Bioaccumulation of Lead in *Neogobius melanostomus* within Lake Macatawa

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ABSTRACT

The various trophic levels within the Great Lakes have been modified from the introduction of various invasive species, including Dreissenid mussels and Round Goby (Neogobius melanostomus). This shift in the food web creates a new pathway for contaminants to be transferred up trophic levels by the process of bioaccumulation. Dreissenid mussels are consumed by N. melanostomus, which is consumed by higher trophic level fish species. Heavy metals enter aquatic environments by multiple pathways. Prior to the phase out of lead based fuel, contaminants entered into the environment from the combustion of fuel, particularly in boat marinas, and also by boat maintenance products. To characterize the heavy metal transfer through the food web from lead contaminants in aquatic environments from boating usage, a study of lead concentrations in N. melanostomus and benthic sediment, their spatial relationship, and any attributed exacerbation of lead within marina regions was conducted as a Hope College Environmental Science research project. Sampling sites included marinas (n=3 Goby; n=4 sediment) and control sites (n=1 Goby; n=3 sediment) proximal to shoreline along Lake Macatawa in Holland, Michigan, USA. Round Goby samples (n≥12) were taken from each sampling site, dried, powdered, and subjected to microwave-assisted nitric acid digestion prior to atomic absorption spectroscopy (AAS) analysis. Sediment samples (n=9) were collected from each study site, dried, sieved, and subjected to microwave-assisted nitric acid digestion prior to AAS analysis. Our results indicate a significant difference in Round Goby lead concentrations between the control site and marina site samples.

Index words: Round Goby, Lake Macatawa, marinas, Lead, bioaccumulation, trophic transfer

INTRODUCTION

Invasive Species and Nutrient Cycling within the Great Lakes

Since European settlement in North America, more than 135 nonindigenous species have been introduced into the Great Lakes basin (Mazak et al. 1997). Once government agencies and scientists found that many invasive species were entering the Great Lakes through ballast water of cargo ships, regulations on ballast water exchange were mandated. Most of these regulations have been implemented within the past few decades. The most noticeable of the nonindigenous species introduced into the Great Lakes waterways include Zebra Mussel (*Dreissena polymorpha*), Round Goby (*Neogobius melanostomus*) and most recently, Quagga Mussel (*Dreissena rostriformis*). Invasions of these species affect the aquatic cycling of contaminants within the food webs, specifically allowing contaminants normally found within sediments to be appropriated to higher trophic levels such as fish, birds and humans (Kwon et al. 2006). Presently, 362 contaminants have been identified within the Great Lakes, and 11 of these are considered to be critical pollutants, including alkylated lead (Fields 2005). Through analysis of sediment, many of these contaminants have been traced back to industrial sites that surround the lakes, particularly in Chicago and Milwaukee (Fields 2005). Through bioaccumulation, which is defined as "the process in which the chemical concentration in an organism achieves a level that exceeds that in the organism's diet, due to dietary absorption" (Gobas & Morrison 2000), substances such as alkylated lead can reach toxic concentrations by moving up the food chain, and can negatively influence the health of animals and humans. This process has been accelerated through the Round Goby, which has been shown to bioaccumulate high concentrations of many toxic substances.

Round Goby Invasion, Characteristics and Relationship to Bioaccumulation

The Round Goby (*N. melanostomus*), shown below in Figure 1, entered the Great Lakes through ballast water discharge from the Caspian and Black Seas, and was discovered in the St. Clair River in 1990 (Glassner-Shwayder 2000). This species did not invade the five Great Lakes as quickly as the Quagga and Zebra mussel. In 1993, the Round Goby began expanding its range, and by 1995 the species had invaded all of the Great Lakes. This benthic species is characterized as a competitive fish (Glassner-Shwayder 2000), has high fertility, can adapt in many different environments, and a highly developed lateral line, which detects movement and vibrations in the water and is comprised of superficial neuromasts. This contrasts most Great Lakes fish which have an enclosed lateral line system (Kornis et al. 2012), allowing the Round Goby to be able to feed at night (Glassner-Shwayder 2000). The most noticeable characteristic of this species is the ability for the Round Goby to feed almost exclusively on Zebra mussels (Figure 2) and Quagga mussels (Figure 3) using pharyngeal teeth to crush the mussels. In a laboratory setting, Round gobies have been observed consuming up to 100 mussels a day (Weimer & Keppner 2000). The overabundance of mussels within the Great Lakes region, along with the evidence that few other species feed on the invasive mussels (Weimer & Keppner 2000), allows for the Round Goby to have a large food resource and grow up to 10 inches (Glassner-Shwayder 2000). Round Goby reproduce several times a year, have aggressive behavior, and have been introduced into the Great Lakes with genetic diversity due to the ballast water of different ships, leading to the Goby's success as an invasive species (Kornis et al. 2012). In previous studies, levels of lead concentration for Round Goby have ranged from 0.1 to 1.0 ppm in a Lake Erie study (Hogan et al. 2007), and from <35-91 ppm in another study focusing on several Lake Erie sites (Painter et al. 2001).

One study found that Dreissenid mussels account for 64.5% (mass) of the Round Goby diet (Kornis et al. 2012), with the greatest preference of mussel size to be 8-11mm in length. It is important to note that the Round Goby can thrive in conditions without Dreissenid mussels, and instead specialize on chironomids and amphipods, types of isopods (Kornis et al. 2012). However, the impact of Round Goby upon Dreissenid species is significant. A study observing the relationships between Zebra mussel density and Round Goby presence showed that larger gobies (>67mm) preferred to consume Zebra mussels, while smaller gobies (<67mm) preferred to consume amphipods, isopods, and chironomids (Kuhns & Berg 1999).

The relationship between Round Goby and Dreissenid mussels is important to discuss, as Dreissenid mussels live in pelagic sediment, and feed on the sediment and algae within this layer. Dreissenid mussels have the ability to filter one or more liters of water every day, removing phytoplankton and other zooplankton from the water column, allowing for greater water clarity (Glassner-Shwayder 2000). Research reveals that Dreissenid mussels accumulate concentrations of heavy metals in tissues at densities ten times higher than native mussels posing a significant threat to the fish that consume them (Fields 2005), with Dreissenid mussels having lead concentrations ranging from 0.3-2.0 ppm in different Great Lakes sites (Hogan et al. 2007, Kwan et al. 2003), and up to 5ppm in Lake Erie (Rutzke et al. 2000). However, Dreissenid mussels do not store all ingested heavy metals due to their ability to excrete a portion of the metals through pseudofeces, which returns to the water column and sediment (Hogan et al. 2007). Because Round Goby prey mainly upon Dreissenid mussels which have directly taken up contaminants from the water column and sediments, it can be inferred that the Round Goby will have higher concentrations of metals from bioaccumulation compared to the Dreissenid mussels.

Figure 1. The Round Goby (*Neogobius melanostomus)*

http://gallery.nanfa.org/v/members/Uland/Exotics/Neogobius+melanostomus++Round+Goby.jpg.html?g2_imageViewsIndex=1

Figure 2. Zebra Mussel (*Dreissena polymorpha)*
http://nas.er.usgs.gov/queries/factsheet.aspx?speciesid=5

Figure 3. Quagga Mussel (*Dreissena rostriformis)*
http://nathistoc.bio.uci.edu/Molluscs/Dreissena%20rostriformis%20bugensis/DSC_6535b.jpg

Presence of Sediment Heavy Metal Pollution from Boat Operation and Maintenance

Heavy metals enter lake water through a variety of sources. High levels can be attributed to sources related to the boating industry. A leading metal contaminant caused by boating is lead. The combustion of leaded gasoline is assumed to be one of the major sources of lead in the aquatic environment (Edgington and Robbins 1976, Graney et al. 1995). Boat engine exhaust is released from the vessel at the atmosphere - aquatic interface, allowing lead deposition directly into the water (Edgington and Robbins 1976). Through core sampling, it was observed in Lake Michigan that with the increase in usage of leaded gasoline, the levels of lead present increased (Edgington and Robbins 1976). Lead has since been removed from gasoline, including marine gasoline, beginning in early 1970s and completed by 1996 (U.S. EPA 1995).

Marinas are the locations of most frequent boat usage and petroleum storage, leading to increased subjectivity to pollution (An and Kampbell 2003). If the marina has been in operation prior to the ban of leaded fuel, pollution from exhaust will be in question. In addition to boat exhaust from engines using leaded fuel, lead, along with other heavy metals (copper and zinc), have been detected from boat maintenance and upkeep, including the application of antifouling paints (An and Kampbell 2003, New Hampshire 2006, Orange 2007). The presence of lead can be found from sources outside boat operations. Lead deposition can be traced to atmospheric inputs of coal combustion, as well as runoff from terrestrial pollution (Edgington and Robbins 1976). Sediments are the sink of metals, including lead, in freshwater environments (An and Kampbell 2003). A study by Thompson et al. (1984) shows correlation between metal concentrations in sediment and organisms, and organisms as source identifiers for heavy metal pollution. Levels of sediment lead concentration were found to be near 10 ppm in Lake Erie (Hogan et al. 2007), and ranging from 1-92 ppm in a Lake Macatawa study (Greve et al. 2013).

Herein, we characterize the significant amounts of lead bioaccumulation in Lake Macatawa and the possibility that its presence could be due to high boating activity in certain locations. We determine whether significant concentrations of lead found within sediments and Round Gobies are associated with marinas. In addition, we search for a spatial association of lead deposition in sediment based on proximity to the marinas.

METHODS

Study Sites

This study occurred on Lake Macatawa, Ottawa County, near Holland, Michigan. Study sites were comprised of four experimental sites and three control sites (Figure 4). The experimental sites were nearby four selected marinas on the lake. These locations and depths are as follows: (1) Anchorage Marina (42.791325° N, 86.183544° W) (6ft), (2) Crescent Shores Marina (42.784701° N, 86.130795° W) (3ft), (3) Eldean Shipyard (42.769852° N, 86.201228° W) (3ft), and (4) Yacht Basin Marina (42.789453° N, 86.183935° W) (4ft). The control sites were utilized to obtain a general overview of Lake Macatawa as a whole, in order to compare levels of lead to the marina sites. These locations are as follows: (1) Howard B. Dunton Park Pier (42.795456° N, 86.120871° W) (4ft), (2) Kollen Park (42.789436° N, 86.121950° W) (6ft), and (3) Holland State Park (42.772534° N, 86.206530° W) (18ft).

Sample Collection

Round Gobies were caught using a standard fishing technique of hook and line, baited with night crawlers following the protocol of Hogan et al. 2007 and Kwon et al. 2006. Fishermen were placed along seawalls of sample sites due to the notable presence of Round Goby. For each site, it was necessary to obtain at least 12 Round Goby for adequate sample site following the protocol of Hogan et al. 2007. Extra catch was included in order to minimize error. Once collected, Round Gobies were immediately killed and measured to sort into size categories (5-10cm and >10cm), then placed in separate plastic storage bags for each sampling location (Kwon et al. 2006). Upon returning to laboratory facilities in A. Paul Schaap Science Center, Round Goby samples were stored in a refrigerator at 4°C until processing.

Sediment samples were collected at all locations in which fishing occurred. Three samples were collected at each site to obtain an accurate sampling due to the lack of uniformity in sediment lead concentrations in a sampling site. A cleaned, steel Ekman grab sampler was used to collect sediments to limit destruction to the benthic zone. Contents were emptied into a clean container, and then sampled from the upper 5 cm with a clean, stainless steel trowel, following the protocol of Chen and Folt (2000). Sediments were placed into clean, 1L plastic sediment sampling containers and stored in a refrigerator at 4°C (following the protocol of Hogan et al. (2007).

Sample Processing

Whole-body samples of Round Gobies were used to be consistent with predation patterns on the species (Hogan et al. 2007). Round Gobies were ground into a homogeneous mixture using a standard food processor with RO water, if necessary (Kwon et al. 2006). After, dehydration of the samples was conducted at 90°C using a Thermo Scientific Precision Econotherm Oven. Weights were recorded daily until a constant weight was obtained to ensure that moisture was absent from the samples (U.S. EPA 2007). Individual dried samples of Round Goby were stored in clean glass beakers with watch glass and placed in a desiccator until analysis.

Sediment samples were transferred to clean glass beakers and were dried at 90°C using a Thermo Scientific Precision Econotherm Oven. Weights were recorded daily until a constant weight was obtained to ensure that moisture was absent from the samples (Hogan et al. 2007). Sediments were sieved to remove all grains and debris greater than 1mm in diameter and returned to the oven for storage until analysis.

Sample Digestion

Round Goby samples were prepared for analysis using Microwave-Assisted Acid Digestion with nitric acid. Samples were powdered using mortar and pestle, then weighed to 0.15g, or under to prevent explosion from excess organic material, and placed in digestion vessel. 10 mL of trace metal grade nitric acid was added to the sample in the vessel. 14 samples of each location were digested due to the apparatus limitations. A method was adapted from EPA Method 3051 and developed for Round Goby, entitled “FISH 1” (U.S. EPA 2007). This method digested the sample at 200°C and 300 psi at 300W for 30 minutes with a cool-down period. The liquefied sample was funneled into labeled 10 mL centrifuge tubes and stored in a refrigerator at 4°C until Atomic Absorption Spectroscopy.

Sediment samples were prepared using the same equipment as the Round Goby samples. Samples were weighed to 0.20g, or under to prevent explosion from excess organic material, and placed in digestion vessel. 10 mL of trace metal grade nitric acid was added to the sample in the vessel. Three trials of each sample at each sample site (9 samples per site in total) were digested. An existing method was developed to digest sediments. Using EPA Method 3051 “EPA 3051X-OMNI 1500”, the sample was digested at 175°C and 1200 psi at 1200W for 10 minutes with a cool-down period (U.S. EPA 2007). The liquefied sample was funneled into labeled 10 mL centrifuge tubes and stored in a refrigerator at 4°C until Atomic Absorption Spectroscopy.

Sample Analysis

Lead concentrations in Round Goby were analyzed using Atomic Absorption Spectroscopy (AAS). Due to the use of whole-body samples, there is significant interference in absorption of lead. To counter this, and achieve accurate measurements, a method of standard additions was used for each sample. For each sample tested, a 2 mL portion of digested Round Goby was combined with a standard solution containing 100 ppm lead. A series of five concentrations were used in AAS, with the standard additions of 0 mL, 0.15 mL, 0.30 mL, 0.45 mL, and 0.60 mL. Standard protocol for use of AAS was completed and it was confirmed that the optimization gain was within the range of 50-75%. Each sample tested was run under the lead lamp and low blue flame for three repetitions (3 seconds per repetition). The output yielded absorbance values which were plotted on Beer's Law plots to calculate the actual concentration of lead in each sample of Round Goby. The diluted lead concentrations were calculated by deriving the slope and intercept from the Beer's Law plot and then dividing the intercept by the slope of the line. This resulting value was then divided by the grams of fish used in digestion, and then multiplied by the grams of nitric acid used in digestion, resulting in a final lead concentration for the Round Goby.

Lead concentrations in sediments were also analyzed with AAS. The procedure for sediments differed from the fish as high interference was not a factor. Standards were created for a Beer's Law plot using a standard solution containing 100 ppm lead and trace metal free nitric acid. Standards of 0ppm, 25ppm, 50ppm, 75 ppm, 100 ppm were tested for their absorbance. Sediment samples were tested on the AAS and their corresponding absorbance were plotted on the Beer's Law plot to calculate concentrations of lead in the sediment samples. The method used to determine the final lead concentration of sediment using the diluted lead concentration followed the method previously mentioned for lead concentration determination for Round Goby.

The error associated with the lead concentrations for Round Goby and sediment samples was calculated by taking into account the mass balance error, along with the volumetric error from the dilutions. From these values, a propagation of error method was utilized in order to determine the uncertainty in the final lead concentration values.

Statistical Analysis

A one-way Analysis of Variance (ANOVA) was used to look at differences of lead concentration between control and marina sites for sediment and Round Goby samples, along with differences in lead concentrations within different Round Goby size classes. General linear models (GLM) were used to test for the effects of control and marina sites and trophic levels on the total lead concentrations. Site was used as a categorical variable, while trophic level was used as a covariate variable. All statistical tests were run using PASW Statistics 18.

RESULTS

Lead bioaccumulated with increasing trophic level (sediment → Round Goby) at Anchorage Marina (GLM, $p<.001$, Table 1), Eldean Shipyard (GLM, $p<.001$, Table 1), Crescent Shores Marina (GLM, $p<.001$, Table 1) and Holland State Park (GLM, $p<.001$, Table 1), where both sediment and fish were collected. Lead concentrations in the sediment were 34% of the lead concentrations found in Round Goby. Levels of lead concentration in Round Goby differed from the control site to marina sites (ANOVA, $p<.001$, Table 2).

The average lead concentrations found in Round Goby for marina sites was 90.1 ppm, with the control site having an average lead concentration of 53.3 ppm (Figure 5, Figure 6, Table 4). Lead concentrations significantly differed when comparing the larger size class of Round Goby (>10 cm) caught at Crescent Shores Marina compared to the smaller size class of Round Goby (5-10 cm), with the larger gobies having higher lead concentrations (ANOVA, $p<.001$, Table 2, Figure 5). No differences in lead concentrations were detected between the different size classes from Eldean Shipyard.

The average lead concentrations found in sediment samples for marina sites was 29.2 ppm, and the control sites having an average lead concentrations of 28.5 ppm (Figure 7, Figure 8, Table 5). The concentration in sediment samples differed from Yacht Basin to each control site: Holland State Park (ANOVA, $p<.001$, Table 3), Dunton Park (ANOVA, $p<.05$, Table 3) and Kollen Park (ANOVA, $p<.05$, Table 3). No differences in lead concentrations were detected between sediments and Round Goby among the three other marina sites.

DISCUSSION

Exact proportions of various lead contributors to Lake Macatawa are largely undetermined, although unpublished findings by Greve et al. (2013) indicate petroleum combustion as a definite contributor of lead to the lake. Along with combusted petroleum, Greve et al. (2013) also raises suspicion of lead contribution from a history of fuel spills and leaking underground fuel storage tank. Robbins et al. (1977) determined benthic isopods are capable of reworking bioaccumulated lead in near-surface sediment (upper 3-6 cm) within Lake Huron at a degree high enough to surpass effects of sedimentation. The maintenance of lead concentrations near the sediment-water interface is largely due to these macroinvertebrates, and therefore we can assume similar processes occur within the benthic environment of Lake Macatawa.

Painter et al. (2001) notes that possible sources of contaminants may also include agricultural runoff and sewage disposal. Both agricultural runoff and residential runoff are known to be major sources of contamination to the Macatawa Watershed, although the presence of lead within these is not verified.

Lead concentrations in Round Goby collected in marina sites were significantly different than those within the control site, in which case Round Goby associated with marinas contained lead concentrations with a minimum exceedance of 17.1 ppm in comparison to the control site. Additionally, lead concentrations in Round Goby averaged 36.8 ppm higher in marina sites than the control site. Lead concentrations in Round Goby from our study were exceptionally higher than those of Hogan et al. (2007), who observed concentrations ranging between 0.1 - 1.0 ppm in Lake Erie Round Goby. Due to inadequate collection of Round Goby samples from other projected sampling locations necessary for whole-sample analysis, only one control site was available. This poses concern and confirms larger error despite the significantly higher lead concentrations determined in Round Goby associated within marinas. Nonetheless, Round Goby lead concentrations were exceptionally high in marina sites compared to the control site at Holland State Park along the channel, which may over-represent lead concentrations of an optimal control since this narrow channel region contains the highest boat traffic within the lake. In addition, there is question as to the distances travelled for each individual Round Goby. From these assumptions, a more optimal control site would provide lower lead concentrations in Round Goby, and further increase the lead concentration differences between marina and control sites.

Adequate Round Goby sample size in Crescent Shore Marina and Eldean Shipyard allowed samples to be partitioned into intervals based on total length (5-10 cm; >10 cm), which was assumed as a general proxy for relative age. We expected longer – and consequently older – Round Goby to bioaccumulate more lead than the smaller samples. This relationship was observed from Crescent Shore Marina samples, where longer Round Goby bioaccumulated significantly higher lead concentrations than the short samples. This relationship, however, was not observed in Eldean Shipyard samples. Many factors may contribute to this unexpected relationship, which possibly include parameters of current turbidity, proximity to, and timing of, dredging, as well as the degree to which dredging affects sediment facies within marinas.

Lead concentrations failed to significantly differ between the marina and control sites with the exception of Yacht Basin Marina, which was 19.6 ppm higher than the control site containing the highest lead concentration (Kollen Park). Removal of sediment via large-scale dredging occurs and may contribute agitation to the upper portion of the sediment facies, and therefore, eliminate any accurate representation of lead present in marinas. Lead concentrations were always higher in Round Goby compared to sediment within the study sites where both samples were obtained. From this, we conclude Lake Macatawa Round Goby are capable of significant lead bioaccumulation. We interpret this uptake is largely attributed to the Round Goby's dominant benthic activity where they are exposed to highest lead concentrations within the lake's stratigraphic column. Dreissenid mussels were incorporated with sediment samples at Holland State Park and Eldean Shipyard sampling sites and are very likely a dominant contributor to the lead consumed in Round Goby. These mussels become less common (and may not be present) eastward of these locations, and therefore may not be a major contribution to our overall findings of lead concentrations in Round Goby.

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APPENDIX

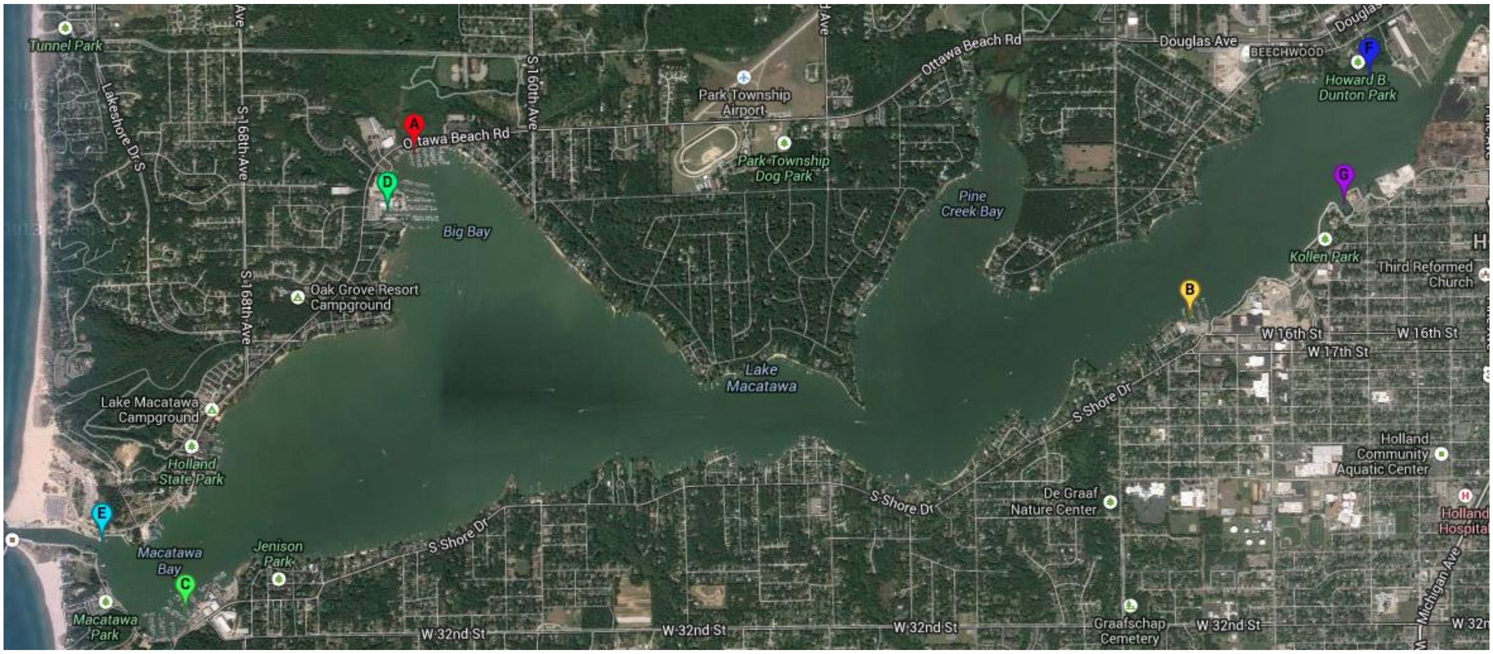

Figure 4. Locations of sampling sites: (A) Anchorage Marina, (B) Crescent Shores Marina, (C) Eldean Shipyard, (D) Yacht Basin Marina, (E) Howard B. Dunton Park Pier, (F) Kollen Park, (G) Holland State Park.

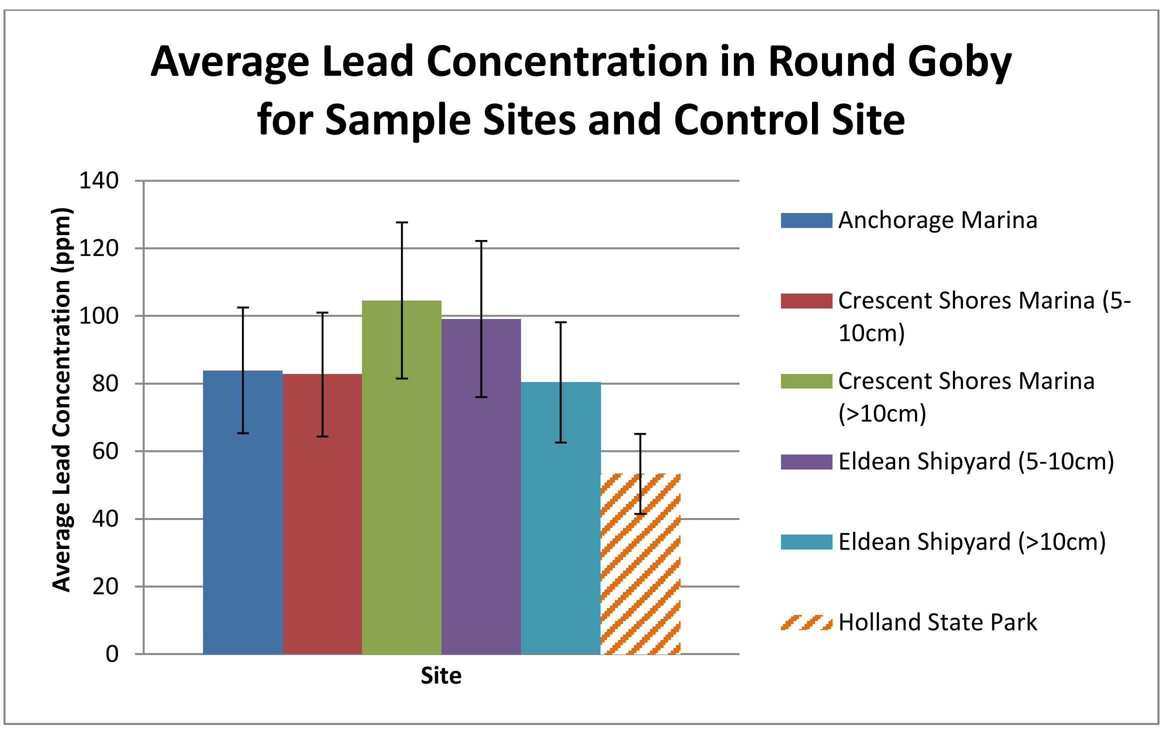

Figure 5. Average lead concentrations (in ppm) with calculated error in Round Goby for sample and control sites within Lake Macatawa.

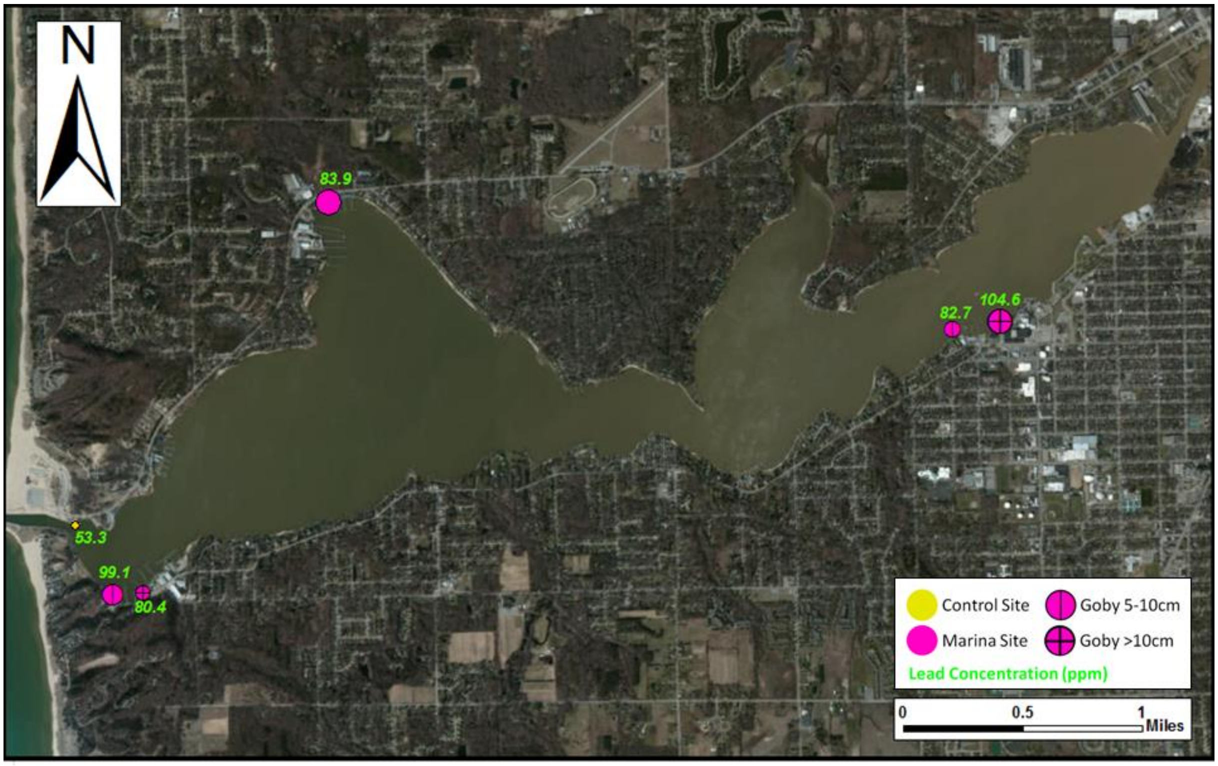

Figure 6. Aerial map of Lake Macatawa showing the spatial distribution of lead concentrations for Round Goby within the control site and marina sample sites.

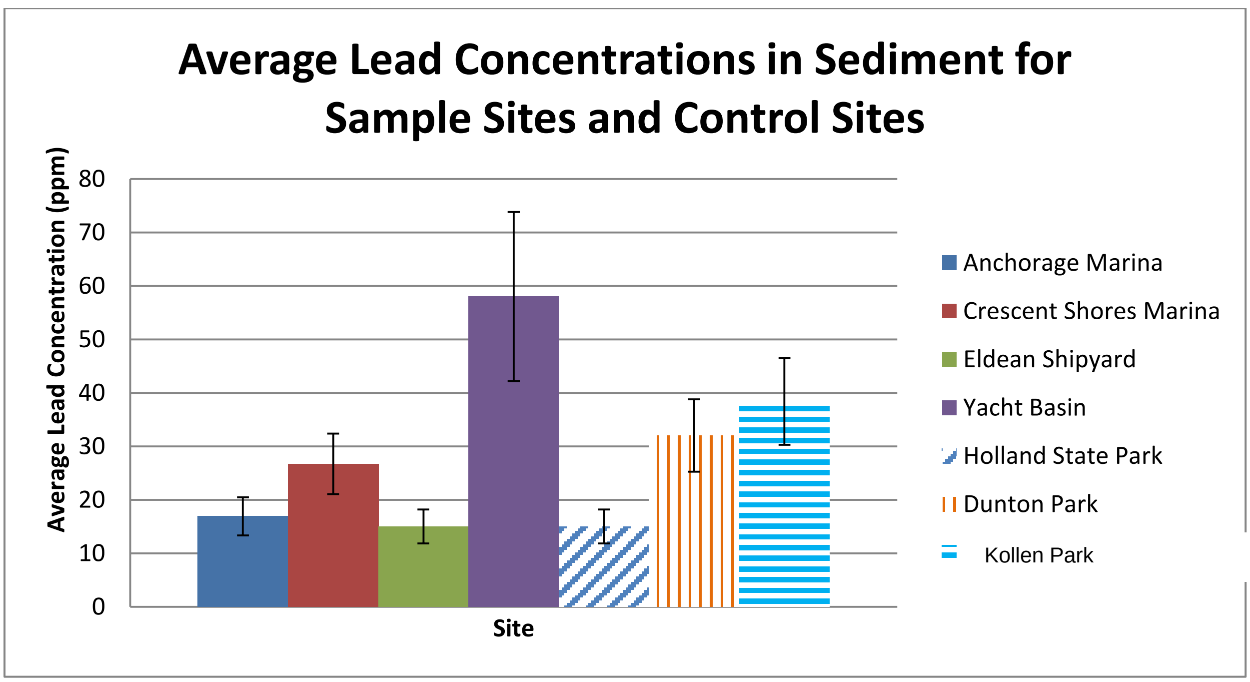

Figure 7. Average lead concentrations (in ppm) with calculated error in sediment for sample and control sites within Lake Macatawa.

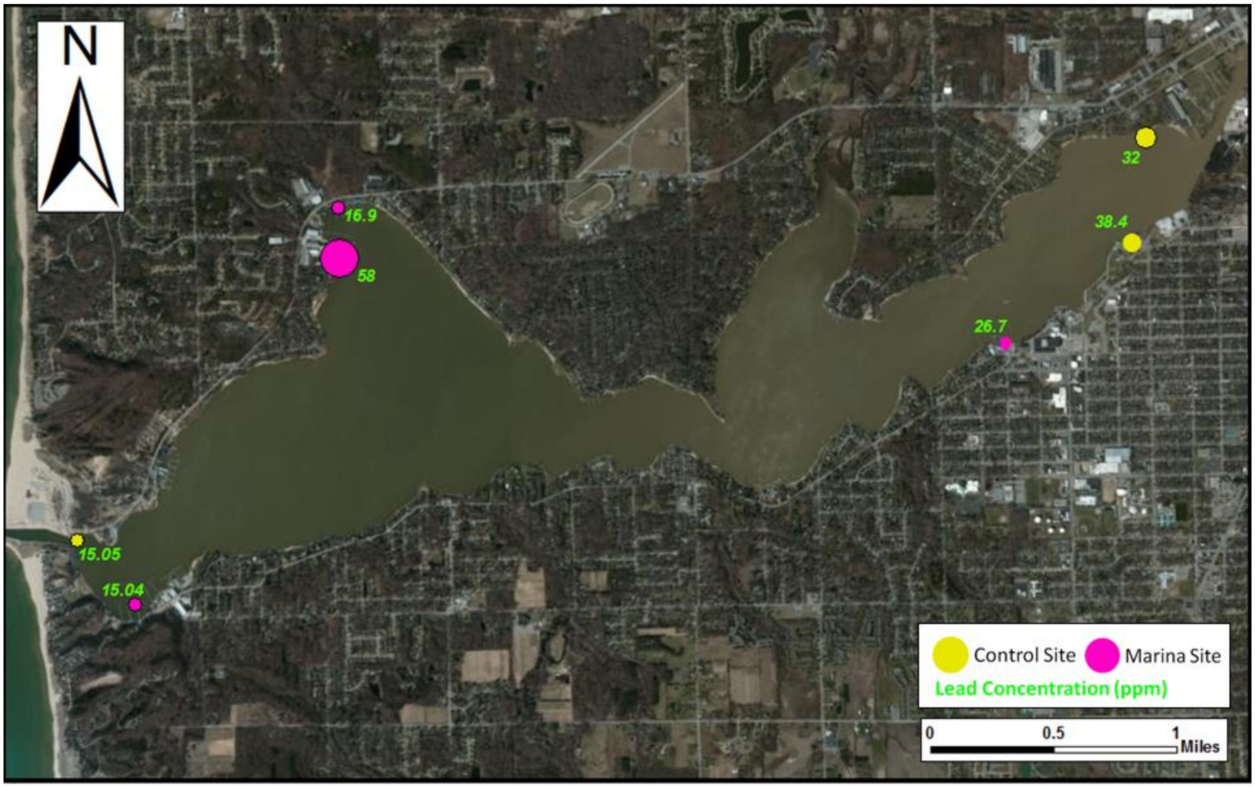

Figure 8. Aerial map of Lake Macatawa showing the spatial distribution of lead concentrations for sediment within the control sites and marina sample sites.

Table 1. Results of the GLM tests for effects of trophic level (sediment and Round Goby), and site on total lead concentrations.

Sample Site	df	F value	Significance
Anchorage Marina	1	2971.3	<.001
Crescent Shore Marina	1	245.4	<.001
Eldean Shipyard	1	487.8	<.001
Holland State Park	1	479.2	<.001

Table 2. Results of ANOVA testing for differences in lead concentrations in Round Goby between the three marina sites compared to the control site.

Sample Site	Mean Difference	Standard Error	Significance
Anchorage Marina	30.51	0.61	<.001
Crescent Shore Marina			<.001
5-10 cm	29.37	0.34	<.001
>10 cm	51.26	0.29	<.001
Eldean Shipyard			<.001
5-10 cm	45.71	0.29	<.001
>10 cm	27.10	0.38	<.001

Table 3. Results of ANOVA testing for differences in lead concentrations in sediment samples between Yacht Basin and the three control sites.

Sample Site	Control Site	Mean Difference	Standard Error	Significance
Yacht Basin	Holland State Park	59.5	12.2	<.001
	Dunton Park	42.6	12.2	<.05
	Kollen Park	45.2	12.6	<.05

Table 4. Average lead concentrations found in Round Goby for the three marina sites compared to the control site, along with the average error for each site.

Sample Site	Average ppm	Average Error
Anchorage Marina	83.9	18.6
Crescent Shores Marina (5-10cm)	82.7	18.3
Crescent Shores Marina (>10cm)	104.6	23.1
Eldean Shipyard (5-10cm)	99.1	21.9
Eldean Shipyard (>10cm)	80.4	17.8
Holland State Park	53.3	11.8

Table 5. Average lead concentrations found in sediment for the four marina sites compared to the three control sites, along with the average error for each site.

Sample Site	Average ppm	Error
Anchorage Marina	16.9	3.58
Crescent Shores Marina	26.7	5.66
Eldean Shipyard	15.0	3.19
Yacht Basin	58.0	15.8
Holland State Park	15.0	3.19
Dunton Park	32.0	6.78
Kollen Park	38.4	8.13