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In situ studies on the bioaccumulation of microcystins in the phytoplanktivorous silver carp (*Hypophthalmichthys molitrix*) stocked in Lake Taihu with dense toxic *Microcystis* blooms

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Abstract

The phytoplanktivorous silver carp is an important biomanipulation fish to control cyanobacterial blooms and is also a food fish with the greatest production in China. The accumulation of the hepatotoxic microcystins (MCs) determined by LC-MS in various organs of silver carp was studied monthly in Lake Taihu dominated by toxic *Microcystis aeruginosa*. Average recoveries of spiked fish samples were 78% for MC-RR and 81% for MC-LR. The highest content of MCs was found in the intestine (97.48 $\mu g\ g^{-1}$ DW), followed by liver (6.84 $\mu g\ g^{-1}$ DW), kidney (4.88 $\mu g\ g^{-1}$ DW) and blood (1.54 $\mu g\ g^{-1}$ DW), and the annual mean MC content was in the order of intestine > liver > kidney > blood > muscle > spleen > gallbladder > gill. Silver carp could effectively ingest toxic *Microcystis* cells (up to 84.4% of total phytoplankton in gut contents), but showed fast growth (from 141 g to 1759 g in 1 year in mean weight). Silver carp accumulated less microcystins in liver than other animals in the same site or other fish from different water bodies at similar level of toxin ingestion. There was possible inhibition of the transportation of the most toxic MC-LR across the gutwall. Muscle of silver carp in Lake Taihu should not be consumed during period of dense *Microcystis* blooms while viscera were risky for consumption in more months.
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1. Introduction

The occurrence of toxic cyanobacterial blooms in eutrophic fresh and brackish waters has been a worldwide problem (Paerl et al., 2001). Among cyanotoxins, microcystins (MCs) are considered to be the most common and dangerous group (Chorus and Bartram, 1999).

MCs are known to be potent hepatotoxins (Codd, 1995; Dawson, 1998) and tumor promoter (Nishiwaki-Matushima 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; Malbrouck and Kestemont, 2006). 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.

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There have been extensive studies on MC bioaccumulation in fishes under laboratory condition (e.g., Råbergh et al., 1991; Williams et al., 1995; Sahin et al., 1996; Kotak et al., 1996; Pflugmacher et al., 1998; Wiegand et al., 1999; Lawrence and Menard, 2001; Malbrouck et al., 2003; Jang et al., 2003; Mohamed and Hussein, 2006; Soares et al., 2004; Li et al., 2005; Xie et al., 2004; Shen et al., 2005; Cazenave et al., 2005, 2006). On the other hand, there are only a few studies conducted in field. Magalhàes et al. (2001, 2003) studied seasonal changes of MC in liver, muscle and viscera of *Tilapia rendalli* in a lagoon and in muscle of fish (no name given) in Sepetiba Bay of Brazil. Mohamed et al. (2003) examined MC content in liver, kidney, gut and muscle of *Oreochromis niloticus* in an Egyptian fish farm in June. Cazenave et al. (2005) detected MC in liver, gill, muscle and brain of *Odontesthes bonariensis* collected from a reservoir of Argentina on two sampling dates. Xie et al. (2005) measured MC in gut, liver, kidney, muscle, blood and bile of eight species of fish in Lake Chaohu of China in September. Jang et al. (2003) measured MC content in body tissue of two native fishes stocked in small cages (2 m × 1 m × 1 m) in Hoedong Reservoir for 6 days. Therefore, information on seasonal dynamics of MC in various organs of wild fish is very limited, in spite of its practical importance in public health management. In addition, in these field studies, MCs in fish tissue were measured by ELISA or HPLC.

The phytoplanktivorous fishes are especially important to humans because of their role in aquatic ecosystems as direct consumers of phytoplankton primary production, their importance as food fish and their potential for biological management of cyanobacterial blooms (Opuszynski and Shireman, 1995; Xie and Liu, 2001). Silver carp (*Hypophthalmichthys molitrix*) is one of the most important phytoplanktivorous fish in China, and has been introduced worldwide for aquaculture, comprising as much as 12% of the total freshwater fish production of the world (FAO, 1991). A sub-chronic toxicity experiment in two 1000 L tanks was conducted to examine tissue (intestine, blood, liver and muscle) distribution and depuration of MC in silver carp fed with fresh *Microcystis* cells (collected from a pond) during a course of 80 days (Xie et al., 2004). Shen et al. (2005) determined the final MC contents in muscle and liver of silver carp after 28-days' feeding experiment in two 100 L tanks using fresh *Microcystis* cells collected from a pond. Recently, Zhang et al. (2006) studied the effects of the silver carp on plankton and microcystins in an enclosure experiment in a eutrophic lake and demonstrated that silver carp can be an efficient biomanipulation fish to reduce nuisance toxic cyanobacterial

blooms. However, there is little knowledge about dynamics and distribution pattern of MC in organs of silver carp under chronic, natural exposure situations.

Taihu Lake (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 lake 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. This area is of historical importance in trade, politics, agriculture and culture. There are six large cities 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, characteristic of extremely dense accumulation of toxic *Microcystis* blooms by wind in the summer (Cai et al., 1997; Qin et al., 2004).

The main purposes of this study are 1) to examine the seasonal changes in the tissue distribution of three common MCs (MC-LR, -RR and -YR) in silver carp cultured in a large net cage in the Meiliang Bay of Lake Taihu where dense toxic cyanobacterial blooms occur, and 2) to discuss the possible mechanisms underlying these patterns with recommendation on the potential risk for human consumption of this fish species containing MCs. This study tries to provide basis for the control of cyanotoxin contamination through fish biomanipulation and also for safe consumption of these biomanipulation fish by humans.

2. Materials and methods

To test the applicability of using phytoplanktivorous fishes to counteract cyanobacteria, two phytoplanktivorous fishes, silver carp and bighead carp, were stocked in a large net cage (1.088 km^2) in the Meiliang Bay of Lake Taihu during the period from April 2004 until March 2005.

Silver carp were collected monthly from Meiliang Bay during all the study period. A total of 20 fish were randomly collected each month for the measurements of total length and body weight, and five of them were used for toxin analysis. The fish were killed by a blow to the head. Blood of the fish were collected from a cut across the tail of the fish. After sampling the blood, we dissected the fish into seven parts: intestine, liver, kidney, spleen, gallbladder, gill and muscle (taken from the
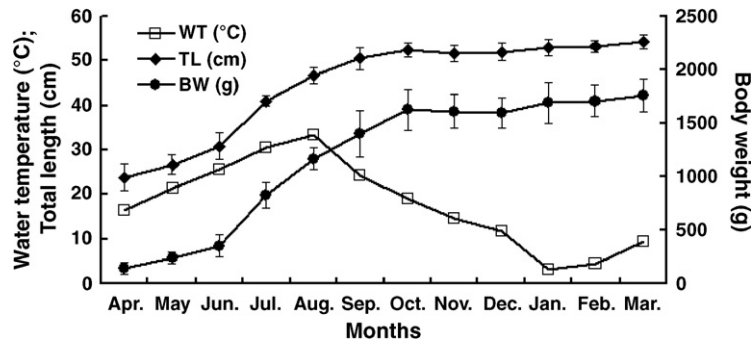
Fig. 1. Annual changes of water temperature (WT), and total length (TL) and body weight (BW) of silver carp in a large net cage in the Meiliang Bay of Lake Taihu.

back). The collected organs were separately washed carefully by distilled water to avoid cross contamination, and then 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 for some organs (e.g., kidney, spleen, gallbladder) especially in the earlier months of the experiments. Therefore, we pooled, respectively, all intestine, liver, kidney, spleen, gallbladder, gill, blood and muscle of five dissected fish on each sampling date (from Apr. 2004 to Mar. 2005). Thus, each value represents an average amount of MCs in the organs of five individuals. Blood sample on each sampling date was c.a. 40 ml in volume. In addition, during September 2004 and March 2005, we divided the intestine into six parts: fore-gut wall, mid-gut wall, hind-gut wall, fore-gut contents, mid-gut contents and hind-gut contents. Since during this period of time, the size of the fish was large enough for individual analysis, the data of MC in gut contents and gut walls were from one fish on each sampling site.

There were two sampling sites located inside the net cage for the analysis of MC in phytoplankton (intracellular MC) and quantification of phytoplankton community. 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 MC extraction was after Park et al. (1998).

Phytoplankton in both the water column and the fore-gut contents of silver carp were preserved with Lugol's iodine solution immediately after sampling. Sub-samples for phytoplankton were concentrated to 30 ml after sedimentation for 48 h. After mixing, 0.1 ml concen-

trated samples or gut content sample were counted directly under 400× magnification. Colonial *Microcystis* cells were separated using a high-speed blender (Ultra-Turrax) and counted as above. Taxonomic identification was made according to Hu et al. (1979) and biomass was estimated from approximate geometric volumes of each taxon, assuming that 1 mm^3 equals 10^{-6} µg fresh weight. The percentage of cyanobacteria in total phytoplankton was calculated.

Samples of intestine, liver, kidney, spleen, gallbladder, gill, blood and muscle were lyophilized using a Alpha 2–4 Freeze Dryer (Martin Christ, German). Extraction of 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).

Qualitative and quantitative analysis of MCs in both fish organs 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
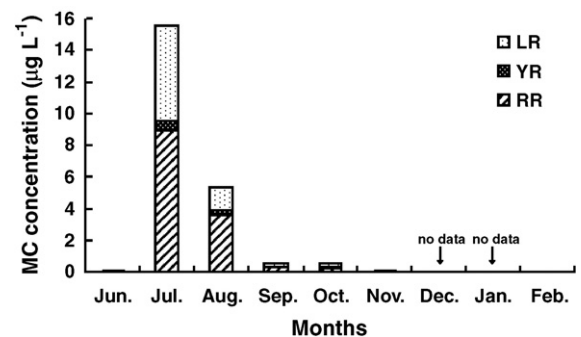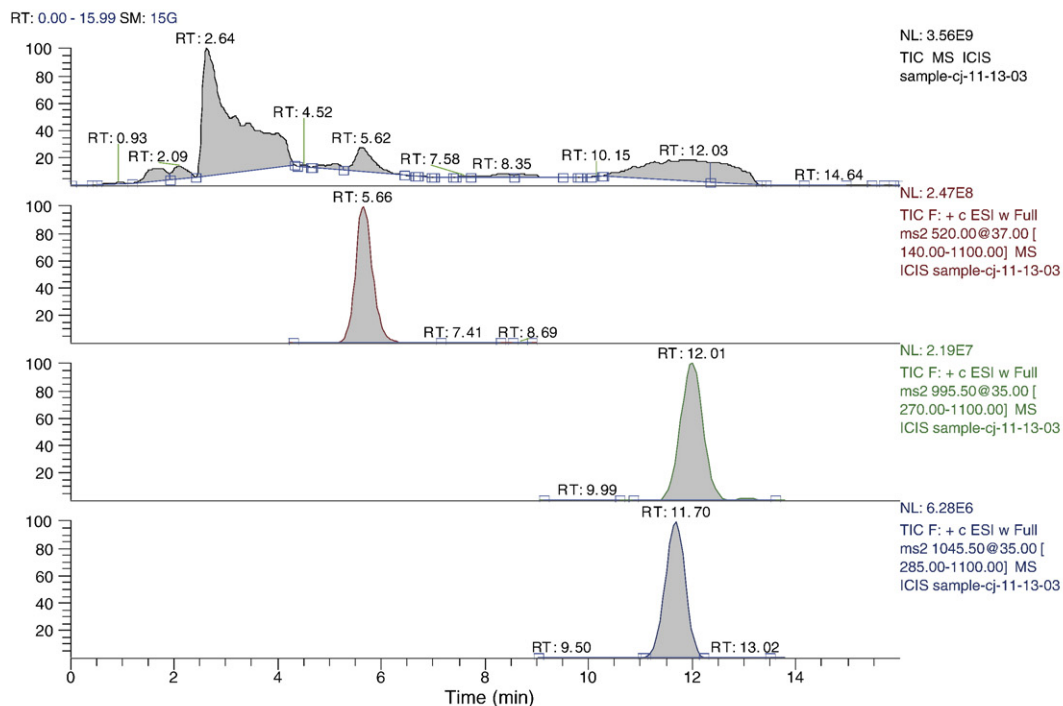

Fig. 2. Seasonal changes of intracellular MC-LR, -YR and -RR concentrations (µg L^{-1}) (detected by ESI LC/MS^2) in the water column of the large net cage where silver carp were stocked. Each value was the mean of two sampling sites.

(a) total ion and mass chromatograms monitored at m/z 520, 1045.5, 995.5

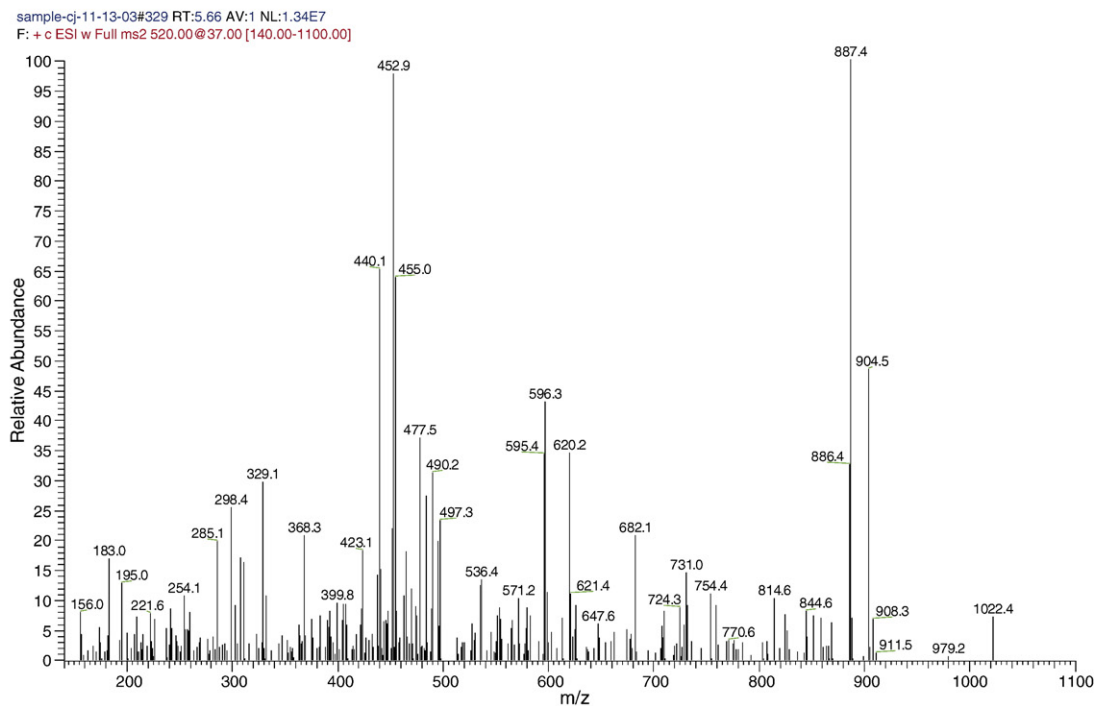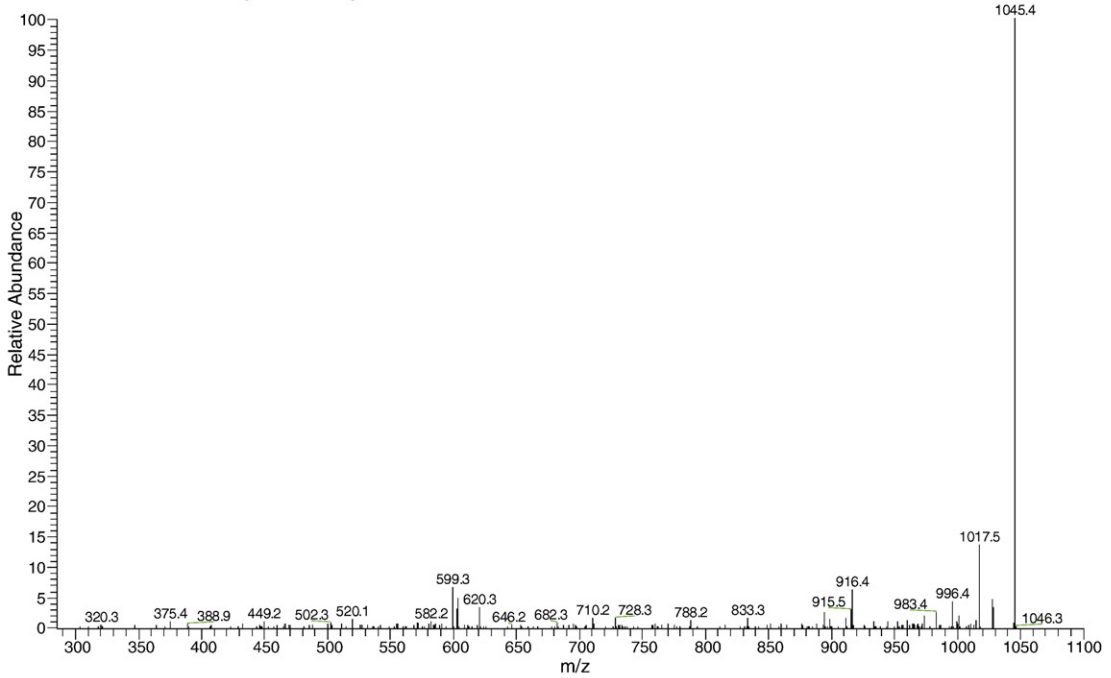

(b) ESI mass spectrum at 5.66 min (MC-RR)

Fig. 3. ESI LC/MS[2] analysis of MCs in the liver of silver carp (July 2004).

(c) ESI mass spectrum at 11.70 min (MC-YR)

sample-cj-11-13-03#613 RT:11.70 AV:1 NL:1.02E7
F: +c ESI w Full ms2 1045.50@35.00 [285.00-1100.00]

(d) ESI mass spectrum at 12.01 min (MC-LR)

sample-cj-11-13-03#629 RT:12.01 AV:1 NL:6.88E6
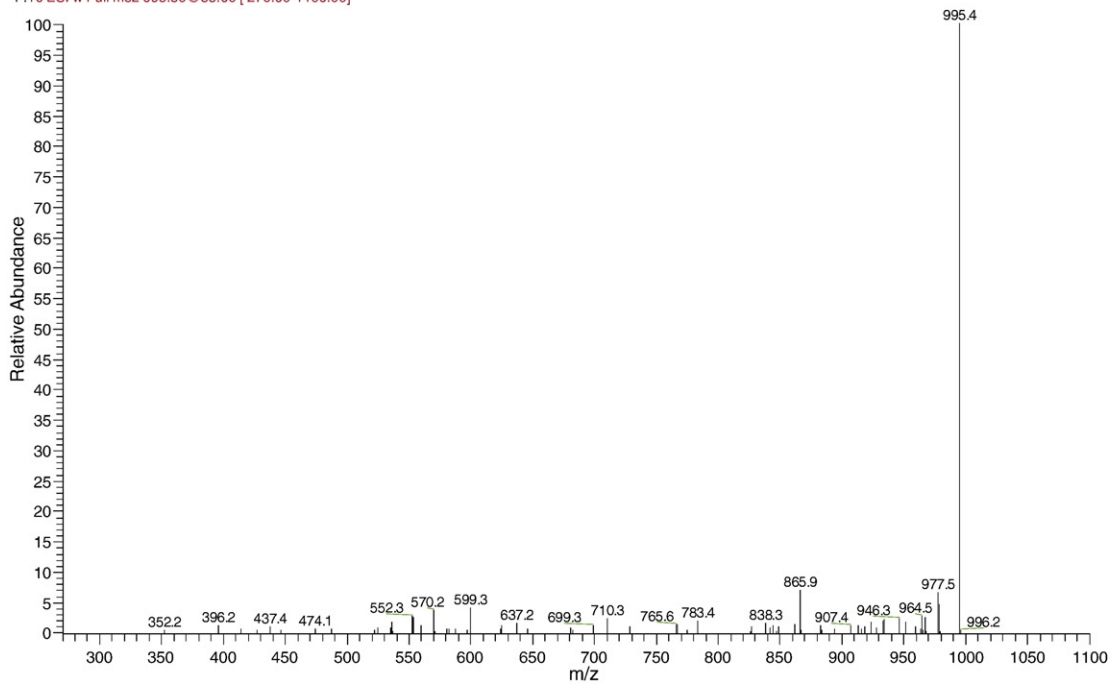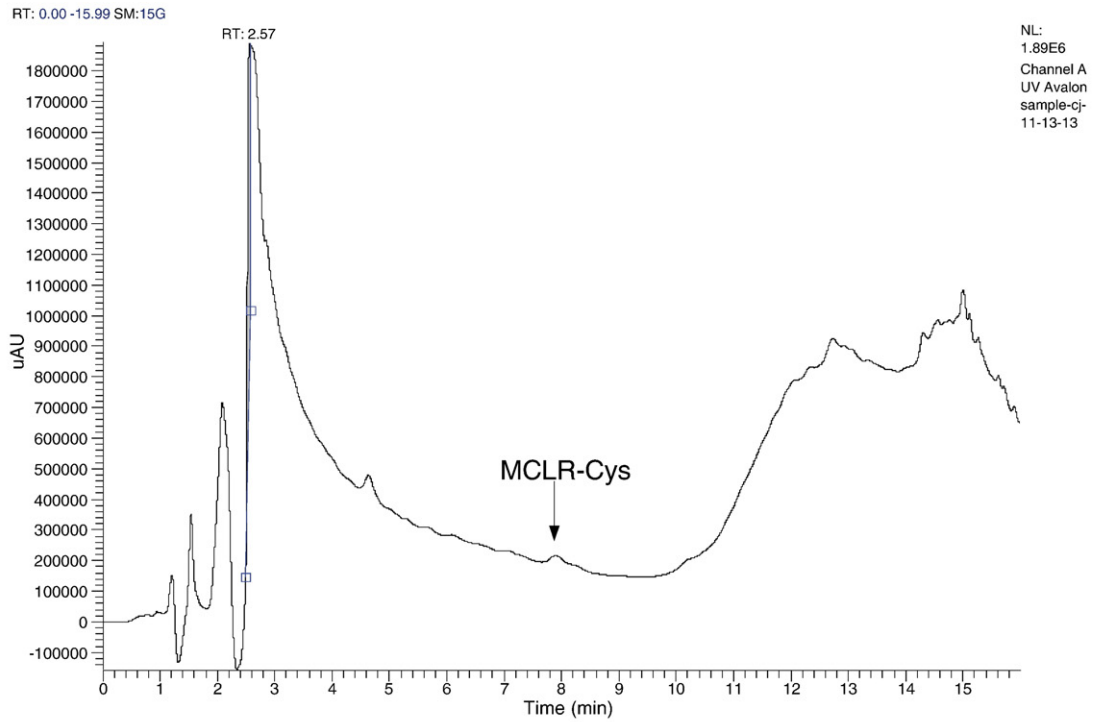F:+c ESI w Full ms2 995.50@35.00 [270.00-1100.00]

Fig. 3 (*continued*).

(a) high performance liquid chromatogram monitored at 238nm
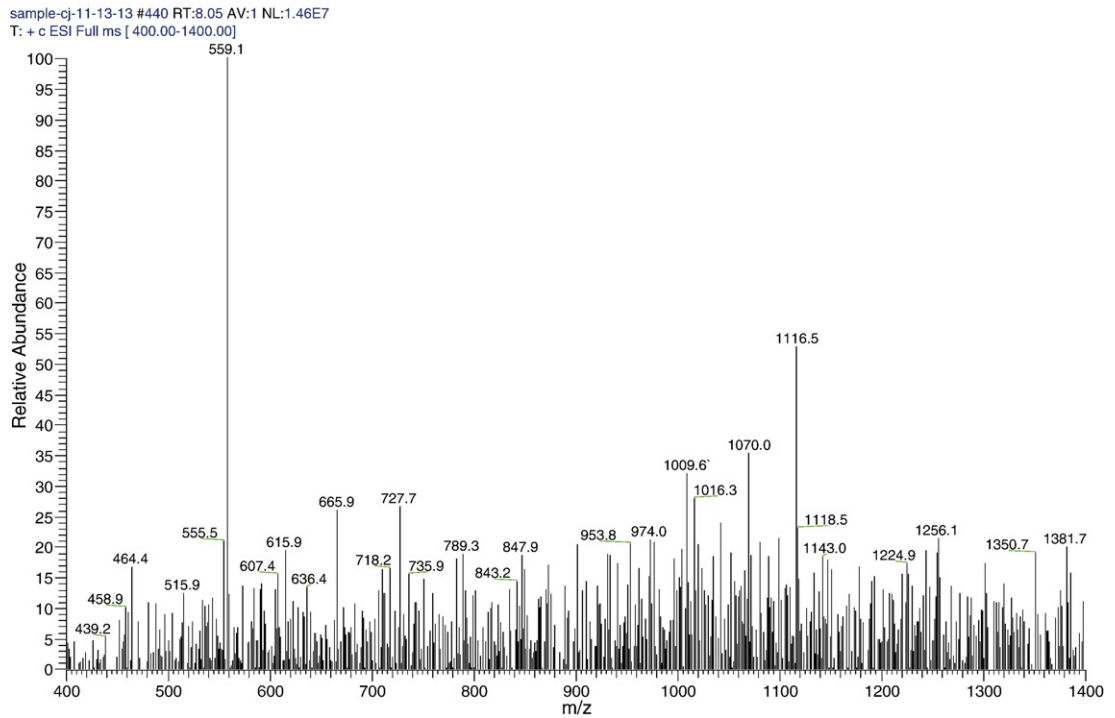
MCLR-Cys

(b) ESI mass spectrum at 8.05 min (MCLR-Cys)

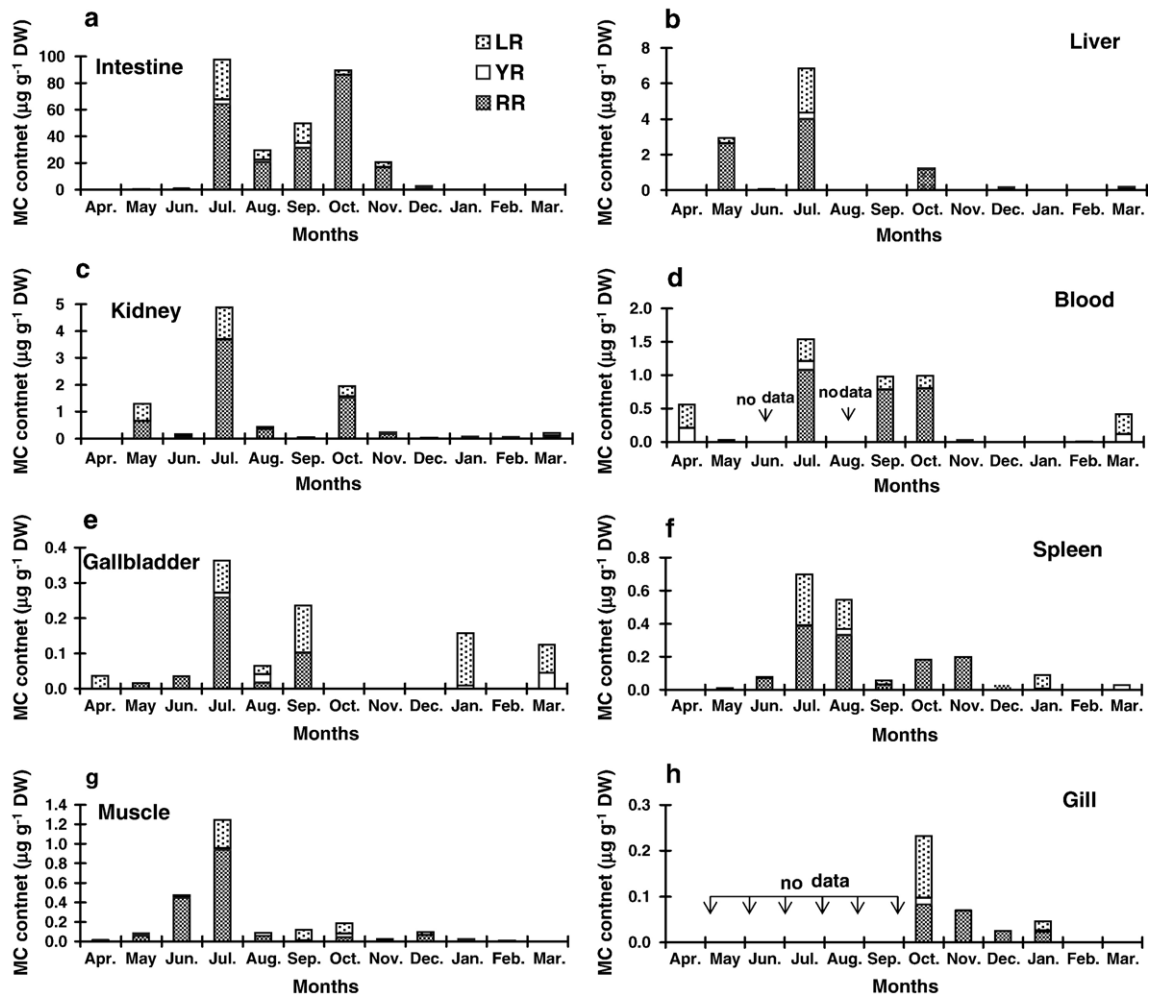Fig. 4. ESI LC/MS analysis of the kidney of silver carp (September 2004).

Fig. 5. The seasonal changes of MC-LR, -YR and -RR concentrations (μg g^{-1} DW) in (a) intestine, (b) liver, (c) kidney, (d) blood, (e) gallbladder, (f) spleen, (g) muscle and (h) gill of silver carp collected from a large net cage in the Meiliang Bay of Lake Taihu detected by ESI LC/MS^2. Each value represents a mean of five individuals.

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 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 mul-

tiplier 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; (4) full scan mode as same as segment 1 in the rest time. All tissue samples were analyzed in duplicate from the same extract and all the values present in the text were measured by ESI-LC/MS^2.

Table 1
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 silver carp collected from Meiliang Bay of Lake Taihu

Tissue	Dry weight	Mean MCs (range)	LR/ RR	Rd[1]	Rd[2]
	%	µg/g		%	%
Intestine	7.71	24.297 (0.018–97.480)	0.45	93.04	
Liver	1.83	0.957 (0.000–6.840)	0.41	0.87	12.50
Kidney	0.89	0.782 (0.004–4.884)	0.30	0.35	4.97
Spleen	0.25	0.159 (0.001–0.698)	0.28	0.02	0.28
Gallbladder	0.23	0.086 (0.000–0.364)	0.61	0.01	0.14
Gill	3.30	0.062 (0.000–0.233)	0.62	0.10	1.47
Blood	2.01	0.379 (0.001–1.537)	0.14	0.38	5.44
Muscle	53.47	0.197 (0.000–1.244)	0.46	5.24	75.22

Rd: relative distribution; [1]including intestines; [2]excluding intestines.

Three standards of MC variants (MC-LR, MC-YR and MC-RR) used in the detection were purchased from Wako Pure Chemical Industries–Japan.

Recovery experiments were performed in quintuplicate spiking 500 mg of homogenized freeze-dried fish samples (liver 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.

Linear correlations between MC contents of different tissues and between MC concentrations in the fore-gut contents and the percentage of *Microcystis* in the total phytoplankton in the fore-gut were conducted by using Statistica 6.0 Software (StatSoft, Inc.). No data transformation was performed prior the analysis.

3. Results

Seasonal changes in water temperature, total body length and body weight of silver carp are shown in Fig. 1. During the study period, water temperature varied between 3.2 (January) and 33.4 (August) °C. Silver carp weighed 141 (±59) g (23.9±3.0 cm in total length) at stocking (in April 2004), and increased to 1759 (±155) g (54.2±1.6 cm) after a growth period of a year (in March 2005). The increase in body weight of silver carp was as high as 12.5 times. In summer, there

were dense toxic cyanobacterial blooms (mainly composed of *Microcystis aeruginosa*) in the lake water. Intracellular MCs in the water column averaged 4.16 µg L^{-1} with a range of 0–15.58 µg L^{-1} (Fig. 2).

Fig. 3 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 5.66 min was derived from MC-RR. Similarly, peaks obtained at 11.70 min and 12.01 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 kidney (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 only LR-Cys was found in the samples of kidney and hind-gut contents.

The monthly changes of MC contents in various organs of silver carp are showed in Fig. 5. During the
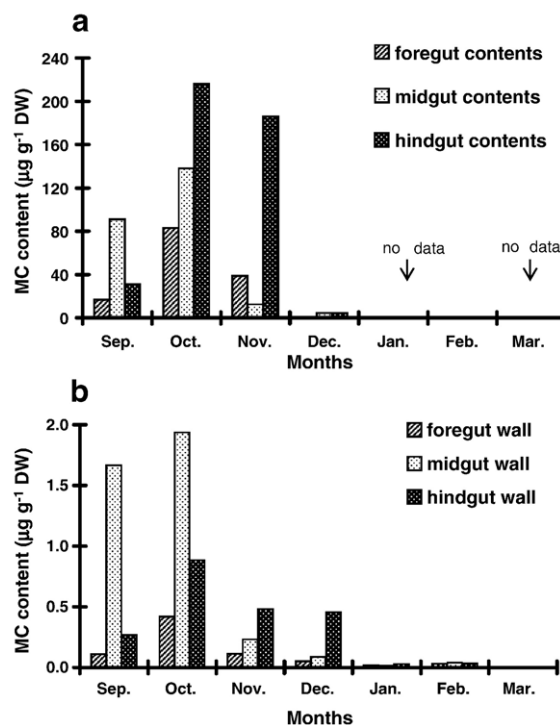

Fig. 6. The seasonal changes of MC concentrations (µg g^{-1} DW) in (a) fore-gut contents, mid-gut contents, hind-gut contents and (b) fore-gut wall, mid-gut wall, hind-gut wall of silver carp collected from a large net cage in the Meiliang Bay of Lake Taihu detected by ESI LC/MS[2].

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 97.48 µg g^{-1} DW), followed by liver (6.84 µg g^{-1} DW), kidney (4.88 µg g^{-1} DW) and blood (1.54 µg g^{-1} DW), and the annual mean MC content was in the order of intestine > liver > kidney > blood > muscle > spleen > gallbladder > gill (Table 1).

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 ($r=0.85$, $P<0.01$) and kidney ($r=0.96$, $P<0.01$).

There were great variations in MC contents in various parts of the intestines (Fig. 6). The highest content of MCs was found in the hind-gut contents (mean 87.59, range 0.08–216.30 µg g^{-1} DW; LR/RR 0.51), followed by the mid-gut contents (mean 49.30, range 0.00–138.18 µg g^{-1} DW; LR/RR, 0.49) and fore-gut contents (mean 16.57, range 0.02–45.39 µg g^{-1} DW; LR/RR, 0.06). Relatively, only small amounts of MCs were detected in the gut walls, the order was mid-gut wall (mean 0.57, range 0.00–1.94 µg g^{-1} DW; LR/RR 0.22) > hind-gut wall (mean 0.31, range 0.03–0.88 µg g^{-1} DW; LR/RR 0.43) > fore-gut wall (mean 0.11, range 0.00–0.42 µg g^{-1} DW; LR/RR 0.09). Apparently, the LR/RR ratio increased significantly from fore-gut contents to mid- and hind-gut contents, and from fore-gut wall to mid- and hind-gut walls. There were also great differences in the ratio of LR/RR between gut contents and gut walls in mid-gut.

In the lake water inside the cage, in terms of annual mean biomass, dominant phytoplankton were cyanobacteria (mainly *M. aeruginosa*), green algae, diatoms and *Cryptomonas*, and cyanobacteria comprised 58.2% of total phytoplankton biomass. Seasonally, phytoplankton community was dominated by *Cryptomonas* in March, by *Cryptomonas + Ulothrix* in April, by *Ulothrix* in May–June, by *Microcystis* in July–August, and by *Microcystis + Melosira* in September–October. During the study period, in the fore-gut contents, percentage of *Microcystis* biomass in total phytoplankton biomass varied from 0.02% to 84.4% (in August) with an average of 25.08%. There was a good correlation between MC concentrations in the fore-gut contents and the percentage of *Microcystis* biomass in the total phytoplankton biomass in the fore-gut ($r=0.897$, $P<0.01$).

In terms of toxin burden, intestine (93.04%) ranked the first, followed by muscle (5.24%), liver (0.87%) and kidney (0.35%), whereas spleen, gallbladder, gill, blood had altogether less than 0.51% of the total toxin. If intestines are excluded, up to 75.22%, 12.50% and 4.97% of the toxin burden were located in the muscle, liver and kidney, respectively (Table 1).

The average recoveries ($n=10$) from different parts of fish samples (liver and muscle) were 78% (range, 77–82%) for MC-RR with relative standards deviations (RSDs) between 9 and 13%, and 81% (range, 72–88%) with RSDs between 9 and 11% for MC-LR. Results obtained for liver were better than those obtained for muscle.

4. Discussion

In the present study, high percentage of *Microcystis* cells was found in the intestinal content of silver carp (e.g., ca. 84.4% in biomass in August), and there was a good correlation between MC concentrations in fore-gut contents and the percentage of *Microcystis* in total phytoplankton in the fore-gut, indicating that silver carp could effectively ingest toxic *Microcystis* cells in natural conditions.

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 hind-gut contents (mainly composed of feces) reached as high as 216.3 µg g^{-1}. The excretion of MCs through feces verified here has already been reported in the literature and can be due to bile excretion, considered as the main excretion route for these toxins (Råbergh et al., 1991; Sahin et al., 1996). It is possible that some MCs present in feces were bound to glutathione, as we detected MCLR-Cys in the hind-gut content samples in several months. In the present study, MCs were detected in the gallbladder of silver carp in most months and the MC content in gallbladder was less than 1/10 that in liver. In a laboratory experiment, high concentrations of MCs were detected in the gallbladder of rainbow trout gavaged with toxic cyanobacteria, and the MC content in gallbladder was several folds higher than that in liver (Sahin et al., 1996). It is suggested that bile plays an important role in the elimination and recirculation of excess microcystins from the liver of fish (Tencalla and Dietrich, 1997).

In the present study, the LR/RR ratio decreased significantly from mid-gut contents (0.49) to mid-gut wall (0.22) and blood (0.14). As the mid-gut wall was the major site for MC absorption, it is likely that there might be an active degradation of MC-LR during the process of digestion whereas MC-RR in the gut fluids was massively transported across the intestines and embedded in the fish body. It is known that once absorbed by intestinal epithelia, MC can be rapidly transported via the blood stream and distributed to various organs or tissues, the

significantly low MC-LR/MC-RR ratio in the blood suggests that little MC-LR could be transported across the intestines and consequently little MC-LR could accumulate in the internal organs (especially highly blood-irrigated organs like liver, kidney etc.) of the fish. Previous studies on rainbow trout (*O. mykiss*) showed that the intestinal tract represents an important barrier to MC-LR: 4.4% and 1.5% of the applied dose reached the blood and liver in 3 days (Tencalla and Dietrich, 1997), and only ca. 0.28–1.29% of the applied dose in the liver and muscle in 24 h (Bury et al., 1998). Such functioning as a barrier to MC-LR might have made great development in the intestinal tract of silver carp. It is likely that although silver carp ingested much MCs by feeding, they might be able to avoid intoxification through efficient inhibition of MC-LR transportation across gut walls and through massive excretion of the toxins as feces. This might be evolutionarily important for phytoplanktivorous fish like silver carp because MC-LR is much more toxic than MC-RR.

The present study indicates that there were no significant correlations in MC concentration between the intestine and all the other organs of silver 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). On the other hand, MC concentration in the kidney and muscle had a strong correlation with the concentration in the liver. Similarly, in an Egyptian fish farm, there were significant correlations in MC concentration among the liver and kidney, muscle of the tilapia *O. niloticus* (Mohamed et al., 2003).

In the present study, average MCs in the intestine, liver and muscle of silver carp were 24.3, 0.957 and 0.197 $\mu g\ g^{-1}$ 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 $\mu g\ g^{-1}$ DW, respectively (Chen and Xie, 2005b). Apparently, silver carp ingested much more toxins than bivalves, but accumulated less in liver and muscle. In Lake Chaohu, MCs in the gut of the silver carp (137 $\mu g\ g^{-1}$ DW) was 20 times more than those in the gut of carnivorous and omnivorous fishes (<6.5 $\mu g\ g^{-1}$ DW), however, MCs in the liver of silver carp (1.16 $\mu g\ g^{-1}$ DW) was significantly lower than those of carnivorous and omnivorous fishes (1.76–11.6 $\mu g\ g^{-1}$ DW) (Xie et al., 2005). Thus, it is likely that compared with mussels and other fishes, silver carp ingest more MCs but accumulate less MCs in liver, probably accounting for their greater resistance to microcystins.

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, for glutathione S-transferases (GST) and glutathione peroxidase (GPx) (Kondo et al., 1992, 1996; Ding et al., 2000). MC-LR can conjugate with GSH and ulteriorly 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). Kondo et al. (1996) identified the presence of this conjugate in the livers of rats treated with MC-LR, Pflugmacher et al. (1998) detected conjugation of MC-LR to GSH in enzyme extracts containing GST of aquatic macrophyte, invertebrates, fish eggs and fish. In the present study, MCLR-Cys was detected in most months (Jul.–Dec. 2004) in the kidney samples, and in the hind-gut content samples in several months, suggesting that conjugation of MC-LR with cysteine in the kidney and/or intestine might be an important route for the detoxification and excretion of MC-LR in silver carp. Similarly, an immunostaining study showed that the injected conjugates (MCLR-GSH and MCLR-Cys) were prominently observed in the intestine and kidney of mice (Ito et al., 2002). In their experiment, no effective accumulation of the injected conjugates in the liver was found in spite of the larger dosage, and the toxins might be effectively eliminated by an appropriate system such as the GS-X (ATP-dependent glutathione S-conjugate exported) pump (Suzuki et al., 1997). This may explain why we could not find MCLR-Cys in the liver samples of silver carp in the present study. 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.

In the present study, the progressive increase of MCs in intestine between August and October did not follow the evolution of MCs in the water column. This might be due to different degrees of digestion in the intestines at sampling or heterogeneity of food resources. On the other hand, significant amounts of MCs were present in muscle in June while only tiny MCs were detected in the lake water, suggesting that silver carp might be able to selectively collect *Microcystis* colonies (which are large in size than other phytoplankton) when the proportion of *Microcystis* was low in phytoplankton community. Similarly, in a Brazil lagoon, *T. rendalli* accumulated substantial amounts of MC in muscle, liver and viscera just after detection of MC in the seston sample, and even during periods when water blooms declined, and there was no MC detectable in seston

samples, MC continued to be detected in fish muscle and liver (Magalhàes et al., 2001). It should be noted, however, that MCs content in the different organs might be the result of an integration of consumed *Microcystis* over several days or weeks, thus one single sample of water for MCs quantification for a whole month might not be representative of MCs presence in water during that month. Therefore, it might be necessary to obtain several water samples during several days preceding silver carp sampling in our future study, so as to obtain reliable conclusions relating MCs in the environment and MCs in silver carp.

WHO proposed a provisional tolerable daily intake (TDI) of 0.04 $\mu g \ kg^{-1}$ bw per day for MC-LR (Chorus and Bartram, 1999). We estimated for the fish muscle the critical amount (g wet weight) that is necessary to ingest to reach the TDI for MC. According to our measurements, mean water contents in the muscle of silver carp was 80.4% (± 0.89%, $n = 5$), and therefore a coefficient of 5 was used to convert dry weight to wet weight of fish muscle. 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. In the present study, MCs in muscle of silver carp ranged from 0.25 to 96.52 (average 16.13) ng LR$_{equivalent}$/g fresh weight and intracellular MC averaged 4.16 $\mu g \ L^{-1}$. Considering an adult of 60 kg, who ingests, on the average, 100 g of fish muscle a day, 2 of the 12 analyzed muscle samples (16.7%) were above the TDI recommended by WHO. In a coastal Lagoon of Brazil, MC in seston averaged 4.7 (maximum 17.1) $\mu g \ L^{-1}$, while MC in muscle samples of *T. rendalli* averaged 27.5 (maximum 337.3) ng g^{-1}, and during the entire study period, 71.7% of fish muscle samples had MC concentrations close to or above the advisable TDI value (Magalhàes et al., 2001). In an Egyptian fish farm where toxic bloom of *M. aeruginosa* occurred, MCs in phytoplankton was 1.12 mg/g dry weight, and MCs in the muscle of *O. niloticus* reached 45.7–102 ng/g fresh weight (Mohamed et al., 2003). It seems that under similar eutrophic status, silver carp accumulate less MCs in muscle than other fish species (e.g., tilapia), indicating a less risk for human consumption.

The present study indicates that in Lake Taihu, silver carp muscle should not be consumed during period of dense cyanobacterial blooms (e.g., June and July in 2004). Also, the high amounts of MCs in intestines between the months of July and October could be a potential risk if consumption of silver carp includes viscera. To obtain more reliable conclusion

for public health management actions, future works need to be done to offer enough data to establish a good predictive model between cyanobacterial densities (or MCs concentration in water column) and MCs in muscle.

5. Conclusion

The large net cage experiment conducted in Lake Taihu where dense toxic cyanobacterial blooms occurred shows that the phytoplanktivorous silver carp could effectively ingest toxic *Microcystis* cells (up to 84.4% in total phytoplankton), but showed fast growth (from 141 g to 1759 g in one year in mean weight). Silver carp accumulated less microcystins in liver than other animals in the same site or other fish from different water bodies at similar level of toxin ingestion. There was possible inhibition of the transportation of the most toxic MC-LR across the gut-wall. It is recommended that muscle of silver carp in Lake Taihu should not be consumed during period of dense *Microcystis* blooms while viscera could be a potential risk for consumption in more months.

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