

Accumulation of microcystins in various organs of the freshwater snail *Sinotaia histrica* and three fishes in a temperate lake, the eutrophic Lake Suwa, Japan

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Abstract

So far, there has been only one study to examine microcystin (MC) contents in various organs of snails in a subtropical Chinese lake. In this study, tissue distribution and seasonal dynamics of MC-RR and -LR were investigated in various organs of a freshwater snail (*Sinotaia histrica*) in a temperate eutrophic lake, Lake Suwa, Japan. Accumulation of microcystins in some fish was also investigated. There was marked temporal variation in the MC content of various organs of the snail. The digestive tract had the highest MC content (mean $9.03 \mu\text{g g}^{-1}$ DW and range $3.74\text{--}23.2 \mu\text{g g}^{-1}$ DW), followed by the gonad (mean $6.90 \mu\text{g g}^{-1}$ DW and range $0.07\text{--}22.7 \mu\text{g g}^{-1}$ DW) and hepatopancreas (mean $5.38 \mu\text{g g}^{-1}$ DW and range $1.08\text{--}8.79 \mu\text{g g}^{-1}$ DW), whereas the foot had the least (mean $2.48 \mu\text{g g}^{-1}$ DW and range $0.04\text{--}4.45 \mu\text{g g}^{-1}$ DW). The disappearance of MC-LR in the hepatopancreas indicated that *S. histrica* is able to depurate MC-LR efficiently. MC-RR was detected in the muscle of three species of fish, with the highest content in *Carassius auratus* ($79.4 \mu\text{g kg}^{-1}$ BW). Because of substantial MC accumulation in these edible aquatic animals in Lake Suwa, it is recommended that regular monitoring of MCs should be undertaken in both cyanobacteria and aquatic animals.

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Keywords: Microcystins; Freshwater snail; Fish; Tissue distributions; Lake Suwa

1. Introduction

A frequent consequence of eutrophication in fresh waters is the mass development of cyanobacteria. The occurrence of these blooms can create a significant water quality problem, as certain species of cyanobacteria are capable of producing toxins. Microcystins (MCs), the ones most commonly

reported, occur in numerous variants, many of which are potent hepatotoxins (Carmichael, 1997).

MCs are usually associated with freshwater environments, and their accumulation by aquatic animals, including mussels, snails, zooplankton, shrimps, frogs and fish, has been reported by several authors (Amorim and Vasconcelos, 1999; Ozawa et al., 2003; Ferrão-Filho et al., 2002; Chen et al., 2004; Gkelis et al., 2006; Xie et al., 2004, 2005). Snails are an important food source not only for fish, but also for waterfowl, crayfish and amphibians, and serious implications exist for the transfer

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of MCs to a higher trophic level through the food web (Ozawa et al., 2003).

So far, field studies on MC accumulation in snails have been mostly limited to MC contents in the whole body or in the intestine and hepatopancreas, and only Chen and Xie (2005) examined MC contents in different organs of the freshwater snail *Bellamya aeruginosa* in a subtropical Chinese lake. No such reports are found from other regions of the world (e.g., temperate lakes).

Lake Suwa, situated in the center of Nagano, Japan, is the largest lake in this prefecture. Since the 1970s, toxic *Microcystis* blooms have occurred during the summer (Park et al., 1998) and there have been a number of reports relevant to this. These include temporal changes in the concentrations of MCs (Park et al., 1998), historical changes in dioxins and polycyclic aromatic hydrocarbon inflows in the sediment (Ikenaka et al., 2005), the influence of nitrate and phosphate concentration on *Microcystis* species composition and MCs concentration, and the relationship between *Microcystis* species composition and MC concentration (Honma and Park, 2005a,b). Yokoyama and Park (2002) suggested that in Lake Suwa MCs persist throughout winter in the freshwater bivalve *Unio douglasiae*. Katagami et al. (2004) described the seasonal changes of MC concentration in *Stenopsyche marmorata* larvae from the Tenryu River (the outflow of the hypertrophic Lake Suwa). However, until now, no paper dealt with the MCs bioaccumulation in other aquatic animals, such as snail.

The main purpose of the study reported here was to investigate the seasonal changes in MC concentration in a resident snail (*Sinotiaia histrica*) in Lake Suwa, together with an assessment of the relative contribution to this of the various organs of the snail. Accumulation of MCs in some fish was also examined. The various observations are considered with respect to the potential risk of MCs to human health.

2. Materials and methods

2.1. Sampling site

Lake Suwa (36°3'N, 138°5'E), a typical hypertrophic shallow lake, is located in Nagano Prefecture, central Honshu, Japan, at an altitude of about 760 m. The surface area of the lake is 13.3 km², with a maximum depth of 6.8 m and an average depth of about 5 m (Fig. 1). Because of the

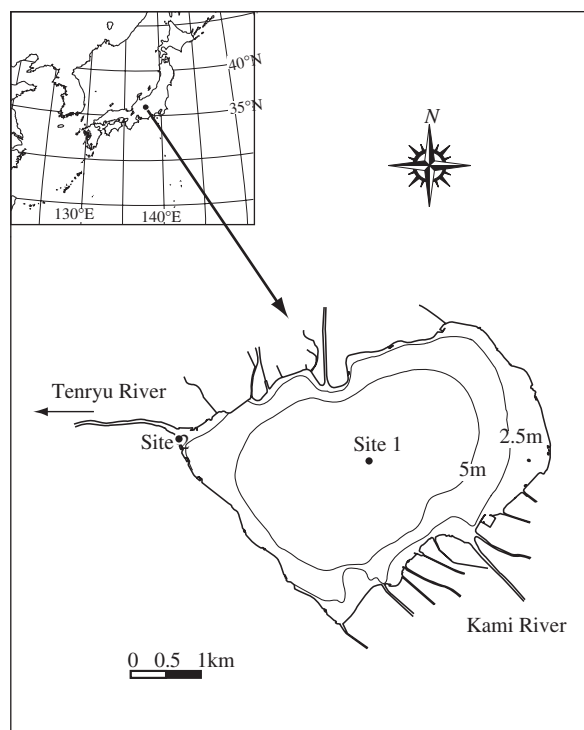


Fig. 1. Map of Lake Suwa, Site 1 and Site 2 mean the sampling sites St. 1 and St. 2.

shallow depth, vertical mixing by wind occurs even during the summer. A dense bloom of *Microcystis* containing MC-RR and MC-LR has occurred during summer in the lake since the 1970s (Park et al., 1998).

2.2. Sample collection

Samples of the three fish species, *Silurus glanis* (three specimens), *Carassius auratus* (three specimens) and *Cyprinus carpio* (three specimens) were provided by local fishermen on 16 November 2005. At the same time, cyanobacterial blooms for determination of MCs content were collected from the center of Lake Suwa (St. 1), using a plankton net (40 μm mesh size). To count cyanobacterial blooms cells, the samples were agitated by gentle ultrasonication to split the colonies into single cells. Cells were counted microscopically using an improved Fuchs–Rosenthal hemacytometer (KAYAGAKI works grid volume: $\frac{1}{16}$ sqmm², depth $\frac{1}{5}$ mm).

Snails were collected at a point in the south littoral zone (about 1 m depth, St. 2). An investigation was carried out monthly from June to September 2005. Water temperature, electric

conductivity (EC) (DKK HEC-110, Japan), pH (DKK HPH-110, Japan) and dissolved oxygen (DKK HEC-110, Japan) were measured at the surface in the same site. To determine the concentrations of MCs in the suspended solids (SS), which were not measured in June, the water sample was filtered through a glass microfiber filter (GF/C, Whatman, UK), and the filter was homogenized in a mortar and extracted three times with 5% acetic acid and the supernatant was applied to an HLB cartridge (0.5 g, Oasis[®], Waters, Milford, Massachusetts, USA).

2.3. Sample preparation and toxin extraction

The samples were stored immediately in a portable refrigerator (around 0 °C) and then transported to the laboratory. They were then immediately frozen at -40 °C, and freeze-dried. Freeze-dried snails were removed from their shell and dissected into foot, hepatopancreas, intestine and gonad for MC analysis. The fish was dissected into intestines, muscle, liver and kidneys.

The method used to extract the MCs from snail and fish tissue was as follows. The lyophilized sample was homogenized in a mortar and extracted three times with 10 ml of BuOH:MeOH:H₂O (1:4:15), sonicated for 3 min (30% amplitude, 60 W, 20 kHz, Branson Digital Sonifier M-250, CT, USA), then centrifuged at 4000 rpm (3200g) for 20 min at room temperature; this was repeated three times. The dry weight of the extracted tissue ranged from 40 to 50 mg.

The supernatant was transferred to a pear-shaped glass flask and evaporated at 35 °C to about 5 mL using a rotary-evaporator, then applied directly to an HLB cartridge (0.5 g, Oasis[®], Waters, Milford, Massachusetts, USA), which was previously conditioned with MeOH (10 mL) and distilled water (10 mL). The column was then eluted with MeOH,

and the eluate containing the toxin was collected. The MC-containing fraction was evaporated to dryness and the residue re-dissolved in MeOH. This solution was applied to a silica gel cartridge (2 g)/plus silica gel (0.69 g) tandem cartridge (SepPak, Massachusetts, USA) to remove impurities in the first extraction step, which had been preconditioned with MeOH. The column containing the toxins was washed with MeOH and then eluted with 70% MeOH. This elution fraction was also evaporated to dryness and the residue was dissolved in MeOH. The resulting solution was injected into a high-performance liquid chromatograph (HPLC) for analysis. Recovery percentages were evaluated from spiked samples, and were always over 80%.

The HPLC system consisted of a Shimadzu (Kyoto, Japan) LC-9A pump coupled to a SPD-10A set at 238 nm, a SPD-M10A photodiode array detector, and a C-R6A integrator and an ODS column (Cosmosil 5C18-AR; 4.6 mm × 150 mm, Nakalai, Japan). 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 MC concentration was quantified against MC-RR and MC-LR standards (Wako Ltd., Japan).

3. Results

Table 1 shows the seasonal changes in environmental factors and MC concentrations in the SS of Lake Suwa (St. 2). Water temperature was highest in August. MC-RR and MC-LR were detected in the SS, and the MC concentration in July was the highest (MC-RR: 26.6 µg g⁻¹ DW and MC-LR: 32.0 µg g⁻¹ DW, respectively). The dominant cyanobacteria were generally *Microcystis aeruginosa*, *M. wesenbergii*, *M. ichthyoblabe* and *Aphanizomenon flos-aquae*.

Table 1
Seasonal changes in environmental factors and microcystins concentration at St. 2 in Lake Suwa

Date	WT (°C)	pH	DO (mg L ⁻¹)	EC (µS cm ⁻¹)	MC in the suspended solids (µg g ⁻¹ DW)		
					MC-RR	MC-LR	Total
050614	23.3	7.17	4.68	215	— ^a	— ^a	— ^a
050712	21.2	8.64	9.69	132	26.6	32.0	58.6
050812	28.7	8.76	5.22	139	20.8	16.2	37.0
050906	23.1	7.07	4.24	171	25.9	16.6	42.5

^aMeans did not measure.

The chromatograms of the MC-LR and MC-RR standards and the extracts of hepatopancreas of *S. histrica* are compared (Fig. 2). It is clear that the toxins were taken up and a part was extractable.

The monthly changes in the MC contents of *S. histrica* are shown in Fig. 3. During the study period, there were great temporal variations in the MC contents of various organs of the snails. Analyses of the digestive tract showed the presence of cyanobacterial cells, providing evidence for the ingestion of bloom material by *S. histrica*. MC concentration in the digestive tract was highest on the first sampling date in June and declined thereafter, ranging from 3.74 to 23.2 $\mu\text{g g}^{-1}$ DW with an average of 9.03 $\mu\text{g g}^{-1}$ DW throughout the experi-

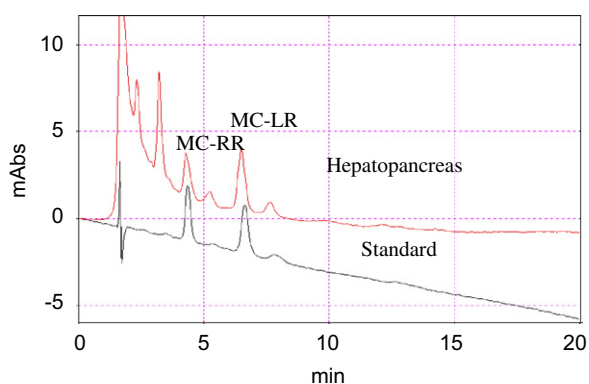


Fig. 2. A comparison of the chromatograms (monitored at 238 nm) of the standard of MC-LR and MC-RR and the extract of hepatopancreas of *S. histrica* collected from Lake Suwa in June 2005.

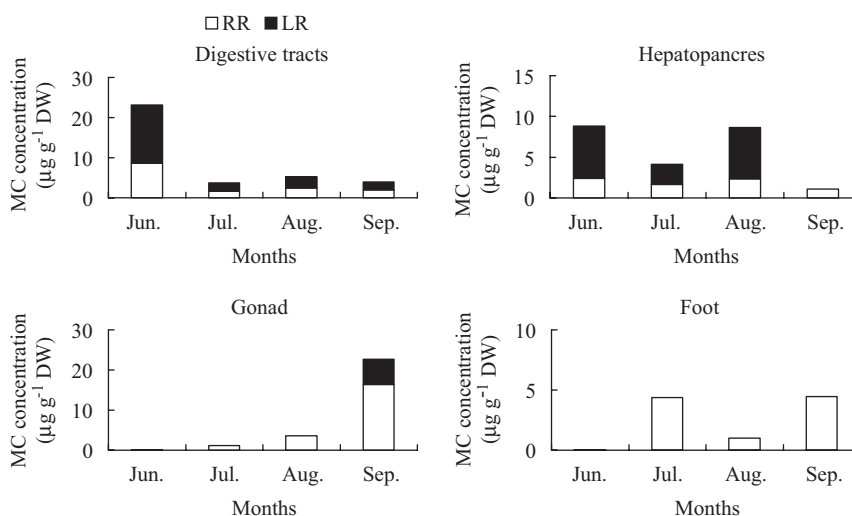


Fig. 3. The seasonal changes of MC-RR and MC-LR concentrations ($\mu\text{g g}^{-1}$ DW) in digestive tracts, hepatopancreas, gonad and foot of *S. histrica* in Lake Suwa during June and September, 2005.

ment. The highest MC concentration in the hepatopancreas was 8.79 $\mu\text{g g}^{-1}$ DW, and the MC content ranged between 1.08 and 8.79 $\mu\text{g g}^{-1}$ DW with an average of 5.38 $\mu\text{g g}^{-1}$ DW. In September, despite MC-LR still being present in the digestive tract (2.05 $\mu\text{g g}^{-1}$ DW), MC-LR was not detectable in the hepatopancreas. In the gonads, the MC content showed a rapid increase (with a maximum of 22.7 $\mu\text{g g}^{-1}$ DW on September 6) during the first 3 months, whereas no MC-LR was detectable afterwards. In the foot, except for June, MCs were present in samples obtained in all months, and the average concentration was 2.48 $\mu\text{g g}^{-1}$ DW. However, no MC-LR was detected in any samples of the foot.

Table 2 shows the MC contents in the different organs of the fish. MCs were not detected in the kidney of any species. In the liver, MC-RR was detected only in *C. auratus* and *Cy. carpio*, reaching 0.82 and 2.06 $\mu\text{g g}^{-1}$ DW, respectively. MC-RR was detected in muscle of all three fish species. No MC-LR was detected in any organ of the fish. The highest MC content was found in *C. auratus*, reaching 79.4 $\mu\text{g kg}^{-1}$ BW.

4. Discussion

In natural environments, snails consume colonies or filaments of *Microcystis* and *Nodularia* present on the sediment in littoral areas during dense blooms (Zurawell et al., 1999; Sipiä et al., 2001). In the present study, the contents of MCs in the

Table 2
MC contents in the different organs of fish in Lake Suwa

Fish name	Wet weight (g)	Liver ($\mu\text{g g}^{-1}$ DW)		Kidney ($\mu\text{g g}^{-1}$ DW)		Muscle ($\mu\text{g g}^{-1}$ DW) ^a		Total conc.fish ⁻¹ (μg)
		MC-RR	MC-LR	MC-RR	MC-LR	MC-RR	MC-LR	
<i>Silurus glanis</i>	1180	ND	ND	ND	ND	0.14	ND	22.4
<i>Carassius auratus</i>	120	0.82	ND	ND	ND	0.49	ND	79.4
<i>Cyprinus carpio</i>	1818	2.06	ND	ND	ND	0.27	ND	46.3

^aAs the muscle is usually the edible part of fish, we just calculated the total MC concentration in the muscle. A coefficient of 5 was used to convert dry weight to wet weight for fish. A coefficient of 80/100 was used to convert muscle to the body weight (BW) of the fish.

digestive tract of the snails averaged $9.04 \mu\text{g g}^{-1}$ DW, while MCs in the SS averaged $46.0 \mu\text{g g}^{-1}$ DW, indicating that MC accumulation was probably a result of direct ingestion.

The study showed that a high concentration of MC accumulated in *S. histrica*, and there was a significant difference in the seasonal pattern of MC accumulation between digestive tracts and gonad (*F*-test, $P = 0.03$). It has been reported that the hepatopancreas is the main organ that accumulates MCs (Chen and Xie, 2005). In this study the hepatopancreas of *S. histrica* also accumulated a high content of MCs during the sampling period. However, MC-LR disappeared from the hepatopancreas in September in accordance with the decline of MCs in SS, although MC-LR was still present in the digestive tract ($2.05 \mu\text{g g}^{-1}$ DW), suggesting that *S. histrica* is able to deplete MC-LR efficiently. A number of studies have demonstrated that MCs can be excreted quickly. Yokoyama and Park (2002) reported that *U. douglasiae* accumulated MC rapidly in response to a slight rise of the toxin in SS in May 1997 and depurated quickly as the level of the toxin decreased in the SS. In a laboratory experiment, during a depuration period of 15 days, *S. histrica* depurated a considerable amount of MC-LR ($297\text{--}172 \mu\text{g g}^{-1}$ DW) that had accumulated during the uptake period (Ozawa et al., 2003). Gérard et al. (2005) suggested that partial degradation of MCs might occur in the snail (*Lymnaea stagnalis*), with a higher rate for adults than for juveniles. This differs from results obtained for the snail *B. aeruginosa* in a Chinese Lake (Lake Chaohu), where MC-LR may be more resistant to degradation in the snail (Chen and Xie, 2005).

In the present study, a substantial amount of MC accumulated in the gonad of *S. histrica* (mean $6.90 \mu\text{g g}^{-1}$ DW). Similar results have also been obtained for other snails and mussels. The fresh-

water snail *B. aeruginosa* accumulated high MC levels in the gonad (mean $0.715 \mu\text{g g}^{-1}$ DW) (Chen and Xie, 2005). The freshwater mussel *U. douglasiae* in Lake Suwa (Japan) accumulated $1.19 \mu\text{g g}^{-1}$ DW in the gonad (Watanabe et al., 1997). Chen and Xie (2005) also found that the gonad of two shrimps (*Palaemon modestus* and *Macrobrachium nipponensis*) and a crayfish (*Procambarus clarkia*) accumulated high amounts of MCs. Chen and Xie (2005) suggested that the reproductive systems of freshwater invertebrates are the second most important target organ of MCs. In the present study, MC-LR was detected in the gonad of *S. histrica* only on the last sampling date, indicating that this snail tended to accumulate MC-RR. Similarly, in Lake Suwa, *Anodonta woodiana* was found to contain only MC-RR in the hepatopancreas on August 10, 1992 (Watanabe et al., 1997). Yokoyama and Park (2002) found that *A. woodiana* and *Cristaria plicata* selectively accumulated MC-RR (up to 90% of total MC).

MC levels in the snail investigated here were within the range of values in the literature (Table 3). *S. histrica* in Lake Suwa accumulated a high content of MC-RR in the edible foot, 2.47 times that reported for *B. aeruginosa* in Lake Chaohu (Chen and Xie, 2005). Yokoyama and Park (2002) suggested that the different accumulation patterns in three bivalves in Lake Suwa might represent an interspecific difference in selective ingestion, reproductive season, MC metabolism and depuration rate. It seems likely that there are considerable differences in the accumulation patterns of MCs among different species of snail.

Gastropods may form a substantial dietary component of many freshwater and terrestrial species (Dillon, 2000). High MC concentrations in *S. histrica* may indicate the potential for transfer of MCs to higher trophic levels, although there is no evidence for biomagnification of MCs (Ibelings

Table 3

A comparison of microcystins contents in snails reported both in literatures and in the present study (DW, dry weight; FW, fresh weight)

Species	Analysis Method	Location	Organ	Concentration ($\mu\text{g g}^{-1}$ DW)	Reference
<i>Sinotaia historica</i>	HPLC	Lake Suwa Japan	Hepatopancreas	35.3	Yokoyama (2003)
<i>Semisulcospira reiniana</i>	HPLC	Lake Suwa Japan	Hepatopancreas	1.86	Yokoyama (2003)
<i>Lymnaea stagnalis</i>	HPLC	Little Beaver	Whole body	54	Kotak et al. (1996)
<i>Hellsoma trivolvis</i>		Little Beaver		11	
<i>Physa gyrina</i>		Lake Driedmeat Canada		121	
<i>Lymnaea stagnalis</i>	HPLC	Lakes of Alberta	Whole body	Up to 140	Zurawell et al. (1999)
<i>Hellsoma trivolvis</i>				Up to 40	
<i>Physa gyrina</i>				Up to 77	
<i>Sinotaia historica</i>	HPLC	Lake Biwa Japan	Hepatopancreas	Up to 3.20	Ozawa et al. (2003)
			Digestive tracts	Up to 19.5	
<i>Bellamyia aeruginosa</i>	HPLC	Lake Chaohu China	Hepatopancreas	4.14	Chen et al. (2004)
			Digestive tracts	1.69	
			Gonad	0.715	
			Foot	0.01	
<i>Lymnaea stagnalis</i>	ELISA	Laboratory	Whole body	5.07 (ng g^{-1} FW)	Gérard et al. (2005)
<i>Viviparus contectus</i>	HPLC	Lake Pamvotis Greece	Whole body	3.5	Gkelis et al. (2006)
<i>Sinotaia historica</i>	HPLC	Lake Suwa	Hepatopancreas	5.08	Present study
			Digestive tracts	9.04	
			Gonad	6.9	
			Foot	2.47	

et al., 2005), a potentially critical issue for human consumption.

In the present study, MC-LR was not detected in any organ of the fish collected from Lake Suwa, although MC-LR was still present at the same time in the surface cyanobacterial blooms (MC-RR: $19.2 \mu\text{g g}^{-1}$ DW, MC-LR: $16.6 \mu\text{g g}^{-1}$ DW, St. 1). Most of the MCs in livers were probably bound covalently to protein phosphatases (Toivola et al., 1994). Chen et al. (2006) found that the inhibition activities of various MCs on PP-1 and PP-2A were compared according to their respective IC_{50} values. Among them, MC-LR is found to be the strongest, with similar IC_{50} values of 0.3 and 0.4 nM toward PP-1 and -2A, respectively, while the IC_{50} values of the MC-RR increase to 1.7 and 58 nM, which were a 6- and 145-fold higher than those of MC-LR. It revealed that MC-RR was less bound covalently to protein phosphatases. Williams et al. (1997a,b) found that only 24% of the total MC-LR from Atlantic salmon liver was extractable with methanol. So the apparent absence of MC-LR might be due to the fact that the amount of MC-LR in fish

was not high enough to be detected by our current HPLC method. This agrees with Kotak et al. (1996), who reported that MC-LR was not detectable in the livers of fish collected from Driedmeat Lake, despite the fish being exposed to high concentrations of MC-LR in the phytoplankton. These facts suggest that MCs found in fish tissues from Lake Suwa are probably underestimated.

The WHO have proposed a tolerable daily intake (TDI) of $0.04 \mu\text{g g}^{-1}$ DW per day for MC-LR (Chorus and Bartram, 1999). According to the calculation of Chen and Xie (2005), a human adult weighing 60 kg, who ingests, on average, 24.3 g of *S. historica*, or 429 g *Si. glanis*, 123 g *C. auratus*, and 222 g *Cy. carpio* from Lake Suwa will be above this limit. Because of substantial MC accumulation in the edible aquatic animals in Lake Suwa, it is recommended that regular monitoring of MCs in both cyanobacteria and aquatic animals should be undertaken. There is also an urgent need to control eutrophication in order to minimize the growth of toxic cyanobacteria and reduce exposure to cyanotoxins.

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