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Transfer, distribution and bioaccumulation of microcystins in the aquatic food web in Lake Taihu, China, with potential risks to human health

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ABSTRACT

In this paper, accumulation and distribution of microcystins (MCs) was examined monthly in six species of fish with different trophic levels in Meiliang Bay, Lake Taihu, China, from June to November 2005. Microcystins were analyzed by liquid chromatography electrospray ionization mass spectrometry (LC-ESI-MS). Average recoveries of spiked fish samples were 67.7% for MC-RR, 85.3% for MC-YR, and 88.6% for MC-LR. The MCs (MC-RR+MC-YR+MC-LR) concentration in liver and gut content was highest in phytoplanktivorous fish, followed by omnivorous fish, and was lowest in carnivorous fish; while MCs concentration in muscle was highest in omnivorous fish, followed by phytoplanktivorous fish, and was lowest in carnivorous fish. This is the first study reporting MCs accumulation in the gonad of fish in field. The main uptake of MC-YR in fish seems to be through the gills from the dissolved MCs. The WHO limit for tolerable daily intake was exceeded only in common carp muscle.

1. Introduction

Eutrophication in lakes, reservoirs, and recreational water with frequent occurrence of cyanobacterial blooms has been recognized as a worldwide problem (Codd, 1995). Many of the bloom-forming cyanobacteria share the ability to produce a wide variety of bioactive compounds including neurotoxins, hepatotoxins, cytotoxins, and lipopolysaccharide endotoxins, which create a significant water quality problem (Carmichael, 1997). Among cyanotoxins, microcystins (MCs) are considered to be the most common and one of the most dangerous groups (Chorus and Bartram, 1999).

Microcystins are known to be potent hepatotoxins (Codd, 1995) and tumor promoter (Nishiwaki-Matsushima et al., 1992). So far, more than 80 structural variants of MCs have been identified (Babica et al., 2006). Exposure to MCs represents a health risk to aquatic organisms, wild life, domestic animals, and humans upon drinking or ingesting cyanobacteria in the water (Malbrouck and Kestemont, 2006). Chronic toxic effects from exposure through drinking water or contaminated food are particularly probable, especially if there is long-term frequent exposure. In a recent study by Chen et al. (in press), microcystins were identified for the first time in the serum of a chronically exposed human population (fishermen at Lake Chaohu, China) together with indication of hepatocellular damage. The high incidence of primary liver cancer in Haimen City (Jiangsu Province) and Fusui County (Guangxi Province) in China is considered to be related to microcystins in drinking water (Yu, 1995). Moreover, it is reported that microcystins can be transferred along food chain (Ibelings et al., 2005; Smith and Haney, 2006), suggesting a potential risk to high trophic level species and human consumption of contaminated aquatic products.

Extensive laboratory experiments and field investigations have been conducted to document bioaccumulation and

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distribution of MCs in a variety of aquatic organisms (Xie et al., 2004; Chen and Xie, 2005a,b; Zhang et al., 2007). But studies on the transfer and accumulation of MCs in the food web are scarce (Ibelings et al., 2005; Xie et al., 2005; Smith and Haney, 2006).

The major objectives of this study were 1) to examine bioaccumulation and distribution of three common microcystins (MC-RR, -YR, and -LR) in some food web compartments, 2) to suggest possible/potential routes for the transfer of MCs among the different food web compartments, 3) to measure bioaccumulation of MCs in fish gonad, and 4) to evaluate the potential threat of the contaminated fish to human health.

2. Materials and methods

2.1. Sample collection and preparation

2.1.1. Study area

Lake Taihu, the third largest freshwater lake in China, is located in the east part of China. It has a total surface area of approximately 2338 km^2, with a mean water depth of 1.9 m and a maximum depth of about 2.6 m. This lake is an important resource for drinking water, irrigation, aquaculture and industrial water, and about 35 million people inhabit the 36,500 km^2 watershed of Taihu Lake. During the past decades, with the rapid development of economy and serious industrial and agricultural pollution, the lake has undergone a steady increase in eutrophication, and with a regular occurrence of cyanobacterial surface blooms in the warm seasons each year (Pu et al., 1998a,b). The present study was carried out in Meiliang Bay, the most eutrophic part of Lake Taihu, with a total surface area of 125 km^2 (Fig. 1(a)). Dense scum of toxic cyanobacterial blooms (primarily *Microcystis*) occurs frequently in the water surface of the bay in the warm seasons every year.

In order to eliminate the toxic blooms, a large fish pen was built in the Meiliang Bay in 2003, with a total area of 1.08 km^2 and a mesh size of 2 cm × 2 cm (Fig. 1(b)). From December 2004 to January 2005, 24,775 kg of silver carp and 8005 kg of bighead carp fingerlings were stocked into the fish pen, respectively.

2.1.2. Sampling of phytoplankton and collection of cyanobacterial blooms

Water samples for phytoplankton and MCs analysis were collected monthly from the surface and near the bottom using a 5-L nodified Patalas's bottle sampler from June to November 2005. Each integrated water sample was a mixture of two subsamples: one from 0.5 m below the surface and one from 0.5 m above the bottom. We preserved 50 ml of the integrated water sample with 5% formaldehyde for the cell counting of *Microcystis* spp. In the laboratory, colonial *Microcystis* spp. cells were separated by sonication for 1 min with an ultrasonic probe (250 W, 20 KHz; Sonics JY88-||; Ningbo Scientz Biotechnology Co., Ltd, China) and 0.1 ml above samples were counted directly under a magnification of 600×.

To estimate MCs concentration in the lake water (intracellular and extracellular toxins), one liter of the integrated water sample was filtered through a Whatman glass-fiber filter ([GF/C], Whatman, Brentford, UK). Seston onto the glass-fiber filter and the filtrate were used to detect intracellular and extracellular toxins, respectively.

Cyanobacterial blooms were collected from the surface water with a plankton net (mesh size, 64 μm; diameter of net opening, 20 cm) from June to October 2005, and immediately frozen at -20 °C and then freeze-dried for toxin analysis.

2.1.3. Sampling of fish

Six species of fish were captured monthly from the fish pen by multi-mesh gillnets from June to November 2005. The fishes were classified into three types according to their food habits: the phytoplanktivorous silver carp *Hypophthalmichthys molitrix*, the omnivorous species (*Carassius auratus*, *Cyprinus carpio*), and the carnivorous species (*Culter ilishaeformis*, *Neosalanx taihuensis*, and *Coilia ectenes*). All six species are native to Lake Taihu and the latter five species were abundant in the lake. Silver carp was artificially stocked into the pen in January 2005, for the purpose of counteracting cyanobacterial blooms. The other species of fish were wild

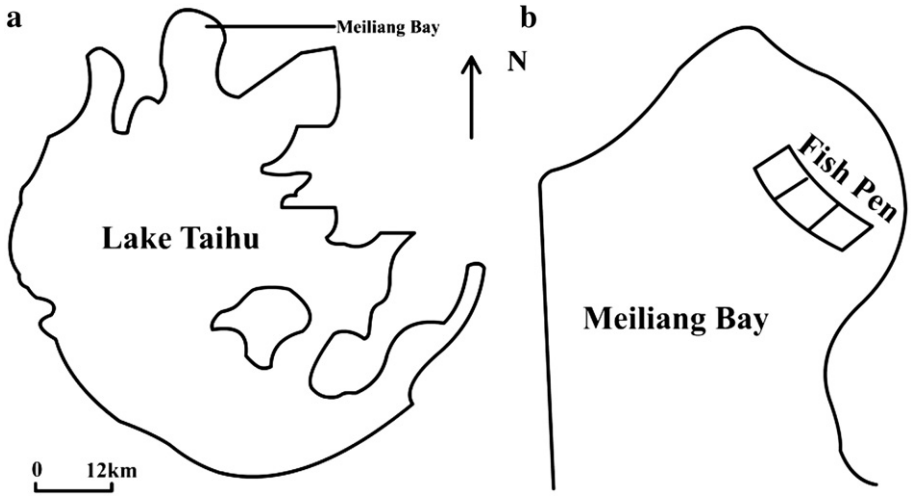

Fig. 1 – The sketch of Lake Taihu (a) and the location of fish pen (b) in Meiliang Bay.

species inhabiting in the pen. The collected fish were measured, weighed, and sacrificed immediately. Silver carps with a mean wet body weight of 1021±325 g and a body length of 34.5±4.8 cm for *H. molitrix*. *C. auratus* had a mean wet body weight of 93±38 g and a body length of 14.9±1.1 cm, *C. carpio* had a mean wet body weight of 167±84 g and a length of 19.9±2.9 cm, *C. ilishaeformis* had a mean wet body weight of 216±54 g and a length of 23.6±1.5 cm, *N. taihuensis* had a mean wet body weight of 2.0±0.8 g and a length of 7.5±1.1 cm, and *C. ectenes* had a mean wet body weight of 15±7 g and a length of 16±2 cm. These fish were dissected in the field into intestines, gut content, muscle, liver, and gonad (*C. auratus* and *C. carpio*), and then frozen at −20 °C immediately.

2.2. *Microcystin analysis*

MCs in the seston of water column and bloom samples were analyzed quantitatively according to Park and Lwami (Park and Lwami, 1998).

All samples of fish were lyophilized prior to MCs analysis. Extraction and analysis of MCs in the animal organs basically followed the method of Zhang et al. (2007) with minor modification: lyophilized samples (~0.3 g DW for each tissue) were homogenized and extracted three times with 25 ml of butanol: methanol: water (1:4:15) for 24 h while stirring. The extract was centrifuged at 36,290 x*g* and the supernatant was diluted with water. This diluted extract was directly applied to a C_{18} reversed- phase cartridge (5 g), which had been preconditioned by washing with 50 ml of 100% methanol and 50 ml of distilled water. The column was washed with 50 ml of water and 100 ml of 20% methanol. Elution from the column with 100 ml of 90% methanol yielded the MC-containing fraction. The MCs containing fraction was evaporated to dryness, and the residue was then redissolved in methanol. This solution was applied to a silica gel cartridge (2 g) that had been preconditioned by 10 ml of 100% methanol. The column containing the MCs was washed with 10 ml of 100% methanol and then eluted with 20 ml of 70% methanol. The MC-containing fraction also was evaporated to dryness. This fraction was dissolved with 100 μl of distilled water and used for the final detection and identification of MCs by liquid chromatography-mass spectrometry (LC-MS).

Qualitative and quantitative analysis of MCs was performed using a Finnigan LC-MS system equipped with a Thermo Surveyor auto sampler, a Surveyor mass spectrum (MS) pump, a Surveyor photo diode array (PDA) system, and a Finnigan LCQ-Advantage MAX ion trap mass spectrometer (Thermo Electron, Waltham, MA, USA) equipped with an atmospheric pressure ionization fitted with an electrospray ionization source (ESI) (Thermo Electron). The instrument control, data processing, and analysis were conducted by using Xcalibur software (Thormo Electron). An Agilent Zorbax SB-C_{18} column (length, 100 mm; inner diameter, 2.1 mm; film thickness, 3.5 μm; Agilent Technologies, Santa Clara, CA, USA) was used for LC separation. The column oven temperature was 45 °C. The mobile phase consisted of solvent A [water+0.05% (v/v) formic acid]/solvent B [acetonitrile+0.05% formic acid]. The linear gradient elution program was described as follow: 0 min 25% B, 8 min 55% B, 13 min 60% B, 14 min 70% B, 15 min 25% B, 20 min 25% B. The flow rate was held at 0.2 ml/min at analyze stage, and was increased to 0.3 ml/min for 5 min before the next injection to renew the initial condition rapidly. The volume of sample injected was 10 μl. Mass spectrum tuning and optimization were achieved by infusing microcystin-RR and monitoring the $[M+2H]^{2+}$ ion at *m/z* 520. The MS analytical 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; and tube lens offset, 55v. Data acquisition was in the positive ionization centroid mode. The MS detection was operated in four segments: First, full scan mode with a mass range from 400 to 1400, 4.2 min duration; second, two scan events: full scan mode as same as segment 1 and MS^2 mode with a mass range from 140 to 1100, parent ion: 520; isolation width: 1; normalized collision energy: 37%; 4.8 min duration; third, three scan events: full scan mode as same as segment 1 and MS^2 mode with mass range from 270 to 1100 and 285 to 1100, respectively; parent ion: 995.5 and 1045.5, respectively; isolation width: equal for both, normalized collision energy equal for both, 35%; 4.8 min duration; fourth, full scan mode as same as segment 1.

Standards of pure MCs (MC-LR, -YR, and -RR) were purchased from the Pure Chemical Industries, Osaka, Japan. The limit of detection for the MCs in the tissues of fish was 0.003 μg/g DW.

2.3. *Recovery experiment*

Recovery experiments were carried out in quadruplicate spiking 300 mg of freeze-dried fish samples (liver and muscle) with mixed MCs solution of the three commercial standards (MC-RR, MC-YR and MC-LR) at 1 μg/g. The extraction and analysis was performed as described previously, and the recovery and the relative standard deviation of the analytical method were calculated.

3. Results

3.1. *Microcystis biomass and intracellular and extracellular toxins in the water column and concentration in surface blooms*

During the study period, *Microycstis aeruginosa* comprised a large proportion of phytoplankton community (73.8%–97.3%), and *Microcystis* biomass ranged from 0.56 to 10.31 mg/L with a maximum in July (Data not showed).

The MCs (MC-RR+MC-YR+MC-LR) concentration in surface cyanobacterial blooms ranged from 328.0 μg/g DW to 780.2 μg/g DW with an average of 540.7 μg/g DW from June to October, and the mean ratio of MC-RR to MC-LR in cyanobacterial blooms was 1.83 (Fig. 2). Microcystins concentration in seston varied considerably among months, ranging from 0.31 μg/L to 1.37 μg/L (in September), with an average of 0.78 μg/L, and the mean ratio of MC-RR to MC-LR in seston was 1.68 (Fig. 2). Dissolved MCs in the water column was relatively low with a maximum level of 0.042 μg/L in July, and the proportion of MC-YR in total dissolved MCs varied from 19.1% to 71.8% with an average of 46.8%, and the highest MC-YR concentration in the water column was found in July (Fig. 2).

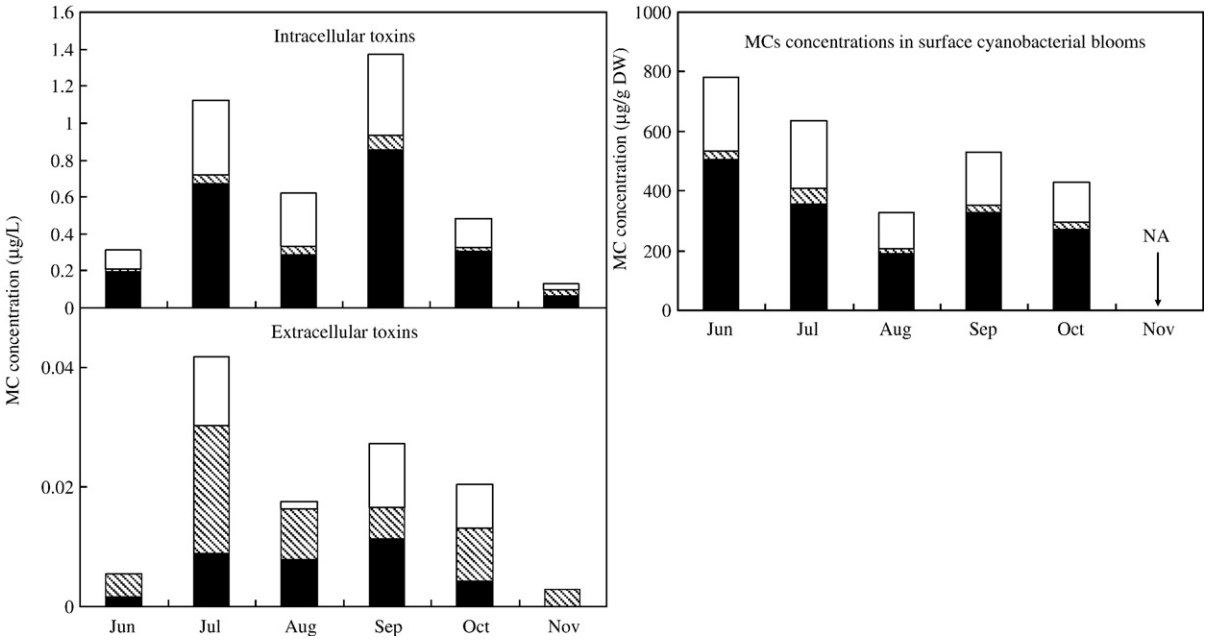

Fig. 2 – Intracellular toxins and extracellular toxins concentration in the water column, and MCs concentration in surface cyanobacterial blooms in fish pen of Lake Taihu. –□– LR; – ▨–YR; –■– RR, NA = not analysed.

3.2. *Distribution and bioaccumulation of MCs in organs of various aquatic organisms*

3.2.1. *Carnivorous fish*

Microcystins concentration was very low in the whole body of *N. taihuensis* (the maximum<0.008 μg/g DW) (Fig. 3). MCs concentration was low in both visceral organs (0.007 μg/g DW in June and 0.045 μg/g DW in October) and muscle (below detection limit — 0.0074 μg/g DW) of *C. ectenes* (Fig. 3). MCs concentration of *C. ilishaeformis* varied between 0.019 μg/g DW and 0.039 μg/g DW in gut content, and from undetectable levels to 0.047 μg/g DW in liver, and, form undetectable concentration to 0.027 μg/g DW in muscle (Fig. 3). Only MC-RR was detected in the muscle of the above fishes. Generally, MCs concentration in the carnivorous fish was relatively low.

3.2.2. *Phytoplanktivorous fish*

MCs concentration in gut content, liver, muscle, and intestinal walls of *H. molitrix* showed great monthly variations (Fig. 4). MCs concentration in gut content was relatively high, ranging from 39.0 μg/g DW to 233.5 μg/g DW during the period from August to October, and the mean proportion of MC-LR in total MCs was 28.1%. MCs concentration in liver varied between 0.020 μg/g DW and 0.10 μg/g DW with an average of 0.048 μg/g DW and the maximum in July. Moreover, in liver, concentration of MC-LR was higher than that of MC-RR, and the proportion of MC-LR in total MCs was 51.6%. MCs concentration in intestinal wall ranged from undetectable concentration to 5.36 μg/g DW with an average of 1.72 μg/g DW, and the proportion of MC-RR in total MCs was more than 98% except for the sample in November when no MC-RR was detected. Total MCs concentration ranged from undetectable concentration to 0.075 μg/g DW with an average of 0.026 μg/g DW, whereas no MC-LR was detectable in muscle.

3.2.3. *Omnivorous fish*

MCs concentration in *C. auratus* and *C. carpio* are shown in Figs. 5 and 6. The highest peak of MCs concentration in gut content of *C. auratus* (73.27 μg/g DW) was much higher than that of *C. carpio* (8.69 μg/g DW). The MCs concentration in liver, intestinal wall, gonad and muscle of *C. auratus* ranged from 0.016 to 0.15, from 0.019 to 0.21, from 0.0026 to 0.13, and from undetectable level to 0.013 μg/g DW, respectively (Fig. 5), and the average MCs concentrations in these organs were 0.070, 0.068, 0.034, and 0.024 μg/g DW, respectively. In *C. carpio*, the MCs concentration in liver, intestinal wall, gonad, and muscle varied between 0.0031 and 0.17, between 0.011 and 0.064, between 0.0014 and 0.083, and between 0.0023 and 0.17 μg/g DW, respectively, and with average values of 0.025, 0.032, 0.027, 0.067 μg/g DW, respectively (Fig. 6).

3.3. *Accumulation patterns of different species of fish*

Mean MCs concentration in gut content, liver and intestinal wall was the highest in *H. molitrix*, followed by *C. auratus* and *C. carpio*, and was the lowest in *C. ilishaeformis* (MCs were not examined in intestinal wall of *C. ilishaeformis*). However, the mean MCs concentration in muscle was the highest in *C. carpio*, followed by *C. auratus*, *H. molitrix*, *C. ilishaeformis*, and was the lowest in *C. ectenes* and *N. taihuensis* (Table 1).

3.4. *Recovery experiment*

The average recoveries from fish ($n=4$) liver and muscle ($n=4$) were 61.5% (ranging from 52.3% to 73.4%) and 74% (ranging from 67.8% to 78.2%) for MC-RR, respectively, with relative standards deviations (RSDs) between 6 and 8%, 83.7% (ranging from 79.2% to 88.4%) and 86.8% (ranging from 83.7 to 89.7%) respectively, with relative standards deviations between 2 and

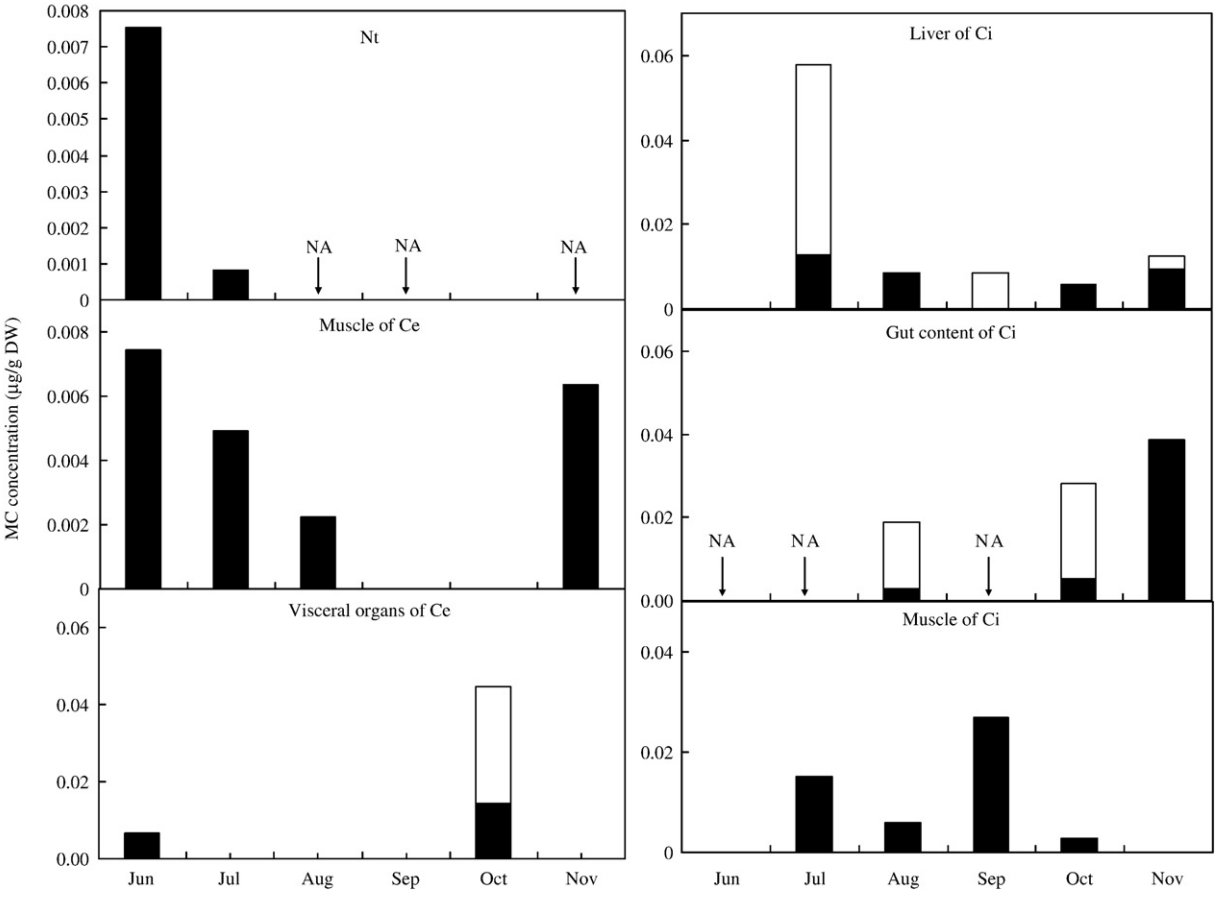

Fig. 3 – Seasonal variations of MCs concentration in whole tissue of *Neosalanx tangkahkeii taihuensis*, visceral organs and muscle of *Coilia ectenes*, gut content, liver, and muscle of *Culter ilishaeformis* from Lake Taihu. NA = not analysed; Nt = *Neosalanx tangkahkeii taihuensis*; Ce = *Coilia ectenes*; Ci = *Culter ilishaeformis*; –□– LR; –▧–YR; –■– RR.

10% for MC-YR, and 85.7% (ranging from 81.8 to 90.3%) and 91.4% (ranging from 86.9% to 94.6%) respectively, with RSDs between 8 and 10% for MC-LR.

4. Discussion

In the present study, MCs concentration in liver and gut content was the highest in phytoplanktivorous fish, followed by omnivorous fish, and was lowest in carnivorous fish; whereas MCs concentration in muscle showed a different pattern: MCs concentration was highest in omnivorous fish, followed by phytoplanktivorous fish, and was the lowest in carnivorous fish. These results suggest that MCs could be transferred through the food web, and that MCs concentration in internal organs of fish was dependent on MCs level in their food (gut content). Similar results have been found in previous studies. Ibelings et al. (2005) studied the distribution of microcystins in the food web of Lake IJsselmeer and found that transfer of microcystins within the food web takes place, despite no evidence for biomagnification. Smith and Haney (2006) examined MCs concentrations in three levels (phytoplankton, zooplankton, and sunfish (*Lepomis gibbosus*)) of an aquatic food web and found evidence for the direct transfer of MCs from zooplankton to sunfish and the subsequent accumulation of toxin in the liver tissue. In contrast, Xie et al. (2005) reported that MCs showed a general tendency to accumulate up the food chain in Lake Chaohu: MCs concentration in the liver and muscle was the highest in carnivorous fish, followed by omnivorous fish, and was the lowest in phytoplanktivorous and herbivorous fish; but in the gut MCs concentration generally showed a reversed pattern: concentration was the highest in the phytoplanktivorous fish, followed by omnivorous and carnivorous fish. It is necessary to point out that microcystins concentration of fish tissues in the present study refers to free microcystins, however, a great majority of microcystins in the tissues of aquatic organisms are bound covalently in a complex with protein phosphatases (Williams et al., 1997a,b), and the covalent microcystin-PPase complex could not be extracted with the method used in the present study. Moreover, the toxicity of the covalent microcystin-PPase complex may be in the order of the microcystin-glutathione conjugates (Ibelings et al., 2005). Hence, the contribution of covalent microcystin-PPase in the food web is so far uncertain.

There have been several studies to examine MCs accumulation in gonad of aquatic invertebrates. Microcystins accumulation was found in the gonad of a gastropod (*Bellamya aeruginosa*) and two shrimps from Lake Chaohu (Chen and Xie, 2005b; Zhang et al., 2007). Zhang et al. (2007) found correlation

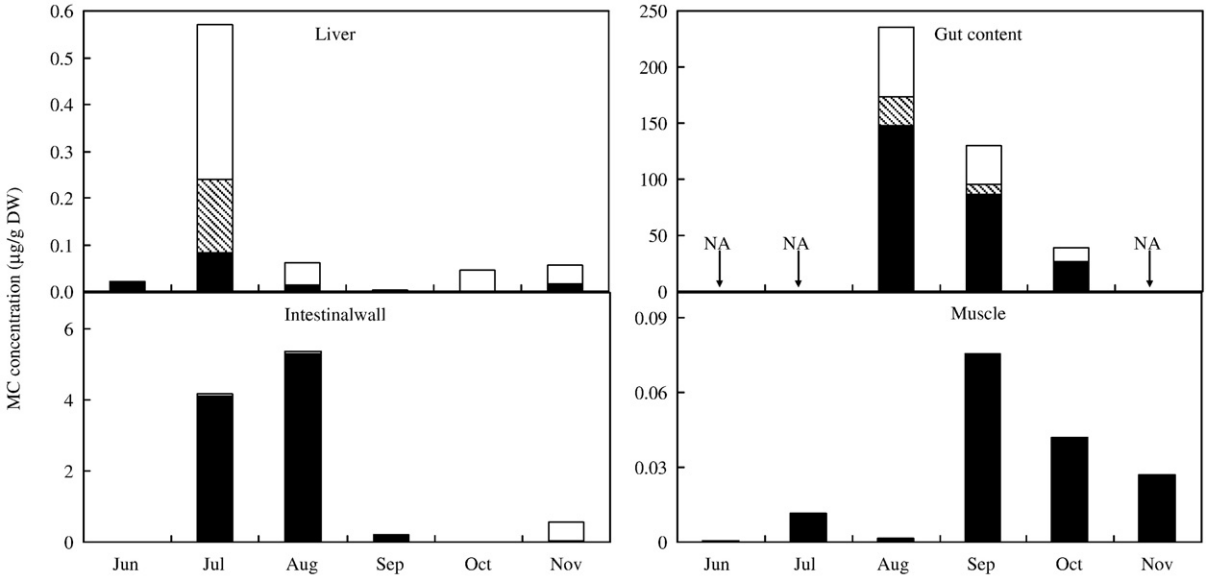

Fig. 4 – Seasonal variations of MCs concentration in gut content, liver, muscle, and intestine of *Hypophthalmichthys molitrix* from Lake Taihu, NA = not analysed; –□– LR; – ▨–YR; –■– RR.

in MCs concentration between gonad and offspring of *B. aeruginosa* (Lake Taihu) and indicated that MCs were likely transferred from adult females to their young snails with physiological connection. The present study is the first to confirm the presence of MCs in gonads of two fish species (*C. carpio* and *C. auratus*), suggesting a potential threats to reproductive system of fish in natural conditions.

There were several studies on the effect of microcystins to the reproductive system of animals. Ding et al. (2006) pointed out that when male mice exposed intraperitoneally to 3.33 or

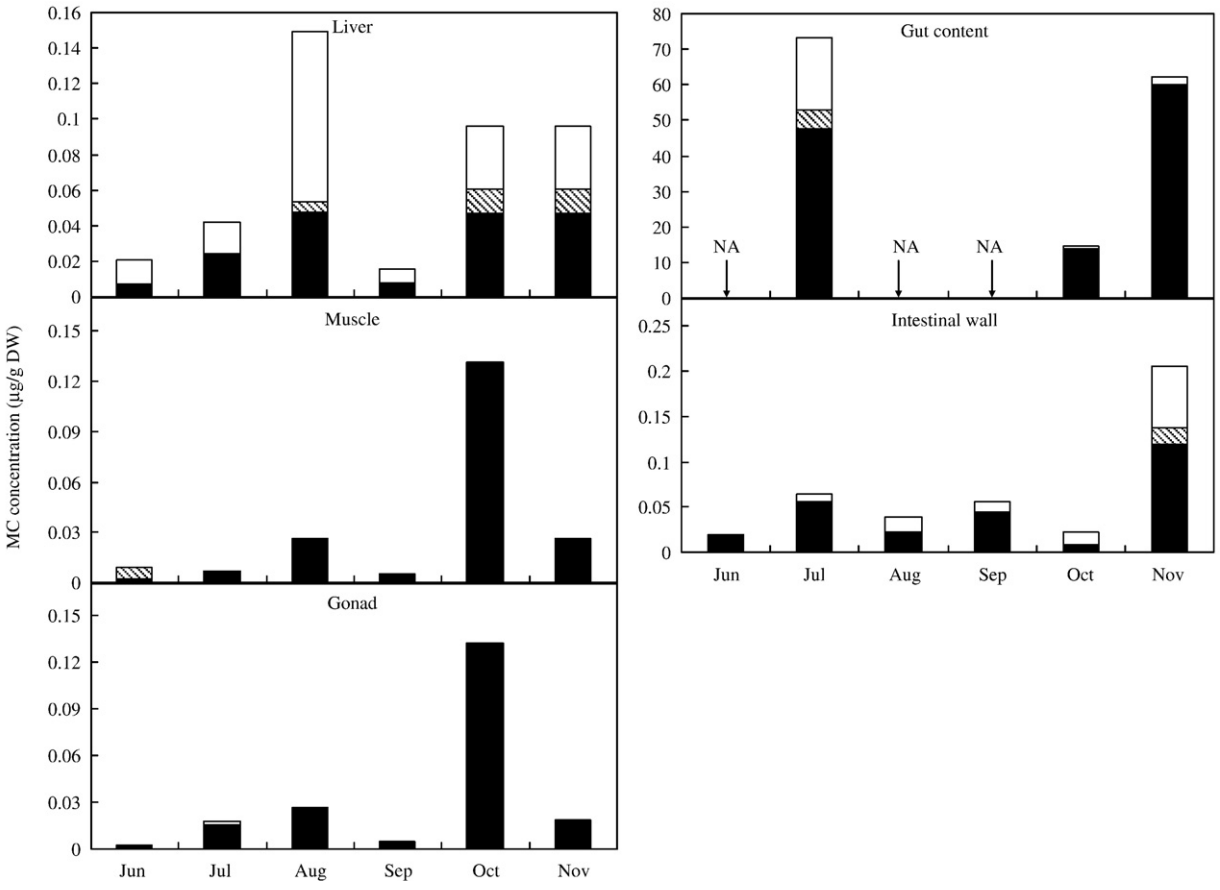

Fig. 5 – Seasonal variations of MCs concentration in gut content, intestine, liver, gonad, and muscle of *Carassius auratus* from Lake Taihu, NA = not analysed; –□– LR; – ▨–YR; –■– RR.

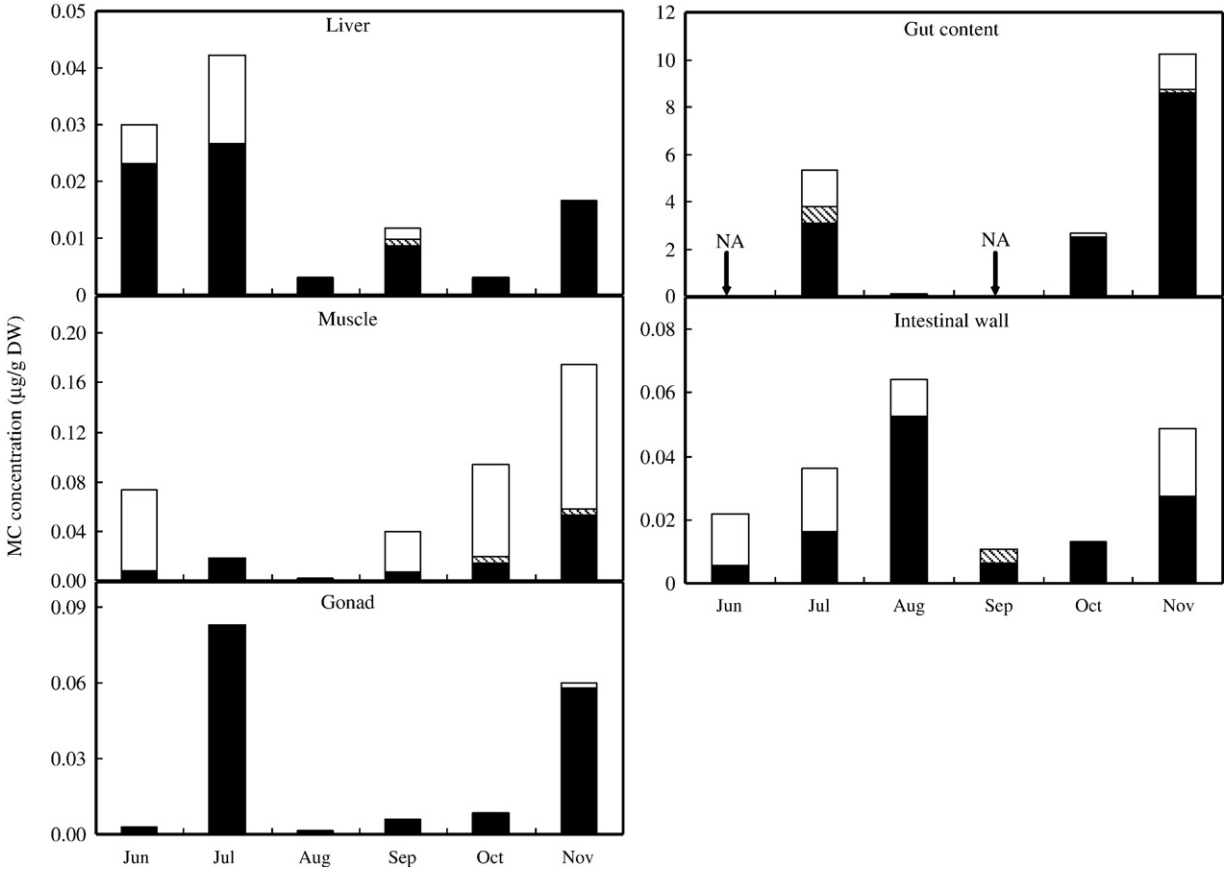

Fig. 6 – Seasonal variations of MCs concentration in gut content, intestine, liver, gonad, and muscle of *Cyprinus carpio* from Lake Taihu, NA = not analysed; –□– LR; – ▨–YR; –■– RR.

6.67 μg/g microcystins/kg body weight for 14 days, their mean body weight decreased, seminiferous tubules were damaged, the quality of mature sperm in the seminiferous tubules was decreased and the motility and viability of the sperm were reduced. Gérard et al. (2005) reported that when the pond snail *L. stagnalis* were exposed to 33 μ g/L MC-LR for a period of 6 weeks, survival, growth, and locomotion of the snail were not affected, but their fecundity decreased to half. Baganz et al. (1998) reported that when zebrafish (*Danio rerio*) were exposed to 50 μg/L MC-LR, their spawning activity and success were reduced. According to the present study, microcystins in the gonad of common and crucian carp represents a potential risk to their reproduction. This may occur during long-term exposure to low MCs concentrations.

Many studies indicate that microcystins can cause various histopathological changes in various tissues (liver, intestine, kidney, spleen, heart, gills) of fish (Zimba et al., 2001; Qiu et al., 2007). Qiu et al. (2007) examined the pathological changes in the liver of fish (*H. molitrix*, *C. auratus*, and *C. ilishaeformis*) studied in the present study, and found that liver of *C. ilishaeformis* was damaged more seriously, followed by *C. auratus*, and *H. molitrix*. However, MCs concentration in the liver of different fish showed a reverse pattern in comparison with the pathological changes of different fish: the highest concentration in liver was found in *H. molitrix*, followed by *C. auratus*, and was lowest in *C. ilishaeformis*. These results suggest that phytoplanktivorous fish may have developed special mechanisms to counteract MCs in their history of evolution and can resist to MCs efficiently, but the carnivorous fish are more sensitive to MCs.

It is well known that the intestinal wall of fish forms an important barrier to microcystins transport (Tencalla et al.,

Table 1 – Mean MC content (μg/g DW) in various organs of *Hypophthalmichthys molitrix* (Hm), *Carassius auratus* (Ca), *Cypinus carpio* (Cc), *Culter ilishaeformis* (Ci), *Coilia ectenes* (Ce), and *Neosalanx taihuensis* (Nt) during the study period

	Hm	Ca	Cc	Ci	Ce	Nt
Gut content	135±98.1	50.1±31.11	4.58±4.34	0.029±0.010	NA	NA
Liver	0.126±0.219	0.070±0.053	0.018±0.016	0.016±0.021	NA	NA
Intestinal wall	1.72±2.39	0.068±0.070	0.032±0.021	NA	NA	NA
Muscle	0.026±0.029	0.034±0.049	0.067±0.063	0.008±0.011	0.004±0.003	0.003±0.004

NA = not analysed.

1994; Tencalla and Dietrich, 1997; Bury et al., 1998; Xie et al., 2004; Chen et al., 2006, 2007). Tencalla et al. (1994) estimated that rainbow trout only absorbed 10% of MCs from toxic *Microcystis aeruginosa* cells in 24 h, and Bury et al. (1998) found evidence for only 0.3% transfer when fish were gavaged with purified MC-LR.

In the present study, the average MCs concentration in gut content of *H. molitrix*, *C. auratus*, *C. carpio*, and *C. ilishaeformis* was 134.85, 50.10, 4.33, 0.029 μg/g DW, respectively, while in liver, where microcystins mainly accumulate, the mean concentration was 0.13, 0.07, 0.018, 0.016 μg/g DW, respectively, demonstrating that the intestinal wall of the fish (*H. molitrix*, *C. auratus*, *C. carpio*) which was in contact to toxic cells directly, can inhibit the transport of microcystins into the internal organs efficiently. In contrast, it seems unlikely that the intestinal wall of *C. ilishaeformis* can inhibit the transport of MCs efficiently. This may be due to different uptake routes and metabolisms of MCs and feeding type of fish. Xie et al. (2004) indicated that routes other than the gastrointestinal tract (e.g. gills) are important for the uptake of MCs by carnivorous fish such as *P. pekinensis* and *C. ilishaeformis* from Lake Chaohu. The intestinal wall of *S. histrica* can also form a barrier to MCs: MCs concentration in hepatopancreas was only 1/6 that in intestine of a resident snail (*S. histrica*) in Lake Biwa in October (Ozawa et al., 2003). However, the intestinal wall of *B. aeruginosa* had no effect on MCs transport, since the MCs concentration in hepatopancreas was higher than that in intestine (including gut content) from June to October 2005 (Zhang et al., 2007). These results suggest that different aquatic animals may have different metabolism routes and accumulation pattern of MCs.

In the present study, MCs were present in intestinal wall of silver carp, common carp, and crucian carp, and variable amounts of MCs were present in the gut wall, suggesting that MCs were mainly absorbed via gastrointestinal tract into the internal organs (especially highly blood-irrigated organs like liver, kidney etc) of these three fish. Tencalla et al. (1994) demonstrated that it was nontoxic to trout when they were exposed to aquarium water containing 8–16 mg of freeze-dried algae/L, but trout died within 96 h when gavaged with 1440 mg of freeze-dried algae per kg body weight (BW) (equivalent to 6.6 mg of MCs kg^{-1} BW).

In the present study, only very much low concentration of MC-YR was detected in the fish in comparison with MC-RR and MC-LR. Moreover, in the intestinal wall of silver carp, common carp, and crucian carp, MC-YR was detectable in only one sample from each species, but in the internal organs (liver, muscle), during some months, MC-YR was detected when it was not detected in intestinal wall. In July, the highest MC-YR concentration occurred in liver (*C. auratus*), and there was a maximum concentration of dissolved MC-YR (0.021 μg/L) in the lake water. This suggests that the main uptake of MC-YR in *C. auratus* was not via the gastrointestinal tract, but through the gills from the dissolved MCs or other mechanisms.

In China, people used to take fish gonad and muscle as delicious food, however, toxic cyanobacterial blooms occurred frequently in eutrophic freshwater bodies, so it is potentially dangerous for human to ingest fish or other aquatic animals in these lakes. To avoid potential health risk, the World Health Organization (WHO) established the tolerable daily intake (TDI) of 0.04 μg/kg body weight per day for MC-LR (Chorus and Bartram, 1999). As is described by Gupta et al. (2003), the intraperitoneal (i.p.) medium lethal dose (LD_{50}) in mice for MC-RR and -YR is about 5-fold and 2.5-fold that for MC-LR, corresponding to 0.2 and 0.4 MC-LR equivalents, respectively. Here, coefficients of 4, 4.55, 3.87, 4, 4.22, 4.68, 3.92, and 6.24 were used to convert dry weight to wet weight of gonad and muscle of common carp, gonad and muscle of crucian carp, muscle of *H. molitrix*, *C. ilishaeformis* and *C. ectenes*, and tissue of *N. taihuensis*, respectively. Assuming that a 60 kg person would consume 300 g edible tissues (gonad or muscle) per day, the TDI (2.4 μg/d) is exceeded only in the case of common carp (Jun. 185%, Oct. 217%, Nov. 355% of TDI; slightly<TDI in Sep. 93% of TDI). TDI was not exceeded with other muscle material (from below detection limit to 82% of TDI), nor with gonads (from 0.9 to 85% of TDI). Therefore, fish (except common carp) from Lake Taihu were safe to eat. There are however potential risks from long-term exposure to the MC-contaminated fish product that can not be ignored. Finally, it is necessary to point out that only uptake of microcystins from fish was used to evaluate exposure risk of human to MCs. However, drinking water is often the major route of human exposure to microcystins. In the present study, concentration of MC-$LR_{equivalent}$ in the water samples ranged from 0.059 to 0.657 μg/L. Although these values were all below the safety limit of 1 μg/L MC-LR, the combined risks from MC-contaminated drinking water and fish product to human health cannot be overlooked in the region around Taihu Lake.

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