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Possibility for Water Quality Biocontrol: Observation of Microcystin Transfer in the "Cyanobacteria–Cladohorn–Fish" Food Chain

Qin-Ming Xie [1,2,3], Yan-Wei Zhang [1,2] and Ta-Jen Chu [1,2,*]

1 Fisheries College, Jimei University, Xiamen 361021, China; qmxie@jmu.edu.cn (Q.-M.X.); zhang202206yw@163.com (Y.-W.Z.)
2 Fujian Provincial Key Laboratory of Marine Fishery Resources and Eco-Environment, Fisheries College, Jimei University, Xiamen 361021, China
3 Key Laboratory of Healthy Mariculture for the East China Sea, Ministry of Agriculture and Rural Affairs, Fisheries College, Jimei University, Xiamen 361021, China
* Correspondence: chutajen@gmail.com

Abstract: Microcystins appear to be considered one of the most dangerous cyanobacterial toxins in the world. The accumulation and change of microcystins MC-LR and MC-RR in the "cyanobacteria–cladocera–fish" food chain were studied. *Microcystis aeruginosa* was fed to *Moina macrocopa* at three densities, 5.0×10^3, 5.0×10^5, and 5.0×10^6 cells/mL, and then passed to *Cyprinus flammans*. The total amount of MCs in the cyanobacteria cell extract increased with increasing density. The content of MCs in *M. macrocopa* increased with the feeding density of *M. aeruginosa*. In the final stage of experiments, MC-RR was the only MC that could be transmitted by *M. macrocopa* and persisted in red carp. In this study, changes in the concentrations of MC-LR and MC-RR in the liver of red carp seem to indicate some kind of transformation or degradation mechanism. It shows the possibility of MCs concentration-controlled biodefense in eutrophic waters.

Keywords: *Microcystins*; *Microcystis aeruginosa*; *Moina macrocopa*; *Cyprinus flammans*

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1. Introduction

Eutrophication has been one of the major ecological problems of global surface water in the past decades [1–3]. It is the process by which water bodies have excessive nutrient enrichment, often resulting in overgrowth of macrophytes, algae, or cyanobacteria [4]. In this context, harmful algal blooms (HABs), observed worldwide, have attracted great attention and warnings [5,6], as they pose a serious threat to human health and natural resources, causing huge economic losses in global aquaculture and fisheries [7–10]. One of the HABs, cyanobacteria, are photosynthetic prokaryotes that are commonly found in some aquatic environments, such as lakes, reservoirs, ponds, streams, rivers, wetlands, etc. [11,12]. As mentioned earlier, eutrophication results in frequent cyanobacterial blooms with severe negative impacts on water quality and aquatic communities. Unfortunately, these blooms are often accompanied by the production of a variety of cyanotoxins, including microcystins (MCs), cylindrospermopins, anatoxins, saxitoxins, and nodularins [12,13]. Likewise, it is classified by target organs, including hepatotoxins (liver), neurotoxins (nervous system), and dermatotoxins (skin) [14]. Among these cyanotoxins, MCs appear to be considered one of the most dangerous worldwide [14–19].

Microcystis aeruginosa, a bloom-forming cyanobacterial species, is common in eutrophic freshwater around the world, and blooms often form in large areas, attracting worldwide attention and causing serious disasters [20]. Some *M. aeruginosa* strains produce MC, a cyclic peptide that can produce both neurotoxins (lipopolysaccharides (LPSs)) [21] and hepatotoxins [22,23]. More than 300 MC analogues have been identified [14,24,25]. Many

MCs have a ring structure of seven amino acids, which compose one unique phenyl decadienoic acid, four invariable D-amino acids, and two variable L-amino acids [26], the most common of which is MC-LR (MC lysine, arginine) [27], which is the most toxic, the largest, and the most studied [28]. Other variants that also occur frequently include MC-RR (MC arginine, arginine), MC-YR, and MC-LA [29]. MC-RR and MC-M(O)R (methionine sulfoxide, arginine) have been studied and considered less toxic [30]. *Microcystis* blooms have been reported in at least 108 countries, with MCs present in at least 79 countries [31]. MC is biosynthesized via mixed non-ribosomal peptide synthetase/polyketide synthase (NRPS/PKS) pathways. It is speculated that MCs play important physiological roles, due to genes related to the pathway constituting a large part of the cyanobacterial genome [12].

M. aeruginosa is favored by warm temperatures [32]. The high abundance of this species predominates in water temperatures ranging from 24.7 to 33.9 °C [33,34]. In laboratory experiments culturing *Microcystis* species at different temperatures, *M. aeruginosa* grew significantly faster at high temperatures (30 and 35 °C) compared to other species [35]. Peng et al. [35] mentioned that toxicity and maximal growth rate are not fully coupled since cyanobacteria have the highest laboratory growth rate at 32 °C and are most toxic at 20 °C. Toxicity decreases with increasing temperature above 28 °C [35]. Therefore, water temperature is not only one of the important controlling factors for bloom development [36], but also for the seasonal succession of *Microcystis* species [34]. Based on this, changing the water temperature is an important factor in controlling the composition of cyanobacteria [37]. Therefore, changes in water temperature in summer may be an important environmental factor for the alternate succession of *Microcystis* species [34]. This is also the season when managers should pay attention and take measures accordingly.

The effect of *M. aeruginosa* on fish is mainly manifested by its secondary metabolite, microcystin [38]. At present, most of the studies are carried out by direct feeding, injection, or exposure to environments with MCs [26,39–45]. However, there are few studies on the indirect effects on fish through the food chain. In this paper, under laboratory conditions, *Moina macrocopa* is used as the intermediate carrier of the entire food chain of "cyanobacteria–fish" to observe the physiological and biochemical changes from liver tissue of *Cyprinus flammans*, and further explore the metabolic process of MCs' delivery in the aquatic ecological food chain. The purpose is to clarify the mechanism of MCs' transport and provide the basis for the protection of residents' health and management.

2. Materials and Methods

2.1. Test Materials and Preliminary Treatment

2.1.1. *M. aeruginosa* Cultivation

Strain *M. aeruginosa* FACHB-905 (purchased from the Chinese Academy of Sciences' Institute of Hydrobiology) was cultivated with M-11 medium ($NaNO_3$ 100 mg, K_2HPO_4 10 mg, $MgSO_4 \cdot 7H_2O$ 75 mg, $CaCl_2 \cdot 2H_2O$ 40 mg, $NaCO_3$ 20 mg, ferric citrate ($C_6H_5FeO_7$) 6 mg, Na_2-EDTA$\cdot 2H_2O$ 1 mg, distilled water 1000 mL, pH 8.0) [46–49] at 23 ± 1 °C, under a light intensity of 4000 l× and a 12 h light:12 h dark cycle. Stock cultures were shaken at regular intervals (3 times a day) to prevent the algae from wall growth and settling down. The number of cyanobacteria cells was counted every 2 days. To count 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 FuchsRosenthal hemocytometer (KAYAGAKI warks grid volume: 1/16 mm^3, depth 1/5 mm). The number of algal cells was counted every 2 days. Before the experiment, the cells were diluted to 5.0×10^3, 5.0×10^5, and 5.0×10^6 cells/mL, respectively.

2.1.2. Cladoceran Cultivation

Cladocera *Moina macrocopa,* commonly known as water fleas, are the main components of freshwater zooplankton [50]. These small water fleas are not only excellent bait for fish, but also ideal for biological testing [51]. They are a characteristic inhabitant of small, usually ephemeral, water bodies from temperate to tropical regions, which are often

rich in dissolved organic carbon [50–52]. Our clone was collected from the pond at the headquarters of Jimei University, Fujian Province, according to the relevant collection method [53]. Since then, it has been successfully used to study the effects of *M. aeruginosa* on the feeding behavior of *M. macrocopa* [54]. The isolated *M. macrocopa* were cultured and fed with laboratory-grown *Scenedesmus* sp. The temperature of the culture water was controlled at 23.0 ± 1.0 °C, and the light conditions were natural light and aerated culture.

2.1.3. *C. flammans* Cultivation and Test Conditions

The test fish were selected from healthy juvenile red carp *C. flammans* with an average body weight of 1.90 ± 0.23 g and an average body length of 3.65 ± 0.68 cm. All the experimental fish were acclimated in the laboratory for 10 days before the experiment. Before the test, juvenile red carp with lively action, glossy body color, fully stretched fins, strong appetite, and basically the same size were selected. The test water was tap water with aeration for 24 h (the water was tested, and no MC was detected). The test container was a self-made glass jar with a size of 30 × 20 × 20 cm. The test temperature was room temperature (15–21 °C), and a natural light cycle was adopted. During the experiment, the water was changed once every evening from 17:00 to 18:00 PM before feeding and the fish excrement was cleaned up, and 1/3 of the water was replaced each time.

2.2. Experiment and Test Method

2.2.1. Feeding and Toxicity Test of *Moina macrocopa*

The cultivated *M. macrocopa* were induced to gather by light from 17:00 to 19:00 PM every evening. Then, the required amount was taken into the prepared M-11 culture medium and placed in a light incubator, under a light intensity of 4000 l× and starvation for 12 h. The next morning, the starvation-cultured *M. macrocopa* were placed in the same amount of *M. aeruginosa* solution with a density of 5.0×10^3, 5.0×10^5, and 5.0×10^6 cells/mL, respectively, for the poisoning test. The experimental conditions were the same as the culture conditions of *Microcystis*, and the filter feeding time of *M. macrocopa* was 8 h.

2.2.2. Toxicity Test of Red Carp

The selected 135 juvenile red carp were randomly divided into 5 groups, 3 parallel groups were set in each group, and 9 red carp were placed in each parallel group. There was no significant difference in the average length and weight of each parallel group ($p > 0.05$) (Table 1). The red carp were fed regularly every day, and the feed was fed at 0.5 g/day, about 180 cladocerans were fed per tank on average (wet weight was about 0.5 g), and the test period was 30 days.

Table 1. The MC content in red carp was designed in different experimental groups.

Trial Group	Treatment
F_{ck1}	fed with special feed *
F_{ck2}	*M. macrocopa* fed with 5.0×10^5 cells/mL of *Scenedesmus* sp.
F_{A1}	*M. macrocopa* fed with 5.0×10^3 cells/mL of *M. aeruginosa*
F_{A2}	*M. macrocopa* fed with 5.0×10^5 cells/mL of *M. aeruginosa*
F_{A3}	*M. macrocopa* fed with 5.0×10^6 cells/mL of *M. aeruginosa*

*: The content includes 5% Peruvian fish meal, 10% barley, 40% soybean cake, and 45% wheat bran. It also adds multivitamins, inorganic salts, lysine, and methionine in appropriate amounts, and the feed coefficient was 2.3–2.4 and the code is 8441.

2.2.3. Extraction and Detection Methods of MCs

Solid-phase extraction (SPE) is usually used for the extraction and concentration of samples in aqueous solutions, and the determination of MCs in water by HPLC-VWD after SPE pretreatment is listed as the China National Standard Method (GB/T 20466-2006) [55–57]. The chromatographic conditions were as follows: column temperature: 40 °C, mobile phase: methanol and phosphate buffer (adjusted to pH 3.0 with 20% phosphoric acid solution) in

a volume ratio of 57:43, flow rate: 1 mL/min, and detector: UV-Vis detector wavelength 238 nm.

The extraction procedure was as follows [58]: The lyophilized tissue was dissolved in 1 mL of methanol (85%), disrupted with an ultrasonic cell crusher (Model 450; Brason Ultrasonics, Danbury, CT, USA) for 5 min. The homogenized tissue was then extracted for 6 h, at 4 °C. The extracted homogenized tissue was centrifuged at 12,000 r min^{-1} for 10 min at 4 °C, and the supernatant was collected. The above operation was repeated for the pellet after centrifugation, and the supernatant was collected. The two supernatants were mixed and extracted with an equal volume of n-hexane for 4 h. The upper layer of n-hexane was removed and dried with a Nitrogen Blowing Instrument (model: EYELA MG-2200). The dried extract was redissolved in 100 μL of 50% methanol and analyzed by liquid chromatography.

2.3. Statistical Analysis

One-way ANOVA was performed to compare the extract contents of MC-LR and MC-RR of different densities of *M. aeruginosa.* All statistical analyses were performed with the statistical package of SPSS-16 (SYSTA, Telford, PA, USA).

3. Results

3.1. Change of MC-LR and MC-RR in the M. aeruginosa

The contents of MC-LR and MC-RR extract of different densities of *M. aeruginosa* are shown in Table 2. One-way ANOVA indicated that MC-LR, MC-RR, and MC total were statistically significant at three different densities. In the high-concentration group (5.0×10^5 and 5.0×10^6 cells/mL), the content of MC-LR was higher than that of MC-RR. The ratio of the two toxins, MC-LR and MC-RR, was related to the external environmental factors and the biomass of algal cells. In addition, the total amount of MCs in the cyanobacteria cell extract increased with the increasing density.

Table 2. ANOVA test and MC contents among different densities of *M. aeruginosa*.

M. aeruginosa Density (cells/mL)	MC-LR (μg/mL)	MC-RR (μg/mL)	MC Total (μg/mL)
5.0×10^3	0.029 ± 0.018	0.090 ± 0.006	0.119 ± 0.024
5.0×10^5	0.673 ± 0.037	0.162 ± 0.199	0.835 ± 0.233
5.0×10^6	0.873 ± 0.020	0.164 ± 0.241	1.037 ± 0.260
	$F = 6.910$, $p = 0.028$ *	$F = 17.971$, $p = 0.003$ **	$F = 17.092$, $p = 0.003$ **

Note: * $p < 0.05$; ** $p < 0.01$.

3.2. Change of MC-LR and MC-RR in the M. macrocopa

In the *M. macrocopa* toxicity transfer experiments, a total of four experimental groups included three densities of *M. aeruginosa* (as shown in Table 3), and one group that was not fed. One-way ANOVA indicated that in addition to MC-LR, MC-RR and MC total were statistically significant at the three different densities. In different groups, approximately 180 individuals in each group of *M. macrocopa* were fed 1 mL of *M. aeruginosa* for 8 h. MCs were then extracted from these *M. macrocopa* and analyzed by HPLC. The contents of MC-LR and MC-RR are shown in Table 3.

F_{ck2} group was set for feeding *Scenedesmus* for 8 h. F_{A1}–F_{A3} groups indicated feeding different densities of *M. aeruginosa* for 8 h.The contents of MCs were non-detectable, at 0.120, 0.36, and 0.450 μg/mL in the different groups, respectively (Table 3). MC-LR and MC-RR were detected in the extracts of the three treatment groups. This indicated that the contents of MCs in *M. macrocopa* increased with the increase of the density of the *M. aeruginosa*. Clearly, the content of MC-LR and MC-RR in *M. macrocopa* followed the content of *M. aeruginosa* cells. There appears to be a correspondence between the toxin content and feeding density. That is, the higher the density of *M. aeruginosa*, the higher the toxin content passed to *M. macrocopa*. Similarly, this result also pointed out that once

M. aeruginosa bloom occurs in the natural environment, the density increases rapidly at this time, and the content of MCs transmitted to *M. macrocopa* is higher.

Table 3. ANOVA test and MC contents of *M. macrocopa* among different experimental groups.

Trial Group (cells/mL)	MC-LR (μg/mL)	MC-RR (μg/mL)	MC Total (μg/mL)
F_{ck2}	/	/	/
F_{A1}	0.040 ± 0.044	0.080 ± 0.017	0.120 ± 0.061
F_{A2}	0.196 ± 0.042	0.155 ± 0.040	0.351 ± 0.014
F_{A3}	0.319 ± 0.026	0.131 ± 0.038	0.450 ± 0.038
	F = 3.046, p = 0.122	F = 52.714, p = 0.000 ***	F = 48.260, p = 0.000 ***

Note: *** $p < 0.001$.

3.3. Change of MC-LR and MC-RR in the Red Carp

According to the conditions of different experimental groups (Table 1), the contents of MCs in red carp were obtained. In total, 135 juvenile red carp were randomly selected for 5 groups, 3 parallel groups were set in each group, and 9 red carp were placed in each parallel group. After the experiment, the contents of MCs in the extract of *C. flammans* liver from different experimental groups were as shown in Table 4. One-way ANOVA indicated that MC-RR and MC total were statistically significant at the three different densities.

Table 4. ANOVA test and MC contents of *C. flammans* liver among different experimental groups.

Trial Group (cells/mL)	MC-LR (μg/mL)	MC-RR (μg/mL)	MC Total (μg/mL)
F_{ck1}	/	/	/
F_{ck2}	/	/	/
F_{A1}	/	0.342 ± 0.027	0.342
F_{A2}	/	1.053 ± 0.160	1.053
F_{A3}	/	2.540 ± 0.288	2.540
		F = 103.278, p = 0.000 ***	

Note: *** $p < 0.001$.

In Table 4, neither F_{ck1} nor F_{ck2} detected MCs. In addition, the total MCs obtained in the other three groups F_{A1}, F_{A2}, and F_{A3}, were 0.342, 1.053, and 2.540 μg/mL, respectively. Only MC-RR was detected in the red carp liver extract. In particular, MC-RR is the only MCs that can be transmitted by *M. macrocopa* and persist in red carp. The transferred amount of MCs to the liver of red carp was related to the MC content of *M. macrocopa*, that is, the density of the filtered *M. aeruginosa*. The results showed that the enrichment of MCs in red carp liver increased with the increase of cyanobacteria density.

Comparing Table 4 with Table 2, it can be seen that the MCs in red carp liver were 2.87, 1.26, and 2.45 times higher than the MCs in *M. aeruginosa* in the corresponding group, respectively. Additionally, comparing Table 4 with Table 3, the MCs in the red carp liver were 2.85, 4.14, and 5.64 times higher than those in *M. macrocopa* in the corresponding group, respectively. To sum up, the accumulation of MCs in red carp at the end of the food chain was observed, which was much higher than the toxin content in *M. aeruginosa* and *M. macrocopa*.

4. Discussion

4.1. The Effect of MCs on Fish

Cyanobacterial toxins enter the fish body mainly through three pathways, including gills, skin, and intestines. Xie et al. [59] studied the effect of *M. aeruginosa* on the liver tissue and gill tissue of the *C. flammans* under the laboratory conditions, and observed the histopathology of the red carp. The results showed that the control group (non-toxic) hepatocytes had regular round nuclei, uniform cytoplasm, intact cell walls, and obvi-

ous hepatocyte cords. However, the results of the experimental group (different density) showed a different damage situation. In the low-density group, the nuclei were deformed, vacuoles appeared in the cytoplasm, and some damaged hepatocytes appeared. In the medium-density group, the shape of the cells disappeared completely, the nucleus was severely atrophied and deformed, the membrane of some liver cells was dissolved, and the cytoplasm began to agglutinate. In the high-density group, most of the nuclei disappeared, the cell membranes of most cells were completely dissolved, and the cytoplasm was granular [59]. These results indicated that the MCs could be transmitted to the red carp through the *M. macrocopa*, and the liver tissues of the fish were damaged. The degree of damage was directly proportional to the amount of MCs enriched in the fed *M. macrocopa* [59]. In addition, effects on the gills of red carp were also observed. Compared with the control group, the cells in the experimental group were swollen, but the whole cell shape was basically intact. Different degrees of apoptosis occurred in the high-algae density group, which was not only related to the enriched *M. macrocopa* in fish, but also possibly related to the amount of MCs.

Some studies also focused on the effect of MCs on the histopathology of fish liver [41,60–62]. The liver of common carp (*Cyprinus carpio*), rainbow trout (*Oncorhynchus mykiss*), and brown trout was damaged by gavage or intraperitoneal injection of MCs, mainly including hepatocyte separation, apoptosis cell death, and delayed cell repair. Fischer et al. [60] showed that the accumulation of MCs and subsequent cell morphological changes, PP inhibition, and hepatocyte necrosis represent the major events in microcystin-induced hepatotoxicity. In the study of Fischer and Dietrich [44], it was demonstrated that the mechanism of cell death is mainly apoptosis. Li et al. [41] reported that when carp free hepatocytes were immersed in 50 μg/L of MC-LR solution, the cell membrane was formed, the nucleus was shrunken and deformed, the rough endoplasmic reticulum membrane was vesicle-like and folded, the cells were swollen, and cytoskeleton rearrangement occurred. Wei et al. [62] described that intraperitoneal injection of MC-LR could not only cause mitochondrial edema and endoplasmic reticulum expansion, but could also widen the junctional gap in the liver of grass carp (*Ctenopharyngodon idella*) and cause cholestasis, inflammatory cell infiltration, a large number of lipid droplets in the cytoplasm, lipofuscin, lysosome increase, and a series of lesions. In the study of Atencio et al. [26], tilapia (*Oreochromis* sp.) injected with two MC variants, MC-LR and MC-RR, caused changes in the activities of acid and alkaline phosphatase (ACP and ALP) in vital organs, and showed different response patterns. Fish injected with MC-LR showed that the liver and kidneys were particularly affected. MC-RR induced a pronounced increase of ACP in the kidney and a significant increase of ALP in the liver. Both MC variants caused pathological lesions in hepatic tissues [26]. The effect of MCs on the organs in fish also showed a time–dose relationship; that is, the longer the time, the more obvious the pathological changes. Xie et al. [59] mention that the cells dispersed at 24 h, and the hepatocyte membrane began to disappear at 48 h, along with the condensation or apoptosis of the nucleus, the condensation of the cytoplasm, and the disappearance of the cell connection between the hepatocytes.

In our study, we found that as the cyanobacteria density increased, the time to fish liver damage was accelerated and shortened. However, with the increase of time, the red carp liver in the experiment gradually recovered. From Table 4, it can be seen that this may be related to the changes in the concentrations of LR and RR. Fischer and Dietrich [44] also found slower pathological development in carp compared to pathological events in salmon exposed to MCs. Carp showed slower pathological development and less necrotic cell death. This may be due to the fact that carp effectively absorbed toxin. In particular, after 21 days of injection of MC-LR, the hepatic cell connection of grass carp returned to normal [63]. Xie et al. mentioned that these lesions can be gradually repaired over time [59].

4.2. Concentration Changes of MC-LR and MC-RR in Fish

In our study, MC-LR was not detected in red carp liver, leaving only MC-RR (Table 4). In the study by Xie et al. [63], a sub-chronic toxicity experiment was conducted to examine MC-LR and MC-RR tissue distribution and depuration in the phytoplanktivorous filter-feeding silver carp during a period of 80 days. The results showed that MC-LR was not detected in the muscle and blood samples of silver carp, and a relatively small amount was detected in the liver. Therefore, they found that silver carp may have a mechanism to actively degrade MC-LR and inhibit MC-LR transport across the intestines. The depuration of MC-RR concentrations occurred slower than uptakes in blood, liver, and muscle. The study by Pflugmacher et al. [64] showed that MCs in animal liver are converted to compounds associated with the depletion of cellular glutathione pools. This conjugate appears to be the first step in the detoxication of a cyanobacterial toxin in aquatic organisms [64]. The process of MCs' biotransformation begins with the conjugation of glutathione to the intracellular tripeptide glutathione, catalyzed by glutathione S-transferase enzymes. This conjugate is then further broken down into cysteine conjugates, enhancing intracellular transport and excretion of conjugated toxins in the organisms [65]. In our study, changes in the concentrations of MC-LR and MC-RR in the liver of red carp seem to indicate some kind of transformation or degradation mechanism. This may also be related to the degraded forms of *M. macrocopa* toxin with different contents of MCs.

4.3. Changes in MC-LR/MC-RR Ratios in Natural Waters

Xie et al. [66] reported that MC concentrations were correlated with the amounts of phytoplankton. In natural waters, two factors were observed, where higher light intensity and total nitrogen concentration may lead to increased MC production. They found that the MC-LR/MC-RR ratio varied with the source of the sample, and the total phosphorus concentration may be one of the reasons for the difference in the ratio. In species of *Planktothrix agardhii*, the MC-LR and MC-RR ratio was affected by photon irradiance [67] or amino acid availability (leucine and arginine) [68]. Monchan et al. [69] showed that total nitrogen, water temperature, ammonium, and dissolved organic nitrogen affected the cyanobacterial community structure, which in turn led to differences in major MC congeners and overall toxicity.

4.4. The Effect of M. aeruginosa on M. macrocopa

Xie et al. [54] showed that the lethal time of *M. macrocopa* decreased as the density of *M. aeruginosa* increased. Stangenberg [70] reported that both the time and concentration of MCs affect the growth of cladocerans (*Daphnia longispina* and *Eucypris virens*). However, some studies have confirmed that *M. aeruginosa* colonies can produce a colloidal protective film composed of polysaccharides. Under the protection of this colloidal film, *M. aeruginosa* can still maintain a complete cellular structure after passing through the intestinal tract of zooplankton. Thus, reducing the digestibility of zooplankton to *M. aeruginosa* [71].

4.5. Possibility of MC Concentration Control in Eutrophic Waters

MC-LR and MC-RR are the two most common MCs, and the presence of the two kinds of MCs in water, especially in drinking water, is of great concern since chronic exposure to low concentrations of MCs may promote tumor growth [13,72,73]. Thus, the World Health Organization (WHO) recommends that the MC-LR concentration in drinking water should not exceed 1 μg/L [74].

There are possibilities for MC concentration control in eutrophic waters, such as silver carp, which have a mechanism to actively degrade MC-LR and inhibit the transport of MC-LR in the intestine. Therefore, this mechanism can be exploited for MC control by using phytoplanktivorous fish in eutrophic waters [63]. In addition, a bacterial strain EMS (*Stenotrophomonas* sp.) with the capability of degrading MCs was isolated from Lake Taihu, China. The EMS in an alkaline environment can completely consume MC-LR and MC-RR within 24 h, at concentrations of 0.7 and 1.7 μg/mL, respectively [75]. Addi-

tionally, an indigenous MC-degrading bacterium designated MC-LTH2 (*Stenotrophomonas acidaminiphila*) was successfully isolated from Lake Taihu. MC-LR and MC-RR could be completely degraded by the MC-LTH2 strain within 8 days with the degradation rates of 3.0 and 5.6 mg/(L·d), respectively. The degradation rates of MCs were dependent on temperature, pH, and initial MC concentration [76]. The above results suggest that certain organisms have significant potential in the bioremediation of waters polluted by MC-LR and MC-RR.

5. Conclusions

The total enrichment of MCs in the liver of red carp increased with the density of the cyanobacterium *M. aeruginosa* fed by *M. macrocopa*. However, MCs in the liver of red carp only exist in the form of MC-RR, which may be related to the function caused by the detoxification and degradation of the liver. Changes in the concentrations of MC-LR and MC-RR in red carp seem to indicate some kind of transformation or degradation mechanism and show the possibility of MCs' biodefense in eutrophic waters.

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