

## Chapter 3

### Hydrocarbons in mussels, intertidal sediment, and passive samplers

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#### Introduction

More than 100 km of coastline on Unalaska Island were oiled by the *Selendang Ayu* (*S. Ayu*) grounding creating both short- and long-term biological impacts, such as death of thousands of oiled birds at the time of the spill and biochemical evidence of continued oil exposure through 2008 in harlequin ducks (Anonymous. 2005; Anonymous. 2007; Flint et al., 2009). The objectives of this chapter were to study long-term oil retention, determine the weathering state and bioavailability of remaining oil, and evaluate potential biological effects [Objectives 1-3 of this report (Chapter 1)]. To accomplish this, three distinct intertidal areas were sampled: the *S. Ayu* oil spill area, a reference area, and a human-impacted area. Of the 23 oiled beach segments under study, 15 (65%) failed to reach final cleanup criteria and an additional 3 were subjected to alternative treatment techniques (in 2005) such as berm relocation and tilling. Three types of sampling were used: collection of indigenous mussels, intertidal sediment, and passive samplers deployed for about one month. Mussel and passive sampler data were quantitative. Quantitative analysis of sediments was precluded by patchy oil distribution both within beaches and on individual oiled clasts, hence target oil sampling essentially provided only hydrocarbon composition and source information. See Chapter 1 for greater design and sampling detail and Chapter 2 for complete site description.

Thermodynamically driven differential hydrocarbon loss from stranded oil, known as weathering, was anticipated and is important because it is a mechanism for organism exposure to hydrocarbons via water. The rate at which polynuclear aromatic hydrocarbons (PAHs) move from stranded whole oil into water is dependent on molecular weight; smaller, less-substituted molecules are lost most rapidly, consistent with first order loss rate kinetics (Short and Heintz 1997). This mechanism explains the characteristic changes in composition as oil weathers and is well documented (Bence and Burns 1995; Short and Heintz 1997; Boehm et al., 2004; Boehm et al., 2005; Page et al., 2006). Molecules that leave oil enter air or water and subsequently can enter living tissue. Because oil constituents are highly insoluble in water and highly soluble in lipids, living organisms (and passive samplers) typically concentrate low aqueous hydrocarbon levels by a factor of 1000 or more, again a pattern driven by thermodynamics (Carls et al., 2004). Thus, stranded oil sets up a mechanism for biological contamination even in the absence of direct contact and this is detectable at very low concentrations. Furthermore, PAHs are toxic (Sundberg et al., 2006; Incardona et al., 2008), often at low concentrations (Rhodes et al., 2005; Farwell et al., 2006; Carls and Meador 2010) thus biological contamination resultant from weathering processes may have negative consequences.

Our approach to understanding the implications of residual *S. Ayu* oil was to demonstrate the presence of stranded oil, identify its source, and link this information to any evidence of biological availability (PAH concentration and composition) in indigenous mussels and passive

samplers. Direct identification of source oil in organisms and passive samplers can be difficult or impossible because composition changes as oil constituents move from oil to water to organism and because sequestered molecules are subsequently degraded by enzymes (such as cytochrome P450). Thus, source identification often must be indirect in biological samples and passive samplers. Nonetheless, some source evidence often remains, enough to at least roughly distinguish petrogenic and pyrogenic sources. Additional evidence for the source of contamination in tissue requires knowledge of the distribution of stranded *S. Ayu* oil, and this was accomplished visually, chemically, and by reference to previously mapped distributions. The environmental stability of biomarkers in *S. Ayu* oil, particularly hopanes, provided a definitive tool to confirm the origin of lingering oil.

## Methods

### Passive Samplers (PEMDs)

The bioavailability and composition of mobile oil constituents from remaining oil was assessed in ambient water with low-density polyethylene membrane sampling devices [PEMDs (Carls et al., 2004)]. These passive samplers were polyethylene plastic strips (~98  $\mu\text{m} \times 4.9 \text{ cm} \times 50 \text{ cm}$ ) housed in aluminum canisters (11.5 cm diameter  $\times$  6.6 cm) with perforated aluminum endplates (3 mm holes spaced 4.8 mm apart). Fig. 3.1 illustrates a PEMD deployed on the shoreline. The PEMDs were placed on ten oiled beach segments within the core spill area and fourteen reference beach segments (Table 3.1). The PEMDs were placed in arrays of three, one intertidal and two nearshore. Passive sampling arrays were allocated to oiled zones within beach segments and were proportional to the alongshore length of that zone (Table 3.1), except no more than ten arrays were located in a given segment.

Most PEMDs were anchored to groundlines (68%) extending perpendicularly across the shoreline and into the water. These were typically anchored supratidally with bolts (approximately 76%); others were anchored with driftwood or once to historic debris (in Chernofski Harbor). A few (10%) were anchored with tarred longline anchors placed supratidally to avoid potential contamination of PEMDs. Most intertidal PEMDs (81%) were placed in 0.6  $\times$  0.9 m polyethylene bags (3 mm cord, 25 mm mesh) loaded with beach gravel collected nearby (roughly 40 kilograms [kg]/bag) and did not move appreciably. Containment bags were typically fastened to 8 mm leaded groundline with a clove hitch and the PEMD was tied to the groundline and bag with 6 mm nylon rope. See Appendix A for additional detail.

The two nearshore PEMDs in each array were 0.5 m from the surface and the seafloor (benthic). Danforth anchors (8 to 10 kg) with chain (8 mm  $\times$  3 m) were used for anchoring in the water well beyond the nearshore PEMDs (typically about 100 m). Nearshore PEMDs were anchored on the groundline with two 4.5 kg lead weights and 10 mm polypropylene rope with 13 cm diameter  $\times$  30 cm buoys. Groundlines were set with an 11 m boat powered by outboard engines; to avoid contamination the boat was not operated in sample positions, rather it was landed downwind to pull groundlines offshore. Nearshore PEMDs and associated floats and anchors were not placed on the line until the boat was typically about 100 m from shore. Nearshore and intertidal PEMDs at remaining installations (32%) were independent; in these cases, benthic PEMDs were generally tied about 1 m from the anchor chains and installation was completed

with a small inflatable raft powered by a small outboard engine. The engine was turned off and fumes and slicks were allowed to dissipate before gear and PEMD deployment. See Appendix B for a detailed comparison of PEMD placement relative to elevation.

The PEMDs were retrieved about one month after deployment (mean 28 days, range 23 to 30 days; 98% were collected within  $\pm 2$  days of the mean deployment time). To prevent contamination, the raft was paddled (with no engine) to collect the nearshore PEMDs. As during installation, the number of landing sites on beaches was minimized, and landings were displaced from and downwind of adjacent PEMDs. The PEMDs were collected with clean disposable gloves and tools, packaged in two layers of aluminum foil, sealed in two ziplock bags, and further sealed in garbage bags. The PEMDs were frozen onboard and later air freighted overnight to the Auke Bay Laboratory where they remained frozen until processing. (This handling was essentially the reverse of deployment conditions.) Passive sampler air blanks, packed in jars, were opened at each beach segment for about 1 minute either during deployment or retrieval. Two unopened PEMDs served as trip blanks and additional laboratory blanks were never shipped. Additional samples were collected to characterize conditions on the boat (interior air, outboard exhaust, and outboard effluent). Shortly after arrival at the laboratory, the aluminum canisters were opened and the PEMDs were transferred to hydrocarbon-free glass jars with Teflon lined lids and frozen for chemical analysis.

General conditions during PEMD deployment (28 June – 2 August 2008) were inferred from buoy 46072 (National Data Buoy Center) located in the central Aleutians. Mean wind speed was 5.8 m/second (s) (range 0 to 13 m/s), air temperature was 8.6°C (range 5.5 to 11.4°C), mean sea surface temperature was 9.0°C (range 6.8 to 11.6°C), and significant wave height was 1.6 m (range 0.6 to 3.0 m). The surrogate surface temperature closely matched actual sea surface temperature measurements at nearshore sample locations (8.8°C, range 5.6 to 11.3°C,  $n = 63$ ). Mean salinity was 23.6 practical salinity units (psu) (range 1.5 to 31.1 psu); further site-specific detail is provided in Appendix B.

## **Mussels**

Blue mussel tissue samples were collected using an opportunistic design, with a minimum of three composite invertebrate samples from within, or seaward of each oiled zone, when available. Mussel tissues were collected as a composite of 16-25 live mussels 3-5 cm in length from an average of three intertidal sites per zone, as close as possible to the shoreline passive sampler arrays, if there was suitable habitat. All tissue sampling was completed prior to tidal inundation of intertidal sediments disturbed during excavation. Each sampling site was photographed prior to sample collection. Live mussels were pried from the substrate by hand wearing clean surgical gloves for each sampling. Whole organisms were wrapped together in aluminum foil and placed in a ziplock bag with a label on waterproof paper. Upon return to the vessel each day, the sample and label were placed in a second ziplock bag. The mussel samples were frozen onboard and later air freighted overnight to the Auke Bay Laboratory where they remained frozen until processing for analysis.

## Sediments

Samples of surface and subsurface oiled sediments were collected from test pits (Chapter 2) to represent the range of visual oiling conditions and to evaluate the weathering state of remaining oil within each identified oiled zone. Because of the coarseness of the sediments (mostly pebbles to boulders), sediment samples consisted of individual clasts with visible oil patches or coating. Where the oil occurred as a specific layer, a composite sediment sample was collected from the oiled layer. Most of the time, the oil occurrence was very patchy; therefore, individual oiled pebbles (4-64 mm) were collected. In many cases, oiled clasts were picked out during pit excavation, placed on a clean surface, and photographed prior to sampling. Fig. 3.2 provides representative photographs of the different types of subsurface oiling. Volcanic ash deposited throughout oiled and reference areas by an erupting volcano during the study, was also collected for hydrocarbon analysis for quality assurance purposes. Most of the sediment samples were placed in a pre-cleaned 8-ounce glass jar; large samples (small cobbles greater than 64 mm) were wrapped in aluminum foil and placed, with labels, in ziplock bags. All sample jars were placed into the original cardboard boxes, and the boxes placed in wetlock boxes. The sediments were frozen onboard and later air freighted overnight to the Auke Bay Laboratory where they remained frozen until processing for analysis.

Of the 138 samples collected, 59 of the glass jars broke during shipment from Dutch Harbor to Juneau, Alaska. Samples were contained primarily by the original glass jars and cardboard dividers between jars; the latter generally remained clean (about 92%) and there was no evidence of sample cross-contamination. There were intact samples for all segments; two zones (KFP-01A and KFP-01C) did not have any intact samples. Only the 78 intact samples were analyzed. The original study plan called for initial analysis of 60 sediment samples to be selected so that there was a sample from every zone and additional samples from the most heavily oiled zones. The intact samples mostly met these priorities (Table 3.1).

## Historical Sediment and Oil Samples

Some 55 oil ( $n = 23$ ) or sediment ( $n = 22$ ) samples collected from beaches oiled by the *S. Ayu* about the time of the spill were analyzed to provide an interpretive frame of reference. Frozen samples from the specific beach segments examined in this study were shipped frozen from the original collection agencies (NOAA, ADEC, and USCG) to the Auke Bay Laboratory. Nearby beach segments were selected to represent oiling for beach segments sampled in 2008 with no previous collections (Table 3.2). The median sample date was 27 January 2005 (range 15 December 2004 to 16 May 2005).

## Sample Processing and Hydrocarbon Extraction

Mussel tissue samples were dissected so that the tissue did not contact external shell surfaces, mechanically macerated 3 minutes (min) with a Tekmar tissuemizer, spiked with 500  $\mu$ L of perdeuterated surrogate recovery standard (Table 3.3), and extracted with dichloromethane in an accelerated solvent extractor (ASE 200; Dionex Corporation)(Larsen et al., 2008). The extract was dried with sodium sulfate and concentrated to 1 ml in hexane. The sample was fractionated into aliphatic and aromatic compounds on a chromatography column (10 g 2% deactivated alumina over 20 g 5% deactivated silica gel). The aliphatic compounds were eluted with 50 ml

pentane and the aromatic compounds were eluted with 250 ml of a 1:1 mixture of pentane and dichloromethane. Aromatic fractions were further purified by a high pressure liquid chromatograph (HPLC) equipped with phenogel size exclusion column (22.5 mm x 250 mm, 100 angstrom pore size). Both the aliphatic and the aromatic extracts were reduced to 1 ml in hexane, spiked with internal standards, dodecylcyclohexane and hexamethylbenzene, respectively, and stored at -20° C pending analysis.

Sediment samples were thawed, homogenized with a spatula, spiked with 500 µL of perdeuterated surrogate recovery standard (Table 3.3) and 100 µL of a 20 µg/ml solution of a deuterated biomarker recovery surrogate, C27 ααα(20R)-cholestane, and were extracted with dichloromethane in an ASE 200. Oil samples were homogenized or scraped from rock, if needed, dissolved in dichloromethane, and spiked as a sediment sample. Extracts were dried with sodium sulfate and concentrated to 1 ml in hexane. Samples were fractionated into aliphatic and aromatic compounds on a chromatography column (3 g 100% activated silica gel). The aliphatic and biomarker compounds were eluted with 6 ml pentane and the aromatic compounds were eluted with 10 ml of a 1:1 mixture of pentane and dichloromethane. Both the aliphatic and the aromatic extracts were reduced to 1 ml in hexane, spiked with internal standards, dodecylcyclohexane and hexamethylbenzene, respectively, and stored at -20°C pending analysis.

Passive samplers were wiped clean to remove gross surface contamination, placed in centrifuge tubes, and spiked with 500 ul of a solution equivalent to half the concentration of the perdeuterated surrogate recovery standard listed in Table 3.3, PAHs only. The spike solvent (hexane) was allowed to evaporate and the PEMDs were extracted in a sonic bath with 100 ml of 80:20 mixture of pentane:dichloromethane for 120 min (three 20 min sonications with a 30 min rest between each sonication). The PEMDs were immediately rinsed with pentane as they were removed after the final sonication. The extracts were dried with sodium sulfate and concentrated to 1 ml hexane. The extracts were purified on a chromatography column (1.5 g 5% deactivated silica gel). Samples were eluted with 22 ml of a 1:1 mixture of pentane and dichloromethane. Extracts were spiked with the internal standard, hexamethylbenzene and stored at -20°C pending analysis.

The aromatic fractions of tissue, sediment, oil samples, and the PEMD extract were analyzed for PAHs by gas chromatograph equipped with a mass selective detector (GC/MSD). The data were acquired in selected ion monitoring (SIM) mode and concentrations were determined by the internal standard method (Short et al., 1996; Carls et al., 2004). Experimentally determined method detection limits (MDLs) were generally 0.7 ng/g for tissue, 0.6 ng/g for sediment, and 0.2 to 3.9 ng/g in PEMDs. The accuracy of the PAH analyses was about ± 15% based on comparison with National Institute of Standards and Technology values, and precision expressed as coefficient of variation was usually less than about 20%, depending on the PAH. Total PAH (TPAH) concentrations were calculated by summing concentrations of individual PAH (Table 3.4). Relative PAH concentrations were calculated as the ratio of PAH<sub>i</sub> / TPAH.

Surrogate recoveries varied by analyte and matrix. Recoveries in sediment and passive samplers was fairly uniform, typically about 85 to 86% (95% confidence interval) for all analytes. Naphthalene recovery was smaller in previously analyzed sediment and tissue (means were 38 and 39%, respectively); means ranged from 52 to 79% for all other analytes. Less than 1% of the

recoveries were <25% (18 of 2754 measurements); all of these were naphthalene and nearly all from tissue (n = 13). All samples were included in the analyses; exclusion of samples with low recovery did not change conclusions.

Hydrocarbon measurements for a few mussel samples were repeated for quality control purposes (n = 8 of 105 samples) and were only used to verify that measurements were repeatable. Evidence of repeatability included high correlation of TPAH concentration and proportions of naphthalenes, fluorenes, dibenzothiophenes, phenanthrenes, chrysenes, and higher molecular weight PAHs; the coefficient of determination ( $r^2$ ) ranged from 0.973 to > 0.999.

The aliphatic fractions of tissue, sediment, and oil samples were analyzed for n-alkanes by gas chromatograph equipped with a flame ionization detector (GC/FID). Analyte concentrations were determined by the internal standard method<sup>5</sup>. Experimentally determined MDL were generally 19 ng/g for tissue, and 10 ng/g for sediment. The accuracy of the alkane analyses was  $\pm 15\%$  based on a spiked blank processed with each set of samples, and precision expressed as coefficient of variation was usually less than about 20%. All surrogate standard recoveries were acceptable (26 to 125%). Total alkane concentrations were calculated by summing concentrations of individual calibrated alkanes (Table 3.5). Relative alkane concentrations were calculated as the ratio of  $\text{alkane}_i$  / total alkanes.

The aliphatic fractions of sediment and oil samples were analyzed for biomarkers by GC/MSD. The data were acquired in SIM mode, and concentrations were determined by the internal standard method with response factors (RF) based on two representative compounds, 17 $\alpha$ (H),21 $\beta$ (H)-hopane (H30) and 5 $\alpha$ (H),14 $\alpha$ (H),17 $\alpha$ (H)-cholestane. The accuracy of the biomarker analyses was  $\pm 15\%$  based on a spiked blank processed with each set of samples, and precision expressed as coefficient of variation was usually less than about 20%, depending on the biomarker. Biomarker concentrations were not corrected for recovery; in only 3% of the samples did the surrogate recovery fall below 70%. Total biomarker concentrations were calculated by summing concentrations of individual biomarkers (Table 3.6). Relative biomarker concentrations were calculated as the ratio of  $\text{biomarker}_i$  / total biomarkers.

## ***Fingerprinting and Statistics***

The source of hydrocarbons in sediments, mussels, and PEMDs was inferred by multiple methods. First, oiling records from the time of the spill through the cleanup process provided primary evidence that any oil discovered in this study originated from the *S. Ayu*. This evidence included previous SCAT surveys and associated data. To further inform and authenticate such inferences, oil or oiled sediment samples collected on or nearby specific oiled beaches about the time of the spill were analyzed for comparison (Table 3.2) to account for possible differences among beaches in source oils or source oil ratios from various *S. Ayu* tanks. Known *S. Ayu* source oils were light intermediate fuel oil (IFO<sub>L</sub>), heavy IFO (IFO<sub>H</sub>), and marine diesel oil (MDO). These source oils are included in the analysis.

Oil weathering was anticipated (Appendix C), thus typical weathering patterns were considered in data interpretation (e.g., the model of Short and Heintz, 1997). An algorithm that summarizes three independent oil recognition models and two pyrogenic recognition models (Carls 2006)

was also used to ensure that interpretation was not confounded by pyrogenic sources and to understand how oil sources were distributed throughout the study area. Results of these models were combined to yield scores ranging from -6 (pyrogenic) to +6 (petrogenic). Highly conserved compounds such as n-C<sub>40</sub> and biomarkers provided additional assessment independent of PAHs and were also used for source interpretation.

Composition of hopanes, steranes, and triterpanes were independently examined to determine whether composition in field-collected samples matched source oil composition. Each sample was scored for fit; if normalized concentrations were within the normalized concentration bounds defined by IFO<sub>L</sub> and IFO<sub>H</sub> (or within  $\pm 0.05$  of those bounds), then that compound was considered a match. This was repeated for each of the 22 hopane compounds. Scores were divided by the number of compounds to yield a proportion. Hopane scores  $\geq 0.85$  were considered a match to S. Ayu oil; smaller scores were not a match. Similar comparisons were made among 14 normalized steranes and among 11 normalized triterpanes; the acceptance criterion for each of these was 0.50. Discrimination among sources was best for hopanes; scores for IFO ranged from 0.89 to 1.00 (i.e., 89 to 100% fit of 22 compounds) and were all 1.00 when acceptance bounds were increased by 0.05. Continuing with the relaxed ( $\pm 0.05$ ) criteria, marine diesel oil from the S. Ayu scores were 0.16 to 0.21 (n = 2) and anchor tar was 0.32, thus neither of these matched S. Ayu IFO characteristics. Discrimination between IFO and anchor tar was poor with steranes and discrimination between IFO and MDO was poor with triterpanes. Hence hopane results discriminated best among sources and were used to determine if field samples represented IFO from the S. Ayu. Hopane modeling results were compared to the originally documented extent and pattern of oil on beaches coated by S. Ayu oil, visible presence or absence of oil, PAH concentration and composition model results, and alkane concentration and composition.

Similarities and dissimilarities in hydrocarbon composition were also inferred using principal component analysis. In mussels, all raw PAH concentrations normalized to TPAH concentration except C<sub>4</sub>-fluoranthene, C<sub>2</sub>-, C<sub>3</sub>-, and C<sub>4</sub>-chrysenes were analyzed as a correlation matrix to determine the first two principal components. (None of the excluded analytes were present in detectable quantities.) Procedures were the same for PEMDs and biomarkers.

Estimated above-background threshold TPAH concentrations were calculated from the concentration distributions at reference sites. Above-background estimates were based on 85<sup>th</sup> percentile tolerance intervals, estimated with 95% confidence (Prins et al., 2008):

$$\bar{x} + 1.483 \cdot s$$

See Appendix D for further detail.

Raw TPAH concentrations were used for analysis of passive sampler data. However, mean blank concentrations were subtracted (by each individual PAH analyte) before statistical analysis; differences less than zero were set to zero. Total PAH concentration in blanks did not vary significantly among areas ( $p = 0.125$ ), thus the subtracted values were the same for each PAH among all areas. Conclusions reached with blank-corrected data were the same as those reached with uncorrected data. Because certain TPAH concentrations were much higher than

others, natural log concentrations were compared with ANOVA and geometric mean concentrations are presented for passive sampler data.

The toxic potential of PAH in sediment was estimated by observing the presence or absence of key analytes, primarily fluorenes, dibenzothiophenes, and phenanthrenes (Incardona et al., 2005). These compounds are more environmentally persistent and inherently toxic, thus pose more risks of chronic toxicity than the initially abundant naphthalenes; relatively few higher molecular weight PAHs beyond C<sub>2</sub>-chrysenes dissolve in water, thus may be less biologically available. We assumed that fluorenes, dibenzothiophenes, and phenanthrenes are potentially toxic. Furthermore, the biological availability of these compounds was inferred from examination of PAH composition in PEMDs and mussel tissues and correlation between TPAH in these matrices and degree of site oiling.<sup>a</sup> Toxic potential was also inferred from the magnitude of TPAH bioaccumulated in mussels or accumulated in PEMDs.

## Results

### PAHs in Mussels Collected in Summer 2008

Quantities of PAHs available to mussels in the summer of 2008 were low (range 0.5 to 326 ng/g wet weight across all areas). Of the detected PAHs, 46% were above MDLs in the oiled area, 28% in the reference area, and 66% in Chernofski Harbor. To avoid potential loss of information, further comparison of hydrocarbons in mussel tissue among areas was based on raw data (without MDL adjustment).

Mean raw TPAH concentrations in mussel tissue were low but marginally greater in the oil spill area ( $30 \pm 4$  ng/g dry wt) ( $\pm$ SE) than in the reference area ( $19 \pm 4$  ng/g dry wt;  $s = 24.620$ ,  $p = 0.056$ ; Figs. 3.3 and 3.4). The estimated above-background TPAH concentration was  $19 + 1.483 \cdot 24.620 = 56$  ng/g dry wt. Although significantly greater than the reference concentration, the mean concentration at oiled sites was less than the estimated above-background concentration. Concentrations in two samples from the oiled area were greater than the above-background estimate but less than the maximum reference concentration (125 ng/g dry wt). The incidence of above-background TPAH was least in oiled areas (3%) compared to 6% in the reference area, and 36% in Chernofski Harbor.

The TPAH concentrations in mussels in the historical human impact area (Chernofski Harbor) were significantly greater than in reference areas ( $P_{\text{ANOVA}} < 0.001$ ; Fig. 3.3). The mean TPAH concentration in tissue from Chernofski Harbor was  $65 \pm 5$  ng/g dry wt ( $\pm$ SE), range 20 to 197 ng/g (excluding one clearly contaminated outlier, 2780 ng/g). Nearly all samples where TPAH was  $> 100$  ng/g dry wt were collected in Chernofski Harbor (5 of 6, including the outlier; Fig. 3.4).

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<sup>a</sup> We recognize that actual toxicity estimates require knowledge of both composition and concentration. Toxicity could not be measured directly because concentration measurement in sediments would require considerably more time and labor to collect and process the large volume samples than possible for this study. In addition, movement of oil or oil constituents into biota of interest (e.g., mussels, birds, and otters) from oil in sediment would be required to understand how oiling relates to toxicity; these mechanisms may include aqueous dissolution, whole oil particles in water, oiled food, encounter of oil in the surface microlayer, and ingestion by preening or grooming, and or direct contact with oil during foraging.



## PAH Sources in Mussels Collected in Summer 2008

Composition of PAHs in mussel tissue differed between oiled and reference areas. Pyrogenic sources were more likely in reference areas and petrogenic sources were more likely in the spill area ( $P_{ANOVA} < 0.001$ ; Figs. 3.5 and 3.6). Model estimates of PAH sources (Carls 2006) were -1.2, -1.9, and +1.8 among reference, historically contaminated, and oiled areas (Fig. 3.6)<sup>b</sup>; negative estimates are pyrogenic, estimates around 0 are neutral, and positive estimates are petrogenic (maximum possible scale range is -6 to +6). Pyrogenic sources were more likely in Chernofski Harbor than in reference areas ( $P_{ANOVA} = 0.032$ ; Fig. 3.6), further evidence that PAH sources in this area may be different than in other study areas.

Consistent with the differences among areas suggested by modeling, the first and second principal components divided PAH composition in mussels into three distinct groups with limited overlap: Chernofski Harbor, the reference area, and an oiled area (Fig. 3.7). Although these two components only explained 31% of the variance, they corroborated the composition differences determined with source modeling. Weathering in oiled tissues (where model scores were  $\geq 2$ ) was related to component scores, particularly component two (Fig. 3.7b). Weathering was not directly estimable in tissue samples because not all PAHs required for modeling were detected. However, changes in relative homologue concentrations provided an alternative method of estimating weathering; more naphthalenes indicate less weathering. Percent naphthalenes were inversely correlated with the second component ( $r = -0.879$ , two-phase exponential decay model) and phenanthrenes were positively correlated with it ( $r = 0.652$ , power model). Thus, the oiled point cloud in principal component space graded from less to more weathered (Fig. 3.7b).

Alkane data did not provide evidence of oil-related differences but did indicate possible biological differences among areas. See Appendix E for further detail.

## Total PAH concentrations in Passive Samplers (PEMDs)

Low quantities of PAHs accumulated in PEMDs and varied by area (oiled, reference, and Chernofski Harbor) and zone (subtidal, intertidal, and surface water). Mean concentrations were consistently highest in Chernofski Harbor and lowest in reference areas. In the oiled area, TPAH concentrations were significantly elevated in all three zones compared to corresponding reference concentrations [subtidal ( $p \leq 0.046$ ), intertidal ( $p < 0.001$ ), and water surface ( $p = 0.007$ ); Fig. 3.3]. In Chernofski Harbor, the historical human impact area, TPAH concentrations were also significantly elevated in all three zones ( $p < 0.001$ ; Fig. 3.3). Concentration differences among areas were least for the subtidal samples (Fig. 3.3). The highest TPAH concentrations (440 to 1785 ng/device) were observed in Chernofski Harbor (7 of 209 PEMDs). Relative TPAH concentration patterns in passive samplers were the same as those in mussels (Fig. 3.3). The above-background estimate for TPAHs in PEMDs was 145 ng/device (or 65 ng/g). The frequency of above-background concentrations in PEMDs ranged from 88% (SKS12) to 0% (Table 3.7).

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<sup>b</sup> Computed with raw data (recommended when interpreting low-level contamination)<sup>4</sup>.

Total PAH concentrations in intertidal PEMDs were correlated with paired mussel samples (power analysis). Correlation was moderate ( $r = 0.657$ ) with all data pairs present ( $n = 53$ );  $p < 0.001$  (Fig. 3.9). However, there were two high leverage points in this data set: 1) a previously identified mussel outlier from Chernofski Harbor, with a corresponding PEMD concentration of 940 ng/device; and 2) a Chernofski Harbor sample with a PEMD concentration of 1,255 ng/device and a mussel concentration of 176 ng/g dry weight. Progressive removal of these two leverage points (Fig. 3.9) reduced  $r$  to 0.492, yet regressions remained significant ( $p < 0.001$ ). The unusual data pairs are of interest. One pair was apparently influenced by proximity of mussels to the contamination source (the sample was collected from wooden pilings, likely creosote-coated). In the other unusual pair, TPAH concentration was relatively high in the PEMD but about 7 times smaller in the mussel sample. Hydrocarbon chemistry in Chernofski Harbor is clearly interesting and merits additional attention. With or without the unusual data pairs, a significant relationship between mussel and PEMD pairs is apparent and consistent with area-wide summaries (Fig. 3.3).

## PAH sources in passive samplers

Petrogenic sources were significantly more likely in the *S. Ayu* spill area than in reference and historical impact areas; sources were more likely pyrogenic in the latter areas ( $P_{ANOVA} < 0.001$ ; Fig. 3.6). The PAH sources in the reference area and Chernofski Harbor were weakly pyrogenic or ambiguous, and did not vary significantly between these two areas. The source in the oiled area was consistently petrogenic in subtidal, surface, and intertidal water (Fig. 3.6). The spatial distribution of PAH sources in PEMDs was similar to that in mussels (compare Fig. 3.5 with Fig. 3.10). Consistent with this area-wide perspective, PAH sources were correlated between mussel and PEMD pairs ( $r^2 = 0.603$ ;  $P < 0.001$ ; linear regression).

Three distinct groupings were evident when normalized PAH matrices were analyzed with principal components analysis in intertidal and subtidal zones; only two groups were distinct in surface water (Fig. 3.11). Composition in Chernofski Harbor PEMDs was always distinctly different from those in reference and oiled areas (Fig. 3.11). Composition in oiled intertidal and subtidal areas was distinct from that in corresponding reference areas, consistent with source modeling (Fig. 3.6). However, the first and second principal components failed to distinguish oiled and reference areas in surface water (Fig. 3.11). Separation of these two areas by the third principal component was better but remained incomplete (Fig. 3.11). In addition, the third component separated exhaust gas generated by the boat used for sampling from all other sources, identifying this potentially confounding source as unique.

Weathering in passive samplers (where model scores were  $\geq 2$ ) was related to principal component scores (Fig. 3.11b). As in mussel tissue, weathering was not directly estimable in passive samplers, hence relative naphthalene concentrations served as surrogate measures. Percent naphthalenes were inversely correlated with the second component in subtidal and intertidal zones ( $r = -0.746$  and  $0.565$ , respectively) and phenanthrenes were positively correlated with either component one ( $0.621$ , subtidal) or component two ( $r = 0.632$ , intertidal). Correlation between principal components and percent naphthalenes, best for component three ( $r$

= -0.522), was weaker in surface water. Nonetheless, weathering generally graded from least to most in each distribution (Fig. 3.11b).

### **Total PAH concentrations in sediments (2008)**

Total PAH concentrations in sediment samples were generally meaningless because sampling procedures both in the field and in the laboratory targeted oil deposits for examination of PAH composition. However, (raw) TPAH concentrations in all PTN03 sediments (1, 47, and 886 ng/g wet weight) were several orders of magnitude less than typical concentrations (median =  $1.2 \times 10^7$  ng/g). No oil was observed in the first two of these; oil coat (CT) was recorded for the latter. Low concentrations were also measured in one HMP05D sample (pit 2; 84 ng/g; no oil observed), two SKS12A samples (944 to 1117 ng/g; pits 12 and 21; no oil observed) and one KFP01F sample (pit 17; 2130 ng/g; trace oil visible). In contrast, TPAH concentration in all other samples ranged from  $8.8 \times 10^3$  to  $3.5 \times 10^7$  ng/g. Total PAH concentrations adjusted by MDLs were >99% of unadjusted values in the 2008 samples, thus nearly all analytes were above method detection limits.

### **PAH Sources and Weathering in oil and Sediment (2008)**

Nearly all sediment samples from oiled areas had petrogenic signatures (PAH source model scores  $\geq 5$  for 74 of 79 samples; Figs. 3.12 and 3.13). There was no evidence of oil or other contamination in 3 of the remaining 5 samples (scores = 2); all of these had low concentrations [TPAH < 100 ng/g; PTN03 pits 7 and 20, and HMP05-D pit 2] and they were described as no oil observed in the field. The other two unusual samples were collected at SKS12A; one of these possibly contained highly weathered oil (pit 21; TPAH = 1,117 ng/g); oil residue was not visible in this sample. The hydrocarbon source in a second SKS12A sample was ambiguous (pit 12; TPAH = 944 ng/g).

Oil weathering accounted for most inter- and intra-site variation; PAH composition was not distinguishable among oiled sites by principal components analysis except for PTN03 (Fig. 3.13). Weathering effects were prominent; the first four principal components (which explained 80% of the variation) were correlated with weathering ( $w$ );  $r = 0.933, -0.914, -0.827, \text{ and } 0.439$  ( $c1$  to  $c4$ , respectively;  $P < 0.001$ ;  $4 \leq F_{\text{observed}} / F_{\text{critical}} \leq 90$ ). All sites except one were represented in the weathering pattern (fitted curve in lower left portion of Fig. 3.13); 72 of 79 data pairs formed this pattern. However, none of the PTN03 sediments were grouped within the typical weathering pattern, though the PAH composition was petrogenic in one of these and a minor amount of oil was visible in the sample (Fig. 3.13). Composition in four additional samples did not conform to the typical weathering pattern: one from HMP05D (pit 2), one from KFP01F (pit 17), and two from SKS12A (pits 12 and 21). All of these outlying samples had low TPAH concentrations.

The degree of oil weathering varied within sites and overlapped closely among sites (Fig. 3.14). Weathering at KFP beaches spanned the entire estimable weathering range, from -0.1 (least weathered) to 6.9 (most weathered;  $n = 28$ ; Figs. 3.13 to 3.15). Sites SKS04 and MKS14 also had relatively broad weathering ranges (2.1 to 6.7 and 0.3 to 3.2, respectively;  $3 \leq n \leq 8$ ). The

weathering range was least at SKN11 ( $n = 1$ ), HMP05 ( $n = 3$ ), and SKS12 ( $n = 2$ ); the number of samples where  $w$  was estimable influenced these ranges.

### **PAH sources in sediment about the time of the spill (2004 to 2005)**

The PAH composition of oiled and reference sediments collected during the spill (from 2004-2005) was distinctly different and did not overlap (Fig. 3.16). Modeling provided no evidence of oil in reference samples (score ranged from -1 to 2;  $n = 19$ ). All reference samples were separated from oiled samples by principal components one and two. All historically oiled sediment samples were identified as oiled by modeling ( $n = 32$ ); the score was 6 in all except one where the score = 5. All oil samples collected directly from the *S. Ayu* were identified as oiled by the model (score = 6;  $n = 4$ ). Weathering effects were prominent in oiled samples; the first three principal components (which explained 69% of the variation) were correlated with weathering ( $w$ );  $r = 0.906, 0.887$ , and  $0.876$  ( $c_1$  to  $c_3$ , respectively;  $P < 0.001$ ;  $27 \leq F_{\text{observed}} / F_{\text{critical}} \leq 37$ ). Some undetected analytes ( $C_4$  naphthalenes) precluded estimation of  $w$  in a single low-concentration oiled sediment sample (21 ng/g); visual interpretation of the chromatogram indicated this sample was rather weathered, consistent with the principal components analysis (Fig. 3.16).

Weathering in 2004 to 2005 sediment and oil samples ranged from unweathered to weathered and varied both among and within sites along a continuum (Fig. 3.16). Oil collected directly from the *S. Ayu* was the least weathered ( $-3.1 \leq w \leq -0.7$ ); of these, a low-viscosity liquid oil sample was the least weathered (STBD MDO service tank; Fig. 3.17). Though weathering was similarly low in one beach sample (PTN06,  $w = -1.0$ ), samples collected from beaches and streams in 2004 to 2005 tended to be somewhat more weathered, ranging from -0.3 to 7.3 (median = 0.3). Weathering spanned this entire range in the vicinity of SKN11 (SKN11 and SKN14). Expressed in principal component space, all oiled samples fell along a single, continuous curve (Fig. 3.16).

### **PAH sources in oiled sediment combined across time (2004 to 2008)**

The PAH composition in oiled sediment collected during the first year of the *S. Ayu* spill (2004 to 2005) combined with contemporary samples (2008) was coherently described as continuous by weathering and principal component analysis (Fig. 3.18a). Excluding three outliers, all other data ( $n_{\text{total}} = 123$ ) formed a declining exponential curve in principal component space. Samples collected in 2004 and 2005 were the least weathered and graded into those collected in 2008. Weathering changed predictably across this curve;  $w$  was closely correlated with the first principal component (which explained 50% of the variation)  $r = 0.910$ ;  $P < 0.001$ ;  $F_{\text{observed}} / F_{\text{critical}} = 300$ ). Weathering was not estimable the three outlier samples, hence they were not included in this analysis. Weathering ( $w$ ) increased significantly ( $P_{\text{ANOVA}} < 0.001$ ) from 2004 and 2005 (0.66) to 2008 (2.59), confirming the patterns observed in PCA analysis (Fig. 3.18a).

An alternative way to demonstrate PAH weathering is to choose information available directly from the composition data without modeling. The proportion total chrysenes, an environmentally persistent homologous family, accomplishes this. Specifically, once oil is

released into the environment, the proportion of total chrysenes will increase because they are lost from oil (or oiled sediment) more slowly than lower molecular weight compounds (Short and Heintz 1997). The proportion of total chrysenes increased significantly ( $P_{ANOVA} < 0.001$ ) from 2004 and 2005 (0.03) to 2008 (0.07). The proportion of total chrysenes was correlated with modeled PAH weathering ( $r = 0.938$ , linear model,  $n = 119$ ) and with the first principal component ( $r = 0.942$ , exponential model,  $n = 123$ ). This step is illustrated to demonstrate parallelism with the novel approach to summarizing alkane weathering. The visual outcome in PCA space is functionally the same when coded either by modeled weathering or percent chrysenes (compare Figs. 3.18a and 3.18b).

### **Alkanes in oil and sediment samples**

The alkane composition in oiled sediment collected during the first year of the *S. Ayu* spill (2004 to 2005) combined with contemporary oil samples (2008) was coherently described as continuous by principal component analysis (Fig. 3.19). Samples collected in 2004 and 2005 were generally more similar to source oil than those collected in 2008. Reference samples formed a separate point cloud; two oiled samples were also mapped to this area, both with low or very low alkane concentrations (89 to 384 ng/g) and thus likely represented mixed signals. (Typical total calibrated alkane concentrations from targeted oil in 2008 ranged from  $4.7 \times 10^6$  to  $7.7 \times 10^6$  ng/g wet weight.) Alkanes in newly erupted volcanic ash were consistent with reference samples (10 ng/g). Alkanes in anchor tar (originating from a different source) were not distinguishable from those collected from oiled beaches. These results were essentially identical when isoprenoid compounds (pristane and phytane) were excluded from PCA analysis. Across the C1 PCA gradient, moving from fresh oil to the most weathered samples (see Figs. 3.19 and 3.20 a-f), alkane composition shifted from a full spectrum of compounds (C9- to C36 n-alkanes plus isoprenoids) dominated by lower mass compounds (roughly C14- to C21 n-alkanes plus isoprenoids) to composition devoid of lower mass compounds and dominated by high mass compounds (roughly C32- to C36-alkanes; Fig. 3.20). Principal component 1 was correlated with PAH weathering ( $r = -0.858$ ).

The proportion of environmentally persistent alkanes (C27 and above) was least in source oil and greatest in samples least similar to source oil (Fig. 3.19). Furthermore, alkanes were less weathered in 2004 and 2005 samples than in 2008 samples; the proportion of environmentally persistent alkanes increased significantly ( $P_{ANOVA} < 0.001$ ) from 0.69 to 0.96. Moreover, the proportion of environmentally persistent alkanes was correlated with PAH weathering ( $r = 0.798$ , linear model,  $n = 106$ ) and percent chrysenes ( $r = 0.905$ , reciprocal exponential model,  $n = 112$ ), hence the proportion of environmentally persistent alkanes was inversely proportional to alkane weathering. The proportion of environmentally persistent alkanes was correlated with the first principal component ( $r = -0.959$ , cubic model,  $n = 112$ ).

### **Biomarkers in source oil and sediment**

Biomarkers added three compound classes (triterpanes, hopanes, and steranes) to the compound classes previously examined (PAHs and alkanes). Although isoprenoids (pristane and phytane) were included in the alkane analysis, isoprenoids are also considered biomarkers (pristane, norpristane, and phytane) and were independently measured a second time along with other

biomarkers. The following presentation of biomarker results begins with all four biomarker classes combined, then refines biomarker results by examination of subsets and individual classes.

Composition of all biomarker classes (dominated by isoprenoids in 2004 and 2005) changed after oil was released from the *S. Ayu* and this change was consistent with weathering. Samples collected from the vessel in December 2004 or January 2005 were clustered in principal component space, roughly between IFO<sub>L</sub> and IFO<sub>H</sub> source oil ( $n = 4$ ; Fig. 3.21). The few non-oiled samples ( $n = 4$ ) were also grouped together but one of these (fresh volcanic ash) overlapped *S. Ayu* source oil. The remaining oiled samples formed a point cloud extending outward from the 2005 samples. The biomarker composition of anchor tar, analyzed for quality control purposes, was distinctly different. The first principal component, which explained 72% of biomarker variance, was correlated with PAH weathering ( $w_{PAH}$ ;  $r = 0.947$ , Boltzman model; Fig. 3.22). None of the remaining principal components, which explained 11, 6, and 4% of the variance, were correlated with  $w_{PAH}$  ( $r = 0.184, 0.281, \text{ and } 0.044$ , respectively; linear models).

Loss of isoprenoids accounted for this time-dependent change in biomarker composition (Fig. 3.23). The mean proportion of isoprenoids (with respect to all four biomarker classes) dropped significantly ( $P_{ANOVA} < 0.001$ ) from  $0.71 \pm 0.03$  ( $n = 22$ ) in 2004 and 2005 to  $0.35 \pm 0.03$  ( $n = 77$ ) in 2008. For this reason, hopanes and steranes were reanalyzed to better understand the relationship between source oil and these compounds in spilled oil.

Hopane and sterane composition was consistent and stable among oiled sediment samples and IFO<sub>H</sub> and IFO<sub>L</sub> source oil and distinctly different in marine diesel oil (MDO), reference samples, and anchor tar (Fig. 3.24). The IFO source oil and oiled sediment all fell along a line in principal component space (Fig. 3.24), separate from MDO, reference samples, and anchor tar. (Volcanic ash was excluded from this analysis; none of these compounds were detected in it.) Hopane and sterane composition in oiled samples in 2004 or 2005 was about the same as in 2008 (Fig. 3.24). Inclusion of replicate measures did not change the relationships in this analysis; they are included to demonstrate measurement error had little influence.

Triterpane, hopane, and sterane compositions did not change significantly with time, indicating these large, complex compounds were environmentally persistent and stable. One way to demonstrate this stability is to compare distributions in 2004 and 2005 with those in 2008; matches indicate stability. Both IFO<sub>L</sub> and IFO<sub>H</sub> source oil were used to define minima (-5%) and maxima (+5%) for each compound (normalized to total concentration); this was completed independently for each of the three compound classes (triterpanes, hopanes, and steranes). *S. Ayu* oil was positively and independently identified in 2004 and 2005 by hopane, sterane, and triterpane distributions in 21, 21, and 19 of 21 samples, respectively. Results were about the same in 2008, with *S. Ayu* IFO mixtures present in 77, 76, or 75 of 78 oiled samples. If differential weathering had modified these patterns, the frequency of matches would have declined in 2008.

The composition gradients determined by PCA likely represent a sorting of samples by IFO mixture rather than weathering. Biomarker composition varied across the C1-C2 gradient (examples a-f in Fig. 3.24), (Fig. 3.25); Tm, H29, H31S, H31R, and NOR30H generally

increased relative to H30, and DIA27S, DIA27R, C27R and possibly C27S decreased relative to H30. Unlike the situation for PAHs and alkanes, 2004-2005 and 2008 samples closely overlapped across this gradient, additional evidence of hopane and sterane stability.

The two primary *S. Ayu* source oils, IFO<sub>L</sub> and IFO<sub>H</sub> were typically present in nearly equal quantities in oiled sediment samples. Based on hopane distributions in samples with likely and confirmed *S. Ayu* oil, mean IFO<sub>L</sub> content was 0.48 (n = 100; range 0.33 to 0.59; source samples were not included in this analysis). Results were nearly identical when restricted to the 78 oiled samples collected in 2008 (mean 0.48, range 0.34 to 0.59). Mixture did not vary significantly between years ( $P_{ANOVA} = 0.863$ ). In contrast, and for verification of modeling accuracy, estimated IFO<sub>L</sub> content was 0.04 to 0.11 in two replicate IFO<sub>H</sub> source oil samples (compared to a theoretical 0.0) and the mean estimated IFO<sub>L</sub> content was 0.82 (range 0.76 to 0.89) in IFO<sub>L</sub> source oil samples (compared to a theoretical 1.0). Thus, discrimination between these two sources is imperfect but reasonable. Dibenzothiophene content relative to phenanthrene content was greater in IFO<sub>H</sub> than IFO<sub>L</sub> (Table 3.8), thus providing another test of the hopane model. Regression of  $\Sigma D/\Sigma P$  and estimated IFO<sub>L</sub> content was significant ( $P < 0.001$ ,  $F_o/F_c = 20$ ,  $r = -0.67$ ,  $n = 100$ ), i.e., proportionate dibenzothiophene content decreased significantly as IFO<sub>L</sub> content increased.

Source oils were distinguishable multiple ways (Table 3.8). Relative biomarker content was greatest in IFO<sub>H</sub>, IFO<sub>L</sub> had the greatest relative PAH content, and MDO had the greatest relative alkane content. Dibenzothiophene content (relative to phenanthrene content) was greatest in IFO<sub>H</sub> and least in IFO<sub>L</sub>. Weathering was greatest in IFO<sub>H</sub> and least in MDO; these weathering relationships are also evident in proportions of naphthalenes, chrysenes, and heavy alkanes.

## Discussion

Residual oil was biologically available in areas oiled by the *S. Ayu* in 2008 and contained toxic tricyclic aromatic constituents, demonstrated by accumulation of PAH in mussels from oiled areas. These accumulations were distinguished by both composition and concentration. Modeling indicated a petrogenic source and distinct differences in PAH composition in mussels among areas was confirmed with principal component analysis. Total PAH concentrations in mussels from the oiled area were low yet marginally greater than in those from the reference area ( $P = 0.056$ ). The bioavailability of oil was confirmed with passive samplers; PAH concentrations were significantly elevated intertidally, in surface water, and subtidally (in order of greatest to smallest) and were correlated with those in nearby mussels. A petrogenic source was consistently and significantly likely in passive samplers deployed in the oiled area; composition was unrecognizable or tended toward pyrogenic in the reference area. Principal component analysis of PAHs in passive samples also distinguished oiled and reference areas. The sources of these biologically available PAHs were likely the hydrocarbons present in corresponding shorelines.

Multiple lines of evidence converged to identify *S. Ayu* oil in all oiled beach segments examined in 2008 except PTN-03. Hopane composition provided the most definitive proof that *S. Ayu* oil was present; composition matched *S. Ayu* IFO composition in 77 of 78 oiled samples. Composition of PAH and concentration, alkane composition, concentration, and unresolved complex mixture concentration, sample locations, and sample appearances all supported the conclusion that *S. Ayu* oil was present in these 78 field samples. Most of the remaining six 2008 field samples did not contain *S. Ayu* oil. One of these was fresh volcanic ash. Four more were collected from pits with no oil observed and no evidence of oil based on PAHs, alkanes, and biomarkers. The last had a substantial, but relatively low TPAH concentration (886 ng/g), no indication of contamination based on alkanes, and was not verified as *S. Ayu* oil with biomarker modeling. Thus, *S. Ayu* oil was positively identified in all beach segments except PTN-03. There was no evidence that potentially introduced hydrocarbons from tarred anchors or support vessels contributed to the hydrocarbons observed in environmental samples.

Weathering of *S. Ayu* oil was the most likely source of biologically available hydrocarbons at previously oiled beaches. Thermodynamically driven loss of hydrocarbons from oil to water and from water to organism (or passive sampler) is likely a primary contamination mechanism. Differential loss of smaller molecules is defined as weathering and that process explains the observed time-dependent changes in PAH and alkane composition. The PAH composition changes between 2005 and 2008 were consistent with weathering; for example, the proportion of chrysenes, which are relatively large and environmentally persistent increased significantly. By 2008, most samples were more weathered than could be explained by composition of the most weathered source oil (IFO<sub>H</sub>), thus the statistically significant time-dependent change was caused by active weathering. Similarly, time-dependent shifts in alkane composition were significant, consistent with weathering, and exceeded weathering in all source oils, thus could not be explained as an unchanging consequence of source oil mixing.

Principal component analysis allowed examination of weathering processes in PAHs and alkanes and provided an unbiased method to distinguish oil sources and non-oiled samples. The well-



documented weathering of PAHs (Bence and Burns 1995; Marty et al., 1997; Boehm et al., 2004; Neff et al., 2006), summarized by  $w$  (Short and Heintz 1997), was related to principal components, demonstrating the mathematical link between the two approaches. Increased PAH weathering can also be described by increased proportions of the most persistent PAHs such as chrysenes – and modeled weathering and principal components were related to chrysene proportions. Furthermore, the results directly support a relationship between PAH and alkane weathering; preferential loss of smaller alkanes was obvious (Fig. 3.20), consistent with known weathering patterns (Wang and Stout 2007). In addition,  $w_i$  of smaller biomarker molecules (isoprenoids) was also related to  $w_{\text{PAH}}$  (Fig. 3.22) and loss patterns (Fig. 3.23) were consistent with known biomarker weathering (Wang and Stout 2007). Distribution patterns in principal component space were essentially the same for each class of compounds; the least weathered oils, collected directly from the *S. Ayu*, were at one extreme and each point cloud extended away, ending with the most weathered patterns in each compound class. Clear compositional gradients existed across each distribution, hallmarks of weathering and in every case the first principal component was correlated with weathering metrics. The significant time-dependent shifts along these weathering gradients confirmed that the PCA analysis detected active weathering and was not simply sorting data based on unchanging mixtures of source oil. Thus, principal component analysis provided a consistent framework to understand and explore these data and, coupled with changing composition patterns, demonstrated PAH and alkane weathering.

Unlike the time-dependent weathering in PAHs and alkanes (including isoprenoids), biomarkers (triterpanes, hopanes, and steranes) did not weather during the three year observation period. Biomarker composition in 2008 was essentially identical to composition in 2005 with no evidence of change. Composition of these compounds varied among source oils, notably between the two largest reservoirs (IFO<sub>L</sub> and IFO<sub>H</sub>), and composition in environmental samples reflected mixtures of these two sources, generally about half IFO<sub>L</sub>. In this case, the gradients evident in PCA reflected unchanging biomarker mixtures and composition did not shift between 2005 and 2008 (Fig. 3.24). Biomarker composition in spilled marine diesel oil was clearly different than in IFO but when relative oil volumes (96% IFO, 4% MDO), densities (0.964 and 0.852 g/ml for IFO and MDO, respectively), and total biomarker content in each source are accounted for, the potential influence of marine diesel oil on composition in spilled oil was < 1% and was not detected.

The unchanging biomarker composition provided a definitive tool for source identification. Of the three biomarker classes, hopane composition provided the most definitive and consistent information; emphasis on these compounds in an alternative analytical approach (Nordtest) is wise. However, we caution those who use the Nordtest to remember that ratios chosen for this test are designed to be unchanging, thus the Nordtest approach cannot be used to understand weathering processes.

Toxic PAH constituents were present in oil in 2008 and these molecules were mobilized by weathering. For example, oil in sediments contained fluorenes, dibenzothiophenes, phenanthrenes, and pyrenes, all toxic to fish embryos (Incardona et al., 2004). Weathering removes lower molecular weight compounds most rapidly, preferentially leaving the heavier and more toxic PAHs (compare Figs. 3.17 and 3.15), yet weathering was not so extensive that mobile, intermediate-sized, toxic PAHs were gone from the oil. Furthermore, measured PAH

concentrations in the oil were above previously established method detection limits in most samples (93%), thus verifying the presence of oil.

The mobility, hence bioavailability, of toxic constituents from *S. Ayu* oil is supported by mussel and passive sampler data, confirming the differential loss of oil constituents as dictated by thermodynamics (Short and Heintz 1997). Toxic PAHs were evident in mussel tissue, though generally at low concentrations. Modeling did not unambiguously identify oil in mussels from oiled areas, rather only indicated a proclivity towards oil compared to reference mussels. However, aqueous transfer and subsequent uptake by living organisms tends to modify PAH composition, making interpretation more difficult (Bence and Burns 1995; Carls 2006). Less hydrophobic compounds leave oil and enter water more rapidly than more hydrophobic compounds, explaining weathering and resulting in an aqueous PAH composition biased toward lower molecular weight PAHs with respect to oil (Short and Heintz 1997; Carls et al., 2004). Conversely, living organisms (and passive samplers) preferentially accumulate the more hydrophobic PAHs and animals, including mussels, are capable of metabolizing PAHs (Livingstone 1991). Composition of PAHs in oiled mussels suggested the presence of oil constituents; in particular, phenanthrene distributions in tissue were frequently consistent with a petroleum source. Oil patterns were also apparent in the less frequently observed fluorenes, dibenzothiophenes, and fluoranthene-pyrenes.

Risk posed by water-borne oil constituents in summer 2008 was likely small in areas oiled by the *S. Ayu*. The low PAH concentrations in mussel tissue (6 to 71 ng/g dry weight) and passive samplers in summer 2008 indicate that aqueous concentrations were low. Bioconcentration factors for petroleum hydrocarbon mixtures for the common mussel, *Mytilus edulis* are approximately  $2 \times 10^5$  for seawater concentrations of 1 to 400 µg/L (Burns and Smith 1981; Livingstone 1991). This implies the mean aqueous TPAH concentration in oiled areas was roughly 0.15 ng/L (parts per trillion) if they were in equilibrium (which is unlikely given the immense dilution factor represented by surrounding water). Estimates based on passive samplers (Carls et al., 2004) placed intertidally were similar, 0.14 ng/L [= (49 ng/device / 2.2 g/device) /  $1.6 \times 10^5$  ml/g]. Estimates based on bioaccumulation factors estimated for suspension feeding yield similar results ( $1.8 \times 10^5$  for pristane in mussels (Short 2005)). Although the potential influence of nonequilibrium conditions on these estimates is unknown, such as how concentration might increase in occasional erratic pulses of unusually contaminated water, the implication is that mean aqueous TPAH concentrations in summer 2008 in areas oiled by the *S. Ayu* spill were probably several orders of magnitude smaller than concentrations damaging to sensitive early life stages such as embryonic fish (Marty et al., 1997; Carls et al., 1999; Heintz et al., 1999; Heintz et al., 2000; Carls et al., 2005).

The PAH concentrations in mussels due to *S. Ayu* oil in summer 2008 were unlikely to cause damage to mussels even though molluscs can be adversely affected by low quantities of oil. Invertebrate immune systems are very sensitive to environmental contaminants (Hannam et al., 2009). For example, exposure via contaminated plankton may interfere with hemocyte maturation or differentiation in soft shell clams (*Mya arenaria*) at PAH concentrations of 0.05 µg/L (Pichaud et al., 2008). Exposure to produced water inhibited cell viability, phagocytosis and cytotoxicity at 0.4 µg/L total PAH in *M. edulis*; this water also contained heavy metals and other contaminants, so relative contributions are unknown (Hannam et al., 2009). Hemocyte counts

and protein levels were elevated and cell membrane stability and phagocytosis were reduced in Arctic scallop (*Chlamys islandica*) at 0.3 to 2.2 µg/L TPAH, resulting in tissue burdens of 3365 and 5700 ng/g dry weight (Hannam et al., 2009); significant effects were more frequent at the higher concentration. Lysosomal destabilization was observed at 2100 ng/g TPAH in oysters (*Crassostrea virginica*) fed oiled food (Hwang et al., 2008). Mussels (*M. trossulus*) collected from an oiled area in Prince William Sound with 655 ng/g dry weight TPAH burdens in tissue were significantly less tolerant to air exposure than reference mussels (Thomas et al., 1999); 39 of the 44 PAHs measured in this study were measured in the former study. The mean TPAH burden observed in Unalaska mussels from the oiled area, 26 ng/g dry weight, is consistent with background levels in Prince William Sound, about 2.5 to 28 ng/g dry weight (Boehm et al., 2004; Carls and Harris 2005). Mean TPAH burdens in mussel tissue from oiled areas in summer 2008 were 25 times smaller than the smallest known detrimental burden (Thomas et al., 1999); the maximum was 9 times smaller, suggesting that any PAH effects caused by residual oil in Unalaska mussels were likely negligible in 2008. In contrast, concentrations in one Chernofski Harbor mussel sample (2780 ng/g dry weight) were high enough to suspect impairment and several were <5 times the minimum known impairment level.

Consistent corroboration among data sets demonstrates that meaningful chemical information is present at low PAH concentrations. This correspondence included corroboration of mussel data by passive samplers and vice versa including similarity of total PAH concentrations, modeled source composition estimates, and area separation by principal components analysis. Furthermore, the low-level patterns in passive samplers were repeated in three different zones, intertidal, subtidal, and surface water. In addition, a consistent relationship between principal components and weathering emerged in mussels and passive samplers that was consistent with the same pattern in whole oil samples, where concentrations typically substantially exceeded method detection limits. Further corroborative evidence was provided by the existence of a third unique signature in a historically contaminated area (Chernofski Harbor). Moreover, the same pattern of petrogenic sources in oiled areas and a tendency toward pyrogenic sources in Chernofski Harbor was evident in mussel samples collected the previous winter and independently analyzed by a different laboratory (Alpha Woods Hole) (Mauseth et al., 2008).

A third distinct, historically impacted area was discovered during the chemical survey, separated from the reference area by greater mean PAH concentrations and pyrogenic composition. Chernofski Harbor was likely contaminated as a result of World War II activities when it was used as a seaplane base; the area was also used for sheep ranching. As of 2005, the military cleanup status of Chernofski Harbor was pending (Anonymous 2005). Remnants of buildings and docks are evident in the area. Perhaps creosote used to preserve pilings may explain at least in part the pyrogenic signature observed in this harbor, an inference strengthened by a mussel sample collected directly from a wooden piling with a clear pyrogenic pattern and order of magnitude higher TPAH concentration than in any other sample.

The multi-year persistence of intertidal oil deposits from the *S. Ayu* and continued bioavailability of PAHs are consistent with other spill histories. Oil effects can persist for many years in sediment (Teal and Howarth 1984; Carls et al., 2001; Carls et al., 2004; Short et al., 2008). For example, intertidally deposited *Exxon Valdez* oil has persisted for more than 20 y and in some places is nearly as toxic as it was the first few weeks after the spill (Short et al., 2006; Short et

al., 2007; Springman et al., 2007; Short et al., 2008; Springman et al., 2008). Oil from the *Florida* barge spill in West Falmouth, Massachusetts has persisted > 30 y in marsh sediment and will likely persist indefinitely; toxic, substituted triaromatic and higher ring number aromatics are degraded slowly (Blumer et al., 1973; Reddy et al., 2002). Hydrocarbons from the *Florida* induced cytochrome P4501A in mummichogs (*Fundulus heteroclitus*) 8-20 y later, signaling continued biological availability and incomplete habitat recovery (Stegeman 1978; Teal et al., 1992). Estimated residence time for *Metula* oil in the Strait of Magellan was 15-30 y in low energy sand and gravel beaches and >100 yr in sheltered tidal flats and marshes (Gundlach et al., 1981). Weathered Bunker C oil was detected in Chedabucto Bay, Nova Scotia, 20 y after the *Arrow* spill (Vandermeulen and Sing 1994). After 6-7 y, clams (*Mya arenaria*) from an oiled location in Chedabucto Bay remained stressed and species diversity was uniformly lower at oiled locations than at reference locations (Gilfillan and Vandermeulen 1978). Given this context, discovery of remnant *S. Ayu* oil, its bioavailability, and potential toxicity after about 4 y is unsurprising.

Prediction of long-term, persistent toxicity of *S. Ayu* oil from these chemical data is not possible. Toxicity is a function of the exposure composition and concentration and both are controlled by weathering rate. Persistence of potential toxicity of intertidal *S. Ayu* oil deposits into the future might be estimated from weathering, though there are insufficient observations across time for adequate prediction. Mean PAH weathering in oiled areas increased from 0.4 (in 2004 to 2005) to 2.6 (in 2008), with broad, overlapping ranges in both groups. If the progression were linear, about six more years would be required to reach a mean *w* of 7, and evidence from the most weathered *S. Ayu* samples indicates toxic PAHs would remain (e.g., Fig. 3.15, bottom panel). However, weathering may be a nonlinear process as suggested by principal component analysis (e.g., Fig. 3.18); if so, predicting future weathering without more time-series data is not possible. Estimation of the time *S. Ayu* oil will persist on the shorelines is not possible from available chemical data because contemporary concentrations are unknown, precluded by sampling decisions to target oil to characterize source and degree of weathering instead of collecting very large samples designed to characterize concentration distributions. However, long-term oil retention can roughly be predicted from visual assessment of the tidal elevation and degree of subsurface oil; in supratidal zones and sheltered shorelines, oil residue may persist for decades (Michel et al., 2008).

## Summary

Some *S. Ayu* oil remains on beach segments within the core spill area. This oil has weathered to various degrees; rates of weathering were highly variable within and among beach segments. The PAHs and alkanes weathered; biomarkers (triterpanes, hopanes and steranes) were stable. However, combined analysis of weathering across years (2004 to 2008) clearly demonstrates that weathering progresses through time and is consistent with a single oiling event. Toxic PAH constituents remain in intertidal oil deposits including fluorenes, dibenzothiophenes, phenanthrenes, and pyrenes. The oil continues to weather, thus toxic compounds remain biologically available. Although biologically available oil constituents were detected in mussels, observation with passive samplers provided the most definitive proof of bioavailability. However, the amount of mobile oil was relatively small, yielding uptake concentrations that are likely inconsequential for mussels.

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**Table 3.1.** List of sampled zones, oiled zone statistics, number of samples collected by type, and sampling dates. (The number of sediment samples analyzed follow in parentheses. All mussel and passive sampler samples were analyzed). Type codes are: E – No Endpoint Attainment, A – Alternative Treatment, H – Harlequin Duck Sampling Site, S – Subsistence Sampling, NOO – No Oil Observed Segment.

| Segment              | Zone | Type | Oil Band Width (m) | Oil Band Buffered Width (m) | Oil Zone Length (m) | Oil Zone Area (m <sup>2</sup> ) | 1 <sup>st</sup> Survey Date | 2 <sup>nd</sup> Survey Dates | Pits | Sediment Samples | Mussel Samples | Passive Arrays |
|----------------------|------|------|--------------------|-----------------------------|---------------------|---------------------------------|-----------------------------|------------------------------|------|------------------|----------------|----------------|
| <b>Oiled beaches</b> |      |      |                    |                             |                     |                                 |                             |                              |      |                  |                |                |
| HMP-05               | B    | H    | 2.5                | 6.5                         | 60                  | 390                             | 6/30/08                     | 7/28/08                      | 10   | 3 (1)            | 2              | 2              |
| HMP-05               | C    | H    | 3.5                | 7.5                         | 160                 | 1200                            | 6/30/08                     | 7/28/08                      | 40   | 7 (2)            | 5              | 2              |
| HMP-05               | D    | H    | 8                  | 12                          | 20                  | 240                             | 6/30/08                     | 7/28/08                      | 10   | 1 (1)            | 2              | 2              |
| KPF-01               | A    | E    | 2                  | 6                           | 100                 | 600                             | 7/01/08                     | 7/29/08                      | 20   | 2 (0)            | 2              | 1              |
| KPF-01               | B    | E    | 10                 | 14                          | 25                  | 350                             | 7/01/08                     | 7/29/08                      | 10   | 3 (1)            | 2              | 1              |
| KPF-01               | C    | E    | 2                  | 6                           | 30                  | 180                             | 7/01/08                     | 7/29/08                      | 10   | 4 (0)            | 2              | 1              |
| KPF-01               | D    | E    | 13                 | 17                          | 22                  | 374                             | 6/28/08                     | 7/29/08                      | 10   | 4 (2)            | 2              | 1              |
| KPF-01               | E    | E    | 25                 | 29                          | 50                  | 1450                            | 6/28/08                     | 7/29/08                      | 40   | 8 (4)            | 2              | 1              |
| KPF-01               | F    | E    | 3                  | 7                           | 150                 | 1050                            | 6/28/08                     | 7/25/08                      | 40   | 15 (11)          | 3              | 2              |
| KPF-01               | G    | E    | 10                 | 14                          | 200                 | 2800                            | 6/28/08                     | 7/25/08                      | 80   | 16 (11)          | 3              | 2              |
| MKS-14               | A    | E    | 15                 | 19                          | 100                 | 1900                            | 6/29/08                     | 7/27/08                      | 36   | 9 (3)            | 3              | 2              |
| MKS-14               | B    | E    | 15                 | 19                          | 50                  | 950                             | 6/29/08                     | 7/27/08                      | 20   | 6 (5)            | 2              | 1              |
| MKS-16               | B/C  | E    | 10                 | 14                          | 100                 | 1400                            | 6/29/08                     | 7/27/08                      | 45   | 12 (10)          | 3              | 3              |
| MKS-16               | F    | E    | 4                  | 8                           | 50                  | 400                             | 6/29/08                     | 7/27/08                      | 10   | 2 (1)            | 3              | 1              |
| PTN-03               | A    | H    | 2                  | 6                           | 400                 | 2400                            | 6/30/08                     | 7/26/08                      | 80   | 3 (3)            | 3              | 3              |
| SKN-05               | A    | A    | 15                 | 19                          | 400                 | 7600                            | 6/30/08                     | 7/31/08                      | 80   | 11 (8)           | 1              | 3              |
| SKN-11               | A    | S    | 5                  | 9                           | 30                  | 270                             | 7/01/08                     | 7/24/08                      | 10   | 1 (1)            | 2              | 1              |
| SKN-15               | B    | E    | 5                  | 9                           | 30                  | 270                             | 7/01/08                     | 7/30/08                      | 10   | 3 (2)            | 2              | 1              |
| SKN-15               | C    | E    | 4                  | 8                           | 25                  | 200                             | 7/01/08                     | 7/30/08                      | 10   | 5 (3)            | 4              | 1              |
| SKN-15               | D    | E    | 15                 | 19                          | 60                  | 1140                            | 7/01/08                     | 7/30/08                      | 40   | 3 (2)            | 3              | 2              |
| SKN-15               | E    | E    | 15                 | 19                          | 60                  | 1140                            | 7/01/08                     | 7/30/08                      | 40   | 10 (1)           | 3              | 2              |
| SKS-04               | A    | S, A | 1                  | 5                           | 235                 | 1175                            | 6/28/08                     | 7/24/08                      | 40   | 5 (3)            | 2              | 2              |
| SKS-12               | A    | H, A | 0.2                | 4.2                         | 250                 | 1050                            | 6/28/08                     | 7/24/08                      | 33   | 3 (4)            | 3              | 3              |

**Table 3.1.** Cont.

| Segment                       | Zone | Type | Oil Band Width (m) | Oil Band Buffered Width (m) | Oil Zone Length (m) | Oil Zone Area (m <sup>2</sup> ) | 1 <sup>st</sup> Survey Date | 2 <sup>nd</sup> Survey Dates | Pits       | Sediment Samples | Mussel Samples | Passive Arrays |
|-------------------------------|------|------|--------------------|-----------------------------|---------------------|---------------------------------|-----------------------------|------------------------------|------------|------------------|----------------|----------------|
| <b>Reference beaches</b>      |      |      |                    |                             |                     |                                 |                             |                              |            |                  |                |                |
| PME-12                        | 1    | NOO  | -                  | -                           | -                   | -                               | 7/02/08                     | 8/01/08                      | -          | -                | 1              | 1              |
| PME-13                        | 1    | NOO  | -                  | -                           | -                   | -                               | 7/02/08                     | 8/01/08                      | -          | -                | 3              | 1              |
| PME-13                        | 2    | NOO  | -                  | -                           | -                   | -                               | 7/02/08                     | 8/01/08                      | -          | -                | 3              | 1              |
| PME-14                        | 1    | NOO  | -                  | -                           | -                   | -                               | 7/02/08                     | 8/01/08                      | -          | -                | 3              | 1              |
| PME-15                        | 1    | NOO  | -                  | -                           | -                   | -                               | 7/02/08                     | 8/01/08                      | -          | -                | 3              | 1              |
| PME-15                        | 2    | NOO  | -                  | -                           | -                   | -                               | 7/02/08                     | 8/01/08                      | -          | -                | 3              | 1              |
| PME-18                        | 1    | NOO  | -                  | -                           | -                   | -                               | 7/02/08                     | 8/01/08                      | -          | -                | 2              | 1              |
| PME-18                        | 2    | NOO  | -                  | -                           | -                   | -                               | 7/02/08                     | 8/01/08                      | -          | -                | 2              | 1              |
| PMN-22                        | 1    | NOO  | -                  | -                           | -                   | -                               | 7/02/08                     | 8/01/08                      | -          | -                | 1              | 2              |
| PMN-22                        | 2    | NOO  | -                  | -                           | -                   | -                               | 7/02/08                     | 8/01/08                      | -          | -                | 3              | 2              |
| PMS-13                        | 1    | NOO  | -                  | -                           | -                   | -                               | 7/02/08                     | 8/01/08                      | -          | -                | 3              | 1              |
| PMS-13                        | 2    | NOO  | -                  | -                           | -                   | -                               | 7/02/08                     | 8/01/08                      | -          | -                | 3              | 1              |
| <b>Human Impacted beaches</b> |      |      |                    |                             |                     |                                 |                             |                              |            |                  |                |                |
| CHER-01                       | 1    | NOO  | -                  | -                           | -                   | -                               | 7/03/08                     | 8/02/08                      | -          | -                | 3              | 1              |
| CHER-02                       | 1    | NOO  | -                  | -                           | -                   | -                               | 7/03/08                     | 8/02/08                      | -          | -                | 2              | 1              |
| CHER-03                       | 1    | NOO  | -                  | -                           | -                   | -                               | 7/03/08                     | 8/02/08                      | -          | -                | 3              | 1              |
| CHER-04                       | 1    | NOO  | -                  | -                           | -                   | -                               | 7/03/08                     | 8/02/08                      | -          | -                | 3              | 1              |
| CHER-05                       | 1    | NOO  | -                  | -                           | -                   | -                               | 7/03/08                     | 8/02/08                      | -          | -                | 3              | 1              |
| CHER-06                       | 1    | NOO  | -                  | -                           | -                   | -                               | 7/03/08                     | 8/02/08                      | -          | -                | 2              | 1              |
| <b>Totals</b>                 |      |      |                    |                             |                     |                                 |                             |                              | <b>724</b> | <b>136</b>       | <b>105</b>     | <b>60</b>      |

**Table 3.2.** Samples collected at or near study segments were used to establish original *S. Ayu* oil characteristics for data interpretation. Target beach segments are in parentheses at the beginning of each section. Asterisks indicate previously quantified data.

|                         | SIN     | Date     | Matrix         | Location      | Latitude  | Longitude   | Zone            | Collector |
|-------------------------|---------|----------|----------------|---------------|-----------|-------------|-----------------|-----------|
| Makushin (MKS14, MKS16) |         |          |                |               |           |             |                 |           |
|                         | 1902408 | 01/27/05 | sediment       | MKS05         | 53.776720 | -166.959530 | Stream          | Zelo      |
|                         | 1902410 | 01/27/05 | sediment       | MKS05         | 53.773460 | -166.952800 | Stream          | Zelo      |
|                         | 1902411 | 01/27/05 | sediment       | MKS05         | 53.773140 | -166.950030 | Stream          | Zelo      |
|                         | 1600245 | 03/20/05 | sediment       | MKS05         |           |             | Stream          | Carls*    |
|                         | 1600246 | 03/20/05 | Oil+rock       | MKS05         |           |             | Stream          | Carls*    |
|                         | 1600501 | 03/20/05 | sediment       | MKS05         |           |             | Stream          | Carls*    |
|                         | 1600250 | 03/20/05 | sediment       | MKS05         |           |             | Stream          | Carls*    |
|                         | 1600504 | 03/20/05 | sediment       | MKS05         |           |             | Stream          | Carls*    |
|                         | 1600503 | 03/20/05 | sediment       | MKS05         |           |             | Stream          | Carls*    |
|                         | 1600530 | 04/15/05 | sediment       | MKS05         |           |             | Stream          | Carls*    |
|                         | 1902401 | 01/31/05 | sediment       | MKS10         | 53.771780 | -166.984100 | Stream          | Zelo      |
|                         | 1902405 | 01/31/05 | sediment       | MKS10         | 53.772550 | -166.983090 | Stream          | Zelo      |
| Humpback Bay (HMP05)    |         |          |                |               |           |             |                 |           |
|                         | 1902420 | 01/04/05 | oil            | HMP05         | 53.739580 | -166.870480 | Intertidal      | Cubit     |
|                         | 1902422 | 01/04/05 | oil            | HMP05         | 53.739870 | -166.869060 | Intertidal      | Cubit     |
|                         | 1902435 | 01/12/05 | oil            | HMP06         | 53.743250 | -166.861583 |                 | Hahn      |
|                         | 1902442 | 01/05/05 | oil            | Humpback Bay  |           |             | Intertidal      | Adkins    |
|                         | 1902443 | 12/15/04 | oil            | Humpback Cove | 53.755000 | -166.873611 | Intertidal      | Carlson   |
| Portage Bay (PTN03)     |         |          |                |               |           |             |                 |           |
|                         | 1902402 | 01/31/05 | sediment       | PTN02         | 53.727640 | -166.710410 | Stream          | Zelo      |
|                         | 1902403 | 01/31/05 | sediment       | PTN02         | 53.726990 | -166.710520 | Stream          | Zelo      |
|                         | 1600248 | 03/20/05 | bank, tar ball | PTN06         |           |             | Stream          | Carls*    |
| Kof Point (KFP01)       |         |          |                |               |           |             |                 |           |
|                         | 1902415 | 12/28/04 | oil            | KFP05         | 53.668450 | -167.059533 | Intertidal      | DiPinto   |
|                         | 1902428 | 12/28/04 | sediment       | KFP05         | 53.667620 | -167.062030 | Intertidal      | DiPinto   |
|                         | 1902431 | 12/28/04 | sediment       | KFP05         | 53.668450 | -167.060960 | Intertidal      | DiPinto   |
|                         | 1902419 | 12/31/04 | oil            | KFP09         | 53.687470 | -167.065410 | Intertidal      | Cubit     |
|                         | 1902429 | 01/07/05 | oil            | KFP10         | 53.689850 | -167.063400 | high intertidal | Hahn      |
| Skan Bay north (SKN15)  |         |          |                |               |           |             |                 |           |
|                         | 1902437 | 01/06/05 | oil            | SKN15         | 53.652750 | -167.032067 |                 | Hahn      |
|                         | 1902436 | 01/11/05 | oil            | SKN14         | 53.647217 | -167.007700 | Bank            | Hahn      |
|                         | 1600236 | 03/19/05 | sediment       | SKN14         |           |             | Stream          | Carls*    |
|                         | 1600526 | 04/12/05 | sediment       | SKN14         |           |             | Stream          | Carls*    |
|                         | 1600718 | 05/16/05 | sediment       | SKN14         |           |             | Stream          | Carls*    |
|                         | 1902448 | 12/28/04 | oil            | SKN-14        |           |             | Intertidal      | BIS       |
|                         | 1600527 | 04/13/05 | sediment       | SKN14t        |           |             | Stream          | Carls*    |
|                         | 1600528 | 04/14/05 | sediment       | SKN14t        |           |             | Stream          | Carls*    |
|                         | 1902447 | 12/25/04 | oil            | beach 14      | 53.387060 | -167.004140 | Intertidal      | BIS       |
|                         | 1902450 | 05/04/05 | oil            | SKN14         | 53.644150 | -166.993367 | Stream          | Bauer     |

|  |            |             |               |                        |                 |                  |                 |                  |
|--|------------|-------------|---------------|------------------------|-----------------|------------------|-----------------|------------------|
| Skan Bay north (SKN11)   |            |             |               |                        |                 |                  |                 |                  |
|  | 1902426    | 12/27/04    | Sediment      | SKN11                  | 53.642220       | -167.007340      | Mid-tidal       | Zelo             |
|  | 1902430    | 12/27/04    | Sediment      | SKN11                  | 53.642220       | -167.007480      | Mid-tidal       | Zelo             |
|  | 1902412    | 01/28/05    | oil           | SKN11                  | 53.640360       | -167.007740      | Supra tidal     | Zelo             |
|  | <b>SIN</b> | <b>date</b> | <b>matrix</b> | <b>Location</b>        | <b>latitude</b> | <b>longitude</b> | <b>Zone</b>     | <b>collector</b> |
| Skan Bay north (SKN05)   |            |             |               |                        |                 |                  |                 |                  |
|  | 1902414    | 01/18/05    | sediment      | SKN05                  | 53.622160       | -166.996870      | Supratidal      | Iadanza          |
|  | 1600216    | 03/16/05    | sediment      | SKN04                  |                 |                  | Stream          | Carls*           |
|  | 1600215    | 03/16/05    | sediment      | SKN04                  |                 |                  | Stream          | Carls*           |
|  | 1600521    | 04/12/05    | sediment      | SKN04                  |                 |                  | Stream          | Carls*           |
|  | 1600522    | 04/13/05    | sediment      | SKN04                  |                 |                  | Stream          | Carls*           |
|  | 1600523    | 04/14/05    | sediment      | SKN04                  |                 |                  | Stream          | Carls*           |
| Skan Bay south (SKS12)   |            |             |               |                        |                 |                  |                 |                  |
|  | 1902416    | 01/08/05    | oil           | SKS12                  | 53.652750       | -167.032067      | high intertidal | Hahn             |
|  | 1902409    | 01/28/05    | sediment      | SKS12                  | 53.598410       | -167.048590      | intertidal      | Zelo             |
| Skan Bay south (SKS04)   |            |             |               |                        |                 |                  |                 |                  |
|  | 1902441    | 12/24/04    | sediment      | SKS04                  |                 |                  | intertidal      | Gilpin           |
|  | 1902449    | 12/24/04    | oil           | SKS04                  |                 |                  | intertidal      | Gilpin           |
|  | 1902451    | 12/27/04    | soybean       | SKS04                  |                 |                  | intertidal      | Gilpin           |
| Outer Pumicestone (PMN20)  |            |             |               |                        |                 |                  |                 |                  |
|  | 1600516    | 04/11/05    | sediment      | PMN20                  |                 |                  | stream          | Carls*           |
|  | 1600517    | 04/11/05    | sediment      | PMN20                  |                 |                  | stream          | Carls*           |
|  | 1600518    | 04/11/05    | sediment      | PMN20                  |                 |                  | stream          | Carls*           |
|  | 1600519    | 04/11/05    | sediment      | PMN20                  |                 |                  | stream          | Carls*           |
|  | 1902417    | 01/21/05    | sediment      | PMN20/21               | 53.571550       | -167.129410      | stream          | Iadanza          |
|  | 1902425    | 01/21/05    | sediment      | PMN20/21               | 53.571370       | -167.129870      | stream          | Iadanza          |
| Pumicestone Bay (reference; PMS13, PMS15, PME13, PME14, PEM15, PME18)                    |            |             |               |                        |                 |                  |                 |                  |
|  | 1600511    | 04/11/05    | sediment      | PMS16                  |                 |                  | stream          | Carls*           |
|  | 1600512    | 04/11/05    | sediment      | PMS16                  |                 |                  | stream          | Carls*           |
|  | 1600513    | 04/11/05    | sediment      | PMS16                  |                 |                  | stream          | Carls*           |
|  | 1600514    | 04/11/05    | sediment      | PMS16                  |                 |                  | stream          | Carls*           |
|  | 1902432    | 01/13/05    | sediment      | PMS16/17/18            | 53.529480       | -166.978800      | stream          | Hahn             |
|  | 1902433    | 01/13/05    | sediment      | PMS16/17/18            | 53.529300       | -166.978200      | stream          | Hahn             |
| Chernofski Harbor (human habitation reference; CFE09, CFE10, CFE14, CFE17, CFW17, CFW20) |            |             |               |                        |                 |                  |                 |                  |
|  | 1902424    | 12/27/04    | sediment      | CHN9                   | 53.403280       | -167.508060      | intertidal      | DiPinto          |
|  | 1902427    | 12/27/04    | sediment      | CHN10                  | 53.400590       | -167.527640      | intertidal      | DiPinto          |
|  | 1902421    | 12/27/04    | sediment      | CHN12                  | 53.410760       | -167.524040      | intertidal      | DiPinto          |
|  | 1902423    | 12/27/04    | oil           | CHN12                  | 53.408900       | -167.529860      | intertidal      | DiPinto          |
| M/V <i>S. Ayu</i>  |            |             |               |                        |                 |                  |                 |                  |
|  | 1902446    | 12/19/04    | oil           | #4 Port IFO tank       |                 |                  | <i>S. Ayu</i>   | Carlson          |
|  | 1902444    | 12/31/04    | oil           | PST-5 F #3C            |                 |                  | <i>S. Ayu</i>   | Atkinson         |
|  | 1902452    | 01/05/05    | oil           | STBD MDO service tank  |                 |                  | <i>S. Ayu</i>   | Atkinson         |
|  | 1902445    | 01/05/05    | oil           | STBD HFO settling tank |                 |                  | <i>S. Ayu</i>   | Atkinson         |

**Table 3.3.** Deuterated surrogate polynuclear aromatic hydrocarbon (PAH) standards and concentrations in spike used for passive samplers (PEMDs), tissue, and sediment. Spike volumes were 500 µL for tissue and sediments and half the equivalent for PEMDs, PAHs only. Spike solvent was hexane.

| (µg/ml) | Surrogate                               |
|---------|---|
| 2.0     | naphthalene-d <sub>8</sub>              |
| 2.0     | acenaphthene-d <sub>10</sub>            |
| 2.0     | phenanthrene-d <sub>10</sub>            |
| 2.0     | chrysene-d <sub>12</sub>                |
| 2.0     | perylene-d <sub>12</sub>                |
| 2.0     | benzo[ <i>a</i> ]pyrene-d <sub>12</sub> |
| 9.9     | n-dodecane-d <sub>26</sub>              |
| 9.7     | n-hexadecane-d <sub>34</sub>            |
| 9.7     | n-eicosane-d <sub>42</sub>              |
| 9.8     | n-tetracosane-d <sub>50</sub>           |
| 9.7     | n-triacontane-d <sub>62</sub>           |

**Table 3.4.** Polynuclear aromatic hydrocarbon (PAH) analytes, abbreviations, deuterated surrogate references, molecular mass, and the log of the octanol-water partition coefficient ( $K_{ow}$ ). Deuterated surrogates were naphthalene- $d_8$  (1), acenaphthene- $d_{10}$  (2), phenanthrene- $d_{10}$  (3), chrysene- $d_{12}$  (4), perylene- $d_{12}$  (5), and benzo[*a*]pyrene- $d_{12}$  (6). Asterisks indicate interpolated log  $K_{ow}$  estimates.

| PAH                           | Abbreviation | Surrogate | Molecular mass (g/mole) | log $K_{ow}$ |
|-------------------------------|--------------|-----------|-------------------------|--------------|
| naphthalene                   | N0           | 1         | 128.2                   | 3.36         |
| C-1 naphthalenes              | N1           | 1         | 142.2                   | 3.80         |
| C-2 naphthalenes              | N2           | 2         | 156.2                   | 4.30         |
| C-3 naphthalenes              | N3           | 2         | 170.3                   | 4.80         |
| C-4 naphthalenes              | N4           | 2         | 184.3                   | 5.30         |
| biphenyl                      | BIP          | 2         | 154.2                   | 3.80         |
| acenaphthylene                | ACN          | 2         | 152.2                   | 3.22         |
| acenaphthene                  | ACE          | 2         | 154.2                   | 4.01         |
| fluorene                      | F0           | 2         | 166.2                   | 4.21         |
| C-1 fluorenes                 | F1           | 2         | 180.3                   | 4.72         |
| C-2 fluorenes                 | F2           | 2         | 194.3                   | 5.20         |
| C-3 fluorenes                 | F3           | 2         | 208.3                   | 5.70         |
| C-4 fluorenes                 | F4           | 2         | 222.3                   | *6.20        |
| dibenzothiophene              | D0           | 3         | 184.2                   | 4.53         |
| C-1 dibenzothiophenes         | D1           | 3         | 198.3                   | 4.96         |
| C-2 dibenzothiophenes         | D2           | 3         | 212.3                   | 5.42         |
| C-3 dibenzothiophenes         | D3           | 3         | 226.3                   | 5.89         |
| C-4 dibenzothiophenes         | D4           | 3         | 240.3                   | *6.34        |
| phenanthrene                  | P0           | 3         | 178.2                   | 4.57         |
| C-1 phenanthrenes/anthracenes | P1           | 3         | 192.3                   | 5.04         |
| C-2 phenanthrenes/anthracenes | P2           | 3         | 206.3                   | 5.46         |
| C-3 phenanthrenes/anthracenes | P3           | 3         | 220.3                   | 5.92         |
| C-4 phenanthrenes/anthracenes | P4           | 3         | 234.3                   | 6.32         |
| anthracene                    | ANT          | 3         | 178.2                   | 4.53         |
| fluoranthene                  | FLU          | 3         | 202.3                   | 5.08         |
| pyrene                        | PYR          | 3         | 202.3                   | 4.92         |
| C-1 fluoranthenes/pyrenes     | FP1          | 3         | 216.3                   | 5.48         |
| C-2 fluoranthenes/pyrenes     | FP2          | 3         | 230.3                   | 6.15         |
| C-3 fluoranthenes/pyrenes     | FP3          | 3         | 244.3                   | 6.60         |
| C-4 fluoranthenes/pyrenes     | FP4          | 3         | 258.3                   | *7.22        |
| benzo(a)anthracene            | BAA          | 4         | 228.3                   | 5.89         |
| chrysene                      | C0           | 4         | 228.3                   | 5.71         |
| C-1 chrysenes                 | C1           | 4         | 242.3                   | 6.14         |
| C-2 chrysenes                 | C2           | 4         | 256.3                   | 6.43         |
| C-3 chrysenes                 | C3           | 4         | 270.4                   | 6.94         |
| C-4 chrysenes                 | C4           | 4         | 284.4                   | 7.36         |
| benzo(b)fluoranthene          | BBF          | 6         | 252.3                   | 6.27         |
| benzo(k)fluoranthene          | BKF          | 6         | 252.3                   | 6.29         |
| Benzo(e)pyrene                | BEP          | 6         | 252.3                   | 6.44         |
| Benzo(a)pyrene                | BAP          | 6         | 252.3                   | 6.11         |
| Perylene                      | PER          | 5         | 252.3                   | 6.44         |
| indeno(1,2,3-cd)pyrene        | ICP          | 6         | 276.3                   | 6.72         |
| dibenzo(a,h)anthracene        | DBA          | 6         | 278.4                   | 6.71         |
| benzo(ghi)perylene            | BZP          | 6         | 276.3                   | 6.51         |

**Table 3.5.** Measured alkanes and their abbreviations. Pristane and phytane are branched isoprenoids; all others are straight-chain compounds.

| <b>Abbreviation</b> | <b>Alkane</b>                               |
|---------------------|---|
| C9alk               | n-nonane                                    |
| C10alk              | n-decane                                    |
| C11alk              | n-undecane                                  |
| C12alk              | n-dodecane                                  |
| C13alk              | n-tridecane                                 |
| C14alk              | n-tetradecane                               |
| C15alk              | n-pentadecane                               |
| C16alk              | n-hexadecane                                |
| C17alk              | n-heptadecane                               |
| Prist               | 2,6,10,14-tetramethylpentadecane (pristane) |
| C18alk              | n-octadecane                                |
| Phyt                | 2,6,10,14-tetramethylhexadecane (phytane)   |
| C19alk              | n-nonadecane                                |
| C20alk              | n-eicosane                                  |
| C21alk              | n-heneicosane                               |
| C22alk              | n-docosane                                  |
| C23alk              | n-tricosane                                 |
| C24alk              | n-tetracosane                               |
| C25alk              | n-pentacosane                               |
| C26alk              | n-hexacosane                                |
| C27alk              | n-heptacosane                               |
| C28alk              | n-octacosane                                |
| C29alk              | n-nonacosane                                |
| C30alk              | n-triacontane                               |
| C31alk              | n-hentriacontane                            |
| C32alk              | n-dotriacontane                             |
| C33alk              | n-tritriacontane                            |
| C34alk              | n-tetratriacontane                          |
| C35alk              | n-pentatriacontane                          |
| C36alk              | n-hexatriacontane                           |



**Table 3.6.** Measured biomarkers, their abbreviations and target ions (m/z).

| Abbreviation | Biomarker   | Target Ions |
|--------------|---|-------------|
| norprist     | norpristane   | 57          |
| prist        | 2,6,10,14-tetramethylpentadecane (pristane)                                   | 57          |
| phyt         | 2,6,10,14-tetramethylhexadecane (phytane)                                     | 57          |
| TR23         | C23 tricyclic terpane   | 191         |
| TR24         | C24 tricyclic terpane   | 191         |
| TR25a        | C25 tricyclic terpane (a)   | 191         |
| TR25b        | C25 tricyclic terpane (b)   | 191         |
| TET24        | C24 tetracyclic terpane   | 191         |
| TR26a        | C26 tricyclic terpane (a)   | 191         |
| TR26b        | C26 tricyclic terpane (b)   | 191         |
| TR28a        | C28 tricyclic terpane (a)   | 191         |
| TR28b        | C28 tricyclic terpane (b)   | 191         |
| TR29a        | C29 tricyclic terpane (a)   | 191         |
| TR29b        | C29 tricyclic terpane (b)   | 191         |
| Ts           | 18 $\alpha$ (H),21 $\beta$ (H)-22,29,30-trisnorhopane                         | 191         |
| Tm           | 17 $\alpha$ (H),21 $\beta$ (H)-22,29,30-trisnorhopane                         | 191         |
| H28          | 17 $\alpha$ (H),18 $\alpha$ (H),21 $\beta$ (H)-28,30-bisnorhopane             | 191         |
| NOR25H       | 17 $\alpha$ (H),21 $\beta$ (H)-25-norhopane                                   | 191         |
| H29          | 17 $\alpha$ (H),21 $\beta$ (H)-30-norhopane                                   | 191         |
| C29Ts        | 18 $\alpha$ (H),21 $\beta$ (H)-30-norneohopane                                | 191         |
| M29          | 17 $\alpha$ (H),21 $\beta$ (H)-30-norhopane (normoretane)                     | 191         |
| OL           | 18 $\alpha$ (H) and 18 $\beta$ (H)-oleanane                                   | 191         |
| H30          | 17 $\alpha$ (H),21 $\beta$ (H)-hopane   | 191         |
| NOR30H       | 17 $\alpha$ (H)-30-nor-29-homohopane  | 191         |
| M30          | 17 $\beta$ (H),21 $\alpha$ (H)-hopane (moretane)                              | 191         |
| H31S         | 22S-17 $\alpha$ (H),21 $\beta$ (H)-30-homohopane                              | 191         |
| H31R         | 22R-17 $\alpha$ (H),21 $\beta$ (H)-30-homohopane                              | 191         |
| GAM          | Gammacerane   | 191         |
| H32S         | 22S-17 $\alpha$ (H),21 $\beta$ (H)-30,31-bishomohopane                        | 191         |
| H32R         | 22R-17 $\alpha$ (H),21 $\beta$ (H)-30,31-bishomohopane                        | 191         |
| H33S         | 22S-17 $\alpha$ (H),21 $\beta$ (H)-30,31,32-trishomohopane                    | 191         |
| H33R         | 22R-17 $\alpha$ (H),21 $\beta$ (H)-30,31,32-trishomohopane                    | 191         |
| H34S         | 22S-17 $\alpha$ (H),21 $\beta$ (H)-30,31,32,33-tetrakishomohopane             | 191         |
| H34R         | 22R-17 $\alpha$ (H),21 $\beta$ (H)-30,31,32,33-tetrakishomohopane             | 191         |
| H35S         | 22S-17 $\alpha$ (H),21 $\beta$ (H)-30,31,32,33,34-pentakishomohopane          | 191         |
| H35R         | 22R-17 $\alpha$ (H),21 $\beta$ (H)-30,31,32,33,34-pentakishomohopane          | 191         |
| S22          | C <sub>22</sub> 5 $\alpha$ (H),14 $\beta$ (H),17 $\beta$ (H)-sterane          | 217,218     |
| DIA27S       | C <sub>27</sub> 20S-13 $\beta$ (H),17 $\alpha$ (H)-diasterane                 | 217,218     |
| DIA27R       | C <sub>27</sub> 20R-13 $\beta$ (H),17 $\alpha$ (H)-diasterane                 | 217,218     |
| C27S         | C <sub>27</sub> 20S-5 $\alpha$ (H),14 $\alpha$ (H),17 $\alpha$ (H)-cholestane | 217,218     |

**Table 3.6.** Cont.

| <b>Abbreviation</b> | <b>Biomarker</b>   | <b>Target Ions</b> |
|---------------------|--|--------------------|
| C27BBR              | C <sub>27</sub> 20R-5 $\alpha$ (H),14 $\beta$ (H),17 $\beta$ (H)-cholestane    | 217,218            |
| C27BBS              | C <sub>27</sub> 20S-5 $\alpha$ (H),14 $\beta$ (H),17 $\beta$ (H)-cholestane    | 217,218            |
| C27R                | C <sub>27</sub> 20R-5 $\alpha$ (H),14 $\alpha$ (H),17 $\alpha$ (H)-cholestane  | 217,218            |
| C28S                | C <sub>28</sub> 20S-5 $\alpha$ (H),14 $\alpha$ (H),17 $\alpha$ (H)-ergostane   | 217,218            |
| C28BBR              | C <sub>28</sub> 20R-5 $\alpha$ (H),14 $\beta$ (H),17 $\beta$ (H)-ergostane     | 217,218            |
| C28BBS              | C <sub>28</sub> 20S-5 $\alpha$ (H),14 $\beta$ (H),17 $\beta$ (H)-ergostane     | 217,218            |
| C28R                | C <sub>28</sub> 20R-5 $\alpha$ (H),14 $\alpha$ (H),17 $\alpha$ (H)-ergostane   | 217,218            |
| C29S                | C <sub>29</sub> 20S-5 $\alpha$ (H),14 $\alpha$ (H),17 $\alpha$ (H)-stigmastane | 217,218            |
| C29BBR              | C <sub>29</sub> 20R-5 $\alpha$ (H),14 $\beta$ (H),17 $\beta$ (H)-stigmastane   | 217,218            |
| C29BBS              | C <sub>29</sub> 20S-5 $\alpha$ (H),14 $\beta$ (H),17 $\beta$ (H)-stigmastane   | 217,218            |
| C29R                | C <sub>29</sub> 20R-5 $\alpha$ (H),14 $\alpha$ (H),17 $\alpha$ (H)-stigmastane | 217,218            |

**Table 3.7.** Percentages of TPAH concentrations above-background (145 ng/device) in PEMDs.  
Reference locations are marked with an asterisk.

| <b>Segment</b>    | <b>percent</b> | <b>n &gt; backgrnd</b> | <b>n total</b> |
|-------------------|----------------|------------------------|----------------|
| SKS12             | 88             | 7                      | 8              |
| Chernofski Harbor | 72             | 13                     | 18             |
| SKS04             | 50             | 3                      | 6              |
| SKN05             | 25             | 2                      | 8              |
| MKS14             | 17             | 2                      | 12             |
| KFP01             | 10             | 3                      | 29             |
| Pumicestone Bay*  | 6              | 2                      | 33             |
| SKN15             | 5              | 1                      | 20             |
| PMN22*            | 0              | 0                      | 8              |
| HMP05             | 0              | 0                      | 18             |
| MKS16             | 0              | 0                      | 9              |
| PTN03             | 0              | 0                      | 9              |
| SKN11             | 0              | 0                      | 3              |

**Table 3.8.** Attributes of source oils; intermediate fuel oil heavy (IFO<sub>H</sub>), intermediate fuel oil light (IFO<sub>L</sub>), and marine diesel oil (MDO). Proportionate composition of polynuclear aromatic hydrocarbons pPAH, alkanes (pAlk), and biomarkers (pBio) are tabulated. Proportionate dibenzothiophene content relative to phenanthrenes ( $\Sigma D/\Sigma P$ ) distinguishes IFO<sub>H</sub> from IFO<sub>L</sub>. Weathering (w, estimated by modeling; Short and Heintz 1997) summarizes PAH composition; w is least where proportions of naphthalenes (pNaph) are greatest and proportions of chrysenes (pChrys) are least. Weathering is also reflected in proportions of heavy alkanes ( $\geq C_{27}$ ) relative to total alkanes. Weathering in this context reports source oil condition and does not imply environmental change.

| Name           | type             | pPAH | pAlk | pBio  | $\Sigma D/\Sigma P$ | w     | pNaph | pChrys | pHeavyAlk |
|----------------|------------------|------|------|-------|---------------------|-------|-------|--------|-----------|
| PST-11-C       | IFO <sub>H</sub> | 0.27 | 0.71 | 0.019 | 0.94                | 1.17  | 30.69 | 4.31   | 15.32     |
| PST-11-C       | IFO <sub>H</sub> | 0.28 | 0.70 | 0.019 | 0.92                | 1.18  | 30.69 | 4.38   | 13.84     |
| PST-5-F        | IFO <sub>L</sub> | 0.58 | 0.41 | 0.003 | 0.38                | -0.71 | 51.51 | 1.56   | 2.56      |
| PST-5-F        | IFO <sub>L</sub> | 0.61 | 0.39 | 0.003 | 0.36                | -0.71 | 51.86 | 1.54   | 2.39      |
| PST-9-A        | IFO <sub>L</sub> | 0.59 | 0.41 | 0.003 | 0.35                | -0.82 | 52.02 | 1.49   | 2.49      |
| PST-9-A        | IFO <sub>L</sub> | 0.59 | 0.41 | 0.003 | 0.34                | -0.82 | 51.55 | 1.47   | 2.29      |
| PST-9-A        | IFO <sub>L</sub> |      |      |       | 0.34                | -0.82 | 51.63 | 1.49   |           |
| #4 Port<br>IFO | IFO <sub>L</sub> | 0.58 | 0.42 | 0.003 | 0.36                | -0.68 | 51.06 | 1.58   | 2.76      |
| #4 Port<br>IFO | IFO <sub>L</sub> | 0.60 | 0.39 | 0.003 | 0.35                | -0.68 | 51.31 | 1.55   | 2.64      |
| #4 Port<br>IFO | IFO <sub>L</sub> |      |      |       | 0.35                | -0.68 | 51.29 | 1.58   |           |
| PST-8-A        | MDO              |      |      |       | 0.86                | -3.08 | 62.00 | 0.47   |           |
| PST-8-A        | MDO              | 0.21 | 0.79 | 0.000 | 0.71                | -3.08 | 62.71 | 0.44   | 2.21      |