

Toxics-focused Biological Observation System (T-BiOS), Puget Sound Ecosystem Monitoring Program (PSEMP)

# Stormwater Action Monitoring 2015/16 Mussel Monitoring Survey

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Data from the Stormwater Action Monitoring (SAM) program mussel monitoring will be available on Ecology's Environmental Information Management (EIM) website at <u>www.ecy.wa.gov/eim/index.htm</u>. Search Study ID, RSMP\_PMNM2015. Data from Pierce County will be under Study ID RSMP\_PC\_PMNM2015.

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# **Executive Summary**

Toxic contaminants enter the Puget Sound from a variety of pathways including non-point sources such as stormwater runoff, groundwater releases, and air deposition, and point sources like marinas, industrial and sewage treatment plant outfalls, and combined sewer overflows. However, stormwater is considered one of the biggest contributors to water pollution in the urban areas of Washington State because it is ongoing and damages habitat, degrades aquatic environments, and can have serious impacts on the health of the Puget Sound. Monitoring pollutants and their effects on the marine biota of Puget Sound is critical to inform best management practices and remediation efforts in this large and diverse estuary. In the winter of 2015/16 the Washington Department of Fish and Wildlife (WDFW), with the help of citizen science volunteers, other agencies, tribes, and non-governmental organizations, conducted the first of a series of biennial, nearshore mussel monitoring efforts under the new Stormwater Action Monitoring (SAM) program. SAM is a new collaborative stormwater program funded by municipal stormwater permit holders. This monitoring survey for SAM was intended to characterize the spatial extent of tissue contamination in nearshore biota residing inside the urban growth areas (UGAs) of Puget Sound, using mussels as the primary indicator organism. Future biennial SAM surveys will continue to track mussel tissue contamination in the Puget Sound nearshore to answer the question: "Is the health of biota in the urban nearshore improving, deteriorating, or remaining the same related to stormwater management?"

In this study we used native mussels (*Mytilus trossulus*) as indicators of the degree of contamination of nearshore habitats. We transplanted relatively uncontaminated mussels from a local aquaculture source to over 70 locations along the Puget Sound shoreline, covering a broad range of upland land-use types from rural to highly urban. At the end of the study we measured the concentration of several major contaminant classes in mussels: polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs, or flame retardants), chlorinated pesticides (including dichlorodiphenyltrichloroethane compounds, or DDTs) and six metals (lead, copper, zinc, mercury, arsenic, cadmium). We also determined the mortality and condition of mussels at the end of the exposure period.

Overall, the mussel survey was a success. We recovered mussels from 90% of the sites and survival was over 78%. The most abundant organic contaminants measured were PAHs, PCBs, PBDEs, and DDTs. PAHs and PCBs were detected in mussels from every site, and the concentrations were significantly higher in Puget Sound's most urbanized areas, as measured both by municipal land-use classification (i.e., cities and unincorporated-UGAs) and by the percent of impervious surface in upland watersheds adjacent to the nearshore (Table 3). Although lower in overall concentration, PBDEs and DDTs followed a similar pattern. In addition, most of these organic contaminants were elevated in areas near marinas and ferry terminals. The other organic contaminants were detected in mussels at only a few study sites and at low levels. Five of the six metals (lead was the exception) were found in mussels from all the study sites, though their concentrations were relatively low. Though zinc and lead were the only two metals that were significantly related to land-use in our testing, our power to detect differences in most of the metals (mercury, arsenic, cadmium, copper and lead) was often low.

These findings suggest toxic contaminants are entering the nearshore food web of the greater Puget Sound, especially along shorelines adjacent to highly urbanized areas. Based on the results of this survey and a number of power analyses, WDFW recommends the following:

- 1. To identify the major sources of contamination into the greater Puget Sound, and to better understand temporal trends and mechanisms, we recommend a) long-term nearshore mussel monitoring, and b) incorporation of our findings with other SAM monitoring studies.
- 2. In order to improve our ability to identify patterns and track changes in nearshore contamination SAM should relocate some of the mussel sites to better cover of the full spectrum of urbanization in Puget Sound. To accomplish this, SAM could introduce substrata into the GRTS model used to assign sites, utilizing mean impervious surface in nearshore watersheds to delineate the substrata. Depending on the number of substrata (three to four), between five to 10 sites would be required per substratum (20-40 sites total) to give sufficient power to detect changes in nearshore contamination in the future.
- 3. Once the new sites are selected, all of the mussel sites should become permanent SAM nearshore sites (i.e., index sites) to be revisited and resampled in future surveys for time trend analyses.
- 4. Considering the low power to detect differences in some of the metals during this first round of monitoring, SAM should commission a literature review of the efficacy of using mussels to detect changes in different types of metals and either drop or retain them from the list as appropriate.
- 5. Given recent evidence of contaminants of emerging concern (CECs) in Puget Sound fish (Peck et al., 2011, Johnson et al., 2008; Fiest et al., 2011), we recommend adding some CECs to the list of contaminants analyzed. We further recommend seeking guidance from PSEMP's Toxics Workgroup on which stormwater-related CECs are relevant to the Puget Sound and measurable via current methods.

The success of this large-scale, field-intensive monitoring study was due in large part to lessons learned from a separate WDFW pilot study using caged mussels (Lanksbury et al., 2014), expertise gained from the National Status and Trends' <u>Mussel Watch Program</u>, and the hundreds of hours of help from many citizen science volunteers. We recommend WDFW be retained to continue the SAM Mussel Monitoring program, and that volunteers continue to be utilized for this monitoring. In addition, partner groups sponsored a number of additional sites (25) to this study, including many locations outside the UGA, where SAM had no sites. These sponsors brought a benefit to SAM Mussel Monitoring by allowing for additional comparisons between UGAs and non-UGAs in this first survey.

# Acknowledgements

This study would not have been possible without substantial help from individual volunteers and volunteer groups. Prior to the start of the field deployment, these partners and volunteers helped evaluate potential monitoring sites, and measured and bagged thousands of mussels. Over the course of evening low tides volunteers helped deploy and retrieve mussel cages at 73 sites throughout the greater Puget Sound. Then after retrieving the cages, volunteers helped shuck and process thousands of mussels in the WDFW laboratory. Over 100 volunteers spent well over 500 hours helping us to execute this study and we are grateful for their efforts.

SAM and WDFW recognizes the following organizations, their staff and volunteers for their assistance:

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We also extend a special thank you to the agencies and groups below, who together sponsored an additional 25 sites for this study:

City of Bellingham, King County, Kitsap County Public Works, Snohomish County MRC, Stillaguamish Tribe, the Washington Department of Natural Resources Aquatic Reserves Program, and Whatcom County MRC. WDFW also sponsored a number of additional sites in this study.

The Puget Sound Partnership provided support to WDFW in managing the financial contracts for a number of these sponsored sites and we thank them for their assistance.

# Laboratory Partners

Analysis of all organic analytes and lipids in this report was conducted by NOAA's Northwest Fisheries Science Center (NWFSC) <u>Environmental Chemistry Program</u>, in Seattle Washington. WDFW and NOAA/NWFSC have worked together for the past twenty years developing and applying methods for measuring contaminants in Puget Sound's marine organisms, and evaluating the health effects from such exposure. This study was greatly enhanced by WDFW's long-standing partnership with NOAA. The authors particularly thank NOAA's chemistry lab director, Ms. Gina Ylitalo, as well as Ms. Bernadita Anulacion, Catherine Sloan, Daryle Boyd, Keri Baugh, Jennie Bolton, Richard Boyer, Ronald Pearce, and Jonelle Herman. Analysis of all metals was conducted by the <u>King County Environmental Lab</u> in Seattle Washington. The authors particularly thank Fritz Grothkopp and Diane McElhany for high quality analysis and advice about processing and analyzing mussel samples for metals.

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# Mussel Bagging

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We extend a special thanks to Penn Cove Shellfish of Whidbey Island, which generously donated the mussels and aquaculture bags used in this study. In addition, 34 volunteers worked diligently during the mussel-bagging phase of the study at Penn Cove; their efforts were central to the success of this study. We give special thanks to Kestutis Tautvydas as lead of the Sound Water Stewards of Island County, whose volunteers made up the majority of our baggers, and to the student volunteers from Western Washington University who joined our efforts as well.

# Peer Review

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#### Volunteer Poetry

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The following poem, submitted to SAM by one of the Sound Water Stewards of Island County, offered spirited insight for future mussel monitoring efforts:

*Just imagine yourself hiking off to retrieve a mussel cage on a black night under the stars.* 

On the walk down to the beach through the howling wind, only the path is lit by your light. All else is black.

*You see the surf breaking white on the black water, the black cage nearby with its small reflector.* 

What you don't see are the rapidly gathering clouds. As you approach the cage over the slippery black rock,

*it begins to rain, mostly sideways. It's a struggle in the blackness to pull on your vinyl gloves over wet hands.* 

Bending over the cage, you recall using a lot of cable ties (black ones) to secure the lid and keep the starfish out. The rain picks up, not quite as sideways as before.

So you begin to cut the cable ties with your dikes, which have a high visibility red handle, and black tips. It's now RAINING straight down, despite the wind.

As you rotate around the cage cutting cable ties, you block the light from the lantern held by your partner, plunging all the black components: the cage, the cable ties, the dikes...into blackness.

And just as your back pocket is filling with water and your boots are feeling the wind driven tide that isn't supposed to be there right now, you have a blinding revelation...

Why don't we make these damn cable ties white next year?

- Mark Kennedy, 2016

# Introduction

Toxic contaminants enter the greater Puget Sound from a variety of pathways. These include stormwater runoff, industrial outfalls, municipal sewage treatment outfalls and combined sewer overflows (CSOs), municipal and agricultural non-point runoff, groundwater releases, and air deposition. In the past, Puget Sound has been subject to contamination from a number of now-banned persistent and toxic chemicals, including polychlorinated biphenyls (PCBs) and dichlorodiphenyltrichloroethanes (DDTs). A reservoir of PCBs and DDTs are considered "legacy" contaminants, meaning they persists in the sediments and biota of Puget Sound (Long et al., 2005, O'Neill and West, 2009; Ross et al., 2000; West et al., 2011a; West et al., 2011b; West et al., 2001; West et al., 2008). In addition, ongoing contaminants to Puget Sound (Hobbs et al. 2015; Herrera, 2011; Milesi, 2015). Stormwater runoff is considered one of the biggest water pollution problems in urban areas of Washington State. The volumes and entrained contaminants in stormwater damages habitat, degrades aquatic environments, exacerbates flooding, and plays a major role in Puget Sound's deteriorating health (PSAT, 2005). Monitoring pollutants in the nearshore and their effects on the marine biota of Puget Sound is critical to inform best management practices used to manage stormwater and remediation efforts in this large and diverse estuary (Hamel, 2015).

# Background

The Puget Sound Ecosystem Monitoring Program (PSEMP) Stormwater Work Group (SWG) is a formal stakeholder coalition comprised of federal, tribal, state, and local governments, business, environmental, and agricultural entities, and academic researchers, all with interests and a stake in the Puget Sound watershed. The SWG was convened in October 2007 at the request of municipal stormwater permittees, the Washington State Department of Ecology (Ecology), and the Puget Sound Partnership (PSP) to develop a regional stormwater monitoring strategy and to recommend monitoring requirements in National Pollutant Discharge Elimination System (NPDES) municipal stormwater permits issued by Ecology. In 2010, the SWG finalized an overall strategy for monitoring, in a document entitled "2010 Stormwater Monitoring and Assessment Strategy for the Puget Sound Region (SWAMPPS)" (SWG, 2010). It promoted an integrated approach to quantifying stormwater pollutant impacts in Puget Sound, providing information to efficiently, effectively, and adaptively manage stormwater and reduce harm to the ecosystem.

A result of the SWG's overall strategy was the formation of a new Regional Stormwater Monitoring Program, recently renamed, and hereafter referred to, as Stormwater Action Monitoring (SAM; Ecology website, 2017). SAM includes three study components: 1) Status and Trends in Receiving Waters, 2) Effectiveness Monitoring of Stormwater Management Program Activities, and 3) Source Identification Information Repository. The Status and Trends in Receiving Waters component of SAM monitors changes in Puget Sound Iowland streams and Puget Sound urban shoreline areas in relation to stormwater management. Contaminant monitoring of mussels in the urban growth areas of Puget Sound's marine nearshore, hereafter referred to as SAM Mussel Monitoring, is part of SAM's Status and Trends in Receiving Waters.

# Purpose of Survey

SAM Mussel Monitoring surveys are intended to assess the tissue contaminant concentrations of nearshore biota in the *urban areas* of Puget Sound, defined as being along shorelines of established Urban Growth Areas (UGAs). Here we document the current geographic patterns of nearshore contamination, as seen in the winter of 2015/16. Future biennial surveys will provide data to describe changes in nearshore contamination over time. The purpose of SAM Mussel Monitoring is to identify existing stormwater-related challenges to the health of nearshore biota and, where possible, provide data to help target contaminant sources. This survey will support nearshore research activities by making uniformly collected, high quality data available to assist the SWG, the PSP, the state of Washington, and all Puget Sound stakeholders in measuring the success of stormwater and other environmental management programs.

# Objectives

In this study, our objectives were to:

- 1) Characterize the spatial extent of tissue contamination in nearshore biota residing inside the UGA sampling frame using mussels (*Mytilus* sp.) as the primary indicator organism.
- 2) Track changes in tissue contamination over time inside the UGA sampling frame to answer the question; "Is the health of biota in the urban nearshore improving, deteriorating, or remaining the same related to stormwater management?"

# Leveraging Existing State and Federal Efforts

From 1986 to 2012 NOAA's National Status and Trends' <u>Mussel Watch</u> Program tracked chemical and biological contaminant trends in naturally occurring bivalves (mussels and oysters) across the U.S. and in Puget Sound (Apeti et al., 2009; Center for Coastal Monitoring and Assessment, 2014). Mussel Watch data from 1986 to 2012 indicated a strong link between urbanization and certain persistent organic pollutants in Puget Sound (Kimbrough et al., 2008; Mussel Watch - unpublished data from 2009 - 2012). In the winter of 2012/13 the Washington Department of Fish and Wildlife (WDFW) conducted a broad-scale, synoptic assessment of toxic contaminants in the nearshore called the Mussel Watch Pilot Expansion (MWPE) study. Though similar to Mussel Watch, this pilot study expanded the footprint of monitoring to a much larger scale, including over 100 study sites in the Puget Sound, and utilized transplanted (i.e., caged) mussels at the study sites, instead of sampling naturally occurring mussels as the Mussel Watch program had in the past. The MWPE study was funded through a grant from the US Environmental Protection Agency's (EPA) <u>National Estuary Program</u> and relied heavily on volunteers and partners to accomplish the fieldwork portion of the study. Through this study, WDFW concluded that toxic contaminants are entering the nearshore food web of the Puget Sound, especially along shorelines adjacent to highly urbanized areas.

In tandem with the MWPE, the Tacoma-Pierce County Health Department (TPCHD) conducted a complementary gradient study, funded by Ecology, which included a high density of mussel cages placed along two Tacoma sites with different land use types. The overall goal of the project was to make progress toward defining the length of shoreline that represents a "site" for mussel contamination sampling and to measure impacts of land-use on nearshore biota (Hanowell et al. 2014). The study authors placed nine

cages along roughly 800 meters of shoreline in a residential/commercial area (Ruston Way) and in an industrial area (Hylebos Waterway) of Tacoma. Results indicated that mussels from the Hylebos Waterway sites had consistently higher concentrations of organic contaminants than those from the Ruston Way sites. The researchers concluded that land-use likely had an important influence on contaminant loading to mussels in the intertidal zone. They cited the many current and historical local nearshore activities in Tacoma and discharge of upland contaminants through stormwater outfalls as likely sources (Hanowell et al. 2014).

Following the success of the MWPE and TPCHD studies, the SWG approached WDFW to manage the SAM Mussel Monitoring. WDFW was able to recruit a number of the same volunteers who helped with the MWPE study to help with SAM Mussel Monitoring. WDFW also expanded the monitoring by soliciting partner groups (i.e., other state and local agencies, tribes, and marine resource committees; see Acknowledgements) interested in sponsoring additional mussel sites in their areas of interest. In addition, the use of "Citizen Science" volunteers to accomplish the majority of the field work realized a significant cost savings to the SAM program.

# Leveraging Pierce County's Efforts

Ecology's 2013-2018 permits that outline the scope of the SAM pooled resources program included a second option for jurisdictions to conduct monitoring in their area and contribute the data, but not pay-in. Pierce County selected this option. WDFW was retained by Pierce County to provide consistent protocols and lab analysis with the larger SAM Mussel Monitoring study, and as such, this report includes data on the Pierce County sites. In this report, we did not distinguish between the SAM and the Pierce County mussels sites. We treated them as one dataset for the statistical analyses (see Spatial Weighting of SAM and Pierce County Mussel Monitoring Sites), and assigned appropriate weights to the sites in the cumulative frequency distribution plots of the contaminants (see Appendices 5 - 14), to describe the entire Puget Sound nearshore biotic condition.

# Study Area and Site Selection

Details on the study design, study area, field and laboratory methods described in brief below are available in the <u>Quality Assurance Project Plan (QAPP)</u> for this study (Lanksbury and Lubliner, 2015), as well as in <u>Appendix 1</u>.

# Study Area

Our study took place in the greater Puget Sound, which is a fjord-like marine estuary on the northwestern coast of Washington State with many interconnected marine waterways and basins. Puget Sound is connected to the Pacific Ocean via the Strait of Juan de Fuca and is part of the larger Salish Sea, which stretches into Canada. Repeated advances and retreats of continental ice sheets shaped Puget Sound's geology. Its estuarine nature is strongly influenced by freshwater input through major river systems like the Skagit and Snohomish Rivers in the north, and the Puyallup and Nisqually Rivers in the south. Washington's Office of Financial Management estimates that five million people will live and work in the Puget Sound region by 2020 (Ecology, 2017).

Monitoring for this nearshore survey focused on a single landscape scale, the shoreline parallel to cities and established UGAs of the Puget Sound. A shoreline-sampling frame was defined to include the basins,

channels, and embayments of Puget Sound from the US/Canada border to the southernmost bays and inlets near Olympia and Shelton, to Hood Canal, and to portions of Admiralty Inlet, the San Juan Islands, and the eastern portion of the Strait of Juan de Fuca.

### Site Selection

The 2015/16 SAM and Pierce County nearshore monitoring site locations were selected using a probabilistic random stratified sampling design that targeted the land-based UGA boundaries of Puget Sound. This sampling framework was based on the EPA's spatially balanced, <u>generalized random</u> <u>tessellation stratified (GRTS)</u> multi-density survey design and is described by Stevens (1997, 2003), and Stevens and Olsen (1999, 2004). Sitka Technology Group, LLC using the GRTS design, generated a linear Puget Sound shoreline sampling frame. The result was 2,048 possible nearshore sites in Puget Sound, each representing approximately 800 meters (m) of UGA shoreline. We chose an 800 m length of shoreline to represent a mussel site based on criteria used by the Mussel Watch Program (pers. comm., D. Apeti, National Status & Trends Mussel Watch, March, 2015), which was supported by results from the TPCHD study (Hanowell et al., 2014). Meta-data for the Puget Sound shoreline sampling frame are on the Pacific Northwest Aquatic Monitoring Partnership's (PNAMP) Monitoring Resources website titled <u>Sample Design: Puget Sound Mussel Monitoring Pilot</u>.

Of the 2,048 possible sites in the Puget Sound UGAs, 40 locations were required for SAM Mussel Monitoring in 2015/16. WDFW staff and volunteers evaluated candidate sites from all counties on this list, except in Pierce County where WDFW evaluated only sites within *incorporated* Pierce County. Pierce County staff evaluated sites that were within *unincorporated* Pierce County. Sites were evaluated in numerical order from lowest to highest. WDFW confirmed 45 sites for SAM Mussel Monitoring. The five extra, confirmed sites provided a number of reserve (i.e., contingency) sites, in case one of the original 40 sites was rejected on the date of deployment. Pierce County confirmed eight qualifying shoreline sites in their own unincorporated UGAs, and had two extra sites in reserve on the date of deployment.

A number of partner groups joined the SAM Mussel Monitoring study, sponsoring 25 additional mussel sites (hereafter referred to as Partner sites) in their areas of interest. These sponsors together contributed nearly \$66,500 to this study, making it a cooperative mussel monitoring effort on an even larger scale.

# Results and Discussion

# Overview of Sampling Efforts

A virtual army of volunteers and partners helped to execute the various stages of this study, which included the safety and accessibility evaluations of the randomly selected SAM Mussel Monitoring sites, the predeployment measuring and bagging of mussels, the mussel cage deployments and retrievals, and the laboratory shucking and processing of mussels (see Acknowledgements). Over 100 volunteers spent well over 500 hours helping us implement this study and we are grateful for their efforts.

WDFW staff, volunteers, and partners deployed mussel cages to 73 monitoring sites: 40 SAM sites, 8 Pierce County sites, and 25 Partner sites. Mussel cages were recovered from 66 of those sites (i.e., 90%): 36 SAM sites, 7 Pierce County sites, and 23 Partner sites (Table 1, Figure 1, Figure 2, Figure 3, and Figure 4). Unfortunately, we lost mussel cages from the following seven monitoring sites due to storms:

- 1. SAM Site #20 (Port Angeles Harbor)
- 2. SAM Site #34 (Elliott Bay, Harbor Island, Pier 17)
- 3. SAM Site #36 (Ediz Hook)
- 4. SAM Site #40 (Fort Worden)
- 5. Pierce County Site #185 (Browns Point)
- 6. Partner Site "CPS\_MIAR" (Maury Island Aquatic Reserve, Old Marine Park)
- 7. Partner Site "SJI\_OINS" (North Shore, Orcas Island)

Mussel cages were deployed during low tide on the evenings of October 26 - 29, 2015. WDFW also collected six replicate samples from the Penn Cove Shellfish aquaculture facility at the start of the study, on October 29, 2015; these samples are hereafter referred to as the Baseline Site mussels. Exposure to local conditions at each mussel-monitoring site lasted approximately three months. The deployed mussel cages were recovered during low tides on the evenings of February 5 - 10, 2016.

Source	Site ID Site Name		Latitude	Longitude	County
SAM	WB_PC	Baseline (Penn Cove)	48.2176	-122.7086	Island
SAM	Site #2	Arroyo Beach	47.5017	-122.3860	King
SAM	Site #3	Brackenwood Ln	47.6823	-122.5065	Kitsap
SAM	Site #4	Cherry Point North	48.8584	-122.7407	Whatcom
SAM	Site #5	Salmon Beach	47.2947	-122.5305	Pierce
SAM	Site #6	Eagle Harbor Dr.	47.6189	-122.5275	Kitsap
SAM	Site #8	Chimacum Creek delta	48.0490	-122.7723	Jefferson
SAM	Site #10	Fletcher Bay, Fox Cove	47.6445	-122.5762	Kitsap
SAM	Site #11	South Bay Trail	48.7257	-122.5063	Whatcom
SAM	Site #13	Ruston Way	47.2927	-122.4950	Pierce
SAM	Site #14	Point Heron East	47.5701	-122.6069	Kitsap
SAM	Site #15	Tugboat Park	48.4893	-122.6761	Skagit
SAM	Site #16	Meadowdale Beach	47.8545	-122.3352	Snohomish

Table 1. Sixty-six (66) nearshore mussel sites were successfully monitored in this study (43 SAM and 23 Partner sites).

Source	Site ID Site Name		Latitude	Longitude	County
SAM	Site #17 Budd Inlet, West Bay		47.0689	-122.9195	Thurston
SAM	Site #18	Seahurst	47.4632	-122.3691	King
SAM	Site #19	Skiff Point	47.6612	-122.4991	Kitsap
SAM	Site #21	Point Defiance Ferry	47.3061	-122.5146	Pierce
SAM	Site #22	Beach Dr. E	47.5593	-122.5970	Kitsap
SAM	Site #23	Wing Point	47.6222	-122.4966	Kitsap
SAM	Site #24	S of Skunk Island	48.0276	-122.7503	Jefferson
SAM	Site #25	Blair Waterway	47.2758	-122.4174	Pierce
SAM	Site #26	N of Illahee State Park	47.6033	-122.5966	Kitsap
SAM	Site #27	Chuckanut, Clark's Point	48.6907	-122.5042	Whatcom
SAM	Site #28	Oak Harbor	48.2721	-122.6398	Island
SAM	Site #29	Liberty Bay	47.7375	-122.6507	Kitsap
SAM	Site #30	Kitsap St Boat Launch	47.5416	-122.6403	Kitsap
SAM	Site #31	Eastsound, Fishing Bay	48.6939	-122.9106	San Juan
SAM	Site #35	Williams Olson Park	47.6658	-122.5669	Kitsap
SAM	Site #37	Saltar's Point	47.1703	-122.6108	Pierce
SAM	Site #38	Rocky Point	47.6026	-122.6700	Kitsap
SAM	Site #39	Smith Cove, Terminal 91	47.6324	-122.3787	King
SAM	Site #42	Evergreen Rotary Park	47.5755	-122.6280	Kitsap
SAM	Site #43	N Avenue Park	48.5211	-122.6153	Skagit
SAM	Site #46	Appletree Cove	47.7873	-122.4947	Kitsap
	Cherry Point Aquatic Reserve,				
SAM	Site #47	Birch Bay South	48.8956	-122.7825	Whatcom
SAM	Site #48	Naketa Beach	47.9278	-122.3093	Snohomish
SAM	Site #49	Donkey Creek Delta	47.3378	-122.5902	Pierce
SAM	Site #61	Dash Point Park	47.3197	-122.4269	Pierce
Pierce	ou				
County	Site #161	Purdy, Dexters	47.3857	-122.62/3	Pierce
County	Site #353	Purdy, Nicholson	47.3761	-122,6249	Pierce
Pierce			1710701	12210210	
County	Site #481	Gig Harbor Boat Launch	47.3379	-122.5828	Pierce
Pierce					
County	Site #625	Gig Harbor, Mulligan	47.3306	-122.5755	Pierce
Pierce	Site #697	Browns Point Wolverton	17 2982	-122 4368	Pierce
Pierce	5110 #057		47.2302	122.4500	Tieree
County	Site #953 Browns Point, Carlson 47.3077 -122.4		-122.4352	Pierce	
Partner	CPS_EF	Edmonds Ferry	47.8142	-122.3822	Snohomish
Partner	CPS_HCV	Port Madison, Hidden Cove	47.6933	-122.5447	Kitsap
Partner	CPS_MASO	Manchester, Stormwater Outfall	47.5562	-122.5428	Kitsap

Source	Site ID	Site Name	Latitude	Longitude	County
Partner	CPS_PNP	Point No Point	47.9086	-122.5267	Kitsap
Partner	CPS_QMH	Quartermaster Harbor	47.4052	-122.4398	King
Partner	CPS_SB	Salmon Bay	47.6663	-122.4018	King
Partner	CPS_SHLB	Shilshole Bay	47.6714	-122.4065	King
Partner	CPS_SQSO	Suquamish, Stormwater Outfall	47.7296	-122.5504	Kitsap
Partner	EB_ME	Elliott Bay, Myrtle Edwards	47.6186	-122.3611	King
Partner	HC_FP	Fisherman's Point	47.7822	-122.8344	Kitsap
Partner	НС_НО	Hood Canal, Holly	47.5704	-122.9716	Kitsap
		Bellingham Bay, Little Squalicum			
Partner	NPS_BLSC	Creek	48.7639	-122.5175	Whatcom
		Cherry Pt Aquatic Reserve 4,			
Partner	NPS_CPAR4	Conoco Phillips	48.8208	-122.7101	Whatcom
Partner	NPS_DHCC	Drayton Harbor, California Creek	48.9621	-122.7327	Whatcom
		Fidalgo Bay Aq Reserve,			
Partner	NPS_FBAR	Weaverling Spit	48.4827	-122.5855	Skagit
Partner	SPS_HIAP	Hammersley Inlet-Arcadia Point	47.1990	-122.9395	Mason
		Nisqually Rch Aq Rsv, Anderson			
Partner	SPS_NRQR	ls	47.1496	-122.6764	Thurston
Partner	SPS_PBL	Purdy, Burley Lagoon	47.3870	-122.6367	Kitsap
Partner	WB_CB	Cavalero Beach Co. Park	48.1744	-122.4758	Snohomish
Partner	WB_KP	Kayak Point	48.1339	-122.3660	Snohomish
Partner	WPS_IC	Illahee Creek	47.6159	-122.5949	Kitsap
Partner	WPS_PB	Point Bolin	47.6943	-122.5959	Kitsap
Partner	WPS_SVD Sliverdale, Dyes Inlet		47.6429	-122.6967	Kitsap



Figure 1. Nearshore mussel sites located in the northern regions of the great Puget Sound, including Whatcom, San Juan, Skagit, Island, Snohomish, Jefferson, and Clallam counties. Site labels correspond to "Site ID" column in Table 1, UGA = urban growth area.



Figure 2. Nearshore mussel sites located in the central regions of Puget Sound, including Snohomish, King, Kitsap and Jefferson counties. Site labels correspond to "Site ID" column in Table 1, UGA = urban growth area.



Figure 3. Nearshore mussel sites located in the Pierce County regions of the Puget Sound, including some sites in King County. Site labels correspond to "Site ID" column in Table 1, UGA = urban growth area.



Figure 4. Nearshore mussel sites located in the southern regions of Puget Sound, including Kitsap, Pierce, Thurston and Mason counties. Site labels correspond to "Site ID" column in Table 1, UGA = urban growth area.

A number of the potential GRTS nearshore sites were rejected for SAM Mussel Monitoring for reasons mostly related to safety or accessibility (see Site Selection Criteria section of Appendix 1). Table 2 lists the rejected sites and their reasons for rejection. Additional information about the Pierce County site selection and results is available from the following link

https://www.co.pierce.wa.us/ArchiveCenter/ViewFile/Item/5489).

Table 2. GRTS nearshore sites that were evaluated and rejected for SAM Mussel Monitoring.

Site ID	County	Nearest City	Reason for Rejection
Site #1	Thurston	Olympia	Sucking mud poses danger at this site.
Site #7	King	Seattle	Site inaccessible due to cliffs.
Site #9	Pierce	Tacoma	Unable to access beach at this location.
Site #12	Island	Oak Harbor	Navy Base - site access restricted and unexploded ordinance on beach.
Site #32	Snohomish	Everett	Site is on a cleanup location owned by Port of Everett, which denied us access.
Site #33	Pierce	DuPont	Sucking mud poses danger at this site.
Site #41	Pierce	Tacoma	One of three potential sites in Blair Waterway, dropped due to oversampling of area.
Site #44	Island	Langley	Sucking mud poses danger at this site.

# Spatial Weighting of SAM and Pierce County Mussel Monitoring Sites

For all of the analyses reported herein, data from Pierce County sites are included with data from the SAM sites (n = 43 successfully monitored sites all together). Though the SAM and Pierce County mussel sites were selected from a random list of locations along the UGAs of Puget Sound, the Pierce County sites came from a much smaller substratum of the original UGA sample frame than the rest of the SAM nearshore sites: the Pierce County sites were selected only from unincorporated-UGA shorelines within Pierce County. Because of this difference in geography, the spatial weights of the regional SAM nearshore sites and the Pierce County nearshore sites are different. Each SAM Mussel Monitoring site had a weight of 33,432 meters (20.8 miles) of shoreline and each Pierce County sites rejected from the random UGA list and those whose cages were lost during the course of the study.

The difference in spatial weight between the SAM and Pierce County nearshore sites affected the *combined data* in the cumulative frequency distribution (CFD) plots for each contaminant type (*black lines* in Appendices 5 - 14) in that the Pierce County sites carry a much lower weight relative to the SAM nearshore sites for the entire Puget Sound. However, spatial weighting of sites was not appropriate for the statistical analyses described in the "Spatial Extent of Nearshore Contamination" and the "Ranges and Concentrations of Organic Contaminants and Metals" sections below, thus no weighting was applied to those analyses.

# Spatial Extent of Nearshore Contamination

Overall, PAHs, PCBs, PBDEs, and DDTs were the most abundant organic contaminants measured in this study (Figure 5, Appendix 2). PAHs and PCBs were detected in mussels from all 43 SAM and Pierce County sites (hereafter referred to simply as SAM Mussel Monitoring sites), PBDEs were detected at 36/43 (84%)

of the sites, and DDTs at 37/43 (86%) of the sites. Three other organic contaminants were rarely detected; chlordanes were detected at 2/43 (5%) sites, and dieldrin and hexachlorocyclohexanes (HCHs) each were detected at 1/43 (2%) sites. The remaining organic contaminants, hexachlorobenzene (HCB), Mirex, aldrin, and endosulfan 1, were not detected at any sites.

PAHs and PCBs were detected in all of the Baseline Site replicate samples (n = 6), but the concentration of PBDEs and DDTs were below the limit of quantitation (LOQ) in all of those samples (i.e., they were not detected). Chlordanes, dieldrin, aldrin, HCHs, HCBs, Mirex, and endosulfan 1 were also not detected above the LOQ in any of the Baseline Site samples. Information about the treatment and use of LOQ data in this study is located in the Data Analyses section.

PAHs, PCBs, PBDEs, and DDTs were the most abundant organic contaminants detected at the Partner sites (i.e., sites sponsored by groups outside the SAM) as well. PAHs and PCBs were detected at 100% of the Partner sites, PBDEs at 18/23 (78%) sites, and DDTs at 20/23 (87%) sites. Dieldrin and HCHs were detected at 1/23 (4%) Partner sites and chlordanes was detected at 2/23 (9%) of the sites (see separate sections on Chlordanes, Dieldrin, and HCHs below for details). HCB, Mirex, aldrin, and endosulfan 1, were not detected at any of the Partner sites.



Figure 5. Range of concentrations of the four most frequently detected organic contaminants at SAM Mussel Monitoring sites; whiskers are 1.5 IQR, single points are outliers, percent of sites where contaminant was detected is indicated above each range.

All six of the metals were detected in mussel from this study (Figure 6). Mercury, arsenic, cadmium, copper and zinc were detected in mussels from all 43 SAM Mussel Monitoring sites, lead was detected at 37/43 (86%) of the sites.

All of the metals were detected in all of the Baseline Site samples (n = 6). Mercury, arsenic, cadmium, copper and zinc were detected in mussels from all of the Partner sites, and lead was detected at 21/23 (91%) of the Partner sites (Appendix 3).



Figure 6. Range of concentrations of metals detected at SAM mussel sites; whiskers are 1.5 IQR, single points are outliers, ercent of sites where metal was detected is indicated above each range.

# Statistical Analyses of Mussel Contaminant Concentrations on SAM Sites

Unless otherwise indicated, the statistical analyses described below were conducted only on the SAM and Pierce County mussel sites, collectively referred to as the SAM Mussel Monitoring sites (n=43). We investigated the impact of land-use, in-water sources of contaminants, and geological features on nearshore contamination. To begin we investigated the effect of land-use at several different geographic scales, below is a list of the three approaches we utilized to investigate differences in nearshore contamination related to land use:

- 1. Municipal land-use designation
- 2. Watershed land use (i.e., effect of nearshore adjacent watersheds)
- 3. Shoreline land use (i.e., effect of land use within 200 m of the shoreline)

#### Municipal Land-Use Designation

Under guidance from the Growth Management Act (GMA), municipalities use urban growth boundaries as regional borders to help control urban sprawl. Washington State Law instructs counties to "designate an urban growth area [UGA] or areas within which urban growth shall be encouraged and outside of which growth can occur only if it is not urban in nature" (RCW 36.70a.110). Cities and towns are located within UGAs. Areas outside of city boundaries that are becoming more urbanized are called "unincorporated-UGAs". We investigated whether these two existing municipal land-use designations correlated with nearshore contamination in mussels.

#### Watershed Land Use

To characterize land use on a watershed scale we overlaid land cover data from the National Land Cover Dataset (Homer et al., 2015) onto predefined, watershed catchment areas adjacent to the Puget Sound shoreline. These watershed catchment areas were originally developed by Ecology for another purpose (Stanley et al., 2011), but were determined to be of a size appropriate for use in this study (median area of 8.8 kilometer<sup>2</sup> or 3.4 mile<sup>2</sup>). To investigate effects of impervious surface on nearshore contamination we calculated the average value (i.e., intensity in percent) of impervious surface within each watershed adjacent to mussel sites. We also determined the percent of land area in each watershed covered by urbanization, forest, agriculture, and wetland to investigate the influence of each on nearshore contamination.

#### Shoreline Land Use

In contrast to the largescale analysis using watersheds, we tested for the effect of land use at a smaller, nearshore scale. We determined the percent of land area covered by urbanization, forest, and agriculture within 200 m (656 feet) of the shoreline adjacent to each mussel site. For this analysis, we used data from NOAA's C-CAP Land Cover Atlas, which reports land use related to discrete shoreline segments. The C-CAP shoreline segments are a modification of the Salmon and Steelhead Habitat Inventory and Assessment Project (SSHIAP) "GeoUnit" attribute, which used the WDNR ShoreZone and a variety of other sources and methods to develop the segments (McBride et al., 2009).

### In-Water Sources and Geological Features

We explored whether in-water and onshore point sources affected nearshore contamination using GIS data on the locations of marinas and ferry terminals in Puget Sound. For these analyses, we considered marinas and ferry terminals present if they were within 2 kilometers (1.2 miles) of a mussel site; only marinas and ferry terminals along an adjoining shoreline to a mussel site, not across a waterway, were included. We also tested for the presence of creosote, based on a systematic review of site data provided by the volunteers when they installed the mussel cages. We considered creosote present if there was any within 200 meters (656 feet) of the mussel cage, either in the water or on the shoreline.

Lastly, we investigated the potential effects of natural geographical and geological features on nearshore contamination. First we classified mussel sites by shoreline form, dividing them into those occurring in an embayment and those occurring along an open shoreline. Second, based on information provided by volunteers on our deployment datasheets we divided the substrate at each mussel site into one of two broad classes; coarse vs. depositional. We defined coarse substrate as dominated by cobble, gravel, and sand, and depositional substrate as containing mostly mud or silt.

# **Overview of Statistical Results**

Of all the factors tested, municipal land-use designation and mean percent impervious surface in the adjacent watershed showed the strongest relationship with observed concentrations of pollutants in mussels (Table 3). Both factors describe urban development in slightly different ways, and both affected concentrations of PAHs, PCBs, PBDEs, and DDTs in nearshore mussels. Figures 7 and 8 depict these land cover types in the central Puget Sound region in relation to the mussel monitoring sites. Following are discussions of the findings for each of the factors tested in this study.

_		Significant Results (α <0.05)		
Туре	Test	Organic Contaminants	Metals	
Municipal land-use planning designations	UGA vs. Baseline Site	PAHs, PCBs, PBDEs, DDTs	NS	
	UGA class (city vs. unincorporated-UGA)	PAHs, PCBs, PBDEs, DDTs	Zinc	
Watershed land use* measured in adjacent watersheds with an average area 8.8 km <sup>2</sup> (3.4 miles <sup>2</sup> )	mean % Impervious Surface	PAHs, PCBs, PBDEs, DDTs	NS	
	% Urban area	PBDEs, DDTs	NS	
	% Forested area	NS	NS	
	% Agricultural area	PCBs, PBDEs, DDTs	Lead	
	% Wetland area	NT	NT	
Shoreline land use <sup>+</sup> measured up to 200 meters (656 ft.) inland from shoreline	% Urban area	NS	NS	
	% Forested area	NS	NS	
	% Agricultural area	NS	NS	
In-water point sources	Marina/ferry terminal presence	PAHs, PCBs, DDTs	Lead	
	Creosote observed	NS	NS	
Natural geographical/geological features	Shoreline form (bay vs. open)	NS	Lead	
	Substrate (depositional vs. coarse)	NS	Lead	

Table 3. Impact of a land-use and point source factors on the concentration of contaminants in nearshore mussels.

UGA = urban growth area, NS = not significant, NT = not tested due to lack of replicates

\* Data from National Land Cover Dataset 2011

<sup>+</sup> Data from NOAA's C-CAP Land Cover Atlas shoreline characterization



Figure 7. View of 2015/16 mussel monitoring sites in the central Puget Sound in relation to municipal land use coverages, and locations of marinas and ferry terminals. UGA = urban growth area.



*Figure 8. View of 2015/16 mussel monitoring sites in the central Puget Sound in relation to mean impervious surface (NLDC, 2011) coverage in nearshore watersheds, and locations of marinas and ferry terminals.* 

## Municipal Land-Use Designations

Overall, municipal land designation had a significant effect on the amount of nearshore organic contaminants in mussels. Organic contaminants were higher in the entire UGA as compared to the Baseline Site. In addition, levels of organic contaminants were generally higher in the city-UGAs relative to the unincorporated-UGAs. The UGA boundaries used in this analysis were taken from Ecology's "City and Urban Growth Areas" Spatial Dataset (Ecology, 2017).

#### Comparison of entire UGA to Baseline Site

Mussels from within the entire UGA had significantly higher concentrations of organic contaminants (PAHs, PCBs, PBDEs, and DDTs) than mussels from the Baseline Site (i.e., six replicate mussel composites from Penn Cove, Whidbey Island). Concentrations of PAHs and PCBs were 100 and 10 times higher, respectively, in UGAs than at the Baseline Site (Table 4). Though none of the metals were significantly higher in the UGAs, the power to detect differences in mercury, arsenic, cadmium, copper, and lead was very low (less than 9%), likely due to the high amount of variability among sites within the UGAs.

Table 4. There were significant differences in contaminant concentrations between mussels at the start of the study (i.e., Baseline Site) and mussels from within the UGA (urban growth area) at the end of the study. Concentrations reported are geometric means, t-statistics and p-values reported for pooled variance.

Chemical	Baseline Site (n = 6)	UGA (n = 43)	t(47)	p-value	Statistical
	Conc. (ng/g,			FOWEI (70)	
PAHs	30.6	383	-6.77	<0.001	100
PCBs	5.41	37.4	-5.74	<0.001	100
PBDEs	<1.27*	5.79	18.3†	<0.001	100
DDTs	<1.27*	2.92	12.7†	0.001	99
	Conc. (mg/kg	, dry wt.)			
Mercury	0.044	0.039	0.66	0.510	5
Arsenic	6.10	6.49	1.18	0.246	9
Cadmium	1.70	1.69	0.25	0.808	5
Copper	7.55	7.13	0.50	0.622	9
Lead	0.333	0.463	-1.52	0.135	5
Zinc	84.1	85.5	-0.17	0.864	51

\*Concentration below limit of quantitation. †ANOVA F-ratio; T-test does not accept zeros.

#### Comparison within UGA: City vs. Unincorporated-UGA

We also investigated differences in nearshore contamination *within* the UGA. We approached this part of the municipal land designation analysis in two different ways:

- 1. We used the municipal land designation nearest the shoreline to assign SAM mussel sites to groups (Table 5, Figure 9),
- 2. We used the dominant municipal land designation in the watershed adjacent to the shoreline (i.e., the municipal land designation that occupied the largest percent of the watershed) to assign the SAM mussel sites to groups (Table 6, Figure 10).

First, we assigned the mussel sites to groups based solely on which shoreline-type was closest to the site. This method gave us two mussel site categories (unincorporated-UGA and city-UGA), and emphasized the municipal land designation near the shoreline. Second, we assigned mussel sites to the municipal land designation category that covered the majority of the upland watershed adjacent to the shoreline. The average size of the watersheds used here was 3.4 miles<sup>2</sup> (8.8 km<sup>2</sup>; Stanley et al., 2012). This method resulted in three SAM mussel site categories (outside the UGA, unincorporated-UGA, and city-UGA). In this second approach some SAM sites (n = 6) were actually categorized as "outside the UGA" because they were adjacent to watersheds with a majority dominated by non-UGA land.

Both of these approaches demonstrated that municipal land-use designation accounts for over 30% of the variability in nearshore organic contaminant concentrations (Table 5 and Table 6). Whether we parsed mussel sites by municipal land use near the shoreline, or within the adjacent watershed the concentration of contaminants went up with increasing levels of urban development (Figure 9 and Figure 10). This was not surprising given the increase in urban land use and decrease in forested cover among the different municipal land-use classifications (Figure 11). However, the watershed approach gave us a slight advantage in discrimination of PAH data in the mussels - it suggests that unincorporated-UGAs are intermediate between areas outside the UGA (OUGA) and cities. In contrast, the shoreline approach could only discriminate between unincorporated-UGAs and cities. However, the watershed approach did not give any additional insight into PCB data and it actually obscured differences in PBDEs and DDTs.

Zinc was significantly different in the shoreline approach, but was not different between groups in the watershed approach (Table 5 and Table 6). However, results were difficult to interpret because the lowest zinc values occurred in the unincorporated-UGA area, with higher values in both the city-UGA and the Baseline sites. Both of the statistical tests lacked the ability to distinguish differences between groups for the rest of the metals, with power well below 80% for mercury, arsenic, cadmium, copper and lead. Power to detect differences in the PAHs, PCBs, PBDEs, and zinc was 100% and for DDTs was >70%.

Chemical	Baseline Site (n = 6)	Unincorporated- UGA (n = 17)	City-UGA (n = 26)	ANOVA values		
	Geometric mean concentration (ng/g, dry wt.)			r <sup>2</sup>	F-ratio	p-value
PAHs	30.6 (A)	259 (B)	494 (C)	0.557	28.930	<0.001
PCBs	5.41 (A)	29.2 (B)	44.1 (B)	0.448	18.663	<0.001
PBDEs	<1.27* (A)	3.27 (B)	8.41 (C)	0.439	17.963	< 0.001
DDTs	<1.27* (A)	2.16 (A)	3.56 (B)	0.303	10.014	<0.001
	Geometric mean concentration (mg/kg, dry wt.)					
Zinc	84.1 (B)	74.3 (A)	93.8 (B)	0.257	7.974	0.001

Table 5. Municipal land use designations near the shoreline affected the contaminant concentrations in mussels at nearshore SAM sites. Groups compared included, 1) mussels from the Baseline Site, 2) mussels from shorelines inside unincorporated-UGAs, and 3) mussels from shorelines inside city-UGAs. Letters signify similar concentrations, ANOVA F-ratio df = 2, 46.

UGA = urban growth area, \*concentration below limit of quantitation.


Figure 9. Municipal land use designations near the shoreline affected the concentrations of PAHs, PCBs, PBDEs, and DDTs in mussels at nearshore SAM sites. Dots are geometric means, bars are 95% confidence intervals, different letters (A, B, C) indicate significantly different concentrations, UGA = urban growth area, \*LOQ = limit of quantitation.

Table 6. Municipal land use designation in the adjacent watershed affected the contaminant concentrations in mussels at nearshore SAM sites. Groups compared included, 1) mussels from the Baseline Site, 2) mussels from shorelines of watersheds outside the UGA, 3) mussels from shorelines of watersheds inside unincorporated-UGAs, and 4) mussels from shorelines of watersheds inside city-UGAs. Letters signify similar concentrations, ANOVA F-ratio df = 3, 45.

Chemical	Baseline Site (n = 6)	Outside UGA (n = 6)	Unincorporated UGA (n = 10)	City UGA (n = 27)	A	NOVA val	ues
	Geometric	mean concentra	tion (ng/g, dry wei	ight)	r <sup>2</sup>	F-ratio	p-value
PAHs	30.6 (A)	187 (B)	268 (BC)	513 (C)	0.591	21.705	<0.001
PCBs	5.41 (A)	20.2 (AB)	30.8 (AB)	46.1 (B)	0.493	14.559	<0.001
PBDEs	<1.27* (A)	2.42 (AB)	5.16 (B)	7.33 (B)	0.388	9.496	<0.001
DDTs	<1.27* (A)	1.71 (AB)	2.49 (B)	3.50 (B)	0.314	6.874	0.001
	Geometric mean concentration (mg/kg, dry weight)						
Zinc	84.14	73.79	79.80	90.57	0.123	2.110	0.112

UGA = urban growth area



Figure 10. Municipal land use designation in watersheds adjacent to the shoreline affected the concentrations of PAHs, PCBs, PBDEs, and DDTs in mussels at nearshore SAM sites. Dots are geometric means, bars are 95% confidence intervals, different letters (A, B, C) indicate significantly different concentrations, UGA = urban growth area, \*LOQ = limit of quantitation.



SAM sites assigned by municipal land use designation at the adjacent shoreline

Figure 11. Land uses at SAM sites in different municipal land-use classifications, UGA = urban growth area, OUGA = outside the UGA.

## Watershed Land Use

We examined other land-use effects on SAM mussel sites. The land use factors tested included the average percent of impervious surface in the watershed, as well as the percent of land covered by upland development, forest, agriculture, and wetland within the watersheds (NLCD data).

#### Mean impervious surface in the watershed

The Encyclopedia of Puget Sound reports that there are 357,840 acres of impervious surfaces in the Puget Sound drainage basin, and that each year the Puget Sound basin receives an average of more than 370 billion gallons of stormwater runoff from these surfaces (Milesi, 2015). In this study the amount of impervious surface in the watershed adjacent to monitoring sites had the largest effect on mussel contaminant concentrations. For this analysis we divided the watersheds into those with an average impervious surface of <20%, 21-50%, and 51-100%. The concentrations of PAHs, PCBs, PBDEs, and DDTs were significantly higher in mussels adjacent to watersheds with high (51-100%) average impervious surface as compare to those adjacent to watersheds with low (<20%) impervious surface (Table 7, Figure 12).

Though we saw no differences in metals, the power to detect differences with this test was very low for all but zinc, which was not significant. Mean impervious surface in the adjacent watershed impacted mussel contaminant concentrations in the 2012/13 Mussel Watch Pilot Expansion study as well (Lanksbury et al., 2014). There WDFW demonstrated significant positive correlations between nearshore watershed land development and the concentrations of PAHs, PCBs, PBDEs, and DDTs in mussels.

In this study, variability in the concentration of contaminants was greater in the high impervious surface category (51-100%) than in the other two categories (Figure 12). However, the number of replicates (n = 3) in the high impervious surface category was also very low, potentially contributing to this high variability. However, WDFW found that variability in mussel contaminant concentration increased with increasing impervious surface in the Mussel Watch Pilot Expansion study as well (Lanksbury et al, 2014). To strengthen the statistical power of future tests on the effects of impervious surface, we recommend future SAM Mussel Monitoring include an equal distribution of nearshore sites along watersheds with differing levels of impervious surface (see "Recommendations for Future SAM Monitoring" section).

Table 7. The intensity (mean value) of percent impervious surface in the adjacent upland watershed affected contaminant
concentrations in mussels. ANOVA factors tested include watersheds with an average impervious surface of <20%, 21-50%, and
51-100%. Letters signify similar concentrations, ANOVA F-ratio df = 2, 40.

Chemical	<20% (n = 20)	21-50% (n = 23)	51-100% (n = 3)	AN	IOVA va	lues
	Geometric	mean conc. (ng	g/g dry weight)	r <sup>2</sup>	F-ratio	p-value
PAHs	277 (A)	413 (A)	1350 (B)	0.203	5.107	0.011
PCBs	25.9 (A)	43.2 (AB)	99.5 (B)	0.200	4.986	0.012
PBDEs	3.49 (A)	7.14 (B)	20.1 (B)	0.240	6.321	0.004
DDTs	1.91 (A)	3.24 (B)	14.7 (C)	0.497	19.726	<0.001
	Geometric	mean conc. (m	g/kg dry weight)			
Zinc	84.9	84.9	93.8	0.013	0.258	0.774



Average impervious surface in the watershed



#### Area of urban upland in the watershed

We used the 2006 USGS National Land Cover Dataset's (Xian et al., 2011) "Developed" class (which includes high, medium and low intensity development and developed open space) to test whether the area of urban development in adjacent watersheds had an effect on mussel contamination. For this analysis we divided the watersheds into those covered by 21-50%, 51-80%, and 81-100% developed area. These developed areas encompassed commercial/industrial uses, apartment complexes, row houses, single-family housing, and developed open spaces (e.g., parks, golf courses, ballfields, and other open areas of planted vegetation). In keeping with the SAM streams analysis, we also conducted a follow-up test in which our urban area categories *did not* include developed open spaces. Results with and without developed open space did not differ appreciably, thus we report only on the results of the first test here.

Area of urbanized upland within the watershed had a marginal effect on the concentration of contaminants in mussels (Table 8, Figure 13). Concentrations of PBDEs and DDTs were significantly higher in areas with the most land area covered by development (81-100%), as compared to areas with the least development (21-50%). PCBs tended to be higher in areas of medium to high development (p-value = 0.068, Table 8). This trend was also apparent with PAHs, though the differences measured between groups were not significant. As with impervious surface, the power to detect differences in the metals was very low (except for zinc), and none of the differences were significant.

Table 8. The area of urbanized (developed) upland in the watershed affected the contaminant concentrations in mussels. ANOVA testing between watersheds covered by 21-50%, 51-80%, and 81-100% developed area. Letters signify similar concentrations, ANOVA F-ratio df = 2, 40.

Chemical	21-50% (n = 15)	51-80% (n = 16)	81-100% (n = 12)	AN	OVA va	lues
enermean	Geometric me	an conc. (ng	/g dry wt.)	r <sup>2</sup>	F-ratio	p-value
PAHs	290	446	443	0.053	1.125	0.335
PCBs	25.8	50.2	40.3	0.126	2.876	0.068
PBDEs	3.36 (A)	6.75 (AB)	9.31 (B)	0.180	4.402	0.019
DDTs	1.90 (A)	3.01 (AB)	4.84 (B)	0.264	7.166	0.002
	Geometric mean conc. (mg/kg dry wt.)					
Zinc	89.1	85.3	81.5	0.025	0.504	0.608



Figure 13. The area of developed upland affected the concentrations of PAHs, PCBs, PBDEs, and DDTs in mussels at nearshore SAM sites. Dots are geometric means, bars are 95% confidence intervals, and letters signify similar concentrations.

## Area of agriculture, forest, and wetlands in the watershed

There were nine SAM sites with measurable levels of agriculture. Seven of these had very small percentages of agriculture in their watersheds (1-4% cover), while two sites had much larger agriculture coverage. These were Site #47 (Cherry Point Aquatic Reserve, Birch Bay South) with 16% agriculture cover and Site #4 (Cherry Point North) with 46% agriculture cover. When all nine sites were pooled together into a single group (1-46% cover), we found significantly lower concentrations of PCBs, PBDEs, DDTs, and lead in mussels from shorelines adjacent to the watersheds with agriculture (Table 9). When the two Cherry Point sites were excluded from the analysis, leaving only the seven sites with 1-4% agriculture, PCBs (t(39) = 3.68, p = 0.001) and PBDEs (t(39) = 2.19, p = 0.034) remained significantly lower in mussels from watersheds with agriculture, but DDTs and lead were no longer significantly different.

The area of watershed covered by forest did not have an effect on the concentration of contaminants at SAM Mussel Monitoring sites. Due to the low number of sites near measurable wetland areas, we were not able to test for a wetland effect on nearshore contamination in mussels.

Chemical	1-46% Agriculture (n = 9)	No Agriculture (n = 34)	t(41)	p-value
Chemical	Geometric mean co	nc. (ng/g, dry		
PCBs	14.4	48.2	4.92	<0.001
PBDEs	2.47	7.24	3.17	0.003
DDTs	1.92	3.27	2.01	0.051
	Geometric mean cor	nc. (mg/kg, dry		
Lead	0.34	0.50	2.15	0.038

Table 9. The presence of agriculture in the watershed affected the contaminant concentrations in mussels. T-statistics and p-values reported for pooled variance.

# Shoreline Land Use

To determine whether upland activities *near the shoreline* had a significant effect on nearshore contamination, we measured the percent of area covered by urbanization, forest, and agriculture within 200 meters (656 ft.) of the shoreline near each of the SAM Mussel Monitoring sites. None of these small-scale upland variables had a significant effect on mussel contamination. This was not surprising given that stormwater and other contaminant sources are often delivered from areas much farther away (i.e., miles) than the nearshore.

## In-Water Point Sources

Recognizing the notion that nearshore contamination in Puget Sound is likely the result of a myriad of sources, including those not directly related to stormwater, we examined the effect of several other potential contaminant sources on the SAM Mussel Monitoring sites.

## Marinas and ferry terminals

The concentration of PAHs, PCBs, DDTs, and lead was higher in mussels placed within two kilometers (1.2 miles) of a marina or ferry terminal (Table 10). These results are not surprising as we found elevated levels of PCBs, PBDEs, DDTs, lead, copper, and zinc in mussels near marinas from the 2012/13 Mussel Watch Pilot Expansion study (WDFW, unpublished data).

Table 10. The presence of a marina or ferry terminal within 2 km (1.2 miles) of a site affected the contaminant concentrations in mussels. T-statistics and p-values reported for pooled variance.

Chemical	Marina or Ferry Terminal (n = 18) Geometric mean cor	None (n = 25)	t(41)	p-value
PAHs	646	263	-3 76	0.001
PCBs	53.2	29.0	-2.54	0.015
DDTs	3.89	2.38	-2.29	0.027
	Geometric mean con	. (mg/kg, dry wt.)		
Lead	0.566	0.401	-2.26	0.029

Petroleum products (e.g., diesel, gasoline, motor oil, hydraulic fluids, etc.) from moored vessels in marinas are a likely source of PAHs in nearby mussels. Fingerprint analysis data from the Mussel Watch Pilot Expansion study showed that mussels from areas with a high concentration of marinas (e.g., Thea Foss and Hylebos Waterways, Salmon Bay, Bremerton Shipyard) were receiving a higher proportion of petrogenic PAHs (i.e., unburned petroleum products) than mussels from other locations in Puget Sound.

PCBs, once common in anti-fouling paints worldwide (Jensen et al. 1972), may also be elevated around marinas in Puget Sound. In the 1990s, researchers in Australia showed that areas within or immediately adjacent to shipping facilities and marinas had a higher incidence of PCBs in their sediments (Burt and Ebell, 1995). Although US production of PCBs was banned in 1979, the Toxic Substances Control Act allows for a limited amount of PCBs in products like sealants, pigments, and dyes (Herrick et al. 2007), which are often used on marine vessels. Together these products comprise a relatively large source of PCBs in Washington State (Davies et al. 2015). A number of studies have linked high levels of DDT in Chinese fishing harbor and shipyard sediments to the use of DDT-containing antifouling paints (Lin et al., 2009; Liu et al., 2012; Bao et al., 2012; Guo et al., 2013). To our knowledge DDT-containing paint is not sold in the US, but it is possible that vessels treated with these paints have made their way into Puget Sound over the last several decades. Lead contamination of sediment in estuaries in England has been attributed to peeling paint from abandoned boats (Rees et al., 2014; Turner, 2014). Research has also shown elevated levels of lead and other metals associated with antifouling paints in sediments near marinas and boat-repair yards (Singh and Turner, 2009; Maharachpong et al., 2006; Turner, 2013).

#### Creosote Presence, Substrate Type, and Shoreline Form

Volunteers reported on the presence of creosote near the deployment sites and on the type of substrate present under the mussel cage during the study. We divided the substrate type reported into two broad classes: coarse (n = 32) and depositional (n = 11). We also classified mussel sites by shoreline form, dividing them into those occurring in bays (n = 28) and those occurring in open sites (n = 15). Analysis indicated no significant difference in mussels from SAM sites with (n = 11) or without (n = 32) creosote present. There was also no significant effect of substrate type or shoreline form on SAM mussel contaminant concentrations.

## Power of Statistical Tests to Distinguish Extent of Nearshore Contamination

To check whether the non-significant findings for metals in this survey were due to a lack of statistical power, we conducted post hoc power analyses on a number of the statistical tests. For the following

three comparisons of mussel sites, we conducted power analyses with SYSTAT 12 (power set at 0.80 and  $\alpha$  = 0.05) using the means from the two-sample t-Tests for each:

- all UGA sites vs. Baseline samples (Table 11),
- all UGA sites vs. sites outside the UGA (Table 12),
- City-UGA sites vs. Unincorporated-UGA sites (Table 13).

In addition, we conducted a post hoc power analyses (power set at 0.80 and  $\alpha$  = 0.05), on the ANOVA test of differences between UGA sites near watersheds with either <20%, 21-50%, or 51-100% mean impervious surface (Table 14).

The first power analysis confirmed that the study had sufficient power to detect differences in PAHs, PCBs, PBDEs, and DDTs between the UGA and Baseline mussels, and that as few as three to four mussel sites in each group would have been sufficient to detect differences at an 0.80 power (Table 11). However, the power to detect differences, if there was one, was much lower for zinc (0.51) and the other metals (<0.10; Table 11). Between 110 to 800,000 mussel sites would have been required to detect a significant difference in copper, arsenic, cadmium, lead, or mercury between UGA and Baseline mussels (Table 11).

Table 11. Power of t-Tests to detect differences in mussel contaminant concentrations between UGA sites (n = 43) and the Baseline Site (n = 6). Power analyses conducted with SYSTAT 12 (Power Analysis: Two-Sample t-Test), using mean values from 2015/16 mussel survey data,  $\alpha = 0.05$ , with sample size of n = 6.

Chemical Group	Power*	Sample size required for 0.80 power (per group)
PAHs	1	3
PCBs	1	3
PBDEs	1	4
DDTs	.99	4
Zinc	.51	11
Copper	.09	110
Arsenic	.09	118
Cadmium	.05	>76,000
Lead	.05	>1,300
Mercury	.05	>800,000

\*Standard acceptable power for ecological studies is 0.80 or higher.

We also ran power analyses on our ability to detect differences between the UGA sites (SAM) and the study sites outside the UGA (Partner-sponsored sites). The test's ability to detect differences in PAHs, PCBs, PBDEs, and zinc was high (Table 12). However, the power to detect differences was much lower for DDTs (0.34), and the number of sites that would be needed to detect differences in copper, arsenic, cadmium, lead, and mercury were very high.

Table 12. Power of t-Tests to detect differences in mussel contaminant concentrations between sites within the urban growth area (UGA, n = 43) and outside the UGA (includes non-random, Partner sites, n = 13). Power analyses conducted with SYSTAT 12 (Power Analysis: Two-Sample t-Test), using 2015/16 mussel survey data,  $\alpha = 0.05$ , with sample size of n = 13.

Chemical		Sample size
Group	Power*	required for 0.80
		power (per group)
PAHs	1	3
PCBs	1	3
PBDEs	.97	8
DDTs	.34	40
Zinc	1	3
Copper	.16	105
Arsenic	.16	103
Cadmium	.06	>1,000
Lead	.05	>3,000
Mercury	.05	>430,000

\*Standard acceptable power for ecological studies is .80 or higher.

The third power analyses tested our ability to detect differences between the City-UGA and the Unincorporated-UGA sites (all SAM sites). This analysis showed high probability of detecting differences in PAHs, PCBs, PBDEs, DDTs, zinc and copper and that three to 17 mussel sites per category would have been sufficient (Table 13). However, many more sites would have been required to detect differences, if there were any, in arsenic, cadmium, lead, or mercury.

Table 13. Power of t-Tests to detect differences in mussel contaminant concentrations between areas designated as City-UGA (n= 17) and Unincorporated-UGA (n = 26). Power analyses conducted with SYSTAT 12 (Power Analysis: Two-Sample t-Test), using 2015/16 mussel survey data,  $\alpha = 0.05$ , and a sample size of n = 17.

Chemical	Power*	Sample size required for
Group	FOWEI	0.80 power (per group)
PAHs	1	3
PCBs	1	3
PBDEs	1	3
DDTs	.80	17
Zinc	1	3
Copper	1	5
Arsenic	.40	44
Cadmium	.05	>19,000
Lead	.05	>5000
Mercury	.05	>50,000

\*Standard acceptable power for ecological studies is .80 or higher.

The last power analyses tested our ability to detect differences between UGA sites near watersheds with three different levels of impervious surface (SAM sites, Table 14). These power analyses included a small sample size (n = 3) due to the low amount of mussel sites that fell into the 51-100% impervious surface category. However, there was still a high probability of detecting differences in PAHs, PCBs, PBDEs, DDTs,

zinc and copper with this design (Table 14). As with the other tests, the power to detect differences in cadmium, arsenic, lead and mercury was very low.

Table 14. Power of ANOVA to detect differences in mussel contaminant concentrations between UGA shorelines characterized as having watersheds with an average impervious surface value of <20%, 21-50%, and 51-100% (n = 20, 23, 3 respectively). Power analyses conducted with SYSTAT 12 (Power Analysis: One-Way ANOVA), using 2015/16 mussel survey data,  $\alpha$  = 0.05, and sample size of n = 3.

Chemical Group	Power*	Sample size required for 0.80 power (per group)
PAHs	1	3
PCBs	1	3
PBDEs	1	3
DDTs	1	3
Zinc	1	3
Copper	.14	17
Cadmium	.05	>2200
Arsenic	.05	>2900
Lead	.05	>2100
Mercury	.05	>300,000

\*Standard acceptable power for ecological studies is .80 or higher.

## Ranges and Concentrations of Organic Contaminants and Metals in Mussels

An overview of the findings for the organic contaminants and metals is detailed in an earlier section of this report (Spatial Extent of Nearshore Contamination), which summaries data on the most abundant organic contaminants measured in this study (PAHs, PCBs, PBDEs, and DDTs) and describes the overall results for the metals measured. The following sections detail the ranges and concentrations of the organic contaminant groups and metals analyzed in SAM, Pierce County, and Partner mussel sites from this study (n = 66). Where applicable, we compare mussel contaminant concentrations at monitoring sites to human health consumption thresholds and screening levels, and contrast findings with mussel data from previous surveys in Puget Sound.

#### Units Reported

We report mussel concentrations in both wet weight format, for comparison with human health screening levels (see below), and in dry weight format for comparisons between sites. We prefer to use the dry weight conversion to compare contaminant concentrations between sites because it is more accurate, given that the amount of water in mussel tissue can vary widely between individuals. The organic contaminants are reported in parts per billion (ppb) as ng/g, that is nanogram of contaminant per gram of mussel tissue. The metals are reported in parts per million (ppm) as mg/kg; that is milligram of contaminant per kilogram of mussel tissue. The wet and dry contaminant concentrations from every site are listed in Appendices <u>2</u> and <u>3</u>.

#### Thresholds and Screening Levels

Although this study was not designed to evaluate seafood safety, seafood-contaminant screening levels provide a reference for comparison to help judge the significance of the contaminant levels we report herein. It is beyond the scope of this study to summarize the complex seafood thresholds available for

all the chemicals we reported, however we have selected several that seem particularly applicable for reference. When possible, we compare mussel contaminant concentrations (wet weight) from this study to human health screening values from the US Environmental Protection Agency (EPA) and the Washington Department of Health (WADOH).

The WADOH fish consumption advisory thresholds (FCATs) and fish consumption advisory screening levels (SLs) used here are based on a consumption rate of 59.7 grams fish/day for general consumers and on 175 grams fish/day for high consumers (pers. comm., D. McBride, Office of Environmental Public Health Sciences, Washington State Department of Health, April 2017). Since the mussels used in this study were transplanted and exposed to local contaminants for only three months, we consider these findings conservative relative to conditions in wild mussels from the same locations (i.e., those growing there naturally). Wild mussels from the same locations likely have similar, or possibly higher, concentrations than the transplanted mussels because wild mussels are exposed over their entire lives and often are located closer to potentially contaminated sediments than the caged mussels.

#### PAHs

Polycyclic aromatic hydrocarbons or polyaromatic hydrocarbons (PAHs) are found in oil, coal, and tar. They are produced by the incomplete combustion of organic matter and are found in non-combusted fuels. Ecology released a <u>Chemical Action Plan (CAP) for PAHs</u> in 2012 that addressed uses and releases of PAHs in Washington State (Davies et al., 2012). The CAP found that the largest anthropogenic sources of PAHs in Washington, including the Puget Sound, are from wood burning stoves, creosote treated wood, and automobile emissions, which includes tire wear, motor oil leaks, and improper oil disposal.

We detected PAHs (i.e.,  $\Sigma_{38}$  PAHs or sum of 38 PAH analytes) at concentrations above the starting condition at 100% of the study sites (Table 15, Figure 14). The highest concentrations of PAHs for each group of sites occurred at SAM Site #39 (Smith Cove, Terminal 91), Pierce County Site #697 (Browns Point, Wolverton), and Partner Site EB-ME (Elliott Bay, Myrtle Edwards; Table 16). The lowest concentrations occurred at SAM Site #8 (Chimacum Creek delta), Pierce County Site #353 (Purdy, Nicholson), and Partner Site HC\_HO (Hood Canal, Holly; Table 16). Sites with the highest and lowest PAH concentrations from all the study sites are listed in Table 17. PAH concentrations from every mussel site are listed in <u>Appendix 2</u>. The cumulative frequency distributions for SAM and Pierce County sites are listed in <u>Appendix 5</u>.

			PAHs (ng/g, dry wt.)	
Sites	n	Min	Average	Max
Baseline	6	21.4	35.5	86.2
SAM	36	94.9	728	7350
Pierce County*	7	164	343	540
Partner	23	48.8	629	3820
All	66	48.8	653	7350

Table 15. Range and average concentration of total PAHs ( $\Sigma_{38}$  PAHs) in mussels from the sites in this study. \*Unincorporated Pierce County mussel sites.

	Site ID	Site Name	Conc. PAH (ng/g, dry wt.)
	HC_HO	Hood Canal, Holly	48.8
	HC_FP	Fisherman's Point	54.3
Bottom	Site #8	Chimacum Creek delta	94.9
10%	Site #47	Cherry Pt Aq Rsv, Birch Bay S	95.3
	SPS_NRQR	Nisqually Rch Aq Rsv, Anderson Is	112
	Site #4	Cherry Point North	124
	Site #6	Eagle Harbor Dr.	1820
	CPS_SHLB	Shilshole Bay	2040
Ten 100/	Site #43	N Avenue Park	2140
Top 10%	Site #23	Wing Point	3100
	EB_ME	Elliott Bay, Myrtle Edwards	3820
	Site #39	Smith Cove, Terminal 91	7350

Table 16. Mussel sites with the highest and lowest total PAH concentrations (10<sup>th</sup> percentile) of 66 monitoring sites.



Figure 14. Map of the relative concentrations of  $\Sigma_{38}$  PAHs from all the 2015/16 SAM Mussel Monitoring sites.

The PAH concentrations in this study (48.8 - 7350 ng/g dry wt.) were similar to those found during the 2012/13 Mussel Watch Pilot Expansion (MWPE) study (29 - 5030 ng/g dry wt.), where PAHs were also detected at 100% of the mussel sites. The concentration of PAHs was highest at MWPE site EB\_ME (Elliott Bay, Myrtle Edwards; 5030 ng/g dry wt.), which was the site with the second highest PAH concentration in this study (3820 ng/g dry wt.). Regression analyses from the MWPE study revealed a significant positive correlation ( $r^2 = 0.372$ , p<0.0001) between PAH concentrations in mussels and the average percent impervious surface in the adjacent upland watershed (Lanksbury et al., 2014), which supports the findings from this study as well.

Exposure to PAHs in humans is linked to cardiovascular disease, poor fetal development, and cancer, and exposure in fish has been directly linked to liver disease (Myers et al., 1994; Myers et al., 2003; Myers et al., 2005). In the U.S., a large percent of PAH exposure in humans occurs through food sources, with the majority of dietary exposure for the average person coming from vegetables and cereal grains (Phillips, 1999). The WADOH fish consumption advisory threshold (FCAT) values for benz(a)pyrene, a PAH analyte considered carcinogenic based on strong and consistent evidence in animals and humans (U.S. EPA, 2017a), is 0.2 ppb wet weight for the general consumers and 0.05 ppb wet weight for high consumers. In this study 14/66 (21%) of the mussel sites had benz(a)pyrene wet weight concentrations that exceeded both of the FCAT values (Table 17).

Table 17. Locations from this study where the wet weight concentration of benz(a)pyrene in mussel tissues exceeded the
Washington Department of Health's fish consumption advisory threshold (FCAT) values of 0.2 ppb wet weight for low consumers,
and 0.05 ppb wet weight for high consumers.

Site ID	Site Name	Benz(a)pyrene (ng/g or ppb, wet wt.)
Site #30	Kitsap St Boat Launch	1.20
CPS_EF	Edmonds Ferry	1.20
Site #49	Donkey Creek Delta	1.40
Site #21	Point Defiance Ferry	1.50
CPS_PNP	Point No Point	1.50
Site #31	Eastsound, Fishing Bay	1.90
CPS_HCV	Port Madison, Hidden Cove	2.40
CPS_SB	Salmon Bay	3.00
CPS_SHLB	Shilshole Bay	4.70
Site #43	N Avenue Park	5.40
Site #6	Eagle Harbor Dr.	6.10
EB_ME	Elliott Bay, Myrtle Edwards	9.70
Site #23	Wing Point	10.00
Site #39	Smith Cove, Terminal 91	21.00

PAHs also have a negative impact on a mussel's scope for growth; Widdows et al. (1997) demonstrated significant negative correlations between scope for growth and tissue concentrations of petroleum hydrocarbons, PCBs, DDT and HCH in mussels (*M. galloprovincialis*). Declines in scope for growth of 50-80% for *M. edulis* have been attributed to PAH contamination (Widdows et al., 2002), and their survival is significantly lowered at higher tissue concentrations of PAHs and PCBs (Smaal et al., 1991). In Puget

Sound, Kagley et al. (1995) associated impaired growth, reduced fecundity, and altered age-structure patterns in mussels with elevated levels of PAHs, PCBS, and DDTs in highly urbanized areas.

## PCBs

Polychlorinated biphenyls (PCBs) are persistent organochlorine compounds once widely used as coolant fluids in electrical devices, in carbonless copy paper, and in heat transfer fluids. They were also used as plasticizers in paints and cements, stabilizers in PVC coatings, and in sealants for caulking and adhesives. Although the manufacture of PCBs in the United States was banned in 1979, they are still found in significant amounts in the Puget Sound basin (e.g., in building paints and caulks), and continue to find their way into stormwater (EnviroVision Corporation et al., 2008; Hart Crowser, 2007; Herrera Environmental Consultants Inc., 2009; Science Applications International Corporation, 2011). Ecology released a <u>PCB</u> <u>Chemical Action Plan (CAP)</u> in 2015, to guide Washington's strategy to find and remove PCBs and reduce PCB exposure (Davies et al., 2015).

We detected PCBs (estimated total PCBs) at concentrations above the starting condition at 100% of the study sites (Figure 15). The highest concentrations of PCBs for each group of sites occurred at SAM Site #39 (Smith Cove, Terminal 91), Pierce County Site #697 (Browns Point, Wolverton), and Partner Site EB-ME (Elliott Bay, Myrtle Edwards; Table 18). The lowest concentrations occurred at SAM Site #4 (Cherry Point North), Pierce County Site #61 (Dash Point Park), and Partner Site HC\_FP (Fisherman's Point; Table 18). Sites with the highest and lowest PCB concentrations from all the study sites are listed in Table 19. PCB mussel concentrations from every site are listed in <u>Appendix 2</u>. Cumulative frequency distribution plots for the SAM and Pierce County sites are shown in <u>Appendix 6</u>.

			PCBs (ng/g, dry wt.)	
Sites	n	Min	Average	Max
Baseline	6	4.81	5.42	5.82
SAM	36	6.16	51.9	236
Pierce County*	7	31.0	45.1	62.9
Partner	23	6.33	55.0	197
All	66	6.16	52.3	236

Table 18. Range and average concentration of estimated total PCBs in mussels from the sites in this study. \*Unincorporated Pierce County mussel sites.

			Conc. PCBs
	Site ID	Site Name	(ng/g, dry wt.)
	Site #4	Cherry Point North	6.16
	HC_FP	Fisherman's Point	6.33
Bottom 10%	Site #47	Cherry Pt Aq Rsv, Birch Bay S	6.64
Bottom 10%	HC_HO	Hood Canal, Holly	7.69
	Site #27	Chuckanut, Clark's Point	10.1
	Site #31	Eastsound, Fishing Bay	10.6
	Site #49	Donkey Creek Delta	125
	CPS_SHLB	Shilshole Bay	157
Top 10%	Site #30	Kitsap St Boat Launch	157
100 10%	CPS_SB	Salmon Bay	182
	EB_ME	Elliott Bay, Myrtle Edwards	197
	Site #39	Smith Cove, Terminal 91	236

Table 19. Mussel sites with the highest and lowest estimated total PCB concentrations (10<sup>th</sup> percentile) of 66 monitoring sites.

The PCB concentrations in this study (6.16 - 236 ng/g dry wt.) were very similar to those from the MPWE study, though the highest concentration in the MWPE study occurred in the Hylebos Waterway in Tacoma. Regression analyses from the MWPE study revealed a significant positive correlation ( $r^2 = 0.193$ , p<0.0001) between mussel PCB concentrations and the average percent impervious surface in the adjacent upland watershed (Lanksbury et al., 2014), which supports the findings of this study.

According to the EPA, PCBs cause cancer in animals, impairment to animal immune systems, behavioral alterations, and impaired reproduction (U.S. EPA, 1996). PCBS are probable carcinogens in humans, are known endocrine disruptors (interfere with hormone systems), and have neurotoxic effects (Lauby-Seretan et al., 2013; Ludewig et al., 2008; Safe, 1989). The WADOH fish consumption advisory screening level for total PCBs are 23 ppb wet weight for general consumers, and 8 ppb wet weight for high consumers. In this study 3/66 (5%) of the sites had mussel PCBs concentrations that exceeded the general population screening level, and 24/66 (36%) of the sites had concentrations that exceeded the high consumer screening level (see PCB wet weights concentrations in <u>Appendix 2</u>). PCBs have also been shown to have a negative impact on mussels, reducing scope for growth, fecundity, and survival (Smaal et al., 1991; Kagley et al. 1995; Widdows et al. 1997).



Figure 15. Map of the relative concentrations of estimated total PCBs from all the 2015/16 SAM Mussel Monitoring sites.

#### PBDEs

Polybrominated diphenyl ethers (PBDEs) are persistent organobromine compounds used as flameretardants in a wide variety of products including building materials, plastics, foams, electronics, furnishings, and vehicles. We detected PBDEs (i.e.,  $\Sigma_{11}$  PBDEs or sum of 11 PBDE congeners) at concentrations above the starting condition at 54/66 (82%) of the sites in this study (Figure 16). The highest concentrations of PBDEs for each group of sites occurred at SAM Site #25 (Blair Waterway), Pierce County Site #697 (Browns Point, Wolverton), and Partner Site CPS\_SB (Salmon Bay; Table 20). PBDEs were not detected at six SAM sites, at Pierce County Site #161 (Purdy, Dexters), and at five of the Partner sites (Table 21). Mussel sites with the highest and lowest concentrations of total PBDEs (10<sup>th</sup> percentile) for the entire study are listed in Table 21. PBDE mussel concentrations from every site are listed in <u>Appendix 2</u>. Cumulative frequency distributions of PBDEs for the SAM and Pierce County sites are shown in <u>Appendix 7</u>.

Table 20. Range and average concentration of detected PBDEs ( $\Sigma_{11}$  PBDEs) in mussels from the sites in this study. Sites where PBDE values fell below the limit of quantitation (LOQ) were not included in this table. \*Unincorporated Pierce County mussel sites.

		PBDEs (ng/g, dry wt.)		
Sites	n	Min	Average	Max
Baseline	6	ND	ND	ND
SAM	36	2.12	10.3	30.0
Pierce County*	7	1.89	8.62	20.9
Partner	23	1.96	10.3	39.2
All	66	1.89	10.1	39.2

ND - not detected; limit of quantitation was 1.27 for Baseline samples.

Table 21. Mussel sites where total PBDEs were not detected above the limit of quantitation (LOQ) and with the highest total PBDE concentrations (10<sup>th</sup> percentile) of 66 monitoring sites.

	Site ID	Site Name	Conc. PBDEs (ng/g, dry wt.)
	Site #4	Cherry Point North	ND
	Site #8	Chimacum Creek delta	ND
	Site #15	Tugboat Park	ND
Sites where	Site #24	S of Skunk Island	ND
PBDEs were	Site #31	Eastsound, Fishing Bay	ND
not detected	Site #47	Cherry Pt Aq Rsv, Birch Bay S	ND
above the limit	Site #161	Purdy, Dexters	ND
of quantitation	HC_FP	Fisherman's Point	ND
( <loq)< td=""><td>HC_HO</td><td>Hood Canal, Holly</td><td>ND</td></loq)<>	HC_HO	Hood Canal, Holly	ND
	NPS_CPAR4	Cherry Pt Aq Rsv 4, Conoco Phillips	ND
	NPS_DHCC	Drayton Harbor, California Creek	ND
	WB_CB	Cavalero Beach Co. Park	ND
	EB_ME	Elliott Bay, Myrtle Edwards	22.0
	Site #30	Kitsap St Boat Launch	26.1
Top 10%	Site #25	Blair Waterway	30.0
	CPS_SHLB	Shilshole Bay	37.1
	CPS_SB	Salmon Bay	39.2

#### ND - not detected; limit of quantitation ranged from 1.76 to 2.45 ng/g, dry weight.

PBDE concentrations in mussels from this study (1.89 – 39.2 ng/g dry wt.) were very similar to those from the 2012/13 MWPE study (1.7 - 35 ng/g dry wt.). PBDEs were detected at 78% of the mussel sites in the MWPE study and the highest concentration occurred at a Bremerton Shipyard site near Charleston Beach. Regression analyses from the MWPE study site also revealed a significant positive correlation ( $r^2 = 0.215$ , p<0.0001) between mussel PBDE concentrations and the average percent impervious surface in the adjacent upland watershed (Lanksbury et al., 2014), which supports findings from this study.

PBDEs are ubiquitous in the environment and have been shown to reduce fertility in humans at levels found in household dust (Meeker et al., 2009; Harley et al., 2010). According to the EPA, exposure to PBDEs may pose a health risk to the human liver, thyroid, and brain. The WADOH fish consumption advisory screening levels (SLs) for total PBDEs are 117 ppb wet weight for general consumers, and 40 ppb wet weight for high consumers. None of the mussel sites in this sstudy had PBDE concentrations that exceeded these SLs (see PBDE wet weights concentrations in <u>Appendix 2</u>). Ecology released a <u>PBDE</u> <u>Chemical Action Plan</u> in 2006 and recommended a number of actions including restricting the use of eight flame retardants commonly used in children's products and furniture, and two flame retardants used in textiles, and requiring that manufacturers report their use of flame retardants in consumer products (Ammann et al., 2006).



Figure 16. Map of the relative concentrations of  $\Sigma_{11}$  PBDEs from all the 2015/16 SAM Mussel Monitoring sites.

## DDTs

Dichlorodiphenyltrichloroethanes (DDTs) are a group of persistent organochlorine insecticides that were banned in the U.S. in 1972. We detected total DDTs (i.e.,  $\Sigma_6$  DDTs or sum of 6 DDTs isomers/metabolites) at 57/66 (86%) of the sites in this study (Figure 17). We did not detected DDTs above the limit of quantitation (LOQ) at the Baseline Site. The ranges and average concentrations of DDTs, at sites where they were detected, are listed in Table 22. The highest concentrations of DDTs for each group of sites occurred at SAM Site #39 (Smith Cove, Terminal 91), Pierce County 2 Site#697 (Browns Point, Wolverton), and Partner Site CPS\_SB (Salmon Bay; Table 23). DDTs were not detected at six SAM sites, the lowest concentration for the Pierce County group occurred at Site #161 (Purdy, Dexters). DDTs were not detected at three of the Partner sites. Mussel sites with the highest and lowest concentrations of total DDTs (10<sup>th</sup> percentile) for the entire study are listed in Table 23. DDT mussel concentrations from every site are listed in <u>Appendix 2</u>. Cumulative frequency distributions of DDTs for the SAM and Pierce County sites are shown in <u>Appendix 8</u>.

Table 22. Range and average concentration of detected total DDTs ( $\Sigma_6$  DDTs) in mussels from the sites in this study. Sites where total DDT values fell below the limit of quantitation (LOQ) were not included in this table. \*Unincorporated Pierce County mussel sites.

		DDTs (ng/g, dry wt.)		
Sites	n	Min	Average	Max
Baseline	6	ND	ND	ND
SAM	36	2.08	5.08	50.4
Pierce County*	7	1.98	4.09	10.4
Partner	23	1.87	7.04	45.7
All	66	1.87	5.65	50.4

ND - not detected; limit of quantitation was 1.27 for Baseline samples.

Table 23. Mussel sites where total DDTs were not detected above the limit of quantitation (LOQ) and with the highest total PBDE concentrations (10<sup>th</sup> percentile) of 66 monitoring sites.

			Conc. DDTs
	Site ID	Site Name	(ng/g, dry wt.)
	Site #4	Cherry Point North	ND
Sites where	Site #8	Chimacum Creek delta	ND
DDTs were	Site #15	Tugboat Park	ND
not	Site #24	S of Skunk Island	ND
above the	Site #31	Eastsound, Fishing Bay	ND
limit of	Site #47	Cherry Pt Aq Rsv, Birch Bay S	ND
quantitation	HC_FP	Fisherman's Point	ND
( <loq)< td=""><td>HC_HO</td><td>Hood Canal, Holly</td><td>ND</td></loq)<>	HC_HO	Hood Canal, Holly	ND
	WB_CB	Cavalero Beach Co. Park	ND
	Site #697	Browns Point, Wolverton	10.4
	EB_ME	Elliott Bay, Myrtle Edwards	16.7
Top 10%	CPS_SHLB	Shilshole Bay	32.8
	CPS_SB	Salmon Bay	45.7
	Site #39	Smith Cove, Terminal 91	50.4

ND - not detected; limit of quantitation ranged from 0.955 to 2.00 ng/g, dry weight.

As with the PBDEs, the range of concentration of DDTs in mussels from this study (1.87 – 50.4 ng/g dry wt.) was very similar to that found in the 2012/13 MWPE study (1.1 - 46 ng/g dry wt.). However, DDTs were detected at 100% of the MWPE sites, where here they were only detected at 86% of sites. As with the PCBs, the highest DDT concentration in the MPWE study occurred in the Hylebos Waterway in Tacoma. Regression analyses from the MWPE study showed a significant positive correlation ( $r^2 = 0.248$ , p<0.0001) between DDT concentrations in mussels and the average percent impervious surface in the adjacent upland watershed (Lanksbury et al., 2014), which also supports findings from this study.

DDT is toxic to a wide range of marine animals including invertebrates, fish, and birds. It is an endocrine disruptor in humans and is considered a likely carcinogen. The WADOH fish consumption advisory screening levels (SLs) for total DDTs are 3 ppb wet weight for general consumers, and 1.2 ppb wet weight for high consumers. In this study 3/66 (5%) of the sites had mussel DDTs concentrations that exceeded the general population screening level, and 7/66 (11%) of the sites had concentrations that exceeded the high consumer screening level (see DDT wet weight concentrations in <u>Appendix 2</u>).



Figure 17. Map of the relative concentrations of  $\Sigma_6$  DDTs from all the 2015/16 SAM Mussel Monitoring sites.

## Chlordanes

Chlordanes (i.e.,  $\Sigma_8$  Chlordanes or sum of 8 chlordane isomers) are persistent organochlorine insecticides that were used in the U.S. until 1988, when the EPA banned them. Chlordanes were detected at only four sites (6%), which included SAM Site #39 (Smith Cove, Terminal 91 at 5.06 ng/g, dry wt.), Pierce County site #697 (Browns Point, Wolverton at 2.11 ng/g, dry wt.), and Partner sites CPS\_SB (Salmon Bay at 14.92 ng/g, dry wt.) and CPS\_SHLB (Shilshole Bay at 6.99 ng/g, dry wt.). The limit of quantitation for chlordanes ranged from 0.828 to 2.35 ng/g, dry wt. Chlordane mussel concentrations from every site are listed in <u>Appendix 2</u>.

Chlordanes are highly toxic to fish. In humans, chlordanes are considered a risk factor for type-2 diabetes and a number of cancers (Purdue et al., 2007). The WADOH fish consumption advisory screening levels (SLs) for chlordane are 3 ppb wet weight for general consumers, and 1.1 ppb wet weight for high consumers. In this study, none of the mussel sites had chlordane concentrations that exceeded the general consumer SL, but site CPS\_SB (Salmon Bay) had a concentration that exceeded the high consumer SL (see Chlordane wet weight concentrations in <u>Appendix 2</u>). Chlordanes were detected at 21% of the MWPE sites in 2012/13 at slightly lower concentration (0.88 - 11.42 ng/g dry wt.) than found in this study (2.11 - 14.9 ng/g dry wt.). In both studies, the highest concentration of chlordanes occurred at CPS\_SB (Salmon Bay; Lanksbury et al., 2014).

## Dieldrin

Dieldrin is a persistent organochlorine insecticide classified as a probable human carcinogen by the EPA (1986) and is linked to early onset of Parkinson's disease (Kanthasamy et al., 2005). Dieldrin was banned in the 1970s but still lingers in some places in the Puget Sound. Dieldrin was detected at three sites (5%) in this study and the dry weight concentrations were as follows: SAM Site #39 (Smith Cove, Terminal 91 at 3.03 ng/g, dry wt.), Partner Site CPS\_SB (Salmon Bay at 3.00 ng/g, dry wt.), and Partner Site CPS\_QMH (Quartermaster Harbor at 2.19 ng/g, dry wt.). Dieldrin was not detected at any of the other sites, and the limit of quantitation was 0.802 to 2.19 ng/g, dry weight. Dry weight concentrations of Dieldrin in mussels from the study sites are listed in <u>Appendix 2</u>.

The WADOH fish consumption advisory screening levels (SLs) for Dieldrin are 0.07 ppb wet weight for general consumers, and 0.03 ppb wet weight for high consumers. The limit of quantitation for Dieldrin in this study ranged from 0.088 to 0.33 ng/g, ppb wet weight (i.e., above the screening values), thus we could not detect concentrations of Dieldrin at those SLs. However, at the three mussel sites where Dieldrin was detected the concentrations exceeded both the general population and the high consumer screening levels (see Dieldrin *wet weight* concentrations in <u>Appendix 2</u>). Dieldrin was detected at 17% of the MWPE sites in 2012/13, at slightly lower concentrations (0.95 - 2.59 ng/g dry wt.) than found in this study (2.19 - 3.03 ng/g dry wt.). The limit of quantitation for chlordanes for the MWPE study was 2.1 ng/g dry wt. (Lanksbury et al., 2014).

# HCHs

Hexachlorocyclohexanes (i.e.,  $\Sigma_3$  HCHs or sum of 3 HCH isomers) are persistent byproducts of the production of the insecticide Lindane, which has not been produced or used in the U.S. since 1985. HCHs are linked to Parkinson's and Alzheimer's disease (Richardson et al., 2009; Singh et al., 2013; Chhillar et al., 2012). The only HCH isomer detected in mussels from this survey was alpha-HCH ( $\alpha$ -HCH) at Site#39 (Smith Cove, Terminal 91) at a value of 0.42 ng/g, ppb wet wt. (2.83 ng/g dry wt.). This wet wt. value exceeded both of the WADOH fish consumption advisory screening levels (SLs) for  $\alpha$ -HCH, which are 0.19

ppb wet wt.for general consumers, and 0.06 ppb wet wt. for high consumers. The limit of quantitation for HCHs ranged from 0.801 to 2.19 ng/g, dry wt. HCHs were not detected in the MWPE study, which had a limit of quantitation that ranged from 0.52 – 2.94 ng/g dry wt. (Lanksbury et al., 2014).

# Other Organic Pollutants

Hexachlorobenzene (HCB), Mirex, aldrin, and endosulfan 1 were not detected in mussels from any of the study sites. HCB was detected at two sites (Manchester Stormwater Outfall, 1.75 ng/g dry wt. and Hylebos Waterway, 1.53 ng/g dry wt.) in 2012/13 during the MWPE study, while Mirex was detected at one site (Sinclair Inlet Marina, 1.6 ng/g dry wt.; Lanksbury, et al. 2014). The limit of quantitation for both HCB and Mirex was 2.1 ng/g dry wt. for that study.

#### Mercury

Mercury is released into the environment from natural sources (volcanoes) and by human activity (e.g., coal combustion, gold production, smelting, cement production, waste disposal/incineration, and caustic soda production). We detected mercury (i.e., total mercury, including methylmercury) in mussels from 100% of the study sites (Figure 18). The highest concentrations of mercury for each group of sites occurred at SAM Site #6 (Eagle Harbor Dr.), Pierce County Site #953 (Browns Point, Carlson), and Partner Site NPS\_BLSC (Bellingham Bay, Little Squalicum Creek; Table 24). The lowest concentration of mercury was found at SAM Site #31 (Eastsound, Fishing Bay), Pierce County Site #161 (Purdy, Dexters), and at Partner Site NPS\_DHCC (Drayton Harbor, California Creek; Table 24). Mussel sites with the highest and lowest concentrations (10<sup>th</sup> percentiles) of mercury for the entire study are listed in Table 25. Interestingly, the sites with the lowest concentrations of mercury occurred at six of the seven Pierce County sites, in either Gig Harbor, near Purdy, or just north of Commencement Bay. Mercury mussel concentrations from every site are listed in <u>Appendix 3</u>. Cumulative frequency distributions of mercury for the SAM and Pierce County sites are in <u>Appendix 9</u>.

		Mercury (mg/kg, dry wt.)			
Sites	n	Min	Average	Max	
Baseline	6	0.038	0.044	0.048	
SAM	36	0.032	0.046	0.058	
Pierce County*	7	0.015	0.020	0.044	
Partner	23	0.032	0.049	0.084	
All	66	0.015	0.044	0.084	

Table 24. Range and average concentration of mercury in mussels from the sites in this study. \*Unincorporated Pierce County mussel sites.

			Conc. Mercury
	Site ID	Site Name	(mg/kg, dry wt.)
	Site #161	Purdy, Dexters	0.015
	Site #697	Browns Point, Wolverton	0.015
Bottom	Site #481	Gig Harbor Boat Launch	0.016
10%	Site #61	Dash Point Park	0.016
	Site #353	Purdy, Nicholson	0.017
	Site #625	Gig Harbor, Mulligan	0.020
	EB_ME	Elliott Bay, Myrtle Edwards	0.057
	Site #6	Eagle Harbor Dr	0.058
Top 10%	NPS_CPAR4	Cherry Pt Aq Rsv 4, Conoco Phillips	0.061
100 10%	CPS_PNP	Point No Point	0.063
	CPS_MASO	Manchester, Stormwater Outfall	0.066
	NPS_BLSC	Bellingham Bay, Little Squalicum Creek	0.084

Table 25. Mussel sites with the highest and lowest mercury concentrations (10<sup>th</sup> percentile) of 66 monitoring sites.

The narrow range of mercury concentrations in mussels from this study (0.015 – 0.084 mg/kg dry wt.) was nearly the same as in the 2012/13 MWPE study (0.03 – 0.11 mg/kg dry wt.). Similar to this study, mercury was detected at 100% of the MWPE mussel sites and, like arsenic, cadmium, and lead, the highest concentration occurred at the Edmonds Ferry site in 2012/13. No significant relationship was found between mercury and impervious surface in adjacent watersheds in either study (Lanksbury et al., 2014).

Mercury, especially methylmercury, is toxic to animals and can cause damage to the brain, kidneys, and lungs. Shellfish and fish concentrate mercury in their bodies, but due to biomagnification the highest mercury concentrations generally occur in fish species high in the food chain (e.g., shark, swordfish, tuna, tilefish). The EPA's human health screening value for mercury is 0.3 ppm wet weight. None of the mussels sampled in this study exceeded this threshold (Appendix 3). Because the WADOH human health screening values are just for methylmercury and our values are for total mercury, we could not compare our data to the WADOH screening values.



Figure 18. Map of the relative concentrations of mercury from all the 2015/16 SAM Mussel Monitoring sites.

## Arsenic

Arsenic is released naturally into the environment via volcanic ash, mineral and ore weathering, and through mineralized groundwater. We detected arsenic in mussels from 100% of the study sites (Figure 19). The highest concentrations of arsenic for each group of sites occurred at SAM Site #2 (Arroyo Beach), Pierce County Site #625 (Gig Harbor, Mulligan), and Partner Site NPS\_BLSC (Bellingham Bay, Little Squalicum Creek; Table 26). The lowest concentration of arsenic was found at SAM Site #43 (N Avenue Park), Pierce County Site #161 (Purdy, Dexters), and at Partner Site CPS\_HCV (Port Madison, Hidden Cove; Table 26). Mussel sites with the lowest and highest concentrations of arsenic (10<sup>th</sup> percentile) for the entire study are listed in Table 27. Similar to the mercury findings, five of the lowest arsenic sites were from Pierce County, including sites in Gig Harbor, near Purdy, and just north of Commencement Bay. Arsenic mussel concentrations from every site are listed in <u>Appendix 3</u>. Cumulative frequency distributions of arsenic for the SAM and Pierce County sites are in <u>Appendix 10</u>.

Table 26. Range and average concentration of arsenic in mussels from the sites in this study. \*Unincorporated Pierce County mussel sites.

		A	rsenic (mg/kg, dry w	/t.)
Sites	n	Min	Average	Max
Baseline	6	5.39	6.14	7.59
SAM	36	5.54	6.76	7.89
Pierce County*	7	4.77	5.36	6.51
Partner	23	5.82	6.89	9.45
All	66	4.77	6.65	9.45

Table 27. Mussel sites with the highest and lowest arsenic concentrations (10<sup>th</sup> percentile) of 66 monitoring sites.

	Site ID	Site Name	Conc. Arsenic
	Site #161	Purdy, Dexters	4.//
	Site #697	Browns Point, Wolverton	4.81
Bottom	Site #481	Gig Harbor Boat Launch	4.91
10%	Site #61	Dash Point Park	4.98
	Site #353	Purdy, Nicholson	5.07
	Site #43	N Avenue Park	5.54
	CPS_SB	Salmon Bay	7.53
	HC_FP	Fisherman's Point	7.61
Top 10%	Site #2	Arroyo Beach	7.89
100 10%	SPS_NRQR	Nisqually Rch Aq Rsv, Anderson Is	7.99
	WB_CB	Cavalero Beach Co. Park	8.57
	NPS_BLSC	Bellingham Bay, Little Squalicum Creek	9.45

Arsenic concentrations in mussels from this study (4.77 - 9.45 mg/kg dry wt.) were nearly the same as those found in the 2012/13 MWPE study (4.83 - 8.02 mg/kg dry wt.). Arsenic was detected at 100% of the MWPE sites and, like cadmium, mercury, and lead, the highest concentration occurred at the Edmonds Ferry site. As with this study, no significant relationship was found between arsenic and

impervious surface in adjacent upland watersheds in 2012/13 (Lanksbury et al., 2014). Historically the highest values of arsenic (16 ppm, dry wt.) were found in naturally occurring mussels taken from the Cape Flattery Mussel Watch site, on the outer coast (Kimbrough et al., 2008).

Arsenic is primarily used by humans in alloys of lead (e.g., in car batteries and ammunition) and as a feed additive in poultry and swine production. In the past, it has also been used as a wood preservative and in various agricultural insecticides and poisons. Arsenic has been linked to a number of cancers in humans and the maximum concentration allowed in drinking water is 0.01 ppm (EPA, 2017b). Because the WADOH human health screening values are just for inorganic arsenic and our values are for total arsenic (including organic and inorganic), we could not compare our data to the WADOH screening values.



Figure 19. Map of the relative concentrations of arsenic from all the 2015/16 SAM Mussel Monitoring sites.

# Cadmium

Cadmium occurs naturally in the earth's crust and is used in batteries, pigments, and metal coatings and alloys. We detected cadmium in mussels from 100% of the study sites, but the range of detected values for cadmium was very narrow (Figure 20). The highest concentrations of cadmium for each group of sites occurred at SAM Site #11 (South Bay Trail), Pierce County Site #161 (Purdy, Dexters), and Partner Site SPS\_PBL (Purdy, Burley Lagoon; Table 28). The lowest concentration of cadmium was found at SAM Site #31 (Eastsound, Fishing Bay), Pierce County Site #697 (Browns Point, Wolverton), and at Partner Site CPS\_SB (Salmon Bay; Table 28). Mussel sites with the highest and lowest cadmium concentrations (10<sup>th</sup> percentile) for the entire study are listed in Table 29. Cadmium mussel concentrations from every site are listed in <u>Appendix 3</u>. Cumulative frequency distributions for cadmium for the SAM and Pierce County sites are in <u>Appendix 11</u>.

Table 28. Range and average concentration of cadmium in mussels from the sites in this study. \*Unincorporated Pierce County mussel sites.

		Cadmium (mg/kg, dry wt.)		
Sites	n	Min	Average	Max
Baseline	6	1.56	1.71	1.94
SAM	36	1.29	1.71	2.14
Pierce County*	7	1.38	1.61	1.86
Partner	23	1.52	1.77	2.11
All	66	1.29	1.72	2.14

*Table 29. Mussel sites with the highest and lowest cadmium concentrations (10<sup>th</sup> percentile) of 66 monitoring sites.* 

			Conc. Cadmium
	Site ID	Site Name	(mg/kg, dry wt.)
	Site #31	Eastsound, Fishing Bay	1.29
Bottom 10%	Site #161	Purdy, Dexters	1.38
	Site #15	Tugboat Park	1.45
	Site #38	Rocky Point	1.46
	Site #353	Purdy, Nicholson	1.47
	Site #43	N Avenue Park	1.49
	NPS_DHCC	Drayton Harbor, California Creek	1.98
	WB_CB	Cavalero Beach Co. Park	2.01
Top 10%	Site #25	Blair Waterway	2.04
100 10%	SPS_PBL	Purdy, Burley Lagoon	2.11
	Site #46	Appletree Cove	2.12
	Site #11	South Bay Trail	2.14

Cadmium concentrations in mussels from this study (1.29 – 2.14 mg/kg dry wt.) were generally lower than the values found in the 2012/13 MWPE study (1.59 – 4.07 mg/kg dry wt.). Cadmium was detected at 100% of the MWPE sites and, like arsenic, mercury, and lead, the highest concentration occurred at the Edmonds Ferry site. However, the national Mussel Watch program reported high concentrations of cadmium (11 ppm, dry wt.) in naturally occurring mussels from their Cape Flattery site, at the northwest corner of Washington (Kimbrough et al., 2008). As with this study, no significant relationship was found between cadmium and impervious surface in adjacent watersheds in 2012/13 (Lanksbury et al., 2014).

Cadmium is found in low levels in many foods, but cadmium levels in shellfish are generally higher (up to 1 ppm) than in other types of fish or meat. In humans, high levels of cadmium can cause damage to the kidneys and fragility in bones. The WADOH fish consumption advisory screening levels (SLs) for cadmium are 1.17 ppm wet weight for general consumers, and 0.400 ppm for high consumers. In this study, none of the mussel sites had cadmium concentrations that exceeded the general consumer SL, but Pierce County Site #697 (Browns Point, Wolverton; 0.402 ppm) had a concentration that reached the high consumer screening level (see cadmium wet weight concentrations in <u>Appendix 3</u>).



Figure 20. Map of the relative concentrations of cadmium from all the 2015/16 SAM Mussel Monitoring sites.

#### Copper

Copper occurs naturally in soils and is used in electrical wire, roofing and plumbing, in industrial machinery, in anti-biofouling paints on boat hulls, and in automotive brake pads. We detected copper in mussels from 100% of the study sites (Figure 21). The highest concentrations of copper for each group of sites occurred at SAM Site #14 (Point Heron East), Pierce County Site #953 (Browns Point, Carlson), and Partner Site CPS\_MASO (Manchester, Stormwater Outfall; Table 30). The lowest concentration of copper was found at SAM Site #31 (Eastsound, Fishing Bay), Pierce County Site #161 (Purdy, Dexters), and at Partner Site NPS\_DHCC (Drayton Harbor, California Creek; Table 30). Mussel sites with the highest and lowest concentrations of copper (10<sup>th</sup> percentile) for the entire study are listed in Table 31.

Similar to the mercury and arsenic findings, all six of the lowest concentration copper sites occurred in Pierce County at in Gig Harbor, near Purdy, and just north of Commencement Bay. Copper mussel concentrations from every site are listed in <u>Appendix 3</u>. Cumulative frequency distributions of copper for the SAM and Pierce County sites are in <u>Appendix 12</u>.

		Copper (mg/kg, dry wt.)		
Sites	n	Min	Average	Max
Baseline	6	6.65	7.60	8.62
SAM	36	5.75	7.98	12.6
SAM Opt 2	7	3.51	4.33	6.20
Partner	23	5.82	7.91	12.7
All	66	3.51	7.57	12.7

Table 30. Range and average concentration of copper in mussels from the sites in this study. \*Unincorporated Pierce County mussel sites.

			Conc. Copper
	Site ID	Site Name	(mg/kg, dry wt.)
	Site #161	Purdy, Dexters	3.51
	Site #481	Gig Harbor Boat Launch	3.52
Bottom 10%	Site #353	Purdy, Nicholson	3.75
B0110111078	Site #61	Dash Point Park	4.12
	Site #697	Browns Point, Wolverton	4.25
	Site #625	Gig Harbor, Mulligan	4.96
	Site #39	Smith Cove, Terminal 91	9.66
	Site #28	Oak Harbor	9.85
Top 10%	Site #30	Kitsap St Boat Launch	10.5
100 10%	NPS_CPAR4	Cherry Pt Aq Rsv 4, Conoco Phillips	12.2
	Site #14	Point Heron East	12.6
	CPS_MASO	Manchester, Stormwater Outfall	12.7

Table 31. Mussel sites with the highest and lowest copper concentrations (10<sup>th</sup> percentile) of 66 monitoring sites.

The range of copper concentrations in this study (3.51 - 12.7 mg/kg dry wt.) was nearly the same as the range from the 2012/13 MWPE study (4.05 - 10.5 mg/kg dry wt.). As in this study, copper was detected at 100% of the MWPE mussel sites, though the highest concentration occurred at the EB\_SB (Salmon Bay) site. Though regression analyses from the MWPE study site showed a weak positive correlation ( $r^2 = 0.098$ , p<0.0001) between copper concentrations in mussels and impervious surface in the adjacent watershed (Lanksbury et al., 2014), no significant relationship was found between the two in this study.

Copper can have toxic effects on fish and other aquatic organisms as well, especially in freshwater systems where copper can be toxic to the salmon olfactory system at very low concentrations. Copper-exposed juvenile salmon become insensitive to chemical signals in their environment, including cues that a predator is nearby (McIntyre et al., 2012). In 2010, Washington passed a law (<u>SB6557</u>) mandating a reduction in the amount of copper used in automotive brake pads. In 2011, Washington passed another law (<u>SB5436</u>) that restricts the use of copper paint on the bottom of boats, beginning on January 1, 2018.

Copper is an essential trace element for humans and combines with protein and iron in hemoglobin, but very high doses of copper can cause liver and kidney damage (ATSDR 2004). There are no WADOH human health screening levels for copper. In a study by Grout and Levings (2001) copper adversely affected juvenile mussel (*Mytilus edulis*) growth at tissue concentrations above 20 mg/kg dry weight, and survival and condition indexes declined when mussels bioaccumulated more than 40 mg/kg dry weight. However, none of the mussels in this study had copper concentrations above these values (see copper dry weight concentrations in <u>Appendix 3</u>).


Figure 21. Map of the relative concentrations of copper from all the 2015/16 SAM Mussel Monitoring sites.

#### Lead

Lead is a naturally occurring element in soil, though it has also been released into the environment through widespread use of leaded gasoline, lead-containing pesticides, lead-based paint, and emissions from smelters. We detected lead in mussels from 58/66 (88%) of the all sites in this study (Figure 22). The highest concentrations of lead for each group of sites occurred at SAM Site #6 (Eagle Harbor Dr.), Pierce County Site #953 (Browns Point, Carlson), and Partner Site NPS\_BLSC (Bellingham Bay, Little Squalicum Creek; Table 32). The lowest concentration of lead was found at SAM Site #47 (Cherry Pt Aq Rsv, Birch Bay S), and lead was not detected above the reporting detection limit (RDL) at six of the seven Pierce County sites or at the Partner sites NPS\_DHCC (Drayton Harbor, California Creek) and CPS\_SHLB (Shilshole Bay; Table 33).

Mussel sites with the highest and lowest concentrations of lead (10<sup>th</sup> percentile) for the entire study are listed in Table 33. Similar to the findings for mercury, arsenic, and copper, six of the eight lowest lead sites occurred in Pierce County at Gig Harbor, near Purdy, and just north of Commencement Bay. Lead mussel concentrations from every site are listed in <u>Appendix 3</u>. The cumulative frequency distributions for the SAM and Pierce County sites are shown in <u>Appendix 13</u>.

Table 32. Range and average concentration of lead in musse	ls from the sites in this study.	*Unincorporated Pierce Cou	nty mussel
sites.			
	1 / / .		

		Lead (mg/kg, dry wt.)			
Sites	n	Min	Average	Max	
Baseline	6	0.252	0.342	0.468	
SAM	36	0.210	0.457	0.977	
Pierce County*	7	0.261	0.261	0.261	
Partner	23	0.182	0.399	0.986	
All	66	0.182	0.433	0.986	

Table 33. Mussel sites where lead was not detected above the reporting detection limit (RDL) the highest lead concentrations (10<sup>th</sup> percentile) of 66 monitoring sites.

			Conc. Lead
	Site ID	Site Name	(mg/kg, dry wt.)
	Site #353	Purdy, Nicholson	ND
Sites where	Site #61	Dash Point Park	ND
lead was not	Site #161	Purdy, Dexters	ND
above the	Site #481	Gig Harbor Boat Launch	ND
reporting	Site #697	Browns Point, Wolverton	ND
detection	Site #625	Gig Harbor, Mulligan	ND
limit ( <rdl)< td=""><td>NPS_DHCC</td><td>Drayton Harbor, California Creek</td><td>ND</td></rdl)<>	NPS_DHCC	Drayton Harbor, California Creek	ND
	CPS_SHLB	Shilshole Bay	ND
	Site #24	S of Skunk Island	0.803
	Stie #49	Donkey Creek Delta	0.816
Top 10%	Site #11	South Bay Trail	0.921
	Site #6	Eagle Harbor Dr	0.977
	NPS_BLSC	Bellingham Bay, Little Squalicum Creek	0.986

ND - not detected, reporting detection limit ranged from 0.087 to 1.27 mg/kg, dry weight.

The narrow range of lead concentrations in this study (0.182 - 0.986 mg/kg dry wt.) was nearly the same as the range from the 2012/13 MWPE study (0.13 - 1.38 mg/kg dry wt.). Lead was detected at more of the study sites (100%) in 2012/13, and, like arsenic, cadmium, and mercury, the highest concentration was at an Edmonds Ferry site. Regression analyses from the MWPE study site showed a significant positive correlation ( $r^2 = 0.198$ , p<0.0001) between lead concentrations in mussels and the average percent impervious surface in adjacent watersheds (Lanksbury et al., 2014), but we found no significant relationship between lead and impervious surface in this study.

Lead is a highly poisonous metal and affects almost every organ and system in the human body. In children lead can cause behavior and learning problems, damage to the central nervous system and kidneys, reductions in growth and hearing, and anemia (ATSDR 1999). Ingestion of paint chips, contaminated soil and house dust, and homes with old plumbing are the main pathways of lead exposure in children. There are no WADOH screening levels for lead, which is considered unsafe at any level of consumption (EPA, 2017c; CDC, 2017). Ecology released a Lead Chemical Action Plan (CAP) in 2009 (Bergman et al., 2009), which made a <u>number of recommendations</u> to reduce lead, including actions to reduce exposures from old paint and plumbing in homes and schools, and reducing lead in current products and processes. In 2009, Washington State banned the installation of lead wheel weights on vehicles, and in 2010, WDFW banned the use of lead fishing tackle in 13 Common Loon nesting lakes.



Figure 22. Map of the relative concentrations of lead from all the 2015/16 SAM Mussel Monitoring sites.

#### Zinc

Zinc is an element that occurs naturally in the earth's soil, and we detected zinc in mussels from 100% of the study sites (Figure 23). The highest concentrations of zinc for each group of sites occurred at SAM Site #21 (Point Defiance Ferry), Pierce County Site #953 (Browns Point, Carlson), and Partner Site CPS\_PNP (Point No Point; Table 34). The lowest concentration of zinc was found at SAM Site #31 (Eastsound, Fishing Bay), at Pierce County Site #697 (Browns Point, Wolverton), and at Partner Site NPS\_DHCC (Drayton Harbor, California Creek; Table 34). Mussel sites with the highest and lowest concentrations of zine (10<sup>th</sup> percentile) for the entire study are listed in Table 35. Similar to the findings for mercury, arsenic, copper, and lead, five out of the six lowest zinc sites occurred in Pierce County in Gig Harbor, near Purdy, and just north of Commencement Bay. Zinc mussel concentrations from every site are listed in <u>Appendix 3</u>. Cumulative frequency distributions of zinc for the SAM and Pierce County sites are in <u>Appendix 14</u>.

Table 34. Range and average concentration of zinc in mussels from the sites in this study. \*Unincorporated Pierce County mussel sites.

			Zinc (mg/kg, dry wt	.)
Sites	n	Min	Average	Max
Baseline	6	77.3	84.3	94.0
SAM	36	76.2	93.5	122
Pierce County*	7	47.2	56.0	75.3
Partner	23	62.0	77.0	95.4
All	66	47.2	83.8	122

Table 35. Mussel sites with the highest and lowest zinc concentrations (10<sup>th</sup> percentile) of 66 monitoring sites.

			Conc. Zinc
	Site ID	Site Name	(mg/kg, dry wt.)
	Site #697 Browns Point, Wolverton		47.2
	Site #161	Purdy, Dexters	48.2
Bottom 10%	Site #481	Gig Harbor Boat Launch	49.5
BOLLOIN 10/0	Site #353	Purdy, Nicholson	50.7
	Site #61	Dash Point Park	54.4
	NPS_DHCC	Drayton Harbor, California Creek	62.0
	Site #17	Budd Inlet, West Bay	101
	Site #13	Ruston Way	103
Top 10%	Site #22	Beach Dr E	104
100 10%	Site #30	Kitsap St Boat Launch	106
	Site #49	Donkey Creek Delta	109
	Site #21	Point Defiance Ferry	122

Zinc ranged from 47.2 - 122 mg/kg dry wt. in this study, which was nearly the same as the range from the 2012/13 MWPE study (68 – 137 mg/kg dry wt.). As in this study, zinc was detected at 100% of the MWPE mussel sites, though the highest concentration occurred at a Silverdale, Dyes Inlet site. Unlike the results from this study, zinc concentrations in the 2012/13 MWPE mussels were weakly correlated ( $r^2 = 0.055$ , p = 0.016) with impervious surface in adjacent watersheds (Lanksbury et al., 2014).

Zinc is an essential trace element for humans and is used as an ingredient in vitamin supplements, sun block, diaper rash ointment, deodorant, in topical medicines and in anti-dandruff shampoos (ATSDR 2005). Zinc is also used in cathodic protection of metal surfaces (i.e., an anti-corrosion and galvanizing agent), and soils can be contaminated with zinc from mining and refining. Absorption of too much zinc can suppress copper and iron absorption in humans, and free zinc ions in solution are highly toxic to plants, invertebrates, and fish (Fosmire, 1990; Eisler, 1993). The WADOH fish consumption advisory screening levels (SLs) for zinc are 352 ppm wet weight for general consumers and 120 ppm for high consumers. In this study, none of the mussel sites had zinc concentrations that exceeded these SLs (see zinc wet weight concentrations in <u>Appendix 3</u>).



Figure 23. Map of the relative concentrations of zinc from all the 2015/16 SAM Mussel Monitoring sites.

## **Biological Endpoints Overview**

#### Mortality

On average, mussel mortality in this study was around 22% (Table 36). The highest mortality for each group of sites occurred at SAM Site #49 (Donkey Creek Delta), Pierce County Site #625 (Gig Harbor-Mulligan), and Partner Site SPS\_HIAP (Hammersley Inlet, Arcadia Point). The lowest mortality was found at SAM Site #31 (Eastsound, Fishing Bay) and Site #47 (Cherry Point Aquatic Reserve, Birch Bay South), Pierce County Site # Site 481 (Gig Harbor Boat Launch), and Partner Site CPS\_MASO (Manchester, Stormwater Outfall). Mussel sites with the highest and lowest mortalities (10<sup>th</sup> percentiles) for the entire study are listed in Table 37. Percent mortality of mussels from every site are listed in <u>Appendix 4</u>.

Table 36. Range and average mortality in mussels from the various groups of sites in this study. We could not calculate the mortality of mussels from the Baseline Site because those mussels were sampled at the beginning of the study (i.e., starting condition) as the source of mussels for transplant. \*Unincorporated Pierce County mussel sites.

		Mortality (%)				
Sites	n	Min	Average	Max		
SAM	36	14.1	25.7	39.1		
Pierce County*	7	12.5	22.3	31.3		
Partner	23	7.8	20.4	43.8		
All	66	7.8	22.2	43.8		

Table 37. Mussel sites with the highest and lowest mortality (10<sup>th</sup> percentile) of 66 monitoring sites.

	Site ID	Site Name	Mortality (%)
	Site #481	Gig Harbor Boat Launch	7.8
	CPS_MASO	Manchester, Stormwater Outfall	7.8
Bottom	HC_FP	Fisherman's Point	7.8
10%	WB_KP	Kayak Point	9.4
	WB_CB	Cavalero Beach Co. Park	10.9
	EB_ME	Elliot Bay, Myrtle Edwards	12.3
	Site #5	Salmon Beach	32.8
	Site #18	Seahurst	32.8
Top 10%	Site #13	Ruston Way	34.9
100 10%	Site #17	Budd Inlet, West Bay	37.5
	Site #26	N of Illahee State Park	39.1
	Site #49	Donkey Creek Delta	43.8

Within the UGA, mortality was significantly higher at SAM and Pierce County sites with some agriculture in the upland watershed (25% mortality, n = 35) than from sites without any agriculture (19% mortality, n = 8; Mann-Whitney U Test Statistic = 208, p = 0.034). Though there was a tendency for mortality to be

higher at city sites (25%, n = 26) than at unincorporated-UGA sites (21%, n = 17), the difference was not significant (Mann- Whitney U Test Statistic = 289, p = 0.091). However, we note that mortality of mussels from the 2012/13 MPWE study was weakly correlated with percent impervious surface in adjacent watersheds (Lanksbury et al., 2014). None of the other upland or in-water point sources tested in this study (Table 3) had a significant impact on mortality.

#### Condition Index

We calculated the Condition Index (CI) of mussels from each of the study sites to investigate differences in growth related to food availability. Although the concentrations of contaminants measured in mussel tissues are a function of bioavailable pollutant levels, accumulation is also effected by growth, which is in turn related to food in the local environment. Condition indices function to normalize biological changes over time and can help assess the role of seasonal fluctuations in environmental factors (e.g., food availability, temperature). Condition index can also serve as an indication of the impact of reproductive status on biological and chemical measurements in the mussels (Benedicto et al., 2011; Kagley et al., 2003; Roesijadi et al., 1984). We determined CI on twelve randomly selected mussels from each site using the method reported by Kagley et al. (2003), as follows:

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

At the end of the study, the CI of transplanted mussels from the SAM and Pierce County sites (2.09 gm/mm, n = 42 sites), and from all the study sites combined (2.20 gm/mm, n = 65 sites) were significantly lower than the starting CI from the Baseline Site 3.15 gm/mm (Baseline Site, Penn Cove; n = 70 mussels); SAM sites  $t_{(46)} = 10.175$ , p<0.0001, all sites together  $t_{(69)} = 10.552$ , p<0.0001. None of the upland or inwater point sources tested in this study had a significant effect on CI, and CI and mortality were not correlated (r = 0.143, n = 42, p = 0.367). Mussel CIs from the 2012/13 MPWE study also were not correlated with impervious surface in adjacent watersheds (Lanksbury et al., 2014).

The average CI for each group of sites is shown in Table 38. The highest CI for each group of sites occurred at SAM Site #49 (Donkey Creek Delta), Pierce County Site #353 (Purdy, Nicholson), and Partner Site NPS\_BLSC (Bellingham Bay, Little Squalicum Creek). The lowest CI was found at SAM Site #37 (Saltar's Point), Pierce County Site # Site 953 (Browns Point, Carlson), and Partner Site CPS\_EF (Edmonds Ferry). Mussel sites with the highest and lowest CI (10<sup>th</sup> percentile) in the entire study are listed in Table 39. Condition index of mussels from every site are listed in <u>Appendix 4</u>.

	Condition Index (gm/mm) dry					
	n	Min	Average	Max		
Baseline	6	2.83	3.15	3.45		
RSMP	35	1.71	2.11	2.59		
Pierce County*	7	1.74	2.01	2.36		
Partner	23	1.80	2.14	2.75		
All	71	1.71	2.20	3.45		

Table 38. Range and average condition index of mussels from the various groups of sites in this study. \*Unincorporated Pierce County mussel sites.

	Site ID	Site Name	<b>Condition Index</b>
	Site #37	Saltar's Point	1.71
	Site #953	Browns Point, Carlson	1.74
Pottom 10%	CPS_EF	Edmonds Ferry	1.80
BOLLOIN 10%	Site #39	Smith Cove, Terminal 91	1.82
	Site #11	South Bay Trail	1.83
	Site #22	Beach Dr E	1.83
	Site #15	Tugboat Park	2.45
	Site #35	Williams Olson Park	2.49
Top 10%	Site #29	Liberty Bay	2.51
100 10%	Site #49	Donkey Creek Delta	2.59
	WPS_PB	Point Bolin	2.60
	NPS_BLSC	Bellingham Bay, Little Squalicum Creek	2.75

Table 39. Mussel sites with the highest and lowest condition index (10<sup>th</sup> percentile) of 66 monitoring sites.

The CI of Baseline mussels from this study (3.15 gm/mm) were higher than the CIs reported for the Baseline mussels in the 2012/13 MWPE study (MWPE CI = 2.51 gm/mm), but the ending average CI for this study (2.20 gm/mm) was similar to the ending CI from the MWPE study (2.30 gm/mm; Lanksbury et al., 2014). The overall decline in CI of mussels from the start to the end of both studies was likely a normal response to winter conditions. Kagley et al. (2003) reported a reduction in CI of wild mussels during the winter months in Puget Sound. During the winter, phytoplankton growth (i.e., primary production) declines due to limitations in sunlight and photosynthesis, leading to a decline in food for filter-feeding organisms like mussels.

# Tracking Changes Over Time

#### Power of Statistical Tests to Track Changes in Nearshore Contamination

This is the first survey in a long term, biennial mussel monitoring program designed to answer the question; "Is the health of biota in the urban nearshore improving, deteriorating, or remaining the same related to stormwater management?" We anticipate that the current survey design will allow us to detect differences in mussel contaminant concentrations in the UGA of Puget Sound between surveys. However, it would be good to know how big of a change in concentration (i.e., what magnitude) we can expect to detect between surveys. To this end, we estimated the number of sites required to detect small to large changes (2 to 100% differences) in UGA contaminant concentrations from the 2015/16 survey to the next survey in 2017/18 (Table 40). We included both small and large numbers in our range of potential future concentrations because long-term WDFW/PSEMP monitoring has detected small changes (~8%) in organic contaminant concentrations in Puget Sound English sole and herring tissues over the last two decades (1990-2010; West et al., 2017).

The power analyses described below were conducted with SYSTAT 12 (Power Analysis: Two-Sampled t-Test, power set at 0.80, and  $\alpha$  = 0.05), using data from the 2015/16 SAM sites (UGA sites). We used the mean contaminant concentrations from the SAM sites in 2015/16 as the "Mean 1" values, and then calculated ±2 to 100% changes for the "Mean 2" values (i.e., the ranges of projected concentrations for 2017/18). For example, the mean PAH value for the SAM and Pierce County sites combined (all UGA) was 665 ppb, so the projected values used in the power analysis for the 2017/18 SAM mussels were ±679 ppb (2% change), 732 ppb (10% change), 998 ppb (50% change), etc. (Table 40). In addition, we use the standard deviation (SD) of each contaminant from the 2015/16 dataset as the pooled SD in the analyses.

Results indicated that the current study design, with 40 SAM sites in the UGA, is likely sufficient to detect an increase or decrease of at least 3% in zinc, 4 to 5% in PAHs, 10% in PCBs and arsenic, 20% in PBDEs, copper and cadmium, and just over 40% in DDTs (Table 40). However, with 40 SAM sites in the UGA we would likely only be able detect an increase or decrease of just over 75% in lead and would not be able to detect even a 100% change in mercury.

Table 40. Estimated number of sample sites required in UGA to have an 80% chance of detecting changes in mussel contaminant concentrations, if they occur, between the 2015/16 and the 2017/18 SAM Mussel Monitoring survey. Power analyses conducted with SYSTAT 12 (Power Analysis: Two-Sample t-Test, power = 0.80,  $\alpha$  = 0.05), using UGA mean values and pooled SD from 2015/16 (SAM and Pierce County sites combined) and projecting a range of mean values for each chemical group in 2017/18.

		± Change in Concentration							
Chemical Group	UGA mean (2015/16)	2%	3%	5%	10%	20%	30%	50%	100%
PAHs	665 ppb	107	48	18	6	3	3	3	3
PCBs	50.8 ppb	675	299	108	28	8	5	3	3
PBDEs	8.37 ppb	NT	NT	655	161	41	19	8	3
DDTs	4.21 ppb	NT	NT	NT	673	170	75	28	8
Zinc	87.4 ppm	88	40	15	5	3	3	3	3
Copper	7.39 ppm	NT	NT	107	55	15	8	4	3
Cadmium	1.69 ppm	NT	NT	216	100	26	13	6	3
Arsenic	6.53 ppm	NT	NT	354	29	8	5	3	3
Lead	0.389 ppm	NT	NT	NT	NT	650	290	105	28
Mercury	0.042 ppm	NT	NT	NT	NT	NT	NT	432	109

NT = Not tenable; estimated number of samples needed to detect change is over 1000.

#### Recommendations for Future SAM Mussel Monitoring

#### Monitoring Modifications

Based on the discussed results, WDFW makes several recommendations for future SAM nearshore mussel monitoring:

- This study highlights increased bioaccumulation of organic contaminants (PAHs, PCBs, PBDEs, DDTs) and metals (zinc and lead) in nearshore mussel tissues in relation to urban growth areas of Puget Sound. This result may be due, in part, to contaminants carried by municipal stormwater, municipal and agricultural non-point runoff, atmospheric deposition, and circulation patterns within the Puget Sound. To identify the major sources of contamination, and to better understand temporal trends and mechanisms, we recommend the following future studies:
  - a. Long-term nearshore mussel monitoring this will help us describe what factors regulate contamination in mussels and elucidate how and why they change over time in Puget Sound.

- b. Incorporation of our findings with other SAM monitoring studies this will improve our ability to evaluate the impact of stormwater and other management practices on the health of Puget Sound.
- 2. SAM should relocate some of the sites to represent the full spectrum of urbanization in Puget Sound. This would require the relocation of some sites while retaining others. To attain this goal SAM could either:
  - a. allow WDFW to choose the locations based on best professional judgement, or
  - b. introduce three to four *substrata*, based on intensity of urban development, into the GRTS model and allow it to randomly select sites within them, with the goal of retaining as many of the 2015/16 sites as possible. Depending on the scale, this option would require about 5 to 10 sites per substratum (20-40 sites total). Substrata should be selected using either the mean impervious surface in watersheds or municipal land use designations.
- 3. Once the nearshore sites are selected based on recommendation #2 they should be revisited during each survey for time trend analysis (i.e., they become index sites).
- 4. Considering the low power to detect differences in most of the metals in this first round of mussel monitoring, SAM should commission a literature review of the efficacy of using mussels to detect changes in metals and either drop or retain metals from the analysis list based on the findings.
- 5. Given recent evidence of contaminants of emerging concern (CECs) in Puget Sound fish (Peck et al., 2011, Johnson et al., 2008; Fiest et al., 2011), we recommend adding some CECs to the list of contaminants analyzed by the SAM Mussel Monitoring effort. We further recommend seeking guidance from PSEMP's Toxics Workgroup on which stormwater-related CECs are relevant to the Puget Sound and measurable via current methods.

If the SWG decides to incorporate substrata into the GRTS model (recommendation 2b), WDFW recommends using the most recent (2011) NLCD percent developed imperviousness dataset (Xian, et al. 2011) as the basis for defining the substrata. We further recommend that definition of impervious surface substrata should be coordinated between the SAM Status and Trends in Receiving Waters monitoring components (i.e., Streams, Nearshore Sediment, Shoreline Bacteria) with the goal of a unified approach.

Impervious surface is a useful, and quantifiable, proxy for urban development and is directly linked to stormwater runoff. Research demonstrates that an impervious surface coverage of 10% or less within a watershed typically leads to measurable and often permanent loss of function in aquatic ecosystems (Booth and Reinelt, 1993). The empirically derived NLCD percent developed imperviousness dataset uses Landsat satellite data with a spatial resolution of 30 meters. Not only does it describe landscape urbanization at a fine scale in Puget Sound, future NLCD scans will allow us to describe how urbanization is changing over time. In addition, substrata defined by impervious surface will likely provide enough replication to allow for a roll-up into the larger municipal land-use classifications (compare Figure 7 to Figure 8 in Overview of Statistical Results section), though the reverse situation would not be likely.

#### Future of Cooperative Monitoring

It was the intent of this study to monitor contaminants in biota from the UGA nearshore, and compare the results to data from prior WDFW mussel monitoring along non-UGA shorelines. Though here we compared SAM UGA site results to those from the Baseline Site at Penn Cove (i.e., starting condition), data on non-UGA sites was added to the study through sponsoring partners. Data on those sponsored sites, which is included in this report benefits those sponsors, giving them a larger context in which to compare their results. However, the sponsored site data also provides a benefit to SAM as it allows for comparison to some non-UGA sites during the same study period, which would otherwise not be possible given SAM's focus on site selection only within the UGA strata. WDFW recommends that SAM continue to encourage this model of cooperative mussel monitoring in the nearshore, which benefits all involved.

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# Appendix 1: Materials and Methods

Methods for this study followed those detailed in the <u>Quality Assurance Project Plan (QAPP) for Status and</u> <u>Trends Monitoring of Marine Nearshore Mussels, for the Regional Stormwater Monitoring Program and</u> <u>Pierce County</u> (Lanksbury and Lubliner, 2015). The sections below summarize the methods employed for this study, for more details please refer to the QAPP.

### Site Selection and Evaluation

The initial list of required candidate sites for SAM and Pierce County (SAM Option 2) were verified by a field crew to determine suitability for sampling. In order to evaluate the accessibility, safety, and suitability of the site, candidate sites were visited in the daylight during low tide, well in advance of monitoring.

#### Site Selection Criteria

The suitability of a mussel site was determined using the criteria outlined below. Field crews evaluated the suitability criteria at the site center. See QAPP for details on site layout and location of site center. If the site center was not suitable, then the field crew evaluated conditions up to 400 m (1312 feet or 0.25 mile) in either direction along the shoreline until the *closest suitable location* relative to the site center was found.

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

- Condition 1 the site was NOT within a marina or port (i.e., where multiple motorized vessels are kept in the water), and
- Condition 2 the site could be safely accessed and worked on in the winter, during night-time low tides, and
- Condition 3 permission of property owners and/or tenants was granted prior to sampling, and
- Condition 4 there was suitable substrate or a location for anchoring/securing a mussel cage at the site.

See the QAPP for further details on the accessibility criteria (p. 18) and intertidal physical criteria (p. 19).

If a location other than the site center was chosen, then the reason for disqualification of the site center was documented and the alternate site coordinates recorded. If all 800 m of a candidate site were not suitable, then the reason for disqualification was documented, including photos, and alternate candidate sites were visited, in numerical order from the site list, and verified for replacement.

#### Site Evaluations

Site evaluators verified all sites given the suitability criteria above. Table 1 in the Overview of Sampling Efforts section lists the decisions and reasons for site selection or disqualification resulting from site evaluations. For details on the Site ID and Location Name naming conventions, refer to the QAPP (p. 20).

## Study Specimen and Sample Units

Naturally occurring mussel populations of sufficient size were lacking at many of the desired Puget Sound locations, thus we chose to transplant mussels to monitoring sites for this study. We used cultured, pre-reproductive bay mussels (*Mytilus trossulus*) from Penn Cove Shellfish, Inc., Whidbey Island, Washington. *M. trossulus* is native to Puget Sound, is tolerant of low temperatures, spawns in early spring, and is readily available in large quantities via local aquaculture cultivation. Mussels used were between 50 - 60 mm in shell length and estimated to be 11 months old (Penn Cove Shellfish LLC, 2012, pers. comm.). Exposure to contaminants in Penn Cove was expected to be minimal and because the animals had not yet reproduced, we assumed no differences in initial contaminant load related to sex.

Prior to deployment at the study site, groups of mussels were placed into heavy duty, high density polyethylene mesh bags with 16 mussels per bag; bags were divided into two pouches with eight mussels on each side. To increase likelihood of survival after handling, bagged mussels were allowed to rest in the water at Penn Cove for approximately 20 days prior to deployment at the study sites. At deployment four bags of mussels were hung horizontally in plastic-coated, wire mesh cages designed to exclude large predators while optimizing water flow (64 mussels per cage). Each mussel cage was anchored to the intertidal substrate at a height of approximately zero feet mean lower low water, with mussels suspended approximately 35 cm above the substrate within the cage. This tidal elevation was selected to simulate natural conditions experienced by mussels in the intertidal zone during the winter in Puget Sound. In addition, a subset of 100 mussels was collected prior to transplantation for analysis. These mussels were split into six replicate composite samples (n = 6) for chemical analysis and reflected the starting condition, they were denoted in this report as the "Baseline Site" mussels.

#### Exposure

Because we were particularly interested in nearshore contamination via watershed processes (e.g., stormwater), we timed our mussel deployments to match the likely period of maximum surface water runoff into the Puget Sound. Precipitation index data from the <u>National Climatic Data Center</u> (National Oceanic and Atmospheric Administration, 2014a) over the last 50 years (1962-2012) indicates annual rainfall in the Puget Sound lowland generally peaks in the months of November through January. Thus, one mussel cage was deployed to each of the study sites during evening low tides between October 26<sup>th</sup> to the 29<sup>th</sup>, 2015. It is generally agreed that 60 to 90 days is sufficient time for transplanted mussels to equilibrate with the range of contaminants in their location (ASTM International, 2007). The target duration of exposure of transplanted mussels in this study was three months (~90 days), so the caged mussels were retrieved between February 5<sup>th</sup> to the 10<sup>th</sup>, 2016.

#### Laboratory Processing

The following sections describe the laboratory measurement processes for biological endpoints and chemical analyses conducted by WDFW staff and volunteers. The lab forms used (Specimen Form and Tissue Resection Logs), equipment cleaning procedures, and sample storage methods are provided in the QAPP (p. 39 - 43).

#### **Biological Endpoints**

Mortality and condition index (CI) were assessed for a subset of the mussels from each study site. Condition indices were used to assess the role of fluctuations in environmental factors (e.g., food availability, temperature) and reproductive status on biological and chemical measurements in mussels.

#### Mortality

WDFW lab staff assessed individual mussel bags for dead or moribund mussels within 36 hours of receiving the mussels at the end of the exposure period. Dead or moribund mussels were counted, recorded and removed. A mussels was considered moribund if it was unable to tightly close its valves when stimulated. A mussel was considered dead if there was no soft tissue inside the shell, or if the soft tissue inside the shell was putrefied.

#### **Condition Index**

After dead mussels were removed, condition index was 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.

Shell length, soft tissue removal, and dry weight methods are provided in the QAPP (pgs. 40-41). Total shell length, tissue wet weight and tissue dry weight were recorded on the Specimen Form.

#### **Chemical Analyses**

A composite of 32 individual mussels (200g of soft tissue) per site (cage) was selected to optimize the amount of tissue available for analyses at two chemistry laboratories. This mass is based on previous experience with the same laboratories, and allowed enough tissue for reanalyses (if needed) and to archive small (20 g) subsamples of tissue. The number of mussels per composite was selected to balance representativeness of the population with labor and time constraints related to processing samples.

#### Composite sample preparation

Composite samples were prepared using the clean equipment procedures described in the QAPP (p. 40) and T-BiOS clean techniques. Previously frozen mussels were thawed and cleaned for tissue resectioning. Using a scalpel, the shells were spread apart at the hinge and all soft tissues were scraped into a clean stainless steel cup. Each mussel's tissue weight was recorded on the Tissue Resection Log as it was added to the cup. After 32 mussels were added to the cup, the total tissue weight was recorded. The combined soft tissue was blended using a hand-held blender until a homogenous mixture was achieved. The mixture was distributed into clean glass sample jars for the two labs and for sample archiving.

#### Analytical methods

Laboratory QA/QC requirements of the analytical chemistry methods are outlined in the QAPP (pgs. 24-26) and are detailed in the Puget Sound Estuary Program protocols (PSEP, 1986, 1997a, b, c) and in the peer-reviewed standard operating procedures (SOPs) for each test. The Northwest Fisheries Science Center Laboratory at Montlake conducted the analyses for organic chemicals, the King County Environmental Laboratory (KCEL) performed metal analyses, and stable isotopes of carbon (13C) and nitrogen (15N) were measured at the University of Washington. All three labs are located in Seattle, Washington. Unfortunately, the stable isotope results were not available in time for publication of this report. Homogenized composite mussel tissue samples were shipped to the analytical labs frozen. The analytical labs thawed and thoroughly mixed the tissue samples to ensure adequate homogeneity prior to sample preparation for chemical analysis. Persistent organic pollutants (POPs), metals, conventionals, and stable isotopes were analyzed as described in the QAPP (pgs. 44-46). POPs measured included polychlorinated biphenyl (PCB) congeners, polybrominated diphenylethers (PBDEs) congeners, organochlorine pesticides (OCPs), and polycyclic aromatic hydrocarbons (PAHs). Metals measured included mercury, arsenic, cadmium, copper, zinc and lead. Conventional parameters measured included lipid content (% total extractables) and dry weight (%).

#### Data Analyses

#### **Contaminant Concentrations**

Mussel contaminant data are presented as summed concentrations (e.g.,  $\Sigma_6$ DDTs) for analyte groups (Table 41), except in cases with fewer than two analytes per group. Summed analytes are the sum of all detected values, with zeroes substituted for non-detected analytes, within each group. In cases where all analytes in a group were not detected the greatest limit of quantitation (LOQ) for any single analyte in the group was used as the summation concentration, and the value was preceded by a "<" (less than) qualifier. An estimated total PCB concentration was calculated by summing the detected concentrations for 17 commonly detected congeners and multiplying the result by two, according to Lauenstein and Cantillo (1993). Summaries of the contaminant concentrations of mussel composites (n = 32 mussels) made for this study are provided in Appendices 2 and 3. Individual results for each congener or analyte were uploaded to Ecology's Environmental Information Management (EIM) database, where they are available on-line. Though contaminant concentrations are reported in both wet and dry weight, all statistical tests were conducted using only dry weight (dry wt.) contaminant concentrations. Appendices 5 – 14 include cumulative frequency distribution plots for each contaminant type that was detected in mussels from at least 80% of the study sites.

Table 41. Analyte and	d congener	groups summed	for this study.
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Sum 2 HCHs Sum 8 Chlordanes		Estimated Total	Sum 6	Sum 11	Sum of 38 Polycyclic Aromatic Hydrocarbons (PAHs)		
Sulli S HCHS	Sum & Chiordanes	PCBs*	DDTs	PBDEs	Low Molecular Weight	High Molecular Weight	
alpha hexachlorocyclohexane	alpha chlordane	PCB018	pp-DDD	PBDE028	Naphthalene (NAP)	fluoranthene (FLA)	
beta hexachlorocyclohexane	beta chlordane	PCB028	pp-DDE	PBDE047	C1-naphthalenes	pyrene (PYR)	
lindane	cis nonachlor	PCB044	pp-DDT	PBDE049	C2-naphthalenes	C1-fluoranthenes/pyrenes	
	heptachlor	PCB052	op-DDD	PBDE066	C3-naphthalenes	C2-fluoranthenes/pyrenes	
	heptachlor epoxide	PCB095	op-DDE	PBDE085	C4-naphthalenes	C3-fluoranthenes/pyrenes	
	nonachlor3	PCB101	op-DDT	PBDE099	acenaphthylene (ACY)	C4-fluoranthenes/pyrenes	
	Oxychlordane	PCB105		PBDE100	acenaphthene (ACE)	benz[ <i>a</i> ]anthracene (BAA)	
	trans Nonachlor	PCB118		PBDE153	fluorene (FLU)	chrysene (CHR) <sup>a</sup>	
		PCB128		PBDE154	C1-fluorenes	C1-benzanthracenes/chrysenes	
		PCB138		PBDE155	C2-fluorenes	C2-benzanthracenes/chrysenes	
		PCB153		PBDE183	C3-fluorenes	C3-benzanthracenes/chrysenes	
		PCB170			dibenzothiophene (DBT)	C4-benzanthracenes/chrysenes	
		PCB180			C1-dibenzothiophene	benzo[b]fluoranthene (BBF)	
		PCB187			C2-dibenzothiophenes	benzo[k]fluoranthene (BKF) <sup>b</sup>	
		PCB195			C3-dibenzothiophenes	benzo[ <i>e</i> ]pyrene (BEP)	
		PCB206			C4-dibenzothiophenes	benzo[ <i>a</i> ]pyrene (BAP)	
		PCB209			phenanthrene (PHN)	perylene (PER)	
					anthracene (ANT)	indeno[ <i>1,2,3-cd</i> ]pyrene (IDP)	
					C1-phenanthrenes/anthracene	dibenz[ <i>a,h</i> ]anthracene (DBA) <sup>c</sup>	
					C2-phenanthrenes/anthracenes	benzo[g,h,i]perylene (BZP)	
					C3-phenanthrenes/anthracenes		
					C4-phenanthrenes/anthracenes		

\*Sum of 17 congeners, then multiplied by two, a coelutes with triphenylene, b coelutes with benzo[j]flouranthene, c coelutes with dibenz[a,c]anthracene

#### Data Transformations and Statistical Analyses

All organic contaminants and metals were reported by the analytical labs on a wet weight basis, however to maintain consistency with the majority of published mussel contaminant studies we converted wet weight to dry weight using the percent moisture value derived from the analytical process. In addition, all contaminant data were log<sub>10</sub>-transformed prior to analysis to achieve normality and equality of variances for statistical testing. Minor violations of the normality and equality of variances assumptions after transformation were ignored if they were near the acceptable threshold (p = 0.05). In a few cases, transformation was not required to achieve normality or homoscedasticity; however we log<sub>10</sub>-transformed all contaminant data for consistency. All means and standard errors generated via two-sample *t*-testing and ANOVA were back calculated and reported as geometric means and 95% confidence intervals. Condition Index data were not transformed before parametric analysis (*t*-testing and ANOVA). Percent mortality data also were not transformed, but were analyzed using non-parametric Kolmogorov–Smirnov and Mann-Whitney U tests.

The cumulative frequency distributions of the combined SAM and Pierce County data (Appendices 5 – 14) include weight-adjusted contaminant values for the two groups of sites to account for differences in the size of the different sample frames used to draw the random samples for those groups; see Spatial Weighting of SAM and Pierce County sites for the resulting weights.

We do not present lipid-adjusted concentrations by dividing wet or dry contaminant concentration by lipid percent in this report; overall the lipid concentrations in our mussels were low and ranging narrowly from 0.80 - 1.85% wet weight. This low and narrow range was not surprising considering mussels do not feed at maximum capacity during the winter and generally lose weight during this season (Kagley et al., 2003). Lipid concentrations below 1% are difficult to measure accurately, and very low lipid concentrations have a large effect when computing contaminant concentrations on a lipid basis. In addition, small inaccuracies in quantitation in the range we encountered can contribute to spurious conclusions. For these reasons we did not lipid-normalize the mussel contaminant data in this study, but instead used lipid concentrations as a covariate in our statistical models. This approach follows protocols from other monitoring programs such as the Massachusetts Water Resource Authority (MWRA) mussel monitoring program, who originally normalized their mussel contaminant data with lipids through 1998, then dropped the practice after they discovered lipid normalization did not substantially alter the mussel contaminant trends when compared to non-lipid-normalized data (Hunt and Slone, 2010; Mitchell et al., 1998).

# Appendix 2: Concentration of Organic Contaminants in Mussels by Site

Dry Weight Concentrations of Organic Contaminants

\* Mean of six replicate samples from Penn Cove, Whidbey Island aquaculture source of mussels (i.e., starting condition) < Indicates the concentration was not measured above the limit of quantitation (LOQ), which is the value reported in this case

		Concentrations in ng/g, dry weight (ppb)								
Site ID	Site Name	5 0411	TROP		5 007.		5	<b>D</b> <sup>1</sup> .1.1.1.1		
		∑ <sub>38</sub> PAHs	TPCBs	$\sum_{11}$ PBDES	$\sum_{6}$ DDTs	∑ <sub>8</sub> Chlordanes	∑ <sub>3</sub> HCHS	Dieldrin		
WB_PC	Baseline Site (n = 6)*	35.5	5.4	<1.27	<1.27	<1.27	<1.27	<1.25		
Site #2	Arroyo Beach	290.2	41.7	12.4	3.4	<2.03	<0.801	<0.801		
Site #3	Brackenwood Ln	435.2	31.8	7.1	2.3	<1.09	<1.02	<1.02		
Site #4	Cherry Point North	123.9	6.2	<2.08	<1.70	<1.01	<1.01	<1.01		
Site #5	Salmon Beach	223.1	30.9	6.9	2.1	<0.857	<0.857	<0.857		
Site #6	Eagle Harbor Dr.	1821.8	116.7	3.0	3.2	<0.828	<0.828	<0.828		
Site #8	Chimacum Creek delta	94.9	12.5	<2.09	<1.20	<1.20	<1.20	<1.20		
Site #10	Fletcher Bay, Fox Cove	256.0	55.2	5.9	3.3	<0.920	<0.920	<0.858		
Site #11	South Bay Trail	382.1	22.4	8.0	3.1	<1.12	<1.12	<1.12		
Site #13	Ruston Way	446.8	48.2	10.1	3.0	<0.991	<0.991	<0.991		
Site #14	Point Heron East	185.7	61.4	7.6	2.1	<0.955	<0.955	<0.955		
Site #15	Tugboat Park	192.3	10.6	<1.83	<1.50	<0.997	<0.997	<0.997		
Site #16	Meadowdale Beach	532.6	40.9	13.4	2.3	<0.828	<0.828	<0.828		
Site #17	Budd Inlet, West Bay	368.3	36.9	3.7	2.2	<1.39	<1.34	<1.39		
Site #18	Seahurst	177.3	28.3	8.1	2.7	<1.21	<1.21	<1.22		
Site #19	Skiff Point	286.2	32.4	8.6	2.6	<2.12	<1.03	<1.03		
Site #21	Point Defiance Ferry	693.1	65.2	10.1	2.9	<1.56	<1.56	<1.51		
Site #22	Beach Dr. E	268.5	79.7	14.4	2.8	<2.19	<2.19	<2.19		
Site #23	Wing Point	3100.7	71.3	10.1	8.4	<1.45	<1.45	<1.45		
Site #24	S of Skunk Island	192.6	16.2	<2.10	<1.53	<1.53	<1.53	<1.53		
Site #25	Blair Waterway	643.4	37.1	30.0	10.2	<2.35	<1.71	<1.71		
Site #26	N of Illahee State Park	191.3	46.0	5.7	2.4	<1.65	<1.65	<1.65		
Site #27	Chuckanut, Clark's Point	275.2	10.1	16.6	2.5	<1.85	<1.85	<1.85		
Site #28	Oak Harbor	179.5	10.7	2.1	3.3	<2.12	<2.04	<2.04		
Site #29	Liberty Bay	599.3	51.3	14.5	3.1	<1.85	<1.85	<1.85		
Site #30	Kitsap St Boat Launch	747.1	157.2	26.1	5.8	<1.17	<1.17	<1.17		

		Concentrations in ng/g, dry weight (ppb)								
Site ID	Site Name	∑ <sub>38</sub> PAHs	TPCBs	∑ <sub>11</sub> PBDEs	∑₅ DDTs	∑ <sub>8</sub> Chlordanes	∑₃ HCHs	Dieldrin		
Site #31	Eastsound, Fishing Bay	1199.1	10.6	<1.79	<1.73	<0.974	<0.974	<0.974		
Site #35	Williams Olson Park	285.2	56.5	5.8	3.0	<0.924	<0.924	<1.91		
Site #37	Saltar's Point	198.8	36.2	6.0	2.4	<1.09	<1.09	<1.09		
Site #38	Rocky Point	285.0	82.1	7.7	3.4	<2.03	<1.04	<2.03		
Site #39	Smith Cove, Terminal 91	7349.0	236.0	21.9	50.4	5.1	2.8	3.0		
Site #42	Evergreen Rotary Park	521.7	112.3	12.3	6.2	<1.94	<1.94	<1.94		
Site #43	N Avenue Park	2135.3	23.7	7.7	4.6	<0.966	<1.82	<0.909		
Site #46	Appletree Cove	214.3	27.6	6.3	2.4	<1.13	<1.13	<1.13		
Site #47	Cherry Pt Aq Rsv, Birch Bay S	95.3	6.6	<1.99	<1.63	<1.03	<1.03	<1.03		
Site #48	Naketa Beach	551.6	32.4	9.9	2.9	<1.21	<1.21	<1.21		
Site #49	Donkey Creek Delta	668.3	125.2	6.0	3.6	<1.53	<1.53	<1.53		
Site #61	Dash Point Park	292.0	31.0	10.3	2.9	<1.59	<1.59	<1.59		
Site #161	Purdy, Dexters	242.0	32.8	<1.57	2.0	<1.46	<1.46	<1.46		
Site #353	Purdy, Nicholson	164.2	36.7	1.9	2.1	<1.53	<1.53	<1.53		
Site #481	Gig Harbor Boat Launch	399.0	55.0	2.3	2.3	<1.81	<1.81	<1.81		
Site #625	Gig Harbor, Mulligan	444.9	48.8	3.1	2.3	<1.48	<1.48	<1.48		
Site #697	Browns Point, Wolverton	540.0	62.9	20.9	10.4	2.1	<1.17	<1.17		
Site #953	Browns Point, Carlson	316.2	48.7	13.3	6.7	<1.92	<0.512	<0.512		
CPS_EF	Edmonds Ferry	666.1	42.9	13.5	3.2	<1.86	<0.959	<0.959		
CPS_HCV	Port Madison, Hidden Cove	892.0	91.2	3.2	3.3	<1.50	<1.50	<1.50		
CPS_MASO	Manchester, Stormwater Outfall	199.5	30.7	5.4	2.0	<1.70	<1.70	<1.64		
CPS_PNP	Point No Point	1450.2	29.6	6.0	2.6	<1.29	<1.29	<1.29		
CPS_QMH	Quartermaster Harbor	451.3	47.1	5.7	4.0	<2.07	<0.877	2.2		
CPS_SB	Salmon Bay	1800.8	182.5	39.2	45.7	14.9	<2.74	3.0		
CPS_SHLB	Shilshole Bay	2042.7	156.6	37.1	32.8	7.0	<0.926	<0.926		
CPS_SQSO	Suquamish, Stormwater Outfall	350.7	49.1	8.8	2.9	<1.21	<1.21	<1.21		
EB_ME	Elliott Bay, Myrtle Edwards	3822.0	197.5	22.0	16.7	<2.22	<1.34	<1.34		
HC_FP	Fisherman's Point	54.3	6.3	<1.97	<0.955	<0.955	<0.955	<0.955		
НС_НО	Hood Canal, Holly	48.8	7.7	<1.76	<1.56	<1.56	<1.56	<1.56		
NPS_BLSC	Bellingham Bay, Little Squalicum Creek	286.8	30.7	2.5	2.7	<1.16	<1.16	<1.16		
NPS_CPAR4	Cherry Pt Aq Rsv 4, Conoco Phillips	156.4	17.9	<2.12	1.9	<1.16	<1.16	<1.16		
NPS_DHCC	Drayton Harbor, California Creek	142.1	14.9	<2.05	2.4	<1.12	<1.12	<1.12		
NPS FBAR	Fidalgo Bay Ag Rsv, Weaverling Spit	307.3	15.1	2.0	3.1	<0.759	<0.759	<0.759		

		Concentrations in ng/g, dry weight (ppb)								
Site ID	Site Name	∑ <sub>38</sub> PAHs	TPCBs	∑ <sub>11</sub> PBDEs	∑ <sub>6</sub> DDTs	∑ <sub>8</sub> Chlordanes	∑₃ HCHs	Dieldrin		
SPS_HIAP	Hammersley Inlet, Arcadia Point	133.9	43.5	5.0	2.1	<1.15	<1.15	<1.15		
SPS_NRQR	Nisqually Rch Aq Rsv, Anderson Is	111.7	31.3	3.7	2.0	<0.802	<0.802	<0.802		
SPS_PBL	Purdy, Burley Lagoon	176.2	55.1	3.0	2.5	<1.37	<1.37	<1.37		
WB_CB	Cavalero Beach Co. Park	430.5	13.8	<2.45	<2.00	<1.34	<1.34	<1.34		
WB_KP	Kayak Point	224.5	18.0	6.1	2.6	<1.51	<1.51	<1.51		
WPS_IC	Illahee Creek	135.4	44.8	5.4	2.1	<1.15	<1.15	<1.15		
WPS_PB	Point Bolin	253.9	65.0	10.0	3.2	<1.12	<1.12	<1.12		
WPS_SVD	Sliverdale, Dyes Inlet	321.2	74.8	6.7	3.0	<1.27	<1.27	<2.09		

# Wet Weight Concentrations of Organic Contaminants

\* Mean of six replicate samples from Penn Cove, Whidbey Island aquaculture source of mussels (i.e., starting condition) < Indicates the concentration was not measured above the limit of quantitation (LOQ), which is the value reported in this case

	Site Name		Concentrations in ng/g, wet weight (ppb)								
Site iD	Site Name	∑ <sub>38</sub> PAHs	TPCBs	∑ <sub>11</sub> PBDEs	∑ <sub>6</sub> DDTs	∑ <sub>8</sub> Chlordanes	∑₃ HCHs	Dieldrin			
WB_PC	Baseline Site (n = 6)*	7.6	1.2	< 0.33	< 0.33	< 0.33	< 0.33	< 0.27			
Site #2	Arroyo Beach	47.1	6.8	2.0	0.6	< 0.33	< 0.13	< 0.13			
Site #3	Brackenwood Ln	64.0	4.7	1.1	0.3	< 0.16	< 0.15	< 0.15			
Site #4	Cherry Point North	19.7	1.0	< 0.33	< 0.27	< 0.16	< 0.16	< 0.16			
Site #5	Salmon Beach	36.4	5.0	1.1	0.3	< 0.14	< 0.14	< 0.14			
Site #6	Eagle Harbor Dr.	307.9	19.7	0.5	0.5	< 0.14	< 0.14	< 0.14			
Site #8	Chimacum Creek delta	15.0	2.0	< 0.33	< 0.19	< 0.19	< 0.19	< 0.19			
Site #10	Fletcher Bay, Fox Cove	41.8	9.0	1.0	0.5	< 0.15	< 0.15	< 0.14			
Site #11	South Bay Trail	51.3	3.0	1.1	0.4	< 0.15	< 0.15	< 0.15			
Site #13	Ruston Way	67.7	7.3	1.5	0.5	< 0.15	< 0.15	< 0.15			
Site #14	Point Heron East	31.1	10.3	1.3	0.4	< 0.16	< 0.16	< 0.16			
Site #15	Tugboat Park	34.7	1.9	< 0.33	< 0.27	< 0.18	< 0.18	< 0.18			
Site #16	Meadowdale Beach	83.6	6.4	2.1	0.4	< 0.13	< 0.13	< 0.13			
Site #17	Budd Inlet, West Bay	53.1	5.3	0.5	0.3	< 0.20	< 0.20	< 0.2			
Site #18	Seahurst	27.8	4.4	1.3	0.4	< 0.19	< 0.19	< 0.19			
Site #19	Skiff Point	44.5	5.0	1.3	0.4	< 0.16	< 0.16	< 0.16			
Site #21	Point Defiance Ferry	119.7	11.3	1.7	0.5	< 0.27	< 0.27	< 0.26			
Site #22	Beach Dr. E	40.5	12.0	2.2	0.4	< 0.33	< 0.33	< 0.33			
Site #23	Wing Point	578.6	13.3	1.9	1.6	< 0.27	< 0.27	< 0.27			
Site #24	S of Skunk Island	30.2	2.5	< 0.33	< 0.24	< 0.24	< 0.24	< 0.24			
Site #25	Blair Waterway	90.2	5.2	4.2	1.4	< 0.33	< 0.24	< 0.24			
Site #26	N of Illahee State Park	29.0	7.0	0.9	0.4	< 0.25	< 0.25	< 0.25			
Site #27	Chuckanut, Clark's Point	43.2	1.6	2.6	0.4	< 0.29	< 0.29	< 0.29			
Site #28	Oak Harbor	24.6	1.5	0.3	0.5	< 0.29	< 0.28	< 0.28			

	Site Name	Concentrations in ng/g, wet weight (ppb)								
Site iD		∑ <sub>38</sub> PAHs	TPCBs	∑ <sub>11</sub> PBDEs	∑ <sub>6</sub> DDTs	∑ <sub>8</sub> Chlordanes	∑₃ HCHs	Dieldrin		
Site #29	Liberty Bay	87.6	7.5	2.1	0.5	< 0.27	< 0.27	< 0.27		
Site #30	Kitsap St Boat Launch	114.7	24.1	4.0	0.9	< 0.18	< 0.18	< 0.18		
Site #31	Eastsound, Fishing Bay	221.6	2.0	< 0.33	< 0.32	< 0.18	< 0.18	< 0.18		
Site #35	Williams Olson Park	49.4	9.8	1.0	0.5	< 0.16	< 0.16	< 0.33		
Site #37	Saltar's Point	31.0	5.6	0.9	0.4	< 0.17	< 0.17	< 0.17		
Site #38	Rocky Point	46.4	13.4	1.3	0.6	< 0.33	< 0.17	< 0.33		
Site #39	Smith Cove, Terminal 91	1090.0	35.0	3.3	7.5	0.8	0.4	0.45		
Site #42	Evergreen Rotary Park	88.9	19.1	2.1	1.1	< 0.33	< 0.33	< 0.33		
Site #43	N Avenue Park	375.9	4.2	1.4	0.8	< 0.17	< 0.32	< 0.16		
Site #46	Appletree Cove	32.1	4.1	1.0	0.4	< 0.17	< 0.17	< 0.17		
Site #47	Cherry Point Aq Reserve, Birch Bay South	15.8	1.1	< 0.33	< 0.27	< 0.17	< 0.17	< 0.17		
Site #48	Naketa Beach	86.6	5.1	1.6	0.5	< 0.19	< 0.19	< 0.19		
Site #49	Donkey Creek Delta	104.5	19.6	0.9	0.6	< 0.24	< 0.24	< 0.24		
Site #61	Dash Point Park	47.6	5.1	1.7	0.5	< 0.26	< 0.26	< 0.26		
Site #161	Purdy, Dexters	41.5	5.6	< 0.27	0.3	< 0.25	< 0.25	< 0.25		
Site #353	Purdy, Nicholson	26.9	6.0	0.3	0.3	< 0.25	< 0.25	< 0.25		
Site #481	Gig Harbor Boat Launch	61.7	8.5	0.4	0.4	< 0.28	< 0.28	< 0.28		
Site #625	Gig Harbor, Mulligan	75.1	8.2	0.5	0.4	< 0.25	< 0.25	< 0.25		
Site #697	Browns Point, Wolverton	92.1	10.7	3.6	1.8	0.4	< 0.20	< 0.2		
Site #953	Browns Point, Carlson	54.4	8.4	2.3	1.2	0.3	< 0.088	< 0.088		
CPS_EF	Edmonds Ferry	118.1	7.6	2.4	0.6	< 0.33	< 0.17	< 0.17		
CPS_HCV	Port Madison, Hidden Cove	142.3	14.5	0.5	0.5	< 0.24	< 0.24	< 0.24		
CPS_MASO	Manchester, Stormwater Outfall	31.7	4.9	0.9	0.3	< 0.27	< 0.27	< 0.26		
CPS_PNP	Point No Point	235.9	4.8	1.0	0.4	< 0.21	< 0.21	< 0.21		
CPS_QMH	Quartermaster Harbor	72.1	7.5	0.9	0.6	< 0.33	< 0.14	0.35		
CPS_SB	Salmon Bay	210.0	21.3	4.6	5.3	1.7	< 0.32	0.35		
CPS_SHLB	Shilshole Bay	242.6	18.6	4.4	3.9	0.8	< 0.11	< 0.11		
CPS_SQSO	Suquamish, Stormwater Outfall	60.9	8.5	1.5	0.5	< 0.21	< 0.21	< 0.21		
EB_ME	Elliott Bay, Myrtle Edwards	568.6	29.4	3.3	2.5	< 0.33	< 0.20	< 0.2		
HC_FP	Fisherman's Point	9.1	1.1	< 0.33	< 0.16	< 0.16	< 0.16	< 0.16		

	Site Name	Concentrations in ng/g, wet weight (ppb)								
Site iD	Site Name	∑ <sub>38</sub> PAHs	TPCBs	∑ <sub>11</sub> PBDEs	∑ <sub>6</sub> DDTs	∑ <sub>8</sub> Chlordanes	∑₃ HCHs	Dieldrin		
HC_HO	Hood Canal, Holly	7.5	1.2	< 0.27	< 0.24	< 0.24	< 0.24	< 0.24		
NPS_BLSC	Bellingham Bay, Little Squalicum Creek	42.1	4.5	0.4	0.4	< 0.17	< 0.17	< 0.17		
NPS_CPAR4	Cherry Pt Aq Res 4, Conoco Phillips	24.3	2.8	< 0.33	0.3	< 0.18	< 0.18	< 0.18		
NPS_DHCC	Drayton Harbor, California Creek	22.9	2.4	< 0.33	0.4	< 0.18	< 0.18	< 0.18		
NPS_FBAR	Fidalgo Bay Aq Reserve, Weaverling Spit	48.6	2.4	0.3	0.5	< 0.12	< 0.12	< 0.12		
SPS_HIAP	Hammersley Inlet, Arcadia Point	22.1	7.2	0.8	0.4	< 0.19	< 0.19	< 0.19		
SPS_NRQR	Nisqually Rch Aq Rsv, Anderson Is	18.1	5.1	0.6	0.3	< 0.13	< 0.13	< 0.13		
SPS_PBL	Purdy, Burley Lagoon	25.7	8.0	0.4	0.4	< 0.2	< 0.2	< 0.2		
WB_CB	Cavalero Beach Co. Park	58.0	1.9	< 0.33	< 0.27	< 0.18	< 0.18	< 0.18		
WB_KP	Kayak Point	29.7	2.4	0.8	0.3	< 0.2	< 0.2	< 0.2		
WPS_IC	Illahee Creek	21.2	7.0	0.9	0.3	< 0.18	< 0.18	< 0.18		
WPS_PB	Point Bolin	40.7	10.4	1.6	0.5	< 0.18	< 0.18	< 0.18		
WPS_SVD	Sliverdale, Dyes Inlet	50.6	11.8	1.1	0.5	< 0.2	< 0.2	< 0.33		

# Appendix 3: Concentrations of Metals in Mussels by Site

# Dry Weight Concentrations of Metals

\* Mean of six replicate samples from Penn Cove, Whidbey Island aquaculture source of mussels (i.e., starting condition) < Indicates the concentration was not measured above the reporting detection limit (RDL), which is the value reported in this case

	Site Name	Concentrations in mg/kg, dry weight (ppm)								
Site ID		Mercury	Arsenic	Cadmium	Copper	Lead	Zinc			
WB_PC	Baseline Site (n = 6)*	0.0440	6.14	1.71	7.60	0.34	84.3			
Site #2	Arroyo Beach	0.0533	7.89	1.95	7.95	0.30	97.0			
Site #3	Brackenwood Ln	0.0559	7.35	1.96	6.71	0.29	85.7			
Site #4	Salmon Beach	0.0404	6.87	1.56	6.56	0.32	85.3			
Site #5	Eagle Harbor Dr.	0.0578	6.78	1.66	8.77	0.98	97.1			
Site #6	Fletcher Bay, Fox Cove	0.0424	6.63	1.70	8.31	0.51	91.0			
Site #8	South Bay Trail	0.0501	6.88	2.14	8.79	0.92	97.1			
Site #10	Ruston Way	0.0543	6.95	1.71	7.53	0.61	103.2			
Site #11	Point Heron East	0.0423	6.49	1.57	12.63	0.48	97.1			
Site #13	Tugboat Park	0.0374	6.25	1.45	6.52	0.25	90.8			
Site #14	Meadowdale Beach	0.0501	6.63	1.76	8.69	0.31	89.4			
Site #15	Budd Inlet, West Bay	0.0432	7.41	1.73	7.69	0.33	100.7			
Site #16	Seahurst	0.0427	6.62	1.74	8.03	0.27	87.9			
Site #17	Skiff Point	0.0475	7.01	1.83	7.27	0.38	96.8			
Site #18	Point Defiance Ferry	0.0491	7.49	1.62	9.65	0.68	121.6			
Site #19	Wing Point	0.0467	7.26	1.72	7.85	0.49	97.3			
Site #21	Blair Waterway	0.0433	6.79	2.04	8.18	0.39	86.9			
Site #22	Chuckanut, Clark's Point	0.0485	6.52	1.56	7.48	0.24	91.6			
Site #23	Oak Harbor	0.0540	7.29	1.71	9.85	0.22	77.2			
Site #24	Liberty Bay	0.0482	6.29	1.67	8.29	0.56	99.3			
Site #25	Kitsap St Boat Launch	0.0516	6.96	1.67	10.47	0.67	106.1			
Site #26	Williams Olson Park	0.0358	7.01	1.64	7.96	0.47	98.8			
Site #27	Saltar's Point	0.0401	6.05	1.66	6.60	0.28	82.4			
Site #28	Smith Cove, Terminal 91	0.0493	6.33	1.60	9.66	0.70	99.3			
Site #29	Evergreen Rotary Park	0.0536	6.53	1.69	7.00	0.69	95.3			

Sita ID	Site Name -	Concentrations in mg/kg, dry weight (ppm)								
Site iD		Mercury	Arsenic	Cadmium	Copper	Lead	Zinc			
Site #30	N Avenue Park	0.0375	5.54	1.49	6.78	0.44	79.5			
Site #31	Naketa Beach	0.0496	6.56	1.61	7.64	0.25	85.4			
Site #35	Donkey Creek Delta	0.0375	6.84	1.59	9.37	0.82	109.5			
Site #37	Cherry Point North	0.0371	5.94	1.89	7.23	0.27	93.7			
Site #38	Chimacum Creek delta	0.0480	6.67	1.56	6.79	0.31	89.9			
Site #39	Beach Dr. E	0.0542	7.33	1.83	8.73	0.59	104.0			
Site #42	S of Skunk Island	0.0466	6.75	1.78	7.90	0.80	93.6			
Site #43	N of Illahee State Park	0.0416	6.32	1.81	7.71	0.36	90.2			
Site #46	Eastsound, Fishing Bay	0.0317	6.63	1.29	5.75	0.35	76.2			
Site #47	Rocky Point	0.0497	6.96	1.46	8.51	0.50	88.8			
Site #48	Appletree Cove	0.0532	6.78	2.12	6.29	0.21	86.3			
Site #49	Cherry Pt Aq Rsv, Birch Bay S	0.0346	6.69	1.77	6.27	0.21	94.6			
Site #61	Dash Point Park	0.0157	4.98	1.60	4.12	<0.089	54.4			
Site #161	Purdy, Dexters	0.0148	4.77	1.38	3.51	<0.090	48.2			
Site #353	Purdy, Nicholson	0.0167	5.07	1.47	3.75	<0.087	50.7			
Site #481	Gig Harbor Boat Launch	0.0156	4.91	1.56	3.52	<0.091	49.5			
Site #625	Gig Harbor, Mulligan	0.0201	6.51	1.60	4.96	<0.102	66.7			
Site #697	Browns Point, Wolverton	0.0151	4.81	1.86	4.25	<0.091	47.2			
Site #953	Browns Point, Carlson	0.0443	6.51	1.82	6.20	0.26	75.3			
CPS_EF	Edmonds Ferry	0.0426	6.50	1.64	7.88	0.33	85.6			
CPS_HCV	Port Madison, Hidden Cove	0.0472	5.82	1.64	6.12	0.27	74.5			
CPS_MASO	Manchester, Stormwater Outfall	0.0657	7.16	1.77	12.69	0.78	81.5			
CPS_PNP	Point No Point	0.0633	6.56	1.89	9.14	0.48	95.4			
CPS_QMH	Quartermaster Harbor	0.0459	6.05	1.94	6.46	0.29	79.5			
CPS_SB	Salmon Bay	0.0525	7.53	1.52	8.20	0.33	87.1			
CPS_SHLB	Shilshole Bay	0.0471	6.21	1.84	9.22	<0.127	68.6			
CPS_SQSO	Suquamish, Stormwater Outfall	0.0465	6.54	1.92	8.01	0.37	70.5			
EB_ME	Elliott Bay, Myrtle Edwards	0.0571	7.12	1.83	7.31	0.58	86.5			
HC_FP	Fisherman's Point	0.0384	7.61	1.63	8.34	0.67	79.8			
HC_HO	Hood Canal, Holly	0.0525	6.95	1.78	7.01	0.25	67.7			
NPS_BLSC	Bellingham Bay, Little Squalicum Creek	0.0842	9.45	1.95	8.36	0.99	91.1			
NPS_CPAR4	Cherry Pt Aq Rsv 4, Conoco Phillips	0.0607	6.93	1.64	12.21	0.65	71.2			

	Site Name	Concentrations in mg/kg, dry weight (ppm)							
Site ID		Mercury	Arsenic	Cadmium	Copper	Lead	Zinc		
NPS_DHCC	Drayton Harbor, California Creek	0.0324	7.02	1.98	5.82	<0.118	62.0		
NPS_FBAR	Fidalgo Bay Aq Rsv, Weaverling Spit	0.0427	6.16	1.70	6.75	0.23	70.0		
SPS_HIAP	Hammersley Inlet, Arcadia Point	0.0373	6.94	1.69	7.18	0.21	72.9		
SPS_NRQR	Nisqually Rch Aq Rsv, Anderson Is	0.0443	7.99	1.69	7.32	0.40	74.4		
SPS_PBL	Purdy, Burley Lagoon	0.0374	6.32	2.11	7.53	0.22	78.7		
WB_CB	Cavalero Beach Co. Park	0.0517	8.57	2.01	7.07	0.31	91.8		
WB_KP	Kayak Point	0.0526	6.57	1.56	6.40	0.36	77.5		
WPS_IC	Illahee Creek	0.0354	5.98	1.70	6.62	0.24	68.8		
WPS_PB	Point Bolin	0.0406	5.96	1.73	7.34	0.18	69.6		
WPS_SVD	Sliverdale, Dyes Inlet	0.0511	6.44	1.60	8.95	0.24	66.6		
### Wet Weight Concentrations of Metals

\* Mean of six replicate samples from Penn Cove, Whidbey Island aquaculture source of mussels (i.e., starting condition) < Indicates the concentration was not measured above the reporting detection limit (RDL), which is the value reported in this case

Site ID	Site Name	Concentrations in mg/kg, wet weight (ppm)						
		Mercury	Arsenic	Cadmium	Copper	Lead	Zinc	
WB_PC	Baseline Site (n = 6)*	0.0074	1.03	0.29	1.27	0.06	14.1	
Site #2	Arroyo Beach	0.0088	1.31	0.32	1.32	0.05	16.1	
Site #3	Brackenwood Ln	0.0082	1.08	0.29	0.99	0.04	12.6	
Site #4	Cherry Point North	0.0059	0.94	0.30	1.15	0.04	14.9	
Site #5	Salmon Beach	0.0066	1.12	0.26	1.07	0.05	13.9	
Site #6	Eagle Harbor Dr.	0.0099	1.16	0.28	1.50	0.17	16.6	
Site #8	Chimacum Creek delta	0.0076	1.06	0.25	1.08	0.05	14.3	
Site #10	Fletcher Bay, Fox Cove	0.0070	1.10	0.28	1.38	0.08	15.1	
Site #11	South Bay Trail	0.0070	0.96	0.30	1.23	0.13	13.6	
Site #13	Ruston Way	0.0084	1.07	0.26	1.16	0.09	15.9	
Site #14	Point Heron East	0.0072	1.11	0.27	2.16	0.08	16.6	
Site #15	Tugboat Park	0.0069	1.15	0.27	1.20	0.05	16.7	
Site #16	Meadowdale Beach	0.0080	1.06	0.28	1.39	0.05	14.3	
Site #17	Budd Inlet, West Bay	0.0062	1.06	0.25	1.10	0.05	14.4	
Site #18	Seahurst	0.0067	1.04	0.27	1.26	0.04	13.8	
Site #19	Skiff Point	0.0073	1.08	0.28	1.12	0.06	14.9	
Site #21	Point Defiance Ferry	0.0084	1.28	0.28	1.65	0.12	20.8	
Site #22	Beach Dr. E	0.0081	1.10	0.28	1.31	0.09	15.6	
Site #23	Wing Point	0.0087	1.35	0.32	1.46	0.09	18.1	
Site #24	S of Skunk Island	0.0073	1.06	0.28	1.24	0.13	14.7	
Site #25	Blair Waterway	0.0059	0.93	0.28	1.12	0.05	11.9	
Site #26	N of Illahee State Park	0.0064	0.97	0.28	1.18	0.06	13.8	
Site #27	Chuckanut, Clark's Point	0.0075	1.01	0.24	1.16	0.04	14.2	
Site #28	Oak Harbor	0.0073	0.99	0.23	1.34	0.03	10.5	
Site #29	Liberty Bay	0.0070	0.92	0.24	1.21	0.08	14.5	
Site #30	Kitsap St Boat Launch	0.0076	1.03	0.25	1.55	0.10	15.7	

Site ID	Site Name –	Concentrations in mg/kg, wet weight (ppm)						
		Mercury	Arsenic	Cadmium	Copper	Lead	Zinc	
Site #31	Eastsound, Fishing Bay	0.0057	1.20	0.23	1.04	0.06	13.8	
Site #35	Williams Olson Park	0.0060	1.17	0.27	1.33	0.08	16.5	
Site #37	Saltar's Point	0.0061	0.93	0.25	1.01	0.04	12.6	
Site #38	Rocky Point	0.0080	1.12	0.24	1.37	0.08	14.3	
Site #39	Smith Cove, Terminal 91	0.0073	0.94	0.24	1.43	0.10	14.7	
Site #42	Evergreen Rotary Park	0.0091	1.11	0.29	1.19	0.12	16.2	
Site #43	N Avenue Park	0.0064	0.95	0.26	1.16	0.08	13.6	
Site #46	Appletree Cove	0.0078	0.99	0.31	0.92	0.03	12.6	
	Cherry Point Aq Reserve, Birch Bay							
Site #47	South	0.0057	1.11	0.29	1.04	0.03	15.7	
Site #48	Naketa Beach	0.0078	1.03	0.25	1.20	0.04	13.4	
Site #49	Donkey Creek Delta	0.0059	1.08	0.25	1.48	0.13	17.3	
Site #61	Dash Point Park	0.0034	1.08	0.35	0.89	< 0.0194	11.8	
Site #161	Purdy, Dexters	0.0032	1.04	0.30	0.77	< 0.0197	10.5	
Site #353	Purdy, Nicholson	0.0037	1.12	0.32	0.83	< 0.0193	11.2	
Site #481	Gig Harbor Boat Launch	0.0034	1.07	0.34	0.77	< 0.0198	10.8	
Site #625	Gig Harbor, Mulligan	0.0039	1.25	0.31	0.95	< 0.0195	12.8	
Site #697	Browns Point, Wolverton	0.0033	1.04	0.40	0.92	< 0.0197	10.2	
Site #953	Browns Point, Carlson	0.0074	1.08	0.30	1.03	0.04	12.5	
CPS_EF	Edmonds Ferry	0.0068	1.04	0.26	1.26	0.05	13.7	
CPS_HCV	Port Madison, Hidden Cove	0.0078	0.96	0.27	1.01	0.05	12.3	
CPS_MASO	Manchester, Stormwater Outfall	0.0085	0.93	0.23	1.65	0.10	10.6	
CPS_PNP	Point No Point	0.0096	0.99	0.29	1.38	0.07	14.4	
CPS_QMH	Quartermaster Harbor	0.0074	0.97	0.31	1.04	0.05	12.8	
CPS_SB	Salmon Bay	0.0094	1.34	0.27	1.46	0.06	15.5	
CPS_SHLB	Shilshole Bay	0.0072	0.95	0.28	1.41	< 0.0194	10.5	
CPS_SQSO	Suquamish, Stormwater Outfall	0.0073	1.02	0.30	1.25	0.06	11.0	
EB_ME	Elliott Bay, Myrtle Edwards	0.0089	1.11	0.29	1.14	0.09	13.5	
HC_FP	Fisherman's Point	0.0063	1.24	0.27	1.36	0.11	13.0	
HC_HO	Hood Canal, Holly	0.0088	1.16	0.30	1.17	0.04	11.3	
NPS_BLSC	Bellingham Bay, Little Squalicum Creek	0.0123	1.38	0.29	1.22	0.14	13.3	
NPS_CPAR4	Cherry Pt Aq Res 4, Conoco Phillips	0.0074	0.85	0.20	1.49	0.08	8.7	

Site ID	Site Name	Concentrations in mg/kg, wet weight (ppm)						
		Mercury	Arsenic	Cadmium	Copper	Lead	Zinc	
NPS_DHCC	Drayton Harbor, California Creek	0.0055	1.20	0.34	1.00	< 0.0202	10.6	
	Fidalgo Bay Aq Reserve, Weaverling							
NPS_FBAR	Spit	0.0068	0.99	0.27	1.08	0.04	11.2	
SPS_HIAP	Hammersley Inlet, Arcadia Point	0.0063	1.18	0.29	1.22	0.04	12.4	
SPS_NRQR	Nisqually Rch Aq Rsv, Anderson Is	0.0073	1.31	0.28	1.20	0.07	12.2	
SPS_PBL	Purdy, Burley Lagoon	0.0056	0.95	0.32	1.13	0.03	11.8	
WB_CB	Cavalero Beach Co. Park	0.0076	1.26	0.30	1.04	0.05	13.5	
WB_KP	Kayak Point	0.0094	1.17	0.28	1.14	0.06	13.8	
WPS_IC	Illahee Creek	0.0056	0.94	0.27	1.04	0.04	10.8	
WPS_PB	Point Bolin	0.0055	0.81	0.24	1.00	0.02	9.5	
WPS_SVD	Sliverdale, Dyes Inlet	0.0068	0.86	0.21	1.19	0.03	8.9	

## Appendix 4: Table of Mortality and Condition Index Data by Site

Mortality and condition index of mussels from nearshore monitoring sites

\* Mean of six replicate samples from Penn Cove, Whidbey Island aquaculture source of mussels (i.e., starting condition)

NA indicates data not available

Site ID	Site Name	Condition Index (CI) n = 12 (unless otherwise indicated)	Mortality (%) n = 64
WB_PC	Baseline Site (n = 6)*	3.15 (n = 70)	NA
2	Arroyo Beach	NA	20.00
3	Brackenwood Ln	2.12	27.69
4	Cherry Point North	2.23 (n = 10)	31.75
6	Eagle Harbor Dr.	2.42	15.63
8	Chimacum Creek delta	2.27	14.06
10	Fletcher Bay, Fox Cove	1.92	19.05
15	Tugboat Park	2.45	18.75
17	Budd Inlet, West Bay	1.97 (n = 10)	37.50
22	Beach Dr. E	1.83	25.00
23	Wing Point	1.90	19.05
25	Blair Waterway	1.88 (n = 11)	25.40
27	Chuckanut, Clark's Point	1.95 (n = 10)	31.75
31	Eastsound, Fishing Bay	2.32	12.50
42	Evergreen Rotary Park	2.42	29.69
46	Appletree Cove	1.84 (n = 11)	28.13
47	Cherry Point Aq Reserve, Birch Bay South	1.85	12.50
49	Donkey Creek Delta	2.59 (n = 4)	43.75
5	Salmon Beach	2.15 (n = 10)	32.81
11	South Bay Trail	1.83	26.56
13	Ruston Way	1.93 (n = 9)	34.92
14	Point Heron East	2.10	26.56
16	Meadowdale Beach	2.08	31.75
18	Seahurst	2.17 (n = 10)	32.81
19	Skiff Point	2.11	15.63
21	Point Defiance Ferry	1.92 (n = 11)	23.33
24	S of Skunk Island	2.06	16.13
26	N of Illahee State Park	2.29 (n = 7)	39.06
28	Oak Harbor	1.96	14.06
29	Liberty Bay	2.51	28.13
30	Kitsap St Boat Launch	2.06	23.81

Site ID	Site Name	Condition Index (CI) n = 12 (unless otherwise indicated)	Mortality (%) n = 64	
35	Williams Olson Park	2.49 (n = 10)	31.25	
37	Saltar's Point	1.71 (n = 10)	26.56	
38	Rocky Point	2.28	18.75	
39	Smith Cove, Terminal 91	1.82 (n = 11)	20.63	
43	N Avenue Park	2.17	23.44	
48	Naketa Beach	2.14	18.75	
61	Dash Point Park	2.33	13.11	
161	Purdy, Dexters	1.84	14.06	
353	Purdy, Nicholson	2.36	19.35	
481	Gig Harbor Boat Launch	1.96	7.81	
625	Gig Harbor, Mulligan	1.98	29.69	
697	Browns Point, Wolverton	1.89	19.05	
953	Browns Point, Carlson	1.74	25.81	
CPS_EF	Edmonds Ferry	1.80	16.13	
CPS_HCV	Port Madison, Hidden Cove	2.17 (n = 8)	15.63	
CPS_MASO	Manchester, Stormwater Outfall	1.97	7.81	
CPS_PNP	Point No Point	2.11 (n = 10)	19.05	
CPS_QMH	Quartermaster Harbor	2.14 (n = 11)	22.58	
CPS_SB	Salmon Bay	2.08	12.50	
CPS_SHLB	Shilshole Bay	2.16 (n = 10)	23.44	
CPS_SQSO	Suquamish, Stormwater Outfall	2.23	20.31	
EB_ME	Elliott Bay, Myrtle Edwards	1.88	12.28	
HC_FP	Fisherman's Point	2.33	7.81	
HC_HO	Hood Canal, Holly	2.01	17.74	
NPS_BLSC	Bellingham Bay, Little Squalicum Creek	2.75	29.69	
NPS_CPAR4	Cherry Pt Aq Res 4, Conoco Phillips	2.13	25.40	
NPS_DHCC	Drayton Harbor, California Creek	2.28 (n = 11)	25.81	
NPS_FBAR	Fidalgo Bay Aq Reserve, Weaverling Spit	2.22 (n =1 1)	21.88	
SPS_HIAP	Hammersley Inlet, Arcadia Point	2.08 (n =10)	31.25	
SPS_NRQR	Nisqually Rch Aq Rsv, Anderson Is	1.89	15.63	
SPS_PBL	Purdy, Burley Lagoon	2.06	26.56	
WB_CB	Cavalero Beach Co. Park	1.94	10.94	
WB_KP	Kayak Point	1.95	9.38	
WPS_IC	Illahee Creek	2.18	26.09	
WPS_PB	Point Bolin	2.60	26.56	
WPS_SVD	Sliverdale, Dyes Inlet	2.20	14.06	











## Appendix 7: PBDEs Cumulative Frequency Distribution Plots







# Appendix 8: DDTs Cumulative Frequency Distribution Plots

107



Percent

## Appendix 9: Mercury Cumulative Frequency Distribution Plots





## Appendix 10: Arsenic Cumulative Frequency Distribution Plots

109



## Appendix 11: Cadmium Cumulative Frequency Distribution Plots

Percent



# Appendix 12: Copper Cumulative Frequency Distribution Plots



# Appendix 13: Lead Cumulative Frequency Distribution Plots



# Appendix 14: Zinc Cumulative Frequency Distribution Plots

## Appendix 15: Laboratory Data Quality Review

### Organohalogens

Surrogate recoveries for the whole body mussel samples were within the guidelines detailed in the laboratory's Quality Assurance Plan (QAP; Sloan et al. 2006). Standard Reference Material (SRM) analyses also indicated that results met the criteria in the QAPP for all sample sets. A method blank was analyzed for persistent organic pollutants (POPs) with each sample set and laboratory QAP for method blanks were met. In addition, continuing calibration verification standards were analyzed at the start, middle, and end of the GC/MS analytical sequence and all of the results met the continuing calibration criteria detailed in the "Quality Assurance Plan for Analyses of Environmental Samples for Polycyclic Aromatic Compounds, Persistent Organic Pollutants, Fatty Acids, Stable Isotope Ratios, Lipid Classes, and Metabolites of Polycyclic Aromatic Compounds," by Sloan et al. 2006.

### Polycyclic Aromatic Hydrocarbons

Surrogate recoveries for whole body mussel samples and all quality assurance samples (method blanks and NIST SRM 1974c) associated with the analyses of these samples were within the guidelines detailed in the QAP (Sloan et al. 2006). SRM analyses also indicated that results met the criteria in the QAPP for all sample sets. However, the case narrative noted inadequate performance of one of the C2-naphthalenes [2,6-dimethylnaphthalene (DMN)], because they measured variable concentration of this analyte at 18 to 43 times higher than the NIST reference value, indicating a high bias. As a result of the high bias for that analyte, the laboratory recommend that for each field sample, the concentration of DMN reported in the sample be subtracted from the concentration reported for C2-NPH. We followed the recommendations of the laboratory in this regard.

A method blank was analyzed for PAHs with each sample set and laboratory QAP (Sloan et al. 2006) for method blanks were met. Continuing calibration verification standards were analyzed at the start, middle, and end of the GC/MS analytical sequence and all of the results met the continuing calibration criteria detailed in the "Quality Assurance Plan for Analyses of Environmental Samples for Polycyclic Aromatic Compounds, Persistent Organic Pollutants, Fatty Acids, Stable Isotope Ratios, Lipid Classes, and Metabolites of Polycyclic Aromatic Compounds," by Sloan et al. 2006.

A number of PAH analyte values were censored with an "i", which indicated an interference and that the concentration should be considered an overestimate because one (or more) significant peak(s) within the elution range of the homolog group had a retention time that did not match those in a known PAH pattern.

#### Data Censorship

We applied the censorship steps outlined below to the raw laboratory organohalogen data. A sample run usually included 12 samples.

- 1. If a method blank for a sample run had a detected value, then any sample value less than three times the detected blank was replaced with the applicable limit of quantitation (LOQ) for the run.
- 2. If a detected value was less than or equal to the highest LOQ for the analyte, then that value was replaced with the highest LOQ value for the run.

3. Dry weight values were calculated from the wet weight values after the above steps were completed.

We applied the censorship steps outlined below to the raw laboratory PAH data:

- 1. 2,6-dimethylnaphthalene (DMN) was subtracted from the C2-naphthalenes (C2NPH).
- 2. If a method blank for a sample run had a detected value, then any sample value less than three times the detected blank was replaced with the applicable limit of quantitation (LOQ) for the run.
- 3. If a detected value was less than or equal to the highest LOQ for the analyte, then that value was replaced with the highest LOQ value for the run.
- 4. Any analyte that had more than 18% "i" flags (i.e., overestimates) in the dataset were deleted; this resulted in the deletion of C4-naphthalenes (C4NPH), C3-fluorenes (C3FLU), C3-phenanthrenes (C3PHN), and C1-chrysenes (C1CHR) from the dataset.
- 5. Dry weight values were calculated from the wet weight values after the above steps were completed.

The limit of quantitation (LOQ) for most organic contaminants fell within expected ranges; for details see the QAPP for Status and Trends Monitoring of Marine Nearshore Mussels, for the Regional Stormwater Monitoring Program and Pierce County (June 2015). All metals with the exception of lead were detected above the reporting detection limit (RDL). As mentioned in the Contaminant Concentrations section of <u>Appendix 1</u>, the summed analytes used in this study are the sum of all detected values, with zeroes substituted for non-detected (<LOQ) analytes, within each group. In cases where all analytes in a group were not detected the greatest LOQ for all the analytes in the group was used as the summation concentration, and the value was preceded by a "<" (less than) qualifier to indicate it was not detected. In most cases summed totals were dominated by substantial concentrations of a number of individual analytes, thus substituting zero for non-detects did not substantially alter comparison results for the summed analytes.



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