Mechanism and Prediction for Contamination of Freshwater Bivalves (Unionidae) with the Cyanobacterial Toxin Microcystin in Hypereutrophic Lake Suwa, Japan

Atsushi Yokoyama, Ho-Dong Park

Department of Environmental Sciences, Faculty of Science, Shinshu University, Matsumoto 390-8621, Japan

Received 17 September 2001; revised 10 April 2002; accepted 21 May 2002

ABSTRACT: Seasonal changes of microcystin (MC) bioaccumulation in three freshwater Unionid bivalves, *Anodonta woodiana, Cristaria plicata,* and *Unio douglasiae,* were investigated in the hypereutrophic Lake Suwa. Total MC concentrations (MC-RR and -LR) as determined by reverse-phase high-performance liquid chromatography were at high levels in the hepatopancreas of *C. plicata* and *U. douglasiae,* with maxima at 297 and 420 μg/g dry weight, respectively. The amounts and seasonal changes in the accumulated MC concentration differed in all species. The total MC concentration of *A. woodiana* was always less than that of other species (maximum concentration of 12.6 μg/g dry weight). The toxin concentration of *C. plicata* remained very low in summer, when the *Microcystis* bloom occurred, but increased rapidly in autumn, when the toxic bloom disappeared. For *U. douglasiae,* simple regression analyses were performed to clarify the relationship between MC bioaccumulation and environmental parameters such as water temperature, chlorophyll *a*, suspended solids (SS), intracellular MC per unit volume of lake water and per-unit weight of SS and extracellular MC. The toxin concentration of *U. douglasiae* correlated more closely with qualitative factors, with intracellular toxin per SS ($p < 0.001$, $R^2 = 0.72$) than with quantitative factors such as chlorophyll *a* and intracellular toxin per unit volume of lake water. No correlation could be found between MC in the tissues and extracellular MC. These results indicate that a long-term survey is needed to assess the safety of bivalves. The study should take into consideration both interspecific differences in toxin content and what is the optimal monitoring parameter.
 Environ Toxicol 17: 424–433, 2002; Published online in Wiley InterScience (www.interscience.wiley.com). DOI 10.1002/tox.10075

Keywords: cyanobacteria; cyanotoxin; microcystin; freshwater bivalve; Unionidae, bioaccumulation

INTRODUCTION

Toxic cyanobacterial blooms occur worldwide in eutrophic waters and cause a range of problems in human health, water management, and fishery and livestock farming (Billings, 1981; Skulberg et al., 1984; Andersen et al., 1993; Pouria et al., 1998; Codd et al., 1999; Pitois et al., 2000). Among several cyanobacterial toxins, microcystin (MC) is a well-recognized hepatotoxin produced by *Microcystis, Anabaena, Oscillatoria,* and *Nostoc* (Sivonen, 1996). MC is a cyclic heptapeptide consisting of five common amino acids and two variable L–amino acids (Botes et al., 1984). Sixty-five analogues of MC have been identified so far

Correspondence to: Ho-Dong Park; e-mail: pparkhd@gipac.shinshu-u.ac.jp

(Rinehart et al., 1994; Park et al., 2001). MC may have adverse effects on various aquatic organisms and livestock. Administration of MC to mammals and fish potently inhibits protein phosphatases 1 and 2A in hepatocytes in the same manner as okadaic acid, followed by destruction of the hepatic cytoskeleton, leading to liver necrosis, apoptosis, and hemorrhage (Sugaya et al., 1990; Yoshizawa et al., 1990; Råbergh et al., 1991; MacKintosh et al., 1995; Tencalla and Dietrich, 1997; Solter et al., 1998; Fischer et al., 2000). In some exposure experiments with MC, zooplankton and fish exposed to the toxin showed death or sublethal effects such as developmental and behavioral abnormalities (Peñaloza et al., 1990; DeMott et al., 1991; Baganz et al., 1998; Oberemm et al., 1999).

Bioaccumulation of MC in aquatic organisms also has been demonstrated in laboratory experiments and field investigations. Several studies have shown that MC is accumulated by those zooplankton which are primary consumers of toxic cyanobacteria (Watanabe et al., 1992; Laurén-Määttä et al., 1995; Kotak et al., 1996; Thostrup and Christoffersen, 1999). Williams et al. (1997a) reported that the larvae of *Cancer magister* accumulated MC from their diet containing cyanobacteria and may play a role as a food chain vector for the introduction of this toxin into Atlantic salmon, which had accumulated the toxin in livers infected with netpen liver disease(Andersen et al., 1993). To elucidate MC bioaccumulation in aquatic ecosystems, many researchers have thoroughly studied mollusks. Table I summarizes the maximum concentrations of MC in mollusks reported in the literature. Although the concentration of bioaccumulated toxin was found to vary with the individual experimental procedure and analytical method and between species, bivalves in general seem to be the organisms that tend to accumulate this toxin. The results of several laboratory experiments have suggested that filter-feeding bivalves accumulated cyanobacterial toxin by ingestion of toxic cyanobacteria (Eriksson et al., 1989; Lindholm et al., 1989; Vasconcelos, 1995; Williams et al., 1997b; Amorim and Vasconcelos, 1999). Moreover, bivalves actually accumulated toxin at sites where toxic cyanobacterial bloom occurred (Falconer et al., 1992; Prepas et al., 1997; Watanabe et al., 1997; Williams et al., 1997b). A few studies have investigated the relationship between MC bioaccumulation and environmental parameters throughout the year. Kotak et al. (1996) studied the influence of environmental parameters on the MC-LR concentration of zooplankton during the warm season for 2 years in lakes of varying trophic states. Zurawell et al. (1999) suggested that the uptake of MC by gastropods was associated with toxin in the phytoplankton ($r = 0.37–0.57$). However, this relationship has not yet been established clearly. To elucidate the bioaccumulation mechanism of MC by bivalves and predict their contamination in a natural lake, we monitored the toxin concentrations in bivalves beginning prior to the occurrence of *Microcystis* bloom until the subsequent disappearance of the bloom; this revealed the environmental parameters associated with the bioaccumulation of MC.

MATERIALS AND METHODS

Study Site and Water Quality

Lake Suwa, a typical hypereutrophic shallow lake, is in central Honshu, Japan. A dense bloom of *Microcystis* containing MC-RR and -LR has occurred in this lake during the summer since the 1970s (Park et al., 1993, 1998). An investigation was carried out from April to December in both 1997 and 1998 at a point in the south littoral zone (about 1 m in depth); this was done monthly in 1997 and biweekly in 1998. The bottom material is sand, and several species of freshwater bivalves such as Unionidae inhabit this zone. Electric conductivity (HEC-110, DKK, Japan), pH (Model PH81, Yokogawa, Japan), water temperature, and dissolved oxygen (Model 55, YSI, USA) were measured at the surface. To determine the concentrations of chlorophyll *a*, suspended solids (SS), intra- and extracellular MC, water samples were taken from the surface water. For measurement of chlorophyll *a* concentration, the water sample was filtered through a glass microfiber filter (GF/C, Whatman, UK), and the filter was extracted with methanol for 24 h at 4°C in the dark. After centrifugation of the extract, the concentration of chlorophyll *a* was determined spectrophotometrically according to Marker et al. (1980). A heat-treated (450°C, 30 min) and weighed glass fiber filter (GF/C, Whatman, UK) was used for filtration of the surface water sample to measure the concentration of SS. After filtration the filter was dried at 70°C for 48 h. The concentration of SS was determined from the difference between weight of the filter before and after filtration.

Analysis of MC in Lake Water

MC in lake water was fractionated to intra- and extracellular MC. These toxins were evaluated according to Park et al. (1998). The intracellular toxin was extracted from cyanobacterial cells on the glass-fiber filters (GF/C, Whatman, UK) through which the lake water sample (5 L) had been filtered. The intracellular MC was expressed as micrograms per liter (μg/L) of lake water or as micrograms per gram (μg/g) of SS. The filtered water (about 5 L) was used to measure the extracellular toxin expressed as micrograms per liter of lake water. Both extra- and intracellular MC were quantified by reverse-phase high-performance liquid chromatography (HPLC).

Extraction of MC from the Hepatopancreas of Bivalve

Three Unionid bivalves, *Anodonta woodiana, Cristaria plicata* and *Unio douglasiae*, were collected monthly at the site

TABLE I. Microcystin (MC) concentrations in aquatic organisms, as reported in the scientific literature

	Organism	Organ	Toxin	Concentration[a]	Analysis Method	Location	Reference
Mollusca							
Gastropoda	*Helisoma trivolvis*	Whole	MC-LR	37 μg/g DW	HPLC	Lake Driedmeat, Alberta, Canada	Kotak et al., 1996
		Whole	MC-LR	40 μg/g DW	HPLC	Lake Little Beaver, Alberta, Canada	Zurawell et al., 1999
	Lymnaea stagnalis	Whole	MC-LR	96 μg/g DW	HPLC	Lake Driedmeat, Alberta, Canada	Kotak et al., 1996
		Whole	MC-LR	140 μg/g DW	HPLC	Lake Steele, Alberta, Canada	Zurawell et al., 1999
	Physa gyrina	Whole	MC-LR	121 μg/g DW	HPLC	Lake Driedmeat, Alberta, Canada	Kotak et al., 1996
		Whole	MC-LR	129 μg/g DW	HPLC	Lake Driedmeat, Alberta, Canada	Zurawell et al., 1999
Bivalvia	*Mytilus edulis*	Whole	MC	0.022 μg/g WW	PPase assay	Campbell Rever, B.C., Canada	Williams et al., 1997b
		Whole	MC	63.4 μg/g WW	Remieux oxidation GC/MS	Campbell Rever, B.C., Canada	Williams et al., 1997b
		Whole	MC	0.204 μg/g WW	PPase assay	Laboratory experiment	Williams et al., 1997b
		Whole	MC	336.9 μg/g WW	Remieux oxidation GC/MS	Laboratory experiment	Williams et al., 1997b
	Mytilus galloprovincialis	Whole	MC-LR	10.5 μg/g DW	HPLC	Laboratory experiment	Vasconcelos, 1995
		Digestive tract	MC-LR	27.6 μg/g DW	HPLC	Laboratory experiment	Vasconcelos, 1995
		Whole	MC	16 μg/g DW	ELISA	Laboratory experiment	Amorim and Vasconcelos, 1999
	Anodonta cygnea	Whole	*Oscillatoria* toxin	30 μg/g DW	HPLC	Laboratory experiment	Lindholm et al., 1989
		Whole	*Oscillatoria* toxin	70 μg/g DW	HPLC	Laboratory experiment	Eriksson et al., 1989
		Hepatopancreas	*Oscillatoria* toxin	136 μg/g DW	HPLC	Laboratory experiment	Eriksson et al., 1989
	Anodonta grandis simpsoniana	Whole	MC-LR	1.35 μg/g DW	PPase assay	Lake Driedmeat, Alberta, Canada[b]	Prepas et al., 1997
	Anodonta woodiana	Hepatopancreas	MC-RR	0.2 μg/g WW	HPLC	Lake Suwa, Japan	Watanabe et al., 1997
	Unio douglasiae	Hepatopancreas	MC-RR, -LR	2.7 μg/g WW	HPLC	Lake Suwa, Japan	Watanabe et al., 1997

[a] Maximum concentration of toxin in the literature expressed as micrograms of toxin per gram dry weight (DW) or wet weight (WW); [b] Sample of field experiment.

on Lake Suwa. Their shell lengths were 134 ± 13.6 mm, 208 ± 17.0 mm, and 69 ± 3.8 mm (mean ± SD), respectively. The bivalves were dissected and the hepatopancreas isolated from other organs. The tissues were frozen at −30°C and weighed after lyophilization. After homogenization of the hepatopancreas in a glass mortar, 0.2 g of the tissue (dry weight) was stirred and extracted for 24 h with 10 mL of a butanol:methanol:water solution (5:20:75) as reported by Eriksson et al. (1989). After centrifugation (1 h, 18,000 rpm, 4°C), the supernatant was pooled at 4°C in the dark. The pellet was then reextracted twice using the same procedure. When the 3-day extraction was finished, three pooled supernatants were combined and diluted with water to twice the volume. The sample was applied to an ODS (octadecylsilane) silica gel cartridge (5 g; Chromatorex ODS, 10–200 mesh, packed into a polypropylene cartridge), which was preconditioned with methanol and water. The cartridge was rinsed with water and 20% methanol–water and then eluted with 90% methanol–water. The eluate was evaporated to dryness, and the residue was dissolved in 5 mL of methanol. The methanol solution was applied to a silica gel cartridge (2 g; SepPak), which was preconditioned with methanol, and the cartridge was rinsed with methanol. The eluate from the cartridge with 70% methanol–water was evaporated to dryness, and then the residue was dissolved in methanol. The methanol solution was subjected to HPLC analysis.

HPLC Analysis

The sample methanol solution was applied to a reverse-phase HPLC system equipped with an ODS column (Cosmosil 5C18-AR, 4.6 × 150 mm, Nacalai, Japan). The HPLC system consisted of a Shimadzu (Kyoto, Japan) LC-9A pump coupled to a SPD-10A set at 238 nm and a SPD-M10A photodiode array detector and a C-R6A integrator. The sample was separated with a mobile phase consisting of methanol:0.05 M phosphate buffer (pH of 3.0, 58:42) at a flow rate of 1 mL/min. The MC concentration was quantified with standard MC-RR and -LR provided by Dr. K.-I. Harada, Meijo University (Japan).

Statistical Analysis

Simple regression analyses were performed with Microsoft® Excel 97 (Microsoft Corp., Redmond, WA) for prediction of the concentration of MC in the hepatopancreas of *U. douglasiae* in relation to environmental factors and the toxin concentration in lake water. Standardized residuals were also calculated with Microsoft® Excel 97 to measure the accuracy of the estimates of the regression model.

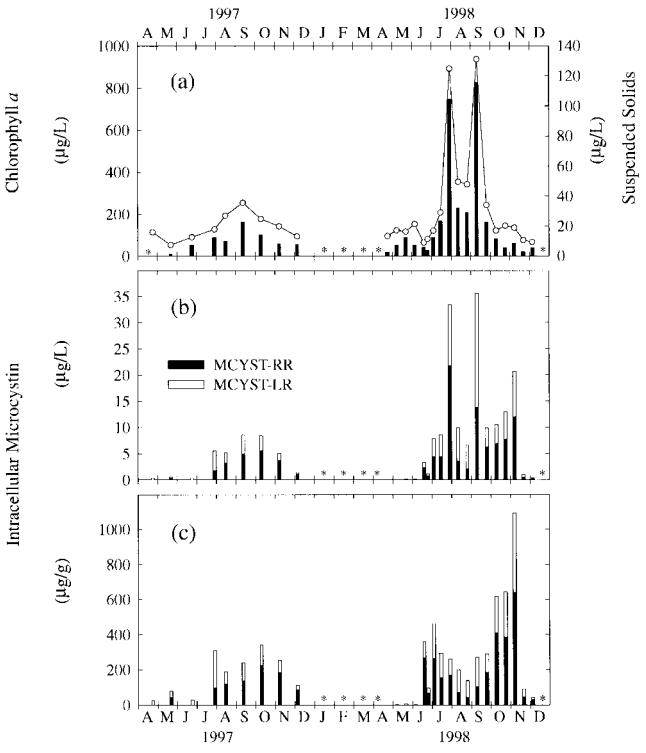

Fig. 1. Seasonal changes of (a) chlorophyll *a* (solid bars) and SS (open circles), (b) intracellular MC (μg/L) in lake water, and (c) intracellular MC (μg/g) in SS at the sampling site for bivalves during April to December (1997–1998); *not determined.

RESULTS

Ambient Water Conditions for Bivalves

Figure 1 shows seasonal variations in ambient water conditions for bivalves, such as chlorophyll *a*, SS, and intracellular MC. Chlorophyll *a* concentration is considered an index of phytoplankton biomass in lakes. Higher concentrations of chlorophyll *a* were observed from July to early October, with maxima of 162 and 827 μg/L in September 1997 and 1998, respectively [Fig. 1(a)]. It appears that *Microcystis* blooms occurred from July to early October in both 1997 and 1998 because the phytoplankton biomass associated with chlorophyll *a* declined in November to the concentration before the bloom. Suspended solids were closely related to chlorophyll *a* ($r = 0.99$, $p < 0.001$, $df = 25$). This shows that the SS comprised mainly organic matter from phytoplankton and represented an abundance of food for bivalves in Lake Suwa. The values were 19.4 ± 8.62 and 31.5 ± 36.4 mg/L (mean ± SD) in 1997 and 1998, respectively [Fig. 1(a)]. Intracellular MC, expressed as micrograms per liter, is a quantitative factor of MC in lake water. As shown in Figure 1(b), total intracellular MC (MC-RR and -LR) increased to more than 5 μg/L in July and then remained at this level in 1997 until November.

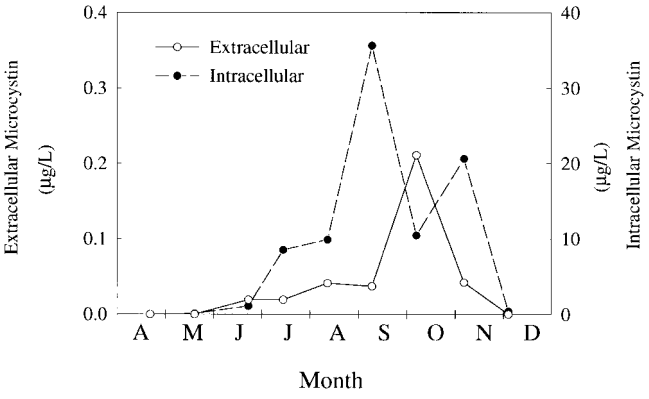

Fig. 2. Seasonal changes in extra- and intracellular MC concentrations in surface water of Lake Suwa from April to December 1998.

However, in 1998 the toxin concentration varied markedly, with three peaks at 33.4 and 35.6 μg/L on July 29 and September 9, respectively, and 20.6 μg/L on November 4. Although the intracellular MC in lake water in 1998 was high until November, in contrast with the decreasing chlorophyll *a* concentration, changes in the toxin corresponded to those of chlorophyll *a* ($r = 0.87$, $p < 0.001$, $df = 25$).

Figure 1(c) shows the qualitative factor of intracellular MC in suspended solids expressed as micrograms per gram. This value indicates the toxin content in food for filter-feeding bivalves. Overall, the toxin concentration in 1997 was lower than that in 1998, but its pattern of seasonal changes corresponded to 1998. The total intracellular MC (MC-RR and -LR) was low from April to June in both years. From then until November higher toxin concentrations were observed, ranging from 189 to 340 μg/g and from 94.8 to 1093 μg/g in 1997 and 1998, respectively. In 1998 the toxin concentration declined on August but then increased and reached its highest concentration on November 4. The toxin concentration in December of both years returned to the concentration in the spring. The pattern of seasonal changes in the microcystin of the suspended solids did not coincide with that of chlorophyll *a* ($r = 0.04$, $p > 0.05$, $df = 25$). The mean ratio of MC-LR to total MC was 52.5 ± 25% [Fig. 1(c)].

Figure 2 indicates the seasonal changes of extra- and intracellular MC concentrations in surface water in 1998. Extracellular MC was very low compared with intracellular toxin, and its ratio to total microcystins (extracellular MC plus intracellular MC) in lake water was always less than 2% during the investigation. The seasonal changes in extracellular MC were related to the occurrence and decay of the *Microcystis* bloom. From April to September the extracellular toxin increased gradually with the increase in the intracellular toxin. The extracellular toxin reached a maximum of 0.211 μg/L in October, when intracellular toxin decreased rapidly. Thereafter, extracellular toxin in December fell to the level in the spring.

Bioaccumulation of MC in Three Bivalves

As shown in Figure 3, all three Unionids, *A. woodiana, C. plicata,* and *U. douglasiae*, bioaccumulated MC into the hepatopancreas. Figure 3 also indicates periods of reproduction recognized by observations of the outer gill lamella, which contain eggs or glochidia during the reproductive season. The seasonal changes in the accumulated toxin concentration differed between species. In *A. woodiana* total toxin (MC-RR and -LR) concentration was always less than in the other two species, and maximum value (12.6 μg/g dry weight) was observed in November 1997. There was no significant trend in seasonal changes of toxin accumulation by *A. woodiana* [Fig. 3(a)]. However, in *C. plicata* and *U. douglasiae* the

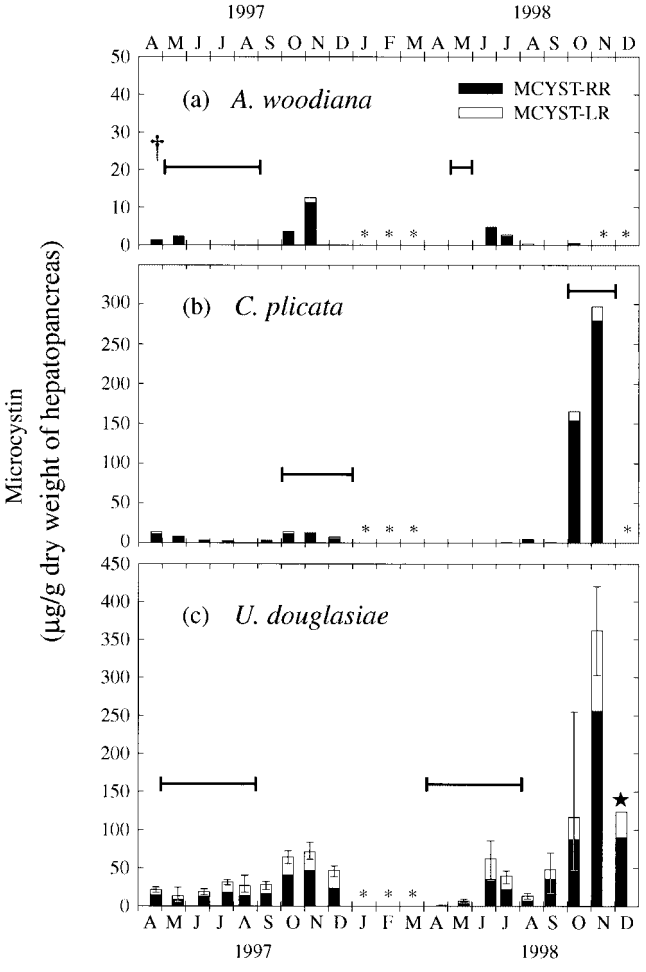

Fig. 3. Seasonal changes of MC concentration in the hepatopancreas of (a) *Anodonta woodiana*, (b) *Cristaria plicata,* and (c) *Unio douglasiae* from Lake Suwa. Data of *U. douglasiae* show mean values. *not determined; †the period during which eggs or glochidia were observed in brood chambers of the outer gill lamella; ★the value derives from one sample taken on December 3, 1998. Vertical bars in panel c represent the range of total MC (MC-RR and -LR) concentrations in two or three samples.

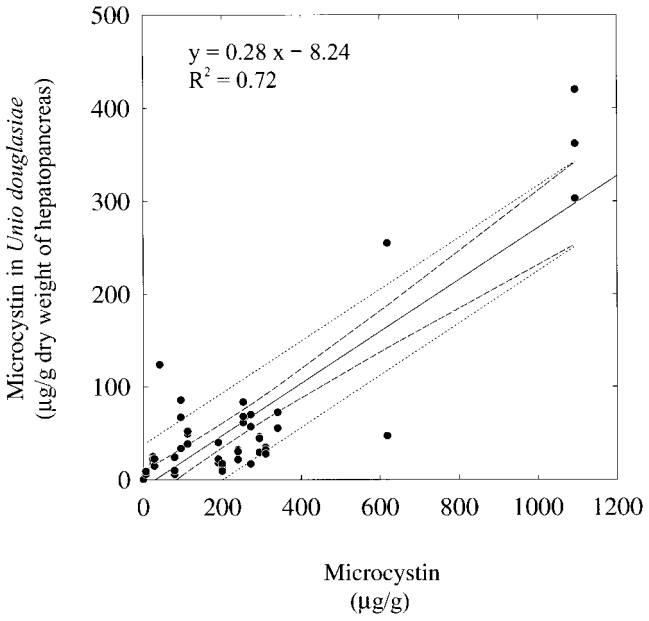

Fig. 4. Linear regression between the tissue concentration of MC in *Unio douglasiae* versus MC in SS during 1997–98. A solid line is a regression line for all the data; dashed and dotted lines indicate 95% confidence limits for the regression and standard errors for the predicted values, respectively.

maximum individual concentration of total toxins was 297 and 420 μg/g dry weight, respectively, in November 1998. The MC concentration of *C. plicata* was low during the *Microcystis* blooming season but increased when autumn came with the season for reproduction [Fig. 3(b)]. Especially in 1998 there was a great difference in accumulated toxin concentration in *C. plicata* between summer and autumn. In *U. douglasiae* MC was detected in all samples collected during the investigation period [Fig. 3(c)]. In spring, when there was no bloom, toxin was detected in the hepatopancreas of *U. douglasiae* in both 1997 and 1998. Thereafter, toxins were accumulated rapidly into the tissue with the occurrence of bloom but subsequently decreased in August. Toxins increased again in the bivalve in the autumn, reaching mean peaks of 71.1 and 362 μg/g dry weight in November 1997 and 1998, respectively. Despite the disappearance of the bloom, the bivalve had a higher toxin concentration in December than in summer. Figure 3(c) also shows that *U. douglasiae* had reproduced from spring to summer in Lake Suwa.

The ratios of MC-LR to total MC in *A. woodiana* and *C. plicata* were 9.8 ± 19.7 and 8.9 ± 10.1% (mean ± SD), respectively, and were significantly lower than the 38.6 ± 17.4% of *U. douglasiae* ($p > 0.001$, Scheffe's multiple range test).

Relationship Between Environmental Parameters and MC Bioaccumulation by *U. douglasiae*

The MC concentration of *U. douglasiae* was predicted significantly ($p < 0.05$) by water temperature or intracellular MC concentration expressed as μg/L and μg/g, respectively (Table II). There was a negative relationship between the toxin in the hepatopancreas and water temperature, whereas both the intracellular MC showed a positive relationship with toxin in the tissue. Although standard regression coefficients were significant in the regression analysis for MC versus the parameters of water temperature and intracellular toxin per unit volume of lake water (μg/L), these R^2 values were low. Thus, these parameters were inappropriate for strict prediction of toxin concentration in the tissue. Intracellular MC in SS could predict toxin concentration in the tissue correctly, as the standard regression coefficient and R^2 value were very high (Fig. 4, Table II). Other parameters, such as chlorophyll *a,* which represents the extent of the *Microcystis* bloom in summer, and SS, which indicates the abundance of food for bivalves in Lake Suwa, could not predict bioaccumulation of MC in the bivalve adequately.

Seasonal variation of MC-RR and -LR in *U. douglasiae* corresponded well to their analogues in SS (the R^2 values of MC-RR and -LR were 0.732 and 0.561, respectively, $p < 0.001$, simple regression analysis).

DISCUSSION

Environmental Parameters Affecting on Seasonal Variation of MC Bioaccumulation in *Unio douglasiae*

Swan mussels (*Anodonta cygnea*) accumulated *Oscillatoria* toxin when exposed to *Oscillatoria*-rich surface water in

TABLE II. Standard regression coefficients and R^2 values in simple linear regression analysis for MC concentration in *Unio douglasiae* versus environmental parameters

Parameter	Standard Regression Coefficient	R^2 [a]
Temperature (°C)	−0.35*	0.10
pH	−0.01	−0.02
Electric conductivity (μS/cm at 25°C)	−0.11	−0.03
Dissolved oxygen (mg O_2/L)	−0.27	0.03
Chlorophyll *a* (μg/L)	−0.07	−0.02
Suspended solids (mg/L)	−0.06	−0.02
Intracellular MC (μg/L)	0.41**	0.15
Intracellular MC (μg/g SS)	0.85***	0.72
Extracellular MC (μg/L)	0.22	0.01

[a] Coefficient of determination adjusted for the degrees of freedom; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Lake Östra Kyrksundet but did not accumulate the toxin when exposed to clear water at a depth of 12 m (Lindholm et al., 1989). Kotak et al. (1996) showed that accumulation of MC-LR in zooplankton correlated with intracellular toxin expressed (as micro g/L ($r = 0.69$). Prepas et al. (1997) studied the accumulation of MC-LR by the freshwater clam *Anodonta grandis simpsoniana* in lakes of three trophic states and showed that the MC-LR concentrations in the clams reflected the toxin concentration (μg/L) in lake phytoplankton. These findings suggest a relationship between bioaccumulation and the quantitative occurrence of MC in lake water. However, in the present study it was impossible to predict the toxin concentration in *U. douglasiae* correctly by quantitative factors such as chlorophyll *a* (μg/L), SS (mg/L), intracellular MC in lake water (μg/L), and extracellular MC (μg/L). The bioaccumulation could be predicted only by a qualitative factor, intracellular MC in SS (μg/g; Table II, Fig. 4). Extracellular MC had no relationship to toxin concentration in *U. douglasiae* (Table II). When *A. grandis simpsoniana* was exposed to a high concentration of extracellular MC-LR (50 μg/L) for 3 days, it did not accumulate the toxin (Prepas et al., 1997). Microcystin-LR concentration in gastropods did not relate to the extracellular MC ($r = 0.14–0.19$) in Canadian lakes (Zurawell et al., 1999). De Maagd et al. (1999) found that the log n-octanol:water distribution ratio (log D_{ow}) of MC-LR was low, ranging from -1.76 to 2.18. These results suggest that MC can scarcely be accumulated through the epithelia of aquatic organisms. Therefore, we conclude that bivalves accumulate MC mainly via the oral route from food rather than via gill from water.

U. douglasiae accumulated MC in Lake Suwa to 28% of the concentration in food particles (Fig. 4). To predict the MC bioaccumulation in *U. douglasiae* through ingestion of food particles contaminated with toxin, it is not appropriate to use the simple equilibrium partitioning bioaccumulation model, which is based on the difference in fugacity capacity between biota and the ambient water, which in turn depends on the physicochemical properties of the contaminant. To predict more accurately the accumulation in bivalves, it is necessary to use the physiologically based (PB) model (Björk and Gilek, 1997), which considers physiological processes such as clearance, filtration, and ingestion, all of which vary in response to feeding conditions. According to the PB model, the important factor for toxin uptake is the exposure rate, calculated as the ingestion rate by the bivalve times the toxin concentration in food particles. The exposure rate is a saturation function of food concentration as well as the ingestion rate at a constant concentration of the toxin in food particles (Björk and Gilek, 1997). In other words, the exposure rate also depends on the qualitative factor, the toxin concentration in food when the bivalve ingests food particles at the maximum rate. During the warm season at Lake Suwa, when primary productivity was high, suspended solids were also high [Fig. 1(a)] and similar to the level (10–30 mg/L) at which the filtration rate of bivalves is generally saturated (Hornbach et al., 1984; Burky et al., 1985; Iglesias et al., 1996; Lei et al., 1996). Iglesias et al. (1996) showed that the ingestion rate became saturated in parallel with the filtration rate with increasing particulate matter concentration at high organic content of the particles. Concerning the significant relationship between chlorophyll *a* and SS in Lake Suwa [Fig. 1(a)], the organic content of SS was considered high. It is assumed that the ingestion rate had reached a maximum level. Thus, the MC exposure rate of *U. douglasiae* was affected strongly by the qualitative factor, intracellular MC in SS (food particles for bivalves), and correlated closely with this factor. For low phytoplankton productivity, quantitative factors, such as *Microcystis* abundance and intracellular MC in lake water, may be adequate for prediction of MC bioaccumulation in bivalves.

Microcystin bioaccumulation responded quickly to changes in the intracellular MC in SS [Figs. 1(c) and 3(c)]. For example, *U. douglasiae* accumulated MC rapidly in response to a slight rise of the toxin in SS in May and in 1998 depurated quickly with decreasing toxin in the food particles from June to August. However, in December 1997 and 1998 the toxin in the tissue remained high despite the low concentration of toxin in the SS. For hydrophobic contaminants, which have a lower depuration rate coefficient (k_d) than hydrophilic contaminants, a time lag has been observed between values in the tissue and the surrounding water, as shown by a field experiment that investigated uptake and depuration of various agrichemicals in shellfish (Uno et al., 1997). Although MC bioaccumulation was predicted significantly by the intracellular MC concentration in SS (Table II and Fig. 4), there was a seasonal trend in the standardized residuals between observed and predicted values (Fig. 5). This indicates that using only the toxin concentration in SS is insufficient to predict MC bioaccumulation. The standardized residuals were negative in summer, when the water temperature was above 20°C and thereafter increased with a fall in the water temperature to less than 15°C in autumn. Thus, we presume that MC bioaccumulation was enhanced relatively in autumn because it is thought that k_d decreases with a decrease in water temperature. Assuming that the k_d value is lower in winter, MC in the tissue can not be depurated easily and will remain till spring. The standardized residuals were also high in spring at a time when the water temperature was higher than in autumn. At least in April the toxin in the tissue might have been residual MC that had accumulated during the previous year; however, we cannot rule out the possibility of MC uptake in spring. Spring is the reproductive season for *U. douglasiae*. Some bivalves are known to increase their filtration rate in the reproductive period (Hornbach et al., 1984; Burky et al., 1985), so *U. douglasiae* may also enhance its filtration and ingestion rates in spring. The exposure rate probably accelerated, thus increasing MC bioac-

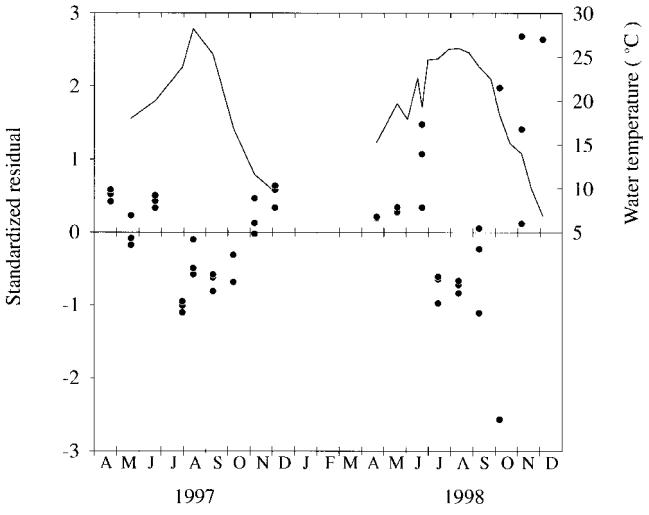

Fig. 5. Seasonal changes in surface water temperature (solid lines) and time-series plots of standardized residuals of the simple regression between tissue concentration of MC in *Unio douglasiae* versus MC in SS (solid circles).

cumulation during this period. Therefore, the standardized residuals increased, particularly in June, although the water temperature was relatively high (about 20°C).

Differences in the Bioaccumulation Patterns of the Three Bivalve Species

MC-RR and -LR were detected in various organs of *U. douglasiae* on July 22. *A. woodiana* in Lake Suwa were found to contain only MC-RR in the hepatopancreas on August 10, 1992 (Watanabe et al., 1997), and on July 19, 1992, no MCs were detected in *C. plicata*. However, MC bioaccumulation by these bivalves has not yet been identified in detail. In the study reported here, MCs were detected at much higher concentrations in *C. plicata* and *U. douglasiae* than in other bivalves (Table I). Furthermore, the study revealed that the MC bioaccumulation pattern differed between the three bivalves. In *A. woodiana* the concentration of bioaccumulated toxin was always very low, and its seasonal pattern showed no relationship with environmental parameters. In *C. plicata* the toxin in the tissue was low in summer, when the *Microcystis* bloom occurred, and increased in autumn as the bloom decayed. In this bivalve MC sometimes accumulated at a very high concentration in autumn. In *U. douglasiae* bioaccumulation was at a higher concentration than in two other bivalves and could be predicted by the intracellular MC in SS and by the water temperature. The different seasonal bioaccumulation patterns in the three bivalves may be a result of interspecific differences in selective ingestion, reproductive season, MC metabolism, and depuration rate. Bougrier et al. (1997) showed that the oyster *Crassostrea gigas* ingested flagellates selectively rather than diatoms depending on differences in algal shape and flexibility. *A. woodiana* might reject *Microcystis* as pseudofeces prior to ingestion. Two species of Unionidae, *Actinonaias ligamentina* and *Amblema plicata,* were found to have different seasonal patterns of physiology and biochemical composition (Baker and Hornbach, 2001). Baker and Hornbach (2001) indicated that larval blooding affected the physiology of *A. ligamentina.* The reproductive season for bivalves would affect the MC bioaccumulation pattern. In *C. plicata*, increasing bioaccumulation in autumn resulted from enhancement of the exposure rate in the reproductive season from October to December, as mentioned above. The mean ratio of MC-LR to total MC differed significantly among the three bivalves. These bivalves tend to accumulate MC-RR because their ratios of MC-RR were higher than that in the SS. In particular, *A. woodiana* and *C. plicata* accumulated MC-RR selectively up to about 90% of the total MC. It is clear that these two bivalves differ from *U. douglasiae* in MC metabolism, which is involved with uptake and depuration. The depuration rate also differs among bivalves. The Mytilidae, *Mytilus galloprovincialis* and *M. edulis* apparently eliminate completely in 10–13 days at 10°C–16°C (Vasconcelos, 1995; Williams et al., 1997b). On the other hand, Unionidae are considered to have a low ability for depuration because *Anodonta cygnea* and *A. grandis simpsoniana* were found to not eliminate completely in 2 months or 21 days at 18°C–20°C (Eriksson et al., 1989; Prepas et al., 1997). Presumably, Unionidae tend to depurate the toxin more slowly. In the study reported here, *C. plicata* and *U. douglasiae* contained MC at a relatively high concentration in spring. This might be a residual portion of toxin accumulated in the previous year.

The present study has shown that a high concentration of MC is accumulated by Unionidae, which are numerous in Lake Suwa, and there is an interspecific difference in the seasonal pattern of bioaccumulation. It has been shown that MC bioaccumulation in *U. douglasiae* is influenced not only by intracellular MC in SS but also by water temperature and reproduction. At present, the three species of bivalve in Lake Suwa are a food source for few people. This study has made clear the following problems concerning risk assessment of edible bivalves contaminated by cyanobacterial toxin: (1) the bioaccumulation pattern must be known for each species; otherwise it will be especially difficult to predict MC bioaccumulation in species such as *C. plicata*, in which bioaccumulation did not necessarily correspond to the abundance of *Microcystis* and/or MC; (2) although previous researchers only have reported on bioaccumulation in the bloom season, a long-term survey that begins prior to a bloom and continues until the bloom disappears is necessary to assess the safety of contaminated bivalves considering both their lifecycle and seasonal changes in their k_d; and (3) when bioaccumulation is monitored, optimal monitoring parameters, such as quantitative

or qualitative factors, should be selected depending on the trophic state of the lake.

REFERENCES

Amorim Á, Vasconcelos V. 1999. Dynamics of microcystins in the mussel *Mytilus galloprovincialis*. Toxicon 37:1041–1052.

Andersen RJ, Luu HA, Chen DZ, Holmes CF, Kent ML, Le Blanc M, Taylor FJ, Williams DE. 1993. Chemical and biological evidence links microcystins to salmon 'netpen liver disease.' Toxicon 31:1315–1323.

Baganz D, Staaks G, Steinberg C. 1998. Impact of the cyanobacteria toxin, microcystin-LR on behaviour of zebrafish, *Danio rerio*. Water Res 32:948–952.

Baker SM, Hornbach DJ. 2001. Seasonal metabolism and biochemical composition of two unionid mussels, *Actinonaias ligamentina* and *Amblema plicata*. J Moll Stud 37:407–416.

Billings WH. 1981. Water-associated human illness in northeast Pennsylvania and its suspected association with blue-green algae blooms. In: Carmichael WW, editor. The water environment: Algal toxins and health. New York: Plenum Press. p 243–255.

Björk M, Gilek M. 1997. Bioaccumulation kinetics of PCB 31, 49 and 153 in the blue mussel, *Mytilus edulis* L. as a function of algal food concentration. Aquat Toxicol 38:101–123.

Botes DP, Tuinman AA, Wessels PL, Viljoen CC, Kruger H, Williams DH, Santikarn S, Smith RJ, Hammond SJ. 1984. The structure of cyanoginosin-LA, a cyclic heptapeptide toxin from the cyanobacterium *Microcystis aeruginosa*. J Chem Soc Perkin Trans 1:2311–2318.

Bougrier S, Hawkins AJS, Héral M. 1997. Preingestive selection of different microalgal mixtures in *Crassostrea gigas* and *Mytilus edulis*, analysed by flow cytometry. Aquaculture 150:123–134.

Burky AJ, Benjamin RB, Conover DG, Detrick JR. 1985. Seasonal responses of filtration rates to temperature, oxygen availability, and particle concentration of the freshwater clam *Musculium partumeium* (Say). Am Malacol Bull 3:201–212.

Codd GA, Bell SG, Kaya K, Ward CJ, Beattie KA, Metcalf JS. 1999. Cyanobacterial toxins, exposure routes and human health. Eur J Phycol 34:405–415.

De Maagd PG-J, Hendriks AJ, Seinen W, Sijm DTHM. 1999. pH-Dependent hydrophobicity of the cyanobacteria toxin microcystin-LR. Water Res 33:677–680.

DeMott WR, Zhang Q-X, Carmichael WW. 1991. Effects of toxic cyanobacteria and purified toxins on the survival and feeding of a copepod and three species of *Daphnia*. Limnol Oceanogr 36:1346–1357.

Eriksson JE, Meriluoto JAO, Lindholm T. 1989. Accumulation of a peptide toxin from the cyanobacterium *Oscillatoria agardhii* in the freshwater mussel *Anodonta cygnea*. Hydrobiologia 183: 211–216.

Falconer IR, Choice A, Hosja W. 1992. Toxicity of edible mussels (*Mytilus edulis*) growing naturally in an estuary during a water bloom of the blue-green alga *Nodularia spumigena*. Environ Toxicol Water Qual 7:119–123.

Fischer WJ, Hitzfeld BC, Tencalla F, Eriksson JE, Mikhailov A, Dietrich DR. 2000. Microcystin-LR toxicodynamics, induced pathology, and immunohistochemical localization in livers of blue-green algae exposed rainbow trout (*Oncorhynchus mykiss*). Toxicol Sci 54:365–373.

Hornbach DJ, Way CM, Wissing TE, Burky AJ. 1984. Effects of particle concentration and season on the filtration rates of the freshwater clam, *Sphaerium striatinum* Lamarck (Bivalvia: Pisidiidae). Hydrobiologia 108:83–96.

Iglesias JIP, Urrutia MB, Navarro E, Alvarez-Jorna P, Larretxea X, Bougrier S, Heral M. 1996. Variability of feeding processes in the cockle *Cerastoderma edule* (L.) in response to changes in seston concentration and composition. J Exp Mar Biol Ecol 197:121–143.

Kotak BG, Zurawell RW, Prepas EE, Holmes CFB. 1996. Microcystin-LR concentration in aquatic food web compartments from lakes varying trophic status. Can J Fish Aquat Sci 53: 1974–1985.

Laurén-Määttä C, Hietala J, Reinikainen M, Walls M. 1995. Do *Microcystis aeruginosa* toxins accumulate in the food web: a laboratory study. Hydrobiologia 304:23–27.

Lei J, Payne BS, Wang SY. 1996. Filtration dynamics of the zebra mussel, *Dreissena polymorpha*. Can J Fish Aquat Sci 53:37–39.

Lindholm T, Eriksson JE, Meriluoto JAO. 1989. Toxic cyanobacteria and water quality problems—examples from a eutrophic lake on Åland, south west Finland. Water Res 23:481–486.

MacKintosh RW, Dalby KN, Campbell DG, Cohen PTW, Cohen P, MacKintosh C. 1995. The cyanobacterial toxin microcystin binds covalently to cysteine-273 on protein phosphatase 1. FEBS Lett 371:236–240.

Marker AFH, Crowther CA, Gunn RJM. 1980. Methanol and acetone as solvents for estimating chlorophyll *a* and phaeopigments by spectrophotometry. Arch Hydrobiol Beih Ergebn Limnol 14:52-69.

Oberemm A, Becker J, Codd GA, Steinberg C. 1999. Effects of cyanobacterial toxins and aqueous crude extracts of cyanobacteria on the development of fish and amphibians. Environ Toxicol 14:77–88.

Park H-D, Iwami C, Watanabe MF, Harada K-I, Okino T, Hayashi H. 1998. Temporal variabilities of the concentrations of intra- and extracellular microcystin and toxic *Microcystis* species in a hypertrophic lake, Lake Suwa, Japan (1991–1994). Environ Toxicol Water Qual 13:61–72.

Park H, Namikoshi M, Brittain SM, Carmichael WW, Murphy T. 2001. [D-Leu^1] microcystin-LR, a new microcystin isolated from waterbloom in a Canadian prairie lake. Toxicon 39:855–862.

Park H-D, Watanabe MF, Harada K-I, Suzuki M, Hayashi H, Okino T. 1993. Seasonal variations of *Microcystis* species and toxic heptapeptide microcystins in Lake Suwa. Environ Toxicol Water Qual 8:425–435.

Peñaloza R, Rojas M, Vila I, Zambrano F. 1990. Toxicity of a soluble peptide from *Microcystis* sp. to zooplankton and fish. Freshwat Biol 24:233–240.

Pitois S, Jackson MH, Wood BJB. 2000. Problems associated with the presence of cyanobacteria in recreational and drinking waters. Int J Environ Health Res 10:203–218.

Pouria S, de Andrade A, Barbosa J, Cavalcanti RL, Barreto VTS, Ward CJ, Preiser W, Poon GK, Neild GH, Codd GA. 1998. Fatal microcystin intoxication in haemodialysis unit in Caruaru, Brazil. The Lancet 352:21–26.

Prepas EE, Kotak BG, Campbell LM, Evans JC, Hrudey SE, Holmes CFB. 1997. Accumulation and elimination of cyanobacterial hepatotoxins by the freshwater clam *Anodonta grandis simpsoniana*. Can J Fish Aquat Sci 54:41–46.

Råbergh CMI, Bylund G, Eriksson JE. 1991. Histopathological effects of microcystin-LR, a cyclic peptide toxin from the cyanobacterium (blue-green alga) *Microcystis aeruginosa*, on common carp (*Cyprinus carpio* L.). Aquat Toxicol 20:131–146.

Rinehart KL, Namikoshi M, Choi BW. 1994. Structure and biosynthesis of toxins from blue-green algae (cyanobacteria). J Appl Phycol 6:159–176.

Sivonen K. 1996. Cyanobacterial toxins and toxin production. Phycologia 35:12–24.

Skulberg OM, Codd GA, Carmichael WW. 1984. Toxic blue-green algal blooms in Europe: A growing problem. Ambio 13:244–247.

Solter PF, Wollenberg GK, Huang X, Chu FS, Runnegar MT. 1998. Prolonged sublethal exposure to the protein phosphatase inhibitor microcystin-LR results in multiple dose-dependent hepatotoxic effects. Toxicol Sci 44:87–96.

Sugaya Y, Yasuno M, Yanai T. 1990. Effects of toxic *Microcystis viridis* and isolated toxins on goldfish. Jpn J Limnol 51:149–153.

Tencalla F, Dietrich D. 1997. Biochemical characterization of microcystin toxicity in rainbow trout (*Oncorhynchus mykiss*). Toxicon 35:583–595.

Thostrup L, Christoffersen K. 1999. Accumulation of microcystin in *Daphnia magna* feeding on toxic *Microcystis*. Arch Hydrobiol 145:447–467.

Uno S, Shiraishi H, Hatakeyama S, Otsuki A. 1997. Uptake and depuration kinetics and BCFs of several pesticides in three species of shellfish (*Corbicula leana*, *Corbicula japonica*, and *Cipangopludina chinensis*): Comparison between field and laboratory experiment. Aquat Toxicol 39:23–43.

Vasconcelos VM. 1995. Uptake and depuration of the heptapeptide toxin microcystin-LR in *Mytilus galloprovincialis*. Aquat Toxicol 32:227–237.

Watanabe MF, Park H-D, Kondo F, Harada K-I, Hayashi H, Okino T. 1997. Identification and estimation of microcystins in freshwater mussels. Nat Toxins 5:31–35.

Watanabe MM, Kaya K, Takamura N. 1992. Fate of the toxic cyclic heptapeptides, the microcystins, from blooms of *Microcystis* (cyanobacteria) in a hypertrophic lake. J Phycol 28:761–767.

Williams DE, Craig M, Dawe SC, Kent ML, Holmes CFB, Andersen RJ. 1997a. Evidence for a covalently bound form of microcystin-LR in salmon liver and Dungeness crab larvae. Chem Res Toxicol 10:463–469.

Williams DE, Dawe SC, Kent ML, Andersen RJ, Craig M, Holmes CFB. 1997b. Bioaccumulation and clearance of microcystins from salt water mussels, *Mytilus edulis*, and *in vivo* evidence for covalently bound microcystins in mussel tissues. Toxicon 35: 1617–1625.

Yoshizawa S, Matsushima R, Watanabe MF, Harada K-I, Ichihara A, Carmichael WW, Fujiki H. 1990. Inhibition of protein phosphatases by microcystin and nodularin associated with hepatotoxicity. J Cancer Res Clin Oncol 116:609–614.

Zurawell RW, Kotak BG, Prepas EE. 1999. Influence of lake trophic status on the occurrence of microcystin-LR in the tissue of pulmonate snails. Freshwat Biol 42:707–718.