

Quality Assurance Project Plan

Toxic Contaminant Monitoring in Mussels: Phase 1

Publication ##, December 14, 2011

Prepared by

Jennifer Lanksbury and James West

Washington State Department of Fish and Wildlife
Marine and Nearshore Protection and Restoration Program

Prepared for and Funded by the
U.S. Environmental Protection Agency



Grant Number PC-00J29801-0

Publication Information

This study was funded by grant money from the National Estuary Program of the U.S. Environmental Protection Agency, with in-kind contributions from: Washington Department of Fish and Wildlife (Puget Sound Assessment and Monitoring Program), Snohomish County Marine Resources Committee, Washington Sea Grant, National Oceanic and Atmospheric Administration, Grays Harbor County Marine Resources Committee, Olympic Coast National Marine Sanctuary, Pacific County Marine Resources Committee, Port Townsend Marine Science Center, Seattle Aquarium, Whatcom County Marine Resources Committee, US Navy Marine Environmental Support Office, and a number of citizen science volunteers across western Washington.

Author and Contact Information

Jennifer Lanksbury
Washington Department of Fish and Wildlife
Jennifer.Lanksbury@dfw.wa.gov
360-902-2820

James West
Washington Department of Fish and Wildlife
james.west@dfw.wa.gov
phone 1: (360) 902-2842
phone 2: (206) 302-2427

Quality Assurance Project Plan

Toxic Contaminant Monitoring in Mussels: Phase 1

December 2011

Approved by:

Signature: Patricia Jatzak Date: 12/20/11
Patricia Jatzak, Client

Signature: Margen Carlson Date: 12/19/11
Margen Carlson, Client's Supervisor/Manager

Signature: James West Date: 12/19/11
James West, Author/Project Manager

Signature: Jennifer Lanksbury Date: 12/19/11
Jennifer Lanksbury, Author/Principal Investigator

Signature: Craig Burley Date: 12/19/11
Craig Burley, Author's Supervisor/Manager

Signature: William Kammin Date: 12/14/11

William Kammin, Quality Assurance Officer, Washington State
Department of Ecology

Table of Contents

	<u>Page</u>
2.0 Abstract.....	5
3.0 Background.....	6
4.0 Project Description.....	7
5.0 Organization and Schedule	8
6.0 Quality Objectives	10
7.0 Sampling Process Design (Experimental Design)	12
8.0 Sampling Procedures	15
9.0 Measurement Methods.....	21
10.0 Quality Control (QC) Procedures	23
11.0 Data Management Procedures	23
12.0 Audits and Reports.....	24
13.0 Data Verification.....	24
14.0 Data Quality (Usability) Assessment.....	24
15.0 References.....	25
16.0 Figures.....	27
17.0 Tables.....	27
18.0 Appendices.....	28
Appendix A. Mussel Sampling Equipment/Supply List.....	28
Appendix B. Sample Data Sheet.....	28
Appendix C. Sample Bag Labels	29
Appendix D. Sample Chain of Custody Form	30
Appendix E. Using and calibrating a salinity refractometer.....	31
Appendix F. Thermometer Accuracy Check: Ice Point Method	33
Appendix F. Mussel Watch Analyte List.....	34
Appendix G. Glossary, Acronyms and Abbreviations, Units.....	36

Distribution List

Patricia Jatzak
Puget Sound Marine & Nearshore EPA Grant Program Manager
Washington Department of Fish and Wildlife
1111 Washington St
Olympia, WA 98501-1091
(360) 902-2597
Patricia.Jatzak@dfw.wa.gov

Margen Carlson
Puget Sound Policy Lead
Washington Department of Fish and Wildlife
1111 Washington St
Olympia, WA 98501-1091
(360) 902-2229
Margen.Carlson@dfw.wa.gov

Brad Sele
Operations Manager
Washington Department of Fish and Wildlife
1111 Washington St
Olympia, WA 98501-1091
(360) 902-2778
Brad.Sele@dfw.wa.gov

Margaret McKeown
Puget Sound Marine & Nearshore EPA Grant Program Manager
Washington Department of Natural Resources
1111 Washington Street SE
Olympia, WA 98504-7000
(360) 902-1072
Margaret.Mckeown@dnr.wa.gov

Dennis Apeti, PhD
Physical Scientist
National Oceanic and Atmospheric Administration
National Centers for Coastal Ocean Science - Mussel Watch Program
(301) 713-3028 x132
dennis.apeti@noaa.gov

Kathleen Herrmann
Marine Resources Program Manager
Snohomish County Public Works, Surface Water Management Division
3000 Rockefeller Ave.
Everett, WA 98201
(425) 388-6414
kathleen.herrmann@co.snohomish.wa.us

Janice Mathisen
Community Outreach Coordinator
Seattle Aquarium
1483 Alaskan Way
Seattle, WA 98101
(206) 386-4365
J.Mathisen@seattleaquarium.org

Jean Walat
Citizen Science/Volunteer Coordinator
Port Townsend Marine Science Center
Fort Worden State Park
532 Battery Way
Port Townsend, WA 98368
(360) 385-5582 x112
JWalat@ptmsc.org

William Kammin (QA Officer) and Tom Gries (NEP QC Coordinator)
Ecology, PO Box 4600, Olympia, WA 98504-7600
(360) 407-6964 and (360) 407-6327
Wkam461@ecy.wa.gov and tgri461@ecy.wa.gov

2.0 Abstract

The primary objective of this study is to collect blue mussels (*Mytilus* spp.) in support of the national NOAA Mussel Watch Program, to satisfy sampling requirements for the 2011/2012 winter season. This effort is meant to fill a gap in an otherwise 25-year progression of monitoring toxic contaminants in selected nearshore locations in Puget Sound. WDFW will collect mussels from approximately twenty locations in Puget Sound (including three reference areas along the Washington Coast). Custody of the samples will then be transferred to NOAA for histopathological and chemical analysis.

This project is the first phase of an effort to expand contaminant monitoring in nearshore habitats of Puget Sound. Although contaminants in several species of marine and anadromous fish have been monitored by WDFW's Puget Sound Assessment and Monitoring Program (PSAMP) for over 20 years, tracking the status of contaminants in nearshore biota has been lacking. A separate scope of work is currently being developed to take the next steps towards augmenting NOAA's mussel coverage in nearshore waters, with the ultimate goal of developing a broad network of sampling locations and stakeholder-partners to track contaminant conditions in nearshore waters. It is also intended that these efforts will ultimately link to Ecology's Stormwater Work Group in support of their draft municipal stormwater permit (see appendix 12 and Appendix 10 of Phase 1 and Phase 2 permits). Additionally, a companion field-based effort evaluating the extent and magnitude of chemical contamination in submerged aquatic vegetation is concurrently being developed by WADNR.

3.0 Background

The Washington Department of Fish and Wildlife (WDFW) has played a central role in evaluating the status and trends of toxic contaminants in the Puget Sound Ecosystem since 1989. As a participant in the Puget Sound Assessment and Monitoring Program (PSAMP), WDFW has tracked contaminants of concern in key species in the ecosystem, identifying where harm to biota has occurred, the extent and magnitude of problems, and whether conditions are improving or degrading. This work informs decisions regarding best management practices for prevention, control and cleanup of contaminants in Puget Sound.

Contaminant conditions in nearshore biota have long been recognized as a gap in coverage for contaminant monitoring in Puget Sound. Because Puget Sound's nearshore waters receive stormwater, groundwater, and other sources of terrestrial pollution, these habitats and their resident biota can be exposed to high contaminant loads. Understanding the fate and transport of chemical contaminants in these waters, especially relative to their infiltration of the marine food web, is needed to make cost-effective decisions regarding mitigation of the harm pollution causes Puget Sound's biota.

Blue mussels (*Mytilus* spp.) and other sessile, filter-feeding bivalves have been used to monitor water quality and the health of nearshore ecosystems worldwide. The National Oceanic and Atmospheric Administration's (NOAA) national Mussel Watch program (MW) has been active in Puget Sound since 1986, sampling mussels in approximately 17 locations across the Salish Sea (20 locations including the Pacific Coast) (Figure 2). The MW program monitors the status and trends of chemical contaminants in all US coastal waters (nearly 300 sites around the country) through biennial collection and analysis of mussels and/or oysters, depending on their availability and location. Mussel Watch has been an important complement to Washington's ongoing contaminant monitoring efforts and PSAMP scientists have placed a high value on the utility of MW for regional contaminant assessments. PSAMP has long reported MW data and results, along with status and trends information from its own sentinel species, to present a more complete contaminant status and trends story for Washington State.

In recent years NOAA has sought sampling partnerships with State and local entities to promote the relevance of its program at regional levels and help ensure its long-term viability. PSAMP staff partnered with the MW in 2009 and 2010 to conduct field sampling of MW sites in Washington. The MW sites, as well as three additional sites added by PSAMP staff, were successfully sampled and NOAA covered the laboratory costs for chemical and histopathological analysis of all samples. The results of this work are documented in Lanksbury et al. (2010).

NOAA has approached PSAMP staff again for assistance in collecting samples for the 2011/12 field season. Although the ultimate goal of PSAMP is to develop an expanded Mussel Watch-type observation program in Puget Sound, the scope of work for this QAPP is limited to field sampling of MW program sites during the current 2011/12 sampling season (December 2011 - March, 2012).

4.0 Project Description

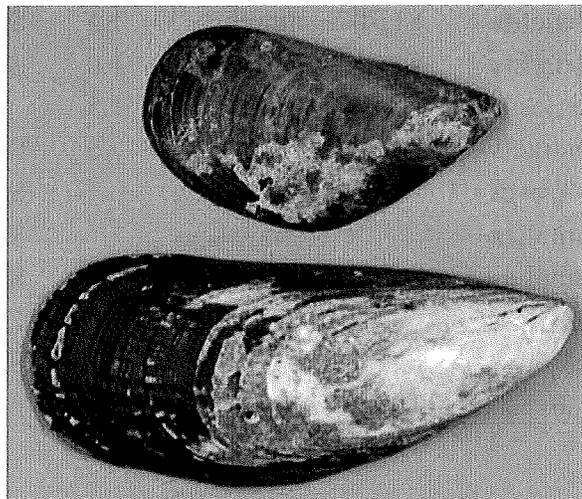
The project described in this QAPP is limited to field work only. The goal of this project is to fill a monitoring gap, in an otherwise 25-year progression of monitoring toxic contaminants in select nearshore locations in Puget Sound, by sampling for the 2011/12 MW program field season. To accomplish this goal, our objective is to collect whole mussel samples from up to 20 established MW sites, including multiple locations around the Salish Sea and three outer coast sites, and send those samples to two NOAA-contracted laboratories, which have a long history of participation in the MW program, for analysis. Tracking, processing, and analysis of samples (both chemical and histopathological) will be the responsibility of, and paid for by, NOAA. Field and laboratory analytical methods will follow NOAA's protocols.

Key points of the WDFW field sampling plan include:

- Use the existing PSAMP *Toxics in Fish* program as a platform for infrastructure and operational support
- Coordinate with existing local Mussel Watch-type programs that will sample at MW sites local to them:
 - Snohomish County's Marine Resources Committee (SCMRC)
 - US Navy's ENVironmental inVESTment (ENVVEST) program
- Rely on a network of citizen science volunteers to assist in field sampling at select MW sites
- Send samples to NOAA for chemical and histopathological analysis of samples

Bivalves collected for this study will typically include blue mussels; *Mytilus galloprovincialis/trossullus* and *M. californianus* (Figure 1). Following the Mussel Watch sampling protocol, mussel populations will be sampled during their reproductively quiescent period, prior to spawning, over the winter months (December to March), to avoid variability in contaminant tissue residues related to reproduction.

Figure 1. *Mytilus galloprovincialis/trossullus* (top) and *M. californianus* (bottom). Photo courtesy of National Mussel Watch Program unpublished report.



At each MW sampling site live mussels will be collected at three replicate locations (stations) using the MW sampling protocol described in Sections 0 and 0. Depending on the size of mussels available, between 210 to 660 individual mussels will be collected in total at each MW site. The mussels will be shipped live via overnight express, on ice, to the NOAA-contracted laboratories.

5.0 Organization and Schedule

Management of the project will be carried out by PSAMP's Toxics in Fish lead, James E. West, and the project work will be carried out by a WDFW Fish Biologist (Jennifer Lanksbury). All work will be supported by existing PSAMP staff and resources, as well as volunteer organizations and affiliated citizen science volunteers.

Setting the project schedule will necessitate assessment of appropriate low-tide targets for sampling. Sampling dates must fall within a three-week target collection date for each site, as set by the MW program (Table 2). After selection of target sample dates/times, PSAMP staff will coordinate with former MW volunteer organizations for assistance with sampling, where possible.

Sampling limitations include frequent night-time sampling (low tides in the winter frequently occur after sunset) and availability of volunteers. Sampling at several of the more remote MW sites (i.e. PRPR, BBSM, WBNA, GHWJ, and JFCF; see Table 3) will require overnight stays in local hotels or other accommodations. In addition, as volunteer participation may vary between sites, additional PSAMP staff may be required to complete sampling at some locations.

Table 1. Projected budget for 2011/12 Mussel Watch sampling.

Object	Cost per Unit	Unit	No. of Units	Total Cost
Bio 3 Salary	\$4,627	month	1.5	\$6,941
Technician Salary	\$2,971	month	1.0	\$2,971
Bio 3 Benefits	\$1,707	month	1.5	\$2,561
Technician Benefits	\$1,460	month	1.0	\$1,460
Computer lease	\$45	month	1.5	\$68
Site Lead Support Contracts	\$1,000		3	\$3,000
Travel				\$2,000
Volunteer supplies				\$1,000
Shipping/supplies	\$145	site	20	\$2,900
SubTotal				\$22,900
Indirect (23.51%)	0.2351			\$5,384
SubTotal				\$28,283

Table 2. Mussel Watch (MW) site sampling schedule for 2011/12 field season. Standard MW protocol indicates that sites should be sampled within a three week window on either side of the target collection date. See Figure 2 map.

Site Name (Code)	MW Target Collection Date	2011/12 Target Sample Date	Staff and/or Volunteers
Whidbey Island, Possession Point (WIPP)	11-Dec	4-Dec-2011	PSAMP
Sinclair Inlet, Waterman Point (SIWP)	11-Dec	5-Dec-2011	PSAMP, ENVVEST
Elliott Bay, Four-Mile Rock (EBFR)	11-Dec	6-Dec-2011	Seattle Aquarium
Commencement Bay, Tahlequah Point (CBTP)	11-Dec	6-Dec-2011	PSAMP
South Puget Sound, Budd Inlet (SSBI)	5-Jan	20-Dec-2011	PSAMP
Puget Sound, Edmonds Ferry (PSEF)	22-Dec	17-Jan-2012	SCMRC (5 days outside collection target)
Puget Sound, Everett Harbor (PSEH)	9-Jan	17-Jan-2012	SCMRC
Puget Sound, Mukilteo Ferry (PSMF)	21-Dec	17-Jan-2012	SCMRC (5 days outside collection target)
Elliott Bay, Duwamish Head (EBDH)	9-Jan	3-Jan-2012	PSAMP, Seattle Aquarium
Puget Sound, Port Townsend (PSPT)	8-Jan	8-Jan-2012	PSAMP, PTMSC
Puget Sound, Hood Canal (PSHC)	8-Jan	9-Jan-2012	PSAMP, PTMSC
Puget Sound, Port Angeles (PSPA)	8-Jan	10-Jan-2012	PSAMP, Iccle Seafoods
Point Roberts, Point Roberts (PRPR)	10-Jan	16-Jan-2012	PSAMP
Bellingham Bay, Squalicum Marina Jetty (BBSM)	9-Jan	17-Jan-2012	PSAMP, WCMRC
South Puget Sound, Kopachuck Park (SSKP)	5-Feb	30-Jan-2012	PSAMP
South Puget Sound, Tolmie Park (SSTP)	7-Feb	31-Jan-2012	PSAMP
Willapa Bay, Nahcotta (WBNA)	6-Feb	6-Feb-2012	PSAMP, PCMRC
Elliott Bay, Myrtle Edwards (EBME)	22-Feb	13-Feb-2012	PSAMP, Seattle Aquarium
Grays Harbor, Westport Jetty (GHWJ)	21-Feb	14-Feb-2012	PSAMP, PCMRC
Juan de Fuca Strait, Cape Flattery (JFCF)	3-March	6-March-2012	PSAMP, PCMRC, OCNMS, Makah Tribe

ENVVEST – ENVIRONMENTAL IN VESTMENT (US NAVY PROGRAM) VOLUNTEERS

SCMRC – SNOHOMISH COUNTY MARINE RESOURCES COMMITTEE VOLUNTEERS

PTMSC – PORT TOWNSEND MARINE SCIENCE CENTER VOLUNTEERS

WCMRC – WHATCOM COUNTY MARINE RESOURCES COMMITTEE VOLUNTEERS

PCMRC – PACIFIC COUNTY MARINE RESOURCES COMMITTEE VOLUNTEERS

OCNMS – OLYMPIC COAST NATIONAL MARINE SANCTUARY VOLUNTEERS

6.0 Quality Objectives

Sections 6.5 and 6.6 need some work - there is confusion re: definition of sensitivity and bias.

6.1 Measurement Quality Objectives

Following are the field sampling measurement quality objectives for NOAA's MW program (Table 3).

Table 3. Measurement quality objectives (MQOs) for NOAA's Mussel Watch Program

Field Measurement	MQOs
Salinity	± 1.0 ppt
Temperature	± 1.0 °C
GPS coordinates	0.000001 decimal degrees (0.111 m/0.364 ft)

ppt = permille, parts per thousand (‰), grams salt/kilogram solution
 °C = degrees Celsius

Although the MW program asks for GPS coordinates to the nearest 0.000001 decimal degrees (0.111 m/0.364 ft), the hand-held GPS units (Garmin, GPSmap 76C, and GPSmap 176) available to PSAMP staff report coordinates to the nearest 0.00001 decimal degrees (1.11 m/3.64 ft). The 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 (see Section 8.1 Field measurement and sample collection SOP). 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, we assert that a GPS position reported to the nearest 0.00001 decimal degrees (1.11 m/3.64 ft) will provide adequate representation of the physical location of collected mussels.

Once the mussels are collected and shipped to the NOAA-contracted laboratories, they will no longer be under PSAMP control. At that point the NOAA Mussel Watch program and its contracted labs will have control of the samples and take responsibility for any further measurement quality objectives (i.e. laboratory MQOs). Data quality assurance associated with NOAA's Mussel Watch Program is described by Cantillo (1995).

6.2 Comparability

Mussel samples collected in this field season will be directly comparable with mussels collected at the same MW sites over the last 25 years, because we will be following the same standardized sampling techniques and methods for the timing of collection, distribution of stations (replicates) and handling of mussels that have been used by MW scientists/field workers since 1986. All staff and citizen science volunteers are trained to ensure consistency. The program used to train citizen science volunteers in the Mussel Watch sampling techniques is described in Lanksbury et al (2010).

6.3 Representativeness

Mussels from each Washington MW site will be representative of environmental conditions in the winter season at that site. Mussels will be taken from naturally occurring populations and are meant to

represent ambient conditions at each site. For this reason mussels will not be collected directly off creosote-treated wood. Following the standard MW protocol, mussel samples will be collected from three separate stations (replicates) at each site. When feasible, replicates will be located between 25 – 250 meters (82 – 820 feet) from one another, to avoid sampling a single non-representative “clump” of mussels at any one site.

6.4 Completeness

Population density and individual mussel sizes can vary greatly at any one location over time. Lack of sufficient mussels at MW sites in the past has led to cancellation of sampling at those sites in select years. This study will be considered a success if 18 of the 20 MW sites (i.e. 90% of those listed on Table 1) are collected and shipped to the NOAA-contracted laboratories.

6.5 Sensitivity

Although the MW program sampling protocol calls for GPS coordinates to the nearest 0.000001 decimal degrees (0.111 m/0.364 ft), the GPS *accuracy* required is not specified. Hand-held GPS units (Garmin, GPSmap 76C, and GPSmap 176) used by PSAMP staff report coordinates to the nearest 0.00001 decimal degrees (1.11 m/3.64 ft) and each have a position accuracy of <15 m (49 ft), 95% typical. Although greater accuracy, 3-5 m (10-16 ft, 95% typical), can be achieved using differential GPS (DGPS), additional equipment and training would be required to use DGPS. Future efforts at developing a broad network of sampling locations, to augment NOAA’s mussel coverage in nearshore waters, will involve investigation of DGPS as a potential improvement in GPS accuracy.

6.6 Bias

In order to minimize bias between the population mean and the true value, mussel samples will be collected from three separate stations (replicates) at each site. When feasible, replicates will be located between 25 – 250 meters (82 – 820 feet) from one another, to avoid sampling a single non-representative “clump” of mussels at any one site. In addition, to avoid bias from point sources of contaminants, no mussels will be collected from creosote treated surfaces (i.e. creosote pilings or logs). This describes sampling bias but not instrument.

In order to minimize instrument bias, the refractometers (ZGRS-10ATC Illumination Refractometer) used to measure salinity, and the mercury or alcohol thermometers use to measure temperature will be checked and calibrated at the beginning of the field season, before measurements are taken in the field. Since the optical components of a refractometer can change slightly at different temperatures, refractometer calibration will be checked (i.e. verify it reads 0 for distilled water) once at every site, before field readings are taken. Instructions on how to use and calibrate the refractometer used in this study are described in Appendix E. Instructions on how to check a mercury or alcohol thermometer, using the ice-point method (Strouse et al. 2010), are detailed in Appendix F.

6.7 Precision

At each station (replicate sampling location), water temperature and salinity will be recorded so that three replicate measurements of each parameter will be made for every Mussel Watch site (see *Mussel Watch Program Data Sheets* in Appendix B). Acceptable precision of salinity and temperature measurements will fall within ± 1.0 ppt and ± 1.0 °C respectively.

7.0 Sampling Process Design (Experimental Design)

7.1 Study Design

The mussel species *Mytilus galloprovincialis/trossullus* and *M. californianus* are typically found at MW sites in Washington State. Either species is acceptable for use by the MW program. Mussel Watch sites are typically located 10 - 100 km apart along US coastlines, in shellfish beds large enough to sustain repeated sampling. National MW monitoring sites were selected by NOAA to provide an assessment of the ambient conditions over broad coastal areas, to allow comparison among very large water bodies. Hence municipal sewage outfalls, industrial effluents, and other known point pollution sources are avoided. In addition, only naturally occurring bivalves are collected from natural substrates or concrete; creosote- or other treated pilings are avoided. The distribution of bivalves is not manipulated with transplantation.

Mussels are sampled during their reproductively quiescent winter months, (prior to spawning) to avoid variability in contaminant tissue residues related to reproduction. They are collected from intertidal zones by hand and removed from their substrates by cutting their byssal threads. The collected bivalves are then rinsed, using water from the collection site, and immediately packed in ice to keep the samples alive until they reach the laboratory. Samples are shipped within two days of collection to NOAA-contracted analytical laboratories for analysis of chemical contaminants and for assessment of gonadal index and histopathology.

Analyses at these labs will include determination of over 140 chemical contaminant residues in the soft tissues. Of the more than 140 organic compounds and metals included in MW analyses, approximately 17 are toxic trace elements, including metals and metalloids. The organic compounds regularly quantified by the program include polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), dichloro-diphenyl-trichloro-ethane (DDT) and its metabolites, organo-tins, chlordanes, Dieldrin and its related compounds, hexachlorocyclohexanes (HCHs), and various other chlorinated pesticides (see Appendix E for list of analytes). The MW program also assesses the gonadal index and histopathology of sampled mussels. The gonadal index/ histopathology component verifies reproductive state and measures the prevalence of nearly 70 diseases and parasites.

We wish to emphasize here that once the mussels have been collected and shipped to the NOAA-contracted laboratories they will no longer be under PSAMP control. *At that point the MW program and NOAA-contracted labs will have control of the samples and responsibility for analyzing them, verifying/validating the results, determining data usability, and entering the results into the Environmental Protection Agency's (EPA) Storage and Retrieval (STORET) database.* Although the MW program has guaranteed that the final data generated by this effort will be made available to EPA's STORET database, the timing of submission of samples for chemical analysis will be subject to availability of NOAA funds, and maximum turnaround time for chemical analysis of data generated from these samples will be approximately one year from time of submission.

Table 4. Location of Mussel Watch site centers (GPS datum set to NAD 1983). Samples are collected on a biennial basis (once every two years in the winter months). See Figure 2 map.

Site Name (Code)	County	Latitude	Longitude
Whidbey Island, Possession Point (WIPP)	Island	47.90568	-122.37722
Sinclair Inlet, Waterman Point (SIWP)	Kitsap	47.55083	-122.62700
Elliott Bay, Four-Mile Rock (EBFR)	King	47.63917	-122.41230
Commencement Bay, Tahlequah Point (CBTP)	King	47.33583	-122.50160
South Puget Sound, Budd Inlet (SSBI)	Thurston	47.10050	-122.91210
Puget Sound, Edmonds Ferry (PSEF)	Snohomish	47.81398	-122.38229
Puget Sound, Everett Harbor (PSEH)	Snohomish	47.97383	-122.23700
Puget Sound, Mukilteo Ferry (PSMF)	Snohomish	47.94968	-122.30158
Elliott Bay, Duwamish Head (EBDH)	King	47.57583	-122.41800
Puget Sound, Port Townsend (PSPT)	Jefferson	48.10300	-122.76500
Puget Sound, Hood Canal (PSHC)	Jefferson	47.83167	-122.68660
Puget Sound, Port Angeles (PSPA)	Clallam	48.13967	-123.42010
Point Roberts, Point Roberts (PRPR)	Whatcom	48.98167	-123.02160
Bellingham Bay, Squalicum Marina Jetty (BBSM)	Whatcom	48.75417	-122.49950
South Puget Sound, Kopachuck Park (SSKP)	Pierce	47.3109	-122.68723
South Puget Sound, Tolmie Park (SSTP)	Thurston	47.12087	-122.7753
Willapa Bay, Nahcotta (WBNA)	Pacific	46.50800	-124.00600
Elliott Bay, Myrtle Edwards (EBME)	King	47.62583	-122.37273
Grays Harbor, Westport Jetty (GHWJ)	Grays Harbor	46.91250	-124.11750
Juan de Fuca Strait, Cape Flattery (JFCF)	Clallam	48.33832	-122.68468

Parameters to be determined at each MW station (replicate sampling location) include field measurements of water temperature (°C) and salinity (ppt). In addition, descriptions of the current weather, site conditions (including a description of any potential sources of contamination), site conditions (including a description of the physical conditions at each replicate station), and the substrate from which mussels are collected will be recorded (see example field log in Appendix B). Photos of each MW replicate station, as well as an overview of the site when possible, will also be taken.

7.2 Assumptions underlying design

Mussel Watch sites were selected to represent large coastal areas that can be used to construct a nationwide assessment (Kimbrough et al. 2008). Sites that were selected for monitoring by MW, generally 10 to 100 km apart along the entire US coastline, are meant to *represent ambient conditions within broad-scale regions of Washington State*. Where possible, sites were selected to coincide with historical mussel and oyster monitoring locations from other programs, such as the EPA's Mussel Watch sites sampled from 1976 to 1978 (Goldberg et al., 1983).

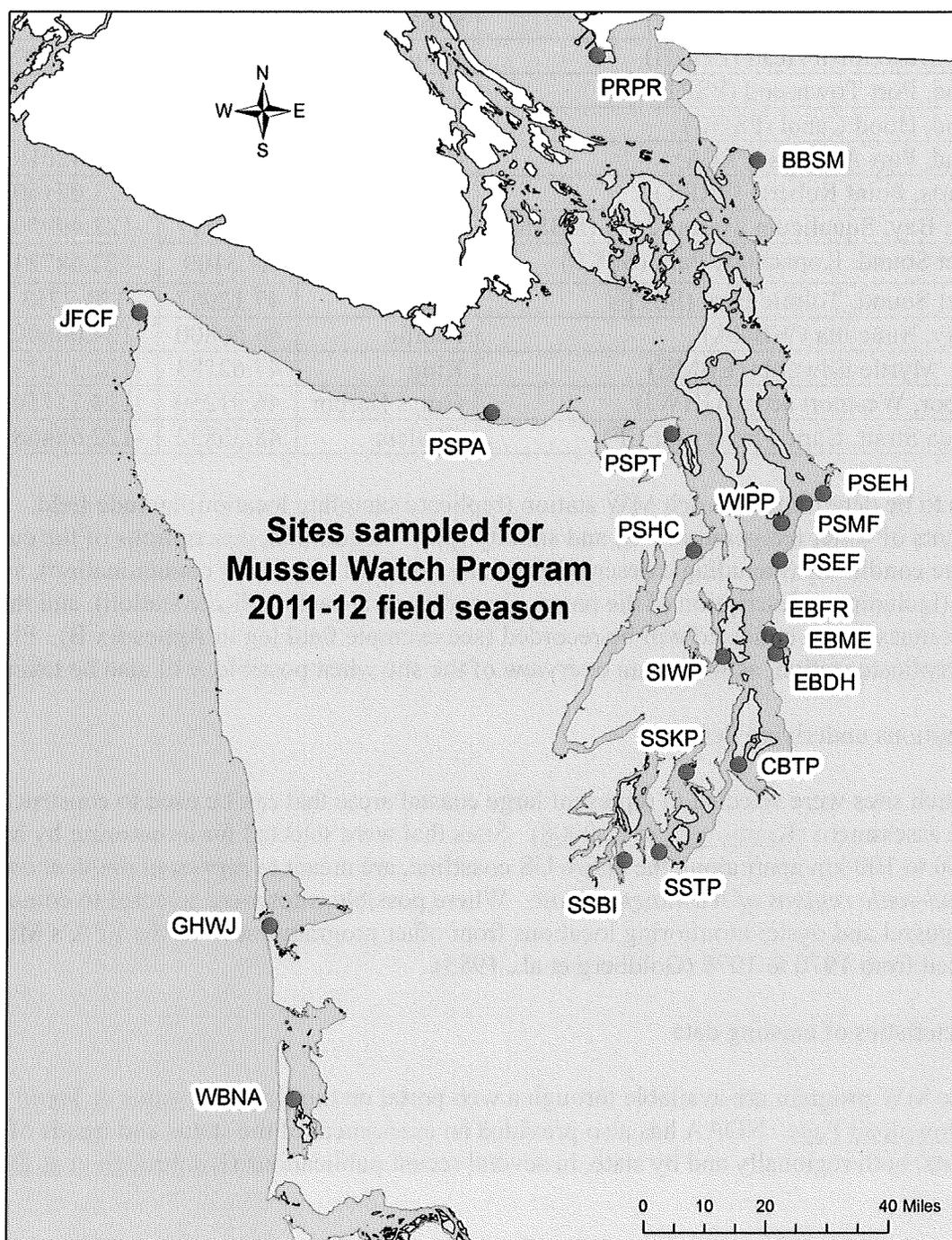
7.3 Characteristics of existing data

Data for the MW program are available through a web portal on the [National Status & Trend \(NS&T\) Program Download Page](#). NOAA has also provided an assessment of the status and trends of MW program data, both regionally and by state, in several recent publications (Kimbrough et al. 2009;

Kimbrough et al. 2008). The data (1986-2005) used to generate these assessment reports, and more recent data (from 2009/2010), is available at the NS&T web portal. This field sampling effort will provide data for the 2011/12 assessment year of the national MW program.

Since its inception, the field and laboratory methods for the Mussel Watch program have undergone some changes. The methods described in the next sections are equal to/consistent with the most recent NOAA protocols.

7.4 Figure 2 - Map of Mussel Watch sites to be sampled in Washington State during the 2011/12 field season. See Tables 2 or 4 for site code names.



8.0 Sampling Procedures

Field personnel will have been trained in the sampling methods specified in this QAPP and detailed in the SOP below. A description of the training program is contained in Lanksbury et al (2010). All samplers will wear Nitrile or latex gloves while handling mussels and all mussels will be rinsed on site, in local marine water, before being placed in Ziploc bags for collection.

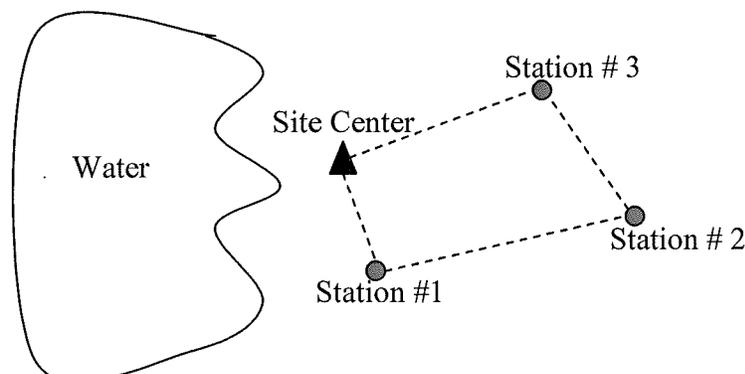
8.1 Field measurement and sample collection SOP

Below is the Standard Operating Procedure (SOP), adapted from the MW program, which will be used for sampling (and available on-site) at all MW sites in Washington State:

- 1) Find the established Site Center as indicated in the MW Local Site Description using a GPS unit:
 - a) Record the latitude and longitude (GPS datum set to NAD 1983) of the Site Center, or as close as you can get to it (may be offshore a bit) at the top of the Mussel Watch Data Sheet (Appendix B).
 - b) Record the date, time of arrival, weather conditions, and mussel watch collectors and data recorder on data sheet (see Appendix B).
 - c) Record site conditions and description, noting any sources of contamination, on back of data sheet.
 - d) Record any additional observations, notes or comments in the space provided.
 - e) Take an overview photo of the Site Center.

- 2) Establish three distinct Stations (i.e. replicate sampling locations) for mussel collection around, or to either side, of the Site Center (Figure 3):
 - a) Site Center can serve as Station #1 if mussels are available there.
 - b) Try spacing Stations between 25 – 250 meters (82 – 820 feet) from one another, if possible.
 - c) If no mussels are found near the Site Center then search for mussels can 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 should stop if the habitat characteristics change significantly from the Site Center. Do not proceed onto substantially different substrates or environments (e.g., if the Site Center is in marina, do not leave the marina, and vice versa).

Figure 3. Example of possible distribution of Stations (i.e. replicate sample locations) near a MW site center.

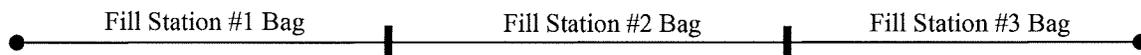


NOTE: If it is not possible to delimit three separate Stations (i.e., not enough mussels)* then collection can be spread out along the shoreline (i.e. along a transect, see Figure 4):

- Clearly note change in sampling technique on data sheet.
- Note latitude and longitude of starting and ending points of the line sampled (see Step #3 below).
- Mussels should still be separated into the three Station bags (see Step #4 below) based on relative spatial distance, to avoid sampling a single non-representative “clump”, by following along the shoreline and filling bags (see figure 4 below).

***Only choose this option if absolutely necessary.**

Figure 4. Example of linear distribution of Stations (replicate sample locations) along the shoreline near MW site center.

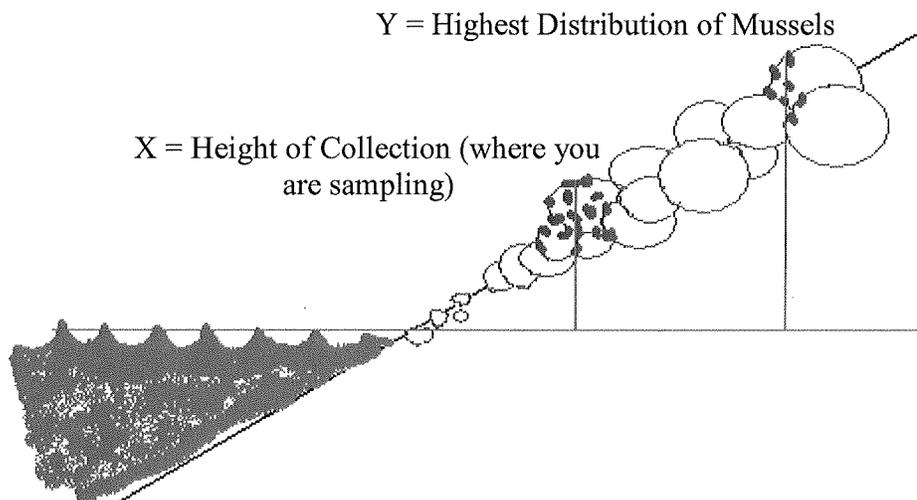


3) At each Station (i.e. replicate sampling location):

- a) Record GPS coordinates and start time.
- b) Measure water temperature using a calibrated thermometer (mercury or alcohol) at the shoreline of the station, in approximately one foot of water.
- c) Measure salinity using a calibrated refractometer (Appendix E) at the shoreline of the station, at an approximate depth of one foot.
- d) Write a description of the Station, including, for instance, its location relative to the Site Center or other landscape features, and the type of intertidal habitat in that area.
- e) Describe the substrate to which mussels are attached (e.g., boulder, cement, pilings, sand, cobble, etc). *Be as descriptive as possible.*
 - **Note: DO NOT collect from creosote-treated wood.**
- f) Estimate and record the height of mussels collected, relative to the height of seawater at the time of collection (“Height of Collection” on the data sheet) and the highest overall distribution of mussels available, even if none are collected there (“Highest Distribution of Mussels” on the data sheet). See figure 5 below.

(Note: the Height of Collection and Highest Distribution of Mussels may be the same if you are collecting mussels from the highest area in which they occur.)

Figure 5. Diagram illustrating the height of collection vs. highest distribution of mussels.



- g) Take photos of the Station, its surroundings, and the substrate.
- h) Collect mussels.

4) To collect mussels:

- a) ALWAYS wear disposable laboratory gloves when handling mussels, bags, and bag tags.
- b) At each Station (replicate location) mussels need to be collected and placed into two (2) different bags for the two (2) separate analyses:
 1. Use pre-labeled **gallon** Ziploc bags for mussels for **chemical analysis**. **At each Station, collect between 50 – 200 mussels**, depending on size.
 - 2 inch – 3 inch long mussels (ideal size): collect 50 mussels
 - ½ inch – 2 inch mussels: collect 100 – 150 mussels
 - Less than ½ inch mussels: collect 150 - 200 mussels
 2. Use pre-labeled **quart** Ziploc bags for mussels for **histology analysis**. **At each Station collect exactly 20 mussels**, independent of size.

****Be sure to use the appropriately labeled bag (Appendix C) for collections at each Station. All Ziploc bags should have WA Mussel Watch, the Site Name and Acronym, the Date, the type of analysis the bag will be sent for (i.e. CHEMISTRY or HISTOPATH) and Station # written on the outside with a Sharpie. The appropriate Rite-in-the-Rain bag tag should be placed inside each bag.****

- c) To collect mussels cut their byssal threads (do not tear off substrate), brush off sediment and rinse in a bucket of marine water collected near each Station.
 - Be sure to change bucket of seawater between Stations.
- d) Double bag the mussels to prevent ice melt leakage from contacting the mussels.
 - Each gallon Ziploc bag with mussels goes into another gallon bag – so chemistry bag from each Station gets double bagged by itself.
 - All three quart Ziploc bags go into a single gallon bag – so histology bags from all three Stations get double bagged *together* into one gallon bag.
 - Place ALL sealed bags into a plastic garbage bag and immediately place on ice in a cooler. **Remember to always use gloves when handling mussels, labels, and bags.**

5) After sampling is complete, record the time on the data sheet (“Time Leave”).

6) Be sure to note on the Chain-of-Custody form (Appendix D) if the final collection of mussels changes hands between collection and shipping (i.e. if someone other than Site Lead keeps the mussels overnight before shipping).

8.2 Containers, preservation methods, holding times

Consistent with standard MW program protocols, samples will be placed in a refrigerator on ice overnight(s) before being shipping in two separate coolers to B&B Laboratories (chemistry) and Rutgers Haskin Shellfish Lab (histopathology). Coolers will be shipped via FedEx *Priority Overnight* either the day of collection, if collection occurs in the morning, or the next day. They should arrive the next business day to the laboratories.

Bivalves can survive in storage for many days if the conditions are properly maintained; double-bagged samples of mussels stored in coolers filled with ice works well to keep mussels alive, provided melt water is allowed to drain and does not touch the mussels. Because sampling will generally occur on Sundays through Tuesdays, shipping will occur within 24 to 48 hours of collection and arrive Tuesdays through Thursdays (i.e. the next business day) at the laboratories. However, if sampling is delayed and occurs on a Thursday through Saturday, mussels will be held over the weekend and shipped the following Monday, so as to avoid arrival at the lab on a Friday or over the weekend. No samples will be shipped to arrive on a holiday.

Below (Figure 6) are illustrated directions that will be used for packing MW samples to be shipped - note that a *copy* of the MW datasheet and the *original* Chain-of-Custody form (Appendix D) go in a Ziploc bag at the top of each cooler:

Figure 6. Instructions and photographs describing proper packaging and mailing of MW shipments to laboratories.

1) Bagged ice is placed in a layer at the bottom of the cooler.



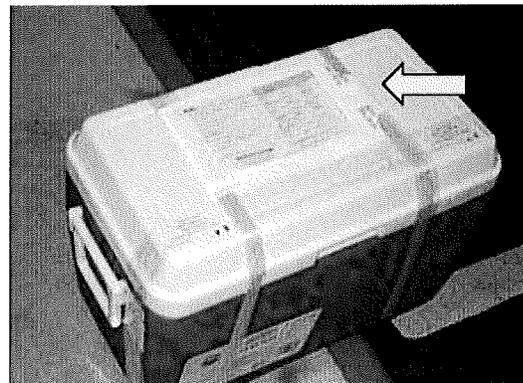
2) Double-bagged mussel samples are placed on top of the ice layer.



3) Bags of mussels with bags of ice are layered on top of each other and the voids are filled with remaining ice. A *copy* of the MW datasheet and the *original* Chain-of-Custody form are placed into a Ziplock bag at the top of each cooler.



4) The FedEx packing label is attached to the top of the cooler using sticker backing. At least two bands of nylon fiber tape will be used to secure sides of label and seal cooler (yellow arrow). Bands of clear tape will be wrapped around the lip of the cooler (to help seal in coldness) as well as around its width.



8.3 Invasive species evaluation

All field sampling gear that comes into contact with marine water or beach sediments (i.e. boots) will be inspected after field sampling for potential invasive species. All sampling gear and equipment will be cleaned, drained, and rinsed with potable water after each sampling effort and before proceeding to the next MW site. This protocol will accomplish level one decontamination, as recommended by the WDFW Aquatic Invasive Species' small gear decontamination protocol.

8.4 Sample ID

The MW program has established Sample IDs for each of their sites (see Tables 2 or 3), which we will use for this study.

8.5 Chain-of-custody, if required

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

8.6 Field log and data sheets

A bound, waterproof field log notebook will be maintained during the duration of the project to record observations and experiences. 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 and datum)
- GPS Make/Model
- Weather
- Collectors and recorder
- Tidal information (tide height, time of low tide)
- Station (replicate) description, site conditions, sampling substrate
- Station (replicate) water temperature and salinity
- Height of collection and highest distribution of mussels
- Other notes/comments

9.0 Measurement Methods

9.1 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 Watch 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 the refractometer used in this study are described in Appendix E. Instructions on how to check a mercury or alcohol thermometer are detailed in Appendix F.

9.2 Laboratory Measurements

This project is limited to field work only. Once the mussels have been collected and shipped to the NOAA-contracted laboratories they will no longer be under PSAMP control. At that point the NOAA Mussel Watch program and its contracted labs will have control of the samples and responsibility for measurement methods.

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.

9.2.1 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.

9.2.2 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.

9.2.3 Gonadal index and histopathology

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.

9.2.4 Lab(s) accredited for method(s)

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. In addition, TDI-Brooks International, with assistance from the National Institute of Standards and Technology (NIST), has conducted yearly intercalibration studies to ensure data are accurate and precise (Kimbrough et al. 2008). Below is an excerpt from the TDI-Brooks website:

“In support of marine monitoring measurement programs, the National Institute of Standards and Technology (NIST), in cooperation with the NOAA National Status and Trends Program (NS&T), and the EPA Environmental Monitoring and Assessment Program (EMAP), has conducted yearly interlaboratory comparison exercises to provide one mechanism for participating laboratories (and monitoring programs) to evaluate their quality and comparability of performance in measuring selected organic contaminants in environmental samples.”

10.0 Quality Control (QC) Procedures

Field instruments will be checked and calibrated at the beginning of the field season, prior to use, to ensure accuracy and to 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).

Although the MW program asks for GPS coordinates to the nearest 0.000001 decimal degrees (0.111 m/0.364 ft), the hand-held GPS units (Garmin, GPSmap 76C, and GPSmap 176) available to PSAMP staff report coordinates to the nearest 0.00001 decimal degrees (1.11 m/3.64 ft). However, the 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 (see Section 8.1 Field measurement and sample collection SOP). 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, we assert that 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.

This project is limited to field work only. Once the mussels have been collected and shipped to the NOAA-contracted laboratories they will no longer be under PSAMP control. At that point the NOAA Mussel Watch program and its contracted labs will have control of the samples and responsibility for quality control (QC) procedures. The MW program data quality objectives, required lab QC samples and data QA processes are all described in NOAA documents available on the internet. See Section 9.0 for links.

11.0 Data Management Procedures

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, PDF copies will be sent to MW headquarters staff, 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.

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.

11.1 Data recording/reporting requirements

Once the mussels have been collected, they will be shipped to NOAA-contracted laboratories. The NOAA Mussel Watch program and its contracted labs will then have control of the samples and

responsibility for laboratory data management procedures. Data management, reporting and quality assurance associated with NOAA's Mussel Watch Program is described by Cantillo, A. Y. (1995).

11.2 Data upload procedures

Although the MW program has guaranteed that the final data generated by this effort will be made available to EPA's STORET database, the timing of submission of samples for chemical analysis will be subject to availability of NOAA funds, and maximum turnaround time for chemical analysis of data generated from these samples will be approximately one year from time of submission. NOAA will notify WDFW and WDFW will notify Ecology when the 2011-2012 Mussel Watch results become available in STORET.

12.0 Audits and Reports

Ecology's NEP QA Coordinator may conduct a field audit of sampling operations. If this is done, a water-proof field audit form will be completed, discussed with the field lead, and filed with other project documents.

Upon project completion, WDFW (Jennifer Lanksbury) 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. This report will be distributed to the people listed on the Distribution List of this QAPP (see pages 3-4).

13.0 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.

This project is limited to field work only. Once the mussels have been collected and shipped to the NOAA-contracted laboratories they will no longer be under PSAMP control. At that point the NOAA Mussel Watch program and its contracted labs will have control of the samples and responsibility for any laboratory data verification.

14.0 Data Quality (Usability) Assessment

The verified field data will be reviewed and assessed for completeness, indications of non-representative sampling, and comparability. Findings will determine if project objectives have been met.

This project is limited to field work only. Once the mussels have been collected and shipped to the NOAA-contracted laboratories they will no longer be under PSAMP control. At that point the NOAA Mussel Watch program and its contracted labs will have control of the samples and responsibility for any data quality (usability) assessment.

15.0 References

- Cantillo, A.Y. 1995. Quality Assurance in Long Term Coastal Monitoring. NCCOS General Technical Report RM-GTR-284 182-188.
<http://www2.coastalscience.noaa.gov/publications/detail.aspx?resource=N+s+tQyTv4sTZR7ii2SrMw==>
- Ecology, 2004. Guidance for the Preparation of Quality Assurance Project Plans for Environmental Studies. <http://www.ecy.wa.gov/biblio/0403030.html>
- Goldberg, E.D., M. Koide, V. Hodge, A.R. Flegal, and J. Martin. 1983. U.S. Mussel Watch: 1977-1978 results on trace metals and radionuclides. *Estuarine Coastal Shelf Science* 16:69-93.
- Kim, Y., K.A. Ashton-Alcox, and E.N. Powell. 2006. Histological Techniques for Marine Bivalve Molluscs: Update. Silver Spring, MD. NOAA Technical Memorandum NOS NCCOS 27. 76 pp.
<http://ccma.nos.noaa.gov/publications/histopathtechmemofinal.pdf>
- Kimbrough, K.L., W.E. Johnson, G.G. Lauenstein, J.D. Christensen and D.A. Apeti. 2009. An assesment of polybrominated diphenyl ethers (PBDEs) in sediments and bivalves of the U.S. coastal zone. Silver Spring, MD. NOAA Technical Memorandum NOS NCCOS 94. 87 pp.
<http://ccma.nos.noaa.gov/about/coast/nsandt/pbdereport.aspx>
- Kimbrough, K.L., W.E. Johnson, G.G. Lauenstein, J.D. Christensen and D.A. Apeti. 2008. An assesment of two decades of contaminant monitoring in the nation's coastal zone. Silver Spring, MD. NOAA Technical Memorandum NOS NCCOS 74. 105 pp.
<http://ccma.nos.noaa.gov/publications/MWTwoDecades.pdf>
- Kimbrough, K. L., and G. G. Lauenstein (Editors). 2006. Major and Trace Element Analytical Methods of the National Status and Trends Program: 2000-2006. Silver Spring, MD. NOAA Technical Memorandum NOS NCCOS 29, 19 pp.
<http://ccma.nos.noaa.gov/publications/nsandtmethods.pdf>
- Kimbrough, K. L., G. G. Lauenstein and W. E. Johnson (Editors). 2006. Organic Contaminant Analytical Methods of the National Status and Trends Program: Update 2000-2006. NOAA Technical Memorandum NOS NCCOS 30. 137 pp.
<http://www.ccma.nos.noaa.gov/publications/organicmethods.pdf>
- Lanksbury, J., J. E. West, et al. 2010. Washington State 2009/10 Mussel Watch Pilot Project: A Collaboration between National, State and Local Partners Olympia, WA, Puget Sound Partnership: 283 pp. <http://wdfw.wa.gov/publications/pub.php?id=01127>.
- McDonald, S. J., D. S. Frank, J. A. Ramirez, B. Wang, and J. M. Brooks. 2006. Ancillary Methods of the National Status and Trends Program: 2000-2006 Update. Silver Springs, MD. NOAA Technical Memorandums NOS NCCOS 28. 17 pp.
<http://coastalscience.noaa.gov/documents/ancillarymethodsnsandt.pdf>
- Strouse, G., D. Cross, W. Miller and D. Ripple. 2010. User-Friendly Guidance on the Replacement of

Mercury Thermometers. Gaithersburg, MD. EPA (PDF document), contract EP-W-04-021. 36 pp. <http://www.epa.gov/mercury/pdfs/nistuserfriendlyguide.pdf>

USEPA, 1997. Glossary of Quality Assurance Terms and Related Acronyms. <http://www.ecy.wa.gov/programs/eap/qa.html>

USEPA, 2006. Guidance on Systematic Planning Using the Data Quality Objectives Process EPA QA/G-4. <http://www.epa.gov/quality/qs-docs/g4-final.pdf>

USGS, 1998. Principles and Practices for Quality Assurance and Quality Control. Open-File Report 98-636. <http://ma.water.usgs.gov/fhwa/products/ofr98-636.pdf>

16.0 Figures

Figure 1. *Mytilus galloprovincialis/trossullus* (top) and *M. californianus* (bottom). Photo courtesy of National Mussel Watch Program unpublished report.

Figure 2. Map of MW sites to be sampled in Washington State during the 2011-12 field season. See Tables 2 or 4 for site code names.

Figure 3. Example of possible distribution of Stations (i.e. replicate sample locations) near a MW site center

Figure 4. Example of linear distribution of Stations (replicate sample locations) along the shoreline near MW site center.

Figure 5. Diagram illustrating the height of collection vs. highest distribution of mussels.

Figure 6. Instructions and photographs describing proper packaging and mailing labels for MW shipments to laboratories.

17.0 Tables

Table 1. Projected budget for 2011/12 Mussel Watch sampling.

Table 2. Mussel Watch (MW) site sampling schedule for 2011-12 field season. Standard MW protocol indicates that sites should be sampled within a three week window on either side of the target collection date. See Figure 2 map.

Table 3. Measurement quality objectives (MQOs) for NOAA's Mussel Watch Program

Table 4. Location of Mussel Watch site centers (GPS datum set to NAD 1983). Samples are collected on a biennial basis (once every two years in the winter months). See Figure 2 map.

18.0 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 C. Sample Bag Labels

Date: _____	_____
Station #: _____	_____

NS&T Mussel Watch Site Washington State	HISTOPATHOLOGY

Date: _____	_____
Station #: _____	_____

NS&T Mussel Watch Site Washington State	CHEMISTRY

Date: _____	_____
Station #: _____	_____

NS&T Mussel Watch Site Washington State	HISTOPATHOLOGY

Date: _____	_____
Station #: _____	_____

NS&T Mussel Watch Site Washington State	CHEMISTRY

Date: _____	_____
Station #: _____	_____

NS&T Mussel Watch Site Washington State	HISTOPATHOLOGY

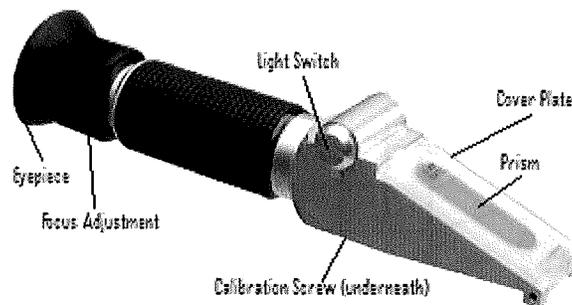
Date: _____	_____
Station #: _____	_____

NS&T Mussel Watch Site Washington State	CHEMISTRY

Appendix E. Using and calibrating a salinity refractometer

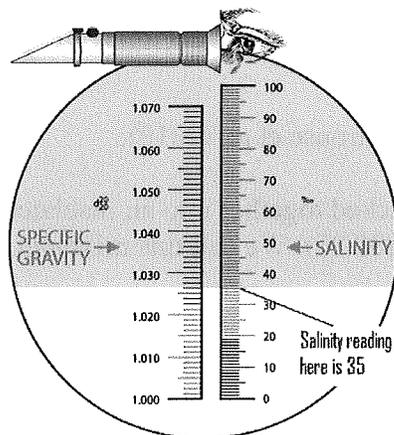
Model used in this study is ZGRS-10ATC, manufactured by Sino Science & Technology Co., Ltd.

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 F. 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. Mussel Watch Analyte List.

Major and trace elements

Symbol	Element	Symbol	Element	Symbol	Element
Al	Aluminum	Si	Silicon	Cr	Chromium
Mn	Manganese	Fe	Iron	Ni	Nickel
Cu	Copper	Zn	Zinc	As	Arsenic
Se	Selenium	Sn	Tin	Sb	Antimony
Ag	Silver	Cd	Cadmium	Hg	Mercury
Tl	Thallium	Pb	Lead		

Polycyclic aromatic hydrocarbons

Analytes	CAS Numbers*	Analytes	CAS Numbers*
Acenaphthene	83-32-9	Fluoranthene	206-44-0
Acenaphthylene	208-96-8	Fluorene	86-73-7
Anthracene	120-12-7	Indeno[1,2,3- <i>cd</i>]pyrene	193-39-5
Benz[<i>a</i>]anthracene	56-55-3	1-Methylnaphthalene	90-12-0
Benzo[<i>a</i>]pyrene	50-32-8	2-Methylnaphthalene	91-57-6
Benzo[<i>e</i>]pyrene	192-97-2	1-Methylphenanthrene	832-69-9
Benzo[<i>b</i>]fluoranthene	205-99-2	Naphthalene	91-20-3
Benzo[<i>k</i>]fluoranthene	207-08-9	Perylene	198-55-0
Benzo[<i>ghi</i>]perylene	191-24-2	Phenanthrene	85-01-8
Biphenyl	92-52-4	Pyrene	129-00-0
Chrysene	218-01-9	1,6,7-Trimethylnaphthalene	2245-38-7
Dibenz[<i>a,h</i>]anthracene	53-70-3	2,6-Dimethylnaphthalene	581-42-0

*Chemical Abstracts Service Registry Numbers

Chlorinated pesticides determined

Analytes	CAS Numbers*
Aldrin	309-00-2
<i>cis</i> -Chlordane	5103-71-9
2,4'-DDD	53-19-0
4,4'-DDD	72-54-8
2,4'-DDE	3424-82-6
4,4'-DDE	72-55-9
2,4'-DDT	58633-27-5
4,4'-DDT	50-29-3
Dieldrin	60-57-1
Endrin	72-20-8
Heptachlor	76-44-8
Heptachlor epoxide	1024-57-4
Hexachlorobenzene	118-74-1
gamma-HCH	58-89-9
Mirex	2385-85-5
<i>trans</i> -Nonachlor	39765-80-5

Polychlorinated biphenyls

Individual congeners	IUPAC Numbers	CAS registry numbers*
2,4'-Dichlorobiphenyl	8	34883-43-7
2,2',5-Trichlorobiphenyl	18	37680-65-2
2,4,4'-Trichlorobiphenyl	28	7012-37-5
2,2',3,5'-Tetrachlorobiphenyl	44	41464-39-5
2,2',5,5'-Tetrachlorobiphenyl	52	35693-99-3
2,3',4,4'-Tetrachlorobiphenyl	66	32598-10-0
3,3',4,4'-Tetrachlorobiphenyl	77(110*)	32598-13-3 (38380-03-9)
2,2',4,5,5'-Pentachlorobiphenyl	101	37680-73-2
2,3,3',4,4'-Pentachlorobiphenyl	105	32598-14-4
2,3',4,4',5-Pentachlorobiphenyl	118	31508-00-6
3,3',4,4',5-Pentachlorobiphenyl	126	57465-28-8
2,2',3,3',4,4'-Hexachlorobiphenyl	128	38380-07-3
2,2',3,4,4',5'-Hexachlorobiphenyl	138	35065-28-2
2,2',4,4',5,5'-Hexachlorobiphenyl	153	35065-27-1
2,2',3,3',4,4',5-Heptachlorobiphenyl	170	35065-30-6
2,2',3,4,4',5,5'-Heptachlorobiphenyl	180	36065-29-3
2,2',3,4',5,5',6-Heptachlorobiphenyl	187	52663-68-0
2,2',3,3',4,4',5,6-Octachlorobiphenyl	195	52663-78-2
2,2',3,3',4,4',5,5',6-Nonachlorobiphenyl	206	40186-72-9
2,2',3,3',4,4',5,5',6,6'-Decachlorobiphenyl	209	2051-24-3

*Chemical Abstracts Service Registry Numbers

Organometallic compounds

Organotins	CAS Numbers*
Monobutyltin trichloride	1118-46-3
Dibutyltin dichloride	683-18-1
Tributyltin chloride	1461-22-9
Tetrabutyltin	1461-25-2

*Chemical Abstracts Service Registry Numbers

Substituted polycyclic aromatic hydrocarbons.

Analytes	Analytes
C1 - Naphthalenes	C4- Phenanthrenes + anthracene
C2 - Naphthalenes	Dibenzothiophene
C3 - Naphthalenes	C1 - Dibenzothiophenes
C4 - Naphthalenes	C2 - Dibenzothiophenes
C1 - Fluorenes	C3 - Dibenzothiophenes
C2 - Fluorenes	C1 - Fluoranthene + pyrenes
C3 - Fluorenes	C1 - Chrysenes
C1 - Phenanthrenes + anthracene	C2 - Chrysenes
C2 - Phenanthrenes + anthracene	C3 - Chrysenes
C3 - Phenanthrenes + anthracene	C4 - Chrysenes

Appendix G. Glossary, Acronyms and Abbreviations, Units

Glossary

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

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

Ambient: Background or away from point sources of contamination.

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

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

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.

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

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

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.

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

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

Data Quality Objectives (DQO) - Data Quality Objectives are qualitative and quantitative statements derived from systematic planning processes that clarify study objectives, define the appropriate type of data, and specify tolerable levels of potential decision errors that will be used as the basis for establishing the quality and quantity of data needed to support decisions. (USEPA, 2006)

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

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 (i.e. replicate location) 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 (i.e. replicate location). (Comparison of the above two values gives the National Mussel Watch project an estimate of where within the intertidal zone mussels were collected.)

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

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

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

Pollution: Such 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.

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

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

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

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

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

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 (0/00 , ppt). (Seawater typically measures around 35 ppt, which is roughly equivalent to 35 pounds of salt per 1,000 pounds of seawater.)

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

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

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

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

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

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

Station – replicate locations where mussels are collected at each site. Mussels are collected at three (3) stations (replicates) 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.

Acronyms and Abbreviations

Following are acronyms and abbreviations used frequently in this report.

COAST	NOAA’s Coastal Ocean Assessments, Status and Trends program
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
GPS	Global Positioning System
i.e.	In other words
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
SOP	Standard operating procedures
STORET	STOrage and RETrieval; a repository for water quality, biological, and physical data managed by the EPA
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