

MICROCYSTIN ACCUMULATION IN FRESHWATER BIVALVES FROM LAKE TAIHU, CHINA, AND THE POTENTIAL RISK TO HUMAN CONSUMPTION

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Abstract—The potential risk through ingestion of microcystins (MC) in contaminated mollusks has not been well studied. The present paper studied seasonal changes of MC content (determined by liquid chromatography–mass spectrometry) in various organs of three species of bivalves (*Cristaria plicata*, *Hyriopsis cumingii*, and *Lamprotula leai*) in Lake Taihu, China, where toxic cyanobacterial blooms occurred. Coinciding with peaks of seston MC (maximum, 5.7 µg/L) and MC in cyanobacterial blooms (maximum, 0.534 mg/g), most organs showed sharp MC peaks during the summer, indicating both fast uptake and fast depuration by bivalves. Because hepatopancreas and intestine had considerably higher MC content than other organs, they are the most dangerous for human consumption. Both the present and previous studies show that the hepatopancreatic MC and total tissue MC often are correlated in various aquatic invertebrates. During the peak of the cyanobacterial blooms, *C. plicata* had higher hepatopancreatic MC content than the other bivalves, whereas *H. cumingii* had higher intestinal MC content than the other bivalves. Estimated daily intakes for humans from the consumption of whole tissues of the three bivalves were 0.48 to 0.94 µg MC-LR equivalent/kg body weight (12- to 23.5-fold the tolerable daily intake value proposed by the World Health Organization), which indicates a high risk for humans consuming these bivalves.

Keywords—Microcystins Seasonal variation Bivalves Lake Taihu Human consumption

INTRODUCTION

Cyanotoxins produced by toxic cyanobacteria, such as *Microcystis*, *Anabaena*, *Oscillatoria*, and *Nostoc* spp., in eutrophic freshwaters are causing serious ecological and human health problems worldwide [1]. Among these toxins, the hepatotoxic, cyclic peptides called microcystins (MCs) probably are the most important in terms of human health, because they are potent inhibitors of protein phosphatases 1 and 2A and are considered to be potent tumor promoters [2,3]. Microcystins are named according to their variable L-amino acids. For example, MC-LR contains leucine (L) and arginine (R), MC-RR contains two molecules of arginine (R), and MC-YR contains tyrosine (Y) and arginine (R). More than 70 variants of MCs have been reported, but MC-LR, MC-RR, and MC-YR are the most common species, with an order of toxicity of MC-LR > MC-YR > MC-RR based on laboratory toxicity tests [4,5]. So far, research has focused mainly on MC exposure via drinking water [5], whereas the risk of chronic intoxication through ingestion of products from contaminated fisheries (e.g., fish and mollusks) has not been well studied [6,7].

Limited information exists regarding MC accumulation by freshwater mollusks in the field [8–11], probably because freshwater mollusks usually are not consumed by humans in Europe and North America. Mollusks are considered to play an important role in the transfer of MCs to humans through food consumption in estuaries of eutrophic rivers [12,13] and also may affect terrestrial food webs [8]. Conversely, a recent study indicates that no biomagnification of MCs occurs in food chains (including seston, zooplankton, mollusks, and fish) [11]. Up to now, studies with bivalves frequently have been limited

to MC accumulation in the hepatopancreas [10] or the whole body [8,11], whereas little attention has been paid to MC distribution in various organs.

Many freshwater bivalves are consumed in Asia. For instance, in Japan, *Cobocula sandai*, a species of Corbiculidae, is an important organism for the local fish industry and a food resource for people who live around Lake Biwa [10,14]. In China, freshwater bivalves commonly are used not only for human consumption but also as feed for domestic fowl or crab culture. Asian immigrants in other countries also consume freshwater bivalves. At present, little is known concerning MC accumulation in freshwater bivalves in China, where freshwaters are threatened by heavy MC contamination.

The present study was conducted in a large, shallow, eutrophic Chinese lake, Lake Taihu, and had three objectives: To describe seasonal dynamics in MC content (MC-LR, MC-RR, and MC-YR) of various organs (e.g., intestine, hepatopancreas, and foot) in three freshwater, filter-feeding bivalves (*Hyriopsis cumingii*, *Cristaria plicata*, and *Lamprotula leai*), to discuss the possible mechanisms underlying the distribution pattern of MCs, and to evaluate the possible risk of consumption of MC-contaminated mussels by humans.

MATERIALS AND METHODS

Sample collection

Lake Taihu is a large (surface area, 2,338 km²), shallow (mean depth, 1.9 m), eutrophic lake in Jiangsu Province in subtropical China. During recent decades, the lake has experienced a regular occurrence of cyanobacterial blooms during the warm seasons of each year [15,16]. Meiliang Bay (surface area, 125 km²), a part of Lake Taihu, is the most eutrophic part of the lake, with dense *Microcystis* blooms during the summer. Annual chlorophyll *a* concentrations average 36.6

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$\mu\text{g/L}$ (Q. Zhou and Z.X. Ke, Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan, People's Republic of China, unpublished data). Several commercially important freshwater bivalves are in this area, including *H. cumingii*, *C. plicata*, and *L. leai*. The annual mean water temperature in Meiliang Bay of Lake Taihu was 17.8°C , with a minimum (3.2°C) during January and a maximum (33.4°C) during August. From May to October, water temperature varied between 19.1 and 33.4°C .

In Meiliang Bay, algal cells from surface cyanobacterial blooms were collected with a plankton net (mesh size, $64\ \mu\text{m}$; diameter of net opening, $20\ \text{cm}$). The average duration of time spent towing the net varied from several seconds to a couple of minutes, depending on the concentration of *Microcystis* cells. The algal samples were immediately frozen at -25°C and then freeze-dried for toxin analysis (MC concentrations in algal blooms). Water samples were collected from the surface and near the bottom using a 5-L, modified Patalas' bottle sampler, and 1-L mixed water sample (including algal cells) was filtered on glass-fiber filter (GF/C; Waterman, Brentford, UK). The filter was homogenized for subsequent toxin analysis (MC concentrations in seston). Both types of samples were collected from May to October/November 2004 when visible cyanobacterial blooms occurred.

Hyriopsis cumingii, *C. plicata*, and *L. leai* were collected monthly from surface sediment at one site in Meiliang Bay during October 2003 and September 2004. The collected animals were immediately frozen at -20°C and then dissected in the laboratory into six parts: Intestine (including intestinal walls), hepatopancreas, visceral mass (excluding hepatopancreas and intestines and, thus, mainly composed of gonad), gill, foot, and remaining tissue (excluding the above five parts and the shell). The collected organs were frozen at -80°C before MC analysis. We pooled, respectively, all intestine, hepatopancreas, visceral mass, gill, foot, and remaining tissue of five dissected animals (body length: *L. leai*, $100 \pm 6.5\ \text{mm}$; body wt: *L. leai*, $59.6 \pm 7.3\ \text{g}$; *H. cumingii*, $89.3 \pm 12.5\ \text{g}$; *C. plicata*, $114.9 \pm 27.8\ \text{g}$) (Table 1). Each value represents an average amount of MCs in the organs of five individuals.

Toxin extraction and analysis

Extraction and analysis of the three major forms of MCs (MC-LR, MC-RR, and MC-YR) in the organs ($\sim 0.5\ \text{g}$ of lyophilized sample for each organ) of the bivalves followed methods described previously [7]. Briefly, qualitative and quantitative analysis of MCs (MC-LR, MC-RR, and MC-YR) in the bivalves of Lake Taihu were performed using a Finnigan (Waltham, MA, USA) liquid chromatography–electrospray ionization–mass spectrum system.

Both freeze-dried algal cells ($0.1\ \text{g}$) and filters were extracted according to the methods described by Zheng et al. [17], and the final extraction samples were detected and identified by the above-mentioned liquid chromatography–mass spectrum method.

Recovery experiments

To evaluate the accuracy of the detection method used in the present study, we conducted recovery experiments in triplicate through spiking $500\ \text{mg}$ of homogenized, freeze-dried bivalve samples (hepatopancreas of *L. leai*, *H. cumingii*, and *C. plicata*) with a mixed MC solution of the two commercial standards (MC-RR and MC-LR) at $2.5\ \mu\text{g/g}$. Before spiking,

Table 1. Mean microcystin contents ($\mu\text{g/g}$ dry wt) in the digestive tract (D_{MC}), hepatopancreas (H_{MC}), and total body (T_{MC}) of various aquatic invertebrates^a

Species	BL (mm)	BW (g)	$D_{\text{MC}}/H_{\text{MC}}$ (D_{max})	H_{MC} (H_{max})	$H_{\text{MC}}/T_{\text{MC}}$ (%)	$H_{\text{wt}}/T_{\text{wt}}$ (%)	Reference
Bivalves							
<i>Lamprotula leai</i>	100 ± 6.5	59.6 ± 7.3	0.22 (2.52)	4.25 (13.23)	48.8 ^b	6.1 ^b	Present study ^c
<i>Hyriopsis cumingii</i>	190 ± 12.4	89.3 ± 12.5	1.12 (20.65)	3.42 (12.5)	55.4 ^b	8.3 ^b	Present study ^c
<i>Cristaria plicata</i>	240 ± 13.5	114.9 ± 27.8	0.29 (5.64)	5.79 (38.48)	53.9 ^b	2.9 ^b	Present study ^c
<i>Anadonta cygnea</i> ^d	90 ± 20	ND	ND	1.30	40.0 ^e	3.5 ^e	Eriksson et al. [24] ^f
<i>Unio douglasiae</i> ^d	ND	ND	0.48 (1.31)	2.72	ND	ND	Watanabe et al. [9] ^f
Snails							
<i>Bellamya aeruginosa</i>	21.6 ± 3.9	4.3 ± 0.56	0.41 (4.54)	4.14 (7.42)	46.7 ^b	7.0 ^b	Chen et al. [6] ^c , unpublished data
<i>Sinotaita histrica</i>	ND	ND	14.0 (19.5)	0.53 (3.2)	ND	ND	Ozawa et al. [14] ^e
Shrimps							
<i>Palaemon modestus</i>	64.0 ± 4.5	4.7 ± 0.63	1.06 (9.83)	4.29 (8.40)	41.0 ^b	6.3 ^b	Chen and Xie [7] ^e
<i>Macrobrachium nipponensis</i>	66.5 ± 3.5	5.8 ± 0.68	5.50 (12.42)	0.53 (1.67)	41.4 ^b	14.2 ^b	Chen and Xie [7] ^e

^a H_{wt} and T_{wt} denote dry weights of hepatopancreas and total body, respectively, and D_{max} and H_{max} denote maximum microcystin contents ($\mu\text{g/g}$ dry wt) in the digestive tract and hepatopancreas, respectively. BL = body length; BW = body weight; ND = no data.

^b Excluding intestine.

^c Field study.

^d Only one sample was measured.

^e Including digestive tract.

^f Laboratory experiment.

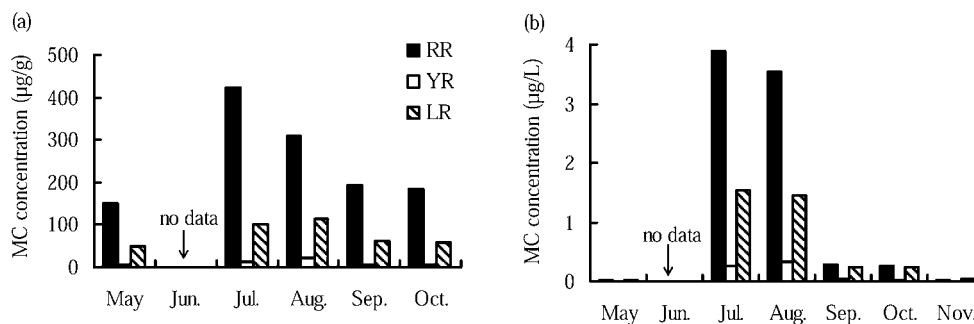


Fig. 1. Seasonal changes of (a) microcystin containing leucine and arginine (MC-LR), microcystin containing tyrosine and arginine (MC-YR), and microcystin containing two molecules of arginine (MC-RR) concentrations in cyanobacterial bloom ($\mu\text{g/g}$) and (b) MC concentrations in seston ($\mu\text{g/L}$) of Meiliang Bay in Lake Taihu, China, from May to October/November 2004.

the hepatopancreas used for the recovery experiments already contained MCs (0.04–0.576 μg MC-RR/g dry wt and 0.084–0.748 μg MC-LR/g dry wt), and these values were subtracted from the data before recovery calculations. The extraction was performed as described previously [7], and the recovery and relative standard deviation (RSD) of the analytical method were calculated. The average recoveries ($n = 9$) from hepatopancreas of the three species of bivalves (*L. leai*, *H. cumingii*, and *C. plicata*) were 75% (range, 63–82%; RSD range, 11–17%) for MC-RR and 81% (range, 76–87%; RSD range, 9–13%) for MC-LR.

Estimation of tolerable daily intake

We estimated the critical amount (g wet wt) of bivalves to ingest that would reach the tolerable daily intake (TDI) for MC according to the assumptions provided by the World Health Organization (WHO; total daily intake, 0.04 $\mu\text{g/kg}$ body wt, or 2 μg for an adult of 60 kg body wt) for MC-LR [4,5]. Ratios of dry weight to wet weight for various organs were calculated on a subset of eight individuals, and the ratios (mean \pm standard deviation) of the hepatopancreas, intestine, gonad, gill, foot, and the remaining tissue were 0.16 ± 0.01 , 0.19 ± 0.03 , 0.21 ± 0.02 , 0.14 ± 0.01 , 0.17 ± 0.02 , and 0.20 ± 0.03 , respectively. A coefficient of five was used to convert the dry weight of bivalve tissue to wet weight. The intraperitoneal median lethal dose in mice for MC-RR and MC-YR is approximately 5.0- and 2.5-fold higher, respectively, than that for MC-LR [18]; therefore, coefficients of 0.2 and 0.4 were used to convert MC-RR and MC-YR, respectively, into the MC-LR equivalent (MC-LReq).

Statistical analysis

Pearson correlation analysis was conducted to determine the relationship of MC content between different tissues of the same bivalve using SPSS® for Windows (Ver 11.5; SPSS, Chicago, IL, USA). No data transformation was performed before analysis.

RESULTS

Three MC analogues in seston were identified, with MC-RR being the main component and MC-LR and MC-YR present in lesser quantities. The mean ratio of MC-LR to total MC (summation of the three identified MC analogues) in cyanobacterial blooms and in seston was 0.23 and 0.45, respectively. During the study period, great temporal variations in MC concentrations were observed in cyanobacterial blooms and in seston. Microcystin in cyanobacterial blooms varied between 205 and 534 $\mu\text{g/g}$, with an average of 339 $\mu\text{g/g}$ (Fig. 1a), and

MC concentrations in seston varied between 0.047 and 5.698 $\mu\text{g/L}$, with an average of 2.044 $\mu\text{g/L}$ (Fig. 1b). A significant correlation was found between MC in seston and MC in cyanobacterial blooms ($r = 0.982$, $p < 0.01$, $n = 5$).

Great seasonal variations of MC-LR, MC-YR, and MC-RR content were observed in various organs/tissues of the bivalves *L. leai* (Fig. 2), *H. cumingii* (Fig. 3), and *C. plicata* (Fig. 4). Most organs (hepatopancreas, intestine, gill, and remaining tissue) showed maximum MC content during the summer (July or August), when MC concentrations in lake water and MC concentrations in cyanobacterial blooms reached maximum (Fig. 1), whereas the highest MC contents in foot and visceral mass were found during months of different seasons (e.g., May, August, or September). The highest MC contents were 38,478.1 ng/g dry weight for hepatopancreas (*C. plicata*), 20,647.2 ng/g dry weight for intestine (*H. cumingii*), 1,699.6 ng/g dry weight for visceral mass (*L. leai*), 640.2 ng/g dry weight for gill (*L. leai*), 584.2 ng/g dry weight for foot (*H. cumingii*), and 605.9 ng/g dry weight for remaining tissue (*L. leai*), respectively.

The mean MC content was considerably higher in hepatopancreas and intestine than in other organs/tissues and ranged between 3,415.7 ng/g dry weight (*H. cumingii*) and 5,787.7 ng/g dry weight (*C. plicata*) in hepatopancreas, between 925.2 ng/g dry weight (*L. leai*) and 3,829.1 ng/g dry weight (*H. cumingii*) in intestine, between 193.4 ng/g dry weight (*C. plicata*) and 410.3 ng/g dry weight (*L. leai*) in visceral mass, between 92.8 ng/g dry weight (*C. plicata*) and 224.0 (*L. leai*) ng/g dry weight in gill, between 110.8 (*C. plicata*) and 207.7 ng/g dry weight (*H. cumingii*) in foot, and between 96.5 ng/g dry weight (*C. plicata*) and 242.0 ng/g dryweight (*H. cumingii*) in remaining tissue.

In terms of toxin burden (excluding intestines), most MCs accumulated in the hepatopancreas (49–55%), with visceral mass (28–36%) ranking second and gill, foot, and the remaining tissue comprising only a minor part (11–17%) (Fig. 5). Therefore, hepatopancreas was the major target organ of MC accumulation in bivalves.

Because MCs are taken up by bivalves through food ingestion, the relationship between MC content in the intestine and that of other organs/tissues is important in risk prediction for human consumption. Microcystin content in intestine was significantly correlated with that in hepatopancreas (*L. leai*: $p < 0.05$, $r = 0.872$, $n = 6$; *H. cumingii*: $p < 0.01$, $r = 0.965$, $n = 6$; *C. plicata*: $p < 0.05$, $r = 0.753$, $n = 9$) and with that in visceral mass for *L. leai* ($p < 0.05$, $r = 0.863$, $n = 6$). No significant correlation was found between MC content in intestine and that in other organs/tissues. On the other hand, the

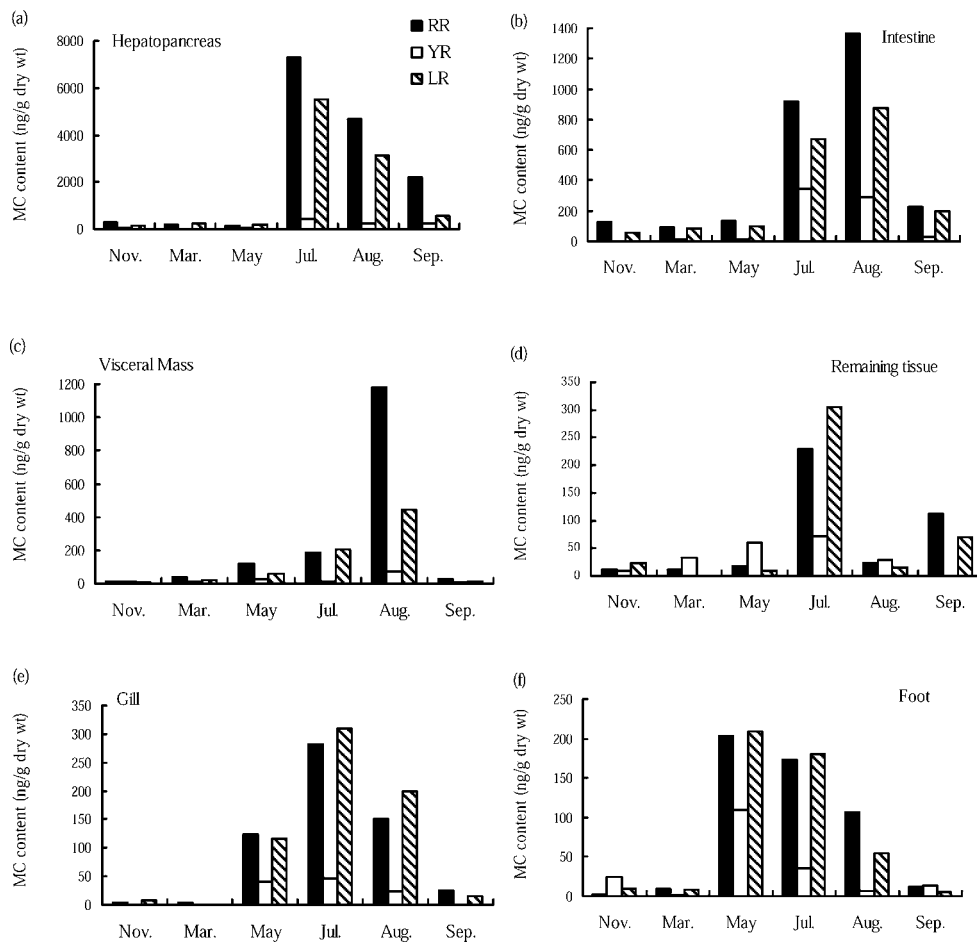


Fig. 2. The seasonal changes of microcystin (MC)-LR, MC-YR, and MC-RR content (ng/g dry wt) in (a) hepatopancreas, (b) intestine, (c) viscera mass, (d) remaining tissue, (e) gill, and (f) foot of the freshwater bivalve *Lamprotula leai*. For definitions, see Figure 1.

average ratio of intestinal MC to hepatopancreatic MC showed great variation, between 0.22 (*L. leai*) and 1.12 (*H. cumingii*). These ratios indicate that linear correlation between intestinal MC and hepatopancreatic MC may be species-specific.

Significant correlation was found between MC in cyanobacterial blooms and that in some bivalve organs/tissues, and significant correlations also were found between MC in seston and that in some bivalve organs. Microcystin in cyanobacterial blooms was significantly correlated with MC in hepatopancreas of *L. leai* ($p < 0.01$, $r = 0.987$, $n = 4$) and intestine of *C. plicata* ($p < 0.05$, $r = 0.974$, $n = 4$), and MC in seston was only significantly correlated with MC in intestine of *L. leai* ($p < 0.05$, $r = 0.964$, $n = 4$) and *C. plicata* ($p < 0.01$, $r = 0.986$, $n = 4$). This indicates that MC concentrations in cyanobacterial blooms or seston are valuable for predicting MC accumulation in some bivalves in Lake Taihu. It should be noted, however, that the small sample size (n) might have contributed, in part, to the interspecific variation of this correlation.

In various organs/tissues, MC-RR comprised a major part of total MCs: Between 46.6% (gill) and 68.7% (remaining tissue) for *H. cumingii*, between 46.5% (remaining tissue) and 64.1% (intestine) for *C. plicata*, and between 39.4% (remaining tissue) and 63.9% (visceral mass) for *L. leai*. Microcystin-LR (the most toxic analogue) ranked second: Between 21.6% (foot) and 43.8% (hepatopancreas) for *H. cumingii*, between 26.1% (intestine) and 36.7% (remaining tissue) for *C. plicata*, and between 30.3% (visceral mass) and 48.0% (gill) for *L.*

leai. Microcystin-YR generally was a minor part of total MCs: Between 3.4% (hepatopancreas) and 30.1% (gill) for *H. cumingii*, between 3.0% (hepatopancreas) and 16.8% (remaining tissue) for *C. plicata*, and between 3.9% (hepatopancreas) and 19.6% (remaining tissue) for *L. leai*. The mean ratio of MC-LR to total MC (summation of the three identified MC analogues) in various organs/tissues of the three bivalves varied from 0.29 to 0.39, which is within the range of concentrations in cyanobacterias and seston.

DISCUSSION

In the present study, MC content in most organs/tissues of three bivalve species reached a sharp peak in the summer, coinciding with peaks of MC concentrations in both cyanobacterial blooms and seston of the water column. These sharp peaks indicate both fast uptake and fast depuration of MCs by bivalves in our study lake. The coincidence of the rise and fall of MC amount in seston and bivalve organs/tissues indicates that dynamics of MC in these benthic bivalves were basically driven by intracellular MC in the water column, although the correlation between MC in bivalve organs/tissues and that in cyanobacterial bloom/seston showed substantial variations. Similarly, in an estuary, hepatotoxicity of the edible mussel *Mytilus edulis* increased during a dense bloom of the cyanobacterium *Nodularia spumigena* but decreased rapidly after the bloom [19]. Previous laboratory experiments (water temperatures, 12–25°C) indicated that the half-life of MC elimination is quite short in mollusks (bivalves, 2.5–4.9 d; snails,

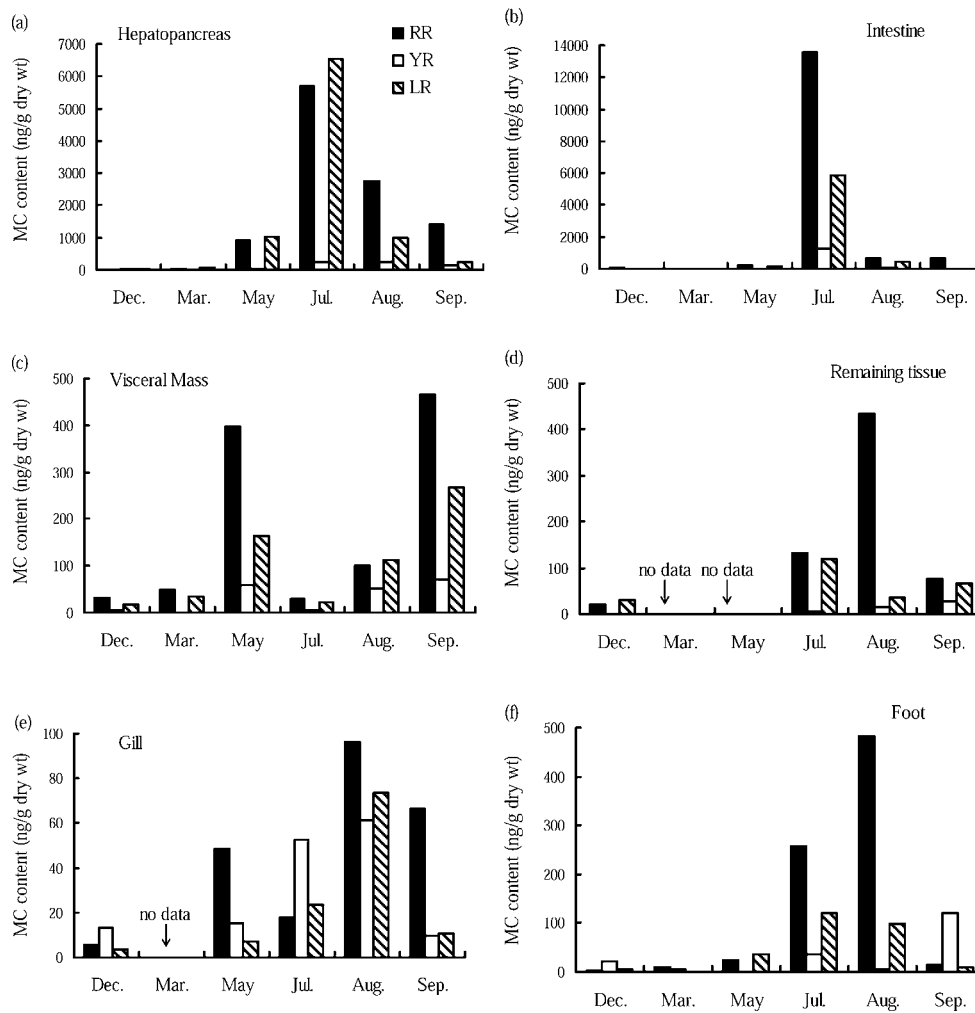


Fig. 3. The seasonal changes of microcystin (MC)-LR, MC-YR, and MC-RR content (ng/g dry wt) in (a) hepatopancreas, (b) intestine, (c) viscera mass, (d) remaining tissue, (e) gill, and (f) foot of the freshwater bivalve *Hyriopsis cumingii*. For definitions, see Figure 1.

8.4 d) and that the half-life of MC uptake is approximately 3 to 5 d for bivalves [20].

In the present study, substantial variation in MC accumulation was found among different species of bivalves; for example, the average ratio of intestinal MC to hepatopancreatic MC varied from 0.22 (*L. leai*) to 1.12 (*H. cumingii*). This may be a result of interspecific differences in selective ingestion, reproductive season, MC metabolism, and depuration rate [10,21,22]. Therefore, the bioaccumulation pattern must be known for each species when conducting a risk assessment of edible bivalves contaminated by MCs.

Generally, good correlations are observed between the MC content of aquatic mollusks and that of phytoplankton. The content of MC-LR in the tissue (whole body excluding shell) of three gastropods was correlated with the toxin in phytoplankton of the water column based on log-log transformed data in seven Canadian lakes [23]. The MC content in hepatopancreas of *Unio douglasiae* was linearly correlated with intracellular MCs in phytoplankton expressed as $\mu\text{g/L}$ or $\mu\text{g/g}$ ($p < 0.05$) in Lake Suwa [10]. In the present study, significant correlation in MC content was found in Lake Taihu between cyanobacterial blooms and hepatopancreas of *L. leai* ($p = 0.01$) and intestine of *C. plicata* ($p = 0.03$) and between seston and intestine of *L. leai* ($p = 0.04$) and *C. plicata* ($p = 0.01$). These results indicate that MC accumulation in benthic

mollusks is substantially regulated by MC level of planktonic cyanobacteria.

Distribution patterns of MC in the organs of various aquatic invertebrates are an important basis both for the understanding of MC intoxication mechanisms and for risk assessment. Interestingly, a comparison of the mean MC content in the digestive tract (D_{MC}), hepatopancreas (H_{MC}), and total body (T_{MC}) with the maximum MC content ($\mu\text{g/g}$ dry wt) in the digestive tracts (D_{max}) and the hepatopancreas (H_{max}) in various aquatic invertebrates (bivalves, snail, and shrimps) shows that $H_{\text{MC}}/T_{\text{MC}}$ remained relatively stable (40–55.4%), although $D_{\text{MC}}/H_{\text{MC}}$, D_{max} , and H_{max} showed great variations (0.22–5.50, 1.31–20.65, and 1.67–38.48 $\mu\text{g/g}$ dry wt, respectively) and the ratio of hepatopancreatic dry weight to total body weight varied greatly (2.9–14.2%) (Table 1). This indicates that the hepatopancreas is the most important target organ of MC in aquatic invertebrates and that the MC burden of the hepatopancreas in the total body is quite similar among aquatic invertebrates despite their significant differences in feeding mode, physiological structure, and toxin uptake and elimination kinetics.

The relationship between hepatopancreatic and intestinal MC of invertebrates has been a focus of recent studies. Actually, such a relationship is quite variable among different groups of invertebrates. In the present study, a good, positive correlation ($r = 0.753\text{--}0.965$, $p < 0.05$) was found between

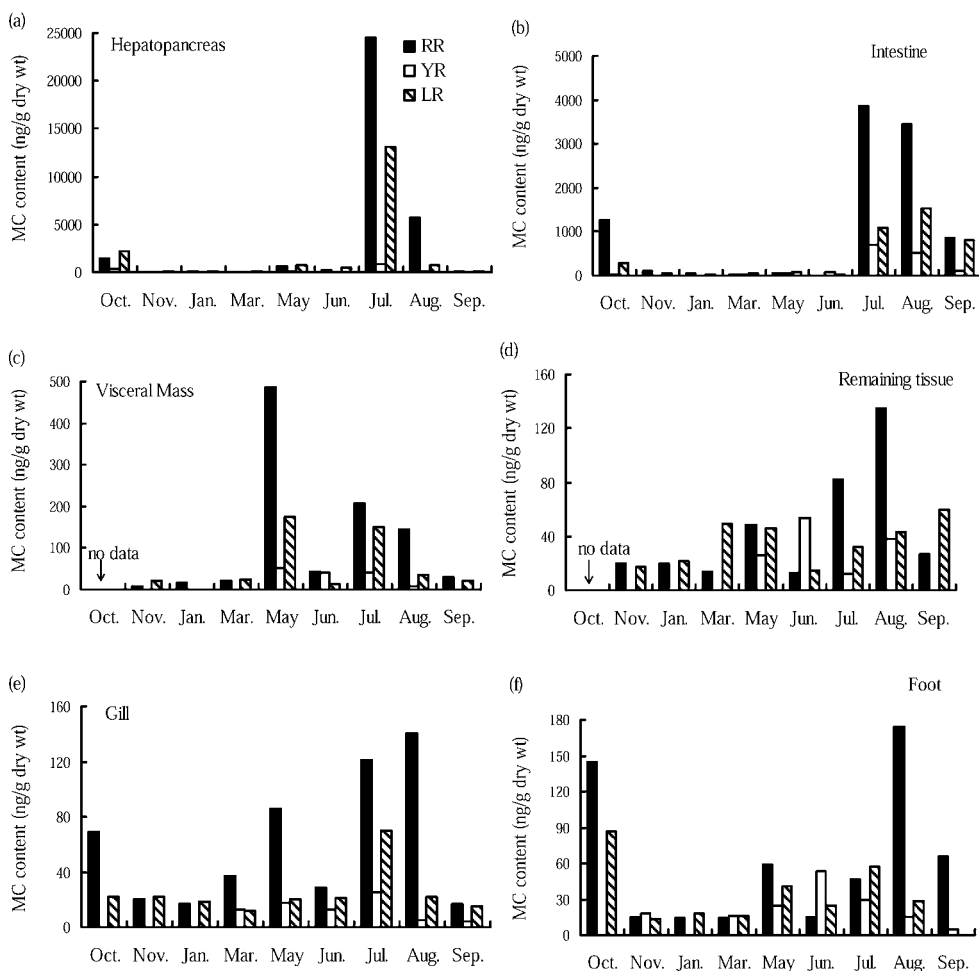


Fig. 4. The seasonal changes of microcystin (MC)-LR, MC-YR, and MC-RR content (ng/g dry wt) in (a) hepatopancreas, (b) intestine, (c) visceral mass, (d) remaining tissue, (e) gill, and (f) foot of the freshwater bivalve *Crastaria plicata*. For definitions, see Figure 1.

MC content of intestine and that of hepatopancreas of the three bivalves in Lake Taihu. In Lake Chaohu, no significantly positive correlation was observed between MC of digestive tracts and that of hepatopancreas of the snail *Bellamya aeruginosa* ($r = 0.6399$, $p = 0.171$) [6], and two shrimp species (*Palaeomon modestus* and *Macrobrachium nipponensis*) also showed no correlation between MCs in the stomach and those in the hepatopancreas [7]. Such a greatly different relationship between MCs in digestive tracts and in the hepatopancreas might occur either because of different mechanisms of uptake and depuration among different groups of animals or because the MC content in the digestive tracts is greatly affected by various factors (e.g., sampling time and procedure, temperature at sampling, or heterogeneity of food resources) [7].

The WHO proposed a provisional TDI of 0.04 $\mu\text{g}/\text{kg}$ body weight/d for MC-LR [5]. Traditionally, soft parts of bivalves are eaten whole in Chinese foods after the gut contents are emptied out for a couple of days in clean water, but cases also exist in which humans consume specific organs, such as feet. During the study period, the overall mean MC content for *H. cumingii*, *C. plicata*, and *L. leai* was 0.188, 0.096, and 0.131 μg MC-LReq/g wet weight, respectively. Considering an adult of 60 kg who ingests, on average, 300 g/d of bivalve (e.g., five individuals of *L. leai*, three individuals of *H. cumingii*, or three individuals of *C. plicata*), the mean daily intakes from *H. cumingii*, *C. plicata*, and *L. leai* reached 0.94, 0.48, and 0.66 μg MC-LReq/kg body weight, respectively (e.g., 23.5-

12-, and 16.5-fold, respectively, the TDI proposed by the WHO). If we removed the high-risk organs (hepatopancreas and intestines) before consumption, then the mean MC content for *H. cumingii*, *C. plicata*, and *L. leai* were 0.061, 0.034, and 0.064 μg MC-LReq/g wet weight, respectively, and the mean daily intakes from *H. cumingii*, *C. plicata*, and *L. leai* still reached 0.30, 0.17, and 0.32 μg MC-LReq/kg body weight, respectively (e.g., 7.5-, 4.2-, and 8.0-fold, respectively, the TDI proposed by the WHO). Removal of hepatopancreatic tissues before human consumption seems to be technologically infeasible, but depuration (Lake Taihu water should not be used) will likely be the preferred method for decontamination of the whole bivalves. It should be noted, however, that quantitative models based on laboratory depuration experiment of a specific bivalve are needed to make accurate predictions for the consumption risk of MC in bivalves in future studies.

The present study indicates that in Meiliang Bay of Lake Taihu, hepatopancreas and intestine are the organs of bivalves that are most dangerous for human consumption. In addition, the overall order of hepatotoxicity of bivalves throughout the study period was *H. cumingii* > *L. leai* > *C. plicata*. The most dangerous months for bivalve consumption (for most organs/tissues) were July and August; even when the MC concentration in seston of the water column declined to rather low levels (e.g., September), a substantial amount of MC still remained in organs/tissues of the bivalves, probably because of either a time lag in MC depuration by bivalves or their con-

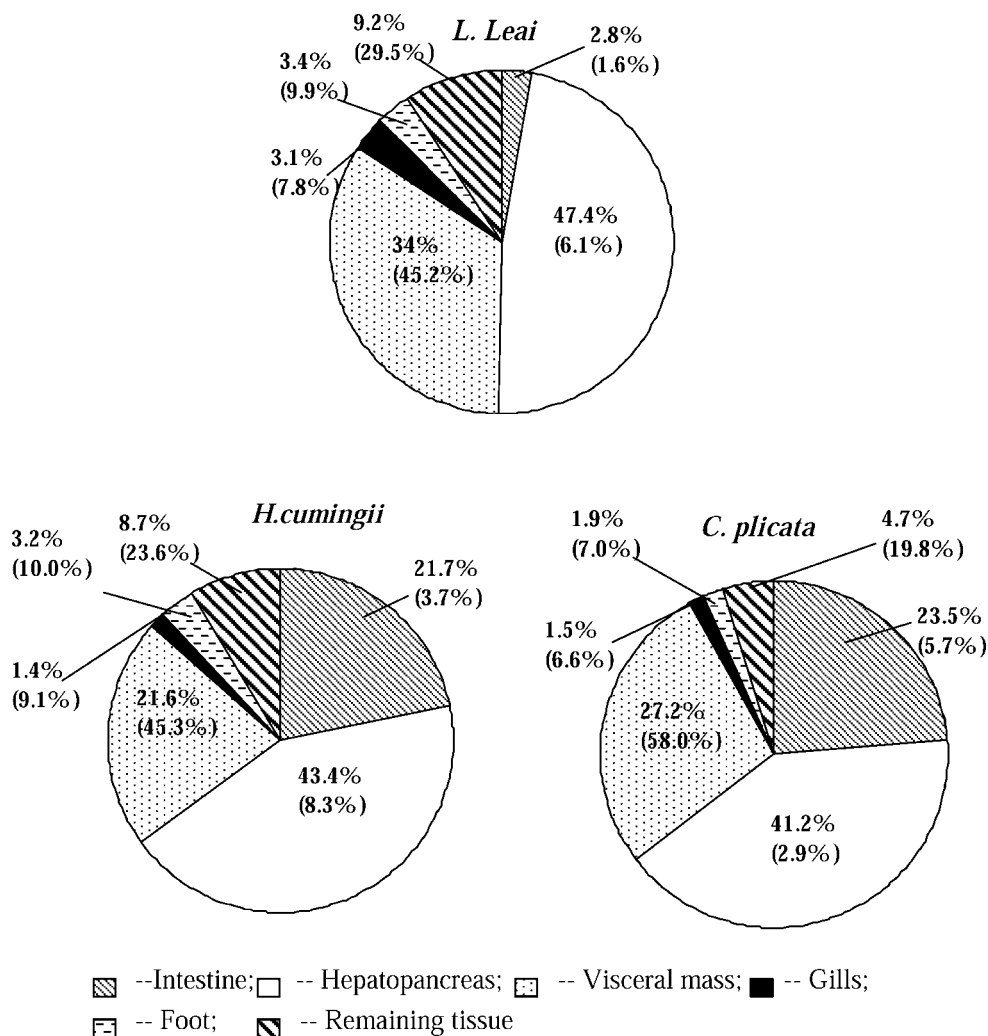


Fig. 5. The percentage of toxins present in the different organs of the freshwater bivalves *Lamprotula leai*, *Hyriopsis cumingii*, and *Cristaria plicata* collected from Meiliang Bay of Lake Taihu, China, during October 2003 and September 2004. Numbers in parentheses are the dry weight of each organ as a percentage of the total weight.

tinuous feeding on cyanobacteria associated with surface sediment.

In conclusion, the present results indicate that the local fishermen of Lake Taihu are at high risk, because they usually consume high amounts of bivalves or other fisheries products and, at the same time, also use the lake water contaminated with MCs. Therefore, it is recommended that in Lake Taihu, edible mussels should not be collected for human consumption during toxic cyanobacterial blooms, and clearly, the long-term impact of MCs to the aquatic organism and public health cannot be overlooked.

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REFERENCES

- Paerl HW, Fulton RS, Moisaner PH, Dyble J. 2001. Harmful freshwater algal blooms, with an emphasis on cyanobacteria. *The Scientific World Journal* 1:76–113.
- MacKintosh C, Beattie KA, Klumpp C, Cohen C, Codd GA. 1990. Cyanobacterial microcystin-LR is a potent and specific inhibitor of protein phosphatase 1 and 2A from both mammals and higher plants. *FEBS Lett* 264:187–192.
- Nishiwaki-Matsushima R, Ohta T, Nishiwaki S, Saganum M, Kohyama K, Ishikawa T, Carmichael WW, Fujiki H. 1992. Liver cancer promotion by the cyanobacterial cyclic peptide toxin microcystin-LR. *J Cancer Res Clin Oncol* 118:420–424.
- Falconer IR, Burch MD, Steffensen DA, Choice M, Coverdale OA. 1994. Toxicity of the blue-green alga (cyanobacterium) *Microcystis aeruginosa* in drinking water to growing pigs, as an animal model for human injury and risk assessment. *Environ Toxicol Water Qual* 9:131–139.
- Chorus I, Bartram J. 1999. *Toxic Cyanobacteria in Water: A Guide to Public Health Consequences, Monitoring, and Management*. E&FN Spon, London, UK.
- Chen J, Xie P, Guo LG, Zheng L, Ni LY. 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. *Environ Pollut* 134:423–430.
- Chen J, Xie P. 2005. 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. *Toxicol* 45:615–625.
- Prepas EE, Kotak BG, Campbell LM, Evans JC, Hrudey SE, Holmes CFB. 1997. Accumulation and elimination of cyanobacterial hepatotoxins by the freshwater clam *Anodonta grandis simpsoniana*. *Can J Fish Aquat Sci* 54:41–46.

9. Watanabe MF, Park HD, Kondo F, Harada K, Hayashi H, Okino T. 1997. Identification and estimation of microcystins in freshwater mussels. *Nat Toxins* 5:31–35.
10. Yokoyama A, Park HD. 2002. Mechanism and prediction for contamination of freshwater bivalves (Unionidae) with the cyanobacterial toxin microcystin in hypereutrophic Lake Suwa, Japan. *Environ Toxicol* 17:424–433.
11. Ibelings BW, Bruning K, Jonge JD, Wolfstein K, Pires LMD, Postma J, Burger T. 2005. Distribution of microcystins in a lake food web: No evidence for biomanipulation. *Microbiol Ecol* 49:487–500.
12. Williams DE, Dawe SC, Kent ML, Andersen RJ, Craig M, Holmes CFB. 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.
13. Amorim A, Vasconcelos M. 1999. Dynamics of microcystins in the mussel *Mytilus galloprovincialis*. *Toxicon* 37:1041–1052.
14. Ozawa K, Yokoyama A, Ishikawa K, Kumagai M, Watanabe MF, Park HD. 2003. Accumulation and depuration of microcystin produced by the cyanobacterium *Microcystis* in a freshwater snail. *Limnology* 4:131–138.
15. Pu PM, Hu WP, Wang GX, Zhang SZ, Hu CG., Yan JS. 1998. 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.
16. Pu PM, Hu WP, Yan JS, Wang GX, Hu CG. 1998. A physico-ecological engineering experiment for water treatment in a hypertrophic lake in China. *Ecological Engineering* 10:79–90.
17. Zheng L, Xie P, Li YL, Yang H, Wang SB, Guo NC. 2004. Variation of intracellular and extracellular microcystins in a shallow, hypereutrophic subtropical Chinese lake with dense cyanobacterial blooms. *Bull Environ Contam Toxicol* 73:698–706.
18. Gupta N, Pant SC, Vijayaraghavan R, Lakshmana R, Rao PV. 2003. Comparative toxicity evaluation of cyanobacterial cyclic peptide toxin microcystin variants (LR, RR, YR) in mice. *Toxicology* 188:285–296.
19. Falconer IR, Choice A, Hosja W. 1992. Toxicity of edible mussels (*Mytilus edulis*) growing naturally in an estuary during a water bloom of the blue-green alga *Nodularia spumigena*. *Environ Toxicol Water Qual* 7:119–123.
20. Xie P. 2006. *Microcystin in Aquatic Animals with Potential Risk to Human Health*. Science Press, Beijing, China.
21. Baker SM, Hornbach DJ. 2001. Seasonal metabolism and biochemical composition of two unionid mussels, *Actinonaias ligamentina* and *Amblema plicata*. *J Molluscan Stud* 37:407–416.
22. Pires LMD, Karlsson KM, Meriluoto JAO, Kardinaal E, Visser PM, Siewertsen K, Donk EV, Ibelings BW. 2004. Assimilation and depuration of microcystin-LR by the zebra mussel, *Dreissena polymorpha*. *Aquat Toxicol* 69:385–396.
23. Zurawell RW, Kotak BG, Prepas EE. 1999. Influence of lake trophic status on the occurrence of microcystin-LR in the tissue of pulmonate snails. *Freshw Biol* 42:707–718.
24. Eriksson JE, Meriluoto JAO, Lindholm T. 1989. Accumulation of a peptide toxin from the cyanobacterium *Oscillatoria agardhii* in the freshwater mussel *Anodonta cygnea*. *Hydrobiology* 183:211–216.