Science of the Total Environment 407 (2009) 3317–3322

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Simultaneous determination of microcystin contaminations in various vertebrates (fish, turtle, duck and water bird) from a large eutrophic Chinese lake, Lake Taihu, with toxic *Microcystis* blooms

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ARTICLE INFO

Article history:
Received 11 November 2008
Received in revised form 31 January 2009
Accepted 2 February 2009
Available online 26 February 2009

Keywords:
Microcystin accumulation
Aquatic vertebrates
Lake Taihu
Microcystis blooms

ABSTRACT

This is the first to conduct simultaneous determination of microcystin (MC) contaminations in multi-groups of vertebrates (fish, turtle, duck and water bird) from Lake Taihu with *Microcystis* blooms. MCs (-RR, -YR, -LR) in *Microcystis* scum was 328 μg g^{-1} DW. MCs reached 235 μg g^{-1} DW in intestinal contents of phytoplanktivorous silver carp, but never exceeded 0.1 μg g^{-1} DW in intestinal contents of other animals. The highest MC content in liver of fish was in *Carassius auratus* (150 ng g^{-1} DW), followed by silver carp and *Culter ilishaeformis*, whereas the lowest was in common carp (3 ng g^{-1} DW). In livers of turtle, duck and water bird, MC content ranged from 18 to 30 ng g^{-1} DW. High MC level was found in the gonad, egg yolk and egg white of *Nycticorax nycticorax* and *Anas platyrhynchos*, suggesting the potential effect of MCs on water bird and duck embryos. High MC contents were identified for the first time in the spleens of *N. nycticorax* and *A. platyrhynchos* (6.850 and 9.462 ng g^{-1} DW, respectively), indicating a different organotropism of MCs in birds. Lakes with deaths of turtles or water birds in the literatures had a considerably higher MC content in both cyanobacteria and wildlife than Lake Taihu, indicating that toxicity of cyanobacteria may determine accumulation level of MCs and consequently fates of aquatic wildlife.

1. Introduction

Toxic cyanobacterial blooms are worldwide problems as they produce various natural toxins, especially the hepatotoxic microcystins (Codd, 1995; Chorus and Bartram, 1999; Havens, 2003). There have been over 80 analogues of MCs (Dietrich and Hoeger, 2005), among which MC-LR (leucine arginine), -RR (arginine arginine) and -YR (tyrosine arginine) are most commonly present. MCs are referred to as hepatotoxins because liver is the primary target in cases of animal and human poisonings (Codd et al., 2005). In 1996, an episode of human intoxications by MCs was first confirmed after an outbreak of acute liver failure that resulted in the deaths of 76 patients at two dialysis centers in Caruaru, Brazil (Carmichael et al., 2001; Azevedo et al., 2002). Recently, microcystins were identified for the first time in the serum (average 0.228 ng MC-LReq/ml) of a chronically exposed human population (fishermen at Lake Chaohu, China) together with indication of hepatocellular damage (Chen et al., 2009).

In recent years, there have been increasing studies to evaluate MC contamination in aquatic vertebrates from natural waters with cyanobacterial blooms, but mainly focusing on fishes (Vasconcelos, 1999; Magalhães et al., 2001, 2003; Mohamed et al., 2003; Ibelings et al., 2005; Xie et al., 2005; Chen et al., 2006; Deblois et al., 2008; Wilson et al., 2008). Similar information is relatively rare for other aquatic vertebrates, although MCs-producing cyanobacteria have been associated with deaths of wild birds and turtles over recent years around the world (Kenya (Krienitz et al., 2003; Metcalf et al., 2006), Tanzania (Lugomela et al., 2006), Japan (Matsunaga et al., 1999), Canada (Park et al., 2001; Murphy et al., 2003), Algeria (Nasri et al., 2008) and Belgium (Wirsing et al., 1998)). There was only one study to detect MC contents in two species of turtles from Lake Oubeira (Algeria) where deaths of the two terrapin species were observed during a toxic *Microcystis* bloom (Nasri et al., 2008). Krienitz et al. (2003) reported mysterious deaths of lesser flamingos around Lake Bogoria in Kenya, and determined MC contents in stomach content and feces of the flamingos, but no MC data were available for other organs of this water bird. Sipïa et al. (2006, 2008) analyzed nodularin in liver and muscle of eider (*Somateria mollissima*), but no study was conducted to determine MC level in domestic ducks living on water with toxic cyanobacterial blooms. Although Gkelis et al. (2006) reported the presence of MCs in the freshwater fauna of Greek (including eight species of fish, a frog, a mussel and a water snail), comparative studies on patterns of MC accumulation among multiple groups of vertebrates such as fish, birds and reptiles are still very rare.

The main purposes of this study were to compare patterns of MC accumulation among different groups of vertebrates (fish, turtle, duck

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doi:10.1016/j.scitotenv.2009.02.005

and water bird) from Lake Taihu during the summer season, when large *Microcystis* blooms occur, and to discuss the possible effects of toxic *Microcystis* blooms on the survivals of these wildlife also with evaluation on the potential risk for human consumption.

2. Materials and methods

Lake Taihu is the third largest freshwater lakes in China, and has a surface area of 2338 km^2, a mean water depth of 1.9 m. During the past decades, the lake has had a regular occurrence of *Microcystis* surface blooms (sometimes occupying about 1000 km^2) in the warm seasons of each year (Xie, 2008). Meiliang Bay (surface area 125 km^2), a part of Lake Taihu, is the most eutrophic part of the lake, characteristic of dense accumulation of toxic *Microcystis* blooms by wind in the summer (Qin et al., 2004).

In August 2005, four species of fish, the phytoplanktivorous *Hypophthalmichthys molitrix* (silver carp, 9 specimens), the benthivorous *Cyprinus carpio* (common carp, 9 specimens), the omnivorous *Carassius auratus* (15 specimens), and the carnivorous *Culter ilishaeformis* (15 specimens), one species of turtle, *Pelodiscus sinensis* (3 specimens), one species of bird, water bird black-crowned night heron, *Nycticorax nycticoraxs* (3 specimens), and one species of domestic duck, *Anas platyrhynchos* (3 specimens) were collected from Meiliang Bay of Lake Taihu. The ducks were bought from local fishermen on the day of sampling, which were cultured near the shore of lake, and were mainly feeding naturally on aquatic organisms of the lake water. Body lengths, body weights and number of collected specimens of these vertebrates are listed in Table 1.

We collected a total of 57 specimens from domestic duck (3 specimens), water bird (3 specimens), turtle (3 specimens) and fish (48 specimens). Duck, water bird, turtle and fish were dissected into 14, 14, 8 and 5 parts, respectively. Due to limited number of specimens, each organ sample of duck, water bird or turtle was only from one specimen. As we collected more specimens of fish, three specimens of Hm or Cc, and five specimens of Ca or Ci were combined to form one sample of a specific organ, respectively. Therefore, a total of 168 organ samples were prepared out of 57 specimens from 7 vertebrates (Table 1). The organ samples were immediately frozen at −20 °C and then freeze-dried for later microcystins analysis.

Extraction and analysis of the three major forms of MCs (MC-LR, MC-RR, and MC-YR) in the organs (~0.5 g lyophilized sample for each organ) of the study animals followed the method of Chen et al. (2006). Briefly, qualitative and quantitative analysis of MCs (MC-LR, MC-RR, and MC-YR) in the seven vertebrates of Lake Taihu was performed using a Finnigan (Waltham, MA, USA) liquid chromatography-electrospray ionization-mass spectrum system (LC-ESI-MS). Each sample (for each organ) had three replicates, and the results given in the text were the average values of the three replicates.

Recovery experiments were performed in quintuplicate through spiking 500 mg of homogenized freeze-dried duck and fish samples (liver and muscle) with mixed MCs solution of the three commercial standards (MC-RR, MC-LR and MC-YR, Wako Pure Chemical Industries-Japan) at 0.5 μg g^{-1}. The extraction was performed as described previously, and the recovery and the relative standard deviation of the analytical method were calculated.

3. Results

3.1. A comparison of MC contents among fishes, turtle, duck and water bird

Total MC (-RR, -YR, -LR) concentration in cyanobacterial scum was 328 μg g^{-1} DW. There was an apparent difference in MC concentration in the intestinal contents among the investigated animals. MCs in the intestinal contents of the phytoplanktivorous silver carp was as high as 235 μg g^{-1} DW, very close to the MC concentration in cyanobacterial scum (Fig. 1a). In the intestinal contents of other animals, MC concentration did not exceed 100 ng g^{-1} DW, and in other words, there was a difference of over 2000 times. Similar difference was also observed in MC concentration of intestinal walls between silver carp (up to 5358 ng g^{-1} DW) and other animals (less than 65 ng g^{-1} DW) (Fig. 1b).

In spite of high MCs in the intestine of silver carp, MC content in their livers was very close to that of other animals (Fig. 1c). The highest MC concentration was found in *C. auratus* (150 ng g^{-1} DW), followed by silver carp and *C. ilishaeformis*, whereas the lowest was found in common carp (3 ng g^{-1} DW). And in livers of turtle, duck and water bird, the MC concentration ranged from 18 to 30 ng g^{-1} DW.

It is interesting to see that in spite of the high MC concentration in the intestinal contents of silver carp, MC concentration in their muscle was the lowest (only 2 ng g^{-1} DW), while the highest MC concentration in muscle was observed for *C. auratus* (Fig. 1d).

There was also substantial accumulation of MCs in the reproductive organ gonad (Fig. 1e). The MC level in gonad was generally close to that of the muscle in these animals. The highest MC concentration was also found in *C. auratus* (27 ng g^{-1} DW).

3.2. Variations of MC contents in various organs of water bird, duck and turtle

There was great variation in the MC concentration among various organs of the water bird (Fig. 2a). The highest value was found in small intestine contents. Except for intestine and stomach, organs with relatively high MC content were liver and egg yolk, followed by gonad and pancreas, whereas only small amounts of MCs were detected in heart, lung and egg white. MC contents in digestive and reproductive organs were generally higher than those in the other organs.

When we dissected the digestive systems of one water bird, we found two small fish in its stomach, and also observed high percentage of *Microcystis* cells in its intestinal content. These indicate that *N. nycticoraxs* accumulated substantial microcystins through the consumption of both cyanobacterial cells and MC-contaminated food such as small fish.

There was also great variation in MC content among various organs of the domestic duck (Fig. 2b). Like water bird, the highest MC concentration was also observed in small intestine (36.4 ng g^{-1} DW). Except for intestine and stomach, the highest MC content was found in

Table 1
Mean body weight (BW), mean body length (BL) number of specimens (*N*) and parts of the dissection of seven vertebrates collected from Meiliang Bay of Lake Taihu in August.

Species	BW (g)	BL (cm)	*N*	Parts of the dissection
Hypophthalmichthys molitrix (Hm)	447	27.9	9	5 parts: liver, gonad, muscle, intestinal wall, intestine contents
Carassius auratus (Ca)	122	15.1	15	5 parts: liver, gonad, muscle, intestinal wall, intestine contents
Culter ilishaeformis (Ci)	234	24.1	15	5 parts: liver, gonad, muscle, intestinal wall, intestine contents
Cyprinus carpio (Cc)	1376	38.8	9	5 parts: liver, gonad, muscle, intestinal wall, intestine contents
Pelodiscus sinensis (Tu)	137	20.7	3	8 parts: liver, intestine, gallbladder, kidney, heart, lung, gonad, muscle
Anas platyrhynchos (Du)	1509	–	3	14 parts: liver, stomach, small intestine, large intestine, pancreas, gallbladder, kidney, heart, lung, spleen, gonad, egg yolk, egg white, muscle
Nycticorax nycticorax (Wb)	603	–	3	14 parts: liver, stomach, small intestine, large intestine, pancreas, gallbladder, kidney, heart, lung, spleen, gonad, egg yolk, egg white, muscle

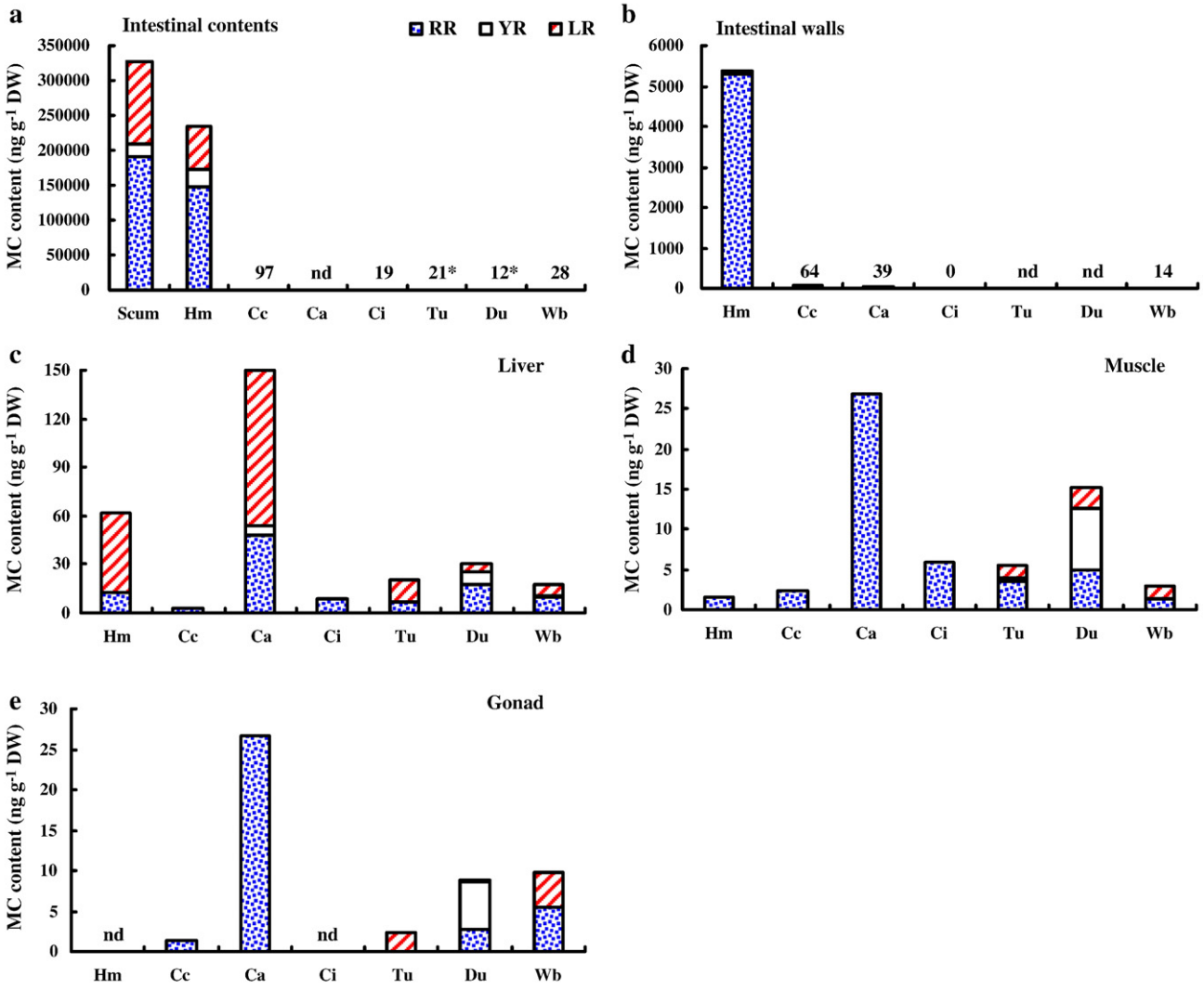

Fig. 1. Mean microcystin contents in cyanobacterial scum and the intestinal contents (a), intestinal walls (b), livers (c), muscles (d) and gonads (e) of various vertebrates in Lake Taihu. Hm — *Hypophthalmichthys molitrix*, Cc — *Cyprinus carpio*, Ca — *Carassius auratus*, Ci — *Culter ilishaeformis*, Tu — turtle (*Pelodiscus sinensis*), Du — duck (*Anas platyrhynchos*), and Wb — water bird (*Nycticorax nycticorax*), nd — no data, * — whole intestine.

liver (30.5 ng g^{-1} DW), followed by muscle and pancreas, whereas only small amounts of MCs were detected in heart and gallbladder.

A between-organ variation in MC concentration was also observed in turtle (Fig. 2c). MC concentration reached highest in liver (20.8 ng/g DW) and intestine (19.7 ng/g DW), and decreased in the order of kidney > lung > muscle > gallbladder > heart > gonad.

3.3. Recovery

The average recoveries from duck and fish samples (liver and muscle) were 83.1% (range, 79.3–95.2%) for MC-RR with relative standards deviations (RSDs) between 5.7 and 9.3%; 89.0% (range, 80.1–96.4%) with RSDs between 3.4 and 11.0% for MC-LR; and 86.7% (range, 83.0–90.7%) with RSDs between 4.1 and 8.7% for MC-YR.

Table 2
Comparison of microcystins distribution in different organs of *Pelodiscus sinensis*, *Anas platyrhynchos* and *Nycticorax nycticorax* collected in Lake Taihu (China) in August 2005.

Organs	*P. sinensis* (ng g^{-1} dry weight)	*A. platyrhynchos* (ng g^{-1} dry weight)	*N. nycticorax* (ng g^{-1} dry weight)
Liver	20.785 (25.60%)	30.495 (18.23%)	17.583 (9.99%)
Intestine	19.721 (24.29%)	50.674 (30.30%)	81.991 (46.59%)
Gonad	2.326 (2.87%)	8.865 (5.30%)	9.786 (5.56%)
Muscle	5.511 (6.79%)	15.170 (9.07%)	3.026 (1.72%)
Other organs	32.844 (40.45%)	62.045 (37.10%)	63.592 (36.14%)

The value in the brackets represents the percentage of microcystins in each tissue compared to the total level of microcystins.

4. Discussion

It is interesting to note that in the present study, although the phytoplanktivorous silver carp directly fed on toxic cyanobacteria, and therefore ingested much microcystins (as indicated by the extremely high MC concentrations in the intestinal contents and intestinal walls), they did not accumulate considerably more MCs in their organs or tissues (such as liver and muscle) than other fishes, turtle, domestic duck or water bird. These results together with our previous studies (Chen et al., 2006, 2007) indicate that phytoplanktivorous fish (silver carp; bighead carp) is probably more resistant to MC exposure than other fishes, turtle, domestic duck or water bird and thus it is quite possible to use such fish to counteract toxic cyanobacterial blooms.

P. sinensis is a carnivorous and carrion feeder with remains of fish, crustaceans, mollusks and insects in its stomachs (Pope, 1935). *A. platyrhynchos* consume a wide variety of foods, including vegetation, insects, worms, gastropods and arthropods (Moon, 1994). *N. nycticoraxs* is an opportunistic feeder, and its diets consist mainly of fish, though it is frequently rounded out by other items such as

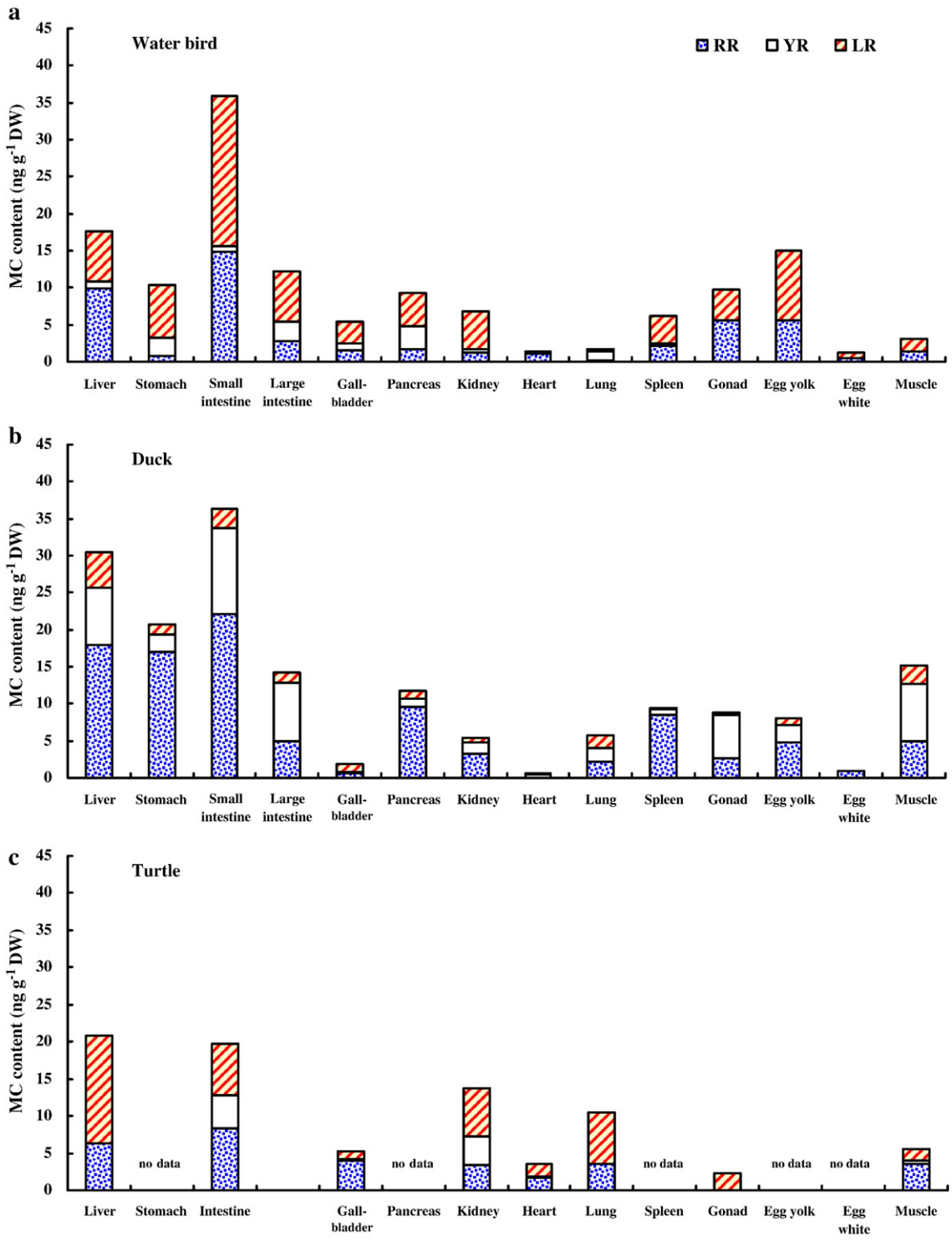

Fig. 2. Mean microcystin contents in various organs of the water bird (a), the domestic duck (b) and the turtle (c) in Lake Taihu.

leeches, earthworms, aquatic and terrestrial insects (Davis, 1993). It seems difficult to discriminate clearly the food niches of these vertebrates. However, the present study indicates that MC level in both intestine (mainly consists of intestinal contents) and gonad were much higher in *N. nycticorax* and *A. platyrhynchos* than in *P. sinensis*, suggesting that exposure risks with potential reproductive toxicity of water bird and domestic duck were higher than that of turtle (Table 2). MCs were also found in the egg yolk and egg white of *N. nycticorax* and *A. platyrhynchos*, which suggests the potential effect of microcystins on water bird and duck embryos. The high MC level in the gonad of *Carassius auratus* also suggests a potentially high risk of the toxin on their reproduction. Huynh-Delerme et al. (2005) reported that when embryos of medaka (*Oryzias latipes*) at stage 19 were microinjected with a dose of 0.2 pg MC-LR/vitellus, MC-LR induced histopathological modifications of the alimentary system (i.e. digestive tract, pancreas, liver) in newly hatched embryos. An exposure of the fertilized eggs of southern catfish to crude extracted MCs (10–100 μg MC-LReq/L) obviously retarded egg and larval development, reduced

hatching rate, caused high malformation rate and hepatocytes damage in larvae (Zhang et al., 2008). On the other hand, MC level in liver and muscle did not show the same pattern with that in intestine and gonad among these three groups of vertebrates, and the possible mechanisms for such a difference remain unclear and need further investigations.

Takahashi and Kaya (1993) reported a lower LD_{50} value (256 μg/kg) for quails compared to 600 μg/kg for mice (intraperitoneal injection of MC-RR), and observed that the spleen was enlarged to a doubling of the control in the quails in contrast to liver damage in mice and rats, indicating a different organotropism of microcystins in birds. In the present study, we identified for the first time high MC contents in the spleens of *N. nycticorax* and *A. platyrhynchos* (6.850 and 9.462 μg/kg, respectively), and in terms of toxin burden, 3.51% and 5.66% of the toxin were presented in the spleen of *N. nycticorax* and *A. platyrhynchos*, in contrast to fish, whereas less than 0.02% of the total toxin were present in the spleen of *Hypophthalmichthys molitrix* and *Aristichthys nobilis* (Chen et al., 2006, 2007). These indicate that toxicological impacts of MCs on spleen of birds should be evaluated experimentally in future studies.

Nasri et al. (2008) reported case of turtle deaths during a toxic *Microcystis* bloom in Lake Oubeira, Algeria. Based on methanol extraction-protein phosphatease method, MC content in *Microcystis* scum was 1.12 mg MC-LR_{eq} g^{-1} DW, and MC-LR_{eq} content in liver, muscle and viscera of *Emys orbicularis* was 300, 1 and 6 ng g^{-1} DW, respectively, and the figures were 270, 20 and 20 ng g^{-1} DW for *Mauremys leprosa*. In the present study, based on LC/MS, MCs (-RR, -YR, -LR) content in *Microcystis* scum was only 0.328 mg g^{-1} DW, and MCs content in liver and muscle of turtle (*P. sinensis*) was only 20.8 and 5.5 ng g^{-1} DW. It appears that as *Microcystis* blooms were much more toxic in Lake Oubeira than in Lake Taihu, liver of turtles also accumulated much more MCs in Lake Oubeira than in Lake Taihu, and consequently deaths of two terrapin species were observed during the *Microcystis* bloom in Lake Oubeira. On the other hand, MC level in muscle of turtles was similar between both lakes, and of course, as MC content of the turtle muscle was low in both studies, difference in methodology might have some influences on the results. According to Krienitz et al. (2003), there was mysterious deaths of lesser flamingos (*Phoenicopterus minor*) around Lake Bogoria in Kenya, and microcystins in cyanobacterial mat samples on the shore of the lake ranged from 221 to 845 μg MC-LR_{eq} g^{-1} DW, in stomach content of the flamingos, MCs (MC-LR, -RR, -LF and -YR) were 0.196 μg g^{-1} FW, and there also MCs present in intestine as well as faces. Assuming a dry weight to wet weight ratio of 0.1, MCs in the stomach content, intestine and faces of the flamingos were 1.96, 0.36 and 0.48 μg g^{-1} DW. In the present study, MCs in the stomach contents and small intestinal contents of the water bird were only 14.7 and 42.0 ng g^{-1} DW, respectively. This may explain why deaths of turtles and water bird were observed in Lake Oubeira and Lake Bogoria but not in Lake Taihu. It appears that toxicity of cyanobacteria may determine accumulation level of MCs in aquatic wildlife and consequently their fates, although such effects may have species-specific difference.

In the present study, toxin content in muscle samples of the seven vertebrates varied between 0.1 and 1.1 (mean 0.5) ng MC-LR_{eq} g^{-1} wet weight (a coefficient of 5 was used to convert dry weight to wet weight). The provisional tolerable daily intake (TDI) suggested by WHO is 0.04 μg kg^{-1} of body weight or 2.4 μg for an adult human weighing 60 kg (Kuiper-Goodman et al., 1999). Assuming an adult human ingests 100 g of fish or turtle or water bird or duck muscle per day from Lake Taihu, daily uptake of MC-LR_{eq} would be only 0.01–0.11 μg, much lower than TDI proposed by WHO, and that consumption of the muscle of these animals was still safe to human health. However, this may not be always so, as both abundance and toxicity of cyanobacteria may show temporal variation, and also as intracellular MC concentration in the lake water in our study month was only 0.15 μg L^{-1}, while the maximum MC concentration in Meiliang Bay of Lake Taihu reached as high as 10.4 μg L^{-1} in July of 2001 (Shen et al., 2003). It is recommended that regular monitoring of MC contamination in these aquatic vertebrates is needed from the viewpoint of human health protection.

5. Conclusion

The present study is the first to compare possible difference of MC accumulation among multiple vertebrate groups (including fishes, turtle, domestic duck and water bird) through simultaneous sampling in a lake. Our study is also the first to report MC contamination in domestic duck and identified for the first time high MC concentrations in the spleen of duck and water bird. The results indicate a general trend that liver was the target organ of all animals, while muscle and gonad could also accumulate substantial MCs. Although there were great variations in MC contents of a specific organ among different groups, MC levels in turtle, domestic duck and water bird were generally within the ranges of fishes. This indicates that fish is never the only vertebrates to be affected by microcystins, and that ecological effects of toxic cyanobacterial blooms on turtle, domestic duck and water bird with potential risks to human consumption should be also evaluated with equal importance with fishes in those water bodies with toxic cyanobacterial blooms.

Acknowledgements

We wish to express our sincere thanks to Prof. J.O. Nriagu and two anonymous reviewers for their very useful comments and suggestions on our manuscript. This research was supported by the National Natural Science Foundation of China (Grant No. 30700077) and National Basic Research Program of China (973 Program) (Grant No. 2008CB418101).

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