

RESEARCH PAPER

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Accumulation and depuration of microcystin produced by the cyanobacterium *Microcystis* in a freshwater snail

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Abstract Seasonal changes in microcystin concentrations in a resident snail (*Sinotaia histrica*) and an edible clam (*Corbicula sandai*) in Lake Biwa were surveyed. To clarify both the accumulation and depuration of microcystins, experimental studies with microcystin were also carried out on the snail. In the field investigation, microcystin was detected from the hepatopancreas and intestine of *S. histrica* (up to $3.2\mu\text{g g}^{-1}$ dry weight and $19.5\mu\text{g g}^{-1}$ dry weight, respectively); however, no microcystin was detected in the hepatopancreas of *C. sandai*. In the laboratory experiment, the microcystin-LR concentration in the hepatopancreas of *S. histrica* reached a value of $436\mu\text{g g}^{-1}$ dry weight on day 10 of 15 days of uptake, and a high value persisted despite a depuration period of 15 days. The depuration rate constant of microcystin and its biological half-life were 0.0828 day^{-1} and 8.4 days, respectively. These results indicate that *S. histrica* has a high ability to accumulate microcystin in its tissue. Because *S. histrica* is predated by fish and water fowl, it is likely to play an important role as a vector for microcystin in lakes with dense blooms of toxic cyanobacteria.

Key words *Microcystis* · Microcystin · Accumulation · *Sinotaia histrica* · Lake Biwa

Introduction

The occurrence of toxic cyanobacterial blooms in eutrophic lakes, reservoirs, and recreational waters has become a worldwide problem. In Japanese lakes, the toxic cyanobacteria are mainly composed of *Microcystis* (Park et al. 1998), which commonly produces microcystin. Microcystin is known to be a potent hepatotoxin (Carmichael 1994) and tumor promoter (Nishiwaki-Matsushima et al. 1991, 1992). Microcystin is composed of seven amino acids, including a unique amino acid called Adda [3-amino-9-methoxy-2, 6, 8-trimethyl-10-phenyldeca-4, 6-dienoic acid] (Botes et al. 1984) (Fig. 1), and 65 variants of microcystin have been reported to date (Rinehart et al. 1994; Park et al. 2001a).

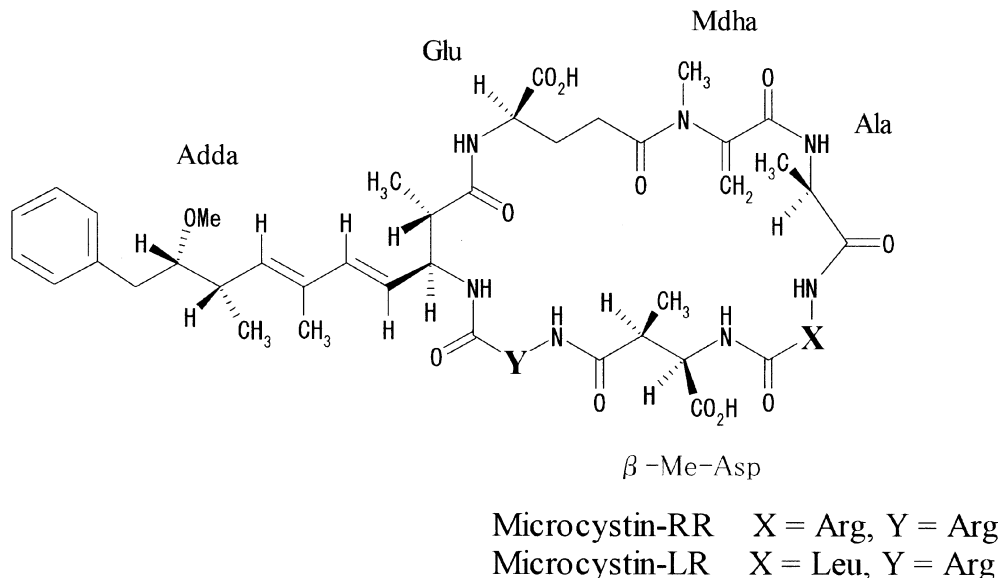
Microcystins are mainly retained in cyanobacterial cells during the growth and steady phase of blooms. However, microcystin is released into the surrounding water body by senescence of the bloom and lysis of *Microcystis* (Park et al. 1998). It is important to trace the fate of microcystin dissolved in the water body where a dense bloom has occurred. Microcystin is chemically stable in laboratory conditions (Dawson 1998; Park et al. 2001b) and it is predicted that microcystin is increasing from year to year in water bodies experiencing dense blooms. However the dissolved microcystin concentration in the natural environment is kept at a low level (Park et al. 1998, 2001b) as a result of dilution by large excesses of influent water (Harada and Tsuji 1998), adsorption by clay particles (Rapala et al. 1994; Harada and Tsuji 1998; Morris et al. 2000; Tsuji et al. 2001), thermal decomposition aided by temperature and pH (Harada et al. 1996; Harada and Tsuji 1998), photolysis (Tsuji et al. 1994; Harada and Tsuji 1998), and bacterial degradation (Rapala et al. 1994; Jones et al. 1994; Takenaka and Watanabe 1997; Harada and Tsuji 1998; Park et al. 2001c). A wide range of aquatic organisms is affected by oral uptake of toxic cyanobacterial cells as food. Microcystins are accumulated in aquatic organisms such as fish (Andersen et al. 1993; Magalhães et al. 2001; Zimba et al. 2001), zooplankton (Watanabe et al. 1992; Kotak et al. 1996; Ferrão-Filho et al. 2002), bivalves (Prepas et al. 1997;

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Fig. 1. Chemical structure of microcystin-RR and microcystin-LR. Microcystins mainly differ in two amino acids, indicated by the international abbreviation: *L*, leucine; *R*, arginine



Watanabe et al. 1997; Williams et al. 1997; Yokoyama and Park 2002), and gastropods (Kotak et al. 1996; Zurawell et al. 1999). The accumulation of microcystin in bivalves has been confirmed by laboratory experiments (Eriksson et al. 1989; Vasconcelos 1995; Amorim and Vasconcelos 1999; Yokoyama and Park 2003). Vasconcelos et al. (2001) reported that the crayfish *Procambarus clarkii* accumulated up to 2.9 µg microcystin per gram dry crayfish weight. Because these organisms are an important food source not only for waterfowl, birds, and fish but also for mammals, including humans, microcystins may be transferred to a higher trophic level through the food web. It is therefore important to investigate the concentration of microcystin in aquatic organisms.

The aim of the present study was to investigate the seasonal changes in microcystin concentration in a resident snail (*Sinotaia histrica*) and an edible clam (*Corbicula sandai*) in Lake Biwa. *S. histrica* is a common species in eutrophic lakes and ponds in Japan. *C. sandai* is one of the native species of Lake Biwa and is an important food for the local fish industry. The study also included an accumulation and depuration experiment on microcystin in the snail *S. histrica*.

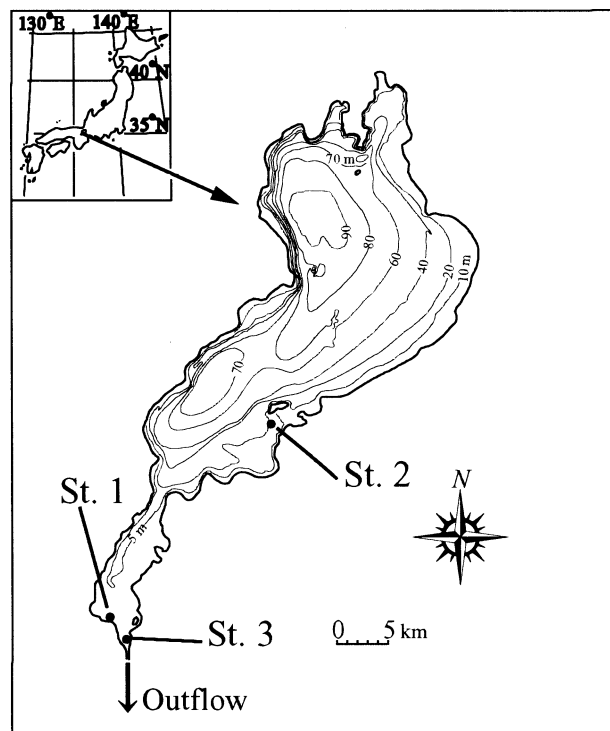


Fig. 2. Location of sampling stations (circles) in Lake Biwa

Materials and methods

Field investigation

Lake Biwa, located in central Honshu, is the largest lake in Japan. It is characterized by two morphologically distinct basins: a deep northern basin and a shallow southern one (Fig. 2). Since 1950, eutrophication of the lake has increased and cyanobacterial blooms of *Anabaena* sp. and *Microcystis* sp. have occurred since 1983, especially in the southern basin (Kumagai and Roberts 1996).

Water samples were collected in a 10-l plastic vessel for determination of phytoplankton biomass from surface

water near Hamaotsu Harbor in the southern basin (Fig. 2) in September 1998 and 1999 and from July to October 2000. Biomass was estimated as chlorophyll *a* determined from a water sample that was filtered through a glass fiber filter (GF/C, Whatman, Maidstone, UK). The filter was frozen and extracted in methanol overnight in a dark freezer. The concentration of chlorophyll *a* was calculated spectrophotometrically (Marker et al. 1980). Phytoplankton concentrated with a plankton net were identified by light microscopy (Olympus BH-12, Tokyo, Japan). A water

sample filtered through a preweighed GF/C filter was used to determine suspended solids (expressed as milligrams per liter). The filter was dried for 48h at 70°C before reweighing. Water temperature and pH were measured with a portable pH meter (DKK HPH-110, Tokyo, Japan).

Phytoplankton for determination of cellular microcystin concentration were collected with a 40- μ m-mesh plankton net and concentrated on a GF/C filter. The filter was immediately frozen at -40°C. To quantify the microcystin concentration in the lake water, a water sample was filtered through a GF/C filter. The filter was also frozen at -40°C.

The freshwater snail *Sinotaia histrica* was collected from Hamaotsu Harbor (St. 1) in September 1998 and 1999 and from July to October 2000 and the freshwater edible clam *Corbicula sandai* was collected from the central (St. 2) and southern basin (St. 3) from August to October 2000 to quantify their accumulated microcystin. The collected molluscs were immediately frozen at -40°C until microcystin analysis.

Microcystin analysis

Microcystin was quantitatively analyzed according to Park et al. (1998). The frozen phytoplankton and the GF/C filter were lyophilized and extracted three times with 10ml of 5% aqueous acetic acid for 30min with stirring. The extract was centrifuged at 3000rpm for 30min and the supernatant was applied directly to an octadecylsilane (ODS) cartridge (0.5g) preconditioned with methanol and water. The cartridge was rinsed with distilled water followed by 20% methanol, and the toxin was finally eluted from the cartridge with a trifluoroacetic acid- (TFA) methanol solution. This eluate was completely evaporated to dryness and the residue was suspended in methanol. The methanol solution was subjected to high-performance liquid chromatography (HPLC) equipped with an ODS column (Cosmosil 5C18-AR; 4.6 \times 150mm, Nacalai, Kyoto, Japan). The HPLC system consisted of a Shimadzu (Kyoto, Japan) LC-9A pump coupled with an SPD-10A set at 238nm, an SPD-M10A photodiode array detector, and a C-R6A integrator. The sample was separated with a mobile phase consisting of methanol: 0.05M phosphate buffer (pH 3.0, 58:42) at a flow rate of 1mlmin⁻¹. The microcystin concentration was quantified by standard microcystin-RR and microcystin-LR solutions provided by Dr. K-I. Harada, Meijo University (Harada et al. 1988).

Freeze-dried molluscs were removed from their shell and dissected to obtain the hepatopancreas and intestine. The tissue was ground with a mortar and extracted three times using a butanol:methanol:water solution (1:4:15, v/v) for 24h with stirring. After the extract was centrifuged at 18000rpm for 1h at 4°C, the supernatants were diluted with distilled water and applied to an ODS cartridge (5g) activated with methanol. The ODS cartridge was rinsed with distilled water and 20% methanol, and eluted with 90% methanol. This eluate was completely evaporated to dryness and the residue was suspended in methanol. This solution was cleaned with a silica gel cartridge (2g).

After rinsing the cartridge with methanol, the cartridge was eluted with 70% methanol. This eluate was completely evaporated to dryness and the residue was suspended in methanol and analyzed by HPLC.

Uptake and depuration of microcystin-LR in the freshwater snail *Sinotaia histrica*

Uptake and depuration experiments of microcystin in *S. histrica* were performed for 30 days in the laboratory to confirm its ability to accumulate microcystin produced by the cyanobacteria. The snails were collected from Hamaotsu Harbor (St. 1) in October 2000 and brought to the laboratory. The snails were reared in an incubation room controlled at 20°C and were acclimated in dechlorinated water for 2 months. The physicochemical characteristics of the culture water during the uptake and depuration experiment were: average water temperature 19.0° \pm 0.3°C, average pH 7.56 \pm 0.07, and average dissolved oxygen concentration 7.01 \pm 0.49mg l⁻¹ with continuous aeration. The incubation water was changed every day and the snails were fed daily on boiled lettuce.

Uptake experiment

Three aquaria containing 6l of dechlorinated water and 185 snails (average shell height 15.7mm ind⁻¹, average shell weight 0.971g wet weight ind⁻¹) were used for this experiment. The snails were divided into the three aquaria and the water in the aquaria was continuously aerated and changed every day. The snails were fed daily on the toxic *Microcystis* strain for 15 days with the final cell density adjusted to 1.0 \times 10⁶ cells ml⁻¹. The toxic *Microcystis* strain *M. ichthyoblabe* (TAC 95) was semicontinuously cultured in a 10-l vessel containing MA medium (Ichimura 1979) in an incubation room controlled at 21.5°C. The culture conditions of the strain were continuous white fluorescent light (40 μ E m⁻² s⁻¹) with continuous aeration. The strain produces high amounts of microcystin-LR (Watanabe et al. 1991). Any dead snail was immediately removed from the aquarium during the experiment. Fifteen snails were collected for microcystin analysis as a control before the start of the experiment and then fifteen snails were collected randomly for microcystin analysis after 1, 3, 5, 10, and 15 days. The samples were frozen at -20°C until toxin analysis. The used incubation water and the residue 24h after adding the cells were taken for microcystin quantification and the *Microcystis* cell count, respectively. The incubation water used for microcystin analysis was filtered through GF/C filters and frozen at -20°C until microcystin analysis. *Microcystis* cell density was counted with the EKDS hemocytometer (Kayagaki Works, Tokyo, Japan). The incubation water was reduced corresponding to the samples collected to maintain a constant *Microcystis* cell concentration per snail.

Depuration experiment

The depuration experiment was performed after the uptake experiment. The incubation water was changed to non-*Microcystis* dechlorinated water and the snails were again fed on boiled lettuce. About fifteen snails were collected randomly for microcystin analysis after 1, 3, 5, 10, and 15 days. The snails collected from the aquarium were analyzed as described above.

Kinetic models

The depuration rate constant and biological half-life of microcystin-LR were evaluated using a simple first-order, one-compartment model (Spacie and Hamelink 1982; Yokoyama and Park 2003). Under steady-state conditions, the equation describing the kinetic uptake and depuration can be written as:

$$dC_f/dt = k_u C_w - k_d C_f \quad (1)$$

where k_u ($\text{mlg}^{-1}\text{day}^{-1}$) is the uptake rate constant, k_d (day^{-1}) is the depuration rate constant, C_w (ngml^{-1}) is the microcystin concentration in water, and C_f (ngg^{-1} dry weight) is the microcystin concentration in the tissue.

After the snails are transferred to clean water, the depuration rate constant k_d can be derived from the slope of the first-order depuration equation:

$$dC_f/dt = -k_d C_f \quad (2)$$

Integration of Eq. 2 gives:

$$\ln C_f(t) = \ln C_f(0) - k_d t \quad (3)$$

where $C_f(t)$ is the toxin concentration in the tissue at time t , $C_f(0)$ is the toxin concentration at the beginning of the depuration period, and t is time (day).

The biological half-life ($t_{1/2}$) of microcystin was calculated from the equation:

$$t_{1/2} = \ln 2/k_d \quad (4)$$

Results

Seasonal changes in environmental factors and microcystin concentration

Table 1 shows the seasonal changes in environmental factors and microcystin concentration in Lake Biwa. Water temperature was highest in August and gradually decreased until October. The dominant species were generally cyanobacteria (*Microcystis aeruginosa*, *Oscillatoria kawamurae*, *Anabaena spiroides*), except in September (*Volvox aureus*). In October *M. aeruginosa* was also observed followed by *A. spiroides*.

Microcystin-RR and microcystin-LR were detected in phytoplankton collected in July and October. The microcystin concentration in phytoplankton in October was higher ($284\mu\text{gg}^{-1}$ dry weight) than that in July ($51.8\mu\text{gg}^{-1}$ dry weight). Microcystin was not detected in the sample collected in August, when *Oscillatoria* was dominant, or in September, when no cyanobacterial species were observed. The microcystin concentration in lake water was low in July ($0.473\mu\text{gl}^{-1}$) and October ($0.467\mu\text{gl}^{-1}$).

Microcystin concentrations in snails and clams

Table 2 shows the microcystin concentrations in snails and clams collected from Lake Biwa. In *S. histrica*, microcystin-RR was detected in the intestine but not in the hepatopancreas in July and August. In September, both microcystin-RR and microcystin-LR were detected in the intestine and microcystin-RR was detectable in the hepatopancreas. In October the microcystin in the snails' hepatopancreas and intestine increased to $3.2\mu\text{gg}^{-1}$ dry weight and $19.5\mu\text{gg}^{-1}$ dry weight, respectively, reaching the highest concentration of the observation period.

Although material of a blue-green color similar to cyanobacteria was infrequently observed in the intestine of *C. sandai*, no microcystin was detected from any samples through the investigation period.

Uptake and depuration of microcystin-LR in freshwater snail *S. histrica*

No difference was observed in snail mortality between the uptake and depuration periods. During the uptake period,

Table 1. Seasonal changes in environmental factors and microcystin concentration at St. 1 in Lake Biwa

Date	WT (°C)	pH	Chl <i>a</i> (μgl^{-1})	SS (mg l^{-1})	MCs ($\mu\text{g g}^{-1}$ DW) ^a			MCs ($\mu\text{g l}^{-1}$) ^b			Dominant species
					RR	LR	Total	RR	LR	Total	
21-Jul-00	29.0	9.62	3.01	9.14	30.2	21.5	51.8	0.276	0.197	0.473	<i>Microcystis aeruginosa</i>
16-Aug-00	31.4	9.31	8.79	5.80	ND	ND	ND	ND	ND	ND	<i>Oscillatoria kawamurae</i>
18-Sep-00	27.2	6.98	2.73	1.48	ND	ND	ND	ND	ND	ND	<i>Volvox aureus</i>
11-Oct-00	23.0	7.37	9.68	2.36	190	93.5	284	0.286	0.190	0.476	<i>Anabaena spiroides</i> , <i>M. aeruginosa</i>

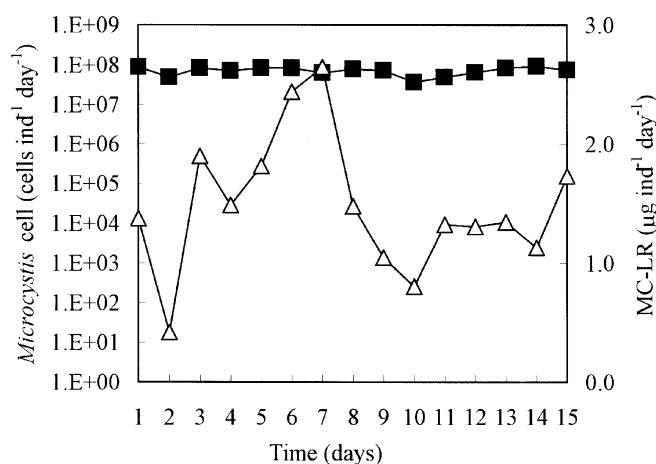
WT, water temperature; Chl *a*, chlorophyll *a*; SS, suspended solids; MC, microcystin; DW, dry weight; RR, Arg-Arg; LR, Leu-Arg; ND, microcystins not detected

^aMicrocystin concentration per dried algae collected with a plankton net

^bMicrocystin concentration per liter of lake water

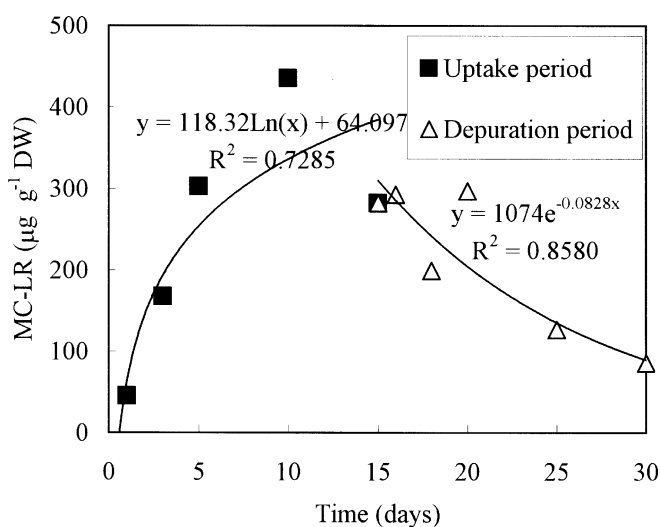
Table 2. Microcystin concentration in snails and clams and clams collected from Lake Biwa

Species	Date	Sampling site	Organ	MCs ($\mu\text{g g}^{-1}$ DW)		
				RR	LR	Total
Snail						
<i>Sinotaia histrica</i>	7-Sep-98	St. 1	Hepatopancreas	ND	ND	ND
	10-Sep-99	St. 1	Hepatopancreas	ND	ND	ND
	12-Jul-00	St. 1	Hepatopancreas	ND	ND	ND
			Intestine	2.70	ND	2.70
	16-Aug-00	St. 1	Hepatopancreas	ND	ND	ND
			Intestine	2.59	ND	2.59
	18-Sep-00	St. 1	Hepatopancreas	Trace	ND	Trace
			Intestine	0.531	4.29	4.82
	11-Oct-00	St. 1	Hepatopancreas	1.66	1.54	3.20
			Intestine	12.8	6.70	19.5
Clam						
<i>Corbicula sandai</i>	4-Aug-00	St. 2	Hepatopancreas	ND	ND	ND
	16-Aug-00	St. 2	Hepatopancreas	ND	ND	ND
	14-Sep-00	St. 2	Hepatopancreas	ND	ND	ND
	11-Oct-00	St. 3	Hepatopancreas	ND	ND	ND
	13-Oct-00	St. 2	Hepatopancreas	ND	ND	ND

**Fig. 3.** Daily uptake rates of *Microcystis ichthyoblabe* cells (squares) and microcystin-LR (MC-LR) (triangles) during the uptake period

the snails produced feces containing *Microcystis* cells soon after being fed and the dissected snails had blue-green contents in their intestines. The uptake rates of *Microcystis* cells and microcystin were not the same throughout the uptake experiment. The average cell concentration of *Microcystis* added during the uptake period was $1.04 \pm 0.11 \times 10^6$ cells ml^{-1} and the average concentration of microcystin to which the snails were exposed was $20.1 \pm 6.1 \mu\text{g l}^{-1}$. Twenty-four hours after addition of the *Microcystis*, the average cell concentration in the aquaria decreased to $3.81 \pm 1.08 \times 10^5$ cells ml^{-1} and the average concentration of microcystin decreased to $6.64 \pm 2.49 \mu\text{g l}^{-1}$. The average daily uptake rate of *Microcystis* cells during the uptake period was $7.2 \pm 1.6 \times 10^7$ cells $\text{ind}^{-1} \text{day}^{-1}$ and the rate of cell uptake was constant (Fig. 3). In contrast, the uptake rate of microcystin-LR ranged from 0.42 – $2.65 \mu\text{g ind}^{-1} \text{day}^{-1}$ and the average was $1.48 \pm 0.57 \mu\text{g ind}^{-1} \text{day}^{-1}$ (Fig. 3).

Figure 4 shows the changes in microcystin-LR concentration accumulated in the hepatopancreas during the experi-

**Fig. 4.** Changes in microcystin-LR (MC-LR) concentration in the hepatopancreas of *Sinotaia histrica* during the uptake and depuration experiment. MC-LR was not detected on day 0. DW, dry weight

mental period. After the uptake experiment started, the microcystin-LR concentration in the hepatopancreas increased rapidly, although no microcystin-LR was detected in snails sampled before *Microcystis* was added as food. The microcystin-LR concentration in the hepatopancreas on day 10 was the highest ($436 \mu\text{g g}^{-1}$ dry weight) during the uptake period. The microcystin concentration in the hepatopancreas reached a steady condition on 5 day judging from the calibration curve (Fig. 4).

The depuration experiment was performed subsequently. After the incubation water of snails was switched to non-*Microcystis* water, the concentration of microcystin-LR in the hepatopancreas remained roughly constant for 5 days (Fig. 4). From day 20 to day 30, the microcystin-LR concentration slowly decreased, but a relatively high

amount of microcystin ($127\mu\text{g g}^{-1}$ dry weight) remained even after 15 days. The depuration rate constant (k_d) of microcystin calculated from our data was 0.0828 day^{-1} and the biological half-life ($t_{1/2}$) was 8.4 days.

Discussion

Field investigation

Watanabe et al. (2000) reported that the main components of microcystin in samples collected from the southern basin of Lake Biwa in 1996 were microcystin-RR and microcystin-LR, and the concentrations ranged from $0.66\text{--}5.8\mu\text{g l}^{-1}$. They detected approximately $1500\mu\text{g}$ microcystin in 1g dry weight of net sample. The concentrations of microcystin in lake water and phytoplankton obtained from our study were lower than those found by Watanabe et al. (2000).

To estimate the concentration of microcystin during the bloom, a GF/C filter through which the lake water sample had been filtered was analyzed. Essentially, when estimating the total microcystin concentration in lake water, it is necessary to evaluate the aqueous, dissolved microcystin, i.e., that which is typically released from cyanobacterial cells during natural senescence. However, almost all the microcystin was confined within the cyanobacterial cells, and there was little dissolved microcystin in the water because of dilution, adsorption, physicochemical decomposition, and bacterial decomposition (Harada and Tsuji 1998; Park et al. 2001c). The GF/C sample represents the total microcystin concentration in the lake water. In our study, the microcystin concentration in phytoplankton collected with a plankton net was also analyzed and this value indicates the toxicity of the cyanobacteria. It is considered that the effect of microcystin in phytoplankton may be severe for aquatic plankton feeders. Therefore, both the total microcystin concentration in the water and the microcystin concentration in phytoplankton are important indices for risk assessment of aquatic biota and humans in eutrophic lakes and rivers. Zurawell et al. (1999) showed that the concentration of microcystin-LR in three species of gastropod (*Lymnaea stagnalis*, *Physa gyrina*, and *Helisoma trivolvis*) collected from a eutrophic-hypereutrophic lake in Canada was correlated with toxin in the phytoplankton. Our results showed that microcystin in the lake water was at a low level ($0.473\text{--}0.476\mu\text{g l}^{-1}$), even though microcystin in phytoplankton changed throughout the investigation period (Table 1). The amount of microcystin in the hepatopancreas of *S. histrica* was highest in October. These results indicate that there was no correlation with microcystin in lake water, but that the microcystin in phytoplankton strongly influenced the accumulation of microcystin by aquatic organisms.

It is known that cyanobacterial toxin is accumulated in bivalves. Nodularin, another type of cyanobacterial hepatotoxin, was detected in blue mussels (*Mytilus edulis*) during a bloom of *Nodularia spumigena* in a brackish/marine water

estuary (Falconer and Choice 1992). Microcystin-RR and microcystin-LR were identified in three species of freshwater mussels (*Unio douglasiae*, *Anodonta woodiana*, and *Cristaria plicata*) collected from Lake Suwa and were especially concentrated in the hepatopancreas tissue (Watanabe et al. 1997; Park et al. 2001b; Yokoyama and Park 2002). In our investigation, however, no microcystin was detected in the edible clam *C. sandai* (Table 2). The reason for this result is unclear because there is little information about dominant phytoplankton species and the microcystin concentration in the lake water at Sts. 2 and 3, where the clams were collected. The clam is an important organism for the local fish industry and a food resource for people who live around Lake Biwa. Thus we concluded that it is necessary to continue monitoring the microcystin concentration in the clams.

Uptake and depuration experiment

The degradation rate of microcystin in the hepatopancreas was quite slow after the start of the depuration experiment. There was a change in microcystin level between days 3 and 5 (Fig. 4). This kind of phenomenon coincides with the results of experimentally exposing mussels (Vasconcelos 1995; Amorim and Vasconcelos 1999) and crayfish (Vasconcelos et al. 2001) to *M. aeruginosa*. It has been suggested that this may be caused by cycles in the production and degradation of protein phosphatases to which microcystin is bound (Vasconcelos et al. 2001), but there is not much evidence to support this, and further study is needed.

It is known that microcystin is irreversibly bound to protein phosphatases in animal tissue (Robinson et al. 1991a,b; MacKintosh et al. 1995). Williams et al. (1997) provided *Mytilus edulis* with microcystin produced by *M. aeruginosa*, and the mussel protein phosphatase assay results were compared with the Lemieux oxidation gas chromatography-mass spectrometry analysis. Less than 0.1% of the total microcystin burden in the mussel tissue was detected by protein phosphatase assay. They concluded that microcystin was not extractable with methanol because microcystin was covalently bound to protein phosphatase in the mussel tissue. The fact that microcystin accumulated in animal tissue was not estimated correctly is an important problem and it is hoped that a new analytical method to estimate microcystin in animal tissue will be developed very soon.

Here we discuss a few points about the residence time of microcystin in molluscs. The accumulation of microcystin has been reported by experimentally exposing a freshwater or intertidal mussel to toxic cyanobacteria (Eriksson et al. 1989; Vasconcelos 1995; Amorim and Vasconcelos 1999). Vasconcelos (1995) exposed a freshwater mussel, *Mytilus galloprovincialis*, to *M. aeruginosa* for 16 days and the accumulated microcystin was depurated by rearing in non-*Microcystis* water. Microcystin was not detectable on day 13 of the depuration experiment. Amorim and Vasconcelos (1999) performed a similar experiment and confirmed that

microcystin was almost undetectable on day 14 of the depuration period. On the other hand, Eriksson et al. (1989) exposed a freshwater mussel, *Anodonta cygnea*, to a toxic strain of cyanobacterium, *Oscillatoria agardhii*, and reported that toxin was detected in the mussel after 65 days in nontoxic water. In our depuration study, from day 1 to day 5 of depuration, the amount of microcystin remained roughly constant ($200\text{--}297\ \mu\text{g g}^{-1}$ dry weight), but then decreased rapidly by day 10 of depuration (Fig. 4), and decreased slowly from day 10 to day 15. A high level of microcystin-LR ($85.8\ \mu\text{g g}^{-1}$ dry weight) remained in the hepatopancreas of *S. histrica* on day 15 of the depuration experiment. Yokoyama and Park (2003) reported the depuration kinetics of microcystin in a freshwater bivalve, *Unio douglasiae*, and calculated the depuration constants for microcystin with a first-order compartment model (Spacie and Hamelink 1982; Yokoyama and Park 2003). Compared with this paper, the depuration rate constant (k_d) of *S. histrica* was lower ($0.0828\ \text{day}^{-1}$) than that of *U. douglasiae* ($0.142\ \text{day}^{-1}$ at 15°C and $0.226\ \text{day}^{-1}$ at 25°C). The biological half-life ($t_{1/2}$) of microcystin in *S. histrica* was 8.4 days, whereas that for *U. douglasiae* was 4.9 days at 15°C and 3.1 days at 25°C (Yokoyama and Park 2003). These values indicate that *S. histrica* has a high ability to accumulate microcystin and that microcystin remains in its tissue for some time. We observed *Microcystis* cells coated with mucus in the alimentary canal of *S. histrica* when samples for analysis were dissected. This suggests that a large amount of microcystin was also contained in the alimentary canal of *S. histrica*. Furthermore, microcystin ($10.1\ \mu\text{g g}^{-1}$ dry weight) was detected in the hepatopancreas of *S. histrica* in May 1999 in Lake Suwa before the cyanobacterial bloom had developed (Yokoyama and Park, personal communication). We suspect that residual microcystin accumulated in the autumn–winter season is still detectable the following spring and that microcystin remains in the snails for several months. Because *S. histrica* is predated by fish and water fowl, it is likely to play an important role as a vector for microcystin in lakes with dense blooms of toxic cyanobacteria.

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