Biological Sampling and Analysis in Sinclair and Dyes Inlets, WA

FY2005 Quality Assurance Project Plan

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Water Body Numbers

WA-15-0040 Sinclair Inlet
WA-15-0050 Dyes Inlet

Photo by Greg Williams (PNL-BMSL)

Prepared by
Puget Sound Naval Shipyard & Intermediate
Maintenance Facility Project ENVVEST

For

Washington State Department of Ecology
Assessments Sections

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October 4, 2002

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WA-15-0040 Sinclair Inlet
WA-15-0050 Dyes Inlet

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1. Introduction

The Sinclair/Dyes Inlets watershed is located in Kitsap County, Washington. The boundaries of the watershed include the receiving waters of Sinclair and Dyes Inlets extending out from the Inlets into the passages that connect them with the main reaches of the Puget Sound and the surrounding landscape that drains into the inlets (Figure 1). Both Sinclair Inlet and Dyes Inlets were listed on the State of Washington's 1998 Section 303(d) list of impaired waters because of fecal coliform contamination in marine waters and tributary streams, heavy metals and toxic organics in the bottom sediments, and polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyl (PCBs), aldrin, dieldrin, Hg, and As in the tissues of marine organisms (WDOE 1998). As part of a cooperative watershed project for the Inlets the Puget Sound Naval Shipyard & Intermediate Maintenance Facility (PSNS & IMF), the Environmental Protection Agency (EPA), the Washington State Department of Ecology (Ecology), and other technical stakeholders are cooperating in an ENVironmental inVESTment (ENVVEST) project know as Project ENVVEST (see Final Project Agreement) to develop Total Maximum Daily Loads (TMDL) and assess ecological risk within the watershed (U.S. Navy, U.S. EPA, and Ecology 2000, ENVVEST 2001a).

Currently studies are underway to support a fecal coliform TMDL for the Inlets and tributary streams (May et al. 2004, see Sincliar/Dyes Water Cleanup Plan Home Page). Storm event sampling is being conducted to collect representative data on stream and storm water runoff quality as a function of hydrology, land use, and land cover with the watershed (ENVVEST 2002a, ENVVEST 2004a, May et al. 2004, Johnston et al. 2005), an integrated watershed and receiving water model is being developed to simulate loading and runoff from the watershed (Johnston et al. 2003, Skahill 2004, Johnston and Wang 2004), and sediment contamination levels are being addressed by the sediment metals (Kohn et al. 2004) and organics (Kohn et al. 2005) verification studies. Additional studies are being conducted by the Navy's installation restoration program to monitoring the effectiveness of remediation and navigational dredging to cleanup PCB and Hg contamination in the sediments of the Inlets (Ginn 2004, Johnston et al. 2005). Investigations on arsenic contamination in fish and shellfish tissues (Johnson and Roose 2002) and an initial evaluation of contaminant levels in demersal fish and macroinvertebrates from the 2003 PSAMP sampling (ENVVEST 2004b) has also been conducted for the Inlets. Ultimately, how effective pollution abatement, cleanup, and restoration programs are will be reflected by the health and status of marine organisms living within the Inlets. Furthermore, information about bioaccumulation and ecological stress of organisms resident in the Inlets will support decisions about setting priorities for implementing TMDLs and determining what contaminants should be on the 303(d) List.

This sampling plan describes specific sampling activities to obtain data necessary to characterize bioaccumulation and ecological stress to marine resources within the Inlets, identify potential sources of ecological stress, and assess ecological risks to ecological receptors within the Inlets (Table 1). This document identifies the objectives, procedures, and quality assurance/quality control (QA/QC) requirements for the biological sampling to be completed by Project ENVVEST for 2005.

2. Objectives

The objectives of this sampling plan are to:

1. Assist in field collection of demersal fish and macro-invertebrate samples from Sinclair Inlet and selected reference locations in the Puget Sound for chemical analysis and obtain live tissue samples of the specimens collected for chemical analysis to evaluate potential biological effects from exposure to contaminants.

2. Conduct a caged mussel bioassay at seven locations in Sinclair and Dyes Inlets to evaluate potential biological effects from ambient exposures to marine organisms.

The data obtained from this sampling effort will be used to characterize bioaccumulation and ecological stress to marine resources within the Inlets, identify potential sources of ecological stress, and assess ecological risks to ecological receptors within the Inlets.

3. Background

An alternative model for developing and implementing new environmental regulations within the clean water act is being tested through an ENVironmental inVESTment Project Agreement (ENVVEST) among PSNS&IMF, EPA and Ecology. This model is specifically addressing the development of Total Maximum Daily Loads (TMDL)s for the Sinclair/Dyes Inlet surface water system adjacent to PSNS&IMF and assisting the Shipyard in meeting current and future National Pollution Discharge and Elimination System (NPDES) requirements (ENVVEST 2002a, b). Understanding and addressing all sources of pollution coming into the Inlets will help regulatory agencies prioritize pollution control and water cleanup plans and focus resources on obtaining measurable improvements in the quality of the environment. Both point and nonpoint pollution sources are being quantified because they will have a direct bearing on setting allowable discharges for industrial activities at the Shipyard.

For a complete summary of current and ongoing work for Project ENVVEST please access the following web pages:

- 2005 Storm Event Sampling and Logistics
- Sediment Contamination in Sinclair and Dyes Inlet Watershed
- Fecal_Coliform_TMDL_Study_Final_Report_(May_et_al_2005)
- Watershed Studies
- Biological Studies
- Data Animations and Model Simulation Results

4. Overview of Sampling

The deposition and transport of contaminants within Sinclair and Dyes Inlets and assessing the impact of these contaminants on the local marine biota requires the cooperation of the various stakeholders conducting environmental monitoring programs in the region. The Puget Sound Ambient Monitoring Program (PSAMP) is a multi-agency effort to monitor the health of Puget Sound. The spatial and temporal trends in contaminant exposure in Puget Sound fish and macro-invertebrates and the effects of contaminant exposure on the health of these resources is assessed by trawl sampling at various sampling stations throughout Puget Sound (WDFW 2003). In order to increase the data yield from these efforts, Project ENVVEST is proposing to collaborate with WDFW to collect additional biological samples for contaminant bioaccumulation analysis and the collection of selected tissue samples to measure biomarkers of sublethal stress. An additional effort will involve the deployment of filter-feeding bivalves at stations within the Sinclair/Dyes region and selected reference locations, which will be analyzed for contaminant bioaccumulation and sublethal measures of stress.

The demersal fish and macro invertebrates sampling will be scheduled to occur along with the PSAMP otter trawl sampling during the late spring and early summer of 2005 (Figure 2). The mussel deployment will be scheduled to occur post-spawning during the summer of 2005 for 60 to 90 days at seven locations within Sinclair and Dyes Inlets (Figure 3).

5. Sampling Design

5.1 Trawl Sampling

The purpose of trawl sampling is to obtain data on the abundance, biomass, diversity, health status, and disease prevalence of demersal fish and macroinvertebrate assemblages at selected locations in the Puget Sound. It is also used to collect fish and macroinvertebrates for tissue contaminant analysis and tissue collection for biomarker measurements. This information is useful in characterizing possible anthropogenic effects on demersal fish and macroinvertebrate populations. The PSAMP protocols will be followed for trawling, species identification, enumeration, length and weight determinations, and tissue collection for chemical analysis (WDFW 2003). The WDFW study assesses five factors that are indicators of the health of demersal fish and invertebrates in the Puget Sound. The focus of the WDFW's study for 2005 is to measure PCBs, Hg, and PAHs in English sole, quillback, and crabs (Sandie O'Neil, WDFW, personal communication). The effort being conducted by ENVVEST will provide complimentary information on contaminant levels in other representative species collected from Sinclair Inlet and the reference locations. In addition, samples will be collected to evaluate damage to cell deoxyribonucleic acids (DNA) as a sublethal indicator of the onset of cell damage that can be caused by a variety of environmental contaminants, including metals, pesticides, and PAHs (Shugart 1988).

The objective of this task will be to assist WDFW in field collection of samples from Sinclair Inlet and selected reference locations for chemical analysis and also to obtain live tissue samples of the specimens collected for chemical analysis to evaluate potential biological effects from exposure to contaminants by evaluating samples of DNA. Samples for DNA analysis will be collected from live specimens selected for chemical analysis by Project ENVVEST, cryogenically preserved, and transported back to the laboratory for analysis. The data obtained by ENVVEST will be used to assess the potential for ecological effects from contaminant exposure in fish and invertebrates, screen for potential human health exposure scenarios, and help better delineate contaminant mass balance and biological availability of contaminants in the study area.

It is anticipated that the 2005 PSAMP Demersal Fish Trawl sampling will be conducted in May-June 2005 at the eight baseline stations within the Puget Sound Region (Sandie O'Neil, WDFW, personal communication). In addition to Sinclair Inlet, samples will be collected at the following baseline stations: Strait of Georgia, Vendovi (Lummi Island), Port Gardner, Hood Canal, Elliot Bay, Commencement Bay, and Nisqually Reach (Figure 2). English sole samples will be collected at each of the baseline stations following PSAMP sampling protocols. During the sampling, a member of the ENVVEST Technical Team will assist WDFW with sample collection and processing. In addition to the PSAMP sampling protocols, live liver and gill tissue samples will be collected from 25 of the english sole specimens collected at each of the stations. The 25 english sole specimens will be a randomly-selected subset of english sole samples processed by WDFW for chemical and biological analysis. The live tissue specimens will be cryogenically preserved for subsequent DNA comet analysis.

During the sampling, WDFW will prepare selected english sole specimens for chemical analysis by excising 5 g of liver and 15 g of fillet tissues to create a composite samples for each station. Approximately six of these specimens from Sinclair Inlet and six specimens from the Strait of Georgia station will be selected for whole body chemical analysis by obtaining the remaining carcass and viscera of the specimens and transferring them to ENVVEST for processing. Care will be taken to assure that the individuals selected for whole body chemical analysis are also sampled for DNA, histopathology, age analysis, and other morphometeric analyses.

In addition to english sole, ENVVEST will also obtain specimens of six other species for whole body residue analysis from Sinclair Inlet and from one of other reference stations (Table 2). From historical trawl catch records the following fish species are expected to be collected: English Sole (*Parophrys vetulus*), Pacific Staghorn Sculpin (*Leptocottus armatus*), Spotted Ratfish (*Hydrolagus collieii*), Sand Sole *(Psettichthys melanostictus*) and Rock Sole (*Lepidopsetta bilineata*). Some invertebrate species are also routinely collected at the trawl stations, Sea Cucumber (*Parasticopus californicus*) and, Graceful Crab (*Cancer gracilis*). Approximately, six specimens of each species will be selected from both the Sinclair Inlet and the reference station and care will be taken such that the specimens from each station are similar in size and weight. The target species to be sampled are listed below:

Demersal Fish and Macro-Invertebrate Target Species

Sinclair Inlet	**Reference Site[1]**
Demersal Fish	
English Sole (carcass and viscera)	English Sole (carcass and viscera)
Sand Sole	Sand Sole
Rock Sole	Rock Sole
Staghorn Sculpin	Staghorn Sculpin
Midwater Fish	
Shiner Surfperch	Shiner Surfperch
Macro-Invertebrates	
Sea Cucumber	Sea Cucumber
Crab	Crab

[1]. Specimens will be collected from one of the other sampling sites.

ENVVEST will conduct whole body residue analysis of PCB congeners, total metals (Hg, Ag, As, Cd, Cr, Cu, Fe, Ni, Pb, and Zn), lipids, and water content.

5.1.1 Examination for Gross Pathology

The specimens selected for whole-body chemical analysis will be examined for gross pathology. This involves scanning each individual organism for anomalies and noting any observed pathology.

The following anomalies will be noted for fish:

1) fin (and tail) erosion

2) tumors

3) external parasites (e.g., copepods, isopods, leeches)

4) color anomalies (ambicoloration, albinism) (Mearns and Haaker 1973)

5) skeletal deformities (Valentine 1975)

6) lesions

7) other anomalies

Fin erosion can be found on the dorsal, anal, and caudal fins of flatfishes, and on the lower caudal fin and pelvic fins of bilaterally symmetrical fishes. Tail erosion occurs on the top and bottom of the caudal fins of bilaterally symmetrical fishes. Tumors can be smooth and rounded (angioepithelial nodules) or furrowed (epidermal papillomas). Externally obvious copepod parasites occur on the eye, fins, or body of fish. Cymothoid isopods occur in the gill cavities of fish or on the body; which may be difficult to detect because they often fall off. Leeches occur on the body of some flatfishes. Skeletal deformities include crooked backs, snub noses, or bent fin rays. Lesions include sores that do not appear to be due to net damage.

5.1.2 Shipboard Safety

Collection of samples in field surveys is inherently hazardous and this danger is greatly compounded in bad weather. Thus, the safety of the crews and equipment is of paramount importance throughout the project. Each person working onboard a vessel during a field effort should take personal responsibility for his or her own safety.

Many accidents at sea are preventable. Safety awareness by the Boat Captain and all crewmembers is the greatest single factor that will reduce accidents at sea. ENVVEST personnel will defer to the judgment of the Boat Captain and crew of the vessels they work on. Sampling should be canceled or postponed during hazardous weather conditions. The Boat Captain, who is responsible for the safety of everyone onboard, will make the final decisions. As with any field program, the first priority is the safety of the people onboard, followed by the safety of the equipment, and the recovery of the data.

5.2 Deployed mussel study for Sinclair and Dyes Inlets

Caged mussels have been shown to be an effective means of evaluating both exposure and effects to marine organisms and establishing the link between the bioavailability of contaminants in sediments and water their uptake and accumulation in organisms (Salazar and Salazar 2004, Bervoets et al. 2005). The objective of this task is to conduct a caged mussel study at seven locations in Sinclair and Dyes Inlets (Figure 3) to evaluate potential biological effects from ambient exposures to marine organisms. The caged mussel study will be conducted in conjunction with the Project ENVVEST 2005 storm event sampling (ENVVEST 2005, Johnston et al. 2005) currently being conducted in Sinclair and Dyes Inlet. The mussel deployment will be scheduled to occur post-spawning during the summer of 2005 for 60 to 90 days between June and September 2005. Mussels will be obtained from a local source (T0) and deployed using procedures similar to the ASTM guide for conducting field bioassays with marine bivalves

(ASTM 2001, Salazar and Salazar 2004). Three replicate cages will be placed at each location and moored to remain about 1 meter from the bottom. Following retrieval individual mussels will be evaluated for growth and condition and prepared for chemical residue analysis. A subset of specimens from each deployment cage will also be selected for collecting live tissue samples for DNA analysis (Table 2). Samples of indigenous bivalves (mussels or other bivalves) will also be collected from three of the caged mussel stations (P1, SN12, and M2) for analysis of size, condition, DNA, and prepared for chemical residue analysis (Table 2).

Mussels will be deployed for 60-90 days between June and September 2005. Prior to deployment 20 representative individuals from the deployment population pool will be randomly selected for a time zero (T0) sampling. Approximately 18 mussels, 6 from each station replicate, will be sampled from each station at the end of the deployment for DNA analysis (Table 2).

5.3 Evaluating Ecological Effects

Traditional monitoring methods for demersal fish and macroinvertebrates may not have the sensitivity to assess effects from exposure to contaminant. While measurements of population and assemblage parameters have high ecological relevance, these measures are also strongly affected by environmental variables such as water temperature and prey abundance that can reduce the ability to identify impacts related to contaminant exposure. An alternative approach for examining impacts on fish and macroinvertebrates is to measure changes at the cellular or biochemical level indicative of contaminant stress. In the last decade numerous biomarkers have been developed that are sensitive indicators of contaminant exposure, which precede and accompany higher order growth/reproductive impairment in marine organisms (Huggett et al. 1992).

Damage to a cell's deoxyribonucleic acids (DNA) is a sublethal indicator of the onset of cell damage that can be caused by a variety of environmental contaminants, including metals, pesticides, and PAHs (Shugart 1988). Many contaminants have been shown to cause significant increases in DNA strand breaks in a dose-dependant manner (Tice 1996). In addition to a linkage with cancer, increases in DNA damage precede or correspond with reduced growth, abnormal development, and reduced survival of adults, embryos, and larvae (Shugart et al. 1992, Lee et al. 1999, Steinert 1999). One of the most prevalent types of genetic damage is DNA single strand breaks. Significantly elevated levels of single strand breaks have been reported in cells from fish collected at polluted sites, compared to those from reference sites (Pandrangi et al. 1995, OCSD). Similar results have been found in fish exposed to polluted sediments in the laboratory (Roy et al. 2003), and cultured fish cells exposed to field collected water samples (Avishai et al. 2004).

Over the past decade a method referred to as the single cell gel electrophoresis or Comet assay, has gained acceptance worldwide as a sensitive method for measuring DNA damage. It detects DNA strand breaks and alkali labile sites by measuring the migration of DNA from immobilized nuclear DNA (Singh et al. 1988). Advantages of the comet assay for assessing DNA damage in aquatic animals includes: (1) damage to the DNA in individual cells is measured; (2) only small number of cells are needed to carry out the assay (<10,000); (3) the assay can be performed on virtually any eukaryotic cell type; and (4) it is a very sensitive method for detecting DNA damage (Leroy et al. 1996, Collins et al. 1996). Another advantage of the

Comet assay is that the assay can be used to determine the presence of many different types of DNA lesions.

DNA strand break monitoring using the Comet assay has been demonstrated as a useful environmental monitoring tool (Lee & Steinert 2003). DNA damage can be induced by a broad spectrum of stressors but is not significantly affected by environmental variables such as water temperature, salinity, prey, or food abundance. DNA damage can be expressed in some tissues and not in others, or may be expressed as a particular type of DNA lesion that can be specifically identified using various enzyme or chemical treatments. Therefore effected tissues and lesion type can be used to determine the mechanism and character of contaminants present.

In regional ocean monitoring surveys conducted throughout the Southern California Bight and in the vicinity of the Orange county sewage outfall, DNA damage monitoring has been successfully utilized to assess condition information of fish collected in order to gather species abundance, species distribution and size (age) distribution information (SCCWRP 2002, OCSD 2001). The Southern California Bight 1998 Regional Survey (Bight98) was the first study in which widespread DNA damage monitoring of fish was attempted. Though limited to only sampling blood, the objective of the DNA damage-monitoring portion of the study was to assess the regional extent of sublethal effects in southern California fishes and to evaluate the potential applicability of biomarker monitoring to a regional assessment. Fish contamination was found to be widespread in the Southern California Bight, but there were few observed effects at the community level. DNA damage monitoring using the Comet assay was found to be informative and its use in future surveys was recommended (SCCWRP 2002).

Since the Bight98 survey the database of DNA damage in flatfish tissues has expanded as a result of subsequent coastal ocean monitoring efforts with the Orange County Sanitation District in the summers of 2000-2004 and the winter of 2004, and in laboratory exposure experiments. It has been found that damaged fish blood cells are so efficiently removed from circulation that only under acutely toxic conditions can persistent damage be found in them. However, liver tissue DNA has been found to be very sensitive to low contaminant exposure levels (OCSD, Roy et al. 2003). Results from monitoring the Orange County sewage outfall effects on feral flatfish populations have identified subtle effects even when contaminant levels were very low. DNA damage monitoring has shown the outfall contribution to be minor in comparison to shore runoff. On a regional level, baseline levels of liver DNA damage in hornyhead turbot and English sole show a more significant response to storm water runoff than the influence of the sewage outfall (Steinert & Armstrong In prep.).

In addition, this method has been used successfully on the tissues of bivalves deployed in the field as sentinels of contaminant exposure (Steinert et al. 1998A and Steinert et al. 1998B). A number of recent studies have reported the results of in situ deployment of a variety of test organisms (Ireland et al. 1996, Chappie & Burton 1997, Gunther et al. 1999, Ringwood et al. 1999, Schulz & Liess 1999, Tucker & Burton 1999, Beckvar et al. 2000, Steinert et al. 1998A, Steinert et al. 1998B). These studies have been successful in demonstrating the effectiveness of in situ deployments, especially in the ability to account for variables not typically replicated in the laboratory, such as ultraviolet photoinduced toxicity (Ireland et al. 1996, Steinert et al. 1998B) and turbidity (Tucker & Burton 1999). Results of in situ deployments also have been shown to agree with responses of the same species native to the deployment sites (Schulz & Liess 1999, Steinert et al. 1998A). Not only has the utility of in situ bivalve deployment been successfully used in assessment studies but also DNA damage has been successfully used as a sensitive sublethal indicator of contaminant bioavailability (Ringwood et al. 1999, Steinert et al. 1998A and Steinert et al. 1998B, Ringwood et al. 1998).

5.4 Tissue Collection

5.4.1 Equipment and Procedures

Demersal fish, macroinvertebrates, and mussels obtained during this study will be collected and dissected on board the collection vessel or in a clean processing area set up on shore. Dissections will be done in areas that are as free as possible from sources of PAH's (e.g., diesel fumes). Standard length, species, sex, maturity, time of dissection, and condition of fish will be recorded for each specimen. In fish, blood will be preserved by gently mixing and freezing a small volume (<100 ul) of whole blood in 1 ml of ice-cold cryopreservation solution, phosphate buffered saline/10% DMSO. Small sections of liver and gonad (collected from male fish) are placed in 1 ml of ice cold cryopreservation solution, chilled on ice and within 20 minutes all samples will be frozen in liquid nitrogen. For deployed and native bivalves ~100 ul of hemolymph will be withdrawn from the posterior adductor muscle with a 26 gauge needle on a tuberculin syringe and transferred to 1 ml of ice cold cryopreservative. The mussel will be split open with a clean stainless steel knife and a small 3 mm^3 portion of digestive gland removed and placed in 1 ml cryopreservative. These samples are then processed in the same way as fish tissue samples. Samples will be transported to the CSC Biomarker Laboratory, San Diego, CA and kept in liquid nitrogen until analyzed.

The Comet assay procedures are those published and approved by ASTM and Standard Methods (ASTM 2000, Standard Methods 2001). To prepare samples for DNA damage analysis, cryopreserved samples are thawed on ice; 10-50 µl of blood is added to 140 µl ice cold PBS; liver is homogenized using dissection scissors and 25 µl of suspended cells added to 100 µl ice cold PBS; and 10 µl cryopreservation solution from a gonad sample vial added to 140 µl ice cold PBS, in a clean 1.5 ml centrifuge tube. Cells are pelleted at 600 x g for 2 min., supernatant discarded and the pellet resuspended in 50-600 µl 0.7% low melting temperature agarose (FisherBiotech, low melting DNA grade agarose) in PBS at 30oC (PBS/LMA). Twenty-five microliters of the resuspended cells is then transferred onto GelBond plastic-backing and the cell/agarose suspension allowed to solidify on an ice-chilled stainless steel tray for 3 minutes, then covered with a top-coat of 25 µl PBS/LMA. After solidifying for 3 minutes the slides are placed in 4 oC lysing solution (LS), 2.5 M NaCl, 10 mM Tris, 0.1 M EDTA, 1% Triton X-100, and 10% DMSO, pH 10.0 (LS) in polycarbonate trays and incubated at 4oC for at least 1 hr.

Slides are then transferred from LS to trays filled with distilled water, the water replaced with fresh every 2 minutes for 3 rinses. For SSB determination the slides are placed in a submarine gel electrophoresis chamber filled with 300 mM NaOH, 1 mM EDTA, and the DNA denatured under alkaline conditions for 15 min. After denaturation electrophoresis is performed at 300 mA, 25 V for 4 V-hrs. The slides are then neutralized with three 2 min. rinses in 0.4 M Tris, removed from the rinsing tray and excess solution blotted away, and fixed in ice cold ethanol for 5 min. The fixed slides are then dried in an oven at 37^{o}C for 20 min. and transferred to slide boxes. Standards and replicates are run with each set of slides to insure consistency between sets.

For analysis, the DNA is stained with 15 µl of a 20 µg/ml solution of ethidium bromide in distilled water (EtBr), and covered with a coverslip. Stained slides are analyzed by viewing at 200X magnification with an epifluorescent microscope (excitation filter 510-560 nm green light, barrier filter 590 nm) with an attached CCD camera and image analysis software (Komet image analysis system, Kinetic Imaging, Ltd U.K.). The fluorescent "head" or nucleus diameter and the length (µm) of any accompanying trailing DNA "tails" resulting from strand breakage are measured for each nucleus analyzed. Measurements are made in five sectors on each slide, counting 5-10 nuclei in each sector randomly positioning the lens above each sector and counting left to right from the upper left-hand corner of the field of view. Overlapping nuclei or tails are not counted. The image system calculates a large number of quantitative parameters for each nuclei the most important being the total intensity of each comet (comet optical intensity), the % DNA in the tail, and the tail moment, which is the product of the tail length multiplied by the %DNA in the tail /100. To insure the calculated analysis values are consistent with those calculated in previous studies, analyzed samples from a previous study, one with a minimum of DNA damage and another with moderate levels of damage, are analyzed to calibrate the system prior to the analysis of samples for the current study.

Analysis of variance (ANOVA) is conducted to determine possible differences between stations. Bartlett's test of homogeneity is conducted to verify homogeneity of variance. In some cases if the variance is not homogeneous, Kruskal-Wallis non-parametric comparisons will be performed. A $P<0.05$ is considered significant, post hoc tests will be performed to single out treatments responsible for the statistical differences.

6. Laboratory Measurements and Quality Control Procedures

Analytical chemistry analysis of samples for trace metals and organics will be performed by Battelle Marine Sciences Laboratory (BMSL), Sequim WA and Battelle Duxbury Operations, Duxbury, MA. The following documents the QA/QC requirements of this study.

6.1 Quality Objectives and Criteria for Measurement Data

6.1.1 Data Quality Objectives (DQO)

The data quality objectives for this study identify project objectives and define how the data will be used to make project decisions (Table 1). The DQOs provide the basis for determining the following:

- Objectives of the intended sampling and analysis;
- Underlying design assumptions for each sample type and matrix;
- How each data type will be assessed;
- Methods that will be used to determine whether or not the data support the design assumptions; and
- How the data will be used in interpretation.

6.1.2 Measurement Quality Objectives

Measurement quality objectives for the analyses conducted for this study can be expressed in terms of accuracy, precision, completeness, and sensitivity goals. Accuracy and precision are monitored through the analysis of quality control samples. Analytical Parameters, Method Detection Limits (MDLs), and Reporting Limits are provided in Table 3.

Accuracy is defined as the degree of agreement between an observed value and an accepted reference value. Accuracy includes a combination of random error (precision) and systematic error (bias) components that are due to sampling and analytical operations.

Precision is defined as the degree to which a set of observations or measurements of the same property, obtained under similar conditions, conform to themselves. Precision is usually expressed as standard deviation, variance, or range, in either absolute or relative terms.

Completeness is the amount of data collected as compared to the amount needed to ensure that the uncertainty or error is within acceptable limits. The goal for data completeness is

100%. However, the project will not be compromised if 90% of the samples collected are analyzed with acceptable quality.

Comparability is a measure of the confidence with which one data set can be compared to another. This is a qualitative assessment and is addressed primarily in sampling design through use of comparable sampling procedures or, for monitoring programs, through accurate re-sampling of stations over time. In the laboratory, comparability is assured through the use of comparable analytical procedures and ensuring that project staff is trained in the proper application of the procedures. Within-study comparability will be assessed through analytical performance (QC samples).

Representativeness is the degree to which data accurately and precisely represent a characteristic of a population. This is a qualitative assessment and is addressed primarily in the sample design, through the selection of sampling sites, and procedures that reflect the project goals and environment being sampled. It is ensured in the laboratory through (1) the proper handling, homogenizing, compositing, and storage of samples and (2) analysis within the specified holding times so that the material analyzed reflects the material collected as accurately as possible.

Sensitivity is the capability of a test method or instrument to discriminate between measurement responses representing different levels (*e.g.*, concentrations) of a variable of interest. Sensitivity is addressed primarily through the selection of appropriate analytical methods, equipment, and instrumentation. The methods selected for the Sinclair and Dyes Inlet Study were chosen to provide the sensitivity required for the end-use of the data. This is a quantitative assessment and is monitored through the instrument calibrations and calibration verification samples and the analysis of procedural blanks with every analytical batch.

Method Detection Limits (MDLs) for organic compounds must be determined annually according to Code of Federal Regulations 40 CFR Part 136 Appendix B for each method of interest by instrument, matrix, and compound of interest. Sediment MDLs are determined by spiking clean sediment or a solid matrix such as pre-baked sodium sulfate with all parameters of interest and processing them according to the methods defined in Section 3.4. MDLs for water samples are determined by spiking ASTM type II (MilliQ) water with all parameters of interest and processing them according to the methods defined in Section 3.4. MDLs for gas chromatography/electron-capture detector (GC/ECD) analysis are determined on the primary column. MDLs for PCBs and pesticides must also be determined on a confirmation column if data from confirmatory analyses will be reported. In these instances, the MDLs determined from confirmation column analysis must be less than those determined from the primary column.

The MDLs for trace metals are determined annually according to 40 CFR Part 136 Appendix B for each method of interest by instrument, matrix, and compound of interest. Because completely metal-free matrices for sediment do not exist, MDLs for metals in sediment samples are calculated from the MDLs generated by a fresh water MDL study, taking into account the anticipated sample dilution factors that would be used in actual sediment samples. MDLs for fresh water samples are determined by spiking deionized water with all metals of interest and processing them according to the methods defined in Section 3.4.

Reporting Limits (RLs) for organic compounds are empirical values based on instrument sensitivity and day-to-day operations. For organic compounds, the RL is calculated as:

RL = (Low Standard Concentration)(Pre-injection volume)(Dilution Factors)(1/ Sample Size)

The actual reporting limit can be lowered by increasing the sample size and decreasing the pre-injection volume of the sample. Detected values that are less than the reporting limit will be qualified as estimates and used with caution during any assessment.

For trace metals, the RL is calculated by multiplying the target analyte MDL by 3.18. The value 3.18 is based on the Student's-t value for 7 to 10 replicates, the number of replicates usually analyzed to generate the MDL. The data qualifier "J" will be added to any reported values that are less than the RL at the direction of the PSNS&IMF Project ENVVEST Manager.

6.2 Sample Handling and Custody

Biological samples will be wrapped in ashed aluminum foil and labeled by species and location. Sample ID numbers will be assigned by Washington Department of Fish and Wildlife (WDFW). The samples will be shipped (or delivered) to Battelle Marine Sciences Laboratory (BMSL) for subsampling and homogenization. Samples will be stored frozen at -20°C until removed for sorting and selection. The approach to sorting will consist of selecting fish of comparable size and length from each of the sites. Samples selected for processing and analysis will include a single organism or a composite of organisms with similar classifications (weight or length, etc.). The selected samples will then be composited, homogenized and assigned a new identification number to represent the new composite sample generated. This study is designed to evaluate the ecological risk of selected contaminants; therefore, the whole organism will be included in the composite.

6.2.1 General Preparation Procedure:

- All homogenization equipment will be decontaminated between each sample using a laboratory detergent, methanol rinse, and deionized water (DI) rinse. Gloves and work surface papers will be changed between samples.

- Blender parts and tools will be cleaned between samples by washing in hot water with laboratory detergent and rinsing successively with methanol and DI water.

- Samples will be partially thawed in the refrigerator prior to homogenization. Excess liquid that collected during thawing will be discarded.

- Ceramic cutting knives and a Teflon blocks will be used to cut the fish into smaller pieces that fit in the titanium tissue homogenizer.

- Homogenized samples will be placed in three separate containers with the following priorities:

    1. Organics split - approximately 30g in a pre-cleaned 4 oz. glass jar

2. Metals split - approximately 20g in a pre-tarred, precleaned 4 oz. SPEX jar

3. Archive split - remaining sample in a 4 oz. Pre-cleaned glass jar.

Homogenized samples for organic analysis will be shipped to Battelle Duxbury Operations. Homogenized samples for metals analysis and archive will be retained at MSL.

6.2.2 Sample Custody

Sample custody records are the administrative records associated with the physical possession and/or storage history of each individual sample from the purchase and preparation of each sample container and sampling apparatus to the final analytical result and sample disposal. MSL-A-002, *Sample Chain-of-Custody*, (Battelle, 2000) defines field and laboratory custody procedures.

Sample containers will be labeled with waterproof, adhesive-back labels. Sample labels must provide sufficient detail to identify each storm event and station to allow tracking to field activities. Sample labels must include a unique sample identification number, station ID, sample event, sample type, collection date/time, and analysis codes.

Sample custody will be documented from "cradle to grave". Samples should not be left unattended unless properly secured. Each laboratory has a formal, documented system designed to provide sufficient information to reconstruct the history of each sample, including preparation of sampling containers, sample collection and shipment, receipt, distribution, analysis, storage or disposal, and data reporting within the laboratory. Laboratory documentation must provide a record of custody for each sample (versus a sample batch) throughout processing, analysis, and disposal.

6.2.3 Sample Receipt

Immediately upon receipt by a laboratory, the condition of samples must be assessed and documented. The contents of the shipping container must be checked against the information on the chain-of-custody form for anomalies. If any discrepancies are noted, or if laboratory acceptance criteria or project-specific criteria are not met, the laboratory must contact the Field Manager for resolution of the problem. The discrepancy, its resolution, and the identity of the person contacted must be documented in the project file. The following conditions may cause sample data to be unusable and must be communicated to the laboratory team leader:

- The integrity of the samples is compromised (e.g., leaks, cracks, grossly contaminated container exteriors or shipping cooler interiors, obvious odors, etc.);

- The identity of the container cannot be verified;

- The proper preservation of the container cannot be established;

- Incomplete sample custody forms (*e.g.,* the sample collector is not documented or the custody forms are not signed and dated by the person who relinquished the samples);

- The sample collector did not relinquish the samples; and,

- Required sample temperatures were not maintained during transport (4°C ± 2).

The custodian must verify that sample conditions, amounts, and containers meet the requirements for the sample and matrix. A unique sample identifier must be assigned to each sample container received at the laboratory, including multiple containers of the same sample.

6.2.4 Sample Handling

Sample holding conditions and holding times are defined in Table 4. Holding times are to be calculated from the time of sample collection. Documentation must be sufficient to track sample holding, processing, and analysis times to ensure that holding times are met. Documentation of sample collection must include both date and time.

Samples will be held for one year after sample collection unless directed otherwise by the ENVVEST program manager. Disposal records for unextracted samples, extracted samples, sample containers, and sample extracts must be sufficient to provide tracking from collection, through laboratory receipt, to sample disposal in a waste drum that is directly traceable to a disposal manifest.

6.3 Analytical Methods

6.3.1 Organic Chemistry

Battelle Duxbury Operations will perform the analysis of PCBs according to low-level methods developed for the National Oceanic and Atmospheric Administration (NOAA) Status and Trends Program (NS&T), as described in Battelle Duxbury Operations SOP 5-190, Tissue Extraction for Trace Level Semi-Volatile Organic Contaminant Analysis. Approximately 30 g of tissue will be spiked with surrogates and extracted three times with dichloromethane using tissuemizer and shaker table techniques. The combined extract will be dried over anhydrous sodium sulfate, concentrated, and an aliquot removed for lipid content determination. The extract will then be processed through an alumina chromatography cleanup column, concentrated, and further purified by GPC/HPLC. The post-HPLC extract will be concentrated, solvent exchanged into hexane, fortified with recovery internal standards (RIS) and submitted for analysis. Extracts will be analyzed using gas chromatography/electron capture detection (GC/ECD), following general NS&T methods (Battelle SOP 5-128, Identification and Quantification of Polychlorinated Biphenyls (By Congener and Aroclor) and Chlorinated Pesticides by Gas Chromatography/Electron Capture Detection. Sample data will be quantified by the method of internal standards, using the RIS compounds.

6.3.2 Metals Chemistry

Composite, homogenized tissue samples will be freeze dried and milled to provide an additional homogenization in accordance with MSL-C-003, Percent Dry Weight and Homogenizing Dry Sediment, Soil, and Tissue. Dried tissue samples will be analyzed for nine metals including: silver (Ag), arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), lead (Pb), mercury (Hg), nickel (Ni), and zinc (Zn). Tissue samples will be digested according to Battelle SOP MSL-I-024, Mixed Acid Tissue Digestion. An approximately 500-mg aliquot of each dried, homogeneous sample will be combined with nitric and hydrochloric acids (aqua regia) in a Teflon vessel and heated in an oven at 130ºC (±10ºC) for a minimum of eight hours. After heating and cooling, deionized water is added to the acid-digested tissue to achieve analysis volume and the digestates were submitted for analysis by two methods.

Digested samples will be analyzed for Hg by cold-vapor atomic absorption spectroscopy (CVAA) according to Battelle SOP MSL-I-016, Total Mercury in Tissues and Sediments by Cold Vapor Atomic Absorption, which is based on EPA Method 245.6, Determination of Mercury in Tissue by Cold Vapor Atomic Absorption Spectrometry.

Digested samples will be analyzed for all other metals using inductively coupled plasma-mass spectrometry (ICP-MS) according to Battelle SOP MSL-I-022, Determination of Elements in Aqueous and Digestate Samples by ICP/MS. This procedure is based on two methods modified and adapted for analysis of solid sample digestates: EPA Method 1638, Determination of Trace Elements in Ambient Waters by Inductively Coupled Plasma-Mass Spectrometry and EPA Method 200.8, Determination of Trace Elements in Water and Wastes by Inductively Coupled Plasma-Mass Spectrometry. Subsamples of tissue homogenate will also be analyzed for total lipids and water content.

6.4 Quality Control Requirements

This section defines the quality control (QC) program for this study. Appropriate laboratory QC procedures are designated in order to assess data quality through the measures of accuracy and precision. If data fall outside the specified accuracy or precision criteria defined for a procedure or measurement, or if problems affecting comparability are identified, the chemistry task leader must contact the PNNL Program Manager and the ENVVEST Program Manager to discuss options available for rectifying the out-of-control situation.

6.4.1 Analytical Laboratory

6.4.1.1 Quality Control Samples

The study design and QC samples are intended to assess the major components of total study error, which facilitates the final evaluation of whether environmental data are of sufficient quality to support the related decisions. The QC sample requirements are designed to provide measurement error information that can be used to initiate corrective actions with the goal of limiting the total measurement error.

The QC samples and frequency applicable to analytical chemistry laboratories are detailed in Table 5. Table 6 defines the required accuracy and precision for QC samples, along with corrective actions that must be implemented if QC criteria are not met. Table 7 provides formulas for the calculation of QC sample assessment statistics. All QC sample failures and associated corrective actions will be documented. If data must be reported with failing QC results, then data qualifiers will be assigned to the QC sample data.

6.5 Instrumentation/Equipment Testing, Inspection, and Maintenance

6.5.1 Laboratory Equipment

All analytical instruments and equipment are to be maintained according to SOPs and the manufacturers' instructions. Equipment and instrument and maintenance and frequency are defined in SOPs and are summarized in Table 8 and Table 9. All routine maintenance and non-routine repairs are to be documented in a bound logbook. The information recorded should include analyst initials, date maintenance was performed, a description of the maintenance activity, and (if the maintenance was performed in response to a specific instrument performance problem) the result of re-testing to demonstrate that the instrument performance had been returned to acceptable standards prior to re-use. The return to analytical control is demonstrated by successful calibration.

6.6 Documentation and Records

6.6.1 Laboratory Documentation

Documentation of all activities is critical for tracking data and evaluating the success of any activity. Laboratory documentation requirements are defined in Battelle MSL standard operating procedures (Battelle 2000a, b).

6.6.2 Documentation Standards

All data generated during the course of this project must be able to withstand challenges to their validity, accuracy, and legibility. To meet this objective, data are recorded in standardized formats and in accordance with prescribed procedures. The documentation of all environmental data collection activities must meet the following minimum requirements.

- Data must be entered directly, promptly, and legibly. All reported data must be uniquely traceable to the raw data. All data reduction formulas must be documented.

- Handwritten data must be recorded in ink. All original data records include, as appropriate, a description of the data collected, units of measurement, unique sample identification (ID) and station or location ID (if applicable), name (signature or initials) of the person collecting the data, and date of data collection.

- Any changes to the original (raw data) entry must not obscure the original entry. The reason for the change must be documented, and the change must be initialed and dated by the person making the change.

- The use of pencil, correction fluid, and erasable pen is prohibited.

Any changes to the QAPP or FSP (*e.g.*, QA procedures, analytical procedures, sampling locations and frequencies, etc) must be documented in writing and approved by the PNNL QA Officer, PNNL Program Manager, prior to implementation of the changes.

Minor deviations from the QAPP or FSP (*e.g.,* those that would not impact the study objectives, design, or data quality) will be reported to and approved by the appropriate team leader and the PNNL Project Manager. Major deviations (*e.g.,* those that could impact the study objectives, design, or data quality) will additionally be reported to the PNNL Program Manager, the PNNL QA Manager, and the ENVVEST Project Manager.

6.7 Data Management

Data generated in support of the Sinclair and Dyes Inlet Study will be tracked and reviewed by the PNNL Program Manager. Data management (*e.g.,* paper flow; data tracking, data entry, etc.) and data assessment (*e.g.,* verification, validation, and Data Quality Assessment (DQA)) activities require adequate QC procedures to ensure that the SOPs will be followed and result in records and reports that are accurate and appropriate. QC procedures include peer review of each step and management review of a certain percentage of the data.

6.7.1 Laboratory Data

Data management at the laboratory begins with the receipt of samples. Samples are logged in and assigned unique identification numbers that are used to identify samples throughout storage, processing, analysis, and reporting. A combination of hand-recorded and electronically captured data is generated. Hand-recorded data include sample processing and spiking procedures. Hand-recorded data are transcribed to spreadsheets using established formats. (The raw data are maintained in the project files and the transcribed data are 100% verified). Data will be entered into an EDD using a format supplied by the ENVVEST Technical Coordinator.

6.8 DATA VALIDATION AND USABILITY

Data review includes data verification, validation, and oversight, as well as reconciliation of the data quality with user requirements. The data verification process includes the initial review of the data packages to ensure that the analyses requested have been provided. Data validation is the process of reviewing data and accepting, qualifying, or rejecting data on the basis of sound criteria. Data will be reviewed by the Chemistry Task Leader to assure that it is complete. Data qualifier codes are provided in Table 10.

7. Summary

This sampling plan describes specific sampling activities to obtain data necessary to characterize bioaccumulation and ecological stress to marine resources within the Sinclair and Dyes Inlets, identify potential sources of ecological stress, and assess ecological risks to ecological receptors within the Inlets (Table 1). This document identifies the objectives, procedures, and quality assurance/quality control (QA/QC) requirements for the biological sampling to be completed by Project ENVVEST for 2005. The data obtained by this study will be used to assess the potential for ecological effects from contaminant exposure in fish and invertebrates, screen for potential human health exposure scenarios, and help better delineate contaminant mass balance and biological availability of contaminants in the study area.

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9. Tables

Table 1. Data quality objectives for biological sampling in Sinclair and Dyes Inlets.

Biological Sampling Data Quality Objectives
STEP 1: State the Problem Sinclair and Dyes Inlets are listed as impaired waterbodies on 303d list due to contamination from heavy metals, toxic organics, and low dissolved oxygen. Data are needed to characterize bioaccumulation and ecological stress to marine resources within the Inlets, identify potential sources of ecological stress, and assess ecological risks to ecological receptors within the Inlets.
STEP 2: Identify the Decision 1. Are contaminants being accumulated to harmful levels in marine organisms within the Inlets? 2. Is there evidence of ecological stress to marine resources within the Inlets?
STEP 3: Identify Inputs to the Decision 1. Within the context of greater Puget Sound region, are contaminants being accumulated in resident populations of organisms within the Inlets? 2. Evaluate both exposure and effects to marine organisms and establish a link between the bioavailability of contaminants in sediments and water their uptake and accumulation in organisms 3.
STEP 4: Define the Study Boundaries Spatial boundaries are Sinclair and Dyes Inlets and the Greater Puget Sound region.
STEP 5: Develop a Decision Rule Data on contaminant accumulation and ecological effects will be compared to reference locations and other areas within the greater Puget Sound.
STEP 6: Evaluate Decision Errors Data will be evaluated to assure accuracy, precision, completeness, comparability, and representativeness.
STEP 7: Optimize the Design for Obtaining Data Develop a sampling program to sample (resident) demersal fish and macro-invertebrates from the Inlets and other reference locations within the Puget Sound. Establish a network of stations for conducting an in situ bioassay study using deployed mussels to measure exposure and effects from ambient exposures within the Inlets. aluate both bioaccumulation and ecological effects to determine whether there is a linkage between exposure and ecological effects.

Table 2. Summary of samples to be collected and analyses to be performed for the (A) demersal fish trawl and (B) caged mussel stations.

A. DEMERSAL FISH TRAWLS						Analyses to be Conducted				
Stations	**Events**	**Species**	**Reps**	**Tissue Types[a]**		**DNA[1]**	**PCB[2]**	**PAH**	**Hg[3]**	**Metals[4]**
Sinclair	1	7	3			0	21		21	21
Georgia Strait	1	4	3			0	12		12	12
Port Gardner	1	2	3			0	6		6	6
Nisqually Reach	1	2	3			0	6		6	6
Sinclair English Sole*	1	1	6			0	6		6	6
PSAMP specimens	8	1	25	1		200				
B. CAGED MUSSELS										
Stations		**Sites/ Samples**	**Reps**	**Tissue Types[b]**						
T0 (Predeployment)		1	3	1		20	3	3	3	3
Sinclair		4	12	1		72	48	48	48	48
Dyes		1	3	1		18	3	3	3	3
PO Passage		1	3	1		18	3	3	3	3
Rich Passage		1	3	1		18	3	3	3	3
Indigenous bivalves		3	9	1		54	27	27	27	27
Total Analysis						400	138	87	138	138

* Carcus and viscera split with WDFW

[a] Liver (gill tissue will also be collected)

[b] Digestive Gland (gill and hemocyte tissue will also be collected)

[1] Comet Assay

[2] NOAA 18 congeners

[3] Total Hg

[4] Ag, As, Cd, Cr, Cu, Fe, Ni, Pb, Zn

Table 3. Typical Method Detection Limits (MDL) and associated Reporting Limits (RL) for chemical analysis of biological samples.

Analytes	Laboratory Values for Method Detection Limit	Tissue Analysis Reporting Limit
Trace Metals, MSL MSL-I-022 (metals) MSL-I-0016 (total Hg)	**mg/Kg (dry weight)**	**mg/Kg**
Ag	0.04	0.13
As	0.2	0.6
Cd	0.03	0.1
Cr	0.1	0.3
Cu	0.08	0.3
Ni	0.05	0.2
Pb	0.04	0.13
Hg	0.005	0.02
Zn	0.9	2.9
Organic Compounds	**ng/g (wet weight)**	**µg/kg (weight)**
PCB Congener (NOAA NS&T 20 congeners)	0.036 – 1.033	Sample specific
Total PCBs	NA	NA
PAH Compounds (16 parent PAHs)	0.036 – 1.033	Sample specific

Table 4. Sample Containers, Sample Size, Preservative Requirements, and Holding Time for Analytical Samples.

Parameter	Method Container [1]	Minimum Sample Size	No. of Field Samples	Sample Preservative	Holding Time [2] x/y
TISSUE - Organic Compound Analysis					
PCBs	G	30 g		-20º±2°C	One year frozen /40 days
TISSUE - Inorganic Compound Analysis					
Metals and Hg	P	10 g		-20º±2°C	One year frozen

[1] Container Types: G = Amber glass with Teflon-lined lid; P = Polycarbonate

[2] "x" days/"y" days refer to the maximum number of days from sampling to extraction/the maximum number of days from extraction to analysis, once samples are identified for analysis.

Table 5. Definitions, Requirements, and Frequency for Typical Quality Control Samples.

QC Sample	Definition	Frequency
LABORATORY QUALITY CONTROL		
Method or Procedural Blank (MB)	A combination of solvents, surrogates, and all reagents used during sample processing, processed concurrently with the field samples. Monitors purity of reagents and laboratory contamination.	1/sample batch[2] A processing batch MB must be analyzed with each sequence.
Standard Reference Material (SRM)	An external reference sample which contain a certified level of target analytes; serves as a monitor of accuracy. Extracted and analyzed with samples of a like matrix (not available for all analytes)	1/ batch[2]
Matrix Spike (MS)	A field sample spiked with the analytes of interest is processed concurrently with the field samples; monitors effectiveness of method on sample matrix; performed in duplicate.	1/sample batch[2]
Duplicate Sample	Second aliquot of a field sample processed and analyzed to monitor precision; each sample set should contain a duplicate.	1/sample batch[2]
Recovery Internal Standards (RIS)	All field and QC samples are spiked with recovery internal standards just prior to analysis; used to quantify surrogates to monitor extraction efficiency on a per sample basis.	Each sample analyzed for organic compounds
Surrogate Internal Standards (SIS)	All field and QC samples are spiked with a known amount of surrogates just prior to extraction; recoveries are calculated to quantify extraction efficiency.	Each sample analyzed for organic compounds

[1] The field duplicate is a collocated sample defined as a sample collected as near in space and time to the original field sample as the sampling equipment and procedure allows.

[2] A batch is defined as 20 field samples processed simultaneously and sharing the same QC samples.

Table 6. Measurement Quality Criteria.

QC Parameter	Acceptance Criteria	Corrective Action
Accuracy		
Method Blank (MB)	MB<RL If MB>RL; sample values ≤10X MB, then perform corrective action Method criteria for all other parameters	Perform corrective action re-process (extract, digest) sample batch. If batch cannot be re-processed, notify client and flag data.
• *Standard Refernce Material* *(SRM)*	Metals: ≤20% PD. Determined vs. certified range. Analyte concentration must be 10xMDL to be used for DQO. Method criteria for all other parameters	Review data to assess impact of matrix. Reanalyze sample and/or document corrective action. If other QC data are acceptable then flag associated data if sample is not reanalyzed.
• *Matrix Spike (MS)/MS Duplicate (MSD)*	Organic compounds: 40 - 120% recovery Metals: 70 - 130% recovery Method criteria for all other parameters	Review data to assess impact of matrix. If other QC data are acceptable and no spiking error occurred, then flag associated data. If QC data are not affected by matrix failure or spiking errors occurred, then re-process MS. If not possible, then notify client and flag associated data.
• *Surrogate Spike (SIS)*	Organic compounds: 40 - 120% recovery	Review data. Discuss with Project Manager. Reanalyze, re-extract, and/or document corrective action and deviations.
• *Laboratory Control Sample (LCS)*	Organic compounds: 40 - 120% recovery Metals: 70 - 130% recovery Method criteria for all other parameters	Perform corrective action. Re-analyze and/or re-process sample batch. If batch cannot be re-processed: notify client, flag data, discuss impact in report narrative.
Precision: *Laboratory Duplicates*	Organic compounds (MSD): <30% RPD Metals: <30% RPD Method criteria for all other parameters	Review data to assess impact of matrix. If other QC data are acceptable, then flag associated data. If QC data are not affected by matrix failure, then re-process duplicate. If not possible, then notify client and flag associated data.

Table 7. Calculation of Quality Control Assessment Statistics.

Percent Recovery

The percent recovery is a measurement of accuracy, where one value is compared with a known/certified value. The formula for calculating this value is:

$$Percent\ \ Recovery = \frac{amount\ \ detected}{amount\ \ expected}\ x\ 100$$

Percent Difference

The percent difference (PD) is a measurement of precision as an indication of how a measured value is difference from a "real" value. It is used when one value is known or certified, and the other is measured. The formula for calculating PD is:

$$Percent\ \ Difference = \frac{X_2 - X_1}{X_1}\ x\ 100$$

where: X_1 = known value (*e.g.*, SRM certified value)

X_2 = determined value (*e.g.*, SRM concentration determined by analyst)

Relative Percent Difference

The relative percent difference (RPD) is a measurement of ***precision***; it is a comparison of two similar samples (matrix spike/matrix spike duplicate pair, field sample duplicates). The formula for calculating RPD is:

$$RPD = \left| \frac{2\ x\ (X_1 - X_2)}{(X_1 + X_2)} \right|\ x\ 100$$

where: X_1 is concentration or percent recovery in sample 1

X_2 is concentration or percent recovery in sample 2

Note: Report the absolute value of the result -- the RPD is always positive.

Relative Standard Deviation

The relative standard deviation (RSD) is a measurement of ***precision***; it is a comparison of three or more similar samples (*e.g*., field sample triplicates, initial calibration, MDLs). The formula for calculating RSD is:

$$\%RSD = \frac{\textit{Standard Deviation of All Samples}}{\textit{Average of All Samples}} \times 100$$

Table 8. Maintenance Procedures for General Laboratory Equipment

Equipment	Activity	Frequency
Deionized water system	Replace seals Replace cartridges	As needed for leaks and to maintain resistivity > 18 mOhms
MilliQ deionized water system	Replace seals Replace cartridges	Every 6 months or as needed for leaks and to maintain resistivity > 18 mOhms
Electronic balances	Clean	As needed
Freezers/refrigerators	Clean Defrost	As needed
Ovens	Clean	As needed
Glass thermometers	Store in protective case	Always except when in use
Digital thermometer	Avoid bending thermocouples	Always

Table 9. Maintenance Procedures for Analytical Instruments.

Equipment	Activity	Frequency
GC/MS Maintenance		
Rough pumps Turbomolecular pump Diffusion pumps	Routine service (service contract) Check fluid levels	Six months Weekly
Foreline traps Helium gas traps	Inspect trap pellets for color change Replace adsorbent pellets	Routinely 6-12 months, as needed
Injection port septum	Replace	As needed to maintain EPC pressure
Injection port liners	Replace	Approximately every 30-40 samples
Precolumn	Replace	As needed to improve peak shape, resolution, or sensitivity
Calibration vial (PFTBA)	Refill	4 months or as needed
Back grills of the MS	Vacuum dust	6 months or as needed
Ion source	Clean	As indicated when usage-dependent surface deposits degrade ion source function
GC Maintenance		
Injection port	Replace	Weekly (~50 injections) or as needed
Injection port liner	Replace	Weekly or as needed
Injection port	Clean	Monthly or as needed
Column	Clip	As needed to maintain performance
Precolumn	Replace	As needed when chromatographic degradation is observed
Gas cylinders	Replace	When PSI is < 300
Autosampler rinse vial	Fill	Prior to analysis
Autosampler syringe	Replace/align	As needed
Ferrule	Replace	As needed for leaks
Gas drying/purification traps	Replace	Annually or as needed
Column, detector	Bakeout	As needed
ICP-MS Maintenance		
Argon supply	Check and record; replace as needed	Daily
Vacuum	Check and record	Daily
Cooling chiller	Check and record temperature	Daily
Nebulizer flow	Check and adjust	Daily or as needed
Sensitivity and stability	Check and record	Daily
Auto sampler tubing	Change	As needed

Maintenance of Analytical Instruments (continued).

Equipment	Activity	Frequency
Cones	Clean or change	As needed
GFAA Maintenance		
Graphite furnace tube	Check and replace (~500 burns)	Daily and as needed
Contact cylinders	Check and replace as needed (10,000 burns)	Daily and as needed
Windows	Clean	Whenever tubes are changed or as needed
Water recirculator fluid level	Check and refill	Daily
CVAA Maintenance		
Soda lime	Check and change	Checked daily, changed weekly
Reagents (SnCl,3% HNO_3, rinse water)	Check and change	Checked daily, changed weekly
Carbon trap	Check and change	Checked daily, changed bimonthly
Filters	Check and change	Checked daily, changed bimonthly
Sample injection syringe	Check and change	Checked weekly, changed as needed
Tubing	Check and change	Checked weekly, changed as needed
Connectors	Check and change	Checked weekly, changed as needed
Lamp	Check and change	Checked weekly, changed as needed
Autosampler arm	Lubricate	Bimonthly

Table 10. Data qualifier codes.

Method Qualifiers	
A	Method qualifier - Flame AA
AV	Method qualifier - Automated cold vapor
C	Method qualifier - Manual spectrophotometric
CV	Method qualifier- Manual cold vapor
F	Method qualifier - Furnace AA
NR	Method qualifier - Analyte was not required
P	Method qualifier - ICP
X	Method qualifier – XRF screening
I	Method qualifier – Immunoassay screening
Data Qualifiers	
B	Analyte found in both sample and associated blank. The "B" will be reported on the result associated with the field samples, not the blank
C	Presence confirmed by GC/MS (Pesticides only)
D	Dilution run. Initial run outside linear range of instrument. Organics only.
E	Estimate, result outside linear range of instrument. GC/MS only
J	Estimated concentration between the MDL and RL
U	The concentration is less than the MDL, or the analyte was not detected
W	Post-digestion spike out of control limits
Quality Control Qualifiers	
M	Duplicate inject precision did not agree, organics only
N	Spiked sample recovery not within control limits
&	Accuracy result not within control limits (outside recovery of SRM)
*	Precision result not within control limits

10. Figures

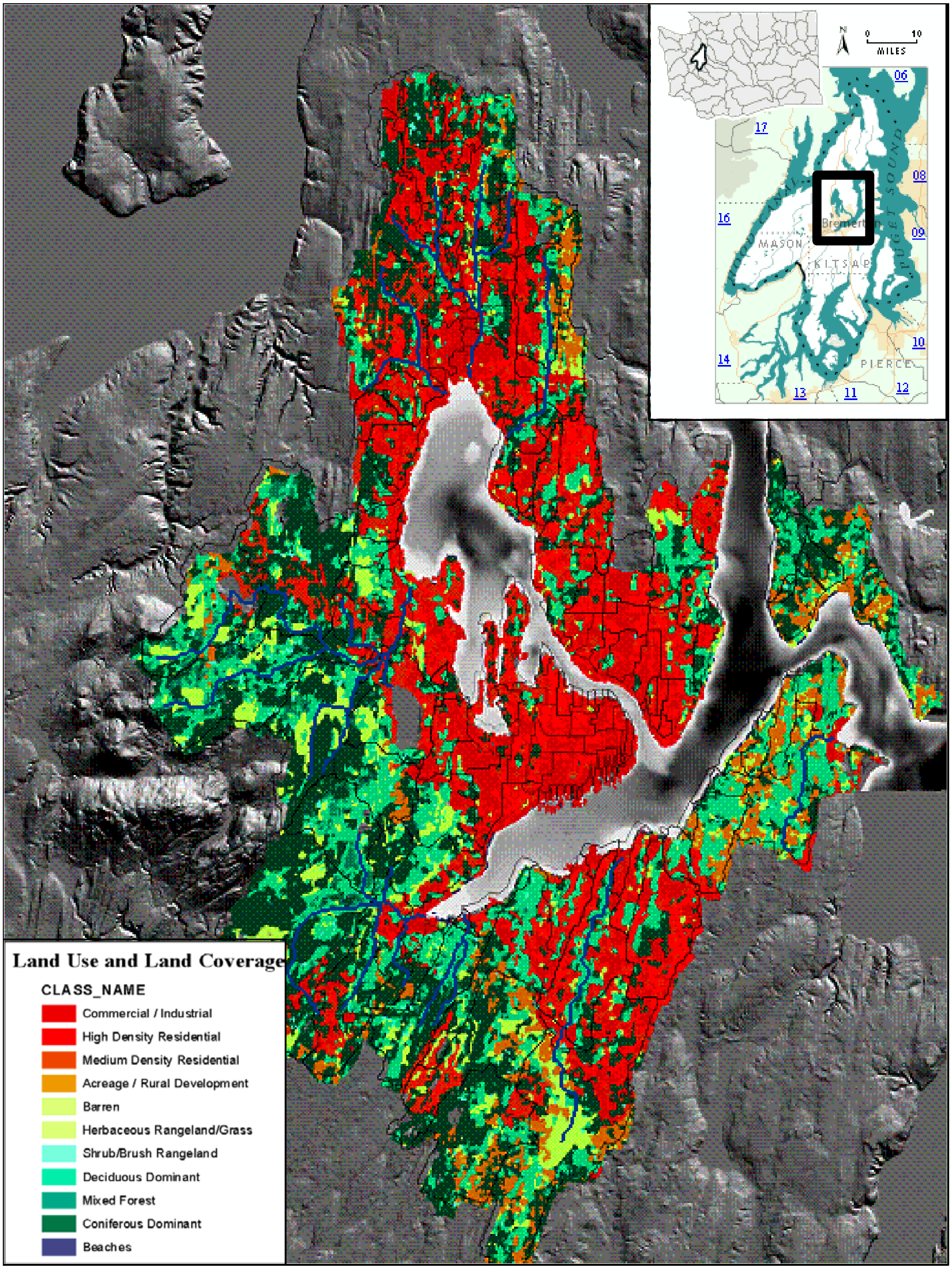


Figure 1. The land use and land cover classification for the watershed draining into Sinclair and Dyes Inlets.

Figure 2. The proposed demersal fish sampling stations are the baseline stations monitored by PSAMP (http://www.wdfw.wa.gov/fish/psamp/solesample.htm).

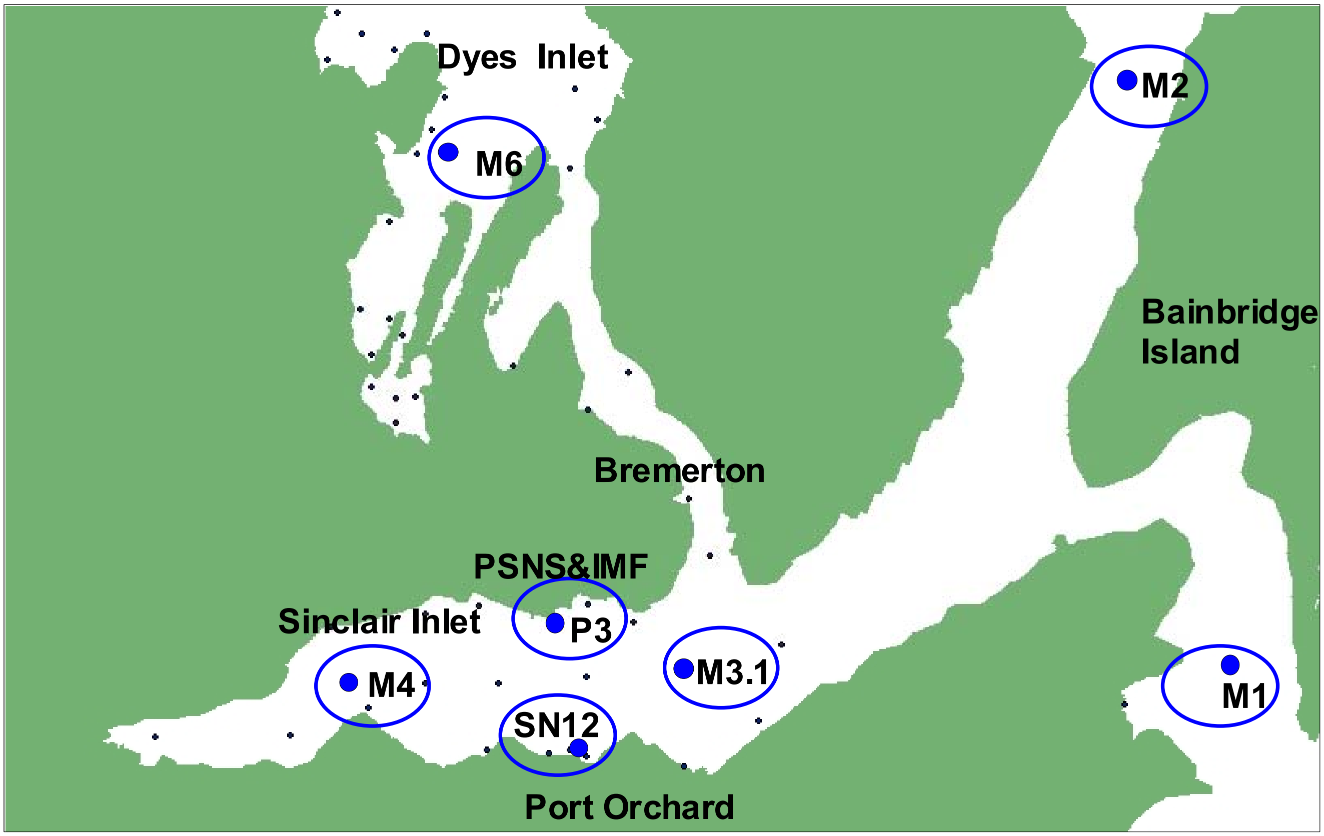
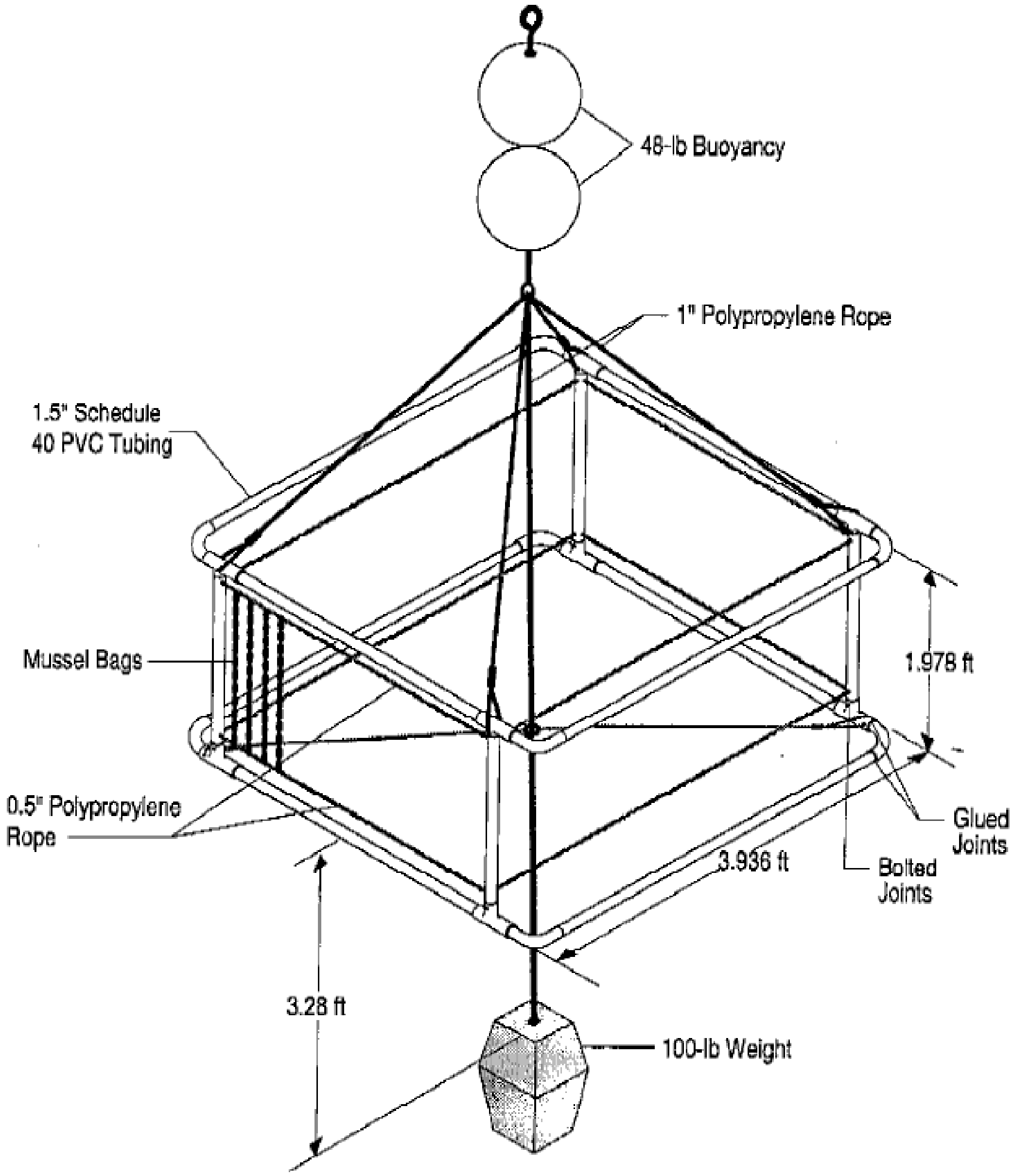


Figure 3. Proposed deployed mussel stations.



Notes: PVC tubing fixed with glue and stainless steel bolts with nylon threaded nuts so that frame can be dismantled to lie flat when not in use. PVC tubing has 0.25" holes for water drainage. Mussel bags affixed to rope with tie wraps.

Figure 4. Diagram of mussel cage mooring for open water deployments.