Quality Assurance Project Plan Elements

Assessment of nearshore mussel (*Mytilus* spp.) tissue contamination for regional stormwater monitoring in Washington State

Jennifer A. Lanksbury, James E. West
Washington Department of Fish and Wildlife
Puget Sound Assessment and Monitoring Program
600 Capitol Way N
Olympia, WA 98501

james.west@dfw.wa.gov
v1 360.902.2842
v2 206.302.2427
fax 360.902.2944
http://wdfw.wa.gov/conservation/research/projects/marine_toxics/index.html

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Abstract

This report was commissioned by the Stormwater Work Group (SWG) to gather elements of a Quality Assurance Project Plan (QAPP) for monitoring toxic contaminants in sessile bivalve mollusks such as blue mussels (*Mytilus* spp). The purpose of the information presented here is to support development of a program to monitor the status and trends of toxic contaminants in Puget Sound's nearshore marine biota. We present elements of a sampling plan to establish a baseline for assessing the status and trends of chemical contamination of mussels (*Mytilus* spp) in the marine nearshore waters of the Puget Sound, Hood Canal, Strait of Juan de Fuca, San Juan Islands and the Strait of Georgia. Such a program will support management decisions regarding control of pollution sources in Puget Sound, as well as provide a context for future, additional studies designed to measure the effectiveness of pollution control efforts.

Because the program has not been funded or established yet, these elements should be viewed as support for design of a future plan. In fulfillment of our Interagency Agreement with Ecology, we have submitted previous reports to the SWG evaluating (a) the statistical power of a sampling design comparing contamination of mussels from shorelines bounded by Urban Growth Areas (UGA) with non-UGA shorelines, (b) the potential distribution and availability of blue mussels along Puget Sound shorelines based on a desktop survey of existing mussel data and high resolution shoreline photos, and (c) recommended revisions to program hypotheses proposed by the SWG.

In this report we recommend establishing a network of mussel monitoring stations across a range of urban marine nearshore environments within the Puget Sound, Hood Canal, San Juan Islands, and Straits of Juan de Fuca and Georgia to characterize the chemical contamination in biota. We suggest using coverage of upland impervious surfaces as a tractable, quantifiable proxy for "urbanization", assigning shorelines one of three classes; low, medium and high. Sampling effort would be distributed across these three strata based on the extent of each stratum. In addition, priority would be given to locations where other organisms or sediments are being monitored for toxic chemicals. Coordinating with other, similar mussel-monitoring efforts will provide an additional key to setting up a successful network of mussel monitoring stations in the greater Puget Sound.

The sampling design recommended in this report describes the first cut of an effort aimed at establishing long-term status and trend monitoring sites. Such a first cut would provide a quantitative evaluation of the geographic extent of contamination of mussels, and the magnitude of their contamination. Moreover, a first cut, broad-scale evaluation would facilitate re-distribution, or fine-tuning of locations for future monitoring, to maximize efficiency of limited sampling funds.

The QAPP elements presented in this report also include a comprehensive set of standard operating procedures for field sampling mussels, originally developed by NOAA's long term national Mussel Watch program. These elements are organized and recast in Ecology's QAPP format, to facilitate use of these elements in a future QAPP.

Introduction

Increasing human population growth and its associated lowland and coastal development have brought about major changes in land use and a decline in the biological integrity of streams, rivers, lakes and marine waters in the Puget Sound Basin. Expanding urban land cover, including impervious surfaces, has been linked to increases in toxic stormwater runoff, which has been identified as one of the most important threats to the biological integrity of the Puget Sound marine environment. Washington's Stormwater Work Group (SWG) has recommended a regional stormwater monitoring program that includes status and trends monitoring in the marine nearshore. The recommended approach included contaminant sampling using mussels (*Mytilus* spp.), which have been demonstrated as a useful sentinel species for inputs of toxic contaminants to nearshore waters.

Mussels (*Mytilus* spp.) are permanently attached, filter-feeding organisms that concentrate pollutants from seawater (Farrington 1983). Many contaminants that they absorb undergo minimal metabolic transformation and the concentrations in their tissues change in response to nearby environmental levels, making them good indicators of recent local contamination (Roesijadi et al., 1984). Since 1986 the National Mussel Watch project, managed under the National Oceanic and Atmospheric Administration (NOAA), has collected and analyzed mussels to characterize the spatial extent and temporal trends in coastal pollution nationwide (Kimbrough et al., 2008; Kimbrough et al. 2009). Scientists and resource managers in Washington place a high value on the utility of Mussel Watch for large-spatial-scale contaminant assessments, but also recognize the need to adapt the program to better answer smaller scale, regional questions (Lanksbury et al., 2010).

In addition to 26 stations regularly sampled by NOAA Mussel Watch in Washington State, two regional entities also monitor nearshore contamination using mussels. The US Navy partnered with the US Environmental Protection Agency (EPA), Washington Department of Ecology and other regional stakeholders, to sample mussels and other bivalves in Sinclair and Dyes Inlets (Bremerton area) in a program called ENVironmental inVESTment (ENVVEST). In addition, Snohomish County's Marine Resources Committee (SCMRC) partnered with Mussel Watch to add more mussel sampling stations to the Snohomish County shoreline, allowing for finer-scale evaluation of contaminants in county waters and monitoring for the effectiveness of cleanup strategies.

During the winter of 2009/10 a team made up of multiple agencies/groups (Washington Department of Fish and Wildlife, the Puget Sound Partnership, SCMRC, Snohomish County Public Works-Surface Water Management, Washington Sea Grant, and NOAA's Mussel Watch) led field-sampling for Mussel Watch in Washington and demonstrated the use of citizen science volunteers as a primary resource for conducting field work. Local volunteer groups were trained in sample collection protocols and successfully sampled mussels from 14 sites, significantly reducing the amount of time professional staff were needed in the field, and also contributing valuable local knowledge and natural history.

Project Description

One of the ultimate goals of monitoring mussels is to track contaminants conveyed via stormwater to Puget Sound receiving waters by monitoring contaminant loads in nearshore resident organisms that integrate water-column contaminant conditions over a short period of time (several months). Blue mussels (*Mytilus* spp.) and similar bivalve mollusk species, such as oysters, are effective organisms for tracking nearshore water quality, and have been used for decades for that purpose in many areas across the United States. A network of mussel monitoring sites in Puget Sound will improve our understanding of stormwater runoff-related issues in both urban growth areas (UGAs) and undeveloped shorelines.

The study design proposed below addresses the following questions:

1. What is the geographic extent and magnitude (tissue concentration) of chemical contamination in mussels across a gradient of land-use in UGAs?
2. What land-use characteristics (factors) are most highly correlated with contamination?
3. Can the relative abundance of contaminants (*i.e.*, fingerprint patterns) in mussels be used to help identify sources of contamination?
4. How are these conditions changing through time?

A study design addressing these questions applies limited resources to establish mussel stations systematically across the full range of nearshore land-use conditions, from highly urbanized to relatively undeveloped (Figure 1). Its aim would be to select mussel locations that represent as wide a range of stormwater-influenced conditions across UGA and non-UGA shorelines as possible, allowing for exploration and identification of stormwater-mussel contaminant patterns and adjustment of sample locations in future years.

In order to maximize compatibility with NOAA Mussel Watch and other sampling efforts, mussel sampling frequency and timing will follow the NOAA protocols: one sampling event at each of the mussel watch sites would occur every two years (i.e. biennial sampling). The next NOAA Mussel Watch sampling event for Washington is scheduled to occur during the winter of 2011/12. Subsequent biennial sampling would occur over the winters of 2013/14, 2015/16, 2017/18, etc.

Mussels would be collected from December-February during their reproductively quiescent winter months (prior to their spawning season), which reduces contaminant variability associated with rapidly changing reproductive condition during summer months. Additional studies could be commissioned to investigate seasonal variation in contaminant exposure. Winter sampling occurs during periods of high stormwater runoff, a few months after the antecedent summer dry period and the first-flush rain events of the fall. Accumulation of contaminants during this period may represent a worst-case scenario.

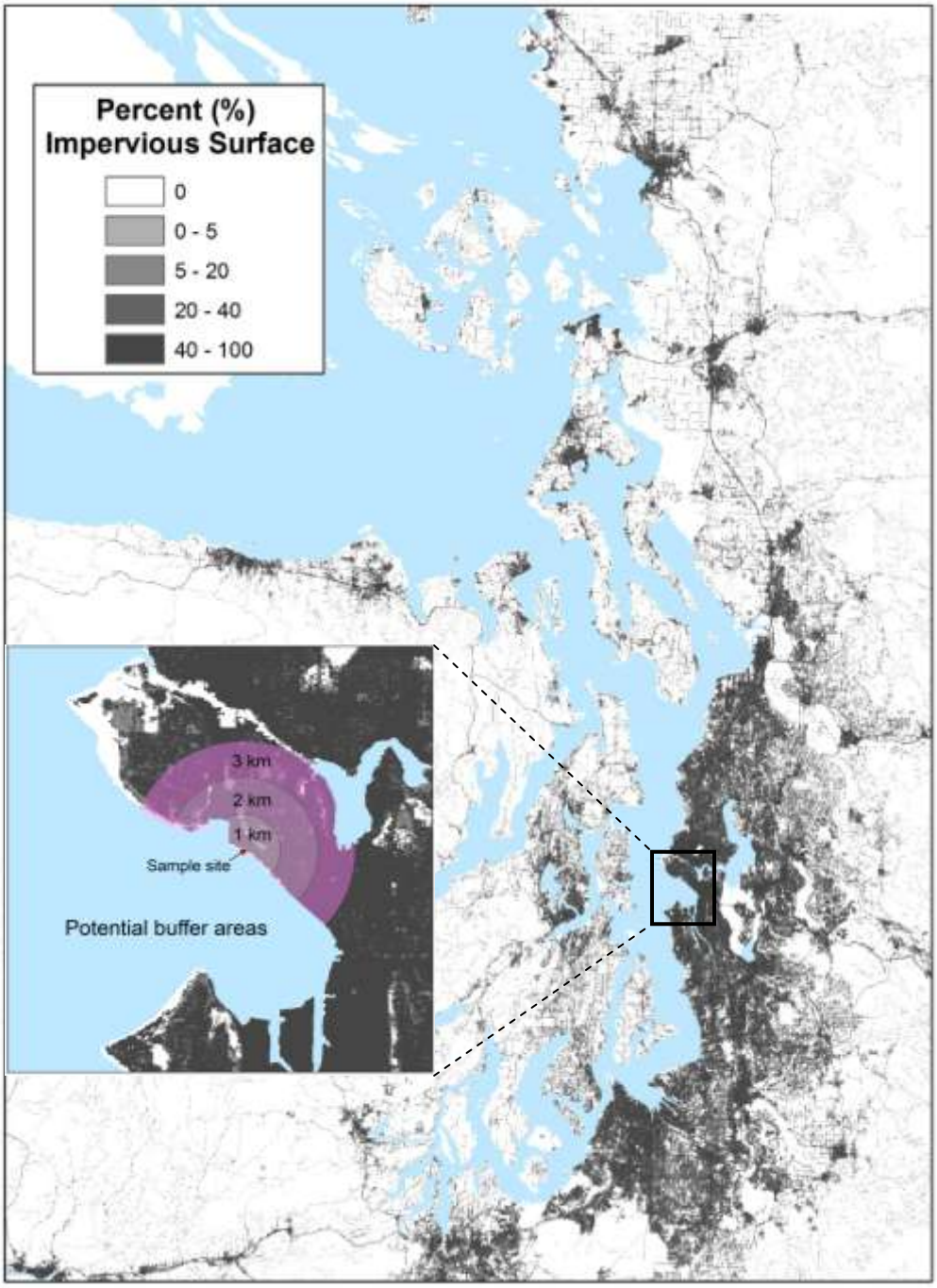

Figure 1. Overview: US Geological Survey Land Cover data (2006) showing ranges of upland impervious surface in the Puget Sound, Strait of Juan de Fuca and Strait of Georgia. Inlay: Buffer areas around a potential mussel monitoring site in Elliott Bay.

Objectives

The objectives of this sampling plan are to establish a baseline to assess the status and trends of chemical contamination of mussels (*Mytilus* spp) in the urban marine nearshore waters of the Puget Sound, Hood Canal, Strait of Juan de Fuca, San Juan Islands and the Strait of Georgia, to support stormwater management decisions in these areas. The expectation is that findings from mussel monitoring will be used in conjunction with regional stormwater effectiveness monitoring and source identification to determine the impact of urban stormwater management strategies and actions. This sampling plan is designed to answer the following questions:

1. **What is the geographic extent and magnitude (tissue concentration) of chemical contamination in mussels across a gradient of land-use in UGAs?**

The aim of this effort is to gain some knowledge of existing contamination in mussels across the full range of shoreline conditions within the UGAs of the study area. This requires a careful, systematic selection of sampling sites based on pre-existing knowledge of contamination in other organisms or sediments and an evaluation of suspected contaminant inputs from the adjacent upland area. Sites will be selected to represent as wide a range of upland land-use conditions as possible - from highly urbanized to undeveloped - and distributed across UGAs. Results from this effort will provide a context for evaluating the scope of contamination and for the following questions.

2. **What land-use characteristics (factors) are most highly correlated with contamination**?

We will develop a systematic list of potential contamination factors related to nearby land-use that can be used to classify or characterize each sampling site ("site type"). Statistical power for detecting differences between sites will be optimized by using contamination factors to stratify the allocation of sample sites, and to explain variance in statistical tests (e.g., ANOVA). Predictive relationships between site type and mussel contamination will be developed when feasible. This method will be used to explain variability in contaminant loads and compare locations regardless of their UGA status, based on adjacent land-use type, and to identify contaminant patterns that can inform refinement of study design.

Additional explanatory covariates such as nearshore current patterns, rainfall, and stormwater discharge can be applied in *post hoc* analyses, as data become available, to inform the development of more specific testable hypotheses. This adaptive, flexible approach increases overall understanding of these unknown relationships allowing the evolution of a better status and trends monitoring design, but also, importantly, informs the design and potential feasibility of future effectiveness monitoring studies using mussels or other shellfish.

3. **Can the relative abundance of contaminants (*i.e.*, fingerprint patterns) in mussels be used to help identify sources of contamination?**

Potential contaminant sources will be evaluated forensically by comparing the relative abundance of diagnostic chemicals in mussels with source constituents. This will be accomplished using simple ratios or multivariate dimensional scaling analyses.

4. **How are these conditions changing through time?**

Understanding temporal trends and patterns in contaminants will be achieved though long-term monitoring. Once a clearer grasp of the extent and magnitude of contaminants and their patterns is achieved, *index sites* may be selected to track trends on a greater frequency than at other locations.

Sampling Process Design (Experimental Design)

A network of monitoring stations will be established to represent a variety of urban marine nearshore environments within the Puget Sound, Hood Canal, San Juan Islands, and Straits of Juan de Fuca and Georgia to characterize the effects of regional scale chemical contamination on biota. Mussel sites will be selected using a three-step process, described below.

As a first cut, sites would be distributed across the Puget Sound shoreline based on degree of impervious surface coverage of adjacent uplands, with sample sizes allotted to a minimum of three impervious surface classes (high, medium, and low), and sample density related to relative abundance of the three classes. In addition to impervious surface, the utility of other land-use data such local roads (traffic density) and commercial property types (which have been linked to high stormwater runoff-related morality of spawning coho salmon in the Puget Sound -- Feist et al. 2011), may be evaluated.

Although locations of many stormwater outfalls have been mapped, key characteristics relating to their volume, type and timing of discharge, and drainage area are lacking. In many cases the pathway of stormwater conveyance pipes is unknown. Because of these uncertainties, position of outfalls will not be used on a large scale to locate mussel monitoring sites. Sampling sites may be co-located with particular outfalls to answer separate questions focused on such outfalls.

Mussel sampling sites will be chosen to represent a wide range of inland impervious surface conditions and capture the full spatial extent of both large, contiguous UGAs and smaller, isolated UGAs. Non-UGA reference sites will also be selected to provide background/baseline levels for comparison. The density of sample sites in each stratum will be determined by the relative contribution of the stratum to the overall shoreline distance. Additionally, priority will be given to locations where other stormwater monitoring sites are being sampled for sediment chemistry or where other long-term biota monitoring sites exist. The existing NOAA Mussel Watch, ENVVEST and SCMRC sites located in Puget Sound UGAs will be classified and used in this sampling scheme as well (Figure 2).

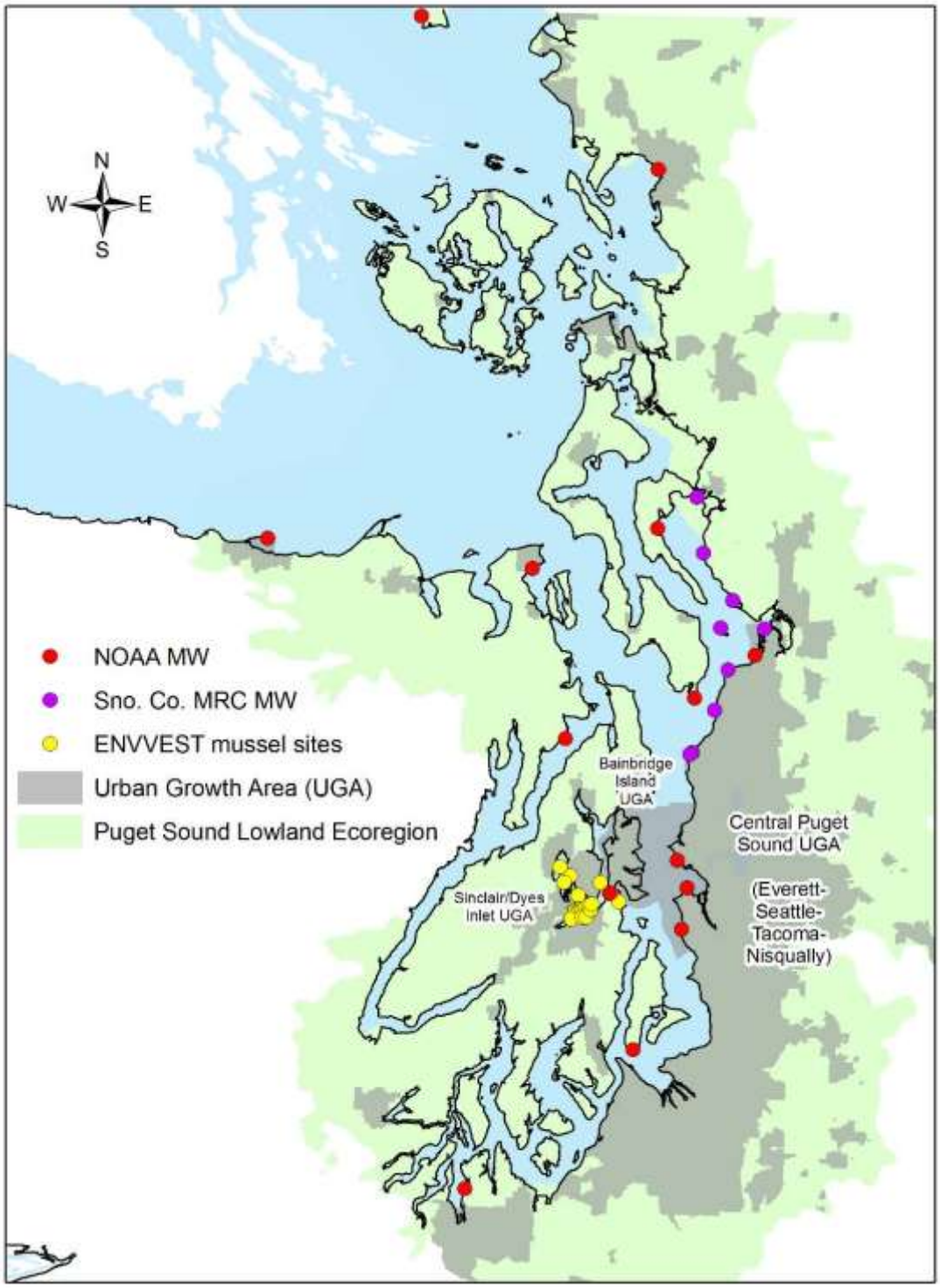

Figure 2. Puget Sound region urban growth areas (UGAs) and location of current NOAA Mussel Watch (MW), Snohomish County Marine Resources Committee (SCMRC), and ENVVEST program mussel sampling locations.

Details of Mussel Sample Site Selection Process:

The site selection process described below assumes a budget for sampling 50 sites, based on feedback we received during SWG meetings. Fifty sites are insufficient to fully characterize the geographic extent of Puget Sound for the purposes of a nearshore toxics status and trends monitoring program. Based on the statistical power analyses previously submitted to the SWG and best professional judgment, roughly 100 sites would be needed for a "first cut" evaluation of contaminant conditions. Evaluation of first cut data may indicate the need for more or fewer sampling sites.

The selection process is weighted towards urban areas, but retains a small number of sites to characterize background conditions, and to capture urbanization of rural areas.

Step1: *Large UGAs* - Select multiple sites within the central Puget Sound UGA (n=12~17, Figure 2) and along the shores of the Bainbridge Island UGA (n = 2~4), to provide representative, systematic coverage of their relatively large contiguous shorelines. Sites in the central Puget Sound UGA already sampled by the NOAA Mussel Watch project or SCMRC will be incorporated into the site list, to provide historical context for analyses. Similarly, sampling will be coordinated with existing ENVVEST mussel contaminant monitoring in the Sinclair/Dyes Inlet UGA. Among the remaining, unrepresented areas of the central Puget Sound UGA and the entire Bainbridge Island UGA, sites will be selected to represent a range of impervious surface conditions, capture the full spatial extent of the UGA, and, where possible, co-locate sites near other SWG study locations and long-term PSAMP biota contaminant monitoring stations.

Smaller, isolated UGAs - Because the smaller, isolated UGAs are numerous (27 total) and funding is limited, we propose sampling some to most (total n = 13~24, Figure 2), but not all of them. We will group the isolated UGA shorelines based on shared oceanographic basins or sub-basins and then characterize the UGAs within each group by their relative proximity to one another and their likelihood of connectedness, based on nearshore drift patterns. The number of mussel watch sites within each group will reflect several factors, including the number of UGAs within that group. As with the larger UGAs, sites will be selected to represent a range of impervious surface conditions, ensure outlying regions are represented, to capture the extent of contamination within the entire study area, and co-locate sites, where possible, near long-term biota contaminant monitoring stations. Isolated UGAs that are already sampled by the NOAA Mussel Watch project (i.e. Port Angeles, Port Townsend, and Bellingham) will be evaluated to determine whether those sites adequately represent the surrounding UGA conditions.

Non-UGA reference sites – A few (n = 3~5, Figure 2) undeveloped reference sites will be chosen in non-UGA areas. Although NOAA Mussel Watch samples in some non-UGA areas that can be used as reference locations, several additional reference sites may be selected in basins where an urban to reference comparison is also desirable (i.e. San Juan Islands, north Whidbey Island, south and/or central Puget Sound). Reference sites are important in monitoring studies because they provide data on natural/baseline conditions and can help in the identification of potential contaminant sources.

Step 2: This step involves using 2006 U.S. Geological Survey (USGS) Land Cover data (Fry et al. 2011) to characterize each sampling site by the associated land-use within a 1-3 km radius (buffer) of the site center, limiting the buffer extent to local watershed boundaries where appropriate (Figure 1). Sites would be placed into one of the following four strata, based on percent (%) impervious surface coverage of the associated upland buffer:

- High
- Moderate
- Low
- Reference

The validity of initial assumptions and decisions related to these coverage thresholds and buffer areas will be evaluated and adjusted accordingly.

Step 3: After site characterization, the number of sites within each land-use category will be evaluated. If representation is not balanced, either more sites will be added or sites will be relocated to provide for the most balance sampling design achievable and enhance power for statistical analysis.

Initial sample sites will be selected in this manner, but ground-truthing will be required to verify presence of mussels at each site. If mussels are not available, we will select alternate sites based on the three-step process outlined above.

Once a first cut evaluation has been conducted, sample site placement will be re-evaluated, and recommendations made for implementing future monitoring. A fully implemented monitoring plan would be conducted on a semi-annual basis to establish trends associated with stormwater management strategies and actions. The sampling will be coordinated with ongoing local (Ecology, Washington Department of Health (DOH), Snohomish County, ENVVESST) and national (NOAA Mussel Watch) sampling projects being conducted within Washington State. Any variations necessary to meet the specific goals and objectives of each survey will be described in a biennial QA Project Plan addendum, generated prior to each sampling event.

Sampling Procedures

Overview

Bivalves collected for this study will typically include blue mussels; *Mytilus galloprovincialis/trossullus* and *M. californianus* (Figure 3). Mussel populations will be sampled during their reproductively quiescent period (prior to spawning) during winter months, to avoid variability in contaminant tissue residues related to reproduction.

Figure 3. *Mytilus galloprovincialis/trossullus* (top) and *Mytilus californianus* (bottom). Photo courtesy of NOAA Mussel Watch Project, unpublished report.

Briefly, at each sampling location (site) live mussels will be collected at three replicate locations (stations) using a sampling protocol adapted from the NOAA Mussel Watch Project (see below). The mussels will be collected by cutting their byssus threads from the substrate, brushing off sediment or other debris on the outside of their shells, rinsing them in a bucket of water obtained locally on site, and placing them into labeled Ziploc bags. Depending on the size of mussels available, between 210 - 660 individual mussels will be collected in total at each MW site. The mussels will be shipped live, on ice, to two separate laboratories contracted with the NOAA Mussel Watch Project. Analyses at these labs will include determination of 1) chemical residues in the soft tissues, and 2) reproductive state (gonadal index).

Sampling Protocol

Initial site assessment - upon arrival at the sampling site the following information will be determined and recorded on data sheets:

1. Latitude and longitude at a central location, around which sampling will occur, using a hand-held global positioning system (GPS) device, which will be set to datum NAD 1983.
   - NOAA Mussel Watch sites have established site centers with specific coordinates.
2. Date, time of arrival, weather conditions, mussel collectors and data recorder
3. Site description, conditions, noting any sources of contamination (i.e. creosote pilings, visible oil/petroleum, outfalls – including size, catch basin, filters -, metals, garbage etc.), and any additional observations, notes or comments
4. Photograph of the site center

Establishing sampling stations - three (3) distinct replicate stations for mussel collection will be established around, or to either side, of the site center (Figure 4):

1. The site center can serve as Station #1 if mussels are available there.
2. Stations will be spaced between 25 - 250 meters (82 - 820 feet) from one another, if possible.
3. If no mussels are found near the site center then the search for mussels will proceed up to 800 meters (~ 3000 feet or ½ mile) from the site center in either direction, as long as the habitat remains consistent:

- IMPORTANT: the search for mussels will stop if habitat characteristics change significantly from those present at the site center. Sampling will not proceed onto substantially different substrates or environments.

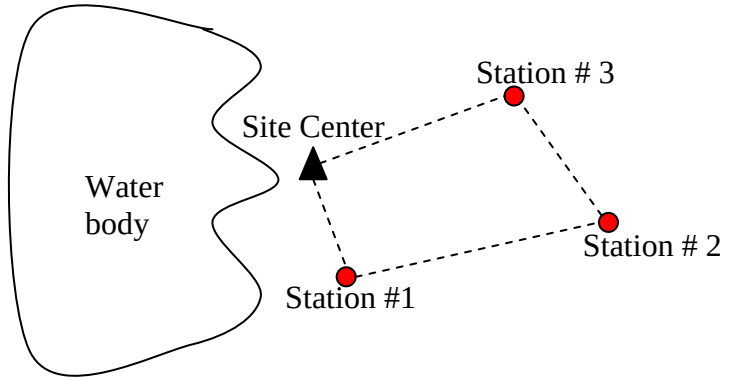

Figure 4. Example of mussel sampling (replicate stations) around a site center.

4. If it is not possible to delimit three separate stations (i.e., not enough mussels) then collection will be spread out linearly along the shoreline (Figure 5):
    a. This option will only be chosen if absolutely necessary
    b. The change in sampling technique will be clearly noted on the data sheet
    c. Latitude and longitude of starting and ending points of the line sampled will be noted
    d. Mussels will still be separated into the three (3) "stations" based on relative spatial distance, to avoid sampling a single non-representative clump, by filling bags as mussels are collected along the shoreline (Figure 5)

Figure 5. Example of mussel sampling (replicate transects) along the shoreline.

<u>Station description</u> - at each replicate station the following information will be recorded:

1. GPS coordinates and mussel collection start time
2. Water temperature and salinity, sampled at approximately one (1) foot water depth
3. A description of the station, including location relative to the site center or other landscape features, and the type of intertidal habitat in that area
4. Description of substrate to which mussels are attached (e.g., boulder, cement, pilings, sand, cobble, etc.)
5. Height where mussels will be collected, relative to the height of seawater at the time of collection, and the highest overall distribution of mussels available, even if none are collected there (Figure 6)
6. Photos of each station, its surroundings, and the substrate will also be taken

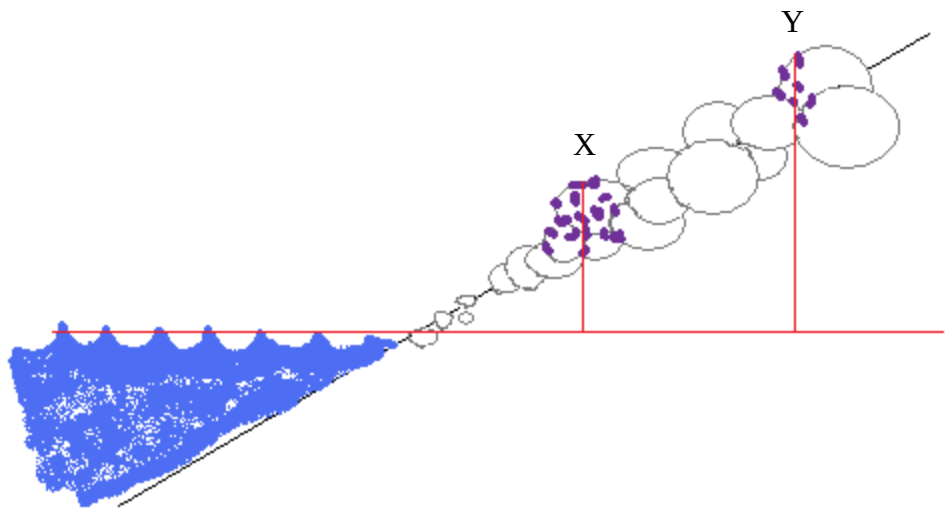

Figure 6. Example of a mussel collection station: X = height of collection (where you are sampling), Y = highest distribution of mussels (further up the shore).

Collecting mussels -

1. Disposable laboratory gloves (Nitrile or latex) will always be worn when handling mussels, collection bags and labels/tags
2. Mussel will be collected by cutting their byssal threads (not torn off substrate), brushing off any sediment on the shells and rinsing them in a bucket of marine water collected at the waterline nearest the station
    - Buckets of seawater will be changed between each station
2. Mussels will NOT be collected directly off creosote-treated wood
3. At each **station** two (2) sets of mussels will be collected in pre-labeled Ziploc bags for – *1*.) chemical residue analysis and *2*.) to determine their reproductive state:
    a. For **chemical residue analysis between 50 – 200 mussels** will be collected, depending on size, into pre-labeled, gallon sized Ziplock bags
    b. For determination of **reproductive state 20 mussels**, of average size for that station, will be collected into pre-labeled, quart sized Ziploc bags
4. All Ziploc bags will be labeled on the outside (permanent marking pen) and inside (waterproof paper tag) with the following information:
    - Program name
    - Site name and acronym
    - Date
    - Station number
    - Chemical Analysis *OR* Reproductive Assessment
5. All mussels will be double-bagged (to prevent ice melt leakage from contacting the mussels) and immediately placed into a cooler(s) on ice
6. At the end of sampling at one site (three replicate stations) there will be three (3) bags of mussels for chemical analysis and three (3) bags of mussels for assessment of reproductive state = 6 bags total.
7. After sampling is complete, the leave time will be recorded on the data sheet

Packaging and shipment of samples -

Assuming separate labs will be used for the chemical residue analysis and the assessment of reproductive state, the bags of mussels from each site will be split into two separate coolers accordingly for shipment. Since the labs may be in far away States with warm climates, plastic (not Styrofoam) coolers will be use for shipping and the coolers will be well packed with ice and thoroughly sealed. Coolers will be shipped via FedEx Priority Overnight as soon as possible, to arrive the next business day, though shipping to arrive on a weekend will be avoided. If sampling occurs on a Friday or Saturday, the mussels will be held on ice until Monday morning for shipping. If necessary, samples may be placed in a refrigerator on ice overnight(s), though samples will not be frozen (i.e. mussels must arrive alive). Mussels packaging details are below.

1) A layer of bagged ice will be placed into the bottom of the cooler.

2) The double-bagged mussel samples will be placed on top of the ice layer.

3) Bags of mussels and ice will be alternated and the voids filled with ice. A *copy* of the datasheet and the *original* Chain-of-Custody form will be placed into a Ziplock bag at the top, *inside* of the cooler.

4) A FedEx packing label will be attached to the top of the cooler. Nylon fiber tape will secure the sides of the label and seal the cooler (yellow arrow). Tape will wrap around the lip of the cooler (to seal in coldness) as well as around its width.

Figure 7. Details of packing and shipping techniques for mussels to be sent for laboratory analyses.

Sample ID

Unique Sample identification codes will be developed for each of the unique sample sites to be included in this mussel monitoring program.

Chain-of-custody
Chain-of-custody forms will be utilized for handling and shipment of all MW site samples.

Field logs and data sheets
A bound, waterproof field log notebook will be maintained during the duration of the project to record observations and experiences. Information included in the field log will include:

- Name and location of project
- Field personnel
- Sequence of events
- Any changes to plan
- Environmental conditions
- Date, time, location, ID, and description of each sample
- Instrument calibration procedures
- Field measurement results
- Identity of QC samples
- Unusual circumstances that may affect interpretation of results

In addition, *Mussel Watch Program Data Sheets* (Appendix B) will be completed for each MW site and kept in a bound notebook at PSAMP headquarters. Data recorded at each MW site will include:

- Site name and code
- Date, time, location (latitude/longitude), datum, accuracy
- GPS Make/Model
- Weather
- Collectors and recorder
- Water temperature and salinity
- Tidal information (tide height, time of low tide)
- Station (replicate) description, site conditions, sampling substrate
- Height of collection and highest distribution of mussels
- Other notes/comments

Measurement Procedures

Field Measurements
Field measurements will include GPS coordinates (datum NAD 1983) recording at the site center and at each station (i.e. replicate sampling location). In addition, water temperature (alcohol thermometer) and salinity (refractometer) will be recorded at each station, so that three replicate measurements of each parameter will be made for every mussel sampling site (see *Mussel Watch Program Data Sheets* in Appendix B). To address the potential for sensitivity, field instruments will be checked and calibrated before measurements are taken in the field. Instructions on how to use and calibrate a refractometer are described in Appendix D. Instructions on how to check a mercury or alcohol thermometer are detailed in Appendix E.

Laboratory Measurements

We expect that the measurement of chemical contaminants and evaluation of reproductive stage of mussels will shift from the NOAA Mussel Watch project's laboratories, located outside of Washington State, to local laboratories. It is assumed this switch will result in cost-savings for future tissue analyses, though this assumption will need to be verified through cost estimates from any new laboratory considered. If and when this switch in analytical labs occurs, a one-time lab comparison (i.e. duplicate samples sent to the old and new labs) will be necessary to allow for comparison of future results with historic NOAA Mussel Watch data.

The MW program contracts with B&B Laboratories, an affiliate of TDI-Brooks International, located in College Station, Texas, for analyzing organic compounds and major and trace elements in mussel tissue. A list of B&B Laboratories' Standard Operating Procedures (SOP's) can be found at http://www.tdi-bi.com/analytical_services/sop_main.html. Rutgers' Haskin Shellfish Research Laboratory, located in Port Norris, NJ, assesses gonadal index and histopathology of mussels for MW. Although these laboratories are not accredited, they have a long history of participation in NOAA's Mussel Watch program.

The MW program uses a performance-based system approach to obtain the best possible data quality and comparability, and requires laboratories to demonstrate precision, accuracy, and sensitivity to ensure results-based performance goals and measures (Kimbrough et al. 2008). Mussel Watch contracted laboratories, analytical methods, matrices, list of analytes, number of samples, MDLs, sample preparation methods, and expected range of results are all described in NOAA documents available at online. McDonald, et al. (2006) describe methods for determination of dry weight and percent lipids in mussels.

A more detailed description of the analytical methods employed by the NOAA Mussel Watch project can be found at NOAA's Coastal Ocean Assessments, Status and Trends (COAST), Mussel Watch Contaminant Monitoring - Methods Documents website. The procedures outlined below describe the basic laboratory techniques utilized by the NOAA Mussel Watch Project for analyses of the core organic contaminants, major and trace elements, and reproductive stage (state of gonadal development) of mussels. A list of the analytes evaluated by the Mussel Watch project is detailed in Appendix C.

Core organic contaminants

The laboratory methods required for analyzing organic compounds in mussel tissue can be found in Kimbrough, et al. (2006). In summary, to determine the organic contaminant levels in mussels, analytes are extracted, isolated, and concentrated from the soft tissues. The tissue extracts require extensive purification to remove lipids from the matrix, which cause analytical interferences. Shell length and volume are determined for all mussels collected at each sampling site. The mussels are then shucked and homogenized and aliquots of the homogenized samples are chemically dried using Hydromatrix® and extracted in dichloromethane using a Dionex Accelerated Solvent Extractor. The extracts are then purified using alumina/silica gel chromatography columns. Further purification of the eluant is achieved using a gel permeation column coupled to a high performance liquid chromatograph. The volume of the resultant eluant is then reduced and analyzed for aromatic and chlorinated hydrocarbons and polybrominated flame retardants by gas chromatography.

Major and trace elements
Kimbrough and Lauenstein (2006) describe the analytical methods used to determine major and trace elements in mussel tissue. In summary, sample preparation to allow the accurate and precise determination of major and trace elements in mussel tissue emphasizes homogenization and total digestion steps that minimize contamination. Analysis methods utilized include inductively coupled plasma - mass spectrometry, inductively coupled plasma - optical emission spectrometry, hydride generation - atomic fluorescence spectrometry, and cold vapor - atomic absorption spectrometry (Kimbrough and Lauenstein 2006). The atomic spectroscopy techniques include a full suite of quality assurance and quality control samples, with an emphasis on certified reference materials, in order to produce reliable data. These methods allow measurement of both background and elevated concentrations in mussel tissue samples.

Reproductive stage
Kim et al. (2006) describe the histological techniques used for assessment of gonadal index and histopathology in MW. In summary, determination of reproductive stage for mussels is based on a histological evaluation of the maturation stages of the gonads, most of which are located in the mantle (Kim et al. 2006). The histological approach uses a semi-quantitative numerical assignment to rank the reproductive stage of five (5) specimens chosen from each site. The mussels are first preserved whole, in shell their shells, for one week. After fixation the anterior-posterior length of each mussel is measured using a ruler, then the soft tissue is carefully removed from the shell and a 5-mm thick, dorsal-ventral cross-section slice is taken. Tissue slices are embedded in paraffin, sectioned, and stained using a pentachrome staining protocol. Stained sections are examined under a compound microscope, and sex and the state of gonadal development is determined.

ENVVEST Laboratories
The ENVVEST program, which analyzes contaminants in mussels from Sinclair and Dyes Inlets, utilizes Battelle Marine Sciences Laboratory (BMSL) in Sequim, WA for chemical analysis of organics and trace metals (Robert Johnston, pers. comm.). Analyses of conventional parameters and nutrients are performed by Columbia Analytical Services (CAS) in Kelso, WA, while pesticide and herbicide analysis is performed by Ecology's Manchester Environmental Laboratory (MEL) in Port Orchard, WA.

PSAMP Biota
The Puget Sound Assessment and Monitoring Program's (PSAMP) Toxics in Biota unit utilizes NOAA's Northwest Fisheries Science Center in Seattle, WA for chemical analysis of organics in fish and invertebrate tissues. They utilized the King County Environmental Laboratory in Seattle, WA for trace metals analysis. A list of the analytes evaluated by the PSAMP Toxics in Biota is detailed in Appendix C.

PSAMP Sediments uses
The PSAMP Sediments unit utilizes Ecology's Manchester Environmental Laboratory (MEL) in Port Orchard, WA for chemical analysis of sediment samples. A list of the analytes evaluated by the PSAMP Sediments is detailed in Appendix C.

Quality Control Procedures

Field

Field personnel will be trained in the sampling methods specified in this QAPP. All samplers will wear Nitrile or latex gloves while handling mussels and all mussels will be brushed off and rinsed on site, in local marine water, before being placed in Ziploc bags for collection. All completed sample labels, chain-of-custody forms, and field logs will be double-checked by members of the field crew after sample collection.

Field instruments will be checked and calibrated to ensure accuracy and minimize bias before measurements are recorded at any site. Instrument check and calibration procedures for the refractometer (salinity) and thermometer (temperature) are listed in Appendices E and F, respectively. In addition, field salinity and temperature measurements will be assessed at every station (replicate sampling location); thus three (3) replicate measurements of each parameter will be made for every Mussel Watch site (see *Mussel Watch Program Data Sheets* in Appendix B).

Hand-held GPS units (Garmin, GPSmap 76C) available to PSAMP staff report coordinates to the nearest 0.00001 decimal degrees (1.11 m/3.64 ft). GPS coordinates for each station (replicate) represent the *central point* of a collection area; mussels are collected from a number of rocks/boulders/etc. *around* the station center. In addition, stations (replicates) are to be located a distance of 25 – 250 meters (82 – 820 feet) from one another, whenever possible. Given these parameters, a GPS accuracy of 0.00001 decimal degrees (1.11 m/3.64 ft) will provide adequate representation of the physical location of collected mussels.

Backup GPS units (same make and model) will be available in the field should the unit currently in use fail. Additional calibrated and checked refractometers and thermometers will also be available for backup in case one of those instruments fails or is broken in the field.

Laboratories

The NOAA Mussel Watch program uses a performance-based system approach to obtain the best possible data quality and comparability, and requires laboratories to demonstrate precision, accuracy, and sensitivity to ensure results-based performance goals and measures (Kimbrough et al. 2008). Mussel Watch contracted laboratories, analytical methods, matrices, list of analytes, number of samples, MDLs, sample preparation methods, and expected range of results are all described in NOAA documents available at online. McDonald, et al. (2006) describe methods for determination of dry weight and percent lipids in mussels.

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Haskin Shellfish Research Laboratory, located in Port Norris, NJ, assesses gonadal index and histopathology of mussels for MW.

Data Management Procedures

Field Data and Observations

Field data and observations will be recorded on *Mussel Watch Program Data Sheets* (Appendix B), which will be printed on waterproof paper. A new data sheet will be completed at every site, including those that are rejected. Original copies of these data sheets will be kept by PSAMP staff in Washington and paper copies will be sent to the participating laboratories with mussel shipments. Digital photos taken at each MW site will be stored in PSAMP staff data files dedicated to Washington State MW data.

EIM data upload procedures

When WDFW receives the final, verified and validated data from NOAA, the PM will coordinate with Ecology staff to ensure they will be entered into EIM. Maximum turnaround time for chemical analysis of data generated from the National MW program is typically approximately one year from time of submission. Any data from newly contracted laboratories in Washington State will also undergo verification and validation with the PM before being entered into the EIM database as well.

Reports

Upon project completion, WDFW staff will prepare a brief summary report, which shall include, at a minimum: a description of the work completed, the status and completion date for the project activities, and future recommendations. The report will summarize the basic project accomplishments and identify key lessons related to planning, design, execution and evaluation.

Data Verification

Data Verification

Measurements recorded in field logs will be reviewed by the Project Manager. The PM will determine if instruments were properly calibrated, if field measurements meet the MQOs for precision and bias.

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. Mussel Sampling Equipment/Supply List

Due to the timing of low tides during the winter season, mussel sampling in the nearshore intertidal zone occurs at night. Sampling Supply List for ONE SITE:

Site Access Materials

- Directions to Site Center and Contacts list
- GPS unit
- Flashlights and/or headlamps
- Propane lantern(s), propane, and matches (useful, but optional)
- Cell phone(s)

Mussel Sampling Materials

- 1 to 3 plastic containers or buckets (for washing mussels)
- 1 to 3 small coolers/ buckets with ice (to carry mussels while sampling)
- 3 scrub brushes
- 3 knives (or more, depending on number of samplers)
- Small/medium/large disposable laboratory gloves (Nitrile or latex)
- Glove liners or knit gloves (worn under laboratory gloves to keep hands warm)

Mussel Bagging Materials – note all samples are DOUBLE-BAGGED (for shipping)

- 7 – gallon-sized Ziploc bags:
- 3 – quart-sized Ziploc bags:
- 6 bag labels (1 for each chemistry and histology bag)
- 1 garbage bag

Water Quality Measurement Devices

- Refractometer + small amount of distilled water
- Thermometer

Documentation and Recording Materials

- Digital camera
- Clipboard
- Sharpies

Appendix B. Sample Data Sheet

MUSSEL WATCH PROGRAM DATA SHEET
(WASHINGTON STATE)

Version 4 – 2009

Site: _____ Site Code: _____

Date: _____ Time Arrive: _____ Time Leave: _____

Latitude: _____ Longitude: _____ Accuracy (±): _____

Weather: _____

Mussel Collectors: _____

Data Recorder: _____ GPS Make/Model (Datum NAD83): _____

SITE WATER PARAMETERS

Tidal Station: _____

Time of Low Tide: _____ Height of Low Tide (MLLW): _____ ft. ☐ m. ☐

STATION DESCRIPTIONS

STATION 1

Latitude: _____ Longitude: _____ Accuracy (±): _____ Start Time: _____

Water Temp. (°C): _____ Salinity (ppt): _____

Station Description: _____

Substrate: _____ Height of Collection: _____ ft. ☐ m. ☐

Highest Distribution of Mussels *(compared to water level at time of collection)*: _____

STATION 2

Latitude: _____ Longitude: _____ Accuracy (±): _____ Start Time: _____

Water Temp. (°C): _____ Salinity (ppt): _____

Station Description: _____

Substrate: _____ Height of Collection: _____ ft. ☐ m. ☐

Highest Distribution of Mussels *(compared to water level at time of collection)*: _____

STATION 3

Latitude: _____ Longitude: _____ Accuracy (±): _____ Start Time: _____

Water Temp. (°C): _____ Salinity (ppt): _____

Station Description: _____

Substrate: _____ Height of Collection: _____ ft. ☐ m. ☐

Highest Distribution of Mussels *(compared to water level at time of collection)*: _____

Check Boxes for Site Conditions:

✓	Condition	Description
	Creosote	
	Oil on water	
	Oil on beach	
	Garbage	
	Other	

Observations and General Notes (i.e. interesting or unusual conditions, information, comments, etc):

Appendix C. Analytes evaluated in three toxics monitoring programs in Washington State

Class	Compound	CAS	IUPAC	MW Project	PSAMP Sediments	PSAMP Biota	Mussel Watch Method	Sediments Methods	Biota Methods
Polycyclic Aromatic Hydrocarbons (PAHs) (Low Molecular Weight)	1,6,7-Trimethylnaphthalene	2245-38-7		X	X		GC/MS-SIM	GC/MS†	
	1-Methylnaphthalene	30-12-0		X	X		GC/MS-SIM	GC/MS†	
	1-Methylphenanthrene	832-69-9		X	X		GC/MS-SIM	GC/MS†	
	2,6-Dimethylnaphthalene	581-42-0		X	X		GC/MS-SIM	GC/MS†	
	2-Methylnaphthalene	91-57-6		X	X		GC/MS-SIM	GC/MS†	
	2-Methylphenanthrene	2531-84-2			X			GC/MS†	
	Acenaphthene	83-32-9		X	X		GC/MS-SIM	GC/MS†	
	Acenaphthylene	208-96-8		X	X		GC/MS-SIM	GC/MS†	
	Anthracene	120-12-7		X	X		GC/MS-SIM	GC/MS†	
	Biphenyl	92-52-4		X	X		GC/MS-SIM	GC/MS†	
	Dibenzothiophene	132-65-0		X	X		GC/MS-SIM	GC/MS†	
	Fluorene	86-73-7		X	X		GC/MS-SIM	GC/MS†	
	Naphthalene	91-20-3		X	X	X (metabolite)	GC/MS-SIM	GC/MS†	GC/ECD
	Phenanthrene	85-01-08		X	X	X (metabolite)	GC/MS-SIM	GC/MS†	GC/ECD
	Retene	483-65-8			X			GC/MS†	
PAHs (High Molecular Weight)	Benz(*a*)anthracene	56-55-3		X	X		GC/MS-SIM	GC/MS†	
	Benzo(*a*)pyrene	50-32-8		X	X	X (metabolite)	GC/MS-SIM	GC/MS†	GC/ECD
	Benzo(*b*)fluoranthene	205-99-2		X	X		GC/MS-SIM	GC/MS†	
	Benzo(*e*)pyrene	192-97-2		X	X		GC/MS-SIM	GC/MS†	
	Benzo(*ghi*)perylene	191-24-2		X	X		GC/MS-SIM	GC/MS†	
	Benzo(*k*)fluoranthene	207-08-9		X	X		GC/MS-SIM	GC/MS†	
	Chrysene	218-01-9		X	X		GC/MS-SIM	GC/MS†	
	Dibenzo(*ah*)anthracene	53-70-3		X	X		GC/MS-SIM	GC/MS†	
	Fluoranthene	206-44-0		X	X		GC/MS-SIM	GC/MS†	

Class	Compound	CAS	IUPAC	MW Project	PSAMP Sediments	PSAMP Biota	Mussel Watch Method	Sediments Methods	Biota Methods
PAHs (High)	Indeno(1,2,3-*cd*)pyrene	193-39-5		X	X		GC/MS-SIM	GC/MS†	
	Perylene	198-55-0		X	X		GC/MS-SIM	GC/MS†	
	Pyrene	129-00-0		X	X		GC/MS-SIM	GC/MS†	
Substituted PAHs	C1-Dibenzothiophenes			X			GC/MS-SIM		
	C2-Dibenzothiophenes			X			GC/MS-SIM		
	C3-Dibenzothiophenes			X			GC/MS-SIM		
	C1-Fluorenes			X			GC/MS-SIM		
	C2-Fluorenes			X			GC/MS-SIM		
	C3-Fluorenes			X			GC/MS-SIM		
	C1-Naphthalenes			X			GC/MS-SIM		
	C2-Naphthalenes			X			GC/MS-SIM		
	C3-Naphthalenes			X			GC/MS-SIM		
	C4-Naphthalenes			X			GC/MS-SIM		
Substituted PAHs	C1-Phenanthrenes + anthracene			X			GC/MS-SIM		
	C2-Phenanthrenes + anthracene			X			GC/MS-SIM		
	C3-Phenanthrenes + anthracene			X			GC/MS-SIM		
	C4-Phenanthrenes + anthracene			X			GC/MS-SIM		
	C1-Fluoranthene + pyrenes			X			GC/MS-SIM		
	C1-Chrysenes			X			GC/MS-SIM		
	C2-Chrysenes			X			GC/MS-SIM		
	C3-Chrysenes			X			GC/MS-SIM		
	C4-Chrysenes			X			GC/MS-SIM		
Chlorinated Pesticides	Aldrin	309-00-2		X	X	X	GC/ECD	GC-DDC/ECD	GC/ECD
	alpha-HCH	319-84-6				X			GC/ECD
	beta-HCH	319-85-7				X			GC/ECD
	cis-Chlordane (alpha-Chlordane)	5103-71-9		X	X	X	GC/ECD	GC-DDC/ECD	GC/ECD

Class	Compound	CAS	IUPAC	MW Project	PSAMP Sediments	PSAMP Biota	Mussel Watch Method	Sediments Methods	Biota Methods
Chlorinated Pesticides	2,4'-DDD	5319-0		X	X	X	GC/ECD	GC-DDC/ECD	GC/ECD
	2,4'-DDE	3424-82-6		X	X	X	GC/ECD	GC-DDC/ECD	GC/ECD
	2,4'-DDT	58633-27-5		X	X	X	GC/ECD	GC-DDC/ECD	GC/ECD
	4,4'-DDD	72-54-8		X	X	X	GC/ECD	GC-DDC/ECD	GC/ECD
	4,4'-DDE	72-55-9		X	X	X	GC/ECD	GC-DDC/ECD	GC/ECD
	4,4'-DDT	50-29-3		X	X	X	GC/ECD	GC-DDC/ECD	GC/ECD
	delta-HCH	58-89-9				X			GC/ECD
	Dieldrin	60-57-1		X	X	X	GC/ECD	GC-DDC/ECD	GC/ECD
	Endosulfan I (alpha Endosulfan)	959-98-8			X	X		GC-DDC/ECD	GC/ECD
	Endosulfan II (beta Endosulfan)	33213-65-9			X	X		GC-DDC/ECD	GC/ECD
	Endosulfan Sulfate	1031-07-8			X	X		GC-DDC/ECD	GC/ECD
	Endrin	72-20-8		X	X		GC/ECD	GC-DDC/ECD	
	Endrin Aldehyde	7421-93-4			X			GC-DDC/ECD	
	Endrin Ketone	53494-70-5			X			GC-DDC/ECD	
	Heptachlor	76-44-8		X	X	X	GC/ECD	GC-DDC/ECD	GC/ECD
	Heptachlor epoxide	1024-57-4		X	X	X	GC/ECD	GC-DDC/ECD	GC/ECD
	Hexachlorobenzene (HCB)	118-74-1		X	X	X	GC/ECD	GC-DDC/ECD	GC/ECD
	gamma-HCH (gamma-BHC, Lindane)	58-89-9		X	X	X	GC/ECD	GC-DDC/ECD	GC/ECD
	Methoxychlor	72-43-5				X			GC/ECD

Class	Compound	CAS	IUPAC	MW Project	PSAMP Sediments	PSAMP Biota	Mussel Watch Method	Sediments Methods	Biota Methods
Chlorinated Pesticides	Mirex	2385-85-5		X	X	X	GC/ECD	GC-DDC/ECD	GC/ECD
	Nonachlor III					X			GC/ECD
	Oxychlordane	27304-13-8			X	X		GC-DDC/ECD	GC/ECD
	Toxaphene	8001-35-2			X	X		GC-DDC/ECD	GC/ECD
	trans-Chlordane (gamma-Chlordane)	5103-74-2			X			GC-DDC/ECD	
	trans-Nonachlor	39765-80-5		X		X	GC/ECD		GC/ECD
Butyltins	Monobutyltin trichloride	1118-46-3		X			GC/FPD		
	Dibutyltin dichloride	683-18-1		X			GC/FPD		
	Tributyltin chloride	1461-22-9		X			GC/FPD		
	Tetrabutyltin	1461-25-2		X			GC/FPD		
Metals & Metalloids	Aluminum (Al)	7429-90-5		X			ICP-MS and/or ICP-OES		
	Antimony (Sb)	7440-36-0		X			ICP-MS		
	Arsenic (As)	7440-38-2		X	X	X	ICP-OES and/or HG-AFS	ICP-MS	IC/APS
	Cadmium (Cd)	7440-43-9		X	X		ICP-MS	ICP-MS	
	Chromium (Cr)	7440-47-3		X	X		ICP-MS and/or ICP-OES	ICP-MS	
	Copper (Cu)	7440-50-8		X	X	X	ICP-OES	ICP-MS	IC/APS
	Iron (Fe)	7439-89-6		X			ICP-OES		
	Lead (Pb)	7439-92-1		X	X	X	ICP-MS	ICP-MS	GFAA
	Manganese (Mn)	7439-96-5		X			ICP-OES		
	Mercury (Hg)	7439-97-6		X	X	X	CVAA	CVAA	CVAA
	Nickel (Ni)	7440-02-0		X	X		ICP-MS and/or ICP-OES	ICP-MS	
	Selenium (Se)	7782-49-2		X	X		HG-AFS	ICP-MS	
	Silicon (Si)	7440-21-3		X			ICP?		
	Silver (Ag)	7440-22-4		X	X		ICP-MS and/or ICP-OES	ICP-MS	

Class	Compound	CAS	IUPAC	MW Project	PSAMP Sediments	PSAMP Biota	Mussel Watch Method	Sediments Methods	Biota Methods
Metals	Thallium (Tl)	7440-28-0		X			ICP-MS?		
	Tin (Sn)	7440-31-5		X	X		ICP-MS	ICP-MS	
	Zinc (Zn)	7440-66-6		X	X		ICP-OES	ICP-MS	
Polychlorinated biphenyls (PCBs)	Aroclor 1016	12674-11-2			X			GC-DDC/ECD	
	Aroclor 1221	11104-28-2			X			GC-DDC/ECD	
	Aroclor 1232	11141-16-5			X			GC-DDC/ECD	
	Aroclor 1242	53469-21-9			X			GC-DDC/ECD	
	Aroclor 1248	12672-29-6			X			GC-DDC/ECD	
	Aroclor 1254	11097-69-1			X			GC-DDC/ECD	
	Aroclor 1260	11096-82-5			X			GC-DDC/ECD	
	Aroclor 1262	37324-23-5			X			GC-DDC/ECD	
	Aroclor 1268				X			GC-DDC/ECD	
	2,4'-Dichlorobiphenyl	34883-43-7	8	X	X		GC/ECD	GC-DDC/ECD	
	2,2',4-Trichlorobiphenyl	37680-66-3	17			X			ECD
	2,2',5-Trichlorobiphenyl	37680-65-2	18	X	X	X	GC/ECD	GC-DDC/ECD	ECD
	2,4,4'-Trichlorobiphenyl	7012-37-5	28	X	X	X	GC/ECD	GC-DDC/ECD	ECD
	2,4',5-Trichlorobiphenyl	16606-02-3	31			X			ECD
	2',3,4-Tetrachlorobiphenyl	38444-86-9	33			X			ECD
	2,2',3,5'-Tetrachlorobiphenyl	41464-39-5	44	X	X	X	GC/ECD	GC-DDC/ECD	ECD
	2,2',4,5'-Tetrachlorobiphenyl	41464-40-8	49			X			ECD

Class	Compound	CAS	IUPAC	MW Project	PSAMP Sediments	PSAMP Biota	Mussel Watch Method	Sediments Methods	Biota Methods
Polychlorinated biphenyls (PCBs)	2,2',5,5'-Tetrachlorobiphenyl	35693-99-3	52	X	X	X	GC/ECD	GC-DDC/ECD	ECD
	2,3',4,4'-Tetrachlorobiphenyl	32598-10-0	66	X	X	X	GC/ECD	GC-DDC/ECD	ECD
	2,5,3',4'-Tetrachlorobiphenyl	32598-11-1	70			X			ECD
	2,4,4',5-Tetrachlorobiphenyl	32690-93-0	74			X			ECD
	3,3',4,4'-Tetrachlorobiphenyl	32598-13-3 (38380-03-9)	77 (110)	X	X		GC/ECD	GC-DDC/ECD	
	2,2',3,3',4-Pentachlorobiphenyl	52663-62-4	82			X			ECD
	2,5,2',3',4'-Pentachlorobiphenyl	38380-02-8	87			X			ECD
	2,5,2',3',6'-Pentachlorobiphenyl	38379-99-6	95			X			ECD
	2,2',4,4',5-Pentachlorobiphenyl	38380-01-7	99			X			ECD
	2,2',4,5,5'-Pentachlorobiphenyl	37680-73-2	101	X	X	X	GC/ECD	GC-DDC/ECD	ECD
	2,3,3',4,4'-Pentachlorobiphenyl	32598-14-4	105	X	X	X	GC/ECD	GC-DDC/ECD	ECD
	2,3,3',4',6-Pentachlorobiphenyl	38380-03-9	110			X			ECD
	2,3',4,4',5-Pentachlorobiphenyl	31508-00-6	118	X	X	X	GC/ECD	GC-DDC/ECD	ECD
	3,3',4,4',5-Pentachlorobiphenyl	57465-28-8	126	X	X		GC/ECD	GC-DDC/ECD	
	2,2',3,3',4,4'-Hexachlorobiphenyl	38380-07-3	128	X	X	X	GC/ECD	GC-DDC/ECD	ECD
	2,2',3,4,4',5'-Hexachlorobiphenyl	35065-28-2	138	X	X	X	GC/ECD	GC-DDC/ECD	ECD
	2,2',3',4,5,6'-Hexachlorobiphenyl	38380-04-0	149			X			ECD
	2,3,5,6,2',5'-Hexachlorobiphenyl	52663-63-5	151			X			ECD
	2,2',4,4',5,5'-Hexachlorobiphenyl	35065-27-1	153	X	X	X	GC/ECD	GC-DDC/ECD	ECD
	2,3,3',4,4',5-Hexachlorobiphenyl	38380-08-4	156			X			ECD
	2,3,3',4,4',6-Hexachlorobiphenyl	74472-42-7	158			X			ECD
	3,3',4,4',5,5'-Hexachlorobiphenyl	32774-16-6	169		X			GC-DDC/ECD	

Class	Compound	CAS	IUPAC	MW Project	PSAMP Sediments	PSAMP Biota	Mussel Watch Method	Sediments Methods	Biota Methods
Polychlorinated biphenyls (PCBs)	2,2',3,3',4,4',5-Heptachlorobiphenyl	35065-30-6	170	X	X	X	GC/ECD	GC-DDC/ECD	ECD
	2,2',3,3',4,4',6-Heptachlorobiphenyl	52663-71-5	171			X			ECD
	2,2',3,3',4',5,6-Heptachlorobiphenyl	52663-70-4	177			X			ECD
	2,2',3,4,4',5,5'-Heptachlorobiphenyl	36065-29-3	180	X	X	X	GC/ECD	GC-DDC/ECD	ECD
	2,2',3,4,4',5',6-Heptachlorobiphenyl	52663-69-1	183			X			ECD
	2,2',3,4',5,5',6-Heptachlorobiphenyl	52663-68-0	187	X	X	X	GC/ECD	GC-DDC/ECD	ECD
	2,3,3',4,4',5',6-Heptachlorobiphenyl	74472-50-7	191			X			ECD
	2,2',3,3',4,4',5,5'-Octachorobiphenyl	35694-08-7	194			X			ECD
	2,2',3,3',4,4',5,6-Octachorobiphenyl	52663-78-2	195	X	X	X	GC/ECD	GC-DDC/ECD	ECD
	2,2',3,3',4',5,5',6-Octachorobiphenyl	52663-75-9	199			X			ECD
	2,3,3',4,4',5,5',6-Octachorobiphenyl	74472-53-0	205			X			ECD
	2,2',3,3',4,4',5,5',6-Nonachlorobiphenyl	40186-72-9	206	X	X	X	GC/ECD	GC-DDC/ECD	ECD
	2,2',3,3',4,5,5',6,6'-Nonachlorobiphenyl	52663-77-1	208			X			ECD
	2,2',3,3',4,4',5,5',6,6'-Decachlorobiphenyl	2051-24-3	209	X	X	X	GC/ECD	GC-DDC/ECD	ECD
Polybrominated diphenyl ethers (PBDEs)	2-Monobrominated diphenyl ether	7025-06-1	1	X*			GC/ECD		
	3-Monobrominated diphenyl ether	6876-00-2	2	X*			GC/ECD		
	4-Monobrominated diphenyl ether	101-55-3	3	X*			GC/ECD		
	2,4-Dibrominated diphenyl ether		7	X*			GC/ECD		
	2,4'-Dibrominated diphenyl ether	147217-71-8	8	X*			GC/ECD		
	2,6-Dibrominated diphenyl ether		10	X*			GC/ECD		
	3,3'-Dibrominated diphenyl ether	6903-63-5	11	X*			GC/ECD		
	3,4-Dibrominated diphenyl ether		12	X*			GC/ECD		
	3,4'-Dibrominated diphenyl ether	83694-71-7	13	X*			GC/ECD		
	4,4'-Dibrominated diphenyl ether	2050-47-7	15	X*			GC/ECD		
	2,2',4-Tribrominated diphenyl ether	147217-75-2	17	X*			GC/ECD		
	2,3',4-Tribrominated diphenyl ether		25	X*			GC/ECD		

Class	Compound	CAS	IUPAC	MW Project	PSAMP Sediments	PSAMP Biota	Mussel Watch Method	Sediments Methods	Biota Methods
Polybrominated diphenyl ethers (PBDEs)	2,4,4'-Tribrominated diphenyl ether	41318-75-6	28	X*		X	GC/ECD		GC/MS
	2,4,6-Tribrominated diphenyl ether		30	X*			GC/ECD		
	2,4',6-Tribrominated diphenyl ether		32	X*			GC/ECD		
	2',3,4-Tribrominated diphenyl ether		33	X*			GC/ECD		
	3,3',4-Tribrominated diphenyl ether		35	X*			GC/ECD		
	3,4,4'-Tribrominated diphenyl ether		37	X*			GC/ECD		
	2,2',4,4'-Tetrabrominated diphenyl ether	5436-43-1	47	X*	X	X	GC/ECD	Capillary GC/MS-SIM	GC/MS
	2,2',4,5'-Tetrabrominated diphenyl ether		49	X*	X	X	GC/ECD	Capillary GC/MS-SIM	GC/MS
	2,3',4,4'-Tetrabrominated diphenyl ether		66	X*	X	X	GC/ECD	Capillary GC/MS-SIM	GC/MS
	2,3′,4′,6-Tetrabrominated diphenyl ether	189084-62-6	71		X			Capillary GC/MS-SIM	
	2,4,4',6-Tetrabrominated diphenyl ether		75	X*			GC/ECD		
	3,3',4,4'-Tetrabrominated diphenyl ether	93703-48-1	77	X*			GC/ECD		
	2,2',3,4,4'-Pentabrominated diphenyl ether	182346-21-0	85	X*		X	GC/ECD		GC/MS
	2,2',4,4',5-Pentabrominated diphenyl ether	60348-60-9	99	X*	X	X	GC/ECD	Capillary GC/MS-SIM	GC/MS
	2,2',4,4',6-Pentabrominated diphenyl ether	189084-64-8	100	X*	X	X	GC/ECD	Capillary GC/MS-SIM	GC/MS
	2,3,4,5,6-Pentabrominated diphenyl ether		116	X*			GC/ECD		
	2,3',4,4',5-Pentabrominated diphenyl ether		118	X*			GC/ECD		
	2,3',4,4',6-Pentabrominated diphenyl ether		119	X*			GC/ECD		
	3,3',4,4',5-Pentabrominated diphenyl ether		126	X*			GC/ECD		
	2,2',3,4,4',5'-Hexabrominated diphenyl ether		138	X*	X		GC/ECD	Capillary GC/MS-SIM	

Class	Compound	CAS	IUPAC	MW Project	PSAMP Sediments	PSAMP Biota	Mussel Watch Method	Sediments Methods	Biota Methods
Polybrominated diphenyl ethers (PBDEs)	2,2',4,4',5,5'-Hexabrominated diphenyl ether	68631-49-2	153	X*	X	X	GC/ECD	Capillary GC/MS-SIM	GC/MS
	2,2',4,4',5,6'-Hexabrominated diphenyl ether		154	X*	X	X	GC/ECD	Capillary GC/MS-SIM	GC/MS
	2,2',4,4',6,6'-Hexabrominated diphenyl ether		155	X*			GC/ECD		
	2,3,4,4',5,6-Hexabrominated diphenyl ether		166	X*			GC/ECD		
	2,2',3,4,4',5,6-Heptabrominated diphenyl ether		181	X*			GC/ECD		
	2,2',3,4,4',5',6-Heptabrominated diphenyl ether	207122-16-5	183	X*	X	X	GC/ECD	Capillary GC/MS-SIM	GC/MS
	2,2',3,4,4',6,6'-Heptabrominated diphenyl ether		184		X			Capillary GC/MS-SIM	
	2,3,3',4,4',5,6-Heptabrominated diphenyl ether	68928-80-3	190	X*			GC/ECD		
	2,3,3',4,4',5',6'-Heptabrominated diphenyl ether		191		X			Cap-GC/MS	
	Decabrominated diphenyl ether	1163-19-5	209		X			Cap-GC/MS	
Chlorinated Aromatic Chemicals	1,2,4-Trichlorobenzene	120-82-1			X	X		Cap-GC/MS	GC/MS
	1,2-Dichlorobenzene	95-50-1			X	X		Cap-GC/MS	GC/MS
	1,3-Dichlorobenzene	541-73-1			X	X		Cap-GC/MS	GC/MS
	1,4-Dichlorobenzene	106-46-7			X	X		Cap-GC/MS	GC/MS
	2-Chloronaphthalene	91-58-7			X			Cap-GC/MS	
Miscellaneous Extractable Chemicals	Benzoic Acid	65-85-0			X			Cap-GC/MS	
	Benzyl Alcohol	100-51-6			X			Cap-GC/MS	
	Beta-coprostanol				X			Cap-GC/MS	
	Carbazole	86-74-8			X			Cap-GC/MS	
	Cholesterol				X			Cap-GC/MS	
	Dibenzofuran	132-64-9			X			Cap-GC/MS	
	Hexachlorobutadiene	87-68-3			X			Cap-GC/MS	
	Isophorone	78-59-1			X			Cap-GC/MS	
	Pentachlorophenol	87-86-5			X			Cap-GC/MS	

Class	Compound	CAS	IUPAC	MW Project	PSAMP Sediments	PSAMP Biota	Mussel Watch Method	Sediments Methods	Biota Methods
Recently added (Sediments)	Bisphenol A	80-05-7			X			Cap-GC/MS	
	Tris(2-chloroethyl)Phosphate (Tcep)	115-96-8			X			Cap-GC/MS	
	Triclosan	3380-34-5			X			Cap-GC/MS	
	Triethyl citrate	77-93-0			X			Cap-GC/MS	
Organo-nitrogen	9(H)Carbazole	86-74-8			X			Cap-GC/MS	
	Caffeine	58-08-2			X			Cap-GC/MS	
	N-Nitrosodiphenylamine	86-30-6			X			Cap-GC/MS	
Phenols	2,4-Dimethylphenol	105-67-9			X			Cap-GC/MS	
	2-Methylphenol	95-48-7			X			Cap-GC/MS	
	4-Methylphenol	106-44-5			X			Cap-GC/MS	
	Phenol	108-95-2			X			Cap-GC/MS	
	Phenol, 4-Nonyl-	104-40-5			X			Cap-GC/MS	
Phthalate Esters	Bis(2-Ethylhexyl) Phthalate	117-81-7			X			Cap-GC/MS	
	Butylbenzylphthalate	85-68-7			X			Cap-GC/MS	
	Diethylphthalate	84-66-2			X			Cap-GC/MS	
	Dimethylphthalate	131-11-3			X			Cap-GC/MS	
	Di-N-Butylphthalate	84-74-2			X			Cap-GC/MS	
	Di-N-Octyl Phthalate	117-84-0			X			Cap-GC/MS	

* Not monitored biennially; measured in samples from 1996 and 2004-2007
† Manchester modification with capillary gas chromatograph/mass spectrometer in selected ion mode (GC/MS-SIM) isotopic dilution analysis
Cap-GC/MS - capillary gas chromatograph/mass spectrometer
CVAA - cold vapor atomic absorption spectrometry
GC-DDC/ECD - gas chromatograph-diethyldithiocarbamate/electron capture detector
GC/ECD - gas chromatograph/electron capture detector
GC/FPD - gas chromatograph/flame photometric detector
GC/MS - gas chromatograph/mass spectrometer
GC/MS-SIM - gas chromatograph/mass spectrometer in selected ion mode
GFAA - Graphite furnace atomic absorption spectrometry
HG-AFS - hydride generation atomic fluorescence spectrometry
IC/APS - inductively coupled argon plasma spectrophotometry
ICP-MS - inductively coupled plasma - mass spectrometry
ICP-OES - inductively coupled plasma - optical emission spectrometry
X (metabolite) -metabolite of the parent compound measured as fluorescing aromatic compound extracted from fish bile and used as biomarker indicating PAH exposure

Appendix D. Using and Calibrating a Salinity Refractometer

Model to be used in this study is ZGRS-10ATC, manufactured by Sino Science & Technology Co., Ltd.

Refractometer Parts

How to measure salinity with the refractometer; paraphrased from manufacturer's operation manual:

1. Verify that the refractometer has been calibrated by testing to see if distilled water reads as zero (0) - *see calibration instructions below*.

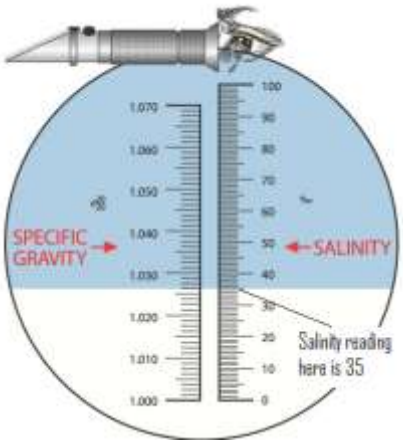
2. Open the cover plate, use a clean dropper from the case to place several drops of seawater* on the clean prism surface; gently close the cover plate and press lightly so seawater spreads across the entire surface of the prism without air bubbles or dry spots.

    - Obtain seawater from the middle of water column (not at the surface), in as deep water as your boots allow you to wade (i.e. 1 – 2 feet of water).

3. Allow the seawater to remain on the prism for approximately 30 seconds, keeping the refractometer level so as not to drain the seawater away.

4. Turn on the light switch to illuminate the prism and look into the eyepiece. Note on the *right side* of the scale where the white and blue boundary lies - this value is the SALINITY (‰, permille, ppt [parts per thousand], grams salt/kilogram solution).

    - Focus using the focus adjustment, just in front of the eyepiece.



5. After measurement, clean away the seawater on the surface of the prism and cover plate using a cloth or paper towel. Put it back into its container after it is dry and store in safe location.

How to calibrate the refractometer; paraphrased from manufacturer's operation manual:

1. Place distilled water in a sealed in a seawater bath to bring to approximately the same temperature as the seawater you will be measuring. This should take about 3-5 minutes.

2. Removed the distilled water vial from seawater bath and wipe outside of vial dry, so as not to contaminate with seawater.

3. Open refractometer cover plate, use dropper from case to place several drops of the distilled water onto the clean prism surface; gently close the cover plate and press lightly so water spreads across the entire surface of the prism without air bubbles or dry spots.

4. Allow the distilled water to remain on the prism for approximately 30 seconds, keeping the refractometer level so as not to drain the water away.

5. Turn on light switch to illuminate the prism; look into refractometer and find where the white and blue boundary lies (see illustration above).

    - Focus the scale using the focus adjustment near the eyepiece.

6. Use the small screwdriver in the refractometer case to adjust the *calibration screw under the prism* until the white and blue boundary is just on the zero (0) mark on the right side.

7. After calibration, clean away the distilled water on the surface of the prism and cover plate using a cloth or paper towel. You are now ready to take a salinity reading of seawater…see directions above.

Appendix E. Thermometer Accuracy Check: Ice Point Method

Method taken directly from Strouse et al. (2010):

"When ice and water are packed together into an insulated container, the mixture has a temperature of nearly 0 °C (32 °F). We call this mixture of ice and water the ice melting point.

The important steps in preparing an ice point are:

1. Use water that is distilled, de-ionized, or purified by reverse osmosis for both the water and the ice.

2. Be sure that the ice pieces are no bigger than a gumdrop - about 1 cm or 0.5 in.

3. Pack the insulated flask so that there is an ice-water mixture from top to bottom.

4. When inserting the thermometer, make sure that it is clean, that it is immersed at least 10 cm to 15 cm (approximately 4 in. to 6 in.) (if possible), and that the probe tip is at least 2 cm (approximately 1 inch) from the flask walls and about 5 cm (approximately 2 in.) from the bottom of the flask.

The test thermometer should read 0 °C (32 °F). Any difference from these values is the measured error."

Appendix F. Glossary, Acronyms, and Abbreviations

Glossary

Ambient: Background or away from point sources of contamination.

Analyte - An element, ion, compound, or chemical moiety (pH, alkalinity) which is to be determined.

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

Chain-of-Custody Form: documentation of custody and transfer of samples. After mussel collection, this form should be filled out and signed when the mussels change hands. The original Chain-of-Custody form should be included in the cooler when the mussels are sent to the labs for processing, as the receiving labs will be the last group to sign these forms.

Conductivity: A measure of water's ability to conduct an electrical current. Conductivity is related to the concentration and charge of dissolved ions in water.

Gonadal Index: a measure of sperm and egg development. This analysis is performed to determine whether mussels were in pre- or post-spawning (reproductive) state when they were collected. This determination is essential to ensure accurate interpretation of mussel contaminant results, as mussels "dump" contaminants into their sperm and eggs and are thus expected to have lower contaminant levels after spawning.

Height of Collection - height above water level (at time of collection) where mussels are actually collected. This measurement is made at each Station and may vary between Stations.

Highest Distribution of Mussels - height above water level (at time of collection) of the highest distribution of mussels at each Station. (Comparison of the above two values gives the National Mussel Watch project an estimate of where within the intertidal zone mussels were collected.)

Pollution: Contamination or other alteration of the physical, chemical, or biological properties of any waters of the state. This includes change in temperature, taste, color, turbidity, or odor of the waters. It also includes discharge of any liquid, gaseous, solid, radioactive, or other substance into any waters of the state. This definition assumes that these changes will, or is likely to, create a nuisance or render such waters harmful, detrimental, or injurious to (1) public health, safety, or welfare, or (2) domestic, commercial, industrial, agricultural, recreational, or other legitimate beneficial uses, or (3) livestock, wild animals, birds, fish, or other aquatic life.

Refractometer – an instrument used to measure the concentration or refractive index of liquids. It measures how much the speed of light is reduced when it passes through a liquid (in this case, seawater) and projects the result onto a salinity scale set to read in parts per thousand (‰, permille, ppt [parts per thousand], grams salt/kilogram solution).

Site Center - the designated site location around which sampling will occur.

Station - the specific location(s) of mussel collections at each site. Mussels are collected at three (3) stations near the site center. Stations will be spaced between 25 - 250 meters (82 - 820 feet) apart. Mussels are collected at three separate stations to spread out collections and avoid sampling a single, non-representative "clump" of mussels at any site.

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.

Watershed: A drainage area or basin in which all land and water areas drain or flow toward a central collector such as a stream, river, or lake at a lower elevation.

Acronyms and Abbreviations

Following are acronyms and abbreviations used frequently in this report.

ANOVA	Analysis of variance (a statistical test)
BMP	Best management practices
BMSL	Battelle Marine Sciences Laboratory
CAS	Columbia Analytical Services
COAST	NOAA's Coastal Ocean Assessments, Status and Trends program
DOC	Dissolved organic carbon
DOH	Washington Department of Health
e.g.	For example
Ecology	Washington State Department of Ecology
EIM	Environmental Information Management database
ENVVEST	Environmental Investment program
EPA	U.S. Environmental Protection Agency
et al.	And others
GIS	Geographic Information System software
GPS	Global Positioning System
i.e.	In other words
MEL	Manchester Environmental Laboratory
MQO	Measurement quality objective
NOAA	National Oceanic and Atmospheric Administration
PBDE	polybrominated diphenyl ethers
PBT	persistent, bioaccumulative, and toxic substance
PCB	polychlorinated biphenyls
PSAMP	Puget Sound Assessment and Monitoring Program
QA	Quality assurance
SCMRC	Snohomish County Marine Resources Committee
SRM	Standard reference materials
SWG	Stormwater Work Group

UGA	Urban growth area
USGS	U.S. Geological Survey
WAC	Washington Administrative Code
WDFW	Washington Department of Fish and Wildlife
WSTMP	Washington State Toxics Monitoring Program

Units of Measurement

°C	degrees Celsius
dw	dry weight
ft	feet
km	kilometer, a unit of length equal to 1,000 meters.
m	meter
mi	mile
ppt	permille, parts per thousand (‰), grams salt/kilogram solution
ww	wet weight