Toxic contaminants in bay mussels (*Mytilus trossulus*) transplanted to Port Gamble Bay, Washington before cleanup and restoration (2015-2017)

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1.0 Introduction and Background

Port Gamble Bay is located in Washington State on the north end of the Kitsap Peninsula. It is approximately two square miles in size and generally shallow, with depths up to 60 feet, and is connected at its north end to the Hood Canal. The former Pope & Talbot Inc. sawmill is located along the western shoreline of the mouth of Port Gamble Bay and manufactured forest products there from 1853 to 1995. Historic mill operations at Pope & Talbot Inc. included wood chipping, log rafting, wood burning, and storage activities, and resulted in contamination of Port Gamble Bay. Contaminants of most concern related to human health from these activities include carcinogenic polycyclic aromatic hydrocarbons (cPAHs), polychlorinated biphenyls (PCBs), dioxins/furans and metals (arsenic, cadmium, copper, and mercury). The Washington State Department of Ecology (Ecology) is currently managing a cleanup of contaminated sediments in the Port Gamble Bay, including removal of thousands of existing creosote-treated pilings, dredging of contaminated sediments, and capping with clean sediments employed as source control measures (Washington Department of Ecology, 2013).

In a study in 2000, caged mussels (*Mytilus galloprovincialis*) were deployed in Port Gamble Bay as part of a larger study to provide a Puget Sound-wide perspective on PAH exposures in documented herring spawning grounds (Applied Biomonitoring, 2002). Mussels were transplanted to two sites in Port Gamble, near the Saw Mill and at Little Boston Harbor, and at one site outside the bay at Teek Bluff for a two month period (April – June). At the end of the study the PAH tissue burdens in mussels were assessed. The Port Gamble mussels, especially those placed near the Saw Mill, showed consistently high PAH signals compared to most of the other sites and exhibited end-of-test total PAHs that were significantly higher than the beginning-of-test concentrations: Saw Mill mean = 427.03 µg/kg dry weight (dw) vs. T_0 mean = 87.18 µg/kg dw (Applied Biomonitoring, 2002). The authors concluded that more studies were needed in Port Gamble to determine if "bioavailable PAHs are affecting herring egg development".

The focus on potential effects of PAHs on herring egg development is linked to the fact that unusually high rates of herring embryo mortality (>20%) have been observed in Port Gamble Bay since the early 1980s (WDFW, unpublished data). Since that time efforts have been made to assess whether herring embryo mortality is linked to contamination (Kocan, 1987), and to compare PAH concentrations and herring embryo health with other spawning stocks (Hershberger et. al., 2005). These concerns are further motivated by the fact that prior to 2000, the Port Gamble Bay herring stock was considered one of the larger spawning stocks in Puget Sound, and since that time its spawning biomass has steadily declined from 2,459 tons to 208 tons in 2008 (Stick and Lindquist, 2009).

The most recent examination of PAHs in Port Gamble Bay mussels was conducted as part of a larger study in the winter of 2012-2013. In that study, mussels (*M. trossulus*) were transplanted to two

locations in Port Gamble Bay (near the previous Saw Mill site and across the bay at Point Julia). Although the 2012/13 mussels exhibited lower total PAHs (205.2 and 180.3 µg/kg dw for Port Gamble, West and Point Julia respectively; **Figure 1**) than the *M. galloprovincialis* mussels from the 2000 study, the PAH content of the Port Gamble Bay mussels at the end of the 2012/13 study were higher than the starting condition (71.36 µg/kg dw; **Figure 1**) of mussels for that study, suggesting a biologically available source of PAHs still exists within the bay (Figure 1; Lanksbury et al., 2014).

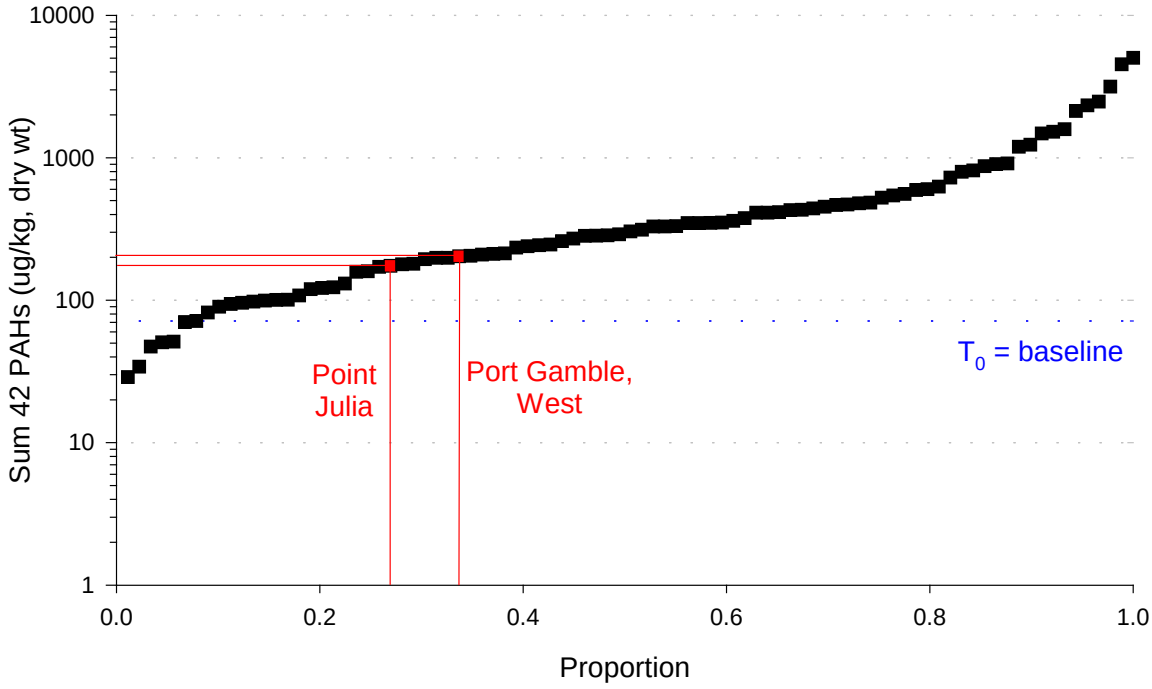

Figure 1. Cumulative frequency distribution of total PAHs in transplanted mussels from 89 sites in the Puget Sound sampled in 2013; adapted from Lanksbury et al. 2014. Values for the Port Gamble, West and Point Julia sites are color-coded in red.

Study purpose and objective

The purpose of this study was to synoptically evaluate the geographic extent and magnitude of contamination in bay mussels (*Mytilus trossulus*) during the winter of 2014-15, prior to a large-scale remediation project scheduled in Port Gamble Bay. The objective was to compare the extent, magnitude and pattern of contamination (specifically PAHs, PCBs, cadmium, and dioxins/furans) in *M. trossulus* mussels exposed to different locations within and outside of the Sediment Management Areas (SMAs; Figure 2) identified in the Port Gamble Bay Remedial Investigation (Ecology 2012). Specifically we will compare; 1) the extent of contamination across the bay before remediation, 2) the magnitude of contamination in the different areas, including a reference area, before remediation, and 3) the patterns of contamination (i.e. PAH analyte fingerprints) before remediation.

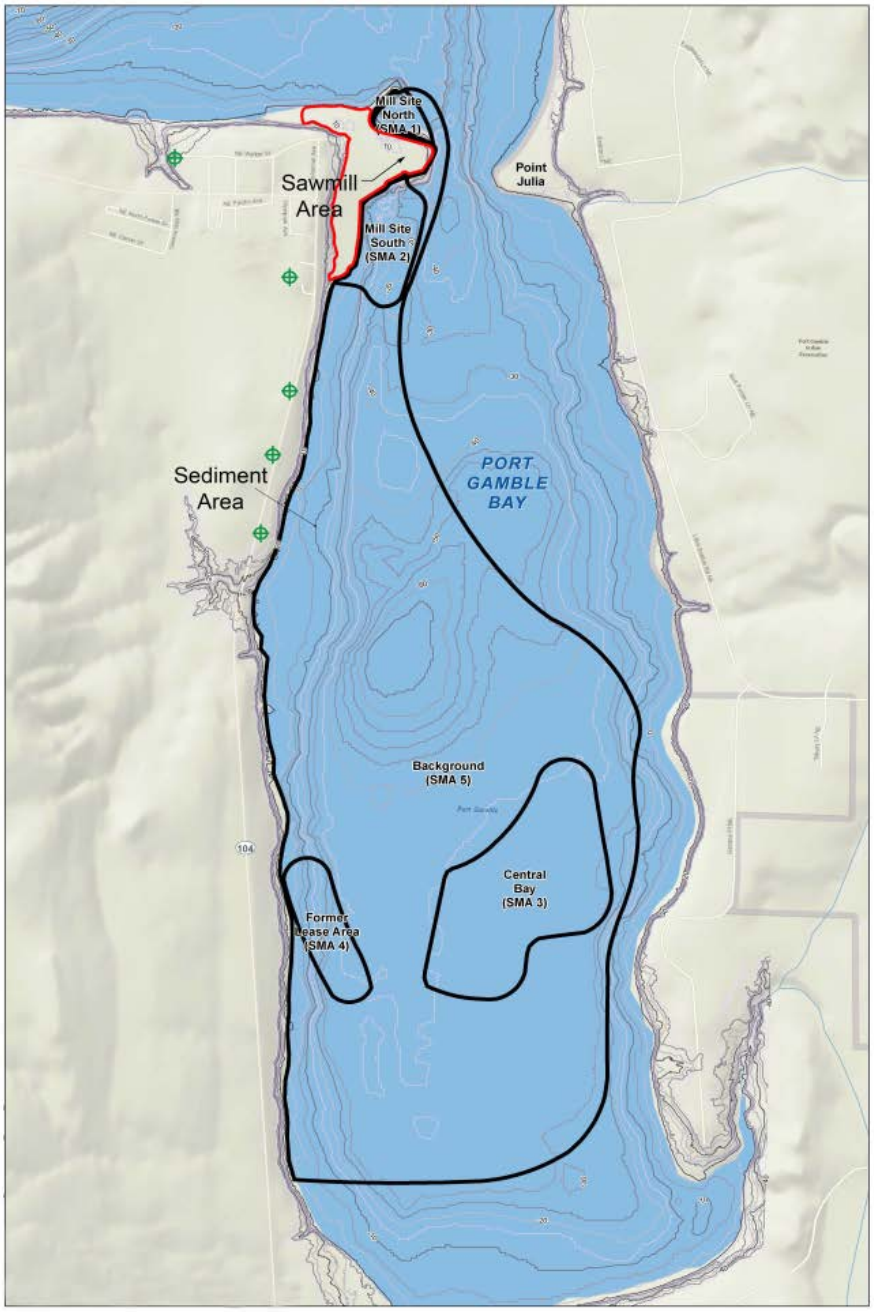

Figure 2. Five Sediment Management Areas (SMAs) identified by Ecology in the Final Cleanup Action Plan for Port Gamble Bay (2013).

2.0 Materials & Methods

Study Area and Site Selection

The Port Gamble Remedial Investigation (Ecology 2012) identified five SMAs based on similarity of contaminant types, toxicity of sediments, geography, and hydrology (Figure 2). Replicate mussel population units (i.e. mussel cages) were transplanted at or near these SMAs, where remediation actions will occur, as well as in non-SMA locations within the bay. The locations of most of the mussel cages overlapped the locations of Pacific herring (*Clupea pallasii*) egg cages placed previously in Port Gamble for a separate contaminant study (West, 2014).

Mussel cages were placed in seven sampling areas. Five of these areas were within the SMAs (see also Figure 3):

1. Mill Site North (MSN) in SMA-1,
2. Mill Site South (MSS) in SMA-2,
3. Central Bay (CB) in SMA-3,
4. Former Lease Area (FLA) in SMA-4,
5. Northwest Shore (NWS) in SMA-5.

Three of these locations were in non-SMA areas within Port Gamble Bay (Figure 3):

6. Head of Bay (HOB),
7. Southeast Shore (SES),
8. Northeast Shore (NES).

In addition, mussel cages were placed off of Hood Head (HH), which served as a reference area outside the bay (**Figure 4**).

The Port Gamble S'Klallam Tribe (PGST) was a partner in this study and provided assistance in the deployment and retrieval of the mussel cages. As a supplement to this study, the PGST placed extra mussel cages in areas of particular interest to the tribe, including both SMA and non-SMA areas in Port Gamble Bay. Therefore additional mussel cages were placed in SMA-5 (Figure 3):

1. Central Mill Site (CMS),
2. MSN Perimeter (MSN-P),
3. MSS Perimeter (MSS-P),
4. Dump Site Shoreline (DSS).

Four other non-SMA areas within Port Gamble Bay received mussel cages as well (Figure 3):

1. North of Jetty (NJ),

2. West of Jetty (WJ),
3. Point Julia North (PJN),
4. Point Julia South (PJS).

Due to the high cost of chemical analysis, mussel composites from a subset of the deployment locations (48 of 55) were analyzed for chemical content (Figure 3 and Figure 4, Table 6).

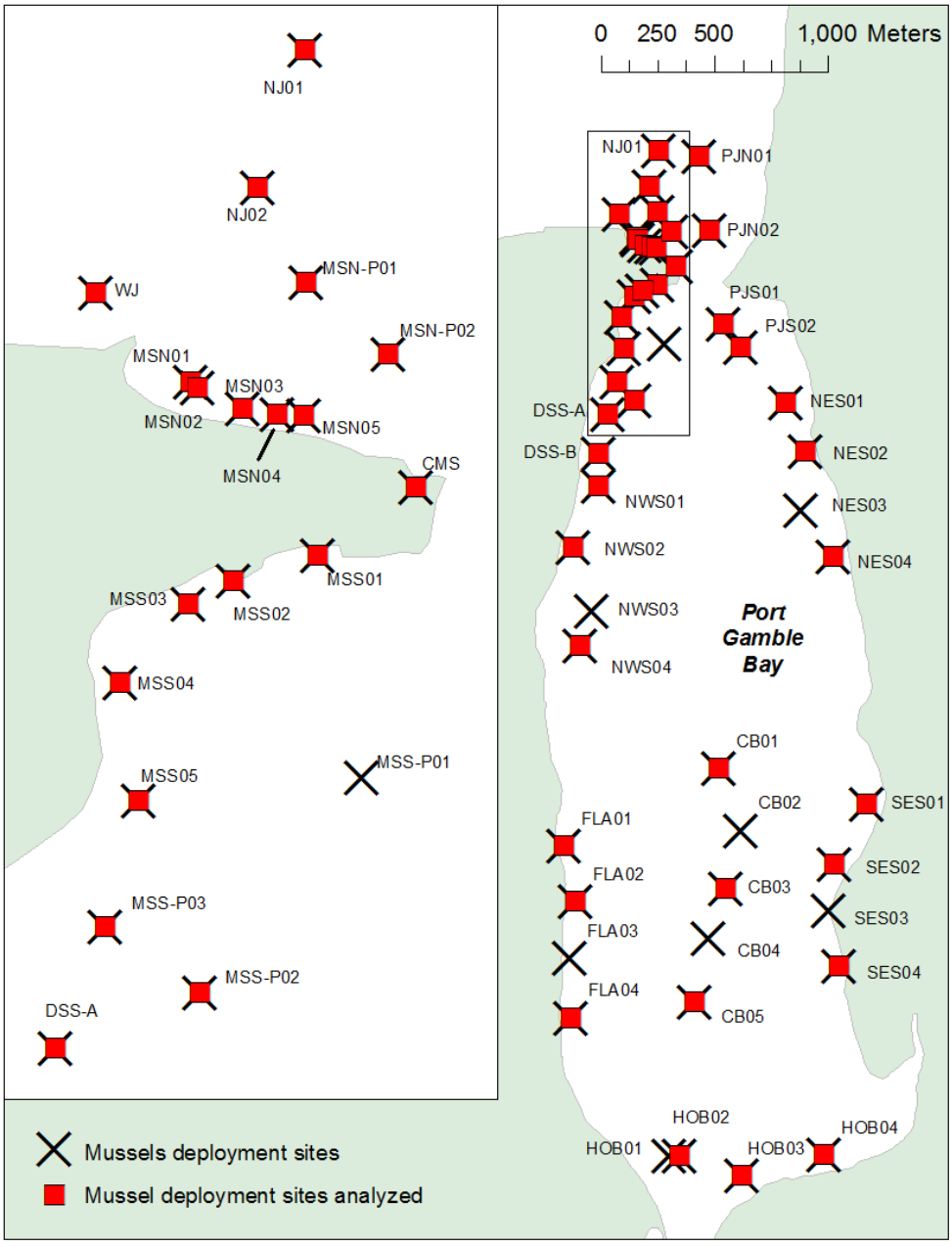

Figure 3. Locations of mussel monitoring cages deployed (X) and mussel composites analyzed for chemicals (red squares) in Port Gamble Bay, Washington. Acronyms for sites listed in Table 6.

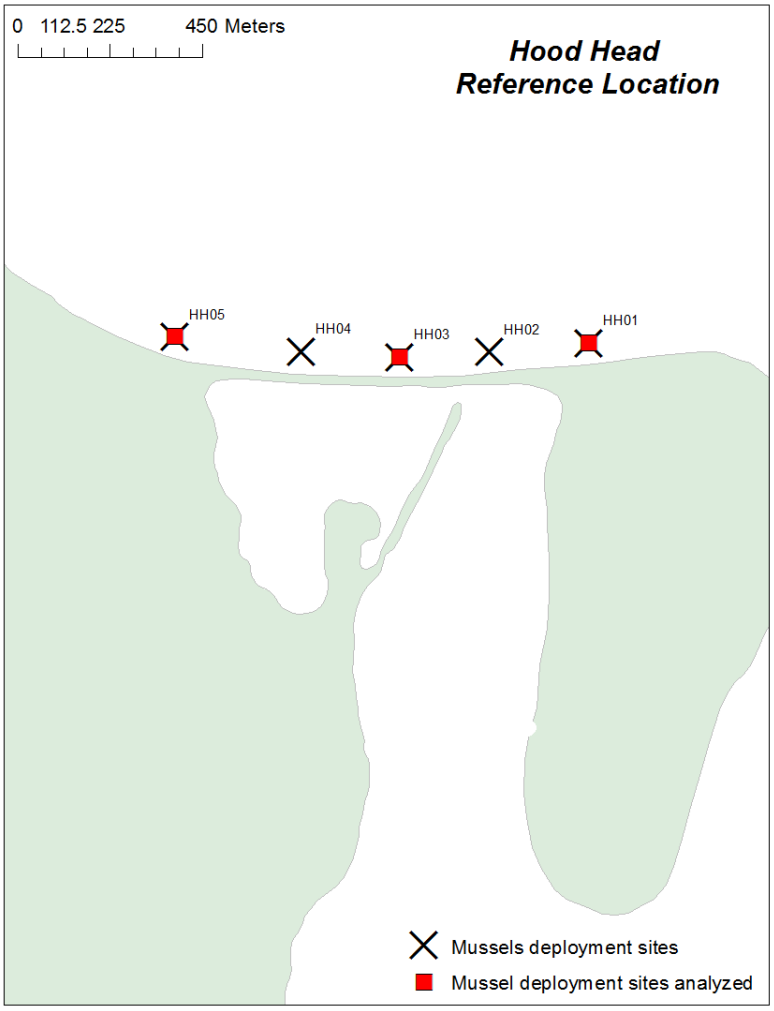

Figure 4. Locations of mussel monitoring cages deployed (X) and mussel composites analyzed for chemicals (red squares) at Hood Head (HH), Washington.

Study specimen

Pacific blue mussels (*Mytilus trossulus*), which are indigenous to intertidal habitats in the Puget Sound, were used for this study. The mussel source was Penn Cove Shellfish, Inc. an aquaculture facility in Penn Cove, Whidbey Island, Washington. The target size and age of mussels was 50 – 60 mm in shell length and approximately 11 months of age, respectively. All the mussels used in this study had not yet reproduced in their lifetime.

Sample Units

A sample unit in this study (hereafter called a "mussel cage") consisted of 64 pre-measured *M. trossulus* mussels, suspended in four sealed aquaculture bags, and hung inside a predator-exclusion cage (**Figure 5**). All

mussels were screened for health, measured for size consistency, and bagged at Penn Cove Shellfish from November 17-19, 2014 (see QAPP for details). Each aquaculture bag held 16 mussels and was divided into two sections with eight mussels each. After bagging the mussels were placed back into Penn Cove under an aquaculture raft and held for approximately 12 days prior to deployment. This period of time was intended to allow them a rest from handling after bagging and prior to deployment (Andral et al, 2011; Benedicto et al, 2011; Galgani et al, 2011).

Figure 5. Bags of mussels hanging inside a predator-exclusion just before deployment into Port Gamble Bay.

On the date of deployment, each mussel cage was outfitted with weighted ballast bars (pieces of rebar attached to the bottom edges of the cage with cable ties) and a floating line with a marker buoy. Each cage received four mussel bags (i.e. 64 mussels per cage). The bags were affixed to the inside of the cage approximately 35 cm above the bottom and stretched across the cage from one side to the other (**Figure 5**). Filled cages were closed and the entire unit was lowered to the substrate.

Timing and Placement of Sample Units

A single mussel cage was deployed to each study site on December 2 and 3, 2014. The cages were retrieved approximately 2 months later, on February 9 - 11 and 23, 2015. All cages, except those in the Central Bay area, were deployed at a subtidal depth of approximately minus 5 feet mean lower low water (MLLW), to match the depth of transplanted herring egg cages from a prior study (West, 2014). Mussel cages placed in the Central Bay area were deployed in approximately 30 feet MLLW. A number of the supplemental cages also exceeded the minus 5 feet depth, including the cages at Mill Site Perimeter North (approximately 15 feet MLLW), Mill Site

Perimeter South (approximately 30 feet MLLW), North of Jetty (approximately 10 feet MLLW), and the Central Mill Site (approximately 30 feet MLLW).

Three to five replicate cages were deployed at each of the nine designated sampling areas; in addition, a subset of bagged mussels were removed from Penn Cove and processed into three baseline, i.e. starting condition, samples on the second day of deployment. A number of additional mussel cages (n = 1 or 2) were deployed to areas adjacent to the SMAs and at other select locations of interest to the Port Gamble Bay S'Klallam Tribe (PGST) in Port Gamble Bay (Figure 3).

Biological Endpoints

Mortality

After retrieval, mussels from each cage were assessed for mortality. Individual mussels from each cage were assigned into one of three categories (1. healthy, 2. dead or moribund, and 3. missing) depending on their condition. Mussels were considered "healthy" when they were whole and in good condition, including some with shells that may have been cracked from handling. Only healthy, uncracked mussels were used for chemical analyses, while some of the mussels that may have been cracked during retrieval were used in the assessment of condition index. "Dead or moribund" included whole empty shells, matched broken shells and hinges, whole rotting mussels, or gaping mussels that would not close their shells. "Missing" mussels included mussels that were simply gone, which may have resulted from a miscount during the bagging phase, or could have occurred if a mussel became fragmented and its shell pieces fell through the cage mesh.

Condition Index

To account for differences in growth related to food availability in this study, we calculated the Condition Index (CI) of mussels from each site. We determined CI on twelve randomly selected mussels from each cage according to a method reported by Kagley et al. (2003) as follows:

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

Chemical Analysis

Thirty-two mussels from each cage were set aside and frozen for later chemical analysis. After shucking, the soft tissues of these 32 mussels were combined into a single composite sample that was blended to create a homogenous mixture (see QAPP for details of composite sample preparation). Each mussel composite was analyzed for 42 PAH compounds, comprising 22 low molecular weight analytes and 20 high molecular weight analytes, 40 PCBs, 11 PBDEs, and 25 organoclorine pesticides (Table 1). In addition, mussels were analyzed for total dioxins and furans (Table 2), metals (Table 3), and total extractible lipids and percent solids (Table 4). All sample data met QA/QC criteria as outlined in the study QAPP, except for minor violations of holding time for mercury, which were considered inconsequential.

Table 1 Persistent organic pollutants measured at the Northwest Fisheries Science Center (NWFSC), Seattle, WA.

Persistent organic pollutants:	No. Analytes	Method	Limit of Quantitation - LOQ (wet weight)	Expected Range (wet weight)
Polycyclic Aromatic Hydrocarbons (PAHs)	42	Sloan et al. 2004	0.2-0.8 ng/g	LOQ to 20 ng/g
Polychlorinated biphenyl (PCB) congeners	40	Sloan et al. 2004[a]	0.2-0.8 ng/g	LOQ to 20 ng/g
Polybrominated diphenylethers (PBDEs) congeners	11	Sloan et al. 2004	0.2-0.8 ng/g	LOQ to 20 ng/g
Organochlorine pesticides (OCPs)	25	Sloan et al. 2004	0.2-0.8 ng/g	LOQ to 20 ng/g

[a]Sloan, C. A., D. W. Brown, et al. (2004). Extraction, cleanup, and gas chromatography/mass spectrometry analysis of sediments and tissues for organic contaminants., U.S. Dept. Commerce. NOAA Tech. Memo. NMFS-NWFSC-59.

Table 2. Dioxins and furans measured at AXYS, Sidney, B.C.

Dioxins (PCDD)	Furans (PCDF)
2,3,7,8 Tetrachlorobenzodioxin (TCDD)	2,3,7,8 Tetrachlorobenzofuran (TCDF)
Total TCDD	Total TCDF
1,2,3,7,8 Pentachlorodibenzodioxin (PeCDD)	1,2,3,7,8 Pentachlorodibenzofurn (PeCDF)
Total PeCDD	2,3,4,7,8 PeCDF
	Total PeCDF
1,2,3,4,7,8 Hexachlorodibenzodioxin (HxCDD)	1,2,3,4,7,8 Hexachlorodibenzofuran (HxCDF)
1,2,3,6,7,8 HxCDD	1,2,3,6,7,8 HxCDF
1,2,3,7,8,9 HxCDD	1,2,3,7,8,9 HxCDF
Total HxCDD	2,3,4,6,7,8 HxCDF
	Total HxCDF
1,2,3,4,6,7,8 Heptachlorodibenzodioxin (HpCDD)	1,2,3,4,6,7,8 Heptachlorodibenzofuran (HpCDF)
Total HpCDD	1,2,3,4,7,8,9 HpCDF
	Total HpCDF
Octachlorodibenzodioxin (OCDD)	Octachlorodibenzofuran (OCDF)

Table 3. Metals measured at the King County Environmental Lab (KCEL), Seattle, WA.

Metals	No. Analytes	Method	Method Detection Limit (wet weight)	Expected Range (wet weight)
Total mercury (Hg)	1	EPA method 245.5	0.005 µg/g	MDL to 5 µg/g
Lead (Pb)	1	EPA method 200.8	0.004 µg/g	MDL to 5 µg/g
Arsenic (As)	1	EPA method 200.8	0.004 µg/g	MDL to 5 µg/g
Zinc (Zn)	1	EPA method 200.8	0.004 µg/g	MDL to 5 µg/g
Copper (Cu)	1	EPA method 200.8	0.004 µg/g	MDL to 5 µg/g
Cadmium (Cd)	1	EPA method 200.8	0.002 µg/g	MDL to 5 µg/g

Table 4 Conventionals measured at the Northwest Fisheries Science Center (NWFSC), Seattle, WA.

Conventional	No. Analytes	Method	Method Detection Limit (wet weight)	Expected Range (wet weight)
Lipid content (% total extractibles)	1	gravimetric	0.1%	0.5 to 3%
Dry Weight (%)	1	gravimetric	0.1%	10-20%

Analytical methods

The PAHs, PCBs, and PBDEs were analyzed at the Northwest Fisheries Science Center (NWFSC) in Seattle, WA according to Sloan et al. (2004). In brief, this method comprises three steps: (a) extraction, (b), cleanup by silica/aluminum columns and size-exclusion high-performance liquid chromatography (SEC HPLC), and (c) quantitation of chlorinated hydrocarbons (CHs) and aromatic hydrocarbons (AHs) using gas chromatography /mass spectrometry (GC/MS) with selected-ion monitoring (SIM). Percent lipids and solids (dry weight) in each sample were also determined according to Sloan et al. 2004. See study QAPP for further details.

All dioxin and furan analyses were performed by AXYS Analytical Services Ltd. in Sidney, BC using a modification of EPA Method 1613B; tetra- through octa-chlorinated dioxins and furans by isotope dilution HRGC/HRMS (AXYS Analytical Services Ltd., 2012). All metals analyses were performed by the King County Environmental Laboratory (KCEL). Mercury was analyzed via automated cold vapor atomic absorption spectrometry following King County Environmental Laboratory Standard Operating Procedure (KCEL SOP) 604v6. Lead, arsenic, zinc, copper and cadmium were analyzed via Thermo Elemental X Series II CCT (Collision Cell Technology) Inductively Coupled Plasma Mass Spectrometer (ICP-MS) following KCEL SOP 624v2.

Data Analysis

Mussel contaminant data are presented as summed concentrations (e.g., Σ_{42}PAHs) for analyte groups (Table 5), 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 (eTPCB) 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).

We report on the total dioxins and the total furans for mussels in Port Gamble Bay. Here total dioxins and total furans are the summations of the detected values of the four homolog groups (tetra-, penta, hexa-, and hepta-) of dioxins and furans, as well as total octa-dioxins and total octa-furans; i.e. $\sum_{5HLs}$Dioxins and $\sum_{5HLs}$Furans.

Table 5. Groupings used for estimated totals (PCBs) and summations (all others) in this study.

Sum 3 HCHs	Sum 8 Chlordanes	*Estimated Total PCBs	Sum 6 DDTs	Sum 11 PBDEs	Sum 42 Polycyclic Aromatic Hydrocarbons (PAHs)		Sum 5 Dioxin homolog groups	Sum 5 Furan homolog groups
					Low Molecular Weight PAHs	High Molecular Weight PAHs		
alpha hexachlorocyclohexane	alpha chlordane	PCB018	ppDDD	PBDE028	naphthalene	fluoranthene (FLA)	tetra-dioxins	tetra-furans
beta hexachlorocyclohexane	beta chlordane	PCB028	ppDDE	PBDE047	C1-naphthalenes	pyrene (PYR)	penta-dioxins	penta-furans
lindane	cis nonachlor	PCB044	ppDDT	PBDE049	C2-naphthalenes	C1-fluoranthenes/pyrenes	hexa-dioxins	hexa-furans
	heptachlor	PCB052	opDDD	PBDE066	C3-naphthalenes	C2-fluoranthenes/pyrenes	hepta-dioxins	hepta-furans
	heptachlor epoxide	PCB095	opDDE	PBDE085	C4-naphthalenes	C3-fluoranthenes/pyrenes	octa-dioxin	octa-furan
	nonachlor3	PCB101	opDDT	PBDE099	acenaphthylene (ACY)	C4-fluoranthenes/pyrenes		
	Oxychlordane	PCB105		PBDE100	acenaphthene (ACE)	benz[*a*]anthracene (BAA)		
	trans Nonachlor	PCB118		PBDE153	fluorene (FLU)	chrysene (CHR)[a]		
		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)[b]		
		PCB195			C3-dibenzothiophenes	benzo[*e*]pyrene (BEP)		
		PCB206			C4-dibenzothiophenes	benzo[*a*]pyrene (BAP)		
		PCB209			phenanthrene (PHN)	perylene (PER)		
					anthracene (ANT)	indeno[*1,2,3-cd*]pyrene (IDP)		
					C1-phenanthrenes/anthracene	dibenz[*a,h*]anthracene (DBA)[c]		
					C2-phenanthrenes/anthracenes	benzo[g,h,i]perylene (BZP)		
					C3-phenanthrenes/anthracenes			
					C4-phenanthrenes/anthracenes			

* Estimated total PCBs is the sum of 17 congeners, then multiplied by two; HMW PAHs - [a] coelutes with triphenylene; [b] coelutes with benzo[*j*]fluoranthene; [c] coelutes with dibenz[*a,c*]anthracene

Data Transformations and Statistical Analysis

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 log10-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 transformed all log10-contaminant data for consistency.

We do not present lipid-adjusted concentrations by dividing contaminant concentration by percent lipid in this report. Based on previous studies (Lanksbury et al. 2014) we expected the overall lipid concentrations in our mussels to be low (<2%). 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.

Summary statistics were calculated for each area in which two or more composites were analyzed (i.e. $n > 1$, see Table 1) and for the Penn Cove Baseline (PCBASE, $n = 3$) composites. Summaries include minimum (Min) and maximum (Max) values, arithmetic means, and standard deviations (±SD), when applicable. Comparisons of contaminant concentrations among areas with three or more replicates were performed using parametric analysis of variance (ANOVA; SYSTAT) with area (MSN, MSS, CB, FLA, NWS, HOB, SES, NES, HH, PCBASE) as the categorical variable. Lipids and CI were included as covariates in the ANOVA models, but mortality was not included as a covariate because this data was not applicable for the PCBASE mussels. For each contaminant type we computed multiple ANOVAs, removing or adding factors in a stepwise fashion, until arriving at the most parsimonious model. Tukey's Honestly-Significant Difference (THSD) post hoc test was used for pairwise location comparisons of least square means generated by the ANOVAs. Because a smaller number of samples ($n = 16$) were analyzed for dioxins and furans and there were not enough replicates available, no ANOVA results are reported those contaminants.

Pattern Analysis of PAHs

The chemical composition (analyte fingerprint) of PAHs has been used as a diagnostic tool to help infer sources of PAH pollution sources in mussels (Amin et al., 2011; Francioni et al., 2007; Guinan et al., 2001; Maioli et al., 2010; Palma-Fleming et al., 2008; Palma-Fleming et al., 2012; Payne et al., 2008; Soriano et al., 2006; Tobiszewski and Namieśnik, 2012). PAH profiles found in mussels and sediment samples from the same area are often similar (Guinan et al., 2001), and mussels have been used successfully in the past to identify nearshore PAH contamination related to harbor pollution (Baumard et al., 1999b), creosote pilings (Dunn and Stich, 1975; Hyötyläinen et al., 2002), and as a result of major oil spills (Apeti et al., 2013; Babcock et al., 1996; Carls et al., 2001; Neff and Burns, 1996).

PAH fingerprints can shed light on whether contamination in a sample came from petrogenic (related to unburned petroleum) or pyrogenic (generated by the combustion of fossil and other fuels, including coal and

wood, or from creosote) sources. For instance, the fraction of parent PAHs (C_0) to their alkylated homologs (C_1, C_2, C_3, or C_4) is used extensively to infer sources in natural resource damage assessments for oil spills: petrogenic sources typically have a greater percentage of alkyl PAHs (C_1, C_2, C_3, or C_4) compared to their parent compounds (C_0), while pyrogenic sources, or highly weathered oil, tend to have a predominance of parent PAHs compared to their alkylated homologs (da Silva and Bícego, 2010; Lima et al., 2005; Payne et al., 2003; Tobiszewski and Namieśnik, 2012; Yunker et al., 2002). Using histogram plots we investigated and compared the overall percent of individual PAH analytes among the mussel sites.

We further summarized and quantified the patterns of PAH analytes by examining the homolog series maximum for three of the most frequently detected analyte pairs, anthracene(ANT)/ phenanthrene(PHN), fluoranthene(FLA)/pyrene(PYR), and benz[a]anthracene(BAA)/chrysene(CHR). In addition, we calculated the ratio of the sum of alkylated PHNs to PHN (Σalkylated-PHNs/PHN) which has been used forensically to distinguish petrogenic PAHs (from an oil spill) from background pyrogenic PAHs in Pacific herring embryos. Following the example of Incardona et al. (2012), we regarded mussels that exhibited a Σalkylated-PHNs/PHN ratio greater than two as indicative of petroleum exposure, and mussels with a ratio less than two as indicative of pyrogenic PAH exposure. Together the PAH fingerprint histograms, the homolog series maxima, and the Σalkylated-PHNs/PHN ratio were used in a "weight of evidence" approach to characterize the different areas of Port Gamble Bay as primarily exposed to pyrogenic or petrogenic PAH sources, or both.

3.0 Results & Discussion

Overview

Of the 55 cages deployed at the start of the study 53 were retrieved intact, thus 96% of the mussel cages were successfully recovered. We were not able to retrieve one of the cages at the Southeast Shoreline (SES03) and one of the Mill Site South Perimeter cages (MSS-P01; Figure 3). Mussels from all recovered cages were processed into soft tissue composites and frozen, however only a subset of these mussel composites (48) were analyzed for biological and chemical endpoints (Table 6).

Table 6. Summary of mussel tissue composites analyzed for chemical contaminants. Ecology – Washington State Department of Ecology; SMA – Sediment Management Areas identified in the Port Gamble Bay Remedial Investigation (Ecology 2012); PGB – Port Gamble Bay; PGST – Port Gamble S'Klallam Tribe. See Figures 3 and 4 for locations on maps.

Funding Source	Location or Purpose	Area	Collection date(s)	Composites analyzed (n)
Ecology	Starting Condition (Baseline)	Penn Cove Shellfish, Whidbey Island	12-3-2014	3
Ecology	SMA-1	Mill Site North (MSN)	2-9-2015	5
Ecology	SMA-2	Mill Site South (MSS)	2- 9 & 10 -2015	5
Ecology	SMA-3	Central Bay (CB)	2- 9 to 10 -2015	3
Ecology	SMA-4	Former Lease Area (FLA)	2-9-2015	3
Ecology	SMA-5	Northwest Shore (NWS)	2- 9 & 11 -2015	3
Ecology	Non-SMA in PGB	Head of Bay (HOB)	2-9-2015	3
Ecology	Non-SMA in PGB	Southeast Shore (SES)	2-10-2015	3
Ecology	Non-SMA in PGB	Northeast Shore (NES)	2- 10 & 23 -2015	3
Ecology	Reference Area outside PGB	Hood Head (HH)	2-10-2015	3
PGST	SMA-5	Central Mill Site (CMS)	2-10-2015	1
PGST	SMA-5	MSN Perimeter (MSN-P)	2- 9 & 23 -2015	2
PGST	SMA-5	MSS Perimeter (MSS-P)	2- 9 & 23 -2015	2
PGST	Non-SMA in PGB	North of Jetty (NJ)	2- 9 & 10 -2015	2
PGST	SMA-5	Dump Site Shoreline (DSS)	2-23-2015	2
PGST	Non-SMA in PGB	Point Julia North (PJN)	2- 9 & 10 -2015	2
PGST	Non-SMA in PGB	Point Julia South (PJS)	2- 10 & 11 -2015	2
PGST	Non-SMA in PGB	West of Jetty (WJ)	2-10-2015	1

Statistical analyses of differences in biological condition and contaminant concentrations among areas are described below. In all the ANOVA tests exploring differences among areas (tested factors included mortality, PAHs, PCBs, PBDEs, and DDTs) neither CI nor lipids were significant covariates, thus they were not included in the final ANOVA models presented below.

Because mussels were deployed for two months, they were not expected to represent contaminant conditions in wild mussels or other bivalve shellfish in Port Gamble Bay. The most appropriate use of the data herein is as a comparison of mussel conditions from different areas of Port Gamble Bay and the Hood Head reference area prior to remediation, and as a baseline against which to compare future mussel conditions after remediation.

Biological Endpoints

Survival and Mortality

On average 19% of the mussels deployed at each site (i.e. cage) died by the end of the study (Table 7, Figure 6). Though mortality varied slightly (range was 14 to 30.3%) among the areas with three to five replicates (testable

via ANOVA), there were no significant differences among these areas ($F_{8,22} = 2.209$; $r^2 = 0.667$; $p = 0.068$; Figure 6). The high survival rate (81%) in this study matched that seen in a recent, large-scale, mussel study in Puget Sound (2012/13) where 80% of transplanted mussels survived to the end of the study (Lanksbury et al., 2014).

Table 7. Mortality of transplanted mussels for each area at the end of the study period.

		Mortality (%)			
Area	**# Samples analyzed**	**Min.**	**Max.**	**Mean**	**SD**
Hood Head Reference	3	17	22	20.0	2.65
Central Bay	3	6	17	11.3	5.508
Former Lease Area	3	12	27	18.7	7.638
Head of Bay	3	8	16	11.7	4.041
Mill Site North	5	17	23	20.4	2.608
Mill Site South	5	10	31	20.6	9.370
Northeast Shore	3	12	14	12.7	1.155
Northwest Shore	3	21	48	30.3	15.308
Southeast Shore	3	14	19	16.7	2.52
Central Mill Site	1	-	-	14.0*	-
MSN Perimeter	2	14	27	20.5	-
MSS Perimeter	2	20	22	21.0	-
North of Jetty	2	14	16	15.0	-
Dump Site Shoreline	2	15	29	22.0	-
Point Julia North	2	11	20	15.5	-
Point Julia South	2	19	37	28.0	-
West of Jetty	1	-	-	28*	-

* Number is a single mortality value (n = 1), not a mean.

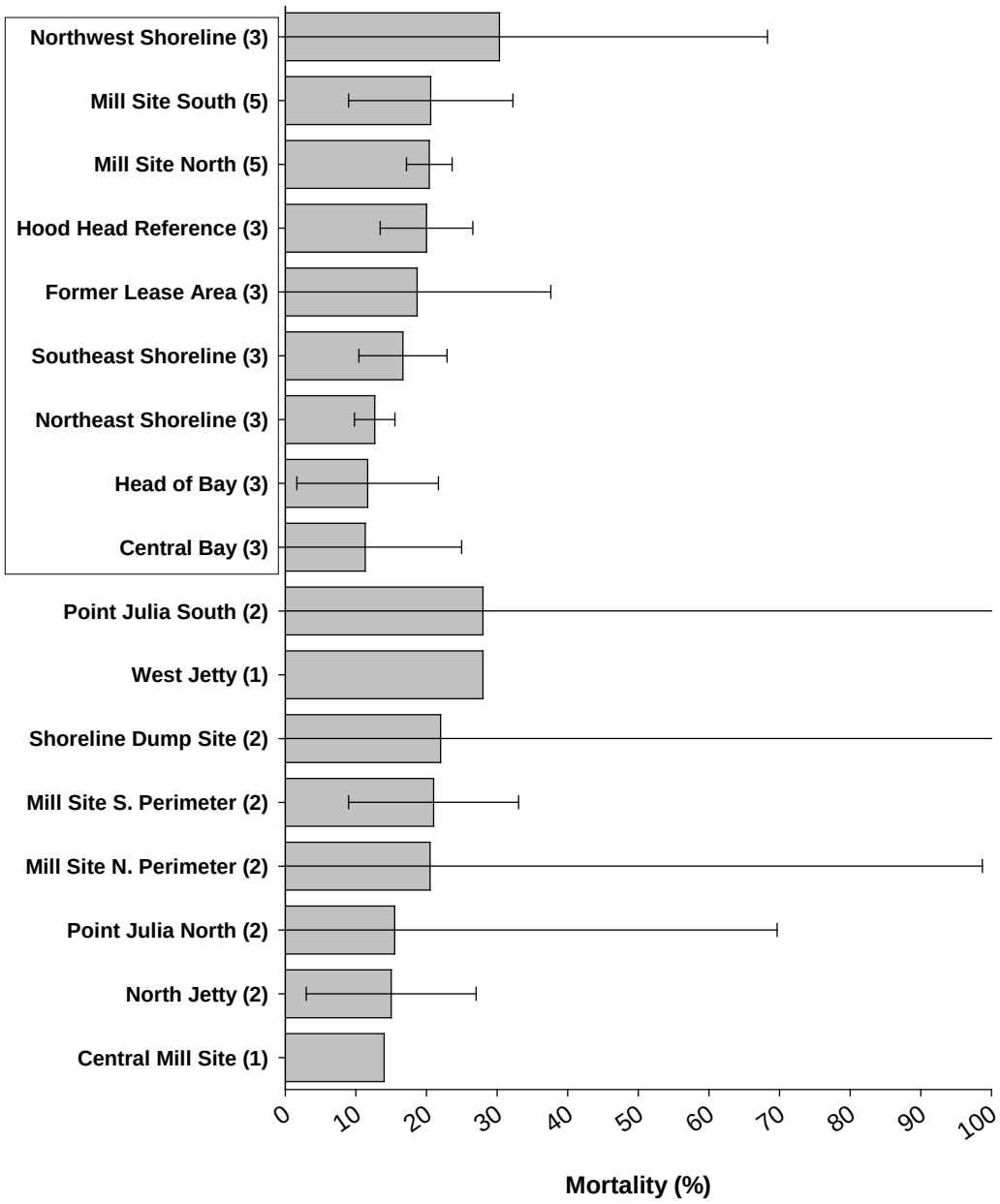

Figure 6. Percent mortality of mussels at the end of the study period in each area. ANOVA performed on the 9 areas in box; replicates analyzed in parentheses; no significant differences were found.

Lipids and Condition Index (CI)

The lipid concentrations of mussels from Port Gamble Bay and the Hood Head Reference areas were low and ranged narrowly (1.02 to 1.36% wet weight;

Table 8). There were significant differences in lipids ($F_{9,24} = 6.153$; $r^2 = 0.835$; $p < 0.001$); specifically, mussels at the start of the study (Penn Cove Baseline, 1.42%) had significantly higher lipids than all mussels at the end of the study, except those from the Southeast Shoreline (Figure 7).

Table 8. Percent lipids detected in mussels from this study.

			Lipids (%, ww)			
Area	**# Samples analyzed**	**n detects**	**Min.**	**Max.**	**Mean**	**SD**
Penn Cove Baseline	3	3	1.35	1.50	1.419	0.0727
Hood Head Reference	3	3	0.93	1.06	1.014	0.0729
Central Bay	3	3	0.91	1.02	0.972	0.0564
Former Lease Area	3	3	0.90	1.08	1.011	0.0992
Head of Bay	3	3	0.91	1.10	0.985	0.1054
Mill Site North	5	5	0.94	1.26	1.057	0.1193
Mill Site South	5	5	1.03	1.27	1.148	0.1005
Northeast Shore	3	3	1.01	1.26	1.123	0.1271
Northwest Shore	3	3	1.08	1.14	1.100	0.0348
Southeast Shore	3	3	1.13	1.22	1.167	0.0465
Central Mill Site	1	1	-	-	1.18*	-
MSN Perimeter	2	2	1.09	1.14	1.113	-
MSS Perimeter	2	2	1.06	1.10	1.083	-
North of Jetty	2	2	0.97	1.11	1.040	-
Dump Site Shoreline	2	2	1.01	1.05	1.033	-
Point Julia North	2	2	1.06	1.11	1.088	-
Point Julia South	2	2	1.10	1.36	1.233	-
West of Jetty	1	1	-	-	1.21*	-

* Number is a single lipid value (n = 1), not a mean.

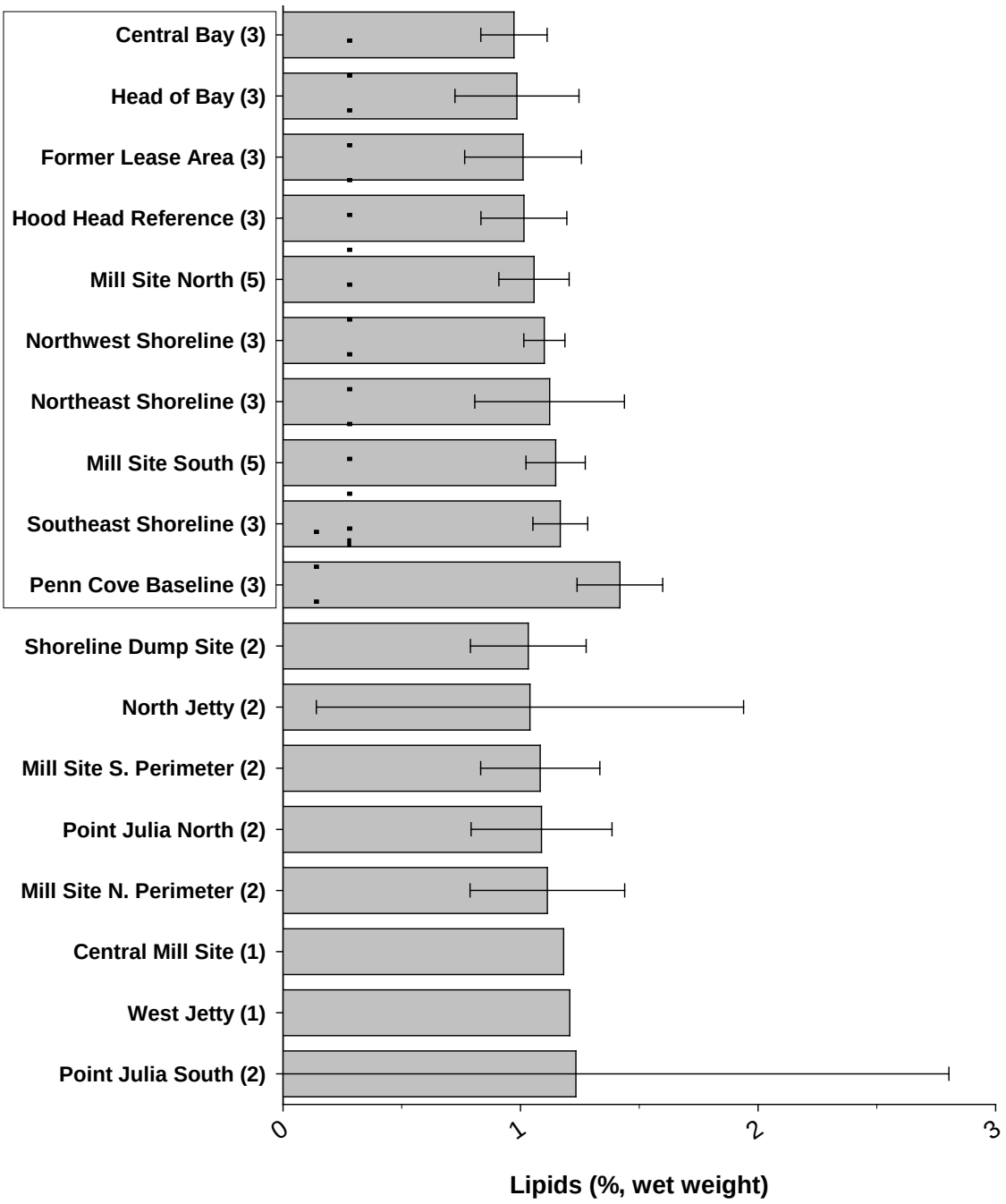

Figure 7. Lipid content of mussels. ANOVA performed on 10 areas in box; replicates analyzed in parentheses; dotted lines indicate areas that were not significantly different from one another (p>0.05).

The CI of mussels from Port Gamble Bay and Hood Head Reference areas ranged from mean 1.77 to 2.4 (Table 9). Similar to the situation with the lipids, there were significant differences in CI ($F_{9,24} = 6.967$; $r^2 = 0.850$; $p < 0.001$; Figure 8). Specifically, mussels from the start of the study had significantly higher CI (2.8) than all other

mussels, except those from the Central Bay (2.4), which were also significantly higher than mussels from the Northwest Shoreline (1.77).

The overall decline in lipids and CI in mussels from the start to the end of the study was likely a normal response of mussels to winter conditions. A similar response was seen in the recent study of transplanted mussels in Puget Sound during the winter months of 2012/13 when mussels from 72% of the study sites showed a decline in CI over their starting condition (Lanksbury et al., 2014). A similar drop in CI was shown by Kagley et al. (2003) during the winter months in Puget Sound in the early 2000s. Though we did not measure chlorophyll concentrations at our study sites, it is common knowledge that during the months of December through February (i.e. winter) in Puget Sound, phytoplankton growth (i.e. primary production) declines relative to summer conditions due to limitations in sunlight. Since mussels primarily feed on phytoplankton, this limitation in food supply likely leads to reductions in mussel growth and fat stores during the winter months.

Table 9. Condition Index of transplanted mussels at the end of the study period; Penn Cove Baseline shows starting condition index.

		Condition Index (CI)			
Area	**# Samples analyzed**	**Min.**	**Max.**	**Mean**	**SD**
Penn Cove Baseline	3	2.7	3.1	2.82	0.8861
Hood Head Reference	3	2.1	2.4	2.21	0.1644
Central Bay	3	2.2	2.5	2.37	0.1238
Former Lease Area	3	1.8	2.3	2.07	0.2385
Head of Bay	3	2.0	2.3	2.16	0.1559
Mill Site North	5	2.0	2.4	2.15	0.2095
Mill Site South	5	1.8	2.2	2.07	0.1731
Northeast Shore	3	2.1	2.2	2.16	0.0163
Northwest Shore	3	1.5	2.0	1.77	0.2336
Southeast Shore	3	2.0	2.3	2.13	0.1479
Central Mill Site	1	-	-	2.1*	-
MSN Perimeter	2	1.8	2.1	1.95	-
MSS Perimeter	2	1.9	2.2	2.06	-
North of Jetty	2	1.8	2.2	1.98	-
Dump Site Shoreline	2	2.0	2.1	2.06	-
Point Julia North	2	2.2	2.4	2.29	-
Point Julia South	2	2.1	2.1	2.08	-
West of Jetty	1	-	-	2.4*	-

* Number is a single CI value (n = 1), not a mean.

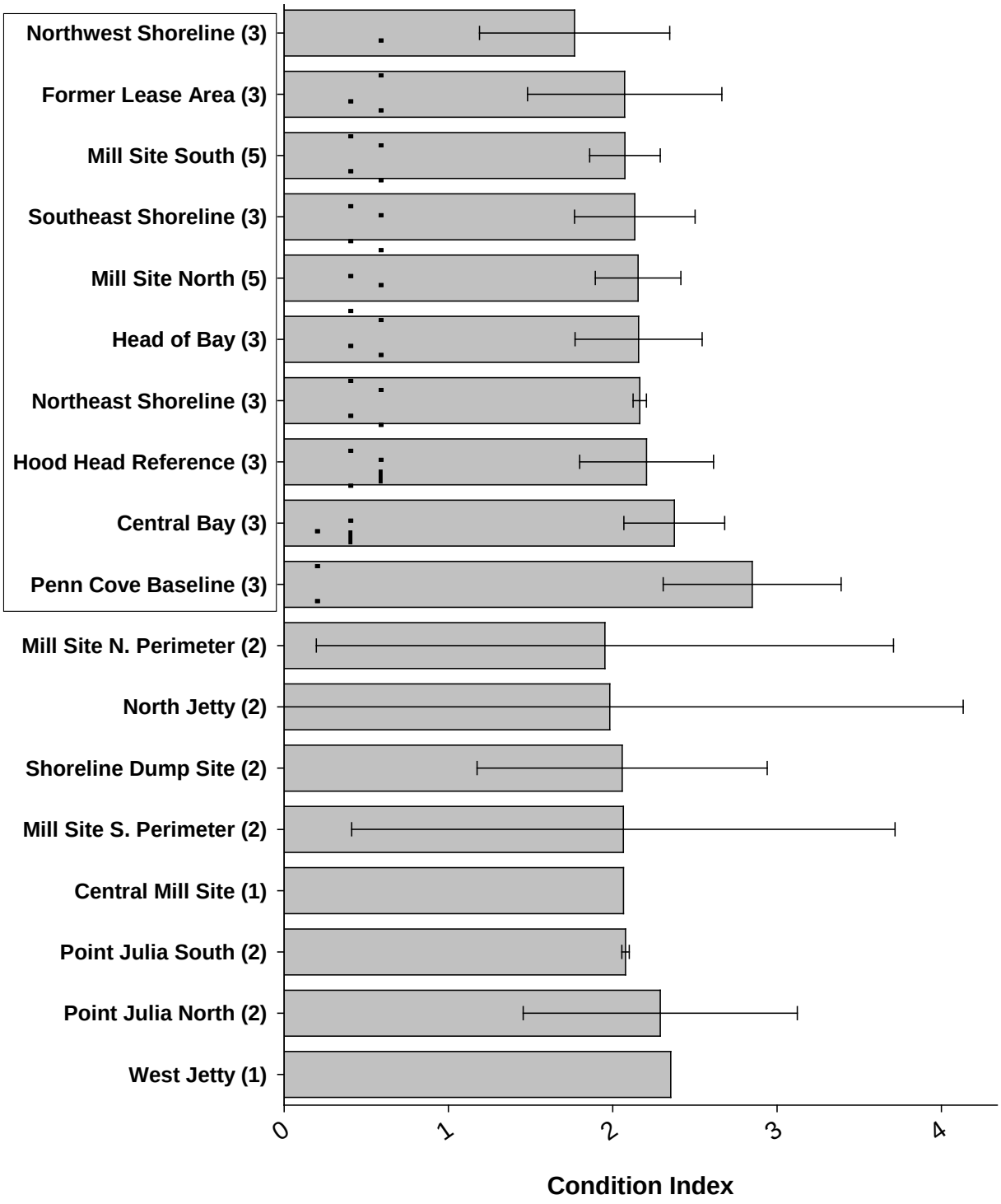

Figure 8. Condition Index (CI) of mussels. ANOVA performed on 10 areas in box; replicates analyzed in parentheses; dotted lines indicate areas that were not significantly different from one another ($p>0.05$).

Not surprisingly, there was a correlation between lipids and CI (n = 34, correlation coefficient = 0.447, p = 0.009; Figure 9). This correlation was driven by mussels from the start of the study (Penn Cove Baseline), which had significantly higher lipids and CI than nearly all of the mussels from the end of the study. When the baseline mussels were removed from the analysis the correlation broke down (n = 31, correlation coefficient = -0.109, p = 0.561). However, because the Penn Cove Baseline mussel samples were included in the ANOVAs exploring contaminant concentrations, lipids and CI were each tested separately as covariates in all the models that follow.

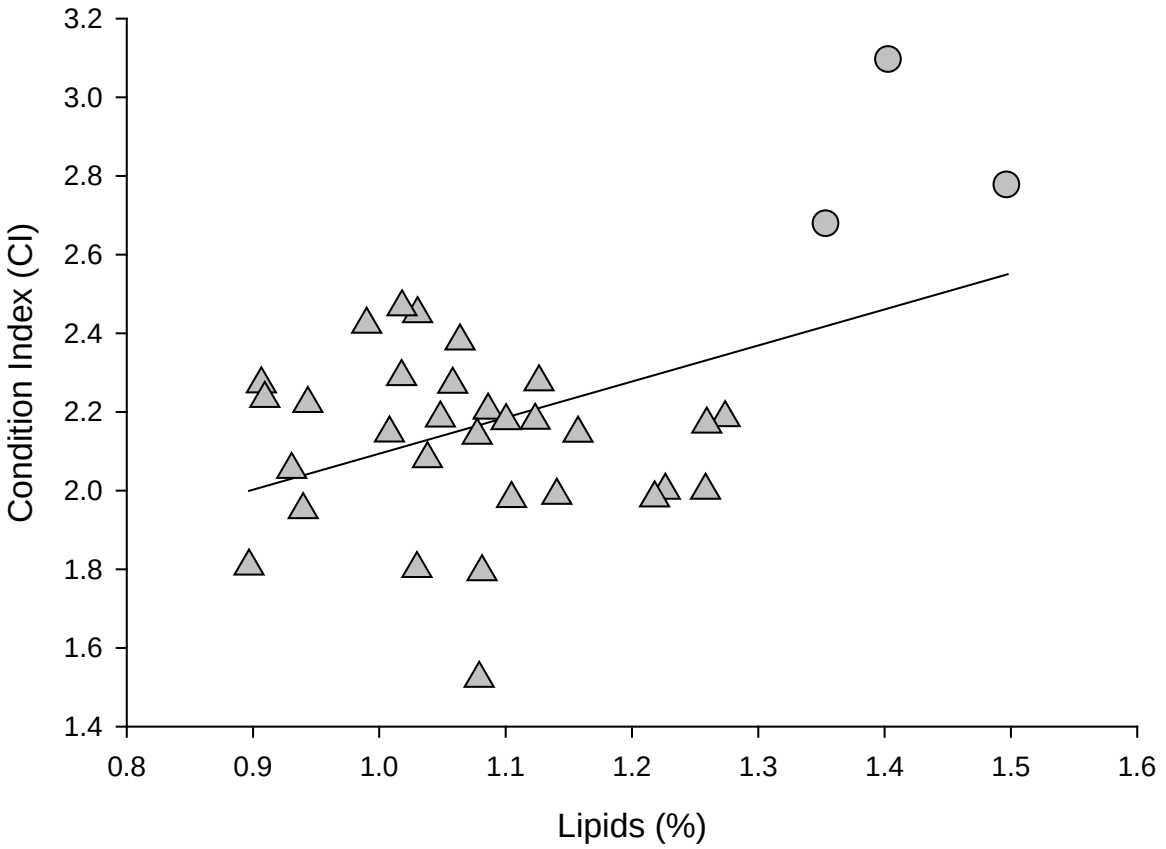

Figure 9. Correlation between lipid and condition index (CI) among mussels used in this study. Circles are mussels from the start of the study (Penn Cove Baseline), triangles are mussels from all sites at the end of the study.

Organic Contaminants

Results for the PAHs, PCBs, PBDEs, DDTs, and "Other Organic Contaminants" are reported in parts per billion, dry weight (ng/g dw). Results for the dioxins and furans are reported in parts per trillion, dry weight (pg/g dw).

Total PAHs

When summed as a group PAHs ($\sum_{42}$PAHs) were detected in mussels from 100% of the study sites, including the Hood Head Reference area. Though some individual PAH analytes were not detected in some composites (LOQs ranged from 0.999 to 8.853 ng/g dw), all samples included a range of low and high molecular weight PAH analytes. The $\sum_{42}$PAH concentrations in mussels transplanted to areas in Port Gamble Bay ranging from 123 to 2,778 ng/g dw (Table 10 and Figure 10). Mussel transplanted to the Hood Head Reference area were similar to the starting condition (mean 75.4 vs. 51.7 ng/g dw, respectively). Both the reference and starting condition mussels had PAH concentrations that were an order of magnitude lower than most of the mussels from Port Gamble Bay, and two orders of magnitude lower than mussels from Mill Site North (mean 1,029.35 ng/g dw), Mill Site South (1,565.66 ng/g dw), and the Central Mill Site (n = 1; 2,778 ng/g dw; Table 10).

Area accounted for nearly all of the differences in total PAH concentrations in mussels ($F_{8,22}$ = 13.124; r^2 = 0.926; $p < 0.001$; Figure 10). Mill Sites North and South (means 1,029 ±304.8 and 1,566 ±1837 ng/g dw, respectively) accumulated significantly higher total PAHs than mussels from all other Port Gamble Bay sites and the Hood Head reference site, and had significantly higher concentrations than mussels at the start of the study (Tukey's honest significance tests; Figure 10). There were no significant differences between mussels from the other Port Gamble locations and the reference site, or as compared to the starting condition. Although not included in the ANOVA, the PAH value of the mussel composite from the Central Mill Site (n = 1; concentration 2,778 ng/g dw) exceeded the concentrations at both Mill Sites North and South (Figure 10).

Table 10. Concentrations (ng/g dry weight) of the sum of 22 low, 20 high, and 42 total PAHs detected in mussels from the start of the study (Penn Cove Baseline) and from all transplanted areas at the end of the study.

			Low Molecular Weight PAHs (Σ_{22}PAHs)				High Molecular Weight PAHs (Σ_{20}PAHs)				Total PAHs (Σ_{42}PAHs)			
Area	**Samples analyzed**	**n detects ‡**	**Min.**	**Max.**	**Mean**	**SD**	**Min.**	**Max.**	**Mean***	**SD**	**Min.**	**Max.**	**Mean**	**SD**
Penn Cove Baseline	3	3	31.6	34.5	32.77	1.552	17.5	20.1	18.97	1.352	51.0	52.2	51.74	0.6372
Hood Head Reference	3	3	39.1	43.2	41.23	2.068	32.4	35.6	34.22	1.655	71.5	78.8	75.44	3.716
Central Bay	3	3	40.9	56.1	50.99	8.773	64.7	76.2	71.85	6.247	105.6	132.3	122.84	14.990
Former Lease Area	3	3	77.9	80.0	78.71	1.157	69.9	86.2	79.78	8.687	149.9	164.3	158.49	7.570
Head of Bay	3	3	53.5	91.2	66.27	21.581	63.1	95.6	75.21	17.757	117.2	186.8	141.47	39.265
Mill Site North	5	5	234.7	510.0	354.15	106.41	435.5	962.5	675.20	201.03	670.2	1472.4	1029.35	304.76
Mill Site South	5	5	146.1	1113.0	434.17	417.30	228.1	3542.4	1131.49	1421.67	374.1	4655.4	1565.66	1837.18
Northeast Shore	3	3	63.4	111.6	80.91	26.659	82.7	105.5	90.33	13.129	146.1	217.1	171.24	39.758
Northwest Shore	3	3	72.2	92.8	84.97	11.180	105.0	126.4	114.61	10.892	184.6	216.4	199.58	15.992
Southeast Shore	3	3	76.5	93.2	84.02	8.478	70.5	91.3	79.25	10.764	152.5	184.5	163.27	18.380
Central Mill Site	1	1	-	-	974.4*	-	-	-	1804*	-	-	-	2778*	-
MSN Perimeter	2	2	114.3	185.0	149.64	-	162.6	203.2	182.86	-	276.8	388.2	332.51	-
MSS Perimeter	2	2	111.3	146.1	128.69	-	149.9	232.6	191.24	-	261.2	378.7	319.93	-
North of Jetty	2	2	85.5	89.9	87.68	-	119.1	160.1	139.63	-	209.0	245.6	227.31	-
Dump Site Shoreline	2	2	89.3	95.5	92.42	-	94.9	95.3	95.13	-	184.2	190.9	187.55	-
Point Julia North	2	2	68.0	92.7	80.34	-	85.7	97.0	91.35	-	153.7	189.6	171.69	-
Point Julia South	2	2	77.9	97.9	87.91	-	79.9	90.6	85.25	-	157.8	188.5	173.17	-
West of Jetty	1	1	-	-	93.19*	-	-	-	96.05*	-	-	-	189.3*	-

* Number is a single detected value (n = 1), not a mean.

‡ n detects values were the same for Low, High and Total PAHs

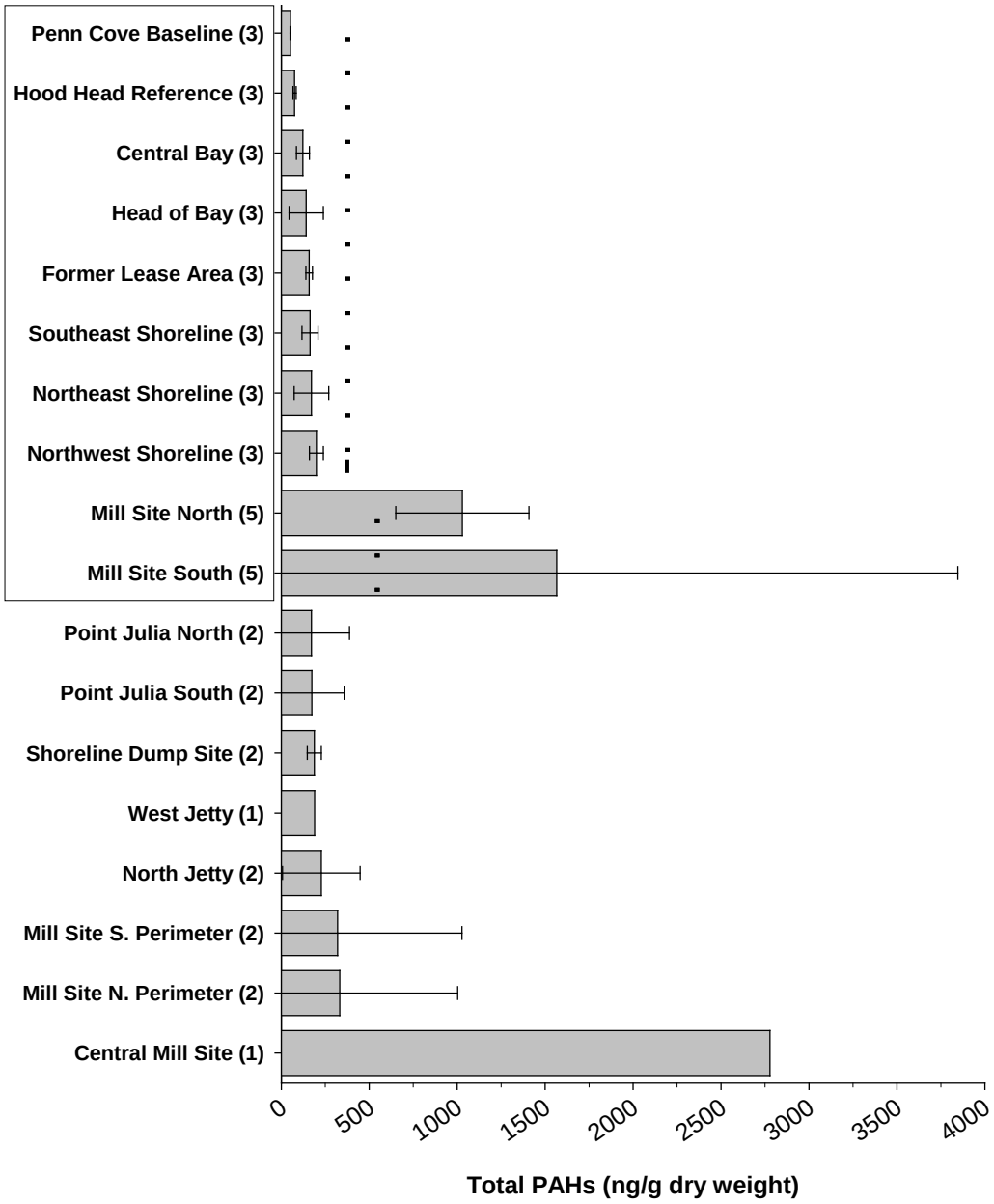

Figure 10. Concentration of total PAHs ($\sum_{42}$PAHS) in mussels. ANOVA performed on 10 areas in box; replicates analyzed in parentheses; dotted lines indicate areas that were not significantly different from one another (p>0.05).

Pattern Analysis of PAHs

The PAHs found in mussels from most of the study sites were dominated by three- and four-ring compounds, namely phenanthrene (PHN), anthracene (ANT), fluoranthene (FLA), pyrene (PYR), and their alkylated homologs (Appendix C). Other four-ring PAHs, chrysene (CHR) and benz[*a*]anthracene (BAA), and five-ring PAHs,

benzo[*b*]fluoranthene (BBF), benzo[*k*]fluoranthene (BKF), and benzo[*e*]pyrene (BEP) were also common, though at lesser concentrations, across most areas.

The proportion of low molecular weight to high molecular weight PAHs (LMW:LMW+HMW) detected in the mussels from the start of the study (Penn Cove Baseline) was different than the proportion detected at the end of the study for the Port Gamble Bay mussels. Specifically, the LMW PAHs were higher in the Penn Cove Baseline (ratio 0.63) and Hood Head Reference mussels (ratio 0.55) relative to nearly all of the mussels transplanted to Port Gamble Bay (Figure 11). Area accounted for nearly all of the differences in this ratio ($F_{8,22}$ = 13.124; r^2 = 0.926; p <0.001), with Mill Site South having significantly more HMW PAHs than all other areas except Mill Site North. Though Mill Site North did not differ significantly from Mill Site South, it also did not differ from a number of the other sites (Figure 11). The sites most similar to the starting condition in terms of this ratio included the Hood Head Reference area, the Southeast Shoreline and the Former Lease Area. The Central Mill Site (n=1) had a PAH ratio very similar to Mill Sites (N and S).

Visual inspection of the PAH histograms revealed a similar pattern across most of the areas, especially for the most often detected homolog series (PHN/ANT, FLA/PYR, and BAA/CHR; Appendix C - **Figure 24**, **Figure 25**, and **Figure 26**). In mussels from all of the Port Gamble Bay areas, the highest concentrations of these three analyte groups occurred at the parent analyte (i.e. C_0) with concentrations declining as degree of alkylation increased (i.e. $C_0 > C_1 > C_2 > C_3 > C_4$). A declining concentration of alkylated homologs from C_0 through C_4 is often used as evidence of pyrogenic PAHs or highly weathered oil sources (Lima et al., 2005; Payne et al., 2003; Tobiszewski and Namieśnik, 2012). In addition, the ratio of Σalkylated-PHN/PHN in mussels from Port Gamble Bay and the Hood Head Reference area ranged from 0.93 to 1.53; the Penn Cove Baseline mussels had a ratio of 0.52. These ratios, which are well below the threshold of two, suggest a dominance of pyrogenic PAHs as well.

Although the overall PAH patterns at all of the Port Gamble Bay areas was similar, there were some differences in the histograms of the Mill Sites (N and S) and the Central Mill Site area mussels. Mussels from these three areas contained fluorene (FLU) and its alkylated homologs (C_1-C_3 FLU) and dibenzothiophene (DBT) and its alkylated homologs (C_1-C_4 DBT) (

Table 11; **Figure 24** – top three graphs). Mussels from almost all other areas had none of those PAH analytes and their homologs (**Figure 24**, **Figure 25**, and **Figure 26**). The exceptions, where one of the parent or one of the alkylated homologs appeared, were the of Head of Bay (C_2 fluorene = 1.87 ng/g dw), Mill Site North-Perimeter (fluorene = 4.40 ng/g dw), and Point Julia North (C_2 fluorene = 1.81 ng/g dw) area mussels. However, this finding should be taken with the caveat that other areas with lower overall PAH concentrations may have had fluorenes and dibenzothiophenes present below the detection limit (LOQs range of 2.00 to 7.49 for both analytes and their alkylated homologs) for this study.

Table 11. Concentrations of parent PAH analytes fluorene (C_0) and dibenzothiophene (C_0) and their alkylated homologs (C_1-C_4) at the three locations where nearly all were detected.

Area	Fluorene (ng/g dw)				
	C_0	**C_1**	**C_2**	**C_3**	**C_4**
Mill Site North	9.5	5.30	8.38	0	-
Mill Site South	11.35	4.64	5.25	5.47	-
Central Mill Site	30.02	16.21	18.01	17.41	-
	Dibenzothiophene (ng/g dw)				
	C_0	**C_1**	**C_2**	**C_3**	**C_4**
Mill Site North	3.57	1.42	5.65	1.29	1.08
Mill Site South	5.38	4.62	6.62	6.24	2.43
Central Mill Site	16.21	13.217	16.21	11.41	5.76

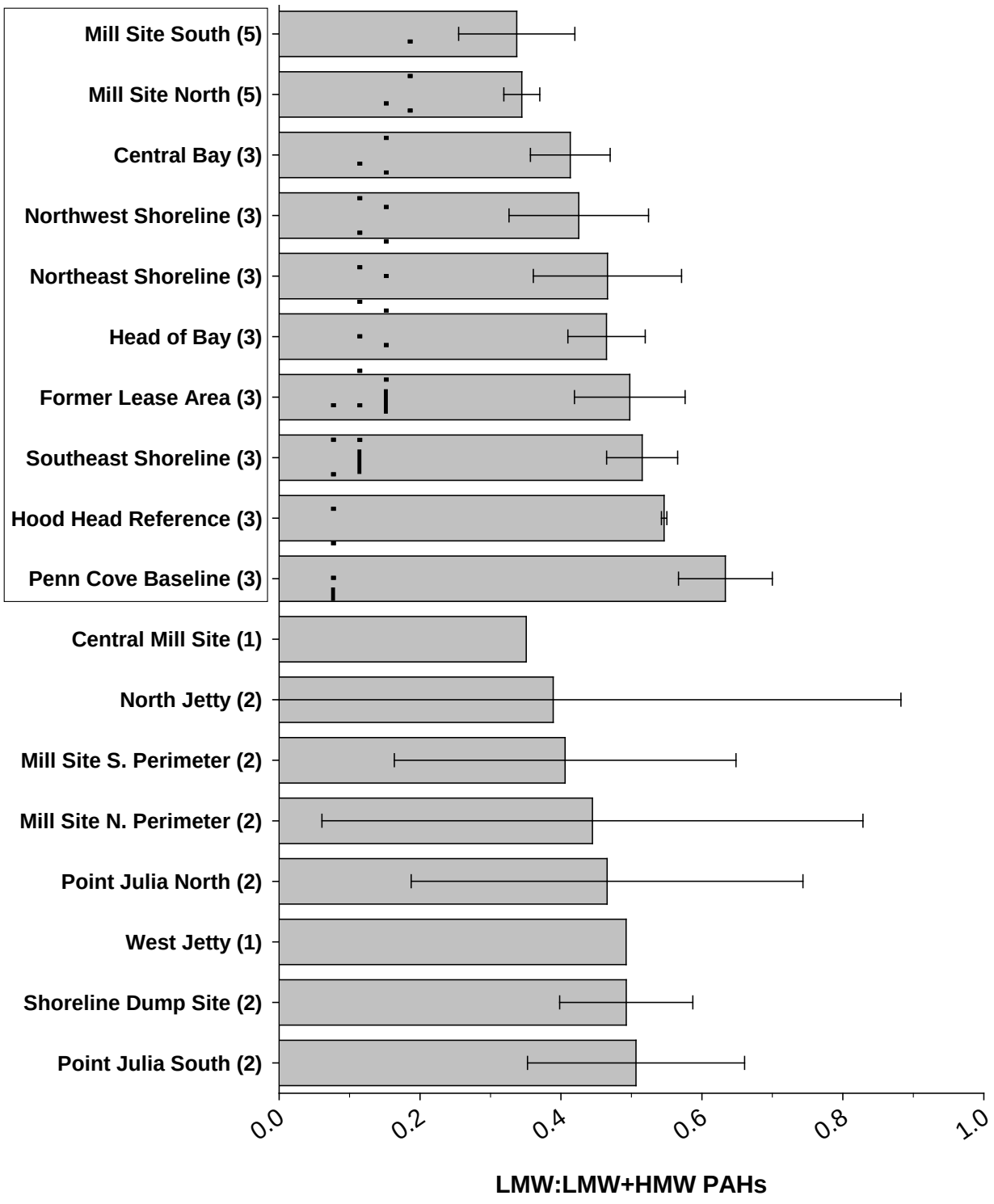

Figure 11. Ratio of low molecular weight (LMW) to high molecular weight (HMW) PAHs in mussels. ANOVA performed on 10 areas in box; replicates analyzed in parentheses; dotted lines indicate areas that were not significantly different from one another ($p>0.05$).

Total PCBs

Estimated total PCBs (eTPCBs) were detected in mussels from 100% of the study sites, though some individual PCB congeners were not detected in various samples (range of LOQs was 0.599 to 1.496 ng/g dw). Estimated total TPCB concentrations in mussels transplanted to Port Gamble Bay ranged from 15.74 to 60.52 ng/g dw (Table 12 and Figure 12). Estimated total PCBs in mussels transplanted to the Hood Head Reference area (mean 19.4 ng/g dw) were also higher than the starting concentration from Penn Cove (Penn Cove Baseline, mean 12.61 ng/g dw).

Area accounted for nearly all of the differences in eTPCB concentrations in mussels from this study ($F_{9,24}$ = 15.097; r^2 = 0.922; p <0.001). Mill Sites North and South (means 44.5 ±8.657 and 60.5 ±20.40 ng/g dw, respectively) accumulated significantly higher eTPCBs than mussels from the Hood Head Reference area and almost all the other areas in Port Gamble Bay, except the Former Lease Area (mean 40.58 ±4.080 ng/g dw; Tukey's honest significance tests, Figure 12). Though the Former Lease Area was grouped with the two Mill Sites, it was not significantly different from three of the five remaining Port Gamble Bay test areas. In addition, the eTPCB value of the Central Mill Site (n=1, concentration 41.9 ng/g dw) mussels was slightly higher than the mean for the Former Lease Area.

Table 12. Concentrations of estimated total PCBs (eTPCBs) in mussels from the start of the study (Penn Cove Baseline) and from all transplanted areas at the end of the study.

			eTPCBs (ng/g dw)			
Area	**# Samples analyzed**	**n detects**	**Min.**	**Max.**	**Mean**	**SD**
Penn Cove Baseline	3	3	8.0	15.9	12.61	4.1281
Hood Head Reference	3	3	16.6	22.8	19.40	3.1509
Central Bay	3	3	19.0	26.2	21.52	4.0124
Former Lease Area	3	3	37.6	45.2	40.58	4.0796
Head of Bay	3	3	12.3	18.5	16.37	3.5439
Mill Site North	5	5	31.3	55.1	44.50	8.6570
Mill Site South	5	5	25.2	75.0	60.52	20.4038
Northeast Shore	3	3	14.5	17.9	15.74	1.8760
Northwest Shore	3	3	18.8	22.3	20.30	1.8081
Southeast Shore	3	3	18.7	21.8	20.08	1.5611
Central Mill Site	1	1	-	-	41.9*	-
MSN Perimeter	2	2	17.6	22.2	19.87	-
MSS Perimeter	2	2	16.0	19.0	17.53	-
North of Jetty	2	2	18.5	26.9	22.71	-
Dump Site Shoreline	2	2	19.1	23.6	21.33	-
Point Julia North	2	2	17.9	27.9	22.92	-
Point Julia South	2	2	20.8	21.1	20.93	-
West of Jetty	1	1	-	-	23.3*	-

* Number is a single detected value (n = 1), not a mean.

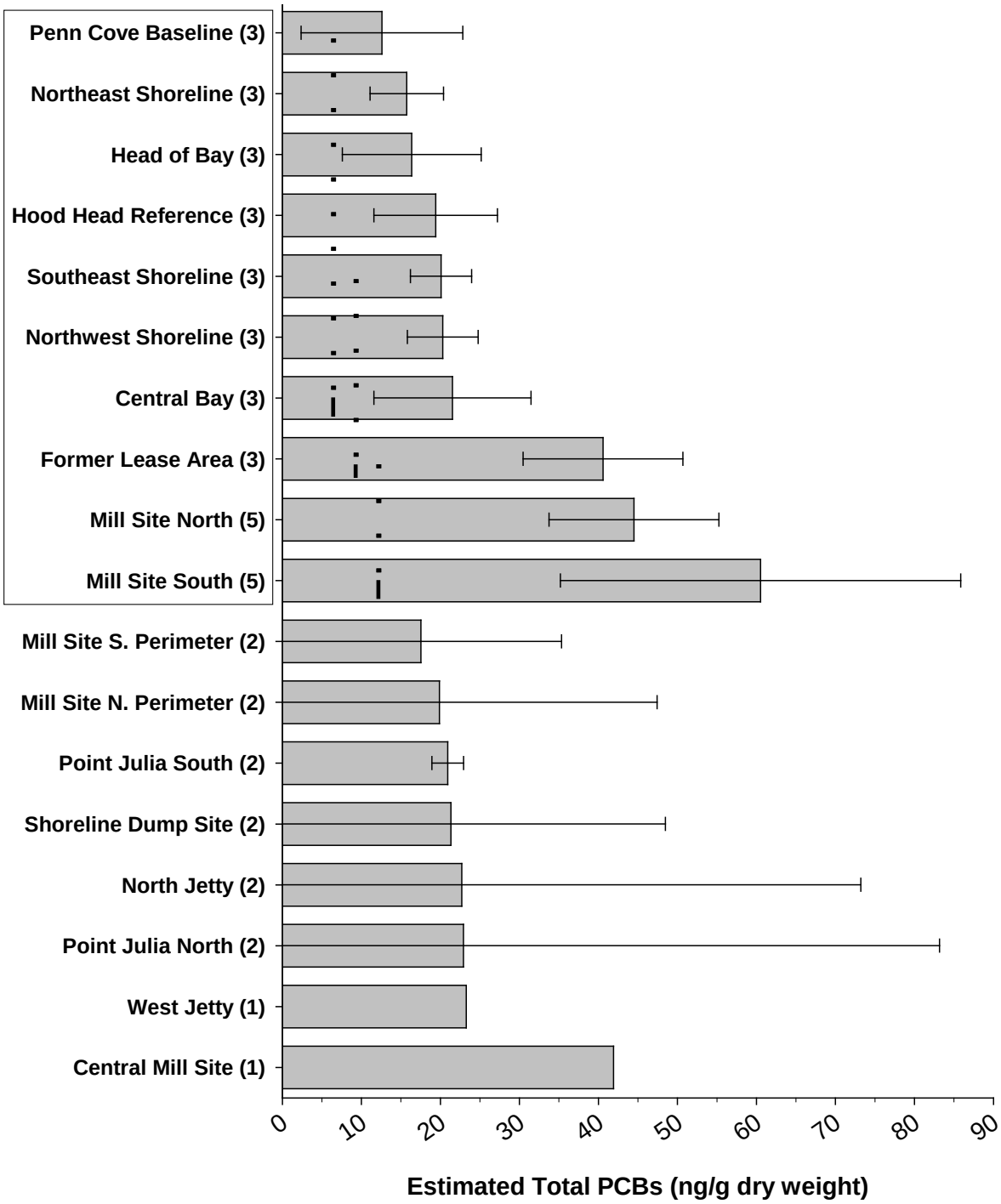

Figure 12. Concentration of estimated total PCBs (eTPCBs) in mussels. ANOVA performed on 10 areas in box; replicates analyzed in parentheses; dotted lines indicate areas that were not significantly different from one another ($p>0.05$).

Total PBDEs

Though there were individual PBDE congeners that were not detected in various samples (range of LOQs was 0.599 to 1.496 ng/g dw), when added together as a group, PBDEs ($\sum_{11}$PBDEs) were detected in mussels from 100% of the study sites. At the start of the study, the mean concentration of $\sum_{11}$PBDEss was 1.11 ng/g dw; at end of the study concentrations in mussels transplanted to Port Gamble Bay and the Hood Head Reference area ranged from 1.98 to 4.78 ng/g dw (Table 13).

As with the PAHs and PCBs, there was a significant difference in $\sum_{11}$PBDE concentrations among mussels from the different areas of this study ($F_{9,24} = 5.544$; $r^2 = 0.822$; $p < 0.001$). Mill Sites North and South, the Former Lease Area, and the Hood Head Reference area had significantly higher $\sum_{11}$PBDEs than mussels at the start of the study (Figure 13). The $\sum_{11}$PBDE concentration in the Central Mill Site (n = 1; concentration 4.4 ng/g dw) mussels was similar to the mean for Mill Sites and the Former Lease Area.

Table 13. Concentrations of the sum of 11 PBDEs ($\sum_{11}$PBDEs) detected in mussels from the start of the study (Penn Cove Baseline) and from all transplanted areas the end of the study.

			$\sum_{11}$PBDEs (ng/g dw)			
Area	**# Samples analyzed**	**n detects**	**Min.**	**Max.**	**Mean**	**SD**
Penn Cove Baseline	3	3	1.0	1.2	1.11	0.0792
Hood Head Reference	3	3	2.2	7.3	4.18	2.7313
Central Bay	3	3	1.6	4.3	2.49	1.5335
Former Lease Area	3	3	3.2	6.7	4.63	1.8135
Head of Bay	3	3	1.4	5.7	3.07	2.3026
Mill Site North	5	5	4.2	5.5	4.78	0.4992
Mill Site South	5	5	3.9	4.9	4.48	0.4316
Northeast Shore	3	3	1.7	3.1	2.17	0.8179
Northwest Shore	3	3	2.7	3.2	2.98	0.2547
Southeast Shore	3	3	1.8	2.4	2.17	0.2803
Central Mill Site	1	1	-	-	4.4*	-
MSN Perimeter	2	2	3.0	4.2	3.55	-
MSS Perimeter	2	2	3.1	4.3	3.72	-
North of Jetty	2	2	2.6	4.8	3.65	-
Dump Site Shoreline	2	2	1.4	3.1	2.25	-
Point Julia North	2	2	1.6	3.0	2.32	-
Point Julia South	2	2	1.9	2.0	1.98	-
West of Jetty	1	1	-	-	2.5*	-

* Number is a single detected value (n = 1), not a mean.

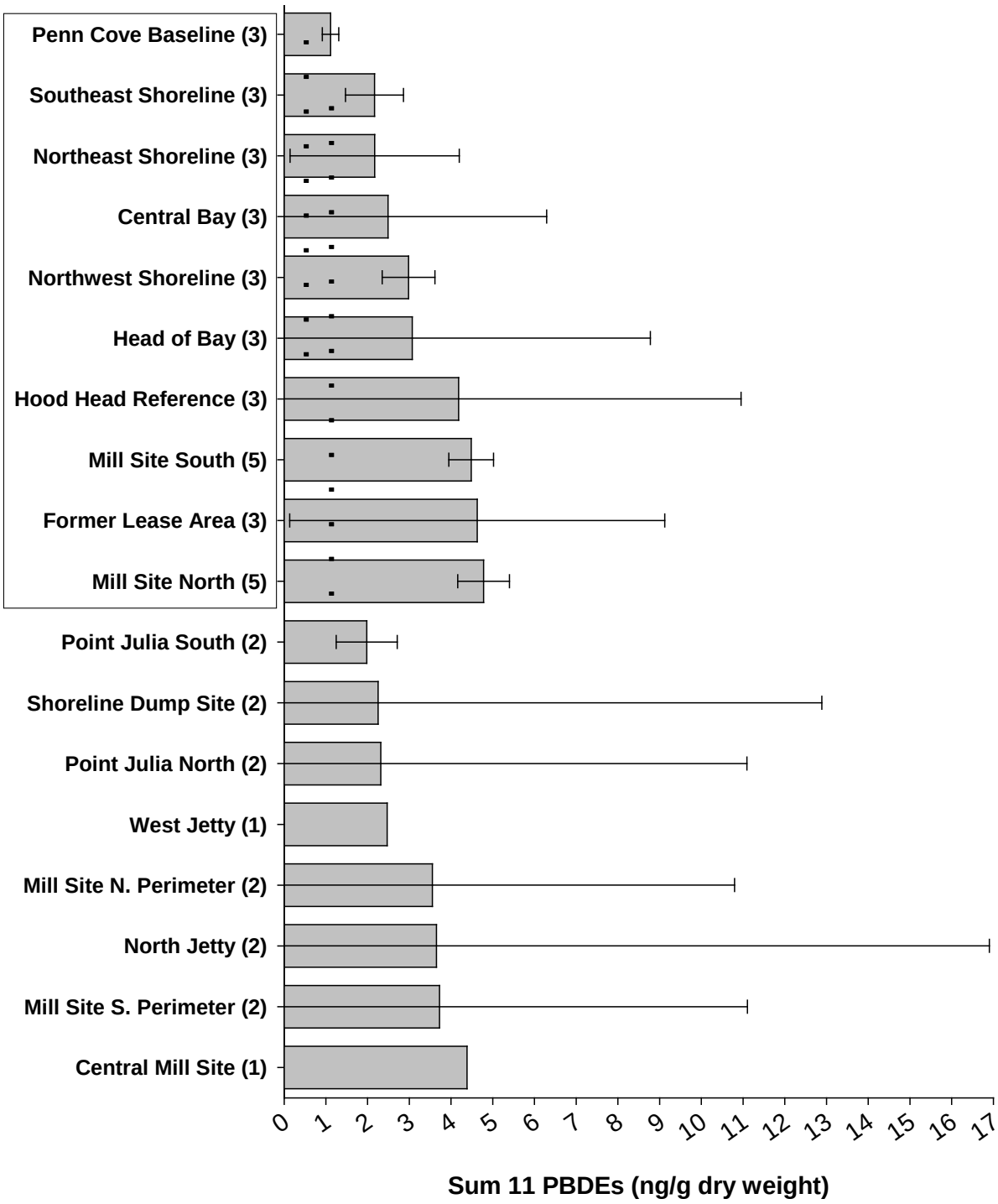

Figure 13. Concentrations of 11 PBDEs ($\sum_{11}$PBDEs) in mussels. ANOVA performed on 10 areas in box; replicates analyzed in parentheses; dotted lines indicate areas that were not significantly different from one another ($p>0.05$).

Total DDTs

Although there were individual DDT isomers that were not detected in various samples (range of LOQs was 0.593 to 1.496 ng/g dw), when added together as a group, DDTs ($\sum_6$DDTs) were detected in all of the Port Gamble Bay and Hood Head Reference area mussel tissue samples at the end of the study, and in two of the three samples from the start of the study (Table 14). The mean concentration of $\sum_6$DDTs at the start of the study was 1.05 ng/g dw, while mussels from Port Gamble Bay and the Hood Head Reference area ranged from 1.40 to 2.7 ng/g dw at the end of the study (Table 14).

There were significant differences in the concentrations of $\sum_6$DDTs in mussels from the different areas of this study ($F_{9,24} = 3.160$; $r^2 = 0.736$; $p = 0.012$). Mill Sites North and South, the Central Bay, and the Hood Head Reference area had significantly higher $\sum_6$DDTs than mussels at the start of the study (Penn Cove Baseline; Figure 13). As with PAHs, the $\sum_6$DDT concentration in mussels from the Central Mill Site (n = 1; concentration 2.7 ng/g dw) was the highest of all areas.

Table 14. Concentrations of the sum of 6 DDTs ($\sum_6$DDTs) detected in mussels from the start of the study (Penn Cove Baseline) and from all transplanted areas at the end of the study.

			$\sum_6$DDTs (ng/g dw)			
Area	**# Samples analyzed**	**n detects**	**Min.**	**Max.**	**Mean**	**SD**
Penn Cove Baseline	3	2	1.0	1.1	1.05	-
Hood Head Reference	3	3	1.6	2.0	1.79	0.1741
Central Bay	3	3	1.7	1.8	1.79	0.0744
Former Lease Area	3	3	1.6	1.7	1.65	0.0675
Head of Bay	3	3	1.5	1.7	1.60	0.0841
Mill Site North	5	5	1.7	1.8	1.71	0.0631
Mill Site South	5	5	1.5	4.0	2.16	1.0117
Northeast Shore	3	3	1.5	1.6	1.59	0.0497
Northwest Shore	3	3	1.5	1.6	1.58	0.0588
Southeast Shore	3	3	1.4	2.1	1.72	0.3936
Central Mill Site	1	1	-	-	2.7*	-
MSN Perimeter	2	2	1.7	1.8	1.73	-
MSS Perimeter	2	2	1.3	1.5	1.39	-
North of Jetty	2	2	1.5	1.8	1.67	-
Dump Site Shoreline	2	2	1.4	1.6	1.53	-
Point Julia North	2	2	1.7	1.8	1.71	-
Point Julia South	2	2	1.4	1.4	1.40	-
West of Jetty	1	1	-	-	1.7*	-

* Number is a single detected value (n = 1), not a mean.

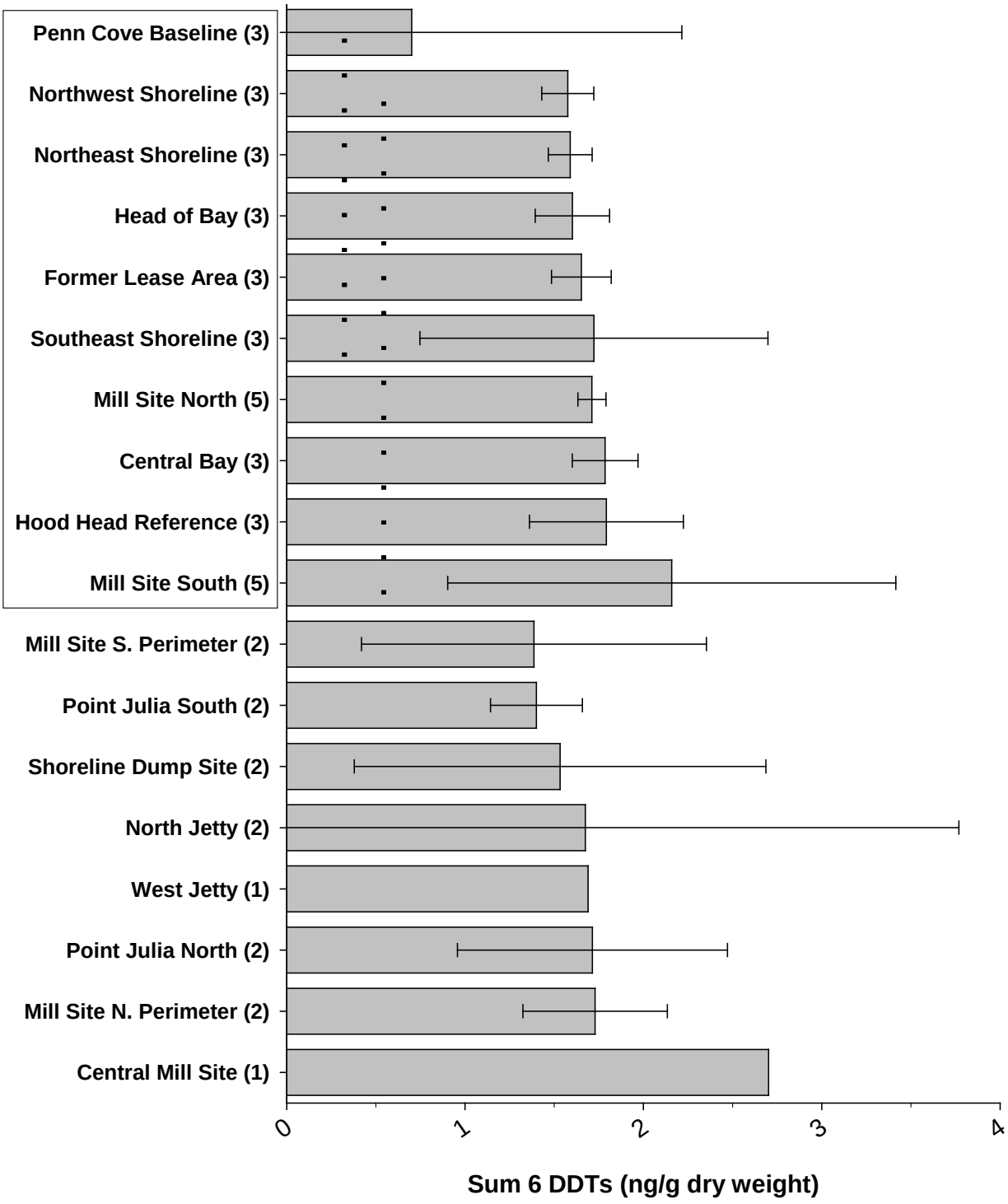

Figure 14. Concentrations of 6 DDTs ($\sum_6$DDTs) in mussels. ANOVA performed on 10 areas in box; replicates analyzed in parentheses; dotted lines indicate areas that were not significantly different from one another ($p>0.05$).

Other Organic Contaminants

Small amounts of dieldrin were detected at 24% (4 out of 17) of the transplanted mussel areas; the Southeast Shoreline (sites #1, #2, and #4), the Central Mill Site, Point Julia South (site #2), and West of Jetty (Table 15). Dieldrin was not detected in the Penn Cove Baseline samples. The detected values of dieldrin ranged in concentration from 0.861 to 0.91 ng/g dw, however, we caution that these concentrations fell within the range of detection limits for dieldrin in this study (i.e. LOQs ranged from 0.680 to 1.43 ng/g dw).

HCHs were detected in two of the 17 transplanted mussel areas (12%) at the end of the study; no HCHs were detected in the Penn Cove Baseline mussels (Table 15). Of the two composites where HCHs ($\sum_3$HCHs) were detected, one came from the Mill Site North #2 site (n = 1; 1.12 ng/g dw) and one from the Central Mill Site (n = 1; 3.72 ng/g dw). The range of LOQs for the $\sum_3$HCHs was 0.599 – 1.43 ng/g dw.

Chlordanes were only detected at the Southeast Shore #4 site (n = 1; 1.83 ng/g dw; Table 15). Mirex was detected only in the Central Mill Site mussels (n = 1; 1.08 ng/g dw). The range of LOQs for both the chlordanes and Mirex was from 0.599 to 1.43 ng/g dw. No $\sum_8$chlordanes or Mirex was detected in the Penn Cove Baseline starting mussels. Due to the low number and range of detected values, dieldrin, HCHs, chlordanes, and Mirex were not evaluated further in this study.

Aldrin, endosulfan 1, and hexachlorobenzene were not detected above the LOQ in the Penn Cove Baseline or any of the transplanted mussels during this study. The LOQs for aldrin, endosulfan 1, and hexachlorobenzene ranged from 0.599 – 1.43 ng/g dw.

Table 15. Concentrations (ng/g dw) of dieldrin, sum of 3 HCHs ($\sum_3$HCHs), sum of 8 chlordanes ($\sum_8$chlordanes), and Mirex detected in mussels from the start of the study (Penn Cove Baseline) and from all transplanted areas at the end of the study.

		Dieldrin					$\sum_3$HCHs		$\sum_8$Chlordanes		Mirex	
Area	**# Samples analyzed**	**n detects**	**Min.**	**Max.**	**Mean**	**SD**	**n detects**	**Mean**	**n detects**	**Mean**	**n detects**	**Mean**
Penn Cove Baseline	3	0	-	-	-	-	0	-	0	-	0	-
Hood Head Reference	3	0	-	-	-	-	0	-	0	-	0	-
Central Bay	3	0	-	-	-	-	0	-	0	-	0	-
Former Lease Area	3	0	-	-	-	-	0	-	0	-	0	-
Head of Bay	3	0	-	-	-	-	0	-	0	-	0	-
Mill Site North	5	0	-	-	-	-	1	1.12*	0	-	0	-
Mill Site South	5	0	-	-	-	-	0	-	0	-	0	-
Northeast Shore	3	0	-	-	-	-	0	-	0	-	0	-
Northwest Shore	3	0	-	-	-	-	0	-	0	-	0	-
Southeast Shore	3	3	0.77	0.92	0.861	0.0773	0	-	1	1.83*	0	-
Central Mill Site	1	1	-	-	0.901*	-	1	3.72*	0	-	1	1.08*
MSN Perimeter	2	0	-	-	-	-	0	-	0	-	0	-
MSS Perimeter	2	0	-	-	-	-	0	-	0	-	0	-
North of Jetty	2	0	-	-	-	-	0	-	0	-	0	-
Dump Site Shoreline	2	0	-	-	-	-	0	-	0	-	0	-
Point Julia North	2	0	-	-	-	-	0	-	0	-	0	-
Point Julia South	2	1	-	-	0.84*	-	0	-	0	-	0	-
West of Jetty	1	1	-	-	0.91*	-	0	-	0	-	0	-

* Number is a single detected value (n = 1), not a mean.

Dioxins and Furans

The range of LOQs fell between 0.339 to 0.422 pg/g dw for dioxins, and between 0.339 to 0.788 pg/g dw for furans. Not all homolog groups were detected in every composite analyzed; the most frequently detected homolog groups for the dioxins were the tetra-dioxins and octa-dioxin (both 100%) and the least detected group was the penta-dioxins (50%; Table 16). The most frequently detected homolog group for the furans was the tetra-furans (100%) and the least detected was the hexa-furans (44%).

When the homolog groups (HLGs) were summed together, $\sum_{5HLGs}$dioxins and $\sum_{5HLGs}$furans were detected in all of the mussel samples from the start of the study, and from all transplanted areas at the end of the study (Table 17 and Figure 15). The mean concentration of $\sum_{5HLGs}$dioxins in mussels from Port Gamble Bay ranged from 23.2 to 656.7 pg/g dw with Mill Site South having the highest mean value; the mean for the Hood Head Reference area mussels was 13.7 pg/g dw, while the Penn Cove Baseline mussels were 15.2 pg/g dw. The mean concentration of $\sum_{5HLGs}$furans in mussels from Port Gamble Bay was lower and ranged from 5.14 to 42.1 pg/g dw with Mill Site South again having the highest mean value; the mean for Hood Head Reference area was 7.88 pg/g dw, and Penn Cove Baseline mussels were 2.26 pg/g dw.

The $\sum_{5HLGs}$dioxins appeared to be dominated by octa-dioxin in all areas, especially along the Southeast Shoreline (Figure 16). Conversely, the $\sum_{5HLGs}$furans appeared to be dominated by the tetra-furans (Figure 17). However, the two samples from Mill Site South appear to be less dominated by tetra-furan, with a greater amount of hepta-furans and octa-furan than all the other areas. Not all homolog groups were represented in all areas.

Table 16. Number of samples in which the different homolog groups of dioxins and furans were detected in mussels from the start of the study (Penn Cove Baseline) and from all transplanted areas at the end of the study.

		Number of samples where any member of a homolog group was detected									
		Dioxin homolog groups					Furan homolog groups				
Area	# Samples analyzed	Tetra-	Penta-	Hexa-	Hepta-	*Octa-	Tetra-	Penta-	Hexa-	Hepta-	*Octa-
Penn Cove Baseline	2	2	0	2	2	2	2	0	0	1	1
Hood Head Reference	2	1	0	0	2	2	2	2	0	1	0
Central Bay	2	1	2	2	2	2	2	1	1	1	1
Former Lease Area	2	2	0	1	2	2	2	2	0	1	2
Head of Bay	1	1	0	1	1	1	1	1	0	1	1
Mill Site North	2	2	2	2	2	2	2	2	2	1	1
Mill Site South	2	2	2	2	2	2	2	2	2	2	2
Northeast Shoreline	1	1	1	1	1	1	1	0	0	1	0
Northwest Shoreline	3	2	1	3	3	2	3	2	1	1	0
Southeast Shoreline	1	1	1	1	0	1	1	1	0	1	0
Central Mill Site	1	1	0	1	1	1	1	1	1	1	0
MSN Perimeter	2	2	1	2	2	2	2	2	0	2	0
MSS Perimeter	2	2	0	2	2	2	2	2	0	1	0
North of Jetty	2	2	0	2	2	2	2	1	1	1	1
Dump Site Shoreline	2	2	2	2	2	2	2	0	1	2	1
Point Julia North	2	2	0	2	2	1	2	1	0	2	0
Point Julia South	2	2	0	2	2	2	2	2	1	2	1
West of Jetty	1	1	1	0	1	1	1	1	0	0	0
% of samples where homolog group was detected		100	50	89	94	100	100	83	44	94	50

* Octa- prefix represents a single homolog, not a group.

Table 17. Concentrations of total dioxins and total furans detected in mussels from the start of the study (Penn Cove Baseline) and from all transplanted areas at the end of the study. Totals are sums of the homolog groups show in Table 15.

		Total Dioxins (pg/g dw)					Total Furans (pg/g dw)				
Area	# Samples analyzed	n detects	Min.	Max.	Mean	SD	n detects	Min.	Max.	Mean	SD
Penn Cove Baseline	2	2	12.4	18.0	15.2	-	2	2.15	2.37	2.26	-
Hood Head Reference	2	2	12.9	14.5	13.7	-	2	5.18	10.6	7.88	-
Central Bay	2	2	35.7	38.3	37.0	-	2	8.17	16.9	12.6	-
Former Lease Area	2	2	23.8	32.3	28.0	-	2	8.88	9.20	9.04	-
Head of Bay	1	1	-	-	40.2*	-	1	-	-	13.2*	-
Mill Site North	2	2	92.9	98.1	95.5	-	2	10.8	11.0	10.9	-
Mill Site South	2	2	399.8	913.6	656.7	-	2	40.9	43.4	42.1	-
Northeast Shoreline	1	1	-	-	25.9*	-	1	-	-	5.70*	-
Northwest Shoreline	3	3	7.10	45.7	27.6	19.4	3	6.52	8.15	7.59	0.920
Southeast Shoreline	1	1	-	-	25.6*	-	1	-	-	7.50*	-
Central Mill Site	1	1	-	-	88.5*	-	1	-	-	10.9*	-
MSN Perimeter	2	2	30.5	44.5	37.5	-	2	7.82	8.59	8.20	-
MSS Perimeter	2	2	39.1	65.0	52.1	-	2	6.80	8.75	7.77	-
North of Jetty	2	2	29.9	53.4	41.7	-	2	5.98	12.4	9.19	-
Dump Site Shoreline	2	2	21.4	31.2	26.3	-	2	2.39	7.90	5.14	-
Point Julia North	2	2	14.5	31.8	23.2	-	2	6.19	9.81	8.00	-
Point Julia South	2	2	28.4	31.3	29.8	-	2	9.98	10.7	10.3	-
West of Jetty	1	1	-	-	33.7*	-	1	-	-	8.00*	-

* Number is a single detected value (n = 1), not a mean.

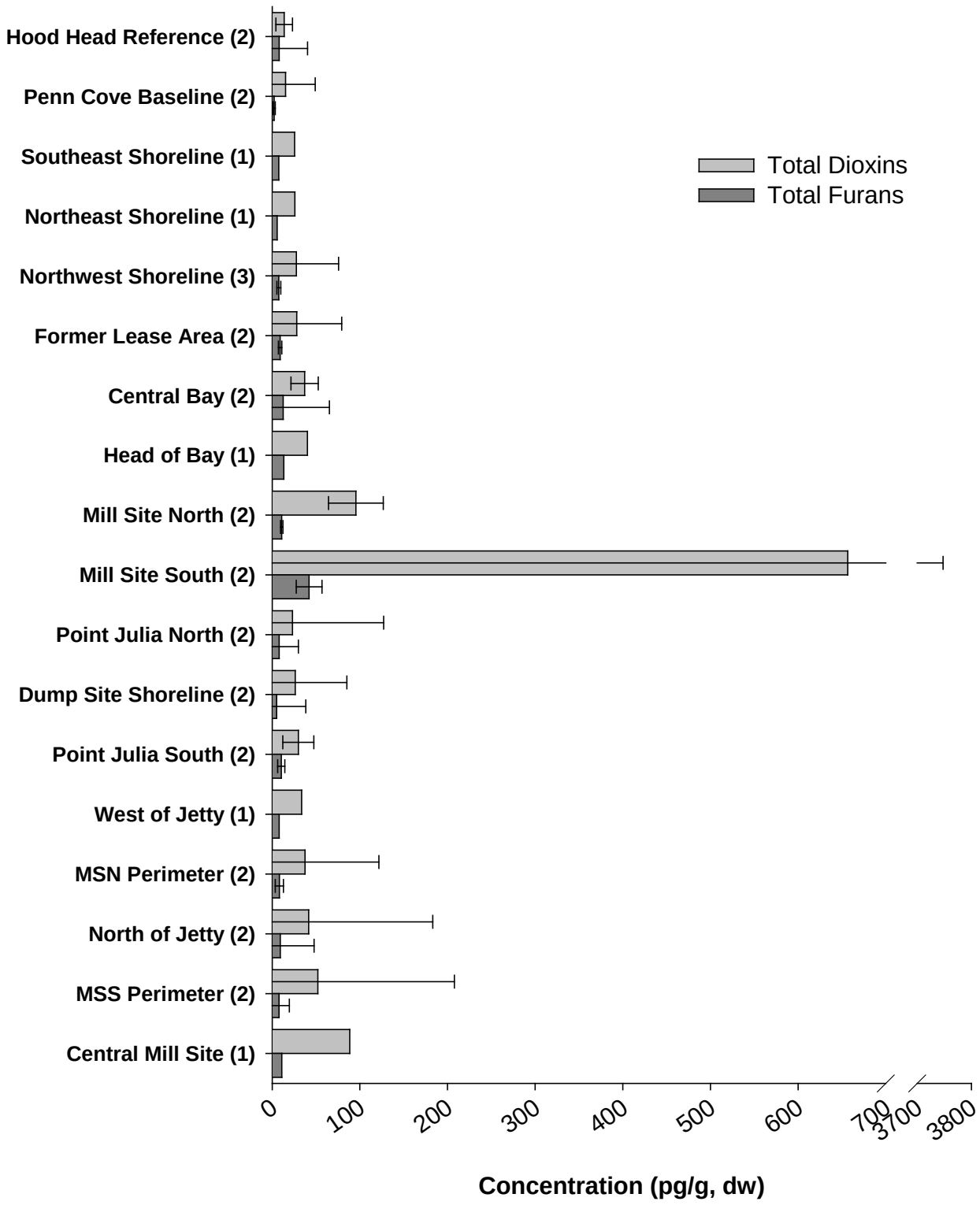

Figure 15. Total dioxins ($\sum_{5HLGs}$dioxins) and furans ($\sum_{5HLGs}$furans) in mussels from the start of the study (Penn Cove Baseline) and from all transplanted areas at the end of the study. Totals are sums of four homolog groups (HLGs) - see Table 16 for groupings.

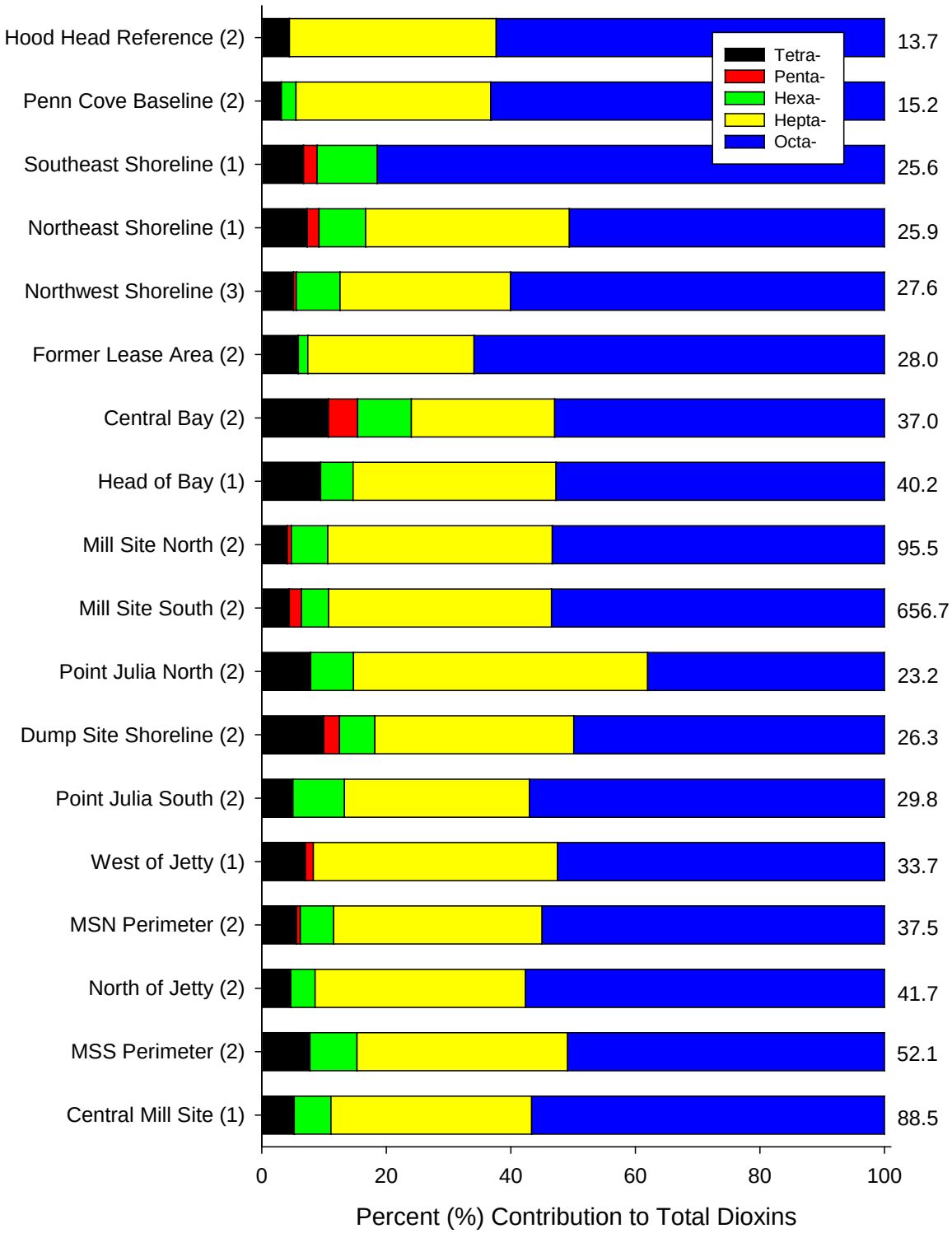

Figure 16. Percent (%) contribution of five homolog groups to the total dioxins detected in mussels from the start of the study (Penn Cove Baseline) and from all transplanted areas at the end of the study. Concentration of total dioxins (pg/g dw) in mussels from each area are shown on the right. See Table 16 for homolog groups used in totals.

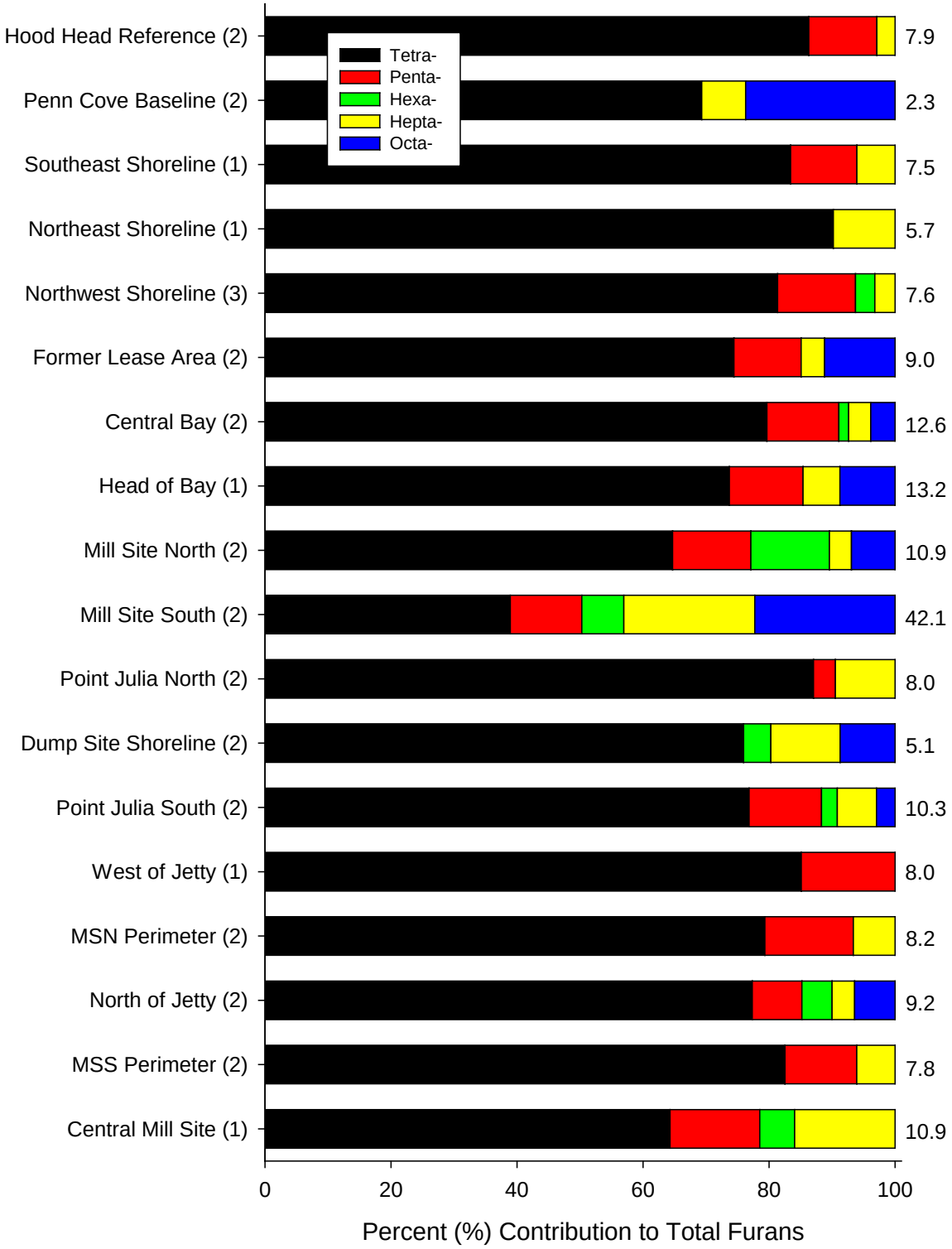

Figure 17. Percent (%) contribution of the different homolog groups to the total furans detected in mussels from the start of the study (Penn Cove Baseline) and from all transplanted areas at the end of the study. Concentration of total furans (pg/g dw) in mussels from each area are shown on the right. See Table 16 for homolog groups used in totals.

Metals

All metal concentrations are reported in parts per thousand, dry weight (mg/kg dw).

Mercury

Mercury was detected in all of the mussels at start of the study (0.023 mg/kg dw) and in mussels from all of the Port Gamble Bay areas and the Hood Head Reference area at the end of the study. The mean concentration of mussels from Port Gamble Bay and the Hood Head Reference area ranged narrowly from 0.033 to 0.051 mg/kg dw at the end of the study (Table 18).

There were significant differences in the concentrations of total mercury in mussels from the different areas of this study ($F_{9,24} = 35.794$; $r^2 = 0.965$; $p < 0.001$). Mussels from all the Port Gamble Bay and Hood Head Reference area had significantly higher mercury than mussels from the start of the study (Figure 18). In addition, mussels from Mill Sites North and South, and the Northwest Shoreline had significantly higher mercury than mussels from the Central Bay, Head of Bay, and the Former Lease Area; however, most of the other areas had intermediate mercury values that did not differ significantly from one another (Figure 18).

Table 18. Concentrations of total mercury detected in mussels from the start of the study (Penn Cove Baseline) and from all transplanted areas at the end of the study..

			Total Mercury (mg/kg dw)			
Area	**# Samples analyzed**	**n detects**	**Min.**	**Max.**	**Mean**	**SD**
Penn Cove Baseline	3	3	0.022	0.024	0.023	0.001
Hood Head Reference	3	3	0.039	0.042	0.040	0.001
Central Bay	3	3	0.028	0.038	0.033	0.005
Former Lease Area	3	3	0.034	0.042	0.037	0.004
Head of Bay	3	3	0.032	0.035	0.033	0.002
Mill Site North	5	5	0.043	0.052	0.047	0.004
Mill Site South	5	5	0.045	0.050	0.047	0.002
Northeast Shore	3	3	0.043	0.044	0.044	0.001
Northwest Shore	3	3	0.045	0.048	0.046	0.002
Southeast Shore	3	3	0.039	0.042	0.041	0.001
Central Mill Site	1	1	-	-	0.046	-
MSN Perimeter	2	2	0.045	0.046	0.045	-
MSS Perimeter	2	2	0.042	0.048	0.045	-
North of Jetty	2	2	0.040	0.045	0.043	-
Dump Site Shoreline	2	2	0.050	0.052	0.051	-
Point Julia North	2	2	0.043	0.048	0.045	-
Point Julia South	2	2	0.047	0.048	0.048	-
West of Jetty	1	1	-	-	0.048	-

* Number is a single detected value (n = 1), not a mean.

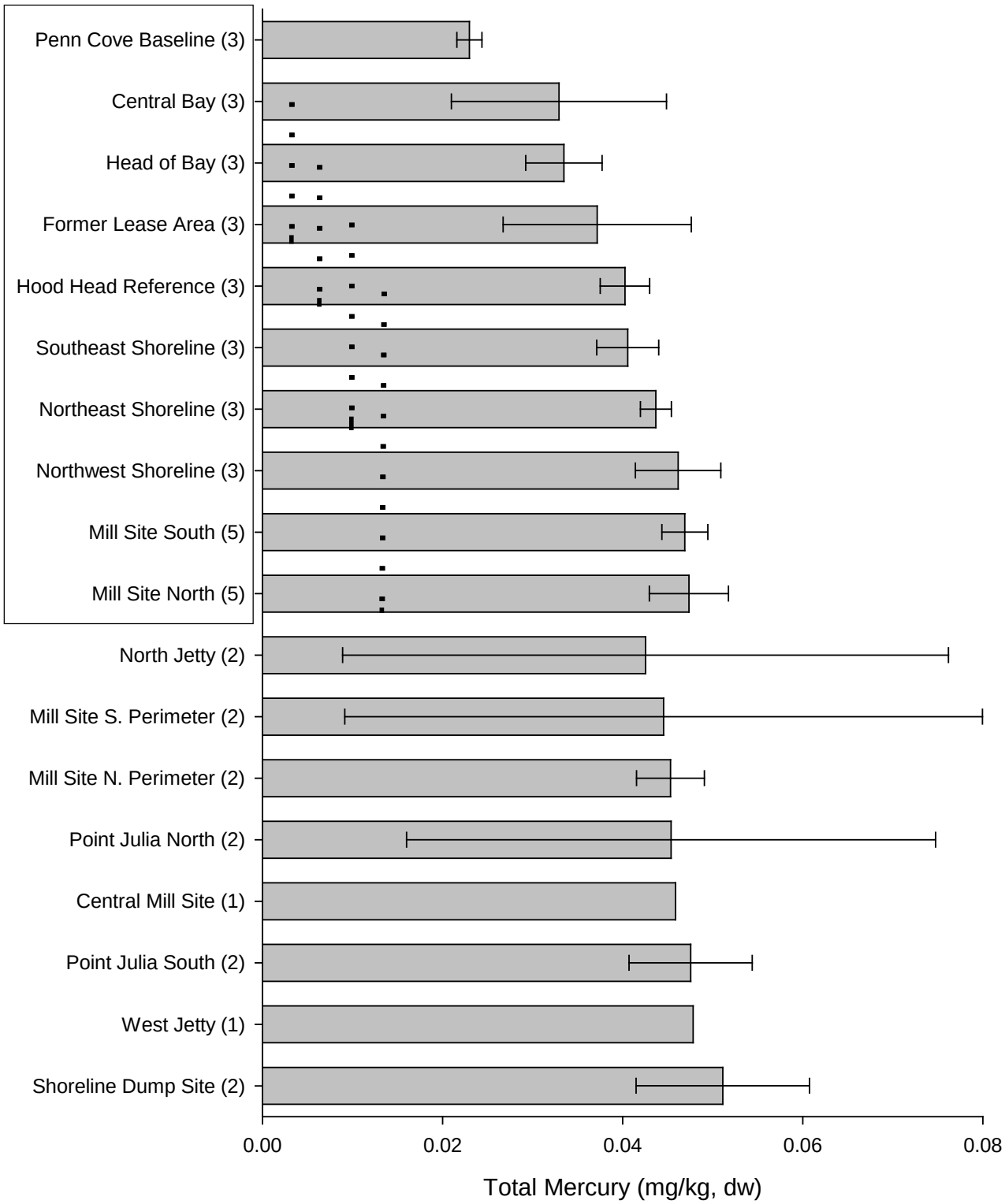

Figure 18. Concentrations of total mercury in mussels. ANOVA performed on 10 areas in box; replicates analyzed in parentheses; dotted lines indicate areas that were not significantly different from one another ($p>0.05$).

Lead

Lead was detected in all of the mussels at start of the study (0.175 mg/kg dw) and in all mussels from Port Gamble Bay and the Hood Head Reference area. The mean concentration of mussels from Port Gamble Bay and the Hood Head Reference area ranged narrowly from 0.217 to 0.457 mg/kg dw (Table 19).

There were significant differences in the concentrations of lead in mussels from the different areas of this study ($F_{9,24} = 15.626$; $r^2 = 0.924$; $p < 0.001$). Mussels from all the Port Gamble Bay areas, but not the Hood Head Reference area, had significantly higher lead than mussels from the start of the study (Figure 19). Also, mussels from Mill Site South and the Central Bay had significantly higher lead than mussels from Mill Site North, the Southeast Shoreline, the Former Lease Area, the Northwest Shoreline, and the Hood Head Reference Area. Mussels from the Northeast Shoreline and the Head of Bay had intermediate concentrations of lead.

Table 19. Concentrations of lead detected in mussels from the start of the study (Penn Cove Baseline) and from all transplanted areas at the end of the study.

			Lead (mg/kg dw)			
Area	**# Samples analyzed**	**n detects**	**Min.**	**Max.**	**Mean**	**SD**
Penn Cove Baseline	3	3	0.170	0.182	0.175	0.007
Hood Head Reference	3	3	0.195	0.245	0.217	0.026
Central Bay	3	3	0.930	0.478	0.429	0.045
Former Lease Area	3	3	0.244	0.305	0.279	0.032
Head of Bay	3	3	0.276	0.346	0.312	0.035
Mill Site North	5	5	0.236	0.269	0.259	0.014
Mill Site South	5	5	0.327	0.619	0.457	0.119
Northeast Shore	3	3	0.277	0.328	0.298	0.027
Northwest Shore	3	3	0.258	0.299	0.280	0.021
Southeast Shore	3	3	0.251	0.288	0.268	0.019
Central Mill Site	1	1	-	-	0.236	-
MSN Perimeter	2	2	0.236	0.241	0.239	-
MSS Perimeter	2	2	0.285	0.306	0.296	-
North of Jetty	2	2	0.260	0.435	0.348	-
Dump Site Shoreline	2	2	0.282	0.335	0.309	-
Point Julia North	2	2	0.263	0.263	0.263	-
Point Julia South	2	2	0.232	0.274	0.253	-
West of Jetty	1	1	-	-	0.245	-

* Number is a single detected value (n = 1), not a mean.

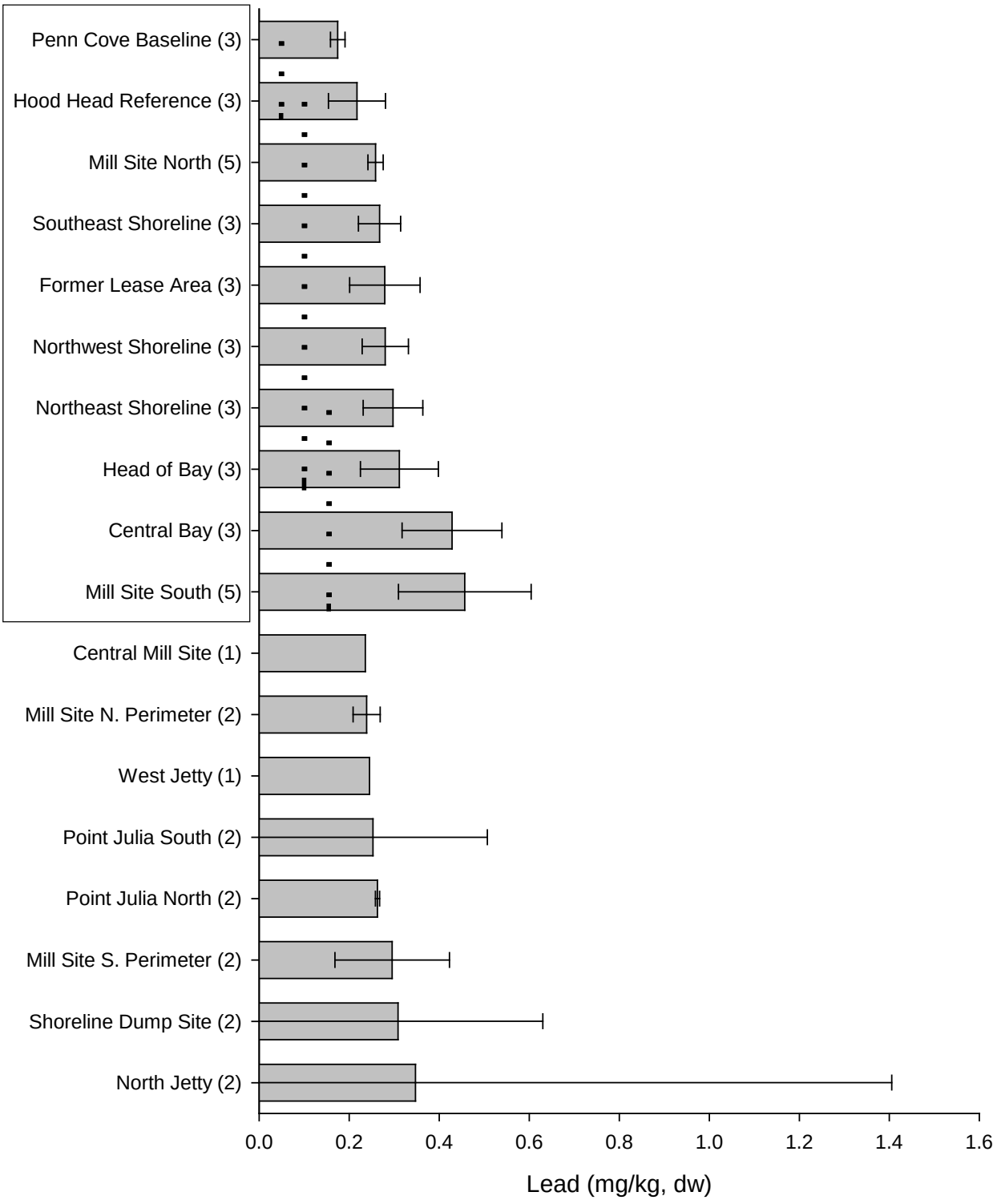

Figure 19. Concentrations of lead in mussels. ANOVA performed on 10 areas in box; replicates analyzed in parentheses; dotted lines indicate areas that were not significantly different from one another ($p>0.05$).

Arsenic

Arsenic was also detected in all of the mussels in this study. The starting concentration in the Penn Cove Baseline mussels was 5.34 mg/kg dw, while the concentrations in mussels from Port Gamble Bay and the Hood Head Reference area at the end of the study ranged from 6.925 to 8.457 mg/kg dw (Table 20).

There were significant differences in the concentrations of total arsenic in mussels from the different areas of this study ($F_{9,24} = 32.169$; $r^2 = 0.961$; $p < 0.001$). Mussels from all the Port Gamble Bay areas and the Hood Head Reference area had significantly higher total arsenic than mussels from the start of the study (Figure 20). In addition, mussels from the Central Bay had significantly higher total arsenic than mussels from the Hood Head Reference Area, and mussels from all the other Port Gamble Bay areas had intermediate concentrations of arsenic.

Table 20. Concentrations of total arsenic detected in mussels from the start of the study (Penn Cove Baseline) and from all transplanted areas at the end of the study.

			Total Arsenic (mg/kg dw)			
Area	**# Samples analyzed**	**n detects**	**Min.**	**Max.**	**Mean**	**SD**
Penn Cove Baseline	3	3	5.215	5.421	5.340	0.110
Hood Head Reference	3	3	7.212	7.547	7.380	0.168
Central Bay	3	3	7.547	8.621	8.384	0.354
Former Lease Area	3	3	7.677	7.895	7.822	0.125
Head of Bay	3	3	7.879	8.598	8.163	0.382
Mill Site North	5	5	7.516	8.405	7.871	0.336
Mill Site South	5	5	7.707	8.452	8.054	0.306
Northeast Shore	3	3	7.784	8.642	8.093	0.477
Northwest Shore	3	3	7.651	8.627	8.148	0.489
Southeast Shore	3	3	7.798	8.323	8.091	0.268
Central Mill Site	1	1	-	-	7.471	-
MSN Perimeter	2	2	7.375	7.500	7.438	-
MSS Perimeter	2	2	7.640	8.625	8.132	-
North of Jetty	2	2	6.522	7.329	6.925	-
Dump Site Shoreline	2	2	8.269	8.645	8.457	-
Point Julia North	2	2	7.239	7.764	7.502	-
Point Julia South	2	2	7.563	8.735	8.149	-
West of Jetty	1	1	-	-	7.453	-

* Number is a single detected value (n = 1), not a mean.

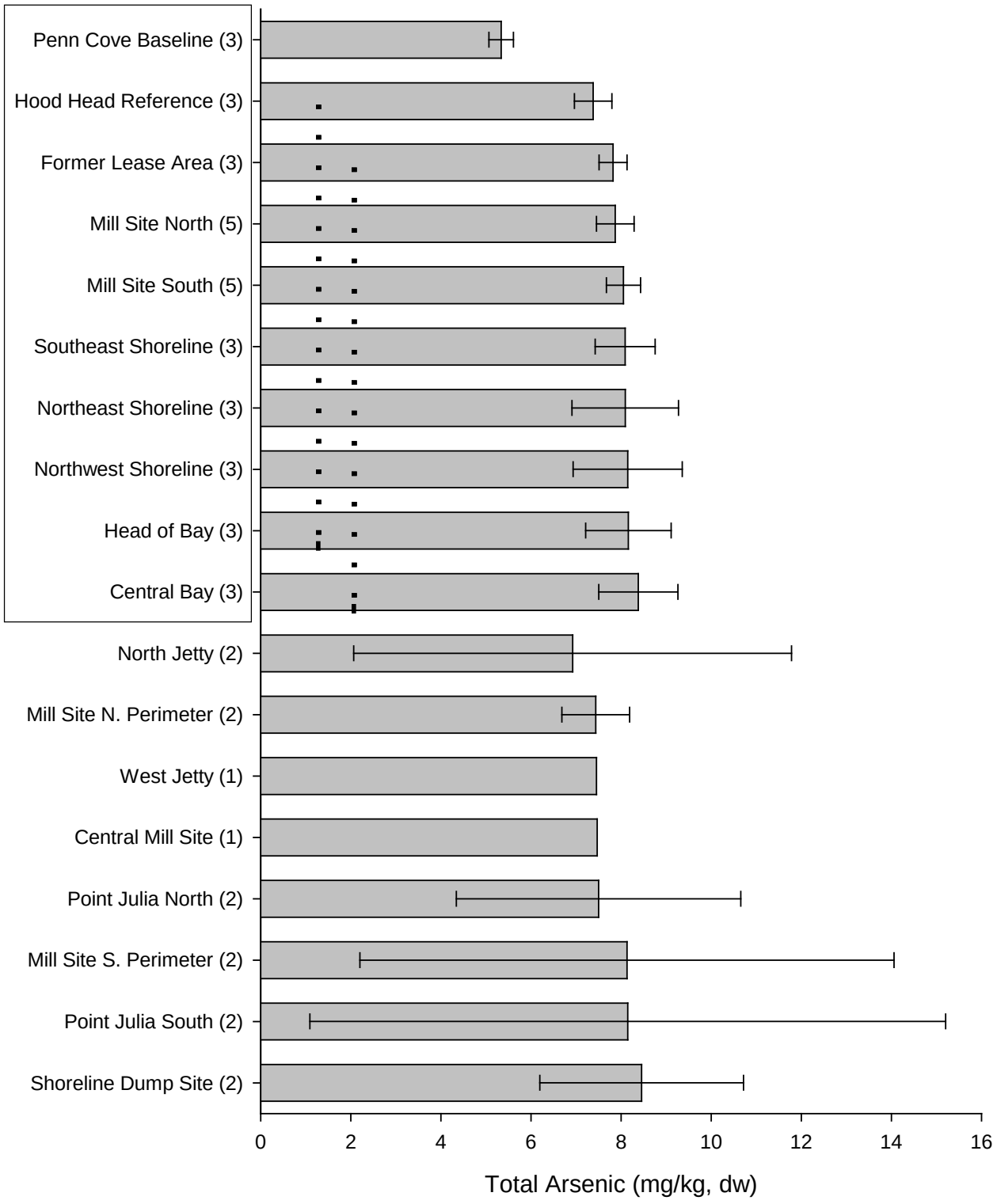

Figure 20. Concentrations of total arsenic in mussels. ANOVA performed on 10 areas in box; replicates analyzed in parentheses; dotted lines indicate areas that were not significantly different from one another ($p>0.05$).

Cadmium

Cadmium was detected in all of the mussels in this study. The starting concentration in the Penn Cove Baseline mussels was 2.208 mg/kg dw, and the concentrations in mussels from Port Gamble Bay and the Hood Head Reference area at the end of the study ranged from 2.556 to 3.028 mg/kg dw (Table 21).

There were significant differences in the concentrations of cadmium in mussels from the different areas ($F_{9,24}$ = 3.571; r^2 = 0.757; p = 0.006). Mussels from Mill Sites North and South, the Northwest Shoreline, and the Central Bay had significantly higher cadmium than mussels from the start of the study (Figure 21). Mussels from all the other Port Gamble Bay areas and the Hood Head Reference area had intermediate concentrations of cadmium.

Table 21. Concentrations of cadmium detected in mussels from the start of the study (Penn Cove Baseline) and from all transplanted areas at the end of the study.

		Cadmium (mg/kg dw)				
Area	**# Samples analyzed**	**n detects**	**Min.**	**Max.**	**Mean**	**SD**
Penn Cove Baseline	3	3	2.120	2.308	2.208	0.095
Hood Head Reference	3	3	2.606	2.744	2.664	0.072
Central Bay	3	3	2.312	3.524	2.913	0.606
Former Lease Area	3	3	2.643	2.987	2.758	0.199
Head of Bay	3	3	2.485	2.696	2.574	0.109
Mill Site North	5	5	2.553	3.233	2.861	0.261
Mill Site South	5	5	2.780	3.200	2.989	0.189
Northeast Shore	3	3	2.569	2.706	2.655	0.075
Northwest Shore	3	3	2.759	2.980	2.905	0.126
Southeast Shore	3	3	2.387	2.764	2.562	0.190
Central Mill Site	1	1	-	-	2.718	-
MSN Perimeter	2	2	2.800	2.913	2.856	-
MSS Perimeter	2	2	2.646	2.837	2.742	-
North of Jetty	2	2	2.317	2.795	2.556	-
Dump Site Shoreline	2	2	2.826	3.231	3.028	-
Point Julia North	2	2	2.571	2.632	2.602	-
Point Julia South	2	2	2.794	2.904	2.849	-
West of Jetty	1	1	-	-	2.857	-

* Number is a single detected value (n = 1), not a mean.

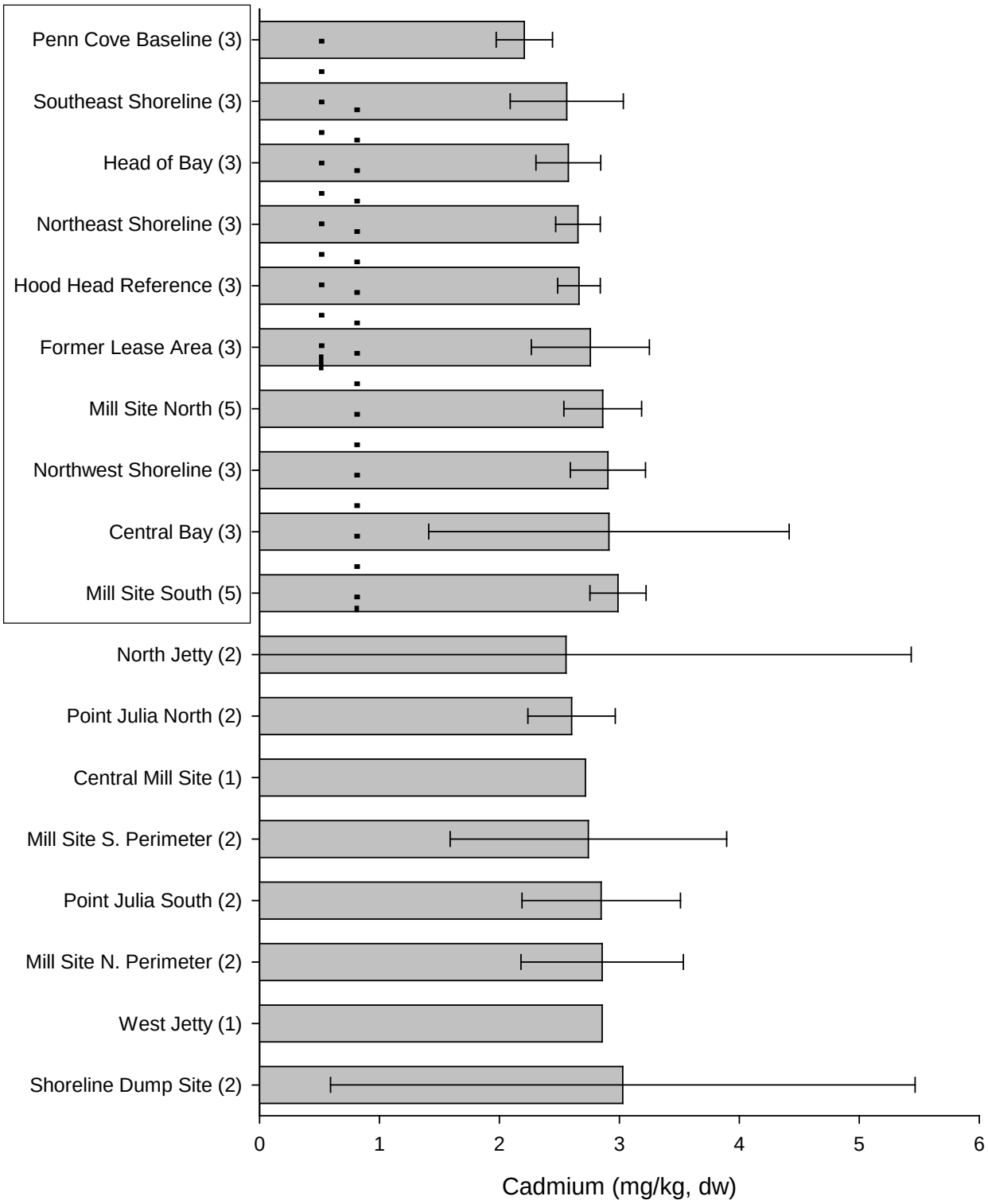

Figure 21. Concentrations of cadmium in mussels. ANOVA performed on 10 areas in box; replicates analyzed in parentheses; dotted lines indicate areas that were not significantly different from one another ($p>0.05$).

Copper

Copper was also detected in all of the mussels in this study. The starting concentration in the Penn Cove Baseline mussels was 5.071 mg/kg dw. The concentrations in mussels from Port Gamble Bay and the Hood Head Reference area at the end of the study ranged from 7.014 to 9.795 mg/kg dw (Table 22).

There were significant differences in the concentrations of copper in mussels from the different areas of this study ($F_{9,24} = 14.750$; $r^2 = 0.920$; $p < 0.001$). Mussels from all of the Port Gamble Bay areas and the Hood Head Reference area had significantly higher copper than mussels from the start of the study (Figure 22). In addition, mussels from Mill Site South had significantly higher concentrations of copper than mussels from Mill Site North and the Hood Head Reference area, but mussels from all other areas had intermediate concentrations of copper.

Table 22. Concentrations of copper detected in mussels from the start of the study (Penn Cove Baseline) and from all transplanted areas at the end of the study.

			Copper (mg/kg dw)			
Area	**# Samples analyzed**	**n detects**	**Min.**	**Max.**	**Mean**	**SD**
Penn Cove Baseline	3	3	5.024	5.140	5.071	0.061
Hood Head Reference	3	3	6.727	7.233	7.014	0.260
Central Bay	3	3	7.283	9.724	8.300	1.271
Former Lease Area	3	3	7.895	8.645	8.145	0.433
Head of Bay	3	3	7.888	8.293	8.040	0.220
Mill Site North	5	5	7.267	8.405	7.800	0.491
Mill Site South	5	5	8.408	10.839	9.795	0.928
Northeast Shore	3	3	7.485	9.383	8.547	0.969
Northwest Shore	3	3	7.470	8.924	8.253	0.734
Southeast Shore	3	3	7.826	9.107	8.447	0.641
Central Mill Site	1	1	-	-	7.059	-
MSN Perimeter	2	2	6.813	7.063	6.938	-
MSS Perimeter	2	2	6.832	7.625	7.229	-
North of Jetty	2	2	6.832	7.764	7.298	-
Dump Site Shoreline	2	2	7.179	8.258	7.719	-
Point Julia North	2	2	7.301	8.323	7.812	-
Point Julia South	2	2	7.813	8.434	8.123	-
West of Jetty	1	1	-	-	7.453	-

* Number is a single detected value (n = 1), not a mean.

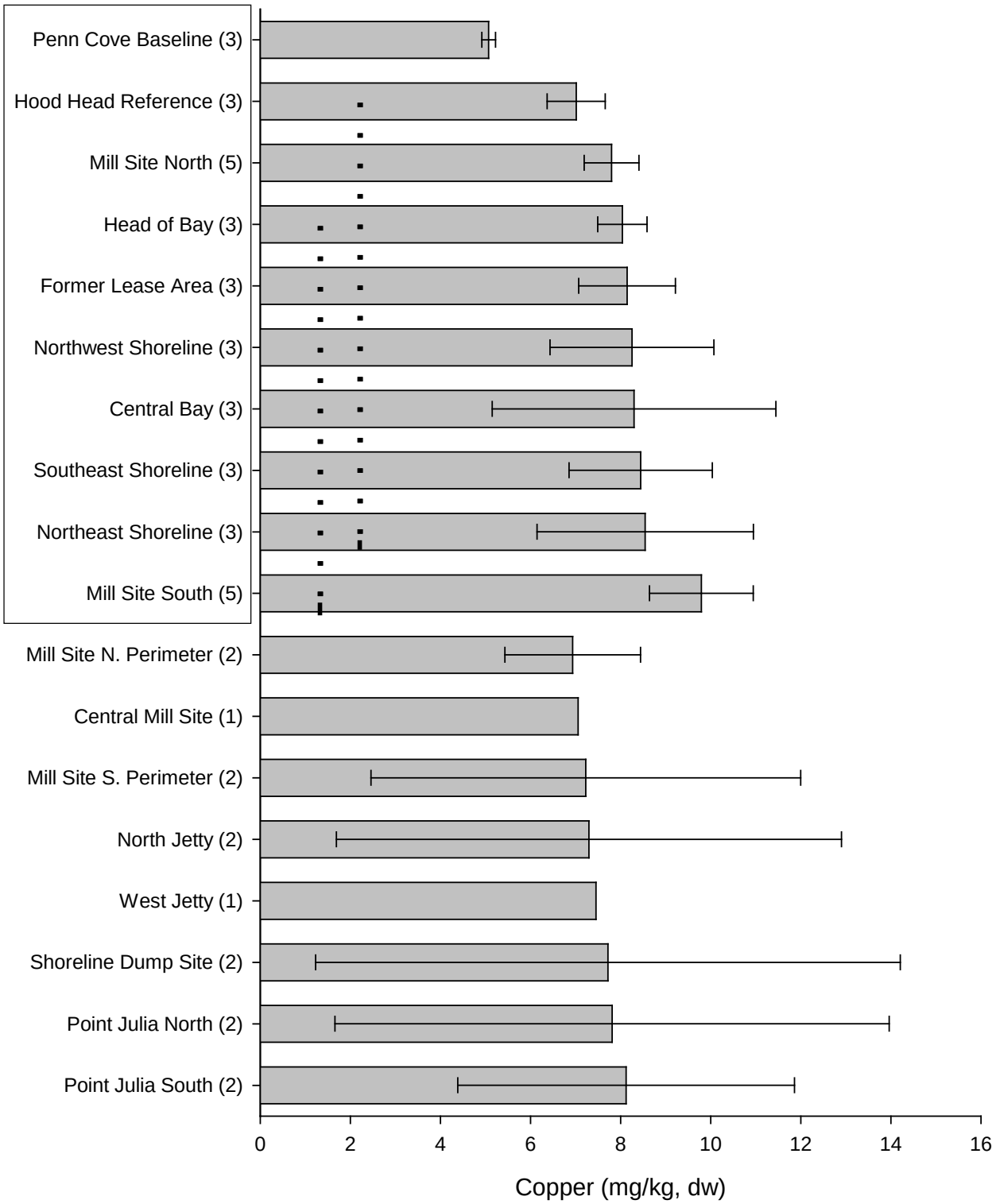

Figure 22. Concentrations of copper in mussels. ANOVA performed on 10 areas in box; replicates analyzed in parentheses; dotted lines indicate areas that were not significantly different from one another ($p>0.05$).

Zinc

Zinc was also detected in all of the mussels in this study; the mean zinc concentration in the Penn Cove Baseline mussels was 71.835 mg/kg dw. Zinc concentrations in mussels from Port Gamble Bay and the Hood Head Reference area at the end of the study ranged from 99.367 to 114.118 mg/kg dw (Table 22).

Mussels from all the Port Gamble Bay areas and the Hood Head Reference area had significantly higher concentrations of zinc than mussels from the start of the study ($F_{9,24} = 8.518$; $r^2 = 0.873$; $p < 0.001$; Figure 23). However, there were no significant differences in the concentrations of zinc among mussels from the Port Gamble Bay areas or the Hood Head Reference area.

Table 23. Concentrations of zinc detected in mussels from the start of the study (Penn Cove Baseline) and from all transplanted areas at the end of the study.

			Zinc (mg/kg dw)			
Area	**# Samples analyzed**	**n detects**	**Min.**	**Max.**	**Mean**	**SD**
Penn Cove Baseline	3	3	67.290	76.923	71.835	4.840
Hood Head Reference	3	3	98.182	109.434	102.539	6.040
Central Bay	3	3	97.688	121.084	106.028	13.065
Former Lease Area	3	3	102.581	108.772	105.539	3.105
Head of Bay	3	3	95.152	107.927	103.304	7.081
Mill Site North	5	5	93.789	115.337	103.694	8.258
Mill Site South	5	5	105.806	122.024	113.988	7.408
Northeast Shore	3	3	101.277	120.370	108.796	10.181
Northwest Shore	3	3	107.843	123.418	113.232	8.826
Southeast Shore	3	3	90.476	112.739	101.693	11.132
Central Mill Site	1	1	-	-	114.118	-
MSN Perimeter	2	2	102.500	105.000	103.750	-
MSS Perimeter	2	2	103.106	108.125	105.615	-
North of Jetty	2	2	99.379	119.876	109.627	-
Dump Site Shoreline	2	2	96.154	102.581	99.367	-
Point Julia North	2	2	102.454	113.043	107.749	-
Point Julia South	2	2	101.250	106.024	103.637	-
West of Jetty	1	1	-	-	113.043	-

* Number is a single detected value (n = 1), not a mean.

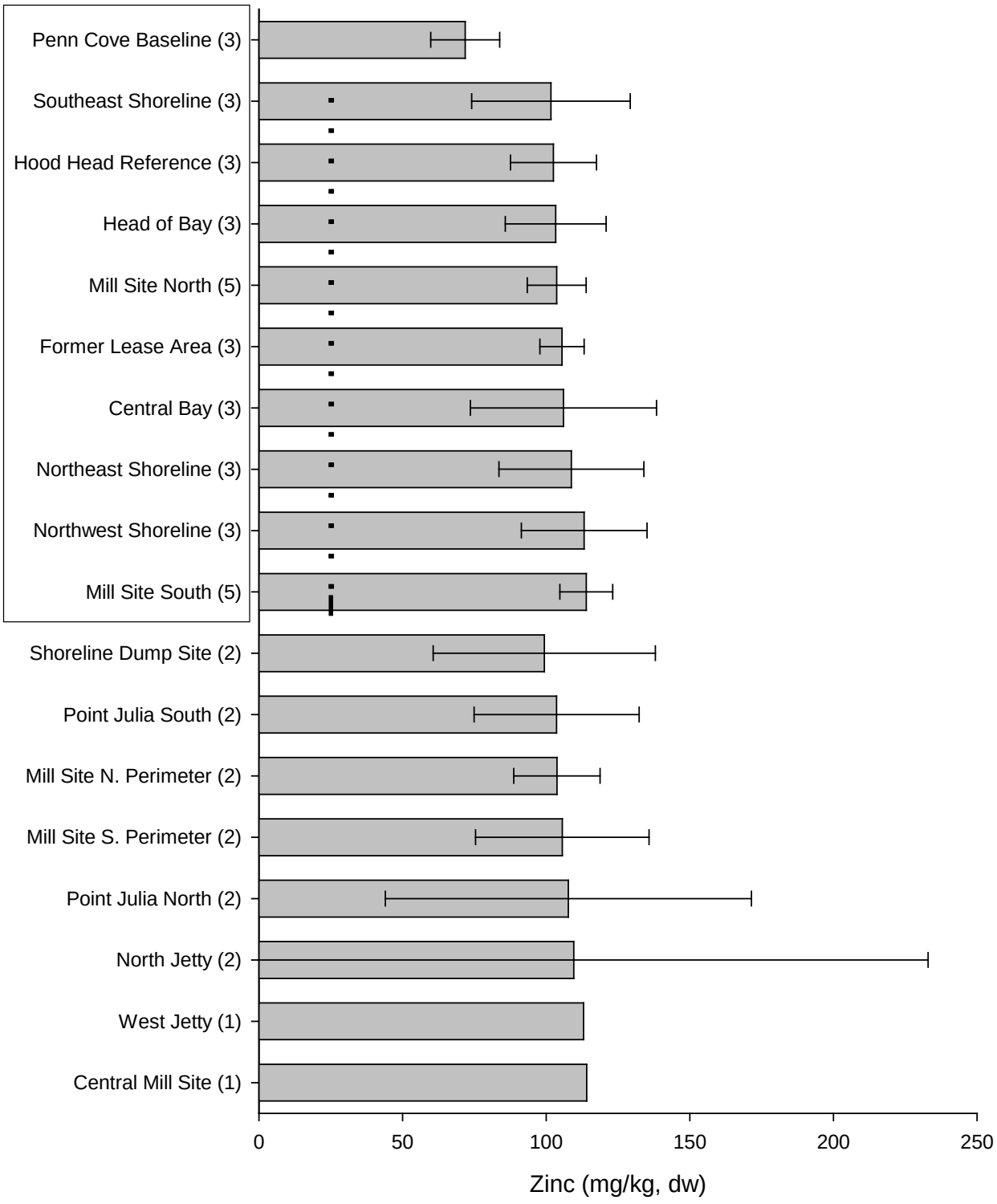

Figure 23. Concentrations of zinc in mussels. ANOVA performed on 10 areas in box; replicates analyzed in parentheses; dotted lines indicate areas that were not significantly different from one another ($p>0.05$).

4.0 Conclusions

This study provides a synoptic evaluation of the geographic extent and magnitude of contamination in a native mussel (*Mytilus trossulus*) in Port Gamble Bay prior to a large-scale remediation project. The data herein are intended as a baseline against which future conditions in Port Gamble Bay mussels can be compared.

The most significant observations from this study were the disproportionately high accumulations of PAHs and PCBs in mussels transplanted to the Mill Site North (SMA 1) and Mill Site South (SMA 2) areas of Port Gamble Bay. Mussels from Mill Sites North and South had concentrations of total PAHs that were an order of magnitude greater than mussels from all other sites in Port Gamble Bay, with the exception of the Central Mill Site. Though not statistically tested due to lack of replicates (n = 1), we noted that the mussel composite from the Central Mill Site was frequently similar to or higher in organic contaminant concentrations than mussels from Mill Sites North and South (e.g. for PAHs, PCBs, PBDEs, DDTs). This similarity was not surprising given the proximity of the Central Mill Site mussel cage to Mill Sites North and South (Figure 3). In addition, Mill Sites North and South had higher concentrations of total dioxins and total furans, compared to other areas of Port Gamble Bay.

The PAH fingerprints and diagnostic ratios in mussels from Port Gamble Bay suggest either combustion (i.e. burning of wood, coal, or fossil fuels) and/or highly weathered oil are the dominant source(s) of PAHs in the bay. In addition, there was some evidence to suggest mussels from Mill Sites North and South and the Central Mill Site accumulated more fluorenes and dibenzothiophenes than mussels from the rest of Port Gamble Bay, though we qualify this finding by noting that this result may be an artifact of the combined laboratory detection limits for PAHs in this study and the high overall concentrations of PAHs at those three locations.

Mussels in Port Gamble Bay almost always had concentrations of metals that were significantly higher than the starting condition, with the exception of cadmium. Though the concentrations of metals differed somewhat among the Port Gamble Bay sites, there were no obvious patterns. However, we noted that mussels from the Dump Site Shoreline exhibited the highest concentrations of mercury, arsenic, and cadmium.

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- King County Environmental Laboratory (Seattle, WA) for all metals,
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7.0 Appendices

Appendix A. Glossary

Adductor muscle – a muscle in the interior of a bivalve mollusk which close the valves.

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

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

Analyte - An element, ion, compound, or chemical moiety (pH, alkalinity) which is to be determined. (Kammin, 2010)

Benthic - Living on or closely associated with the bottom of a body of water. Or relating to, or living in a benthos, which is the sediment-water interface of an ocean, sea, or lake.

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

Bivalves - A taxonomic class of marine and freshwater mollusks that have a laterally compressed body enclosed by a shell in two hinged parts. This class includes the clams, oysters, mussels, scallops and numerous other families.

Blank - A synthetic sample, free of the analyte(s) of interest. For example, in water analysis, pure water is used for the blank. In chemical analysis, a blank is used to estimate the analytical response to all factors other than the analyte in the sample. In general, blanks are used to assess possible contamination or inadvertent introduction of analyte during various stages of the sampling and analytical process. (USGS, 1998)

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

Check standard - A substance or reference material obtained from a source independent from the source of the calibration standard; used to assess bias for an analytical method. This is an obsolete term, and its use is highly discouraged. See Calibration Verification Standards, Lab Control Samples (LCS), Certified Reference Materials (CRM), and/or spiked blanks. These are all check standards, but should be referred to by their actual designator (i.e. CRM, LCS, etc.) (Kammin, 2010; Ecology, 2004).

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

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

Composite – Predetermined number of individuals consisting of one species specific matrix (i.e., muscle tissue) created through homogenous mixing to represent a location or field replicate in chemical analysis.

Condition Index (CI) - serves as an indication of the influence of seasonal fluctuations, such as temperature and food availability, on the physiological status of bivalves. It allows for better comparison of mussels from different locations by normalizing biological changes over time and minimizing the influence of internal factors (e.g. mussel growth rates). CI = dry weight (g) of soft tissue/shell length (mm) X 100.

Contaminant - A substance that makes something dirty, polluted, or toxic

Continuing Calibration Verification Standard (CCV) - A QC sample analyzed with samples to check for acceptable bias in the measurement system. The CCV is usually a midpoint calibration standard that is re-run at an established frequency during the course of an analytical run. (Kammin, 2010)

Control chart - A graphical representation of quality control results demonstrating the performance of an aspect of a measurement system. (Kammin, 2010; Ecology 2004)

Control limits - Statistical warning and action limits calculated based on control charts. Warning limits are generally set at +/- 2 standard deviations from the mean, action limits at +/- 3 standard deviations from the mean. (Kammin, 2010)

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

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

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

Dataset - A grouping of samples, usually organized by date, time and/or analyte. (Kammin, 2010)

Data validation - An analyte-specific and sample-specific process that extends the evaluation of data beyond data verification to determine the usability of a specific data set. It involves a detailed examination of the data package, using both professional judgment, and objective criteria, to determine whether the MQOs for

precision, bias, and sensitivity have been met. It may also include an assessment of completeness, representativeness, comparability and integrity, as these criteria relate to the usability of the dataset. Ecology considers four key criteria to determine if data validation has actually occurred. These are:

- Use of raw or instrument data for evaluation
- Use of third-party assessors
- Dataset is complex
- Use of EPA Functional Guidelines or equivalent for review

Examples of data types commonly validated would be:

- Gas Chromatography (GC)
- Gas Chromatography-Mass Spectrometry (GC-MS)
- Inductively Coupled Plasma (ICP)

The end result of a formal validation process is a determination of usability that assigns qualifiers to indicate usability status for every measurement result. These qualifiers include:

- No qualifier, data is usable for intended purposes
- J (or a J variant), data is estimated, may be usable, may be biased high or low
- REJ, data is rejected, cannot be used for intended purposes (Kammin, 2010; Ecology, 2004)

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

Detection limit (limit of detection) - The concentration or amount of an analyte which can be determined to a specified level of certainty to be greater than zero. (Ecology, 2004)

Duplicate samples - two samples taken from and representative of the same population, and carried through and steps of the sampling and analytical procedures in an identical manner. Duplicate samples are used to assess variability of all method activities including sampling and analysis. (USEPA, 1997)

Field blank - A blank used to obtain information on contamination introduced during sample collection, storage, and transport. (Ecology, 2004)

Initial Calibration Verification Standard (ICV) - A QC sample prepared independently of calibration standards and analyzed along with the samples to check for acceptable bias in the measurement system. The ICV is analyzed prior to the analysis of any samples. (Kammin, 2010)

Laboratory Control Sample (LCS) - A sample of known composition prepared using contaminant-free water or an inert solid that is spiked with analytes of interest at the midpoint of the calibration curve or at the level of

concern. It is prepared and analyzed in the same batch of regular samples using the same sample preparation method, reagents, and analytical methods employed for regular samples. (USEPA, 1997)

Limit of Quantitation (LOQ) – In organic analyses, the LOQ is the concentration that would be calculated if that analyte had a GC/MS response area equal to the area of the lowest level calibration standard used in that calibration. Similar to a Detection Limit (DL) in metals analyses.

Matrix spike - A QC sample prepared by adding a known amount of the target analyte(s) to an aliquot of a sample to check for bias due to interference or matrix effects. (Ecology, 2004)

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

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

Method - A formalized group of procedures and techniques for performing an activity (e.g., sampling, chemical analysis, data analysis), systematically presented in the order in which they are to be executed. (EPA, 1997)

Method blank - A blank prepared to represent the sample matrix, prepared and analyzed with a batch of samples. A method blank will contain all reagents used in the preparation of a sample, and the same preparation process is used for the method blank and samples. (Ecology, 2004; Kammin, 2010)

Method Detection Limit (MDL) - This definition for detection was first formally advanced in 40CFR 136, October 26, 1984 edition. MDL is defined there as the minimum concentration of an analyte that, in a given matrix and with a specific method, has a 99% probability of being identified, and reported to be greater than zero. (Federal Register, October 26, 1984)

Organic - Material derived from the remains or products of living entities.

Percent Relative Standard Deviation (%RSD) - A statistic used to evaluate precision in environmental analysis. It is determined in the following manner:

> Percent relative standard deviation, %RSD = (100 * s)/x where s = sample standard deviation, and x = sample mean (Kammin, 2010)

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

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

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

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

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

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

Relative Percent Difference (RPD) - RPD is commonly used to evaluate precision. The following formula is used:

Abs(a-b)/((a+b)/2) * 100

Where a and b are 2 sample results, and abs(XX) indicates absolute value. RPD can be used only with 2 values. More values, use %RSD. (Ecology, 2004)

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

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

Resect - To remove part or all of an organ or tissue.

Resection - Excision of a portion or all of an organ or other structure.

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

Sample (statistical) – A finite part or subset of a statistical population. (USEPA, 1997)

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

Shellfish - An exoskeleton or shell bearing aquatic invertebrate, includes various species of mollusks (bivalves), crustaceans, and echinoderms.

Spiked blank - A specified amount of reagent blank fortified with a known mass of the target analyte(s); usually used to assess the recovery efficiency of the method. (USEPA, 1997)

Spiked sample - A sample prepared by adding a known mass of target analyte(s) to a specified amount of matrix sample for which an independent estimate of target analyte(s) concentration is available. Spiked samples can be used to determine the effect of the matrix on a method's recovery efficiency. (USEPA, 1997)

Split Sample – The term split sample denotes when a discrete sample is further subdivided into portions, usually duplicates. (Kammin, 2010)

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

Surrogate – For environmental chemistry, a surrogate is a substance with properties similar to those of the target analyte(s). Surrogates are unlikely to be native to environmental samples. They are added to environmental samples for quality control purposes, to track extraction efficiency and/or measure analyte recovery. Deuterated organic compounds are examples of surrogates commonly used in organic compound analysis. (Kammin, 2010)

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Appendix B. Acronyms, Abbreviations and Units of Measure

Following are acronyms and abbreviations used frequently in this report.

%IS	Percent Impervious Surface
AHs	Aromatic Hydrocarbons
ASE	Accelerated solvent extraction
CHs	Chlorinated Hydrocarbons
COC	Chain of Custody
DNR	Washington Department of Natural Resources
DOH	Washington State Department of Health
e.g.	For example
Ecology	Washington State Department of Ecology
EIM	Environmental Information Management database
EPA	U.S. Environmental Protection Agency
et al.	And others
GC/MS	Gas Chromatography / Mass Spectrometry
GIS	Geographic Information System software
GPS	Global Positioning System
i.e.	In other words or that is
LOQ	Limit of Quantitation
MQO	Measurement quality objective
NIST	National Institute of Standards and Technology
OCPs	Organochlorine pesticides
PBDEs	Polybrominated diphenyl ethers
PCBs	Polychlorinated biphenyls
POPs	Persistent organic pollutants
PSEMP	Puget Sound Ecosystem Monitoring Program
PSP	Puget Sound Partnership
PSWQA	Puget Sound Water Quality Authority
QA	Quality assurance
QC	Quality control
RPD	Relative percent difference
RSD	Relative standard deviation
SEC HPLC	Size-exclusion high-performance liquid chromatography
SOP	Standard operating procedure
SRM	Standard reference material
WDFW	Washington Department of Fish and Wildlife

Units of Measurement

°C	degrees Centigrade
dw	dry weight
ft	feet
g	gram, a unit of mass
kg	kilograms, a unit of mass equal to 1,000 grams.
km	kilometer, a unit of length equal to 1,000 meters.
m	meter
mg	milligram
mg/Kg	milligrams per kilogram (parts per million)
mm	millimeter
ng/g	nanograms per gram (parts per billion)
ng/Kg	nanograms per kilogram (parts per trillion)
pg/g	picograms per gram (parts per trillion)
ug/g	micrograms per gram (parts per million)
ug/Kg	micrograms per kilogram (parts per billion)
ww	wet weight

Appendix C. PAH Fingerprints (see Table 5 for PAH acronym names on X-axis)

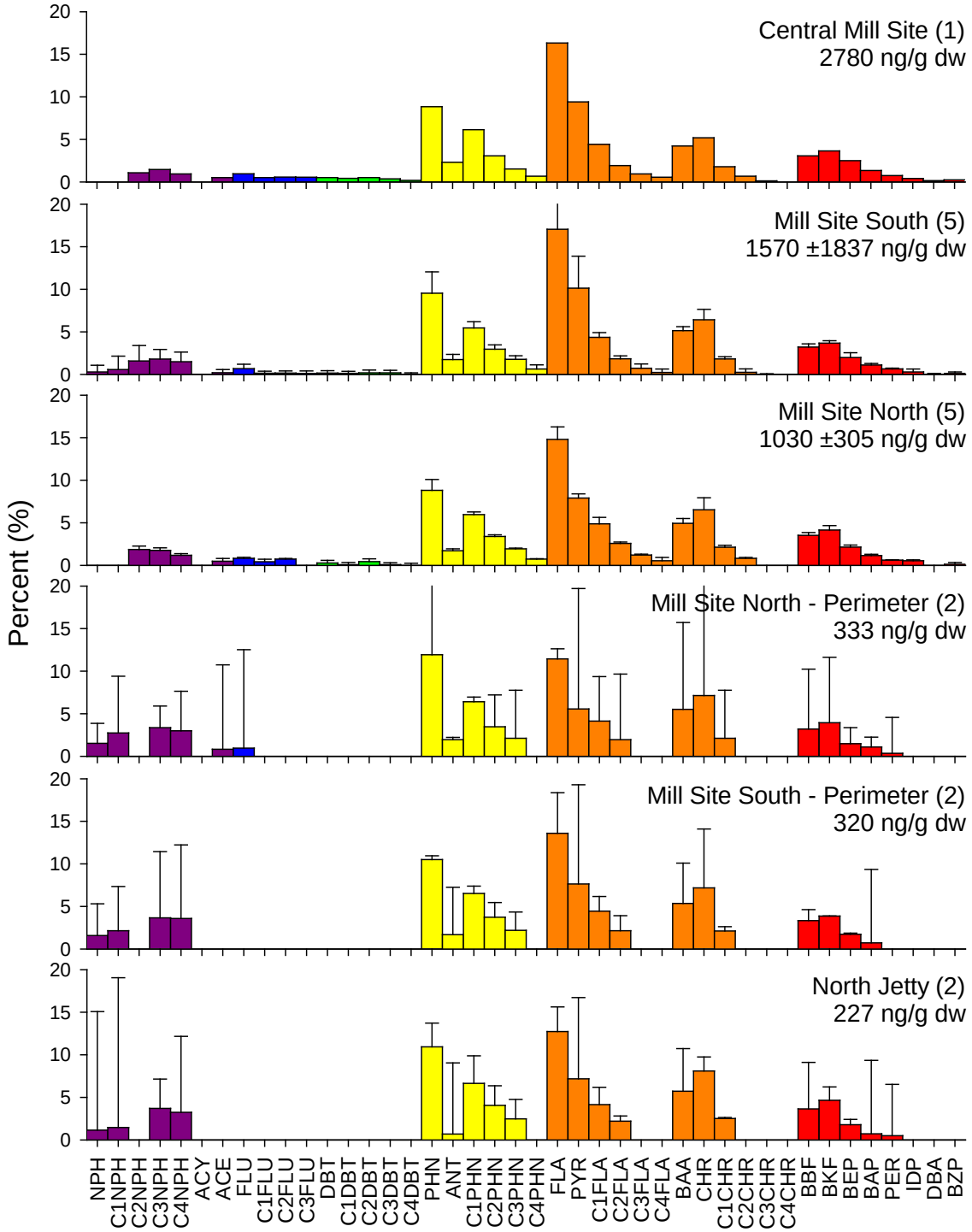

Figure 24. Proportion of individual PAHs (i.e. PAH fingerprints) in mussels transplanted to Port Gamble Bay. Replicates per area are shown in parentheses; total PAHs presented for areas with 1 sample, mean for 2-5 replicates and standard deviation (±SD) for 5 replicates.

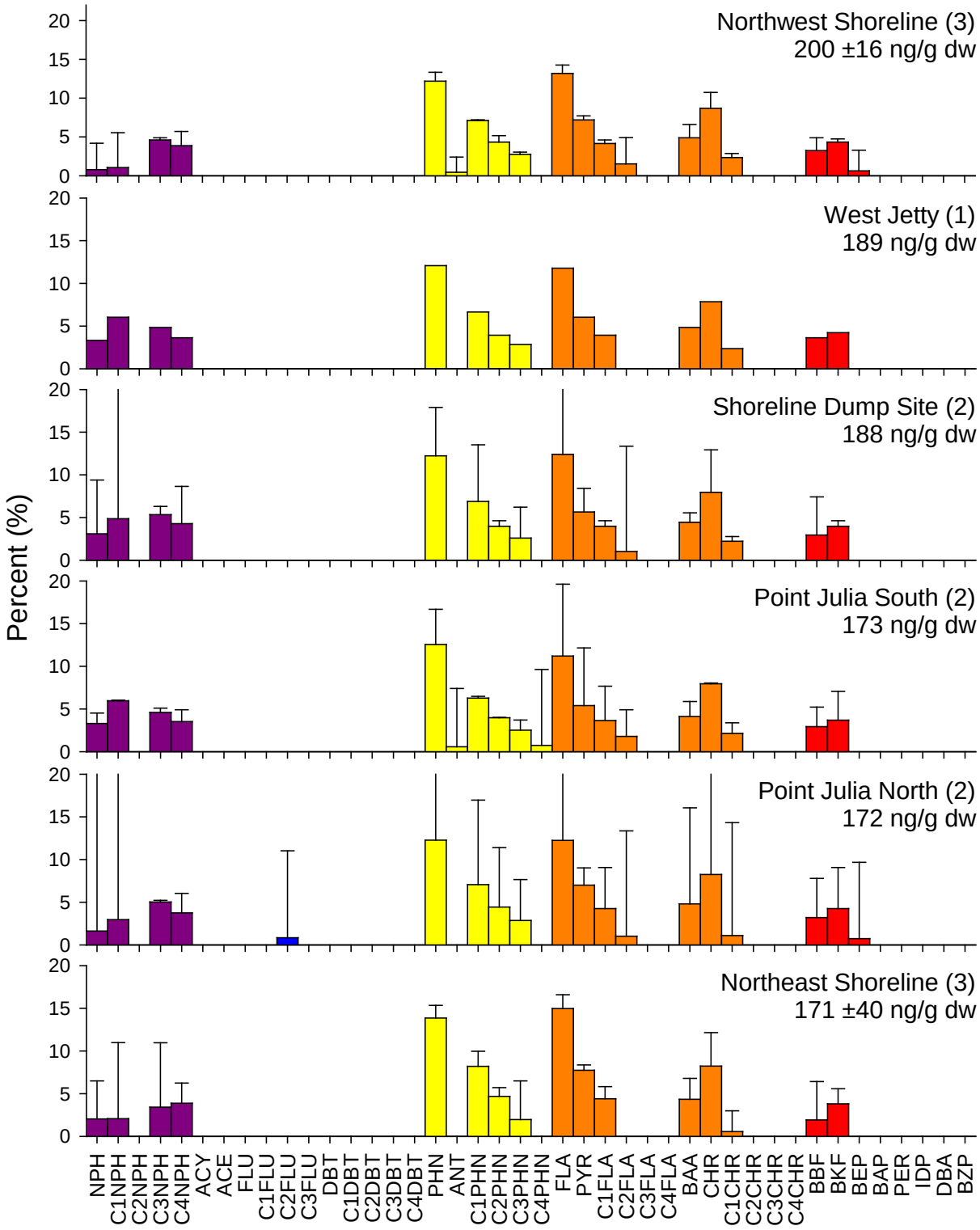

Figure 25. Proportion of individual PAHs (i.e. PAH fingerprints) in mussels transplanted to Port Gamble Bay. Replicates per area in shown in parentheses; total PAHs presented for areas with 1 sample, mean for 2-3 replicates and standard deviation (±SD) for 3 replicates.

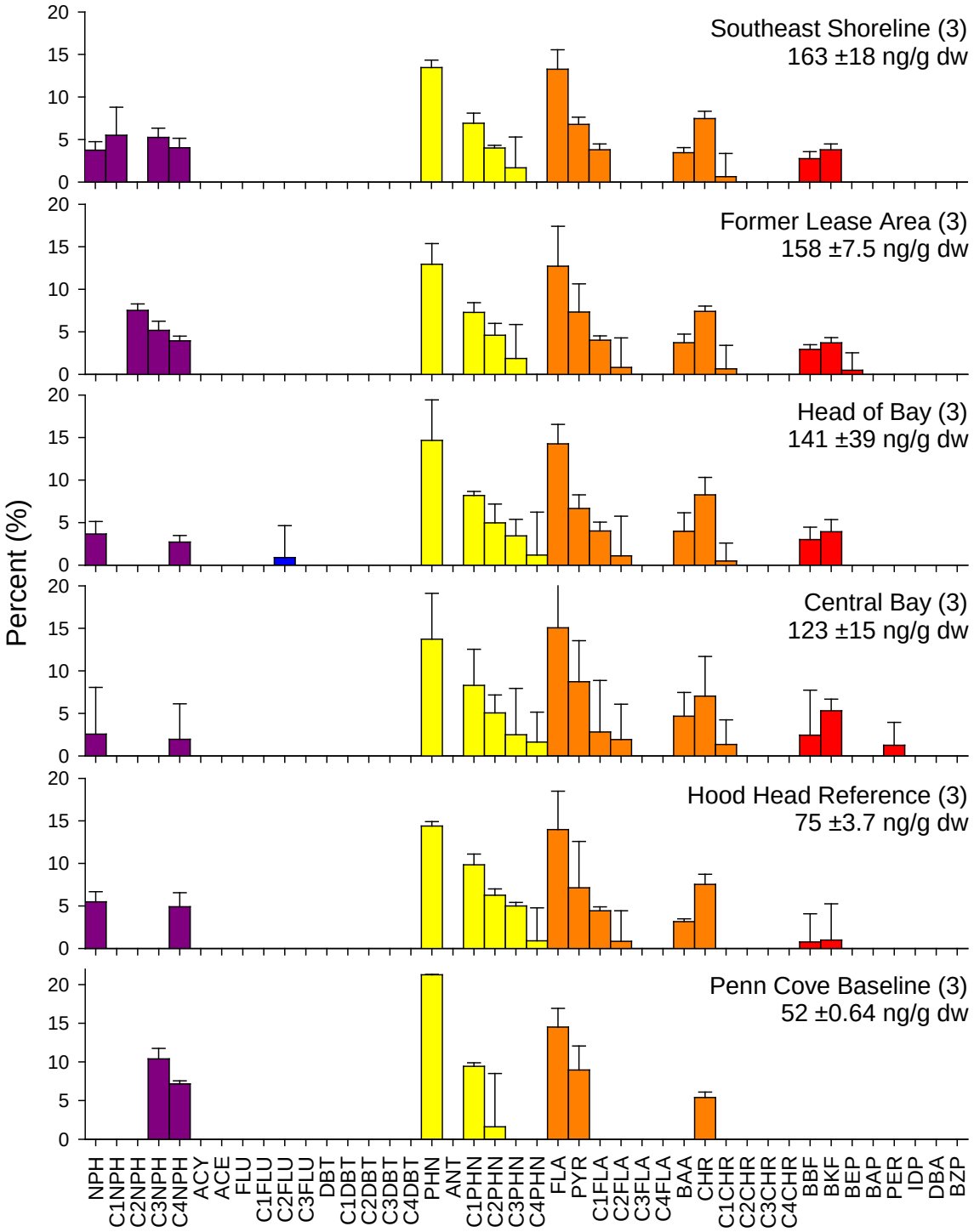

Figure 26. Proportion of individual PAHs (i.e. PAH fingerprints) in transplanted mussels; all histograms, except Hood Head (reference area) and Penn Cove Baseline (starting condition), depict mussels transplanted to areas in Port Gamble Bay. Replicates per area in shown in parentheses; mean total PAHs and standard deviation (±SD) presented.