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Biochemical and ultrastructural changes of the liver and kidney of the phytoplanktivorous silver carp feeding naturally on toxic *Microcystis* blooms in Taihu Lake, China

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Abstract

Many experimental studies have documented the impact of microcystins (MC) on fish based on either intraperitoneal injection, or oral gavaging via the diet, but few experiments were conducted by MC exposure through natural food uptake in lakes. In this study, the phytoplanktivorous silver carp were stocked in a large pen set in Meiliang Bay of Taihu Lake where toxic *Microcystis* blooms occurred in the warm seasons. Fish samples were collected monthly and MC concentrations in liver and kidney of the fish were determined by LC–MS. The maximum MC concentrations in liver and kidney were present in July when damages in ultrastructures of the liver and kidney were revealed by electron microscope. In comparison with previous studies on common carp, silver carp showed less damage and presence of lysosome proliferation in liver and kidney. Silver carp might eliminate or lessen cell damage caused by MC through lysosome activation. Recovery in the ultrastructures of liver and kidney after *Microcystis* blooms was companied with a significant decrease or even disappearance of MC. Catalase and glutathione S-transferase in liver and kidney of silver carp during *Microcystis* blooms were significantly higher than before and after *Microcystis* blooms. The high glutathione pool in liver and kidney of silver carp suggests their high resistance to MC exposure. The efficient antioxidant defence may be an important mechanism of phytoplanktivorous fish like silver carp to counteract toxic *Microcystis* blooms.

Keywords: Phytoplanktivorous silver carp; *Microcystis*; Microcystin; Liver; Kidney; Biochemical and ultrastructural changes; LC–MS; Large fish pen

1. Introduction

The occurrence of toxic cyanobacterial blooms in eutrophic lakes, reservoirs, and recreational waters has become a worldwide problem (Paerl et al., 2001). Among cyanotoxins, microcystins (MC) are considered to be one of the most dangerous groups, which are known to be potent hepatotoxin (Codd,

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1995; Dawson, 1998) and tumor promoter (Nishiwaki-Matsushima et al., 1992). Exposure to MC represents a health risk to animals (Carmichael, 1996; Malbrouck and Kestemont, 2006) and humans (Yu, 1995; Pouria et al., 1998). The toxicity of MC has been attributed to the highly specific inhibition of serine/threonine phosphatases (PP1/PP2A) (MacKintosh et al., 1990) and/or to the increased formation of reactive oxygen species (ROS) (Ding et al., 1998, 2001; Li et al., 2003). It has been suggested that either of the above mechanisms could induce the cytoskeletal damage leading to loss of cell morphology (Toivola and Eriksson, 1999; Ding and Ong, 2003).

Many experimental studies have documented the impact of MC on fish based on either intraperitoneal (i.p.) injection (Råbergh et al., 1991; Kotak et al., 1996; Malbrouck et al., 2004), or oral gavaging via the diet (Tencalla and Dietrich, 1997; Fischer and Dietrich, 2000; Li et al., 2004), or immersion in water containing purified MC or lysates or whole cells of cyanobacteria (Carbis et al., 1996). In acute toxic experiment, when fishes were treated with high doses of MC, liver pathology was characterized by disruption of the liver structure, condensed cytoplasm, the appearance of massive pyknotic/apoptotic nuclei (Råbergh et al., 1991; Kotak et al., 1996; Tencalla and Dietrich, 1997; Fischer and Dietrich, 2000). Råbergh et al. (1991) had reported degenerative changes in the epithelial cells of tubule, glomeruli and interstitial tissue in the kidney of common carp (*Cyprinus carpio* L.) exposed i.p. with a sublethal dose of to MC-LR (150 μg MC-LR kg^{-1}). Kotak et al. (1996) also showed renal lesions that consisted of coagulative tubular necrosis and dilation of Bowman's space in the kidney from the two longest surviving rainbow trout (*Oncorhynchus mykiss*) in the 1000 μg/kg dose group. So far, all these studies were limited to acute toxicity experiments and by contamination routes that cannot reflect the uptake route under natural environments. Therefore, toxic effects on fish from MC exposure through natural food uptake need to be evaluated experimentally, especially if there is long-term, frequent exposure in natural environments.

Recently, evaluations of oxidative stress in organisms exposed to toxins are becoming increasingly important in the field of ecotoxicology. Previous studies have demonstrated that cyanobacterial extracts and pure MC induce oxidative stress through increasing ROS and oxidative damage products such as lipid peroxides in many organisms including fish (Ding et al., 1998, 2001; Li et al., 2003; Jos et al., 2005; Pinho et al., 2005). Both ROS and lipid peroxides are known to be reduced (scavenged) by non-enzymatic defence chemicals such as vitamins E, C, A and glutathione (GSH) and specific antioxidant enzymes such as catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPX), glutathione S-transferase (GST) (Filho, 1996). To the extent of our knowledge, effects of MC on these antioxidant defence systems of fish have only been measured in *Danio rerio* embryos (Wiegand et al., 1999; Best et al., 2002), in isolated hepatocytes of common carp (Li et al., 2003), liver of juvenile goldfish (*Carassius auratus* L.) and tilapia fish (*Oreochromis* sp.) either injected intraperitoneally with MC-LR, or fed with toxic cyanobacterial cells (Malbrouck et al., 2004; Jos et al., 2005). However, such studies are still lacking from fish in field experiment.

This paper aims to examine seasonal variations of major biochemical parameters including CAT, SOD, GPX, GST, and GSH, and morphological changes in liver and kidney of silver carp (*Hypophthalmichthys molitrix*) collected from a fish pen located in Meiliang Bay of Taihu where heavy toxic cyanobacterial blooms (mainly *Microcystis aeruginosa*) frequently accumulated densely in the warm seasons by wind. Silver carp, one of the most intensively cultured freshwater phytoplanktivorous fish, is especially important to humans because of its roles in aquatic ecosystems as a direct consumer of phytoplankton and zooplankton, its potential for biological management of cyanobacterial blooms and its value as fish food (Opuszynski and Shireman, 1995; Xie and Liu, 2001).

2. Materials and methods

2.1. Sampling sites and samples collection

Taihu Lake, the third largest freshwater lake in China, is located in Jiangsu province, eastern China. Meiliang Bay (water surface area 135 km^2), a part of Taihu Lake, accommodates municipal and industry wastewater from Wuxi City, but also acts as a principal water source for the city. Meiliang Bay is the most eutrophic part of the lake, characteristic of extremely dense accumulation of toxic *Microcystis* blooms by wind in the summer.

A large fish pen was built in Meiliang Bay in 2003 (120°12′46″–120°13′07″E, 31°29′07″–31°29′55″N)

(Fig. 1), with a total area of 1.08 km^2 and a mesh size of 2 cm × 2 cm. Silver carp with a mean weight of 133 ± 3.3 g were bought from a local fishery and put into the pen at the beginning of March 2004. Totally, approximately 16,000 kg of silver carp fingerlings were stocked. During the first couple of weeks, dead fish were occasionally found due to injury of transportation of the fingerlings. Afterwards, no visible fish death was found in the fish pen. From April 2004 to March 2005 (12-month period), five silver carps in the pen were captured monthly using gill nets, and then measured, weighed, sacrificed immediately. Samples of liver and kidney were excised and divided into two parts: one was immediately frozen in liquid nitrogen before storage at −80 °C for determination of toxin content and biochemical analysis, and the other one was fixed for microscopic examination (samples for electron microscopic examination were merely prepared in July and December). Water temperature was recorded by a WMY-01 digital thermometer in the field. Dissolved oxygen and pH were determined with an Orion 810 dissolved oxygen meter and PHB-4PH meter, respectively.

For the analysis of MC in phytoplankton (intracellular MC) and quantification of phytoplankton community, we set two sampling sites in the fish pen. The samples were taken monthly from June 2004 to February 2005 using a 5 L modified Patalas's bottle sampler. At each site, water samples were collected from the surface and near the bottom, and 1 L mixed water sample was filtered on a filter (Waterman GF/C, UK). The filter was homogenized and the MC extraction was after Park et al. (1998).

2.2. Phytoplankton analysis

For phytoplankton estimation, 1 L of water was preserved with 1% acidified Lugol's iodine solution and concentrated to 30 ml after sedimentation for 48 h. Sub-samples were counted with a microscope under 400 × magnifications. Taxonomic identification was according to Hu et al. (1979), and biomass was estimated from approximate geometric volumes of each taxon.

2.3. Determination of MC concentration in fish organs and phytoplankton

Extraction of MC in liver and kidney of silver carp basically followed the method of Chen and Xie (2005). Qualitative and quantitative analysis of MC in both organs of fish and phytoplankton were performed using a Finnigan LC–MS system comprising a thermo surveyor auto sampler, a surveyor MS pump, a surveyor PDA system, and a Finnigan LCQ-Advantage MAX ion trap mass spectrometer equipped with an atmospheric pressure ionization fitted with an electrospray ionization source (ESI).

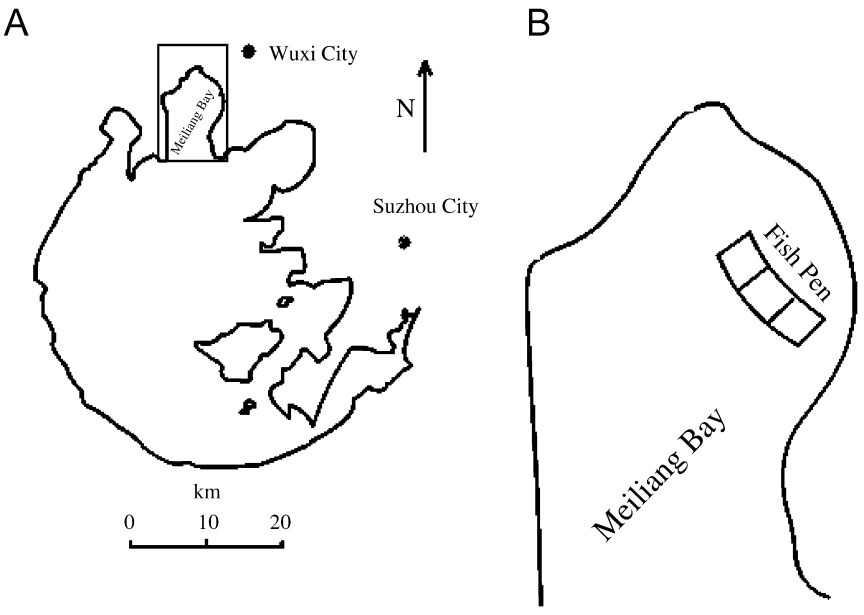

Fig. 1. Sketch map of Taihu Lake and the sampling sites in Meiliang Bay.

The instrument control, data processing, and analysis were conducted by using Xcalibur software. Separation was carried out under the reversed phase on Hypersil GOLD 5 μm column (2.1 mm i.d. × 150 mm). The isocratic mobile phase consisted of solvent A [water + 0.05% (v/v) formic acid]/ solvent B [acetonitrile + 0.05% formic acid]. The linear gradient programme: 0 min 30% B, 2 min 30% B, 7 min 50% B, 11 min 100% B, 14 min 100% B, 15 min 30%B, 25 min 30%B. Sample injection volumes were typically 10 μL. MS tuning and optimization were achieved by infusing microcystin-RR and monitoring the $[M+2H]^{2+}$ ion at m/z 520. MS conditions were as follows: ESI spray voltage 4.54 kV, sheath gas flow rate 20 unit, auxiliary gas flow rate 0 unit, capillary voltage 3.36 V, capillary temperature 250 °C, and multiplier voltage −853.19 V. Tube lens offset, 55 V. Data acquisition was in the positive ionization centroid mode with full mass mode at a mass range between 400 and 1400.

2.4. Transmission electron microscopy

Specimens of liver and kidney 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. Ultrathin 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.

2.5. Biochemical analyses

Tissue samples were homogenized (1:10, w/v) in a cold (4 °C) buffer solution containing tris base (20 mM), EDTA (1 mM), dithiothreitol (1 mM; Sigma), sucrose (0.5 mM) and KCl (150 mM) and phenylmethylsulfonyl fluoride (1 mM; Sigma), with pH adjusted to 7.6. Homogenates were centrifuged at 9500g (4 °C) for 20 min and the supernatants were used as enzyme source. CAT activity was determined spectrophotometrically by measuring the disappearance rate of hydrogen peroxide (H_2O_2) at 240 nm according to the method of Aebi (1984) and expressed as μmoles H_2O_2/min/mg protein. SOD activity assay was based on the method described by Bayer and Fridovich (1987). One SOD unit was defined as 50% inhibition of the nitroblue tetrazolium (NBT) photoreduction to blue formazan and expressed as specific activity (units/mg protein). GPX activity was measured as described by Drotar et al. (1985), using GSH as substrate, and expressed in nmoles/min/mg protein. GST activity was detected using the method of Habig et al. (1974) by evaluating the conjugation of GSH (1 mM, Sigma) with the standard model substrate 1-chloro-2,4-dinitrobenzene (CDNB) (1 mM, Sigma) and expressed in nmoles/min/mg protein. GSH concentration was measured by the method of Griffith (1980) and expressed as μg/mg protein. Protein contents were determined by the Coomassie blue method (Bradford, 1976) using bovine serum albumin as a standard. All the experiments were carried out in triplicate.

2.6. Stastistical analyses

Statistical analysis was undertaken using SPSS 11.5 for Windows. Values were expressed as mean ± SD. The data were tested for normality and homogeneity, and then analyzed by multifactorial analysis of variance (MANOVA) to determine statistical differences among different periods regarding biochemical parameters (CAT, SOD, GPX, GST, and GSH). Differences were considered to be significant at level $P<0.05$. A Pearson's linear correlation has been used to present the relationship between antioxidant components ($n = 12$, $^*P>0.05$, $^{**}P>0.01$).

3. Results

3.1. Physical parameters and phytoplankton biomass in fish pen

According to occurrence and disappearance of *Microcystis* blooms in fish pen, we divided the study period into three phases before, during, and after *Microcystis* blooms (Table 1). The biomass of total phytoplankton, cyanobacteria and *Microcystis* in the fish pen during *Microcystis* blooms were obviously higher than those before and after *Microcystis* blooms. During July to October, 52–89% of the total algal biomass was from *Microcystis aeruginosa*: water temperature increased progressively, while dissolved oxygen content decreased slightly during *Microcystis*

Table 1
The mean values of the physical parameters and the phytoplankton biomass in the fish pen before, during, and after *Microcystis* blooms

Parameters	Before *Microcystis* blooms (2004.4–2004.5)	During *Microcystis* blooms (2004.6–2004.10)	After *Microcystis* blooms (2004.11–2005.3)
Total phytoplankton biomass (mg/L)	7.44±2.46	21.90±20.87	2.90±1.84
Cyanobacterial biomass (mg/L)	0.18±0.23	17.62±19.81	0.26±0.40
Microcystis biomass (mg/L)	0	16.99±19.21	0.21±0.40
Temperature (°C)	21.2±4.36	26.87±6.56	8.57±4.47
Dissolved oxygen (mg/L)	8.09±0.69	7.67±0.80	9.04±2.59
PH	8.01±0.62	8.77±0.95	8.17±0.17

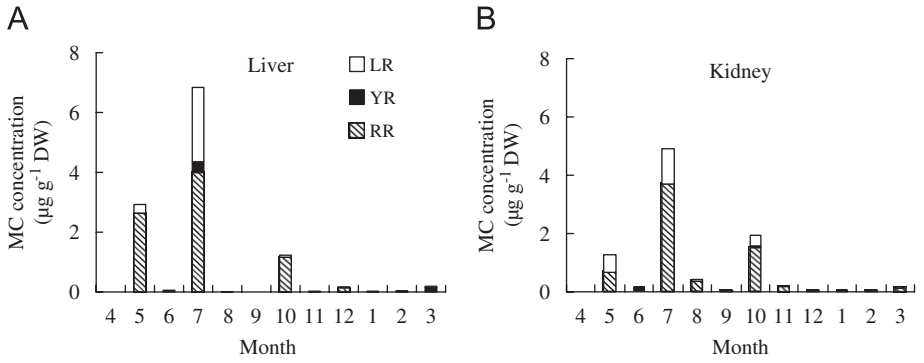

Fig. 2. The monthly changes of MC concentrations in the liver and kidney of silver carp collected from the fish pen in Meiliang Bay of Taihu Lake during April 2004 and March 2005.

blooms. During the whole study period, pH was maintained stably.

3.2. Intracellular MC in the water column and MC concentration in liver and kidney of silver carp

During the study period, intracellular MC in the water column reached the maximum 15.58 $\mu g\,L^{-1}$ in July 2004 and then decreased progressively. Intracellular MC concentration averaged 4.16 $\mu g\,L^{-1}$ with a range of 0–15.58 $\mu g\,L^{-1}$.

During the study period, there were similarly temporal variations in MC (MC-LR + MC-YR + MC-RR) contents in liver and kidney of silver carp, and both liver and kidney had its maximum MC contents in July (6.84 and 4.88 $\mu g\,g^{-1}$ DW, respectively) (Fig. 2). MC in liver varied between 0 and 6.84 with an annual average of 0.957 $\mu g\,g^{-1}$ DW. In kidney, MC contents were between 0.004 and 4.88 $\mu g\,g^{-1}$ DW with an annual average of 0.782 $\mu g\,g^{-1}$ DW. However, there was no significant correlation between intracellular MC in the water column and MC contents in liver or kidney of silver carp.

3.3. Ultrastructural observations

Under transmission microscope, the liver of silver carp showed augmentation of lipid droplets with decrease of cellular organelles and presence of extremely large membrane-bound vacuole containing granular material, proliferation of lysosomes and formation of many myeloid-like bodies in July (Fig. 3A and B), but in December, we observed recovery of hepatocytes, extensive organelle-containing portions of cytoplasm around the centrally located nucleus and big fields of glycogen at the edge of hepatocytes with abundant mitochondria and stacks of endoplasmic reticulum in December (Fig. 3C and D). Ultrastructural changes in kidney in July was characterized by a dilation of Bowman's capsule, partial inosculation of foot processes of epithelial cell (Fig. 4A and B), and increase and proliferation of lysosomes (Fig. 4C), while in December, a normal subcellular structure of

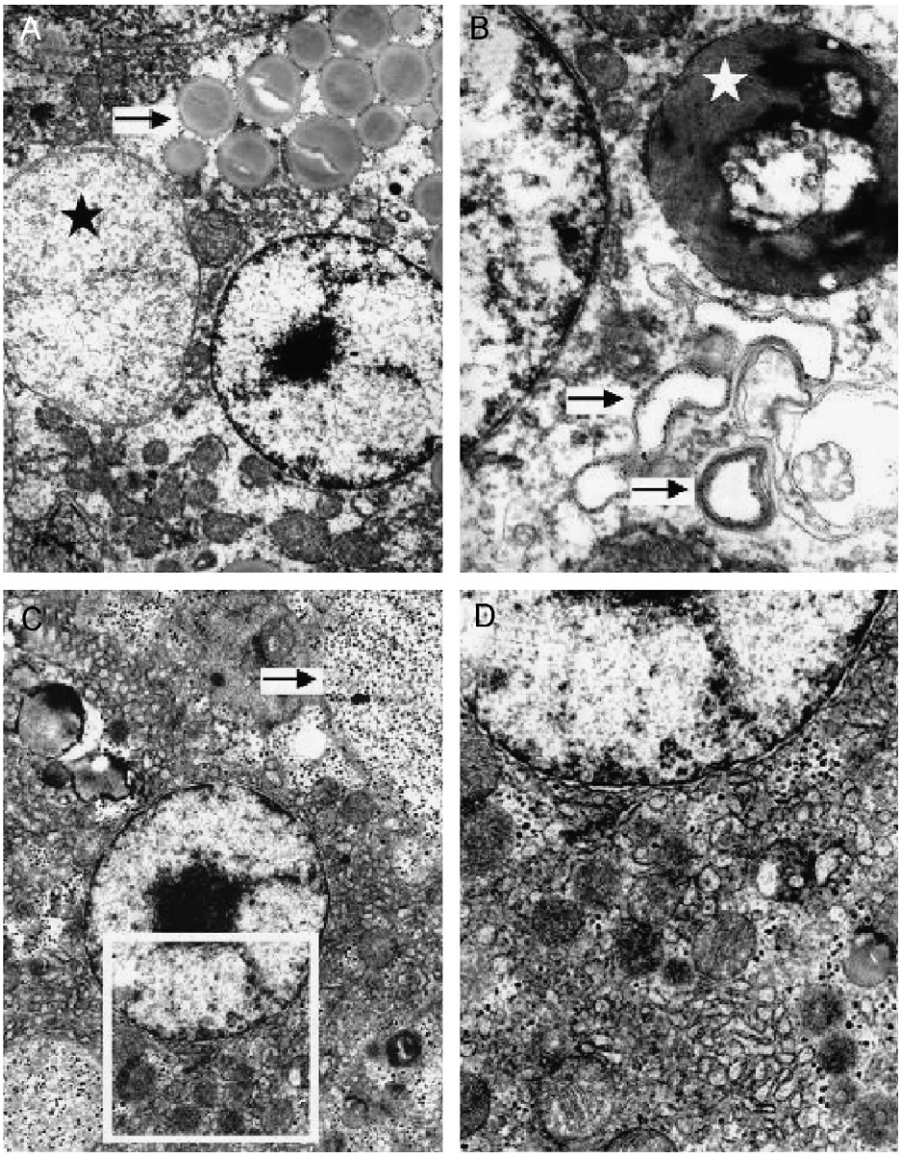

Fig. 3. Transmission electron micrograph of liver of silver carp collected from a large pen in Meiliang Bay of Taihu Lake during July (A and B) and December (D and E), 2004. (A) shows augmentation of lipid droplets (arrow) with decrease of cellular organelles and presence of extremely large membrane-bound vacuole containing granular material (black asterisk), 8000 ×. (B) shows proliferation of lysosomal elements (white asterisk) and formation of many myeloid-like bodies (arrows), 15,000 ×. (C) shows recovery of hepatocytes, extensive organelle-containing portions of cytoplasm around the centrally located nucleus and big fields of glycogen at the edge of hepatocytes (arrow), 6000 ×. (D) the magnification of box in Fig. 3C, shows abundant mitochondria and endoplasmic reticulum, 20,000 ×.

kidney was observed including foot processes in epithelial cell of glomeruli and elliptical nuclear and rich mitochondria in proximal tubular cells (Fig. 4D and E).

3.4. Biochemical analysis

During the study period, there were great temporal variations in the activities of antioxidant enzymes CAT, SOD, GPX, GST, and the concentration of GSH in liver and kidney of silver carp (Fig. 5). From June to October when toxic *Microcystis* blooms occurred, silver carp showed higher activities of CAT, SOD, and GST and lower concentrations of GSH than before or after *Microcystis* blooms in both liver and kidney. The GPX activity in liver reached the maximum in June 2004 and then decreased progressively. In contrast, GPX

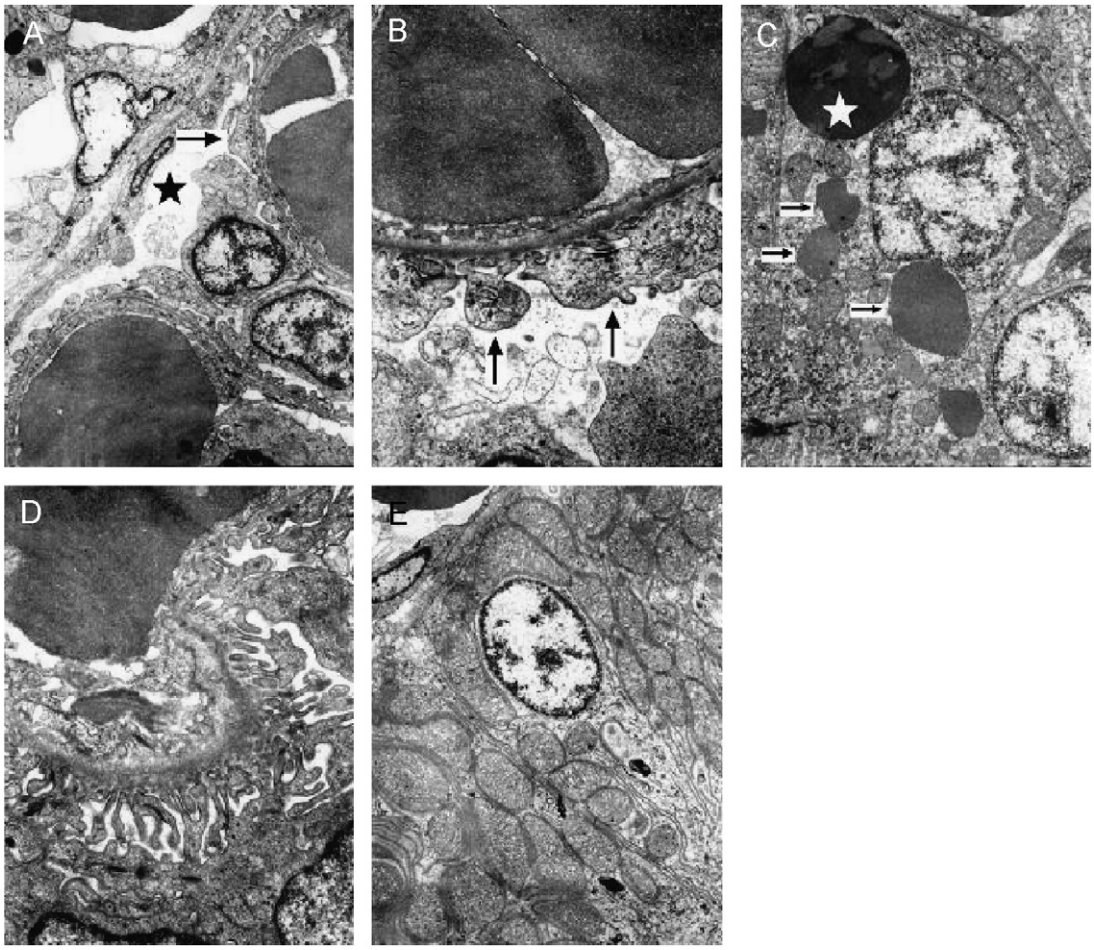

Fig. 4. Transmission electron micrograph of the kidney of silver carp collected from fish pen in Meiliang Bay of Taihu Lake during July (A and B) and December (C and D), 2004. (A) shows dilation of Bowman's space in the glomeruli (black asterisk), 6000 ×; (B) inosculation of foot processes in epithelial cell of glomeruli (arrows), 10,000 ×; (C) increase (arrows) and proliferation of lysosomes (white asterisk) in the proximal tubules, 6000 ×; (D) normal morphology of foot processes in epithelial cell of glomeruli, 10,000 ×; (E) healthy tubules with normal epithelial cells, 6000 ×.

in kidney showed a complex fluctuation during the study period.

Significant differences in activities of CAT and GST in liver and kidney were observed in before, during, and after *Microcystis* blooms (Table 2). On the other hand, the activity of the antioxidant enzymes was strongly tissue dependent: biochemical parameters (CAT, GST, and GSH) had higher activities in liver than in kidney ($P<0.001$ in CAT, $P<0.01$ in GST and GSH). And the GPX activity in kidney was significantly higher than in liver ($P<0.001$). No statistical difference in SOD activity was found between liver and kidney.

Correlation analyses between antioxidant component activities in liver and kidney of silver carp during the study period are presented in Table 3. Significantly positive correlations between CAT and GST, SOD and GPX were found in liver and kidney. In kidney, there was also significantly positive correlation between CAT and GPX.

4. Discussion

In the present study, *M. aeruginosa* dominated the phytoplankton communities in Meiliang Bay of Taihu Lake during the warmer seasons (from June to October). Meanwhile, our microscopic examinations found that the major phytoplankton species in the gut content of silver carp were similar with those in the lake water, with *Ulothrix* from February to May, *Microcystis* from June to October, and *Melosira*, *Cyclotella* and *Microcystis*

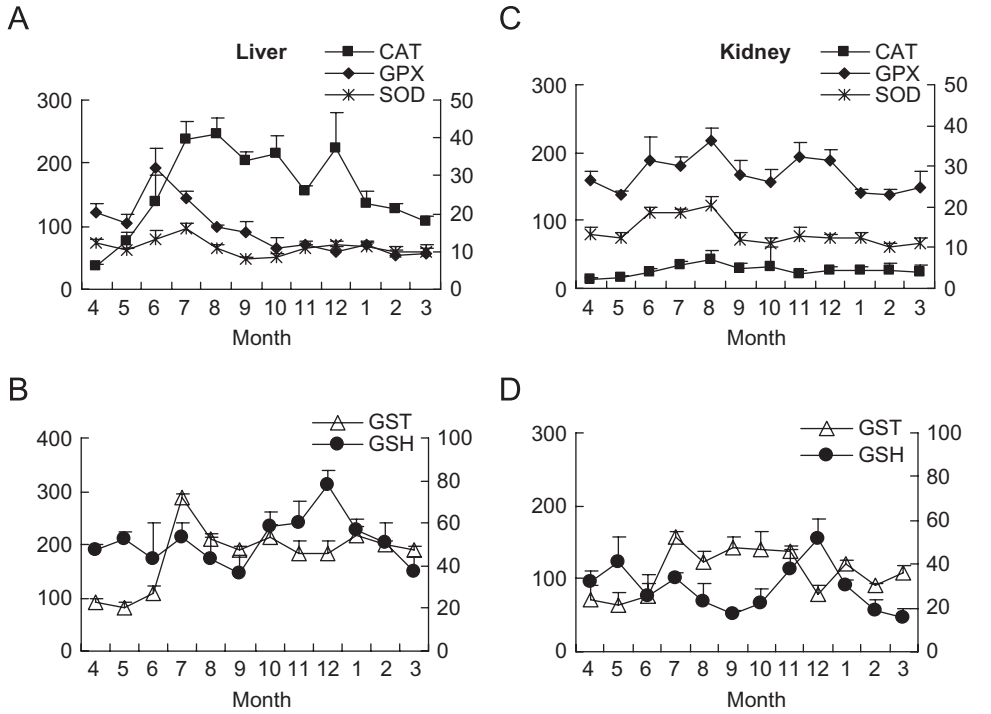

Fig. 5. Seasonal variations of the activities of CAT (μmoles/min/mg protein), SOD (U/mg protein), GPX (nmoles/min/mg protein) and GST (nmoles/min/mg protein) and GSH concentrations (μg/mg protein) in liver (A and B) and kidney (C and D) of silver carp collected from a fish pen in Meiliang Bay of Taihu Lake during April 2004 and March 2005. Results were expressed mean ± SD ($n = 5$).

Table 2
The activities of antioxidant enzymes CAT, SOD, GPX, GST, and GSH levels in the liver and kidney of silver carp collected from a fish pen in Meiliang Bay of Taihu Lake during three periods (before, during, and after *Microcystis* blooms)

		CAT	SOD	GPX	GST	GSH
Liver	Before *Microcystis* blooms (2004.4–5)	56.1 ± 28.9^{a}	11.4 ± 1.4^{a}	113.0 ± 11.1^{ab}	86.6 ± 6.1^{a}	50.0 ± 3.5^{a}
	During *Microcystis* blooms (2004.6–10)	208.5 ± 42.0^{b}	11.0 ± 3.4^{a}	117.9 ± 51.5^{a}	202.2 ± 64.1^{b}	46.9 ± 8.6^{a}
	After *Microcystis* blooms (2004.11–2005.3)	150.2 ± 45.1^{ab}	10.7 ± 0.7^{a}	62.1 ± 8.3^{b}	195.0 ± 13.7^{b}	56.6 ± 14.9^{a}
Kidney	Before *Microcystis* blooms (2004.4–5)	15.4 ± 1.5^{a}	12.9 ± 0.7^{a}	149.4 ± 14.3^{a}	68.5 ± 5.5^{a}	36.6 ± 6.1^{a}
	During *Microcystis* blooms (2004.6–10)	32.8 ± 6.9^{b}	16.1 ± 4.3^{a}	182.5 ± 22.5^{a}	128.0 ± 31.6^{b}	24.5 ± 6.1^{a}
	After *Microcystis* blooms (2004.11–2005.3)	25.1 ± 3.0^{ab}	11.7 ± 1.1^{a}	161.8 ± 24.3^{a}	107.0 ± 23.7^{ab}	30.9 ± 4.6^{a}
ANOVA effects		(B) $P < 0.01$	(B)N.S	(B) $P < 0.05$	(B) $P < 0.01$	(B) NS
		(T) $P < 0.001$	(T) N.S.	(T) $P < 0.001$	(T) $P < 0.01$	(T) $P < 0.01$
		B*T $P < 0.01$	B*T N.S.	B*T N.S.	B*T N.S.	B*T N.S.

Results are expressed as mean ± SD. Number of replicates for each group was 25, except for the period before *Microcystis* blooms ($n = 10$). The statistical significance was analyzed by two-way ANOVA with blooms (B) and tissue (T) as factors. Post hoc comparison for the effect of cyanobacteria blooms: Bonferroni's test, $P < 0.05$. Similar letters mean absence of statistical differences ($P > 0.05$).

from November to January 2005. On average, Cyanophyta, Chlorophyta, Bacillariophyta, other phytoplankton constituted, respectively, 52.6%, 38.3%, 7% and 2.1% of phytoplankton contents in the guts of silver carp. In July and August, more than 80% of the phytoplanktons in the gut contents of silver carp were from *Microcystis aeruginosa*, indicating massive ingestion of *Microcystis* (unpublished data of Dr. Ke ZX). Thus, the ultrastructural lesions in liver and kidney of silver carp during *Microcystis* blooms are likely due to intestinal absorption of MC following ingestion of *Microcystis* cells and MC accumulation in both organs.

Table 3
Correlation between biochemical parameters in the liver (below diagonal) and kidney (above diagonal) of silver carp collected from a fish pen in Meiliang Bay of Taihu Lake before, during, and after *Microcystis* blooms

	CAT	SOD	GPX	GST	GSH
CAT		0.49	0.64*	0.60*	−0.46
SOD	0.08		0.77**	0.11	0.10
GPX	−0.05	0.63*		0.21	0.12
GST	0.74**	0.17	−0.29		−0.47
GSH	0.11	0.41	0.06	−0.07	

Statistical significance: $^{*}P<0.05$, $^{**}P<0.01$.

In the present study, silver carp displayed only slight pathological changes in spite of higher accumulation of MC in their livers during *Microcystis* blooms. These ultrapathological changes included an increase of lipid droplets in both number and size, proliferation of lysosomes and vacuolation of cytoplasm. So far, there have been a few studies describing histopathological changes of fish liver by oral uptake of MC (Carbis et al., 1996; Fischer and Dietrich, 2000; Li et al., 2004), but only one study examined ultrastructural changes of hepatocytes (Li et al., 2004). When common carp were fed with bloom scum at a dose of 50 μg MC-LR kg^{-1} body weight (BW) for 28 days, they displayed ultrastructural lesions such as a widespread swelling and vacuolization of the hepatocytic endomembrane system (mainly consisting of endoplasmic reticulum (ER), mitochondria and Golgi body) (Li et al., 2004). In comparison with these pathological changes in common carp where the severe injury possibly caused hepatocytic necrosis (Li et al., 2004), there were no obvious changes in mitochondria, rough ER and Golgi body of the hepatocytes of silver carp in this study. In the present study, lysosomal proliferation occurred in hepatocytes of silver carp during *Microcystis* blooms, which agrees with previous in vitro studies on fish cell lines (RTG-2 and PHLC-1), where there was a very potent concentration-dependent stimulation of the lysosomal function by MC-LR (Pichardo et al., 2005), probably related to cytoskeletal modifications and the induction of oxidative stress (Ding and Ong, 2003; Moreno et al., 2005). Braunbeck (1998) reported proliferation of lysosomes in the hepatocytes of rainbow trout following *in vivo* and *in vitro* sublethal exposure to xenobiotics (e.g. 4-chloroaniline), which indicates a general adaptation to compensate for increased turnover of cellular components under conditions of toxicant-induced stress. Therefore, the proliferation of lysosomes in our study may indicate an adaptive mechanism of silver carp to eliminate or lessen cell damage caused by MC through lysosome activation.

When common carp were fed with bloom scum at a dose of 50 μg MC-LR kg^{-1} BW for 28 days, they accumulated 261.0 ± 108.3 ng MC-LReq/g fresh hepatopancreas (Li et al., 2004). In the present study, a coefficient of 5 was used to convert dry weight to wet weight (WW), and since i.p. LD_{50} in mice for MC-RR and MC-YR are about 5 times and 2.5 times higher than that for MC-LR, respectively (Gupta et al., 2003), coefficients of 0.2 and 0.4 were used to convert MC-RR and MC-YR into the MC-LR equivalent, respectively. In the present study, silver carp fed on *Microcystis* blooms for over five months and accumulated 2 μg MC-LReq/g WW in liver (annual average) with a maximum of 17.15 μg MC-LReq/g WW in July 2004, which was 65 times higher than that in liver of common carp in the study of Li et al. (2004). Apparently, in comparison to common carp, silver carp accumulated higher MC in liver but displayed less pathological changes.

In the present study, a substantial amount of MC also accumulated in the kidney of silver carp, suggesting the possibility of chronic toxicosis. Kidney tubular cells may possess a transport mechanism similar to that of the hepatocytes (the multispecific bile acid transport system), which is responsible for the uptake of the toxin into the cell (Suchy, 1993). In mice, renal damages are seldom observed and this can be explained by the short survival time (1–2 h) to lethal dose (Kotak et al., 1993). In acute experiments of fish, MC induces dilation of Bowman's capsule in glomeruli and necrotic tubular cells with pycnotic nuclei (Råbergh et al., 1991; Kotak et al., 1996). In our study, kidney lesions were indicated by dilation of Bowman's capsule, partial inosculation of foot processes of epithelial cell and proliferation of lysosomes in proximal tubular cells. To our knowledge, this is the first study to examine ultrastructural changes of fish kidney by MC exposure through natural food uptake and to describe the chronic nephrotoxic damages characterized by inosculation of foot processes of epithelial cell in glomeruli and the proliferation of lysosomes.

In the present study, following a decrease or even disappearance of MC in both organs after *Microcystis* blooms, ultrastructural recovery of liver and kidney was observed. This indicates that the toxic effects of MC on liver and kidney of silver carp were reversible. Similarly, the activities of the antioxidant enzymes CAT, SOD, GPX, and GST in liver and kidney of silver carp showed temporal variations with an increase during the period of *Microcystis* blooms followed by a decrease after *Microcystis* blooms. In the present study, activities of CAT and GST in liver of silver carp were significantly higher during *Micorcystis* blooms than before or after *Microcystis* blooms, and there were also significantly positive correlations between CAT and GST, and between SOD and GPX in both liver and kidney. It appears that MC-induced-oxidative stress activated the antioxidant enzymes, which probably cooperated to eliminate ROS so as to protect cells from lesions. Similar results are also observed in the liver of loach (*Misgurnus mizolepis*) that were orally exposed to a low dose of *Microcystis* (equal to 10 μg MC-RR/kg BW) through dietary supplementation with bloom scum and in liver, kidney, and gill tissues of tilapia (*Oreochromis* sp.) exposed during 21 d to cyanobacterial cells mixed with their food (Li et al., 2005; Jos et al., 2005). On the other hand, the enhanced GST activities during the period of *Microcystis* blooms in our study could be due to enhanced biotransformation reactions and higher rates of MC conjugation, suggesting the importance of MC detoxification by GSH pathway. Pflugmacher et al. (1998) showed the existence of the conjugation of microcystin with GSH, in a phase II reaction catalyzed by GST, in several aquatic organisms like plants (*Ceratophyllum demersum*), invertebrates (*Dreissena polymorpha* and *Daphnia magna*) and fish (*Danio rerio*). Augmented GST activity has been utilized to point out MC detoxification in various aquatic organisms ranging from microalgae, plants, invertebrates to fish (Wiegand et al., 1999; Beattie et al., 2003; Pflugmacher, 2004; Pinho et al., 2005).

The GSH content is a critical factor for preserving normal cellular redox balance and protecting cells against oxidative stress (Ding and Ong, 2003). GSH depletion or formation of the glutathione disulfide by exposure to MC limits the ability of an organism to detoxify MC or prevent oxidative damage (Gehringer, 2003; Peuthert and Wiegand, 2004). In an *in vitro* experiment, acute depletion of GSH was observed in common carp hepatocytes exposed to high concentrations of MC-LR (10 μg MC-LR/L) with apoptosis and even necrosis of cells (Li et al., 2003). Ding et al. (2000) observed a biphasic change of intracellular GSH concentrations, with a significant increase in the initial stage followed by a decrease after prolonged treatment, suggesting an increase in GSH levels in order to protect cells from the cytotoxicity and cytoskeletal changes. However, the present in vivo study with silver carp was carried out in natural conditions and after a relatively long exposure to *Microcystis* blooms (5 months), no statistically significant difference in GSH concentration was observed between three different periods (before, during, and after *Microcystis* blooms), possibly because of the high basic level of GSH in liver and kidney of silver carp. In the present study, the annual mean concentration of GSH in liver and kidney of silver carp was 51.32 μg/mg protein, more than two times that in the liver of the goldfish *Carassius auratus* (22.16 ± 1.60 μg/mg protein) (Liu et al., 2004). When goldfish were injected i.p. with purified MC-LR (125 μg/kg BW), no decrease in GSH levels was observed, which was explained by the important basic GSH concentration in goldfish liver (Malbrouck et al., 2004). Thus, the high GSH pool in liver and kidney of silver carp in our study suggests high resistance to MC exposure. The phytoplanktivorous silver carp has strong resistance to toxic *Microcystis* blooms, probably due to their efficient antioxidant defence mechanisms, and can be used as a biomanipulation fish to counteract toxic *Microcystis* blooms.

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