Variation of Microcystin Concentrations in Fish Related to Algae Blooms in Lake Erie, and Public Health Impacts

THESIS

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Abstract

Lake Erie is an important economic and cultural resource that is threatened by recurring blooms of cyanobacteria which produce toxins such as microcystin (MC). This potent liver toxin, which has been linked to human and animal illness and death, has been found in fish from Lake Erie, sometimes in excess of World Health Organization guidelines for safe consumption. Even so, few studies have examined the variation of MC concentrations in fish within Lake Erie, and these past studies have derived conflicting results as to the risk these concentrations pose to public health. This uncertainty likely exists because of the extremely variable nature of the algae blooms from year to year, and because of the different species on which each study has focused.

To address this gap in knowledge, I used ELISA to analyze the toxin content of muscle tissue from three of the most commonly harvested sport and commercial fish in Lake Erie: Walleye (*Sander vitreus*, n=33), Yellow Perch (*Perca flavescens*, n=52), and White Perch (*Monroe Americana*, n=55), collected during summer 2013. Additionally, remote sensing was used to compare toxin concentration to bloom conditions at the time of harvest. Results demonstrated that toxin concentrations in walleye (mean = 85 ng MC / g wet weight), white perch (mean = 37 ng MC / g), and yellow perch (mean = 8.1 ng MC / g) were significantly different. This variation is possibly because of differences in feeding habits among these species. MC concentrations in white perch were sensitive to bloom conditions, whereas those in walleye and yellow perch were not. While few of the fish harvested for this
study exceeded WHO levels of MC established for safe consumption, results indicate that more intense blooms in the future may increase MC in fish to levels that are a threat to public health.
For my family in New Hampshire, who let me leave, but didn’t let go (in a good way);
For my family in Rochester, who took me in when I was first far from home (and eventually became a home for me);
And for my family in Columbus, who continue to guide me in this experiment in independence.
I feel that learning to stand on my own two feet has been at least as much about discovering you all as it has been about discovering myself.
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# Table of Contents

Abstract ................................................................................................................................. ii

Acknowledgments .................................................................................................................... v

Vita ........................................................................................................................................ vi

Fields of Study ....................................................................................................................... vi

Table of Contents ................................................................................................................... vii

List of Tables .......................................................................................................................... ix

List of Figures ........................................................................................................................ x

Sample Collection ................................................................................................................ 11

Toxin extraction and Quantification .................................................................................... 12

Determination of Cyanobacterial Blooms .......................................................................... 14

Additional parameters .......................................................................................................... 16

Data Analysis ......................................................................................................................... 16

Public Health Impact ............................................................................................................ 18

Data collected as part of this study are available in Appendix A. ........................................ 23

Extraction Efficiency ............................................................................................................ 23
Sample Variability................................................................................................................................. 24

Table 2 provides information about the fish collected during this study: .................. 24

Microcystin in All samples .................................................................................................................. 24

White Perch ......................................................................................................................................... 26

Yellow Perch ......................................................................................................................................... 28

Walleye .................................................................................................................................................. 29

Comparison with Public Health Thresholds ...................................................................................... 31

Appendix A: Data Collected for This Study ..................................................................................... 46
List of Tables

Table 1: Extraction efficiency of microcystins from control samples .................................. 23
Table 2: Age, Length, and Bloom Conditions experienced by fish collected for this study
.................................................................................................................................................. 24
Table 3: Threshold values of microcystin in fish tissues according to various sources, and
the number of fish of each species in this study exceeding these values .................... 32
Table 4: Data for White Perch ..................................................................................................... 46
Table 5: Data for Yellow Perch ................................................................................................... 49
Table 6: Data for Walleye .............................................................................................................. 52
List of Figures

Figure 1: Fish for this study were collected from 13 sampling locations in western Lake Erie during August and September 2013. ................................................................. 20

Figure 2: Cyanobacterial bloom (measured as CI) and sites sampled for white and yellow perch in August ........................................................................................................ 21

Figure 3: Cyanobacterial bloom (measured as CI) and sites sampled for white and yellow perch in September .................................................................................................. 22

Figure 4: Toxin concentration (ng microcystin / g wet weight of fish) for all fish examined in this study ........................................................................................................ 25

Figure 5: A pairwise Wilcox test with the Holm p-adjustment method demonstrated that mean microcystin concentrations in all species were significantly different from each other ................................................................................................................ 26

Figure 6: A Wilcox test determined that toxin concentrations in white perch caught on different dates were significantly different ........................................................................ 27

Figure 7: MC in white perch correlated with CI, Date Caught, and their cross ............... 28

Figure 8: MC in yellow perch was not correlated with CI ............................................. 29

Figure 9: MC in walleye was not correlated with MC in the water column ................. 30
Introduction

Cyanobacterial blooms and their associated health threats are a rapidly growing concern worldwide, due to changes in climate and increases in anthropogenic nutrient loading.\textsuperscript{1–3} These blooms can have significant environmental impacts due to shading, hypoxia, and alterations of aquatic food webs.\textsuperscript{4} Additionally, cyanobacteria are known to create several varieties of toxin, and therefore represent a human health threat.\textsuperscript{5} The most widespread and best-studied cyanotoxin is microcystin\textsuperscript{a} (MC), a potent hepatotoxin and a suspected tumor promoter.\textsuperscript{5,6} This toxin has been found worldwide, and has been implicated in human and animal sickness and death.\textsuperscript{6}

The World Health Organization (WHO) has established provisional guidelines for microcystin content in food, drinking water, and in water used for recreational purposes.\textsuperscript{6} The latter two routes of exposure are regularly controlled in the United States, via beach closures and advanced water treatment. Therefore, in developed countries like the United States, food consumption is the most likely route for microcystin exposure. However, few studies have explored the presence of microcystin in commercially and recreationally harvested aquatic organisms, and no regulation has addressed this concern. Herein, I

\textsuperscript{a} Cyanobacteria are known to produce about 80 varieties of microcystin. However, as differentiation among all of these congeners is difficult, they are often lumped and talked of as one toxin. This approach has been taken in this paper.
address this gap in knowledge by determining concentrations of microcystin in several ecologically and commercially important Lake Erie fishes.

The link between microcystin levels in water or algae and those in higher consumers (e.g., fish) is obscure, and dependent on the species and system under study. Therefore, there is a need to examine links between cyanobacterial blooms, which are readily observable, and health risk via fish consumption. This study examines commercially and recreationally important fish taken from the western basin of Lake Erie in 2013 (Figures 1 – 3), and correlates levels of toxin found in edible tissue with local bloom intensity, as well as fish species and size. By comparing these data with previous studies on the toxin content of fish from Lake Erie, I attempt to determine factors that affect the risk of microcystin exposure to humans via fish consumption.
Background and Literature Review

Lake Erie is important economically and culturally, and provides numerous ecosystem services, including opportunities for recreation by swimming, boating, and fishing, as well as support for associated tourism activities. The sport fishing industry alone accounts for as much as $1.2 billion per year in economic activity. Additionally, the lake is the source of drinking water for 11 million people. However, in recent years the lake has been experiencing regular and persistent harmful algal blooms (HABs) that threaten these services. In addition to aesthetic problems related to the presence of the blooms, the organisms that comprise these blooms release toxins into the water. In particular, the blooms on Lake Erie have been dominated by cyanobacteria of the genus *Microcystis*, which are known to produce liver toxins known as microcystins. These toxins have been implicated in human and animal sicknesses and deaths, and have also been linked to tumor formation. As a result, the WHO has set provisional guidelines for safe levels of microcystins in drinking water, for recreational contact, and for food. Though the United States Environmental Protection Agency has set no official limits, New York and Ohio have both recently issued numerous human contact advisories following WHO guidelines, and recent reports indicate that Lake Erie waters experiencing these blooms regularly exceed the WHO threshold for recreational contact (e.g., Watson et al.).
Additionally, there has been concern regarding the consumption of fish from waters with *Microcystis* blooms related to concentrations of microcystin in edible fish tissue. For microcystins (MC) in general, The WHO has detailed a provisional lifetime total daily intake (TDI) amount of 0.04 µg MC / kg body weight. The lifetime TDI is the amount that an individual should be able to consume daily for the rest of his or her life with no ill effects. This particular threshold is based on a 13-week mouse study\(^\text{11}\) that found a No Observable Adverse Effects Level (NOAEL) of 40 µg / kg. To determine the threshold for human consumption, uncertainty factors of 10 were each applied for intra-species variability, inter-species variability, and for a lack of chronic toxicity data, for a total uncertainty factor of 1000; thus, a lifetime TDI of 0.04 µg / kg. This value was further supported by a 44-day study in pigs.\(^\text{12}\) However, according to Ibelings & Chorus\(^\text{5}\), the uncertainty factor associated with a lack of chronic toxicity data is intended to transform the dose from a short-term amount (as necessitated by the length of the studies) to a lifetime amount. They suggest that this last factor should be ignored for events such as algae blooms in temperate lakes, which only occur seasonally; this results in a “seasonal” TDI of 0.4 µg MC / kg body weight. They also suggest a maximum safe value of 2.5 µg MC / kg body weight for a single exposure event, derived from the results of several acute toxicity studies.\(^\text{13}\)

Starting from this recommendation, threshold values for concentrations of MC in fish may be derived. For Lake Erie, Dyble *et al.*\(^\text{14}\) started with the WHO guideline of 0.04 µg / kg and, assuming a 70 kg individual and various relevant consumption rates, derived thresholds ranging from 431 ng microcystin / g fish for the average consumer to
8.5 ng microcystin / g fish for very high fish consumers. Mulvenna et al.\textsuperscript{15} used the 97.5\textsuperscript{th} percentile of fish consumption in Australia and New Zealand to derive safe thresholds for microcystin content in fish tissue: humans of age >17 could consume fish with 39 ng MC / g fish tissue, and humans of age 2 – 16 could consume fish with 24 ng MC / g fish tissue (these authors based their estimates on a lifetime TDI of 0.2 µg MC / kg, which they had derived using a procedure similar to that of the WHO). The US EPA has also issued guidelines for microcystin exposure that are an order of magnitude lower than those from the WHO; however, they are in a draft provisional report which is not to be cited or quoted.\textsuperscript{16}

However, despite this work in delineating guidelines, it is generally uncertain whether microcystins in fish present a threat to public health: while there is strong evidence that microcystin consumption is dangerous, observed concentrations of microcystin in fish vary greatly. In turn, several studies have found little to no danger of microcystin intoxication via fish consumption (Kopp et al.\textsuperscript{17}, fish from several ponds and reservoirs in the Czech Republic; Niedzwiadek et al.\textsuperscript{18}, fish and shrimp sold commercially in Canada). In contrast, Poste et al.\textsuperscript{19} found potentially dangerous levels of microcystin in fish from both temperate and tropical lakes. Schmidt et al.\textsuperscript{20} include a table summarizing the results of several studies on microcystin levels in fish, which range from 0.5 – 1960 ng MC / g dry weight.

In addition to uncertainties about the MC concentrations in fish, it also is unclear what factors affect these concentrations. In a study in Lake Ijsselmeer, the Netherlands, Ibelings et al.\textsuperscript{21} showed that MC is transferred up the food web, from cyanobacteria to
zooplankton to fish, in concentrations high enough to cause liver damage in the fish studied. Microcystin content in fish livers was higher than that in the organisms upon which they fed; however, because the concentration of microcystin in the overall fish biomass was lower, the authors concluded that biomagnification of microcystin does not occur in this system. In general, studies have found that microcystin content of fish livers is much higher than that in muscle (reviewed in Ibelings and Chorus, but see Kozlowsky-Suzuki et al.). However, as toxins in liver are not relevant to public health unless fish are consumed whole, my study only focused on microcystin content in muscle.

This conclusion about the trophic transfer of microcystin is echoed more broadly by a meta-analysis performed by Kozlowsky-Suzuki et al. After reviewing 42 studies on microcystin concentrations in food webs, these authors concluded that biodilution rather than biomagnifications of microcystins was the norm. Regular exceptions included zooplankton and zooplanktivorous fishes, with the authors concluding that feeding habits likely played a large role in the relative magnification of microcystins. However, there is great variability of toxin concentration within feeding guilds in the reviewed literature. While it seems logical that phytoplanktivorous fish would have much greater exposure to MC due to the biodilution of microcystin in food webs and their sometimes greater food intake than carnivorous fishes, studies have generally shown higher levels of MC in carnivorous and zooplanktivorous fish. Wood et al. recently used stable isotope analysis to investigate trophic status and microcystin transfer in the James River Estuary foodweb, and found that predators with benthic feeding habits had lower concentrations.
of microcystin than those with pelagic feeding habits. Other studies have suggested that the length and alkalinity of the gastrointestinal tract of fish under study also play important roles.\textsuperscript{5,17,26}

The history of an organism’s exposure to microcystin also appears to be important, with animals that are routinely exposed to microcystin perhaps better adapted to eliminate the toxin. Gustafsson \textit{et al.}\textsuperscript{27} and Sarnelle and Wilson\textsuperscript{28} show evolution of microcystin resistance across generations in \textit{Daphnia}, while Smith and Haney\textsuperscript{29} show possible induction of a depuration pathway in sunfish. Because of this, the age of fish may be a significant factor in MC contamination of fish tissues, with fish old enough to have lived through large blooms better able to reduce their internal MC burdens. While Smith and Haney found that age did not affect MC concentration in Pumpkinseed Sunfish, no others have explicitly studied this factor. One study does mention, however, that depuration may be more prevalent in younger fish, due to a higher activity of detoxification enzymes.\textsuperscript{30}

Additionally, the bloom dynamic plays a role in fish toxin exposure. While studies seem to indicate that fish are primarily exposed via food consumption,\textsuperscript{5,31} some exposure may occur through direct uptake of toxins from the water.\textsuperscript{32,33} Water-borne toxin concentration is likely to be higher after bloom senescence, as the cyanobacteria lyse and release internally-stored MCs into the water column\textsuperscript{4,6,26}, therefore, fish collected after the peak of the bloom may have been exposed to higher levels of MC, and exhibit higher MC concentrations in their muscle tissue.
The complexity and confusion of mechanisms that cause microcystin to be present in fish tissue means that local studies are necessary to establish the health risk in a particular area. Given that monitoring has shown MC concentrations in Lake Erie water regularly reaching 10 – 20 µg / L (10 times the WHO limit for drinking water), and occasionally as high as 1000 µg / L, MC exposure via fish consumption should be a concern for fish harvested from this lake.

Of the important commercial and recreationally fished species, yellow perch (Perca flavescens) has been most studied for MC contamination: the general conclusion has been that it exhibits levels of MC below WHO thresholds. Wilson et al.\textsuperscript{16} performed a comprehensive study on microcystin levels in yellow perch from Lake Erie in 2006; fish were collected from several sites, and MCs were extracted from both muscle and liver tissues. Fish were collected at three different times (June, July, and August) for a total of \( n = 68 \) samples. In addition, seston samples were collected and analyzed for MC. The authors found low overall concentrations of microcystin in muscle samples (0.4 – 4.02 ng MC / g dry weight). Additionally, they detected a negative correlation between seston MC and fish muscle MC concentrations, and therefore concluded that there was negligible threat to public health due to MC content in yellow perch from the lake. However, this study was only performed during one year. As algal blooms can vary widely from year-to-year, there is a need for studies performed during additional years, to better understand how annual variation in algal blooms affects fish toxicity. Dyble et al.\textsuperscript{14} studied the accumulation of microcystin in yellow perch, and found that the fish rapidly eliminated MCs within 24 hours of exposure. They therefore concluded that MCs
were unlikely to be a concern. However, the fish were exposed to microcystin only once via an oral dose, rather than over a prolonged period, as might occur in the field.

Poste et al.\textsuperscript{19} quantified MC concentration in fish taken from several lakes experiencing algal blooms in 2006 and 2007, including Lake Erie, and also found low toxin concentrations in yellow perch. They also studied white perch (\textit{Monroe americana}), another important sportfish in Lake Erie, and found toxin concentrations on par with yellow perch, albeit with a larger range and higher variability. White perch is particularly important because changes in climate are projected to increase its abundance and range in the great lakes,\textsuperscript{35} thereby increasing its dominance relative to other Lake Erie sportfish.

However, in the Poste \textit{et al.} study, several species of fish from Lake Erie exhibited microcystin concentrations greater than 20 ng / g wet weight; the highest concentrations in Lake Erie were found in walleye (\textit{Sander vitreus}) (5.3 – 41.2 ng / g wet weight). The authors concluded that an individual consuming an average daily amount of fish solely in walleye from Lake Erie would exceed the maximum lifetime TDI recommended by the WHO. Walleye is one of the most important commercial fish caught in Lake Erie – catch rates regularly exceed 3 million fish annually, and the walleye fishing economy has an estimated value or tens of millions of dollars\textsuperscript{36} – so the safety of this fish is of particular importance.

Unfortunately, all of the fish surveyed in the Poste \textit{et al.}\textsuperscript{19} study had small sample sizes (n = 2 – 6), and the fish were not analyzed with respect to the time or location at which they were taken. This thesis starts to fill these gaps in knowledge by examining
microcystin concentration in these three fish species of particular commercial and recreational importance in Lake Erie: white perch, yellow perch, and walleye.

The *objectives* of the study are:

1) To determine whether any of the species posed a threat to public health related to microcystin exposure during the summer of 2013.

2) To examine what factors affect microcystin concentrations in fish, notably:
   a. Location caught (inside or outside the bloom)
   b. Time caught (before or after the peak of the bloom)
   c. Bloom intensity when and where caught
   d. Age and size

The *central hypotheses* are that microcystin concentrations in fish tissue will vary by species, with higher concentrations found in walleye; and that the intensity of the bloom will be positively related to microcystin concentrations in fish tissue.

Conclusions focus on the health threat posed by algal toxins in the fish, and impacts of algal blooms on the MC burden in fish distributed spatially and temporally throughout the lake.
Methods

Sample Collection

White Perch (n = 55), and yellow perch (n=52) were collected by the Ohio Department of Natural Resources-Division of Wildlife from 17 different locations across the Western basin of Lake Erie (Figures 1 – 3). The sampling effort was the same for all sites: a 10-minute bottom trawl at 1.6 – 1.7 knots. Fish were collected from both the August and September trawls, which occurred on August 19, 2013 and September 24, 2014, respectively, and span the period in which the bloom peaked and then began to senesce (as confirmed by observation of the National Oceanic and Atmospheric Administration Great Lakes Environmental Research Laboratory Harmful Algae Bloom Bulletin\textsuperscript{34}). These trawls were part of annual fishery-independent trawl surveys conducted during late August and again in late September, and they do a good job of sampling white and yellow perch of all ages. Fish were shipped on ice and frozen at -20 °C before sample evaluation.

Walleye (n = 33) were collected by charter boat captains participating in water quality monitoring supervised by Stone Laboratory at the Ohio State University. Fish locations were not determined in advance, but were reported by the captains. Harvestable fish were filleted on the boat, and samples from the muscle of the belly flap were placed
on ice. Samples were shipped to Stone Lab, where they were stored at -20°C until they were shipped to Columbus, where they were stored at -20°C until processing.

Toxin extraction and Quantification

White perch and yellow perch, received whole, were weighed, measured for standard length, and filleted, and a piece of fillet from the dorsal-anterior end of the fish was selected for analysis. Walleye samples, from the belly flap, were processed to remove the skin and the belly lining.

Fish tissue was analyzed for microcystins using methanol extraction followed by Enzyme-Linked Immunosorbant Assay (ELISA), in a manner derived from that of Hu et al.\textsuperscript{37} and Moreno et al.\textsuperscript{38} Tissue samples were diced, placed in ceramic dishes, and then placed in a drying oven at 60°C for 24 hours. Samples were weighed before and after drying to determine wet-to-dry conversion factors for each individual sample. Dried tissues were homogenized using a mortar and pestle and then extracted with 75% methanol for 2 hours, at room temperature with stirring. When possible, 0.45 – 0.55 g of each sample was used; smaller samples were not supplemented by additional fillets. Extracts were centrifuged in a Fischer Scientific Centrifuge centrifuge with a hanging bucket rotor, at ~4,750 rpm for 15 minutes. Supernatant was removed, and the solids resuspended in 75% methanol for a second extraction. This process was repeated for a total of three extractions. The supernatant from all extractions was pooled and diluted to one-quarter strength with deionized water; the resulting solution was concentrated by passing through a SepPak® C18 column (Waters corporation, Milford,
Massachusetts). The supernatant was passed through the column twice, the second time at double speed; total contact time was about 75 minutes per sample. Microcystin was eluted from the column with 5 mL of 100% methanol. The sample was then diluted to <5% methanol, according to the directions provided with the ELISA kit.\(^25\)

Finally, the sample was centrifuged and then analyzed using the Microcystins/Nodularins (ADDA), ELISA kit (catalog number PN520011, Abraxis Inc, Warminster, Pennsylvania). This is an indirect competitive ELISA that targets the ADDA group, a peptide conserved among microcystin congeners. ELISA is a sensitive and low-cost method suitable for high throughput screening, but it is not as precise as HPLC or LC/MS.\(^17\) Dry-weight concentrations were converted to wet-weight concentrations using the previously determined conversion factor. The method was verified by running both negative and positive controls. Lower limits of detection varied by sample, and ranged from 1.27 ng MC / g to 30.29 ng MC / g (mean 9.64 ng MC / g) for white and yellow perch; for walleye the range was 7.48 ng MC /g to 86.36 ng MC / g, with a mean of 22.18 ng MC / g. Several walleye samples were well below the desired mass of 0.5 g, which dramatically raised the lower limit of detection.

Percent recovery was estimated by spiking tissue samples with Microcystis colony extract. Microcystis colonies grown in the lab were collected on a CF/C membrane. A sample of this paper was cut up and extracted in a mixture of 90% methanol by volume in deionized water for 2 hours. The extract was centrifuged at 12,000 rpm for 2 minutes. The supernatant was diluted to 30% methanol with deionized water and was passed through a SepPak® C18 column (Waters corporation, Milford, Massachusetts) in a
manner identical to that described above. Microcystin was eluted from the column with 5 mL of 100% methanol, and then left in the fume hood for several days to dry. Final drying was accomplished on a heating block at 40 °C. It was then re-suspended in 1.5 mL of 18.2 MΩ water, with 2 minutes of sonication. Solution was stored at -20 °C until needed. MC concentration of this solution was determined by ELISA.

Controls were run on store-bought white lake perch and store-bought walleye fillets. Muscle tissue from both of these samples tested below the detection limit for microcystins (6.9 – 7.3 µg MC / g wet weight) before spiking (negative controls). Spiked samples were prepared and run exactly as other samples, except that before the first extraction, 50 µL or 75 µL of *Microcystis* extract was added by micropipettor to the homogenized fish tissue in the extraction flask. The MC content of the solution used to spike the controls was determined by the same ELISA used to determine MC content in the spiked samples. However, given the range of extraction efficiencies found in this study (Table 1), they were not taken into account when calculating the final toxin concentrations in samples, which limits the conclusions that can be drawn.

*Determination of Cyanobacterial Blooms*

Bloom intensity at sampling locations was determined by analysis of remote sensing imagery. This was only possible for white and yellow perch, as the exact sampling locations for walleye are not known. Pictures were captured by the Moderate Resolution Imaging Spectroradiometer (MODIS) on NASA’s Aqua satellite, which has a spatial resolution of 1 km². Images were processed to extract the “cyanobacterial index”
according to Wynne et al. This involves determining the spectral shape around the band at 678 nm according to the calculation:

\[ S_{2d}(678) = \rho_2(678) - \rho_2(667) - \{\rho_2(678) - \rho_2(667)\} \frac{(678 - 667)}{(748 - 667)} \]

where \( S_{2d}(678) \) represents the second derivative, \( \rho_2 \) is the Rayleigh-corrected reflectance, and 667, 678, and 748 are the numeric values associated with these wavelengths (expressed in nm). The Cyanobacterial Index is then calculated as

\[ CI_{\text{MODIS}} = -S_{2d}(678) \]

Previously, Wynne et al. had used images from the Medium Resolution Imaging Spectrometer (MERIS) on the ESA’s Envisat satellite to construct a similar index for cyanobacterial blooms, and had correlated this index to field measurements of Microcystis spp. cell counts in Western Lake Erie. After the loss of the MERIS sensor, the authors devised the above relationship for use with the MODIS system, and further derived the relationship

\[ CI_{\text{MODIS,corr}} = 1.3 \times CI_{\text{MODIS}} \]

where \( CI_{\text{MODIS,corr}} \) is approximately equivalent to the index derived from MERIS measurements (though the measure from MODIS is liable to saturate at high biomass intensities). This allows the cyanobacterial index derived from MODIS imagery to be related to Microcystis spp. cell counts, and therefore to cyanobacterial blooms.

Therefore, in this study the values of this index were used as a proxy for Microcystis bloom intensity. Images used were from within one day of the sampling dates (depending on data availability due to cloud cover). In addition to the continuous
variable that denotes the intensity of the cyanobacterial bloom, a binary variable was constructed to indicate whether a “significant” cyanobacterial bloom had occurred at a specific location and time. This variable was constructed from $C_{I_{MODIS,corr}}$ using the threshold value of 0.001 given by Stumpf et al.\textsuperscript{42}, which is roughly equivalent to $10^5$ cells per mL.

Additional parameters

Water samples associated with walleye fillets were collected by charter boat captains using a two-meter integrated tube sampler. These samples were collected weekly, and may not correspond well to the location or time at which walleye were caught. Samples were transported on ice to Stone Laboratory at Ohio State, and there analyzed for microcystin, chlorophyll a, and total phosphorous. Fish ages for samples of all species were determined by the Sandusky Fish Research Station from fish lengths, species, and dates and locations caught.

Data Analysis

The data was analyzed with MC concentration as a response variable, and all other variables as explanatory variables (factors). Samples that tested below the detection limit for MC were replaced by a number drawn from a uniform distribution between 0 and the lower limit of detection for that sample. Basic hypothesis testing was used to examine the effects of categorical variables, while ordinary least squares regression was used to determine the effects of continuous variables. Hypothesis testing
and graphing of data was performed in R version 3.0.1 -- “Good Sport”. As the data was not normally distributed, non-parametric Wilcoxon-Mann-Whitney tests were used to determine whether the samples, among different levels of these factors, came from similar distributions. When testing differences among species, pairwise Wilcoxon tests with Holm p-adjustment were used. All tests were performed at a significance of $\alpha = 0.05$. The available explanatory variables for toxin concentration were different for walleye than for white and yellow perch, due to different sources of the data. Therefore, while differences in toxin concentration by species were tested with the complete dataset, further testing was done on data separated by species. This is further justified by the finding that all pairs of species exhibited significant differences in toxin concentration.

Linear models were constructed in JMP 10.0 using Ordinary Least Squares regression, and unless noted residuals exhibited a good fit to a normal distribution. For white and yellow perch, the continuous variable describing the intensity of the cyanobacterial bloom – the Cyanobacterial Index (CI) – was tested along with the date caught and the age of the fish. For walleye, date caught, microcystin, chlorophyll a, and total phosphorous were tested by multiple linear regression. Models were constructed using stepwise comparison with the corrected Akaike Information Criterion ($AIC_c$) as a standard; models with $\Delta AIC_c < 2$ were considered insignificantly different, and those with $\Delta AIC_c > 10$ were considered important.
Public Health Impact

Public health exposure was estimated by calculating maximum allowable levels of MC in fish tissues. These thresholds were calculated by Dyble et al.\textsuperscript{15} using the WHO lifetime Total Daily Intake (TDI) value of 0.04 µg / kg body weight. The authors assumed as 70 kg person, and estimated daily consumption values for populations in the Lake Erie area from a variety of sources\textsuperscript{44}; from this, the average concentration of MC in fish tissue at which an individual will exceed the TDI can be calculated (Table 3). Concentrations in fish tissues from this study may then be compared to these threshold values to determine the risk to public health.

However, as pointed out by Ibelings and Chorus\textsuperscript{5}, the WHO value of 0.04 µg / kg body weight represents a \textit{lifetime} TDI value, whereas in temperate climates, such as experienced by Lake Erie, algae blooms are usually seasonal events. Indeed, microcystin in Lake Erie is generally only measurable from July through October, with peaks in August and September.\textsuperscript{34} Therefore, most individuals need not worry about lifetime consumption of microcystin, but only consumption over a period of several months. Ibelings and Chorus therefore suggest a “seasonal TDI” of 0.4 µg / kg body weight. This value was calculated by removing the safety factor of 10 for extrapolating from a several-week-long study to a lifetime TDI exposure limit. The original calculations are: a NOAEL of 40 µg / kg body weight, divided by a factor of 10 for inter-species variability (the study was performed on mice, not on humans), another factor of 10 for intra-species variability, and one more factor of 10 for extrapolation to lifetime exposure; this yields a lifetime TDI of 0.04 µg / kg body weight. The “lifetime threshold values” of MC
concentrations in fish derived by Dyble et al. may be converted to “seasonal threshold values” by removing this last safety factor, which amounts to multiplying the threshold values by 10.

As an additional relevant level of consumption, the Ohio Department of Health currently recommends only eating one meal (170 g) per week of sportfish, those following this recommendation will consume 24.3 g / day of fish. Additional lifetime and seasonal threshold values of microcystin in fish tissue were derived from this consumption rate according to the methods of Dyble et al. and used for comparison in this study (Table 3).
Figure 1: Fish for this study were collected from 13 sampling locations in western Lake Erie during August and September 2013.
Figure 2: Cyanobacterial bloom (measured as CI) and sites sampled for white and yellow perch in August. Bloom picture is from August 18, 2013; fish were collected on August 19, 2013.
Figure 3: Cyanobacterial bloom (measured as CI) and sites sampled for white and yellow perch in September. Bloom picture is from September 24, 2013; fish were collected this same day. Note that start and endpoints on the scale for CI are different than those in Figure 2.
Results

Data collected as part of this study are available in Appendix A.

*Extraction Efficiency*

Table 1 displays the extraction efficiencies that were obtained:

<table>
<thead>
<tr>
<th>Species</th>
<th>Amount of <em>Microcystis</em> extract added</th>
<th>Microcysts in <em>Microcystis</em> extract</th>
<th>Microcysts added to sample</th>
<th>Microcysts recovered from sample</th>
<th>Extraction efficiency (% recovery)</th>
</tr>
</thead>
<tbody>
<tr>
<td>White Perch</td>
<td>50 µL</td>
<td>598 µg / L</td>
<td>29.9 ng</td>
<td>202.5 ng</td>
<td>677%</td>
</tr>
<tr>
<td>White Perch</td>
<td>75 µL</td>
<td>598 µg / L</td>
<td>44.85 ng</td>
<td>208.75 ng</td>
<td>465%</td>
</tr>
<tr>
<td>Walleye</td>
<td>50 µL</td>
<td>598 µg / L</td>
<td>29.9 ng</td>
<td>30 ng</td>
<td>100%</td>
</tr>
<tr>
<td>Walleye</td>
<td>75 µL</td>
<td>598 µg / L</td>
<td>44.85 ng</td>
<td>66.25 ng</td>
<td>148%</td>
</tr>
</tbody>
</table>

*Table 1: Extraction efficiency of microcystins from control samples*
Sample Variability

Table 2 provides information about the fish collected during this study:

<table>
<thead>
<tr>
<th>Species</th>
<th>Age (years)</th>
<th>Standard Length (in.)</th>
<th>Bloom Exposure (Microcystis cells/ mL)</th>
<th>MC Exposure (µg / L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Range</td>
<td>Mean</td>
<td>Range</td>
</tr>
<tr>
<td>White Perch</td>
<td>1.8</td>
<td>1 - 5</td>
<td>6.0</td>
<td>4.44 - 7.91</td>
</tr>
<tr>
<td>Yellow Perch</td>
<td>3.31</td>
<td>1 - 7</td>
<td>6.45</td>
<td>4.75 - 9.34</td>
</tr>
<tr>
<td>Walleye</td>
<td>5.79*</td>
<td>3 - 12*</td>
<td>21.11*</td>
<td>18 - 25*</td>
</tr>
</tbody>
</table>

Table 2: Age, Length, and Bloom Conditions experienced by fish collected for this study. CI was transformed to Microcystis cells per mL according to Wynne et al. Note that CI is only available for white and yellow perch, and MC exposure is only available for walleye. *Ages and lengths were not available for all walleye, so these numbers do not represent the entire sample.

Microcystin in All samples

Toxin content in fish ranged from below the detection limit to 203 ng MC / g wet weight, with an average of 37.6 ng MC / g wet weight (Figure 4). The lower detection limit ranged from 7.51 ng MC / g wet weight to 50.0 ng MC / g wet weight, with a mean of 14.48 ng MC / g wet weight, for samples with concentrations below the limit. Mean toxin concentrations varied significantly by species (Figure 5) (walleye vs. white perch: p = 1.6e-11; walleye vs. yellow perch: p = 1.6e-11, white perch vs. yellow perch: p = 4.6e-11). Therefore, further analyses were carried out on data for individual species.
Figure 4: Toxin concentration (ng microcystin / g wet weight of fish) for all fish examined in this study. Color and shape distinguishes species, and the lines represent the mean for each species. Means were calculated by replacing the non-detect values with 0.
Figure 5: A pairwise Wilcoxon test with the Holm p-adjustment method demonstrated that mean microcystin concentrations in all species were significantly different from each other.

White Perch

White perch exhibited an average concentration of 37.5 ng MC / g wet weight. Of the 55 white perch analyzed, 50 had concentrations above the detection limit; these exhibited an average concentration of 40.73 ng MC / g wet weight. The lower limit of
detection ranged from 7.83 to 23.5 ng MC / wet weight, with a mean of 12.8 ng MC / g wet weight, for those samples below the limit. The date caught showed a significant effect on toxin concentration in fish tissue ($W = 120$, $p = 0.0003852$, figure 6), but the binary variable of bloom occurrence was not correlated with fish toxin concentrations. Neither length, nor weight, nor age was significantly correlated with fish toxicity.

Figure 6: A Wilcoxon test determined that toxin concentrations in white perch caught on different dates were significantly different. White perch caught on September 24th had higher muscle tissue concentrations than those caught on August 19th.
The best model for MC in white perch had CI, date caught, and their cross as explanatory variables, with $R^2_{adj} = 0.337$ (Figure 7). However, further analysis revealed that there was a significant difference between CI at sites where white perch were caught in August, and those in which they were caught in September (Wilcoxon-Whitney-Mann test, $W = 6$, $p << 0.001$). Therefore, for white perch, date caught and CI are correlated.

**Figure 7**: MC in white perch correlated with CI, Date Caught, and their cross. 

$$R^2_{adj} = 0.337$$

*Yellow Perch*

Yellow perch exhibited an average concentration of $11.80 \text{ ng MC / g wet weight}$. From a total of 52 yellow perch, 25 had concentrations above the detection limit; these exhibited an average concentration of $16.90 \text{ ng MC / g wet weight}$. The lower limit of
detection ranged from 7.51 to 30.3 ng MC / wet weight, with a mean of 12.4 ng MC / g wet weight, for those samples below the limit.

None of the factors examined were significantly correlated with MC concentrations in yellow perch: the best model (lowest AICc) had no factors. MC in yellow perch modeled as a function of CI gives an insignificant model with $r^2_{adj}$ of -0.00587(Figure 8). However, the residuals of this model are not normally distributed.

Figure 8: MC in yellow perch was not correlated with CI. $r^2_{adj} = -0.00587$

**Walleye**

The 33 walleye samples collected exhibited a range of toxin concentrations from below the detection limit to 203 ng MC / g wet weight, with an average of 86.6 ng MC / g wet weight. Only three samples had concentrations below the detection limit, which
ranged from 17.94 to 49.96 ng MC / g wet weight, with a mean of 36.1 ng MC / g wet weight for those samples.

None of the factors examined were significantly correlated with MC concentrations in walleye: the best model (lowest AICc) had no factors. MC in fish modeled as a function of MC in water gave an insignificant model with $r^2_{\text{adj}} = -0.0271$ (Figure 9).

Figure 9: MC in walleye was not correlated with MC in the water column.

$r^2_{\text{adj}} = -0.0271$.  


Comparison with Public Health Thresholds

Of the 55 white perch sampled, 5 samples exceeded the threshold value for the lifetime TDI at consumption common to Lake Erie anglers; 36 exceeded the threshold for lifetime TDI for tribal members, and 50 exceeded the threshold for lifetime TDI, and two the threshold for seasonal TDI, for very high fish consumers. Of the 52 yellow perch sampled, one sample exceeded the threshold value for the lifetime TDI at consumption common to Lake Erie anglers; 10 exceeded the threshold for lifetime TDI for tribal members; and 21 exceeded the threshold for lifetime TDI for very high fish consumers. None exceeded the threshold for seasonal TDI of any population under consideration. Of the 33 walleye sampled, 9 samples exceeded the threshold value for lifetime TDI at consumption suggested by the ODH advisory level. Nineteen exceeded the threshold for the lifetime TDI for a Lake Erie angler. Twenty-nine exceeded the threshold for the lifetime TDI, and four that for the seasonal TDI, for a tribal member with traditionally high fish consumption. Thirty exceeded the threshold for lifetime TDI, and 16 that for the seasonal TDI, for very high fish consumers. Half of the walleye sampled in this study would therefore cause very high fish consumers to exceed the seasonal TDI. These results are summarized in Table 3, below.
Table 3: Threshold values of microcystin in fish tissues according to various sources, and the number of fish of each species in this study exceeding these values. Sources of daily fish consumption data: a. derived from Dyble et al. (15). b. derived from the ODNR advisory for consumption of Lake Erie sportfish (39). c. quoted in Dyble et al. (15).

<table>
<thead>
<tr>
<th>Population of Interest</th>
<th>Average daily fish consumption</th>
<th>Level of concern (lifetime TDI) (ng MC / ng wet weight)</th>
<th>Level of concern (Seasonal TDI) (ng MC / ng wet weight)</th>
<th># White Perch exceeding</th>
<th># Yellow Perch exceeding</th>
<th># Walleye exceeding</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n = 55</td>
<td>n = 52</td>
<td>n = 33</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lifetime</td>
<td>Seasonal</td>
<td>Lifetime</td>
<td>Seasonal</td>
<td>Lifetime</td>
<td>Seasonal</td>
</tr>
<tr>
<td>Average U.S. Consumer</td>
<td>6.5 g / day&lt;sup&gt;a&lt;/sup&gt;</td>
<td>431 ng / g</td>
<td>4310 ng / g</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>ODH Advisory Level</td>
<td>24.3 g / day&lt;sup&gt;b&lt;/sup&gt;</td>
<td>115 ng / g</td>
<td>1150 ng / g</td>
<td>0</td>
<td>0</td>
<td>9</td>
</tr>
<tr>
<td>Lake Erie Angler</td>
<td>40 g / day&lt;sup&gt;c&lt;/sup&gt;</td>
<td>70 ng / g</td>
<td>700 ng / g</td>
<td>5</td>
<td>0</td>
<td>19</td>
</tr>
<tr>
<td>Tribal Member—low estimate</td>
<td>190 g / day&lt;sup&gt;c&lt;/sup&gt;</td>
<td>14.7 ng / g</td>
<td>147 ng / g</td>
<td>46</td>
<td>0</td>
<td>29</td>
</tr>
<tr>
<td>“Very high fish consumers”</td>
<td>328 g / day&lt;sup&gt;c&lt;/sup&gt;</td>
<td>8.5 ng / g</td>
<td>85 ng / g</td>
<td>50</td>
<td>2</td>
<td>30</td>
</tr>
</tbody>
</table>

<sup>a</sup>Dyble et al. (15).  
<sup>b</sup>Derived from ODNR advisory (39).  
<sup>c</sup>Quoted in Dyble et al. (15).
Discussion

From the comparison tests, it is clear that MC accumulates differently to different species, with higher concentrations in white perch than in yellow perch, and higher concentrations in walleye than in both white perch and yellow perch. This is similar to the results of Poste et al., though the MC concentrations reported for white perch and for walleye in this study are much higher than in that one. While yellow perch exceeding the concentrations reported in Poste et al. were caught in this study, the difference in mean MC concentration is only 6 ng / g wet weight. This finding indicates that fish can have different MC burdens in different years, and provides further incentive for long-term studies. However, comparisons with other studies may not be very accurate, given the range of extraction efficiencies found in this study.

The intensity of the algae bloom at the location in which the fish were caught has a significant effect on MC concentration in white perch, but not in yellow perch, as indicated by the significance of the Cyanobacterial Index in linear models (a similar index for cyanobacteria bloom was not available for walleye samples, as their sampling locations are not well known). There are several reasons why this might be so, but perhaps the most compelling is that yellow perch consume a large proportion of their diet from benthic sources.46,47 White perch, by contrast, have zooplankton as a larger component of their diet.46 Similarly, Kozlowski-Suzuki et al. found that
zooplanktivorous fish were more likely to concentrate MC than fish of other feeding guilds (phytoplanktivorous, carnivorous, and omnivorous).\textsuperscript{22} Ibelings \textit{et al.}\textsuperscript{21} also found higher microcystin concentrations in smelt (planktivorous) livers than in perch or ruffe (predatory and benthivorous, respectively) livers. They also found higher levels in zooplankton than in \textit{Dreissena}, which make up a significant portion of the yellow perch diet in Lake Erie.\textsuperscript{47}

This fits with the pattern in consumers observed by Wood \textit{et al.}\textsuperscript{25}, that organisms with largely benthic diets accumulated lower concentrations of microcystins than those organisms with largely pelagic diets, as determined by stable isotope analysis. The authors suggest several possible reasons for this observation, including degradation of MCs over time and adsorption of MCs by sediments. Rinta-Kanto \textit{et al.} \textsuperscript{48}, in analyzing both contemporary and archived sediment samples from Lake Erie, found \textit{Microcystis sp.} but no microcystins, and attributed this to possible adsorption to clay sediments.

The observation that MC concentration in white perch was correlated with date caught could indicate the importance of MC uptake from the water, but as this variable was not directly measured for white and yellow perch, this conclusion remains very speculative. Prolonged exposure of fish to MC in the intervening month could also have a part to play, and it could be that MC burdens in white perch rise as the season progresses Ibelings and Chorus mention that concentrations from lysed blooms are high but short-lived due to processes like mixing and adsorption.\textsuperscript{5}

No water-quality variables were seen to correlate with MC concentration in walleye. Walleye are largely piscivorous, so the exposure of their prey, and therefore of
walleye themselves, is potentially less sensitive to the bloom in their immediate surroundings than those fish that consume largely sessile or pelagic organisms. Algae blooms have high spatial diversity, and in mobile creatures such as fish past exposure to MC may not be correlated to the levels in their immediate environment. Nonetheless, the discovery of high microcystin concentrations in piscivorous fishes agrees with Xie et al.\textsuperscript{24} Unfortunately, the mechanism for this remains unclear.

Fish size, weight, and age appeared to have no significant effect on MC concentrations in any species. This indicates that long-term (multi-annual) exposure likely does not have an effect on fish MC concentrations: fish likely are not retaining toxins from year to year. This conforms with the relatively short depuration times found by others, such as Dyble et al.\textsuperscript{14} More interesting is what this says about long-term acclimation to algal toxins: if fish that have a history of exposure to microcystins, such as those that survived the intense bloom of 2011, have more effective depuration systems,\textsuperscript{29} they might be expected to accumulate less MC during contemporary blooms. However, it may be more appropriate to examine this issue along with metrics of yearly cumulative exposure. These questions could also be better answered by analyzing fish over multiple years, to more thoroughly investigate the effect of the bloom from the previous year.

While no appreciable threat was posed to average consumers due to fish-borne microcystins in 2013, a potential threat existed for very high consumers of walleye, as half of the fish sampled contained MC concentrations that exceeded the safe threshold for very high seasonal consumption. Additionally, the trends observed in this study suggest potential for threats in the future. White perch were observed both to have higher MC
concentrations than yellow perch, and to be more susceptible to the effects of the blooms. They therefore have a greater potential for public health threat. Further, due to changing climactic conditions, it is likely not only that white perch will become more dominant in Lake Erie, but also that extreme blooms, such as the one observed in 2011, will become more common. Therefore, under projected climate change conditions, it is more likely to observe particularly large blooms in a lake dominated by white perch, which may pose a greater health threat to fish consumers than that identified in this study.

In contrast to the highest concentration of microcystin observed in this study (203 ng MC / g wet weight), the level of concern for lifetime exposure of the average fish consumer in the United States was calculated to be 430.77 ng MC / g wet weight. The level of concern for the average angler in Lake Erie is 70 ng/g wet weight; 19 walleye, five white perch, and one Yellow Perch exhibited concentrations above this level of concern. However, if the “seasonal” TDI for microcystin is adopted, the level of concern for the average angler is 700 ng / g wet weight, and no samples represented a significant threat. In fact, the only population group identified by Dyble et al. that would potentially receive a dose exceeding the seasonal TDI is the “Very High Fish Consumers,” with 18 samples exceeding the threshold of 85 ng / g wet weight. Using the seasonal TDI, no samples represented a threat to an individual who follows the guidelines for sportfish consumption from the Ohio Department of Natural Resources. Therefore, given the large gaps between the toxin concentrations observed in the samples and the seasonal TDI values identified by Ibelings and Chorus, there is no indication that the fish
studied posed a threat of microcystin intoxication to the general public in the summer of 2013.

These results are confounded slightly by the range of extraction efficiencies found in this study. Extraction efficiencies varied considerably relative to species, and were never lower than 100%. Percent recoveries for walleye are comparable to those in the literature,\textsuperscript{16} but those for white perch are significantly in excess of those published for other species. Nonetheless, concerns about extraction efficiencies do not affect the finding that toxin levels in white perch are responsive to bloom intensity. Additionally, as the efficiencies for walleye tissue were much lower than those for white perch tissue, it can still be confidently asserted that walleye exhibited higher toxin concentrations than white perch. As no yellow perch samples were available to use as controls, the relative extraction efficiency for this species could not be evaluated. However, the range of toxin concentrations in yellow perch was in line with previous studies of the species. Concerns over extraction efficiencies of different methods do, however, confound attempts to compare the results of different studies. Confirmation of the toxin concentrations found in this study using established methods such as HPLC would help to shed light on this issue.

Results, strictly speaking, are in terms of total extractable microcystins, but are treated as microcystin-LR equivalents in accordance with common practice.\textsuperscript{9,16} As microcystin-LR is the most toxic of the known microcystins, this tends to over-report the threat from MC intoxication. At the same time, estimates of MC are likely conservative, as some studies on microcystin content in animals have found a large portion of
microcystin (38 - 99%) to be covalently bonded to tissues,\textsuperscript{21,49} and these will not be captured in methanol extraction. However, the bioavailability and toxicity of these covalently bound toxins to consumers is debated.\textsuperscript{50} No extraction efficiency is below 100%, so results presented here are likely overestimates. In summary, estimates of public health threat are almost certainly exaggerated, in the sense that the actual threat is unlikely to be larger than that presented here. Confirmation of these results with HPLC should precede any educational or policy actions.
Conclusions

The results presented here indicate that, of the three species of sportfish studied, there was significant health threat from algal toxins only to individuals with very high consumption of walleye from Lake Erie in the summer of 2013. However, it should be noted that the safe threshold values presented in this study were figured for healthy adults; at-risk populations such as children and the immune-compromised may be susceptible to lower doses of microcystin.\(^{14}\)

The age and size of the fish did not appear to affect toxin concentrations regardless of species, indicating that toxin neither accumulates preferentially to younger or older fish, nor accumulates from year to year. However, microcystin concentrations in white perch were not only significantly more toxic than yellow perch, but were positively related to the severity of the algal blooms. Additionally, both white perch and walleye showed higher levels of toxicity in 2013 than reported for 2007, though this conclusion is tenuous because of uncertainty regarding the relative extraction efficiency of different methods. As blooms are predicted to become more severe with climate change,\(^{3}\) it is recommended to continue monitoring the toxin burden of recreationally and commercially important fish from Lake Erie, so that information from several years may be compared. As blooms vary on an annual basis, it would be informative to examine
how the toxin burdens in white perch and walleye, in particular, change with annual changes in bloom duration and intensity.
Works Cited


# Appendix A: Data Collected for This Study

## Table 4: Data for White Perch

<table>
<thead>
<tr>
<th>Sample</th>
<th>Species</th>
<th>Site</th>
<th>Weight (g)</th>
<th>Standard Length (in.)</th>
<th>Cyanobacterial Index</th>
<th>Bloom</th>
<th>Toxin (ng MC / g wet weight)</th>
<th>Age (years)</th>
<th>Date Caught</th>
</tr>
</thead>
<tbody>
<tr>
<td>WP3</td>
<td>White Perch</td>
<td>35-888</td>
<td>120</td>
<td>7.125</td>
<td>0.002337</td>
<td>TRUE</td>
<td>27.62137</td>
<td>2</td>
<td>09/24/2013</td>
</tr>
<tr>
<td>WP4</td>
<td>White Perch</td>
<td>34-879</td>
<td>142</td>
<td>7.6875</td>
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<td>FALSE</td>
<td>37.60426</td>
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<td>08/19/2013</td>
</tr>
<tr>
<td>7</td>
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<td>54</td>
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</tr>
<tr>
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<tr>
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<td>White Perch</td>
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<td>47.36745</td>
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<td>5.25</td>
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<td>30.88574</td>
<td>1</td>
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Table 4: Continued

<table>
<thead>
<tr>
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