Quality Assurance Project Plan

Measuring Mercury Trends in Freshwater Fish in Washington State

by

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Abstract

Concern about mercury in our environment has increased due to the persistent, bioaccumulative, and toxic nature of this substance. Mercury was chosen as the first pollutant to be addressed in the state's Persistent and Bioaccumulative Toxins Reduction Strategy. A Mercury Chemical Action Plan was developed in 2003 by the Departments of Ecology and Health with assistance from an advisory committee representing business, health, environmental, and local government organizations.

The Mercury Chemical Action Plan identified the need for improved understanding of mercury's behavior in the environment in order to guide management of this environmental toxicant. The 2005 Legislature provided funds to begin long-term monitoring of mercury in the freshwater environments of Washington. This document is the initial plan for long-term monitoring of mercury in fish tissue.

The primary goal of this project is to monitor mercury levels in edible tissue from freshwater fish at six sites per year on a five-year frequency (30 sites total) in order to characterize temporal trends in fish tissue mercury levels. Target fish species for trend monitoring are bass and walleye. Ancillary data on the fish and sites will be collected to help understand patterns, dynamics, and changes in fish tissue mercury levels over space and time. Such data include: fish length; weight; sex; age; and physical and chemical characteristics of sites such as morphometry, water chemistry, and surficial sediment mercury levels.

A secondary goal of this project is to provide information about mercury levels in fish species other than bass and walleye in order to help the Department of Health craft more informative recommendations for fish consumption advisories. For two other species per site, three composite samples of 3-5 fish per composite sample will be collected and analyzed for mercury.

Background

Introduction

While mercury is a naturally occurring substance, human activity has increased the release of mercury into the environment. Consequences of this include increased health risks to humans and wildlife due to the persistent, bioaccumulative, and toxic nature of this substance. Concerns about these risks have led governments at international, national, state, and local levels to recognize and address the problems associated with humanity's use and disposal of mercury.

In Washington, mercury was chosen as the first priority pollutant to be addressed in the state's Persistent and Bioaccumulative Toxins (PBT) Reduction Strategy (Gallagher, 2000). This focus on mercury resulted in development of the Washington State Mercury Chemical Action Plan (Peele et al., 2003). This Mercury Chemical Action Plan (CAP) was developed in 2003 by the Departments of Ecology (Ecology) and Health (DOH) with assistance from an advisory committee representing business, health, environmental, and local government organizations.

The Mercury CAP provides a thorough description of mercury in the environment including: natural and anthropogenic sources, occurrence and biogeochemical cycling in environment, mercury use and emissions in Washington, a summary of health effects and concerns, and fish consumption advisories in Washington due to mercury-contaminated fish. Other information in the Mercury CAP addresses: Clean Water Act Section 303d listings of waterbodies impaired by mercury, a review of research projects looking at mercury in Washington, the regulatory structures and numerical criteria that address mercury, and recommendations for reducing mercury emissions in Washington.

One of the goals of the PBT Strategy and Mercury CAP was to develop information needed for understanding the behavior of PBTs in the environment and reaching decisions on measures to reduce PBTs. While several studies have helped to initially characterize mercury levels in Washington's environment, these studies and the Mercury CAP recognized and stated the need for a long-term commitment to monitoring mercury in the environment. Monitoring contaminant trends over time has also been one goal of the Washington State Toxics Monitoring Program (Seiders and Yake, 2002), yet lack of resources have prevented implementation of such monitoring. The information gained from this long-term monitoring effort of mercury in fish tissue will be useful in understanding the fate of mercury in our environment and will be useful in future efforts that may be developed for determining the effectiveness of Washington's Mercury CAP.

In 2005, the Legislature provided funds to begin long-term monitoring of mercury in the environment. This funding was to address specific monitoring efforts:

- Determine mercury levels in edible tissue from ten individual fish of the same species (bass and/or walleye) from six sites per year for long-term trend characterization. Sampling at each of these sites will be repeated every five years such that a total of 30 sites will be sampled over a five-year period.

- Sediment cores from three lakes per year will be collected to assess depositional history of mercury in Washington. This sediment coring effort is being developed as a separate, yet related, project and scheduled to begin in the spring of 2006.

This document is the plan for long-term monitoring of mercury in freshwater fish tissue in Washington. This plan should be revised within five years as new information is gained and resources are developed to better understand mercury in the environment. Development of this quality assurance project plan followed guidance described by Lombard and Kirchmer (2004).

Studies on Mercury in Washington

Several studies described the extent and severity of mercury contamination in fish throughout Washington, many of which led to issuance of fish consumption advisories. Continued monitoring across Washington is needed to better characterize mercury contamination in fish and changes in mercury levels over time.

Fischnaller et al. (2003) examined mercury in bass and sediment from 20 sites across Washington. Samples of muscle tissue from bass confirm that elevated levels of mercury are prevalent across Washington. The study recommended developing and implementing a long-term monitoring plan for mercury in fish.

Mercury concentrations were positively correlated with fish size, increasing with fish age, weight, and length in about 90% of sites sampled. These findings were stated to be consistent with other studies, demonstrating that bioaccumulation of mercury occurs in upper trophic level predatory species, such as bass. A weak, positive correlation was found between mercury concentrations and lipids such that lipids analysis in future studies was deemed unnecessary. The technique of adjusting fish tissue mercury concentrations to a standard fish size was useful in comparing tissue mercury levels among sites.

Many fish exceeded one or more criteria for protection of human health. About 23% of 185 fish representing 70% of 20 sites exceeded the EPA Recommended Fish Tissue Criterion of 300 ug/kg wet weight (EPA, 2001). A single ten-year old fish from Samish Lake had a muscle tissue mercury level of 1280 ug/kg wet weight (ww). This result exceeded the National Toxics Rule criterion of 825 ug/kg ww (CFR, 2004) and FDA's Action Level of 1000 ug/kg ww (FDA, 1985). The Action Level criterion is used to remove fish from commercial markets. This study was the basis of DOH's issuance of a statewide fish consumption advisory for large- and smallmouth bass (McBride, 2003).

Norton (2004) investigated mercury levels in the surficial sediments and sediment cores of Lake Whatcom. Findings suggest that mercury levels began increasing around 1900, may have peaked in the late 1990s, and are currently declining. The study recommended that mercury levels in fish from Lake Whatcom be monitored periodically to determine if mercury levels decline over time. The study also recommended monitoring of bottom waters for methyl mercury and total mercury to help evaluate compliance with a water quality target concentration in the lake that would prevent excessive bioaccumulation of mercury in fish.

Serdar et al. (2001) examined mercury concentrations in 273 fish from six finfish and one crayfish species in Lake Whatcom. Mercury levels were particularly elevated in smallmouth bass. The Lake Whatcom fish tissue mercury data were used in development of a fish consumption advisory for Lake Whatcom (Lake Whatcom Cooperative Management Program, 2001). Serdar et al. (2001) recommended a monitoring program to routinely characterize mercury levels in fish throughout Washington.

Munn et al. (1995) investigated mercury and other metals in walleye, bass, and trout from Lake Roosevelt. Elevated mercury levels in walleye led DOH to issue a fish consumption advisory in Lake Roosevelt (USGS, 1997).

Problem Statement

The lack of a long-term monitoring effort for mercury in fish tissue hampers efforts to understand the scope of fish tissue contamination and develop reasonable expectations for managing mercury sources to reduce their levels in freshwater environments. A long-term monitoring effort of mercury in freshwater fish tissue is needed to:

- Identify temporal and spatial patterns in fish tissue mercury levels.
- Identify factors affecting pollutant loading such as source, transport, and fate mechanisms.
- Develop understanding of contaminant behavior to inform decision making.
- Educate the public, public health authorities, and natural resource managers.
- Provide guidance for efforts to improve environmental conditions.
- Meet requirements of CWA Section 303d to assess the quality of Washington's waters.
- Assess the effectiveness of pollutant management actions.

Project Description

Goal and Objectives

The goal of this project is to monitor mercury levels in edible tissue from freshwater fish over time in order to characterize temporal trends in mercury levels. Specific objectives of this project are to:

- Determine mercury concentrations in ten individual fish from six sites per year on an approximate five-year sampling frequency. Thirty different sites will be sampled over a single five-year period. Target fish species are bass (primary) and walleye (secondary).

- Collect ancillary data on the fish and sites to better understand patterns, dynamics, and changes in fish tissue mercury levels over space and time. Examples of ancillary data are: fish length, weight, sex, and age; physical and chemical characteristics of sites such as morphometry, water chemistry, and surficial sediment mercury levels; and fish community information, where available.

The primary goal of this project is to determine temporal trends in fish tissue mercury levels. The detection and quantification of such trends will require many years of monitoring. A critical factor for the success of this project will be sustaining funding over time.

A secondary goal of this project is to provide information about mercury levels in fish species other than bass and walleye. This information will help DOH craft more informative recommendations for fish consumption advisories. Data from other species may also provide additional information about mercury trends at each site. The objective for meeting this goal is to:

- Determine mercury concentrations in composite samples from two other fish species that are present at the sites where bass and/or walleye are collected. Species commonly targeted by consumers will be selected. For each species, three composite samples of 3-5 fish per composite sample will be collected. Again, fish from a total of 30 sites will be collected over each five-year period.

Site and Fish Species Selection

The spatial extent of this project encompasses the entire state of Washington. Sites for long-term trend monitoring will be selected based on various considerations (Table 1). Candidate sites for long-term monitoring will be evaluated to determine how well site characteristics contribute to meeting project objectives and a balance among the primary and secondary considerations will be sought.

Table 1. Considerations in Selecting Sites for Fish Tissue Mercury Trend Project.
Primary Considerations
Ability to collect target species at adequate size and numbers (e.g. boat access, min. fish length 10").
Stability of fish community (e.g. target species likely to be there for decades, long-term Washington Department of Fish and Wildlife (WDFW) management, waterbody size).
Historical issue with contamination (e.g. Roosevelt, Whatcom fish consumption advisories, 303d listing for Hg in tissue).
Proximity (or distance from) to local mercury point sources and urban areas (e.g. coal power plant, incinerators, other point sources).
Statewide distribution to represent varied site and regional characteristics (e.g. urban, rural, ag, forestry, reference, lake, reservoir, river).
Secondary Considerations
Ability to obtain info on fish community status, productivity, food chain length, and changes over time (e.g. WDFW surveys).
Availability of historical data (e.g. sampled during 2002 screening study).
Ability to obtain current/historical water quality data (e.g. DO profile, seasonal dynamics/stratification, reducing env. at sed/water interface).
Ability to track changes in watershed, lake mgmt, etc. (e.g. info/help from Lake Mgmt groups, etc.).
Potential complement to other work with mercury (e.g. sediment, loon, grebe, raptor, national, and regional monitoring effort).
Ability to leverage sampling and data resources from other entities (federal sampling, WDFW surveys, academia WQ info).

Figure 1 shows candidate sites of initial interest for long-term monitoring of mercury in bass. The reference numbers relate to the first 42 of 68 candidate sites given in Table 2. Sites will be selected each year after additional site characterization and review of considerations given in Table 1. The list of candidate sites will be refined as more information about mercury sources and other considerations is gathered. Sites 1-9 (bold type in Table 2) were targeted in 2005 concurrent with fish collection efforts for other projects such as the Washington State Toxics Monitoring Program.

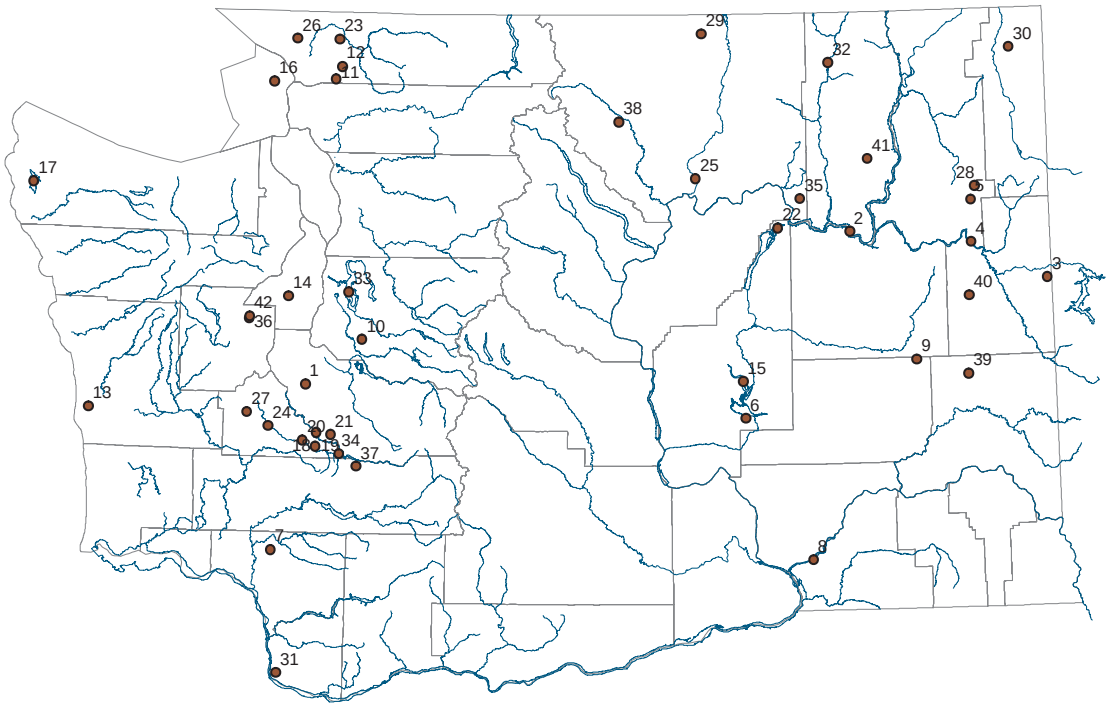

Figure 1. Candidate Sites of Initial Interest for the Fish Tissue Mercury Trend Project.

Candidate sites where composite samples from three other species are collected will be selected annually in conjunction with the planning of other monitoring efforts. Fish collected from other projects may be analyzed for mercury to meet the needs of this project, allowing for more efficient use of resources.

The target species for long-term trend monitoring are bass and walleye. These species are selected because of their known propensity to accumulate mercury. Largemouth bass (*Micropterus salmoides*) and smallmouth bass (*M. dolomieui*) are the primary target species at all sites. Bass are widespread across Washington and are frequently targeted by recreational anglers. Historical data are available for bass at many sites investigated for mercury in fish (Fischnaller et al., 2003; Serdar et al., 2001). Walleye (*Sander vitreus* – formerly *Stizostedion vitreum*) will be sampled at some sites due to their abundance, popularity, and management as a fishery by WDFW. Table 2 indicates sites where individual walleye may be collected in addition to individual bass.

Table 2. Candidate Sites for Fish Tissue Mercury Trend Project.

Ref #	Site	Location Description	County	Rank: Initial Interest	Bass	Walleye
1	**American L**	Steilacoom	Pierce	1	y	
2	**Columbia R, L Roosevelt**	Roosevelt L lower, nr Sand Hills	Ferry/Lincoln	1	y	y
3	**Liberty L**	E of Spokane	Spokane	1	y	y
4	**Long L**	Spokane R	Spokane	1	y	
5	**Loon L**	20 mi S Chewelah	Stevens	1	y	
6	**Potholes Res.**	10 mi SW of Moses Lake	Grant	1	y	y
7	**Silver L**	12 mi NE of Longview	Cowlitz	1	y	
8	**Snake R @ IceHarbor Dam**	10 mi E Tri-Cities	Franklin/Walla Walla	1	y	
9	**Sprague L**	nr Ritzville	Adams/Lincoln	1	y	y
10	Meridian L	10 mi E Federal Way	King	1	y	
11	Samish L	6 mi SE Bellingham	Whatcom	1	y	
12	Whatcom L	Bellingham	Whatcom	1	y	
13	Duck L	Ocean Shores	Grays Harbor	1	y	
14	Kitsap L	3 mi W of Bremerton	Kitsap	1	y	
15	Moses L	at Moses Lake	Grant	1	y	y
16	Cascade L	San Juan Is, Moran SP	San Juan	1	y	
17	Ozette L	NW coast	Clallam	1	y	
18	Clear L	25 mi ENE Centralia	Thurston	1	y	
19	Harts L	25 mi NE Centralia	Thurston	1	y	
20	Lawrence L	20 mi NE Centralia	Thurston	1	y	
21	Silver L	30 mi NE Centralia	Thurston	1	y	
22	Banks L	S of Grand Coulee dam	Grant	2	y	y
23	Fazon L	NW of Bellingham	Whatcom	2	y	
24	Offut L	Thurston Co.	Thurston	2	y	
25	Okanogan R	nr Omak	Okanogan	2	y	
26	Terrel L	5 mi W Ferndale	Whatcom	2	y	
27	Black L	Olympia	Thurston	2	y	
28	Deer L	nr Chewelah	Stevens	2	y	
29	Palmer L	10 mi W Oroville	Okanogan	2	y	
30	Sullivan L	5 mi SE Metaline Falls	Pend Oreille	2	y	
31	Vancouver L	Vancouver	Clark	2	y	
32	Curlew L	5 mi NE Republic	Ferry	2	y	
33	Washington L	Seattle	King	2	y	
34	Alder L	10 mi S Eatonville	Pierce	2	y	
35	Buffalo L	8 mi NE Grand Coulee dam	Okanogan	2	y	
36	Haven L	18 mi SW of Bremerton	Mason	2	y	
37	Mineral L	40 mi E Centralia	Lewis	2	y	
38	Patterson L	3 mi W Winthrop	Okanogan	2	y	
39	Rock L	20 mi S Cheney	Whitman	2	y	
40	Silver L	by town of Medical L	Spokane	2	y	y
41	Twin L, South	30 mi SW Kettle Falls	Ferry	2	y	
42	Wooten L	18 mi SW of Bremerton	Mason	2	y	
43	Lacamas L	2 mi N of Camas	Clark	3	y	
44	Leland L	5 mi N of Quilcene	Jefferson	3	y	
45	Long L	7 mi SE of Bremerton	Kitsap	3	y	
46	Sammamish L	5 mi E Bellevue	King	3	y	
47	Scooteney Res	12 mi SE Othello	Franklin	3	y	y
48	Wildcat L	5 mi NW orf Bremerton	Kitsap	3	y	
49	Yakima R @ Horn Rapids	12 mi W Richland	Benton	3	y	
50	Walla Walla R	Walla Walla	Walla Walla	4	y	
51	Diamond L	10 mi SW Newport	Pend Oreille	4	y	
52	Eloika L	30 mi N Spokane	Spokane	4	y	
53	Gillette L	Little Pend Oreille Lakes chain	Stevens	4	y	
54	Seep Lakes Wildlife Area	12+ lakes 5-10 mi N of Othello	Grant/Adams	4	y	
55	Tiger L	10 mi SW of Bremerton	Kitsap	4	y	
56	West Medical L	town of Medical L	Spokane	4	y	y
57	Bonaparte L	E of Tonasket	Okanogan	5	y	
58	Newman L	NE Spokane	Spokane	5	y	y
59	Columbia R, lower	CR blw. Longview*	Wahkiakum	5	y	
60	Crab Creek lower	nr confluence w Columbia R	Grant	5	y	
61	Ferry L	10 mi S Republic	Ferry	5	y	
62	Frenchman Hills L	10 mi W of Potholes Res	Grant	5	y	
63	Green L	Seattle	King	5	y	
64	Lone L	Whidbey Island	Island	5	y	

Target species where mercury will be determined in tissue from composite samples include many freshwater species in Washington that represent varied trophic levels (Table 3). These other species are included in the project so that DOH can better inform the public about risks and benefits of consuming species other than bass and walleye. The more popular fish species sought by anglers will be targeted for collection. Species in Table 3 are listed in general order of preference for collection.

Table 3. Target Fish Species for Fish Tissue Mercury Monitoring.

Common name	Scientific name	Habitat	Feeding	Water temp.	Family name
Largemouth bass	*Micropterus salmoides*	water col.	piscivore	warm	Centrarchidae
Smallmouth bass	*Micropterus dolomieu*	water col.	piscivore	cool	Centrarchidae
Walleye	*Sander vitreus**	water col.	piscivore	cool	Percidae
Rainbow trout	*Oncorhynchus mykiss*	hider	invert/piscivore	cold	Salmonidae
Brown trout	*Salmo trutta*	hider	invert/piscivore	cold	Salmonidae
Cutthroat trout	*Oncorhynchus clarki*	water col.	invert/piscivore	cold	Salmonidae
Kokanee salmon	*Oncorhynchus nerka*	water col.	invertivore	cold	Salmonidae
Lake trout	*Salvelinus namaycush*	benthic	piscivore	cold	Salmonidae
Brook trout	*Salvelinus fontinalis*	hider	invert/piscivore	cold	Salmonidae
Yellow perch	*Perca flavescens*	water col.	invert/piscivore	cool	Percidae
Black crappie	*Pomoxis nigromaculatus*	water col.	invert/piscivore	warm	Centrarchidae
White crappie	*Pomoxis annularis*	water col.	invert/piscivore	warm	Centrarchidae
Pumpkinseed	*Lepomis gibbosus*	water col.	invert/piscivore	cool	Centrarchidae
Bluegill	*Lepomis macrochirus*	water col.	invert/piscivore	warm	Centrarchidae
Channel catfish	*Ictalurus punctatus*	benthic	invert/piscivore	warm	Ictaluridae
Lake whitefish	*Coregonus clupeaformis*	water col.	invertivore	cold	Salmonidae
Burbot	*Lota lota*	benthic	piscivore	cold	Gadidae
Common carp	*Cyprinus carpio*	benthic	omnivore	warm	Cyprinidae
Rock bass	*Ambloplites rupestris*	water col.	invert/piscivore	warm	Centrarchidae
Warmouth	*Lepomis gulosis*	water col.	invert/piscivore	warm	Centrarchidae
Green sunfish	*Lepomis cyanellus*	water col.	invert/piscivore	warm	Centrarchidae
White sturgeon	*Acipenser transmontanus*	benthic	invert/piscivore	cold	Acipenseridae
Mountain whitefish	*Prosopium williamsoni*	benthic	invertivore	cold	Salmonidae
Northern pikeminnow	*Ptychocheilus oregonensis*	water col.	invert/piscivore	cool	Cyprinidae
Peamouth	*Mylocheilus caurinus*	water col.	invertivore	cool	Cyprinidae
Brown bullhead	*Ameiurus nebulosus*	hider	invert/piscivore	warm	Ictaluridae
Yellow bullhead	*Ameiurus natalis*	hider	invert/piscivore	warm	Ictaluridae

NOTE: Species are listed in general order of preference. Other considerations are availability of fish, size, historical data available, mix of families/trophic levels per site, angler use, cooperation with other studies.

* formerly *Stizostedion vitreum*

Other considerations during collection are adequate numbers of fish, size ranges, historical data available, mix of families/trophic levels per site, angler use, and cooperation with other studies. (Bass and walleye are included in Table 3 for completeness).

Target Analytes for Fish

Fish tissue will be analyzed for total mercury. Total mercury was the target analyte used in other fish tissue studies in Washington, largely due to the relative simplicity and lower cost as compared to methylmercury. Methylmercury, the bioaccumulative and toxic form of mercury in fish tissue, accounts for more than 95% of the mercury in fish tissue where it is associated with muscle proteins (Bloom, 1995; Driscoll et al., 1994).

Physical characteristics of fish are critical to help explain variability in tissue mercury levels and increase the sensitivity of trend analyses. The total length, weight, sex, and age will be determined for each fish analyzed for mercury. Fish condition indices and growth rates (Nielson, et al., 1983) may also be determined using size and age information.

While fish tissue mercury concentrations generally increase with size and age, there can be shifts in this relationship as the food source of fish changes throughout their life. Driscoll et al. (1994) reported shifts in the relationship between mercury and fish size in yellow perch from 16 Adirondack Lakes. A shift toward higher mercury concentrations in fish seems to occur as young fish shift to being more piscivorous. Growth dilution may occur in older, larger fish after a certain age as the rate of weight gain exceeds that of mercury uptake in the food. Thus older and faster growing fish may exhibit a decline in mercury concentrations.

Lipids will not be analyzed in individual fish used for the trend monitoring component based on the recommendation of Fischnaller et al. (2003) who found that lipids did not correlate well enough with mercury levels to be useful in accounting for variance in trends analyses. Review of other studies of mercury show that lipids were not analyzed even though studies were trying to discern spatial and temporal trends as well as sources of variability in fish tissue mercury.

Target Analytes for Waterbodies

While atmospheric deposition is a major source of mercury, site-specific processes convert mercury to methylmercury which then enters the food chain. While lake water and sediment chemistry are important to mercury accumulation in fish, fish community and food web effects are also important. Rose et al. (1999) suggests that properties of individual lakes appear to be more important in determining fish tissue mercury levels than do small-scale ecoregional differences. Evers (2005) summarized attributes of mercury sensitive waters from 21 research papers. These attributes are:

- Water chemistry: high acidity, low acidic neutralizing capacity, and high sulfate.
- Physical: abundant wetlands, small lake with large drainage area, and water level fluctuations greater than six feet.
- Biological: low zooplankton abundance, low nutrient levels, and numerous trophic levels in food chain.

This study will gather data on these various factors in order to better understand their roles and relationships to fish tissue mercury levels across 30 sites over many decades of monitoring.

Water Chemistry

Grab samples from the epilimnion and hypolimnion will be analyzed for dissolved organic carbon (DOC), alkalinity, and chlorophyll *a*. Vertical profiles of dissolved oxygen (DO), pH, temperature, and conductivity will be measured in-situ. Grab samples will help characterize factors that seem to influence mercury levels in fish such as acid neutralizing capacity and productivity. Temperature and DO measurements will help determine the level of lake stratification and hypolimnetic hypoxia.

Dissolved organic carbon appears to play a complicated role in the transport and bioavailability of mercury in lake systems (Driscoll, et al., 1994). Water column mercury levels appear to correlate positively with DOC (Watras, et al., 1995; Driscoll et al., 1994) and negatively with pH (Watras et al., 1995; Evers, 2005). These correlations were particularly strong in a study of 23 Wisconsin lakes: DOC accounted for about 90% of the variability in mercury and 64% of variability in methylmercury among lakes, and pH accounted for about 25% of the variability among lakes (Watras et al., 1995).

Low pH, high DOC, and low productivity have been commonly correlated with elevated mercury levels in fish (Watras et al., 1995; Evers, 2005). One 16-lake study in the Adirondack region of New York found a negative correlation between mercury concentrations in yellow perch and lake pH, yet the authors also report that relationships among fish mercury levels and DOC are inconsistent across many studies (Driscoll et al., 1994).

Anoxic waters and sediments are important sources of methylmercury likely due to the methylation process of sulfate-reducing bacteria (Morel et al., 1998). In a 24-lake study in the Adirondack region, lakes with an anoxic hypolimnion during summer stratification showed high concentrations of methylmercury in water. Methylmercury in these lakes was also a higher proportion of total mercury (20%) than in oxic lakes (10%). These findings seem to be consistent with other work suggesting greater methylmercury production under anaerobic conditions (Driscoll et al., 1994). Watras et al. (1995) also reported significantly higher MeHg in water samples taken in fall versus those taken in the spring. Spring methylmercury levels were 60-80% of those found in the fall, implying a 20-40% gain over the summer months. The relative contributions of hypolimnetic anoxia and water temperature to increased water column mercury are not clear. Watras et al. (1995) reports suggestions that smaller and warmer lakes may be more efficient at methylating mercury than are larger lakes which tend to be cooler.

Lake productivity (as quantified by Carlson's Trophic Status Index - TSI) was not a strong predictor of mercury in three fish species in a study of 24 lakes in Massachusetts (Rose et al., 1999). The authors also note that other studies suggest that while availability of mercury may be affected by trophic status, other factors are also important and likely confound relationships between mercury levels in fish and lake productivity.

While other studies found weak relationships between productivity and fish mercury levels, this study will estimate the productivity of the waters sampled using Carlson's TSI because Washington's lakes, rivers, and reservoirs are more diverse in their characteristics than those examined in other studies. These data may help understand factors affecting mercury occurrences in fish and help in trends analyses.

Water column constituents that have been analyzed in other studies will not be monitored for this project because their value is questionable at this time: their role in the bioaccumulation of mercury in fish has not been consistently demonstrated. Among these constituents are sulfate, aluminum, selenium, and various mercury species. The complex behavior of mercury species in the water column and their relationships to fish tissue mercury levels is not well understood. Also, the sampling and analyses for mercury require use of ultra-clean procedures to avoid sample contamination: the cost for determining mercury in water is also relatively high (~$125/sample for total mercury and ~$250/sample for methylmercury). Inclusion of these or other constituents in water column monitoring may be considered in the future as this project matures.

Sediment Chemistry

Surface sediment from the deeper parts of sites will be collected and analyzed for total mercury, total organic carbon, and grain size. Sediments can be an important source of methylmercury in waterbodies, particularly in anoxic environments where sulfate-reducing bacteria convert available mercury forms to methylmercury (Morel et al., 1998). While sediments may play a dominant role in the methylation of mercury, the importance of this and other processes varies among sites due to site-specific characteristics. Fischnaller et al. (2003) did not find a consistent pattern between sediment and fish tissue mercury levels across 20 sites sampled. Some sites with elevated levels of sediment mercury appeared to correspond to sites where fish tissue had elevated levels of mercury; yet, other sites did not correspond suggesting the importance of other factors in site characteristics or sampling strategy.

Physical Lake and Watershed Characteristics

This study will characterize the physical aspects of sites selected for long-term monitoring of mercury in fish tissue. These characteristics include: lake morphometry such as depth, area, and volume; watershed size, soils, land use, and wetland area; hydrologic characterization such as flow and retention times; and site classification as drainage, reservoir, seep, or riverine.

Watershed size, soil characteristics, land use, groundwater discharge, and wetlands are important factors affecting mercury loading to waterbodies (Jeremiason, 2001). Rose et al. (1999) found that mercury levels in bass correlated with the size of the watershed and lake surface area and the percentage of watershed occupied by wetlands.

Basin and wetland hydrology, particularly water level fluctuations, seem to play an important role in DOC transport (Driscoll et al., 1994; Watras et al., 1995). Seepage lakes in an Adirondack Lakes study had the lowest concentrations of total mercury in the water column (< 1 ng/L) while drainage lakes had mercury concentrations ranging from 1- 5 ng/L (Driscoll et al., 1994). A study of 18 lakes in Washington found that fish from reservoirs had lower mercury levels than fish from other lakes (Fischnaller et al., 2003).

Biological Characteristics

This study should consider the effects of fish community and food web structure in determining trends in fish mercury levels. Yet the ability to obtain such information needs further exploration. Entities that conduct fish community studies as a means to improve management of the fishery (e. g. WDFW, academia) may generate information about fish communities. Other techniques to characterize or quantify food web structure, such as use of stable carbon and nitrogen isotopes may need to be pursued. Apparent changes in fish tissue mercury levels could be the result of changes in food web structure over time rather than a change due to managing anthropogenic mercury emissions.

The structure of the food web determines how efficiently methylmercury is transferred from algae to the top predators. The presence of certain planktivores that increase the number of trophic levels in the aquatic ecosystem leads to higher concentrations in top predators. (Morel et al., 1998). Jeremiason (2001) also related that longer food webs and slower-growing fish tend to result in higher Hg levels in top predator fish. Longer food chains generally result in higher mercury levels at the top of the food chain.

Organization and Schedule

Organization

Name	Organization	Phone Number	Role
Keith Seiders	EAP-WES-TSU	360-407-6689	Project Lead
Darren Alkire	EAP-WES-TSU	360-407-6060	Project Assistance
Patti Sandvik	EAP-WES-TSU	360-407-7198	Project Assistance
Casey Deligeannis	EAP-WES-TSU	360-407-7395	Project Assistance
Dale Norton	EAP-WES-TSU	360-407-6765	Unit Supervisor
Mike Gallagher	SWFAP-HQ	360-407-6868	Client
Maria Peeler	HWTRP-HQ	360-407-6704	Client
Dean Momohara	Manchester Lab	360-871-8808	Unit Supervisor
Stuart Magoon	Manchester Lab	360-871-8801	Lab Director
William Kammin	EAP	360-407-6964	Ecology QA Officer

Schedule

The major tasks and timeframes related to this trend monitoring component will occur over a two year period on an annual basis as described below. The major tasks for this project are: site selection, sample collections, fish sample processing, laboratory analyses, obtaining ancillary information about sites such as water quality and site characteristics, data management and reports, data quality reviews, data summaries and analyses, and report development.

Two schedules are given here: an Operations Schedule which describes the timeframe for major tasks of the project, and a Project Tracker schedule which contains items used in Ecology's "Project Tracker" database.

Project Operations Schedule

Annual Site Selection and Sampling Plan	Yr 1	March-April*
Water Sampling	Yr 1	August-September**
Fish Collection	Yr 1	September-November*
Fish Tissue Processing	Yr 1	December
Laboratory Analyses	Yr 2	January
Laboratory Data to Project Officer	Yr 2	March
Data Entry in EIM	Yr 2	April
Draft Annual Report	Yr 2	June
Final Annual Report	Yr 2	September (year after sampling)

*Some sites may require fish collections during spring in order to address seasonal effects and/or compare data that was historically collected during the spring months (e.g Lake Whatcom). When this occurs, dates will shift for tissue preparation, lab analyses, etc.

**Water sampling will likely be independent of fish collections because the timing of water sampling will likely be driven by site-specific seasonal conditions whereas the timing of fish collection will likely be driven by when historical data were collected (usually October) and coordination with other state and federal fish collection efforts.

Project Tracker Schedule

Environmental Information System (EIM) Data Set	
EIM Data Engineer	Darren Alkire
EIM User Study ID	HgFish05 ("HgFish06" for 2006, etc.)
EIM Study Name	Mercury Trends in Freshwater Fish 2005 (year changes accordingly)
EIM Completion Due	Annual, April 2006
Final Report	
Report Author Lead	Keith Seiders
Schedule:	
Report Supervisor Draft Due	Annual, June 2006
Report Client/Peer Draft Due	Annual, July 2006
Report External Draft Due	Annual, August 2006
Report Final Due (original)	Annual, September 2006

Quality Objectives

Measurement Quality Objectives

Manchester Environmental Laboratory (MEL) is expected to meet the measurement quality objectives shown in Table 4.

Table 4. Measurement Quality Objectives for Fish Tissue Mercury Trend Project.

Parameter	Matrix	Reporting Limit	Accuracy	Check Standard (% recovery limit)	Duplicate Sample (RPD)	Matrix Spike (% recovery limit)	Lowest Concen-tration of Interest
Laboratory Analyses							
Mercury, total	tissue	0.017 mg/kg, wet	+/- 15% of SRM value	80-120%	<20%	75-125%	0.020 mg/kg, wet
Dissolved Organic Carbon	water	1 mg/L	N/A	80-120%	<20%	75-125%	1 mg/L
Alkalinity	water	5 mg/L	N/A	80-120%	<10%	N/A	5 mg/L
Chlorophyll a	water	0.05 ug/L	N/A	80-120%	<20%	N/A	0.05 ug/L
Mercury, total	sed	0.005 mg/kg, dry	N/A	90-110%	<15%	85-115%	0.005 mg/kg, dry
Total Organic Carbon	sed	0.1%	N/A	80-120%	<20%	75-125%	0.1%
Grain Size	sed	1%	N/A	N/A	<20%	N/A	N/A
Field Measurements							
Dissolved Oxygen	water	0.2 mg/L	+/- 0.2 mg/L	N/A	< 10%	N/A	0.2 mg/L
pH	water	1.0 SU	+/- 0.3 pH units	N/A	< 10%	N/A	4.0 SU
Conductivity	water	5 uS/cm	+/- 5 uS/cm	N/A	< 10%	N/A	20 uS/cm
Temperature	water	0.0 C	+/- 0.2 C	N/A	< 10%	N/A	0.0 C
Secchi Disc (20 cm dia)	water	1/4 foot	+/- 1/4 foot	N/A	< 10%	N/A	1/4 foot

N/A = not applicable

Sampling Process Design (Experimental Design)

Sampling Process

Fish tissue, water, and sediment samples for the trend monitoring component will be collected from six sites each year. Sites will be re-sampled every five years such that there will be 30 sites in the trend monitoring study over a five-year period. Sites will be selected each year after review of considerations previously described. A preliminary list of candidate sites is given in Table 2. This list will be refined as more information about mercury sources and other considerations is gathered. Figure 1 shows sites that are of primary interest at this time.

For the trend component, ten individual bass and/or walleye will be collected from each of six sites in the fall of each year. Bass is the primary target species at all sites while walleye may be a secondary target at some sites. Table 2 indicates sites where individual walleye will likely be collected in addition to bass for the trend component.

In order to characterize mercury in non-trend species, three composite samples (3-5 fish of the same species per composite) from up to two other species will be collected from the six selected sites each fall. A minimum of three composite samples for each species should meet the needs of DOH in evaluating contaminant levels for possible human health issues (McBride, 2006). Results from ten individual fish will also meet this need. Individual fish and composite samples will be analyzed for total mercury.

Water quality will be determined at the deepest part of the waterbody as determined from bathymetric maps. In-situ continuous measurements from the surface to the bottom will be made for pH, dissolved oxygen, conductivity, and temperature. Water samples from the epilimnion and hypolimnion will be analyzed for DOC, alkalinity, and chlorophyll *a*.

Additional information about site and watershed characteristics will be collected to help understand factors that affect levels of mercury in environments. Bioaccumulation of mercury is driven by many factors and the relative contributions of each are poorly defined; however, these mechanisms may be better understood as information is gained during this project.

The main assumptions of the study design are that funding and resources will be available for this long-term monitoring effort and that the same species and size range of fish will be available at the same sites over time.

Representativeness

Fish, water, and sediment samples are expected to be representative of conditions due to the timing and manner of their collection. Fish will be sampled in the fall of each year to coincide with other fish collection efforts by Ecology and other agencies. Fall sampling will also allow use of data from the 20-lake study by Fischnaller et al. (2003). Fish will be collected from suitable habitats within the waterbody since fish collection techniques include shoreline (electrofishing) and open water habitats (gillnetting).

Relationships among physical characteristics and mercury concentrations can be helpful to explain sampling variance and add strength to some statistical methods for trend detection. While a range of sizes is preferred to help establish such relationships, too wide a range may confound analyses. Keeping the size range as tight as possible can help reduce variance in estimates of mean concentrations. A balance between a broad and narrow range of fish sizes will be sought as fish are collected while also considering other factors.

The target size range for individual bass and walleye will be determined by considering historical data, fishing regulations, and angler-preferred size ranges. Where historical data have been collected on individual fish, the size range of the historical data set will be duplicated as best possible in new collections. For sites lacking historical data, the *75% Rule* recommended for composite samples by EPA (2000) will be used as a rough guide in selecting fish to retain for analyses of individuals (i.e. the length of the smallest fish should be at least 75% the length of the largest fish). In general, the target size range for bass will be about 10 to 18 inches and the target size range for walleye will be about 16 to 28 inches.

The minimum size for fish to be used in composite samples of non-trend fish species will be determined by reviewing state regulations and considering what size could reasonably be expected to be kept by anglers. Again, the *75% Rule* recommended by EPA will guide selection of fish to keep for composite samples.

Water and sediment samples for the trend monitoring component will be collected in the late summer of each year at the same sites where fish will be collected. Water and sediment samples will be collected during the latter period of thermal stratification (if present) and before fall turnover in order to represent potentially low-oxygen conditions. More precise timing of sample collection for each site will be determined after consulting others having local knowledge about the timing of thermal stratification and fall turnover because the time of these occurrences are unique to each site.

In order to characterize mercury levels in sediments exposed to anoxic or hypoxic water, surficial sediment (top two cm) at each site will be collected from three areas that lay within the extent of the hypolimnion. Sample sites will be determined by review of bathymetric charts and results from vertical profiling of temperature and dissolved oxygen.

Comparability

The selected sampling and analytical methods should ensure that data are comparable over the life of the project. Where possible, fish for the trend monitoring component will be collected from the same area and be of a similar size range as fish previously collected for this project. Water and sediment samples will also be collected from the same sites previously sampled for this project. Standard Operating Procedures (SOPs) will be developed for field sampling efforts and SOPs exist for laboratory analyses. Potential changes in field and/or lab methods will be reviewed by the project officer to ensure comparability of results over time.

Completeness

The completeness goal laboratory analytical data is 100%. Any loss of fish tissue data or inability to collect sufficient numbers will decrease the ability to detect trends at sites where data needs are not met. The completeness goal for field measurements is also 100%. Loss of in-situ water quality measurements may affect the ability to collect water and/or sediment samples during desired conditions and from desired locations.

Trend Assessment

Assessment of temporal trends will employ various statistical procedures and be guided by the nature of data collected. The strength of trend assessment may vary among approaches used, e.g. from simple qualitative reviews (e.g. plot of contaminant levels over time) to more rigorous evaluations using hypothesis testing and multivariate regression.

For trend monitoring, the sample size of ten fish per site was selected by balancing several factors: available funding, spatial coverage, and ability to detect trends. Earlier reviews of the variability found in fish tissue data and the ability to detect trends in fish tissue guided the determination of sample size.

Ehinger (2002) examined tissue mercury results from Lake Whatcom smallmouth bass to define relationships among covariates and describe which relationships could be used to reduce variance and so improve the ability to detect trends. The use of ten fish in the mercury screening survey by Fischnaller et al. (2003) appeared adequate in most cases for defining relationships among covariates at individual sites.

Yake (2002) built on results from Ehinger's examination and estimated the effectiveness of several approaches to trend detection involving different sample sizes and the use of composite samples and samples of individual fish (Appendix A). As expected, the ability to detect trends could be improved by increasing the number of fish samples and/or removing variance than can be attributed to fish size (or other factors). Samples consisting of individual fish were deemed to provide better information and thus improve trend detectability than were the use of composite samples.

Yake (2002) evaluated five options of sampling techniques to determine trends in fish tissue and determined that for mercury, the use of ten individual fish per sampling event provided the optimum balance of cost and ability to detect trends. The detectable difference between two sample events was estimated to be about 25-35% of the mean value of the ten fish sampled. This estimate was based on removing the variance due to length and using a standard t-test (alpha = 0.05) to compare the means of the samples (Appendix A).

Sampling Procedures

Fish

Methods for the collection, handling, and processing of fish tissue samples for analysis will be guided by methods described in EPA's *Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories* (EPA, 2000). Most fish will be collected using a combination of methods incuding electrofishing, netting, and angling. Fish may also be collected through cooperative efforts with other agencies, such as the Washington State Department of Fish and Wildlife (WDFW) fish population surveys.

Upon capture in the field, fish will be identified to species and target species retained; non-target species will be released. Fish that are retained will be inspected to ensure that they are acceptable for further processing (e.g. proper size, no obvious damage to tissues, skin intact).

For the trend monitoring component at each site, ten fish of target species will be collected and analyzed as individual samples for tissue mercury concentrations. At approximately one site each year, bass and walleye will be sampled (ten individuals per species). For mercury characterization in two other species per site, adequate numbers of fish will be collected to form three composite samples of 3-5 fish per composite for each species.

Fish to be kept will be euthanized by a blow to the head with a dull object, rinsed in ambient water to remove foreign material from their exterior, weighed to the nearest gram, and their total lengths measured to the nearest millimeter. Individual fish will then be double-wrapped in foil and placed in a plastic zip-lock bag along with a sample identification tag. The sample tag will include the date, the site, and the field ID assigned to the individual fish. The bagged specimens will be placed on ice in the field. Fish may remain on ice for a maximum of 24-72 hours and then they will be frozen to –20 C at Ecology facilities in Lacey, Washington.

Skin-on fillets will be used for individual fish and for most fish used in composite samples (catfish are one exception). Fish will be removed from the freezer, partially thawed, slime and scales removed, rinsed in tap water followed by a rinse in deionized water. Fish will then be filleted with the skin left on. Fillets will be cut into small cubes and passed three times through a Kitchen-Aid food grinder. The ground tissue will be homogenized by stirring to a consistent texture and color. Subsamples from the homogenate will be taken and placed into appropriate containers and transported to the laboratory for analyses. Excess homogenate will be placed into an appropriate container, labeled, and archived frozen at –20 C.

After fillets are removed, the sex of the fish will be determined and recorded. Species-appropriate structures (e.g. otoliths, scales, opercula) will be removed and sent to WDFW biologists who will determine the age of individual fish. Prior to filleting, a section of the caudal or other fin will be removed and preserved in ethanol and sent to WDFW for DNA archiving. This archive sample is taken for WDFW only because it is a requirement of the WDFW Scientific Collection Permit.

Non-lethal sampling of fish tissue may be pursued in the future. One method involves removing a small plug of muscle tissue (about one cubic centimeter) from individual fish for the sample. The wound is then closed and dressed, and the fish released. This method is being pioneered by other researchers and would require development for this project.

Water

Water quality sampling will occur in late summer only at sites where fish are collected for the trend monitoring component. Water will be sampled using two different techniques: in-situ measurement for field measurements and grab samples for laboratory measurements. In-situ measurements will be made using a continuously recording datalogger such as a Hydrolab for depth, temperature, conductivity, and dissolved oxygen. The instrument will be prepared and calibrated according to manufacturer's directions. Field measurements and sample collection will be guided by protocols developed by Ecology's Watershed Assessments Section (Cusimano, 1993).

Grab samples from the epilimnion and hypolimnion will be collected using a Kemmerer bottle or similar device after reviewing the vertical profile data to determine the degree of stratification. These grab samples will be analyzed for total and/or dissolved organic carbon, alkalinity, and chlorophyll *a*. The epilimnetic sample will be taken at a depth of one meter while the hypolimnetic sample will be taken about one meter above the lake bottom.

The sampling device will be lowered to the selected depth, then triggered to collect the sample, and recovered. Upon recovery, appropriate sample containers will be filled. Samples needing filtering will be filtered before transfer to the final sample container. Filtering may take place before leaving the site, such as at the boat launch area. Samples will then be kept in an iced cooler until transport to the laboratory.

Location of water sampling will be determined prior to field sampling after reviewing available water quality data and bathymetric information. Generally, in-situ measurements and sample collection will be done from the deepest part of the waterbody. While one location per site will be selected for grab samples and vertical profiles, additional locations on larger lakes may need to be sampled. Sites that are believed to be representative of the lake's water quality will be chosen.

Sediment

The locations where sediments are collected will be guided by review of a bathymetric chart for the site and the water quality data from the vertical profile of temperature and dissolved oxygen. The objective is to gather sediment from areas exposed to anoxic or hypoxic waters. Generally, the three sites will be in the deeper areas of the waterbody and represent the greatest spatial coverage while staying within the extent of the hypolimnion and waters with lower DO levels. Bathymetric maps will be reviewed prior to sampling and areas for potential sediment sampling identified. The extent of the hypolimnion will be determined from the temperature and dissolved oxygen profiles and also used as a guide in selecting sites to collect sediment samples.

Sediment samples will be collected with a 0.05 square meter stainless steel petite ponar grab sampler. The sampler will be lowered by hand or winch wire to collect a sample at each designated site. Upon retrieval, the overlying water will be siphoned off and, if the sample is deemed acceptable, the top 2 centimeters of sediment will be removed with a stainless steel spoon. Sediment will be spooned into an 8 oz sample container and then homogenized to uniform color and consistency. Subsamples will be removed from the homogenate and placed in containers for TOC and grain size analyses. Sediments in contact with the side of the grab-sampler will not be used. Sample containers will be kept in an iced cooler or refrigerator until transport to the laboratory.

Decontamination Procedures

All utensils used for processing tissue samples will be cleaned in order to prevent contamination of the sample. Utensils include bowls and knives of stainless steel and tissue grinding appliances having plastic, wood, bronze, and stainless steel parts. All utensils for fish tissue sampling will be cleaned with the following procedure: soap (Liquinox) and hot water wash, hot tap water rinse, 10% nitric acid rinse, and a final deionized water rinse. Utensils will be air-dried and then packaged in aluminum foil until used. Fish will be filleted and tissues processed on aluminum foil that covers a nylon cutting board laid on the workbench. The foil will be placed such that fish contact only the dull side of the foil.

Water sampling devices will be cleaned between sites with soap and tap water, rinsed with tap water, and finally rinsed with deionized water.

Sediment sampling devices and utensils will be cleaned in order to prevent contamination of the sample. All utensils coming in contact with the sample will be cleaned with the following procedure: soap (Liquinox) and hot water wash, hot tap water rinse, 10% nitric acid rinse, and a final deionized water rinse. Utensils will be air-dried and then packaged in aluminum foil until used.

Sample Handling and Field Records

The identification of water and tissue samples will be maintained from the time of collection to the time of reporting of results. For water samples, the sample container will be tagged and labeled with a unique laboratory identifier. Sample identification will be recorded in field notes.

Tissue and water samples will be stored, preserved, and transported following procedures designed to maintain the integrity, quality, and identification of the sample. Pre-cleaned sample containers will be obtained prior to field sampling efforts with containers for metals possessing Quality Assurance Certification from the supplier (e.g. I-Chem 200 series or equivalent). Sampling containers, sample preservation, and holding times for fish and water laboratory samples are described in Table 5.

Field notes will be kept for each sampling event. Notes will be entered in a field notebook and include: date and time, sampling personnel, general sampling location for fish collection areas and latitude/longitude coordinates of water and sediment sample collection sites, general weather conditions, method of sampling, fish species collected, weights and lengths for individual specimens, and results from field measurements. Latitude and longitude coordinates, and their datum, will be obtained with a hand-held Global Positioning System device and use of maps. A fish processing benchsheet form will be used to record various data during processing, such as: processing date, processing crew, lab sample ID names, lab sample numbers, fillet weights, DNA sample vial number, sex of individual fish, age structure container references, and any relevant comments.

Table 5. Containers, Preservation, and Holding Times for Laboratory Samples for Fish Tissue Mercury Trend Project.

Parameter	Matrix	Minimum Amount Required	Sample Container	Preservation	Holding Time
Mercury, total (mg/kg)	tissue	5 gm	2 oz. precleaned glass jar w/teflon lid, I-CHEM Series 200	freeze, -20 C	6 months [1]
Dissolved Organic Carbon (mg/L)	water	60 mL	60 mL pre-acidified PE bottle. Filter with Whatman Puradixc 25 PP; 25 mm dia; 0.45 um pore size	refrigerate, 4 C, H2SO4 to pH <2	28 days
Alkalinity (mg/L)	water	500 mL	500 mL amber PE bottle	refrigerate, 4 C	14 days
Chlorophyll a	water	500 mL	500 mL PE bottle	refrigerate, 4 C	24 hours before filtration; 28 days after filtration
Mercury, total (mg/kg)	sed	100 gm	8 oz. precleaned glass jar w/teflon lid, I-CHEM Series 200	refrigerate, 4 C	28 days (or 6 months if frozen)
Total Organic Carbon (mg/kg)	sed	25 gm	2 oz. precleaned glass jar w/teflon lid, I-CHEM Series 200	refrigerate, 4 C	14 days
Grain Size	sed	100 gm	8 oz polyethylene jar	refrigerate, 4 C	6 months

1 - See text for discussion of holding time.

Holding Time for Analysis of Mercury in Fish Tissue and Sediment

This project will use a six month holding time for tissue and sediment samples. The holding time is the period between the time of sample collection to the time of analysis. For fish tissue, this decision is based on review of varied opinions about the proper holding time for fish tissue samples and the practical need to store fish samples for extended periods in order to maximize efficiency of field and laboratory operations. Nationally, the USGS's National Water Quality

Assessment (NAWQA) program uses six months as a holding time for biota (Crawford and Luoma, 1993). Bloom (1995) also states that biota samples for mercury analysis may be stored indefinitely when frozen. Ecology's Manchester Environmental Laboratory (MEL) uses a maximum holding time of 28 days from the date tissue is removed from the fish and ground or macerated (Momohara, 2006). This 28-day timeframe appears to be based on the recommendations of the PSEP Protocols.

While Puget Sound Estuary Program Protocols (PSWQAT, 1997) recommend a 28-day holding time for mercury in tissue, the protocols note that EPA has no holding time criteria for metals in fish tissue. The PSEP protocols also note that a number of unpublished studies have demonstrated that freezing tissue samples may increase the holding time for mercury analysis up to 6 months. One unpublished study described in the PSEP protocols was by WDFW and King County. This study found no significant differences in fish muscle tissue mercury levels from samples analyzed at six different times ranging from 4 to 86 days after sample collection. Samples were frozen at -20 C. Based on these results, WDFW and King County suggested that a holding time of 3 to 6 months for frozen tissue would be acceptable (PSWQAT, 1997).

The PSEP Protocols recommend a holding time for sediment samples of 28 days unfrozen and note that a number of unpublished studies have demonstrated that freezing sediment samples may increase the holding time for mercury analysis up to 6 months.

Measurement Procedures

Laboratory and field measurement methods are described in Tables 6 and 7.

Table 6. Laboratory Methods for Fish Tissue Mercury Trend Project.

Parameter	Matrix	# Samples: Annual Timeframe	Expected Range of Results	Reporting Limit	Method Description	Method Reference
Mercury, total	tissue	n=106: Dec*	0.020-1.50 mg/kg wet	0.017 mg/kg, wet	CVAA	EPA 245.6; MEL SOP**
Dissolved Organic Carbon	water	n=13: Aug-Sept	1-5 mg/L	1 mg/L	Combustion NDIR	EPA 415.1
Alkalinity	water	n=13: Aug-Sept	5-300 mg/L	5 mg/L	titrimetric	EPA 310.2
Chlorophyll a	water	n=13: Aug-Sept	0.05-30 ug/L	0.05 ug/L	fluorometric	SM 20th Ed; 10200 H3
Mercury, total	sed	n=13: Aug-Sept	0.005-0.500 mg/kg wet	0.005 mg/kg, dry	CVAA	EPA 245.5; MEL SOP**
Total Organic Carbon	sed	n=19: Aug-Sept	0.1% - 35%	0.1%	Combustion NDIR	PSEP Protocol
Grain Size	sed	n=19: Aug-Sept	1% - 100%	1%	Sieve and Pipette	PSEP Protocol

**MEL modifications to analytical methods are documented in their Standard Operating Procedures.

Table 7. Field Measurement Methods for Fish Tissue Mercury Trend Project.

Parameter	Matrix	# Samples: Annual Timeframe	Expected Range of Results	Reporting Limit	Method Description	Method Reference
Dissolved Oxygen	water	n=6: Aug-Sept	0.0 - 14.0 mg/L	+/- 0.2 mg/L	electrometric	SM 16th Ed; 421F
pH	water	n=6: Aug-Sept	6.0 - 9.5 SU	+/- 0.2 SU	electrometric	EPA 150.1
Conductivity	water	n=6: Aug-Sept	40 - 400 uS/cm	+/- 5 uS/cm	electrometric	EPA 120.1
Temperature	water	n=6: Aug-Sept	4.0 - 25.0 C	+/- 0.2 C	thermistor	thermistor
Secchi Disc (20 cm)	water	n=6: Aug-Sept	1/4 - 50 feet	1/4 foot	visual observation	Ecology's Lake Monitoring Guidance

The estimated annual analytical costs for fish tissue and water samples for this project are shown in Table 8. Costs include the 50% discount for Manchester Lab's pricing scheme.

Table 8. Estimated Annual Laboratory Analytical Costs for the Fish Tissue Mercury Trend Project for Sampling Six Sites per Year.

Parameter	Matrix	Cost per Sample	No. Samples	QA Field dup	QA lab dup	QA MS	QA SRM	Total QA	Total Cost	Cost QA Only
Total Mercury (individual fish samples)	tissue (bass)	$ 40	60	0	3	3	2	8	**$ 2,720**	$ 320
Total Mercury (individual fish samples)	tissue (walleye)	$ 40	10	0	1	1	0	2	**$ 480**	$ 80
Total Mercury (3 composites per site)	tissue (other species 1)	$ 40	18	0	1	1	1	3	**$ 840**	$ 120
Total Mercury (3 composites per site)	tissue (other species 2)	$ 40	18	0	1	1	0	2	**$ 800**	$ 80
Total Organic Carbon	water	$ 30	12	1	1	0	0	2	**$ 420**	$ 60
Dissolved Organic Carbon	water	$ 32	12	1	1	0	0	2	**$ 448**	$ 64
Chlorophyll A	water	$ 48	12	1	1	0	0	2	**$ 672**	$ 96
Alkalinity	water	$ 16	12	1	2	0	0	3	**$ 240**	$ 48
Total Mercury	sed	$ 40	18	1	1	1	0	3	**$ 840**	$ 120
Total Organic Carbon	sed	$ 40	18	1	1	1	0	3	**$ 840**	$ 120
Grain Size (3 fractions)	sed	$ 90	18	1	0	0	0	1	**$ 1,710**	$ 90
								Total Cost:	**$ 10,010**	$ 1,198

Quality Control Procedures

Field

Field quality control procedures will include blank samples and field replicate samples for water only. Replicate samples for fish tissue will not be taken because ten individual fish will be collected from each site; this sample size will be adequate to estimate a mean tissue mercury level for that species and site.

About 10% of water samples will be blanks or field replicates submitted *blind* to the laboratory. For blanks, contaminant-free water will be obtained from MEL, transported to the sample site, transferred to sampling device, and then transferred from the sampling device to a sample container. Such blank samples will include a filter blank for DOC.

Replicate samples of water and sediment will be taken for about 10% of the sites sampled. A replicate water or sediment sample will consist of a separate sample collected in the same manner as the first sample on the same day of sampling. The laboratory will be asked to perform their duplicate analysis (split sample) on the first sample of the replicate pair. This will allow separation of sampling variability from analytical variability.

Laboratory

Laboratory quality control procedures will include various analyses such as calibration standards, lab control samples, matrix spikes, standard reference materials, and duplicate analyses to evaluate the quality of data that are generated. For water samples, check standards will be used to estimate analytical accuracy and bias. Bias may be estimated by finding the difference between the mean of the check standard results and the true value of the check standard. Method blanks may be analyzed to assess contamination from laboratory procedures.

Precision will be estimated using laboratory and field duplicate analyses for tissue and water by calculating the Relative Percent Difference (RPD) of the results. For tissue, water, and sediment samples, matrix spikes may be used to indicate the presence of bias due to the sample matrix. The project officer may indicate which samples should be used for matrix spikes.

For tissue analyses for mercury, Standard Reference Material 1946 (Lake Superior fish tissue) from the National Institute of Standards and Technology will be analyzed by the laboratory as a regular sample. This reference material has a mean total mercury concentration of 433 ug/Kg ww with an approximate 95% Confidence Interval of +/- 9 ug/Kg ww.

Data Management Procedures

Data management for this project will include written and electronic media generated from field and laboratory activities. Field notes and observations will be recorded by hand onto prepared field forms and/or notebooks. Pertinent data collected in field books will be transferred to electronic media using Microsoft Office products (Word, Excel, and Access) and ArcView GIS. After entry into electronic media, the electronic data will be reviewed and compared to handwritten data to check and correct data entry errors. After these reviews, pertinent field data will be entered into Ecology's electronic Environmental Information Management (EIM) system. Hardcopy and electronic data not entered into EIM will be retained in a file system maintained by the project officer.

Laboratory analyses of samples generate data recorded in handwritten and electronic formats. These data will be examined by designated laboratory personnel for quality control, completeness, accuracy, errors, and usefulness. Laboratory data generated by MEL will be entered into the Laboratory Information System (LIMS) by MEL staff. Project staff will then access LIMS data and load appropriate data into EIM.

Audits and Reports

Oversight of project components will occur through established practices within Ecology. The laboratories employed for sample analysis participate in audits that include review of laboratory facilities, capabilities, and analytical performance through various federal and state audit programs. Laboratories will report the analytical results and data quality through a case narrative, typically provided for each batch of samples analyzed by a specific procedure.

The content of annual reports will be limited during the first five years of the project because there will be little historical data available for trend analyses. After that, there will always be historical data that can be used to evaluate changes in fish tissue mercury levels. So, initial reports will briefly summarize data and provide limited interpretation. From the sixth year onward (2011), annual reports will be more comprehensive and include analyses and interpretation of trends. As the project matures, alternate frequencies of reporting and levels of complexity may be considered in order to meet new needs.

Each type of report will provide the following, at a minimum, in varying levels of detail:

- Describe the project and methods used.
- Describe the locations of sampling sites.
- Assess the quality of the data and its limitations.
- Summarize the data using tables and graphs.
- Present analyses of the data including temporal and spatial patterns with description of statistical procedures used.
- Discuss significant findings and recommend follow-up actions.

Data Verification and Validation

Data Verification

Hard copy and electronic forms of data will be reviewed and examined for errors, omissions, and legibility. Field data will be examined by the field leader prior to leaving the sampling site. Laboratory data are reviewed by qualified staff at MEL before they are entered into the LIMS and released to the project officer. Where errors or omissions in the data are found, the source of the data (e.g. field sampling personnel, laboratory technician) will be consulted to determine the correct value or form of the data in question. Corrections or qualifications will be made where possible.

Data verification will be determined by examining the quality control information for each set of data. The project officer will examine field data while qualified laboratory staff will examine laboratory data and document findings in a case narrative. Laboratory staff may be consulted in order to review QC data that are normally retained by MEL.

Data Validation

The project officer will be responsible for validating all data by examining the complete data record and determining whether the methods and procedures described in this Quality Assurance (QA) Project Plan were used. Results from the quality control procedures used in the laboratory and field will be used to determine how well the data comply with the Measurement Quality Objectives described in Table 6.

Data Quality (Usability) Assessment

The project officer will determine whether the data generated by the project can be used to meet project objectives by examining the data and quality control information associated with it. The procedures described in the above sections will guide the project officer in making this determination. Other staff may be consulted where their expertise can be of value (e.g. quality assurance staff, laboratory staff).

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Appendix A

An Estimate of the Effectiveness of Several Approaches to Trend Detection

(using mercury data from Lake Whatcom smallmouth bass fillets)

Final
March 8, 2002
Bill Yake

The trend component of Washington State Toxics Monitoring Program will evaluate trends in bioaccumulative toxics that accumulate in edible fish tissue. Design of this component requires decisions about sample size which, in turn, depend on the magnitude of trends to be detected. This document provides information analysis in support of this design issue.

Fillet tissue from ninety-five smallmouth bass collected from Lake Whatcom were analyzed for mercury (Serdar, *et al.*, 2001). Bill Ehinger (2002, unpublished) evaluated these data and determined that the variance of the $\log_{10}$-transformed data was 0.071. After removing the variance due to length and location (basin) this variance was reduced to 0.020.

The present analysis converts this information into a form that allows one to compare the change (delta=d) in sample means that is 'detectable' (alpha=0.05) using a standard t-test. Delta is estimated for sample sizes of 10 and 30 fish, before and after variance due to length (and location) is accounted for.

Mercury data from Lake Whatcom smallmouth bass fillets appear to be log-normally distributed, with the following estimates of central tendency:

Table 1 – Central Tendency of Mercury Data – Lake Whatcom

Arithmetic Mean	486.7 ug Hg/Kg
Median	409 ug Hg/Kg
Geometric Mean	406.0 ug Hg/Kg

The following analysis used the equations and tables in Steele and Torrie (1960). With the initial variance of 0.071, and a sample size of 10, a delta of 0.250 can be detected. The mean of the logs for this sample set was 2.609, so a sample with a mean of logs <2.459 or >2.859 would be 'detectably different' from 2.609. This translates into a geometric mean of <229 or >723; a decrease of 43.7% or an increase of 78.0% from original geometric mean.

A similar evaluation was made assuming a sample size of 30 fish.

Table 2 summarizes the information for the data before residuals are determined, and variance due to length and location removed:

Table 2 – Detectable Increases and Decreases Using Unmodified Data (Variance = 0.071)

Sample Size	'Detectable' Decrease	'Detectable' Increase
10 fish	43.7%	78.0%
30 fish	27.1%	37.6%

These estimates of delta are a little higher than would be expected to be found in fish from a single location (basin), but according the Ehinger (personal communication) the variance attributable to location was quite small compared to that attributable to length.

After residuals for fish length and location (basin) were calculated and removed, the variance decreased to 0.020. Conducting the same analysis using this reduced variance yields the following results:

Table 3 – Detectable Increases and Decreases Using Data with Variance Due to Length Removed (Variance = 0.020)

Sample Size	'Detectable' Decrease	'Detectable' Increase
10 fish	26.3%	36.0%
30 fish	15.4%	18.5%

For this example, removing variance attributable to length improved ability to detect decreases in mercury concentration by 40-42%, while improving ability to detect increases in mercury concentrations by 50-55%.

Increasing the sample size from 10 to 30 fish improved ability to detect decreases in mercury concentration by 38-40%, while improving ability to detect increases in mercury concentrations by 47-52%.

Taken together, increasing the sample size from 10 to 30 fish, and stripping out the variance due to length and location improved ability to detect decreases in mercury concentrations by about 65%, while improving the ability to detect increases in mercury concentrations by about 76%.

Comparison of Trend Detection Capabilities: Individual vs. Composite Samples

Although a direct comparison between the use of individual sample results (as above) and the results of composite samples to detect differences in populations raises some difficulties, what follows is an attempt to estimate the relative effectiveness of these different techniques.

One of the main difficulties with this comparison is that use of composite samples works best if results are normally distributed. The evaluation tools I have found require an assumption of normality, although – as we've seen above – the distribution of mercury results from Lake Whatcom smallmouth bass probably is closer to log-normal.

The following composite analysis evaluation assumes Lake Whatcom that smallmouth bass data are normally distributed.

Data subsets were generated from the largest and smallest fish from basins 1, 2, and 3, such that the smallest fish in each subset was no less than 75% the length of the longest fish in that subset.

This created 6 subsets – the largest basin 1, 2 and 3 fish; the smallest Basin 1, 2 and 3 fish. The subsets for Basins 2 and 3 included nearly all the fish, but for Basin 1, many for the middling length fish were left out. So a 7th subset, consisting of middling length fish from Basin 1, was created. Again, for this 7th subset, the smallest fish was no less than 75% the length of the longest.

The results of these analyses are shown in Table 4.

Table 4. Variance in Mercury Concentrations – Lake Whatcom Fish

Species	Location	Length Range (mm)	Number of Fish	Mean Hg Concentration (ug/Kg ww)	Standard Deviation	Coefficient of Variance
Smallmouth Bass	Basin 1	249-332	10	200.7	67.7	33.7%
Smallmouth Bass	Basin 1	289-386	19	411.9	172.4	41.9%
Smallmouth Bass	Basin 1	365-486	14	724.9	396.3	54.7%
Smallmouth Bass	Basin 2	249-325	15	245.5	103.3	42.1%
Smallmouth Bass	Basin 2	330-440	16	536.1	224.6	41.9%
Smallmouth Bass	Basin 3	255-330	15	374.7	152.7	40.7%
Smallmouth Bass	Basin 3	352-468	13	806	305.2	37.9%
Smallmouth Bass	All Basins	249-486 All Lengths	95	486.7	306.5	63.0%

The coefficient of variance (COV = standard deviation/mean) for results from all smallmouth bass from all basins is 63%. The COV for samples from individual basins that meet the "75% rule"[1] range from 34-55% with a mean of about 42%. (As in Bill Ehinger's analysis, it's apparent that restricting the size range of the fish reduces the variance.)

To estimate the difference in means that could be detected by using composite samples, I used Opticomp software (Rohlf, et al, 1996). Using a coefficient of variance of 42%, a sample consisting of 3 composites of 10 fish each should, on average, be able to detect a delta of about 41% (alpha =0.05, power = 0.80). Whether one is evaluating increases or decreases in mercury concentration, this delta remains the same because the distribution is assumed to be normal.

The table below summarizes some of the characteristics of the five approaches to trend detection discussed above. In addition to the characteristics included in the table, there are other considerations:

- Analysis of individual fish allows statistical procedures that conform with the actual distribution of the sample population.
- Analysis of individual fish retains information about the populations that is obscured by composite sampling.
- Analysis of individual fish will probably make data evaluation easier if the characteristics of sample populations (length, size, age) shift between sampling events.

[1] Smallest fish in composite sample is no less than 75% the length of the longest fish.

Based on all considerations: sampling difficulty, analytical cost, and ability to detect trends – the best option appears to be #3, although there may be situations where an option more like #4 is warranted.

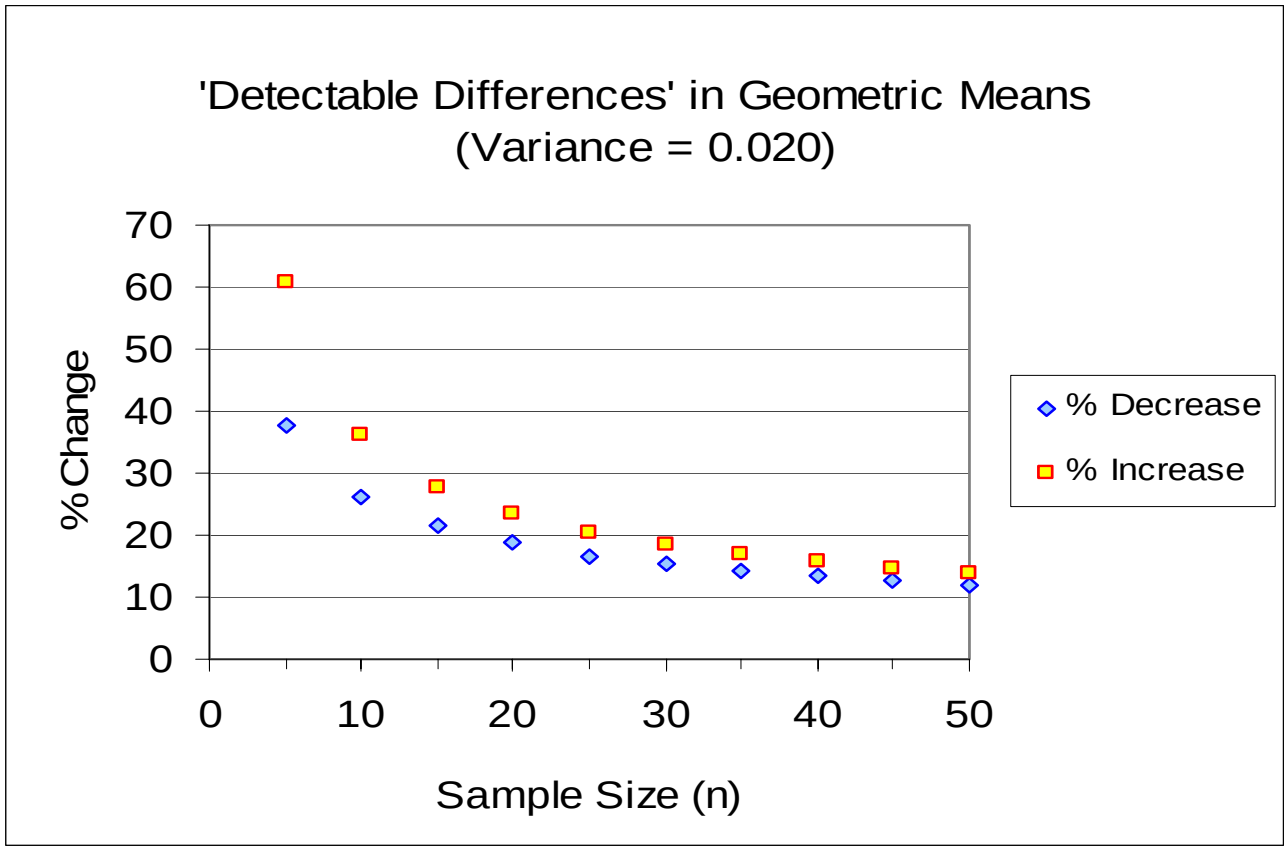
This conclusion is likely to be different for analytes such as chlorinated pesticides and dioxin - for which the analytical costs are much higher, and the relationships between size and contaminant concentration are substantially weaker.

Table 5. Comparison of Five Options for Trend Detection Using Mercury Data from Lake Whatcom Smallmouth Bass

Technique Number	Sample Type	Number of fish collected	Variance	Estimated Mean[2] Change Detectable (Delta)	Estimated fish-processing time	Analytical Cost per Sampling Event	Sampling Difficulty
1	Individual fish	10	Unadjusted	61%	1 person-day	$480	Moderate
2	Individual fish	30	Unadjusted	32%	3 person-days	$1440	Difficult
3	Individual fish	10	Variance due to length removed.	31%	1 person-day	$480	Moderate
4	Individual fish	30	Variance due to length removed.	17%	3 person-days	$1440	Difficult
5	Composite, 3 samples of 10 fish each.	30	Variance reduced using '75% Rule.'	41%	1.5 person-days	$144	Very Difficult

The figure below shows the differences in geometric means that are detectable (alpha = 0.05, variance = 0.020) when applying the t-test to samples ranging in size from 5 to 50. This figure may be helpful in estimated sample sizes in future studies once the target delta has been determined.

[2] For techniques 1-4, this is the average of the 'detectable increase' and the 'detectable decrease.'



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