African Journal of Biotechnology Vol. 11(13), pp. 3192-3196, 14 February, 2012
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.3469
ISSN 1684–5315 © 2012 Academic Journals

Full Length Research Paper

Dynamics of photosynthetic activity of cyanobacteria after gut passage through crucian carp (*Carassius auratus gibelio*) and goldfish (*Carassius auratus auratus*)

Qing-fei Zeng[1*], Xiao-hong Gu[1], Yin-ping Wang[2], Zhi-gang Mao[1,3], Ming-bo Sun[1,3], and Xian-kun Gu[1,3]

[1]State Key laboratory of Lake Science and Environment, Nanjing Institute of Geography and Limnology, Chinese Academy of Sciences, Nanjing 210008, P. R. China.
[2]Henan Agriculture University, Zhengzhou 450002, P. R. China.
[3]Graduate School of Chinese Academy of Sciences, Beijing 100049, P. R. China.

Accepted 18 January, 2012

The photosynthetic activity of cyanobacteria (predominantly *Microcystis* spp.) after passage through crucian fish and goldfish was determined by photo-PAM (pulse-amplitude-modulated) during a 17-day cultivation of fish faeces in filtered lake water. The *Microcystis* exhibited a significant reduction of activity after passage through both crucian carp and goldfish, whereas there was a significant stimulation of photosynthetic activity of diatom and green algae following the depressed cyanobacteria during cultivation. The mainly stimulated eukaryotic algae species were *Fragilariaceae* and *Scenedesmus obliquus* by microscopy. Our results provide experimental evidence that *Microcystis* is damaged by crucian carp and goldfish digestion. That may be useful as a complementary method for combating cyanobacterial blooms and utilizing them for crucian carp and goldfish nutrition.

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

INTRODUCTION

Cyanobacterial blooms are a growing problem in tropical-water bodies due to increasingly high incidence of eutrophication. It has been documented that stocking phyto-planktivorous fish is the most effective management technique to reduce nuisance blooms of large algal specie in lakes (Zhang et al., 2008). The silver and bighead carp have been widely stocked in many *Microcystis*-infested Chinese lakes (Ke et al., 2007). However, many studies reported that the introduction of planktivorous fish into “blooming” waters did not always bring about the desired effect. Those with the opposite view demurred that planktivorous fish decreased zooplankton biomass and body size and consequently increased the total chlorophyll a (Attayde and Hansson, 2001; Figueredo and Giani, 2005).

Several studies have reported that some colonial and filamentous cyanobacteria remain viable after the intestinal tract of planktivorous (herbivorous) fishs and even increase their specific photosynthetic activity. Kolmakov and Gladyshev (2003) showed that the passage of cyanobacteria through the intestine of Cyprinidae (crucian carp) increased the photosynthesis and growth rates of some species of cyanobacteria. Studies with Atlantic menhaden (*Brevoortia tyrannus* Latrobe) (Friedland et al., 2005), silver carp, and Nile tilapia (Gavel et al., 2004), showed that passage through these fish did no damage to cyanobacterial cells with mucous cover, and only the nutrients from attached bacteria were definitely assimi- lated (Kamjunke and Mehner, 2001).

Nevertheless, most researchers reported the growth of cyanobacteria after passage through the intestinal tract of

*Corresponding author. E-mail: qfzeng79@yahoo.com.

obligate filter-feeding fish, such as silver carp and Nile tilapia. Few studies have considered the omnivorous fish. The aim of this study was to investigate what effect gut passage through crucian carp 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, goldfish.

MATERIALS AND METHODS

Eighteen 2-year-old crucian carp (*Carassius auratus gibelio*) (SL 21.6±4.3 cm, W 166.93±7.21 g) and 18 goldfish (*Carassius auratus auratus*) (SL 12.1±2.4 cm, W 36.52±3.43 g) were used for this 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. Fish were allowed to acclimatize 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 17 days in filtered lake water by Whatman GFF glass fiber filters (pore size 0.7 µm) 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 species were identified according to Hu et al. (1980).

Fluorescence was measured using a multiwavelength 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. *F* was the fluorescence in the light when part of the reaction centers was open. 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.

$$Fv/Fm = (Fm-F0)/Fm$$
$$Yield = (Fm'-F)/Fm'$$

Fv/*Fm* is the maximum optical quantum yield and *Yield* is the actual photosystem II quantum yield.

RESULTS

The photosynthetic activity of *Microcystis* colonies from goldfish faeces was reduced significantly following gut passage, and decreased to zero at the 3rd day during incubation in filtered lake water (Figure 1). But the *Fv*/*Fm* and *Yield* of diatom and chlorophyte was detected at the 4th and 8th day during incubation, respectively (Figure 2). In the final sample from the goldfish faeces flasks, *Fragilariaceae* and *Scenedesmus obliquus* dominated, and their biomass practically equal to that of *Microcystis* in the control sample. As for crucian carp, there was 50-79% inhibition of photosynthetic activity in *Fv*/*Fm* and *Yield* when compared with the control samples during the 12-day incubation period (Figure 1). The difference in the photosynthetic activity of cyanobacteria from crucian carp and the control samples was significant ($P<0.01$) for all measurement intervals. The photosynthetic activity, *Fv*/*Fm*, of diatom and chlorophyte from crucian carp guts after the 5th and 9th day became significantly higher than that of *Microcystis* in the control (Figure 2).

Figure 3 shows the mean concentrations of phytoplankton chlorophyll *a* in the control and experimental cultures. The chlorophyll concentrations in the first several days were decreased in both experimental and control variants, but increased on the 8th day and remained higher in the experimental cultures than in the control during the following incubation days. The growth of chlorophyll a in experimental flasks occurred primarily due to an increase in biomass of *Fragilariaceae* and *S. oblique.*

DISCUSSION

The present study is the first experimental confirmation of the ability of crucian carp and goldfish to depress the photosynthetic activity of *Microcystis* colonies during gut passage. Our findings confirm that omnivorous filter-feeding fishes might be useful as a complementary method for combating cyanobacterial blooms. In fact, planktivorous fishes have been introduced worldwide for both aquaculture fish production and algal control (Figueredo and Giani, 2005). However, stocking of silver carp as a biomanipulation tool to reduce phytoplankton biomass in lakes not always brings about the desired effect, namely, a decrease in phytoplankton (Zhang et al., 2008). One possible reason why silver carp may have no effect on phytoplankton is that some microalgae and cyanobacteria pass through the fish intestine undamaged, without loss of viability (Friedland et al., 2005). The role of fish gut passage in enhancing cyanobacteria productivity has recently been reported for a number of species residing in eutrophic lakes.

Many studies have revealed viable cyanobaceteria after their passage through the alimentary tract of these cyprinids. 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 the intestine of silver carp increases the photosynthesis and growth rates of some species of cyanobacteria (Kolmakov et al., 2006; Jančula et al., 2008). Friedland et al. (2005) demonstrated that only cyanobacteria was found in the hindgut of juvenile Atlantic menhaden with epifluoresence microscopy. They considered that the mucous-possessing *Microcystis* can directly use the high phosphorus concentrations in guts without being digested, which makes the *Microcystis* show high viability after passage (Lewin et al., 2003).

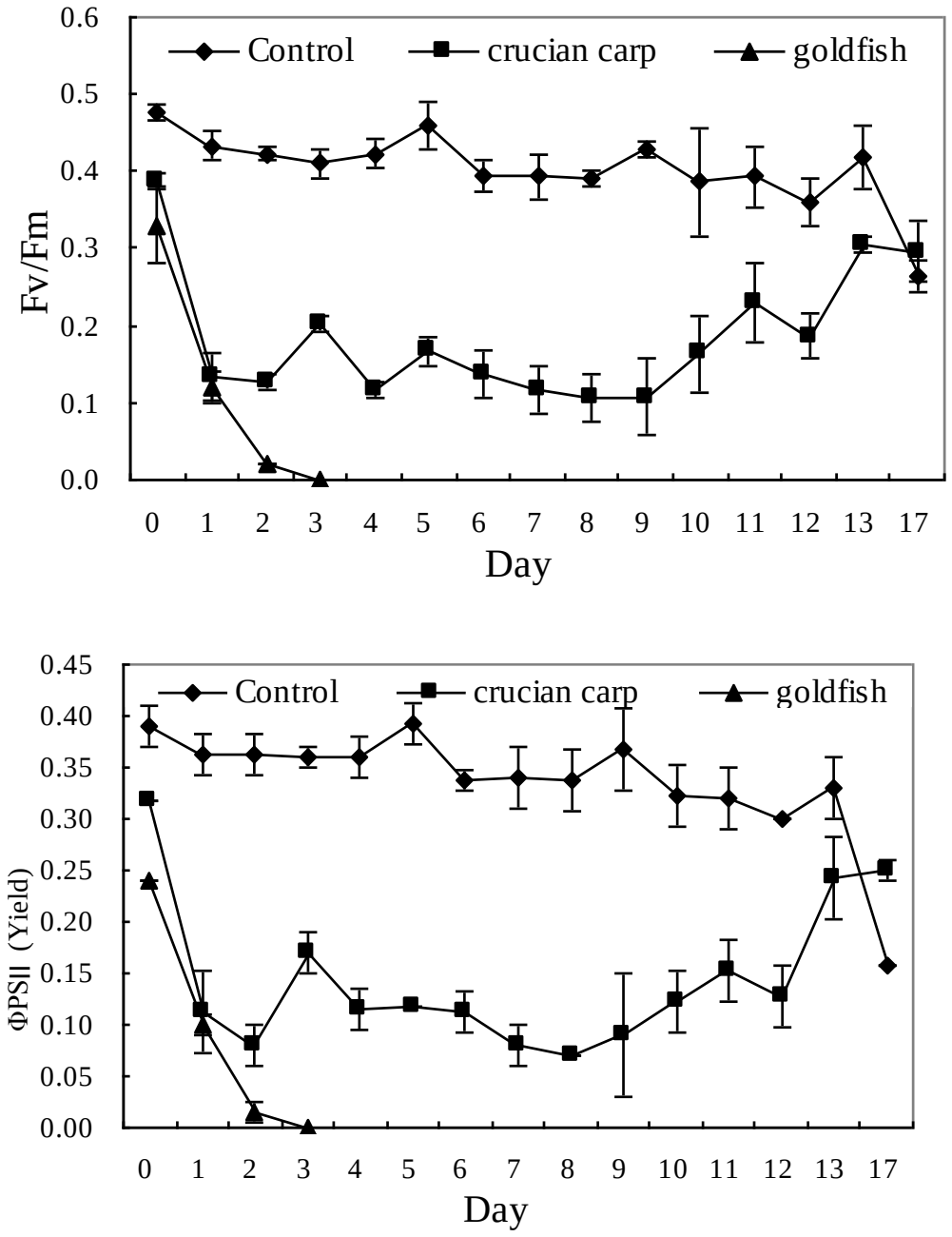

Figure 1. Time dependent course of *Microcystis* fluorescence activity after passage through the digestive tract of fish compared with colonies in control phytoplankton samples.

In the experiment, the photosynthetic activity of *Microcystis* was suppressed. Jančula et al. (2008) also demonstrated that cyanobacteria colonies exhibited a 92-95% reduction of photosynthetic activity after passage through Nile tilapia. It may come from their specific digestive tract features. Moriarty (1973) considered damage to cyanobacterial cells after their passage through the gut of Nile tilapia (*Oreochromis niloticus L*). Extremely low pH values (pH<1) in the stomach of Nile tilapia, as well as their ability to lyse algal and cyanobacterial cell walls by acid secretion in the stomach have been reported (Adámek et al., 1996). Unfortunately, the required pH values were not detected in the crucian carp and goldfish stomach.

Interesting phenomenon found that the photosynthetic activity of diatom and green algae was stimulated in 5 and 9 days during cultivation of crucian carp faeces in filtered lake water, respectively. The same result was also found in goldfish, which the fluorescence of diatom and green algae was detected in 4 and 8 days during cultivation,

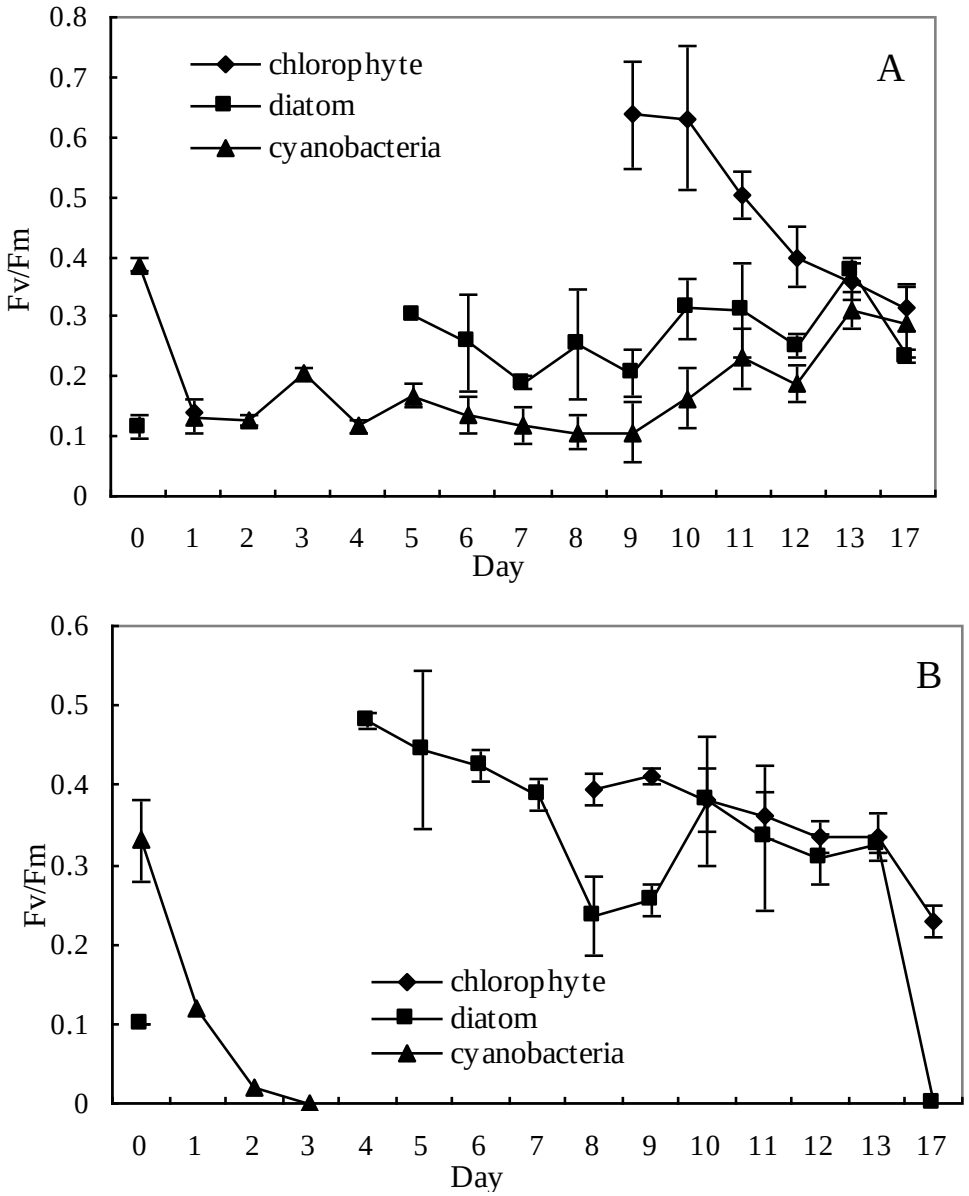

Figure 2. Time dependent course of chlorophyte and diatom fluorescence activity after passage through the digestive tract of fish compared with colonies in control *Microcystis* samples. "A" represents an experiment with crucian carp and "B" with goldfish.

respectively. *Fragilariaceae* and *S. oblique* may contribute to the increase of chlorophyll *a* concentrations after 4 days of incubation in the experimental cultures by microscope. Little information had been reported on the photosynthetic activity of other kinds of algae during the cultivation. It is possible that *Microcystis* can be digested and assimilated by fish but other microalgae, eucaryotic microalgae may seem to be unaffected by the transit through intestine (Kolmakov and Gladyshev, 2003). The growth of undigested algae is enhanced when they return to the water. Concentrations of inorganic nitrogen and phosphorus during cultivation were high (0.42 mg L^{-1} of NH_4^+ and 0.028 mg $L^{-1}$of PO_4^{3-}) and are unlikely to limit growth of algae.

Although, this study suggested the potential efficicacy of crucian carp and goldfish 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, in a field study that MC content in the liver was the highest in carnivorous fish, followed by omnivorous fish, and was the lowest in phytoplanktivorous and herbivorous fishes. Qiu et al. (2007) demonstrated that silver carp displayed only slight ultrastructural changes in liver during the cyanobacterial blooms, while

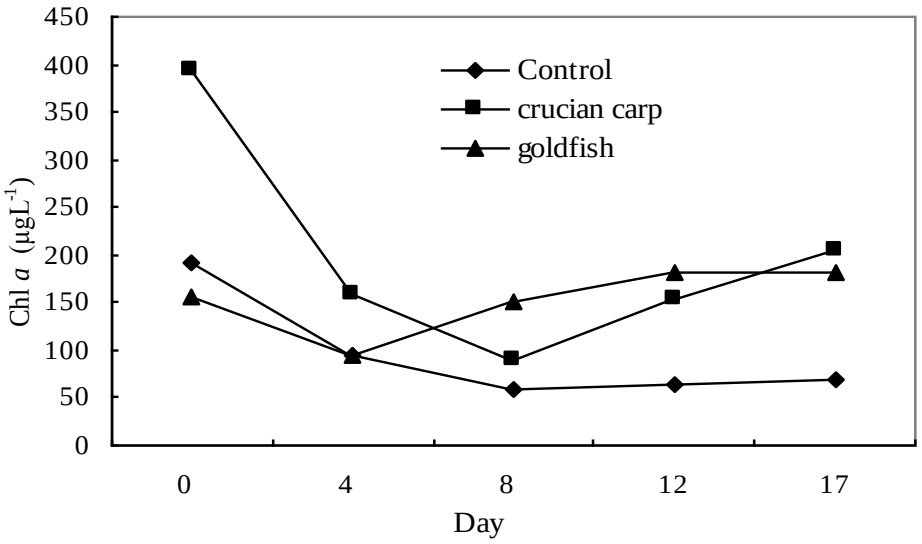

Figure 3. Concentration of chlorophyll *a* in the control and experimental cultures.

the crucian carp presented morphologic alterations in nuclei and production of a lot of lipid droplets. The juvenile gold fish injected with purified MC-LR at a dose of 125 mg/kg body weight displayed no significant changes in GSH content in liver during an experiment of 96 h (Malbrouck et al., 2004). 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. Gold fish is a kind of fish for entertainment and no risk to human health. So it might be a more suitable use for cyanobacteria bloom control.

ACKNOWLEDGEMENTS

This work was supported by the National Natural Science Foundation of China (No. 30900207), Jiangsu Natural Science Foundation (No. SBK 201021370) and the Regional Joint Research Program of the Chinese Academy of Sciences (Y1YD 11031).

REFERENCES

Adámek Z, Stibranyiová I, Siddiqui MA (1996). The course of pH values in the digestive tract of some commercial fishes. Bull. VÚRH Vodňany (original in Czech with English summary). 1: 26-31.

Attayde J, Hansson L (2001). Fish-mediated nutrient recycling and the trophic cascade in lakes. Can. J. Fish Aquat. Sci. 58: 1924-1931.

Figueredo CC, Giani A (2005). Ecological interactions between Nile tilapia (*Oreochromis niloticus L*.) and the phytoplanktonic community of the Furnas Reservoir (Brazil). Freshwater Biol. 50: 1391-1403.

Friedland KD, Ahrenholz DW, Haas LW (2005). Viable gut passage of cyanobacteria through the filter-feeding fish Atlantic menhaden, *Brevoortia tyrannus*. J. Plankton Res. 27(7): 715-718.

Gavel A, Maršálek B, Adámek Z (2004). Viability of *Microcystis* colonies is not damaged by silver carp (*Hypophthalmichthys molitrix*) digestion. Algol. Stud. 113: 189-194.

Hu H, Li Y, Wei Y, Zhu H, Chen J, Shi Z (1980). Freshwater algae in China. Shanghai Sci. Technol. Press, Shanghai, China.

Jančula D, Míkovcová M, Adámek Z, Maršálek B (2008). Changes in the photosynthetic activity of *Microcystis* colonies after gut passage through Nile tilapia (*Oreochromis niloticus*) and silver carp (*Hypophthalmichthys molitrix*). Aquacult. Res. 39: 311-314.

Kamjunke N, Mehner T (2001). Coupling the microbial food web with fish: are bacteria attached to cyanobacteria an important food source for under yearling roach? Freshwater Biol. 46: 633-639.

Ke ZX, Xie P, Guo LG, Liu YQ, Yang H (2007). In situ study on the control of toxic *Microcystis* blooms using phytoplanktivorous fish in the subtropical Lake Taihu of China: a large fish pen experiment. Aquaculture, 265: 127-138.

Kolmakov VI, Gladyshev MI (2003). Growth and potential photosynthesis of cyanobacteria are stimulated by viable gut passage in crucian carp. Aquat. Ecol. 37: 237-242.

Kolmakov VI, Gladyshev MI, Kravchukb ES, Chuprov SM, Anishchenko OV, Ivanova EA, Trusova MY (2006). Species-Specific Stimulation of Cyanobacteria by Silver Carp *Hypophthalmichthys molitrix* (Val.). Dokl. Biol. Sci. 408: 223-225.

Lewin WC, Kamjunke N, Mehner T (2003). Phosphorus uptake by *Microcystis* during passage through fish guts. Limnol. Oceanogr. 48(6): 2392-2396.

Mohamed ZA, Carmichael WW, Hussein AA (2003). Estimation of microcystins in the freshwater fish Oreochromis niloticus in an Egyptian fish farm containing a *Microcystis* bloom. Environ. Toxicol. 18: 137-141.

Malbrouck C, Trausch G, Devos P, Kestemont P (2004). Effect of microcystin-LR on protein phosphatase activity and glycogen content in isolated hepatocytes of fed and fasted juvenile goldfish (*Carassius auratus* L.). Toxicon. 44: 927-932.

Moriarty DJW (1973). The physiology of digestion of blue green algae in the cichlid fish, Tilapia nilotica. J. Zool. 171: 25-29.

Papista E, Acs E, Boeddi B (2002). Chlorophyll-a determination with ethanola critical test. Hydrobiologia, 485: 191-198.

Qiu T, Xie P, Ke ZX, Li L, Guo LG (2007). In situ studies on physiological and biochemical responses of four fishes with different trophic levels to toxic cyanobacterial blooms in a large Chinese lake. Toxicon, 50: 365-376.

Xie LQ, Xie P, Guo L, Li L, Miyabara Y, Park HD (2005). Organ distribution and bioaccumulation of microcystins in freshwater fish at different trophic levels from the eutrophic Lake Chaohu, China. Environ. Toxicol. 20: 293-300.

Zhang X, Xie P, Huang XP (2008). A Review of Nontraditional Biomanipulation. Sci. World J. 8: 1184-1196.