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In situ studies on physiological and biochemical responses of four fishes with different trophic levels to toxic cyanobacterial blooms in a large Chinese lake

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Abstract

Physiological and biochemical responses of four fishes with different trophic levels to toxic cyanobacterial blooms were studied in a large net cage in Meiliang Bay, a hypereutrophic region of Lake Taihu. We sampled four fishes: the phytoplanktivorous *Hypophthalmichthys molitrix* and *Aristichthys nobilis*, the omnivorous *Carassius auratus*, and the carnivorous *Culter ilishaeformis*. Alterations of the antioxidant (GSH) and the major antioxidant enzymes (CAT, SOD, GPx, GST) in livers were monitored monthly, and the ultrastructures of livers were compared between the bloom and post-bloom periods. During the cyanobacterial blooms, the phytoplanktivorous fishes displayed only slight ultrastructural changes in liver, while the carnivorous fish presented the most serious injury as swollen endomembrane system and morphologically altered nuclei in hepatocytes. Biochemically, the phytoplanktivorous fishes possessed higher basal GSH concentrations and better correlations between the major antioxidant enzymes in liver, which might be responsible for their powerful resistance to MCs. This article provided physiological and toxicological evidences for the possible succession of fish communities following occurrence of toxic cyanobacterial blooms and also for the applicability of using phytoplanktivorous fish to counteract toxic cyanobacterial blooms in natural waters.

Keywords: Cyanobacterial blooms; Fish; Liver; Antioxidants system; Ultrastructure

1. Introduction

The occurrence of heavy cyanobacterial blooms in eutrophic freshwater ecosystems has been a worldwide problem (Carmichael, 1992). Moreover, 50–75% of the cyanobacterial blooms were detected to be toxic (Word Health Organization, 2003). Microcystins, the predominant toxins of cyanobacterial blooms, are associated with mortality and illness in both animals and human (Carmichael and Falconer, 1993). Fish, one of the main inhabitants in aquatic systems, is frequently exposed to MCs directly and passively, which consequently causes fish kill or fish-poisoning episode (Andersen et al., 1993; Zimba et al., 2001; Jewel et al., 2003).

MCs have been characterized as potent inhibitors of protein phosphatases 1 and 2A in hepatocytes of animals (Eriksson et al., 1990; Falconer and Yeung,

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1992). The inhibition leads to unbalance of protein phosphorylation, which consequently causes disruption of the cell cytoskeleton and may induce liver disease or even liver necrosis finally (Falconer and Yeung, 1992; Ito et al., 1997, 2000).

Oxidative stress induced by MC exposure is considered to be involved in the toxicity of MCs as well. Intracellular tripeptide glutathione (GSH) plays a key role in cellular defense against oxidative damage and participates in the detoxification of many xenobiotics (e.g. microcystins) by serving as a substrate of glutathione S-transferases (GST) and glutathione peroxidase (GPx). Conjugation of MCs to GSH, mediated by GST is the first step in the detoxification of this toxin in a wide range of aquatic organisms (Pflugmacher et al., 1998; Takenaka, 2001). Hepatic GST and GSH concentration may be privileged biomarkers of contamination with MCs.

So far, there have been many acute experiments to document the toxicity of MC exposure to fishes with different feeding types. However, few studies were conducted to document post-event investigations on wild animal poisonings due to long-term and/or frequent exposure to toxic cyanobacteria. Therefore, toxic effects on fish from MC exposure in natural conditions need to be evaluated experimentally.

In addition, the sensitivity of a fish to MCs is probably influenced by its natural habitat and feeding mode. Species native to oligotrophic habitats are more vulnerable than species native to eutrophic ones, where MC exposure is more frequent (Snyder et al., 2002). The planktivorous fish, which feed more frequently on toxic cyanobacteria, might have developed special mechanisms to counteract MCs in the history of evolution. However, no information is available for a comparative study concerning the responses of fishes (with different trophic levels) exposed to MC simultaneously in the field, which may provide direct evidences for the better resistance of phytoplanktivorous fish to MCs.

The main aims of this study were to examine physiological and biochemical responses in livers of fishes with different trophic levels to toxic cyanobacterial blooms in a natural lake, and to compare the difference in sensitivity among these fishes with discussion on the possible mechanisms. Activities of the major antioxidant enzymes (CAT, SOD, GPx, GST) and the concentration of an antioxidant (GSH) were monitored monthly in 2005, and the ultrastructures of hepatocytes of the fish livers were compared between the stages with and without cyanobacterial blooms.

2. Materials and methods

2.1. Sampling site and fish collection

Lake Taihu (30°5′–32°8′N and 119°8′–121°55′E) is located in the east part of China. It is the third largest freshwater lakes in China, and has a suface area of 2338 km^2, a mean water depth of 1.9 m and a maximum depth of about 2.6 m (Qin et al., 2004). This area is of historical importance in trade, politics, agriculture and culture. There are seven large cities (Wuxi, Suzhou, Changzhou, Jiaxin, Huzhou, Hangzhou and Shanghai) and about 35 million people inhabiting the 36,500 km^2 watershed of Taihu Lake. During the past decades, the lake has witnessed a steady increase in eutrophication, characteristic of a regular occurrence of cyanobacterial surface blooms in the warm seasons of each year (Pu et al., 1998a, b). Meiliang Bay (water surface area 125 km^2), a hypertrophic region of Lake Taihu (Dokulil et al., 2000), accommodates municipal and industry wastewater from Wuxi City and acts as principal water source for the city. Vigorous and long-lasting cyanobacterial blooms frequently cover the water surface in the bay. A large fish pen was built in the Meiliang Bay (Fig. 1) in 2003, with a total area of 1.08 km^2 and a mesh size of 2 cm × 2 cm. Sampling was carried out in the pen during 2005. Water condition in the pen was monitored monthly at 10 locations. Each water sample was a mixture of two sub-samples—one from 0.5 m below the surface and one from 0.5 m above the bottom. Water temperature was recorded by a WMY-01 digital thermometer. Dissolved oxygen (DO) and pH were determined with an Orion 810 dissolved oxygen meter and PHB-4PH meter, respectively. One liter of lake water was fixed with 5% formalin, and *Microcystis* cells were separated with ultrasonic before microscopical examination. *Microcystis aeruginosa* biomass (expressed as mg fresh wt/l) was estimated from its geometric cell volume, assuming a mean density of 1 mg/mm. Seston was filtered from 1 l lake water on the glass-fiber filter (GF/C, Waterman, UK) and the toxins in the seston were examined by HPLC according to Park and Lwami (1998).

We studied four species of fish that can be classified into three trophic levels: the phytoplanktivorous silver carp [*Hypophthalmichthys molitrix*

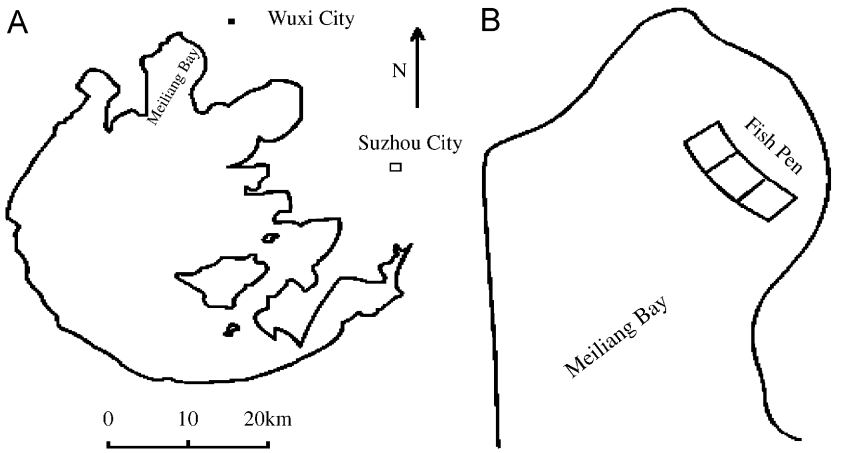

Fig. 1. The sketch of Lake Taihu (A) and the location of the fish pen in Meiliang Bay (B).

(Hm)] and bighead carp [*Aristichthys nobilis* (An)], the omnivorous *Carassius auratus* (Ca), and the carnivorous *Culter ilishaeformis* (Ci). All four species are native to Lake Taihu and the latter two species were abundant in the lake. Silver and bighead carps with mean wet body weight 162 ± 28 g and length of 26.8 ± 2.9 cm for the *H. molitrix* and with mean wet body weight 186 ± 30 g and length of 24.8 ± 1.2 cm for the *A. nobilis* were artificially stocked into the pen at the beginning of the year, for the purpose of counteracting cyanobacteria. The *C. auratus* and *C. ilishaeformis* were wild species inhabiting in the pen, with mean wet body weight 150 ± 27 g and length of 22.7 ± 5.6 cm for the *C. auratus* and with mean wet body weight 230 ± 30 g and length of 30.9 ± 3.7 cm for the *C. ilishaeformis*. Five individuals per species were randomly captured from the pen by gill net, and then measured, weighed, and sacrificed immediately. Liver was rapidly dissected out, frozen in liquid nitrogen, and maintained at $-80\,^{\circ}C$ until being processed for analyses. Samples for histopathological observation were merely collected in August and December.

2.2. Biochemical analysis

For enzyme measurement, the liver was homogenized (1:10) in a cold (4 °C) buffer solution containing sucrose (250 mM), phenylmethylsulfonyl fluoride (1 mM), and EDTA (1 mM), with pH adjusted to 7.5. Homogenates were centrifuged at 5000*g* (4 °C) for 10 min, keeping the supernatants. Then they were centrifuged at 10,000*g* (4 °C) for 15 min. The supernatants of the final centrifugation were used for analysis.

Catalase activity (CAT) was measured according to Claiborne (1985) with slight modifications. The reaction mixture in a total volume of 3 ml contained 67 mM sodium phosphate buffer (pH 7.0) and 15 mM H_2O_2. The reaction was initiated by the addition of 0.01 ml of enzyme extract. CAT activity was determined by measuring the rate of disappearance of H_2O_2 at 240 nm for 3 min. Superoxide dismutase (SOD) activity assay was based on the method described by Bayer and Fridovich (1987). One unit of the enzyme activity was defined as the amount of enzyme required to result in a 50% inhibition of the rate of nitro blue tetrazolium reduction measured at 560 nm. GST activity was measured according to the method of Habig et al. (1974) and Habig and Jakoby (1981) by evaluating the conjugation of GSH (1 mM, Sigma) with the standard model substrate 1-chloro-2, 4-dinitrobenzene (CDNB) (1 mM, Sigma). GPx activity was determined according to Drotar et al. (1985), using H_2O_2 as substrate. GSH content was measured according to Griffith (1980). The activity or the content of antioxidants was calculated in terms of the protein content of a sample (Bradford, 1976). Each assay was carried out by triplicate.

2.3. Transmission electron microscopic observation

For transmission electron microscopic study, specimens of the liver tissues were prefixed in 2.5% glutaraldehyde solution, diced into 1 mm^3, followed by three 15 min rinses with 0.1 M phosphate buffer (pH 7.4). Post-fixation was in cold 1% aqueous osmium tetroxide for 1 h. After rinsing with phosphate buffer again, the specimens were

dehydrated in a graded ethanol series of 50–100% and then embedded in Epon 812. Ultra-thin sections were sliced with glass knives on a LKB-V ultramicrotome (Nova, Sweden), stained with uranyl acetate and lead citrate and examined under a HITACHI, H-600 electron microscope.

2.4. Statistical analysis

Values were expressed as means ± standard deviation (S.D.). The data were tested for normality and homogeneity. A pearson's correlation was used to present the relationship between antioxidant enzymes throughout their fluctuation during the year ($n = 5$, $^{*}P<0.05$, $^{**}P<0.01$). Based on the appearance of cyanobacteria, the experiment was divided into three stages, the stage before cyanobacterial blooms, the stage during cyanobacterial blooms and the stage after cyanobacterial blooms. Variations of water conditions among stages were tested with one-way analysis of variance. Multifactorial Analysis of Variance (MANOVA) was used to determine statistical differences of the average antioxidant enzymatic activities (SOD, CAT, GPx and GST) and the average GSH concentration among different periods. The level of significance was set at 0.05. Principal component analysis (PCA) was used in the ordination method to analyze antioxidant enzyme activities and GSH concentrations within the tissues in the stage during the cyanobacterial blooms. Statistical analysis was carried out with SPSS 10.0.

3. Results

No fish-kill episode was observed throughout the whole period of the year. Temperature, MC content in seston, biomass of cyanobacteria and *Microcystis* all significantly differed with stages. However, pH and oxygen concentration did not change much (Table 1). In cyanobacteria, *Microsystis* was dominant. During the cyanobacterial blooms, the average temperature increased to 25.4 °C, and the maximum biomass of cyanobacteria and *Microcystis* in August reached 14.3 and 12.5 mg/l, respectively, when MC content in seston was as high as 1.53 μg/l (Fig. 2). The relatively stable concentration of DO in the lake water in spite of the occurrence of cyanobacterial blooms was probably due to frequent stirring of the lake water by strong wind in this bay (Fig. 1).

Biochemical parameters of the fish liver showed a complex fluctuation during the year. Peaks of CAT activity were observed in July for *H. molitrix* and *A. nobilis*, but in October for *C. auratus* and *C. ilishaeformis*. SOD activity was higher from July to October than in other months for all four species of fish. From April to July, GPx activities were enhanced obviously for *H. molitrix* and *A. nobilis,* but not for *C. auratus* and *C. ilishaeformis*. GST

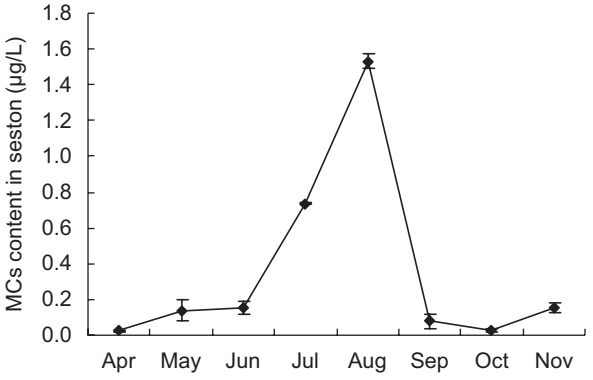

Fig. 2. Seasonal variation of MCs content in seston in the fish pen.

Table 1
The mean values of physical and chemical parameters of the lake water in three different periods

	Before cyanobacterial blooms	During cyanobacterial blooms	After cyanobacterial blooms
Temperature (°C)	5.6 ± 2.7	25.4 ± 4.8*	12.4 ± 7.0
Oxygen concentration (mg/l)	10.4 ± 2.1	7.8 ± 1.2	7.9 ± 0.4
pH	8.1 ± 0.9	8.5 ± 0.4	7.4 ± 0.4
MCs content in seston (μg/l)	0	0.5 ± 0.5*	0.1 ± 0.1
Biomass of Cyanobacteria (mg/l)	0.014 ± 0.020	6.30 ± 8.04*	0.27 ± 0.08
Biomass of *Microcystis* (mg/l)	0	5.99 ± 6.46*	0.27 ± 0.08
Time (month)	January, February, March	April, May, June, July, August, September	October, November, December

$^{*}P<0.05$, $^{**}P<0.01$.

activities of the four fishes were higher in June and July than in other months. GSH contents showed no discernible characters for all fishes throughout the year (Fig. 3).

Correlation analysis between major antioxidant enzymes in liver showed different patterns in the four species of fish. In the liver of *H. molitrix*, there were positive correlations between GPx and GST ($r = 0.618$, $P < 0.05$), SOD and GST ($r = 0.683$, $P < 0.05$), CAT and GST ($r = 0.821$, $P < 0.01$), SOD and CAT ($r = 0.854$, $P < 0.01$). Similarly, in the liver of *A. nobilis*, there were also positive correlations between GST and GPx ($r = 0.604$, $P < 0.05$), GST and CAT ($r = 0.702$, $P < 0.05$), SOD and CAT ($r = 0.723$, $P < 0.05$). However, in the liver of *C. auratus*, significantly positive correlation was observed only between SOD and CAT ($r = 0.861$, $P < 0.01$). As to *C. ilishaeformis*, the antioxidant enzymes showed no obvious correlation between each other.

The MANOVA analysis revealed differences of the antioxidant (GSH) and the major antioxidant enzymes among the three stages. CAT activity was significantly elevated merely in *A. nobilis* as the cyanobacterial blooms occurred. GST activities were prominently enhanced in *H. molitrix*, *A. nobilis* and *C. ilishaeformis* during the cyanobacterial blooms, whereas the absolute value of GST activity of *C. ilishaeformis* was much lower than other three species of fish during the same period. The fluctuations of the other parameters did not differ significantly among the three periods. The annual

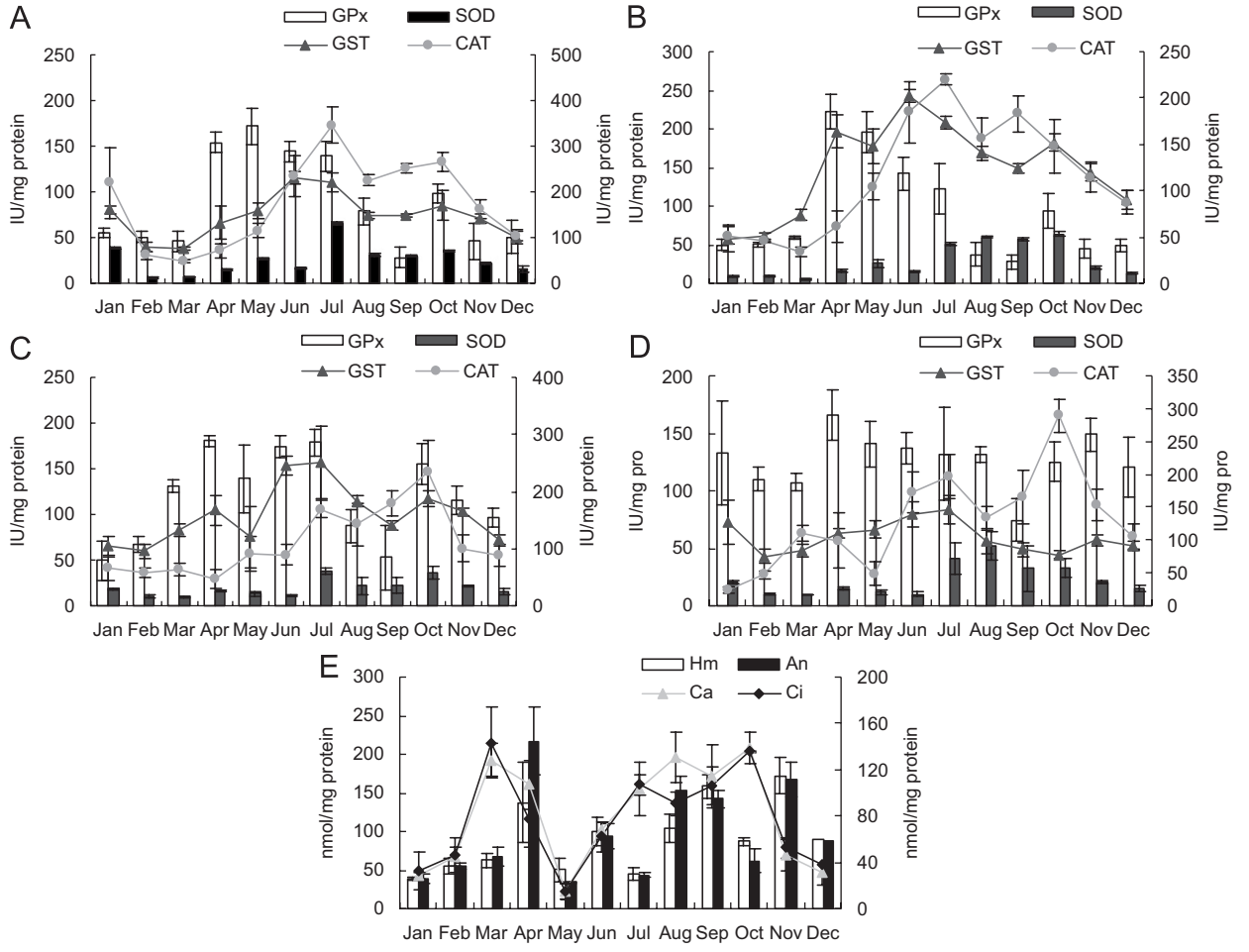

Fig. 3. Biochemical changes of livers of four fishes during the year. In (A), (B), (C), (D), the left axis is applied to the activities of GPx and SOD, while the right axis is applied to the activities of GST and CAT. (A) Activities of antioxidant enzymes in the liver of *H. molitrix*. (B) Activities of antioxidant enzymes in the liver of *A. nobilis*. (C) Activities of antioxidant enzymes in the liver of *C. auratus*. (D) Activities of antioxidant enzymes in the liver of *C. ilishaeformis*. (E) Contents of glutathione in livers of *H. molitrix* (Hm), *A. nobilis* (An), *C. auratus* (Ca) and *C. ilishaeformis* (Ci).

average contents of GSH in livers of *H. molitrix, A. nobilis, C. auratus* and *C. ilishaeformis* were 91.9, 97.0, 79.6 and 75.7 nmol/mg protein, respectively. It is evident that GSH contents in livers of *H. molitrix* and *A. nobilis* were much higher than those of the other two species (Table 2).

PCA was applied to analyze antioxidant enzyme activities (CAT, SOD, GPx, GST) and GSH concentrations in livers of fish during the cyanobacterial blooms. PCA showed that PC1 and PC2 explained 78.4% of the total variance of the data, with 43.7% for the first principal component and 34.7% for the second. PC1 was characterized by CAT, SOD and GPx, while PC2 was characterized by GSH and GST (Fig. 4). PCA on the data scores showed that *H. molitrix* and *A. nobilis* were close to each other, while *C. auratus* and *C. ilishaeformis* were far from them, suggesting a higher similarity in the activity of antioxidants in liver between the two phytoplanktivorous fishes (Fig. 5).

Ultrastructural alterations of liver in different fishes were studied in August during cyanobacterial blooms and in December after cyanobacterial blooms. In August, the hepatocytes of *H. molitrix* and *A. nobilis* merely presented several lipid droplets and lysosomes, while other subcellular organs were in good condition as round nuclei, intact mitochondria and clear stacks of endoplasmic reticulum (Fig. 6A–C, F and G). However, in August, all the examined specimens of *C. auratus* and *C. ilishaeformis*

Table 2
Activities of antioxidant enzymes and the glutathione content in livers of the four fishes and the results of multifactor analysis of variance (MANOVA)

	Before cyanobacterial bloom	During cyanobacterial bloom	After cyanobacterial bloom
Catalase[a]			
H. molitrix	55.0±5.6	207.4±90.7	177.3±66.6
A. nobilis	39.7±6.2	**151.8±39.1***	116.4±21.8
C. auratus	60.3±3.0	119.8±47.7	140.8±66.7
C. ilishaeformis	78.8±31.2	136.1±49.9	182.7±77.8
Superoxide dismutase[a]			
H. molitrix	6.4±0.6	30.9±16.7	24.2±7.8
A. nobilis	7.4±2.1	37.6±18.7	32.4±22.8
C. auratus	9.9±0.3	20.3±8.8	24.5±8.8
C. ilishaeformis	10.1±0.1	27.2±15.9	22.9±7.3
Glutathione S-transferase[a]			
H. molitrix	78.3±2.0	**173.8±38.7***	137.0±28.8
A. nobilis	62.0±11.7	**158.8±25.3****	120.6±25.6
C. auratus	113.5±18.3	184.4±49.4	155.8±30.1
C. ilishaeformis	79.4±4.2	**116.1±21.4***	89.5±9.4
Glutathione peroxidase[a]			
H. molitrix	48.3±2.5	119.3±49.7	64.6±23.3
A. nobilis	54.7±4.4	124.8±73.1	62.2±22.4
C. auratus	99.3±32.0	135.4±49.3	122.3±24.1
C. ilishaeformis	108.7±1.5	130.2±27.4	131.4±12.3
Glutathione[b]			
H. molitrix	59.1±3.9	99.4±41.2	116.3±39.2
A. nobilis	62.0±6.2	114.1±63.9	105.7±44.7
C. auratus	86.7±41.5	89.6±38.3	72.0±47.8
C. ilishaeformis	94.8±48.5	76.5±31.6	75.7±42.7

Mean values±standard deviations. Asterisks indicate significant differences between the sampling periods.
*$P<0.05$, **$P<0.01$.
[a]IU/mg protein.
[b]nmol/mg protein.

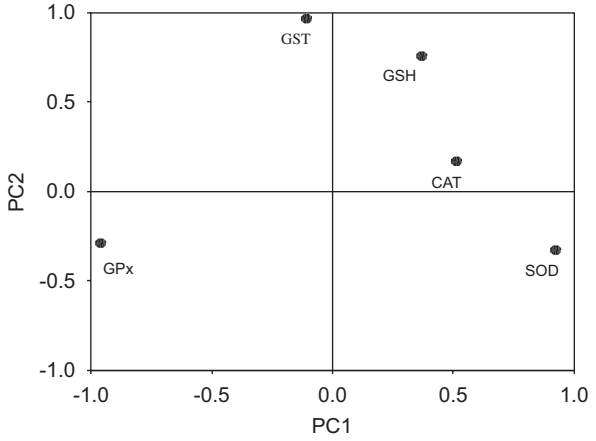

Fig. 4. PCA of the antioxidants activity and the GSH concentration in livers of fishes during the cyanobacterial blooms showing the loading of the variables on PC1 and PC2.

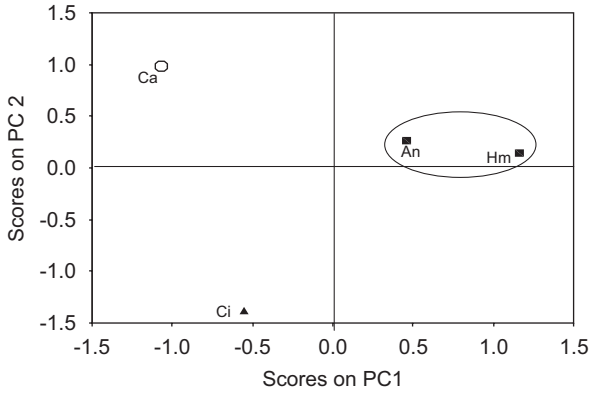

Fig. 5. PCA of the antioxidants activity and the GSH concentration in livers of fish (*H. molitrix* (Hm), *A. nobilis* (An), *C. auratus* (Ca) and *C. ilishaeformis* (Ci)) during the cyanobacterial blooms showing the data scores labeled as species.

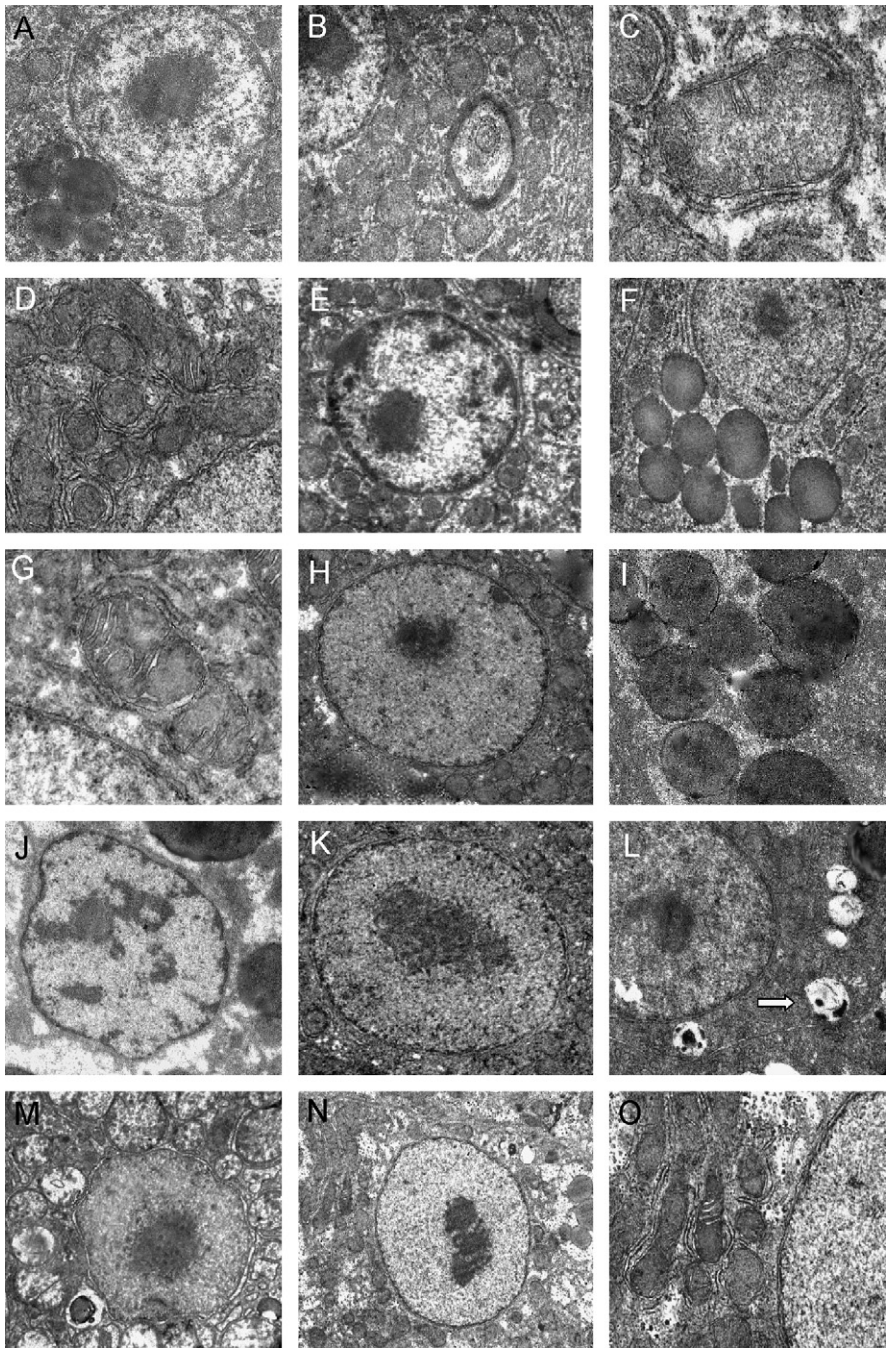

Fig. 6. Ultrastructures of livers of four fishes during the cyanobacterial blooms and the post-bloom period. (A–E) Ultrastructures of the liver of *H. molitrix* during the cyanobacterial blooms and the post-bloom period. During the cyanobacterial blooms: (A) nuclear and lipid droplet (8000 ×), (B) lysosome (6000 ×), (C) mitochondrion and rough endoplasmic reticulum (40,000 ×); during the post-bloom period: (D) mitochondrion (40,000 ×), (E) nuclear (8000 ×). (F–H) Ultrastructures of the liver of *A. nobilis* during the cyanobacterial blooms and the post-bloom period. During the cyanobacterial blooms: (F) nuclear and lipid droplet (8000 ×), (G) mitochondrion (30,000 ×); during the post-bloom period: (H) nuclear (12,000 ×). (I–K) Ultrastructures of the liver of *C. auratus* during the cyanobacterial blooms and the post-bloom period. During the cyanobacterial blooms: (I) lipid droplet (8000 ×), (J) nuclear (12,000 ×); during the post-bloom period: (K) nuclear (12,000 ×). (L–O) Ultrastructures of the liver of *C.ilishaeformis* during the cyanobacterial blooms and the post-bloom period. During the cyanobacterial blooms: (L) nuclear and damaged mitochondrion (arrow) (8000 ×), (M) the swelling and vesiculation of the organelles (10,000 ×); during the post-bloom period: (N) nuclear (12,000 ×), (O) mitochondrion (30,000 ×).

revealed seriously pathological changes in hepatocytes such as ubiquitous deformation of the nuclear outline (Fig. 6J and M), and in hepatocytes of 80% *C. auratus* examined, a lot of lipid droplets were widespread with overcast subcellular organs (Fig. 6I). As to *C. ilishaeformis*, the swelling of the hepatocytic endomembrane system (mainly including endoplasmic reticulum (ER), mitochondria and nuclear envelope) was quite conspicuous in all specimens (Fig. 6L and M). Loss of cristae and matrix in mitochondria was commonly observed in the hepatocytes. In December, the hepatocytes of *H. molitrix* and *A. nobilis* presented no remarkable changes from those in August (Fig. 6D, E and H). However, the hepatocytes of *C. auratus* and *C. ilishaeformis* showed considerable recovery in December, for normal subcellular organs were usually observed in all specimens during the same time (Fig. 6K, N and O).

4. Discussion

There have been several field studies to document fish impairment associated with cyanobacterial blooms (English et al., 1994; Carbis et al., 1997; Zimba et al., 2001). The present experiment observed for the first time that damage of fish liver by toxic cyanobacterial blooms varied with trophic levels, i.e., the carnivorous *C. ilishaeformis* was the most seriously damaged fish, as indicated by its swollen hepatocytic endomembrane system and the morphologically altered nuclei, followed by the omnivorous *C. auratus* with morphologic alterations in nuclei and production of a lot of lipid droplets, whereas the phytoplanktivorous *H. molitrix* and *A. nobilis* were the least affected fish, with normal subcellular organs and merely a few lipid droplets and lysosomes.

Jewel et al. (2003) investigated a fish kill episode concomitant to cyanobacterial blooms and pointed out that the fish kill was possibly caused by oxygen deficiency or toxins secreted from cyanobacteria or by the combination of both. In the present study, dissolved oxygen concentration in the lake water remained at relatively high levels throughout the study period, while MCs of cyanobacterial blooms were likely be an important factor responsible for the pathological changes in fish liver. The prominent swelling of the endomembrane system observed in *C. ilishaeformis* in the present study was compatible with previous in vivo studies on toxicity of microcystins on bighead carp and common carp. Bighead carp injected with extracted MCs at a dose of 500 MC-LR_{eq} μg/kg bw displayed swollen organelles in hepatocytes within 24 post-injection (Li et al., 2005a, b). After common carp were exposed to 50 μg MCs/kg bw through feeding on bloom scum for 28 days, there were also obvious ultrastructural changes in hepatocytes: swollen endomembrane system, numerous electron-lucent membrane-bound vacuoles and concentric membrane whorls transformed form ER. The typical morphologic alterations in nuclei of *C. auratus* in the present study were similar to those of bighead carp injected with extracted MCs at a dose of 200 MC-LR_{eq} μg/kg bw (Li et al., 2005a, b).

In the present study, it was obvious that the phytoplanktivorous fish were better resistant to and the carnivorous fish were more vulnerable to toxic cyanobacterial blooms. Carnivorous fish kill episodes associated with toxic blooms have already emerged in previous documents. In Lake Peipsi, a fish kill associated with strong cyanobacterial bloom in the summer of 2002 revealed that the dead fishes were dominated by carnivorous ruffe. Both English et al. (1994) and Zimba et al. (2001) reported channel catfish deaths due to toxic blooms. Aquatic animals in eutrophic fresh waters may be killed by microcystins, 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. 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. Therefore, in the present study, the most serious damage in the carnivorous fish might be due to their higher accumulation of MCs.

Various degrees of alteration in pathology were likely due to different capability in detoxification of MCs among fish species, since phytoplanktivorous fishes like silver and bighead carps were reported to be possibly more tolerant to high microcystins than other fishes from an evolutionary point of view (Xie et al., 2004). Antioxidant components and antioxidant enzymes, which are generally ubiquitous in aquatic organisms, counteract endogenous and exogenous oxidative stress and play an important role in decomposing some toxic xenobiotics. In the present study, better correlations among antioxidant enzymes were present in the livers of *H. molitrix* and *A. nobilis*, compared with other two species, suggesting that *H. molitrix* and *A. nobilis*

probably have more powerful ability to counteract oxidative stress by MC. Also, the annual average contents of GSH in the livers of *H. molitrix* and *A. nobilis* were much higher than those of the other two species, and PCA analyses pointed out a higher similarity in the antioxidant (GSH) and in the antioxidant enzymes of the liver between the phytoplanktivorous *H. molitrix* and *A. nobilis* during cyanobacterial blooms.

The detoxification of microcystins in liver is known to occur via conjugation to GSH by GST firstly (Pflugmacher et al., 1998; Takenaka, 2001). Nevertheless, the responses of GST activity to MCs in fish have been quite variable so far (Table 3). It appears that exposure time, routes, composition of MCs and the physiological characters of the selected tissue all cast influences on the responses of GST when fish are exposed to MCs. GST activities in liver, gill, intestine and brain were all inhibited when *Corydoras paleatus* was exposed to dissolved MC-RR at concentrations of 0.5, 2, 5 and 10 μg/l, respectively, for 24 h (Cazenave et al., 2006a, b). Also, significant decrease in GST activity in liver was recorded in the first hours when juvenile goldfish were injected with purified MC-LR at a dose of 125 μg/kg bw, but the activity returned to a normal level as the time went on (Malbrouck et al., 2004). On the contrary, increased soluble GST activity was reported in embryos of zebrafish exposed to MC-LR at 0.5 μg/l for 24 h, while the microsomal GST remained stable (Best et al., 2002). The stable GST activity was also obtained in hepatocytes of common carp exposed to MC-LR at a dose of 10 μg/l for 2, 4 and 6 h, respectively (Li et al., 2003) and in the liver of juvenile gold fish, *C. auratus* L., injected with purified MC-LR at a dose of 125 μg/kg bw throughout the experiment for 96 h (Malbrouck et al., 2004). In the present study, all fishes enhanced GST activity during the cyanobacterial blooms, especially *H. molitrix*, *A. nobilis* and *C. ilishaeformis*.

GSH is involved in a number of crucial cellular functions, such as ROS scavenging, detoxification of electrophiles, maintenance of thiol-disulfide status and signal transduction (Halliwell and Gutteridge, 1999; Dröge, 2002). Reduced GSH is the main nonprotein thiol and one of the main reductants found in cells (Siegers, 1989). Pflugmacher et al. (1998) detected conjugation of MC-LR to GSH in enzyme extracts containing GST of aquatic

Table 3
Effect of microcystins (MC) on GST activity and GSH level in fish[a]

Subjects	Exposure route and dose	Effect on antioxidants	References
Hepatocytes of Common carp	MC-LR 10 μg/l; Exposure time: 2 h, 4 h, 6 h	After 6 h, decreased GSH levels, no discernable GST activity changes	Li et al. (2003)
Liver of silver carp (*Hypophthalmichthys molitrix*)	Exposed in living natural population of cyanobacterial water bloom; exposure time: 25 days	Significant increase in GSH level	Blaha et al. (2004)
Liver of juvenile goldfish (*Carassius auratus* L.)	I.p. administration of purified MC-LR 125 μg/kg bw; exposure time: 6 h, 24 h, 48 h, 96 h	Not significant changes of GSH content and GST activity in both fed and fasted groups throughout the experiment	Malbrouck et al. (2004)
		Significant decrease in GST during the first hours; but after 24 h, merely below the control individuals but not significantly	Malbrouck et al. (2003)
Liver, gill, intestine and brain of *Corydoras paleatus*	Exposed to dissolved MC-RR at 0.5, 2, 5, 10 μg/l; exposure time: 24 h	Inhibited GST in all studied organs at most MC-RR concentrations used	Cazenave et al. (2006a, b)
Embryos of (prim six stage) Zebrafish (*Danio rerio*)	MC-LR 0.5 μg/l; MC-LR 0.5 μg/l + LPS 0.5 μg/l; exposure time: 24 h	In MC-LR exposure group: increased sGST activity but no change of mGST activity. In MC-LR + LPS exposure group: reduced mGST and sGST activity	Best et al. (2002)

[a]MC-LR = microcystin-LR; MC-RR = microcystin-RR; LPS = lipopolysaccharides; GSH = glutathione; GST = glutathione-S-transferase; sGST = soluble glutathione-S-transferase; cGST = cytosolic glutathione-S-transferase; mGST = microsomal glutathione-S-transferase.

macrophyte, invertebrates, fish eggs and fish. MC-LR can conjugate with GSH and ulteriorly be degraded to MCLR-Cys, which can neutralize the electrophilic sites of MC-LR and increase water solubility, consequently reducing the toxicity and enhancing excretion of MC-LR (Kondo et al., 1992). Studies on toxicity of MC-LR on animal hepatocytic antioxidant systems also directly demonstrate that antioxidant systems, mainly GSH, could be relevant indices in explaining the sensitivity of some vertebral species to MC (Takenaka and Otsu, 1999). Furthermore, supplement with GSH provides animals' higher resistance to MC toxicity. Hermansky et al. (1991) revealed that pretreatment of mice with GSH protected them against MC-LR lethality and Takenaka and Otsu (1999) also reported that the mixture of GSH and MC-LR solution weakened acute toxicity of MC-LR than intact MC-LR to mice. Similar results were obtained in *Ctenopharngodon idellus*, which presented fewer ultrastructural changes by pretreatment with GSH, compared with the fish subjected to MC-LR directly (Zhang et al., 1996). Therefore, it is likely that GSH level in organisms is closely related to their resistance to the toxicity of MCs. The conjugation of microcystin with GSH is expected to induce a leak of this tripeptide from the intracellular pool, but previous results showed different results (Table 3). Decreased GSH levels were observed in hepatocytes of common carp exposed to MC-LR at a dose of 10 μg/l for 2, 4 and 6 h, respectively (Li et al., 2003), while a significant increase in GSH level was recorded in liver of silver carp exposed to the living natural population of cyanobacterial water bloom for 25 days (Blaha et al., 2004). However, the juvenile gold fish *C. auratus* injected with purified MC-LR at a dose of 125 μg/kg bw displayed no significant changes in GSH content in liver during an experiment of 96 h (Malbrouck et al., 2004). In the present study, there was no discernable difference in GSH level among periods for all fish species. Malbrouck et al. (2004) attributed the lack of GSH alteration in liver of goldfish i.p. administered with purified MC-LR to its important basic GSH concentration. In light of the above discussions, the higher annual average content of GSH in the livers of *H. molitrix* and *A. nobilis* might have favored their coexistence with the toxic cyanobacterial blooms.

In the present study, *C. auratus* and *C. ilishaeformis* showed serious histological damage during cyanobacterial blooms, but most components of the antioxidant systems had little change except GST in the liver of *C. ilishaeformis*. Bury et al. (1997) reported that necrotic cells appeared in livers of brown trout and rainbow trout receiving a dose of 25 μg/l MC-LR, without apparent changes in plasma liver enzyme levels. Pinho et al. (2003) found that crab orally injected with MCs (176 μg/l) also showed histological damage with only a few enzymes (GST, CAT) of the antioxidant defense system activated and LPO levels maintaining stable. These results indicate that histopathological features are probably more sensitive indicators of MC hepatotoxicosis.

In conclusion, the phytoplanktivorous fishes are especially important to humans because of their role in aquatic ecosystems as direct consumers of phytoplankton primary production and their potential for biological management of cyanobacterial blooms (Opuszynski and Shireman, 1995; Xie and Liu, 2001). In the present study, the phytoplanktivorous fish presented higher resistance to toxic cyanobacterial blooms than omnivorous and carnivorous ones, which might be due to their higher basal GSH concentrations and better cooperation among antioxidant components in liver. These results provide physiological and toxicological evidences for the applicability of using phytoplanktivorous fish to counteract toxic cyanobacterial blooms in natural waters. However, it is needed to clarify the detailed mechanisms underling the higher resistance of the phytoplanktivorous fish to toxic cyanobacterial blooms in our future study.

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References

Andersen, R.J., Luu, H.A., Chen, D.Z.X., Holmes, C.F.B., Kent, M.L., Le Blanc, M., Taylor, F.J.R., Williams, D.E., 1993. Chemical and biological evidence links microcystins to salmon netpen liver disease. Toxicon 31, 1315–1323.

Bayer, W.F., Fridovich, J.L., 1987. Assaying for superoxide dismutase activity: some large consequences of minor changes in conditions. Anal. Biochem. 161, 559–566.

Best, J.H., Pflugmacher, S., Wiegand, C., Eddy, F.B., Metcalf, J.S., Codd, G.A., 2002. Effects of enteric bacterial and cyanobacterial lipopolysaccharides, and of microcystin-LR, on glutathione-S-transferase activities in zebra fish *(Danio rerio*). Aquat. Toxicol. 60, 223–231.

Blaha, L., Kopp, R., Simkova, K., Mares, J., 2004. Oxidative stress biomarkers are modulated in silver carp (*Hypophthalmichthys molitrix* Val) exposed to microcystin-producing cyanobacterial water bloom. Acta Vet. Brno. 73, 477–482.

Bradford, M.M., 1976. A rapid and sensitive method for the quantification of microgram quantities of proteins utilizing the principle of protein–dye binding. Anal. Biochem. 72, 248–254.

Bury, N.R., McGeer, J.C., Eddy, F.B., Codd, G.A., 1997. Liver damage in brown trout, *Salmo trutta* L., and rainbow trout, *Oncorhynchus mykiss* (Walbaum), following administration of cyanobacteria hepatotoxin microcystin-LR via the dorsal aorta. J. Fish Dis. 20, 209–215.

Carbis, C.R., Rawlin, G.T., Grant, P., Mitchell, G.F., Anderson, J.W., McCauley, I., 1997. A study of feral carp, *Cyprinus carpio* L., exposed to *Microcystis aeruginosa* at Lake Mokoan, Australia, and possible implication for fish health. J. Fish Dis. 20, 81–91.

Carmichael, W.W., 1992. Cyanobacteria secondary metabolites—the cyanotoxins. J. Appl. Bacteriol. 72, 455–459.

Carmichael, W.W., Falconer, I.R., 1993. Diseases related to freshwater blue-green algal toxins, and control measures. In: Falconer, I.R. (Ed.), Algal Toxins in Seafood and Drinking Water. Academic Press, London, pp. 187–209.

Cazenave, J., Bistoni, M.A., Pesce, S.F., Wunderlin, D.A., 2006a. Differential detoxification and antioxidant response in diverse organs of *Corydoras paleatus* exposed to microcystin-RR. Aquat. Toxicol. 76, 1–12.

Cazenave, J., Bistoni, M.A., Zwirnmann, E., Wunderlin, D.A., Wiegand, C., 2006b. Attenuating effects of natural organic matter on microcystin toxicity on zebra fish (*Danio rerio*) embryos—benefits and costs of microcystin detoxification. Environ. Toxicol. 21, 22–32.

Claiborne, A., 1985. Catalase activity. In: Greenwald, R.A. (Ed.), CRC Handbook of Methods in Oxygen Radical Research. CRC Press, Boca Raton, FL, pp. 283–284.

Dokulil, M., Chen, W., Cai, Q., 2000. Anthropogenic impacts to large lakes in China: the TaiHu example. Aquat. Ecosyst. Health Manage. 3, 81–94.

Drotar, A., Phelps, P., Fall, R., 1985. Evidence for glutathione peroxidase activities in cultured plant cells. Plant Sci. 42, 35–40.

Dröge, W., 2002. Free radicals in the physiological control of cell function. Phys. Rev. 82, 47–95.

English, W.R., Schwedler, T.E., Dyck, L.A., 1994. *Aphanizomenon flos-aquae*, a toxic blue-green alga in commercial catfish, *Ictalurus punctatus*, ponds: a case history. J. Appl. Aquaculture 3, 195–209.

Eriksson, J.E., Toivola, D., Meriluoto, J.A.O., Karaki, H., Han, Y., Hartshorne, D., 1990. Hepatocyte deformation induced by cyanobacterial toxin reflects inhibition of protein phosphatases. Biochem. Biophys. Res. Commun. 173, 1347–1353.

Falconer, I.R., Yeung, D.S.K., 1992. Cytoskeletal changes in hepatocytes induced by *Microcystis* toxins and their relation to hyperphosphorylation of cell proteins. Chem. Biol. Interact. 81, 181–196.

Griffith, O., 1980. Determination of glutathione and glutathione disulfide using glutathione reductase and 2-vinylpyridine. Anal. Biochem. 106, 207–212.

Habig, W.H., Jakoby, W.B., 1981. Assays for differentiation of glutathione S-transferases. Methods Enzymol. 77, 398–405.

Habig, W.H., Pabst, M.J., Jakoby, W.B., 1974. Glutathine-S-transferases: the first enzymatic step in mercapturic acid formation. Biol. Chem. 249, 7130–7139.

Halliwell, B., Gutteridge, J.M.C., 1999. Free Radicals in Biology and Medicine, third ed. Clarendon Press, Oxford.

Hermansky, S.J., Stohs, S.J., Eldeen, Z.M., Roche, U.F., Mereish, K.A., 1991. Evaluation of potential chemoprotectants against microcystin-LR hepatotoxicity in mice. J. Appl. Toxicol. 11, 65–74.

Ito, F., Kondo, F., Harada, K.I., 1997. Hepatic necrosis in aged mice by oral administration of microcystin-LR. Toxicon 35, 231–239.

Ito, F., Kondo, F., Harada, K.I., 2000. First report on the distribution of orally administered microcystin-LR in mouse tissue using an immunostaining method. Toxicon 38, 37–48.

Jewel, M.A., Affan, M.A., Khan, S., 2003. Fish mortality due to cyanobacterial blooms in an aquaculture pond in Bangladesh. Pakistan J. Biol. Sci. 6, 1046–1050.

Kondo, F., Ikai, Y., Oka, H., Masanao, O., Ishikawa, N., Harada, K.I., Matsuura, K., Murata, H., Suzuki, M., 1992. Formation characterization and toxicity of the glutathione and cysteine conjugates of toxic heptapeptide microcystins. Chem. Res. Toxicol. 5, 591–596.

Li, X.Y., Liu, Y.D., Song, L.R., Liu, J.T., 2003. Responses of antioxidant systems in the hepatocytes of common carp (*Cyprinus carpio* L.) to the toxicity of microcystin-LR. Toxicon 42, 85–89.

Li, X.Y., Chung, I., Kim, J., Lee, J., 2005a. Oral exposure to *Microcystis* increases activity-augmented antioxidant enzymes in the liver of loach (*Misgurnus mizolepis*) and has no effect on lipid peroxidation. Comp. Biochem. Physiol. C 141, 292–296.

Li, L., Xie, P., Chen, J., 2005b. *In vivo* studies on toxin accumulation in liver and ultrastructural changes of hepatocytes of the phytoplanktivorous bighead carp ip-injected with extracted microcystins. Toxicon 46, 533–545.

Malbrouck, C., Trausch, G., Devos, P., Kestemont, P., 2003. Hepatic accumulation and effects of microcystin-LR on juvenile goldfish *Carassius auratus* L. Comp. Biochem. Phys. C 135, 39–48.

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.

Opuszynski, K., Shireman, J.V., 1995. Merbivorous Fishes—Culture and Use for Weed Management. CRC Press, Boca Raton, FL, USA.

Park, H.D., Lwami, C., 1998. Temperal variabilities of the concentrations of intra- and extracellular microcystin and toxic microcystis species in a hypertrophic lake, Lake Suwa, Japan (1991–1994). Environ. Toxicol. Water Qual. 13, 61–72.

Pinho, G.L., Moura da, R.C., Yunes, J.S., Yunes, J.S., Bianchini, A., Monserrat, J.M., 2003. Toxic effects of microcystins in the hepatopancreas of the estuarine crab *Chasmagnathus granulatus* (Decapoda, Grapsidae). Comp. Biochem. Phys. C 135, 459–468.

Pflugmacher, S., Wiegand, C., Oberemm, A., Beattie, K.A., Krause, E., Codd, G.A., Steinberg, C.E.W., 1998. Identification of an enzymatically formed glutathione conjugate of the cyanobacterial hepatotoxin microcystin-LR: the first step of detoxication. Biochim. Biophys. Acta 1425, 527–533.

Pu, P.M., Hu, W.P., Wang, G.X., Zhang, S.Z., Hu, C.G., Yan, J.S., 1998a. The new strategy for improving the aqua-ecological environmental in Taihu Lake Basin, China. How can we solve the problem of lakc of qualified water and deterioration of environment and natural resources in Taihu Lake basin. J. Lake Sci. 10, 47–58.

Pu, P.M., Hu, W.P., Yan, J.S., Wang, G.X., Hu, C.G., 1998b. A physico-ecological engineering experiment for water treatment in a hypertrophic lake in China. Ecol. Eng. 10, 79–90.

Qin, B.Q., Hu, W.P., Chen, W.M., 2004. Processes and Mechanisms of the Changes in Aquatic Environments in Lake Taihu. Science Press, Beijing (in Chinese).

Siegers, C.P., 1989. Glutathione and glutathione dependent enzymes. Progr. Pharmacol. Clin. Pharmacol. 7, 171–180.

Snyder, G.S., Goodwin, A.E., Freeman, D.W., 2002. Evidence that channel catfish, *Ictalurus punctatus* (Rafinesque) mortality is not linked to ingestion of the hepatotoxin MC-LR. J. Fish Dis. 25, 275–285.

Takenaka, S., Otsu, R., 1999. Effects of L-cysteine and reduced glutathione on the toxicities of microcystin-LR: the effect for acute liver failure and inhibition of protein phosphatase activity. Aquat. Toxicol. 48, 65–68.

Takenaka, S., 2001. Covalent glutathione to cyanobacterial hepatotoxin microcystin-LR by F344 rat cytosolic and microsomal glutathione-S-transferases. Environ. Toxicol. Pharm. 9, 135–139.

World Health Organization, 2003. Algae and cyanobacteria in freshwater. In: Guidelines for Safe Recreational Water Environments, vol. 1: Coastal and Fresh Waters. Geneva, Switzerland, pp. 136–158.

Xie, P., Liu, J.K., 2001. Practical success of biomanipulation using filter-feeding fish to control cyanobacteria blooms: synthesis of decades of research and application in a subtropical hypereutrophic lake. Sci. World J. 1, 337–356.

Xie, L.Q., Xie, P., Ozawa, K., Honma, T., Yokoyama, A., Park, H.D., 2004. Dynamics of microcystins-LR and -RR in the phytoplanktivorous silver carp in a sub-chronic toxicity experiment. Environ. Pollut. 127, 431–439.

Xie, L.Q., Xie, P., Guo, L., Li, L., Miyabara, Y., Park, H.-D., 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.

Zimba, P.V., Khoo, L., Gaunt, P.S., Brittain, S., Carmichael, W.W., 2001. Confirmation of catfish, *Ictalurus punctatus* (Rafinesque), mortality from *Microcystis* toxins. J. Fish Dis. 24, 41–47.

Zhang, Y.Y., Xu, L.H., Zhou, B.S., Xu, Y., 1996. Preiliminary studies on the role of GSH in detoxification of microcystin-LR in fish. Acta Hydrobiol. Sinica 20, 284–286.