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Growth and potential photosynthesis of *Microcystis* colonies after gut passage through crucian carp (*Carassius auratus gibelio*) and koi carp (*Cryprinus carpiod*)

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Growth and potential photosynthetic activity of cyanobacteria (predominantly *Microcystis* spp.) passed through intestine of crucian carp and koi carp were compared with those of phytoplankton taken directly from lake during a 9 day cultivation of fish faeces in algal BG-11 medium. The cyanobacteria exhibited a significant reduction of activity in both maximum optical quantum yield (F_v/F_m) ($P<0.001$) and non-photochemical quenching value (NPQ) ($P<0.001$) after passage through the experimental cyprinid fishes. The difference in the photosynthetic activity of the cyanobacteria from crucian carp and koi carp faeces was not significant ($P>0.05$) for all the measurement intervals. The results provide experimental evidence that *Microcystis* can be damaged by crucian carp and koi carp digestion. That may be a complementary method using bio-manipulation to control cyanobacterial blooms.

Key words: *Microcystis* colonies, photosynthetic activity, crucian carp, koi carp, gut passage.

INTRODUCTION

Non-traditional bio-manipulation is widely used to control nuisance algal blooms in tropical lakes that are highly productive and hard to reduce nutrient concentrations to low levels. Chen et al. (2006) discovered that silver carp could effectively ingest toxic *Microcystis* cells up to 84.4% of total phytoplankton, but showed fast growth in Lake Taihu. Long-term observations in Lake Donghu and Lake Qiandaohu have documented that silver carp (*Hypophthalmichthys molitrix*) and bighead carp (*Hypophthalmichthys nobilis*) (two filter-feeding planktivorous species commonly used in water bloom formation control) can suppress *Microcystis* blooms efficiently (Zhang et al., 2008a). However, some studies found no evidence of reversed effects when blue-green algae were controlled by stocking planktivorous fish. Those with opposite view considered that some colonial and filamentous cyanobacteria remain viable after the intestinal tract of planktivorous (herbivorous) fishes and even increase their specific photosynthetic activity (Miura and Wang, 1985). Kolmakov et al. (2006) demonstrated

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that some species of cyanobacteria are not suppressed when passing through the fish intestine and even enhanced when they return to the water. Studies with bighead carp (Datta and Jana, 1998), silver carp (Gavel et al., 2004; Jančula et al., 2008) and Atlantic menhaden (Friedland et al., 2005), showed that passage through these fish did no damage to cyanobacterial cells. Lewin et al. (2003) demonstrated that *Microcystis* is undamaged after guts passage of roach because of mucous cover protection and can directly use the phosphorous supplied in the fish guts during passage.

Many studies have reported the growth of cyanobacterial after passage through the intestinal tract of obligate filter-feeding fish, such as silver carp and Nile tilapia. Few studies have considered the omnivorous fish (Gavel et al., 2004). The aim of this study was to investigate what effect gut passage through crucian carp (*Carassius auratus gibelio*) had on the photosynthetic activity of *Microcystis* spp. using chlorophyll fluorescence, and to compare this with the effects of gut passage through another omnivorous fish, koi fish (*Cryprinus carpiod*).

MATERIALS AND METHODS

Eighteen 2 years old crucian carp (*Carassius auratus gibelio*) (SL 21.6 ± 4.3 cm, W 166.93 ± 7.21 g) and 18 common carp (*Carassiu*) (SL 18.1 ± 2.7 cm, W 136.52 ± 4.49 g) were used for experiments. The fish were placed in 120 L glass aquaria (six fish in each) with continuous aeration. Fish were caught from a reservoir in the morning by a gill net. Fishes were allowed to acclimate to experimental conditions during a 72 h starvation period. A *Microcystis*-dominated phytoplankton sample from Taihu Lake was then added to each aquarium. Floating fresh faeces were collected gently from the surface water during 24 h after adding the cyanobacterial bloom. A sample of non-ingested phytoplankton served as the control. Samples were incubated for 9 days in algal BG-11 medium (Stanier et al., 1971) at 25°C with a light: dark cycle of 12: 12. Chlorophyll *a* concentrations were determined spectrophotometrically after extraction in 90% hot ethanol (Papista et al., 2002). Phytoplankton abundance counts were carried out under an inverted microscope by the Utermöhl technique. Phytoplankton samples were preserved with Lugol's iodine. Algal growth rate μ_t was calculated using the formula:

$$\mu_t = (\ln N_{t2} - \ln N_{t1})/(t_2 - t_1) \quad (1)$$

Where N_{t1} and N_{t2} are cell abundances (cells ml^{-1}) at time t_1 and t_2, respectively in Equation (1).

Fluorescence was measured using a multi-wavelength phytoplankton pulse-amplitude-modulated fluorometry (Phyto-PAM) (Walz, Effeltrich, Germany). The Phyto-PAM fluorometry can distinguish between differently pigmented algal groups, such as cyanobacteria, chlorophytes, and diatoms/dinoflagellates, by applying four different excitation wavelengths (665, 645, 520, and 470 nm). F_0 was determined as the fluorescence of dark-adapted cells stimulated by a weak probe light immediately following 15 min of darkness. F_m was the maximum fluorescence signal following the closure of all reaction centers by a 600 ms pulse of saturating irradiance. Simultaneously, F_m' was the maximum fluorescence signal in the light adapted state. Fluorescence parameters were calculated according to the following equations after subtraction of blank fluorescence value obtained by measuring the fluorescence of a 0.22 µm filtered sample using:

$$F_v/F_m=(F_m - F_0)/F_m \quad (2)$$

$$NPQ=(F_m - F_m')/F_m' \quad (3)$$

Where F_v/F_m is the maximum optical quantum yield in Equation (2) and NPQ is the non-photochemical quenching value in Equation (3)

RESULTS AND DISCUSSION

In this study, the photosynthetic activity of *Microcystis* colonies from the cyprinid faeces was generally reduced following gut passage. The ratio of F_v/F_m provides a measure of the efficiency of excitation capture by active photosystem II (PSII) reaction centers (Genty et al., 1989). The F_v/F_m ratio is about 0.65 for algae under optimum conditions (Kolber et al., 1988). The NPQ is a relative measure of heat dissipation. There was 9 to 58% inhibition of photosynthetic activity (P<0.001) in F_v/F_m between control samples and both carps at all measurement intervals (Figure 1). F_v/F_m ratios of *Microcystis* from crucian carp and koi carp faeces were indistinguishable from each other throughout the cultivation (P>0.05). F_v/F_m of phytoplankton after gut passage recovered gradually at the first two days and decreased sharply in the following days. NPQ of *Microcystis* form both of the carp faeces was significantly lower than those of phytoplankton from the control samples during the cultivation period (P<0.001). NPQ of *Microcystis* from cyprinid guts displayed parallel variation patters: NPQ rose gradually at the first five days and then decreased (Figure 1). Furthermore, there was no distinguishable difference between NPQ of crucian carp and koi carp faeces. The result showed that the passage of cyanobacterial through the cyprinid fish led to the damage of *Microcystis*. Similar phenomenon also happened in Nile tilapia. Jančula et al. (2008) found a 92 to 95% reduction of photosynthetic activity of cyanobacterial. They contributed to the extremely low pH values (pH<1) in the stomach of Nile tilapia. The present study is the first experimental confirmation of the ability of crucian carp and koi carp to damage the photosynthetic activity of *Microcystis* colonies during gut passage. It may contribute to their specific digestive tract features.

Chlorophyll *a* concentrations in the experiments decreased gradually during the cultivation period (Figure 2). Graphs of growth rate of cyanobacteria are given in Figure 3. The growth rates of phytoplankton from both faeces and nature showed similar variation patterns, and the growth of cyanobacteria after gut passage was generally lower than that in the control sample. There exited a light increase at the first 48 h in cultivation.

The introduction of planktivorous fish to eliminate blue-green algae has been widely used in hypereutrophic lakes. However, it does not always bring about the

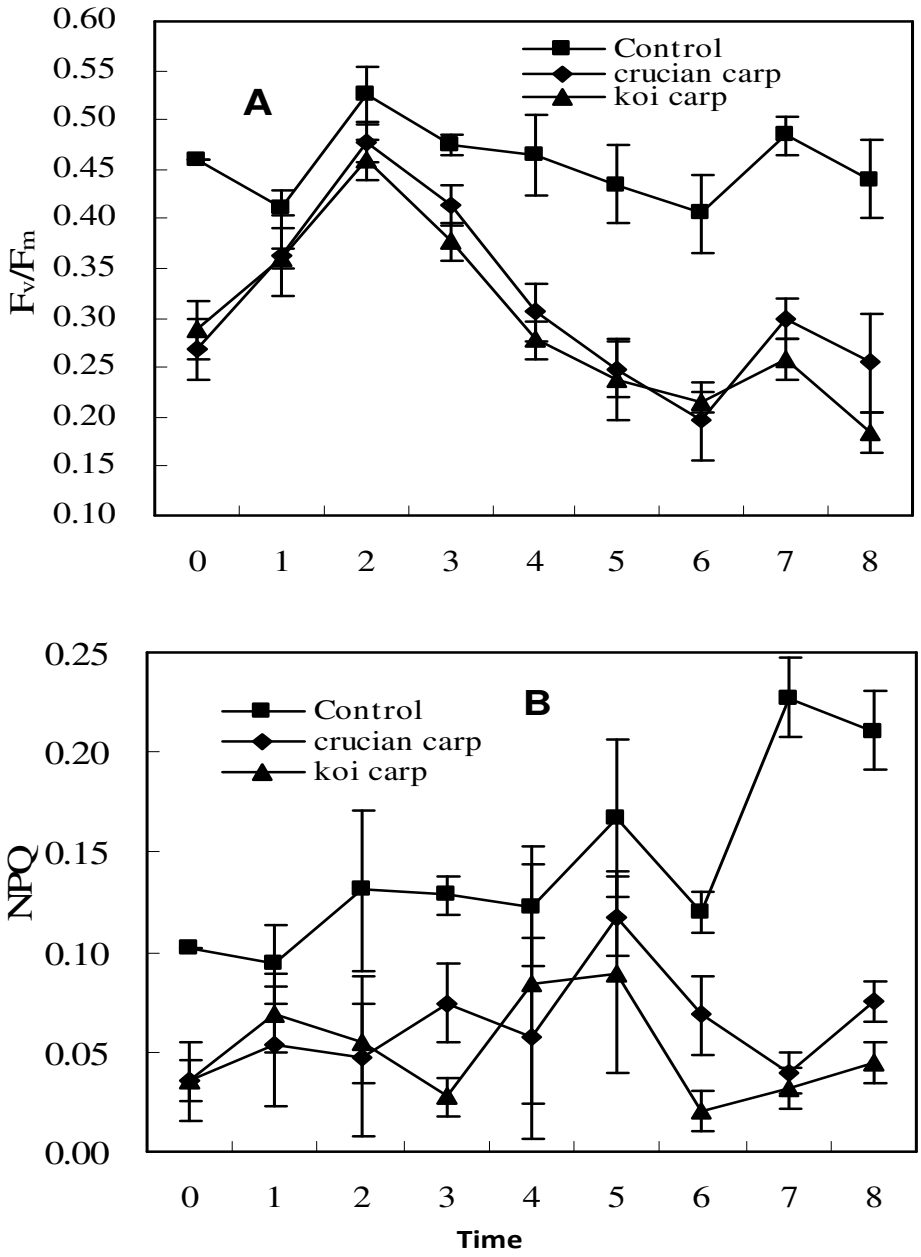

Figure 1. Time dependent course of cyanobacteria fluorescence activity after passage through the digestive tract of fish compared with colonies in control phytoplankton samples.

desired effect, on the contrary, an increase in picophytoplankton biomass (Mátyás et al., 2003; Kolar et al., 2005). We performed experiments to test the assumption that cyanobacteria passing through the intestine of crucian carp and koi carp may be stimulated. The variable chlorophyll *a* fluorescence analysis has become an important tool for studying photosynthesis in natural phytoplankton populations (Oliver and Whittington, 1998; Zhang et al., 2008b). The technique provides information on the major processes in light capture and electron transport, which together provide an estimate of the rate of photosynthesis (Regel et al., 2004).

Many researches demonstrated that the stimulation of *Microcystis* by the passage through the fish intestine was crucial for the initiation of the “cyanobacterial bloom” in water. Kolmakov and Gladyshev (2003) found that the growth rate and final crop of chlorophyll after gut passage of roach significantly exceeded those of “free-living” phytoplankton from the reservoir. The passage through he intestine of silver carp increased the photosynthesis and growth rates of some species of cyanobacteria

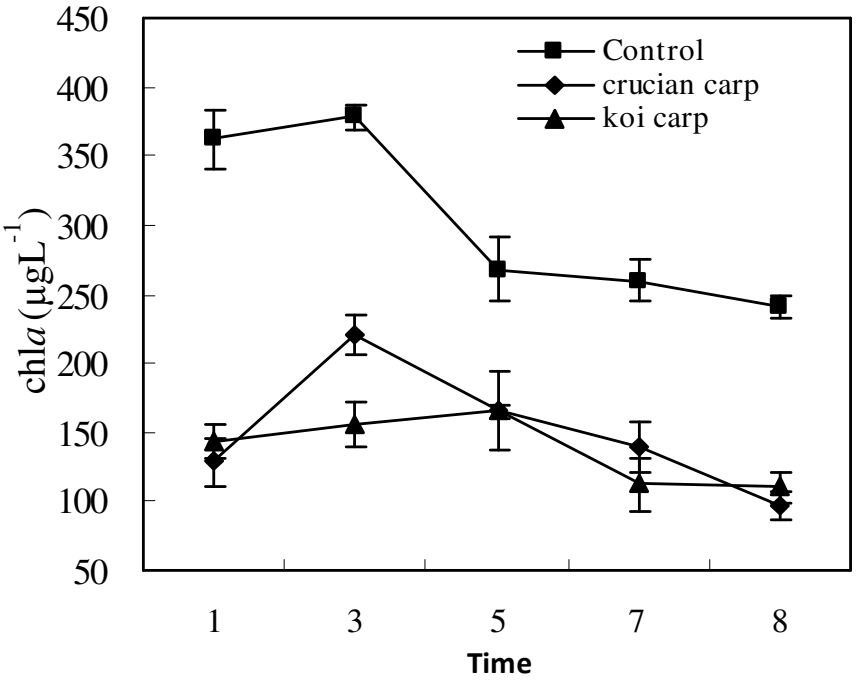

Figure 2. Concentrations of chlorophyll *a* in the control and experimental cultures.

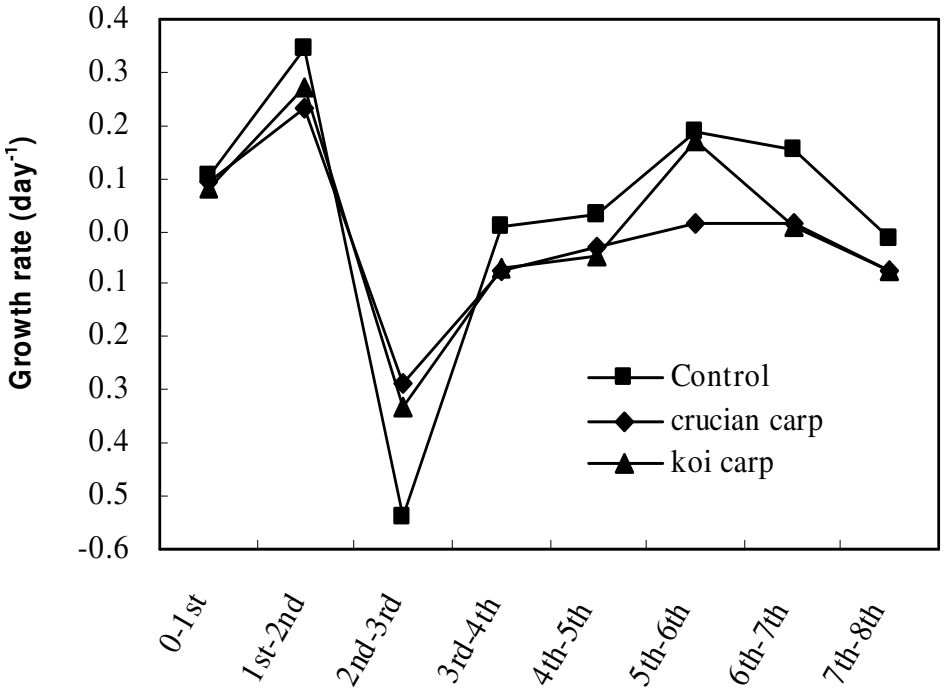

Figure 3. Variations of growth rates of cyanobacteria in the control and experimental cultures.

(Kolmakov et al., 2006; Jančula et al., 2008). Friedland et al. (2005) demonstrated that only cyanobacteria were found in the hindgut of juvenile Atlantic menhaden with epifluoresence microscopy. The role of fish gut passage in enhancing phytoplankton productivity depends on biochemical properties of cyanobacterial colonies. Some species without mucous cover are suppressed when passing through the fish intestine; their nutrients can be

assimilated by fish (Vőrős et al., 1997; Kamjunke et al., 2002a, b). Moreover, their growth is enhanced when they return to the water (Lewin et al., 2003). Mucilaginous species like *Microcystis* seem to pass the fish gut rather unaffected (Vőrős et al., 1997; Datta and Jana, 1998), and only the nutrients from attached bacteria were definitely assimilated (Kamjunke and Mehner, 2001). In the study, the species of algae were determined by microscopy, about 98% dominant by *Microcystis* spp.

Feeding on cyanobacteria has been reported from herbivorous and omnivorous fish species as well (Kolmakov and Gladyshev, 2003; Kolmakov et al., 2006; Kamjunke et al., 2002a, b). Both the crucian carp and common carp are omnivorous fishes, which include cyanobacteria and detritus in their food in Lake Taihu (Qiu et al., 2007; Liu, 2008). The Koi carp *C. carpiod*, a close congener to the common carp *C. carpio*, is usually stocked in ponds of park for sightseeing because of its colorful appearance. It possesses a more wide food selection and strong adaptability than the common carp. In these experiments, growth and potential photosynthetic activity of *Microcystis* passed through intestine of crucian carp and koi carp were compared to not ingested. It suggested a potential efficacy of crucian carp and koi carp for use in the control of cyanobacterial blooms.

Although, the present study suggested the potential efficacy of crucian carp and koi carp to counteract toxic cyanobacterial blooms, there are some potentially negative considerations. In the natural environment, microcystins (MCs) are found to accumulate in a wide range of aquatic animals such as fish (Mohamed et al., 2003). Xie et al. (2005) observed MC content in the liver was high in omnivorous fish in a field study. Qiu et al. (2007) demonstrated that silver carp displayed only slight ultrastructural changes in liver during the cyanobacterial blooms, while the crucian carp presented morphologic alterations in nuclei and production of a lot of lipid droplets. But in many cases, the toxicity is sublethal and the animals can survive long enough to accumulate the toxins and transfer them along the food chain. Koi carp is a kind of fish for entertainment and no risk to human health and as such, it might be a more suitable use for cyanobacterial bloom control.

Conclusion

We performed experiments to test the assumption that cyanobacteria passing through the intestine of crucian carp and koi carp may be stimulated. The photosynthetic activity and growth rate of cyanobacteria from the cyprinid faeces were detected compared with the undigested during a 9 day cultivation. The F_v/F_m ratios and NPQ of *Microcystis* from cyprinid faeces were suppressed significantly following gut passage. No significant difference in photosynthetic activity was found between crucian carp and koi carp faeces. The growth of cyanobacteria after gut passage was generally lower than that in the control sample, though, there exited a light increase at the first 48 h in cultivation. The results provide experimental evidence that *Microcystis* can be damaged by crucian carp and koi carp digestion, which may be useful as a complementary method for cyanobacterial blooms control.

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