Environmental Factors Affecting Methylmercury in Fish of the Laurentian Great Lakes Region

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By

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I HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER MY SUPERVISION BY Joel James Harvey ENTITLED Environmental Factors Affecting Methylmercury in Fish of the Laurentian Great Lakes Region BE ACCEPTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF Master of Science.

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ABSTRACT

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Monomethylmercury (MMHg) can accumulate in fish to concentrations that pose a threat to the health of fish, piscivorous organisms, and humans who eat fish. A variety of environmental factors have been hypothesized to influence either the methylation of inorganic Hg or the bioaccumulation and magnification of MMHg. This study investigates the influence of selected environmental factors on MMHg concentrations in freshwater fish across a regional scale, most of the U.S. portion of Laurentian Great Lakes region. Fish MMHg was correlated with proton deposition, sulfate deposition, nitrate deposition, mercury deposition, pH, watershed area, and Secchi depth. Only proton deposition was positively correlated with MMHg concentrations in walleye, largemouth bass, and northern pike, underscoring the fundamental role played by protons in fish MMHg accumulation in the Great Lakes region. Lake water pH showed a significant correlation with fish MMHg only in Largemouth bass, suggesting proton deposition plays a role in Hg transport.

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Statistical studies such as this one are only possible with free access to data. I would like to thank the following government agencies for collecting the fish mercury data and allowing me to use it in my thesis, New York State Department of Environmental Conservation, Wisconsin Department of Natural Resources, Minnesota Pollution Control Agency, and the Michigan Department of Environmental Quality. I would like to acknowledge the R Foundation for Statistical Computing for writing the R program and making it freely available. And finally, acknowledgment is due to my thesis committee, Bruce Monson, Mark Sandheinrich, David Dominic, and Chad Hammerschmidt, for reviewing this manuscript.

Introduction

Monomethylmercury (MMHg) can accumulate in fish to concentrations that pose a threat to the health of fish, piscivorous organisms, and humans who eat fish. At exposures encountered in some natural waters, MMHg can impair reproduction of fish (Hammerschmidt et al., 2002; Drevnick et al., 2003; Depew et al., 2012), and neurochemical, reproductive, and behavioral effects have been well documented for amphibians and fish-eating birds and mammals (Scheuhammer et al., 2007; Burke et al., 2010). Human MMHg exposure from fish consumption increases the risk for diminished neurological development and fetal growth (Mahaffey et al., 2009; Karagas et al., 2012; Goldman et al., 2001). Accordingly, understanding the environmental processes influencing MMHg production and its bioaccumulation are important for potentially mitigating these exposures and effects.

Methylmercury is produced from inorganic Hg by microorganisms. Inorganic Hg is introduced into the environment from geologic and anthropogenic sources (Pacyna et al., 2010). About two-thirds of atmospheric Hg loadings are from anthropogenic sources (Fitzgerald et al., 1998). Microorganisms, particularly sulfate and iron reducing bacteria, transform inorganic Hg to MMHg (Gilmour et al., 1992; Fleming et al., 2006; Kerin et al., 2006; Parks et al., 2013). MMHg is accumulated by biota and biomagnified with successive trophic transfer in food webs, resulting in piscivorous fish often having the highest concentrations in aquatic ecosystems (Wiener et al., 2003).

A variety of environmental factors have been hypothesized to influence either the methylation of inorganic Hg or the bioaccumulation and magnification of MMHg, including, for example, atmospheric Hg deposition, pH, the cycling of nitrogen and sulfur, and water body productivity (Winfrey et al., 1990, Gilmour et al., 1991, Benoit et al., 1999, Hammerschmidt et al., 2004, Chen et al., 2005, Hammerschmidt and Fitzgerald, 2006). Both correlational and comparative field studies have provided a foundation for most of the hypothesized connections between MMHg and particular environmental factors affecting either its production or bioaccumulation in fish. While many of these studies have been highly localized, for example, comparing MMHg in fish among lakes within a small geographic proximity (Wiener et al., 2006; Grieb et al., 1990; Drevnick et al., 2007), others have looked for bioaccumulation patterns on regional (Kamman et al., 2005) and semi-continental scales (Hammerschmidt and Fitzgerald, 2006). Such studies often yield similar findings, including that MMHg concentrations in fish frequently are negatively related to surface water pH (Grieb et al., 1990; Cope et al., 1991), although there is limited mechanistic understanding of how acidity directly affects either production or bioaccumulation of MMHg (Hammerschmidt and Fitzgerald, 2006). However, other investigations have provided contradictory results, such as the role of sulfate in affecting MMHg production and bioaccumulation. Drevnick et al. (2007), for example, found that fish MMHg levels declined over time in Isle Royale lakes, coincident with a reduction of atmospheric sulfate deposition, whereas no relationship was found between atmospheric sulfate deposition and MMHg in largemouth bass

(*Micropterus salmoides*) across the continental U.S. (Hammerschmidt and Fitzgerald, 2006). A likely explanation for the difference in findings between the two studies is the geographical scale and gradient of parameter variation that exists within a particular study area. Whereas primary drivers of MMHg bioaccumulation are likely to be more evident across the broadest ranges, such as atmospheric Hg deposition on a continental scale (Hammerschmidt and Fitzgerald, 2006), secondary and tertiary controls on bioaccumulation will be more evident on smaller spatial scales where the primary driver may be relatively constant in magnitude and influence.

I investigated the influence of selected environmental factors on MMHg concentrations in freshwater fish across a regional scale; most of the U.S. portion of Laurentian Great Lakes region, including the states of New York, Michigan, Minnesota, and Wisconsin. I hypothesized that within- and among-state differences in atmospheric Hg deposition would not be large enough to account for the variation of MMHg concentrations in fish, and by extension, secondary regional controls on MMHg bioaccumulation could be evaluated. This was done by examining the distribution of total Hg concentrations in the fillets of largemouth bass, walleye (*Sander vitreus*), and northern pike (*Esox lucius*) compiled from each state. The highest concentration of Hg is in the muscle tissue as MMHg, where is complexes with amino acids methionine and cysteine (Bloom et al., 1989; Chan et al., 2003). These fish species were selected because they are common top predators in the Great Lakes region, popular with recreational anglers, and extensively monitored for Hg because of human health concerns related to their consumption. I used data compiled from each of the four states to examine potential relationships between fish MMHg and atmospheric fluxes of Hg, nitrate, sulfate, and protons, as well as water-body specific water chemistry (pH, sulfate and nitrate concentration, Secchi depth).

Experimental Section

Fish Hg. Largemouth bass, northern pike, and walleye were sampled, processed, and analyzed for total Hg concentrations in skin-on fillets by state agencies in Michigan, Minnesota, Wisconsin, and New York. MMHg concentrations often increase with the size and age of fish (Grieb et al., 1990; Simoneau et al., 2005). Accordingly, I used fish length as a proxy for age and adjusted for differences of mean fish length between lakes by calculating a weighted mean. Mercury concentrations in largemouth bass length were adjusted to a standard length of 35 cm, walleye length to 40 cm, and to 55 cm in northern pike. These lengths were selected because they are comparable to the average length of each species in my data. I analyzed fish Hg data only from lakes that had at least of five fish of the same species, and only fish sampled between 2001 and 2009 were included to minimize potential effects of temporal changes in environmental factors, a previous study has found a general decrease in fish MMHg concentration between 1970 and 2009 (Monson et al., 2011). The combined data set included 325 lakes for largemouth bass, 500 lakes for northern pike, and 355 lakes for walleye; data for fish in rivers and reservoirs were excluded from analysis. The weighted mean Hg concentration was calculated for each species in each lake by dividing the Hg concentration of a fish by its length to find the length-specific mercury concentration for each lake-species-sample. This value was then averaged for all lake-species-samples in a given lake and this average was multiplied by the species-specific standard length to find the weighted mean Hg concentration for each species-lake (Monson, 2009 Supplemental Information).

Wet Atmospheric Deposition. Annual mean wet atmospheric deposition fluxes of Hg, sulfate, nitrate, and protons were estimated for each of the fish-containing lakes, based on results from the National Atmospheric Deposition Program (NADP, 2013) and Mercury Deposition Network (MDN, 2013). These networks have 36 stations that measure atmospheric flux of sulfate, nitrate, and acid and 18 that measure Hg in the four-state region. All available results from 2001 to 2009 from the monitoring stations were averaged to give a yearly mean depositional flux. Georeferenced flux results from the sampling stations were interpolated with a kriging function (ArcGIS, version 9.2) to estimate the flux to each lake from which fish were monitored for Hg.

Physicochemical Factors of Lakes and Watersheds. Many different biogeochemical factors are hypothesized to influence either MMHg production or bioaccumulation in fish. Due to the unavailability of information on some of these variables, comparisons in this study were limited to the most commonly quantified physiochemical factors. These included water pH, sulfate and nitrate concentrations, watershed area, and Secchi depth, which is a proxy for biological productivity (Swift et al., 2006). State environmental agencies often monitor these variables and have extensive databases. I only used data sampled from the years 2001-2009.

Statistical Analysis. The computer program R (version 2.14) was used to calculate the mean value of water chemistry variables for water chemistry data for each lake and to determine the weighted average for MMHg concentration for each species at each lake. Spearman rank order correlation (SigmaPlot, version 9) was used to identify any correlation between environmental factors and MMHg accumulation in largemouth bass, northern pike, and walleye because the fish data for each species failed the normality test (Shapiro-Wilk).

Results and Discussion

Fish Mercury

The median length adjusted largemouth bass Hg concentration over the entire Great Lakes region was 0.376 µg/g. Largemouth bass met selection criteria from 325 lakes across the Great Lakes region. Of these lakes, 209 had a weighted mean average above the 0.3 µg/g EPA human-health criterion limit (EPA website).

In my study northern pike had a length adjusted mean Hg concentration median of 0.300 µg/g. Data was available for 500 lakes, in 253 of these lakes the length-adjusted mean was over the EPA limit of 0.3 µg/g.

Walleye met selection criteria at 355 lakes. The length adjusted mean Hg concentration had a median of 0.28 µg/g and 170 lakes were above the EPA human-health criterion limit. These MMHg concentrations are high enough to be of toxicological concern not only in humans but also in wildlife. Yellow perch (*Perca flavescens*) MMHg concentrations of 0.21 µg/g have been associated with a 50% decrease from maximal production of common loon (*Gavia immer*) chicks (Wiener et al., 2012).

Atmospheric Mercury Flux

Concentrations of MMHg in northern pike and walleye were related negatively to estimated fluxes of Hg in wet atmospheric deposition among the four Great Lakes states (Figures 1, 2). A previous study demonstrated a significant positive correlation between atmospheric Hg deposition and fish MMHg on a continental scale (Hammerschmidt and Fitzgerald, 2006). A possible explanation for this negative correlation at the regional scale is that Hg flux is auto correlated with another environmental factor that has a greater influence on fish MMHg; one such relationship could exist between Hg flux and proton flux. MMHg concentrations in all three species of fish were positively correlated with proton fluxes in wet deposition. At locations where pike were sampled atmospheric Hg deposition and proton deposition had a correlation of $r=-0.702$ and a $p<0.01$, at locations where walleye were sampled these two variables had an $r=-0.527$ and a $p<0.01$. This negative correlation may account for the negative correlation between fish MMHg and atmospheric Hg flux. Atmospheric fluxes of protons and Hg were positively correlated among locations where largemouth bass were sampled and MMHg in largemouth bass was unrelated to atmospheric Hg flux (Table 1.). It could be possible that the combustion of fossil fuels that result in high concentrations of protons also produce lower quantities of Hg.

In Hammerschmidt and Fitzgerald (2006), the workers compared largemouth bass MMHg concentration to a five-fold range of wet Hg deposition values and found a positive correlation. In my study, the Great Lakes region had only approximately a 2.5-fold range in wet Hg deposition. I didn't expect that this smaller range of values would be sufficient to account for the variation found in fish MMHg. While atmospheric Hg deposition didn't show a significant correlation with largemouth bass MMHg, the northern pike and walleye both had significant negative correlations (Figure 1, 2). This difference could be explained by the positive correlation between proton flux and fish Hg and the negative correlation between Hg flux and proton flux. It is possible that a higher proton flux facilitates the transport of Hg from the watershed into the lakes, resulting in higher MMHg in the fish. A higher proton concentration could result in more Hg available for methylation despite of a lower atmospheric Hg flux.

Mercury dry deposition is not as well understood as wet deposition. It has been assumed to be about equal to wet deposition and could vary widely across the Great Lakes region. Even though dry deposition could be an important factor in fish MMHg accumulation, due to a paucity of data, it was not included in this study.

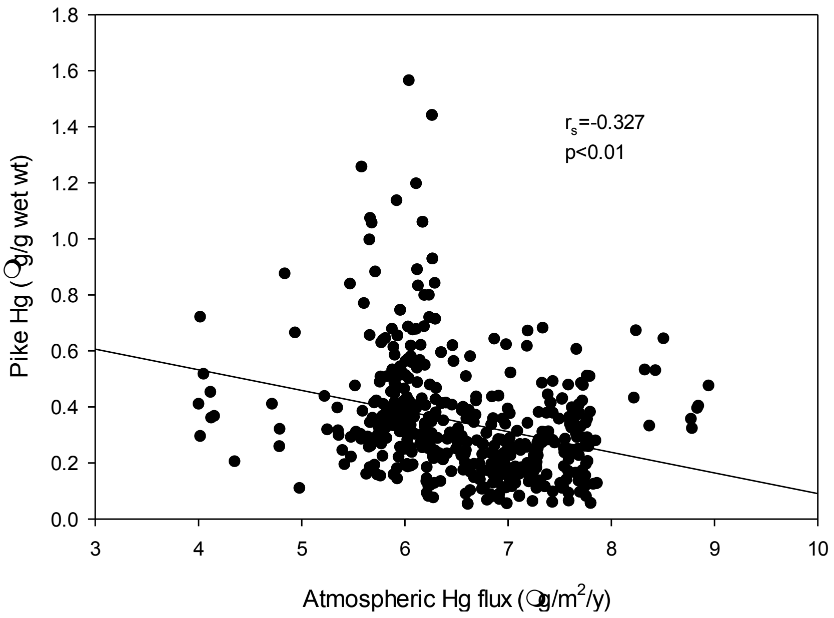

FIGURE 1. Relation between depositon of inorganic mercury and concentrations of mercury in axial muscle of 55-cm (standard length) northern pike. Data from 500 lakes in the Laurentian Great Lakes region of the United States (i.e. Minnesota, Wisconsin, Michigan, and New York) from 2001-2009.

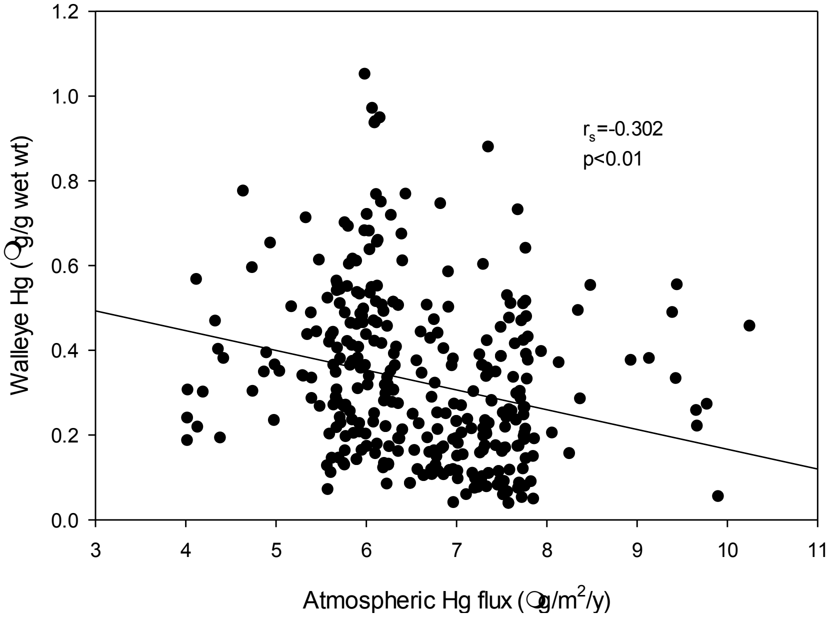

FIGURE 2. Relation between deposition of inorganic mercury and concentrations of mercury in axial muscle of 40-cm (standard length) walleye. Data was from 355 lakes in the Laurentian Great Lakes region of the United States (i.e. Minnesota, Wisconsin, Michigan, and New York) from 2001-2009

Atmospheric Proton Flux

MMHg concentrations in walleye, largemouth bass, and northern pike were positively correlated to wet atmospheric flux of protons (Figures 3, 4, 5). Atmospheric proton flux was the only environmental factor included in this study that was significantly correlated with MMHg concentrations in all three fish species. A hypothesis to explain the importance of proton deposition is that a higher proton flux would act as an “acid wash”. A high proton concentration would facilitate transport of inorganic Hg from the watershed into the water bodies. Instead of being buried in soil within the watershed, more Hg would be transported into lakes and be available for methylation and, in turn, accumulated in fish. A way to test this hypothesis would be to see if lakes in high proton deposition areas had more Hg dissolved in the water, compared to similar lakes with low proton deposition.

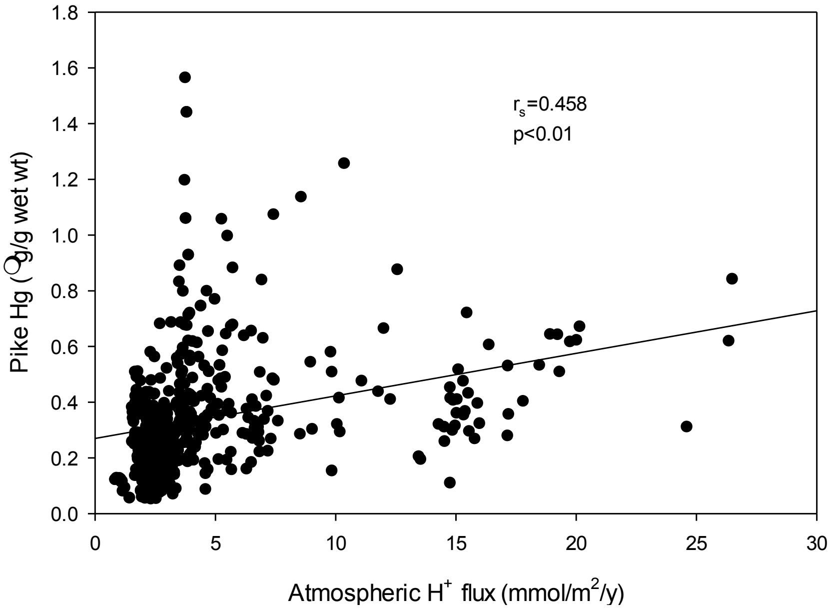

FIGURE 3. Relation between deposition of protons and concentrations of mercury in axial muscle of 55-cm (standard length) northern pike. Data was from 500 lakes in the Laurentian Great Lakes region of the United States (i.e. Minnesota, Wisconsin, Michigan, and New York) from 2001-2009.

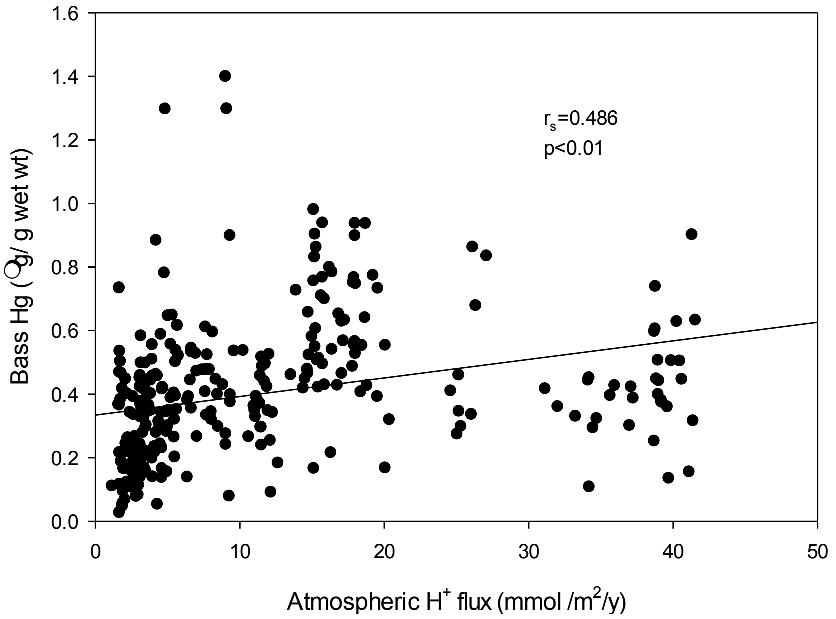

FIGURE 4. Relation between deposition of protons and concentrations of mercury in axial muscle of 35-cm (standard length) largemouth bass. Data was from 325 lakes in the Laurentian Great Lakes region of the United States (i.e. Minnesota, Wisconsin, Michigan, and New York) from 2001-2009.

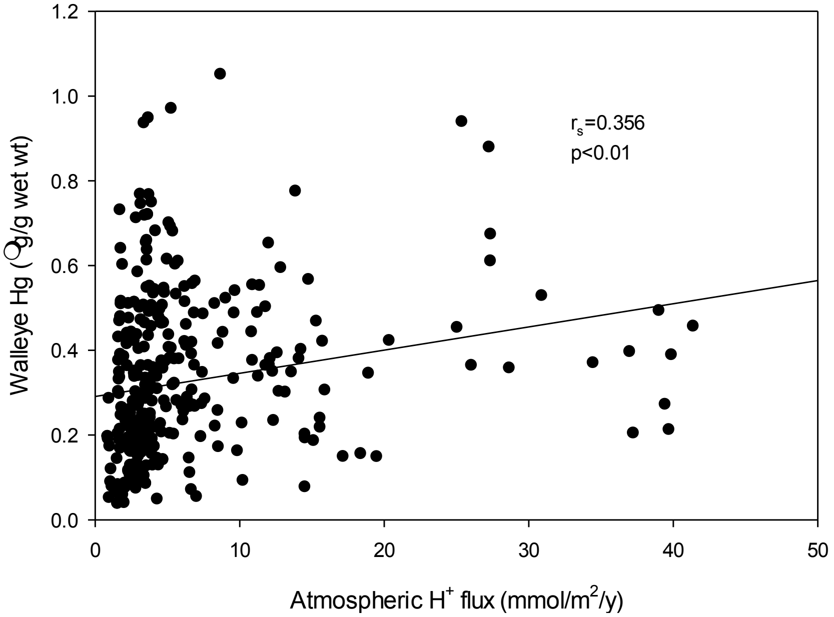

FIGURE 5. Relation between deposition of protons and concentrations of mercury in axial muscle of 40-cm (standard length) walleye. Data was from 355 lakes in the Laurentian Great Lakes region of the United States (i.e. Minnesota, Wisconsin, Michigan, and New York) from 2001 to 2009.

Lake Water pH

When fish MMHg concentrations were compared to lake pH, only largemouth bass had a significant correlation (Figure 6). MMHg concentrations in walleye (p=0.305, 56 lakes sampled) and northern pike (p=0.112, 31 lakes sampled) were unrelated to lake pH. Lake water pH is an important environmental factor in influencing MMHg accumulation in biota (Kelley et al., 2003, Ramial et al., 1985, Ponce et al., 1991, Hammerschmidt and Fitzgerald, 2006). Given that fish MMHg concentrations were positively correlated with atmospheric proton flux, I would have expected walleye and northern pike to also have a negative correlation with lake pH. One explanation for why MMHg in walleye and northern pike was related to proton flux but not lake pH may be the result of having limited lake pH data. The GIS analysis estimated an atmospheric proton flux for every location that fish were sampled. In contrast, lake pH was only measured at a limited number of lakes. Another possible explanation for this discrepancy is the source of the water in the lake. A high concentration of protons in precipitation may not necessarily translate to a low lake water pH. The pH of the water could change before it reached the lake. For example, if a lake were primarily precipitation or stream fed with a relatively small watershed, then one would expect that the lake pH would more closely reflect the proton concentration in wet deposition. In contrast, if a lake was primarily feed by groundwater, the terrestrial environment could affect the pH of the water entering the lake.

The exact mechanism between fish MMHg accumulation and pH remains unknown. Proton activity has no significant effect on the activity of sulfate-reducing bacteria (Rudd et al., 1986). A study has suggested that increased proton activity may enhance the uptake of inorganic Hg by methylating microorganisms (Kelley et al., 2003).

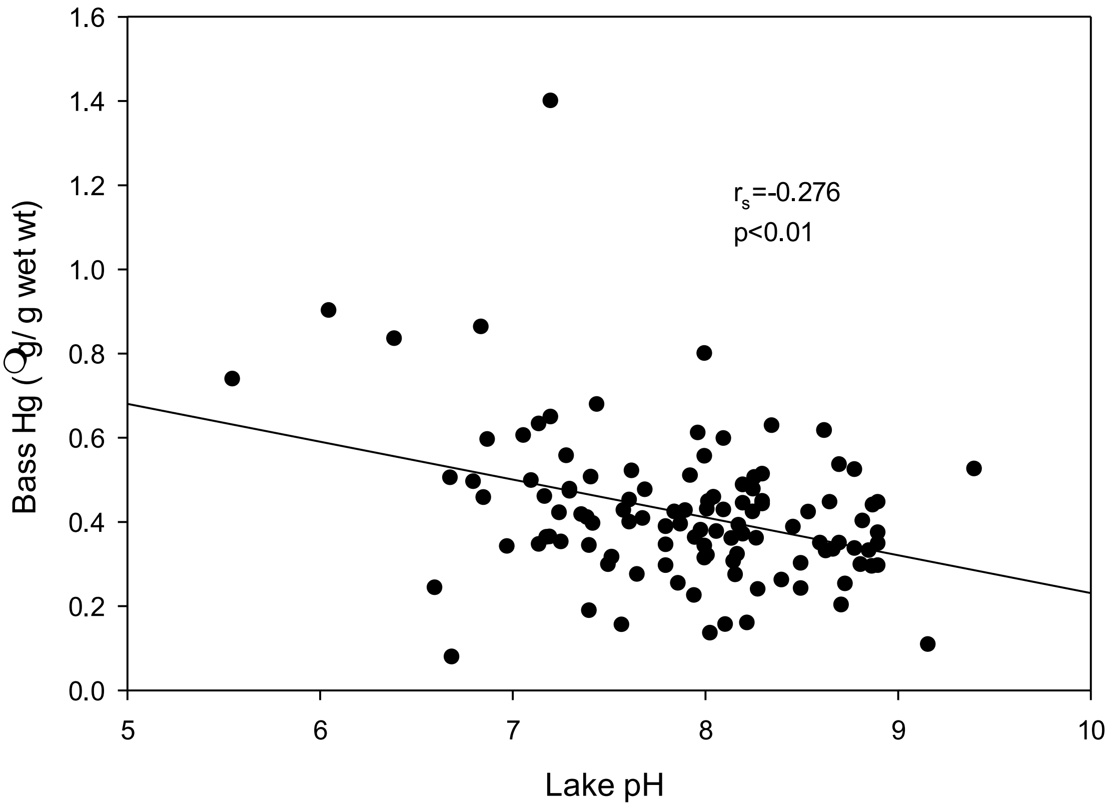

FIGURE 6. Relation between lake water pH and concentrations of mercury in axial muscle of 35-cm (standard length) largemouth bass. Data was from 112 lakes in the Laurentian Great Lakes region of the United States (i.e. Minnesota, Wisconsin, Michigan, and New York) from 2001-2009.

Atmospheric Sulfate Flux

Only largemouth bass MMHg concentrations had a significant positive correlation between sulfate flux and fish Hg concentration (Figure 7). MMHg in walleye (p=0.09) and northern pike (p= 0.206) were unrelated to sulfate deposition. Previous studies have demonstrated the important role played by sulfate in Hg methylation and accumulation in fish (Gilmour et al., 1992; Jeremiason et al., 2006; Drevnick et al., 2007; Coleman et al., 2012). Sulfate-reducing bacteria are presumed to be the primary methylators of inorganic Hg (Gilmour et al., 1992). These bacteria require sulfate as a terminal electron acceptor to drive their metabolism. Anoxic sediments provide the redox conditions necessary for sulfate-based metabolism and, in turn, the production of MMHg. In addition to its role in Hg methylation, sulfate metabolism produces sulfide as a waste product. Sulfide can complex with inorganic Hg to form less soluble cinnabar (HgS). An increased sulfate flux could drive a higher metabolism in sulfate-reducing bacteria, which could reduce the soluble Hg in the pore water, reducing the concentration of inorganic Hg available for methylation and reducing MMHg available for bioaccumulation (Benoit et al., 1999; Wolfenden et al., 2005). Sulfate's antagonistic effects on MMHg production could explain why no significant correlation was found in northern pike or walleye in my study. Decreasing the concentration of available inorganic Hg negated the effect of increased sulfate metabolism. Largemouth bass could prefer lakes where the balance between methylation of Hg is favored over the removal of inorganic Hg from the water column by sulfide.

Drevnick et al. (2007) found a temporal trend between decreasing atmospheric sulfate deposition and MMHg in northern pike from lakes at Isle Royale, Michigan. While MMHg concentrations in northern pike caught from reference lakes at Isle Royale were unchanged in the decade before the study, the northern pike caught from advisory lakes had a significant decline in MMHg. Drevnick et al. (2007) demonstrated a decrease in pike Hg that coincided with a reduction in atmospheric sulfate deposition since the passage of new environmental regulations. This result suggests that sulfate is an important control on MMHg accumulation in fish, at least at Isle Royale. The largemouth bass data used in this study had the widest distribution within the Great Lakes region of the three species. The lakes where largemouth bass were collected also had a wide range of sulfate flux, approximately five-fold across the Great Lakes region, as well as proton flux, approximately ten-fold, as compared to an approximately 2.5-fold Hg flux.

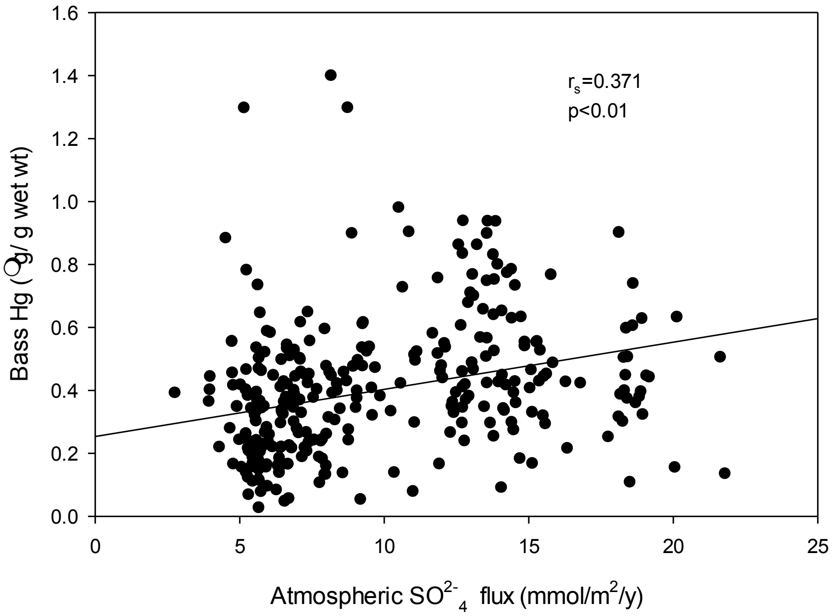

FIGURE 7. Relation between deposition of sulfate and concentrations of mercury in axial muscle of 35-cm (standard length) largemouth bass. Data was from 325 lakes in the Laurentian Great Lakes region of the United States (i.e. Minnesota, Wisconsin, Michigan, and New York) from 2001-2009.

Atmospheric Nitrate Flux

The northern pike MMHg had a significant negative correlation with nitrate flux (Figure 8). The largemouth bass MMHg concentration data showed a significant positive correlation with atmospheric nitrate flux (Figure 9). Walleye MMHg did not have a significant correlation with atmospheric nitrate flux ($p=0.971$).

Microorganisms that use nitrate in metabolic redox reactions will often have an ecological advantage over sulfate-reducing bacteria (Zehnder and Stumm, 1988). Sulfate-reducing bacteria are presumed to be the primary mercury methylators (Gilmour et al., 1992; Gilmour et al., 2013); as such sulfate-based metabolism only begins in pore waters after the dissolved nitrate is exhausted. Todorova et al. (2009) demonstrated that increasing the nitrate load on a lake can reduce MMHg concentrations in pore-water. Given nitrates role in reducing MMHg concentration in pore water, it is interesting that a different result was found in each of the three species of this study. An explanation for the difference between the fish species is the relationship between the wet deposition of sulfate and nitrate. These two anions have a very high significant positive correlation with each other in my data (at locations where northern pike were sampled $r=0.870$, $p<0.01$). Largemouth bass was the only species in this study that had a positive significant correlation with sulfate flux. Because of this high correlation between sulfate and nitrate, perhaps the sulfate flux was large enough to overcome the increased flux of nitrate. This could explain a positive correlation between nitrate deposition and largemouth bass Hg concentration. Whereas the largemouth bass demonstrated a positive correlation and the northern pike had a negative correlation, the walleye didn’t have a significant correlation at all. It is possible that nitrate is only autocorrelated with Largemouth bass MMHg because it increases with sulfate flux. This discrepancy could also be the result of interspecies differences in each species natural history, perhaps in diet or preferred habitat. Fish that prefer to live near the shore may have a diet that includes terrestrial organisms, which may change the fishes Hg concentration.

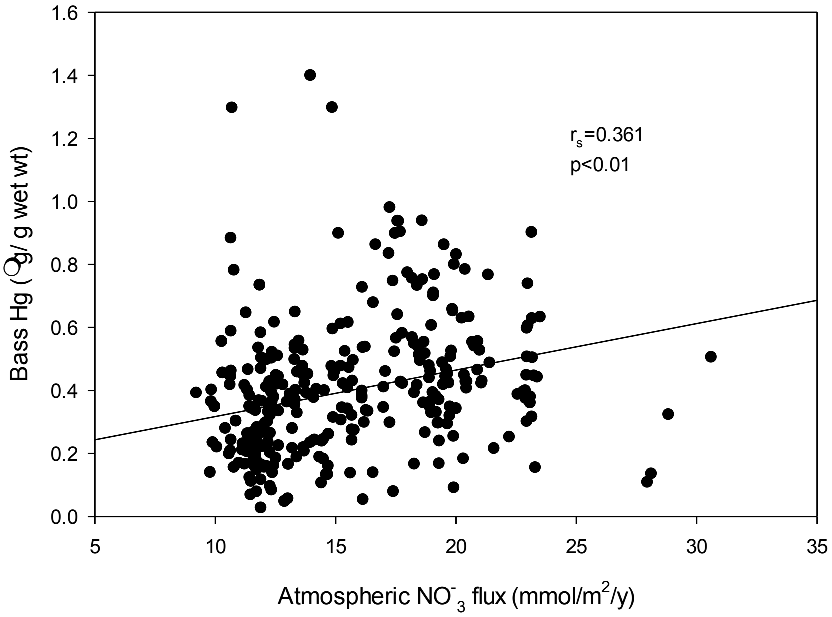

FIGURE 8. Relation between deposition of nitrate and concentrations of mercury in axial muscle of 35-cm (standard length) largemouth bass. Data was from 325 lakes in the Laurentian Great Lakes region of the United States (i.e. Minnesota, Wisconsin, Michigan, and New York) from 2001 to 2009.

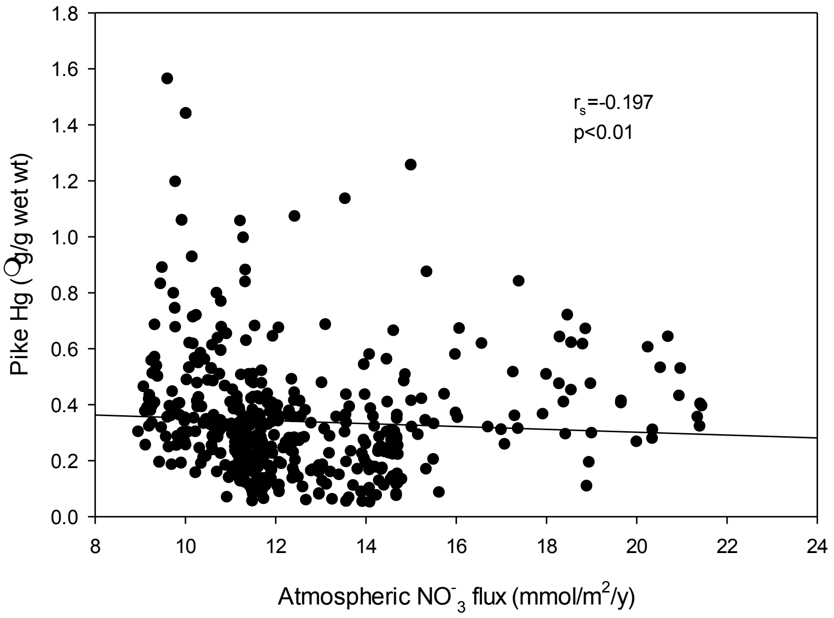

FIGURE 9. Relation between deposition of nitrate and concentrations of mercury in axial muscle of 55-cm (standard length) northern pike. Data was from 500 lakes in the Laurentian Great Lakes region of the United States (i.e. Minnesota, Wisconsin, Michigan, and New York) from 2001-2009.

Secchi Depth

MMHg in northern pike and walleye was positively correlated with Secchi depth (Figure 10, 11). Secchi depth was used as a proxy for lake primary productivity, a lower depth having a higher primary productivity. Previous studies have suggested that greater biological productivity in a lake could translate into lower MMHg concentrations higher in the food web due to biological dilution (Pickhardt et al., 2002; Chen and Folt, 2005, Karimi et al., 2007, Simonin et al., 2008, Chen et al., 2011). Eutrophic lakes may also have chemical characteristics, such as higher lake water pH, that reduce bioavailability of MMHg (Driscoll et al., 2007). A New York study using chlorophyll a to measure productivity, found that lakes containing yellow perch above 5 ng/g mercury also had unusually low chlorophyll a concentrations (Simonin et al., 2008). The largemouth bass had no significant correlation. A possible explanation for the difference between largemouth bass and the other two species is that largemouth bass prefer warmer water, which may have higher biological productivity. Given higher productivity, Secchi depth variation may not be great enough to account for bass Hg concentrations.

Secchi depth would also reflect, not only primary productivity, but also the dissolved organic carbon (DOC) concentration within a lake. Driscoll et al. (1994) suggested that DOC is an important environmental factor in transporting inorganic Hg from the watershed into a lake. This study also suggests that DOC could bind with MMHg and inhibit its bioaccumulation. This study did not use examine chlorophyll a or

DOC. Further research is necessary to understand the difference between DOC and primary productivity.

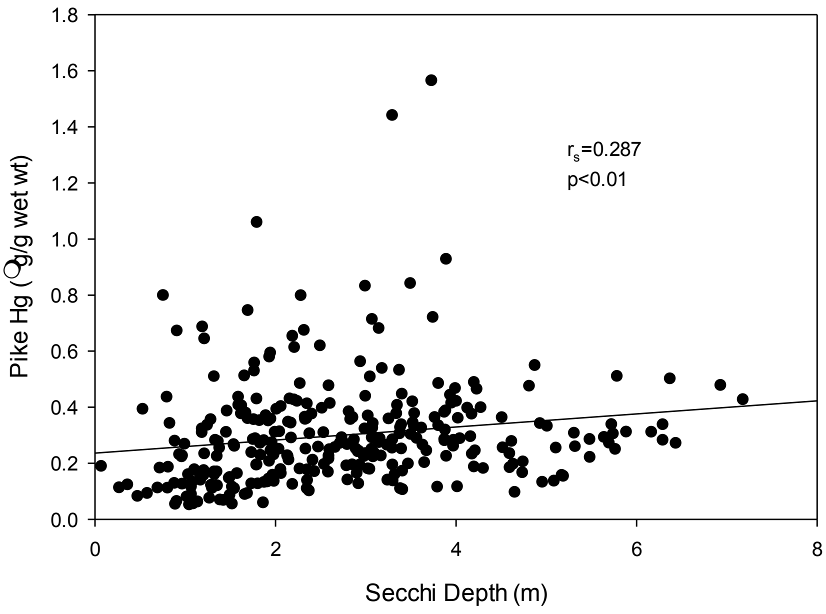

FIGURE 10. Relation between Secchi depth and concentrations of mercury in axial muscle of 55-cm (standard length) northern pike. Data was from 298 lakes in the Laurentian Great Lakes region of the United States (i.e. Minnesota, Wisconsin, Michigan, and New York) from 2001 to 2009.

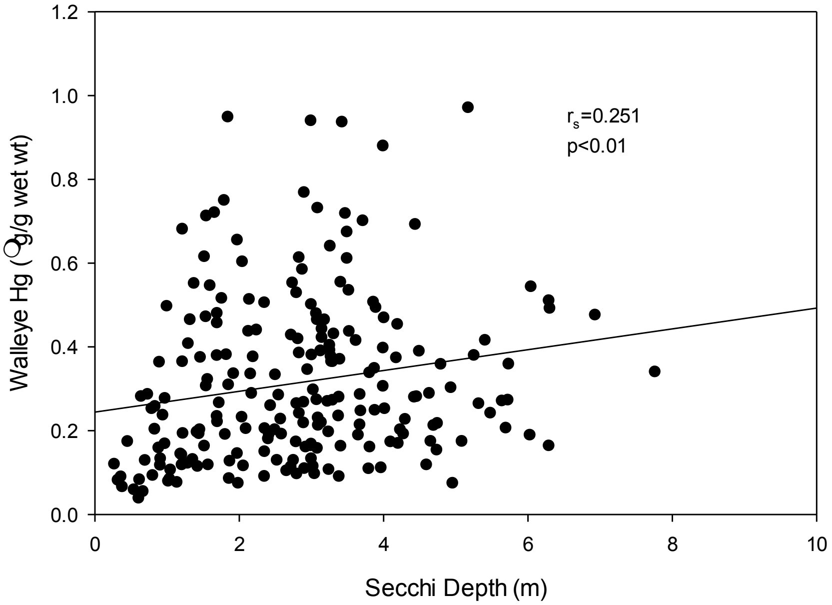

FIGURE 11. Relation between Secchi depth and concentrations of mercury in axial muscle of 40-cm (standard length) walleye. Data was from 214 lakes in the Laurentian Great Lakes region of the United States (i.e. Minnesota, Wisconsin, Michigan, and New York) from 2001 to 2009.

Watershed Area

MMHg concentrations in largemouth bass were negatively correlated with watershed area ($r=-0.3$, $p<0.01$) and MMHg in walleye and northern pike were unrelated to the size of the watershed. Given the fundamental role of atmospheric Hg flux, it can be inferred that the greater the land area that drains into a lake, relative to the size of the lake, the greater the total amount of inorganic Hg that will be available for methylation.

An important consideration is that wetlands are significant sites of Hg methylation (Mitchell et al., 2008). While wetland area was not measured in this study, it is conceivable that a watershed containing a large area of wetland may have higher fish MMHg than another watershed with a more limited wetland area. Wetland area could be important environmental factor in fish MMHg accumulation. Further study is required to resolve this possibility. This study only correlated the total watershed area to fish MMHg concentration; a future study could take into account the ratio of the watershed to lake volume, or lake surface area.

Conclusion

Among the variables I included in my study, dissolved sulfate and nitrate didn't have a significant correlation with MMHg in any fish species (Table 1.). The atmospheric fluxes of sulfate and nitrate both had significant positive correlations in largemouth bass and nitrate had a significant negative correlation in northern pike. Given the wet deposition of theses two anion's significance in fish MMHg, I would have expected that their dissolved values would also be important. No distinction was made for the time of year the samples were collected. It is possible that the variation of dissolved sulfate and nitrate concentrations through the year make a correlation impossible.

One of the trends of my study is the number of environmental factors significantly correlated both walleye and northern pike MMHg concentrations. MMHg concentrations in both species showed positive correlations with proton deposition and Secchi depth and negative correlations with atmospheric Hg deposition. The only variable in which one species is correlated and the other is not is northern pikes correlation with atmospheric nitrate flux. Largemouth bass is associated with another set of factors. Only largemouth bass has a correlation with atmospheric sulfate flux and pH. Why northern pike and walleye are similar to each other and not largemouth bass is likely due to differences in natural history. The northern pike and walleye prefer lower water temperatures than do the largemouth bass. Walleye and pike may have diets that are more similar to each other than either is to the bass. Whatever the reason, clear differences exist in the environmental factors affecting fish Hg between species in the same geographic area. In order to understand and potentially mitigate the environmental impact of MMHg, these interspecies differences must be taken into account.

A number of variables could have an important role in fish MMHg in the Laurentian Great Lakes region that were not included in this study. Due to a lack of available data wetland area associated with each lake was not factored into this study. Gradient of the land draining into the lake is could also have a significant correlation with fish MMHg. Dissolved aluminum has been correlated with fish MMHg in New York (Simonin et al., 2008) and was the only variable significantly correlated with mercury in three- to five-year-old yellow perch (Driscoll et al., 1994). This correlation may not be causative, the Al and Hg could both be weathered from the same rocks within the environment.

Many of the variables used in my study confound one another. For example, wet atmospheric proton deposition is correlated with both wet atmospheric nitrate deposition and wet atmospheric sulfate deposition. Sulfate is generally associated with higher MMHg production while nitrate is associated with lower MMHg production. These conflicting forces make correlating fish MMHg with one variable a much more difficult task. More study is required before the complex problem of Laurentian fish MMHg is fully understood.

The most significant finding of my study was the role played by proton wet deposition on fish MMHg. Of all the environmental factors considered in this study, only proton deposition had a significant positive correlation in all three species. Proton deposition, at a ten-fold gradient, had a wider range than the atmospheric deposition of sulfate, nitrate, or Hg across the Great Lakes region. My hypothesis is that proton flux facilitates the transport of inorganic Hg from the surrounding environment into the water body where it is methylated and ultimately bioaccumulated by fish.

A number of environmental factors could be examined in future studies of methylmercury accumulation in fish. While this study found a negative correlation between watershed area and largemouth bass MMHg concentration, I only took into account the total area of the watershed. Future researchers could consider how the ratio of watershed area to lake volume correlates with fish MMHg concentration. Another area that was not considered in this study are the physical characteristics found within the watershed (i.e. soil type, wetland area, land use practices). Wetlands are thought to be a significant area of Hg methylation (Mitchell et al., 2008).

Another variable not considered is water temperature. Water temperature can affect primary productivity as well as the flux of Hg between the lake and atmosphere. While water temperature was not a variable considered in this study, it is doubtful that the climatic range found in the Great Lakes region would account for a significant portion of variation of fish MMHg.

Previous studies have noted a positive correlation between dissolved aluminum (Al) and fish MMHg (Driscoll et al., 1994). This correlation may not be causative. In this study I propose the hypothesis that a higher atmospheric proton flux could facilitate the transport of Hg from the environment into a lake. It is possible that this same principle accounts for the higher concentration of Al in the lake water.

Finally, this study considered most the United States portion of the Laurentian Great Lakes region. The results of this study are a statistical generalization of the entire region. Future researchers could narrow the geographic scope of their study to focus on a smaller land area. The range of atmospheric fluxes would be smaller over a more constrained geographic area and would be expected to account for a smaller portion of the variation found in fish MMHg concentration. By analyzing data from a smaller geographic area, future researchers may identify secondary and tertiary controls on MMHg accumulation in fish in the Great Lakes region.

Fish Species	Largemouth Bass	Northern Pike	Walleye
Atmospheric Hg	r=-0.072, P>0.01, n=325	r=-0.327, P<0.01, n=500	r=-0.302, P<0.01, n=355
Atmospheric SO_4	r=0.371, P<0.01, n=325	r=-0.056, P>0.01, n=500	r=0.089, P>0.01, n=355
Atmospheric NO_3	r=0.361, P<0.01, n=325	r=-0.197, P<0.01, n=500	r=-0.001, P>0.01, n=355
Atmospheric H^+	r=0.486, P<0.01, n=325	r=0.458, P<0.01, n=500	r=0.356, P<0.01, n=355
pH	r=-0.276, P<0.01, n=112	r=-0.290, P>0.01, n=31	r=-0.139, P>0.01, n=56
Dissolved SO_4	r=0.065, P>0.01, n=71	r=-0.136, P>0.01, n=117	r=0.114, P>0.01, n=84
Dissolved NO_3	r=-0.099, P>0.01, n=109	r=0.218, P>0.01, n=26	r=0.119, P>0.01, n=55
Secchi Depth	r=0.007, P>0.01, n=210	r=0.287, P<0.01, n=298	r=0.251, P<0.01, n=214
Watershed Area	r=-0.298, P<0.01, n=151	r=-0.229, P>0.01, n=59	r=-0.274, P>0.01, n=82

TABLE 1. A statistical summarization of environmental factors influencing methylmercury accumulation in fish of the Laurentian Great Lakes region.

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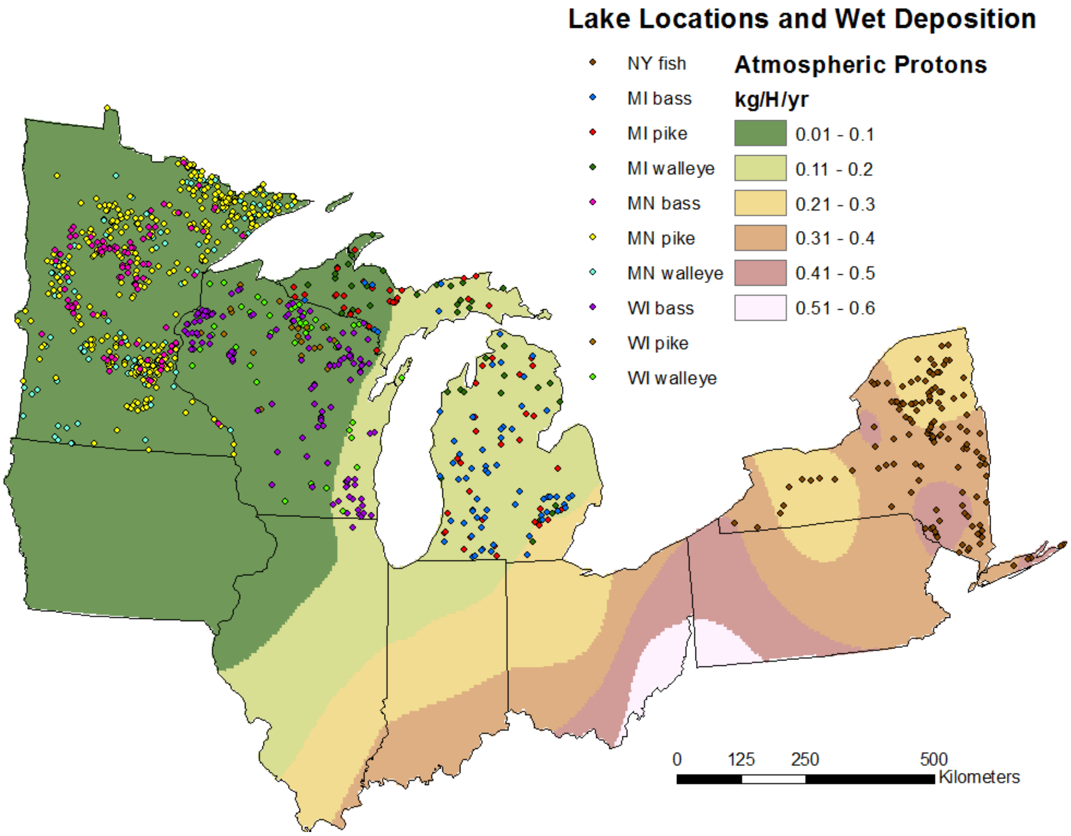
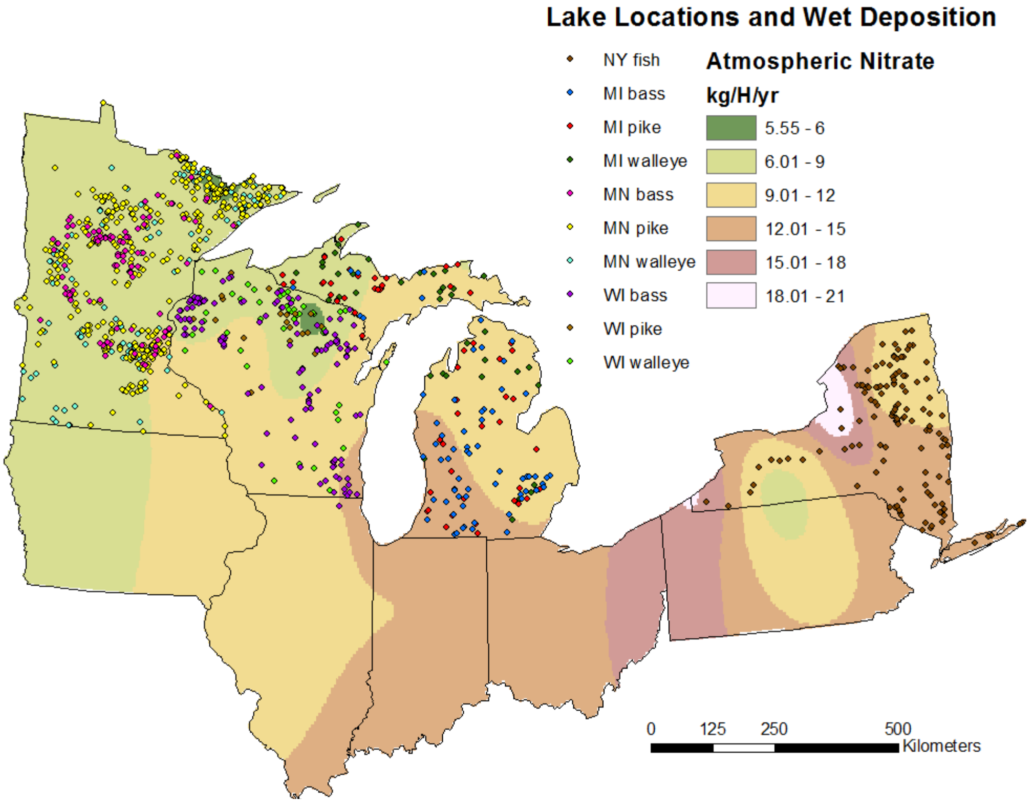
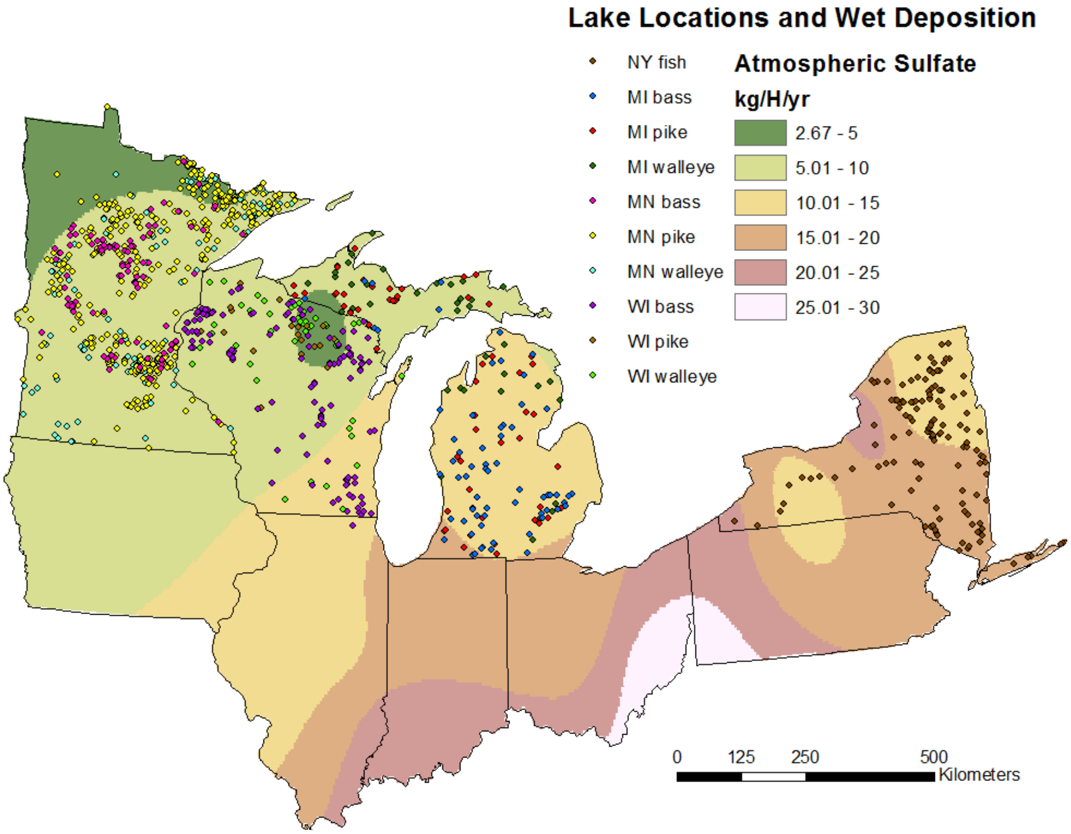
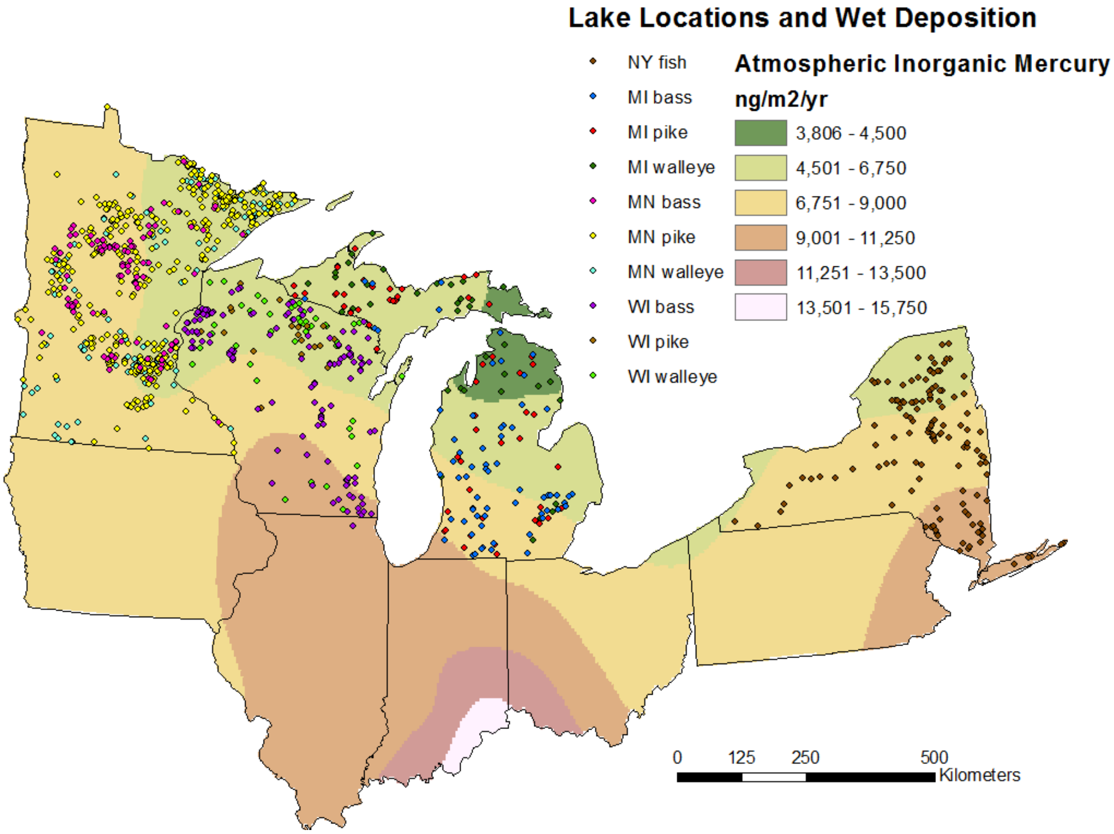
APPENDIX ONE

This appendix is a collection of maps used in this study. These maps show the expected atmospheric deposition based on data collected by the National Atmospheric Deposition Program and the Mercury Deposition Network. Superimposed on the calculated fluxes, are the location of the lakes that were used in this study.


Lake Locations and Wet Deposition
NY fish
MI bass
MI pike
MI walleye
MN bass
MN pike
MN walleye
WI bass
WI pike
WI walleye
Atmospheric Protons
kg/H/yr
0.01 - 0.1
0.11 - 0.2
0.21 - 0.3
0.31 - 0.4
0.41 - 0.5
0.51 - 0.6
0
125
250
500
Kilometers


Lake Locations and Wet Deposition
NY fish
MI bass
MI pike
MI walleye
MN bass
MN pike
MN walleye
WI bass
WI pike
WI walleye
Atmospheric Nitrate
kg/H/yr
5.55 - 6
6.01 - 9
9.01 - 12
12.01 - 15
15.01 - 18
18.01 - 21
0
125
250
500
Kilometers


Lake Locations and Wet Deposition
NY fish
MI bass
MI pike
MI walleye
MN bass
MN pike
MN walleye
WI bass
WI pike
WI walleye
Atmospheric Sulfate
kg/H/yr
2.67 - 5
5.01 - 10
10.01 - 15
15.01 - 20
20.01 - 25
25.01 - 30
0
125
250
500
Kilometers


Lake Locations and Wet Deposition
NY fish
MI bass
MI pike
MI walleye
MN bass
MN pike
MN walleye
WI bass
WI pike
WI walleye
Atmospheric Inorganic Mercury
ng/m2/yr
3,806 - 4,500
4,501 - 6,750
6,751 - 9,000
9,001 - 11,250
11,251 - 13,500
13,501 - 15,750
0
125
250
500
Kilometers

APPENDIX TWO

This appendix consolidates all the data used in this study into one table. A mean fish Hg (wet weight) value was taken for each species if 5 or more fish of that species were sampled from a given lake. Using data from the National Atmospheric Deposition Network and the Mercury Deposition Network I interpolated the likely average wet atmospheric deposition at the locations of each lake for the years 2001–2009. I also took the average of available water data for each lake. This data was provided by State agencies that keep extensive databases on lake water chemistry.