

Pilot study on control of phytoplankton by zooplankton coupling with filter-feeding fish in surface water

Hua Ma, Fuyi Cui, Zhiquan Liu and Zhenqiang Fan

ABSTRACT

A pilot-scale facility was originally designed to control phytoplankton in algae-laden reservoir water characterized by summer cyanobacteria blooms (mainly *Microcystis flos-aquae*). The system made good use of the different food habits of *Daphnia magna* and silver carp. Zooplankton (*Daphnia magna*), filter-feeding fish (silver carp), and zooplankton (*Daphnia magna*) were stocked in three separated tanks in sequence, respectively. Thus, single-cell phytoplankton and some *Microcystis flos-aquae* in small size were first grazed by *Daphnia magna* in the first tank, and in the second tank phytoplankton larger than 10 μm were filtered by silver carp, and the concentration of the remaining phytoplankton was further reduced to a rather low level by *Daphnia magna* in the third tank. The results showed that the system had good removal efficiencies of phytoplankton and chlorophyll *a*, 86.85% and 59.41%, respectively, and permanganate consumption (COD_{Mn}) and turbidity were lowered as well. A high phytoplankton removal efficiency and low cost indicated that the system had a good advantage in pre-treating algae-laden source water in drinking water works.

Key words | *Daphnia magna*, drinking water treatment, filter-feeding fish, *Microcystis flos-aquae*, phytoplankton, silver carp, zooplankton

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INTRODUCTION

Excessive growth of phytoplankton caused by continuing eutrophication in surface source water posed increasing problems to drinking water works. The direct algae-related problems in such waters were unpleasant tastes, odors and high treatment cost (Hu & Chiang 1996; Sugiura *et al.* 1998). In addition, phytoplankton and extra-cellular product could be potential trihalomethane (THM) precursors of disinfection by-products (Oliver & Shindler 1980; Graham *et al.* 1998), and some species of cyanobacteria could produce toxins which contaminate drinking water sources and threaten public health (Codd 1995; Lankoff *et al.* 2004; Hoeger *et al.* 2005; Huang *et al.* 2007). To reduce the concentration of phytoplankton in raw water becomes the focus of recent research.

The technology of using biological control to reduce the level of phytoplankton in water source showed a great

prospect. Biomanipulation, the biological control method being used to control lake eutrophication, was a very popular lake management technique, which artificially changed the food web through the use of large-scale structure of zooplankton grazing on the phytoplankton to achieve the purpose of reducing phytoplankton (Shapiro *et al.* 1975). Large sized zooplankton, especially the most effective grazer *Daphnia*, were able to consume a wider range of phytoplankton species than small zooplankton. Therefore when they were dominant in water body, phytoplankton biomass would be kept to a very low level (Brook & Dodson 1965). But some studies showed that biological control was not able to fundamentally eliminate the formation of water bloom (Carpenter *et al.* 1995). *Daphnia* filtration can induce the change of inedible phytoplankton (Gliwicz 1975). Additionally, a high

proportion of colonies would also reduce the grazing efficiency of zooplankton (Hessen & Van 1993).

Silver carp, one of the most intensively cultured fish in the world, was considered to be the most promising tool in the control of eutrophication in the future (Starling 1993). Though silver carp could not completely control phytoplankton smaller than 10 μm (Smith 1989; Vörös et al. 1997), it showed effectiveness in dealing with phytoplankton larger than 10 μm (Radke & Kahl 2002), especially colony-forming cyanobacteria (Xie & Liu 2001). The application conditions and stocking densities of silver carp had been fully studied (Domaizon & Dévaux 1999; Radke & Kahl 2002).

Based on the characteristics of the food habits of zooplankton and silver carp, high phytoplankton removal efficiency can be achieved if they are well used. However, research in the area of drinking water treatment is rarely reported in literature. Owing to the predator–prey relationship between silver carp and zooplankton, silver carp will dramatically reduce the zooplankton density; consequently, the roles of the two controlling phytoplankton cannot be well played. In consideration of their different food habits and complementarity on phytoplankton removal, it is of high interest to find a way to make full use of their functions in phytoplankton control and algae-laden raw water pre-treatment.

In this study, we designed a pilot-scale facility, in which *Daphnia magna* and silver carp were stocked in separated tanks that were linked up in sequence. The objective of this study is to completely remove phytoplankton from algae-laden source water through this system consisting of zooplankton and algal-grazing fish. The phytoplankton removal efficiency of the proposed system was investigated, and phytoplankton species composition and concentration were analyzed in detail. In addition, the stability and application of the system were discussed.

MATERIALS AND METHODS

Field site and source water quality

The pilot study was conducted in a drinking water plant in Tianjin, China, between September and October in 2007. Source water used in our test was from Yu-Qiao Reservoir,

the main water source of drinking water plants in Tianjin, China. The reservoir was characterized by summer cyanobacteria blooms (mainly *Microcystis flos-aquae*) from June to October. Observational results by microscopy demonstrate that the reservoir water contains principally *Synechocystis*, *Merismopedia*, *Scenedesmus*, and *Cyclotella* etc, apart from *Microcystis flos-aquae*. The raw water quality during the experiment period was listed (Table 1).

Experiment design and pilot-scale facility

The experimental facility consists of three semi-underground tanks in series (Figure 1). Each tank was 4 m \times 2 m \times 2.5 m, with an effective capacity of 20 m³. Two steel meshes were set in the two cross sections of the flow of the adjacent water tanks to prevent silver carp in tank 2 from running to the other two tanks. Raw water was pumped into the bottom of tank 1 through a PVC pipe with Φ 40 mm, and then overflowed in tank 3. The three tanks were pre-filled with raw water. The hydraulic retention time (HRT) in each tank was 24 h, and the total HRT was 72 h in the facility. *Daphnia magna* appeared in the three tanks two weeks later, and then seven silver carp were stocked in tank 2. The average weight was 342.9 g, with stocking density 120 g/m³. Related water quality monitoring work was carried out 6 days later.

ANALYSIS METHODS

Phytoplankton counting: 1,000 mL water sample was collected, and 15 mL Lugols solution (40 g iodine was dissolved in 1,000 mL aqueous solution containing 60 g potassium

Table 1 | Raw water quality during the experiment period

Index	Value
Temperature ($^{\circ}\text{C}$)	21–24
Turbidity (NTU)	5–8
pH	8.0–8.4
Total phosphorus (mg/L)	0.02–0.04
COD _{Mn} (mg/L)	3.2–3.8
Algae concentration (cells/L)	3×10^6 – 2×10^7
Chlorophyll a (mg/m ³)	6.1–15.2

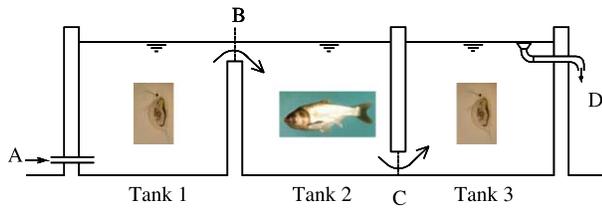


Figure 1 | The pilot-scale facility of the system.

iodide) was added, then the water sample was filtered with cellulose acetate membrane (0.65 μm). Put the membrane into 50 mL beaker, and add 30 mL distilled water. Oscillate the beaker containing membrane in ultrasonic cleaning for 10 min so as to move the cells from the membrane into the distilled water. Draw 0.1 mL sample into the cell count box, and count all algae at $400\times$ using a microscope (Olympus BX41), in which colony-forming *Microcystis* was also counted by cell count and 50 visions were examined. Calculate the number in 1 L water samples by the following formula:

$$N = \frac{A}{A_c} \times \frac{V_w}{V} n$$

where N is phytoplankton concentration in water (cell/L), A is the area of cell count box (mm^2), A_c is the area of 50 visions (mm^2), V_w is the volume of concentrated water (30 mL), V is the volume of cell count box (0.1 mL), n is the cell number (cell). Another same water sample was counted as a duplicate, and the average of two numbers was obtained as phytoplankton concentration. If the difference value of the two numbers was above 15%, count the third sample, and the average of the similar two numbers was obtained as phytoplankton concentration.

Daphnia magna density microscope counting: 2,500 mL water sample was collected, and 25 mL Lugols solution was added, then the water sample was filtered with cellulose acetate membrane (1.2 μm). Count all *Daphnia magna* at $100\times$ using a microscope (Olympus BX41), and calculate the density of *Daphnia magna* in water samples. The counting was carried out by triplicate like phytoplankton counting.

Permanganate consumption (COD_{Mn}) used acid method according to Chinese Standard methods (Yu et al. 2007). Samples for chlorophyll *a* (chl *a*) analysis were

analyzed by visible spectrophotometry (see U.S. EPA method 446.0). Turbidity was measured using a 2,100 N Turbidimeter (HACH).

RESULTS

Phytoplankton concentration, chl *a* in the inflow and outflow

Concentrations of phytoplankton and chl *a* of inflow and outflow in the system from September 19 to October 6, were determined. Experiment was conducted between late summer and early fall mainly because the highest phytoplankton concentration was obtained during this time. During the experiment algal concentration in raw water or inflow ranged from 3.1×10^6 cell/L to 2.01×10^7 cell/L, with a down trend. Phytoplankton concentration in outflow was always in a stable and low level, with a maximum value of 1.9×10^6 cell/L and the average value of 9.0×10^5 cell/L (Figure 2). The removal efficiency of

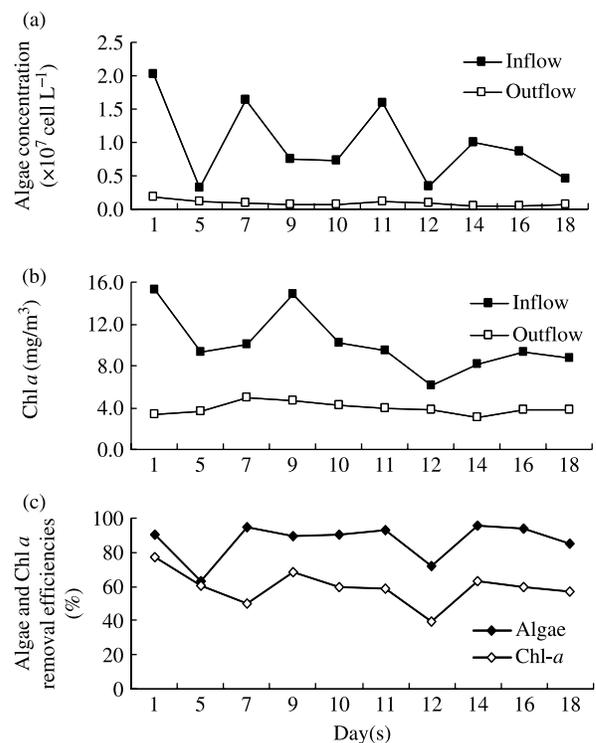


Figure 2 | Algae and chl *a* in inflow and outflow, and their removal efficiencies in the system.

phytoplankton, averagely 86.85%, shared the same trend with that of chl *a*, averagely 59.41%.

COD_{Mn} and turbidity

COD_{Mn} and turbidity in outflow in the system were significantly reduced compared with that in inflow (Figure 3). COD_{Mn} in inflow ranged from 3.19 to 3.82 mg/L, with a mean value of 3.45 mg/L, while that in outflow maintained at 3 mg/L, with a mean of 2.97 mg/L. Turbidity in outflow was about 2.0 NTU, and the average removal rate was 67.91%.

Phytoplankton analysis and *Daphnia magna* density

Species composition and amount of phytoplankton, chl *a* and *Daphnia magna* densities in inflow and outflow of the system were determined on September 19, September 27 and October 2. Results showed that phytoplankton in raw water (point A) were mainly composed of *Cyanophyta*, *Chlorophyta* and *Bacillariophyta*, with the percentage of 80.32%, 65.29%, and 77.59% respectively (Figure 4). Most of the *Chlorophyta* and *Bacillariophyta* were removed in tank 1. It should also be noted that 30–40% of *Cyanophyta* removal was achieved in the first tank. In tank 2 the concentration of *Chlorophyta* in outflow was further reduced to a lower level (below 1.0×10^6 cell/L). What is more worth noting is that there was an obvious increase of

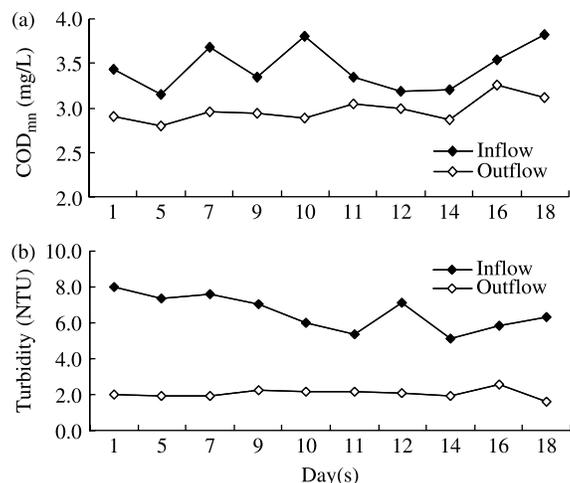


Figure 3 | Permanganate consumption (COD_{Mn}) and turbidity in inflow and outflow in the system.

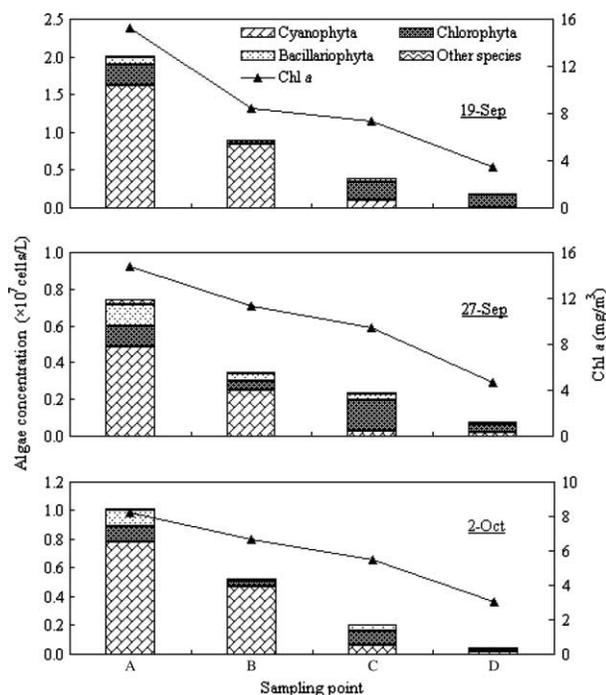


Figure 4 | Changes in algal species composition, amount, and chl *a* in each sampling point (A–The inflow of tank 1 (raw water); B–The outflow of tank 1; C–The outflow of tank 2; D–The outflow of tank 3 (the outflow of the system)).

Chlorophyta concentration in tank 2. The outflow concentration of all the phytoplankton decreased to a minimum value through this system. The sample concentrations of the outflow phytoplankton were 1.9×10^6 cell/L, 1.8×10^5 cell/L and 4.3×10^5 cell/L, respectively. The system reduced *Cyanophyta*, *Chlorophyta* and *Bacillariophyta* by 96.56%, 64.27% and 86.15%, respectively.

Changes in chl *a* indicated that chl *a* was gradually reduced as the total phytoplankton concentrations were reduced. The concentrations of chl *a* in raw water (point A) were 15.21 mg/m^3 , 14.83 mg/m^3 and 8.19 mg/m^3 , respectively, corresponding to that in outflow (point D) of 3.41 mg/m^3 , 4.70 mg/m^3 and 3.03 mg/m^3 , and the removal efficiencies reached 77.58%, 69.31% and 63.00%, respectively.

Daphnia magna densities in the three tanks were monitored on September 19, September 27, and October 2 (Table 2). Water samples were taken at the depth of 0.5 m, 1.0 m, and 2.0 m, respectively, and densities of the samples were counted and calculated. The average of the three figures was the *Daphnia magna* density in this tank.

Table 2 | *Daphnia magna* density in each tank on each sampling date

Sample collection date	<i>Daphnia magna</i> density (ind/L)		
	Tank 1	Tank 2	Tank 3
19 September 2007	27	1	10
27 September 2007	26	1	33
2 October 2007	22	2	48

Obviously, the density of *Daphnia magna* in tank 2 was much lower compared with that in tank 1 and tank 3. In tank 1 and tank 3 *Daphnia magna* grazing phytoplankton were free to grow due to the absence of silver carp, so *Daphnia magna* densities in these two tanks were higher than in tank 2. It was observed that the *Daphnia magna* density in tank 1 was relatively stable, while that in tank 3 showed a growing trend, increasing from 10 ind/L to 48 ind/L.

Single-cell phytoplankton and *Microcystis flos-aquae*

Concentrations of single-cell phytoplankton (mainly *Chlorophyta*, *Bacillariophyta*, and some single-cell cyanobacteria) in each sampling point were determined (Figure 5). The results showed that the concentrations of single-cell phytoplankton, smaller than 10 μm and larger than 10 μm , decreased obviously, while the two types of phytoplankton in the outflow of tank 2 both increased. However, in tank 3, there was a sharp decrease in the two types of phytoplankton. The concentration of single-cell phytoplankton in the outflow of the system got an obvious decrease, compared to that in the raw water.

The *Microcystis flos-aquae* concentration in raw water showed a wild fluctuation in the range of 1.33×10^7 cell/L to 4.9×10^6 cell/L, while decreased significantly in tank 1 and tank 2. And it was reduced below the limit of determination method in the outflow of tank 3 (Figure 6).

DISCUSSION

A number of studies on how to remove excessive water-bloom algae or how to prevent eutrophication in lakes and reservoirs had already been carried out (Drenner & Hambright 1999; Meijer et al. 1999; Mehner et al. 2002). Most of these studies were based on the principle of

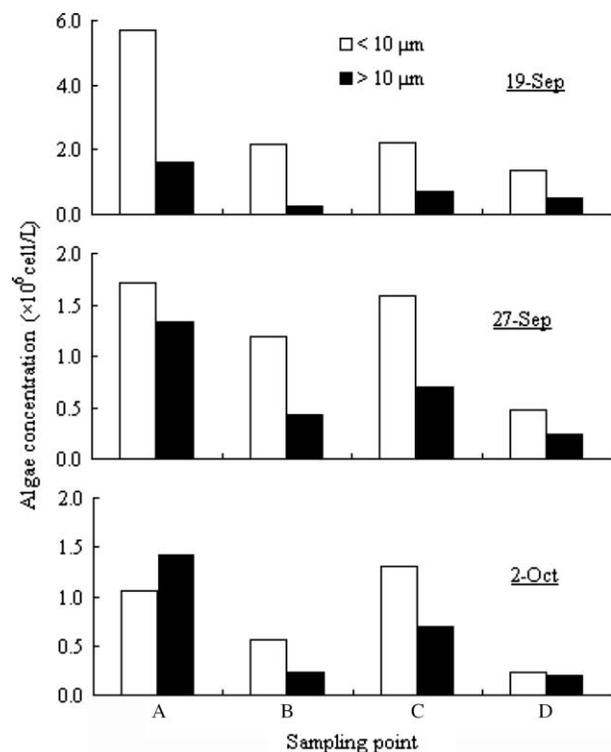


Figure 5 | Changes of single-cell algae (<math>< 10 \mu\text{m}</math> and $> 10 \mu\text{m}$) concentration in each sampling point (A–The inflow of tank 1 (raw water); B–The outflow of tank 1; C–The outflow of tank 2; D–The outflow of tank 3 (the outflow of the system)).

biomanipulation, but it was usually difficult to meet the conditions of successful application of biomanipulation (Benndorf et al. 2002). Similarly, just stocking silver carp in eutrophic water body could not completely remove phytoplankton (Chen et al. 1990; Laws & Weisburd 1990; Starling & Rocha 1990). The raw water used in our study is typical mesotrophic reservoir water in north of China, which is the main source of drinking water. Since the 1,980 s, every year

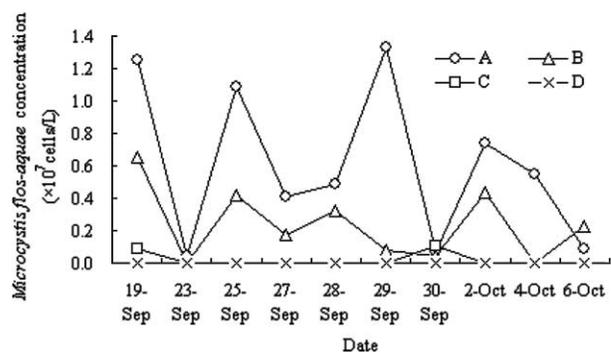


Figure 6 | Changes of *Microcystis flos-aquae* concentration in each sampling point on each sampling date.

in summer and autumn water-bloom appears and seriously affects the normal operation of water treatment process.

Permanganate consumption (COD_{Mn}) can not only be used to evaluate the concentration of ordinary organic compounds, but also phytoplankton biomass (He 1989), so the decrease of phytoplankton in the outflow often results in the reduction of permanganate consumption. In summer and autumn high concentration of phytoplankton in water causes water turbidity, therefore the decrease of phytoplankton in outflow can also reduce water turbidity. Apart from phytoplankton, *Daphnia magna* also filtered particles that caused turbidity in tank 3. Therefore, in tank 3 the concentration of phytoplankton was decreased, and the turbidity in outflow was stabilized at a very low level (about 2 NTU) as well.

Daphnia magna filtered food particles by its filter comb (Brendelberger & Geller 1985). It was observed that *Daphnia magna* size was generally 2 to 3 mm in microscope counting for tank 1 and tank 2, and with this size *Daphnia magna* was able to well graze and ingest most size classes of phytoplankton smaller than 60 μm , consequently in tank 1 and tank 3 the number of single-cell phytoplankton as well as chl *a* had been significantly reduced. At the same time the colony-forming *Microcystis flos-aquae* with small size would also be grazed by *Daphnia magna* in tank 1 and tank 3, so the total concentration of *Microcystis flos-aquae* declined (Figure 6). The remaining *Microcystis flos-aquae* not grazed by *Daphnia magna* in tank 1 would be filtered by silver carp in tank 2.

Food particle selection by silver carp was a mechanical function of gill rake morphology (Smith 1989), and its gill rakes determined that its food particle size was larger than 10 ~ 20 μm , which obviously was higher than that of *Daphnia magna*, so in theory silver carp could well filter *Microcystis flos-aquae* and other large phytoplankton larger than 20 μm . However, the size-selective filtering of silver carp was unsuitable for smaller species, and the presence of silver carp also led to a very small number of *Daphnia magna* in tank 2, therefore, in this tank the number of the single-cell phytoplankton increased (Figure 5). The phytoplankton entering into tank 3 was mainly single-cell *Chlorophyta* and *Bacillariophyta*. As a result of the absence of silver carp *Daphnia magna* became the dominant species. Single-cell phytoplankton was reduced to a very

low level under the grazing pressure. Through series of tanks, the phytoplankton concentration of raw water was lowered to a level below 1.9×10^6 cell/L, and chl *a* was also below 5 mg/m^3 , and this phytoplankton concentration almost had no impact on the traditional drinking water treatment process.

Although the mean retention time of water in each tank was only 24 hours, the velocity of water flow is less than 0.005 cm/s. At this water flow velocity, very fewer *Daphnia* can be taken into next tank with water flow, so *Daphnia* in the system do not need a high growth rate. On the other hand, *Daphnia* generally has very long life (more than 30 days), so under a very low growth rate, the system can maintain in a steady state for a very long time. Therefore, *Daphnia magna* density in the tank 1 and tank 3 will be steady in several days, but will vary due to the change of edible phytoplankton biomass in flow water.

Due to its high removal efficiency for phytoplankton, low cost, simple operation and management and absence of chemicals addition, the system shows a great prospect in pre-treating algae-laden raw water in drinking water works. Optimal system parameters can be achieved to lower the cost by further study of the elements of phytoplankton removal efficiency, retention time, phytoplankton concentration, surface area and depth, and phytoplankton growth rates.

CONCLUSION

- (1) The results of this study manifest that the system, in which zooplankton coupling with filter-feeding fish silver carp are stocked to control phytoplankton, can reduce the amount of phytoplankton by an average of 86.85% and chlorophyll *a* by an average of 59.41% for reservoir water characterized by summer cyanobacteria blooms (mainly *Microcystis flos-aquae*). COD_{Mn} and turbidity were also obviously lowered.
- (2) Because the system can operate stably and has many advantages, such as high removal efficiency for phytoplankton, low cost, simple operation and absence of chemicals addition, it shows a great prospect in pre-treating algae-laden raw water in drinking water works.

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