ELSEVIER

In situ studies on the distribution patterns and dynamics of microcystins in a biomanipulation fish − bighead carp (*Aristichthys nobilis*)

Jun Chen, Ping Xie*, Dawen Zhang, Hehua Lei

Donghu Experimental Station of Lake Ecosystems, State Key Laboratory of Freshwater Ecology and Biotechnology of China, Institute of Hydrobiology, Chinese Academy of Sciences, Donghu South Road 7, Wuhan, Hubei 430072, PR China

Bighead carp is resistant to microcystins and can be used as biomanipulation fish to counteract cyanotoxin contamination.

Abstract

The distribution and dynamics of microcystins in various organs of the phytoplanktivorous bighead carp were studied monthly in Lake Taihu, which is dominated by toxic cyanobacteria. There was a good agreement between LC-MS and HPLC-UV determinations. Average recoveries of spiked fish samples were 63% for MC-RR and 71% for MC-LR. The highest MC contents in intestine, liver, kidney and spleen were 85.67, 2.83, 1.70 and 1.57 $\mu g\ g^{-1}$ DW, respectively. MCs were much higher in mid-gut walls (1.22 $\mu g\ g^{-1}$ DW) than in hind- and fore-gut walls (0.31 and 0.18 $\mu g\ g^{-1}$ DW, respectively), suggesting the importance of mid-gut wall as major site for MC absorption. A cysteine conjugate of MC-LR was detected frequently in kidney. Among the muscle samples analyzed, 25% were above the provisional tolerable daily intake level by WHO. Bighead is strongly resistant to microcystins and can be used as biomanipulation fish to counteract cyanotoxin contamination in eutrophic waters.
© 2006 Elsevier Ltd. All rights reserved.

1. Introduction

Toxic cyanobacterial blooms occur worldwide in eutrophic lakes, rivers and reservoirs (Paerl et al., 2001; Havens, 2003; Havens et al., 2003). Among cyanotoxins, microcystins (MCs) are considered to be one of the most dangerous groups, which are known to be potent hepatotoxin (Codd, 1995; Dawson, 1998) and tumor promoter (Nishiwaki-Matsushima et al., 1991, 1992). Exposure to MCs represents a health risk to aquatic organisms, wild life, domestic animals, and humans upon drinking or ingesting algae in the water (Carmichael, 1996). No case of human deaths caused by oral consumption of cyanobacteria toxins has yet been documented, whereas chronic toxic effects from exposure through food need to be considered, especially if there is long- term frequent exposure.

In the natural environment, MCs are found to accumulate in a wide range of aquatic animals such as fish (Magalhàes et al., 2001; Mohamed et al., 2003), shrimps (Chen and Xie, 2005a), gastropods (Zurawell et al., 1999; Chen et al., 2005), and bivalves (Williams et al., 1997; Chen and Xie, 2005b), and the toxins are present not only in the viscera but also in the edible muscle/foot. This means that consumption of such aquatic animals containing microcystins may pose a risk to human health. There have been extensive studies on MC bioaccumulation in fishes (Råbergh et al., 1991; Sahin et al., 1996; Williams et al., 1997; Pflugmacher et al., 1998; Wiegand et al., 1999; Lawrence and Menard, 2001; Magalhàes et al., 2001, 2003; Malbrouck et al., 2003; Soares et al., 2004; Li et al., 2005; Xie et al., 2004, 2005). However, most of these studies were performed in laboratory.

---

* Corresponding author. Tel./fax: +86 27 6878 0622.
  E-mail address: xieping@ihb.ac.cn (P. Xie).

The phytoplanktivorous fishes are of economic importance to humans because of their importance as food fish and their potential for biological management of cyanobacterial blooms (Opuszynski and Shireman, 1995; Xie and Liu, 2001). Among various groups of fishes, they are most frequently exposed to toxic cyanobacterial blooms, and therefore are at high risk of accumulating microcystins. Up to now, there has been only one field study to report organ distribution and bioaccumulation of MCs in phytoplanktivorous fish (silver carp *Hypophthalmichthys molitrix*, from Lake Chaohu in September 2003) (Xie et al., 2005).

Bighead carp (*Aristichthys nobilis*), one of the most important freshwater phytoplanktivorous fish, comprises not only much of the production of Chinese aquaculture (Liang, 1981; Tang, 1981) but also a substantial proportion (e.g. 6% in 1989) of the total world catch in inland waters (FAO, 1991). Bighead carp is suggested to be able to suppress and graze out toxic *Microcystis aeruginosa* blooms in non-traditional biomanipulation (Xie and Liu, 2001).

The main purposes of this study are: 1) to examine the distribution patterns and the seasonal dynamics of three common microcystins (MC-LR, -RR and -YR) in various organs (intestine, liver, kidney, spleen, gallbladder, gill, blood and muscle) of bighead carp cultured in a large net cage in the Meiliang Bay of Lake Taihu where dense toxic cyanobacterial blooms occurred; and 2) to discuss the possible mechanisms underlying these patterns with emphasis on how bighead is resistant to MCs. Comments are also given on the potential risk to human health when this fish are consumed.

2. Materials and methods

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 surface 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., 2004a). 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 part of Lake Taihu, accommodates municipal and industry wastewater from Wuxi City, and acts as principal water source for the city. Meiliang Bay is the most eutrophic part of the lake (Table 1), characteristic of extremely dense accumulation of toxic *Microcystis* blooms by wind in the summer (Cai et al., 1997; Qin et al., 2004b).

To test the applicability of using phytoplanktivorous fishes to counteract toxic cyanobacteria, two phytoplanktivorous fishes, silver carp and bighead

Table 1
Physical-chemical parameters of the lake water in Meiliang Bay of Lake Taihu during April 2004 and March 2005 (unpublished data of Zhou Q and Ke ZX)

Parameters	Mean ± S.E.	Range
T (°C)	17.8 ± 9.8	3.0−33.6
pH	8.3 ± 0.66	7.6−10.1
Chl-a (mg/L)	36.6 ± 65.2	3.4−251.7
TP (mg/L)	0.22 ± 0.15	0.05−0.59
TN (mg/L)	5.48 ± 2.13	3.27−10.36

carp, were stocked in a large net cage (1.088 km^2) in the Meiliang Bay of Lake Taihu during April 2004 and March 2005.

During the study period, bighead carp were collected monthly from Meiliang Bay and on each sampling date, a total of 20 fish were sampled for the measurements of total length and body weight. After sampling the blood (by cutting the tail), we dissected the fish into seven parts: intestine, liver, kidney, spleen, gallbladder, gill and muscle. The collected organs were immediately frozen at −20 °C in a field research station. In the laboratory, the collected organs were frozen at −80 °C prior to microcystin analysis. For the analyses of fish tissues, resources did not allow analysis of individual replicate fish. Therefore, we pooled, respectively, all intestine, liver, kidney, spleen, gallbladder, gill, blood and muscle of five dissected fish (from Apr. 2004 to Mar. 2005). Thus, each value represents an average amount of MCs in the organs of five individuals. In addition, during September 2004 and March 2005, we divided the intestine into six parts: foregut wall, mid-gut wall, hindgut wall, foregut contents, mid-gut contents and hindgut contents.

Extraction and analysis of the MCs in the organs (≈0.5 g lyophilized sample for each organ) of the study animals basically followed the method of Chen and Xie (2005a): the toxin-containing fraction was subjected to a HPLC equipped with an ODS column (Cosmosil 5C18-AR, 4.6 × 150 mm, Nacalai, Japan) and a SPD-10A UV-vis spectrophotometer set at 238 nm. MC concentrations were determined by comparing the peak areas of the test samples with those of the standards available (MC-LR, MC-YR and MC-RR, Wako Pure Chemical Industries-Japan). The limit of detection and quantification for the MCs were 0.02 and 0.07 μg/g, respectively.

Qualitative and quantitative analysis of MCs were also 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). 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 was 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. MS detection was operated in four segments: (1) full scan mode with a mass range between 400 and 1400, 4.2 min; (2) two scan events: full scan mode as same as segment 1 and MS^2 mode with a mass range between 140 and 1100, parent ion: 520; isolation width: 1; normalized collision energy: 37%; 4.8 min; (3) three scan events: full scan mode as same as segment 1 and MS^2 mode with mass range between 270 ∼ 1100 and 285 ∼ 1100, respectively; parent ion: 995.5 and 1045.5, respectively; isolation width: equal for both, 1; normalized collision energy: equal for both, 35%; 4.8 min; and (4) full scan mode as same as segment 1 in the rest time. All the values present in the text were measured by ESI-LC/MS^2.

Recovery experiments were performed in quintuplicate spiking 500 mg of homogenized freeze-dried fish samples (liver, kidney and muscle) with mixed MCs solution of the two commercial standards (MC-RR and MC-LR) at 2.5 μg g^{-1}. The extraction was performed as described previously, and the recovery and the relative standard deviation of the analytical method were calculated.

3. Results

Seasonal changes in water temperature, total body length and body weight of bighead carp are shown in Fig. 1. During the study period, water temperature varied between 3.2 (January) and 33.4 (August) °C. Bighead carp weighed 264 (±24) g (25.5 ± 4.7 cm in total length) at stocking (in April), and
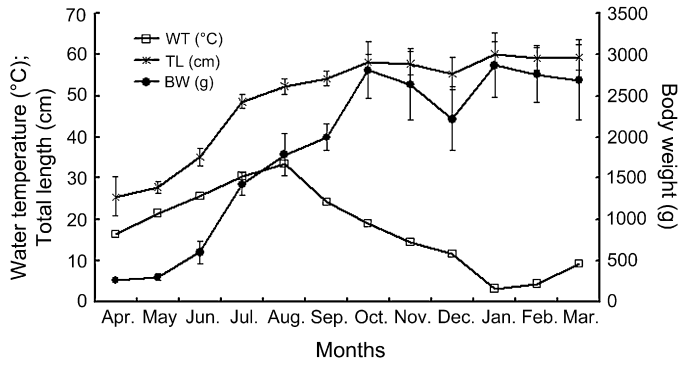
Fig. 1. Annual changes of water temperature (WT), and total length (TL) and body weight (BW) of bighead carp collected from a large net cage in the Mei-liang Bay of Lake Taihu.

increased to 2811 ($\pm$345) g (58.1 $\pm$ 2 cm) after a growth period of half year (in October). The increase in body weight of bighead carp was as high as 10.6 times. In the lake water inside the cage, dominant phytoplankton were cyanobacteria (mainly *Microcystis aeruginosa*), green algae, diatoms and *Cryptomonas*, and cyanobacteria comprised 58.2% of total phytoplankton biomass (unpublished data of Liu YQ).

MCs in various organs (liver, gallbladder, spleen, kidney, gill, blood, muscle and intestines) of bighead carp were

determined by LC-ESI-MS and HPLC. Fig. 2 shows an ESI LC/MS^2 measurement of MCs in the liver. Based on total ion chromatogram, mass chromatograms monitored at m/z 520, and the presence of $[M + H]^+$ ion at m/z 452 and 887, it is confirmed that peak obtained at 6.18 min was derived from MC-RR. Similarly, peaks obtained at 11.66 min and 11.96 min were derived from MC-YR and MC-LR, respectively, as the peaks were detected by monitoring with m/z 1045.5 and m/z 995.5, respectively, and the mass chromatogram showed $[M + H]^+$ ion at m/z 1045.5 and 599 for MC-YR and m/z 995.5 and 599 for MC-LR, respectively. In addition, ESI LC/MS revealed that a cysteine conjugate of MC-LR (m/z 1116) was present in both kidney (Fig. 3) and hindgut contents (Fig. 4). It should be pointed out that we monitored all of the samples at m/z 1302 (LR-GSH); 1352 (YR-GSH); 1345 (RR-GSH); 1116 (LR-Cys); 1166 (YR-Cys) and 1159 (RR-Cys), respectively, but LR-Cys was found only in the samples of kidney and hindgut contents.

The monthly changes of MC contents in various organs of bighead carp are showed in Fig. 5. During the study period, there were great temporal variations in MC contents in each organ. The highest content of MCs was found in the intestine (as high as 85.67 μg g^{-1} DW in Nov. 2004), followed by liver (2.83 μg g^{-1}
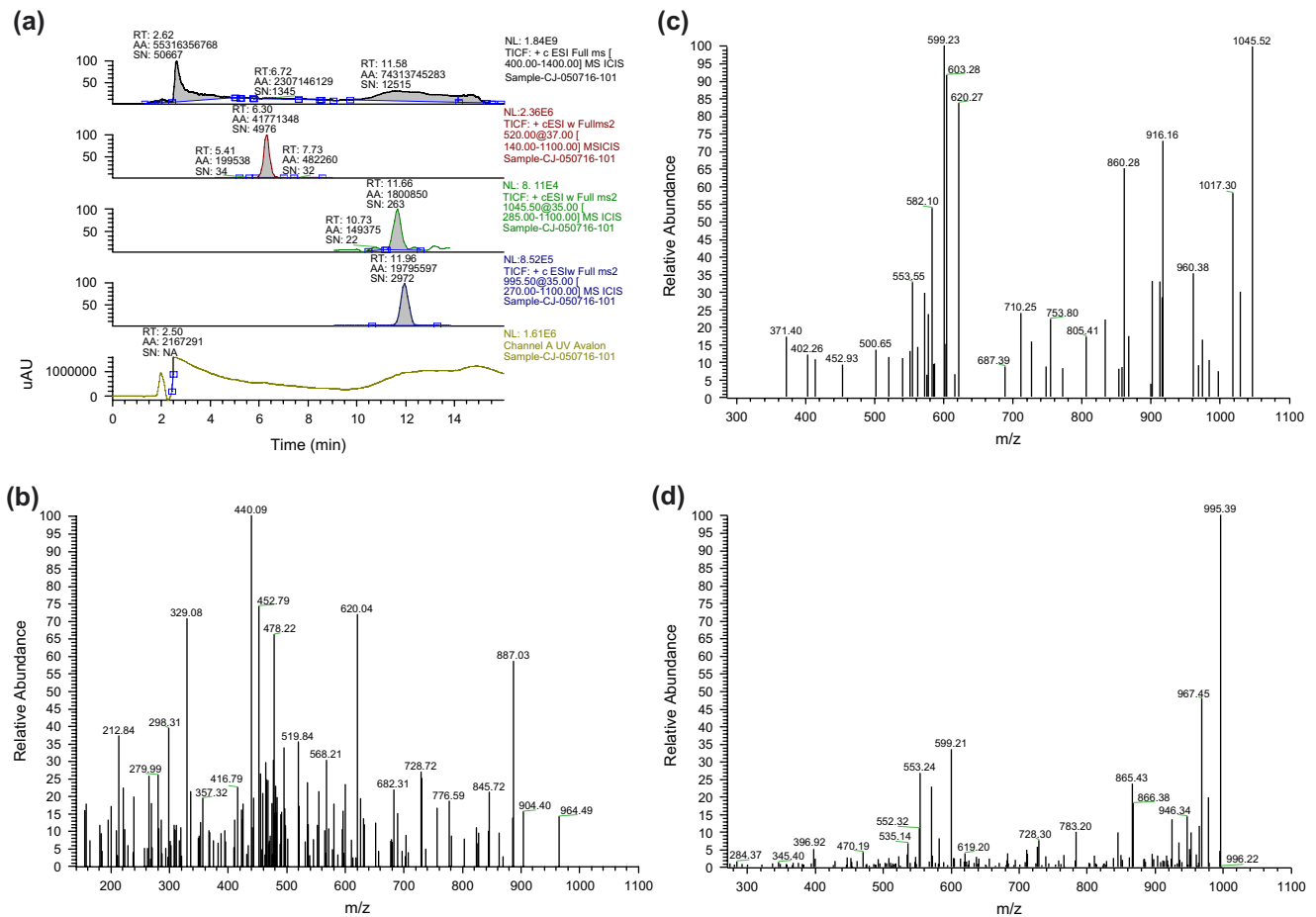

Fig. 2. ESI LC/MS^2 analysis of MCs in the liver of bighead carp (October 2004). Shown are: (a) total ion and mass chromatograms monitored at m/z 520, 1045.5, 995.5; (b) ESI mass spectra for MC-RR; (c) ESI mass spectra for MC-YR; and (d) ESI mass spectra for MC-LR.
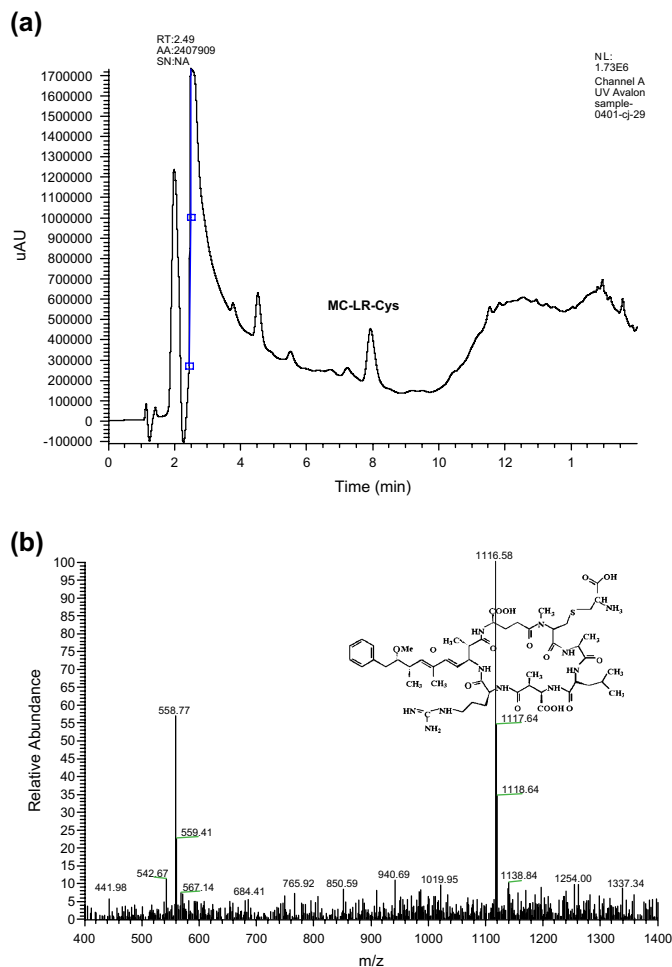
(a)

(b)

Fig. 3. ESI LC/MS analysis of the kidney of bighead carp (August 2004). Shown are: (a) high performance liquid chromatogram monitored at 238 nm; and (b) ESI mass spectra for MC-LR-Cys.
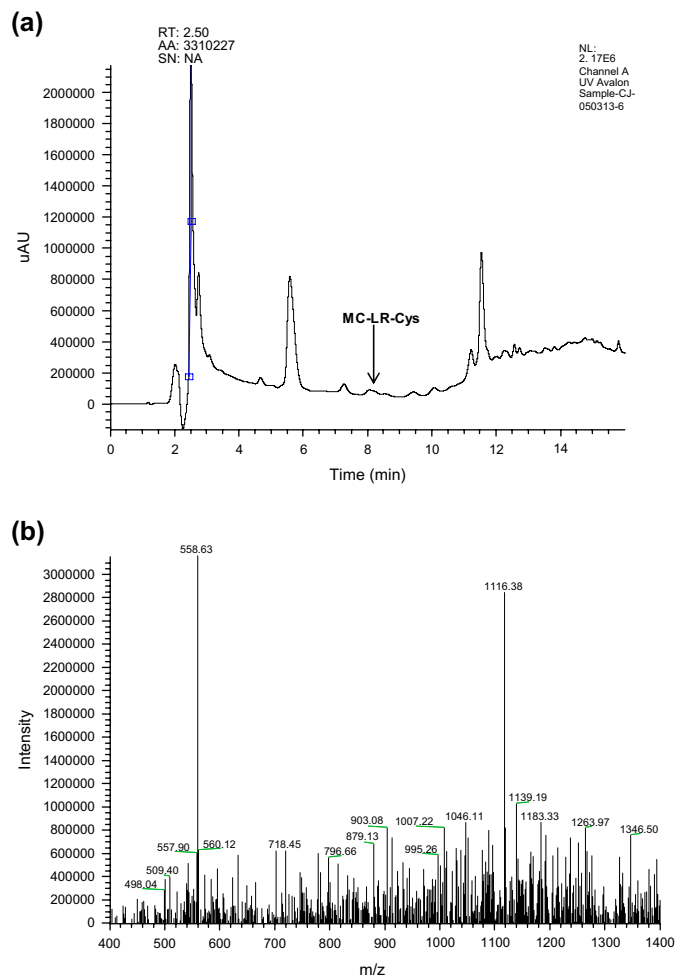
(a)

(b)

Fig. 4. ESI LC/MS analysis of the hindgut contents of bighead carp (Sep. 2004). Shown are: (a) high performance liquid chromatogram monitored at 238 nm; and (b) ESI mass spectra for MC-LR-Cys.

DW in Jul. 2004), kidney (1.70 µg g^{-1} DW in Sep. 2004) and spleen (1.57 µg g^{-1} DW in Sep. 2004), and the annual mean MC content was in the order of intestine > kidney > liver > spleen > gallbladder > muscle > blood > gill (Table 2).

The statistical analysis revealed that there were no significant correlations in MC concentration between the intestine and all the other organs. While, MC content in the liver had a strong correlation with that in the muscle ($r = 0.94$, $P < 0.01$), but there were no significant correlations in MC concentrations between liver and other remaining organs.

There were great variations in MC contents in various parts of the intestines. The highest content of MCs was found in the hindgut contents (mean 124.65, range 0.00–478.28 µg g^{-1} DW; LR/RR 0.37), followed by the mid-gut contents (mean 41.57, range 0.25–103.16 µg g^{-1} DW; LR/RR, 0.05) and foregut contents (mean 16.57, range 0.02–45.39 µg g^{-1} DW; LR/RR, 0.02). Relatively, only small amounts of MCs were detected in the gut walls, the order was mid-gut wall (mean 1.22, range 0.02–5.02 µg g^{-1} DW; LR/RR 0.83) > hindgut wall (mean 0.31, range 0.03–1.44 µg g^{-1} DW; LR/RR 0.84) > foregut wall (mean 0.18, range 0.00–0.62 µg g^{-1} DW; LR/RR 0.20) (Figs. 5c,d). Apparently, the LR/RR ratio

increased significantly from fore- and mid-gut contents to hindgut contents, and from foregut wall to mid- and hindgut walls. There were also great differences in the ratio of LR/RR between gut contents and gut walls in both mid- and hind-guts.

In terms of toxin burden, intestine (89.54%) ranked the first, followed by muscle (7.22%), liver (1.05%) and kidney (0.79%), whereas spleen, gallbladder, gill, blood and the rest of the tissues had altogether less than 1.41% of the total toxin. If intestines are excluded, up to 77.3%, 11.3% and 8.5% of the toxin burden were located in the muscle, liver and kidney, respectively (Table 2).

All samples were analyzed independently by both LC-UV and LC-MS for comparison. The results are shown in Fig. 6 and Table 3, and there was a good agreement between the two methods. This indicates that the less expensive LC-UV method can be used to do routine monitoring of aquatic samples while confirmation of positive samples can be accomplished using LC-MS.

The average recoveries ($n = 15$) from three different parts of fish samples (liver, kidney and muscle) were 63% (range,
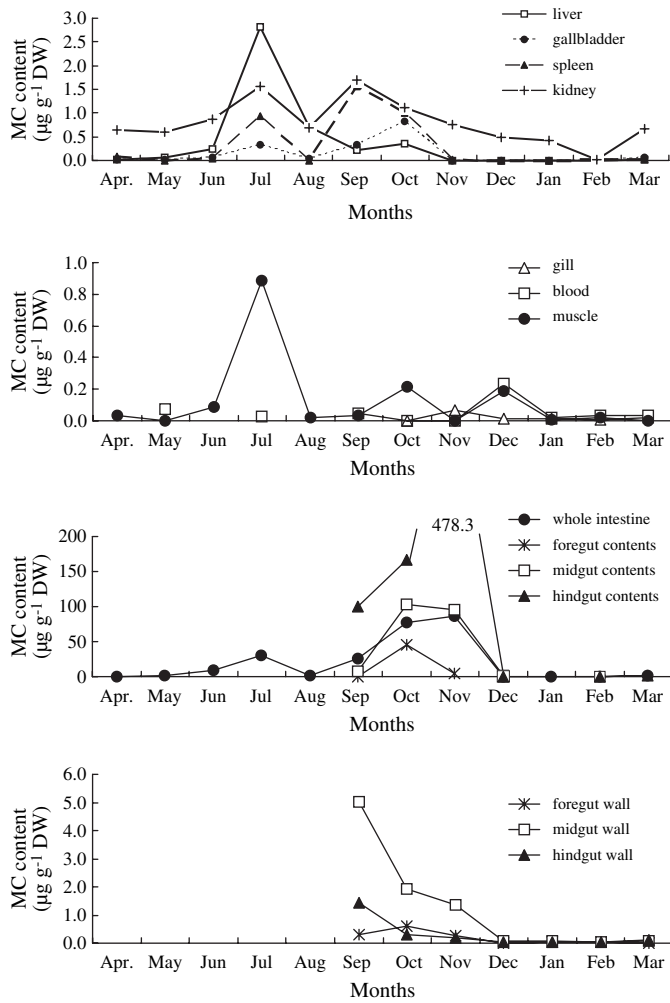
Fig. 5. The seasonal changes of MC concentrations ($\mu g\ g^{-1}$ DW) in (a) liver, gallbladder, spleen, kidney; (b) gill, blood, muscle; (c) whole intestine, foregut contents, mid-gut contents, hindgut contents; and (d) foregut wall, mid-gut wall, hindgut wall of bighead carp collected from a large net cage in the Meiliang Bay of Lake Taihu detected by ESI LC/MS[2].

47−82%) for MC-RR with relative standards deviations (RSDs) between 11 and 16%, and 71% (range, 62−87%) with RSDs between 9 and 11% for MC-LR. Results obtained for liver were better than those obtained for kidney and muscle.

4. Discussion

There have been several experimental and field studies on MC accumulation in phytoplanktivorous fishes. Magalhàes et al. (2001) studied the seasonal changes of MC concentration in the liver, muscle and viscera of the phytoplanktivorous *Tilapia rendalli* in a coastal Lagoon of Brazil. Mohamed et al. (2003) examined MC contents in the gut, liver, kidney and muscle of a tilapia fish (*Oreochromis niloticus*) in June from an Egyptian fish farm containing toxic bloom of *Microcystis aeruginosa*. Xie et al. (2005) examined the MC content in the gut, liver, kidney, bile, muscle, and blood of eight fish species (including the phytoplanktivorous silver carp) in September from a Chinese lake. In a subchronic laboratory experiment, Xie et al. (2004) documented the changes of MCs in the gut, liver, muscle and blood of silver carp fed with fresh toxic *Microcystis* cells. Li et al. (2005) documented the dynamics of MCs in the liver of bighead carp after i.p. injection with MCs equivalent to 200 and 500 MC-LReq. $\mu g\ kg^{-1}$ bw, respectively. In all the above studies, quantitative determination of MC content in the organs was based on HPLC or ELISA methods. The present study is the first to determine MC content in phytoplanktivorous fish through LC-MS method. MCs in organs of bighead carp are within the range of the reported values from literatures and the parallel results of toxins determined by HPLC-UV were in good agreement ($r = 0.866−0.997$, $P < 0.01$).

It is interesting to note that in the present study, there were significant increases in MCs when the ingested food moved from fore- to hind-guts, and the maximum MC concentrations in the hindgut contents (mainly composed of faeces) reached as high as 478.3 $\mu g\ g^{-1}$. There are probably two reasons for this. First, food digestion by the sampled fish was usually more advanced (also with much more digestive enzymes) in the anterior part than in the posterior part of the intestines. Second, as bighead carp only digests part of their ingested algae (Xie, 2001), the presence of substantial amount of intact cyanobacterial cells in the hindgut may account for the high contents of MCs. The excretion of MCs through faeces in fish is also attributed to bile excretion, and is considered as the main excretion route of these toxins (Råbergh et al., 1991; Sahin et al., 1996). The much higher MCs in the midgut walls (1.22 $\mu g\ g^{-1}$ DW) than in the hind-and fore-gut

Table 2

Dry weight of the different organs as a percentage of total weight, MC contents (detected by ESI LC/MS[2]) and percentage of toxins present in the different organs of bighead carp collected from Meiliang Bay of Lake Taihu

Tissue	Dry weight (%)	MCs (µg/g)	LR/RR	Rd[1] (%)	Rd[2] (%)
Intestine	4.64	19.318 (0.052−85.674)	0.207	89.54	
Liver	2.82	0.374 (0.000−2.827)	0.540	1.05	11.29
Kidney	0.99	0.797 (0.029−1.696)	0.458	0.79	8.51
Spleen	0.18	0.311 (0.006−1.568)	0.207	0.06	0.61
Gallbladder	0.19	0.144 (0.000−0.829)	0.287	0.03	0.29
Gill	3.71	0.019 (0.000−0.068)	0.000	0.07	0.77
Blood	2.17	0.052 (0.000−0.238)	0.004	0.11	1.22
Muscle	58.20	0.124 (0.000−0.887)	0.420	7.22	77.32

Rd: relative distribution; [1]Including intestines; [2]Excluding intestines.

(a)

(b)
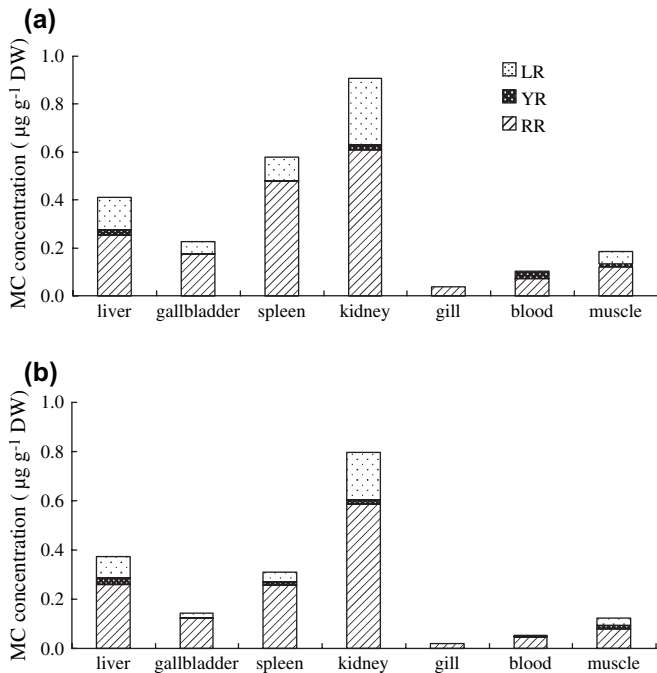
Fig. 6. The annual average values of MC-LR, -YR and -RR concentrations (μg g^{-1} DW) in different organs (liver, gallbladder, spleen, kidney, gill, blood and muscle) of bighead carp detected by: (a) ESI LC/MS^2; and (b) LC-UV.

walls (0.31 and 0.18 μg g^{-1} DW, respectively) suggest that the mid-gut wall might be the major site for MC absorption, whereas the hind- and fore-gut walls were much less important. It seems that although bighead carp ingest much microcystins by feeding, they are able to avoid intoxification through efficient inhibition of MC-LR transportation across gut walls (Xie et al., 2004) and through massive excretion of the toxins as feces.

The present study indicates that there were no significant correlations in MC concentration between the intestine and all the other organs of bighead carp, suggesting that MC contents in the intestine may be significantly affected by various factors (e.g., digesting degree at sampling or heterogeneity of food resources). In Sepetiba Bay of Brazil, a significant correlation was observed between MC concentration in seston samples and in the muscle of *Tilapia rendalli*, a phytoplanktivorous fish ($r = 0.96$, $P < 0.05$) (Magalhàes et al., 2003). The relationship in MC contents between digestive tract and the hepatopancreas is quite variable among different groups of invertebrates. In Lake Chaohu, there was roughly a positive

correlation in MC contents between digestive tracts and hepatopancreas of the snail *B. aeruginosa* ($r = 0.64$, $P = 0.17$) (Chen et al., 2005), whereas both shrimp species (*Palaemon modestus* and *Macrobrachium nipponensis*) showed no correlation in MC contents between stomach and hepatopancreas (Chen and Xie, 2005a). On the other hand, in the present study, MC content in the liver of bighead carp had a strong correlation with that in the muscle ($r = 0.94$, $P < 0.01$). Similarly, in an Egyptian fish farm, there was a significant correlation in MC concentration between the liver and muscle of a tilapia, *Oreochromis niloticus* ($r = 0.96$) (Mohamed et al., 2003).

In the present study, average MCs in the intestine, liver and muscle of bighead carp were 19.3, 0.374 and 0.124 μg g^{-1} DW, respectively, while in the same study site, average MCs in the intestine, hepatopancreas and foot of four bivalves were 0.93–3.83, 1.54–5.79 and 0.072–0.21 μg g^{-1} DW, respectively (Chen and Xie, 2005b). Apparently, bighead carp ingested much more toxins than bivalves, but accumulated less in liver and muscle. The liver of bighead carp collected in July showed little histopathologic changes under light microscopy although MCs content in liver reached the maximum (Personal communication of Dr. L. Li). In Lake Chaohu, MCs in the gut of the phytoplanktivorous silver carp (137 μg g^{-1} DW) was 20 times more than those in the gut of carnivorous and omnivorous fishes (<6.5 μg g^{-1} DW), however, MCs in the liver of silver carp (1.16 μg g^{-1} DW) was significantly lower than those of carnivorous and omnivorous fishes (1.76–11.6 μg g^{-1} DW) (Xie et al., 2005). These suggest that phytoplanktivorous fish could be more resistant to microcystins than other fishes due to less toxin accumulation in liver, the target organ of the toxins.

In the present study, a cysteine conjugate of MC-LR (MCLR-Cys) was detected in most months (July 2004–March 2005) in the kidney samples. It is known that MC-LR can conjugate with glutathione (GSH) and ultimately degrades to MCLR-Cys, or directly conjugate with cysteine, and this compound 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). Therefore, conjugation of MC-LR with cysteine or GSH in the kidney might be an important route for the detoxification and excretion of MC-LR in bighead carp. We also detected MCLR-Cys in the hindgut content samples in several months. However, we could not find MCLR-Cys in the liver samples of bighead carp. An immunostaining study (Ito et al., 2002)

Table 3
Correlation analysis of microcystin contents (μg g^{-1} DW) in various tissue samples of bighead carp between two analytical methods (LC-UV with ESI LC/MS^2) ($n = 118$)

Analytical method	LC-UV			ESI LC/MS^2		
Toxins	MC-RR	MC-YR	MC-LR	MC-RR	MC-YR	MC-LR
Average contents	27.33	0.56	4.71	25.25	0.46	4.82
r and P for RR	$r = 0.997$; $P < 0.01$					
r and P for YR	$r = 0.866$; $P < 0.01$					
r and P for LR	$r = 0.981$; $P < 0.01$					

showed that the injected conjugates (MCLR-GSH and MCLR-Cys) were prominently observed in the intestine and kidney of mice, whereas effective accumulation and bleeding were not found in the liver in spite of the larger dosage. They presented two reasons for this: firs, the transport system to the liver does not function well, second, the transported toxins are effectively eliminated by an appropriate system such as the GS-X (ATP-dependent glutathione S-conjugate exported) pump (Suzuki et al., 1997). They also reported that MCLR-Cys was detected in kidney soon after the injection and continued to be strongly detected for 1 h, and concluded that MCLR-Cys was the most actively excreted from the kidney. Kondo et al. (1996) conducted a laboratory experiment with mouse and rat i.p. injected respectively with MC-RR and -LR, and identified the presence of MCRR-Cys and MCLR-Cys in mouse and rat livers, respectively; and conjugates of MCs with GSH were also observed. In a field study, a GSH conjugate of MC-LR was detected in the foot sample of the bivalve *Cristaria plicata* in Lake Taihu (Chen and Xie, 2005b). Through in vitro experiment, a GSH conjugate of MC-LR was detected in a macrophyte (*Ceratophyllum demersum*), invertebrates (*Dreissena polymorpha*, *Daphnia magna*), fish eggs and fish (*Danio rerio*) (Pflugmacher et al., 1998). However, all the above studies were limited to qualitative identification, and therefore, for a better understanding of the role of MC-GSH and MC-Cys conjugates in the detoxification of MCs in animals, quantitative evaluations of such conjugates in animal tissues are needed in future study.

The present study also shows that MCs in spleen reached up to $1.568\ \mu g\ g^{-1}$ (average $0.311\ \mu g\ g^{-1}$), and 0.61% of the toxin burden was located in the spleen if intestines are excluded. This finding may be explained by the process known as presystematic hepatic elimination, which prevents, or at least minimizes, the distribution of foreign chemicals to other parts of the body. However, when this process (presystematic hepatic elimination) is overwhelmed or bypassed by exposure to toxins such as MC, it may allow MC to circulate to these other organs (Klaassen and Watkins, 1984).

A coefficient of 5 was used to convert dry weight to wet weight, and since i.p. LD_{50} in mice for MC-RR and -YR is about 5 times and 2.5 times higher than MC-LR, respectively (Gupta et al., 2003), coefficients of 0.2 and 0.4 were used to convert MC-RR and -YR into MC-LR$_{equivalent}$, respectively. Considering an adult human weighing 60 kg ingesting 300 g of fish muscle, for the entire study period, 25% of fish muscle samples had MC concentrations close to ($0.043\ \mu g\ kg^{-1}$ of body weight, October) or above ($0.375\ \mu g\ kg^{-1}$ of body weight, July and $0.123\ \mu g\ kg^{-1}$ of body weight, December) the provisional tolerable daily intake (TDI) ($0.04\ \mu g\ kg^{-1}$ of body weight) suggested by WHO (Chorus and Bartram, 1999). Therefore, consumption of bighead muscle in these months is risky to human health.

Conclusively, the phytoplanktivorous bighead carp is strongly resistant to microcystins as evidenced by: (i) its fast growth in the large net cage of Lake Taihu where dense toxic cyanobacterial blooms occurred; (ii) less accumulation of microcystins in liver, a target organ of these toxins; and (iii) perhaps efficient detoxification and excretion mechanisms through conjugation of MCs with GSH or Cys in the kidney. It is therefore quite possible to use such phytoplanktivorous fish to counteract cyanotoxin contamination in eutrophic waters.

Acknowledgements

References

Cai, Q.M., Gao, X.Y., Chen, Y.W., Ma, S.W., Dokulil, M., 1997. Dynamic variations of water quality in Taihu Lake and multivariate analysis of its influential factors. Journal of Chinese Geography 7, 72–82.

Carmichael, W.W., 1996. Toxic *Microcystis* and the environment. In: Watanabe, M.F., Harada, K.-I., Carmichael, W.W., Fujiki, H. (Eds.), Toxic *Microcystis*. CRC Press, Boca, Raton, FL, pp. 1–11.

Chen, J., Xie, P., 2005a. Tissue distributions and seasonal dynamics of the hepatotoxic microcystins-LR and -RR in two freshwater shrimps, *Palaemon modestus* and *Macrobrachium nipponensis*, from a large shallow, eutrophic lake of the subtropical China. Toxicon 45, 615–625.

Chen, J., Xie, P., 2005b. Seasonal dynamics of the hepatotoxic microcystins in various organs of four freshwater bivalves from the large eutrophic Lake Taihu of subtropical China and the risk to human consumption. Environmental Toxicology 20, 572–584.

Chen, J., Xie, P., Guo, L.G., Zheng, L., Ni, L.Y., 2005. Tissue distributions and seasonal dynamics of the hepatotoxic microcystins-LR and -RR in a freshwater snail (*Bellamya aeruginosa*) from a large shallow, eutrophic lake of the subtropical China. Environmental Pollution 134, 423–430.

Chorus, I., Bartram, J., 1999. Toxic Cyanobacteria in Water. A Guide to Public Health Consequences, Monitoring and Management. E & FN Spon, London. 416.

Codd, G.A., 1995. Cyanobacterial toxins: occurrence, properties and biological significance. Water Science and Technology 32, 149–156.

Dawson, R.M., 1998. The toxicology of microcystins. Toxicon 36, 953–962.

FAO, 1991. Fishery Statistics, Catches and Landing, FAO Yearbook. Food and Agriculture Organization of the United Nations, Rome.

Gupta, N., Pant, S.C., Vijayaraghavan, R., Lakshmana Rao, P.V., 2003. Comparative toxicity evaluation of cyanobacterial cyclic peptide toxin microcystin variants (LR, RR, YR) in mice. Toxicology 188, 285–296.

Havens, K.E., 2003. Phosphorus-algal bloom relationships in large lakes of South Florida: implications for establishing nutrient criteria. Lake and Reservoir Management 19, 222–228.

Havens, K.E., James, T., East, T.L., Smith, V.H., 2003. N:P ratios, light limitation, and cyanobacterial dominance in a subtropical lake impacted by non-point source nutrient pollution. Environmental Pollution 122, 379–390.

Ito, E., Takai, A., Kondo, F., Masui, H., Imanishi, S., Harada, K., 2002. Comparison of protein phosphatase inhibitory activity and apparent toxicity of microcystins and related compounds. Toxicon 40, 1017–1025.

Klaassen, C.D., Watkins, J.B., 1984. Mechanisms of bile formation, hepatic uptake, and biliary excretion. Pharmacological Reviews 36, 1–67.

Kondo, F., Ikai, Y., Oka, H., Okumura, M., 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. Chemical Research in Toxicology 5, 591–596.

Kondo, F., Matsumoto, H., Yamada, S., Ishikawa, N., Ito, E., Nagata, S., Ueno, Y., Suzuki, M., Harada, K., 1996. Detection and identification of metabolites of microcystins formed in vivo in mouse and rat livers. Chemical Research in Toxicology 9, 1355–1359.

Lawrence, J.F., Menard, C., 2001. Determination of microcystins in blue-green algae, fish and water using liquid chromatography with ultraviolet detection after sample clean-up employing immunoaffinity chromatography. Journal of Chromatography A 922, 111–117.

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

Liang, Y.L., 1981. The apparatus of filtering and feeding of silver carp and bighead carp. Journal of Dalian Fisheries College 1, 13–33 (in Chinese).

Magalhães, F.V., Soares, R.M., Azvedo, S.M.F.O., 2001. Microcystin contamination in fish from Jacarepaguá Lagoon (Rio de Janeiro, Brazil): ecological implication and human health risk. Toxicon 39, 1077–1085.

Magalhães, V.F., Marinho, M.M., Domingos, P., Oliveira, A.C., Costa, S.M., Azevedo, L.O., Azevedo, S.M.F.O., 2003. Microcystins (cyanobacteria hepatotoxins) bioaccumulation in fish and crustaceans from Sepetiba Bay (Brasil, RJ). Toxicon 42, 289–295.

Malbrouck, C., Trausch, G., Devos, P., Kestemont, P., 2003. Hepatic accumulation and effects of microcytin-LR on juvenile goldfish *Carassius auratus* L. Comparative Biochemistry and Physiology C 135, 39–48.

Mohamed, Z.A., Carmichael, W.W., Hussein, A.A., 2003. Estimation of microcystins in the freshwater fish *Oreochromis niloticus* in an Egyptian fish farm containing a *Microcystis* bloom. Environmental Toxicology 18, 137–141.

Nishiwaki-Matsushima, R., Nishiwaki, S., Ohta, T., Yoszawa, S., Suganuma, M., Harada, K., Watanabe, M.F., Fujiki, H., 1991. Structure-function relationships of microcystins, liver-tumor promoters, in interaction with protein phosphatase. Japanese Journal of Cancer Research 82, 993–996.

Nishiwaki-Matsushima, R., Ohta, T., Nishiwaki, S., Suganuma, M., Yoszawa, S., Kohyama, K., Ishikaawa, T., Carmichael, W.W., Fujiki, H., 1992. Liver tumor promotion by the cyanobacterial cyclic peptide toxin microcystin-LR. Journal of Cancer Research and Clinical Oncology 118, 420–424.

Opuszynski, K., Shireman, J.V., 1995. Food habits, feeding behavior and impact of triploid bighead carp, *Aristichthys nobilis*, in experimental ponds. Journal of Fish Biology 42, 517–530.

Paerl, H.W., Fulton, R.S., Moisander, P.H., Dyble, J., 2001. Harmful freshwater algal blooms, with an emphasis on cyanobacteria. The Scientific World Journal 1, 76–113.

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. Biochimica et Biophysica Acta (BBA) – General Subjects 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 lack of qualified water and deterioration of environment and natural resources in Taihu Lake basin. Journal of Lake Science 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. Ecological Engineering 10, 79–90.

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

Qin, B.Q., Hu, W.P., Gao, G., Luo, L.C., Zhang, J.S., 2004b. The dynamics of resuspension and conceptual mode of nutrient release from sediments in large shallow Lake Taihu, China. Chinese Science Bulletin 49, 54–64.

Råbergh, C.M.I., Bylund, G., Erikssonl, J.E., 1991. Histopathological effects of microcystin-LR, a cyclic peptide toxin from the cyanobacterium (blue-green alga) *Microcystis aeruginosa*, on common carp (*Cyprinus carpio* L.). Aquatic Toxicology 20, 131–146.

Sahin, A., Tencalla, F.G., Dietrich, D.R., Naegeli, H., 1996. Biliary excretion of biochemically active cyanobacteria (blue-green algae) hepatotoxins in fish. Toxicology 106, 123–130.

Soares, R.M., Magalhães, V.F., Azevedo, S.M.F.O., 2004. Accumulation and depuration of microcystins (cyanobacteria hepatotoxins) in *Tilapia rendalli* (Cichlidae) under laboratory conditions. Aquatic Toxicology 70, 1–10.

Suzuki, M., Mori, M., Niwa, T., Hirata, R., Furuta, K., Ishikawa, T., Noyori, R., 1997. Chemical implications for antitumor and antiviral prostaglandins: reaction of Δ^7-prostaglandin A1 and prostaglandin A1 methyl esters with thiols. Journal of the American Chemical Society 119, 2376–2385.

Tang, Y., 1981. Evaluation of balance between fishes and available fish foods in multispecies fish culture ponds in Taiwan. Transaction of America Fisheries Society 99, 708–717.

Wiegand, C., Pflugmacher, S., Oberemm, A., Meems, N., Beattie, K.A., Steinberg, C.E.W., Codd, G.A., 1999. Uptake and effects of microcystin-LR on detoxication enzymes of early life stages of the zebrafish (*Danio rerio*). Environmental Toxicology 14, 89–95.

Williams, D.E., Dawe, S.C., Kent, M.L., Andersen, R.J., Craig, M., Holmes, C.F.B., 1997. Bioaccumulation and clearance of microcystins from salt water mussels, *Mytilus edulis*, and in vivo evidence for covalently bound microcystins in mussel tissues. Toxicon 35, 1617–1625.

Xie, P., 2001. Gut contents of bighead carp (*Aristichthys nobilis*) and the processing and digestion of algal cells in the alimentary canal. Aquaculture 195, 149–161.

Xie, P., Liu, J.K., 2001. Practical success of biomanipulation using filter-feeding fish to control cyanobacteria blooms: a synthesis of decades of research and application in a subtropical hypereutrophic lake. The Scientific World Journal 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. Environmental Pollution 127, 431–439.

Xie, L.Q., Xie, P., Guo, L.G., 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. Environmental Toxicology 20, 293–300.

Zurawell, R.W., Kotak, B.G., Prepas, E.E., 1999. Influence of lake trophic status on the occurrence of microcystin-LR in the tissue of pulmonate snails. Freshwater Biology 42, 707–718.