

Quality Assurance Project Plan

Mussel Watch Pilot Expansion Project

WDFW Contract No. of 11-1916

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1.0 Title Page/TOC/Distribution List

Quality Assurance Project Plan

Mussel Watch Pilot Expansion Project

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2.0 Abstract

The following *Mussel Watch Pilot Expansion Project* is a broad-scale assessment of toxic contaminants in the nearshore biota of the greater Puget Sound. This expands spatial coverage of the National Oceanic and Atmospheric Administration's (NOAA's) Mussel Watch program in Puget Sound with a one-season synoptic survey. It combines results and experience from NOAA's long-term monitoring program, as well as previous DFW feasibility projects, with a long-term goal of developing a regional plan for mussel-monitoring in Puget Sound. This Quality Assurance Project Plan describes the objectives and operating procedures for this study.

[NOAA's Mussel Watch \(MW\) program](#) monitors contaminant conditions in Washington State mussels (*Mytilus* spp.) at approximately 17 locations across the Puget Sound. Although the MW data are useful to broadly characterize ambient contaminant conditions, expanded spatial distribution and additional mussel monitoring sites are needed to address regional questions regarding the fate, transport, and effects of chemical contaminants in the Puget Sound's nearshore urbanized waters. This study will use Pacific blue mussels (*Mytilus trossulus*) as a representative species to evaluate the geographic extent and magnitude of contamination in nearshore biota. Additionally we will compare contamination patterns of mussels with land use patterns of adjacent shorelines and watersheds, compare contaminant uptake between mussels and eelgrass taken in a companion study, and provide recommendations for a long-term, nearshore status and trends monitoring program.

Mussels from a common source will be transplanted in predator-exclusion cages to over 110 sites along the shoreline, including areas affected by an array of upland land-use types. Areas to be covered include the southern and central Puget Sound, Whidbey and Bellingham Basins, San Juan Archipelago, Strait of Georgia, and Admiralty Inlet. Mussel cages will be placed within the middle intertidal zone during the winter months (November – January). Upon retrieval the condition index of mussels from each site will be determined and a composite of the mussel soft tissue will be prepared for chemical analysis. Each composite will be analyzed for a range of organic contaminants and metals.

Upon completion of the study, the Washington State Department of Fish and Wildlife (WDFW)-Puget Sound Ecosystem Monitoring Program (PSEMP) will produce a final report and an oral presentation of the study findings. The PSEMP final report will be posted to the internet and all data will be submitted for uploading into Ecology's EIM database.

3.0 Background

Toxic substances enter Puget Sound from a variety of pathways including (1) non-point sources such as surface water runoff, groundwater releases, and air deposition, (2) point sources including discharges from wastewater treatment plants and combined sewer overflows (CSOs) and (3) focal non-point sources such as marinas and ferry terminals. These toxic substances can cause harm to people, fish, other animals and plants. Controlling toxic chemicals is a Puget Sound recovery priority. Tracking toxic contamination in fish (Toxics in Fish) is one of a set of Puget Sound recovery indicators recently adopted by the Puget Sound Partnership. However the condition of contaminants in nearshore biota has long been recognized as a monitoring gap in Washington State. Understanding how contaminants enter and move through the marine food web (the fate and transport of chemicals), and what damage they cause once they are there, would improve our ability to make cost-effective decisions to mitigate the harm pollution causes Puget Sound's animals and plants.

Blue mussels (*Mytilus* spp.) and other sessile, filter-feeding bivalves have been used to monitor contaminant conditions in nearshore biota worldwide (O'Connor and Lauenstein, 2006). The National Oceanic and Atmospheric Administration's (NOAA) national Mussel Watch program has been active in Washington since 1986, sampling mussels in approximately 17 locations across the Puget Sound and 3 locations along the Pacific Coast. The Mussel Watch program originally selected their monitoring sites to characterize average conditions across the States, for comparison on a national scale. Although the Mussel Watch data from Washington have been useful to broadly characterize ambient contaminant conditions in nearshore biota, data from these sites alone cannot be used to answer regional questions regarding the fate, transport, and effects of chemical contaminants in the Puget Sound's nearshore urbanized waters. An expanded spatial distribution and additional mussel monitoring sites are needed to address these regional questions.

Over the past three years, PSEMP's *Toxics in Biota* team has worked with the NOAA Mussel Watch program to adapt and expand the core Mussel Watch design to accommodate regional needs and interests. The emerging model adds new monitoring sites in Puget Sound to the existing national Mussel Watch sites, in order to evaluate status and track trends of contaminants on a watershed or land-use scale.

This emerging model, using data from mussel tissue to evaluate status and track trends of contaminants on a watershed or land-use scale, is of interest to organizations that are responsible for managing regional stormwater and other aspects of water quality, as well as the release of toxic chemicals. Some applications of an expanded Mussel Watch would include the following:

- Fill the existing gap in tracking toxics in nearshore biota
- Mussel Watch sampling will be required by the Department of Ecology's (Ecology) Stormwater Work Group for Puget Sound's National Pollutant Discharge Elimination System (NPDES) permits, beginning in 2015 (see Draft Western Washington Phase II Municipal Stormwater Permit, appendix 10, page 4).

- Mussel Watch data would provide WDFW's and Ecology's oil spill programs with an understanding of baseline conditions, to assist in natural resource damage assessments. Mussel Watch data are of interest to the Department of Natural Resources (WDNR) in its ongoing assessment of pollution from outfalls to state-owned aquatic lands, as detailed in a companion proposal titled "Outfall Assessment and the Effects on Critical Nearshore Habitats".
- Mussels and other similar biota can be used to monitor or assess the effectiveness of pollutant reduction actions.

The following project details specific procedures and quality assurance guidelines proposed by the PSEMP - *Toxics in Biota* unit, under the Washington Department of Fish and Wildlife (WDFW), to implement a short-term *Mussel Watch Pilot Expansion Project*. This work also builds off recent studies conducted by *Toxics in Biota* addressing the use of mussels as nearshore contaminant sentinels, including a desktop survey of mussel distribution and potential availability in Puget Sound, a power analysis to predict sample sizes required to detect spatial trends, and a detailed recommendation for initial sampling approaches (Lanksbury and West, 2011).

PSEMP's *Toxics in Biota* unit is well suited to conduct this work because it has played a central role in assessing the status of and trends in the health of Puget Sound fishes and macro-invertebrates, as related to their exposure to toxic contaminants, since 1989 (Monitoring Management Committee 1988a). The *Toxics in Biota* component of PSEMP (a) monitors the status and trends of chemical contamination in Puget Sound biota, (b) evaluates the effects of contamination on the health of these resources and (c) provides information to public health officials for assessing if Puget Sound seafood is safe to eat (Stern 1989). Their staff are recognized as regional leaders in designing and conducting long-term assessment and monitoring programs to track and report on toxic contaminants in biota. In addition, Lanksbury, West et al. (2010) conducted a pilot study during the 2009/10 national Mussel Watch sampling period as a first step in partnering with NOAA, and successfully demonstrated the feasibility of using citizen scientist volunteers for field sampling.

4.0 Project Description

Project goals

This project represents the next logical step in a series of efforts aimed at developing an expanded network of sites for monitoring toxics in nearshore biota. The primary goal of this study is to use blue mussels as an indicator to evaluate contaminant conditions in Puget Sound's nearshore biota. The design involves distributing cage-protected mussels from a common source (aquaculture facility) along Puget Sound's shoreline to synoptically evaluate the geographic extent and magnitude of contamination across a wide range of upland land-use types including rural, undeveloped, agricultural, urban, and industrial areas. This work is linked to a companion proposal targeting the effects of outfall contaminants on eelgrass health in Puget Sound, as well as to the development of status and trends monitoring of contaminants in nearshore waters in support of Ecology's comprehensive National Pollutant Discharge Elimination System (NPDES) in Puget Sound.

Project objective

The objectives of this project are to:

1. Evaluate the geographic extent of chemical contamination in shoreline biota, using Pacific blue mussels (*Mytilus trossulus*) as the primary indicator organism.
2. Measure the magnitude of contamination where it occurs.
3. Compare contamination patterns in mussels with adjacent shorelines, covering a wide range of land-use types .
4. Compare contaminant uptake between mussels and plants (eelgrass).
5. Provide recommendations for long-term status and trends monitoring.
6. Deliver an oral briefing to Washington Department of Ecology, the Stormwater Work Group and stakeholders describing the extent and magnitude of contamination in nearshore biota.

Information needed and sources

We will be generating baseline data on toxic contaminants in Pacific blue mussels, presented as wet weight concentration, over a regional network of sites, many of which have been previously untested. Pre-existing NOAA Mussel Watch and PSEMP contaminant data on *Mytilus* sp. will be incorporated when pertinent, for context. Organic chemical contaminants and metals have been measured in *Mytilus* sp. from various locations in the greater Puget Sound and along the Washington Pacific Coast for over 20 years by the National Mussel Watch Program. Results from the National Mussel Watch Program (Kimbrough, Johnson et al., 2008), a pilot study during the 2009/10 National Mussel Watch sampling period (Lanksbury, West et al. 2010), and a desktop survey of mussel distribution and potential availability in Puget Sound, a power analysis to predict sample sizes required to detect spatial trends, and a detailed recommendation for initial sampling approaches (Lanksbury and West, 2011) informed the design of the current study.

Target population

The target population for this study is the Washington State native Pacific blue mussel (*Mytilus trossulus*), cultured at the Penn Cove Shellfish aquaculture farm in Penn Cove, Whidbey Island, and distributed throughout Puget Sound.

Study boundaries

The geographic scope of this project is the greater Puget Sound. The study boundaries are listed below and in the project Scope of Work:

- Southern Puget Sound
- Central Puget Sound
- Whidbey Basin
- Bellingham Basin

Additional sites in the Strait of Georgia, San Juan Archipelago, Strait of Juan de Fuca, Admiralty Inlet, and Hood Canal have been included through sponsorship by outside entities.

Tasks required

Tasks involved in this study include:

- Developing a partnership with an aquaculture facility to supply mussels for the study
- Soliciting partnerships with Citizen Science volunteer groups to help with site reconnaissance and sample deployment/retrieval
- Site selection
- Approval of this QAPP
- Deployment of caged mussels
- Retrieval of caged mussels
- Sample preparation
 - Tissue resection
 - Sample homogenization and compositing
 - Delivery of samples to contract analytical lab
- Contract with labs for analysis of samples
- QA/QC review
- Formatting of data for relational database
- Transfer of data to STORET and EIM
- Analysis of data for PSEMP/DFW report
- Communicate results to decision makers at Ecology and other entities

Practical constraints

The most pertinent constraints here relate to (a) sample timing, (b) numerous and various sample locations, (c) obtaining permission to deploy cages along shorelines with a wide range of ownership, (d) reliance on partner/volunteer groups to help deploy and retrieve transplanted mussels, and potential loss or theft of cages during the course of the study. In order to avoid variability related to seasonal differences in contaminant exposure and the length of exposure time among individual organisms, all test organisms will be transplanted to the various study sites in mid-November and collected in mid-January. Adult *M. trossulus* are reproductively quiescent and available for transplantation from Penn Cove Shellfish aquaculture farm during these months. In order to minimize variability related to size or age of the animals, we plan to transplant adult *M. trossulus* (within a 10 mm size range) to all the study sites for this project.

Sample timing is a constraint. Because the primary goal of this study is to evaluate contamination in the nearshore, mussel cages will be transplanted into the mid- to low-intertidal zone. To access this area we will have to be on the beaches during the mean lower low water (MLLW) time, which occurs after dark in the late fall and winter in Puget Sound. Thus we will be doing most of the deployment and retrieval (i.e. sampling) work in the dark, during cold and potentially inclement weather.

The numerous sample locations needed for this study presents a constraint. Because we will be placing mussel cages along more than 1000 miles of Puget Sound coastline, we will need to gain the permission of a variety of property owners, including private citizens, businesses, cities, counties and state agencies, to access desired sample sites. We will require a *Hydraulic Project Approval* (HPA) and a *Shellfish Transfer Permit* from our own agency (WDFW) to legally do this work. We will be required to enter into a *Memorandum of Understanding* (MOU) with the WDNR to access state-owned aquatic lands. In addition, we will require a *Scientific Research Permit* to sample within Washington State Park boundaries. Other permits may be required from individual county and/or city agencies.

Reliance on partners and volunteers groups is a constraint. Because a large number of sites over a very large geographic area need to be sampled within a short period of time for this study, we will rely heavily on the help of up to 30 separate partners and citizen science volunteer groups. Coordination of these various groups will require careful planning and tracking. A detailed protocol and instructions will be required to insure that the various partners and volunteers deploy and retrieve the caged mussels correctly, and do so according to the schedule required for this study. Additional protocols and on-site trainings will be required for any partners/volunteers who come to our laboratory to help measure and prepare mussels for analysis.

Loss of sample units from some sites due winter storms, vandalism or theft is a constraint. Because we will be placing mussel cages along more than 1000 miles of Puget Sound coastline, we expect some cages will be lost during the course of the study. To help diminish vandalism and theft, we will place identification plates on each cage. The ID plates will have the WDFW logo, "Contaminant Monitoring Study – Please Do Not Disturb", and the phone number of the Project Manager on them. In addition,

the cages will be exposed only during low tide after dark, which should minimize visibility and potential for theft. Cages lost within the first two weeks of deployment *may* be replaced, depending on the number lost, the reason for the loss, and the feasibility of replacing them at any particular site.

5.0 Organization and Schedule

Key individuals and their responsibilities

Table 1. Organization of project staff and responsibilities.

Name	Title	Phone #	Email	Responsibilities
James E. West	Senior Research Scientist	360.902.2842	james.west@dfw.wa.gov	Principal Investigator and Lead Author
Jennifer A. Lanksbury	Fish and Wildlife Biologist 3	360.902.2820	jennifer.lanksbury@dfw.wa.gov	Project management, data analysis and co-author
Laurie A. Niewolny	Fish and Wildlife Biologist 2	360.902.2687	laurie.niewolny@dfw.wa.gov	Project management, co-author, sample processing, and data review & analysis
Stefanie Orlaineta	Part-time temporary technician	360.902.2657	stefanie.orkaineta@dfw.wa.gov	field and lab support
Tom Gries,	NEP QA Coordinator	360.407.6327	tgri461@ecy.wa.gov	reviews QAPP and draft report
William Kammin	Ecology QA Officer	360.407.6964	wkam461@ecy.wa.gov	approves QAPP

Project schedule

Table 2. Proposed schedule for completing field and laboratory work.

Field and laboratory work	Due date	Lead staff
Field work completed	January, 2013	Jim West
Laboratory analyses completed	August, 2013	
Quarterly reports		
Author lead	James West	
Schedule		
QAPP completion – 30 Sep, 2012 (see section 5.3)		
Complete lab analysis – 31 August, 2013		
Final Report -- 31 July 2014		
1 st quarterly report	Short progress report with invoice	
2 nd quarterly report	Short progress report with invoice	
3 rd quarterly report	Short progress report with invoice	
4 th quarterly report	Short progress report with invoice	
Final report		
Author lead and support staff	James West, Jennifer Lanksbury, and Laurie Niewolny,	
Schedule		
Draft due to supervisor	31 April, 2013	
Final report due	31 July, 2014	

Limitations on schedule

Because of delays in establishing the contract for this project, and changes in hiring practices that have significantly slowed the hiring process, a WDFW Biologist was hired almost three months after the initial expected project start date. In addition, an unexpected death of a Fish and Wildlife Biologist in the PSEMP Toxics in Biota team in September left us short-staffed at a critical time in the development of this project. Although short-staffed, existing personnel have been working on obtaining permit applications, work contracts with outside groups that are sponsoring additional sites, gathering reconnaissance data for site selection, and ordering and modification of equipment necessary for the study.

In addition, unexpected delays in sample analysis occur from time to time, potentially delaying data availability. Examples include national emergencies such as the Deepwater Horizon oil spill, which resulted in a reprioritization sample analysis at many commercial and research labs throughout the country.

Budget and funding

This project is supported by a grant from the WDFW and WDNR as Lead Organizations for Marine and Nearshore Habitat Restoration and Protection efforts that are funded by EPA's National Estuary Program

(NEP). Match for this study is provided by Washington Department of Fish and Wildlife in the form of staff time and laboratory supplies.

The grant mentioned above will fund analysis of 60 mussel samples for this study. Funding for up to 55 additional samples from sites both within and outside of our main focus areas will be provided by a number of outside groups. Groups contributing funds for additional sites and/or additional data analyses include, but are not limited to, WDFW – Puget Sound Ecosystem Monitoring Program, WDNR – Aquatic Reserves Program, Washington Department of Ecology, US Navy Marine Environmental Support Office, Kitsap County Public Works, King County, Tacoma Pierce County Public Health District, City of Bellingham – Department of Public Works, Stillaguamish River Clean Water District, San Juan County MRC, Port Townsend AirWatchers, Puget Soundkeeper Alliance, Stillaguamish Tribe of Indians, Tulalip Tribes, and the Port Gamble S’Klallam Tribe.

Table 3 Proposed budget for 2012/13 mussel sampling, and data analysis and processing.

Object	Cost per Unit	Unit	No. of Units	Total Cost	Total Match
Research Sci 2 Salary	\$6069	month	3.625		\$22,000
Research Sci 2 Benefits	\$1922	month	3.625		\$6,247
Bio 3 Salary	\$4627	month	12.5		\$57,838
Bio 3 Benefits	\$1701	month	12.5		\$21,338
Bio 2 Step G Salary	\$3,800	month	21.0	\$39,900	
Bio 2 Benefits	\$1,584	month	21.0	\$16,632	
Technician Salary	\$2,971	month	3.0	\$8,913	
Technician Benefits	\$1,460	month	3.0	\$4,380	
Personnel Svcs	\$23	month	10.5	\$242	
Computer lease	\$65	month	21.0	\$1,365	
Site Lead Support Contracts	\$1,000	group	5	\$5,000	
Travel	\$300		20	\$6,000	
Boat/fuel	\$250	day	20	\$5,000	
Vehicle	\$9	day	60	\$740	
Volunteer supplies				\$1,000	
Volunteer time	\$700	site	30		\$21,000
Supplies	\$67	site	60	\$4,000	
Chemical analysis	\$1,001	sample	60	\$60,060	
Subtotal				\$153,232	
FY12 Indirect (23.51%)	0.2351			\$1,985	
FY13 Indirect* (28.36%)	0.2836			\$25,118	
					\$128,423
Total				\$178,961	

6.0 Quality Objectives

The general quality objective of this study is to collect tissue samples from caged mussels in numbers sufficient to evaluate the spatial distribution and magnitude of chemical contamination in nearshore biota, across a broad range of shore-land use types, on a one time basis, during a season of peak stormwater inputs to Puget Sound. The objective for analytical chemistry is to employ methods sufficient to evaluate the target analytes, with limits of detection sufficient to identify and measure the analytes, at a cost that maximizes geographic coverage.

Table 4. Summary of mussel tissue composites to be collected and analyzed for chemical contaminants during this study.

Purpose	Location	Timing of collection	Composites	Replicates
Baseline samples	Aquaculture source	November deployment	6	6
Study sites	Various	January retrieval	112 (max)	1 per site
Deployment control samples	Aquaculture source	January retrieval	6	6
Lab QC samples	Various	Aliquots taken during resection	20	20 ^a
Total			144	

^a two QA samples per batch of 12

Measurement Quality Objectives

WDFW staff and volunteers will be asked to record the GPS coordinates of the cage at each deployment site, both at the time of cage deployment and upon cage retrieval. We are not able to supply GPS units for each of our more than 110 sites, many of which will be visited on the same night. Instead we will ask our volunteers to use their own GPS unit. Each field worker will record the make and model of the GPS unit, and the accuracy of the GPS reading when taken. In addition, we will require all GPS devices used in this study to be set to North American Datum 83 ([NAD83](http://www.gps-basics.com)) for comparability, and coordinates will be recorded in decimal degree format. The specifications for many GPS receivers indicate accuracy within 3 to 15 meters (10 to 50 feet) 95% of the time (<http://www.gps-basics.com>). Since many of our sites are placed miles apart from one another, this level of accuracy is acceptable for our study purposes.

Following are three tables listing the minimum QA criteria for organic chemicals and metals analyzed in *M. trossulus* for this study.

Table 5. Quality assurance criteria for PCBs, PBDEs, PAHs, and OCPs. Reproduced from Sloan et al. (2006).

Table 8. Minimum analytical quality assurance criteria: Polycyclic aromatic compounds (PACs) and persistent organic pollutants (POPs) by gas chromatography/mass spectrometry (GC/MS).		
Quality assurance element	Minimum frequency	Acceptance criteria
Instrument calibration	Once every batch of samples or once every two batches in one continuous analytical sequence	Analyte concentrations are to be calculated using point-to-point calibration with at least four concentration levels of calibration standards.
Continuing calibration	At start and end of every analytical sequence and every 10 or fewer field samples	The RSD of the analyte responses relative to the internal standard is to be $\leq 15\%$ for the repetitions.
Reference materials: Sediment: NIST SRM 1944, NIST SRM 1941b Mussel tissue: NIST SRM 1974b Blubber: NIST SRM 1945 Fish tissue: NIST SRM 1946, NIST SRM 1947	One with every batch of 20 or fewer field samples	Concentrations of $\geq 70\%$ of individual analytes are to be within 30% of either end of the 95% confidence interval of the reference values. These criteria do not apply to analytes with concentrations below their lower LOQ when the lower LOQ is within or greater than the 95% confidence interval, nor to those analytes known to have coeluting compounds.
Method blank	One with every batch of 20 or fewer field samples	No more than 5 analytes in a method blank are to exceed $2 \times$ lower LOQ. Samples are not corrected for analytes found in the blank.
Sample replicates (i.e., duplicates or triplicates)	One with every 20 or fewer field samples	RSDs are to be $\leq 15\%$ (equivalent to relative percent difference $\leq 30\%$ for duplicates) for $\geq 90\%$ of the analytes that have concentrations ≥ 1 ng/g.
Internal standards/surrogates	At least one internal standard/surrogate is added to every sample	The recoveries are to be 60–130%.
Interlaboratory comparisons	At least one per year	In conjunction with the NIST or the IAEA.

Table 6. Required batch quality control measures and quality assurance criteria for mercury via CVAA.
 Reproduced from KCEL SOP 604v6.

Quality Control Element	Description of Element	Frequency of Implementation	Control Limit		
			Liquid	Solid	Tissue
Method Blank (MB)	Interference-free matrix to assess overall method contamination	1 per sample batch	± MDL	± MDL	± MDL
Spike Blank (SB)	Interference-free matrix containing all target analytes	1 per sample batch	85 - 115%	85 - 115%	85 - 115%
Standard Reference Material (SRM)	Certified reference material from NIST or NRCC, that is digested with samples.	1 per solid or tissue sample batch, if applicable	NA	80-120% ^c	80-120% ^c
Laboratory Control Sample (LCS)	Certified reference material from a source other than NIST or NRCC	1 per solid or tissue sample batch, if applicable	NA	80-120% ^c	80-120% ^c
Matrix Spike (MS)	Sample matrix spiked with all/subset of target analytes prior to digestion	1 per sample batch	70-130%	75 - 125%	75-125%
Matrix Spike Duplicate (MSD) ^a	Sample matrix spiked with all/subset of target analytes prior to digestion	1 per sample batch	70 - 130% RPD ≤ 20%	75 - 125% RPD ≤ 20%	75 - 125% RPD ≤ 20%
Lab Duplicate (LD) ^{a, b}	Self explanatory	1 per sample batch	RPD ≤ 20%	RPD ≤ 20%	RPD ≤ 20%
Filtration Blanks ^d	Method blank for the filtration process, when samples filtered in the lab	2 per sample batch	± MDL		

^a No calculation performed when both sample and duplicate values < RDL

^b LD are only analyzed with QA1 sediments and when required by specific projects

^c Or varies due to control charting

^d Entered to LIMS as an MB

Table 7. Required batch quality control measures and quality assurance criteria for the ICP-MS metals As, Cd, and Pb.

Reproduced from KCEL SOP 624v2.

Quality Control Element	Description of Element	Frequency of Implementation	Control Limit
			Liquid
Method Blank (MB)	Interference-free matrix to assess overall method contamination	1 per QC batch	< MDL & > -MDL
Spike Blank (SB)	Interference-free matrix containing all target analytes	1 per QC batch	85% - 115%
Matrix Spike (MS)	Sample matrix spiked with all/subset of target analytes prior to digestion	1 per QC batch	75% -125%
Matrix Spike Duplicate (MSD)	Sample matrix spiked with all/subset of target analytes prior to digestion	1 per QC batch or (LD) – Ultra Low level analysis only.	75% -125% %Recovery 20% RPD
Lab Duplicate (LD) ^a	Self explanatory	1 per QC batch or MSD – Routine level analysis only.	≤ 20% RPD, when at least one value is > RDL
Filtration Blanks (Routine)	Method blank for the filtration process if samples filtered in the lab	2 per QC batch	< MDL & > -MDL
Filtration Blank (Ultra-low)	Method blank for the filtration process	1 per QC batch	< MDL & > -MDL

^a No calculation performed when both sample and duplicate values < RDL

Precision

Precision is monitored and controlled within batches using laboratory replicates of field samples and across batches by analyzing Standard Reference Materials (SRM) of applicable matrix i.e., tissue. Cross-batch precision is expressed as the relative standard deviation (RSD) for repeated measurements. The RSD of analyte responses relative to the internal standard must be ≤ 15% for the repetitions.

Bias

Bias or accuracy of samples is evaluated by comparing measured SRM values with National Institute of Standards and Technology (NIST) certified values. In addition for POPs, concentrations of ≥70% of individual analytes are to be within 30% of either end of the 95% confidence interval of the reference values.

Sensitivity

The Lower Limit of Quantitation (LOQ) for all POPs in this study is “the concentration that would be calculated if that analyte had a GC/MS response area equal to its area in the lowest level calibration standard used in that calibration. When an analyte is not detected in a sample or it has a response area that is smaller than its area in the lowest level calibration standard used, the concentration of the analyte in that sample is reported to be less than the value of its lower LOQ.” (Sloan et al. 2006).

Typically LOQ values for POPs that have been reported to PSEMP by this method are in the range of 0.2 to 0.8 ng/g wet weight. In this study, the POPs' LOQs are given as a range because tissue sample LOQs are affected by the field sample mass used. The LOQ is the lowest concentration at which a POPs sample result will be reported.

EPA defines Method Detection Limit (MDL) in Appendix A to 40 CFR Part 136 as the “minimum concentration of a substance that can be measured and reported with 99 percent confidence that the analyte concentration is greater than zero and is determined from analysis of a sample in a given matrix containing the element”. In this study, the metal's MDLs are concentrations that cannot be detected or detected at a concentration less than the associated method detection limit considering tissue sample detection limits are affected by the sample mass used, matrix and polyatomic/isobaric interferences. The MDL is the lowest concentration at which a sample result will be reported. **Error! Reference source not found.** Table 6 and Table 7 lists the respective method detection limits for the four metals of concern (Hg, As, Cd, and Pb). They range from 0.002 to 0.005 µg/g wet weight.

Comparability

The SOPs described in this document (Sloan, Brown et al. 2004; Sloan, Brown et al. 2006) are consistent with other concurrent and future sampling efforts that could be used as comparison for *M. trossulus*. In addition, methods detailed here are consistent with ongoing PSEMP monitoring of contaminants in other Puget Sound species.

Although not necessary for the current project, comparability with historical NOAA Mussel Watch or other data will require some targeted evaluation. The performance-based nature of current analytical procedures is designed to allow the broadest comparability with other similar programs, however some difficulties will arise, especially as outdated methods are replaced. In particular, the congener based approach detailed herein is not directly comparable to Aroclor-based data for PCBs. This issue will be addressed in future efforts to fully expand and establish a mussel-monitoring program in Puget Sound.

Representativeness

Mussels used for this study will be of the species *Mytilus trossulus*, which is indigenous to intertidal habitats in the Puget Sound. As recommended in the *Standard Guide for Conducting In-situ Field Bioassays with Caged Bivalves* (ASTM E2122-02, 2007), mussels for this study will come from an aquaculture facility. The source will be Penn Cove Shellfish in Penn Cove, Whidbey Island, Washington. The advantage of using mussels from this facility is that all individuals will be of similar ages from the same population, will have a similar genetic and environmental history and are expected to be relatively uncontaminated. In addition, Penn Cove Shellfish is the only local aquaculture farm that raises *M. trossulus*.

The target size of mussels selected for transplantation will be based on the median size (± 5 mm) of 100 randomly selected adult (approximately 11 months old and larger than 45 mm) mussels available the day before bagging begins. Based on previous measurements taken at Penn Cove Shellfish on August, 2012, mussels selected for transplantation will likely measure between 50 – 60 mm in shell length.

The sampling design for this study is aimed at representing contaminant conditions, as tissue residues, in nearshore biota in the greater Puget Sound. To that end the design incorporates spatial coverage that

is representative of nearshore areas potentially affected by a range of upland land-use types including rural, undeveloped, agricultural, urban, and industrial areas.

Since the Puget Sound on average receives its highest amount of rainfall in the winter months, the sampling period chosen for this study (November – January) represents a period when input of contaminants from stormwater runoff is at its potential highest. Mussel cages will be placed on the intertidal substrate between 0 to -1.5 feet mean lower low water (MLLW), with mussels suspended approximately 40 cm above the substrate. The placement of cages is meant to simulate contaminant conditions experienced by most nearshore biota in the intertidal zone during the winter in Puget Sound.

Completeness

The goal of this study is to collect and analyze mussel tissue from >110 different sites representing different conditions in the greater Puget Sound. We expect loss of some sample units from some sites due winter storms, vandalism or theft. Cages lost within the first two weeks of deployment may be replaced.

Based on the number of individuals used to determine the condition of mussels from National Mussel Watch Program sites (Kim et al. 2006), a sample size of ten mussels from each site will be selected for determination of Condition Index (CI). If fewer than ten mussels are available for CI analysis from any cage for any reason, power analyses may be conducted to evaluate whether the incomplete sample size was sufficient to differentiate cage-populations with statistical rigor.

For tissue chemistry analysis a composite size of about 30 individuals (200g grams of soft tissue) per site (cage) was selected to optimize the amount of tissue available for analysis at the two chemistry laboratories. This mass is based on previous experience with the same laboratories, and allows enough tissue for reanalysis (if needed) and archiving small (20 gm) subsamples. The number of animals per composite was selected to balance representativeness of the population with the labor and time constraints related to processing samples. Our goal will be achieved if we are able to create a tissue composite from every site.

If the mussel cage at any site is no longer available (lost) at the end of the study in January, then native mussels may be sampled at that site as a replacement. Only mussels that are 1) within the same size range as those selected for transplantation, 2) found no further than 1 km away from the cage along the same shoreline, 3) fall within an Assessment Unit (AU) of the same mean percent impervious surface (%IS) (see Section 7.2.1) as the caged location, and 4) can be collected between January 7 – 13, 2013 will be used as replacements for caged mussels. Use of native mussels as replacement samples will be determined on a site-by-site and as-needed basis and will be subject to availability of volunteers or staff to find and collect the mussels. The basic technique for native mussel collection will follow the protocol outlined in the “Toxic Contaminant Monitoring in Mussels: Phase 1” QAPP, with the exception of mussel size selection and location of the site. A standard *Mussel Watch Retrieval Data Sheet* will also be filled out at that site, noting the difference in mussel origin. Once collected the native mussels will be placed on ice and processed for laboratory analysis in an identical fashion as the caged mussels for this study (see Section 8.5).

7.0 Sampling Process Design (Experimental Design)

Study Design

This study is designed to provide a qualitative reconnaissance survey to evaluate the geographic extent and magnitude of contamination in nearshore biota, as potentially affected by a wide range of upland land-use types. As noted in the Standard Guide for Conducting In-situ Field Bioassays with Caged Bivalves, “qualitative surveys are often conducted in areas where little is known about contamination patterns” (ASTM E2122-02, 2007). Thus, the sampling design for this study is meant to represent nearshore areas potentially affected by a wide range of upland land-use types including rural, undeveloped, agricultural, urban, and industrial areas. Thus the sites selected for this study are *representative of a wide range of expected conditions*, not randomly selected.

Using cultured and transplanted mussels for this study is meant to provide as much control over potentially confounding biological covariates (e.g., age, size, reproductive loss of POPs, and contaminant exposure history) as possible. Age of mussels for this study will be known and roughly equal (approximately 11 months), and all the mussels will have the same history of contamination exposure at the growing facility. Animals will be selected within a narrow size range. Contamination exposure of these mussels is expected to be minimal, and will be measured prior to caging and deployment. In addition, because the animals have not yet reproduced and whole-body tissues will be analyzed, there should be no differences in contaminant load related to sex.

In order to maximize probability for survival of transplanted mussels we will use the native mussel species *Mytilus trossulus* for this study. The target size of mussels selected for transplantation will be based on the median size (± 5 mm) of 100 randomly selected adult (larger than 45 mm) mussels available the day before bagging begins. Based on previous measurements taken at Penn Cove Shellfish on August, 2012, mussels selected for transplantation will likely measure between 50 – 60 mm in shell length. In order to achieve synoptic sampling all the mussels will be transplanted to their sample sites in mid-November (Nov. 11 – 19) and will be retrieved in mid-January (Jan. 7 - 13), allowing for a sample period of approximately two months. This period was selected to coincide with the period of maximum average rainfall in the Puget Sound, when the input of contaminants from stormwater runoff is at its potential highest.

Mussel cages will be anchored on intertidal substrate between 0 to -1.5 feet mean lower low water (MLLW), with mussels suspended approximately 35 cm above the substrate within the cage. This tidal elevation will result in occasional exposure to air during the tidal cycle in the months of deployment. Such placement is meant to simulate natural conditions experienced by mussels in the intertidal zone during the winter in Puget Sound.

Equipment

The equipment and materials used in this study are based on recommendations from the *Standard Guide for Conducting In-situ Field Bioassays with Caged Bivalves* (ASTM E2122-02, 2007). See Figure 1, Figure 5, Figure 3, and Figure 4 for photos of the hardware.



Figure 1 Cage system.
Cubic cage measuring 40.6 cm (16") on a side with lid (inside cage); screw anchor measuring 76.2 cm (30 inch); bent-tip rebar measuring approximately 1.2 m (4 foot).



Figure 2 Screwing in 76.2 cm (30 inch) screw anchor.



Figure 3 Mussel bags being zip-tied to edges of cage.

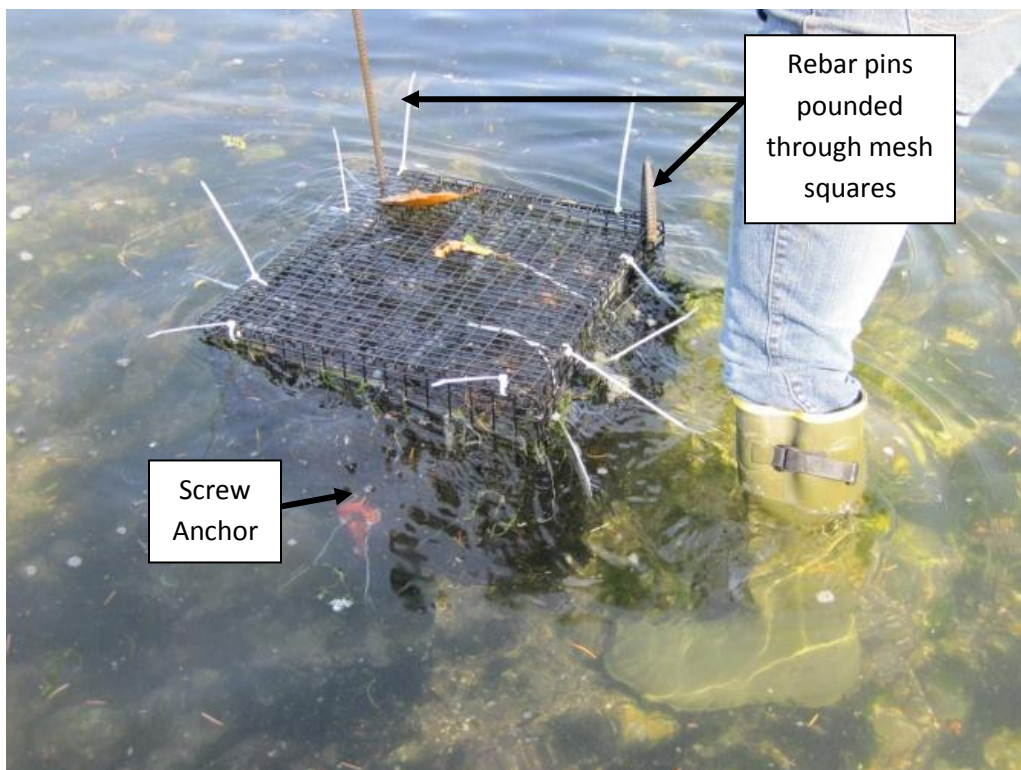


Figure 4 Anchoring cage with rebar and screw anchor. Cable tie ends will be trimmed.

Plastic-coated, wire mesh cages, designed for Washington Department of Health's biotoxin monitoring program were modified slightly for this study. The cages are manufactured by McKay Shrimp & Crab in Brinnon, Washington, and are designed to exclude predators such as seastars and crabs from reaching mussels, while optimizing water flow through the cage. The mesh opening is 1.25 x 2.5cm and the cages are coated in a vinyl alkyd material, which is equivalent to [TT-E-2124](#). Each mussel cage will have a stainless-steel identification plate attached including the WDFW logo, study title, and WDFW Program Manager phone number (see Figure 5).

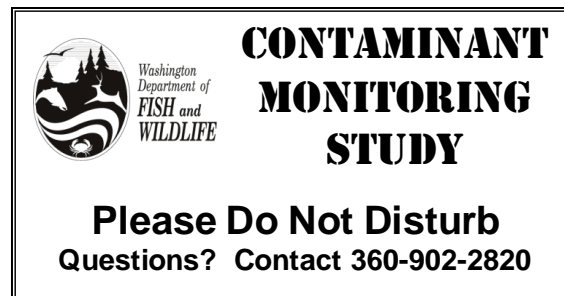


Figure 5 Metal identification plate attached to each cage.

Heavy duty mesh bags (manufactured by Norplex) made from extruded high density polyethylene (HDPE) will be used to contain groups of mussels within the cages. These mesh bags are used by the aquaculture industry for growing bivalves. Mussels will be sorted and selected at the Penn Cove Shellfish facility ten days prior to deployment, placed into bags in two groups of eight and left on-site (re-immersed) at Penn Cove Shellfish for at least ten days prior to deployment. This will ensure they have time to re-cluster after they are handled during sorting, measurement, and bagging (Andral et al, 2011; Benedicto et al, 2011; Galgani et al, 2011).

At deployment the bags of mussels will be placed into the wire mesh cages and affixed at either end with cable ties, at the top of the cage. Bags will be suspended across the top of the cage, just under the lid, to provide a uniform distance from the sediments (approximately 35 cm) for all mussels. The whole cage will be anchored to the substrate with a combination of screw anchors, rebar stakes, and/or concrete blocks, depending on the situation at each sample location. If possible, some cages may be tied (using large nylon cable ties) to steel or concrete pilings or other fixed points on-site. No cages will be affixed to or placed next to creosote-treated material.

The *Standard Guide for Conducting In-situ Field Bioassays with Caged Bivalves* (ASTM E2122-02, 2007), recommends immersing empty cages and anchoring materials in water at least 24 hours in advance of mussel placement, to dissipate any potential surface contaminants. We will soak all cages and materials in one saltwater location for 24 hours prior to distributing materials to the sample sites, or wash the cages with a high pressure hose using fresh water.

Site Selection

Shoreline sites for this study were selected to represent a wide range of adjacent land-use characteristics. Because land-use patterns are highly complex, representing a wide range of potential contamination sources and pathways, we simplified the classification by using percent impervious surface (%IS) as an easily quantifiable proxy, as described in Lanksbury and West (2011). We determined the mean %IS for predefined watershed catchment areas, called *Assessment Units* (AU), along the Puget Sound shoreline. These predefined AUs were originally developed by Ecology (Stanley et al., 2011) and were determined to be of a size (median area of 8.8 km² (3.4 mile²) appropriate for this study.

We used “percent developed imperviousness” measures from the [National Land Cover Database 2006 \(NLCD2006\)](#), with a spatial resolution of 30 meters, to calculate the *mean* %IS within each shoreline AU in basins defined for this study. Mean %IS ranged from zero to 94%, and most AU values fell below 15% (Figure 9). From this distribution we created four %IS classes: 0-5%, 6-15%, 16-50% and 51-94%, ranging from mostly undeveloped to highly developed. We then allocated the 60 sites supported by the current NEP grant across the four basins such that each basin was assigned at least one site in all the %IS classes available in that basin. Not all %IS classes were present in all basins, and we placed additional sites in the most urbanized embayments to provide a greater capacity to evaluate greater contaminant inputs.

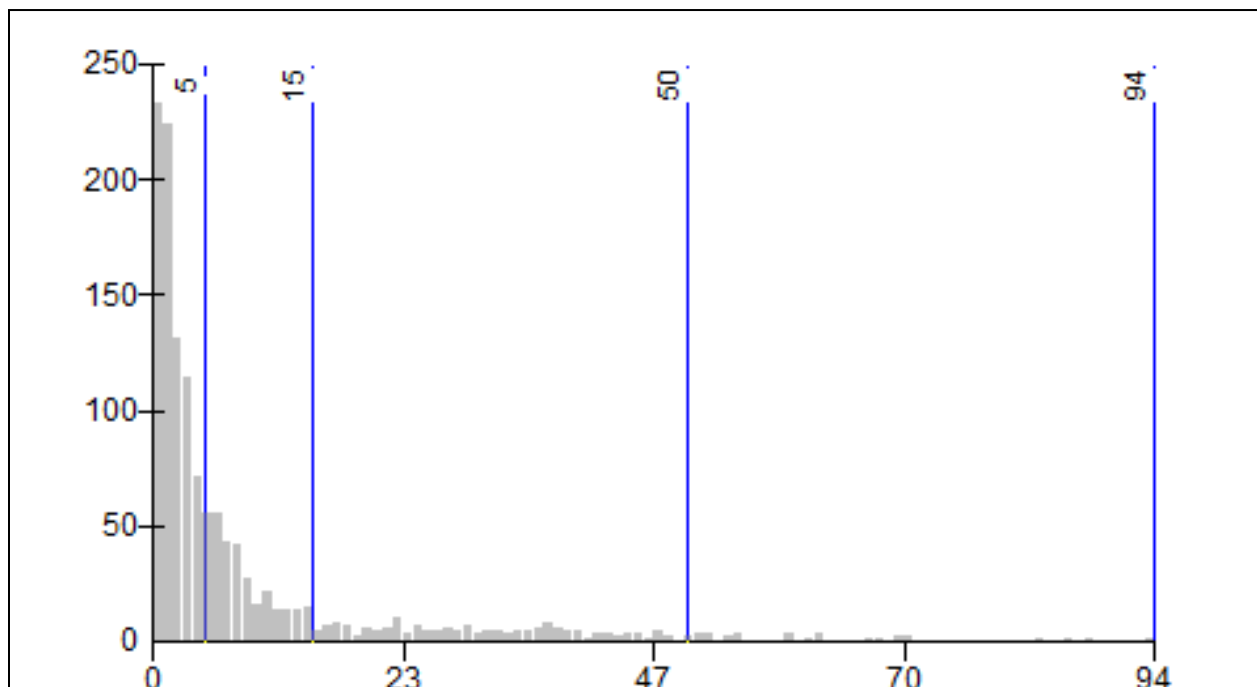


Figure 6. Frequency distribution of impervious surface (IS) for assessment units (AU) with shorelines where caged mussel will be deployed.

The distribution shows number of AUs (y axis) as a function of their mean % IS (%IS (x axis). The vertical delineate four %IS classifications: 0-5%; >5≤15%; >15≤50%; and >50≤94%.

Within each basin sites were distributed as widely as possible to represent the greatest geographic coverage. Other factors considered when locating a site along a shoreline included ecological factors such as presence of eelgrass, forage fish spawning areas, and shellfish beds. Also considered was

whether site could be placed in areas with a history of contaminant monitoring (for data comparison) and/or a significant need for National Resource Damage Assessment (NRDA) baseline data in the area. All these factors influenced the final placement of sites, with a preference to co-locate whenever possible.

Approximately 55 additional sites were established with additional resources from external partners, comprising tribes, Washington Department of Natural Resources, city and county governments, academic institutions and others. These sites were located to satisfy the needs of the sponsoring entities. In some cases placement of NEP sites was adjusted to create more representative shoreline coverage as externally funded sites were added. NEP funded sites are identified as stars in Figure 10, and externally funded sites are identified with crosses.

Mussel cages will be placed on the intertidal substrate between 0 to -1.5 feet mean lower low water (MLLW). Cages will be anchored to the substrate or tied to pilings or other fixed points on site, as long as the fixed point is not constructed of creosote-treated material.

All the mussels will be transplanted to their sample sites in mid-November (Nov. 11 – 19) and will be retrieved in mid-January (Jan. 7 – 13). Cages will be visited at the first available lower low tide period available after deployment to determine whether the cage remains on site. If the cage is no longer on site, it may be replaced over the following two evenings, following the deployment protocol outline in this QAPP. Any cage replaced at this period will be left on site later than the other retrieved cages, to approximate the same length of soak time as those for the rest of the study.



Parameters to be determined

Parameters to be determined related to sample deployment/retrieval include depth and location of the caged mussels and a description of the environmental conditions at the site. Approximate cage depth will be determined by recording of the exact time of deployment and measuring the water depth at the cage or the distance of the waterline from the cage. This information will be used in conjunction with data from the nearest tidal station to approximate the depth of the cage relative to MLLW. Digital photographs of the site, substrate, and installed cage will also be taken.

Parameters to be determined in the laboratory related to mussels include percent mortality per cage and the Condition Index (CI -- Section 8.5.1) of select mussels per cage. The CI can serve as an indication of the influence of seasonal fluctuations, such as temperature and food availability, on the physiological status of bivalves (Kagley, 2003; Benedicto et al. 2011). Calculation of condition index will allow for better comparison of mussels from different locations by allowing us to normalize biological changes over time and minimizing the influence of internal factors (e.g. mussel growth rates). Mussel soft tissue will be measured for concentration of organic contaminants, metals, and conventional parameters such as lipids and stable isotopes (see Table 8 through Table 10).

Field measurements

Separate datasheets (Figure 7), printed on Rite-in-the-Rain paper, will be filled out at the time of mussel cage deployment and retrieval.

DEPLOYMENT DATA SHEET		MUSSEL WATCH PILOT EXPANSION STUDY	
Site Name: _____	Bag #'s: _____	Date: _____	
Cage & Mussel Deployer(s) - please print: _____			
Data Recorder - please print: _____			
GPS Make/Model (set to Datum NAD83): _____			
Latitude: _____		Longitude: _____	
		Accuracy: _____ (± feet)	
Deployment Details			
Time cage was anchored: _____			
Cage Elevation (approximate): depth of water on cage _____ (inches) OR distance to water line _____ (feet).			
Anchoring method(s): No. of Rebar Used _____ Screw Anchor: (circle one) Yes No			
Comments regarding deployment: _____			
Conditions at Deployment Site			
Time of low tide: _____		Height of Low Tide in Feet (MLLW): _____	
Eelgrass present (circle one): Yes No		Substrate: _____	
What else is present around the area of the cage? (Bulkheads, stream, docks, etc....) _____			
Obvious sources of pollution? (oil slicks, pilings, seeps, derelict boats or pipes, etc....) _____			
Other observations: _____			
			
Please take photos of deployed cage.			



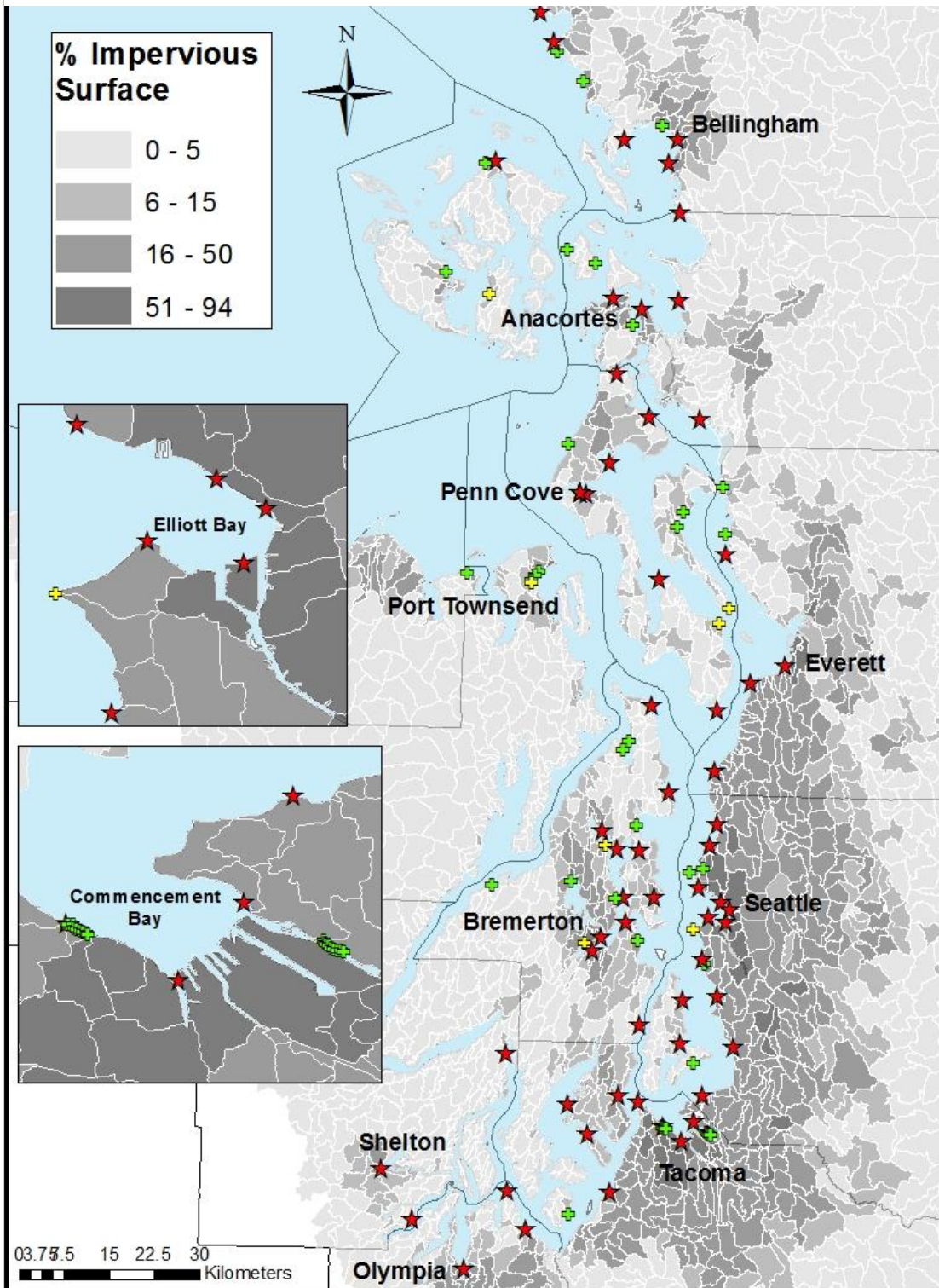
RETRIEVAL DATA SHEET		MUSSEL WATCH PILOT EXPANSION STUDY	
Site Name: _____	Bag #'s: _____	Date: _____	
Cage & Mussel Retriever(s) - please print: _____			
Data Recorder - please print: _____			
GPS Make/Model (set to Datum NAD83): _____			
Latitude: _____		Longitude: _____	
		Accuracy: _____ (± feet)	
Retrieval Details			
Time cage was removed: _____			
Cage Elevation (approximate): depth of water on cage _____ (inches) OR distance to water line _____ (feet).			
Comments regarding retrieval: _____			
Conditions at Retrieval Site			
Time of low tide: _____		Height of Low Tide in Feet (MLLW): _____	
Eelgrass present (circle one): Yes No		Substrate: _____	
What else is present around the area of the cage? (Bulkheads, stream, docks, etc....) _____			
Obvious sources of pollution? (oil slicks, pilings, seeps, derelict boats, pipes, etc....) _____			
Other observations: _____			
			
Please take photos of cage before removal.			

Figure 7. Deployment and Retrieval Datasheet forms for recording field data.

Maps or diagram

Figure 8. Draft map of mussel cage sites in the greater Puget Sound



Points represented by red stars are funded by this study. Points represented by crosses are funded by outside sponsors; green are already funded, yellow are potentially funded. Specific locations of some sponsored sites may be moved. Gray polygons represent mean percent impervious surface (%IS) calculated within each assessment unit (AU).

Assumptions underlying design

The primary assumption of this study is that mussels transplanted in cages along the intertidal zone experience a similar degree of chemical contaminant exposure as naturally occurring mussels do in those same areas. This assumption is supported by a number of studies comparing contaminant uptake between native and transplanted mussels (Baumard et al. 1999; Piccardo et al. 2001; Bervoets et al. 2004; Nigro et al. 2006).

We assume that mussels from Penn Cove Shellfish have a relatively low level of contamination, and that contaminant levels in mussels from the farm will be relatively uniform. We will test this assumption by analyzing six composites of our bagged mussels, held back for this purpose at the time of deployment. These samples will represent the baseline condition of contaminants in mussels being deployed.

We also assume that the adult mussels used for this study and taken from Penn Cove Shellfish are all approximately 11 months of age, have not yet spawned, and will not spawn during the study period. This information is provided by Ian Jefferds, aquaculture professional and owner of Penn Cove Shellfish, and based on the known biological cycles of *M. trossulus* in Washington State.

We anticipate that because the test period begins in the fall, when the heavy rains generally begin in the Puget Sound, the mussels will be exposed to contaminants transported to Puget Sound via stormwater runoff. Thus the study period of approximately two months should coincide with a period when yearly stormwater input into the Puget Sound is at its highest.

Characteristics of existing data

National Mussel Watch Program surveys of *Mytilus* sp. in Puget Sound from 1986 - 2005, and from 2007 - 2010 indicated urban signals of certain persistent organic pollutants (POPs) in mussel tissue (Kimbrough et al. 2008; WDFW unpublished data). These data were used to inform the current study in terms of the range of locations selected for monitoring. A summary and analysis of the WDFW unpublished data will be provided and discussed in the final report for this study.

8.0 Sampling and Lab Procedures

Field measurement and field sampling Standard Operating Procedures

The following sections describe the procedure for harvesting, measuring, and bagging mussels at a commercial aquaculture facility (Penn Cove Shellfish) in preparation for subsequent deployment in predator-exclusion mesh cages at sites around the greater Puget Sound.

The protocols described below are based on procedures outline in the *Standard Guide for Conducting In-situ Field Bioassays with Caged Bivalves* (ASTM E2122-02, 2007). Although the *Standard Guide* initially mentions several possible cage types for in-situ field tests with caged bivalves, the majority of their subsequent field measurement and sampling methods are based on the assumption that the researcher is using individually compartmentalized mussels in cages suspended in the water column. In this study our mussels will not be individually compartmentalized; they will be grouped together within their cages. In addition, our cages will be deployed in the intertidal zone on the substrate, not suspended in the water column. Thus although our methods are based on guidance from the *Standard Guide for Conducting In-situ Field Bioassays with Caged Bivalves* we have made modifications where necessary to accommodate the specific needs of our study design.

Mussel Transplant Size Range Determination

The target size of mussels selected for bagging and subsequent transplantation will be based on the median size (± 5 mm) of 100 randomly selected adult (approximately 11 months old and larger than 45 mm) mussels available the day before bagging begins. Based on previous measurements taken at Penn Cove Shellfish on August, 2012, mussels selected for transplantation will likely measure between 50 – 60 mm in shell length.

Mussel Presort

The presort, measuring, and bagging described below will take place from October 22 – November 1, 2012, allowing extra time for inclement weather.

WDFW staff will obtain live mussels for cage deployment during normal, periodic harvest operations conducted by Penn Cove Shellfish staff. This company grows mussels attached to 20 foot sections of rope hanging under floating docks. Penn Cove staff harvest mussels by removing them from the ropes and cleaning them with specially designed brushes aboard a harvesting vessel tied up to whichever floating platform is scheduled for harvest. WDFW staff will divert live, cleaned mussels from this operation to a nearby beach, where sorting, measuring and bagging will occur.

The beach sorting, measuring and bagging area will have tables, chairs and a canopy to provide shade so mussels are not exposed to direct sunlight during sorting. Mussels will be held in ambient seawater in coolers while they await processing. Using a knife or scissors we will select mussels that fall within the desired size range (see Section 8.1.2), separating them from one another by cutting their byssal threads. Care will be taken not to pull or tear the byssal threads, so as not to damage the byssal glands. The cleaned and separated mussels will then be replaced into a cooler filled with ambient Penn Cove seawater.

We will monitor the water temperature inside this seawater holding cooler with a thermometer, to ensure it stays within $\pm 5^{\circ}$ C of current Penn Cove surface temperature, and change water as needed to maintain suitable water quality.

Measuring and Bagging

We will take presorted mussels from the holding cooler and measure their shell length. Only intact mussels with no cracks in their shells and that respond to physical stimulation by tightly closing their shells will be selected for measuring and bagging. Mussels that do not meet these requirements will be discarded.

Measuring

Mussels will be randomly select from the holding cooler. We will measure shell length (umbo to farthest posterior margin) using a digital caliper with measurement accuracy of 0.1 mm. Length measurements will be manually recorded onto a waterproof paper data sheet.

Bagging

Sixteen (16) measured mussels will be placed into heavy duty mesh bags measuring 20 inches in length. We will use a cable tie to secure one end of the bag, place eight mussels into the bag, then cable tie the center of the bag, sealing those mussels into a section. We will then place eight more mussels in the remaining section of the bag and use a cable tie to close the end of the bag, making a second section. The finished mussel bags will have two separated sections with ample space for eight mussels to feed and grow, for a total of 16 mussels per bag.

We will affix a plastic identification tag with a unique number to each finished bag. This number will be noted alongside the measurements of the mussels for that specific bag. Once the identification tag is affixed to the filled mussel bag the bag will be placed into another holding cooler filled with ambient Penn Cove seawater. The seawater in these coolers will be maintained in the same fashion as described above in Section 8.1.1.

Presoak period

Once a sufficient number of mussel bags have been processed, we will affix them to a 20-foot weighted line, spaced approximately six inches from each other. Approximately 40 bags will be placed along each line. When a line is filled with bags, Penn Cove Shellfish staff will hang the line under one of their aquaculture platforms. Each line of bagged mussels will be marked with an identification flag indicating the range of bag ID numbers hanging on that line. The location of the line will be noted in the Mussel Watch Field Notebook.

The finished mussel bags will be left to soak at Penn Cove Shellfish for at least 10 days before they are removed from the water for deployment in mesh cages. The 10+ day period following mussel bagging is intended to allow the mussels a resting period after they are separated, sorted, cleaned and bagged. This allows them time to re-cluster prior to deployment (Andral et al, 2011; Benedicto et al, 2011; Galgani et al, 2011).

Deployment

In preparation for deployment each volunteer (deployer) will be given a written protocol detailing the procedure below. The protocol will include all the pertinent steps involved in deployment of a cage and mussel bags, a “Mussel Watch Deployment Data Sheet”, and a “Chain of Custody” form. WDFW staff will review this protocol with volunteer leads to be sure all steps are understood and followed during deployment. In addition, a pre-filled sample data sheet and photos of a properly deployed cage will be provided for reference. Some volunteer groups will deploy mussel cages at more than one site, either on the same or consecutive evenings depending on distance between sites.

Cage and Mussel Deployment

Deployment groups will come to Penn Cove Shellfish on one or several evenings, depending on how many cages they are deploying, during evening low tides from November 11 – 19, 2012. WDFW staff will assign each deployer a deployment kit(s), which will include a 16”x16”x16” wire mesh cage and anchoring devices, cable ties, laboratory gloves, deployment and retrieval data sheets, a cooler with ice, and four bags of mussels per site. The four mussel bags will be placed into a large plastic bag marked with the name of the site where the cage will be deployed. The bagged mussels will be placed on separately bagged ice, as a double barrier to be certain they do not come into contact with ice melt water during transportation.

At this time the following information will be recorded in the Mussel Watch Field Notebook next to each mussel bag ID number: site name to which that bag is assigned, deployer taking possession of that bag, and the number of dead mussels found in each bag. Mussels will be considered dead if there is no soft tissue inside. WDFW staff and the deployer will also fill out and sign a “Chain of Custody” (COC) form.

The bagged mussels will be transported directly to the deployment site(s) and deployed on the same night they were taken from Penn Cove, to minimize time out of the water (ASTM E2122-02, 2007). We anticipate a length of between 2-4 hours from the time of mussel pick and deployment on site. The exact time will be determined from the COC form and the *Mussel Watch Deployment Data Sheet*.

At the deployment site the cage will be anchored to the substrate, a fixed pier, rock, or other fixed object in the intertidal zone between 0 to -1.5 feet MLLW. Depending on the substrate available at each site, cages may be anchored using a screw anchor and rebar stakes, secured with a large cable tie to a non-cresote fixed object (i.e. piling or pole), or to cement blocks that will act as weighted anchors.

The mussel bags will be affixed to the top 1/3 of the cage, spaced evenly apart, using cable ties to secure the end of each bag to the side of cage, so that the bags are suspended across the middle of the cage (Figure 3). After mussel bags are secured inside the cube the mesh lid will be secured in place with at least two nylon conduit ties per edge.

The GPS coordinates of the cage location time of deployment, approximate height of the water next to the cage or the distance of the cage from the water line, as well as all other items outlined on the *Mussel Watch Deployment Data Sheet* provided by WDFW will be recorded by the deployer. GPS readings will be taken with a wide range of instruments – each volunteer group will secure its own instrument. To validate accuracy and insure consistency among units, WDFW staff will instruct field staff to record the make and model of the GPS unit and accuracy at the time of reading. The datum for

each unit will be set to NAD 83 and positions recorded in decimal degrees. The deployer will also take digital photos confirming proper deployment of the mussel bags in the cage. The datasheet and photos will be sent to WDFW within two days after deployment.

Deployers will check the status of cages on the following night to evaluate the integrity of the cage. If the mussel cage is no longer there (i.e. lost or stolen), the deployer will immediately contact Jennifer Lanksbury and arrange to get another cage. On one of the following evenings, the deployer will pick up a new set of bagged mussels and a new cage and deploy them together on the same night, following the above protocols. If the cage is damaged it will be repaired on site or replaced with a new cage.

Baseline and Control Samples

Baseline biological and chemical conditions of the population of sorted/cleaned/bagged mussels will be estimated prior to deployment by sub-sampling the pre-deployment group. At the mid-point of the deployment period (November 14), a subset of 20 bags of mussels will be removed from Penn Cove Shellfish to serve as a baseline group for biological characteristics and contaminant levels. In addition, 20 bags of mussels will also remain at the Penn Cove Shellfish (not deployed) as a control group for the study.

The 20 bags of mussels removed at the outset of the deployment process will serve as an estimate of the initial, or baseline condition of biological and chemical metrics for the deployed population. These samples will be processed for all biological and chemical metrics according to protocols used on deployed mussels (see Section 8.1.6 below). One hundred mussels will be processed for biological metrics (condition index) and six tissue composite samples (30 mussels per composite, 6 replicates) will be prepared for analytical chemistry. Biological and chemical measurements of mussels from the deployed population will be compared against the mean initial (baseline) condition of mussels to calculate the net increase or decrease in biological metrics (such as Condition Index) and concentration of analytes.

This process will be repeated for the 20 bags of mussels held at Penn Cove Shellfish for the duration of the study. These mussels will serve as growth and chemical uptake controls for deployed mussels. As with the baseline samples, One hundred mussels will be processed for biological metrics (condition index and growth) and six tissue composite samples (30 mussels per composite, 6 replicates) will be prepared for analytical chemistry.

Check on Mussels and Redeployment if Necessary

WDFW staff and volunteers will check on the deployed mussels at each site during the lowest low tide from November 25 – December 2. If the mussel cage is no longer present at that site, WDFW staff will determine whether it is feasible to replace the cage. A sufficient number of bagged mussels (extras) will be kept at Penn Cove Shellfish for this purpose. If it is determined that the cage can be replaced, deployment will occur immediately on the following evening(s). Deployment of the replacement cage will follow the protocol outlined above and new COC and datasheets will be made for that site, with a note highlighting the delayed deployment of that cage. Retrieval of replaced mussel cages will be delayed by one low tide cycle, to allow for an eight week deployment period for those cages.

Retrieval and End-of-Test Measurements

Mussel retrieval will take place during MLLW periods from January 7 – 13, 2013.

Upon arrival at the caged mussel site, the volunteer (retriever) will take a digital photo of the cage, to document its condition, including structural integrity and degree of biofouling. The retriever will remove the bags of mussels from the cage, keeping the mesh intact, and place the bagged mussels immediately into large (1-Gallon), pre-labeled Ziplock bags. The Ziplock bag(s) will be placed into a cooler with bagged ice. This double barrier bagging method will ensure that mussels do not come into contact with any ice melt water during holding. The cages and all anchoring devices will then be removed from the beach.

The retriever will fill out a *Mussel Watch Retrieval Data Sheet* and a Chain of Custody form. They will then hold the mussels overnight on ice in the cooler, taking care not to allow mussels to freeze during holding. The following morning the retriever will deliver the cooler with live mussels, the cage(s), datasheet(s) and COC form(s) to the WDFW Marine Resources Laboratory on the 6th floor of the Natural Resources Building at 1111 Washington Street SE, Olympia WA 98501. WDFW staff will sign and keep the COC form when they take possession of the mussels.

The control group of reserved mussels at Penn Cove Shellfish will also be taken during this retrieval period, and treated in the same manner as field-deployed mussels.

Chain-of-Custody

Two Chain of Custody (COC) forms will be used. The field Chain of Custody (Figure 9), printed on Rite-in-the-Rain paper, will be used to transfer samples from field staff to lab staff, and the PSEMP Task Order (Figure 10) will be used to track the chain of custody of samples from the lab to the analytical lab.



**DEPLOYMENT CHAIN OF CUSTODY
MUSSEL WATCH PILOT EXPANSION STUDY**

Relinquished by: (release of mussel bags from Penn Cove, please record bag numbers)	Print and Sign NAME	DATE	TIME
	_____	_____	_____
	_____	_____	_____
Received by: (Primary mussel possession, please record bag numbers)	Print and Sign NAME	DATE	TIME
	_____	_____	_____
	_____	_____	_____
Received by: (Secondary mussel possession, record the bag numbers)	Print and Sign NAME	DATE	TIME
	_____	_____	_____
	_____	_____	_____
Received by: (Secondary mussel possession, record the bag numbers)	Print and Sign NAME	DATE	TIME
	_____	_____	_____
	_____	_____	_____
Received by: (Secondary mussel possession, record the bag numbers)	Print and Sign NAME	DATE	TIME
	_____	_____	_____
	_____	_____	_____

Page ____ of ____



**RETRIEVAL CHAIN OF CUSTODY
MUSSEL WATCH PILOT EXPANSION STUDY**

Instructions: Remove mussel bags from cage. Do not open the mesh bags. Place whole mussel bags in prelabelled Ziploc storage bag, seal, and put on ice in a cooler for overnight. DO NOT FREEZE! Remove cage, anchoring devices and sample debris from site. Deliver mussels and gear to the WDFW Marine Lab on the 6th floor of the Natural Resources Building (1111 Washington St. SE, Olympia, WA 98501) the following morning after retrieval.

SITE NAME:			
BAG ID NUMBERS:	#	#	#
Retriever Took Possession:			
Print and Sign NAME		DATE	TIME
Observations or Comments:			
Retriever Relinquished Possession:			
Print and Sign NAME		DATE	TIME
Observations or Comments:			
WDFW Lab Took Possession:			
Print and Sign NAME		DATE	TIME
Observations or Comments:			

Figure 9 Deployment and Retrieval Chain of Custody (COC) form for transferring samples between the field and laboratory.

WASHINGTON DEPARTMENT OF FISH AND WILDLIFE
TASK ORDER FOR SAMPLE ANALYSIS
 IN ACCORDANCE WITH INTERAGENCY AGREEMENT # xx-xxxx

Fiscal Year: _____

Task Order: _____ Date Delivered: _____

Further deliveries for sample set: YES NO

When more than one delivery for a sample set, reference the original Task Order number, appending it with a text character, using "A" for the first delivery, "B" for the second delivery, etc....

Work to be done under this Task Order:

See attached page(s) for an itemization of the work to be done.

Task Order Cost: Total cost for this Task Order shall not exceed _____ \$0 _____ or the total cost per sample, whichever is less, nor the total budget as specified within the agreement.

ACKNOWLEDGMENT OF RECEIPT OF SAMPLES:

National Marine Fisheries Service

Washington Dept. of Fish and Wildlife

 Representative Date

 Project Representative Date

AGREEMENT TO FULFILL TASK ORDER:

US Department of Commerce
 National Marine Fisheries Service

State of Washington
 Department of Fish and Wildlife

 Contracts Officer Date

 Contracts Officer Date

 Project Representative Date

 Project Representative Date

NOTICE TO PROCEED

TASK ORDER FOR SAMPLE ANALYSIS
 INTERAGENCY AGREEMENT #xx-xxxx

SURVEY:							TASK ORDER: 0	
	STATION/SAMPLE ID	SPECIES	SAMPLE DATE	TYPE	NUMBER OF CONTAINERS	NUMBER OF CASSETTES	ANALYSES	
1								
2								
3								
4								
5								
6								
7								
8								
9								
10								
11								
12								
13								
14								
15								
16								
17								
18								
19								
20								
21								
22								
23								
24								
25								
26								
27								
28								
29								
30								

Sample Type: Muscle Tissue (M) Liver Tissue (L) Whole fish (W) Gonad (G) Bile (B) Blood plasma (P) Spleen (S) Other _____ ()

Analyses: Fluorescent Aromatic Compounds in bile (FAC), Cytochrome P450 in liver tissue (CYP1A), DNA adducts (DNA), Pesticides (Pest), PCBs (PCB), PBDEs (PBDE)
 % Solids (%S), %Lipids (%L)
 Metals: Mercury (Hg), Lead (Pb), Arsenic (As), Copper (Cu), Other _____ ()
 Histopathology: H&E stain (H&E), _____ ()

Figure 10 Task Order Chain of Custody form for transferring samples from the WDFW Resection Lab to the Analytical Lab.

Field log requirements

The lead scientist will maintain a spiral bound Rite-in-the-Rain Mussel Watch Field Notebook with detailed notes for each day's activities. Information recorded will include:

- Name and location of project
- Field personnel
- Sequence of events
- Any changes to plan (i.e. replacement of any cages or sampling of native mussels)
- Unusual circumstances that may affect interpretation of results

In addition, the following information will be recorded in the field notebook next to each mussel bag ID number: site name to which that bag is assigned, deployer taking possession of that bag, number of dead mussels found in each bag at the time of deployment. Mussels will be considered dead if there is no soft tissue inside the shell.

Entries are made in the daily log either in permanent ink or pencil.

Preparation for Tissue Resection and Processing in the Lab

Equipment, reagents, and supplies

Lab coat

Apron

Nitrile gloves – talc-free

Eye protection

Terg-A-Zyme®

Deionized (DI) Water -

Tap water

Isopropyl Alcohol - B&J Brand® Multipurpose ACS, HPLC

Teflon Squeeze bottles

Heavy duty aluminum foil – Reynolds 627 (60.96 cm wide x 0.94 mm thick)

Scissors - stainless steel

Forceps - stainless steel

Spatulas – stainless steel, flat blade/round blade

Scalpels with stainless steel blades

Mixing spoons – stainless steel

Calipers – stainless steel (Figure 3)

Stainless steel mixing bowls

Plastic colanders

Metal trays

Aluminum weigh dishes with finger grips

Bamix hand held mix/grind motor with stainless steel cutting blade (Figure 13)

Bench scale – A&D HP-22K 20,000 x 0.1gram range (Figure 14)

Balance - A&D EK-6000H, 6,000 x 0.1 gram range (Figure 15)

Sample jars – clear, short, wide mouth jars, I-CHEM Certified 200-0250 series, Type III glass with Teflon-lined polypropylene lid (Figure 12), various sizes

Sample jar labels – cryogenic, laser printer ready, Diversified Biotech LCRY-2380 0.94in. x 0.50in and LCRY-1258 2.625in x 1.0in.

Freezers – walkin freezer at -20°C, chest freezer at -15°C

GCA Precision Gravity Convection Oven set to 120°C (Figure 16)



Figure 11. Stainless steel caliper with digital readout to 0.01 mm.

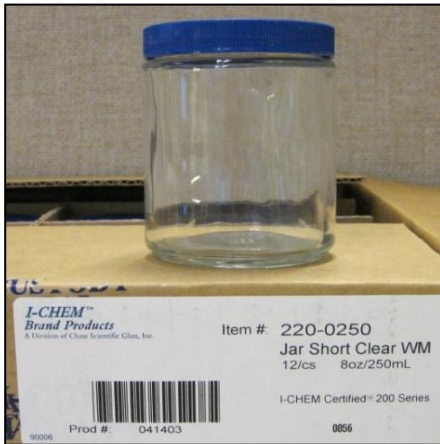


Figure 12. Pre-cleaned Series 200 I-Chem sample jar.

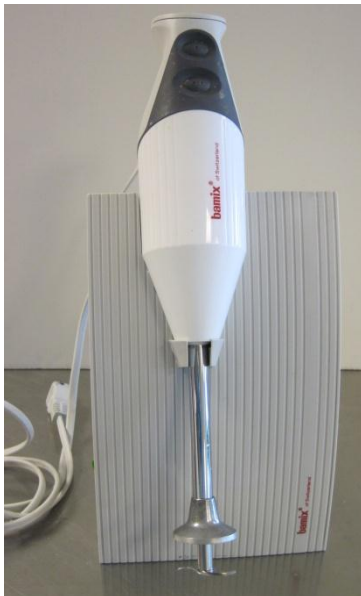


Figure 13. Hand-held Bamix tissue grinder

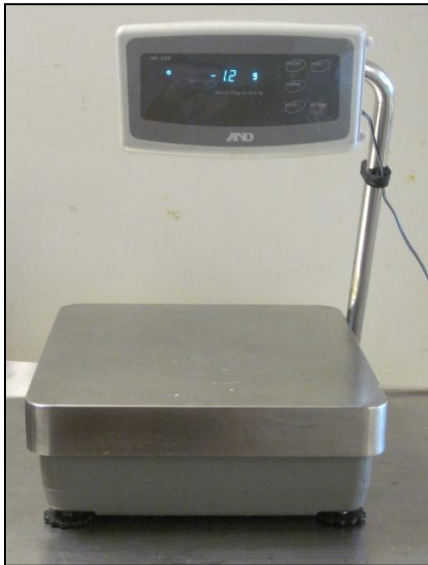


Figure 14. 20-kg capacity bench scale for weighing large specimens



Figure 15. 6-kg capacity bench scale for weighing small specimens.

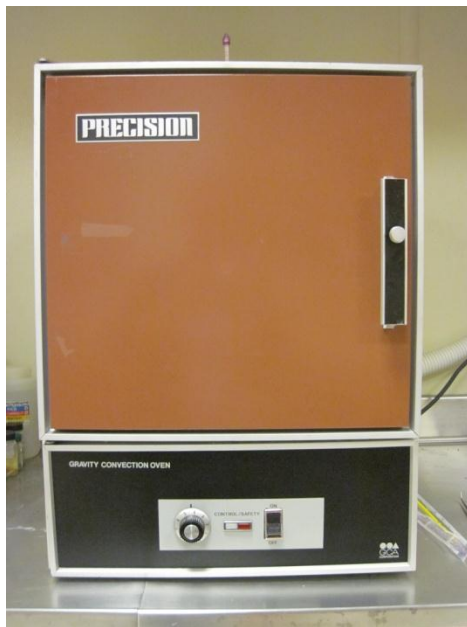


Figure 16. GCA Precision Gravity Convection Oven

Preparation of Lab Record Forms

Two forms are used to track mussel samples as they are processed in the lab. A Specimen Form (Figure 17) records collection information and biological metrics of each individual mussel that is processed for a composite sample. Resection Logs (Figure 18) are used to document which individual mussel specimens are included in each composite sample. In addition a daily log (lab notebook) of operations is maintained to record each day's activity (number of samples processed, observations, problems, resolutions, and so on).

Sampling Codes - Use and Creation

Data codes are assigned based on a coding system devised for and used by the PSEMP Toxics in Biota program. These include (but are not limited to) the following:

SurveyID

Each survey carried out by the PSEMP unit is assigned a SurveyID to differentiate it from surveys of the past and future. The PSEMP database manager creates a unique alpha numeric code that identifies the survey type and the year. The SurveyID for this project will be MusselWatch12.

StationID

Each station sampled by PSEMP is assigned a StationID code to help differentiate it from other locations sampled in the past, present and future. The database manager compares the latitude/longitude information for the sampling location in question against those of StationIDs listed in the database to determine if the location has been sampled in the past. A new location is assigned a descriptive name that is unique from all other StationIDs (using all capital letters for the text in the code) and a location which has been sampled in the past is assigned the same SampleID as the past sampling effort(s).

FishID

Each individual mussel collected by PSEMP that will contribute to a sample is assigned a FishID; a six digit numeric code that is unique from all past, present or future FishIDs. Each FishID consists of two parts, the first two digits represent the year and the last 4 digits are part of a sequential number series running from 0001 through 9999; allowing up to 9,999 FishIDs to be assigned in any one year. Through an informal agreement between PSEMP and NOAA's Environmental Conservation Division, each year PSEMP is assigned 0001 through 4999 and NOAA is assigned 5000 through 5000 to ensure that neither group duplicates the other's FishIDs.

To assign FishIDs:

1. Determine what FishID numbers for the current year have not already been assigned to specimens collected by PSEMP. Unassigned numbers are available for use.
2. By station, from the specimen collected, determine how many will be used for the composite sample and assign a sequential series of available FishIDs to them.

SampleID

All samples created by PSEMP are assigned a unique SampleID code that differentiates each sample from similar samples collected in the past, present or future. Whereas a FishID identifies an individual biological unit, SampleIDs are more analogous to a jar of tissue to be submitted for analysis. A SampleID may identify a composite of tissues from multiple FishIDs, or from a single FishID. A SampleID is a unique alpha-numeric code that is assigned to an analytical sample. Each SampleID consists of six parts, a two-character year code, followed by a two or more character site code, a dash, a two-character species code, a one or two-character matrix code and either a two-digit (composite sample) or 4-digit (individual FishID) sample number.

An example of a unique SampleIDs, created by concatenating numbers of label acronyms is as follows:

- Two digit year (“12” for 2012),
- Two or three (typically) digit station identifier (“EB” for Elliott Bay)
- A dash “-”
- Two digit species (“MT” for *Mytilus trossulus*)
- Single digit matrix (“W” for whole)
- A sequential number

In this case, 12EB-MTW01

Forms - Use and Creation

Once the database manager has determined the sampling codes, he/she then prepares the Specimen Forms and Tissue Resection Logs for use in the lab. The forms are printed on waterproof paper to facilitate use in the lab environment.

Specimen Form

Specimen Forms (Figure 9) are used to record information for each individual specimen processed for samples for a given station and survey. The information recorded includes station information, biological metrics, the SampleID (if assigned) and pertinent observations about the condition of the specimen. The following information is captured on a Specimen Form:

1. Station Information

SurveyID – database manager provides, preprinted on form

StationID – database manager provides, preprinted on form

Collection Date – preprinted on form

2. Specimen Information

Species – preprinted on form

Effort – Enter the EffortID if one has been assigned or a general description of the effort (e.g. Tow-1, Tow-2, Set-1, Set-2, Etc.)

FishID – database manager provides, preprinted on form

Sex – enter sex, if easily distinguishable from gross observation

Length – enter the length to the nearest 0.1 millimeter

Weight – enter the weight to the nearest 0.1 grams

Type of Sample – Indicate with a "Y" (Yes) or an "N" (No) whether or not a sample type indicated on the form was taken.

SampleID – database manager provides, preprinted on the form.

3. Observations: Note any unusual physical aberrations, lesions, parasites, etc.

Tissue Resection Log

A Tissue Resection Log (TRL) (Figure 21) uses SampleID as a unique identifier to document the location, species, tissue matrix, and amount of tissue contributed by each individual specimen to the composite. Each TRL form (front and back) is to reference only one station. Lab staff record each FishID for the specimens contributing tissue to the composite samples, the amount of tissue (tissue weight) contributed by each, the total weight of tissue in the composite sample (sample weight), the Sample ID for each composite sample, the date the specimens were collected (date collected), the date each sample was resected (resection date), the number of days the specimens were held before resection (days to resection) and any pertinent observations regarding the compositing procedure.

Mussel tissues and resected tissue samples will be stored in a WDFW Marine Laboratory freezer. The location and conditions of these samples will be recorded in a standard laboratory notebook used to track tissue samples for the PSEMP program. The temperature of this freezer is set at -20° C and is continuously monitored through data loggers tracked by [Washington State Enterprise Services](#). Any temperature anomalies will trigger an alarm, triggering on-site maintenance staff to contact a laboratory supervisor from a priority list of supervisors, for immediate attention. In addition, this freezer is backed up by emergency generators in case of power outage.

Labeling of Composite Sample Jars

To facilitate identification of composite samples compiled in glass jars, corresponding labels are attached to both the lid and the jar. Both labels are printed on cryogenic, laser printer ready labels produced by Diversified Biotech. The lid label has the SampleID printed on it and the jar label has the Year, Station, Species, Matrix, SampleID, Date (capture), jar Weight (empty weight with lid on) and tissue weight.

Equipment Cleaning Procedure

When processing specimens for contaminant analysis, anything (work-surfaces, instruments, etc.) that may contact those portions of a specimen that are subject to contaminant analysis must be cleaned before use.

A “clean” work-surface, means a surface (lab counter, cutting board, sorting tray, etc.) covered by aluminum foil fresh off the roll. The work surface is covered with at least one layer of aluminum foil and the foil must be changed between composites.

"Clean" instruments means stainless steel dissection tools and grinding apparatus (hand grinder and cutting blades) that have been washed in warm soapy water (Terg-A-Zyme®), thoroughly rinsed three times under warm running tap water, followed by a rinse with deionized water (held in Teflon squeeze bottle), solvent rinsed using isopropyl alcohol (held in a Teflon squeeze bottle) and then placed on aluminum foil for air drying.

The same clean instruments/surface can be used repeatedly, without re-cleaning, on specimens contributing to the same composite. They must be subjected to the complete cleaning procedure between composites. Lab personnel must change nitrile gloves between composites or anytime they feel the glove has touched a “dirty” item.

Mussel Processing in the Lab for Biometrics and Chemistry

Each site will be represented by a cage that contains four individually numbered bags of mussels (64 individuals). PSEMP lab staff will receive cages and bags of mussels from volunteers the day after retrieval, signing off on the field COC. Lab staff will then determine the mortality in each bag and select a random set of ten mussels from the four bags to measure condition index. The remaining portion of live mussels will be stored in a labeled plastic Ziploc type bag at -20°C until tissue resectioning for chemical analysis can take place at a later date, not to exceed three months from the date of retrieval.

Biometrics - Initial Assessment

Mortality

PSEMP lab staff will assess individual bags for dead or moribund mussels before retrieval. Dead or moribund mussels will be counted, recorded and removed. Mussels will be considered moribund if the animal is unable to tightly close its valves. Dead animals will be obvious if there is no soft tissue inside the valves, or if the mussel soft tissue inside is putrefied.

Condition Index

After dead mussels have been removed, condition index will be determined on ten randomly selected mussels, according to a method reported by Kagley (2003) as follows:

$$\text{Condition index (CI)} = \text{dry weight (g) of soft tissue/shell length (mm)} \times 100.$$

The ten mussels will be prepped for measuring condition index by the following procedure. If needed, byssal threads and barnacles will be removed from the shell of the mussels prior to measuring, to prevent exterior debris from interfering with measurements. Shell length will be measured from the umbo to the farthest posterior margin (Figure 19) to the nearest tenth of a millimeter (0.1 mm) using a digital caliper. Total Shell Length (TSL) will be recorded on Specimen forms (Figure 17).

Mussels will be opened by inserting a scalpel blade between the bivalve shells, severing the posterior and anterior adductor muscles (Figure 22). The shells will be spread apart at the hinge to reveal the soft tissue. At this point, the remaining byssal fibers will be cut from the byssal gland using scissors. Then using a Teflon squeeze bottle filled with DI water, the tissue will be gently rinsed of sediment and foreign material with care not to lose pieces of tissue. After draining excess water, a scalpel will be used to scrape all the mussel soft tissue (including the adductor muscle) from the shell onto a preweighed drying pan. The wet weight of the soft tissue will be measured to the nearest tenth of a gram (0.1g)

using a bench scale and recorded on the specimen form. The pans of tissue will then be placed in a drying oven (Figure 16) set at 120°C until the weight is constant (approximately 18 hours). After cooling to room temperature the resulting dry weight will then be recorded to the nearest tenth of a gram (0.1g) on the Specimen form.

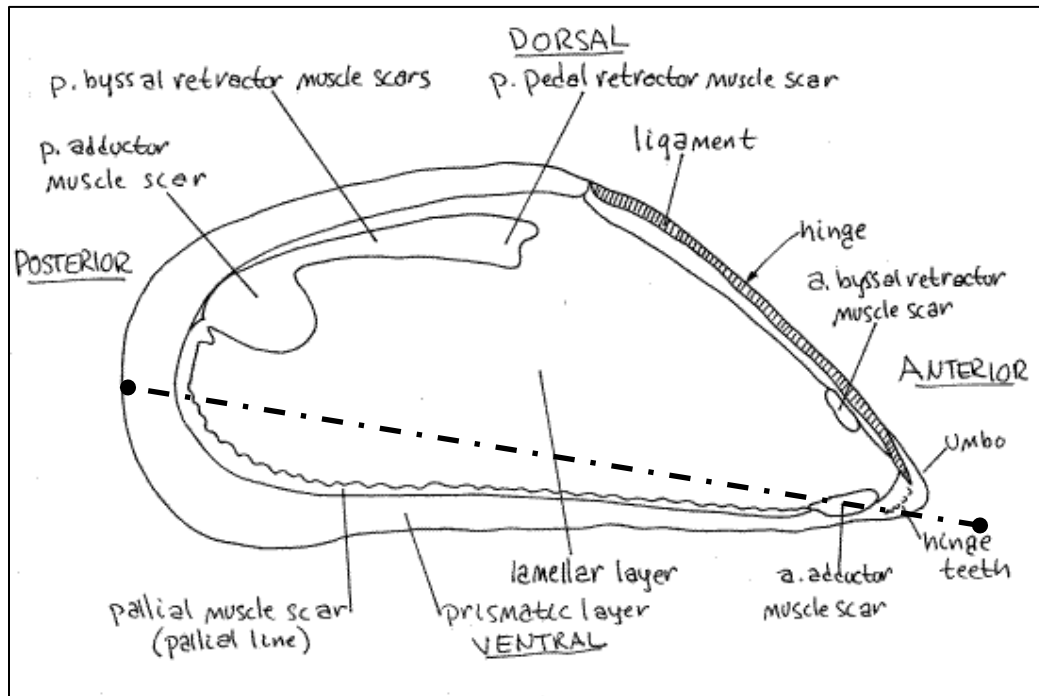


Figure 19. External anatomy of *Mytilus edulis* (Ruppert, Fox, and Barnes 2004)

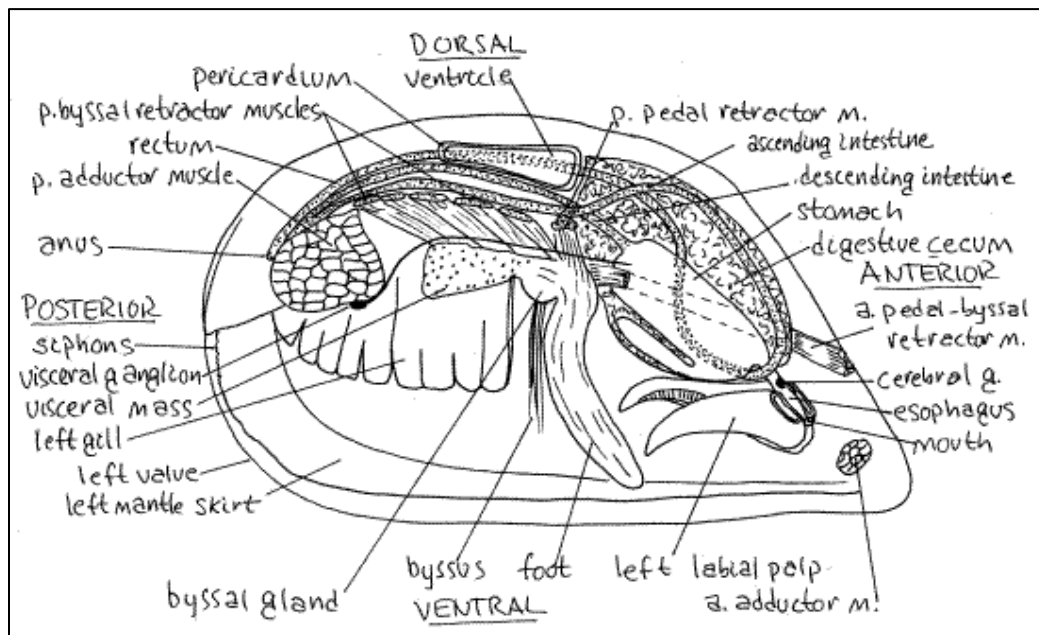


Figure 20. Internal anatomy of *Mytilus edulis* (Ruppert, Fox, and Barnes 2004)

Processing Mussels for Chemical Analysis

Resectioning Mussel Soft Tissue – Composite Samples

Previously frozen mussels will be thawed and prepared for tissue resectioning by the following procedure, which is a modification of Field Procedure 11.7 from the *Standard Guide for Conducting In-situ Field Bioassays with Caged Bivalves* (ASTM E2122-02, 2007). Lab staff will wear clean nitrile gloves and change gloves between each sample. Lab staff will maintain two sets of instruments per site; one set of tools to open the mussel, and one set of tools to remove tissue from the shell to the jar.

Prior to shucking the mussels for the soft tissue, byssal threads, sediment, biofouling, and barnacles will be removed from the shell of the mussels using scissors and gloved hands. Also, mussels will be rinsed several times with DI water to further remove external debris to reduce the risk of cross contamination after the mussels are opened.

Once cleaned and thawed sufficiently, lab staff will open each mussel by inserting a clean scalpel blade between the bivalve shells, severing the posterior and anterior adductor muscles. The shells will be spread apart at the hinge to reveal the soft tissue. The remaining byssal fibers will then be trimmed from the byssal gland using scissors. Then using a Teflon squeeze bottle filled with DI water, the tissue will be gently rinsed of sediment and foreign material with care not to lose pieces of soft mussel tissue. Excess water will be allowed to drain from the specimen. Using a scalpel, all soft tissue will be scraped (including the adductor muscle) into a clean I-CHEM (Class 200) glass sample jar.

Tissue from approximately 30 individual mussels from each site will be combined into a single pre-labeled composite sample jar, with the goal of collecting approximately 200 grams of tissue for each composite sample. Mussel tissue weight will be recorded as each specimen is added to the jar by taring the jar on the scale between individuals. Each composite sample will then be frozen for later homogenization. Unused whole mussels and cleaned (empty) mussel shells will be placed into a Ziploc bag with original bag-tags and re-frozen until the conclusion of the study.

Homogenizing Samples

After creation of composite samples, tissues will be ground in their original jars to a homogenous mixture. Partially thawed samples will be ground, using a Bamix hand mixer (Figure 13), to a consistency resembling pudding. Homogeneity will be determined by visual inspection. Samples will be labeled accordingly and frozen to -20°C until transfer to the analytical lab. Subsamples may be placed in separate, pre-labeled smaller I-Chem jars for convenience, to allow easier distribution of samples between labs, and for an archive sample.

Chemical Analyses -- Measurement Methods

Analytes

Table 8 Persistent organic pollutants to be measured in this study.

Persistent organic pollutants:	No. Analytes	Method	Limit of Quantitation - LOQ (wet weight)	Expected Range (wet weight)
Polychlorinated biphenyl (PCB) congeners	40	Sloan et al. 2004 ^a	0.2-0.8 ng/g	LOQ to 20 ng/g
Polybrominated diphenylethers (PBDEs) congeners	11	Sloan et al. 2004	0.2-0.8 ng/g	LOQ to 20 ng/g
Organochlorine pesticides (OCPs)	25	Sloan et al. 2004	0.2-0.8 ng/g	LOQ to 20 ng/g
Polycyclic Aromatic Hydrocarbons (PAHs)	45	Sloan et al. 2004	0.2-0.8 ng/g	LOQ to 20 ng/g

^aSloan, C. A., D. W. Brown, et al. (2004). Extraction, cleanup, and gas chromatography/mass spectrometry analysis of sediments and tissues for organic contaminants., U.S. Dept. Commerce. NOAA Tech. Memo. NMFS-NWFSC-59.

Table 9. Metals to be measured in this study.

Metals	No. Analytes	Method	Method Detection Limit (wet weight)	Expected Range (wet weight)
Total mercury (Hg)	1	KCEL SOP 604v6 ^b	0.005 µg/g	MDL to 5 µg/g
Lead (Pb)	1	KCEL SOP 624v2 ^c	0.004 µg/g	MDL to 5 µg/g
Arsenic (As)	1	KCEL SOP 624v2	0.004 µg/g	MDL to 5 µg/g
Zinc	1	KCEL SOP 624v2	0.004 µg/g	MDL to 5 µg/g
Copper	1	KCEL SOP 624v2	0.004 µg/g	MDL to 5 µg/g
Cadmium (Cd)	1	KCEL SOP 624v2	0.002 µg/g	MDL to 5 µg/g

^b KCEL SOP 604v6: King County Environmental Laboratory Standard Operating Procedure 604v6 - Instrumental Analysis for Mercury in Environmental Samples by Cold Vapor Atomic Absorption Spectrometry (see Appendix E)

^c KCEL SOP 624v2: King County Environmental Laboratory Standard Operating Procedure 624v2 - ICPMS Analysis of Water, Wastes, Sediments and Tissues by the Thermo X Series II CCT (see Appendix E)

Table 10 Conventional to be measured in this study.

Metals	No. Analytes	Method	Method Detection Limit (wet weight)	Expected Range (wet weight)
Lipid content (% total extractibles)	1	gravimetric	0.1%	0.5 to 3%
Dry Weight (%)	1	gravimetric	0.1%	10-20%
δ^{15} Nitrogen	1	See section 9.5		
δ^{13} Carbon	1	See section 9.5		

Matrix

Composited somatic mussel tissue will be the only matrix analyzed for chemical contaminants.

Number of samples

The maximum number of samples to be submitted for chemical analysis in this study is expected to be 124, which is 112 sites, 6 baseline samples and 6 control samples. WDFW and volunteers will continuously monitor to meet study goals and reduce loss of cages.

Expected range of results

The range of concentrations for persistent organic pollutants (POPs) in this study is from the Limit of Quantitation (LOQ) -- typically between 0.2 and 0.8 ng/g wet weight) to 20 ng/g wet weight for individual PCB or PBDE congeners, OCP isomers, or PAH analytes.

The range of concentration of metals should be from the limit of detection (approximately 0.005 to $\mu\text{g/g}$) to 5 $\mu\text{g/g}$ wet weight.

Analytical methods

Persistent Organic Pollutants

All POPs in this study will be analyzed according to Sloan et al. (2004). This analytical method is consistent with previous WDFW/PSEMP studies. In brief, this method comprises three steps: (a) extraction, (b), cleanup by silica/aluminum columns and size-exclusion high-performance liquid chromatography (SEC HPLC), and (c) quantitation of chlorinated hydrocarbons (CHs) and aromatic hydrocarbons (AHs) using gas chromatography /mass spectrometry (GC/MS) with selected-ion monitoring (SIM). Samples are extracted using accelerated solvent extraction (ASE with methylene chloride), which provides an extract that can be used for AH, CH recovery and gravimetric lipid evaluation. This method also includes alterations to typical GC/MS methods to stabilize the instrument and improve accuracy such as chemical ionization filaments (to increase source temperature), employing a cool on-column injection system in the GC, a guard column before the analytical column,

and point-to-point calibration to improve data fit over the full range of GC/MS calibration standards (Sloane et al. 2004).

Metals

All metals analyses will be performed by the King County Environmental Laboratory (KCEL). Appendix E contains the standard operating procedures for sample preparation and metals analyses. The metals mercury, arsenic, cadmium, and lead will be analyzed by two methods. Mercury will be analyzed via automated cold vapor atomic absorption spectrometry following King County Environmental Laboratory Standard Operating Procedure (KCEL SOP) 604v6. This SOP incorporates elements of EPA 245.1 revision 3, SW-846 7470, 7471B and PSEP 1997. Arsenic, cadmium, and lead will be analyzed via Thermo Elemental X Series II CCT (Collision Cell Technology) Inductively Coupled Plasma Mass Spectrometer (ICP-MS) following KCEL SOP 624v2. This SOP incorporates elements of EPA 200.8 revision 5.4, SW-846 6020A February 2007, ILM05.3 Exhibit D part B, and PSEP 1997. Total solids will be analyzed via KCEL SOP 307v3 to facilitate reporting metals data in both dry and wet weight concentrations.

Stable Isotopes

Stable isotopes of carbon (^{13}C) and nitrogen (^{15}N) will be measured by Mass Spectrometry (following Herman et al. 2005) after preparation as follows:

1. Homogenized tissue samples freeze-dried overnight
2. Freeze-dried tissue pulverized in a micro-ball mill
3. 0.4 to 0.6 mg powder of each sample placed into separate tin cups, in triplicate
4. Combusting samples in a Costech elemental analyzer attached to a Thermo-Finnegan Delta Plus Isotope Ratio Mass Spectrometer

Values are calibrated with internal standards every ten samples. Unenriched histidine is used as a control material to evaluate set-to-set reproducibility, analyzed after every 25 samples. Stable isotope results are expressed in “delta” (δ) notation in ‰:

$$\delta Z = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 1000 (1),$$

where Z is ^{15}N or ^{13}C ,

R_{sample} is the ratio $^{15}\text{N}/^{14}\text{N}$ or $^{13}\text{C}/^{12}\text{C}$ for the tissue sample, and

R_{standard} is the ratio of $^{15}\text{N}/^{14}\text{N}$ or $^{13}\text{C}/^{12}\text{C}$ of standards (atmospheric air for nitrogen and Pee Dee Belemite limestone for carbon).

Percent Lipids

Percent lipids in each sample are represented by total extractibles, according to Sloan et al. 2004. Briefly samples from the extraction step of the POP analyses (Section 9.5.1) will be evaporated and compared to the mass of the original, unextracted sample (paraphrasing from Sloan et al. 2004):

The pan containing the sample for total extractables from Section 3 is placed on a covered rack in the hood and the solvent is allowed to completely evaporate (approximately 1–2 hours). The pan is dried in a 50°C oven for 2 hours, then cooled in a desiccator overnight. The pan is weighed to the nearest 0.0001g and the weight is recorded as the “Pan w/TE” weight. The percent total extractables (% TE) content of the sample is calculated as follows:
% TE = ((Pan w/TE – Pan) x (ASE Vial w/Extract – ASE Vial) x 100%)/
((ASE Vial w/Extract – ASE Vial w/o TE Extract) x Sample Weight).

Percent solids (Dry Weight) Determination

The percent of the sample as dry weight is determined by simple drying of tissues according to Sloan et al. 2004 (paraphrasing):

Pre-homogenized tissue (1 + 0.5 g) is placed into the pan, and the pan is weighed to the nearest 0.001 g. The weight is recorded as the “Pan w/Wet Sample” weight. The pan is placed in a drying oven at 120°C for 24 hours then cooled in a desiccator for 30 minutes. The pan is weighed to the nearest 0.001 g, and the weight is recorded as the “Pan w/Dry Sample” weight. The percent dry weight of the sample is determined as follows:

$$\% \text{ Dry Weight} = ((\text{Pan w/Dry Sample} - \text{Pan}) \times 100\%) / (\text{Pan w/Wet Sample} - \text{Pan}).$$

Sensitivity/Method Detection Limit (MDL)

The Lower Limit of Quantitation (LOQ) for all POPs in this study is “the concentration that would be calculated if that analyte had a GC/MS response area equal to its area in the lowest level calibration standard used in that calibration. When an analyte is not detected in a sample or it has a response area that is smaller than its area in the lowest level calibration standard used, the concentration of the analyte in that sample is reported to be less than the value of its lower LOQ.” (Sloan et al. 2006). Typically LOQ values for POPs that have been reported to PSEMP by this method are in the range of 0.2 to 0.8 ng/g wet weight.

EPA defines Method Detection Limit (MDL) in Appendix A to 40 CFR Part 136 as the “minimum concentration of a substance that can be measured and reported with 99 percent confidence that the analyte concentration is greater than zero and is determined from analysis of a sample in a given matrix containing the element”. In this study, the metal’s MDLs are concentrations that cannot be detected or detected at a concentration less than the associated method detection limit considering tissue sample detection limits are affected by the sample mass used, matrix and polyatomic/isobaric interferences. The MDL is the lowest concentration at which a sample result will be reported. **Error! Reference source not found.** lists the respective method detection limits for the four metals of concern (Hg, As, Cu, Zn, Cd, and Pb). They range from 0.002 to 0.005 µg/g wet weight.

Sample preparation method(s)

Mussels will be resection and prepared for tissue analysis according to Section 8.5.2. Mussel tissue samples will be shipped to the analytical labs frozen. The analytical labs will thaw and thoroughly mix the tissue samples with clean utensils to ensure adequate homogeneity prior to sample preparation for chemical analysis.

9.0 Quality Control (QC) Procedures

Quality control procedures, quality assurance criteria and corrective actions for POPs data are detailed in Sloan et al. (2006). Briefly, precision is monitored and controlled within batches using laboratory replicates of field samples (2 replicates run for every batch of 12 samples) and across batches by analyzing Standard Reference Materials (SRMs –one per batch). Cross-batch precision is expressed as the relative standard deviation (RSD) for repeated measurements. The RSD of analyte responses relative to the internal standard must be $\leq 15\%$ for the repetitions.

For POPs analysis, accuracy of samples is evaluated by comparing measured SRM values with National Institute of Standards and Technology (NIST) certified values. A SRM of applicable matrix will be selected to be analyzed i.e., tissue. Concentrations of $\geq 70\%$ of individual analytes are to be within 30 % of either end of the 95% confidence interval of the reference values. One method blank is run for every 20 or fewer field samples. No more than 5 analytes in a method blank are to exceed 2x the lower LOQ before corrective action is taken. The corrective action will be to re-extract and re-analyze the affected samples and if necessary, qualify the sample data. At least one internal standard (surrogate) is added to each sample, with acceptable recoveries ranging from 60 to 130%.

Quality control measure and quality assurance criteria for metals data are detailed in Table 6 and Table 7. Briefly, precision is monitored and controlled within batches using laboratory replicates of field samples and matrix spike duplicates (one per batch). Accuracy of analysis is evaluated by comparing measured standard reference material (SRM) values and a laboratory control sample (LCS) with the respective certified values. A SRM of applicable matrix will be selected to be analyzed i.e., tissue. Method blanks and spikes are evaluated for overall run and process contamination. These are run every batch as is applicable.

10.0 Data Management Procedures

Data recording/reporting requirements

Data are received from analytical laboratories in Excel spreadsheets in various formats. PSEMP staff format these data into a structure compatible with the Toxics in Biota (TIB) database. The TIB database is a relational format created in Access, with separate tables for (1) field effort data, (2) biological characteristics of individuals used to create samples, (3) many-to-many cross reference for individuals-to-composites, (4) sample tracking, condition and summary statistics, and (5) chemical analyses. The TIB database is stored on a WDFW server, which is backed up nightly as part of an automated network backup service provided by WDFW Information Technology (IT) Services.

Data are examined visually using Excel filters and sorting procedures to identify gross formatting or transcription errors. Raw analyte concentrations are compared with expected ranges to identify potential outliers. In addition preliminary tables of summary statistics, scatter plots, and time trend plots are created to examine the new data.

EIM data upload procedures

All data generated by this project will be submitted to Ecology's EIM database.

11.0 Audits and Reports

Frequency of Audits

The NWFSC analytical lab participates in annual NIST or IAEA interlab comparison studies. The King County Environment Lab is an accredited with Washington Department of Ecology (ECY) and is audited based on the ECY schedule.

Responsibility for reports

WDFW staff will submit final reports and data packages EPA's STORET and to Ecology's EIM database as detailed in the Scope of Work. James E. West is responsible for these products.

12.0 Data Verification and Validation

Field data verification, requirements, and responsibilities

All sample location data for this study are verified by comparing GIS-plotted latitude and longitude data with field notes provided by samplers. Deployment and retrieval information are also validated with photographic evidence from the site. Size distributions of mussels will be checked at the end of each daily effort in the laboratory to identify any size outliers.

Lab data verification and validation

Data generated by the analytical lab are reviewed for out-of-bounds values, transcription errors and other problems by at least two chemists. Final review is conducted by a lab manager who approves data before they are released to the client. Prior to database entry the client reviews data by comparing results with similar species or matrices in the PSEMP database. Individual data, means, and standard deviations are plotted and putative outliers evaluated for validity. Evaluation of the validity of putative outliers includes reviewing all collection, biological, and analytical data for potential transcription errors, communication with analytical labs to verify reported values are correct, and evaluation of biological covariates that might explain otherwise unanticipated values. PSEMP does not currently conduct data validation by a third party reviewer.

13.0 Data Quality (Usability) Assessment

Process for determining whether project objectives have been met

The success of meeting data quality objectives is evaluated based on the outcome of quality control procedures during analytical procedures. Typically if QC criteria are not met the problem is identified, corrected, and sample (or extract) re-run. In cases where QC criteria have not been met and there is not enough tissue to be reanalyzed, the data are to be censored with appropriate qualifiers to allow an objective evaluation of the usability of the final record. Rejected data are censored with an “R” or equivalent qualifier. Based on (1) a long history of employing these methods to measure target analytes in a wide range of Puget Sound biota matrices, (2) the range of data values we expect in this study, and (3) appropriate (tenth-of-ppb) limits of quantitation, we expect rejected data to be rare, with the singular possible exception of potential blank contamination for naphthalene-compounds.

Adequacy of sample number will be evaluated during the statistical analysis of analytes. We have predicted that five replicates per class will provide enough power to distinguish spatial trends in most analytes, however a final evaluation of sample size adequacy will be made after this analysis.

Data analysis and presentation methods

Toxics data collected for this study are part of a long-running tissue residue monitoring program. This program has a long history of data analysis and presentation, which will be continued in the present study. Analysis and presentation of contaminant and covariate data will be conducted using programs commonly employed by PSEMP to compare spatial distribution of contaminants. This includes a General Linear Model that compares contaminant concentrations across geographic locations while adjusting for potentially obfuscating covariates such as condition index and trophic level.

Treatment of non-detects

Non-detected analytes are censored with a “<LOQ” or “U” qualifier. The value reported for non-detected analytes will be the LOQ or Method Detection Limit, depending on analytical procedure. It is the responsibility of data users to decide how to use data censored as not-detected. Because the current study will primarily report **analyte sums** or totals for major groups and compared across a wide range of conditions from highly contaminated to relatively pristine, we anticipate substituting zero for “U” qualified data in contaminant-class summations. Previous experience with data from similar studies for the target analytes in this study suggest that summed totals will be dominated by substantial concentrations of a number of individual analytes. Substituting zero, or any trivial or nominal concentration, is not anticipated to change comparison results for summed analytes.

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15.0 Appendices

APPENDIX A. GLOSSARY, ACRONYMS, AND ABBREVIATIONS

Glossary

Adductor muscle – a muscle in the interior of a bivalve mollusk which close the valves.

Accreditation - A certification process for laboratories, designed to evaluate and document a lab's ability to perform analytical methods and produce acceptable data. For Ecology, it is "Formal recognition by (Ecology)...that an environmental laboratory is capable of producing accurate analytical data." [WAC 173-50-040] (Kammin, 2010)

Accuracy - the degree to which a measured value agrees with the true value of the measured property. USEPA recommends that this term not be used, and that the terms precision and bias be used to convey the information associated with the term accuracy. (USGS, 1998)

Analyte - An element, ion, compound, or chemical moiety (pH, alkalinity) which is to be determined. The definition can be expanded to include organisms, e. g. fecal coliform, Klebsiella, etc. (Kammin, 2010)

Assessment Units (AU) - predefined watershed catchment areas used for this study, originally developed by Ecology, with a median area of 8.8 km² (3.4 mile²).

Benthic - Living on or closely associated with the bottom of a body of water. Or relating to, or living in a benthos, which is the sediment-water interface of an ocean, sea, or lake.

Bias - The difference between the population mean and the true value. Bias usually describes a systematic difference reproducible over time, and is characteristic of both the measurement system, and the analyte(s) being measured. Bias is a commonly used data quality indicator (DQI). (Kammin, 2010; Ecology, 2004)

Bivalves - A taxonomic class of marine and freshwater mollusks that have a laterally compressed body enclosed by a shell in two hinged parts. This class includes the clams, oysters, mussels, scallops and numerous other families.

Blank - A synthetic sample, free of the analyte(s) of interest. For example, in water analysis, pure water is used for the blank. In chemical analysis, a blank is used to estimate the analytical response to all factors other than the analyte in the sample. In general, blanks are used to assess possible contamination or inadvertent introduction of analyte during various stages of the sampling and analytical process. (USGS, 1998)

Calibration - The process of establishing the relationship between the response of a measurement system and the concentration of the parameter being measured. (Ecology, 2004)

Check standard - A substance or reference material obtained from a source independent from the source of the calibration standard; used to assess bias for an analytical method. This is an obsolete term, and its use is highly discouraged. See Calibration Verification Standards, Lab Control Samples (LCS), Certified Reference Materials (CRM), and/or spiked blanks. These are all check standards, but should be referred to by their actual designator. (i. e. CRM, LCS, etc.) (Kammin, 2010; Ecology, 2004))

Comparability - The degree to which different methods, data sets and/or decisions agree or can be represented as similar; a data quality indicator. (USEPA, 1997)

Completeness - The amount of valid data obtained from a data collection project compared to the planned amount. Completeness is usually expressed as a percentage. A data quality indicator. (USEPA, 1997)

Composite – Predetermined number of individuals consisting of one species specific matrix (i.e., muscle tissue) created through homogenous mixing to represent a location or field replicate in chemical analysis.

Condition Index (CI) - serves as an indication of the influence of seasonal fluctuations, such as temperature and food availability, on the physiological status of bivalves. It allows for better comparison of mussels from different locations by normalizing biological changes over time and minimizing the influence of internal factors (e.g. mussel growth rates). $CI = \text{dry weight (g) of soft tissue/shell length (mm)} \times 100$.

Contaminant - A substance that makes something dirty, polluted, or toxic

Continuing Calibration Verification Standard (CCV) - A QC sample analyzed with samples to check for acceptable bias in the measurement system. The CCV is usually a midpoint calibration standard that is re-run at an established frequency during the course of an analytical run. (Kammin, 2010)

Control chart - A graphical representation of quality control results demonstrating the performance of an aspect of a measurement system. (Kammin, 2010; Ecology 2004)

Control limits - Statistical warning and action limits calculated based on control charts. Warning limits are generally set at +/- 2 standard deviations from the mean, action limits at +/- 3 standard deviations from the mean. (Kammin, 2010)

Data Integrity- A qualitative DQI that evaluates the extent to which a dataset contains data that is misrepresented, falsified, or deliberately misleading. (Kammin, 2010)

Data Quality Indicators (DQI) - Data Quality Indicators (DQIs) are commonly used measures of acceptability for environmental data. The principal DQIs are precision, bias, representativeness, comparability, completeness, sensitivity, and integrity. (USEPA, 2006)

Data Quality Objectives (DQO) - Data Quality Objectives are qualitative and quantitative statements derived from systematic planning processes that clarify study objectives, define the appropriate type of

data, and specify tolerable levels of potential decision errors that will be used as the basis for establishing the quality and quantity of data needed to support decisions. (USEPA, 2006)

Dataset - A grouping of samples, usually organized by date, time and/or analyte. (Kammin, 2010)

Data validation - An analyte-specific and sample-specific process that extends the evaluation of data beyond data verification to determine the usability of a specific data set. It involves a detailed examination of the data package, using both professional judgment, and objective criteria, to determine whether the MQOs for precision, bias, and sensitivity have been met. It may also include an assessment of completeness, representativeness, comparability and integrity, as these criteria relate to the usability of the dataset. Ecology considers four key criteria to determine if data validation has actually occurred.

These are:

- Use of raw or instrument data for evaluation
- Use of third-party assessors
- Dataset is complex
- Use of EPA Functional Guidelines or equivalent for review

Examples of data types commonly validated would be:

- Gas Chromatography (GC)
- Gas Chromatography-Mass Spectrometry (GC-MS)
- Inductively Coupled Plasma (ICP)

The end result of a formal validation process is a determination of usability that assigns qualifiers to indicate usability status for every measurement result. These qualifiers include:

- No qualifier, data is usable for intended purposes
- J (or a J variant), data is estimated, may be usable, may be biased high or low
- REJ, data is rejected, cannot be used for intended purposes (Kammin, 2010; Ecology, 2004)

Data verification - Examination of a dataset for errors or omissions, and assessment of the Data Quality Indicators related to that dataset for compliance with acceptance criteria (MQO's). Verification is a detailed quality review of a dataset. (Ecology, 2004)

Detection limit (limit of detection) - The concentration or amount of an analyte which can be determined to a specified level of certainty to be greater than zero. (Ecology, 2004)

Duplicate samples - two samples taken from and representative of the same population, and carried through and steps of the sampling and analytical procedures in an identical manner. Duplicate samples are used to assess variability of all method activities including sampling and analysis. (USEPA, 1997)

Field blank - A blank used to obtain information on contamination introduced during sample collection, storage, and transport. (Ecology, 2004)

Impervious surfaces – artificial structures, such as pavements and rooftops, which are covered by impenetrable materials such as asphalt, concrete, brick, and stone. Soils compacted by urban development are also considered impervious.

Initial Calibration Verification Standard (ICV) - A QC sample prepared independently of calibration standards and analyzed along with the samples to check for acceptable bias in the measurement system. The ICV is analyzed prior to the analysis of any samples. (Kammin, 2010)

Laboratory Control Sample (LCS) - A sample of known composition prepared using contaminant-free water or an inert solid that is spiked with analytes of interest at the midpoint of the calibration curve or at the level of concern. It is prepared and analyzed in the same batch of regular samples using the same sample preparation method, reagents, and analytical methods employed for regular samples. (USEPA, 1997)

Limit of Quantitation (LOQ) – In organic analyses, the LOQ is the concentration that would be calculated if that analyte had a GC/MS response area equal to the area of the lowest level calibration standard used in that calibration. Similar to a Detection Limit (DL) in metals analyses.

Matrix spike - A QC sample prepared by adding a known amount of the target analyte(s) to an aliquot of a sample to check for bias due to interference or matrix effects. (Ecology, 2004)

Measurement Quality Objectives (MQOs) - Performance or acceptance criteria for individual data quality indicators, usually including precision, bias, sensitivity, completeness, comparability, and representativeness. (USEPA, 2006)

Measurement Result - A value obtained by performing the procedure described in a method. (Ecology, 2004)

Method - A formalized group of procedures and techniques for performing an activity (e.g., sampling, chemical analysis, data analysis), systematically presented in the order in which they are to be executed. (EPA, 1997)

Method blank - A blank prepared to represent the sample matrix, prepared and analyzed with a batch of samples. A method blank will contain all reagents used in the preparation of a sample, and the same preparation process is used for the method blank and samples. (Ecology, 2004; Kammin, 2010)

Method Detection Limit (MDL) - This definition for detection was first formally advanced in 40CFR 136, October 26, 1984 edition. MDL is defined there as the minimum concentration of an analyte that, in a given matrix and with a specific method, has a 99% probability of being identified, and reported to be greater than zero. (Federal Register, October 26, 1984)

Organic - Material derived from the remains or products of living entities.

Percent Relative Standard Deviation (%RSD) - A statistic used to evaluate precision in environmental analysis. It is determined in the following manner:

Percent relative standard deviation, %RSD = $(100 * s)/x$ where s = sample standard deviation, and x = sample mean (Kammin, 2010)

Parameter - A specified characteristic of a population or sample. Also, an analyte or grouping of analytes. Benzene, nitrate+nitrite, and anions are all “parameters”. (Kammin, 2010; Ecology, 2004)

Population - The hypothetical set of all possible observations of the type being investigated. (Ecology, 2004)

Precision - The extent of random variability among replicate measurements of the same property; a data quality indicator. (USGS, 1998)

Protandric - Hermaphrodite animals that develop and function as males then undergo a transformation into females for the remainder of their lives.

Quality Assurance (QA) - A set of activities designed to establish and document the reliability and usability of measurement data. (Kammin, 2010)

Quality Assurance Project Plan (QAPP) - A document that describes the objectives of a project, and the processes and activities necessary to develop data that will support those objectives. (Kammin, 2010; Ecology, 2004)

Quality Control (QC) - The routine application of measurement and statistical procedures to assess the accuracy of measurement data. (Ecology, 2004)

Relative Percent Difference (RPD) -. RPD is commonly used to evaluate precision. The following formula is used: $Abs(a-b)/((a+b)/2) * 100$

Where a and b are 2 sample results, and abs() indicates absolute value. RPD can be used only with 2 values. More values, use %RSD. (Ecology, 2004)

Replicate samples - two or more samples taken from the environment at the same time and place, using the same protocols. Replicates are used to estimate the random variability of the material sampled. (USGS, 1998)

Representativeness - The degree to which a sample reflects the population from which it is taken; a data quality indicator. (USGS, 1998)

Resect - To remove part or all of an organ or tissue.

Resection - Excision of a portion or all of an organ or other structure.

Sample (field) – A portion of a population (environmental entity) that is measured and assumed to represent the entire population. (USGS, 1998)

Sample (statistical) – A finite part or subset of a statistical population. (USEPA, 1997)

Sensitivity - In general, denotes the rate at which the analytical response (e.g., absorbance, volume, meter reading) varies with the concentration of the parameter being determined. In a specialized sense, it has the same meaning as the detection limit. (Ecology, 2004)

Shellfish - An exoskeleton or shell bearing aquatic invertebrate, includes various species of mollusks (bivalves), crustaceans, and echinoderms.

Spiked blank - A specified amount of reagent blank fortified with a known mass of the target analyte(s); usually used to assess the recovery efficiency of the method. (USEPA, 1997)

Spiked sample - A sample prepared by adding a known mass of target analyte(s) to a specified amount of matrix sample for which an independent estimate of target analyte(s) concentration is available. Spiked samples can be used to determine the effect of the matrix on a method's recovery efficiency. (USEPA, 1997)

Split Sample – The term split sample denotes when a discrete sample is further subdivided into portions, usually duplicates. (Kammin, 2010)

Standard Operating Procedure (SOP) – A document which describes in detail a reproducible and repeatable organized activity. (Kammin, 2010)

Surrogate – For environmental chemistry, a surrogate is a substance with properties similar to those of the target analyte(s). Surrogates are unlikely to be native to environmental samples. They are added to environmental samples for quality control purposes, to track extraction efficiency and/or measure analyte recovery. Deuterated organic compounds are examples of surrogates commonly used in organic compound analysis. (Kammin, 2010)

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Acronyms and Abbreviations

Following are acronyms and abbreviations used frequently in this report.

%IS	Percent Impervious Surface
AHs	Aromatic Hydrocarbons
ASE	Accelerated solvent extraction
AU	Assessment Unit
CHs	Chlorinated Hydrocarbons
COC	Chain of Custody
DNR	Washington Department of Natural Resources
DOH	Washington State Department of Health
e.g.	For example
Ecology	Washington State Department of Ecology
EIM	Environmental Information Management database
EPA	U.S. Environmental Protection Agency
et al.	And others
GC/MS	Gas Chromatography / Mass Spectrometry
GIS	Geographic Information System software
GPS	Global Positioning System
i.e.	In other words or that is
LOQ	Limit of Quantitation
MQO	Measurement quality objective
NIST	National Institute of Standards and Technology
OCPs	Organochlorine pesticides
PBDEs	Polybrominated diphenyl ethers
PCBs	Polychlorinated biphenyls
POPs	Persistent organic pollutants
PSEMP	Puget Sound Ecosystem Monitoring Program
PSP	Puget Sound Partnership
PSWQA	Puget Sound Water Quality Authority
QA	Quality assurance
QC	Quality control
RPD	Relative percent difference
RSD	Relative standard deviation
SEC HPLC	Size-exclusion high-performance liquid chromatography
SOP	Standard operating procedure
SRM	Standard reference material
STORET	STorage and RETrieval data warehouse – EPA's repository and framework for sharing ecological monitoring data
WDFW	Washington Department of Fish and Wildlife

Units of Measurement

°C	degrees Centigrade
dw	dry weight
ft	feet
g	gram, a unit of mass
kg	kilograms, a unit of mass equal to 1,000 grams.
km	kilometer, a unit of length equal to 1,000 meters.
m	meter
mg	milligram
mg/Kg	milligrams per kilogram (parts per million)
mm	millimeter
ng/g	nanograms per gram (parts per billion)
ng/Kg	nanograms per kilogram (parts per trillion)
pg/g	picograms per gram (parts per trillion)
ug/g	micrograms per gram (parts per million)
ug/Kg	micrograms per kilogram (parts per billion)
ww	wet weight

APPENDIX B -- STANDARD OPERATING PROCEDURES FOR ANALYSIS OF METALS BY KING COUNTY ENVIRONMENTAL LABS.

Double-click on each file to open it.

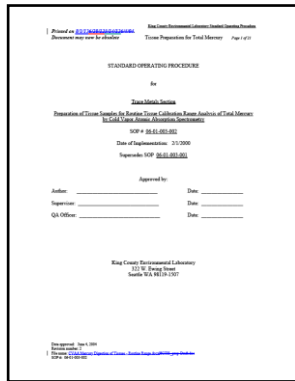


Figure 23. Preparing samples for routine mercury analysis

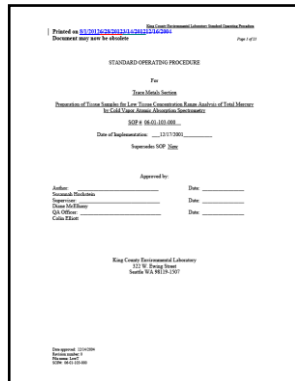


Figure 25. Preparing samples for low-level mercury analysis

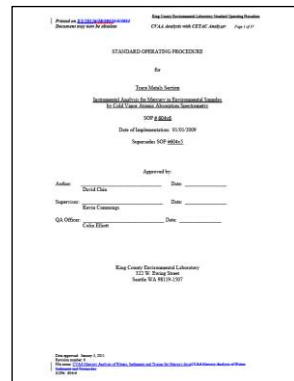


Figure 24. Cold Vapor Atomic Absorption (CVA) method for mercury analysis.

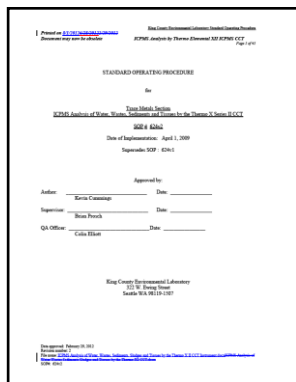


Figure 22. Inductively Coupled Plasma Mass Spectrometry (ICP-MS) method for analysis of metals.

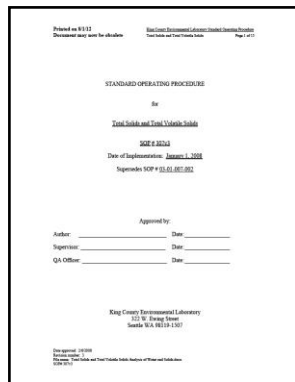


Figure 21. Method for determining total solids in a sample.

1.0 Title Page/TOC/Distribution List

Quality Assurance Project Plan

Mussel Watch Pilot Expansion Project

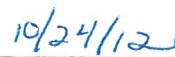
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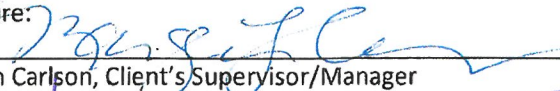


Patricia Jatzak, Client

Date:

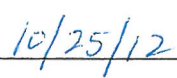


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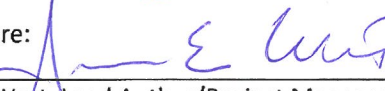


Margen Carlson, Client's Supervisor/Manager

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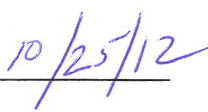


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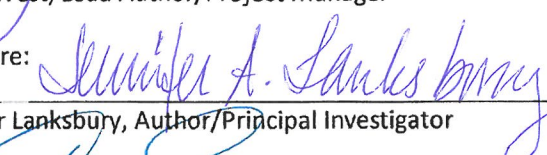


James West, Lead Author/Project Manager

Date:

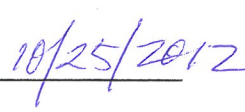


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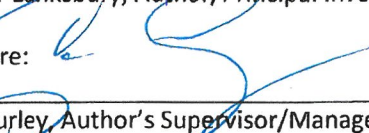


Jennifer Lanksbury, Author/Principal Investigator

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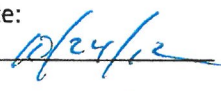


Signature:



Craig Burley, Author's Supervisor/Manager

Date:



Signature:



William Kammin, Quality Assurance Officer, Ecology:

Date:

