Fish Mercury Long Term Monitoring
Annual Data Reports - Methods

Massachusetts Department of Environmental Protection
Office of Research and Standards

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Cover photo: Lake Lashaway, by Jane Rose

LIST OF ABBREVIATIONS

BCR ref. std.	European Commission's Community Bureau of Reference Standard
Ca	calcium
Cl	chloride
Fe	iron
K	potassium
LMB	largemouth bass (*Micropterus salmoides*)
LT	lake trout *(Salvelinus namaycush)*
m	meter
MassDEP	Massachusetts Department of Environmental Protection
Mg	magnesium
mg/kg	milligrams per kilogram
Mn	manganese
Na	sodium
NH_3	ammonia
NO_2	nitrite
NO_3	nitrate
ORS	MassDEP's Office of Research and Standards
pH	the negative log (base 10) of the molal concentration of hydrogen ions (H^+) in solution (acidity measure)
SO_4	sulfate
Std. dev or s	standard deviation
Tot. P	total phosphorus
YP	yellow perch (*Perca flavescans*)

1.0 PROGRAM OVERVIEW

MassDEP's Office of Research and Standards started a research program on mercury in freshwater fish in 1994, which continues today. The work is distinct from more limited fish sampling for a variety of contaminants conducted under the auspices of the Massachusetts Interagency Committee on Fish Toxics for the purposes of determining whether or not fish consumption advisories for lakes are needed.

In 2001, MassDEP established a long-term monitoring research program to track changes in mercury contamination of fish. The program objective is to document the magnitude and direction of year-to-year and long-term changes in edible muscle total mercury concentrations in LMB and YP in designated monitoring lakes. The initial stages of this effort coincided with reductions in mercury use and emissions in Massachusetts and the surrounding region.

This report presents the standard methods used throughout the program, along with background materials. Data from 1999 – 2004 are presented in Hutcheson et al (2006). Data for subsequent years will be available and posted as Annual Data Reports in the mercury section of MassDEP's website (http://www.mass.gov/dep/). The Annual Data Reports do not include an interpretation of the fish mercury testing results. The purpose of the reports is to document the results of fish mercury testing. Interpretation of the data will be provided in a future report.

A number of complementary studies were also conducted. A study of seasonal variation in fish tissue mercury concentrations provided perspective on intra-annual variation in mercury and helped better design monitoring studies (MassDEP, 2006). A comparative food web mercury study in two similar lakes located near each other, but with different levels of mercury in top predator fish increased understanding of the ecological basis for varying patterns of mercury bioaccumulation seen in different lakes (MassDEP 2003a).

Wildlife are an integral part of any pond ecosystem. A first step toward addressing the risks mercury poses to animals that live in and around the water, for example, fish-eating birds such as loons, is to better understand their exposure from the food chain. As part of its overall program, Massachusetts has compiled information on mercury in wildlife (Pokras, et al 2006).

Data from studies on mercury in popular freshwater fish allow widespread screening of Massachusetts lakes for potential human health risks. The Massachusetts Department of Public Health (DPH) issues fish consumption advisories to address health hazards posed by eating mercury-contaminated fish.

Sediment and water quality of the lakes where fish have been studied were analyzed, and that data along with individual fish tissue mercury concentrations are available from a database access portal (http://public.dep.state.ma.us/fish/). The database contains total mercury concentrations in edible tissues (dorsal muscle). The identified reports may be consulted for details of analytical methodologies employed in particular parts of the program.

Fish tissue data, water quality data and lake physical data from ORS's research program are made available in the Annual Data Reports from the Long-Term Fish Monitoring Research Program.

2.0 MATERIALS AND METHODS

The program objective is to document the magnitude and direction of long-term changes in edible muscle total mercury concentrations in LMB and YP in the monitoring lakes. Approximately half the lakes designated as long term monitoring lakes are sampled on a rotating annual cycle. Dependent upon the degree of interannual variation observed between years in the initial stages of the program and available financial resources, the duration between repeat samplings may be changed in subsequent years. To date, in some years, additional numbers of lakes were sampled in regions of the state of particular interest, specifically the high mercury deposition area (Hutcheson et al. 2008) encompassing the northeast part of Massachusetts, in order to give more temporal and spatial resolution.

The criteria used to select long term monitoring lakes led us to choose lakes that:

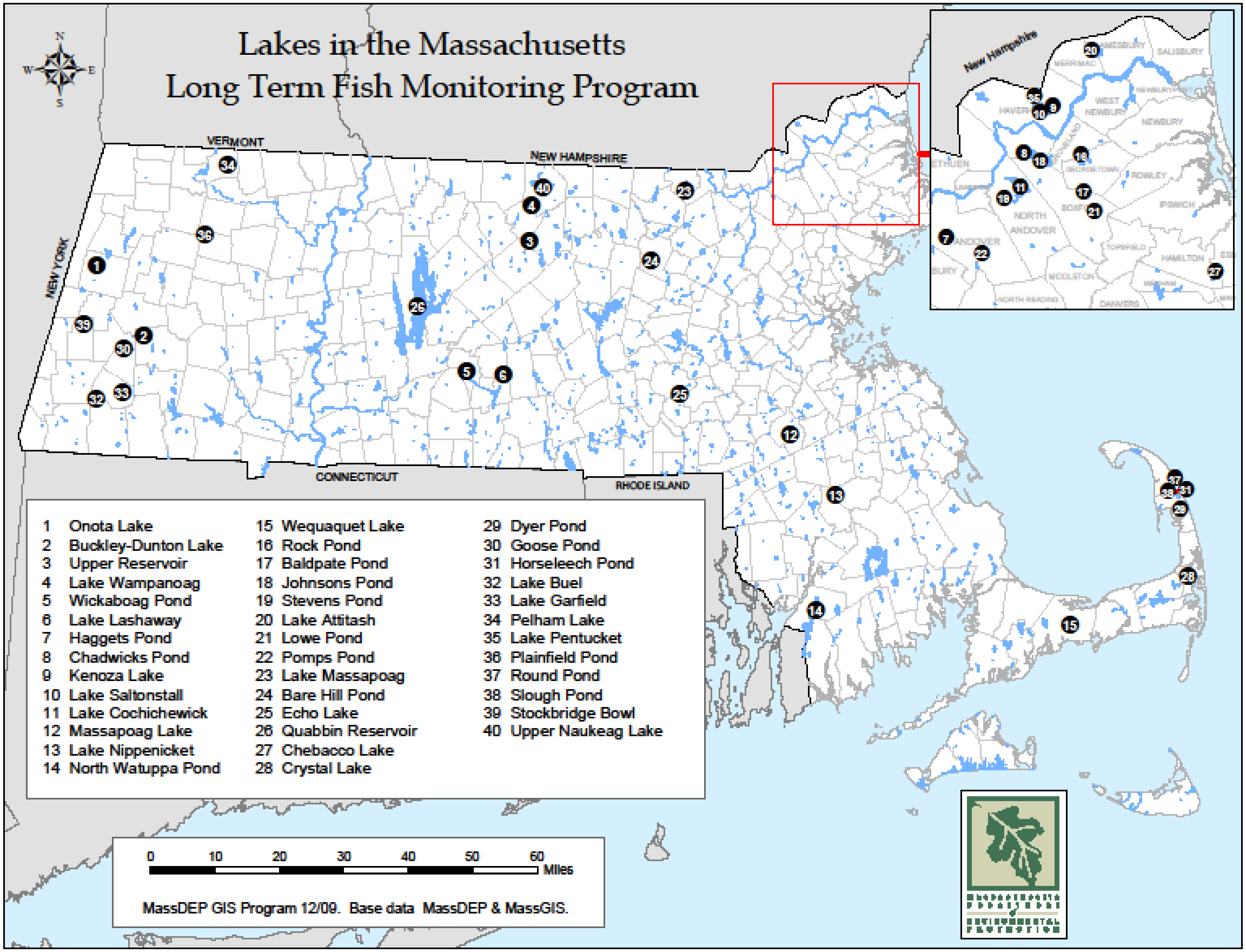
- are in representative ecoregions of the state;
- are in the predicted high mercury deposition area in northeast Massachusetts;
- span the West–to-East distance across the state to reflect possible out-of-state long-range atmospheric inputs with prevailing winds;
- are positioned in urban and rural areas of the state;
- were recommended by Massachusetts Basin Team leaders;
- have protected watersheds;
- are heavily fished;
- provide habitat for species higher on the food chain than fish, such as loons, beavers and turtles.
- have been sampled previously.

2.1 FIELD SAMPLING

Fish are collected with box nets, gill nets, trot lines, electroshocking and rod and reel. They are 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.

Fish are collected in the spring of each year to control for the variability which can be introduced by seasonal changes in fish tissue mercury concentrations (MassDEP 2005). In order to provide robust

Figure 1. Locations of Long-Term Monitoring Lakes



size/age ranges of LMB, a size spectrum of fish is collected. YP greater than 20-25 cm total length are sought 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 data from our previous studies were used along with a desired confidence level of 0.10 and power of 80% to calculate required sample sizes. Our calculations and consideration of practical issues including analytical costs and concerns over potential overharvesting of resident fish populations, led us to seek 30 replicate YP per lake per sampling event and 12-15 LMB. These sample sizes were estimated to have an ability to identify differences in means of approximately 40-50% in LMB and 15-20% in YP. In practice, there are occasions when it is not possible to obtain the desired numbers of fish.

Basic water quality measurements are 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 are measured. Dependent upon whether or not the water column is stratified at the time of sampling, either mid-epilimnion and hypolimnion water samples are taken or a single mid-depth sample is taken for analysis.

2.2 LABORATORY PROCEDURES

2.2.1 Tissue Mercury Analysis

Fish are processed for analysis of mercury in lateral muscle in accordance with US EPA procedures (U.S. Environmental Protection Agency 1993). Total fish lengths and wet weights are recorded. The sex and reproductive condition of each fish was assessed by visual examination of gonads and classification as: Immature; Developing; Ripe; and Spent. Fish are occasionally classified as “resting” and “ripe and running”. Gonad wet weights are determined. Scales are removed from the fish for age analysis. Other details of handling and sample preparation are identical to those described in Rose et al. (1999).

Fish tissue mercury concentrations expressed on a wet weight basis are determined in accordance with US EPA procedures (U.S. Environmental Protection Agency 1993) and are described elsewhere ((Hutcheson *et al.* 2008)). For analyses performed through 2004 according to US EPA Method 245.6 (US EPA 1991), accuracy (i.e., Hg percent recovery from Hg-spiked fish samples) and precision (i.e., Hg relative percent difference among duplicate fish samples) in the analyses of fish samples were 103 ± 9.1 % and 4.0 ± 3.8 % (means ± 1 s) respectively. The accuracy of analyses of a mercury fish tissue reference standard consisting of freeze-dried tuna tissue (BCR ref. std #463) was 103 ± 4.7 % recovery. Mercury in all laboratory reagent blanks was less than the method detection limit (MDL) of 0.02 mg/kg. The analytical method was changed in 2005 to one following US EPA Method 7473 (US EPA 2007) using a Milestone DMA80 direct mercury analyzer. Details on an intercomparison of mercury concentrations determined with the two methods on the same tissue samples are available from the Office of Research and Standards. This method employs sample thermal decomposition, mercury amalgamation, and atomic absorption spectrophotometry. The method detection limit is 0.002 mg/kg and the reporting limit is 0.006 mg/kg.

2.2.2 Water Analysis

Water samples are analyzed for 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 and MassDEP (2005).

2.2.3 Quality Control And Quality Assurance

Sample spikes and replicate analyses are conducted to determine precision. Freeze-dried tuna tissue (BCR ref. std. #463) is analyzed for mercury as a reference standard. All recoveries and precision estimates are within the EPA Method 7473 Acceptance Criteria. Mercury in all laboratory reagent blanks is less than the method detection limit.

Table 1. Analytical Methods for Water Quality

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	Standard Methods. 4500-NH_3 F
Tot. P	0.001	Standard Methods. 4500-P E

2.3 DATA ANALYSIS

Bivariate plots of individual fish mercury concentrations versus total fish length for each species for each lake are examined for outliers. Outliers are either corrected, if representing a data entry error, or excluded if outlying the sphere of the remainder of the data. The criterion for exclusion is a subjective determination that a data point falls well outside the range of others in the data set and/or represents a mercury/size relationship at odds with all the other data. A positive linear correlation of fish length with tissue mercury concentrations exists in most cases. In order to adjust for the effects of this covariate prior to examining mercury concentration differences between years, individual fish mercury concentrations are adjusted to the concentration of a standard-sized fish of that species. A "standard-sized fish" is defined as the arithmetic mean fish length over all fish sampled (339 mm for LMB; 243 mm for YP) in our study of mercury concentrations in fish from northeastern Massachusetts (MassDEP 2003b). Size-standardized tissue Hg concentrations are determined by first regressing all individual fish mercury concentrations on total body lengths for the fish species from a lake in a year, 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 are examined for linearity: most of the mercury – length relationships approximate linearity. In order to retain individually-based fish data in analyses, thereby getting maximal statistical benefit from the sample size, individual fish mercury concentrations are size-adjusted to the mercury concentration of a standard-sized fish. The rationale 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 is then available for any subsequent comparisons between groups. All statistical evaluations in this study are performed with the Statistica/W©, Version 5.0 software package (StatSoft, Tulsa, OK, USA).

4.0 REFERENCES

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APPENDICES

APPENDIX 1. Annual Sampling Schedule and Catch

Numbers represent total largemouth bass and yellow perch sampled.

Lake	'94	'99	'01	'02	'03	'04	'05	'06	'07	'08	Lake Totals
Baldpate Pond	0	20	0	0	0	19	0	15	0	16	70
Bare Hill Pond	0	20	0	0	0	42	0	45	45	0	152
Chadwicks Pond	0	21	0	0	0	45	0	12	0	29	107
Cochichewick	0	18	72	39	0	44	0	45	0	45	263
Forest Lake	0	18	0	0	0	0	0	0	0	0	18
Haggetts Pond	0	17	0	0	42	45	43	0	45	0	192
Johnsons Pond	0	20	0	0	0	49	0	30	45	0	144
Lake Attitash	0	18	0	0	0	42	0	45	0	45	150
Lake Pentucket	0	10	0	0	0	0	0	0	0	0	10
Lake Saltonstall	0	9	0	0	12	0	0	0	0	0	21
Long Pond	0	18	0	0	0	0	0	0	0	0	18
Lowe Pond	0	18	0	0	0	33	0	7	0	41	99
Massapoag Dunstable	0	18	0	0	0	42	0	45	45	0	150
Newfield Pond	0	18	0	0	0	0	0	0	0	0	18
Pomps Pond	0	16	18	0	0	15	0	0	16	0	65
Rock Pond	0	20	0	0	0	44	45	0	45	0	154
Stevens Pond	0	18	12	0	0	11	0	0	0	0	41
Kenoza	0	0	87	50	0	43	0	115	0	45	340
North Watuppa Pond	19	0	63	60	0	42	45	0	45	0	274
Onota	0	0	71	47	0	36	0	42	0	42	238
Upper Reservoir	27	0	48	44	0	6	0	0	16	0	141
Wampanoag	26	0	68	9	0	44	0	45	0	45	237
Wequaquet	0	0	102	60	0	42	0	45	0	45	294
Buckley Dunton Lake	29	0	0	0	32	29	0	30	0	33	153
Lake Lashaway	0	0	0	0	27	0	45	0	29	0	101
Lake Nippenicket	0	0	0	0	42	0	45	0	45	0	132
Massapoag Sharon	0	0	0	0	42	0	46	0	45	0	133
Wickaboag Pond	0	0	0	0	42	0	45	0	45	0	132
Echo Lake	0	0	0	0	0	31	0	18	0	35	84
Quabbin Reservoir	0	0	0	0	0	0	25	0	0	0	25
Plainfield Pond	27	0	0	0	0	0	0	40	0	30	97
Ashfield Pond	27	0	0	0	0	0	0	0	0	0	27
Bog Pond	27	0	0	0	0	0	0	0	0	0	27
Somerset Reservoir	20	0	0	0	0	0	0	0	0	0	20
Upper Naukeag	27	0	0	0	0	0	0	0	42	0	69
Laurel Lake	28	0	0	0	0	0	0	0	0	0	28
Lake Garfield	0	0	0	0	0	0	0	42	0	45	87
Chebacco	0	0	0	0	0	0	0	42	0	45	87
Stockbridge Bowl	0	0	0	0	0	0	0	42	0	45	87
Goose Pond	0	0	0	0	0	0	0	9	0	41	50
Lake Buel	0	0	0	0	0	0	0	37	0	45	82
Pelham Lake	0	0	0	0	0	0	0	30	0	37	67
Dyer Pond	0	0	0	0	0	0	0	0	30	0	30
Slough Pond	0	0	0	0	0	0	0	0	42	0	42
Crystal Lake	0	0	0	0	0	0	0	0	15	0	15
Horseleach Pond	0	0	0	0	0	0	0	0	0	45	45
Round Pond (East)	0	0	0	0	0	0	0	0	0	45	45
All Groups	549	354	541	309	239	704	339	781	595	799	5210

APPENDIX 2. INFORMATION FOR ALL LAKES SAMPLED IN THE LONG TERM MONITORING PROGRAM

Water body	*Town*	*PALIS#*	*Watershed*	*Acres*	*Lat./ Long.*
Attitash	Amesbury	84002	Merrimack	368	42.851, -70.983
Baldpate	Boxford	91001	Merrimack	59	42.699, -71.002
Bare Hill	Harvard	81007	Nashua	768	42.490, -71.598
Buckley Dunton	Becket	32013	Westfield	145	42.313, -73.138
Chadwicks	Haverhill, Boxford	84006	Merrimack	173	42.742, -71.080
Chebacco	Essex, Hamilton	93014	No. Shore	207	42.611, -70.808
Cochichewick	North Andover	84008	Merrimack	573	42.704, -71.097
Crystal Lake	Orleans	96050	Cape Cod	33	41.774, -69.983
Dyer	Wellfleet	96070	Cape Cod	10	41.937, -70.007
Echo	Milford, Hopkinton	72035	Charles	105	42.192, -71.512
Goose	Lee	21043	Housatonic	238	42.284, -73.191
Haggetts	Andover	84022	Merrimack	210	42.648, -71.199
Horseleech	Truro	96144	Cape Cod	23	41.969, -70.006
Johnsons	Groveland Boxford	84027	Merrimack	193	42.733, -71.052
Kenoza	Haverhill	84028	Merrimack	240	42.792, -71.050
Buel	Monterey	21014	Housatonic	194	42.171, -73.280
Garfield	Monterey	21040	Housatonic	256	42.183, -73.195
Lashaway	North & East Brookfield	36079	Chicopee	274	42.236, -72.046
Lowe	Boxford	92034	Ipswich	35	42.676, -70.985
Massapoag	Dunstable	81081	Nashua	111	42.649, -71.495
Massapoag	Sharon	73030	Neponset	389	42.103, -71.177
Nippenicket	Bridgewater	62131	Taunton	375	41.970, -71.039
North Watuppa	Fall River	61004	Mount Hope Bay	1700	41.706, -71.104
Onota	Pittsfield	21078	Housatonic	646	42.471, -73.279
Pelham Lake	Rowe	33016	Deerfield	79	42.699, -72.889
Pentucket	Haverhill	84051	Merrimack	37	42.791, -71.073
Plainfield	Plainfield	33017	Deerfield	63	42.542, -72.957
Pomps	Andover	83014	Shawsheen	25	42.636, -71.152
Quabbin Reservoir	Multiple towns	36129	Chicopee	24462	42.447, -72.272
Rock	Georgetown	91012	Parker	49	42.730, -71.006
Round(E)	Truro	96260	Cape Cod	6	41.971, -70.010
Saltonstall	Haverhill	84059	Merrimack	44	42.783, -71.066
Slough	Truro	96298	Cape Cod	28	41.966, -70.012
Stevens	North Andover	84064	Merrimack	22	42.691, -71.108
Stockbridge Bowl	Stockbridge	21105	Housatonic	383	42.336, -73.317
Upper Naukeag	Ashburnham	35090	Millers	31	42.658, -71.927
Upper Reservoir	Westminster	35091	Millers	304	42.536, -71.968
Wampanoag	Ashburnham Gardner	81151	Nashua	225	42.616, -71.965
Wequaquet	Barnstable	96333	Cape Cod	573	41.670, -70.341
Wickaboag	West Brookfield	36166	Chicopee	314	42.246, -72.156

PALIS# = Massachusetts pond and lake identification system number