rate through silver carp filter-feeding was much higher than the nutrient discharge rate through excretion of silver carp. At higher stocking levels, increased grazing of phytoplankton by silver carp meant that food availability became the main limiting factor. Here, to maintain the normal activities of silver carp, the amount of nutrients excreted form fish was higher than the uptake amount form water. Therefore, under excessive grazing pressure, the growth of other species in the food chain was stimulated (Brooks & Dodson 1965). When the silver carp biomass is increased further, the lack of nutrients and dissolved oxygen might reduce the activities of silver carp and the filtration efficiency of phytoplankton. The decrease of grazing pressure was conducive to the growth of larger algae, which meant a decrease in the proportion of small phytoplankton cells. Thus, when silver carp is used to remove phytoplankton in the pre-treatment of algae-loaded raw water, the selection of biomass parameter should be mainly based on the initial phytoplankton community structure and nutrient levels.

It should be noted that all experiments in this study were conducted in water tanks with relatively short HRT, in which the biological phase was simple and controllable, and nutrient concentrations in the raw water were not high. This situation meant that only the feature of silver carp filter-feeding was utilized as a bio-processor for pre-treating algae-laden raw water. In contrast, large eutrophic lakes or reservoirs have high concentrations of nitrogen and phosphorus in the water, nutrient-rich sediment, the presence of complex biological phases and very long HRTs. It has usually been difficult to achieve satisfactory results by silver carp stocking in such water bodies.

CONCLUSIONS

Due to the effective filter-feeding of silver carp for *Microcystis flos-aquae*, the average removal efficiency exceeded 50% at all stocking biomass levels (20–120 g/m^3) used in experiments in the *Microcystis*-dominated period. However, during the non-*Microcystis*-dominated period the ineffective filter-feeding for small green algae resulted in the increase of small single algae, which led to the negative removal of chlorophyll *a*, and when the biomass was higher, the negative removal was more significant. Total phosphorus removal efficiencies could exceed 50% at silver carp biomass stocking levels of 60–80 g/m^3 during both the non-*Microcystis*-dominated period and the *Microcystis*-dominated period.

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