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Abstract

Cyanobacteria toxins, called microcystins (MCs), were found in components of twelve, stratified New Hampshire (USA) lakes of varied trophic status during the summer of 1998. A sensitive ELISA method detected MC levels in whole lakewater, grazable phytoplankton fractions (<30 mm), net phytoplankton (> 375 μm), and isolated copepod and cladoceran (Daphnia sp.) samples. Whole lakewater (WLW) and net phytoplankton MC concentrations ranged between 9 and 165 ng MC L-1 and 0.2 and 2031 mg MC g-1-dry wt, respectively. Lakewater MC concentrations correlated with total epilimnetic phosphorus and total epilimnetic chlorophyll a concentrations and inversely with Secchi disk depth.

The filter-feeding cladoceran (daphnid) and omnivorous copepod components of the zooplankton were separated and assayed independently for MCs. The cladoceran component accumulated between 7 and 2800 μg MC g-1-dry wt. in 10 of the lakes. The copepod component accumulated similar levels (4 and 2400 μg MC g-1 dry-wt.) in all lakes. Toxin accumulation by zooplankton directly correlated with lakewater and net phytoplankton MC concentrations. The highest levels were found in Silver Lake, a productive lake where Microcystis aeruginosa blooms frequently occur. It is particularly noteworthy that MC levels were also detected by ELISA methods in Russell Pond, a pristine, deep mountain lake of low productivity. The results emphasize the importance of including oligotrophic lakes and water supplies in monitoring programs for MCs to ensure the safety of animals and humans utilizing them for drinking and recreation.

Introduction

Limnological features commonly associated with cyanobacteria blooms in freshwater ecosystems include eutrophic, or hypereutrophic status, high light intensity, thermal stratification, prolonged hydraulic retention time, and warm surface water temperatures. The frequency and intensity of blooms is increasing worldwide, and their

occurrence is often associated with increasing eutrophication (Watanabe et al., 1996; Carmichael, 1992). In addition to the unsightliness, foul taste and odor caused by such blooms, cyanobacteria may produce secondary metabolites, or biotoxins, that have been associated with advanced trophic status. More than 40 species of cyanobacteria have been found to produce toxins (Carmichael, 1996; Skulberg, 1993), some of which are neurotoxic, but more commonly are hepatotoxic to vertebrate animals. The liver toxins called microcystins (MCs) were first characterized from Microcystis aeruginosa (Botes et al., 1982), a species commonly found in freshwater ecosystems worldwide (Carmichael, 1992). MCs are also produced by species in the genera Oscillatoria, Anabaena and Nostoc (Rapala et al. 1997; Rinehart et al., 1994), all of which are present in various New Hampshire lakes. MCs have also been reported in the small (< 2 μm) picoplankton, Aphanocapsa spp., from a Brazilian drinking

water supply (Domingos et al., 1999).

MCs have been known for a long time to cause breakdown of liver hepatocytes and intra-hepatic hemorrhaging (Foxall, 1980, Foxall & Sasner, 1982; Runnegar and Falconer, 1981). In addition, MCs irreversibly inhibit, serine/ threonine protein phosphatase enzymes (PP1 and 2A) and result in the hyper-phosphorylation of target proteins, similar to okadaic acid (MacKintosh, 1990, Chen et al., 1993, Honkanen et al., 1991). Cells may become mitotically active resulting in uncontrollable cell division and accelerated tissue growth. Chronic doses of MCs promote liver tumor growth and cancer formation in mice (Falconer et al., 1991; Fujiki et al., 1996) and in rats (Nishiwaki-Matsushima et al., 1992). Blooms of MC-producing cyanobacteria pose a health threat to wild and domesticated vertebrates, aquatic organisms, as well as humans through the use of water for drinking and recreation. Surveys have shown that 25 - 95% of cyanobacteria blooms are either neurotoxic or hepatotoxic to a variety of animal species (Rapala et al., 1997; Sivonen, 1996). Microcystis is the most common bloom-forming cyanobacterium associated with human and animal poisonings and has been linked to the death of cattle, sheep, waterfowl, dogs and trout (Watanabe et al., 1996; Carmichael, 1992), while fish, muskrat and bird deaths have been linked to blooms of Oscillatoria agardhii (Eriksson et al. 1986). The reported LD50 for MC-LR in mammals is 50 mg kg-1 body weight (Carmichael, 1992).

MCs have impacted human health via water supplies in Australia (Steffensen and Nicholson, 1994) and in Caruaru, Brazil where more than 100 kidney patients suffered from acute liver damage following hemodialysis treatment with MC- contaminated water (Jochimsen et al., 1998). MCs in drinking water have also been linked to human primary liver cancer (PLC) in China (Ueno et al., 1996a). In Australia and England, guidelines of 1 mg MC L-1 have been suggested for safe exposure to drinking and recreational waters (Steffensen and Nicholson, 1994). Ueno et al. (1999) propose levels of 0.01mg MCs L-1 as a maximum level in drinking water. This level was suggested due to reported incidences of liver carcinoma in connec

tion to China's drinking water supplies. The United States has acquired no such drinking water safety level guideline to date.

Detection of MCs has routinely used high performance liquid chromatography (HPLC). A recent study has shown that various plastic field collection devices may interfere with the HPLC determination of MCs (Ikawa et al., 1999), possibly indicating inaccurate assessments of MC quantification, depending upon the collection and storage devices used. A novel monoclonal antibody against MC-LR was produced, which has a high affinity for MC and a high cross-reactivity with various MC derivatives, (Nagata et al., 1995) and ELISA (enzyme-ligand immunosorbant assay) methods were developed to detect MCs in water samples (Chu et al., 1989; Ueno et al., 1996b). The ELISA method is two to three orders of magnitude more sensitive than HPLC with a detection level in the picogram range. This allows for easy detection of MCs in components of the food web that was, heretofore, difficult or impossible to measure by other methods.

Previous studies have focused on the zooplankton accumulation of MCs in eutrophic and hypereutrophic lakes where blooms are common and toxins are accumulated by zooplankton (Watanabe et al., 1992, Capron, 1995; Kotak et al., 1996). Accumulated MCs in zooplankton may serve as a pathway to higher trophic levels of the food web. Zooplankton grazers, with accumulated MCs, have the potential of passing the toxin to predacious zooplankton and planktivorous fishes. Piscivores may then be affected and potentially pass the toxin on to birds and mammals that feed on piscivores. Field studies indicate that MCs may also be present in some oligotrophic lakes (Christoffersen 1996; Prepas et al, 1997; Haney, unpublished). These recent findings have led us to ask whether these toxins (MCs) accumulate in various compartments of the food web of less productive lakes.

This study examines and compares the presence of MCs in whole lakewater (WLW), and phyto and zooplankton from twelve NH lakes with diverse physical features and varied trophic status. The study employs a sensitive ELISA method for

the assay of MCs. Field samples from the twelve lakes were assayed for the accumulation of MCs. Zooplankton was separated from the net phytoplankton of each lake, and divided into copepod and Daphnia subsamples. While studies have focused on productive lakes with significant cyanobacteria populations, we tested the increased sensitivity of the ELISA method for MCs by also including oligotrophic lakes, where cyanobacteria may be sparse and blooms do not occur.

Materials and Methods

Study Sites - Twelve New Hampshire lakes were chosen based upon varied trophic status, physical features, and location (Table 1). The determination of trophic status for each lake was based on the Forsberg and Ryding Index (1980). The lakes were sampled during the period of summer vertical stratification (early July – mid-August), allowing for comparisons of the chemical, physical, and biological parameters during the most productive season. Lakewater, phytoplankton and zooplankton samples, nutrient and physical data were collected from a deep site in each lake (Table 1). Water transparency was determined using a Secchi disk and view scope.

Integrated lake water sampling - Integrated epilimnetic water samples were collected for analysis of total phosphorus, chlorophyll a, and MC concentration. Triplicate water samples were collected in 4 L cubitainers using a peristaltic pump and Tygon tubing lowered through the epilimnion. Samples for MC analysis were fractionated into whole unfiltered lakewater (WLW) and a filtered zooplankton-grazable (< 30 mm) fraction. The grazable fraction was prepared by pouring WLW samples through a 30 mm Nitex net, and storing the filtrate in HDPE sample jars (200 mL). The samples were put on ice in the field, and stored frozen (-20 ∞C) in the laboratory. Total phosphorus water samples were preserved with 20 N H2SO4 (1 mL), put on ice in the field and then frozen (-20 ∞C) in the laboratory until analyzed. For chlorophyll a determinations, 500 mL of integrated lake water sample was filtered (0.45 μm, 4.7 cm Millipore filter), placed in a darkened desiccator, and stored dried until analysis. Methods for total phosphorus and chlorophyll a determinations were in accordance with the quality assurance project plan (QAPP) for watershed and limnological survey conducted by the NH Lakes Lay Monitoring Program in September 1996 (APHA,

Table 1. Features of 12 NH study lakes. Data obtained from NH Lake Survey Database. *HRT = Hydraulic Retention Time. N/A = data not available.

Lake/Pond/Reservoir Town	Lake Key	Max. depth (m)	Sample depth (m)	Shore length (m)	Area (Ha)	*HRT (Yr^{-1})	Volume (Ha-m)	Watershed (Ha)
Russell Pond Woodstock	RP	23.7	23.1	1600	15.8	1.58	129.6	147.9
Merrymeeting Lake New Durham	ML	41.2	24.8	17400	339.7	0.20	6809.8	2907.6
Pleasant Lake Deerfield	PL	19.8	16.3	7200	199.7	2.55	1397.9	895.0
Stonehouse Pond Barrington	SHP	16.8	13.3	1000	5.8	0.90	42.2	75.1
Swains Lake East & West Basins, Barrington	SLE SLW	8.0	8.0 6.0	11400	170.2	1.10	469.8	1113.7
Northeast Pond Milton	NEP	14.9	12.5	10600	228.5	0.04	617	29597.5
Townhouse Pond Milton	THP	10.7	10.1	9800	48	0.01	158.4	29525.9
Barbadoes Pond Madbury	BP	14.6	13.5	1000	5.8	0.50	34.9	38.8
Great Pond Kingston	GP	14.6	13.6	6600	82.6	0.33	371.7	2175.6
Old Durham Reservoir Durham	ODR	4.0	3.9	182.6	0.143	N/A	N/A	N/A
Silver Lake Hollis	SL	7.5	7.2	1800	13.6	0.53	36.7	124.1

1998). Total phosphorus was analyzed by using a persulfate digestion and ascorbic acid indicator (Std. Method 4500-PB.3-E). Chlorophyll a analysis (Std. Method APH 10200H.2) consisted of dissolving the filter and grinding the sample with 95% acetone with MgCO3 in a glass tissue grinder. The sample was centrifuged and placed at 4 ∞C for 2 hr to extract the chlorophyll a. The sample was then centrifuged for 15 min at 1600 rpm, transferred to a cuvet (~ 12 mL), and read at 663 nm and 750 nm on a spectrophotometer [Milton Roy (Spectronic), 1001+]. Acetone (95%) was used as a blank.

Plankton Sampling for Identification, Separation, and Enumeration - Samples for the determination of phyto- and zooplankton population densities were collected from a deep site of each lake. Triplicate vertical integrated samples were collected using a plankton net (30 cm diameter, 53 mm mesh) towed from one meter above the sediments to the surface. Plankton samples were preserved in 4% formalin/sucrose and identified and enumerated within 8 months. Zooplankton and phytoplankton densities were determined using light microscopy. Phytoplankton was identified and counted using an inverted microscope (40X) and a Sedgwick-Rafter counting cell. Zooplankton was counted in a Bogrov chamber using a dissecting microscope at 20X. Identification of Daphnia species was based on Brooks (1957) and Brandlova et al. (1972). Subsamples, of both phytoplankton and zooplankton, were taken using a 1 mL Hensen Stemple pipet until at least 100 of the most dominant forms were counted.

Plankton Sampling for ELISA Analysis - Zooplankton and phytoplankton collections and field separation of samples was modified from Capron (1995). Triplicate plankton samples were collected using a plankton net (0.5 m diameter, 375 mm mesh) and prepared for MC analysis by ELISA. Ten water column tows were performed for each replicate from a deep site of each lake and stored in a cubitainer (4 L) in the dark until separation of phyto and zooplankton was accomplished. The net zooplankton-phytoplankton (> 375 μm) separation procedure was as follows. Plankton samples were

zooplankton replicates were placed in 4 L cubitainers containing Chlorella vulgaris (5 X 104 cells mL-1) in aerated well water (1 hr) to clear the digestive tracts of recently ingested cyanobacteria. Each replicate was then passed through a 50 mm mesh net (approximately 2.5 cm square) fastened to the end of a funnel to collect the captured zooplankton. The mesh nets were placed in scintillation vials (20 mL) in the field, transported on dry ice, and stored frozen (-20 ∞C) in the laboratory.

The net phytoplankton (> 375 μm) was kept in the darkened separatory funnel (1 hr) to allow for a visible surface layer to be collected and the subsurface water was discarded through the spigot. In cases where no visible phytoplankton layer developed, the remaining water was passed through the mesh nets (50 mm) to concentrate the phytoplankton samples. The mesh nets were placed in scintillation vials (20 mL) in the field, transported on dry ice, and stored frozen (–20 oC) in the laboratory.

Prior to preparation for ELISA analysis for MCs, zooplankton replicates were removed from the freezer, thawed and placed in HDPE sample jars containing cold distilled water. A pipette was used to transfer a small volume (~ 1 mL) of zooplankton under the dissecting scope into a glass petri dish for zooplankton separation. Dissecting tweezers were used to separate the copepods from the cladocerans. Copepods were more abundant in the samples (>1000 individuals) than the daphnids (< 200 individuals). For each sample 500 copepods were picked, combining large and small copepods. All adult daphnids present in the samples were picked (100 – 200 animals) from each replicate sample. The copepod and daphnid sub-samples were immediately placed in separate sample jars containing cold distilled water and collected on 50 mm mesh nets (~ 2.5 cm sq.), placed in scintillation vials (20 mL) and refrozen.

Sample Preparation for ELISA - Whole lakewater and the grazable phytoplankton fractions (<30 mm) were freeze-thawed three times, filtered (13 mm, 0.2 mm syringe filter), and assayed using the ELISA method for MCs. When toxin levels were below the detection range, the samples were concentrated (40 X), using a Speed Vac Concentrator (Savant Inc., 100H). Twenty mL of water was

mm Millipore filter in the bottom and the samples were brought to dryness. Another 10 mL were added (total 20 mL) and the drying process was repeated. Once dried, the residue was re-suspended in 0.5 mL of PBS [(1.5 mM KH2PO4, 8.1 mM Na2HPO4), pH 7.4, containing 0.137 M NaCl and 2.7 mM KCl]. These were extracted overnight on a shaker (New Brunswick Scientific, G76) at room temperature to ensure removal of the MCs from the Millipore filter. The concentrated sample was removed from the scintillation vial by washing the sides of the vial with the sample before filtration (13 mm, 0.2 mm PTFE syringe filter) and stored frozen (-20 oC) until ELISA analysis. Concentrated water samples above the upper detection limit (1600 pg mL-1) of the ELISA assay were diluted (20X) with PBS and re-analyzed.

   One of the goals was to develop a protocol for preparing phytoplankton and zooplankton field samples for the ELISA. Sample weights of copepod, Daphnia and phytoplankton were determined using a CAHN microbalance (C-31 model, sensitivity ± 0.1 μg). Samples were thawed, removed from the mesh nets and placed in pre-weighed aluminum boats, with spatula and tweezers, and dried in a desiccator under vacuum at room temp (~48-96 h). Sample dry weight was determined, then transferred to a 1.6 mL vial, moistened with 10 - 20 μL of dH2O plus 250 μL of methanol (100%), and extracted overnight at room temperature. The extracted phytoplankton samples were diluted 1:10 with PBS (2.25 mL), filtered (13 mm, 0.2 μm PTFE syringe filter), and stored frozen (-20 oC) until ELISA analysis. The extracted zooplankton samples were dried in a Speed Vac Concentrator until dryness occured (~1 hr). Copepod and Daphnia subsamples were re-suspended in 0.25 mL of PBS, filtered (4 mm, 0.2 μm PTFE syringe filter), and stored frozen (-20 oC) until ELISA analysis.

   ELISA MC Analysis - MC analysis was performed on the lake water, phytoplankton, and copepod and Daphnia samples using the MC ELISA kit (Wako BioProducts, Richmond, Va.). The MC-coated micro-titer plate, primary and secondary antibodies, chromogen and stop solution, wash buffer, citrate buffer, assay buffer and standards (50, 100, 200, 400 and 1600 pg MC mL-1) were

body (50 μL) was placed into each well followed by the addition of 50 μL of the sample standard. The plate was covered with parafilm and incubated at 4 ∞C for 16-24 h. This incubation allowed for the primary antibody and MC competitive binding. Following the primary incubation, wells were washed three times with ≥ 200 mL of wash buffer. Horseradish peroxidase-conjugated secondary antibody (100 μL) was added to each well, covered with parafilm, and incubated at room temperature for 2 h. Following the incubation, wells were washed again three times with wash buffer (≥200 μL). Chromogen (100 μL) (diluted 1:99 with citrate buffer) was added to each well, covered with parafilm, and incubated for 15 min. Stop solution (100 μL) was added and the plate was read using a Microplate Reader with a 450 nm filter (Bio-tek, Winooski, Vt; EL800, sensitivity ± 0.010 Abs.).

Statistical Analysis - Statistical analysis (t- test, ANOVA, Tukey's Post Hoc test) were performed using Systat. MC analysis for the lakewater, phytoplankton and zooplankton samples utilized KC Junior, a companion program to the Bio-tek Microplate Reader that produces a standard curve and interpolates the concentration of MC in each sample.

Results

   Description of the 12 Lakes - Tables 1 and 2 describe the physical and chemical features of the twelve lakes included in the study. The physical data in Table 1 was obtained from the NH Lake Survey database. The designation of trophic status (Table 2) was based on the Forsberg and Ryding (1980) index for categorizing lakes on the basis of total phosphorus (TP), chlorophyll a (Chl-a), and water clarity. Lakes with low TP (<15 ppb), low Chl-a (< 3 ppb) and high water clarity (> 4 m) are designated as oligotrophic. Lakes with relatively high TP (> 25 ppb), high Chl-a (> 7 ppb) and low clarity < 2.5 m) are termed eutrophic. Intermediate values between these two are termed mesotrophic. According to the index, the study included 4 oligotrophic lakes; 6 mesotrophic lakes and 2 eutrophic lakes covering a range of surface areas, depths, volumes and hydraulic retention times.

MCs in Lake Water - MCs were detected in whole lakewater (WLW) samples and the grazable fraction (< 30 μm) of each lake and no significant differences were measured between them in 11 of the 12 study sites (p > 0.05). The exception was

The sensitivity of the ELISA assay detected MCs in all of the study lakes. Silver L. had the highest MC concentration in the net phytoplankton (2031.05 ± 242.20 µg MC g-1 d.w.), which was

Table 2. Chemical features, water clarity and trophic status of 12 NH lakes in the summer of 1998.

Lake Key (See Table 1.)	Sample Date 1998	Total phos. (ppb)	Chl *a* (ppb)	Secchi disk depth (m)	K_{ext}	Trophic Status
RP	8/11	2.58± 0.16	0.69± 0.02	11.45± 0.10	0.20± 0.01	Oligo
ML	8/4	2.68± 0.62	0.91± 0.13	10.83± 0.15	0.28± 0.01	Oligo
PL	8/12	4.06± 0.44	1.62± 0.02	7.20± 0.03	0.53± 0.02	Oligo
SHP	7/22	6.14± 0.57	2.71± 0.18	3.70± 0.12	0.99± 0.04	Oligo/Meso
SLE	8/6	8.39± 0.31	2.29± 0.29	4.38± 0.06	0.93± 0.03	Oligo
SLW	8/6	9.95± 0.09	2.05± 0.10	3.30± 0.06	1.40± 0.04	Oligo/Meso
NEP	8/13	9.95± 0.43	2.95± 0.16	4.23± 0.14	0.97± 0.02	Oligo/Meso
THP	7/16	11.33± 0.57	2.14± 0.08	3.37± 0.04	0.91± 0.06	Oligo/Meso
BP	7/28	11.94± 0.15	3.83± 0.15	3.08± 0.04	0.78± 0.02	Oligo/Meso
GP	7/23	13.40± 0.31	5.55± 0.14	2.20± 0.01	1.07± 0.09	Oligo/Meso/Eu
ODR	7/7	27.94± 0.23	4.88± 0.23	2.70± 0.04	1.83± 0.02	Meso/Eu
SL	7/30	10.03± 0.09	3.62± 0.28	3.92± 0.02	0.85± 0.03	Oligo/Meso

Silver L. (p = 0.028) where the WLW MC concentration was almost 4 X higher than in the grazable fraction (Table 3). Silver L. had the highest total MC concentration in the WLW (165.60 ± 17.94 ng MC/L) and differed significantly from all other lakes (p < 0.0001). Other lakes, like Russell P., Merrymeeting L. and Pleasant L., are oligotrophic and had the lowest WLW levels ranging between approximately 9 and 10.3 ng MC L -1, respectively. However, the latter were only significantly different from Old Durham Res. and Barbadoes P. (p < 0.05). Because of the greater variability in replicates no lakes differed significantly in their grazable fraction concentrations (p > 0.05). With a few exceptions, the MC concentration of WLW was generally related to trophic status (Table 2). The more productive lakes (Silver L., Barbadoes P., and Old Durham Res.) had higher MC concentrations in the WLW than the less productive lakes (Russell P., Pleasant L., and Merrymeeting L.).

MCs in Phytoplankton - The ELISA assay was performed on small quantities (0.71 to 10.88 mg dry wt) of net phytoplankton (> 375 µm) ranging.

approximately 700 times higher than the second highest lake, Townhouse P. (3.17 mg MC g-1 d.w.), (Table 3). The Silver L. net phytoplankton concentration differed significantly (p > 0.05) from all other lakes, while Townhouse P., Barbadoes P, and Great P. had higher total MC levels than the remaining lakes ranging from 1.74 to 3.17 mg MC g-1 d.w. It is noteworthy that Old Durham Res., a mesotrophic to eutrophic lake often experiencing blooms, had an intermediate level (0.66 ± 0.37 µg MC g-1 d.w.) that was similar to MC levels in oligotrophic Russell P. with few cyanobacteria and where blooms do not occur (Table 3). While the presence of MCs were expected in the former (ODR), they were not expected in the latter (RP) and beg the question as to the source of MCs in pristine Russell P.

MC Accumulation in Zooplankton - Zooplankton samples extracted for MC analysis ranged from 0.17 to 4.17 mg dry weight (d.w.). MCs were detected in all copepod and Daphnia samples from the study sites (Table 3). Silver L. copepod and

Daphnia MC accumulation differed significantly (p < 0.05) from the other lakes. Silver L. had a copepod MC accumulation (2440.58 ± 908.22 ng MC g-1 tissue d.w.) approximately seven times higher than the second highest copepod accumulation of Old Durham Res. (342.62 ± 99.48 ng MC g-1 copepod d.w.). The Daphnia MC accumulation in Silver L. (2822.59 ± 154.48 ng MC g-1 d.w.) was approximately 15 times higher than the second highest Daphnia accumulation in Barbadoes P. (215.54 ± 6.49 ng MC g-1 Daphnia d.w.). Daphnia accumulations were significantly correlated to the MC concentration in the whole lake water (WLW). As MC concentration increased, Daphnia accumulation increased linearly. Silver L., differed significantly from all other lakes in WLW, grazable fraction, net phytoplankton and zooplankton MC concentrations. Surprisingly, there was no significant correlation (p > 0.05) between the MC concentration in the grazable (<30 μm) fraction with the MC accumulation in the Daphnia or the copepods. Daphnia accumulation was also directly correlated to the net phytoplankton (> 375 μm) MC concentration. As the MC concentration in the net phytoplankton increased, Daphnia accumulation also increased. Copepod MC accumulation was also directly correlated with WLW MC levels. As WLW concentration increased, copepod MC accumulation also increased. Copepod MC accumulation was also directly correlated to the net phytoplankton MC concentration. (r2 = 0.98; p = 0.0001).

Phytoplankton/Cyanobacterial Composition - Phytoplankton samples from the deep site of study lakes showed that Old Durham Res. had the highest percentage of cyanobacteria in the net plankton, followed by Silver L., Townhouse P. Swain's L. East, Northeast P., and Great P. In addition, Old Durham Res., also had the highest percentage of MC- producing species (Microcystis, Anabaena, and Oscillatoria) within the net phytoplankton (25.67 ± 5.28 %).

Silver L. had higher densities of Microcystis colonies (9812.72 ± 2290.59 L-1) than the other lakes containing Microcystis and also possessed a high density of Anabaena filaments (972.02 ± 487.66 L-1), both of which produce MCs. Old Durham Res. had the highest density of Oscillatoria filaments (1602.09 ± 781.77 L-1) and the second highest level of Microcystis colonies (646.85 ± 86.74 L-1). Great P. had the second highest density of Oscillatoria filaments (980.59 ± 299.83 L-1) and low densities of both Anabaena filaments and Microcystis colonies. Other lakes had lower densities of MC- producing cyanobacteria with densities lower than 500 L-1. Silver L., Swain's L. East and West, Northeast P., Townhouse P., and Merrymeeting L. all had additional genera of cyanobacteria present which are not reported to produce MCs (e.g., Aphanothece, Merismopedia, Coelosphaerium, and Arthrospira).

Zooplankton Composition of the Study Sites - Lakes had higher copepod densities than daphnids with the exception of Old Durham Res., which had more Daphnia (262.65 ± 26.05 L-1) than copepods (97.18 ± 13.40 L-1), Barbadoes P., Swain's L. East and West, and Great P. had the highest densities of copepods ranging from 45.29 to 77.74 L-1. All other lakes had densities below 23 copepods L-1. Daphnia densities for all lakes besides the Old Durham Res. had lower densities at <19 L-1.

Old Durham Res. had only cyclopoid copepods present while Russell P. had only C. diapto mus present. All other lakes had a combination of calanoid copepods (Diaptomus sp.) and cyclopoid copepods. Species of Daphnia found in the study lakes were D. catawba, D. ambigua, D. schodleri, D. pulex, D. galeata, and D. retrocurva.. Half of the lakes (Stonehouse P., Russell P., Swain's L. West, Great P., Northeast P., and Townhouse P.) had only one species of Daphnia present, while the remainder of the lakes contained two or more species. Other zooplankton observed in the study lakes but not examined for MC accumulation were Diaphanosoma, Bosmina, Ceriodaphnia, and Holopedium . Copepod nauplii were counted but not included due to their size. Barbadoes P. had the highest densities of Diaphanosoma, and Bosmina that ranged from 46.45 ± 5.61 to 105.68 ± 19.65 L-1, respectively.

Table 3.   Microcystin (MC) levels (by ELISA) in whole lake water (WLW), grazable fraction (<30 μm), and dried phytoplankton and zooplankton. N/A indicates Merrymeetng Lake and Townhouse Pond samples were insufficient for analysis. Note that WLW and grazable fractions are per liter and phytoplankton and zooplankton values are per gram.

Lake Key (SeeTable 1)	Sample Date 1998	WLW (ng MC L^{-1})	Phyto Fraction <30 μm, grazable (ng MC L^{-1})	Phyto Fraction >375 μm (μg MC g^{-1} d.w.)	Copepod (ng MC g^{-1} d.w.)	Daphnia (ng MC g^{-1} d.w.)
RP	8/11	9.98± 0.73	8.93± 1.35	0.77± 0.27	81.62± 1.58	46.18± 5.76
ML	8/4	9.17± 0.47	8.15± 1.32	0.24± 0.04	38.32± 14.52	N/A
PL	8/12	10.30± 0.24	19.18± 6.48	0.30± 0.07	15.41± 1.38	28.84± 3.35
SHP	7/22	20.85± 3.41	47.50± 21.53	0.11± 0.02	14.00± 3.07	6.98± 2.17
SLE	8/6	25.15± 3.95	47.12± 10.27	0.83± 0.30	4.38± 0.16	19.19± 2.71
SLW	8/6	24.14± 3.78	74.69± 22.53	0.57± 0.14	4.38± 1.26	119.99± 9.80
NEP	8/13	19.27± 2.08	40.78± 15.81	0.41± 0.04	35.10± 14.52	88.60± 21.95
THP	7/16	22.26± 4.98	39.74± 12.23	3.17± 0.72	8.24± 1.10	N/A
BP	7/28	58.29± 15.43	25.87± 4.53	2.18± 0.25	126.14± 33.45	215.54± 6.49
GP	7/23	38.00± 13.76	28.49± 8.38	1.74± 0.62	122.91± 24.86	113.17± 9.66
ODR	7/7	53.77± 8.44	82.44± 29.36	0.66± 0.37	342.62± 99.49	100.33± 4.61
SL	7/30	165.60± 17.94	44.08±18.90	2031.05± 242.24	2440.58± 908.22	2822.59± 154.48

Discussion

Daphnia are nonselective filter feeders that can ingest single cells and small colonies of cyanobacteria (Lampert and Sommer, 1997). Calanoid copepods are herbivorous grazers, while the cyclopoid copepods are highly selective omnivores that feed on phytoplankton, as well as other zooplankton (Adrian, 1991). Therefore, MC accumulation in calanoid copepods may be due to the direct ingestion of cyanobacteria while that of the cyclopoid copepods may be due to either the ingestion of cyanobacteria and/or ingestion of other zooplankton prey that accumulated MCs. Well-fed cladocerans and copepods contain large lipid reserves that could allow the lipid soluble MCs to accumulate in the body. A preliminary laboratory study suggests that MC accumulation in the livers of planktivorous fish (golden shiners) my result from a diet of Daphnia that were raised on Microcystis (Nowak, 2002). Kotak et al. (1996) found no measurable accumulation of MCs in livers of northern pike or white suckers, two fish species not likely to feed on zooplankton. Other studies have revealed MC accumulation in mussel (Eriksson, 1989; Nye, 1997), gastropods (Kotak et al., 1996; Nye, 1997), and crayfish (Hathaway, 2001).

To date, studies have focused on MC accumulation in net zooplankton living in eutrophic and hypereutrophic lakes where Microcystis blooms commonly occur. Watanabe et al. (1992) indirectly measured accumulation (75-1387 μg MC g-1 d.w.) in net zooplankton from Lake Kasumigaura, Japan, by analyzing whole lakewater MC levels and subtracting out the net phytoplankton MC concentration and the estimated gut contents. Capron (1995) found accumulated MC levels in Silver Lake (Hollis, NH) net zooplankton ranging from 1 to 60 μg MC g-1 d.w. during the summers of 1991-1993. Kotak et al. (1996) found up to 67 mg MC- LR g-1 biomass in hypereutrophic lakes in Alberta, Canada. The studies noted above all used HPLC to measure MC levels. The ELISA assay used in this study, is more sensitive than the HPLC analysis by approximately three orders of magnitude, thus permitting the examination of MCs in lakes of varied trophic status, including those that do not experience cyanobacteria blooms.

Daphnia MC accumulation directly correlated with whole lake water MC concentration (Table 3) and also directly correlated with the net phytoplankton MCs (> 375 μm). However, MC accumulation in the Daphnia did not correlate with the concentrations found in the grazable water fraction. However, MC accumulation is a cumulative process and therefore, may not correlate with the grazable standing crop. In addition, the large variance in the MC concentration for the grazable fractions may have obscured possible correlations. Since the Daphnia accumulation of MC in Silver L. was approximately 2.5 μg MC g-1 d.w., the question arises as to whether predators could accumulate MCs in the food chain. Wu and Culver (1994) performed a study on yellow perch predation on D. galeata and found that they ingested as many as 213 daphnids d-1 in mid-August in western Lake Erie. If yellow perch in Silver L. ingest daphnids at similar rates, we estimate the potential rate of MC ingestion to approximate 5.0 μg MC d-1 and suggest that they could act as sources of MCs to the fish community.

As lakes increase in their productivity, as indicated by total epilimnetic phosphorus, the percentages of MC-producing cyanobacteria increased. In contrast to the expected, pristine Russell P. had higher levels of MC in the net phytoplankton than the more productive Old Durham Reservoir. Copepod MC accumulation in many of the oligotrophic lakes (Merrymeeting L., Russell P., Stonehouse P. and Pleasant L.) was similar to or higher than that of some of the more productive lakes (Swain's L. East and West, Northeast P. and Townhouse P.). Russell Pond's Daphnia accumulation was higher than in the more productive Swain's L. East.

The question arises as to the source of the toxins in the case of less productive lakes, like Russell P. The high copepod and Daphnia MC accumulation could be caused by the zooplankton feeding on cyanobacteria that occur in deep stratified layers in the water column. The deeper light penetration in less productive lakes may allow for Microcystis or other MC-producing cyanobacteria to grow at high enough densities in such deep layers to accumulate in the zooplankton (e.g. Daphnia) that migrate downward during the day to avoid fish predation.

Even with the colder temperatures slowing the feeding rates of the zooplankton, the light levels that reach these depths may be sufficient to allow significant MC production and accumulation in the zooplankton. On the other hand, smaller-sized primary producers are often the dominant phytoplankton in oligotrophic lakes (Lampert and Sommer, 1997). Perhaps the zooplankton graze on these MC-producing picoplankton and nanoplankton and accumulate their MCs for passage up the food chain.

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