Quality Assurance Project Plan

Persistent Organic Pollutants in Three Guilds of Pelagic Marine Species from the Puget Sound

Washington Department of FISH and WILDLIFE

December 14, 2009

DEPARTMENT OF ECOLOGY
State of Washington

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Publication No. 09-10-099

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Persistent Organic Pollutants (POPs) in Three Guilds of Marine Pelagic Species from Puget Sound

December 14, 2009

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Abstract

Each Washington State Department of Ecology (Ecology) study that generates new data must have an approved Quality Assurance (QA) Project Plan. This document is that plan. This QA Plan describes the objectives of the study and the procedures to be followed to achieve those objectives. After completion of the study, a final report describing the study results will be posted to the Internet.

This project is designed to evaluate the extent and magnitude of Persistent Organic Pollutant (POP) exposure in the pelagic ecosystem of Puget Sound, and to gain a better understanding of the pathways of contaminants in this food web. To this end, non-migratory, pelagic forage fish species, including the Pacific whiting (*Merluccius productus*) and walleye pollock (*Theragra chalcogramma*) will be sampled and analyzed for contaminants. The primary prey of these pelagic predators, *Euphausia pacifica*, or krill, will also be sampled, as will phytoplankton, primary producers at the base of the Puget Sound pelagic food web. Harbor seals (*Phoca vitulina*) are upper trophic level predators and can serve as an informal sentinel of marine ecosystem contamination by integrating contaminant information from the food chain upon which it depends. Harbor seals are a relatively long-lived species and consume a variety of fish. They do not migrate, so they provide a 'local' signal of contamination.

To determine geographically where POPs are infiltrating the pelagic food web, samples will be gathered from a range of locations representing worst case, best case, and intermediate environments across the eight major basins of the Puget Sound, Strait of Juan de Fuca, and Strait of Georgia, as well as within several urban bays of the central Puget Sound for both fish and plankton. Associating contaminant levels and pathogens to foraging areas of harbor seals in Puget Sound will take place in three new areas: Central Puget Sound basin, Hood Canal, and Whidbey basin.

This project will focus on measuring levels of polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), polycyclic aromatic hydrocarbons (PAHs – in plankton only), chlorinated pesticides, and mercury. Samples will also be analyzed for percent lipids and solids. Stable isotopes of carbon and nitrogen will be used to evaluate trophic status.

Background

Over the past 20 years, researchers from the Puget Sound Assessment and Monitoring Program (PSAMP) and from the Washington Department of Fish and Wildlife (WDFW) have monitored and assessed a wide range of bioaccumulative and other Persistent Organic Pollutants (POPs) in a number of species that represent important ecological guilds in Puget Sound. These efforts have provided a picture of the geographic extent of ecosystem contamination by POPs, the magnitude of contamination, and temporal trends in these patterns. In addition, monitoring and assessment studies have raised questions regarding the pathways by which POPs from terrestrial

sources find their way into the Puget Sound food web, and why Puget Sound's pelagic food web exhibits an unusually high exposure to some POPs (O'Neill and West, 2009; West, *et al.*, 2008).

Long-term PSAMP and other WDFW studies support the hypothesis that benthic (bottom-dwelling) species reflect contaminant conditions in sediments. However, assessments of pelagic (open water) species, such as Pacific herring (*Clupea pallasi*), suggest that the pelagic food web is more directly linked to POPs that occur in Puget Sound's waters and pelagic biota (rather than sediments). Pacific herring hold unusually high tissue burdens of bioaccumulative POPs (*e.g.*, polychlorinated biphenyls (PCBs), an observation that is not typically predicted from sediment-as-source models. In addition, other research indicates that PCBs and polybrominated diphenyl ethers (PBDEs) have biomagnified in Puget Sound's harbor seals (*Phoca vitulina*) and killer whales (*Orcinus orca*) to levels that have impaired their health (Hickie, 2007; Ross, *et al.*, 2000; Ross, *et al.*, 2004).

Ecology's Phase 1 and Phase 2 toxics loading and modeling studies found that stormwater and aerial deposition represent the primary conveyance mechanisms for PCBs, PBDEs, and polycyclic aromatic hydrocarbon congeners (PAHs) from terrestrial sources into Puget Sound. These toxicants represent three important POP classes to which Puget Sound biota are exposed in high enough doses to impair their health. Several of these POPs bioaccumulate through the pelagic food web to high-level predators such as salmon, harbor seals, killer whales, seabirds, and humans. However, the pathways of contaminant flow from their abiotic sources to these predators are unclear, making it difficult to prioritize management actions aimed at reducing loading of toxicants, remediating contaminated habitats, or reducing exposure of biota to toxicants. To better protect these biota, we must evaluate: a) where (geographically) POPs enter the pelagic food web from stormwater and the atmosphere; b) the pathways of toxic contaminants within the pelagic food web; and c) the sources of POPs to species at the highest trophic levels (marine mammals, seabirds, and humans).

Project Description

In the summer of 2009, WDFW scientists from the PSAMP and the Marine Mammal Research Program will conduct pilot surveys of plankton, pelagic fish and harbor seals to evaluate the extent and magnitude of POP exposure in representative pelagic species of the Puget Sound. These species or guilds are meant to provide: a) a broad scale evaluation of POP exposure at the lowest trophic levels – *i.e.*, plankton, the putative point of entry of POPs into the food web; b) a better understanding of the role of residency in Puget Sound as a risk factor for POP exposure in pelagic predators; and c) an expanded geographic coverage of exposure and health effects of POPs on harbor seals.

Plankton

Two plankton guilds will be targeted: 1) phytoplankton, representing a seasonally abundant, lipid-rich pelagic medium at the base of the food web, to which POPs may sorb directly; and 2) the macrozooplankton species which graze on phytoplankton and serve as the dominant prey for pelagic fish species. Size selective netting will be used to obtain phytoplankton samples.

However, the nets will retain other organisms (micro-heterotrophs, *e.g.*, microzooplankton) as well as inorganic and organic particles that cannot be sorted out. Hence, although dominated by phytoplankton, we will refer hereafter to these samples as Particulate Organic Matter (POM).

Macrozooplankton that represent the primary prey of the intermediate pelagic predators mentioned above will also be sampled. Target species will be determined during surveys, depending on their abundance and distribution and on the feasibility of collection. Ideal taxa will represent both holoplankton, such as krill (*Euphausia pacifica* and *Thysanoessa* sp.), and meroplankton, such as larvae of abundant crab or benthic fish species.

Fish

Fish species that are both important predators of the zooplankton guild described above and prey of harbor seals will be targeted. Ideal candidates will include gadoid cod fishes, such as Pacific whiting (*Merluccius productus*) and walleye pollock (*Theragra chalcogramma*), as well as other pelagic forage fish species including Pacific herring (*Clupea pallasi*) and Pacific salmon species (*Oncorhynchus* spp).

Pelagic taxa will be sampled across the full extent of Puget Sound in the summer and autumn. To help determine geographically where POPs may infiltrate the pelagic food web, we will target South Puget Sound, Central Puget Sound, Hood Canal, Admiralty Inlet, Whidbey Basin, Strait of Juan de Fuca, San Juan Archipelago, and Strait of Georgia, as well as several urban bays in Central Puget Sound (Commencement Bay, Elliott Bay, and Sinclair/Dyes Inlets). Analytes for all samples will include PCBs, PBDEs, PAHs, chlorinated pesticides, mercury, percent lipids and solids, and stable isotopes of carbon (C) and nitrogen (N).

Harbor seals

Samples from up to eight harbor seal (*Phoca vitulina*) pups will be collected during August and September 2009 in three new areas: Central Puget Sound basin, Hood Canal, and Whidbey basin. Harbor seal pups at our long term study area at Gertrude Island in the South Puget Sound basin may also be sampled if funding allows. Concentrations and patterns of POPs in biota, including seals, are confounded by age (POPs increase with age), sex (reproductive females transfer POPs to their offspring), and condition (POPs concentrate in diminished blubber reserves). We therefore take great care to ensure maximum comparability among our study animals. By selecting pups of comparable size and age, we limit the variation in POP results and maximize our ability to rigorously interpret results. Results will be evaluated against contaminant concentrations and compared with results obtained from previous sampling in Puget Sound as well as additional British Columbia sites. This collaborative effort will help to document the implications of high POP levels in Puget Sound for the health of high trophic level marine mammals, and provide important new insight into the overall 'health' of Puget Sound.

Organization and Schedule

The following people are involved in this project. All (except the client) are employees of the Washington State Department of Fish and Wildlife (WDFW).

Table 1. Organization of project staff and responsibilities.

Staff	Title	Responsibilities
James M. Maroncelli Department of Ecology Phone: (360) 407-6588	Client	Clarifies scopes of the project. Provides internal review of the QAPP and approves the final QAPP.
James West WDFW Fish Program Natural Resource Bldg Phone: (360) 902-2842	Project Manager	Designs the study and writes the QAPP. Oversees field sampling and transportation of samples to the laboratory.Conducts QA review of data, analyzes and interprets data. Writes the draft and final report.
Steven Jeffries WDFW Wildlife Program 7801 Phillips Rd. SW Lakewood, WA 98498 Phone: (253) 589-7235	Project Manager	Designs the study and administers the contract and budget. Oversees field sampling. Conducts QA review of data, analyzes and interprets data. Co author of draft and final report.
Jennifer Lanksbury WDFW Fish Program Natural Resource Bldg Phone: (360) 902-2820	Principal Investigator	Helps write the QAPP. Conducts field sampling and transportation of samples to the laboratory. Conducts QA review of data, analyzes and interprets data. Co author of draft and final report.
Monique Lance WDFW Wildlife Program 7801 Phillips Rd. SW Lakewood, WA 98498 Phone: (253) 589-7235	Principal Investigator	Assists with study design. Oversees field sampling. Conducts QA review of data, analyzes and interprets data. Co author of draft and final report.
Steve Quinnell WDFW Fish Program Natural Resource Bldg Phone: (360) 902-2849	Field Assistant	Helps collect and process samples and records field information.
Anne Marshall WDFW Fish Program Natural Resource Bldg Phone: (360) 902-2769	Field Assistant	Helps collect and process samples and records field information.
Stefanie Orlaineta WDFW Fish Program Natural Resource Bldg Phone: (360) 902-2859	Field Assistant	Helps collect and process samples and records field information.

QAPP – Quality Assurance Project Plan.

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

Field and laboratory work	Due date	Lead staff
Field work completed	September 2009	Jennifer Lanksbury (plankton/fish) Monique Lance (harbor seals)
Laboratory analyses completed	December 2009 (plankton, fish) April 2010 (harbor seals)	
Quarterly reports		
Author lead	James West	
Schedule		
1st quarterly report	Oct 2009	
2nd quarterly report	Jan 2010	
3rd quarterly report	Apr 2010	
4th quarterly report	Jul 2010	
Final report		
Author lead and support staff	James West/J. Lanksbury (plankton, fish) Steve Jeffries/Monique Lance (harbor seals)	
Schedule		
Draft due to supervisor	Dec 2009 (plankton/fish) May 2010 (harbor seals)	
Final report due to Ecology	Feb 2010 Sep 2010	

Quality Objectives

The data collected in these studies will:

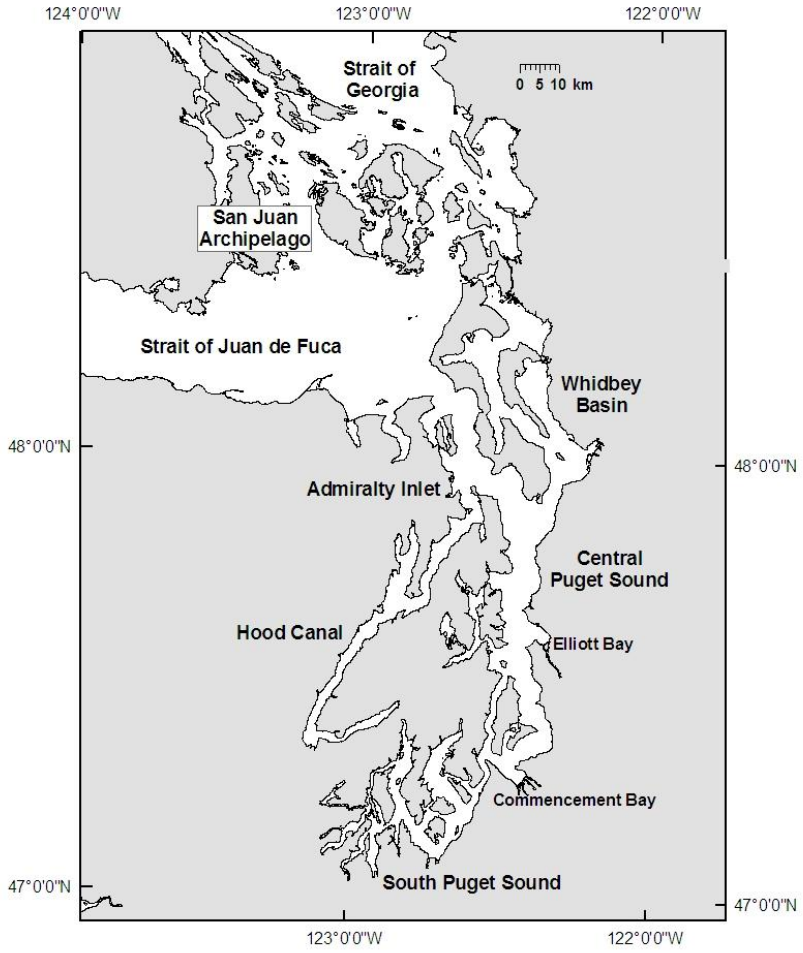
- Provide a first look at where, geographically, POPs initially enter Puget Sound's pelagic food web by:
    - Measuring POP exposure in pelagic biota across a wide geographic range to evaluate Ecology's *Control of Toxic Chemicals in Puget Sound -- Phase 1: Initial Estimate of Loadings* conclusions regarding the relatively high inputs and broad distribution of PBDEs via atmospheric deposition to Puget Sound's waters.
    - Providing exposure data to Ecology's modeling efforts to compare land use patterns (POP sources) with where POPs may initially become entrained in Puget Sound's food web.
- Provide a better understanding of contaminant exposures in some of the most contaminated pathways of the food web to aid in evaluating whether environmental quality standards are protective of Puget Sound's aquatic resources, including:
    - Providing a first assessment of the exposure of Puget Sound's lowest trophic levels to POPs.
    - Combining these results with previous PSAMP food web study findings to provide a clearer understanding of how bioaccumulative POPs move from their source (loading) through the food web to Puget Sound's highest trophic levels.
- Inform strategic efforts to reduce harm from toxicants to Puget Sound's biota by:
    - Initiating efforts to compare broad geographic-scale sources of POPs loaded to surface waters with sediment-sources, which will
    - Facilitate more accurate prediction of the impact, efficacy, and relative merit of remediation efforts, such as source control and sediment cleanup.

Sampling Process Design (Experimental Design)

Approximately 60 samples, representing several species of fish, macrozooplankton, and Particulate Organic Matter (POM, comprising primarily phytoplankton) as described above will be collected across as wide a geographic range in Puget Sound as possible. Samples will be taken from a range of regions thought to receive low, medium, and high contaminant inputs and that represent major oceanographic basins.

Sampling locations will likely occur in each of the following major areas of interest for this study, including but not limited to South Puget Sound, Central Puget Sound, Hood Canal, Admiralty Inlet, Whidbey Basin, Strait of Juan de Fuca, San Juan Archipelago, and Strait of Georgia, as well as several urban bays (Commencement Bay, Elliott Bay, Sinclair/Dyes Inlets, Everett Harbor, and Bellingham Bay).

Figure 1. Major areas of interest for this study; the eight major basins that comprise Puget Sound.



Locations for fish sampling will include previously occupied PSAMP survey stations, as well as locations that become available through opportunistic research cruises. Station locations for macrozooplankton sampling (specifically krill) will include sites from previous studies (Cooney, 1971). Although the POM sample collection will be attempted at all sites of macrozooplankton sampling, other sites determined to be potentially fruitful through observation (*i.e.*, reported plankton blooms) will also be targeted. Samples for each guild will be taken as synoptically as possible, to reduce seasonal effects.

Sampling for harbor seals will take place in Central Puget Sound basin, Hood Canal, and the Whidbey Basin.

Sampling Procedures

Fish Collection
A mid-water rope trawl, utilized during regular PSAMP surveys, will be used to capture most of the fish for this study. Occasionally other mid-water rope trawl or bottom trawling gear will be used to capture fish on opportunistic surveys with other vessels. Once captured, the contents of the net-cod end will be released onto a large sorting table. Pelagic fish of appropriate species and size will be separated from other taxa and placed together (by species) in labeled Ziploc bags. Bag labels will include survey ID, date, location, species, and total number of fish. Bagged samples will be either frozen immediately or held on ice for no longer than 2 days prior to freezing. Fish will be held frozen at -20 ºC until they are prepared for composites.

Whole Fish Composite Preparation
For each basin, three whole-body composites of pre-reproductive (<25cm fork length, FL) and/or three whole-body composites of mature (>25cm FL) fish will be made for all species collected. The number of individuals per composite will vary depending on availability of fish, but no more than 20 fish will be included in any one composite. Some large individuals will be composited and analyzed individually.

After morphometric measurements are taken on thawed individuals in the laboratory (see Measurement Procedures), fish will be refrozen in preparation for grinding. Each fish in a composite will be ground (still frozen) in a Hobart 4812 meat chopper. To ensure complete homogenization, all fish in a composite will be sent through a large-bore (½ inch) grinding plate twice. When grinding is complete, each composite will also be stirred with a pre-cleaned spatula.

Once completely homogenized, a pre-cleaned spatula will be used to scoop the fish homogenate into a clean (EPA standards), labeled, 8-oz glass jar (I-Chem brand) and held at -20 °C until submitted for chemical analysis. Jars will be filled to approximately three-quarter volume (~200 gm) to allow a space for a final mixing prior to removing the sample for analysis.

After each composite has been completed, all instruments that came into contact with fish material, including the grinder component parts, bowl for catching homogenized material, and all utensils, will be brushed and cleaned in warm tap water mixed with Terg-a-zyme lab soap, rinsed repeatedly (at least five times) with tap water, and lastly rinsed with isopropyl alcohol (reagent-

residue analysis grade) before being set aside under clean foil to dry. This cleaning technique will be repeated between each composite.

Macrozooplankton Collection
Krill, the target macrozooplankton for this study, will be collected using a modified Kvichak net (Figure 2). The net mesh size is widest at the mouth 10 mm) and becomes progressively smaller towards the cod end, ending with ~3-mm size at the zippered cod end. The net is suspended between two horizontal beams that open during deployment by means of floats on the upper beam and 260 lbs of weight on the bottom beam. Sampling will consist of targeted horizontal and oblique tows from near-bottom to the surface made from a 30-ft research vessel, using pre-marked Kevlar towing line wound on a hydraulic winch.

Figure 2. Modified Kvichak net being launched for macrozooplankton sampling.

A sonar depth sounder (Furuno or SIMRAD) set at 50 and 200 kHz will be used to locate deep scattering layers (potential krill "sign"). Once krill sign is seen on the depth sounder, a target sampling depth will be determined and length of trawl line calculated. A ratio of approximately 2:1 (line:net depth) will be used to reach the target depth. The net will be fished at approximately 2 knots (boat speed) for 5 to 30 minutes, depending on the strength of the signal observed. A *ReefNet Inc. Sensus Ultra* dive data logging device, attached to the Kvichak's upper net beam, will be used to record the actual depth sampled. Recorded depth information will be downloaded and viewed immediately after each tow to verify sampling depth and make any needed corrections for subsequent tows.

Once on deck, the contents of the cod end will be released into a pre-cleaned sorting basin. The collected organisms will be held in seawater obtained on site and will be immediately sorted, using pre-cleaned stainless steel sieves (>3-mm mesh), to remove large, unwanted organisms. Although krill will be the targeted taxa for sample collection, the cod end contents will also be scanned for crab megalopae, which may be retained as secondary target taxa for this study.

Whole Macrozooplankton Composite Preparation
Once larger, unwanted organisms (*e.g.*, fishes, algae, and jellies) and debris are removed, the remaining krill and other smaller taxa will be concentrated by passing the sample through other

pre-cleaned, stainless steel sieves varying in mesh size from 500µm to 3,000 µm. This mass will then be transported to a clean working table where the krill taxa will be isolated, using pre-cleaned tweezers and/or stainless steel spatulas, and placed into 2-oz, pre-cleaned I-Chem brand sample jars (Figure 3). Multiple sample jars (up to six composites) will be collected per sample site. Sample jars will be labeled, placed on ice immediately, and frozen to at least -20 °C within 72 hours of collection. Composites will remain frozen until analyzed in the laboratory.

Figure 3. Isolated krill taxa (sample jar at bottom) picked from a mixture of macrozooplankton.

A voucher sample will be collected at each site for verification of species present. The voucher samples will be collected in 1-oz sample jars and preserved in a 5% buffered formalin solution (see Measurement Procedures).

All sampling gear will be washed using soap and freshwater between sampling basins, and stored in covered containers.

Particulate Organic Matter Collection

Particulate organic matter, dominated by phytoplankton, will be collected using conical phytoplankton nets designed to retain phytoplankton with as little damage to the cells as possible (Figure 4). The two phytoplankton nets used in this study measure: a) 25-cm mouth diameter by

60 cm length, and b) 30-cm mouth diameter by 100 cm length. Both nets are attached to a stainless steel ring, have 20-µm mesh, and are equipped with closed cod end jars. Each net ring is attached to a 3-point bridle which is secured to a 30-m nylon line.

Figure 4. Conical plankton sampling net (20 µm mesh pore size) used to sample particulate organic matter.

Each sampling effort will consist of vertical lifts from near bottom or a depth of 25 m (whichever is shallower) to the surface. Lifts will be made from a drifting boat to minimize the effects of currents on net performance. The boat engine will be switched off prior to sampling to avoid contamination of gear and samples by exhaust fumes. Depth will be measured using marks on the nylon line and volume filtered will be calculated using the measured depth. After the net ring breaks the surface, the net will be lifted out of the water slowly enough to allow some drainage of seawater through the mesh.

Particulate Organic Matter Composite Preparation

Once on deck, the phytoplankton net will be suspended until all visible POM is concentrated in the cod end. After the POM sample has been concentrated in the cod end, the cod end will be detached and its contents poured through: 1) a pre-cleaned 1-mm stainless steel sieve to filter out jellyfish and other large plankton, and 2) a 20-µm sieve to retain POM. The resulting green "paste" of POM will be gently scooped out of the 20-µm sieve and placed into a 2-oz, pre-cleaned I-Chem brand sample jar using a pre-cleaned stainless steel spatula (Figure 5). Multiple lifts may be needed to obtain sufficient volume of POM for a sample. POM samples will be labeled, placed on ice immediately, and frozen to at least -20 °C within 72 hours of collection. Composites will remain frozen until analyzed in the laboratory.

Figure 5. Collection of particulate organic matter (POM) paste concentrated on a 20-µm sieve.

The phytoplankton net will be cleaned between sampling areas and transported on the boat in a sealed tote to minimize the risk of contamination from boat-engine exhaust fumes. All sampling gear will be washed using soap and freshwater between sampling basins.

Harbor Seal Sample Collection

The primary method of harbor seal pup capture will be by beach seine, salmon landing net, or tangle nets deployed from boats. Additional animals may also be captured by hoop net.

Figure 6. Open circles indicating areas for harbor seal pup captures and blubber sampling.

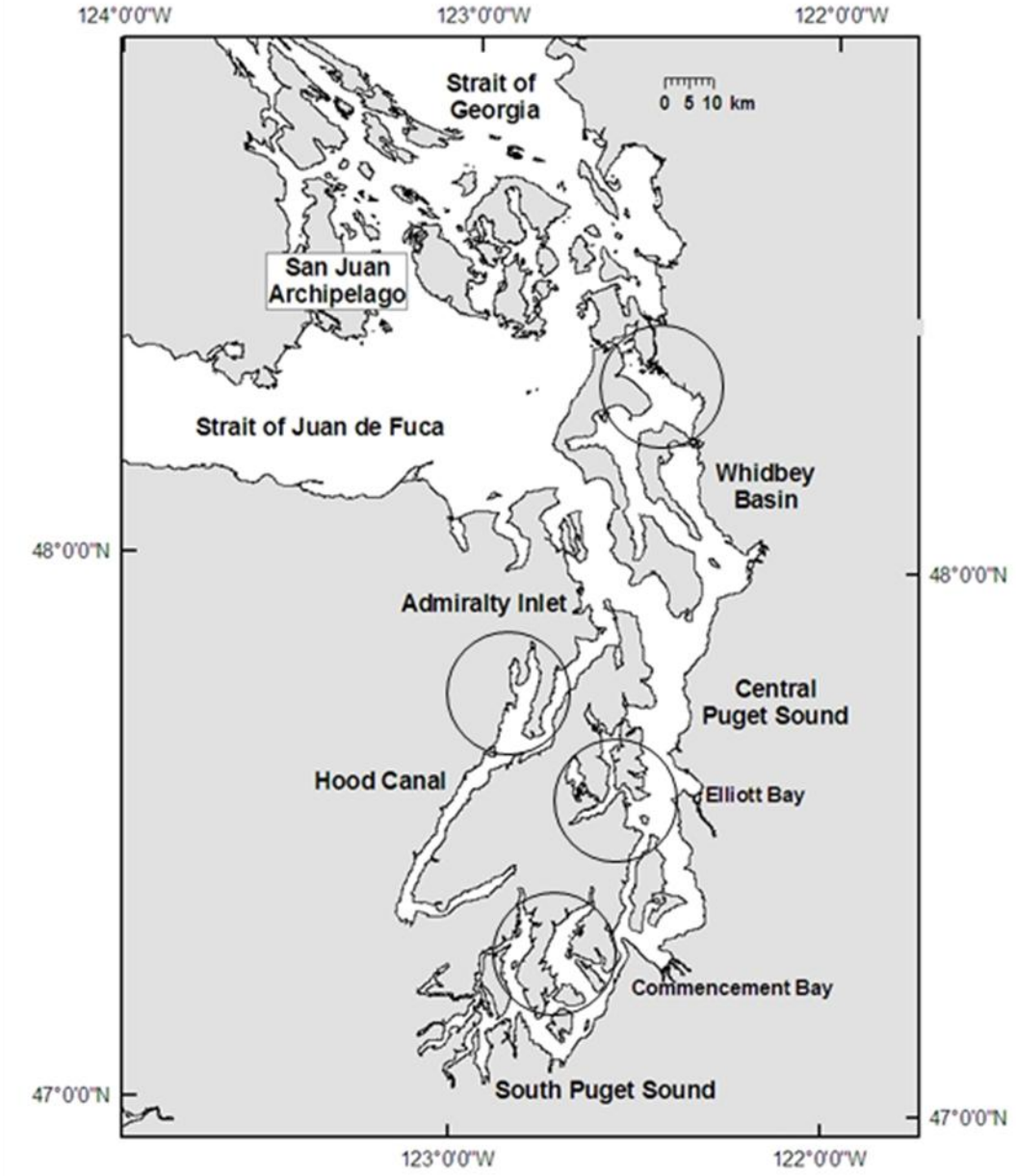


Figure 7. Capture team preparing for boat rush capture of harbor seal pups with salmon landing nets at Orchard Rocks in Central Puget Sound.

We will sample only animals greater than 20 kg in weight. We will take length and girth measurements and flipper tag all pups. Blood samples will be drawn in designated plasma or serum vacuum collection tubes from the extra-dural sinus. These samples will be stored approximately 4 hours before centrifugation and decanting of serum or plasma. These samples will be frozen on liquid nitrogen and stored at -80 °C until analysis. Blood collected will not exceed 1 ml per kg for each animal. Blubber and skin biopsies will be accomplished using sterile 3.5 and 8.0 mm biopsy punches. Blubber core samples will be collected from the left side of the animal in the pelvic region. The biopsy site will be prepared by shaving the hair with an electric razor, rinsing with isopropyl alcohol, scrubbing with Betadine, and rinsing a second time with isopropyl alcohol. Hair will be collected for stable isotope analysis in 1.5 mL cryovials and stored at room temperature. The 8.0-mm biopsy will be wrapped in foil, placed into a 5 mL cryovial, and transferred immediately into a dewar filled with liquid nitrogen. These samples will be used for Vitamin A and POP analysis. The 3.5-mm biopsy will be rinsed with buffered saline solution to remove the blood, and placed immediately in pre-labeled tubes containing RNAlater solution. These genomic samples will be kept in a cooler for 24 hours and then placed in the refrigerator at -20 °C until analysis. A complete cross section biopsy of blubber from skin to muscle will be taken. Wounds resulting from the biopsy punch will be filled with antiseptic cream and left open to allow drainage. Special attention will be paid to time of capture and time of sampling for biopsy samples. Two whiskers will be collected to measure recent exposure to

mercury. These samples will be stored at room temperature until analysis by laser ablation ICP-MS. Harbor seal research activities will be conducted under Marine Mammal Protection Act Research Permit 782-1702-05.

Figure 8. Harbor seal pup length being measured in the bow of capture boat.

Figure 9. .Research biologist taking 8-mm blubber sample from harbor seal pup.

Measurement Procedures

Analytes

The POPs to be measured in the composited fish and plankton tissue samples include analytes of polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), polycyclic aromatic hydrocarbon congeners (PAHs), and chlorinated pesticides by gas chromatography/mass spectrometry (GC/MS) according to (Sloan, *et al.*, 2004). Forty PCB and ten PBDE congeners, 43 PAH compounds and their alkylated homologs, and 13 chlorinated pesticides will be quantitated using low resolution GC/MS for all plankton. Up to 209 PCB congeners (some as co-eluters) will be measured in the pelagic fish samples. The percent lipid and solids, and stable isotopes of carbon and nitrogen, will also be measured for each sample (Tables 3).

Table 3. Analytes to be measured in whole body fish composites.

Analyte	Number of Expected Samples			Reporting Limit			Expected Range		
	Fish	MZ	POM	Fish	MZ	POM	Fish	MZ	POM
PCB congeners (ng/g)	60	18	30	0.5	0.5	0.05	<RL-30	<RL-3	<RL-0.3
PBDE congeners (ng/g)	60	18	30	0.5	0.5	0.05	<RL-25	<RL-3	<RL-0.3
PAH (ng/g, each cpd)	60	18	30	0.5	0.5	0.05	<RL-10	<RL-1	<RL-0.1
Chl. Pest. (each,ng/g)	60	18	30	0.5	0.5	0.05	<RL-5	<RL-0.5	<RL-0.05
Total Lipids (%)	60	18	30	0.01	0.01	0.01	NA	NA	NA
Solids (%)	60	18	30	0.01	0.01	0.01	NA	NA	NA
^{13}Carbon ($^{0}/_{00}$)	60	18	30	(-13)-(-23)	(-13)-(-23)	(-13)-(-23)	NA	NA	NA
^{15}Nitrogen ($^{0}/_{00}$)	60	18	30	12-16	7-11	5-7	NA	NA	NA

MZ – Macrozooplankton
Chl. Pest. – Chlorinated Pesticides
cpd – compound

Fish Measurements

In order to make morphometric measurements, collected frozen fish will be thawed and rinsed in cold tap water from the Natural Resources Building/Marine Resources Lab sink for 5 seconds to remove any debris. Each fish will be measured for fork length (nearest mm) and weight (to nearest 0.1 gm). The sex of fish will be determined from a visual examination of gonads via a small incision in the abdomen. Otoliths (used to estimate fish age) will be removed from a small oblique slice made at the dorsal surface of the head, cleaned by rubbing on a paper towel, dipped in ethyl alcohol to remove moisture, and placed in a capped vial labeled with the individual Fish ID.

Macrozooplankton Measurements

Wherever composite samples of macrozooplankton are collected, a voucher sample of specimens will also be collected to allow for verification of the species present. The voucher samples will be collected in labeled, 1- or 2-oz sample jars and preserved in a 5% buffered formalin solution. Data collected from these voucher specimens will include species identification and a measure of body length (nearest mm).

Particulate Organic Matter (Phytoplankton) Measurements

A voucher sample will be collected for later identification of species composition. Collections will be made by gently pipetting the concentrated phytoplankton from the cod end jar into a 20-mL or 2-oz sample jar. Buffered formalin solution will then be added to make a ~0.8 to 1% formaldehyde solution for preservation. Data collected from these voucher specimens will include identification to the lowest possible taxonomic level and relative abundance of each identifiable taxon.

Harbor Seal Sample Measurements

Stable isotopes in fur

Fur (0.1 g) will be rinsed in 2:1 chloroform:methanol, then freeze-dried for 48 to 72 hours. Bulk stable carbon and nitrogen isotope ratio (15N:14N and 13C:12C) measurements will be carried out at the University of Winnipeg in Winnipeg, Manitoba, with equipment and standards described elsewhere (Cullon, *et al.*, 2005). Isotopic composition will be expressed in ‰ notation as the proportional deviation in parts per thousand (‰) of the isotope ratio in a sample from that of a standard:

$$‰X = (R_{sample}/R_{standard} - 1)\ (1{,}000)$$

where X is ^{13}C or ^{15}N, and R_{sample} and $R_{standard}$ are the ratios of ^{13}C:^{12}C or ^{15}N:^{14}N for the sample and standard (Hobson, *et al.*, 1997). The isotope mass spectrometer will be calibrated with reference material in each batch.

Genomic analysis in blubber samples

Tissue Homogenization. All blubber samples will be separated from the skin and a 20-mg (approximate) piece will be cut longitudinally and homogenized in TRIzol reagent (Invitrogen Canada Inc., Toronto, Ontario) using a Retsch MM301 mixer mill as described by Veldhoen and Helbing (2001). Each blubber sample will be homogenized in a 1.5-mL microcentrifuge tube with the addition of 700 µL TRIzol and a 3-mm diameter tungsten-carbide bead. Blubber samples will be homogenized 2 times using 5-minute intervals, at a frequency of 20 Hz. For any given sample, an additional 3 minutes of mixing will be performed if un-homogenized tissue remaines. All samples will be cooled on ice between homogenization intervals.

Isolation of Total RNA. Total RNA will be isolated from the tissue homogenates in TRIzol reagent as described by the manufacturer. After phase separation by centrifugation at 12,000 x g for 10 min, 1 mL glycogen (Roche Diagnostics, Laval, Quebec) will be added in a clean 1.5 mL micro-centrifuge tube to each retained aqueous phase from blubber homogenates. Chloroform (140 µL) will be added to separate the solution into an organic and an aqueous RNA-containing phase. Tubes will be inverted 20 times, incubated for 2 min at room temperature, and centrifuged for 15 min at 4 °C to obtain phase separation. The aqueous phase containing RNA will be transferred into a new tube, and RNA will be precipitated with the addition of isopropanol (350 µL) a 10-min incubation at room temperature, and centrifugation at 12,000 x g

for 10 min at 4 °C. The RNA precipitate will appear as a translucent gel-like pellet at the bottom of the Eppendorf tube. The supernatant will then be discarded, and the total RNA pellet will be washed with 700 µL of 75% ethanol (made with diethyl pyrocarbonate-treated distilled, deionized H_20 (DEPC-dH_20)). After a 5-min centrifugation at 7,500 x g at 4 °C, total RNA will be re-suspended in 30 µL of DEPC-dH_20, incubated in a 55 °C water bath for 10 min, and stored at -80 °C.

cDNA Translation. Total cDNA will be produced using Superscript II RNase H-reverse transcriptase, as described by the manufacturer (Invitrogen, Canada). Spectrophotometry will be used to determine RNA concentration, and 2 µg of total RNA will be used in the preparation of cDNA. The final cDNA solutions will be diluted 40-fold using DEPC-dH_2O prior to quantification.

Real-Time Quantitative Polymerase Chain Reaction (PCR) Assay. Seven qRT-PCR primers specific for harbor seal (*Phoca vitulina*) were designed and assessed for their ability to amplify a single specific DNA amplicon. Quantitative DNA amplification reactions (15 µl) will be performed on a Realplex4 Eppendorf system (Eppendorf in Westbury, New York) as described previously (Crump, *et al.*, 2002) using gene-specific primers. The thermocycle program for most gene targets includes an initial enzyme activation step at 95 °C (9 min) followed by 40 cycles of 95 °C denaturation (15 sec), 60 °C annealing (30 sec), and 72 °C elongation (45 sec). Quadruplicate reactions will be performed for each sample, and data will be averaged and normalized to the expression of the gene encoding the ribosomal protein rpL8 using the comparative (ΔΔCT) method (Livak and Schmittgen, 2001). This transcript passed the Bestkeeper method of normalizer determination (Pfaffl, *et al.*, 2004), and the expression of this gene was invariant in blubber tissue in this study.

Thyroid hormone assay

The concentrations of TT_4, FT_4, TT_3, and FT_3 will be measured in pups using enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturer's recommended protocol (Calbiotech, California). Frozen (undiluted) serum samples will be thawed on wet ice, and four TH measurements of each sample will be obtained within 6 hours in order to avoid repeated freeze-thaw cycles, which can lower the quality of samples. Serum samples will be incubated with horseradish peroxidase- (HRP-) labeled hormones in anti-TH antibody coated polystyrene microtiter plates. HRP-labeled hormone and native hormones competitively bind to the antibodies on the wells. After washing off the unbound hormones, the amount of enzyme-labeled hormones will be measured by adding substrate: a mixture of 3,3',5,5'- tetramethylbenzidine (TMB), which changes color by reacting with the HRP. The color intensity of seal serum samples and TH standards will be measured at 450 nm on a MRX microplate reader (Dynatech Laboratories Inc. in Chantilly, Virginia). For each ELISA, reactions will be prepared in triplicate, and the sample data will be subsequently averaged and compared to the standard curve in order to obtain representative TH concentration values.

Inter-assay variation will be evaluated in two ways. The first method will employ the regular inclusion of a reference pooled seal serum sample, whereby results will be accepted for an assay only when standard results are ± 20% of expected values. In the second method, total hormone measurements (TT3 and TT4) will be validated using the manufacturer's reference standard

(Thyroid Cal-ver™ reagent; Casco Neal, Portland, Maine), and results will be accepted for an assay only when concentrations are within ± 5% of the expected values.

No purified harbor seal thyroid hormones are commercially available. With this in mind, we validated the thyroid hormone assays for harbor seals by conducting analyses of serial dilutions within a fixed sample volume, and using incremental spikes of seal serum with Thyroid Cal-ver™ reagent. Responses of serial dilutions of seal serum and standard additions of seal serum with the reference standard both produced linear results. More detailed methods can be found in Tabuchi, *et al.*, (2006).

Contaminant analyses

Each frozen (-80 °C) 8 mm tissue biopsy will be cut vertically, and the upper skin layer (approximately 2mm) will be removed. A portion of each blubber sample (100 mg to 300 mg wet weight) will be used for measuring POPs in two methods. Firstly, all congeners of PCBs, as well as specific congeners of PCDDs and PCDFs, will be analyzed at the Fisheries and Oceans Canada LEACA (Laboratory of Excellence in Aquatic Chemical Analysis, Institute of Ocean Sciences, Sidney, British Columbia). Briefly, the blubber sample will be ground with anhydrous sodium sulphate and spiked with a mixture of $^{13}C_{12}$-labeled PCBs, PCDDs, and PCDFs (Cambridge Isotope Laboratories, Andover, Maine). Using dichloromethane/hexane (1:1 ratio), the sample will be extracted from a glass column. The extracts will be evaporated to dryness and weighed. Total lipid concentrations will be determined related to the original sample weight. The residues will be resuspended in dichloromethane/hexane (1:1), and analyzed by using high resolution gas chromatography and high resolution mass spectrometry (HRGC-HRMS) analysis. Details of the chromatography and mass spectrometry conditions, the criteria used for chemical identification and quantification, and the quality assurance and quality control practices have been previously described (Ikonomou, 2001).

Vitamin A

Skin and blubber biopsy samples require hydrolysis to extract the retinoids quantitatively due to the small sample size and rough texture. In this method, as established by Vahlquist, *et al.* (1990), all retinol-esters are hydrolysed into all-trans retinol. Several compounds then appear in a common "total retinol" HPLC peak, improving the detection limit of the assay. Also, there is no need for further homogenization. Briefly, samples will be saponified in ethanolic KOH solution (10 mL ethanol and 1.6 g KOH per gram sample) in the presence of 0.1% butylated hydroxytoluene to prevent oxidative degradation and TMMP as an internal standard. Tubes will be flushed with nitrogen and sealed before a 30-minute incubation in a water bath of approximately 80 °C. Processed tissue samples will be immediately cooled afterwards, and an equal amount of DMQ will be added to stop the reaction. Retinol will be extracted twice by adding *n*-hexane (2 mL) and shaking for 3 minutes. A known amount of the organic layer will be evaporated, dissolved in methanol:dichloromethane (9:1), and immediately used for HPLC-analysis. All work will be performed under yellow light.

Mercury analysis in fur

To remove any external contamination, all hair samples will be rinsed in series with acetone, de-ionized water, and acetone and left to dry at room temperature, and then stored in a dessicator until analysis. About 1 mg of hair will be analyzed for total mercury using a Zeeman atomic absorption spectrometer RA-915+ coupled with a PYRO-915 attachment (Lumex, St. Petersburg, Russia). The detection limit will be 0.002 µg/g dry weight. Details on the methods and instrumentation can be found elsewhere (Sholupov, *et al.*, 2004).

Two standards will be used: a sediment standard NIST 2709 (National Institute of Standards and Technology, Gaithersburg, West Virginia), and a human hair standard NIES 13 (National Institute for Environmental Studies, Ibaraki, Japan). Each of the seal hair samples will be run in triplicate. One NIST 2709 and one NIES 13 standard will be run every six samples to ensure that there is no deviation from the calibration curve.

Shipping

Iced or frozen samples of whole fish and/or zooplankton composites and POM samples in jars will be transported in coolers to either WDFW's laboratory at the Natural Resource Building in Olympia or NOAA's Northwest Fisheries Science Center in Seattle for storage in -20° C freezers. Some samples may be shipped to analytical laboratories via a commercial shipping firm, such as FedEx. If so, sample jars will be packed in Styrofoam coolers and sealed with enough dry ice to keep them frozen during transit. If a shipping firm is used, the principal investigator will obtain a waybill to track progress of the shipment and status of the samples.

The Institute of Ocean Sciences (IOS) in Sidney, British Columbia will perform high resolution analysis of PBTs on all fish composites. The IOS is a research facility under the management of the Pacific Region of Fisheries and Oceans Canada. Low resolution analysis of PBTs in all plankton samples will be undertaken at the NOAA Fisheries Montlake Laboratory in Seattle, Washington.

Iced or frozen samples from harbor seals will be shipped via FedEx to IOS. Samples will be packed in Styrofoam coolers and sealed with enough wet ice or dry ice to keep a consistent temperature during transit. Samples will be tracked via tracking number and communication with Canadian customs and contacts at IOS.

Quality Control Procedures

Field

Duplicate or Triplicate Composites per Area

When possible, target organisms will be sampled in duplicate or triplicate at each basin. The total hake and/or walleye pollock collected in each basin will be split into two size classes (< 25 or >25 cm FL) and then divided into three groups of up to 20 fish/group. Each of these groups will be homogenized (see Whole Fish Composite Preparation) to create a total of three separate

composites per basin and fish species/size class. For example, from the Hood Canal basin up to 20 hake of <25cm FL will be homogenized into each of three composites.

Due to the aggregating nature of krill, we anticipate that enough krill will be collected in each basin to fill at least three separate 2-oz sample jars. Although duplication of POM samples will be attempted, we anticipate that sufficient amounts of POM may only be available in areas experiencing phytoplankton blooms.

Wet-Lab Blanks
A lab blank will be collected each day using tap water to test the cleaning procedure for the grinding equipment. This blank will be collected at the end of each day after the last cleaning, and will consist of a sample of tap water that is run through the Hobart 4812 meat chopper, including the large-bore (½-inch) grinding plate, and collected in a clean (EPA standards), labeled, 8-oz jar. This water blank will be held at -20° C for later possible chemical analysis, and will be analyzed only if results from the analytical laboratory indicate a potential wet-lab contamination.

Laboratory

Analysts will contact the principal investigator with any QC issues that are not resolved by re-running invalid or problematic samples. Quality control samples will be run with each GC/MS batch as follows.

Check Standards - Each laboratory will run at least one Standard Reference Material (SRM) sample in each batch. SRMs are certified by the NIST and will be selected to best represent the tissue being analyzed and the range of expected analyte concentrations.
http://ts.nist.gov/MeasurementServices/ReferenceMaterials/DEFINITIONS.cfm

Analytical Duplicates - Each laboratory will run one duplicate or triplicate aliquot per GC/MS batch.

Laboratory Blanks - One lab blank, consisting solely of solvent, will be run per GC/MS batch.

Harbor seal sample laboratory analyses
Stable isotope analyses will take place at the Department of Geography, Stable Isotope Lab, where the contact is Dr. Bill Buhay, University of Winnipeg, Winnipeg, Manitoba. Contaminant analyses will take place at Fisheries and Oceans Canada, Laboratory of Excellence in Aquatic Chemical Analysis, where the contact is Dr. Andrew Ross, IOS, Sidney, British Columbia. Vitamin A, thyroid, and genomics analyses will take place at Fisheries and Oceans Canada, Ecotoxicology Lab, where the contact is Dr. Peter Ross, IOS, Sidney, British Columbia. Mercury analyses will take place at Department of Earth and Ocean Sciences, where the contact is Dr. Kevin Telmer, University of Victoria, Victoria, British Columbia.

Data Management Procedures

Field notes will be taken during all sampling activities. The following records will be maintained throughout the field and laboratory phases of the survey: Field Log Book, Haul Position Form (Appendix B), Plankton Survey Form (Appendix C), Specimen Forms and Tissue Resection Log (fish composites only).

The Field Log Book will include notes on research cruise vessels, staff, dates and times, weather, fish/krill "sign", and the success or failure of sampling efforts and techniques. Haul Position Forms will be used during fish sampling and will record survey and station names, date, time, location (GPS), station depth, sample depth, and length of tow. During POM sampling, the Plankton Survey Form will take the place of the Haul Position Form. The Plankton Survey Form includes multiple rows to record the time, location (GPS), station depth, and sample depth for each tow of a POM sample (multiple tows/sample).

Specimen Forms and Tissue Resection Logs will be used in the laboratory during fish compositing. Specimen Forms record data on fish morphometrics (FishID, length, weight, sex, otolith removal), while Tissue Resection Logs keep track of which fish (FishID) are included in each composite, as well as the total weight of tissue per composite. Specimen forms will also be used to record the species ID and body length of all krill in voucher samples. Specimen ID and relative abundance of phytoplankton in the POM samples will be recorded on an Excel spreadsheet.

Harbor seal data management
Data will be collected on field data sheets and during sample processing in the laboratory (Appendix D). Data will be received from the various laboratories in Excel spreadsheet format and will be summarized into a single workbook with data in multiple worksheets. As part of DFO national scientific data management policy, all data will be backed up in its original form on network servers. DFO's report writing contributors will include Dr. Peter S. Ross and Marie Noel, MSc.

Data Quality (Usability) Assessment

The principal investigators will review and verify analytical data to determine whether the data are valid by comparing results with other similar studies, evaluating expectations relative to known correlations of analytes with biological metrics, and checking for exceedances of reasonable, expected bounds (*e.g.*, decimal errors). Questionable or outlying data will be scrutinized by checking the entire stream of data, including looking for errors in biological metrics, assignments of location, and unusual circumstances documented in field notes. Additionally, analytical laboratory staff may be contacted to check errors in data processing, transcription, and interpretation.

Method detection limits will be reported for each sample/analyte for non-detects, and non-detected results will be censored with appropriate qualifiers. Other censoring qualifiers may be applied.

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Appendices

Appendix A. Glossary, Acronyms, and Abbreviations

Bioaccumulate: The buildup of contaminants in an organism's tissues (usually fatty tissue) through ingestion, or contact with the skin or respiratory tissue. Contaminants that bioaccumulate may also biomagnify in the food web, resulting in higher tissue concentrations in predators relative to ambient environmental concentrations.
Holoplankton: Organisms which are planktonic for their entire life cycle, such as krill or copepods.
Macrozooplankton: Animals in the water which drift with the currents and are large enough to be visible, usually between 2 to 20 mm in length.
Meroplankton: organisms which are planktonic for only a part of their life cycles, usually the larval stage, such as crab megalopae.
Microzooplankton: Animals in the water which drift with the currents and range in size from 20 to 200 microns (µm).
Plankton: Passively floating animal and plant life in the water that drifts with the currents.
Persistent Organic Pollutant: Organic compounds resistant to degradation that persist in the environment, are capable of long-range transport, and often bioaccumulate in living tissue.
Stormwater: The portion of precipitation that does not naturally percolate into the ground or evaporate but instead runs off roads, pavement, and roofs during rainfall or snow melt. Stormwater can also come from hard or saturated grass surfaces such as lawns, pastures, playfields, and from gravel roads and parking lots.
Particulate Organic Matter: Material of plant or animal origin that is suspended in water.
Toxicant: A toxic agent (chemical compound or mixture) that presents a risk of death, disease, injury, or birth defects in organisms that ingest or absorb it. Toxicants are typically introduced into the environment by human activity.

Acronyms and Abbreviations

C	carbon
cDNA	complementary deoxyribonucleic acid
DEPC-dH_20	diethyl pyrocarbonate-treated distilled, deionized water
DNA	deoxyribonucleic acid
Ecology	Washington State Department of Ecology
EIM	Environmental Information Management database
ELISA	enzyme linked immunosorbent assay
EPA	U.S. Environmental Protection Agency
FL	fork length
GC/MS	gas chromatography/mass spectrometry
GPS	global positioning system
HPLC	high performance liquid chromatography
HRGC-HRMS	high resolution gas chromatography and high resolution mass spectrometry
HRP	horseradish peroxidase
IOS	Institute of Ocean Sciences, Sidney, British Columba
KOH	potassium hydroxide

LEACA	Laboratory of Excellence in Aquatic chemical Analysis, Institute of Ocean Sciences, Sidney, British Columbia
MMP	Marine Mammal Program
MZ	macrozooplankton
N	nitrogen
NOAA	National Oceanic & Atmospheric Administration
PAH	polycyclic aromatic hydrocarbon
PBDE	polybrominated diphenyl ether
PBT	persistent, bioaccumulative, and toxic substance
PCB	polychlorinated biphenyl
PCDD	polychlorinated dibenzo-p-dioxin
PCDF	polychlorinated dibenzofuran
POM	particulate organic matter
POP	persistent organic pollutant
PSAMP	Puget Sound Assessment and Monitoring Program
QA	quality assurance
QC	quality control
QPCR	quantitative polymerase chain reaction
RNA	ribonucleic acid
SRM	standard reference materials
TH	thyroid hormone
TMB	3,3',5,5'- tetramethylbenzidine
WDFW	Washington Department of Fish and Wildlife

Units of Measurement

cm	centimeter
ft	feet
gm	gram
km	kilometer
kHz	kilohertz
mL	milliliters
mm	millimeters
ng/g	nanograms per gram (parts per billion)
oz	ounces
°C	degrees centigrade
µm	micrometer
$^{0}/_{00}$	permille (parts per thousand)

Appendix B. Haul Position Form for fish sample collections.

HAUL POSITION

SURVEY ID: _____ **DATE:** _____ **VESSEL:** _____

STATION ID: _____ **HAUL:** _____ **A B C D E** **GIS NO.** _____

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

GPS START POSITION

LATITUDE:										
					.					

LONGITUDE:										
						.				

GPS END POSITION

LATITUDE:										
					.					

LONGITUDE:										
						.				

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

TIME: **Start tow:**_____ **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

Appendix C. Plankton Survey Form for macrozooplankton and POM sample collections.

PLANKTON SURVEY Page ___ of ___

SURVEY ID: _____ STATION ID: _____ EFFORT ID: _____ EFFORT DATE: _____ GEAR TYPE: _____

START POSITION		END POSITION	
LATITUDE	LONGITUDE	LATITUDE	LONGITUDE

	Bottom Depth (m) (ft)	Acoustic Layer Depth (m) (ft)	Time	WIRE Out (m) (ft)	WIRE Angle	VESSEL Speed (k)	VESSEL RPM	Estimated Gear Depth (m) (ft)	COMMENTS
1									
2									
3									
4									
5									
6									
7									
8									
9									
10									
11									
12									
13									
14									
15									
16									
17									
18									

PSAMP 7/09

Appendix D. Harbor seal field data sheet.

Sample collection sheet: harbour seal capture 2009

Date: _____ Capture time: _____ Release time: _____

Seal #: _____

Location: _____ Site: _____

Capture method: _____ Capture crew_____

Sex:_____ Length: _____ Girth: _____

Body weight (gross): _____-net_____ = _____ (kg or lbs)

Blood sample taken at _____ Number of pokes: ____

Number of vacutainer tubes: Green ____ Green/Black ____ Red ____ Red/Black ____

Age estimate: _____ Umbilicus: _____

Skin/blubber biopsy (yes/no): _____

Number/size of biopsies: 8mm _____ 6mm _____ 3.5mm _____

Anesthetic: _____ Vocalization: _____

Presence/absence of mother: _____

Condition (eyes, teeth, flippers): _____

_____

Comments: _____

_____

Name of note taker: _____ Name of blood sampler: _____

	Plasma	Serum
Blood processing time-start		
Time in liquid Nitrogen		
Centrifuge speed		
Centrifuge duration		
Lipemic? (yes or no)		
Degree of lipemia (0-100)		
Colour		
Obtained volume		
Comment		