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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

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Abstract

So far no information is available on microcystin (MC) contents in shrimps, prawns or crayfish from natural freshwaters. Tissue distributions and seasonal dynamics of the hepatotoxic MC-LR and -RR in two freshwater shrimps, *Palaemon modestus* and *Macrobrachium nipponensis* were studied monthly (during June–November, 2003) in a Chinese lake containing toxic cyanobacterial blooms. The shrimps *P. modestus* and *M. nipponensis* accumulated high MCs not only in the hepatopancreas (mean 4.29 and 0.53 μg g^{-1} DW, respectively) but also in the gonad (mean 1.17 and 0.48 μg g^{-1} DW, respectively), and the crayfish *Procambarus clarkii* accumulated as much as 0.93 μg g^{-1} DW in the gonad. This indicates that gonads of these invertebrates are the second important target organ of MCs. *P. modestus* apparently accumulated more MCs in their organs than *M. nipponensis*, which might be a reflection of their difference in trophic niche. Eggs of the shrimps accumulated 8.4% (*M. nipponensis*, 0.27 μg g^{-1} DW) and 29.0% (*P. modestus*, 2.34 μg g^{-1} DW) of total toxin burden, indicating that MCs had been transferred into offspring from their adults. Among the shrimp muscle samples analyzed, 31% were above the provisional WHO TDI level, suggesting the risk of consuming shrimps in Lake Chaohu.

Keywords: Microcystin-RR and -LR; Shrimps; Crayfish; Hepatopancreas; Gonad and egg; Muscle; Human consumption; Lake Chaohu

1. Introduction

Serious eutrophication accompanied with the presence of massive cyanobacterial blooms and the associated cyanotoxins has been documented in many inland waters worldwide (Paerl et al., 2001). Among cyanotoxins, the hepatotoxic microcystins (MCs) are considered to be one of the most dangerous groups (Carmichael, 1997; Chorus and Bartram, 1999), because they are highly toxic to mammals, e.g. the LD_{50} of MC-LR i.p. or i.v. in mice and rats ranged between 36 and 122 μg kg^{-1}, comparable to the toxicity of the chemical organophosphate nerve reagents (Dawson, 1998). The exposure to MCs has been implicated in acute death of terrestrial animals and through haemodialysis also caused death of humans (Carmichael et al., 2001; Azevedo et al., 2002). The long-term exposure to MCs is related to chronic human intoxication such as primary liver cancer (Yu, 1989, 1995). Now, MCs are of great concern to public due to their potential risk to human health.

Since it is a common belief that human exposure to MCs is mainly through drinking water and recreation, numerous researches have focused on intracellular and extracellular

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MCs of phytoplankton in the water (Chorus and Bartram, 1999); and also as freshwater products are rarely used for human consumption in western countries, little information in the scientific literatures is available on contamination of microcystins in aquatic animals of natural waters. There have been only occasional reports on microcystin contents in edible wild aquatic animals such as fish in a Brazil lagoon (Magalhàes et al., 2001) and an Egyptian fish farm (Mohamed et al., 2003). MC contents were also measured occasionally for mussels in Lake Suwa (Japan) (Watanabe et al., 1997; Yokoyama and Park, 2002) and snails in several Canadian lakes (Kotak et al., 1996) and Lake Biwa (Japan) (Ozawa et al., 2003) because of the concern that MCs may be transported to terrestrial food web (Prepas et al., 1997; Ozawa et al., 2003).

However, so far no information is available on MC contents in shrimps, prawns or crayfish collected from natural freshwater environments. There are some immersion bioassay studies on the toxicity (indicated by LC_{50} or EC_{50}) of MCs on brine shrimp (*Artemia salina*) (Kiviranta et al., 1991; Delaney and Wilkins, 1995; Metcalf et al., 2002; Sabour et al., 2002) or fairy shrimp (*Thamnocephalus platyurus*) (Blom et al., 2001; Keil et al., 2002). In a laboratory experiment, toxin accumulation and depuration kinetics were studied by injecting prawn with MC-LR at a dose of 86 ng g^{-1} body weight, and maximums in the hepatopancreas and muscle reached 130 and 5 ng g^{-1}, respectively, while the majority of MC-LR in the hepatopancreas and muscle was depurated within a few hours (Kankaanpää et al., 2004).

There were only a few experimental studies on MC accumulation in crayfish. In laboratory, 12 signal crayfish (*Pacifastacus leniusculus*) (collected from a crayfish farm in southern Sweden) were fed with a toxic strain of *Planktothrix agardhii* with a MC content of 3.6 mg g^{-1} DW, and MCs were detected in the hepatopancreas of six of 12 of the animals, but the amounts of MCs accumulated in the hepatopancreas could not be quantified accurately because of interference from other components (Liras et al., 1998). In the laboratory, the crayfish *Procambarus clarkii* were fed with toxic *Microcystis aeruginosa* strain containing a MC content of 2.3 mg g^{-1} DW, and their whole body accumulated up to 2.9 μg MC g^{-1} DW (determined by ELISA method) at the end of an uptake period (2 weeks) with 53, 38 and less than 0.1% of the MC in the intestine, hepatopancreas and muscle (edible part), respectively (Vasconcelos et al., 2001).

In China, freshwater shrimps are commercially important because they are widely used for human consumption. They are not only cultured in ponds but also abundantly present in many natural freshwater lakes. However, during the past decades, eutrophication in Chinese lakes has progressed rapidly, resulting in frequent outbreak of toxic cyanobacterial blooms in many large lakes such as Lake Chaohu and Lake Taihu where production of freshwater shrimps are an important industry. It is quite likely that oral consumption of these shrimps exposed to high MC levels could lead to chronic human intoxication. Therefore, it is urgently needed to clarify whether MCs are able to accumulate in these shrimps or not, and to evaluate quantitatively consumptive risk for humans.

The present research was conducted on two freshwater shrimps, *Palaemon modestus* and *Macrobrachium nipponensis*, with occasional sampling of the red swamp crayfish *P. clarkii*, in a large shallow, eutrophic subtropical freshwater lake (Lake Chaohu) where heavy cyanobacterial blooms occur in the warm seasons of every year. *P. modestus* and *M. nipponensis* are high in production and used directly for human consumption; and there was a 6-fold increase in shrimp harvest during 1979–2001. The purposes of this study are mainly to examine distributions and seasonal changes of microcystins-LR and -RR in various organs (stomach, hepatopancreas, gonad, muscle, egg and gill) of the shrimps and to evaluate the relative importance of different organs in the accumulation of MCs with comments on the potential risk to human health when these are consumed.

2. Materials and methods

Lake Chaohu, located in Anhui Province in the southeastern China, is among the five largest freshwater lakes in China. It is a subtropical lake with a surface area of 760 km^2, a mean depth of 3.06 m, and a mean retention time of 136 days. During the past decades, the lake has witnessed a steady increase in eutrophication, characteristic of a regular occurrence of cyanobacterial surface blooms (mainly composed of *Microcystis* spp. and *Anabaena* sp.) in the warm seasons of each year (Deng, 2004). In this lake, *P. modestus* and *M. nipponensis* are two important freshwater shrimps, and the red swamp crayfish *P. clarkii* are minor in the crustacean catch.

P. modestus has a natural range from Fujian Province of China in the south to Siberia of Russia in the north, and *M. nipponensis* is widely distributed in China with natural occurrence also in Vietnam and Siberia of Russia (Li et al., 2003). Generally, *M. nipponensis* prefers littoral habitats, dominating in macrophytic shallow lakes like Lake Honghu (Sun et al., 1999), while *P. modestus* prefers pelagic habitats, dominating in lakes like Lakes Chaohu and Taihu where there are more pelagic habitats (Shi, 1995).

The red swamp crayfish *P. clarkii* is one of the most widespread freshwater crayfish in the world. It is a native species from South America and used in many countries as a food resource (Perry and LaCaze, 1969; Vasconcelos et al., 2001). *P. clarkii* was transplanted into Japan in the early 20th century; from Japan, it was introduced into China in the late 1930s, and now is widely dispersed in natural waters of China (Guo and Zhu, 1997) perhaps due to its high migratory ability, resistance to environmental changes and high ability to tolerate low water quality (Johnson and

Avault, 1982; Vasconcelos et al., 2001). *P. clarkii* is also popularly used as food for humans in China.

During June and November 2003, the *P. modestus* (62.0±4.5 mm in body length, 4.7±0.63 g in body weight) and *M. nipponensis* (66.5±3.5 mm in body length, 5.8±0.68 g in body weight) were collected monthly near Zhongmiao where surface cyanobacterial blooms frequently accumulated densely by wind. The collected animals were immediately frozen at −20 °C, and then dissected into seven parts (stomach, intestine, hepatopancreas, gonad, muscle, egg and gill) in the laboratory. Since intestines of *P. modestus* and *M. nipponensis* are very small in weight, we could not collect enough samples to measure MCs, and therefore there is no data on MC contents in the intestines of the shrimps. The collected organs were frozen at −80 °C prior to microcystin analysis. Since there were insufficient toxins in the stomach, hepatopancreas, intestine, gonad, gill and muscle to allow for individual analysis, we pooled, respectively, all stomach, hepatopancreas, intestine, gonad, gill and muscles (abdomen) of 50 dissected animals. Thus, each value represents an average amount of microcystins in the organs of 50 individuals.

Occasional (in June and July) samplings were also undertaken in the same sampling site for the red swamp crayfish *P. clarkii* (71.8±4.4 mm in body length, 14.9±2.5 g in body weight). Twenty individuals were collected in June and July, respectively. The collected crayfish were dissected into stomach, intestine, gonad, hepatopancreas, muscle and gill in laboratory.

Extraction and analysis of the microcystins in the organs of the study animals basically followed the method of Xie et al. (2004): lyophilized samples (ca. 0.5 g DW for each organ) were homogenized and extracted three times with 10 ml of BuOH:MeOH:H_2O (1:4:15) for 24 h while stirring. The extract was centrifuged at 18,000 rpm and the supernatant was diluted with water. This diluted extract was directly applied to 5 g of a reversed phase ODS cartridge, which had been preconditioned by washing with 50 ml of 100% MeOH and 50 ml of H_2O. The column was washed with water (50 ml), followed by water–MeOH (4:1, 100 ml). Elution from the column with 90% MeOH (100 ml) yielded the toxin-containing fraction. The toxin-containing fraction was evaporated to dryness. Then the residue was dissolved with 100% MeOH (5 ml) and then eluted with 70% MeOH (20 ml), the toxin-containing fraction was also evaporated to dryness. This fraction was dissolved with 100% MeOH and the methanol solution was subjected to a reverse-phase high-performance liquid chromatography (HPLC) equipped with an ODS column (Cosmosil 5C18-AR, 4.6×150 mm, Nacalai, Japan) and a SPD-10A UV–vis spectrophotometer set at 238 nm. A gradient starting at 50% (v/v) aqueous methanol with 0.05% trifluoroacetyl (TFA) was increased to 70% (v/v) in 25 min at a flow rate of 1 ml min^{-1}. MC concentrations were determined by comparing the peak areas of the test samples with those of the standards available (MC-LR and MC-RR, Wako Pure Chemical Industries—Japan).

Qualitative analysis of MCs was performed using a Finnigan LC-MS system comprising a thermo surveyor auto sampler, a surveyor MS pump, a surveyor PDA system, and a Finnigan LCQ-Advantage MAX ion trap mass spectrometer equipped with an atmospheric pressure ionization fitted with an electrospray ionization source (ESI). The instrument control, data processing, and analysis were conducted by using Xcalibur software. Separation was carried out under the reversed phase on Hypersil GOLD 5 μm column (2.1 mm i.d.×150 mm). The isocratic mobile phase consisted of solvent A [water+0.05% (v/v) trifluoroacetic acid (TFA)]/solvent B [acetonitrile+0.05% TFA]. The gradient went from 15% B (stand 2 min) to 95% B (stand 7 min) over the first 13 min, followed by a decrease to 15% B (stand 5 min) over the subsequent 3 min. Sample injection volumes were typically 10 μl. MS tuning and optimization were achieved by infusing microcystin-RR and monitoring the $[M+H]^+$ ion at *m/z* 520. MS conditions were as follows: ESI spray voltage 4.54 kV, sheath gas flow rate 30 units, auxiliary gas flow rate 0 unit, capillary voltage 45.67 V, capillary temperature 230 °C, and multiplier voltage −801.62 V. Data acquisition was in the positive ionization centroid mode with full mass mode at a mass range between 500 and 1100.

3. Results

The chromatograms of the MC-LR and -RR standards, the extracts of stomach, hepatopancreas, gonad and eggs of *P. modestus* are compared in Fig. 1. This shows that the toxins were taken up by the shrimp and a part was extractable in the organs. Fig. 2 shows the ESI LC/MS analysis of microcystins in the hepatopancreas of *P. modestus*. Based on total ion chromatogram, mass chromatograms monitored at *m/z* 1038, and the presence of $[M+H]^+$ ion at *m/z* 1038, it is confirmed that peak A was derived from MC-RR. Similarly, peak B was deduced to be derived from MC-LR, as the peak was detected by monitoring with *m/z* 995, and the mass chromatogram showed $[M+H]^+$ ion at *m/z* 995.

The monthly changes in MC contents of both shrimps were showed in Figs. 3 and 4. During the study period, there were great temporal variations in MC contents in various organs of both shrimps, and the stomachs of both shrimps showed remarkably high peaks in August, whereas the highest MC peaks in the hepatopancreas appeared in different months (June for *M. nipponensis* and October for *P. modestus*, respectively).

The ratio of MC contents in hepatopancreas/stomach was much higher in *P. modestus* (0.95) than in *M. nipponensis* (0.18), but the ratio of MC contents in

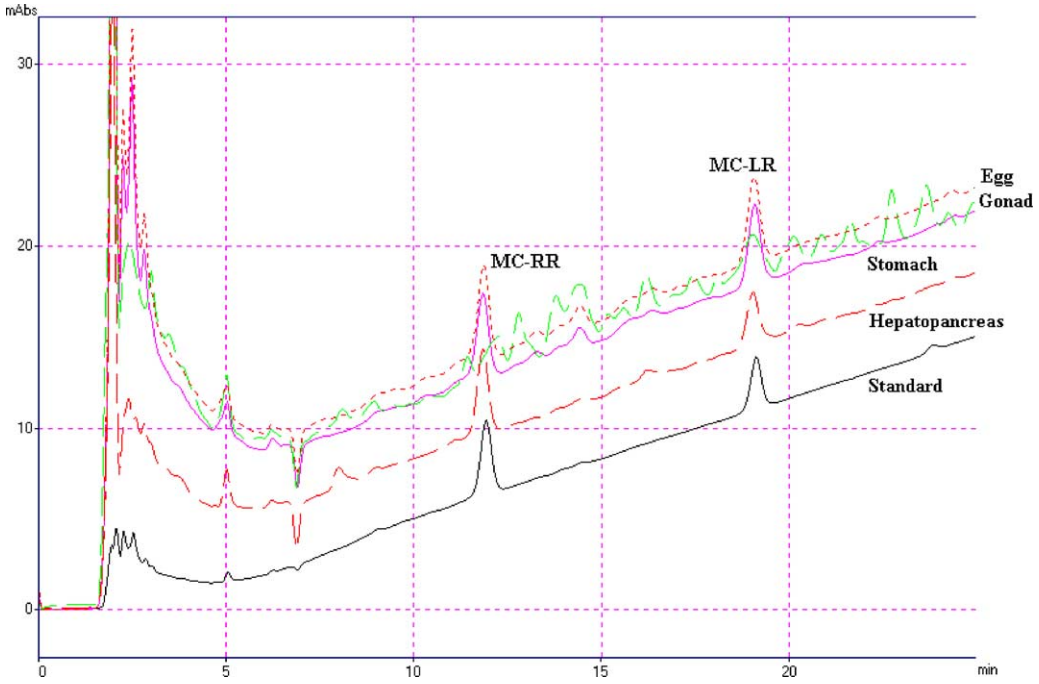


Fig. 1. A comparison of the chromatograms (monitored at 238 nm) of the standard MC-LR and -RR, the extracts of stomach, hepatopancreas, gonad and egg of the freshwater shrimp *Palaemon modestus* collected from Lake Chaohu in June of 2003. (Note on HPLC analysis: a gradient starting at 50% (v/v) aqueous methanol with 0.05% trifluoroacetyl (TFA) was increased to 70% (v/v) in 25 min at a flow rate of 1 ml min^{-1}).

gonad/hepatopancreas was much lower in *P. modestus* (0.27) than in *M. nipponensis* (0.91), and the ratio of MC contents in muscle/hepatopancreas was lower in *P. modestus* (0.03) than in *M. nipponensis* (0.08). This may indicate different metabolism mechanisms of MCs between these two species.

On the average, *P. modestus* accumulated much more MCs in their organs than *M. nipponensis*. Mean MC levels decreased in the order of stomach > hepatopancreas > gonad > gill > muscle, while the difference between stomach and hepatopancreas was much greater in *M. nipponensis* than in *P. modestus*. Except for stomach where *M. nipponensis* had the highest MC level, the maximum MC contents in other organs were also observed for *P. modestus*. For *P. modestus*, the maximum MCs were observed in August for stomach, but in October for hepatopancreas and muscle; while for *M. nipponensis*, the maximum MCs of stomach, hepatopancreas and muscle were in August, June and October, respectively. In terms of toxin burden (excluding stomach), hepatopancreas ranked the first for both shrimps (41.0% for *P. modestus* and 41.4% for *M. nipponensis*), followed by gonad and eggs, and muscle, whereas gills were the least (Table 1).

There was no correlation between MCs in the stomach and those in the hepatopancreas (for *P. modestus*: $r=0.480$, $p=0.414$; for *M. nipponensis*: $r=-0.159$, $p=0.764$) or muscle (for *P. modestus*: $r=-0.371$, $p=0.539$; for *M. nipponensis*, $r=-0.297$, $p=0.568$). Also, no correlation was found between MCs in the hepatopancreas and those in the muscle for both *P. modestus* ($r=0.509$, $p=0.381$) and *M. nipponensis* ($r=0.391$, $p=0.443$).

In the sample of June, high MC contents were detected in various organs of the crayfish *P. clarkii*, and the order of decreasing MC contents was stomach > intestines > gonad > gills > muscle (Table 2). In terms of toxin burden, digestive tracts contained the highest share (67.6%), followed by gonads (27.5%). If digestive tracts are excluded, up to 84.8% of the toxin burden were allocated in the gonad. However, in the sample of July, MC was detected only in the stomachs, and the quantity was also very low (0.05 μg MC-RR g^{-1} DW and 0.06 μg MC-LR g^{-1} DW).

Average proportion of MC-LR in total MCs showed great variation in present study. In *P. modestus*, the average ratio of MC-LR/MCs decreased in the order of gonad (93.5%) > stomach(60.8%) > egg (55.5%) > hepatopancreas (29.7%) > muscle (4.5%) > gill (0%), and in *M. nipponensis*, this ratio was in the order of stomach (70.7%) > muscle (47.8%) > hepatopancreas (39%) > egg (38.9%) > gonad (33.4%) > gill (0%). In the June sample of *P. clarkii*, the order was muscle (100%) > intestine (67.6%) > stomach (58.1%) > gonad (57%) and gills (51.9%), and it should be noted, however, that toxin level in the muscle was very low.

4. Discussion

The present study is the first to measure MC contents in shrimps collected from a natural freshwater environment. Kankaanpää et al. (2004) measured (through ELISA method) the seasonal (December–April) changes of mixed hepatotoxins (MC-LR, MC-LA, MC-RR, MC-YR, and NODLN) in the marine prawn *Penaeus monodon* that

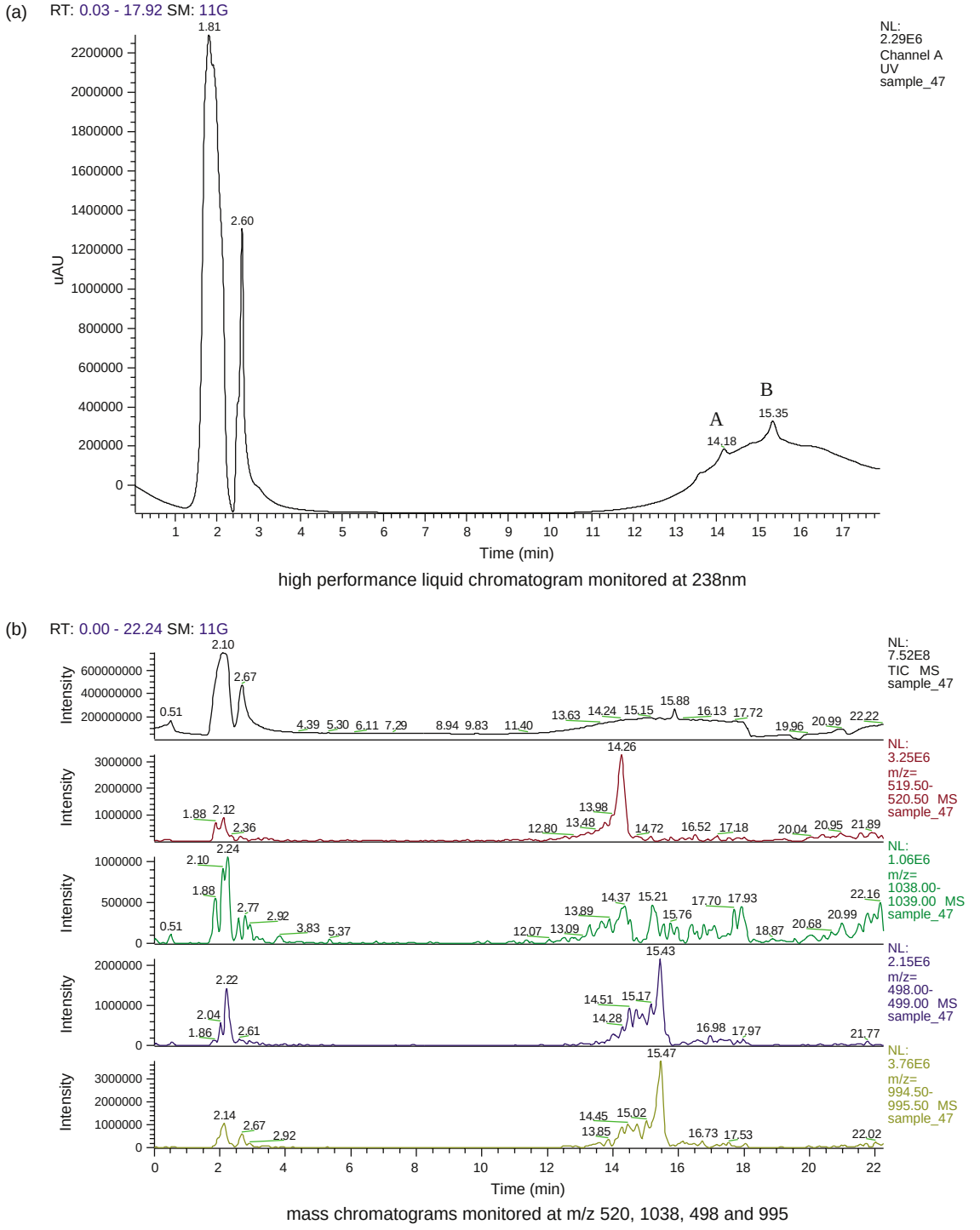
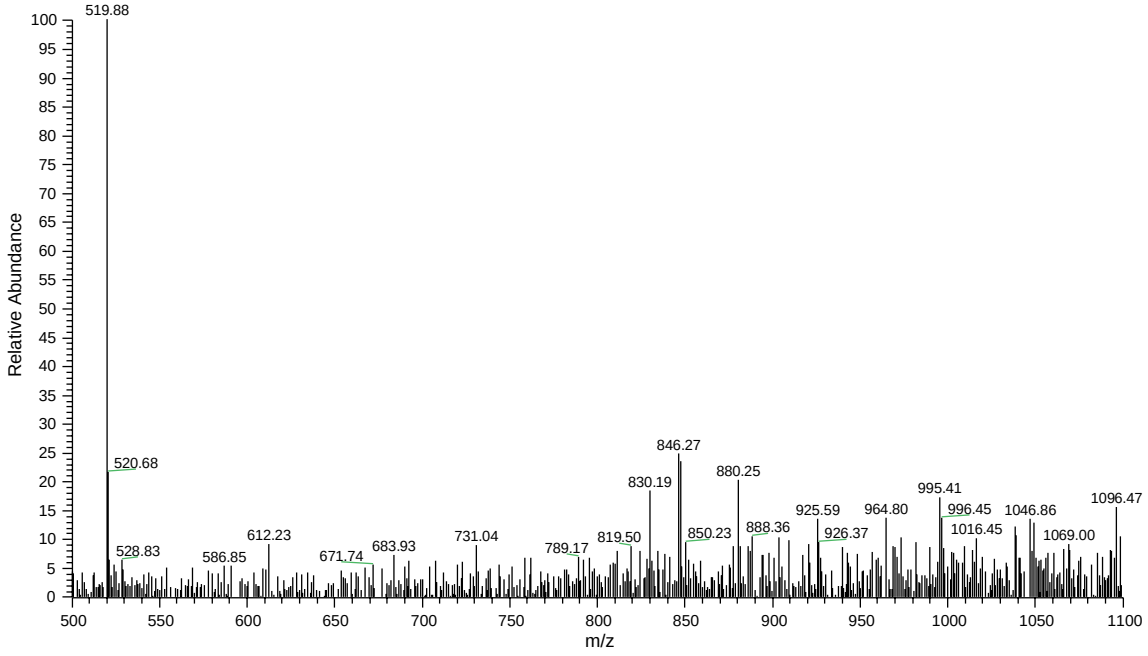


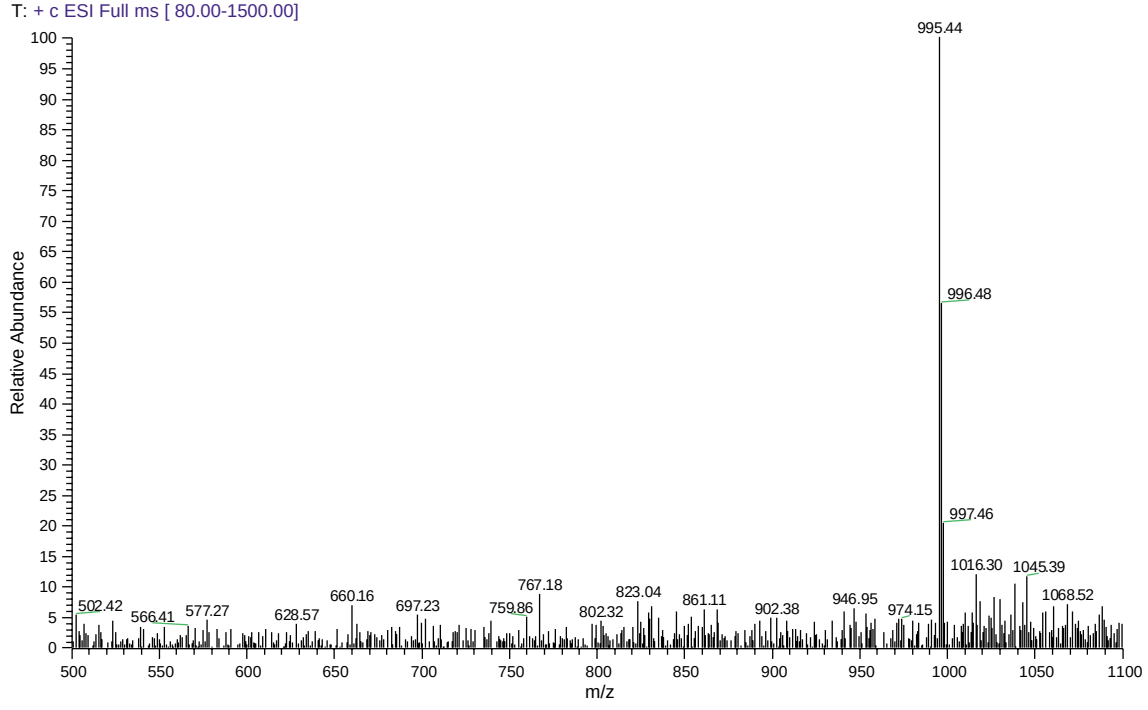
Fig. 2. ESI LC/MS analysis of microcystins in the hepatopancreas of *Palaemon modestus* (October 2003).

(c) sample_47 #676-700 RT: 13.94-14.43 AV: 25 SB:30014.80-17.47, 9.58-13.07 NL: 1.70E6
T: + c ESI Full ms [80.00-1500.00]



ESI mass spectrum at 14.26 min (microcystin-RR)

(d) sample_47 #737-757 RT: 15.19-15.60 AV: 21 SB: 20115.97-17.51, 11.53-14.10 NL: 2.77E6
T: + c ESI Full ms [80.00-1500.00]



ESI mass spectrum at 15.47 min (microcystin-LR)

Fig. 2 (*continued*)

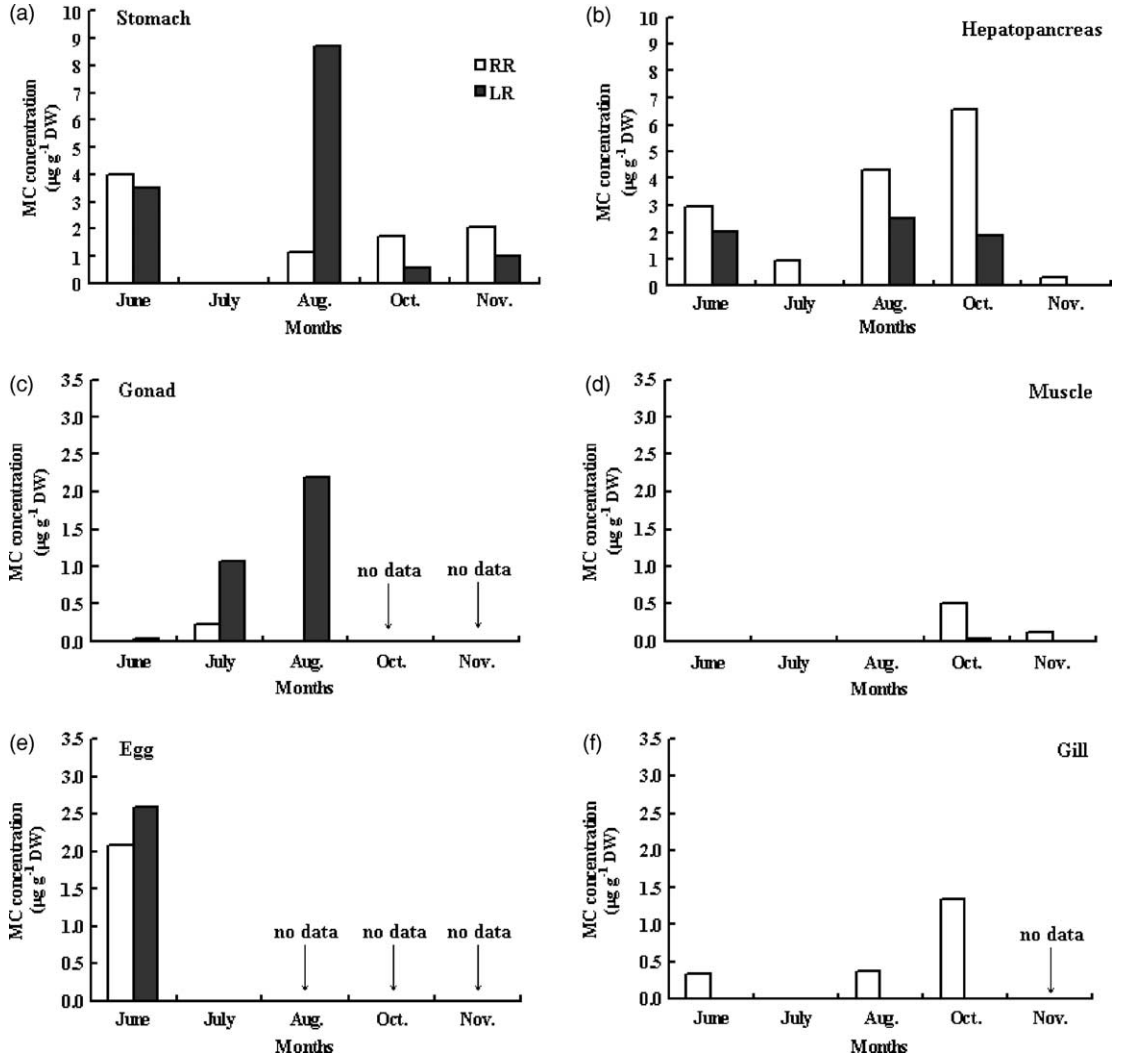


Fig. 3. The seasonal changes of MC-LR and -RR concentrations (μg g^{-1} DW) in (a) stomach, (b) hepatopancreas, (c) gonad, (d) muscle, (e) egg and (f) gill of the freshwater shrimp *Palaemon modestus* in Lake Chaohu in 2003.

were cultured in several Australian brackish ponds with a salinity of ca. 31–33 g l^{-1}. In their study, the total hepatotoxins in prawn tissue varied between 6.4 and 81.6 ng g^{-1} DW, although it reached a maximum of 1200 ng g^{-1} DW for phytoplankton; but no data were available for the content of MCs in the total hepatotoxins. Magalhàes et al. (2003) studied seasonal changes of MC contents in a marine shrimp (no name was given) through ELISA method in Sepetiba Bay of Brazil, and found that the MC contents were always less than 10 ng g^{-1} ww. Our study is also the first to report the accumulation of MCs in crayfish from natural environment.

Generally, accumulation of MCs in aquatic animals depends on MC level in the food resources. Magalhàes et al. (2003) report that in Sepetiba Bay of Brazil, a significant correlation was observed between MC concentration in seston samples and in fish muscle ($r=0.96$, $p<0.05$). Zurawell et al. (1999) report that the concentration of MC-LR in the tissue (whole body excluding shell) of three gastropods was correlated with toxin in the phytoplankton of the water column based on log–log transformed data in seven Canadian lakes. A field investigation in Lake Suwa showed that the MC content in the hepatopancreas of *Unio douglasiae* was linearly correlated with intracellular MCs in phytoplankton expressed as μg l^{-1} or μg g^{-1} ($p<0.05$) (Yokoyama and Park, 2002). However, in our study lake, both shrimp species showed no correlation between MCs in the stomach and in the hepatopancreas, suggesting that MC contents in the stomach may be significantly affected by

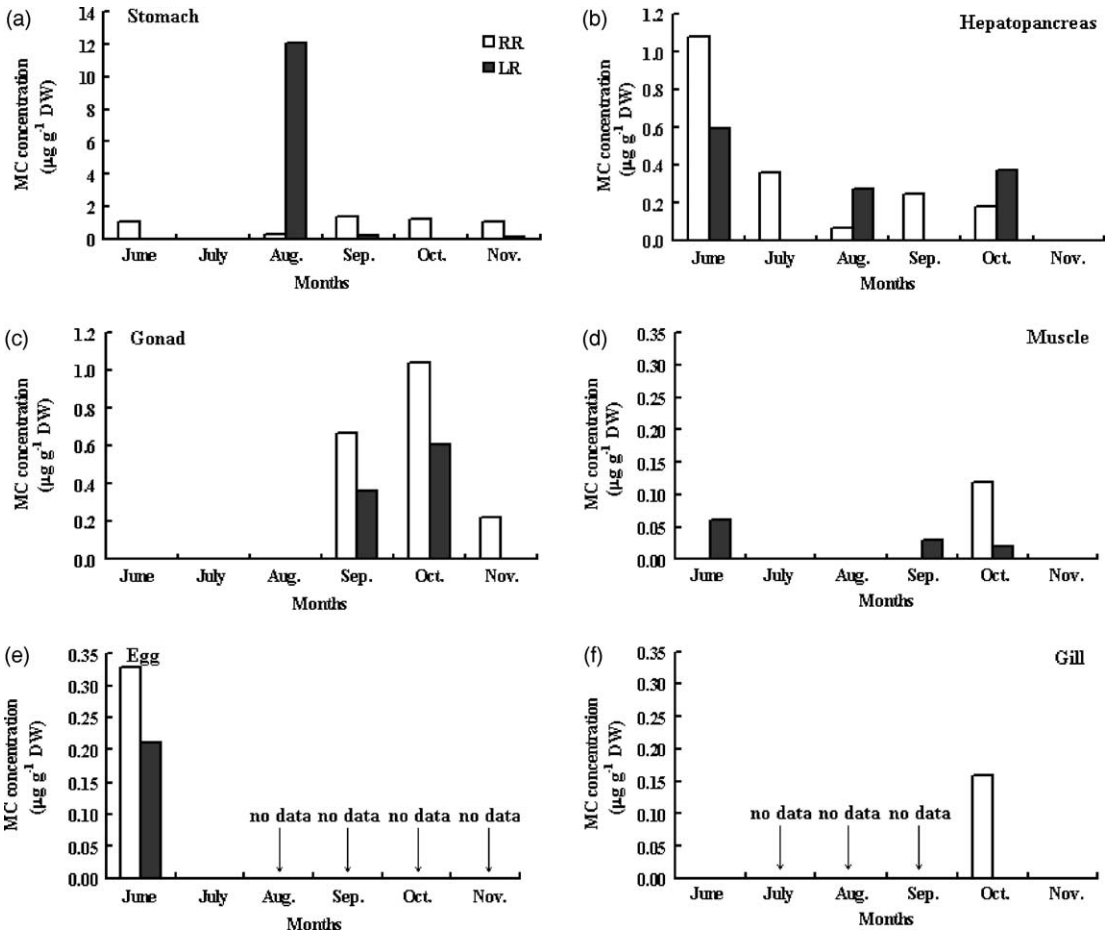


Fig. 4. The seasonal changes of MC-LR and -RR concentrations (μg g^{-1} DW) in (a) stomach, (b) hepatopancreas, (c) gonad, (d) muscle, (e) egg and (f) gill of the freshwater shrimp *Macrobrachium nipponensis* in Lake Chaohu in 2003.

various factors (e.g. sampling time and procedure, digesting degree at sampling or heterogeneity of food resources).

In the present study, *P. modestus* apparently accumulated more MCs in their organs than *M. nipponensis*, which might be a reflection of their difference in food sources. In Lake Chaohu, phytoplankton (mainly cyanobacteria) are the main primary producers, whereas macrophytes are only distributed in some littoral areas. In the fisheries practice, *M. nipponensis* contributes the majority of the shrimp catch in littoral areas while *P. modestus* dominated in the pelagic catch. Stable isotope analysis on the food web of Lake Chaohu during the same period showed that *P. modestus* had a δ^{13}C value closer to phytoplankton and δ^{13}C value of *M. nipponensis* was closer to that of macrophytes, whereas there was little difference in δ^{15}N values between these two shrimps (Personal communication with Dr Jun Xu). This suggests that *P. modestus* might have ingested more cyanobacteria (thus more MCs), but *M. nipponensis* might have ingested more macrophytes (thus less MCs). Such a differentiation in trophic niche (space and food resources) may explain why *P. modestus* accumulated more MCs in their stomach than *M. nipponensis*. This indicates that species in different genera of the same family may accumulate quite different MC levels, and therefore, to predict bioaccumulation of MCs in various shrimps, accumulation pattern must be known for each species. Similarly, in Lake Suwa (Japan), three freshwater mussels in the family Unionidae accumulated quite different MC levels in their hepatopancreas, with maximums of 420, 297 and 12.6 μg MC g^{-1} DW for *U. douglasiae*, *Cristaria plicata* and *Anodonta woodiana*, respectively; and it is suggested that the different seasonal bioaccumulation patterns in the three bivalves may be a result of interspecific differences in selective ingestion, reproductive season, MC metabolism, and depuration rate (Yokoyama and Park, 2002). Since they only measured toxins in the hepatopancreas, it is unknown whether such different patterns were due to different food sources.

In mussels, reproduction is also related to increased MC levels in the hepatopancreas. Yokoyama and Park (2002) report that in Lake Suwa, the increased MC bioaccumulation in the hepatopancreas of *U. douglasiae* in spring was

Table 1
Dry weight of the different shrimp organs as a percentage of total weight, MC contents and percentage of toxins present in the different organs of two freshwater shrimps collected from Lake Chaohu during June and November 2003

Species	Tissue	Dry weight (%)	MCs (μg g^{-1})	Toxins[a] (%)	Toxins[b] (%)
P. modestus	Stomach	6.9	4.53 (0–9.83)	32.2	
	Hepatopancreas	6.3	4.29 (0.33–8.40)	27.8	41.0
	Gonad	7.9	1.17 (0.03–2.19)	9.6	14.1
	Egg	8.2	2.34 (0–4.67)	19.7	29.0
	Muscle	67.4	0.13 (0–0.53)	9.0	13.3
	Gills	3.4	0.51 (0–1.34)	1.8	2.6
M. nipponensis	Stomach	7.5	2.92 (0–12.42)	54.6	
	Hepatopancreas	14.2	0.53 (0–1.67)	18.8	41.4
	Gonad	14.1	0.48 (0–1.65)	16.9	37.2
	Egg	5.7	0.27 (0–0.54)	3.8	8.4
	Muscle	55.5	0.04 (0–0.14)	5.5	12.2
	Gills	3.0	0.05 (0–0.16)	0.4	0.8

[a] Including stomach.
[b] Excluding stomach.

attributed to an accelerated exposure rate as the mussel may enhance its filtering and ingestion rates in the reproductive period (Hornbach et al., 1984; Burky et al., 1985). In the present study, the shrimps *P. modestus* and *M. nipponensis* accumulated high MCs not only in the hepatopancreas (mean 4.29 and 0.53 μg g^{-1} DW, respectively) but also in the gonad (mean 1.17 and 0.48 μg g^{-1} DW, respectively), and also occasionally in eggs (2.34 and 0.27 μg g^{-1} DW, respectively); and the crayfish *P. clarkii* accumulated as much as 0.93 μg g^{-1} DW in the gonad. Similar results were also found for snail and mussels. During the same study period, the freshwater snail *Bellamya aeruginosa* also accumulated high MC contents in both hepatopancreas (mean 4.14 μg g^{-1} DW) and gonad (mean 0.715 μg g^{-1} DW)(Chen et al., 2005). Similarly, a freshwater mussel (*U. douglasiae*) in Lake Suwa (Japan) accumulated 2.72 and 1.19 μg MC g^{-1} DW in the hepatopancreas and gonad, respectively, although there was only one sample (in June) in their study (Watanabe et al., 1997). These results indicate that the reproductive systems of freshwater invertebrates (e.g. shrimps, snails and mussels) are the second important target organ of MCs.

It has been well illustrated that the liver is the prime target of the microcystins: after i.v. or i.p. injection of sublethal doses of variously radiolabelled microcystins in mice and rats, about 70% of the toxin was rapidly localized in the liver as a result of active uptake by hepatocytes (Falconer et al., 1986; Runnegar et al., 1986; Brooks and Codd, 1987; Robinson et al., 1989, 1991; Meriluoto et al., 1990; Lin and Chu, 1994; Nishiwaki et al., 1994). However, in aquatic invertebrates, toxin burden of hepatopancreas may not be as high as in mammals, but that of gonad is important, e.g. in the present study, 41.4% (*M. nipponensis*) and 41.0% (*P. modestus*) of the toxins were in the hepatopancreas, whereas 37.2% (*M. nipponensis*) and 14.1% (*P. modestus*) of the toxins were in the gonad. So far there has been no any report on bioaccumulation of MCs in reproductive organs of mammals.

It is surprising that in the present study, eggs of the shrimps accumulated 8.4% (*M. nipponensis*) and 29.0% (*P. modestus*) of total toxin burden. This indicates that MCs, in high contents, had been transferred into offspring from their adults, possibly exerting significant effects on reproduction and thus population dynamics of these

Table 2
MC contents (μg g^{-1}) and percentage composition of dry weight and MC burden in various organs of the crayfish *Procambarus clarkii* collected from Lake Chaohu in June

Tissues	MC-RR	MC-LR	Total MCs	Dry weight (%)	Toxins[a] (%)	Toxins[b] (%)
Stomach	4.18	5.79	9.97	6.7	62.8	
Intestine	0.73	1.52	2.25	2.1	4.8	
Hepatopancreas	0.00	0.08*	0.08*	2.7	0.2	0.7
Gonad	0.40	0.53	0.93	28.5	27.5	84.8
Muscle	0.00	0.05	0.05	53.9	2.8	8.6
Gills	0.13	0.14	0.27	6.8	1.9	5.9

*This value might be underestimated because we could only collect a pooled hepatopancreas sample of 0.08 g DW for toxin analysis, whereas a 0.5 g DW sample for each of other organs was collected except intestinal sample (only 0.2 g DW).
[a] Including stomach.
[b] Excluding stomach.

animals. The high MCs in the eggs of these invertebrates suggest that potential transport of MC into offspring of mammals should be investigated.

WHO proposed a provisional tolerable daily intake (TDI) of 0.04 μg kg^{-1} bw per day for MC-LR (Chorus and Bartram, 1999). We estimated for the shrimps the critical amount (g wet weight) that is necessary to ingest to reach the TDI for MC. A coefficient of 5 was used to convert dry weight to wet weight, and since i.p.LD_{50} in mice for MC-RR is about 5 times higher than MC-LR (Gupta et al., 2003), a coefficient of 0.2 was used to convert MC-RR into MC-LR equivalent. Considering an adult of 60 kg, who ingests, on the average, 300 g of shrimp muscle a day, 4 of the 13 analyzed muscle samples (31%) were above this limit. For instance, in October 2003, concentration of MC-LReq in *P. modestus* muscle sample reached 0.026 μg g^{-1} ww, representing an estimated daily intake of 0.13 μg kg^{-1} of body weight. This is 3.25 times the TDI value suggested by WHO. During the study period, the mean daily intakes from muscles of *P. modestus*, *M. nipponensis* and *P. clarkii* were estimated to be 0.031, 0.022 and 0.025 μg MC-LR equiv. kg^{-1} bw, respectively, and the maximum daily intakes were 0.13, 0.06 and 0.05 μg MC-LR equiv. kg^{-1} bw, respectively. On the other hand, *P. modestus* and *M. nipponensis* are often eaten as a whole by local residents, and during the study period, the overall mean MC-content for these two shrimps were 0.114 and 0.051 μg MC-LR equiv. g^{-1} ww, respectively. This indicates that the daily intakes from *P. modestus* and *M. nipponensis* reached, respectively, 0.57 and 0.255 μg MC-LR equiv. kg^{-1} bw (14.2 and 6.4 times the TDI value suggested by WHO!) when the shrimps are eaten as a whole. Therefore, the risk of consuming shrimps in MCs-contaminated lakes like Lake Chaohu cannot be overlooked and regular monitoring of MC levels in shrimps should be conducted to protect health of the public who regularly consume. It is also needed in our future studies to evaluate the potential harmful effects of MCs on human health by multiple exposure routes through aquatic food, drinking water and swimming in lakes with toxic cyanobacterial blooms.

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