Quality Assurance Project Plan

Investigation of Background Concentrations of Organic and Inorganic Arsenic in Freshwater Fish Tissue in Four Washington Lakes

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Waterbodies addressed in this study:

Black Lake, Waterbody No. 265WMV; Lake Gillette, Waterbody No. 325IJJ; Conner's Lake, Waterbody No. 896HNZ; Forde Lake, Waterbody No. none available; Mountain Lake, Waterbody No. 115RIM; Cascade Lake, Waterbody No. 642DEC; Ozette Lake, Waterbody No. 258HMF; Lake Pleasant, Waterbody No. 871WBX

Ecology EIM number: RJAC001

Approvals

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Abstract

This study plan describes the rationale and methods for characterizing inorganic and organic arsenic in freshwaters and associated fish tissues from four lakes throughout Washington State. The appropriate definition of natural conditions will assist in describing the magnitude of ambient arsenic in waters and fish. The form arsenic takes in the environment is important because different arsenic species have markedly varying toxicities.

Eight lakes have been identified for planning purposes; however, only four will be sampled. The availability of fish, by electroshocking and/or gill nets, will determine the final sampling locations.

Samples will be collected for total and dissolved arsenic during two different seasons, for a total of four water samples from each lake. Water will be collected using a depth integrating sampler. Phytoplankton, a potential seasonal reservoir of arsenic, will be sampled twice from each lake, once in the summer and again in the late fall. The phytoplankton data will determine if seasonal cycling is potentially occurring. If so, this media may require further evaluation and/or future water sampling may need to account for seasonal phytoplankton uptake or release. Two sediment grabs will be collected immediately after the water sampling.

Two different fish species will be sampled. One will be a salmonid and the other a species of opportunity based upon availability. In general, larger specimens will be emphasized. No anadromous or species stocked within the past five years will be sampled. Equal weight aliquots of descaled, skin-on fillet tissue will be mixed into equal sized composites and analyzed for organic and inorganic arsenic species. More complex arsenosugars and arsenobetaine will not be analyzed, but tissues will be archived for this possible future analysis.

Background/Problem Statement

Arsenic (As) is a common element in the earth's crust, where it is frequently found in association with iron and in minerals containing copper or lead. Arsenic is leached from rocks and minerals by surface water and storm runoff and released as fine dust during smelting. Dust emissions may travel many miles prior to deposition. Moore and Ramamoorthy (1984) estimate that weathering contributes 30% of global arsenic emissions while 70% are derived from anthropogenic releases. These releases have led to measurable increases in arsenic concentrations in some surface waters.

Arsenic has been acknowledged as a human poison for centuries. Ingestion of As has been linked with skin, liver, bladder, and prostate cancer. Many health agencies have recognized arsenic as a human carcinogen (ATSDR, 2000). People are exposed to arsenic from a variety of sources including air, soil, and water. Another source, which may be significant, is fish consumption. Fish and shellfish, particularly marine species, are known to contain high concentrations of arsenic. Historically much of these data have been for total arsenic only.

Understanding the form of this arsenic is important due to the varying toxicity of arsenic when ingested via different compounds (Donohue and Abernathy, 2002).

The inorganic forms of arsenic are most poisonous to humans, while the organic forms vary in their toxicity. Mono-and-dimethyl-arsenic have low toxicity (ATSDR, 2000) while the more complex arsenobetaine, arsenocholine, and other arsenosugars are considered inert when ingested by mammals (Neff, 1997). Organic arsenicals are hydrophilic by nature and have little tendency to bioaccumulate in human tissue.

Quantitative data on As concentrations and speciation in fish tissue are sparse. The currently available literature was reviewed by Donohue and Abernathy (2002). They found very few samples of freshwater fish tissue were analyzed specifically for inorganic arsenic. Most prior sampling by the Department of Ecology, Environmental Assessment Program (EAP), has also not distinguished between arsenic species in freshwater species. Data on ambient concentrations and ratios of inorganic to organic arsenic in freshwater fish tissue are critical in describing the magnitude of naturally occurring arsenic concentrations in water and estimating the potential health concerns associated with fish ingestion from those waters.

Project Description

This study proposes to evaluate the levels and forms of arsenic in water, phytoplankton, sediments, and freshwater fish tissue. This evaluation will be used to begin developing a database of typical, arsenic concentrations in these media. The study will establish ambient concentrations in these media from four lakes located upstream of current anthropogenic As inputs. The study media will also be used to evaluate the relative magnitude of possible seasonal arsenic cycling.

The study will analyze all media for organic arsenic and inorganic arsenic by USEPA method 1632. This method quantifies the faction of inorganic arsenic via hydride generation quartz furnace atomic absorption spectrometry. Laboratories typically determine total As via ICP(MS) and then calculate organic arsenic by difference as part of this method.

A variety of lakes in differing geologic and hydrologic settings have been selected for study. Each of these lakes is upstream from all known anthropogenic arsenic sources, such as orchards, except for global fugitive dusts. Some of these lakes are below historic mining activities in the upper watersheds. For the selected lakes, however, historic mining activities are not known to have significant arsenic discharges (Roeder, R. personal communication). The elimination of every possible source of anthropogenic arsenic is not possible given atmospheric sources and the intensity of prospecting and historic mining in Washington State. The database of abandoned mine sites under development by the Washington Department of Natural Resources (DNR) was reviewed to document potential historic anthropogenic arsenic sources (Norman, 2000). Also reviewed were U.S. Geological Survey Land Use Coverages, previous Ecology sampling activities for natural background in soils (San Juan, 1994), and sampling at abandoned mine sites (Raforth R.L. et al., 2000).

Arsenic concentrations in lentic systems are known to vary seasonally in response to lake stratification, oxidation-reduction potential (Eh), changes in phytoplankton composition, and/or alterations in the As:phosphate ($H_2PO_4^+$) ratio (Anderson and Bruland, 1991). Phytoplankton are the most significant mechanism for As methylation and/or biotranformation (Maeda, 1994; Baker et al., 1983). This study will analyze surface waters during two seasons (mid-summer and late fall).

The first surface water sampling event should be during any stratification, while the second sampling event will be after fall turn-over. Concurrent with the phytoplankton arsenic analysis, waters will be sampled for total phosphorus and chlorophyll (a). The arsenic:phosphate ration is an important determinant in estimating the relative amount of arsenic uptake in algal communities. However, there is more than one uptake mechanism for phosphate in plants (including algae) and thus there is no linear or universal relationship between arsenic and phosphate, despite their chemical similarity (Andreae and Klumpp, 1979; Macnair and Cumbes, 1987; Meharg and Macnair, 1990). Chlorophyll (a) concentrations will also be measured in waters to estimate the relative primary productivity of the algal community relative to the typically abundant diatom community. These analyses are secondary to the study objectives, but they may partially explain any observed variability in phytoplankton As concentrations.

The first sampling event will be during the period of greatest growth in fish and high phytoplankton biomass. The second will be after fall turnover and during a period of low phytoplankton biomass and reduced fish growth. The differences in phytoplankton concentrations and mass may be used to develop an estimate of the magnitude of seasonal cycling. Should seasonal cycling be pronounced, subsequent investigations may be required to determine other As reservoirs, including possible seasonal sediment sampling.

The sampling of surficial sediments for As will provide a snapshot of the size of the arsenic pool. Arsenic is known to cycle seasonally between sediments and the water column, but the magnitude of this cycling is unknown for Washington waters. These data may be used to evaluate the magnitude of this reservoir.

Fish are the relevant exposure media for this study's purposes and they are assumed to integrate seasonal variations in As concentrations within their tissues. Fish fillets will be analyzed as these are the most relevant tissue for human exposure.

None of the selected lakes have been stocked by the Washington Department of Fish and Wildlife within the past three years (WDFW Hatchery Stocking Plans). Backup locations have also been selected, in case fish collection is hampered at primary sites. Some of the backup locations have been stocked in the past. If collection efforts are required using these sites, stocked species will not be collected.

Responsibilities

Richard Jack, Ecology Project Manager. Responsible for field sampling, sample preparation, and preparation of draft and final Ecology report describing results of chemical and biological analyses.

Morgan Roose, EAP Field and Sampling Assistance. Responsible for field sampling, sample preparation.

Cheryl Niemi, WQ Client and Project Coordinator. Responsible for review of QA (Quality Assurance) Project Plan and final reports for the project.

Jim White, WA Department of Health. Liaison for sharing data with Department of Health.

Schedule

Finalize QA Project Plan	July 2002
Collect Surface Water, Phytoplankton and Fish Tissue	Mid August 2002
Laboratory Analysis	Late August and September 2002
Collect Surface Water and Phytoplankton	November 2002
Laboratory Analysis	December 2002
Draft Report Development	February 2003
Final Report	April 2003

Data Quality Objectives and Decision Criteria

This study will be used to define prominent forms of arsenic naturally present in surface waters, phytoplankton, sediment, and freshwater fish tissue in four lakes. One sample of certified reference material (CRM) (Dogfish muscle) will be analyzed for arsenic to estimate possible analytical bias. Unfortunately, available CRMs are restricted to total arsenic only. Table 1 summarizes the analytical accuracy, bias, and precision goals for the project, while table 2 shows the necessary reporting limits.

Table 1. Analytical Goals by Media for the Background Freshwater Fish Tissue Arsenic Investigation.

Parameter	Accuracy (% Deviation from True Value)	Bias	Precision (RSD)
Arsenic, Species (surface waters)	70%	±20%	25%
Arsenic, Totals and Species (fish tissues and phytoplankton)	70%	±20%	25%
Total Phosphorus	30%	±10%	10%
Chlorophyll (a)	60%	±20%	20%
% lipids	60%	±20%	20%
Total As in Sediment	40%	20%	10%
Total Organic Carbon	N/A[a]	N/A[a]	20%
Grain Size	40%	20%	10%

RSD = Relative Standard Deviation
N/A[a] = Evaluated Qualitatively

Table 2. Necessary Reporting Limits for the Arsenic in Freshwater Fish Tissue Investigation.

Matrix	Analyte	Required Reporting Limit (Maximum)
Surface Water	Total Arsenic	0.01 µg/L
	Arsenic Species	0.05 µg/L for DMA, 0.01 µg/L for all others
	Total Phosphorus	0.01 mg/L
	Chlorophyll (a)	0.05 µg/L
Phytoplankton Tissue	Total Arsenic	0.1 µg/g
	Arsenic Species	0.1 µg/g each
Fish Tissue	Total Arsenic	1.0 µg/g
	Arsenic Species	0.05 µg/g for MMA, 0.1 µg/g for all others
Sediment	Total Arsenic	0.5 mg/Kg
	Grain Size	±0.5% for each fraction
	TOC	0.5%

Study Design

Four sampling sites have been selected based upon soils, geology, surrounding land use, the lack of or minimal mining and agricultural activity upstream, fish species presence, and access. A location map is provided as Figure 1. Figures 2 and 3 illustrate the sampling sites, their location in the watershed, current Department of Natural Resources (DNR) permitted mines or gravel pits, and mining activity locations from the three principle sources in the DNR database of historic mines. Each site is comprised of two lakes: one primary sampling location and one secondary location. Secondary locations will not be sampled if primary locations have sufficient fish. The primary and their secondary locations are listed in Table 3.

Table 3. Primary and Secondary Sampling Locations with Elevations for the Arsenic in Freshwater Fish Tissue Investigation.

County	Primary Location (elevation)	Secondary Location (elevation)
Okanogan	Forde Lake (1560 ft)	Conner's Lake (1557 ft)
Stevens	Black Lake (3701 ft)	Lake Gillette (3160 ft)
San Juan	Mountain Lake (914 ft)	Cascade Lake (346 ft)
Clallam	Ozette Lake (29 ft)	Lake Pleasant (390 ft)

Representativeness

The objective for this study is to describe, to the extent practical, ambient As concentrations in surface waters, phytoplankton, sediment, and fish tissue in four Washington lakes. The lakes span a range of elevations, and one each are located in Okanogan, San Juan, Stevens, and Clallam counties. There are no locations in the

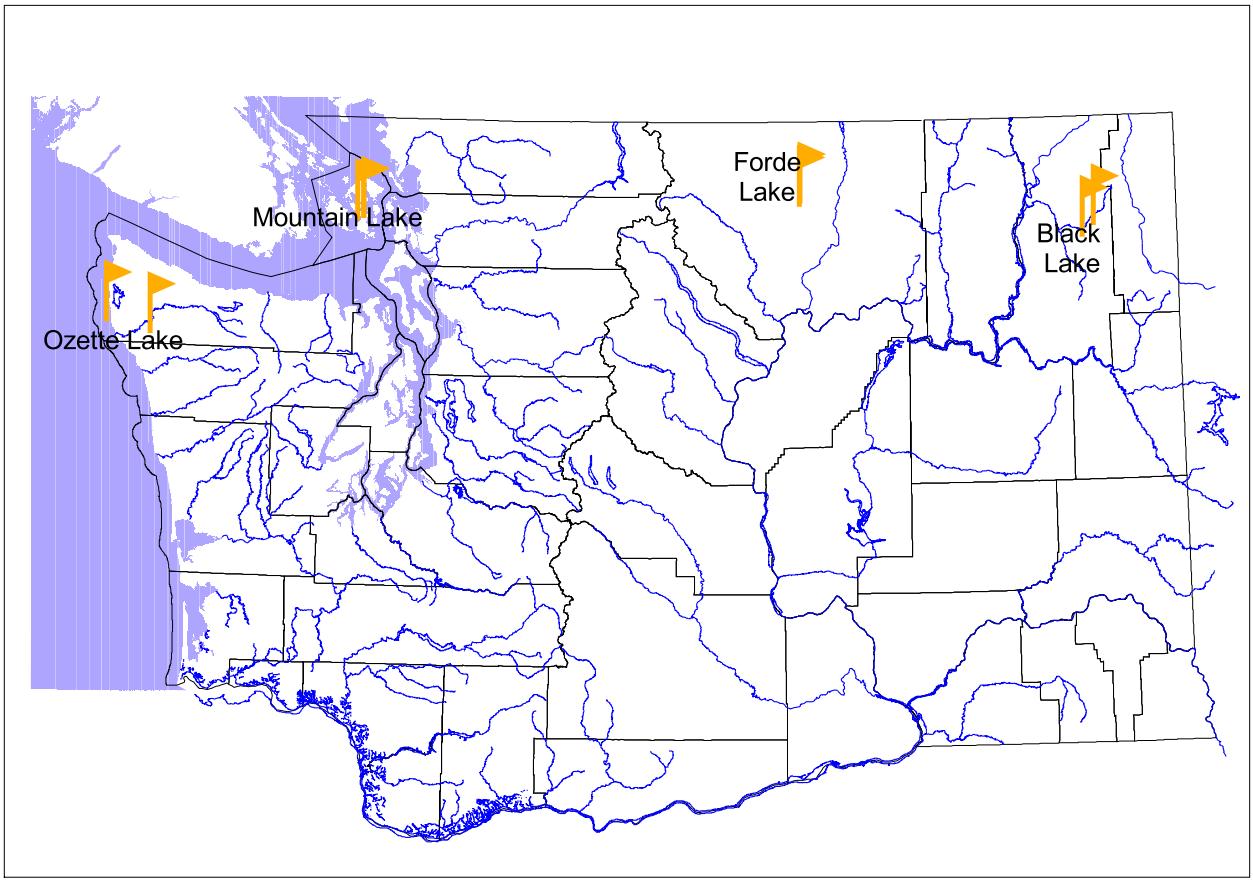
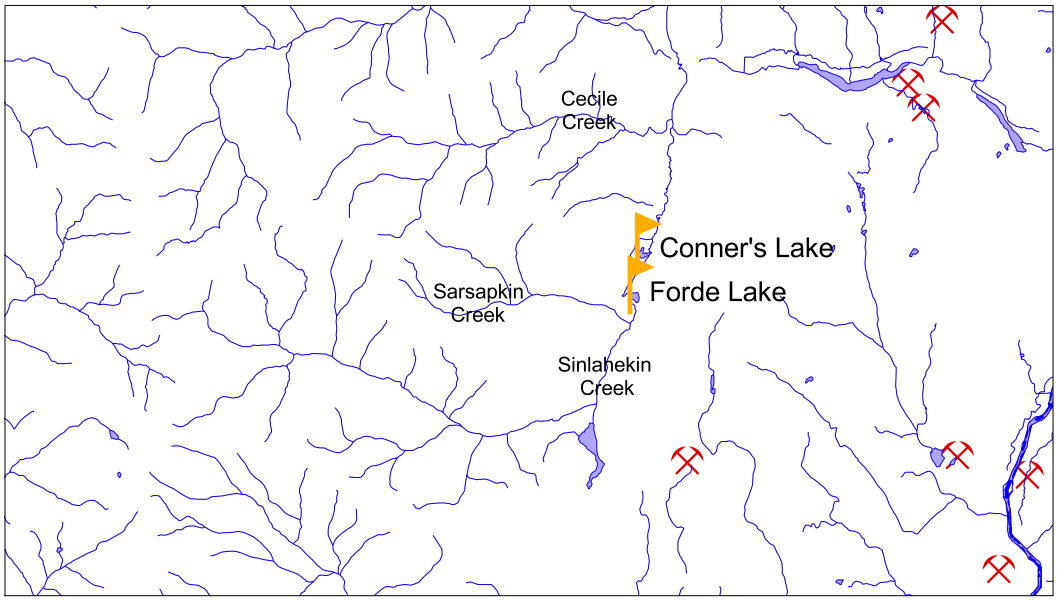
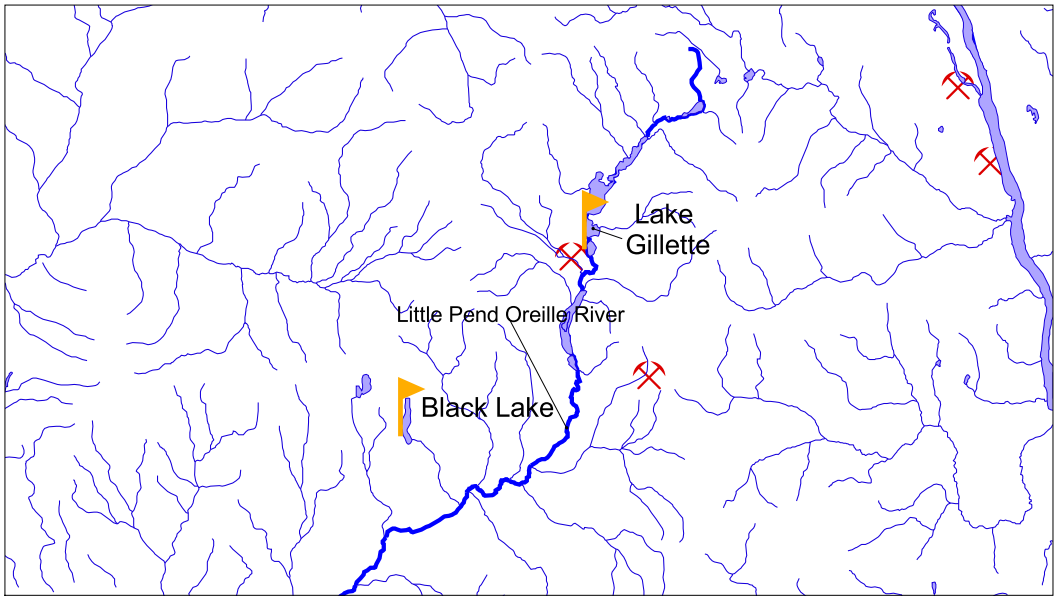


Figure 1. Freshwater Fish Arsenic Primary Sampling Location Map.



Forde and Conner's Lakes, Okanogan County

3 0 3 6 Miles



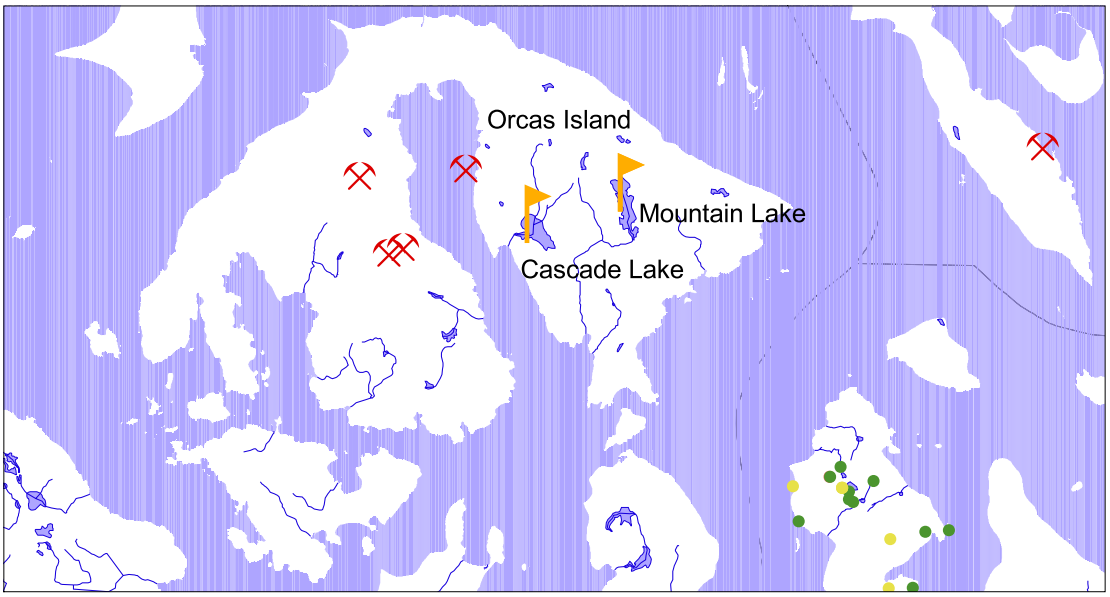
Black Lake and Lake Gillette, Stevens County

2 0 2 4 Miles

Mines from Huntting (1956)
Mines from McFaul et al (2000)
Mines from Derkey et al. (1990)
Current DNR Surface Mines
Fish Arsenic Sampling Location
Rivers and streams

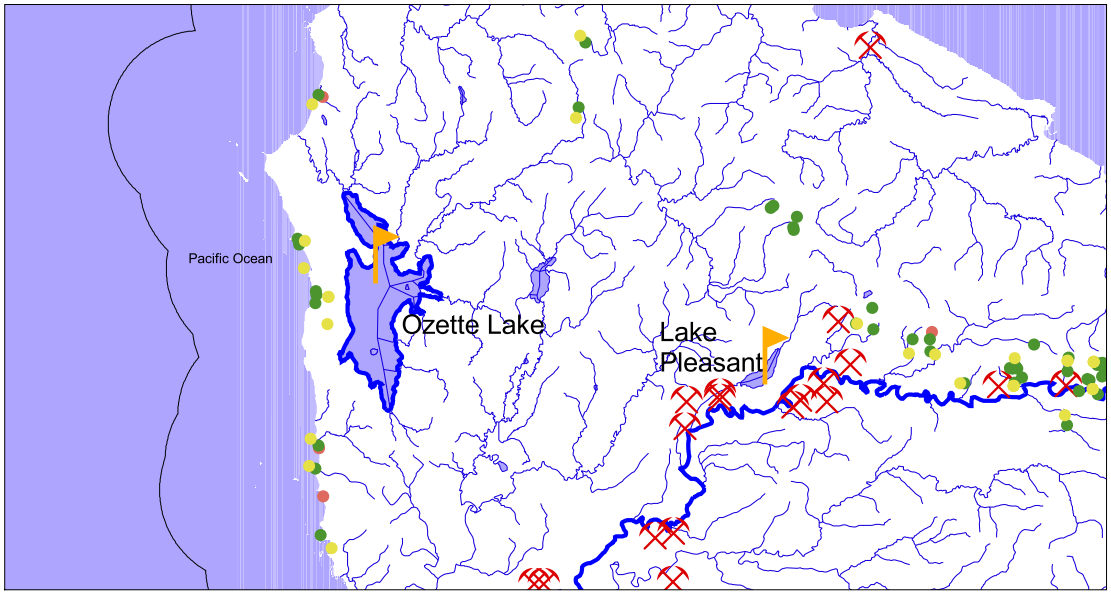
N

Figure 2. Eastside Arsenic Study Lakes
Okanogan and Stevens Counties



Mountain Lake and Cascade Lake,
San Juan County

2 0 2 4 Miles



Ozette Lake and Lake Pleasant,
Clallam County

4 0 4 8 Miles

Mines from Huntting (1956)
Mines from McFaul et al (2000)
Mines from Derkey et al. (1990)
Current DNR Surface Mines
Fish Arsenic Sampling Location
Rivers and Streams
N

Figure 3. Westside Arsenic Study Lakes,
San Juan and Clallam Counties

southern half of Washington State because those regions are often downstream of agricultural and/or mining activities, or they are within known smelter plumes.

Surface water samples will be collected in two seasons: late summer and fall. The first water sampling event represents the period during which fish feed and grow the most. The second event represents non-growing season conditions. Phytoplankton will be sampled during both events. The first event should represent the period of greatest plankton biomass. The chlorophyll (a) and total phosphorus concentrations will be measured concurrently with the surface water and phytoplankton As concentrations. Sediment samples will be collected immediately following the collection of surface waters.

Two species of fish will be collected concurrent with the first plankton and water sampling event. The species will be chosen based on availability. If previous stocking has occurred, that fish species will be avoided if possible. Desirable species are included in Table 4.

Table 4. Preferred Fish Species for Freshwater Fish Arsenic Investigation.

Common Name	Scientific Name	Family
Rainbow Trout	*Salmo gairdneri*	Salmonidae
Brown Trout	*Salmo trutta*	Salmonidae
Mountain Whitefish	*Prosopium williamsoni*	Salmonidae
Kokanee	*Oncorhynchus nerka*	Salmonidae
Largemouth Bass	*Micropterus salmoides*	Centrarchidae
Smallmouth Bass	*Micropterus dolomieui*	Centrarchidae
Black Crappie	*Pomoxis nigromaculatus*	Centrarchidae
Walleye	*Stizostedion vitreum vitreum*	Percidae
Yellow Perch	*Perca flavescens*	Percidae
Largescale Suckers	*Catostomus macrocheilus*	Catostomidae
Brown Bullhead	*Ictalurus nebulosus*	Ictaluridae
Burbot	*Lota lota*	Gadidae

The species listed in Table 4 represent fish commonly caught for human consumption. Two species will be collected from each lake. If possible, one species will be from the salmonid family (trouts, salmons, and whitefishes). The other species will be from a different family. The preferred species are higher in Table 4 than less desirable species. Final species selection will be decided in the field.

Fish fillets will be sampled, representing the commonly consumed portion. Fillets will be homogenized individually, and then equal aliquots of tissue will be blended to form the composite sample. Composites will be used to represent the average arsenic concentration in each species' fillet. There are three composite samples of each species from each lake. These three samples represent field replicates which will be used to estimate the variance of the mean As concentration.

Comparability

Fish will be collected during the time of year which is typical for EAP tissue sampling. The fish will be collected using a combination of electrofishing and gill nets. Both of these methods have been utilized by EAP before and they should not bias this study in any unique way (USEPA, 1997). Lake water will be collected from the deepest part of each lake using a depth integrating sampler. The DH-76 sampler has also been used in previous EAP investigations. These results should be comparable to previous EAP investigations of metals concentrations in waters and tissues.

Ecology has not sampled freshwater phytoplankton for arsenic in the past. However, suitable standard sampling equipment is available and has been used in marine investigations. The use of standardized marine phytoplankton sampling techniques and equipment will allow any subsequent investigations to replicate the chosen methodology.

The sediment sampling will use a petite ($0.02m^2$) Ponar sampler. The top 2 cm of sediment will be collected and composited from 2 grabs. The top 2 cm represents the more labile fraction of the sediment arsenic pool. Inter-year variability or trends in As concentration or speciation in phytoplankton, chlorophyll (a), total phosphorus concentrations, and sediment or fish tissue will not be investigated in this study.

Field Procedures

Water samples will be integrated across the entire water column using a DH-76 sampler lowered into the deepest part of the lake on a rope. The retrieved water will then be transferred into pre-cleaned polyethylene bottles. The sampler bottle will be cleaned with Liquinox® detergent, followed by rinses with tap water, 10% nitric acid and deionized water prior to the beginning of field work. Before actual sampling, the sampler will be rinsed three times with surficial lake water.

One water sample will be field filtered to remove microorganisms which might alter arsenic speciation. Each sample will use a dedicated 0.45µm filter-funnel with vacuum pump. After filtration, 3mL of 6M HCl/L will be added for preservation. For each water sampling event, water will be collected once from the deepest part of each lake. Total As samples will only have the 6M HCl added. In addition to the arsenic species, the filtered water will also be analyzed for total phosphorus. Arsenic and phosphates are absorbed across plant cell membranes via similar or the same active transport pathways (Andreae and Klumpp, 1979). By analyzing the phosphate concentration, estimates of variation in uptake between lakes may be derived and sources of variation in bioaccumulation factors may be segregated. Total phosphorus and chlorophyll (a) will be analyzed in surface water grab samples into appropriate containers.

Fish will be collected using a combination of electrofishing and gill netting. Two gill nets will be set in the evening prior to electrofishing. Gill nets will be set perpendicular to shore with the smaller mesh inshore. The collection crew will then sample shallow areas using a Smith-Root 16' aluminum electroshocking boat. The boat will use standardized power outputs as developed by the Washington Department of Fish and Wildlife. These outputs should minimize spinal

injuries and hemorrhaging in salmonids. The guidelines specify pulsed DC current with wattages ranging from 50 to 3000 depending on lake water conductivity (Bonar et al., 2000).

Every two hours, the electrofishing crew will pause to retrieve and inspect the gill nets. Target fish will be removed and retained. Non-target fish will be released if they appear healthy. If their vigor is poor, non-target fish will be retained in the electrofishing livewell until they revive. The gill net will be reset after inspection and electrofishing will continue.

At the completion of the sampling night, the largest target fish will be retained for arsenic analysis. A minimum of 15 fish and a maximum of 30 fish will be used from each target species. Whenever possible, the total length of the smallest fish will be no less than 75% of the largest.

Fish will be killed through a blow to the skull and packaged in aluminum foil and polyethylene bags. Fish will be maintained on ice for transport to Ecology Headquarters. Upon delivery, fish will be frozen at -18^0 C until processing.

Phytoplankton will be collected using a plankton tow with 20 micron mesh and a 25 cm diameter hoop. A flow meter will be attached upstream from the hoop to quantify the amount of water filtered. Additionally, water samples will be analyzed for chlorophyll(a) to estimate the primary productivity of the waters. Preliminary estimates of phytoplankton mass suggest that only about 10 liters of water need to be filtered to collect the minimum amount of phytoplankton mass required for analysis. However, freshwater experience has shown that the bulk of this mass may be comprised of diatoms, which are likely to have a different affinity of arsenic than the algal community. The chlorophyll analysis will allow for estimating the sources of variability in phytoplankton arsenic concentrations between lakes. Two tows will be conducted from each lake. A handheld GPS will be used to locate the starting and ending points of each tow. Each tow sample will be combined in the field into one composite sample. This combination will produce a mass weighted composite. While mass-weighting is not appropriate for fish tissue (Fabrizio, M.C. et al., 1995), in this case the sample unit is the lake phytoplankton concentration and such compositing is appropriate for determining its average.

Water and phytoplankton will be field packaged in pre-cleaned jars and bottles as specified in Table 5 (USEPA, 1990). Coolers will be chilled with ice and samples transported to Manchester lab via courier. Only the chlorophyll (a) and total phosphorus will be analyzed by Manchester, the As analyses will require the use of a commercial contract laboratory. Sample containers, collection times, and holding times are also shown on Table 5.

The petit ($0.1m^2$) Ponar sampler will be used to collect two grabs from the deepest part of each lake. The sampler will be lowered into the deepest part of each lake in the vicinity of the depth integrated water sample on a rope. The top 2 cm of two grabs will be composited in a stainless steel mixing bowl, cleaned as per the DH-76 sampler.

Table 5. Freshwater Fish Arsenic Background Investigation Sample Containers and Holding Times.

Media	Sample Size	Container	Number of Jars	Date Collected	Holding Time
Surface Water	1 L	1 L HDPE	20	8 in August, 2002 8 in November, 2002 1 field blank and 1 field duplicate for each event	As Species, 28 days at pH<2
	500 mL	1 L amber polyethylene	10	8 in August, 2002 1 field blank and 1 field duplicate	Chlorophyll (a) 24 hours to filtration
	125 mL	125 mL amber polyethylene	10	8 in August, 2002 1 field blank and 1 field duplicate	Total phosphorus 28 days at pH<2 and 4^0 C
Phytoplankton	2 gms	8 oz. of concentrate, Depending on boat speed and time	9	4 in August 2002 4 in November 2002, one sample from 2 tows (field composite) per event plus one field duplicate	28 days
Fish Tissue	100 gm/wet	4 oz. glass jar	25	12 Salmonid 12 other species in August, 2002 1 field duplicate	28 days
Sediment	50 gms	4 oz. glass jar	10	4 in August, 2002 4 in November, 2002 1 MS/MSD and 1 duplicate for each event	6 months at 4^0 C
	100 gms	8 oz. plastic jar	10	4 in August, 2002 4 in November, 2002 1 MS/MSD and 1 duplicate for each event	Grain size, 6 months
	20 gms	2 oz. glass jar	10	4 in August, 2002 4 in November, 2002 1 MS/MSD and 1 duplicate for each event	28 days for TOC at 4^0 C
	100 gms	2 oz. glass jar	10	4 in August, 2002 4 in November, 2002 1 MS/MSD and 1 duplicate for each event	7 days for % solids at 4^0 C

Laboratory Procedures

After collection, the entire concentrate of phytoplankton will be transferred to a pre-cleaned 8 oz. glass jar and held in the dark at 4^0 C. Upon delivery of the concentrate to the contract laboratory, the phytoplankton concentrate will then be vacuum extracted through a 5-micron polycarbonate filter. Following this process, a suitable (about 1 gm/ww) phytoplankton mass will be removed from the filter surface with a spatula and analyzed.

Blank filter media will also have lab deionized water run through them to determine if the filtration process/media has introduced any contamination to the phytoplankton samples.

Fish fillets will be randomly composited by species into three samples. Each sample will be comprised of an equal number of fillets with an equal mass of tissue from each fillet. The exact number of fish in each composite will depend upon fishing success. However, a minimum of five fillets will be used in each composite. The preferred composite size is ten fillets. To produce these composite sizes, fifteen to thirty fish of each species will be required from each lake.

The quantity of interest is the average fillet As concentration. Thus, each fillet will be removed (see below), and homogenized separately. Then an equal mass aliquot of each fillet's tissue will be combined with the other fillets. This compositing method will produce a physical average concentration of As for the fillets without weighting (Fabrizio, M.C. et al., 1995).

Fish will be washed with tap water to remove slime, descaled, and rinsed with deionized water. Fish will be filleted on dedicated aluminum foil, covering a polyethylene cutting board. Each fillet will be removed using stainless steel implements cleaned with Liquinox® detergent, 10% nitric acid and deionized water. The skin will remain on fillets, but the inclusion of scales and bones will be minimized. Fillets will be ground using a Kitchenaide meat grinder cleaned with detergent and 10% nitric as per the fillet knives.

Each composite sample will have a minimum of 5 and a maximum of 10 fish. All composites will have the same number of fish. Reviews of the previous freshwater fish arsenic speciation data (Donohue and Abernathy, 2002), have not yielded sufficient numbers of organic arsenic analyses to compute the variance of freshwater fish tissue arsenic species concentrations. Without knowledge of the variance, it is not possible to compute the expected power of the 3 sample composites.

Chlorophyll (a) is measured by filtering water onto a glass fiber filter disk and then extracting the pigments from the filter disk using acetone. The concentration of chlorophyll (a) is then measured colormetrically.

Sediment will be analyzed for total arsenic via ICP-MS. Grain size, percent solids and TOC will also be analyzed via PSEP methods.

Table 6. Freshwater Fish Arsenic Investigation Preparation Methods by Media.

Media	Preparation Methods	Method References
Surface Water, As species	Field addition of 6M HCL/L for preservation as shown in the analytical methods	USEPA Method 1632
Chlorophyll (a)	Filtration onto glass fiber disk, acetone extraction	APHA Standard Method 10200H(3)
Total Phosphorus	None	4500 P-I
Phytoplankton	Removal from water using 5 µm polycarbonate vacuum filter, HCL acid digestion per methods	USEPA Method 1632 (Rev. a)
Fish Tissues	HCL digestion per methods	USEPA Method 1632 (Rev. a)
Sediment	Acid digestion per methods for ICP-MS	USEPA (1997)
	Dried at 104^0 C with Acid cleanup for TOC	USEPA (1997)

The arsenic speciation will be performed at a contract laboratory. Table 7 illustrates the available reporting limits for Method 1632, Hydride Generation Quartz Furnace Atomic Absorption Spectrometry and the other analytic methods and reporting limits. The contract laboratory will be requested to report values between the detection limit and the reporting level whenever feasible (i.e. to not censor data below the reporting limit). Any data at these low levels would be flagged (probably as estimated). Table 8 lists the number of samples of each media and the estimated analytical costs of the project.

Table 7. Freshwater Fish Arsenic Background Study, Analytical Methods, and Available Method Reporting Limits.

Analyte	Analytical Method	Analytical Method Reference	Reporting Limit, Solids	Reporting Limit, Waters
Arsenic Species	HG-CT-GC-AAS, EPA Method 1632	USEPA, 2001	0.1 µg/g	0.01 to 0.05 ug/L depending on the species
Chlorophyll (a)	EPA Method 10200H(3)	APHA, 1991	n/a	0.05 µg/L
Total Phosphorus	EPA Method 365.3	USEPA, 1993	n/a	0.01 mg/L
Percent Lipids	Gravimetric	USEPA, 1990b	0.1%	N/A
Total Arsenic in Sediment	ICP-MS	EPA Method 600/4-79-020	0.2 mg/Kg	N/A
TOC in Sediment	Combustion	USEPA, 1997	0.02%	N/A
% Solids	Gravimetric	USEPA 160.3	0.1%	N/A
Grain Size	Sieve and Pipette	USEPA, 1996	0.1%	N/A

Table 8. Sample Numbers and Estimated Analytical Costs for the Background Freshwater Fish Arsenic Investigation.

Surface Water	# Samples	Cost	Total
As Species (total and dissolved)	16	$320.00	$5,120.00
Field Blanks	2	$320.00	$640.00
Field Duplicates	2	$320.00	$640.00
Contracting Fee	25% of	$6400.00	$1,600.00
Total Phosphorus	8	$12.00	$96.00
MS/MSD for Total Phosphorus	2	$12.00	$24.00
Bottles	30	$14.00	$420.00
Precleaned Filters	16	$21.00	$336.00
6M HCL for Preservation	20	$7.00	$140.00
		Subtotal	**$9,016.00**
Fish Tissue			
As Species	24	$350.00	$8,400.00
Field Duplicate	1	$350.00	$350.00
Reference Material Analysis	1	$350.00	$350.00
Contracting Fee	25% of	$9100.00	$2275.00
Dogfish Muscle, CRM Cost	1	$150.00	$150.00
Containers, including for archiving samples	50	$14.00	$700.00
		Subtotal	**$12,225.00**
Phytoplankton	8	$450.00	$3600.00
Field Duplicate	1	$450.00	$450.00
Contracting Fee	25% of	$4050.00	$1012.50
Chlorophyll (a)	8	$39.00	$312.00
MS/MSD for Chlorophyll (a)	2	$39.00	$78.00
Amber Polyethylene Bottles	10	$15.00	$150.00
5 µm Polycarbonate Filters	9	$22.00	$198.00
		Subtotal	**$5,800.50**
Sediment Total As via ICP-MS	8	$34.00	$272.00
Field Duplicates	2	$34.00	$68.00
MS/MSD for As	4	$34.00	$136.00
Preparation Charges	14	$17.00	$238.00
TOC	8	$33.00	$264.00
Field Duplicates	2	$33.00	$66.00
% Solids	8	$10.00	$80.00
Field Duplicates	2	$10.00	$20.00
Grain Size	8	$100.00	$800.00
Field Duplicates	2	$100.00	$200.00
		Subtotal	**$2,144.00**
		Grand Total =	**$29,185.50**

Quality Control Procedures

Field Quality Control

Field quality control will consist of the use of pre-cleaned bottles and sampling equipment which are dedicated to each lake. Field blanks for surface waters will be collected at a frequency of one per sampling event (summer and fall). One blank will be analyzed during the initial surface water sampling, and one blank will be analyzed during the second surface water sampling. These blanks will be collected using lab supplied deionized water, transferred across any sampling equipment into sample bottles in the field. Blanks will then be filtered and preserved as if actual samples.

Matrix spikes and matrix spike duplicates are included by the analytical contract laboratory and will be conducted by the contract laboratory at least once per batch. One blank and one field replicate each will be collected for total phosphorus and chlorophyll (a). Sediment analysis will include one field duplicate from each sampling event.

Lab Quality Control

For surface waters, one field blank will be conducted and one field duplicate will be collected per sampling event. For As, one matrix spike and spike duplicate (MS/MSD) per batch have been factored into the estimated contract lab prices quoted in Table 8. For chlorophyll (a) and total phosphorus analyzed by Manchester, MS/MSDs (one for each analyte) have been shown in Table 8.

For fish tissue, one field duplicate will be conducted. In addition, a certified reference material (CRM) will also be analyzed for total arsenic. Total arsenic results from the CRM shall be within 20% of the analytical windows specified by the supplier. As with the surface water, the cost for conducting at least one MS/MSD per batch have been factored into the estimated contract lab prices quoted in Table 7.

Phytoplankton will have one field duplicate. As with the surface water and fish tissues, the cost for conducting at least one MS/MSD per batch have been factored into the estimated contract lab prices quoted in Table 7.

For sediments, As by ICP-MS will include one matrix spike/matrix spike duplicate per sampling event. Grain size, TOC, and percent solids will not receive additional QA analysis beyond the standard methods.

Table 9. Statewide Freshwater Fish Arsenic Background Investigation, Quality Control Samples, and Required Frequencies.

Parameter	Check Standards	Method Blanks	Analytical Duplicates	Matrix Spike & Duplicate	Reference Materials
Surface Water	10% or more	1 per batch	1 per batch	1 per batch	None
Fish Tissue	10% or more	1 per batch	1 per batch	1 per batch	1 per batch
Phytoplankton	10% or more	1 per batch	1 per batch	1 per batch	None
% lipids	10% or more	1 per batch	1 per batch	1 per batch	None
Sediment	10% or more	1 per batch	1 per batch	1 per batch	None
TOC	10% or more	1 per batch	1 per batch	None	None
% Solids	None	None	1 per batch	None	None
Grain Size	None	None	1 per batch	None	None

Data Review and Validation

Both the Manchester Laboratory and the project manager will review all data and analytical narratives for completeness, bias, and precision goals. The data will be verified against the method performance criteria and the data quality objectives stated above.

Data Quality Assessment

Data quality will be summarized based on the review and validation described above. The mean, and the 95% upper confidence limit of that mean, will be calculated. Limitations of the data will be described.

Data will be tabulated and a draft report will be prepared by EAP. The report will include:

1) A map of the study area showing sample sites.
2) Photographs of site conditions during sampling activities.
3) Documentation of potential upstream anthropogenic arsenic contributions as described in DNR and literature databases (Huntting, 1956; Derkey et al., 1990; McFaul et al., 2000).
4) Discussion of data quality and any significant analytical problems.
5) Summary tables of analytical data.
6) Summary statistics of: the As concentrations, the inorganic/organic As ratio, As to phosphorus ratio. The 95% upper confidence limits of the As means will be calculated using the formula provided in Fabrizio et al. (1995) and *sensu* Gilbert (1987).
7) Descriptions of pertinent biotransformations through sampled media.
8) Recommendations for follow-up work including potential seasonality, critical parameters, media, or arsenic species as warranted.
9) An appendix of case narratives.

Project data will be entered into Environmental Information Management (EIM) prior to completion of the final report. Fish data will be given coordinates corresponding to the center of each water body. A handheld GPS will be used to determine the latitude and longitude of surface water sampling stations and the midpoint of the phytoplankton tow will be used for these media.

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