Washington Department of FISH and WILDLIFE

DEPARTMENT OF ECOLOGY
State of Washington

Quality Assurance Project Plan

Status and Trends Monitoring of Marine Nearshore Mussels in the Puget Lowland Ecoregion for Stormwater Action Monitoring (SAM) 2021-2025

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1. Quality Assurance Project Plan Signatures

Status and Trends Monitoring of Marine Nearshore Mussels in the Puget Lowland Ecoregion for Stormwater Action Monitoring (SAM)

January 2022

Approved by:

Signature:	**Date:**
Mariko M. Langness, Marine nearshore mussels study lead/WDFW Author **Signature:**	**Date:**
James E. West, WDFW co-author **Signature:**	**Date:**
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Table of Contents

List of Figures and Tables

Page

Figures

Tables

2. Abstract

This Quality Assurance Project Plan (QAPP) updates a long-term status and trends monitoring study for toxic contaminants in nearshore habitats of Puget Sound. This program is directed by the Stormwater Action Monitoring (SAM), the regional stormwater monitoring program funded by the Phase I Municipal Stormwater permit and the Western Washington Phase II Municipal Stormwater permittees. SAM and the Washington Department of Fish and Wildlife's (WDFW) Toxics-Biological Observation System (TBiOS) implement the monitoring program under a long-standing interagency agreement.

This monitoring program in the Puget Lowland Ecoregion is focused on the health of biota in the marine nearshore, and is designed to provide a regional assessment of whether collective stormwater management actions implemented in the region are leading to improved nearshore contaminants levels.

Mussels were selected as the indicator species, or sentinel, to monitor contaminant conditions in the nearshore. As filter feeders, they ingest particles from the water and accumulate contaminants. This allows them to be used as a means to integrate measurable contaminant conditions over time, overcoming many of the difficulties and limitations related to measuring contamination in receiving waters directly.

Beginning in 2021 and onward this study will continue a previous, successful SAM/WDFW mussel monitoring program, using translocated, caged mussels deployed for 3-month periods every-other-year throughout the Puget Lowland nearshore.

The expansion in scale of this program from the Urban Growth Area to the Puget Lowland nearshore required a redesign, to represent the whole nearshore area in the region and to ensure maximizing statistical power to detect trends in a cost-effective way.

This mussel study design employs a random probabilistic sampling scheme, like the previous monitoring in 2016-2020, with some design adjustments to increase statistical power and monitoring efficiency. Beginning in 2020, the core monitoring design has been modified as follows:

- the study area is extended to cover the whole Puget Lowland nearshore from the Urban Growth Area of Puget Sound,
- candidate sampling sites (master points) in the Puget Sound shoreline have been redrawn using updated high-resolution National Hydrography Dataset (NHD) layer,
- the study area is stratified into four different groups (strata) by estimates of impervious surfaces in watersheds to each master point, with sampling sites selected for each stratum,

- sampling will be conducted every other year at selected sites. Thirty-three sites will be selected each sampling year, comprising a combination of new and revisited sites to improve status assessment and trend detection power.

- reference condition sites will be monitored in each sampling event to establish a better comparison of the results from the sampling sites to a 'least disturbed' condition.

3. Introduction

3.1. Scope of this Quality Assurance Project Plan

This Quality Assurance Project Plan (QAPP) defines the status and trends mussel monitoring in the Puget Sound nearshore (hereafter called mussel monitoring) to be conducted by the Washington Department of Fish and Wildlife (WDFW) staff and volunteers recruited for this purpose. This QAPP describes the geographic scope of the study, study participants, objectives and goals, design, field sampling procedures, laboratory processing of mussels, analytical chemistry, and measurement quality objectives.

3.2. Study Area and Surroundings

Western Washington, particularly the Puget Lowland ecoregion is experiencing increased human population pressure, land-use changes, and urban development. The NPDES Phase I and II municipal stormwater permits and NPDES municipal stormwater permit for WSDOT within the Phase I and II areas (herein 'permits') require flow control and treatment for new and re-development to reduce stormwater runoff and pollutants to receiving waters. Other permit requirements aim to find and control sources of pollutants to the stormwater system. By implementing multiple stormwater management activities, Ecology and the permittees are attempting to reduce stormwater contamination impacts in Puget Sound. The Puget Lowland ecoregion captures much of the urban and urbanizing areas within Phase I and II western Washington coverage and is the focus area of this study (Figure 1).

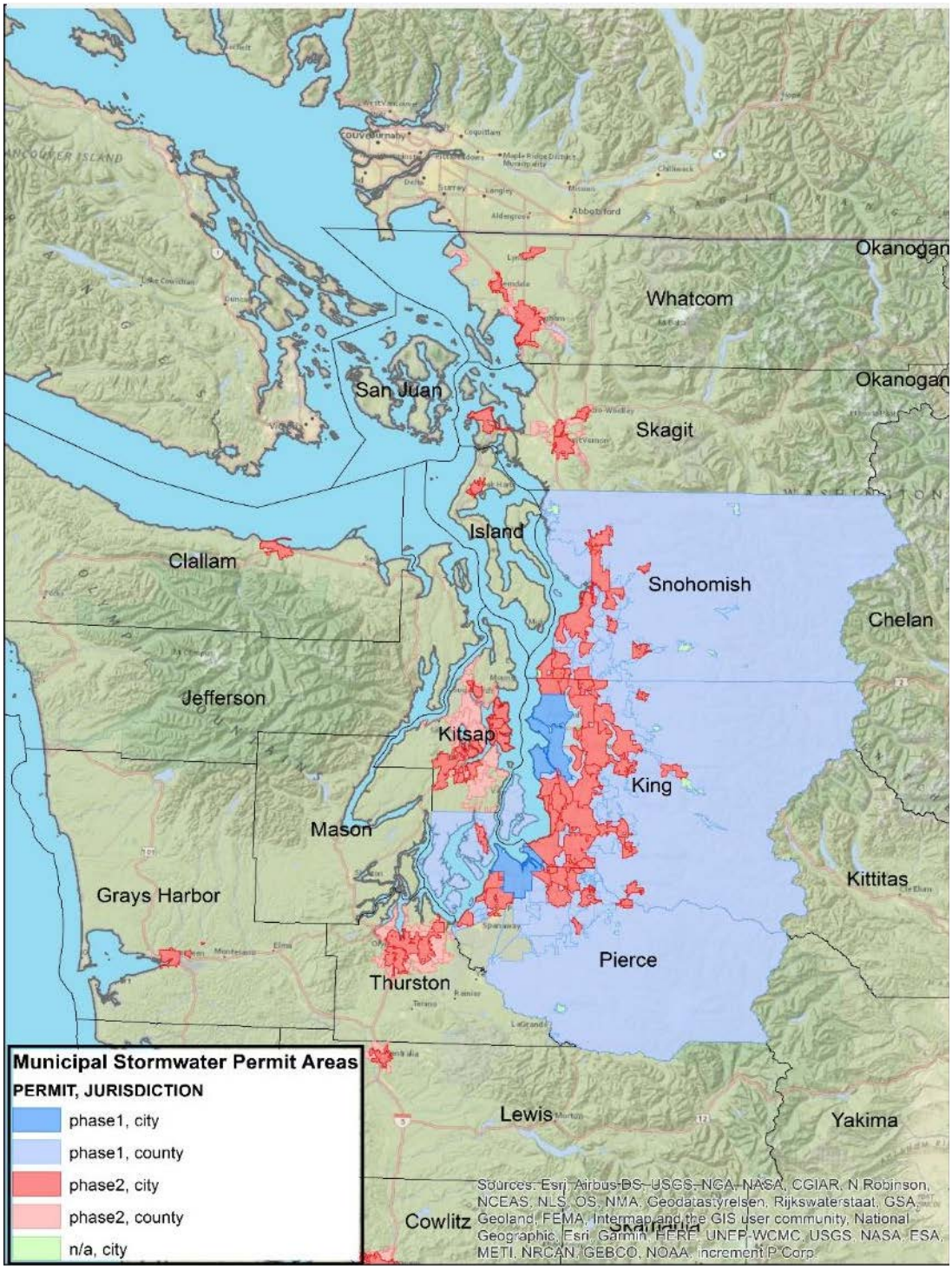

Figure 1. Western Washington Municipal Permit Areas within Puget Sound region.

3.3 Logistics of Field Sampling

Paramount to the success of this mussel monitoring is careful planning to ensure the safety and well-being of its many participants under sometimes challenging field conditions. The foundation of this monitoring program comprises a large, coordinated field effort to deploy and retrieve caged mussels during night-time low tides in the winter, at dozens of sampling sites along the Puget Sound nearshore. This involves coordinating up to over 100 volunteers from a wide range of governmental and non-governmental entities for both the deployment and retrieval of mussel cages. Naturally occurring blooms of harmful algae near the aquaculture facility where mussels originate for this study may delay or prevent the translocation of those mussels. Although this QAPP outlines the planned deployment and retrieval activities, such logistical challenges and unpredictable conditions may necessitate altering those plans. Any significant alterations will be discussed and only implemented under agreement with all program team members.

3.4. Summary of Previous Studies and Existing Data

Under the 2007-2012 Phase I Municipal Stormwater Permit, six permittees and their co-permittees were required to characterize the stormwater quality and quantity from representative municipal stormwater discharges from three urban land uses (commercial, residential and industrial) (Hobbs et al., 2015). The compiled data findings reported high frequencies of detection of conventional parameters, including TSS and nutrients, metals (except mercury), total PAHs, PCB and bis(2-ethylhexyl) phthalate. Most parameters showed significant concentration differences between those land uses and seasonality with higher concentrations during the dry season (May to September) (Hobbs et al., 2015).

Mussels and other sessile bivalve mollusks have been used for decades to monitor toxic contaminants in marine and freshwater habitats in the US and Europe. US The National Oceanographic and Atmospheric Administration (NOAA) began tracking contaminants in US coastal (including Puget Sound) and Great lakes waters in 1986, and NOAA monitored toxics in Puget Sound mussels from that year through 2011. In 2012 NOAA shifted its national Mussel Watch program from directly monitoring mussels to track ambient conditions on a national scale, to supporting sampling design characteristics that address more local or regional needs.

The transition from national to regional scope for mussel monitoring in Puget Sound occurred from 2009, when WDFW conducted the last sampling of naturally occurring mussels (Lanksbury et al. 2012) to 2013, when WDFW conducted a Sound-wide pilot survey to test the concept of deploying/retrieving transplanted mussels (in anti-predator cages) for a prescribed period of time (Lanksbury et al. 2013). The transition to deploying caged mussels allowed greater control over where toxics could be monitored in nearshore habitats. WDFW partnered with SAM soon thereafter to meet toxics monitoring goals for the SAM-NPDES permitting system.

WDFW and SAM have conducted four deployments since 2012, in the winters of 2012/13 (Lanksbury et al. 2014), 2015/16 (Lanksbury et al 2017), 2017/18 (Langness and West 2020), and 2019/20 (Final report in preparation).

Although WDFW and other local sponsors such as tribes, counties, cities, and non-governmental organizations support additional sampling locations and chemical analytes, this QAPP covers only elements supported by the SAM-WDFW interagency agreement.

3.5. Parameters of Interest and Potential Sources

Parameters of key interest in this study are primarily toxic chemicals found in stormwater and able to be passively sampled using mussels. The toxic chemicals analyzed in mussels currently include 6 metals (total mercury, total arsenic, cadmium, copper, zinc, and lead), and 110 organic compounds including 42 polycyclic aromatic hydrocarbons (PAHs), 41 polychlorinated biphenyls (PCBs), 11 polybrominated diphenyl ether (PBDE) flame-retardants, and 17organochlorine pesticides, including six dichlorodiphenyltrichloroethane (DDT) compounds.

These chemicals originate from multiple human and natural sources, and all can be transported from their initial source to Puget Sound waters via stormwater or other transport pathways. All metals measured in this study are naturally occurring elements, but may be altered in form to toxic states or concentrated to toxic levels by human activities. The organic compounds are typically referred to as persistent organic compounds (POPs). PAHs originate from burning fossil fuels (pyrogenic) or directly from petroleum (petrogenic). All PCBs, most PBDEs, and all DDTs are synthetic compounds, created by humans for various industrial, agricultural, or other purposes.

With these chemical parameters, biological metrics of mussels and landscape information describing site-specific or watershed level characteristics (e.g., shoreline type, substrate type, and potential visible contaminant sources) are collected to help interpret the chemical results.

3.6. Criteria and Standards for Status Assessment

Mussels are used to measure contaminants in nearshore habitats because they bioconcentrate many chemical compounds, integrating conditions organisms experience over a period of time. These tissue contaminant levels are ecologically relevant, representing realistic body burdens similar species may experience in this habitat.

The deployment duration for mussels in this study is three months. Although three month long deployed mussels may not capture the true body burdens that may accumulate in resident species over longer period of time, three months in wet season represents a period of time sufficient for mussels to accumulate measurable contaminants (if present) carried by stormwater, while minimizing the risk of losing the cages to storms, theft, and other disturbances. Tissue contaminant results in mussels deployed for this three-month period are most appropriately analyzed by comparing tissue concentrations:

- with the initial condition (concentration) of each contaminant at deployment (to estimate the amount of the 3-month accumulation),
- with contaminant concentrations in mussels deployed at reference sites thought to be the least contaminated in the region (to estimate contaminant accumulation, accounting for all metabolic processes and field conditions occurring in the deployment period),

- to the condition of mussels, including mortality, body condition, and growth (to correlate mussel condition with contaminant loads), and
- between sites across the full range of urbanization to evaluate correlations between contaminants and possible sources.

Deploying pre-reproductive mussels of similar size and identical background (initial) conditions synoptically over the nearshore sampling region over a fixed period minimizes variability in contaminants related to these factors, which may otherwise reduce the statistical power to recognize spatial or temporal trends, when they exist.

SAM identified two least-disturbed reference condition sites that have consistently exhibited low contamination levels in previous monitoring (Langness and West 2020), to represent least-disturbed baseline condition. These sites will be included in all deployments and will follow the same monitoring procedures described herein. A third reference condition site will be determined after completion of the 2021-2022 survey.

Table 1. List of reference sites in the Puget Lowland sampling region.

Site Name	Latitude	Longitude	County
Penn Cove	48.21423	-122.71897	Island
Hood Canal (Holly)	47.57060	-122.97170	Kitsap
TBD			

4. Project Overview

4.1. Project Goals

The goal of the SAM nearshore mussel monitoring is to provide statistically valid estimates of status and trends of chemical and biological conditions in stormwater receiving waters in the nearshore of the Puget Lowland ecoregion.

The probabilistic study design, selected monitoring parameters and indicators, and frequency of monitoring are designed to develop unbiased regional assessment of the health of biota in these receiving waters in a cost-effective way.

Findings will inform the permittee's stakeholders, and the public on the stormwater impact to nearshore and which contaminants are delivered by stormwater in high concentrations, so that stormwater management decisions can be adapted and implemented to protect nearshore ecosystems including biota.

4.2. Project Objectives

Using the system of deployed mussels and a probabilistic sampling design described herein, the specific objectives of this monitoring are to:

- describe the geographic patterns of contamination in nearshore biota, represented by bay mussels,
- identify the full range of contamination across the study area,
- establish location-specific contaminant status baselines conditions,
- determine whether contaminants are increasing or declining in the nearshore over time,
- correlate contaminant patterns with land-use patterns to inform evaluation of contamination sources and best management practices to reduce contamination of stormwater,
- correlate contamination with mussel health metrics to infer potential contaminant impacts on mussels.

5. Organization and Schedule

5.1. Key Individuals and Their Responsibilities

WDFW is responsible for implementing the authorized SAM project as described in this document. WDFW staff and the volunteers they manage conduct all monitoring activities, lab analyses, data analysis, and report writing. Table 2 lists key WDFW and Ecology staff responsible for activities detailed in this QAPP.

Table 2. Organization of monitoring team members and responsibilities.

Staff	Title	Responsibilities
Mariko Langness WDFW - TBiOS Mariko.Langness@dfw.wa.gov 360.688.4837	WDFW Project Lead	Coordinates project objectives, budgets, and study design with SAM staff. Implements and oversees all WDFW staff, volunteers, and project activities including site evaluations, mussel deployments and retrievals, tissue preparation, and analytical chemistry. Conducts QA review of data with WDFW Data Coordinator. Completes data analysis and writes QAPP and all technical reports with review input from SAM staff. Completes oral and written presentations of survey results.
James E. West WDFW - TBiOS James.West@dfw.wa.gov 360.870.8303	WDFW Project co-lead	Oversees and supports Project Manager

Staff	Title	Responsibilities
Louisa Harding WDFW – TBiOS Louisa.Harding@dfw.wa.gov 360.480.2882	WDFW Data Coordinator	Assists with the compilation and QA review of chemistry data received from analytical laboratories. May provide support with data analysis.
Danielle Nordstrom WDFW – TBiOS Danielle.Nordstrom@dfw.wa.gov 360.628.0971	WDFW Field and Lab Coordinator	Coordinates project field planning including evaluating new sites, obtaining site permits/permissions, recruiting and managing volunteers for mussel bagging, cage deployment/retrieval and tissue preparation. Transcribes any paper field and lab (tissue preparation/CI/mortality) data sheets into spreadsheets. Conducts QA review of site data/coordinates. Submits QA reviewed data to Ecology's Environmental Information Management (EIM) system.
Rob Fisk WDFW – TBiOS Robert.Fisk@dfw.wa.gov 360.688.4841	WDFW Field and Lab Staff	Assists with implementing field work including mussel bagging, cage deployment/retrieval, and tissue preparation.
Andrew Beckman WDFW – TBiOS Andrew.Beckman@dfw.wa.gov 360.485.5410	WDFW Field and Lab Staff	Assists with implementing field work including mussel bagging, cage deployment/retrieval, and tissue preparation.
Keunyea Song Ecology-WQP Keunyea.Song@ecy.wa.gov 360.407.6158	SAM scientist / Project manager	Manages the study, select sampling sites, write QAPP, and manage study related contracts, and provide technical support.

WDFW will coordinate with an aquaculture facility to provide mussels for the monitoring sites. WDFW will contract and coordinate with analytical laboratories for all mussel tissue chemistry analyses.

WDFW will obtain a Hydraulic Project Approval (HPA), a Shellfish Transfer Permit, and a Memorandum of Understanding (MOU) with the Washington Department of Natural Resources (DNR) to access State-Owned Aquatic Lands (SOAL) for all mussel monitoring activities. WDFW staff and volunteers will perform reconnaissance and verification of the sites, respectively, and acquire any *other* permits or permissions (outside those listed above) necessary

to access their approved sites, including but not limited to permission to access privately-owned, city, county, port, or tribal property, or state or federal park lands.

WDFW will process all mussels for biological and chemical analysis, compile the results, conduct a quality assurance (QA) and quality control (QC) review of the data, and submit the QC'ed data to EIM. SAM project manager will review uploaded EIM data and notify WDFW of any problems regarding data quality.

5.2. Special Training and Certifications

The WDFW will provide training for WDFW staff and volunteers regarding mussel cage deployment, retrieval and the field survey. This training will take the form of a webinar or document (i.e., self-train) to ensure comparability of results for both programs. WDFW staff and/or volunteers are required to have the means to transport their mussels to the WDFW Marine Resources Laboratory in Olympia for processing.

Any necessary training for study design, statistical tools, analyses or data evaluation are given by WDFW staff or SAM staff as needed throughout the monitoring period as technology evolves or as staff changes.

5.3. Key Monitoring Activities and Reports

Table 3. Proposed schedule for key field and laboratory activities, and reports.

Activities/Reports	Description	Target Dates 2021-2022 Survey	Target Dates 2023-2024 Survey
Site evaluation	Site suitability for sampling including permission, accessibility and other criteria. Evaluate and finalize the sampling site list through GIS image checks and site visits.	September 2021	June - August 2023
Final site list/map	Memo summarizing site evaluation process and final site list with detailed information including landscape site names and coordinates of confirmed and rejected sites. Map of confirmed sites.	October 2021	September 2023
Preparation of mussels for cages	Measure and bag mussels supplied to WDWF staff by the aquaculture facility. Bagged mussels re-hung at aquaculture floats to acclimate before deployment.	October 2021	October - November 2023
Mussel cage deployment	Mussels deployed; subsamples taken for evaluation of initial contaminant condition	November 2021	November – December 2023
Mussel cage retrieval	All mussels retrieved from the field and transported to WDFW's laboratory in Olympia, WA. Mussel subsamples taken for biometric measurements, all remaining frozen for later processing.	January/February, 2022	January/February 2024
Composite tissue samples created	Frozen mussels are thawed, composite samples created, and samples frozen for analytical chemistry	February - April, 2022	February – April 2024
Samples submitted to contract labs	Organics to NOAA Fisheries, metals to King County Environmental Labs	April 2022	April 2024
Data posted to WDFW	WDFW receives contaminant data from contract labs.	September - October 2022	September – October 2024
Data reviewed and analyzed	Data undergoes QA/QC and submitted to EIM. Meet with SAM staff to review/discuss data	November - December 2022	November - December 2024

Monitoring report draft to SAM for review	SAM and WDFW staff review draft survey report	April 2023	April 2025
Final report published	Final report published on WDFW web site	June 2023	June 2025

6. Study Design

6.1. Study Boundaries

This QAPP details a new sampling area starting in 2021 which targets the entire nearshore of the Puget Lowland ecoregion. Past sampling conducted between 2016 and 2020 covered the SAM-defined Urban Growth Area (UGA) with ca. 1638 km nearshore length, a portion of Puget Sound nearshore of 3632 km.

The total nearshore length and area was identified using the high-resolution (1:24k or higher) National Hydrography Dataset (NHD) layer (Figure 2). The coastline (redline in Figure 2) is a representation of the Mean High Water (MHW) as calculated by NOAA.

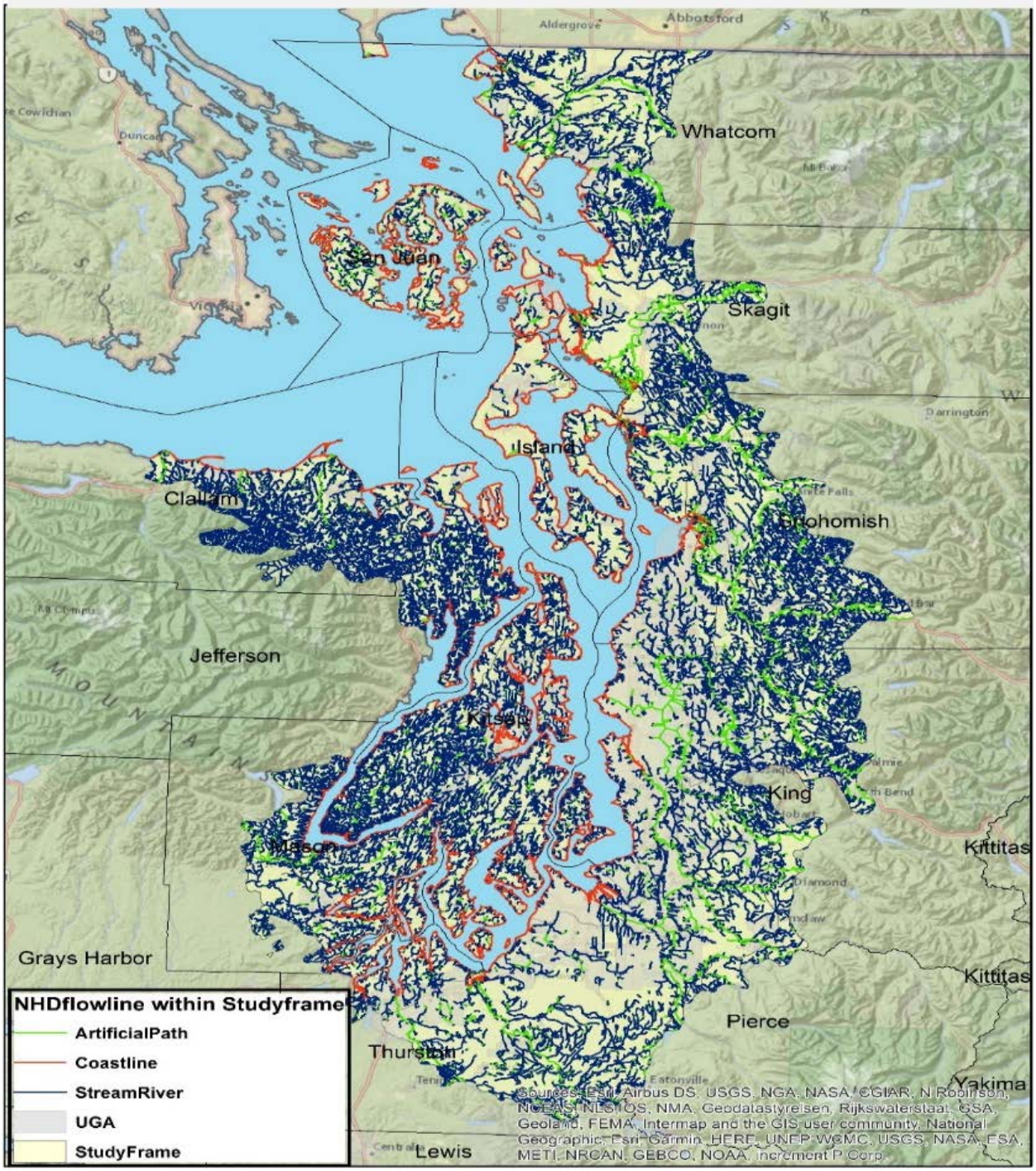

Figure 2. NHD flow line within the study frame, Puget lowland ecoregion.

6.2. Site Selection

6.2.1. Probabilistic Sampling Design

The SAM Nearshore Mussel study will continue to use EPA's Generalized Random Tessellation Stratified (GRTS) survey design, as was done in previous monitoring, to select spatially balanced random sampling sites in the study frame.

The GRTS study design facilitates unbiased extrapolation of any measured indicators, mussel tissue contamination level in this study, from the sites sampled to estimates of the status of the extent of the whole represented the region, that is, the nearshore of Puget lowland ecoregion (Figure 2).

6.2.2. Target Population and Site Selection Process

Master points

The master sample points are potential sampling sites generated at every 800 meters along the coastline of the Puget lowland region, which means each point represents an average coastline length of 800 meters; a GRTS-computed weight for each site became 799.8942 m. WDFW advised to use an 800 m length of shoreline to represent a mussel site based on criteria used by the National Centers for Coastal Ocean Science's COAST National Status & Trends Mussel Watch Contaminant Monitoring program. This shoreline length was also supported by results from a mussel contaminant study conducted in 2012/13 by the Tacoma Pierce County Health Department in collaboration with WDFW (Callahan, Hanowell, Jensen, 2014).

Ecology recently (2019) re-generated the Washington State Master sample points using the high-resolution (1: 24k or higher) National Hydrography Dataset (NHD) layer (Figure 2). The study boundary covers 4540 nearshore master points (Figure 3). In previous monitoring, the SAM nearshore studies used the master sample generated at every 800 meters from a medium-resolution DNR generated flow line layer (1:100K).

Final target population points were selected by filtering through site selection criteria listed below.

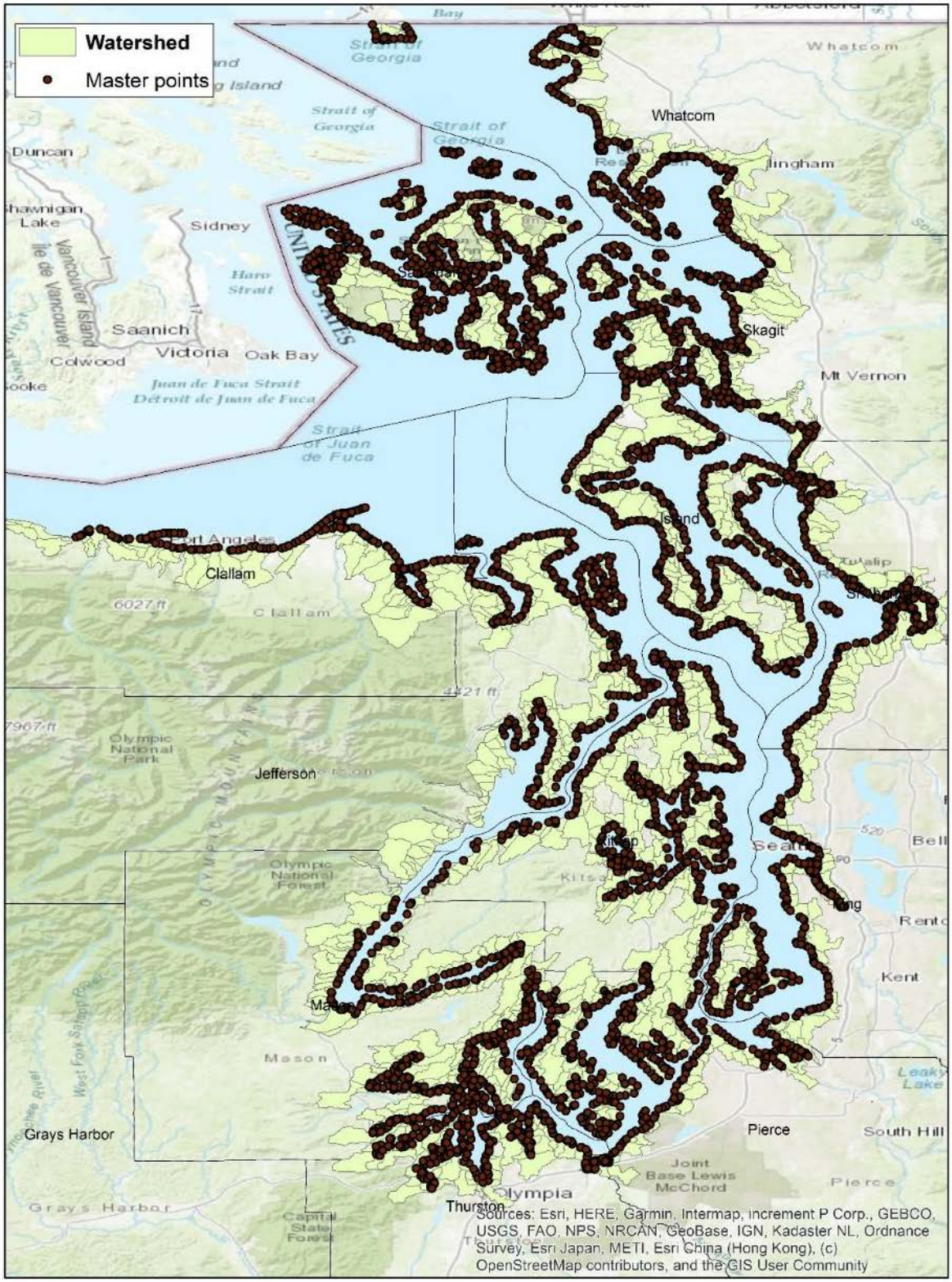

Figure 3. Puget Sound nearshore master points from 2020

Contributing watershed delineation and land cover information

The contributing watersheds to each nearshore sample points are the subset of assessment units in the Puget Sound Characterization project (Hume et al., 2019). The assessment units in the Puget Sound Characterization project were modified from the Salmon and Steelhead Habitat Inventory and Assessment (SSHIAP) catchments. These nearshore assessment units in the Puget Sound Characterization projects represent the local nearshore geomorphic and hydrological properties such as tidal influence, longshore currents and interfluvial influences well.

The land-use characteristics including land-use type and averaged impervious cover (%) were determined for each watershed using 2016 National Land Cover Data. Metadata for nearshore watersheds and their land-use characteristics are available through USGS web portal, ScienceBase (https://doi.org/10.5066/P9MIRRWL; Headman, 2020).

Freshwater influenced areas

As the NHD coastline represents Mean High Water (MHW) level, some coastlines with high tidal influence reach upstream freshwater bodies where salinity levels are likely too low to support bay mussels (*Mytilus trossulus*). Unfortunately, there is no available information or GIS layer that shows salinity levels or another coastal waterline; therefore, freshwater influenced areas by high tidal activities were identified using best professional judgment between the SAM scientist and the WDFW project lead. These high freshwater influence areas are mainly estuarine wetland areas. This criterion removed 362 master points, resulting in a total of 4178 points available for the next site selection process.

Marinas, Ports, and Other potential contamination sources

Marinas, ports, and other nearshore activities or structures may be significant local point sources of contamination that could mask stormwater contamination originating from NPDES sources. Any shoreline points near (100 m radius) a marina or a port were removed from the candidate sampling site list.

Accessibility

Some small islands in Puget Sound such as Waldron Island are not easily accessible by commercial ferries. Points on islands lacking ferry service will not be sampled. Shoreline points on Point Roberts that require Canadian border crossing were also excluded from the candidate sampling site list.

Watershed size criteria (0.5-70km^2)

The three criteria above removed 1079 master points, making a total of 3461 points available for watershed size criteria application.

This SAM nearshore mussel study focuses on relatively small watershed areas to increase the chance of detecting stormwater impacts on contaminant levels and recovery signals related to stormwater management efforts.

Another related SAM receiving water monitoring study in the region, the SAM Puget Small Streams called PSS, targets small streams with watershed sizes ranging from 0.5 to 70 km^2. This study will follow the same watershed size criteria. While the PSS study mainly excluded points in large watersheds, the watersheds of nearshore points are much smaller compared to stream points, with a maximum watershed size of 20.44 km^2. Therefore, this watershed size criterion filtered the master sample points in a watershed less than 0.5km^2.

After applying this criterion, the total number of candidate sites within the study frame was reduced to 3221 (Table 4, Figure 4).

Table 4. Summary of final candidate points within the study area.

Watershed size (km^2)	# Of points	Min	1st Quantile	Median	Mean	3rd Quantile	Max
SAM candidate points with watershed areas greater than 0.5 km^2	3221	0.67	2.00	2.75	3.26	3.66	20.44

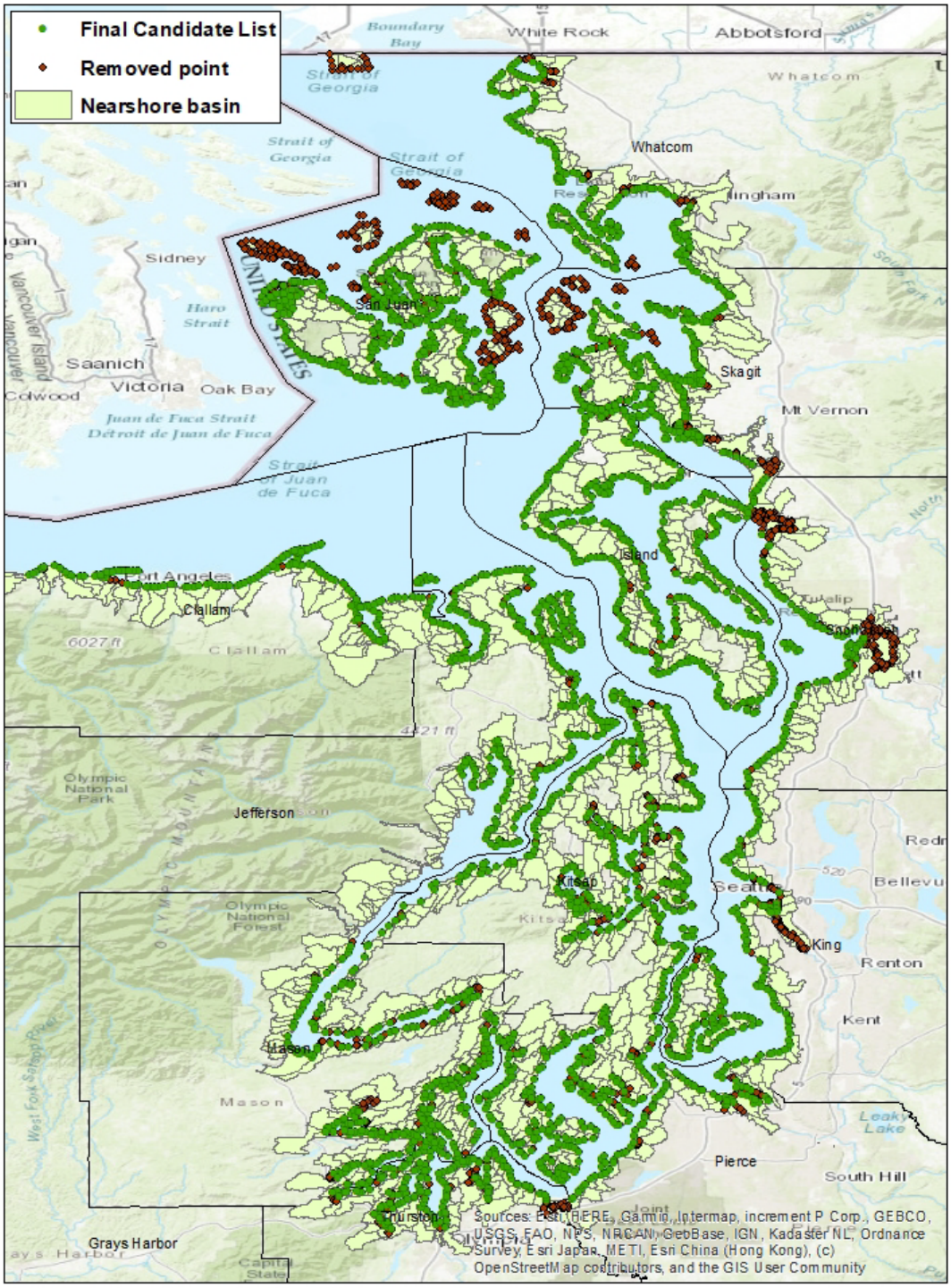

Figure 4. Location of final candidate sites and dropped sites.

6.2.3. The Previous Sampling Site Selection

The previous sampling sites sampled in 2015/16, 2017/18 and 2019/20 were from a DNR flow line layer with medium resolution. Given that revisiting previous sampling sites improves the time-trend detection power of the ongoing study, the previous sampling sites that meet the site selection criteria are included in this monitoring starting from 2021.

The past sampling sites were evaluated in the same way as described above for candidacy in the new candidate sampling list. Land-use and impervious cover of watershed of each of previous sampling sites were also incorporated using 2016 NLCD. As a result, 39 sites out of 43 previously sampled sites were included in the candidate sampling site list. Four sites were removed due to history of mussel cage loss related to strong currents.

6.2.4. Stratification

Impervious surface cover can serve as an indicator of stormwater influence to receiving waters. For this study, the candidate sites as well as selected past sampling sites have been stratified into four strata using average percentage of total impervious cover of the contributing watershed (Figure 5).

The four strata for impervious surface cover are:

- Least: <10 %
- Low: 10 to <20 %
- Medium: 20 to <40 %
- High: 40 to 100 %

This stratification was necessary because most of the study area is still undeveloped, with 76% of sites classified as exhibiting the "least" impervious cover (Table 5), and where contamination is likely low. This characteristic of the study area is due to unique geography of the region, with relatively small shoreline lengths draining large urbanized, residential, or agricultural watersheds, and most of the Puget Sound shoreline draining large areas of forested and undeveloped areas. In order to focus in on contamination in a cost-effective way, we used the impervious cover attribute for all the candidate sites to describe four strata which are used to ensure an adequate number of streams in medium and highly developed areas are sampled every year.

Table 5. Number of candidate sites in each percent impervious surface strata.

Strata	0 - <10 %	10 - <20 %	20 - <40 %	40 – 100 %	Total
Number of new SAM candidate points	2457	346	290	128	3221
Carry forward past sampling sites	8	8	14	9	39

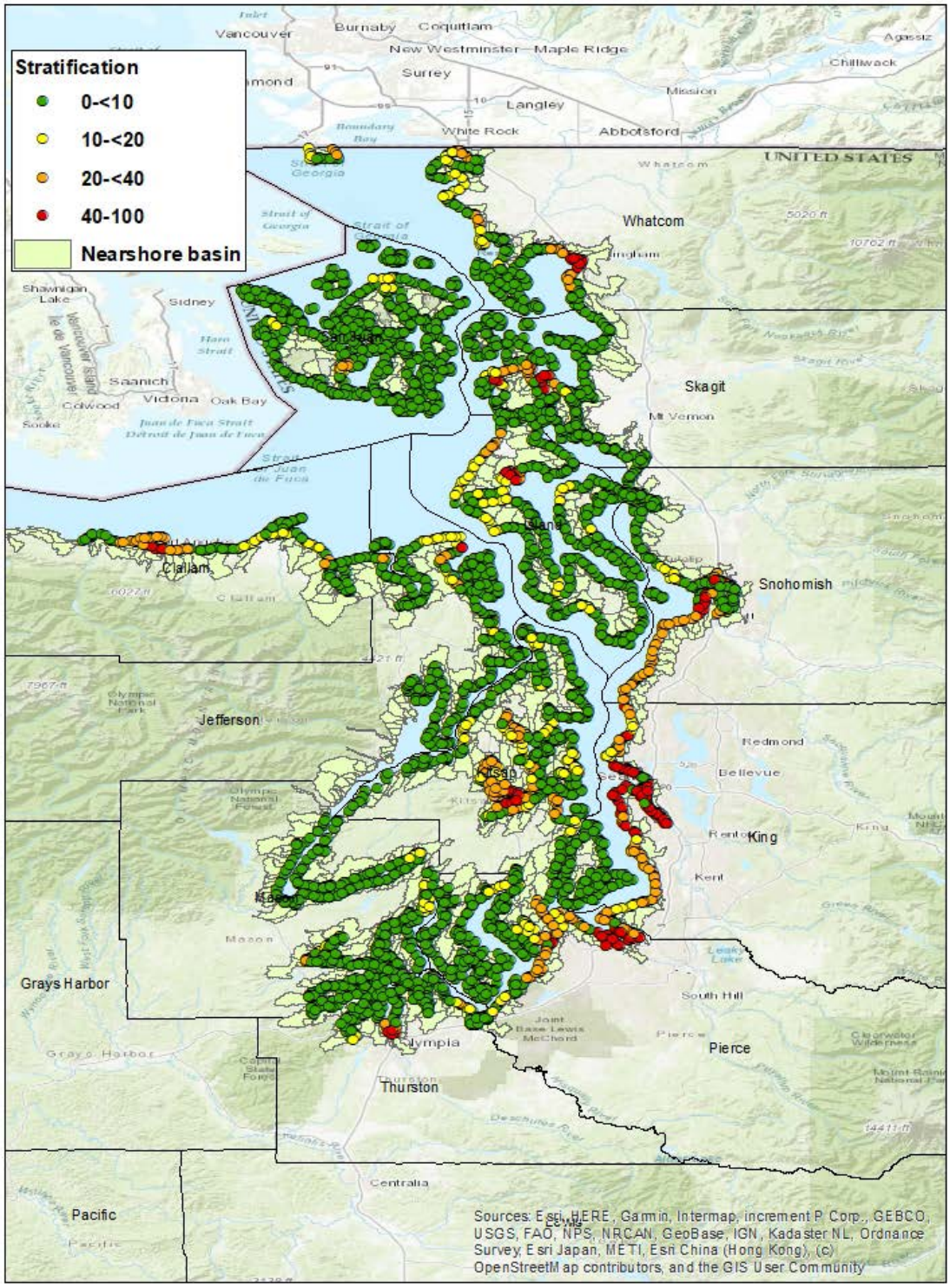

Figure 5. Location of points distributed among four impervious surface strata.

6.2.5. Study Panel Design

A panel of sites is a set of sites that are all visited together at their initial sampling year, and then all or a portion of it are revisited in specific subsequent years. Each panel will be sampled three times on six-year intervals. Each year, new sites (identified in a new panel) from the master point list are continually added (Table 6). This panel design is meant to adequately characterize the large nearshore shoreline length in the region with continuous addition of new sites, while maximizing power for trend detection by revisiting each site over a 12-year period.

This panel design selects spatially balanced sites from each impervious cover-based stratum as well as alternative sites in case selected sites are not suitable or rejected during the candidate site evaluation process.

The Panel 1 site list, which will be sampled in 2021, 2027 and 2033 is a "transition" panel; this will be a combination of past sampling sites and new sites. Having past sampling sites in Panel 1 will provide continuity with past sampling conducted between 2015 and 2020, enabling a longer time-trend tracking period. Given that past sampling sites were selected using the same GRTS principle, it is safe to assume that they could represent the nearshore in the sampled region. However, the past sampling study frame was limited to Urban Growth Area (UGA) which is a smaller area of the Puget lowland nearshore, and so prevents fully evaluating trends with the new extended entire Puget lowland nearshore. Therefore, when past sampling sites are sampled, which is for Panel 1, it will always be a combination of past sampling sites and new sites. As a result, only 15 past sampling sites will be visited in 2021 out of 39 available past sampling sites.

Table 6. Panel design for the monitoring*

Year	Past	2021	2023	2025	2027	2029	2031	2033	2035	2037	2039	2041	2043	2045
Panel														
1	43	15, 18			22			11						
2			33			22			11					
3				33			22			11				
4					11			11			11			
5						11			11			11		
6							11			11			11	
7								11			11			11
8									11			11		
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*Each year starting from 2021, a total of 33 sites will be sampled. Panel 1 in 2021 is a combination of a subset of past sampling sites (colored in red), and new selected sites (colored in blue). Only a portion of panel 1 to panel 3 will be revisited between 2027 to 2031 in order to add new panels for each given year's sampling. Numbers in blue indicate new sites added for each sampling event whereas numbers in green represent third and last visit of the same panel. Once a site is visited three times, it will be dropped from the study design. Second visit sites are displayed in black.

6.3. Site Evaluation

The suitability of a mussel sample site will be further determined using the criteria outlined below. An initial assessment of each candidate site will be conducted remotely via computer (maps, public records, etc.) to see if there are any obvious criteria that cannot be met. If the site is deemed viable, field crews will visit the candidate site and further evaluate the suitability criteria at the site center. If the site center is not suitable, then the field crew will evaluate conditions up to 400 meters (1312 feet or 0.25 mile) in either direction along the shoreline until the closest suitable location relative to the site center is found.

If a location other than the site center is chosen, then the reason for disqualification of the site center must be documented and the alternate site coordinates must be recorded. If all 800 m of a candidate site are not suitable, then the reason for disqualification must be documented in the site evaluation form and/or field log notebook including photos. Alternate candidate sites must then be visited in numerical order under each stratum from the candidate site list and verified for replacement.

The list of candidate sites in Table 7 will be evaluated for sampling in 2021 and the list of candidate sites in Table 8 will be evaluated in 2023. Finalized sampling sites will be posted on the SAM status and trends webpage.

Suitability of a candidate site is determined by the following criteria:

- Condition 1 - the site can be safely accessed and worked on in the winter, during night-time low tides, and
- Condition 2 - permission of property owners and/or tenants is granted prior to sampling, and
- Condition 3 - there is suitable substrate or a location for anchoring/securing a mussel cage at the site.

Accessibility Criteria

These criteria concern whether access to a candidate site is permitted by the landowners, and if the site can be safely accessed and sampled throughout the year. A site may also be deemed unsuitable or impracticable for sampling if more than one hour is required to access the site from the nearest parking location.

Permission

If the mussel cage is to be placed on private or commercially owned tidelands, or private property must be traversed to gain access to public tidelands, permission must be granted from the landowner(s) prior to monitoring. Useful shoreline information can be gained from a remote evaluation of candidate sites via computer (e.g., search Google maps, public records, etc.) and a good-faith effort to contact owners or tenants. In some cases, it might be necessary to obtain a special license, easement, or other legal document from a commercial or government property (e.g., Port Authority, City/County park, Tribe, etc.) to access and place a mussel cage on their property.

Property owners will be contacted well in advance of (i.e., several months before) cage deployment. This will ensure adequate time to explain the needs and timing of the study and to obtain permission to access

the property during night-time low tides. In some cases, keys or gate codes may be necessary to allow field crew access after business hours. Property owners should be reminded the day before mussel cage deployment and removal that workers will be on their property soon.

Permits

WDFW will obtain a blanket HPA, Shellfish Transfer Permit, and Memorandum of Understanding with the DNR to access SOAL for all SAM mussel monitoring activities. WDFW is also responsible for obtaining any *other* permits or permissions (outside those listed above) necessary to conduct mussel monitoring work at the SAM approved sites, including but not limited to site access permits for privately-owned, city, county, port authority, or tribal properties, or state or federal lands. For instance, a Scientific Research Permit is required when conducting research (including mussel monitoring) within the boundaries of a Washington State Park. Application for this permit must be sent to Washington State Parks (http://www.parks.wa.gov/stewardship/) at least two weeks prior to mussel monitoring.

Safety

Field work, particularly in coastal environments, has an inherent risk of danger and environmental conditions can often be unpredictable. Mussel site reconnaissance, deployment, and retrieval pose several potential safety hazards including unstable terrain (e.g., deep mud or cobbles/boulders), incoming tides, breaking waves, exposure to extreme temperatures, and sudden changes in weather. Field crews will evaluate each candidate site for safety. Appropriate reasons for disqualifying a candidate site for monitoring may include:

- route of entry or intertidal area is unstable or unsafe (e.g., sucking mud, quicksand),
- hostile people or animals are present.

Intertidal Physical Criteria

These criteria concern the conditions of the intertidal substrate at a candidate site for mussel monitoring. To be considered suitable for mussel cage placement, the intertidal area at the candidate site's center (or within 400 meters of the site center) must:

- have a substrate (i.e., mud, sand, cobble) into which a helical/screw anchor or rebar stakes can be driven, to secure the mussel cage, OR
  have some kind of structure to which the mussel cage can be tied or secured (e.g., steel or concrete pilings or other fixed points on-site) – this is especially important in high energy environments. *However, no cages will be affixed to or placed next to creosote-treated material.*

Table 7. Candidate site list for 2021-2022 sampling.

No.	Strata	Location ID	Site Name	Longitude	Latitude	Watershed (km^2)	Impervious (%)	County	Past or New sampling site?
1	[0,10]	SAM-1001	Williams Olson Park	-122.56698	47.66586	1.49	2.85	Kitsap	Past
2	[0,10]	SAM-1002	Brackenwood Ln	-122.50640	47.68234	4.10	6.52	Kitsap	Past
3	[0,10]	SAM-1003	S of Skunk Island	-122.75076	48.02667	2.63	5.27	Jefferson	Past
4	[0,10]	SAM-1004	Chuckanut, Clark's Point	-122.50425	48.69068	3.71	5.77	Whatcom	Past
5	[0,10]	SAM-1040	Dungeness	-123.09987	48.13662	2.81	8.48	Clallam	New
6	[0,10]	SAM-1041	Discovery Bay	-122.86012	48.06718	1.94	5.10	Jefferson	New
7	[0,10]	SAM-1042	Squaxin Island	-122.90465	47.17650	1.05	0.00	Mason	New
8	[0,10]	SAM-1043	Eld Inlet	-123.00398	47.07439	2.42	2.58	Thurston	New
9	[0,10]	SAM-1044	Polnell Point	-122.55966	48.27247	5.85	7.05	Island	New
10	(10,20]	SAM-1009	Salmon Beach	-122.53053	47.29464	1.98	12.36	Pierce	Past
11	(10,20]	SAM-1010	Admiralty Inlet	-122.76217	48.13083	1.24	19.39	Jefferson	Past
12	(10,20]	SAM-1011	Skiff Point	-122.49884	47.66142	2.88	10.37	Kitsap	Past
13	(10,20]	SAM-1012	Eastsound, Fishing Bay	-122.90985	48.69368	2.33	12.07	San Juan	Past
14	(10,20]	SAM-1314	North Camano	-122.51211	48.25451	1.86	14.77	Island	New
15	(10,20]	SAM-1315	Reach Island	-122.82253	47.34649	2.03	11.18	Mason	New
16	(10,20]	SAM-1316	Fort Worden	-122.77387	48.14311	1.24	19.39	Jefferson	New
17	(10,20]	SAM-1317	Friday Harbor	-123.01830	48.50669	2.37	11.82	San Juan	New
18	(10,20]	SAM-1318	Tulalip Reservation	-122.29978	48.06979	3.25	14.16	Snohomish	New
19	(20,40]	SAM-1017	N Avenue Park	-122.61531	48.52108	2.40	37.20	Skagit	Past
20	(20,40]	SAM-1018	Port Angeles Yacht Club	-123.45715	48.12823	2.20	37.52	Clallam	Past
21	(20,40]	SAM-1019	Kitsap St Boat Launch	-122.64034	47.54167	2.41	30.32	Kitsap	Past
22	(20,40]	SAM-1020	Rocky Point	-122.66992	47.60255	3.42	22.89	Kitsap	Past
23	(20,40]	SAM-1588	Blaine	-122.75053	48.98704	1.95	32.60	Whatcom	New
24	(20,40]	SAM-1589	Three Tree Point	-122.37077	47.44853	2.54	24.68	King	New
25	(20,40]	SAM-1590	Priest Point Park	-122.89893	47.06997	2.99	25.19	Thurston	New
26	(20,40]	SAM-1591	Cap Sante	-122.59955	48.51879	2.40	37.20	Skagit	New
27	(20,40]	SAM-1592	Locust Beach, Bellingham	-122.53787	48.77637	3.43	30.87	Whatcom	New
28	(40,100]	SAM-1031	Elliott Bay, Harbor Island, Pier 17	-122.35065	47.58766	1.64	94.39	King	Past
29	(40,100]	SAM-1032	Arroyo Beach	-122.38593	47.50161	4.90	40.04	King	Past
30	(40,100]	SAM-1033	Blair Waterway	-122.41730	47.27568	8.93	77.19	Pierce	Past
31	(40,100]	SAM-1862	Harbor Island Shipping	-122.34605	47.58223	1.64	94.39	King	New
32	(40,100]	SAM-1863	West Bay Park	-122.91257	47.05337	1.96	41.01	Thurston	New
33	(40,100]	SAM-1864	Lions Park	-122.64146	47.58335	4.75	43.69	Kitsap	New

Table 8. Candidate site list for 2023-2024 sampling.

No.	Strata	Location ID	Site Name	Longitude	Latitude	Watershed (km^2)	Impervious (%)	County	Past or New sampling site?
1	[0,10]	SAM-1045	TBD	-122.70356	47.91770	3.04	6.12	Jefferson	New
2	[0,10]	SAM-1046	TBD	-122.45389	47.39477	1.76	8.66	King	New
3	[0,10]	SAM-1047	TBD	-122.51007	48.44753	4.64	2.60	Skagit	New
4	[0,10]	SAM-1048	TBD	-122.97786	48.60677	2.22	2.52	San Juan	New
5	[0,10]	SAM-1049	TBD	-122.68510	47.84649	2.21	0.82	Jefferson	New
6	[0,10]	SAM-1050	TBD	-122.58444	47.56942	3.93	9.17	Kitsap	New
7	[0,10]	SAM-1051	TBD	-122.45216	48.22565	3.02	4.99	Island	New
8	[0,10]	SAM-1052	TBD	-122.87905	48.62828	1.66	1.02	San Juan	New
9	[0,10]	SAM-1053	TBD	-123.05138	47.45363	2.97	0.84	Mason	New
10	(10,20]	SAM-1319	TBD	-122.50862	47.67040	2.88	10.37	Kitsap	New
11	(10,20]	SAM-1320	TBD	-122.60842	47.70776	5.78	12.22	Kitsap	New
12	(10,20]	SAM-1321	TBD	-122.65536	48.24818	2.74	15.16	Island	New
13	(10,20]	SAM-1322	TBD	-122.73131	47.10239	3.04	14.52	Thurston	New
14	(10,20]	SAM-1323	TBD	-122.37584	47.73764	2.84	14.05	King	New
15	(10,20]	SAM-1324	TBD	-122.58941	47.90763	2.07	14.05	Kitsap	New
16	(10,20]	SAM-1325	TBD	-122.76890	48.90521	1.86	13.08	Whatcom	New
17	(10,20]	SAM-1326	TBD	-122.54632	47.31479	1.98	12.36	Pierce	New
18	(10,20]	SAM-1327	TBD	-122.74831	47.71869	1.69	14.34	Kitsap	New
19	(20,40]	SAM-1593	TBD	-122.35751	47.33703	2.91	31.23	King	New
20	(20,40]	SAM-1594	TBD	-123.40999	48.14173	2.20	37.52	Clallam	New
21	(20,40]	SAM-1595	TBD	-122.76563	48.04089	0.85	24.31	Jefferson	New
22	(20,40]	SAM-1596	TBD	-122.53699	47.56724	6.17	20.13	Kitsap	New
23	(20,40]	SAM-1597	TBD	-122.57250	47.26014	4.27	23.73	Pierce	New
24	(20,40]	SAM-1598	TBD	-123.05093	48.08490	11.98	20.93	Clallam	New
25	(20,40]	SAM-1599	TBD	-122.32638	47.90011	1.87	37.32	Snohomish	New
26	(20,40]	SAM-1600	TBD	-122.68014	47.57422	3.22	34.77	Kitsap	New
27	(20,40]	SAM-1601	TBD	-122.43848	47.30650	3.00	35.69	Pierce	New
28	(40,100]	SAM-1865	TBD	-122.36715	47.49161	4.90	40.04	King	New
29	(40,100]	SAM-1866	TBD	-122.38062	47.70829	1.85	42.19	King	New
30	(40,100]	SAM-1867	TBD	-122.39423	47.26798	4.10	72.75	Pierce	New
31	(40,100]	SAM-1868	TBD	-122.35479	47.57311	1.64	94.39	King	New
32	(40,100]	SAM-1869	TBD	-122.65716	48.28306	11.09	47.19	Island	New
33	(40,100]	SAM-1870	TBD	-122.22015	48.00328	3.80	52.75	Snohomish	New

7. Field Sampling Procedures

The following sampling procedures are outlined in time-sensitive order. Field activities should be conducted by at least two people, although activities can be parsed into tasks to be accomplished by one or more persons at a given time.

7.1. Preparation for Field Work

Safety

Mussel site reconnaissance, deployment, and retrieval pose several potential safety hazards to field crew, including unstable terrain (i.e., deep mud or cobbles/boulders), incoming tides, breaking waves, exposure to extreme temperatures, and sudden changes in weather. A contact person will be designated at the office to which field personnel report at pre-designated times.

WDFW staff/volunteers will develop a site-specific safety plan including at a minimum the following elements. To ensure their safety, all field crew members are required to follow these safety guidelines:

- Do not go to the monitoring site alone; use a minimum of two people.
- Wear appropriate clothing for thermal and water protection.
- Be alert to breaking waves - wear a life jacket if appropriate.
- Avoid falls - wet rocks and logs are slippery.
- Avoid getting stuck in deep (i.e., sucking) mud.
- Wear gloves: protect hands from cuts and samples from contamination.
- Bring a cell phone or other means of two-way communication to call for emergency response in the field if needed.

It is possible that during deployment or retrieval, invasive species (e.g., benthic invertebrates or marine plants) could collected on equipment or clothing (e.g., boot treads). All boots and other field gear, and materials not retained for analyses or archiving will be rinsed and inspected before leaving the sampling location to minimize the risk of translocating invasive aquatic species.

7.2. Equipment Preparation

Cages

WDFW will obtain plastic-coated, wire mesh cages (anti-predator cages, Figure 6) with the following attributes:

- Size = 16 x 16 x 16 inches (length x width x height)
- Mesh opening = 1.25 x 2.5cm
- Removable lids.

Acceptable cages are sold at McKay Crab and Shrimp Gear, in Brinnon, Washington.

Figure 6. Anti-predator mussel monitoring cage (lid shown inside cage) with 30-inch screw anchor and bent-tip rebar stake.

To dissipate any potential surface contaminants, cage owners will either 1) soak cages and anchoring materials to be used for monitoring in water for 24 hours prior to use, or 2) wash the cages and anchoring materials with a high-pressure hose using fresh water.

Anchors

WDFW will obtain anchoring devices suitable for anchoring their cages into the substrate at their individual monitoring sites. WDFW recommends using a screw anchor with a 30-inch shaft and four bent-tip rebar stakes to anchor cages in mud, sand or sand/cobble beaches. Large cable ties (3 to 5 foot long) may be used as alternate anchoring devices to secure cages to fixed objects like non-creosote pilings or boulders. In addition, cinder blocks may be used in combination with cable ties and/or rebar stakes as anchoring devices.

Field Log

The lead scientist at WDFW will maintain water-resistant field logs with detailed notes for each major monitoring-related activity detailed below. Information recorded will include:

- Name and location of project
- Field personnel
- Sequence of events and/or changes in plans or procedures
- Unusual circumstances that may affect interpretation of results
- *in-situ* condition data

If a candidate mussel monitoring site is found to be unsuitable, the reasons for rejecting the site must be recorded in the Field Log. Alternate candidate sites must be visited and verified.

7.3. Mussel Preparation

The following sections describe the procedure WDFW will follow for harvesting, measuring, and bagging mussels at Penn Cove Shellfish, Inc. a commercial aquaculture facility, in preparation for subsequent deployment in anti-predator mesh cages at sites around the greater Puget Sound.

The protocols described below are based on procedures outlined in the *Standard Guide for Conducting In-situ Field Bioassays with Caged Bivalves* (ASTM E2122-02, 2007).

Determination of Mussel Size Range

The target size of mussels selected for bagging and subsequent transplantation will be based on the median size (± 5 mm) of 100 randomly selected adult (approximately 11 months old and larger than 45 mm) mussels available the day bagging begins. Based on previous measurements taken at Penn Cove Shellfish, Whidbey Island during prior SAM/WDFW survey years, mussels selected for transplantation will likely measure between 50 - 60 mm in shell length.

Mussel Presort

The presorting, measuring, and bagging described below will take place during October, prior to deployment, allowing time for inclement weather.

WDFW staff and volunteers will obtain live mussels for cage deployment during normal, periodic harvest operations conducted by Penn Cove Shellfish, Inc. aquaculture staff. Penn Cove Shellfish, Inc. grows mussels attached to 20-foot sections of polypropylene line hanging under floating docks. Penn Cove staff harvest mussels by removing them from the ropes and cleaning them with specially designed and automated brushes aboard a harvesting vessel. WDFW staff and volunteers will divert live, cleaned mussels from this operation to a nearby shoreline location (beach or park facility), where sorting, measuring and bagging will occur.

During the beach sorting, measuring and bagging mussels will be conducted in the shade, so as not exposed them to direct sunlight for long periods of time. Mussels will be held in ambient seawater in coolers while they wait for processing. Using a knife or scissors we will separate the mussels from one another by cutting their byssal threads. Care will be taken not to pull or tear the byssal threads, so as not to damage the byssal glands. Separated mussels will then be further sorted, by selecting mussels within the desired size range by comparing them to mussel length (50 – 60 mm) templates constructed from wood (allowing for quick rough sorting). The sorted mussels will then be placed into a cooler filled with ambient Penn Cove seawater.

WDFW will monitor the water temperature inside this seawater holding cooler with a thermometer, to ensure it stays within ±5° C of current Penn Cove surface temperature, and change water as needed to maintain suitable water quality.

Measuring and Bagging

On each day of mussel bagging, WDFW staff and volunteers will take presorted mussels from the holding cooler and measure the shell length of 100 total mussels, 50 mussels at the beginning of the morning bagging shift and 50 mussels in the afternoon shift. Shell length (umbo to farthest posterior margin) will be measured using a digital caliper with measurement accuracy of 0.1 mm. Length measurements for these mussels will represent the average starting length of mussels used in the survey.

Only intact mussels with no cracks in their shells and that respond to physical stimulation by tightly closing their shells will be selected for measuring and bagging. Mussels that do not meet these requirements will be discarded.

Bagging

Twenty (20) measured mussels meeting the size requirement will be placed into a heavy-duty polyethylene mesh bag measuring 20 inches in length. WDFW staff and volunteers using a cable tie will divide the bag into two sections with ten mussels in each section. The finished mussel bags will have two separate sections providing ample space for the mussels to feed and grow.

The filled mussel bags will be placed into another holding cooler filled with ambient Penn Cove seawater. The seawater in these coolers will be maintained in the same fashion as described above.

Presoak period

Once a sufficient number of mussel bags have been processed, WDFW staff and volunteers will affix them to a 20-foot weighted line, spaced approximately six inches from each other. Approximately 40 bags will be placed along each line. When a line is filled with bags, Penn Cove Shellfish staff will hang the line under one of their aquaculture platforms. Each line of bagged mussels will be marked with an identification flag indicating the number of bags hanging on that line. The location of the line will be noted in the Field Notebook.

The finished mussel bags will be left to soak at Penn Cove Shellfish for at least 10 days before they are removed from the water for deployment in mesh cages. The 10+ day period following mussel bagging is intended to allow the mussels a resting period after they are separated, sorted, cleaned and bagged. This allows them time to re-cluster and form new byssal thread attachments prior to deployment (Andral et al, 2011; Benedicto et al, 2011; Galgani et al, 2011).

7.4. Chain-of-Custody

A *Mussel Chain-of-Custody form* (part of the Site Deployment/Retrieval datasheet, Figure 15) will be used to track mussel possession during the field and laboratory portion of the study. The chain-of-custody (COC) will be initiated by WDFW for each monitoring site to track possession of mussel bags (i.e., start of monitoring) and will be maintained by each party responsible for the mussels until all samples are relinquished to the WDFW Marine Resources Laboratory in Olympia.

7.5. Mussel Cage Deployment and Retrieval

WDFW staff and volunteers will place their pre-bagged mussels in wire mesh cages that will be anchored to the substrate with a combination of screw anchors, rebar stakes, and/or concrete blocks as described below. If necessary and possible, some cages may be tied (using large nylon cable ties) to steel or concrete pilings or other fixed points on-site. *No cages will be affixed to or placed near creosote-treated material.*

Deployment/Retrieval Dates

WDFW staff and volunteers will deploy and retrieve their caged mussels during low tide times in the late fall (November) and late winter (January – February), respectively.

Baseline Tissue Sampling

At the time of deployment WDFW will sub-sample the bagged mussels from the aquaculture facility to assess the baseline biological and chemical conditions of the starting population.

7.5.1. Deployment

WDFW staff and volunteers deploying mussel cages (hereafter referred to collectively as "deployers") must be on site to deploy the mussel cage at the time of the zero MLLW on the night of deployment. Proper timing ensures that the field crew can place the mussel cage at 0 to -1.5 feet MLLW (i.e., at the water line at the moment of, or just after, the daily lowest low tide) with plenty of time to work before the incoming tide.

Pick Up and Transport Mussels to the Monitoring Site

Deployers will go to Penn Cove Shellfish, Inc. on Whidbey Island on the afternoon of the low tide on which they will deploy the cage. Deployers will provide a cooler(s) of sufficient size, half filled with ice, to transport the mussels on the date of pick-up. Each deployer will get four bags of mussels (20 mussels per bag) per mussel cage to be deployed. The four mussel bags will be placed into a large plastic Ziploc bag(s) marked with the name of the site(s) where the cage(s) will be deployed. The bagged mussels will be placed in the cooler on bagged ice. Mussels must not come into contact with ice melt water during transportation.

At this time WDFW will initiate a COC form unique to each monitoring site for which mussels are being transferred. The deployers must keep these forms for later use upon retrieval and delivery of mussels to the WDFW processing laboratory.

Deployers will transport the bagged mussels on ice directly to the deployment site(s) and deployed on the same night they were received from the aquaculture facility, to minimize time out of the water.

Secure the Mussels into the Cage

Deployers must wear powder-free nitrile laboratory gloves when handling the mussel bags. At the mussel site, deployers will affix the four mussel bags to the top quarter (¼) of the anti-predator cage, so that they span the width of the cage and are spaced evenly apart (Figure 7). Once installed the mussel bags should hang well above the bottom of the cage. Use 8-inch cable ties to secure the end of each bag to the sides of cage, so that the bags are stretched across the middle of the cage and all mussels are an equal height above the bottom (Figure 7). After the mussel bags are fastened inside the cage, secure the cage's lid in place with at least eight 8-inch cable ties (two per edge, Figure 8). Sea stars can get through relatively small (0.5 x 1 inch) openings, so it is important not to leave any gaps. If desired, cable ties can be trimmed to about one inch length after they have been fastened.

Figure 7. Mussel bags affixed to the top quarter (1/4) of an anti-predator cage, lid not shown.

Figure 8. Anti-predator cage lid secured in place with at least two 8-inch cable ties per edge (red circles).

Secure the Cage to the Substrate

Once the mussels are attached inside the cage and the lid is secured, deployers will anchor the cage to the substrate in the intertidal zone between 0 to -1.5 feet MLLW. Timing is critical to ensure proper placement relative to tidal height; the cage must be installed at or just below the water line when the lowest low tide of the day reaches zero feet.

Whenever possible cages should be anchored to the substrate using a screw anchor (30-inch shaft recommended) and four rebar stakes. The helical anchor must be screwed as deeply into the substrate as possible, leaving only a few inches of the shaft and the top eye hole visible.

Screwing in the anchor will require a lever (to turn the anchor) and substantial downward pressure. Figure 9 illustrates use of the lever. Heavy-duty gloves are recommended for installing the screw anchor and the rebar stakes.

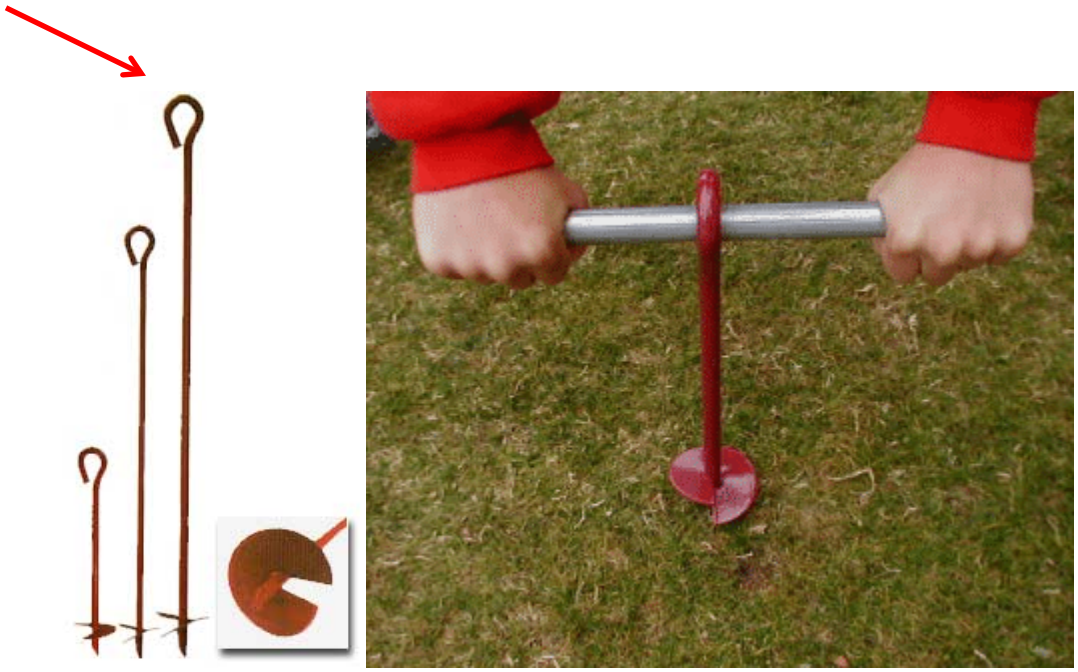

Figure 9. Helical, earth or screw anchors and lever used to screw anchor into the substrate. The red arrow indicates the 30-inch-long anchor shaft that is recommended.

Once the anchor is installed, the cage will be placed next to the helical anchor and secured to the anchor using two 8-inch cable ties. In addition, rebar stakes should be pounded through the top and/or sides of the cage, taking care to avoid driving the stakes through the mussel bags. Deployers may also cable tie the stakes to the cage (Figure 10).

Figure 10. Mussel monitoring cage driven through with bent-tip rebar stakes (on the far end) and secured to a helical anchor with cable ties. For better cage anchoring, 3-4 rebar stakes are recommended.

If a screw anchor and rebar stakes are not adequate and more or different anchoring is needed, the cage may be secured with large (3 to 5 foot long) cable ties to a non-creosote, fixed object (i.e., piling or pole) or secured to a cement block(s) that will act as a weighted anchor (Figure 11). No cages should be affixed to creosote-treated material.

Figure 11. Examples of additional cage anchoring methods.

7.5.2. Retrieval

Mussel retrieval will take place during MLLW periods within a specific range of dates to be announced by WDFW. WDFW staff and volunteers (hereafter collectively called the "retrievers") must remove their monitoring cages during the WDFW designated low tide period. Arriving on site at the time of MLLW ensures that retrievers can find and remove the mussel cage when it is totally exposed, with plenty of time to work before the incoming tide.

Upon arrival at the caged mussel site, the retrievers will take a digital photo of the cage, to document its condition, including structural integrity and degree of biofouling. Afterwards the retrievers will fill out the small retrieval section of the *Mussel Monitoring Site Datasheet*.

After field measurements, while wearing nitrile laboratory gloves, the retrievers will remove the four bags of mussels from the cage, keeping the mussels in the bags and the mesh intact, and place the bagged mussels immediately into a large, pre-labeled Ziploc bag(s). The Ziploc bag(s) will be placed into a cooler with bagged ice. This double barrier bagging method will ensure that mussels do not come into contact with any ice melt water during holding.

The cages and ALL anchoring devices and other paraphernalia will be removed from the beach; nothing from the monitoring project should be left behind. Upon finishing the removal, the retrievers will fill out the Chain of Custody (COC) section of the *Mussel Monitoring Site Datasheet,* which will be kept with the cooler until it is delivered to the WDFW Marine Resources Laboratory in Olympia the following morning (see address below).

Mussel Transport

Retrievers will hold the mussels overnight on ice in a cooler. Care will be taken to avoid freezing the mussels during holding (i.e., do not leave the cooler outside if the temperature drops below freezing). The retrievers will deliver the live mussels and matching Mussel Site Datasheet/COC form to WDFW for processing the morning following retrieval. Mussels should be delivered as early as possible to the WDFW Marine Resources Laboratory in Olympia (see address below), to ensure adequate time to process the mussels in the laboratory, especially if multiple cages are to be processed in one day.

Deliver mussels to:

WDFW - Marine Resources Laboratory

1111 Washington St SE, 6th Floor

Olympia, WA 98504-3150

Unsecured Cages / Early Retrieval

In the event a mussel cage is found unsecured from the original site coordinates before the end of the exposure period, the location (coordinates) and retrieval date as well as the condition of the cage and mussels should be reported to the WDFW field and lab coordinator. Any remaining mussel bags will be removed from the cage and placed in plastic bags over ice until transported to a freezer (-20° C if available) for storage before final delivery to the WDFW Marine Resources Lab freezer. The mussel cage and any remaining anchoring equipment will be returned to WDFW. WDFW and SAM staff will evaluate the condition of the retrieved mussels and total exposure period and upon mutual agreement will determine if the collected mussels will be further processed and used in the study analysis.

7.6. Decontamination, Prevention of Spread of Invasive Species

WDFW will conduct field work and clean equipment to prevent the spread of invasive species. Staff and equipment that contact multiple surface waters will, at a minimum, be cleaned according to Ecology's SOP EAP070, *Minimizing the Spread of Aquatic Invasive Species* (Ecology, 2012). These procedures will be followed at the end of each workday or upon leaving a water body before entering another. Some areas are designated to be of "Extreme Concern"; these areas are shown in several maps at the following link:
www.ecy.wa.gov/programs/eap/InvasiveSpecies/AIS-PublicVersion.html

8. Laboratory Processing of Mussels

This section describes the laboratory measurement processes to be conducted by WDFW staff and volunteers. Data generated as described in this section will be entered into Excel spreadsheets and verified for accuracy. Results will be entered into WDFW's TBiOS database by WDFW staff.

8.1. Equipment Cleaning Procedure

Anything that may contact portions of a mussel subject to contaminant analysis will be cleaned before use. A "clean" work surface (lab counter, cutting board, sorting tray, instruments, etc.) will be covered by at least one layer of new aluminum foil or bench paper, which will be changed between composites. "Clean" stainless steel dissection tools and grinding apparatus (hand grinder and cutting blades) will be 1) washed in warm soapy water (Micro90®), 2) thoroughly rinsed three times under warm running tap water, 3) rinsed with deionized water (held in Teflon squeeze bottle), 4) rinsed with isopropyl alcohol (held in a Teflon squeeze bottle), and then 5) placed on aluminum foil for air drying.

The same clean instruments/surfaces will be used repeatedly, without re-cleaning, on mussels contributing to the same composite. Afterwards, these instruments/surfaces will be subjected to the complete cleaning procedure prior to the processing of a new composite. Lab personnel will change nitrile gloves between composites.

8.2. Processing Mussels for Mortality, Condition Index, and Analytical Chemistry

Each mussel site will be represented by a cage that contains four bags of mussels (80 individuals). WDFW lab staff will receive cages and bags of mussels the day after retrieval and complete the field transportation portion of the COC form. WDFW lab staff will then determine the mortality in each mussel bag and remaining live mussels will be stored in a labeled plastic Ziploc type bag at -20°C until tissue resectioning for chemical analysis and measure of condition index can take place. The length of mussel storage between retrieval and chemical analysis will not exceed three months.

8.2.1. Mortality Check

WDFW lab staff will assess individual mussel bags for dead or moribund mussels within 36 hours of receiving the mussels. Dead or moribund mussels will be counted, recorded and removed. Mussels will be considered moribund if the animal is unable to tightly close its valves when stimulated. Mussels will be considered dead if there is no soft tissue inside the valves, or if the mussel soft tissue inside is putrefied.

8.2.2. Condition Index Measurement

Condition index will be determined on 12 randomly selected mussels, according to the method reported by Kagley (2003) as follows:

Condition index (CI) = dry weight (g) of soft tissue/shell length (mm) X 100.

If needed, byssal threads and barnacles will be removed from the shell of the mussels prior to measuring, to prevent exterior debris from interfering with measurements. Shell length will be measured from the umbo to the farthest posterior margin (Figure 12) to the nearest tenth of a millimeter (0.1 mm) using a digital caliper. Total Shell Length (TSL) will be recorded on the *Lab Processing Form*, *Condition Index* section (Figure 16).

Mussels will be opened by inserting a scalpel blade between the bivalve shells and severing the posterior and anterior adductor muscles (Figure 13). The shells will be spread apart at the hinge to reveal the soft tissue. At this point, the remaining byssal fibers will be cut from the byssal gland using scissors. Then, if necessary, the tissue will be gently rinsed of sediment and foreign material with care not to lose pieces of tissue, using a Teflon squeeze bottle filled with DI water.

After draining excess water, a scalpel will be used to scrape all the mussel soft tissue (including the adductor muscle) from the shell onto a pre-weighed drying pan. The wet weight of the soft tissue will be measured to the nearest tenth of a gram (0.1g) using a bench scale and recorded on the Specimen Form. Pans of mussel tissue will then be placed in a drying oven set at 120°C until the weight is constant (approximately 18 hours). After cooling to room temperature, the resulting dry weight will then be recorded to the nearest tenth of a gram (0.1g) on the *Lab Processing Form* (Figure 16).

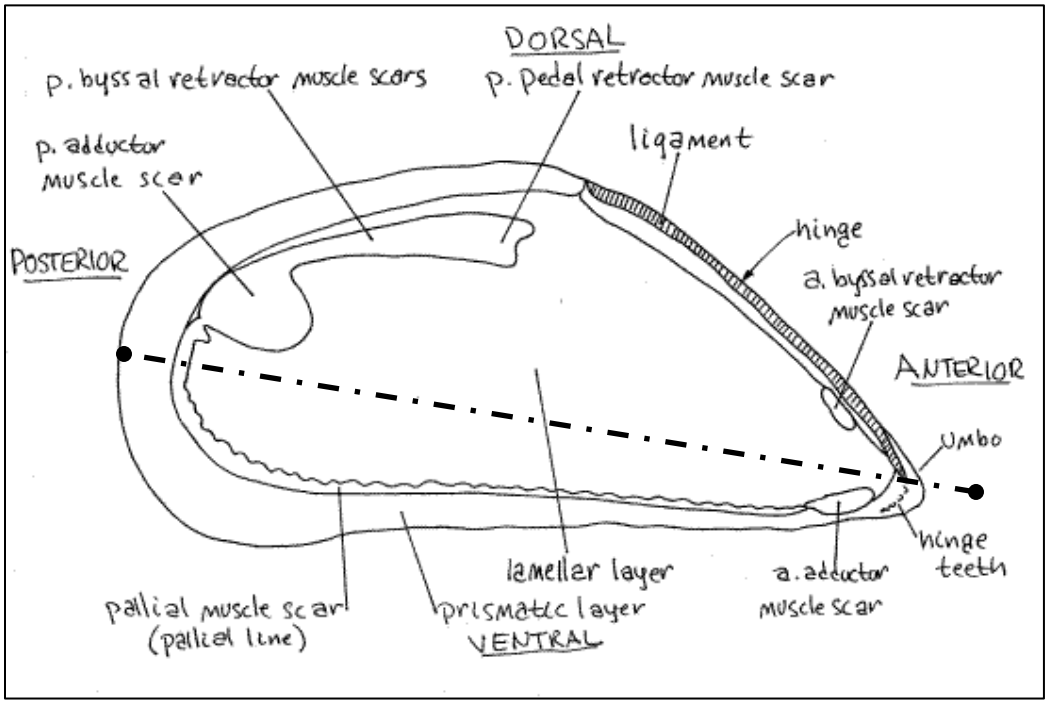

Figure 12. External anatomy of *Mytilus edulis* (Ruppert, Fox, and Barnes 2004).

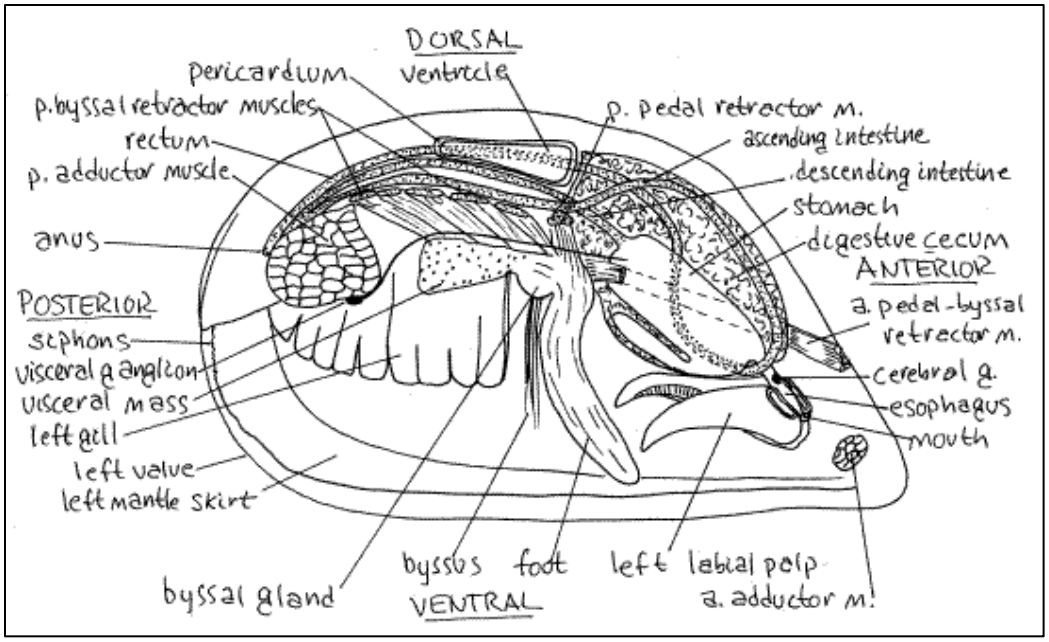

Figure 13. Internal anatomy of *Mytilus edulis* (Ruppert, Fox, and Barnes 2004).

8.2.3. Preparing Composite Samples for Chemical Analysis

Previously frozen mussels will be thawed and prepared for tissue resectioning using the following procedure, which is a modification of Field Procedure 11.7 from the *Standard Guide for Conducting In-situ Field Bioassays with Caged Bivalves* (ASTM E2122-02, 2007). WDFW lab staff will wear clean nitrile gloves and change gloves between each sample. Lab staff will also maintain two sets of instruments per site; one set of tools to open the mussel, and one set of tools to remove tissue from the shell into the jar.

Prior to shucking the mussels for the soft tissue, byssal threads, sediment, biofouling, and barnacles will be removed from the shell of the mussels using scissors and gloved hands. Mussels will be rinsed several times with DI water to further remove external debris to reduce the risk of cross contamination after the mussels are opened.

Once cleaned and thawed sufficiently, lab staff will open each mussel by inserting a clean scalpel blade between the bivalve shells, severing the posterior and anterior adductor muscles (Figure 13). The shells will be spread apart at the hinge to reveal the soft tissue. The remaining byssal fibers will then be trimmed from the byssal gland using scissors. If necessary, the tissue will be gently rinsed of sediment and foreign material with care not to lose pieces of soft mussel tissue using a Teflon squeeze bottle filled with DI water. Excess water will be allowed to drain from the specimen. Using a scalpel, all soft tissue (including the adductor muscle) will be scraped into a clean stainless steel mixing cup.

Tissue from approximately 32 individual mussels from each site will be combined into a single composite sample, with the goal of collecting approximately 200 grams of tissue. For each mussel, the tissue weight will be recorded on the *Lab Processing Form, Tissue Chemistry*

Composite section (Figure 16) as it is shucked and added to the mixing cup. After 32 mussels are added to the cup, the total tissue weight will be recorded. Tissues will then be ground using a Bamix hand mixer to a consistency resembling pudding. Homogeneity will be determined by visual inspection. Once homogenized, sample aliquots will be placed in clean I-CHEM (Class 200) glass sample jars to allow for distribution of samples between several labs and for sample archiving. Samples will be stored in a freezer kept at -20°C until delivery to chemistry labs for analysis. Mussel shells (shucked) remaining from the tissue composite process will be discarded. Unused whole mussels will be placed into a labeled Ziploc bag and kept frozen until the conclusion of the study.

A total of 39 composite samples will be created, 3 baseline condition samples (mussels collected from aquaculture source), 33 Puget Sound monitoring site samples, and 3 least-disturbed site (reference condition) samples (Table 9).

Table 9. Summary of mussel tissue composites to be collected and analyzed for chemical contaminants during this study.

Purpose	Location	Timing	Composites	Replicates
Baseline samples	Aquaculture source	November	3	3
SAM mussel sites	Various: throughout Puget Sound lowlands	January/February	33	1 per site
Least-disturbed sites (reference)	Penn Cove and Hood Canal	January/February	3	1 per site
Total			39	

8.2.4. Sample Storage

All mussel samples will be labeled and frozen to -20ºC and held in a WDFW Marine Laboratory freezer until transfer to the analytical labs or their final archival destination. The location and conditions of all mussel composite samples will be recorded in a standard laboratory notebook

used to track tissue samples for the WDFW-TBiOS program. The temperature of the WDFW-TBiOS program freezer is set at -20° C and is continuously monitored through data loggers tracked by Washington State Enterprise Services. Any temperature anomalies will trigger an alarm, triggering on-site maintenance staff to contact a laboratory supervisor from a priority list of supervisors, for immediate attention. In addition, this freezer is backed up by emergency generators in case of power outage.

8.3. Chemical Analyses

8.3.1 Overview

All organic chemicals analyses, lipid measurements, and dry-weight determinations will be made by NOAA's Northwest Fisheries Science Center Environmental Chemistry Laboratory (NWFSC/ECL) in Seattle WA. All metals will be analyzed by the King County Environmental Lab (KCEL), also in Seattle, WA. This section lists the chemicals and identifies the standard operating procedures (SOPs) these labs use to analyze the chemicals for this study. Quality assurance and quality control procedures are detailed in the following section, Measurement Quality Objectives. Analytes in these sections are grouped according to common SOPs for the analytical procedures used to measure them as follows:

- Extraction and clean-up procedures common to PAHs and HOCs (NWFSC/ECL)
- PAHs (NWFSC/ECL),
- Halogenated Organic Compounds (NWFSC/ECL)
- Conventionals (Percent lipids and Percent solids -- NWFSC/ECL)
- Metals (KCEL)

Please refer to the cited SOPs for details regarding instrument models, system accessories, solvents, reagents, purity testing, standard solutions, standard reference materials, and column packing materials.

8.3.2 Extraction Procedure for Organic Compounds

NWFSC/ECL employs a single accelerated solvent extraction (ASE) procedure for all organics measured in mussel tissues as detailed in Sloan (2014) and described herein. This procedure applies a solvent under high pressure and temperature to extract desired compounds for PAHs and HOCs. Batches of 12 to 14 tissue samples are prepared as follows:

- Each thawed, homogenized sample is manually remixed with a spatula in its original jar.
- An aliquot of tissue (typically < 2g mass for low-lipid tissues like mussels) is transferred to a separate glass jar for extraction.
- A separate aliquot is removed and dried to gravimetrically determine percent dry weight (percent solids; Section 8.3.5)
- 15 cc sodium sulfate and 15 cc of magnesium sulfate are added to the tissue aliquot to absorb water.
- The sample/drying agent mixture as loaded into the ASE cell, with glass fiber filters at the bottom and top of each cell.
- Standards are added to each vial as follows, with volumes varying depending on the amount of extract planned for injecting on the Gas Chromatography/Mass Spectrometer (GC/MS —See Sloan et al., 2014 for details)
- 150 µl each of PAH and HOC surrogate standards are added to the top filter in each cell.

    - PAH surrogate standards contain 1.7 ng/µL each of naphthalene-d8, acenaphthene-d10, and benzo[*a*]pyrene-d12.
    - HOC surrogate standards contain 1 ng/µL each of PCB 103 and dibromooctafluorodiphenyl.
- Separate PAH and HOC Standard Check Solutions are prepared and loaded into separate GC vials by adding 50 µl of isooctane to 150 µl of PAH surrogate standard and 50 µl isooctane to 150 µl of HOC surrogate standard.
- These volumes may be altered depending on whether tissue samples require micro- or macro-determinations of total extractables.

After samples are extracted in the ASE (ASE 200 accelerated solvent extractor Dionex, Salt Lake City, UT), contents of the ASE cells are discarded and extracts in ASE collection vials are retained. At this stage a portion of the extract is removed and the percent total extractables (lipids) is determined (Section 8.3.5), and the remainder is further prepared (cleaned-up) for analysis by GC/MS as follows:

- Each sample extract is filtered through a gravity-flow silica/alumina column to remove highly polar compounds.
- Each filtered sample is concentrated and then a portion is chromatographed on a size-exclusion high-performance liquid chromatography (HPLC) column using dichloromethane to remove lipids
- Dichloromethane is replaced with isooctane and each sample is reduced in volume to approximately 100 µL.

8.3.3 Measurement of PAHs

PAHs analytes are separated on a 60 m DB-5 gas chromatography (GC) capillary column and then detected on an electron impact mass spectrometer (MS) in selected-ion monitoring mode. Analytes are quantitated relative to the internal surrogate standards using multiple concentration levels of GC/MS calibration standards.

Forty-two PAH compounds are typically quantitated in this study according to Sloan et al. 2014; this suite of PAHs comprises 22 low molecular weight compounds and 20 high molecular weight compounds (Table 10). Nineteen analytes are parent PAH compounds, and 23 analytes are alkylated homologs. The expected lower limit of quantitation (LOQ) for PAH compounds ranges from 0.2 to 1.2 ng/g wet weight. With expected measurements in field samples from <LOQ to over 500 ng/g wet weight. The concentration of each analyte is reported on a ng/g wet weight basis.

Table 10. List of PAHs (Low Molecular Weight and High Molecular Weight Compounds) to be quantitated in the study.

LMW Compounds	HMW Compounds
Naphthalene (NPH)	Fluoranthene (FLA)
C_1NPH	Pyrene (PYR)
C_2NPH	C_1 FLA/PYR
C_3NPH	C_2 FLA/PYR
C_4NPH	C_3 FLA/PYR
Acenaphthylene (ACY)	C_4 FLA/PYR
Acenaphthene (ACE)	Benz[*a*]anthracene (BAA)
Fluorene (FLU)	Chrysene (CHR)[a]
C_1FLU	C_1CHR
C_2FLU	C_2CHR
C_3FLU	C_3CHR
Dibenzothiophene (DBT)	C_4CHR
C_1DBT	Benzo[*b*]fluoranthene (BBF)
C_2DBT	Benzo[*k*]fluoranthene (BKF)[b]
C_3DBT	Benzo[*e*]pyrene (BEP)
C_4DBT	Benzo[*a*]pyrene (BAP)
Phenanthrene (PHN)	Perylene (PER)
Anthracene (ANT)	Dibenz[*a,h*]anthracene (DBA)[c]
C_1PHN/ANT	Indeno[*1,2,3cd*] pyrene (IDP)
C_2PHN/ANT	Benzo[*z*]pyrene (BZP)
C_3PHN/ANT	
C_4PHN/ANT	

[a]coelutes with triphenylene
[b]coelutes with benzo[*j*]fluoranthene
[c]coelutes with dibenz[*a,c*]anthracene

8.3.4 Measurement of Halogenated Organic Compounds (HOCs)

HOC analytes are separated on a 60 m DB-5 gas chromatography (GC) capillary column and then detected on an electron impact mass spectrometer (MS) in selected-ion monitoring mode. Analytes are quantitated relative to the internal surrogate standards using multiple concentration levels of GC/MS calibration standards. The concentration of each analyte is reported on a ng/g wet weight basis.

Seventy-three halogenated organic compounds (HOCs) including 40 polychlorinated biphenyls (PCBs), six dichlorodiphenyltrichloroethanes (DDTs), eight chlordanes, and others will be analyzed in mussels according to Sloan et al. 2014 (Table 11 reprinted from Sloan et al. 2014).

Table 11. List of halogenated compounds to be quantitated in this study.

PCB 17	PCB 206
PCB 18	PCB 208
PCB 28	PCB 209
PCB 31	2,4'-DDD (*o,p'*-DDD)
PCB 33	4,4'-DDD (*p,p'*-DDD)
PCB 44	2,4'-DDE (*o,p'*-DDE)
PCB 49	4,4'-DDE (*p,p'*-DDE)
PCB 52	2,4'-DDT (*o,p'*-DDT)
PCB 66	4,4'-DDT (*p,p'*-DDT)
PCB 70	Aldrin
PCB 74	*cis*-Chlordane
PCB 82	*trans*-Chlordane
PCB 87	Dieldrin
PCB 95	Endosulfan I
PCB 99	Heptachlor
Sum of PCB 101 and PCB 90[c]	Heptachlor epoxide
PCB 105	Hexachlorobenzene
PCB 110	*alpha*-Hexachlorocyclohexane
PCB 118	*beta*-Hexachlorocyclohexane
PCB 128	*gamma*-Hexachlorocyclohexane (lindane)
Sum of PCB 138, PCB 163, and PCB 164[c]	Mirex
PCB 149	*cis*-Nonachlor
PCB 151	*trans*-Nonachlor
Sum of PCB 153 and PCB 132[c]	Nonachlor III
PCB 156	Oxychlordane
PCB 158	PBDE 28
PCB 170	PBDE 47
PCB 171	PBDE 49
PCB 177	PBDE 66
PCB 180	PBDE 85
PCB 183	PBDE 99
Sum of PCB 187, PCB 159, and PCB 182[c]	PBDE 100
PCB 191	PBDE 153
PCB 194	PBDE 154
PCB 195	PBDE 155
PCB 199	PBDE 183
PCB 205	

[a] PCB congeners are numbered according to Ballschmiter et al. 1992.
[b] PBDE congeners are numbered as is done for PCBs according to Ballschmiter et al. 1992.
[c] These analytes are quantitated and reported as the sum of their concentrations because they coelute during GC/MS analysis; the first congener is present in the calibration standard, whereas the additional congeners are not.

8.3.5 Conventional Analytes

Percent Lipids

Percent lipids in each sample are represented by total extractables, according to Sloan et al. 2014. Samples from the extraction step of the organics analyses will be evaporated and compared to the mass of the original, unextracted sample (paraphrasing from Sloan et al. 2014):

- The pan containing the sample for total extractables from Section 3 is placed on a covered rack in the hood and the solvent is allowed to completely evaporate (approximately 1–2 hours).
- The pan is dried in a 50°C oven for 2 hours, then cooled in a desiccator overnight.
- The pan is weighed to the nearest 0.0001g and the weight is recorded as the "Pan w/TE" weight.
- The percent total extractables (% TE) content of the sample is calculated as follows:

  % TE = [(Pan w/TE – Pan) x (ASE Vial w/Extract – ASE Vial) x 100%]/[(ASE Vial w/Extract – ASE Vial w/o TE Extract) x Sample Weight].

Percent Solids (Dry Weight) Determination

The percent of the sample as dry weight is determined by simple drying of tissues according to Standard Methods 2540-G (paraphrasing from Sloan et al. 2014):

- Pre-homogenized tissue (1 + 0.5 g) is placed into the pan, and the pan is weighed to the nearest 0.0001g. The weight is recorded as the "Pan w/Wet Sample" weight.
- The pan is placed in a drying oven at 105°C for 4 hours to overnight, then cooled in a desiccator for at least an hour. The pan is weighed to the nearest 0.0001g, and the weight is recorded as the "Pan w/Dry Sample" weight.
- The percent dry weight of the sample is determined as follows:

% Dry Weight = [(Pan w/Dry Sample – Pan) x 100%]/(Pan w/Wet Sample – Pan).

Dry weight of a sample is determined by placing approximately 1 g of sample in a pre-weighed aluminum pan, drying in an oven at 120 deg. C. for 24 hrs., cooling in a desiccator for 30 minutes, and then weighing the contents. Total extractible lipids are measured by evaporating solvent from a portion of the extract and measuring the remaining material (Sloan et al 2014).

8.3.6 Measurement of Metals

Total mercury is analyzed at KCEL using KCEL SOP #604v8, for cold vapor atomic absorption (CVAA) spectrometry. A summary from the SOP states:

> **An aqueous sample digestate is treated with stannous chloride to reduce the mercury** (*sic*) **in the digestate to elemental mercury. The elemental mercury is volatilized from micro-droplets to a gaseous state in the gas-liquid separator. This gas is carried through to a sample cell where its atomic absorption is measured and quantitated.**

All other metals are analyzed at KCEL according to their SOP #623v1, by inductively coupled plasma mass spectrometry (ICP-MS, using an Agilent 7900 spectrometer. A summary from the SOP states:

> **Aqueous samples / digestates are nebulized into a spray chamber where a stream of argon carries the sample aerosol through a quartz torch and injects it into an R.F. plasma. There the sample is decomposed and desolvated. The ions produced are entrained in the plasma gas and by means of a water cooled, differentially pumped interface, introduced into a high-vacuum chamber that houses a quadrupole mass spectrometer. The ions are sorted according to their mass-to-charge ratio and measured with a detector.**

9. Measurement Quality Objectives

The measurement quality objectives (MQOs) for nearshore mussel monitoring described here are designed to ensure sufficient numbers of high-quality mussel tissue samples are collected to meet the goals and objectives of the SAM program (Section 4.2). In addition, this section describes quality control procedures, quality assurance criteria and corrective actions defined by analytical labs to ensure the performance, accuracy, and precision of organic chemicals and metals.

9.1. Field Measurements

WDFW staff and volunteers will record the GPS coordinates of the mussel cage at each deployment site with individual GPS units. Each field team will record the make and model of their GPS unit and the accuracy of the GPS reading when taken. In addition, all GPS devices used in this study will be set to North American Datum 83 (NAD83) for comparability and coordinates will be recorded in decimal degree format. The specifications for many GPS receivers indicate accuracy within 3 to 15 meters (10 to 50 feet) 95% of the time.

Measurements of tidal stage, site location, habitat (visible from mussel cage), and anthropogenic structures at shoreline (visible from mussel cage) are taken by field staff during a sample collection event. WDFW staff and volunteers must meet measurement quality objectives (MQOs) listed in Table 12.

Table 12. Measurement quality objectives for field measurements.

Parameter	Expected Range Of Results	Technique/ Instrument	Measurement Method	QA/QC
Time of cage deployment and retrieval	12:00 – 24:00	Clock	Read from clock and reported in military time	Careful observation
GPS coordinates	N/A	GPS device or mobile device with GPS application	Set GPS device to NAD83, record in decimal degrees (e.g., 47.5893, -122.3953)	Record accuracy of coordinates at reading (e.g., ±15ft)
Wave energy	Flat, calm, wind chop, swells, breaking waves	Visual examination	Visual examination of sea near cage	Careful observation
Beach exposure level	Exposed, moderately exposed, sheltered	Visual examination	Visual examination of beach within ½ mile in either direction of cage	Careful observation
Time zero tide (MLLW)	12:00 – 24:00	NOAA tides and currents website https://tidesandcurrents.noaa.gov/	Read from harmonic or subordinate tidal gauge station nearest to monitoring site	Accurate reading of information from website
Majority (>50%) Substrate Type	Bedrock-hardpan, cobble-gravel mix, sand-gravel mix, sand, sand-mud mix, mud-silt	Visual examination	Visual examination within 200-foot radius of cage	Careful observation
Freshwater inputs	Natural streams, rivers, outfalls	Visual examination	Visual examination within 200-foot radius of cage	Careful observation, may include mix of types
Erosion control structures	None, hard, soft. Includes materials used	Visual examination	Visual examination of beach within ½ mile in either direction of cage	Careful observation and documentation
Abandoned or derelict structures	No/Yes, type	Visual examination	Visual examination of beach within ½ mile in either direction of cage	Careful observation and documentation

Parameter	Expected Range Of Results	Technique/ Instrument	Measurement Method	QA/QC
Current shoreline use	Wide range of choices (see Figure 13)	Visual examination	Visual examination of beach within ½ mile in either direction of cage.	Careful observation, may include mix of types
Construction of structures on beach touching water	Treated wood, concrete, steel, other	Visual examination	Visual examination of beach within ½ mile in either direction of cage.	Careful observation, may include mix of types
Tires	No/Yes	Visual examination	Visual examination of beach within ½ mile in either direction of cage	Careful observation and documentation
Outfalls	No/Yes	Visual examination	Visual examination of beach within ½ mile in either direction of cage.	Careful observation, may include mix of types
Potential sources of pollutants	N/A	Visual examination	Visual examination of beach within ½ mile in either direction of cage.	Careful observation, may include mix of types

*Field-measured parameters follow manufacturer's website guidelines for calibrations.

Field Quality Control

Field personnel will follow measurement and QC methods specified in Table 12, to obtain consistent field measurements specified in this QAPP. Training on mussel deployment, retrieval, and how to take field measurements will be provided by WDFW staff. This training will take the form of a slideshow document (i.e., self-train), to ensure comparability of results between WDFW staff and volunteers.

Field personnel will ensure photos are taken of the fully installed mussel cage, for verification of proper technique. In addition, field personnel are expected to fill in ALL sections of the combined *SAM Mussel Monitoring Site Datasheet*/Chain of Custody Form (Figure 15) in this QAPP Field personnel will perform in-field reviews of their datasheets before leaving the study site, to ensure all data is recorded correctly.

A GPS accuracy of 5-10 meters (15-30 feet) will provide adequate representation of the physical location of collected mussels. Field personnel will ensure that backup GPS units are available in the field should the unit currently in use fail.

Field notes and any notifications from field staff/volunteers of changes in protocols will be reviewed by the WDFW study lead to determine if samples should be flagged for any potential contamination or other outcomes (e.g., reduced exposure time, mussel caged retrieved early due to washing onshore).

9.2. Analytical Laboratory Measurements

9.2.1 Organics at NWFSC/ECL

For analytical chemistry at the NWFSC/ECL, quality control procedures, quality assurance criteria and corrective actions are detailed in Sloan et al. (2006, 2014). The following Table 13 (taken from Sloan et al., 2006), lists the minimum quality assurance criteria for PAHs and HOCs (termed “organics” here) analyzed in mussel tissue for this study.

Table 13. Quality assurance criteria for PCBs, PBDEs, PAHs, and OCPs. Reproduced from Table 8 in Sloan et al. (2006).

Quality assurance element	Minimum frequency	Acceptance criteria
Instrument calibration	Once every batch of samples or once every two batches in one continuous analytical sequence	Analyte concentrations are to be calculated using point-to-point calibration with at least four concentration levels of calibration standards.
Continuing calibration	At start and end of every analytical sequence and every 10 or fewer field samples	The RSD of the analyte responses relative to the internal standard is to be ≤15% for the repetitions.
Reference materials: Sediment: NIST SRM 1944, NIST SRM 1941b Mussel tissue NIST SRM 1974b Blubber: NIST SRM 1945 Fish tissue: NIST SRM 1946, NIST SRM 1947	One with every batch of 20 or fewer field samples	Concentrations of ≥70% of individual analytes are to be within 30% of either end of the 95% confidence interval of the reference values. These criteria do not apply to analytes with concentrations below their LOQ with the lower LOQ is within or greater than the 95% confidence interval, nor to those analytes known to have coeluting compounds.
Method blank	One with every batch of 20 or fewer field samples	No more than 5 analytes in a method blank are to exceed 2x lower LOQ. Samples are not corrected for analytes found in the blank.
Lab sample replicates (i.e., duplicates or triplicates split from a single tissue aliquot)	One with every 20 or fewer field samples.	RSDs are to be ≤15% (equivalent to relative percent difference ≤30% for duplicates) for ≥90% of the

Quality assurance element	Minimum frequency	Acceptance criteria
		analytes that have concentrations ≥1 ng/g.
Internal standards/surrogates	At least one internal standard/surrogate is added to every sample	The recoveries are to be 60-130%.
Interlaboratory comparisons*	As they are offered by NIST and IAEA	In conjunction with the NIST or the IAEA.

*The NWFSC-ECL participates in interlaboratory comparison exercises as offered by the National Institute of Standards and Technology (NIST) and the International Atomic Energy Agency's Environment Laboratories (IAEA-NAEL). Such exercises are conducted to allow participating laboratories to evaluate the quality and comparability of their performance in measuring selected organic contaminants in environmental samples.

Precision

Precision is monitored and controlled within batches using laboratory replicates of field samples (two replicates run for every batch of 12 samples) and across batches by analyzing Standard Reference Materials (SRM) of applicable matrix i.e., tissue. Cross-batch precision is expressed as the relative standard deviation (RSD) for repeated measurements. The RSD of analyte responses relative to the internal standard must be ≤ 15% for the repetitions, and across batches by analyzing Standard Reference Materials (SRMs – one per batch). For this study, NIST SRM 1974c will be used for all organics[1].

Bias

Bias, or accuracy of samples is evaluated by comparing measured SRM values with National Institute of Standards and Technology (NIST) certified values. Analyte concentrations of ≥70% of individual analytes are to be within 30% of either end of the 95% confidence interval of the reference values.

Sensitivity/Method Detection Limit (MDL)

The lower Limit of Quantitation (LOQ) for all organic analytes in this study is the concentration that would be calculated if that analyte had a GC/MS response area equal to its area in the lowest level calibration standard used in that calibration. When an analyte is not detected in a sample or it has a response area that is smaller than its area in the lowest level calibration standard used, the concentration of the analyte in that sample is reported to be less than the value of its lower LOQ." (Sloan et al. 2006). An LOQ concentration is calculated for each sample based on sample mass and instrument performance for each batch of samples (Sloan et al. 2014). LOQ values for PAHs and HOCs reported in herring embryos by this method typically range from 0.2 to 0.8 ng/g wet weight of original tissue.

[1] SRM 1974b is no longer available from NIST.

Detection of Analytes in Method Blanks

One method blank is run for every 20 or fewer field samples. No more than five analytes in a method blank may exceed 2x the lower LOQ before corrective action is taken by the lab. The corrective action will be to re-extract and re-analyze the affected samples. Data are reported by the analytical laboratory without blank correction. If there is a detect in the blank sample, the sample is reported to EIM with a "B" flag, defined by Ecology as an analyte detected in sample and method blank and the reported result is sample concentration without blank correction or associated quantitation limit.

9.2.2 Metals at KCEL

Quality assurance parameters, control limits, precision, bias and sensitivity for the KCEL CVAA analysis of total mercury and ICP-MS metals analysis are detailed in KCEL SOP 608v7 and SOP 616v4.

9.2.3 All Biological and Analytical Metrics

Comparability

The SOPs described in this document (Sloan et al. 2014; Sloan, Brown et al. 2004; Sloan, Brown et al. 2006, KCEL #SOP 608v4, and KCEL SOP #616v4) are consistent with other concurrent and future sampling efforts that could be used as comparison for mussels. In addition, methods detailed here are consistent with ongoing WDFW monitoring of contaminants in other Puget Sound species.

Although not necessary for the current project, comparability with historical NOAA Mussel Watch or other data will require some targeted evaluation. The performance-based nature of current analytical procedures is designed to allow the broadest comparability with other similar programs; however, some discrepancies will exist with new vs. older mussel monitoring programs. For example, PCB Aroclors vs. PCB congeners that may be used in this study. This issue will be addressed in future efforts to fully expand and establish a mussel-monitoring program in Puget Sound.

Representativeness

Mussels used for this study will be of the species *Mytilus trossulus* (bay or foolish mussel), which is indigenous to intertidal habitats in the Puget Sound. As recommended in the *Standard Guide for Conducting In-situ Field Bioassays with Caged Bivalves* (ASTM E2122-02, 2007), mussels for this study will come from an aquaculture facility. The source will be Penn Cove Shellfish, Inc. in Penn Cove, Whidbey Island, Washington. The advantage of using mussels from this facility is that all individuals will be of similar ages from the same population, will have a similar genetic and environmental history and are expected to be relatively uncontaminated. In addition, Penn Cove Shellfish, Inc. is the only local aquaculture farm that raises *M. trossulus.*

The target size of mussels selected for transplantation will be based on the median size (± 5 mm) of 100 randomly selected adult (approximately 11 months old and larger than 45 mm) mussels available when bagging begins. Based on previous measurements taken at Penn Cove Shellfish on August 2012, mussels selected for transplantation will likely measure between 50 – 60 mm in shell length.

Since the Puget Sound on average receives its highest amount of rainfall in the winter months, the sampling period chosen for this study (October – January/February) represents a period when input of contaminants from stormwater runoff is at its potential highest. Mussel cages will be placed on the intertidal substrate between 0 to -1.5 feet mean lower low water (MLLW), with mussels suspended approximately 40 cm above the substrate. The placement of cages is meant to simulate contaminant conditions experienced by most nearshore biota in the intertidal zone during the winter in Puget Sound.

10. Data Management

WDFW will format all digitized field and laboratory data into a structure compatible with the PSEMP-Toxics in Biota (TIB) database. The TIB database is a relational database created in Access, with separate tables for (1) field effort data, (2) biological characteristics of individuals used to create samples, (3) one-to-many cross reference for individuals-to-composites, (4) sample tracking, condition and summary statistics, and (5) chemical analyses. The TIB database is stored on a WDFW server, which is backed up nightly as part of an automated network backup service provided by WDFW Information Technology (IT) Services.

10.1. Data Recording

Field Data

WDFW staff and volunteers will be collecting and managing data from field work during site evaluations, and deployment and retrieval of mussel samples. All data will be managed and stored by the field personnel responsible for each site. A new field datasheet and additional notes in a separate field log will be completed at every mussel monitoring site evaluated on location for suitability of cage placement (Figure 14). Another field datasheet will be completed for each site where cages are deployed and later retrieved (Figure 15). Completed datasheets and field log will be reviewed by the WDFW study lead after each sampling, scanned, and an electronic version stored on internal servers that are backed up regularly.

Field data will be digitized (placed into Excel spreadsheets) and all entries will be independently verified for accuracy by another data reviewer (field/lab staff member). This data will be incorporated into annual reports and electronic reports by WDFW. Reports and data will be submitted to Ecology in the format required.

2021/22 Mussel Monitoring Survey Field Site Evaluation Site Evaluator:_____
Date: _____ Time:_____

Site ID _____

Site Coordinates: Lat._____ Long._____

Site County: _____

Please take photos of site and access points!

Is the site within a marina/port where motorized vessels are kept in the water? (Y/N)_____

Safety/Accesibility

How long does it take to walk/access the site? _____

Describe the terrain when walking or accessing the site (ex. Deep mud, wooded trail):

_____

Are there hostile people/animals present? (Y,N)_____

Safety/Accesibility Comments:

Include instructions on how to get to the site, where to park, etc.

_____

_____

_____

Suitability

At 0 to -1.5 ft mean lower low water line

Can a helical anchor or rebar stakes be driven into substrate to secure cage? (Y/N) _____

Is there a structure that the cage can be tied to that doesn't include creosote covered material (ex. Steel or concrete pilings)? (Y/N) _____ Specify Structure _____

Is the site high energy? (Y/N) _____

Site Comments: _____

_____

Alternate Coordinates

If the above criteria has not been met, alternate coordinates can chosen if the site is within 400 m along the shoreline on either side of the initial site

Alternate Site Coordinates Lat._____ Long. _____

Reason for choosing an alternative site: _____

_____

Please attach photos of old and new site along with access location.

Figure 14. Mussel monitoring field candidate site evaluation form.

Washington State 2021/22 SAM
Mussel Monitoring Site Datasheet

DEPLOYMENT INFORMATION			
Site ID:	Site Name:		
Deployers name(s):			
Recorder name:			
Deployment date:			
Estimated time of zero tide:		Time cage anchored:	
Cage GPS location (decimal degrees)	Latitude:	Longitude:	Accuracy (± XX feet):
GPS make/model or app name: (please set to datum NAD83)			
Anchors Used (type and number):			

HABITAT (visible from mussel cage)

Sea Conditions: ☐ Flat ☐ Calm ☐ Wind chop ☐ Swells ☐ Breaking waves

Beach Exposure: ☐ Exposed ☐ Moderately exposed ☐ Sheltered

Substrate – select ONE that describes the majority (50%) of substrate around cage: ☐ Bedrock, hardpan ☐ Cobble-gravel mix ☐ Sand-gravel mix ☐ Sand ☐ Sand-mud mix ☐ Mud, silt

Stream or River present: ☐ No ☐ Yes

Other Habitat Comments/Observations:

ANTHROPOGENIC STRUCTURES AT SHORELINE (visible from mussel cage)

Erosion Control/Shoreline Armoring: ☐ None ☐ Hard (bulkhead, riprap, etc.) ☐ Creosote Included

Abandoned/Derelict Structures on Beach (e.g. old pilings, docks, etc.) ☐ No; ☐ Yes, describe:

Current Shoreline Use (check all that apply): ☐ Boat ramp/launch; ☐ Boathouse/shed; ☐ Bridge; ☐ Breakwater; ☐ Dock/pier/wharf; ☐ Floating home; ☐ Marina; ☐ Mooring buoy; ☐ Outfall; ☐ Piling/dolphin; ☐ Raft/float; ☐ Road; ☐ Shipyard or terminal; ☐ Utilities; ☐ Other:

Dock/Pier/Wharf/Piling Material (if present): ☐ Creosote ☐ Other treated wood; ☐ Concrete; ☐ Steel; ☐ Other:

Tires present: ☐ No ☐ Yes

Outfall Present (pipe, culvert, point of flow onto beach): ☐ No ☐ Yes

Other obvious sources of pollution (oil slicks, seeps, etc.):

Additional comments/observations (it's a good idea to note landmarks that will help you find the cage later!):

TAKE PHOTOS of the deployed cage and surrounding substrate, including any interesting observations!

Washington State 2021/22 SAM
Mussel Monitoring Site Datasheet

RETRIEVAL INFORMATION (TAKE PHOTO of the mussel cage BEFORE removal, to document condition of cage.)			
Site ID:	Site Name:		
Retrievers name(s):			
Recorder name:			
Retrieval date:		Time cage removed:	
Cage GPS location (decimal degrees)	Latitude:	Longitude:	Accuracy (± XX feet):
GPS make/model or app name: (must be set to datum NAD83)			
ANY NEW obvious sources of pollution (oil slicks, seeps, etc.)?			
Additional comments/observations (including condition of CAGE on retrieval, major changes in habitat or structures around cage):			

Mussel Chain of Custody Signatures

Mussel Cage Retriever: _____ Date : _____

Mussel Runner: _____ Date : _____

Mussel Runner: _____ Date : _____

WDFW Personnel: _____ Date : _____

2

Figure 15. Mussel monitoring site deployment and retrieval datasheet with Chain of Custody signatures.

Laboratory Data

A new lab processing datasheet will be completed for each mussel monitoring site and will include sections for mortality assessment, condition index, and tissue chemistry composite (Figure 16). The sample processing data collected in the lab will be digitized (placed into Excel spreadsheets) and all entries will be independently verified for accuracy by another data reviewer (field/lab staff member). Data received from the analytical laboratories will be in Excel spreadsheets in various formats. WDFW staff will format these data into a structure compatible with the TIB database and incorporate the data accordingly. All entries will be independently verified for accuracy by the data coordinator and project manager.

Lab Processing Datasheet for Mussel Monitoring

Site Name:_____

Site ID:_____ Retrieval Date:_____

MORTALITY ASSESSMENT

Assess mussels in each bag Today's Date_____ Recorder_____

Total Live Mussels:_____

Comments (specify if a live, cracked mussel was used in the Condition Index Log or if any unique bycatch was observed):

CONDITION INDEX

Recorder:			Recorder:	
Today's Date:			Today's Date:	
Fish ID#	Shell Length	Other Observations	Dry Tissue Weight (includes pan)	Dry Tissue Weight - Final (minus pan)
	0.1 mm		0.1 g	0.1 g
	Date & Time in Oven:		Date & Time out of Oven:	

Lab Processing Datasheet for Mussel Monitoring

TISSUE CHEMISTRY COMPOSITE

Today's Date:_____

Site Name:_____ Recorder:_____

Site ID:_____ Shucker:_____

Wet Tissue Weight (tare scale each time)	Comments	Wet Tissue Weight (tare scale each time)	Comments	Wet Tissue Weight (tare scale each time)	Comments

			Sample Wt (g):	Sample Comments
SampleIDs (include incremental numbers and/or letters, exactly as it is shown on jar):		-MTW01A		
		-MTW01B		
		-MTW01C		
		-MTW01D		
		-MTW01Z		

Page 2 of 2

Figure 16. Lab processing datasheet for mussel monitoring: mortality assessment, condition index, tissue chemistry composite.

10.2. Data Storage

All datasheets, photographs, and printed or electronic data generated for this project will be stored by WDFW in organized filing systems for paper and electronic files. These files may be sought by Ecology for permit compliance review and audit purposes and must be maintained according to the records retention requirements for all documents related to the permits. Location and measurement data will be evaluated through the data verification process outlined in this QAPP. Acceptable results will be used by scientists to prepare a summary report and entered Ecology's EIM database.

Key deliverables, reports and summary results will be posted on the SAM status and trends webpage.

10.3. Electronic Transfer Requirements

After each survey and completing all necessary QC review and correction procedures, the final field and laboratory data will be loaded to Ecology's EIM database by the WDFW project lead and/or the field and lab coordinator with assistance from the Ecology EIM coordinator and SAM project manager.

10.4. Data Reporting Requirements

The project lead will submit reports as deliverables to SAM project manager. The SAM Mussel Monitoring report will include a complete discussion of the monitoring effort. The Table 3 provides a list of reports and target dates.

10.5. Audits

The WDFW mussel monitoring lead will routinely coordinate all activities with staff and volunteers to ensure the field sampling locations are suitable, deployment and retrieval of mussels and the COC form is properly filled out. Laboratories will inform the WDFW lead if timeframes are not met, or samples are lost. The WDFW will take corrective actions where necessary to ensure adequate timeframes and safe sample delivery.

11. Data Verification and Quality Assessment

WDFW project leads will examine and verify all field-generated data to ensure:

- Specified methods and protocols were followed.
- Data are consistent, correct, and complete, with no errors or omissions.
- Data specified in the *Sampling Process Design* section were obtained.
- Results for QC samples as specified in the *Measurement Quality Objectives* and *Quality Control* sections accompany the sample results.
- Established criteria for QC results were met.
- Data qualifiers are properly assigned where necessary.

11.1. Field Data

Throughout the duration of field sampling, the field personnel leads and crew members are responsible for implementation of sample-collection procedures. The field lead is also responsible for a systematic review of all field documentation generated (e.g., datasheets, field logs, chain-of-custody sheets, sample labels) to ensure data entries and labels are consistent, correct, and complete, with no errors or omissions. This review should be completed prior to leaving the site where the measurements were made.

Data usability assessment follows verification. This involves a detailed examination of the data package using professional judgment to determine whether the quality objectives have been met. WDFW and project managers will examine the complete field data packages (i.e., hard copy datasheets and Excel spreadsheets) to determine compliance with procedures outlined in this QAPP and referenced SOPs. WDFW and project managers will also ensure that the MQOs have been met and determine if the quality of the field data is usable for the SAM objectives.

11.2. Laboratory Data

Data generated by the analytical labs will be reviewed by analytical lab staff for out-of-bounds values, transcription errors and other problems by at least two chemists.

The success of meeting data quality objectives is evaluated based on the outcome of quality control procedures during analytical procedures. Typically, if QC criteria are not met the problem is identified, corrected, and sample (or extract) re-run. In cases where QC criteria have not been met and there is not enough tissue to be reanalyzed, the data are to be censored with appropriate qualifiers to allow an objective evaluation of the usability of the final record. Rejected data are censored with an “R” or equivalent qualifier. Based on (1) a long history of employing these methods to measure target analytes in a wide range of Puget Sound biota matrices, (2) the range of data values we expect in this study, and (3) appropriate (tenth-of-ppb)

limits of quantitation, we expect rejected data to be rare, with the singular possible exception of potential blank contamination for naphthalene-compounds.

Non-detected analytes will be censored with a "<LOQ" or "U" qualifier. The value reported for non-detected analytes will be the LOQ or Method Detection Limit, depending on analytical procedure. It is the responsibility of data users to decide how to use data censored as not detected. Previous experience with data from similar studies for the target analytes in this study suggest that summed totals will be dominated by substantial concentrations of a number of individual analytes.

Final lab review is conducted by a lab manager who approves data and summarizes the process in the form of a case narrative before they are released to WDFW. Prior to entering data into EIM, WDFW will review the case narrative and conduct the final review of the data to ensure all QA/QC criteria are met.

12. Adaptive management of this QAPP

If a need is identified for adaptive changes to the monitoring protocols or data analysis approaches specified in this QAPP, the proposed revision(s) to this QAPP must be detailed. Minor changes (corrections, personnel updates, reference site updates) may be written in a separate memo which provides justification for the change(s) and the expected results and impacts to data usability for the monitoring that has been conducted to date and that will be conducted in the future. Any proposed changes must be approved by the SAM project manager prior to implementation. Moderate to major changes (change of laboratory, missed season, parameter changes, study design updates) will undergo an approval process that may include discussion(s) with the Stormwater Workgroup or Status & Trend Subgroup and other interested parties, and reauthorization signatures. These may be captured as addenda.

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