# Effects of silver carp (*Hypophthalmichthys molitrix*) on spring phytoplankton community structure of Three-Gorges Reservoir (China): results from an enclosure experiment

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### ABSTRACT

Serious phytoplankton blooms occurred repeatedly every spring in many bays of the Three-Gorges Reservoir. However, little was known about the impact of phytoplanktivorous fish on spring phytoplankton community structure in the Three-Gorges Reservoir. In this study, an enclosure experiment was conducted to assess the impact of silver carp (Hypophthalmichthys molitrix) on the plankton community structure and water quality of the Three-Gorges Reservoir during 2 April to 2 May 2008. The field experiment was performed in six enclosures. Stocking silver carp into enclosures caused a dramatic change in the aquatic ecosystem. For example, pH, transparency, DO and phosphate were reduced, while chlorophyll-a concentration and turbidity increased dramatically. Furthermore, during the enclosure experiment, some zooplankton such as rotifer and copepoda were significantly reduced, and some phytoplankton biomass through reducing the densities of some zooplankton which directly fed on phytoplankton. It was concluded that silver carp was not suitable for clearing spring phytoplankton (<20  $\mu$ m) blooms in the Three-Gorges Reservoir at the present time.

Key words: phytoplanktivorous fish, biomanipulation, phytoplankton, Three-Gorges Reservoir, Xiangxi Bay

# 1. INTRODUCTION

The Three-Gorges Dam, the largest water conservancy project ever built in the world, is a multi-purpose hydro-development project producing comprehensive benefits mainly in flood control, power generation and navigation improvement. Now the Three-Gorges Reservoir have been used to meet drinking water requirement of the middle and lower reaches of the Yangtze River, and will supply freshwater resource for northern China by the planned South-to-North Water Diversion Project. Therefore, the water quality of the Three-Gorges Reservoir absolutely should not been polluted. However, the Three-Gorges Reservoir showed a serious deterioration of water quality with algal blooms occurring every spring after the damming in the year of 2003. The organisms causing algal blooms belong to genera Peridiniopsis, Cyclotella, Asterionella, Stephanodiscus, Rhodomonas, Pandorina, Eudorina, Aphanizomenon, Microcystis, Mallomonas, Volvox, etc. The excessive growth of phytoplankton can deteriorate the water quality, damage water natural functions, and even threaten to whole aquatic ecosystem (Pan et al. 2006). Some algal blooms can also secrete toxins or odorous compounds thus causing various health hazards in aquatic organisms and in humans (Codd 2000; Li et al. 2007). Therefore, it is very urgent to develop safe and efficient ways to control blooms occurred in the Three-Gorges Reservoir.

Many approaches have been used to control growth of phytoplankton and one of the extensively used approaches is biomanipulation, using filter feeders, including zooplankton, silver carp and bighead (Liu et al. 2009). In fact, the use of the filter-feeding fish as a biomanipulation tool to reduce phytoplankton biomass in lakes and reservoirs is still controversial (Domaizon & Dévaux 1999b; Radke & Kahl 2002). Filter-feeding fish are selective phytoplankton grazers that can suppress phytoplankton directly through algal ingestion or enhance phytoplankton indirectly by suppressing herbivorous zooplankton and by increasing nutrient availability (Drenner et al. 1987). At present time, silver carp is suggested to control algal blooms in some bays of the Three-Gorges Reservoir. However, few researches have investigated the applicability of the biomanipulation theory to the Three-Gorges Reservoir, and the effects of silver carp on plankton communities are still poorly understood. Therefore, prior to implementing this biomanipulation technique at the wholereservoir scale, it is essential to assess the effects of silver carp on plankton communities of the Three-Gorges Reservoir. Furthermore, ascertaining the effects of silver carp on plankton communities is an important step in algal blooms control and water environment protection in the Three-Gorges Reservoir.

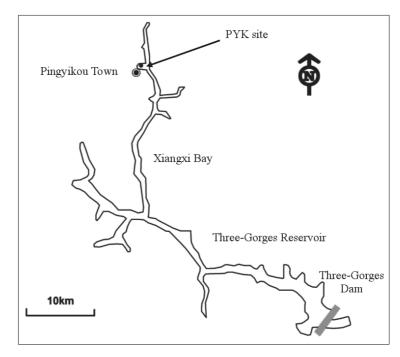


Fig. 1 Location of PYK sampling site in the Xiangxi Bay of the Three-Gorges Reservoir.

The Xiangxi River is the largest tributary of the Three-Gorges Reservoir in Hubei Province, and its water quality could directly influence the water quality of the Three-Gorges Reservoir (Tang et al. 2004; Fu et al. 2006). After impoundment in the year of 2003, the downstream region of the Xiangxi River became a long bay, with current speed of approximate 0.009 m s<sup>-</sup> (Wang *et al.* 2007). The Xiangxi River was subject to serious phosphorus and nitrogen pollution provided by untreated multiple industrial wastewater, domestic sewage and waste water drainage from land with intensive fertilization (Fang et al. 2006; Li et al. 2008). The new formed Xiangxi Bay was thus considered as a representative of most eutrophic bays of the Three-Gorges Reservoir (Cai & Hu 2006). The mean concentrations of total nitrogen and total phosphorus in the Xiangxi Bay, which had already exceeded the threshold values of eutrophic state, were 1.29 mg L<sup>-1</sup> and 0.153 mg L<sup>-1</sup>, respectively (Cao et al. 2006; Xu et al. 2010). The Xiangxi Bay had suffered repeatedly from algal blooms every spring after the impoundment of the Three-Gorges Reservoir (Zhou et al. 2010). A new species of freshwater dinoflagellates, Peridiniopsis niei, was the most abundant species (Liu et al. 2008; Xu et al. 2010), and sometimes Cyclotella sp. and Chroomonas acuta dominated the phytoplankton community in spring in the Xiangxi Bay (Zhou et al. 2010). This research was performed in spring 2008 in the Xiangxi Bay of Three-Gorges Reservoir.

The main objectives of this research were to test the effects of silver carp on the plankton community and water quality, and such information can be used effectively to improve our understanding of the control of algal blooms in the Three-Gorges Reservoir.

# 2. METHODS

### 2.1. Experimental design

PYK (31°10'N, 110°45'E) site of the Xiangxi Bay is situated in Pingyikou town with a distance of 26 km from the mouth of the Xiangxi bay (Fig. 1). Spring phytoplankton blooms occurred frequently at this site due to slow current speed and mass nutrients input. Six of 1.5 m (length)  $\times$  1.5 m (width)  $\times$  2.5 m (depth) enclosures were set up in PYK site. The exposition depth of enclosures was 0.25 m. The enclosures of polyethylene sheets were open to air above and sealed underneath. The enclosures were filled with water pumped from the bay (0.5 m) using a submerged pump and the volume of the water in each enclosure was about 5 m<sup>3</sup>. On 2 April 2008, three enclosures were randomly selected and each was stocked with three silver carp (Hypophthalmichthys *molitrix*) which individual biomass was  $141 \pm 19$  g. The resulting initial biomass in enclosures with fish was 84.6  $\pm$  11.4 g m<sup>-3</sup>. The remaining three enclosures served as controls with no fish. To prevent fish from jumping in or out, enclosures were covered with a 1 cm mesh net.

#### 2.2. Data collection

Mixed samples of 0.5 m and 2 m were collected every three days by using 5 L sampler between 2 April and 2 May 2008. Physical factors of water such as temperature, pH, transparency, conductivity and turbidity were determined using *in situ* instruments. Nitrate and phosphate were measured by spectrophotometric methods (Huang *et al.* 1999; SEPB 2002). Chlorophyll-*a* concentration was extracted from the filter for

**Tab. 1.** Results of laboratory and field measurements, and countings of organisms in enclosures without (NF) and with fish stock (F) on the first day of the experiment. N = 3, NF = No Fish, F = with Fish; *t*-Test for comparisons of means, *P*-value = calculated probability value, significant if  $P \le 0.05$ ; DO = Dissolved Oxygen, Total abundance = cell sum of all phytoplankton organisms; Ind.= Indviduals.

|  | Treatment | Mean±SD            | t-Value | P-value       |
|--|-----------|--------------------|---------|---------------|
| Temperature (°C)   | F         | 17.0±0             | 1.606   | 0.184         |
| Temperature ( C)   | NF        | 16.8±0.3           |         |               |
| рН   | F         | 9.13±0.06          | 1.000   | 0.374         |
|  | NF        | 9.10±0             |         |               |
| Conductivity (µS cm <sup>-1</sup> )                              | F         | 243.3±2.1          | -1.250  | 0.279         |
|  | NF        | $245.0\pm1.0$      |         |               |
| Transparency (cm)  | F         | $145\pm0$          | -       | -             |
| Transparency (eni)   | NF        | $145\pm0$          |         |               |
| Turbidity (NTU)  | F         | 5.23±0.23          | -0.671  | 0.539         |
| fullolatly (1(10)  | NF        | 5.33±0.12          |         |               |
| DO (mg $L^{-1}$ )  | F         | 8.73±0.15          | 0.000   | 1.000         |
| DO (ling L )   | NF        | 8.73±0.12          |         |               |
| Chlorophyll- $a (mg m^{-3})$                                     | F         | 15.2±3.3           | -0.972  | 0.386         |
| Chlorophyn- <i>a</i> (hig m <sup>-</sup> )                       | NF        | 17.5±2.6           |         |               |
| Nitrate (mg L <sup>-1</sup> )                                    | F         | $0.02 \pm 0.01$    | -0.945  | 0.398         |
|  | NF        | $0.03 \pm 0.01$    |         |               |
| Phosphate (mg L <sup>-1</sup> )                                  | F         | 0.12±0.004         | 0.500   | 0.643         |
|  | NF        | 0.12±0.002         |         |               |
|  | F         | 8.55±0.25          | 1.221   | 0.289         |
| Total abundance $(10^6 \text{ cells } \text{L}^{-1})$            | NF        | 8.17±0.49          |         |               |
| Cyclotella meneghiniana (10 <sup>6</sup> cells L <sup>-1</sup> ) | F         | 3.90±0.52          | 0.839   | 0.449         |
|  | NF        | 3.62±0.24          |         |               |
|  | F         | 0.21±0.04          | 0.756   | 0.492         |
| <i>Cyclotella</i> sp. $(10^6 \text{ cells } \text{L}^{-1})$      | NF        | 0.18±0.05          |         |               |
|  | F         | 1.67±0.32          | -0.182  | 0.865         |
| Cryptomonas sp.(10 <sup>6</sup> cells L <sup>-1</sup> )          | NF        | $1.71\pm0.24$      | 0.102   | 0.000         |
| <i>,</i> ,   | F         | $0.23\pm0.05$      | 1.789   | 0.148         |
| Peridiniopsis niei (10 <sup>6</sup> cells L <sup>-1</sup> )      | NF        | 0.18±0.02          | 1.709   | 0.140         |
|  | F         | 2.45±0.22          | -0.105  | 0.922         |
| <i>Carteria</i> sp. $(10^6 \text{ cells } \text{L}^{-1})$        | NF        | 2.46±0.06          | -0.105  | 0.922         |
|  | F         | 2.40±0.00<br>0±0   | -       | -             |
| Actinastrum sp. $(10^6 \text{ cells } \text{L}^{-1})$            | NF        | $0\pm0$<br>$0\pm0$ | -       | -             |
|  | F         |                    | 1.061   | 0.349         |
| Synedra sp. $(10^6 \text{ cells } \text{L}^{-1})$                |           | $0.06 \pm 0.06$    | 1.001   | 0.349         |
|  | NF        | $0.01 \pm 0.02$    |         |               |
| Scenedesmus sp. (10 <sup>6</sup> cells L <sup>-1</sup> )         | F         | 0±0                | -       | -             |
| 1 ( )  | NF        | 0±0                | 2 010   | 0.042         |
| Protozoa (Ind. L <sup>-1</sup> )                                 | F         | 38775±4365         | -2.919  | 0.043         |
|  | NF        | 56650±9668         | 0.055   | 0.00 <b>-</b> |
| Rotifer (Ind. L <sup>-1</sup> )                                  | F         | 2408±582           | -2.257  | 0.087         |
|  | NF        | 3200±173           |         |               |
| Cladocera (Ind. L <sup>-1</sup> )                                | F         | 0±0                | -       | -             |
|  | NF        | $0\pm0$            |         |               |
| Copepoda (Ind. L <sup>-1</sup> )                                 | F         | $0.2\pm0.1$        | 2.000   | 0.116         |
| copopour (mu. L.)  | NF        | 0.1±0.1            |         |               |

24 h with 90% acetone, centrifuged for 10 min at 3000 rpm and analyzed spectrophotometrically following the method described in SEPB (2002). Samples for phytoplankton, protozoa and rotifer were preserved with 1.5% lugol's iodine solution and counted with a microscope after sedimentation for 24 hours. Samples for cladocera and copepoda were obtained by filtering 10 liter of mixed water through a 64  $\mu$ m mesh plankton net and preserved by 5% formaldehyde and counted in a counting chamber.

## 2.3. Statistical analysis

A *t*-test was performed to test the differences between control and treatment enclosures of all measured variables at the beginning of the experiment, and repeated-measures ANOVA was performed to test the effects of fish presence and time on ten sampling dates. Statistical analysis was carried out using the SPSS 16.0 package. Levels of Significance used were 5% and 1%, corresponding to "significant" and "highly significant", respectively. Data were presented as the mean  $\pm$  standard deviation unless otherwise stated.

# 3. RESULTS

At the beginning of experiment, there were no differences between control and treatment enclosures for all measured variables, except for densities of protozoa, which were slightly higher in the control enclosures (p < 0.05) (Tab. 1). There were no marked changes in the body weights of fishes prior to and after the experiment.

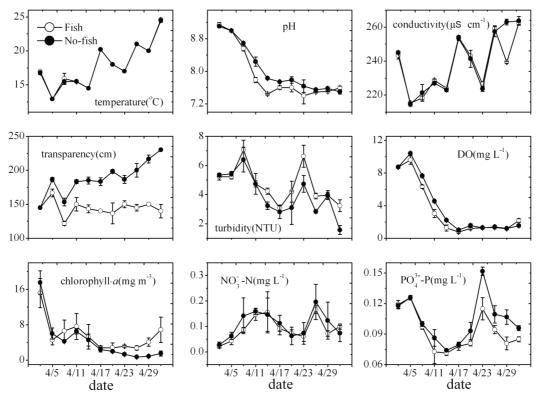


Fig. 2. Time course of laboratory and field measurements in enclosures without (black dots) and with fish stock (open dots). Dots = means of 3 enclosures, Bars = standard deviation; DO = Dissolved Oxygen; for statistical analyses see table 2.

# 3.1. Environmental parameters and chlorophyll-a concentration

During the experiment, no significant differences were found in temperature, conductivity, nitrate between control and treatment enclosures. Chlorophyll-*a* concentration and turbidity were significantly higher in treatment than those in control enclosures. Whereas, pH, transparency, DO and phosphate were significantly lower in treatment compared to those in control enclosures (Fig. 2, for the statistical analysis see Tab. 2). Transparency increased gradually in control enclosures while kept low in treatment enclosures. Other parameters changed simultaneously in both control and treatment enclosures during the experiment (Fig. 2).

### 3.2. Phytoplankton analysis

Organisms of six algal genera and of two algal species accounted for more than 10% of the total cell densities in at least one sample and they were *Cyclotella meneghiniana*, *Cyclotella* sp., *Cryptomonas* sp., *Peridiniopsis niei*, *Carteria* sp., *Actinastrum* sp., *Synedra* sp. and *Scenedesmus* sp., respectively. Total algal densities and densities of *Cryptomonas* and *Synedra* were significantly higher in treatment than those in control enclosures. The densities of other six dominant species were relatively but not statistically higher in the fish enclosures than in the no-fish enclosures (Tab. 2, Fig. 3).

**Tab. 2.** Statistical analysis of the time course of laboratory and field measurements, and countings of organisms in enclosures without and with fish stock in the period from 5 April to 2 May 2008. 3 parallel enclosures each, 10 sampling dates; F = calculated value of the repeated measure ANOVA-test, *P*-value = calculated probability value, significant if  $P \le 0.05$ ; DO = Dissolved Oxygen, Total abundance = cell sum of all phytoplankton organisms; Ind. = Indviduals.

|   | F       | Р       |
|---|---------|---------|
| Temperature (°C)  | 0.962   | 0.382   |
| pH  | 65.428  | 0.001   |
| Conductivity ( $\mu$ S cm <sup>-1</sup> )               | 5.361   | 0.082   |
| Transparency (cm)                                       | 873.095 | < 0.001 |
| Turbidity (NTU)   | 21.209  | 0.010   |
| $DO(mg L^{-1})$   | 28.352  | 0.006   |
| Chlorophyll- <i>a</i> (mg m <sup>-3</sup> )             | 15.444  | 0.017   |
| Nitrate (mg $L^{-1}$ )                                  | 1.569   | 0.279   |
| Phosphate (mg $L^{-1}$ )                                | 28.909  | 0.006   |
| Total abundance (cells L <sup>-1</sup> )                | 136.907 | < 0.001 |
| <i>Cyclotella meneghiniana</i> (cells L <sup>-1</sup> ) | 3.946   | 0.118   |
| Cyclotella sp. (cells L <sup>-1</sup> )                 | 5.322   | 0.082   |
| Cryptomonas sp. (cells L <sup>-1</sup> )                | 51.958  | 0.002   |
| Peridiniopsis niei (cells L <sup>-1</sup> )             | 5.469   | 0.080   |
| <i>Carteria</i> sp. (cells $L^{-1}$ )                   | 2.269   | 0.206   |
| Actinastrum sp. (cells L <sup>-1</sup> )                | 6.261   | 0.067   |
| Synedra sp. (cells $L^{-1}$ )                           | 51.020  | 0.002   |
| Scenedesmus sp. (cells L <sup>-1</sup> )                | 0.062   | 0.815   |
| Protozoa (Ind. L <sup>-1</sup> )                        | 8.745   | 0.042   |
| Rotifer (Ind. L <sup>-1</sup> )                         | 13.662  | 0.021   |
| Cladocera (Ind. L <sup>-1</sup> )                       | 6.721   | 0.061   |
| Copepoda (Ind. L <sup>-1</sup> )                        | 56.916  | 0.002   |

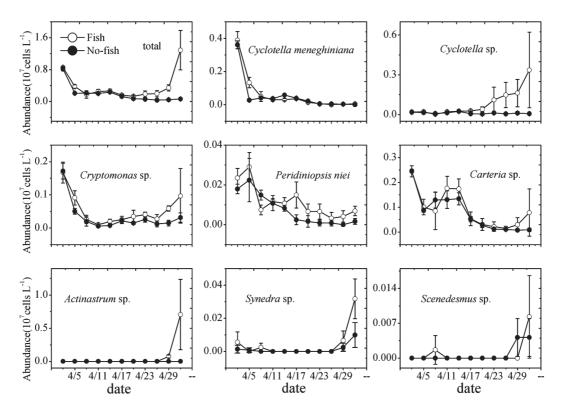


Fig. 3. Time course of phytoplankton countings in enclosures without (black dots) and with fish stock (open dots). Dots = means of 3 enclosures, Bars = standard deviation; for statistical analyses see table 2; total = cell sum of all phytoplankton organisms.

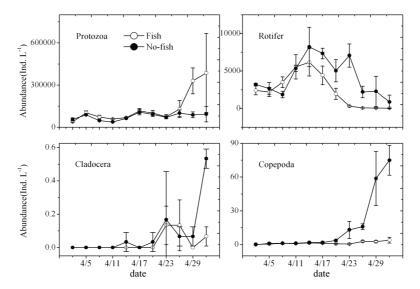


Fig. 4. Time course of zooplankton countings in enclosures without (black dots) and with fish stock (open dots). Dots = means of 3 enclosures, Bars = standard deviation; for statistical analyses see table 2; Ind. = individuals.

### 3.3. Zooplankton analysis

No significant difference in the cladocera density was detected between control and treatment enclosures. Protozoa densities were significantly higher in treatment enclosures than in control enclosures. Whereas, rotifer and copepoda densities were significantly lower in treatment enclosures than in control enclosures (Tab. 2, Fig. 4).

### 4. DISCUSSION

In this study, introduction of phytoplanktivorous fish (silver carp *Hypophthalmichthys molitrix*) into enclosures caused drastic reduction of large rotifer and copepoda populations, but increase in the abundance of small algae and protozoa. These results showed that silver carp failed to control the growth of algae in the pre-

sent enclosure experiment. In consistent with our study, the inability of silver carp to control phytoplankton effectively had been observed in several experimental studies (Burke et al. 1986; Miura 1990; Lieberman 1996; Wu et al. 1997; Radke & Kahl 2002). It was well known that silver carp fed selectively on algae >8-20  $\mu$ m × 15-33  $\mu$ m (Liu et al. 1993) and thus phytoplankton <10 µm could not be filtered effectively (Smith 1989; Dong & Li 1994; Vörös et al. 1997). In our study, most of algae species was less than 10 µm and maybe that was the reason that silver carp failed to control phytoplankton biomass in treatment enclosures. Although some algae species such as dinoflagellates (about 20 µm) could be filtered by silver carp, short-term high retention of dinoflagellates by silver carp indicated that the clearance effect did not last long due to a high rate of defaecation of undigested dinoflagellates. Furthermore, the undigested dinoflagellates in the faecal matter revealed a significant contribution to the rise of the dinoflagellates population of the enclosures. Protozoa also could not be filtered effectively by silver carp due to little volume. Silver carp significantly reduced the rotifer densities and copepod densities and that was in concordance with previous studies (Drenner et al. 1987; Levebter & Teltsch 1990; Domaizon & Devaux 1999a). Zooplankton suppression by fish enhanced the competitive advantage of fast growing phytoplankton, released from zooplankton grazing pressure, thus resulting in the increase of phytoplankton populations. In the present study, silver carp had no significant effect on cladocera population and similar case was once reported in previous study (Li et al. 1993). The main reason maybe was the high random errors caused by low cladocera densities in the enclosures.

In the Yangtze River, no phytoplankton blooms were reported before the building of the Three-Gorges Dam. However, algal blooms were observed every spring in many bays of the Three-Gorges Reservoir after impoundment in the year of 2003. To prevent potential destroy to aquatic ecosystem, it was very urgent to develop safe and efficient ways to control algal blooms. Though eco-hydraulic regulation and several physical methods have been recommended to control algal blooms in the Three-Gorges Reservoir, the control through stocking and management of herbivorous fishes may be the most ecologically sound management strategy. Based on the present study, when phytoplankton (<20 µm) blooms occurred in spring, however, introduction of silver carp not only failed to control algal bloom, but increased the extent of algal bloom. Similar situation has once been reported in previous studies (Domaizon & Dévaux 1999a; Radke & Kahl 2002). It was concluded that silver carp was not suitable for clearing phytoplankton (<20 µm) blooms in spring in the Three-Gorges Reservoir because of its low filter effect. Rotifer densities and copepoda densities were significantly higher in control enclosures than those in treatment enclosures, whereas phytoplankton densities and chlorophyll-a concentration were significantly lower. Furthermore, transparency of control enclosures also increased gradually during experiment period. All of these results showed that herbivorous zooplankton increase by fish lack could effectively control phytoplankton population, resulting in increase of water transparency and water quality. Therefore, large herbivorous zooplankton could be used to reduce phytoplankton biomass in spring in the Three-Gorges Reservoir. The use of biomanipulation strategies fostering zooplankton grazing was appropriate because zooplankton could reduce nuisance blooms of small phytoplankton species.

Many previous studies reported that silver carp could effectively control cyanobacteria blooms in the world (Starling & Rocha 1990; Starling 1993; Datta & Jana 1998). In China, many experiments were also carried out in many lakes and reservoirs (Li et al. 1993; Liu & Xie 1999; Lu et al. 2002). For example, the disgusting cyanobacteria bloom occurred in Donghu lake had disappeared since the year of 1985 due to introduction of phytoplanktivorous fish (Liu & Xie 1999). The probable reason was that silver carp could filter directly large cyanobacteria colonies. The phytoplankton community was dominated by some small species such as Peridiniopsis and Cyclotella in the Three-Gorges Reservoir in the last several years. However, cyanobacteria blooms began occasionally to appear in recent years in some bays such as the XiaoJiang Bay (spring 2007), the DaNing Bay (winter 2008) and the Xiangxi Bay (summer 2008). The cyanobacteria bloom-forming species in the Three-Gorges Reservoir such as Microcystis, Anabeana and Aphanizomenon were large or colonial algae. Maybe silver carp was the most efficient grazer of cyanobacteria bloom and was suitable for clearing cyanobacteria from the Three-Gorges Reservoir because of its high feeding rates. However, further experimental works are necessary in the future to evaluate the potential of silver carp in control of excess cyanobacteria in reservoir, to understand the knowledge of fish relations in the food web, to determine the range of appropriate fish biomass at which significant long-term water quality improvement can be expected, and to examine the ecological consequences of these fish introductions into cyanobacteria bloom infested water bodies.

### 5. CONCLUSIONS

During the enclosure experiment, some zooplankton such as rotifer and copepoda were significantly reduced, while some phytoplankton and protozoa were significantly increased by silver carp. It was concluded that silver carp was not suitable for clearing spring phytoplankton (<20  $\mu$ m) blooms in the Three-Gorges Reservoir at the present time.

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