Article

Bioaccumulation of microcystins in the food web: a field study of four Swedish lakes

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Received 29 May 2013; accepted 9 October 2013; published 28 January 2014

Abstract

The transfer of microcystins (MC) up the food chain was measured in 4 lakes in central Sweden; Ekoln, Lilla Ullfjärden, Valloxen, and Storsjön. In lakes Ekoln and Valloxen, *Microcystis aeruginosa* was the dominant cyanobacterium, while the oscillatorian species *Planktothrix prolifica* form dense blooms in Lake Lilla Ullfjärden. The cyanobacterial composition in Lake Storsjön was more diverse with several *Microcystis* and *Dolichospermum* species. All dominant taxa are well-known producers of hepatotoxic MC. The highest recorded MC in the water samples from Lake Lilla Ullfjärden was measured in the bloom of *P. prolifica* (35 μg L^{-1}). The highest MC content was measured in invertebrates; however, the MC concentration was usually low in fish. Maximum levels were 9 μg g^{-1} dry weight (dw) in zooplankton, 10 μg g^{-1} dw in benthos, and 2.7 μg g^{-1} dw in fish (smelt) liver. In fish muscle the highest recorded levels were 0.10–0.18 μg g^{-1} dw in bleak and smelt from Lake Lilla Ullfjärden and in pike-perch and roach from Lake Storsjön. Based on the World Health Organization's tolerable daily intake value of 0.04 μg kg^{-1} body weight, we conclude that any risk related to MC from human consumption of fish from these lakes is minimal.

Key words: bioaccumulation, cyanobacteria, ELISA, food web, microcystin, Swedish lakes

Introduction

Cyanobacterial blooms are common in most countries, particularly in agricultural areas (Chorus and Bartram 1999, Codd et al. 2005, Carmichael 2008). In Sweden, about 50% of cyanobacterial blooms are toxic, and the most common toxin-producing genera are *Dolichospermum*, *Aphanizomenon*, *Microcystis*, and *Planktothrix* (Willén et al. 1995, 2000, Willén and Mattsson 1997).

Microcystins (MC) are the most common of the hepatotoxins, and to date about 70 variants with varying toxicity have been identified (Carmichael 1997, Haande et al. 2007). A prerequisite to MC production is the presence of the *mcy* gene PCC7806 (Tillett et al. 2013). Genetic variability and differences in environmental conditions can result in different toxicities of species between lakes, seasons, and years (Janse et al. 2004, Wilson et al. 2005).

Although cyanobacterial toxicity has been well studied, investigations on the bioaccumulation of MC in common aquatic organisms are less common; however, toxicity levels in freshwater mussels (Eriksson et al. 1989, Williams et al. 1997), crayfish (Miles et al. 2013), zooplankton (Watanabe et al. 1992), and fish have been investigated (Dawson 1998, Ibelings and Chorus 2007, Poste et al. 2011). Two recent reviews by Ferrao-Filho and Kozlowsky-Suzuki (2011) and Kozlowsky-Suzuki et al. (2012) reported data on MC accumulation in a wide variety of taxa. They found accumulation in zooplankton and zooplantivorous fish, whereas biodilution of MC was evident for most primary consumers.

Laboratory experiments have shown that MC can be toxic to fish (Tencalla et al. 1994); however, some of these experiments were performed with unrealistically high toxin concentrations (e.g., 6600 μg kg^{-1} MC body weight).

DOI: 10.5268/IW-4.1.627

It is therefore necessary to study bioaccumulation directly in the field. To our knowledge, no previous studies have presented data on MCs in aquatic organisms other than phytoplankton from Swedish lakes.

This research investigated the bioaccumulation of MC in samples of zooplankton, zoobenthos, and fish in 4 Swedish lakes. Phytoplankton samples were taken concurrently to enable analysis of MC concentration in the water column to be compared with the data of zooplankton, benthos, and fish. The study addressed the following questions:

1. What levels of MC are present in zooplankton, zoobenthos, and fish under natural conditions in these 4 Swedish lakes?
2. Are the levels of MC in fish livers positively correlated to the size of the fish?
3. Is there a relation between MC levels in phytoplankton and fish?
4. Does occasional consumption of fish from these lakes pose a health risk based on the World Health Organization (WHO)'s daily tolerable intake values?

Studied lakes and cyanobacterial characteristics

The 4 lakes sampled (Ekoln, Lilla Ullfjärden, Valloxen, and Storsjön), are situated in the boreonemoral region of the eastern part of central Sweden and differ in size, nutrient state, and dominance of various cyanobacterial taxa (Table 1 and 2).

Lake Ekoln is a large basin of Lake Mälaren, Sweden's fourth largest lake. While it remains eutrophicated, it has been the focus of remedial measures that have halved Lake Ekoln's phosphorus concentrations and algal biomasses and shortened its summer algal blooms to 2 months (Aug and Sep). *Microcystis aeruginosa* (Kützing) Kützing continues to be the dominant species (Willén 2001).

Lake Lilla Ullfjärden is a groundwater-fed, well-separated headwater basin of Lake Mälaren, classified as mesotrophic. It is 22 m deep, and this in combination with its sheltered location creates long periods of stable stratification. Lake Lilla Ullfjärden is almost always dominated by cyanobacteria, especially the oscillatorian, red-colored *Planktothrix prolifica* (Gomont) Anagn. & Komárek. The species develops during winter in the surface water and forms spectacular reddish blooms under the ice. It moves downward during summer stratification to avoid bright light and usually reaches its biomass maximum in the metalimnion. This species is a common MC producer in Swedish lakes (Willén and Mattsson 1997, Chorus and Bartram 1999).

Lake Valloxen is quite eutrophic and is situated in the drainage area of Lake Mälaren. It has a mixed cyanophyte community in summer, with *Microcystis*-dominated blooms the most common.

Lake Storsjön belongs to a smaller northerly draining area. It is characterized by a large area in relation to its depth, resulting in mixing in the water column and a lack of stable summer stratification. The development of cyanophytes begins in June and continues over the summer season until late September. Especially rich taxa diversity has been recorded, with 14 species of *Dolichospermum* and 4 species of *Microcystis* (Söderhielm 1998); *Microcystis* is usually the prevailing taxon in late summer. Notable among the toxin-producing taxa are the frequently occurring *M. viridis* (A. Braun) Lemmerm.; *Dolichospermum circinale* (Rabenh.) Wacklin, L. Hoffm. & Komárek; and *D. flos-aquae* (Bréb. ex Bornet & Flahault) Wacklin, L. Hoffm. & Komárek. (Komárek and Anagnostidis 1999a, 1999b, Komárek 2013; preprints to these flora were used for determinations).

Methods

Sampling

Phytoplankton were sampled 3 times in Lake Ekoln, 5 times in Lake Lilla Ullfjärden, and once in Lake Storsjön in 1998 (Table 2a). In 2001 they were sampled 5 times in Lake Ekoln and Lake Lilla Ullfjärden, 4 times in Lake Valloxen, and 5 times in Lake Storsjön (Table 2b). Samples were taken at the surface layer (0–1 m), except from Lake Lilla Ullfjärden, where we also collected water from the metalimnic accumulation of *Planktothrix*.

Samples were filtered on glass-fiber filters (MGC, Munktell filter AB) for MC and chlorophyll *a* (Chl-*a*), analysis, and the filters and an aliquot of the filtrate were kept in a freezer (−20 °C) until analysis. Samples for analysis of Chl-*a*, MC, and dominant phytoplankton per volume of water were taken from all 4 lakes. Chl-*a* in Lake Storsjön was sampled only once in 2001 (Table 3), and therefore we used Chl-*a* and MC data from 1997 for that lake (Söderhielm 1998), assuming that few differences would have occurred over such a short time (Fig. 1). Subsamples of all phytoplankton samples were preserved with Lugol's solution, supplemented with acetic acid for later identification and enumeration.

Zooplankton were sampled in Lake Lilla Ullfjärden 3 times in 1998 and 4 times in 2001, and once in Lake Ekoln in 2001. At the beginning of the season, when zooplankters were rare, sufficient numbers were obtained by pumping water directly into the plankton net from a depth of 0–5 m. We concentrated the zooplankton using an 80 µm plankton net in 1998 and a 160 µm plankton net in 2001. Cladocerans and copepods sampled in 2001 were

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Table 1. Morphometric and chemical characteristics of the 4 Swedish lakes (from Söderhielm 1998, Willén et al. 2000). Department of Aquatic Sciences and assessment database, Swedish University of Agricultural Sciences; "—" represents no samples.

Parameter	L. Ekoln (basin of Mälaren)	L. Lilla Ullfjärden	L. Valloxen	L. Storsjön (w. basin)
Coordinates N	59°45′	59°37′	59°44′	60°33′
Coordinates E	17°36′	17°30′	17°50′	16°42′
Surface area (km^2)	30	2	3	39
Catchment area (km^2)	100	9	30	1200
Mean depth (m)	15	22	3.8	4.5
Max depth (m)	50	52	9	15
Alkalinity (meq L^{-1}	2.1	1.8	2.6	0.31
Secchi depth (m)	2.3	2.7	—	1.6
Colour (mg Pt L^{-1})	59	13	29	80
pH	8.2	8.7	8.1	7.4
Total-P (µg L^{-1})	50	25	53	39
Total-N (µg L^{-1})	1610	600	1050	540

Table 2a. Dominant cyanobacteria during summer 1998 in 3 Swedish lakes.

Lakes	Dates	Depth (m)	Dominating taxa
Ekoln	29 July	0	*Microcystis aeruginosa* (sparse), diatoms dominating
	12 Aug	0	*Microcystis aeruginosa* (sparse), diatoms dominating, green algae and dinoflagellates (subdominants)
	26 Aug	0	*Microcystis aeruginosa*, *Planktothrix agardhii*, *Aphanizomenon flos-aquae* (no visible cyano-bloom due to mixed water column)
Lilla Ullfjärden	29 July	0.5	*Planktothrix prolifica*, *Aphanizomenon* spp. (= *flos-aquae*, skujae), *Limnothrix* spp., *Planktolyngbya limnetica*
		6.5	*Planktothrix prolifica*
	12 Aug	0.5	*Planktothrix prolifica*, *Limnothrix* spp., *Pseudanabaena* sp. (subdominants)
		6.5	*Planktothrix prolifica*, *Aphanizomenon* spp.
	26 Aug	0.5	*Planktothrix prolifica*
		7.0	*Planktothrix prolifica*
	9 Sep	0.5	*Planktothrix prolifica*, *Limnothrix* spp. *Planktolyngbya limnetica*, *Aphanizomenon* spp. (small amounts)
		7.0	*Planktothrix prolifica*, *Limnothrix* spp. *Planktolyngbya limnetica*, *Aphanizomenon* spp. (small amounts)
	14 Oct	0.5	*Planktothrix prolifica*, *Limnothrix* spp., *Planktolyngbya limnetica*
		1.0	*Planktothrix prolifica*, *Limnothrix* spp. *Planktolyngbya limnetica*
Storsjön	27 Aug	0	*Microcystis viridis* (subdominant, diatoms prevail)

manually separated with forceps and kept frozen with the phytoplankton samples. Functional groups were not separated in 1998. Some of the concentrated zooplankton was preserved with Lugol's solution supplemented with acetic acid for size analysis.

We collected 3 species of zoobenthos (*Asellus aquaticus* L., *Bithynia tentoculata* L., and *Viviparus* sp.) in Lake Lilla Ullfjärden in 1998. The sample amounts were 8, 1.3, and 2.5 g, respectively, for the specimens of each collected, and the sample amounts for enzyme linked im-

munosorbent assay (ELISA) analyses were between 24.7 and 26.7 mg.

Fish were collected from Lake Lilla Ullfjärden once in 1998 and twice in 2001. Lakes Ekoln and Valloxen were sampled in 2001, using 3 multimesh gillnets with several sections of different mesh sizes and a total area of 36 × 1.5 m. Lake Storsjön was sampled in both 1998 and 2001 using 3 smaller multimesh nets with an area of 30 × 1.0 m and a net with a mesh-size of 50 mm to catch pike-perch (*Lucioperca lucioperca* L.). From the total catch, pieces of livers and dorsal muscle tissue were taken from 3–5 fishes of various sizes and species. All samples were freeze-dried before MC analyses.

Analyses

Chl-*a* analyses were conducted according to ISO 10260:1992(E) (ISO 1992), using warm 95% ethanol for extraction.

Microcystins were analyzed using ELISA (EnviroGard Microcystin Plate Kit, Strategic Diagnostics) according to the standard operating procedure (Meriluoto and Codd 2005). The limit of detection (LOD) is at 0.1 µg L^{-1}. Phytoplankton and zooplankton were thawed and frozen 3 times before the extraction with 75% methanol (Fastner et al. 1998). Pieces of fish liver and muscles (micro-balance) were ground before extraction. Phosphate buffer was used

Table 2b. Dominating cyanobacteria during summer 2001 in 4 Swedish lakes.
* Different sampling sites.

Lakes	Dates	Depth (m)	Dominating taxa
Ekoln	30 July	0	*Microcystis aeruginosa*, *M. flos-aquae*, *Dolichospermum* spp., *Aphanizomenon* flos-aquae
	21 Aug	0	*Microcystis aeruginosa* (a heavy bloom drifted to the beach)
	5 Sep	0	*Microcystis aeruginosa* (conspicuous dominance ≈ 95%)
	24 Sep*	0	*Microcystis aeruginosa*
	24 Sep*	0	*Microcystis aeruginosa*, *M. botrys* (both sparse)
Lilla Ullfjärden	15 June	0	*Planktothrix prolifica*, *Aphanizomenon* spp. (= *flos-aquae*, skujae)
		6	*Planktothrix prolifica*
	16 July	0	*Planktothrix prolifica*, *Aphanizomenon* spp., *Limnothrix* spp.
		9	*Planktothrix prolifica*
	1 Aug	0	*Limnothrix* spp., *Planktothrix prolifica*, *Aphanizomenon* spp.
		9	*Planktothrix prolifica*,
	16 Aug	0	*Aphanizomenon* spp., *Limnothrix* spp., *Planktothrix prolifica*
		9	*Planktothrix prolifica*
	2 Oct	0	*Planktothrix prolifica*, *Limnothrix* spp.
		12	*Planktothrix prolifica*
Valloxen	9 July	0	*Aphanizomenon* spp.
	19 July	0	*Microcystis aeruginosa* (but diatoms and green algae prevail)
	19 Aug	0	*Microcystis* spp. (=*aeruginosa*, *botrys*, *viridis*), *Aphanizomenon* spp., *Dolichospermum* spp. (subdominants).
	4 Sep	0	*Aphanizomenon* spp.
Storsjön	21 Aug	0	*Planktolyngbya limnetica* (dominance), *Aphanizomenon flos-aquae*, *Cuspidothrix issatschenkoi*, *Microcystis viridis*, *Anabaena mendotae*, *Woronichinia naegeliana*
	5 Sep	0	*Aphanizomenon flos-aquae* (dominance), *Microcystis viridis*, *M. wesenbergii*, *Planktolyngbya limnetica*.
	20 Sep	0	*Aphanizomenon flos-aquae*, *Microcystis wesenbergii*
	7 Oct	0	Diatoms (small amounts)
	23 Oct	0	*Aphanizomenon flos-aquae* (sparse), diatoms

 DOI: 10.5268/IW-4.1.627

if samples needed dilution (Chu et al. 1990). The samples were analyzed in duplicate.

All phytoplankton samples were analyzed under an inverted microscope for dominant cyanophytes (Table 2a and b). Zooplankton lengths (mean of 20 individuals) in the samples collected in 2001 were measured under an inverted microscope. The length (µm) was converted to biomass (m, µg g^{-1} dw) using equations described by Dumont et al. (1975):

Cladocerans	$m = 1.5 \times 10^{-8} \times length^{2.84}$
Calanoida copepods	$m = 7.9 \times 10^{-7} \times length^{2.33}$
Cyclopoida copepods	$m = 1.1 \times 10^{-7} \times length^{2.59}$

Phytoplankton samples, 6 from 1998 and 19 from 2001, were dried on filters and analyzed for MC (Table 3a and b). Water samples, 10 from 1998 and 15 from 2001 (Table 3a and b); dried zooplankton samples, 10 from 1998 and 8 samples from 2001 (Fig. 2a and b) and 7 dried zoobenthos samples from 1998 (Fig. 2c); 41 dried fish liver samples from 1998 and 50 samples from 2001 (Tables 4, 5a and b, 6, 7a and b); dried dorsal muscle samples, 24 from 1998 and 26 from 2001 (Tables 5a and b, 6, 7a and b) were also analyzed for MCs. All fish were separated in species and classed by size.

Results

Phytoplankton

Microcystis aeruginosa was dominant in Lake Ekoln and *Planktothrix prolifica* in Lake Lilla Ullfjärden in both sampled years (Table 2a and b). Lake Valloxen was dominated by *Aphanizomenon* spp., while Lake Storsjön showed a more diverse cyanobacterial assemblage over the season (Table 2b).

Concentrations of Chl-*a* and MC varied considerably between lakes and over the season (Table 3a and b; Fig. 1a–e). The MC quantities from concentrated phytoplankton on filters were lower than those from raw water samples, except for one sample from Lake Ekoln. This difference was expected because the raw water samples contained both particulate and dissolved MC.

The Chl-*a* concentration 1998 was lowest in Lake Ekoln (0.9–11 µg L^{-1}), while Lake Lilla Ullfjärden had much higher values on most of the sampling occasions that year (16–58 µg L^{-1}; Table 3a). In 2001, the Chl-*a* concentration was lowest in the surface sample of Lake Lilla Ullfjärden but considerably higher in the metalimnion and in Lake Ekoln (Table 3b; Fig. 1). The high values of Chl-*a* and MC in Lake Ekoln (700 and 210 µg L^{-1}, respectively) were measured on 21 August at a popular beach close to the town of Uppsala. The wind had concentrated the heavy bloom of *M. aeruginosa* close to a bridge where the

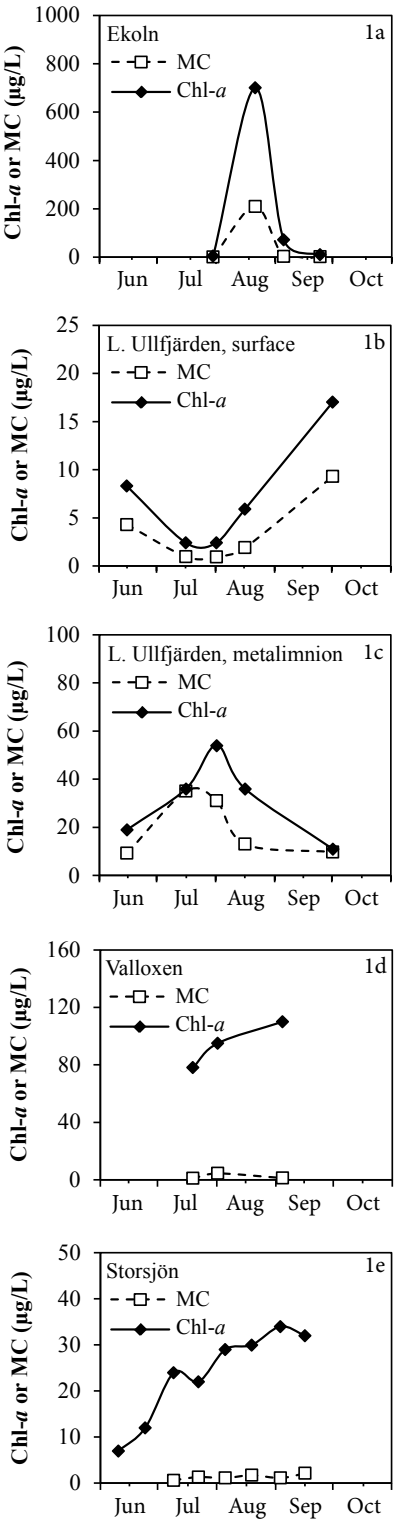

Fig. 1. Chlorophyll *a* (Chl-*a*; full line) and microcystin (MC; broken line), sampled in the surface layer of (a) Lake Ekoln during summer 2001; (b) Lake Lilla Ullfjärden suface during summer 2001; (c) Lake Lilla Ullfjärden metalimnion during summer 2001; (d) Lake Valloxen surface during summer 2001; and (e) Lake Storsjön surface during summer 1997 (Söderhielm 1997).

sampling took place, but the value decreased considerably due to storm mixing 3 weeks later (Table 3b). The Chl-*a* concentrations were high in Lake Valloxen and low in Lake Storsjön, while the MC concentrations were relatively low in both lakes (Table 3b; Fig. 1).

Zooplankton and benthos

The mixed zooplankton collected from Lake Lilla Ullfjärden in 1998 contained relatively high levels of MC ranging from 1.5 to a maximum of 8.7 µg g^{-1} dw (Fig. 2a). Similar levels were found in 2001 with MC from 0–6.9 µg g^{-1} dw in cladocerans and 0–3.5 µg g^{-1} dw in copepods (Fig. 2b). All sampled zooplankton in 1998 reached MC values higher than the LOD, whereas 5 samples in 2001, including the single sample from Lake Ekoln, had concentrations lower than the LOD (Fig. 2a and b). The 3 zoobenthos genera *Asellus*, *Bithynia*, and *Viviparus*, sampled in September 1998, contained high levels of MC, 2.3–8.3 µg g^{-1} dw (Fig. 2c).

Fish

We collected 8 fish species from Lake Ekoln (Table 4), 9 from Lake Lilla Ullfjärden (Table 5a and b), 4 from Lake Valloxen (Table 6), and 8 from Lake Storsjön (Table 7a and b). Most fish liver samples from lakes Ekoln and Lilla Ullfjärden (August and October) contained MC (Table 4 and 5). In Lake Valloxen, 5 of the 12 analyzed fish livers had microcystins ranging from 0.05 to 1.1 µg g^{-1} dw; the other values were below the LOD (Table 6). Six of the 15 (40%) fish liver samples from Lake Storsjön had MC values lower than the LOD in 1998 and 6 of 17 (35%) in 2001 (Table 7a and b). Microcystins were measured in the fish muscles samples of bleak, smelt, roach, ruffe, and pike-perch from Lake Lilla Ullfjärden and Lake Storsjön (Table 5a and b, 7a).

The mean MC content in livers from Lake Lilla Ullfjärden was not significantly different between the 2 sampling dates in Lake Lilla Ullfjärden 2001 (P = 0.09, mean and CV = 0.86 ± 0.58 and 0.54 ± 0.19, respectively), whereas the MC levels were significantly higher in zooplankton than in fish both years (P = 0.0002, mean and CV = 5.3 ± 2.7 and 0.17 ± 0.039, respectively). Similarly, MC levels in benthos were much higher than in fish (P = 0.022). Only phytoplankton data from Lake Lilla Ullfjärden were sufficient to run a regression test between MC results for phytoplankton and fish. This regression test indicated no significant relationship existed between MC levels in phytoplankton and fish in that lake (RSquare = 0.26, P = 0.16, n = 9).

Discussion

This study demonstrates that although the liver toxin MC is accumulated in the food web, it is concentrated at the highest levels in zooplankton and zoobenthos (Fig. 2) and at lower levels in fish livers and muscles (Table 4–7).

Table 3a. Chlorophyll *a* (Chl-*a*) and microcystin (MC) concentrations of phytoplankton in 2 Swedish lakes, 1998; "—" represents no samples.

Lakes	Date	Depth (m)	Chl-*a* (µg L^{-1})	MC (µg L^{-1})	
				Filter	Raw-water
Ekoln					
	29 July	0	11.4	0.008	0.1
	12 Aug	0	10.5	—	0.1
	26 Aug	0	0.9	—	0.1
L. Ullfjärden					
	29 July	0.5	23.3	—	1.4
		6.5	58.4	1.5	4.3
	12 Aug	0.5	16.3	—	—
		6.5	51.5	—	—
	26 Aug	0.5	26.6	0.22	1.8
		7.0	37	0.37	4.1
	9 Sep	0.5	17.4	—	2.3
		7.0	22.5	—	4.0
	14 Oct	0.5	26.7	0.22	2.8
		1.0	32.6	0.66	—

 DOI: 10.5268/IW-4.1.627

Table 3b. Chlorophyll *a* (Chl-*a* and microcystin (MC) concentrations of phytoplankton in the 4 Swedish lakes 2001.
* Different sampling place. "—" represents no samples

Lakes	Date	Depth (m)	Chl-*a* (µg L^{-1})	Microcystins (µg L^{-1})	
				Filter	Raw-water
Ekoln					
	30 July	0	4.7	0.36	0.48
beach	21 Aug	0	700	>53	210
beach	5 Sep	0	71	4.5	3.5
	24 Sep*	0	16	0.92	2.5
	24 Sep*	0	3.2	>0.25	0.5
L. Ullfjärden					
	15 June	0	8.3	1.2	4.3
		6	19	7.3	9.2
	16 July	0	2.4	0.24	0.97
		9	36	13	35
	1 Aug	0	2.4	0.30	0.9
		9	54	19	31
	16 Aug	0	5.9	1.1	1.9
		9	36	6.4	13
	1 Oct	0	17	2.9	9.3
		12	11	2.0	9.7
Valloxen					
	19 July	0	78	0.97	—
	1 Aug	0	95	4.4	—
	4 Sep	0	110	1.2	—
Storsjön					
	21 Aug	0	14	0.5	—

Table 4. Average and SD of microcystin (MC) content in fish liver from Lake Ekoln, 25 Sep 2001. No analyses were made of fish muscles.

Species in size classes	Length (cm)	n	MC (µg g^{-1}dw)
Perch (*Perca fluviatilis* L.)	11.5–12.0	3	0.09 ± 0.01
	16.4–17.6	3	0.22 ± 0.21
	23.2	1	0.69
Pike-perch (*Sander lucioperca* L.)	8.2–9.0	3	0.15 ± 0.03
	25.1–29.8	2	0.14 ± 0.05
Roach (*Rutilus rutilus* L.)	10.9–13.9	3	0.11 ± 0.04
	24.3–26.4	3	0.21 ± 0.23
Bleak (*Alburnus alburnus* L.)	12.7–13.1	3	0.26 ± 0.25
Bream (*Abramis brama* L.)	13.5–16.0	3	0.11 ± 0.06
	21.2–23.7	3	0.08 ± 0.02
White bream (*Abramis bjoerkna* L.)	11.3–12.1	3	0.12 ± 0.04
	15.9–18.4	3	0.08 ± 0.01
Ruffe (*Gymnocephalus cernuus* L.)	9.2–9.8	3	0.14 ± 0.07
Smelt (*Osmerus eperlanus* L.)	8.2–10.5	3	0.17 ± 0.04

A generalized illustration of toxin distribution calculated for Lake Lilla Ullfjarden in 1998 (Fig. 3) indicates that the highest content of MC in fish livers was found in Lake Lilla Ullfjärden 2001, where the highly toxic species *P. prolifica* reached considerable biomass, especially in the metalimnion. The lowest contents were recorded in Lake Storsjön, where *cyanobacteria* were subdominant. Both zooplankton and zoobenthos are important foods for all species of young fish. For some planktivorous species such as smelt, roach, and ruffe, the zooplankton and zoobenthos are important over their entire life cycle (Ahlgren et al. 1994).

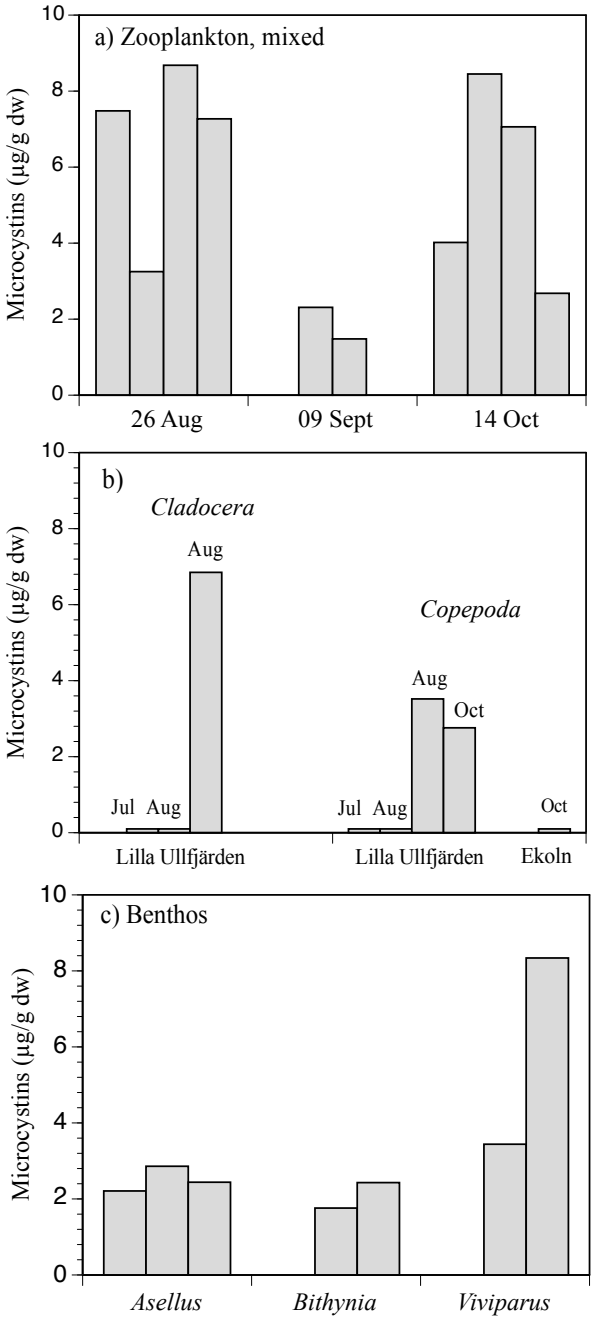

Fig. 2. (a) Microcystin in mixed zooplankton, sampled over 3 months in Lake Lilla Ullfjärden 1998, concentrated through 80 µm net. (b) Microcystin in cladocerans and copepods, sampled during summer 2001 in lakes Lilla Ullfjärden and Ekoln, concentrated through 160 µm net. (c) Microcystin content in zoobenthos, sampled in Lake Lilla Ullfjärden on 21 Sep 1998.

The biomagnification (BMF) factor is a measure of MC accumulation in different organisms and is calculated as the ratio between the MC measured in consumers and that in their diets (Kozlowsky-Suzuki et al. 2012). Because the fish were not sampled on the same dates as zooplankton and benthos, the BMF could only be estimated for our data from ratios between the means of MC in both categories. In addition, MC concentrations for cyanophytes were presented in µg L^{-1} and for zooplankton and fish in µg g^{-1} dw. To resolve this difference, we estimated dry weight (dw) for the cyanophytes using the conversion factors from 2 years data from Lake Norrviken with heavy blooms of cyanophytes (Ahlgren 1983), such as dw/Chl = 70.9, (SD ± 24, n = 13) and dw/fw (fresh weight) = 0.50 (SD = 0.20, n = 11).

BMF was estimated between and zooplankton and cyanophytes, between zooplankton and fish liver, and between fish liver and cyanophytes in 1998 and 2001. In 1998 BMF was estimated between benthos and cyanophytes and between benthos and fish. All samples used for this analysis were collected from Lake Lilla Ullfjärden in September–October 1998 and August–October 2001 (Table 8). When cyanophytes were abundant in the diet, a high degree of biodilution (BMF << 1) was seen but varied considerably, lower in 2001 than in 1998. Zooplankton and benthos as food for fish showed lower BMF in 1998 than in 2001 (Table 8). All data indicated strong biodilution except fish/zooplankton data, which indicated moderate dilution. In a synthesis of 42 studies of aquatic food webs, such as zooplankton, decapods, molluscs, fishes, turtles, and birds, biodilution of MC was evident for most primary consumers (Kozlowsky-Suzuki et al. 2012). Zooplankton and zooplanktivorous fish showed potential for BMF, whereas omnivorous fish tended to show the greatest biodilution.

Several recent studies have been published on accumulation of MC in fish, particularly in Brazil, Uganda, and China (e.g., Magalhaes et al. 2003, Xie et al. 2005, Deblois et al. 2008, Zhang et al. 2009, and Semyalo et al. 2010). Most reported values were low (<1 µg g^{-1} dw) except in China, where some liver values exceeded 10 µg g^{-1} dw, and muscle values reached 2–3 µg g^{-1} dw (Xie et al. 2005). The highest levels were found in planktivorous fish and the lowest in carnivorous fish. European data show reduced growth related to cyanobacterial toxins in brown trout (*Salmo trutta*; Bury et al. 1995). The impact of cyanobacterial toxins on whitefish was also

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Table 5a. Averages and ± SD of microcystin (MC) content in fish liver and muscle. Lake Lilla Ullfjärden 22 Sep 1998. "—" represents no samples; bold represents > detection limit.

Size classes	Length (cm)	n	MC in liver (µg g^{-1} dw)	MC in muscle (µg g^{-1} dw)
Perch (*Perca fluviatilis* L.)	10–10	3	**0.14** ± 0.03	<0.1
	13–18	3	<0.1	—
	22–26	3	<0.1	<0.1
	32–37	3	<0.1	**0.09**
Roach (*Rutilus rutilus* L.)	9	1	**0.16**	<0.1
	12–12	2	**0.45** ± 0.31	<0.1
	19–20	2	**0.21** ± 0.04	—
Bleak (*Alburnus alburnus* L.)	10–10	2	**0.18** ± 0.01	**0.11** ± 0.01
	13–14	1	**0.11**	—
Ruffe (*Gymnocephalus cernuus* L.)	9	1	**0.11**	<0.1
Smelt (*Osmerus eperlanus* L.)	11	1	**0.64**	**0.10**
Vendace (*Coregonus albula* L.)	21–23	2	<0.1	<0.1

Table 5b. Averages and ± SD of microcystin (MC) content in fish liver and muscle. Lake Lilla Ullfjärden 16 Aug 2001 and 2 Oct 2001. * consist of a mixture of 3 samples. Bold represents > detection limit.

Size classes	Length (cm)	n	MC in liver (µg g^{-1} dw)	MC in muscle (µg g^{-1} dw)
16 August				
Perch (*Perca fluviatilis* L.)	8.7–9.2	4	0.77 ± 0.37	<0.03
	17.2–18.3	4	0.50 ± 0.26	<0.03
	25.9–34.5	5	0.14 ± 0.40	<0.03
Pike-Perch (*Sander lucioperca* L.)	100	1	1.14	<0.03
Roach (*Rutilus rutilus* L.)	9.4–10.3	2	0.94 ± 0.64	<0.03
	13.5–14.5	3	0.67 ± 0.33	0.038
Bleak (*Alburnus alburnus* L.)	11.8–12.9	3	0.42 ± 0.31	—
Bream (*Abramis brama* L.)	14.9–17.0	2	0.74 ± 0.078	<0.03
Ruffe (*Gymnocephalus cernuus* L.)	9.6–10.8	9	1.08 ± 0.42	0.035
	13.0–14.0	3	1.35 ± 0.35	—
Smelt (*Osmerus eperlanus* L.)	8.0–9.5	3	2.3 ± 0.41	0.11 ± 0.07
Vendace (*Coregonus albula* L.)	23.9	1	0.25	<0,03
2 October				
Perch (*Perca fluviatilis* L.)	8.7–9.5	3	0.86 ± 0.21	<0.03
	12.9-20.0	3	0.28 ± 0.16	<0.03
	26.0–26.7	3	0.60 ± 0.42	<0.03
Roach (*Rutilus* rutilus L.)	10.3–10.5	3*	0.52	—
	13.5–14.5	3	0.52 ± 0.42	<0.03
Bream (*Abramis brama* L.)	26.6	1		<0.03
White Bream (*Abramis bjoerkna* L.)	9.7–10.7	3*	0.55	<0.03
Ruffe (*Gymnocephalus cernuus* L.)	9.7–9.9	3	0.73 ± 0.15	<0.03
	13.5	1	0.30	—

found in Lake Ammersee in southern Germany, with MC detected in the gut at levels up to 35 µg g^{-1} dw (Ernst et al. 2001). In a study performed of 7 tropical lakes and 2 temperate lakes from 2006 to 2009, Poste et al. (2011) sampled ~80 fish of 33 different species and reported levels of MC from fish muscle or whole small fish (<10 cm in length) as µg kg^{-1} wet weight (ww). Most samples (42) showed levels <10 µg kg^{-1}, but 33 samples showed levels of 20–30 µg kg^{-1} ww. In one crater lake the highest levels of MC were reached in 2 cichlid species (Alluaud's haplo [*Astatoreochromis alluaudi*] and red back scraper [*Haplochromis* spp.]) of 56 and 719 µg kg^{-1} ww, respectively. Recalculated to µg g^{-1} dw (dw/ww = 0.31; Poste et al. 2011) these values correspond to 0.18 and 2.3 µg g^{-1} dw, similar to the MC levels in fish livers observed in the present study.

Studies of MC accumulation in the lower parts of the food chain are less common. Eriksson et al. (1989)

Table 6. Microcystin (M) content in fish liver and muscle from Lake Valloxen, 4 Sep 2001.
* consist of a mixture of 3 samples. "—" represents no samples; bold represents > detection limit.

Species in size classes	Length (cm)	MC in liver (µg g^{-1} dw)	MC in muscle (µg g^{-1} dw)
Roach *(Rutilus rutilus* L.)	7.2–7.5*	<0.12	<0.03
	8.9–9.1*	1.08	<0.03
	11.6	<0.06	—
	13.0	<0.08	
Bleak *(Alburnus alburnus* L.)	10.0–10.4*	0.31	—
	12.5.	0.05	—
	13.4	<0.08	
Bream (*Abramis brama* L.)	24.2	<0.07	—
	25.6	0.06	
Ruffe (*Gymnocephalus cernuus* L.)	9.3–11.2*	0.07	—
Roach (*Rutilus rutilus* L.)	11.4	<0.13	
	13.6	<0.08	—

Table 7a. Averages and ± SD of microcystin (MC) content in fish liver and muscle from Lake Storsjön 27 Aug 1998. Bold represents > detection limit.

Species in size classes	Length (cm)	n	MC in liver (µg g^{-1} dw)	MC in muscle (µg g^{-1} dw)
Perch *(Perca fluviatilis* L.)	11–12	2	**0.15** ± 0.0	<0.1
	13–15	4	<0.1	<0.1
	25–30	3	<0.1	<0.1
Pike-Perch *(Sander lucioperca* L.)	17–18	2	**0.1**	**0.10**
	19–20	3	<0.1	<0.1
	27	1	<0.1	<0.1
	34–34	3	<0.1	<0.1
	38–39	2	<0.1	<0.1
Roach (*Rutilus rutilus* L.)	13	1	<0.1	<0.1
	17	1	**0.14**	**0.11**
Bleak (*Alburnus alburnus* L.)	12	1	**0.28**	<0.1
	14	1	**0.16**	<0.1
Ruffe (*Gymnocephalus cernuus* L.)	6	1	<0.1	<0.1
	8–10	2	**0.18** ± 0.01	<0.1
Pike (*Esox lucius* L.)	47–60	2	<0.1	<0.1

 DOI: 10.5268/IW-4.1.627

Table 7b. Microcystin content in fish liver and muscle from Lake Storsjön 21 Aug 2001. n = 1; "—" represents no samples; bold represents > detection limit.

Size classes	Length (cm)	MC in liver (µg g^{-1} dw)	MC in muscle (µg g^{-1} dw)
Perch *(Perca fluviatilis* L.)	10.5	<0.10	—
	16.0	<0.10	—
	23.1	<0.05	—
	23.8	**0.05**	—
	31.0	**0.05**	—
Pike-perch (*Sander lucioperca* L.)	17.4	**0.05**	**<**0.03
	37.6	<0.06	<0.03
	47.6	<0.06	<0.03
	48.0	<0.10	<0.03
	50.6	**0.05**	**<**0.03
	51.3	**0.05**	<0.03
	51.4	<0.08	<0.03
Roach (*Rutilus rutilus* L.)	13.4	<0.12	—
Bleak (*Alburnus alburnus*) L.)	13.0	<0.08	—
Bream (*Abramis brama* L.)	25.0	<0.08	—
White bream (*Abramis bjoerkna* L.)	17.2	<0.11	—
Ruffe (*Gymnocephalus cernuus* L.)	9.0	**0.05**	—

Table 8. Biomagnification (BMF) stands for ratios between the MC contents in the consumers and their diets. BMF is estimated from data of Lake Lilla Ullfjärden in August–October 1998 and August-October 2001.

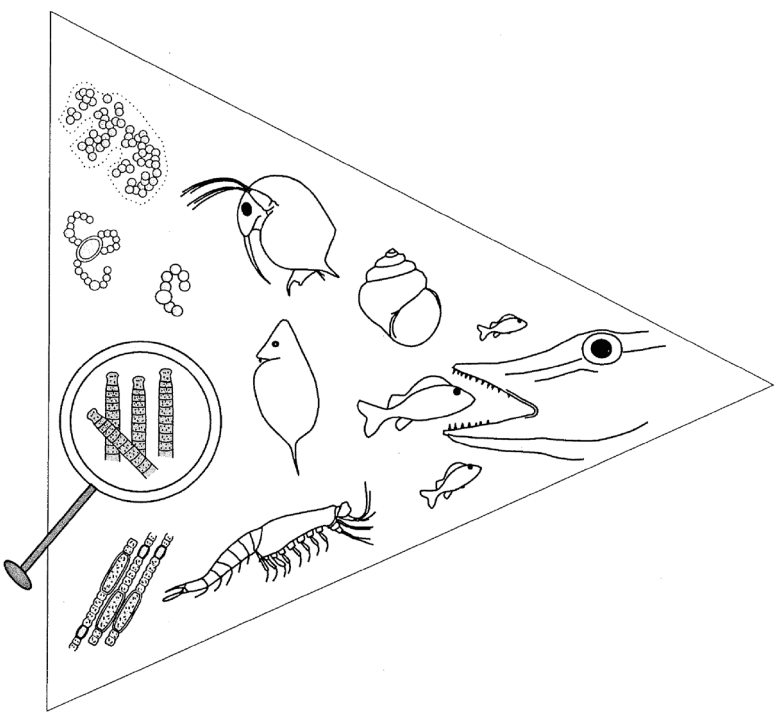
Category	MC contents (µg g^{-1} dw)	SD	n	BMF (Consumers/diets)
1998				
Cyanophytes	1465	589	7	
Zooplankton	5.31	2.7	10	
Benthos	3.34	2.24	7	
Fish-liver	0.167	0.20	12	
Zooplankton/Cyano				5.31/1465 = 0.0036
Fish/Cyano				0.167/1465 = 0.00011
Benthos/Cyano				3.34/1465 = 0.0023
Fish/Zooplankton				0.167/5.31 = 0.031
Fish/Benthos				0.167/3.34 = 0.05
2001				
Cyanophytes	7846	3447	8	
Zooplankton	1.88	2.7	7	
Fish-liver	0.72	0.48	21	
Zooplankton/Cyano				1.88/7846 ≈ 0.00024
Fish/Cyano				0.72/7846 = 0.00009
Fish/Zooplankton				0.72/1.88 = 0.38

showed that swan mussels (*Anadonta cygnea*) could concentrate high amounts of toxins, up to 70 µg g^{-1} dw, from *Oscillatoria agardhii* that contained lower levels (40–60 µg L^{-1}). In hepato-pancreatic tissue, as much as 150 µg g^{-1} dw was found. A maximum MC value of 65 µg L^{-1} (ADDA-ELISA method) was detected in caged freshwater mussels from lakes in New Zealand where phytoplankton had a maximum MC of 760 µg L^{-1} (Wood et al. 2006).

Kotak et al. (1996) studied the food chain in 4 different lakes of varying trophic states and found MC up to 67 µg g^{-1} dw in zooplankton, and correlated MC concentration in zooplankton and phytoplankton. Among 9 groups of macroinvertebrates, MC was detected only in gastropods up to 120 µg g^{-1} dw. It was not further detected in the livers of pike white sucker (*Catostomus commersoni*), although this fish did contain toxic phytoplankton in the stomach. These levels of MC in invertebrates recorded by Kotak et al. (1996) are more than 10 times higher than we found in Swedish invertebrates.

Daphnids have been shown to be less sensitive to MC than copepods (DeMott et al. 1991). In experiments in which toxic *Microcystis* were fed to *Daphnia magna*, reduced growth, survival, and fecundity were recorded over the season (Thostrup and Christoffersen 1999), and the maximum content of MC in *D. magna* was 24.4 µg g^{-1} dw. Ibelings et al. (2005) could not find evidence for BMF in planktivorous fish (smelt). Seasonal averages from 3-year data were 63–211 and 2–12 µg g^{-1} dw, respectively, in zooplankton and in the mussel *Dreissena*, whereas the levels in phytoplankton were much higher, between 70 and 2000 µg g^{-1} dw (mean of 3 years). In contrast, Ferrao-Filho et al. (2002) found a very high concentration factor in zooplankton (× 5400–22 000), which might be a potential risk for transferring microcystins to fish. In a later study, Kozlowsky-Suzuki et al. (2012) reported BMF values in zooplankton up to 12. The MC values ranged from 0.3 to 16 µg g^{-1} dw.

The MC content in perch of edible size (32–37 cm) recorded in Lake Lilla Ullfjärden was 0.1 µg g^{-1} dw, which when converted to ww using the conversion factor of 0.31 (Poste et al. 2011) is approximately equivalent to 28 µg kg^{-1} ww. A human consuming 100 g dorsal muscle of this perch would then ingest 2.8 µg MC. The tolerable daily intake recommended by WHO of 0.04 µg kg^{-1} body-weight would be slightly surpassed (2.4 µg MC)



Cyanobacteria	Zooplankton	Benthic fauna	Small fishes ≤ 20 cm		Piscivores
			liver	muscle	
1000‰	4‰	2‰	0.1‰	traces	?

Fig. 3. Distribution of microcystins in the foodweb of Lake Lilla Ullfjärden. Example from the growth season of 1998. Cyanobacteria, mean July-Oct, Zooplankton mean Aug-Oct, benthic fauna Oct and Fish Oct.

 DOI: 10.5268/IW-4.1.627

when calculated for a person of 60 kg weight (WHO 2003). There is probably little risk for liver intoxication from a daily intake of fish of this kind because the liver is not consumed. For a child weighing 20 kg, however, MC concentration recorded in this study could be a concern, especially considering the child's more sensitive internal organs (Bruckner 2000, concerning chemical toxicity such as pesticides).

These 4 lakes are all popular for recreational fishing. We conclude that the risks of adverse health effects related to MC by occasionally eating freshwater fish from these 4 lakes in central Sweden is minimal for adults, although higher for children. In contrast, the use of small fish from these lakes as food for pets is not recommended. The water concentration of MC in one of the lakes (maximum of 35 μg L^{-1} recorded in Lilla Ullfjärden) is among the highest recorded in Sweden, and at these concentrations the water would be unsuitable for drinking or recreational activity based on guidelines in other countries (Chorus 2012).

Acknowledgements

We are grateful to Dr. Ingemar Ahlgren for his help with some of the references and figures, to two anonymous referees for valuable comments on an earlier version of this paper. Financial support was given from the Magnus Bergvall Foundation, Stockholm.

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