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Bioaccumulation of Microcystins by Freshwater Mussels in Mystic Lake and Middle Pond, MA

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Abstract

The UNH Center for Freshwater Biology investigated a possible relationship between a cyanobacteria bloom and a large-scale die-off of freshwater mussels in Mystic Lake and Middle Pond (Barnstable, MA). Four mussel species, *Elliptio complanata* (Eastern Elliptio), *Pyganodon cataracta* (Eastern Floater), *Leptodea ochracea* (Tidewater Mucket), and *Lampsilis radiata* (Eastern Lampmussel) (Nedeau, 2008), along with water samples, were collected from these lakes on August 9, 2010 (during bloom) and again on September 29 and October 8, 2010 (post-bloom). Hepatopancreas tissue, foot tissue, and water samples were tested for the cyanobacteria toxins, microcystins (MC), using ELISA techniques. MC concentrations in the hepatopancreas were generally higher (171.2 ng MC g^{-1} dry weight (dw)) than in the muscle (foot) tissue (55.8 ng MC g^{-1} dw) for each species. Average microcystin concentrations in mussels sampled during post-bloom tissues were slightly lower (161.6 ng MC g^{-1} dw) than those collected during the cyanobacteria bloom (171.2 ng MC g^{-1} dw). Live mussels were also subjected to a depuration experiment to determine the release of MC from mussels into the water. Mussels that were placed in cyanobacteria-free water depurated 61-90% MC within the first few days demonstrating their ability to release free MC-cyanotoxins into the lake water.

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Introduction

Mystic Lake and Middle Pond of Barnstable, MA experienced severe blooms of cyanobacteria in the summers of 2009 and 2010 (Indian Ponds Association (IPA) website). Cyanobacteria, formerly known as blue green algae, are photosynthetic bacteria that can produce neurotoxins and hepatotoxins such as anatoxin α and microcystins, respectively. According to the IPA in 2009, seven species of freshwater mussels were native to these bodies of water. However, large scale die-offs that occurred during the summer months, concurrent with cyanobacteria blooms, resulted in the extinction of several mussel species from these systems (Fig. 2).

In August, 2010, the Center for Freshwater Biology at the University of New Hampshire was contacted by the Indian Ponds Association (IPA) to test whether mussels had accumulated microcystins from a cyanobacteria bloom that persisted in Middle Pond and Mystic Lake, Barnstable, MA (Fig. 1).

Fig. 1: Google Maps image showing locations of Mystic Lake, Middle Pond, and Hamblin Pond, MA.

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Fig. 2: Dead mussels found along the shoreline of the Indian Ponds in 2009. (Photo by Bob Nichols).

Microcystins (MC) are hepatic (liver) toxins produced by many species of cyanobacteria common to New England waters (Haney and Ikawa 2000, Chorus 2001 and Hudnell 2008). However other cyanotoxins are also ubiquitous and may lead to acute toxicity in animals as well. Since mussels have such a high filtering capacity for lake water, it is plausible that mussels may ingest high levels of cyanobacteria and several studies have addressed the interactions between mussels and cyanobacteria (Williams et al. 1997; Amorim 1998 et al.; Okumus et al. 2002). Still, less is understood on the effects that mussels may have on cyanobacteria and the release of their cyanotoxins into water. As it was not possible to make conclusions on the cause of the mussel die-off, the objectives were to test for the potential bioaccumulation of microcystins in the hepatopancreas and muscle tissues of a subset of the remaining mussel specimens living in the lakes. Further investigation on the ability for live mussels to release microcystins into lake water were trialed to determine the role that mussels may have in releasing extracellular microcystins in lake water.

Materials and Methods

Study Location- The Indian Ponds are comprised of Mystic Lake, Middle Pond, and Hamblin Pond. According to the Indian Ponds website, Mystic Lake, the largest of the three Indian Ponds, covers an area of 149 acres (60.3 ha) and has a maximum depth of 42 ft (12.8 m). It is located northwest of both Middle and Hamblin Ponds. Middle Pond, which is the smallest of the three Indian Ponds, expands over 105 acres (42.5 ha) and reaches depths of 33 ft (10 m). It is located northwest of Hamblin Pond and southeast of Mystic Lake. Hamblin Pond, the second largest of the Indian Ponds, spans an area of 115 (46.5 ha) and is the deepest pond at 62 ft (18.9 m). Hamblin Pond is located to the southeast of both Middle Pond and Mystic Lake (Fig. 1).

Mussels and lake water samples were collected on August 9, 2010 (reported as "bloom"). Mussels were additionally collected on September 29, 2010 and lake water was sampled on October 8, 2012 (reported as "subsided" or "post bloom"). The bloom subsided naturally and not as a result of a water treatment plan to manage cyanobacteria.

Water Collection and Preparation- Water samples were collected from the following sites on both collection dates: the south end of Middle Pond, the south end of Mystic Lake, the north end of Mystic Lake (only collected on August 9th) and Hamblin Pond (only collected on September 29th). All water was collected as near-surface "grab" samples. Water from each collection site was sub-sampled into a 30 mL HDPE container and frozen at -40^{o}C until processing. Sub-samples (10 mL) from each collection site underwent three freeze-thaw cycles, the third of which was a 24-hour freeze drying-rehydration process concentrating the water samples 10-fold. Water samples were then rehydrated with 1 mL of milli-Q filtered water, filtered through a 0.2 μm Whatman filter, and frozen in 1.5 mL centrifuge tubes.

Mussel Collection, Dissection and Preparation- Mussel specimens were collected by IPA volunteers. Deceased mussels were separated by species into plastic bags and were placed in coolers with ice for transport to the lab. Upon arrival at the lab, all deceased mussels were frozen at -40^{o}C until dissection. Live mussels were placed in coolers containing water from their collection site for transport to the lab. The age of each mussel was estimated by the number of rings around the shell. Tissue from the foot and hepatopancreas were removed from each mussel. For each species on each collection

date, 1-3 tissue samples of each type were homogenized to obtain an average MC concentration for the population. A subsample of each homogenized tissue (0.045 g) was placed in a 1.5 mL centrifuge tube containing 150 µl of 80% MeOH. Centrifuge tubes were then sonicated and shaken for 1 min before a 24-hour MeOH extraction. After 24-hours, 1350 µL of phosphate buffer solution was added to the centrifuge tube to dilute the MeOH to <10%. Centrifuge tubes were then sonicated again for 1 min. Tube contents were then filtered through a 0.2 µm filter into new 1.5 mL centrifuge tubes. Tubes were then frozen at -40^{o}C until ELISA analysis.

Depuration- Live mussels, three *Elliptio complanata* (Eastern Elliptio) and four *Leptodea ochracea* (Tidewater Mucket), were collected during the post-bloom conditions (October 8, 2010) to be used for depuration experiments. Each *Elliptio* was placed in a separate 1000 mL glass ball jar with 500 mL of aerated, filtered (Whatman GFC) well water. Muckets were placed in individual 500 mL glass jars with 300 mL of aerated, filtered well water. All jars were gently aerated during the depuration period. Every 2 or 3 days, 5 mL of water was collected from each jar and frozen in vials at -40^{o}C. Water was replaced in each container after sample collection and mussels were fed *Nannochloropsis,* a non-toxic alga (Class Eustigmatophyceae), at approximately 10^6 cells ml^{-1}. On the sixth day of collection, the mussels were removed from the jars, placed in separate labeled bags, and frozen at -40^{o}C until dissection to test bioaccumulation of microcystin in tissues.

MC Analysis- All samples were tested for MC using ELISA (Quantiplate Kit, Envirologix Inc, Portland, ME) with an extended standard curve to enable minimum detection at 25 pg MC mL^{-1}.

Calculations- Below detectable limit (BDL) for water samples was <2.5 ng MC L^{-1} and tissue samples <8.3 ng MC L^{-1}. A concentration of 0 ng MC L^{-1} was used for all concentrations that were BDL when calculating averages for replicates.

MC release rates were calculated using the MC concentrations obtained from the depuration experiment MC concentrations. The total MC release in the depuration bottle was calculated as the MC concentration (ng MC L^{-1}) times the volumes (L) of water in the container. Rates of MC release were estimated per day. To calculate the MC release rate at the time of capture, MC release rates at the first two time periods were extrapolated back to time zero, assuming a linear change in release rate during this unmeasured period. This allowed for an estimate of the MC release rate of individual mussels while they were in the lake.

Results

Water Microcystins- Surprisingly, post-bloom water samples had much higher MC concentrations than those found in bloom water samples (Table 1). Bloom and post-bloom water samples averaged 9.6 ng MC L^{-1} and 301 ng MC L^{-1}, respectively, at the south end of Middle Pond (Table 1). Bloom water samples ranged from 7.4 ng MC L^{-1} at the north end to 9.7 ng MC L^{-1} at the south end of Mystic Lake (Table 1). Post bloom water samples averaged >3000 ng MC L^{-1} at the south end of Mystic Lake (Table 1). No post bloom water sample was collected from the north collection site of Mystic Lake. For reference to MC in the other pond, water samples were also measured from Hamblin Pond and averaged 74.5 ng MC L^{-1}(September 29, 2010).

Table 1: MC detected at all collection locations during both the reported bloom and post bloom.
**BDL = <2.5 ng MC L^{-1}*
**N/A = no water sample collected*

Whole Lake Water	**Water (ng L^{-1})**	
	Bloom	**Post Bloom**
Middle (south)	9.6	301
Mystic (south)	9.7	>3000
Mystic (north)	7.4	NA
Hamblin D	NA	74.5

Tissue Microcystins (Middle Pond)- Four species of mussels including Eastern Lampmussel, Eastern Elliptio, Eastern Floater, and Tidewater Mucket were collected from bloom condition waters. Tissues from these mussels had detectable microcystins, ranging from below detectable limits (BDL= <8.3 ng MC g^{-1}) in the muscle (foot) tissues of both the Eastern Lampmussel and Eastern Elliptio to 171.2 ng MC g^{-1} in the hepatopancreas tissue of the Tidewater Mucket (Table 2). Only two species (Eastern Floater and Tidewater Mucket) were found alive during post bloom conditions (Table 2; noted depurated mussels^). The tissues of the Eastern Floater and Tidewater Mucket were analyzed after depuration and therefore were not representative of MC concentrations in Middle pond during post bloom conditions. Depuration resulted in a general decrease of tissue MC compared to MC in tissues measured during lake conditions.

Tissue Microcystins (Mystic Lake)- During bloom conditions Mystic Lake mussels had no detectable MC except for in the hepatopancreas tissues of the Eastern Lampmussel (33.2 ng MC g^{-1}) and Eastern Floater (76.5 ng MC g^{-1}). Bloom tissue samples ranged from BDL in the muscle of Eastern Lampmussel, Eastern Floater and both the muscle and hepatopancreas tissues of Tidewater Muckets to 76.50 ng MC g^{-1} in the hepatopancreas tissue of Easter Floater (Table 3). MC concentrations were measurable in the hepatopancreas tissue (36.6 +/- 22.1 ng MC g^{-1}) and were not detected in the muscle tissues from these mussels collected during bloom conditions. No post bloom mussel samples were collected from Mystic Lake due to absence of mussels.

Table 2: MC detected in Middle Pond mussel tissues by ELISA. MC data from "Bloom"; n= 3 homogenized mussel individuals. However, due to lack of mussels from "Post Bloom"; n= 1 individual mussel.
**BDL = <8.3 ng MC g^{-1}*
^species depurated from post bloom

Middle Pond				
Mussel Species	**Hepatopancreas (ng MC g^{-1} dw)**		**Muscle (ng MC g^{-1} dw)**	
	Bloom	**Post Bloom**	**Bloom**	**Post Bloom**
Lampsilis radiata (Eastern Lampmussel)	97.87	161.64 +/- 22.11	BDL	55.80 +/- 43.21
Elliptio complanata (Eastern Elliptio)	79.74	87.98 +/- 0.32	BDL	52.00 +/- 24.18
Pyganodon cataracta (Eastern Floater)^	54.33	23.36 +/- 11.75	32.08	BDL
Leptodea ochracea (Tidewater Mucket)^	171.24	BDL	19.71	BDL
Average	100.79	68.25	12.95	26.95
Standard Deviation	25.12	36.27	7.89	15.58

Table 3: MC detected in Mystic Lake mussel tissues by ELISA MC data from Lampmussel; n=3 homogenized mussel individuals. Due to lack of Floater mussels; n=2, Mucket; n=1.
**BDL = <8.3 ng MC g $^{-1}$*
**NA = no mussels collected*

Mystic Lake				
Mussel Species	**Hepatopancreas (ng MC g^{-1} dw)**		**Muscle (ng MC g^{-1} dw)**	
	Bloom	**Post Bloom**	**Bloom**	**Post Bloom**
Lampsilis radiata (Eastern Lampmussel)	33.16	NA	BDL	NA
Elliptio complanata (Eastern Elliptio)	NA	NA	NA	NA
Pyganodon cataracta (Eastern Floater)	76.50	NA	BDL	NA
Leptodea ochracea (Tidewater Mucket)	BDL	NA	BDL	NA
Average	36.55	NA	0.00	NA
Standard Deviation	22.15	NA	0.00	NA

Depuration Results- MC in depurated water reached BDL (below detectable limits, <2.5 ng MC L^{-1}) by 120-h for the Eastern Floater and 288-h for the Tidewater Mucket. One Eastern Floater delayed depuration until 288-h, when it released MC at a concentration of 17.7 ng MC L^{-1} (Fig. 3). All muscle tissues of depurated mussels were BDL by the last day of depuration, indicating the mussels had been purged of MC in 10.75 days.

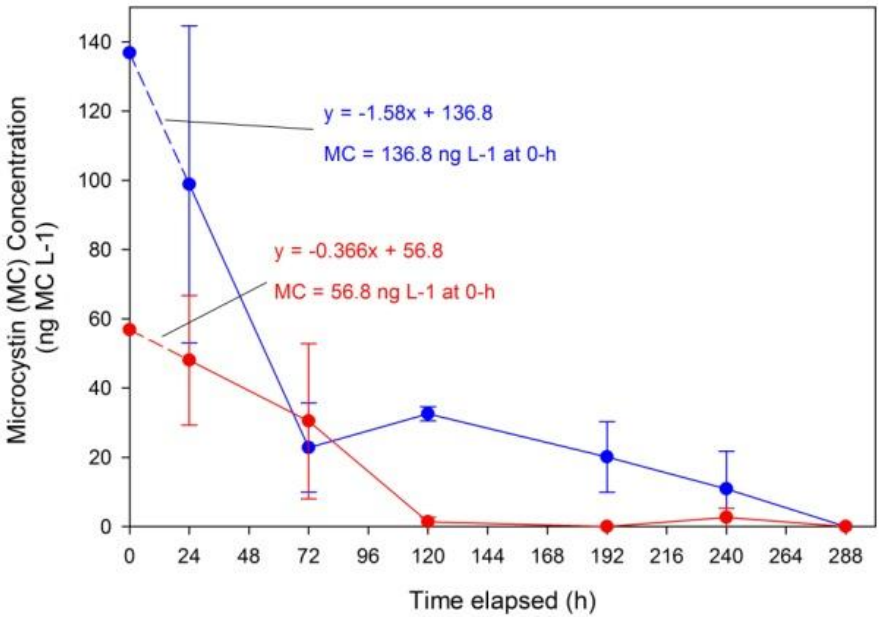

Fig. 3: MC released into water from Tidewater Muckets (blue line) and Eastern Floaters (red line) collected from Middle Pond (post-bloom). MC values at 0-h were extrapolated from start of depuration to the collection time at 24 h (dotted lines). Note: at 120-h, 1343.17 ng L^{-1} was found depurated from one Eastern Floater.

MC Release Rates- MC release rates varied between individual mussels. Eastern Floaters had an average MC release rate of 48.01 +/- 18.70 ng MC L^{-1} in the first 24-h of depuration (Table 4). Tidewater Muckets had a higher average MC release rate at 98.82 +/- 45.80 ng MC L^{-1} after the first 24-h of depuration (Table 4).

Table 4: MC release rates by individual Eastern Floaters and Tidewater Muckets, based on the first 24-h MC depurations.

Microcystin Release Rate (MC/mussel/day)	
Mussel Species	**ng MC L^{-1}**
Pyganodon cataracta (Eastern Floater)	48.0 +/- 18.7
Leptodea ochracea (Tidewater Mucket)	98.8 +/- 45.8

Based on calculated data, Eastern Floaters had the greatest release of MC after 96-h of depuration (Fig. 4).

Tidewater Muckets had the greatest release of MC after the first 24-h. The rate of MC released for Tidewater Muckets, decreased exponentially (with the exception of MC released after 72-h) before ultimately reaching zero release (Fig. 5).

Discussion

Microcystins (MC) in the water taken from bloom conditions were surprisingly lower than water MC detected in the post bloom condition. This is contradictory to many other studies which have shown that MC concentrations are higher in water systems during cyanobacteria blooms (Shen et al. 2002). Although it might be argued that this could be due to release of

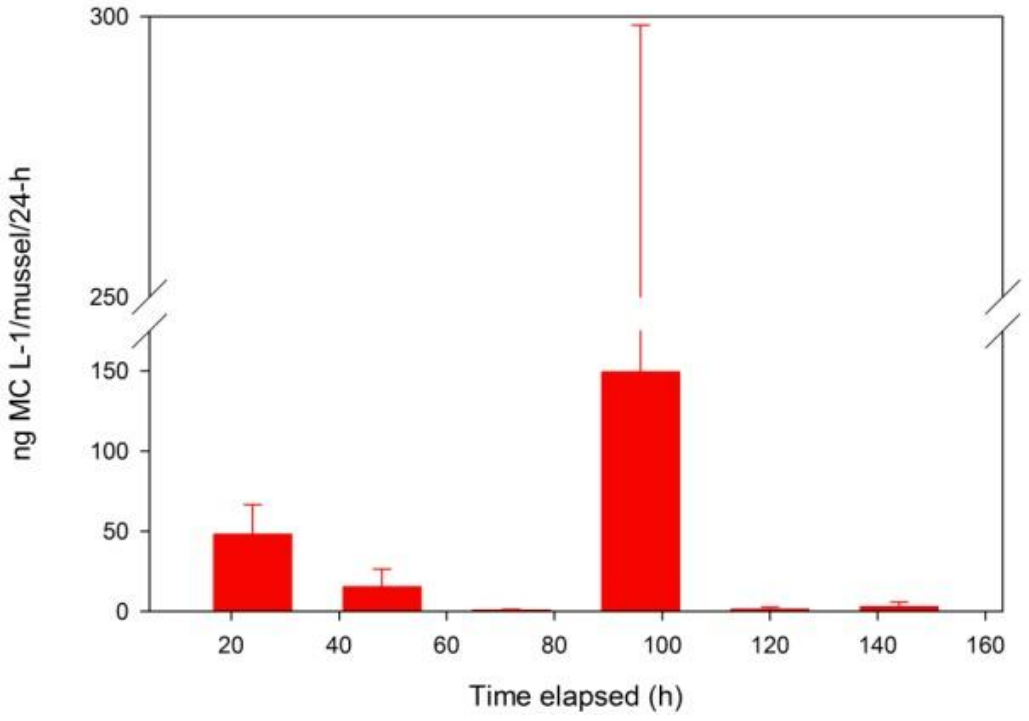

Fig. 4: Average MC released from Eastern Floaters over 24-h periods. N=3
*Calculations based on data collected from depuration experiments.

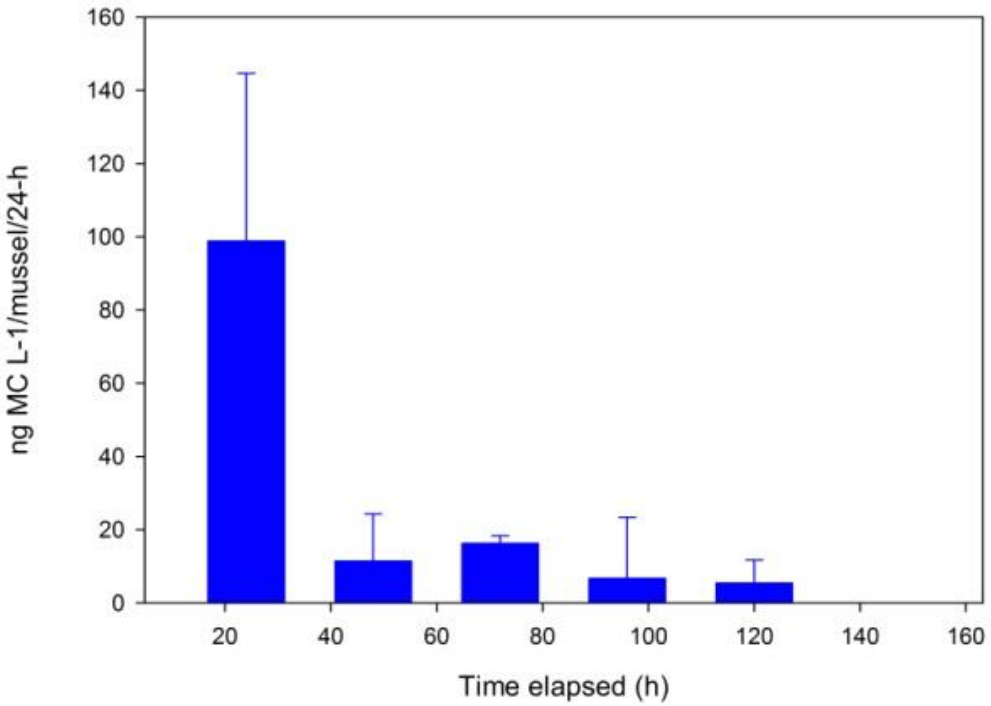

Fig. 5: Average MC released from Tidewater Muckets over 24-h periods. N=3
*Calculations based on data collected from depuration experiments.

MC from decomposing cyanobacteria, this is unlikely since extraction procedures in the laboratory using freeze-thaw and sonication of the cells should have released intracellular MC from both bloom and post-bloom water.

The high concentrations of MC in the mussels collected after the bloom raise some questions concerning the relationship between MC in the mussels and water. Filtration of phytoplankton by mussels is generally viewed as a positive effect on water quality by reducing chlorophyll α levels and increasing transparency (Ministry of Environment and Energy 1997). Some mussels exhibited a delayed release of MC into the water suggesting the potential of mussels to release toxins into a lake after a cyanobacteria bloom has passed. The large release of MC after 120-h of depuration (1343.17 ng MC L^{-1}), from an individual mussel, supports this hypothesis. Assuming mussels release free MC in the lake at rates comparable to those observed in this depuration experiment, mussels might also contribute to an increase in the concentration of MC in the water, effectively reducing water quality.

Muscle tissue MC may be the result of long term exposure of the mussel to toxins. For example, MC extracted by the mussel from food ingested might be accumulated in the muscle tissue over time both as free MC as well as MC covalently bound to protein (Williams et al. 1997), whereas the hepatopancreas would be expected to have a higher turnover rate of MC. Thus, hepatopancreas tissue should provide MC concentrations that correlate with recent conditions to which the mussel was exposed. The hepatopancreas also has a high lipid concentration and therefore has the potential to effectively accumulate the lipid-soluble microcystins. For this reason, the toxins may be intercepted by the hepatopancreas before they can be stored in the muscle tissue, thereby leading to higher MC concentrations found in the hepatopancreas (Watanabe et al. 1997).

Elliptio complanata, may filter cyanobacteria at rates of 1-3 L h^{-1}, depending on the water

temperature and size of the mussel (Bottom 2006, Okumus et al. 2002). The MC release rates described in this paper provide evidence that individual mussels may depurate at varying concentrations. These rates are meant to represent the amount of MC that would have been released into the lake water via mussel during feeding. However, these concentrations could underestimate the actual amount of MC that would have been released in the lake since samples were not collected until the second day of depuration and rates may vary by time. The species of mussel in this experiment did not seem to be an important factor in determining the MC depuration rate and a larger population size would have to be tested to compare. Individual characteristics of the mussel including the age, size, and physiological state of the individual could explain some of the variation in MC release rates (Amorim et al. 1998). In situ, other physical parameters of the system such as water temperature, the amount of cyanobacteria producing MC, and the number of other mussels filtering MC, could also have an influence on the depuration rate.

Conclusion

Microcystins were detected in the tissues of the mussel species present in Mystic Lake and Middle Pond. Thus, all four species appeared to have fed on the toxic cyanobacteria in these lakes.

The concentrations of MC in the Indian Pond mussels (76.84 ng MC g^{-1} dw) were comparable to MC found in Eastern Elliptio in various New England lakes (33.7-738.6 ng MC g^{-1} dw) (Hathaway 2001). Some mussels had high MC concentrations that were comparable to those found in a study performed by Bottom (2006) on Silver Lake (Hollis, NH) mussels, which has high levels of the cyanobacteria *Microcystis aeruginosa* and a stunted population of *Elliptio complanata*. However, the majority of the mussels in the Middle Pond and Mystic Lake had lower MC concentrations, closer to those found in Lake Ossipee, NH, which contains larger *Elliptio complanata* and sparse levels of cyanobacteria (Hathaway 2001, Leuchtner 2006).

It is not possible to conclude that MC was the major factor contributing to the mussel die-off in the Indian Ponds. However, the timing of the bloom events and mussel die-offs that occurred in both Middle Pond and Mystic Lake strongly suggests a causal relationship. Other studies have also shown that the toxins produced by cyanobacteria have serious health consequences and can ultimately lead to death when ingested by animals (Carmichael 1992).

Although concentrations of microcystins were detected in the mussels, this does not exclude the presence of other cyanotoxins such as anatoxin α and neosaxitoxin (neurotoxins) and possible synergistic effects of multiple toxins.

Two intriguing questions raised by this study are 1) the degree to which mussels may at times increase the concentration of microcystins in lake water through natural release and 2) whether delayed release of accumulated cyanotoxins could temporally shift or prolong the period of toxicity causing relatively high concentrations of soluble microcystins after a cyanobacteria bloom has subsided. Monitoring of microcystins in the mussels and water during bloom and non-bloom conditions would help better define and answer these questions. Laboratory feeding experiments could also be performed to assess the ability of the different mussel species present in the Indian Ponds to consume, bioaccumulate, depurate and detoxify microcystins and other cyanotoxins.

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