1.0 Title Page/TOC/Distribution List

Quality Assurance Project Plan

Toxic Contaminants in Dungeness crab (*Cancer magister*) and Spot Prawn (*Pandalus platyceros*) from Puget Sound, Washington, USA

September 2012

Approved by:

Signature: _____ Date: 9/27/12

James West, Lead Author/ Principal Investigator

Signature: _____ Date: 10/2/12

Craig Burley, Author's Supervisor/Manager

Signature: _____ Date: 9/24/12

William Kammin, Quality Assurance Officer, Ecology:

Quality Assurance Project Plan

Toxic Contaminants in Dungeness crab (*Cancer magister*) and Spot Prawn (*Pandalus platyceros*) from Puget Sound, Washington, USA

WDFW Contract No. 12-1100

September 2012

Prepared by: James E. West
Washington Department of Fish and Wildlife

Prepared for:
Washington Department of Ecology

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Toxics in Biota

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2.0 List of Figures

Distribution List

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3.0 Abstract

The following study *Toxic Contaminants in Dungeness Crab and Spot Prawn from Puget Sound* is a broad-scale, Puget Sound-wide assessment of toxic contaminants in Dungeness crab (*Cancer magister*) and spot prawn (*Pandalus platyceros*). This Quality Assurance Project Plan describes study objectives and operating procedures to be followed to achieve the goals of this study. The purposes of this study are to (a) evaluate the geographic extent and magnitude of toxic chemical contaminants in these two shellfish species in Puget Sound and (b) to provide contaminant data to DOH for them to conduct a human health risk assessment.

Geographic coverage of samples reflects the usual fishing grounds in Puget Sound for tribal and non-tribal fisheries, for both Dungeness crab and spot prawns. Both species will be collected in WDFW Recreational Marine Areas (MAs) 6 through 13 and three urban embayments within MAs 10 and 11, Commencement Bay, Elliot Bay, and Sinclair Inlet. This sampling plan covers the entire extent of Puget Sound from Point Roberts in the north to Budd Bay in the south, and west to Port Angeles, including Hood Canal. Additional effort is focused on three urban areas of concern.

One objective is to represent each MA and embayment by sampling up to five replicate samples of crab and shrimp from discrete fishing locations (stations) using individuals taken from test fishery and trawl study efforts. Muscle tissue will be dissected from crab and shrimp, creating composites for each. Each species composite will represent a station. Each composite will be analyzed for the organic contaminants; polychlorinated biphenyls (PCBs), polybrominated diphenyls (PBDEs), chlorinated pesticides, polycyclic aromatic hydrocarbons (PAHs), and the metals; mercury, lead, arsenic, and cadmium. A second objective is to characterize the contaminant load in other tissues that may be also be consumed by humans, namely hepatopancreas in Dungeness crab and the head and thorax (non-tail tissues) of spot prawn. This second objective will rely on analyzing a smaller set of samples, paired with muscle samples, to generate a predictive regression between the two tissue types.

At completion of the study, the Washington State Department of Fish and Wildlife (WDFW)-Puget Sound Ecosystem Monitoring Program (PSEMP) will produce a final report to Ecology and a data report to the Washington State Department of Health (DOH). The DOH will produce a human risk assessment. The PSEMP final report will be posted to the internet. All data will be submitted for uploading into STORET and EIM databases.

4.0 Background

As a member of the Puget Sound Ecosystem Monitoring Program (PSEMP), the Washington Department of Fish and Wildlife (WDFW) assesses status of and trends in the health of Puget Sound fishes and macro-invertebrates related to their exposure to toxic contaminants. This *Toxics in Biota* effort is one component of PSEMP, a multi-agency effort designed to monitor the health of the Puget Sound ecosystem. PSEMP tracks a broad range of status indicators, including submerged aquatic vegetation, sediment health, fecal contamination in shellfish, water quality and several others. WDFW's *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.

Overarching goals, design and implementation of the complete, original PSEMP were written by a committee organized by the Puget Sound Water Quality Authority (PSWQA) in 1988 (Monitoring Management Committee 1988a), in response to Washington State legislation that created the PSWQA to evaluate the health of the Puget Sound ecosystem. The original scope and sampling design of all PSEMP components were updated based on a formal review in 1995 (O'Neill, West et al. 1995; Shen 1995) and several components were reviewed in 2002. The *Toxics in Biota* component was implemented in 1989 (Stern 1989); periodic changes to the *Toxics in Biota* component have subsequently been made related to changing technology, management and policy needs, and variability in budgets. Changes in design or direction have generally been guided and vetted by PSEMP's Steering and Management Committees, and by scientific liaisons from PSEMP's organizing entity (formerly the Puget Sound Water Quality Authority, Puget Sound Action Team, and now Puget Sound Partnership)

The following project details specific procedures and quality assurance guidelines proposed by *Toxics in Biota* staff to implement its short–term project *Toxic Contaminants in Dungeness Crab and Spot Prawn from Puget Sound.*

5.0 Project Description

This project is a broad-scale, Puget Sound-wide assessment of toxic contaminants in Dungeness crab (*Cancer magister*) and spot prawn (*Pandalus platyceros*). In general, this study covers all of Puget Sound from its southern basin to the Canadian border, and westward to approximately Port Angeles.

Funds for this project support collection of new samples from a variety of fishery sources including test fisheries conducted by the Washington Department of Fish and Wildlife's (WDFW), and various Native American Tribes throughout the sound. In addition, crab and spot prawn samples collected from previous efforts (in anticipation of this study) by WDFW's Puget Sound Ecosystem Monitoring Program (PSEMP) will be used. WDFW staff have coordinated and consulted with Washington Department of Health (DOH) staff to ensure adequate spatial coverage, sample size, and other criteria regarding the human health risk assessment as detailed below.

5.1 Project goals

The goal of this study is to characterize the exposure of large, benthic macroinvertebrates in Puget Sound to the toxic chemical contaminants typically observed in biota from this ecosystem. Exposure is measured here as tissue residues (concentration) of contaminants. The study is designed to provide information regarding the health of benthic invertebrates in Puget Sound relative to their exposure to toxic contaminants, and to support an evaluation of seafood safety to be conducted separately by the Washington Department of Health.

5.2 Project objective

The objectives of this study are to (a) collect Dungeness crab and spot prawn from up to nine WDFW Marine Areas and three urban embayments, (b) measure contaminants in their muscle tissue, (c) evaluate the spatial extent and magnitude of contamination in these animals, (d) measure contaminants in a subset of hepatopancreas (Dungeness crab) and head/thorax (spot prawn) and (e) provide tissue data to the Washington Department of Health for their human health risk assessment.

5.3 Information needed and sources

We will be generating new data on toxic contaminants in muscle tissue from Dungeness crab and spot prawn, presented as wet weight concentration. Pre-existing PSEMP contaminant data on these species will be incorporated when pertinent, for context. Organic chemical contaminants were measured in twelve individual Dungeness crab from 2005 and eight composite samples of spot prawn muscle in 2007 (Washington department of Fish and Wildlife, unpublished data). Mercury was measured in 25 Dungeness crab samples from 2005. Results from these pilot efforts informed the design of the current study.

5.4 Target population

The target population for this study is Dungeness crab and spot prawn from Puget Sound, as typically taken in Puget Sound sport or commercial fisheries.

5.5 Study boundaries

Study boundaries are the WDFW Marine Areas of Puget Sound described herein (Section 7.0) and in the project Scope of Work.

5.6 Tasks required

Tasks involved in this study include

- Sample collection
- Sample preparation
    - Tissue resection
    - Sample homogenization and compositing
    - Delivery of samples to contract analytical lab
- Contract lab analysis of samples
- QA/QC review
- Formatting of data for relational database
- Delivery of data to DOH
- Analysis of data for PSEMP/DFW report
- Transfer of data to EIM

5.7 Practical constraints

The most pertinent constraints here relate to (a) sample timing and (b) minimizing contaminant variability related to variable biological characteristics of sample organisms. In order to avoid variability related to seasonal differences in contaminant exposure all samples will be taken in the months of April through June. Spot prawns are only sampled easily during these months, when fisheries are operating, or when prawns are aggregating. Dungeness crab fisheries also operate during this window of opportunity, and the 2011 PSEMP crab samples were taken in the month of May.

In order to minimize variability related to age and sex of the animals, we plan to sample adult female spot prawn (the dominant stage in the fishery), and male Dungeness crab with hardened shells, and of legal size (per WDFW fishery regulations).

Sample location is a constraint. Both crab and spot prawn aggregate in predictable areas and habitat, which precludes random sampling within Marine Areas or urban embayments. WDFW and Tribal test fisheries routinely sample pre-selected, historical index sites, which they have selected after many years of testing. We have adopted these sampling areas from the WDFW test fishery standard operating procedures (section 16.0) for our sampling source. Although we plan to sample as evenly as possible within Marine Areas, we will need to evaluate the spatial coverage of samples after sampling has been completed to decide which Marine Area sites will be analyzed to achieve as representative a coverage as possible.

In some cases it may be impossible to collect enough or any crab or shrimp in a MA because the species simply does not exist there. For example spot prawn are relatively rare in South Puget Sound.

6.0 Organization and Schedule

6.1 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	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
Stephen R. Quinnell	Fish and Wildlife Biologist 2	360.902.2849	stephen.quinnell@dfw.wa.gov	PSEMP database, lab/field lead
Stefanie Orlaineta	Part-time temporary technician	360.902.2657	stefanie.orlaineta@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

6.2 Project schedule

Table 2 Proposed schedule for completing field and laboratory work.

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

6.3 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. This has resulted in a delay in delivering the QAPP for this project to be finalized prior to sample collection. Permission to proceed with sample collection was given by Ecology based on a preliminary Draft QAPP.

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.

6.4 Budget and funding

This project is supported by a grant from the Washington State Department of Ecology (Ecology) as Lead Organization for Toxics and Nutrients Prevention, Reduction, and Control 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.

Table 3 Draft budget for 2012/13 crab and prawn analysis and data processing for DOH human health risk assessment.

						Breakdown by Task			
Object	Cost per Unit	Unit	No. of Units	FTE	Total Cost	Task1 (QAPP)	Task 2 Sample Prep and Collection	Task 3 Submit samples for analysis	Task 4 Data analysis report writing
Bio2 Step G	$3,800	month	13.0	0.50	$24,700	$3,800	$5,700	$5,700	$9,500
Bio 2 Benefits	$1,584	month	13.0	0.50	$10,296	$1,584	$2,376	$2,376	$3,960
Technician Salary	$2,971	month	2.5	1.00	$7,428		$7,428		
Technician Benefits	$1,460	month	2.5	1.00	$3,650		$3,650		
Personnel Services	$23	month	9.0		$207	$12	$81	$46	$69
Supplies	$1,100	project	1		$1,100		$1,100		
Sample Archiving	$20	sample	121		$2,420		$2,420		
Travel	$300	per diem	10		$3,000		$3,000		
Vehicle	$324	project	1		$324		$324		
Organics+conventionals	$881	sample	121		$106,601			$106,601	
Metals	$120	sample	121		$14,520			$14,520	
SubTotal Direct					$174,246	$5,396	$26,078	$129,243	$13,529
Indirect* FY12 (23.51%)	0.2351				$2,882	$1,268	$1,614		
Indirect* FY13 (30%)	0.3				$12,260		$5,764	$2,437	$4,059
TOTAL					$189,387	$6,664	$33,456	$131,680	$17,588

*Indirect fees waived by WDFW for chemical analyses . Will increase to approximately 30% beginning 1 July 2012

7.0 Quality Objectives

7.1 Measurement Quality Objectives

Following are three tables listing the minimum QA criteria for organic chemicals and metals analyzed in Dungeness crab and spot prawn in this study.

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 ≤ 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 ≥ 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 × 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 ≤ 15% (equivalent to relative percent difference ≤ 30% for duplicates) for ≥ 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.

Figure 1 Quality assurance criteria for PCBs, PPBDEs, PAHs, and OCPs.

Reproduced from Sloan et al. (2006).

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

Figure 2 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
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

Figure 3 Required batch quality control measures and quality assurance criteria for the ICP-MS metals As, Cd, and Pb. Reproduced from KCEL SOP 624v2

7.1.1 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. For this study NIST SRM 1974b will be used for al organics[1]. 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.

7.1.2 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.

[1] SRM 1974b is no longer available from NIST. The NOAA lab has enough matrix on hand for this study, however, a suitable alternative may be substituted, at the chemist's discretion.

7.1.3 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. Table 5 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.

7.1.4 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 Dungeness crab and spot prawn.

7.1.5 Representativeness

The sampling design in this study is aimed at representing contaminant conditions as tissue residues in Dungeness crab and spot prawn taken in typical sport fisheries in Puget Sound. To that end the design optimizes spatial coverage that is representative of the fisheries (rather than a random location selection for instance). The location and timing of sampling in this study is congruent with typical sport fisheries. To maintain a low cost, no attempt is made in this design to evaluate catch rates and to weight areas by amount caught.

Sample handling will be consistent so not affect one sample more so than another. Test fishers and samplers were instructed using the same protocol. Animals selected out of the pools of collected specimens will be legal sized and in the case of Dungeness crab, male. Five crabs (5) and twenty (20) spot prawn of legal criteria will be randomly sampled. All samples will be placed on ice in coolers until they can be frozen, which will be no more than eight hours. WDFW will retrieve frozen samples for transport back to the lab where the samples will remain frozen until processed.

7.1.6 Completeness

The goal of this study is to collect and analyze five stations (replicates) of spot prawn and Dungeness crab muscle tissue in each of the Marine Catch Areas (6-13), plus three urban embayments for metals and POPs. This is 12 total areas each, with five replicates totaling 60 field samples of each species. It is impossible to predict whether we will successfully sample this number of replicates from each Marine Area because these species may not occur naturally in every MA or urban embayment. A sample size of five per MA/embayment was selected to optimize power for detecting spatial differences, based on previous experience with the same analytes in other species. The number of animals per composite was selected to balance representativeness of the population with labor and time constraints related to processing samples. Our goal will be achieved if we are able to (1) represent each Marine Area or urban embayment with five replicates, for the areas where people habitually fish in large numbers for these species, and (2) include at least three crab and 15 shrimp per composite.

When processing the samples into composites, lab staff will be inspecting each animal for quality (no punctures or cracks that might expose internal tissues to outside contamination) and meristic characters that satisfy fishery regulations. In the case of crab, we will composite a station with no fewer than three crabs but not more than five. In the case of prawns, we will composite a station with no fewer than 15 but not more than 20.

Chemical analysis data will be reviewed to ensure data quality objectives were met. Data will not be released from the labs unless all data quality objectives are met. The residue concentrations will be inspected for lab contamination and expected trends i.e., urban is greater than rural.

8.0 Sampling Process Design (Experimental Design)

8.1 Study Design

8.1.1 Sampling location and frequency

Samples of crab and shrimp will be taken from each of nine areas designated by the Washington Department of Fish and Wildlife as Marine Areas for fishery management (Figure 4). In addition, three urban areas (Elliott Bay, Sinclair Inlet, and Commencement Bay) will be targeted for sampling. The sampling design is meant to mimic the spatial coverage of typical sport fisheries within these areas, and so sampling areas are pre-determined by historical fishing patterns and species distribution and availability.

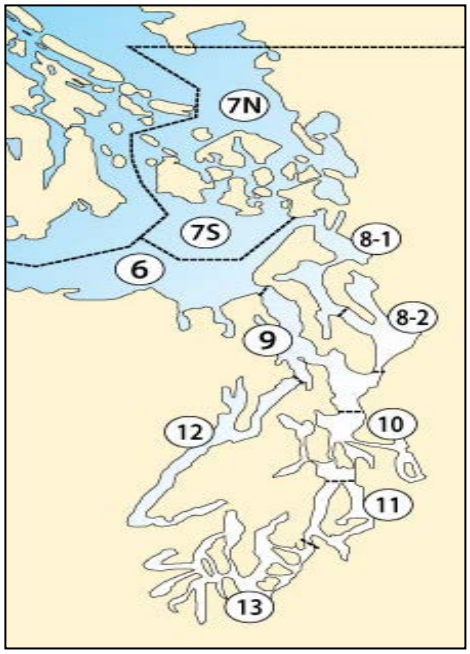


Figure 4 WDFW Marine Areas (MA) designated for Puget Sound. For this study MA 7N and 7S are combined into a single MA (7).

All crab and shrimp samples will be taken from Late March through July (according to schedule). Spot prawn are easily sampled during these months, when fisheries are operating, or when prawns are aggregating. Dungeness crab fisheries also operate during this window of opportunity, and the 2011 PSEMP crab samples were taken in the month of May.

8.1.2 Parameters to be determined

Parameters related to sample collection include date, location (latitude and longitude), depth, species, and tissue concentrations of contaminants listed in Table 4 and Table 5.

8.1.3 Field measurements

Dungeness crab will be measured for carapace length, sexed and tested for shell hardness according to Appendix B. *Washington Department of Fish and Wildlife Protocols for Dungeness Crab Test Fishery*. Size of spot prawn will be measured in the lab (see below).

8.1.4 Maps or diagram

Figure 4 shows the boundaries of the Washington Department of Fish and Wildlife's Marine Areas, which delineate the boundaries of this study.

8.1.5 Assumptions underlying design

The primary assumption of this study is that test fisheries conducted by WDFW and Tribal biologists sample the same population as targeted by fishers, and that their sampling gear has similar selectivity, regarding the size of animals retained by the pots. Some of the test fishery gear has smaller mesh size than legal-size mesh in the sport fishery, however we will retain only specimens that are either of legal size by measurement (carapace width for Dungeness crab) or of a size that would be retained by legal sized mesh (> 29 mm carapace length for spot prawn).

We assume any differences in gear selectivity between bottom trawling and pot-fishery methods would not affect the current study, because sampling locations, animal size, and condition are constrained by the study design. In one case, samples of spot prawn were obtained in the 2011 bottom trawling. This area (Marine Area 13) typically does not support pot-fishing for spot prawn because they are considered rare there. Hence the only spot prawn samples from MA 13 are from bottom trawling. To minimize cost, this study is focused on muscle as a target tissue. However, it is known that some fishers consume internal organs of these animals, including the hepatopancreas (in the case of crab) or the shelled head and thorax (in the case of spot prawn). In this study, we anticipate a demonstrable relationship between muscle and hepatopancreas (Dungeness Crab) or muscle and head (spot prawn) concentration, which would allow prediction of the latter from the former. We assume a sample size of 15 to 20, across a wide range of contaminant loads, will be sufficient to construct a predictive model, using linear or log-linear regression analysis.

8.1.6 Relation to objectives and site characteristics

A consistent number of composite samples is targeted from each of the nine Marine Areas and three urban embayments. However, spot prawn or Dungeness crab may be naturally lacking from these study areas. Efforts will be expended to catch samples in all MAs and urban embayments, however sample objectives may not be met for each.

8.1.7 Characteristics of existing data

Pilot sampling of Dungeness crab and spot prawn from 2007 and 2005 PSEMP surveys indicated urban signals of certain POPs in muscle (spot prawn and crab) and hepatopancreas (crab) (WDFW, unpublished data). These data were used to inform the current study in terms of the range of locations selected for study. In particular they led to the selection of three urban embayments (Elliott Bay, Sinclair Inlet, and Commencement Bay) as sub-areas.

9.0 Sampling Procedures

9.1 Chain-of-custody

Two Chain of Custody (COC) forms are used for the Toxics in Crab and Shrimp study. The Field COC (Figure 5) is used to transfer samples from field staff to lab staff, and the PSEMP Task Order (Figure 6) is used to track the chain of custody of samples from the lab to the analytical lab.

9.2 Field log requirements

The lead scientist for each field survey maintains a spiral bound Rite-in-the-Rain field log with detailed notes for each day's activities. Minimum information recorded is:

- Name and location of project
- Field personnel
- Sequence of events
- Any changes to plan
- Date, time, location, ID, and description of each sample
- Unusual circumstances that may affect interpretation of results

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

9.3 Field measurement and field sampling Standard Operating Procedures

Spot prawn (*Pandalus platyceros*) and Dungeness crab (*Cancer magister*) for the **Toxics in Crab and Shrimp** study may be collected using either of two methods (1) pot sampling, as conducted by Agency or Tribal biologists from annual spot prawn and Dungeness crab test fisheries, with samples donated to this study from those efforts or (2) bottom trawling conducted by WDFW/PSEMP/Toxics in Biota staff. Standard operating procedures for test fisheries are presented below. Spot prawn and Dungeness crab sampled by bottom trawl were retained from the WDFW/PSEMP/Toxics in Biota 2011 bottomfish trawl survey, the most recent of the PSEMP biennial English sole surveys. See Appendix D *Standard Operating Procedure for Bottom Trawl Fishing Gear* and the *2011 PSEMP Groundfish Survey Report.*

9.3.1 Spot Prawn from Test Fisheries

Spot prawn obtained from an Agency or Tribal test-fishery for contaminant analyses will be collected following the method "Spot Prawn Length-frequency Sampling Research Protocol-Draft 6[2]" (Appendix B). This protocol was developed and is used by WDFW and Tribal staff to obtain samples for their annual pre- and post-fishery population studies. They also agreed to retain samples for the **Toxics in Crab and**

[2] This is an unpublished SOP used by WDFW and tribal fishery biologists to sample population abundance and biological metrics of spot prawn both prior to and after setting public fishing seasons.

Shrimp study. The following additional protocol details are communicated to field staff conducting spot prawn test-fisheries to ensure project goals and objectives regarding spot prawn for the **Toxics in Crab and Shrimp** study are met.

For spot prawn, the objective of the **Toxics in Crab and Shrimp** study is to obtain five separate replicate samples of twenty spot prawn, from each of nine WDFW Recreational Marine Areas (MA), and three urban embayments. If more than 20 spot prawn are collected, no more than 20 will be randomly selected for processing. Samples will be taken during late March through July (according to schedule), corresponding to months when the test-fisheries or public fisheries take place, and to match timing of the biennial PSEMP bottom-trawl survey. Sample station distribution will be allotted according to traditionally fished areas, as designated by WDFW's Spot Prawn Length-frequency Sampling Research Protocol – Draft #6 (Appendix B). In cases where more than 5 locations are available from a MA, stations will be selected to optimize broad coverage across the MA.

Spot prawn retained by test-fishers will be equivalent to the size taken in the sport fishery, i.e. legal-sized based on the 1-inch size selection of the spot prawn pot mesh (approximately 29 mm carapace length or larger, Mark O'Toole, WDFW Spot Prawn Test Fishery Manager, personal communication). These represent the largest and oldest individuals in fished populations, and because spot prawns are protandric hermaphrodites sampled animals for this study are assumed to be dominated by females or individuals in transition from male to female (Mark O'Toole, WDFW Spot Prawn Test Fishery Manager, personal communication).

Each group of twenty spot prawn identified as suitable for this study will be removed from the pot into a sample bag. Each bag will be identified by a tag or label that indicates the date and location sampled (e.g., either station ID, waypoint, and/or coordinates). Spot prawn will be placed whole in a 1-gallon Ziploc-type bag and held on ice on the boat until the bags can be placed in a freezer (typically less than 8 hours). Field staff will be instructed to handle the spot prawn to avoid potential contamination sources from the boat, e.g., the boat deck, oily surfaces and water, and engine exhaust. Because spot prawn have sharp rostrums, field staff will wear protective thick vinyl or plastic gloves for personal protection. We assume that the shell and shell membranes of undamaged specimens afford protection from potential environmental contaminants during retrieval from the pot. For these reasons, field staff are not expected to wear nitrile gloves. To optimize the natural shell and membrane protection, specimens with damaged shells will not be accepted as samples.

Spot prawn samples will be placed in coolers on ice immediately and frozen upon return to port. WDFW staff will present a field chain-of-custody (COC) form (Section 8.8, Figure 12) to the field crew staff to record the condition of samples collected and the transfer of samples. If available, WDFW staff will obtain a copy of field data sheets during this transfer. If data sheets are unavailable at that point, they

Washington Department of Fish and Wildlife
Puget Sound Ecosystem Monitoring Program
Primary contact: James West at 360-902-2842 or james.west@dfw.wa.gov

CHAIN OF CUSTODY FORM

SURVEYID:__________________STATIONID:__________________EFFORTID:__________________

SPECIES:______________________COLLECTION DATE:__________________________________

Sample Type /Purpose	Species	Collection Date	Haul ID	Station ID (Location)	Count	Condition	Sample Container	Sample ID	Signature of *Release*	Organization	Date	Signature of *Recipient*	Organization	Date
COMMENTS														
COMMENTS														
COMMENTS														
COMMENTS														
COMMENTS														
COMMENTS														
COMMENTS														

This form is to be filled out for all samples collected, with one sample type per record and each record signed by the person taking custody of the samples. PSEMP 6/12

[1] Sample condition: F - frozen LN - liquid nitrogen I - on ice L - live P - preservative

General Comments:____

Page_____of_____

Figure 5 Chain of custody used for transferring samples from the field to the WDFW lab

WASHINGTON DEPARTMENT OF FISH AND WILDLIFE Fiscal Year: _____

TASK ORDER FOR SAMPLE ANALYSIS

IN ACCORDANCE WITH INTERAGENCY AGREEMENT # xx-xxxx

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:

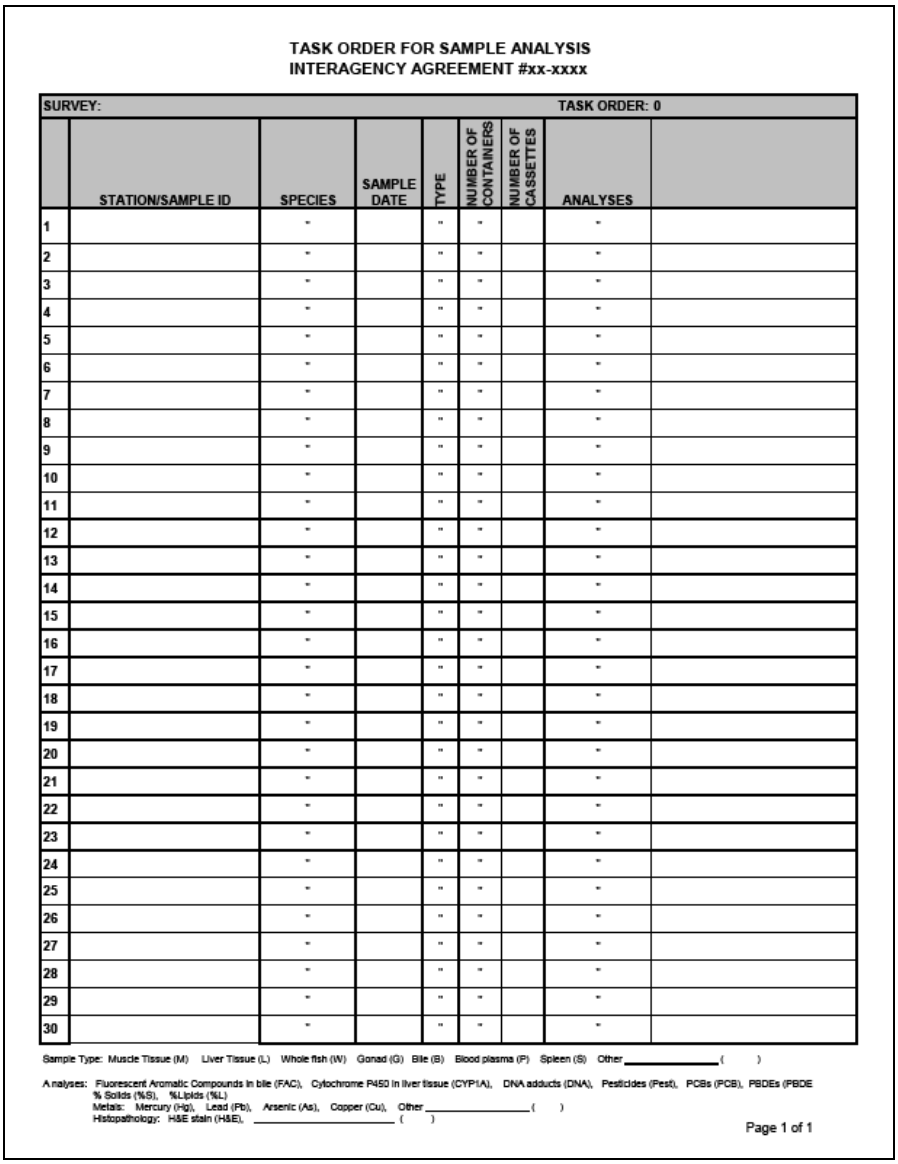
National Marine Fisheries Service		Washington Dept. of Fish and Wildlife	
_____		_____	
Representative	Date	Project Representative	Date

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

Page 1 of 1

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), _____( )

Page 1 of 1

Figure 6 Task Order Chain of custody form used to transfer samples from the WDFW Resection Lab to the Analytical Labs

may be sent later by field staff. Field data and/or COC forms will record time of soak, pot depth, date of collection, and latitude/longitude PSEMP staff will continually monitor test-fishery field staff and schedule sample retrieval to minimize samples being in possession by non-PSEMP staff.

The PSEMP database manager will assign station names (e.g., "Vashon Island" versus 3TreePt.") as unique identifiers within MAs or urban embayments. Spot prawn sample data forms (Appendix B, Figure 1) and GPS waypoint data will be delivered electronically to PSEMP staff for cross reference to the COC and to complete station identification within the PSEMP database. Sample depth, location, and period of immersion (soak time) will be transcribed from test-fishery data into the PSEMP database.

9.3.2 Dungeness Crab from Test Fisheries

Dungeness crab obtained from an Agency or Tribal test-fishery for contaminant analyses will be collected following methods outlined in "Washington Department of Fish and Wildlife Test Fishery Protocol Manual for Hood Cana Dungeness Crab (*Cancer magister* [3]" Appendix C). This protocol was developed and is used by WDFW and Tribal staff to obtain samples for their annual pre- and post-fishery population studies. They also agreed to retain samples for the **Toxics in Crab and Shrimp** study. The following additional protocol details are communicated to field staff conducting Dungeness crab test-fisheries to ensure project goals and objectives regarding Dungeness crab for the **Toxics in Crab and Shrimp** study are met.

For Dungeness crab, the objective of the **Toxics in Crab and Shrimp** study is to obtain five separate replicate samples of five Dungeness crab, from each of nine WDFW Recreational Marine Areas (MA), and three urban embayments. Samples will be taken during April through June, corresponding to months when the test-fisheries or public fisheries take place, and to match timing of the biennial PSEMP bottom-trawl survey. Sample station distribution will be allotted according to traditionally fished areas, as communicated to this study by WDFW crab biologists. In cases where more than 5 locations are available from a MA, stations will be selected to optimize broad coverage across the MA.

Dungeness crab retained by test-fishers for this contaminant study will be equivalent to the size and condition taken in the sport fishery, i.e. legal-sized male crab with a hard shell. Legal size is defined as a minimum carapace width of 6.25 inches (Appendix C, Figure 3). Procedures for sexing and evaluation of shell condition are in Appendix C.

Each of five Dungeness crab identified as suitable for this study will be removed from the pot and placed singly into a sample bag. If there are greater than five crabs that are suitable, five will be chosen randomly either on the boat or in the lab. Each bag will be identified by a tag or label that indicates the date and location sampled (e.g., station ID, waypoint, and/or coordinates). Individual Dungeness crab Ziploc-type bags will be held on ice on the boat until the bags can be placed in a freezer (typically less than 8 hours). Because Dungeness Crab have claws, field staff wore protective thick vinyl or plastic gloves for personal protection. Also, the target tissue for this study is muscle (claw and leg). The shell and shell membranes of undamaged specimens afford protection from potential environmental contaminants during the retrieval from the pot. For these reasons, field staff were not expected to wear nitrile gloves. To optimize the natural shell and membrane protection, specimens with damaged shells will not be accepted as samples. .

Spot prawn samples will be placed in coolers on ice immediately and frozen upon return to port. A chain-of-custody (COC) form (Section 8.8, Figure 12) will be presented by WDFW staff to the field crew staff to record the condition of samples collected and the transfer of samples. If available, WDFW staff will obtain a copy of field data sheets during this transfer. If data sheets are unavailable at that point, they may be sent later by field staff. Field data and/or COC forms will record time of soak, pot depth,

[3] This is an unpublished SOP used by WDFW and tribal fishery biologists to sample population abundance and biological metrics of spot prawn both prior to and after setting public fishing seasons.

date of collection, and latitude/longitude. PSEMP staff will continually monitor test-fishery field staff and schedule sample retrieval to minimize sample possession by non-PSEMP staff.

The PSEMP database manager will assign station names (e.g., "Vashon Island" versus 3TreePt.") as unique identifiers within MAs or urban embayments. Dungeness crab sample data forms (Appendix C, Figure 2) and GPS waypoint data will be delivered electronically to PSEMP staff for cross reference to the COC and to complete station identification within the PSEMP database. Sample depth, location, and period of immersion (soak time) will be transcribed from test-fishery data into the PSEMP database.

9.3.3 Spot Prawn and Crab from WDFW Bottomfish Trawl Surveys

Some samples for this study were taken opportunistically in May, 2011 from WDFW's biennial English sole survey, and in 2012 from WDFW's bottomfish abundance survey. These surveys are typically conducted in the month of May; the former survey provides the primary indicator species for toxics in benthic biota, English sole. Dungeness crab and spot prawn were retained from these surveys in anticipation of current efforts to conduct a Sound-wide survey of toxic in these invertebrates. Retaining these specimens represents a significant cost savings for the present study, but also necessitates a separate description of field sampling methods.

Bottom trawl gear and operations for the bottomfish trawl methods are described in detail in Appendix D. Spot prawn and Dungeness crab were taken from those surveys as follows. Spot prawn and Dungeness crab were separated from the catch and sorted into individual deck bins. They were placed in labeled Ziploc bags and immediately put into an on-deck freezer for later processing in the lab. Female and soft-shelled Dungeness crab were released, and hard-shelled males were retained for measurement. Up to twenty male crab exceeding 6.25 inches carapace width (see Appendix C) were retained for lab processing and chemical analysis. The legs and claws from each retained crab were removed and placed in individual Ziploc-style bags, with a FishID label. Individual bags were then placed in an on-deck freezer. Collection information, including sex and carapace width for each crab was recorded on a Specimen Form (Section 8.2, Figure 10).

At the end of each week all Spot Prawn and Dungeness crab samples were transferred to the lab. Individual Ziploc bags were gathered in groups into larger plastic bags by station, and labeled inside and out with pre-numbered tear-off wire tags (Appendix D). These bags were placed in 96 quart coolers with enough ice to prevent thawing during transport to the lab. Once returned to the WDFW lab at the Natural Resources Building all bags were removed from coolers and placed in a -20° centigrade walk-in or chest freezer.

9.4 Preparation for Tissue Resection and Processing in the Lab

9.4.1 Equipment, reagents, and supplies

Terg-A-Zyme®
Deionized (DI) Water - teflon squeeze bottles
Isopropyl Alcohol - B&J Brand® Multipurpose ACS, HPLC
Tap water
Teflon Squeeze bottles
Heavy duty aluminum foil – Reynolds 627 (60.96 cm wide x 0.94 mm thick)
Scissors - stainless steel
Forceps - stainless steel
Spatula – stainless steel, flat blade/round blade
Mixing spoon – stainless steel
Measuring tape – cloth
Calipers – stainless steel (Figure 3)
Stainless Steel mixing bowl
Plastic colander
Sample jars – clear, short, wide mouth 8 oz jars, I-CHEM Certified 200-0250 series, Type III glass with Teflon-lined polypropylene lid (Figure 8)
Bamix hand held mix/grind motor with stainless steel cutting blade (Figure 9)
Bench scales– A&D HP-22K (20,000 x 0.1grams) Figure 10
A&D EK-6000H (6,000 x 0.1 grams) Figure 10
Sample jar labels – cryogenic, laser printer ready, Diversified Biotech LCRY-2380 0.94in. x 0.50in and LCRY-1258 2.625in x 1.0in (Figure 14) .
Lab coat/apron
Nitrile exam gloves – talc-free
Eye protection
Freezers – walkin freezer at -20°C, chest freezer at -15°C

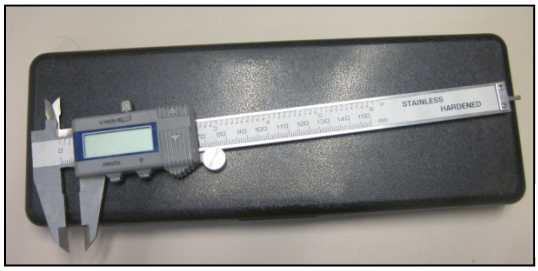


Figure 7 Stainless steel caliper with digital readout to 01.mm.

Figure 8 Pre-cleaned Series 200 I-Chem sample jar.

Figure 9 Hand-held Bamix tissue grinder

Figure 10 20-kg capacity bench scale for weighing large specimens

Figure 11 Six (6)-kg capacity bench scale for weighing small specimens.

9.4.2 Setup and Preparation

9.4.2.1 Preparation of Lab Record Forms

Two forms are used to keep a record of the samples prepared in the lab and the specimens that are used in their preparation -- Specimen Forms (Figure 9) and Resection Logs (Figure 10). In addition a daily log of operations is kept in the lab. A series of codes are assigned and printed on the lab forms; identification code for the survey (SurveyID), station StationID, specimen (FishID) and sample (SampleID).

9.4.2.2 Sampling Codes - Use and Creation

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.

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).

For specimens acquired from a source outside PSEMP (e.g. WDFW test fishery, WDFW survey, Tribal test fishery), if derived from a fixed[4] site, PSEMP uses the sources assigned name as the StationID; however, if the fixed site corresponds to an establish PSEMP station, the PSEMP StationID is used.

FishID: Each specimen (individual fish or invertebrate) 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

[4] fixed site – a specific location that is returned to repeatedly over time.

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 5001 through 9999 and NOAA is assigned 0001 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. A SampleID is a unique alpha-numeric code that is assigned to an analytical sample; either a sample taken from an individual or a composite of individual tissues. Each id consists of six parts, a two-character year code, 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.

Unique SampleIDs are assigned by concatenating numbers of label acronyms as follows:

- Two digit year,
- Two or three (typically) digit station identifier
- A dash "-"
- Two digit species
- Single digit matrix
- A sequential number

An example for a SampleID comprising a composite is **12EB-SPM01**, from 20**12**, **E**lliott **B**ay, **S**pot **P**rawn, **M**uscle, 01.

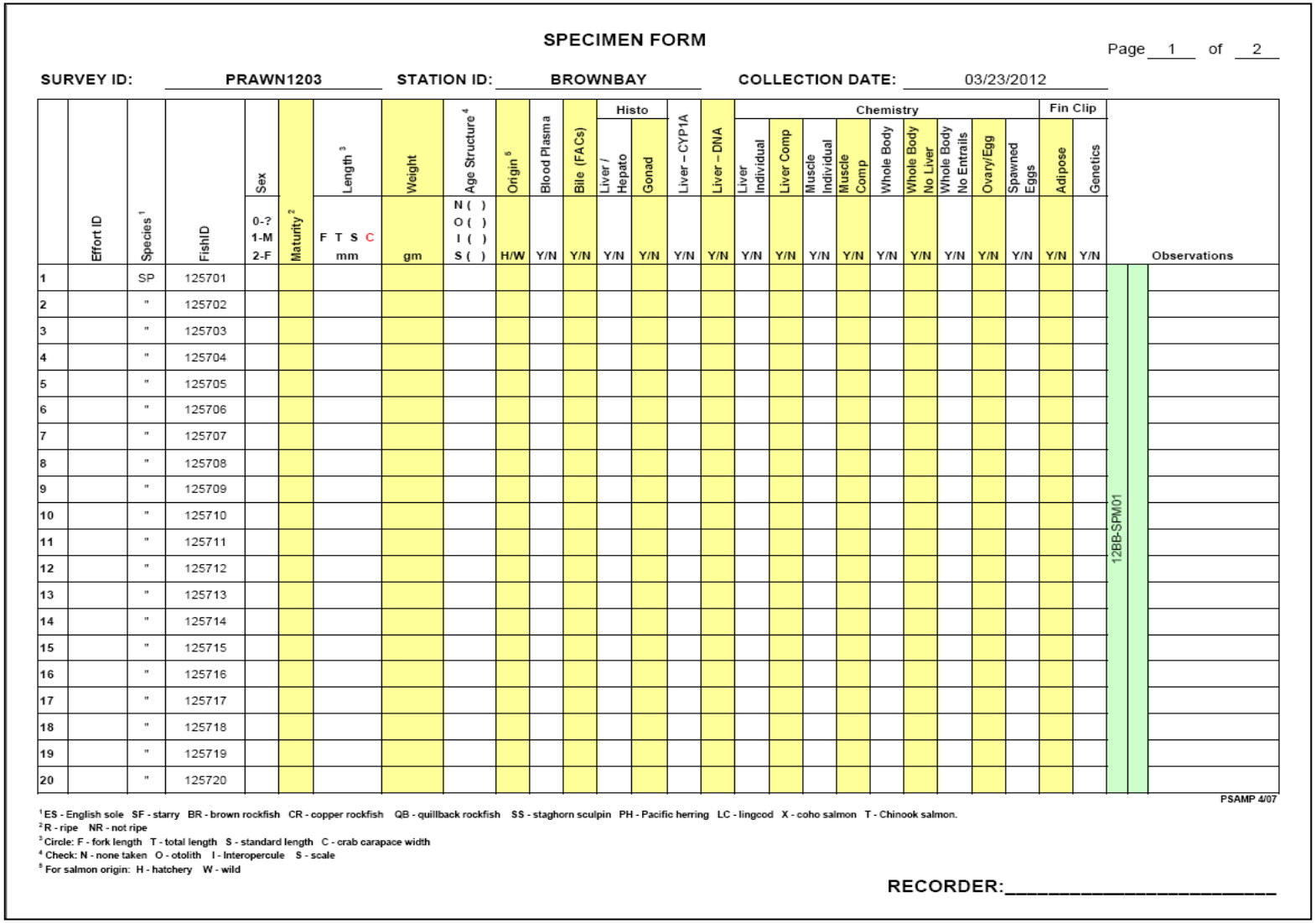
9.4.2.3 Forms - Use and Creation

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 Forms: Specimen Forms (Figure 12) are used to record information for each specimen processed for samples for a given station and survey. The information recorded includes station information, specimen information, 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
    a. SurveyID – database manager provides, preprinted on form
    b. StationID – database manager provides, preprinted on form
    c. Collection Date – preprinted on form and Time?
2. Specimen Information
    a. Species – preprinted on form
    b. 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.)
    c. FishID – database manager provides, preprinted on form
    d. Sex – enter sex if not preprinted on form
    e. length – enter the length to the nearest millimeter except spot prawn to the nearest 0.1 millimeter
    f. weight – enter the weight to the nearest 0.1 grams
    g. Type of Sample – Indicate with a "Y" (Yes) or an "N" (No) whether or not a sample type indicated on the form was taken.
    h. SampleID – database manager provides, preprinted on the form.
3. Observations : Note any unusual physical aberrations, lesions, parasites, etc.



SPECIMEN FORM

Page 1 of 2

SURVEY ID: PRAWN1203 **STATION ID:** BROWNBAY **COLLECTION DATE:** 03/23/2012

	Effort ID	Species[1]	FishID	Sex	Maturity[2]	Length[3]	Weight	Age Structure[4]	Origin[5]	Blood Plasma	Bile (FACs)	Histo		Liver – CYP1A	Liver – DNA	Chemistry									Fin Clip		Observations
												Liver / Hepato	Gonad			Liver Individual	Liver Comp	Muscle Individual	Muscle Comp	Whole Body	Whole Body No Liver	Whole Body No Entrails	Ovary/Egg	Spawned Eggs	Adipose	Genetics	
				0-? 1-M 2-F		F T S C mm	gm	N ( ) O ( ) I ( ) S ( )	H/W	Y/N	Y/N	Y/N	Y/N	Y/N	Y/N	Y/N	Y/N	Y/N	Y/N	Y/N	Y/N	Y/N	Y/N	Y/N	Y/N	Y/N	
1		SP	125701																							12BB-SPM01	
2		"	125702																								
3		"	125703																								
4		"	125704																								
5		"	125705																								
6		"	125706																								
7		"	125707																								
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9		"	125709																								
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18		"	125718																								
19		"	125719																								
20		"	125720																								

PSAMP 4/07

[1] ES - English sole SF - starry BR - brown rockfish CR - copper rockfish QB - quillback rockfish SS - staghorn sculpin PH - Pacific herring LC - lingcod X - coho salmon T - Chinook salmon.
[2] R - ripe NR - not ripe
[3] Circle: F - fork length T - total length S - standard length C - crab carapace width
[4] Check: N - none taken O - otolith I - Interopercule S - scale
[5] For salmon origin: H - hatchery W - wild

RECORDER:_____

Figure 12. Specimen form for recording biological metrics.

TISSUE RESECTION LOG

SURVEY ID: PRAWN1203 STATIONID: BROWNBAY SAMPLE TYPE: Whole Fish ( ) Muscle (X) Liver ( ) Gonad ( ) Bile ( ) Hepato ( )

	Species[1]	FishID	Weight: Tissue[3] (g)	Weight: Empty Jar (g)	Weight: Sample[4] (g)	SampleID	Date Collected	Date Composited	Days to Resection	Observations
1	SP	125701								
2	"	125702								
3	"	125703								
4	"	125704								
5	"	125705								
6	"	125706								
7	"	125707								
8	"	125708								
9	"	125709								
10	"	125710								
11	"	125711				12BB-SPM01				
12	"	125712								
13	"	125713								
14	"	125714								
15	"	125715								
16	"	125716								
17	"	125717								
18	"	125718								
19	"	125719								
20	"	125720								

[1]ES - English sole, SF - starry flounder, SS - staghorn sculpin, CR - copper rockfish, QB - quillback rockfish, BR - brown rockfish, PH - herring, LC – lingcod, SP - spot prawn X - coho salmon, T - chinook salmon, DC - Dungeness crab, GC - graceful crab

[2]Tissue Wt: grams of tissue taken from an individual fish.

[3]Sample Wt: total grams of tissue in a sample.

PSAMP 5/11

Page 1 of 2

Figure 13. Tissue Resection Log for recording the amount and type of tissue taken from a specimen, and the destination composite identification number (SampleID)

Tissue Resection Log: A Tissue Resection Log (TRL) (Figure 13) 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 and time? 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.

9.4.2.4 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.

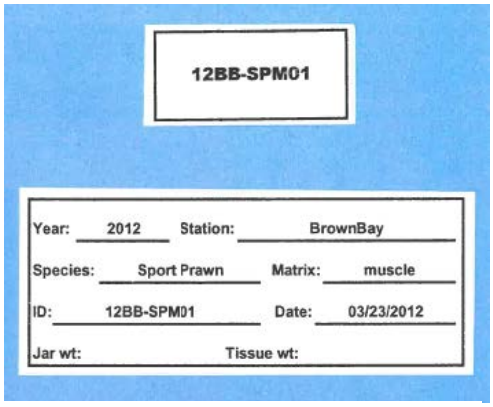

Figure 14. Labels for jar front and lid.

9.4.2.5 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.

9.5 Dungeness Crab

9.5.1 Daily preparation

Sample bags of whole crabs or crab legs will be removed from the freezer at the start of each resection day, and allowed to thaw just enough to allow processing. Lab staff will wear nitrile gloves and change gloves between resecting individual crab. Each crab is removed, inspected for quality, rinsed with tap water to remove large debris and sediment, rinsed with deionzed water and placed on metal tray lined with brown paper towels to dry.

9.5.2 Processing

Crab samples will be processed one-at-a-time; thawing specimens awaiting resection may be placed in a walk-in cooler or freezer to slow thawing. The sex of the crab is verified by inspection of the telson width, and shell hardness is verified (Appendix C). The carapace width of each Dungeness crab will be recorded to the nearest mm using sliding calipers. Carapace width will be measured across the back of the crab from the inside of the last anterolateral spine (see Appendix C, Figure 3). For whole crab the total weight will be measured to the nearest tenth of a gram (0.1g) using a bench scale. Weight will not be recorded for crab supplied by the 2011PSEMP effort because only crab legs and claws were taken. Crab muscle tissue will be removed from the claws and the walking legs.

9.5.3 Resectioning Muscle Tissue

Muscle tissue will be taken from the claws and largest sections of each leg. Individual crab leg sections will be broken from the body or cut from the body using pre-cleaned stainless steel scissors. Each section of the leg will be slit down the flat edge using a pre-cleaned stainless steel scissors. Once muscle tissue is exposed, a pre-cleaned stainless steel forceps or spatula will be used to remove the muscle tissue from all the claw and leg cavities. Forty-grams of tissue will be removed from each of five crab, and placed into a single 8-ounce I-Chem Series 200 composite jar Each composite will then be ground in its jar to a homogenous mixture (paste-texture) using a clean Bamix (Fig 5) hand mixer. Aliquots (subsamples) from each composite may then be removed and distributed to additional labeled jars for analysis of samples by multiple labs, as well as for archiving.. Samples will be labeled and frozen to -20ºC until transfer to the analytical lab

9.5.4 Resectioning Hepatopancreas Tissue

For frozen specimens, hepatopancreas must be removed before complete thawing occurs, otherwise hepatopancreas fluids will drain and be difficult to recover. Hepatopancreas will be taken immediately after the crab carapace is removed. The carapace may be removed by a number of methods while still semi-frozen. The preferred method is to strike the crab against a hard, immovable object (such as the edge of a lab sink) at the base of the telson, where the telson meets the carapace. The carapace can then be pulled upward while holding the body down, to separate the carapace from the body. Once exposed, the hepatopancreas (also known as “crab butter” is observable as a greenish to brownish paired organ, from the anterior margin of the body (and posterior to the mouth parts), extending at the surface of the body posteriorly to about half the distance to the posterior body margin. Hepatopancreas should be resected while the crab is still slightly frozen, to avoid significant mixing of body fluids, or loss of tissue if it becomes fluid. Still-frozen hepatopancreas can be scraped and lifted from the body cavity

using a pre-cleaned spatula directly into a composite jar. The weight of tissue from each crab added to the composite jar is determined by taring jar weight prior to adding the tissue. Samples will be labeled and frozen to -20ºC until transfer to the analytical lab.

9.6 Spot Prawn

9.6.1 Daily preparation

Preparation of Spot Prawn: Sample bags of prawns will be removed from the freezer. While wearing nitrile gloves, each prawn is removed, inspected for quality, and rinsed with tap water to remove large debris and sediment. The prawns are placed in a clean stainless steel bowl, rinsed with deionzed water and strained through a plastic colander. Individual prawns are placed on brown paper towels to dry and each is numbered.

9.6.2 Processing:

Carapace length of each Spot Prawn will be recorded to the nearest tenth of a millimeter (0.1 mm) using a caliper. Carapace length is defined as behind the eye stalk to the beginning of the abdomen (see Appendix B). Total animal weight will be measured to the nearest tenth of a gram (0.1g) using a bench scale.

9.6.3 Resectioning Muscle Tissue

Individual prawns are best resected while still slightly frozen. For this project first separate the tail from the head/thorax. This is done by grasping the each section in one hand and pulling them apart with a twisting motion. This typically cleanly separates muscle tissue from other tissues inside the carapace. To resect muscle tissue from the tail, make a sagittal cut along the ventral surface of the abdomen using a clean stainless steel scissors. A clean forceps will be used to remove the muscle tissue from the abdominal cavity and the tissue will be place in a clean I-CHEM (Class 200) glass sample jar. Individual prawn muscle weight will be recorded. Removed muscle tissue sections accumulated in the sample jar will be ground to a homogenous mixture using a Bamix hand mixer. Homogeneity will be determined by visual inspection. Samples will be labeled and frozen to -20ºC until transfer to the analytical lab

9.6.4 Resectioning Head Tissue

To remove tissues from the head/thorax, hold the still semi-frozen head in one hand (with care to avoid being cut by the rostrum or other head spines) and slide the curved edge of a spatula into the ventral surface of the thorax cavity interior of the walking legs. Once inside the thorax, with legs having been separated from the body by the spatula the spatula is then scraped upward following the inside curve of the carapace. This is repeated for each side of the carapace, after which the carapace and legs can be cleanly separated from the internal organs. The entire contents of the thorax (minus legs, carapace, and any errant shell fragments) is placed in a pre-cleaned composite jar with its weight tared to zero. The weight of each added thorax contents is recorded. Samples will be labeled and frozen to -20ºC until transfer to the analytical lab

10.0 Chemical Analyses -- Measurement Methods

10.1 Analytes

Table 4 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

[a] Sloan, 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 5 Trace 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
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)

10.2 Matrix

Three matrices are targeted for this study (1) somatic muscle tissue from crabs (claw and leg) and spot prawn (tail), (2) hepatopancreas from crab, and (3) head/thorax tissues from spot prawn.

10.2.1 Number of samples

The maximum number of samples to be submitted for chemical analysis in this study is expected to be 120, which is 60 Dungeness crab samples and 60 spot prawn samples. WDFW will continuously monitor

sampling efforts and direct sampling (multiple test fisheries and trawl surveys) to meet study goals. A map will be generated in real time to record sampling activities relative to how many samples of which species have been collected per Marine area and embayment. After all samples are collected and locations identified, it will be determined which replicates will be additionally processed for hepatopancreas/head tissue.

10.2.2 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 µg/g) to 5 µg/g wet weight.

10.2.3 Analytical methods

All POPs in this study will be analyzed according to Sloan et al. (2004), to provide consistency 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).

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.

10.2.4 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. Table 5 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.

10.2.5 Sample preparation method(s)

Tissue samples of crab and spot prawn tissue are homogenized in the Resection Lab (see Section 8). After thawing and prior to extraction each homogenized sample should again be mixed thoroughly with a clean spatula or other utensil.

11.0 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 ≤ 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 for 1974b Blue Mussel. Concentrations of ≥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. Data are reported by the analytical lab without blank correction. It is up to the user to decide if and how to correct data with respect to blank contamination, and how or whether such data should be censored with qualifiers. 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 Figure 2 and

Figure 3. 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.

12.0 Data Management Procedures

12.1 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.

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 summary statistic tables, scatter plots, and time trend plots are created to examine the new data.

12.2 EIM data upload procedures

All data generated by this project will be submitted to Ecology's EIM for later export to EPA's STORET database.

13.0 Audits and Reports

13.1 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.

13.2 Responsibility for reports

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

14.0 Data Verification and Validation

14.1 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. Crab and spot prawn are re-measured in the lab to ensure they represent fished sizes, and to obtain accurate sizes. Size distributions are checked at the end of each daily resection effort before carcasses are discarded, to identify any size outliers.

14.2 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.

15.0 Data Quality (Usability) Assessment

15.1 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.

15.2 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. This includes comparison of results with previously analyzed samples for a wide range of species, including some pilot analyses conducted on Dungeness crab and spot prawn in anticipation of this study. These pilot data from 2005 and 2007 will be presented for comparison to the current study results.

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 animal size and trophic level.

Modeling the relationship between muscle and the other tissue matrices will be accomplished using linear or log-linear regression analysis on paired composites.

15.3 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.

16.0 References

Code Of Federal Regulations, Title 40--Protection Of Environment, Chapter I--Environmental Protection Agency, Subchapter D--Water Programs, Part 136--Guidelines Establishing Test Procedures For The Analysis Of Pollutants, Appendix D To Part 136 --Precision And Recovery Statements For Methods For Measuring Metals.

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US EPA SW-846 Method 6020a Inductively Coupled Plasma-Mass Spectrometry, Office of Waste, Test Methods for Evaluating Solid Waste, Physical/Chemical Methods, U.S. Environmental Protection Agency.

US EPA SW-846 Method 7470a, Mercury In Liquid Waste (Manual Cold-Vapor Technique), Office of Waste, Test Methods for Evaluating Solid Waste, Physical/Chemical Methods, U.S. Environmental Protection Agency

US EPA SW-846 Method 7471b, Mercury In Solid Or Semisolid Waste (Manual Cold-Vapor Technique), Office of Waste, Test Methods for Evaluating Solid Waste, Physical/Chemical Methods, U.S. Environmental Protection Agency.

17.0 Appendix A – Glossary, Acronyms, and Abbreviations

Glossary

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)

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)

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)

Carapace - Hard, chitinous shell covering the bodies of crabs, shrimps, and lobsters.

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.

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)

Crustacean - An animal that is a member of the arthropod class Crustacea, such as lobsters, crabs, and shrimp, etc.

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)

Fishery - The catching, processing, or selling of fish, including the industries and occupations involved in these activities

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)

Hepatopancreas - A glandular digestive organ of some invertebrates and fish that combines the digestive functions of the mammalian liver and pancreas.

Hermaphrodite - An animal that has both male and female reproductive organs and secondary sexual characteristics.

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.

Macroinvertebrate - An invertebrate (i.e., without a backbone) animal large enough to be seen without magnification.

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, crustaceans (i.e., crab and shrimp), 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)

Test Fishery – The professional examination of a fisheries' readiness to open for recreationally and commercially fishing.

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Acronyms and Abbreviations

Following are acronyms and abbreviations used frequently in this report.

AHs	Aromatic Hydrocarbons

ASE	Accelerated solvent extraction
CHs	Chlorinated Hydrocarbons
COC	Chain of Custody
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

18.0 Appendix B. Standard Operating Procedure: Washington Department of Fish and Wildlife Spot Prawn Length-frequency Sampling Research Protocol – Draft #6

Long-term goal – The long term benefit of this study is to improve monitoring of the spot prawn stock and the impacts of fishing on the stock.

Project goal – To obtain more precise information on age composition and stock dynamics of spot prawns to improve population modeling.

Objective – The short term specific task is to estimate the stock structure of spot prawns by collecting length frequency data at selected sites before and after fisheries occur.

The populations of interest are all spot prawns in the areas that are fished extensively. A stratified sample design will be used and the sample unit will be individual spot prawn. Data will be collected on the species, size, and the sexual stage of all spot prawn collected.

Sampling Methods:

a) Gear layout – The general layout for the spot prawn pot sampling is given in Figure 1. There will be two lines of ten pots per line. The pots will be about 40ft apart, with 40ft between the outer pots and the anchors. The buoy lines will be approximately 400 ft in length. The two lines will be set roughly parallel to each other (perpendicular to the shore and depth contours) and a minimum of 300ft apart.

b) Pot design – Ladner **½"** mesh 'San Juan Island' 30" diameter spot prawn pots will be used. These pots are stackable, easily deployed, and are used by many State and Tribal commercial spot prawners (they use mostly 7/8" mesh pots). At sampling sites with considerable tidal current, these lightweight pots will require additional weight to reduce pot movement when set.

c) Bait & Bait jars – Moore Clark spot prawn bait will be used; it is available from http://mckayspot prawnandcrabgear.com/, though it is not in their catalog; you have to call and order it by telephone. This bait is not messy to handle, lasts well in 24 hour soaks, and is the choice of many commercial spot prawners. Scotty brand one quart bait jars will be used. Approximately 40, 6mm holes will be drilled in the bait jars as purchased from the supplier.

The bait swells after soaking in water, thus bait jars will be filled approximately 50% full of the bait pellets for each soak period.

d) Depth - Each of the two lines will be set starting at a depth of approximately 200ft - 250ft and will extend to approximately 300ft - 350ft. Pilot studies will determine whether these depths need to be extended. During the actual sampling, there will be special sampling sites, e.g. Discovery Bay, at which different depths will necessarily be used.

e) Soak time – The standard soak time, defined as the time the pots are set until the time the pots are retrieved, will be approximately 24 hours. Record the soak time to the nearest half-hour. The Canadian researchers insist on 18-24 hour soaks.

f) Frequency of sampling – As a minimum, each sampling site will be sampled twice: once before any fisheries begin in that area and once after all fisheries close in that area. The first sampling can also serve as an ovigery test. Where resources allow, additional sampling before, during and after the 2002 fisheries will provide supplemental information that may increase sampling requirements for subsequent years. Although some areas may be different, as a rule of thumb, the pre-fishery sampling will be in March or April and the post fishery sampling will be in August or September.

g) Setting procedure – The baited jars will be put in the spot prawn pots, and the pots closed and staged for deployment. When necessary, zinc releases will be attached to the buoy lines such that the buoy will be held underwater for the duration of the soak time. This buoy line will be put in the water and an anchor clipped onto the end. The ground line will also be clipped to the anchor and slowly lowered from the boat. Every 40ft a spot prawn pot will be clipped onto the ground line as the line is let out and the boat proceeds into deeper water. After ten pots have been clipped into the ground line the second anchor will be clipped to the end of ground line. The second buoy line will also be clipped to the anchor and approximately 100ft of line slowly let out; the boat will keep tension on the line by continuing towards deeper water. The zinc time-release will be attached about 100ft above the anchor and again about 10ft below the second buoy. As the second line and buoy are let go, the buoy will be pulled under the surface well out of sight. The second line of pots will be similarly set about 300ft away and parallel to the first line of pots. Muckleshoot and Suquamish were the only participants that used zinc time-releasers. Muckleshoot reported problems with one string where neither buoy popped-up due to twisted lines below the zinc timer, thus requiring grappling for the ground line.

h) Hauling procedure – The pots will be retrieved approximately 24 hours after setting, depending on when the zinc timers release the buoys to the surface. Depending on the prevailing wind and tidal current, the ‘downstream’ buoy line will be hauled first. After that line and the first anchor are onboard the pots will be retrieved and, depending on the number of crew available, the pots will either be stacked consecutively until all ten pots are retrieved, or if there are enough people, each pot will be emptied of prawns as they are brought aboard.

In either case, the prawns and by-catch from each pot will be put into separate marked containers.

i) Measuring – Every prawn collected will be measured (to the nearest mm) with calipers. The carapace length is defined as follows: from the top back edge of the carapace to the base of the eye-stalk (posterior mid-dorsal margin to the posterior most part of the eye-stalk orbit. By-catch will not be measured but will be classified into small, medium, large categories, dependent on the species. Additional data on the by-catch, for instance, sex of any crabs caught, will be noted on data sheets, as available. A list of species that may be encountered as by-catch will be developed and distributed to participants.

j) Sexing – Every prawn collected will have its sex determined (i.e. male, transitional, female) and, in the case of females, whether it is: a female with eggs in the carapace (the eggs must be spherical formed, not just a mass of orange-brown tissue in the 'head'), female with eggs on the abdomen, female recently spawned (this designation also requires more detailed description to be useful). If there are logistical problems (e.g. time constraints) with sexing the prawns on the sampling day, then they may be bagged, tagged and frozen (one pot per bag).. Also a dissecting microscope may be necessary to determine the appropriate sex category, particularly for late-transitionals vs. females. The sexing standard used is: "If the male appendage is the same size or larger than the female appendage AND the male appendage has some setae on it , then the spot prawn is a male. If the male appendage is 25% or less the size of the female appendage, the spot prawn is a female."

Pilot studies are underway to investigate egg maturity and the possibility of unfertilized eggs…*For females with eggs on the abdomen, the degree of egg maturity will be determined by….color chart?.* "The colour of the egg changes from a dark orange when freshly extruded to brown at time of hatching" (Butler, 1970). It should be noted if the eggs have eyes.

k) Data recording – Data, including the species (see figure *x*), carapace length see figure *y*) and sex (see figure *z*), for each spot prawn will be recorded on a data sheet for each pot (Figure__) and entered into an Excel spreadsheet .

Note: Depending on the size of the spot prawn bed at each location, there may be several sampling sites at that location. Two lines of pots will be set at each sampling site. Exact latitude and longitude coordinates of each line of pots will be specified, along with depth of the shallow anchor and depth of the deep anchor..

Assumptions:

1. The areas sampled represent the extensively fished populations of spot prawns.
2. The sampling methodology will catch prawns in the same proportions as they exist in the areas sampled; this includes the sub-assumptions:

a) prawns enter the pots in the same proportions as they exist in the areas sampled. Is there a way to verify this assumption?

b) no prawns escape from the pots as they are retrieved to the boat. This assumption will be tested by having a scuba diver (or camera) observe spot prawn pots being hauled…

Sampling Gear

Table A.1. Gear Inventory and Cost for Spot Prawn Test Fishery

Item	Supplier	Unit	UnitsRequired	Cost per unit	Cost
½" mesh Ladner spot prawn pot	LFS[5]	pot	20	48.64	972.80
5/16" buoy & ground line	LFS	1800 ft	2	86.00	172.00
30 lb. kedge anchors	LFS	1	4	39.95	159.80
Scotty bait jar #652 1 quart	LFS	1	20	1.40	28.00
3/8" x 4" kong snap	LFS	1	8	6.47	51.76
8" trawl buoys	Seattle Marine[6]	1	4	8.50	34.00
24 hour zinc timed release	Seattle Marine	1	40	0.50	20.00
Moore Clark spot prawn bait	McKay Spot prawn Gear[7]	20kg bag	1	39.95	39.95
Other equipment					
Collecting tub(s)					
Calipers					
Water-proof paper	Rite-in-the-rain				
					$1478.31

[5] LFS – Lummi Fish Supply Inc. 1-800-647-2135

[6] Seattle Marine & Fishing Supply Co.- 1-800-426-2783

[7] McKay Spot prawn & Crab Gear – 1-866-375-8020

Figure A.1. Spot Prawn String Data Form

SPOT SHRIMP TEST FISHERY - STRING DATA	
Check: Pre Season	Post Season
Set Date:	Crew:
Set Time:	Boat Used/Operator:
Region:	Hours Soaked:
Location:	Pots Per String: 5
String#:	Bait Used: Skretting Commercial Pellets
Shallow Anchor	**Deep Anchor Information**
Lat:	Lat:
Long:	Long:
Depth (ft):	Depth (ft):
Waypoint#:	Waypoint#:
Gear Retrieval Information	
Pick Date:	Pick Time:
Estimated Pounds Of Spots Per Pot: 1) 2) 3) 4) 5)	
Byctach Per Pot and Denote if Spot Shrimp were NOT Caught	
1)	
2)	
3)	
4)	
5)	
WEATHER/SEA CONDITIONS	
Set Day:	
Pick Day:	

19.0 Appendix C – Standard Operating Procedure: Washington Department of Fish and Wildlife Protocols for Dungeness Crab Test Fishery

WASHINGTON DEPARTMENT OF FISH AND WILDLIFE

TESTFISHERY PROTOCOL MANUAL

For

HOOD CANAL DUNGENESS CRAB (*Cancer magister*)

November, 2010

INTRODUCTION

Hood Canal supports a large Dungeness crab fishery with three user groups: State recreational, Treaty commercial, and Treaty ceremonial and subsistence. This test fishery was originally started in 1999 and conducted monthly to assess the main molting time of legal sized Dungeness crab. A pre season and post season test fishery is now conducted each year (May and September) to provide a relative measure of abundance before and after fisheries occur.

FIELD SAMPLING

INDEX STATIONS

The annual Dungeness crab test fisheries are conducted at 8 standard index stations in Hood Canal (Fig. 1). These index stations were selected based on historical crabbing effort data that indicated these locations were productive crabbing areas. The test fisheries originally started with 6 index stations in 1999 but in 2003 Holly and Squamish Harbor were added (Fig. 1).

FISHING GEAR

The crab pots used in these test fisheries are of commercial grade and approximately 36” in diameter. They are welded 5/8” metal rod wrapped in rubber and covered with stainless steel wire with a mesh size of approximately 2.5”. The access door is on top of the pot, secured with a hook attached to a rubber strap by means of a single strand of cotton twine serving as a biological escapement device (rot cord) – if the pot is lost the twine will decay allowing the door to open. The standard 2 four inch escape rings are wired shut to allow retention of sublegal crab. The total weight of each pot is about 70 lbs. Each pot is baited with 2 Morrison® bait jars, 1 perforated with many holes (to immediately release scent) and 1 with 2 holes (for staying power) filled, with fish, squid, or clams.

TEST FISH GEAR SET UP

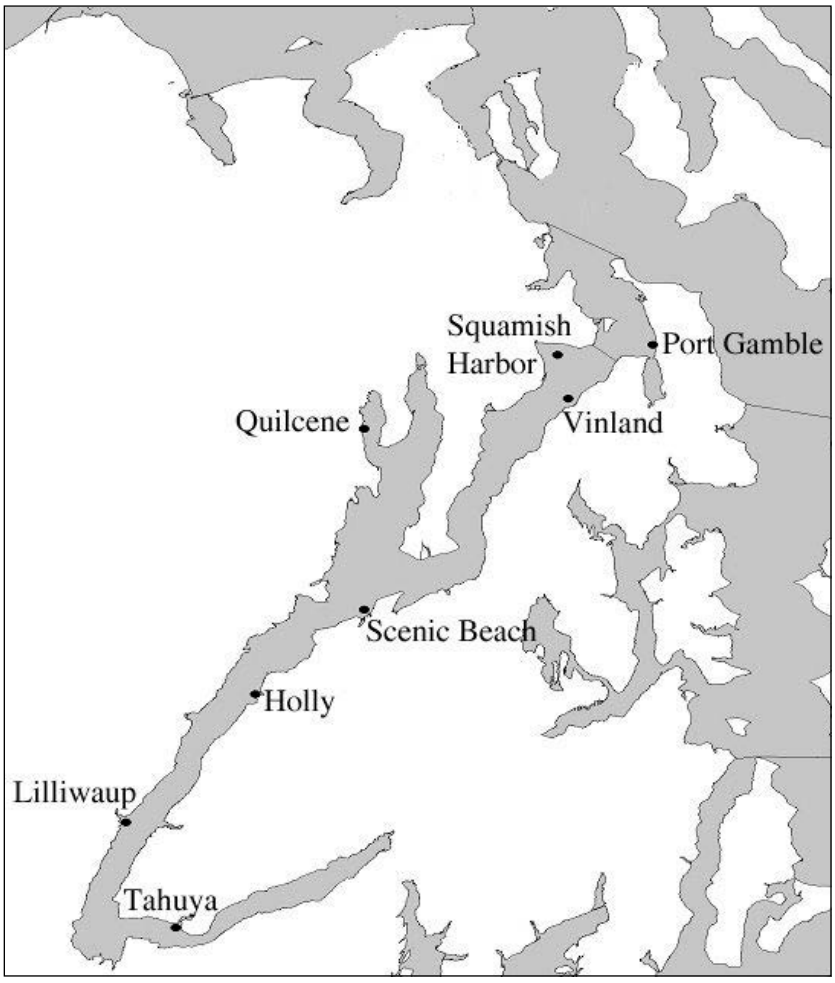
A total of 15 crab pots are set at each index station. Each pot has its own line (depth fished plus 15 ft. of scope) and Polyform® buoy. Three different depth strata (40, 95, and 150 feet) are fished at each station with 5 pots at each depth (at the Tahuya site the deepest set is 130 ft.). The pots are set at each depth using pre set GPS waypoints and are set about 100 feet apart. After each pot is set, sampling criteria such as location, pot number, date, time, depth, and waypoint numbers are recorded on a data sheet (Fig. 2).

SAMPLING REGIME

The soak time for each crab pot varies but falls within 18 to 48 hours, however the target is 24 hours – 48 hour soaks are usually due to weather problems. Crab pots are pulled in the order in which they were set. Upon retrieval, catch data, pull date and time, and bycatch are recorded for each pot. Bycatch species are identified, counted, and returned to the water immediately. For red rock crab *Cancer productus* and graceful crab *Cancer gracilis*, a count and the sex of each individual crab is recorded. Each Dungeness crab is sexed - for female crab the presence/absence of an egg clutch is also recorded. The carapace width (CW) in millimeters of each Dungeness crab is measured inside the last anterolateral spine using Vernier calipers (Fig. 3). A shell condition stage is also assesed for each Dungeness crab using stage categories from 3-2 to 1-1m (Fig. 4). Color and epifaunal growth is assessed qualitatively while carapace firmness is assessed using a pinch test. The pinch test generally begins with the first segment of the largest walking leg and progresses on to the edges of the carapace, the mouthparts, and the top of the carapace until a definite shell condition stage can be assigned. Occasionally weights (in grams) are taken for individual Dungeness crab using an Acculab® field scale. This data is all recorded on a data sheet (Fig. 2) and all crab are released to the water as soon as possible.

Figure 1: Locations of the test fishery index stations in Hood Canal.

Port Gamble, though technically outside the opening to Hood Canal, is considered a Hood Canal station.



DATA ENTRY

A summary of the Dungeness crab data from these test fisheries is entered into an Excel summary file which is distributed to tribal biologists and crab advisors. Each individual Dungeness crab and its' associated data is entered into an Access database file, which includes catch data from test fisheries in Port Townsend and Oak Bays and areas in the eastern Strait of Juan de Fuca (Dungeness Spit and Discovery Bay). To date the catch in 6,600 pots from Hood Canal have been entered.

DUNGENESS CRAB SHELL CONDITION FORM

DATE ___/___/___ SAMPLERS:____________________ VESSEL:__________________ Pg __ of __

WYPT #	SAMPLING LOCATION	REG. #	ZONE #	STA. #	POT #	DEPTH	SET DATE	SET TIME	PULL DATE	PULL TIME	LATITUDE	LONGITUDE

STATION

POT #	SEX	WIDTH (mm)	WEIGHT (gm)	SHELL COND	COMMENTS \ Bycatch \ Eggs (y\n)	POT #	SEX	WIDTH (mm)	WEIGHT (gm)	SHELL COND	COMMENTS \ Bycatch \ Eggs (y\n)

1-1	1-1m	1-2	1-3	2-1	2-2	3-1	3-2	SUB	FEM	TOTAL

Figure 2. Dungeness crab test fishery data sheet

Figure 3: Location at which the carapace width (CW) of each Dungeness crab is measured.

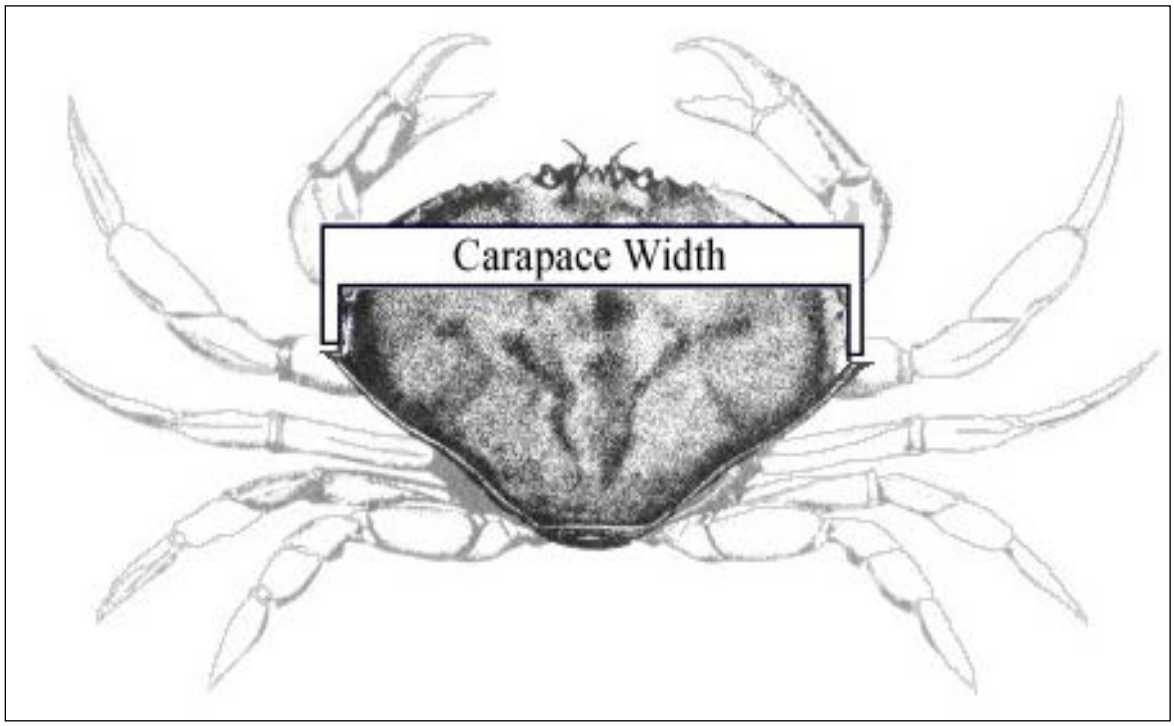


Figure 4. Shell condition stages

DUNGENESS CRAB SHELL CONDITION STAGES

Stage	Shell Condition Description
3-2	Newly molted – The carapace feels like parchment, is very pliable, and can be easily deformed without breaking.
3-1	Recently molted – The entire carapace has begun to harden but can still be easily deformed. The top of the carapace will bend or crush under light pressure. The bottom of the carapace may appear translucent.
2-2	Early intermediate stage – The top of the carapace continues to harden and is now only flexible at the rear, left, and right margins. The leading bottom edge of the carapace and upper segment of the first walking leg are very flexible but will readily spring back into shape after moderate pressure is applied.
2-1	Late intermediate stage – The top of the carapace is now hard. There is little or no flex left to the rear edge of the carapace. The leading bottom edge of the carapace and the upper segment of the first walking leg are not yet firm.
1-3	New hard shell stage – The entire carapace is now rigid and tissue growth, for the most part, is complete. The carapace is light gray to tan and supports little or no epifaunal growth.
1-2	Late hard shell stage – The leading bottom edge of the carapace and upper segment of the first walking leg are now firm when moderate pressure is applied. The color of the entire exoskeleton is beginning to darken and the crab is in prime quality for market.
1-1	Pre-molt stage – The color of the bottom surface of the carapace is now dark yellow or brown. The crab shows signs of age (i.e. damage to the exoskeleton, epifaunal growth, or separation at the suture line).
1-1m	Late pre-molt stage – The entire carapace is now dark yellow or brown and shows marked signs of age. Epifaunal growth regularly covers over one-third of the top of the carapace.

20.0 Appendix D – Standard Operating Procedure: Bottomfish Trawl Survey

(*beginning next page*)

Standard Operating Procedures

For

Collecting Benthic Fish and Macroinvertebrates

Using a Bottom Trawl in Puget Sound

Washington Department of Fisheries

Puget Sound Ecosystem Monitoring Program

Toxics in Biota

June, 2012

PURPOSE AND SCOPE

This document describes Standard Operating Procedures (SOP) for collecting benthic fish and macroinvertebrates using bottom-trawling gear in Puget Sound. These procedures support biennial surveys to collect biota for long-term status and trends monitoring of toxics in the Puget Sound food web. The primary target species is English sole (*Parophrys vetulus*), although a wide range of other species may be taken as well, including macroinvertebrates such as Dungeness crab (*Cancer magister*) and spot prawn (*Pandalus platyceros*). This SOP describes the fishing operation to the point at which biota are placed on the deck of the vessel for sorting and processing. Procedures for forting and processing catch are described in a a separate SOP.

TRAWL GEAR

To capture benthic and demersal fish and macroinvertebrates, generally found on and just above sand, mud or cobble bottoms, PSEMP uses a 400-mesh Eastern trawl as described in the Puget Sound Estuary Program (PSEP) protocols for sampling bottomfish (PSEP 1990, see "alternative protocol"). This gear has proven efficient in capturing English sole, other benthic fishes, and macroinvertebrates associated with these habitat types.

To carry out a bottom trawl operation, PSEMP employs a chartered fishing vessel capable of pulling a 400-mesh Eastern trawl at the speeds, configurations and depths required to efficiently fish for target species. In addition the vessel must support the staffing and space requirements demanded by PSEMP bottom trawl operations.

The 400-mesh Eastern trawl used by PSEMP is a modified commercial design, composed of synthetic twine (polyethylene) making up 10 cm meshes, with a 21.4 m head rope, a 28.7 m foot rope and has a 3.2 cm mesh codend liner (Table 1, Figures 1 and 2).

When fishing, the width of the net opening ranges between 9 m and 13 m, depending on speed, amount of trawl cable out (wire out) and trawl depth. A vessel speed between 2 to 3 knots is maintained and the net width is maximized by regulating the scope (fathoms of wire out per fathom of depth) of the cable. A 2:1 scope is the minimum allowed, except for shallow tows for which it is increased to allow maximum spreading of the doors.

Personnel and Responsibilities

WDFW supplies a scientific crew to carry out the scientific studies and the vessel contractor provides a vessel and qualified crew to carry out vessel duties.

The scientific crew consists of a lead scientist and three to five biologists or technician who serve in the roles listed below. The scientific crew is staffed primarily with WDFW personnel however volunteers and visitors from other institutions also participate.

The lead scientist is in charge of the field operation (is on-site) and is responsible for dealing with the vessel captain/operator. All scientific crew and visitors are under the direction of the lead scientist; however, vessel operation and safety issues are under the authority of the vessel captain/operator. The lead scientists responsibilities include:

1. ensuring adherence to all aspects of the survey plan and the prescribed sampling procedures;
2. maintaining a daily log of survey activities;
3. ensuring that survey forms are accurate and complete;
4. ensuring that all necessary gear is loaded and any material/equipment needs are made known to the contact person in Olympia;
5. maintaining a positive working relationship between the vessel crew and scientific crew during the survey;
6. conducting a safety orientation in conjunction with the skipper, for scientific personnel and visitors new to the vessel, prior to getting underway and
7. ensuring strict adherence to all safety procedures.

Designated tasks assigned to the scientific crew include:

1. trawlmaster - a designated crew member who monitors electronic equipment in the vessel's wheelhouse, recording tow information on the Haul Position form. Works with lead scientist and skipper to ensure tow location and execution is accurate;
2. weighmaster - a designated crew member who, under the direction of the lead scientist, is responsible for coordinating deck sampling, weighing the catch and recording catch and biological information on the Catch Composition Form (CCF);

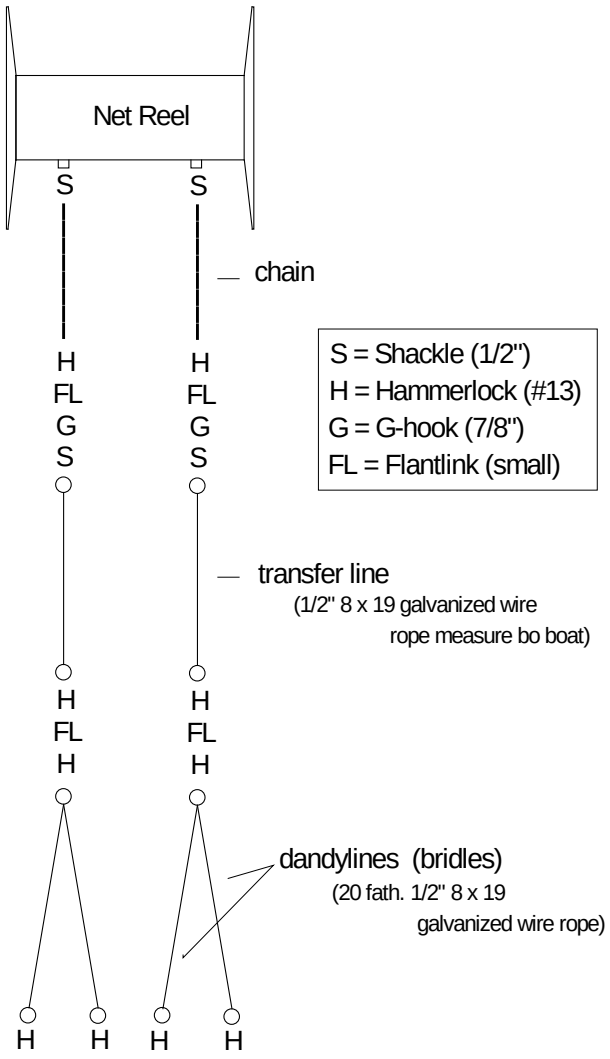


Figure 1. Hardware setup for the 400-mesh Eastern trawl.

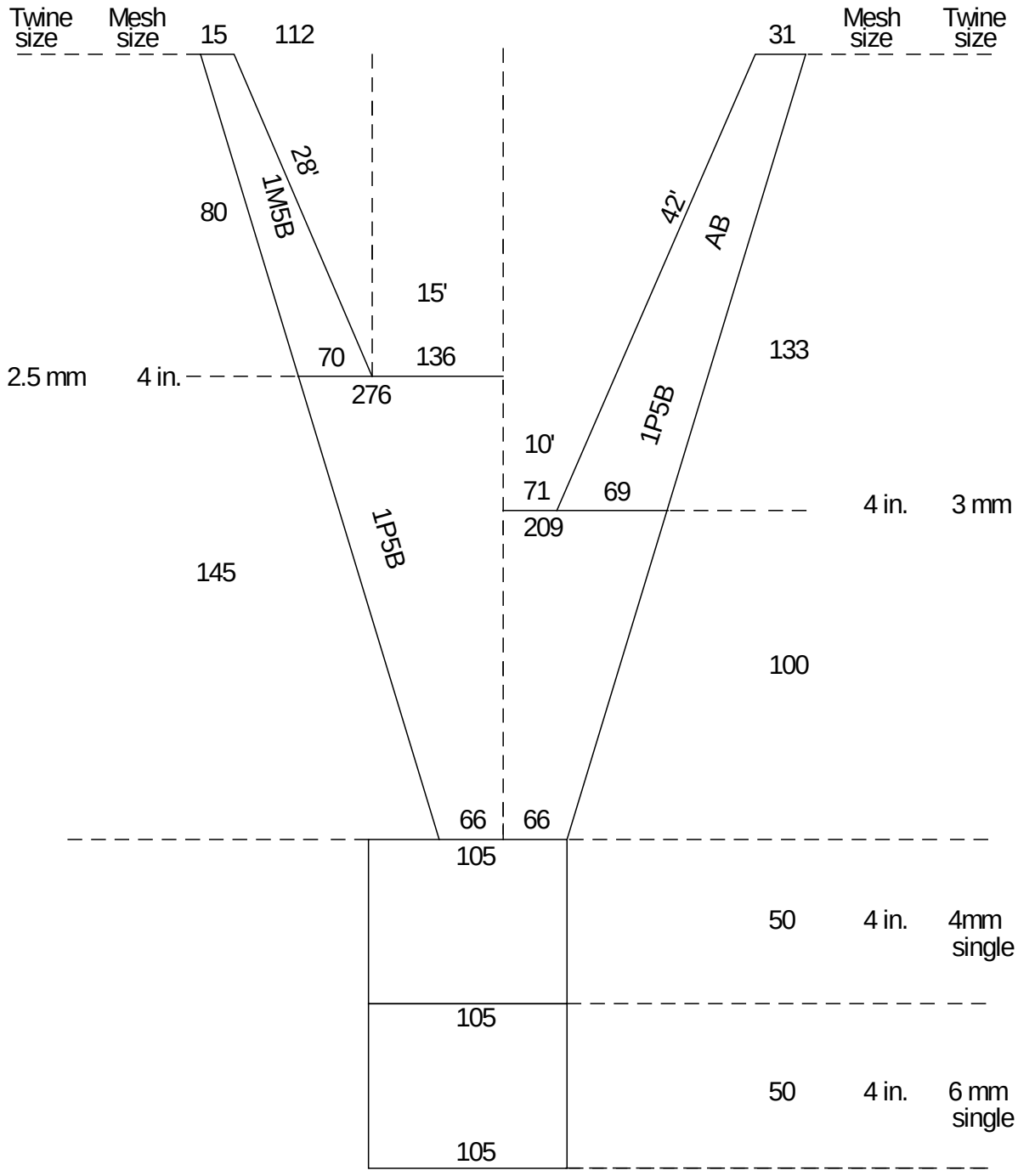


Figure 2. Schematic of the 400-mesh Eastern trawl.

The scientific crew responsibilities also include:

1. sorting the catch by species when called for;

2. reporting catch weight and number by species to weighmaster;

3. collecting the samples;

4. labeling each sample with appropriate tag (including the tow number and date) on the inside and outside of the sample bag;

5. cleaning and washing down sampling equipment and deck area as necessary, at the close of work each day and thoroughly after the final haul of the week;

The vessel contractor will provide crew to operate vessel equipment, maintain nets, and cook meals. Contractor crew is under the direction of the skipper and all gear handling and piloting is their responsibility. Difficulties or disagreements between scientific crew and vessel crew/skipper will be reported directly to the lead scientist for resolution.

Trawl Procedure

The vessel proceeds to station coordinates provided by the lead scientist to the skipper for each survey station. Once in the vicinity of the target station, the vessel operator and lead scientist refer to a nautical chart/ navigational software and observe conditions (vessel traffic, current direction/speed, wind direction/speed, etc.) to determine the course for a tow. The primary criteria to consider are vessel and worker safety, legal navigation and avoiding charted or known obstructions.
Prior to fishing, the vessel operator pilots the vessel over the proposed tow, watching for other vessel traffic, and using a fathometer to observe for obstructions on the sea floor. Under normal conditions the vessel speed will be between 2 and 3 knots during the tow. For most tows, the vessel/net should be oriented into the current.

Tow length is kept short, generally 10 minutes or less, to keep the catch at a size that will not exceed the capacity of the sorting table; generally, less than 1,500 pounds. For all tows, the lead scientist or trawlmaster will complete a Haul Position Form making sure to collect the start and end position (latitude and longitude), minimum and maximum net depth, tow start[8] and haul[9] times, wire out[10] and

[8] time at which the net is on the bottom and the trawl doors are spreading. Generally, after the trawl winches are locked and the vessel reaches its tow speed of 2 to 3 knots. This will vary with depth, from a few seconds in shallow tows to a minute or two at extreme depths.

[9] time that the vessel slows to retrieval speed and the winches are engaged.

[10] the amount (fathoms) of trawl cable fed out

net performance[11]. All station positions will be taken using a global positioning system (GPS) and the make and model of the GPS will be recorded on the Haul Position form along with the Map Datum[12] used. In addition to the listed information, they will also comment on observed sea and weather conditions, problems encountered and any other information that will help reconstruct what was done and why. This information will be useful in selecting stations in the future and in analyzing tow results.

Once a tow is completed, the gear is retrieved and the net and catch are lifted aboard the vessel. Contracted vessel personnel are the only ones allowed to handle the net and operate the fishing equipment. The catch is processed according to the Catch SOP. If one tow was insufficient to capture enough of the target species, more tows may be made, typically not exceeding five for a station.

Packaging of Specimen/samples for transfer to lab

Once onboard processing of specimens has been completed, the resulting whole bodies, carcasses and/or samples are packaged and held for transport to the lab.

Individual specimen (whole bodies or carcasses) that have been assigned a FishID[13] are packed into individual plastic bags along with a FishID label[14] (See Figure 3). To reduce the risk of ink from the FishID label bleeding onto a specimen, each label is contained in a small ziplock bag.

Figure3. FishID label.

Groups of specimens (whole bodies) which have not been assigned a FishID and groups of individually bagged specimens are both packed in large plastic bags and sealed shut by either knotting the end of the bag or closed off with a wire/string tag. Knotting the bag is preferred since it will keep melt-water out of the bag when stored on ice. For each bag, the upper and lower parts of a tear tag are filled out in full (see Figure 4), the lower portion torn off and placed in the bag and the upper portion with the wire tie is attach to the outside of the bag. Each tag has a unique 4 digit id number which is used to track the

[11] the following NOAA ADP codes are used to indicate net performance: 0 – good, 1 – satisfactory, 5 – unsatisfactory, 6 – hung and 7 - ripped net

[12] Generally, PSEMP uses the WGS84 map datum, but some surveys require use of NAD83.

[13] Fish ID - A unique six digit number where the first two digits represent the year (11 represents 2011) and the next four digits a number ranging between 5001 and 9999. Using this format, each specimen used in a PSEMP study will have a unique identifier that is never repeated between years. The range of numbers is assigned in cooperation with the NOAA Environmental Conservation Division, who agrees to use 0001 through 5000 so that between our two units, we will not duplicate IDs for individual specimen.

[14] A Fish ID label is a small waterproof label with a unique six digit id number printed on both sides.

samples while they remain in the bag. For tracking purposes, the tag number should be recorded in the field log and noted on a Chain of Custody Form.

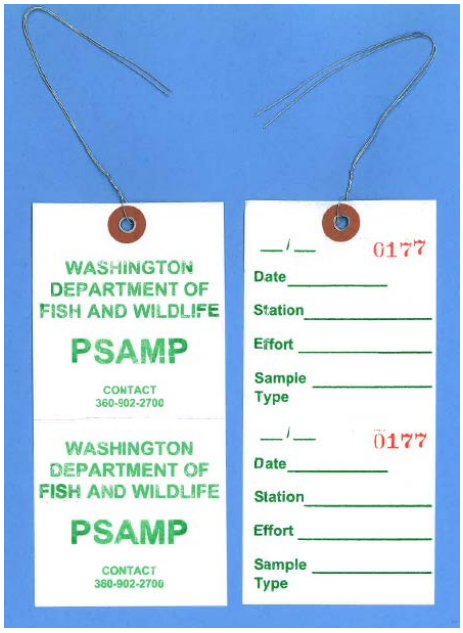
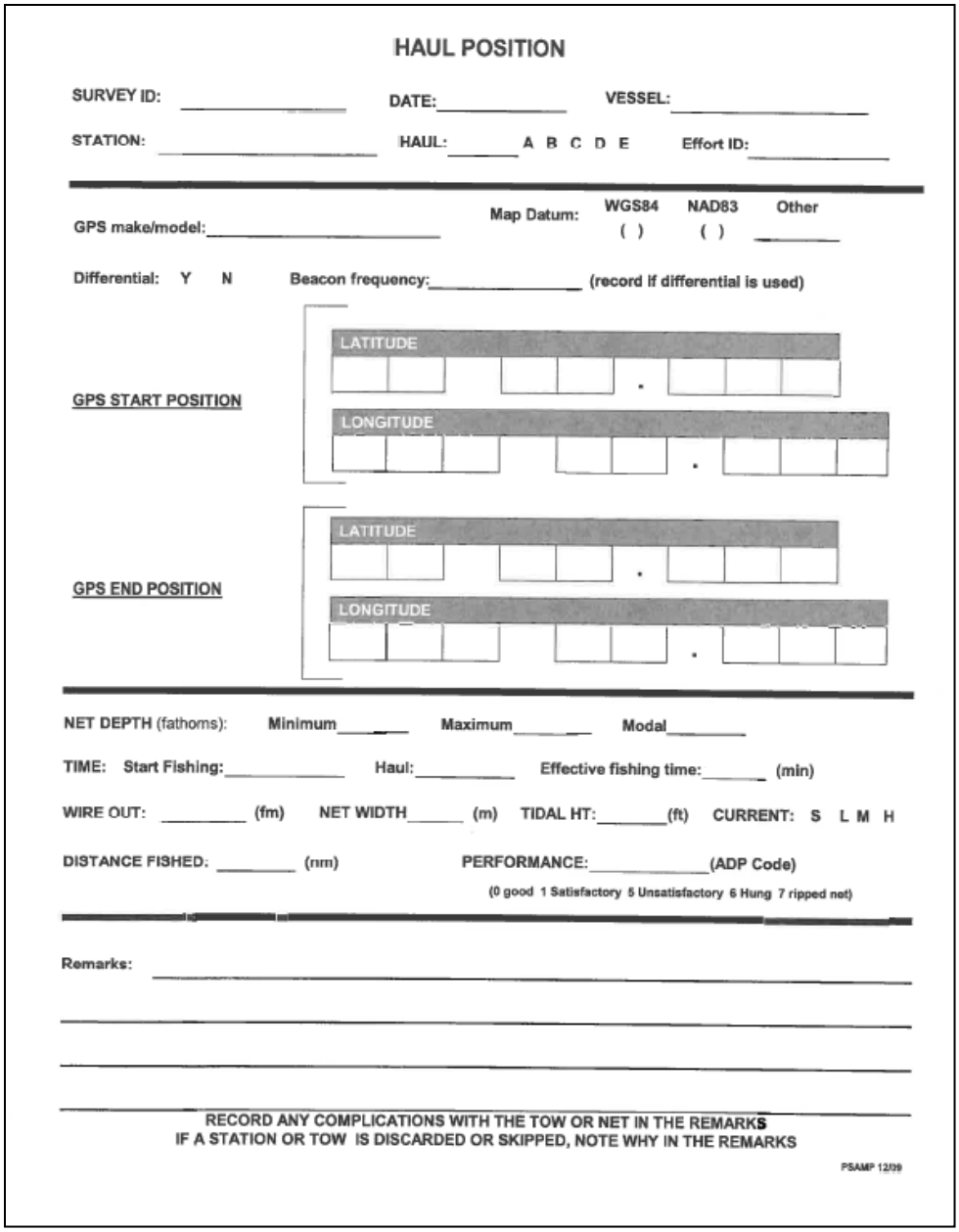


Figure 4. PSEMP tear tag, front and back sides.



HAUL POSITION

SURVEY ID: _____ DATE: _____ VESSEL: _____

STATION: _____ HAUL: _____ A B C D E Effort ID: _____

GPS make/model: _____ Map Datum: WGS84 ( ) NAD83 ( ) Other _____

Differential: Y N Beacon frequency: _____ (record if differential is used)

GPS START POSITION

LATITUDE

LONGITUDE

GPS END POSITION

LATITUDE

LONGITUDE

NET DEPTH (fathoms): Minimum _____ Maximum _____ Modal _____

TIME: Start Fishing: _____ Haul: _____ Effective fishing time: _____ (min)

WIRE OUT: _____ (fm) NET WIDTH _____ (m) TIDAL HT: _____ (ft) CURRENT: S L M H

DISTANCE FISHED: _____ (nm) PERFORMANCE: _____ (ADP Code)

(0 good 1 Satisfactory 5 Unsatisfactory 6 Hung 7 ripped net)

Remarks: _____

RECORD ANY COMPLICATIONS WITH THE TOW OR NET IN THE REMARKS
IF A STATION OR TOW IS DISCARDED OR SKIPPED, NOTE WHY IN THE REMARKS

PSAMP 12/09

Haul Position Form

The Trawl Master is to complete a Haul Position Form (Figure 5) for each station trawled. Positioning data are recorded for the beginning and end of every tow and at each significant change in direction or depth. Detail must be sufficient to allow later surveys to reoccupy the same tract.

Haul: A haul number is assigned to each station as it is encountered, beginning with 1. For consecutive hauls at a station, the first haul is assigned the letter "A", the second "B" etc. For example, if three hauls were made at the third station sampled, they would be numbered 3A, 3B, and 3C.

GPS Start/End Position: Latitude and longitude for the beginning and end of a haul will be taken from the Global Positioning System (GPS). The position information should reflect when the net is on the bottom and starts and ends fishing. Any significant course change should be noted with the latitude and longitude of the turn and the compass bearing.

Wire Out: Record how many fathoms of cable were let out for a tow.

Depth: Give the minimum and maximum depths fished on the tow. The numeric average of actual minimum and maximum depth should be close to the modal depth fished on a tow. On occasion there will be a sudden dramatic change in depth for a short duration. This information should be noted in the remarks section. A dramatic depth change, if reported as the minimum or maximum, misrepresents the average depth fished during the tow. Use your judgment as to what depth to report in the Modal depth space.

Net Width: Using the estimated “Modal” depth for the tow in question, look it up in the “depth” column of **Error! Reference source not found.**. Using the “Wire Out” for the tow, compare it to the value in the “Break Wire” column. If it is less than the Break Wire value, use the minimum net width. If it is equal to or greater than the Break Wire Value, use the maximum net width.

Distance Fished: Record from the way-points of the GPS system.

Performance: Evaluate the performance of the net during the haul and select the appropriate coded value from the list on the form. Do not leave this field blank. If the tow is satisfactory, at least enter 0.

Remarks: Note equipment failures, unusual characteristics of the area, or any event associated with a tow that affects the fishing performance. Anything is ok, just write it down. **If a station is not used, document the reason why**. This information is important and omitting it has been one of the most common errors.

Chain of Custody

The Chain of Custody Form (CCF) is a chain-of-custody record used to identify and track specimens and samples collected during a survey (Figure 6). Whenever specimens/samples are collected, on the CCF enter a checkmark under the specimen/sample type, record the number collected under “Count”, indicate how they were stored under “Condition” and list the Fish ID or Sample ID numbers included in the sample set under “ID Numbers”. The person packaging/storing the sample is responsible for entering the sample on the CCF. It is important that everyone carry through on this responsibility

Figure 6. Chain of Custody Form.

CHAIN OF CUSTODY PAGE: OF

SURVEY ID: TRWO0104 **STATION ID:** Elliott Bay **HAUL:** 12 **SPECIES:** Rockfish **COLLECTION DATE:** 5-08-01

SAMPLES TAKEN						CHEMISTRY													OFF VESSEL TRANSFER			LAB TRANSFER		
BLOOD PLASMA	BILE – FACS	GONAD HISTOLOGY	LIVER - HISTOLOGY	LIVER - CYP1A	LIVER - DNA ADDUCTS	LIVER - INDIVIDUAL	LIVER – COMPOSITE	LESS LIVER	LESS ENTRAILS	WHOLE FISH	OVARY	DEFORMITIES	FIN CLIP	AGE STRUCTURE	OTHER	COUNT	CONDITION	ID NUMBER(S)	DATE	SIGNATURE	DESTINATION	DATE	SIGNATURE	DESTINATION
Y																3	F	015015, 015016, 015017						
	Y															14	F	015015-015022, 015024, 015026 - 015029, 015032						
		Y	Y													5 5	P P	015015 – 015018 Rockfish Jug						
						Y										4	F	015015 – 015018						
									Y							18	I	015015 – 015032						
													Y			18	P	015015 – 015032 Rockfish boy 1 & 2						

This form is to be filled out for all samples collected, with **one sample type per record** and each record **signed** by the person taking custody of the samples. PSEMP 4/01

Table 1. Specifications for the 400-mesh Eastern trawl net.

Headrope	71 feet with thimbled eyes, of 3/8: 6 x 19 galvanized wire rope served (full wrap) with 3/8" polypropylene rope.
Fishing line (footrope)	94 feet with thimbled eyes of 3/8" 6 x 9 galvanized wire rope served with 3/8" polypropylene rope (web laced or "hung" to the fishing line).
Disc footrope	4" discs (5" – 5 1/2" if 4" don't fit) on 1/2" long link Beacon 7 deck lashing chain.
Breast lines	6 feet of 3/8" 6 x 19 galvanized wire rope served with 3/8" polypropylene rope.
Seams	Side seams shall consist of lacing 3 knots (2 meshes) from each panel with No. 36 nylon twine. Tie each full mesh.
Hanging	Headrope: Wings – 2 meshes t0 6" Bosom – 4 meshes to 5 1/4" Footrope: Wings – 4 bars to 7 9/16" Lower Bosom – 4 meshes to 7"
Pucker rings	5/16" by 2 1/4" galvanized steel (approx. 33 pieces), secured with No. 48 braided polypropylene
Splitting rings	1/2" by 4" galvanized steel (4 pieces) set up 12 meshes from bottom
Liner in bag section	1 1/4" mesh, No. 18 nylon; 360 meshes around, 200 meshes deep (leave about 2 feet of liner extending from end of bag)
Chafing gear	Hula skirt chafing, 8" – 5 mm double bar mesh with 300 pounds hula rope

Restrictors	5 pieces 1” polypropylene rope spaced 4 feet apart
Webbing	4” mesh (including one knot) polyethylene, depth stretched and heat set; twine: 2 1/2 mm top - 3 mm bottom
Guard mesh	4 meshes on fishing line, 4 mm double bar mesh; 4 meshes on breastlines, 4 mm double bar mesh
Floats	15, 8 inch Deep Sea floats, evenly spaced (5.5 lbs buoyancy each)
Dandylines	8, 20 fath. 1/2” 6 x 19 galvanized wire rope
Riblines	two 3/4” coated Duralon riblines on codend and intermediate hung in at 10% of stretched measure of web, secured to web using benzels every 16”

21.0 Appendix E – Standard operating procedures for analysis of metals by King County Environmental Labs

Double-click on each file to open it.

Figure 17. Preparing samples for routine mercury analysis

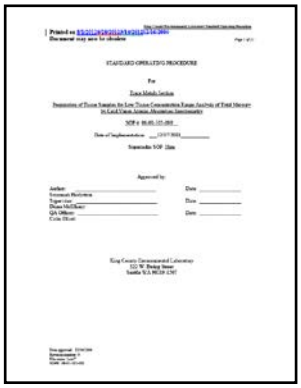


Figure 19. Preparing samples for low-level mercury analysis

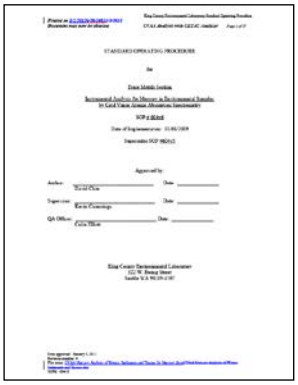


Figure 18. Cold Vapor Atomic Absorption (CVAA) method for mercury analysis.

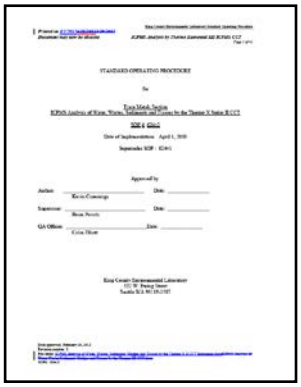


Figure 16. Inductively Coupled Plasma Mass Spectrometry (ICP-MS) method for analysis of metals.

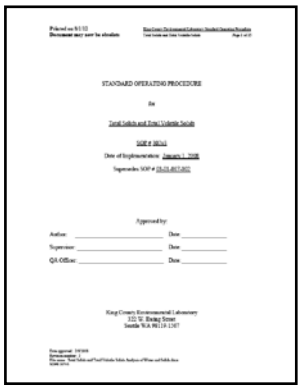


Figure 15. Method for determining total solids in a sample.