

FLORIDA DEPARTMENT OF ENVIRONMENTAL PROTECTION

Division of Environmental Assessment and Restoration

Bureau of Watershed Restoration

Mercury TMDL for the State of Florida

Appendix L

Aquatic Cycling Modeling

Watershed Evaluation and TMDL Section



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Appendix L: Aquatic Cycling Modeling

INTEGRATED REPORT ON AQUATIC MODELING

EXECUTIVE SUMMARY

Introduction

Overview

The Florida Department of Environmental Protection (FDEP) is currently developing a statewide total maximum daily load analysis (TMDL) to define maximum allowable levels of mercury (Hg) loadings to the state's freshwater resources (lakes, streams, and Everglades) such that consumption of fish will not result in unacceptable human health impacts arising from Hg exposure. A critical component of this TMDL is defining the magnitude of freshwater aquatic resources at risk. Because sampling for biota Hg concentrations the over 7,700 lakes greater than 10 acres in size, and 83,457 km of stream and riverine reaches in Florida is both resource- and time-prohibitive, this study used empirical (statistical) models to relate the occurrence of Hg in fish to water chemistry and potentially other variables. Predictive models were constructed for each major freshwater aquatic resource in the state; these models in turn were applied to: (1) water quality data sets compiled for 2009 by the FDEP as part of its Status and Monitoring Network (SMN); and (2) *Gambusia* (mosquitofish) data collected throughout the Everglades by the USEPA during the wet season in 2005 as part of its Regional Environmental Monitoring and Assessment Program (R-EMAP) to predict the statistical distribution of endpoint (largemouth bass or LMB) Hg concentrations across the entire state.

The efficacy of using statistical models to infer fish tissue Hg concentrations in lakes and streams from water quality variables has been demonstrated in numerous studies, including an early study of Hg concentrations in LMB in 53 lakes Florida by Lange *et al.* (1993), subsequent unpublished results from Lange *et al.* for 100 lakes in Florida, and initial analyses designed to evaluate critical water quality variables that influence LMB Hg concentrations in Florida lakes and streams as part of this TMDL effort (Pollman, 2008). These studies all indicate that factors other than total mercury concentrations (which should ultimately reflect input loadings) are important determinants of the variability in biota Hg between lakes. This reflects the fact that the cycling of mercury in aquatic ecosystems is influenced by a number of biogeochemical factors, including pH, dissolved organic carbon, and trophic state, and that the range of some of these factors observed between lakes greatly exceeds the variability in atmospheric loadings of Hg. Using the 100 lakes sampled by Lange *et al.* as an example, the range in LMB Hg concentrations differed by 25-fold across the lakes, while total phosphorus concentrations and atmospheric inputs (based on Mercury Deposition Network [MDN] wet deposition data) differed by 40-fold and less than 2x, respectively.

Key Assumptions

There are several key assumptions inherent in our approach towards using empirical models to construct the distributions of impaired aquatic resources with respect to fish Hg concentrations and how those distributions will respond to changes to Hg loadings. These assumptions include:

Linearity – The approach used to determine load reductions of Hg necessary to reduce ambient fish Hg concentrations to levels below a critical target value assumes that the long-term response of aquatic ecosystems to changes in atmospheric deposition of Hg is linear. For example, a 50% reduction in inputs is assumed to result in a long-term 50% reduction in fish tissue Hg. Although our conceptual understanding of the biogeochemical cycling of Hg includes a number of non-linear interactions and components, simulations using the dynamic Mercury Cycling Model (MCM) in both the Everglades (Atkeson *et al.*, 2003) and Devils Lake, WI (Tetra Tech, Inc. and SAI/ICF Inc., 2006) conducted as part of pilot Hg TMDL studies sponsored by USEPA suggest

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that the long-term response of wetlands and lakes to changes in atmospheric deposition fluxes of Hg is linear. Mesocosm dosing studies conducted in the Everglades by Krabbenhoft *et al.*, 2004 provide empirical support for the linearity assumption, and the assumption is a fundamental component of the load reduction calculations included in the regional New England states TMDL (Connecticut Department of Environmental Protection *et al.*, 2007) and the statewide Hg TMDL conducted by the Minnesota Pollution Control Agency (2007).

Steady State – The empirical modeling assumes that status of lakes and streams is currently in steady-state or equilibrium with respect to atmospheric inputs of Hg. This is not expected to be a major issue for the most sensitive lakes within Florida, particularly given the emerging paradigm over the past decade (both through research in the Everglades and through METAALICUS in the Canadian Experimental Lakes Area) that ecosystem response is largely driven by new Hg inputs and that the Hg signal in wet deposition in Florida has essentially remained constant between 1999 and 2008 decade (Pollman, unpublished data). For systems such as streams and lakes where riparian wetlands are likely the major source of methyl Hg, disequilibrium between current atmospheric inputs and methyl Hg production and export rates may be a source of error.

Seasonality – Generally, seasonal variations are an explicit component of TMDL's. Because the concentrations of Hg in LMB at any point in time reflects integration of the Hg signal over time, temporal dynamics on a seasonal scale are dampened and less important to consider – particularly if the fish of interest are older than a couple of years. Target concentrations for LMB were developed for fish normalized to 15" in Florida's lakes and streams, and 14" in the Everglades; these lengths translate to ages averaging 3.4 and 2.6 years, respectively for lakes and streams (lengths between 14.5 and 15.5") and the Everglades (lengths between 13.5 and 14.5").

Sampling Design

The empirical models for lakes and rivers/streams were developed using data collected at random from a matrix of waterbodies segregated by key physical and chemical lake characteristics, with the goal of obtaining an even distribution of study sites spanning a wide range of the target variables. Preliminary analyses identified a set of three target variables (pH, color, and chlorophyll *a* for lakes; pH, color, and nitrate for streams) that were important determinants of the variability of fish tissue Hg concentrations; the selection of lake and stream sites for sampling in turn was based on the joint distribution of these three target variables across five concentration intervals for each variable. This yielded a possible 125 unique variable interval combinations (5 x 5 x 5) or sampling bins. Additional lakes and streams were sampled at random from individual bins to produce a total *N* of lake and stream reaches or segments of 130 lakes and 128 streams for each waterbody type. Prospective lakes and streams were selected from the FDEP SMN database.

Data

Stream and lake sampling began in September, 2008 and concluded in September 2010. Water quality variables sampled included:

- Major ions (pH, calcium, magnesium, sodium, potassium, chloride, sulfate, and alkalinity)
- Nutrients and trophic state variables (soluble reactive phosphorus, total phosphorus, nitrate ($\text{NO}_2^- + \text{NO}_3^-$), ammonia, total Kjeldahl nitrogen, dissolved organic carbon, color, and chlorophyll *a*)

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- Field parameters (dissolved oxygen, temperature, oxidation-reduction potential, Secchi disk transparency, and specific conductance)
- Mercury species (total Hg, filtered total Hg, total methyl Hg, and filtered methyl Hg).

In addition, surficial sediments were collected from pelagic stations in 129 lakes and analyzed for the following suite of sediment parameters:

- Trace elements (aluminum, iron, chromium, magnesium, manganese, nickel, potassium, strontium, titanium, antimony, arsenic, cadmium, copper, lead, and selenium)
- Nutrients (total carbon, total organic carbon, total phosphorus, and total Kjeldahl nitrogen)
- Mercury species (total Hg and methyl Hg)
- Physical (percent total solids)

Fish sampling focused on LMB, with the goal of collecting 12 fish from each waterbody. For waterbodies where LMB were either absent or only small numbers were collected (primarily streams in the Panhandle), the fish sampling was augmented through collecting spotted sunfish (SPSU). An empirical model was used to relate Hg concentrations in SPSU to concentrations in LMB and was used to infer LMB Hg concentrations in streams with low or absent collected numbers of LMB.

Statistical Models

Fish Normalization

Because Hg concentrations in fish vary as a function of age or size, and because the age-Hg or size-Hg relationship typically varies between different lakes and different streams, three sets of empirical models were developed (*i.e.*, models specific each for lake, rivers and streams, and the Everglades) to eliminate size of the fish sampled within a given water body as a confounding variable when comparing fish mercury concentrations across lakes or streams. Normalizing was based on length because LMB growth rates vary, and a consistent length at age relationship cannot be established that is representative of all lakes and streams. As a result, LMB methyl Hg concentrations were normalized to a common length providing a measureable human health endpoint. As mentioned previously, target concentrations for LMB were developed for fish normalized to 15" in Florida's lakes and streams, and 14" in the Everglades. The choice of 15" as the target end point originates with Florida's tiered system of harvest restrictions with a focus on minimum length limits (MLL) for LMB. The smaller length for the Everglades was selected because recruitment to larger sizes is limited and ample numbers of LMB of smaller sizes are produced within the Everglades. As a result, a human health endpoint for the Everglades region is more appropriately established for 14", which also represents the worst-case scenario for the majority of angler caught fish from the Everglades.

Lake Empirical Model

The final sets of models describing the relationship between LMB Hg and lake water chemistry included five variables: alkalinity, chlorophyll *a*, sulfate (transformed to linearize its unimodal relationship between methylation potential), and two variables related to sediment chemistry obtained from principal components analysis. These two sediment chemistry variables were interpreted to represent an urban runoff disturbance component, and a component related to atmospheric deposition. Multiple linear

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regression (MLR) was used to initially construct the lake model, which in turn was tested to establish model robustness and overall linearity of the modeled relationships and validated for its ability to predict “out-of-sample” LMB Hg concentrations. The MLR modeling was followed by developing a final model with the same variables using generalized linear modeling (GLM); the advantage of using GLM vs. MLR for this study is that the GLM approach enabled modeling LMB Hg concentrations directly. More specifically, in order to improve linearity with the independent variables, the MLR model used log-transformed concentrations of LMB Hg to construct the model, and back-transforming the predicted log-transformed concentrations of LMB Hg from the MLR model imposes a bias on the predicted values that is avoided when using GLM. The sequence of importance of independent variable contributions to the overall variability in LMB Hg in the study lakes was:

Alkalinity > chlorophyll *a* > urban runoff disturbance > atmospheric deposition > sulfate

Streams and Rivers Empirical Model

Initial efforts using linear approaches such as MLR to model the relationship between LMB Hg concentrations and stream water quality variables produced relatively poor results, and indicated that the relationships may be complex and non-linear. As a result, classification and regression tree analysis (CART) was used to model LMB Hg concentrations in the Hg TMDL streams. CART is a non-parametric approach and, as a result, it makes no assumption that the underlying relationships between the predictor variables and the dependent variable are linear. Final variables in the CART modeling included pH, conductivity, SO₄, color, TP, TKN, water resource type (large river or stream) and percent dissolved oxygen saturation. Validation of the model was conducted using *k*-fold (5 folds) cross-validation. The sequence of importance of independent variable contributions to the overall variability in LMB Hg in the study streams and rivers was:

pH >> DO % saturation > Conductivity > TKN > SO₄ > TP

Everglades Empirical Model

The objective behind the Everglades empirical model was to take advantage of data collected by USEPA's REMAP program to make inferences about the distribution of LMB Hg throughout the Everglades Protection Area (Loxahatchee National Wildlife Refuge, Water Conservation Areas 2A, 2B, 3A and 3B, and Everglades National Park). The REMAP program utilized a randomized stratified sampling program that was designed with purpose of inferring the distribution of critical water quality parameters throughout the Everglades. Although LMB were not collected as part of REMAP, samples of *Gambusia* were collected and analyzed for Hg. As a result, MLR was used to initially develop a model for predicting LMB Hg concentrations as a function of *Gambusia* Hg concentrations using data from 54 sites with co-located data on Hg concentrations for both types of fish. Similar to the modeling conducted for the TMDL study lakes, the MLR modeling was revised using GLM to eliminate bias related to back-transforming predicted log-transformed LMB Hg concentrations.

Predicted Distributions of LMB Hg Concentrations in Florida's Aquatic Resources

Water quality and sediment chemistry (lakes) data from the FDEP SMN for 2009 were used in conjunction with the respective empirical models to infer the statewide distribution of Hg concentrations in LMB (normalized to 15"). The SMN utilizes a stratified randomized sampling approach that, because of its probabilistic approach towards sampling, enables inferential estimates of the magnitude of an aquatic resource type with fish tissue concentrations above or below a concentration level of interest. Moreover, these inferential estimates can be developed with a known degree of

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confidence. Sampling weights were provided by FDEP based on: (1) surface area for large lakes; (2) numerical abundance for small lakes; and reach length for rivers and streams (each as a separate resource).

Uncertainty in model predictions was addressed by calculating 90th percentile confidence limits on each of model predictions of the mean LMB Hg for a given set of input parameters. Because the 90th percentile model confidence limits define an interval, the 90th percentile upper confidence limit defines with 95% certainty an upper bound that the mean LMB Hg concentrations predicted by the model are expected to lie below. In addition, the effects of uncertainty in the distribution estimates of LMB Hg concentrations inherent in using the SMN probabilistic samples to construct the weighted population distributions were evaluated. This latter uncertainty was superimposed on the empirical model predicted results by constructing confidence intervals on the cumulative frequency distribution based on the number of samples in the sampling frame and the individual sampling weights.

The predicted distributions of LMB Hg concentrations indicate that the most sensitive freshwater aquatic resources with respect to Hg are streams and small lakes, followed closely by large rivers and the Everglades. 90th percentile concentrations for the distribution of LMB Hg were as follows (all values in mg/kg): streams – 1.167; small lakes – 1.027; rivers – 0.949; the Everglades – 0.915; and large lakes – 0.389. The much lower concentration distribution of LMB Hg observed for large lakes reflects both characteristically higher alkalinities and higher productivity compared to small lakes. When the effects of both model prediction uncertainty coupled with uncertainty in the inferred distributions are considered, the 90th percentile concentrations for the distribution of LMB Hg were as follows: streams – 1.295; small lakes – 1.319; rivers – 1.136; the Everglades – 1.071; and large lakes – 0.694.

Load Reductions Necessary to Achieve the TMDL Target Level

As stated earlier, load reductions were calculated by assuming that the long-term response between changes in Hg loading to Florida's aquatic ecosystems and fish tissue Hg concentrations is linear. Load reductions also were based on several other assumptions: (1) the target value for LMB Hg concentrations protective of the most sensitive component of the Florida populace at risk to excessive Hg exposure due to consuming fish is 0.3 mg/kg; (2) the target goal of Hg reductions is to reduce the 90th percentile concentration for the distribution of LMB Hg in Florida's freshwater aquatic resources to levels below 0.3 mg/kg; and (3) loadings of Hg to Florida's aquatic ecosystems is overwhelmingly from atmospheric deposition. This latter assumption is supported by comparing the mass flux of Hg released from point sources in Florida (25 kg in 2009) to the estimated loadings from atmospheric deposition (6,043 kg in 2009). In addition, the load reduction calculations assumed that only contributions of anthropogenic sources to Hg deposition in Florida are controllable. Calculations for the requisite load reductions thus considered different contributions from background natural sources to the Hg deposition signal in Florida. Calculations assuming no uncertainty in the predicted LMB Hg distribution estimates indicate that, for an assumed background natural source contribution of 30%, the requisite load reductions ranged from 33% (large lakes) to 106% (streams). When the aggregated effects of model uncertainty and uncertainty in the distribution estimates are factored into the calculations, the range of requisite reductions increases to 81% (large lakes) and 110% (streams).

The contributions of Hg emission sources within Florida to the current (2009) distributions of Hg in Florida rivers, streams, and lakes were estimated using the CMAQ modeled output predicted for each site. CMAQ estimates of the fractional contributions of Florida sources to Florida's lakes and rivers/streams was generally below 5% (mean

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contribution = 2.1%; median = 3.1%). Only ~4% of the sites had contributions in excess of 10%, and the maximum estimated contribution was 24.6%. As a result, the effects of eliminating the fraction of atmospheric Hg loadings to Florida lakes and streams/rivers was predicted to be quite small, with (weighted) reductions averaging about 0.01 and 0.02 mg/kg for large and small lakes, respectively and about 0.01 and 0.02 mg/kg for rivers and streams, respectively.

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Introduction

Overview

The Florida Department of Environmental Protection (FDEP) is currently developing a statewide total maximum daily load analysis (TMDL) to define maximum allowable levels of mercury (Hg) loadings to the state's freshwater resources (lakes, streams, and Everglades) such that consumption of fish will not result in unacceptable human health impacts arising from Hg exposure. A critical component of this TMDL is defining the magnitude of freshwater aquatic resources at risk. Because sampling biota Hg concentrations for the over 7,700 lakes greater than 10 acres in size, and 83,457 km of stream and riverine reaches in Florida is both resource- and time-prohibitive, this study used empirical (statistical) models to relate the occurrence of Hg in fish to water chemistry and potentially other variables. Predictive models were constructed for each major freshwater aquatic resource in the state; these models in turn were applied to: (1) water quality data sets compiled for 2009 by the FDEP as part of its Status and Monitoring Network (SMN); and (2) *Gambusia* (mosquitofish) data collected throughout the Everglades by the USEPA during the wet season in 2005 as part of its Regional Environmental Monitoring and Assessment Program (REMAP) to predict the statistical distribution of endpoint (largemouth bass or LMB) Hg concentrations across the entire state. These distributions in turn form the basis for determining reductions in Hg loadings to Florida that are necessary to reduce Hg exposure to the element of Florida's population considered to be a greatest risk – women of childbearing age – below a critical threshold or target value defined by FDEP (as a function of fish Hg concentration and fish consumption rate) as part of the TMDL.

The efficacy of using statistical models to infer fish tissue Hg concentrations in lakes and streams from water quality variables has been demonstrated in numerous studies, including an early study of Hg concentrations in LMB in 53 lakes Florida by Lange *et al.* (1993), subsequent unpublished results from Lange *et al.* for 100 lakes in Florida, and initial analyses designed to evaluate critical water quality variables that influence LMB Hg concentrations in Florida lakes and streams as part of this TMDL effort (Pollman, 2008). These studies all indicate that factors other than total mercury concentrations (which should ultimately reflect input loadings) are important determinants of the variability in biota Hg between lakes. This reflects the fact that the cycling of mercury in aquatic ecosystems is influenced by a number of biogeochemical factors, including pH, dissolved organic carbon, and trophic state, and that the range of some of these factors observed between lakes greatly exceeds the variability in atmospheric loadings of Hg. Using the 100 lakes sampled by Lange *et al.* as an example, the range in LMB Hg concentrations differed by 25-fold across the lakes, while total phosphorus concentrations and atmospheric inputs (based on Mercury Deposition Network [MDN] wet deposition data) differed by 40-fold and less than 2x, respectively.

Key Assumptions

There are several key assumptions inherent in our approach towards using empirical models to construct the distributions of impaired aquatic resources with respect to fish Hg concentrations and how those distributions will respond to changes to Hg loadings. These assumptions include:

Linearity – The approach used to determine load reductions of Hg necessary to reduce ambient fish Hg concentrations to levels below a critical target value assumes that the long-term response of aquatic ecosystems to changes in atmospheric deposition of Hg is

linear. For example, a 50% reduction in inputs is assumed to result in a long-term 50% reduction in fish tissue Hg. Although our conceptual understanding of the biogeochemical cycling of Hg includes a number of non-linear interactions and components, simulations using the dynamic Mercury Cycling Model (MCM) in both the Everglades (Atkeson *et al.*, 2003) and Devils Lake, WI (Tetra Tech, Inc. and SAI/ICF Inc., 2006) conducted as part of pilot Hg TMDL studies sponsored by USEPA suggest that the long-term response of wetlands and lakes to changes in atmospheric deposition fluxes of Hg is linear. Mesocosm dosing studies conducted in the Everglades by Krabbenhoft *et al.*, 2004 provide empirical support for the linearity assumption, and the assumption is a fundamental component of the load reduction calculations included in the regional New England states TMDL (Connecticut Department of Environmental Protection *et al.*, 2007) and the statewide Hg TMDL conducted by the Minnesota Pollution Control Agency (2007).

Steady State – The empirical modeling assumes that status of lakes and streams is currently in steady-state or equilibrium with respect to atmospheric inputs of Hg. This is not expected to be a major issue for the most sensitive lakes within Florida, particularly given the emerging paradigm over the past decade (both through research in the Everglades and through METAALICUS in the Canadian Experimental Lakes Area) that ecosystem response is largely driven by new Hg inputs and that the Hg signal in wet deposition in Florida has essentially remained constant between 1999 and 2008 decade (Pollman, unpublished data). For systems such as streams and lakes where riparian wetlands are likely the major source of methyl Hg, disequilibrium between current atmospheric inputs and methyl Hg production and export rates may be a source of error.

Seasonality – Generally, seasonal variations are an explicit component of TMDL's. Because the concentrations of Hg in LMB at any point in time reflects integration of the Hg signal over time, temporal dynamics on a seasonal scale are dampened and less important to consider – particularly if the fish of interest are older than a couple of years. Target concentrations for LMB were developed for fish normalized to 15" in Florida's lakes and streams, and 14" in the Everglades; these lengths translate to ages averaging 3.4 and 2.6 years, respectively for lakes and streams (lengths between 14.5 and 15.5") and the Everglades (lengths between 13.5 and 14.5").

Organization of this Report

The primary goal of this report is to present the development and application of the statistical models used to infer LMB Hg concentrations and their distribution in Florida's freshwater aquatic ecosystems. The initial three major chapters of the report (following this Introduction) lay the predicate for developing the inferential statistical models used to predict LMB Hg concentrations. The first of these three chapters (Section 2) presents an overview of the sampling design that was developed and implemented to select lake and stream sites that formed the basis for empirical model development; this chapter also discusses basic processing of the field data as a necessary prerequisite for constructing the statistical models. This overview is followed by a detailed discussion of the modeling approaches used for and the results obtained from the fish normalization (Section 3). The last of the initial three chapters presents transformations of two key variables – sulfate and pH in streams - that were developed to improve the linearity between these two variables and LMB Hg concentrations (Section 4).

The next three chapters present the development of the actual statistical models used to predict LMB Hg concentration. Each chapter is devoted to a specific type of resource: lakes – Section 5; streams – Section 6; and the Everglades – Section 7.

The report concludes with three chapters that are related to load reduction calculations, and uncertainty in those estimates. The first of this last suite of chapters is a discussion

of the calculations involved in predicting weighted distributions (*e.g.*, distributions that consider the effect of lake size of the distribution of LMB Hg concentrations) for each of three major freshwater ecosystem resources (Section 8). Section 8 is followed by a chapter on calculating the reductions in Hg loading necessary to reduce the overall (90th percentile) distribution to the TMDL target concentration (Section 9). The final chapter (Section 10) in turn discusses the effects of two sources of uncertainty – uncertainty in the statistical model predictions, and uncertainty in the estimates of weighted cumulative frequency distributions – on both critical LMB Hg concentrations and the requisite reduction in Hg loadings required to meet the TMDL objectives of protecting the most sensitive element of Florida's populace – women of childbearing age.

Sampling Design and Data Overview

TMDL Lakes, Streams and Rivers

Sampling Design

The empirical models for lakes and rivers/streams were developed using data collected at random from a matrix of waterbodies segregated by key physical and chemical lake characteristics, with the goal of obtaining an even distribution of study sites spanning a wide range of the target variables. Preliminary analyses (Pollman, 2008) identified a set of three target variables (pH, color, and chlorophyll *a* for lakes; pH, color, and nitrate for streams) that were important determinants of the variability of fish tissue Hg concentrations; the selection of lake and stream sites for sampling in turn was based on the joint distribution of these three target variables across five concentration intervals for each variable. This yielded a possible 125 unique variable interval combinations ($5 \times 5 \times 5$) or sampling bins. Additional lakes and streams were sampled at random from individual bins to produce a total *N* of 133 lakes and 130 stream reaches. These water bodies included three lakes and two stream sites for which only water chemistry data were collected, or in one case (WBID = S148), only three LMB and no spotted sunfish or spotted bass were collected. Eliminating these observations results in a total of 130 lakes and 128 streams with both full water chemistry data and fish tissue Hg data. Appendix A includes a list of all the lake and streams sites sampled, including location (latitude and longitude coordinates). Prospective lakes and streams were selected from the FDEP SMN database.

Data Collection Overview

One of the goals of the TMDL was to develop an analysis that estimates the magnitude of impairment of freshwater aquatic resources and mercury loadings during 2009. With that goal in mind, and because of the intensity of effort required to sample approximately 260 bodies of water with the appropriate rigor that sampling for Hg in the field requires, stream and lake sampling began in September, 2008 and concluded in September 2010. Water quality variables sampled included:

- Major ions – pH, calcium, magnesium, sodium, potassium, chloride, sulfate, and alkalinity;
- Nutrients and trophic state variables – soluble reactive phosphorus, total phosphorus, nitrate ($\text{NO}_2^- + \text{NO}_3^-$), ammonia, total Kjeldahl nitrogen, dissolved organic carbon, color, and chlorophyll *a*;
- Field parameters – dissolved oxygen, temperature, oxidation-reduction potential, Secchi disk transparency, and specific conductance; and
- Hg species – total Hg, filtered total Hg, total methyl Hg, and filtered methyl Hg.

In addition, surficial sediments were collected from pelagic (open water) stations in 129 lakes and analyzed for the following suite of sediment parameters:

- Trace elements – aluminum, iron, chromium, magnesium, manganese, nickel, potassium, strontium, titanium, antimony, arsenic, cadmium, copper, lead, and selenium;
- Nutrients – total carbon, total organic carbon, total phosphorus, and total Kjeldahl nitrogen;
- Hg species – total Hg and methyl Hg; and
- Physical – percent total solids.

Fish sampling focused on LMB, with the goal of collecting 12 fish from each waterbody. For waterbodies where LMB were either absent or only small numbers were collected (primarily streams in the Panhandle), the fish sampling was augmented through collecting spotted sunfish (SPSU). An empirical model was used to relate Hg concentrations in SPSU to concentrations in LMB and was used to infer LMB Hg concentrations in streams with low or absent collected numbers of LMB.

Data Reduction for Resampled Sites

During the course of field sampling and analysis, analytical difficulties experienced while analyzing the samples suggest that some stream and lake samples for water column total Hg concentrations (both filtered and total) were compromised. These compromised samples were identified, for example by high field blanks exceeding the method practical quantitation limit (PQL), which may have been due to contamination of the peristaltic pump sampling line. In addition, a small number of lake and stream water quality samples were flagged because of either temperature exceedances inconsistent with FDEP quality assurance (QA) protocols, or improper sample acidification. In total, water quality QA concerns were identified in a total of 24 lakes and 9 streams. The lakes and streams flagged for sampling issues were resampled.

The integrated data set includes results from both the original and resampling events. A working data set for statistical analyses was created by consolidating the reported results for the resampled water bodies, so that each lake and stream is represented in the working data set as a single observation. Details of how the original and the resampled data events were consolidated to produce a single observation are presented for each water body in Appendix B.

The original integrated data set also included observations for one lake (WBID = L616) which had duplicate samples collected for sediment chemistry. These duplicate values were averaged to produce a single observation for L616 in the working data set for statistical analysis.

Data Processing for Largemouth Bass Hg Normalization Calculations

The basic data set used to conduct normalization calculations for the TMDL lakes and streams was transmitted to *Aqua Lux Lucis, Inc.* by Ted Lange (Florida Fish and Wildlife Conservation Commission or FFWCC) on January 11, 2011. Several minor corrections to the data set subsequently noted by Lange were used to produce the final data set of raw fish tissue Hg concentrations. These corrections included:

1. Changed selected observations for samples labeled with WB# S002 to S02
2. Changed selected observations for samples labeled with WB# S2012 to S643
3. Changed selected observations for samples labeled with WB# S053 to S061

One additional change to the working data set was made as follows:

1. Changed samples labeled with WB# S008 to S08

Data for spotted bass (SPBA) were combined with data for largemouth bass (LMB) to increase the number of observations available to conduct normalization calculations for individual sites. This decision was based on plots prepared by Ted Lange of Hg concentration vs. total length comparisons for co-located samples. These plots (Figure 1 through Figure 3) demonstrate that essentially the same concentration-length relationships hold for both species across sites. Data for SPBA were collected from six stream sites, and aggregating SPBA with LMB data changed the total number of *N* for the sites as follows (Table 1):

Table 1. Listing of stream sampling sites for which data for SPBA mercury concentrations were combined with data for LMB mercury concentrations. Table shows number of fish specimens collected for LMB alone and LMB plus SPBA combined for each of the sites with combined data.

WBID #	<i>N</i> – LMB only	<i>N</i> - LMB combined with SPBA
S062	10	13
S081	1	2
S1377	6	12
S290	6	14
S431	10	12
S824	10	12

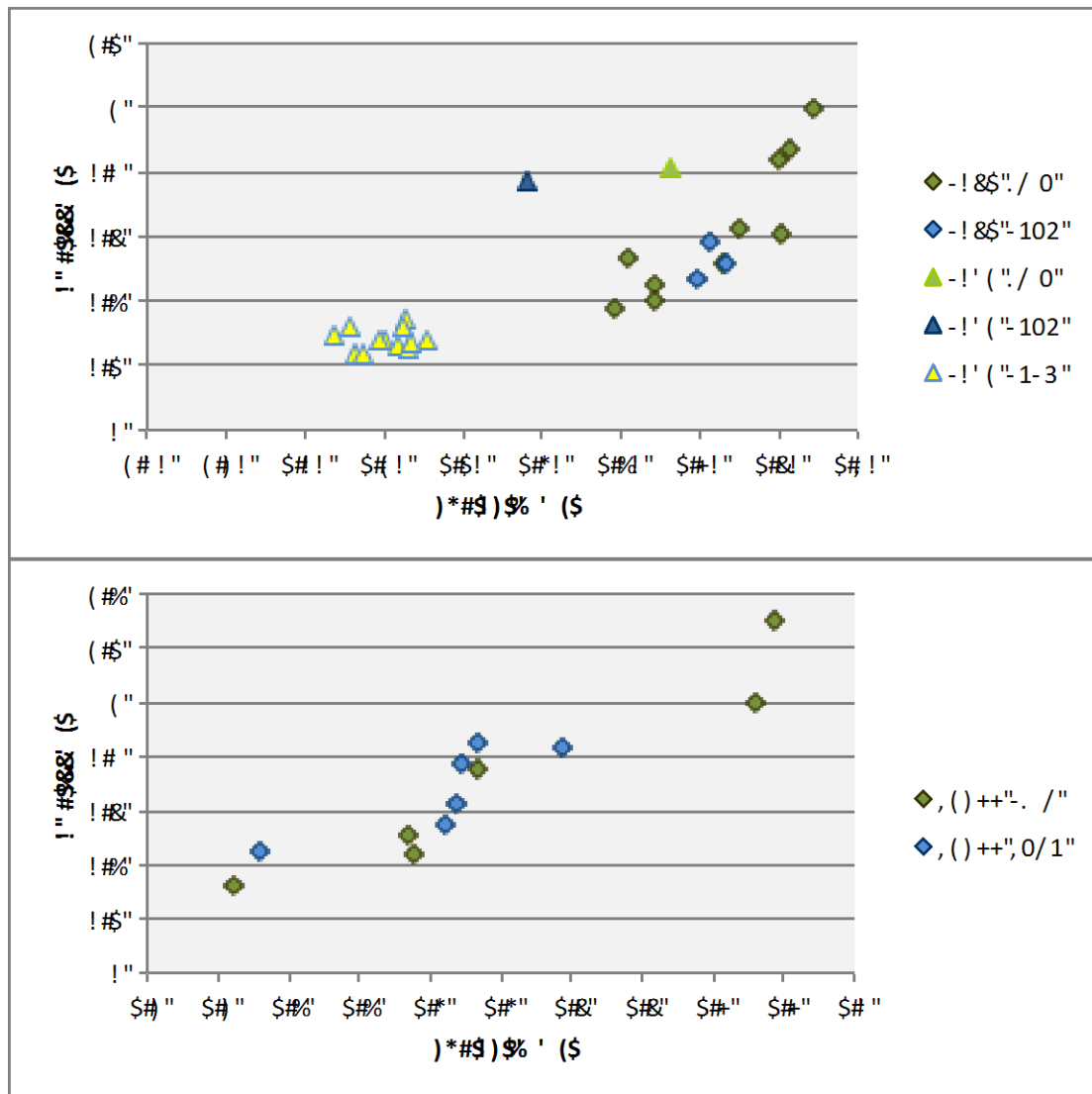


Figure 1. Fish tissue mercury concentrations as a function of total length (log-transformed). Included are data for largemouth bass (LMB), spotted bass (SPBA), and spotted sunfish (SPSU). Upper panel – stream sites S062 and S081; lower panel – stream site S1377. Figure courtesy of T. Lange (Florida FWC).

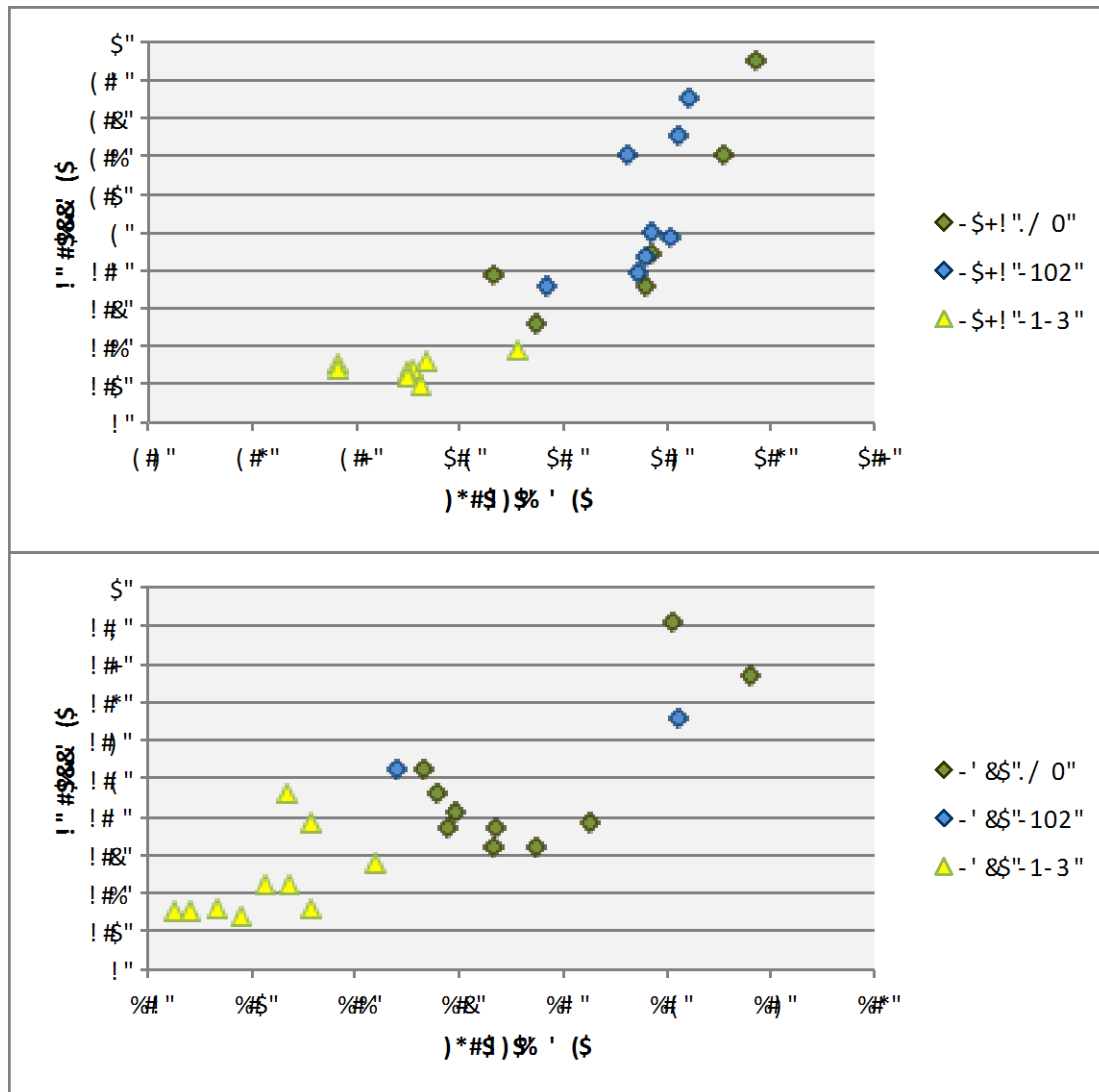


Figure 2. Fish tissue mercury concentrations as a function of total length (log-transformed). Included are data for LMB, SPBA, and SPSU. Upper panel – stream site S290; lower panel – stream site S431. Figure courtesy of T. Lange (Florida FWC).

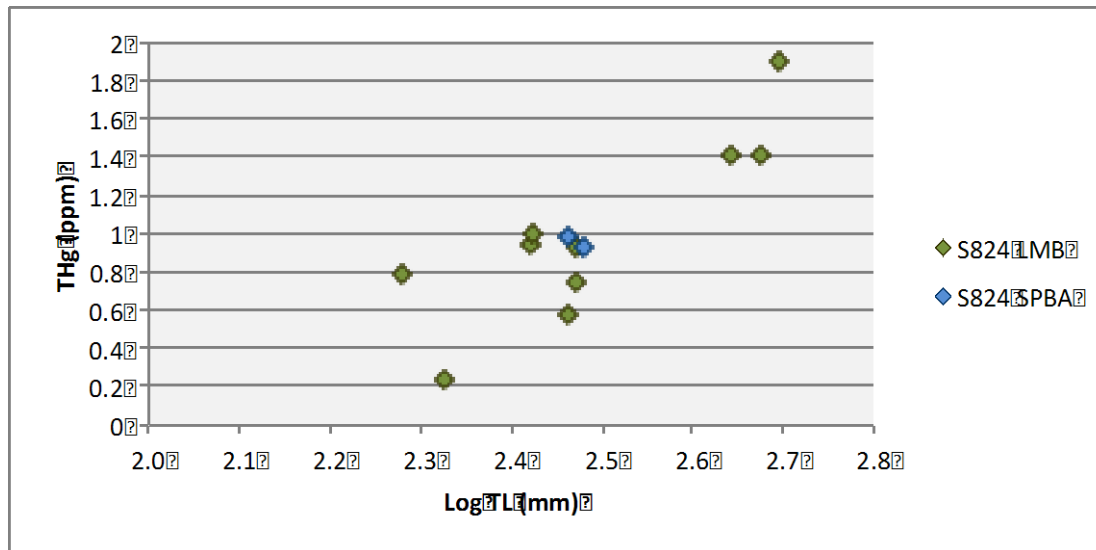


Figure 3. Fish tissue mercury concentrations as a function of total length (log-transformed). Included are data for LMB and SPBA for stream site S824. Figure courtesy of T. Lange (Florida FWC).

Everglades

Overview

As part of its Regional Environmental Monitoring and Assessment Program (R-EMAP), the USEPA collected water quality, sediment, and biota samples throughout the Everglades marsh to establish its status with respect to ecosystem health and provide a basis for determining the effectiveness of efforts designed to restore the Everglades (Scheidt and Kalla, 2007). Sampling began in 1993 and included sampling conducted during both the wet and dry seasons (each season comprising a cycle). A total of eight sampling cycles were conducted (four wet and four dry), with the most recent wet and dry cycles conducted in 2005. Similar to the FDEP SMN, the REMAP program utilized a randomized stratified sampling program for selecting sites that was designed with the purpose of inferring the distribution of critical water quality and other ecosystem health-related parameters throughout the Everglades.

Although LMB were not collected as part of REMAP, samples of *Gambusia* (mosquitofish) were collected and analyzed for Hg. Because the FFWC also has been collecting fish samples for Hg analysis for a variety of species including LMB throughout the Everglades during the same overall time frame as REMAP, the objective of this aspect of the study was to take advantage of data collected by USEPA's REMAP program to make inferences about the distribution of LMB Hg throughout the Everglades Protection Area (Loxahatchee National Wildlife Refuge, Water Conservation Areas 2A, 2B, 3A and 3B, and Everglades National Park). Such inferences require developing a model that relates variability in LMB Hg concentrations in the Everglades to variability in *Gambusia* Hg concentrations, and then using the R-EMAP *Gambusia* Hg data to infer the distribution of LMB Hg. The ability to construct such a model in turn is predicated on co-located or proximally located sampling sites for both types of fish that were also sampled reasonably concurrently in time.

Data Processing

R-EMAP data for all eight cycles were obtained directly from Peter Kalla with the USEPA (personal communication, March 20, 2008) and can be downloaded from the internet as follows:

- Cycles 0 – 5:
<http://www.epa.gov/region4/sesd/reports/epa904r98002/sf1data.html>
- Cycles 6 and 7:
<http://www.epa.gov/region4/sesd/reports/epa904r07001.html>

Data for Hg concentrations in LMB collected throughout the Everglades were obtained from Ted Lange (FFWCC, personal communication, January 25, 2011), who compiled data on length, weight, age, and location (as well as sex for LMB) information in addition to Hg concentrations for 27 different species (mostly fish, including LMB) and 31 different locations within the Everglades Protection Area, the Big Cypress National Preserve, and the Everglades Agricultural Area. This data set includes a total number of 3,981 observations, contains 1,800 observations for LMB at 28 sites. The years sampled range from 1993 to 2010, with the number and frequency of sites sampled varying within any given year. A total of 1,712 observations include reported values for

total length, total weight and total Hg concentrations each. Appendix C provides a summary of the number of LMB samples available for a given site within a given year. Lange also compiled supplemental data on Hg concentrations for *Gambusia* collected from sites that were monitored extraneous to the R-EMAP program. These data, includes a total of 11 sites co-located with sites that were sampled for LMB. This equated to 89 different combinations of site, year, and month, with a total $N = 257$. These data were transmitted to *Aqua Lux Lucis* as part of the LMB data file sent on January 25, 2011.

Based on location and knowledge of site characteristics, Lange also developed a list of REMAP sites that were assessed to be sufficiently proximal and similar in general habitat and flow dynamics to be considered as sites *co-located* with a given LMB monitoring site. These REMAP sites and the corresponding LMB monitoring sites are identified in Appendix C (Table C-2).

The supplemental *Gambusia* data were combined with the R-EMAP data to produce a single integrated file of *Gambusia* Hg concentrations. Because R-EMAP sampling was conducted to represent dry and wet periods, each observation in the supplemental *Gambusia* data file was assigned to the appropriate hydroperiod to match the R-EMAP data as follows: months between June and November inclusive were considered “wet”; the remaining months (December through May) were considered “dry”. Supplemental sites with more than one month sampled during a given year-hydroperiod combination were averaged across the combination. This yielded 84 unique site-year-hydroperiod combinations for the supplemental data.

Gambusia data from the co-located REMAP sites likewise were averaged across site (LMB monitoring co-located site), year and hydroperiod. This also yielded 84 (including 73 observations with recorded *Gambusia* Hg concentrations) unique site-year-hydroperiod combinations for the co-located REMAP data. The two files then were combined based on location (co-located LMB monitoring location), year the sample was collected, and hydroperiod. The integrated data set included a total of 148 observations with *Gambusia* Hg data for a unique site-year-hydroperiod combination. For observations where both R-EMAP and supplemental data were available (9 observations), the two values were averaged to yield a single value for the observation.

Fish Normalization

Introduction and Selection of Normalization Endpoint

Because Hg concentrations in fish vary as a function of age or size, and because the age-Hg or size-Hg relationship typically varies between different lakes and different streams, three sets of empirical models were developed (*i.e.*, models specific each for lake, rivers and streams, and the Everglades) to eliminate size of the fish sampled within a given water body as a confounding variable when comparing fish mercury concentrations across lakes or streams. Normalizing was based on length because LMB growth rates vary, and a consistent length at age relationship cannot be established that is representative of all lakes and streams. As a result, LMB methyl Hg concentrations were normalized to a common length providing a measureable human health endpoint. Target concentrations for LMB were developed for fish normalized to 15" in Florida's lakes and streams, and 14" in the Everglades. The choice of 15" as the target end point originates with Florida's tiered system of harvest restrictions with a focus on minimum length limits (MLL) for LMB. In central Florida, the MLL is 14" whereby only LMB $\geq 14"$ in total length (TL) may be harvested. Due to slower growth in the Panhandle region, waters west of and including the Suwannee River and its tributaries, have an MLL of 12" TL, while in the Everglades region there are restrictive regulations allowing anglers to keep only 1 LMB $\geq 14"$ in TL while the remainder must be $< 14"$. Although regulations allow consumptive use of LMB, the fishery has a high level of catch and release with harvest commonly well less than 10% of catch. In addition, size specific exploitation (size of LMB harvested) is difficult to measure and most estimates are for select fisheries only during peak fishing periods and may not be representative of the diverse types and sizes of lakes and streams sampled during this study. Therefore, the endpoint length for normalizing fish methyl Hg concentrations was selected based on results of recent and ongoing exploitation studies, judgment by Florida Fish and Wildlife Conservation Commission (FWC) fishery managers, and estimates of percent composition of harvestable size LMB from representative lakes and streams. Based on data from exploitation studies and best judgment by FWC fisheries managers, the majority of LMB harvested are from the 14"-16" size class. Similarly, for 33 LMB populations surveyed between 2007 and 2009, the majority of LMB available for harvest (65%) were less than 17" TL while the 14" size group was the largest with 28% of all LMB. Both estimates of harvest as well as the availability of LMB for harvest support the use of a 15" TL LMB for normalizing individual water bodies.

The smaller length for the Everglades was selected because recruitment to larger sizes is limited and ample numbers of LMB of smaller sizes are produced within the Everglades. As a result, a human health endpoint for the Everglades region is more appropriately established for 14", which also represents the worst-case scenario for the majority of angler caught fish from the Everglades.

Normalizing Largemouth Bass Hg Concentrations – TMDL Lake, Rivers, and Streams

Statistical Model

Normalizing LMB Hg concentrations to a standard length of 15" was conducted for the TMDL study lakes using robust regression (StataCorp, 2011). In order to optimize the linearity of the relationship between Hg concentrations in LMB as the dependent variable and length (mm) as the independent variable, both variables were *ln*-transformed. Because the relationship between fish length and Hg concentration varies across lakes, the model also incorporated site – *ln* length as an interaction term to produce independent slope and intercepts for each site. Robust regression is an iterative procedure that selectively weights observations as part of the regression model fitting procedure in order to minimize the effect of individual points on the fitted model. Robust regression was selected in favor of ordinary least squares regression (OLS) because it is less sensitive to the leverage effects of outlying observations. This can be particularly important since individual slope terms were being developed for individual sites, and the number of observations within each site (12) can be sensitive to leverage by individual specimens.

Robust regression was conducted in a similar manner for stream sites, with the exception that only sites with a total number of combined LMB and SPBA equal to four or more specimens were included in the analysis. A minimum number of four specimens was set because preliminary calculations indicated less stable predictions for $N = 3$. The resulting intercepts and slopes from the robust regression (Appendix E for lakes and Appendix F for streams) were then used to compute *ln*-transformed concentrations for LMB Hg normalized to 15 inches length for each lake and stream site used in the analysis by substituting the value 5.9428 (the value of 15" converted to mm and then *ln* transformed) for the independent variable denoting length in each site specific equation. Early during the stream sampling, it became apparent that collecting the desired target number of 12 individual LMB specimens¹ was going to be difficult for some stream sites – particularly small, shallow segments found in the upper reaches of streams in the Panhandle. This necessitated using a surrogate species to infer LMB concentrations in stream sites where the number of LMB specimens collected were well below 12. Because spotted sunfish (SPSU) are ubiquitous in streams throughout Florida, including headwater reaches, SPSU specimens were collected from a total of 77 sites with joint collections of LMB and/or SPBA to develop an inferential model. SPSU specimens were also collected from 9 additional sites where LMB/SPBA specimens were absent. Developing a model to infer LMB concentrations of Hg as a function of SPSU Hg concentrations first requires developing concentrations of SPSU Hg normalized to a given length. The length selected as the normalization endpoint was 124 mm, which is the median length of the 933 specimens collected during the study. Similar to the process used for LMB, the normalization for SPSU was conducted using robust regression for all sites where the total number of SPSU specimens was greater than or equal to 4. This equates to 81 sites included in the robust regression analysis. *Ln*-

¹ The Florida Department of Health (DOH) conducts human health risk assessments for a number of fresh and marine fish species, including LMB. In fresh water systems, DOH health assessments currently are based on the average mercury concentration from a representative sample of harvestable size fish which is 12 individuals per species. Lesser numbers of fish are on occasion utilized based on the "best professional judgment" of staff. Therefore, for consistency with current state protocol, samples of 12 harvestable size LMB were targeted to characterize individual water body mercury concentrations in fish.

transformed Hg concentration in SPSU was modeled as the dependent variable, and $\ln$ -transformed length (mm) and site were used as the independent variables, with site – $\ln$ length as an interaction term in the model to produce independent slope and intercepts for each site. The resulting intercepts and slopes from the robust regression (Appendix G) were then used to compute $\ln$ -transformed concentrations for SPSU Hg normalized to 124 mm length for each of the 81 stream sites used in the analysis.

Regression analysis was then used to relate normalized SPSU concentrations to normalized LMB/SPBA concentrations of Hg. A total of 59 observations were available with joint robust regression estimates of normalized Hg for SPSU and LMB/SPBA. The following summarizes the fit statistics for the robust regression model:

Table 2. Summary statistics for robust regression model developed to predict normalized Hg concentrations in LMB ($\ln$ -transformed) as a function of normalized concentrations of Hg in SPSU. Total N = 59. $F(1, 57) = 56.42$; probability > $F < 0.0001$.

	Model Coefficient	Std. Err.	t	P> t	Coefficient 95% Confidence Interval	
$\ln$ SPSU Hg_{std}	0.7302157	0.0972145	7.51	0	0.535547	0.9248844
Intercept	0.5714757	0.1631175	3.5	0.001	0.2448385	0.8981129

where $\ln$ SPSU Hg_{std} is the $\ln$ -transformed concentration of Hg in SPSU normalized to 124 mm. Figure 4 plots the bivariate relationship between the two variables, as well as the resultant fits obtained from both robust and ordinary least squares (OLS) regression.

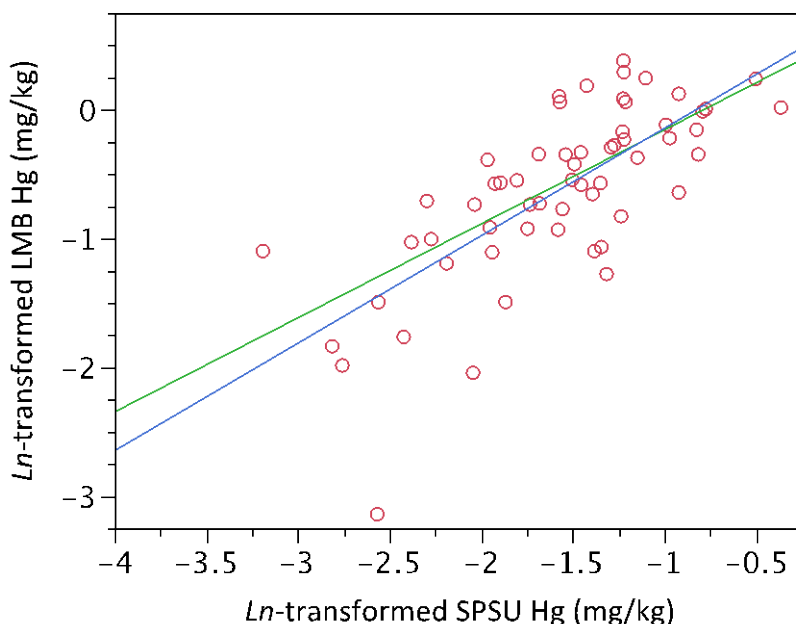


Figure 4. Normalized LMB Hg concentrations (normalized to 15 inches length and $\ln$ -transformed) vs. normalized SPSU Hg concentrations (also $\ln$ -transformed) in Hg TMDL

streams. Normalized values derived from robust regression for sites with $N \geq 4$ specimens for each species at a given site. Solid green line shows calculated relationship derived from robust regression; solid blue line shows the same relationship obtained from ordinary least squares regression.

Inferred values from the SPSU model were used to estimate the final normalized (15 inch) values for LMB Hg for those sites where the number of LMB/SPBA specimens were jointly less than seven for a given site *and* less than the number of SPSU specimens. These constraints yield a total number of inferred values equal to 35. This final data set on normalized values was then integrated with the water and sediment chemistry data set. Figure 5 compares the results from the normalization to 15 inches with observed concentrations for fish (both LMB and SPBA) collected from both lake and stream sites and with a total length within ± 0.5 inches of the standard length of 15 inches.

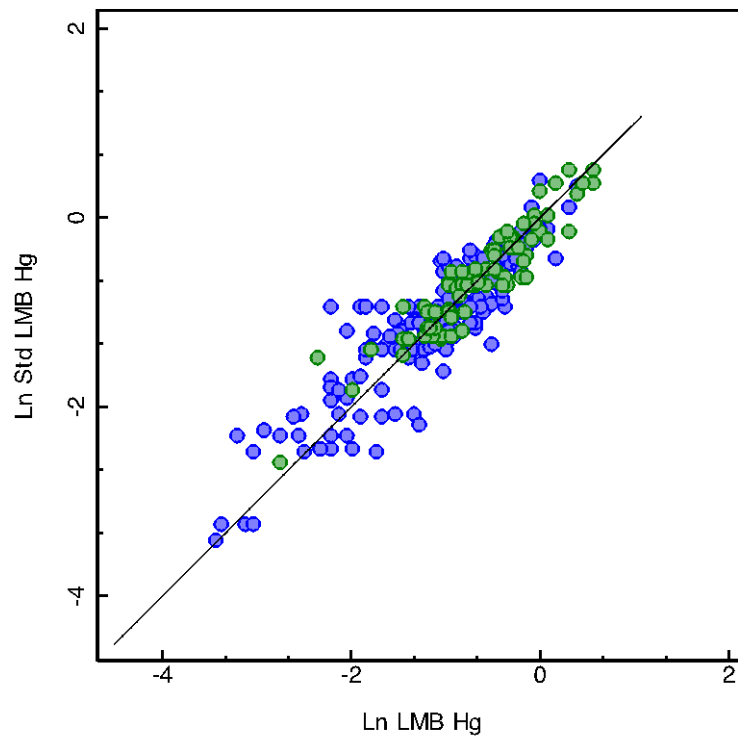


Figure 5. Comparison of modeled LMB Hg (ln-transformed) concentrations standardized to 15 inches length vs. observed (ln-transformed) concentrations for LMB and SPBA with total length between 14.5 and 15.5 inches. Blue filled circles – samples collected from lakes; green filled circles – samples obtained from streams. Solid line shows 1:1 agreement line.

Normalizing Largemouth Bass Hg Concentrations – Everglades

Statistical Model

Similar to the calculations conducted for the TMDL lakes and streams (Section 3.2.1), LMB Hg concentrations for each Everglades site were normalized to a standard length using robust regression (StataCorp, 2011). As described in Section 3.1, 14" was selected as the normalization endpoint. Normalization was conducted using the statistical software package Stata/MP using the following model:

$$\begin{aligned} \ln \text{ LMB Hg} = & \text{Location} + \ln \text{ TL} + \text{Location} * \ln \text{ TL} + \text{Location} * \text{Year} + \text{Year} * \ln \text{ TL} + \\ & \text{Year} * \text{Year} + \ln \text{ TL} * \ln \text{ TL} \end{aligned} \quad (1)$$

where TL is total length (mm). Because the modeling between *Gambusia* and LMB Hg concentrations includes data collected between 1995 and 2009, and because LMB Hg concentrations have declined during that period, particularly before ~ 2000 (BOZO SFER REFERENCE), the year the samples were collected was included in the model both as an explicit term and as interaction terms to account for both site-specific temporal dynamics and changes in length-Hg concentration related to time.

The normalization analysis was conducted by using only those sites with a minimum of four LMB collected during a given month. Total *N* for the analysis equaled 1,637. In order to minimize the effect of individual influential observations on the regression (which is important because of the large number of interaction terms), the analysis was conducted using Stata's implementation of robust regression. Significance of the model fit is given by the F statistic:

$$F(64, 1572) = 74.81; p < 0.0001.$$

Coefficients for the individual interaction terms are presented in Appendix D. Figure 6 compares the resulting predicted (ln-transformed) concentrations of Hg in Everglades LMB with observed values, while Figure 7 shows the same comparison for individual specimens with total lengths between 13.75" and 14.25".

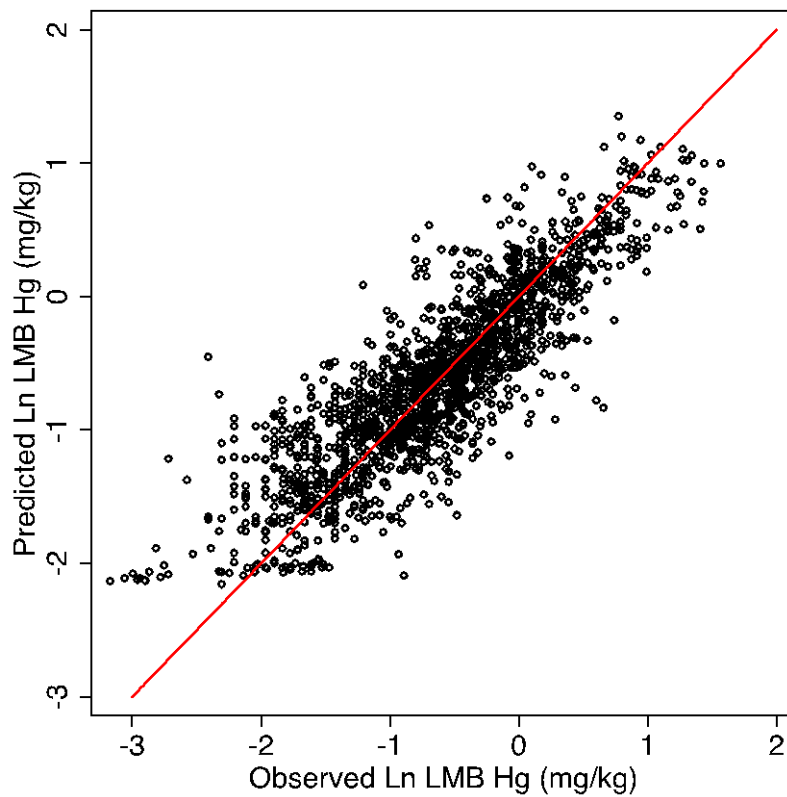


Figure 6. Predicted vs. observed In-transformed LMB Hg concentrations from FFWC large fish monitoring stations in the Florida Everglades. N = 1,637. Red line shows line of 1:1 correspondence.

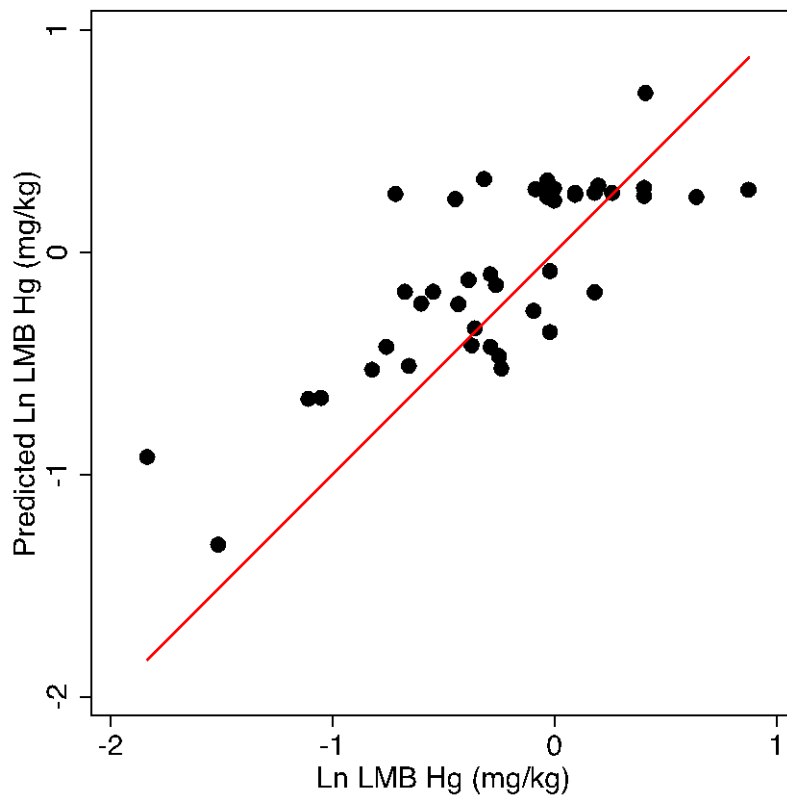


Figure 7. Comparison of predicted In-transformed LMB Hg concentrations with observed In-transformed LMB Hg concentrations. Data shown are for LMB with observed total lengths ranging between 13.75 and 14.25 inches (inclusive). Red line shows line of 1:1 correspondence.

Data Transformations

Transformation of Sulfate

Sulfate dynamics can play a critical role in mercury cycling because methylation of mercury in freshwater aquatic ecosystems is governed in large part by the activity of sulfate-reducing bacteria that use sulfate as the terminal electron acceptor to metabolize organic matter under anaerobic conditions (Gilmour, 2011). Early work by Gilmour and Henry (1991) hypothesized that the relationship between sulfate and mercury (Hg) methylation was non-linear and essentially unimodal, with the optimal concentration of sulfate supporting maximal rates of methylation in the range of 200 – 500 μM (20 – 50 mg/L). According to this paradigm, sulfate availability at concentrations below the optimal concentration limits the activity of sulfate reducing bacteria, which in turn limits rates of methylation. At concentrations above the optimum, sulfate reduction produces sulfide at levels which inhibit sulfate reduction, primarily through the sequestration of labile Hg(II) which is a necessary substrate to support methylation. Restated, from a theoretical perspective there is a critical sulfate concentration that is optimal for maximizing mercury methylation; at sulfate concentrations increasingly below (because of sulfate limitation) or above (because of sulfide inhibition) this threshold, methylation is expected to decrease.

While subsequent work in freshwater aquatic ecosystems suggests that the optimal concentration of sulfate supporting methylation is lower and narrower in range ($< 200 \mu\text{M}$ or 20 mg/L), the unimodal nature of the relationship is readily apparent from cross-sectional data from both the Everglades, and lakes and streams in Florida (Figure 8 and Figure 9). For both the freshwater lakes and streams combined, and for the Everglades, maximal Hg concentrations for either *Gambusia* or LMB follow similar unimodal patterns, and suggest that a maximum potential fish Hg concentration can be defined for any given sulfate level, which in turn can be modified as a function of ambient water quality and biogeochemical factors. The goal of this phase of the analysis was to attempt to linearize the underlying relationship so that the effects of sulfate on LMB Hg concentrations can be explicitly and appropriately considered in linear model frameworks such as MLR or GLM.

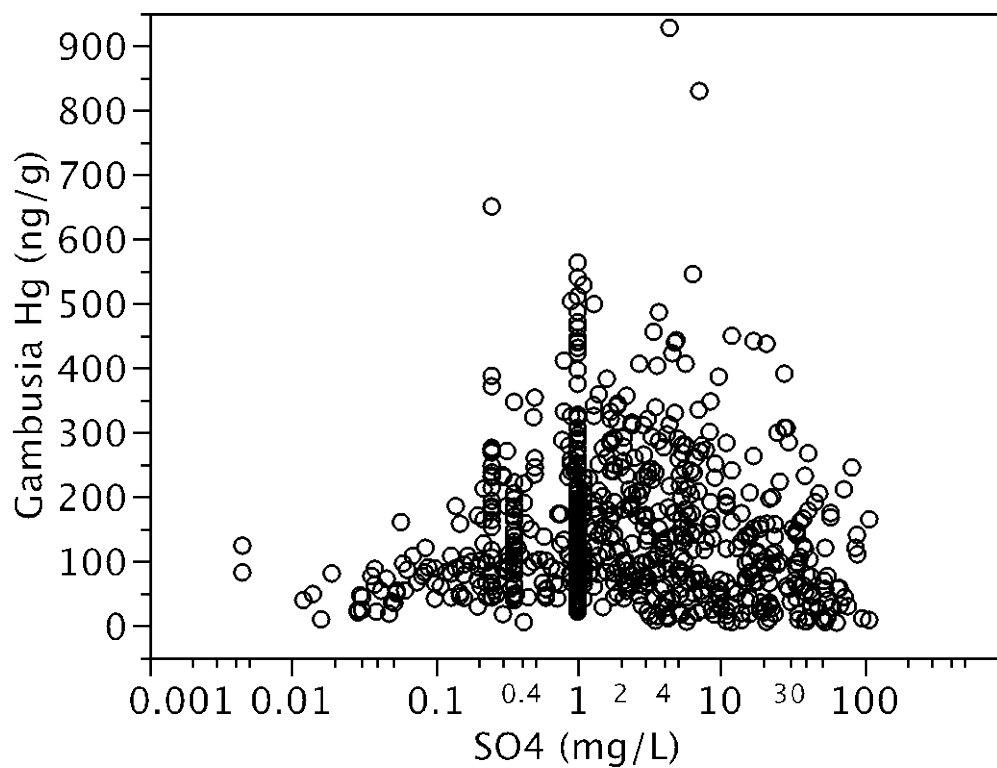


Figure 8. Mercury concentrations in *Gambusia* as a function of water column sulfate concentrations in the Florida Everglades. Data from the USEPA R-EMAP program for the Everglades, Cycles 0 – 7.

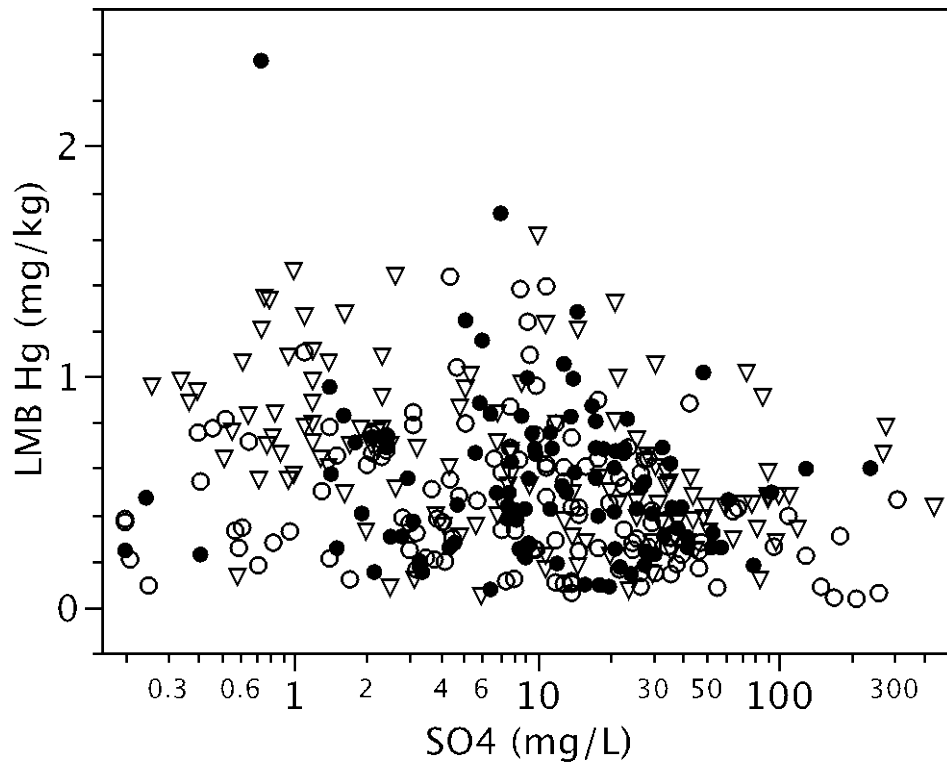


Figure 9. Largemouth (LMB) Hg concentrations in Florida lakes as a function of water column sulfate concentrations. Open circles – LMB concentrations normalized to a standardized length of 381 mm (15") in Florida Hg TMDL lakes (N = 130). Open triangles – LMB concentrations normalized to a standardized length of 381 mm (15") in Florida Hg TMDL streams (N = 128). Filled circles – LMB concentrations normalized to standardized age of 3 years in 104 Florida lakes sampled by Lange et al. (unpublished data).

In order to capture the non-linear response inferred by the maximal response envelope, *ln*-transformed sulfate concentrations were aggregated into the following intervals:

Hydro_Type	N Rows	Interval Midpoint	Interval Minimum	Interval Maximum	Median Concentration
Lake	6	1.50	1.25	1.75	1.45
Lake	3	2.00	1.75	2.25	2.14
Lake	8	2.50	2.25	2.75	2.53
Lake	3	3.00	2.75	3.25	3.00
Lake	12	3.50	3.25	3.75	3.44
Lake	23	4.00	3.75	4.25	4.06
Lake	26	4.63	4.25	5.00	4.59
Lake	133	6.00	5.00	7.00	5.81
Lake	20	8.00	7.00	9.00	7.67
Stream	1	1.50	1.25	1.75	1.69
Stream	3	2.00	1.75	2.25	2.04
Stream	8	2.50	2.25	2.75	2.57
Stream	16	3.00	2.75	3.25	3.04
Stream	8	3.50	3.25	3.75	3.51
Stream	12	4.00	3.75	4.25	3.91
Stream	12	4.63	4.25	5.00	4.68
Stream	50	6.00	5.00	7.00	6.25
Stream	17	8.00	7.00	9.00	7.54
Stream	1	10.00	9.00	11.00	9.12

Median values of LMB Hg concentrations were obtained for each interval, and plotted against the interval midpoint (Figure 10). Both cubic spline and piece-wise regression models were fit to both sets of points (Figure 11). The piecewise regression was conducted with knots set at *ln*-transformed SO₄ (μeq/L) = 3.5 (lakes and streams both) and 4.625 (streams only).

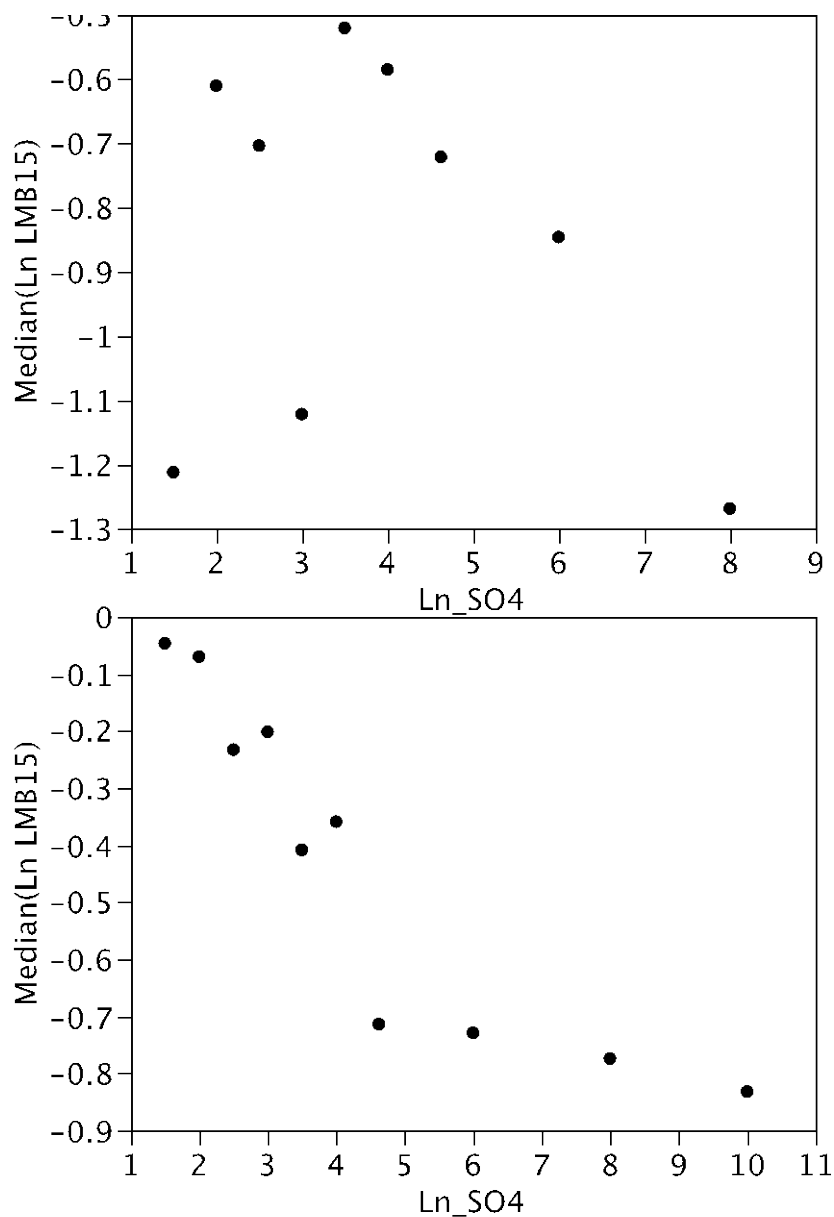


Figure 10. Median LMB Hg concentrations (ln-transformed) as a function of sulfate concentration interval (also ln-transformed; original units $\mu\text{eq/L}$). Upper panel – Hg TMDL lakes and Lange et al. study lakes; lower panel – Hg TMDL streams.

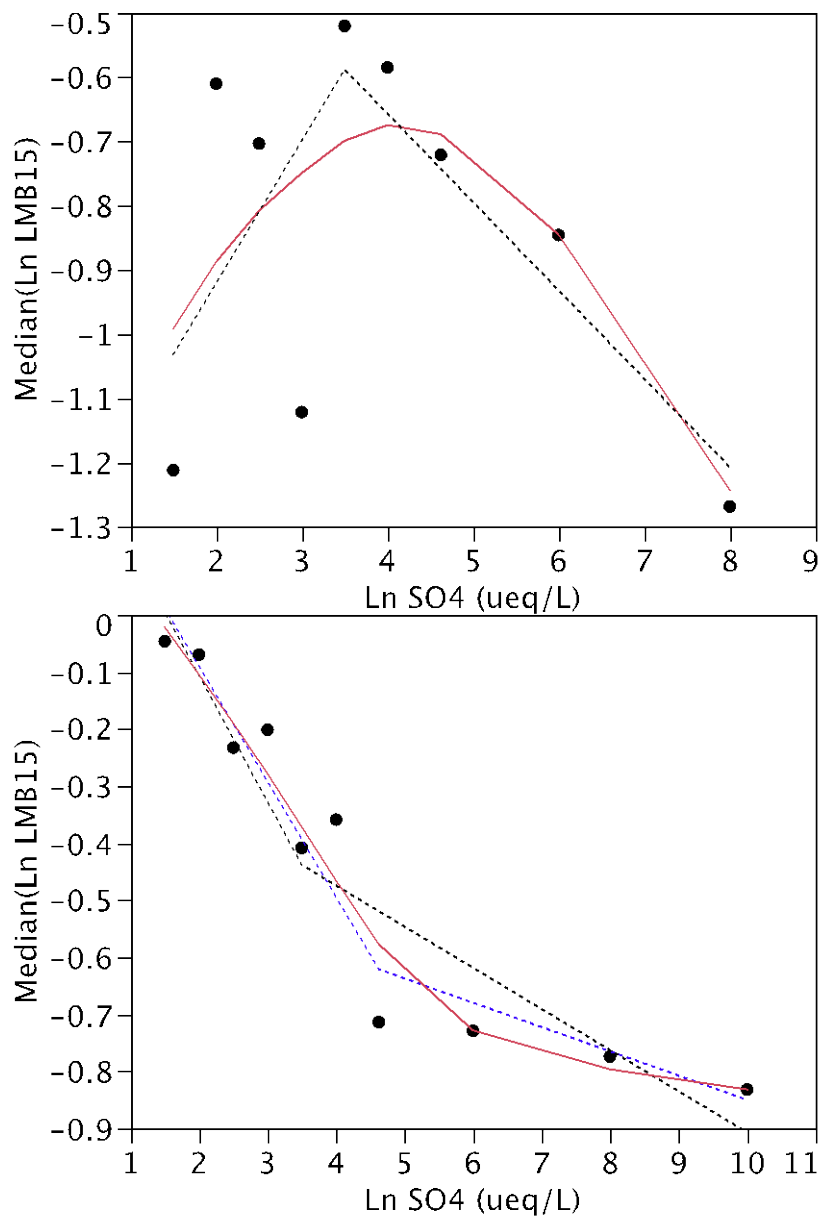


Figure 11. Same as Figure 10, except that each plot shows fitted relationship obtained from smoothing spline (red solid line) and from piecewise regression with knots at $\ln \text{SO}_4 (\mu\text{eq/L}) = 3.5$ (black dashed line) and 4.625 (blue dashed line, Hg TMDL streams only).

Transformation of pH

Initial analyses examining the relationship between pH and LMB Hg concentrations indicated that the relationship for the TMDL lakes was reasonably linear, while for the TMDL streams the relationship was clearly non-linear. This is evidenced by examining the bivariate plots for both lakes and streams, as well as plots of the residuals obtained from regressing LMB Hg as a function of pH (Figure 12 and Figure 13, respectively).

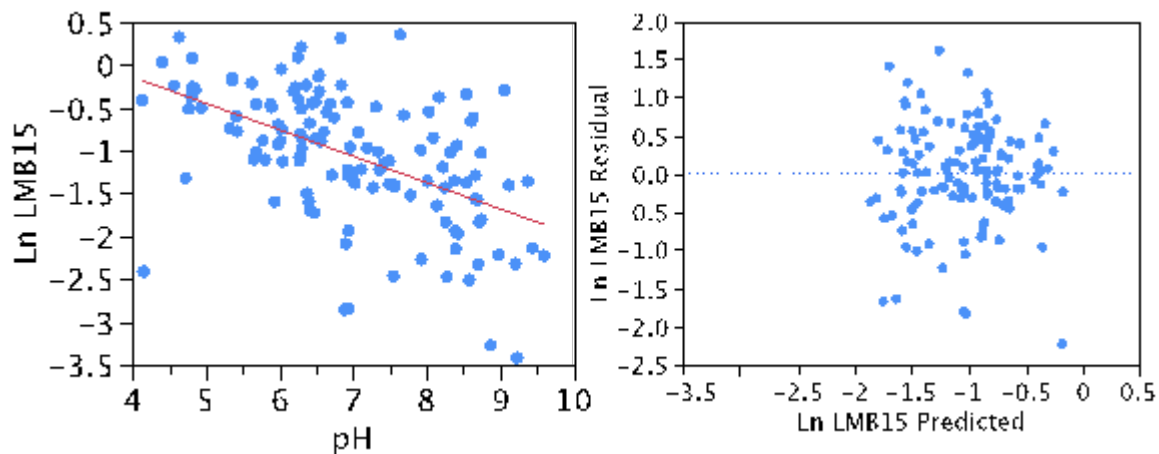


Figure 12. Bivariate relationship between pH and ln-transformed LMB Hg concentrations in Hg TMDL lakes. Left hand panel: fitted linear model (red line). Right hand panel: residuals from fitted linear model plotted against predicted values.

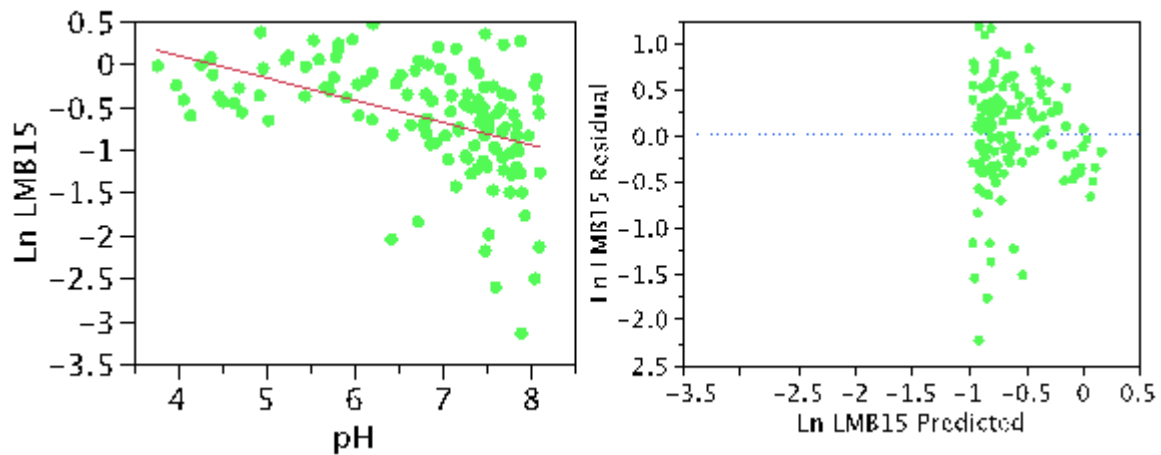


Figure 13. Bivariate relationship between pH and ln-transformed LMB Hg concentrations in Hg TMDL streams. Left hand panel: fitted linear model (red line). Right hand panel: residuals from fitted linear model plotted against predicted values.

Sites deviating from linearity in the bivariate relationship appear to be systems with low to moderate organic carbon and low concentrations of methyl Hg in the water column (see Figure 14 through Figure 16).

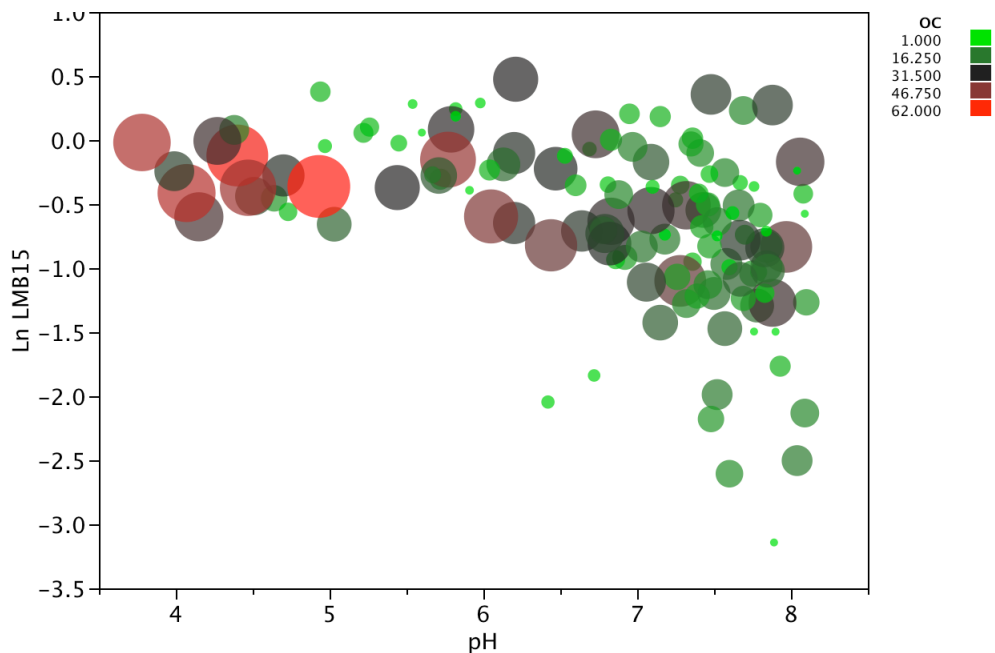


Figure 14. Bubble plot showing relationship between In-transformed LMB Hg, pH and organic carbon in Hg TMDL streams.

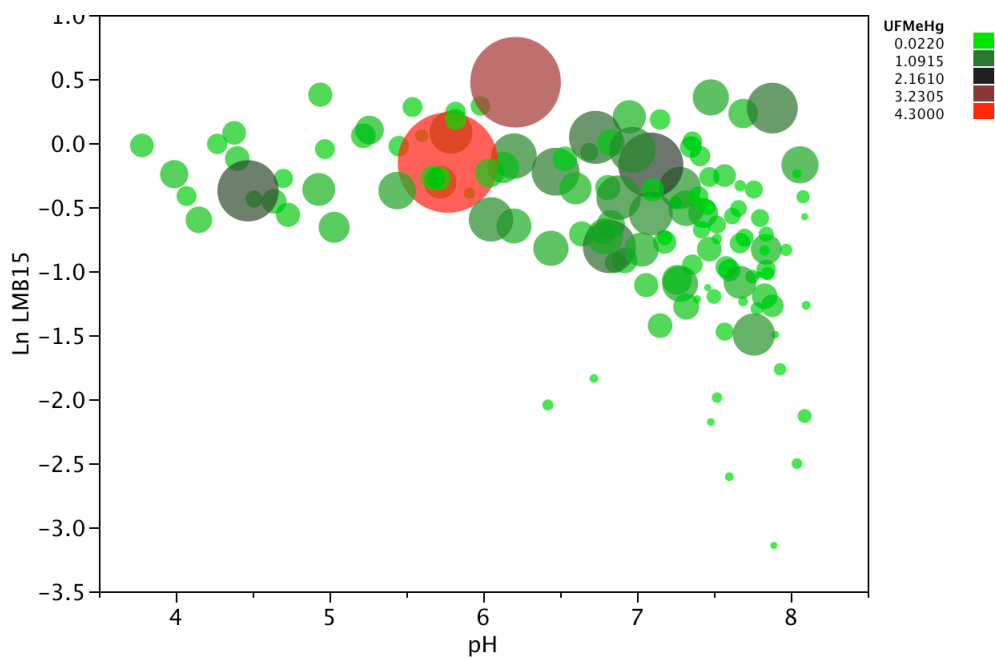


Figure 15. Bubble plot showing relationship between In-transformed LMB Hg, pH and unfiltered methyl Hg in Hg TMDL streams.

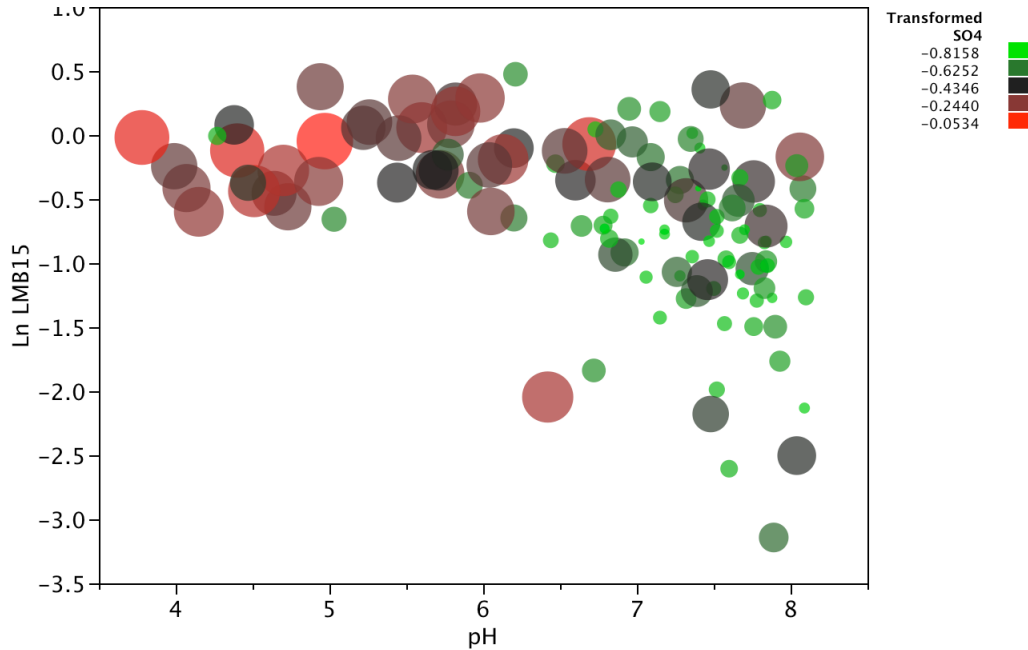


Figure 16. Bubble plot showing relationship between In-transformed LMB Hg, pH and transformed SO4 in Hg TMDL streams.

The variable pH was subsequently transformed to produce a variable that was more linear in its relationship with LMB Hg. pH was initially transformed by computing its inverse, pH_{INV} :

$$pH_{INV} = \frac{1}{pH}$$

pH_{INV} was then transformed using a Monod-type expression:

$$pH_{transinv} = \frac{\alpha \cdot pH_{INV}}{\beta + pH_{INV}}$$

where $\alpha = -0.120409584124109$ and $\beta = -0.112009287460566$.

The following plot (Figure 17) shows the improved relationship using transformed pH instead of pH in the bivariate relationship with LMB Hg:

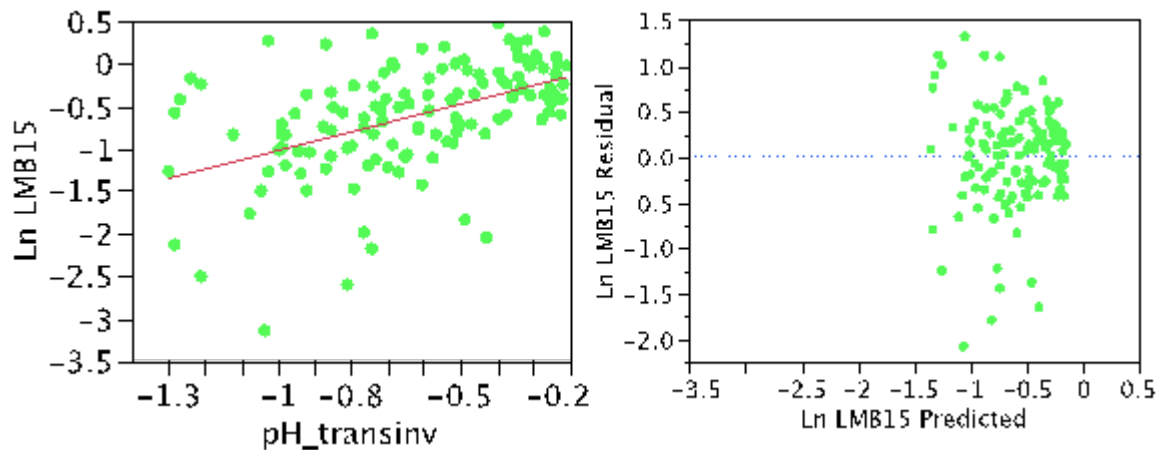


Figure 17. Bivariate relationship between transformed pH and ln-transformed LMB Hg concentrations in Hg TMDL streams. Left hand panel: fitted linear model (red line). Right hand panel: residuals from fitted linear model plotted against predicted values. See Figure 13 for similar plot for untransformed pH.

TMDL Lakes Statistical Model

The development of the statistical lake model was conducted as a three-step process. The first stage, which was suggested by preliminary analyses of the lake sediment chemistry indicating it could have an influence on the expression of Hg in fish tissue, involved developing a set of sediment variables through principal components analysis (PCA) that are indicative of overall processes such as watershed weathering and anthropogenic disturbance. The second stage involved developing an appropriate algorithm to better represent the expected non-linear relationship between lakewater concentrations of sulfate and LMB Hg concentrations. The third stage involved developing a multivariate model that both integrates the components from the first two stages and includes other water quality variables in building a predictive model for LMB Hg concentrations in lakes throughout Florida. All analyses with LMB Hg concentrations utilized concentrations normalized to a standard 15" (381 mm) length.

Sediment Modeling

The lake sediment data for mercury and other analytes collected from the TMDL lakes were analyzed to determine if various sources and processes that may be reflected in sediment chemistry such as anthropogenic enrichment or watershed weathering are important determinants of mercury accumulation in fish in Florida's lakes. One difficulty with analyzing sediment chemistry is that the overall variance in sediment Hg as well as other constituent concentrations may be related more strongly to factors other than atmospheric deposition or local sources of contamination. This concept is illustrated by Figure 19, which plots sediment Hg concentrations in the Florida Hg TMDL lakes as a function of sediment total organic carbon (TOC). In brief, Figure 19 suggests that sediment Hg variability is driven by TOC dynamics or, restated, the factors that govern the accumulation of TOC also are largely responsible for governing the accumulation of Hg. One might leap to the conclusion that trophic state is such a factor, but that hypothesis has two problems. First, although sedimentary total Kjeldahl nitrogen (TKN) and TOC are very highly correlated ($\log\text{-log } r^2 = 0.882$, $n = 129$; $p < 0.0001$), variations in sedimentary N and C are only weakly related with trophic state in Florida lakes (Brenner and Binford, 1988). The second problem relates to particle dilution of Hg concentrations. If higher sediment TOC concentrations are largely a function of trophic state then, all other things being equal, biodilution should result in *lower* concentrations of Hg corresponding with *higher* concentrations of sedimentary TOC. Indeed, when we examine surface water chemistry data relationships with LMB Hg concentrations, we do see evidence of biodilution (Figure 18).

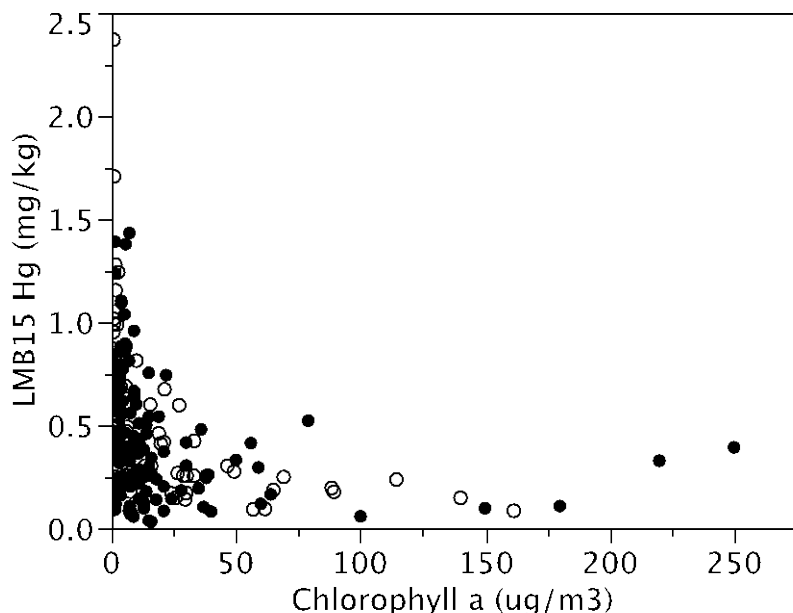


Figure 18. Hg concentrations in LMB normalized to 15 inches length as a function of chlorophyll a concentrations in Florida statewide Hg TMDL lakes and lakes from the Lange et al. (unpublished data) data set. Filled circles – Hg TMDL lakes; open circles – Lange et al. lakes.

The overall inter-lake variability in sedimentary TOC and Hg is more likely a product in differences in the lacustrine depositional environment where the samples were collected. Concentrations of sediment organic carbon and contaminants such as Hg reflect a variety of processes including effects of selective size sorting and deposition of fine vs. coarse grained sediments in more pelagic vs. littoral environments (Håkanson and Jansson, 1983), watershed weathering inputs, and enrichment due to anthropogenic inputs (both direct atmospheric and watershed-related – Hg and other contaminants). One approach towards quantifying enrichment of sediment contaminants is to normalize sediment concentrations to constituents such as aluminum (*cf.* Bertine and Goldberg, 1977; Förstner and Wittman, 1979) that are assumed to be natural in origin, and whose variations in concentration reflect the differential effects of particle dynamics (erosion, transportation, and deposition) characteristic of the lacustrine environment. An alternative approach is to use principal components analysis (PCA), which is a multivariate statistical method that seeks to maximize the variance explained in a set of variables through a smaller set of indices or components. Each component reflects different linear combinations of the original variables and is uncorrelated with the other components. The association of the original variables with different components extracted from the analysis can lead to interpretable underlying processes and the method (and subsequent variants) has a rich history of use in conducting source apportionment for atmospheric aerosols beginning with Thurston and Spengler's (1985) analysis quantifying major aerosol pollution source classes affecting a monitoring site in metropolitan Boston, MA. More recently, Rubio *et al.* (2000) used PCA to identify both sources and background concentrations of trace elements in sediments of the coastal Ria de Vigo ecosystem in northwest Spain.

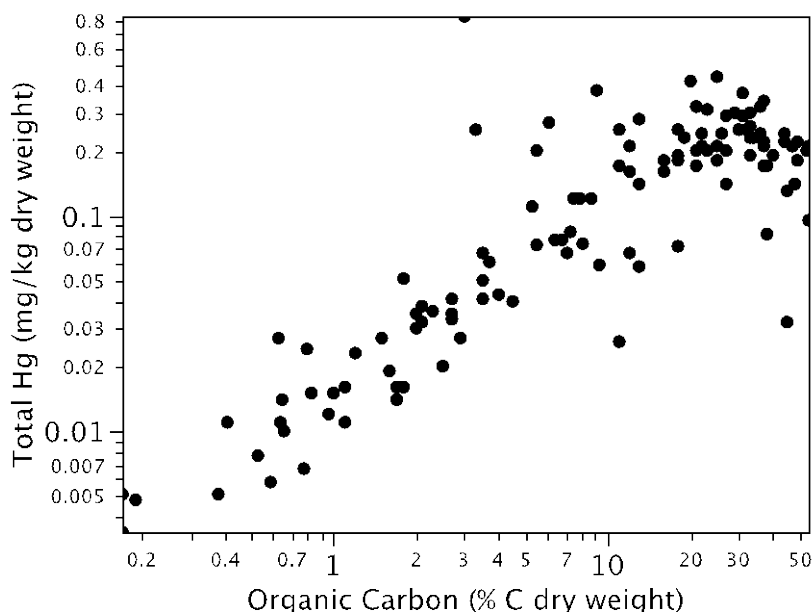


Figure 19. Sediment Hg concentrations as a function of sediment organic carbon.

Imputation of Below Detection Limit Values for As, Cu, and Pb

One of the inherent assumptions underlying the application of PCA is that the observations for each variable are normally distributed. Of the variables that were included in the PCA (Table 3), three in particular exhibit significant tailing at the lower end of the distribution – arsenic (As), copper (Cu), and lead (Pb) – due to relatively large numbers of samples with non-detectable concentrations. Concentrations for the lower end of the distributions for each of these three variables (*i.e.*, for samples with sediment qualifier codes containing the code *U* for undetected) were estimated or imputed using linear regression modeling. Stepwise regression was conducted using sediment parameters that included all those listed in Table 3 (but excluding Cu, Pb and As), as well as magnesium (Mg), manganese (Mn), vanadium (V), and titanium (Ti). Both forward and backward stepwise regression was conducted, with model selection for imputation based on minimizing the Akaike information criterion (AIC) and the Bayesian information criterion (BIC) statistics. The resulting model was then used to impute the concentrations for observations flagged with the code *U* for a given parameter. One criticism of imputation used in this manner is that the MLR models used to impute values for As, Cu, and Pb observed below detectable levels were extended beyond the limits of the data used to construct the models. The sensitivity of modeling using imputed values vs. using the original data (with values flagged as “*U*” kept at their original reported values) was evaluated by conducting PCA using both sets of variables and then comparing the results obtained from regressing the extracted components against both sediment Hg (*ln*-transformed, Table 4) and LMB Hg concentrations (*ln*-transformed; Table 5). The analysis also considered the results obtained from imputing the flagged values for As, Cu, and Pb using a restricted maximum likelihood estimation (REML) procedure (SAS, 2011) that uses the values for the entire suite of other measured sediment variables to impute the flagged values.

Comparison of the model coefficients and *t* statistics indicate that imputing using REML or MLR yields very similar results. In addition, there was little difference between the imputed model results and the results obtained from using the original data with no imputation for the sediment Hg model. For the LMB Hg model, the choice to impute also had little bearing on the resulting extracted component coefficients when compared to the original data results. Similarly, direct comparison of the extracted components for urban runoff and for atmospheric disturbance indicate that extracted components are fundamentally the same regardless of imputation technique or whether or not imputation was conducted *vis-à-vis* the original data (Figure 20). The LMB Hg model coefficients were appreciably different, however, when the concentrations of the flagged observations for As, Cu, and Pb were set equal to ½ the MDL. This may suggest that setting the flagged concentrations to ½ the MDL may artificially impose upon the data distribution a cluster of points with values too low. As a result, imputed values based on MLR were used for sediment As, Cu, and Pb concentrations flagged as “U”. The implementation of MLR to impute the flagged concentrations of As, Cu, and Pb are detailed in the immediately following sections.

Table 3. Overview of subset of parameters measured in surficial lake sediments sampled as part of the Florida statewide Hg TMDL that were included in principal components analysis (PCA). Table indicates number of samples with no QA qualifying code, or were coded with or A (indicating that the reported value was the average of multiple analyses) or I (value is between the method detection limit [MDL] and the practical quantitation limit [PQL]). The table also indicates whether the variable is included as a routine parameter monitored in lake sediments as part of the FDEP Status and Monitoring Network (SMN).

Sediment Parameter	Included in SMN	Included in PCA	No QA code	No QA Code + A or I
Pb	Yes	Yes	67	96
Cu	Yes	Yes	76	104
As	Yes	Yes	84	115
Zn	Yes	Yes	87	115
Ni	Yes	Yes	83	119
Hg	Yes	Yes	80	126
Cr	Yes	Yes	93	126
OC	Yes	Yes	111	128
TC	Yes	Yes	102	129
Al	Yes	Yes	121	129
Fe	Yes	Yes	121	129
TKN	Yes	Yes	127	129
TP	Yes	Yes	128	129

Table 4. Summary of results obtained from different methods of treating concentrations of As, Cu, and Pb measured below the method detection limit (MDL). Each analysis represents the results of computing a set of four components extracted from PCA using the sediment data (ln-transformed) and then regressing (via multiple linear regression) sediment Hg (ln-transformed) concentrations as a function of the extracted components. Note that for the data set with values below the MDL transformed by assuming the concentration equals ½ the MDL, both 4 and 5 PCA component models were evaluated.

	Impute via MLR	No imputation - drop values < MDL	Impute via REML	Original data	Original data - 1/2 MDL	Original data - 1/2 MDL - 5 PCA
<i>Model coefficient</i>						
Selective Size Sorting	0.8727	0.4737	0.8690	0.8569	0.8783	0.8448
Watershed weathering	0.5923	0.3038	0.5893	0.5827	0.6102	0.5600
Urban runoff	0.3885	0.1128	0.3705	0.3898	0.5266	0.3930
Atmospheric deposition	0.3973	0.2485	0.4335	0.4455		0.4571
Phosphorus					0.0783	0.1951
<i>t Ratio</i>						
Selective Size Sorting	20.04	9.36	20.08	19.82	20.44	19.64
Watershed weathering	13.60	6.00	13.56	13.48	14.20	13.02
Urban runoff	8.92	2.23	8.25	9.01	12.25	9.14
Atmospheric deposition	9.13	4.91	9.76	10.30		10.63
Phosphorus					1.82	4.54
<i>Probability > t </i>						
Selective Size Sorting	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
Watershed weathering	<.0001	6.0000	<.0001	<.0001	<.0001	<.0001
Urban runoff	<.0001	0.0283	<.0001	<.0001	<.0001	<.0001
Atmospheric deposition	<.0001	<.0001	<.0001	<.0001		<.0001
Phosphorus					0.0708	<.0001

<i>N</i>	129	94	129	129	129	129
<i>r</i> ²	0.86	0.63	0.86	0.86	0.86	0.86

Table 5. Same as Table 4 except that each analysis represents the results of computing a set of four components extracted from PCA using the sediment data (ln-transformed) and then regressing (via multiple linear regression) LMB Hg (ln-transformed) concentrations as a function of the extracted components.

	Impute via MLR	No imputation - drop values < MDL	Impute via REML	Original data	Original data - 1/2 MDL	Original data - 1/2 MDL - 5 PCA
<i>Model coefficient</i>						
Selective Size Sorting	0.0741	0.1897	0.0708	0.0734	0.1415	0.1102
Watershed weathering	-0.1349	0.0104	-0.1524	-0.1450	-0.0092	-0.0468
Urban runoff	-0.3454	-0.3730	-0.3569	-0.3690	-0.2293	-0.3398
Atmospheric deposition	0.2843	0.3188	0.3132	0.3137		0.2062
Phosphorus					-0.4221	-0.3003
<i>t Ratio</i>						
Selective Size Sorting	1.37	3.30	1.33	1.43	2.71	2.1400
Watershed weathering	-2.49	1.00	-2.85	-2.82	-0.18	-0.9100
Urban runoff	-6.37	-6.48	-6.45	-7.19	-4.40	-6.6100
Atmospheric deposition	5.25	5.54	5.72	6.11		4.0100
Phosphorus					-8.09	-5.8400
<i>Probability > t </i>						
Selective Size Sorting	0.1739	0.0014	0.1864	<.0001	0.0076	0.0341
Watershed weathering	0.0141	0.8575	0.0051	0.0055	0.8597	0.3646
Urban runoff	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
Atmospheric deposition	<.0001	<.0001	<.0001	<.0001		<.0001

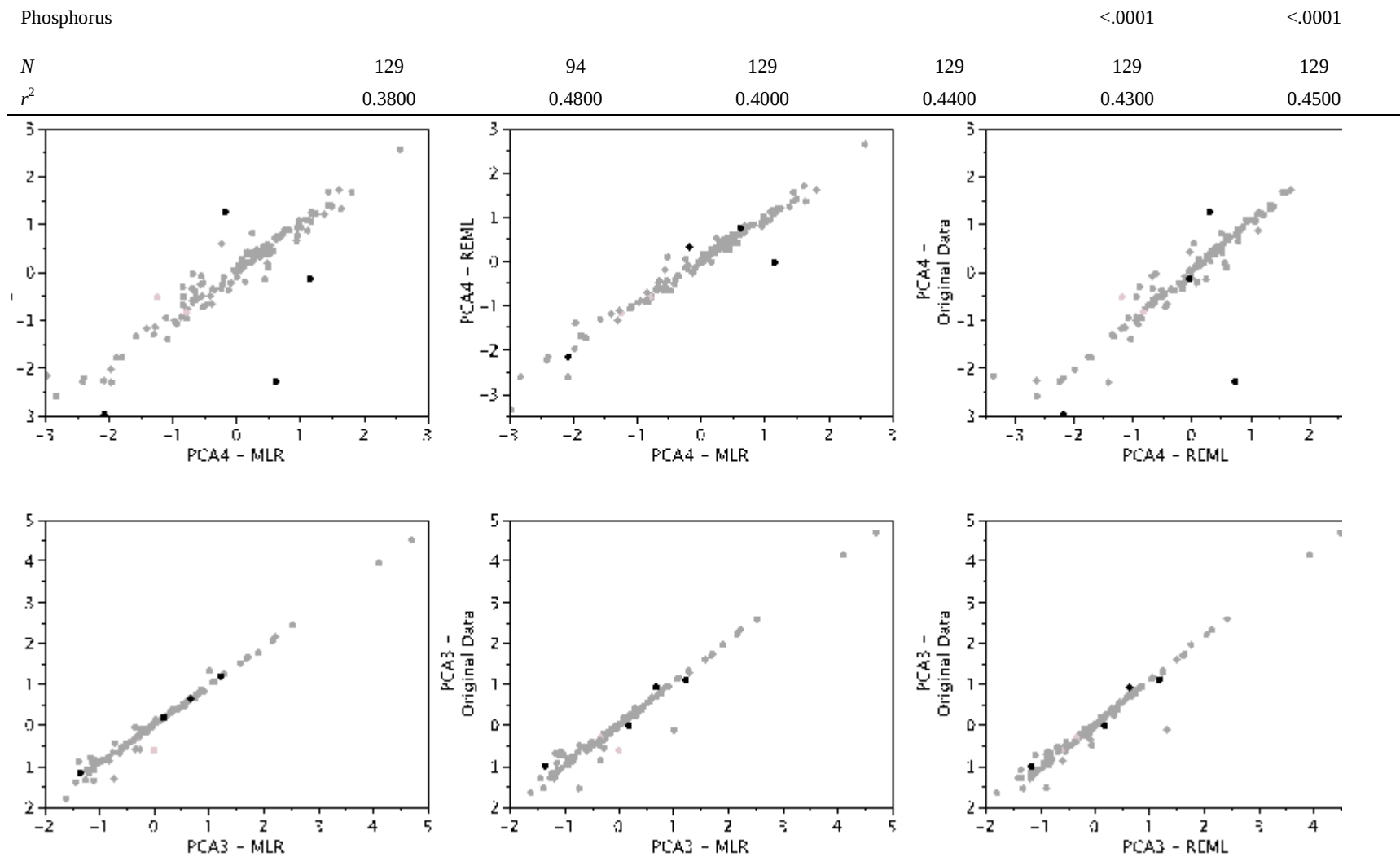


Figure 20. Comparison of extracted components (third and fourth components following Varimax rotation for a four component model) conducted using (1) the original reported values for sediment As,

Cu, and Pb for observations flagged as “U”; (2) MLR imputed values for flagged As, Cu, and Pb concentrations; and (3) REML imputed values for flagged As, Cu, and Pb concentrations. Analysis includes only the third and fourth extracted components because only these components were subsequent, significant contributors to the MLR model for LMB Hg.

Lead (Pb)

The imputation model for undetected sediment Pb concentrations was constructed using forward stepwise regression. The imputation model was constructed by excluding all samples with the qualifier code U for sediment Pb concentrations, yielding a total set of $N = 98$ samples to construct the model. Results for the forward regression model are shown in Figure 21, while the model coefficients and significance are shown in Table 6. The results shown in Figure 21 indicate that the imputation model is essentially linear and fits the data reasonably. As expected, the comparison of modeled and observed results shows that imputation produces a substantially broader range of $\ln$ -transformed concentrations. As a result, substituting the imputed for flagged values improves the lower tail of the frequency distribution for sediment Pb concentrations such that it better approximates a normal distribution than the original values (Figure 22).

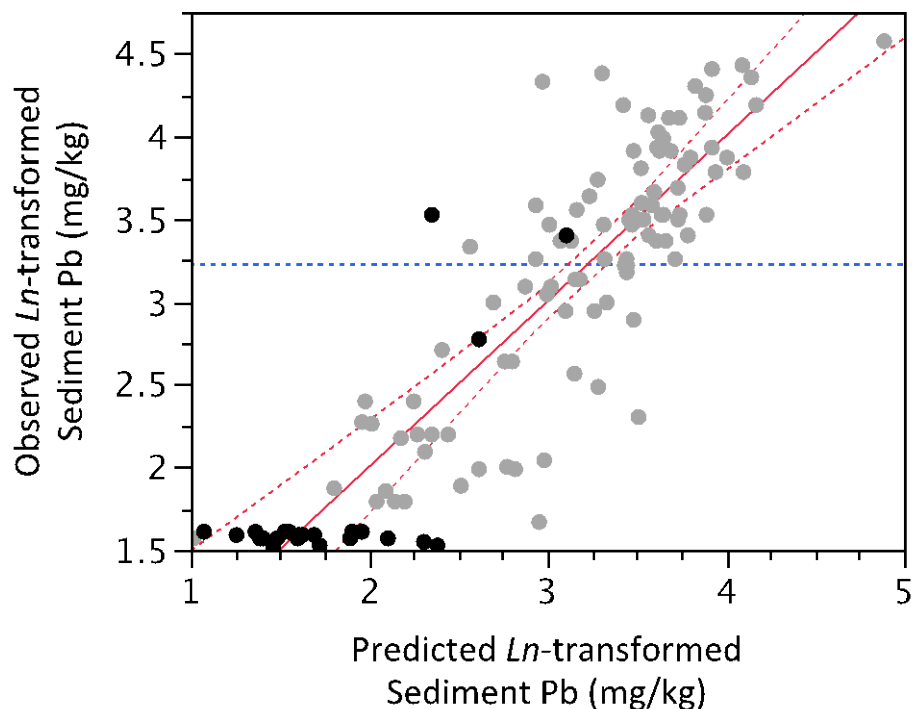


Figure 21. Model fit between actual and predicted sediment Pb concentrations based on MLR model obtained from forward stepwise regression. Highlighted circles are flagged observations with concentrations below the method detection limit (qualifier code containing U) that were excluded from fitting the regression model. $R^2 = 0.690$ ($p < 0.0001$). Total N of unflagged values = 98.

Table 6. Fitted coefficients, standard errors, and *t* statistics for parameter coefficient significance for the MLR model used to impute values for sediment Pb concentrations reported as below the MDL.

Term	Estimate	Std Error	t Ratio	Prob> t
Intercept	1.1087401	0.811616	1.37	0.1752
Ln_Sed_Zn	0.4268162	0.068274	6.25	<.0001
Ln_Sed_TOC	0.493807	0.091003	5.43	<.0001
Ln_Sed_TKN	-0.273434	0.098975	-2.76	0.0069
Ln_Sed_Ti	0.2418139	0.06289	3.85	0.0002

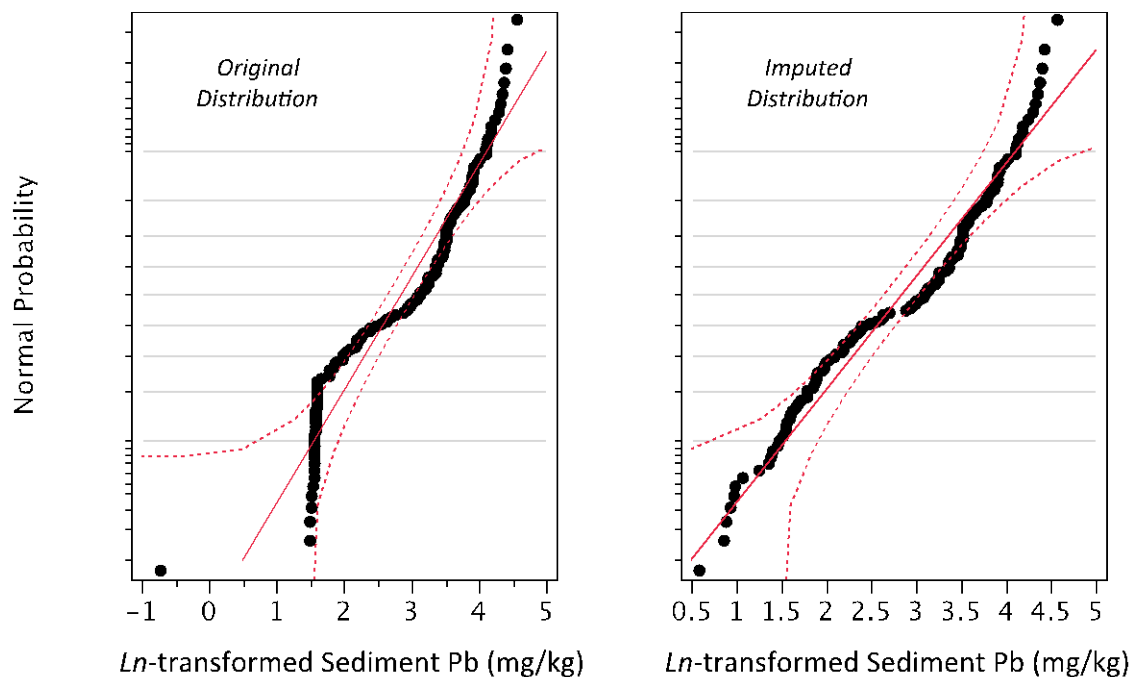


Figure 22. Normal probability plot of ln-transformed sediment Pb concentrations. Left hand panel – original data; right hand panel – distribution with imputed values for flagged observations with concentrations below the MDL.

Copper (Cu)

Initial stepwise regression analyses indicated that the sediment Cu concentration for Ward Lake (WB# = 1501) was an outlier that exerted significant leverage on the overall regression model (Cook's D for the forward regression model, which included Iron (Fe), Nickel (Ni), Zinc (Zn), and Mg as independent variables, equals 0.357 compared to a critical value of 0.038). Cook's D was used as a metric to identify potential outliers because it calculates the overall influence the observation exerts on the regression (Hamilton, 2009). As a result, this observation (along with values flagged as below the method detection limit) was excluded from developing the imputation model. The total number of observations used to develop the imputation model was thus 103. Identical results were obtained from forward and backward stepwise regression. Results for the regression model are shown in Figure 23, and the details of the fitted model are presented in Table 7). Similar to Pb, imputing values for Cu concentrations below the MDL results in an improved distribution of values (Figure 24).

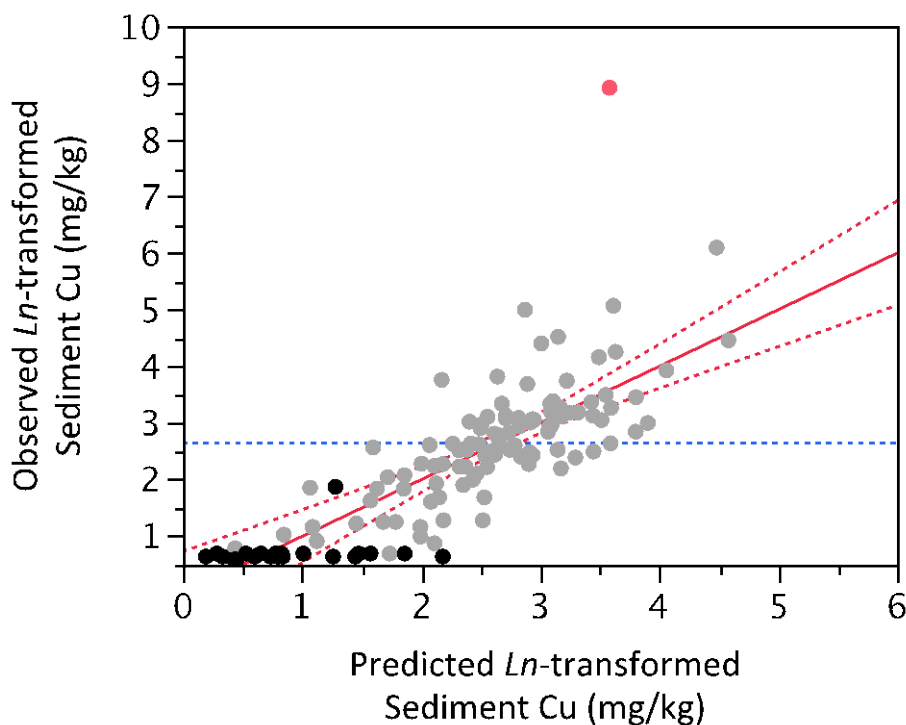


Figure 23: Model fit between actual and predicted sediment Cu concentrations based on MLR model obtained from stepwise regression. Note that the same model was obtained from both forward and backward stepwise regression. Black highlighted circles are flagged observations with concentrations below the method detection limit (qualifier code containing U) that were excluded from fitting the regression model. Red highlighted circle is for WB# = 1501 (Ward Lake), which was also excluded as an outlier based on Cook's

D analysis. $R^2 = 0.577$ ($p < 0.0001$). Total N of values used in the model = 103.

Table 7. Fitted coefficients, standard errors, and *t* statistics for parameter coefficient significance for the MLR model used to impute values for sediment Cu concentrations reported as below the MDL.

Term	Estimate	Std Error	t Ratio	Prob> t
Intercept	1.3127613	0.579799	2.26	0.0258
Ln_Sed_Fe	-0.434676	0.103946	-4.18	<.0001
Ln_Sed_Ni	0.5805828	0.135392	4.29	<.0001
Ln_Sed_Zn	0.5350138	0.09698	5.52	<.0001
Ln_Sed_Mg	0.2724009	0.086324	3.16	0.0021

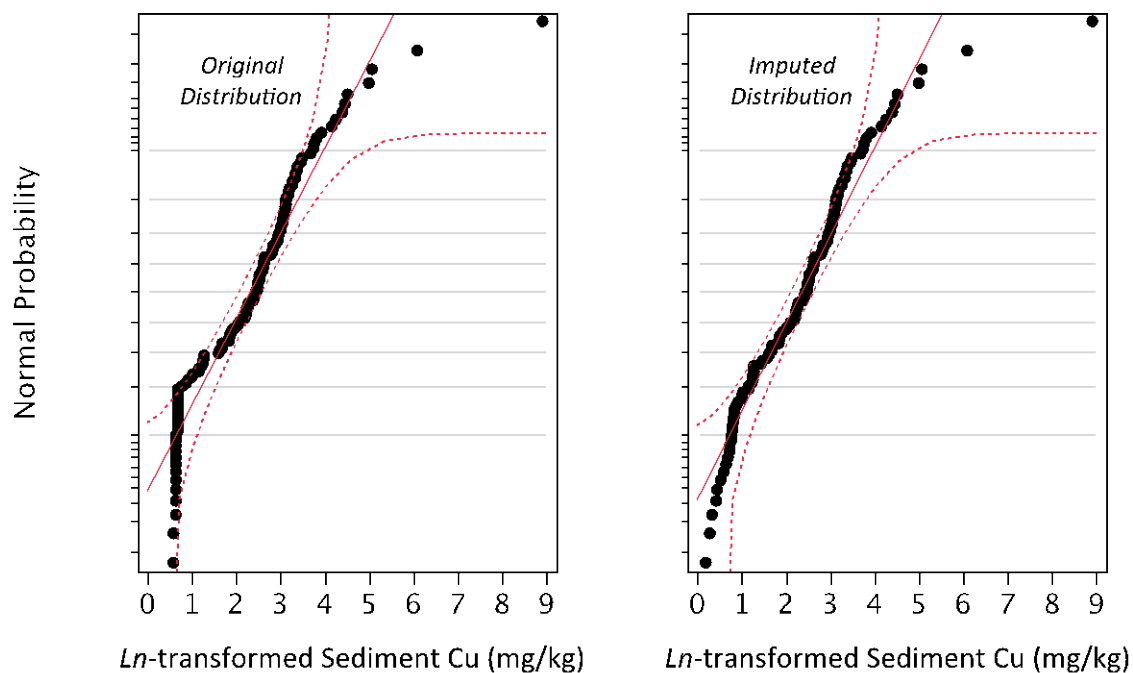


Figure 24: Normal probability plot of ln-transformed sediment Cu concentrations. Left hand panel – original data; right hand panel – distribution with imputed values for flagged observations with concentrations below the MDL.

Arsenic (As)

A forward stepwise regression model was selected to impute As sediment concentrations below the MDL because the forward stepwise regression model (which differed from the backward regression model by the inclusion of V as a variable) yielded a slight improvement in AIC and BIC. N for the analysis equals 115. Overall results of the As imputation regression are shown in Figure 25, while the overall fit statistics are given in Table 8. Figure 26 compares the distributions of the original As sediment concentrations with the distribution resulting from including imputed values for concentrations below the MDL.

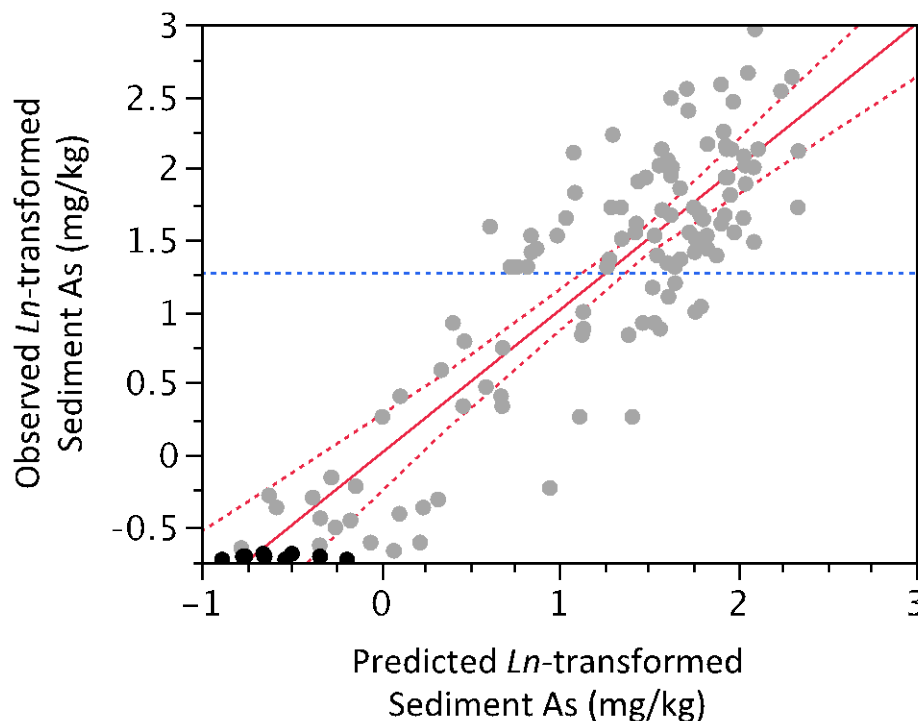


Figure 25. Model fit between actual and predicted sediment As concentrations based on MLR model obtained from forward stepwise regression. Highlighted circles are flagged observations with concentrations below the method detection limit (qualifier code containing U) that were excluded from fitting the regression model. $R^2 = 0.725$ ($p < 0.0001$). Total N of unflagged values = 115.

Table 8. Fitted coefficients, standard errors, and *t* statistics for parameter coefficient significance for the MLR model used to impute values for sediment As concentrations reported as below the MDL.

Term	Estimate	Std Error	t Ratio	Prob> t
Intercept	-2.401463	0.625571	-3.84	0.0002
Ln_Sed_Fe	0.2330226	0.072977	3.19	0.0018

Term	Estimate	Std Error	t Ratio	Prob> t
Ln_Sed_Ni	0.2920415	0.108049	2.70	0.0080
Ln_Sed_TKN	0.2299958	0.050402	4.56	<.0001
Ln_Sed_Ti	-0.247429	0.073121	-3.38	0.0010
Ln_Sed_V	0.2588059	0.115588	2.24	0.0272

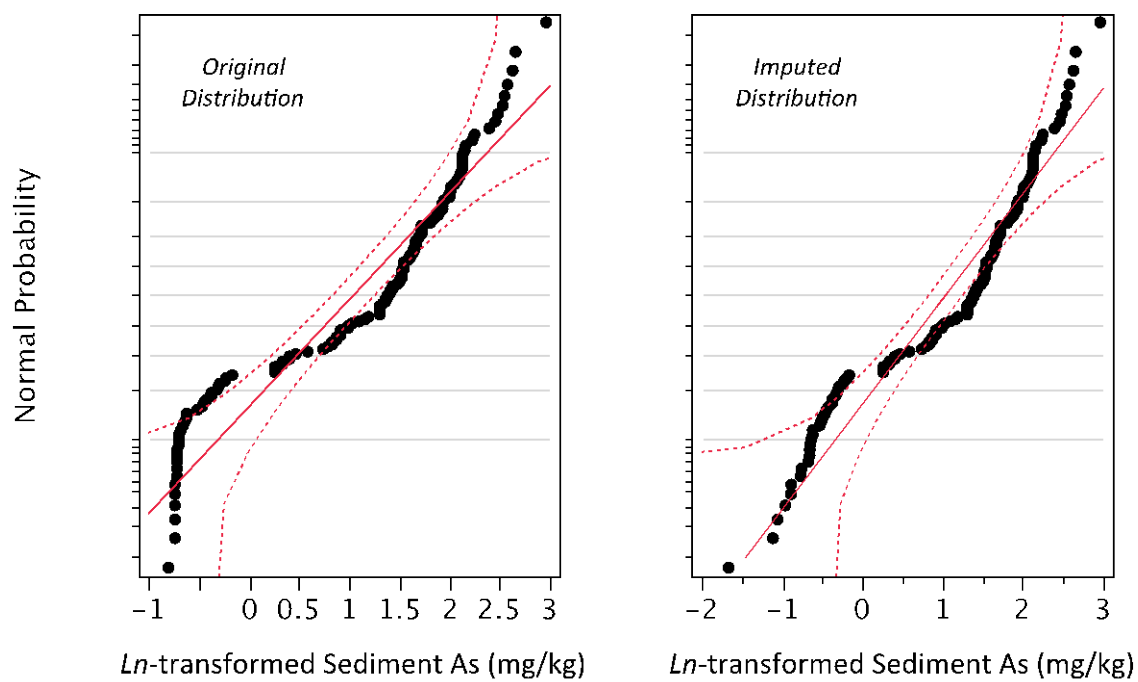


Figure 26. Normal probability plot of ln-transformed sediment As concentrations. Left hand panel – original data; right hand panel – distribution with imputed values for flagged observations with concentrations below the MDL.

Initial Sediment PCA Model

Sediment principal components model building was first conducted excluding sediment Hg as a variable to determine if the variability in sediment Hg concentrations could be explained by any of the components extracted from the PCA. A four-component model was developed which allowed for separation of disturbance into two components (Table 9). The components were extracted using a Varimax rotation, which produces independent (orthogonal) components that are uncorrelated with each other. Table 10 shows the variance and the percentage of the total variance explained by each extracted component.

Table 9. Variable loadings on extracted principal components using Varimax rotation. Only loadings with a magnitude (absolute value) greater than 0.3 are shown. *N* = 129.

Sediment Analyte	PCA 1	PCA 2	PCA 3	PCA 4
Ln_Sed_Al	0.398	0.783		
Ln_Sed_Cr		0.861	0.334	
Ln_Sed_Fe	0.484	0.771		
Ln_Sed_Ni	0.316	0.655	0.521	
Ln_Sed_Zn	0.437	0.454	0.577	0.410
Ln_Sed_TOC	0.889	0.323		
Ln_Sed_Tot C	0.872	0.316		
Ln_Sed_TKN	0.850	0.321		
Ln_Sed_TP	0.475	0.641	0.438	
Imputed Ln_Sed_Pb	0.513	0.464	0.337	0.596
Imputed Ln_Sed_Cu			0.886	
Imputed Ln_Sed_As	0.598	0.534	0.445	

Based on the various components loadings the extracted components shown in Table 9 are interpreted as follows:

- PCA 1 – Selective size sorting/sediment redistribution. Indicated by very high loadings of total and organic C, as well as TKN².

² To reiterate, the first component is interpreted to relate to selective-size sorting rather than lake trophic state because of the weak relationship previously noted between sediment carbon and nitrogen and lake trophic state. This interpretation is further supported by the increases in TOC (and N and P) typically observed in more stable depositional environments within a given lake (Hakanson and Jansson, 1983). The weathering component would be expected to contribute most prominently to large drainage lakes and also to lakes in the Florida panhandle (where, for example, the ultisol soils contain somewhat higher Fe concentrations than the entisol soils characteristic of the central Florida ridge) or where the Hawthorn formation is exposed or close to the soil surface. The selective-size sorting component is expected to vary randomly across the state. The weathering component would be expected to contribute most prominently to large drainage lakes and also to lakes in the Florida panhandle (where, for example, the ultisol soils contain somewhat higher Fe concentrations than the entisol soils characteristic of the central Florida ridge) or where

- PCA 2 – Watershed weathering. Indicated by high loadings of aluminum and iron, which are both constituents derived from weathering.
- PCA 3 – Anthropogenic disturbance/urban runoff. Indicated by high loading of copper, nickel, zinc, lead, and arsenic, coupled with high loadings of phosphorus.
- PCA 4 – Anthropogenic disturbance/direct atmospheric inputs. Indicated by high loadings of lead and zinc.

The PCA indicates that nearly 2/3 of the variance in the sediment constituents analyzed can be attributed to selective size sorting (~ 1/3) and weathering related inputs (~1/3).

Table 10. Variance, percent of total variance, and cumulative percent of total variance explained by each component extracted from the PCA summarized in Table 9.

Factor	Variance	%	Cumulative %
Factor 1	3.9166	32.639	32.639
Factor 2	3.8896	32.414	65.052
Factor 3	2.2773	18.977	84.030
Factor 4	0.8654	7.212	91.241

Principal Component Regression Model to Predict Sediment Hg

The extracted components obtained from the PCA summarized in Table 9 were used as independent variables to predict sediment Hg concentrations, using MLR as the model framework. As shown in Figure 27, the resultant model predicts sediment Hg rather well, accounting for 86% of the variance in sediment Hg concentrations (Table 11; $r^2 = 0.858$, $p < 0.0001$; $N = 129$). Standardized β coefficients, which reflect the change in ($\ln$ -transformed) Hg concentrations in response to a change in the respective independent variable by one standard deviation, indicate that, consistent with the relationship between sediment Hg and TOC shown in Figure 19, variations in selective size sorting or the dynamics of sediment erosion and net settling exert the greatest degree of influence on sediment Hg concentrations. The contributions from both sediment disturbance components are essentially equivalent.

the Hawthorn formation is exposed or close to the soil surface. The selective-size sorting component is expected to vary randomly across the state.

Table 11. Fitted coefficients (including standardized β coefficient), standard error, and t statistics for parameter coefficient significance for the calibration MLR model to predict sediment Hg (ln-transformed) concentrations. Note that the variance inflation factor (VIF) equals 1 for each component because the components by definition are orthogonal (extracted using Varimax rotation). This model uses imputed values (imputed by MLR) for sediment As, Cu, and Pb concentrations flagged as below the method detection limit (MDL).

Term	Estimate	Std Error	t Ratio	Prob> t	Std Beta	VIF
Intercept	-2.5692	0.0434	-59.2300	<.0001	0.0000	.
PCA1	0.8727	0.0435	20.0400	<.0001	0.6781	1
PCA2	0.5923	0.0435	13.6000	<.0001	0.4602	1
PCA3	0.3885	0.0435	8.9200	<.0001	0.3019	1
PCA4	0.3973	0.0435	9.1300	<.0001	0.3087	1

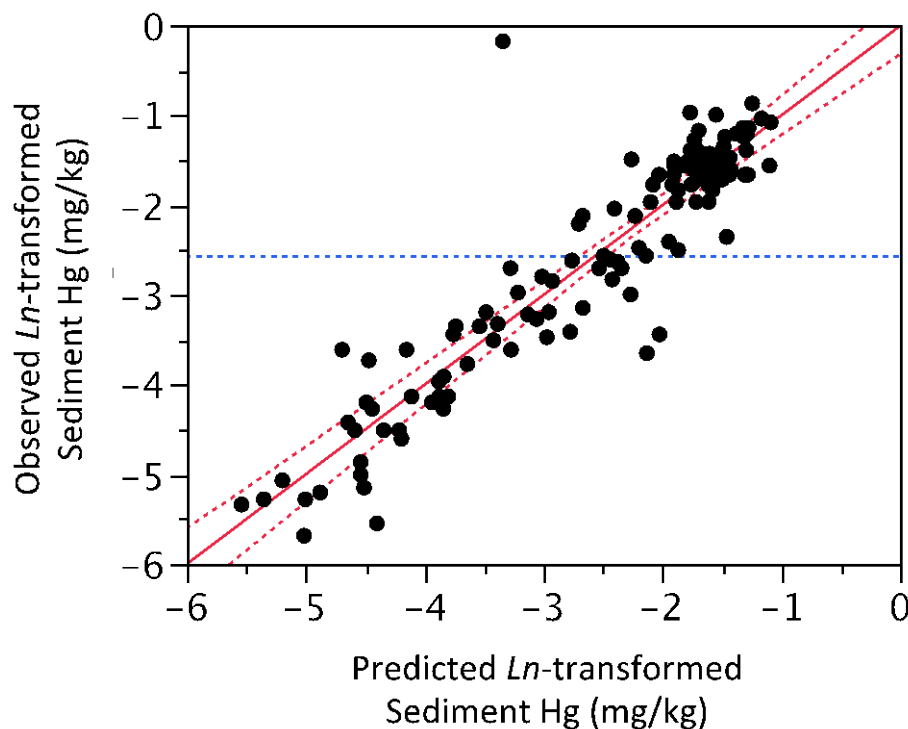


Figure 27. Initial sediment Hg model calibration using Hg TMDL study lakes.

Validation of calibrated model for predicting sediment Hg

Validation of the calibrated model for predicting sediment Hg was conducted via two independent processes. The first approach used cross-validation by conducting N different regressions using the four extracted principal components listed in Table 9 as independent variables, and $\ln$ -transformed sediment Hg as the dependent variable. Each of the N simulations excluded a single observation from constructing the regression model. The resultant model was then used to predict sediment Hg concentration for the withheld observation. This process was repeated for each observation in the calibration data set (hence the N simulations), and the root mean square error (RMSE) was computed for the N simulations. This statistic is also known as the PRESS (predicted residual sum of squares) statistic. The PRESS RMSE for the model was 0.504, which agrees quite well with the calibration data set RMSE (0.493). In other words, when applied to “out-of-sample” observations, the overall predictive error for the model is nearly as good as the overall error in the predictions for the calibration data set.

The second approach takes advantage of existing data collected as part of the FDEP Status and Monitoring Network (SMN). As mentioned previously (Section 5.1), the selection of sediment variables for inclusion in building the initial PCA model considered whether the variable was also included as a routine sediment variable monitored as part of the SMN. The data set obtained from FDEP of 747 observations was initially screened to remove flagged values (qualifier codes J, Y, and Q)³, yielding a set of 619

³ FDEP applies J qualifier codes to designate estimated samples. The flag may be applied for a variety of reasons, including failure of the sample to meet quality control criteria, or improper field or laboratory

observations. The principal component regression model presented in Section 5.1.2.1 was used to predict sediment Hg in the SMN data set (Figure 28), which resulted in “out-of-sample” predictions for a total of 618 observations (619 observations not flagged with qualifier codes J, Y, and Q, and 1 of the 619 observations with incomplete data). The root mean square error (RMSE) for the validation data set is 0.489, which is virtually identical to the RMSE of the calibration data set (0.493). The actual mean error is 0.015, which is statistically indistinguishable from 0 (t test: $p = 0.4364$).

A test of the slope between the observed and predicted values in the SMN validation set suggests that slope is slightly less than 1 (slope = 0.967; 95% confidence interval = 0.954 to 0.980). Because the SMN sediment data used in this validation exercise were collected between 2004 and 2006, and the TMDL sediment data were collected between 2009 and 2010, an appreciable deviation of the slope from unity would suggest that the Hg signal in the sediments had changed over time. The fact that the actual mean error is essentially zero, and the slope is 0.967 suggests that, all other factors being equal, the sediment signal for Hg has changed only slightly since 2004. These results are consistent with the idea that the study lakes are at steady-state with respect to Hg. However, because the sediments sampled represent an integrated signal over a number of years (surficial sediments were collected using a petit Ponar dredge, which composites surficial sediment over several cm depth or more, compared to annual net sedimentation rates often on the order of 2 mm/yr or less), the comparison is expected to be sensitive to only relatively large shifts in the signal.

protocols. Y qualifier codes are used to designate improperly preserved samples, while samples designated as Q were held beyond the accepted holding time.

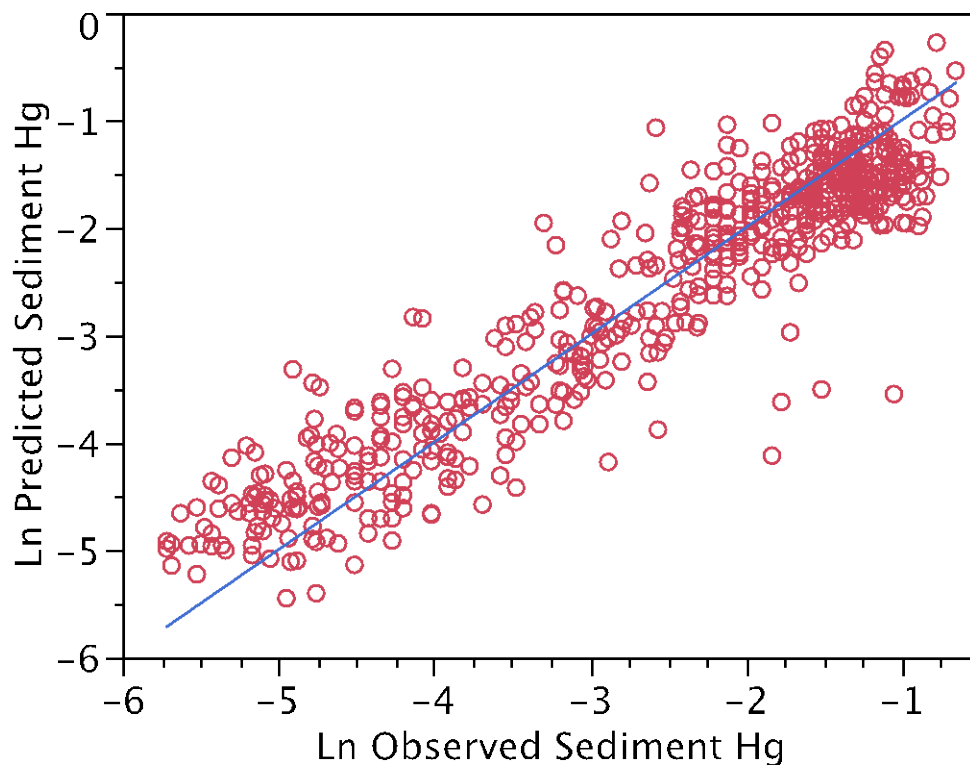


Figure 28. Model validation plot showing agreement between predicted (based on the principal component regression model presented in Section 5.1.2.1) and observed sediment Hg concentrations in the screened SMN sediment chemistry data set. N = 618.

PCA Sediment Model with Hg

The PCA model was subsequently extended to include sediment Hg as one of the component variables. As before (Table 9), four components were retained, producing both nearly identical loadings for the variables common to both models (Table 12), and similar variance characteristics (Table 13). Consistent with the PCA-MLR results shown in Table 11, sediment Hg loads or correlates most highly with the selective size sorting component, followed by watershed weathering. Sediment Hg loads most weakly with the urban runoff component.

Plots of the spatial distribution of PCA scores for both sediment anthropogenic disturbance components confirm their basic interpretations. The scores for PCA4 (atmospheric inputs of Hg; see Figure 29) cluster with the generally highest values for lakes in the Florida panhandle and along the Trail Ridge (north central Florida) and Florida Ridge (central Florida). Lakes in these regions are predominantly seepage lakes, which derive the majority of their hydrologic income (and likely Hg inputs as well) from atmospheric deposition directly to the lake surfaces. High values are also observed for a cluster of lakes north of Tampa, and may reflect elevated inputs of Hg from local emissions. The highest scores for PCA3 are located in the urban regions of Tampa,

Orlando, and along the Miami, Ft. Lauderdale, and West Palm Beach corridor, which is consistent with its' interpretation reflecting urban runoff (Figure 30). Also consistent with the interpretation of PCA2 as reflecting weathering is Figure 31, which shows the cumulative distribution for the calculated component scores for lakes defined as either large or small (based on the definition used by the FDEP Status and Monitoring Network). From a hydrologic perspective, small lakes tend to include more seepage lakes while large lakes generally include more drainage lakes (Dickinson *et al.*, 1982); as a result – and, as shown in Figure 31 – large lakes should show generally higher scores for variables that reflect weathering-related inputs.

Table 12. Same as Table 9, except that sediment Hg is included as a variable in the PCA analysis. N = 129.

	Factor 1	Factor 2	Factor 3	Factor 4
Ln_Sed_Hg	0.714	0.445		0.326
Ln_Sed_Al	0.414	0.780		
Ln_Sed_Cr		0.860	0.337	
Ln_Sed_Fe	0.492	0.769		
Ln_Sed_Ni	0.331	0.652	0.523	
Ln_Sed_Zn	0.451	0.457	0.590	0.357
Ln_Sed_TOC	0.899	0.318		
Ln_Sed_TC	0.876	0.313		
Ln_Sed_TKN	0.856	0.315		
Ln_Sed_TP	0.474	0.641	0.435	
Imputed Ln_Sed_Pb	0.536	0.464	0.353	0.554
Imputed Ln_Sed_Cu			0.887	
Imputed Ln_Sed_As	0.601	0.532	0.447	

Table 13. Same as Table 10, except values presented are keyed to the analysis presented in Table 12.

Factor	Variance	%	Cumulative %
Factor 1	4.5418	34.937	34.937
Factor 2	4.0679	31.291	66.228
Factor 3	2.4042	18.494	84.722
Factor 4	0.8149	6.269	90.991

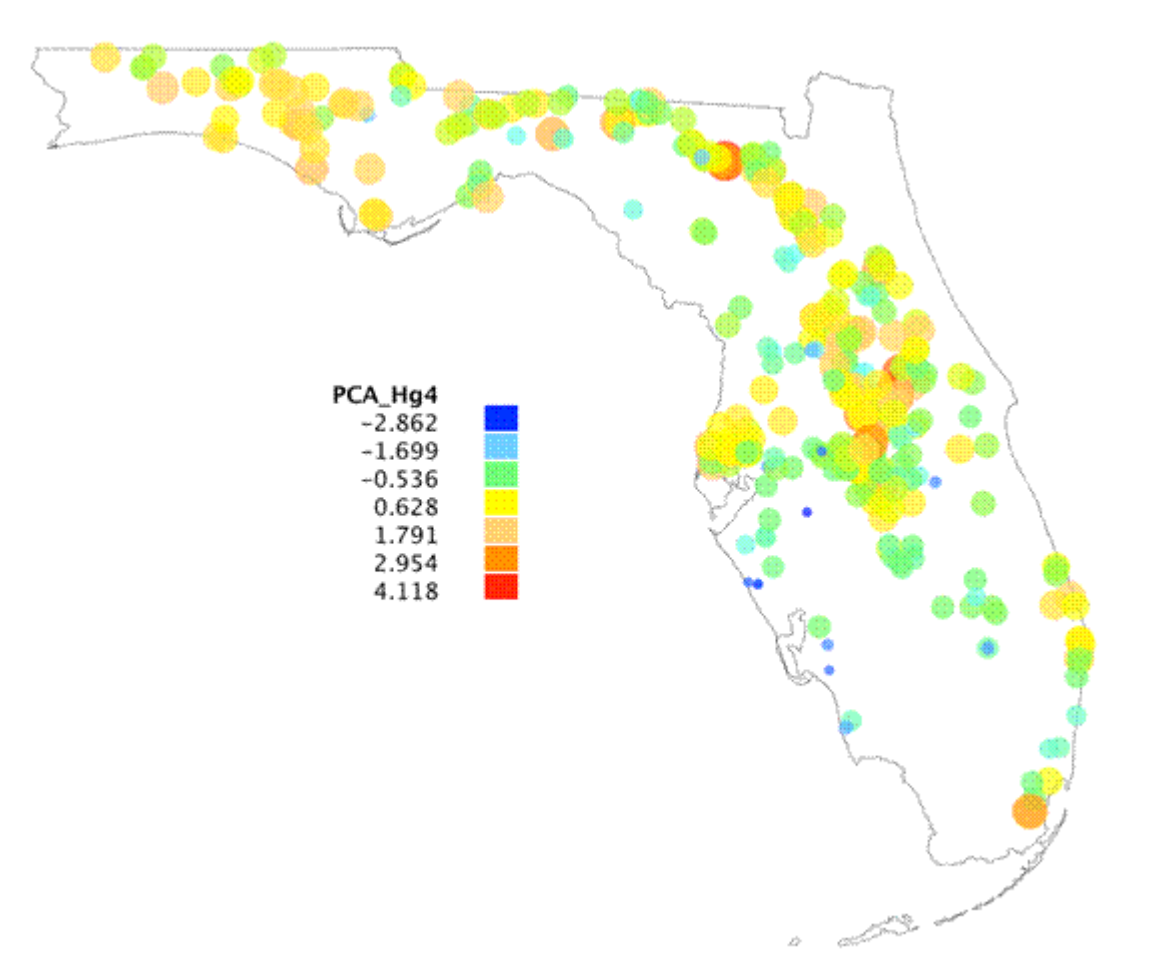


Figure 29. Spatial distribution of sediment PCA4 (anthropogenic disturbance/atmospheric deposition). See Table 12 for actual sediment variable loadings.

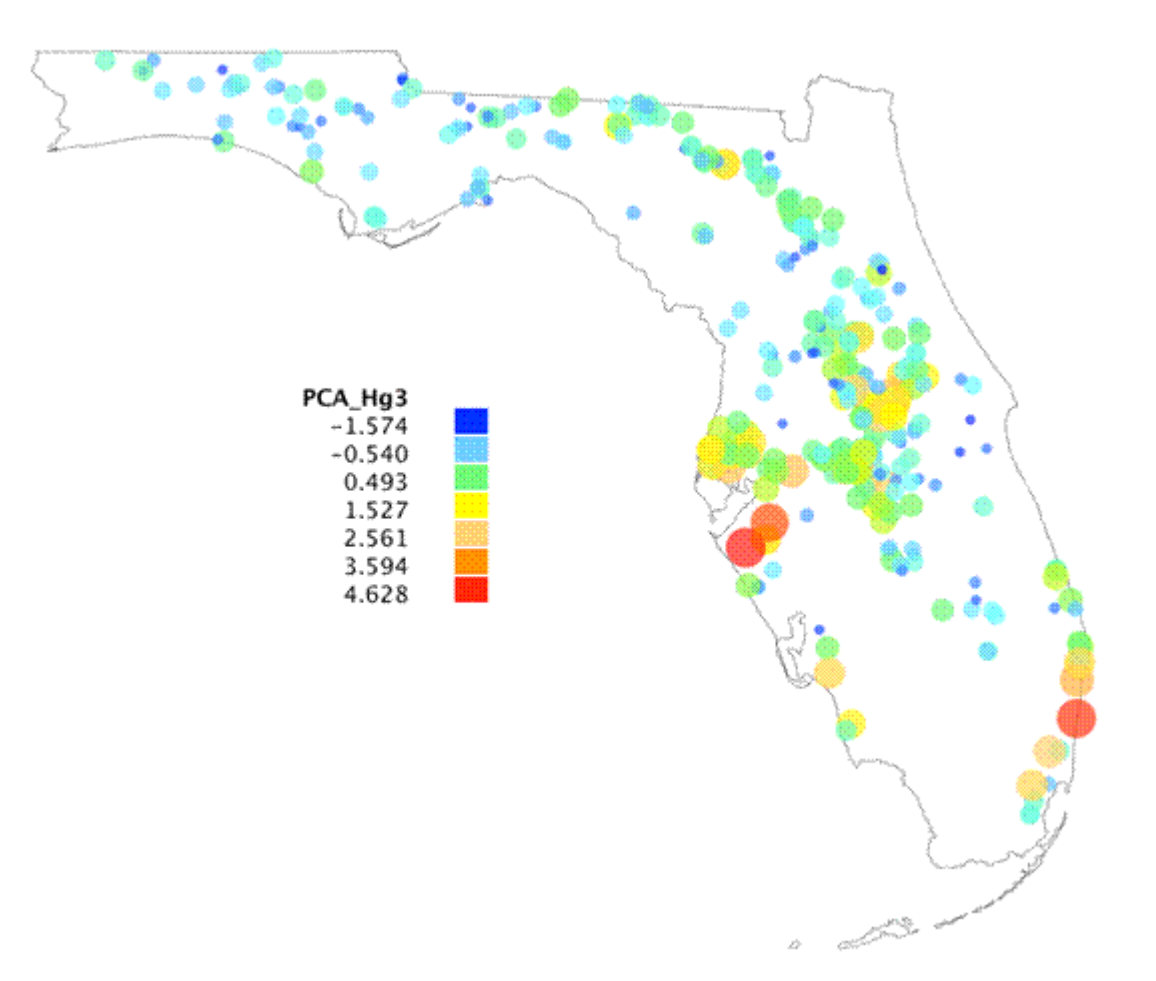


Figure 30. Same as Figure 29, except plot shows distribution of PCA3 (anthropogenic disturbance/urban runoff).

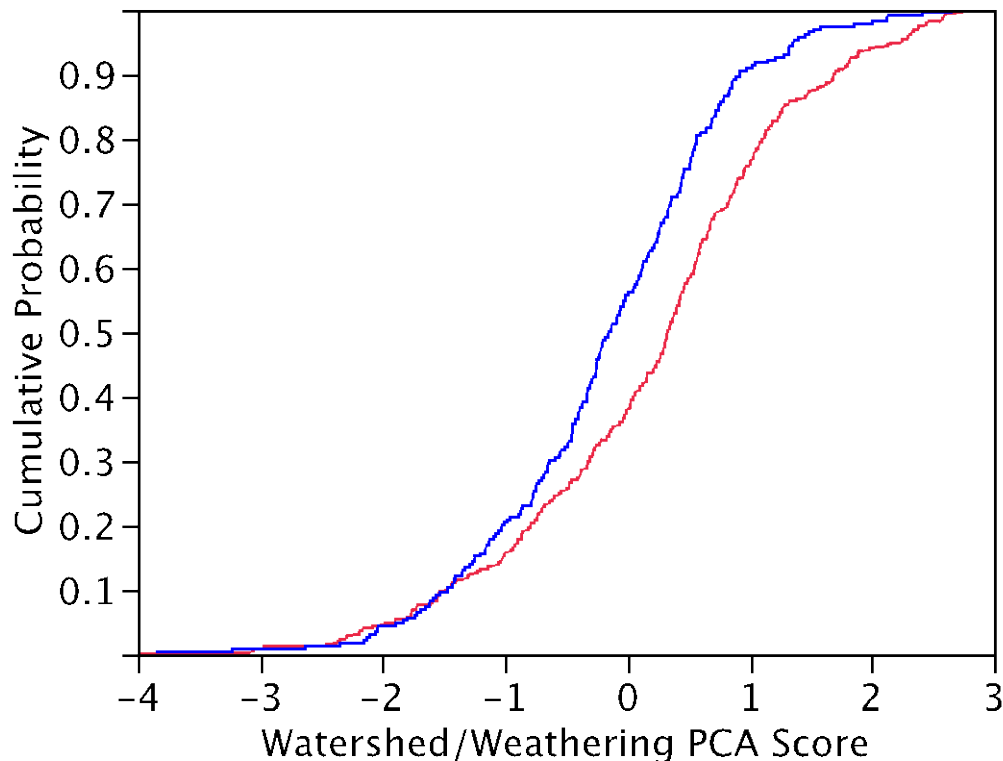


Figure 31. Cumulative probability distribution of PCA2 scores (watershed/weathering; see Table 12) for large and small SMN lakes. Red line – large lakes; blue line – small lakes.

Relationship of Sediment Principal Components with Largemouth Bass Hg Concentrations

Preliminary analyses using the Hg TMDL sediment data indicated that extracted components from principal components analysis have explanatory power with respect to LMB Hg concentrations normalized to 15". This is illustrated by regressing LMB Hg as a function of the extracted components from the PCA model presented in Table 12 and in Figure 32. Inspection of the model coefficients indicates that LMB Hg concentrations are influenced by both anthropogenic disturbance components, and by impacts from watershed weathering. As expected, both watershed weathering inputs and anthropogenic disturbance characterized as urban runoff promote lower fish tissue Hg concentrations, in all likelihood through particle dilution effects and trophic state impacts (Table 14).

The factor ascribed to atmospheric deposition impacts exerts a positive (magnifying) impact on fish tissue Hg, which would suggest that lakes receiving greater contributions from this factor in turn have higher fish tissue Hg concentrations than would otherwise be expected. The spatial distribution of values for the direct atmospheric deposition principal component suggests that high values reflect primarily the occurrence of seepage lakes and, to a lesser extent, locally elevated signals in major urban areas such as Tampa and Orlando (Figure 29). Seepage lakes receive nearly all their hydrologic income from direct atmospheric deposition, while drainage lakes receive a significant and often dominant fraction of their hydrologic income from surface runoff. Because a

large fraction of the atmospheric signal of Hg is retained in the soils of terrestrial watersheds, and because drainage lakes in Florida characteristically are both more alkaline and eutrophic, seepage lakes should be sensitive to atmospheric fluxes of Hg.

Table 14. Fitted coefficients, standard error, and *t* statistics for parameter coefficient significance for the MLR model obtained by fitting the extracted components from the PCA model presented in Table 12 to LMB Hg (*ln*-transformed) as the dependent variable.

Term	Estimate	Std Error	t Ratio	Prob> t
Intercept	-1.050579	0.0535	-19.64	<.0001*
PCA_Hg1	0.086804	0.053709	1.62	0.1086
PCA_Hg2	-0.137499	0.053709	-2.56	0.0117*
PCA_Hg3	-0.339786	0.053709	-6.33	<.0001*
PCA_Hg4	0.2973048	0.053709	5.54	<.0001*

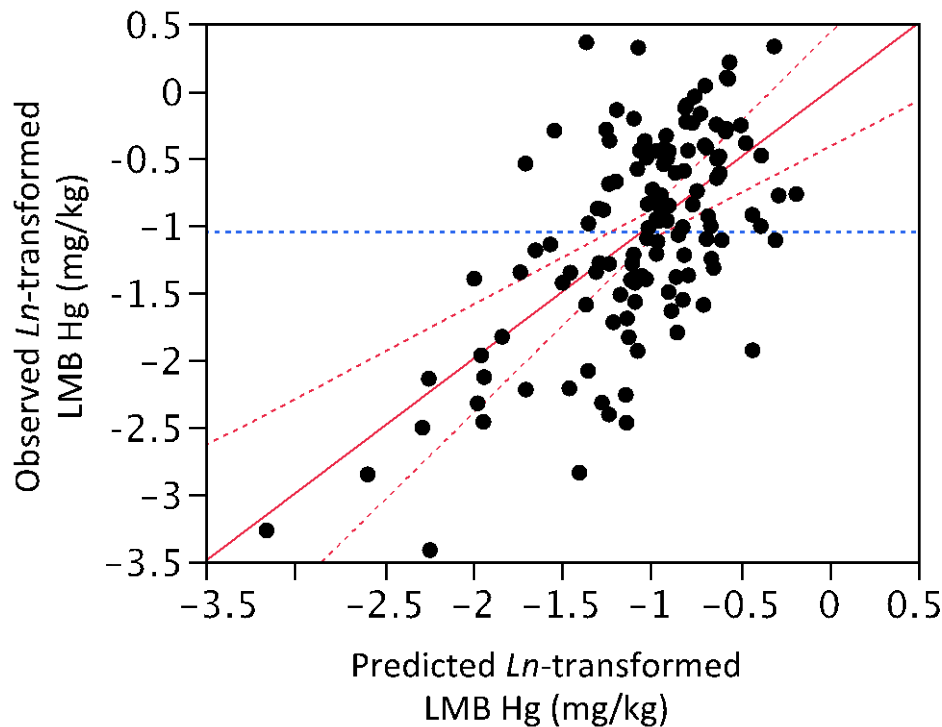


Figure 32. Observed vs. predicted *ln*-transformed LMB Hg concentrations. Predicted values based on MLR constructed from sediment PCA (including Hg) model. $R^2 = 0.39$; RMSE = 0.6076.

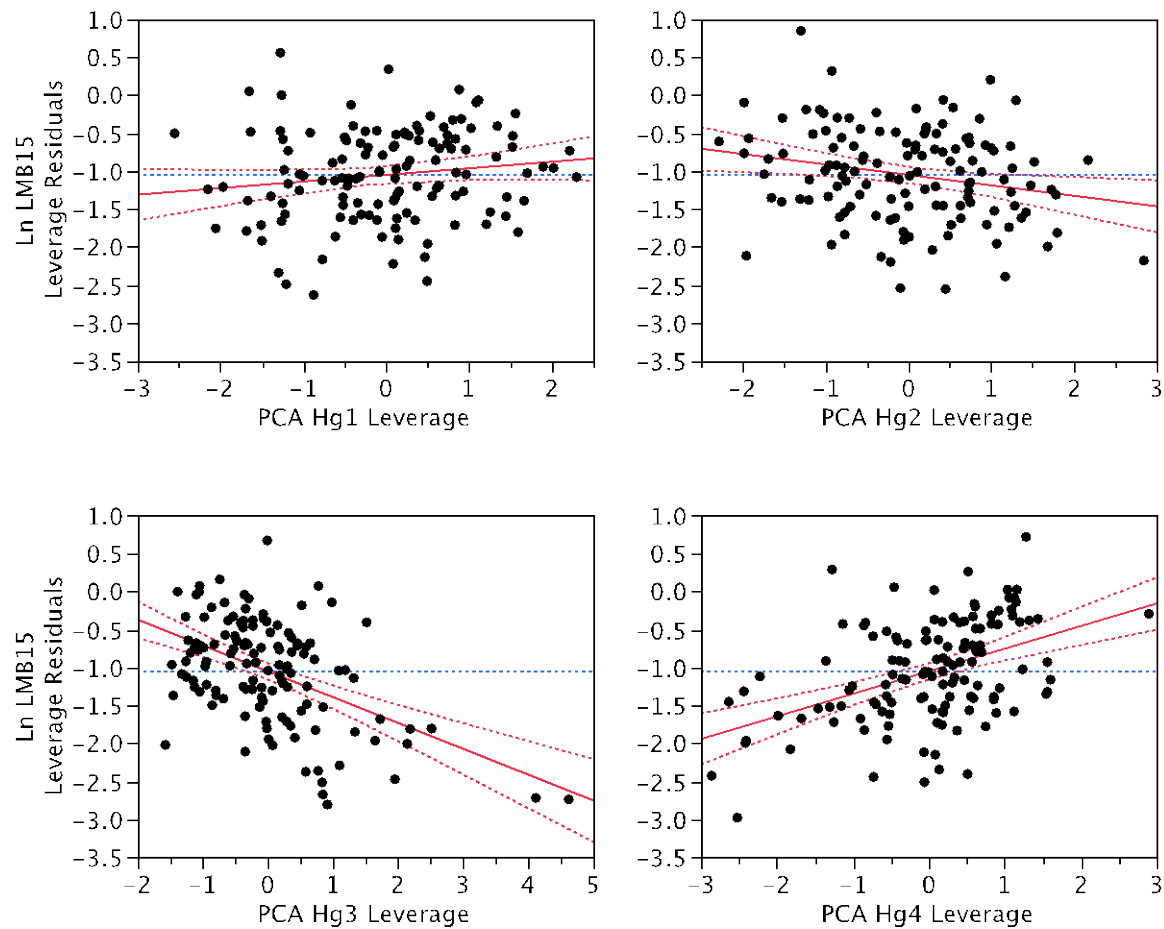


Figure 33. Individual model component leverage plot for independent variables in MLR model predicting LMB Hg concentrations (ln-transformed) as a function of sediment PCA extracted components summarized in Table 14.

Modeling Largemouth Bass Hg Concentrations in Hg TMDL Lakes

Statistical Methods

Multiple Linear Regression Model

Modeling the relationship between LMB Hg concentrations and other water quality and sediment chemistry variables initially was conducted using MLR. A schematic summarizing key aspects of the overall process used for model development, evaluation and validation is presented in Figure 34. MLR was selected initially for model development for several reasons. First, MLR allows LMB Hg concentrations to be modeled as a continuous response. This allows flexibility in choosing and modifying the TMDL target Hg concentration at a later date. Because LMB Hg concentrations can be defined as either below or above a defined target concentration, LMB Hg concentrations potentially could be modeled as a dichotomous variable via logistic regression. However, because logistic regression requires defining the threshold level in advance of the analysis, should FDEP decide to change the target level at some future point in time, the logistic regression modeling would need to be redone. In addition, using logistic regression to infer the distribution of the likelihood of exceeding target levels for lakes throughout the state after reductions in inputs of atmospherically deposited Hg is not as straightforward as using MLR. For example, with MLR (and other approaches such as neural network modeling that predict Hg concentrations as a continuous variable), the model can be used to initially predict the concentration of LMB Hg for lakes lacking measured LMB Hg concentrations but with appropriate water chemistry data. By invoking the joint assumptions of current steady state conditions and a long-term linear response to changes in Hg inputs, the fish tissue concentration response to changes in Hg inputs is thus equivalent to the fractional reduction of the deposition flux. With logistic regression, the perhaps best approach to predicting changes in the statewide distribution of lakes to changes in atmospheric inputs would be to first reduce the mercury concentrations in LMB Hg concentrations in the model calibration data set in a linear fashion, and then refit the logistic regression model to the revised target classification (dichotomous) variable. Evaluating different management scenarios thus would require an equivalent number of model recalibrations, including model evaluation, diagnostics and validation.

Secondly, MLR is a popular statistical technique that is used broadly across a number of disciplines (Tabachnick and Fidell, 2007), including limnological studies examining the relationship between watershed, water quality, and/or biotic variables with fish biota Hg concentrations (e.g., Lathrop *et al.*, 1991; Dittmann and Driscoll, 2009; Shanley *et al.*, 2005), including a study of 53 Florida lakes conducted by Lange *et al.* (1993). MLR assumes that a linear relationship exists between the modeled and the independent variables, which may require transformations either of the dependent variable, some or all of the independent variables, or both to reasonably approximate linearity. MLR is sensitive to the choice of independent variables and, in part because correlation between independent variables can lead to nonsensical model dependencies, the ideal solution to parameter choice should be guided by minimizing the number of uncorrelated independent variables included in the model (*cf.* Tabachnick and Fidell, 2007). MLR is well developed, and there are a large suite of diagnostic tools that has been developed and incorporated into commercial statistics packages such as the two used in this study (Stata and JMP; StataCorp, 2012 and SAS Institute Inc., 2011 respectively) to evaluate model robustness and conduct validation exercises.

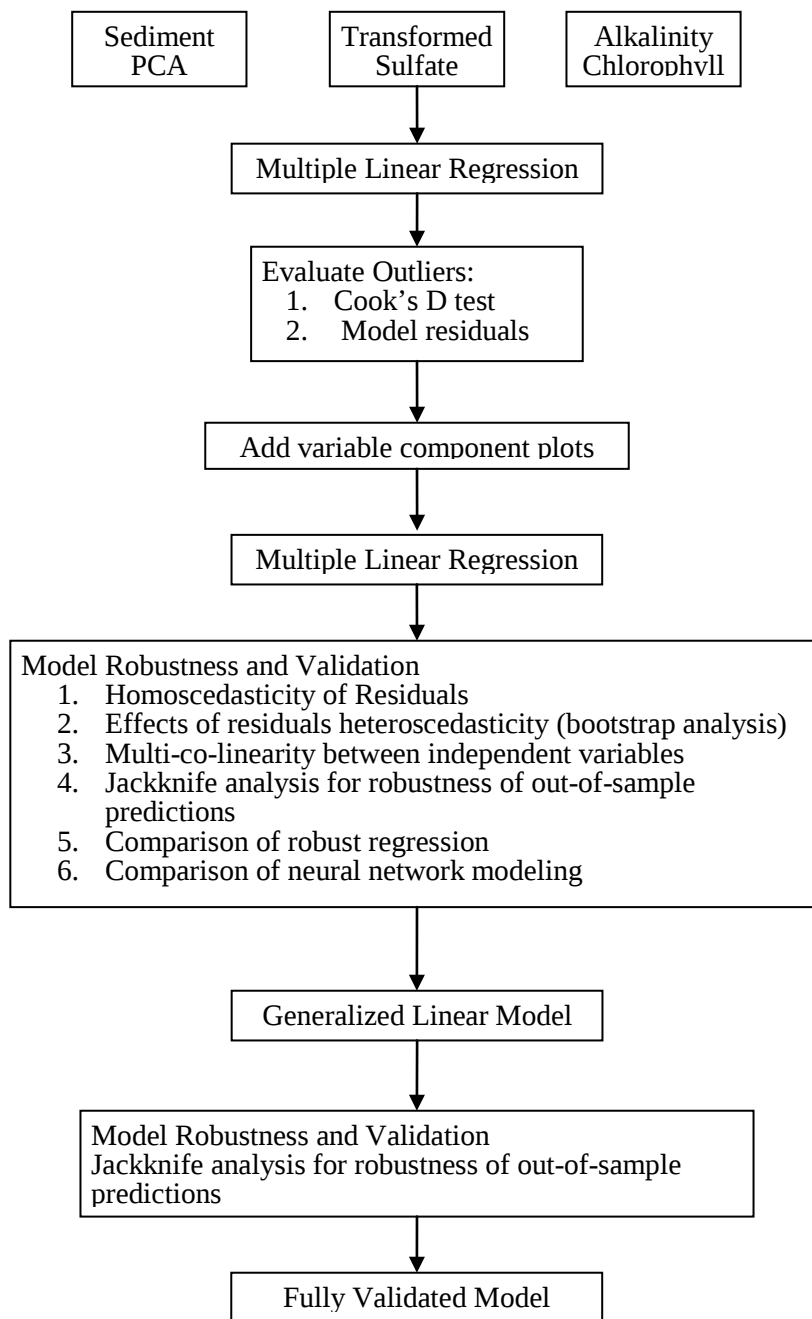


Figure 34. Schematic diagram depicting construction predictive model for LMB Hg concentrations in Hg TMDL lakes.

Artificial Neural Network Model

The essential linearity of the independent variable relationships with LMB Hg was further verified by conducting artificial neural network (ANN) modeling. ANN modeling is an approach that has its roots in simulating biological neural networks, and is capable of handling linear and non-linear relationships. It has been used in a number of environmental applications, including modeling water quality in streams (Schleiter *et al.*, 1999) and coastal ecosystems (Palani *et al.*, 2008), and phytoplankton dynamics in lakes (Talib *et al.*, 2007). An ANN is a set of nonlinear equations that are used to predict a response variable given a set of input variables. Underlying the ANN is a series of layers, including an input layer, an output layer, and a “hidden” layer that consists of a series of nodes that link the two other layers. The implementation of ANN in JMP (SAS, 2011), which is the software package used to conduct the ANN analyses, uses a single hidden layer for which the number of nodes can be specified. Each node in the hidden layer uses a so-called “activation function” – characteristically a sigmoidal type relationship to map the relationship between the input and the output layers. Because the method is non-linear and because a large number of nodes in the hidden layer can be specified, overfitting the ANN is a primary concern, and emphasis on model validation is critical.

Generalized Linear Model

An important aspect of the MLR modeling was transforming LMB Hg concentrations by using a logarithmic transformation to optimize the linearity between the dependent and independent variables and improve the distribution of the model residuals. Implementing such a transformation requires in turn back-transforming predicted $\ln$ LMB Hg values to untransformed values in order to construct cumulative frequency distribution (*cdf*) curves necessary for the actual TMDL assessment. If, however, the errors in the original regression are not incorporated in the calculation, the back-transformation imposes a bias in the predicted results (Newman, 1993). Generalized linear modeling (GLM) allows direct modeling of untransformed LMB Hg concentrations using a link function; as a result back-transformation is not necessary and the associated problem of bias is avoided. GLM modeling was thus conducted of LMB Hg concentrations using the same suite of independent variables implemented in the MLR modeling.

Data Screening

The TMDL lake chemistry data were initially screened to identify samples that may include potential analytical artifacts (e.g., concentrations for key variables that appear inconsistent with values observed for other variable, thus indicating the possibility of analytical problems) by examining a series of bivariate relationships, including:

1. An evaluation of the cation-anion balance (Figure 35). If all the major species contributing to the cationic and anionic composition of the sample have been analyzed, and if the analyses are accurate, the sum of the cation concentrations should balance the sum of the anion concentrations if the concentrations are expressed on an equivalent basis;
2. A comparison of calculated conductivities based on measured major ion concentrations and their associated equivalent conductance values with measured specific conductance values (Figure 36). Assuming that all the major cationic and anionic components have been analyzed, this comparison is a useful test in determining the overall integrity of the reported values for major ion chemistry.
3. Comparing measured sodium (Na) and chloride (Cl) concentrations (Figure 37). This relationship characteristically follows that of marine aerosols, although deviations of higher Na concentrations are not unusual in Florida; and
4. Analyzing the relationship between alkalinity (including the effects of the acid anions, dissolved organic carbon (DOC) and SO₄), the two primary cations contributing to carbonate alkalinity, calcium (Ca) and magnesium (Mg) (Figure 38). From a data quality perspective, sums of the equivalent concentrations of alkalinity + DOC + SO₄ should match and not exceed the sum of Ca + Mg in systems where carbonate weathering is the primary source of alkalinity, and the primary sources of sulfate are marine aerosols and atmospheric deposition.

The bivariate plot that compares the sum of the anions with the sum of the cations suggests three potential outliers (Figure 35; sum of anions clearly in excess of the sum of cations) – Riggins, T-Pit, and Basin Bayou lakes. Because the measured and predicted conductivities closely match (Figure 36), and because the computation of predicted conductivity does not include DOC (there are no published equivalent conductance values for DOC, and the contribution to conductivity is generally low [Epstein, 1988]), these results suggest that the reported DOC concentrations for these three lakes may be high. Inspection of the plot for the sum of alkalinity + DOC + SO₄ as a function of Ca + Mg (Figure 38) provides further evidence that the DOC concentrations of Riggins and T-Pit lakes are erroneously high (the deviation of Basin Bayou above the expected line is not problematic because the deviation is likely due to high inputs of NaSO₄ – see also Figure 37). As a result Riggins and T-Pit lakes were flagged as potential outliers.

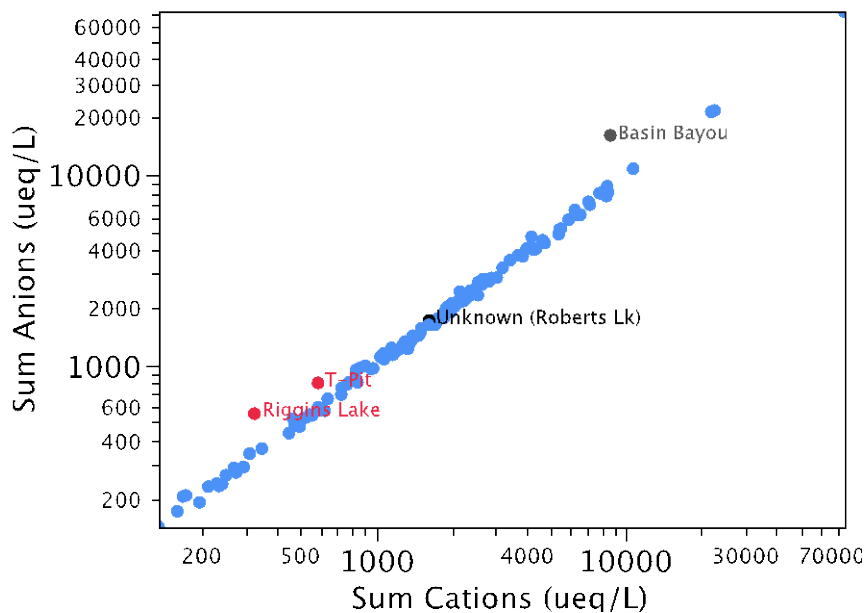


Figure 35. Plot of the sum of anions vs. sum of cations in Hg TMDL lakes. Concentrations of each ionic constituent is first expressed in microequivalents per liter before computing the sum of cations or anions.

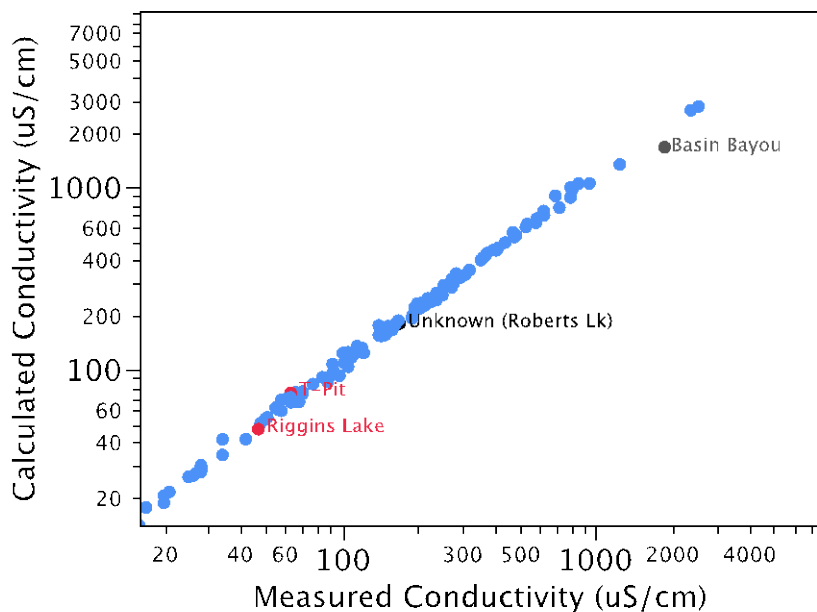


Figure 36. Plot of the predicted conductivity (based on equivalent conductances of individual ionic constituents) vs. measured conductivity in Hg TMDL lakes.

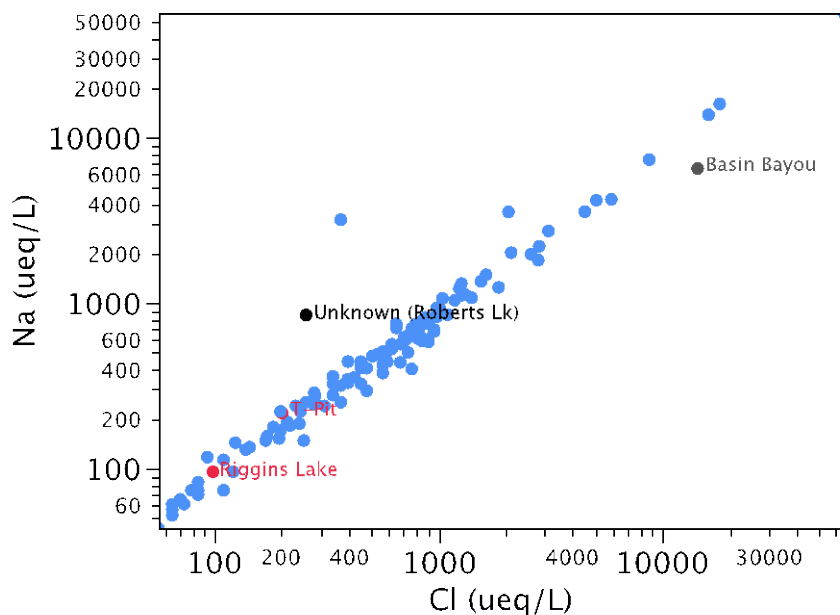


Figure 37. Plot of Na vs. Cl concentrations in Hg TMDL lakes.

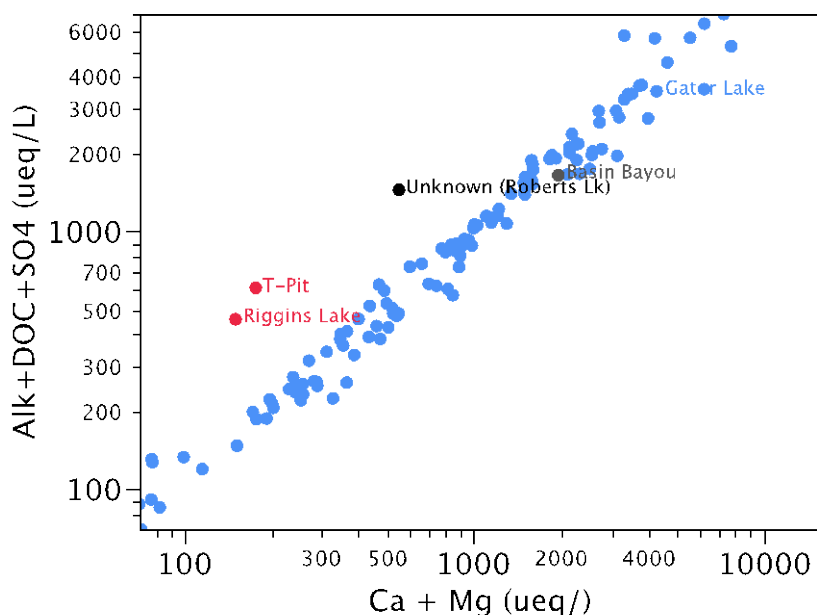


Figure 38. Plot of alkalinity plus DOC, SO₄ and OH⁻ concentrations vs. Ca plus Mg concentrations.

Multiple Linear Regression Modeling Results

Initial model testing indicated that a MLR model for LMB Hg concentrations in the Hg TMDL lakes that included chlorophyll *a* and alkalinity (both *ln*-transformed), cubic spline transformed sulfate to represent methylation potential (Section 4.1), and the two anthropogenic disturbance components obtained from the PCA model of sediment metals and nutrient-related parameters yielded promising results. The coefficient of determination for the initial model was 0.536, with a RMSE of 0.533 (Figure 39). The PRESS RMSE was 0.549.

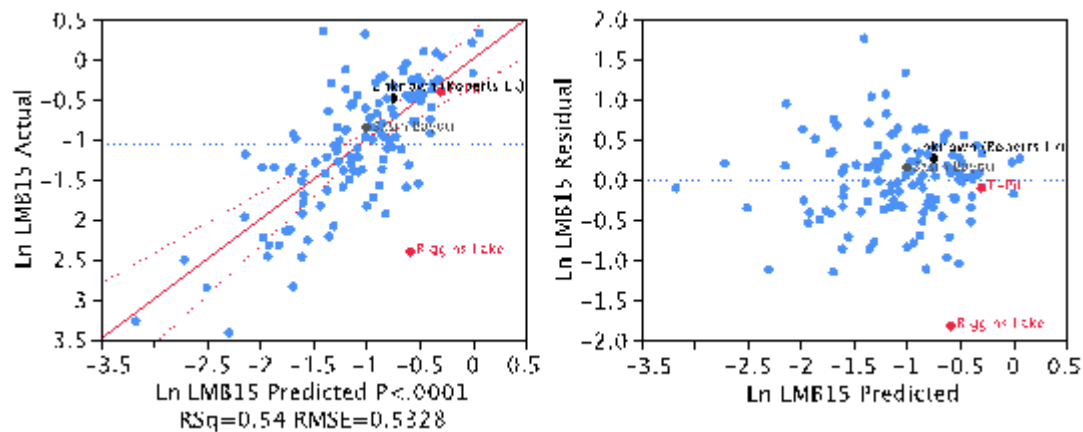


Figure 39. Preliminary multivariate model fit results. Left hand panel – observed vs. predicted results. Right hand panel – model residuals vs. predicted results. N = 129.

Table 15. Fitted coefficients, standard error, and *t* statistics for parameter coefficient significance for the preliminary MLR model to predict LMB Hg (*ln*-transformed) concentrations. See Figure 39 for a comparison between observed and predicted concentrations.

Model variable	Estimate	Std Error	t Ratio	Prob> t
Intercept	0.7047267	0.311371	2.26	0.0254
PCA Hg3	-0.255325	0.048962	-5.21	<.0001
PCA Hg4	0.1484302	0.052050	2.85	0.0051
<i>Ln</i> Chlorophyll <i>a</i>	-0.132466	0.042655	-3.11	0.0024
<i>Ln</i> Alkalinity (µeq/L)	-0.125553	0.029502	-4.26	<.0001
Sulfate methylation potential	0.9400847	0.336523	2.79	0.0060

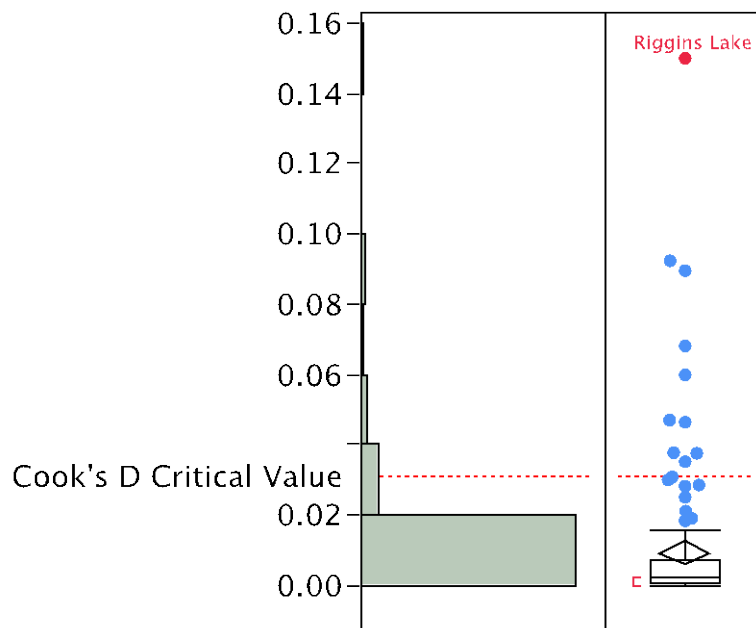


Figure 40. Distribution of Cook's D influence statistic for preliminary model fit shown in Figure 39.

Inspection of the distribution of calculated values for the Cook's D influence statistic, is a measure of the relative influence a given observation influences the regression model as a whole (Hamilton, 2009), indicates that Riggins Lake is a clear outlier (Figure 40). This fact, coupled with the fact that Riggins Lake also may be an outlier from an analytical perspective (Section 5.2.2), resulted in deleting this observation from further model analysis. Refitting the same MLR model without including Riggins Lake resulted in further improvement of the model, both with respect to the coefficient of determination (Figure 41) and the PRESS RMSE ($r^2 = 0.57$ compared to 0.54; PRESS RMSE = 0.522 compared to 0.549).

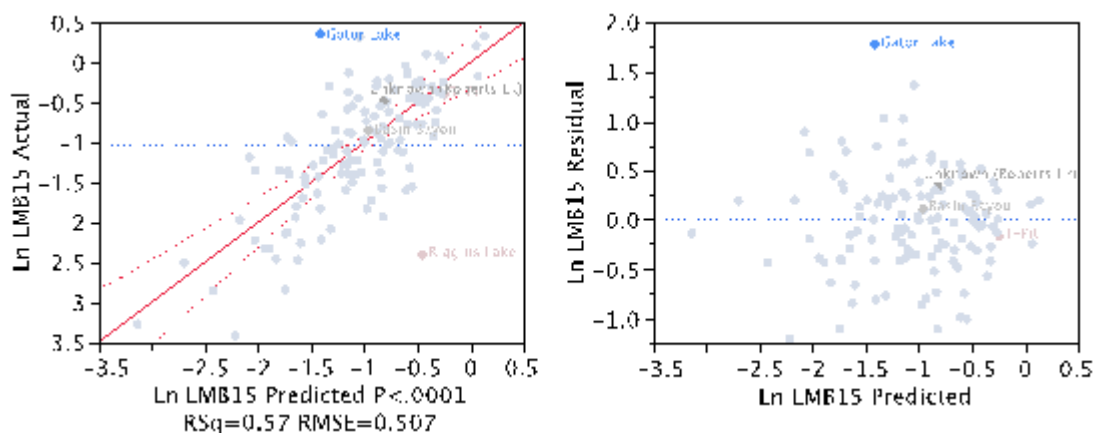


Figure 41. Same as Figure 39, except that Riggins Lake has been excluded from the analysis. PRESS statistic = 0.522. N = 128.

Both the plot of the model fit, and the plot of model residuals as a function of predicted (*ln*-transformed) LMB Hg concentrations, suggest that Gator Lake also is an outlier (Figure 41), although it is not obvious that there are analytical difficulties that are contributory. Excluding Gator Lake results in further improvement in the model (Figure 42, $r^2 = 0.61$, RMSE = 0.482, PRESS RMSE = 0.496). Analysis of the distribution of model residuals from the fitted model indicates residuals are normally distributed (Shapiro-Wilks test $p = 0.8294$; Figure 43).

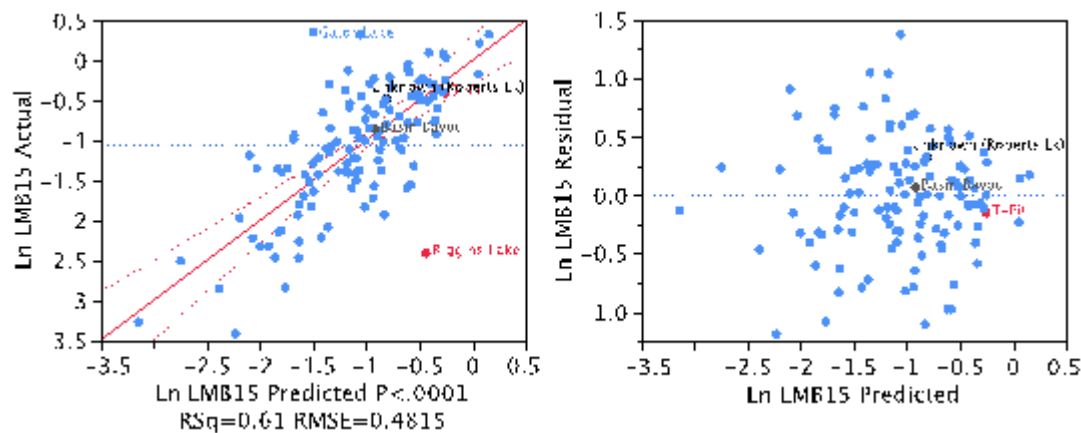


Figure 42. Same as Figure 41, except that Gator Lake has been removed. PRESS statistic = 0.496. N = 127.

Table 16. Fitted coefficients, standard error, and *t* statistics for parameter coefficient significance for the preliminary MLR model to predict LMB Hg (*ln*-transformed) concentrations with Riggins and Gator Lakes removed as outliers. See Figure 42 for a comparison between observed and predicted concentrations.

Model variable	Estimate	Std Error	t Ratio	Prob> t
Intercept	0.6647129	0.282356	2.35	0.0202
PCA Hg3	-0.258281	0.044249	-5.84	<.0001
PCA Hg4	0.1443104	0.047586	3.03	0.0030
<i>Ln</i> Chlorophyll <i>a</i>	-0.146803	0.038886	-3.78	0.0002
<i>Ln</i> Alkalinity (μeq/L)	-0.152702	0.027134	-5.63	<.0001
Sulfate methylation potential	0.6736446	0.308052	2.19	0.0307

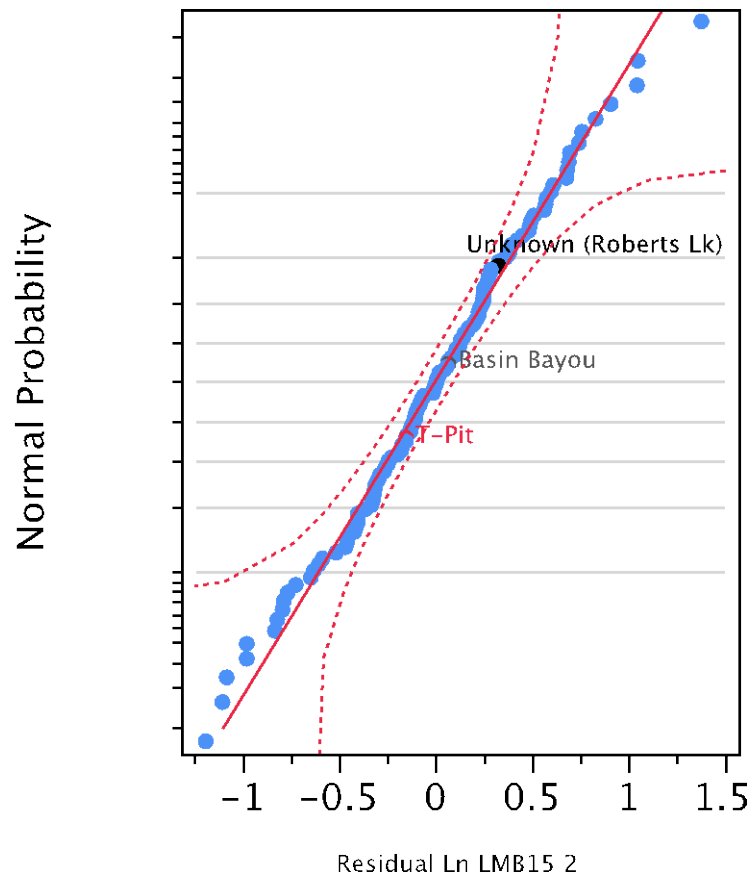


Figure 43. Analysis of distribution of model residuals from fitted model shown in Figure 42. Shapiro-Wilks test indicates residuals are normally distributed ($p = 0.8294$).

Multiple Linear Regression Model Validation and Robustness

Model validation and robustness were evaluated through a series of different analyses. The Ramsey Regression Equation Specification Error Test (RESET; StataCorp, 2011) test of independent variables is a test for model specification errors, including omitted variables, or the incorrect functional form of the variables. The RESET test is implemented by testing whether non-linear combinations of the fitted values, or alternatively, independent variables help explain the dependent variable; if shown to be the case, the model is considered *mis-specified*. Results from the RESET test for the independent variables indicate that the model is well-specified ($p = 0.4387$ and 0.7099 for fitted value and independent variable tests, respectively).

An important consideration in regression analysis is whether the independent variables are indeed truly independent. When multicollinearity occurs among the independent variables, the fitted parameter coefficients can become unreliable, and their interpretation suspect. The severity of multicollinearity can be evaluated computing the variance inflation factor (VIF) for each of the independent variables. The inverse of the VIF indicates how much the variance of the variable of interest is independent of the other independent variables in the model; as a result values can range upwards from a

minimum value of 1 (the variable is completely independent). Opinions vary regarding threshold values for problematic VIF; for example, Hamilton (2009) cites a value of 10 based on Chatterjee *et al.* (2000) and Haan (2002) notes that some researchers use values of 5. Computing the VIF for the independent variables used in the MLR model indicates that multicollinearity is essentially absent (VIF values all ≤ 1.27).

An underlying assumption in ordinary least squares regression that the variance of the model residuals is constant (*i.e.*, the residuals are homoskedastic) was evaluated by formally testing for the presence of heteroskedascity (*i.e.*, the residuals are *not* homoscedastic). Two procedures were conducted to evaluate heteroskedascity: the Cook-Weisberg test (Stata procedure *hettest*), the results from which indicate that heteroskedascity is present ($p = 0.0269$); and White's generalized test for heteroskedascity (Stata procedure *imtest*, $p = 0.1762$), which suggests that heteroskedascity is not present. Plotting the model residuals against the independent variables in the model suggest that problems with heteroskedascity likely lie with the variable *PCA Hg3* and the clustering of two site with high *PCA Hg3* scores above 4; in contrast, the scores for the remaining sites were all below 3 (Figure 44).

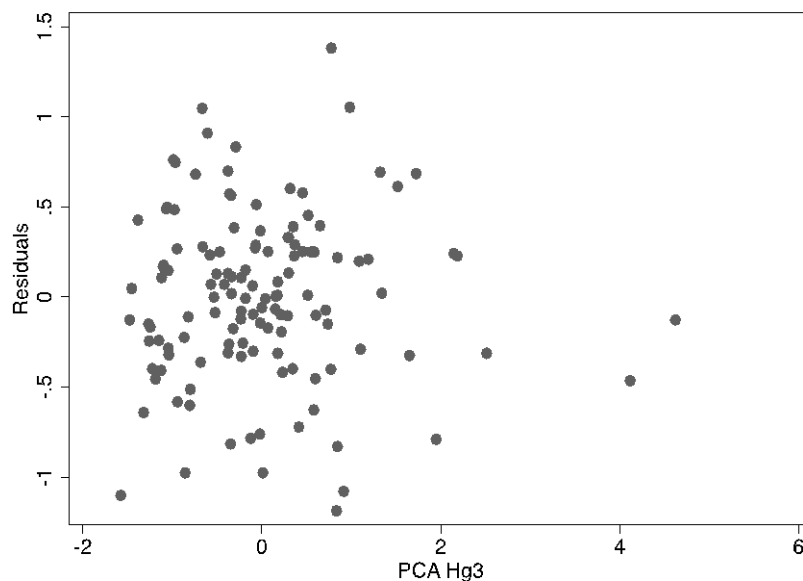


Figure 44. Plot of model residuals against independent variable *PCA_Hg3*.

To test the possible effects of heteroskedascity on the model coefficients, robust estimates of variance (and significance of the coefficients) were obtained by conducting a bootstrap analysis with 1,000 replications. Results, which are shown in Table 17, indicate that heteroskedascity does not particularly affect the significance of the model coefficients.

Further examination of the robustness of the MLR model was conducted by comparing the predicted results obtained from the ordinary least squares regression model (with the two outliers excluded) with predicted results obtained from robust regression indicates that the two model approaches yield virtually identical results, and further demonstrates that no additional observations are exerting undue leverage on the OLS model (Figure 45 and Table 18).

Table 17. Comparison of model coefficient standard errors obtained normally from linear regression (N observations = 127) with robust estimate of standard error using a bootstrap analysis (N replications = 1,000).

Model variable	Model coefficient	Coefficient Std. Error		$p > t $	
		Regular	Bootstrapped	Regular	Bootstrapped
<i>Ln</i> chlorophyll <i>a</i>	-0.1468033	0.0388857	0.042365	0.000	0.001
<i>Ln</i> alkalinity	-0.1527018	0.0271344	0.025639	0.000	0.000
PCA Hg3	-0.2582815	0.0442495	0.0504117	0.000	0.000
PCA Hg4	0.1443104	0.0475855	0.0502844	0.003	0.004
SO4 methylation potential	0.6736446	0.3080517	0.3432258	0.031	0.05
Intercept	0.6647129	0.2823564	0.296759	0.02	0.025

Model validation was performed through comparing the RMSE obtained from the MLR model fitted with the full ($N=127$) data set with the RMSE obtained from a jackknife analysis of the same model conducted by withholding a single observation and predicting the *ln-transformed* LMB Hg concentration for the out-of-sample observation (*i.e.*, the PRESS statistic). The PRESS RMSE is 0.496, which compares quite favorably with the RMSE of the model fitted with the full set of observations (RMSE = 0.482). Model validation was also performed by conducting a variant of the five-fold cross validation analysis. In this analysis 20% of the samples are withheld at random from the model calibration, and the withheld observations are used to validate the calibration model predictions. This process is repeated 1,000 times and the RMSE values for the out-of-sample predictions are compiled from each simulation. The average RMSE (0.492) across the 1,000 simulations also agrees quite well with the PRESS statistic.

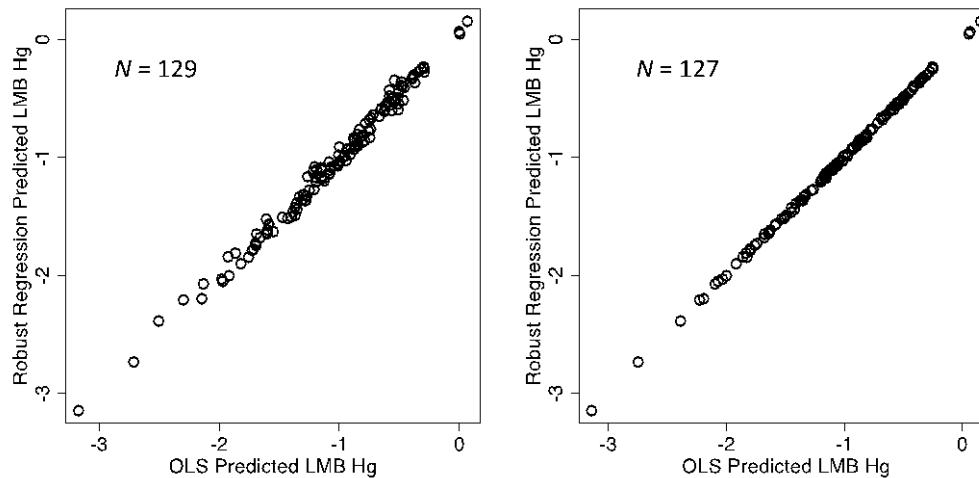


Figure 45. Comparison of predicted values obtained from OLS with results obtained from robust regression. Left hand panel – N = 129 observations for both regression models; right hand panel – outliers removed (N =127) for the OLS regression, while the robust regression was conducted using the full suite of observations (N = 129).

Table 18. Comparison of model coefficients obtained from OLS with outliers removed (N =127) with coefficients obtained from robust regression using the full suite of observations (N = 129).

Model Parameter	Model Coefficient	
	OLS	Robust Regression
<i>Ln chlorophyll a</i>	-0.1468033	-0.1398124
<i>Ln alkalinity</i>	-0.1527018	-0.1563925
PCA Hg3	-0.2582815	-0.2613731
PCA Hg4	0.1443104	0.1388469
SO4 methylation potential	0.6736446	0.6587628
Constant	0.6647129	0.6619409

Artificial Neural Network Modeling Results

Artificial neural network (ANN) modeling was conducted in part as an alternate and comparative exercise to test whether the MLR model that was developed to predict LMB Hg concentrations in the Hg TMDL lakes (Section 5.2.3) could be improved through nonlinear representations of the independent variables. In this exercise, ANN modeling was conducted with the same suite of independent variables as used in the MLR model, viz. alkalinity and chlorophyll *a* (both *ln*-transformed), PCA_Hg3, PCA_Hg4, and spline transformed SO4. The modeling was conducted using 2/3 of the suite of 127 observations (*i.e.*, 84 observations) selected randomly as the model training or calibration data set, and the remaining 43 observations as a validation data set. Overall results with respect to model fit are shown Figure 46 and summarized in Table 19. Figure 47 plots the profiles of the functional relationship between LMB Hg concentrations and each independent variable for fixed values of the other independent variables. The results show that that little, further (non-linear) transformation is invoked for the independent variables and, as a result, the overall fit is similar to the results obtained from the MLR model.

Table 19: ANN modeling results for model calibration and validation for neural network modeling of LMB Hg in Hg TMDL lakes using alkalinity and chlorophyll *a* (both *ln*-transformed), PCA_Hg3, PCA_Hg4, and spline transformed SO4 as independent variables.

Variable	Data Set (Calibration or Validation)	
	Calibration	Validation
r^2	0.6338534	0.5430764
RMSE	0.4803568	0.4336062
Mean Absolute Deviation	0.3675729	0.3515202
SSE	19.382381	8.0846158
<i>N</i>	84	43

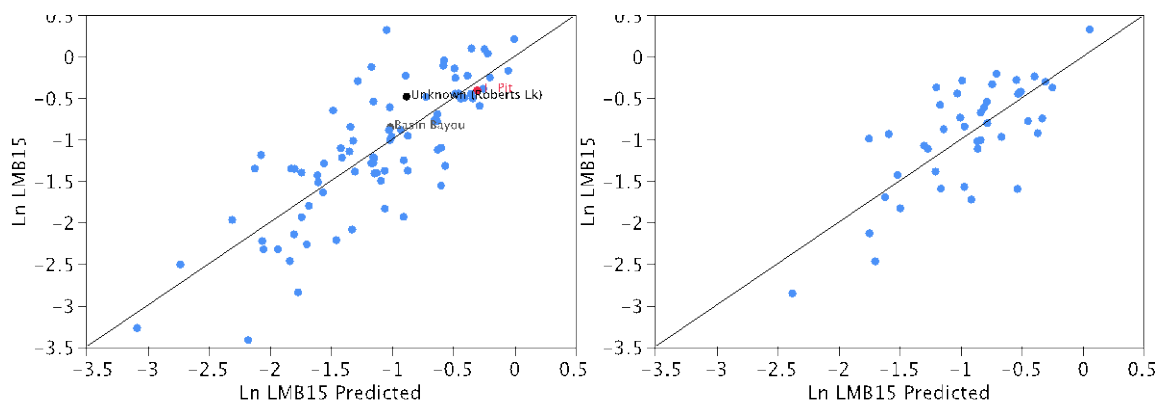


Figure 46. Observed vs. predicted results from neural network modeling of LMB Hg in Hg TMDL lakes using alkalinity and chlorophyll *a* (both *ln*-transformed), PCA_Hg3, PCA_Hg4, and spline transformed SO₄ as independent variables. Left hand panel – training or calibration data set results (N = 83); right hand panel – results for the validation data set (N = 43).

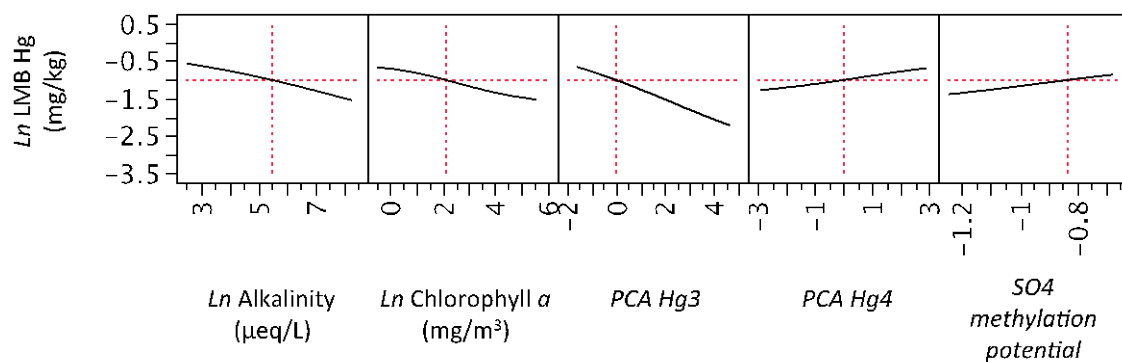


Figure 47. Profile plot showing the functional shapes of the fitted relationship between the input variables and LMB Hg concentrations (*ln*-transformed) for the neural network model shown in Figure 46 and summarized in Table 19.

Generalized Linear Model Results

As discussed in Section 5.2.1, when ordinary least squares models such as MLR are constructed using an initial transformation of the dependent variable, a back-transformation can results unless the residual errors from the OLS model are incorporated in the back-transformation. For the lakes LMB Hg MLR model, this error (observed – predicted) averages 0.024 mg/kg and, when compared to the TMDL target concentration for LMB Hg concentrations of 0.3 mg/kg, equates to an average bias towards underprediction of 8%.

Generalized linear modeling provides a framework for modeling the dependent variable directly through a link function (such as a logarithmic transformation); in contrast, OLS models the transformed variable directly. As a result, GLM models avoid the back-transformation bias inherent in OLS models using transformed dependent variables that require back-transformation.

The lakes LMB Hg MLR model was refitted using the same set of independent variables and excluding the same outliers identified in the development of the MLR model. Significance based on calculated chi-square statistics was $p < 0.0001$. Estimates for the fitted independent variable coefficients and their significance are presented in Table 20.

Table 20. Fitted coefficients, standard error, and z statistics for parameter coefficient significance for the GLM model to predict LMB Hg concentrations with Riggins and Gator Lakes removed as outliers.

Model variable	Estimate	Standard Error	z	P> z
<i>Ln</i> Alkalinity	-0.1418379	0.0222771	-6.37	0
<i>Ln</i> Chlorophyll <i>a</i>	-0.1424010	0.0385794	-3.69	0
PCA Hg3	-0.1301117	0.0506704	-2.57	0.01
PCA Hg4	0.1485547	0.0499472	2.97	0.003
SO4 methylation potential	0.8012827	0.3223066	2.49	0.013
Intercept	0.8154923	0.2612538	3.12	0.002

Using GLM to directly model LMB Hg concentrations essentially eliminates the bias in the predicted estimates (mean residual = - 0.011 mg/kg). This is illustrated by Figure 48, which compares the predictions obtained from both models, and shows that the MLR predictions are rather consistently lower than the GLM predictions (mean difference = 0.035 mg/kg).

Analysis of whether the residuals approximate a normal distribution was conducted by constructing a normal quantile plot of the model *deviance* residuals (Hardin and Hilbe, 2007). For GLM models with an assumed normal distribution of errors, the deviance residuals are equivalent to the observed – predicted value. The residual normal quantile plot shows appreciable tailing and deviation from the expected distribution (assuming normality) imposed by two observations at the upper end of the distribution (Figure 49). As a result, the robustness of the model coefficient estimates for the independent variables was evaluated by conducting a bootstrap analysis (number of replications =

1,000) to calculate the standard of the model coefficient estimates. Results on the significance and the confidence intervals obtained from both the original GLM analysis and the bootstrap analysis are shown in Table 21. The bootstrap analysis indicates that the estimates of the model coefficients are indeed robust.

Similar to the MLR model, validation of the GLM model for predicting “out-of-sample” LMB Hg concentrations was performed by conducting a jackknife analysis ($N=127$). Comparison of the GLM model RMSE (0.197) with the RMSE obtained from the jackknife analysis (0.202) verifies that the GLM model predictive capabilities are robust.

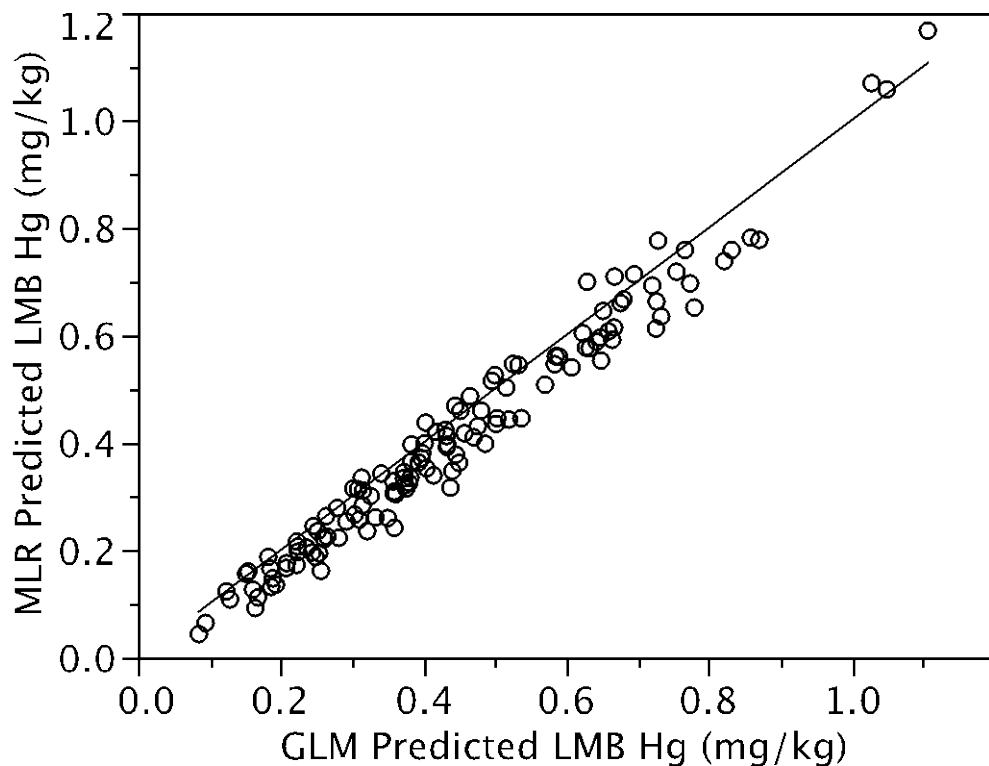


Figure 48. Comparison between predicted LMB Hg concentrations obtained from the MLR model (back-transformed with no error correction), and from GLM. Solid line shows 1:1 agreement.

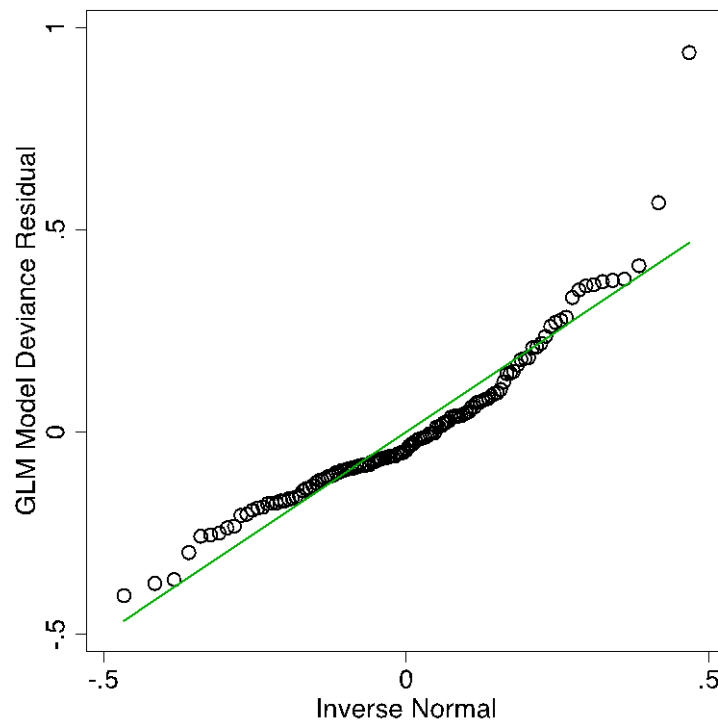


Figure 49. Normal quantile plot of the GLM model deviance residuals. Solid green line shows the expected distribution of the residuals if the residuals are normally distributed.

Table 21. Significance and confidence limits for parameter coefficient estimates obtained from both the original GLM model and bootstrap analysis (N replications = 1,000).

	95% Confidence Interval				
Model variable	Standard Error	z	P> z	LCL	UCL
Original GLM Model Estimates					
Ln Alkalinity	0.0223	-6.37	0	-0.1855	-0.0982
Ln Chlorophyll a	0.0386	-3.69	0	-0.2180	-0.0668
PCA Hg3	0.0507	-2.57	0.01	-0.2294	-0.0308
PCA Hg4	0.0499	2.97	0.003	0.0507	0.2464
SO4 methylation potential	0.3223	2.49	0.013	0.1696	1.4330
Intercept	0.2613	3.12	0.002	0.3034	1.3275
Robust Estimates					
Ln Alkalinity	0.0249	-5.69	0	-0.1907	-0.0930
Ln Chlorophyll a	0.0374	-3.81	0	-0.2157	-0.0691
PCA Hg3	0.0505	-2.57	0.01	-0.2292	-0.0311
PCA Hg4	0.0563	2.64	0.008	0.0381	0.2590
SO4 methylation potential	0.3101	2.58	0.01	0.1935	1.4091
Intercept	0.2493	3.27	0.001	0.3269	1.3040

Discussion of Lake Model Statistical Results

The estimates for the parameter coefficients, including standardized β coefficients, for the MLR model relating alkalinity, chlorophyll *a*, transformed sulfate, and the two sediment principal components relating to anthropogenic disturbance are summarized in Table 22. The standardized β coefficients indicate that the variables in the MLR model assume the following order of greatest importance:

Alkalinity > chlorophyll *a* > urban runoff disturbance > atmospheric deposition > sulfate

Examination of the semipartial correlation coefficient for the MLR model independent variables indicates that the variables with the greatest degree of independent (*i.e.*, non-overlapping with other variables) correlation with LMB Hg concentrations are alkalinity and the sediment principal component indicative of urban runoff impacts (Table 23). The inverse relationship suggested by the MLR modeling between alkalinity and fish tissue Hg concentrations has been noted by a number of studies, including studies in Wisconsin (Spry and Wiener, 1990), Scandinavia (Hakanson *et al.*, 1988), the northeastern United States (Kamman *et al.*, 2004; Dittmann and Driscoll, 2009), South Carolina (Glover *et al.*, 2010), and early studies in Florida by Lange *et al.* (1993). The negative association between pH and biota Hg concentrations has been attributed to a variety of mechanisms, and may be either direct or indirect. For example, Gilmour and Henry (1991) hypothesized that the association between increased methylation and reduced pH in acidified lakes could be explained, at least in part, by the stimulation of sulfate reducing bacteria (and resultant increases in Hg methylation) driven by increases in sulfate typically responsible for lake acidification. Accordingly, declines in fish tissue Hg in lakes in Wisconsin (Hrabik and Watras, 2002) and Sweden (Johansson *et al.*, 1991) may be linked to reductions in sulfate loading as well as reductions in atmospheric loadings of Hg. Low pH also stimulates methylation (Kelly *et al.* 2003; Golding *et al.* 2008). Spry and Wiener (1991) suggest that the greater bioaccumulation at acidic pH levels (< 6.0 – 6.5) may also be due to greater aqueous abundances of biologically available forms of and increased membrane permeabilities to methyl Hg resulting from lower concentrations of aqueous calcium.

Changes in food web structure and function and oligotrophication induced by acidification also has been hypothesized to contribute to elevated biota Hg concentrations at lower pH levels (Hakanson, 1980), although it should be noted that the evidence for acidification-induced oligotrophication is controversial (Olson and Petersson, 1993). As has been noted for largemouth bass in Florida lakes, lower pH levels can lead to reduced condition factors for fish and reduced body masses (Pollman and Canfield, 1991); conversely, higher pH levels are less stressful and can result in comparatively lower biota Hg concentrations though faster growth rates or “growth dilution” (Dittmann and Driscoll, 2009).

The inverse association of chlorophyll *a* concentrations and LMB Hg concentrations in this study is also consistent with results from other studies. For example, early studies on the distribution of LMB Hg concentrations in Florida lakes conducted by Ware *et al.* (1990) indicate that hypereutrophic lakes have generally much lower fish tissue Hg concentrations, and subsequent work by Lange *et al.* (1993) indicated that lakes with chlorophyll *a* concentrations below 10 $\mu\text{g/L}$ have significantly higher concentrations than other systems. Similarly, results from this study show higher LMB Hg concentrations for lakes with chlorophyll *a* < 10 $\mu\text{g/L}$ (Figure 50); the results also show greater variance in

LMB Hg concentrations for the same group of lakes. The compressed variance for lakes with higher chlorophyll *a* values suggests that trophic state becomes increasingly dominant as a control on biota Hg concentrations in more eutrophied systems; for lakes with chlorophyll *a* < 10 µg/L, the higher variance suggests that other biogeochemical factors assume much more importance than trophic state. The negative association of LMB concentrations with increasing trophic state most likely represents several phenomena, including: biodilution related to increased phytoplankton and zooplankton densities (Chen and Folt, 2005); growth dilution (faster growth rates; Bjornberg *et al.*, 1988); and the simplification of food webs characteristic of more eutrophied lakes. While the relationship between alkalinity and biota Hg generally appears to be inverse, Hongve *et al.* (2012) have suggested that increases in alkalinity relating to lake recovery from acidification may actually promote higher concentrations of biota Hg because of associated changes in the dynamics of organic carbon (DOC). DOC, which is a polyprotic organic acid, is more soluble at higher pH levels; in addition, higher levels of ionic strength characteristic of acidified soils promote flocculation and retention of DOC (Hongve *et al.*, 2012). As a result, progressive acidification for a given lake should result in reduced DOC, and increases in lakewater pH should result in higher DOC concentrations. DOC is a critical variable in the aquatic cycling of Hg, including serving as a metabolic substrate supporting the activity of sulfate reducing bacteria, and transport of methyl Hg and total Hg from riparian wetlands. In a recent study of lakes and impoundment in South Dakota, Stone *et al.* (2011) found that increasing alkalinity was *positively* related to increasing fish tissue Hg, apparently due to increasing concentrations of DOC that accompany increasing pH levels.

Table 22. Parameter coefficient estimates, including standardized β , t ratio statistics, and variance inflation factor (VIF) for the MLR model predicting LMB Hg concentrations in Florida Hg TMDL lakes.

Term	Estimate	Std Error	t Ratio	Prob> t	Std Beta	VIF
Intercept	0.664713	0.282356	2.35	0.0202	0.0000	.
<i>Ln</i> Alkalinity (µeq/L)	-0.152702	0.027134	-5.63	<.0001	-0.3620	1.2747
<i>Ln</i> Chlorophyll <i>a</i>	-0.146803	0.038886	-3.78	0.0002	-0.2310	1.1539
PCA Hg3	-0.258281	0.044249	-5.84	<.0001	-0.3456	1.0800
PCA Hg4	0.144310	0.047586	3.03	0.0030	0.1915	1.2288
SO4 methylation potential	0.673645	0.308052	2.19	0.0307	0.1350	1.1742

Table 23. Parameter significance, partial and semipartial correlation coefficients for the MLR model predicting LMB Hg concentrations in Florida Hg TMDL lakes.

Variable	Partial Correlation	Semipartial Correlation	Partial r ²	Semipartial r ²	Significance
<i>Ln</i> Alkalinity (µeq/L)	-0.4555	-0.3206	0.2074	0.1028	0.0000
<i>Ln</i> Chlorophyll <i>a</i>	-0.3246	-0.2151	0.1054	0.0463	0.0002
PCA Hg3	-0.4687	-0.3325	0.2197	0.1106	0.0000
PCA Hg4	0.2658	0.1728	0.0706	0.0299	0.0030
SO4 methylation potential	0.1950	0.1246	0.0380	0.0155	0.0307

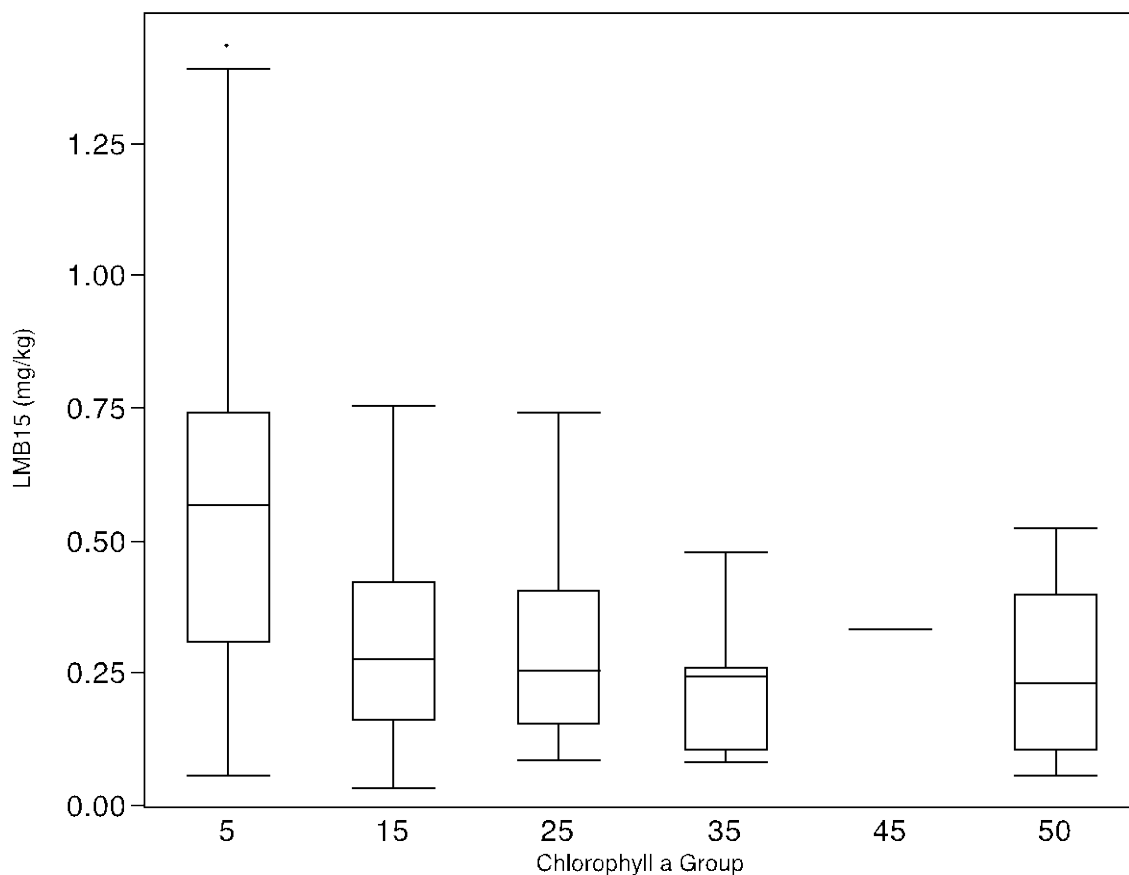


Figure 50. Box plot of LMB Hg concentrations as a function of chlorophyll a concentrations for the Hg TMDL study lakes. Chlorophyll a groups are defined as follows: The group number for each chlorophyll a values

indicates the midpoint of the group concentration range, with each group encompassing a range in concentration of 10 ug/L (e.g., Group 5 $\leq$ 10 ug/L); the exception is Group 50, which includes all lakes with chlorophyll a > 50 ug/L.

Effects of Sulfate

Similar to alkalinity, the effects of sulfate on the aquatic cycling of Hg are both direct (e.g., use of sulfate by sulfate reducing bacteria as the terminal electron acceptor in sulfate reduction) and indirect (incipient acidification driven by increases in excess [anthropogenic] sulfate loadings). The initial analysis of the relationship between LMB Hg concentrations and sulfate (Section 4.1) indicates that the concentration range of lakewater sulfate resulting in maximal net methylation and uptake of Hg lies between 2.3 and 3.8 mg/L (Figure 11). Assuming no associated changes in alkalinity, the direct effect of reducing sulfate concentrations on biota Hg concentrations thus will depend upon both the magnitude of the reduction of sulfate and the initial concentration before implementing reductions. For example, for lakes with sulfate concentrations approximating 8 mg/L, reducing sulfate by 50% would result in ambient levels approaching the net methylation maximum, and would be expected to result in higher LMB Hg concentrations as well. Conversely, reducing sulfate concentrations with SO₄ initially at or below the net methylation maximum should result in lower LMB Hg concentrations.

The indirect effect of sulfate on biota Hg concentrations lies with the associated reductions in alkalinity and pH resulting from acidic deposition and loadings of excess sulfate. This is illustrated in Figure 51, which shows the relationship between the sum of the concentrations of calcium (Ca) and magnesium (Mg), and the sum of alkalinity, DOC, and sulfate. In brief, if the predominant source of alkalinity is due to carbonate (and dolomitic limestone) weathering, in the absence of the titrating effect of any acids such as DOC or sulfuric acid, the concentration of alkalinity should be identical to the summed concentrations of Ca and Mg. As shown in Figure 51, the presence of both DOC and SO₄ result in titrated or reduced alkalinity concentrations below the levels supplied by carbonate weathering. The titrating effect of sulfate alone is illustrated by comparing the left hand panel of Figure 51 (which plots the sum of alkalinity + DOC alone vs. the sum of Ca + Mg) with the right hand panel of the same figure (which includes sulfate in the term for the ordinate). The right hand panel demonstrates that, for the very large majority of lakes, alkalinity is the net function of carbonate weathering offset by inputs of DOC and sulfate. The left hand panel also illustrates that the effect of sulfate as an acidifying influence reducing alkalinity (or more correctly, the sum of alkalinity plus DOC) can be quite large.

While quantifying the effect of reducing sulfate inputs on lakewater concentrations is complex and must consider the effects of lake hydrology, changes in base cation weathering, and sulfate retention – both in the watershed and *in situ* (Pollman, 1990) – the magnitude of excess sulfate concentrations suggests that reductions in excess sulfate would result in both increased lakewater pH and reductions in biota Hg. Figure 52 shows the results of a simplified analysis that illustrates the effects of reducing sulfate concentrations in Florida lakes to levels that are supported by inputs associated with marine aerosols only. The analysis assumes that sulfate retention is constant for a given system and is defined by the ratio of observed to expected sulfate concentrations:

$$R_{SO4} = 1 - \frac{SO4_{obs}}{SO4_{exp}}$$

where R_{SO4} equals the sulfate retention coefficient, $SO4_{obs}$ is the observed lakewater sulfate concentration, and $SO4_{exp}$ is the expected sulfate concentration based on assumed atmospheric inputs of sulfate and chloride, and assuming that effects of evaporative concentration are accounted for by the ratio of observed to wet deposition chloride concentrations:

$$SO4_{exp} = \frac{Cl_{obs}}{Cl_{wet}} \cdot SO4_{wet}$$

Concentrations of sulfate and chloride were based on volume weighted mean wet deposition concentrations measured at National Trends Network (NTN) sites across Florida between 2001 and 2010⁴. For sites where observed sulfate concentrations exceed $SO4_{exp}$, R_{SO4} is assumed equal to zero.

The left hand panel of Figure 52 compares the cumulative distribution of transformed sulfate or methylation potential values for both currently observed sulfate concentrations in the Hg TMDL lakes, and concentrations calculated assuming that sulfate inputs from atmospheric deposition are marine in nature only. Because of the non-linear relationship between sulfate and methylation potential (Figure 11), the comparison indicates that eliminating all excess or non-marine sulfate (which defines an absolute lower limit for ambient sulfate concentrations given that a fraction of the sulfate in atmospheric deposition has a natural component – e.g., volcanic emissions) will result in relatively modest reductions in the number of lakes with high sulfate methylation potentials. On the other hand, the comparison of the distribution of sulfate concentrations for both sulfate scenarios (right hand panel of Figure 52) suggests large reductions in lakewater sulfate concentrations resulting from the elimination of excess sulfate from atmospheric wet deposition which likely would translate to substantial increases in lakewater alkalinity. From a conceptual perspective this analysis thus suggests that sulfate reductions imposed by reducing atmospheric inputs will have very limited efficacy with respect to directly reducing biota Hg concentrations in Florida lakes; rather the benefits would inure primarily from the indirect benefit of mitigating the acidifying effects of excess sulfate.

⁴The average sulfate to chloride ratio for Florida measured between 2001 and 2010 (VWM average across years and sites) is 1.14. This result is based on calculating the average sulfate to chloride ratio weighted by precipitation across sites in each year, and then weighting each year by the total precipitation across sites. Calculating a simple arithmetic average of the ratio across all sites and years together yields a value equal to 1.33.

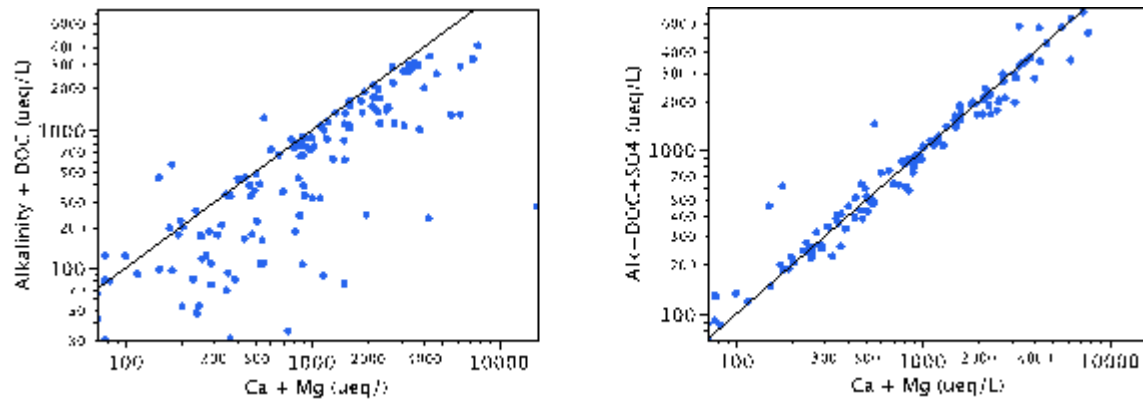


Figure 51. Relationship between Ca plus Mg concentrations (as sources of alkalinity), and alkalinity plus DOC (left hand panel) and alkalinity plus DOC and sulfate in the Hg TMDL lakes. Solid black line shows the expected line for the sum of the anionic species based on the sum of Ca plus Mg.

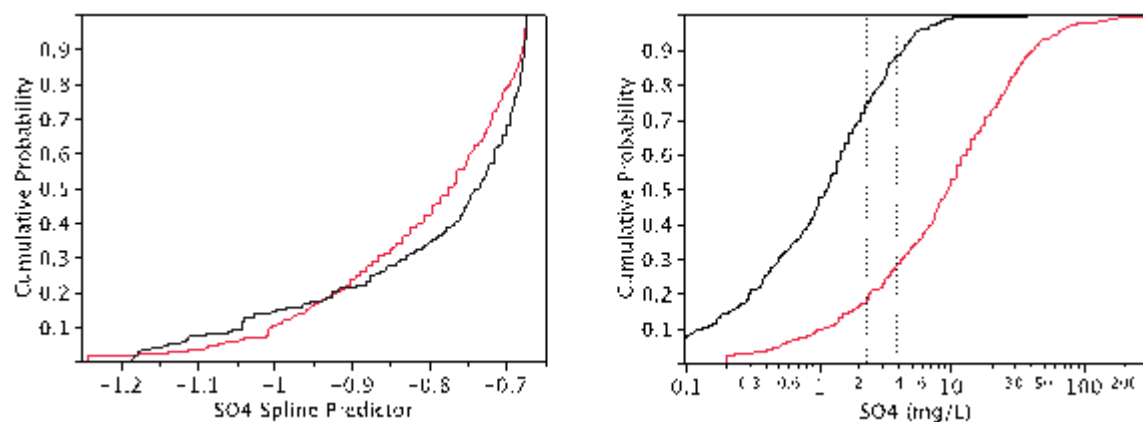


Figure 52. Effects of reducing sulfate concentrations in Florida TMDL lakes to levels supported by marine aerosol inputs only. Left hand panel – spline transformed sulfate or sulfate methylation potential (see Section 4.1); right hand panel – lakewater sulfate concentrations. Red line shows current levels; black line assumes that sulfate concentrations have been reduced to inputs associated with marine aerosols only, and that sulfate retention is both linear and equivalent to values estimated from current lakewater concentrations and an assumed SO4:Cl ratio = 1.14. Vertical dotted lines define the interval of sulfate concentrations where the effect on methylation is expected to be maximal.

TMDL Streams and Rivers Statistical Model

Statistical Methods

As stated in Section 1, one of the goals of the aquatic modeling for both lakes and streams is to develop a tool that can be used to infer LMB Hg concentrations across a wider distribution of water bodies for which water quality data are available, but are lacking in actual measurements of fish tissue Hg concentrations. Preliminary analyses using as independent variables only water chemistry variables collected as part of this study that are collected routinely as part of the FDEP Status and Monitoring Network (SMN)⁵ indicated poor results using MLR. Artificial neural network modeling resulted in better performance in terms of overall fit, presumably because of its ability to incorporate non-linearity into its model relationships; poor cross-validation results however, suggest that improvements in overall fit of the calibrated model came at the cost of overfitting. As result, classification and regression tree analysis (CART) was used to model LMB Hg concentrations in the Hg TMDL streams. CART is a non-parametric approach and, as a result, it makes no assumption that the underlying relationships between the predictor variables and the dependent variable are linear. With a continuous response variable such as LMB Hg concentrations, and continuous independent variables, an initial split of the response variable into two groups is constructed based on maximizing the separation of the two groups of the response variable (as measured by the sums of squares due to differences between the means) and splitting an independent variable into two partitions. Partitioning into a succession of branches can continue until an objective criterion such as the AIC value is optimized. CART methods are particularly useful when the relationship between the response variable and the predictors is not homogeneous within the sample space; however, a disadvantage to CART models is that the CART-model predictions are based on the mean values of discrete partitions rather than a continuous function (Amrhein *et al.*, 1999).

Data Screening

Similar to the water chemistry data for the TMDL lakes, water quality data for the TMDL streams and rivers were initially screened for potential analytical artifacts by examining a series of bivariate relationships, including:

- An evaluation of the cation-anion balance (Figure 53);
- Comparing calculated conductivities based on measured major ion concentrations and their associated equivalent conductance values with measured specific conductance values (Figure 54);
- Comparing measured Na and Cl concentrations (Figure 55); and

⁵ The SMN database comprises samples of lakes and streams collected annually using a stratified, randomized sampling scheme that is designed to facilitate inferential estimates of the statewide distribution for key water quality variables such as nutrients and major ions. The use of the SMN coupled with the lake and stream LMB Hg models is critical for developing appropriately weighted estimates of the cumulative distribution of LMB Hg concentrations as a function of area or number (lakes) or segment length (large and small rivers). As a result, the models developed for both resources ultimately must include only those variables sampled routinely as part of the SMN.

- Analyzing the relationship between alkalinity (including the effects of the acid anions DOC, SO₄ and OH⁻) the two primary cations contributing to carbonate alkalinity, Ca plus Mg (Figure 56).

Comparison of the balance between cations and anions shows an apparent excess of anions measured for approximately six streams (Figure 53). Examination of the relationship between measured alkalinity (plus DOC and SO₄) and Ca + Mg (Figure 56) shows that these same streams appear to contain either an excess amount of sulfate, or the contributions from DOC have been overestimated. While it is not clear which measurement may be in error, the fact that measured and predicted specific conductance agree well suggest that any error is likely due to the concentration in equivalents ascribed to DOC or excess SO₄. Because alkalinities were measured titrimetrically to a color indicator endpoint, negative alkalinities resulting from excess sulfate appear as a positive value recorded as the method detection limit; this consequence of using a colorimetric titration to determine alkalinity (which is a routinely used method for most lakes and streams) accounts for the apparent anion excess.

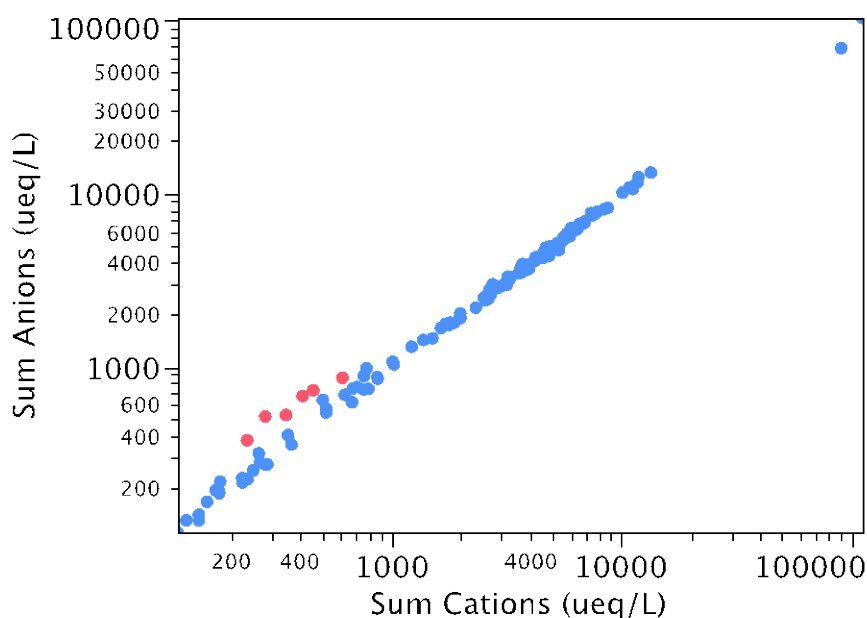


Figure 53. Plot of the sum of anions vs. sum of cations in Hg TMDL streams. Concentrations of each ionic constituent is first expressed in microequivalents per liter before computing the sum of cations or anions.

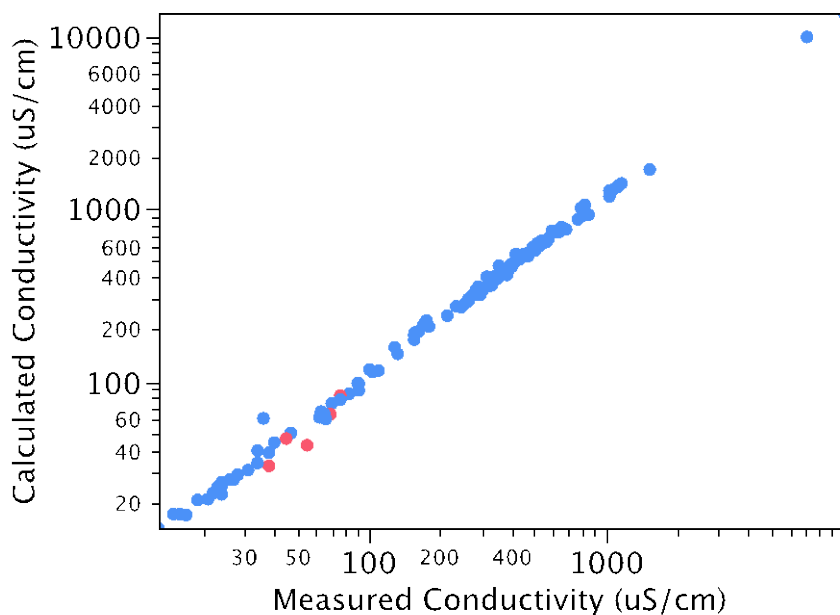


Figure 54. Plot of the predicted conductivity (based on equivalent conductances of individual ionic constituents) vs. measured conductivity in Hg TMDL streams.

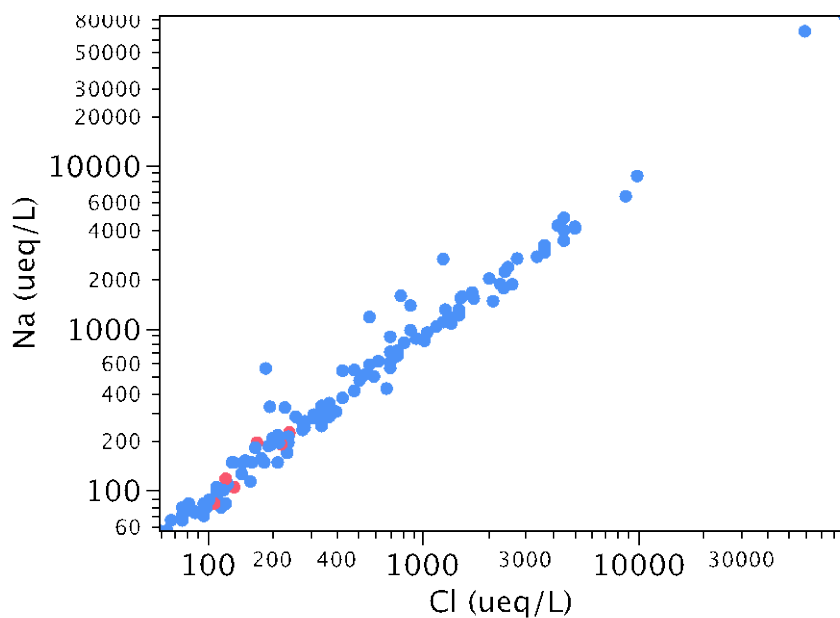


Figure 55. Plot of Na vs. Cl concentrations in Hg TMDL streams.

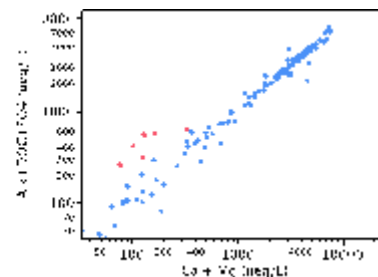


Figure 56. Plot of alkalinity plus DOC and SO4 and concentrations vs. Ca plus Mg concentrations. Left hand panel – alkalinity only; middle panel – alkalinity + SO4; right hand panel – alkalinity + DOC + SO4.

Initial Modeling

Overview

Preliminary modeling efforts indicated that the ability to predict LMB concentrations of Hg were greatly improved if the models included water column concentrations of unfiltered methyl Hg in particular. This is illustrated by comparing the results of conducting stepwise regression using all the water chemistry variables as candidate independent variables to predict LMB Hg concentrations. Water column concentrations for total and methyl Hg were excluded from the analysis, and all variables (with the exception of pH which was further transformed to better account for apparent non-linear effects with fish tissue Hg concentrations; see Section 4.2) were *ln*-transformed. The results, which are summarized in Figure 57 and Table 24, indicate that pH and ionic strength are overall determinants of variability in fish tissue Hg concentrations between different streams, although the strength of the model is poor ($r^2 = 0.26$).

Table 24. Model coefficient estimates, standard error, and significance obtained from stepwise regression model to predict LMB Hg concentrations. Stepwise regression was conducted using all water quality variables monitored in the Hg TMDL streams other than mercury species.

Term	Estimate	Std Error	t Ratio	Prob> t	Std Beta	VIF
Intercept	0.639	0.242	2.64	0.0094	0	
<i>Ln</i> Na (ueq/L)	-0.122	0.046	-2.66	0.0088	-0.2373	1.3601
Transformed pH	0.831	0.202	4.13	<.0001	0.3678	1.3601

Including methyl Hg (unfiltered) as an explicit variable results in both an improved model (Figure 58 and Figure 59), and a shift in variables that enter into the model as significant. Backward stepwise regression results in an improvement in model fit from $r^2 = 0.26$ to $r^2 = 0.44$, and from BIC = 226.4 to BIC= 200.6. The model with methyl Hg as an independent variable no longer included pH as a significant variable, which in all likelihood reflects the effects of pH embedded as an intrinsic variable influencing methylation. Ionic strength is still important, but is represented better by Cl rather than Na in the revised model. In addition, trophic state effects (represented by TP) are now significant.

Table 25. Same as Table 24, except that resulting model reflects inclusion of mercury species in the water column as candidate variables in the stepwise regression.

Term	Estimate	Std Error	t Ratio	Prob> t	Std Beta	VIF
Intercept	0.3054303	0.284677	1.07	0.2854	0	.
<i>Ln</i> Cl (µeq/L)	-0.123947	0.039078	-3.17	0.0019	-0.23939	1.2328097
<i>Ln</i> TP	-0.130538	0.037504	-3.48	0.0007	-0.28078	1.408373
<i>Ln</i> Methyl Hg (unfiltered)	0.3520904	0.046545	7.56	<.0001	0.583065	1.2857778

As evidenced by the standardized β coefficients, variability in methyl Hg concentrations appears to be the strongest determinant of variations in LMB Hg concentrations in the Hg TMDL streams. Because water chemistry can influence both trophic state dynamics and reflect or influence methylation dynamics as well, further modeling was conducted to evaluate what water quality factors appeared to influence stream-water methyl Hg concentrations. Assuming a satisfactory model could be developed, this model would then enable calculations of the residual component of methyl Hg that is not predicted by the water quality model. This residual reflects the effects of unmeasured factors not included in the model (as well as error), including (potentially) the effects of atmospheric deposition of Hg. This residual methyl Hg component can then be used in predictive model building for LMB Hg concentrations.

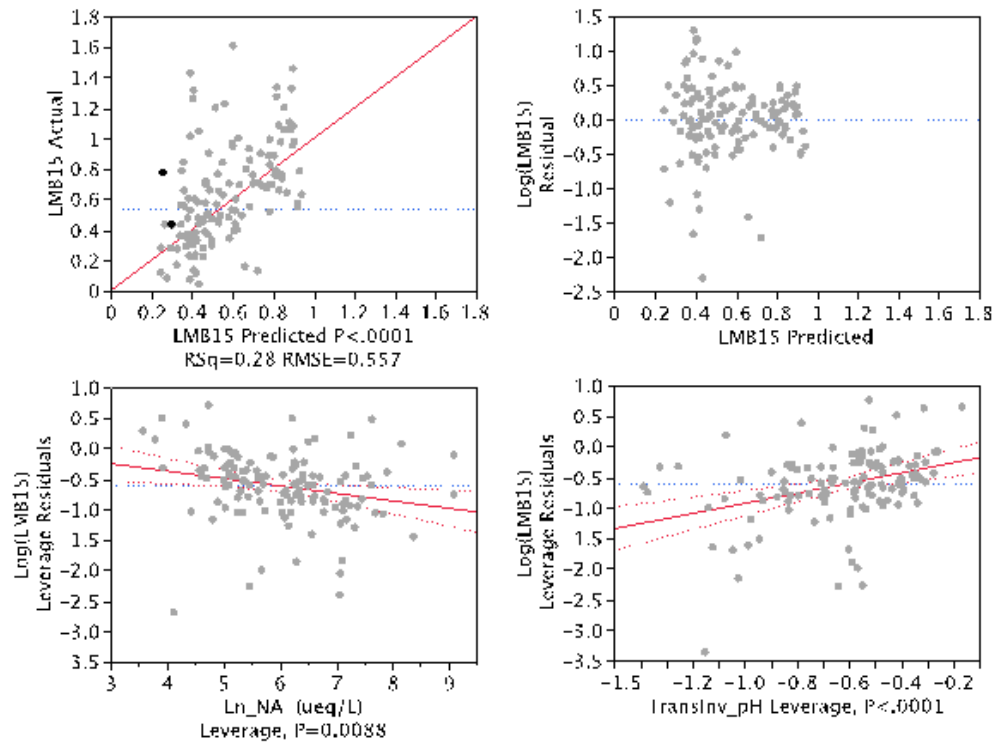


Figure 57. Stepwise regression results for Hg TMDL streams using LMB Hg (ln-transformed) as the dependent variable. Top left hand panel – observed vs. predicted values. Top right hand panel – model residuals (observed – predicted) vs. predicted LMB Hg concentrations. Bottom panels – independent variable leverage plots: Na (ln-transformed, left hand panel); and transformed pH (see Section 4.2, right hand panel). The two filled black circles are sites with specific conductance or measured Cl concentrations in excess of state of Florida water quality standards for freshwater lakes and streams and were excluded from the stepwise regression analysis. N = 126.

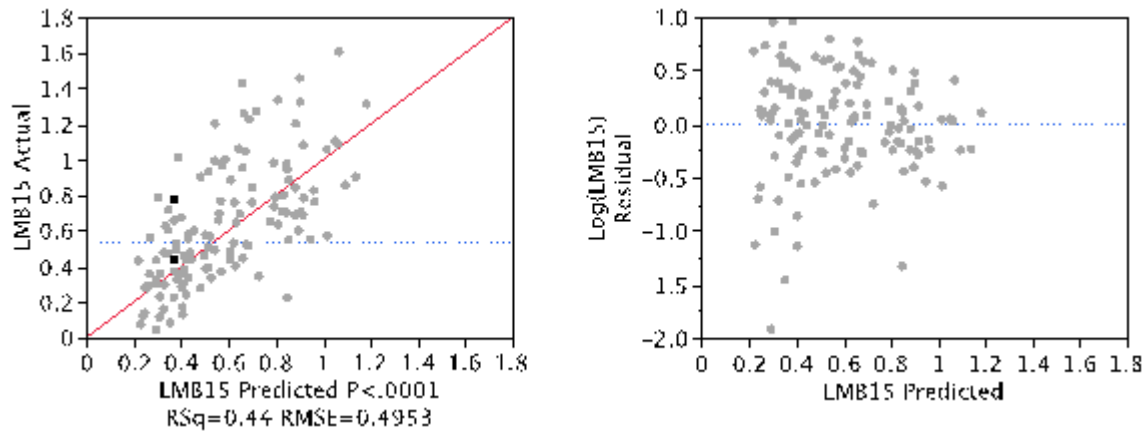


Figure 58. Same as Figure 57, except that stepwise regression was conducted using filtered methyl Hg as one of the candidate independent variables.

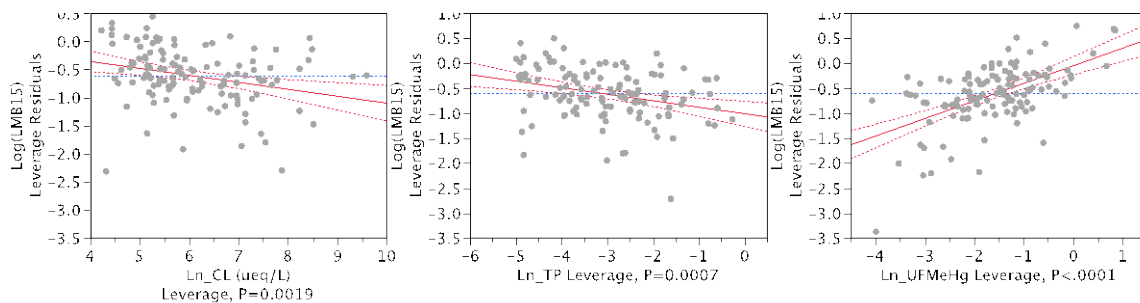


Figure 59. Individual model component leverage plot for independent variables obtained from stepwise regression modeling conducted to predicting LMB Hg (ln-transformed). See Figure 58.

Principal Components Regression Model for Methyl Hg

Principal components regression (which combines PCA with multiple linear regression) was conducted to factor out contributions from watershed processes (as reflected in water quality variables such as color) and other water chemistry effects on in-stream methyl Hg concentrations. PCA initially was conducted using essentially the full suite of water chemistry variables measured during the Hg TMDL study, less mercury species and sulfate (which is used in the subsequent MLR modeling as a separate variable because of its non-linear transformation to better represent Hg methylation potential, see Section 4.1). DOC also was not included in the PCA because it is not included as a routine variable monitored as part of the SMN.

An evaluation of potential multivariate outliers showed three sites as the strongest candidates as outliers based on calculating the Mahalanobis distance (SAS, 2011; Figure 60). Two sites had measured specific conductance values or chloride concentrations in excess of state water quality criteria for fresh waters (specific conductance > 5,000 $\mu\text{S}/\text{cm}$, or $\text{Cl} > 1,500 \text{ mg}/\text{L}$) and were excluded from further analysis. The third outlier was Reedy Creek, which is characterized by dissolved oxygen concentrations well below values observed at other sites (0.06 mg/L compared to the next lowest observed concentration of 1.55 mg/L). Whether to retain or delete the observation for Reedy Creek was evaluated by the conducting PCA both with (Table 26 and Table 27) and without Reedy Creek (results not shown) and comparing the overall results obtained from regressing the retained components from both PCA results against unfiltered methyl Hg concentrations (Table 28). As evidenced by the results shown in Table 28, the choice to include or exclude Reedy Creek as a multivariate outlier had little bearing on the overall fit for the unfiltered methyl Hg model and, as a result, subsequent model development retained this observation.

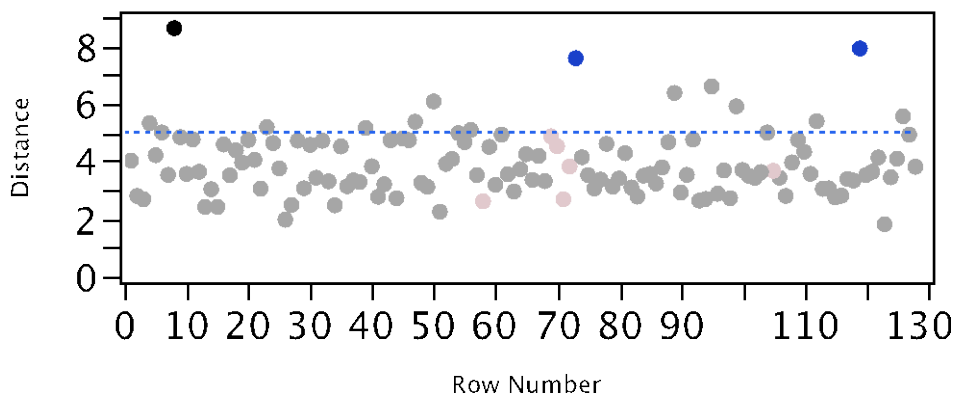


Figure 60. Mahalanobis distance vs. observation for each stream site (N = 128) calculated using the full suite of water quality variables summarized in Table 26). Blue filled circles show sites exceeding FDEP freshwater quality criteria for specific conductance or Cl concentrations; the black filled circle shows Reedy Creek.

Table 26. Principal components results showing extracted components and variable loadings derived from the modeling following Varimax rotation. Only loadings with a magnitude (absolute value) greater than 0.3 are shown. Two observations were excluded because the measured specific conductances or chloride concentrations exceeded state water quality criteria for fresh waters (specific conductance > 5,000 $\mu\text{S}/\text{cm}$, or $\text{Cl} > 1,500 \text{ mg}/\text{L}$). Total $N = 126$. All variables (except pH) were initially $\ln$ -transformed.

Parameter	pH/Ionic Strength	DOC/Methylation	Trophic State	Groundwater
pH	0.867			
Alkalinity ($\mu\text{eq}/\text{L}$)	0.920			
NH_3 ($\mu\text{eq}/\text{L}$)		0.706		
Ca ($\mu\text{eq}/\text{L}$)	0.928			
Cl ($\mu\text{eq}/\text{L}$)	0.839			
Chlorophyll a (mg/m^3)	0.450		0.720	
Color (PCU)		0.846		
Dissolved oxygen (mg/L)				
Mg ($\mu\text{eq}/\text{L}$)	0.889			
TKN (mg/L)		0.791	0.347	
$\text{NO}_2\text{-NO}_3$ ($\mu\text{eq}/\text{L}$)				0.868
K ($\mu\text{eq}/\text{L}$)	0.719	0.336	0.380	
Na ($\mu\text{eq}/\text{L}$)	0.844	0.307		
TP (mg/L)	0.309	0.497	0.410	0.408
Total suspended solids (mg/L)			0.871	

Table 27. Variance, percent of total variance, and cumulative percent of total variance explained by each component extracted from the PCA summarized in Table 26.

Factor	Variance	%	Cumulative %
Factor 1	5.7444	38.296	38.296
Factor 2	2.7559	18.372	56.668
Factor 3	1.8217	12.144	68.812
Factor 4	1.3710	9.140	77.952

Table 28. Comparison of overall model fit for MLR model predicting unfiltered methyl Hg concentrations as a function of retained principal components obtained from PCA of water chemistry variables and transformed SO₄ (see Section 4.1) as independent variables. Comparison contrasts results obtained from either including or excluding the Reedy Creek stream site observation as a multivariate outlier. Coefficients shown for each model variable are the standardized (β) coefficients.

Principal Component (PC) or Variable	Use of Reedy Creek Observation	
	Yes: N = 126	No: N = 125
pH/Alkalinity PC	-0.517	-0.518
Methylation PC	0.572	0.567
Transformed SO ₄	-0.229	-0.229
r ²	0.491	0.488
PRESS RMSE	0.797	0.801

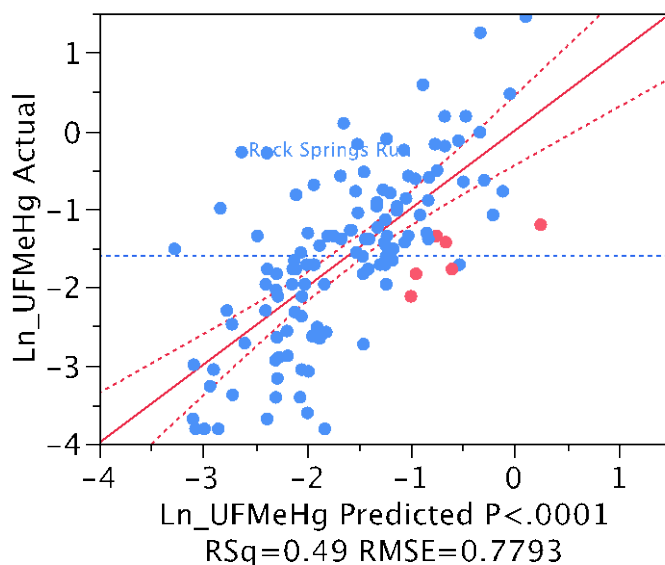


Figure 61. Observed vs. predicted ln-transformed unfiltered methyl Hg concentrations. Predicted values based on MLR constructed from water chemistry PCA model and transformed sulfate as independent variables.

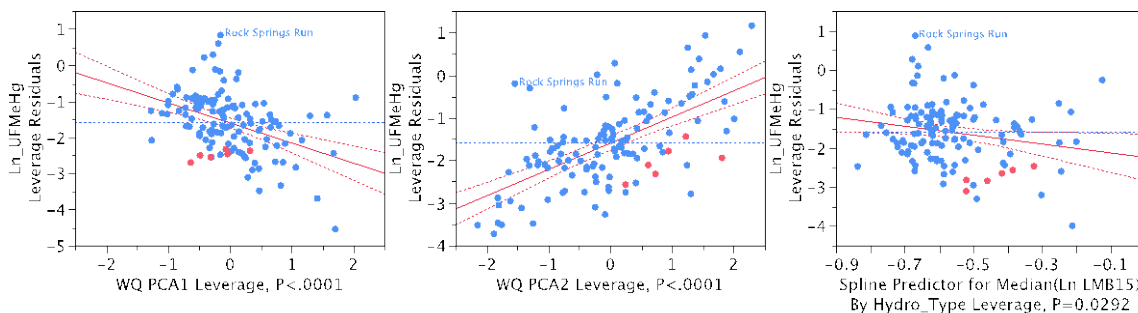


Figure 62. Individual model component leverage plot for independent variables in MLR model predicting unfiltered methyl Hg (ln-transformed) as a function of water quality PCA extracted components (Table 26) and cubic spline transformed sulfate.

The fitted predictive model explains 49% of the variability in (*ln*-transformed) unfiltered methyl Hg concentrations (Figure 61). Key predictive variables include the two principal components for pH/ionic strength and methylation, as well as (cubic spline transformed) sulfate. The standardized β coefficients and the individual component leverage plots (Figure 62) indicate that, as expected, methyl Hg concentrations decline with increasing pH and ionic strength, and increase with contact with riparian wetlands (*i.e.*, higher values of the second extracted principal component in Table 26). The leverage plots also suggest that methylation is positively influenced by increases in sulfate concentrations, although the slope changes at approximately 5 mg/L such that further increases in sulfate in excess of ~ 4 to 7 mg/L result in less efficient increases in methylation potential (Figure 11).

The distribution of residuals from the model for unfiltered methyl Hg (designated as χ , and defined as the difference between observed and predicted values) are shown in Figure 63). Although there is some tailing of the distribution for residuals with the largest (positive) values, the overall distribution still reasonably approximates normality based on the Shapiro-Wilks test ($p = 0.0927$).

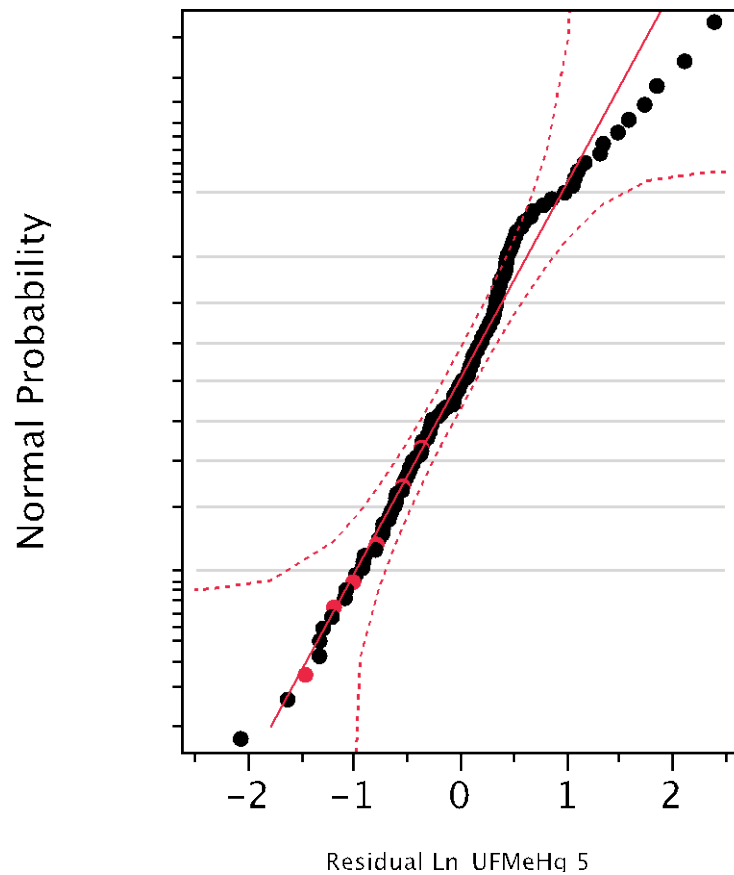


Figure 63. Analysis of distribution of model residuals from fitted model for unfiltered methyl Hg shown in Figure 61. Shapiro-Wilks test indicates residuals reasonably approximate a normal distribution ($W = 0.981895$; $p = 0.0927$).

Stream LMB Hg Model

Initial MLR Modeling

MLR modeling was conducted to predict LMB Hg concentrations using the residual unfiltered methyl Hg signal, χ , as an independent variable. Different model constructs then were evaluated by trying different combinations of multiple independent variables (up to five) and then choosing the model that both minimized the BIC and for which the model parameter coefficients made theoretical sense. This led to a model that included three variables: χ , pH, and $\ln$ -transformed TP (Figure 64). It is important to note that factoring out the watershed factors that influence methyl Hg concentration and using the residual component, χ , results in a somewhat better fit than if methyl Hg ($\ln$ -transformed) concentrations were used in the model instead ($r^2 = 0.45$ vs. 0.43).

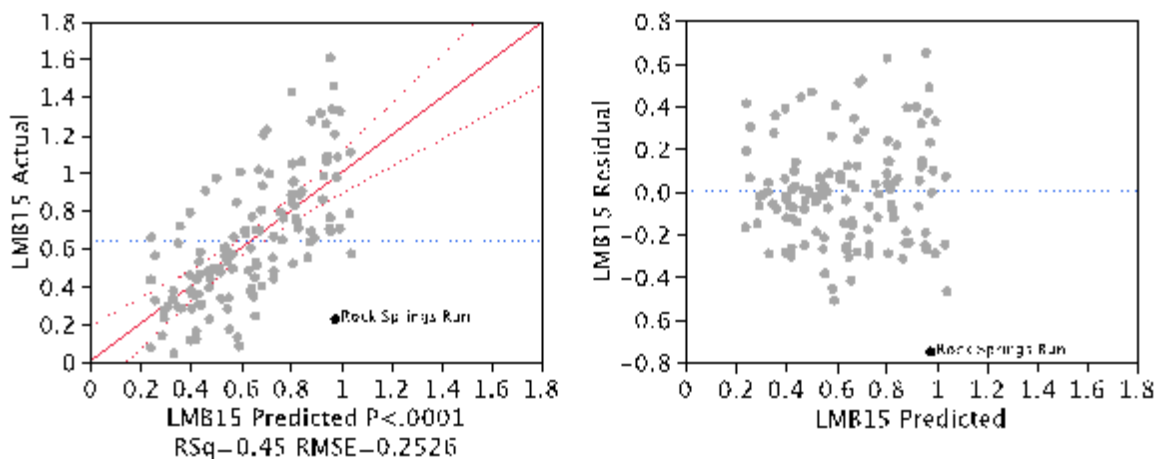


Figure 64. Observed vs. predicted LMB Hg concentrations for Hg TMDL streams. Predicted values based on MLR using pH, $\ln$ -transform TP, and residual unfiltered methyl Hg as independent variables. N = 126.

A comparison of both the actual and predicted LMB Hg concentrations, as well as the plot of the resulting residuals shown in Figure 64 shows that one observation, Rock Springs Run, stands out as a clear outlier. As a result, the MLR model was refitted by excluding Rock Springs Run. This results in an improved fit (Figure 65). Examination of the individual component leverage plots also indicates that the model relationships are well-behaved and reasonably linear after the effects of other independent variables have been factored out (Figure 66). Estimates for the model coefficients, their statistical significance, and VIF values are shown in Table 29. The PRESS RMSE for the model is 0.247, which agrees quite well with the RMSE for the fully fitted model, 0.243.

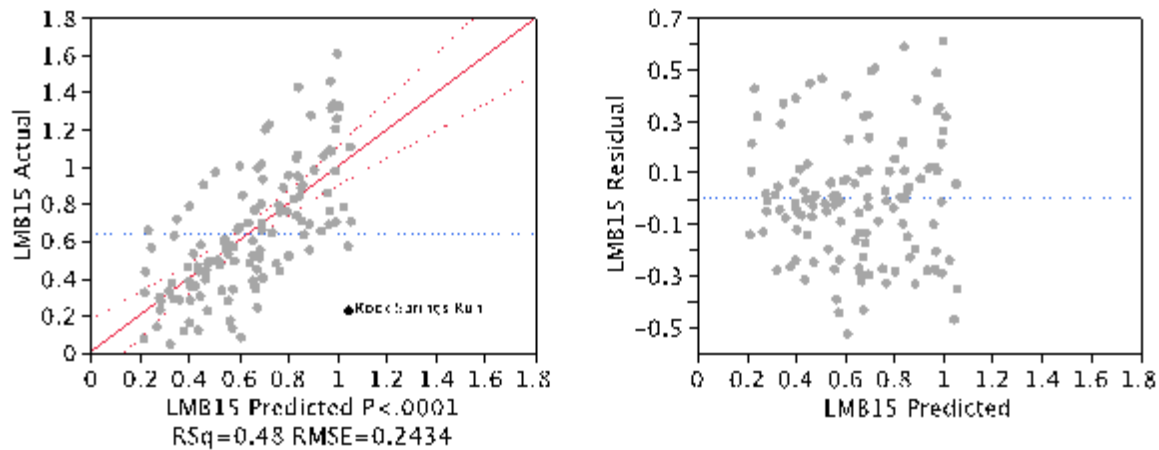


Figure 65. Same as Figure 64, except that Rock Springs Run has been deleted as an outlier.

Table 29. Model coefficient estimates, standard error, and significance obtained for MLR model to predict LMB Hg concentrations with Rock Springs Run deleted as an outlier (see fit shown in Figure 65 and leverage plots shown in Figure 66). $N = 125$.

Term	Estimate	Std Error	t Ratio	Prob> t	Std Beta	VIF
Intercept	1.3301896	0.159051	8.36	<.0001	0	.
$\ln$ TP	-0.05303	0.016463	-3.22	0.0016	-0.22304	1.1227433
pH	-0.125012	0.020163	-6.20	<.0001	-0.42324	1.0911881
χ	0.2363676	0.029913	7.90	<.0001	0.525391	1.0352258

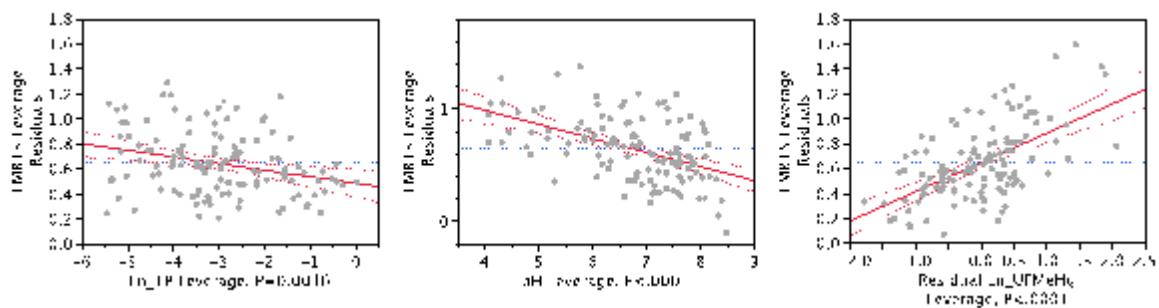


Figure 66. Individual model component leverage plot for independent variables in MLR model predicting LMB Hg concentrations as a function of TP (ln-transformed, pH, and χ . Left hand panel – leverage plot for TP; middle panel – leverage plot for pH; right hand panel – leverage plot for χ .

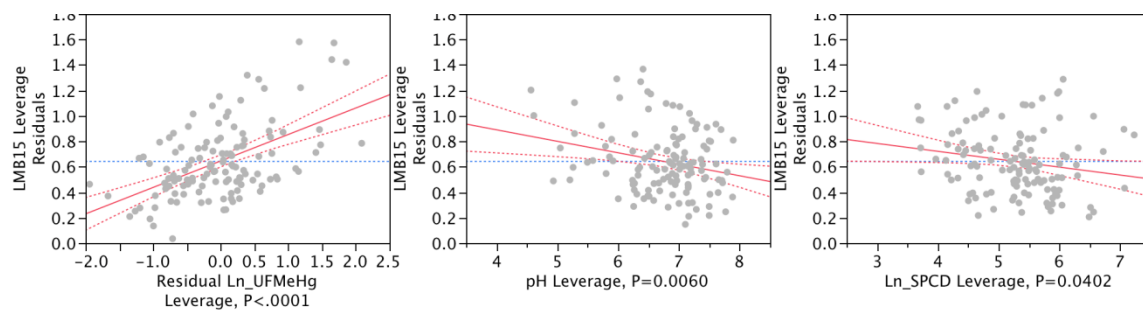


Figure 67. Individual model component leverage plot for independent variables in MLR model predicting LMB Hg as a function of residual methyl Hg, pH, and ln-transformed specific conductance. N = 126.

Neural Network Model

Neural network modeling was conducted as an approach towards integrating underlying nonlinearities in the relationship between key independent variables and LMB Hg concentrations. Neural network modeling is an approach that offers the possibility of mapping such nonlinear relationships, including ones characteristic of aquatic ecosystems (Lek *et al.*, 1996). Neural network modeling was conducted using three hidden nodes to predict LMB Hg ($\ln$ -transformed) as a function of the first three water quality components extracted from PCA (Table 26), sulfate ($\log_{10}$ -transformed), and χ . The fourth extracted component from the water quality PCA (interpreted as groundwater influence) was not included because previous model analysis suggested that this variable had little, if any, effect on fish tissue Hg concentrations. Log-transformed sulfate concentrations were used in the neural network model rather than either of piecewise or cubic spline transformed values derived in Section 4.1 because of the inherent ability of neural network modeling to identify or elucidate nonlinear relationships.

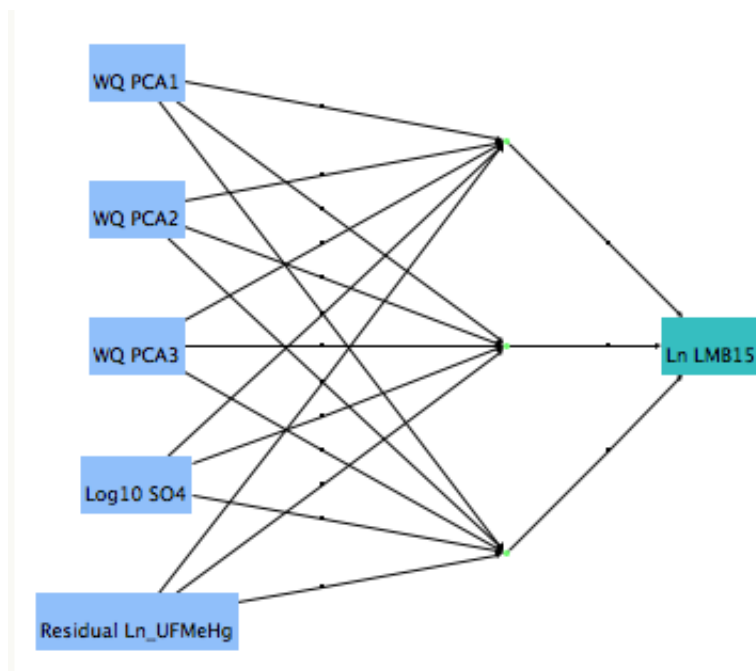


Figure 68. Neural network model structure depicting the relationship between the model input variables and the dependent variable, $\ln$ -transformed LMB Hg, mediated by three nodes in one hidden layer.

The neural network modeling was conducted using a calibration or training data set to construct the model, and a validation data set to evaluate and verify the performance of the model. 100 samples were selected randomly for the training set, and the remaining 26 observations (*i.e.*, the two high conductivity sites that exceeded FDEP water quality criteria were excluded from the model) used for model validation. Results comparing the overall fit, RMSE, and mean absolute deviation are shown in Table 30. Note that the

independent variable profile plot shown in Figure 70 indicates an inflection point for the effect of sulfate (~ 5 mg/L) that corresponds to the estimated change-point derived during the initial process of variable transformation (Section 4.1).

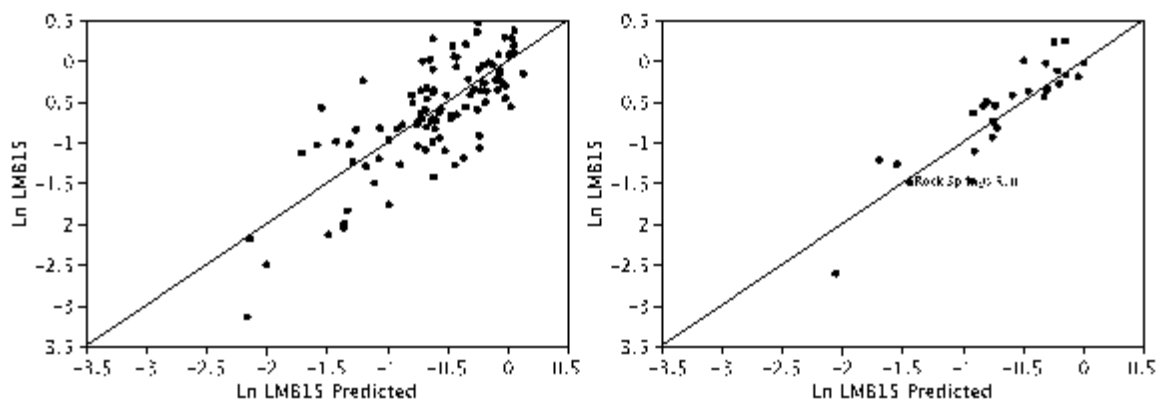


Figure 69. Observed vs. predicted results from neural network modeling of LMB Hg using the model structure shown in Figure 68. Left hand panel – training or calibration data set results (N = 100); right hand panel – results for the validation data set (N = 26).

Table 30. Neural network modeling results for model calibration and validation for model shown in Figure 68.

Variable	Data Set (Calibration or Validation)	
	Calibration	Validation
r^2	0.5915744	0.7961512
RMSE	0.4188257	0.2819952
Mean Abs Dev	0.329199	0.2262228
SSE	17.541496	2.0675535
N	100	26

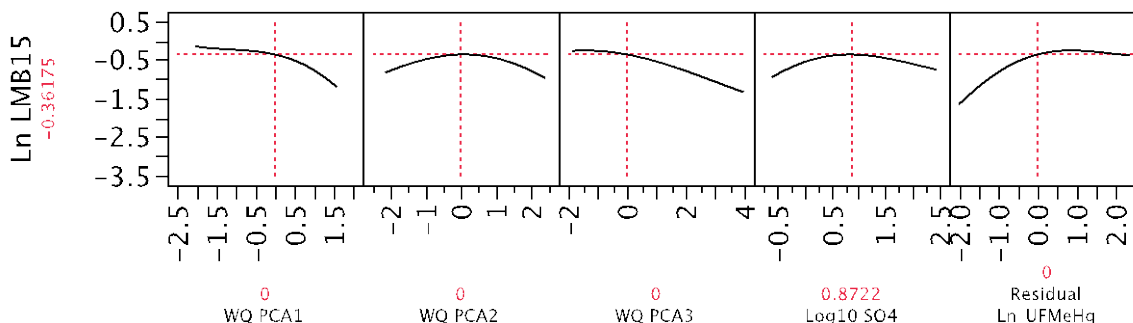


Figure 70. Profile plot showing the functional shapes of the fitted relationship between the input variables and LMB Hg (ln-transformed) for the neural network shown in Figure 68 and summarized in Table 24.

Classification and Regression Tree (CART Model)

Classification and regression tree (CART) analysis was conducted as an alternative approach towards accounting for inherent nonlinearity relationships between LMB Hg and various water quality variables. As described in Section 6.1, CART is a non-parametric approach makes no assumption that the underlying relationships between the predictor variables and the dependent variable are linear. CART recursively partitions the data into a series of dichotomous groups based on the values of the independent variables that best predict a value of the dependent variable (SAS Institute, Inc, 2011).

CART modeling was conducted using JMP 9.0.3 (SAS, 2011). In the implementation of the CART model for TMDL streams, LMB Hg concentration is the dependent variable, with mean values based on the observed data calculated for each partitioned group, along with the standard deviation of the mean. Similar to the previous MLR modeling, three observations were excluded from the analysis – the two high conductivity sites; and Rock Springs Run. Input variables in the final CART model included pH, conductivity, color, sulfate, TP, TKN, chlorophyll a, dissolved oxygen % saturation, and water resource type (whether the site was classified as a river or stream by the SMN). Results from the CART analysis are summarized in Table 29. The choice of when to terminate further model splits was based on minimizing the AIC; this resulted in a model with 14 splits. Figure 71 compares the observed with predicted LMB Hg concentrations, while Figure 72 shows how the coefficient of determination for the full model and the cross-validation (5-fold cross-validation) results changes with the number of splits incorporated into the model. Variable contributions to variance in the LMB Hg followed the following sequence of importance (based on model sum of squares):

Table 31. Summary results for CART modeling LMB Hg concentrations in Hg TMDL streams as a function of pH, sulfate, conductivity, color, and TP concentrations

Calibration/ Validation	r^2	RMSE	SSE	Number of Splits	AICc
Overall	0.655	0.196	4.785	14	-16.074
K-fold	0.444		6.192		

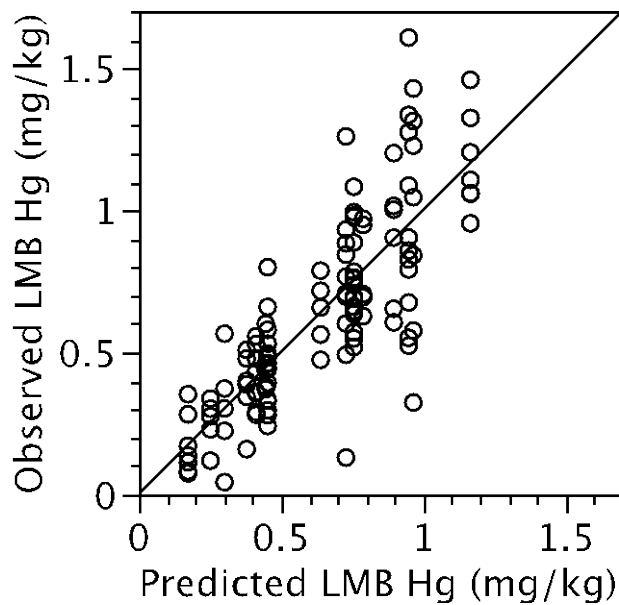


Figure 71. Comparison of observed LMB Hg concentrations with predicted concentrations obtained from the CART model summarized in Table 31.

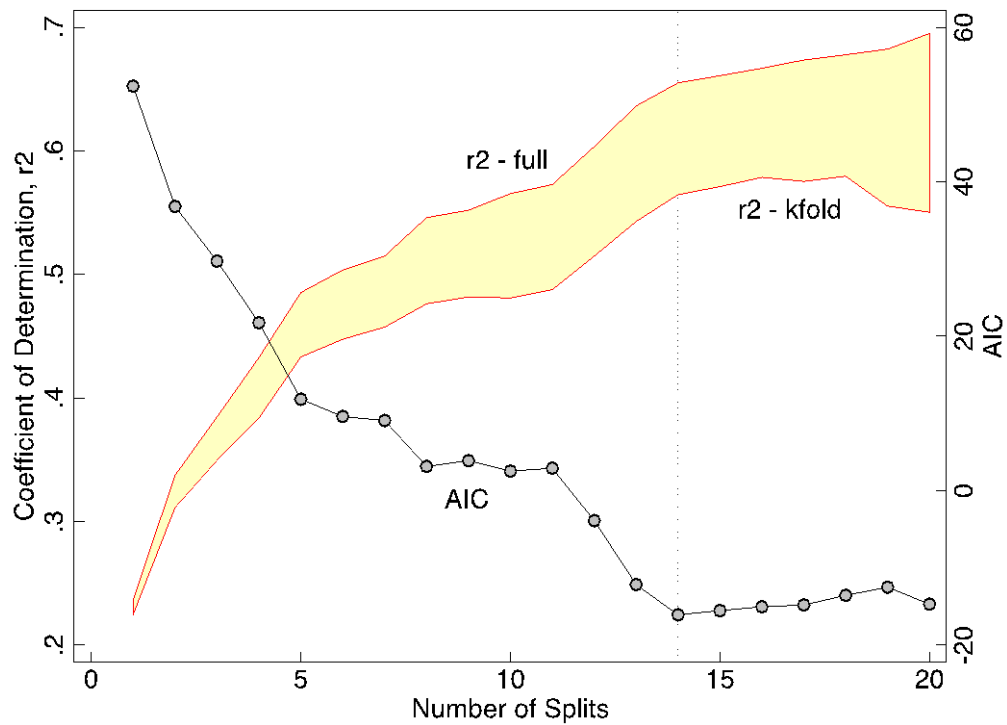


Figure 72. Effect of number of splits implemented in CART model of stream LMB Hg concentrations on fit for both the calibration and validation data sets (k fold cross-validation – 5 folds) and AIC statistic.

Table 32. Summary of independent variable contributions to CART model sum of squares. Total model SS equals 7.8084407.

Model Variable	Number of Splits	Sum of Squares
pH	3	4.2663747
Dissolved oxygen % saturation	1	1.3995281
Specific conductance	3	1.0942215
TKN	1	0.9596817
SO4	2	0.8238349
TP	1	0.5405255

Discussion of Stream Modeling Results

Based on contributions to the model sum of squares, the most important variables in the CART model are:

pH >> DO % saturation > Conductivity > TKN > SO₄ > TP

The initial split in the CART model was for pH equal to 6.42. This break is evident in Figure 73, which shows the distribution of LMB Hg concentrations in the Hg TMDL streams as a function of pH intervals (10% deciles). This break also corresponds fairly closely to the minimum in the density function plot of the frequency of occurrence of stream and river segments (weighted by segment length) as a function of pH. This minimum occurs at ~ pH 5.7 and essentially corresponds to the pH of natural waters where carbonate alkalinity is exhausted, and the ability of the water to resist changes in pH for a given change in acid neutralizing capacity [ANC] is minimal (pH = 5.65; Figure 75; *cf.*, Stumm and Morgan, 1983). The lower pH group of stream segments is further split into two groups based on conductivity (split point equals 23 $\mu\text{S}/\text{cm}$) with higher LMB Hg concentrations associated with the lower conductivity groups. While it is not clear if there is a direct functional relationship between conductivity and LMB Hg concentrations, the low conductance value serves as a clear discriminator for pristine systems located in the Florida panhandle.

The primary split in the high pH group of streams occurs in the CART model based on the DO % saturation (split point equals 30.6%). The lower DO % saturation group is defined by much higher LMB Hg concentrations (0.97 vs. 0.50 mg/kg mean values for low and high DO % saturation groups, respectively). This split almost certainly reflects the effects of riparian wetlands and DOC transport on methyl Hg production and transport, respectively. Further splitting of the high DO % saturation group occurs as a function of sulfate concentrations (split point = 2.5 mg/L). This cut point approximates the shift in slope observed in the relationship between LMB Hg concentrations and sulfate concentrations that occurs between 2.6 and 4.3 mg/L (Figure 11), with higher LMB Hg concentrations corresponding to the lower sulfate concentration group. As the model splits more deeply, interpretations become more difficult because of the increasingly complex nature of the groupings.

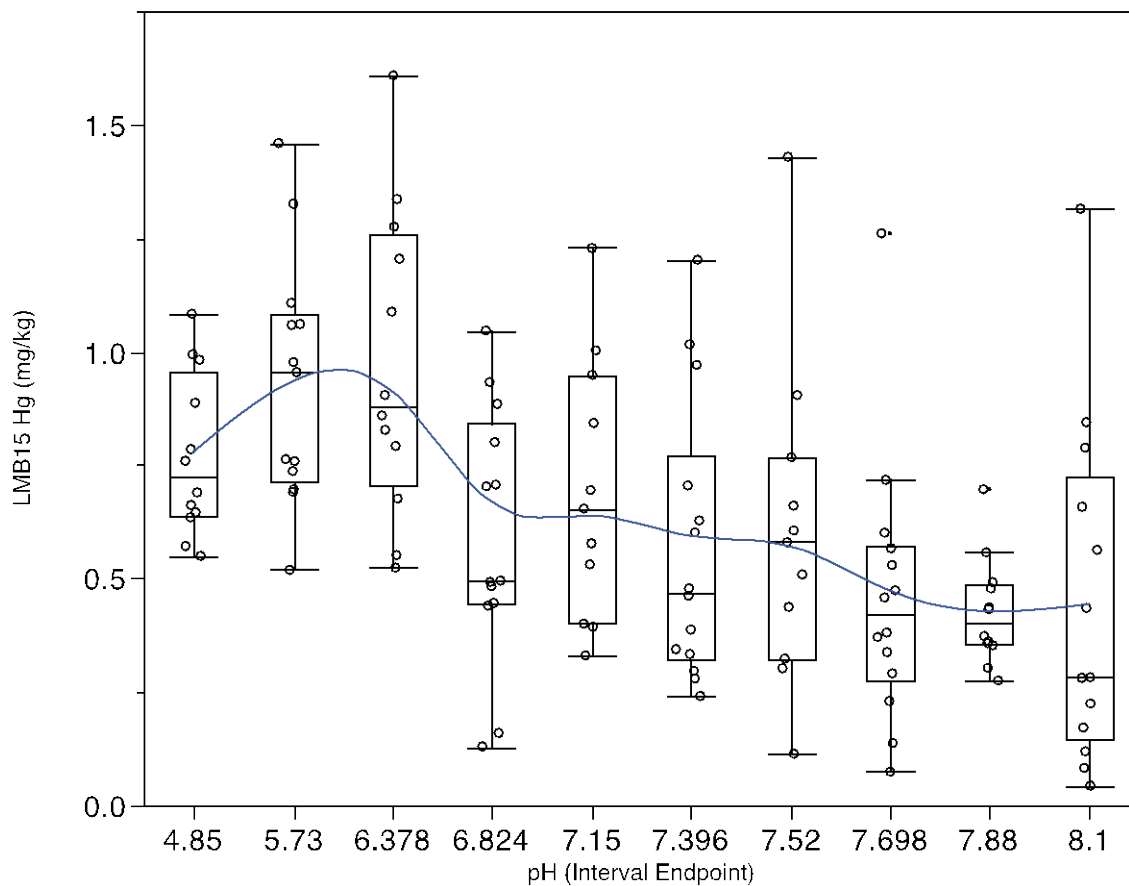


Figure 73. Boxplot of the relationship between LMB Hg concentrations and pH for Hg TMDL streams used in constructing the CART model. pH is shown as intervals, with each interval encompassing ~ 10% of the data sorted by pH. Each interval value shown on the abscissa corresponds to the decile endpoint.

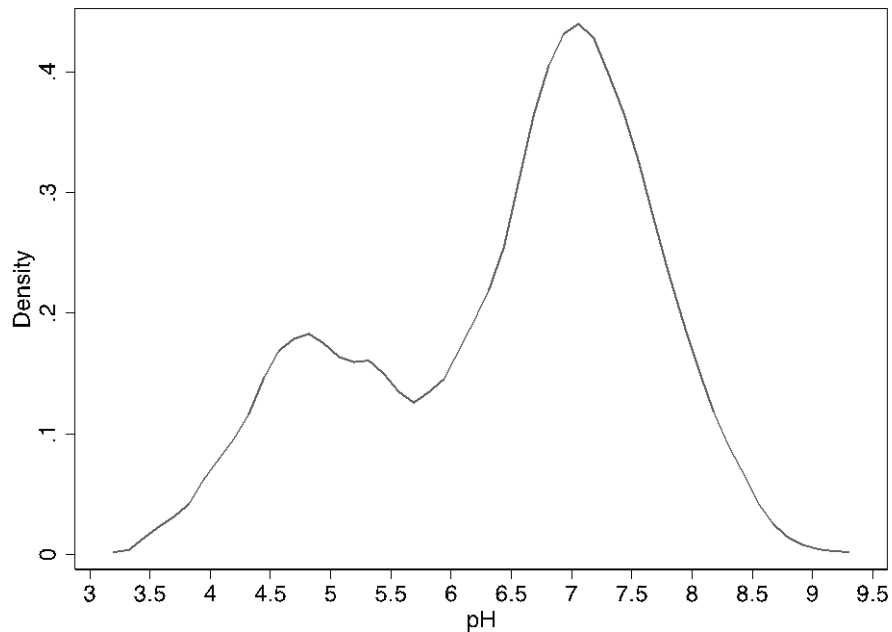


Figure 74. Kernel density estimation of the weighted distribution of pH in SMN streams for 2009 and 2010. Bandwidth for smoothing equals 0.3.

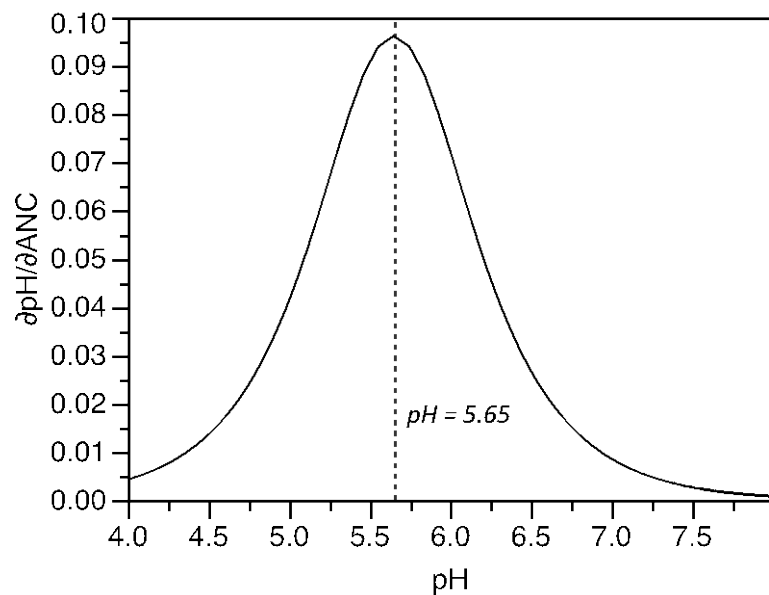


Figure 75. Sensitivity of ambient pH for natural waters controlled by carbonate alkalinity to changes in acid neutralizing capacity (ANC). Units of ANC in the ratio $\partial \text{pH} / \partial \text{ANC}$ are $\mu\text{eq/L}$.

Effects of Sulfate

Similar to lakes, the effects of sulfate on Hg accumulation in Florida streams can be either direct (through stimulation of sulfate reducing bacteria and promoting higher rates of Hg methylation) or indirect (through the acidifying effects of excess sulfate). Unlike previous analyses conducted on both the Hg TMDL lakes (Section 4.1) and the Everglades (Pollman, 2012), however, the results from this study suggest that, if there is a sulfate concentration in streams that correlates with the maximum potential for methylation, it occurs at levels below the minimum values measured in this study (0.26 mg/L). This apparent discrepancy may reflect more the fact that methyl Hg production occurs primarily *in situ* in the Everglades, and at least to some extent *in situ* in lakes (and, in particular, clearwater seepage lakes), while methyl Hg production for Florida streams and rivers most likely occurs predominantly in the flooded soils of riparian wetlands. Since methylation in riparian wetlands will be responding to the pool of sulfate stored in wetland soils rather than streamwater concentrations *per se*, there may be no exact correlation between ambient streamwater concentrations of sulfate and the maximum methylation potential for riparian wetlands.

Plots of the relationship between alkalinity plus DOC and SO₄ as a function of the sum of Ca + Mg further illustrate the magnitude of soil retention of sulfate inputs. When compared to many Florida lakes (Figure 51), sulfate additions contribute to much smaller reductions in alkalinity, particularly for streams and rivers with ambient sulfate concentrations below ~ 1,000 mg/L (Figure 76). This difference is a direct consequence of the relative differences in the characteristic hydrologic flow paths for lakes (and seepage lakes in particular) compared to streams; streamwater concentrations of sulfate reflect contributions from overland runoff and baseflow, while concentrations of sulfate in seepage lakes in particular will reflect direct atmospheric inputs (Pollman *et al.*, 1991). Sulfate concentrations in base flow in turn are attenuated to a very appreciable extent (*cf.* Figure 76) by both adsorption to soil surfaces and removal via sulfate reduction. Because of the inability to identify an optimal sulfate concentration for maximizing Hg methylation in Florida stream and river catchments and the negative correlation of sulfate with methylation potential it is easy to conclude that there likely is no direct benefit to reductions in excess sulfate. Such a conclusion also is suggested by the CART model, which indicates that reductions in sulfate would offer limited or no direct benefit, both because of how sulfate interacts in the model with respect to overall explanatory power and the functional nature (negative association) of the relationship. It is important to recognize, however, that using streamwater concentrations of sulfate to infer how riparian wetland methylation of Hg will respond to mitigation strategies that involve loading reductions of sulfate may be difficult, particularly if the strategy considered involves steep reductions in sulfate. Restated, reductions in excess sulfate loadings *may* indeed result in reduced production rates on methyl Hg in riparian wetlands – certainly there is empirical evidence from other studies to suggest such a possibility (Engstrom *et al.*, 2011); the empirical relationships evaluated in this study between streamwater sulfate concentrations and LMB Hg concentrations, however, provide no real insight into the quantitative dynamics of riparian wetland methylation and sulfate loadings. With respect to the indirect benefits of reducing excess sulfate loadings on alkalinity and thus biota Hg concentrations, however, the evidence is more clear: given the small contributions of sulfate to the alkalinity plots in Figure 76, reducing excess sulfate will not have very substantial benefits with respect to ameliorating stream acidification and, in all likelihood, offer only limited benefits with ameliorating LMB Hg concentrations in most Florida streams as well.

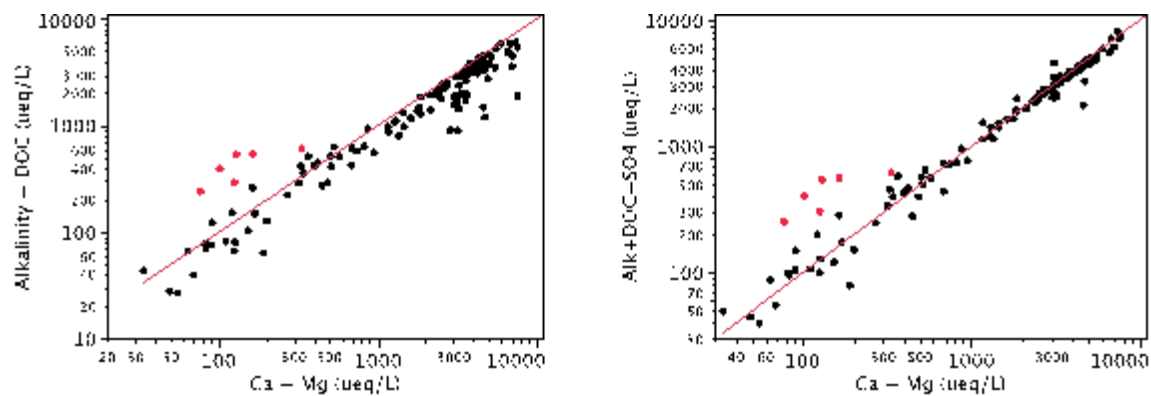


Figure 76. Relationship between Ca plus Mg concentrations (as sources of alkalinity), and alkalinity plus DOC (left hand panel) and alkalinity plus DOC and sulfate in the Hg TMDL streams and rivers. Solid black line shows the expected line for the sum of the anionic species based on the sum of Ca plus Mg.

Everglades Statistical Model

Multiple Linear Regression Model

A data set comprising 112 LMB Hg concentrations (each value corresponding to a unique combination of site, year and hydroperiod) standardized to a length of 14" (Section 3.3.1) was merged with the integrated *Gambusia* data set (Section 2.2.2) by site-year-hydroperiod. This merged or working data set, which included 54 observations with concomitant *Gambusia* Hg and standardized LMB Hg concentrations for the same site-year-hydroperiod, was used to develop the inferential model relating standardized LMB Hg concentrations to *Gambusia* Hg concentration.

The final model relating standardized LMB Hg concentrations as a function of *Gambusia* Hg concentrations had the following form:

$$\ln \text{ LMB Hg} = \text{Year} + \ln \text{ GMB Hg} + \text{LNWR_Flag} \quad (2)$$

where $\ln \text{ GMB Hg}$ is the $\ln$ -transformed concentration of Hg in *Gambusia* at a given site, LNWR_Flag is a dummy variable that indicates whether a given site was located within the Loxahatchee National Wildlife Refuge, and Year defines the year when the sample was collected. The initial model had $r^2 = 0.665$ ($p < 0.0001$), with the RMSE = 0.305; the PRESS RMSE = 0.332. Estimates for the model coefficients and their significance are shown in Table 33.

Table 33. Fitted coefficients, standard error, and t statistics for parameter coefficient significance for the preliminary MLR model to predict LMB Hg (ln-transformed) concentrations. Analysis includes all 54 values of joint observations of LMB and *Gambusia* (aggregated by site, year, and hydroperiod).

Model variable	Estimate	Standard Error	t Ratio	Prob> t
Intercept	96.659437	21.75266	4.44	<.0001
Year	-0.049184	0.010794	-4.56	<.0001
$\ln \text{ GMB Hg}$	0.3162625	0.0673	4.70	<.0001
LNWR Area[0]*	0.1970334	0.050302	3.92	0.0003

*Coefficient estimate for *LNWR Area* when the dummy variable = 0; otherwise, the coefficient is 0.

Figure 77 shows both the fit between observed values and values predicted by the model. As can be seen from the figure, one outlier (site = LNWR 1; year = 1995 and hydroperiod = dry) appears to exert an inordinate amount of leverage on the regression. This is confirmed through the Cook's D test, which is an objective measure of how much a given observation influences the regression model as a whole (Hamilton, 2009). An inspection of the calculated Cook's D test value for the observation in question relative to the distribution of values for the remaining observations clearly identifies the observation as an outlier (Figure 78), and it was subsequently removed from analysis.

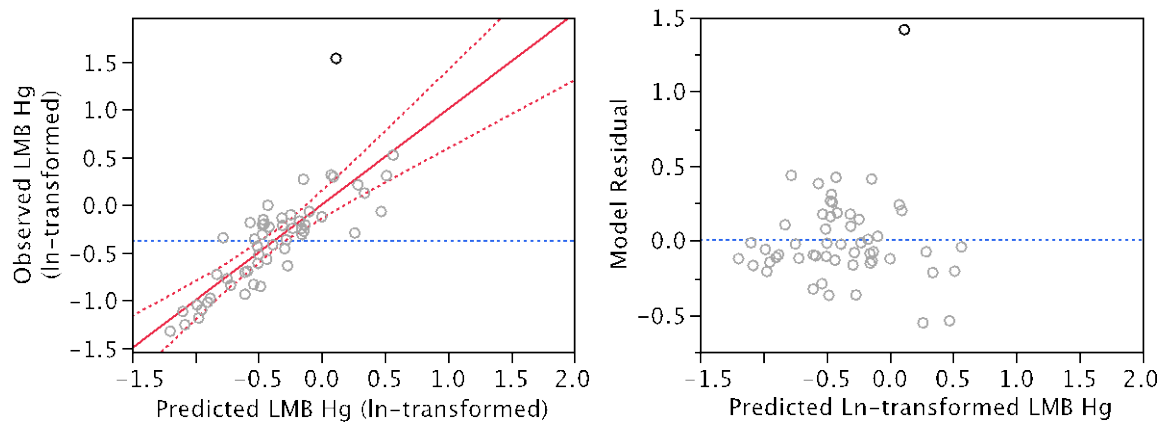


Figure 77. Model results for predicting standardized LMB Hg concentrations as a function of Gambusia Hg concentrations within the Everglades. Left hand panel: Comparison of actual with predicted concentrations. Right hand panel: plot of model residuals as a function of predicted values. Highlighted observation is an outlier defined by the Cook's D influence statistic (see Figure 78).

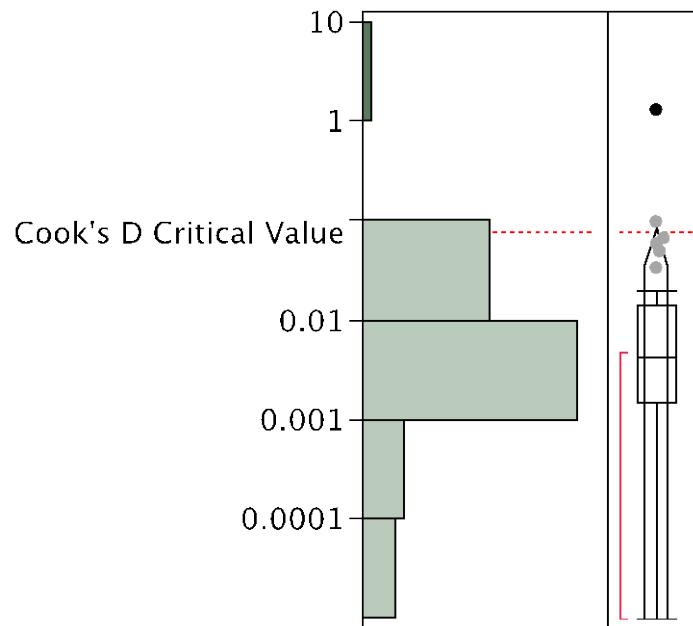


Figure 78. Distribution of calculated values for Cook's D influence statistic for model equation (2) using all 54 observations. Critical value for Cook's D defining potential outliers is given by $4/n$ (0.074).

Removal of the outlier resulted in substantial improvement in model fit ($r^2 = 0.779$), residual behavior (Figure 79) and robustness of predictions (as indicated by the PRESS statistic, which evaluates the predictive error for individual observations left out of the regression model building – *cf.* Freund *et al.* 2003; PRESS RMSE = 0.221, which also agrees closely with the model RMSE of 0.215). The estimated coefficients from the refitted MLR model excluding the single outlier are summarized in Table 34:

Table 34. Same as Table 33, except that the Cook's D outlier identified in Figure 78 has been excluded from the analysis. Resultant N = 53.

Model variable	Estimate	Standard Error	<i>t</i> Ratio	Prob> <i>t</i>
Intercept	86.321422	15.36331	5.62	<.0001
Year	-0.043888	0.007626	-5.76	<.0001
<i>Ln</i> GMB Hg	0.2384072	0.048538	4.91	<.0001
LNWR Area[0]	0.2684308	0.036729	7.31	<.0001

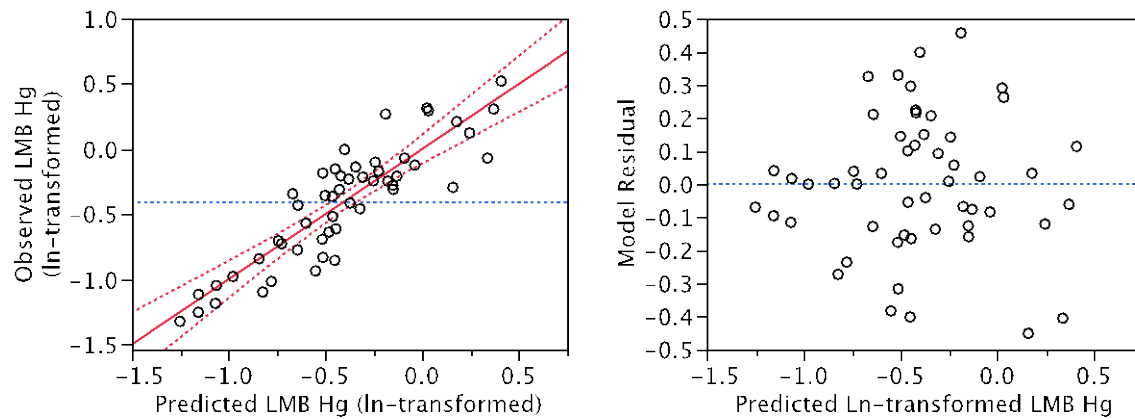


Figure 79. Model results for predicting standardized LMB Hg concentrations as a function of *Gambusia* Hg concentrations within the Everglades with outlier identified by Cook's D analysis removed. Right hand panel: Comparison of actual with predicted concentrations. Left hand panel: plot of model residuals as a function of predicted values.

Generalized Linear Model Results

Similar to the modeling conducted for LMB Hg concentrations in the TMDL lakes (Section 5.2.3), LMB Hg concentrations inferred from *Gambusia* Hg concentrations were modeled using the $\ln$ -transform of LMB Hg concentrations to linearize the modeled relationship. As discussed in Section 5.2.6, back-transformation of the predicted $\ln$ -transformed LMB Hg – which is necessary to compute the weighted cumulative frequency distribution of LMB Hg across the Everglades as a prerequisite to calculating the TMDL load reductions – imposes a bias unless model error is accounted for in the transformation. Although this bias is small (0.013) and not significantly different than 0 ($t = 0.5712$), the model was refit using GLM to compare the results. Not surprisingly, because the bias in the back-transformation of the MLR is comparatively small, the predictions between the two models show quite close agreement (Figure 80).

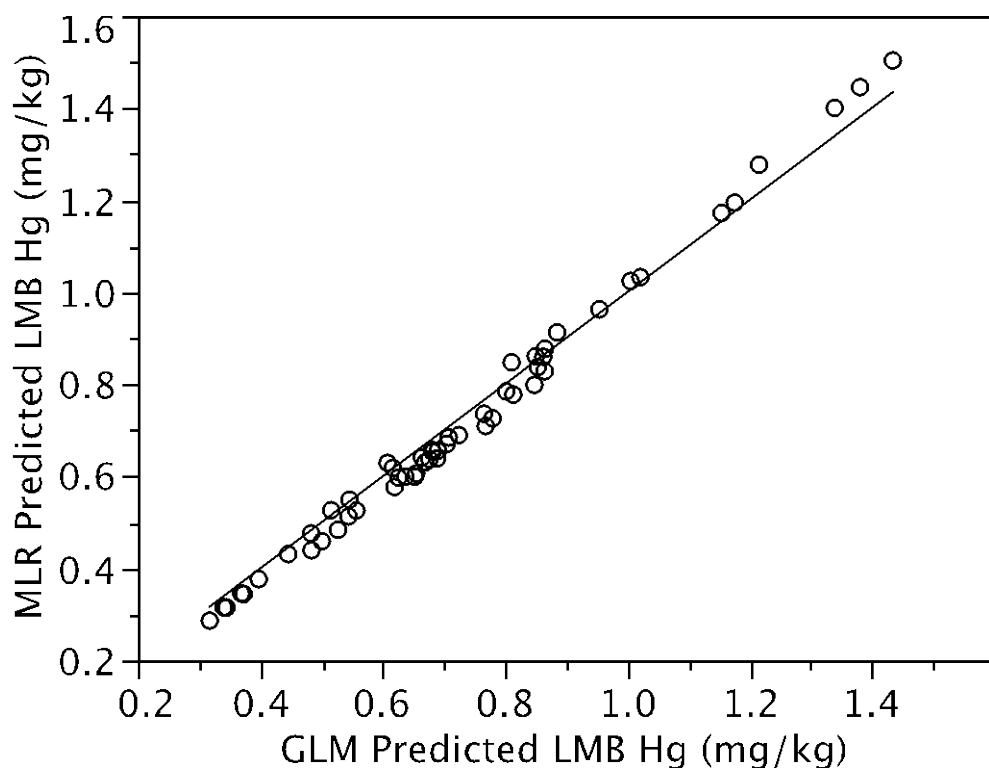


Figure 80. Comparison between predicted LMB Hg concentrations in the Everglades obtained from the MLR model (back-transformed with no error correction), and from GLM. Solid line shows 1:1 agreement.

Using GLM nonetheless by definition eliminates any bias (calculated bias = -0.002 mg/kg), and the GLM model was used in subsequent calculations of weighted distributions of LMB Hg in the Everglades. Estimates of the model coefficients for the GLM model are presented in Table 35.

Table 35. Fitted coefficients, standard error, and z statistics for parameter coefficient significance for the GLM model to predict LMB Hg concentrations in the Everglades as a function of measured Gambusia Hg concentration. Analysis conducted with a single outlier removed; N = 53.

Model variable	Estimate	Standard error	z	P> z
LNWR Flag	-0.5276	0.1162713	-4.54	< 0.0001
Year	-0.0345729	0.0080334	-4.3	< 0.0001
<i>Ln Gambusia</i> Hg	0.2304601	0.0479767	4.8	< 0.0001
Intercept	67.99784	16.20053	4.2	< 0.0001

A normal quantile plot of the GLM model deviance residuals shows tailing and departure from the expected normal distribution at both ends of the distribution (Figure 81). As a result, the robustness of the model estimated parameter coefficients was evaluated by conducting a bootstrap analysis of the deviance residuals. A comparison of the significance of the model coefficients obtained from the bootstrap analysis with the significance obtained from the original GLM estimates verifies that the coefficient estimates are sufficiently robust.

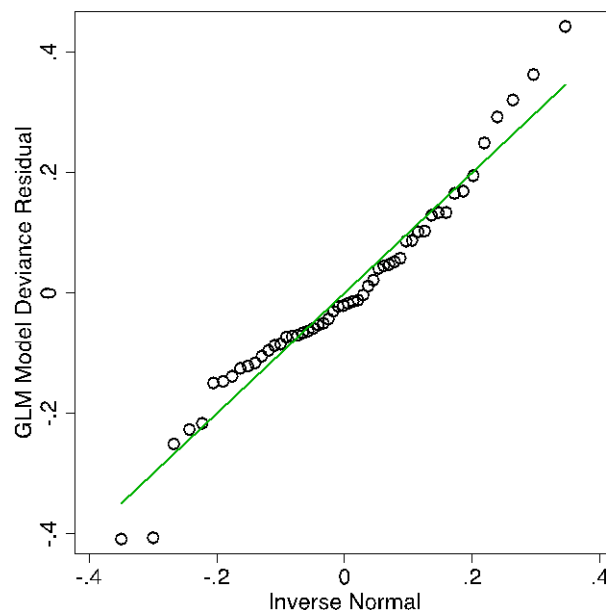


Figure 81. Normal quantile plot of the Everglades LMB GLM model deviance residuals. Solid green line shows the expected distribution of the residuals if the residuals are normally distributed.

Table 36. Significance and confidence limits for parameter coefficient estimates obtained from both the original GLM model and bootstrap analysis (N replications = 1,000).

				95% Confidence Interval	
Model variable	Standard error	z	P> z	LCL	UCL
Original GLM Model Estimates					
LNWR Flag	0.1162713	-4.54	< 0.001	-0.7554876	-0.2997124
Year	0.0080334	-4.3	< 0.001	-0.0503181	-0.0188278
<i>Ln Gambusia</i> Hg	0.0479767	4.8	< 0.001	0.1364275	0.3244927
Intercept	16.20053	4.2	< 0.001	36.24538	99.75031
Robust Estimates					
LNWR Flag	0.0589622	-8.95	< 0.001	-0.6431638	-0.4120361
Year	0.0081601	-4.24	< 0.001	-0.0505663	-0.0185795
<i>Ln Gambusia</i> Hg	0.0512728	4.49	< 0.001	0.1299673	0.3309529
Intercept	16.4079	4.14	< 0.001	35.83895	100.1567

Validation of the GLM model for making “out-of-sample” predictions was performed by conducting a jackknife analysis ($N = 53$). The RMSE from the jackknife predictions was 0.180 agrees reasonably well with the RMSE obtained from the original GLM calibration (0.172).

Estimating the Magnitude of Statewide Resources at Risk

Overview

The statistical models developed previously as part of this project to predict concentrations of Hg in the tissue of LMB normalized to a length of 15 inches (Sections 5, 6, and 7) were used in conjunction with water quality data collected by FDEP as part of its Status Monitoring Network (SMN; FDEP, 2010) to develop cumulative distribution function (*cdf*) plots of LMB Hg concentrations in lakes and streams across Florida. The SMN utilizes a stratified randomized sampling approach that, because of its probabilistic approach towards sampling, enables inferential estimates of the magnitude of a resource type with fish tissue concentrations above or below a concentration level of interest. Moreover, these inferential estimates can be developed with a known degree of confidence. In lieu of sampling the full suite of Florida lakes and streams, utilizing the randomized sampling approach adopted by the SMN allows unbiased estimates. Stratified randomized sampling involves separating the total population of interest into different groups or strata delineated by, for example, lake hydrologic type or size, disturbance category, or ecoregions. Lakes or stream reaches within any given stratum are then selected at random, and weights are then applied to each sample based on the total magnitude of the resource present within the stratum. The sample weights are then used to develop *cdf* curves for variables of interest:

$$f_{i, \text{weighted}} = \frac{W_i}{\sum_{i=1}^{i=N} W_i}$$

where W_i is the sample weight for sample i , N is the total number of samples collected in the sampling frame, and $f_{i, \text{weighted}}$ is the calculated fraction of the total distribution of the resource represented by sample i .

Perhaps the most significant early application of a stratified randomized sampling approach towards developing estimates of population characteristics for lakes and streams was the National Surface Water Survey (NSWS) and the National Streamwater Survey (NSS) conducted by USEPA in the mid-1980's as part of the National Acid Precipitation Assessment Program (NAPAP). As part of the NSWS, USEPA randomly sampled 140 lakes in 1984 that were then used to infer major ion chemical characteristics for a target population of 2,128 lakes (Linthurst *et al.*, 1986; Kanciruk *et al.*, 1986; and Pollman and Canfield, 1991). Pollman (unpublished data) subsequently used the Florida NSWS data to infer LMB Hg concentrations in the NSWS Florida target population. Pollman used a statistical model that related LMB Hg concentrations (normalized to age 3) that he developed from fish and ambient water chemistry data collected by T. Lange and colleagues of the Florida Fish and Wildlife Commission from 80 lakes sampled across Florida between 2000 and 2002. While this early analysis was useful in identifying the potential magnitude of problematically high fish tissue Hg concentrations in Florida lakes, there were two difficulties with directly utilizing this analysis as part of Florida's statewide Hg TMDL. First, the lakes included in the NSWS target population were, by definition, restricted to lakes without obvious sources of watershed disturbance; as a result, the inferential calculation conducted by Pollman ignores the remaining ~ 5,500 lakes present in Florida. Second, the NSWS sampling for lake water chemistry was collected 25 years previous to the target year of 2009 for

which the Florida TMDL has focused its efforts, and it is uncertain whether significant changes in water chemistry have occurred in these lakes in the intervening years. The USEPA has also used stratified randomized sampling to estimate the distribution of key water and soil chemistry variables, as well as *Gambusia* Hg concentrations, in the Everglades as part of its Regional Environmental Monitoring and Assessment Program (R-EMAP; Scheidt and Kalla, 2007).

The SMN separates its surface water resources into two primary categories based on flow dynamics (flowing waters or lotic and still waters or lentic). The FDEP SMN Monitoring Design Document (FDEP, 2010) further defines lotic and lentic waters as follows:

The lotic group consists of rivers, springs, spring runs, and sinking streams. Certain waters are excluded due to saline or marine conditions, such as estuaries and marine waters, while others are removed because other sections of FDEP focus on those resources, e.g., wetlands, springs, and spring runs . . . [T]he lentic group consists of many types of natural lakes, including sandhill lakes, sinkhole lakes, and oxbow lakes, and established reservoirs (i.e. not impoundments used for agriculture). These range in size from less than an acre to over 500,000 acres. Artificial waterbodies, such as stormwater retention ponds, golf course ponds, or other man-made water features that are not waters of the state are common but are not part of the target population, and are removed from the resource list frame.

The definition of lakes by FDEP is further constrained by a minimum depth requirement (at least 1 meter (m) in depth at the deepest part of the lake) and the requirement that any given lake is not in direct contact or influenced by oceanic waters.

The SMN divides lakes into two groups based on surface area:

- Small lakes ($4 < X < 10$ hectares (ha)), where X is the surface area of the lake; and
- Large lakes ($X > 10$ ha)

For lotic waters, the SMN distinguishes between streams and rivers, with rivers initially identified by FDEP and state water management district staff; the remaining lotic waters are subsequently defined as streams.

The SMN collects samples for a variety of water quality parameters, including major ions, nutrients, and physical parameters annually. Sampling locations or sites for each annual cycle are selected randomly using a generalized random Tessellation stratified (GRTS) sampling design. This design, which is supported by EPA's Environmental Monitoring and Assessment Program (EMAP), ensures that the sites selected are representative of the target resources and that their selection is not biased.

The SMN has stratified the state into six non-overlapping geographic zones from which sites for sampling are randomly identified (Figure 83). FDEP incorporated the use of these strata because it optimizes the sampling design based on the variability of the water resource, thus enhancing the power to detect differences in water quality. The target number of samples established by the SMN for rivers, streams, and each of the two resource types within lakes is 60, yielding a total of 120 lake samples, and 120 stream/river samples. With a sample size of 60, the 95% confidence interval for inferring the statewide distribution of a sampled water quality parameter for each of the four surface water groups is $\pm 12\%$.

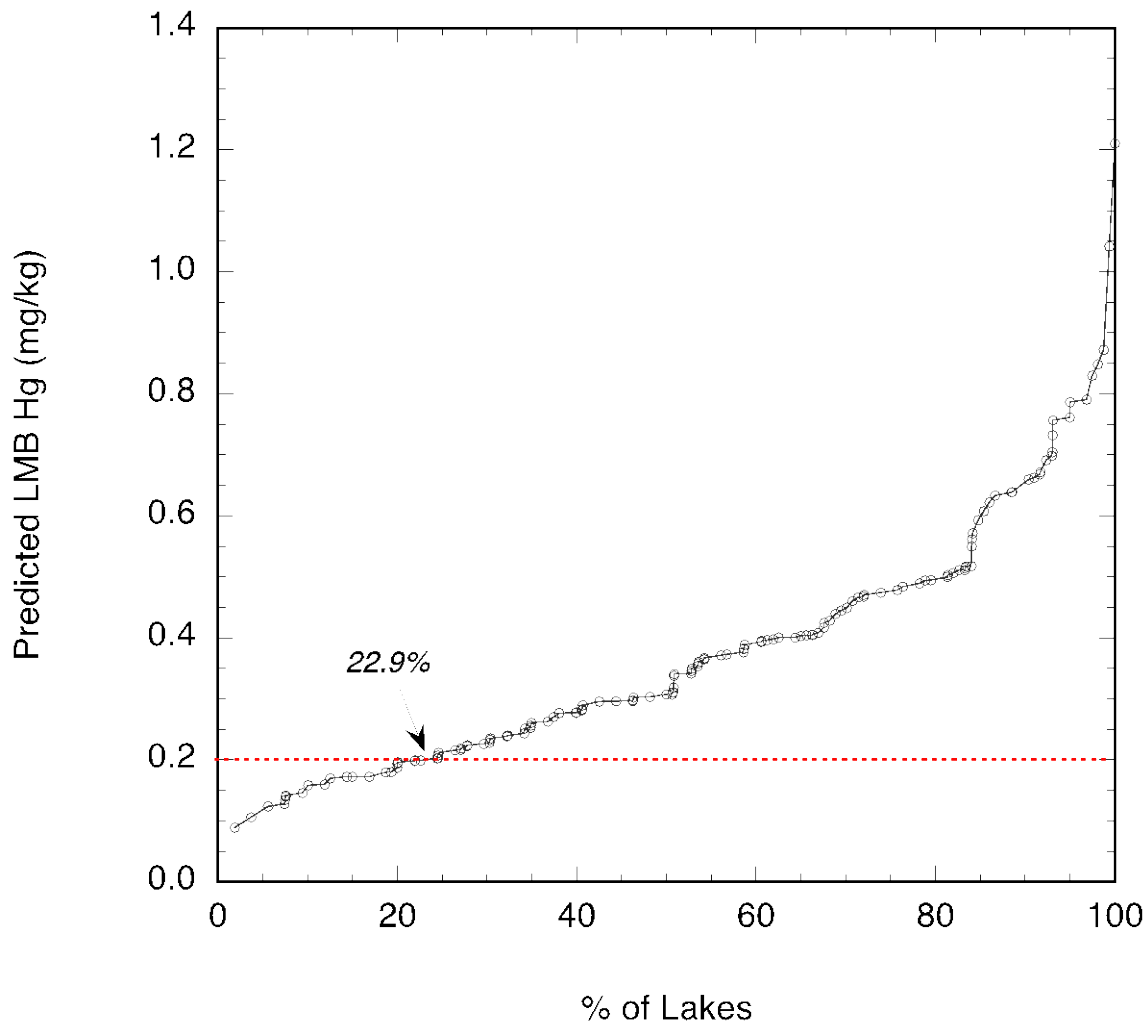


Figure 82. Early estimate of the cdf in undisturbed Florida lakes for Hg concentrations in LMB (normalized to age 3) inferred from empirical model developed by Pollman (unpublished data) from the Lange et al. (unpublished data) 80 lakes study and using water chemistry data collected by the NSW. The weighted cdf indicates that nearly 77% of the lakes in the target population (2,089 lakes in this analysis) have age 3 LMB Hg concentrations in excess of 0.2 mg/kg.

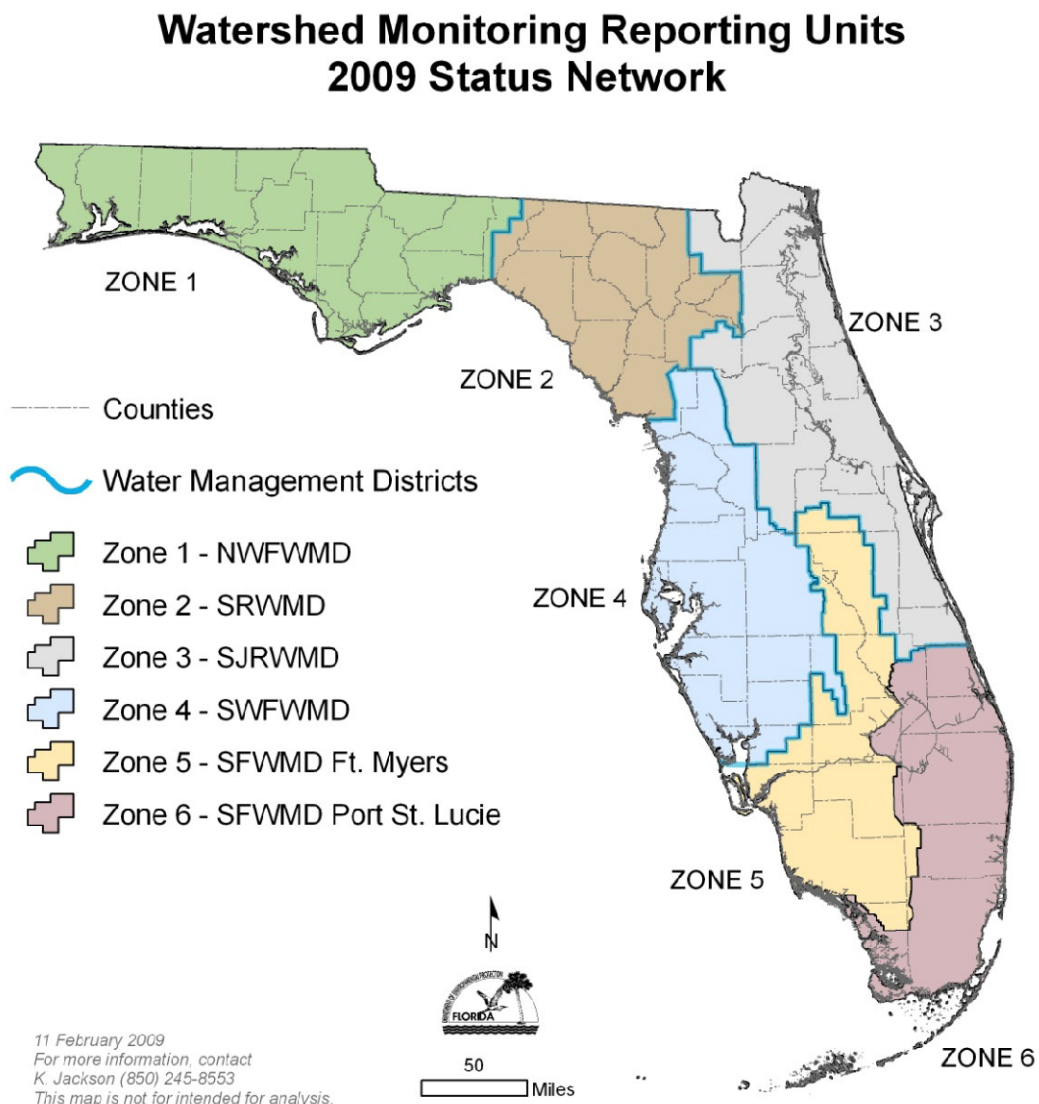


Figure 83. Geographic zones used as sampling strata for the SMN (from FDEP, 2010).

Application of the Lake Empirical Model to SMN Lakes

Applicability of GLM Model to SMN Lakes (2009) Data Set

The final form of the statistical model used to infer LMB Hg concentrations in Florida lakes was a generalized linear model (GLM) that related LMB Hg concentrations to three water quality variables: alkalinity, chlorophyll *a*, and sulfate; and two sediment chemistry variables (an extracted from principal components analysis (PCA; the two components were identified as an urban runoff component and an atmospheric deposition component). A log-link function was used to relate LMB Hg to the independent variables. Alkalinity and chlorophyll *a* were log-transformed, and sulfate was transformed using a cubic spline to better provide linear relationships between the independent variables and LMB Hg in the log-link model. The lake model described in Section 5.2.1.1 was developed using multiple linear regression (MLR) and log-transformed LMB Hg as the dependent variable. As discussed in Section 5.2.1.3, the necessary back-transformation imposed by directly modeling log-transformed LMB Hg introduces a bias that would need to be corrected when predicting the distribution of LMB Hg concentrations. GLM avoids this back-transformation, and was shown to be free of the bias imposed on the MLR results.

The water and sediment chemistry results from the 2009 SMN sampling were used as input to the GLM to infer the statewide distribution of the LMB Hg concentrations in 2009 for both large and small lakes. An issue that is inherent with any statistical model that is used for predictive purposes is the fact that the model is most appropriate when the values for the independent variables input to the model lie within the range of values used to construct the model. The initial sampling design sought to minimize the likelihood of values lying outside the range of values used to construct the model by selecting lakes that encompassed the range of concentrations of the three variables (pH, color, and chlorophyll *a*) initial analyses suggested as most important in determining LMB Hg concentrations (Pollman, 2008). Five concentration ranges spanning the breadth of the observed distribution of each of the three variables were defined using the SMN data base, and a 5 x 5 x 5 matrix was used to define “bins”, with each bin corresponding to one of the 125 mathematically possible joint occurrences. Lakes within the SMN were categorized according to bin, and a lake from within each populated bin was selected at random and sampled.

While the GLM model used chlorophyll *a* and alkalinity (which is fundamentally related to pH) as independent variables, it also used several variables that were not included in the development of the sampling matrix. Table 37 summarizes the range of values (minimum and maximum) for the input variables used to calibrate the GLM and compares these values with the upper and lower 2.5% values for the lakes sampled by the SMN in 2009. Because the overall SMN lakes database includes observations for lakes that are not truly freshwater, the SMN 2009 data set was screened to exclude non-freshwater lakes defined by chloride concentrations exceeding 1,500 mg/L (FDEP, 2008). This screening also was used in developing the target concentration interval bins used to sample lakes to develop the empirical model (Pollman, 2008). All the lakes in SMN 2009 data set met this criterion, and thus all were used in subsequent calculations. Because the target endpoint calculations are based on the 90% percentile distribution of inferred values (see Section 9), the possibility that the extreme tails beyond the upper and lower 2.5% are characterized with less certainty is of less concern. As indicated by Table 37, the calibration data set minimum and maximum input parameter values

encompass the interquantile (upper and lower 2.5%) range of the SMN 2009 lakes, with the exception of the upper limit for sediment atmospheric deposition principal component. Closer inspection of the distribution of values for the sediment atmospheric deposition principal component shows that the upper 5% value (2.64) lies within the range of calibration values, thus indicating that the range of input values for this parameter for the SMN 2009 is not problematic.

Perhaps of even more concern is the possibility that the joint combinations of variables used in developing the GLM model did not encompass the range of combinations embodied in the lakes sampled by the SMN in 2009. One approach towards evaluating this possibility is to compute the Mahalanobis distances for each of the observations in the combined data sets and determine to what extent the range of values in the SMN 2009 data set exceeds the values computed for the GLM model calibration data set. The Mahalanobis distance is a metric devised to identify multivariate outliers and is a measure of the multivariate distance of a given observation from the centroid of the remaining points (Tabachnick and Fidell, 2007). As can be seen in Figure 84, the upper limit of Mahalanobis values calculated for the SMN 2009 lakes data set lies below the upper limit of values calculated for the GLM model calibration data set. This provides further assurance that the range of input values used to infer the statewide distribution of LMB 15 in Florida lakes is appropriate for the GLM model.

Table 37. Comparison of data ranges for input variables used to calibrate the GLM model with the observed lower and upper 2.5 percentile ranges for the SMN 2009 lakes data set used to infer the statewide distribution of LMB Hg concentrations. Units for variables are as follows: chlorophyll *a* – log-transformed (mg/m³); alkalinity – log-transformed (μeq/L); sulfate – cubic spline transformation of sulfate initially log-transformed (μeq/L) (Section 4.1); sediment principal components – scores computed from log-transformed sediment concentrations (Section 5.1.2.3).

	Calibration		2009 SMN Lakes	
	Minimum	Maximum	2.5%	97.5%
Chlorophyll <i>a</i>	-0.46	5.52	-0.33	4.35
Alkalinity	2.56	8.28	2.56	7.99
Sulfate	-1.24	-0.67	-1.10	-0.67
Sediment – urban runoff	-1.57	4.63	-1.22	2.99
Sediment – atmospheric inputs	-2.86	2.90	-1.39	3.28

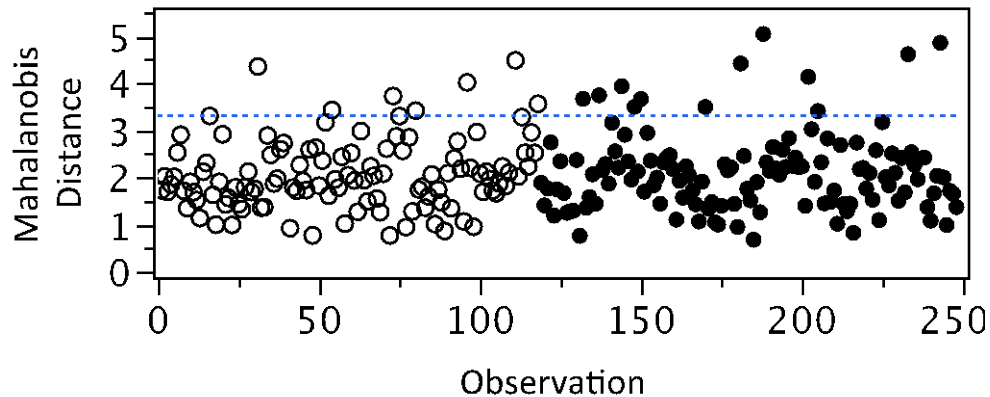


Figure 84. Calculated values for the multivariate Mahalanobis distances (calculated for the five GLM input variables) for the GLM calibration data set (filled circles; N = 127) and the SMN 2009 data set (open circles; N = 116).

Computation of Cumulative Distribution Function curves for the Statewide Distribution of Hg in Largemouth Bass in Florida Lakes

As indicated in Section 8.2.1, data from the SMN for 2009 were used to infer the statewide distribution of Hg concentrations in LMB (normalized to 15"). Weights based on surface area were provided by FDEP for both large and small lakes; in addition, weights based on numerical abundance (number of lakes) were provided for each small lake in the sampling frame. Table 38 summarizes the total values for both the original frame estimates for surface area, number of small lakes, and the sum of the respective sampling weights for each geographical zone used as a sampling stratum by the SMN in 2009.

Table 38. Summary of (1) original sampling frame estimates for large and small lake surface area, and number of small lakes; and (2) sum of respective sampling weights for each geographic zone sampled by the SMN in 2009. Total number of samples collected = 118 (60 large lakes and 58 small lakes).

SMN Zone	Original Frame Estimates			Sample Weights		
	Area (ha)		Number	Area		Number
	Large Lakes	Small Lakes	Small Lakes	Large Lakes	Small Lakes	Small Lakes
Z1	19101.289	2339.841	384	11160.068	553.158	209
Z2	10154.560	1266.076	206	5743.782	39.656	23
Z3	126976.899	8367.893	1361	97814.529	354.072	268
Z4	51605.445	5346.075	878	34066.248	449.091	194
Z5	65843.259	2739.173	448	56160.915	160.328	31
Z6	148053.568	2715.373	431	147466.977	94.180	38
Total (N = 118)	421735.020	22774.431	3708	352412.519	1650.485	762
Total (N 116)				315706.640	1646.645	760

Because sediment chemistry parameters were missing values for two of the SMN lakes, the final *N* used for inferring the *cdf* curves for LMB Hg concentrations using the GLM model was equal to 116 lakes (59 large lakes and 57 small lakes). The revised total sums for both the areal and numerical weights resulting from the two missing samples are included in Table 38. The predicted probability distributions for LMB Hg concentrations using the GLM model for 2009 are shown for both large lakes (weighted by area) and small lakes (weighted by number) in Figure 85. Comparing the two distributions shows very distinct and large differences between the two lake resource types, with LMB Hg much higher throughout almost the entire distribution. For example, the weighted median values for large and small lakes were 0.26 and 0.59 mg/kg, respectively, while the 90% concentrations were 0.39 and 1.03 mg/kg, respectively. These differences are related to several factors, including both higher pH and more enriched trophic state (as reflected in chlorophyll *a*) in large lakes, and higher distributions for both methylation potential (embodied in transformed sulfate) and the atmospheric deposition component in small lakes (Figure 86). The distribution of the urban runoff component is also higher in small lakes, but is a mitigating factor against higher LMB Hg concentrations. While the differences between lake resource type are distinct for each model variable, previous analysis of the contributions of variations in each variable to variations in LMB Hg concentrations (calculated using standardized β coefficients obtained from MLR modeling – see Section 5.2.3) suggest the following order greatest importance:

Alkalinity > chlorophyll *a* > urban runoff disturbance > atmospheric deposition > sulfate

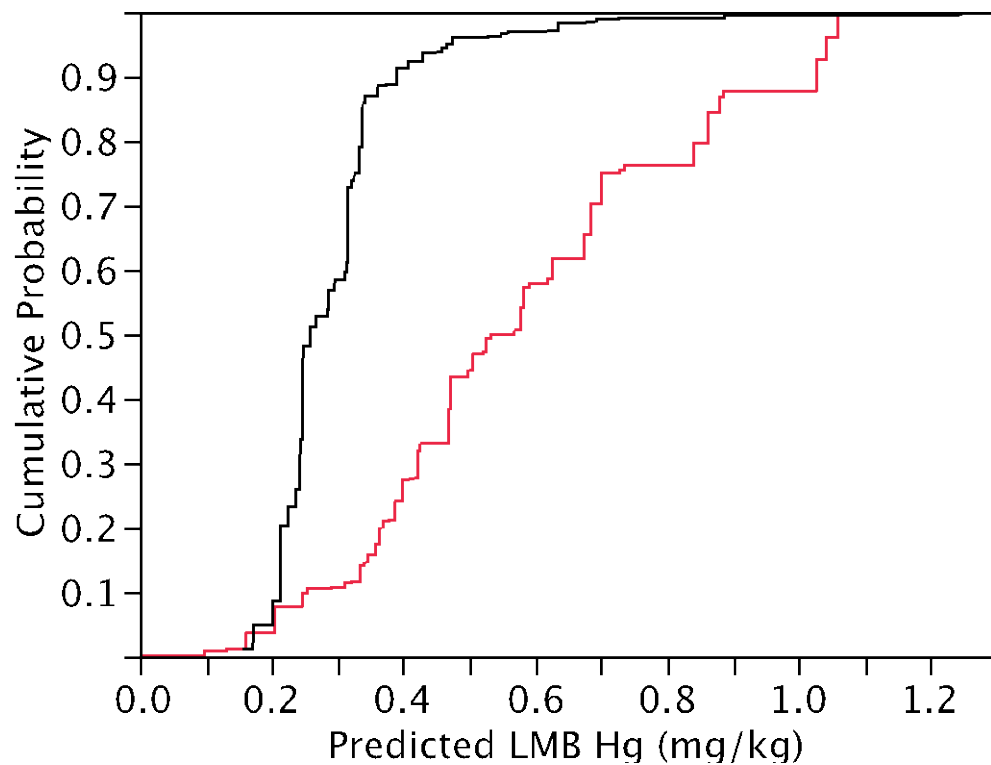


Figure 85. Predicted distribution of LMB Hg concentrations (normalized to 15") obtained with the GLM model coupled with the SMN 2009 data set for water and sediment chemistry. Black line – weighted (by area) distribution for large lakes; red line – weighted (by number) distribution for small lakes.

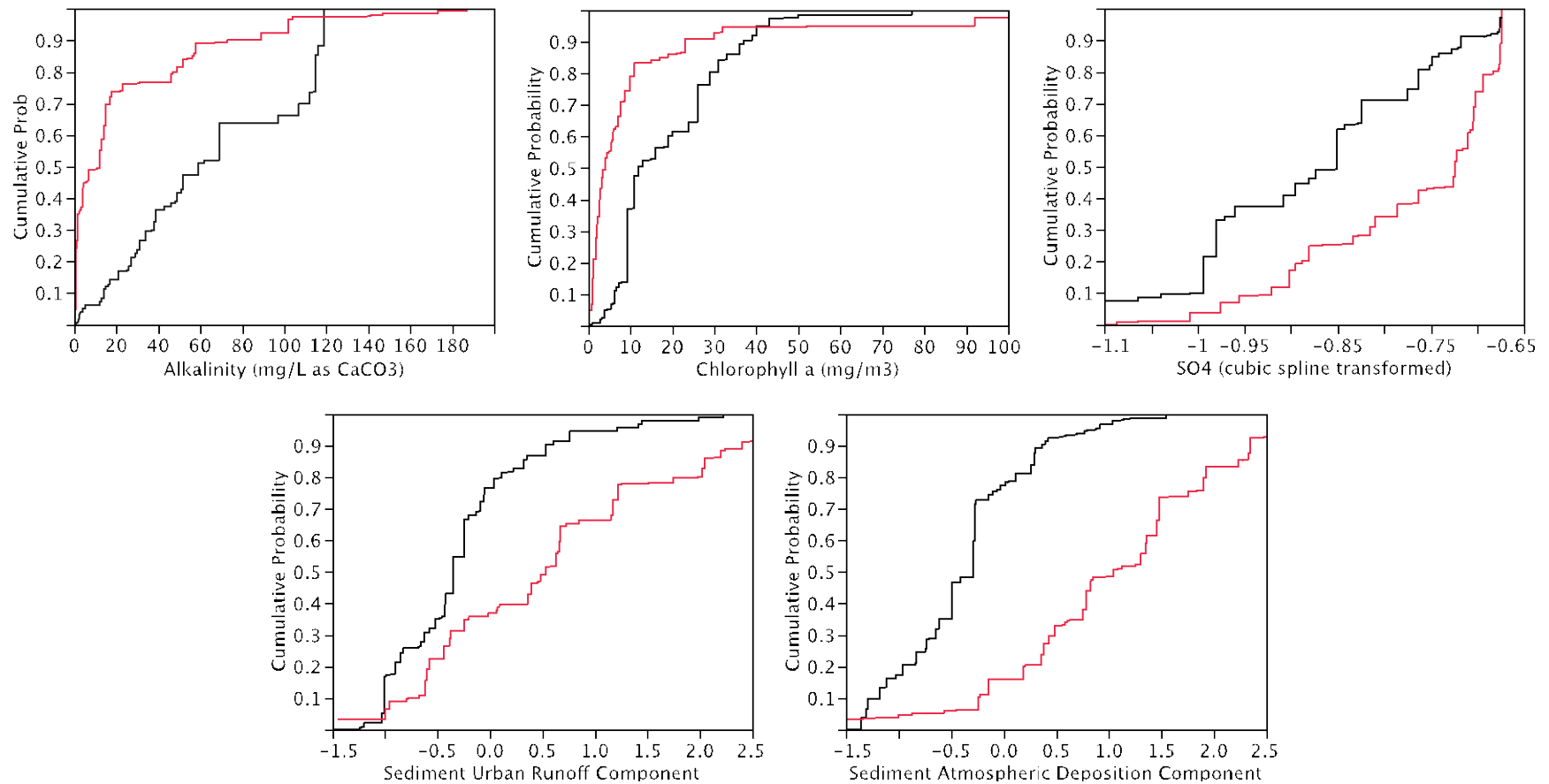


Figure 86. Weighted distributions for GLM model input variables for large lakes (weighted by area; black line) and small lakes (weighted by number; red line) for the SMN 2009 data set.

Application of the Stream Empirical Model to SMN Streams and Rivers

Applicability of CART Model to SMN Streams and Rivers (2009) Data Set

Modeling LMB Hg concentrations in the Hg TMDL streams was conducted using a classification and regression tree analysis (CART) model (6.4.3). CART is a non-parametric approach and, as a result, it makes no assumption that the underlying relationships between the predictor variables and the dependent variable are linear. CART methods are particularly useful when the relationship between the response variable and the predictors is not homogeneous within the sample space; however, a disadvantage to CART models is that the CART-model predictions are based on the mean values of discrete partitions rather than a continuous function (Amrhein *et al.*, 1999). Final variables in the CART modeling included pH, conductivity, SO₄, TP, TKN, and percent dissolved oxygen saturation (DO % saturation).

The SMN 2009 data set used for inferring the statewide distribution of LMB Hg concentrations in Florida streams and rivers with the CART model included a total of 111 sites, including 60 rivers and 51 streams. As was the case for lakes (see Section 8.2.1), the stream/river sampling design incorporated a CI screening level of 1,500 mg/L in developing the target concentration interval bins used to sample streams and rivers and develop the empirical model (Pollman, 2008). As a result, two values from the SMN 2009 streams and rivers data set were excluded from the inferential calculations of LMB Hg distribution.

Table 39. Comparison of data ranges for input variables used to calibrate the GLM model with the observed lower and upper 2.5 and 5 percentile ranges for the SMN 2009 streams and rivers data set used to infer the statewide distribution of LMB Hg concentrations. Ranges exclude two sites with CI concentrations exceeding 1,500 mg/L. N = 109.

Parameter	Calibration		2009 SMN Streams and Rivers			
	Minimum	Maximum	2.50%	97.50%	5%	95%
pH	3.78	8.1	4.175	8.4475	4.34	8.3
Conductivity (µS/cm)	13	1540	16.75	2977.5	20.5	2145
TKN (mg/L)	0.08	2.5	0.1875	4.475	0.23	3.65
SO ₄ (mg/L)	0.26	270	0.585	590	0.885	470
TP (mg/L)	0.004	1.1	0.004	1.09	0.0055	0.72
DO % Saturation	0.7	159.7	13.0	131.2	22.3	107.9

Mahalanobis distances were calculated for the resulting combined CART model calibration and SMN streams/rivers data sets (combined N = 234). Comparing values across the two data sets shows that 10 of the SMN sites have higher Mahalanobis distances than the maximum computed value for the CART model calibration data set.

For 9 sites, the higher values are related primarily to higher conductivity (2 sites), higher sulfate (3 sites), or the combination of conductivity and sulfate concentrations in excess of the calibration data set range (4 sites; Figure 88). For the remaining SMN site, its high Mahalanobis distance appears to be related primarily to high TKN concentrations (4.02 mg/L compared to a maximum of 2.5 mg/L). Because the CART model is inherently non-linear and did not include categories of such high sulfate and conductivity levels, the subsequent inferences of the LMB Hg distribution using the CART model and the SMN 2009 streams and rivers data set combined focused only those sites with Mahalanobis distances less than the maximum value computed for the calibration data set.

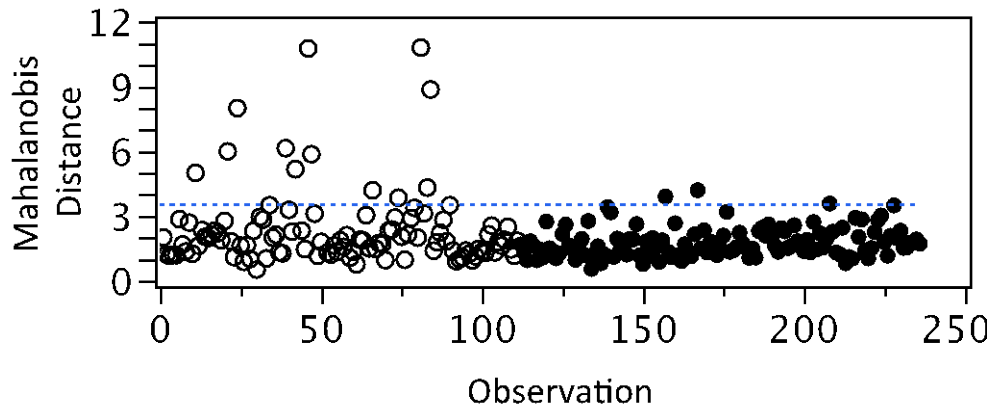


Figure 87. Calculated values for the multivariate Mahalanobis distances (calculated for the six model input variables) for the CART model calibration data set (filled circles; N = 125) and the SMN 2009 data set (open circles; N = 109).

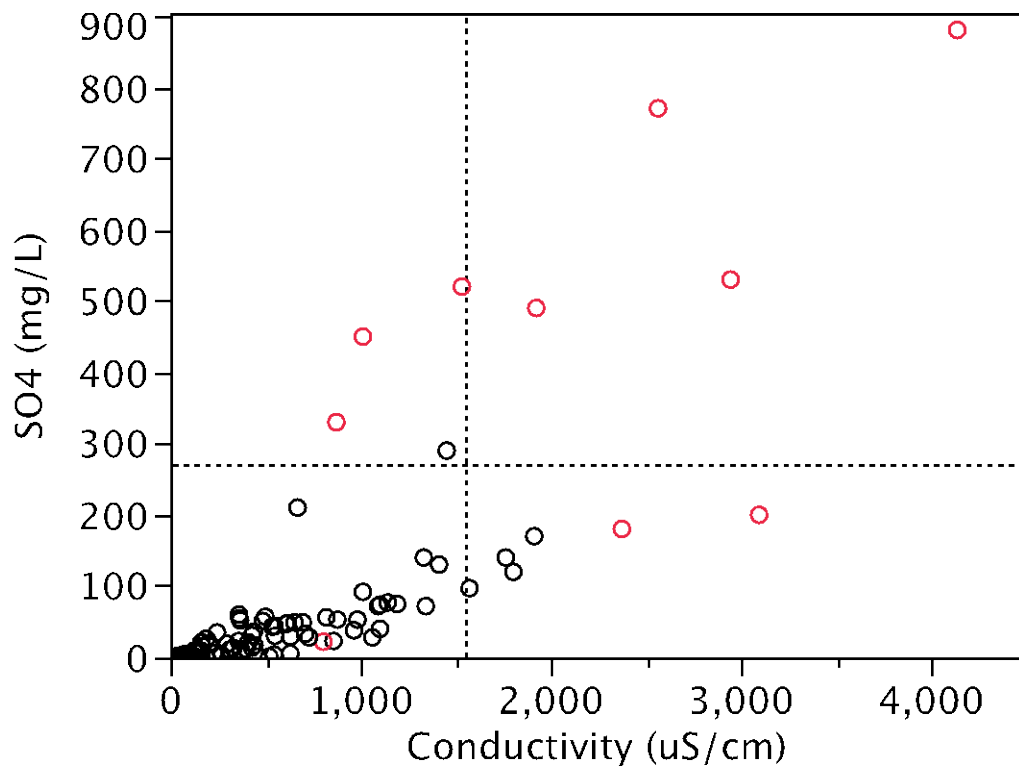


Figure 88. Plot of sulfate concentrations in SMN 2009 streams and rivers data set vs. conductivity. Open red circles show sites with Mahalanobis distances in excess of the computed maximum value for sites used to calibrate the CART model (see Figure 87). Dashed lines correspond to the maximum observed conductivity and sulfate concentration observed in the calibration data set.

Computation of Cumulative Distribution Function curves for the Statewide Distribution of Hg in Largemouth Bass in Florida Streams and Rivers

Similar to lakes (Section 8.2.2), data from the SMN for 2009 were used to infer the statewide distribution of Hg concentrations in LMB (normalized to 15") in Florida rivers and streams. Weights based on reach length were provided by FDEP for both rivers and streams for each site included in the sampling frame. Table 40 summarizes the total values for both the original frame estimates for reach length (km) and the sum of the respective sampling weights for each geographical zone used as a sampling stratum by the SMN in 2009.

Table 40. Summary of (1) original sampling frame estimates for reach length (totals) for rivers and streams; and (2) sum of respective sampling weights for each geographic zone sampled by the SMN in 2009. Total number of samples collected = 111 (60 rivers and 51 streams). Table also includes the sum of the sampling weights for the remaining samples after exclusion of high CI sites (N =2) and sites with Mahalanobis distances exceeding maximum value calculated for the CART model calibration sites (N = 10).

SMN Zone	Original Frame Estimate (reach length in km)		Sample Weights	
	Rivers	Streams	Rivers	Streams
Z1	14311.1581	11087.45479	1301.090909	4106.481482
Z2	862.2776414	2426.702419	507.2352941	323.56
Z3	605.0447	4331.204376	465.3846154	849.254902
Z4	1092.3238	3924.866168	331	106.0783784
Z5	1064.216196	10758.31807	665.125	2501.930233
Z6	1136.865242	11961.14001	1136.9	2917.341463
Totals (N = 111)	19071.88568	44489.68583	4406.735819	10804.64646
Totals (N = 99)			4227.797357	9704.24091

The predicted distributions for LMB Hg concentrations for Florida rivers and streams are (excluding the high Mahalanobis distance sites) are shown in Figure 89. Similar to the results for lakes, LMB Hg concentrations in small streams are consistently higher throughout the entire distribution compared to rivers. The input variable contributions to the CART model sum of squares indicates the following sequence of variable importance:

pH >> DO % saturation > conductivity, TKN, SO4 > TP

with pH and DO % saturation accounting for 62% of the total model sum of squares. A comparison of the weighted distributions for these variables for the SMN 2009 rivers and stream sites shows, not surprisingly, that the distributions of pH and DO % saturation are fundamentally different between rivers and streams. These results are consistent with a greater degree of influence of riparian wetlands on stream water chemistry compared to rivers, including in particular Hg methylation potential suggested by dissolved oxygen dynamics, and the small fraction of river reaches with pH levels below 6.5 (see also Figure 91) reflecting greater contributions of carbonate weathering to alkalinity.

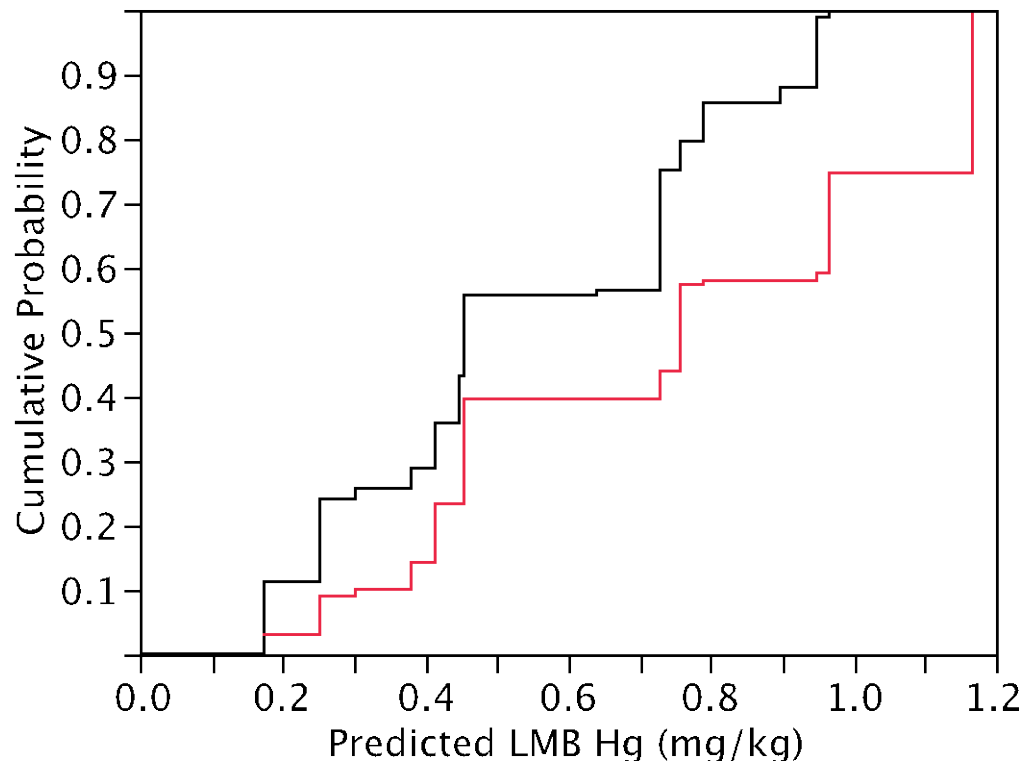


Figure 89. Predicted distribution of LMB Hg concentrations (normalized to 15”) obtained with the CART model coupled with the SMN 2009 data set for water chemistry for Florida rivers and streams. Distributions are weighted by reach length. Black line – rivers; red line – streams. N for inference equal 55 for rivers and 44 for streams.

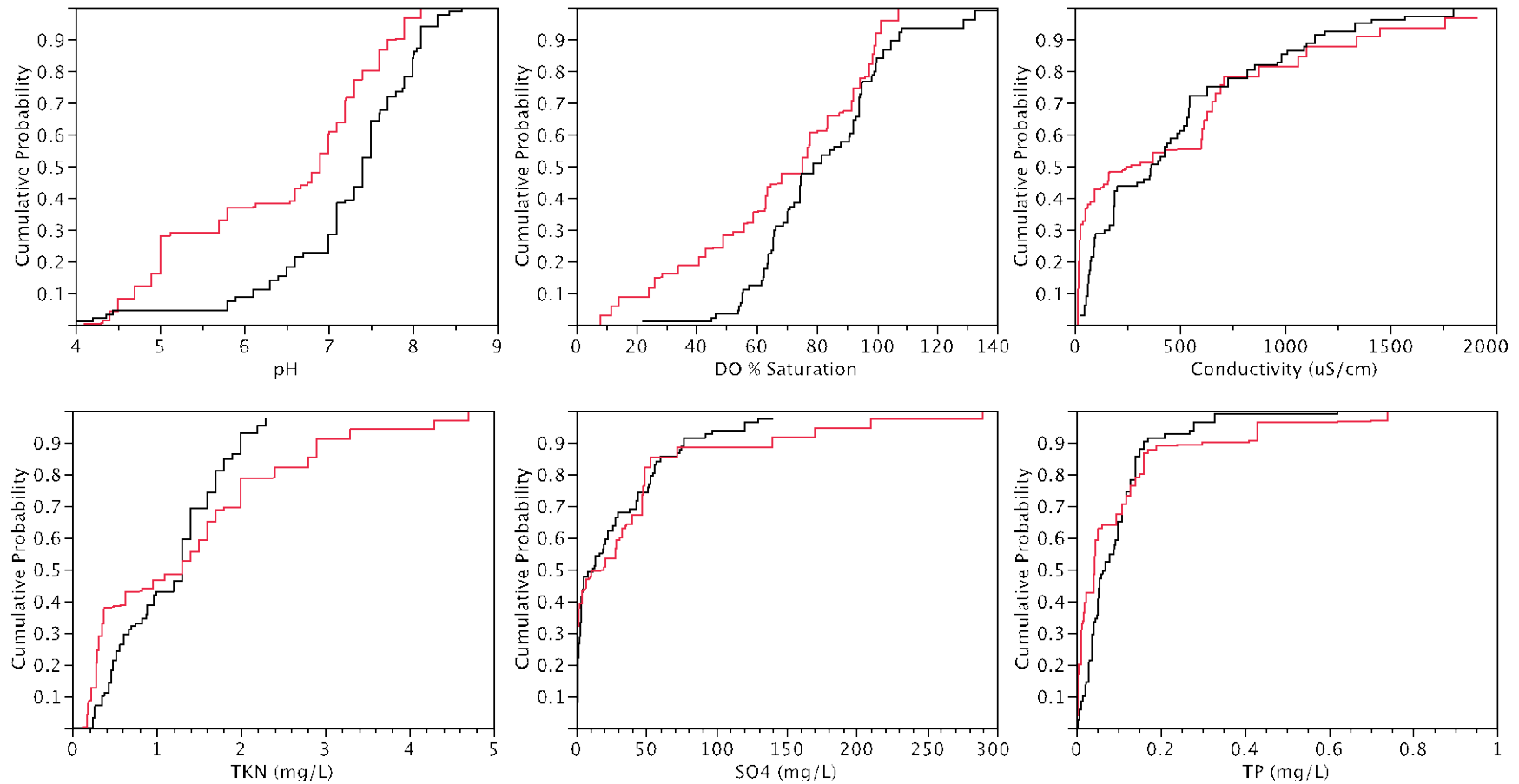


Figure 90. Weighted distributions for CART model input variables for rivers (black line) and streams (red line) for the SMN 2009 data set. All distributions based on sample weightings by reach length.

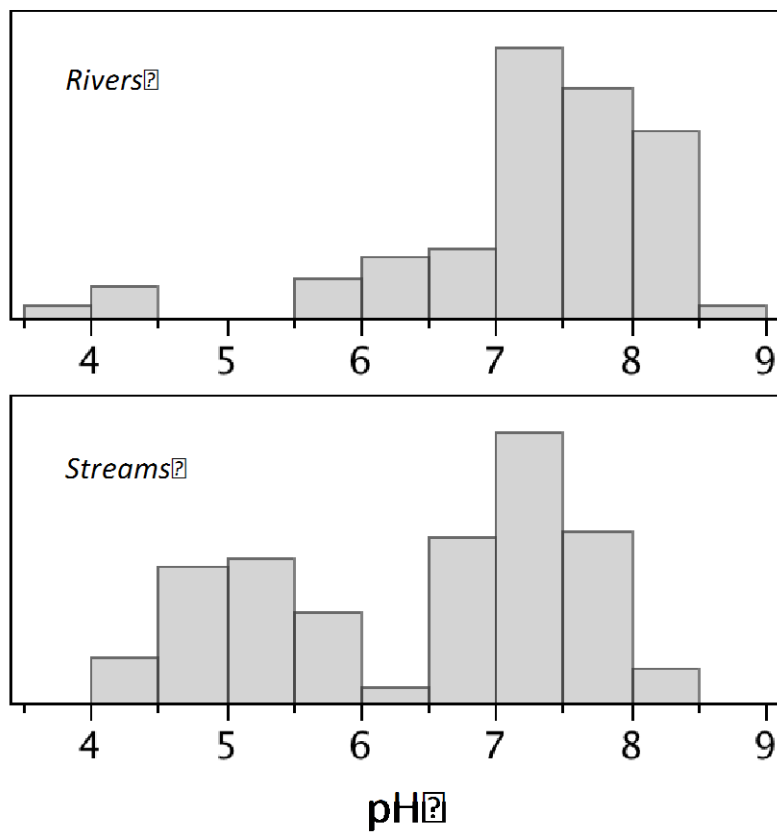


Figure 91. Weighted distributions of pH levels in SMN 2009 rivers (upper panel) and streams (lower panel).

The calculations conducted with the CART model to infer LMB Hg distributions were repeated with the full SMN 2009 data excluding the two high CI sites *only* (i.e., the 10 high Mahalanobis value stream and river sites were included the calculations). A comparison of these results with distribution estimates obtained from excluding the high Mahalanobis value sites indicates that the choice to exclude sites had little or no influence on the results for rivers and streams (Figure 92).

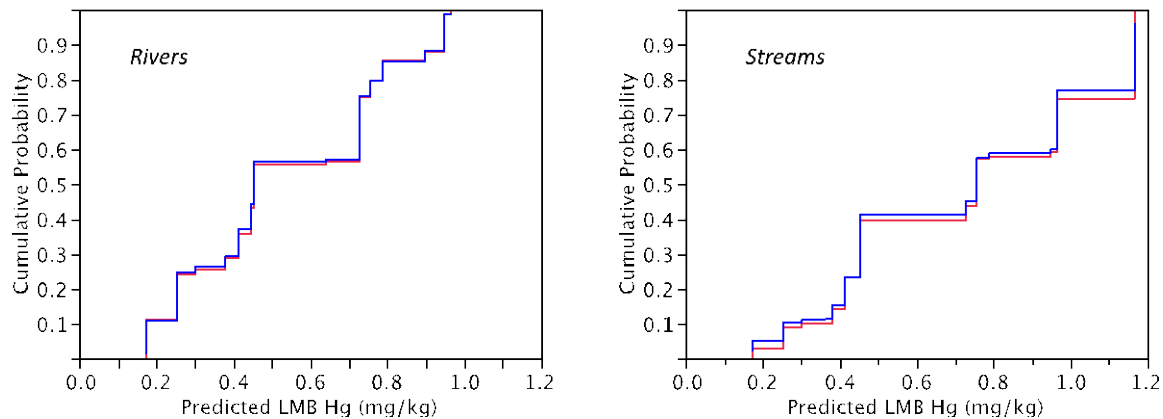


Figure 92. Predicted weighted LMB Hg distributions in SMN 2009 rivers (left hand panel) and streams (right hand panel). Each panel compares the distribution developed using the SMN data for all sites sampled (but excluding the two high CI sites; total N = 109; blue lines) with the distribution developed for those sites only that had Mahalanobis values calculated for the CART model input variables less than the maximum value calculated for the CART model calibration data set (total N= 99; red lines).

Computation of Cumulative Distribution Function curves for the Distribution of Hg in Largemouth Bass in the Everglades

Data from the most recent USEPA REMAP cycles (cycles 6 and 7, May and November 2005) were used to infer the cumulative distribution across the Everglades of LMB Hg concentrations normalized to 14" length. One of the advantages to using the REMAP data is that the REMAP samples were collected from different areas within the Everglades using a stratified random sampling design (Scheidt and Kalla, 2007). As a result, the individual samples can be aggregated to infer the proportional area that has concentrations above or below a given value for a specific variable such as *Gambusia* Hg concentrations in the area or sampling frame sampled. Weights are assigned to each sample based on the area for the corresponding sample frame from which the sample was collected and the total number of random samples collected. The sample frames or regions that were sampled during REMAP include the Loxahatchee National Wildlife Refuge (LNWR), Water Conservation Areas 2 and 3 (WCA2 and WCA3) and the Everglades National Park (ENP). A weighted areal cumulative distribution for a given variable thus can be developed for the entire Everglades (LNWR + WCA2 + WCA3 + ENP) by using the sampling frame weight for each observation in the distribution. Inferred $\ln$ -transformed LMB Hg concentrations were calculated according to the fitted model for each REMAP sampling location with recorded *Gambusia* concentrations (see Section 7.2). Surface areas for each region reflecting the extent of inundated marsh and the associated sample weights were provided by USEPA (P. Kalla, email correspondence to C. Pollman dated 2/2/11 and 2/3/11)⁶ and are summarized in **Table**

⁶ The total inundated surface area of the Everglades region (5281 km²) during REMAP Cycle 7 was adjusted downwards by subtracting the surface area (224 km²) of the Miccosukee Reservation. This was because

41. Weights were adjusted for each area to reflect the smaller number of samples collected for *Gambusia* Hg (compared to the total number of target samples) as follows:

$$\text{Adjusted Weight} = \text{Weight} * N_{\text{tot}}/N_{\text{Gambusia}} \quad (3)$$

Table 41. Areal weights and adjusted weights used to compute areal weighted cumulative distributions for predicted standardized LMB Hg concentrations for the Everglades marsh. Total areas for the wet hydroperiod (Cycle 7) and dry hydroperiod (Cycle 6) are 5057 km² and 3540 km², respectively. Weights provided by P. Kalla (USEPA, email correspondence to C. Pollman dated 2/2/11 and 2/3/11).

Area	N_{Gambusia}	N_{tot}	Weight	Adjusted Weight
<i>Cycle 7 – Wet Hydroperiod</i>				
ENP	41	43	41.75763267	43.79459036
LNWR	12	12	19.59910159	19.59910159
WCA2	10	12	18.61540481	22.33848577
WCA3	48	52	53.89546301	58.38675159
<i>Cycle 6 – Dry Hydroperiod</i>				
ENP	11	35	32.51929399	103.47048086
LNWR	8	13	15.26305192	24.80245937
WCA2	9	13	14.49698553	20.94009020
WCA3	32	48	41.97178488	62.95767732

The resulting cumulative frequency distribution estimate for standardized LMB Hg concentrations are shown for Cycle 7 in Figure 93. The analysis was conducted for the wet cycle only because LMB tend to migrate from shallow marsh areas back into deeper water and the canals during dry periods.

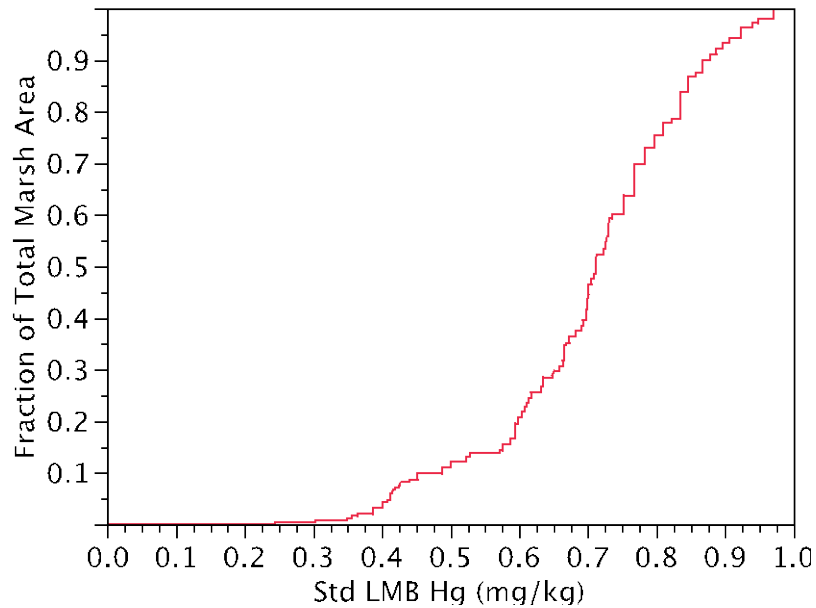


Figure 93. Cumulative weighted distribution of predicted largemouth bass concentrations in the Florida Everglades standardized to 14 inches length based on Model (2). Concentrations inferred from *Gambusia* Hg concentrations measured in the Florida Everglades during REMAP Cycle 7.

Calculation of Requisite Load Reductions to Meet the TMDL Target

Load Reduction Calculations

Load reductions were calculated by assuming that the long-term response between changes in Hg loading to Florida's aquatic ecosystems and fish tissue Hg concentrations is linear. Although our conceptual understanding of the biogeochemical cycling of Hg includes a number of non-linear interactions and components, simulations using the dynamic Mercury Cycling Model (MCM) in both the Everglades (Atkeson *et al.*, 2003) and Devils Lake, WI (Tetra Tech, Inc. and SAI/ICF Inc., 2006) conducted as part of pilot Hg TMDL studies sponsored by USEPA suggest that the long-term response of wetlands and lakes to changes in atmospheric deposition fluxes of Hg is linear. Mesocosm dosing studies conducted in the Everglades by Krabbenhoft *et al.*, 2004 provide empirical support for the linearity assumption, and the assumption is a fundamental component of the load reduction calculations included in the regional New England states TMDL (Connecticut Department of Environmental Protection *et al.*, 2007) and the statewide Hg TMDL conducted by the Minnesota Pollution Control Agency (2007).

Given the assumption of a long-term linear response between Hg loads and biota Hg concentrations, the load reduction, LR , required to reduce biota Hg concentrations to the acceptable target value is given by:

$$LR = \frac{C_{\%ile} - C_{target}}{C_{\%ile}}$$

where $C_{\%ile}$ is the weighted percentile concentration of LMB Hg for a given resource (e.g., lake, river/stream, or the Everglades) and type (e.g., large or small lake; river or stream), and C_{target} is the target concentration of Hg in LMB established by the Florida TMDL as necessary to protect the most sensitive component of the exposed population at risk.

Calculated values for LR have been developed for the statewide distribution of LMB Hg concentrations in Florida's lakes (both large and small), rivers, streams, and the Everglades assuming that reducing the 90th percentile LMB Hg concentration to the target concentration is sufficiently protective. The two previous regional TMDL's established for Hg (Minnesota and the New England states) both use 90th percentile fish tissue Hg concentrations for computing load reductions as well.

Table 42 summarizes the weighted 90th percentile concentration for LMB Hg for each aquatic resource and resource type. Table 42 also includes the requisite load reductions necessary to reduce $C_{90\%}$ values to a target concentration of 0.3 mg/kg. The load reductions (expressed as a % reduction) consider different ranges for the contribution of natural background sources to atmospheric inputs and assume that the natural source contributions are fixed and uncontrollable. The expressed load reductions in the table thus reflect the percentage decrease in the remaining (anthropogenic) fraction of the total load that must occur to reach target levels. For assumed natural background contributions $\geq 30\%$, the analysis indicates that for certain water resource types (small

lakes and streams in particular) ambient $C_{90\%}$ values may not be reduced to an acceptable target value through the elimination of anthropogenic sources alone.

Table 42. Requisite statewide load reductions of Hg inputs necessary to reduce 90th percentile concentrations of LMB Hg concentrations to a target concentration of 0.30 mg/kg for major aquatic resources in Florida (lakes, rivers/stream, and the Everglades). Analysis shows the requisite reduction for different assumed percent contributions of natural sources to atmospheric deposition of Hg in Florida and assumes that the reductions are imposed only on the anthropogenic fraction.

Resource	Sample Frame	Resource Type	Weight	$C_{90\%}$	% Contribution from Natural Background Sources					
					0	10	20	30	40	50
Lakes	2009 SMN	Small lakes	Number	1.027	70.8%	78.7%	88.5%	101.1%	118.0%	141.6%
		Large lakes	Area	0.389	22.9%	25.4%	28.6%	32.7%	38.1%	45.8%
Rivers/Streams	2009 SMN	Streams	Length	1.167	74.3%	82.5%	92.9%	106.1%	123.8%	148.6%
		Rivers	Length	0.949	68.4%	76.0%	85.5%	97.7%	114.0%	136.8%
Everglades	2007 REMAP	Marsh (wet season)	Areal	0.915	67.5%	75.0%	84.4%	96.5%	112.6%	135.1%

Effects of Florida Hg Emission Sources on LMB Hg Concentrations

The contributions of Hg emission sources within Florida to the current (2009) distributions of Hg in Florida rivers, streams, and lakes were estimated using the CMAQ modeled output predicted for each site based on its location (latitude and longitude). CMAQ estimates for Hg wet and dry deposition, including the relative contributions from all tagged Florida emission sources, were provided to *Aqua Lux Lucis, Inc.* by the University of Michigan (S. Sillman, personal communication, May 4, 2012). Computations of the effect of atmospheric sources assumed as a first order analysis that the overwhelming source of Hg to Florida's aquatic resources is from atmospheric deposition. This assumption is supported by comparing the estimated fluxes released to Florida's waters by point sources (24.7 kg/yr; Pollman, 2011) to an estimated total atmospheric deposition flux of Hg of 6,043 kg.⁷ Moreover, it should be noted that 98% of the estimated total point source discharge of Hg is due to a single source – the Davie sewage treatment plant located close to the Atlantic Ocean coast in Broward county; excluding that source from consideration yields a total point source discharge flux of only 0.5 kg/yr. Reductions in Hg loadings thus were calculated by eliminating (or “zeroing-out”) the tagged estimate of Florida source contributions to the total Hg atmospheric deposition flux to each of the SMN 2009 sites.

As shown in Figure 94, the CMAQ estimates of the fractional contributions of Florida sources to Florida's lakes and rivers/streams was generally below 5% (mean contribution = 2.1%; median = 3.1%). Only ~4% of the sites had contributions in excess of 10%, and the maximum estimated contribution was 24.6%. As a result, the effects of eliminating the fraction of atmospheric Hg loadings to Florida lakes and streams/rivers is predicted to be quite small, with (weighted) reductions averaging about 0.01 and 0.02 mg/kg for large and small lakes, respectively (Figure 95) and about 0.01 and 0.02 mg/kg for rivers and streams, respectively (Figure 96).

⁷ This calculation is based on an average total (wet + dry) Hg deposition equal to 35.48 $\mu\text{g}/\text{m}^2\text{-yr}$ obtained from CMAQ for 1,433 stream, river, and lake sites located throughout Florida, and a statewide surface area of 170,312 km^2 (http://www.netstate.com/states/geography/fl_geography.htm).

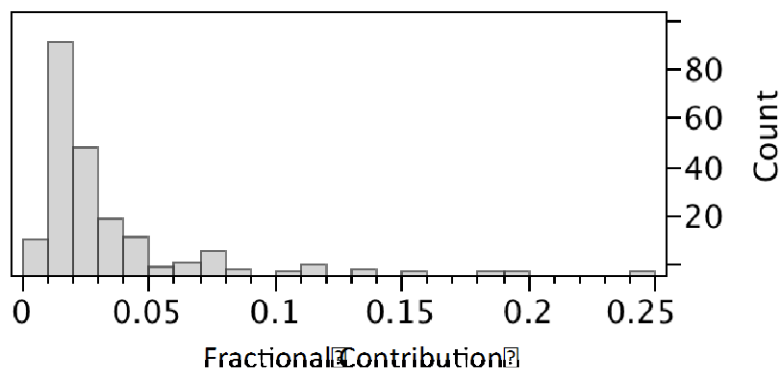


Figure 94. Fractional contributions of tagged Florida emission sources to total atmospheric deposition of Hg at SMN 2009 streams, rivers, and lake sites. Contributions based on CMAQ modeling conducted by the University of Michigan (S. Sillman, personal communication, May 4, 2012).

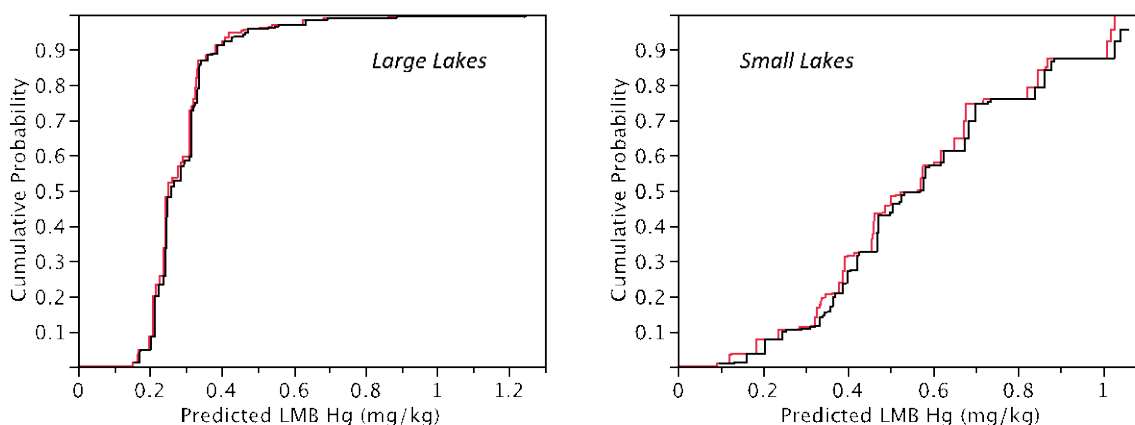


Figure 95. Effects of eliminating atmospheric deposition of Hg attributed to Florida emission sources through CMAQ modeling on the predicted distributions of LMB Hg in Florida lakes (large and small). Distributions are weighted based on sampling probability as a function of area (large lakes) and number (small lakes) length using the SMN 2009 data base. Black line – distribution assuming no reduction in atmospheric deposition; red line – distribution assuming complete elimination of atmospheric deposition of Hg attributable to Florida sources.

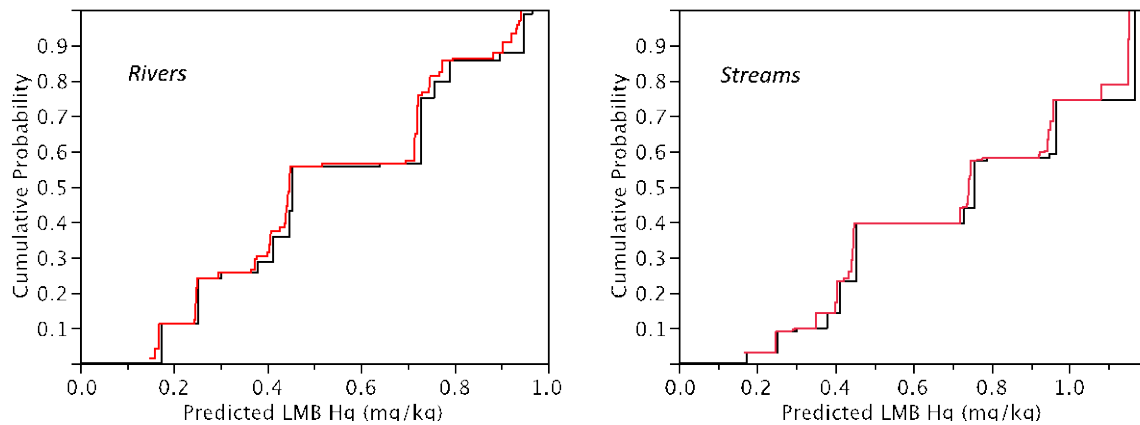


Figure 96. Same as Figure 95, except that the predicted distributions of LMB Hg are for Florida rivers and streams. Distributions are weighted based on reach length and sampling probability using the SMN 2009 data base. Black line – distribution assuming no reduction in atmospheric deposition; red line – distribution assuming complete elimination of atmospheric deposition of Hg attributable to Florida sources.

Effects of Model Uncertainty

Model Confidence Intervals

Model confidence intervals are estimates with a specified degree of confidence of the upper and lower limit for the predicted mean value of the dependent variable for a given set of input variables. Mean confidence intervals were calculated for each of the three models used to infer LMB Hg concentrations as a function of water chemistry (lakes and streams/rivers) and *Gambusia* Hg concentrations (Everglades). Because the goal of the TMDL is to reduce dietary exposure to Hg in fish to levels below a critical target concentration, the focus in constructing model confidence limits is on the upper limit. Model confidence intervals characteristically are constructed to define upper and lower limits; thus 95% confidence intervals indicate that the mean value has a 2.5% probability of lying above the upper confidence interval, and a 2.5% probability of lying below the upper confidence interval. Given the TMDL focus on limiting exposure, 90th confidence intervals were calculated, which gives a 95% upper limit (not to exceed) for the mean value of LMB Hg predicted by the model.

The final model forms for both the TMDL lakes and the Everglades empirical models used generalized linear modeling (GLM) to model LMB Hg concentrations with a log link function to provide both a framework of linear relationships between the dependent variable and the independent variables used in the two respective models, and a model form that avoids the necessity of accounting for model error in back-transforming logarithmically transformed LMB Hg concentrations to untransformed values. Upper limit confidence intervals were constructed using the delta method (StataCorp, 2011). The delta method is a general approach used to estimate model variance when the model function is too complex for analytically computing the variance. The delta method involves creating a linear approximation of the original function, and then computes the variance of the simpler linear function; this variance is then used to construct the model confidence intervals (Long and Freese, 2006).

The TMDL streams empirical model was constructed using classification and regression tree analysis (CART). CART recursively partitions the data into a series of dichotomous groups based on the values of the independent variables that best predict a value of the dependent variable (SAS Institute, Inc, 2011). In the implementation of the CART model for TMDL streams, LMB Hg concentration is the dependent variable, with mean values based on the observed data calculated for each partitioned group, along with the standard deviation of the mean. For each of the 14 groups in the model, 95th percentile confidence upper limits (single tail) were constructed for the mean based on the number of observations in the group and the observed standard deviation (SAS Institute Inc., 2011).

Weighted distributions for both the original model estimates of LMB Hg concentrations and the 90th upper confidence interval for the model predicted concentrations are shown in Table 43 for median, upper quartile (75th), and 90th percentile concentrations.

Utilizing the model 90th upper confidence interval concentrations results in upward shifts in the 90th percentile concentration for the weighted aquatic resources ranging from 10% (the Everglades) to 20% (rivers).

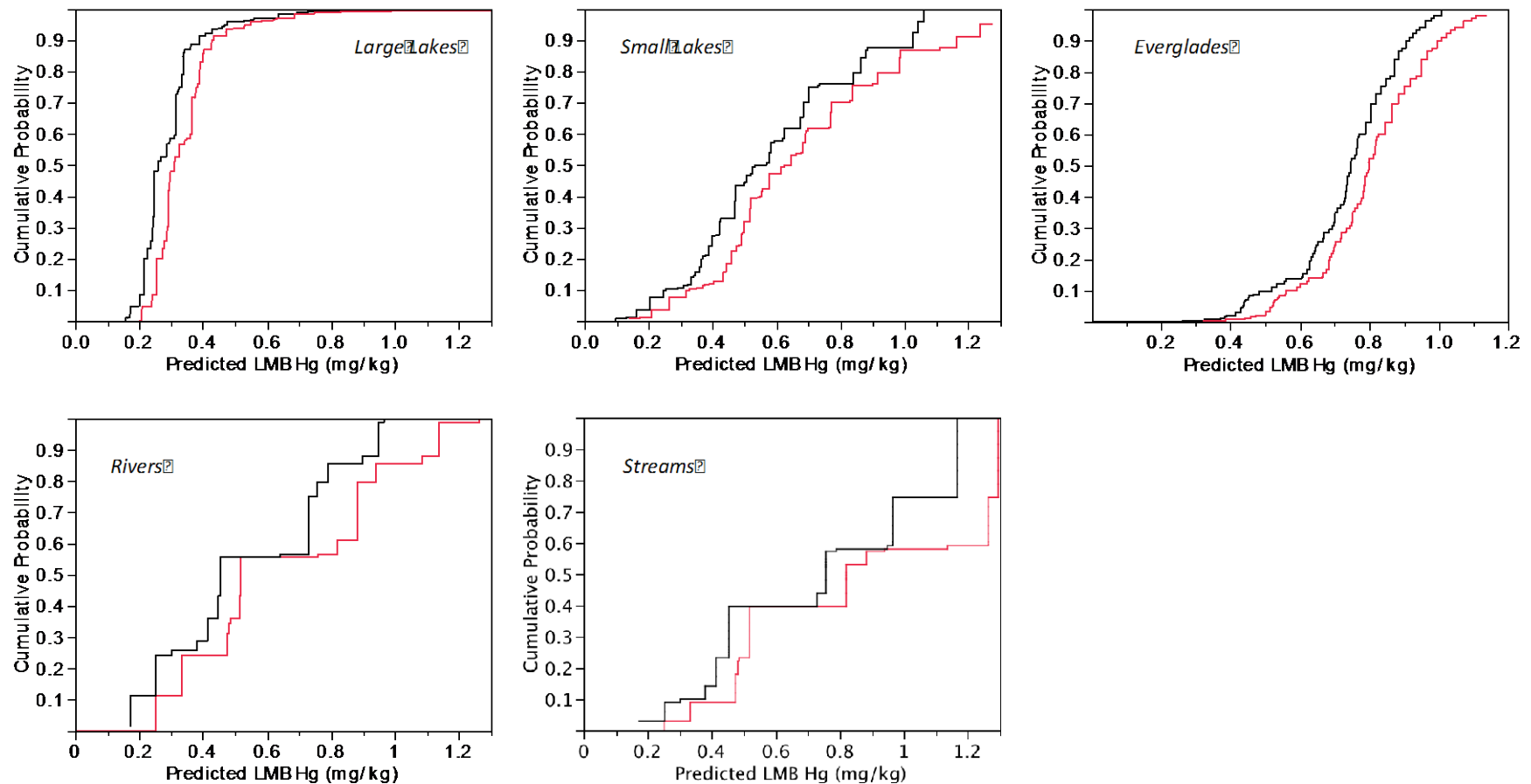


Figure 97. Comparison of weighted distributions for original model predictions of LMB Hg concentrations (black line) with the weighted distributions obtained from estimating the 90th percentile model upper confidence interval (red line).

Table 43. Comparison of original modeled (weighted) estimates of Hg in LMB with weighted estimates based on the 90th upper confidence interval for the model predictions. Comparisons are shown for weighted median, 75th, and 90th percentile concentrations.

LMB Hg Metric	Small Lakes		Large Lakes		Streams		Rivers		Everglades	
	Original Prediction	95% Upper Limit	Original Prediction	95% Upper Limit	Original Prediction	95% Upper Limit	Original Prediction	95% Upper Limit	Original Prediction	95% Upper Limit
Median	0.568	0.645	0.259	0.310	0.757	0.820	0.454	0.518	0.747	0.798
75%	0.729	0.868	0.332	0.383	1.167	1.295	0.729	0.883	0.834	0.901
90%	1.027	1.164	0.389	0.433	1.167	1.295	0.949	1.136	0.915	1.008

Cumulative Frequency Distribution Confidence Intervals

Because the distribution calculations are based on a sampled subset for each water resource (large lakes, small lakes, rivers, streams, and the Everglades), there is uncertainty regarding the resulting distributions that can be estimated as function of both the total number of samples collected and the sampling weights associated with each sample. Because a value of interest is either above or below a given frequency level, one approach towards computing confidence intervals calculates the standard deviation, σ , associated with each sample assuming a binomial distribution:

$$\sigma_i = \sqrt{\frac{P_{cum,i} \cdot (1 - P_{cum,i})}{N}}$$

where $P_{cum,i}$ is the cumulative probability associated with a given value, i (e.g., LMB Hg) in the distribution, and N is the total number of samples.

Student's t values are used to calculate the confidence intervals; however, because the error distribution becomes increasingly skewed towards the minimum and maximum cumulative probabilities of 0 and 1, respectively, the following approximations were used to estimate the lower and upper confidence intervals:

$$LCL_i = P_{cum,i} - 2 \cdot P_{cum,i} \cdot t_{crit} \cdot \sigma_i$$

$$UCL_i = P_{cum,i} + 2 \cdot (1 - P_{cum,i}) \cdot t_{crit} \cdot \sigma_i$$

where LCL_i and UCL_i are the lower and upper confidence limit estimates for the probability of occurrence of value i , and t_{crit} is the value from the Student's t distribution given by N ($N - 1$ degrees of freedom) and the confidence level of interest (http://en.wikipedia.org/wiki/Cumulative_frequency_analysis#Confidence_intervals). For the purposes of this analysis, the confidence level was 90%, which defines the upper (or lower) 5% of the distribution.

The weighted distributions for LMB Hg concentrations obtained from the upper confidence limit (90th percentile; see Figure 97) of the model predictions are shown in Figure 98 for each water resource in conjunction with the upper and lower confidence intervals calculated for each distribution. Shown also in the plot is the intersection between the different distributions with the assumed critical distribution value of 90%. Restated, Figure 98 shows the aggregated effect of two sources of error on the distribution of LMB Hg concentrations – the effect of model uncertainty on the estimated values for LMB Hg, and the effect of overall sampling size and sample weight on the estimation of the probability of occurrence of a given LMB Hg concentration.

The revised estimates for the critical 90th percentile LMB Hg concentration ($C_{90\%}$) that reflects these two sources of uncertainty are summarized in Table 44, which also includes the reductions in anthropogenic inputs of Hg in atmospheric deposition required to reduce $C_{90\%}$ to 0.3 mg/kg. By including these sources of uncertainty into the load reduction calculations, the requisite load reductions (assuming that contributions from background natural sources equals 30%) range from 81% (large lakes) to 110% (streams and small lakes). If the best estimates of $C_{90\%}$ (i.e., without considering the effects of model uncertainty or uncertainty associated with estimating weighted population distribution) are used in the calculations instead, the requisite reductions in anthropogenic loads range from 33% (large lakes) to 106% (streams) (see Table 42).

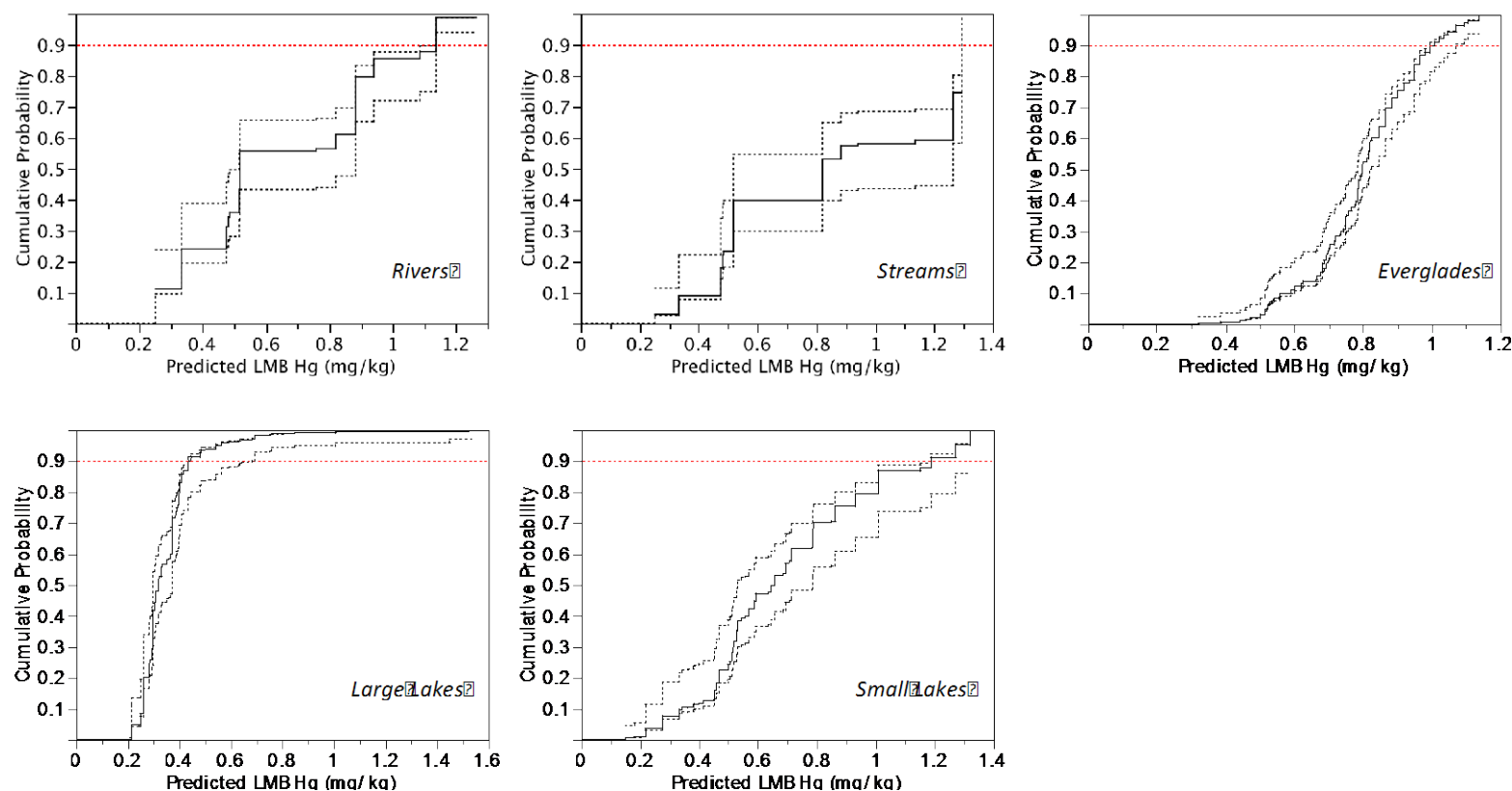


Figure 98. Weighted distributions of LMB Hg in Florida lakes (small and large), rivers, streams, and the Everglades using the upper 90th confidence limits for the respective empirical models to estimate LMB Hg. Plots show both the distribution of the model estimates (solid line) and the 90th percentile confidence intervals for the distribution of the model estimates (black dotted lines). Also shown for reference is the 90th percentile for the cumulative probability curves (red dotted line).

Table 44. Requisite statewide load reductions of Hg inputs necessary to reduce 90th percentile concentrations of LMB Hg concentrations to a target concentration of 0.30 mg/kg for major aquatic resources in Florida (lakes, rivers/stream, and the Everglades), after imposing effects of model and distribution uncertainty on $C_{90\%}$. The analysis shows the requisite reduction for different assumed percent contributions of natural sources to atmospheric deposition of Hg in Florida and assumes that the reductions are imposed only on the anthropogenic fraction.

Resource	Sample Frame	Resource	Weight	$C_{90\%}$	% Reduction of Hg Inputs as Function of Assumed Natural Background Contributions					
					0	10	20	30	40	50
Lakes	2009 SMN	Small lakes	Number	1.319	77.3%	85.8%	96.6%	110.4%	128.8%	154.5%
		Large lakes	Area	0.694	56.8%	63.1%	71.0%	81.1%	94.6%	113.5%
Rivers/Streams	2009 SMN	Streams	Length	1.295	76.8%	85.4%	96.0%	109.8%	128.1%	153.7%
		Rivers	Length	1.136	73.6%	81.8%	92.0%	105.1%	122.7%	147.2%
Everglades	2007 REMAP	Wet season	Areal	1.071	72.0%	80.0%	90.0%	102.8%	120.0%	144.0%

References Cited

- Amrhein, J.F., C.A. Snow, and C. Wible. 1999. Whole-fish analysis versus filet polychlorinated-biphenyl concentrations: an analysis using classification and regression tree models. *Environ. Toxicol. Chem.* **18(8)**: 1817-1823.
- Atkeson T.D., D. Axelrad, C.D. Pollman, and G. Keeler. 2003. Integrating Atmospheric Mercury Deposition and Aquatic Cycling in the Florida Everglades: An approach for conducting a Total Maximum Daily Load analysis for an atmospherically derived pollutant. Integrated Summary. Final report submitted to USEPA. Florida Department of Environmental Protection, Tallahassee, FL.
- Bertine, K.K. and E.D. Goldberg. 1977. History of heavy metal pollution in southern California coastal zone – Reprise. *Environ. Sci. Technol.* **11(3)**: 297- 299.
- Björnberg, A., L. Håkanson, and K. Lundbergh. 1988. A theory on the mechanisms regulating the bioavailability of mercury in natural waters. *Environmental Pollution* **49(1)**:53–61.
- Brenner, M. and M.W. Binford. 1988. Relationships between concentrations of sedimentary variables and trophic state in Florida lakes. *Can. J. Fish. Aquat. Sci.* **45**: 294-300.
- Chatterjee, S. A.S. Hadi, and B. Price. 2000. Regression analysis by example, 3rd edition. Wiley & Sons, New York.
- Chen, C.Y. and C.L. Folt. 2005. High plankton densities reduce mercury biomagnification. *Environ. Sci. Technol.* **39**:115-121.
- Connecticut Department of Environmental Protection, Maine Department of Environmental Protection, Massachusetts Department of Environmental Protection, New Hampshire Department of Environmental Services, New York State Department of Environmental Conservation, Rhode Island Department of Environmental Management, Vermont Department of Environmental Conservation, and New England Interstate Water Pollution Control Commission. 2007. Northeast Regional Mercury Total Maximum Daily Load. <http://www.epa.gov/region1/eco/tmdl/pdfs/ne/Northeast-Regional-Mercury-TMDL.pdf>
- Dickinson, R.E., W. Huber, and J. Heaney. 1982. A Gazetteer of Florida Lakes. Publication No. XX, Water Resources Research Center, Gainesville, FL.
- Dittmann, J.A. and C.T. Driscoll. 2009. Factors influencing changes in mercury concentrations in lake water and yellow perch (*Perca flavescens*) in Adirondack lakes. *Biogeochemistry* **93(3)**: 179-196.
- Epstein, C.B. 1988. A method for the estimation of historical sulfate concentrations in natural waters. *Environ. Sci. Technol.* **22**: 1460-1463.
- Florida Department of Environmental Protection (FDEP). 2010. Monitoring Design Document, Cycle 3, January – December 2009. Florida Department of Environmental Protection Watershed Monitoring Program, Tallahassee, FL.
- Förstner, U. and G.T.W. Wittmann. 1979. Metal pollution in the aquatic environment. Springer-Verlag. Berlin, Heidelberg, New York. 486 pp.
- Freund, R. R. Littell, and L. Creighton. 2003. Regression using JMP. SAS Institute, Cary, NC.
- Haan C.T. 2002. Statistical methods in Hydrology, 2nd edition. Iowa State University Press, Ames, Iowa.

- Håkanson, L. and Jansson, M., 1983. *Principles of Lake Sedimentology*. Springer-Verlag. Berlin, Heidelberg, New York. 316 pp.
- Hamilton, L. 2009. *Statistics with Stata: Updated for Version 10*. Brooks/Cole. Belmont, CA. 499 pp.
- Hardin, J.W. and J.M. Hilbe. 2007. *Generalized linear models and extension*, 2nd edition. Stata Press, College Station, TX.
- Hudson, R.J.M., S.A. Gherini, C.J. Watras, and D.B. Porcella. 1994. Modeling the biogeochemical cycle of mercury in lakes: The Mercury Cycling Model (MCM) and its application to the MTL study lakes. *In* C.J. Watras and J. W. Huckabee [eds.], *Mercury Pollution – Integration and Synthesis*. CRC Press Inc. Lewis Publishers.
- Johansson, K., B. Bergbäck, G. and Tyler. 2001. Impact of atmospheric long range transport of lead, mercury and cadmium on the Swedish forest environment. *Water Air Soil Pollut. Focus* **1**:279–297
- Kamman N.C., P.M. Lorey, C.T. Driscoll, R. Estabrook, A. Major, B. Pientka, and E. Glassford. 2004. Assessment of mercury in waters, sediments, and biota of New Hampshire and Vermont lakes, USA, sampled using ageographically randomized design. *Environ. Toxicol. Chem.* **23**:1172–1186.
- Kanciruk, P., J.M. Eilers, R.A. McCord, D.H. Landers, D.F. Brakke, and R.A. Linthurst. 1986. Characteristics of lakes in the Eastern United States. Vol. 3. Data compendium of site characteristics and chemical variables. US EPA 600/4-86/007C. 374 pp.
- Krabbenhoft, D., C. Gilmour, W. Orem, and G. Aiken. 2004. Unraveling the complexities of mercury methylation in the Everglades: The use of mesocosms to test the effects of “new” mercury, sulfate, and dissolved organic carbon. *RMZ Materials and Geoenvironment* **51**: 1150-1151.
- Lange, T.R., H.E. Royals, and L.L. Connor. 1993. Influences of water chemistry on mercury concentrations in largemouth bass from Florida lakes. *Trans. American Fish. Society* **122**: 74-84.
- Lathrop, R.C., P.W. Rasmussen, and D.R. Knauer. 1991. Mercury concentrations in walleyes from Wisconsin (USA) lakes. *Water Air Soil Poll.* **56**: 295-307.
- Lek, S., Delacoste, M., Baran, P., Dimopoulos, I., Lauga, J., Aulagnier, S., 1996. Application of neural networks to modelling nonlinear relationships in ecology. *Ecol. Model.* **9**: 39–52.
- Linthurst, R.A., D.H. Landers, J.M. Eilers, D.F. Brakke, W.S. Overton, E.P. Meier, and R.E. Crowe. 1986. Characteristics of lakes in the Eastern United States. Vol. 1. Population descriptions and physico-chemical relationships. US EPA 600/4-86/007A. 275 pp.
- Long, J.S. and J. Freese. 2006. *Regression models for categorical dependent variables using Stata*. Second edition. Stata Press, College Station, TX.
- Minnesota Pollution Control Agency. 2007. Minnesota statewide mercury total maximum daily load, final report dated March 27, 2007.
http://www.pca.state.mn.us/index.php/component/option,com_docman/task,doc_view/gid,8507
- Newman, M. 1993. Regression analysis of log-transformed data: statistical bias and its correction. *Environ. Toxicol. Chem.* **12**: 1129-1133.
- Palani, S., S.-Y. Liong, and P. Tkalich. An ANN application for water quality forecasting. *Marine Poll. Bull.* **56**: 1586-1597.

- Pollman, C.D., T.M. Lee, W.V. Andrews, L.A. Sacks, S.A. Gherini, and R.K. Munson. 1991. Preliminary Analysis of the Hydrologic and Geochemical Controls on Acid Neutralizing Capacity in Two Acidic Seepage Lakes in Florida. *Water Resources Research* **27**:2321-2335.
- Pollman, C.D. and D.E. Canfield, Jr. 1991. Florida, pp. 367-416. In D.F. Charles [Ed.], *Acid Deposition and Aquatic Ecosystems: Regional Case Studies*. Springer-Verlag, New York.
- Pollman, C.D. 2008. Task 2.2 Deliverable. Initial water quality-fish mercury data assessment. Final report submitted to Florida Department of Environmental Protection, Tallahassee, FL. *Aqua Lux Lucis*, Inc. Gainesville, FL.
- Tetra Tech, Inc. and SAI/ICF Inc. 2006. Developing A Quantitative Assessment of Mercury Inputs and Cycling in Devils Lake, Wisconsin: A Pilot Study for Conducting a Total Maximum Daily Load Analysis for an Atmospherically Derived Pollutant. Final Report Submitted To U.S. Environmental Protection Agency, Office of Wetlands, Oceans, and Watersheds. Submitted by Tetra Tech, Inc., Lafayette, CA.
- Pollman, C.D. 2011. Florida Statewide Mercury TMDL, Task 5.2.F – Report on Analyses of Point Source Loadings. Final report submitted to Florida Department of Environmental Protection, Tallahassee, FL. *Aqua Lux Lucis*, Inc. Gainesville, FL.
- Rubio, B., M.A. Nombela, and F. Vilas. 2000. Geochemistry of Major and Trace Elements in Sediments of the Ria de Vigo (NW Spain): an Assessment of Metal Pollution. *Marine Poll. Bull.* **40**(11): 968-980.
- SAS Institute, Inc. 2011. JMP Version 9.0.3. SAS Institute, Inc. Cary, NC.
- Scheidt, D.J. and P.I. Kalla. 2007. Everglades ecosystem assessment: water management and quality, eutrophication, mercury contamination, soils and habitat: monitoring for adaptive management: a RE-MAP status report. USEPA Region 4, Athens, GA. EPA 904-R-07-001. 98 pp.
- StataCorp, LP. 2011. Stata/MP 12.0. StataCorp, LP. College Station, TX.
- Schleiter, I.M., D. Borchardt, R. Wagner, T. Dapper, K.-D. Schmidt, H.-H. Schmidt, and H. Werner. 1999. Modelling water quality, bioindication and population dynamics in lotic ecosystems using neural networks. *Ecol. Modelling* **120**: 271-286.
- Shanley, J.B., NC. Kamman, T.A. Clair, and A. Chalmers. 2005. Physical controls on total and methylmercury concentrations in streams and lakes of the northeastern USA. *Ecotoxicology* **14**: 125-134.
- Stumm, W.E. and J.J. Morgan. 1983. *Aquatic chemistry*. 2nd edition. Wiley-Interscience, New York.
- Tabachnick, B.G. and L.S. Fidell. 2007. *Using multivariate statistics*, 5th edition. Pearson Education Inc. Boston. 980 pp.
- Talib, A., F. Recknagel, and D. van der Molen. 2007. Patternising phytoplankton dynamics of two shallow lakes in response to restoration measures by applying non-supervised artificial neural networks. *Environmentalist* **27**: 195-205.
- Tetra Tech, Inc. and SAI/ICF Inc. 2006. Developing A Quantitative Assessment of Mercury Inputs and Cycling in Devils Lake, Wisconsin: A Pilot Study for Conducting a Total Maximum Daily Load Analysis for an Atmospherically Derived Pollutant. Final Report Submitted To U.S. Environmental Protection Agency, Office of Wetlands, Oceans, and Watersheds. Submitted by Tetra Tech, Inc., Lafayette, CA.

Thurston, G.D. and J.D. Spengler. 1985. A quantitative assessment of source contributions to inhalable particulate matter. *Atmos. Environ.* **19(1)**:9-25.



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