Blue Mussels as Indicators of Stormwater Pollution in Nearshore Marine Habitats in Puget Sound
PROPOSED REVISED STATEMENT OF HYPOTHESIS

Washington Department of Fish and Wildlife/Puget Sound Assessment and Monitoring Program
August 26, 2011

EXECUTIVE SUMMARY

This report summarizes the feasibility of applying a probabilistic random sampling design for monitoring the status and trends of toxic contaminants in blue mussels (*Mytilus* spp.) in Puget Sound, and proposes an alternate design and study questions. The original intent of the Puget Sound Stormwater Work Group (SWG) was to apply a probabilistic study design to compare contaminant loads in mussels from urban growth areas (UGAs) to those from NOAA's Mussel Watch program. The Mussel Watch program, which has been conducted for decades, involves collection of samples thought to represent ambient[1] conditions. The SWG recommended the Mussel Watch program in Puget Sound be expanded to improve our understanding of stormwater runoff-related issues in Urban Growth Areas (UGAs). Recently, the Washington Department of Fish and Wildlife (DFW) was tasked with (1), evaluating whether there were sufficient mussel populations in Puget Sound to support a such a monitoring program, (2) estimating how many sample sites would be required for a statistically rigorous UGA/non-UGA comparison and (3) determining whether a statistically valid design could be achieved by combining existing NOAA Mussel Watch stations with 30 to 50 new stations, in a probabilistic stratified random sampling design.

The SWG recommended the funding for these samples come from local jurisdictions through their National Pollutant Discharge Elimination System (NPDES) permit monitoring requirements. Sampling would begin in either December 2015 or December 2017 to coincide with NOAA Mussel Watch sampling timeframes and the SWG-recommended schedule for implementing the broader regional stormwater monitoring program.

A desktop census evaluation of mussels in Puget Sound (Appendix A) indicated a high likelihood that Puget Sound mussel populations are widespread and abundant enough to support a moderate monitoring program. However, mussels were not ubiquitous along all Puget Sound shorelines and uncertainty existed for some key areas, which cannot be resolved without ground-truthing.

Results from power analyses using existing recent data from NOAA's Mussel Watch program indicated sample sizes needed to compare UGA and non-UGA conditions were too large to be feasible (Appendix B). We do not anticipate having enough funds to collect hundreds of samples. To conduct a simple t-test comparison, 100 to 220 stations in total would be required, depending on the contaminant of interest, to detect contaminant differences between UGA and non-UGA mussels. These results highlight the high variability of contamination in mussels within UGAs, which is thought to be related to the wide range of land-use types occurring within those areas. Land use types within Puget Sound UGAs range from highly impacted (developed), to relatively undeveloped (green space).

In addition to NOAA Mussel Watch, two additional efforts have developed a network of Mussel Watch sites in Puget Sound (Figure 1). A previous DFW project proposed a further extended Mussel Watch

[1] the intent behind NOAA's study design is to represent ambient or average conditions, away from urbanized shorelines

network program in Puget Sound. These projects have demonstrated the utility of citizen scientist volunteers to save costs and engage communities in monitoring and recovery efforts. We recommend that the SWG study coordinate with and complement these efforts.

Given these findings, the limitations of contributions from NPDES permittees, and other current constraints for developing a status and trends monitoring program for mussels in Puget Sound, DFW recommends an alternate study design to address the following questions:

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

A study design addressing these questions would apply limited resources to establish mussel stations systematically across the full range of nearshore land-use conditions, from highly urbanized to relatively undeveloped (Figure 2). Its aim would be to select mussel locations that represent as wide a range of stormwater-influenced conditions across UGA and non-UGA shorelines as possible, allowing for exploration and identification of stormwater-mussel contaminant patterns and adjustment of sample locations in future years.

DETAILS

One of the ultimate goals of the SWG is to track contaminants conveyed via stormwater to Puget Sound receiving waters by monitoring contaminant loads in nearshore resident organisms that integrate water-column contaminant conditions over a short period of time. Blue mussels (*Mytilus* spp.) and similar bivalve mollusk species are effective organisms for tracking nearshore water quality, and have been used for decades for that purpose in many areas across the United States. Recently, the study design from NOAA's national Mussel Watch Program has been adapted by local entities seeking to apply Mussel Watch principles on a smaller spatial scale. The Southern California Coastal Water Resources Project (SCCWRP) recently completed an intensive Mussel Watch investigation of coastal waters to evaluate the extent and magnitude of contamination of their nearshore marine waters. In Puget Sound, two regional entities employ adapted Mussel Watch study designs to monitor nearshore contamination. The US Navy has partnered with US EPA, Washington Department of Ecology and other regional stakeholders to develop a plan for improving the environmental quality of Sinclair and Dyes Inlets (Bremerton area) and their associated watersheds in a program called ENVironmental inVESTment (ENVVEST). Snohomish County's Marine Resources Committee (SCMRC) have partnered with NOAA's national Mussel Watch program to evaluate contaminants in county waters and monitor effectiveness of cleanup strategies (Figure 1).

Explicitly linking terrestrial contaminant sources and conveyance pathways to contaminant conditions in mussels across a large (Puget Sound) scale is beyond the scope of the current SWG proposal. Factors that have the potential to effect mussel contamination, including upland impervious surfaces, stormwater, wastewater, combined sewer inputs, marina activities, river inputs, ferry traffic and boating, atmospheric deposition, point sources, contaminated sediment, and spills, are so numerous and complicated that a balanced study design incorporating all or even some of them is unfeasible. However, we propose a strategy to address the four proposed revised questions as follows.

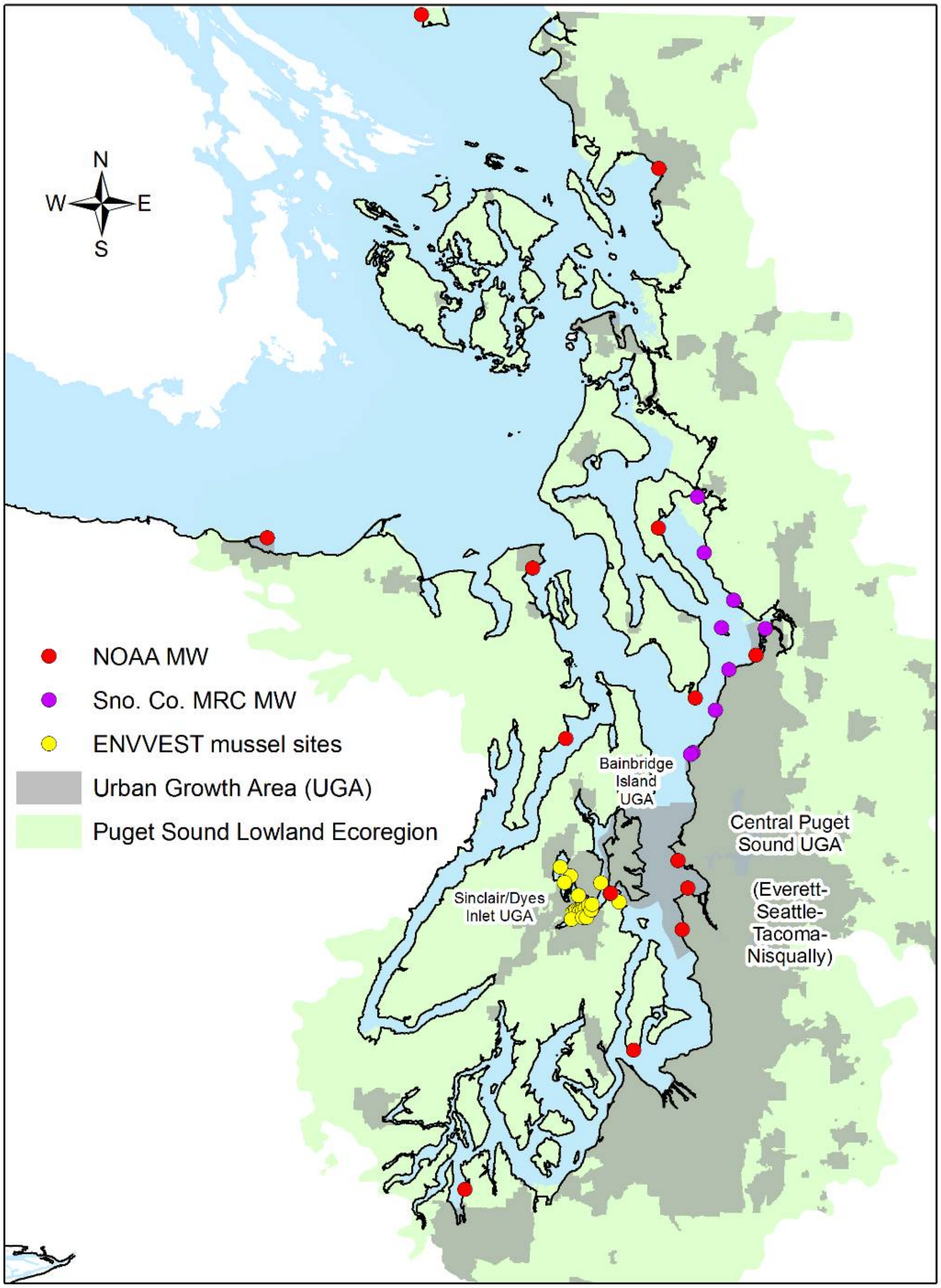

Figure 1. Puget Sound region urban growth areas (UGAs) and location of current NOAA Mussel Watch (MW), Snohomish County Marine Resources Committee (SCMRC), and ENVVEST program mussel sampling locations.

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

The aim of this effort is to gain some knowledge of existing contamination in mussels across the full range of shoreline conditions in Puget Sound UGAs. This requires a careful, systematic selection of sampling sites based on pre-existing knowledge of contamination in other organisms or sediments (for example, using data from the Puget Sound Assessment Monitoring Program (PSAMP)) and an evaluation of suspected contaminant inputs from the adjacent upland area. Sites would be selected to represent as wide a range of upland land-use conditions as possible, from highly urbanized to undeveloped (Figure 2), and distributed across UGAs using considerations described below. Results from this effort would provide a context for evaluating the scope of contamination and for the following questions.

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

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

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

We propose selecting mussel sites using a three-step process, described below. ***As a first cut***, sites would be distributed across the Puget Sound shoreline based on degree of inland impervious surface coverage, with sample sizes allotted to a minimum of three impervious surface classes (high, medium, and low), and sample density related to relative abundance of the three classes. Impervious surface is strongly correlated with many of the other contaminant factors and is one of the most feasible factors to map accurately. Traffic density might also be used. Although the location of many stormwater outfalls in Puget Sound is known and they have been mapped, key characteristics of outfalls relating to their volume and drainage area are lacking. In many cases the pathway of stormwater conveyance pipes is unknown.

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

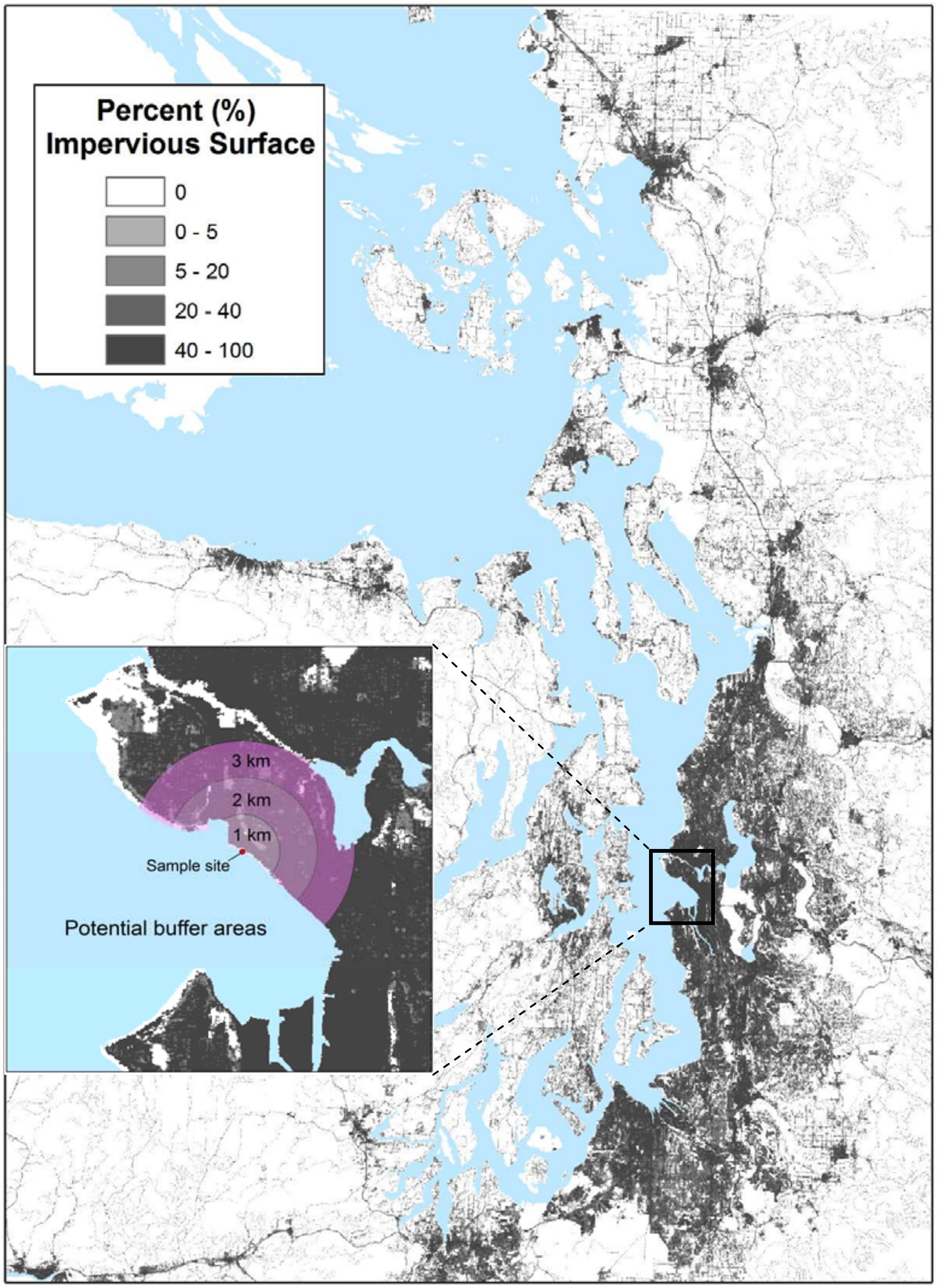

Figure 2. Overview: US Geological Survey Land Cover data (2006) showing ranges of upland impervious surface in the Puget Sound, Strait of Juan de Fuca and Strait of Georgia. Inlay: Buffer areas around a sample mussel monitoring site in Elliott Bay.

There are approximately 458 linear miles of shoreline within the UGAs of the Puget Sound, Strait of Georgia, San Juan Islands and Strait of Juan de Fuca (up to and including Port Angeles), representing shorelines of diverse lengths, and separated from each other by varying distances of undeveloped shoreline (Figure 1, Table 1). We propose here two sampling scenarios based on the number of sites funded for stormwater monitoring (n = 30 vs. n = 50, Table 1). The distribution of samples sites within/among UGAs areas will reflect the size and distribution of those UGAs.

Three large areas, labeled on Figure 1 as (1) Central Puget Sound (Everett-Seattle-Tacoma-Nisqually), (2) Sinclair/Dyes Inlets (Bremerton-Port Orchard), and (3) Bainbridge Island, represent by far the longest, contiguous UGA shorelines in the Puget Sound and cover orders of magnitude more linear miles than the rest of the smaller and isolated UGAs (27 total). The primary difference between the sampling scenarios is the number of samples available to cover isolated UGAs; 13 for scenario 1 and 24 for scenario 2. The distribution of mussel sample sites for each sampling scenario will also take into account the large differences in UGA shoreline length, as well as the number of sites already sampled through other programs (Table 1).

Table 1. Two proposed scenarios based on number of stormwater mussel sampling sites, relative to the length of shoreline and number of sites currently sampled by NOAA Mussel Watch (MW), the Snohomish County Marine Resources Committee (SCMRC), and the ENVironmental inVESTment Project Agreement (ENVVEST).

	Longest, contiguous UGAs shorelines					
	Central PS UGA	Sinclair/Dyes Inlet UGA	Bainbridge Island UGA	Isolated UGAs (27 total)	non-UGA (reference)	Total
Miles of shoreline	198	56	49	155	1936	2394
NOAA MW sites	4	0	0	3	7	14
SCMRC sites	5	0	0	0	4	9
ENVVEST sites	0	22	0	0	1	23
Total sites monitored by other programs	9	22	0	3	12	46
Scenario 1 (n = 30)	12	0	2	13	3	30
Scenario 2 (n = 50)	17	0	4	24	5	50

Puget Sound (PS), Urban Growth Area (UGA), number of proposed sampling sites (n)

Mussel Sample Site Selection Process:

Step1: *Large UGAs* - We would select multiple sites within the central Puget Sound UGA (n=12~17, Table 1) and along the shores of the Bainbridge Island UGA (n = 2~4), to provide representative, systematic coverage of their relatively large contiguous shorelines. Sites in the central Puget Sound UGA already sampled by the NOAA Mussel Watch program or SCMRC would be incorporated. Similarly, SWG sampling could be coordinated with existing ENVVEST mussel contaminant monitoring in the Sinclair/Dyes Inlet UGA. Among the remaining, unrepresented areas of the central Puget Sound UGA and the entire Bainbridge Island UGA, best professional judgment will be used to target a range of impervious surface conditions, systematically capture the full spatial extent of the UGA, and where possible co-locate sites near other SWG study locations and long-term PSAMP biota contaminant monitoring stations.

Smaller, isolated UGAs - Because the smaller, isolated UGAs are numerous (27 total) and funding is limited, we propose sampling some to most (total n = 13~24, Table 1), but not all of them. We would group the isolated UGA shorelines based on shared oceanographic basins or sub-basins and then characterize the UGAs within each group by their relative proximity to one another and their likelihood of connectedness, based on nearshore drift patterns. The number of mussel watch sites within each group would reflect several factors, including the number of UGAs within that group. As with the larger UGAs, best professional judgment will be used to target a range of impervious surface conditions, ensure sites are placed in outlying regions to capture the extent of contamination within the entire study area, and co-locate sites where possible near long-term biota contaminant monitoring stations. Isolated UGAs that are already sampled by the NOAA MW program (i.e. Port Angeles, Port Townsend, and Bellingham) would be evaluated to determine whether those sites adequately represent the surrounding UGA conditions.

Non-UGA reference sites – A few (n = 3~5, Table 1) undeveloped reference sites will be chosen in non-UGA areas. Although NOAA Mussel Watch samples in some non-UGA areas that can be used as reference locations, several additional reference sites may be selected in basins where an urban to reference comparison is also desirable (i.e. San Juan Islands, north Whidbey Island, south and/or central Puget Sound). Reference sites are important in monitoring studies because they provide data on natural/baseline conditions and can help elucidation potential non-point sources of contamination, such as atmospheric deposition as opposed to stormwater signals.

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

- High *(>30%)*
- Moderate *(15 – 30%)*
- Low *(10 – 15%)*
- Reference *(<2%)*

Assumptions related to these coverage thresholds and buffer areas will be investigated and adjusted accordingly.

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

The site selection process outline above is similar to that used by SCCWRP (Keith Mayura, personal communication). Once sample sites are selected in this manner, ground-truthing will be required to verify presence of mussels at the sampling site. If mussels are not available, we will select alternate sites based on the three-step process. Also, although sampling native mussels is more efficient in terms of sampling costs and field time, transplants could be used in some areas to expand spatial coverage. This option has not been fully considered here, but is utilized by other programs (i.e. SCCWRP) in areas where coverage is highly desired but no native mussels are available.

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

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

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

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

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

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

A COORDINATED MUSSEL SAMPLING NETWORK

Currently, the three existing mussel sampling efforts in Washington State track 46 sites across the Puget Sound, and Straits of Georgia and Juan de Fuca, including a variety of sites inside and outside UGAs (Figure 1, Table 1). Mussel sampling sites proposed by the SWG could coordinate with these existing studies, building a network of long-term mussel monitoring sites supported by a diverse set of entities.

NOAA Mussel Watch, Washington State scientists and Puget Sound recovery managers have already been discussing the desirability and feasibility of expanding NOAA Mussel Watch coverage in Puget Sound to help inform recovery efforts. During the winter of 2009/10, DFW teamed with the Puget Sound Partnership, SCMRC, Snohomish County Public Works-Surface Water Management, Washington Sea Grant, and NOAA's Mussel Watch team to conduct field-sampling for the Mussel Watch program in Washington and demonstrate the use of citizen scientists as a primary resource for conducting field work. Local volunteer groups were trained in sample collection protocol and successfully sampled mussels from 14 sites. More than 65 volunteers contributed over 500 hours to sampling mussels in this project, with a value of more than $10,000. Volunteers significantly reduced the amount of time professional staff were needed in the field, provided staff scientists with valuable local knowledge and natural history, and engaged citizens' desire to become involved in Puget Sound's recovery. In a post-project survey 90% of participants indicated a desire to participate again in the program and expressed an interest in expanding Mussel Watch coverage in their region to answer local pollution questions.

It is clear that SWG interests and goals are compatible with those articulated by these other groups, and it makes sense to coordinate with these other programs into a larger, Puget Sound-wide regional mussel monitoring program. Combined sampling efforts between programs could be leveraged to gain efficiencies and increase monitoring output/results for all groups. In addition, interest has been expressed by staff from various other entities (*e.g.*, tribes, county health agencies and Marine Resource Committees, WDFW Oil Spill Team) to participate in such a coordinated effort. We recommend a series of coordination meetings between this and the other programs to understand the level of interest and commitment from these parties and work towards the goal of an expanded mussel watch network to serve multiple needs in the greater Puget Sound.

BUDGET

The first biennium of a mussel status and trends monitoring program with 30 sampling sites would cost approximately $441,400, while support for 50 sites would cost $523,411 (2012/13 biennium, Table 2). Note the cost for this first biennium will be higher than the following biennia because it incorporates a *one-time* lab comparison requirement ($60,000) necessary to allow for a shift to a local laboratory, which is expected to result in cost-savings for future tissue analyses. The budget plan laid out below takes advantage of DFW's existing Puget Sound Assessment and Monitoring Program, including its associated sampling infrastructure and staff with a long history of monitoring toxics in Puget Sound biota. Estimates are provided for potential match or cost sharing opportunities (Table 3).

The current proposal would provide the infrastructure required to add mussels to this monitoring program, one biologist to plan/execute sampling and perform the follow-up analysis/reporting, and pay for basic sampling equipment (coolers, salinity gauges, flashlights, raingear, etc., Appendix C), as well as shipping and laboratory analysis costs. Temporary technicians would be recruited for field sampling and processing tissues.

Duties of the permanent biologist would evolve over the development of the monitoring program. In its early years the program would require a substantial investment of time in bringing together partner-entities, developing the sampling network, organizing volunteers, and selecting and comparing analytical lab(s). In later years biologist time would also be invested in analyzing data and writing reports. Specific activities in a biennial cycle will depend on when the project starts, relative to the fixed sampling schedule (starting in December of odd-numbered years). Initial organization and training of the volunteer network would require approximately six months of full time activity, with part-time attention thereafter. Sampling typically occurs over a 4 month period, from December through March. Recruiting and organizing network partners would require an initial substantial time commitment, and would remain as an activity throughout the course of the program. Data analysis and report writing would begin immediately; starting with a review of NOAA's existing 20+ year Mussel Watch data set, and would continue as new data are generated by the program.

The accuracy of the following proposed budget depends on a number of assumptions:

- chemical analyses could shift from NOAA's Texas contractor (TDI Brooks) if local labs can complete the work for less money,
- NOAA MW staff agree to a change in labs, if needed,

- shifting labs would require a one-time lab comparison study costing approximately $60,000,
- some key chemicals of concern missing in the current NOAA chemical target list will be added,
- the staff biologist and technicians (and perhaps volunteers) will process tissue samples locally, in an effort to reduce cost,
- costs include a 2% annual inflation multiplier,
- this program bears the cost of all analyses in the current proposal, however a key goal of the MW network would be to share costs with partners,
- a network of citizen scientists/volunteers would be established and maintained to help with field sampling, organized under volunteer site leaders,
- one-third of new stations will require boat access (rather than sampling from shore).

Table 2. Projected biennial budget for a Puget Sound mussel-contaminant monitoring program.

Fixed Costs	Cost per	Unit	No. Units (30 sites)	No. Units (50 sites)	Cost per biennium (30 sites)	Cost per biennium (50 sites)
Bio III S&B	$7,300	month	24	24	$175,200	$175,200
Technician S&B	$3,500	month	3	3	$10,500	$10,500
Computer lease	$45	month	24	24	$1,080	$1,080
Travel	$117	site	30	50	$3,510	$5,850
Equipment					$2,500	$2,500
Volunteer support*	$1,500	group	6	6	$9,000	$9,000
Boat charter	$1,000	day	10	17	$10,000	$17,000
Lab Analysis**	$2,623	sample	30	50	$78,690	$131,150
Lab comparison†	$2,000		30	30	$60,000	$60,000
Shipping/supplies	230	site	30	50	$6,900	$11,500
SubTotal					$357,380	$423,780
WDFW overhead††	0.2351				$84,020	$99,631
2012/13 Biennium	(includes one-time lab comparison)				$441,400	$523,411
2014/15 Biennium	(incl. 2% inflation /biennium hereafter)				$374,640	$458,291
2016/17 Biennium					$382,133	$467,457
2018/19 Biennium					$389,775	$476,806
2020/21 Biennium					$397,571	$486,342

* includes incidental costs incurred by volunteer organizations, site leads and volunteers

** based on existing NOAA Mussel Watch program: cost reduction anticipated by shifting to local lab(s)

† one year only (during 12/13 biennium)

†† includes all necessary lab space and freezer storage area

Table 3. Projected cost sharing (match) from implementing Agency and partners.						
Fixed Costs	Cost per	Unit	No. Units (30 sites)	No. Units (50 sites)	Potential Match (30 sites)	Potential Match (50 sites)
PSAMP Lead Scientist S&B	$9,424	month	2	2	$18,848	$18,848
Volunteer time	$600	site	30	50	$18,000	$30,000
SeaGrant Volunteer Coordinator						no estimate yet
WDFW Oil Spill Response boat	$1,000	day	3	5	$3,000	$5,000
NOAA ambient stations (see Table 2)	$2,630	sites	14	14	$36,820	$36,820
SCMRC (see Table 2)*	$2,630	sites	9	9	$23,670	$23,670
ENVVEST (see Table 2)*	$2,630	sites	23	23	$60,490	$60,490
Total per Biennium					$160,828	$174,828

*assumes development of a multi-partite sampling network, with these partners donating data

APPENDIX A: DESKTOP SURVEY OF MUSSEL AVAILABILITY IN PUGET SOUND

Nearshore mussel (*Mytilus* spp.) sampling feasibility study in support of regional stormwater monitoring

PURPOSE

The Washington Department of Fish and Wildlife's (WDFW) Puget Sound Assessment and Monitoring Program (PSAMP) conducted a desk-top study of available data and shoreline images to evaluate the distribution and abundance of mussels (*Mytilus* spp.) in the greater Puget Sound region, i.e. Washington's Salish Sea. This briefing paper describes the known and predicted availability of mussels along the shorelines of all urban growth areas (UGAs), as well as some non-urban areas, of the Puget Sound, Hood Canal, Strait of Juan de Fuca and Strait of Georgia. A searchable ArcGIS map called "PredictedMusselDistribution-WaSalishSea_2011-07-05.mxd" with supporting Geographic Information System (GIS) layers that contains the mussel data is available in digital format as part of this report. Sample images from that map are included here (Figures A1 – A6).

METHODS

UGA Base Map (ArcGIS)

The Urban Growth Areas layer utilized for this feasibility study displays polygons of incorporated and unincorporated UGAs associated either with cities (e.g. Bremerton – Unincorporated UGA, Des Moines – Incorporated UGA) or general regions (e.g. South Kitsap – Unincorporated UGA). The attribute table for this layer indicates that the origin for each polygon was either County or County parcel (Ecology) data, with modifications dating from 2007 - 2010. For this study we characterized mussel habitat potential along both incorporated and unincorporated shorelines, as well as for some areas around and/or near the UGA shorelines. We evaluated all the UGAs contained within a larger "Puget Sound Lowlands Ecoregion" layer that is within the Puget Sound Salmon Recovery Region. Both the "Urban Growth Areas" and "Puget Sound Lowlands Ecoregion" layers were provided by Scott Collyard (Ecology).

Other layers which may prove useful in future stormwater-related sampling considerations are also included in the map. Though they are not shown in the figures in this report, they can be activated and displayed in the digital map version. All outfall layers were provided by People for Puget Sound (PPS), though not all were originally produced by PPS. The "Man made outfalls" layer (4529 records) was created by PPS, which contacted various jurisdictions (i.e. cities and counties) for man-made outfall data in their areas. This layer includes data on: jurisdiction, type (man-made, ditch, culvert), diameter, receiving water body, and material (i.e. concrete, plastic, iron, etc.).

There are two Washington State Department of Transportation (WSDOT) layers: "WSDOT Outfalls 250m" (297 records) and "WSDOT Outfalls ALL" (5063 records). Both layers contain data on the name and location (state route, milepost) for the WSDOT outfalls. The "Natural outfalls" layer (2123 records) was likely taken from Washington Department of Natural Resources (DNR) and displays stream and creek outfalls sites. The "Combined Sewer Overflows" (CSO) layer (93 records) contains data on: jurisdiction, type (CSO), receiving water body, and some location name (mostly streets). Although we do not know the source of the "Surface water wq outfalls PS" layer (963 records) it appears to have been updated recently (2010-2011) and contains data on: facility (i.e. originator), Department of Ecology (Ecology) Water Quality Permit number, feature description, and receiving water body. It is likely this layer originated with Ecology.

Several other supporting layers are provided at the bottom of the map layers list. These include "Bridges", "Counties", and "Marine Water" layers, as well as the base orthophotos "Puget Sound 2009 Orthophoto" and "Washington State 2006 Orthophoto", and a map showing highway and place names called "Washington Hwy Map 2002".

"Puget Sound 2009 Orthophoto" only becomes visible when the scale range is zoomed in beyond 1:24,000, which allows for faster panning at smaller scales. "Washington State 2006 Orthophoto" is older and has poorer resolution than the aforementioned orthophoto from 2009, but it covers a larger area. The default setting for the 2006 orthophoto is off, but it can be activated when zoomed in to areas not covered by the 2009 orthophoto (the San Juan Islands and west of Discovery Bay in Clallam County).

Confirmed Mussel Locations

To find and document confirmed mussel locations, we started by mapping stations already sampled on a regular basis by the NOAA National Mussel Watch Program and the Snohomish County Marine Resources Committee (MRC). Colleagues from the following agencies and groups provided additional data on specific locations where mussel populations have either been sampled or are known to occur (confirmed sites):

- ENVVEST - US Navy, Puget Sound Naval Shipyard (Sinclair and Dyes Inlets)
- Washington State agencies - WDFW, Department of Natural Resources (DNR), Department of Health (DOH), Ecology
- Washington county agencies - King County (including Beach Assessment data), Tacoma-Pierce County Health Department
- University of Washington (Tacoma)
- Tribes - Muckleshoot Tribe, Nisqually Indian Tribe, Swinomish Indian Tribe, Skokomish Tribe, Squaxin Island Tribe
- Seattle Aquarium
- Citizens for a Healthy Bay (Tacoma)

Depending on the source of data, the confirmed mussel locations were distributed into several ArcGIS layers called "Confirmed Mussel Sites (various)", "Confirmed DOH Mussel Sites", "National Mussel

Watch Stations" and "ENVVEST Mussel Sites". It is important to note the following caveat when considering the reliability of these confirmed locations: due to changes in biotic (recruitment, competition, predators, harvest) and abiotic (temperature, salinity, pH, sea level) factors, mussel populations can fluctuate considerably between years at any given location. *Therefore, confirmed mussel sampling sites may not always have populations in sufficient sizes for sampling.* This has occurred at several NOAA National Mussel Watch sampling sites, which were not sampled in some years due to low population numbers.

Potential Mussel Locations

In areas where no confirmed mussel location data were available we used high resolution orthophotos ("Puget Sound 2009 Orthophoto" and "Washington State 2006 Orthophoto") in tandem with matching oblique shoreline photos from Ecology's Washington Coastal Atlas (images from 2006) to inspect shorelines and assess habitat within the mid-intertidal zone for potential mussel habitat. Data from this assessment are housed in the "Potential Mussel Locations" ArcGIS layer.

For this assessment, areas characterized by hard substrate that appeared suitable for byssal thread attachment were considered potential mussel habitat. This included, but was not limited to, shorelines dominated by bedrock, boulders, cobble, large woody debris (LWD), bank armoring, and various man-made structures (i.e. docks, marinas, jetties, net pens, navigational markers, anchorage floats). In general, we considered shoreline areas with greater than or equal to about 30% coverage with boulders and/or cobble mixed with sand or shell hash as potential mussel habitat.

Additional Shoreline Mapping

The DNR Aquatic Division's *ShoreZone Inventory* was also utilized to provide data on mussel populations and to provide a base map feature that we modified for the purposes of this study. The ShoreZone Inventory is an inventory of Washington State's saltwater shorelines. It comprises linear shoreline features that divide the shoreline into homogenous physical segments called 'units' (line data) that are associated with data in a tabular file (table). The ShoreZone Inventory systematically characterizes the shoreline morphology, substrate, wave exposure and biota, allowing a wide range of feature information (including presence of mussel bands) to be illustrated on an interactive map.

ShoreZone Inventory information was collected from a helicopter during low tides from 1994-2000. From the helicopter a geomorphologist and a marine ecologist recorded continuous commentary on the physical and biological features along the shoreline. Following the survey, the videotapes were taken back to the office for interpretation and classification. The geomorphologist divided the shoreline into units on orthophoto maps and described each unit (e.g.,. eroding cliffs, sand and gravel beaches, sand flats, wetlands). Next the marine ecologist added information on the visible macrobiotic living resources in each unit (e.g., wetland grasses, intertidal algae, subtidal vegetation, barnacles, sand-dollars, oysters, <u>mussels</u>). ShoreZone Inventory data on mussel 'bio-bands' (blue color band visible from the helicopter due to aggregates of mussels) in mid-intertidal areas included the following values:

- "P" – patchy mussels, bio-band occurred on less than half of the unit
- "C" – continuous mussels, bio-band occurred on more than half of the unit

- "N" – null, no bio-band was visible from the helicopter

ShoreZone Inventory unit data indicating patchy or continuous mussel distribution was retained for the feasibility study map (ArcGIS). Data units with "N" (null) values within or near UGA areas were examined and re-classified according to mussel habitat suitability into the following categories:

- Present-Confirmed (red) – an outside source has confirmed the presence of mussels along this shoreline
- Present-Estimated (orange) – habitat along this shoreline appears to support mussel attachment
- Absent-Estimated (light green) – habitat along this shoreline does not appear favorable to mussel attachment
- Absent-Confirmed (dark green) – an outside source has confirmed that mussels are not present along this shoreline

These classifications were determined using orthophotos and the Washington Coastal Atlas, as described above. If suitable mussel habitat was seen on only part of a unit, then the unit was subdivided into sections and each section was assigned a separate value. The length (feet) of each unit is recorded in the associated tabular file (last column), which allowed calculation of linear miles of shoreline assigned to each category.

RESULTS

Confirmed Mussel Locations

We identified a number of locations throughout the Puget Sound, Hood Canal, Strait of Juan de Fuca and Strait of Georgia where mussel populations have been observed, and often sampled, either directly from a substrate (described here as "native") or from cages populated specifically for sampling (i.e. DOH). Of the confirmed locations where "native" mussel populations have been found or sampled in the past, 62 occur within UGA areas and 76 occur within non-UGA areas (Figure 1, Table 1).

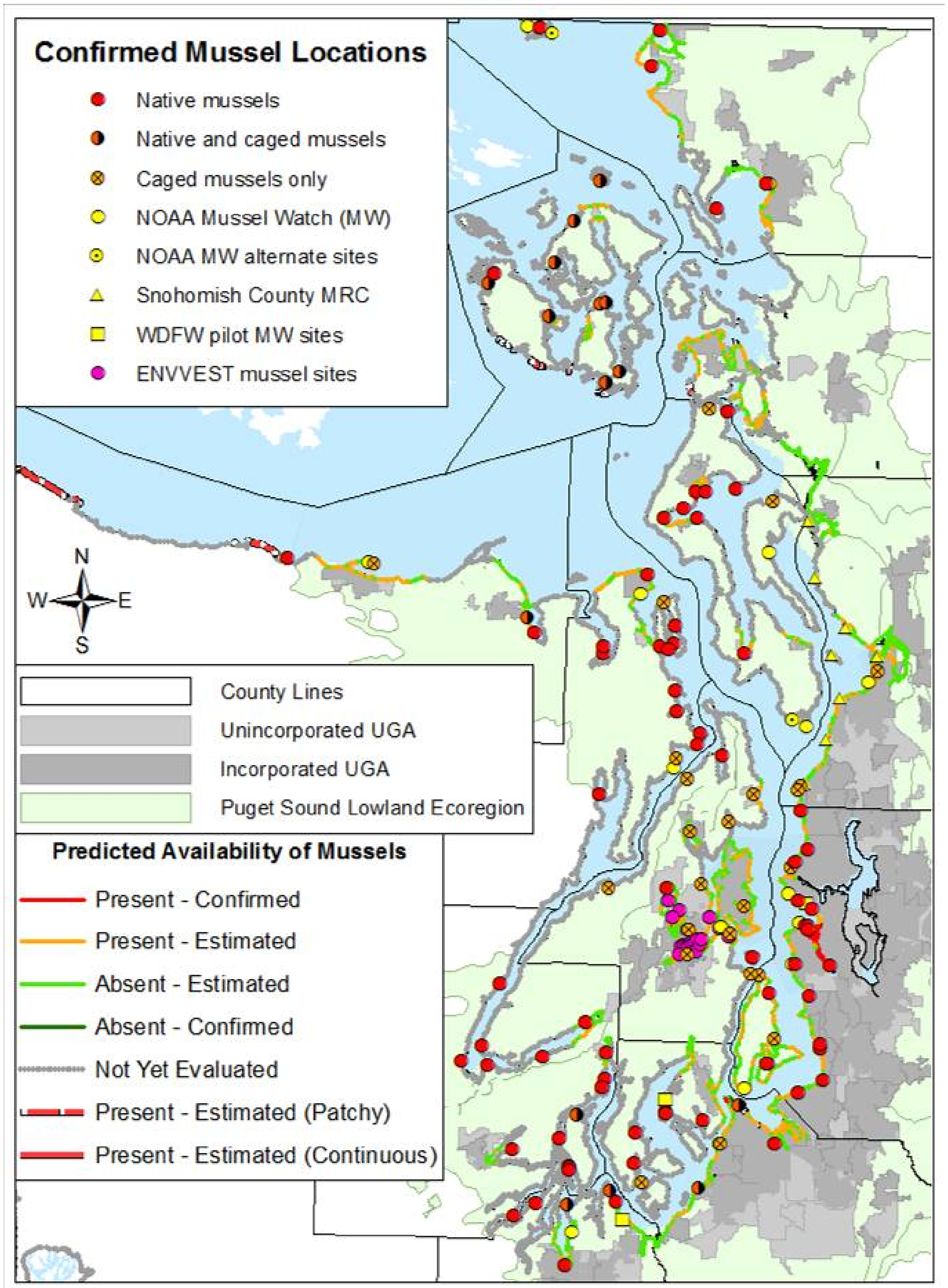

Figure A1. Overview of ArcGIS map showing confirmed and predicted availability of mussels.

Table A1. Number of confirmed mussel locations in counties with shorelines along the Puget Sound, Hood Canal, Strait of Juan de Fuca and Strait of Georgia. Native = mussel population naturally attached to a substrate (rock, concrete, piling, etc.), Native/Caged = both native and caged mussels available, Caged = mussels only available from a cage populated with specimens from another location.

	Urban Growth Areas			Non-Urban Growth Areas		
County	**Native**	**Native/Caged**	**Caged***	**Native**	**Native/Caged**	**Caged**
Whatcom	4	-	-	3	-	-
San Juan	-	1	-	1	8	-
Skagit	-	-	-	-	-	-
Island	1	-	-	10	-	2
Jefferson	2	-	-	12	-	2
Clallam	1	1	1	7	-	-
Snohomish	6	-	3	4	-	-
Kitsap	23[†]	-	5	4	-	6
King	17	1	1	3	-	2
Mason	1	-	-	12	1	-
Pierce	1	2	1	5	-	1
Thurston	1	-	-	4	2	-
Total	**57**	**5**	**11**	**65**	**11**	**13**

*Caged mussels placed, maintained and sampled by the Washington Department of Health.
†High concentration of mussel sampling locations within/around the Puget Sound Naval Shipyard, Bremerton (ENVVEST study).

Potential Mussel Locations

There are approximately 2394 miles of shoreline in the greater Puget Sound, Hood Canal, Strait of Juan de Fuca and Strait of Georgia areas (as measured along the Puget Sound Lowlands Ecoregion layer, Figure 1). We evaluated 753 miles of this shoreline, placing higher priority on UGA areas. We successfully characterized 100% of UGA shorelines and approximately 12% of non-UGA shorelines for potential mussel habitat (Table 2). The additional 1641 miles of non-UGA shoreline have yet to be evaluated by our team. Although at this time we cannot estimated the percent of shoreline in non-UGA areas that may potentially yield suitable mussel sites, there are a number of *confirmed* sampling locations in various non-UGA areas that could be utilized for sampling (Table A1).

Of the evaluated coast, we identified 325 miles of UGA and non-UGA shoreline where mussels are either estimated or confirmed to occur. When we narrowed the search area to within just the UGAs, we identified 240 miles where mussels are estimated or confirmed to occur. Thus, we characterized roughly 52% of the UGA shorelines as potential or suitable mussel sampling sites (Table A2).

Table A2. Linear miles of shoreline evaluated and miles estimated to possess suitable mussel sampling sites.

County	Urban Growth Areas (UGAs)			Non-UGAs
	Shoreline	Mussels Present*	Percent shoreline likely to yield mussel sites	Mussels Present*
Whatcom	42	21	50%	6
San Juan	6	4	67%	6
Skagit	45	24	53%	7
Island	10	6	60%	10
Jefferson†	13	7	54%	5
Clallam††	20	14	70%	6
Snohomish	54	24	44%	5
Kitsap	109	48	44%	8
King	72	49	68%	20
Mason	5	3	60%	5
Pierce	70	35	50%	5
Thurston	12	5	42%	2
Total	**458**	**240**	**52%**	**85**

*Numbers reflect potential (*estimated* and *confirmed*) mussel sources; see map and methods for definitions.

†Only the portion of Jefferson County within the Puget Sound Lowland Ecoregion was evaluated; Pacific Coast portion of county was not included.

††Only the portion of Clallam County within the Puget Sound Lowland Ecoregion was evaluated; Strait of Juan de Fuca shoreline west of Port Angeles and Pacific Coast portion of County was not included.

Accessibility of Potential Mussel Sampling Sites

Many of the shorelines where mussels are predicted to occur are accessible by land. These include a mixture of public (e.g. city, county and state parks, community docks, marinas) and private shore lands (e.g. residential, community, aquaculture, tribal, commercial). Permission to access and sample mussels from privately owned sites will be needed, keeping in mind that sampling will likely occur on a regular basis. Special care and consideration may also be warranted in reporting results of mussel contamination from aquaculture sites that raise mussels for human consumption (e.g. Penn Cove, Kamilche Farms), if sampling is allowed to occur there.

In addition, some stretches with potential mussel habitat include beaches or rocky shorelines at the base of cliffs and/or bluffs (e.g. west side of Point Defiance, Tacoma; many areas in the San Juan Islands). Depending on the distance between land access and the sampling site, several promising mussel sampling locations may only be reasonably accessed via boat.

Some of the potential or confirmed sampling sites identified in this feasibility study represent mussels growing on creosote-treated pilings (especially in marinas). Creosote-treated pilings are not suitable for monitoring in the context of the present study, and the NOAA Mussel Watch protocol stipulates mussel samples may not be taken from such substrates. Mussels growing on creosote-treated pilings have been shown to accumulate contaminants such as polycyclic aromatic hydrocarbons (PAHs) from the pilings, and therefore mask signals from overlying water.

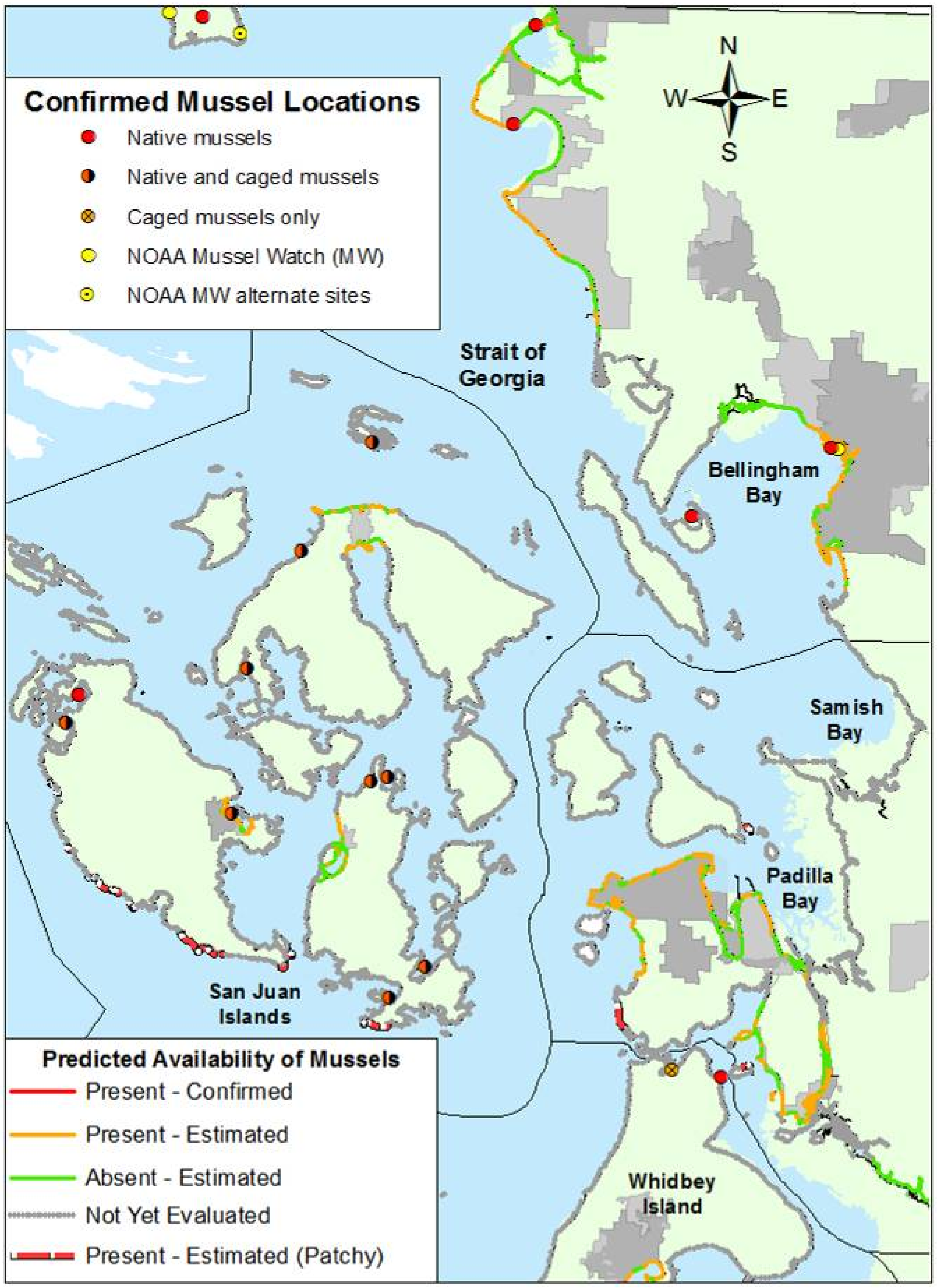

Figure A2. Confirmed and predicted availability of mussels in the Strait of Georgia.

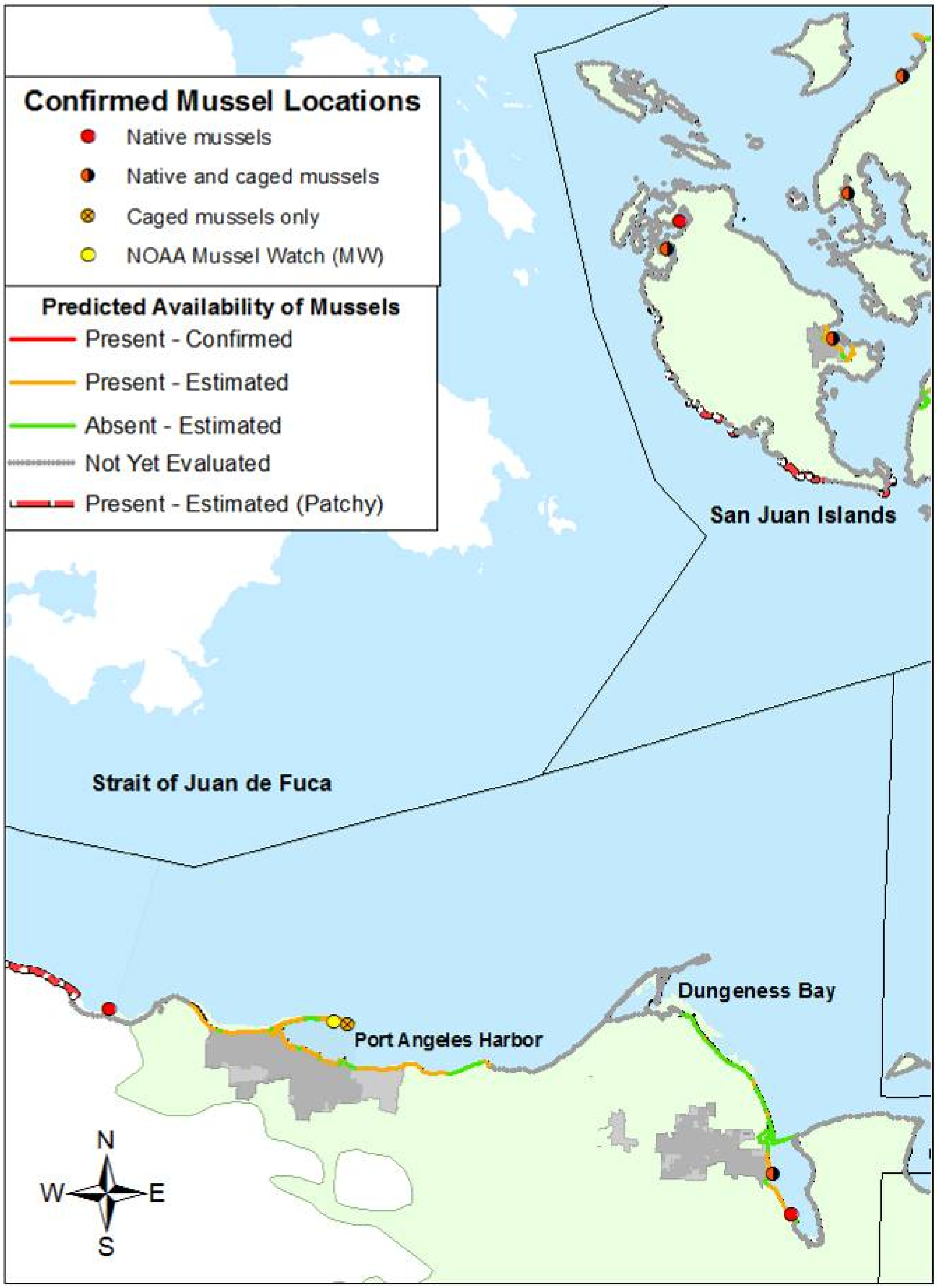

Figure A3. Confirmed and predicted availability of mussels in the Strait of Juan de Fuca.

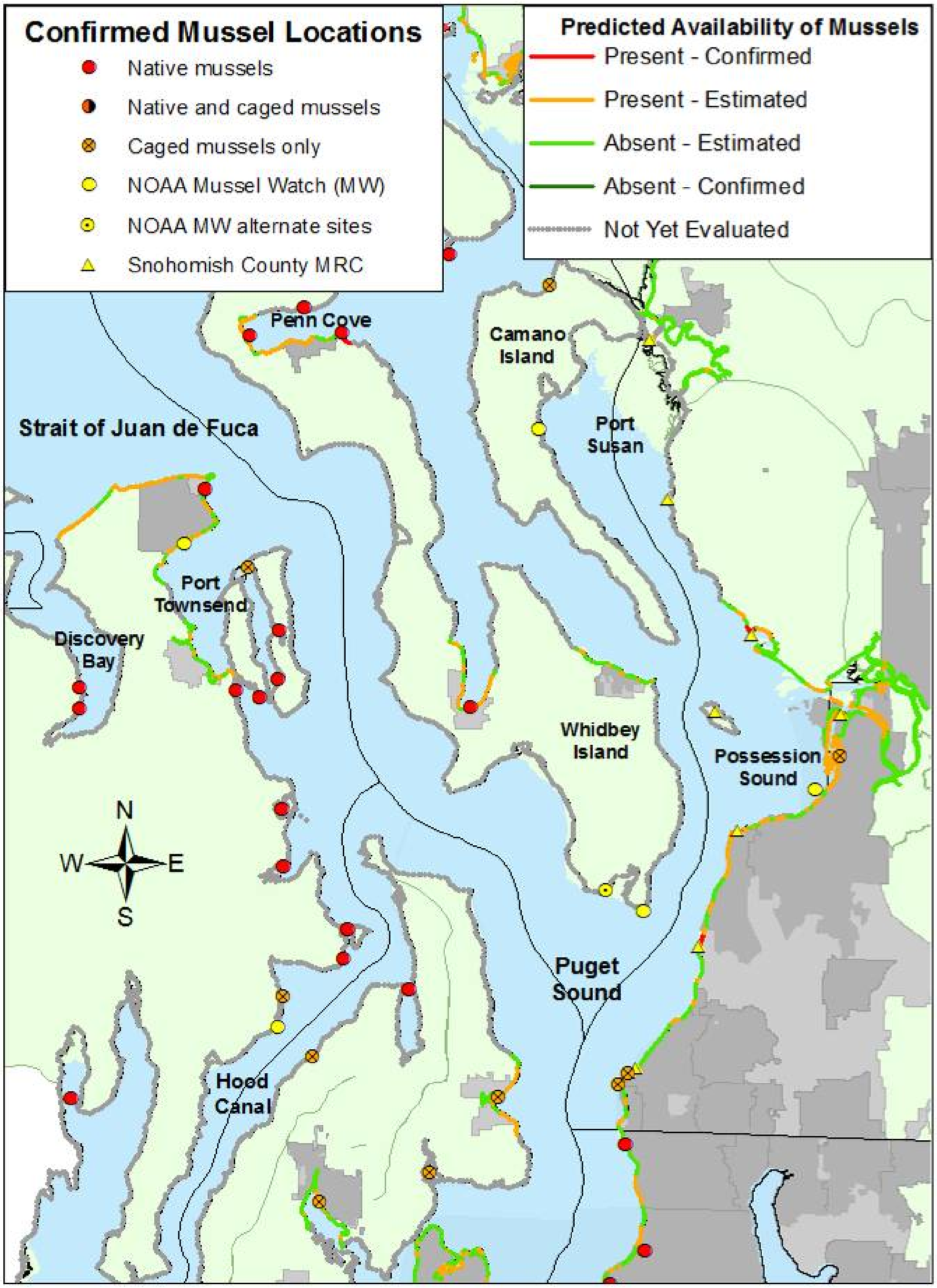

Figure A4. Confirmed and predicted availability of mussels in the north Puget Sound.

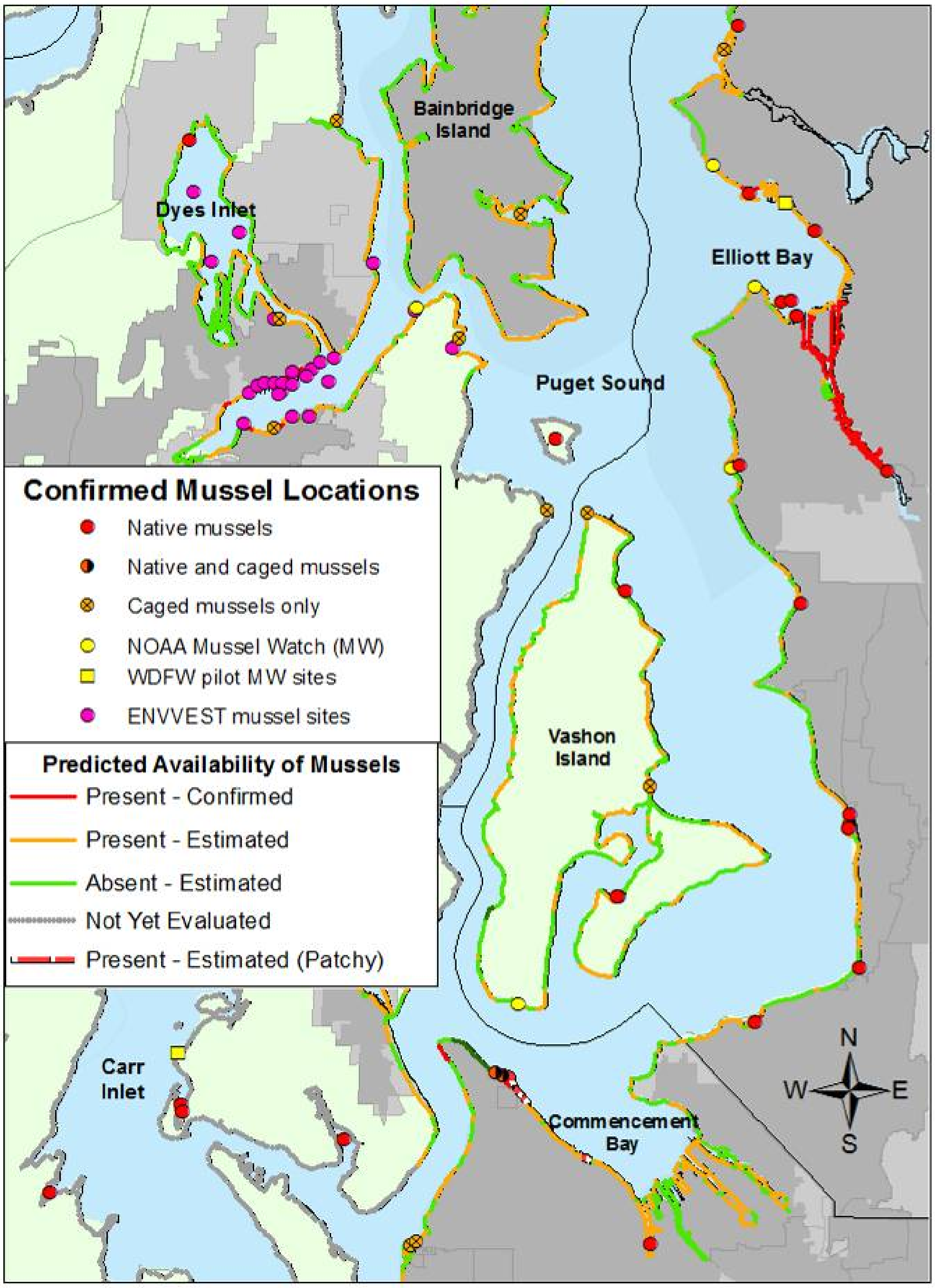

Figure A5. Confirmed and predicted availability of mussels in the central Puget Sound.

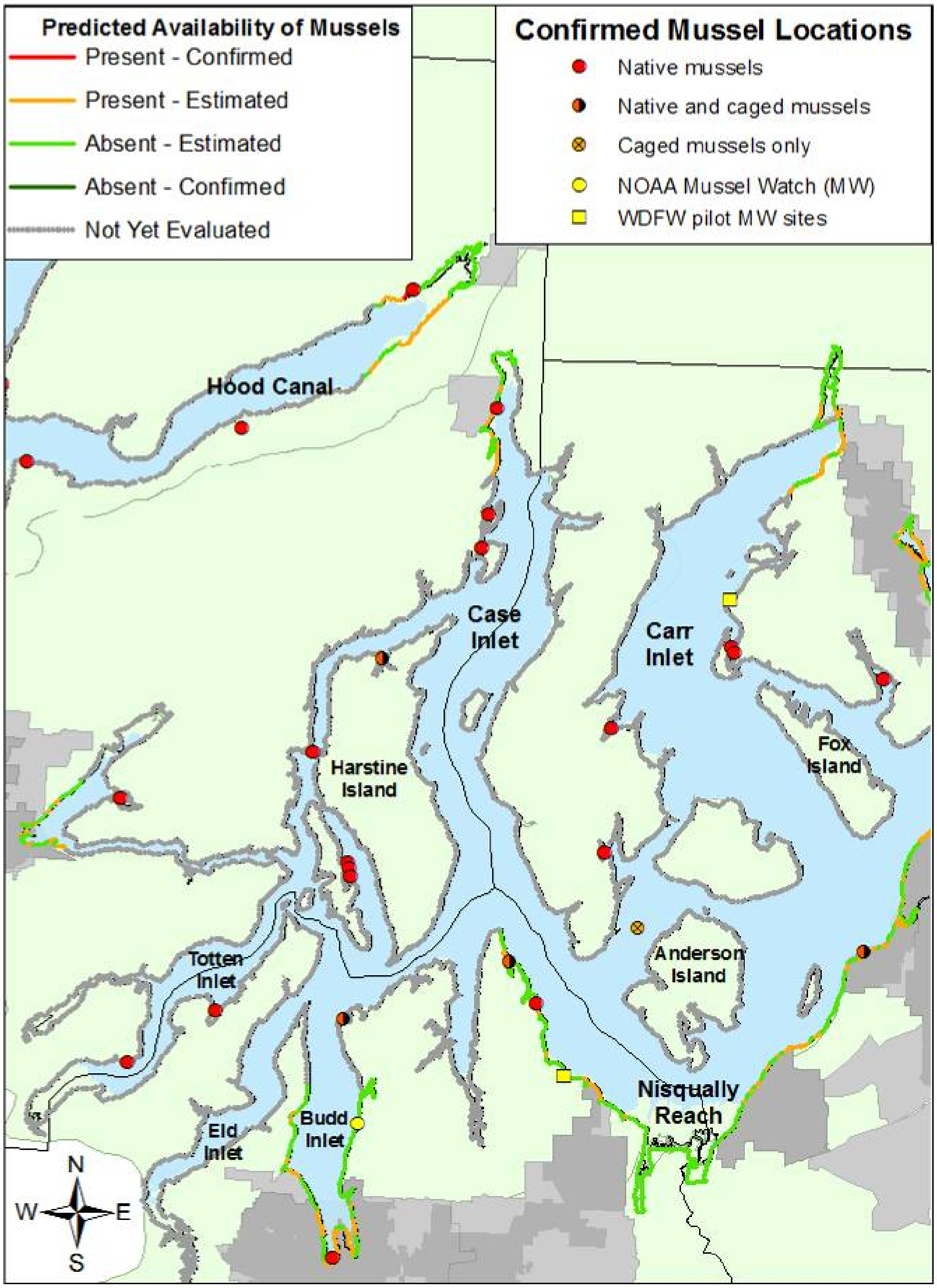

Figure A6. Confirmed and predicted availability of mussels in the south Puget Sound.

CONCLUSIONS

Based on this desktop study of putative mussel distribution along Puget Sound shorelines, we conclude sufficient known or suspected mussel beds exist to warrant continuing with the next steps towards developing a mussel sampling strategy to support Stormwater Working Group monitoring needs. This study suggests that over half of UGA shorelines in Puget Sound support or may support mussel beds, and that mussels are commonly distributed along non-UGA shorelines.

The products from this feasibility study will also serve as a foundation for establishing a mussel-monitoring program. They provide a geospatially-explicit database of mussel presence that will allow comparison or co-location with sediment and other studies, as well as evaluation of important shoreline features that relate to stormwater and other contaminant inputs to Puget Sound. These feasibility studies will support refining questions, developing appropriate hypotheses, and designing sampling schemes for monitoring.

APPENDIX B. POWER ANALYSIS of UGA vs. Non-UGA STUDY DESIGN:

A statistical power analysis is useful because it allows researchers to estimate the sample size needed to detect a significant ($\alpha = 0.05$) difference between groups with a specified power. A standard power for ecological studies is 0.80, which translates into an 80% likelihood of success at detecting difference, when there is one to detect. Power analysis relies on input of *known* or *predicted variance* of the populations of interest; the rest of the variables (α and power) are set at desired levels. We used NOAA's most current Mussel Watch data gathered from the greater Puget Sound- Strait of Georgia- /Juan de Fuca area in power analyses to estimate the number of mussel sample sites needed to detect a difference between UGA and non-UGA using a parametric t-test. To provide contemporary relevance, we limited the data to mussels collected from 2002 to 2008 and performed power analysis for six major contaminants of concern; PCBs, PBDEs, two PAHs (phenanthrene and fluoranthene), copper and mercury.

The power analysis for PCBs in mussels predicted the need for about 50 UGA and 50 non-UGA sites to allow detection of differences in contaminants 80% of the time (Figure B1). The number of sample sites required to adequately monitor the other five classes of pollutants ranged from 50 to 110 per group. ***Thus, at least 100 to 220 stations in total would need to be sampled to adequately compare contamination between UGA vs. non-UGA mussels in the Puget Sound (Table B1).***

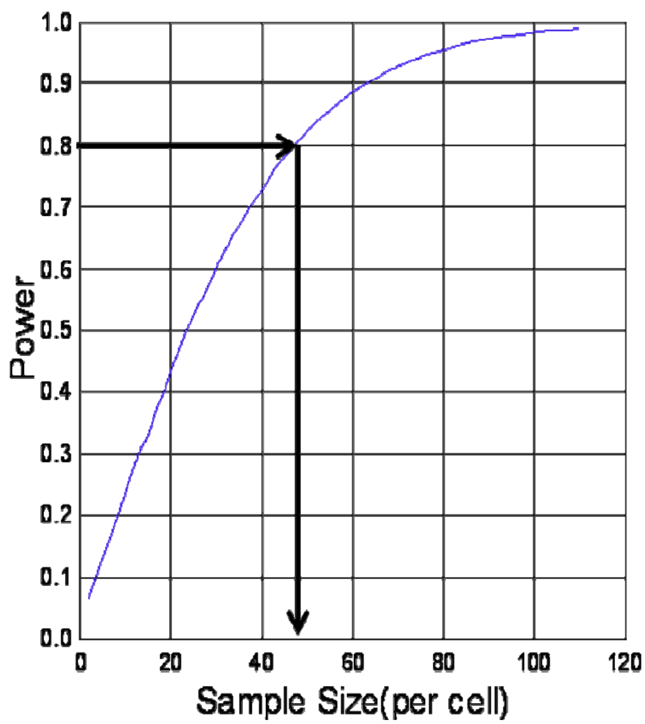

Figure B1. Example of power analysis curve (here for PCBs) indicating that a sample size of approximately 50 is required to gain an 80% power (power = 0.80; $\alpha = 0.050$)

Table B1. Number of sample sites required, per group (UGA; non-UGA) and total, to allow detection of a significant difference in contamination 80% of the time (power = 0.80; $\alpha = 0.05$)

Contaminant	Sample size per group	Total samples required
PCB	50	100
PBDE	75	150
Phenanthrene (PAH)	110	220
Fluoranthene (PAH)	105	210
Copper	50	100
Mercury	105	210

High variance in contamination among the sample population was largely responsible for the sample sizes indicated by the power analyses, and these results call into question whether a difference between UGA and non-UGA mussels actually existed. Graphic examination revealed that mussel contamination patterns did not follow a high - low curve from UGA - “rural” or non-UGA sites (Figure B2; yellow = UGA, green = non-UGA sites) as expected. Instead, sites with high levels of contamination were found in non-UGAs and sites with relatively low levels of contamination were found in UGAs. *These results suggest a stratified random sampling design specifying UGA and non-UGA as the strata is not optimal for monitoring regional contamination in mussels.* Many factors probably have an influence on mussel contamination and classifications of sites using more appropriate categories should be considered.

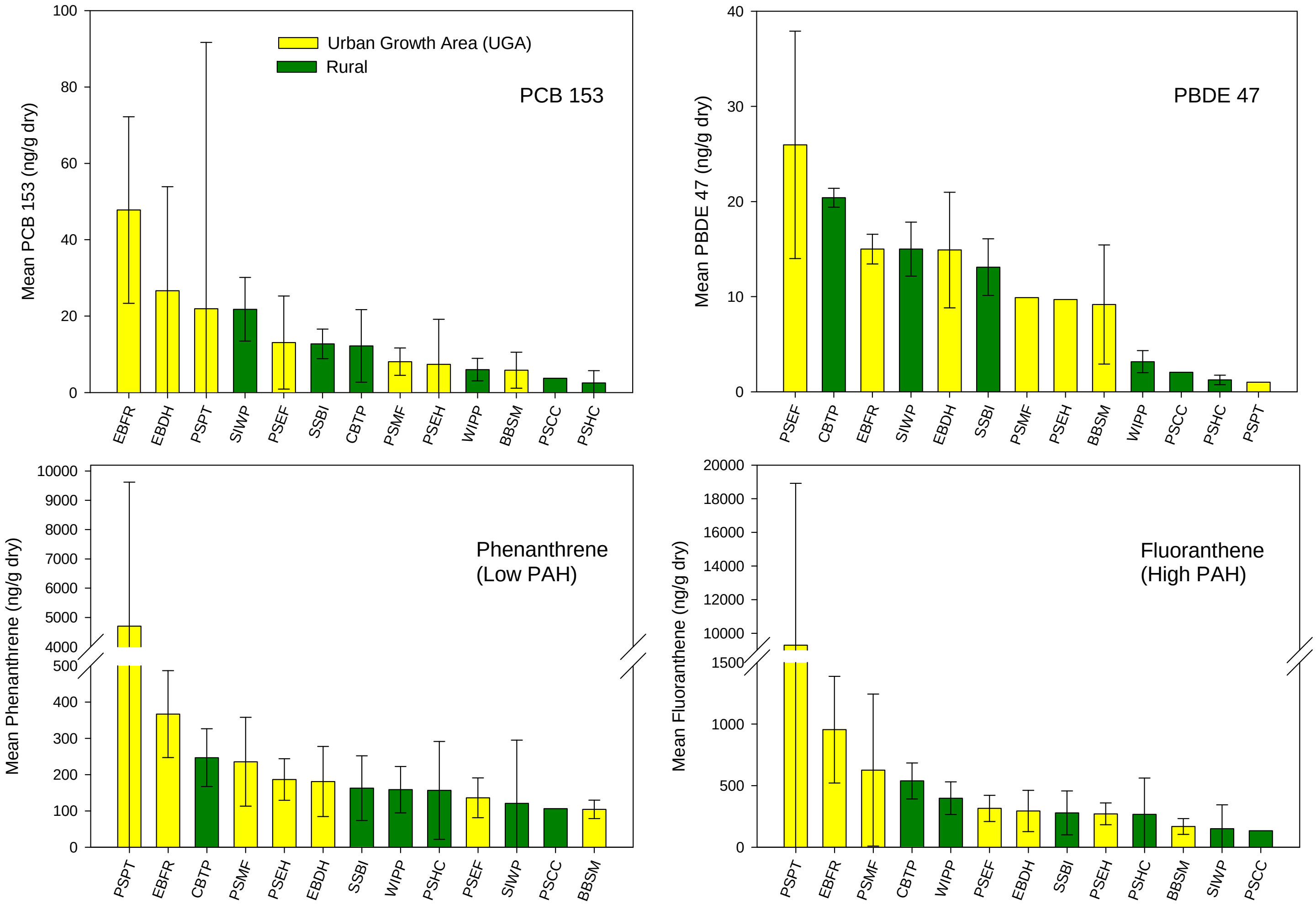

Figure B2. Average contaminant loads (dry weight) in mussel tissue taken from UGA (yellow) and non-UGA (green) areas in Washington. Data from 2004-2008, lines indicate 95% confidence intervals (variance).

Classifying mussel watch sites by land-use strata, such as the amounts of nearby impervious surface, roadway density, forests/wetland coverage, or agricultural development may be worth consideration, especially as they are linked to varying levels of stormwater runoff. In addition, special consideration may be required to adequately account for PAHs related to their sources within nearshore areas, such as from marinas, ferry terminals, and shoreline road and railways.

APPENDIX C: MUSSEL SAMPLING EQUIPMENT AND SUPPLY LIST

Note – due to the timing of low tides during the winter season, mussel sampling in the nearshore intertidal occurs at night.

Sampling –

- Flashlights and/or headlamps
- Batteries
- Propane lantern(s)
- Propane
- GPS unit
- Cell phone and/or two-way radios
- Thermometer
- Refractometer (measures salinity)
- Digital camera (waterproof)
- Plastic containers or small buckets (to wash mussels)
- Small cooler/bucket with ice (to carry mussels)
- Scrub brushes
- Knives
- Disposable laboratory gloves
- Gallon-sized Ziploc bags
- Quart-sized Ziploc bags
- Waterproof paper (data sheets/bag labels)
- Garbage bags
- Clipboard
- Sharpies/Pencils
- Paper towels
- Life jackets
- Raingear and boots

Shipping (per sample site) –

- 48 qt Cooler (holding for overnight/weekend prior to shipping)
- 16 or 28 qt Cooler (shipping container - size dependent on size of mussels)
- Garbage bags
- Ice
- Chain of Custody forms
- FedEx *Priority Overnight* mailing labels
- Nylon reinforced packing tape