Microcystin-LR concentration in aquatic food web compartments from lakes of varying trophic status

Brian G. Kotak, Ron W. Zurawell, Ellie E. Prepas, and Charles F.B. Holmes

Abstract: Microcystin-LR (MC-LR) concentrations were examined in water, phytoplankton, invertebrates, and two fishes for up to 3 years in four central Alberta lakes spanning a trophic gradient in total phosphorus from 15 to over 500 $\mu g \cdot L^{-1}$ in epilimnetic waters. MC-LR was not detected by HPLC in phytoplankton from an oligo-mesotrophic lake, was less than 150 ng cellular toxin$\cdot L^{-1}$ of lake water in a eutrophic–hypereutrophic lake, and was up to 6000 and 11 000 ng$\cdot L^{-1}$ in two hypereutrophic lakes. MC-LR in phytoplankton was strongly correlated with the abundance of the cyanobacterium *Microcystis aeruginosa*, and with aqueous microcystin concentration, determined by protein phosphatase bioassay ($r = 0.83$). MC-LR was also detected in zooplankton (up to 67 $\mu g \cdot g^{-1}$ of biomass) and MC-LR concentration in zooplankton and phytoplankton were correlated ($r = 0.69$). Although nine groups of macroinvertebrates were analyzed, MC-LR was only detected in gastropods (up to 120 $\mu g \cdot g^{-1}$). MC-LR appears to be transferred to invertebrates through grazing activity. MC-LR was not detected in the livers of northern pike (*Esox lucius*) and white sucker (*Catostomus commersoni*) collected from one lake containing toxin-producing phytoplankton. Accumulation of MC-LR in aquatic food webs appears to occur in the primary consumer with probable transfer of the toxin to higher trophic levels.

Résumé : Pendant une période atteignant une durée de 3 ans, on a mesuré les concentrations de microcystine-LR (MC-LR) dans l'eau et le phytoplancton ainsi que chez les invertébrés et chez deux espèces de poissons de 4 lacs du centre de l'Alberta où le gradient trophique de phosphore total épilimnétique allait de 15 à plus de 500 $\mu g \cdot L^{-1}$. La HPLC du phytoplancton du lac oligomésotrophe n'a pas mis en évidence de MC-LR; la concentration de la toxine cellulaire était inférieure à 150 ng$\cdot L^{-1}$ d'eau dans le lac eutrophe–hypereutrophe et elle atteignait 6000 et 11 000 ng$\cdot L^{-1}$ dans les deux hypereutrophes. Par ailleurs, on a noté une forte corrélation entre la concentration de MC-LR phytoplanctonique et l'abondance de la cyanobactérie *Microcystis aeruginosa* et la concentration de microcystine aqueuse, dosée avec une protéine-phosphatase ($r = 0,83$). On a aussi détecté de la MC-LR chez le zooplancton (jusqu'à 67 $\mu g \cdot g^{-1}$ de biomasse) et observé une corrélation entre les concentrations de MC-LR zooplanctonique et phytoplanctonique ($r = 0,69$). On a analysé neuf groupes de macroinvertébrés, mais la MC-LR n'a été détecté que chez les gastéropodes (jusqu'à 120 $\mu g \cdot g^{-1}$). La MC-LR semble passer aux invertébrés par le broutage. On n'en a pas détecté dans le foie du broche (*Esox lucius*) et du meunier noir (*Catostomus commersoni*) prélevés dans l'un des lacs où le phytoplancton est producteur de toxine. La bioconcentation de MC-LR dans les réseaux trophiques aquatiques semble survenir au niveau des consommateurs primaires et il y a probablement transfert de la toxine aux niveaux trophiques plus élevés. [Traduit par la Rédaction]

Introduction

Microcystins are hepatotoxic compounds produced primarily by the cyanobacterium *Microcystis aeruginosa* (Namikoshi et al. 1992; Kotak et al. 1993, 1995). Microcystin production by cyanobacteria is likely a common occurrence in eutrophic and hypereutrophic lakes in western Canada. Kotak (1995) recorded the toxin in 24 of 28 lakes surveyed from 1990 to 1994 in central and northern Alberta, with 360 of the 490 phytoplankton samples collected containing detectable concentrations of microcystin-LR (MC-LR). Although more than 50 microcystins have been structurally characterized, MC-LR is the most common analogue in Alberta lakes (Boland et al. 1993; Craig et al. 1993; Kotak et al. 1993). It is also one of the most toxic of the microcystins, having an LD_{50} of 50 $\mu g \cdot kg^{-1}$ by intraperitoneal injection in mice (Carmichael 1992).

Much of the research on the toxicity of microcystins has traditionally focused on laboratory mammals (e.g., rats, mice). More recently, studies have evaluated the relationship between toxic cyanobacteria and other components of aquatic food webs. However, most of these studies examined only acute toxicity, in either zooplankton (DeMott et al. 1991; Rothhaupt 1991; Gilbert 1994; Jungmann and Benndorf 1994; Reinikainen et al. 1994) or fish (Phillips et al. 1985; Sugaya et al. 1990; Rabergh et al. 1991; Kotak et al. 1996). Only one study has evaluated whether microcystins are accumulated by natural populations of aquatic invertebrates (Watanabe et al. 1992) in freshwater lakes. Recently, MC-LR has also been detected in marine organisms. Chen et al. (1993) reported MC-LR in mussels collected from coastal waters of British Columbia, and Andersen et al. (1993) documented detectable concentrations of MC-LR in aquatic invertebrates fouling Atlantic salmon (*Salmo salar*) net pens, as

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B.G. Kotak, R.W. Zurawell, and E.E. Prepas. Department of Biological Sciences, University of Alberta, Edmonton, AB T6G 2E9, Canada.
C.F.B. Holmes. Department of Biochemistry, University of Alberta, Edmonton, AB T6G 2H7, Canada.

well as in the livers of salmon with severe liver disease (Kent et al. 1988; Kent 1990). The source (producer) of MC-LR in the marine environment, however, is currently unknown. Our study reports the concentration of MC-LR in five compartments (water, phytoplankton, micro- and macro-invertebrates, and to a limited extent, fish) from up to four lakes in central Alberta, ranging in trophic status from oligo-mesotrophic to hypereutrophic. MC-LR concentrations were then related to physical and biological characteristics of the lakes.

Materials and methods

Study lakes

The four lakes chosen for study are located in central Alberta, Canada: Coal, Driedmeat, Little Beaver, and Narrow lakes. Coal and Little Beaver lakes were sampled every 2 weeks from May to the end of September in 1992 and May to the end of August in 1993 and 1994. Driedmeat Lake was sampled similarly, although sampling in 1993 extended until the end of September. Narrow Lake was sampled only in 1994: on 13 May, 14 June, 11 July, and 20 September. The lakes chosen differed in trophic status and, thus, in the potential for MC-LR production. Historically, Narrow Lake is oligo-mesotrophic, Coal Lake is eutrophic, and Driedmeat and Little Beaver lakes are hypereutrophic based on summer total phosphorus and chlorophyll *a* concentrations in the euphotic zone (Mitchell and Prepas 1990; Kotak et al. 1993).

Water quality

On each sampling date an integrated water sample was collected from the surface of the water to a depth of 2 m (except for in Little Beaver Lake where water was collected from the surface to a depth of 1 m) at four sites with Tygon tubing fitted with a one-way valve. These samples are referred to as epilimnetic. Water from these four sites was combined prior to analyses. Water was analyzed for total phosphorus by colorimetric methods after potassium persulfate digestion (Menzel and Corwin 1965, as modified by Prepas and Rigler 1982), although large particles (>250 μm) were not filtered out. Conductivity was measured on a Radiometer Analytical Model CDM83 conductance meter. Alkalinity (as milligrams $CaCO_3$ per litre) was determined on a Mettler DL21 autotitrator (APHA, AWWA, and WPCF 1992). A small subsample (10 mL) of the water was filtered through a pre-rinsed, 0.45-μm Millipore HA filter and frozen for subsequent aqueous microcystin analysis. Phytoplankton biomass (estimated as chlorophyll *a*) was determined from water samples that were filtered onto Whatman GF/C filters, extracted in 95% ethanol, and measured spectrophotometrically (Bergmann and Peters 1980). Phytoplankton were identified and enumerated on an inverted microscope (Prescott 1978). *Microcystis aeruginosa* abundance (cells per millilitre) is reported. Secchi disk depth and water temperature were recorded at one central site in each lake. Surface water temperature was recorded with a YSI Model 51 temperature – O_2 meter. Total phosphorus, conductivity, and Secchi disk depth for 1994 are reported as summer means from May to September (±SE). Mean summer alkalinity (±SE) in Coal, Driedmeat, and Little Beaver lakes are reported for 1993.

Zooplankton

In 1992 and 1993 in Coal, Driedmeat, and Little Beaver lakes and in 1994 in Narrow Lake, epilimnetic zooplankton samples were collected with a 243-μm mesh plankton net. One vertical haul was made at each of four sites per lake and the four samples combined for zooplankton identification. Samples were preserved in 10% buffered formalin and zooplankton were identified and enumerated with a dissecting microscope. Identification was based on descriptions in Pennak (1978). Abundance of each species was then expressed as a percentage of the total abundance in the sample. Zooplankton for microcystin-LR (MC-LR) analysis were collected as described above. The zooplankton samples (which also contained phytoplankton) were then resuspended in 1 L of lake water. Upon arrival at the laboratory, the plankton sample was gently bubbled with CO_2 to anesthetize the zooplankton. The sample was then tightly capped and the zooplankton settled to the bottom of the jar while the cyanobacteria rose to the surface. The cyanobacteria that accumulated along the surface and most of the water were siphoned off, avoiding removal of the zooplankton on the bottom. The remaining zooplankton and phytoplankton were resuspended in distilled water and the process repeated. The samples contained only zooplankton and groups of phytoplankton that settled out quickly (e.g., diatoms). Microscopic examination of the processed sample revealed no detectable *M. aeruginosa*, the species responsible for MC-LR production in Alberta lakes (Kotak et al. 1993, 1995). Thus, any MC-LR detected in these samples was presumably from zooplankton tissue or gut contents. The sample was then frozen and freeze-dried. On several sampling dates, zooplankton biomass was insufficient for MC-LR analysis. As a result, only 68 of 99 zooplankton samples collected were analyzed for MC-LR.

Phytoplankton

In all years, phytoplankton were collected for MC-LR analysis with a 64-μm mesh plankton net at the same locations and in a similar manner as that for zooplankton. The samples collected at the four sites in each lake were combined and later freeze-dried. These samples contained both phytoplankton and zooplankton. To determine the concentration of MC-LR in the phytoplankton alone, MC-LR concentration detected in the separate zooplankton sample was subtracted from the MC-LR concentration detected in the sample containing both phytoplankton and zooplankton. The correction for the presence of zooplankton was not always possible as there were 99 phytoplankton samples but only 68 zooplankton samples.

Macroinvertebrates

In 1994, a suite of aquatic macroinvertebrates was collected from each of the four lakes and analyzed for MC-LR. These included gastropods (*Lymnaea stagnalis*, *Physa gyrina*, *Helisoma trivolvis*), dragonfly (*Ashna* sp.), and damselfly (*Lestes* sp.) larvae, water boatmen (Family Corixidae), backswimmers (*Notonecta* sp.), predacious water beetles (*Dytiscus* sp.), scuds (*Gammarus lacustris*), and leeches (Subclass Hirudinea). Identifications were based on descriptions given in Clifford (1993). These invertebrates were collected with a dip net from the shoreline or from the littoral zone from the side of the boat. In addition, surface sediments were collected from four sites in each lake with an Eckmann dredge and the four samples were combined. Mud was sifted through a 500-μm sieve and chironomids (*Chironomus* spp.) were rinsed and saved for MC-LR analysis. All invertebrates (including zooplankton) were frozen, freeze-dried, and then homogenized with a mortar and pestle.

Fish

On 29 and 30 August 1993, northern pike (*Esox lucius*) and white sucker (*Catostomus commersoni*) were collected from Driedmeat Lake with a combination of gill and seine nets. Driedmeat Lake was chosen as the site for fish collection because we felt the fish had been exposed to high concentrations of MC-LR in the phytoplankton during the previous month. The eight northern pike collected ranged in size from 38 to 46 cm (fork length) and weighed from 445 to 814 g. The 23 suckers collected ranged in size from 32 to 42 cm and weighed from 584 to 1302 g. The fish were immediately frozen and later thawed to remove their livers for MC-LR analysis. The fish were weighed (wet mass), and a limited examination of the gut contents of the fish was made. Livers were freeze-dried prior to MC-LR analysis.

Microcystin analyses

MC-LR in phytoplankton and aquatic invertebrates was quantified

Table 1. Water quality and morphometric characteristics of the study lakes.

Variable	Lake			
	Narrow	Coal	Little Beaver	Driedmeat
Total Phosphorus ($\mu g \cdot L^{-1}$)	15 (2)	71 (17)	207 (26)	530 (119)
Secchi depth (m)	7.2 (0.5)	1.3 (0.1)	0.8 (0.2)	1.1 (0.3)
Conductivity ($\mu S \cdot cm^{-1}$)	231 (41)	474 (12)	826 (65)	574 (14)
Alkalinity ($mg \cdot L^{-1}$ $CaCO_3$)*	155 (1)	196 (6)	332 (11)	210 (10)
Surface area (km^2)	1.1	11	0.75	17
Maximum depth (m)	39	5.5	2.0	3.7

Note: Water quality parameters (total phosphorus, Secchi disk depth, conductivity, and alkalinity) represent seasonal means (May to September, with SE given in parentheses) for 1994 and are based on samples collected from the surface of the water to a depth of 2 m (surface to 1 m for Little Beaver Lake), except where indicated. Lake morphometry data were obtained from Mitchell and Prepas (1990) and Kotak et al. (1993).

*Alkalinity in Narrow Lake is for 1983 (Mitchell and Prepas 1990) and for 1993 in Coal, Little Beaver, and Driedmeat lakes.

by reverse phase high performance liquid chromatography (HPLC). Phytoplankton and zooplankton, and macroinvertebrate subsamples (100 and 500 mg freeze-dried weight, respectively) were sonicated and extracted in 5% acetic acid overnight. The extract was then filtered through Whatman GF/A filters and the microcystins purified and concentrated on preconditioned Supelco LC-18 cartridges. Microcystins were eluted from the cartridge in 5 mL 100% methanol. The methanol was evaporated in a Reactivap heater and the sample resuspended in 2 mL of 10 mM ammonium acetate : acetonitrile (75%:25%, v:v).

Over the 3 years of this program, three different HPLC separation methods were utilized on two separate HPLC systems. In 1992, phytoplankton were analyzed on a HPLC consisting of Waters 6000A pumps with a Hewlett Packard HP 1040A diode array detector and HP85 data processor. Samples were run under isocratic conditions at a flow rate of 1 $mL \cdot min^{-1}$. The mobile phase consisted of 10 mM ammonium acetate and acetonitrile (74:26%, v:v). In 1993 and 1994, phytoplankton were analyzed on a Waters LC Module I HPLC with a model 746 data module. For both HPLCs, MC-LR detection was based on UV absorbance at 238 nm. In 1993, samples were run under isocratic conditions, at a flow rate of 1 $mL \cdot min^{-1}$, and with a mobile phase consisting of 10 mM ammonium acetate: acetonitrile (76:24%, v:v). In 1994, samples were run under linear gradient conditions (20–30% acetonitrile in 10 mM ammonium acetate over 10 min, then at 20% from 10 min until the end of the run, (i.e., 30 min). Flow rate was 1 $mL \cdot min^{-1}$. A comparison of the two HPLCs and the three separation methods (two isocratic, one gradient) indicated no significant difference in the quantity of MC-LR detected, although the gradient method gave much better separation of MC-LR away from other interfering peaks (Kotak 1995). MC-LR concentration in phytoplankton was first calculated as micrograms per gram of phytoplankton biomass based on MC-LR calibration curves. MC-LR was then re-expressed volumetrically, as nanograms cellular toxin per litre (Kotak et al. 1995). This volumetric expression represented MC-LR in the phytoplankton cells per litre of lake water, not as dissolved toxin.

All invertebrate samples (zooplankton and macroinvertebrates) were analyzed for MC-LR on the latter HPLC system under the gradient conditions described above. MC-LR concentrations in the invertebrates are reported as micrograms per gram of biomass. In addition, the protein phosphatase (PP1c) assay (Luu et al. 1993) was also utilized to quantify total microcystin concentration in the zooplankton samples as well as total aqueous (dissolved) microcystin concentration in lake water. This gave an indication of the quantity of MC-LR and other microcystin analogues in the samples. The assay results are expressed as total microcystin concentration (as there may be more than 50 microcystins present in a sample) in MC-LR equivalent mass units. The detection limit of the PP1c assay was less than 10 pg MC-LR equivalents while the detection limit for MC-LR by HPLC was 10–20 ng for a 20-μL injection. For the PP1c assay, subsamples (100–500 mg) of zooplankton were extracted as described above for HPLC, except that the extraction solvent was 100% methanol and microcystins were not preconcentrated on LC-18 cartridges. Raw lake water samples were analyzed without prior treatment.

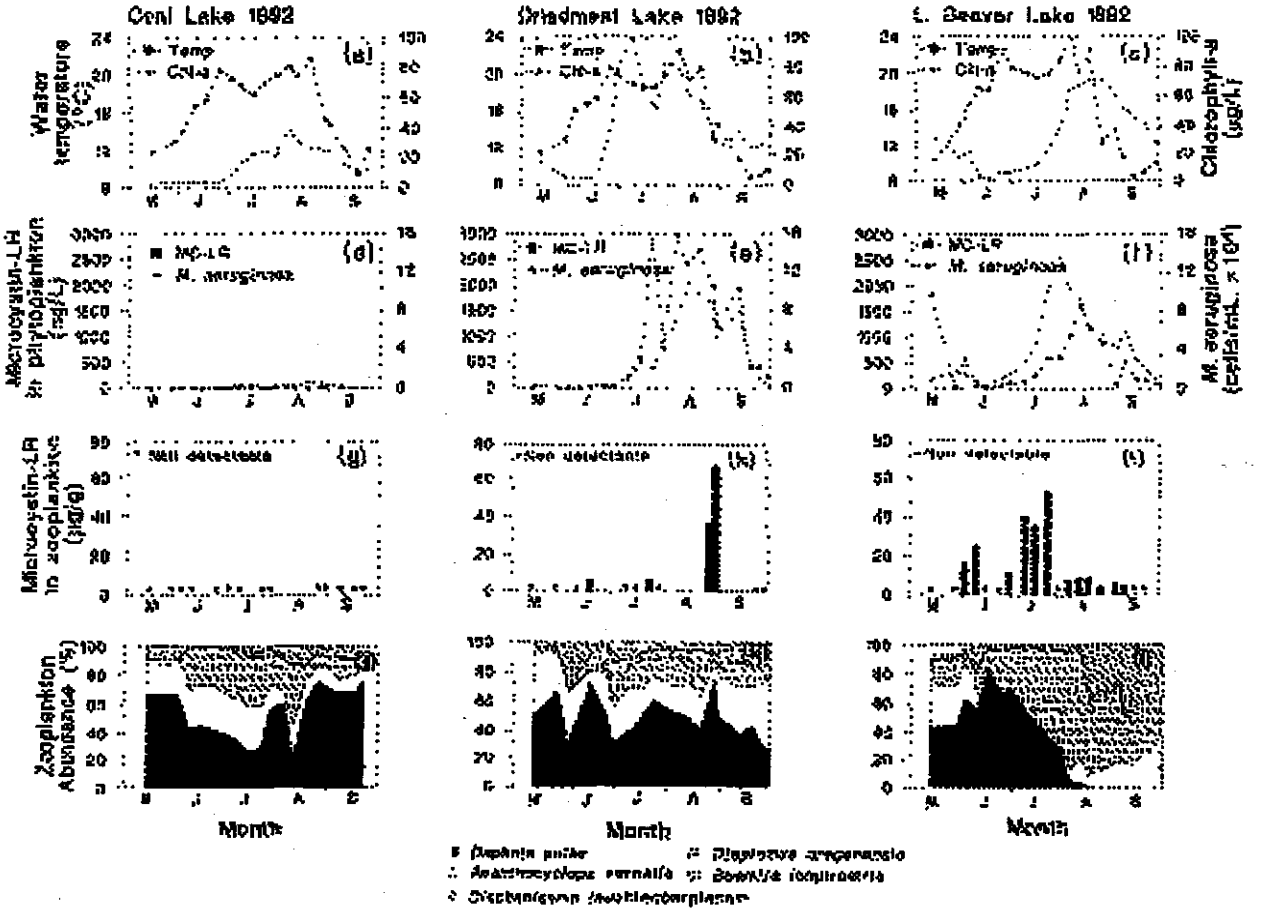
Livers from the fish collected from Driedmeat Lake were homogenized with a mortar and pestle and extracted in 100 mL, of 100% methanol. The methanol was evaporated to approximately 10 mL, and 400 mL of deionized distilled water was added. The sample was then passed through pre-conditioned Supelco C-18 cartridges and the sample eluted in 100% methanol as described earlier. The extract was then filtered through a 0.45-μm Millipore HV filter and injected onto a Waters LC Module I HPLC and run under gradient conditions (described above).

To confirm the presence of MC-LR (and possibly other microcystins) in the various compartments sampled, one sample of phytoplankton (collected from Driedmeat Lake on 17 August 1994), zooplankton (collected from Driedmeat Lake on 11 August 1993), gastropod (*Physa gyrina* collected from Driedmeat Lake on 17 August 1994), and northern pike liver and sucker liver (collected from Driedmeat Lake on 29 and 30 August 1993) were fractionated by HPLC and each fraction analyzed for PP1c inhibition. Samples were run on a Waters LC Module I HPLC under the gradient conditions previously described. Between five and eight (depending on the sample) 0.2- to 1.0-min fractions (i.e., 0.2–1.0 mL fractions) were collected over the 20-min runs. One fraction represented the retention time window where MC-LR was expected to elute from the column, and the other fractions represented other unidentified peaks and also areas in the chromatograms where no peaks occurred. The fractions were then analyzed for the ability to inhibit PP1c. Additionally, the presence of MC-LR in two invertebrate samples (the zooplankton and gastropod identified above) was confirmed by spiking the samples with a known amount of MC-LR standard.

Statistical analysis

MC-LR concentrations in water, phytoplankton, zooplankton and gastropods are also reported as summer means (May to September) (±SE) for the years in which the compartments were sampled.

Fig. 1. Seasonal changes in surface water temperature and chlorophyll *a* (*a–c*), *Microcystis aeruginosa* abundance and MC-LR concentration in the phytoplankton (*d–f*), MC-LR concentration in the zooplankton community (*g–i*), and zooplankton species composition (*j–l*) for Coal (left panels), Driedmeat (middle panels), and Little Beaver (right panels) lakes for 1992. Samples were collected from the 0- to 2-m stratum of the water column in Coal and Driedmeat lakes, and from the 0- to 1-m stratum in Little Beaver Lake. MC-LR was quantified by HPLC.



Correlation analyses were performed on log-transformed data with SPSS for Windows (version 6.0) statistical software.

Results

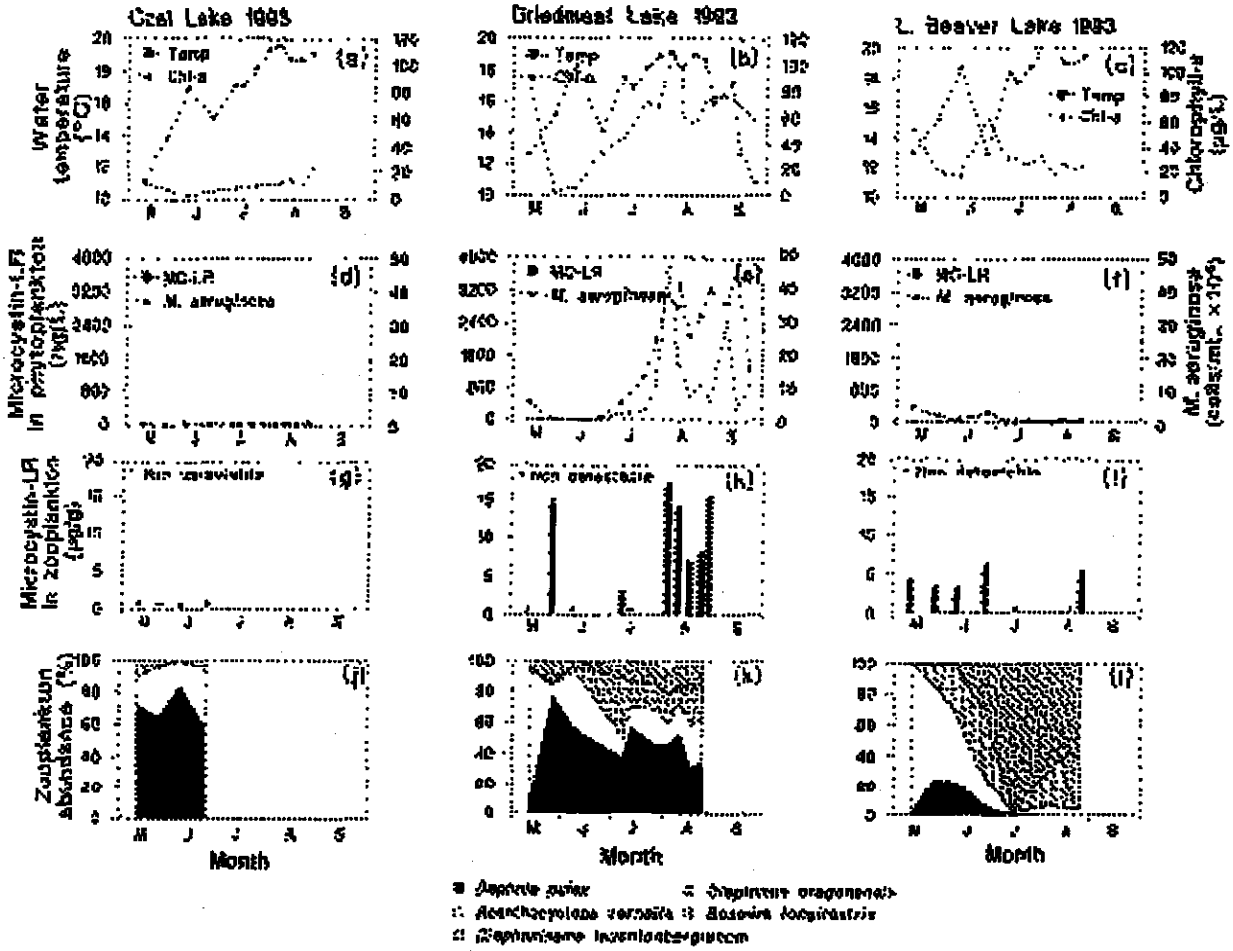
Water quality

In 1994, the only year for which data were collected from all four lakes for this study, Narrow Lake was classified as oligo-mesotrophic based on epilimnetic total phosphorus concentration, and Coal, Driedmeat, and Little Beaver lakes were hypereutrophic but differed substantially from each other (Table 1). Narrow Lake had the most transparent water (Secchi depth averaged 7.2 m in 1994), while Coal, Driedmeat, and Little Beaver lakes were much less transparent, with an average Secchi depth less than 1.3, 1.1, and 0.8 m, respectively. The water in the four lakes ranged from fresh to slightly saline in 1994, and all four lakes contained hard water (Table 1). The hypereutrophic lakes are all shallow (<6 m) and well mixed by the wind while Narrow Lake is deep (maximum depth of 38 m) and thermally stratified during summer.

The three hypereutrophic lakes contained the same pool of cyanobacterial genera over the 3 years and differed markedly from Narrow Lake. From 1992 to 1994 the dominant phytoplankton taxa in the three hypereutrophic lakes was usually cyanobacteria: *Microcystis aeruginosa*, *Aphanizomenon flos-aquae*, *Gomphosphaeria* sp., *Anabaena flos-aquae*, and *Phormidium* sp. (Kotak et al. 1995; Lam 1994). The phytoplankton community in Narrow Lake during 1994 included chlorophytes (*Clamydomonas* sp.), cryptophytes (*Rhodomonas* sp.), and chrysophytes (*Ochromonas* sp.).

Chlorophyll *a* concentrations differed dramatically among study lakes. On the four dates in 1994 when we sampled Narrow Lake, chlorophyll *a* was less than 2 $\mu g \cdot L^{-1}$. Chlorophyll *a* in the three hypereutrophic lakes was lowest in Coal Lake, was fairly consistent among years, and peaked in August (Figs. 1*a*, 2*a*, and 3*a*). Chlorophyll *a* varied most among years in Little Beaver Lake (Figs. 1*c*, 2*c*, and 3*c*), with mean summer chlorophyll *a* of 33 and 30 $\mu g \cdot L^{-1}$ in 1992 and 1993, respectively, and 67 $\mu g \cdot L^{-1}$ in 1994. On 17 August 1994 it exceeded 150 $\mu g \cdot L^{-1}$. Driedmeat Lake generally had the highest chlorophyll *a* concentration of the lakes studied over the 3 years: mean summer concentration of

Fig. 2. Seasonal changes in surface water temperature and chlorophyll *a* (*a–c*), *Microcystis aeruginosa* abundance and MC-LR concentration in the phytoplankton (*d–f*), MC-LR concentration in zooplankton community (*g–i*), and zooplankton species composition (*j–l*) for Coal (left panels), Driedmeat (middle panels), and Little Beaver (right panels) lakes for 1993. Samples were collected from the 0- to 2-m stratum of the water column in Coal and Driedmeat lakes, and from the 0- to 1-m stratum in Little Beaver Lake. MC-LR was quantified by HPLC.



51 $\mu g \cdot L^{-1}$ for all 3 years, with maxima for chlorophyll *a* over 100 $\mu g \cdot L^{-1}$ in July and August of 1992 and 1993 (Figs. 1*b* and 2*b*).

Microcystin-LR in phytoplankton

MC-LR concentration in the phytoplankton was highest in the two most hypereutrophic lakes (Driedmeat and Little Beaver). MC-LR concentration in the phytoplankton from Driedmeat Lake was highest from August to early September (1212 – 2987 $ng \cdot L^{-1}$ in 1992 and 2093 – 3780 $ng \cdot L^{-1}$ in 1993; Figs. 1*e* and 2*e*) and highest near the end of August in 1994 (5778 to 6069 $ng \cdot L^{-1}$ Fig. 3*e*). Peak MC-LR concentration in Driedmeat Lake phytoplankton corresponded to periods when *M. aeruginosa* abundance was at its seasonal high (usually greater than 200 000 cells·mL^{-1} in August; Figs. 1*e*, 2*e*, and 3*e*). Both *M. aeruginosa* abundance and MC-LR concentration in the phytoplankton also differed substantially among the 3 years of study in the two lakes. For example, in Little Beaver Lake, mean summer *M. aeruginosa* abundance and MC-LR concentration in phytoplankton was 43 024 cells·mL^{-1} and 488 $ng \cdot L^{-1}$, respectively, in 1992, decreased 9- and 6-fold, respectively, in 1993, and in 1994 increased almost 15- and 57-fold relative to 1993 (Figs. 2*f* and 3*f*). The highest MC-LR concentration measured during the 3 years was in phytoplankton from Little Beaver Lake (11 216 $ng \cdot L^{-1}$) on 17 August 1994. Both *M. aeruginosa* and MC-LR were virtually absent in the phytoplankton from Coal Lake in all 3 years (Figs. 1*d*, 2*d*, and 3*d*). *Microcystis aeruginosa* was absent from Narrow Lake on three of four sampling dates; on 20 September there were a few cells (1221 cells·mL^{-1}). MC-LR was not detected in phytoplankton samples from Narrow Lake. Seasonal changes in MC-LR concentration in the phytoplankton from all lakes tracked changes in *M. aeruginosa* abundance closely: the two were highly correlated ($r = 0.67$, $P < 0.0001$, df = 121).

Microcystin in lake water

Aqueous microcystin concentration in lake water (expressed as MC-LR equivalents) was highest in the two hypereutrophic lakes. In Driedmeat Lake, aqueous microcystin

Fig. 3. Seasonal changes in surface water temperature and chlorophyll *a* (*a–c*), *Microcystis aeruginosa* abundance and MC-LR concentration in the phytoplankton (*d–f*). MC-LR concentration in three gastropod species (*g–i*) for Coal (left panels), Driedmeat (middle panels), and Little Beaver (right panels) lakes for 1994. Except for gastropods, samples were collected from the 0- to 2-m stratum of the water column in Coal and Driedmeat lakes, and from the 0- to 1-m stratum in Little Beaver Lake. Asterisks in Figs. 3*g–3i* indicate nondetectable MC-LR (<1 $\mu g \cdot g^{-1}$ MC-LR) in all three gastropod species. MC-LR was quantified by HPLC.

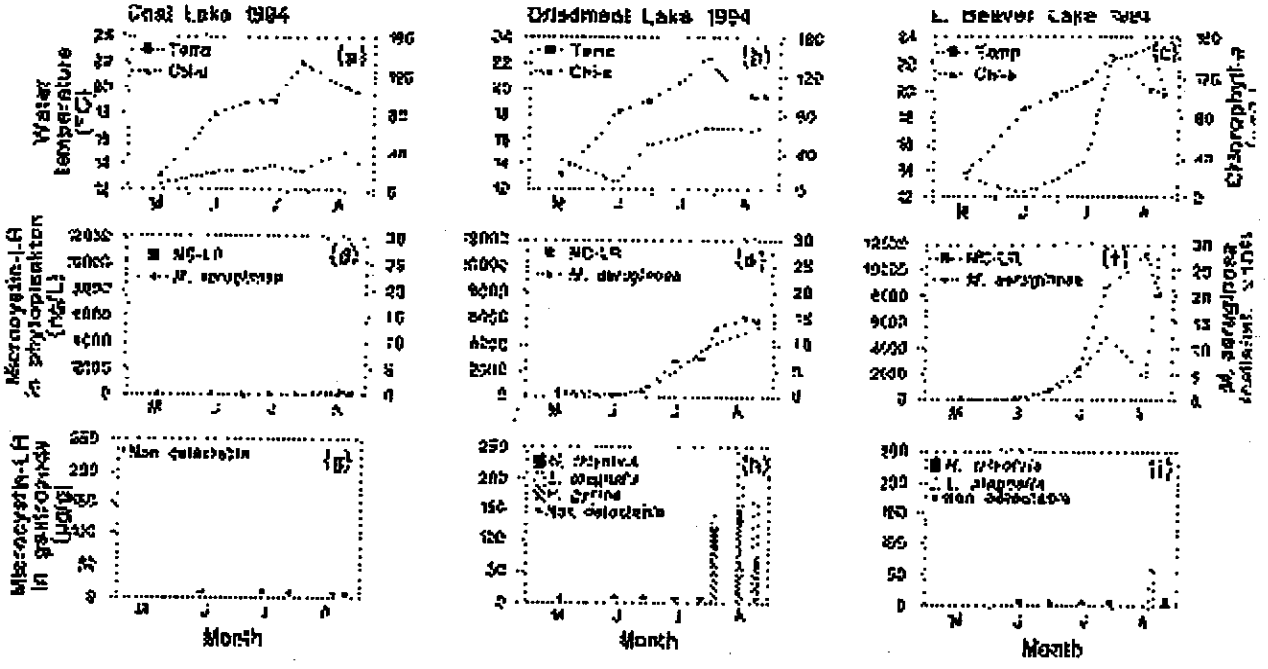
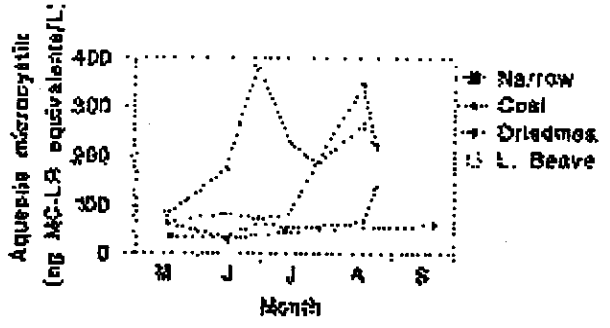


Fig. 4. Seasonal changes in aqueous concentration of total microcystin in the upper 2 m of the water column (Coal, Driedmeat, and Narrow lakes) or the upper 1 m of the water column (Little Beaver Lake) during 1994. Total microcystin concentration was determined by protein phosphatase assay and is expressed as MC-LR equivalent units per litre.



increased over the summer, peaking at 344 $ng \cdot L^{-1}$ in mid-August (Fig. 4). The trend of increasing microcystin concentration in the water paralleled the trend of increasing MC-LR in the phytoplankton in Driedmeat Lake ($r = 0.89$, $P = 0.006$, df = 5; Figs. 4 and 3*e*). In contrast, microcystin concentration in the water from Little Beaver Lake in 1994 peaked on 29 June (284 $ng \cdot L^{-1}$) when MC-LR concentration in the phytoplankton was low (Figs. 4 and 3*f*). Aqueous microcystin concentration was close to our detection limit in Narrow and Coal lakes, generally below 60 $ng \cdot L^{-1}$ (Fig. 4). Aqueous microcystin concentrations for all lakes combined were correlated to MC-LR in the phytoplankton ($r = 0.83$, $P < 0.001$, df = 24).

Zooplankton

MC-LR was detected by HPLC in 27 of the 68 zooplankton samples; all collected from Driedmeat and Little Beaver lakes (the lakes containing the highest MC-LR concentrations in phytoplankton and water). MC-LR concentrations in the zooplankton communities peaked in August in Driedmeat Lake (Figs. 1*h* and 2*h*) but was highest from mid-May to late August in the Little Beaver Lake (Figs. 1*i* and 2*i*). The peaks in MC-LR concentration in the zooplankton communities coincided with peak MC-LR in phytoplankton ($r = 0.69$, $P < 0.001$, df = 66), although there were occasions where peak MC-LR in zooplankton did not correspond to maximum MC-LR concentration in the phytoplankton (Figs. 2*e* and 2*h*).

Substantive seasonal shifts in zooplankton community composition occurred in the lakes. In Little Beaver Lake, these shifts occurred following periods when MC-LR concentration was at its highest in the zooplankton community. MC-LR concentration decreased in the zooplankton during these shifts. For example, up to a 17-fold decrease in MC-LR concentration in the zooplankton community (Fig. 1*j*) coincided with a shift from *Daphnia pulex* (in June and July 1992) to *Diaptomus oregonensis* and *Diaphanisoma leuchtenbergianom* in July and August 1992 (Fig. 1*l*). In contrast, similar shifts in zooplankton community structure were not observed in Driedmeat Lake in 1992 and 1993 (Figs. 1*k* and 2*k*, respectively). In addition, no single zooplankton species was dominant during periods when MC-LR concentration in the zooplankton community peaked.

For the majority of zooplankton samples (i.e., 61 of 68 samples) there was agreement between total microcystin and MC-LR concentrations determined by PP1c assay and HPLC, respectively . These results are consistent with observations that the major microcystin analogues produced by phytoplankton in Alberta lakes is MC-LR (Kotak et al. 1993; Boland et al. 1993; Craig et al. 1993). However, in a small number of the samples, other microcystins may have been present.

Macroinvertebrates

MC-LR was detected in three species of gastropods (*Lymnaea stagnalis*, *Helisoma trivolvis*, and *Physa gyrina*) collected from Driedmeat and Little Beaver lakes, but only in August (Figs. 3*h* and 3*i*), when MC-LR in the phytoplankton was at its highest (Figs. 3*e* and 3*f*). MC-LR was not detected in gastropods collected from Coal and Narrow lakes or in any other macroinvertebrates. On 3 and 17 August in Driedmeat Lake, *P. gyrina* had the highest MC-LR concentration (90 and 121 $\mu g \cdot g^{-1}$, respectively), while on 23 August, *L. stagnalis* had the highest concentration (96 $\mu g \cdot g^{-1}$). In Little Beaver Lake, MC-LR was detected only once in *L. stagnalis* (on 17 August; 54 $\mu g \cdot g^{-1}$) and *H. trivolvis* (on 23 August; 11 $\mu g \cdot g^{-1}$). However, no samples of *H. trivolvis* and *P. gyrina* on 17 August and *L. stagnalis* and *P. gyrina* on 23 August were collected. The nondetectable values, indicated by an asterisk in Figs. 3*h* and 3*i*, represent nondetectable MC-LR for all three gastropod species. Detectable MC-LR concentrations in the gastropods from Driedmeat Lake also coincided with high aqueous total microcystin in the lake water (Fig. 4). However, the same was not true in Little Beaver Lake, where MC-LR was nondetectable in gastropods even when total dissolved microcystin concentration was highest (from late June onward). Therefore, MC-LR detected in gastropods likely originated from the phytoplankton, not from toxin taken up from an aqueous source.

Fish

Northern pike and white sucker were collected from Driedmeat Lake after MC-LR had been at its highest in the phytoplankton for almost 1 month (mean 2849 $ng \cdot L^{-1}$ from 4 to 30 August; Fig. 2*e*). Eight livers from the northern pike and 23 from white sucker were extracted and analyzed for MC-LR by HPLC. MC-LR was not detected in any of the livers from the two species of fish, despite stomach contents that suggested that the fish should be exposed to the toxin through an oral route. For example, the stomachs of the northern pike contained gastropods (which were collected from Driedmeat Lake on the same days as the fish and contained 58 $\mu g \cdot g^{-1}$ MC-LR equivalents by PP1c assay) as did the stomachs of the white sucker, in addition to unidentified phytoplankton.

HPLC-linked PP1c bioassay

To confirm the presence of MC-LR and possibly other microcystins in fish liver, phytoplankton, and invertebrate samples, HPLC-linked PP1c bioassay was used (Figs. 5 and 6). MC-LR was not detected in the fish livers by HPLC or HPLC-linked PP1c assay. In the phytoplankton, zooplankton, and gastropod samples, the main peak in the HPLC chromatogram (and the one with the highest PP1c inhibition) always corresponded to the retention time of MC-LR (Figs. 5 and 6). For example, the HPLC chromatogram for the Driedmeat Lake phytoplankton sample contained one major peak with several smaller peaks (Fig. 5*a*). The largest peak in the chromatogram, with a retention time of 8.60 min, corresponded to the retention time of the MC-LR standard (Fig. 5*b*). Four HPLC fractions inhibited PP1c (Fig. 5*a*). The fraction with the greatest inhibition corresponded to the MC-LR peak. In addition, another peak, with a retention time of 9.07 min also inhibited PP1c. Two other peaks, both preceding MC-LR, had much lower PP1c inhibition (Fig. 5*a*). Therefore, the major microcystin in the phytoplankton was likely MC-LR, although another peak (likely another microcystin) also inhibited PP1c. We have previously shown by fast atom bombardment – mass spectrometry, that the peak in phytoplankton samples corresponding to the retention time of MC-LR standard, has the same molecular mass as that of MC-LR (Kotak et al. 1993).

To further confirm the presence of MC-LR in the zooplankton and gastropod samples, each sample was spiked with MC-LR and rerun on the HPLC (Fig. 6). The peak area of the presumed MC-LR peak in the zooplankton and gastropod samples increased by 1.78- and 1.59-fold, respectively, a recovery of 98.7 and 99.6%, respectively, of the added MC-LR standard. This suggested that the original peak in question in the two samples was MC-LR.

Discussion

Few studies have examined whether cyanobacterial toxins can accumulate in aquatic food webs, at what concentrations they are present in the various compartments, and how the trophic status of a water body may influence toxin concentrations. In companion studies, we have shown that microcystin-LR (MC-LR) concentration in phytoplankton can be highly dynamic, reflecting, at least partially, changes in the abundance or biomass of *M. aeruginosa* within a lake, temporally within and between years, and even over 24 h (Kotak et al. 1993, 1995). Therefore, the occurrence of MC-LR in food web compartments will depend on the occurrence and biomass of *M. aeruginosa* in the phytoplankton. Our data indicate that lakes of low productivity (such as oligo-mesotrophic Narrow Lake) and even some hypereutrophic lakes (such as Coal Lake) may have a low potential for supporting microcystin-producing phytoplankton. Kotak (1995) has shown that consistently low MC-LR concentrations in Coal Lake phytoplankton may be related to seasonal total nitrogen to total phosphorus (TN:TP) ratios that exceed 5:1, whereas higher MC-LR concentrations in Driedmeat and Little Beaver lakes correspond to TN:TP ratios that are often less than 5:1. Trophic status, therefore, likely will influence the occurrence and concentration of MC-LR in aquatic food webs by restricting the distribution of *M. aeruginosa*.

Aqueous microcystin concentration and MC-LR concentration in the phytoplankton were both highest in Driedmeat and Little Beaver lakes. Microcystins are considered endotoxins because the majority of the toxin is found within the cells. When the cell wall of the cyanobacterium is compromised, as a result of chemical treatment (Kenefick et al 1993; Lam and Prepas 1995; Lam et al. 1995*a*, 1995*b*) or natural senescence (Berg et al. 1987), microcystins are released into the water. Despite the much higher MC-LR concentrations in the

Fig. 5. HPLC chromatogram of phytoplankton collected from Driedmeat Lake on 17 August 1994 (*a*) and MC-LR standard (*b*). Analysis was on a Waters LC I HPLC with UV detection at 238 nm. The sample and standard were run under linear gradient conditions (20–30% acetonitrile in 10 mM ammonium acetate in 10 min, then 20% from 10 min to the end of the run (30 min)). Boxes in (*a*) indicate fractions collected for protein phosphatase inhibition. Values above the boxes indicate microcystin concentration of the fraction, as quantified by protein phosphatase assay, and are expressed as nanograms MC-LR equivalents per millilitre. Values <1 ng·mL^{-1} are considered nondetectable.

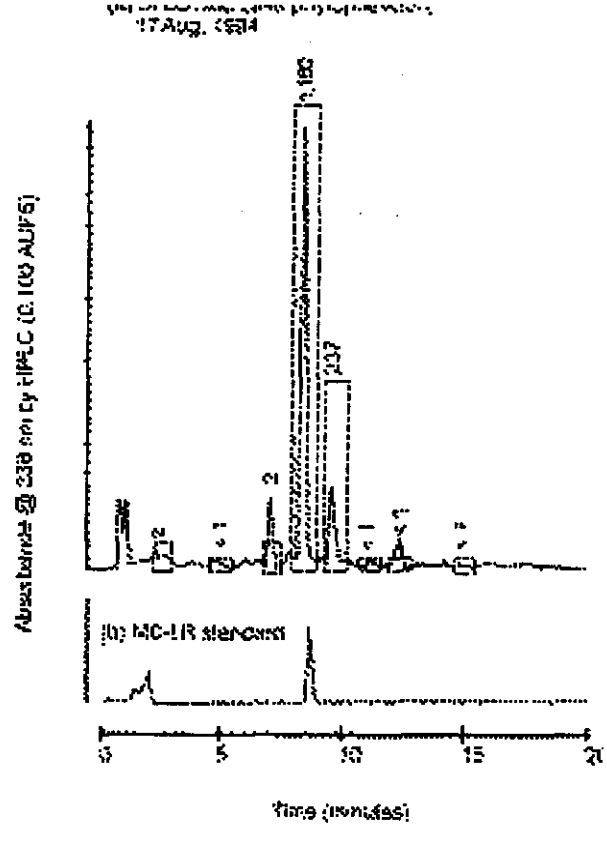
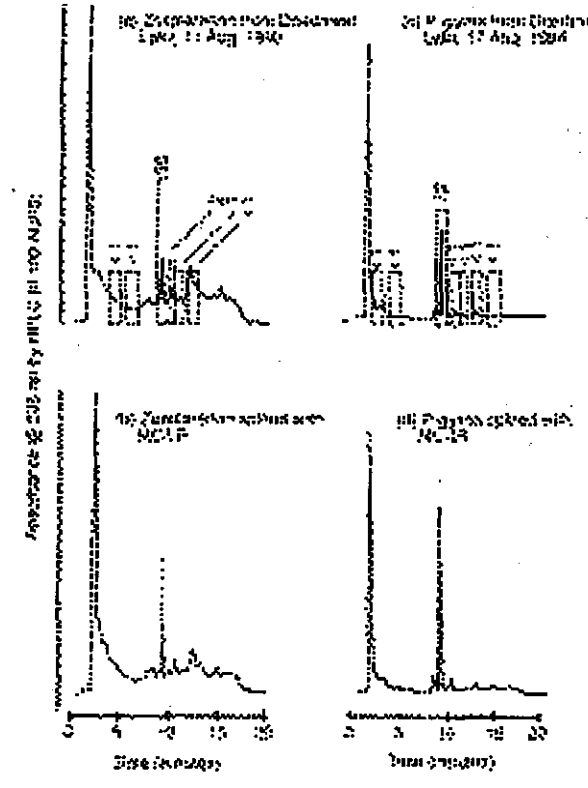


Fig. 6. HPLC chromatogram of zooplankton collected from Driedmeat Lake on 11 August 1993 (*a*), the above zooplankton sample spiked with MC-LR standard (*b*), *Physa gyrina* (gastropod) sample collected from Driedmeat Lake on 17 August 1994 (*c*), and the above gastropod sample spiked with MC-LR (*d*). Analysis was on a Waters LC I HPLC with UV detection at 238 nm. The sample and standard were run under linear gradient conditions (20–30% acetonitrile in 10 mM ammonium acetate in 10 min, then 20% from 10 min to the end of the run (30 min)). Boxes in Figs. 6*a* and 6c indicate fractions collected for protein phosphatase inhibition. Values above the boxes indicate microcystin concentration of the fraction, as quantified by protein phosphatase assay, and are expressed as nanograms MC-LR equivalents per millilitre. Values <1 ng·mL^{-1} are considered nondetectable.



phytoplankton in Little Beaver than in Driedmeat Lake, concentrations of aqueous microcystin in the two lakes were similar. Degradation by microbial action (Lam et al. 1995*a*) and (or) photolysis (Tsuji et al. 1995), as well as dilution, may account for the similar concentrations of aqueous microcystin in these two lakes.

Aquatic invertebrates analyzed for MC-LR by HPLC covered a diversity in trophic feeding levels, from detritivores (such as *Gammarus lacustris* and *Chironomus* spp.), herbivores (zooplankton such as *Daphnia pulex*, *Bosmina longirostris*, and *Diaphanisoma leuchtenbergianom* and gastropods such as *Physa gyrina*, *Lymnaea stagnalis*, and *Helisoma trivolvis*) to predators (zooplankton such as *Acanthocyclops vernalis* and the predacious water beetle, *Dytiscus* sp.). However, MC-LR was only detected in zooplankton communities and gastropods (i.e., in the grazers). In general, the highest MC-LR concentrations in the zooplankton and gastropods corresponded fairly well to high concentrations in the phytoplankton although there were exceptions. The correlation between MC-LR concentration in the zooplankton and phytoplankton (r = 0.69) suggests that MC-LR may be present in the zooplankton as result of filter feeding on the toxin-containing *M. aeruginosa* cells. If so, there should be a relationship between the abundance of herbivorous grazers and MC-LR in the zooplankton. MC-LR concentration in the zooplankton was correlated to the relative abundance of all the grazers combined (*D. pulex*. *B. longirostris*, *D. leuchtenbergianom*, and *D. oregonensis*, r = 0.35, $P < 0.05$, df = 36) but not to individual species ($P > 0.09$).

It is possible that accumulation of MC-LR in zooplankton may also occur by uptake for the toxin in aqueous form, directly from the water. Unfortunately, this hypothesis cannot

be addressed in our study as water and zooplankton samples for MC-LR were not collected in the same years. However, the absence of detectable MC-LR in macroinvertebrates (e.g., damselfly and dragonfly larvae, chironomids) collected in 1994 from Driedmeat and Little Beaver lakes (the lakes with the highest aqueous microcystin concentrations), suggests that either MC-LR is not taken up from the water, or that given its high water solubility, the toxin is rapidly eliminated. DeMott et al. (1991) found that purified, aqueous MC-LR was lethal to *Daphnia pulicaria*, *D. hyalina*, *D. pulex*, and *Diaptomus birgei* in laboratory experiments, and thus, MC-LR must be taken up from the water. However, concentrations of MC-LR used in their trials were unrealistically high (500–50 000 $\mu g \cdot L^{-}$) compared with the total aqueous microcystin concentrations reported in our study lakes (maximum concentration of 0.34 $\mu g \cdot L^{-1}$). Given this evidence, we believe that the MC-LR detected in the zooplankton and gastropods from the lakes in the present study was toxin that was present in the ingested *M. aeruginosa* cells.

Only one other study has quantified microcystin concentrations in freshwater zooplankton communities in situ. Watanabe et al. (1992) detected concentrations of microcystins -LR, -YR, and -RR from 75 to 1387 $\mu g \cdot g^{-1}$ of the three analogues in zooplankton samples collected from Lake Kasumiguara, Japan. These concentrations are up to 9 times higher than the highest total microcystin concentration detected by PP1c and up to 20 times higher than the highest MC-LR concentration detected by HPLC from zooplankton collected during the present study. Watanabe et al. (1992) suggested that *Bosmina fatalis* was the principal zooplankton species responsible for accumulation of the three microcystin variants, although they did not attempt to quantify the relationship between microcystin concentrations and zooplankton species composition.

It is not known whether the concentrations of MC-LR detected in the zooplankton from the two lakes were sufficient to cause mortality in the various zooplankton species and, hence, cause changes in zooplankton community composition. Given the results of DeMott et al. (1991), it is unlikely that the aqueous microcystin concentrations in Driedmeat and Little Beaver lakes would cause mortality in any of the zooplankton species found. In addition, high MC-LR concentrations in the zooplankton in Driedmeat Lake during late August in 1992 (Fig. 1*h*) and during late May and throughout August in 1993 (Fig. 2*h*) were not proceeded by major shifts in zooplankton species composition (Figs. 1*k* and 2*k*, respectively). Therefore, it did not appear that the high MC-LR burdens resulted in a change in zooplankton species composition in Driedmeat Lake. However, large shifts in zooplankton species composition did occur in Little Beaver Lake in 1992 and 1993 following peak MC-LR concentrations in the zooplankton (Fig. 1*i*, 1*l*, 2*i*, and 2*l*). The relative importance of MC-LR in affecting zooplankton community composition, however, is unknown. It has been suggested that cyanobacterial toxins are produced as a defense mechanism against grazing pressure exerted by herbivorous zooplankton (Lampert 1981; DeMott and Moxter 1991) and, therefore, may control zooplankton competitive relations and species composition (Fulton and Paerl 1988). In response, the zooplankton have in turn evolved mechanisms to reduce their susceptibility to the toxins. These mechanisms include increased physiological resistance to the toxins themselves (DeMott et al. 1991), reduction in filter feeding activity (Lampert 1981, 1982), and rejection of toxic forms over nontoxic forms after the cells have been captured (DeMott and Moxter 1991). Any one of these mechanisms could help explain the variable concentrations of MC-LR detected in the zooplankton from Driedmeat and Little Beaver lakes and changes in zooplankton species composition.

MC-LR that was detected in three species of gastropods collected from Driedmeat and Little Beaver lakes in 1994 (Figs. 3h i) was also likely a result of grazing activities. The changes in MC-LR concentration in *Lymnaea stagnalis* and *Physa gyrina* roughly followed the changes in MC-LR concentration in the phytoplankton. In contrast, MC-LR concentration in *Helisoma trivolvis* was highest on the first sampling date in August and declined thereafter (Fig. 3*h*). On the last sampling date, 23 August, many of the *H. trivolvis* were either dead or appeared unhealthy. It is unknown whether the high MC-LR concentrations in the phytoplankton were responsible for the large number of dead *H. trivolvis* on this date. Gastropods in general are not considered filter feeders (Calow 1970; Elder and Collins 1991), preferring to graze on periphyton (attached algae) which adhere to the surface of macrophytes. The source of the MC-LR in the gastropods in the present study is likely from *M. aeruginosa* cells and colonies that settled out of the water column and became trapped in the abundant macrophytes in Driedmeat and Little Beaver lakes. The gastropods were almost always associated with dense growths of *Potamogeton pectinatis*, *P. richardsonii*, and *Myriophyllum spicatum*, which had dense films of *M. aeruginosa* on their surfaces.

Only one study has examined microcystin accumulation in macroinvertebrates. Erikkson et al. (1989) examined the accumulation of a microcystin analogue (detected by HPLC) by the freshwater mussel, *Anodonta cygnea*, exposed to a hepatotoxic laboratory culture of the cyanobacterium *Oscillatoria agardhii*. Although the mussels accumulated high concentrations of the microcystin in the tissue (70 $\mu g \cdot g^{-1}$ after a 15-day exposure), they were also exposed to high concentrations of the microcystin in pure culture (2000–3000 μg toxin$\cdot g^{-1}$ or 40–60 μg cellular toxin$\cdot L^{-1}$). Gastropods from our field study were exposed to much lower concentrations of MC-LR. The highest concentration of MC-LR in the phytoplankton during our study was 11.2 $\mu g \cdot L^{-1}$. Therefore, it may be difficult to extrapolate the results of Eriksson et al. (1989) to natural lake settings.

Accumulation of other protein phosphatase inhibitors have also been reported in marine and brackish-water environments. Nodularin, a cyanobacterial hepatotoxin, was detected in blue mussels (*Mytilus edulis*) during a hepatotoxic bloom the cyanobacterium *Nodularia spumigena* in Peel–Harvey estuary, Western Australia (Falconer et al. 1992). Gut contents of the mussels contained filaments of *Nodularia spumigena*, and the highest toxicity (detected by mouse bioassay) was associated with the gastrointestinal tract of the mussels. No toxicity was observed for non-intestinal tissues. Additionally, motuporin (a derivative of nodularin) was isolated from the marine sponge *Theonella swinhoei* (deSilva et al. 1992), diarrhetic shellfish poisons okadaic acid and dinophysitoxin-1

from marine shellfish (Holmes 1991) and MC-LR from crab larvae, copepods, amphipods, mussels, and pen-reared Atlantic salmon afflicted with netpen liver disease (Andersen et al. 1993; Chen et al. 1993).

MC-LR was not detected in the livers of fish collected from Driedmeat Lake, despite the fish potentially being exposed for more than 1 month in 1993 to high concentrations of MC-LR in the phytoplankton, aqueous microcystin, and MC-LR in food items (e.g., gastropods). Most of the microcystins in the fish livers were likely covalently bound to protein phosphatases (Toivola et al. 1994) and, therefore, would not be detected by our current HPLC method. The absence of detectable microcystin in the liver (or in the flesh or any other organ; data not shown) does not imply that the fish were not exposed to the toxins or that the toxin had no health effect on the fish. Binding of any microcystin to protein phosphatases in the liver is a pathological result in itself. This would likely be manifested as focal, multifocal, or massive zones of coagulative or liquifactive hepatocyte necrosis, as is seen in rainbow trout that have been exposed to MC-LR by intraperitoneal injection (Phillips et al. 1985; Kotak et al. 1996). Although long-term (i.e., >96 h) exposure of fish to microcystins has not been studied, we would hypothesize that chronic exposure of natural fish populations to microcystins may lead to severe necrosis of hepatocytes, loss of liver function, and perhaps liver tumour promotion or mortality. Such physiological stress would clearly have an impact on growth rates in the fish.

There has been one study published that has confirmed that microcystins can have a substantive and, at times, severe impact on commercially important fish populations. Kent et al. (1988) and Kent (1990) reported severe liver lesions, which they termed netpen liver disease (NLD), and mortality in netpen-reared Atlantic salmon since 1986. The liver lesions (Kent et al. 1988; Kent 1990) were identical to those caused by intraperitoneal exposure of the salmon to MC-LR in laboratory experiments (Andersen et al. 1993). The disease in fish held in netpens was not related to diet, could not be experimentally transmitted (therefore, was not due to viral or bacterial action), was not related to anthropogenic contamination and became progressively worse over the summer in each year. Subsequently, Andersen et al. (1993) successfully isolated a compound that was chromatographically indistinguishable from MC-LR from the livers of salmon inflicted with NLD and from biota fouling the netpens, utilizing HPLC-linked PP1c bioassay. MC-LR was not detected in the livers of fish not afflicted with NLD. The more sophisticated extraction and cleanup steps used in the isolation and purification of MC-LR from the salmon livers (including semi-preparative HPLC followed by the use of a microbore analytical column) compared with our method of sample extraction and cleanup and the much greater mass of liver tissue extracted (22.7 g dry weight versus 2–3 g in the present study) may explain why we did not detect MC-LR in the livers of northern pike and white sucker. Andersen et al. (1993) is the first study to document a direct (and severe) effect of MC-LR on fish populations in the field. They suggested that the fish were exposed to the MC-LR via feeding on organisms fouling the netpens.

In summary, the potential for MC-LR to occur in organisms of aquatic food webs in our study lakes was related to the trophic status of the water body, strongly influenced by the occurrence and abundance of the toxin producer (*M. aeruginosa* in this case). MC-LR, accumulated in herbivorous invertebrates, could potentially affect the health of organisms at higher trophic feeding levels (e.g., fish). Although not studied to date, there is also a strong possibility of transfer of MC-LR from aquatic food webs to terrestrial food webs, by waterfowl and shorebirds, for example, feeding on contaminated gastropods and zooplankton. Accidental ingestion of cyanobacterial hepatotoxins in drinking water has repeatedly caused poisonings and death in animals worldwide (Schwimmer and Schwimmer 1968; Edler et al. 1985). Given this scenario, more attention needs to be directed to the ecology of toxin-producing cyanobacteria and the role of the toxins in food webs.

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References

American Public Health Association (APHA), American Water Works Association (AWWA), and Water Pollution Control Federation (WPCF). 1992. Standard methods for the examination of water and wastewater. 19th ed. American Public Health Association, American Water Works Association, and Water Pollution Control Federation, Washington, D.C.

Andersen, R.J., Luu, H.A., Chen, D.Z.X., Holmes, C.F.B., Kent, M.L., LeBlanc, M., Taylor, F.J.R., and Williams, D.E. 1993. Chemical and biological evidence links microcystin-LR to salmon netpen liver disease. Toxicon, **31**: 1315–1323.

Berg, K., Skulberg, O.M., and Skulberg, R. 1987. Effects of decaying toxic blue-green algae on water quality—a laboratory study. Arch. Hydrobiol. **108**: 549–563.

Bergmann, M., and Peters, R.H. 1980. A simple reflectance method for the measurement of particulate pigment in lake water and its application to phosphorus–chlorophyll–seston relationships. Can. J. Fish. Aquat. Sci. **37**: 111–114.

Boland, M.P., Smillie, M.A., Chen, D.Z.X., and Holmes, C.F.B. 1993. A unified bioscreen for the detection of diarrhetic shellfish toxins and microcystins in marine and freshwater environments. Toxicon, **31**: 1393–1405.

Calow, P. 1970. Studies on the natural diet of *Lymnaea pereger obtusa* (Kobelt) and its possible ecological implications. Proc. Malacol. Soc. London, **39**: 203–215.

Carmichael, W.W. 1992. Cyanobacteria secondary metabolites—the cyanotoxins. J. Appl. Bacteriol. **72**: 445–459.

Chen, D.Z.X., Boaland, M.P., Smillie, M.A., Klix, H., Ptak, C., Andersen, R.J., and Holmes, C.F.B. 1993. Identification of pro-

tein phosphatase inhibitors of the microcystin class in the marine environment. Toxicon, **31**: 1407–1414.

Clifford, H. 1993. Aquatic invertebrates of Alberta. University of Alberta Press. Edmonton, Alta.

Craig, M., McCready, T.L., Luu, H.A., Smillie, M.A., Dubord, P., and Holmes, C.F.B. 1993. Identification and characterization of hydrophobic microcystins in Canadian freshwater cyanobacteria. Toxicon, **31**: 1541–1549.

DeMott, W.R., and Moxter, F. 1991. Foraging on cyanobacteria by copepods: responses to chemical defenses and resource abundance. Ecology, **72**: 1820–1834.

DeMott, W.R., Zhang, Q.-X., and Carmichael, W.W. 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.

deSilva, E.D., Williams, D.E., Andersen, R.J., Klix, H., Holmes, C.F.B., and Allen, T.M. 1992. Motuporin, a potent protein phosphatase inhibitor isolated from the Papua New Guinea sponge *Theonella swinhoei* Gray. Tetrahedron Lett. **33**: 1561–1564.

Edler, L., Ferno, S., Lind, M.G., Lundberg, R., and Nilsson, P.O. 1985. Mortality of dogs associated with a bloom of the cyanobacterium *Nodularia spumigena* in the Baltic Sea. Ophelia, **24**: 103–109.

Elder, J.F., and Collins, J.J. 1991. Freshwater molluscs as indicators of bioavailability and toxicity of metals in surface-water systems. Rev. Environ. Contam. Toxicol. **122**: 37–79.

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

Falconer, I.R., Choice, A., and 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.

Fulton, R.S., III., and Paerl, H.W. 1988. Effects of the blue-green alga *Microcystis aeruginosa* on zooplankton competition relations. Oecologia, **76**: 383–389.

Gilbert, J.J. 1994. Susceptibility of planktonic rotifers to a toxic strain of *Anabaena flos-aquae*. Limnol. Oceanogr. **39**: 1286–1297.

Holmes, C.F.B. 1991. Liquid chromatography-linked protein phospatase bioassay: a highly sensitive marine bioscreen for okadaic acid and related diarrhetic shellfish toxins. Toxicon, **29**: 469–477.

Jungmann, D., and Benndorf, J. 1994. Toxicity to *Daphnia* of a compound extracted from laboratory and natural *Microcystis* spp., and the role of microcystins. Freshwater. Biol. **32**: 13–20.

Kenefick, S.L., Hrudey, S.E., Peterson, H.G., and Prepas, E.E. 1993. Toxin release from *Microcystis aeruginosa* after chemical treatment. Water Sci. Technol. **27**: 433–40.

Kent, M.L. 1990. Netpen liver disease (NLD) of salmonid fishes reared in sea water: species susceptibility, recovery, and probable cause. Dis. Aquat. Org. **8**: 21–28.

Kent, M.L., Meyers, M.S., Hinton, D.E., Eaton, W.D., and Elston, R.A. 1988. Suspected toxicopathic hepatic necrosis and megalocytosis in pen-reared Atlantic salmon *Salmo salar* in Puget Sound, Washington, USA. Dis. Aquat. Org. **4**: 91–100.

Kotak, B.G. 1995. Concentration and toxicity of microcystin-LR in compartments and organisms of aquatic food webs. Ph.D. thesis, University of Alberta, Edmonton, Alta.

Kotak, B.G., Kenefick, S.L., Fritz, D.L., Rousseaux, C.G., Prepas, E.E., and Hrudey, S.E. 1993. Occurrence and toxicological evaluation of cyanobacterial toxins in Alberta surface waters. Water Res. **27**: 495–506.

Kotak, B.G., Lam, A.K.-Y., Prepas, E.E., Hrudey, S.E., and Kenefick, S.L. 1995. Variability of the hepatotoxin, microcystin-LR, in hypereutrophic drinking water lakes. J. Phycol. **31**: 248–263.

Kotak, B.G., Semalulu, S., Fritz, D., Prepas, E., Hrudey, S., and Coppock, R. 1996. Hepatic and renal pathology of intraperitoneally-administered microcystin-LR in rainbow trout (*Oncorrhynchus mykiss*). Toxicon. **34**: 517–525.

Lam, A.K-Y. 1994. Occurrence, control and persistence of the cyanobacterial toxin, microcystin-LR. M.Sc. thesis, University of Alberta, Edmonton, Alta.

Lam, A.K.-Y., and Prepas, E.E. 1995. *In situ* evaluation of options for chemical treatment of hepatotoxic cyanobacterial blooms. Can. J. Fish. Aquat. Sci. **52**:

Lam, A.K.-Y., Fedorak, P.M., and Prepas, E.E. 1995*a*. Biotransformation of the cyanobacterial hepatotoxin microcystin-LR, as determined by HPLC and protein phosphatase bioassay. Environ. Sci. Technol. **29**: 242–246.

Lam, A.K.-Y., Prepas, E.E., Spink, D., and Hrudey, S.E. 1995*b*. Chemical control of hepatotoxic phytoplankton blooms: implications for human health. Water Res. **29**: 1845–1854.

Lampert, W. 1981. Toxicity of the blue-green alga *Microcystis aeruginosa*: effective defense mechanism against grazing pressure by *Daphnia*. Verh. Int. Ver. Theor. Angew. Limnol. **21**: 1436–1440.

Lampert, W. 1982. Further studies on the inhibitory effect of the toxic blue-green *Microcystis aeruginosa* on the filtering rate of zooplankton. Arch. Hydrobiol. **95**: 207–220.

Luu, H.A., Chen, D.Z.X., Magoon, J., Worms, J., Smith, J., and Holmes, C.F.B. 1993. Quantification of diarrhetic shellfish toxins and identification of novel protein phosphatase inhibitors in marine phytoplankton and mussels. Toxicon, **31**: 75–83.

Menzel, D.W., and Corwin, N. 1965. The measurement of total phosphorus in seawater based on the liberation of organically-bound fractions by persulfate oxidation. Limnol. Oceanogr. **10**: 280–282.

Mitchell, P.A., and Prepas, E.E. 1990. Atlas of Alberta lakes. University of Alberta Press, Edmonton, Alta.

Namikoshi, M., Rinehart, K.L., Sakai, R., Scotts, R.R., Dahlem, A.M., Beasley, V.R., Carmichael, W.W., and Evans, W.R. 1992. Identification of twelve hepatotoxins from a Homer Lake bloom of the cyanobacteria *Microcystis aeruginosa*, *M. viridis* and *M. wesenbergii*: nine new microcystins. J. Org. Chem. **57**: 866–872.

Pennak, K.W. 1978. Freshwater invertebrates of the United States. John Wiley & Sons, New York, N.Y.

Phillips, M.J., Roberts, R.J., Stewart, J.A., and Codd, G.A. 1985. The toxicity of the cyanobacterium *Microcystis aeruginosa* to rainbow trout, *Salmo gairdneri* Richardson. J. Fish. Dis. **8**: 339–344.

Prepas, E.E., and Rigler, F.H. 1982. Improvements in quanitifying the phosphorus concentration in lake water. Can. J. Fish. Aquat. Sci. **39**: 822–829.

Prescott, G.W. 1978. How to know the freshwater phytoplankton. 3rd ed. Cambridge University Press, Cambridge, U.K.

Rabergh, C.M.I., Bylund, G., and Eriksson, J.E. 1991. Histopathological effects of MC-LR, a cyclic peptide toxin from the cyanobacterium (blue-green alga) *Microcystis aeruginosa*, on common carp (*Cyprinus carpio* L.). Aquat. Toxicol. **20**: 131–146.

Reinikainen, M., Ketola, M., and Walls, M. 1994. Effects of the concentrations of toxic *Microcystis aeruginosa* and an alternative food on the survival of *Daphnia pulex*. Limnol. Oceanogr. **39**: 424–432.

Rothhaupt, K.O. 1991. The influence of toxic and filamentous blue-green algae on feeding and population growth of the rotifer *Brachionus rubens*. Int. Rev. Gesamten Hydrobiol. **76**: 67–72.

Schwimmer, M., and D. Schwimmer. 1968. Medical aspects of phycology. *In* Algae, man and the environment. *Edited by* D.F. Jackson. Syracuse University Press, Syracuse, N.Y. pp. 279–359.

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

Toivola, D.M., Eriksson, J.E., and Brautigan, D.L. 1994. Identification of protein phosphatase 2*a* as the primary target for microcystin-LR in rat liver homogenates. FEBS Lett. **344**: 175–180.

Tsuji, K., Naito, S., Kondo, F., Ishikawa, N., Watanabe, M.F., Suzuki, M., and Harada, K.-I. 1994. Stability of microcystins from cyanobacteria: effect of light on decomposition and isomerization. Environ. Sci. Technol. **28**: 173–177.

Watanabe, M.M., Kaya, K., and 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.