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Dynamics of microcystins-LR and -RR in the phytoplanktivorous silver carp in a sub-chronic toxicity experiment

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"Capsule": *Silver carp are tolerant of cyanobacterial toxins, and might be used to control toxic algal blooms in highly eutrophic lakes.*

Abstract

A sub-chronic toxicity experiment was conducted to examine tissue distribution and depuration of two microcystins (microcystin-LR and microcystin -RR) in the phytoplanktivorous filter-feeding silver carp during a course of 80 days. Two large tanks (A, B) were used, and in Tank A, the fish were fed naturally with fresh *Microcystis viridis* cells (collected from a eutrophic pond) throughout the experiment, while in Tank B, the food of the fish were *M. viridis* cells for the first 40 days and then changed to artificial carp feed. High Performance Liquid Chromatography (HPLC) was used to measure MC-LR and MC-RR in the *M. viridis* cells, the seston, and the intestine, blood, liver and muscle tissue of silver carp at an interval of 20 days. MC-RR and MC-LR in the collected *Microcystis* cells varied between 268–580 and 110–292 μg g^{-1} DW, respectively. In Tank A, MC-RR and MC-LR varied between 41.5–99.5 and 6.9–15.8 μg g^{-1} DW in the seston, respectively. The maximum MC-RR in the blood, liver and muscle of the fish was 49.7, 17.8 and 1.77 μg g^{-1} DW, respectively. No MC-LR was detectable in the muscle and blood samples of the silver carp in spite of the abundant presence of this toxin in the intestines (for the liver, there was only one case when a relatively minor quantity was detected). These findings contrast with previous experimental results on rainbow trout. Perhaps silver carp has a mechanism to degrade MC-LR actively and to inhibit MC-LR transportation across the intestines. The depuration of MC-RR concentrations occurred slowly than uptakes in blood, liver and muscle, and the depuration rate was in the order of blood > liver > muscle. The grazing ability of silver carp on toxic cyanobacteria suggests an applicability of using phytoplanktivorous fish to counteract cyanotoxin contamination in eutrophic waters.

Keywords: Sub-chronic toxicity experiment; Microcystin-LR and -RR; *Microcystis viridis*; Phytoplanktivorous sliver carp; Depuration; Evolution

1. Introduction

Cyanotoxins produced by freshwater cyanobacteria are very common worldwide, and have been reported to cause poisonings and deaths of wild and domestic animals (Codd, 1995; Dawson, 1998; Carmichchael, 2001) and even significant hazards to human health (Yu, 1995; Pouria et al., 1998). One of the most studied groups of cyanotoxins is the cyclic heptapeptide hepatotoxins called microcystins (MC), of which over 60 structural variants are currently known (Sivonen and Jones, 1999). The microcystins differ principally in the two L-amino acids at positions 2 and 4, and the most common form is microcystin-LR (MC-LR) (Dawson, 1998). So far, most documented reports have concerned microcystin toxicity to terrestrial mammals, although toxicity to higher animals does not appear to be of evolutionary adaptive value to the toxic cyanobacteria (Jungmann, 1992; Landsberg, 2002).

In aquatic systems, fish stand at the top of the aquatic food chain, and are possibly affected by exposure to toxic cyanobacteria. Many experimental studies have been conducted to document the toxicity of microcystin exposure through gastrointestinal or blood circular systems on (1) omnivores (animals, plant and detritus-

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eating) fish such as common carp (Rabergh et al., 1991; Fischer and Dietrich, 2000; Li et al., 2001; Wu et al., 2002), and gold fish (Xu et al., 1998), (2) invertebrate-eating carnivores such as Atlantic salmon (Williams et al., 1995, 1997), rainbow trout (Sahin et al., 1996; Bury et al., 1996, 1997; Tencalla and Dietrich, 1997; Fischer et al., 2000), and channel catfish (Zimba et al., 2001), (3) piscivores such as brown trout (Bury et al., 1997), Northern snakehead (Chen et al., 1995), and (4) macrophytes-eating herbivores such as grass carp (Chen et al., 1995; Zhang et al., 1996). However, all these studies were limited to acute toxic experiments, and they were based on either oral gavaging, or intraperitoneal injection, or administration via the dorsal aorta of the toxins, which cannot reflect the uptake route under natural environments. Additionally, the toxins used in these experiments were either purified MC or dried algae containing MC. Therefore, toxic effects on fish from MC exposure through natural food uptake need to be evaluated experimentally, especially if there is long-term, frequent exposure in natural environments.

Probably because of the difficulty to measure MCs in the fish tissues, there were only a few experimental studies on tissue distribution and depuration of MC-LR in fish (Williams et al., 1995, 1997; Tencalla and Dietrich, 1997; Bury et al., 1998). Nevertheless, no other MCs have been examined despite of the wide presence of some toxins (e.g., MC-RR) in natural waters.

Herbivorous fish are among the most abundant fish groups. The phytoplanktivorous fish are especially important to humans because of their role in aquatic ecosystems as direct consumers of phytoplankton primary production, their importance as food fish, and their potential for biological management of algal blooms (Opuszynski and Shireman, 1995; Xie and Liu, 2001). The silver carp and bighead carp are the most important phytoplanktivorous fish in China, and they have been introduced worldwide for aquaculture, comprising as much as 18% (silver carp 12%) of the total freshwater fish production of the world (Xie and Liu, 2001). And both silver carp and bighead carp were suggested to be able to suppress and graze out *Microcystis aeruginosa* blooms (Xie and Liu, 2001). However, little is known for the dynamics of MC in such phytoplanktivorous fish that have specific anatomical and physiological adaptations for feeding on phytoplankton, including toxic cyanobacteria.

The main objectives of this study were, to use a subchronic toxic experiment to examine tissue distribution and depuration of two microcystins (MC-LR and MC-RR) in the phytoplanktivorous filter-feeding silver carp that were fed naturally with fresh *Microcystis viridis* cells in large tanks, to discuss the toxic effect of *Microcystis* blooms on the fish and the possible mechanisms underlying the different patterns of MC distributions between fishes with different habitats.

2. Materials and methods

Two plastic tanks (each 1000 l in volume) were used in the experiment, and the tanks were aerated with an air pump. Sliver carp fingerlings (mean weight of 10 ± 6 g) were purchased from the Saitama fishery company. Prior to the experiment, fish that were fed commercial carp food were acclimated in the two tanks, each containing 800 l dechloroized tap water for 1 week.

Cyanobacteria used as food of sliver carp were collected, using a 40 μm plankton net, from Chikato Pond from July to September. The dominant species (>90% in biomass) was *Microcystis viridis*. Cyanobacterial species was identified according to Komárek (1958, 1991).

There were two treatments (A, B) in the experiment. During July 5 and the end of August, all fish in Treatments A ($n=40$) and B ($n=40$) were fed only with *Microcystis* cells. *Microcystis* cells were added to both treatments in the morning at 5 day intervals, using a daily food ratio of approximately 3–12% of the fish body weight at water temperatures 15–25 °C (Chen, 1990). In Treatment B, after 40 days, we stopped to feed the fish with *Microcystis*, and instead used artificial carp feed (microcystin-free) as food of the fish for the next 40 days. The fish in Treatment A were always fed with *Microcystis* cells throughout the experiment.

Sampling was conducted at an interval of 20 days for the analysis of microcystins in the muscle (taken from the back), liver, blood (collected from a cut across the tail of the fish) and intestine (including intestinal tissues and gut contents) of the silver carp from both treatments. On each sampling date, 10 fish from each treatment were randomly collected and dissected to obtain the liver and intestine. All muscle, liver, blood and intestine samples were immediately frozen and freeze-dried for microcystin analysis. All waters (containing *Microcystis* cells) in both treatments were refreshed on 3 September (just after the 3rd sampling) when the major chemicals reached relatively high in the tanks, which might be harmful to the fish based on our observation. The water volume in the tanks was adjusted to maintain an equal fish density throughout the experiment.

The *Microcystis* cells from the pond and the seston from the tanks were lyophilized and the microcystins were extracted by the methods of Park et al. (1998). The method used to extract the microcystins of fish muscle, blood, liver and intestine was as follows: lyophilized samples 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 directly applied to 2 g of silica gel cartridge, which had been preconditioned by washing with 10 ml of 100% MeOH. The column containing toxins was washed with 100% MeOH (10 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 high performance liquid chromatography (HPLC) equipped with an ODS column (Cosmosil 5C18-AR; 4.6˙˙150 mm, Nakalai, Japan). The HPLC system consisted of a Shimadzu (Kyoto, Japan) LC-9A pump coupled to an SPD-10A set at 238 nm, an SPD-M10A photodiode array detector, C-R6A integrator. The sample was separated with a mobile phase consisting of methanol: 0.05 M phosphate buffer (pH 3.0, 58: 42) at a flow rate of 1 ml min^{-1}. The microcystin concentration was quantified by standard microcystins-RR and –LR provided by Dr. K.-I. Harada, Meijo University (Harada et al., 1988). ESI LC/MS analysis of microcystins was conducted under the following condition, MS: ionization, ESI; CDL temperature, 200 °C; nebulizing gas flow, 1.5 l/min (N_2); gain of detector, 1.5 KV.

There was insufficient toxin in fish blood and liver to allow for individual analysis. Therefore, we pooled, respectively, all livers, blood, muscle and intestines of the dissected fish on the first two sampling dates. Thus, each value represents an average microcystin amount of livers, blood, muscle and intestines. For the last two samplings, we divided the fish in each tank into two size groups: smaller and bigger groups.

Nutrients (N and P) in the tanks were analyzed by Ion Chromatography (Dionex DX120). Electric conductivity (EC) and pH were determined by a DKK HEC-110 EC meter and a pH meter (DKK HPH-110, Japan), respectively.

To determine chlorophyll a in the seston of the tanks, the water sample was filtered through a glass fiber filter (GF/C, Whatman, UK), and the filter was frozen and extracted in methanol over night in a dark refrigerator. The concentration of chlorophyll a was measured by a spectrophotometric method (Marker et al., 1980).

To count *Microcystis viridis* cells in the tank, the samples were agitated by gentle ultrasonication to split the colonies into single cells. *Microcystis* cells were counted microscopically using an improved Fuchs-Rosenthal hemaecytometer (KAYAGAKI warks grid volume: 1/16 mm^3, depth 1/5 mm).

3. Results

During the experiment, water temperature in the tanks varied between 15.5 and 25.3 °C with an average of 20.7 °C. The mean body weight of the sampled fish showed a slight increase tendency but varied greatly on each sampling date (Fig. 2). No fish mortality was observed during the 80 days of exposure time in both tanks.

Changes of major chemical parameters in the tank waters are shown in Table 1. The NO_3–N and PO_4–P concentration showed steady increase in both treatments. Before 3 September, NO_3–N and PO_4–P showed a steady increase in both tanks, while other chemicals varied irregularly. The change of water in the tanks directly led to remarkable declines in NO_3–N, PO_4–P and EC. Generally, there was a positive correlation between Chl-a and the abundance of *M. viridis* cells in both tanks. Chl-a amount reached as high as 366 μg l^{-1} in Tank A (Fig. 1).

During the experiment, the MC content of the collected *Microcystis* cells as food for the silver carp fluctuated between 268 and 866 μg g^{-1} DW (average 628 μg g^{-1} DW), with MC-LR between 110 and 292 (average 227) μg g^{-1} DW and MC-RR between 268 and 580 (average 401) μg g^{-1} DW (Fig. 2). That is, the content of MC-LR was ca. half that of RR in the food *Microcystis* cells (MC-LR/MC-RR = 0.57).

There were large variations in the concentrations of MC-LR and MC-RR in the seston of the experimental tanks (Fig. 1). At the beginning of the experiment (5 July), both MC contents and the MC-LR/MC-RR ratio in seston of the tanks were high, close to the level in the collected *Microcystis* cells. However, MC contents in seston of the tanks declined drastically after 20 days, whereas the MC-LR/MC-RR ratio exceeded 1 during July 24 to September 23 in Tank A. In Tank B, the change of food from *M. viridis* cells to artificial carp feed after the second sampling resulted in the elimination of MC in the seston of the tanks eventually. Based on the chlorophyll-a contents in the seston, the decreased MC contents might have been attributed to the accumulation of fish feces, while the increased MC-LR/MC-RR ratio might have been due to a different metabolism of the toxins by the fish.

We only collected one pooled feces sample of the fish from both tanks on July 30. The mean MC content in the feces was 44.47 (MC-RR 25.4 and MC-LR 19.1) μg g^{-1} DW, and the MC-LR/MC-RR ratio in the feces reached 0.75, considerably higher than that in the collected *Microcystis* cells (0.57).

The percentage of the weight of the intestinal tract in total body weight varied between 5.2 and 8.7% with a mean of 6.7 and 7.3% in Tanks A and B, respectively (Fig. 1). MC intake per intestinal tract varied between 335.6 and 747.7 mg kg^{-1} body weight (fresh wet weight) in Tank A. Since the daily food ration reached 10.8–16.7% at a water temperature of 23.7–27.5 °C, the MC intake per intestinal tract might be lower? than the daily MC intake of silver carp.

In Tank A, the MC in the intestines fluctuated between 49.2 and 115.3 (average 78.8) μg g^{-1} DW, which was only ca. 1/8 that in the original *Microcystis*

cells. MC-RR ranged between 41.5 and 99.5 (average 67.3) μg g^{-1} DW, and MC-LR varied between 6.9 and 15.8 (average 11.5) μg g^{-1} DW. Thus, the ratio of MC-LR/MC-RR in the intestines was only 0.17. This ratio was as low as 0.07 for the first two samplings in Tank B. When the food of silver carp was changed from *Microcystis* cells to artificial carp feed in Tank B, both MC-RR and MC-LR disappeared completely from the intestines in spite of the presence of small quantity of *Microcystis* cells (Table 1).

On the first sampling date, MC-RR in the blood was very low (0.336 μg g^{-1} DW in Tank A) or below limit of detection (in Tank B). In Tank A, it showed a rapid increase with the maximum 49.7 μg g^{-1} DW on September 2, while it declined substantially (30.2 μg g^{-1} DW) at the end of the experiment in spite of an increased MS-RR content in the intestines. In Tank B, the change of food from *Microcystis* cells to carp feed resulted in a rapid elimination of MC-RR from the blood (declining to 3.3 μg g^{-1} DW on 2 September and to zero on 23

Table 1
Changes of nitrogen and phosphorus (mg l^{-1}), electronic conductivity (EC, μS cm^{-1}) and pH in the tank waters

Date	Tank A					Tank B				
	NO_3–N	NO_2–N	PO_4–P	EC	pH	NO_3–N	NO_2–N	PO_4–P	EC	pH
July 5	0	0	0	87.7	8.2	0	0	0	87.9	8.4
July 24	0.07	0.08	0.13	93.0	7.6	0.06	0.10	0.11	95.7	7.6
August 13	6.77	0.10	0.36	86.7	6.5	6.95	0.08	0.39	85.5	6.1
September 2	10.50	0	0.77	119.7	5.7	10.01	0.03	1.82	120.7	6.3
September 23	4.47	0	0.51	76.9	7.4	2.92	0.17	0.43	76.3	7.4

All waters (including *Microcystis* cells) in both tanks were renewed on 3 September.

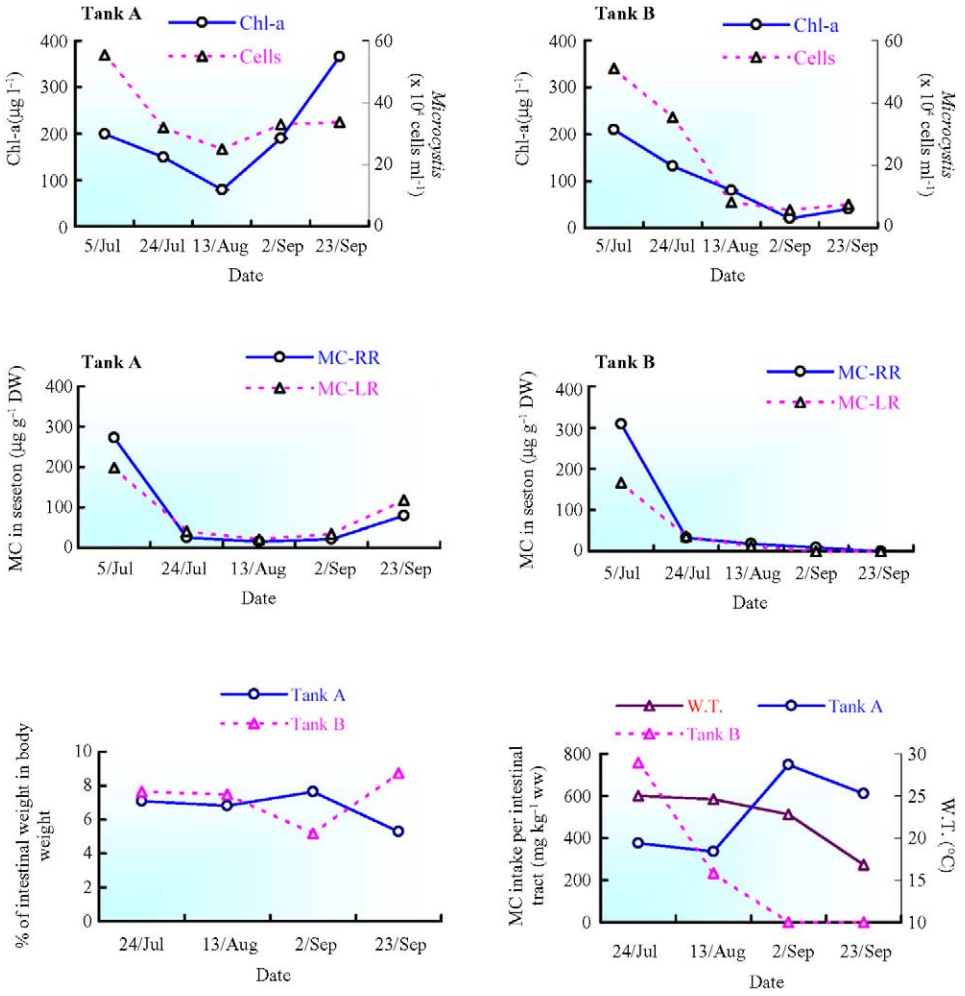


Fig. 1. The dynamics of chlorophyll a (Chl-a) and *Microcystis aeruginosa* cells in the water of the tanks, MC-RR and MC-LR in the dry seston, percentage of intestinal weight in body weight, and MC intake per intestinal tract.

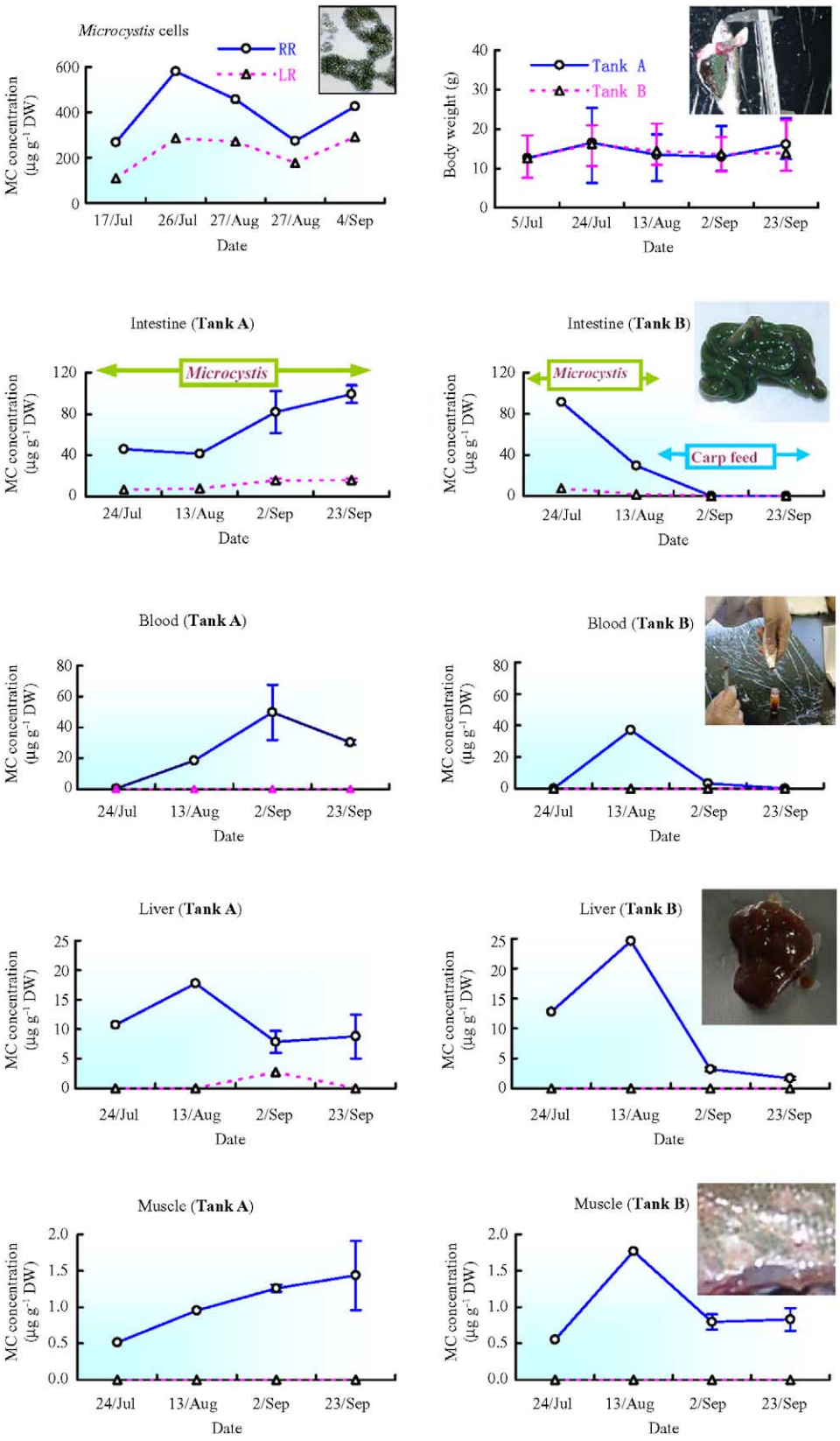


Fig. 2. The changes in body weight of the sampled silver carp, and the dynamics of MC-RR and MC-LR concentrations in the collected *Microcystis aeruginosa* cells, and in the intestinal tract, blood, liver and muscle of silver carp.

September). No MC-LR was detectable in the fish blood samples of both tanks throughout the experiment.

In Tank A, during the first 40 days' exposure, MC-RR contents in the livers showed a rapid increase (with a maximum of 17.8 μg g^{-1} DW on Aug. 13), and then declined substantially to 7.8–8.8 μg g^{-1} DW, in spite of an increased MC-RR contents in the intestines during the same period. MC-LR was detected only on September 2 (2.8 μg g^{-1} DW).

MC-RR content in muscle was relatively low (0.51–1.44 in Tank A, 0.55–1.77 in Tank B). There was a constant increase in MC-RR content of the muscle in Tank A. In Tank B, the change of fish food from *Microcystis* cells to carp feed caused a significant reduction in the MC-RR content of the muscle, but remained still at a relatively high level (ca. half of the maximum) even after a 40 days' depuration. Thus, during the depuration period, the MC-RR dynamics in muscle was obviously different from that in intestines, blood and livers. No MC-LR was detectable in the muscle samples during the experiment.

The presence of MC-RR and MC-LR in the intestine was confirmed by ESI LC/MC (Fig. 3). The mass chromatograms were monitored at m/z 1038 and 995.

4. Discussion

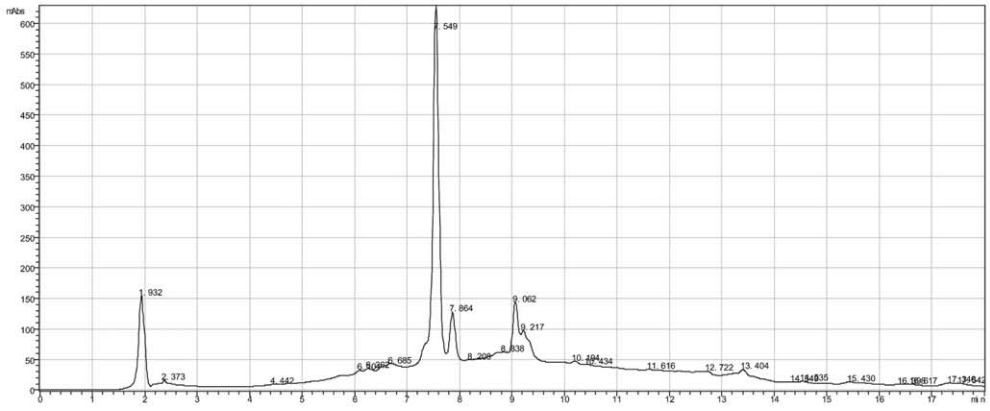
Previous experimental studies on MC dynamics in fish were limited to acute toxic experiment and to MC-LR, either through oral gavaging (Tencalla and Dietrich, 1997; Bury et al., 1998) or through intraperitoneal injection (Williams et al., 1995; 1997). Instead of the cold-water carnivorous salmonids (rainbow trout and Atlantic salmon), a warm-water phytoplanktivorous cyprinid, silver carp, was tested in the present study. The present research was also the first to include a sub-chronic MC toxic experiment in fish, and to use fresh *Microcystis* cells as food of fish for the examination of both MC-LR and MC-RR.

In the present study, it wasn't clear why the MC-LR/MC-RR ratio changed from 0.57 in the collected *Microcystis* cells to 0.07–0.17 in the intestinal tract, to 0.75 in the feces and sometimes over one in the seston of the tanks again! The significantly low MC-LR/MC-RR ratio in the intestinal tract may suggest the possibility of an active degradation of MC-LR during the process of digestion, while the increased MC-LR/MC-RR ratio in the feces and the feces-rich seston may indicate that MC-RR in the gut fluids might have been massively transported across the intestines and embedded in the fish body, whereas the transportation of MC-LR across the intestines was probably inhibited selectively. The satisfactory explanation for such a great difference in the MC-LR/MC-RR ratio needs a further assumption that transportation of MC-RR took place quickly and efficiently only in a minor part of the intestinal tract. Unfortunately, since we did not measure MC in different parts of the long intestinal tract, it is not clear if there was a significant difference in the MC-LR/MC-RR ratio across the intestinal tract of silver carp. MC was demonstrated to be predominantly taken up via the ileum in rats (Dahlem et al., 1989), and a similar mechanism was suggested for fish (Fischer and Dietrich, 2000). Thus, it is likely that different parts of the intestinal tract of silver carp may differ in degradation and transportation of the toxins during food digestion.

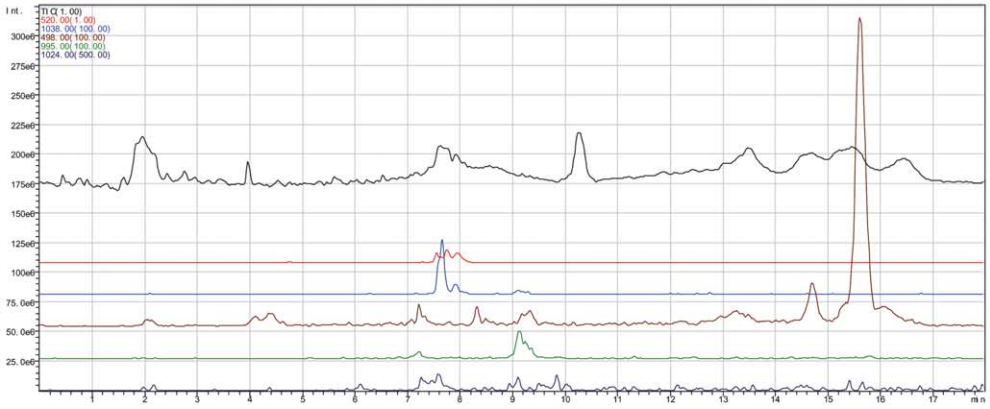
Among the group of MCs, MC-LR is the most toxic and thus best-characterized (Sivonen and Jones, 1999; Fitzgerald et al., 1999). However, in the present study, no MC-LR was detectable in the muscle and blood samples of the silver carp, in spite of the abundant presence of this toxin in the intestines, and for the liver, there was only one case when relatively minor quantity of MC-LR was detected. Physiologically, the fact that MC-LR was not detectable in the blood suggests that little MC-LR could be transported across the intestines of silver carp. This is quite different from the results of previous gavaging experiment on rainbow trout (Tencalla and Dietrich, 1997). MC-LR is transported across the intestine of rainbow trout immediately: at a dose of 5700 μg MC-LR kg w.w.$^{-1}$, MC-LR was detected in both blood and liver at 1 h with the maximum 517 ng ml^{-1} in blood and 524 μg kg w.w.$^{-1}$ in liver at 3 h (Tencalla and Dietrich, 1997), and at a dose of 0.64 (or 5873) μg MC-LR kg w.w.$^{-1}$, after 24 hrs, MC-LR reached 0.739 (or 4100) μg kg w.w.$^{-1}$ in liver, and 0.166 (or 1200) μg kg w.w.$^{-1}$ in muscle (Bury et al., 1998).

The present experiment indicated that in spite of 20 days exposure, little MC-RR (0.336 μg g d.w.$^{-1}$ in Tank A, zero in Tank B) was detected in the blood of silver carp while there had already been quite a lot of MC-RR accumulating in the liver and muscle. It is likely that during the 1st 20 days, transportation of MC-RR from blood to liver might be so transient that it was not detectable in the blood, whereas with the accumulation of MC-RR in the liver and the declining absorption of MC-RR by liver, MC-RR began to accumulate in the blood. This may also explain why MC-LR was absent in the blood but present in minor quantity in the liver (Tank A, 2 September). We have not yet found physiological or ecological explanations on why there were so great differences between MC-LR and MC-RR dynamics in silver carp.

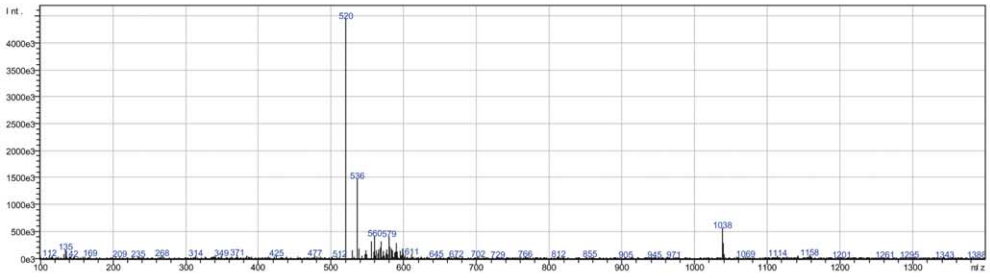
In the present sub-chronic toxicity experiment, the depuration of MC-RR concentrations occurred slowly than uptakes in blood, liver and muscle, and the depuration rate was in the order of blood->liver>muscle: a depuration period of 20 days resulted in MC-RR declines from 37.02 to 3.28 μg g^{-1} (d.w.) in blood, from 24.63 to 3.21 μg g^{-1} (d.w.) in liver, and from 1.77 to 0.8 μg g^{-1} (d.w.) in muscle. This tendency generally agrees with the acute experimental result for MC-LR in the blood and liver of rainbow trout, while



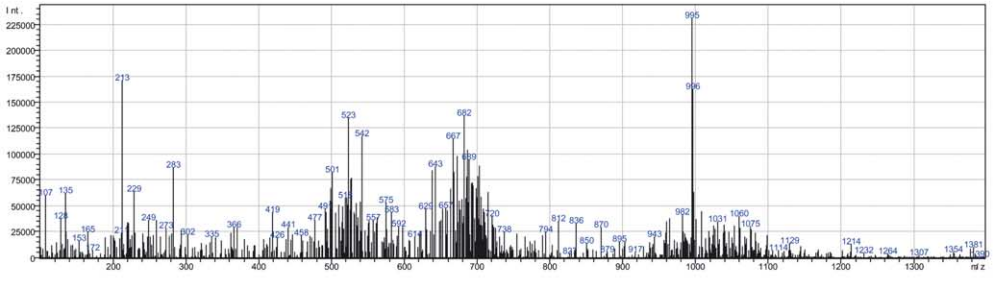
(a) high performance liquid chromatogram monitored at 238nm



(b) mass chromatograms monitored at m/z 520, 1038, 1045 and 995



(c) ESI MS spectrum at 7.665 min (microcystin-RR)



(d) ESI Mass spectrum at 9.136 min (microcystin-LR)

Fig. 3. ESI LC/MS analysis of microcystins in the intestine of silver carp (2 September 2002).

the depuration of MC-LR in rainbow trout is much faster: a depuration period of 3 days resulted in reductions of 100 and 91.6% in blood and liver, respectively (Tencalla and Dietrich, 1997). Apparently, there is a fundamental difference in the kinetics of MC in the tissue between rainbow trout and silver carp, but it seems to be different to attribute such a difference to the changes in dose, food type and analytic method.

The daily doses in our experiment were estimated to be 1202–4807 μg MC-RR and 681–2726 μg MC-LR kg (w.w.)$^{-1}$, assuming a food ratio of 3–12% at water temperatures 15–25 °C (Chen, 1990) and considering the average contents of MC-RR (400.6 μg g d.w.) and MC-LR (227.2 μg g d.w.) in the added *Microcystis* cells. Thus, the MC doses for silver carp were within the range of those for rainbow trout (Tencalla and Dietrich, 1997; Bury et al., 1998). In the previous experiments, freeze-dried *Microcystis aeruginosa* cells containing MC-LR (Tencalla and Dietrich, 1997) or purified MC-LR from freeze-dried *M. viridis* cells (Bury et al., 1998) were introduced directly into the trout stomachs, while in our experiment, silver carp were fed naturally with fresh *Microcystis virids* cells containing both MC-LR and MC-RR. Different analytical methods were used to detect MC in fish tissues; protein phosphatase inhibition assay or the radiolabelled ^{3}H-MC-LR for rainbow trout (Tencalla and Dietrich, 1997; Bury et al., 1998), while the HPLC for silver carp (in the present study).

In this study, a decline in the ratio of MC-LR/MC-RR from 0.57 in the *Microcystis* cells to 0.07–0.17 in the intestines and the absence of MC-LR in the blood of silver carp suggests that (1) there may exist a mechanism to degrade MC-LR actively in the intestinal tract in the process of digestion, and/or that (2) the intestines may selectively inhibit the transportation of MC-LR. Previous studies on rainbow trout show that the intestinal tract represents an important barrier to MC-LR: less than 5% and 1.5% of the applied dose reached the blood and liver in 3 days (Tencalla and Dietrich, 1997), and only ca. 0.28-1.29% of the applied dose in the liver and muscle in 24 h (Bury et al., 1998). Such functioning as a barrier to MC-LR might have made great development in the intestinal tract of silver carp. Little is known about the toxicity of MC-RR to fish, while in spite of massive accumulation of MC-RR in various tissues, no mortality was observed, suggesting that to silver carp, this toxin may be less harmful. This needs to be confirmed in our future study.

The vulnerability of fish to MC, from an evolutionary point of view, is probably influenced by the natures of habitat and feeding mode. Species native to oligotrophic habitats are more vulnerable than species native to eutrophic habitats where MC exposure is more frequent (Snyder et al., 2002). The present study suggests that phytoplanktivorous fish are probably more resistant to MC exposure than other fishes.

Silver carp is native to the flooding plain of East Asia—(from Amur River in the north and to Red River in the south) with abundant distribution in the productive subtropical Yangtze River (Xie and Chen, 2001), and historically is frequently exposed to eutrophic waters containing toxic cyanobacteria. This fish is adapted to an efficient filter-feeding mode mainly on phytoplankton (Xie, 1999, 2001). Thus, it should not be astonished to see an evolutionary development of a stronger resistance to MC in silver carp, but not in rainbow trout that are native to relatively cold and oligotrophic habitats, feeding mainly on zooplankton and zoobenthos.

The stronger resistance of silver carp to the cyanotoxins are evidenced by the experience in a hypereutrophic Chinese lake, Lake Donghu (surface area 32 km^2), which is located in the productive flood plain of the subtropical Yangtze basins with heavy surface blooms of cyanobacteria (mainly *Microcystis*, *Anabaena* and *Oscillatoria*) regularly since 1970s (Xie and Liu, 2001). Although the blooms in 1984–1986 were found to be toxic to mouse (through i.p. injection) with a LD_{50} of 100-370 mg dry *M. aeruginosa* kg^{-1} (He, 1990), a trace experiment using *Microcystis aeruginosa* (>99%) collected from the blooms and then labeled with ^{32}P showed that silver carp assimilated 35–48% of the ingested *M. aeruginosa* (at 26–30 °C) (Zhu and Deng, 1983), and more importantly, the blooms of the whole lake had been completely eliminated through increased stocking of the phytoplanktivorous silver and bighead carps after the mid-1980s, and has never occurred again for a period of 18 years (Xie and Liu, 2001) The grazing ability of silver carp on toxic cyanobacteria suggests an applicability of using phytoplanktivorous fish to counteract cyanotoxin contamination in eutrophic waters.

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