# FISH MERCURY DISTRIBUTION IN MASSACHUSETTS LAKES

## Final Report

by

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Office of Research and Standards
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## ABSTRACT

The sediment, water and 3 species of fish from 24 of Massachusetts' relatively least-impacted waterbodies were sampled to determine the patterns of variation in edible tissue mercury concentrations and the relationships of these patterns to characteristics of the sediment, water and water bodies (lake, wetland and watershed areas). Sampling was apportioned among three different ecological subregions and among lakes of differing trophic status. We sought to partition the variance to discover if these broadly defined concepts are suitable predictors of mercury levels in fish. Average muscle mercury concentrations were 0.14 mg/kg wet weight in the bottom feeding brown bullheads (*Ameiurus nebulosus)* (range=0.01-0.79 mg/kg); 0.31 mg/kg in the omnivorous yellow perch (*Perca flavescens*) (range=0.01-0.75 mg/kg); and 0.40 mg/kg in the predaceous largemouth bass (*Micropterus salmoides)* (range=0.05-1.1 mg/kg). Statistically significant differences in fish mercury concentrations between ecological subregions in Massachusetts existed only in yellow perch, although there was a suggestion of such a relationship in brown bullhead. The productivity level of the lakes (as deduced from Carlson's Trophic status Index) was not a strong predictor of tissue mercury concentrations in any species. pH was a highly (inversely) correlated environmental variable with yellow perch and brown bullhead tissue mercury. Largemouth bass tissue mercury concentrations were most highly correlated with the weight of the fish (+), the weight (+) and mercury concentrations (-) of yellow perch in the same lake and the magnitude of surface areas, watershed and wetland areas associated with lake (+). These results are generally consistent with existing knowledge of freshwater fish tissue mercury dynamics and are notable for demonstrating spatially correlated differences in tissue mercury concentrations across ecological subregions on a scale less than about 150 miles.

## EXECUTIVE SUMMARY

Massachusetts has surveyed contaminants in freshwater fish since 1983, focusing primarily in areas of known or suspected contamination, or where biological effects were observed. These studies have shown that the variation in fish mercury contamination is relatively high. Concentrations have been sufficiently high in some species to warrant the issuance of Fish Consumption Advisories for specific waterbodies. A statewide health advisory cautioning pregnant women of the possible health risk from eating fish from Massachusetts freshwater bodies, excluding stocked and farm-raised fish, has also been issued.

While some previous studies have shown that ecologically-based geographic subdivisions ("ecoregions") account for variation in fish mercury concentrations, others have suggested that lake productivity and lake trophic status affect the accumulation of persistent pollutants in fish. The Massachusetts Department of Environmental Protection conducted a study beginning in 1994, which explored whether these concepts were associated with mercury variation in fish in Massachusetts lakes. We also attempted to determine the relative degrees of influence of lake biological, physical and chemical characteristics on fish tissue mercury concentrations. Yellow perch (*Perca flavescens*), largemouth bass (*Micropterus salmoides*) and brown bullheads (*Ameiurus nebulosus*) were sampled from 24 lakes that did not have active point sources of contamination (e.g., landfills, industrial facilities, hazardous waste sites, wastewater treatment facilities). Another objective of the study was to determine levels of cadmium, lead, selenium, arsenic, polychlorinated biphenyls (PCBs) and chlorinated pesticides in sediments and edible muscle of these species of freshwater fish from Massachusetts lakes.

Three ecological subregions within Massachusetts were selected: the Green Mountain/Berkshire Highlands, the Worcester/Monadnock Plateau, and the Narragansett/Bristol Lowland (Figure 1). Eight lakes were selected from each of these ecological subregions. Watershed, wetland and lake areas were calculated for each water body.

Nine fish of each species within narrow size ranges were targeted for collection from each lake during autumn of the year after the spawning season. Water and sediment samples were obtained in mid summer during periods of lake stratification when lake productivity was most easily characterized. Water samples were analyzed for total phosphorus, dissolved organic carbon, ammonia, nitrate, chloride, calcium, sulfate, and chlorophyll *a* concentrations.

Cumulative frequency distributions of individual species' mercury concentration values were determined. A Pearson's product moment cross-correlation matrix of the environmental data was prepared. The relative importances of the geographical locations of lakes (3 ecoregion levels) and their trophic state (2 levels) were assessed with an analysis of variance (for yellow perch and brown bullhead) and an analysis of covariance (for largemouth bass). Lake trophic states were characterized by calculating Carlson's Trophic State Index. Stepwise multiple regressions were performed to detect significant relationships between physical and chemical variables and fish tissue mercury levels. The data were also analyzed by factor analysis in order to identify which

variables exhibited similar variance patterns, particularly those associated with the bioaccumulation of mercury.

Bottom-feeding brown bullhead generally had the lowest mercury concentrations (mean = 0.14 mg/kg wet weight; range = 0.01-0.79 mg/kg), omnivorous yellow perch (mean = 0.31 mg/kg; range=0.01-0.75 mg/kg) had higher levels and predaceous largemouth bass (mean = 0.40 mg/kg; range 0.05-1.1 mg/kg) the highest. The species had somewhat similar distributions in the concentration range of 0.2-0.6 mg/kg, but the bass distribution had a tail to the right beyond 0.6 mg/kg with upper concentrations up to 1.1 mg/kg. Mercury concentrations measured in yellow perch and largemouth bass were consistent with those of similarly aged fish in the Adirondack Mountains of New York State, the Upper Peninsula of Michigan and Wisconsin. The largemouth bass concentrations were less than those of this species in Florida. Largemouth bass are the only one of the three species in this study which exhibited a significant correlation between fish size and mercury content.

Statistically significant differences in fish mercury concentrations between ecological subregions in Massachusetts existed only in yellow perch, although there was a suggestion of such a relationship in brown bullhead. Regionally, the Narragansett/Bristol Lowlands subecoregion and the Green Mountain/Berkshire Highlands subecoregions had somewhat lower mercury in all species than those form the Worcester Monadnock Plateau subecoregion. Other studies that attributed spatial differences in fish species mercury concentrations to the geographic regions delineated on the basis of ecological, geological and climatic factors have not been completely successful. In some of those studies, differences between regions such as presence of mercury deposits and mining activities have overshadowed ecoregional parameters. Ecoregional differences in Massachusetts may also be overshadowed by past human land use patterns in the state.

Our analyses did not show a compelling association between fish tissue mercury concentrations and lake trophic status. The surrogate variable used for lake trophic status and variables associated with it were identified as having variance patterns which were relatively independent of both fish mercury and pH.

Our analyses resolved a clear link between some of the other environmental variables and elevated mercury concentrations in fish. Low pH of the waterbody was a significant and major predictor of tissue mercury concentrations in brown bullhead and yellow perch. In largemouth bass, pH was not a significant predictor of variation in tissue mercury concentrations. However, the weight and mercury contents of the yellow perch (one of the principal prey species of largemouth bass) were associated with the mercury contents of the bass, thereby indirectly linking bass tissue mercury and low pH. While pH was not a major predictor of yellow perch tissue mercury concentrations, water calcium concentrations were.

Aside from substantiating the association between mercury in fish and acid waters in Massachusetts, the principal contributions of the present study are the confirmation of the relationships seen in other studies and the identification of variation in mercury in one fish species (yellow perch) on so fine a geographic scale as occurs across ecological subregions in a 150-mile

transect in this relatively small state. A difference in lake bedrock alone may account for elevated fish mercury concentrations, when a source of mercury is present, whether the source is mercury associated with acid rain, the earth's crust or historic mercury contamination currently being subjected to the effects of acidic waters.

## INTRODUCTION

A growing awareness of the problem of high mercury concentrations in freshwater fish during the past ten years has generated a proliferation of studies at the international (1,2,3,4,5,6,7,8), national (9,10,11) and state (12,13,14,15,16,17,18,19) levels. A considerable volume of work on the problem has been completed in Canada (20,21,22,23,24,25,26,27,28). Critical reviews and summary articles have added background and perspective to the studies (29,30,31,32,33).

Massachusetts has surveyed contaminants in freshwater fish since 1983 (34), focusing primarily in areas of known or suspected contamination, or where biological effects were observed. These studies have shown that the variation in fish mercury contamination is relatively high in surface waters. Concentrations have been sufficiently high in some species to warrant the issuance of Fish Consumption Advisories for specific waterbodies, as well as a statewide health advisory cautioning pregnant women of the possible health risk from eating fish from Massachusetts freshwater bodies, excluding stocked and farm-raised fish **(**35).

Many factors contribute to the dynamics of contaminant accumulation in fish populations (31). Previous studies have shown that an ecoregional approach partially explains geographic variation in fish mercury concentrations(17). Lake productivity and lake trophic status have been shown in other studies (2) to affect the accumulation of persistent pollutants in fish. The complexity of the definitions of ecoregion and lake trophic status makes these concepts apt descriptors for ecosystems, which are inherently complex systems.

Two ecoregions and 13 ecological subregions have been delineated in Massachusetts (36). Shared components of ecoregions included soils, vegetation, climate, geology and physiography. Patterns of animal migration and land use were also used to delineate ecoregions. The ecoregion concept may prove to be an effective tool for statistical analysis, research, and assessment of environmental resources, because it characterizes relatively homogeneous geographic regions, incorporating more information than individual physical or chemical measurements.

An example of the interaction of physiography and contaminant deposition occurs when lakes in the rain shadow of a mountain range are spared from contaminant deposition, whereas lakes on the windward side of a mountain range may be impacted. Limestone bedrock is another example of a physical attribute that buffers lake waters in some regions. The availability of certain contaminants is likely different in surface waters with limestone lake beds, when compared to poorly buffered, acidic lakes.

Lakes can be categorized according to their trophic status. Many lakes evolve naturally from oligotrophy to eutrophy as they accumulate sediment and nutrients over time, particularly under the influence of human activities. Oligotrophic lakes tend to exhibit low concentrations of phosphorus, and therefore, low production by phytoplankton and low concentrations of chlorophyll *a*. A transparent water column without depletion of dissolved oxygen is typical as well. At the other end of the trophic spectrum, eutrophic lakes exhibit comparatively high concentrations of phosphorus, and during the growing season, high concentrations of chlorophyll *a*, with

corresponding low transparency. In-lake and watershed production of organic matter stimulate the growth of aerobic bacteria which, in turn, deplete dissolved oxygen at depth. Human activity increases nutrient and sediment loading to lake basins, thereby accelerating the rate of eutrophy.

Although many lakes in Massachusetts were formed by receding glaciers, another very common origin of lakes was the construction of dams. In many of these instances, nutrient-rich pastures had been transformed to nutrient-rich lake sediments. Internal nutrient loading of phosphorus was likely assured, as was the growth of aquatic plants and algae.

In this study, yellow perch (*Perca flavescens*), largemouth bass (*Micropterus salmoides*) and brown bullheads (*Ameiurus nebulosus*) were sampled in 24 lakes that did not have active point sources of contamination (e.g., landfills, industrial facilities, hazardous waste sites, wastewater treatment facilities). One objective of the study was to determine levels of mercury, cadmium, lead, selenium, arsenic, PCBs and chlorinated pesticides in edible muscle of these species of freshwater fish from Massachusetts lakes that are typical of lakes used for fishing. We also attempted to determine the relative degrees of influence of geographic location, and lake biological, physical and chemical characteristics on fish tissue chemical concentrations.

## MATERIALS AND METHODS

### *LAKE SELECTION*

The lakes chosen for sampling were identified on the basis of the region of the state in which they were located, and the degree of development on or near the lakes. Three ecological subregions (36) that represent contrasting environmental settings in Massachusetts were selected for the present study (Figure 1). Eight lakes were selected from each of these ecological subregions.

The Green Mountain/Berkshire Highlands, located in northwestern Massachusetts, is characterized by relatively high elevations, reaching approximately 1000-2500 feet above mean sea level. Metamorphic geology composed of schists, gneiss and marbles creates a steep terrain, overlain by thin deposits of glacial till. Forest types include northern hardwoods (maple, beech, birch) and spruce-fir. Surface waters are generally low in phosphorus, with alkalinity under 200 ug/l (36).

The Worcester/Monadnock Plateau is located in the north central part of the state. Moderate elevations of 500-1500 feet above sea level are found in this area. The monadnocks after which the subregion is named are formed of granite plutons that dominate the surrounding geology of metamorphic schists and gneiss. Forest types include transition hardwoods (maple-beech-birch, oak-hickory) and some northern hardwoods. Surface waters are poorly buffered and acidic, with alkalinities generally between 50-100 ug/l (36). Some exhibit moderate to high concentrations of dissolved organic compounds.

The Narragansett/Bristol Lowland is located in the southeastern part of the state. The landscape of this region consists of flat to rolling plains that seldom exceed 200 feet above mean sea level, with numerous wetlands and bogs. Extensive deposits of glacial till and outwash material make up the soils and sediments. Central hardwoods (oak-hickory) are common as well as elm, ash, red maple, cottonwood, white pine and red pine. Phosphorus in surface waters ranges widely, and alkalinities are low to moderate (36).

All lakes within each ecological sub-region were identified on United States Geological Survey, 7.5′ series topographical maps. The suitability of each lake was then assessed using the following exclusion criteria in order to identify twenty four lakes for study:

- surface area less than 10 acres
- proximity to concentrated urban, agricultural or industrial areas
- evidence of impact from human activities based on prior studies (37,38,39)
- potential point or non-point sources of pollution (power lines, highways, cranberry bogs, storm drains, farms, development, etc).

Lake watershed areas were delineated based on US Geological Survey (USGS) topographic quadrangles (40). These area boundaries were transferred onto mylar manuscripts containing USGS interpreted sub-basins and digitized. Wetlands within the watersheds were delineated from US Fish and Wildlife Service National Wetlands Inventory (NWI) Maps (1:24,000). NWI wetlands were delineated using stereoscopic analysis of high altitude aerial photographs. Delineation was based on vegetation, visible hydrography and geography in accordance with 'Classification of Wetlands and Deepwater Habitats of the United States' (41). Surface water features were clipped from MassGeographic Information System (MassGIS) 1:25,000 hydrography coverages, which are based on USGS Digital Line Graph (DLG) data (42). In some cases MassGIS 1:25,000 digital data were unavailable for certain watersheds. Hydrographic features were then digitized from USGS 1:25,000 topographic quadrangles. Ecoregion and subregion boundaries were digitized and linework rectified with MassGIS 1:25,000 state boundaries (43). Watershed, wetland and lake areas were then calculated for each surface water body in the study using the data derived as described above.

*FISH, WATER AND SEDIMENT SAMPLING*

Yellow perch, largemouth bass, and brown bullhead were selected as test species principally because they encompass a range of fish trophic levels. Largemouth bass are fish-eating predators, although their diet also includes invertebrates and amphibians . The native range of largemouth bass extended originally only as far north as Virginia. They exist in Massachusetts lakes as a result of human manipulation of their distribution. The species did not occur in all of the study lakes. Yellow perch are omnivorous, consuming insects, invertebrates and other fish, and brown bullhead are bottom feeding omnivores (44). All three species are favored by anglers. The inclusion of

three species in the study design provided different perspectives of intraspecific and interspecific relationships of bioaccumulation of mercury with physical and geographic parameters.

Nine individuals of each species were targeted for collection from each lake. Fish sampling was conducted in the early fall after summer spawning so as to minimize the potential effects of cyclic changes in the water and fat contents of fish associated with the annual reproductive cycle. Total length criteria of 8-10 inches (20-25 cm) for yellow perch and brown bullhead and 12-14 inches (30-36 cm) for largemouth bass were established. The larger size was selected for largemouth bass because 12 inches is the legal minimum size limit for this species, and may be representative of fish retained for consumption.

Fish were obtained by electrofishing, gill netting, and trot lines. Contamination of fish samples was minimized by not allowing fish slated for inclusion in the sample to rest on the bottom of the boat, or to be handled by the person operating the boat if the boat was powered by an outboard motor or contained any other gasoline operated engine. Fish were rinsed in ambient water, chilled on ice, wrapped individually in aluminum foil, placed inside polyethylene "zip lock" bags and delivered to the laboratory on ice within 24 hours of collection.

Water quality sampling was conducted during midsummer, not coincident with fish sampling, but during the period when lakes would be thermally stratified and measures of degree of eutrophy might be strongest. In stratified lakes, water samples were taken with Van Dorn samplers in the deepest part of the lake at three different depths: 1.5 m below the surface, at mid thermocline, and at 1.5 m above the bottom. The three samples were composited, and then divided into three pre-cleaned glass containers for chemical analyses. Single samples were taken from mixed lakes (nonthermally stratified) 1.5 m below the surface. All water quality sampling and handling was performed in accordance with US EPA protocols (45). The following parameters were measured in the field using a Datasonde Hydrolab: pH, dissolved oxygen (DO), temperature, depth, conductivity. Water clarity was measured using a Secchi disk. Chlorophyll *a* samples were taken at the deepest part of the lake, 1.5 m below the surface. The samples were filtered in the field following EMAP protocols (45). The filters were placed in petri dishes, wrapped in foil, and stored on ice.

Sediments were sampled using an Ekman dredge at two locations in each waterbody: at the deep hole, and half way to a shore. These samples were combined. In addition, a replicate sample was taken at the deep hole. Pre-cleaned wide-mouthed glass jars were inverted and pushed into the portion of sediment sample away from the sides of the dredge, then capped with Teflon-lined caps, and placed on ice for shipment to the lab. All sediment sampling and handling was performed in accordance with US EPA protocols (45).

Fish and sediment samples were analyzed for total mercury, selenium, arsenic, cadmium, lead, chlorinated pesticides and PCBs. Water column samples were analyzed for total phosphorus (Total P), dissolved organic carbon (DOC), ammonia ($NH_3$-N), nitrate ($NO_3$-N), chloride ($Cl^-$), calcium (Ca), sulfate, and chlorophyll.

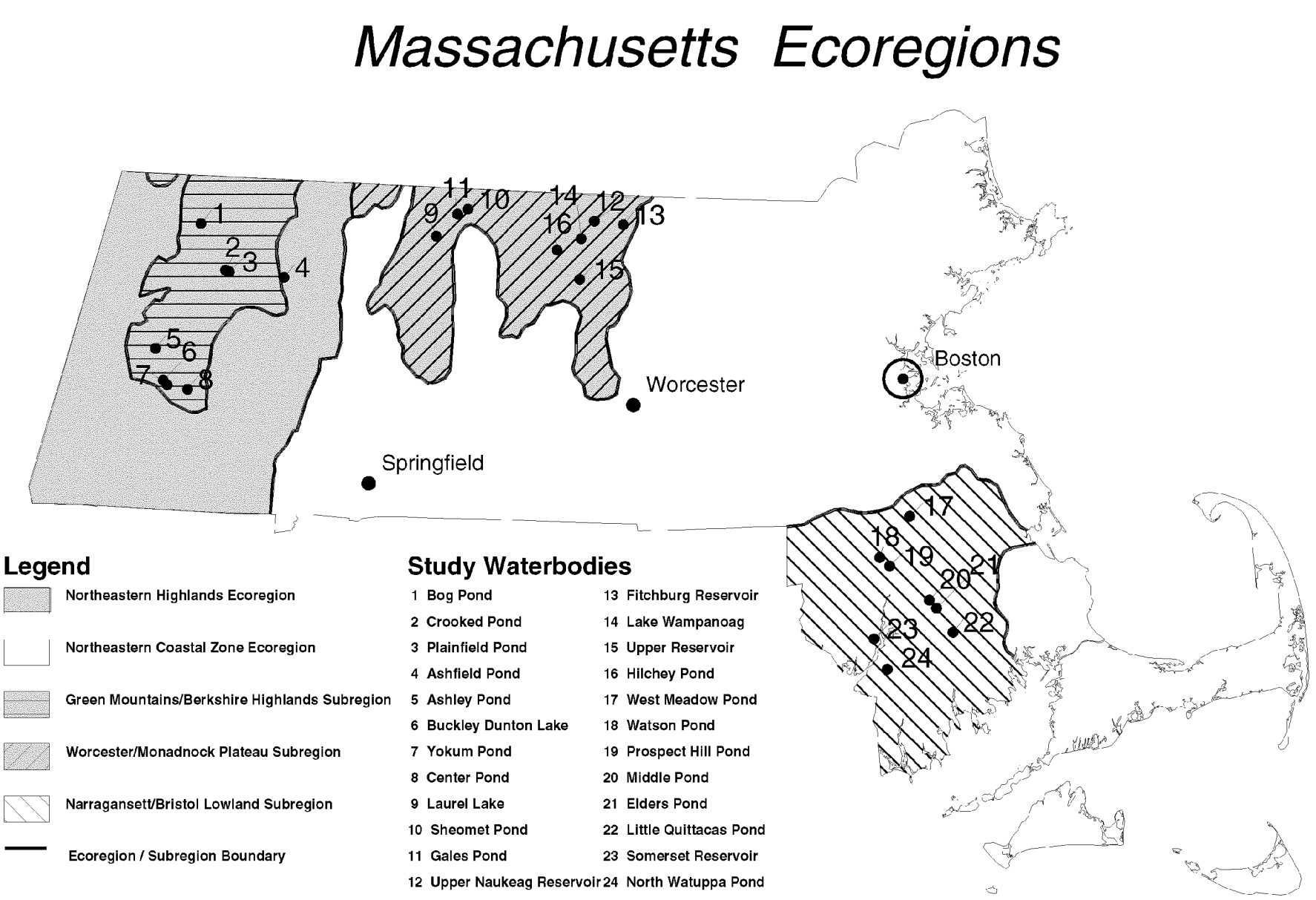



Figure 1. Ecoregions of Massachusetts used in this study.

## *LABORATORY METHODS*

### *Fish specimen processing*

Fish specimens were processed for analysis in accordance with US EPA procedures (46). In the laboratory, all fish specimens were held at 4°C prior to dissection and were dissected within 48 hours of collection. Since only the fish fillets (edible tissue) were analyzed in this study, fish specimens were not frozen prior to dissection in order to avoid possible internal organ rupture and subsequent fillet contamination (46). Fish dissection and tissue homogenization were conducted in a small clean laboratory dedicated for fish processing; it should be noted, however, that this laboratory is not a class-100 clean room (46, 47). While clean room conditions are required for the analysis of trace metals in ambient waters where ng/L concentrations are usually observed (48,49,50), it is generally accepted that such facilities are not necessary for processing fish samples which usually contain µg/g (wet weight) metal concentrations (48).

Prior to use, all fish dissection and tissue homogenization equipment, and fish sample containers were washed with a laboratory-grade detergent, rinsed with tapwater, soaked in 50% reagent-grade nitric acid (Fisher Scientific, Pittsburgh, PA) for 12 to 24 h, rinsed with tapwater, rinsed with reagent water (ASTM Type I) (51), and finally rinsed with pesticide-grade isopropanol (EM Science, Gibbstown, NJ).

Samples were analyzed within their recommended holding time for mercury of 6 months. The fish were placed on precleaned borosilicate glass dissection boards and whole fillets were removed with high quality stainless steel knives. The skin was carefully removed from the underlying muscle tissue after filleting. Sufficient mass of tissue was removed to meet the analytical detection requirements. A duplicate muscle sample from the other side of the fish was taken, frozen and archived. The sample intended for analysis was either immediately digested, or frozen until the analysis took place.

Dissection instruments and work surfaces were decontaminated between each dissection in order to minimize the chances for sample cross-contamination during handling. They were rinsed with tap water, washed with detergent, rinsed with tap water, rinsed with reagent water, and finally rinsed with isopropanol prior to use on another fish specimen. Fillets from individual fish specimens were placed in individual high-density polyethylene cups with tight-fitting covers (VWR Scientific, Boston, MA) and frozen at -20°C. Prior to analysis, the entire fillet sample from each fish specimen was thoroughly homogenized in a food grinder with stainless steel blades (Model HC20, Black & Decker, Shelton, CT).

*Mercury analysis of fish homogenates*

In addition to mercury, the concentrations of cadmium, lead, selenium, arsenic, PCBs and chlorinated pesticides in fish muscle were determined as part of this study, but are not reported here.

Individual fish homogenates were analyzed for total mercury by cold vapor atomic absorption spectrometry using US EPA Method 245.6 (52). All handling of fish homogenates prior to analysis was conducted in a NUAIRE (Plymouth, MN) Model NU-153-624 laminar airflow polypropylene fume hood for trace metal analysis that exceeds federal standard 209B for class-100 clean benches. Trace metal-grade sulfuric and nitric acids (Fisher Scientific, Pittsburgh, PA) were used throughout this study for fish sample digestions. All mercury analyses were conducted with a Varian (Victoria, Australia) Model 1475 atomic absorption spectrometer equipped with a Varian Model VGA-76 vapor generation accessory. The concentration of total mercury in individual fish fillets was expressed in units of µg/g (wet weight) as in previous studies by other investigators (13,53,54,11). The method detection limit (MDL) for mercury analysis in fish tissue of 0.020 mg/kg was experimentally determined using the conventional US EPA procedure (55)

*Analyses of water column samples*

Water column samples were collected in pre-cleaned containers purchased from I-CHEM (Hayward, CA), and analyzed for conductivity, chloride, calcium, sulfate, ammonia-nitrogen, nitrate-nitrogen, total phosphorus, dissolved organic carbon, suspended nitrogen, and suspended carbon. Conductivity was determined with a conductivity meter as per US EPA Method 120.1 (56). Chloride was analyzed by the argentometric method (51). Calcium was analyzed by inductively coupled plasma-atomic emission spectrometry (ICP-AES) using US EPA Method 200.7 (57) and a Perkin-Elmer (Norwalk, CT) Model 6500 ICP spectrometer equipped with a Perkin-Elmer Model AS-50 auto sampler. Sulfate was analyzed by turbidimetric nephelometry using US EPA Method 375.4 (56). Ammonia-N, nitrate-N, and total phosphorus were quantitated by automated colorimetry on a Bran+Luebbe (Elmsford, NY) Model Technicon TrAAcs 800 auto analyzer using US EPA Methods 350.1 (58), 353.1 (56) and 365.4 (56), respectively. Dissolved organic carbon was determined on filtered (glass-fiber filter) samples by ultraviolet (254 nm) absorbence using a Perkin-Elmer Model 554 double-beam spectrophotometer and potassium biphthalate ($C_8H_5KO_4$, Fisher Scientific) as the standard (51,59). Suspended nitrogen and carbon were determined based on the elemental analysis of suspended solids (i.e., solids retained on a glass-fiber filter) in the sample using a Perkin-Elmer Model 2400 CHN elemental analyzer.

*Analyses of sediment samples*

Sediment samples were collected in pre-cleaned containers purchased from I-CHEM, and analyzed for total mercury, arsenic, selenium, cadmium, lead, phosphorus, carbon, and nitrogen. Total mercury was analyzed by cold vapor atomic absorption spectrometry using US EPA Method 7471A (60) and the Varian atomic absorption spectrometer system described above for fish sample

analyses; this method includes the digestion of the sediment sample in aqua regia (i.e., a mixture of 3 volumes of concentrated hydrochloric acid and one volume of concentrated nitric acid) prior to analysis. Trace metal-grade acids (Fisher Scientific) were used for all sediment analyses. For total arsenic, selenium, cadmium, and lead analyses, the sediment samples were digested according to US EPA Method 3050A (1) prior to instrumental analysis. Total arsenic and selenium were analyzed by graphite furnace atomic absorption spectrometry using US EPA Methods 7060A and 7740 (60), respectively, and a Perkin-Elmer Model 5100 PC Zeeman atomic absorption spectrometer equipped with a Perkin-Elmer Model HGA-600 graphite furnace and Model AS-60 auto sampler. Total cadmium and lead were analyzed by flame atomic absorption spectrometry using US EPA Methods 7130 and 7420 (60), respectively, and a Varian Model 1475 atomic absorption spectrometer. Total phosphorus was quantitated in the sediment samples by automated colorimetry using US EPA Method 365.4 (54) as described above for water samples. Total carbon and nitrogen were determined using a Perkin-Elmer Model 2400 CHN elemental analyzer (60). All analyte concentrations in sediment samples were expressed in units of µg/g (dry weight), except for total carbon and nitrogen concentrations which were reported in % dry weight.

*STATISTICAL METHODS*

The number of each species of fish to be sampled in each lake was initially determined using data on mercury concentration sampling variance from ten years of monitoring in Massachusetts (34). Available resources for fish collection and analysis were also taken into consideration when determining optimal sample sizes for the study.

The distributions of individual species' mercury concentration values were graphed and cumulative frequency distribution curves were constructed. The distributions of mean mercury concentrations for each species were also determined for each lake. Missing values resulted in deletion of the case ("casewise deletion"). The data were tested for normality using the Kolmogorov-Smirnov test (61).

Prior to statistical analyses of the raw tissue concentration data, the data were examined for correlations between mercury content and size. While the experimental design of this study called for sampling of uniform sized fish of each species to control for the effect of size on tissue mercury, in practice the fish retained for each species represented several year classes and varied in size. Linear regressions and Pearson's product moment correlations were calculated for each species over all lakes and also for each lake. The equality of slopes of the regression lines between lakes was tested (62) as an additional check for determining the nature of the relationships between mercury content and fish size and whether these relationships were different between lakes.

A Pearson's product moment correlation matrix of the environmental data, without inclusion of the fish mercury levels, was calculated (Table 5).The data were also examined for outliers. The pH value for Prospect Hill Pond, 10.5 (Table 2), was eliminated from further analysis as an outlier, since other chemical values for this pond suggested inconsistencies.

The relative importances of the geographical locations of lakes (3 ecoregion levels) and their trophic state (2 levels) were assessed with fixed constants Model 1 analyses of variance (ANOVA) of mean lake tissue mercury concentrations with replication for both yellow perch and brown bullhead. A separate analysis of covariance of mean lake tissue mercury concentrations across ecoregions and trophic states, using fish weight as a covariate, was performed for largemouth bass due to the observed relationship between weight and mercury concentrations. Weight correlated with mercury more than other size-related variables, such as length or age. Mean mercury values for each species were normally distributed and therefore no data transformation was necessary to satisfy normality assumptions.

Lake trophic states were characterized by calculating Carlson's Trophic State Index (TSI)(63). This index gives a scaled measurement of water quality based on one of three associated parameters: surface chlorophyll *a* concentration, Secchi disk depth, or surface phosphorus concentration. Chlorophyll *a* measurements were used to calculate TSIs using the formula (64): TSI = 30.6 + 9.81 ln Chlorophyll *a* ($mg/m^3$). The TSI scales waterbodies from 0 to 110. An oligotrophic lake falls between 0-39 on the scale, a mesotrophic lake falls between 40-50, and a eutrophic lake falls between 51-110. Lakes were grouped into these three categories. Because of their small number, mesotrophic lakes were grouped with eutrophic lakes for the ANOVAs.

Stepwise multiple regressions were carried out separately for each species using lake mean fish mercury concentrations as the dependent variable and physical and chemical variables (Table 2 and Table 3 ) as independent variables. In addition to the environmental variables, the mean fish weights for largemouth bass were entered into the regression for that species to determine the extent to which size affected mercury concentration. Regressions were run with untransformed and transformed (log10 for all variables except for data in "%" units which were arc $sin^{-1}$ transformed) values for all variables. No improvements in regressions resulted with transformation, so only results on untransformed data are reported.

The data were also analyzed by factor analysis (Appendix A), using a varimax normalized rotation strategy, to assess which physical and chemical parameters might influence regional differences associated with the bioaccumulation of mercury. In factor analysis, the number of variables analyzed is limited by the number of cases. All species of fish were not available in every pond. We collected brown bullhead in 22 lakes, largemouth bass in 19 lakes, and yellow perch in 22 lakes, producing a variable number of cases. Therefore, certain variables needed to be eliminated, and stepwise multiple regressions aided in this task, by identifying variables which were not correlated with fish mercury.

All statistical evaluations were performed with the Statistica/W, Version 5.0 software package (61). Ecoregion was numerically coded for analyses as follows: 1 - Green Mountain/Berkshire Highlands; 2 - Narragansett/ Bristol Lowland; 3 - Worcester/Monadnock Plateau. This order roughly followed the order of increasing mean mercury concentrations seen in the species in the geographical areas. Lake trophic status was numerically coded, using 4 for oligotrophic lakes and 5 for mesotrophic or eutrophic lakes. In the ANOVA/ANCOVA, ecoregion and lake trophic status were treated as categorical variables.

## RESULTS

Summary statistics for mercury concentrations in each species in the 24 lakes are presented in Table 1. The only analytes detected in fish were mercury and selenium. Since concentrations of the other elements and compounds in fish were uniformly extremely low, or at or below analytical detection limits, the methods and results are not reported here, and have not been subjected to interpretation. Raw data are available from DEP's Wall Experiment Station. Unadjusted mean mercury levels in the three species of fish tested in each ecological subregion are presented in Figure 2. The results of physical and chemical sampling and measurement are contained in Table 2 and Table 3.

A total of 198 yellow perch, 169 brown bullhead and 152 largemouth bass were analyzed. Nine individuals of each species were not obtained in all waterbodies, nor were all three species present in every waterbody. Tissue mercury concentrations in each species were lognormally (Figure 3). Brown bullhead generally had the lowest concentrations, with mean tissue mercury concentrations of 0.14 mg/kg wet weight (range = 0.01-0.79 mg/kg). Yellow perch mean tissue mercury concentrations were 0.31 mg/kg (range = 0.01-0.75 mg/kg). Largemouth bass mean tissue mercury was 0.40 mg/kg (range = 0.05-1.1 mg/kg). Largemouth bass tissue mercury distributions were somewhat similar to the distribution of mercury in yellow perch and brown bullhead in the concentration range of 0.2-0.6 mg/kg, but the bass distribution had a tail to the right beyond 0.6 mg/kg with upper concentrations up to 1.1 mg/kg. Statistics for these distributions are presented in Table 4.

Species specific relationships between tissue mercury concentrations and total fish length are illustrated in Figure 4. Similar patterns held for fish wet weight as a size variable. Largemouth bass are the only one of the three species in this study which exhibited a significant correlation between fish size and mercury content. Correlation coefficients for regression equations of mercury on length for each species for individual lakes also generally exhibited the same patterns as the aggregate presented here. The slopes of these regression lines were not equal between lakes (only lakes having >3 fish were included in this analysis) for largemouth bass ($F_{16,116} = 4.74$, $p \leq 0.01$) and brown bullhead ($F_{17,125} = 3.59$, $p \leq 0.01$). They were equal for yellow perch ($F_{20,147} = 1.44$, $p = 0.11$).

Cross-correlation analyses between pairs of environmental variables identified a number of significant positive and negative relationships. Many of these were anticipated because the variables are indicators of the same or interrelated processes (e.g., pH and conductivity, chlorophyll *a* concentration and DO, Secchi disk depth and DOC or trophic status) ( Table 5). Independent measures of potential source area contributors to the amount of mercury in a lake were highly positively intercorrelated (watershed area with both wetlands area and pond area, and pond area with wetlands area). The metals mercury, cadmium and lead in pond sediments all had modest, positive correlations with sedimentary selenium concentrations.

The lake trophic state indicator values ranged from 19 to 75, with 13 lakes falling in the oligotrophic range, 7 lakes in the mesotrophic range and 4 lakes in the eutrophic range ( Table 1).

Analyses of variance showed no significant differences between lakes of different trophic states for any of the three species. The analyses of variance also determined that significant differences (at p = 0.05) in fish mercury concentrations between subecoregions existed only in yellow perch ( Table 6 and Table 7, Figure 2). Regionally, the Narragansett/Bristol Lowlands subecoregion and the Green Mountain/Berkshire subecoregion have somewhat lower mercury in all species than the Worcester/Monadnock Plateau ( Figure 2).

The environmental variables explaining the majority of the observed variance in each species tissue mercury concentrations were ranked by the stepwise multiple regression analysis and are shown in decreasing order of importance in Table 8. Regression coefficients (B) and intercepts are also shown.

The 15 variables entered into the stepwise multiple regression for yellow perch accounted for >95% of the variance in the tissue mercury concentrations (Table 8). Calcium concentrations in lake water were the most significant predictor of mercury in yellow perch tissue, accounting for 32.9% of the variance, and temperature was second most important , accounting for 12.1% of the variance. Sediment cadmium content, chloride and pH were the next most important variables, each accounting for less than 10% of the variance.

The average weight of largemouth bass was the most important determinant of the variance in largemouth bass tissue mercury concentrations, accounting for 39.7% of the variance in that variable. Mercury concentration in yellow perch was the next most significant predictor of the mercury values (30% of variance explained) in largemouth bass. The average weight of yellow perch was the next most important variable, accounting for 9.5% of the variance. The mercury concentrations used in these calculations had not been size-adjusted: instead, fish weights of all three species were included as independent variables in the analysis for this species to determine the relative effects of weight in relation to the other environmental variables.

Table 1. Summary statistics for mercury concentrations in brown bullhead, largemouth bass and yellow perch in Massachusetts lakes.

| Species | Region | Lake | Trophic state | n | Mean Hg (mg/kg) | Hg std. dev. | Mean weight (gm) | Weight std. dev. |
|---|---|---|---|---|---|---|---|---|
| Brown Bullhead | Green Mtn/ Berkshire | Plainfield Pond | oligotrophic | 9 | 0.182 | 0.( | 97.11 | 61.74 |
| | | Ashfield Pond | oligotrophic | 9 | 0.083 | 0.( | 144.89 | 22.25 |
| | | Yokum Pond | oligotrophic | 6 | 0.050 | 0.( | 225.89 | 84.81 |
| | | Buckley Dunton | mesotrophic | 9 | 0.168 | 0. | 185.56 | 60.22 |
| | | Center Pond | oligotrophic | 9 | 0.123 | 0.( | 195.67 | 43.92 |
| | | Ashley Lake | oligotrophic | 10 | 0.099 | 0.( | 175.70 | 54.28 |
| | | Bog Pond | mesotrophic | 9 | 0.149 | 0.( | 72.67 | 10.32 |
| | | Crooked Pond | mesotrophic | 9 | 0.115 | 0.( | 136.94 | 91.78 |
| | Narragansett/ Bristol | Elders Pond | eutrophic | 6 | 0.279 | 0.: | 466.00 | 46.74 |
| | | West Meadow Pond | eutrophic | 8 | 0.074 | 0.( | 515.00 | 110.37 |
| | | Little Quitticas Pond | oligotrophic | 4 | 0.225 | 0. | 470.75 | 304.31 |
| | | Prospect Hill Pond | oligotrophic | 0 | ---- | ---- | ---- | ---- |
| | | North Watuppa | oligotrophic | 2 | 0.100 | 0.( | 563.50 | 37.48 |
| | | Somerset Reservoir | mesotrophic | 2 | 0.187 | 0.( | 733.50 | 78.49 |
| | | Middle Pond | mesotrophic | 3 | 0.026 | 0.( | 416.00 | 164.33 |
| | | Watson Pond | eutrophic | 9 | 0.069 | 0.( | 460.33 | 118.28 |
| | Worcester/ Monadnock | Wampanoag Lake | oligotrophic | 9 | 0.214 | 0. | 105.67 | 29.07 |
| | | Upper Naukeag | oligotrophic | 0 | --- | --- | --- | --- |
| | | Hilchey Pond | eutrophic | 9 | 0.186 | 0.( | 205.62 | 55.55 |
| | | Sheomet Pond | oligotrophic | 9 | 0.097 | 0.( | 66.67 | 16.51 |
| | | Upper Reservoir | oligotrophic | 2 | 0.260 | 0.( | 224.5 | 36.06 |
| | | Laurel Lake | oligotrophic | 9 | 0.116 | 0.( | 329.00 | 135.36 |
| | | Gales Pond | mesotrophic | 9 | 0.322 | 0. | 142.44 | 39.86 |
| | | Fitchburg Res | mesotrophic | 8 | 0.107 | 0.( | 172.00 | 93.28 |

| Species | Region | Lake | Trophic state | n | Mean Hg (mg/kg) | Hg std. dev. | Mean weight (gm) | Weight std. dev. |
|---|---|---|---|---|---|---|---|---|
| Largemouth bass | Green Mtn/ Berkshire | Plainfield Pond | oligotrophic | 9 | 0.626 | 0.2 | 767.75 | 411.57 |
| | | Ashfield Pond | oligotrophic | 9 | 0.468 | 0.3 | 419.11 | 324.61 |
| | | Yokum Pond | oligotrophic | 9 | 0.188 | 0.0 | 374.50 | 147.28 |
| | | Buckley Dunton | mesotrophic | 11 | 0.426 | 0.2 | 572.00 | 298.54 |
| | | Center Pond | oligotrophic | 9 | 0.323 | 0.1 | 729.10 | 248.60 |
| | | Ashley Lake | oligotrophic | 0 | ---- | ---- | ---- | ---- |
| | | Bog Pond | mesotrophic | 9 | 0.413 | 0.1 | 794.44 | 257.60 |
| | | Crooked Pond | mesotrophic | 0 | ---- | ---- | ---- | ---- |
| | Narragansett/ Bristol | Elders Pond | eutrophic | 9 | 0.250 | 0.0 | 555.78 | 197.76 |
| | | West Meadow | eutrophic | 9 | 0.144 | 0.0 | 298.33 | 105.96 |
| | | Little Quitticas | oligotrophic | 5 | 0.280 | 0.1 | 272.60 | 107.53 |
| | | Prospect Hill | oligotrophic | 9 | 0.199 | 0.0 | 541.44 | 92.84 |
| | | North Watuppa | oligotrophic | 9 | 0.724 | 0.1 | 1150.56 | 379.61 |
| | | Somerset Res | mesotrophic | 9 | 0.668 | 0.2 | 713.50 | 305.66 |
| | | Middle Pond | mesotrophic | 10 | 0.330 | 0.1 | 556.80 | 490.64 |
| | | Watson Pond | eutrophic | 9 | 0.309 | 0.0 | 581.22 | 175.66 |
| | Worcester/ Monadnock | Wampanoag Lake | oligotrophic | 9 | 0.439 | 0.1 | 475.11 | 119.05 |
| | | Up Naukeag | oligotrophic | 1 | 0.366 | 0 | 328.00 | 0 |
| | | Hilchey Pond | eutrophic | 0 | ---- | ---- | ---- | ---- |
| | | Sheomet Pond | oligotrophic | 0 | ---- | ---- | ---- | ---- |
| | | Upper Res | oligotrophic | 9 | 0.551 | 0.1 | 488.89 | 441.76 |
| | | Laurel Lake | oligotrophic | 9 | 0.392 | 0.1 | 619.11 | 127.48 |
| | | Gales Pond | mesotrophic | 0 | ---- | ---- | ---- | ---- |
| | | Fitchburg Res. | mesotrophic | 0 | ---- | ---- | ---- | ---- |

| Species | Region | Lake | Trophic state | n | Mean Hg (mg/kg) | Hg std. dev. | Mean weight (gm) | Weight std. dev. |
|---|---|---|---|---|---|---|---|---|
| Yellow perch | Green Mtn/ Berkshire | Plainfield Pond | oligotrophic | 9 | 0.342 | 0.126 | 80.78 | 29.04 |
| | | Ashfield Pond | oligotrophic | 9 | 0.330 | 0.085 | 75.67 | 19.80 |
| | | Yokum Pond | oligotrophic | 9 | 0.105 | 0.046 | 118.11 | 32.32 |
| | | Buckley Dunton | mesotrophic | 9 | 0.272 | 0.145 | 96.33 | 29.11 |
| | | Center Pond | oligotrophic | 9 | 0.181 | 0.079 | 121.44 | 29.85 |
| | | Ashley Lake | oligotrophic | 10 | 0.380 | 0.176 | 104.80 | 27.01 |
| | | Bog Pond | mesotrophic | 10 | 0.284 | 0.071 | 133.11 | 76.37 |
| | | Crooked Pond | mesotrophic | 9 | 0.46 | 0.076 | 139.70 | 27.98 |
| | Narragansett/ Bristol | Elders Pond | eutrophic | 9 | 0.273 | 0.062 | 124.56 | 34.03 |
| | | West Meadow | eutrophic | 0 | ---- | ---- | ---- | ---- |
| | | Little Quitticas | oligotrophic | 9 | 0.272 | 0.139 | 113.89 | 20.78 |
| | | Prospect Hill | oligotrophic | 9 | 0.106 | 0.063 | 122.78 | 29.12 |
| | | North Watuppa | oligotrophic | 9 | 0.338 | 0.163 | 170.88 | 95.28 |
| | | Somerset Res. | mesotrophic | 9 | 0.203 | 0.054 | 32.44 | 22.47 |
| | | Middle Pond | mesotrophic | 9 | 0.155 | 0.052 | 258.00 | 67.58 |
| | | Watson Pond | eutrophic | 9 | 0.195 | 0.065 | 87.89 | 26.52 |
| | Worcester/ Monadnock | Wampanoag | oligotrophic | 9 | 0.439 | 0.067 | 74.88 | 25.53 |
| | | Upper Naukeag | oligotrophic | 9 | 0.547 | 0.091 | 94.67 | 16.02 |
| | | Hilchey Pond | eutrophic | 9 | 0.314 | 0.090 | 142.67 | 24.59 |
| | | Sheomet Pond | oligotrophic | 0 | ---- | --- | ---- | ---- |
| | | Upper Reservoir | oligotrophic | 9 | 0.465 | 0.148 | 103.56 | 33.59 |
| | | Laurel Lake | oligotrophic | 9 | 0.219 | 0.056 | 97.56 | 15.88 |
| | | Gales Pond | mesotrophic | 9 | 0.514 | 0.073 | 91.00 | 24.77 |
| | | Fitchburg Res. | mesotrophic | 9 | 0.326 | 0.088 | 112.22 | 18.01 |

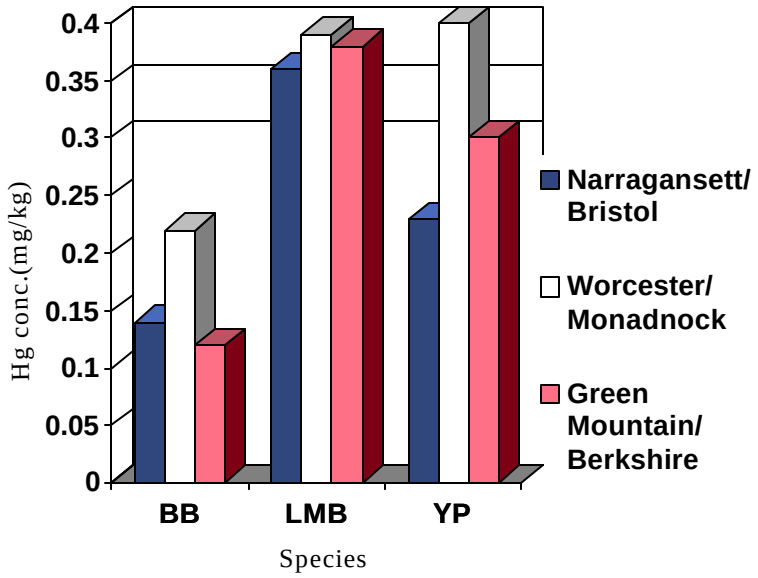
# Table 2. Physical/chemical characteristics of study lakes.

| Lake | pH | Chlorophyll a ($mg/m^3$) | Secchi depth (m) | Depth (m) | Conductivity (mS) | DO (mg/L) | Total P (mg/L) | DOC mg/L | $NH_3$ - N (mg/L) | $NO_3$ -N (mg/L) | $Cl^-$ (mg/L) | Ca (mg/L) | $SO_4$ (mg/L) | Wshed area (W)(acres) | Pond Area (P) (acres) | W/P | Wtld area(acres) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Plainfield | 7.5 | 1.2 | 2.75 | 2.75 | 37 | 8.6 | <=MDL | <=MDL | <=MDL | <=MDL | 4 | 2.3 | <=MDL | 419 | 63 | 7 | 23.5 |
| Ashfield | 8.5 | .5 | 3.1 | 5 | 178 | 8.87 | <=MDL | 0.7 | <=MDL | <=MDL | 28 | 16 | 4 | 709 | 39 | 18 | 4.2 |
| Yokum | 7.2 | .8 | 2.75 | 2.9 | 50.8 | 8 | 0.01 | 0.5 | <=MDL | 0.03 | 1 | 20 | 4 | 399 | 95 | 4 | 6.8 |
| B- D Res. | 5.7 | 5.1 | 1.2 | 3.25 | 29.1 | 6.36 | <=MDL | 9.7 | 0.15 | 0.02 | 2 | 1.8 | 2 | 1435 | 145 | 10 | 42.2 |
| Center Pond | 7.5 | 2.1 | 2.75 | 4.8 | 114 | 8.34 | 0.02 | 0.5 | <=MDL | 0.02 | 19 | 28 | 4 | 632 | 102 | 6 | 23.4 |
| Ashley Lake | 7.9 | 1.9 | 4 | 13.8 | 47.9 | 8.09 | <=MDL | 3.1 | <=MDL | <=MDL | 2 | 3.8 | <=MDL | 428 | 111 | 4 | 10.3 |
| Bog Pond | 6.5 | 3.7 | 1.5 | 2 | 19.2 | 7.21 | <=MDL | 12.1 | <=MDL | <=MDL | 1 | 2.7 | <=MDL | 872 | 37 | 24 | 34.2 |
| Crooked | 6.7 | 2.8 | 2.25 | 2.75 | 23 | 7.13 | <=MDL | 2.2 | <=MDL | <=MDL | <=MDL | 1.4 | <=MDL | 237 | 34 | 7 | 20.5 |
| Elders Pond | 7.1 | 14.3 | 2.9 | 13.8 | 117.4 | 7.85 | <=MDL | 3.4 | <=MDL | <=MDL | 21 | 3 | 8 | 574 | 137 | 4 | 14.6 |
| W. Meadow | 7.6 | 90.8 | 0.04 | 1.5 | 209 | 2.54 | 0.01 | 23.3 | <=MDL | <=MDL | 35 | 8.3 | 6 | 2956 | 72 | 41 | 218.8 |
| L. Quittacas | 7.1 | 1.5 | 2.5 | 3.75 | 102.8 | 7.54 | 0.01 | 8 | <=MDL | <=MDL | 18 | 3.4 | 6 | 1030 | 278 | 4 | 130.5 |
| Prospect H. | 10.5 | 1.9 | 1.25 | 2 | 135.7 | 9.92 | 0.04 | 6.1 | <=MDL | <=MDL | 11 | 0.8 | 17 | 307 | 42 | 7 | 21.5 |
| N. Watuppa | 6.1 | 1.1 | 2.75 | 4.75 | 93.3 | 7 | 0.01 | 4 | <=MDL | <=MDL | 17 | 2.9 | 8 | 7252 | 1730 | 4 | 752.3 |
| Somerset R. | 7.3 | 2.9 | 2.5 | 9.5 | 101.7 | 7.39 | 0.02 | 6 | 0.05 | <=MDL | 13 | 6.6 | 9 | 924 | 164 | 6 | 90.6 |
| Middle | 8.9 | 2.5 | 2.2 | 4.5 | 152.6 | 7.9 | 0.01 | 2.5 | 0.11 | <=MDL | 22 | 7.7 | 4 | 1029 | 24 | 44 | 98.6 |
| Watson | 8.3 | 40.2 | 0.6 | 2.9 | 101.3 | 7.32 | 0.04 | 13 | 0.09 | <=MDL | 16 | 5.6 | <=MDL | 389 | 72 | 5 | 63. 6 |
| Wampanoag | 5.4 | 1.1 | 2.5 | 3.75 | 79.2 | 7.84 | <=MDL | 6.1 | <=MDL | <=MDL | 18 | 2.2 | 2 | 1911 | 224 | 9 | 272.5 |
| U. Naukeag | 5.6 | .4 | 7.5 | 13.75 | 47.8 | 7.64 | <=MDL | 0.1 | <=MDL | <=MDL | 9 | 1 | <=MDL | 1224 | 304 | 4 | 67.1 |
| Hilchey | 7.3 | 13.2 | 0.07 | 2.7 | 152 | 7.13 | 0.01 | 40 | 1.51 | 0.05 | 14 | 5.5 | 4 | 2033 | 12 | 170 | 356.4 |
| Sheomet | 6.8 | 1.8 | 2.25 | 3.2 | 37 | 7.92 | <=MDL | 4.2 | <=MDL | <=MDL | 3 | 2.2 | 3 | 3415 | 31 | 112 | 49.0 |
| U. Reservoir | 4.9 | 2.3 | 0.75 | 1.1 | 45.8 | 7.15 | 0.01 | 58.8 | <=MDL | <=MDL | 6 | 1.8 | <=MDL | 1099 | 41 | 26 | 212.2 |
| Laurel | 6.4 | .3 | 6 | 7.3 | 24.7 | 7.87 | <=MDL | 5 | <=MDL | <=MDL | <=MDL | 5.5 | 2 | 541 | 41 | 13 | 7.6 |
| Gales Pond | 6.1 | 4.4 | 0.75 | 1.3 | 36.7 | 7.19 | 0.01 | 37 | <=MDL | <=MDL | 5 | 2.6 | 2 | 2047 | 11 | 181 | 192.9 |
| Fitchburg R. | 6.3 | 4.1 | 5.25 | 6 | 73.9 | 7.99 | <=MDL | 0.8 | <=MDL | <=MDL | 14 | 1.7 | 4 | 1368 | 150 | 9 | 47.1 |

Table 3. Sediment metal concentrations (mg/kg) from lakes

| Location | Arsenic | Selenium | Mercury | Cadmium | Lead |
|---|---|---|---|---|---|
| Plainfield Pond | 2.95 | 1.80 | 0.200 | 10.0 | 144.0 |
| Ashfield Pond | 2.28 | 1.10 | 0.172 | 8.6 | 84.0 |
| Yokum Pond | 0.44 | 0.32 | 0.030 | ≤MDL | 50.0 |
| Buckley-Dunton Reservoir | 2.29 | 1.34 | 0.290 | 10.0 | 55.0 |
| Center Pond | 0.47 | 0.29 | 0.080 | ≤MDL | 144.0 |
| Ashley Lake | 1.60 | 1.26 | 0.222 | 6.7 | 60.0 |
| Bog Pond | 1.63 | 1.27 | 0.133 | 6.7 | 51.0 |
| Crooked Pond | 2.92 | 1.92 | 0.250 | 13.0 | 127.0 |
| | | | | | |
| Elders Pond | 1.75 | 0.11 | 0.029 | 1.5 | 7.6 |
| West Meadow Pond | 5.41 | 2.81 | 0.366 | 12.3 | 101.5 |
| Little Quitticas Pond | 8.19 | 1.54 | 0.279 | 2.6 | 56.0 |
| Prospect Hill Pond | 27.05 | 1.62 | 0.213 | 50.0 | 112.0 |
| North Watuppa Pond | ≤MDL | ≤MDL | 0.149 | ≤MDL | ≤MDL |
| Somerset Reservoir | 3.40 | 0.76 | 0.215 | 3.1 | 55.0 |
| Middle Pond | 6.05 | 0.76 | 0.128 | 5.9 | 41.0 |
| Watson Pond | 395.00 | 1.98 | 0.425 | 17.0 | 134.5 |
| | | | | | |
| Wampanoag Lake | 6.14 | 1.14 | 0.301 | 4.4 | 62.5 |
| Upper Naukeg Reservoir | 11.48 | 2.31 | 0.148 | 39.0 | 104.0 |
| Hilchey Pond | 5.47 | 0.69 | 0.282 | 6.9 | 9.5 |
| Sheomet Pond | 1.52 | 0.95 | 0.266 | 7.2 | 28.5 |
| Upper Reservoir | 4.17 | 2.05 | 0.215 | 7.2 | 89.5 |
| Laurel Lake | 2.41 | 1.45 | 0.274 | 11.0 | 123.0 |
| Gales Pond | 2.35 | 1.85 | 0.356 | 8.9 | 55.0 |
| Fitchburg Reservoir | 11.27 | 1.06 | 0.260 | 3.9 | 55.5 |

Figure 2. Mean species mercury concentrations (mg/kg) in Massachusetts ecoregions.




BB=Brown bullhead, LMB=Largemouth bass, YP=Yellow perch

Figure 3. Frequency distributions for individual species tissue mercury concentrations.

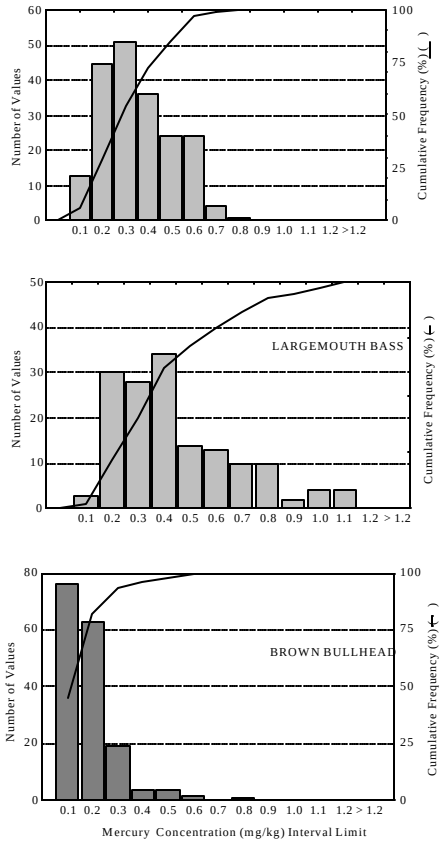



Table 4. Summary mercury concentration statistics for individual species.

| Species | n | Mean ± 1 std. dev | Geometric mean, geom. std. dev. | Distribution Percentile Values (%) | | | |
|---|---|---|---|---|---|---|---|
| | | | | 25 | 50 | 75 | 95 |
| yellow perch | 198 | 0.31 ± 0.16 | 0.26, 1.89 | 0.18 | 0.27 | 0.42 | 0.57 |
| largemouth bass | 152 | 0.40 ± 0.23 | 0.34, 1.82 | 0.21 | 0.33 | 0.54 | 0.91 |
| brown bullhead | 169 | 0.14 ± 0.11 | 0.11, 1.91 | 0.08 | 0.11 | 0.17 | 0.32 |

Figure 4. Tissue mercury concentrations versus total fish length. Linear Regressions: Yellow Perch: Hg = 0.200 + 0.0005*Length, r = 0.09, p for $H_o$:ρ = 0, 0.21; Largemouth bass: Hg = -0.542 + 0.003*Length, r = 0.72, p for $H_o$: ρ= 0, 0.011; Brown bullhead: Hg = 0.14 + 0.000003*Length, r = 0.002, p for $H_{o:}$ ρ = 0, 0.98.

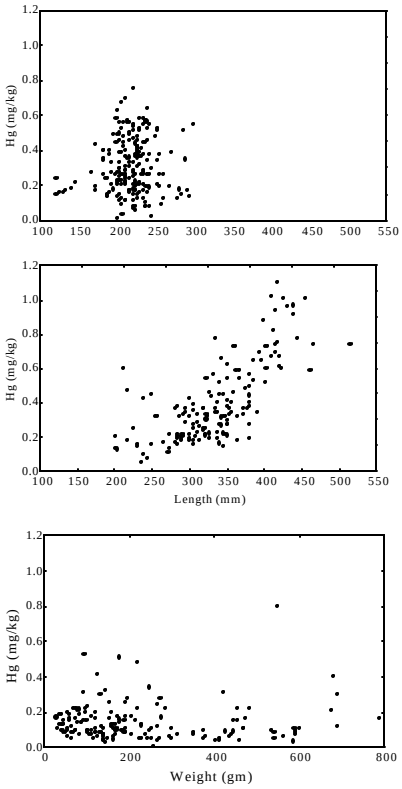


Table 5. Pearson's correlation coefficients between environmental variables.
(*: significant at p<0.05)

| | pH | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| pH | 1.00 | As | | | | | | | | | | | | | | | | | | | | | | |
| As | .30 | 1.00 | Se | | | | | | | | | | | | | | | | | | | | | |
| Se | -.16 | .24 | 1.00 | Hg | | | | | | | | | | | | | | | | | | | | |
| Hg | -.08 | .45* | .63 | 1.00 | Cd | | | | | | | | | | | | | | | | | | | |
| Cd | .15 | .19 | .54* | .16 | 1.00 | Pb | | | | | | | | | | | | | | | | | | |
| Pb | .15 | .32 | .56* | .27 | .43* | 1.00 | CH | | | | | | | | | | | | | | | | | |
| CH | .27 | .35 | .45* | .44* | .06 | .19 | 1.00 | SD | | | | | | | | | | | | | | | | |
| SD | -.15 | -.22 | -.09 | -.34 | .14 | .03 | -.42* | 1.00 | DO | | | | | | | | | | | | | | | |
| DO | .10 | -.02 | -.40 | -.40* | .20 | .06 | -.80* | .36 | 1.00 | DC | | | | | | | | | | | | | | |
| DC | .34 | .04 | .33 | .38 | -.04 | .03 | .24 | -.58* | .37 | 1.00 | Ca | | | | | | | | | | | | | |
| Ca | .45* | -.03 | -.39 | -.42* | -.33 | .22 | .05 | -.01 | .08 | -.20 | 1.00 | CN | | | | | | | | | | | | |
| CN | .62* | .13 | -.11 | -.05 | .03 | .01 | .51* | -.29 | -.18 | -.04 | .44* | 1.00 | S | | | | | | | | | | | |
| S | .54* | -.16 | -.31 | -.19 | .28 | -.23 | .06 | -.16 | .16 | -.15 | .05 | .56* | 1.00 | BP | | | | | | | | | | |
| BP | -.02 | -.11 | .04 | .34 | -.08 | -.17 | .08 | -.42* | -.16 | .58* | -.11 | -.04 | -.08 | 1.00 | WD | | | | | | | | | |
| WD | -.27 | -.15 | -.23 | .11 | -.22 | -.50* | .14 | -.12 | -.37 | .12 | -.18 | .07 | .17 | .26 | 1.00 | PA | | | | | | | | |
| PA | -.24 | -.07 | -.36 | -.17 | -.16 | -.42* | -.10 | .15 | -.09 | -.17 | -.12 | .02 | .23 | -.21 | .81* | 1.00 | WT | | | | | | | |
| WT | -.29 | -.07 | -.20 | .13 | -.22 | -.37 | .11 | -.25 | -.33 | .34 | -.18 | .13 | .17 | .24 | .87* | .80* | 1.00 | DP | | | | | | |
| DP | -.07 | -.12 | -.23 | -.37 | .04 | -.21 | -.19 | .69* | .20 | -.42* | -.06 | -.03 | .01 | -.33 | -.15 | .11 | -.21 | 1.00 | T | | | | | |
| T | .37 | .32 | -.02 | -.02 | .16 | -.08 | .29 | -.09 | .02 | -.15 | -.14 | .54* | .45* | -.26 | -.19 | -.14 | -.15 | .21 | 1.00 | TP | | | | |
| TP | .35 | .61* | .06 | .20 | .39 | .31 | .24 | -.42* | .12 | .14 | .19 | .44* | .51* | -.04 | -.10 | -.01 | .05 | -.27 | .40 | 1.00 | ER | | | |
| ER | -.45* | .02 | .16 | .38 | .15 | -.15 | .03 | .14 | -.08 | .43* | -.42* | -.09 | .04 | .46* | .30 | .03 | .33 | .02 | .16 | .00 | 1.00 | TS | | |
| TS | -.18 | .31 | .44* | .47* | .31 | .16 | .44* | -.70* | -.38 | -.71* | -.22 | .05 | .03 | .40 | .00 | -.23 | .12 | -.53* | -.06 | .41* | .41* | 1.00 | AM | |
| Am | .12 | .01 | -.17 | .16 | -.05 | .01 | .06 | -.32 | -.08 | .42* | -.01 | .11 | .01 | .58* | .08 | -.11 | .29 | -.13 | -.11 | .06 | .23 | .33 | 1.00 | N |
| N | .06 | -.10 | -.37 | -.14 | -.19 | .03 | -.03 | -.26 | -.04 | .23 | .41* | .04 | .00 | .37 | -.03 | -.12 | .12 | -.19 | -.36 | .06 | -.08 | .22 | .78* | 1.00 |
| Cl | .42* | .11 | -.05 | .04 | -.09 | -.07 | .55* | -.19 | -.31 | -.02 | .30 | .92* | .39 | -.06 | .22 | .15 | .27 | .02 | .51* | .22 | .07 | -.03 | .06 | -.09 |

*KEY:*

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| *As=arsenic* | *Cd=cadmium* | *SD=Secchi depth* | *Ca=calcium* | *BP=basin/pond ratio* | *WT=wetlands area* | *TP=total phosphorus* | *Am=ammonia* |
| *Se=selenium* | *Pb=lead* | *DO=dissolved oxygen* | *CN=conductance* | *WD=watershed area* | *DP=depth* | *ER=subecoregion* | *N=nitrate* |
| *Hg=mercury* | *CH=chlorophyll a* | *DC= organic carbon* | *S=sulfate* | *PA=pond area (acres)* | *T=temperature* | *TS=trophic status* | *Cl=chloride* |

Table 6. ANOVA results for effect of region (1), trophic status (2) and their interaction (1,2) on yellow perch and brown bullhead muscle mercury concentrations.

YELLOW PERCH

| Effect | degrees of freedom Effect | Mean Square Effect | degrees of freedom Error | Mean Square Error | F | p-level |
|---|---|---|---|---|---|---|
| 1 | 2 | 0.0470 | 16 | 0.0130 | 3.6195 | 0.05 |
| 2 | 1 | 0.0009 | 16 | 0.0130 | 0.0656 | 0.80 |
| 1,2 | 2 | 0.0057 | 16 | 0.0130 | 0.4377 | 0.65 |

BROWN BULLHEAD

| Effect | degrees of freedom Effect | Mean Square Effect | degrees of freedom Error | Mean Square Error | F | p-level |
|---|---|---|---|---|---|---|
| 1 | 2 | 0.0034 | 15 | 0.0063 | 0.5397 | 0.59 |
| 2 | 1 | 0.0028 | 15 | 0.0063 | 0.4362 | 0.52 |
| 1,2 | 2 | 0.0040 | 15 | 0.0063 | 0.6308 | 0.55 |

Table 7. ANCOVA results for effect of region (1), trophic status (2) and their interaction (1,2) on largemouth bass tissue mercury concentrations.

LARGEMOUTH BASS

| Effect | degrees of freedom Effect | Mean Square Effect | degrees of freedom Error | Mean Square Error | F | p-level |
|---|---|---|---|---|---|---|
| 1 | 2 | 0.0347 | 11 | 0.0143 | 2.4244 | 0.13 |
| 2 | 1 | 0.0004 | 11 | 0.0143 | 0.0251 | 0.88 |
| 1,2 | 2 | 0.0216 | 11 | 0.0143 | 1.509 | 0.26 |

Table 8. Stepwise multiple regression results for each species showing important predictor variables of tissue mercury concentrations.

Dependent Variable: Mercury in yellow perch
R= .9768 $R^2$= .9542 Adjusted $R^2$= .8169
F(15,5)=6.947 p<.021 Std.Error of estimate: .0504

| | Step +in/-out | R-square change | B |
|---|---|---|---|
| Intercept | | | 2.883 |
| Calcium | 1 | .329 | .001 |
| Temperature | 2 | .121 | -.046 |
| Sedimentary cadmium | 3 | .077 | .007 |
| Chloride | 4 | .062 | .003 |
| pH | 5 | .055 | -.085 |
| Nitrate | 6 | .041 | -9.205 |
| Ammonia | 7 | .055 | .244 |
| Watershed area | 8 | .031 | -.000 |
| Wetland area | 9 | .030 | .000 |
| Sedimentary selenium | 10 | .044 | .004 |
| Sulfate | 11 | .034 | .000 |
| Secchi | 12 | .035 | -.059 |
| Trophic status | 13 | .013 | -.176 |
| Depth | 14 | .016 | .012 |
| Basin /pond area ratio | 15 | .010 | .000 |

Dependent Variable: Mercury in largemouth bass
R= .9999 $R^2$= .9999 Adjusted $R^2$= .9999
F(14,1)=2136000 p<.00054 Std.Error of estimate: .0001

| | Step +in/-out | R-square change | B |
|---|---|---|---|
| Intercept | | | -.2267 |
| Average weight of bass | 1 | .397 | .0005 |
| Mercury in yellow perch | 2 | .300 | -.3669 |
| Average weight yellow perch | 3 | .095 | -.0037 |
| Depth | 4 | .050 | -.0016 |
| Sulfate | 5 | .062 | .0438 |
| Selenium in sediments | 6 | .013 | .2072 |
| Mercury in sediments | 7 | .023 | -.5256 |
| Watershed area | 8 | .013 | .0001 |
| DOC | 9 | .016 | .0057 |
| Average weight brown bullhead | 10 | .014 | -.0004 |
| DO | 11 | .009 | .0729 |
| Calcium | 12 | .007 | -.0021 |
| Ammonia | 13 | .001 | .3640 |
| Conductance | 14 | .001 | -.0003 |

Dependent Variable: Mercury in brown bullhead
R= .9848 $R^2$= .9697 Adjusted $R^2$= .9092
F(14,7)=16.026 p<.00057 Std.Error of estimate: .02657

| | Step +in/-out | | R-square change | B |
|---|---|---|---|---|
| Intercept | | | | -.2844 | |
| DOC | 1 | | .391 | | -.0008 |
| pH | 2 | | .158 | | -.0749 |
| Depth | 3 | | .083 | | .0143 |
| DO | 4 | | .046 | | .0681 |
| Secchi depth | 5 | | .051 | | -.0347 |
| Selenium | 6 | | .044 | | .1384 |
| Sulfate | 7 | | .113 | | .0190 |
| Mercury | 8 | | .026 | -.3679 | |
| Basin/pond | 9 | | .015 | | .0006 |
| Lead | 10 | | .011 | | .0007 |
| Trophic state | 11 | | .009 | | .0702 |
| Cadmium | 12 | . | .007 | | -.0062 |
| Calcium | 13 | | .010 | -.0029 | |
| Chloride | 14 | | .007 | | .0012 |

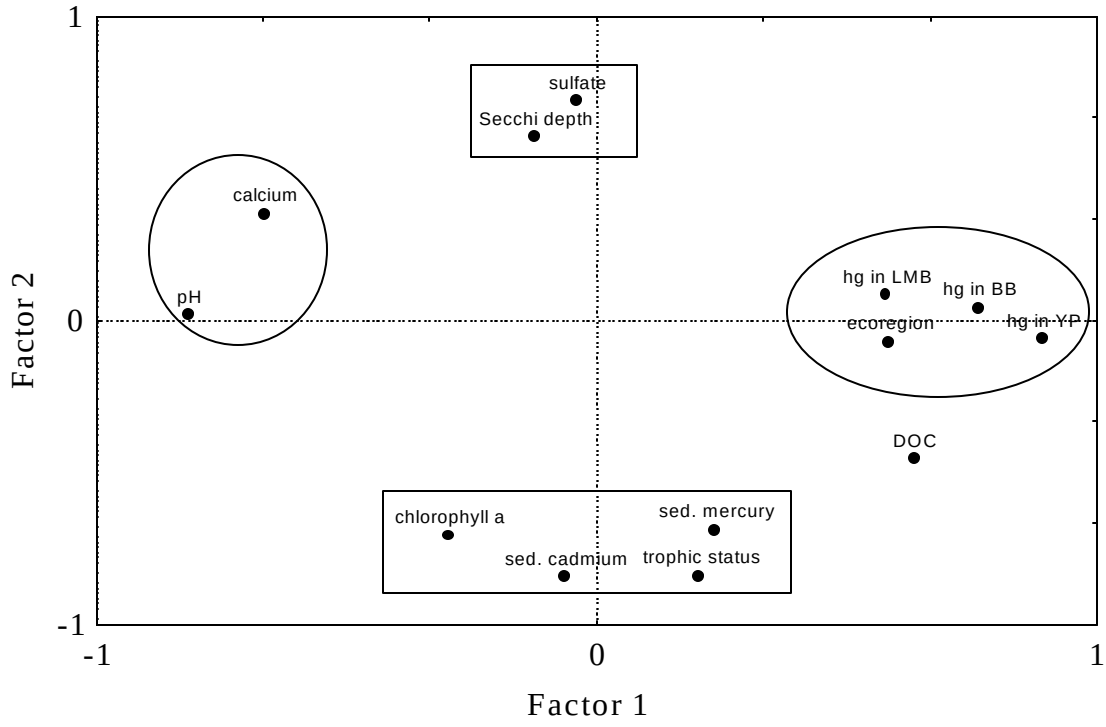
Fourteen environmental variables accounted for >97% of the variance in the brown bullhead tissue mercury concentration data with dissolved organic carbon content being the most important, accounting for 39% of the total, and pH, second most important, accounting for 15.8% of the total (Table 8). All other environmental variables contributed to explaining less than 10% of the variance individually.

Factor analysis provided another perspective on the relationships between the variables by partitioning the variation in a reduced set of variables, including mercury concentrations in each species, into two relatively independent factors explaining about 50% of the variation in the measured variables ( Table 9). Figure 5 graphs the factor scores on these two factors (the center of the graph represents a score of 0 on both Factors). The data points enclosed by ellipses score high (negatively or positively) on Factor 1. Tissue mercury concentrations in all three species are clustered on the high positive end of Factor 1 (Figure 5), along with the variable "ecoregion" (representing ecological subregion). The high negative scores for pH and calcium on Factor 1 indicate that these intercorrelated variables are inversely associated with mercury concentrations in largemouth bass, yellow perch and brown bullhead (Figure 5 and Table 9). Data points enclosed in rectangles score high (or high negative) on Factor 2. Secchi depth scores high on Factor 2, while lake trophic status and chlorophyll *a* score high negative, reflecting the relationship between chlorophyll *a*, Secchi disk readings, and lake trophic status. Sedimentary mercury and cadmium score with trophic status of the lake and chlorophyll on Factor 2, suggesting that sedimentary metals enrichment is associated with lake trophic status. Sulfate also appears to be related to trophic status based on its association with Factor 2 in Figure 5.

Table 9. Factor loadings for variables on two factors extracted by principal components. All species included.

| Variables | Factor 1 | Factor 2 |
|---|---|---|
| Mercury in bullhead | .762 | .041 |
| Mercury in bass | .578 | .085 |
| Mercury in perch | .893 | -.063 |
| pH | -.816 | .018 |
| Mercury in seds. | .235 | -.690 |
| Cadmium in seds | -.064 | -.846 |
| Chlorophyll a | -.295 | -.708 |
| Secchi depth | -.125 | .604 |
| DOC | .634 | -.455 |
| Calcium | -.664 | .348 |
| Sulfate | -.038 | .722 |
| Ecoregion | .582 | -.072 |
| Trophic state | .197 | .851 |
| Chloride | .205 | -.840 |
| Explained Variance | 3.766 | 3.633 |
| Percent of Total | .290 | .279 |

Figure 5. Factor loadings plot for all species and reduced environmental variables data set.




Key: DOC=dissolved organic carbon, hg in LMB, hg in BB, hg in YP=tissue mercury concentrations in largemouth bass, brown bullhead and yellow perch respectively.

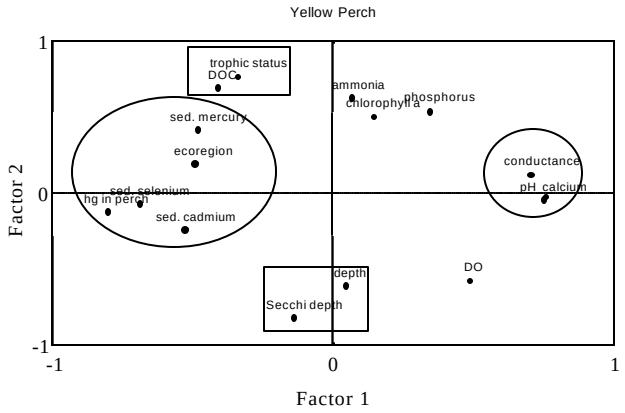
Relative associations of tissue mercury concentrations and environmental variables are shown separately for each species in Figure 6. Fewer cases are deleted (compared to the analysis represented by Table 9) due to missing data when factor analysis is performed on a single species, allowing more variables to be included in the factor analysis matrix. Initially, the factor analysis was computed for two factors. The number of factors was increased iteratively until mercury in the species being analyzed scored high on one factor. Only two factors were required for high scores for tissue mercury concentrations in yellow perch and largemouth bass. Five factors were required before a high score resulted for mercury in brown bullhead.

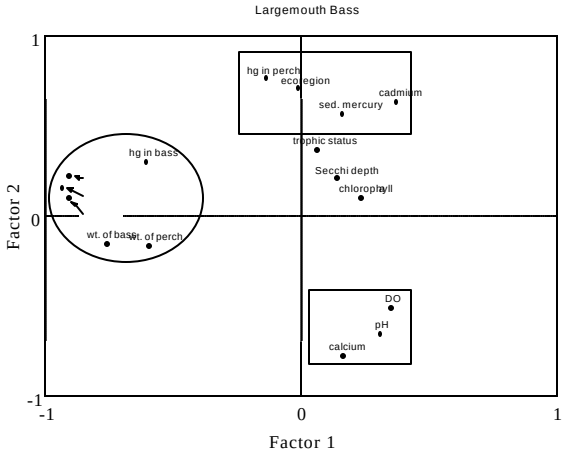
Mercury concentrations in yellow perch score high negatively on Factor 1, with a fairly high score for ecoregion. At the opposite end of the factor are high positive scores for pH, conductance and calcium. Variables loading orthogonal to this factor on Factor 2 are primarily indicators of lake trophic status.

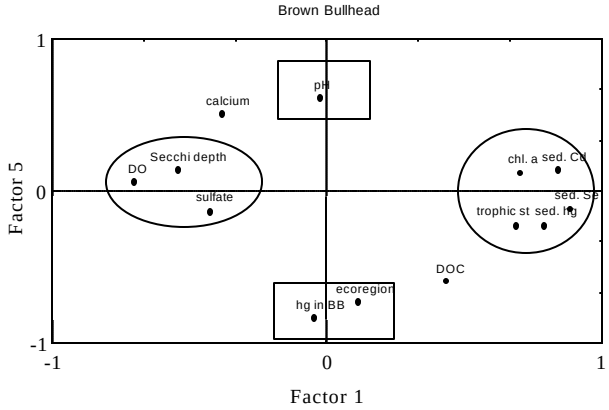
Mercury concentrations in bass are most strongly positively associated with the size of the fish, lake size, and variables representing potential source area contribution sizes (wetlands and watersheds). An interesting link (also identified by multiple regression analysis) between the size of perch, a prey item of largemouth bass, and the size and mercury content of bass is also identified. Neither ecoregion , nor the trophic state variable have similar variance patterns (score highly on Factor 1) to the mercury concentrations in this species. Sedimentary mercury does not have a similar variance to the fish mercury or input areas, but does apparently have some commonality with measures of lake trophic state on the second factor.

Mercury concentrations in brown bullhead tissues and ecoregion classification had similar variance patterns with both being negatively associated with Factor 5 (Figure 6). pH scored strongly positevely on this factor, indicating an inverse relationship between mercury concentrations in brown bullhead and lake pH. Sediment mercury, selenium and cadmium showed similar variance patterns (high positive scores on Factor 1) independent of the variable of interest (mercury in brown bullhead tissues), and inversely related to the trophic status variables of DO and Secchi disk depth.

Figure 6. Rotated factor loading plots for yellow perch, largemouth bass and brown bullhead.



## DISCUSSION

This study of fish mercury concentrations in relatively non-source impacted freshwater lakes in Massachusetts, and possible determinants of these concentrations, revealed that within the size ranges of fish sampled, largemouth bass generally have the highest mercury concentrations, yellow perch the next and brown bullhead the lowest. In order to exert some control on the known effects of fish age/size on tissue mercury concentrations (15,17,65) for our statistical analyses, we sampled narrow size ranges of each species. As a result, the data we report do not reflect the distributions of mercury concentrations across all sizes of each species, but they do represent concentrations in older fish, which are more likely to be retained for human consumption.

The mean yellow perch mercury concentration of 0.31 mg/kg. primarily represented fish in the 4+ and 5+ year classes. Comparable means for these year classes of yellow perch from other studies were 0.36 and 0.43 mg/kg in the Adirondacks (66) and 0.25 and 0.27 mg/kg in the Upper Peninsula of Michigan and Wisconsin (15). Ninety five percent of the measured tissue mercury values in Massachusetts were less than or equal to 0.57 mg/kg (Table 4).

Largemouth bass mercury concentrations were lognormally distributed (Figure 3) and had a mean concentration of 0.40 mg/kg with a 95th percentile concentration of 0.91 mg/kg (Table 4). The maximum measured value was 1.1 mg/kg. The fish sampled were primarily in the 4+ and 5+ year classes. Comparable mean concentrations for similarly aged fish in the Michigan and Wisconsin data set were 0.43 and 0.33 mg/kg (15), and 0.59 and 0.65 mg/kg in Lake Tohopekaliga, Florida (13).

Brown bullhead tissue mercury concentrations were primarily low (mean 0.14 mg/kg) with the majority of fish from the 2+ through 4+ year classes. The 95th percentile mercury concentration was 0.32 mg/kg.

Comparisons of fish of similar ages between studies is equivocal, because aging fish using scales is an inexact science. The methods of aging fish may be different in each study, introducing the potential for an element of uncertainty in comparisons such as the one just presented..

The interspecific differences in tissue mercury concentrations recorded in this study with largemouth bass > yellow perch > brown bullhead were consistent with observations from other studies using the same species or species representing the same trophic level (15,27,7). They are also consistent with a priori considerations of the trophic level at which each species functions.

A geographic gradient of fish mercury concentration for some species was detectable in our analyses, even across the relatively narrowly defined differences between ecological subregions ( Table 10). When all the data are examined together by factor analysis (Figure 5 and Table 9) mercury levels in all three of the species studied and the variable “ecoregion” grouped together, indicating that mercury concentrations in all three of the species vary in a similar manner with respect to geographic variation. However, when species are analyzed individually, only yellow perch mercury concentrations show statistical relationships to ecoregion with most of the statistical approaches used (Table 10). The result seen for the aggregated species data is therefore likely being driven by the yellow perch and possibly

brown bullhead data. No ecoregional differences were apparent in largemouth bass with any of the statistical approaches used and only the factor analysis for brown bullhead suggested a relationship between ecoregion and that species' mercury content. Other efforts to attribute spatial differences in fish species mercury concentrations to geographic regions delineated on the basis of ecological, geological and climatic factors have not been completely successful (17). Differences between regions such as presence of mercury deposits and mining activities have overshadowed ecoregional parameters. Ecoregional differences in Massachusetts may also be overshadowed by past human land use patterns in the state.

Table 10. Summary of significance of test results for ecoregion versus species mercury content. (+ = sig. association; 0 = no assoc.)

| Species | ANOVA | Multiple regression | Factor analysis | |
|---|---|---|---|---|
| | | | individual species | combined species |
| yellow perch | + | 0 | + | |
| largemouth bass | 0 | 0 | 0 | } + |
| brown bullhead | 0 | 0 | + | |

Archival and historic records obtained during this study revealed that many of the lakes sampled had suspected or documented historic point sources of mercury during earlier periods of development (67). Mercury-based preservatives were used to treat felled timber (67, 68). Raw logs and lumber were often treated with mercurial pesticides as a dressing prior to use. The shavings were used in paper manufacturing, wall-board, or as fuel, which, when burned, contributed mercury directly to the atmosphere (68). Sawmill ponds are often "mercury hotspots" (67) and sawmills were common historic industries in Massachusetts. Large areas of Massachusetts that are undeveloped and forested today were deforested and cultivated in the past (69). Mercury-based pesticides, fertilizers and fungicides for seed grain were used and could have contributed significantly to the present load of mercury in the state. The extent to which historic sources such as these contributed to fish mercury concentrations in the lakes we studied is not known, and represents a source of uncertainty in the study.

Our analyses did not show an association between fish tissue mercury concentrations and the lake trophic state index. Lake trophic status was not a significant predictor of the tissue mercury concentrations in the three species of fish studied here (Table 8), nor did the ANOVA and ANCOVA identify significant differences between species mercury concentrations based upon the trophic status of the lakes (Table 6, Table 7). When the results of individual statistical analyses (Table 8, Figure 5 and Figure 6) are examined for associations between the individual physical/chemical variables which are reflective of the trophodynamics of the lakes (i.e., chlorophyll *a* concentration, DO, DOC, Secchi disk depth, nutrient concentrations) and species tissue mercury concentrations, only the brown bullhead mercury concentrations and DOC were significantly related (Table 8, stepwise multiple regression). The first few variables in the multiple regression, which account for the majority of the variance in the dependent variable, are most important. The relative positions of the others may change over time or with additional data.

Factor analyses showed trophic status and variables associated with it as relatively independent of both fish mercury and pH (Figure 5 and Figure 6). For example, chlorophyll *a* and Secchi disk depth, both associated with lake trophic status, did not partition onto the same factor as species mercury values (Figure 5) for all species; Figure 6 for individual species), indicating that variance in trophic state variables was independent of mercury concentrations in most fish tissue.

Three times more lakes in the study were classified as oligotrophic than eutrophic using Carlson's Trophic Status Index. This finding was contrary to our expectations of a historically industrialized and regionally urbanized state. When viewed in the context of all the lakes that have been classified in the Massachusetts Lake Classification Program (70), the study lakes we classified as oligotrophic may fall disproportionately in the classification of high acidity lakes and ponds. Oligotrophic conditions may exist because lakes have never evolved beyond oligotrophy, or because they have regressed in productivity due perhaps to acidification. The ability to distinguish between these routes to oligotrophy is not possible using the index of trophic state we used in the study, Carlson's TSI. The single summer sampling event used to provide the data for trophic state classification may also have been too limited to provide data of sufficient complexity needed to accurately support this classification. The study design itself, being based on relatively uncontaminated lakes, steered us away from selecting hypereutrophic or highly enriched lakes. We may have narrowed the spectrum of lakes we tested unduly, thereby excluding greater extremes in fish mercury concentrations from our data set. Additional variability may have been introduced due to the lake sampling methodology used. The water from stratified lakes was sampled from three different depths, and these samples were combined for analysis and comparison to unstratified lakes. These data were not necessarily comparable, however, introducing additional variability into the water column data set

Richman et al. (23) reviewed the evidence from a number of studies about the influence of lake trophic status on fish mercury concentrations and concluded that while the general availability of mercury within aquatic ecosystems may be affected by trophic status, other abiotic factors interfere with and confound the issue. In addition, studies by Hakanson (71) Lindberg et al. (72) and Allen-Gil et al. (17) have supported this general conclusion.

Every study of mercury in fish has had the underlying objective of identifying the variables responsible for the variation in fish mercury concentrations. Variation may be, in varying degrees, due to biological variability associated with the species themselves (age, size, physiology, diet); chemical variability (water quality and mercury biogeochemistry), physical variability ( e.g., temperature, lake and watershed size), and other influences such as geology, climate and anthropogenic influences. In our study design we sought to control the influence of age (or its surrogates length or weight) by confining our sampling to restricted size ranges of fish. In practice, we obtained fish over a wider range than intended and thereby possibly introduced some size-related variability into the data. However, the lack of correlation between mercury concentration and size in yellow perch or brown bullhead may indicate that our attempt to control for fish size by limiting the size range during capture may have been successful.

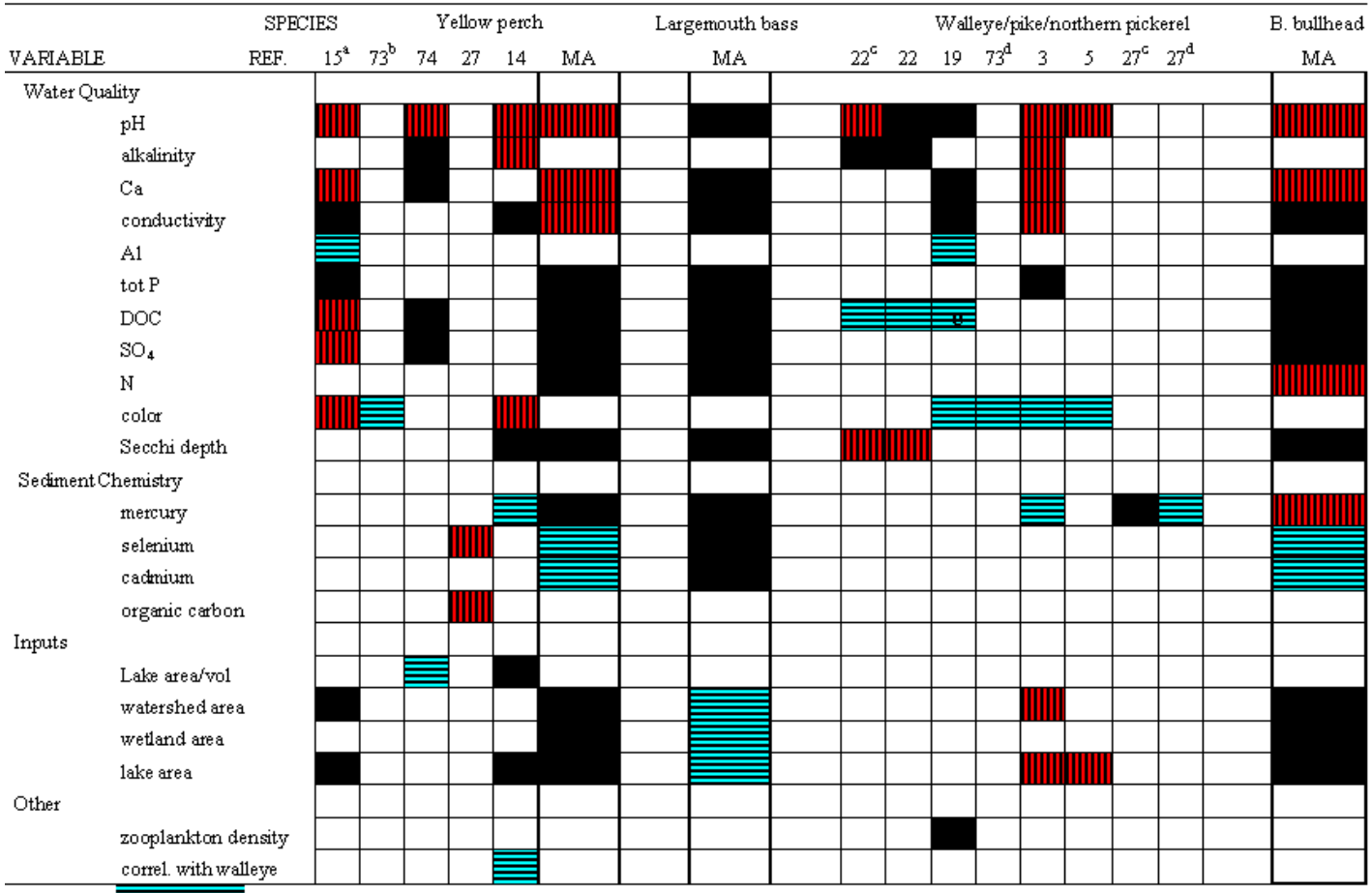
Another possible explanation for the observed relationships with size stems from interspecific variations in the kinetics of mercury bioaccumulation (27). Largemouth bass are long-lived, have the

largest body sizes and probably the lowest rates of growth and metabolism (44). They are also the only species studied here which had a positive, significant correlation between mercury and length. Yellow perch and brown bullhead have smaller body sizes, shorter lifespans (in the case of perch) and presumably higher rates of growth and metabolism. The older, slower growing fish had longer times to accumulate and concentrate (as a result of more uptake than excretion) mercury, because growth dilution of methylmercury is not sufficiently rapid to offset this effect. In the other two species, the higher growth rates probably have resulted in growth dilution of their body burdens of mercury, offsetting possible accelerated mercury uptake due to higher metabolic rates and age-dependent bioaccumulation.

Because of the recognized influence of size on tissue concentrations of many contaminants, raw metal concentrations (mass/mass) are often normalized to a standard sized fish (5,19,73,22). Alternatively, covariance analysis may be used in certain tests to control for the effect of a size-related variable. We found in covariance analyses with tests for mercury-length regression slope parallelism between lakes for each species that slopes were unequal between lakes for largemouth bass and brown bullhead, and were equal for yellow perch. These results were interpreted to mean that fish weight had a differential effect on fish mercury concentrations between lakes for brown bullhead and largemouth bass. Given that the effect of size would not have been removed from the data set for 2 species if covariance analysis had been used to standardize mercury concentrations to a standard sized fish because of non-parallel regression lines (65), we chose to treat fish size as an independent variable in all our subsequent bivariate and multivariate statistical procedures (stepwise multiple regression and factor analysis).

The roles of other possible determinants of freshwater mercury concentrations have been summarized and contrasted with our results in Table 11. Indications of positive, negative or no correlations between variables have been assigned variously from cross-correlation coefficients, signs of regression coefficients and strengths and signs of Factor scores from factor analyses provided in the cited papers and this report.

Table 11. Reported correlations between environmental variables and species specific muscle mercury concentrations.



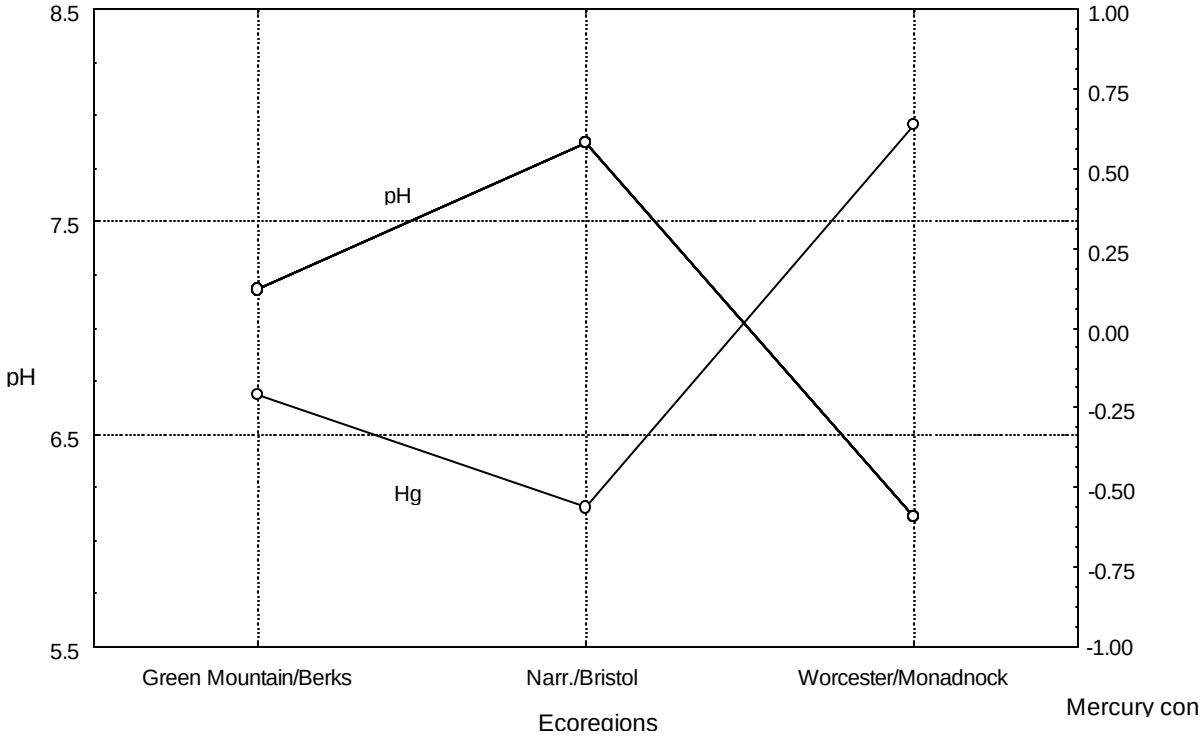
Our analyses resolved a clear link between some of the environmental characteristics and elevated mercury concentrations in fish. Low pH of the waterbody was a significant and major predictor of tissue mercury concentrations in brown bullhead and yellow perch (Table 8 and Figure 6.). In largemouth bass, however, pH was not a significant predictor of variation in tissue mercury concentrations. Mercury in yellow perch was a significant predictor of variation in largemouth bass tissue mercury concentrations, thereby indirectly linking bass tissue mercury and low pH.

The association between high mercury concentrations and low pH is clearly delineated by factor analysis. Factor analysis was employed to identify variables with similar variance patterns. The variables for mercury in each species, and ecoregion (the variable name representing ecological subregion in the graphical renditions of the factor analyses), score high on Factor 1 ( Table 9 and Figure 5). The variables scoring high negatively on this same factor are pH and calcium. The resolution of variables on these two factors suggests that high levels of mercury in fish occur in acidic, low pH lakes.

Variation between ecoregions in lake pH and its relationship to yellow perch tissue mercury concentrations is shown in Figure 7. This figure combines the ANOVA results for mercury in yellow perch and lake pH in 3 ecological subregions. The mirror imagery of the graphed variables suggests that regional differences in fish mercury levels are closely tied to pH. The bivariate correlation between pH and ecoregion was inverse, weak ($r = -0.45$) (Table 5) but statistically significant ($p=.009$). It closely tracked the inverse of tissue mercury concentrations in perch. The lack of a stronger correlation between the variable ecoregion and pH in some of the statistical analyses is likely due to its being a coded, partitioning variable conceived for use in the analysis of variance, rather than a continuous variable associated with field measurements. Trophic status as a variable was also designed to partition data in ANOVA. Some of the continuous variables measured in the field (e.g. Secchi disk depth, chlorophyll *a*, DO) represent measures of trophic status which are perhaps better suited for use in the corrleation and other association tests. The environmental variable most likely to represent ecoregion in our analyses is probably pH. The factor analysis provided complementary information, scoring mercury in perch highly on the same factor as pH, which scored highly negative (Figure 6.). Suns and Hitchin (74) observed a similar relationship in yellow perch from 16 lakes situated on the Precambrian Canadian Shield north of Toronto, Canada.

In Massachusetts, the problem of high mercury in some fish species is part of the family of problems associated with lake acidity. Low pH has been most consistently documented as being responsible for elevated tissue mercury concentrations in freshwater fish (3,14, 15, 19) (Table 11). These studies report correlative relationships between mercury in fish, low pH and alkalinity over a wide range of areas in the northern hemisphere, from Ontario to Florida, and Russia, Norway and Sweden. Possible mechanisms responsible for this relationship include (23):

Figure 7. Mean region-specific yellow perch tissue mercury concentrations and average lake pH.




i) mercury entering watersheds with acid deposition;
ii) mobilization of existing sediment-bound mercury and mercury present in the surrounding watershed by acidification of surface water run-off and lake water leading to increases in the amount of mercury available for methylation and bioaccumulation;
iii) differential production of the more bioavailable monomethylmercury form of mercury at lower pH;
iv) alteration of rates of mercury methylation and demethylation by microorganisms by acidic conditions.

Having reviewed evidence for each of these mechanisms, Richman et al. (23) concluded that they were not mutually exclusive processes and that mercury cycling and uptake into fish tissues was governed by an array of interrelated variables whose relative importance can differ from lake to lake.

Aside from substantiating the association between mercury in fish and acid waters in Massachusetts, the principal contribution of the present study may be the demonstration of the association on so fine a geographic scale as occurs across ecological subregions in a 150-mile transect in this relatively small state. A difference in lake bedrock alone may account for elevated fish mercury concentrations, when a source of mercury is present, whether the source is mercury

associated with acid rain, the earth's crust or historic mercury contamination currently being subjected to the effects of acidic waters.

In Upper Naukeg Pond yellow bullhead populations were present, but brown bullhead were not. In Upper Reservoir, only two brown bullhead were obtained and yellow bullhead were caught. These locations also had the lowest (Upper Reservoir) and third to lowest (Upper Naukeg) pH levels. Notably, fish mercury concentrations in yellow perch and yellow bullhead were also substantially higher than other lakes. We may be seeing a pattern of species displacement, as one species becomes locally rare at the same time as low pHs are recorded We found brown bullhead to be lowest in tissue mercury concentrations, but this finding may be due to the sensitivity of the species to conditions that enhance mercury uptake. This somewhat speculative observation would benefit from future investigation.

Mercury was detected in all sediment samples, ranging from 0.029 ppm to 0.425 ppm (Table 3). Of the three fish species studied in this project, only in largemouth bass and brown bullhead were mercury concentrations related (inversely) to mercury concentrations in sediments (Table 8), accounting for only 2.3% and 2.6 % respectively of the variance in tissue mercury concentrations. Under low pH conditions, leaching of sedimentary metals into surface waters may be facilitated. The relationship between sedimentary mercury and fish tissue mercury concentrations is considerably more complex, being modified by the amount and types of particulate and organic matter in the water column, and the pH and Eh of the sediment (23). In brown bullhead the source of mercury may not be confined to diet, given the bottom-dwelling habitat of the species, and the scaleless, permeable skin. Of the other sedimentary metals, cadmium and selenium concentrations correlated positively (Table 8) with tissue mercury concentrations. These two metals were weak positive predictors of tissue mercury in yellow perch. The direction of this positive relationship is counter to the prevailing theories of the interactions of selenium and mercury in the environment. Selenium can form highly insoluble complexes with mercury and reduce its biological availability, or when absorbed into the body can reduce the toxic effects of methyl mercury (75).

The ratio of basin area to pond area was not a strong predictor or correlate of fish mercury concentrations in any of the species we studied in Massachusetts' lakes ( Table 8, Figure 6). The absence of such a relationship does not support the logic that where the basin (watershed) is much larger than the pond, there should be a tendency to have higher mercury concentration in fish tissue reflective of mercury transport from the watershed (14). In largemouth bass, however, we did find significant correlations between tissue mercury concentrations and the size of the watershed and the lake area, as well as the area in the watershed occupied by wetlands (Figure 6). The relative importance of watershed derived mercury to fish mercury is not consistent in various studies ( Table 11) and sometimes appears to be a function of the types of water inputs to the lakes. In cases where there has been little surface water inflow into lakes (14, 15), no relationship has been seen between fish mercury concentrations and watershed area to lake volume ratios, whereas positive relationships have been seen in lakes with greater surface water inputs from drainage basins (74, 76).

## CONCLUSIONS

Of the three fish species we studied, statistically significant differences in yellow perch tissue mercury concentrations occurred between ecological subregions in Massachusetts.

Regression analysis and factor analysis associate mercury concentrations in the yellow perch and brown bullhead with lakes of low pH. The regional pattern of fish mercury concentrations probably reflects the regional bedrock geology and its interaction with acid rain and lake water. Mercury levels in fish are highest in the Worcester-Monadnock Plateau, where granite is the dominant bedrock in the lakes. Granite is a non-reactive crystalline rock that has no buffering capacity, so waterbodies in the ecological subregion would be most vulnerable to acid rain.

Mercury in largemouth bass correlated with fish weight and age, and the weight and mercury contents of yellow perch. Mercury in largemouth bass also correlated with the size of the lake and the watershed, and the amount of wetlands in the watershed.

Lake trophic status did not correlate consistently with mercury concentrations in fish. An unexpectedly large number of our study lakes were found to be oligotrophic, however. As a result, our study design may have been inadequate to test the relationship between fish tissue mercury levels and lake trophic status.

## ACKNOWLEDGMENTS

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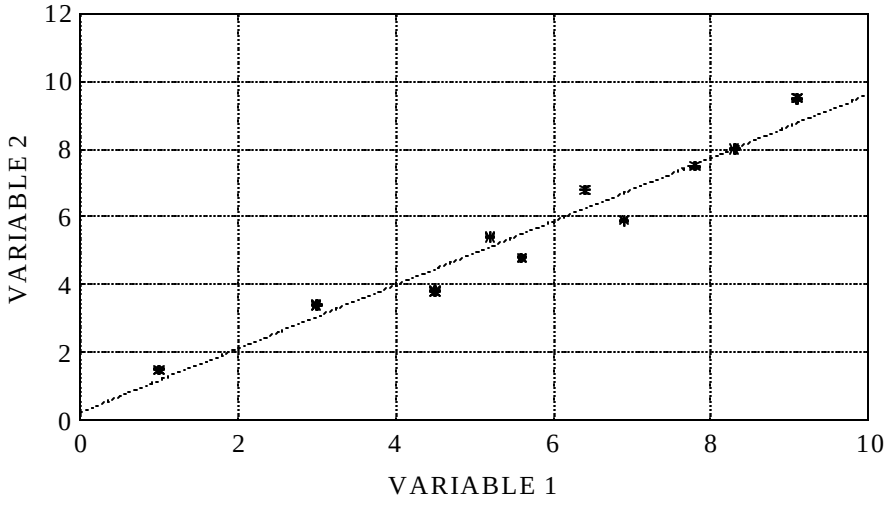
# Appendix A: Factor Analysis Description

# FACTOR ANALYSIS DESCRIPTION

The term “Factor analysis” (FA) actually represents a collection of mathematical techniques which can be used with sets of variables to detect underlying patterns of relationships among the variables or to reduce the size of the data set. Because FA is applied to sets of variables, it is referred to as a multivariate procedure. This brief discussion of Factor Analysis refers to classical Factor Analysis. The reader should consult more detailed statistics texts for discussion of other types of FA which are available.

In order to explain the basis for FA, it is useful to return to the simple correlation concepts used for individual pairs of variables. A regression line on an x-y plot between two variables represents the best summary of the linear relationship between the two variables (Figure A1).

Figure A1. BIVARIATE X-Y PLOT AND LINEAR REGRESSION




If a new variable could be defined that would approximate the regression line of the plot, then it would capture the essence of the correlation between the two variables. Two variables would be reduced to one factor. When interrelationships between more than two variables have to be discerned in data sets, new correlations or factors for each pair can be developed. This sequential, bivariate approach for looking at all possible pairs of variables quickly outstrips our ability to conceptually link all the interrelationship and discern any underlying patterns of variance relationships in the data. FA is a statistical technique which moves beyond the limitations of the bivariate approach, and which provides a

means for identifying intervariable correlation structures among numerous variables by extending the basic idea of the derived factor for a two variable relationship to multiple variables.

FA reduces the size of a data set of variables to one of fewer new variables called Factors. The factors are constructed to be independent of each other in terms of correlations and to represent those original variables in the data set which are most highly correlated in their patterns of variance. For example, a complex data set of variables from an ecology study might includes variables such as a species density in a particular habitat (SPEN), the density of its prey (PREY), the mean annual air temperature (ATEMP), the density of a particular plant species (PLANT), the median grain size of the soil (GRAIN), and the water content of the soil (SH2O). The researcher finds this number of variables too many to interpret when all possible intercorrelations are considered (Table A1) and wonders if any of these variables have similar patterns of variance which would indicate some commonality in the processes which link those groups of intercorrelated variables.

Table A 1. Sample Correlation Matrix for All Variables

| | SPDEN | PREY | PLANT | ATEMP | GRAIN | SH2O |
|---|---|---|---|---|---|---|
| SPDEN | **1.00** | 0.65 | 0.65 | 0.14 | 0.15 | 0.14 |
| PREY | 0.65 | **1.00** | 0.73 | 0.14 | 0.18 | 0.24 |
| PLANT | 0.65 | 0.73 | **1.00** | 0.16 | 0.24 | 0.25 |
| ATEMP | 0.14 | 0.14 | 0.16 | **1.00** | 0.66 | 0.59 |
| GRAIN | 0.15 | 0.18 | 0.24 | 0.66 | **1.00** | 0.73 |
| SH2O | 0.14 | 0.24 | 0.25 | 0.59 | 0.73 | **1.00** |

A FA on the data set eventually extracts or derives two factors (Table A3). Factor 1 is composed of the variance in both the species density and its prey density and density of vegetation . The analysis indicates that these three have similar variance patterns and even can convey whether they are positively or inversely related. In this example, they are all positively related as indicated by positive values in the table. The second factor identified could result from the similar variance patterns in mean annual temperature, soil grain size and water content. This relationship might make intuitive sense from our understanding of ecology, but in other types of data sets, the underlying relationships between variables may not be known and the objective of the analysis would be to identify these patterns and perhaps fortuitously reduce the complexity of the data set.

The sequence of steps in a FA, some of which were omitted for simplification in the description in the previous paragraph, is illustrated by the sample data set just discussed:

i) preparation of a matrix of correlation coefficients between all variables in the data set (e.g., Table A1);

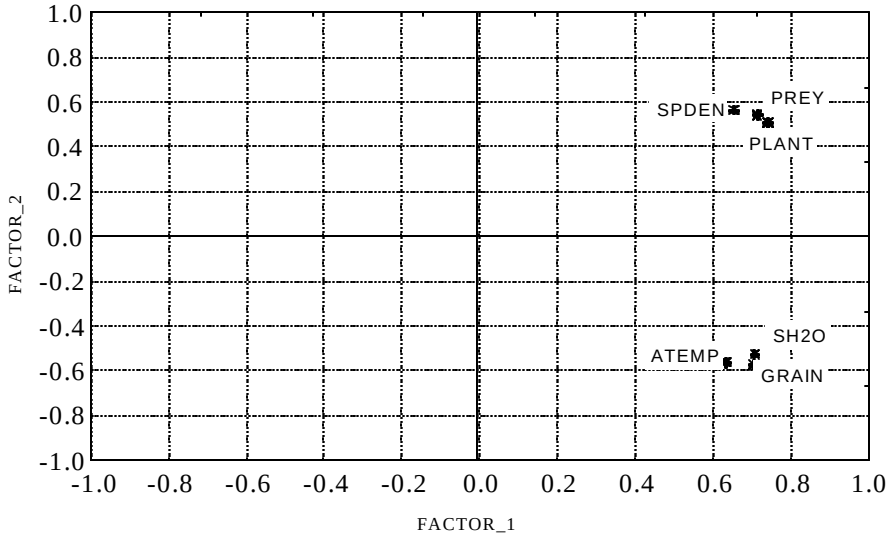
ii) extraction of an initial set of factors on the basis of interrelationships exhibited in the data. Each variable will have a varying correlation with each factor referred to as its factor "score" or "loading" (Table A2).

Table A2. Factor Loadings on Unrotated Axes

| Variable | Factor 1 | Factor 2 |
|---|---|---|
| SPDEN | 0.654 | 0.564 |
| PREY | 0.715 | 0.541 |
| PLANT | 0.742 | 0.508 |
| ATEMP | 0.634 | -0.563 |
| GRAIN | 0.706 | -0.573 |
| SH2O | 0.708 | -0.526 |
| Variance Total | 2.89 | 1.79 |
| Proportion of Total | **0.48** | **0.299** |

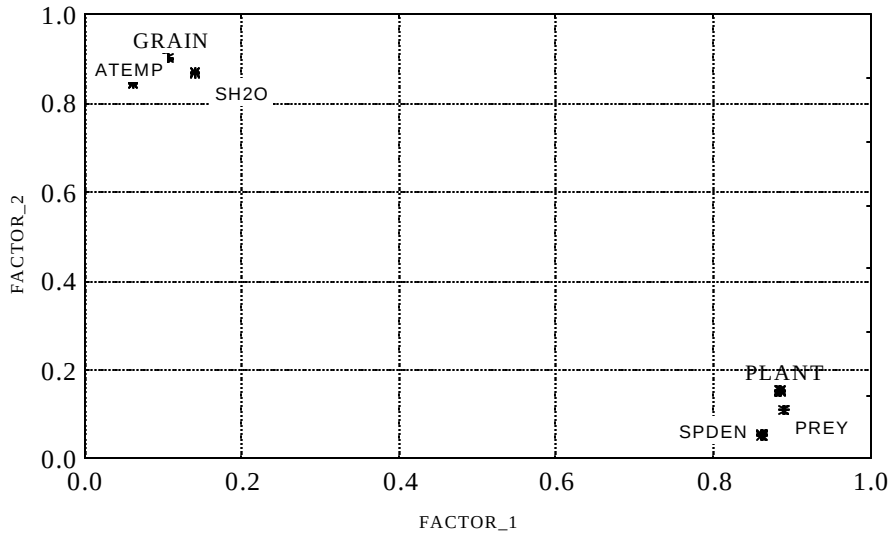
This extraction process is performed so that the factors are independent of (uncorrelated with or orthogonal to) each other. The first extracted factor accounts for the largest possible amount of variance in the data set. Each additional factor extracted accounts sequentially for the largest possible amount of remaining variation independent of the previously derived factors. Note that at this stage of the analysis, each variable may have a relatively high score on both factors. In addition, the proportion of total variance explained by each factor is given at the bottom of each Factor column. These relationships can be graphically represented by bivariate plots of the correlation scores of each original variable on each pair of factors (Figure A2 for Factor 1 versus Factor 2).

Figure A2. Factor 1 and 2 Loadings on Unrotated Axes



iii) rotation in n-dimensional space of the axes for each pair of Factors about the points, while their relative positions are maintained, so as to achieve a simpler and more meaningful factor pattern. Such a pattern is one where the correlations for one set of intercorrelated original variables clearly have high correlations for one factor and low correlations on the other factors (Figure A3).

Figure A3. Rotated Factor Loadings on Factors 1 and 2.




The final product is a rotated factor matrix (Table A3 ) containing values for each variable which are both regression weights and correlation coefficients versus the inferred factor. These loadings represent the regression coefficients of factors to describe a given variable. For example, the equation to describe a specific variable in terms of the new factors could be:

SPDEN = 0.862* Factor 1 + 0.052 * Factor 2

Table 3. Factor Loadings on Rotated Axes.

| Variable | FACTOR_1 | FACTOR_2 |
|---|---|---|
| SPDEN | **0.862** | 0.052 |
| PREY | **0.890** | 0.110 |
| PLANT | **0.886** | 0.153 |
| ATEMP | 0.062 | **0.846** |
| GRAIN | 0.107 | **0.903** |
| SH2O | 0.141 | **0.870** |
| Variance Total | 2.357 | 2.326 |
| Proportion of Total | **0.393** | **0.388** |

In common with regression analysis, the independent variables (i.e., the hypothetical factors) are said to control or account for a certain percentage of the

variance in the dependent variables. The variance of SPDEN due to Factor 1 is the square of the factor score contained in the factor matrix and the total variance in a variable accounted for by all the factors is given by the sum of squares of the respective factor loadings.

It is also possible to determine the importance of a given factor in terms of the amount of total variance in the data set that it accounts for. This is accomplished by squaring each factor score, summing down in the table across variables, and dividing the total by the number of variables in the data set. For example, in the final solution, Factor 1 accounts for 39.3% of the total variance in the data set on the rotated axes (Table A3). Since the vairibles SPDEN, PREY, and PLANT have the highest factor scores, the are responsible for the majority of the variance in Factor 1 and have common patterns of variance themselves.