FINAL REPORT

MASSACHUSETTS FISH TISSUE MERCURY STUDIES:

INVESTIGATIONS OF SEASONAL AND OTHER SOURCES OF VARIATION

by

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PREFACE

The Commonwealth of Massachusetts has been conducting studies on the dynamics and distribution of mercury in tissues of freshwater fish for almost two decades. The primary goal of much of the early work was to identify fish populations which might pose unacceptable health risks to those consuming the fish. Sampling sites have not often been revisited in subsequent years, methods and procedures had not been fully standardized until more recent years, and sampling intensity was not designed to permit more rigorous statistically-based comparisons between samples. In 1994, the first comprehensive statewide examination of mercury in freshwater fish was conducted (MA DEP 1997). This study was followed in 1999 by a study of fish mercury concentrations in a region of the state thought to have regionally high atmospheric deposition of mercury (MA DEP 2003a). This study was complemented by a study of historical mercury deposition into one lake in this region through the analysis of a sediment core using radioisotope dating techniques (Wallace *et al.* 2004).

A number of additional studies have been conducted as part of the Department's continuing efforts to better elucidate the status of the Commonwealth's freshwater fish populations and environments with respect to mercury contamination. The study reported here examines the relative contributions to variance in fish tissue mercury concentration measurements from: seasonally varying factors, degree of tissue hydration and reproductive cycle. This information is intended to help more efficiently design monitoring studies in the future.

A long-term monitoring network of lakes was established in 2001 to provide temporal tracking of changes in the mercury contamination status of fish in the Commonwealth, particularly as comprehensive mercury use and emissions reductions efforts have been implemented both regionally and nationally. The data from this network will also provide a perspective on the scale of natural variability in tissue mercury concentrations for comparison with other sources of variation. The results from the first 5 years of this effort are reported separately (MA DEP 2006) and in particular highlight the changes which have taken place in the state's high mercury deposition area during a period when emissions from major point sources of mercury to the atmosphere in that region have declined substantially.

In order to help elucidate the ecological basis for varying fish mercury patterns seen in different lakes, a comparative food web mercury study was conducted in two similar lakes in close proximity which have different levels of mercury in top predator fish (MA DEP 2003b).

Wildlife are integral parts of the pond ecosystems which we study. Piscivorous birds in particular are at risk from mercury exposure via the food chain. Loons have been a focus of attention in New England for aesthetic and ecological reasons. A first step in the

process for addressing threats of mercury to wildlife in MA is to have an understanding of the state of knowledge of mercury in indigenous non-fish vertebrates in the Commonwealth. A compilation of information on the state of knowledge on mercury in wildlife in the Commonwealth has been performed as part of our overall program (Pokras and Tseng 2001) .

The data generated from these studies on mercury concentrations in edible tissues of popular freshwater fish also permit more widespread screening of the Commonwealth's lakes for potential human health threats posed by eating contaminated fish from these lakes. These threats are addressed through the issuance of fish consumption advisories by the Massachusetts Department of Public Health.

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LIST OF ACRONYMS

Acronyn	Definition
ANCOVA	Analysis of Covariance
ANOVA	Analysis of Variance
Ca	Calcium
$CaCO_3$	Calcium carbonate
CI	Condition index
Cl	Chloride
CV	Coefficient of variation
DO	Dissolved oxygen concentration, mg/L
DOC	Dissolved organic carbon
Epil.	Epilimnion
Fe	Iron
GSI	Gonadal somatic index
Hg	Mercury
Hypol.	Hypolimnion
ICP	Inductively coupled plasma emission spectroscopy
K	Potassium
LMB	Largemouth bass
Mg	Magnesium
Mn	Manganese
MS	Micro Sieman. Unit of specific conductance.
n	Sample size
Na	Sodium
NH_3	Ammonia
NO_2	Nitrite
NO_3	Nitrate
NS	Not statistically significant
s	Standard deviation
SC	Specific conductance
Se	Selenium
SO_4	Sulfate
SS	Size-standardized
Std. dev.	Standard deviation
Temp.	Temperature
TOC	Total organic carbon
Tot. P	Total phosphorus
YP	Yellow perch

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ABSTRACT

Mercury concentrations in edible fish tissues are potentially influenced by a number of parameters not systematically addressed in mercury monitoring studies. Failure to account for these sources of variation may result in the production of data or interpretations of data where true relationships under investigation are obscured by other sources of variance. One potential source of such variance is that introduced by seasonally varying factors. Other sources, somewhat related to seasonal variation, are hydration state of tissues, since most tissue mercury concentration data are reported on a wet weight basis, and the reproductive cycle.

Seven lakes forming part of the state's long-term network of lakes for fish tissue mercury monitoring were sampled during every major season (spring, summer, fall, winter, spring) for 15 months to determine if the edible muscle mercury concentrations in our two principal monitoring species (largemouth bass, *Micropterus salmoides* [LMB] and yellow perch, *Perca flavescens* [YP]) varied seasonally. Sample sizes for the analysis of individual fish used in this study were statistically-based to have a confidence level of 0.10 and a power of 80%. Sampling objectives were to obtain 30 YP and 12-15 LMB per lake. These sample sizes were associated with an ability to correctly distinguish differences in group mean mercury concentrations of approximately 40-50% in LMB and 15-20% in YP. In addition, the role of the degree of tissue hydration on the intrasample variance of tissue mercury concentrations was evaluated by comparing within-sample variance for mercury concentrations expressed on a wet weight basis to those expressed on a dry weight basis. This aspect of the work was performed to examine whether improvements in sample statistics could be achieved, and thereby enhance the power of studies to correctly differentiate differences between sample groups, through control of tissue moisture content. Reproductive indices were examined in relation to fish tissue mercury concentrations. Selenium concentrations were determined to explore possible covariation between selenium and mercury.

To control for the confounding effect of size on tissue mercury concentrations, LMB and YP tissue mercury concentrations were size-adjusted to a common size for each species.

Tissue mercury and selenium concentrations varied significantly between seasons in almost all lakes for YP, with the highest concentrations usually occurring in the spring and the lowest levels in the summer and fall. A similar, though less strong and consistent pattern was seen in LMB. Size-standardized seasonal mercury concentration means for YP ranged from 0.169 - 0.796 mg/kg and those in LMB ranged from 0.191 - 1.075 mg/kg: those non-standardized for selenium were 0.215 – 0.916 for YP and 0.214 – 0.536 mg/kg for LMB. Within any one lake, the seasonal size-standardized maximum mercury concentrations in YP ranged from 29 - 176% of the minimum size-standardized value. The range for LMB was 13 - 59%.

No strong and consistent relationships between tissue selenium and mercury concentrations were seen. The concentrations of selenium were all below the level at which developmental toxicity in fish would likely occur.

Moisture content had no effect on within sample variance estimates when compared to mercury concentrations expressed on a dry weight basis.

Failure to consider these sources of variance in study designs and interpretations of data from fish tissue mercury monitoring studies could have major implications for correct interpretation of the data. Closer attention to the possible contribution of seasonal variation to variance in fish tissue mercury data offers an opportunity to improve the capabilities of field studies to discriminate differences between samples due to the influence of a particular parameter under study and also perhaps to improve the targeting and specificity of fish consumption advisories.

1.0 INTRODUCTION

Mercury concentrations in edible fish tissues may be influenced by a number of factors not specifically examined in our past studies (Rose et al. 1999; MassDEP 1997), nor systematically by others in the literature. These factors likely contribute to the variation inherent in the data in these types of studies and may obscure true relationships under study, possibly leading to erroneous conclusions about trends in fish tissue mercury concentrations. Important among these factors are: year-to-year variation and seasonally-varying factors, perhaps affecting the condition, reproductive state, and mercury accumulation and elimination dynamics in fish.

Seasonal variability complicates the interpretation of fish mercury studies conducted at different times of the year. For example, our two previous studies on fish tissue mercury (Rose et al 1999; MassDEP 2003a) were conducted in the spring and autumn of different years respectively, yet there has been no estimate of the degree of contribution to variability in the data from natural seasonal changes which might be superimposed upon longer-term trends reflective of different environmental processes. Seasonal factors may result in apparent changes in tissue mercury concentrations that could erroneously be attributed to other factors.

Temperature and photoperiod changes throughout the year drive the fish reproductive cycle. The time of year, seasonal temperature, reproductive state of the fish and their physiological condition are interrelated (Hoar and Randall 1988). The relative influences of these factors with respect to interpreting tissue mercury concentration data may be important because as a fish's physiology changes, so might its apparent mercury concentrations in specific tissues. For instance, spawning is a major physiological event in the lifecycle of a fish with the sudden release of gametes and is usually associated with low stores of energy reserves in the form of lipids, protein (muscle tissue) and carbohydrates. Even with a constant tissue mercury burden, apparent concentrations of mercury could seemingly change throughout the spawning cycle. The relationships between all of these factors and variation in tissue mercury concentration or body burden of mercury have not been well examined, nor does the existing data present a clear picture. Some studies have shown no seasonal changes (e.g., Bidwell and Heath 1993; Park and Curtis 1997; Foster *et al.* 2000); some have shown seasonal changes, albeit with seasonal highs at different times of the year (Weis et al. 1986; Meile 1991; Ward and Neumann 1999). Age-adjusted seasonal differences between means have ranged between 26% and 43% (Ward and Neumann 1999).

Another potential source of variance in tissue mercury concentration estimates is the degree of hydration of the tissues. Many aquatic organism tissue metal concentrations are expressed on a dry weight basis, thereby removing the potential influence of variation in the amount of water in tissues. Fish tissue mercury concentrations are usually reported on a wet weight basis. Previous experiences (Hutcheson, unpublished data) with marine invertebrate tissues have shown that the relative standard deviations of mean metal concentration estimates can be reduced substantially by expressing concentrations on a

dry weight basis, rather than on a wet weight basis. From an experimental design viewpoint, reducing the variance in a data set will yield greater power to identify statistically significant differences between samples for the same or lowered sampling effort. Wet weight-based mercury concentrations in fish tissues retaining any variance associated with moisture content may complicate the interpretation of intersample differences.

Mercury accumulation and toxicity in fish muscle are also recognized to have a relationship to selenium presence. In contrast to reported positive correlations between mercury and selenium concentrations in a variety of species, Burger *et al.* (2001) found both positive and negative relationships across several freshwater fish species. Studies have also addressed the potential role of selenium (Se) within muscle tissues for affecting mercury sequestration versus that of Se in the water and sediments affecting mercury methylation (Southworth *et al.* 2000; Chen *et al.* 2001). Selenium measurements were incorporated into this study to provide perspective on another variable potentially affecting tissue mercury dynamics.

The objectives of this work have been to: 1) sample two freshwater fish species (largemouth bass, *Micropterus salmoides* [LMB] and yellow perch, *Perca flavescens* [YP]) caught throughout the year in order to determine if seasonal differences in fish mercury concentrations occur; 2) assess the contribution to tissue mercury variance associated with the reproductive cycle; 3) determine if any reduction in variance associated with mean mercury concentration estimates in edible freshwater fish tissues can be achieved by expressing data on a dry weight basis in preference to a wet weight basis; and 4) determine tissue selenium concentrations to examine whether mercury tissue concentrations were correlated with tissue Se concentrations .

2.0 MATERIALS AND METHODS

2.1 STUDY DESIGN

The concentrations of edible muscle mercury and selenium were determined in LMB and YP from a 7 lake subset of MassDEP's long-term monitoring lakes (Figure 1). Sampling at these lakes distributed throughout the state took place in the spring, summer, and autumn of 2001, and winter and spring of 2002. The original objective was to obtain the spring fish just prior to spawning and immediately after spawning. Sampling around spawning was intended to provide data on whether fish tissue mercury concentrations change during this significant period in the life of fish. In practice, YP spawn in the very early spring, just after ice-out, and we were unable logistically to obtain fish before they spawned. Winter YP could perhaps be considered spring pre-spawn fish. LMB spawn in the late spring and early summer, so we were able to obtain both pre- and post-spawning individuals.

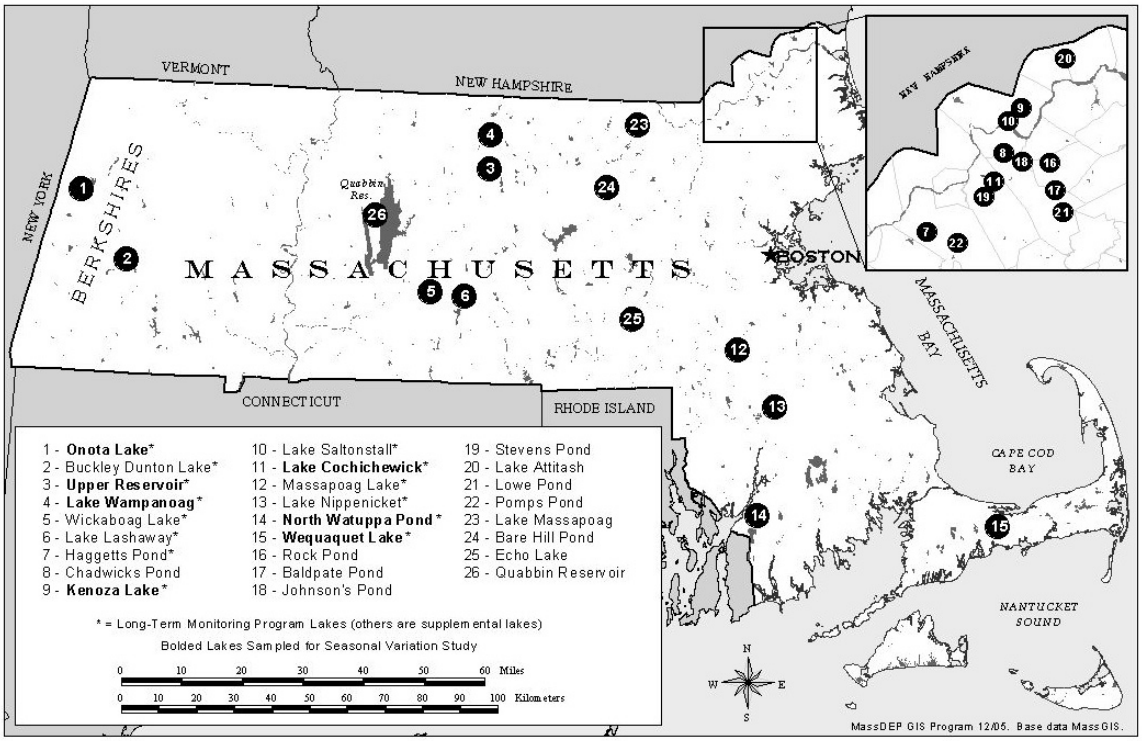


Figure 1. Locations of Sampling Lakes for Seasonal Variation Study (**Bolded**) from MassDEP Long-Term Monitoring Lakes.

2.2 FIELD SAMPLING

The protocol for collecting fish and water samples in the field and subsequent processing in the laboratory is shown in Figure 2.

Fish were collected with box nets, gill nets, trot lines, electroshocking and rod and reel. They were removed from the water, rinsed with ambient water, wrapped individually in aluminum foil, placed in polyethylene Ziploc© bags and placed on ice for delivery to the laboratory within 24 hours of collection.

In order to provide robust size/age ranges of LMB, a size spectrum of fish was collected. We sought to obtain YP greater than 20-25 cm total length to represent those consumed by anglers.

Required numbers of replicate fish were determined using sample size calculation algorithms in Statistica©. Estimates of variance in the edible tissue mercury concentration data from our previous studies were used along with desired confidence level of 0.10 and power of 80% to calculate required sample sizes. Our calculations and consideration of practical realities of potential overharvesting of resident fish populations and analytical costs led us to seek 30 replicate YP per lake per sampling event and 12 -

15 LMB. These sample sizes were associated with an ability to correctly identify differences in means of approximately 15 - 20% in YP and 40 - 50% in LMB. In practice, there were occasions when it was not possible to obtain the desired numbers of fish.

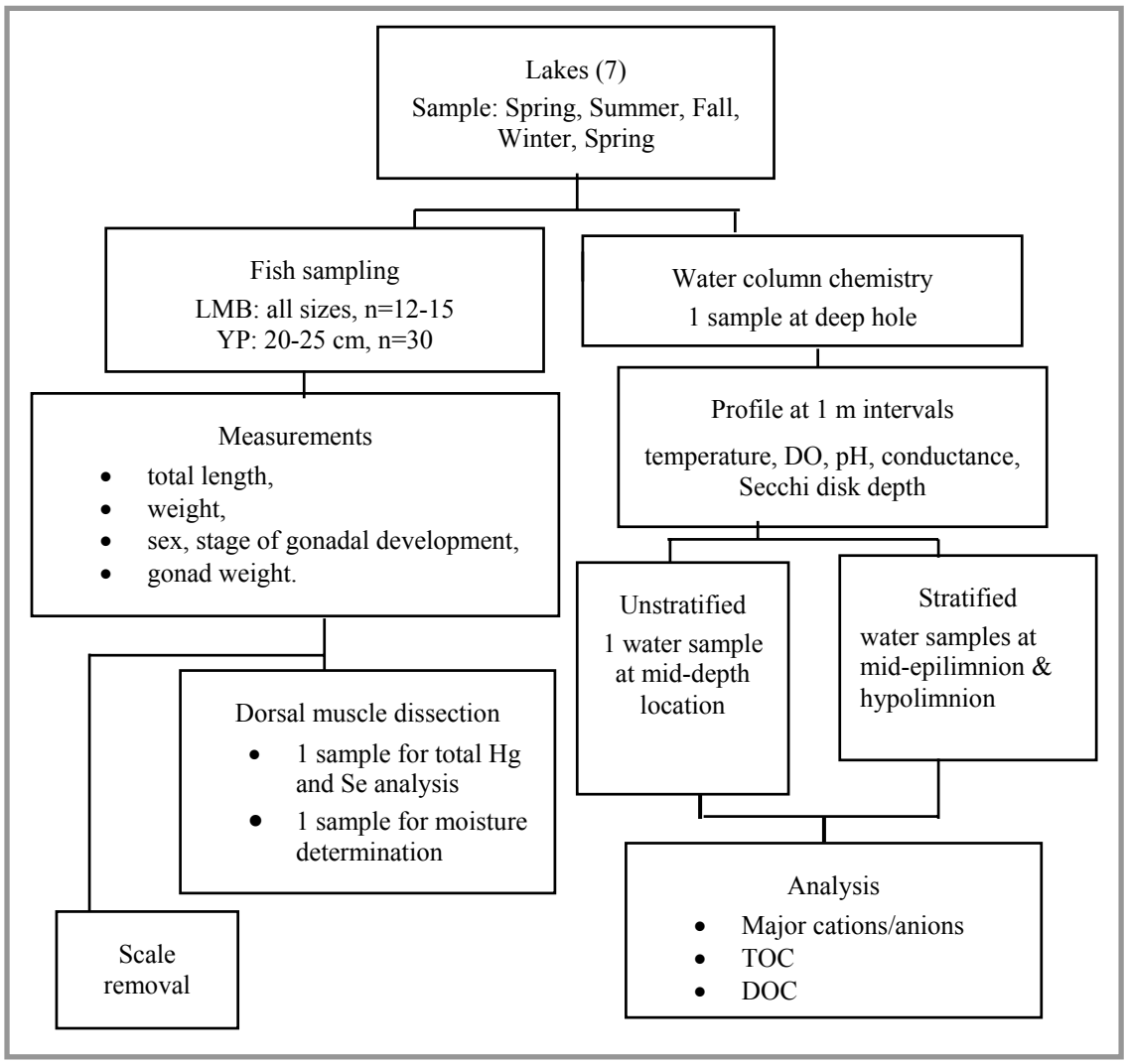


Figure 2. Field and Lab Handling Protocol

Basic water quality measurements were obtained at one station at the deepest part of each lake at 1 m depth intervals with multiprobe field instruments. Temperature, pH, dissolved oxygen concentration and conductivity were measured. Dependent upon whether or not the water column was stratified at the time of sampling, either mid-epilimnion and hypolimnion water samples were taken or a single mid-depth sample was taken for analysis of major cations and anions (Na, K, Ca, Mg, Fe, Mn, SO_4, Cl), dissolved organic carbon content (DOC), total organic carbon content (TOC), nitrate+nitrite nitrogen, total phosphorus, and ammonia. The analytical techniques used for each and associated detection limits are provided in Table 1.

Table 1. Analytical Methods for Water Testing

Analyte	Method Reporting Limit, mg/L	Method
Na	0.02	EPA 200.7
K	0.07	EPA 200.7
Ca	0.01	EPA 200.7
Mg	0.005	EPA 200.7
SO_4	0.06	EPA 300
Cl	0.07	EPA 300
Fe	0.01	EPA 200.7
Mn	0.005	EPA 200.7
TOC	0.2	EPA 415.1
DOC	0.2	EPA 415.1
Alkalinity	0.25	EPA 310.1
NO_2	0.003	EPA 300.0
NO_3	0.002	EPA 300.0
NH_3	0.001	APHA, 1998. Method 4500-NH_3 F
Tot. P	0.001	APHA, 1998. Method 4500-P E

2.3 LABORATORY PROCEDURES

Fish were processed for analysis of mercury and selenium in lateral muscle in accordance with US EPA procedures (U.S. Environmental Protection Agency 1993). Total fish lengths and wet weights were recorded. The sex and reproductive condition of each fish was assessed by visual examination of gonads and classification as : immature; developing; ripe and spent. Gonad wet weights were recorded. Scales were removed from the fish for age analysis. Tissue moisture contents were determined for calculation of the dry weight basis of the mercury content of the tissues (Figure 3). Other details of handling and sample preparation are identical to those described in Rose *et al.* (1999). Mercury in tissues was analyzed using US EPA Method 245.6. A Perkin Elmer Flow Injection Mercury System was used for total mercury analysis. The method detection limit (MDL) was 0.01 mg/kg and the reporting limit (RL) was 0.03 mg/kg. Recovery for mercury- spiked fish samples and precision of the analyses were 96.0 ± 11.1% and 5.5 ± 5.5 % (means ± 1 std. dev.). The reference standard for mercury in fish tissue was freeze-dried tuna tissue (BCR ref. std #463). The accuracy of analyses of that standard was 102.1 ± 12.7%. Mercury in all laboratory reagent blanks was less than the method detection limit.

Tissue selenium concentrations were determined using US EPA Method 200.9 employing graphite furnace atomic absorption spectrophotometry (U.S. Environmental Protection Agency 1994). The MDL was 0.06 mg/kg and RL was 0.08 mg/kg.

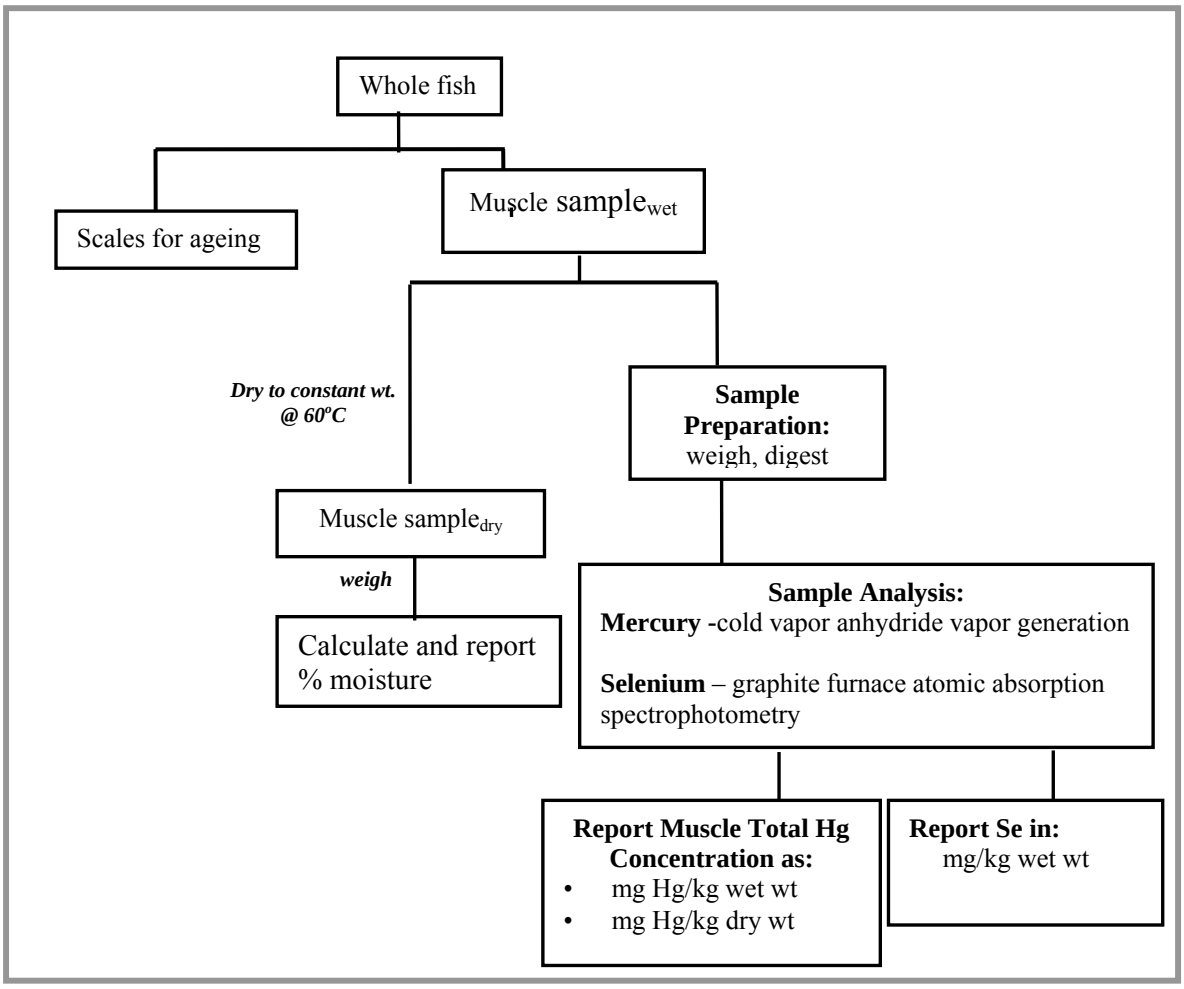


Figure 3. Laboratory Processing Protocol

Moisture content was determined on a duplicate tissue sample of the same size and from the same portion of the fillet as the sample for mercury analysis. Individual samples were gently blotted on laboratory tissue paper and their wet weights determined. They were then dried overnight at 60^{o}C in an aluminum weighing dish and weighed again. The moisture content as a percent was calculated from the wet and dry weights of the tissues. Mercury concentrations of the samples were then calculated using the wet weight and the dry weight values.

2.4 DATA ANALYSIS

For the investigation of seasonal effects, the underlying hypothesis tested was that there were no differences in seasonal mean mercury concentrations for a species in a lake. This hypothesis was tested with either a one-way analysis of variance (ANOVA) or an analysis of covariance (ANCOVA). For species and lakes where there were only 2 seasons of data, a t-test was employed to test the hypothesis that the two seasonal means were equal. The process for performing this overall evaluation consisted of the following steps.

Bivariate plots of individual fish mercury and selenium concentrations versus total fish length for each species for each lake were examined to determine if there were any outliers. Outliers were either corrected if representing a data entry error or excluded if indeed outlying the sphere of the remainder of the data. An examination of these plots revealed that in almost all cases, there was a positive linear correlation of tissue mercury concentrations with fish length. In order to adjust for the effects of this covariate prior to testing for differences between seasons, either an ANCOVA was performed, or individual fish mercury concentrations were adjusted to the concentration of a standard-sized fish of that species. ANCOVAs may be used for this purpose when there is no interaction between the covariate and the independent variable (length) and the classification variable (season). Interaction was determined from tests of parallelism of regression line slopes for concentrations versus lengths for different seasons (Sokal and Rohlf 1995) performed on the data for individual lakes as the first part of the ANCOVA analysis.

In those cases where there was a differential relationship between seasons of the mercury concentrations versus lengths regression slope, the size effect was controlled for by deriving a predicted mercury concentration for a "standard-sized fish", defined as the arithmetic mean fish total length over all fish sampled (339 mm for LMB; 243 mm for YP) in our study of mercury concentrations in fish from northeastern Massachusetts (MA DEP 2003a). In subsequent analyses for comparing data between lakes, the predicted mercury concentration of a standard-sized fish for a lake was used as a basis for comparison. It was determined by regressing individual fish mercury concentrations on body lengths for the fish species from a lake in a season, and then solving the regression equation for the predicted tissue mercury associated with the length of the standard-sized fish. Prior to running the regression analysis, plots of these two variables were examined for linearity: there were no non-linear relationships. In order to retain individually-based fish data in analyses, thereby getting maximal statistical benefit out of the sample size "n" for the lake, individual fish mercury concentrations were also size-adjusted to the mercury concentration of a standard-sized fish. The theory behind this adjustment is that the mercury-size relationship for each individual fish in the lake would follow the same relationship (slope of regression line) as that determined for all fish in the lake (least squares regression line). Lines having the same slope as the overall regression positioned to cross through each data point will have different intersection points with a vertical line

at the standard-sized fish length (representing tissue mercury concentrations). This set of new size-adjusted data points for each fish for each lake was then available for use in subsequent analyses testing for seasonal differences using a one-way ANOVA.

The fish tissue mercury concentration data or size-standardized mercury concentrations for each species for each season for a lake were examined with the following techniques to determine if they met the assumptions implicit in using parametric statistics for analysis of the data: normal distribution of the data; homogeneity of error variances; independence of the means and variance. Normality was assessed through: generation of frequency histograms of individual fish tissue mercury concentrations and application of Kolmogorov-Smirnov test for goodness of fit to normal distribution at $\alpha = 0.05$ (Sokal and Rohlf 1995); and generation of normal probability plots of these mercury concentrations for each lake. Homogeneity of error variances between lake tissue mercury concentrations was assessed with Levene's test (Sokal and Rohlf 1995). Plots of lake mean tissue mercury concentrations or size-standardized mercury concentrations versus associated standard deviations were examined to determine if means were correlated with errors. Violations of these assumptions of normality and errors for any species were addressed by applying $\log_{10}$ transformations to the individual fish tissue mercury concentration data prior to additional testing.

The underlying hypothesis being tested with the tissue moisture data was that the variance in the data for samples (a species in a particular season in a lake) was the same between mercury concentrations expressed on a wet weight and a dry weight basis. The test statistic used was the sample coefficient of variation (CV). The two sample t-test was used to test whether the CVs for wet and dry weight concentrations for the fish from a lake in a season were the same.

A gonadal somatic index (GSI) was calculated for each fish as the ratio of the gonadal weight to the total body weight. A condition index (CI) for each fish was calculated as the ratio: total weight/length^3 x 100.

All statistical evaluations in this study were performed with the Statistica/W©, Version 5.0 software package (StatSoft, Tulsa, OK, USA).

3.0 RESULTS

3.1 MOISTURE CONTENTS

The results of wet weight versus dry weight-based mean mercury concentration variance comparisons are shown in Figure 4. Variances have been normalized to the sample means by expressing them as coefficients of variation (CV). The objective of this portion of the study was to determine if variance estimates for dry weight-based concentrations were less than those for wet weight-based values. Slopes of individual species regression relationships relating dry weight CVs to wet weight CVs for tissue mercury concentrations are essentially similar, so the combined slope is shown in Figure 4. The

slope of this regression line relating dry weight CVs to wet weight CVs for tissue mercury concentrations is not significantly different from 1.0 (t test, p=0.01). Therefore the conclusion from the data is that removal of tissue moisture to produce dry weight-based tissue mercury concentrations does not produce any significant reductions in sample variance estimates for LMB or YP.

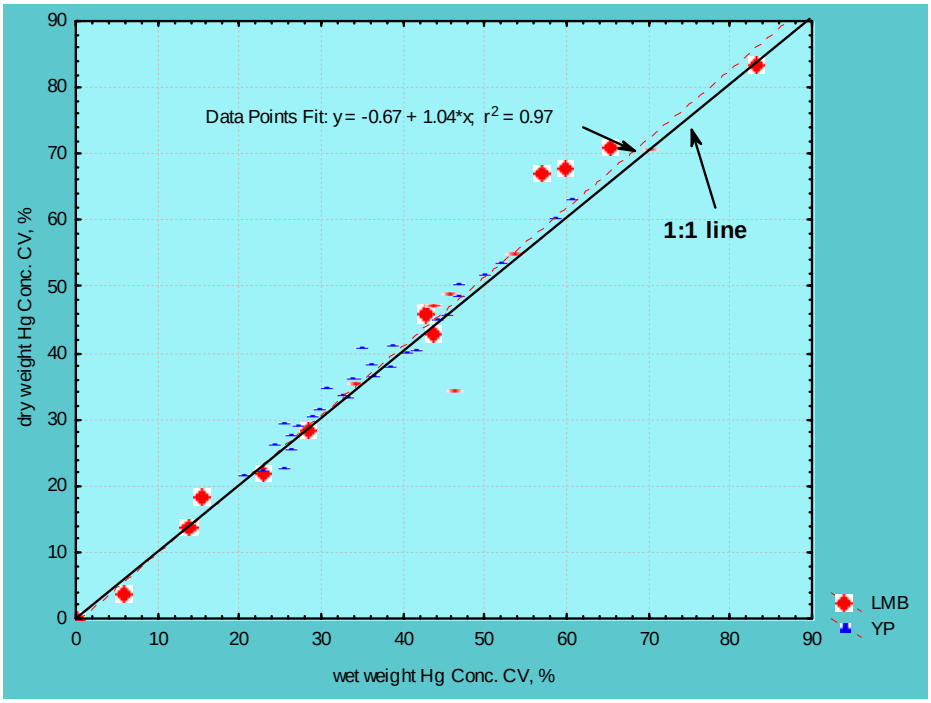


Figure 4. Season/Lake Dry Weight Mercury Concentration Coefficients of Variation Versus Wet Weight Mercury Concentration CVs for LMB and YP.

3.2 SELENIUM

Mean selenium concentrations ranged from 0.215 – 0.916 and 0.214 – 0.536 mg/kg in YP and LMB respectively (Table 2). There was no apparent relationship between tissue selenium concentrations and fish length when scatter plots of these two variables for each lake and season and year were examined (not presented).

When viewed over all fish sampled, there was not a strong correlation between tissue selenium and mercury concentrations (Figure 5) except with some YP. Most of the selenium concentrations were less than approximately 1.0 mg/kg in YP, however some values ranged up to approximately 1.9 mg/kg.

When lake means are plotted and identified, it is apparent that the lake with the high YP selenium concentrations is North Watuppa Pond in southeastern Massachusetts (Figure 6A). These high mean selenium concentrations (>0.8 mg/kg) are associated with the

middle to lower range of mean mercury concentrations in our data set (0.4 – 0.7 mg/kg). Data averaging in a lake tends to smooth out the sharper relationship between individual fish tissue selenium and mercury concentrations as illustrated in Figure 5. No pattern is apparent for LMB (Figure 5 and Figure 6B).

Seasonal selenium means in almost all lakes for each species changed significantly throughout the year (1-way ANOVAs or t-tests at $\alpha = 0.01$). The cycle generally started with higher values in the spring, dropping to the lowest in the summer. Values began to rise in the fall towards the winter and spring highs (Figure 7).

Table 2. Mean Selenium Concentrations (mg/kg wet wt) by Species by Lake and Season

Lake (Abbreviation)	Year	Season	YP			LMB		
			mean	n	std.dev.	mean	n	std.dev.
Lake Cochichewick (Co)	2001	spring	0.437	30	0.105	0.358	12	0.054
	2001	summer	0.324	18	0.073	0.250	12	0.042
	2001	fall		0			0	
	2002	winter	0.351	13	0.149		0	
	2002	spring	0.391	26	0.075		0	
Kenoza Lake (Ke)	2001	spring	0.254	28	0.042	0.238	5	0.023
	2001	summer	0.238	30	0.066	0.214	11	0.051
	2001	fall	0.241	9	0.050	0.245	2	0.064
	2002	winter	0.230	23	0.042		0	
	2002	spring	0.304	27	0.032		0	
North Watuppa Pond	2001	spring	0.821	30	0.248	0.510	9	0.128
(NW)	2001	summer	0.581	12	0.256	0.334	10	0.157
	2001	fall		0			0	
	2002	winter	0.916	30	0.231		0	
	2002	spring	0.851	30	0.328		0	
Onota Lake (ON)	2001	spring	0.259	30	0.047	0.269	21	0.049
	2001	summer	0.215	17	0.042	0.270	3	0.070
	2001	fall		0			0	
	2002	winter	0.468	23	0.066		0	
	2002	spring	0.332	24	0.040		0	
Upper Reservoir (Up)	2001	spring	0.357	30	0.046	0.280	5	0.062
	2001	summer	0.298	12	0.034	0.220	1	0.000
	2001	fall		0			0	
	2002	winter	0.408	24	0.089		0	
	2002	spring	0.349	20	0.041		0	
Lake Wampanoag (Wa)	2001	spring	0.508	30	0.089	0.376	14	0.090
	2001	summer	0.399	10	0.041	0.330	9	0.074
	2001	fall	0.411	5	0.156		0	
	2002	winter	0.618	5	0.171	0.503	4	0.139
	2002	spring		0			0	
Wequaquet Lake (We)	2001	spring	0.538	30	0.069	0.536	30	0.072
	2001	summer	0.449	9	0.050	0.518	10	0.088
	2001	fall	0.518	9	0.049	0.516	14	0.087
	2002	winter	0.475	30	0.067		0	
	2002	spring	0.468	30	0.060		0	

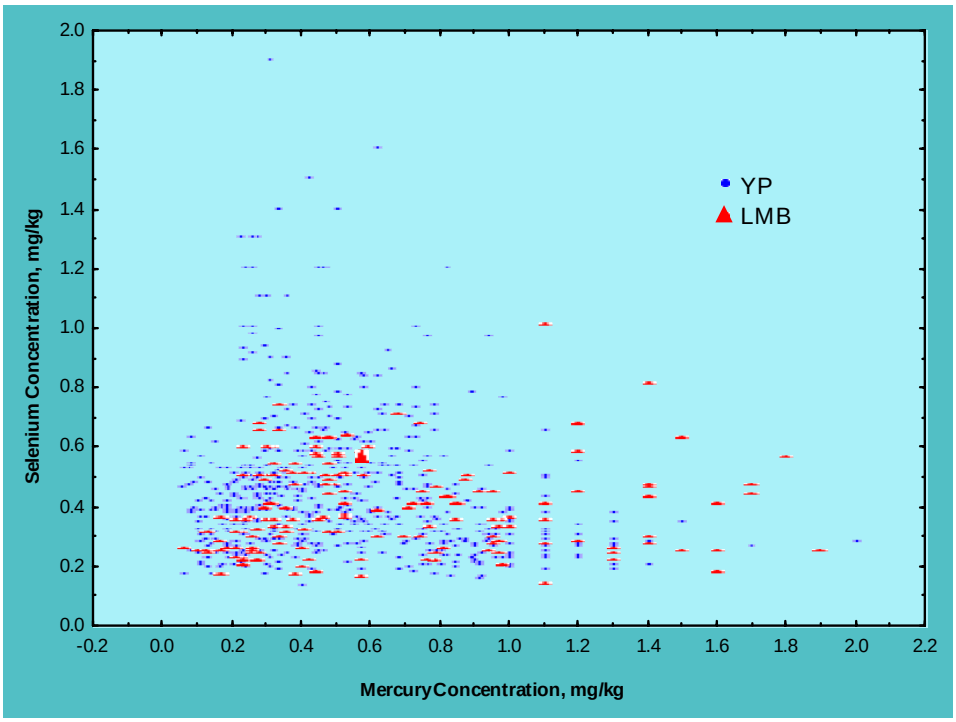


Figure 5. Individual Fish Tissue Selenium Versus Mercury Concentrations for Yellow Perch and Largemouth Bass

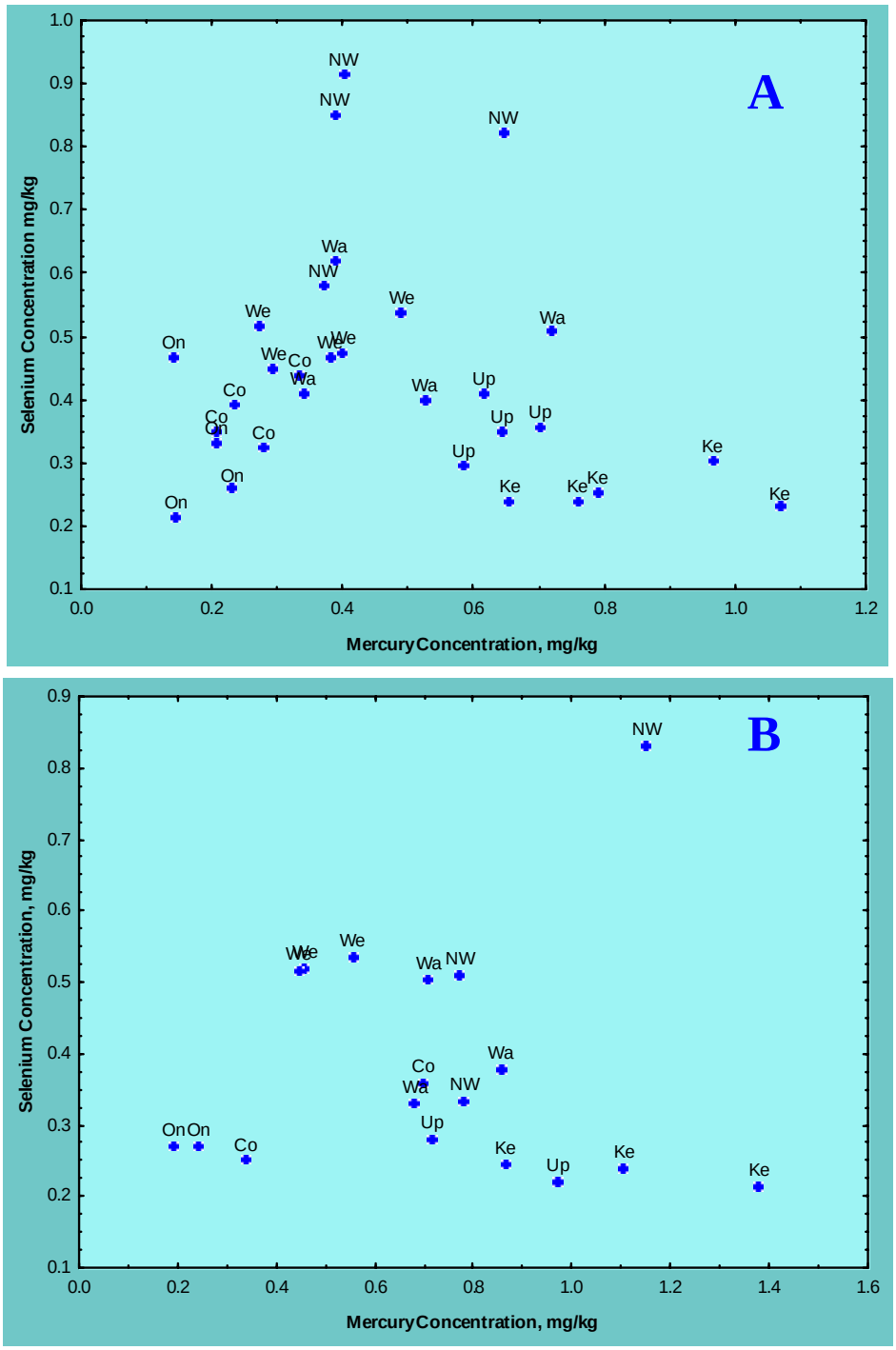
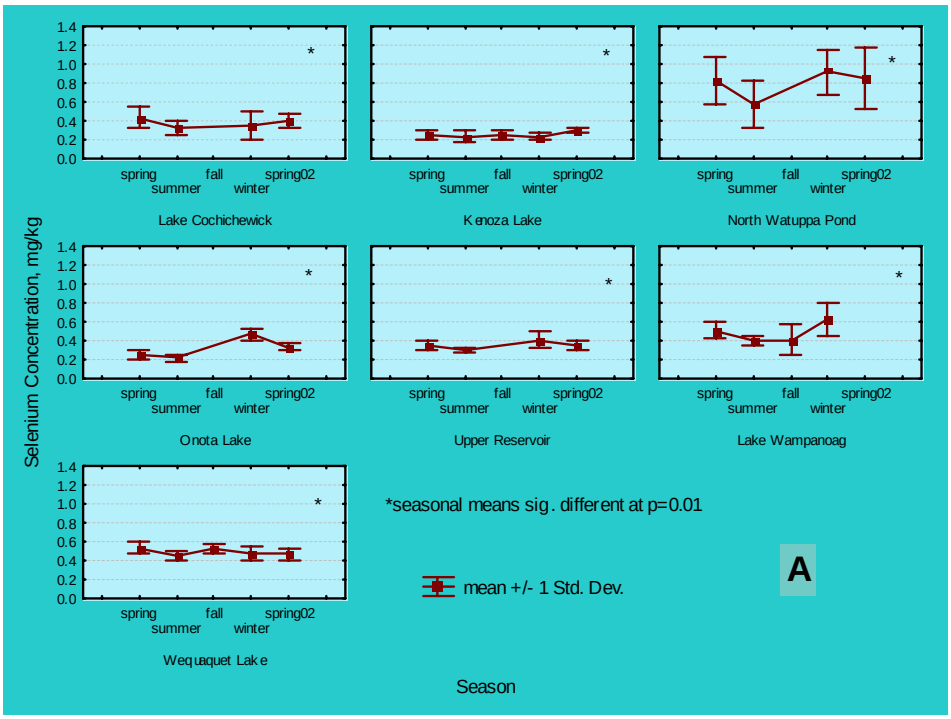


Figure 6 . Lake Seasonal Mean Se Versus Hg Concentrations (see lake name coding in Table 2). A. Yellow Perch; B. Largemouth Bass.



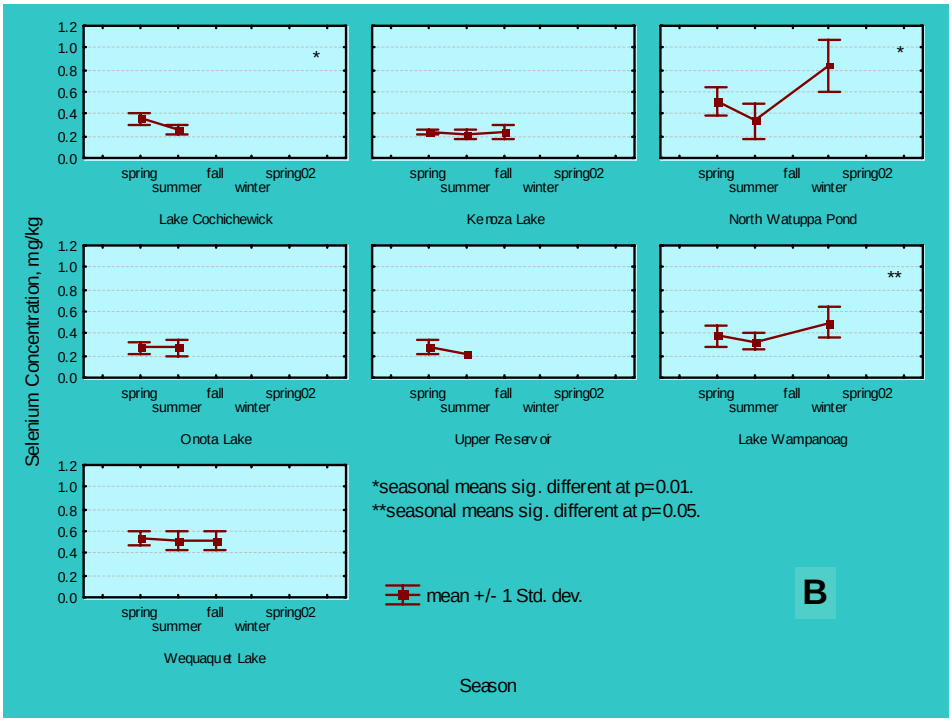


Figure 7. Seasonal Mean Se Concentrations (mg/kg). A. Yellow Perch; B. Largemouth Bass.

3.3 MERCURY SEASONAL DIFFERENCES

Raw and size-standardized seasonal mean mercury concentrations for YP and LMB are presented in Table 3 and 4. For YP, there were marked statistically significant ($p \leq 0.05$) seasonal differences in concentrations of mercury in the edible tissues (Figure 8A). Highest concentrations generally existed in the spring (seen in 6 of 7 lakes). The one exception was in Kenoza Lake where highest values were in the summer and fall. Lowest values were generally in the summer and fall. The raw seasonal means range from 0.140 – 1.068 mg/kg, while the size standardized means ranged from 0.169 - 0.796 mg/kg. Within any one lake the seasonal size-standardized maximum value ranged from 28.8 -175.6% of the minimum size-standardized value.

A similar, though less complete, trend was observed in the LMB because of the difficulty of obtaining fish in the fall and winter in many of the lakes (Figure 8B). Two of the seven lakes had statistically significant seasonal variation in the size-standardized tissue mercury concentrations with the highest values in the spring (Table 4). In two of the other lakes, seasonal high means were also in the spring, but not significantly so. The range of raw tissue mercury concentrations was from 0.190 – 1.377 mg/kg. The range for the size-adjusted values was 0.191 – 1.075 mg/kg, with the seasonal maximum concentrations ranging from 13.1- 59.0 % higher than the seasonal minimum in any one lake.

Water quality data for each lake for each season are presented in Table 5. Most lake pH values were in the 6-7 range, with the exception of some low pH seasonal values in Lake Wampanoag and Upper Reservoir. The ponds with the lowest water conductivities were North Watuppa, Upper Reservoir and Wequaquet Lake (generally < 100 µS). Kenoza and Onota Lakes were at the other end of the conductivity spectrum (high 100s to > 200 µS).

3.4 MERCURY VARIATION ASSOCIATED WITH REPRODUCTION

The seasonal cycles of YP and LMB CI and GSIs are shown in
Figure 9. The YP CI and GSI track each other through the year fairly closely, except for in the spring. CI means were significantly different between seasons (One-way ANOVA, $F=12.1$, $p= 0.01$) with the winter mean being significantly different from all the others (Duncan's test, $p=0.05$). In the first spring of the cycle (2001), the mean condition index was greater than that in the summer, which was the annual minimum. Values started to increase through the fall towards a seasonal maximum in the winter.

The LMB seasonal cycle of CI or GSI were not as pronounced as those of YP, nor did tissue mercury concentrations vary as much (Figure 8B).

Table 3. Yellow Perch Unadjusted And Size-Standardized Seasonal Mean Muscle Mercury Concentrations (mg/kg).

Pond/Lake Season	n	Unadjusted Means	Undajusted Std. dev.	Size Stdz. Means	Size-Stdz. Std. dev.	% Δ: Low to High+	Direction from Spring
Lake Cochichewick*							
spring	30	0.333	0.128	**0.349**	0.103		
summer	18	0.279	0.140	0.344	0.074		
winter	13	0.206	0.125	0.213	0.089		
spring	26	0.235	0.137	0.226	0.074	64.1	down
Kenoza Lake*							
spring	29	0.790	0.370	0.534	0.164		
summer	30	0.759	0.277	**0.652**	0.203		
fall	9	0.653	0.271	**0.621**	0.154		
winter	23	1.068	0.270	0.477	0.194		
spring	27	0.966	0.279	0.497	0.149	36.5	up then down
North Watuppa Pond*							
spring	30	0.646	0.157	**0.532**	0.131		
summer	12	0.371	0.167	0.193	0.088		
winter	30	0.402	0.162	0.274	0.091		
spring	30	0.388	0.089	0.376	0.074	175.6	down
Onota Lake*							
spring	30	0.229	0.082	**0.269**	0.077		
summer	17	0.145	0.051	0.169	0.042		
winter	23	0.140	0.065	0.184	0.051		
spring	24	0.208	0.092	**0.225**	0.089	59.1	down
Upper Reservoir*							
spring	30	0.702	0.209	**0.778**	0.189		
summer	12	0.585	0.194	**0.662**	0.161		
winter	24	0.615	0.167	0.604	0.164		
spring	20	0.642	0.218	**0.739**	0.160	28.8	down
Lake Wampanoag**							
spring	30	0.720	0.236	**0.796**	0.174		
summer	10	0.525	0.272	0.722	0.155		
fall	5	0.340	0.089	0.341	0.089		
winter	5	0.388	0.098	0.479	0.047	133.6	down
Wequaquet Lake*							
spring	30	0.489	0.129	**0.412**	0.094		
summer	9	0.293	0.060	0.305	0.059		
fall	9	0.271	0.083	0.251	0.079		
winter	30	0.399	0.154	0.345	0.084		
spring	30	0.380	0.129	0.330	0.084	64.6	down

+ differences calculated on size-standardized values;
* significant seasonal differences in means at $\alpha = 0.01$;
** significant seasonal differences in means at $\alpha = 0.05$;
Highest statistically significant seasonal size-adjusted means noted in ***bolded italics*** . More than one bolded season at a lake represents no significant difference between those values.

Table 4. Largemouth Bass Unadjusted and Size-Standardized Seasonal Mean Muscle Mercury Concentrations (mg/kg).

Pond/Lake Season	n	Unadjusted Means	Unadjusted Std. Dev.	Size Stdz. Means	Size Stdz. Std. Dev.	% difference: Low to High+	Direction from Spring+
Lake Cochichewick							
spring	12	0.699	0.458	0.626	0.191		
summer	12	0.337	0.280	0.520	0.106	20.3	down
Kenoza Lake							
spring	5	1.104	0.254	0.951	0.207		
summer	11	1.377	0.395	1.075	0.202	13.1	no sig. change
fall	2	0.865	0.120				
North Watuppa Pond							
spring	9	0.772	0.461	0.528	0.124		
summer	10	0.780	0.446	0.640	0.242	21.2	up
winter	2	1.150	0.071				
Onota Lake*							
spring	21	0.241	0.106	**0.298**	0.063		
summer	3	0.190	0.082	0.191	0.014	55.9	down
Upper Reservoir							
spring	5	0.716	0.111	0.726	0.111		
summer	1	0.970	0.000			-	-
Lake Wampanoag							
spring	14	0.856	0.395	0.805	0.201		
summer	9	0.681	0.479	0.669	0.207		
winter	4	0.708	0.243	0.618	0.037	30.1	down
Wequaquet Lake*							
spring	30	0.554	0.297	**0.612**	0.129		
summer	10	0.458	0.201	0.412	0.075		
fall	14	0.445	0.204	0.385	0.089	59.0	down

+ Calculated on size-standardized values;

* significant seasonal differences in means at $\alpha = 0.01$;

Highest statistically significant size-adjusted seasonal means noted in ***bolded italics.*** More than one bolded season at a lake represents no significant difference between those values.

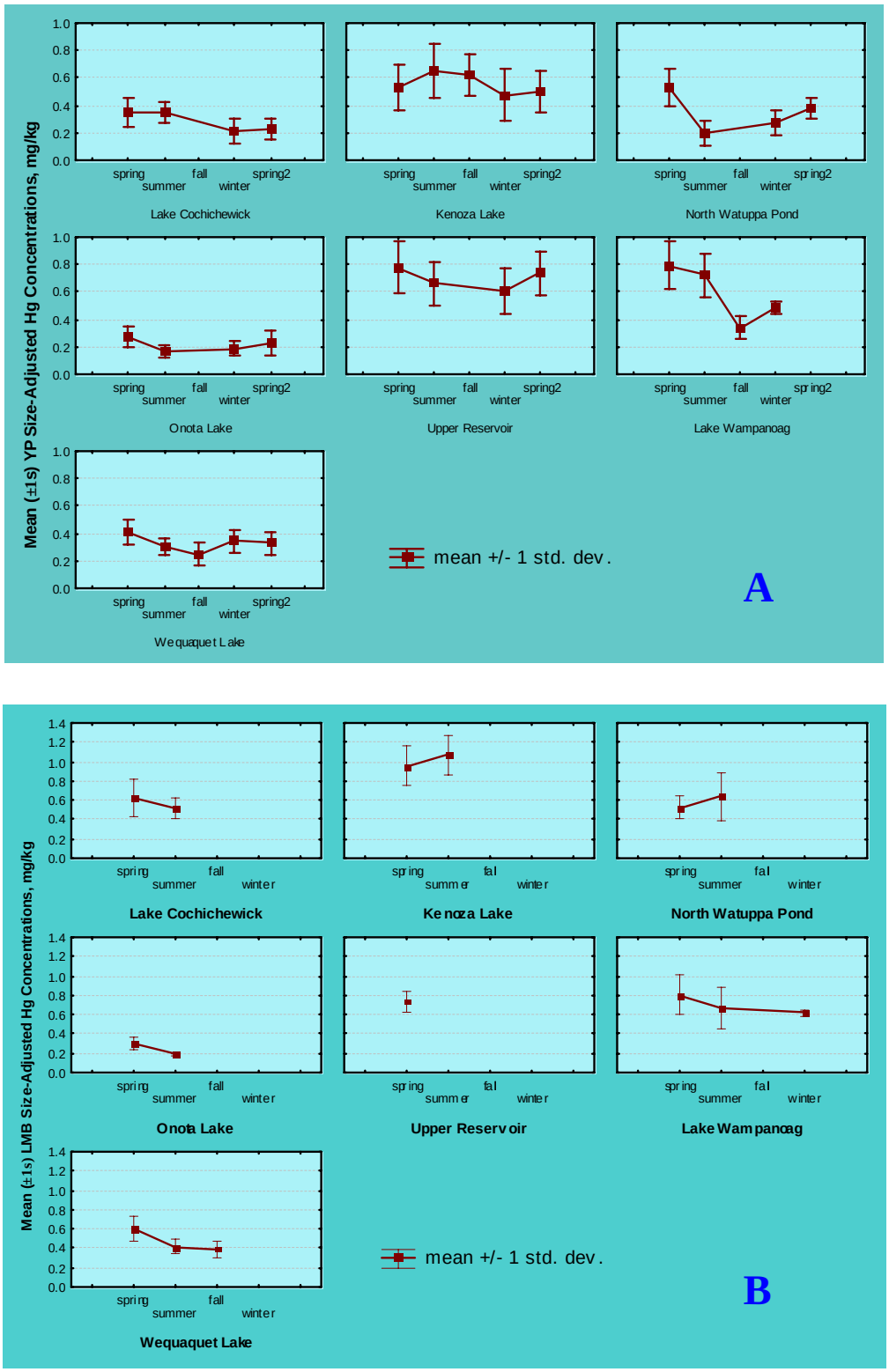


Figure 8. Seasonal Mean Size-Adjusted Tissue Mercury Concentrations (mg/kg) from Spring 2001 to Spring 2002. A. Yellow Perch; B. Largemouth Bass.

Table 5.Water Quality Data (all variable units mg/L unless otherwise noted).

Location	Date	Season	Temp, ^{o}C.	DO	pH	SC, µS	Alkalinity as $CaCO_3$	Ca	Na	K	Mg	Mn	Fe	Cl^-	SO_4
Kenoza Lake	4/23/01	prespawn	7.3	11.4		213.5									
Kenoza Lake	6/6/01	spring	12.4	8.3	6.9	234.1	19.5	11.8	24.6	1.6	2.4	0.02	0.08	48.4	9.5
Kenoza Lake Epil.	8/1/01	summer	23.5	7.5	7.4	243.5	23.0	12.4	25.6	1.6	2.5	0.01	0.08	50.2	8.5
Kenoza Lake Hypol.	8/1/01	summer	9.7	3.4	7.1	210.0	22.1	11.8	24.5	1.6	2.4	0.04	0.12	48.0	8.8
Kenoza Lake	12/16/01	fall	6.2	10.4	7.2	162.8	21.9	12.4	24.7	1.9	2.2	0.03	0.06	46.0	7.6
Kenoza Lake	3/13/02	winter	4.8	12.7	6.8	154.7	21.2	13.2	27.6	1.6	2.7	0.03	0.12	53.2	10.0
Kenoza Lake	4/29/02	postspawn	9.4	10.5	7.4	210.1									
Lake Cochichewick	5/2/01	prespawn	12.1	12.4		162.0									
Lake Cochichewick	6/5/01	spring	17.7	9.9	7.5	106.4	14.4	7.6	17.0	1.7	2.0	0.13	0.12	31.9	8.0
Lake Cochichewick	8/1/01	summer	21.5	5.1	6.7	192.0	14.3	7.9	20.3	2.1	2.4	0.03	0.07	29.9	7.5
Lake Cochichewick	3/13/02	winter	5.1	12.5	7.2	107.6	12.6	8.2	18.6	1.7	2.2	0.05	0.07	33.6	9.0
Lake Cochichewick	4/30/02	postspawn	9.9	11.3	7.5	148.8									
Lake Wampanoag	5/8/01	spring	16.2	8.1		106.2	0.2	2.2	14.6	0.4	0.5	0.13	0.26	25.8	4.4
Lake Wampanoag	8/29/01	summer	24.1	7.0	6.9	125.1	0.1	2.3	16.8	0.7	0.5	0.03	1.06	28.9	3.7
Lake Wampanoag	12/19/01	fall	2.3	12.6	5.6	78.2	0.2	2.5	17.5	0.8	0.5	0.03	0.18	30.3	3.9
Lake Wampanoag	1/21/02	winter	4.3	8.0	4.9	102.6	0.0	2.7	18.5	0.8	0.5	0.03	0.14	31.9	3.9
N. Watuppa Pond	5/22/01	spring	16.6	8.8		75.9	1.6	2.6	8.5	0.4	0.8	0.03	0.07	13.9	7.1
N. Watuppa Pond	9/19/01	summer	21.3	9.2	6.9	91.0	2.4	2.8	9.8	0.7	0.8	0.02	0.03	14.7	6.6
N. Watuppa Pond	1/29/02	winter	3.9	13.3	6.1	48.6	1.8	2.9	9.8	0.6	0.8	0.01	0.03	14.7	7.4
N. Watuppa Pond	5/7/02	postspawn	14.0	11.8	6.8	73.2									
Onota Lake Hypol.	6/5/01	spring	6.0	3.4	6.9	220.8	77.8	21.6	5.1	0.4	7.8	0.20	0.05	8.1	5.3
Onota Lake Epil.	6/5/01	spring	7.5	9.5	7.7	189.8	65.1	18.8	4.5	0.3	6.6	0.01	0.03	7.1	6.1
Onota Lake	9/12/01	summer	14.1	7.0	8.0	162.2	71.0	20.6	4.7	0.5	6.7	0.05	0.04	7.2	5.6
Onota Lake	1/22/02	winter	2.7	11.3	7.2	107.4	72.5	21.1	5.2	0.5	6.9	0.01	0.00	8.3	5.9
Onota Lake	5/22/02	postspawn	9.5	5.3	8.0	208.0									
Upper Reservoir	5/23/01	spring	16.6	4.3	4.6	64.0	-0.4	1.7	6.2	0.5	0.4	0.03	0.32	10.2	4.1
Upper Reservoir	8/29/01	summer	19.0	5.8	5.7	67.0	1.7	2.3	6.7	0.6	0.4	0.04	0.89	10.1	2.8
Upper Reservoir	1/16/02	winter	3.4	7.7	5.1	46.3	0.9	2.6	7.7	1.0	0.5	0.05	0.55	11.9	3.6
Upper Reservoir	5/9/02	postspawn	16.5	9.4	4.9	61.0									
Wequaquet Lake	5/30/01	spring	17.6	5.1	6.6	109.1	3.3	1.2	12.4	1.0	2.0	0.01	0.08	20.0	7.2
Wequaquet Lake	9/19/01	summer	21.2	9.0	6.7	112.0	2.7	0.8	13.0	1.1	1.9	0.01	0.03	20.5	6.6
Wequaquet Lake	11/7/01	fall	11.8	6.9	6.8	113.1	3.1	0.8	13.3	1.2	1.9	0.01	0.04	21.2	6.7
Wequaquet Lake	1/30/02	winter	4.2	13.8	6.6	59.8	3.0	1.0	12.7	1.2	1.8	0.00	0.01	20.0	6.9
Wequaquet Lake	5/8/02	postspawn	14.7	10.9	7.1	67.1									

4.0 DISCUSSION

The purpose of this study was to examine the magnitude of the contribution to variance in fish tissue mercury concentrations from several sources:

- degree of tissue hydration;
- seasonally varying factors;
- reproductive cycle
- and tissue selenium concentrations.

The degree of hydration of muscle tissues could be a potential source of variation in fish tissue mercury concentration estimates, which in turn could produce varying mercury concentration estimates, even when the tissue burden of mercury remains constant. One potential source of this variability could be imprecision associated with water loss from muscle during sample preparation. In addition, it seemed possible that tissue water contents might vary seasonally. Our hypothesis was that by drying the tissues and expressing the tissue mercury burden on a dry weight basis, a reduction in variance would be achieved, as has been our experience with cadmium in marine bivalves (Hutcheson unpublished data). This study found that removal of moisture from fish muscle tissues did not provide any reduction in the variance associated with mean tissue mercury concentration estimates. Going forward, tissue mercury work can continue to be performed on wet tissues and data expressed on a wet weight basis with no additional variance penalty for doing so.

Selenium appears to sometimes play a role in influencing the levels of tissue mercury. The selenium data collected in this study provide a basis for evaluating the potential toxicological significance of this metal in these fish species and for examining possible interactions between selenium and mercury accumulation.

At sufficiently high tissue concentrations (> ~2 mg/kg), selenium can produce developmental toxicity in the offspring of aquatic species (Lemly 1993; Burger *et al.* 2001). Mean tissue concentrations monitored for both species in this study ranged from 0.214 – 0.916 mg/kg wet weight with the highest individual value being in the 1.9 mg/kg range. Therefore, none of the fish in this study lived in conditions where direct toxicity from selenium would be expected. The observed mean selenium concentrations were in ranges characterized as regional background (< 1 mg/kg) or low selenium habitat (~ 0.25 – 2 mg/kg) in other studies (Lemly 1993).

In contrast to the positive age (size) relationship seen with mercury in the fish we collected, selenium showed no such relationships in this study. This finding is similar to that of Chen *et al.* (2001) with walleye and YP.

Tissue selenium and mercury concentrations did not exhibit a clear cross-correlation with each other. Chen et al.(2001) noted that the relationship between mercury and selenium in bioaccumulation has not yet been clarified. Many studies show a positive correlation, while others show an inverse one (Burger *et al.* 2001; Chen et al. 2001).

There was one location in our study (North Watuppa Pond) where high tissue selenium concentrations in fish (YP) were associated with lower to mid-range mercury concentrations (Figure 6). This lake is in a downwind direction from a large coal-fired power plant in southeastern Massachusetts. Combustion of fossil fuels such as coal is the primary source of airborne selenium, followed by incineration of municipal wastes (Agency for Toxic Substances and Disease Registry 2003). In contrast to this location, the lake with the highest tissue mercury concentrations (Kenoza Lake, Figure 6) was in the high mercury deposition area of the state (Merrimack River Valley) where local emissions of mercury were primarily from 4 large waste incineration facilities in the late 1990s. YP from another lake in this same region (Lake Cochichewick, Figure 6) however, had lower mercury concentrations (<~ 0.3 mg/kg) than were recorded in other lakes in this sampling program, making it difficult to produce a single theory to account for the results. Between-lake differences in tissue mercury concentrations were as great as between unwind - downwind deposition areas in this region of the state (MassDEP 2003a). This situation was attributed to differences in lake biogeochemistries, which could also apply to selenium patterns of accumulation.

The contrasting results from one lake (high selenium, moderately high mercury) presumably influenced by emissions from an upwind coal-fired power plant with those from another lake influenced by mercury emissions from waste combustors without the high selenium emissions associated with coal combustion facilities are in agreement with theories of selenium activity in the environment. Some investigators have concluded that high selenium in the aqueous and sedimentary environments seems a more important determinant of fish tissue mercury levels than direct tissue-level competition between mercury and selenium. They hypothesize that selenium inhibits the metabolic activity of sulfate-reducing bacteria who normally methylate mercury, making it more readily available for food chain uptake. When methylation is reduced, uptake is presumably reduced (Southworth *et al.* 2000). Those same authors noted that selenium in bass tissues was not very effective at inhibiting the accumulation of methyl mercury.

Seasonal variability also represents another potentially important source of variation in fish tissue mercury concentration estimates. One of the primary objectives of this study was to determine if the LMB and YP tissue mercury concentrations varied seasonally throughout the year in lakes. The answer to this question has important ramifications for future field study sampling designs for mercury in fish tissues, for interpreting field data and for drawing conclusions about the public health risks posed by mercury contaminated freshwater fish in the environment.

Some of the factors affecting the amount of mercury present in the muscle tissue of fish include: water chemistry affecting mercury form and abundance; prey concentrations of mercury; prey availability; temperature; fish growth and metabolism; internal toxicodynamics of mercury; and selenium presence in water, sediments and tissues. In our study, none of the major water quality indicators used to characterize the physicochemical status of the lakes varied substantially between the earlier and later sampling dates, therefore we do not anticipate that there were geochemically-influenced

seasonal differences in mercury availability. However, throughout the year, some of the other parameters change.

The amount of mercury taken up by fish is a function of the amount of mercury available in the aquatic environment, and the availability of prey organisms. Once taken into the body, mercury is distributed to various body compartments and is stored or slowly eliminated. In predacious organisms, the net result over time is the accumulation of larger and larger amounts of mercury. That mass of mercury in tissues may increase with time or stay constant over defined periods. The mercury mass is traditionally expressed as a concentration in mass Hg per unit of body weight.

During the life of the fish from season to season, its body is a dynamic entity: growing and occasionally "wasting" in periods of scarce food availability or after spawning. Tissue growth with no appreciable change in the tissue mercury burden will have associated with it an apparent decrease in the concentration of tissue mercury which is referred to as "growth dilution"(Slotton *et al.* 1995). This phenomenon was noted by these authors in spring through mid-summer in growing young-of-the-year LMB. During periods of limited food availability or metabolic redirection of energy reserves into the production and eventual loss of gametes, fish will become leaner as they meet their metabolic demands by the sequential metabolism of carbohydrate and lipid reserves. They will then start to use protein reserves which are represented by muscle tissue. As this process progresses, tissue mass decreases, while the mercury body burden remains the same (or changes at a lessened rate from tissue mass). As the "condition" of the fish declines seasonally, the tissue concentration of mercury may appear to increase in a process referred to as "starvation concentration" (Cizdziel *et al.* 2002). Correlations of tissue mercury content with fish condition indices have been observed in striped bass (*Morone saxitilis*) (Cizdziel et al. 2002); bream (*Abramis brama*) (Farkas *et al.* 2003); and northern pike (*Esox lucius*) (Olsson 1976). During periods of starvation, the mercury in muscle can also be transferred via the blood to other tissues such as the liver and kidney as has been seen with flounder (*Platichthys flesus*) (Riisgard and Famme 1988).

Our study found that tissue mercury concentrations varied seasonally in both species studied and that they were usually highest in the spring and lowest in the summer and fall. Seasonality was most prominent in YP which spawn early in the spring in Massachusetts shortly after ice-out. While we were never successful obtaining prespawn adults, winter fish should have been in essentially the same condition. The spent fish that we sampled would therefore be going towards their lowest ebb in terms of energy reserves and body fitness. The GSI annual cycle (Figure 9) illustrates the spent condition of fish in the spring of 2001, with that condition carrying on through the summer. The spring spent fish however appeared to be slightly more robust than later in the summer. The CI graph mimicked the seasonal picture of tissue Hg concentrations seen in most lakes (Figure 8A).

The less pronounced seasonal cycles in LMB CI, GSI (Figure 9B) and tissue mercury concentrations (Figure 8B) may have been due to a combination of factors: our inability

to collect fish for some seasons which resulted in the need to use less sensitive tests of significance on a species that displayed a narrower range of mercury concentrations; and the fact that in the spring some of the fish had already spawned while others were ripe (Figure 10). Those taken later in the spring or summer had usually shed their gametes and were in a spent condition with low gonad weights and GSI values.

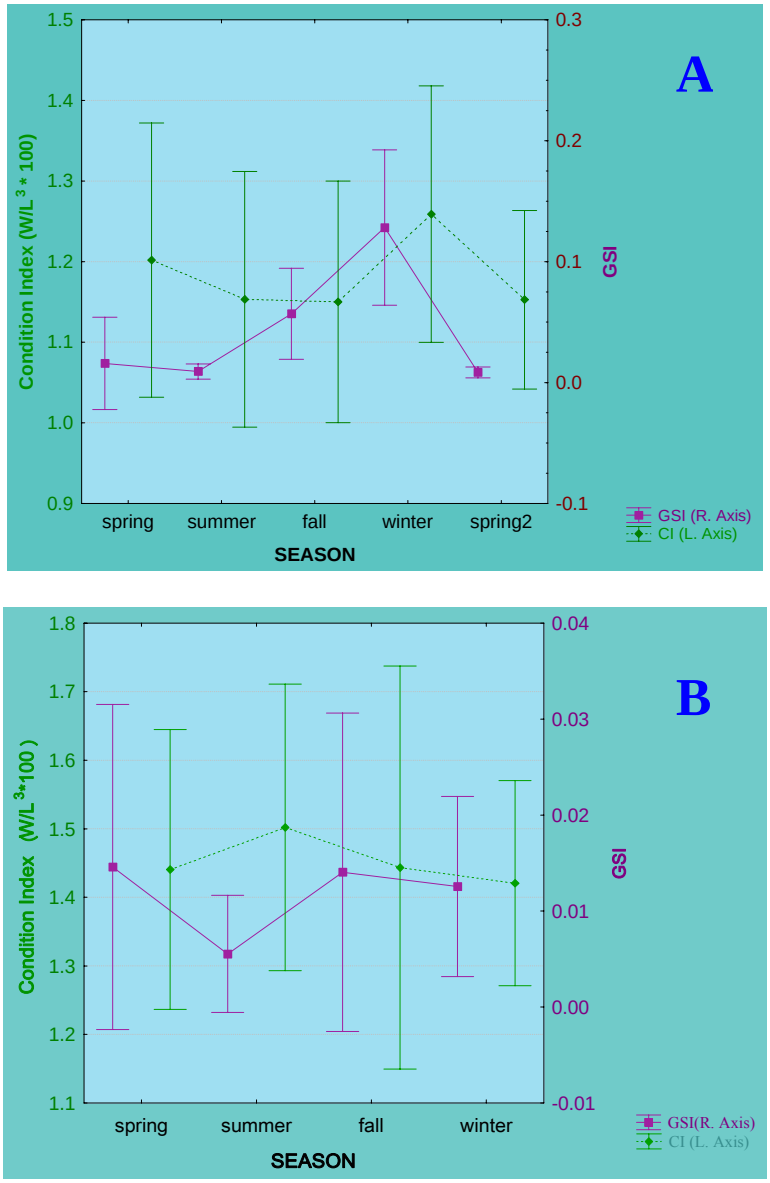


Figure 9. Seasonal Means ± 1 Std. Dev. for Condition Index (W/L^3*100) and GSI (Gonad Weight/Body Weight). Seasonal Bars for GSI and CI Offset for Display Clarity. A. Yellow Perch; B. Largemouth Bass.

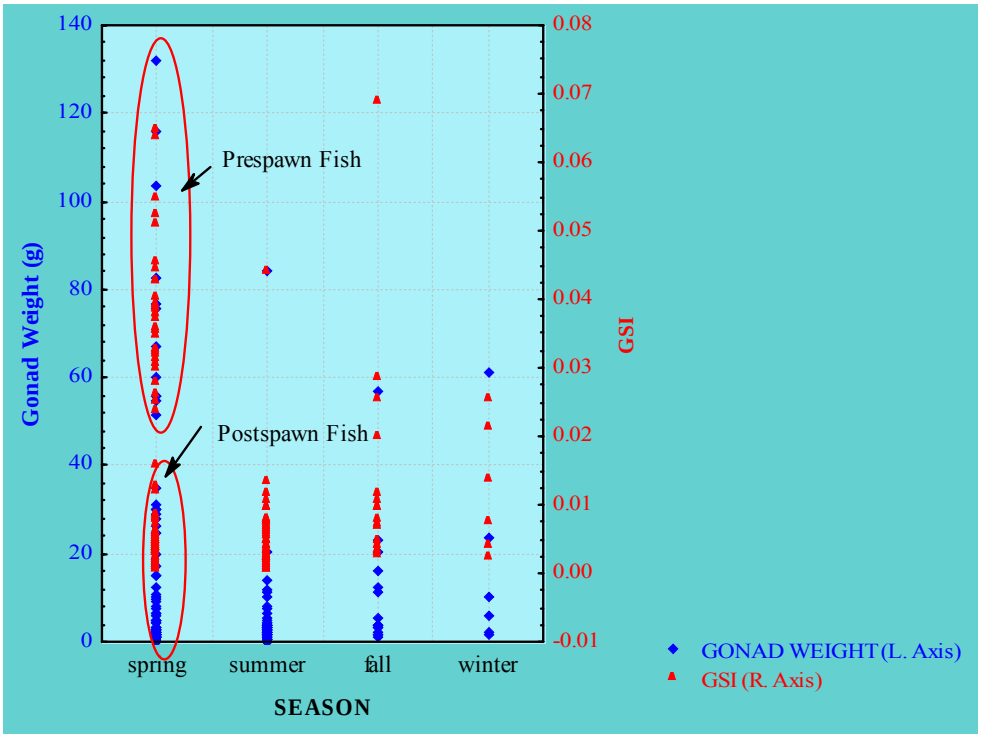


Figure 10. Seasonal Values for LMB Gonad Weights and GSI.

Predaceous fish species show increasing concentrations of tissue mercury as they age as evidenced by the well-recognized positive relationship between tissue mercury concentrations and surrogate measures of fish age such as length or weight. Superimposed upon this progression is a seasonal cycle in the tissue mercury concentration which likely reflects seasonally varying growth and reproductive processes. The high spring mercury concentrations recorded in YP could have reflected "starvation concentration" of the mercury in tissues. These fish would have recently shed their gametes, would be coming off the winter season in a lean condition as a result of lessened food resources and would not have been very far into the summer growth period. The less consistent signal of mercury concentration trends in LMB might have reflected an absence of "starvation concentration" in those individuals that had not yet released their gametes and the result of this process in post spawned individuals. The majority (> ~85%) of the LMB from lakes which had higher tissue mercury concentrations in the spring than summer were spent (data not presented). However, the condition indices of these fish were no different between the spring and summer (ANOVA and Duncan's test: all spring versus summer differences not significant at $p = 0.05$) and the summer fish were not markedly larger than the spring fish. If they were, mercury concentration decreases might have been reflective of "growth dilution".

The scale of variation we found between seasons in within-lake tissue mercury concentrations for the two species that we studied was 13 - 176 % of the seasonal low value. Within the literature, the range of variation for tissue mercury concentrations

across a variety of species in seasonal studies was 22 - 288% with a geometric mean of 79% (Staveland *et al.* 1993; Slotton *et al.* 1995; Olivero and Solano 1998; Farkas *et al.* 2003; Haines *et al.* 2003; Szefer *et al.* 2003; Paller *et al.* 2004). These results indicate that seasonal variation in tissue mercury concentrations can be significant.

Failure to recognize this fact in either the design phase of field sampling studies for fish mercury or during the data interpretation phase could have major implications for the correct interpretation of the data. For example, suppose data are available from Lake A and Lake B. The mean tissue mercury concentrations in a species of fish in Lake A are greater than those in Lake B and it is concluded that this difference is the result of Lake A being located closer to a small industry which uses mercury. However, the analyst overlooks the fact that the samples were taken in different seasons. The differences could have realistically been reflective of seasonal variation.

Another important example of the public health implications of a failure to recognize that seasonal variation exists is in the process of determining whether or not fish consumption advisories are warranted for particular water bodies. It would be possible for fish sampled from waterbodies during the summer and fall to have mercury concentrations which fall below any health-based screening limit for mercury in the edible muscle of that species. Therefore no advisory would be posted and people would continue to utilize that fishery. However, those same fish in the spring may have higher tissue mercury concentrations which could be above the limit and which would warrant an advisory. Therefore the humans consuming fish from that location would be unaware of the potential health risks for eating those fish in the spring. Conversely, if an advisory is based on fish from a spring sampling effort, it is possible that the fish could be consumed with no unacceptable health risks in other seasons of the year. This knowledge can be employed in the future to more efficiently design fish sampling studies which are providing data for advisory screening. Advisories based on sampling may be inappropriate for other times of the year, or decisions made about there being no need for an advisory may be underprotective for other times of the year. Targeting advisories seasonally would have two beneficial results. It could provide additional health protection where there may presently be gaps and it would provide justification for lifting advisories in certain seasons and thus providing additional access to people for use of the fishery.

5.0 ACKNOWLEDGMENTS

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