Microcystin Concentrations in Lake Erie Walleye and Implications for Public Health

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Introduction

Cyanobacterial harmful algal blooms (HABs) degrade water quality of western Lake Erie and create negative economic impacts on an annual basis. Public health is at the forefront of concern because these blooms are often toxic due to an abundance of Microcystis. This genus of cyanobacteria produces the toxin microcystin, which causes gastrointestinal illnesses, damages the liver, and is capable of promoting tumors or death of animals (Poste et al 2011). The World Health Organization (WHO) has set values for microcystin in drinking water, recreational contact, and total daily consumption, but no standards exist for concentrations of microcystin in food. Because microcystin can accumulate in fish tissues, and fishing and fish consumption are important economic and cultural practices in Lake Erie, there is a potential health risk to humans via consumption of fish inhabiting waters with high concentrations of microcystin.

Walleye is one of the most significant sportfish of Lake Erie, and a previous study found this species can have greater microcystin concentrations than yellow perch and white perch studied in the same time period (Wituszynski 2014). For these reasons, this study quantified microcystin levels in walleye tissue using an enzyme-linked immunosorbent assay (ELISA) and compared to public health thresholds used by the Ohio Department of Natural Resources (ODNR). Samples were harvested at different times and from locations in attempt to understand the seasonal correlation between bloom intensity and microcystin concentration, and impacts of variations in bloom intensity at different locations. The effects of chronic exposure of fish to microcystin has not been widely studied for Lake Erie, and no studies presently exist which have examined year-to-year variation in microcystin content in fish. Thus, by comparing this study to a similar study conducted in 2013 (Wituszynski 2014), we can aid in identifying correlation between annual variation in HABS, determine if previous exposure has an effect on

accumulation in fish, and understand when microcystin concentrations in fish tissues may be at its peak when compared to HAB intensity.

Background

Lake Erie is a vital freshwater resource to the nearly 12 million people living along its basin (LEEP 2013). In addition to providing recreation and drinking water, this region has a prominent fishing industry. One of the most popular fish in Lake Erie is walleye (*Sander vitreus*), with an industry valued at tens of millions of dollars annually (U.S. EPA 2009). In 2014, an estimate of 2.869 million walleye was harvested from Lake Erie (Walleye Task Group, 2015). It may be interesting to note that fishing effort from 2013 to 2014 increased by 11% for sport fishing and by 3% in the commercial industry (Walleye Task Group, 2015).

Unfortunately, Lake Erie has been plagued by HABs in recent years. This is especially prominent in the western basin of Lake Erie, which is relatively shallow, allowing most of the region to exist in the photic zone. An abundance of sunlight, warmer temperatures, and nutrient loading of phosphorus create ideal conditions for algal blooms. The excess of phosphorus delivered to western Lake Erie is greatly due to agriculture in the Maumee Watershed and other anthropogenic sources (Bridgeman et al 2013)(LEIA 2012).

While the first noticeable signs of a HAB are the thick layer of greenish cyanobacteria on the surface of the water and the aesthetic and odor issues that follow, HABs greatly affect the ecology of systems. Cyanobacteria can outcompete eukaryotic producers for resources, limiting the growth of beneficial types of algae (Paerl and Huisman 2009). The decomposition of dead algae removes oxygen from the lake and creates a ‘dead zone’ (i.e. a hypoxic region where other organisms, such as fish, cannot survive) in Lake Erie’s central basin. Finally, of most importance

to this research, the cyanobacteria produce toxins, which can cause harm to humans via contact or fish consumption.

While several types of cyanobacteria and toxins exist in HABs, the genus *Microcystis* makes up the majority of the blooms in Lake Erie (LEEP 2013). These species produce the hepatotoxin 'microcystin', which causes gastrointestinal illnesses, damages the liver, and is capable of promoting tumors or death of animals (Poste et al 2011). Microcystin has many congeners, of which MC-LR is the most studied and most toxic (WHO 2003), and a bloom can be more or less noxious depending on which congeners are present. Most of the toxin release occurs as cells lyse. The major routes of human exposure to microcystin include consumption of contaminated drinking water and absorption through skin during contact, such as recreational swimming. Advisories are normally issued when the concentration of microcystin exceeds WHO limits at 20 µg/L for recreational use and 1 µg/L for drinking water. Previous measurements have found levels of microcystin in Lake Erie water above WHO limits, creating cause for concern (LEMNST 2011)(Poste et al 2011). As recent as August 2014, the water supply to Toledo, Ohio was contaminated by microcystin and deemed unsafe to consume, cutting off drinking water for nearly half a million people.

Although the focus of advisories has been on water consumption and recreation, it is possible that fish consumers can also be exposed to the toxin (LEMNST 2011). Microcystin can accumulate in fish tissues, but overall concentration can vary greatly between individuals and between species. The World Health Organization has established a tolerable daily intake value (TDI) for chronic exposure to MC-LR at 0.04 µg/kg body weight. (WHO 2003). Using this guideline and taking into account several factors, Ibelings and Chorus (2007) derived a seasonal TDI value of 300 µg of MC/kg body weight for healthy adults and 40 µg of MC/kg body weight

for children, and lifetime TDI decreases by tenfold. For the purposes of this study, we have utilized ODNR's TDI guidelines, which can be seen in Table 1, and analyzed only edible walleye tissues.

Population of Interest	Average daily fish consumption	Level of concern (lifetime TDI) (ng MC / ng wet weight)	Level of concern (Seasonal TDI) (ng MC / ng wet weight)
Average U.S. Consumer	6.5 g / day[a]	431 ng / g	4310 ng / g
ODNR Advisory Level	24.3 g / day[b]	115 ng / g	1150 ng / g
Lake Erie Angler	40 g / day[c]	70 ng / g	700 ng / g
Tribal Member with traditionally high fish consumption – low estimate	190 g / day[c]	14.7 ng / g	147 ng / g
As above, high estimate – "Very high fish consumers"	328 g / day[c]	8.5 ng / g	85 ng / g

Table 1. Threshold values of microcystin in fish tissues as reported in Wituszynski 2014. Same data is used to allow for direct comparison. Sources of daily fish consumption: a. derived from Dyble et al 2011 b. derived from ODNR Fish Advisory c. quoted in Dyble et al 2011

Microcystin can be transferred to fish tissue through ingestion of *Microcystis*, food web transfer, or passage through gills (Smith and Haney 2006)(Schmidt et al 2013). Several studies found that walleye accumulated more toxins than other fish species (i.e. white and yellow perch) sampled in the same study. Poste et al (2011) studied seven fish species from Lake Erie, including the popular sportfish yellow perch and walleye. The highest microcystin concentrations were observed in walleye tissue, some samples at concerning levels as they exceeded the WHO provisional TDI in place. However, this study had relatively small samples sizes from Lake Erie (n=2-7 per species). Likewise, Wituszynski (2014) found that of 33 walleye

sampled, 9 were above lifetime TDI advisory level at 119 ng MC/g ww and 19 were above the threshold for lifetime TDI for a Lake Erie angler. However, both Poste et al (2011) and Wituszynski (2014) analyses were based on ELISA, which has wide variation in the results and should not be used for direct comparison between species. Because absolute numbers cannot be determined with this method, it is necessary to use HPLC results for clarity. It is also important to note that chronic exposure of walleye to microcystin in Lake Erie has not been well studied, despite its significance in the culture and diet of that region.

Organisms have the capacity to rid themselves of these toxins. Dyble et al (2011) found that fish exposed to microcystin could eliminate the toxin within 24 hours. However, this was from a single oral dose rather than a chronic exposure as fish in an aquatic ecosystem such as Lake Erie would experience. Additionally, Adamovsky et al (2007) performed a 9-week chronic exposure experiment to measure depuration rates in carp. Carp inhabiting waters with an average microcystin concentration of 17 µg/L had greatest concentrations of microcystin in their tissues after four weeks and less significant increases during longer periods of nine weeks. Once moved to clean water, the carp could rapidly eliminate microcystin within two weeks. Interestingly, no obvious correlation exists between microcystin levels in the water column and respective fish tissues (Schmidt et al 2013)(Wituszynski 2014)(Guo and Xie 2006), but the occurrence of a HAB can increase toxicity in certain species (Wituszynski 2014). These studies introduce the need to attempt to identify trends between varying microcystin levels in the water and accumulation in Lake Erie walleye tissue, i.e. a lag may exist in accumulation and a relevant study can identify whether the entire length of exposure or intensity of a bloom may be a greater factor in fish toxicity.

It is still unclear whether prior exposure to algal toxins influences accumulation or depuration rates. In one study, no correlation was seen between age and toxicity, indicating that previous exposure did not influence toxin accumulation (Wituszynski 2014). However, it has also been found that fish can accumulate the toxin during chronic exposure, and then are able to greatly decrease the concentration of microcystin in their liver tissue despite a constant consumption of the toxin (Smith and Haney 2006). This seems to indicate a development of an efficient depuration system, which means that fish may accumulate fewer toxins if they lived through a HAB event before. Exposure to microcystin may also create tolerance over generations (Gustafsson and Hanson 2004).

Overall, the previous studies show the need for continual monitoring, as it is not yet clear what factors influence microcystin concentrations. Discrepancies between data sets, which include the use of different extraction methods and analysis, increase the difficulty of trying to compare across studies. Of most importance to this study, it has been observed that the severity of algal blooms varies annually, the response of organisms with respect to accumulation can vary greatly, and there is a lack of long-term studies.

Procedures and Methodology

This project follows the same procedures as described in Wituszynski 2014, which were influenced by Hu et al. 2008, and Moreno et al 2005. Walleye fillets were obtained from three general sources (provide map with sample locations). First, 16 samples were collected 14 July 2014, 8 August 2014, and 28 August 2014 by charter boats participating in water quality monitoring supervised by Stone Laboratory at OSU. The harvested fish were measured and filleted by the fishermen, after which a portion of the belly flap was placed in ice. Fish were aged using scales, as described in 'Manual of Fisheries Survey Methods II' (2000). Second, 10

samples were collected by ODNR on 9 October 2014. These were measured and aged by ODNR using otoliths, then transported and stored in the Aquatic Ecology Laboratory at OSU at -80°C. The date and location of collection and length of fish were obtained for the charter boat and ODNR samples. Additionally, water quality parameters were measured by the OSU Stone Laboratory from water samples collected by the charter boats. Third, 19 samples were purchased from Columbus-area grocery stores on 16-20 October 2014. For these samples, it was assumed that fish were harvested within a week of purchase of fillets. It was known that these fish were collected on the Canadian side of Lake Erie, because of management regulations on commercial fishing. In addition, fish collection is approximately 1.5 times greater in Management Unit 1 (i.e. the western basin) than all other regions combined, and nearly 3 times greater in MU 1 than MU 2 (Walleye Task Group, 2015). Because of these facts and the prominence of commercial ports in the western basin, it was assumed that the grocery store samples were collected from this region (refer to Figures 2 and 3, which depict the management units and show the collection estimates by MUs).

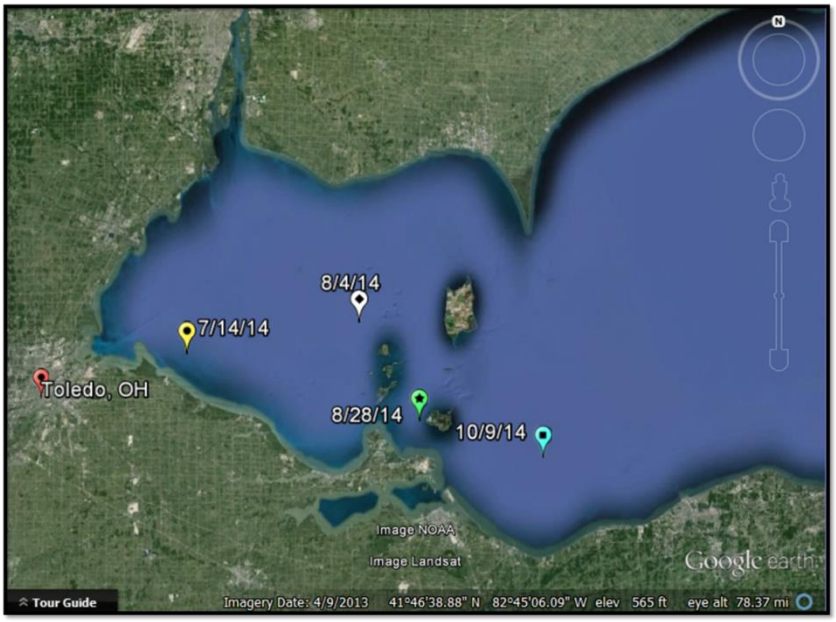


Figure 1. Map of charter boat and ODNR sampling locations in Lake Erie. Charter boat samples include 7/14-8/28/14, and ODNR sampled 10/9/14.

Once received, fillets were assigned a sample number. Skin and belly lining were removed from samples. Remaining tissues were diced, placed in ceramic crucibles, and weighed. Ceramic dishes were weighed prior to addition of sample. Dishes were dried in oven at 60°C for 24 hrs. Samples were weighed after drying, and wet-to-dry conversion factors were determined.

Dried tissues were homogenized using a mortar and pestle. Approximately 0.55 grams of each homogenized sample were measured and placed in flasks. Samples were extracted with 20 mL of 75% methanol for two hours, at room temperature on stirplates. Samples that were smaller than 0.5 grams were extracted with less methanol (e.g. sample of 0.1 grams will be extracted with 5 mL of 75% methanol).

Extracts were removed from the flasks and centrifuged at about 4,750 rpm for 15 minutes. Supernatant were then removed and pooled as appropriate. Solids were resuspended in 75% methanol for another extraction. A total of three extractions were completed for each sample. At the end of the third extraction, the remaining solids were consolidated into one 15 mL centrifuge tube (instead of resuspended) and centrifuged for 15 minutes. This final amount of supernatant was removed and added to the other supernatant tubes as appropriate.

Supernatants from each sample were diluted with three times as much DI water. This solution was passed twice through a SepPak® C18 column (Waters corporation, Milford, Massachusetts). Microcystin was eluted from the column with 5 mL of 100% methanol (or 1mL if sample was extracted with 5 mL of 75% methanol).

Samples were diluted to less than 5% methanol, according to directions provided by the ELISA kit. Samples were centrifuged and analyzed using the Microcystins/Nodularins (ADDA) ELISA kit. Each sample was run in triplicates. Dry-weight concentrations were converted to wet-weight concentrations using appropriate conversion factor. Because the opportunity exists for

matrix effects to occur at low concentrations of microcystin when running an ELISA, a subset of the samples should be measured with a HPLC.

Controls were run using store-bought walleye harvested in April 2014. The first control will be only walleye. The second was spiked with 50 µL of *Microcystis* extract, and a third had 75 µL of the same extract added. These controls were run through the same process and analysis as the samples. Extraction efficiencies are reported in Table 1.

Data Analysis

All data analyses were performed in JMP 11.0 and Microsoft Excel, using primarily ANOVAs and Student's t-tests with $\alpha = 0.05$. Samples that were determined to have concentrations below the lower limit of detection were considered to have 'non-detectable levels.'

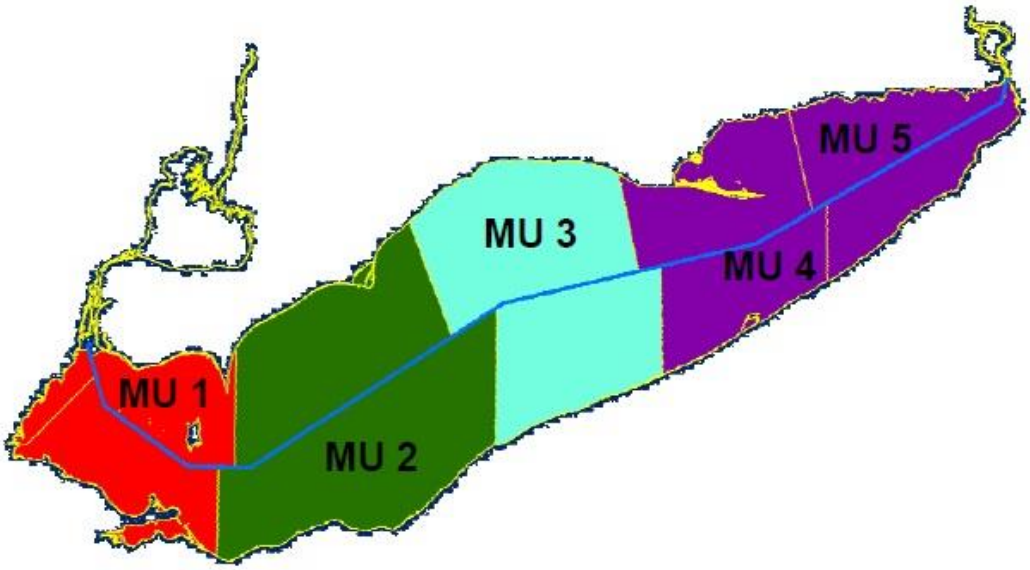


Figure 2. Map of Lake Erie management units as recognized by the Walleye Task Group

	Sport Fishery															Commercial Fishery					
	Unit 1				Unit 2			Unit 3			Units 4 & 5										
Year	OH	MI	ON[a]	Total	OH	ON[a]	Total	OH	ON[a]	Total	ON[a]	PA	NY	Total	Total	Unit 1 ON	Unit 2 ON	Unit 3 ON	Unit 4 ON	Total	Grand Total
1975	77	4	7	88	10	--	10	--	--	--	--	--	--	0	98	--	--	--	--	0	98
1976	605	30	50	685	35	--	35	--	--	--	--	--	--	0	720	113	44	--	--	157	877
1977	2,131	107	69	2,307	37	--	37	--	--	--	--	--	--	0	2,344	235	67	--	--	302	2,645
1978	1,550	72	112	1,734	37	--	37	--	--	--	--	--	--	0	1,771	274	60	--	--	334	2,106
1979	3,254	162	79	3,495	60	--	60	--	--	--	--	--	--	0	3,555	625	30	--	--	655	4,211
1980	2,096	183	57	2,336	49	--	49	24	--	24	--	--	--	0	2,409	953	40	--	--	993	3,402
1981	2,857	95	70	3,022	38	--	38	48	--	48	--	--	--	0	3,108	1,037	119	3	--	1,159	4,268
1982	2,959	194	49	3,202	49	--	49	8	--	8	--	--	--	0	3,259	1,077	134	2	--	1,213	4,470
1983	1,626	146	41	1,813	212	--	212	26	--	26	--	--	--	0	2,051	1,129	167	80	--	1,376	3,427
1984	3,089	351	39	3,479	787	--	787	179	--	179	--	--	--	0	4,445	1,639	392	108	--	2,139	6,584
1985	3,347	461	57	3,865	294	--	294	89	--	89	--	--	--	0	4,248	1,721	432	225	--	2,378	6,627
1986	3,743	606	52	4,401	480	--	480	176	--	176	--	--	--	0	5,057	1,651	558	356	--	2,565	7,622
1987	3,751	902	51	4,704	550	--	550	132	--	132	--	--	--	0	5,386	1,611	622	405	--	2,638	8,024
1988	3,744	1,997	18	5,759	584	--	584	562	--	562	--	--	85	85	6,990	1,866	762	409	--	3,037	10,026
1989	2,891	1,092	14	3,997	867	35	902	434	80	514	--	--	129	129	5,542	1,656	621	386	--	2,663	8,206
1990	1,467	747	35	2,249	389	14	403	426	23	449	--	--	47	47	3,148	1,615	529	302	--	2,446	5,595
1991	1,104	132	39	1,275	216	24	240	258	44	302	--	--	34	34	1,851	1,446	440	274	--	2,160	4,011
1992	1,479	250	20	1,749	338	56	394	265	25	290	--	--	14	14	2,447	1,547	534	316	--	2,397	4,844
1993	1,846	270	37	2,153	450	26	476	372	12	384	--	--	40	40	3,053	2,488	762	496	--	3,746	6,800
1994	992	216	21	1,229	291	20	311	186	21	207	--	--	59	59	1,806	2,307	630	432	--	3,369	5,176
1995	1,161	108	32	1,301	159	7	166	115	27	141	--	--	27	27	1,635	2,578	681	489	--	3,748	5,384
1996	1,442	175	17	1,634	645	8	653	229	27	256	--	89	39	128	2,671	2,777	1,107	589	--	4,473	7,143
1997	929	122	8	1,059	188	2	190	132	5	138	--	89	29	118	1,505	2,585	928	544	--	4,057	5,563
1998	1,790	115	34	1,939	215	5	220	299	5	304	19	125	34	178	2,641	2,497	1,166	462	28	4,153	6,793
1999	812	140	34	986	139	5	144	83	5	88	19	89	23	131	1,349	2,461	631	317	68	3,477	4,827
2000	674	252	34	961	165	5	170	93	5	98	19	78	29	125	1,354	1,603	444	196	48	2,291	3,645
2001	941	160	34	1,135	171	5	176	46	5	51	19	53	15	87	1,449	1,004	310	141	20	1,475	2,924
2002	516	194	34	744	141	5	146	46	5	51	19	22	18	59	1,000	937	309	146	17	1,409	2,409
2003	715	129	34	878	232	5	237	68	5	73	2	44	27	73	1,261	948	283	182	14	1,427	2,688
2004	515	115	34	664	272	2	274	72	0	72	2	20	8	30	1,040	866	334	175	11	1,386	2,426
2005	374	38	27	438	110	2	112	126	0	126	2	20	27	49	725	1,878	625	401	15	2,920	3,645
2006	1,194	306	27	1,526	503	2	505	170	0	170	2	152	37	191	2,392	2,137	784	545	66	3,532	5,924
2007	1,414	166	27	1,607	578	2	580	169	0	169	2	116	29	147	2,502	1,348	450	333	35	2,167	4,669
2008	524	121	44	689	333	2	335	225	0	225	2	74	29	105	1,354	954	335	241	35	1,565	2,919
2009	553	94	44	691	287	2	288	128	0	128	2	42	14	58	1,166	705	212	135	28	1,079	2,244
2010	587	55	44	686	257	2	259	114	0	115	2	54	37	93	1,152	607	184	147	23	962	2,115
2011	224	50	44	318	104	2	106	89	0	90	2	45	32	79	593	736	262	181	29	1,208	1,801
2012	596	87	44	726	233	2	235	93	0	93	2	45	37	84	1,138	834	285	191	28	1,338	2,476
2013	757	54	44	855	190	2	192	136	0	136	2	60	35	97	1,280	737	297	195	31	1,260	2,540
2014	909	42	45	996	177	13	190	218	13	231	13	85	62	160	1,577	756	259	238	40	1,292	2,869
Mean	1,547	269	40	1,856	274	10	280	165	12	174	7	68	36	58	2,346	1,400	436	285	31	2,042	4,389

[a] Ontario sport harvest values were estimated from the 2014 lakewide aerial creel survey. These values are included in Ontario's total walleye harvest, but are not used in catch-at-age analysis.

Figure 3. Annual harvest (in thousands of fish) of Lake Erie Walleye by industry, management unit, and year. Means include data from 1975-2013. 2014 data is most important to this study, in which MU1 had the greatest annual harvest that is approximately three times greater than MU2. This led to the assumption that the store-bought samples are likely from MU1.

Results

All data collected during this experiment can be seen in Table 4 on pages 21 and 22.

Extraction Efficiency

Amount of *Microcystis* Added	Microcystins in *Microcystis* extract	Microcystins added to sample	Microcystins recovered from sample	Extraction efficiency
50 µL	598 µg/L	29.9 ng	14.2 ng	47%
75 µL	598 µg/L	44.85 ng	20.9 ng	47%

Table 2. Extraction efficiency of microcystin in control samples

Concentrations

A total of 44 walleye samples were analyzed, 34 of which had non-detectable concentrations. All charter boat and ODNR samples, which were harvested in July, August, and early October, had non-detectable (ND) concentrations of microcystin when analyzed using ELISA. The average for the store-bought samples was 17.48 ng/g ww and had a range between ND and 88.13 ng/g ww. The lower limits of detection vary with each sample and ranged from 1.17 to 51.22 ng/g ww, with an average of 9.04 ng/g ww. A summary of data is provided in Table 3.

Date Caught/ Bought	Sample Size	Number of Non-Detects	Concentration found in fish (ng MC/g ww)	Lower limit of detection	Sample mass ('wet weight', in grams)	Fish length (inches)	Fish age (years)
7/14/14	5	5	ND	11.102	1.027	21	7
8/4/14	5	5	ND	31.325	0.388	16.583	6
8/28/14	6	6	ND	15.01	1.377	19.729	6.5
10/9/14	10	10	ND	4.466	2.529	19.764	3.1
10/16-10/20/14 *	18	6	17.477	5.288	2.725	N/A	N/A

Table 3. Sample summary table, categorized by date of fish harvesting or date the fish were bought from store, which is indicated by asterisk. Reported values are averages for that date.

Age

The ages of the 16 charter boat samples ranged from 4-11 years and the average was 6.5 years. The ages of the ten ODNR samples ranged from 2-5 years, with an average of 3.1 years. It was not possible to determine the ages of the store-bought samples since scales are removed from the fish before sale.

Discussion

Only trace levels of microcystin was found in the walleye tissue. A total of 34 samples had microcystin concentrations at non-detectable levels. Toxin concentrations in walleye tissues were significantly below the ODNR lifetime TDI provision of 70 ng/g ww in 42 of 44 samples, indicating there is not an apparent threat to public health with respect to microcystin exposure from fish consumption.

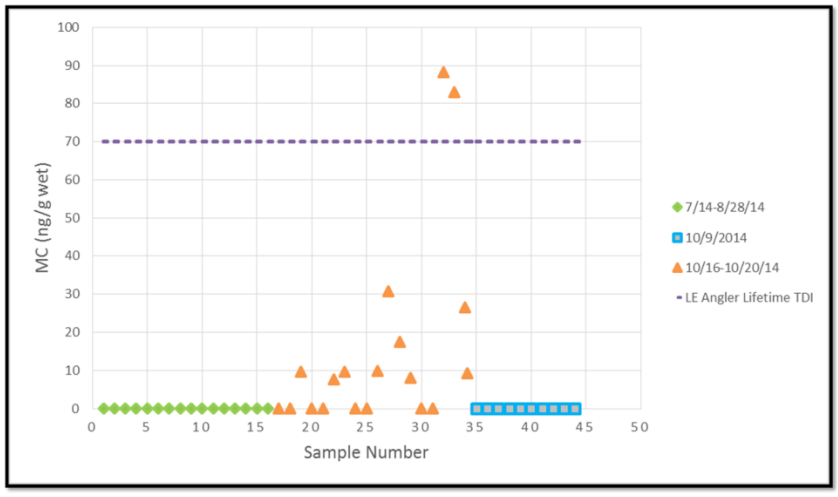


Figure 4. Microcystin concentrations in walleye tissues by sample number, with date caught or bought indicated. The dashed line represents the lifetime TDI for a Lake Erie Angler, set at 70 ng MC/g ww by ODNR. Non-detectable concentrations were set to zero in this graph.

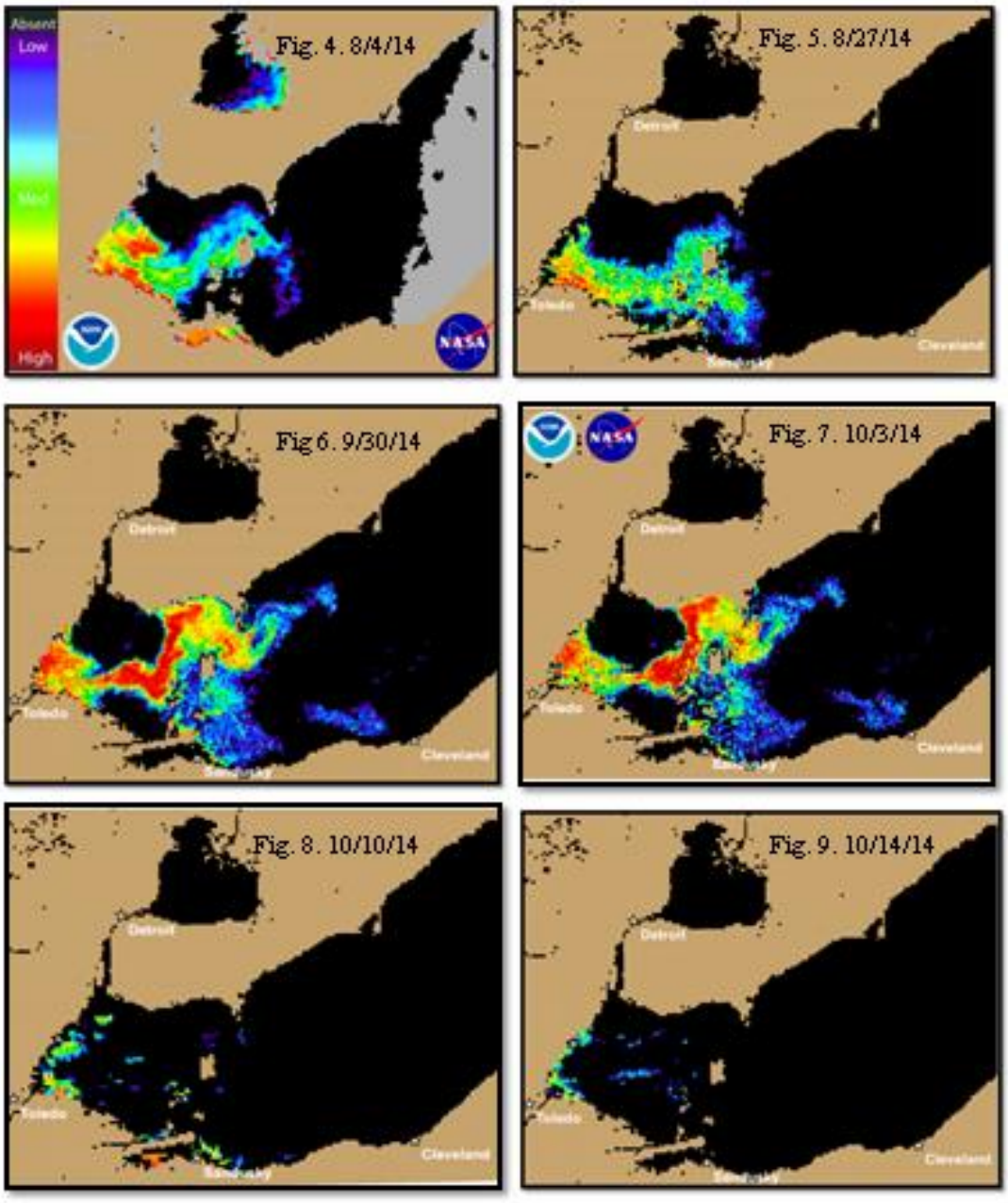
This data set differed greatly from the 2013 data found in the Wituszynski study, which had an average of 85 ng MC/g ww (n= 33) and only three of those samples were below the detection limits. The lower limits of detection in the study were also higher, ranging from 17.94 to 49.96 ng MC/g ww. Because studies have shown that the occurrence of a HAB can increase the amount of microcystin in fish tissues and there are significant differences between two years of study (i.e. 2013 and 2014), it is suspected that bloom severity also plays a role. The 2013 bloom was 'severe,' having a CI value of 8, and the 2014 bloom had a CI value around 4.5 (UM/GLERL 2014). This, along with the fact that age does not appear to factor in toxin concentrations in fish, supports the hypothesis that concentrations in fish tissue are linked to the severity of each seasonal bloom rather than accumulating year-to-year.

Microcystin was detected only in the samples harvested from the western basin after the peak of the HAB, so it is expected that bloom severity at times of collection accounts for the differences between concentrations in samples. Based on NASA's Moderate Resolution Imaging Spectroradiometer (MODIS) satellite imagery, it appears that the 2014 HAB peaked in late September (Figures 4-9). The MODIS imagery from 30 September 2014 and 3 October 2014 shows a high concentration of cyanobacteria in the western basin and especially near the Ontario Ports in MU1. Because the bloom was not severe during expected time of harvest for the store-bought samples as can be seen in Figures 8 and 9, it is then suspected that the microcystin found in the walleye tissue is remaining from the bloom peak. While the ODNR and store-bought samples were likely caught around the same date, the ODNR samples are closer to the central basin and in a region that does not appear to have been greatly affected by HABs (refer to Figures 1 and 4-9).

The findings of this study seem to be in accordance with Adamovsky et al (2007) that found that it takes about two weeks for carp to eliminate microcystin from their muscle. The charter boat water samples at microcystin concentrations approximately half of the constant concentration used in Adamovsky, so it is possible that was a low enough concentration to allow the walleye's metabolism to keep up with the toxin intake. It is difficult to make definite conclusions without having samples from September or later in October.

It should also be noted that detection limits vary with each sample. The charter boat samples generally had higher limits of detection, which means they could have had comparable concentrations to those of the store-bought samples that just did not appear in the ELISA due to the wet weight of those samples. The masses of the charter boat and store-bought samples were significantly different (t-test, p-value= 1.83669E-08), and a small sample mass greatly increases the limit of detection.

Water quality parameters were not available for the store-bought or ODNR samples. Therefore, all data analysis was performed using only 16 samples that had randomly assigned concentrations below the detectable levels. In addition, samples taken from the same location had the same or similar data, meaning several factors can have the same statistical significance in analysis, as is the case with microcystin concentrations in the water column and wave height, for example. With this in mind, the data cannot be used to successfully draw conclusions on the factors contributing to microcystin concentrations and provides weak evidence, at best.



Figures 4-9. MODIS satellite images of HAB severity in western Lake Erie. Warm colors indicate high concentrations of cyanobacteria, while cool colors indicate low concentrations of cyanobacteria. Black coloring indicates no cyanobacteria found. Estimated threshold of detection is 35.000 cells/mL. It appears that the bloom was most severe at the end of September and early October. A map is not seen for the July 14 samples, because there was no bloom reported at the time. Images obtained from GLERL's HAB Bulletin Archive.

Recommendations

It is necessary to continue monitoring Lake Erie HABs and their impacts on microcystin concentrations in sportfish. Therefore, annual analysis on walleye tissues is imperative, but needs to be linked with CI values and complete water quality data sets. A controlled laboratory experiment may provide greater insight in order to verify hypotheses of chronic exposure to microcystin such as there being a lag between a HAB peak (i.e. greatest microcystin concentrations in water at a time) and maximum walleye tissue concentrations, and questions regarding which time a fish might have the greatest toxicity. A relevant study could be created to understand how varying microcystin levels within a season affects depuration rates, and if there is a point that changes the two week approximation. Otherwise, close monitoring of CI values and concentrations with more frequent sampling is necessary.

Additionally, it is necessary to have larger samples or better adjust the amount of methanol used. When increasing the amount of sample relative to the amount of methanol, this decreases the lower limit of detection, and makes the ratio comparable to samples with greater masses.

Conclusion

In an analysis of 44 samples, only trace concentrations of microcystin were found in the walleye tissues, indicating fish consumption with respect to microcystin was likely not a concern to public health in 2014. The current ODNR recommendations to consume one fish meal per week should suffice in protecting against microcystin toxicity. Because higher concentrations were detected using similar methods in 2013, it is suspected that microcystin levels in fish tissue are related to the severity of the annual HAB. Therefore, it is necessary to continue fish tissue analysis to test this hypothesis and continue to safeguard public health.

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Sample	Concentration in fish (ng MC/ g wet weight)	Lower Limit of Detection (ng MC/ g ww)	Source	Date Caught /Bought	Length (inches)	Age (years)	Weight of whole fish (g)	Water Depth (ft)	Microcystin in water (µg/L)	Chlorophyll-a (µg/L)	TP in water (mg P/L)	TN:TP (g/g)	Waves (ft)	Surface Water Temp (F)
1	ND	51.221	C.b.	8/4/2014	19	6		32	7.36	14.44	0.014	46.94	.5	76.7
2	ND	16.313	C.b.	8/4/2014		11		32	7.36	14.44	0.014	46.94	.5	76.7
3	ND	46.494	C.b.	8/4/2014	15.5	5		32	7.36	14.44	0.014	46.94	.5	76.7
4	ND	16.313	C.b.	8/4/2014	15.25	4		32	7.36	14.44	0.014	46.94	.5	76.7
5	ND	26.283	C.b.	8/4/2014		4		32	7.36	14.44	0.014	46.94	.5	76.7
6	ND	23.027	C.b.	7/14/2014	19.25	4		23	<0.15	7.93	0.0263		1	76
7	ND	20.954	C.b.	7/14/2014	21.5	6		24	<0.16	7.93	0.0263		1	77
8	ND	1.543	C.b.	7/14/2014	24	8		25	<0.17	7.93	0.0263		1	78
9	ND	6.731	C.b.	7/14/2014	20	8		26	<0.18	7.93	0.0263		1	79
10	ND	3.253	C.b.	7/14/2014	20.25	9		27	<0.19	7.93	0.0263		1	80
11	ND	1.787	C.b.	8/28/2014	21.5	6		10	6.54	37.65	0.0252	30.39	1-2	74
12	ND	28.785	C.b.	8/28/2014	19.25	6		10	6.54	37.65	0.0252	30.39	1-2	74
13	ND	15.961	C.b.	8/28/2014	20.13	7		10	6.54	37.65	0.0252	30.39	1-2	74
14	ND	8.667	C.b.	8/28/2014	21.5	6		10	6.54	37.65	0.0252	30.39	1-2	74
15	ND	19.220	C.b.	8/28/2014	17.5	7		10	6.54	37.65	0.0252	30.39	1-2	74
16	ND	15.641	C.b.	8/28/2014	18.5	7		10	6.54	37.65	0.0252	30.39	1-2	74
17	ND	6.969	G.s.	10/17/2014										
18	ND	7.285	G.s.	10/17/2014										
19	9.568	8.182	G.s.	10/17/2014										
20	ND	6.694	G.s.	10/17/2014										
21	ND	6.832	G.s.	10/17/2014										
22	7.743	6.961	G.s.	10/16/2014										
23	9.663	6.871	G.s.	10/16/2014										
24	ND	1.389	G.s.	10/16/2014										
25	ND	1.428	G.s.	10/17/2014										
26	9.773	1.294	G.s.	10/17/2014										

Sample	Concentration in fish (ng MC/ g wet weight)	Lower Limit of Detection (ng MC/g ww)	Source	Date Caught /Bought	Length (inches)	Age (years)	Weight of whole fish (g)	Water Depth (ft)	Microcystin in water (µg/L)	Chlorophyll-a (µg/L)	TP in water (mg P/L)	TN:TP (g/g)	Waves (ft)	Surface Water Temp (F)
27	30.784	1.406	G.s.	10/17/2014										
28	17.559	1.241	G.s.	10/27/2014										
29	8.073	7.025	G.s.	10/16/2014										
30	ND	6.966	G.s.	10/16/2014										
31	ND	6.102	G.s.	10/16/2014										
32	88.132	6.378	G.s.	10/20/2014										
33	82.887	6.320	G.s.	10/20/2014										
34	26.527	5.837	G.s.	10/20/2014										
35	ND	1.583	ODNR	10/9/2014	18.39	4	1533	42.65						
36	ND	7.346	ODNR	10/9/2014	17.05	2	922	42.65						
37	ND	6.825	ODNR	10/9/2014	17.28	2	784	42.65						
38	ND	6.921	ODNR	10/9/2014	20.24	2	712	42.65						
39	ND	8.371	ODNR	10/9/2014	18.11	5	3006	42.65						
40	ND	7.555	ODNR	10/9/2014	17.56	3	1406	42.65						
41	ND	1.466	ODNR	10/9/2014	21.73	3	1410	42.65						
42	ND	1.503	ODNR	10/9/2014	21.14	3	874	42.65						
43	ND	1.913	ODNR	10/9/2014	20.51	3	984	42.65						
44	ND	1.173	ODNR	10/9/2014	24.88	4	1585	42.65						

Table 4. Data from walleye tissue analysis and associated parameters. Key for 'source' column: C.b. = charter boat, G.s. = grocery store, and ODNR = Ohio Department of Natural Resources. Cells shaded in gray indicate that data is not available.