Exxon Valdez Oil Spill
Long-Term Monitoring Program "Gulf Watch Alaska" Final Report

Lingering Oil: Extending the Tracking of Oil Levels and Weathering (PAH Composition) in Prince William Sound through Time

Restoration Project 16120114-S
Final Report

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Study History: This project is part of the *Exxon Valdez* Oil Spill Trustee Council's (EVOSTC) Long-term Monitoring Program known as Gulf Watch Alaska. Project 12120114-S was a 5-year project initiated in 2012 and culminated in 2016. The overall goal of the lingering oil project was to extend previous efforts funded by the EVOSTC to track *Exxon Valdez* oil occurrence and chemical composition in Prince William Sound (PWS). Previous EVOSTC-funded studies (projects 02543, 040585, 050620, 070801 and 12120117) had demonstrated that, on some beaches, subsurface oil persisted in a relatively unweathered state longer than expected with an unknown long-term fate. Chemical analyses were conducted during the first 3 years of this project on archived samples and then on newly acquired samples from 2015 field work. The fieldwork portion, which targeted beaches with lingering oil in PWS, was completed in 2015.

Abstract: Relatively small patches of *Exxon Valdez* oil have persisted in the spill area for 26 years. Goals for this project were to provide the EVOSTC with an assessment of persistent *Exxon Valdez* oil in Prince William Sound, describe its chemical characteristics, and initiate a routine for long-term monitoring. These goals were accomplished in two ways: (1) by conducting a retrospective oil chemistry analysis, which included novel pattern matching of petroleum biomarkers, and (2) by conducting a lingering oil survey during the summer of 2015 to revisit a small set of beaches in Prince William Sound known to have persistent *Exxon Valdez* oil. Results of biomarker pattern matching allowed for definitive identification of 25-year-old Alaska North Slope crude oil, however some biomarkers did show some evidence of weathering. Results from the lingering oil survey indicated that *Exxon Valdez* oil remained in subsurface sediments for 26 years and there was little evidence of change in oil area or mass over the last 14 years at these beach segments. This suggests the remaining oil is sequestered and not bioavailable unless disturbed by natural process or human activities and likely will persist in the environment on a decadal scale. Recommendations are to continue monitoring lingering oil for changes in chemical composition, bioavailability, and retention in the environment but on a decadal cycle over which change is more likely to be detected.

Key words: chemical composition, *Exxon Valdez*, Gulf of Alaska, lingering oil, long-term tracking, passive samplers, petroleum biomarkers, polynuclear aromatic hydrocarbons (PAHs), Prince William Sound, weathering

Project Data:

Data description - All chemical analyses were completed at the NOAA NMFS Auke Bay Laboratories. Hydrocarbon concentration and composition data were acquired from previously frozen sediment samples archived at the lab or collected in 2015, using solvent

extraction and measurement by gas chromatography. Data also include gravimetric oil analyses and frequency of oil encounter on beaches surveyed for lingering oil during the 2015 survey in Prince William Sound, Alaska.

Data Location - all data reside online in the publically available AOOS data portal (http://portal.aoos.org/gulf-of-alaska.php#metadata/91b73240-b68d-43d8-bd64-aea4ea14e976/project/files).

Data format - includes a Microsoft Access database of hydrocarbon analyses (*Exxon Valdez* Trustees Hydrocarbon Database - EVTHD) and Excel spreadsheets.

Data Contact – Carol Janzen, 1007 W. 3rd Ave. #100, Anchorage, AK 99501, 907-644-6703, janzen@aoos.org, http://portal.aoos.org/gulf-of-alaska.php.

Data access limitations - These data are archived by the Gulf Watch Alaska's *Exxon Valdez* Oil Spill Trustee Council and NMFS. There are no limitations on the use of the data, however, it is requested that the authors be cited for any subsequent publications that reference this dataset. It is strongly recommended that careful attention be paid to the contents of the metadata file associated with these data to evaluate data set limitations or intended use.

Citation:

Lindeberg, M. R., M. G. Carls, and J. Maselko. 2017. Lingering Oil: Extending the Tracking of Oil Levels and Weathering (PAH Composition) in PWS through Time. *Exxon Valdez* Oil Spill Restoration Project Final Report (Restoration Project 12120014-S), NOAA/NMFS Auke Bay Laboratories, Juneau, Alaska.

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EXECUTIVE SUMMARY

The Gulf of Alaska in the northeastern Pacific Ocean is considered one of the most productive marine ecosystems in the world, with numerous complex interactions and food webs (Spies et al. 2007). In March of 1989, the *Exxon Valdez* oil tanker ran aground on Bligh Reef spilling an estimated 11 million gallons of crude oil into Prince William Sound (PWS) (Rice et al. 2007). The spill impacted coastal marine habitats from PWS to Kodiak and Katmai National Park and Preserve. Following the *Exxon Valdez* oil spill (EVOS), numerous studies have been conducted to understand effects of the EVOS on the region and restore injured resources through work funded by the *Exxon Valdez* Oil Spill Trustee Council (EVOSTC) (Mundy 2005, Harwell et al. 2010). To continue this legacy, the EVOSTC initiated funding for the Gulf Watch Alaska (GWA) Long-Term Monitoring Program in 2012. The intent was to facilitate a twenty-year-long monitoring program managed in five-year increments resulting in publically available datasets, scientific publications, and 5-year final reports. Two lingering oil projects were funded under this program from 2012-16: 1) *Evaluating chronic exposure of harlequin ducks and sea otters to lingering Exxon Valdez oil* (12120114-Q) (Esler et al. 2015, Esler et al. 2014*a*, *b*), and 2) *Extending the tracking of oil levels and weathering (PAH Composition) in PWS through time* (#16120014-S). The second project listed here is the focus of this report.

The overall goal of the lingering oil project was to extend previous efforts to track *Exxon Valdez* oil (EVO) levels and weathering in PWS since the onset of the spill. The main objectives were to: 1) determine the quantity and weathering state of EVO in PWS approximately 25 years after the spill, 2) provide supplemental support for hydrocarbon analyses if needed, 3) maintain the EVOSTC hydrocarbon database, and 4) produce a peer reviewed synthesis publication. To accomplish objective 1, a lingering oil survey using established techniques revisited a small set of the worst case sites in PWS where sequestered oil was known to persist. Sampling techniques allowed for estimation of the amount of remaining oil at specific sites, detailed examination of hydrocarbons present (including chemical biomarkers), verification of hydrocarbon source (identifying oil as EVO), and weathering state of the oil. In addition, an extension of the oil loss time series data (where they exist) could be achieved. Sediment samples and passive samplers also were used to examine hydrocarbon loads to determine if PAHs were biologically available. This effort has set the foundation for establishing long term monitoring sites for the next 20 years.

A great deal of data has been assembled by this project but significant findings can be summarized from two major manuscripts that were drafted, one on petroleum biomarkers as tracers of EVO, Appendix A (Carls et al. 2016) and a second synthesizing results of the lingering oil survey in PWS, Appendix B (Lindeberg et al. 2017). The first manuscript covers petroleum biomarkers, alkanes, and PAHs that have persisted in sequestered EVO in PWS and the Gulf of Alaska for 25 years. Retention of highly conserved petrogenic biomarkers, which are molecular fossils derived from previously living organisms, allowed

definitive identification of Alaska North Slope crude oil (ANSCO). A novel pattern matching model indicated the presence of ANSCO from the time of the spill to present at most sites and distinguished this source from several other potential sources. Biomarkers were conserved in oil relative to other constituents (1989 to 2014), and there was evidence of weathering (e.g., tricyclic triterpane, hopane, and sterane) (Carls et al. 2016). The second manuscript covers the 2015 lingering oil survey in PWS, which revealed that *Exxon Valdez* oil remained in subsurface sediments for 26 years and there was little evidence of change in oil area or mass over the last 14 years at these beach segments. This suggests oil was well sequestered and not bioavailable unless disturbed by natural processes or human activities and will persist in the environment on a decadal scale.

We recommend that future monitoring of lingering EVO have the following two objectives: 1) continue tracking the long-term chemistry and bioavailability of EVO in the environment, and 2) continue documenting subsurface EVO. The likely timescale for conducting this monitoring would be decadal given the sequestered nature of the lingering oil. A suggested approach for lingering oil monitoring would be to offset objectives 1 and 2 by five years in 10-year cycles. A bioavailability and oil chemistry sampling cycle could be initiated first (e.g., 2020; 2030) and then a lingering oil survey with chemistry five years later (e.g., 2025; 2035), which would allow the beach segments to settle before sensitive sampling is repeated. We suggest convening a workshop with agencies and experts prior to lingering oil surveys to discuss what are actionable detection levels of bioavailability.

INTRODUCTION

On Friday March 24th, 1989 the supertanker *Exxon Valdez* ran aground on Bligh reef in PWS, Alaska, releasing at least 11 million gallons of ANSCO. The crude oil contaminated approximately 2,100 km of shoreline. Initial Shoreline Cleanup Assessment Teams (SCAT) in PWS estimated that 40% of the spilled oil made landfall on beaches in PWS (Galt et al. 1991). By 1992, it was estimated that 5-8% of the initial spilled oil had been recovered from beaches resulting from an unprecedented clean-up effort (Wolfe et al. 1994). Follow-up SCAT surveys showed there was a decrease of oiled shoreline in PWS from 783 km in 1989 to 10 km by 1992 (Neff et al. 1995). Given the considerable loss rate observed by 1992, assumptions were the remaining oil would continue to weather and dissipate on a short time scale (Page et al. 1995). However, during the first decade after the EVOS, some site specific observations of oiled shoreline in PWS began to cast doubt on the expected loss rate and EVO was observed persisting in a fairly unweathered state (Brodersen et al. 1998). Entrenched subsurface oil was no longer being removed by natural processes in some places, which suggested loss rates had slowed down.

During the 2000s a new series of comprehensive surveys to update estimates of lingering oil in PWS were initiated by the EVOSTC. National Marine Fisheries Service's (NMFS) Auke Bay Laboratories (ABL) conducted a lingering oil survey in 2001 to provide a quantitative, probability-based estimate of the amount of oil remaining 12 years after the spill (Short et al. 2004, Pella and Maselko 2007). Estimates from this survey revealed a cumulative area of EVO of 11.3 ha and a mass of 55,600 kg of subsurface oil in PWS. The majority of the subsurface oil was located in the mid intertidal zone, although some occurred surprisingly

low in the intertidal. The significance of this oil became apparent when locations of lingering oil from the comprehensive surveys was compared to the areas where sea otter recovery was slower than the rest of the PWS (Bodkin et al. 2014), and supporting evidence of chronic oil exposure and wildlife effects was detected (Iverson and Esler 2010).

Additional surveys conducted in 2003 and 2005 focused on determining the distribution of subsurface oil with respect to tidal elevation and the probability of encountering oil in a heavily oiled region of PWS (Short et al. 2006, 2007). A comparison of survey results between 2001 and 2005 showed the likely rate of decline of oiled beach area within PWS was 3-4 % yr^{-1} and the oil was moderately weathered. Given the quantitative consistency of the surveys conducted in the 2000s, a geomorphic spatial model was developed to predict where subsurface oil was likely to occur in addition to known locations (Michel et al. 2010, Nixon and Michel 2015). Model estimates based on data collected between 2001 and 2015, revealed lingering subsurface oil represented 0.6% of the total spill volume (Michel et al. 2017).

Now that we are into the third decade since the spill, NMFS Auke Bay Laboratories proposed to revisit a small set of beaches in PWS known to have persistent subsurface oil and determine if there has been any measurable loss of oil since they were last visited. A key component was to document any weathering of the oil and use newly acquired biomarker data to verify that the oil is from the *Exxon Valdez*. Another major goal was to determine if there was any measurable loss of persistent oil since the sites were last visited. If oil loss was detected, this would suggest the oil is being naturally removed by biophysical processes. No change in the amount of oil at these sites would suggest the oil has been sequestered. Also of interest is knowing if we can see any changes in oil retention rates during the last 5, 10, or 15 years. This will help determine at what time scale we can detect loss of persistent oil in PWS. Answers to these questions have implications for decision makers and resource managers in the future (e.g. need for further remediation, monitoring toxicity of the oil in the environment and determining recovery of injured resources).

OBJECTIVES

The following lists each objective as proposed in 2012 and minor changes that were made to achieve some objectives in later years.

1. Determine quantity and weathering state of EVO at 12 beaches in PWS:
    a. *Oil Composition* - retrospective analysis of biomarkers in EVO, weathered EVO, and other potential source oils in PWS.
    b. *Lingering Oil Survey* - major field effort to conduct lingering oil survey in PWS. Visit 10-12 beaches in 2015.
        i. Collect sediment samples for PAH concentration and weathering profiles.

ii. Using random quadrats, measure the quantity of oil on specific beaches to estimate the quantity present.

iii. Collect mussels near oil patches to determine bioavailability in tissues. These samples were not acquired by the field crew, passive samplers were used instead (see iv).

iv. Place a limited number of passive samplers on oiled sites to model oil bioavailability. These samplers will be deployed without pit digging disturbance.

v. Begin and end the chemical analyses of samples collected in primary field effort by using state-of-the art Gas Chromatography-Mass Spectrometry (GMS), with chemical biomarkers included.

2. Provide supplemental support analyses as needed. Ten to twenty hydrocarbon samples per year were anticipated from other studies funded by the EVOSTC.

3. Maintain hydrocarbon database:

    a. Add new information to hydrocarbon database. This database contains data from all Natural Resource Damage Assessment [NRDA] hydrocarbon samples from 1989 to present, including numerous data sets from investigators outside ABL.

    b. Prepare a complete Freedom of Information Act package if needed.

4. Produce publication: prepare peer reviewed synthesis manuscript.

METHODS

All project methods can be found in the appendices (A-E). The following brief methods touch on fundamental information associated with the project objectives.

Chemical analyses

Chemical analyses of oil were conducted at National Oceanic and Atmospheric Administration's (NOAA) NMFS ABL following standard operating procedures (SOPs) developed by the lab (Appendix C) and published methodologies (Short et al. 1996, Carls et al. 2004).

Oil Composition

PAHs, alkanes and biomarkers were analyzed to determine the extent to which weathering had occurred since previous surveys and to verify that subsurface oil found on beaches was oil spilled from the *Exxon Valdez*. The EVO has been quantified into a single index, *w* (Short and Heintz 1997), where values range upwards from zero, which reflects the PAH composition of EVO weathered to about 80% of its initial mass (Short and Heintz 1997). The oil weathering index, *w*, and percentage of remaining PAHs were compared with similar values from previous collections to determine if subsurface oil has weathered since

surveys began. The weathering index was calculated for each of the samples collected for PAH analysis. Both *w* and the percentage of total PAH (TPAH) remaining were compared across beaches and years using a two-way ANOVA to test the hypothesis that subsurface oil is degrading over time.

Petroleum biomarkers

Chemical analyses were upgraded to include chemical biomarker data (terpanes, hopanes, and steranes); these compounds are the most recalcitrant compounds to biodegradation and weathering, and yield a more complete picture of the biodegradation/weathering that has occurred over the last 25-years. We analyzed new samples, but also re-analyzed archived samples stored at the lab including *Exxon Valdez* source oil. In addition, biomarkers were measured in a limited number of other known (stored) sources (Constantine Harbor, coal, and Monterey oil) for comparison and contrast with *Exxon Valdez* oil. Sufficient time series data were available for 9 study sites based on the archived samples. Study sites were based on shoreline assessment data gathered by Exxon Corporation and the Alaska Department of Environmental Conservation and were limited to sites with persistent oil and repeated observations. Oil biomarker time-series sample locations are shown in Fig. 1. For more details on biomarker methods see Appendix A.

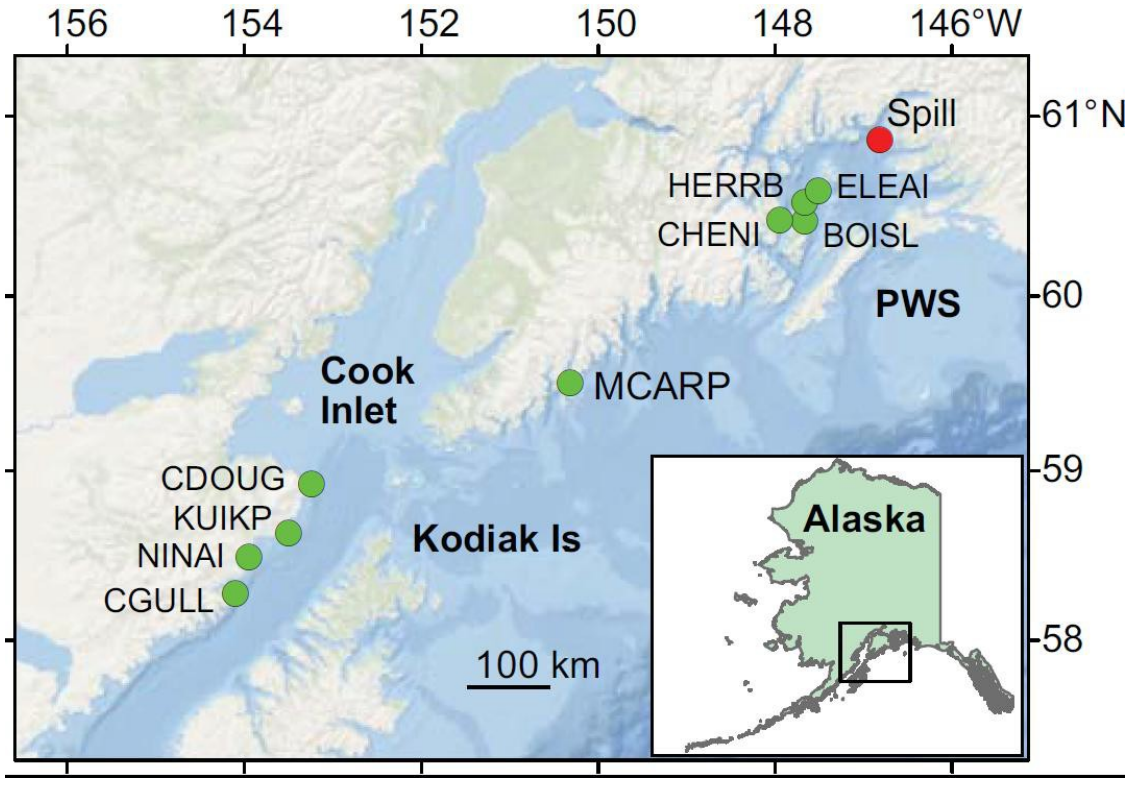

Figure 1. Oil biomarker time-series sample locations included 4 within Prince William Sound (PWS), 4 along the Katmai coast, and one intervening location (McArthur Pass). The red circle marks the location of the *Exxon Valdez* oil spill. See Appendix A for more details.

Hydrocarbon database

The *Exxon Valdez* Trustee Hydrocarbon Database (EVTHD) was updated and is fully functional; it has undergone major revisions, data additions, and quality control routines. Quality control included linking key variables to detect errors, corrections as needed with verification against original records, and data insertions. The data include PAH, alkane, and biomarker concentrations and associated quality control tables. Sample information includes location, latitude, longitude, project name, date, investigator information, project codes, and sample type. The database is publicly available through the AOOS data portal (http://portal.aoos.org/gulf-of-alaska.php#metadata/91b73240-b68d-43d8-bd64-aea4ea14e976/project/files).

Lingering oil survey

Methods for surveying beach segments, discovering oil, and estimating oiled area matched those used by the NMFS ABL 2003 lingering oil survey (Short et al. 2006). These methods were proven to estimate subsurface oil for the entire intertidal zone (mean high water to zero tide height) and they ensured comparison over time for a given site. Detailed lingering oil survey methods, including collection of samples, gravimetric sampling, and passive samplers, are described in Appendix B and complete SOPs are presented in Appendix E.

Site selection

Factors considered for prioritization of beach segments were based on the following: initial oiling, shore types prone to oil retention (Michel and Hayes 1993, Hayes and Michel 1999, Michel et al. 2010), past oil surveys to aid our understanding of loss rates, most recently observed oil in heaviest categories, and a high probability of oil persistence (Nixon and Michel 2015). As a result of the prioritization exercise, 9 beach segments were selected and surveyed in June of 2015 (Fig. 2); these should not be considered as a random or representative selection of beaches. We also acknowledge that sites selected for long-term monitoring have a history of man-made disturbance and that loss of subsurface oil (SSO) could be variable among these sites over time. Since the onset of the spill, oiled beaches have been surveyed and treated by using a gamut of mechanical removal and remediation techniques. Mechanical removal techniques included high-pressure water flushing and berm relocation. Remediation techniques included Inipol EAP22, Customblen, Corexit, and PES-51. Certain sites were identified with persistent SSO and repeatedly excavated in the 2000s by EVOSTC and Exxon Corporation surveys. Additional experimental remediation techniques by Boufadel and Bobo (2011) were applied to a suite of sites in recent years. Due to these factors we estimated loss of SSO at each site individually, not stratified by initial oiling or treatment history.

Passive samplers

The bioavailability and composition of mobile oil constituents 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 µm × 4.9 cm × 50 cm) housed in aluminum canisters (11.5 cm diameter × 6.6 cm) with perforated aluminum endplates (3 mm holes spaced 4.8 mm apart). The PEMDs were placed along one oiled

beach segment (KN0114; n = 18) prior to being surveyed for SSO. The PEMDs were retrieved 10 days after deployment, sealed in ziplock bags, and frozen as soon as practical pending processing. 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 two additional laboratory blanks were never shipped. 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 (Appendix E).

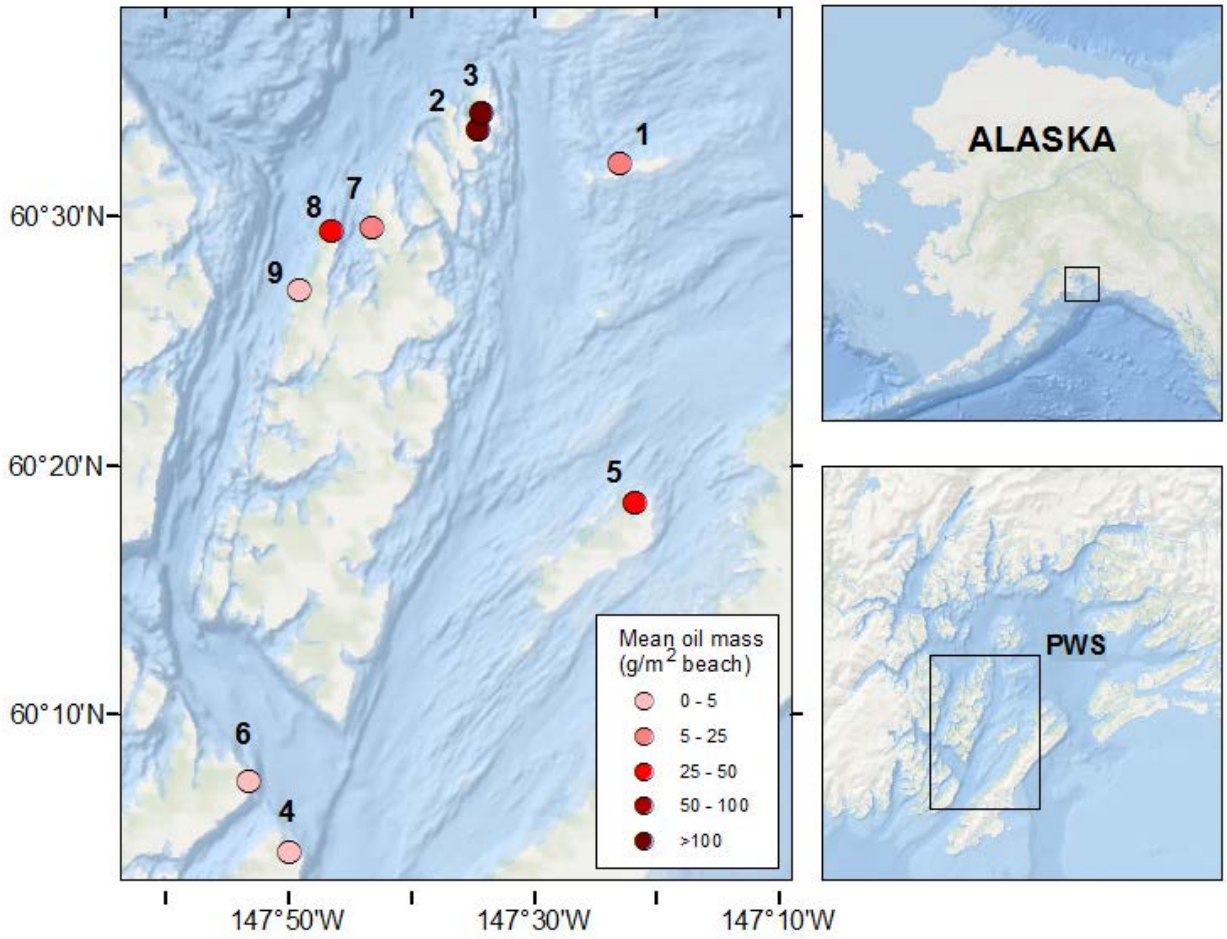

Figure 2. Sites surveyed for subsurface *Exxon Valdez* oil in Prince William Sound, Alaska during the summer of 2015. Light red to darker brown colored icons indicate greater oil discovered on beach segments. Numbers adjacent to icons correspond to prioritized sites. See Appendices B and E for more detail.

RESULTS

Complete results of biomarker analyses and the lingering oil surveys can be found in Appendix A and B including tables and figures. A brief summary of results from the included manuscripts is provided below. Supplemental support analyses for hydrocarbon analyses under objective two were not requested.

Oil composition

Petroleum biomarkers

Biomarker patterns were typically consistent with ANSCO over the entire observation period at contaminated sites (up to 23 years, 2015). The combined result of all classes (triterpanes, hopanes, steranes) matched ANSCO in 77% of these samples (n = 62). ANSCO was definitively present through time at all sites except Chenega Island, where it was not identified in 1995 and 1999, and Cape Gull samples in 1999 and thereafter. Other potential biomarker sources were not plausible alternatives to ANSCO. The combined result for all three types of source oil (Monterey, coal, and Constantine Harbor) was a 0% fit compared to 77% for ANSCO. ANSCO explained the biomarker ratios in the field samples from each site in Nordtest analyses. All regression slopes were near 1 and the 95% confidence interval of these slopes either overlapped 1 or was within 0.01 units of it. ANSCO matched the field data better than any alternative source. Thus, the Nordtest analysis is consistent with the pattern-matching analysis; ANSCO was frequently detected in samples and none of the alternative sources were plausible.

Oil from the 2015 PWS survey

Subsurface oil in PWS was found in a range of weathering states as indicated by the degradation of PAHs and n-alkanes. Values of *w*, the weathering index, ranged from 0 to 6.7 with an overall mean value of 3.0 ± 1.8 (mean ± 1 s.d.). The least weathered oil was found in a LOR residue on Knight Island segment KN0506A. Characteristically, it had high naphthalene concentrations, averaging 43% of TPAH and low chrysene concentrations (1% of TPAH). The most weathered oil was found in an HOR residue on Latouche Island segment LA018A. This oil was characterized by low concentrations of naphthalenes (4% of PAHs) and higher levels of chrysenes (>12% of TPAH). Over all sites, the percent n-alkanes remaining relative to 11 d mousse averaged 4.7 ± 5.8%. The lowest percentage (1.1% retained) of remaining n-alkanes was found on Eleanor Island segment EL056C and the location with the least degraded n-alkanes (28.1% retained) was on Smith Island segment SM006B.

There has been little change in the weathering state of the oils in selected beaches since surveys began. The PAH composition of subsurface oils collected from beach segments EL058B, GR103B, KN0300A-2 and LA018A-1 was characterized for collections made in 2001, 2005 and 2015. The weathering index for these sites was compared by two-way ANOVA with years, sites and their interaction as the main factors. None of these factors had an effect on the average *w* value ($p > 0.120$).

In contrast to the inter-annual comparisons, comparisons of w and the remaining PAHs among tide levels and oiling intensities revealed some evidence of weathering. The two-way ANOVA for *w* detected a marginal effect of oiling intensity ($F_{2, 27} = 3.40$, $p = 0.048$) and no effect of tide stage ($F_{4, 27} = 1.52$, $p = 0.225$) or their interaction ($F_{32, 27} = 0.76$, $p = 0.525$). Pairwise contrasts revealed the greatest difference was between HOR and LOR residues, while the latter was less weathered the difference was not significant ($p = 0.087$).

Hydrocarbon database

A complete SOP (Appendix E) documents database content, structure, and supporting structure. The data is publically available online as a zipped package of files which includes a Microsoft Access database and all supporting metadata and documentation: http://portal.aoos.org/gulf-of-alaska.php#metadata/91b73240-b68d-43d8-bd64-aea4ea14e976/project/files.

No Freedom of Information Act requests were made during the period 2012-2016 but documentation was prepared (project objective 3b.).

Lingering oil survey

Delays acquiring EVOSTC funds and soliciting a federal research vessel required the survey be conducted in 2015 rather than 2014. Increased charter and fuel costs during the third year limited the number of charter days resulting in 9 prioritized beach segments that were surveyed. A thorough summary of survey results can be found in the draft manuscript entitled: *Persistent Exxon Valdez oil on beaches in Prince William Sound 26 years later* (Appendix B). Brief results follow along with results from the passive samplers, which were not presented in the manuscript.

Oil estimates and retention

Oil was readily found in subsurface sediments sampled in 8 of the 9 beaches re-surveyed in 2015. Most of the contaminated sediments were lightly to moderately oiled. Of the 400 pits dug, 20 had lightly oiled residues compared with 19 moderately oiled and 8 heavily oiled residues. The probability of encountering oil on these beach segments, expressed as the number of oiled pits divided by the total number dug, generally agreed with model estimates (Michel et al. 2010, Nixon and Michel 2015) used to select beach segments. Eleanor Island segment EL056C had the largest estimated oiled area at 1,218 m^2 (±112 m^2) as well as the largest total weight of oil estimated at 1,124 kilograms (±470 kg). In contrast, no subsurface oil was encountered in the 50 pits dug on Evans Island segment EV039A.

Comparisons of the estimated oiled areas and weights for six beach segments that were sampled in 2005 and 2015 (six sites) revealed little change in subsurface oil estimates. Based on 95% confidence intervals, there was little evidence for a decline in oiled area or oil weight at the survey sites. There was little if any evidence that oil has been lost from the beaches surveyed in 2001, 2005, and 2015, based on the probability of encountering oil. Estimates of retention for the periods 2001 to 2005, 2001 to 2015, and 2005 to 2015 did not differ from each other (p=0.612). The only significant loss in oil was detected at Eleanor Island on segment EL056C between 2001 to 2015 (95%CI: 0.89-0.998). There has been no appreciable change in the frequency distribution of LOR, MOR and HOR residues since 2001. It is important to note that subsurface oil described here is located only in the upper to mid-tide levels (MVD1-3) for annualized comparisons. Oil previously has been found in lower sections of these beaches (MVD 4) (Short et al. 2006).

Passive samplers

Oil was not present in passive samplers, based on polyaromatic hydrocarbons (PAH) source modeling and concentration (Table 1). Total PAH concentrations were statistically indistinguishable from concentrations in blanks (PANOVA = 0.739) and composition also

was indistinguishable (PANOVA = 0.517). Observed concentrations were ≤ 77 ng/g device. These concentrations are within typical background levels for passive samplers.

Table 1. Total polyaromatic hydrocarbon (TPAH) concentration (ng/g device) and source model results for passive samplers by sample identification number (SIN) and location. Source model values can range from −1 (pyrogenic) to +1 (petrogenic). Values near 0 indicate no definitive source.

SIN	Location	model	TPAH
20150204	KN0114A	0.017	77.14
20150205	KN0114A	-0.083	26.25
20150206	KN0114A	-0.083	54.30
20150207	KN0114A	-0.083	37.05
20150208	KN0114A	-0.050	20.36
20150209	KN0114A	-0.017	7.39
20150210	KN0114A	-0.117	17.74
20150211	KN0114A	-0.067	23.40
20150212	KN0114A	-0.017	8.71
20150214	KN0114A	-0.067	9.25
20150216	KN0114A	-0.067	9.17
20150218	KN0114A	-0.050	14.37
20150220	KN0114A	-0.017	6.56
20150221	KN0114A	0.000	4.94
20150215	KN0114A	-0.017	9.02
20150217	KN0114A	-0.017	8.57
20150219	KN0114A	-0.017	9.22
20150222	KN0114A	0.000	4.43
20150203	Blank, field	-0.017	26.23
20150213	Blank, field	-0.067	57.98
20150201	Blank, lab	0.000	0.00
20150202	Blank, lab	0.000	0.00
20150223	Blank, trip	-0.033	51.41
20150224	Blank, trip	-0.067	18.44

DISCUSSION

Appendix A and B are manuscripts that provide detailed discussion of this project's results. A brief summary of these discussions follows.

Oil composition

Petroleum biomarkers

Unlike contemporary biomarkers, which are measurable substances from a living organism, petroleum biomarkers are molecular fossils derived from previously living organisms and diagenetically altered during the formation of crude oil (Peters et al. 2007, Wang and Stout 2007). The structure of petroleum biomarkers is highly similar to or unchanged from their biogenic precursors (Wang and Stout 2007). Petroleum biomarkers are generally resistant to biodegradation, evaporation, and other weathering processes (Peters et al. 2007), thus, definitive identification of source oils is possible for long periods of time. Biomarkers are so persistent that they can be found in sedimentary rock more than 2 billion years old with identifiable biological origins (Peters et al. 2007). This persistence allows more definitive identification of sequestered oil than with smaller molecules such as previously reported PAHs and n-alkanes. Petroleum biomarker composition is retrospectively analyzed and discussed in Appendix A.

Oil from the 2015 survey

Surveys conducted during the summer of 2015 confirmed the presence of oil in the subsurface sediments of some beaches in PWS 26 years following the *Exxon Valdez* oil spill. This is consistent with earlier geomorphic model predictions for persistent subsurface oil (Michel et al., 2010; Nixon and Michel, 2015). Most recent testing by Nixon and Michel (2017) including data ranging from 2001-2015 (14,000 pit excavations), continues to support estimates and even suggests previous estimates were slightly underestimated changing from 0.25% to 0.6% of the originally spilled mass of oil.

There is little evidence of weathering in the subsurface oil since surveys were initiated in 2001. Moreover, there was little evidence of a difference in the weathering indices at beaches consistently sampled in 2001, 2005 and 2015. Accordingly, Short et al. (2004) reported a median weathering parameter, *w*, equal to 3.3 for the 2001 survey, a value somewhat higher than 2.9, the median observed in 2015.

Subsurface oil collected in 2015 have enriched concentrations of phenanthrenes and chrysenes relative to ANSCO, indicating that buried oil has retained its toxic potential over the last two decades. While acute toxic effects from BTEX (benzene, toluene, ethylbenzene, xylene) compounds were possible in the early days of the spill, these compounds are not present in the sediments; however, chronic toxic effects from environmentally persistent PAHs are still possible.

Hydrocarbon database

The EVTHD database has undergone a major quality control sweep and now contains information on archived samples and analyses conducted by NOAA NMFS Auke Bay Laboratories between 1989 and 2015. Auke Bay laboratories is no longer conducting hydrocarbon analyses and the database will not remain active. The database and supporting materials available online are the final version available to the public.

Lingering oil survey

Oil estimates and retention

Overall, a comparison of oil area and weight for a given site after ten years (2005-2015) revealed little change. The inability to detect change in the estimated oil weight between 2001 and 2015 indicates there is unlikely to be any change in current estimates of the total amount of oil remaining in PWS. These estimates are around 0.6% of the spilled volume (Nixon and Michel, 2017). Based on their recent predictive model Nixon and Michel (2017) concluded present removal rates for these remaining subsurface oil residues have slowed to nearly zero and removal mechanisms will operate over time scales of decades. In addition, there has been little change in how the oil is distributed on the beaches.

Annualized retention rates of oil (MVD 1-3) indicate that oil loss has been undetectable, over the last 14 years. The slightly decreasing trend in oil retention suggests loss of subsurface oil may eventually be detected, but the interval will be on the order of multiple decades. Any detected loss or gain of oil at a site is most likely due to repeated site disturbance and re-excavation of oiled areas resulting in mobilization of the trapped oil and not natural weathering. For example, a history of re-excavation (Short et al. 2004, 2006, 2007, Boehm et al. 2008, Michel et al. 2010) and intensive experimental remediation (Boufadel and Bobo 2011) are most likely the main causes for the Eleanor Island site (EL056C) to have a significant loss in subsurface oil between 2001 and 2015.

Persistence of the oil is likely due to its sequestration from reworking of sediments by hydrologic forces and nutrient depletion. Factors hindering physical removal of oil in PWS include low exposure to wave action, armoring by coarse substrates, and protective small scale geomorphic features (Owens et al. 2008, Hayes et al. 2010, Michel et al. 2016). Oil not removed by physical factors is often remediated by microbial degradation (Venosa et al. 2010), however, oiled beaches in PWS have low dissolved oxygen levels in subsurface layers, which significantly slows microbial degradation by orders of magnitude (Boufadel et al. 2010, Guo et al. 2010, Li and Boufadel 2010, Xia et al. 2010).

Passive samplers

The absence of PAHs in passive samplers deployed in 2015, which are considerably more mobile than biomarkers, is evidence that the oil was not meaningfully biologically available outside the sediment in 2015. In contrast, TPAH concentrations in some passive samplers deployed in previous SCAT projects (2002 to 2004) were orders of magnitude greater than observed in 2015 and composition was consistent with EVOS oil. The low to negligible PAH loss rates estimated by the lingering oil surveys are further evidence that the remaining sequestered oil is relatively isolated from the environment.

CONCLUSIONS

Oil composition

Biomarkers provide an excellent way of definitively identifying the source of spilled oil over long periods of time, yet their concentrations change within the oil over time. Differential weathering may cause composition to slowly shift away from that in the source oil, although such shifts did not preclude identification of ANSCO after 25 years. Biomarkers were clearly retained while other oil constituents were lost, explaining their initial concentration increase, yet concentrations declined over time, indicating removal or destruction by some process, possibly microbial. Isoprenoid loss was substantially greater than tricyclic triterpane, hopane, and sterane loss.

Lingering oil surveys

These findings demonstrate that the estimated 0.6% of oil spilled by the *Exxon Valdez* has remained sequestered in subsurface sediments of PWS for 26 years. Both the estimated area occupied by the oil and its mass have not changed since surveys conducted in 2001. Nor has there been any change in the distribution of oiling intensities or their location on the beach. Consequently, we are unable to detect any loss of oil from these beaches. Sequestration has protected the oil from weathering, allowing it to maintain its potential toxicity, but the absence of any loss suggests it is not currently bioavailable. It would take a rather unusual disturbance event (e.g. earthquake, man-made intervention) to increase the potential for bioavailability at this point. Observations of the prolonged persistence of spilled oil in subsurface sediments have been documented following other spills and, in some cases long-term ecological effects of persistent oil have been identified (e.g. Bodkin et al., 2014) but this is the only attempt to quantify the rate at which oil is being lost over a long-term time period. Viewing this survey in the context or previous surveys makes it clear that claims made after the spill that beaches would clean themselves (Page et al., 1995) were overly optimistic and we now know subsurface EVO can persist in the environment on a decadal scale.

RECOMMENDATIONS

Looking forward, it is anticipated that questions will continue to be asked about oil presence, weathering, bioavailability and long-term persistence. In order to answer these questions, monitoring of lingering EVO needs to continue. Based on the recent findings of this project we recommend future monitoring of lingering EVO have the following two objectives: 1) continue tracking the bioavailability of EVO in the environment, and 2) continue documenting the weathering state of subsurface EVO. The likely timescale for conducting this monitoring would be decadal given the unchanging and sequestered nature of the lingering oil.

A suggested approach for lingering oil monitoring would be to offset objectives 1 and 2 by five years in 10-year cycles. A bioavailability and oil chemistry sampling cycle could be initiated first (e.g. 2020; 2030) and then a lingering oil survey with chemistry five years later (e.g. 2025; 2035). The advantage of this monitoring schedule is that it would allow for bioavailability and oil chemistry sampling to occur independently from survey sampling

during the lingering oil surveys. Goals for objective 1 should be to deploy passive samplers on beaches known to have persistent oil, preferably including sites visited in 2015. Flying out to sites by floatplane to deploy and retrieve the samplers would be a quick, efficient and cost effective way to get sampling done and has been proven in the past (project #040740, 2004). A suite of oil and mussel samples can also be collected at deployment and/or retrieval to maintain long-term oil chemistry datasets. Samples should be analyzed by an institution certified to carry out state of the art hydrocarbon analyses. Analyses should include an assessment of the source of any hydrocarbon signatures detected by the samplers. Source identification should rely on published reports describing the behavior of passive samplers exposed to different hydrocarbon sources (Short et al. 2008). There is a noticeable lack of information on the bioavailability of subsurface oil during high energy times of the year such as late fall and winter. To address this issue a seasonal component of passive sampling could be investigated during the first cycle or two (e.g. summer and fall/winter of 2020).

Should EVO be detected as the source in surveys of bioavailability then more detailed surveys aimed at characterizing the processes leading to mobilization of subsurface oil would be warranted. Goals for objective 2, conducting lingering oil surveys (e.g. 10-15 day charter with a crew of 6-8), should follow methods used by previous surveys (Appendix E) and assess long-term oil retention rates. We suggest convening a workshop with agencies and experts prior to lingering oil surveys to discuss what are actionable detection levels of bioavailability (e.g., TPAH in sediment-tissue, PEMDs, levels of P450 in wildlife, etc.).

There is significant value in continuing to track EVO in the environment. We are now in the unique position of having legacy data sets of persistent oil over a quarter of a century. Information on persistent EVO has been heavily cited ranging from environmental impact statements to supporting legal court cases such as the Deepwater Horizon oil spill. This kind of information is rare and will continue to be valuable to scientists and decision makers in the future.

ACKNOWLEDGEMENTS

Since the *Exxon Valdez* oil spill a wealth of experience and knowledge has been gained at the Auke Bay Laboratories about oil in the environment which has significantly informed scientists and policy makers on a global scale. There are too many people to list here but we would like to thank all the contributors over the last few decades and especially appreciate the long-term support of the EVOSTC and NOAA NMFS. Finally, we would like to thank the GWA program leadership team and Science Review Team.

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Gulf Watch Alaska – http://www.gulfwatchalaska.org/

Gulf of Alaska Data Portal – http://portal.aoos.org/gulf-of-alaska.php

Lingering Oil project webpage – http://www.gulfwatchalaska.org/monitoring/lingering-oil/lingering-oil-weathering-and-tracking/

Lingering Oil project publically avialable data - http://portal.aoos.org/gulf-of-alaska.php#metadata/91b73240-b68d-43d8-bd64-aea4ea14e976/project/files

APPENDIX A: MANUSCRIPT - BIOMARKER TRACERS FOR EVO

Petroleum biomarkers as tracers of *Exxon Valdez* oil

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Abstract

Over the past quarter century, petroleum biomarkers persisted in sequestered *Exxon Valdez* oil in Prince William Sound and the Gulf of Alaska, hence the oil remained identifiable. These biomarkers are molecular fossils derived from biochemicals in previously living organisms. Novel pattern matching indicated the presence of Alaska North Slope crude oil (ANSCO) over the entire observation period at most sites (7 of 9) and distinguished this source from several other potential sources. The presence of ANSCO was confirmed with Nordtest forensics, demonstrating the veracity of the new method. The principal advantage of the new method is that it provides sample-specific identification, whereas the Nordtest approach is based on multi-sample statistics. Biomarkers were conserved relative to other constituents, thus concentrations (per g oil) in initial beach samples were greater than those in fresh oil because they were lost more slowly than more labile oil constituents such as straight-chain alkanes and aromatic hydrocarbons. However, biomarker concentrations consistently declined thereafter (1989 to 2014), though loss varied substantially among and within sites. Isoprenoid loss was substantially greater than tricyclic triterpane, hopane, and sterane loss.

Keywords: Biomarker, *Exxon Valdez* oil, Forensic

Citation:

Carls, M. G., Holland, L., Irvine, G. V., Mann, D. H. and Lindeberg, M. (2016), Petroleum biomarkers as tracers of *Exxon Valdez* oil. Environ Toxicol Chem, 35: 2683–2690. doi:10.1002/etc.3454.

Introduction

Intertidal areas in western Prince William Sound and to the southwest along the Gulf of Alaska were extensively contaminated with *Exxon Valdez* oil when the tanker grounded in 1989. The source of this oil was Alaska North Slope crude oil (ANSCO), transported from the Prudhoe Bay oil field via the trans-Alaskan pipeline and loaded onto the vessel in Port Valdez. Stranded oil in the coastal zone was persistent and has been studied for more than 2 decades [1-5], yet the focus was on polycyclic aromatic hydrocarbons (PAHs), which are toxic, and study of biomarkers was delayed until recently [6].

Although the majority of intertidally sequestered oil residues are from the *Exxon Valdez* oil spill, other sources also exist, such as Monterey oil and background hydrocarbons from natural sources [7, 8]. Thus there is a need for highly conserved source information to accurately discriminate among oil samples. Petroleum biomarkers are a good choice for this role, as they weather very slowly compared to other commonly measured petrogenic hydrocarbons (PAHs and *n*-alkanes), thus they remain recognizable and consistent with the original source for longer periods of time. Unlike contemporary biomarkers, which are measurable substances from a living organism, petroleum biomarkers are molecular fossils derived from previously living organisms and diagenetically altered during the formation of crude oil [9, 10]. The structure of petroleum biomarkers is highly similar or unchanged from their biogenic precursors [10]. Petroleum biomarkers are generally resistant to biodegradation, evaporation, and other weathering processes [9] thus definitive identification of source oils is possible for long periods of time. Biomarkers are so persistent that they can be found in sedimentary rock more than 2 billion years old with identifiable biological origins [9]. This persistence allows more definitive identification of sequestered oil than smaller molecules such as previously reported PAHs and *n*-alkanes.

The purpose of the present study was to investigate if ANSCO can be definitively identified by biomarker content, determine if the biomarkers were weathering, and distinguish oil sources in time-series samples. Four classes of biomarkers were examined in this project, the isoprenoids (acyclic terpenoids), triterpanes (mostly tricyclic), hopanes (pentacyclic triterpanes), and steranes (tetracyclic terpenoids; Table 1). We apply novel pattern-matching procedures to compare samples with ANSCO to verify its presence in specific samples (or not). Alternative hydrocarbon sources were similarly compared to oil from sample beaches to determine if these sources were or were not explanatory. Results were confirmed with Nordtest [11] plots. Despite their persistence, biomarker weathering is possible [10] and evidence of weathering in each biomarker class is presented in the present study.

Methods

Sample locations

Sufficient time series data were available from 9 sites (Figure 1; Table 2). Time series samples spanned 18 to 23 years at 6 sites. Three additional sites with samples spanning < 15 years and with few data points across time (3 to 4) were treated with caution but were ultimately accepted as part of the analysis (biomarker composition and change at these

sites was consistent with those at the other sites). Study sites were based on shoreline assessment data gathered by Exxon and the Alaska Department of Environmental Conservation and were limited to sites with persistent oil and repeated observations.

Sediment and oil sample collection methods were previously reported [4, 5, 7, 12-16]. In brief, these samples were generally collected with a spoon or shovel, following various sampling protocols, placed in hydrocarbon-free jars, and frozen pending analysis. Samples were later processed at the Auke Bay Laboratory at various times (depending on collection times and individual study needs) for aliphatic and aromatic hydrocarbons. Study of biomarkers began more recently (approximately 2011 to 2014 for these samples) and previously archived extracts were used when available. Expected biomarker loss under storage conditions (−20°C) was negligible, an assumption based on the large size of these molecules, limited potential exchange with atmosphere and water in frozen jars, low temperature thus limited potential for microbial decomposition, the inherent resistance of biomarkers to microbial decomposition, and observation that biomarker patterns often remain unaltered even when oil is undergoing active weathering [10].

Sample processing

Sediment and oil samples were dried with anhydrous sodium sulfate and extracted with dichloromethane (DCM). Prior to 2011, samples were exchanged into hexane over steam and separated into aliphatic and aromatic fractions by column chromatography (10 g of 2% deactivated alumina over 20 g of 5% deactivated silica gel). The aliphatic fraction was eluted with 50 ml of pentane and PAHs were subsequently eluted with 250 mL of 1:1 (v/v) pentane:DCM. From 2011 onward, samples extracted with DCM were reduced in volume over steam to approximately 5 ml. The exact total volume was recorded and an aliquot was archived. The DCM was evaporated from the remaining extract to determine the total mass of extracted oil. This information was used to calculate the application of 7 to 10 mg of oil from the archived aliquot to a 6 g silica column. The aliphatic fraction was eluted with 10 ml pentane and PAHs were subsequently eluted with 20 mL of 1:1 (v/v) pentane:DCM. All purified extracts were exchanged into 1 mL of hexane over steam and spiked with instrument internal standards prior to instrumental analysis. Reported units were ng PAH g^{-1} oil (n = 49) using the amount of oil applied to silica column as the divisor or ng PAH g^{-1} sediment (n = 13).

Aliphatic fractions were analyzed for biomarkers by gas chromatography – mass spectroscopy. Many of these extracts were previously analyzed for aliphatics and archived at −20°C. The data were acquired in the selected ion monitoring mode, and concentrations were determined by the internal standard method with response factors (RF) based on 2 representative compounds, 17α (H),21β(H)-hopane (H30) and 5α(H),14α(H),17α(H)-cholestane. The accuracy of the biomarker analyses was approximately ± 15% based on a spiked blank processed with each set of samples, and precision expressed as coefficient of variation was approximately 20%, depending on the biomarker. Biomarker concentrations were not corrected for recovery; surrogate recovery averaged 93% (range 59 to 125%). Reported biomarkers and their abbreviations are listed in Table 1.

Pattern-matching forensics

Biomarker composition in samples was compared to that in ANSCO (obtained from the T/V *Exxon Valdez* in 1989) to determine if they matched source oil composition [17]. Concentrations were normalized to total class concentration before comparison. For example, hopane source oil bounds were set from minimum − 20% to maximum + 20%, expressed in proportional units ($H_i / \sum H_i$), where H_i is the i^{th} hopane concentration and $\sum H_i$ is the total hopanoid concentration. For each sample, the number of $H_i / \sum H_i$ within corresponding source oil bounds was divided by the total number of hopanes (20) to calculate the fraction of analytes consistent with the source oil. Possible outcomes ranged from 0 to 1, where 1 was a perfect match and 0 was a complete mismatch (Supplemental data, Figure S1 and Table S1). The probability that an unknown sample was consistent with ANSCO composition was assessed by reference to results of randomly permuting the source oil data set 10,000 times. The probability of randomly encountering a match > 0.55 was < 0.0001, thus any score > 0.6 was accepted as consistent with ANSCO. Triterpanes and steranes were similarly modeled. Site and time-specific data were considered matched to the source oil when scores in all 3 classes were > 0.6.

Biomarker composition in samples was similarly compared to other potential biomarker sources. These included Monterey oil (spilled as a result of the 1964 Alaska earthquake), coal from the Tyndall Glacier (a possible source of benthic hydrocarbons) [18, 19], and Constantine Harbor sediment, which likely collects material transported into PWS from the Gulf of Alaska [20]. Results for each class (triterpanes, hopanes, and steranes) were independently compared to ANSCO results with Kruskal-Wallis one-way ANOVA on ranks, as data distributions were not normal (groups were ANSCO, Monterey, coal, and Constantine). Overall site-specific matches that considered the combined results of all 3 classes were also compared among the 4 potential sources.

Nordtest forensics

An alternative forensic method, the Nordtest [21], compares pre-determined compound ratios in samples with those in the potential source. The Nordtest uses sample averages (and confidence bounds) to infer if a sample matched the average source pattern. The 14 ratios used were those recommended by Daling [21] (Table 3).

Each ratio was calculated for each sample at a given site and summarized as a mean. Site means (x-axis in the Nordtest plots) were paired with corresponding means from each of the 4 potential sources (y-axis) in 4 separate tests. The Nordtest method states that if the 95% confidence bounds of all diagnostic ratios overlap the diagonal then the sample is a positive match to the source oil. Daling et al. (2002) also indicate that conclusions can be based on regressions between spill and source samples for the selected suite of measured diagnostic ratios.

Weathering

Concentration change over time (per g oil) was examined for each compound with linear regression. Data from all sites were combined for these analyses. Concentrations were log-transformed (natural log). We considered the usefulness of regressions by dividing the observed F value (F_o) by the critical F value (F_c) as suggested by Draper and Smith [22]. Typically a regression is useful if $F_o/F_c \geq 4$, a more restrictive criterion than significance.

Results

Pattern-matching forensics

Biomarker patterns were typically consistent with ANSCO over the entire observation period at contaminated sites (up to 23 years; Table 2). The combined result of all classes (triterpanes, hopanes, steranes) matched ANSCO in 77% of these samples (n = 62; Table 2). ANSCO was definitively present through time at all sites except Chenega Island, where it was not identified in 1995 and 1999, and Cape Gull samples in 1999 and thereafter.

Other potential biomarker sources were not plausible alternatives to ANSCO (Figure 2). When Monterey oil was used as the potential source in the model, class-specific model fits in field samples (n = 62) were consistently poorer than with ANSCO as the source ($P_{ANOVA} < 0.05$ for triterpane, hopane, and sterane results; one-way ANOVA on ANSCO, Monterey, coal, and Constantine; Figure 2A and Supplemental Data, Table S2); the combined result for all 3 classes was a 0% fit compared to 77% for ANSCO (Figure 2B). Similarly, when coal, and Constantine Harbor were also independently modeled as potential sources, model fits in field samples were consistently poorer than with ANSCO as the source, and neither explained the combined pattern (Figure 2B).

Nordtest forensics

ANSCO explained the biomarker ratios in the field samples from each site in Nordtest analyses. All regression slopes were near 1 and the 95% confidence interval of these slopes either overlapped 1 or was within 0.01 units of it (Figure 3 and Supplemental data, Figure S2). Although some error bars did not overlap the diagonal, all were fairly close and regressions were highly significant ($p_{regression} < 0.001$, 9 sites). Error bars were large at sites with few samples, such as Chenega Island, and at Cape Gull where weathering was prominent.

ANSCO matched the field data better than any alternative source (Figure 3). Regression fits were best for ANSCO and worst for Constantine. This was evident by inspecting F-values: medians were 701, 65, 19, and 1 for ANSCO, Monterey, coal, and Constantine, respectively ($p < 0.001$, Kruskal-Wallis one way ANOVA on ranks). Mean Nordtest regression slopes were 0.96, 0.78, 0.96, and 0.36 for ANSCO, Monterey oil, coal, and Constantine, respectively. Although the slope for coal was close to 1, the regression was displaced from $x = y$ and Nordtest ratios were frequently inconsistent with coal as the source (i.e., they did not overlap $x = y$). Nordtest ratios were also frequently inconsistent with $x = y$ for Monterey oil. Constantine ratios were never close to $x = y$. Thus, the Nordtest analysis is consistent with

the pattern-matching analysis; ANSCO was frequently detected in samples and none of the alternative sources were plausible.

Weathering

Initial biomarker concentrations in beached oil were typically greater than in the source oil and declined thereafter (Figure 4 and Supplemental data, Figure S3). However, concentrations were heterogeneous within and among sites, with some remaining relatively high and others declining. For example, H30 concentrations declined at each site (after the initial increase above that in source oil). Calculated independently for each site, the median H30 slope was −0.06 $\log_e$(concentration) year^{-1} and ranged from −0.11 to 0.01 ng g oil^{-1} year^{-1} (Figure 5). The combined slope was similar, −0.05 $\log_e$(concentration) year^{-1}, when all site data were regressed in common; this combined regression was significant ($F_{1,41}$ = 19.000, F_o/F_c = 4.7, $p < 0.001$). Thus, there was a general decline in H30 content in sequestered oil. Similarly, C27bbS concentration declined with time ; the combined slope was negative and significant ($F_{1,41}$ = 16.280, F_o/F_c = 4.0, ≤< 0.001). 2Nearly all slopes for all biomarkers were negative (97%; Figure 5). Estimated loss rates were greater for TR28 through TR29b among triterpanes, NOR25H and OL among hopanes, and from DIA27S through C27bbS among steranes (Figure 5). The same results can be demonstrated with an alternative analysis method (Supplemental data, Figure S4). Median correlation with time for the isoprenoid, triterpane, hopane, and sterane concentrations (ng/g oil) was −0.747, −0.478, −0.566, and −0.533, respectively (combined site data). Isoprenoid loss was substantially greater than for other biomarkers (Figure 5).

Discussion

Biomarkers can be used to identify hydrocarbon sources, thus allowing discrimination among oil sources where multiple contamination events occurred. For example, in PWS, Monterey crude oil was spilled 25 years before the *Exxon Valdez* oil spill, there was limited evidence of site-specific historical contamination within the sound, and coal was a minor source [23]. Biomarkers are also useful for studying the fate, behavior and weathering of oils in a wide variety of environmental conditions [10].

Forensics demonstrated the presence of ANSCO at all sites and generally through time, thus the ensuing changes in biomarker concentration (per gram oil) are records of ANSCO weathering. The implications of this weathering are that biomarkers are initially conserved, or more accurately, are lost slowly with respect to more labile oil constituents such as straight-chain alkanes and aromatic hydrocarbons, hence initial biomarker concentrations increased. This increase occurred early and was generally evident in the earliest intertidal samples. However, with continued time, biomarker weathering was also evident and concentrations often fell below those in the fresh source oil. Weathering was a heterogeneous process with concentrations in some samples remaining relatively high (above initial concentrations) and in other samples falling well below initial concentrations; this scatter was evident within sites as well as among sites (Figure 4). Overall trends were declines in biomarker concentrations from the earliest collections of

stranded oil to present, and this was true whether data from all sites were analyzed collectively or on a site-specific basis.

Biomarker composition at Cape Gull was unusual after 1989 likely because of rapid physical oil loss. Biomarker loss was unusually rapid (Figure 4) and composition was unlike ANSCO in 1999 and thereafter because some triterpanes (TR28a through TR29b) and steranes (DIA27S through C27bbS) were lost. These are the analytes with the highest relative loss rates presented in Figure 5. In addition, the estimated loss rates of these compounds was also high without Cape Gull data. Others have also reported enigmatic conditions at Cape Gull including unusually rapid weathering of PAHs and biomarkers [4-6]. In this analysis, Cape Gull is the most distant site from the spill location and very little surface and subsurface oil remained by 2012 [6]. Remaining oil was highly biodegraded [6]. The remaining biomarkers are unusual. One hypothesis considered by [6] is that Cape Gull biomarkers could represent a secondary contamination event. Given the unusually rapid PAH weathering and oil loss history at this site, we suggest that biomarker weathering may be a more parsimonious explanation for the change in composition but have no explanation for the underlying cause.

The pattern-matching method illustrated in the present study definitively discriminated ANSCO from several other potential sources (Supplemental data, Tables S1 and S2). The pattern-matching method included the normalized value of every reported triterpane, hopane, and sterane analyte, thus making full use of the data (Supplemental data, Tables S1 and S2). It will not, however, perform well for samples with multiple analytes below detection limits, although it can still provide insight into the source if a few analytes are consistently above detection limits. An alternative method, the Nordtest, which compares specific compound ratios [21], uses sample averages to infer if they matched the source pattern (Figure 3 and Supplemental data, Figure S2), whereas the new pattern-matching method provides a specific result for each sample and subsequent statistics can follow. Nordtest outcomes become ambiguous when few samples are available or variance is high because confidence limits become large. The pattern matching approach does not require multiple samples from a site to estimate origins, although several source oil samples are necessary for the model to function (the minimum used in the present study was 5).

Weathering

The biomarker weathering observed in the present study may have been microbial from the point the oil stranded (1989) and was sequestered until present. Rates of evaporation and dissolution were likely negligible for the large complex biomarker molecules under study, although evaporative loss was apparent for smaller compounds ($< C_{16}$) [6]. Microbial removal of n-alkanes was apparent at some sites [6], thus at least some oil constituents were lost by this mechanism. Photooxidation is not likely because the buried oil was shielded from sunlight and resin and asphaltene fractions did not increase [6]. Because microbial degradation and photooxidation are the only 2 natural processes that destroy petroleum hydrocarbons [10] and photooxidation, evaporation, and dissolution were unlikely, microbial degradation may be the most parsimonious explanation for biomarker loss. How microbes manage to remove biomarkers from bulk oil, however, is not obvious, hence there may be other explanations.

The relatively rapid isoprenoid weathering observed in residual oil is consistent with literature reports. Wang and Stout [10] report that they can be severely degraded, although isoprenoids are more recalcitrant than the n-alkanes [24]. Our previous experience suggested isoprenoid loss was too rapid and variable for source modeling and comparison of ratios (e.g., pristane/phytane) indicated differences between the source oil and samples. Such changes are not surprising because these molecules were lost at different rates.

Weathering rates in other biomarkers varied. Triterpanes TR28a through TR29b tended to weather more rapidly than other triterpanes (Figure 5). Steranes DIA27S through C27bbS weathered more rapidly than other steranes (Figure 5). This suggests a way to estimate the weathering of these compounds in future studies. These results are generally consistent with observations from the *Metula* spill that diasteranes, C27 steranes, and tricyclic terpanes weathered relatively rapidly [10]. Weathering rates were approximately the same among all hopanes in our study. In contrast, another study observed H30 and H31 to H34 were degraded relatively rapidly [10, 25].

Conclusions

Biomarkers provide an excellent way of definitively identifying the source of spilled oil over long periods of time, yet their concentrations change within the oil over time. Differential weathering may cause composition to slowly shift away from that in the source oil, although such shifts did not preclude identification of ANSCO after 25 years. Biomarkers were clearly retained while other oil constituents were lost, explaining their initial concentration increase (per unit oil), yet concentrations declined over time, indicating removal or destruction by some process, possibly microbial. Isoprenoid loss was substantially greater than tricyclic triterpane, hopane, and sterane loss.

Acknowledgment

We thank M. Zaleski and E. Pihl for processing samples.

Data availability

Data are available at http://portal.aoos.org/gulf-of-alaska.php#metadata/91b73240-b68d-43d8-bd64-aea4ea14e976/project/files

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FIGURE LEGENDS

Figure 1. Time-series sample locations included 4 within Prince William Sound (PWS), 4 along the Katmai coast, and one intervening location (McArthur Pass). The red circle marks the location of the *Exxon Valdez* spill. See Table 2 for site abbreviations.

Figure 2. Potential biomarker source comparison. Panel A: median model results by class (triterpanes, hopanes, and steranes) across samples from all sites. Perfect model fits = 1.0 (100% match), a complete lack of fit = 0.0. Panel B: combined results for each potential source [Alaska North Slope crude oil (ANSCO), Monterey oil (Monte), coal, and Constantine Harbor (Const)].

Figure 3. Comparison of potential sources using Nordtest plots. Samples in this example are from Ninagiak Island (NINAI). Potential biomarker sources examined were ANSCO, Monterey oil, Constantine Harbor, and coal (panels A – D, respectively). The dotted line is $x = y$. Axes are ratios specified for Nordtest; those from NINAI are on the x-axis and ANSCO, Monterey, Constantine, or coal ratios are on the y-axis. Solid lines are regression fits. Error bars (vertical and horizontal) are 95% confidence bounds.

Figure 4. Loss of H30 (panel A) and C27bbS (panel B) over time. Data from all sites were combined to calculate the illustrated linear regressions. Concentrations in source oil (EVO) are illustrated with black circles and the low and high ranges are marked by horizontal dashed lines. Note that scaling is different between the panels.

Figure 5. Biomarker loss. Slopes are $\log_e$(concentration + 0.1) / day. A slope of 0 indicates no change over time. See Table 1 for biomarker abbreviations. Note that y-axis scaling for isoprenoids (panel A) is different than for all other graphs.

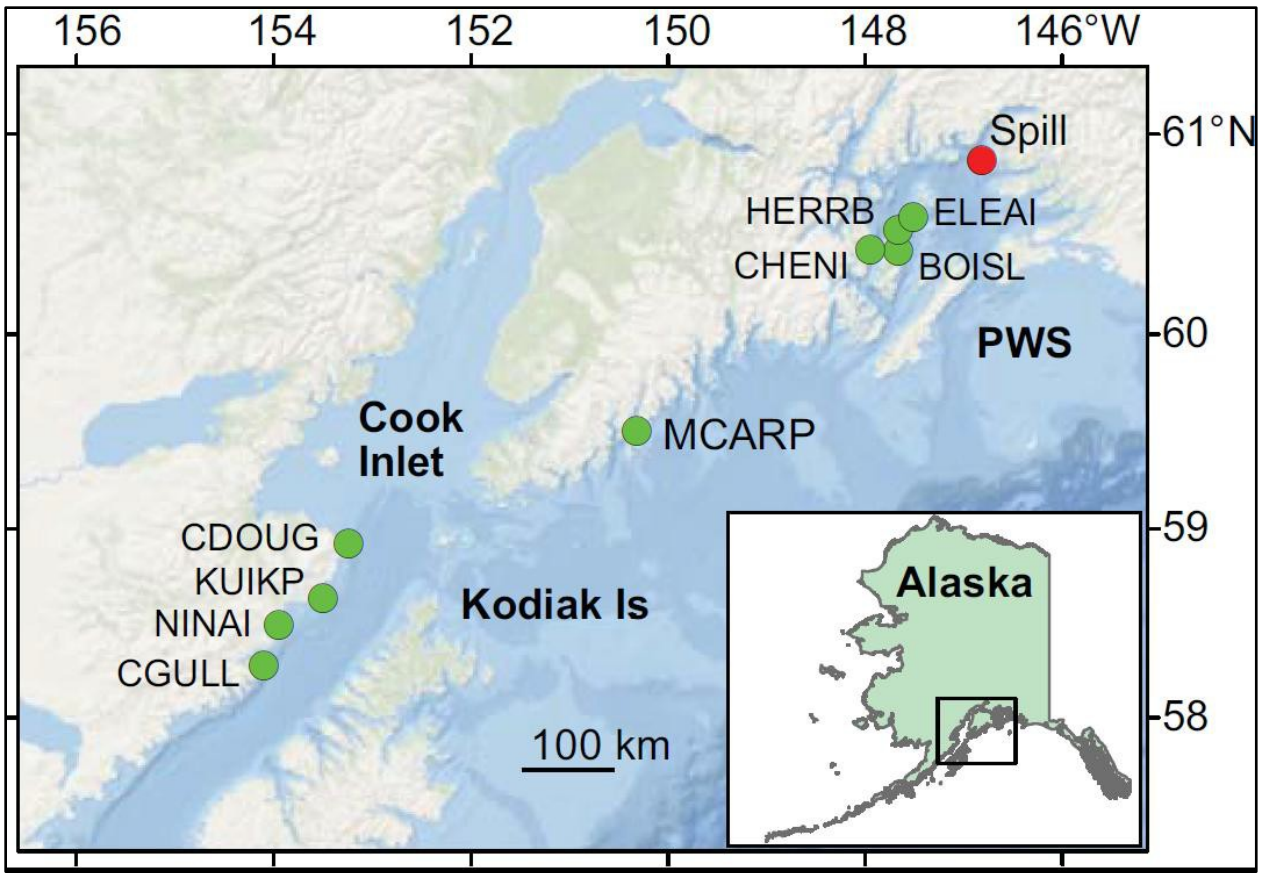

156
154
152
150
148
146°W
61°N
60
59
58
Spill
HERRB
ELEAI
CHENI
BOISL
PWS
Cook
Inlet
MCARP
CDOUG
KUIKP
NINAI
CGULL
Kodiak Is
100 km
Alaska

Figure 1.

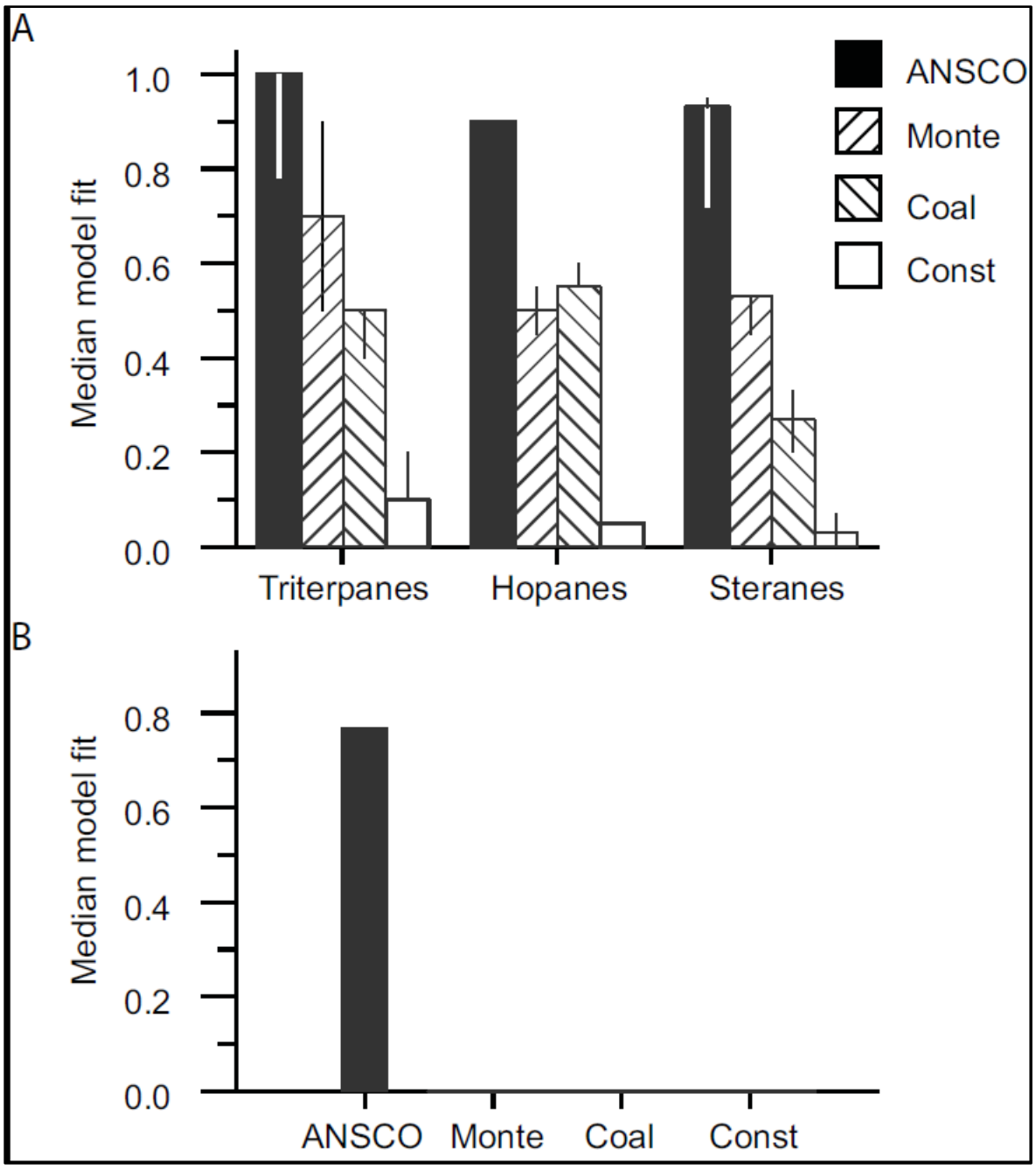

A
Median model fit
1.0
0.8
0.6
0.4
0.2
0.0
Triterpanes
Hopanes
Steranes
ANSCO
Monte
Coal
Const
B
Median model fit
0.8
0.6
0.4
0.2
0.0
ANSCO
Monte
Coal
Const

Figure 2.

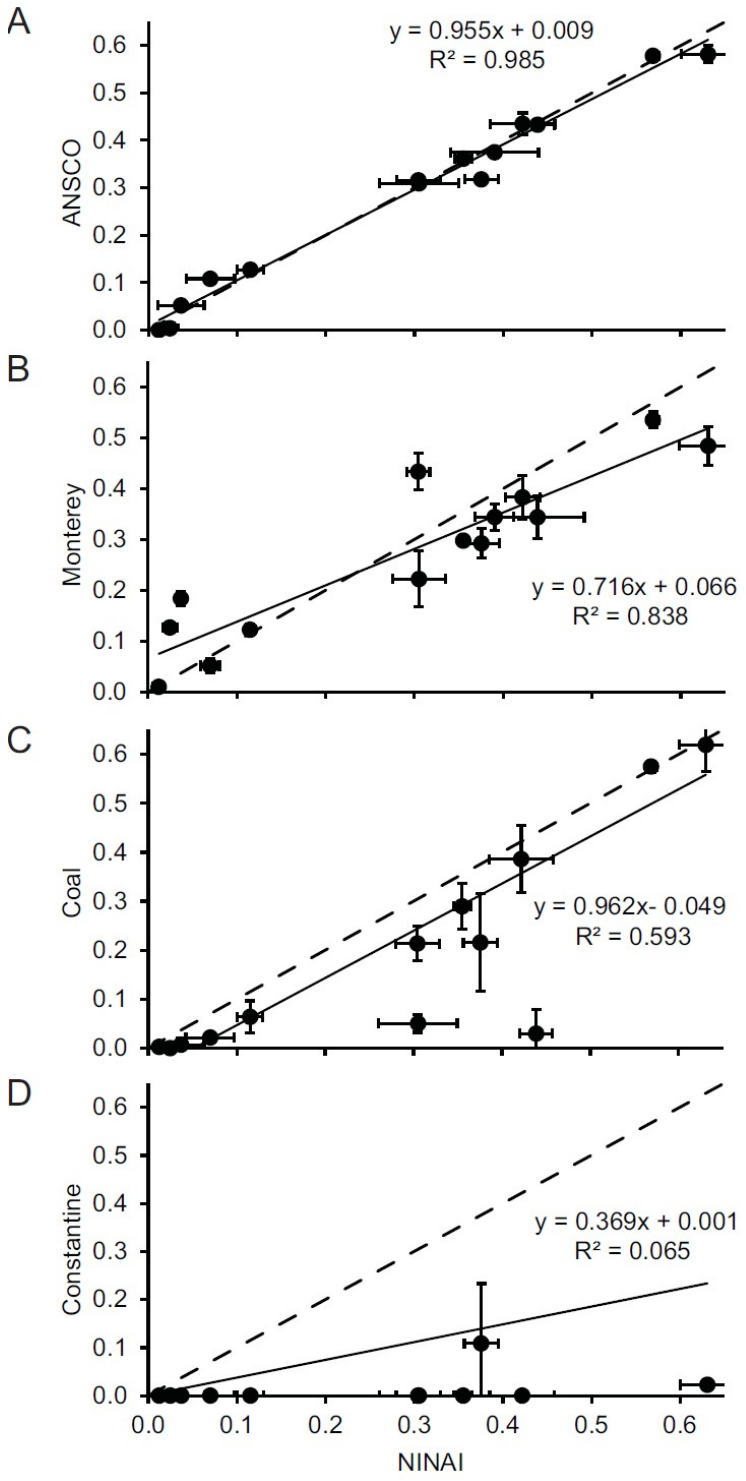
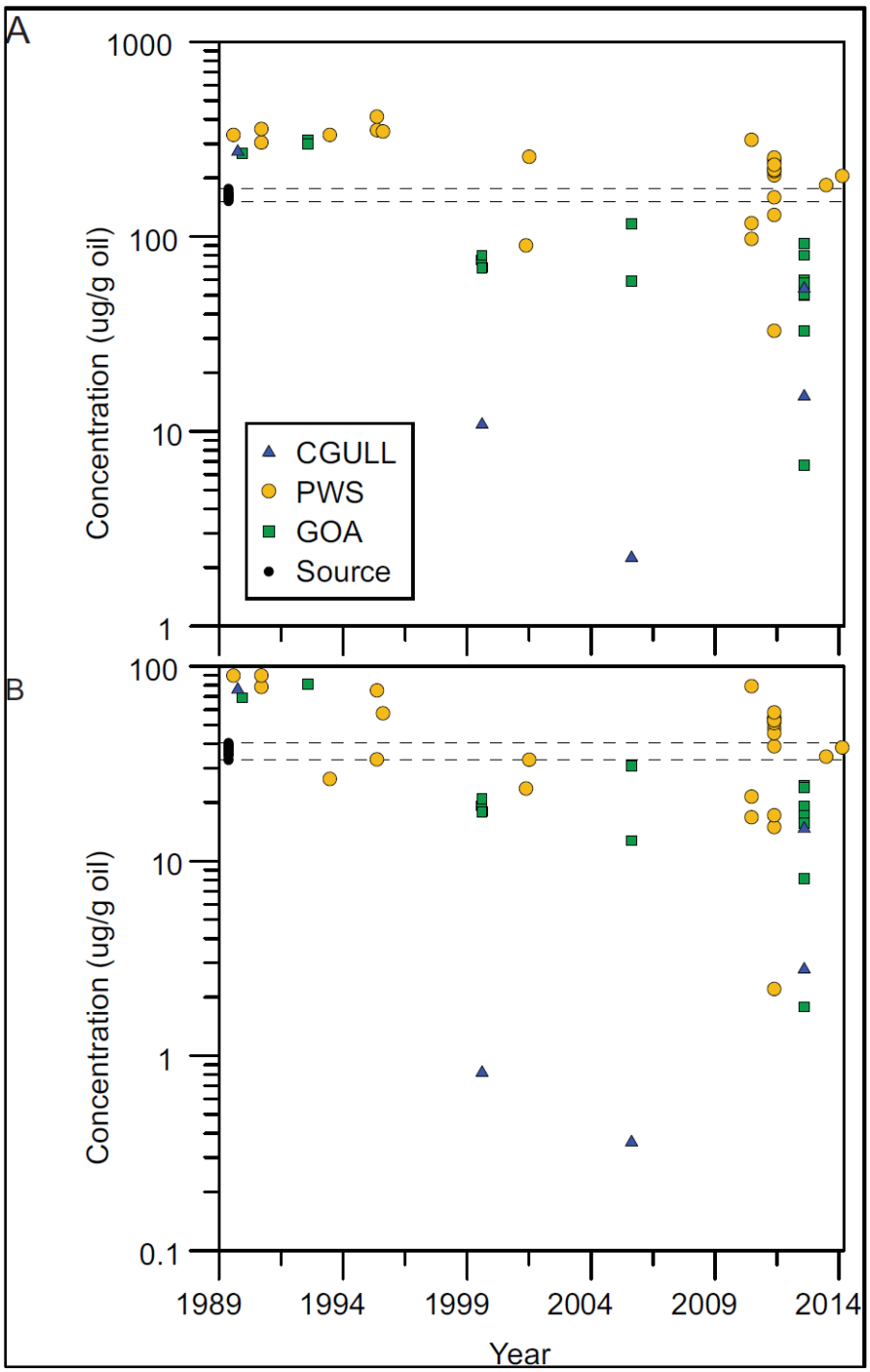

A
y = 0.955x + 0.009
R² = 0.985
ANSCO
B
Monterey
y = 0.716x + 0.066
R² = 0.838
C
Coal
y = 0.962x- 0.049
R² = 0.593
D
Constantine
y = 0.369x + 0.001
R² = 0.065
NINAI
0.0
0.1
0.2
0.3
0.4
0.5
0.6

Figure 3.


A
1000
100
10
1
Concentration (ug/g oil)
CGULL
PWS
GOA
Source
B
100
10
1
0.1
Concentration (ug/g oil)
1989
1994
1999
2004
2009
2014
Year
Figure 4.

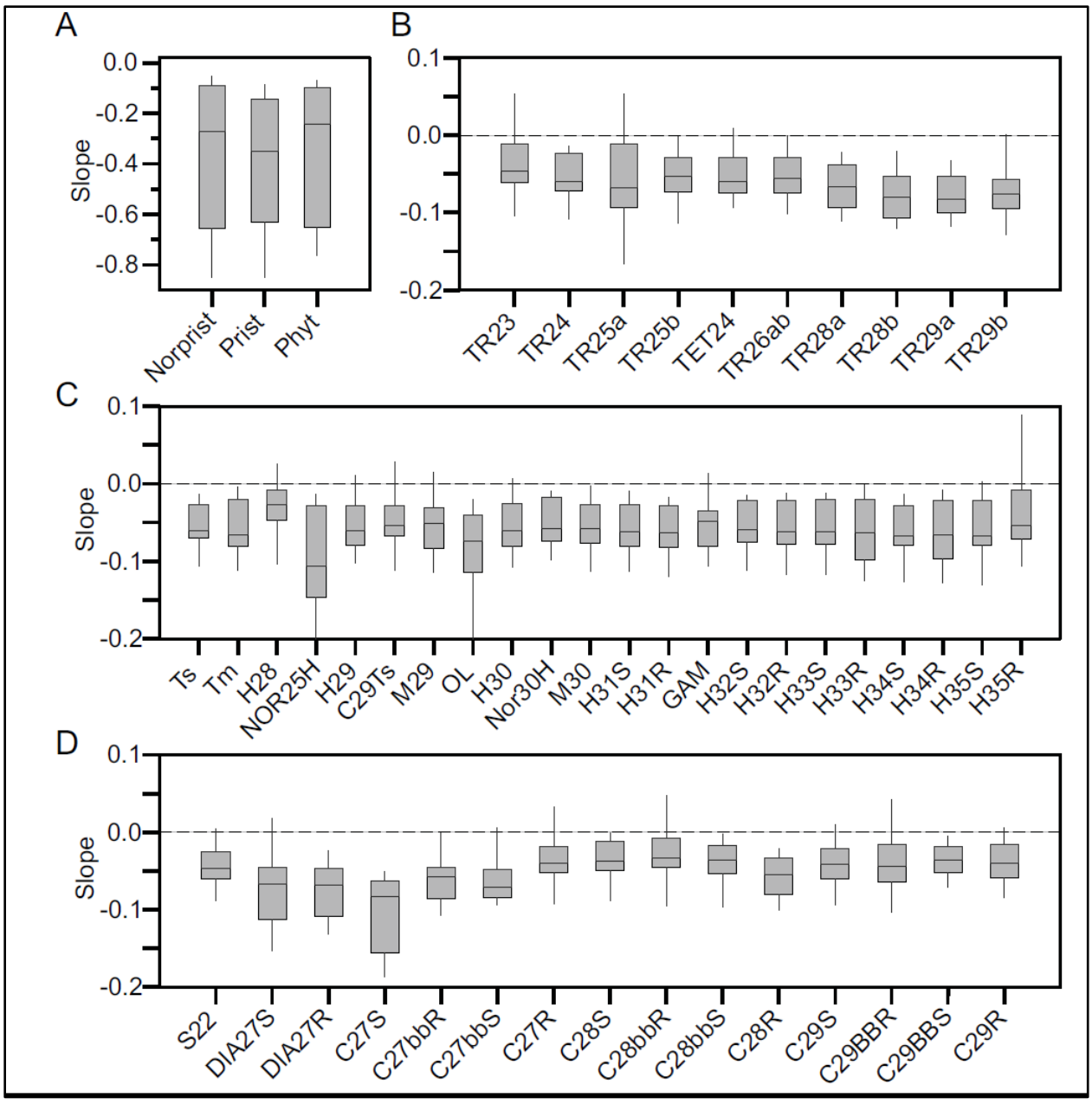

A
B
C
D
Slope
0.0
-0.2
-0.4
-0.6
-0.8
0.1
-0.1
-0.2
Norprist
Prist
Phyt
TR23
TR24
TR25a
TR25b
TET24
TR26ab
TR28a
TR28b
TR29a
TR29b
Ts
Tm
H28
NOR25H
H29
C29Ts
M29
OL
H30
Nor30H
M30
H31S
H31R
GAM
H32S
H32R
H33S
H33R
H34S
H34R
H35S
H35R
S22
DIA27S
DIA27R
C27S
C27bbR
C27bbS
C27R
C28S
C28bbR
C28bbS
C28R
C29S
C29BBR
C29BBS
C29R

Figure 5.

APPENDIX B: MANUSCRIPT - LINGERING EVO 26 LATER

Revised Manuscript submitted to Deep Sea Research II special issue Journal:
Conditions of persistent oil on beaches in Prince William Sound 26 years after the *Exxon Valdez* spill

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Keywords:
Exxon Valdez, Oil spill, persistent oil, Prince William Sound, Alaska

Bounding Coordinates:
West Bounding Coordinate: -147.974 East Bounding Coordinate: -147.299
North Bounding Coordinate: 60.591 South Bounding Coordinate: 60.054

Revised Manuscript submitted to Deep Sea Research II special issue Journal:
Conditions of persistent oil on beaches in Prince William Sound 26 years after the Exxon Valdez spill

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Abstract

On March 24, 1989, the *Exxon Valdez* grounded on Bligh Reef in Prince William Sound, Alaska, spilling an estimated 10.8 million gallons of crude oil. Contrary to early projections, subsequent studies over several decades have shown subsurface oil persisting on impacted beaches. Here we present findings from a lingering oil survey conducted during the summer of 2015 at a small set of beaches in Prince William Sound known to have persistent subsurface *Exxon Valdez* oil. The objectives of the survey were to estimate how much oil remains at these sites, the oil composition, and oil retention rates compared to previous studies. Results from the survey found lingering oil was present at 8 of 9 sites that were revisited. Surveys revealed little evidence of change in oil area or mass over the last 14 years, nor has there been a change in the distribution of oiling intensities or their location on the beach. Detailed analysis of the oil indicated it has not weathered since 2001. Subsurface oils collected in 2015 have enriched concentrations of phenanthrenes and chrysenes relative to oil originating in the cargo hold indicating that buried oil has retained some toxic potential over the last two decades, but it is not currently bioavailable. Subsurface oil appears to be sequestered in sediments and protected from hydrological washing and low oxygen and nutrient levels inhibiting biodegradation. These findings are consistent with previous surveys and predictive geomorphic models suggesting the estimated 0.6% *Exxon Valdez* oil remaining is sequestered and not bioavailable unless disturbed and will likely persist in the environment on a decadal scale.

1. Introduction

The 1989 *Exxon Valdez* oil spill (EVOS) in Prince William Sound (PWS), Alaska, remains one of the largest spills in United States history after the Deep Water Horizon. At least 10,800,000 gallons of crude oil moved southwest into PWS, with significant amounts escaping and contaminating the Kenai and Alaska peninsulas and Kodiak Archipelago (Wolfe et al., 1994). A total of 2,100 km of shoreline was impacted by the spill (Owens, 1991). A spill of this magnitude required a massive shoreline survey effort to determine the extent and degree of oiling and to direct clean-up activities. These surveys were known as the Shoreline Cleanup Assessment Teams (SCAT) conducted between 1989 and 1992. Initial SCAT surveys in PWS estimated 40% of the spilled oil made landfall on beaches in PWS (Wolfe et al., 1994). Over a three-year period (1989-1991) Exxon Corporation employed an unprecedented 10,000 people on clean-up crews, totaling 20 million man hours at a cost of $2 billion dollars (Rice et al., 2007). Clean-up techniques on beaches included manually wiping oil off rocks, high pressure hot-and-cold seawater washing and mechanical tilling resulting in an estimated 30,000 tons of oily solid waste recovered during 1989 alone (Piper, 1993). By 1992, it was estimated that 5-8% of the initial spilled oil had been recovered from beaches (Wolfe et al., 1994). Follow-up SCAT surveys showed there was a decrease of visibly oiled shoreline in PWS from 783 km in 1989 to 10 km by 1992 (Neff et al., 1995). Given the considerable loss rate observed by 1992, the remaining oil was predicted to continue weathering and dissipate over a short time scale, consequently SCAT surveys were terminated (Page et al., 1995).

During the first decade after the EVOS, some site-specific observations of oiled shoreline in PWS began to cast doubt on the expected loss rate of *Exxon Valdez* oil and its persistence in a relatively un-weathered state in the environment (Brodersen et al., 1998; Carls et al., 2001; Hayes and Michel, 1999). Surveys funded by the *Exxon Valdez* Oil Spill Trustee Council (EVOSTC) during 1993 estimated about one-half of the surface oil had decreased from 1991 to 1993, but 7 km of subsurface oil remained on PWS beaches (Gibeaut and Piper, 1998). Entrenched subsurface oil was no longer being removed by natural processes which suggested loss rates had slowed down (Boehm et al., 1995). A 1997 beach cleaning effort in two PWS bays revealed substantial deposits of moderately weathered, subsurface oil (Brodersen et al., 1998). A monitoring effort sponsored by National Oceanic and Atmospheric Administration (NOAA) Hazmat also reported that subsurface oil was persisting on some beaches 8 years after the spill and was likely related to unique coastal geomorphology and other physical factors present at those beaches (Hayes and Michel, 1999).

The second decade following the EVOS marked a new series of comprehensive surveys to update estimates of lingering oil in PWS, determine the subsurface oil distribution geographically, as well as relative to tidal height, and to determine if the lingering oil was distributed throughout the spill area in PWS or was more of a site-specific issue. National Marine Fisheries Service's (NMFS) Auke Bay Laboratories initiated a PWS lingering oil survey in 2001 to provide a probability-based estimate of the amount of oil remaining 12 years after the spill (Pella and Maselko, 2007; Short et al., 2004). Estimates from this survey revealed a cumulative area of *Exxon Valdez* oil of 7.8 ha and a mass of 55,600 kg of subsurface oil in PWS. The majority of the subsurface oil was located in the mid-intertidal zone and some lower in the intertidal than expected. Additional surveys conducted in 2003 and 2005, focused on determining the distribution of oil with respect to tidal elevation and the probability of encountering oil in a heavily oiled region of PWS (Short et al., 2007, 2006), indicated that most of the oil was found in the middle of the intertidal and that it was distributed symmetrically with respect to tide height from that area. Subsurface oil had more than a 3-fold greater encounter probability than surface oil. A comparison of survey results between 2001 and 2005 showed the likely rate of decline of oiled beach area within PWS was 3-4 % yr^{-1} (Short et al., 2007). Given the quantitative consistency of the surveys conducted in the 2000s, a spatial model was developed to predict where subsurface oil is likely to occur in addition to known locations (Michel et al., 2010; Nixon and Michel, 2015). Estimates based on data collected between 2001 and 2008, revealed lingering subsurface oil represented 0.25% of the total spill volume (Michel et al., 2016). Surveys of subsurface oil conducted by Exxon Mobil Corporation in 2002 (Taylor and Reimer, 2008) and 2007 (Boehm et al., 2008) examined oil distribution and weathering at many of the same locations NMFS surveyed. The 2002 survey reported sites with subsurface oil residues represented less than 0.5% of the originally oiled shoreline in PWS (Taylor and Reimer, 2008). Both surveys concluded that oil was most prevalent near the middle of the intertidal, but the conclusion that the distribution in the intertidal was nearly symmetrical with respect to tide height (Short et al., 2006) was contested.

From a management perspective significance of lingering oil in the lower intertidal became apparent when its occurrence (Short et al., 2007, 2006, 2004) was linked to lack of recovery of sea otter and harlequin duck populations in those same areas (Bodkin et al. 2014, Iverson and Esler, 2010). In addition to failed population recovery, populations in these areas displayed evidence of chronic oil exposure (Esler, 2010). Most recently, the evidence of exposure has diminished, populations have recovered and some affected species are no longer considered injured (Esler et al., 2017). Historically, lingering oil following oil spills has not been well documented and is seldom sought. However natural resource trustees have a responsibility to understand the risks posed by lingering oil. One notable exception was

persistence of oil in wetland sediments following the 1969 spill in near West Falmouth in Massachusetts that has been followed by Woods Hole Oceanographic Institute researchers for over three decades (Reddy et al., 2002).

Now in the third decade since the EVOS, we present findings from a lingering oil survey conducted by NMFS's Auke Bay Laboratories in 2015, 26 years after the spill. The intent of the survey was to re-visit a small set of beaches in PWS known to have persistent subsurface oil and determine if there has been any measurable loss of persistent oil since they were last visited. If oil loss was detected this would suggest the oil is being naturally removed, however, no change in the amount or character of the oil at these sites would suggest the oil has been sequestered. We were also interested in knowing if we can see any changes in oil retention rates during the last 5, 10, or 15 years. This will help determine at what time scale we can detect loss of persistent oil in PWS. Very few oil spills in the marine environment have presented the opportunity to assess persistence and loss rates measured throughout such a long time period. Answers to these questions have implications for decision makers and resource managers in the future (e.g. need for further remediation, continued monitoring of the toxicity of oil in the environment, and determining recovery of injured resources).

2. Materials and methods

Nine beach segments in PWS were selected for lingering oil surveys during summer 2015 (Figure 1). Prioritization of beach segments selected for surveying subsurface oil were based on initial oiling (heavy or moderate), a history of being surveyed (Short et al., 2007, 2006, 2004), subsurface oil observed in more recent years, shore types prone to oil retention such as armoring (Hayes and Michel, 1999), and a high probability of finding subsurface oil based on the Nixon and Michel (2015) predictive model (Table 1).

2.1. *Oiled beach surveys*

Methods for surveying beach segments, discovering oil and estimating oiled area were based on the stratified random sampling method used during NMFS's Auke Bay Laboratories' 2003 and 2005 lingering oil surveys (Short et al., 2006; Short et al. 2007). We note that these methods are a more efficient adaptation of the methods employed in 2001 (Short et al. 2003). These methods were proven to estimate subsurface oil for the entire intertidal zone (approximately mean high water to zero tide height) with a similar error structure. Repeating this approach ensured comparison over time for a given site. In brief, survey equipment was used to divide a typical 100 m length of shoreline into 5 contiguous columns each 20 m wide. Shorter shoreline segments were divided into correspondingly fewer 20 m sampling columns. Each column was partitioned into 5 rectangular blocks designated MVD 1 to MVD 5 and defined by 1-m vertical drops or tidal elevation intervals, beginning at + 4.8 m tide height (MVD 1) and extending to down to -0.2 m (MVD 5). Two sampling quadrats (each 0.25 m^2) were randomly located within each block, resulting in 50 quadrats/100 m of shoreline. Each quadrat was excavated to a depth of 0.5 m or less if an impenetrable substrate was encountered (e.g. bedrock).

On each beach segment, representative samples of subsurface oil encountered in a random quadrat or "pit" were collected for chemical analysis to determine source and weathered state of the oil using gas chromatography – mass spectroscopy (Short and Heintz, 1997). Each oiled pit was visually classified using standard categories light, moderate or heavy oil residue: LOR, MOR, HOR (Short et al., 2004). Estimating the volume of classified oil (LOR, MOR, and HOR) was accomplished by weighing the mass of

oil extracted from pit sediments for each category and site. On site, all material from an oiled quadrat or "pit" was placed in 5 gallon buckets and weighed to the nearest 0.5 kg to obtain a total pit weight. Pit material was homogenized in a concrete mixing tub (79 L) and then a subsample (5 to 8 kg) was collected in a 4 L plastic jug (high density polyethylene) for gravimetric analysis in the laboratory.

2.2. *Laboratory analysis of Exxon Valdez Oil*

Oil volume per pit by gravimetric: In the laboratory, all 36 homogenized gravimetric subsamples collected from oiled pits in the field were weighed to the nearest 5 grams. Dichloromethane was added to fill the subsample jugs and sonicated in a water bath for 1 hour. The extracts were decanted through cotton-plugged glass funnels overlain with anhydrous sodium sulfate. The extraction process was repeated until the solution was colorless. The extracts were transferred to tared flasks which were heated on a steam table to remove the dichloromethane. When only crude oil remained, the flask was allowed to cool and vent overnight before final re-weighing to the nearest 0.01 gm.

Analysis for source and weathering state: Oiled sediment samples brought back from the field were processed in the lab to determine oil source, total polycyclic aromatic hydrocarbons (PAH) and weathering state. Sediments were dried with anhydrous sodium sulfate and then extracted with dichloromethane. A 6 gm silica gel column was used to separate the extracted oil into aromatic and aliphatic fractions. Aromatic fractions were analyzed for PAHs by gas chromatography – mass spectroscopy. Data were acquired in selected ion monitoring mode and concentrations (ng analyte/g sediment) were determined by the internal standard method (Short et al., 1996). Concentrations below method detection limits were set to zero and were not used for loss-rate estimates. Aliphatic fractions were analyzed for *n*-alkanes using gas chromatography and flame ionization detection (Short et al., 1996). Analyte concentrations were determined by the internal standard method. Measured normal alkanes ranged from *n*−C9 through *n*−C36 plus pristane and phytane. Concentrations below method detection limits were set to zero.

Analysis of survey data

Oil composition

PAHs, alkanes and biomarkers were analyzed to determine the extent to which weathering had occurred since previous. Weathering describes the process by which compounds are lost from the surface of deposits to surrounding matrices. Consequently, the rate at which a deposit weathers is a function of the ratio between the deposit's surface area and volume. The weathering state of a deposit can be quantified because compounds are lost predictably, according to first-order loss-rate kinetics (Venosa et al., 1996; Short and Heintz, 2007). Thus naphthalenes are lost more rapidly from oil residues than chrysenes (Figure 2). These processes for *Exxon Valdez* oil (EVO) have been quantified into a single index, *w* (Short and Heintz 1997), where values range upwards from zero, which reflects the PAH composition of EVO weathered to about 80% of its initial mass (Short and Heintz 1997).

The oil weathering index, *w*, and percentage of remaining PAHs were compared with similar values from previous collections to determine if subsurface oil has weathered since surveys began. The weathering index was calculated for each of the samples collected for PAH analysis. In addition, PAH and *n*-alkane concentrations were re-expressed as the proportion remaining in the sequestered oil following the method of Short et al., (2007) as an alternative method for evaluating how much the subsurface oil has weathered since the last surveys. The proportion remaining was estimated as summed concentrations

of PAHs (TPAH) or *n*-alkanes normalized to the sum of the chrysenes divided by the same proportions obtained from mousse collected from the sea surface 11 days after the spill. Both *w* and the percentage of TPAH remaining were compared across beaches and years using a two-way ANOVA to test the hypothesis that subsurface oil is weathering over time.

We tested the hypothesis that weathering was not uniform across the beaches. Specifically we hypothesized that the occurrence of residues with differing oiling intensities (HOR, MOR and LOR) reflected patches with different weathering states based on the idea that LOR patches would be more weathered than MOR patches and so on (Boehm et al. 2008). Similarly, we hypothesized that the location of the residues on the beach relative to tide height affected weathering state based on Boehm et al.'s conclusion that low intertidal patches are more accessible to weathering factors than high intertidal patches. We tested these hypotheses using a two-way ANOVA using the weathering index *w* and the percentage of PAH remaining. For each test the fixed main factors were MVD (beach location relative to tide height), oiling intensity and their interaction. We did not test the interaction between them. We also conducted a similar test using the percentage of remaining PAHs as the response.

Oil area and weight calculations

The total estimated area and the weight of subsurface oil found on each beach segment were calculated based on stratified random sampling without replacement sampling design. The area of subsurface oil within *k*th block at a given beach segment is estimated as follows:

$$\hat{\tau}_k = N_k \frac{\sum_{i=1}^{n_k} x_{ki}}{n_k} \qquad (1)$$

where, N_k is the total number of available quadrats in *k*th block and x_{ki} is the oiling status of the ith pit out of n_k total excavated pits, in block *k* (here *i* will be 1 or 2 since only 2 pits per block were excavated). The total amount of oil in a given beach segment is then calculated by summing all B block estimates:

$$\hat{\tau} = \sum_{k=1}^{B} \hat{\tau}_k \qquad (2)$$

The estimated variance of the total area of subsurface oil at a beach is then calculated as follows:

$$\widehat{var}(\hat{\tau}) = \sum_{k=1}^{B} (N_k)(N_k - n_k) \frac{s_k^2}{n_k} \qquad (3)$$

$$s_k^2 = \left(1 - \frac{n_k - 1}{N_k - 1}\right) \left[\frac{\left(\frac{\sum_{i=1}^{n_k} x_{ki}}{n_k}\right)\left(1 - \frac{\sum_{i=1}^{n_k} x_{ki}}{n_k}\right)}{n_k} \right] \qquad (4)$$

Subsurface oil weight is calculated in a similar fashion, except the oiled pits now take on the value of the oil weight based on the gravimetric estimates. The total weight of oil in *k*th block is:

$$\hat{\varphi}_k = \frac{N_k}{n_k} \sum_{r=LOR}^{HOR} \sum_{i=1}^{n_k} x_i I\{r\}[I\{actual\} w_{kri} + I\{estimated\} \overline{w}_r] \qquad (5)$$

where $I\{r\}$ is and indicator function {0,1} of whether the jth pit belonged to oiling oil class *r* (LOR, MOR, or HOR). $I\{actual\}$ is an indicator function {0,1} of whether the oil in the *i*th pit was measured directly

and w_{kri} was the weight of the oil found in the *i*th pit through gravimetric analysis. $I\{estimated\}$ is the indicator function {0,1} of whether the oil in the *i*th pit was only scored and not directly measured with $\bar{w}_r$ being the mean oil weight from gravimetric samples from all beach segments of *r* oil class.
The total weight of oil on a beach is then calculated as the sum of all the blocks:

$$\hat{\varphi} = \sum_{k=1}^{B} \hat{\varphi}_k \qquad (6)$$

The estimated variance of the total weight of oil on a beach is calculated as follows:

$$\widehat{var}(\hat{\varphi}) = \sum_{k=1}^{B} (N_k)(N_k - n_k) \frac{s_{w_k}^2}{n_k} \qquad (7)$$

$$s_{w_k}^2 = \left(1 - \frac{n_k - 1}{N_k - 1}\right) \sum_{r=LOR}^{HOR} \sum_{i=1}^{n_k} x_i I\{r\}\left[I\{actual\} s_{w_r}^2 + I\{estimated\} s_{\bar{w}_r}^2\right] \qquad (8)$$

where $s_{w_r}^2$ is the variance of the *r*th oil class weight that was analyzed from the beach being estimated and $s_{\bar{w}_r}^2$ is the variance of the *r*th oil class weight across all the beach segments sampled. Results are presented as 95% confidence intervals and compared with similar intervals for these same beaches surveyed in 2005. Comparisons with these same beaches in 2001 were not made because the 2001 survey did not include the lower portions of the beach.

Oil retention rate comparison calculations

Pairwise comparisons of annualized retention rates were restricted to tidal elevations MVD 1 through 3 because MVD 4 and MVD 5 were not sampled in 2001. Estimated annualized retention rate denoted $\hat{\theta}$ is the proportion change in the amount of oil per year, where 1 indicates no change, >1 are annual gains, while <1 are losses in oiled areas. We followed (Short et al., 2007) in calculating the annualized retention rate ($\hat{\theta}_{it}$) for each of the *i*th beach segments between the sampling intervals *t* of 4, 10, and 14 years (i.e. 2001, 2005, and 2015):

$$\hat{\theta}_{it} = \left(\frac{p_2}{p_1}\right)^{1/t}, p_2 = \frac{k_2}{n_2}, p_1 = \frac{k_1}{n_1} \qquad (9)$$

Noting that p_j is the proportion of oiled pits found where k_j is the number of observed oil quadrats and n_j is the number of sampled quadrats during the *j*th (j ϵ {1,2}) surveys *t* years apart.

Variance of $\hat{\theta}_{it}$ for each of the ith segments was calculated as follows:

$$\widehat{var}\left(\hat{\theta}_{it}\right) = \left(\frac{1}{t\hat{p}_2}\left(\frac{\hat{p}_2}{\hat{p}_1}\right)^{\frac{1}{t}}\right)^2 \widehat{var}(\hat{p}_2) + \left(\frac{\hat{p}_1}{t}\left(\frac{\hat{p}_2}{\hat{p}_1}\right)^{\frac{1}{t}}\right)^2 \widehat{var}(\hat{p}_1) \qquad (10)$$

The interval specific (t ϵ 4, 10, and 14 years) annualized retention rate ($\hat{\theta}_{it}$) was based on the observed oil found for all beach segments that were sampled in at least two of the years (2001, 2005, and 2015). We used a semi-parametric bootstrap approach to calculate the bootstrap estimators of (θ_i) and subsequently the standard error for each of the t intervals (Efron and Tibshirani, 1993). We used a mixed effects model (Bates et al., 2015) with beach segment treated as a random effect to test for differences in θ_i among the three intervals. All calculations were performed in R statistical software package (R

Development Core Team, 2015). Results are presented as 95% confidence intervals. Comparisons to previous surveys conducted in 2001 and 2005 were based on calculations from raw data and the same method as described above.

3. Results

3.1. *Subsurface oil and composition*

Oil was readily found in subsurface sediments sampled in eight of the nine beaches re-surveyed in 2015; subsurface oil was not found on Evans Island segment EV039A (Table 2). Overall oil was encountered in 11.75% of the 400 pits. The probability of encountering oil on these beach segments, expressed as the number of oiled pits divided by the total number excavated, generally agreed with the predictive model (Michel et al., 2010; Nixon and Michel, 2015) used to select beach segments (Table 2). Similar to other surveys, most of the contaminated sediments were lightly to moderately oiled (Figure 3). Of the 400 pits excavated, 19 were identified as LOR compared with 12 MOR and 8 HOR (Table 2). Oil mass per unit area averaged 0.2 ± 0.2 kg/m^2 in LOR, 1.0 ± 0.3 kg/m^2 in MOR and 2.0 ± 1.0 kg/m^2 in HOR residues. Trace amounts of surface oil, visible prior to pit excavation, were found at Latouche and Smith Island shore segments (asphalt and surface oil residues, respectively). Those residues are not discussed here.

The distribution of the oil patches on the beaches was the same as in other surveys. The oil was primarily located in the middle of the intertidal (MVD 3) with approximately equal amounts of oil located above and below the middle (Figure 3). Only 2.5% of the oiled pits were found in the uppermost section of beach (MVD 1) compared with 12.2% in the next lower section (MVD 2). The lowest section of beach (MVD 5) held 12.2% of the oiled pits compared with 28.2% in the next highest section (MVD 4). Allocating half of the MVD 3 pits to the upper half of the beach results in an estimated 38% of the oil in the upper intertidal and 62% in the lower intertidal.

Comparisons of the oil composition

Subsurface oil in PWS was found in a range of weathering states as indicated by the relative losses of PAHs and *n*-alkanes (Table 3). Values of *w*, the weathering index, ranged from 0 to 6.7 with an overall mean value of 3.0 ± 1.8 (mean ± 1 s.d.). The least weathered oil was found in a MOR residue on Knight Island segment KN0506A. Characteristically, it had high naphthalene concentrations, averaging 43% of TPAH and low chrysene concentrations (1% of TPAH). The most weathered oil was found in an HOR residue on Latouche Island segment LA018A. This oil was characterized by low concentrations of naphthalenes (4% of TPAH) and higher levels of chrysenes (>12% of TPAH). Estimates of the percentage of PAH remaining relative to 11 d mousse mirrored the weathering indices. Values ranged from 3% to 94% with a mean value of 20.2 ± 14.4%. The lowest percentage of remaining PAH relative to 11 d mousse was observed on Latouche Island segment LA018A while the greatest remaining percentage was observed on Knight Island segment KN0506-A. Losses of *n*-alkanes were even greater when compared with those of PAH. Over all sites, the percent *n*-alkanes remaining relative to 11 d mousse averaged 4.7 ± 5.8%. The lowest percentage (1.1% retained) of remaining *n*-alkanes was found on Eleanor Island segment EL056C and the location with the least degraded *n*-alkanes (28.1% retained) was on Smith Island segment SM006B.

There has been little change in the weathering state of the oils in selected beaches since surveys began. The PAH composition of subsurface oils collected from beach segments EL058B, GR103B, KN0300A-2

and LA018A-1 was characterized in 2001, 2005 and 2015. The weathering index for these sites was compared by two-way ANOVA with years, sites and their interaction as the main factors. None of these factors had an effect on the average *w* value ($p > 0.120$). A similar comparison of the average amount of PAH remaining relative to 11 d mousse failed to detect a difference due to year ($F_{1, 24} = 1.99$, $p = 0.171$), site ($F_{3, 24} = 0.026$, $p = 0.994$) or their interactions ($F_{3, 24} = 1.435$, $p = 0.257$). Variability within a site in a given year was typically very high, coefficients of variation within a site for both *w* and the percent PAH remaining averaged around 50%. Note that samples selected to show weathering on segment LA018A-1 in Figure 2 also reflect the high variability in weathering states observed at sites in a given year.

In contrast to the inter-annual comparisons, comparisons of *w* and the remaining PAHs among tide levels and oiling intensities revealed some evidence of weathering. The two-way ANOVA for *w* detected a marginal effect of oiling intensity ($F_{2, 27} = 3.40$, $p = 0.048$) and no effect of tide stage ($F_{4, 27} = 1.52$, $p = 0.225$) or their interaction ($F3_{2, 27} = 0.76$, $p = 0.525$). Pairwise contrasts revealed the greatest difference was between HOR and LOR residues, while the latter was less weathered the difference was not significant ($p = 0.087$) (Figure 4). Similar results were observed in the two-way ANOVA on the percentage of PAHs remaining relative to 11 d mousse (Figure 4). Oiling intensity had a significant effect ($F_{2, 29} = 3.98$, $p < 0.030$) due to a nearly significant ($p = 0.058$) difference of 7% between HOR and LOR. Tide stage had a large effect on the percentage of PAHs remaining ($F_{4, 29} = 27.2$, $p = 0.001$). Pairwise contrasts indicated that this difference was driven by the only sample collected from the highest tide stage (MVD 1), it was a relatively fresh sample with 94% of PAHs remaining. The other tide stages did not differ ($p > 0.220$). There was no interaction between tide stage and oiling intensity ($F_{3, 29} = 0.12$, $p = 0.946$).

3.2. *Estimates of subsurface oil*

Eleanor Island segment EL056C had the largest estimated oiled area at 1,218 m^2 (± 112 m^2) (Table 4) as well as the largest total weight of oil estimated at 1,124 kilograms (±470 kg) (Table 4). In contrast, no subsurface oil was encountered in the 50 pits excavated on Evans Island segment EV039A. Coefficients of variation for the oil area estimates for each beach varied from 5% to 50% with an overall median 13.5%, indicating a reasonable sampling strategy for estimating oiled beach area. The coefficients of variation for the oil weight estimates at each oiled beach segment were much larger, ranging from 21% to 251% with a median of 58.5%.

Comparison of the estimated oiled areas and weights for the six beach segments surveyed in both 2005 and 2015 generally reveal little change in subsurface oil estimates. Comparisons were limited to just the beaches surveyed in both years, 2001 was not included because the lower portions of the beaches were not surveyed. Based on 95% confidence intervals, there was little evidence for a decline in oiled area (Figure 5) or oil weight (Figure 6) at these survey sites. The oiled area at one site, Eleanor Island segment EL058B had significantly greater oiled area in 2015 (Figure 5), but there was no difference in mass (Figure 6). The only location where oil appeared to decrease was at Evans Island segment EV039A, where the oiled area in 2005 was estimated at 154 ± 116 m^2 (Short et al., 2007) and no oil was detected 2015. There was no change in the oiled areas at the remaining four beach segments that were surveyed in 2005 and 2015.

3.3. *Annualized retention rate of subsurface oil*

There was little evidence that oil has been lost from the beaches surveyed in 2001, 2005 and 2015, based on the probability of encountering oil. Retention estimates for the periods 2001 to 2005, 2005 to

2015, and 2001 to 2015 did not differ from each other (p=0.612) (Figure 7). The 95% confidence intervals for the PWS wide annualized retention rates averaged over the sites common to all the surveys included 1.0 for all the intervals, indicating no oil was lost. The same was true when each of the beach segments were examined individually, except that no oil was found at EV039A in 2015 (Figure 8). In fact, retention rate estimates indicated oil accumulation ($\Theta > 1$) at Eleanor Island beach segment EL058B between 2001 and 2015 (95% CI: 1.02-1.22) and Green Island beach segment GR103B for the same period (95% CI: 1.03-1.56), and again at EL058B from 2005 to 2015 (95% CI: 1.04-1.89). The only significant loss in oil was detected at Eleanor Island on segment EL056C between 2001 to 2015 (95% CI: 0.89-0.998) (Figure 8).

4. Discussion

4.1. *Subsurface oil and composition*

Surveys conducted during summer of 2015 confirmed the presence of oil in subsurface sediments of some beaches in PWS 26 years following the *Exxon Valdez* oil spill. The oil's presence and its probability of encounter were consistent with earlier geomorphic model predictions for persistent subsurface oil (Michel et al., 2010; Nixon and Michel, 2015). The model was designed to predict locations where persistent subsurface oil could be found throughout PWS based on surveys conducted between 2001 and 2008, and then updated to included subsequent surveys up through 2015. Previous testing of the model with data from 2001-2008, showed there was good agreement for estimated total area and weight by oil class for the same area (Short et al., 2004). Most recent testing by Nixon and Michel (this issue) including data ranging from 2001-2015 (14,000 pit excavations), continues to support estimates and even suggests previous estimates of the initial amount of oil remaining were slightly underestimated changing from 0.25% to 0.6% of the originally spilled mass of oil. These values are similar to 0.5%, the estimate for subsurface oil in 2002 provided by Taylor and Reimer (2007).

The failure to detect significant change in the estimated oil surface area or weight between 2005 and 2015 along with the observation that loss rates do not differ from zero indicate the 2001 estimate of 7.8 ha of subsurface oil remaining in PWS (Short et al. 2004) has changed little. Trends from previous surveys suggested that *Exxon Valdez* oil would be found during the 2015 survey (Hayes and Michel, 1999; Michel and Hayes, 1993; Peterson et al., 2003; Short et al., 2007, 2006, 2004). Based on their recent model Nixon and Michel (this issue) concluded present removal rates for these remaining subsurface oil residues have slowed to nearly zero and removal mechanisms will operate over time scales of decades. Outside of PWS Irvine et al. (2014) found subsurface *Exxon Valdez* oil to persist for at least 23 years at 4 of the 5 monitoring sites established in coastal areas of Katmai National Park and Preserve and one site in Kenai Fjords National Park.

Coincident with the retention of the oil mass, the distribution of oiling intensities and the location of the oil on the beach has not changed. The frequency distribution of LOR, MOR and HOR residues has remained constant since 2001 (Short et al., 2007, 2006, 2004, Taylor and Reimer, 2008, Boehm et al., 2008). There is remarkable similarity among all the previous surveys. Together they indicate an average of 62% of the residues encountered are LOR, 26% MOR and 11% are HOR. Similarly, surveys conducted in 2002 (Taylor and Reimers, 2008), 2003 (Short et al., 2006) and in 2015 found oil distributed the same way with respect to beach height. Oil is most frequently located in the middle of the intertidal and the frequency of encounter decreases symmetrically with increasing or decreasing tidal elevation from the middle tidal elevation. Surveys conducted in 2001 (Short et al., 2004) and 2007 (Boehm et al., 2008) did

not survey the same beach intervals; the lowest two tidal sections were missed in the former and the highest tidal section in the latter surveys. Regardless, their results are consistent with the general pattern.

The apparent absence of weathering since 2001 provides further evidence for the retention of sequestered oil mass. Our estimates of the proportions of TPAH and *n*-alkanes remaining relative to 11 d mousse are somewhat less than those observed in 2001, which averaged ~25 % and ~12%, respectively (Short et al. 2007). Conversely, Short et al. (2004) reported a median weathering index, *w*, equal to 3.3 (range: 0.94-12.1) for the 2001 survey, a value somewhat higher than 2.9, the median observed in 2015. In addition, estimates of the mass of oil per unit area associated with different residue types is nearly identical to 2001 estimates (Short et al. 2004). Most telling, the small reduction in the percentage of *n*-alkanes remaining since 2001 argues for low rates of biodegradation. Boehm et al. (2008) argued that alkanes had declined to an average 1% in sediments surveyed in 2007. However, their alkane concentrations were compared with those of oil in the cargo hold, which are close to 60% of Alaska North Slope crude (Fingas, 2010), much of which was lost to the atmosphere by evaporation during the first few hours after release. In contrast, alkanes comprised about 6.5% of the 11 d mousse. Comparisons of alkanes in sediments with cargo oil would amplify weathering effects.

The constant area and mass of the oil, the lack of any change in its physical location, the consistent distribution of oiling intensities and the absence of weathering all demonstrate that oil has changed little since it made landfall. Statistically, the surveys conducted since 2001 are all sampling oil from the same distributions. Consequently, the differences in the composition between LOR and HOR are the result of weathering before sequestration. Differences in composition led Boehm et al. (2008) to erroneously conclude that LOR was weathering more rapidly than HOR. However, they did not have the benefit of comparing the compositions over time. We cannot make direct comparisons between weathering states with the Boehm survey because they measured the percent PAH remaining in comparison with cargo oil. However, the percentage of PAH remaining relative to cargo oil in the LOR and MOR residues they encountered (14% and 20%, respectively) are within the confidence intervals presented here.

Sequestration from reworking of sediments by hydrologic forces and nutrient depletion have been described as likely reasons for the persistence of oil on coarse-grained beaches (Owens et al., 2008; Venosa et al., 2010). Natural processes that remove oil from beach environments include physical removal and microbial degradation. Factors hindering physical removal of oil in PWS include low exposure to wave action (Hayes, 1996), armoring by coarse substrates (Hayes et al., 2010; Hayes and Michel, 1999), and protective small-scale geomorphic features (Michel et al., 2016). Some of the beaches sampled here consist of two layers of sediment: a permeable upper layer and less permeable lower layer. The oil is sequestered in the lower less permeable layer (Bobo et al., 2012, Boufadel et al., 2016), which limits physical removal. The conservation of *n*-alkanes since 2001 indicates little biological degradation has occurred. This is explained by the observations that oiled beaches in PWS have low dissolved oxygen levels in subsurface layers, which significantly slow microbial degradation by orders of magnitude (Guo et al., 2010; Li and Boufadel, 2010; Xia and Boufadel, 2011).

4.2. *Estimates of subsurface oil*

The comparison of oil area and weight for a given site after 10 years (2005-2015) revealed no change. These findings were expected due to similar results reported by previous surveys in PWS (Michel et al., 2010; Short et al., 2007). Two beaches appeared to be inconsistent with this finding, Eleanor Island

segment EL058B where oil significantly increased in area and Evans Island segment EV039A where subsurface oil was not found (Evans Is., EV039A). However the results from these sites are consistent with model predictions for oil encounter rates (Nixon and Michel, 2015) and no difference was detected in the estimated oil mass on the Eleanor segment. Increases have also been reported on other surveys (Short et al., 2007). These inconsistencies are the result of sampling error. For example, the probability of encountering oil on segment EV039A was 1-5% and we excavated 50 pits. Thus we were likely to see oil in no more than two of the pits.

Sequestration of the subsurface oil limits its bioavailability, but the lack of weathering indicates that subsurface oil maintains some level of toxic potential. Subsurface oils collected in 2015 have enriched concentrations of phenanthrenes and chrysenes relative to oil in the cargo hold, indicating that buried oil has retained some toxic potential over the last two decades. While acute narcotic effects from BTEX (benzene, toluene, ethylbenzene, xylene) compounds were possible in the early days of the spill, these compounds are not present in the sediments (Wolfe et al., 1996). Consequently the buried oil is unlikely to cause acute toxicity (Page et al., 2002). However, toxic effects of PAH are known to result from other mechanisms, particularly following exposure to environmentally persistent PAHs such as alkylated phenanthrenes (Heintz et al., 2000, Hicken et al., 2011; Incardona et al., 2015, 2013, 2009; Jeong et al., 2015; Ji et al., 2011). Similarly, toxic levels of PAH were found in salt marsh sediments 21 years after being contaminated with No. 2 fuel oil from the Bouchard 65 spill (Peacock et al., 2007). Buried No. 2. fuel oil spilled from the barge Florida in Buzzards Bay, MA has remained toxic for decades (Culbertson et al. 2002). The slow weathering rate of the sequestered oil suggests bioavailability is limited. This is further indicated by the recent recovery of harlequin ducks and sea otters from the spill (Ballachey et al., 2014; Esler et al., 2016) where recovery was determined by evidence demonstrating that these animals were no longer exposed to PAHs.

4.3. *Annualized retention rate of subsurface oil*

Annualized retention rates of oil indicate that neither oil area nor mass has declined over the last 14 years. The slightly decreasing trend in oil retention suggests loss of subsurface oil may eventually be detected, but since the oil first made landfall 26 years ago it is clear that oil persistence will be on the order of decades. It is important to note that the uncorrected loss rates (1 – retention), which correspond to the approach outlined in Short et al. (2007), are even lower than those described here. The corresponding confidence intervals would also include $\Theta = 1$ (i.e. no change). Consideration of the rate at which oil was initially lost from beaches (Taylor and Reimer, 2007) and its persistence in later years indicates a two-phase loss process wherein some stranded oil is lost to hydrological washing and microbial degradation (Owens et al. 2008; Venosa 2010), but other oil is sequestered from these factors (Bobo et al., 2012; Boufadel et al., 2016) and remains unchanged over time. Following that model, we conclude that subsurface oil in PWS is currently in the sequestered phase and current attempts to estimate retention essentially serve to refine error estimates. The marginal ability to resolve differences in the weathering state between surveys is due to the high degree of variability in oil composition on a given beach segment. Any apparent loss or gain of oil at a site is most likely due to repeated site disturbance and re-excavation of oiled areas resulting in mobilization of the trapped oil and not natural weathering. For example, a history of re-excavation (Boehm et al., 2008; Michel et al., 2010; Short et al., 2007, 2006, 2004) and intensive experimental remediation (Boufadel and Bobo, 2011) are most likely the main causes for the Eleanor Island site (EL056C) to have a significant loss in subsurface oil between 2001 and 2015.

Consideration of the conditions under which spilled oil has shown long-term persistence indicates that sequestration of potentially toxic oils is a common feature of major oil spills. The coarse substrates of PWS are but one example. Other examples of subsurface oil in coarse substrates include a 36-year interval following the 1970 *Arrow* spill of Bunker C in Nova Scotia (Owens et al., 2008), and a 20-year interval following the Baffin Island oil spill experiment (Prince et al., 2002). However, coarse substrates are not a prerequisite for sequestration. The persistence of PAH has been described for 30-year-old diesel deposits in anoxic salt marsh sediments in Buzzards Bay, Massachusetts (Reddy et al., 2002), and intertidal marshes 22 years following the *Metula* spill (25-32% weathered) (Wang et al., 2001). While these cases, as with the *Exxon Valdez*, describe retention of masses that are only a small proportion of the initial amount spilled these observations indicate that when oil inundates a sufficiently large portion of shoreline the conditions necessary for long-term persistence will likely be encountered. Thus, decadal scale contamination of some shoreline segments with oil is likely to be an outcome of any major oil spill.

5. Conclusion

Surveys conducted on previously surveyed beaches in PWS revealed that an estimated 0.6% of oil spilled by the *Exxon Valdez* 26 years ago remains sequestered in subsurface sediments of PWS. Both the estimated area occupied by the oil and its mass have not changed since surveys conducted in 2001. Nor has there been any change in the distribution of oiling intensities or their location on the beach. Consequently, we are unable to detect any loss of oil from these beaches. The loss rate estimates demonstrate that most of the oil available for natural cleaning is gone and residual oil is protected from these natural processes. Hence, efforts to measure sequestered oil amount to measuring the inherent error. Coincident with the retention of bulk oil is our observation that there has been negligible change in its composition. In addition, there is no evidence of increased weathering in the lower intertidal. These observations suggest that sequestration limits the bioavailability of the oil despite the fact that it still retains toxic compounds. Viewing this survey in the context or previous surveys makes it clear that claims made after the spill that beaches would clean themselves (Boehm et al., 1995; Page et al., 1995) were overly optimistic.

Acknowledgments

The research described in this paper was supported by the *Exxon Valdez* Oil Spill Trustee Council. However, the findings and conclusions presented by the authors are their own and do not necessarily reflect the views or position of the Trustee Council.

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2015. Oil areas are reported in meters squared and oil weight in kilograms estimated for the whole site. 95% confidence intervals are presented.

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Table 1.

	Location Name	Shore Segment	Initial Oiling	Oil Surveys Excavation History	Most Recent Oil Class	Shore Type Prone to Persistent Oil
1	Smith Is.	SM006B	Heavy 1990-93	1989-92[1], 2001[3], 2008[4]	HOR	armored
2	Eleanor Is.	EL056C	Medium 1990-93	2001[3], 2007[4]	MOR	rubble accumulation
3	Eleanor Is.	EL058B	Heavy 1989	2001[3], 2005[3]	MOR	breakwater
4	Latouche Is.	LA018A-1	Heavy 1990-93	1989-92[1], 2001[3], 2005[3]	HOR	rubble, slope
5	Green Is.	GR103B	Heavy 1990-93	2001[3], 2005[3], 2007[4]	HOR	armored, slope
6	Evans Is.	EV039A	Heavy 1990-93	1993[2], 2005[3]	MOR	edge effect
7	Knight Is.	KN0114A	Heavy 1990-93	2003[3]	HOR	breakwater
8	Knight Is.	KN0300A-2	Medium 1990-93	1993[2], 2005[3]	MOR	breakwater
9	Knight Is.	KN0506A	Heavy 1990-93	2001[3], 2005[3]	LOR	edge effect

Note: excavation history - [1] NOAA Hazmat (now Office of Response & Restoration), [2] EVOSTC Gibeaut et al., 1998, [3] NOAA Auke Bay Laboratories, [4] Nixon and Michel, 2015.

Table 2.

Shore Segment	Length (m)	Estimated Area (m^2)	Total # of Pits Sampled	# of Pits LOR	# of Pits MOR	# of Pits HOR	2015 Probability of Encountering SSO	2008 Model Heavy SSO
SM006B	100	5,488	50	4	0	2	20%	>30%
EL056C	90	2,594	50	4	9	3	40%	>30%
EL058B	51	1,892	30	3	0	1	33%	>30%
LA018A-1	100	3,132	50	1	-	-	2%	5-15%
GR103B	100	4,398	50	1	2	1	12%	1-5%
EV039A	109	4,809	50	-	-	-	0%	1-5%
KN0114A	68	2,676	40	6	-	-	23%	>30%
KN0300A-2	52	2,076	40	-	1	-	3%	1-5%
KN0506A	50	1,960	40	-	-	1	3%	0-1%
Totals	720	29,025	400	19	12	8		

Table 3.

Shore Segment	*w*	% PAHs remaining	% n-alkanes remaining	% ∑Naph	% ∑Fluor	% ∑Dibenzo	% ∑Phenan	% ∑Chrys
EL056C	3.1 ± 1.7	17.8 ± 8.7	2.7 ± 3.1	13.8 ± 7.2	12.2 ± 4.6	27.2 ± 9.1	20.8 ± 5.9	7.8 ± 7.1
EL058B	2.8 ± .9	20.7 ± 3.3	1.7 ± 0.4	18. ± 4.7	12.6 ± .7	29. ± 2.2	24.6 ± 3.1	4.7 ± 0.8
GR103B	2.3 ± 1.9	17.8 ± 9.5	5.7 ± 5.7	14.6 ± 3.4	9.6 ± 2.6	25.3 ± 5.9	25.4 ± 9.1	6.8 ± 3.9
KN0114A	2.7 ± 1.8	19.7 ± 6.7	8.1 ± 6.8	15.8 ± 7.1	11.6 ± 1.9	29.8 ± 2.8	23.6 ± 5.3	5.5 ± 2.6
KN0300A2	3.3	10.2	9.7	12.2	9.4	20.8	19.4	9.3
KN0506A	0	94.4	8.0	43.6	16.3	11.4	21.8	1.0
LA018A-1	6.3	7.6	5.1	4.8	6.9	20.8	24.9	12.4
SM006B	3.5 ± 2.3	20. ± 9.1	6.3 ± 10.7	23.0	10.1 ± 4.5	26. ± 6.2	19.6 ± 4.3	6.1 ± 3.7

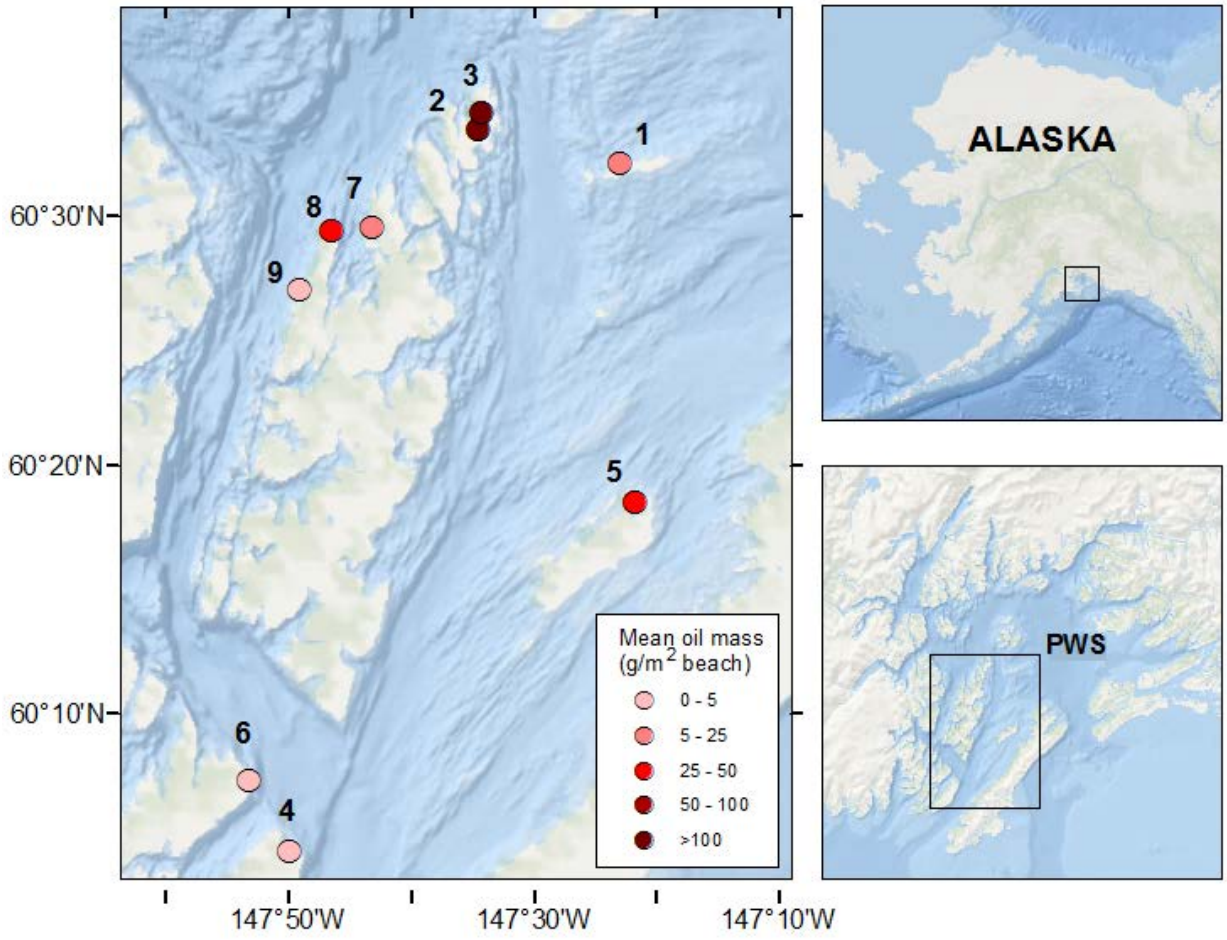
ALASKA
PWS
1
2
3
4
5
6
7
8
9
60°30'N
60°20'N
60°10'N
147°50'W
147°30'W
147°10'W
Mean oil mass
(g/m2 beach)
0 - 5
5 - 25
25 - 50
50 - 100
>100

Figure 1

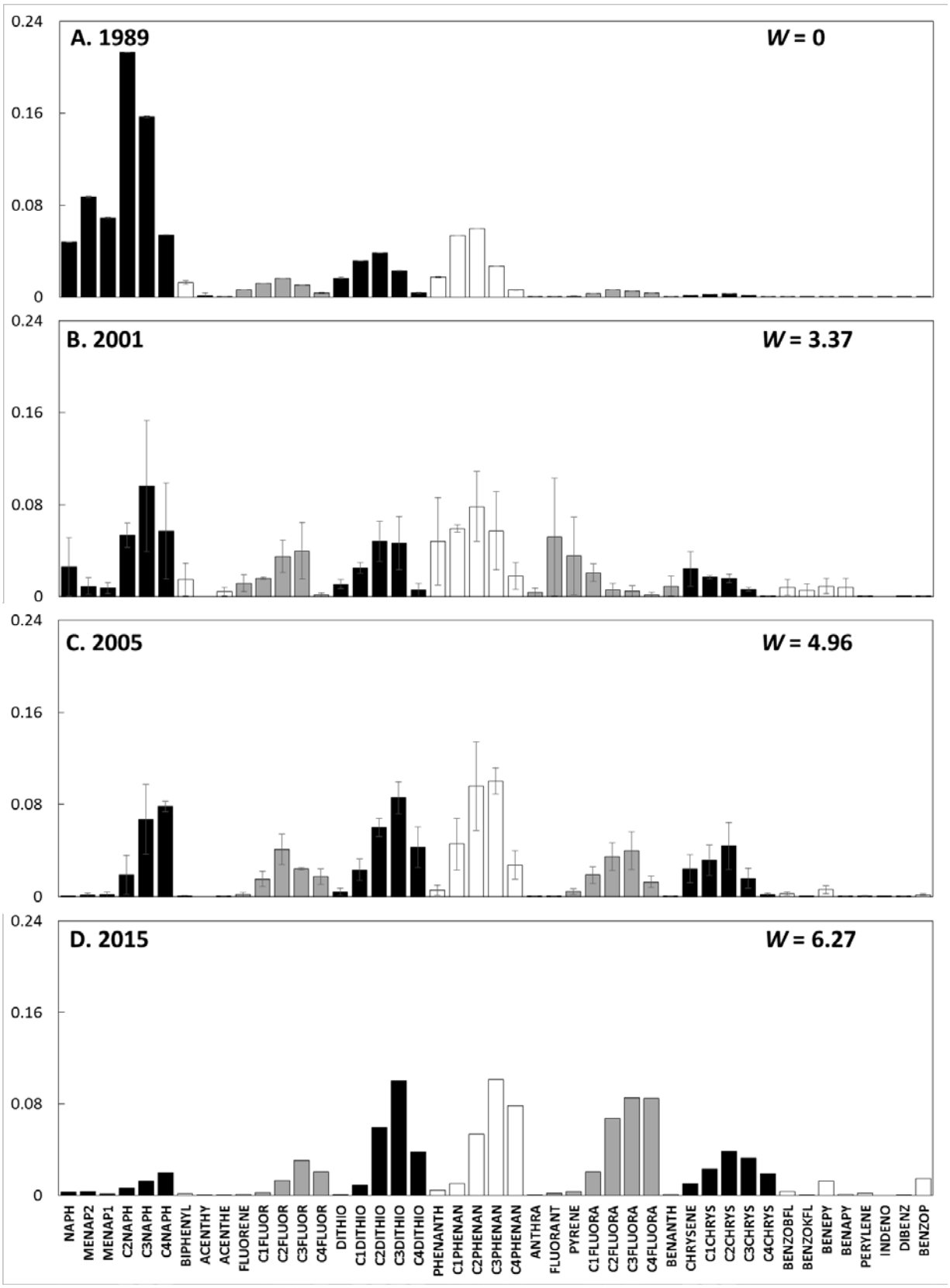
A. 1989
W = 0
B. 2001
W = 3.37
C. 2005
W = 4.96
D. 2015
W = 6.27
0.24
0.16
0.08
0
NAPH
MENAP2
MENAP1
C2NAPH
C3NAPH
C4NAPH
BIPHENYL
ACENTHY
ACENTHE
FLUORENE
C1FLUOR
C2FLUOR
C3FLUOR
C4FLUOR
DITHIO
C1DITHIO
C2DITHIO
C3DITHIO
C4DITHIO
PHENANTH
C1PHENAN
C2PHENAN
C3PHENAN
C4PHENAN
ANTHRA
FLUORANT
PYRENE
C1FLUORA
C2FLUORA
C3FLUORA
C4FLUORA
BENANTH
CHRYSENE
C1CHRYS
C2CHRYS
C3CHRYS
C4CHRYS
BENZOBFL
BENZOKFL
BENEPY
BENAPY
PERYLENE
INDENO
DIBENZ
BENZOP

Figure 2

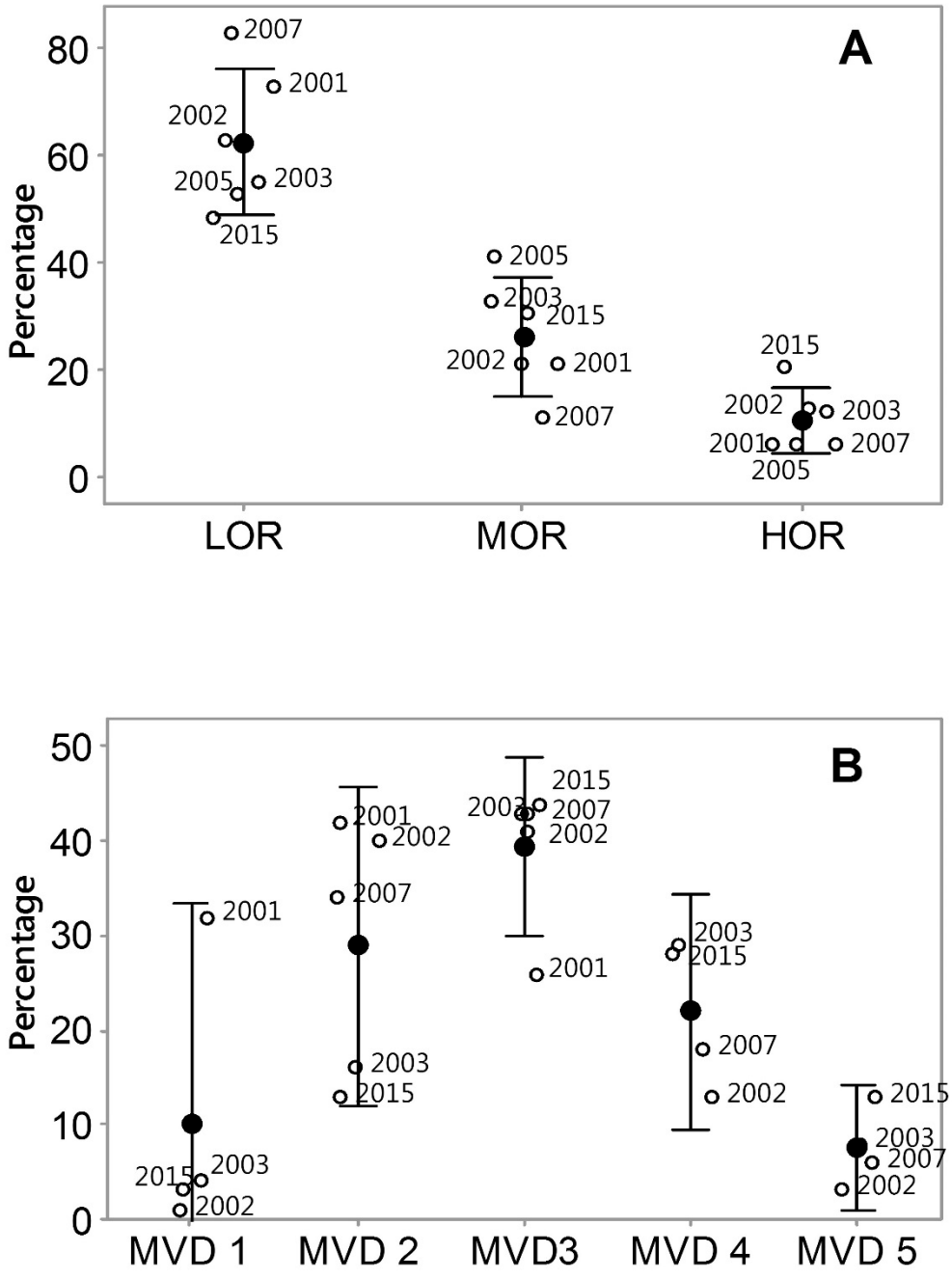
A
Percentage
80
60
40
20
0
2007
2001
2002
2005
2003
2015
2005
2003
2015
2002
2001
2007
2015
2002
2003
2001
2007
2005
LOR
MOR
HOR
B
Percentage
50
40
30
20
10
0
2001
2002
2007
2003
2015
2015
2003
2007
2002
2001
2003
2015
2007
2002
2015
2003
2007
2002
2001
2003
2015
2002
MVD 1
MVD 2
MVD3
MVD 4
MVD 5

Figure 3

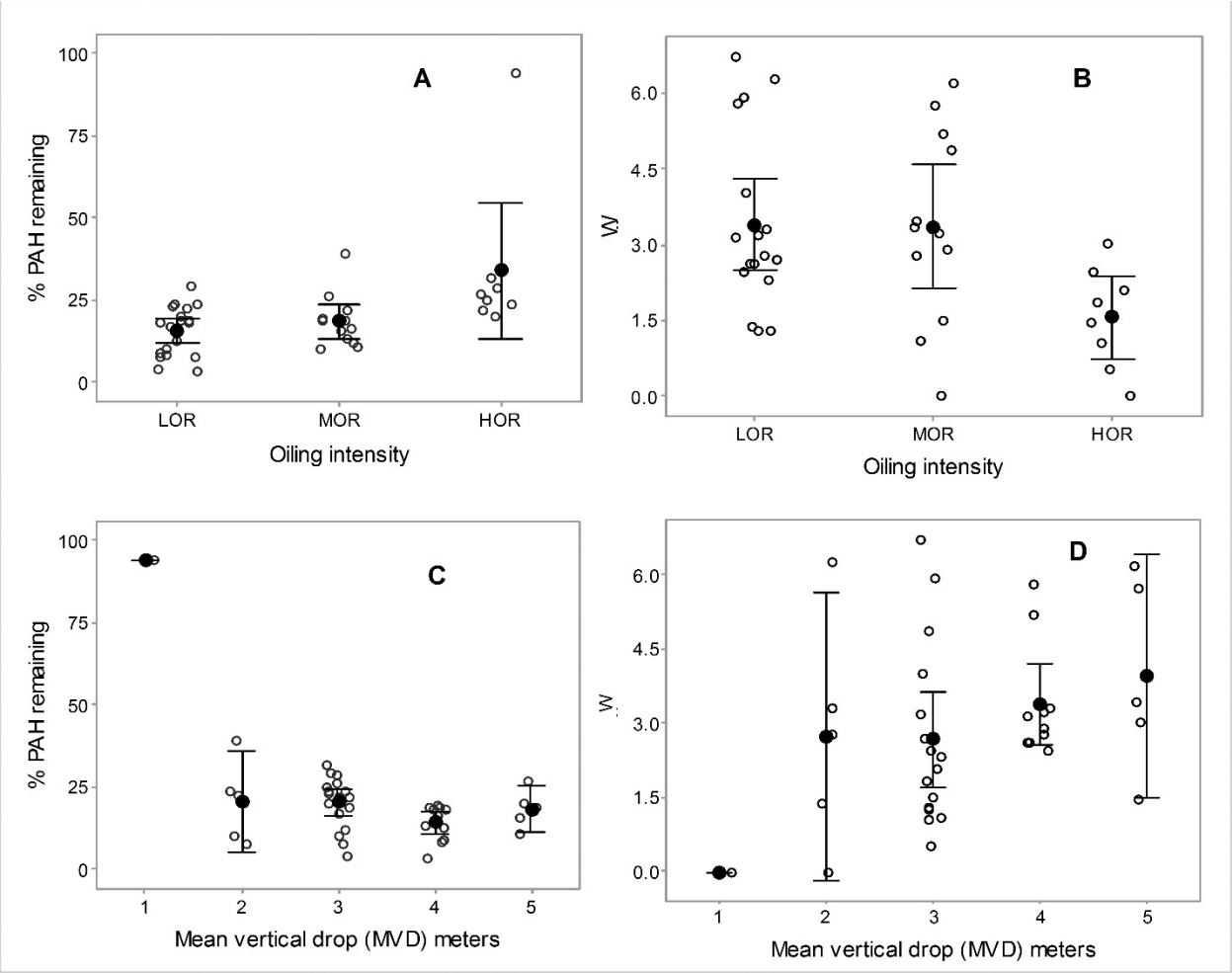
A
% PAH remaining
100
75
50
25
0
LOR
MOR
HOR
Oiling intensity
B
W
6.0
4.5
3.0
1.5
0.0
LOR
MOR
HOR
Oiling intensity
C
% PAH remaining
100
75
50
25
0
1
2
3
4
5
Mean vertical drop (MVD) meters
D
W
6.0
4.5
3.0
1.5
0.0
1
2
3
4
5
Mean vertical drop (MVD) meters

Figure 4

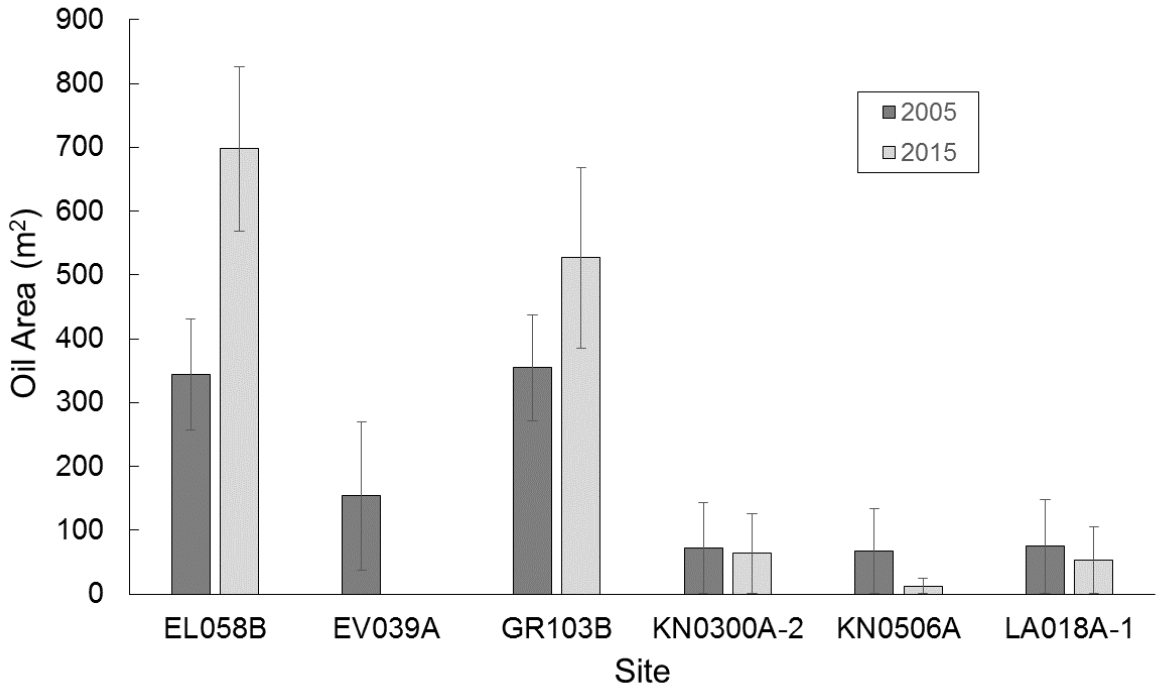
900
800
700
600
500
400
300
200
100
0
Oil Area (m2)
2005
2015
EL058B
EV039A
GR103B
KN0300A-2
KN0506A
LA018A-1
Site

Figure 5

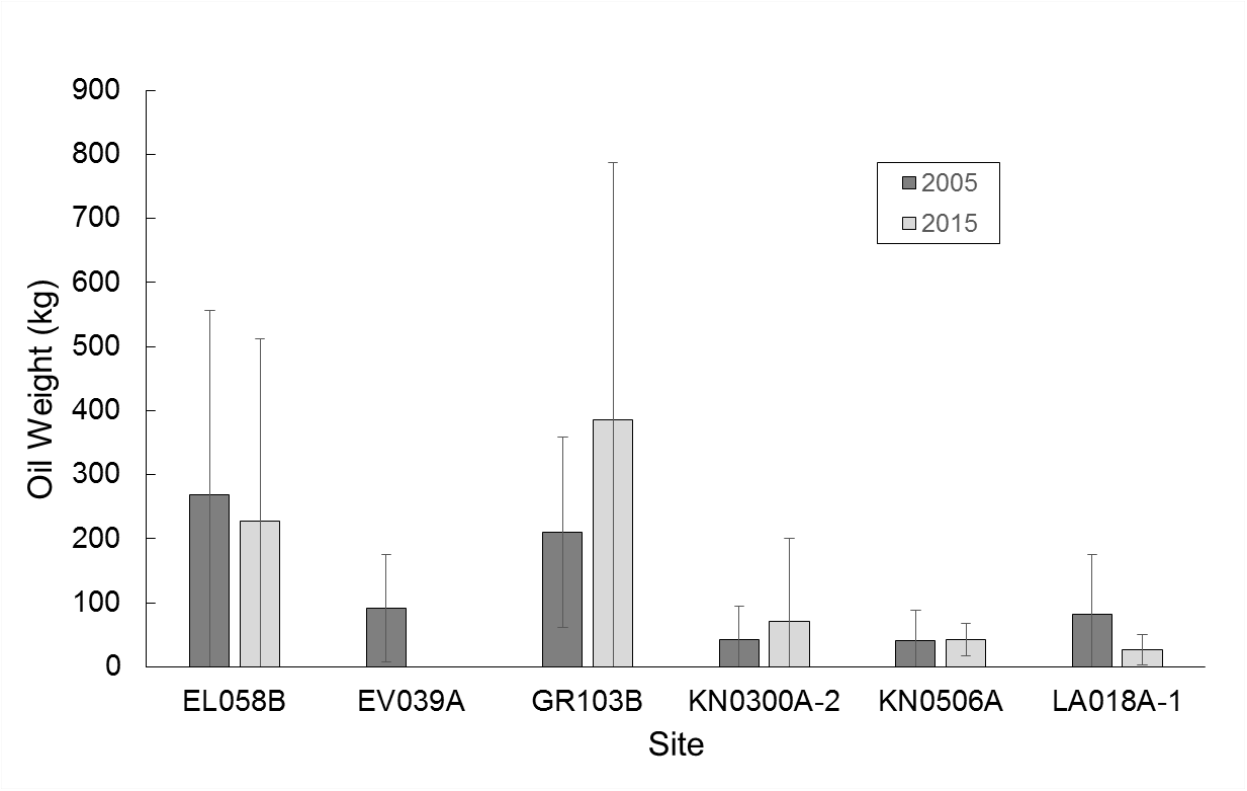
900
800
700
600
500
400
300
200
100
0
Oil Weight (kg)
2005
2015
EL058B
EV039A
GR103B
KN0300A-2
KN0506A
LA018A-1
Site

Figure 6

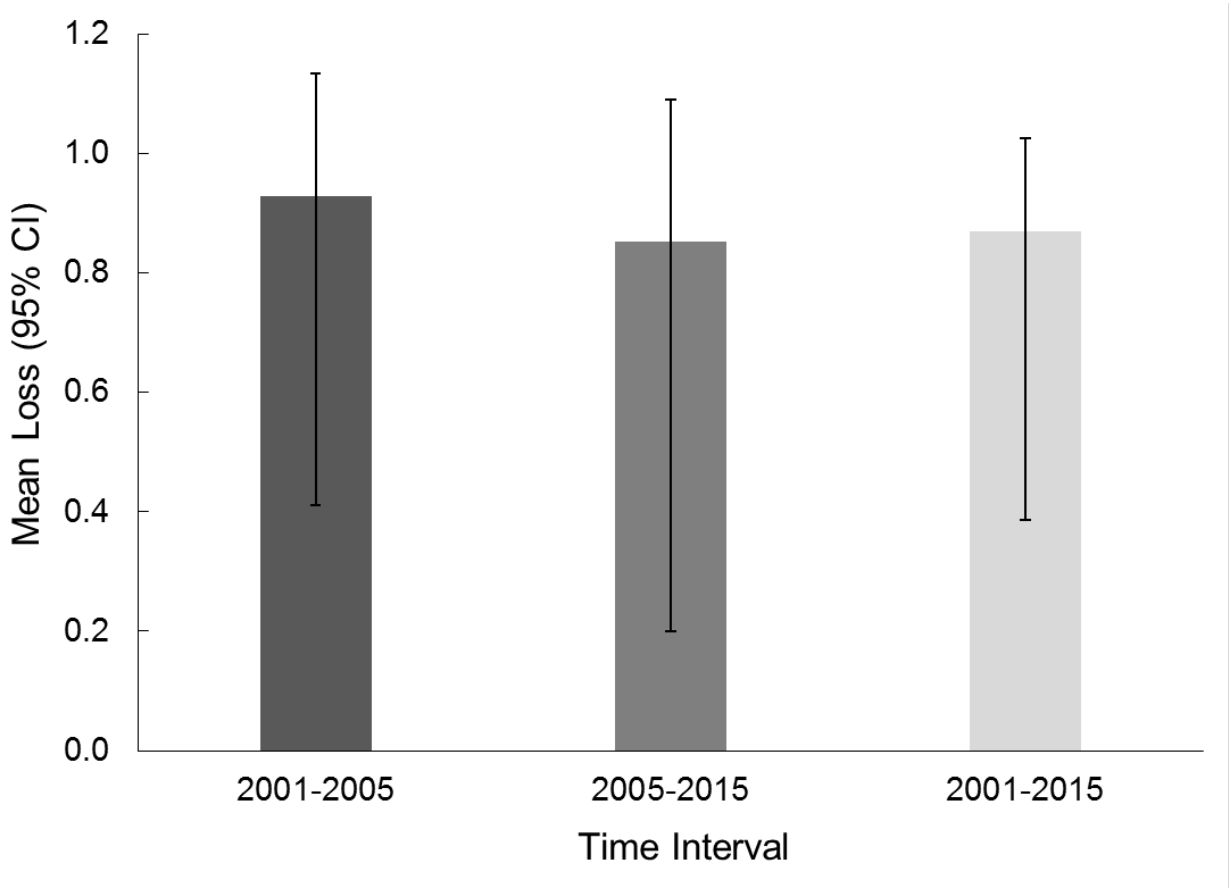
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Mean Loss (95% CI)
2001-2005
2005-2015
2001-2015
Time Interval

Figure 7

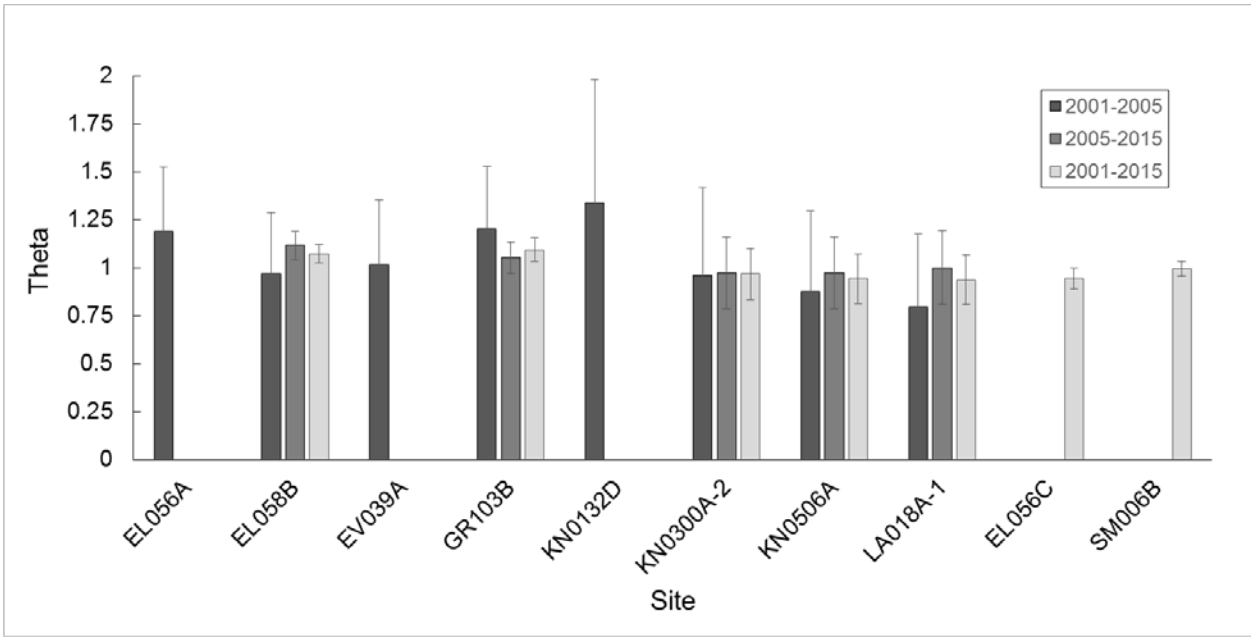
2
1.75
1.5
1.25
1
0.75
0.5
0.25
0
Theta
2001-2005
2005-2015
2001-2015
EL056A
EL058B
EV039A
GR103B
KN0132D
KN0300A-2
KN0506A
LA018A-1
EL056C
SM006B
Site

Figure 8

APPENDIX C: SOP - ANALYSIS OF PETROLEUM HYDROCARBONS

Standard Operating Procedures for the Analysis of Petroleum Hydrocarbons in Seawater, Sediments, Tissue, and Passive Samplers at the Auke Bay Laboratory

by

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May 2016

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INTRODUCTION

The standard operating procedures (SOPs) in this document describe the methods used at the Auke Bay Laboratories (ABL) for analysis of petroleum hydrocarbons in environmental and experimental samples. Samples of marine sediments, seawater, and tissues of various marine biota have been collected to assess the extent of resulting damages to natural resources, to assess the persistence of oil in the environment, and to estimate toxic effects on living marine resources. In addition, passive samplers have been deployed to understand the biological availability of polynuclear aromatic hydrocarbons (PAHs). Passive samplers are low-density polyethylene membrane devices (PEMDs); the plastic central to these devices is the low-density polyethylene (LDPD) and this is the name generally used in the chemistry laboratory. However, when reporting to the database, use PEMD.

The purpose of these SOPs is to provide a complete description of the methods used to analyze these samples for petroleum hydrocarbons, to verify the quality of these analytical data, and to assure the integrity of samples during the analytical process. In addition, these SOP's include a detailed description of data analysis procedures, so that the final data reported may be traced to its origins.

These samples have been collected by several State of Alaska and federal agencies to provide defendable data and legal evidence and have therefore been collected using chain-of-custody procedures to verify the integrity of the samples. These chain-of-custody procedures are maintained through the analytical process and final data reporting, as described in section 2.

Environmental samples are analyzed at ABL, generally in batches of 13, and are analyzed together with quality assurance (QA) samples and calibration standards. One batch of environmental samples, QA samples, and standards is called a sample string. A description of the overall QA strategy is given in section 3.

The details of the analytical methods used to determine petroleum hydrocarbons are described in sections 4 through 12. These methods are adapted from, and in general are very similar to, methods described by the Geochemical and Environmental Research Group at Texas A&M University (1989) for the analysis of similar samples. These methods are summarized by (Short, Jackson et al. 1996). The petroleum hydrocarbons targeted by these methods are listed in Table 1.a.

Data analysis is described in section 13, includes both the calculations used and the computer programs that implement those calculations. Section 13 also includes a description of the computer programs that transfer data among data acquisition and processing stations within the analytical process. The QA criteria used to determine the acceptability of the analytical data produced is given in section 3.

The authors gratefully acknowledge helpful discussions with Dr. Terry Wade and Mr.

Donald Brown, whose advice and assistance facilitated the development and implementation of these SOPs.

Table 1.a
Targeted Petroleum Hydrocarbons

PAHs

Abbr	moleWt	PAH	Abbr	moleWt	PAH
N0	128.17	naphthalene	ANT	178.24	anthracene
N1	142.20	C-1 naphthalenes	FLU	202.26	fluoranthene
N2	156.23	C-2 naphthalenes	PYR	202.26	pyrene
N3	170.25	C-3 naphthalenes	FP1	216.28	C-1 fluoranthenes/pyrenes
N4	184.28	C-4 naphthalenes	FP2	230.31	C-2 fluoranthenes/pyrenes
BPH	154.21	biphenyl	FP3	244.34	C-3 fluoranthenes/pyrenes
ACN	152.21	acenaphthylene	FP4	258.35	C-4 fluoranthenes/pyrenes
ACE	154.21	acenaphthene	BAA	228.29	benzo(a)anthracene
F0	166.22	fluorene	C0	228.29	chrysene
F1	180.25	C-1 fluorenes	C1	242.32	C-1 chrysenes
F2	194.27	C-2 fluorenes	C2	256.34	C-2 chrysenes
F3	208.30	C-3 fluorenes	C3	270.36	C-3 chrysenes
F4	222.30	C4 fluorenes	C4	284.38	C-4 chrysenes
D0	184.20	dibenzothiophene	BBF	252.32	benzo(b)fluoranthene
D1	198.30	C-1 dibenzothiophenes	BKF	252.32	benzo(k)fluoranthene
D2	212.30	C-2 dibenzothiophenes	BEP	252.31	Benzo(e)pyrene
D3	226.30	C-3 dibenzothiophenes	BAP	252.31	Benzo(a)pyrene
D4	240.30	C4 dibenzothiophenes	PER	252.32	Perylene
P0	178.23	phenanthrene	ICP	276.34	indeno(1,2,3-cd)pyrene
P1	192.26	C-1 phenanthrenes/anthracenes	DBA	278.35	dibenzo(a,h)anthracene
P2	206.29	C-2 phenanthrenes/anthracenes	BZP	276.34	benzo(ghi)perylene
P3	220.32	C-3 phenanthrenes/anthracenes			
P4	234.34	C-4 phenanthrenes/anthracenes			

Alkanes

Abbr	Formula	moleWt	Name
C9ALK	C_9H_{20}	128.26	C9- (n-Nonane)
C10ALK	$C_{10}H_{22}$	142.29	C10- (n-DECANE)
C11ALK	$C_{11}H_{24}$	156.31	C11- (n-UNDECANE)
C12ALk	$C_{12}H_{26}$	170.34	C12- (n-DODECANE)
C13ALK	$C_{13}H_{28}$	184.37	C13- (n-TRIDECANE)
C14ALK	$C_{14}H_{30}$	198.40	C14- (n-TETRADECANE)
C15ALK	$C_{15}H_{32}$	212.42	C15- (n-PENTADECANE)
C16ALK	$C_{16}H_{34}$	226.45	C16- (n-HEXADECANE)
C17ALK	$C_{17}H_{36}$	240.48	C17- (n-HEPTADECANE)
PRIS	$C_{19}H_{40}$	268.53	PRISTANE
C18ALK	$C_{18}H_{38}$	254.50	C18- (n-OCTADECANE)
PHY	$C_{20}H_{42}$	282.56	PHYTANE
C19ALK	$C_{19}H_{40}$	268.53	C19- (n-NONADECANE)
C20ALK	$C_{20}H_{42}$	282.56	C20- (n-EICOSANE)
C21ALK	$C_{21}H_{44}$	296.58	C21- (n-HENEICOSANE)
C22ALK	$C_{22}H_{46}$	310.61	C22- (n-DOCOSASNE)
C23ALK	$C_{23}H_{48}$	324.64	C23- (n-TRICOSANE)
C24ALK	$C_{24}H_{50}$	338.67	C24- (n-TETRACOSANE)
C25ALK	$C_{25}H_{52}$	352.69	C25- (n-PENTACOSANE)
C26ALK	$C_{26}H_{54}$	366.72	C26- (n-HEXACOSANE)
C27ALK	$C_{27}H_{56}$	380.75	C27- (n-HEPTACOSANE)
C28ALK	$C_{28}H_{58}$	394.77	C28- (n-OCTACOSANE)
C29ALK	$C_{29}H_{60}$	408.80	C29- (n-NONACOSANE)
C30ALK	$C_{30}H_{62}$	422.83	C30- (n-TRIACONTANE)
C31ALK	$C_{31}H_{64}$	436.86	C31- (n-Hentriacontane)
C32ALK	$C_{32}H_{66}$	450.88	C32- (n-DOTRIACONTANE)
C33ALK	$C_{33}H_{68}$	464.91	C33- (nTritriacontane)
C34ALK	$C_{34}H_{70}$	478.94	C34- (n-TETRATRIACONTANE)
C35ALK	$C_{35}H_{72}$	492.96	C35- (n-Pentatriacontane)
C36ALK	$C_{36}H_{74}$	506.99	C36- (n-Hexatriacontane)

Biomarkers

Abbrev	Formula	Mol wt	Target Ions	Name
Norpristt	$C_{18}H_{38}$	254.494	57	2,6,10-trimethylpentadecane
Prist	$C_{19}H_{40}$	268.521	57	2,6,10,14-tetramethylpentadecane
Phyt	$C_{20}H_{42}$	282.547	57	2,6,10,14-tetramethylhexadecane
TR23	$C_{23}H_{42}$	318.580	191	C_{23} tricyclic terpane
TR24	$C_{24}H_{44}$	332.606	191	C_{24} tricyclic terpane
TR25a	$C_{25}H_{46}$	346.633	191	C_{25}(a) tricyclic terpane
TR25b	$C_{25}H_{46}$	346.633	191	C_{25}(b) tricyclic terpane
TET24	$C_{24}H_{42}$	330.590	191	C_{24} tetracyclic terpane
TR26a	$C_{26}H_{48}$	360.659	191	C_{26}(a) tricyclic terpane
TR26b	$C_{26}H_{48}$	360.659	191	C_{26}(b) tricyclic terpane
TR28a	$C_{28}H_{52}$	388.712	191	C_{28}(a) tricyclic terpane
TR28b	$C_{28}H_{52}$	388.712	191	C_{28}(b) tricyclic terpane
TR29a	$C_{29}H_{54}$	402.739	191	C_{29}(a) tricyclic terpane
TR29b	$C_{29}H_{54}$	402.739	191	C_{29}(b) tricyclic terpane
Ts	$C_{27}H_{46}$	370.654	191	18α(H)-22,29,30-trisnorneohopane
Tm	$C_{27}H_{46}$	370.654	191	17α(H)-22,29,30-trisnorhopane
H28	$C_{28}H_{48}$	384.681	191, 163	17α(H),18α(H),21β(H)-28,30-bisnorhopane
NOR25H	$C_{29}H_{50}$	398.707	191, 177	17α(H),21β(H)-25-norhopane
H29	$C_{29}H_{50}$	398.707	191	17α(H),21β(H)-30-norhopane
C29Ts	$C_{29}H_{50}$	398.707	191	18α(H),21β(H)-30-norneohopane
M29	$C_{29}H_{50}$	398.707	191	17α(H),21β(H)-30-norhopane
OL	$C_{30}H_{52}$	412.734	191, 412	18α(H) & 18β(H)-oleanane
H30	$C_{30}H_{52}$	412.734	191	17α(H),21β(H)-hopane
NOR30H	$C_{30}H_{52}$	412.734	191	17α(H),21β(H)-30-nor-29-homohopane
M30	$C_{30}H_{52}$	412.734	191	17β(H),21α(H)-hopane
H31S	$C_{31}H_{54}$	426.760	191	22S-17α(H),21β(H)-30-homohopane
H31R	$C_{31}H_{54}$	426.760	191	22R-17α(H),21β(H)-30-homohopane

GAM	$C_{30}H_{52}$	412.734	191, 412	Gammacerane
H32S	$C_{32}H_{56}$	440.787	191	22S-17α(H),21β(H)-30,31-bishomohopane
H32R	$C_{32}H_{56}$	440.787	191	22R-17α(H),21β(H)-30,31-bishomohopane
H33S	$C_{33}H_{58}$	454.814	191	22S-17α(H),21β(H)-30,31,32-trishomohopane
H33R	$C_{33}H_{58}$	454.814	191	22R-17α(H),21β(H)-30,31,32-trishomohopane
H34S	$C_{34}H_{60}$	468.840	191	22S-17α(H),21β(H)-30,31,32,33-tetrakishomohopane
H34R	$C_{34}H_{60}$	468.840	191	22R-17α(H),21β(H)-30,31,32,33-tetrakishomohopane
H35S	$C_{35}H_{62}$	482.867	191	22S-17α(H),21β(H)-30,31,32,33,34-pentakishomohopane
H35R	$C_{35}H_{62}$	482.867	191	22R-17α(H),21β(H)-30,31,32,33,34-pentakishomohopane
S22	$C_{22}H_{38}$	302.537	217, 218	C_{22} 5α(H),14β(H),17α(H)-sterane
DIA27S	$C_{27}H_{48}$	372.670	217, 218, 259	C_{27} 20S-13β(H),17α(H)-diasterane
DIA27R	$C_{27}H_{48}$	372.670	217, 218, 259	C_{27} 20R-13β(H),17α(H)-diasterane
C27S	$C_{27}H_{48}$	372.670	217, 218	C_{27} 20S-5α(H),14α(H),17α(H)-cholestane
C27BBR	$C_{27}H_{48}$	372.670	217, 218	C_{27} 20R-5α(H),14β(H),17β(H)-cholestane
C27BBS	$C_{27}H_{48}$	372.670	217, 218	C_{27} 20S-5α(H),14β(H),17β(H)-cholestane
C27R	$C_{27}H_{48}$	372.670	217, 218	C_{27} 20R-5α(H),14α(H),17α(H)-cholestane
C28S	$C_{28}H_{50}$	386.697	217, 218	C_{28} 20S-5α(H),14α(H),17α(H)-ergostane
C28BBR	$C_{28}H_{50}$	386.697	217, 218	C_{28} 20R-5α(H),14β(H),17β(H)-ergostane
C28BBS	$C_{28}H_{50}$	386.697	217, 218	C_{28} 20S-5α(H),14β(H),17β(H)-ergostane
C28R	$C_{28}H_{50}$	386.697	217, 218	C_{28} 20R-5α(H),14α(H),17α(H)-ergostane
C29S	$C_{29}H_{52}$	400.723	217, 218	C_{29} 20S-5α(H),14α(H),17α(H)-stigmastane
C29BBR	$C_{29}H_{52}$	400.723	217, 218	C_{29} 20R-5α(H),14β(H),17β(H)-stigmastane

C29BBS	$C_{29}H_{52}$	400.723	217, 218	C_{29} 20S-5α(H),14β(H),17β(H)-stigmastane
C29R	$C_{29}H_{52}$	400.723	217, 218	C_{29} 20R-5α(H),14α(H),17α(H)-stigmastane

1. Field Preparation

1.1 Preparation of passive samplers (LDPEs = PEMDs) prior to deployment

This section describes low-density polyethylene (LDPE) devices. It does not describe procedures used for semi-permeable membrane devices (SPMDs). SPMDs were used briefly at ABL but have not been used for many years.

Before field or experimental deployment, passive samplers must be constructed, cleaned, and placed in suitable housings.

1.1.1. From the low density polyethylene (LDPE) tubing roll, cut 0.5 meter long pieces and cut lengthwise to produce a 0.5 m by 2" strip. (Clip one end of the 0.5 m piece to a board for easier cutting.) Heat seal both ends into a loop for placement into the device. The outside edge of the loop ends should be 43.2 cm apart with a 3 cm fold for each loop. Use the premeasured board in Lab 201 as a guide for cutting and heat sealing.

1.1.2. Place a maximum of 15 strips in one (1) liter of pentane in a sonic bath and clean with the following schedule:

15 min on
30 min off
15 min on
30 min off
15 min on

1.1.3. Immediately after the last sonication, rinse the strips with pentane as they are removed from the sonic bath, allow to briefly air dry, roll into tight circles using to long forceps, and place in a clean I-Chem jar with Al foil lining the lid.

1.1.4. Store in freezer @ −20°C until deployment. Record the number of LDPE strips and date prepared in the LDPE Logbook.

1.2 Loading the Pucks

1. Prior to being loaded, pucks need to be HC clean. Pucks returning from the field are soap and water washed using Dawn soap. Cleaned pucks undergo puck maintenance.

2. Maintained pucks are fully submerged and agitated in MeCl2 then allowed to air dry on clean Al foil.

3. Once HC clean, pucks are loaded with prepared LDPE strips. Remove an I-Chem jar from the freezer. Using HC clean long forceps, remove a strip from the jar. Hook one looped end onto the first prong in the puck then loosely weave the LDPE around the prongs (see diagram). Hook the second looped end around the final prong; this takes some finagling of the LDPE around the prongs.

4. Once the strip is in the puck, secure the lid of the puck using a power drill.

5. Wrap the puck in Al foil twice.

6. Place the wrapped puck in a zip lock bag and heat seal the bag. Repeat once more.

7. Store at -20° C until ready for deployment.

A successfully loaded puck: the first (F) and last (L) prongs are

1.3 Unloading the Pucks

1. Have I-Chem jars ready with pre-applied and labeled tape (1" wide). The labels should include SIN, project name, PI name, date, and location. The jar lid should be labeled with the SIN.

2. Remove the pucks from the freezer. Keep them in a cooler in the lab so that you can work on one puck at a time while the others stay cold.

3. Open the Ziploc bags and unwrap the puck from the Al foil. Keeping the puck on the Al, unscrew the top off the puck using a power drill with a Phillips head drill bit.

4. In a timely manner, remove the LDPE strip from the puck using HC clean forceps. Scrape off any sediment or biota from the strip. Using two long forceps, roll into a tight circle and place in the labeled I-Chem jar. Store in the freezer until sample processing.

5. Between each puck, clean the forceps with soap and water, dry, then rinse with MeCl2.

1.4 Unloading a Clip Strip

Some LDPE strips are loaded on halibut clips. They are 0.7 m, thus longer than the usual 0.5 m strip, so instead of simply cutting them in half before extraction they need to be properly measured and cut to remove the extra LDPE material. This assures that the extracted HC will have comparable results with those from pucks (as in "parts per 0.25 m strip").

1. Ready your metal tools: 2 long forceps, two-finger prong (attached to the ring stand), 22 cm marker (on the ring stand), and scissors. Soap and water wash each tool, then thoroughly dry and rinse with MeCl2. Place on an unused piece of Al foil.

2. Have I-Chem jars ready with pre-applied and labeled tape (1" wide). The labels should include SIN, project name, PI name, date, and location. The jar lid should be labeled with the SIN.

3. Remove the clips from the freezer. Work only one clip at a time.

4. Open the Ziploc bags and unwrap the clip from the Al foil. In a timely manner, remove the LDPE strip from the clip using HC clean forceps. Scrape off any sediment or biota from the strip.

5. Place the two loops of the strip on the two-finger prong and pull the middle of the strip down past the 22 cm marker. Cut the strip at the level of the marker so that you have three pieces total: 2 long (each with a looped end) and one very short (the middle piece). Discard the short piece. Using the forceps, roll each long piece into tight circles and place in the labeled I-Chem jar. Store in the freezer until sample processing.

6. Between each clip, clean all the tools as described in Step 1.

2. Sample Tracking

Chain of custody documentation provides a recorded history of each sample from the date of TSMRI's receipt of the sample. The Chemistry Lab Data Sheet provides handling information regarding the analytical process. Each custody transfer is documented on chain of custody forms; an example chain of custody form follows as figure 2.a. Documentation includes the sample identification number(s) (SIN) of the samples involved in the transfer, the date and place of transfer, and the signatures of both parties involved in the custody transfer. Specific notes regarding the processing of a sample string are documented on the Chemistry Lab Data Sheet, figure 2.b. The chain of custody sheets provide an audit trail from which a sample's handling can be traced either to its origin or release.

SINs of each sample are assigned by a Sample Custodian and are transferred to each vessel in which the sample is contained throughout the entire analysis. This label maintains the integrity of each samples identity.

2.0 Sample tracking files

Files associated with sample tracking are in directory 2.0 Sample tracking. The purpose of these files is to provide data sheets for field and laboratory record-keeping and forms for data transfer to the hydrocarbon database. These include chain-of-custody (COC) files, chem lab data sheets, quality assurance (QA) spreadsheets, and hydrocarbon database (HCD) data entry files.

Samples cannot be accepted, tracked and processed without appropriate COC sheets and sample identification numbers (SINs). COC sheets and SINs are generated before field or laboratory collections begin (item 1 below). They must be completed and returned to the chem lab before any processing can begin. This information is carried forward from the field or lab COC sheets to batch-specific (sample "string") processing in the lab (item 2 below). Once the samples have been processed, data must be examined for quality (item 3 below). Collection data must be entered in the HCD data entry form before analytical data can be added to the hydrocarbon database (item 4 below). See Section 14 for preparation of analytical data for output to the hydrocarbon database.

Files

1. COC form 2015 with log.xlsx
    a. Chain of custody form with chain of custody log
    b. Assigns SINs to each specific project

2. Chem Lab Data Sheets.xlsx
    a. General hydrocarbon lab data form
    b. LDPE lab data form

3. QAsheet.xls

a. Quality assurance: guidelines for data acceptance

4. HCD data entry form.xlsx
    a. Data entry sheets for output to the hydrocarbon database must be completed before any analytical data is accepted for the database. This information provides sample type, collection time, location, and other details.

2.1 Receipt of Samples at ABL

The Chemistry Laboratory Manager (CLM) of ABL receives custody of the environmental samples from the Principle Investigator (PI) or collection party. The transfer is documented on chain of custody forms (see fig. 2.a.) with signatures of both parties acknowledging the transfer.

2.1.1 Samples are examined to insure a correct SIN is displayed.

2.1.2 Sample integrity is examined. A sample should be frozen and show no indication of decay. Sample containers should not be damaged and custody seal must not be broken.

2.1.3 Samples are stored in a freezer at -20° C until analysis.

2.2 Sample Processing

The CLM organizes samples in analysis batches of about 13 for processing. The transfer is documented on the chain of custody form (see fig. 2.b) with the signatures of both parties and with references to pertinent logbook pages.

2.2.1 Sediment and tissue samples are subsampled by the analyst under the supervision of the CLM; custody remains in with the CLM. Notes regarding the analysis are documented in the logbook of the pertinent analyst.

2.2.2 Water samples are not subsampled but are transferred in their entirety.

2.3 Data Analysis

Following sample processing, the analyst submits the sample extracts for instrumental analysis. References are documented in the instrument logs, and the extracts remain in the custody of the CLM.

2.4 Archival

Analytical data are processed (see section 13) then the final results are submitted to the DBM for entry into the data base.

2.4.1 The CLM submits the final data to the DBM and archives hardcopies of the data reports in secured filing cabinets. The chain of custody forms are archived with their corresponding sample string.

2.4.2 The unused portions of sample extract are archived in a 2 ml glass vial capped with a teflon-lined lid and stored in a freezer at -20° C until the principle investigator determines that no more data are required; the extracts are then discarded.

2.4.3 Raw data are archived on appropriate media, either magnetic tape or floppy disk, and archived under the custody of the instrument operator.

Figure 2.a

Example Chain of Custody Sheet
Receipt at ABL

N.M.F.S. Prince William Sound Oil Assessment Chain of Custody Form

R-2002-__ __ Project RCAC-LTEMP

Page 1 of 1

Serial # 13 0 3 9

NMFS Auke Bay Laboratory
11305 Glacier Highway
Juneau AK 99801

For information contact
Bonita Nelson
(907) 789-6071

Note: Use waterproof ink

Numbering scheme is as follows:assigned serial number of the sheet (13_ _ _) then individual sample number eg: 01 : these last two digits are typed on the sheet already in the 1st column, the serial number is directly above this box

Assigned Sample #	Collector's Sample code	Date Collected	Matrix & Species, organ, etc	Location Collected	Latitude	Longitude	Collection Method	Comments
01		9-July-02	Mussels	AMT-B	61°05.408'	146°24.486'	Handgrab	Rep1-A
02			"	"	~~146°24.486~~	"	"	Rep1-B
03			"	"	"	"	"	Rep2-A
04			"	"	"	"	"	Rep2-B X +
05			"	"	"	"	"	Rep3-A
06			"	"	"	"	"	Rep3-B
07		10 July	Field Blank	AMT-S	61°05.415'	146°23.578'	Field Blank	—
08		"	Sediment	"	61°05.416'	146°23.584'	Van Veen	Rep2 68m
09		"	"	"	61°05.415'	146°23.578'	Grab	Rep3 64m
10		" X	"	"	61°05.404'	146°23.577'	"	Rep1 65m
11		"	Field Blank	GOC-S	61°07.473'	146°29.444'	Field Blank	—
12		" X	Sediment	"	61°07.489'	146°29.414	Van Veen grab	Rep1 39m
13		"	"	"	61°07.469'	146°29.442	"	Rep2 32m
14		"	"	"	61°07.473	146°29.444	"	Rep3 34m
15		"	Mussels	GOC-B	61°07.447'	146°29.769	Hand Grab	Rep1-A

Continue list on back of page -->

Notes:
homo 40 g
* 1/2 ABL
1/2 GERG

\+ 10 g abl only
wt wt homo

X thaw, homo,
50 g to Gerg
50 ABL

Chain of Custody — tissue wt expanded cultures

Collected by: Jeffrey W. Short / NMFS — signature: Jeffrey W. Short — signature date: 10 July 2002

Relinquished by: Jeffrey W. Short — date / time: 1415 h — place: Auke Bay Lab

Received by: Marie Larsen — date / time: 11-July-02 2pm — place: Auke Bay Lab

ABL C of C 1/25/2000

Figure 2.b

Example Chemistry Lab Data Sheet
Sample Analysis

Chemistry Lab Data Sheet

Auke Bay Lab

String ID _____
Project _____

SIN	Repl Num	Matrix	Cat.No.	FID File#	U C M	MSD File	MSD #	SAMPLE WT (g)	% Dry wt.	DRY WEIGHT	COMT.

3. Analytical Quality Assurance

Quality assurance samples are processed with every 13 environmental samples and are used to determine the acceptability of the data from each sample string. The quality assurance samples consist of 2 reference material samples, 1 method blank, and 1 spiked blank and evaluate precision, method cleanliness and accuracy, respectively. See section 4 for composition of the QA samples.

A mid range calibration standard (calibration standard #3) is run near the middle and at the end of each string and is used to evaluate calibration stability. The standards are analyzed as a sample and the results are reviewed for accuracy.

These samples are analyzed on analytical instrumentation along with standards in the following general order, which together constitute a string:

Calibration standards (5 standards, beginning with the lowest concentration standard and run in ascending order to the highest concentration)

Environmental samples (6 samples run consecutively)

Calibration standard #3

Method blank sample (MBLK)

Reference material sample (AREF, replicate 1)

Environmental samples (6 samples run consecutively)

Spiked blank sample (SBLK)

Reference material sample (BREF, replicate 2)

Calibration standard #3

3.1 Calibration

Each string begins with 5 calibration standards (see section 4.5.1 and 4.5.2 for composition) which are used to determine the calibration curve for each analyte contained in the standards. The regression lines of each analyte are reviewed for linearity and comparability among strings.

3.1.1 Linearity

Linearity, as measured by the correlation coefficient (r), must be greater than 0.995, (i.e., $r^2 > 0.990$) for the five point calibration curve of all calibrated alkane and aromatic analytes. The r^2 value for all calibrated analytes are calculated and compiled for review as described in sections 13.4.2 and 13.5.3.

3.1.1.1 Corrective Action

If more than three r^2 values are less than 0.990 the string fails QA criteria and the extracts for that string are reanalyzed on the appropriate instrument, i.e., GC/FID for aliphatic analytes and GC/MS for aromatic analytes.

3.1.2 Comparability

The slope of the regression line for each analyte is compared to the slope of the line for that analyte from all previously analyzed strings of the same matrix. The slope must be within ± 20% of the mean slope of all strings.

3.1.2.1 Corrective Action

If more than three slopes are outside of the ± 20% window specified in section 3.1.2 the string fails QA criteria and the extracts for that string are reanalyzed on the appropriate instrument.

3.1.3 Calibration Stability

The measured concentrations of the calibrated analytes in the two calibration standards run near the middle and end of each string must be within ± 15% of the known concentration of the analyte in the standard. These data are calculated and compiled for review as described in section 13.4.2 and 13.5.3.

3.1.3.1. Corrective Action

If more than 6 standard recovery values are outside the parameters given in section 3.1.3 the string fails QA criteria and the extracts for that string is reanalyzed on the appropriate instrument, i.e. GC/FID for aliphatic analytes and GC/MS for aromatic analytes.

3.2 Method Cleanliness

A method blank (MBLK) is processed with each string and is used to determine the extent of laboratory contamination due to reagents, glassware, and handling techniques. The method blank sample is processed exactly as an environmental sample in the laboratory using identical reagents and techniques but without a matrix included. Contamination levels present in the method blank are subject to QA review for magnitude.

3.2.1 Contamination

The method blank is acceptable if the signal quantitation in an analyte window is less than three times the method detection limit (MDL) for any analyte in the same window. Exceptions may be made for analytes inherent in the solvents, e.g., naphthalene, C-1 naphthalenes.

3.2.1.1 Corrective Action

If contamination levels are greater than 3 times the MDL the string fails QA criteria and the entire string is reprocessed.

3.3 Precision

Two reference material samples (AREF and BREF) are processed with each string, analyzed near the middle and end of each string, and are used to evaluate the precision of the analytical procedure. The results are subject to QA review for comparability within and among all strings. (For composition of reference samples see section 4.2.)

3.3.1 Precision within a string

The precision within a string is acceptable if the amount of each calibrated analyte differs by no more than 15% of the mean of that analyte in the two reference samples.

3.3.1.1 Corrective Action

If more than three analytes are outside of the 15% window described in section 3.3.1 the string fails QA criteria and the sample extracts are reanalyzed on the appropriate instrument. If the string fails precision criteria after reanalysis, the entire string is reprocessed.

3.3.2 Precision among strings

The precision of a string, when compared to previously analyzed strings, is acceptable if the amount of each analyte in the reference samples is within ± 35% of the mean of that analyte in all strings of the same matrix.

3.3.2.1 Corrective Action

If more than six analytes are outside of the 35% window described in section 3.3.2 the string fails QA criteria and the extracts are rerun on the appropriate instrument. If the string fails precision criteria after reanalysis, the entire string is reprocessed.

3.4 Accuracy

A spiked blank (SBLK) is processed with each tissue and sediment string and is used to evaluate the accuracy of the analytical procedures. (For composition of spiked blanks see section 4.3.)

A water string is processed without a SBLK; however, one of the reference samples processed within the string is used to evaluate the accuracy of the analytical procedures. (See section 4.2.3 for composition of water references samples).

3.4.1 Accuracy

The amount of each native analyte found in the spiked blank must be ± 15% of the known amount in the spiked blank.

3.4.1.1 Corrective Action

If more than three analytes are outside of the 15% window described in section 3.4.1 the string fails QA criteria and the extracts are rerun on the appropriate instrument. If the string fails accuracy criteria after reanalysis, the entire string is reprocessed.

3.5 Surrogate Recovery

All environmental and QA samples within a string are spiked with identical amounts of deuterated surrogate standards (see section 4.1.1 and 4.1.2). These standards allow the estimation of native analyte loss through sample processing. Final analyte results are corrected for surrogate standard losses. The surrogate standard recovery is subject to QA review for method efficiency.

3.5.1 Percent Recoveries

The recovery of each surrogate analyte must be greater than 30% and less than 120% of the known surrogate spike amount.

3.5.1.1

If more than 10 recoveries from an entire string fall outside the QA parameter specified in section 3.5.1 the string fails QA criteria and the string is reprocessed. If the majority of the failed recoveries occur in an individual sample only that sample is reprocessed.

3.6 Integrity

All data generated by the methods described in these SOPs are checked for acceptability according to the QA guidelines described in section 3. All data is inspected by two analysts to insure data acceptability and integrity.

Chromatographic anomies periodically cause data to fail specific QA guidelines; however, data may be accepted if professional discretion of the analyst can justify acceptance. Any such justification will be documented and archived with the string.

4. Standards

The standards used in these SOPs and described below include spiking solutions, calibration standards, and QA sample components which are used in the quantitative analysis of environmental samples for petroleum hydrocarbons. These standards are composed of both National Institute of Standards and Technology (NIST) certified solutions and solutions made at ABL. NIST solutions are measured volumetrically and diluted with hexane in a volumetric flask to the appropriate volume. Standards made at ABL include compounds measured gravimetrically or volumetrically, depending on their form, and are diluted with hexane in a volumetric flask to the appropriate volume. All standards are stored at -20° C.

Spiking is performed volumetrically using Hamilton glass syringes after the solutions have equilibrated to room temperature.

4.1 Surrogate Recovery Standards

All samples of a string, environmental and QA, are spiked with deuterated surrogate standards which are used to determine processing losses (see section 3.5).

4.1.1 Tissue/Sediment Surrogate Recovery Standard

All tissue, sediment, and QA samples (MBLK, AREF, BREF, and SBLK) are spiked with 500 µl of the Tissue/Sediment Surrogate Recovery Standard just prior to processing.

Tissue/Sediment Surrogate Recovery Standard

Compound		**Concentration** (ng/µl)
n-Dodecane-d26	d-C12	10.50
n-Hexadecane-d34	d-C16	9.79
n-Eicosane-d42	d-C20	10.40
n-tetracosane-d50	d-C24	9.89
n-Triacontane-d62	d-C30	10.00
Naphthalene-d8		2.50
Acenaphthene-d10		2.50
Phenanthrene-d10		2.00
Chrysene-d12		2.00
Benzo[a]pyrene-d12		2.50
Perylene-d12		2.50

4.1.2 Water Surrogate Recovery Standard

All water and associated QA samples (MBLK, Ali AREF, Ali BREF, Aro AREF, and Aro BREF) (see section 4.2.3 for ali ref and aro ref descriptions) are spiked with 500 µl of the Water Surrogate Recovery Standard just prior to processing.

Water Surrogate Recovery PAH Standard

Compound	Concentration (ng/µl)
Naphthalene-d8	2.50
Acenaphthene-d10	2.50
Phenanthrene-d10	2.00
Chrysene-d12	2.00
Benzo[a]pyrene-d12	2.50
Perylene-d12	2.50

4.1.3 LDPE Surrogate Recovery Standard

All LDPE and associated QA samples (MBLK, AREF, and BREF) are spiked with 200 µL of the deuterated Water Surrogate Recovery PAH Standard just prior to extraction. The deuterated surrogate standard is used to determine method efficiency (section 3.5).

4.2 Reference Material Standards

Each sample string is processed with 2 reference samples (AREF and BREF) which are used to determine method precision (see section 3.3).

4.2.1 Tissue Reference Material Standards

Tissue reference sample is Standard Reference Material (SRM) supplied by NIST, SRM 1974a, Organics in Mussel Tissue. Approximate sample size used in analysis is 8 grams.

4.2.2 Sediment Reference Material Standard

Sediment reference sample is Standard Reference Material (SRM) supplied by NIST, SRM 1944, a freeze dried sediment sample. Approximate sample size used in analysis is 0.5 grams.

4.2.3 Water Reference Standards

Water reference samples, aromatic and aliphatic, are prepared separately. Water samples are not fractionated into aromatic and aliphatic components during sample processing (see section 5.3); therefore, references are prepared separately resulting in 4 reference samples, aro AREF, aro BREF, ali AREF, and ali BREF.

Water reference samples incorporate the equivalent amount of solvent that was used in the sample extraction and the appropriate water reference standard. See section 4.2.3.1 for the Aromatic Water Reference Standard and section 4.2.3.2 for the Aliphatic Water Reference Standard.

4.2.3.1 Water Aromatic Reference Standard

The Water Aromatic Reference Standard is composed of SRM 1491, Aromatic Hydrocarbons, supplied by NIST and dibenzothiophene a component added at ABL. Water QA samples, aro AREF and aro BREF, are spiked with 500 µl Water Aromatic Reference Standard prior to processing.

Water Aromatic Reference Standard

Compound	Concentration (ng/µL)
Naphthalene	2.27
2-methylnaphthalene	2.6
1-methylnaphthalene	2.74
Biphenyl	2.31
2,6-dimethylnaphthalene	2.38
Acenaphthylene	2.3
Acenaphthene	2.4
2,3,5-Trimethylnaphthalene	2.18
Fluorene	2.4
Dibenzothiophene	1.8
Phenanthrene	2.31
Anthracene	2.58
1-Methylphenanthrene	2.31
Fluoranthene	1.95
Pyrene	1.94
Benz[a]anthracene	1.18
Chrysene	2.32
Benzo[b]fluoranthene	1.73
Benzo[k]fluoranthene	1.84
Benzo[e]pyrene	1.85
Benzo[a]pyrene	2.24
Perylene	2.35
Indeno[1,2,3-cd]pyrene	2.08
Dibenz[a,h]anthracene	1.71
Benzo[ghi]perylene	1.75

4.2.3.2 Water Alkane Reference Standard

The Water Alkane Reference Standard contains the aliphatic compounds listed below and is prepared at ABL. Water Alkane Reference Samples ali AREF and ali BREF are spiked with 500 µl of Water Alkane Reference Standard prior to processing.

Water Alkane Reference Standard

Compound		Concentration (ng/µL)
n-Decane	C10	25.5
n-Undecane	C11	24.24
n-Dodecane	C12	25.78
n-Tridecane	C13	26.52
n-Tetradecane	C14	24.5
n-Pentadecane	C15	23.95
n-Hexadecane	C16	26.35
n-Heptadecane	C17	28.94
Pristane		27.1
n-Octadecane	C18	30.82
n-Nonadecane	C19	26.98
n-Eicosane	C20	24.17
n-Heneicosane	C21	23.5
n-Docosane	C22	26.74
n-Tricosane	C23	23.95
n-Tetracosane	C24	24.94
n-Pentacosane	C25	23.52
n-Hexacosane	C26	23.35
n-Heptacosane	C27	9.86
n-Octacosane	C28	26.02
n-Nonacosane	C29	23.3
n-Triacontane	C30	24.6
n-Dotriacontane	C32	23.28
n-Tetratriacontane	C34	23.83
n-Hexatriacontane	C36	24.14

4.2.3 LDPE Reference Standards

Each sample string is processed with 2 reference samples (AREF and BREF) which are used to determine method precision and accuracy (see sections 3.3 and 3.4). LDPE reference samples are composed of a blank LDPE strip cleaned with the same procedure as all environmental LDPEs prior to field deployment (see section 1.1). The reference samples are spiked with the PAH surrogate standard (see sec. 4.1.3) and native PAH analyte solution listed below.

LDPE Native Analyte Solution

Compound	**Concentration (ng/µL)**
Naphthalene	1.65
2-methylnaphthalene	1.89
1-methylnaphthalene	1.99
Biphenyl	1.68
2,6-dimethylnaphthalene	1.73
Acenaphthylene	1.67
Acenaphthene	1.75
2,3,5-Trimethylnaphthalene	1.58
Fluorene	1.74
Dibenzothiophene	1.20
Phenanthrene	1.68
Anthracene	1.88
1-Methylphenanthrene	1.68
Fluoranthene	1.42
Pyrene	1.67
Benz[a]anthracene	0.86
Chrysene	1.69
Benzo[b]fluoranthene	1.26
Benzo[k]fluoranthene	1.34
Benzo[e]pyrene	1.35
Benzo[a]pyrene	1.63
Perylene	1.71
Indeno[1,2,3-cd]pyrene	1.51
Dibenz[a,h]anthracene	1.24
Benzo[ghi]perylene	1.27

4.3 Spiked Blank Standards

Each sample string is processed with a spiked matrix blank (SBLK) which contains an appropriate sample matrix and an enrichment spike. The SBLK is used to determine the accuracy of the analytical method (see section 3.4).

4.3.1 Tissue/Sediment Spiked Blank Standard

The tissue SBLK and the sediment SBLK are composed of an uncontaminated mussel tissue homogenate, "Base Mussel", and an uncontaminated marine sediment, "Admiralty Island Sediment", respectively. These matrices are enriched with the Spiked Blank Standard before sample processing. This standard is prepared at ABL and contains SRM 1491, dibenzothiophene, and an alkane mixture C10-C36.

All tissue and sediment SBLKs are spiked with 500 µl of Spiked Blank Standard prior to extraction.

Tissue/Sediment Spiked Blank Standard

Compound		Concentration (ng/µL)
n-Decane	C10	21.24
n-Undecane	C11	20.2
n-Dodecane	C12	21.48
n-Tridecane	C13	22.1
n-Tetradecane	C14	21.24
n-Pentadecane	C15	19.96
n-Hexadecane	C16	21.96
n-Heptadecane	C17	24.12
Pristane		22.58
n-Octadecane	C18	25.68
n-Nonadecane	C19	22.48
n-Eicosane	C20	20.14
n-Heneicosane	C21	19.58
n-Docosane	C22	22.28
n-Tricosane	C23	19.96
n-Tetracosane	C24	20.78
n-Pentacosane	C25	19.6
n-Hexacosane	C26	19.46
n-Heptacosane	C27	8.22
n-Octacosane	C28	21.68

n-Nonacosane	C29	19.42
n-Triacontane	C30	20.5
n-Dotriacontane	C32	19.4
n-Tetratriacontane	C34	19.86
n-Hexatriacontane	C36	20.12
Naphthalene		1.65
2-methylnaphthalene		1.89
1-methylnaphthalene		1.99
Biphenyl		1.68
2,6-dimethylnaphthalene		1.73
Acenaphthylene		1.67
Acenaphthene		1.75
2,3,5-Trimethylnaphthalene		1.58
Fluorene		1.74
Dibenzothiophene		1.2
Phenanthrene		1.68
Anthracene		1.88
1-Methylphenanthrene		1.68
Fluoranthene		1.42
Pyrene		1.67
Benz[a]anthracene		0.86
Chrysene		1.69
Benzo[b]fluoranthene		1.26
Benzo[k]fluoranthene		1.34
Benzo[e]pyrene		1.35
Benzo[a]pyrene		1.63
Perylene		1.71
Indeno[1,2,3-cd]pyrene		1.51
Dibenz[a,h]anthracene		1.24
Benzo[ghi]perylene		1.27

4.3.2 Water Spiked Blank Standard

The aromatic and aliphatic reference samples serve as the spiked blanks for this matrix (see section 4.2.3).

4.4 Instrumental Internal Standards

4.4.1 HPLC Internal Standard

All tissue and sediment samples and their corresponding QA samples are spiked with 50 µl of the HPLC internal standard just prior to HPLC cleanup (see section 9). This standard is prepared at ABL.

HPLC Internal Standard

Compound	Concentration (ng/µl)
Anthracene-d10	20.0
Benzo[a]anthracene-d12	20.0

4.4.2 GC/FID Internal Standard

All aliphatic fractions of environmental and QA samples are spiked with 50 µl of the GC/FID Internal Standard just prior to GC/FID analysis (see section 10). This standard is prepared at ABL.

GC/FID Internal Standard

Compound	Concentration (ng/µl)
Dodecylcyclohexane (DCH)	42.0

4.4.3 GC/MS Internal Standard

All aromatic fractions of environmental and QA samples are spiked with 25 µl of the GC/MS Internal Standard just prior to GC/MS analysis. This standard is supplied by NIST.

GC/MS Internal Standard

Compound	Concentration (ng/µl)
Hexamethylbenzene (HMB)	80.0

4.5 Calibration Standards

Calibration standards are analyzed with the aliphatic and aromatic fractions of each sample string and are used to create the calibration curves from which the level of hydrocarbon contamination is quantified. Calibration standards are prepared at 5 different concentrations which encompass the expected range of environmental sample contamination levels.

4.5.1 Aliphatic Calibration Standards

Aliphatic calibration standards contain the analytes listed below and are prepared at ABL. The native analyte concentrations range from near the detection limit, approximately 1 ng/µl, to approximately 50 ng/µl. The amounts of the surrogate and internal standard analytes in these standards are equal to the amounts of the surrogate and internal standard analytes added to the environmental samples during processing (see sections 4.1 and 4.4).

The following list of the aliphatic calibration standards demonstrates a grouping of native analytes with a deuterated surrogate compound, e.g., C10, C11, C12, and C13 grouped with d-C12. Each group indicates the deuterated surrogate that is used for the normalization of each native analyte in the quantitative process. (See section 13 for the quantitative process.)

Aliphatic calibration standards are not used with LDPEs

Aliphatic Calibration Standards

Compound		Ali #1	Ali #2	Ali #3	Ali #4	Ali #5 (ng/µl)
n-Decane	C10	1.06	6.37	12.74	26.55	53.1
n-Undecane	C11	1.01	6.06	12.12	25.25	50.5
n-Dodecane-d26	d-C12	5.25	5.25	5.25	5.25	5.25
n-Dodecane	C12	1.07	6.44	12.89	26.85	53.7
n-Tridecane	C13	1.11	6.63	13.26	27.62	55.25
n-Tetradecane	C14	1.06	6.37	12.74	26.55	53.1
n-Pentadecane	C15	1	5.99	11.98	24.95	49.9
n-Hexadecane-d34	d-C16	4.9	4.9	4.9	4.9	4.9
n-Hexadecane	C16	1.1	6.59	13.18	27.45	54.9
n-Heptadecane	C17	1.21	7.24	14.47	30.15	60.3
Pristane		1.13	6.77	13.55	28.22	56.45
Dodecylcyclohexane	DCH	2.1	2.1	2.1	2.1	2.1
n-Octadecane	C18	1.28	7.7	15.41	32.1	64.2
n-Nonadecane	C19	1.12	6.74	13.49	28.1	56.2
n-Eicosane-d42	d-C20	5.22	5.22	5.22	5.22	5.22
n-Eicosane	C20	1.01	6.04	12.08	25.17	50.35
n-Heneicosane	C21	0.98	5.87	11.75	24.47	48.95
n-Docosane	C22	1.11	6.68	13.37	27.85	55.7
n-Tricosane	C23	1	5.99	11.98	24.95	49.9
n-Tetracosane-d50	d-C24	4.94	4.94	4.94	4.94	4.94
n-Tetracosane	C24	1.04	6.23	12.47	25.97	51.95
n-Pentacosane	C25	0.98	5.88	11.76	24.5	49
n-Hexacosane	C26	0.97	5.84	11.68	24.32	48.65
n-Heptacosane	C27	0.41	2.47	4.93	10.27	20.55
n-Octacosane	C28	1.08	6.5	13.01	27.1	54.2
n-Nonacosane	C29	0.97	5.83	11.65	24.27	48.55
n-Triacontane-d62	d-C30	5	5	5	5	5
n-Triacontane	C30	1.03	6.15	12.3	25.62	51.25
n-Dotriacontane	C32	0.97	5.82	11.64	24.25	48.5
n-Tetratriacontane	C34	0.99	5.96	11.92	24.82	49.65
n-Hexatriacontane	C36	1.01	6.04	12.07	25.15	50.3

4.5.2 Aromatic Calibration Standards

Aromatic calibration standards are prepared at ABL and contain NIST and ABL solutions in the concentrations listed below. NIST components include SRM 1491, Aromatic Hydrocarbons; AH-5, deuterated aromatic hydrocarbons; and HMB-6, hexamethylbenzene. ABL components include dibenzothiophene, and the deuterated compounds anthracene, benz[a]anthracene, phenanthrene, and chrysene. The native analyte concentrations range from near the detection limit, approximately 70 pg/µl, to approximately 1700 pg/µl. The amounts of the surrogate and internal standard analytes in these standards are equal to the amounts of the surrogate and internal standard analyte concentrations added to the environmental samples during processing (see sections 4.1 and 4.4).

The following list of the aromatic calibration standards demonstrates a grouping of native analytes with a deuterated surrogate compound, e.g., naphthalene, 2-methylnaphthalene, and 1-methylnaphthalene grouped with naphthalene-d8. Each group indicates the deuterated surrogate that is used for the normalization of each native analyte in the quantitative process. (See section 13 for quantitative process.)

Aromatic Calibration Standards

Compound	Aro #1	Aro #2	Aro #3	Aro #4	Aro #5 (pg/µl)
Naphthalene-d8	1250	1250	1250	1250	1250
Naphthalene	68.9	172.3	344.5	689	1722.5
2-Methylnaphthalene	118	295	590	1180	2950
1-Methylnaphthalene	83	207.5	415	830	2075
Acenaphthene-d10	1250	1250	1250	1250	1250
Biphenyl	70	175	350	700	1750
2,6-Dimethylnaphthalene	72	180	360	720	1800
Acenaphthylene	69.6	174	348	696	1740
Acenaphthene	72.8	182	364	728	1820
2,3,5-Trimethylnaphthalene	66	165	330	660	1650
Fluorene	72.7	181.8	363.5	727	1817.5
Hexamethylbenzene (HMB)	1000	1000	1000	1000	1000
Anthracene-d10	1000	1000	1000	1000	1000
Benz[a]anthracene-d12	1000	1000	1000	1000	1000
Phenanthrene-d10	1000	1000	1000	1000	1000
Dibenzothiophene	80	200	400	800	2000
Phenanthrene	70.1	175.3	350.5	701	1752.5
Anthracene	78.2	195.5	391	782	1955
1-Methylphenanthrene	70	175	350	700	1750
Fluoranthene	59.1	147.8	295.5	591	1477.5
Pyrene	58.9	147.3	294.5	589	1472.5
Chrysene-d12	1000	1000	1000	1000	1000
Benz[a]anthracene	35.9	89.8	179.5	359	897.5
Chrysene	70.3	175.8	351.5	703	1757.5
Benzo[a]pyrene-d12	1250	1250	1250	1250	1250
Benzo-b-fluoranthene	52.5	131.3	262.5	525	1312.5
Benzo-k-fluoranthene	55.7	139.3	278.5	557	1392.5
Benzo-e-pyrene	56.2	140.5	281	562	1405
Benzo-a-pyrene	67.9	169.8	339.5	679	1697.5
Indeno[1,2,3-cd]pyrene	62.9	157.3	314.5	629	1572.5
Dibenz[a,h]anthracene	51.8	129.5	259	518	1295
Benzo[ghi]perylene	52.9	132.3	264.5	529	1322.5
Perylene-d12	1250	1250	1250	1250	1250
Perylene	71.2	178	356	712	1780

4.6 HPLC Calibration Standard

The HPLC calibration standard is used to define the collection time window used in the HPLC purification procedure (see section 9).

HPLC Calibration Standard

Compound	Concentration (ng/µl)
Perylene	5.7
Biphenyl	17.1
Dibromooctoflurobiphenyl	12.1

4.7 Biomarker Calibration Standard

The biomarker calibration standards contain the analytes listed below and are prepared at ABL. The hopane and sterane analytes are made at 5 different levels to encompass the levels expected in environmental samples. The isoprenoid standards are make at 3 different levels to the same end. The amounts of the surrogate and internal standard analytes in these standards are equal to the amounts of the surrogate and internal standard analytes added to the environmental samples during processing (see sections 4.1 and 4.4).

The biomarkers are calculated using response factors rather than by linear regression as the n-alkanes and PAH. Response factors are calculated relative to the Internal Standard, DCH. The isoprenoids are calculated from their respective analyte relative response factor. Hopanes are calculated from the response factor for H30 with the exception of Tm which is calculated with its unique response factor. Steranes are calculated from the response factor for C27S.

Biomarker Calibration Standards
Table 4.7a

	Std:	**1**	**2**	**3**	**4**	**5**
Code	**Compound**					
IS	DCH	2660	2660	2660	2660	2660
Surr	d2-C27aaa(20R)cholestane	2000	2000	2000	2000	2000
Tm	17a(H)-22,29,30-trisnorhopane	10	100	500	1000	5000
M30	17b(H),21a(H)-hopane	10	100	500	1000	5000
H30	17a(H),21b(H)-hopane	10	100	500	1000	5000
C27S	aaa(20S)-cholestane	10	100	500	1000	5000
C27R	aaa(20R,24R)-24-ethylcholestane	10	100	500	1000	5000
		(ng/ml)	(ng/ml)	(ng/ml)	(ng/ml)	(ng/ml)

Isoprenoid Calibration Standards
Table 4.7b

	Std:	**1**	**2**	**3**
Code	**Compound**			
IS	DCH	2660	2660	2660
	pristane	780	4680	9360
	phytane	977	5862	11724
	norpristane	800	5000	10000
		(ng/ml)	(ng/ml)	(ng/ml)

5. Extraction of Samples

5.1 Tissue Extraction

The SOPs in this section describe the methods used at ABL for tissue sample dissection, homogenization, spiking, and extraction.

Tissue samples, depending on the organisms constituting the sample, may require dissection before the sample can be homogenized and extracted. For example, if the sample consists of bivalves, the internal tissues must be separated from the shells. Dissection methods are described in section 5.1.3. Similarly, if the sample consists of whole salmon fry, dissection is not required and sample processing can begin with homogenization as described in section 5.1.4.

5.1.1 Glassware and Apparatus

Glassware and apparatus are cleaned to insure that they are free of hydrocarbons. Glassware is washed with ALCONOX and rinsed with tap water. The glassware is then combusted at 440°C for at least 4 hours. Solvent rinses of acetone and methylene chloride $MeCl_2$ may be substituted for the combustion process if time does not allow for combustion. All combusted glassware are stored in a clean environment and sealed with combusted aluminum foil. Apparatus which are not glass are washed with ALCONOX, rinsed with tap water, rinsed with acetone and $MeCl_2$, and stored in a clean environment.

The following labware are used in these methods for the extraction of marine faunal tissue:

Jars: I-CHEM, wide-mouth glass, 4 oz.
Aluminum foil
Aluminum weighing dishes
Dissection kits: 2 sets
Scalpels: 2 stainless steel
Tissuemizer: TEKMAR, type SDT 1810 S1
Spatulas: nichrome
Beaker: 100 ml, Kimax
Accelerated Solvent Extractor: Dionex, ASE 200
Extraction Cell: Dionex, stainless steel, 33 ml
Collection vial: Dionex, 60 ml Clear collection vials
Centrifuge tubes: KIMBLE, glass, 50 ml, tapered end
Syringe: HAMILTON, 500 µl
Flat bottom flasks: 250 ml
Funnels: glass, short, wide stem
Filter paper: WHATMAN, 12.5 cm glass microfiber, combusted at 440 for 4 hours
Pasteur pipets: glass, capillary, 1 ml
Electronic balance: toploading, sensitivity to 0.01g

Boiling stones: SCIENCEWARE, teflon

5.1.2 Chemicals and Solvents

The following reagents and standards are used in these methods for the extraction of marine faunal tissue:

Diatomaceous Earth: Sigma, Acid-washed; combusted at 440 C for 4 hours

Sodium Sulfate (Na_2SO_4): MALLINCKRODT, analytical grade, anhydrous, granular, combusted 4 hours at 440°C

Solvents: Pesticide grade pentane, hexane, methylene chloride (MeCl2), and methanol

Tissue/Sediment Surrogate Recovery Standard: see section 4.1.1

Tissue Reference Material and Standard: see section 4.2.1

Tissue/Sediment Spiked Blank Material and Standard: see section 4.3.1

5.1.3. Dissection

Tissue samples are received from the SC, (see section 2.2), and a representative sample is dissected. The majority of tissue samples analyzed at ABL consist of mussels; therefore, this dissection standard operating procedure is disposed to that species.

1. The organism(s) of one sample are placed on a sheet of aluminum foil that has been rinsed with $MeCl_2$. A representative sample of organisms is selected for dissection. The number of organisms chosen should result in a sample weight, after dissection, of approximately 20 g.

2. The internal fluids of the organism are part of the sample; therefore, it is helpful to dissect a frozen or partially frozen organism so that these fluids are not lost or contaminated by contacting the outside of the organism.

3. The dissection process must avoid contaminating internal tissues. Two sets of dissection implements and 2 scalpels are used; each set must exclusively contact either internal tissues or external surfaces.

4. A 4 oz. I-CHEM jar is tared on a top loading balance.

6. Each organism in the sample is opened or incised in whatever manner is most facile and the internal tissues are removed. The tissues are placed in the tared jar on the balance and the sample weight is recorded.

7. The composite of tissues are homogenized as described in section 5.1.4.

5.1.4 Homogenization

Tissue samples are homogenized to insure that subsamples taken for hydrocarbon analysis, percent moisture determination, and lipid content analysis are representative of the entire sample.

1. The components of a TEKMAR tissuemizer probe are cleaned as described in section 5.1.1. The probe is reassembled as per manufacturer instructions. This procedure must be repeated with each sample. Rinsing can be facilitated by attaching the reassembled probe to the tissuemizer body, immersing the probe in the appropriate rinse solvents, and operating the probe for several seconds.

2. A sample is homogenized by placing the tissuemizer probe into the sample and operating the tissuemizer at a speed fast enough to macerate the sample but slow enough to avoid sample loss from splattering.

3. The probe is removed from the sample and a clean nichrome spatula is used to push any sample remaining on the tissuemizer probe into the sample jar.

4. Homogenized samples are frozen if they are not scheduled for immediate extraction.

5.1.5 Subsampling tissue samples

Aliquots of the 12 tissue samples of a string are taken for hydrocarbon analysis and percent moisture determination. Aliquots of the QA samples of a string, reference materials and spiked blank matrix (see sections 4.2.1 and 4.3.1, respectively), are taken for hydrocarbon analysis only.

1. Twelve 100 ml beaker,12 aluminum weighing dishes and 12 stainless steel extraction cells are labeled with the sample identification numbers (SINs) appropriate for the sample string. Four beakers and four extraction cells are labeled as AREF, BREF, SBLK, and MBLK.

2. For the environmental samples, a beaker labeled with a SIN is tarred on a top loading balance. A nichrome spatula is used to transfer approximately 4.5 grams of the sample homogenate with the same SIN into the beaker. The sample weight is recorded.

3. For the environmental samples, an aluminum weighing dish labeled with the same SIN as in step 2 is weighed on a top loading balance. The weight is recorded. The aluminum dish is then tarred, and with a nichrome spatula,

approximately 1 gram of the sample homogenate is transferred into the dish. The weight of the tissue is recorded. The aluminum dish with tissue are placed uncovered in an oven at 115° C to dry. The calculation of percent moisture is described in Appendix I.

4. For the QA samples AREF and BREF, the beakers are tared and with a nichrome spatula approximately 3 grams of the tissue reference material, SRM 1974 (see section 4.2.1), are transferred into each beaker. The sample weights are recorded.

5. For the SBLK, the beaker is tared and with a nichrome spatula approximately 5 grams of the spiked blank matrix, Base Mussel (see section 4.3.1), are transferred into the tube. The sample weight is recorded.

6. For the MBLK, the beaker is tared and with a nichrome spatula 5 grams of combusted Diatomaceous Earth is transferred into the baker. The weight is not recorded.

5.1.6 Spiking a tissue string

All samples of a string, environmental and QA, receive a spike of the Tissue/Sediment Surrogate Recovery Standard prior to extraction. QA samples, AREF, BREF, and SBLK, receive an additional spike of an enrichment standard. These spikes are delivered directly onto the sample mixture in the extraction cell
(see section 5.1.7.4) with the exception of the MBLK in which the spike is delivered into the diatomaceous earth in the extraction cell.

1. Three 500 µl Hamilton syringes are cleaned by rinsing at least 3 times with both $MeCl_2$ and hexane.

2. All 16 samples constituting the string (12 environmental and 4 QA) are spiked with 500 µl of Tissue/Sediment Surrogate Recovery Standard (see section 4.1.1).

3. AREF and BREF are spiked with 500 µl of *Exxon Valdez* Crude Oil Solution (see section 4.2.1).

4. SBLK is spiked with 500 µl of Tissue/Sediment Spiked Blank Mixture (see section 4.3.1).

5. MBLK receives no enrichment spike.

5.1.7 Extraction of hydrocarbons from tissue samples using ASE 200 (Accelerated Solvent Extractor)

This extraction procedure must be repeated for each sample in a string. Extracts should be stored at -20° C.

1. A funnel is placed into an extraction cell that has been labeled with SIN. Each of the extraction cells are lined with 1.983 cm Cellulose Filter, grade D28.

2. Approximately 4.5 grams of Acid-washed Diatomaceous Earth are added to the sample in the 100 ml beaker that displays the same SIN as in step 1.

3. With a clean nichrome spatula, the sample and the diatomaceous earth are mix thoroughly. Additional 1 gram of diatomaceous earth is added if needed to the sample mixture until it is almost dry. The homogeneous sample mixture is transferred through the funnel on the extraction cell that displays the same SIN as in step 1.

4. The sample mixture in the extraction cell are spiked with 500 µl Tissue/Sediment Spiking Solution (see section 4.2.1). The QA samples, (AREF and BREF) receive an additional spike of enrichment standard (see section 4.2.1) SBLK receive the spike blank mixture enrichment (see section 4.3.1). MBLK receives no enrichment spike.

5. Approximately 3 grams to 5 grams of combusted sand is added on top of the sample mixture prior to loading the sample on the ASE 200.

6. Twelve collection vials are labeled with sample identification numbers (SINs). The extraction cells with the samples are loaded in the upper carousel tray and the collection vials with SINs are places in the lower carousel tray of the ASE 200 for extraction.

7. For a very wet samples, add ca. 5 grams of Na2SO4 to the extract in the collection vial and filter through a funnel into another tube.

5.1.8 Concentration and solvent exchange

Sample extracts are reduced in volume and the solute is changed from $MeCl_2$ to hexane. This procedure prepares the sample extracts for fractionation and purification by liquid column chromatography (see sections 6 to 8).

1. After extraction, 2-3 boiling stones are added to each sample extract and the extracts are placed on a steam bath at approximately 80° C. The extracts are boiled until the volume is reduced to approximately 10 ml, 1 ml of hexane is added to the extract.

2. The extract is further concentrated to 2 ml and 0.5 ml of hexane is added. The extract is re-concentrated to 1 ml and is removed from the steam bath. The solvent exchange is complete.

5.2 Sediment extraction

The SOPs in this section describe the methods used at ABL for sediment sample homogenization, spiking, and extraction.

5.2.1 Glassware and Apparatus

All glassware and apparatus are cleaned as described in section 5.1.1.

The following labware are used in these methods for the extraction of marine sediment:

Extraction bottles: teflon bottles, 250 ml, with caps and O-ring seals
Amber bottles: QORPAK, glass, 500 ml
Centrifuge tubes: KIMBLE, glass, 50 ml, tapered end
Mechanical tumbler: (must accommodate 16 extraction bottles)
Spatulas: nichrome
Funnels: KIMAX, glass, short, wide stem
Filter paper: WHATMAN, 12.5 cm glass microfiber, combusted 4 hours at 440°
Syringes: HAMILTON, 500 µl
Pasteur pipets: glass, capillary, 1 ml
Boiling chips: SCIENCEWARE, teflon
Electronic Balance: top loading, sensitivity to 0.01g
Aluminum foil
Aluminum weighing dishes

5.2.2 Chemicals and Solvents

The following reagents and standards are used in these methods for the extraction of marine sediments:

Sodium sulfate (Na_2SO_4): MALLINCKRODT, analytical grade, anhydrous, granular, combusted 4 hours at 440°
Solvents: Pesticide grade pentane,hexane,$MeCl_2$,and methanol
Tissue/Sediment Surrogate Recovery Standard: see section 4.1.1
Sediment Reference Material: SRM 1944, see section 4.2.2
Tissue/Sediment Spiked Blank Material and Standard: see section 4.3.1

5.2.3 Homogenization

Sediment samples are homogenized by the analyst to insure that the sub-samples taken for hydrocarbon analysis and percent moisture determination are representative of the entire sample.

1. Sediment samples are removed from the freezer and are allowed to thaw for 3-5 hours.

2. The sediment sample is stirred with a spatula to disseminate any settling gradations. The sample is stirred until the analyst judges it to be homogeneous (1-2 min).

5.2.4 Sub-sampling sediment samples

Aliquots of the 12 sediment samples of a string are taken for hydrocarbon analysis and percent moisture determination. Aliquots of the QA samples of a string, reference materials and spiked blank matrix (see sections 4.2.2 and 4.3.1 respectively), are taken for hydrocarbon analysis only.

1. Twelve teflon extraction bottles and 12 aluminum weighing dishes are labeled with the SINs appropriate for the sample string. Four Teflon extraction bottles are labeled as AREF, BREF, SBLK, and MBLK.

2. For the environmental samples, a Teflon bottle labeled with a SIN is tared on the top loading balance. Approximately 20 grams of the homogenized sample possessing the same SIN is transferred into the Teflon bottle using a nichrome spatula. The sample weight is recorded.

3. For the environmental samples, the aluminum weighing dish with the appropriate SIN is weighed on a top loading balance. The weight is recorded. The aluminum dish is then tared, and with a nichrome spatula approximately 1 gram of the sample homogenate is transferred into the dish. The weight of the sediment is recorded. The aluminum dish and sediment are placed uncovered in an oven at 115° C to dry. For calculation of percent moisture see Appendix I.

4. For the QA samples AREF and BREF, the Teflon bottles are tared and with a nichrome spatula approximately 1 gram of the sediment reference material, QC-SED-1 (see section 4.2.2), is transferred into each bottle. The sample weight is recorded.

5. For SBLK, the Teflon bottle is tared and with a nichrome spatula approximately 5 grams of the spiked blank matrix, Admiralty Island Sediment, (see section 4.3.1), is transferred into the bottle. The sample weight is recorded. For the MBLK 100 ml of $MeCl_2$ is added to the Teflon bottle.

5.2.5 Spiking a sediment string

The spiking procedure for a sediment string is identical to the procedure outlined in section 5.1.6 with one exception. No enrichment spike is required for reference samples in a sediment sample string, i.e., omit step 3.

5.2.6 Extraction

See section 5.4, Accelerated Solvent Extraction (ASE). Extracts are stored at −20° C.

5.2.7 Concentration and solvent exchange

The procedures for concentration and solvent exchange are described in section 5.1.8. This methods prepares the sample extract for fractionation and purification by liquid column chromatography (see section 7).

5.3 Water Sample Processing

The SOPs in this section describe the methods used at ABL for processing water samples. Water samples are received at ABL after a liquid-liquid extraction is performed at the collection site. The samples are composed of a $MeCl_2$ extract with residual water.

Water samples, unlike tissue and sediment samples, are not purified and fractionated by column chromatography. After dehydration and concentration the water extracts are prepared for instrumental analysis. Two aliquots are taken from each extract. One aliquot is analyzed for aromatic hydrocarbons; one is analyzed for aliphatic hydrocarbons.

5.3.1 Glassware and Apparatus

All glassware and apparatus are cleaned as described in section 5.1.1.

The following labware are used in these methods for processing water samples:

Jars: glass, wide mouth, 100ml
Funnels: glass, short, wide stem
Amber bottles: QORPAK, glass, narrow neck, 250 ml
Centrifuge tubes: KIMBLE, glass, 50 ml, tapered end
Syringes: HAMILTON, glass, 25, 50, and 500 µl
Pasteur pipettes: glass, capillary, 1 ml
Filter paper: WHATMAN, 12.5 cm glass microfiber, combusted at 440° C 4 hours
Boiling stones: SCIENCEWARE, Teflon
Screwtop vials: SUPELCO, 2 ml glass with Teflon lined cap
Crimptop vials: HEWLETT PACKARD, 2 ml glass with 100µl tapered inserts and Teflon lined caps
Graduated cylinder: 10 and 100 ml

5.3.2 Chemicals and Solvents

The following reagents and standards are used in these methods for the processing of marine water samples:

Sodium sulfate (Na_2SO_4): MALLINCKRODT, anhydrous, granular, combusted at 440°C overnight and stored at 105°C
Solvents: Pesticide grade acetone, hexane, $MeCl_2$
Water Surrogate Recovery Standard: see section 4.1.2
Water Aromatic Reference Standard: see section 4.2.3.1
Water Alkane Reference Standard: see section 4.2.3.2
GC/FID Internal Standard: see section 4.4.2
GC/MS Internal Standard: see section 4.4.3

5.3.3 Spiking a Water String

All samples of a water string, environmental and QA, receive a spike of the Water Surrogate Recovery Standard prior to their concentration. The QA samples, aro AREF, aro BREF, ali AREF, and ali BREF, receive an additional spike of an enrichment standard. These spikes are delivered directly into the extraction solvent.

1. Three 500 µl Hamilton syringes are cleaned by rinsing at least 3 times with both $MeCl_2$ and hexane.

2. Five 100 ml glass jars are labeled as aro AREF, aro BREF, ali AREF, ali BREF, and MBLK. Approximately 75 ml of $MeCl_2$ are placed each bottle.

3. All 17 samples constituting the string (12 environmental and 5 QA) are spiked with 500 µl of Water Surrogate Recovery Standard (see section 4.1.2)

4. QA samples, aro AREF and aro BREF, are spiked with 500 µl of Water Aromatic Reference Standard (see section 4.2.3.1).

5. QA samples, ali AREF and ali BREF, are spiked with 500 µl of Water Alkane Reference Standard (see section 4.2.3.2).

6. MBLK receives no enrichment spike.

7. After spiking, the samples are capped with Teflon lined lids and are placed in a freezer at -20° C overnight to allow the residual water freeze. This will facilitate removal of the water from the extract (see section 5.3.4).

5.3.4 Water extraction

Water is typically extracted in the field or at the experimental site, not in the chemistry laboratory.

Remove the spiking standard(s) from the freezer.

Collect the sample in a hydrocarbon clean (HC) 4 liter jug and adjust sample volume to approximately 3.5 L +/- 25 mL. Compare height of water to “calibrated jug” for initial volume adjustment.

Rinse a 500 µl volumetric syringe in $MeCl_2$ and dry.

Spike water sample with 500.0 µl of "Surrogate Water Spike" (p.99 in laboratory notebook PWS-342).

Add ~ 90 ml of $MeCl_2$ to the sample jug and shake vigorously for 2 minutes minimum.

Let stand and settle for a minimum of 2-3 minutes.

Decant off the water into a 2nd HC clean 4 liter bottle until 500-700 ml is left in the jug. Transfer the remaining water and solvent to a HC clean 1 liter Teflon separatory funnel. Rinse the 1st 4 liter jug with a small amount of $MeCl_2$ two times and place these rinses into the separatory funnel and let the sample sit for a minute or two (break up emulsions with a HC clean wire or stir rod).

Drain the extract into a "LABELED" HC clean 250 ml amber bottle and pour the remaining water into the 2nd 4 liter jug.

Again add ~90 ml of $MeCl_2$ and shake vigorously for 2 minutes minimum.

Let stand and settle for a minimum of 2-3 minutes

Decant off the water into a bucket (or directly into a 1 L graduate cylinder) until 500-700 ml is left in the jug. Note the number of times the 1 L graduate is filled completely (usually 3 x), and discard the water after the volume has been measured. Transfer the remaining water and solvent in the amber jug to the 1 liter separatory funnel. Rinse the 2nd 4 liter jug with a small amount of $MeCl_2$ two times and place these rinses into the separatory funnel and let the sample sit for a minute or two (break up emulsions with a HC clean wire or stir rod).

Combine the 2nd extract into the same "LABELED" 250 ml amber bottle as in step #8, and transfer the remaining water to the 1L graduate cylinder to get the final volume extracted. Discard the water when the final volume measurement is completed.

Place the extract(s) in the freezer until further processing.

Rinse the separatory funnel and second amber extraction bottle with a minimum amount of $MeCl_2$ (< 20 mL) and transfer rinses to a waste solvent jug.

Repeat process with next sample.

5.3.5 Dehydration of Water Extracts

Residual water must be removed from the water extracts before they are concentrated and prepared for instrumental analysis. Water extracts are removed from the freezer just prior to this dehydration procedure to minimize melting of the residual water. The water freezes to the wall of the container; this allows the $MeCl_2$ extract to be easily decanted away. Further drying is accomplished with anhydrous Na_2SO_4.

1. A funnel is lined with a combusted glass fiber filter paper that has been folded into quarters and opened to form a cone. Approximately 50 grams of combusted Na_2SO_4 is placed in the filter paper. The funnel is placed into a 250 ml amber bottle that has been labeled with a SIN.

2. The sample with the corresponding SIN is removed from the freezer and the extract is decanted into the amber bottle through the lined funnel. The sample container is rinsed 3 to 4 times with 2 ml $MeCl_2$ that has been stored in the freezer. Cold solvent will minimize melting of the residual water. Each rinsate is decanted through the lined funnel. The rinsate is allowed to drip into the amber bottle.

3. The Na_2SO_4, filter paper, and funnel are rinsed with $MeCl_2$. The rinsate is allowed to drip into the flask. The filter paper and its contents are discarded and the funnel is removed from the amber bottle.

4. The extract container is capped with a Teflon lined lid and stored at -20° C.

5. Steps 1-4 are repeated for each sample in the string.

5.3.6 Concentration and Solvent Exchange

The procedures for concentration and solvent exchange are described in section 5.1.8.

5.3.7 Preparation for Instrumental Analysis

Water sample processing includes no purification or fractionation techniques prior to instrumental analysis. After dehydration, concentration, and solvent exchange, the sample

string is prepared for analysis by the addition of internal standards.

1. Syringes, 25 µl and 50 µl, are cleaned by rinsing at least 3 times with both $MeCl_2$ and hexane.

2. Each sample in the sample string, environmental and QA, is spiked with 50 µl of the GC/FID Internal Standard, DCH, (see section 4.4.2).

3. Each sample in the sample string, environmental and QA, is spiked with 25 µl of the GC/MS Internal Standard, HMB, (see section 4.4.3).

4. The samples are mixed for approximately 5 seconds to insure thorough mixing of the internal standard and the sample extract.

5. The extract is transferred to a 2 ml glass screw top vial using a Pasteur pipette. The centrifuge tube is rinsed once with 0.5 ml hexane and the rinsate is added to the 2 ml vial. The vial is sealed with a Teflon lined cap and stored at -20° C.

6. The extracts are further prepared for instrumental analysis by removing the caps from the sample vials and placing them in a fume hood. The sash of the fume hood is situated to optimize airflow over the samples. The extracts are allowed to concentrate to approximately 1 ml.

7. An aliquot of the 1 ml extract is transferred to a crimptop vial fitted with a 100 µl insert. The vial is labeled with the appropriate SIN and is sealed with a Teflon lined cap. The vial is stored at -20° C until designated for instrumental analysis.

5.4 Accelerated Solvent Extraction

The SOPs in this section describe the methods used at ABL for the extraction of tissue and sediment samples with an Accelerated Solvent Extractor (ASE 200) made by the Dionex Corporation. The ASE 200 is a mechanized extraction instrument that uses high pressures and temperatures optimize extraction efficiency.

5.4.1 Glassware and Apparatus

All glassware and apparatus are cleaned as described in section 5.1.1

The following labware are used in these methods for the sample extraction:

Extraction Cells: Dionex, stainless steel, 33ml, with interchangeable caps screw onto each end of the cell body.
Collection vials: I-Chem, glass, clear, 60mL, with caps. Caps have solvent-resistant septa.

Mechanical Extractor: Dionex ASE 200 Accelerated Solvent Extractor
Funnels: Kimax, glass, short, wide stem
Beakers: Kimax, glass, 200 mL
Filter paper: Dionex, D28 grade, 1.983cm in diameter, disposable
Insertion tool: rod supplied by Dionex
Syringes: Hamilton, 500 µl
Separatory funnel: Kimax, 125 mL, with Teflon stopcock
Sand: EM Science, standard, Ottawa, combusted 4 hours at 440°C
Boiling chips: Scienceware, Teflon
Electronic Balance: Top loading, sensitivity to 0.01g
Aluminum foil
Aluminum weighing dishes

5.4.2 Chemicals and Solvents

The following reagents and standards are used in these methods for the extraction of tissue and marine sediment sample.

Sodium sulfate (Na_2SO_4): Mallinckrodt, analytical grade, anhydrous, granular, combusted 4 hours at 440°C
Hydromatrix: Varian, pellet, combusted 4 hours at 440°C
Solvents: Pesticide grade $MeCl_2$, hexane and methanol
Tissue/Sediment Surrogate Recovery Standard: see section 4.1.1
Sediment Reference Material: SRM 1944

5.4.3 Homogenization (see section 5.2.3)

5.4.4 Sample Preparation

Aliquots of the 13 samples are taken for hydrocarbon analysis and percent moisture determination. An aliquot of the QA samples of a string, reference materials (see section 4.2.1 or 4.2.2), is taken for hydrocarbon analysis only.

1.Thirteen stainless steel extraction cells, 13 collection vials and 13 aluminum-weighing dishes are labeled with SINs appropriate for the sample string. Three stainless steel extraction cells and 3 collection vials are labeled as AREF, BREF and MBLK. The labeled collection vials are loaded in the lower carousel of the Accelerated Solvent Extractor (ASE).

2.For the environmental samples, a beaker labeled with SIN is tared on the top loading balance. The appropriate sample is transferred into the beaker using a nichrome spatula. The sample weight is recorded. (Approximately 15 grams sediment; 8 grams tissue.)

3.For environmental samples, the aluminum-weighing dish with appropriate SIN is weighed on a top loading balance. The weight is recorded. The aluminum dish is then tared, and with a nichrome spatula approximately 1 gram of the sample is transferred in to the dish. The weight of the sample is recorded. The aluminum dishes with the samples are placed uncovered in an oven at 115°C to dry. For calculation of percent moisture see Appendix I.

4.For QA samples AREF, BREF, the beakers are tared and with a nichrome spatula the appropriate amount of reference material is transferred into each beaker. The sample weight is recorded.

5.For MBLK, approximately 5 grams of sand (see section 5.3.2) is transferred into the tared beaker. The weight of sand is not recorded.

6.The weighed samples are mixed with enough hydromatrix to dry them.

7.The dried samples are transferred into a stainless steel extraction cell possessing the same SIN using the nichrome spatula and glass funnel. Make sure that disposable filter paper is installed in the cell before transferring the sample. The samples are packed into the cell with a use of the insertion tool.

8.500 µl of the Tissue/Sediment Surrogate Recovery Standard (see section 4.1.1 are added into each the sample in the extraction cell.

9.Top the packed sample in the stainless steel cell with hydrocarbon clean sand and attached the top end caps of the cell. The extraction cells filled with samples are loaded in the upper carousel of the Accelerated Solvent Extractor (ASE) and extracted as per the conditions listed in section 5.4.5.

5.4.5 The ASE extraction parameters are listed below.

The ASE is programmed to extract the samples under the conditions below. The sample extracts are dried with sodium sulfate in a glass separatory funnel. The extracts are stored at –20°C.

Pressure:	2000psi
Temp:	100°C
Preheat:	0 minute
Heat:	5 minutes
Static heat:	5 minutes
Flush:	60%
Purge:	200 sec
Solvent:	$MeCl_2$

5.4.6 Dehydration of ASE Extracts

Residual water must be removed from the extracts before they are concentrated and prepared silica column fractionation.

1. Granular anhydrous Na_2SO_4 is added to the ASE vial containing the sample extracts until the crystals drift freely indicating all the water has been bound.

2. The extract is decanted away from the Na_2SO_4 into a clean centrifuge tube that has been labeled with the appropriate SIN. The Na_2SO_4 is rinsed 3 times with a small volume of $MeCl_2$ and the rinse is decanted into the centrifuge tube to complete the transfer.

3. Steps 1-2 are repeated for each sample in the string.

5.5 LDPE Extraction

5.5.1. Transfer 13 sample LDPE strips from I-Chem jar to a 100 mL extraction centrifuge tube making sure to "unroll" the strip and place in the tube in a random manner to increase the surface exposed to the solvent. Also, place "clean" LDPE strips in extraction tubes labeled AREF, BREF, and MBLK; these are two reference samples and a method blank respectively.

5.5.2. During the transfer to the extraction vessel it is important to remove sediment, biofoul, rust, or other particulate matter that is adhered to the LDPE strip. This is accomplished by wiping the LDPE with a fresh Kimwipe® while holding the strip with a clean forceps prior to placing the sample in the extraction vessel. If this step is done to any environmental samples then it is also done to the QA samples. This step is completed to minimize any potential PAH contribution from the surface particulate matter to the LDPE sample extract.

5.5.3. All samples (environmental & QA) are spiked with 500 µL of tissue/sediment surrogate (see sec. 4.1.1), being careful to place the solution on the LDPE strip.

5.5.4. AREF & BREF are also spiked with 50 µL of PAH native analyte solution being careful to place the solution on the LDPE strip.

5.5.5. Add 100 mL of 80%/20% Pentane/ MeCl2 to each extraction tube.

5.5.6. Place the extraction tubes in the rack of the sonic bath and extract for the following schedule:

20 min on
30 min off
20 min on
30 min off

20 min on

5.5.7. Remove the tubes from the sonic extractor and with a clean forceps remove the LDPE strip from the tube, rinsing with pentane as it is removed.

5.6 LDPE Extract Purification Procedure

5.6.1. Concentrate the extracts in the extraction tube to a volume of around 20-30 mL. Remove the tubes from the steam bath and allow to cool for 5 min, then add 2-4 grams sodium sulfate to each sample to remove any residual water.

5.6.2. Sample extracts are then transferred to a 50 mL centrifuge tube along with 2-3 thorough pentane rinses. Care must be taken to not transfer any sodium sulfate as this will cause "bumping" on the steam bath.

5.6.3. Sample extracts are concentrated on a steam bath to 1-2 mL with a hexane solvent exchange.

5.6.4 Pass extracts through micro columns (section 8).

5.8 Oil Extraction and Partitioning

Purpose: extract oil from sediment for the purpose of analyzing it as oil instead of sediment. Outcomes are expressed as ng/g oil and the matrix in the database is OIL. The advantage of this method is that hydrocarbon concentrations in oil samples can be tracked over time (assuming it is the same source oil), thus weathering processes can be monitored. The disadvantage is that this method does not provide an oil mass per unit sediment, so it does not estimate how oiled the beach substrate was unless sediment mass (or beach area) are measured independently.

Method abstract for manuscript

In brief, samples are thawed, homozenized, and subsampled. One subsample was used to calculate percent moisture (6 to 10 g). These were placed in tared aluminum weighing dishes, dried at 100 °C for 48 h and weighed to the nearest milligram.

The second subsample was used to extract oil. Typically about 40 to 50 g of homogenized sediment was placed in a 250 ml Teflon extraction vessel (range 10 to 100 g depending on oiling) and was dried with enough anhydrous sodium sulfate to eliminate water. The oil was extracted with 50 ml dichloromethane (DCM) with shaking and 30 minutes in an ultrasonic bath. Extracts were poured through a combusted, DCM-rinsed 17 cm glass fiber filter into 250 ml round bottom flasks. The remainder was re-extracted with 40 ml DCM and 20 minutes sonication and added to the first aliquot through the filter. The extraction vessel was rinsed twice with 25 ml DCM and filtered into the flask. Extract volumes were reduced by boiling at 80 °C and quantitatively transferred to 50 ml conical tipped centrifuge tubes and concentrated to approximately 5 ml. Aliquots were removed for gravimetric analysis and gas chromatography and exact total and aliquot volumes were recorded. Gravimetric measures were completed by allowing the DCM to evaporate from a tared aluminum weigh pan. Pans were then placed in a vacuum oven, evacuated to 50 mm Hg and allowed to remain 45 to 60 minutes without heat. The oven was subsequently vented and pans remained at atmospheric pressure for about 15 minutes before weighing in grams to the 5th decimal. The target oil mass for PAH and alkane analysis was 3 to 10 mg; appropriate volumes were removed from remaining aliquots, and spiked with 500 uL deuterated

5.8.1 Materials

The following labware are used to extract oil from sediment:

Aluminum weighing dishes
Spatulas: nichrome
Teflon bottle: 250 Ml, with caps and O-rings
Ultrasonic bath
Flat bottom flasks: 250 mL
Centrifuge tubes: KIMBLE, glass, 50 mL, tapered end
Funnels: glass, short, wide stem
Filter paper: WHATMAN, 15 cm glass microfiber, combusted at 440 °C for 4 hours
Electronic balance: top loading, sensitivity to 0.01 g
Boiling stones: SCIENCEWARE, Teflon, combusted at 440 °C for 4 hours
Syringes: 1 mL

All glassware are cleaned to insure they are free of hydrocarbons. Wash glassware with alconox (SUPELCO laboratory detergent) and tap water, dry, then combust in the muffle furnace for 4 hours at 440 °C. Solvent rinses of methylene chloride ($MeCl_2$) may be substituted for the combustion process if time does not allow for combustion. Store all hydrocarbon clean glassware in a clean environment. Clean Teflon bottles with alconox and water, dry, then rinse twice under a hood with 5 – 10 mL $MeCl_2$ and store in a clean environment.

5.8.2 Chemicals and Solvents

The following reagents and standards are used:

Sodium sulfate (Na_2SO_4): MALLINCKRODT, analytical grade, anhydrous, granular, combusted at 440 °C for 4 hours
Solvents: pesticide grade $MeCl_2$
Tissue/Sediment Surrogate Recovery Standard: see section 4.1.1 of SOP for the Analysis of Petroleum Hydrocarbons in Seawater, Marine Sediments, and Marine Faunal Tissue at the Auke Bay Laboratory (March 2010)
Tissue Reference Material and Standard: see section 4.2.1 of SOP (March 2010)
Tissue/Sediment Spiked Bland Material and Standard: see section 4.3.1 of SOP (March 2010)

5.8.3 Equations

Percent Moisture:

$$\%\, moisture = \frac{net\ wet\ weight\ (g) - \ net\ dry\ wet\ (g)}{net\ wet\ weight\ (g)} \times 100\%$$

Concentration of Oil in Extract:

$$[oil]\ (mg/mL) = \frac{net\ oil\ weight\ (g)}{volume\ of\ extract\ added\ to\ pan\ (mL)} \times \frac{1000\ (mg)}{1\ (g)}$$

Sample Aliquot for PAH Analysis:

$$aliquot\ (mL) = \frac{6\ (goal\ weight, mg)}{[oil](m\,g/mL)}$$

** Find an aliquot volume that maintains the goal weight range of 5 – 8 mg for the PAH sample while staying within a multiple of 50 µL for an accurate syringe transfer. Example: your concentration of oil in the extract is 35 mg/mL, and you start calculating your sample aliquot with a goal weight of 6 mg; this results in an aliquot of 0.1714 mL or ~170 µL. You can play around with that number to see what would be most efficient for transferring via syringe: an aliquot of 100 µL, 150 µL, or 200 µL. A 100 µL aliquot will only give you a sample weight of 3.5 mg, so you'll want to choose between 150 µL with a sample weight of 5.25 mg or 200 µL with a sample weight of 7 mg. The larger of the two might be best for the instrument detection limits.**

5.8.4 Sediment Subsamples

Samples are thawed and homogenized within their jars. Any oil that remains on the side of the jars is incorporated into the sediment. If an unusually large rock is in the sample, it is removed before subsampling.

A subsample is weighed for percent moisture determination: aluminum boats are labeled, weighed, and their weights recorded. A subsample of ~ 6 g is placed in a tared aluminum boat and its wet weight is recorded (net wet weight). The subsample in aluminum boat is then stored in a drying oven at 100 °C for 48 hours. The dry weight is recorded after allowing the subsample to come to room temperature, and the net dry weight is calculated by subtracting the aluminum boat's weight. Percent moisture is calculated (See equation 3.1).

A subsample is removed for extraction: depending on how oiled the sample is, a subsample of ~ 30 – 50 g is placed in a tared Teflon bottle, and the subsample weight is recorded. If the sample is heavily oiled, a smaller subsample is needed; if the sample does not look very oiled, a larger subsample is needed. For those samples where no oil is visible, a subsample of up to 90 g can be weighed out and extracted.

5.8.5 Sediment Subsample Extraction

After the sediment subsample is placed in the tared Teflon bottle and its weight is recorded, ~ 50 g of anhydrous Na_2SO_4 is added to the sediment in the extraction vessel. The contents are swirled vigorously to mix the sediment and the sodium sulfate. The sample is allowed to set for 5 – 10 minutes then checked for moisture; if the sample moves loosely like sand, no additional sodium sulfate is added, but if the sample has clumps, sodium sulfate is added until all clumps are broken up.

Figure 1. A flat-bottom flask with all four extracts poured in through the same filter/funnel combo per subsample.

50 mL of $MeCl_2$ are added to the dry subsample. The lid is placed tightly on the bottle and the sample is shaken to thoroughly mix the contents. The bottle is then placed in an ultrasonic bath for 30 minutes. About halfway through sonicating, the bottle should be shaken to dislodge any air bubbles, then carefully vented to release pressure.

A 15cm glass fiber filter is prepared for decanting the first extract through by placing it in a glass funnel, placing the funnel on top of a 250 mL flat bottom flask, and wetting the filter with a small amount of $MeCl_2$. The first extract is decanted through the filter into the flask. The filter is then rinsed with $MeCl_2$ and allowed to drain into the flask. Both funnel and filter paper stay with the flask for the next extraction (Figure 1).

40 mL of $MeCl_2$ are added to the subsample, which is then capped and shaken. The bottle is placed in an ultrasonic bath for 20 minutes, again allowed to vent part way through.

The filter in the funnel over the flask is rewetted with $MeCl_2$ and allowed to drain, then the second extract is poured through the filter while trying to keep the sediment in the bottle.

A third extract is prepared by adding 25 mL $MeCl_2$ to the subsample, capping and shaking the bottle, then pouring through the filter into the flask.

This is repeated for a fourth extract: 25 mL $MeCl_2$ is added to the subsample, mixed, and poured through the filter into the flask.

The subsample itself is now dumped into the filter paper from the bottle and rinsed with $MeCl_2$ until the extract dripping into the flask appears almost clear.

The extract is concentrated on a steam bath in the flat bottom flask. Once the volume appears to be less than 50 mL, the extract is transferred to a 50 mL centrifuge tube, with 2-3 rinses of $MeCl_2$ for a quantitative transfer. The extract is further reduced to approximately 7 mL.

5.8.6 Extract Partitioning

The ~ 7 mL extract is partitioned into three portions: one for SARA analysis, one for PAH and biomarker analysis, and one for gravimetric analysis. This must be performed efficiently so that as little evaporation as possible occurs since the concentrations should be the same in all three portions.

Prepare your equipment for extract partitioning while bringing the extracts to room temperature. For each sample, label two 2 mL screw top vials with the SIN and "1 of 2" for the SARA sample and "2 of 2" for the PAH sample. Label the backs of 70 mm aluminum weigh boats for each sample, crimp the top edge of the boats so that oil can't travel up over the sides (Figure 2), and record their weights from the 5 decimal analytical scale. Condition the 1 mL syringes with 3 rinses of $MeCl_2$.

For each extract, transfer aliquots of the extract to the vials and weigh boat using the 1 mL syringes. If the extract is heavily oiled (very dark) a smaller aliquot of 0.6 – 0.7 mL each can be used for SARA and PAH analyses; if the extract is very light a larger aliquot of up to 1.0 mL should be transferred for SARA and PAH analysis. The remaining extract in the centrifuge tube should be transferred to the aluminum weigh boat and the volume recorded to the lowest possible order. Rinse the syringe thrice with $MeCl_2$ once you are finished partitioning each sample.

<u>Gravimetric analysis</u>:
Allow the $MeCl_2$ to evaporate from the samples, then place the aluminum weigh boats in a vacuum oven. Evacuate the oven[7] to 50 mmHg and maintain the samples at that level, without heat, for 45 – 60 minutes.

Slowly vent the vacuum oven once the appropriate time has passed[8] and let the pans set for 15 minutes. Weigh and record the pan with oil on the 5 decimal analytical scale. The concentration of oil in the extract can now be determined (Equation 2), which can then used to calculate the total weight of oil in the sample.

See also large-scale gravimetric analysis (section 5.9)

PAH sample size and analysis

Calculate the amount of extract you will use for each PAH aliquot (Equation 3.3). The PAH sample weight goal is 5 – 8 mg, so you may need to finagle the math to find a straight-forward aliquot volume to transfer.

Prepare your equipment for transferring aliquots of the extract reserved for PAH analysis into centrifuge tubes. Label the centrifuge tubes, and clean two syringes (100 µL and 500 µL) with 3 rinses of $MeCl_2$ and lay them out on aluminum foil. Allow the sediment/tissue surrogate to come to room temperature and clean the sed/tis surrogate syringe with 3 rinses of $MeCl_2$ and 3 rinses of hexane.

Transfer the calculated amount of extract for each sample into the centrifuge tubes, rinsing the syringes with $MeCl_2$ between each transfer. Spike each sample with 500 µL tis/sed surrogate. Blow down the spiked sample to about 500 µL using a gentle stream of nitrogen (a strong stream may dry sample to the sides of the centrifuge tube), then solvent exchange the sample with several drops of hexane and again blow it down to ~500 µL, slightly swirling the sample to ensure any $MeCl_2$ on the bottom is evaporated[9].

The spiked samples in centrifuge tubes are now ready to be run through 6 g silica columns and eluted with 10 mL pentane (plus three 1 mL rinses) for the L-fraction and 20 mL 1:1 pentane:$MeCl_2$ for the R-fraction (see section 7)

5.9. Large-scale gravimetric analysis

Purpose. Measure oil content in large sediment samples (roughly 5 to 10 kg) to determine mass of oil in beaches.

Method.

- Weigh sediment sample to the nearest 5 grams.
- Add dichloromethane to just fill the container (4.7 L HDPE Plastic)
- Place in a sonicating bath and sonicate 1 hour
- Decant the extract through a cotton-plugged glass funnel overlain with anhydrous sodium sulfate.
- Collect the extract in a 2 L Erlenmeyer flask.
- Repeat the extraction process until the extract is colorless.
- Transfer the combined extract into a tarred, 3 L round-bottom flask with two side arms.
    - (Record the mass to the nearest 0.01 gram)
- Heat on a steam table to remove dichloromethane.
    - Continue heating until only viscous crude oil remains (the "burble stage")
- Cool the flask and vent overnight to remove remaining dichloromethane
- Weigh to the nearest 0.01 gram (gross mass)
- Subtract the tare mass from the gross mass to calculate oil mass.

6. Column Chromatography: Tissue

There are three types of columns, one for tissue, one for sediment, and one for passive samplers. This section describes the columns used for tissue. Until about 2009, columns used to fractionate sediment were the same as those described for tissue.

Tissue samples are separated into aliphatic and aromatic components by fractionation on a silica gel and alumina chromatography column. Fractionation occurs after sample extraction and concentration; see section 5.1.8.

6.1 Glassware and Apparatus

All glassware and apparatus are cleaned as in section 5.1.1.

The following labware are used in these methods for the fractionation and purification of samples by silica gel/alumina column chromatography:

Liquid chromatography columns: glass, 20 mm id, 300 ml, Teflon stopcock
Centrifuge tubes: KIMBLE, glass, 50 ml, tapered end
Flat bottom flasks: PYREX, 500 ml
Pasteur pipettes: glass, capillary, 1 ml
Glass wool: combusted at 440° 4 hours
Screwtop vials: SUPELCO, 2 ml glass with Teflon lined cap
Electronic balance: top loading, sensitivity to 0.01 g
Glass stirring rod
Beakers: 150 ml and 250 ml
HPLC vials: 620 µl, amber glass, tapered end
Aluminum foil

6.2 Chemicals and Solvents

The following reagents and standards are used in these methods for the fractionation and purification of environmental samples by silica gel/alumina column chromatography:

Alumina: SIGMA Chemical Co., activity grade 1, type WB-2, activation is described in section 6.3.1
Silica gel: DAVISON Chemical Co., basic, 100-200 mesh, grade 923, activation is described in section 6.3.2
Sand: EM Science, #SX0070-3, combusted at 440° C for 4 hours
Sodium Sulfate (NA_2SO_4): MALLINCKRODT, analytical grade, anhydrous, granular, combusted at 440° C for 4 hours
Solvents: pesticide grade pentane, $MeCl_2$, and methanol

Tissue/Sediment Spiked Blank Standard: see section 4.3.1
Copper: granular, activated by stirring in concentrated HCl for 5 minutes, then rinsed with distilled water to neutralize, methanol to dry, and $MeCl_2$ for final solvent
HPLC Internal Standard: see section 4.4.1
GC/FID Internal Standard: see section 4.4.2

6.3 Solid Phase Preparation (tissue)

This SOP describes the methods used to prepare an amount of silica gel and alumina sufficient to fractionate one tissue string. Each batch prepared according to these procedures is evaluated for proper activation before it is used for a sample string.

6.3.0 Copper activation.

a. Cover the desired amount of copper with concentrated (~35%) HCL.
b. Stir the mixture with a glass rod periodically for 5 minutes
c. With water running in hooded sink, pour off HCL
    i. Allow water to run in the sink for at least 5 minutes after discarding the acid to purge the drain.
d. Rinse the copper several times with MeOH to remove the water
e. Rinse the copper several times with methylene chloride ($MeCl_2$)
f. Keep the copper covered in $MeCl_2$ until ready for use.

6.3.1 Alumina activation.

Approximately 200 grams of alumina are baked at 170° C overnight. The exact weight is recorded and the alumina is allowed to absorb 2% of its weight in moisture. It is stored in a tightly stoppered container at 120° C. Activate alumina as described for silica (below), except activate to 2%.

6.3.2 Silica activation

Approximately 400 grams of silica gel are baked at 400° C for 4 hours. The exact weight is recorded and the silica gel is allowed to absorb 5% of its weight in moisture. It is stored in a tightly stoppered container at 120° C.

Silica activation.
a. Pour baked silica onto tared, clean, foil-lined pans.
b. Record weight and calculate 5% of its mass.
c. Place pan in steam bath hood with the bath ON to create steam
d. Allow the silica gel to absorb 5% of its weight in moisture
    i. Gently stir the silica periodically through this process to expose new particles

ii. Be careful not the weigh any moisture that may have condensed on the pan

e. Transfer and store in an airtight reagent bottle in the oven at ~100°C.
   i. Sometimes the top may pop off; cover the stopper and neck with foil and crimp to secure.

6.3.3 Every batch of silica gel and alumina is evaluated for proper activation levels before it is used to fractionate a sample string. A test column is packed and a test solution is fractionated as if it were a sample.

1. A chromatography column is prepared as described in section 6.4 using the new batches of silica gel and alumina.

2. A test sample and a test standard are made by spiking 500 µl aliquots of Tissue/Sediment Spike Blank Standard (see section 4.3.1) into 2 vials each containing approximately 0.5 ml of hexane.

3. The test sample is loaded onto the test column and eluted as described in section 6.5.

4. Both fractions of the test sample are concentrated and the solvent is changed as described in section 5.1.8.

5. The aliphatic fraction is prepared for instrumental analysis by adding the GC/FID Internal Standard as described in section 6.6.2. The aromatic fraction is prepared for instrumental analysis by adding the GC/MS Internal Standard as described in section 9.7. The test standard receives both the GC/FID and the GC/MS Internal Standard.

6. The aliphatic fraction of the test sample and an aliquot from the test standard are analyzed by GC/FID as described in section 10. The aromatic fraction of the test sample and an aliquot from the test standard are analyzed by GC/MS as described in section 11.

7. The percent of each analyte recovered is calculated by the equation below.

$$\text{Test Column Percent Recovery} = \left(\frac{\left(\frac{Area_x}{Area_{IS}} \right)}{\left(\frac{Area_{x_S}}{Area_{IS_S}} \right)} \right) X\ 100$$

Where:

$Area_x$ = Area of Analyte x in the Test Sample

$Area_{IS}$ = Area of Internal Standard in Test Sample

$Area_{xS}$ = Area of Analyte x in the Test Standard

$Area_{ISS}$ = Area of Internal Standard in the Test Standard

8. The batches of silica gel and alumina are acceptable if the recovery of analytes exceeds 85%. Exceptions in this criterion apply to late eluting aromatic analytes whose recovery is greatly influenced by the presence or absence of a matrix. These recoveries are frequently lower than 85%; prudent judgement by the analyst must be employed.

6.4 Packing and conditioning chromatography columns (tissue)

Chromatography columns packed with silica gel and alumina are used to fractionate tissue and sediment strings into their aliphatic and aromatic components. The solid phases must be activated as described in sections 6.3.1 and 6.3.2 and must be determined acceptable according to the criterion in section 6.3.3 step 8. This section describes the methods used for column packing and column conditioning.

1. A small ball of glass wool is packed into the glass chromatography column near the stopcock to inhibit any loss of column packings. The column and glass wool are rinsed with $MeCl_2$. The rinsate is allowed to drain.

2. 20 ml $MeCl_2$ are added to the column but not allowed to drain.

3. 10 g of alumina are weighed and added slowly to the column. A gentle agitation of the column distributes the alumina evenly. The sides of the column are rinsed with 5-10 ml $MeCl_2$.

4. 20 g of silica gel are weighed in a 250 ml beaker. $MeCl_2$ is added to the beaker until a thin slurry is created. The slurry is poured into the column on top of the alumina.

5. The sides of the column are rinsed with $MeCl_2$ and the solvent is drained to about 1 inch above the silica gel. For tissue samples, 5 g of sand is added to the top of the column. For sediment samples, a 2-3 cm layer of Na_2SO_4 is added followed by a 1 cm layer of activated copper granules.

6. 50 mls of $MeCl_2$ are added and drained to a level just above the top of the column packing.

7. 100 mls of pentane are added and drained at a flow rate of approximately 2 mls per minute. The flow is stopped when the pentane level just covers the top of the column packing.

6.5 Sample loading and elution (tissue)

Tissue samples are fractionated into their aliphatic and aromatic components by using the column loading and elution procedures outlined below. The solvent in the columns should never be allowed to drain to a level below the solid phase.

1. Samples from a tissue string, after preparation as described in sections 5.1.8 and 5.2.7, respectively, are transferred onto silica gel/alumina columns for fractionation. The transfer is accomplished by pipetting the sample from the centrifuge tube to the chromatography column. The centrifuge tube is rinsed

3 times with 1 ml of pentane. Each rinsate is added to the column.

2. The level of the solvent is drained to the top of the column solid phase.

3. 50 mls of pentane is added to the column and eluted at a flow rate of approximately 2 mls/min. The eluant is collected in a 50 ml centrifuge tube and constitutes the aliphatic fraction of the sample.

4. The centrifuge tube is labeled with the appropriate SIN and an "L" which designates it as an aliphatic fraction.

5. 250 mls of a 1:1 mixture (by volume) of pentane and $MeCl_2$ are added to the column and eluted at a flow rate of approximately 2 mls/min. The eluant is collected in a 250 ml flat bottomed flask and constitutes the aromatic fraction of the sample.

6. The flask is labeled with the appropriate SIN and with an "R" which designates it as an aromatic fraction.

7. Both fractions of each sample are sealed with foil lined caps or ground glass stoppers and are stored at −20° C.

6.6 Post-fractionation concentrations

After fractionation on the silica gel/alumina column the aliphatic and aromatic fractions are treated differently. The aromatic fraction is concentrated and prepared for further purification by high performance liquid chromatography (HPLC) (see section 9). The aliphatic fraction is concentrated and prepared for analysis by GC/FID (see section 10).

6.6.1 Aromatic fraction concentration

No solvent exchange is required in the preparation of aromatic fractions for HPLC purification.

1. Aromatic fractions are concentrated by adding 2-3 boiling stones to the flat bottom flasks and placing them on a steam bath at approximately 80° C. The extracts are boiled until the volume is reduced to approximately 10 ml.

2. The extract is transferred quantitatively using $MeCl_2$ to a 50 ml centrifuge tube by either pouring or pipeting.

3. The extract is reduced to 0.5 ml.

4. Each extract receives a 50 µl spike of the HPLC Internal Standard (see section 4.4.1). The extract and spike are mixed for 5 seconds to homogenize the

solution.

5. The spiked extracts are transferred into 620 µl HPLC vials and sealed with foil and teflon tape. The extracts are stored at -20° until ready for HPLC purification (see section 9).

6.6.2 Aliphatic fraction concentration

1. Aliphatic fractions are concentrated and the solvent is exchanged as described in section 5.1.8.

2. Each extract is spiked with 50 µl of the GC/FID Internal Standard (see section 4.4.2).

3. The spiked extracts are prepared for analysis by GC/FID by repeating the procedures described in section 5.3.6, steps 4-7.

4. Store all sample extracts at -20° C until ready for instrumental analysis (see section 10).

7. Column Chromatography: Sediment

There are three types of columns, one for tissue, one for sediment, and one for passive samplers. This section describes the columns used for sediment. Until about 2009, columns used to fractionate sediment were the same as those described for tissue (Section 6).

Sediment samples are separated into aliphatic and aromatic components by fractionation on a silica gel column. Fractionation occurs after sample extraction and concentration; see section 5.4 for ASE extraction protocol.

7.1 Glassware and Apparatus

All glassware and apparatus are cleaned as in section 5.1.1.

The following labware are used in these methods for the fractionation and purification of samples by silica gel column chromatography:

Liquid chromatography columns: glass, 12 mm id, 200 ml, Teflon stopcock
Centrifuge tubes: KIMBLE, glass, 50 ml, tapered end
Pasteur pipettes: glass, capillary, 1 ml
Glass wool: combusted at 440° 4 hours
Screwtop vials: SUPELCO, 2 ml glass with Teflon lined cap
Electronic balance: top loading, sensitivity to 0.01 g
Glass stirring rod
Beaker: 100 ml
Aluminum foil

7.2 Chemicals and Solvents

The following reagents and standards are used in these methods for the fractionation and purification of environmental samples by silica gel/alumina column chromatography:

Silica gel: DAVISON Chemical Co., basic, 100-200 mesh, grade 923, activation is described in section 6.3.2
Sodium Sulfate (NA_2SO_4): MALLINCKRODT, analytical grade, anhydrous, granular, combusted at 440° C for 4 hours
Solvents: pesticide grade pentane, $MeCl_2$, and hexane
Copper: granular, activated by stirring in concentrated HCl for 5 minutes, then rinsed with distilled water to neutralize, methanol to dry, and $MeCl_2$ for final solvent
GC/MSD Internal Standard: see section 4.4.3
GC/FID Internal Standard: see section 4.4.2

7.3 Solid Phase Preparation (Sediment)

This SOP describes the methods used to prepare silica gel fractionate a sediment string.

7.3.1 Silica gel is baked at 170° C 16 hours and is stored in a tightly stoppered container at a temperature of 100° C. No deactivation is allowed to occur.

7.4 Packing and conditioning chromatography columns (Sediment)

Chromatography columns packed with silica gel are used to fractionate sediment samples into their saturated and aromatic components. The silica gel must be activated as described in sections 7.3.1. This section describes the methods used for column packing and column conditioning. All packing and conditioning solvent is drained to waste.

1 A small ball of glass wool is packed into the glass chromatography column near the stopcock to inhibit any loss of column packings. The column and glass wool are rinsed with $MeCl_2$. The rinsate is allowed to drain.

2. 5 ml $MeCl_2$ are added to the column but not allowed to drain.

3. 6 g of silica gel are weighed into a 100 ml beaker. About 10 ml of $MeCl_2$ is added to the beaker and stirred with a glass stir rod until any gas bubbles are released. The mixture is poured into the column and the sides of the column are rinsed with $MeCl_2$.

4. The solvent is drained to 5-10 cm above the silica gel. Anhydrous Na_2SO_4 is sprinkled into the column to form a layer of about 1 cm. The sides of the column are rinsed.

5. Copper activated as described in section 6.2 is added to form a layer of about 1 cm.

6. The sides of the column are rinsed and the $MeCl_2$ is drained to just above the copper.

7. 15 mls of $MeCl_2$ are added and drained to a level just above the top of the column packing.

8. Three 1 ml volumes of pentane are added consecutively to rinse the residual $MeCl_2$ from the sides of the glass column. Each 1 ml volume is allowed to drain to the top of the copper before the next is added.

9. Ten (10) mls of pentane are added to the column and allowed to drain to the top of the copper. A flow rate of about 2 ml/min should be maintained to insure the $MeCl_2$ is pushed through.

10. Another 15 mls of pentane is added and allowed to drain. The $MeCl_2$ needs to be completely removed for proper elution. The flow is stopped when the pentane level just covers the top of the column packing.

7.5 Sample loading and elution (Sediment)

Sediment extracts are fractionated into their saturated and aromatic components by using the column loading and elution procedures outlined below. The volume of solvents used should be as accurate as possible to affect proper fractionation. The solvent in the columns should never be allowed to drain to a level below the solid phase.

1. Sediment extracts, after extraction and preparation as described in section 5.4 are transferred onto the silica gel/copper columns for fractionation. The transfer is accomplished by pipetting the sample from the centrifuge tube onto the chromatography column. Care should be taken to drip the extract directly onto the copper minimizing contact between the extract and potential active sights on the glass sides of the column.

2. Drain to waste until the level of the extract is at the top of the copper. Exchange the collection vessel to a clean centrifuge tube labeled with the appropriate SIN and the letter L. (L designates it as the aliphatic/saturated fraction.)

3. Rinse the original extract vessel 3 times with 1 ml volumes of pentane. Transfer each rinse to the column rinsing down the sides of the column to insure all the extract is loaded onto the column. Drain each rinse to the level of the copper before the next is added collecting all in the same collection vessel.

4. Ten (10) mls of pentane is added to the column and eluted at a flow rate of approximately 2 mls/min. (The total volume of eluant is approximately 13 mls, i.e., 3 rinses and bulk elution.) The solvent should be drained down to the level of the copper.

5. The flow should be stopped and the collection vessel exchanged for a centrifuge tube is labeled with the appropriate SIN and the letter R. (R designates it as the aromatic fraction.)

6. Twenty (20) mls 1:1 mixture (by volume) of pentane and $MeCl_2$ is used to elute the aromatic fraction. Several small volumes (approximately 1m) of the

mixture are added and drained to the top of the copper before the bulk of the mixture is added. (There are some components of the oil that are soluble only in the $MeCl_2$ and these initial small volumes will push these components onto the column. If this is not done, these components will dissolve into the elution solvent.) The column is eluted as a flow rate of approximately 2 mls/min. and is allowed to flow until the column is dry.

7. Both fractions of each sample are sealed and are stored at −20° C. Until they are volume reduced, solvent exchanged and spiked with internal standard.

7.6 Post-fractionation concentrations (Sediment)

After fractionation on the silica gel column the saturated and aromatic fractions are volume reduced and solvent exchanged to hexane. Both fractions are spiked with their respective internal standard in preparation for instrumental analysis.

7.6.1 The aromatic fraction is concentrated on a steam bath at a temperature that maintains the extract at a gentle boil. When the extracts have been reduced to 2-3 ml, 1 ml of hexane is added and the volume reduction continues to a volume of 1 ml. The extract is removed from the heat and spiked with the internal standard hexamethylbenzene (HMB) in the amount of 2000 ng. The extract is transferred to a 2 ml screw top vial and stored in the freezer until analysis by GC/MSD for PAH, (see section 11).

7.6.2 The saturated fraction is concentrated and solvent exchanged as described in section 7.6.1 and spiked with the internal standard dodecylcyclohexane (DCH) in the amount of 2570 ng. The extract is transferred to a 2 ml screw top vial and stored in a freezer until GC/FID analysis for alkanes, (see section 10) or GC/MSD analysis for biomarkers (see section12).

8. C Column Chromatography: Passive Samplers

There are three types of columns, one for tissue, one for sediment, and one for passive samplers (LDPEs = PEMDs). This section describes the columns used for LDPEs.

8.1 Glassware and Apparatus

Glassware and apparatus are cleaned to insure that they are free of hydrocarbons and to eliminate them as a source of sample contamination. Glassware is washed with ALCONOX, laboratory detergent by SUPELCO, and rinsed with tap water. The glassware is then combusted at 440°C for at least 4 hours. Solvent rinses of acetone and methylene chloride ($MeCl_2$) may be substituted for the combustion process if time does not allow for combustion. All combusted glassware are stored in a clean environment and sealed with combusted aluminum foil. Apparatus which are not glass are washed with ALCONOX, rinsed with tap water, rinsed with acetone and $MeCl_2$, and stored in a clean environment.

The following labware are used in these methods for the extraction of LDPE strips.

Centrifuge tubes: KIMBLE, glass, 50 mL, tapered end
Glass wool: combusted at 440° 4 hours
Screwtop vials: SUPELCO, 2 mL glass with teflon lined cap
HPLC vials: 620 µL, amber glass, tapered end
Sonic Bath with timer
Kimwipes
Aluminum foil
Centrifuge tubes: thick walled glass, 100 mL
Syringe: HAMILTON, 500 µL, 50 µL, and 25 µL
Pasteur pipets: glass, capillary, 1 mL
Boiling stones: SCIENCEWARE, teflon

8.2 Chemicals and Solvents

The following reagents and standards are used in these methods for the purification of LDPE samples:

Silica gel: DAVISON Chemical Co., basic, 100-200 mesh, grade 923, activation is described in section 6.3.2
Sodium Sulfate (NA_2SO_4): MALLINCKRODT, analytical grade, anhydrous, granular, combusted at 440° C for 4 hours
Solvents: pesticide grade pentane, $MeCl_2$, and methanol
HPLC Internal Standard: see section 4.4
GC/FID Internal Standard: see section 4.4
PAH Surrogate Recovery Standard: see section 4.1

LDPE Native Analyte Solution: see section 4.2

8.3 Solid Phase Preparation (LDPE)

8.3.1 Approximately 200 g of silica gel are baked at 400° C for 4 hours. The exact weight is recorded and the silica gel is allowed to absorb 5% of its weight in moisture. It is stored in a tightly stoppered container at 105° C.

8.3.2 Every batch of silica gel is evaluated for proper activation levels before it is used to purify a sample string. A test column is packed and a test solution is fractionated as if it were a sample.

1. A micro silica column is prepared as described in section 8.4 using the new silica gel.

2. A test sample and a test standard are made by spiking 100 µL aliquots of LDPE Native Analyte Solution (section 4.2.3) into 2 vials each containing approximately 0.5 mL of hexane.

3. The test sample is loaded onto the test column and eluted as described in section 8.5.

4. The purified test sample is concentrated and a 1 mL hexane solvent exchange is completed.

5. The test sample is prepared for instrumental analysis by adding the GC/MS Internal Standard as described in section 4.5.2. The test standard receives the GC/MS Internal Standard also.

6. An aliquot of the test sample and an aliquot of the test standard are analyzed by GC/MS as described in section 11.

7. Percent recovery is calculated as described in 6.3.3 step 7.

8. The batches of silica gel are acceptable if the recovery of analytes exceeds 85%. Exceptions in this criterion apply to late eluting aromatic analytes whose recovery is greatly influenced by the presence or absence of a matrix. These recoveries are frequently lower than 85%; prudent judgement by the analyst must be employed.

8.4 Micro silica columns are prepared as follows:

A. Pack a small plug of glass wool into the tapper of a short disposable pasture pipet.

B. Add 1.5 - 2 grams 5% deactivated silica gel (see sec. 6) to the pipet using another pipet with a bulb.

C. Add ca. 1 cm of sodium sulfate to the top of the silica gel. Tap the column gently.

D. Attach the "solvent reservoir" to the silica column and wash the column with 6-8 mL of 100% MeCl2.

E. "Condition" the column with 6-8 mL of 100& pentane being careful not to let the columns go dry in between solvent additions.

8.5 Sample loading and elution (LDPE)

8.5.1 Apply the concentrated sample extract to the column and replace the waste container with a labeled 50 mL centrifuge tube. Add two small pentane rinses of the sample tube.

8.5.2. Elute the column with a 17-19 mL of 50/50 Pentane/ MeCl2.

8.6 Post-fractionation concentration (LDPE)

8.6.1 Concentrate the samples over a steam bath at medium heat (between 200 - 225° F) and solvent exchange to hexane for a final volume of 1 mL.

8.6.2 Each sample in the sample string, environmental and QA, is spiked with 25 µL of the GC/MS Internal Standard, HMB, (see section 4.4):

A. Clean a 25 µL syringe by rinsing at least 3 times with MeCl2 then hexane.

B. Spike each sample with 25 µL HMB while it is still in the centrifuge tube. Be sure to spike onto the extract and not down the sides of the tube. Take care to keep the syringe from touching the sample or tube.

C. Vortex the samples for approximately 5 seconds to insure thorough mixing of the internal standard and sample extract.

D. Transfer the extracts to labeled 2 mL glass screw top vials using Pasteur pipets. Use a different pipet for each sample. Allow the samples to further concentrate to approximately 1 mL by leaving uncapped in the fume hood. Cap with Teflon lined caps and store at -20° C.

E. Prepare the extracts for instrumental analysis by transferring an aliquot to a brown crimp top vial fitted with a 100 μL insert. The vial should be labeled with the appropriate SIN. Seal the vial with a rubber septa crimp top. Store in the freezer until designated for instrumental analysis.

F. See section 11 for measurement of aromatics by GCMSD.

9. HPLC Purification of Aromatic Hydrocarbon Samples

The aromatic fractions of tissue hydrocarbon samples are purified by High Performance Liquid Chromatography (HPLC) after they are fractionated from their aliphatic components by column chromatography (see section 6). The elution scheme used for fractionation, see section 6.5, allows matrix interferents to coelute with the aromatic component of each sample; therefore, an additional purification procedure is required. The size exclusion HPLC columns used in this procedure separate the aromatic compounds from the matrix interferents. This procedure is used only for tissue samples.

9.1 HPLC System

The HPLC system used by ABL for hydrocarbon sample purification consists of the following components:

GILSON programmable autosampler: model 231
GILSON dilutor: model 401 dilutor
SPECTRA PHYSICS pump: model 8800
PHENOMENEX guard column: phenogel, 7.8 x 50 mm, 100 angstrom, size exclusion
PHENOMENEX HPLC columns: phenogel, 22.5 x 250 mm, 100 angstrom, size exclusion gel, 2 columns in sequence
SPECTRA PHYSICS UV-vis detector: model 100, variable wavelength
ISCO Foxy-200 programmable fraction collector
SPECTRA PHYSICS Chromjet Integrator

9.2 HPLC Operating Parameters

The operating parameters used in these HPLC purification methods are listed below:

Mobile phase: $MeCl_2$
Isocratic Elution
Temperature: 20° C
Flow rate: 7 ml/min
UV wavelength: 254 nm
Chart speed: 0.5 cm/min
Attenuation: 8
Injection volume: 500 µl

9.3 Glassware

All glassware are cleaned as described in section 5.1.1.

HPLC vials: 620 µl, amber glass, tapered end
Centrifuge tubes: KIMBLE, glass, 50 ml, tapered end
Pasteur pipets: glass, capillary, 1 ml
Screwtop Vials: SUPELCO, 2 ml glass with teflon lined cap

9.4 Chemicals and Solvents

The following reagents and standards are used in these methods for the purification of the aromatic fractions of hydrocarbon samples:

Helium: high purity
Solvents: Pesticide grade $MeCl_2$, for mobile phase and autosampler rinsate.
GC/MS Internal Standard: see section 4.4.3
HPLC Calibration Standard: see section 4.6

9.5 Purification of Aromatic Hydrocarbon Samples

All aromatic samples processed by the HPLC methods described in this section are prepared as described in section 6.6.1.

1. The mobile phase ($MeCl_2$) is degassed with helium.

2. The pump is set to a flow rate of 7 ml/min. and the columns are allowed time (approximately 10 minutes) to equilibrate.

3. The UV detector is turned on and the lamp is allowed to warm (approximately 5 minutes).

4. After the HPLC system has stabilized, the detector is zeroed and the HPLC is calibrated to determine collection times. For an initial calibration, the method described in section 9.6, steps 1-8, is used. (This is necessary only the first time samples are analyzed by these SOPs or if there has been change in the system which dramatically changed retention times.) For a routine calibration, the method described in section 9.6, step 8, is used.

5. Each sample is injected into the HPLC system. The aromatic fraction is collected during the time window that has been determined in the calibration process.

6. The autosampler is programmed to inject a HPLC standard after every 4 sample injections. The resultant data is used to verify that no shift in retention time has occurred.

7. If a retention time shift of more than ±0.2 min. occurs, the run is aborted and the system is recalibrated.

8. The aromatic fractions are concentrated as described in section 5.1.8.

9. The aromatic fractions are spiked with 25 µl of GC/MS Internal Standard (see section 4.4.3).

9.6 HPLC Calibration

An initial calibration of the HPLC system typically needs to be completed only once. The initial calibration will establish an approximate collection window that will be adjusted for routine use by employing a less rigorous calibration, i.e., step 9. Both procedures are outlined below.

1. Six HPLC vials are filled with 500 µl of the HPLC Calibration Standard (see section 4.6) and loaded onto the autosampler. The fraction collector is loaded with 20 centrifuge tubes.

2. The HPLC autosampler is programmed to inject the first 5 calibration standards without collecting any eluant. The retention times of the 3 components of the calibration standards are monitored by the integrator and should stabilize to within ±0.1 min. after 3-5 injections. If the retention times do not stabilize, the system should be allowed more time to equilibrate and the calibration procedure should be repeated.

3. The average retention time for biphenyl, the earliest eluting component of the calibration standard, is calculated. The average retention time for perylene, the latest eluting component of the calibration standard, is calculated.

4. The HPLC is programmed to inject the last calibration standard. The fraction collector is programmed to collect 10 consecutive 0.1 minute fractions beginning one minute before the average retention time that was calculated for biphenyl and also to collect 10 consecutive 0.1 minute fractions beginning at the average retention time that was calculated for perylene. The collection window for each fraction is recorded.

5. Each of the 20 fractions collected in step 4 are spiked with 25 µl of HMB, section 4.4.3, and are analyzed by GC/FID.

6. The retention time that began the collection window for the fraction where biphenyl is first detected by the GC/FID is determined. This retention time is designated as T1 on a HPLC trace of the HPLC calibration standard, figure 7.a.

A safety margin of 0.2 minutes is subtracted from T1. The result is the retention time, T2, for the beginning of the hydrocarbon sample collection window.

7. The retention time that ended the collection window for the fraction where perylene is last detected by the GC/FID is determined. This retention time is designated as T3 on figure 7.a. A safety margin of 0.2 minutes is added to T3. The result is the retention time, T4, for the end of the hydrocarbon sample collection window.

8. The total collection time for the purification of hydrocarbon samples is T2 through T4. This collection window is designated as T5 in figure 7.a.

9. Daily calibration of the HPLC for the purification of hydrocarbon samples requires the injection and integration of 4 HPLC calibration standards. If the retention time of biphenyl (T6) has changed more than 0.05 min. from the previous run, the collection window (T5) must be adjusted. If the retention times have increased, the amount of the increase is added to T2 and T4, shifting the collection window later. If the retention times decreased, the amount of the decrease is subtracted from T2 and T4, shifting the collection window earlier. If the average value of either T6 or T7 has changed by more than 0.5 min., a problem with the HPLC system is indicated. Diagnostics and maintenance are then performed according to the manufacturer's specifications. The system is again calibrated before proceeding with hydrocarbon sample purification.

9.7 Concentration of Aromatic Fraction for GC/MS Analysis

1. The samples are concentrated and the solvent is exchanged as described in section 5.1.8, steps 3 and 4.

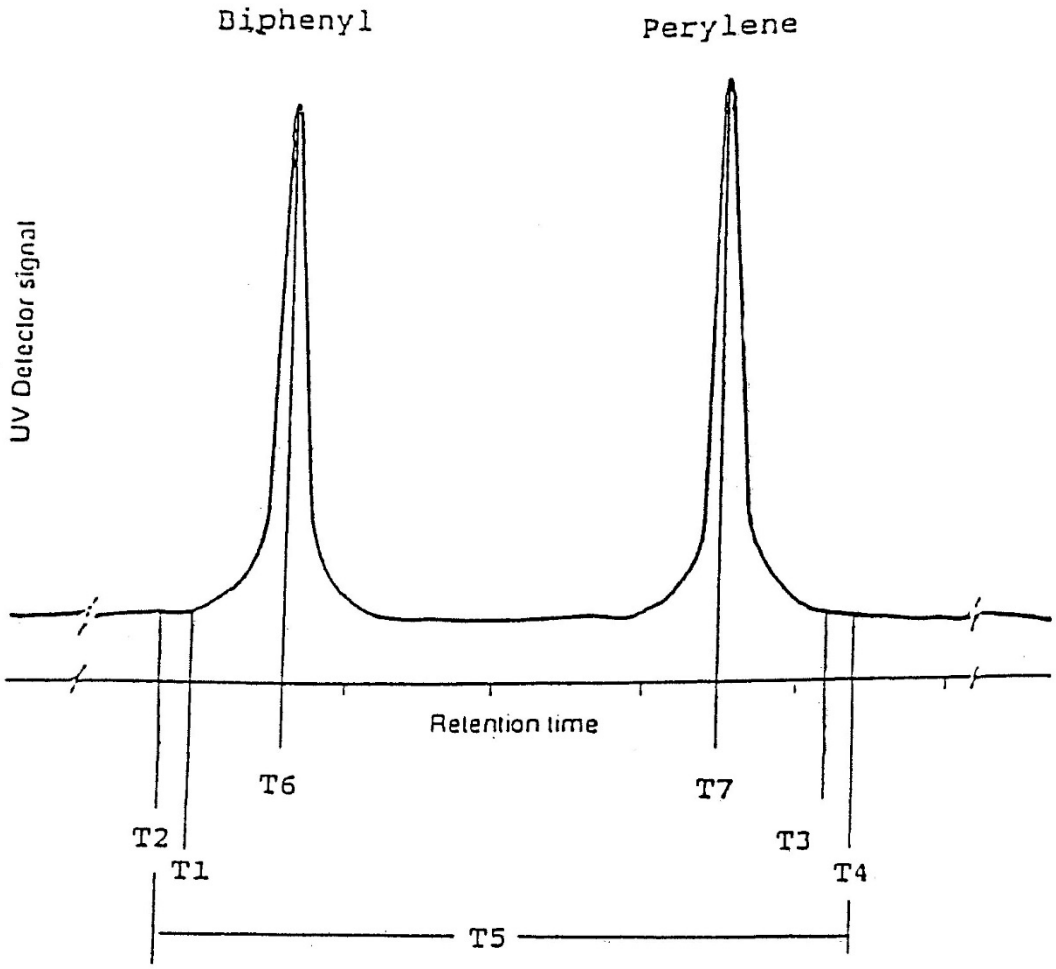
2. Each sample is spiked with 25 µl of GC/MS IS (see section 4.4.3).

3. The spiked extracts are prepared for analysis by GC/MS by repeating the procedures described in section 5.3.6, steps 4-7.

4. Store all sample extracts at -20° C until ready for instrumental analysis (see section 11).

Figure 9.a

HPLC Chromatographic Trace



10. Instrumental Analysis of Aliphatic Hydrocarbons

The instrumental analysis method outlined in this section describes the conditions by which analytical data are acquired from the aliphatic fraction of hydrocarbon samples. Hydrocarbons targeted for analysis include the n-alkanes, C9- C36, as well as pristane and phytane. Each of these compounds, except phytane, is incorporated into the calibration standards, and hence has a calibration curve established for its quantitation. Phytane is quantitated using the mean of the regression statistics of the C18 and C19 calibration curves. These analytes will be referred to as calibrated analytes in these SOPs.

The data acquired by the instrumental analysis method described in this section will be quantitatively analyzed by the methods described in section 13.

10.1 GC/FID System

AGILENT Gas Chromatograph: model 7890A, equipped with a Flame Ionization Detector
AGILENT Auto Injector: model 7683B
AGILENT Communication Software: Chemstation
AGILENT Ultra II capillary column: 5% phenylmethyl-silicone, .20 id, 25 m, .33 micron film thickness

10.2 GC Conditions

The GC is programmed to operate under the following conditions:

Injection volume: 1 µl
Injection mode: splitless
Injector temperature: 300° C
Detector temperature: 320° C
Initial oven temperature: 60° C
Initial hold time: 1.00 min.
Temperature ramping rate: 6° C/min.
Final oven temp.: 300° C
Final hold time: 26.0 min.

Carrier gas: Helium, 0.80 ml/min.
Make-up gas: Nitrogen, 34 ml/min.
Detector gases: Hydrogen, 33 ml/min.
Compressed Air, 410 ml/min.

10.3 Analysis Sequence

A sample string is loaded on to the autosampler and the system is programmed to analyze the samples in the following order:

Analysis Sequence

Hexane Blank
Aliphatic Calibration Standard #1
Aliphatic Calibration Standard #2
Aliphatic Calibration Standard #3
Aliphatic Calibration Standard #4
Aliphatic Calibration Standard #5
6 Environmental Samples
Aliphatic Calibration Standard #3
Method Blank (MBLK)
Aliphatic Reference Material (AREF)
6 Environmental Samples
Spiked Blank (SBLK)
Aliphatic Reference Material (BREF)
Aliphatic Calibration Standard #3

This analysis sequence constitutes an entire sample string and is described in detail in section 3.

The aliphatic fractions of samples analyzed by this sequence are prepared as described in section 6.6.2 for tissue, section 7.6.2 for sediment samples, and in section 5.3.6 for water samples.

Water strings are analyzed by the same analysis sequence but with the deletion of the SBLK.

10.4 Sample Analysis

All the samples of a sample string are analyzed according to the parameters described sections in 10.2 and 10.3. A data file for each sample is generated during analysis. The data file will include the retention time (rt), peak area, and identity of each calibrated analyte found in the sample. The data file will also include the rt and area of each uncalibrated peak as well as the sum of all peak areas. Data from these data files will be extracted and used in the quantitation procedures described section 13.

10.4.1 Calibration

A calibration table is established prior to sample analysis to insure the proper identification of calibrated analytes. The calibration table established here is not used for quantitation, but merely to identify calibrated analytes by their retention times (rt). The calibration table contains an average rt for each calibrated analyte and is set up according to instructions in

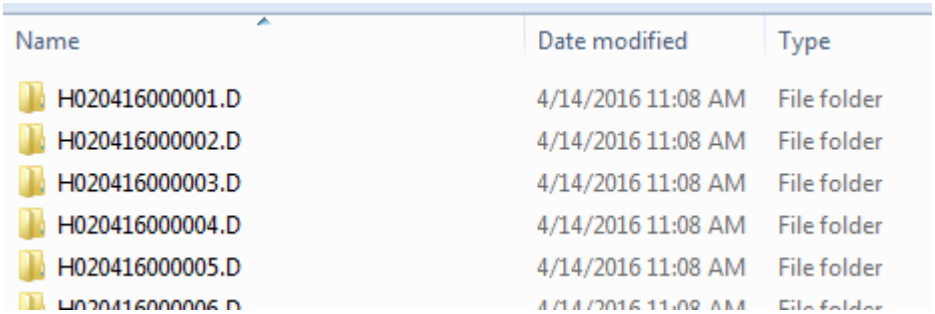
the GC/FID Operator's Manual. A window of ± 0.25 % around the calibrated retention time is searched; if a peak is found, it is identified as the respective calibrated analyte.

10.4.2 Unresolved Complex Mixture

The unresolved complex mixture (UCM) of each sample is calculated after sample analysis. A BASIC program is used to determine the total alkane and UCM area. Isolation of the UCM area from the total alkane area is performed during data analysis, section 13.

10.5 Data files

Data files on the instrument will be similar to the example illustrated below. These are all subdirectories filed under the appropriate string name (20160204LH in this example).



1) Confirm Raw Data (FID hardcopies)
   a) UCMs
      i) Baseline area from a blank or standard is subtracted from each sample and QA sample. Check the math.
   b) ID of analytes and surrogates and Internal Standard (IS.)
      i) Using the retention times in a calibration standard report confirm that the peaks in the samples are flagged appropriately.
         (1) Make corrections in the electronic files if necessary.
   c) copy files to drive and memory stick

See Chapter 13 for quantification procedures.

11. Instrumental Analysis of Polynuclear Aromatic Hydrocarbons (PAH)

The instrumental analysis method outlined in this section describes how analytical data are acquired from the aromatic fraction of hydrocarbon samples. Hydrocarbons targeted for analysis include 25 PAHs and dibenzothiophene. Each of these compounds is incorporated into the calibration standards, and hence has a calibration curve established for its quantitation. These analytes will be referred to as calibrated analytes in these SOPs. The alkylated homologues of 8 of the calibrated analytes are also targeted for analysis. These homologues are quantified, not individually, but as a group per their level of methylation. For example, all monomethylated chrysenes are combined and reported as one number, C1-chrysenes, rather than specific isomers of monomethylated chrysene. These homologue groups are quantified using the calibration curve of their parent analyte and will be referred to as uncalibrated homologues. A complete list of calibrated and uncalibrated analytes targeted by these methods are listed in Table 11.b.

The data acquired by the instrumental analysis method described in this section will be quantitatively analyzed by the methods described in section 13.

11.1 Mass Spectrometer System

AGILENT Mass Selective Detector: model 5975C
AGILENT Gas Chromatograph: model 7890A
AGILENT Data System: Agilent Chemstation
AGILENT Ultra II capillary column: 5% phenylmethyl-silicone, .20 id, 25 m, .33 micron film thickness
AGILENT Auto Injector: model 7683A
Ionization mode: Electron Impact 70 eV
Interface temperature: 240° C
Ionizer pressure: 10^{-6} torr
Scan mode: Selected Ion Monitoring (SIM)
Inlet system: Capillary direct interface

11.2 GC Conditions

The GC is programmed to operate under the following conditions:

Injection volume: 1 µl
Injection mode: splitless
Injector temperature: 300° C
Source temperature: 230° C
Quad temperature: 150° C
Initial oven temperature: 60° C
Initial hold time: 0 min.
Temperature ramping rate: 10°/min.
Final temperature: 300° C
Final hold time: 20 min.
Carrier flow rate: Helium at 50 ml/min.

11.3 Tune Parameters

The Hewlett Packard (HP) mass spectrometer system used in these procedures is equipped with an autotune program which adjusts the electronics of the ion source so that it will operate at optimum sensitivity. The autotune procedure is executed as instructed by the manufacturer just prior to the analysis of each sample string. The autotune report is evaluated for consistency of the tuning parameters among sample strings. Criteria for these tuning parameters are listed below. If these criteria are not acceptable, diagnostics on and maintenance of the instrument are performed according manufacturers specifications and the autotune procedure is repeated.

Autotune Criteria

Profile Scan (voltage)	Spectrum Scan
Ion Focus: < 20	Mass 69 = 100%
Entrance lens: 45-95	Mass 219 > 35%
Repeller: 9.8-10.2	Mass 502 > 1%
	Mass 28 < 3%
	Mass 18 < 3%

11.4 Data Acquisition Parameters

The MSD system is programmed to acquire data in the Selected Ion Monitoring (SIM) scan mode. The ions selected to be monitored include a one quantification ion and one confirmation ion for each calibrated analyte, one confirmation ion for each surrogate and internal standard, and one quantification ion for each group of uncalibrated homologues. The ions monitored and the time window range in which they are monitored are displayed in Table 11.a.

Table 11.a

SIM Tables for PAH Data Acquisition

SIM Group	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
Start time*:	8.9	11.6	13.5	14.2	16.4	17.9	19.2	20.6	22.4	24.0	28.2
(min)											
Masses:	127	141	141	155	152	179	101	101	225	241	252
(amu)	128	142	153	165	165	191	191	202	226	242	269
	136	147	154	166	169	192	193	205	228	243	270
	141	152	155	169	176	193	202	215	229	244	276
	142	153	156	170	178	194	205	216	230	252	277
		154	164	180	179	197	206	220	232	253	278
		155	170	184	180	198	208	221	234	255	279
		156			184	208	211	222	240	256	283
		170			188	211	212	225	242	257	284
		180			194	212	220	226	243	258	
					198		221	229	244	264	
							222	230		269	
							225	232		270	
							226	234		284	
							240	240			

11.5 Analysis Sequence

A sample string is loaded on to the HP autosampler and the system is programmed to analyze the samples in the following order:

Analysis Sequence

Hexane Blank
Aromatic Calibration Standard #1
Aromatic Calibration Standard #2
Aromatic Calibration Standard #3
Aromatic Calibration Standard #4
Aromatic Calibration Standard #5
6 Environmental Samples
Aromatic Calibration Standard #3
Method Blank (MBLK)
Aromatic Reference Material (AREF)
6 Environmental Samples
Spiked Blank (SBLK)
Aromatic Reference Material (BREF)
Aromatic Calibration Standard #3

This analysis sequence constitutes an entire sample string and is described in detail in section 3.

The aromatic fractions of samples analyzed by this sequence are prepared as described in section 9.7 for tissue, section 7.6 for sediment samples, section 5.3.6 for water samples, and 8.6.2 for LDPEs.

Water strings are analyzed by the same analysis sequence but with the deletion of the SBLK.

11.6 Sample Analysis

All the samples of a sample string are analyzed according to the parameters described in sections 11.4 and 11.5. Raw data files are automatically generated as the samples are analyzed.

11.7 Data Processing

The raw data files, as they are acquired in section 11.6, are processed to ensure their compatibility with the quantitation programs in section 13. The processing procedures are outlined below.

11.7.1 Calibration Table

The first 5 calibration standards of the analysis sequence are used to establish a calibration table. The calibration table is a compilation of the retention times and identifying masses of each calibrated analyte. The information in this calibration table is used as described in section 11.7.2 to identify the calibrated analytes in each sample. The calibration table is generated as instructed in the Chemstation Operator's Manual.

11.7.2 Calibrated Analytes

Calibrated analytes are identified by the ion masses constituting their chromatographic peak and by the retention time at which the peak elutes from the capillary column. Each calibrated analyte has 2 ions associated with it, a quantitation ion and a confirmation ion. (The ion masses for each analyte are listed in Table 11.b.) The area of the quantitation ion is used in section 13 to quantify its respective analyte. The ratio of the area of the confirmation ion and the area of the quantitation ion is used to confirm the identity of a chromatographic peak. (See section 11.7.3 for Ion Ratios.) If the quantitation ion and the confirmation ion of an analyte are present within ± 0.15 min. of the retention time specified in the calibration table (section 11.7.1) for the respective analyte, the information is extracted from the raw data. The extracted analyte information is compiled in a Calibrated Analyte Report file.

The Calibrated Analyte Report file contains a list of each calibrated analyte and its corresponding peak area. The Calibrated Analyte Report file also contains a compilation of all ions which were not extracted. These ions are used for determining uncalibrated homologue concentrations (see section 11.7.4).

11.7.3 Ion Ratios

The ratio between the area of the confirmation ion and the area of the quantitation ion is used to confirm the identity of a calibrated analyte. The ion ratio for an analyte must be within ±30% of the expected ratio, (see Table 11.b for expected ion ratios). The Calibrated Analyte Report will indicate if the ion ratio fails to meet the criteria. If the ion ratio fails, the instrument operator must use professional judgement to determine the presence or absence of the analyte. If the analyte is determined to be absent, the data analysis report is edited and the area of the analyte is deleted.

11.7.4 Uncalibrated Homologues

The Calibrated Analyte Report file contains a compilation of data referred to as the Uncalibrated Report that contains the mass, area, and retention time of every peak that was not extracted and identified as a calibrated analyte. This data will be used to quantify the uncalibrated homologues. Each uncalibrated homologue group has a retention time window and a quantitation mass with which they are identified (see Table 11.c). The retention time window for a each homologue group is searched for peaks of the quantitation mass of the respective analyte. If there are peaks of the correct mass and in the correct retention time

window the peak is extracted and summed with every other peak of that homologue group. The final summed area is used for the quantitation of the respective homologue group (see Data Quantitation, section 13).

Table 11.b

Aromatic Analyte Quantitation Masses

Analyte	Quant Ion	Conf Ion	Ion Ratio	Sur STD Rel to
d_8-NAPHTHALENE	136			1
naphthalene	128	127	15	1
2-methylnaphthalene	142	141	88	1
1-methylnaphthalene	142	141	88	1
2,6-dimethylnaphthalene	156	141	67	2
C_2-naphthalenes	156	141	67	2
2,3,5-trimethylnaphthalene	170	155	90	2
C_3-naphthalenes	170	155	90	2
C_4-naphthalenes	184	196	100	2
biphenyl	154	152	28	2
HEXAMETHYLBENZENE	147			IS
acenaphthylene	152	153	13	2
d_{10}-ACENAPHTHENE	164			2
acenaphthene	154	153	99	2
fluorene	166	165	92	2
C_1-fluorenes	180	165	130	2
C_2-fluorenes	194	179	138	2
C_3-fluorenes	208	193	72	2
C_4-fluorenes	222	221	100	2
dibenzothiophene	184	152	15	3
C_1-dibenzothiophenes	198	197	75	3
C_2-dibenzothiophenes	212	211	80	3
C_3-dibenzothiophenes	226	225	70	3
C_4-dibenzothiophenes	240	225	37	3
d_{10}-PHENANTHRENE	188			3
phenanthrene	178	176	19	3
d_{10}-ANTHRACENE	188			
anthracene	178	176	18	3
1-methylphenanthrene	192	191	57	3

C_1-phenanth/anthracenes	192	191	57	3
C_2-phenanth/anthracenes	206	191	50	3
C_3-phenanth/anthracenes	220	205	60	3
C_4-phenanth/anthracenes	234	232	60	3
fluoranthene	202	101	15	3
pyrene	202	101	19	3
C_1-fluoranthene/pyrenes	216	215	70	3
C_2-fluoranthene/pyrenes	230	229	40	3
C_3-fluoranthene/pyrenes	244	243	25	3
C_4-fluoranthene/pyrenes	258	257	25	3
d_{12}-CHRYSENE	240			4
chrysene	228	226	25	4
C_1-chrysenes	242	241	50	4
C_2-chrysenes	256	255	50	4
C_3-chrysenes	270	269	50	4
C_4-chrysenes	284	283	50	4
d_{12}-BENZ-a-ANTHRACENE	240			
benz-a-anthracene	228	226	28	4
benzo-b-fluoranthene	252	253	22	5
benzo-k-fluoranthene	252	253	22	5
benzo-e-pyrene	252	253	23	5
d_{12}-BENZO-a-PYRENE	264			5
benzo-a-pyrene	252	253	24	5
d_{12}-PERYLENE	264			6
perylene	252	253	25	6
indeno-123-cd-pyrene	276	277	24	5
dibenzo-a,h-anthracene	278	279	22	5
benzo-ghi-perylene	276	277	23	5

In the last column of this table, each deuterated surrogate is assigned a number 1 to 6. Each native analyte is assigned the number that reflects which surrogate it is relative to.

HPLC = HPLC Internal Standard
IS = GC/MS Internal Standard

Table 11.c

Uncalibrated Homologue Quantitation Information

Homologue Group Ion	Quantitation time* (amu)	Retention (min)
C-1 naphthalene	142	8.0-13.0
C-2 naphthalene	156	11.0-14.5
C-3 naphthalene	170	12.5-16.5
C-4 naphthalene	184	14.0-17.5
C-1 fluorene	180	15.0-18.0
C-2 fluorene	194	16.5-20.0
C-3 fluorene	208	18.0-21.5
C-4 fluorene	222	21.5-24.0
C-1 phenanthrene/anthracene	192	17.5-20.0
C-2 phenanthrene/anthracene	206	19.0-22.5
C-3 phenanthrene/anthracene	220	20.0-23.0
C-4 phenanthrene/anthracene	234	21.0-23.5
C-1 dibenzothiophene	198	17.0-21.0
C-2 dibenzothiophene	212	18.0-23.0
C-3 dibenzothiophene	226	19.5-23.0
C-4 dibenzothiophene	240	23.0-25.0
C-1 fluoranthene/pyrene	216	20.0-24.0
C-2 fluoranthene/pyrene	230	24.0-26.0
C-3 fluoranthene/pyrene	244	26.0-28.0
C-4 fluoranthene/pyrene	258	28.0-30.0
C-1 chrysene	242	24.5-27.5
C-2 chrysene	256	26.0-28.0
C-3 chrysene	270	27.0-31.0
C-4 chrysene	284	28.0-33.0

*times will vary with column length and age

12. Instrumental Analysis of Biomarkers

12.1 Mass Spectrometer System

The GC/MSD system is as described in section 11.1.

12.2 GC/MSD conditions for the analysis of Biomarkers is outlined below.

Injection volume: 1 µl
Injection mode: splitless
Injector temperature: 280° C
Source temperature: 230° C
Quad temperature: 150° C
Initial oven temperature: 50° C
Initial hold time: 2 min.
Temperature ramping rate: 6°/min.
Final temperature: 300° C
Final hold time: 20 min.
Constant flow rate: Helium at 1 ml/min.

12.3 Tune Parameters

Tune parameters are as described in section 11.3

12.4 Data Acquisition Parameters

The MSD system is programmed to acquire data in the Selected Ion Monitoring (SIM) scan mode. The ions monitored and the time window range in which they are monitored are displayed in Table 12.a.

Table 12.a

SIM Table for Biomarker Data Acquisition

SIM Group:	**1**	**2**
Start time:* (min)	20.0	30.0
Masses: (amu)	57	
	66	
	83	83
	123	123
	135	135
	149	149
	163	163
	177	177
	187	187
	188	188
	191	191
	201	201
	217	217
	218	218
	219	219
	231	231
	259	259
		412

*times will vary with column length

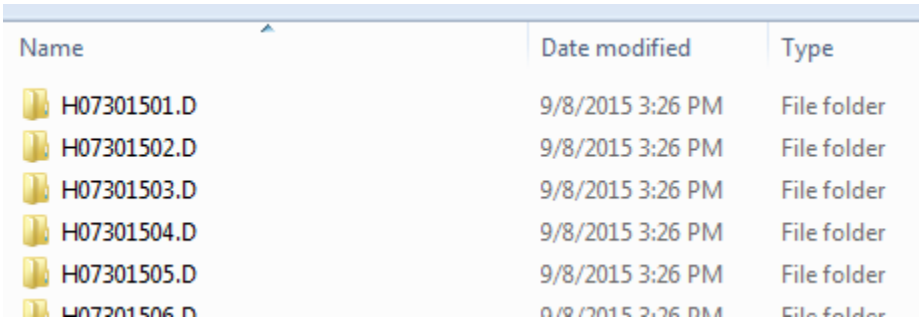
12.5 Raw Data File Evaluation and Manipulation

Data for sample and standards are acquired according to parameters outlined above and the raw data files are evaluated for accurate peak identification and integration by Agilent Chemstation Data Analysis software. Standards are evaluated first to insure retention times are appropriate for proper peak identification. Retention times are changed in the data analysis software if modification is required.

Integrations of standard and sample peaks are evaluated to ensure resolution, baseline assignments, and signal to noise criteria are met. Final data files should contain the area of all target analytes in standards and samples. Chemstation Custom Reports is used to concatenate the area data for the entire string of samples into an Excel spreadsheet for quantitation.

12.6 Data files

Data files on the instrument will be similar to the example illustrated below. These are all subdirectories filed under the appropriate string name (20150730LH in this example).



2) Confirm Raw Data
   a) ID of analytes and surrogates and Internal Standard (IS.)
      i) Using the retention times in a calibration standard report confirm that the peaks in the samples are flagged appropriately.
         (1) Make corrections in the electronic files if necessary.
   b) copy files to drive and memory stick

See Chapter 13 for quantification procedures.

12.6 Quantitation of Biomarkers

Due to the availability and expense of biomarker standards a relatively few number of biomarkers are used to calculate the targeted biomarker analytes. Table 12b shows which analyte RRF is used for each analyte. Calibration standards are made as per tables 4.7a and 4.7b. The relative response factor (RRF) is calculated as per the equation below.

$$RRF = \frac{(area\ \ Analyte_x)_{std}\,(amt\ IS)_{std}}{(area\ \ IS)_{std}\,(amt\ \ Analyte_x)_{std}}$$

where:

(area $Analyte_x$)$_{std}$ = Area of analyte x in a calibration standard

(area IS)$_{std}$ = Area of the internal standard in a calibration standard

(amt $Analyte_x$)$_{std}$ = Amount of analyte x in a calibration standard

(amt IS)$_{std}$ = Amount of the internal standard in a calibration standard

Response factors are calculated over the range of concentrations and the average of the RRF is used to quantitate the targeted analytes as per the equation below.

All response factors are calculated relative to the internal standard, DCH. As per is standard in the industry, a surrogate standard is not used and data are not recovery corrected. Recoveries are generally considered acceptable if the surrogate d-C20 recovery is > 80%. If the recovery is lower, the data should be used with caution. It should be noted that we work under the assumption that the response factor for a broad number of analytes is the same. However the quantitation of Tm from an H30 RRF vs a Tm RRF shows a notable difference. The d-ααα recovery is calculated and used to evaluate the efficiency of the method. If the recovery of d-c20 is less than 80% the data should be used with caution. The data are not recovery corrected. (Since assuming that responses are identical over a broad range.)

Equations below

$$Conc = \frac{\left(area\ \ Analyte_x\right)_{sample}\left(amt\ IS\right)_{sample}}{\left(area\ \ IS\right)_{sample}\left(\overline{RRF}\right)_{analyte}} / \left(sample\ wt\right)$$

where:

(Area Analyte$_x$)$_{sample}$ = Area of analyte x in the sample

(Area IS)$_{sample}$ = Area of the internal standard in the sample

(Amt IS)$_{sample}$ = Amount of internal standard in the sample

($\overline{RRF}$) = Mean RRF for surrogate analyte x

(sample wt) = weight (g) of the sample extracted

	Table 12b	**Quant**	
Code	**Analyte**	**Mass**	**RRF**
	ISOPRENOIDS		
nor	Norpristane	57	**nor**
prist	Pristine	57	**prist**
phy	Phytane	57	**phy**
	TERPANES		
TR23	C_{23} tricyclic terpane	191	**H30**
TR24	C_{24} tricyclic terpane	191	**H30**
TR25a	C_{25} tricyclic terpane (a)	191	**H30**
TR25b	C_{25} tricyclic terpane (b)	191	**H30**
TET24	C_{24} tetracyclic terpane	191	**H30**
TR26a	C_{26} tricyclic terpane (a)	191	**H30**
TR26b	C_{26} tricyclic terpane (b)	191	**H30**
TR28a	C_{28} tricyclic terpane (a)	191	**H30**
TR28b	C_{28} tricyclic terpane (b)	191	**H30**
TR29a	C_{29} tricyclic terpane (a)	191	**H30**
TR29b	C_{29} tricyclic terpane (b)	191	**H30**
Ts	18α(H),21β(H)-22,29,30-trisnorhopane	191	**H30**
Tm	17α(H),21β(H)-22,29,30-trisnorhopane	191	**Tm**
H28	17α(H),18α(H),21β(H)-28,30-bisnorhopane	191	**H30**
NOR25H	17α(H),21β(H)-25-norhopane	191	**H30**
H29	17α(H),21β(H)-30-norhopane	191	**H30**
C29Ts	18α(H),21β(H)-30-norneohopane	191	**H30**
M29	17β(H),21α(H)-30-norhopane (normoretane)	191	**H30**
OL	Oleananes	191	**H30**
H30	17α(H),21β(H)-hopane	191	**H30**
Nor30H	17α(H)-30-nor-29-homohopane	191	**H30**
M30	17β(H),21α(H)-hopane (moretane)	191	**H30**
H31S	22*S*-17α(H),21β(H)-30-homohopane	191	**H30**
H31R	22*R*-17α(H),21β(H)-30-homohopane	191	**H30**
GAM	Gammacerane	191	**H30**
H32S	22*S*-17α(H),21β(H)-30,31-bishomohopane	191	**H30**
H32R	22*R*-17α(H),21β(H)-30,31-bishomohopane	191	**H30**
H33S	22*S*-17α(H),21β(H)-30,31,32-trishomohopane	191	**H30**
H33R	22*R*-17α(H),21β(H)-30,31,32-trishomohopane	191	**H30**
H34S	22*S*-17α(H),21β(H)-30,31,32,33-tetrakishomohopane	191	**H30**
H34R	22*R*-17α(H),21β(H)-30,31,32,33-tetrakishomohopane	191	**H30**
H35S	22*S*-17α(H),21β(H)-30,31,32,33,34-pentakishomohopane	191	**H30**
H35R	22*R*-17α(H),21β(H)-30,31,32,33,34-pentakishomohopane	191	**H30**

	STERANES		
S22	20-methyl-5α(H)- pregnane	217	**C27S**
DIA27S	C_{27} 20*S*- 13β(H),17α(H)-diasterane	217	**C27S**
DIA27R	C_{27} 20*R*- 13β(H),17α(H)-diasterane	217	**C27S**
C27S	C_{27} 20*S*- 5α(H),14α(H),17α(H)-cholestane	217	**C27S**
C27bbR	C_{27} 20*R*- 5α(H),14β(H),17β(H)-cholestane	218	**C27S**
C27bbS	C_{27} 20*S*- 5α(H),14β(H),17β(H)-cholestane	218	**C27S**
C27R	C_{27} 20*R*- 5α(H),14α(H),17α(H)-cholestane	217	**C27S**
C28S	C_{28} 20*S*- 5α(H),14α(H),17α(H)-ergostane	217	**C27S**
C28bbR	C_{28} 20*R*- 5α(H),14β(H),17β(H)-ergostane	218	**C27S**
C28bbS	C_{28} 20*S*- 5α(H),14β(H),17β(H)-ergostane	218	**C27S**
C28R	C_{28} 20*R*- 5α(H),14α(H),17α(H)-ergostane	217	**C27S**
C29S	C_{29} 20*S*- 5α(H),14α(H),17α(H)-stigmastane	217	**C27S**
C29BBR	C_{29} 20*R*- 5α(H),14β(H),17β(H)-stigmastane	218	**C27S**
C29BBS	C_{29} 20*S*- 5α(H),14β(H),17β(H)-stigmastane	218	**C27S**
C29R	C_{29} 20*R*- 5α(H),14α(H),17α(H)-stigmastane, ααα(20R,24R)24-ethylcholestane	217	**C27S**
	STANDARDS		
IS	Dodecylcyclohexane (DCH)	83	
d-C20	d-42 n-eicosane	66	
d-ααα	d-2 C27 ααα(20R)cholestane	219	

13. Data Quantitation

The procedures used for the quantitation of petroleum hydrocarbons in environmental samples processed according to these SOPs are described in this section. The sample files, as they are formatted in sections 10.4 for aliphatic data and 11.7 for aromatic data, are manipulated by an Excel macro. This macro extracts analyte data, i.e., peak areas, from REPORT.TXT sample files and places the data in Excel spreadsheets.

13.0 Data transfer

Copy data string directories from the GCs to your network computer using a memory stick for transfer.

Copy a previous quantification Excel workbook (from QAed data) into the string directory and rename it with the appropriate string name. The data will be incorrect at this point. This workbook must match the matrix and calibration standards used in the new data.

13.1. Load raw data into spreadsheet

Peak area data from an entire sample string are extracted by an Excel macro and added to the spreadsheets. The spreadsheets and macros were originally designed in LOTUS for the quantitation of hydrocarbon data and later transferred to Excel. Spreadsheet formats have been created for the aliphatic data and the aromatic data of both matrices. The design establishes specific blocks within each spreadsheet for calculations, regressions, QA, and sample information.

Information regarding each sample's identity, mass/volume, QCBatch, replicate, etc. are manually entered into the spreadsheets. The information required is located on the chain of custody sheet which accompanies each sample string (see section 2). An example of the data required for each sample follows:

Data Example

Information	Type	Value	Description
SIN	integer	108531	Sample identification number
Replicate	integer	2	no. times submitted
QCbatch	text	F05021	string identification
Dry weight	real	12.4	grams of sample, dry weight
Wet weight	real	20	grams of wet sample
Matrix	text	sediment	matrix
Catalog #	text	NMFS_124	catalog identification
Total pk area	real	331750	sum all alkanes
UCM area	real	404266	UCM area

This information is required in both the aliphatic and aromatic spreadsheets with the exception of the Total peak area and the UCM area. These areas are only pertinent for the aliphatic spreadsheets. The total peak area is the sum of all peaks as described in section 10.4. This area is found at the bottom of each aliphatic data file. The UCM area is calculated by subtracting the total peak area from the uncorrected UCM area as it was calculated in section 10.4.2. Both areas are entered into the spreadsheets manually.

Summary procedure for loading Raw Data into spreadsheet

Check that the surrogate amounts in the calibration standards and samples are correct.

Run macro "load rpt."

Confirm that areas were loaded correctlv.

13.2 Evaluate Data

Check regression output ($R^2 > 0.99$), and accuracy of calibration standards run with the samples ($\pm$ 15% of known amount.)

Make corrections if possible or necessary.

Check precision of references samples (calculated amount ± 15% of their mean and ± 35% of mean of all strings of the same matrix) (make allowances for data below mdl.)
Make corrections if possible or necessary
Check accuracy of reference samples if an SRM was extracted and values available (no certified alkane values exist for many SRMs.)
Make corrections if possible or necessary
Check cleanliness of Mblk (analyte signals should be < 3x MDL.)
Make corrections if possible or necessary
Check surrogate recoveries for acceptability (30% < recovery < 120%.)
Make corrections if possible or necessary
Check final data for anomalies.
Make corrections if possible or necessary
Note QA information on "Guidelines for Data Acceptance" (see 13.3)
Decide if data is acceptable or needs to be rerun or re-extracted
Make hardcopies of QA output and data report for hardcopy file.

13.3 Guidelines for data acceptance

	# out of range:
I. Calibration	
1 Linearity as measured by the correlation coefficient. R^2 > 0.990.	aro -
If more than 3 are < 0.990, rerun instrumental analysis.	ali -
2 Calibrated analytes found in standards analyzed with the string are ± 15% of known amount.	aro -
If more than 6 are out of range, rerun instrumental analysis.	ali -
II. Precision	
1 The amount of each calibrated analyte found in the reference samples is ± 15% of their mean. (exclude analytes outside calibration range)	aro -
If more than 3 are out of range, rerun instrumental analysis. If still out, reprocess entire string.	ali -
2 The amount of each analyte in the reference samples is ± 35% of the mean for that analyte in all strings of the same matrix.	aro -
3 If out of range, reanalyze	ali -
III. Accuracy	
1 The amount of each calibrated analyte found in the spiked blank is ± 15% of the known amount.	aro -

If > 3 are out of range, reprocess string. ali -

IV. Contamination

1 The method blank is acceptable if the analyte windows are void of signals greater than 3x the MDL for any target analyte in that window. aro -

If contamination is not acceptable, reprocess string. ali -

2 The Instrument blank is acceptable if there is no significant signal in the retention time window of the analytes. aro -

If contamination is unacceptable, rerun instrumental analysis. ali -

V. Surrogate Recovery

1 The recovery for each surrogate is >30% and <120%. aro -

If > 10 are out of range, reprocess problem samples. ali -
If majority of string is out of range, reprocess entire string.

Chromatographic anomalies periodically cause data to fail specific QA guidelines; however, data may be acceptable If professional discretion of the QA officer can justify acceptance. Justification will be documented In the following QA comments.

QA comments

13.4 Calculations

13.4.1 Equations

The quantitation of samples is accomplished by manipulating sample data with Excel. The equations used for the quantitation of hydrocarbon data are displayed below.

An initial concentration for each analyte in a sample is calculated using equation 1. This initial concentration is then modified by subtracting from it the initial concentration of the analyte in the MBLK as per equation 2. This results in the final analyte concentration.

Equation 1. Initial analyte concentration

$$C_x = \frac{(I_{ss,x}) \times \left(\left(\frac{(A_x) \times (S_x)}{(A_{ss,x})} \right) + Intercept_x \right)}{M_s}$$

where:

C_x = Initial concentration of analyte x in the sample

$I_{ss,x}$ = Amount of surrogate standard associated with analyte x in sample (ng, section 4.1.1)

A_x = Area of analyte x present in the instrumental data file

S_x = Calibration curve regression slope for analyte x

$A_{ss,x}$ = Area of surrogate standard associated with analyte x in instrumental data file

M_s = Sample mass (g)

$Intercept_x$ = y-axis intercept of analyte x from the calibration curve regression line

Equation 2 Final Analyte Concentration

$$C_{cx} = C_x - \left(\frac{C_{x\ mblk}}{M_s} \right)$$

where:

C_{cx} = Final concentration of analyte x in a sample and corrected for MBLK

C_x = Initial concentration of analyte x in a sample as calculated in equation 1

$C_{x\ mblk}$ = Amount of analyte x in MBLK (ng). This is calculated using equation 1 but omitting M_s

M_s = Mass of the sample (g) whose analyte concentrations are being corrected for the MBLK

The regression line intercept cancels itself by using this equation; therefore, the intercept is not used in the calculation of the final sample concentration, C_{cx}. The final sample concentration, C_{cx}, is the value reported for analyte x.

The final concentrations for the analytes in the MBLK are calculated by equation 3.

Equation 3 Final MBLK Analyte Concentrations

$$C_{c\ mblk} = \frac{\left(\frac{A_{x\ mblk}}{A_{ss,x}} \right) \times (S_x) \times (I_{ss,x})}{Mass_m}$$

where:

$C_{c\ mblk}$ = Final MBLK concentration

$A_{x\ mblk}$ = Area of analyte x in MBLK

$A_{ss,x}$ = Area of surrogate standard associated with analyte x in MBLK

S_x = Calibration curve regression slope of analyte x

$I_{ss,x}$ = Amount of surrogate standard associated with analyte x in the sample (ng, section 4.1.1)

$Mass_m$ = nominal mass (g)

Recovery of the analytes in the Surrogate Recovery Standard are calculated in the Excel spreadsheet and are used to determine method efficiency (see section 3.5). A response factor (RF) is calculated for each surrogate analyte in the calibration standards (equation 4). The mean RF for each surrogate is calculated and used to calculate the recovery of the respective surrogate (equation 5).

Equation 4 Response Factor Calculation

$$RF = \frac{(Area\ Surr_x)_{std}\ (Amt\ I.S.)_{std}}{(Area\ I.S.)_{std}\ (Amt\ Surr_x)_{std}}$$

where:

$(Area\ Surr_x)_{std}$ = Area of surrogate standard x in a calibration standard

$(Area\ I.S.)_{std}$ = Area of the internal standard in a calibration standard

$(Amt\ Surr_x)_{std}$ = Amount of surrogate standard x in a calibration standard (see section 4.1.1)

$(Amt\ I.S.)_{std}$ = Amount of the internal standard in a calibration standard (see section 4.4)

Equation 5 Percent Recovery Calculation

$$Surr\ \%\ Rec = \left(\frac{(Area\ Surr_x)_{samp}\ (Amt\ I.S.)_{samp}}{(Area\ I.S.)_{samp}\ (Amt\ Surr_x)_{samp}\ (\overline{RF_x})_{stnds}} \right) \times 100\ \%$$

where:

$(\text{Area Surr}_x)_{samp}$ = Area of surrogate standard x in an environmental or QA sample

$(\text{Area I.S.})_{samp}$ = Area of the internal standard in an environmental or QA sample

$(\text{Amt Surr}_x)_{samp}$ = Amount of surrogate standard x in an environmental or QA sample (see section 4.1)

$(\text{Amt I.S.})_{samp}$ = Amount of internal standard in an environmental or QA sample (see section 4.4)

$(\overline{RF}_x)_{stnds}$ = Mean RF for surrogate standard x

13.4.2 Quality Assurance

The Excel spreadsheets are designed to extract the final hydrocarbon data calculated for the QA samples AREF, BREF, SBLK, and MBLK. Data are also extracted from the midrange calibration standards analyzed within each string and the regression statistics from the calibration curves. Together the extracted data is transferred to a specific block within the spreadsheet where its compliance with the QA criteria established in section 3 can easily be reviewed.

Final data are submitted to the DBM of NRDA Technical Services #1.

13.5. Quantitation of Water Samples

13.5.1. Data File Manipulation

Data files from water samples must be edited before they are compiled for Excel importation. Water samples are not fractionated during sample processing and hence the aromatic surrogates are integrated and included with the aliphatic data report. The peaks corresponding to the deuterated aromatic surrogates and the aromatic IS are deleted from the aliphatic data file. The aromatic ISs often coelute with aliphatic analytes and are hence removed from the data files. (These analytes frequently include analytes C15, C25, and C18 or phytane.)

Peak area information is extracted by an Excel macro and placed into a spreadsheet as explained in 13.1. Quantitation and evaluation are explained in sections 13.2 and 13.3.

Information regarding the identity of each sample is entered into the Excel spreadsheets manually. The information required is located on the chain of custody sheet which accompanies each sample string (see section 2). An example of the data required for each sample follows:

Data Example

Information	Type	Value	Description
SIN	integer	108531	Sample identification number
Replicate	integer	2	no. times submitted
QCbatch	text	F05021	string identification
Volume	real	0.9	Sample volume, liters
Matrix	text	water	Type of sample
Catalog #	text	NMFS_124	catalog identification
Total pk area	real	331750	sum all alkanes
UCM area	real	404266	UCM area

This information is required in both the aliphatic and aromatic spreadsheets with the exception of the Total peak area and the UCM area. These areas are only pertinent for the aliphatic spreadsheets. The total peak area is the sum of all peaks as described in section 10.4. This area is found at the bottom of each aliphatic data file. The UCM area is calculated by subtracting the total peak area from the uncorrected UCM as described in section 10.4.2. Both areas are entered into the spreadsheets manually.

13.5.2 Equations

The equations used for the quantitation of the aromatic hydrocarbons in water are identical

to those in section 13.4.1. The calculations for the aliphatic hydrocarbons, however require modifications. No surrogate aliphatic analytes exist in water samples; the IS, DCH, serves as the surrogate in the calculation of the calibration curves and in sample quantification equations 1 and 3. Likewise, no RF or percent recoveries are calculated for aliphatic water samples. The volume of the sample is used rather than the mass in equations 1 and 2.

13.5.3 Quality Assurance

The Exel spreadsheets are designed to extract the final hydrocarbon data calculated for the QA samples ali AREF, ali BREF, aro AREF, aro BREF, and MBLK. Data are also extracted from the midrange calibration standards analyzed within each string and the regression statistics from the calibration curves. Together the extracted data is transferred to a specific block within the spreadsheet where its compliance with the QA criteria established in section 3 can easily be reviewed.

14. Database Output and Management

All hydrocarbon concentration data are ultimately put in the hydrocarbon database for future use, study analysis, and manuscript preparation.

14.1. Prepare Data for Submission to Database

After PAH, Alkane, and biomarker data, if applicable, have been generated for a sample string the data are prepared for the database. This involves using the last 2 sheets of the quantitation spreadsheets, titled PAH and PAHQC if it is the aromatic data and ALK and ALKQC if it is alkane data, to transpose the data. In these 2 sheets cell addresses have simply copied the corrected data and QC codes from the data sheet in the vertical format. Below the vertically oriented data is the area where these data are to be copied (values) and transposed and pasted.
Delete the default lines of data in the horizontal area.

Highlight the vertical data to be submitted, copy, and paste special (values and transpose) the pertinent data to the horizontal section. Take this opportunity to delete any unwanted rows of data, eg. If it was not a full string, or if one of the samples was lost, etc. Do this identical cut and paste for the QC codes. Make sure what you delete for the data is the same as what you delete from the QC codes.

Create a new blank spreadsheet. In this spreadsheet create 4 pages- PAH, PAH QC, ALK, and ALK QC. From a past data submission, copy into the respective page the appropriate header information. Copy and paste the horizontal data from as many individual sample strings as wanted for the data submission.

NOTE: The header information does not exactly match between the submission and the quantitation spreadsheets. For the data you must insert a column for LAB identification. (LAB identifier = ABL) For the qc codes you must insert 2 rows, one for QCbatch identification, and one for Rep identification. You must then copy and paste these identifiers, preferably from the data page. The bottom line is that the output fields must exactly match those in the database and they must contain the correct type of information (numbers where they are expected, text where it is expected, etc).

Now is the opportunity to "clean up" some of the information and spot errors.

-Clear the contents of the HPLC surrogate columns (if there is something there it is confusing to user)

-For water samples the volume will be in the WetWt column and should be moved to the appropriate column

-For LDPE samples the Proportion will be in the WetWt column and should be moved.

-Clear the contents of confusing "default" values in the weights, proportions, and volume columns.
-Clear the contents of the calibration standard's uncalibrated peaks (it is an intercept value and will be confusing to a user.
-Look down the columns for obvious errors, eg. A QA sample with a "0" as the type or a sample SIN with a "QC" as the type. These errors are easier to see in the horizontal format than the vertical and especially if you build this submission file to include multiple strings. Sorting the data after multiple strings have been added is also helpful in spotting oddities.

Next, compare the header information between the PAH and the ALK data. Create a working spreadsheet and copy into it the header information from both data sets. Using formulas subtract the PAH SIN column from the ALK SIN column… they should all be zero. Likewise, subtract other numeric columns, like the wet weight and dry weight from each other to be sure they are the same and the result is zero.

Then use freeze pane to bring columns like QCbatch, Matrix, and Catno next to each other to look for anomalies.

Make any corrections that become obvious in the original quantitation spreadsheet and in the data submission file.

[Suggestion: put the data submission together and perform the quality checks in your own drive and do not transfer it to th shared drive until all data and header information are confirmed.]

14.2. Submit data to database manager

Place database submission files in directory ..\hydrocarbon data\Database submissions\YYYY\, where YYYY is the current calendar year. Biomarkers occupy a subdirectory under this structure. Excel submission file names are standardized: DBsubmissionYYYYa, b, c, … where YYYY is the current year and a, b, c, … represent individual submissions within that year.

14.3. Data assembly and quality control by database manager.

The database manager will assemble individual submission files (a, b, c…) into a composite spreadsheet for quality control and eventual transfer to the database. These composite files are also completed yearly. Use the previous version to create new versions. Information in these files has been regularly updated, including how algorithms function, so using the newest version is important.

14.3.1. Procedures for preparing database data.

1 Catalog new samples

Store appropriately in freezer
Place copy of custody sheet on network
File original custody sheet

2 Label new sample boxes or work them into appropriate boxes

Use established box naming scheme (yyyy-nnnn, where yyyy=year and nnn= box number within year)
Enter box data into sheet "Inventory"
Add box inventory information to database

3 Add new sample identification numbers (SINs) to table "Sample Information"

Add sample information to database
Be careful to ensure consistency between new entries and previous naming conventions, such as place name abbreviations. This will provide a more coherent – or at least more easily searchable – long-term database.

4 Add SINs & project data to the queue

New sample locations & abbreviations can be added to database table "location"
New projects can be added to database table "project names"

5 Inspect data for general errors

Are the matrices correct? Remember that LDPEs are reported as PEMDs in the database
Are the units present and correct?
Are volumes, weights, and proportions reported correctly?

6 Inspect PAH data for errors

Copy data into PAH scratchpad and manipulate as needed before loading sheet PAHdata
Use sheet "PAHdata"
Add submitted PAH data, including QCcodes
Inspect deuterated recoveries. Are they acceptable?
Check for erroneous concentration reports where analytes were not detected. If this happens, master quant sheet(s) might need correction
PAH data may be visually inspected using sheet "Graphics PAHs"
Data in the graphics sheet are obtained from sheet "PAHdata"
Be very careful about the data in sheet PAHdata – if rows are moved or deleted then sheet “Graphics PAHs” will not function properly
Add data to database

7 Check accuracy and precision of PAH references

Use sheets -720 PAH REF QCwater, -557 PAH SRM1582, and -55 PAH SRM1944
If other references appear, add appropriate sheets. This information resides under ..\hydrocarbons\hydrocarbon data\QA QC\QA QC April 2015 composit.xlsm. Not enough information was routinely submitted to the database at this level to properly understand the QA consistency over time – so this approach can use improvement. Primary quality assurance procedures are described in section 13.

8 Add string (batch) information to sheet "Project batches"

This information is critical to proper database functioning! The primary purpose of this step was to allow complete extraction of project-specific information. It was more important when subsets were prepared for specific users, such as the *Exxon Valdez* Trustee Council. By 2015, this was relaxed and a streamlined copy of the entire database was supplied to the trustees.

9 Inspect alkane data for errors

Copy data into ALK scratch and manipulate as needed before loading sheet ALKdata
Use sheet "ALKdata
Add submitted alkane data, including QCcodes
Inspect deuterated recoveries. Are they acceptable?
Check for erroneous concentration reports where analytes were not detected.
If this happens, master quant sheet(s) might need correction
Alkane data may be visually inspected using sheet "Graphics Alkanes"
Data in the graphics sheet are obtained from sheet "ALKdata"
Be very careful about the data in sheet ALKdata – if rows are moved or deleted then sheet “Graphics Alkanes” will not function properly
Add data to database

10 Check accuracy and precision of alkane references

This information resides under ..\hydrocarbons\hydrocarbon data\QA QC\QA QC April 2015 composit.xlsm. Not enough information was routinely submitted to the database at this level to properly understand the QA consistency over time – so this approach can use improvement. Primary quality assurance procedures are described in section 13.

11 Inspect biomarker data for errors

Copy data into Biomarker scratch and manipulate as needed before loading sheet Biomarker data
Use sheet "biomarker data"
Add submitted biomarker data
Inspect deuterated recoveries. Are they acceptable?
Check for erroneous concentration reports where analytes were not detected.
If this happens, master quant sheet(s) might need correction

Biomarker data may be visually inspected using sheets "triterpane," "hopanes," & “steranes"
Data in the graphics sheets are obtained from sheet "biomarker data"
Be very careful about the data in sheet biomarker data – if rows are moved or deleted then sheet “Graphics Biomarkers” and several others will not function properly
Add data to database

12 Sheet Graphics PAHs reports summary values and modeling.

See summary info section in orange.
This can be exported to sheet "What is it?" for further analysis

13 Sheet Graphics Alkanes reports summary values and modeling.

See summary info section in orange.
This can be exported to sheet "What is it?" for further analysis

14 Sheet Graphics Biomarkers reports summary values and modeling

See summary info section in orange.
This can be exported to sheet "What is it?" for further analysis

14.4 Database description

The hydrocarbon database is described in detail in a document generally called EVTHD Lexicon (Appendix B).

15. Miscellaneous Calculations

15,1 Percent Dry Weight Calculation

The % dry weight of both tissue and sediment samples is calculated according to the equation below. The % dry weight can then be used to calculate the dry weight of a sample.

The data for this calculation are obtained as described in section 5.1.5, step 3, for tissues and section 5.2.4, step 3, for sediment.

15.2 Lipid Determination

A lipid analysis on tissue samples is completed after the tissues are quantitatively analyzed. The analysis determines the percentage of lipid in the sample. The procedures for determining percent lipid is described below.

Glassware and Apparatus

All glassware and apparatus are cleaned as described in section 5.1.1.

Beakers: 100 ml
Round bottomed flasks: 125 ml
Spatulas: nichrome
Aluminum weighing dishes
Extraction thimbles: LABSOURCE, cellulose, 25 mm x 80 mm
Soxhlet Extractors
Soxhlet Condensers

Chemicals and Solvents

The following reagents are used in this method for lipid determination:

Solvent: $MeCl_2$, pesticide grade
Na_2SO_4: MALLINCKRODT, analytical grade, anhydrous, granular, combusted at 440° C for 4 hours

The lipids for tissue samples are extracted from the sample homogenates they were prepared in section 5.1.4. The extraction procedure is described below and must be repeated for each sample in a string.

1. A 3-5 g aliquot of the tissue sample is weighed into a tared 100 ml beaker. The sample weight is recorded.

2. Approximately 20 g of Na_2SO_4 are added to the beaker and the contents are stirred with a spatula until the sample is dry.

3. The dried sample is transferred into an extraction thimble and the thimble is placed into a soxhlet extractor.

4. The beaker is rinsed with $MeCl_2$. The rinsate is added to the soxhlet extractor.

5. 50 mls of $MeCl_2$ and several boiling stones are added to a 125 ml round bottom flask.

6. The round bottom flask, the soxhlet extractor, and the condenser are assembled and are placed on a hot plate at approximately 70° C.

7. The soxhlet system is allowed to extract and siphon for 2.5 hours.

8. The system is allowed to cool and is dismantled.

9. The extract in the round bottomed flask is placed on a steam bath at 80° C and is reduced in volume to approximately 5 ml.

10. An aluminum weighing dish is weighed and the weight is recorded. The extract is transferred into the aluminum weighing dish.

11. The round bottomed flask is rinsed twice with 1 ml volumes of $MeCl_2$. The rinsates are added to the aluminum weighing dish.

12. The aluminum dish is placed in a fume hood overnight or until all the $MeCl_2$ has evaporated.

13. The aluminum dish with the lipid is weighed and the weight is recorded. The weight of the aluminum pan from step 10 is subtracted from the weight of the dish with the lipid. The difference is the weight of the lipid.

14. The percent lipid is determined with the following equation:

15.3 Method Detection Limits

The method detection limit (MDL) is the minimum concentration of an analyte that can be measured and reported with 99% confidence. The MDL, for each passive sampler device (SPMD or LDPE), has been estimated by ABL to be ca. ½ that of the PAH concentrations in the lowest calibration standards (see sec. 4.4.1.). This estimation is based on evaluation of % accuracy for AREF or BREF and calculated MDL for sediment and tissue matrices that have been determined as specified in the Environmental Protection Agency publication 40 CFR, Appendix B.

For comparison, the range of MDLs for PAHs in tissue, sediment, and water are outlined below.

Tissue	Sediment	Water
(ng/g)	(ng/g)	(ng/l)
0.5-2.0	0.5-2.0	20.0-50.0

References

Aeppli, C., C. A. Carmichael, et al. (2012). "Oil Weathering after the Deepwater Horizon Disaster Led to the Formation of Oxygenated Residues." Environmental Science & Technology 46(16): 8799-8807.

Folk, R. L. (1974). Petrology of sedimentary rocks. Austin, TX, Hemphill Publishing Co.

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Short, J. W., T. L. Jackson, et al. (1996). "Analytical methods used for the analysis of hydrocarbons in crude oil, tissues, sediments, and seawater collected for the natural resources damage assessment of the *Exxon Valdez* oil spill." American Fisheries Society Symposium 18: 140-148.

APPENDIX D: SOP - *EXXON VALDEZ* TRUSTEE HYDROCARBON DATABASE

Standard Operating Procedures for the

***Exxon Valdez* Oil Spill Trustee Council**

Hydrocarbon Database (EVTHD)

2016

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June 2016

Acknowledgements

We thank the *Exxon Valdez* Oil Spill Trustee Council for support of this database for many years. We also thank the developers of the original versions of this database, including Sid Korn, Carol-Ann Manen, Jeffrey W. Short, Bonita D. Nelson, Ron A. Heintz, and Marshal Kenziorek.

Introduction

The purpose of this hydrocarbon database is to record all analyses completed as a result of the *Exxon Valdez* oil spill for the *Exxon Valdez* Oil Spill Trustee Council.

The purposes of this document are to 1) explain the structure of the database and 2) provide summary information on database content. This document is not intended as a primer for Access®, the current database software. Summary descriptions are minimal, intended to provide an overview of content, not analyses of the data. Sections of this manual are introduction, history, content, and structure. Structure is subdivided into an overview, key linking variables, key tables, supporting descriptive tables, and queries.

This hydrocarbon database was initiated after the *Exxon Valdez* oil spill in 1989. The first version was as an RBase database, PWSOIL (Short, Heintz et al. 1996). It migrated to a proprietary structure in 1997, EVTHD (*Exxon Valdez* Oil Spill Trustee Council Hydrocarbon Database) and contained the collection and hydrocarbon analysis information for environmental samples obtained for the *Exxon Valdez* National Resource Damage Assessment and Restoration efforts. The data were organized into three matrix types, tissues, sediment, and seawater. The analytical results included concentrations of 63 hydrocarbons, summary statistics for the evaluation of the hydrocarbon sources and laboratory quality control data. Features of the database included identification of replicate samples, presentation of results in dry or wet weight, optional correction for method detection limits (MDL) of the analytes, and easy identification of samples contaminated with *Exxon Valdez* crude oil. This structure, written in Visual Basic, ceased to function well when Windows operating systems were upgraded to XP and the data were moved to a Microsoft Access format. The 2015 version continues in Access and is described in this manual.

A data analysis tool (EVTHD data analysis tool.xlsm) accompanies the database to further explain the data. This tool highlights deuterated recoveries when they are <30% or >150%. Concentrations less than method detection limits (MDLs) are indicated visually; all summary data are filtered by MDL (values less than MDL are set to zero). The MDLs vary by matrix and the algorithm relies on "analysis type" reported in the database. Investigators using the data should be aware of samples with recovery problems and the implications of below MDL observations and deal with them appropriately.

To use the analysis tool, paste desired records from the database into sheets "PAHdata," "ALKdata," and or "Biomarker data." These records are read and summarized by corresponding sheets "Graphics PAHs," "Graphics Alkanes," and "Graphics Biomarkers."

Moving records in the data sheets will cause changes in the graphics sheets – and moving records in graphics sheets will influence those in modeling sheets, so copy & paste to avoid scrambling records.

The data analysis tool also converts values to dry weight (where appropriate), summarizes data records, provides two PAH composition models (Short and Heintz 1997; Carls 2006; Carls, Larsen et al. 2015), an alkane composition model, and biomarker composition models. The models all use Alaska North Slope crude oil as the comparison source. The Short and Heintz (1997) model produces a weathering factor, w. Samples where $w \leq 0$ are considered unweathered, $0 < w \leq 2$ slightly weathered, $2 < w \leq 8$ moderately weathered, and $w > 8$ highly weathered (Carls, Babcock et al. 2001). The pS3 source model is an update of Carls (2006) (Carls, 2015); values range from -1.0 (pyrogenic) to +1.0 (petrogenic). Chances of randomly observing values <−0.1 or >0.4 are about 5% (Carls, unpublished). Alkane and biomarker composition models determine whether the unknown sample pattern matches the composition pattern in source oil; values range from 0.0 (no match) to 1.0 (perfect match). The large acceptance bounds for *n*-C10 through *n*-C13 are caused by natural weathering in some source samples.

Database Content

Overview

The purpose of this section is to provide a brief overview and accounting of data contained within this database. The number of samples and the fraction analyzed provides a sense of the intensity of research on a yearly basis (Fig. 1). Another measure of general research activity is the number of projects operative in any given year (Fig. 2).

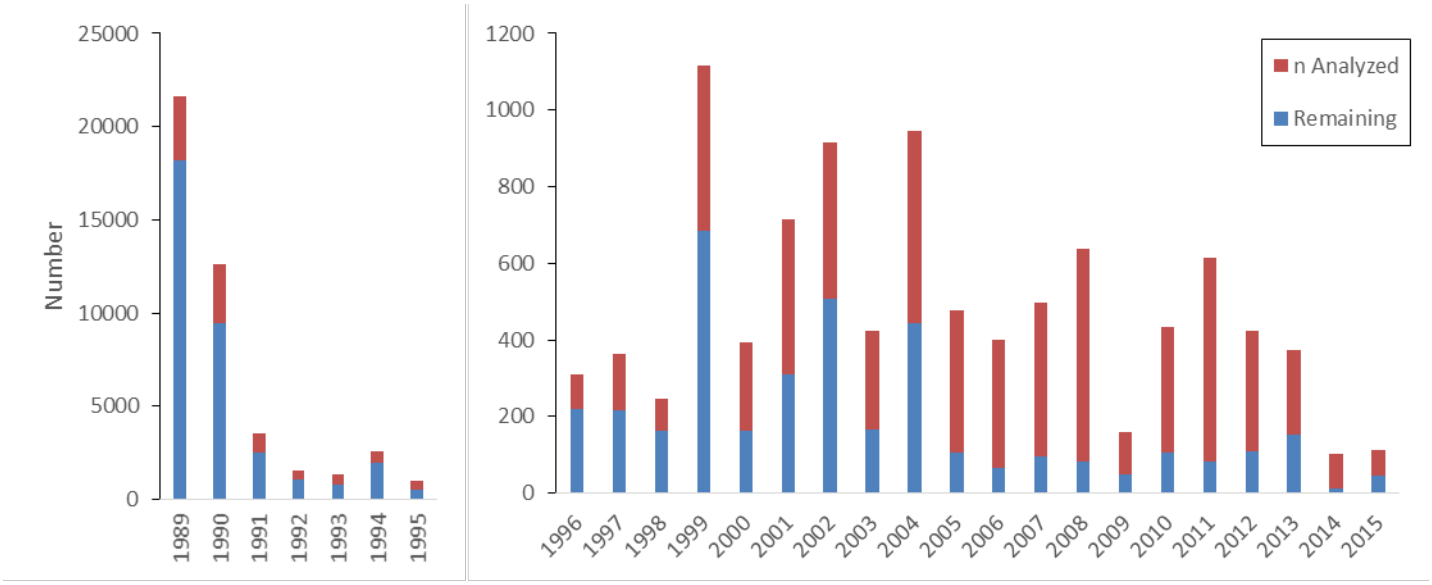


Fig. 1. The total number of samples collected and analyzed by year, subdivided by those analyzed and those not analyzed.

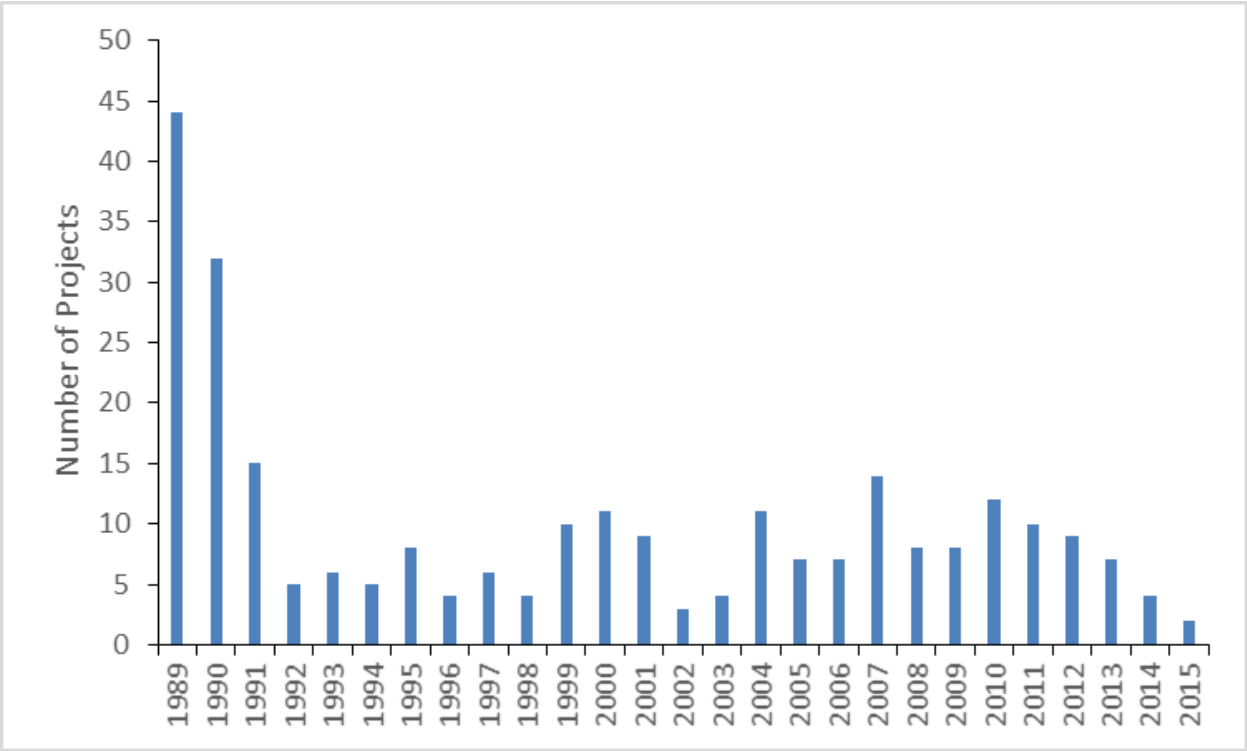


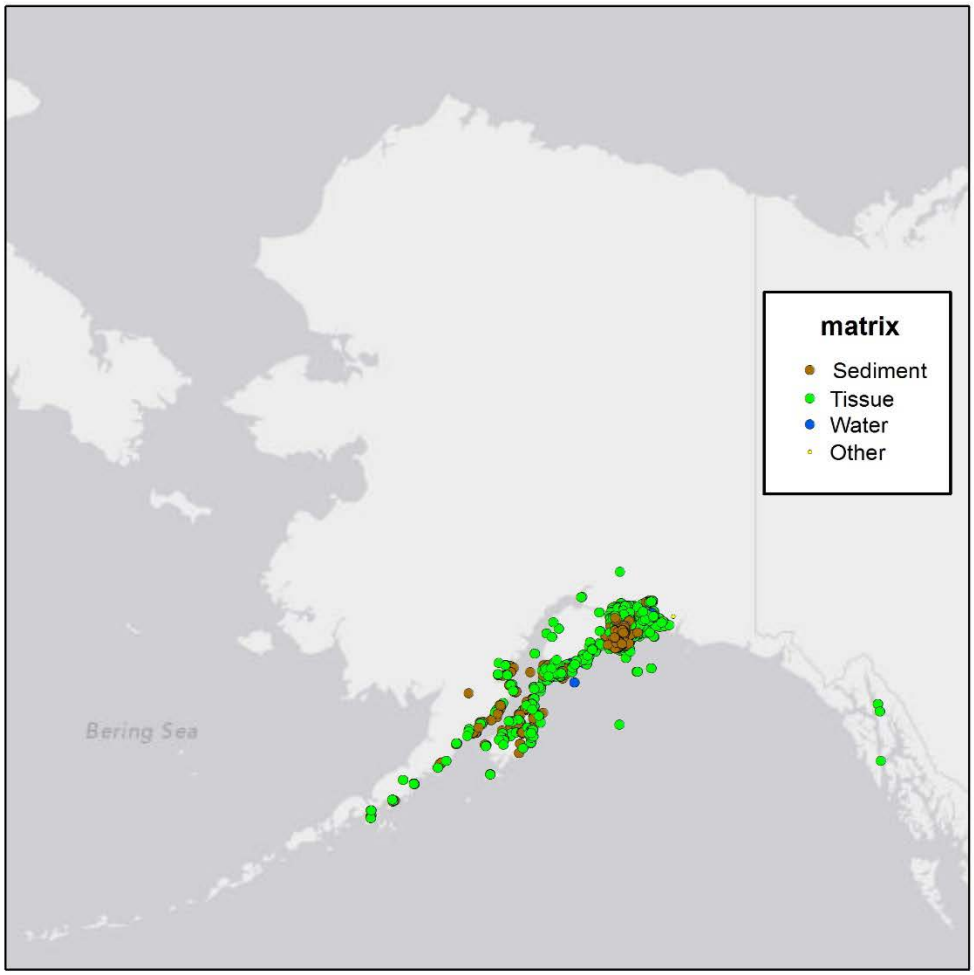
Fig. 2. The number of discrete projects represented in the database by year.

1989

		Matrix						
Year	Project	Sediment	Tissue	Water	PEMD	SPMD	Blanks	Other
1989	AIRWAT2	1509	1				64	
	AIRWAT3	20	492	620			25	
	BIRD01		417					
	BIRD03		150					
	BIRD04		455				15	
	BIRD06		71					
	BIRD07	77	38				2	
	BIRD08		99					
	BIRD09		37					
	BIRD11		141					
	BIRD12		285				1	
	COAHAB1	903	458	23			224	2
	EVO	14 (oil)						
	FSHSHL01		245				10	
	FSHSHL02		14					
	FSHSHL04	369	334	223			26	
	FSHSHL07		9				9	
	FSHSHL08		47				16	
	FSHSHL11		219					
	FSHSHL13	211	136				13	
	FSHSHL14	54	32				3	
	FSHSHL15		60				6	

FSHSHL16		34	2
FSHSHL17		494	
FSHSHL18	4	452	5
FSHSHL20	49		
FSHSHL22	25	49	11
FSHSHL23		385	
FSHSHL24		8477	
FSHSHL25		21	2
FSHSHL26		18	
MARMAM1		98	
MARMAM2		26	
MARMAM4		646	
MARMAM5		1075	
MARMAM6		943	
Pink Habitat Recovery		310	
RMB		3	
TERMAM1		128	
TERMAM3		106	
TERMAM4		66	
TERMAM5		5	
VOLUNTEE	3	25	

1989



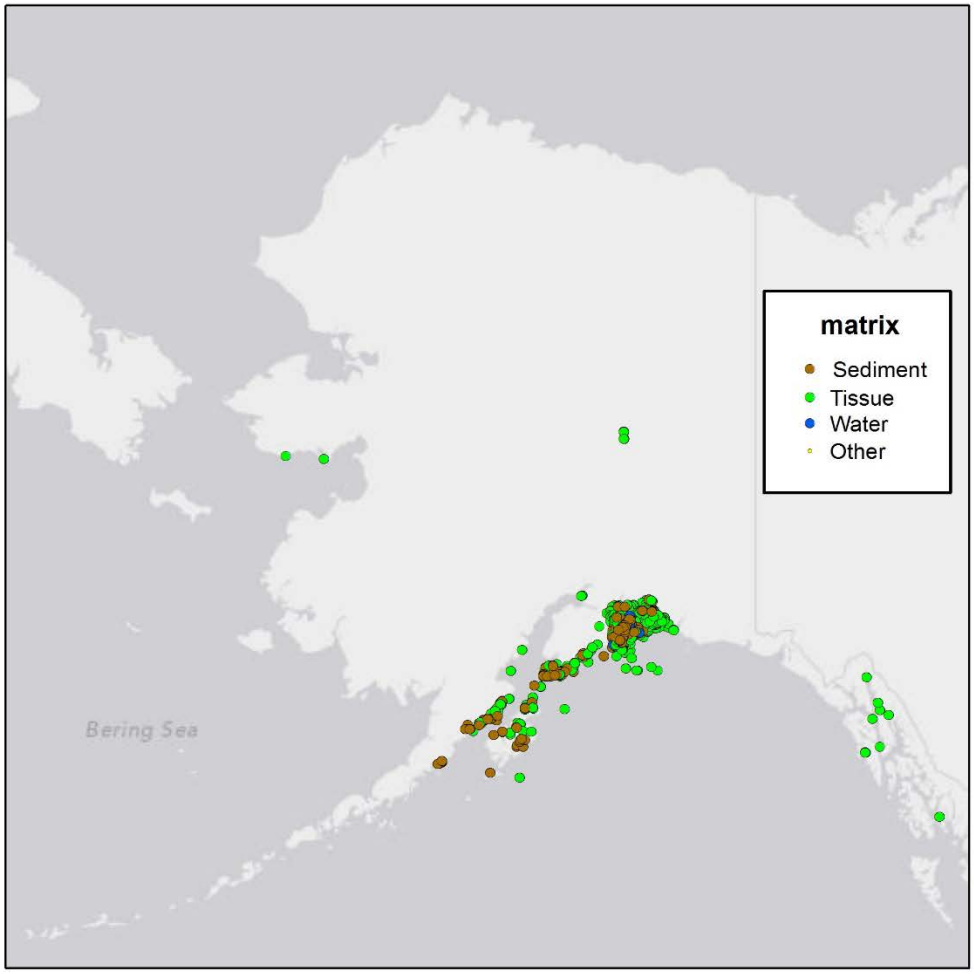
Map illustrates environmental samples only

1990

		Matrix						
Year	Project	Sediment	Tissue	Water	PEMD	SPMD	Blanks	Other
1990	AIRWAT2	1102					105	1
	AIRWAT3	98	245				53	
	BIRD01	1	13					
	BIRD03		39					
	BIRD04		120				4	
	BIRD05		40					
	BIRD06		57					
	BIRD07		36					
	BIRD08		112					
	BIRD09		5				2	
	BIRD11		452					
	BIRD12		37					
	COAHAB1	1155	405	91			273	
	FSHSHL01		259				41	
	FSHSHL02		41				16	
	FSHSHL04	345	122	1			99	
	FSHSHL08		8				8	
	FSHSHL11		150				4	
	FSHSHL13	197	211				29	
	FSHSHL15	1	122				11	
	FSHSHL16		9					
	FSHSHL17	72	377				32	

FSHSHL18		1131		7
FSHSHL22	45	22		17
FSHSHL24		1705		
FSHSHL26	11		14	4
MARMAM2		8		
MARMAM4		15		
MARMAM5		283		
MARMAM6		1882		
Pink Habitat Recovery		25		
TERMAM6	1	886		1

1990

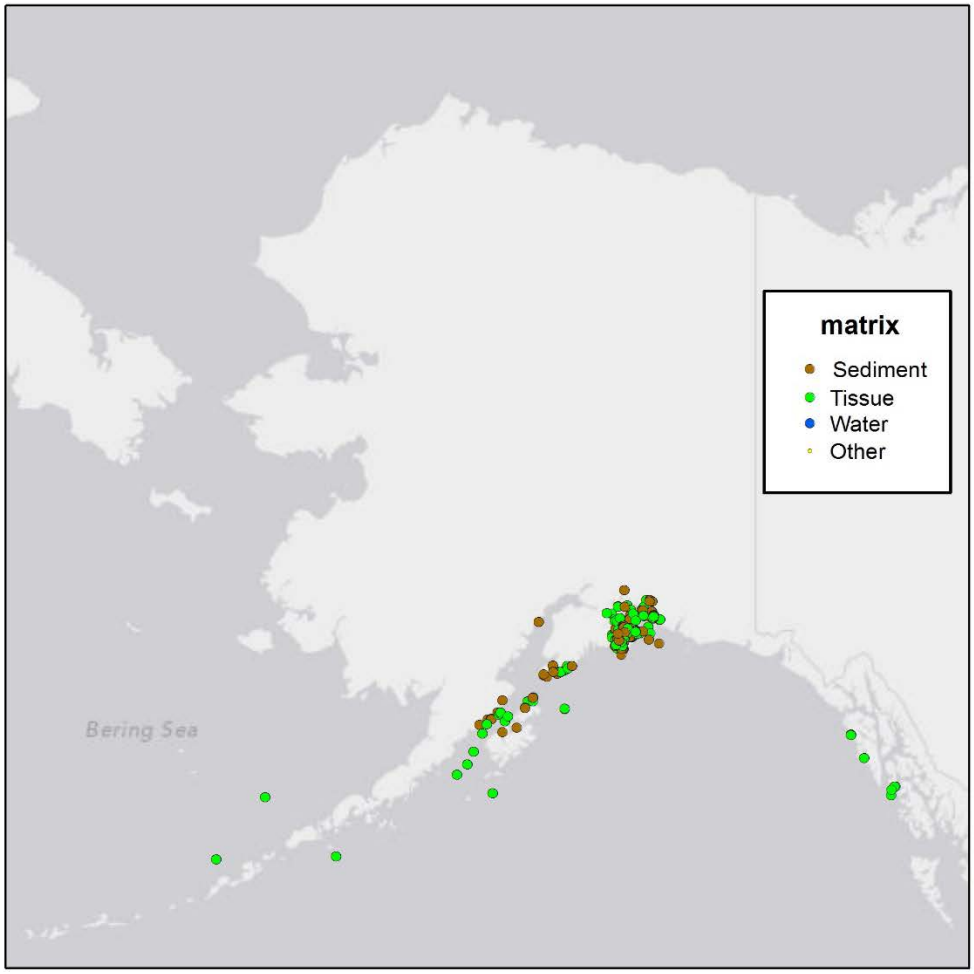


Map illustrates environmental samples only

1991

Year	Project	Matrix						
		Sediment	Tissue	Water	PEMD	SPMD	Blanks	Other
1991	AIRWAT2	429					23	
	AIRWAT3	178	85				18	
	ARCHEOLO	107	34				12	
	BIRD8		48					
	COAHAB1	270	175	9			141	
	FSHSHL11		36					
	FSHSHL13	71	50				6	
	FSHSHL15		63				6	
	FSHSHL17	36	192				18	
	FSHSHL24		772					
	FSHSHL26	19		20				
	FSHSHL4	25	534				5	
	MARMAM6		140					
	MARMAM7		33					
	Pink Habitat Recovery		9					

1991



Map illustrates environmental samples only

1992

		Matrix						
Year	Project	Sediment	Tissue	Water	PEMD	SPMD	Blanks	Other
1992	AIRWAT3	90	11					
	FSHSHL4	60						
	Pink Habitat Recovery	4						
	RMB	642	376				68	
	RPSG	37	20	276				

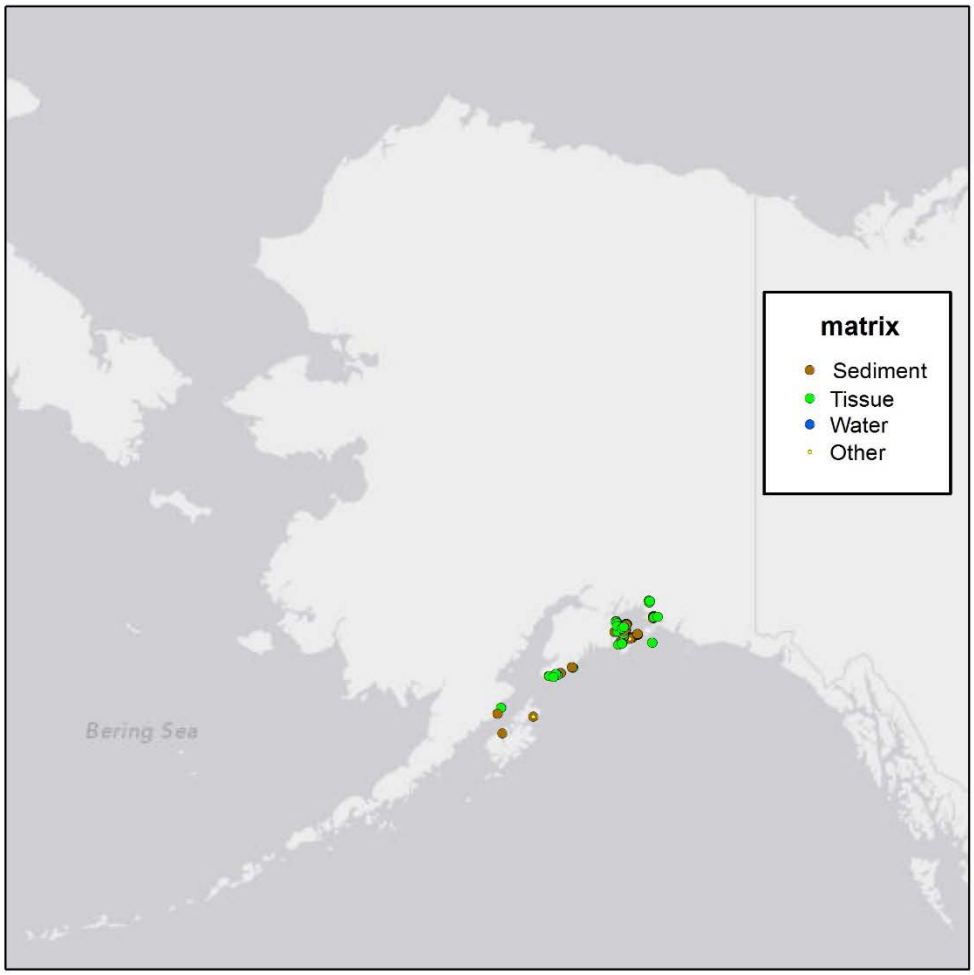
1992

Map illustrates environmental samples only

1993

		Matrix						
Year	Project	Sediment	Tissue	Water	PEMD	SPMD	Blanks	Other
1993	PSG			1				
	RARCH	8					3	
	RDH		114					
	RMB	309	269				25	
	RPSG	31	51	334			7	
	RSUB	221					9	

1993

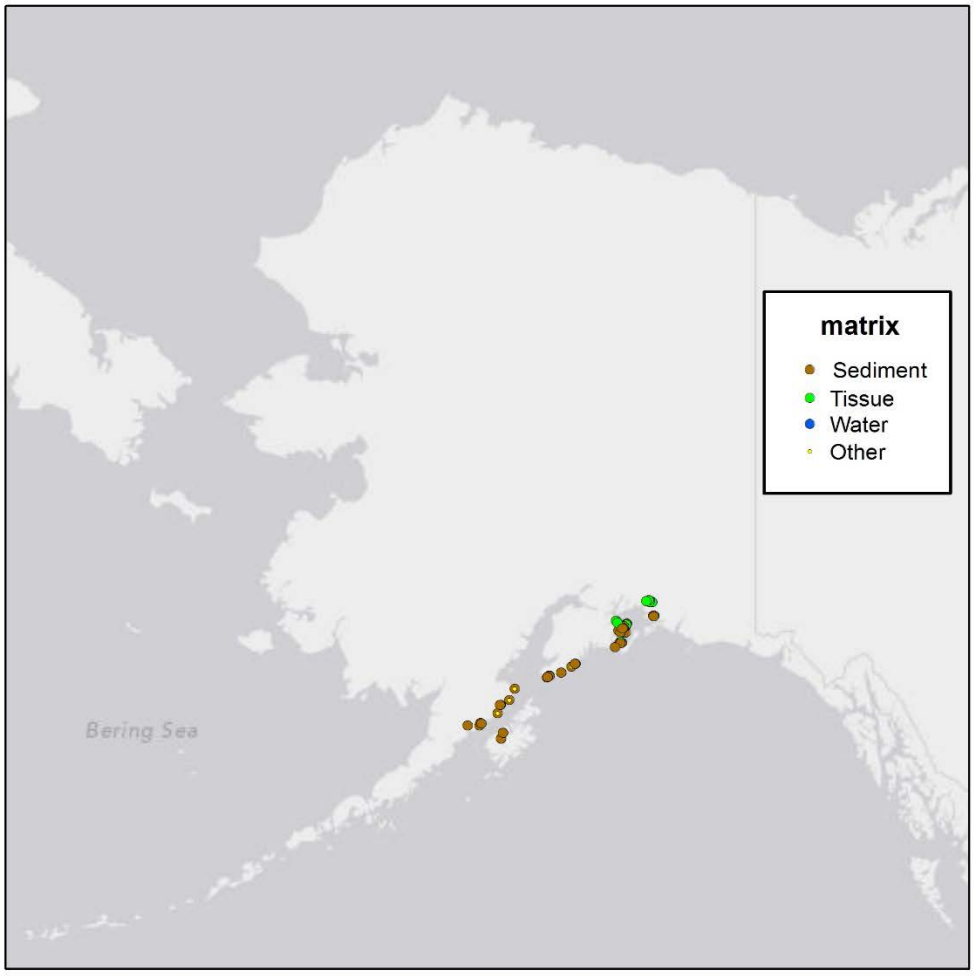


Map illustrates environmental samples only

1994

Year	Project	Matrix Sediment	Tissue	Water	PEMD	SPMD	Blanks	Other
1994	RHERR	33	1497	142			1	
	RMB	322	215				10	1
	RPSG	8	17	99			2	
	RSLA	27					6	
	RSUB	216					3	

1994

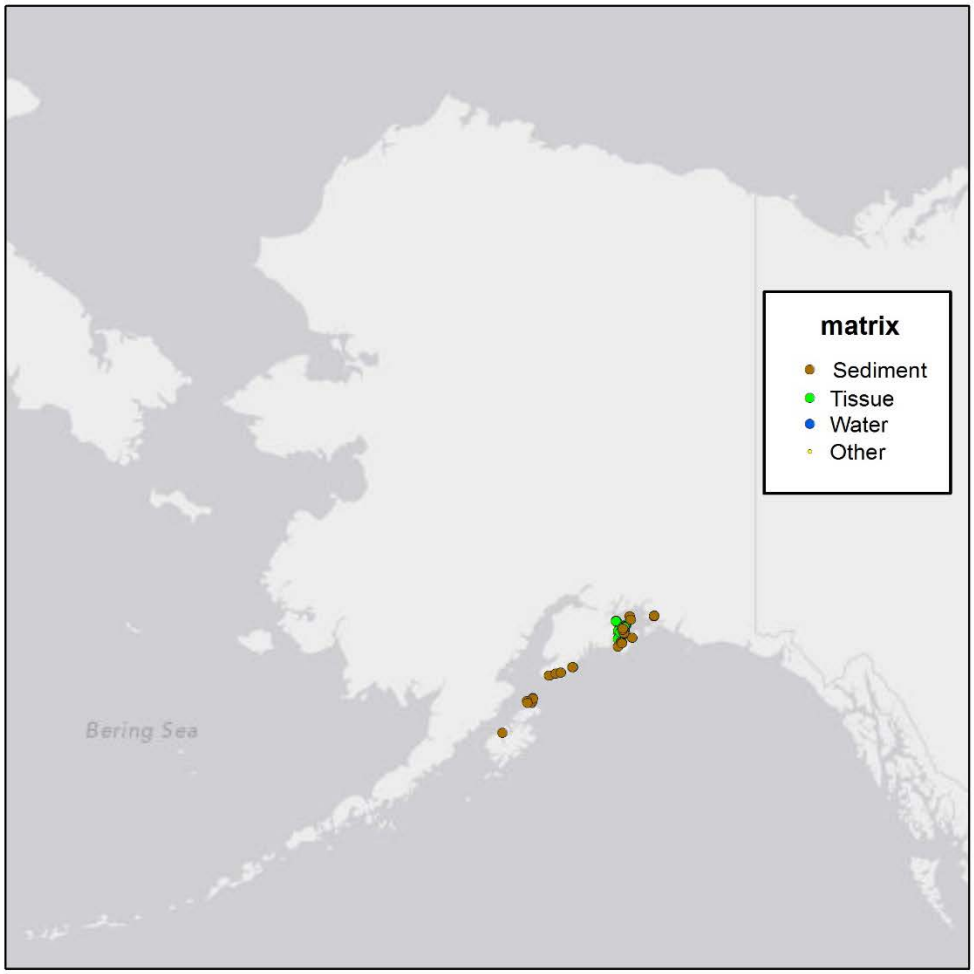


Map illustrates environmental samples only

1995

		Matrix						
Year	Project	Sediment	Tissue	Water	PEMD	SPMD	Blanks	Other
1995	Musselbed/ Pristane	3	3					
	Pink Habitat Recovery	92	5					
	PSTOX			2				
	PSTOX I	10	6	31				
	RHERR95	10	82	126				
	RMB	362	198				10	
	RSLA	13						
	RSUB	48						

1995

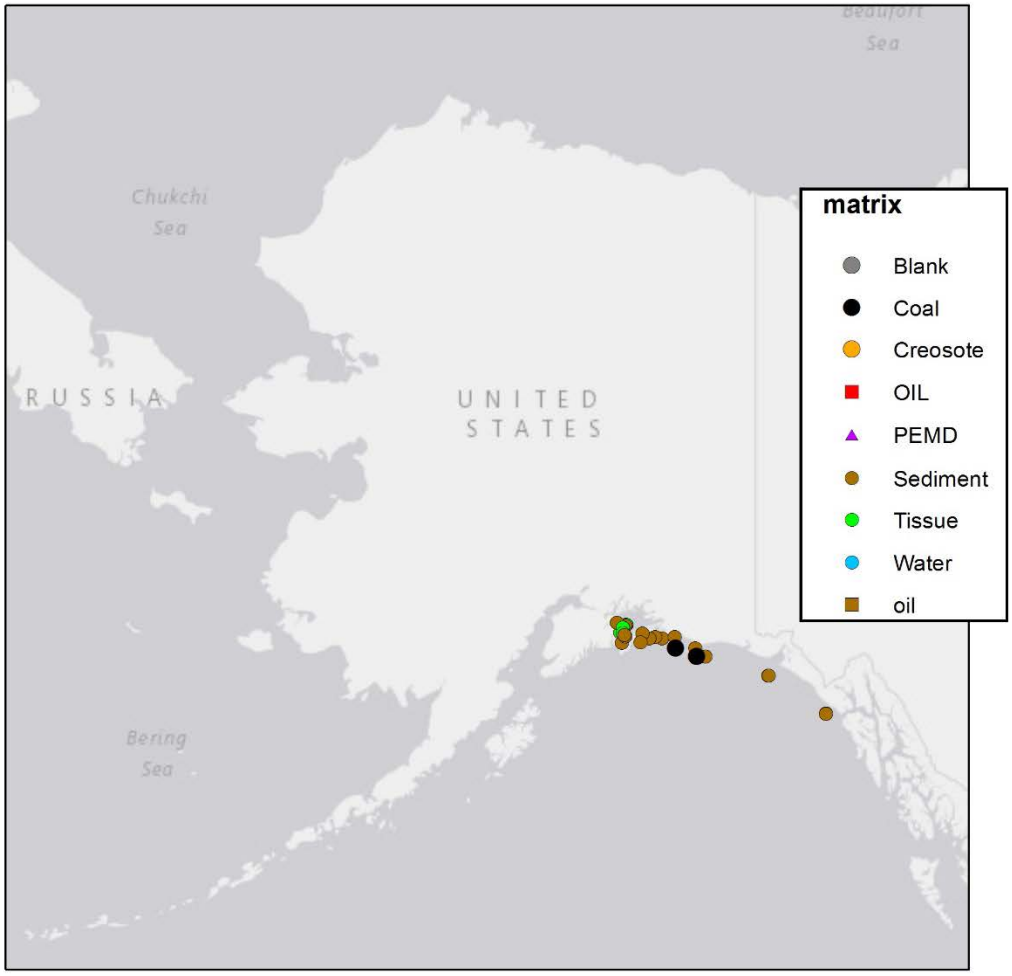


Map illustrates environmental samples only

1996

Year	Project	Matrix Sediment	Tissue	Water	PEMD	SPMD	Blanks
1996	KATALLA	26					
	NVP Mussels		97				
	PSTOX I	3	9	11			
	RMB	102	59				

1996

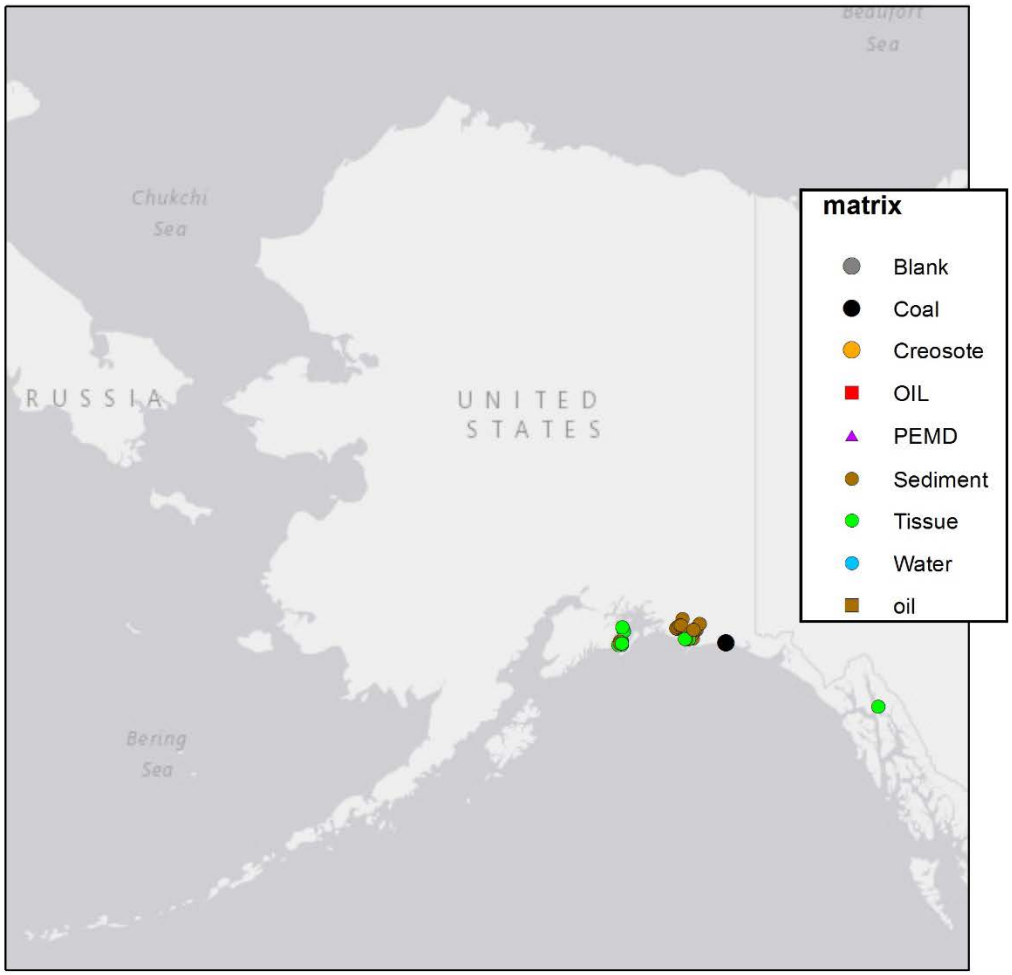


Map illustrates environmental samples only

1997

		Matrix					
Year	Project	Sediment	Tissue	Water	PEMD	SPMD	Blanks
1997	CHENEGA	214	58				
	EVO	3 (oil)					
	KATALLA	24	2	3			
	NVP Mussels		12				
	THOMAS		46				

1997

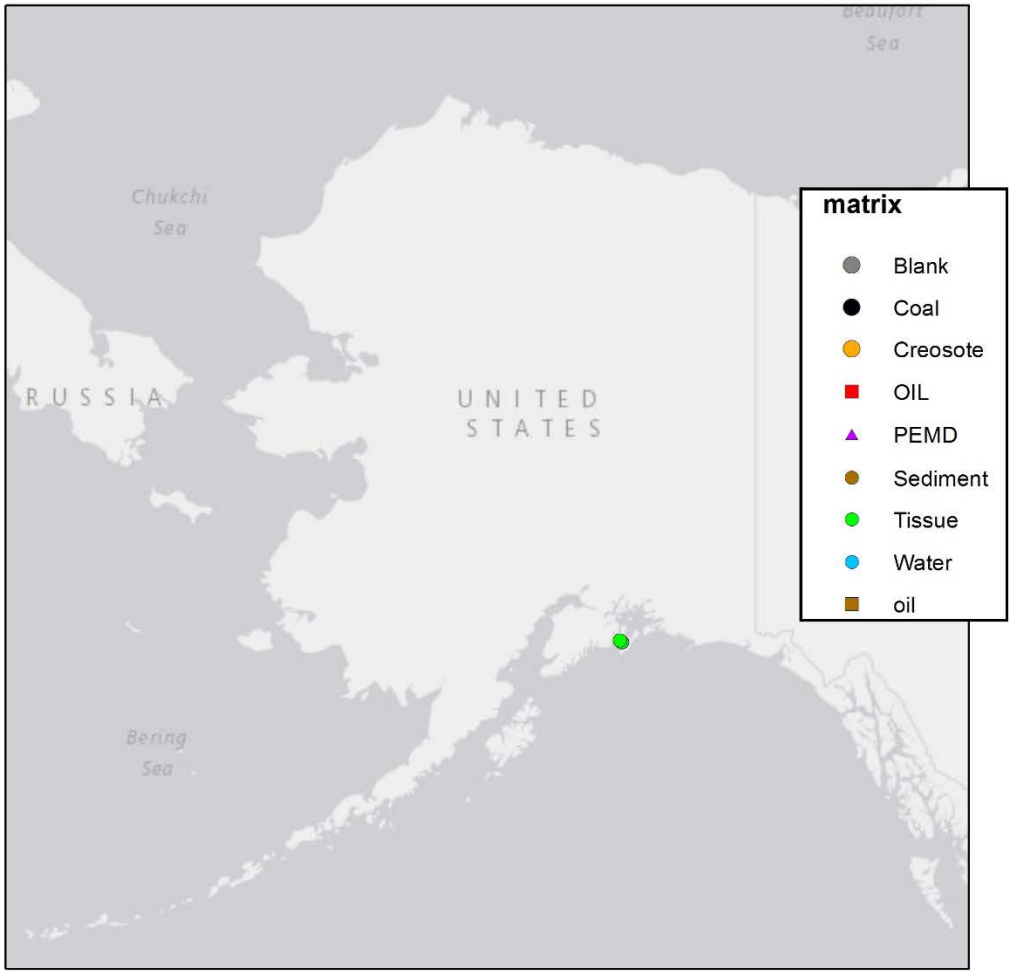


Map illustrates environmental samples only

1998

Year	Project	Matrix Sediment	Tissue	Water	PEMD	SPMD	Blanks
1998	CHENEGA	97	11				
	PSTOX			7			
	PSTOX I	16	12	36			
	RMB	34	34				

1998

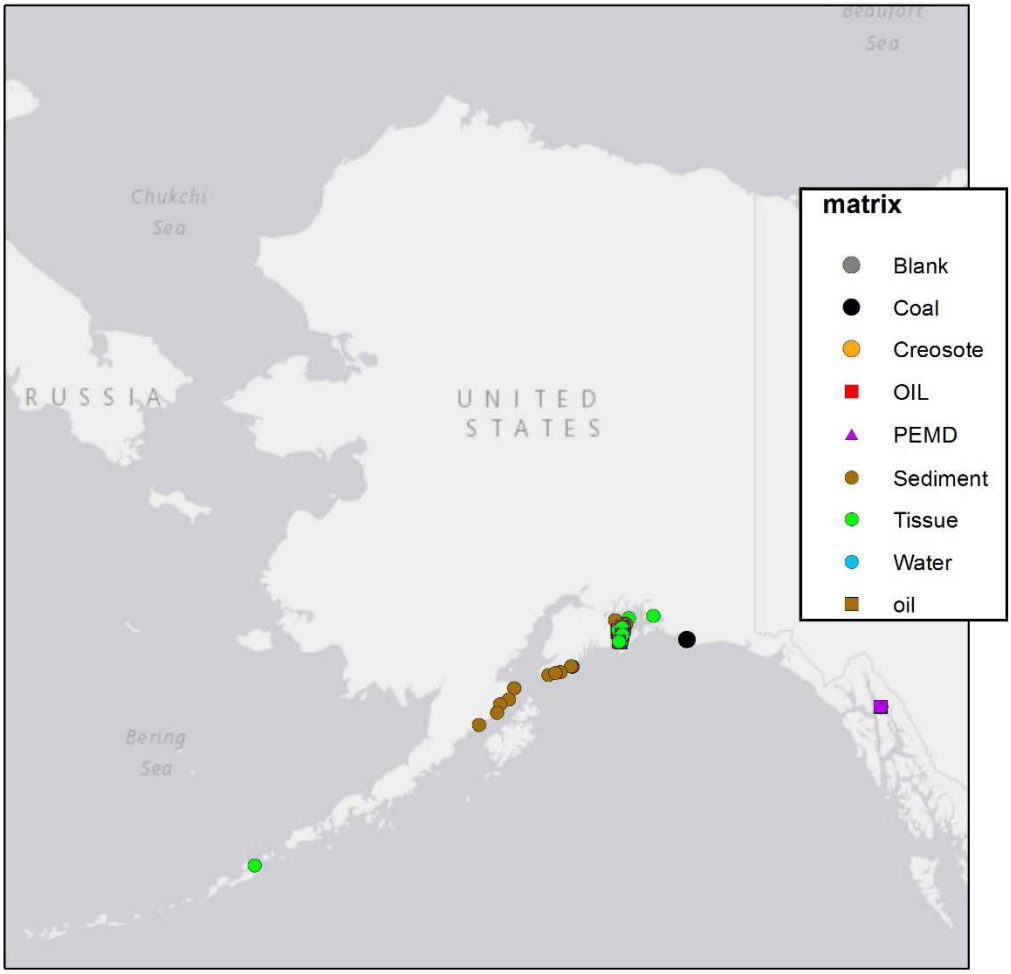


Map illustrates environmental samples only

1999

Year	Project	Matrix Sediment	Tissue	Water	PEMD	SPMD	coal
1999	Auke Lake	4		36	74	15	
	Hawaiian Island Tarballs	3					
	Lingering oil-GOA	12					
	KATALLA						9
	KUROSHIMA Post Spill		3				
	Lingering oil-GOA	12					
	Pink Natal Habitat	119	48		94	24	
	PSTOX I	3	3	10			
	PSTOX II	38	62	64	24	4	
	RMB	311	143				
	ROABMB	6	6				

1999

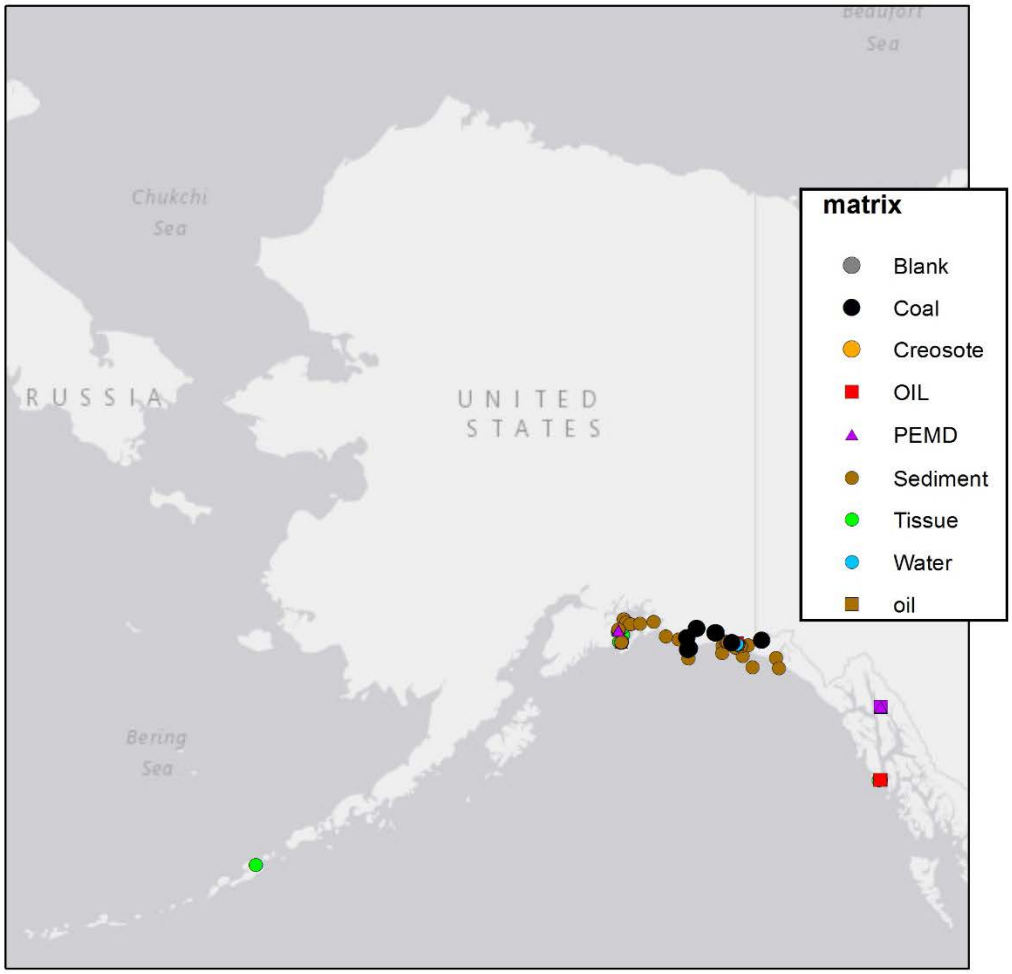


Map illustrates environmental samples only

2000

		Matrix						
Year	Project	Sediment	Tissue	Water	PEMD	SPMD	Oil	Coal
2000	Auke Lake			4	34	5		
	Bering River Coal							12
	DWH/LSU	1						
	Hawaiian Island Tarballs	2	1					
	KUROSHIMA Post Spill		3					
	LPW Oil Spill		13	1			2	
	Pink Natal Habitat	37	17		28		1	
	PSG	9	8	30	11			
	PSTOX II	12	12	12	1			
	STURTGD		21	26				
	Yakataga	51	11				2	

2000

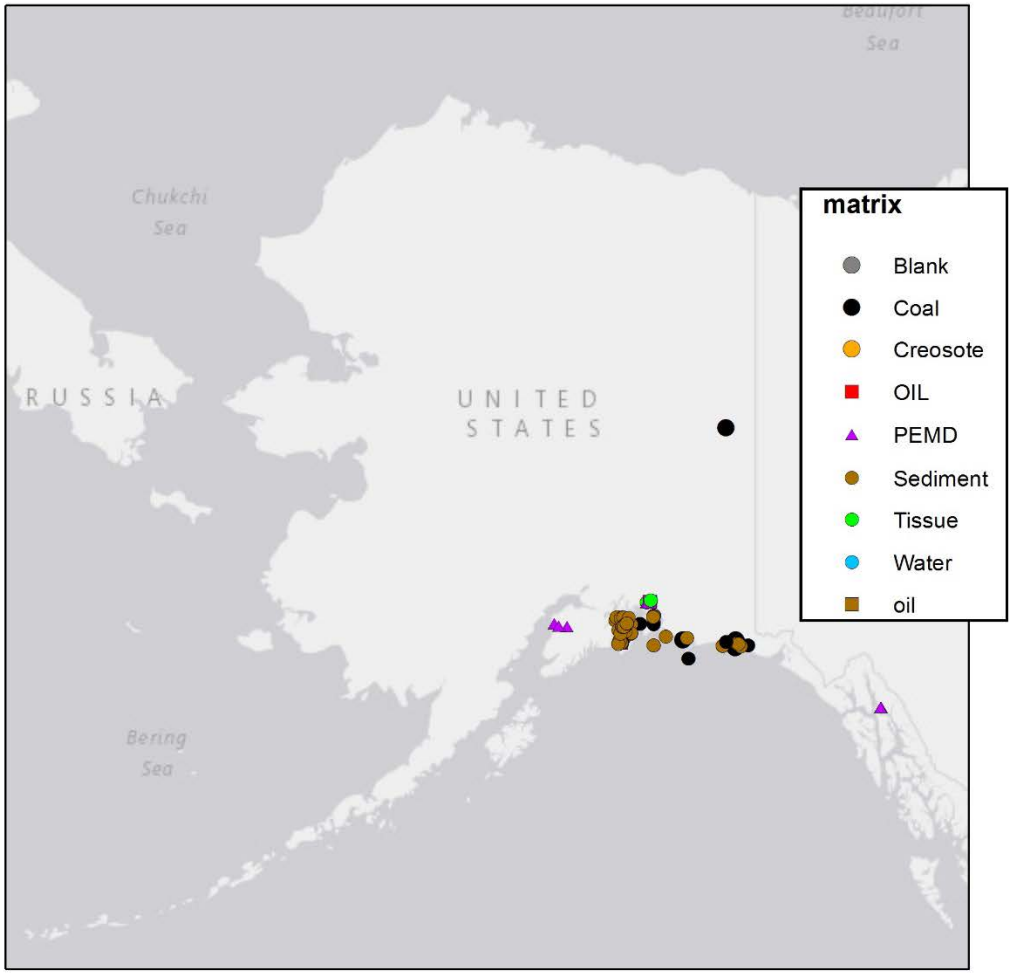


Map illustrates environmental samples only

2001

		Matrix							
Year	Project	Sediment	Tissue	Water	PEMD	SPMD	Oil	PARTICLT	coal
2001	Auke Lake				10				
	EVO						1		
	Kenai				3				
	LTEMP		4	25	18	3		25	
	PHOTOTOX herring		55	27					
	PSG	2	2	5					
	RCAC		19	30	41	6		28	
	SCAT I	311	41	3			1		
	Yakataga	37							19

2001

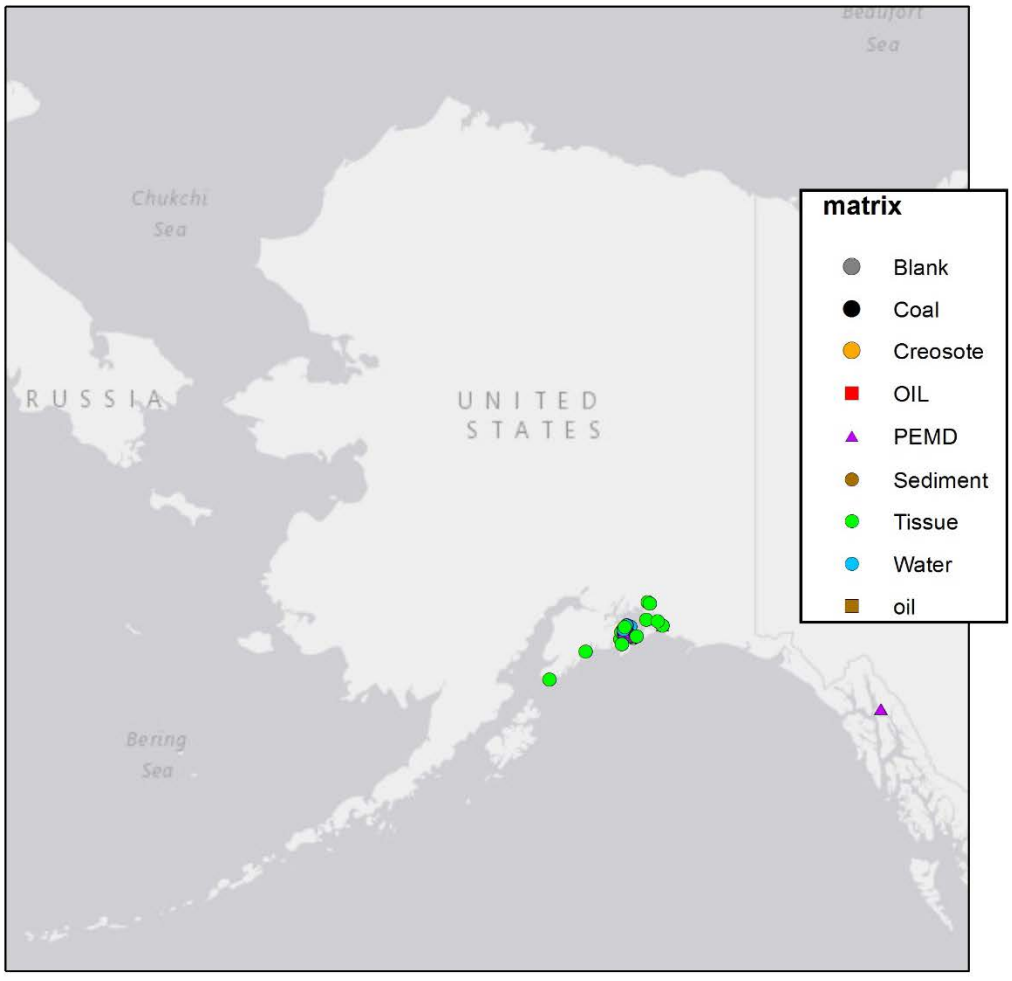


Map illustrates environmental samples only

2002

Year	Project	Matrix: Sediment	Tissue	Water	PEMD	SPMD	Oil
2002	Auke Lake				14		
	LTEMP	9	38				
	SCAT II	251	360	39	168		

2002

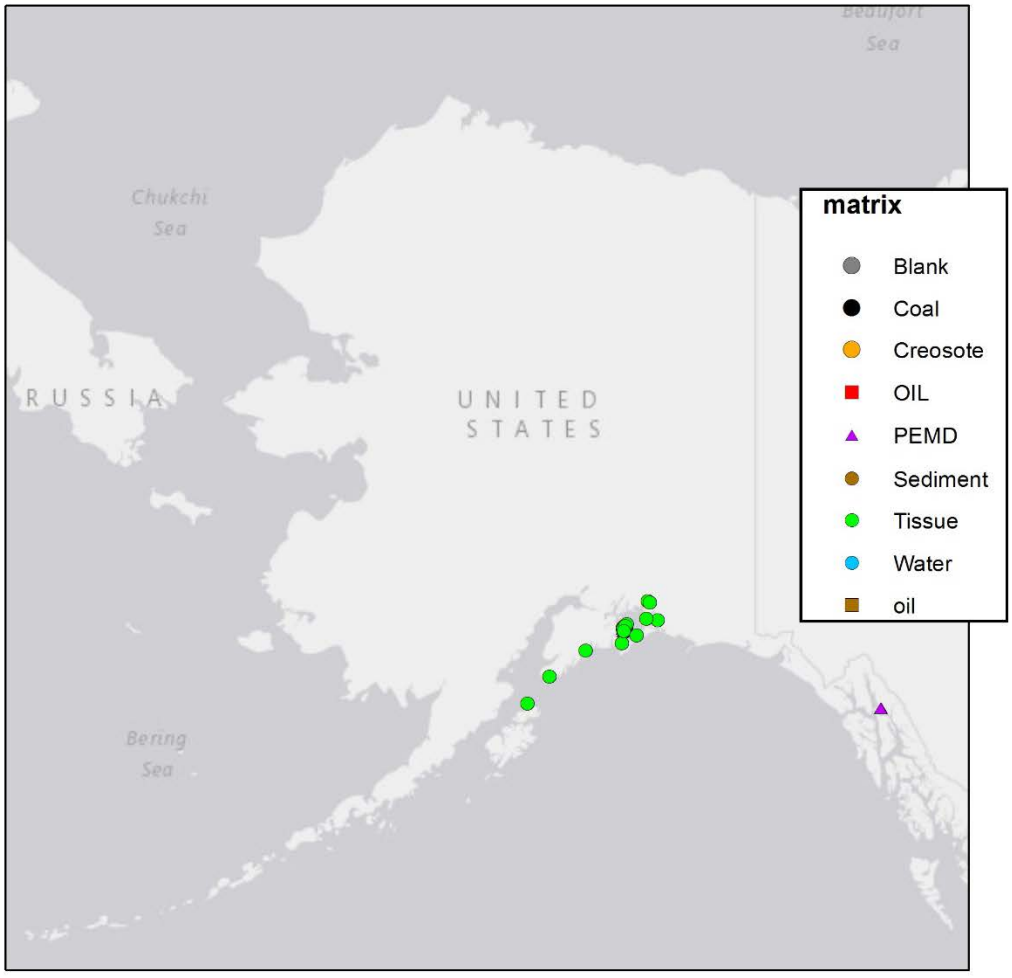


Map illustrates environmental samples only

2003

Year	Project	Matrix Sediment	Tissue	Water	PEMD	Oil	Blank	Other
2003	Auke Lake				74		5	
	LTEMP	19	66				2	4
	PHOTOTOX pink			8		2		
	SCAT III	43	76	2	110			

2003

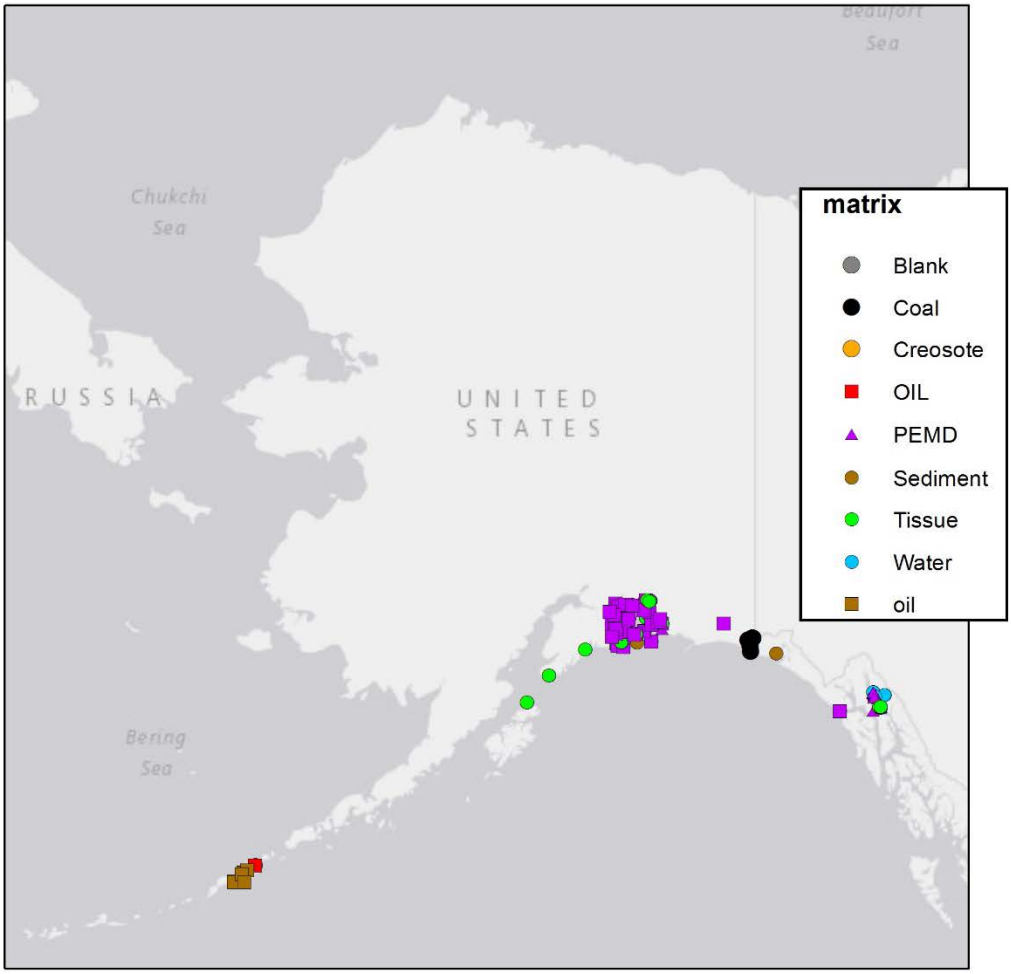


Map illustrates environmental samples only

2004

Year	Project	Matrix: Sediment	Tissue	Water	PEMD	SPMD	Oil	Other
2004	Auke Bay Spill	25	24					
	Auke Lake				2			
	Berners Bay	8	8	4	44			
	KUROSHIMA Post Spill		18				1	
	Laysan Tarball						7	
	LTEMP	41	84					8
	Neocalanus	10	38	22			2	24
	SCAT IV	96	283	10	48	96		
	Selendang						19	
	Tyndall Arm Coal	5						13
	ZEBRA FISH	4		2				

2004



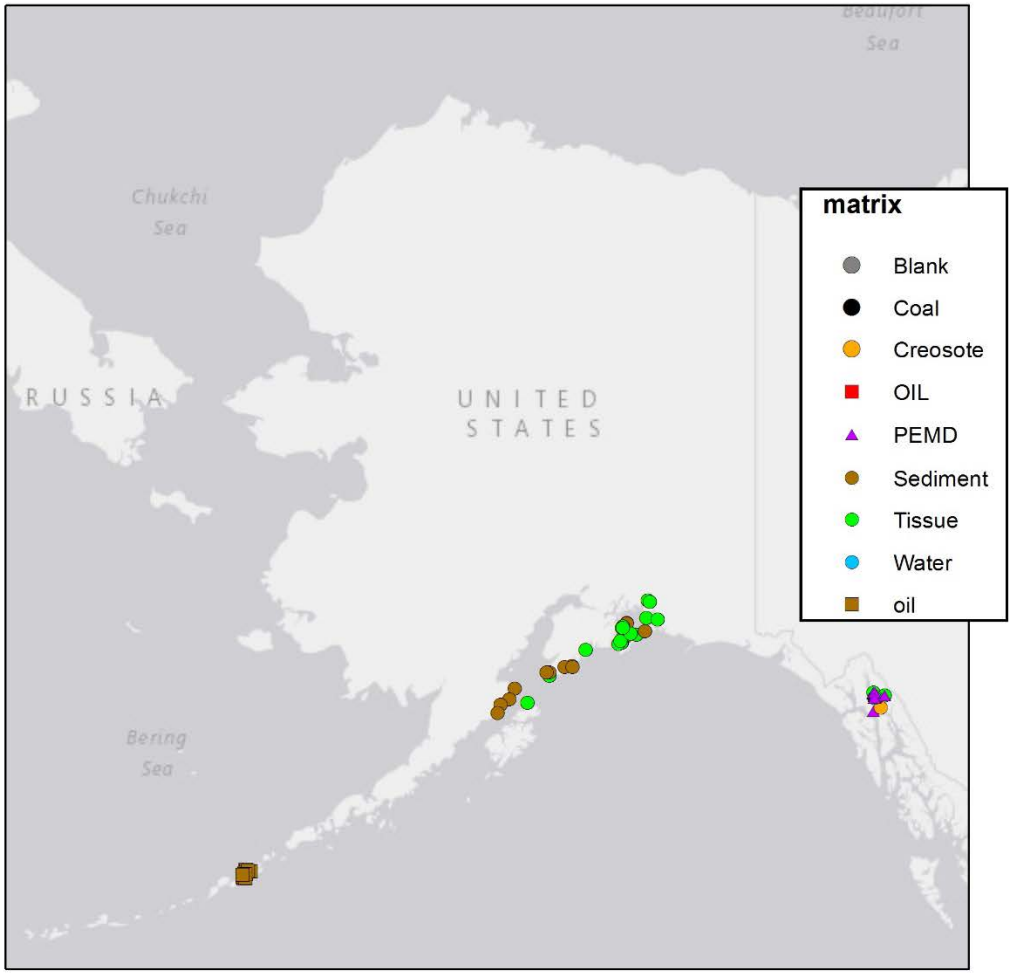
Map illustrates environmental samples only

Magenta squares are SPMDs

2005

		Matrix						
Year	Project	Sediment	Tissue	Water	PEMD	Blank	Oil	other
2005	Berners Bay	4	4	4	48			
	Creosote							1
	Lingering oil-GOA	15	4			9		
	LTEMP	28	69			1		5
	SCAT V	23	18					
	Selendang	56	12		105		34	
	ZEBRA FISH		5	31			2	

2005

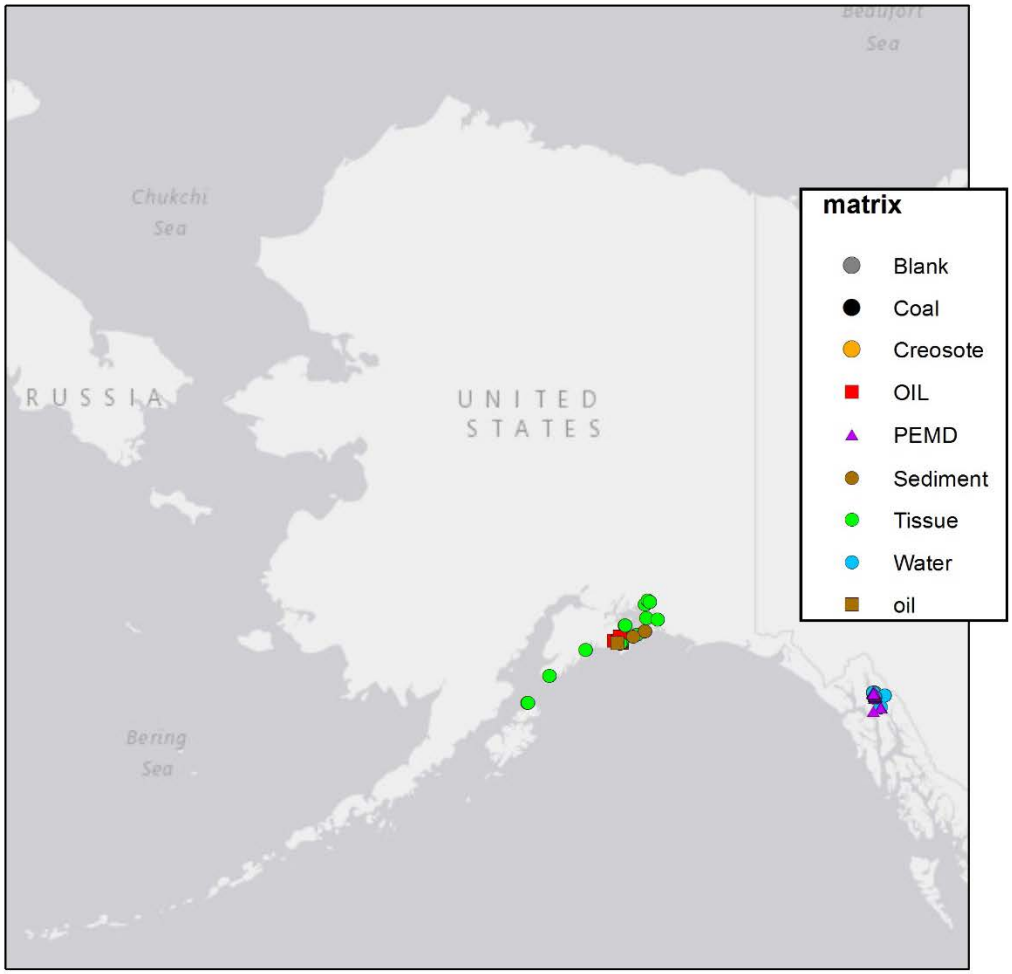


Map illustrates environmental samples only

2006

Year	Project	Matrix: Sediment	Tissue	Water	PEMD	Oil	Other
2006	Auke Lake			1	10		
	Berners Bay	6	6	4	47		
	LTEMP	33	73	2			2
	Particulate Oil Tox	33		60			
	Queens University			70			
	SCAT VI	13	11	2		17	
	ZEBRA FISH			4	4		1

2006

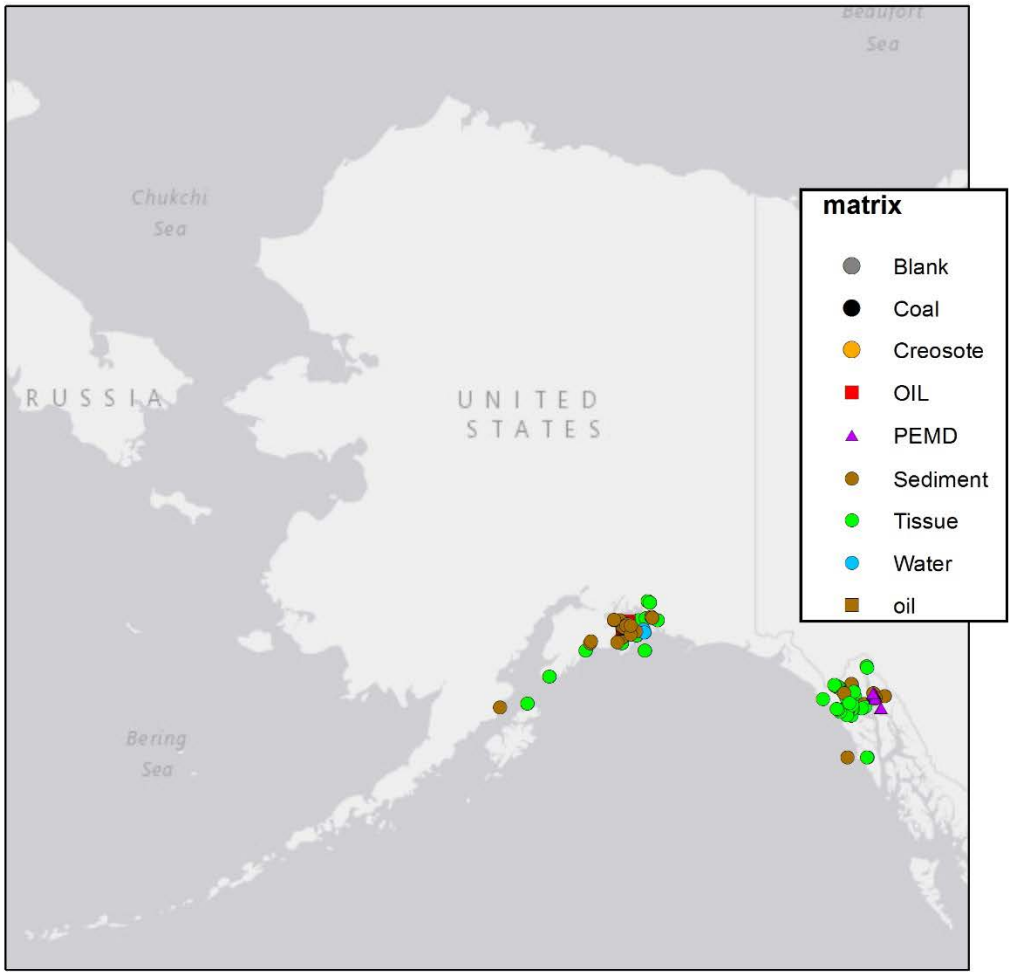


Map illustrates environmental samples only

2007

Year	Project	Matrix					
		Sediment	Tissue	Water	PEMD	SPMD	Other
2007	Auke Lake				14		1
	Berners Bay	4	4	4	44		
	DWH/LSU		6		11		
	Egg Shock	3	16	18			
	Lingering Oil	36					
	LTEMP	34	85	6			
	Nordic Viking Spill Sampling		2				
	NPS	6	53				
	NWC Urban Runoff				8		
	Oiled Rock Column			15			
	Particulate Oil Tox			34			14
	SCAT VII						
	Seward Marine Center			4			
	UAF-Hicken			47			

2007

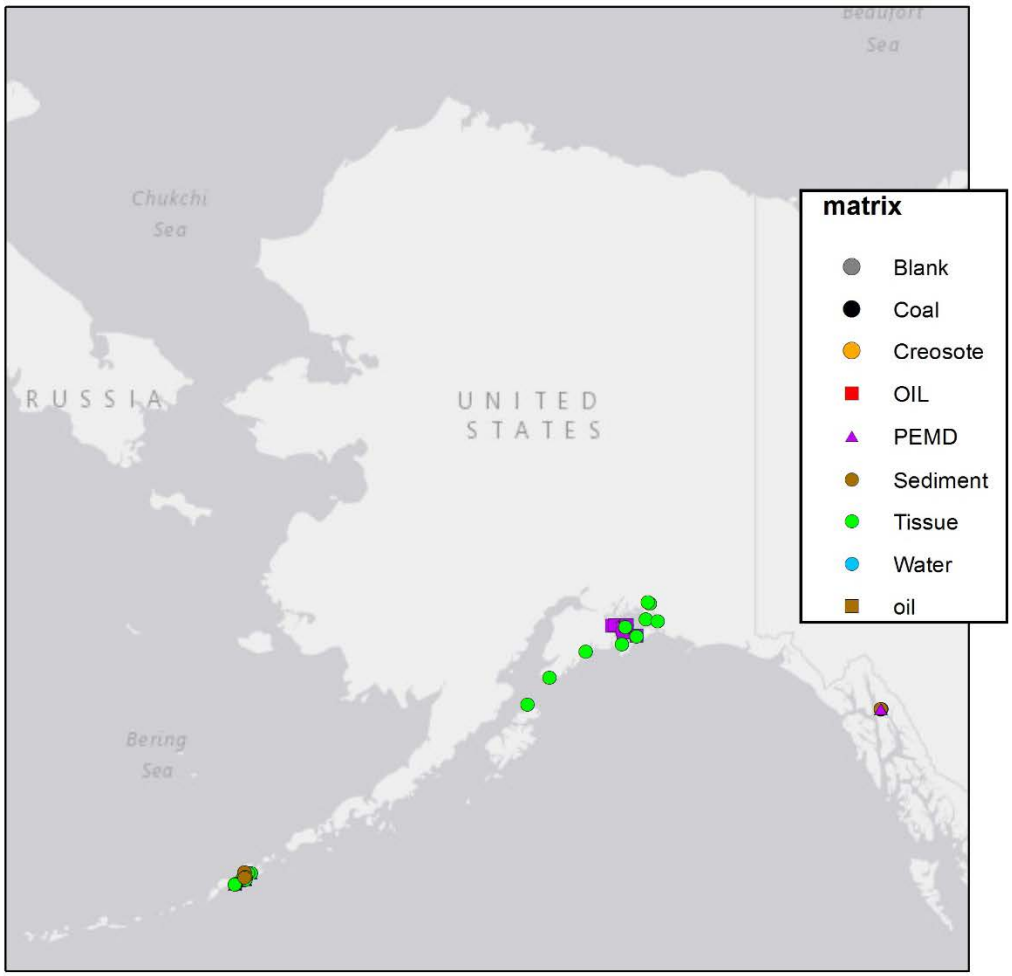


Map illustrates environmental samples only

2008

		Matrix					
Year	Project	Sediment	Tissue	Water	PEMD	SPMD	Other
2008	Aleutian/Prib				5		
	Auke Lake	8		7	9		
	Egg Shock			4			4
	HADU-SPMD PWS					4	
	LTEMP	12	36				5
	Nearshore Otter Duck	120					
	SPMD/HADU	10				12	

2008

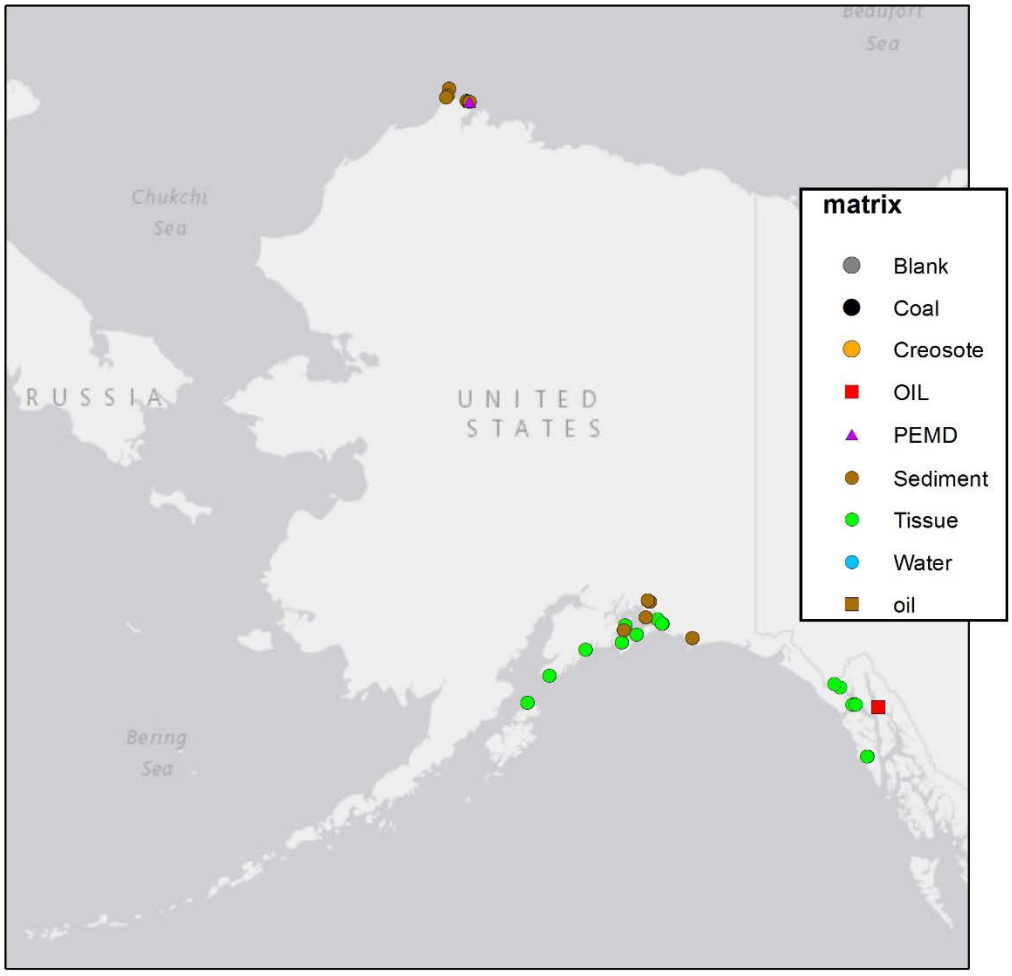


Map illustrates environmental samples only

2009

Year	Project	Matrix						
		Sediment	Tissue	Water	PEMD	SPMD	Oil	Other
2009	Aleutian/Prib				13			
	Arctic	12			3			3
	Cordova-CHILKAT	11	3					
	Lingering Oil-PWS	4						
	LTEMP	32	44					8
	NPS		6					
	NWC Urban Runoff				19			
	Princess Kathleen						1	

2009

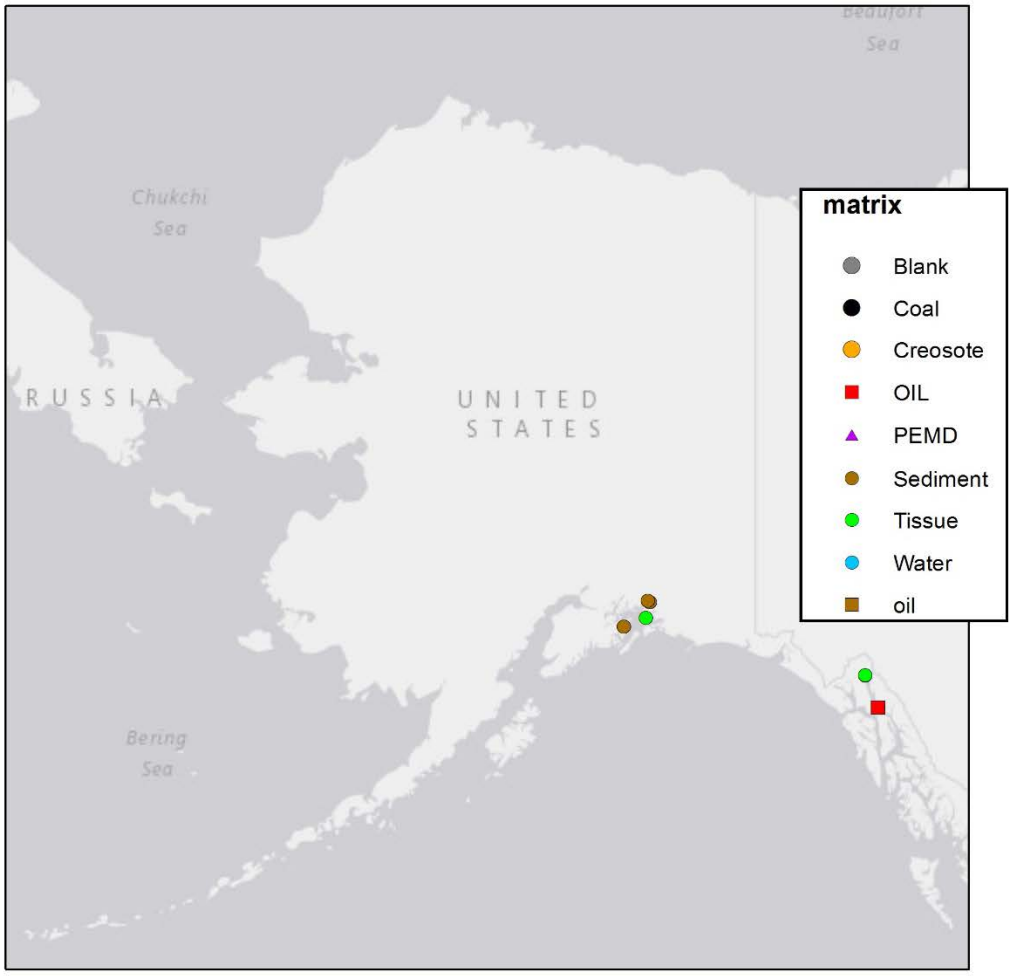


Map illustrates environmental samples only

2010

		Matrix						
Year	Project	Sediment	Tissue	Water	PEMD	Oil	Blank	Other
2010	Cardiac/Herring		6	13				
	Cardiac/pink	15	12	40	6			
	DWH					5		
	DWH/LSU				52			
	DWH/NWFSC			23				
	DWH/SAV				157	5	6	
	Haines Harbor Expansion	3	3					
	Lingering Oil-PWS	6						
	LTEMP	13	9					3
	NWC Urban Runoff				9			
	PEMD Fluorescence		2	7	16			
	Princess Kathleen	8				3		

2010

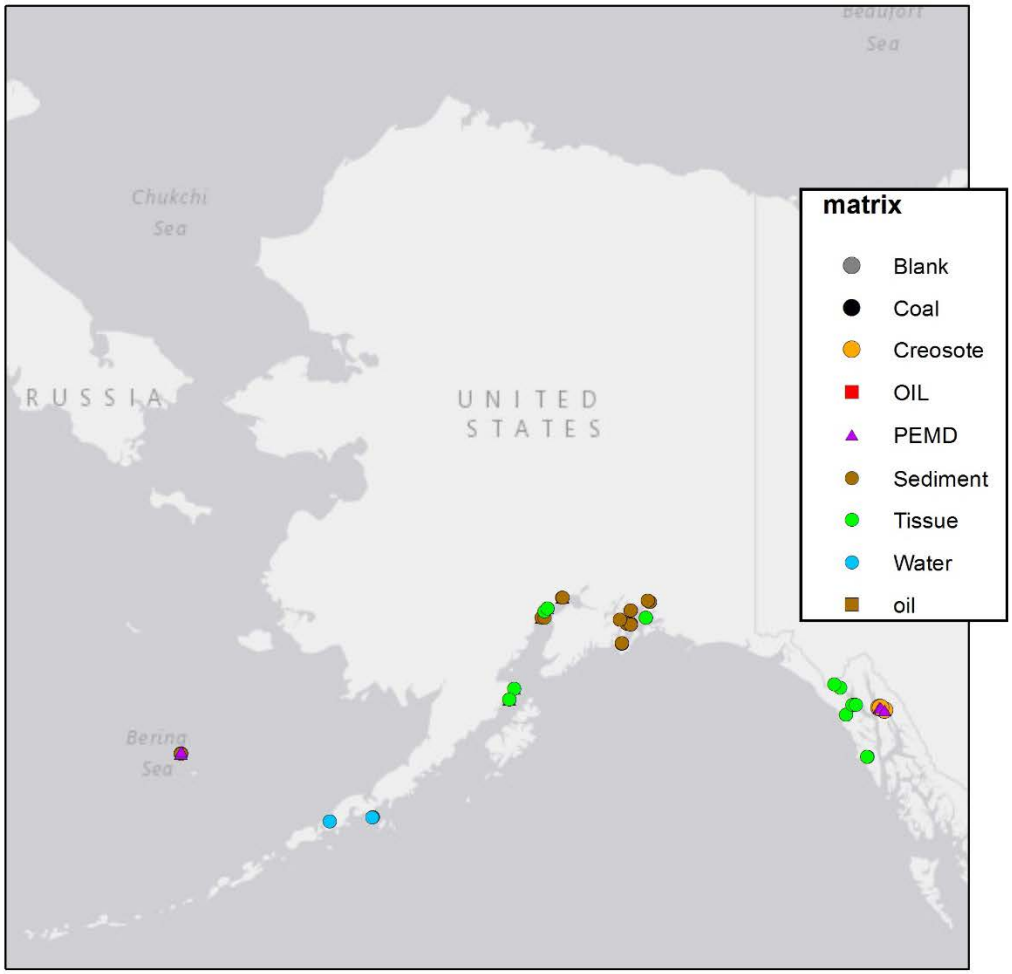


Map illustrates environmental samples only

2011

Year	Project	Matrix					
		Sediment	Tissue	Water	PEMD	Creosote	Other
2011	ADFG Birds	17	8		42		
	Auke Lake				9		
	Bioremediation/PWS	125		1			
	Cardiac/pink		10				
	Creosote Thesis		12	128	89	3	1
	DWH/LSU	38			53		
	Lingering oil-GOA		5		36		
	LTEMP	12	9				3
	NOAA Radionuclide	2	3	3			
	NPS		6				

2011

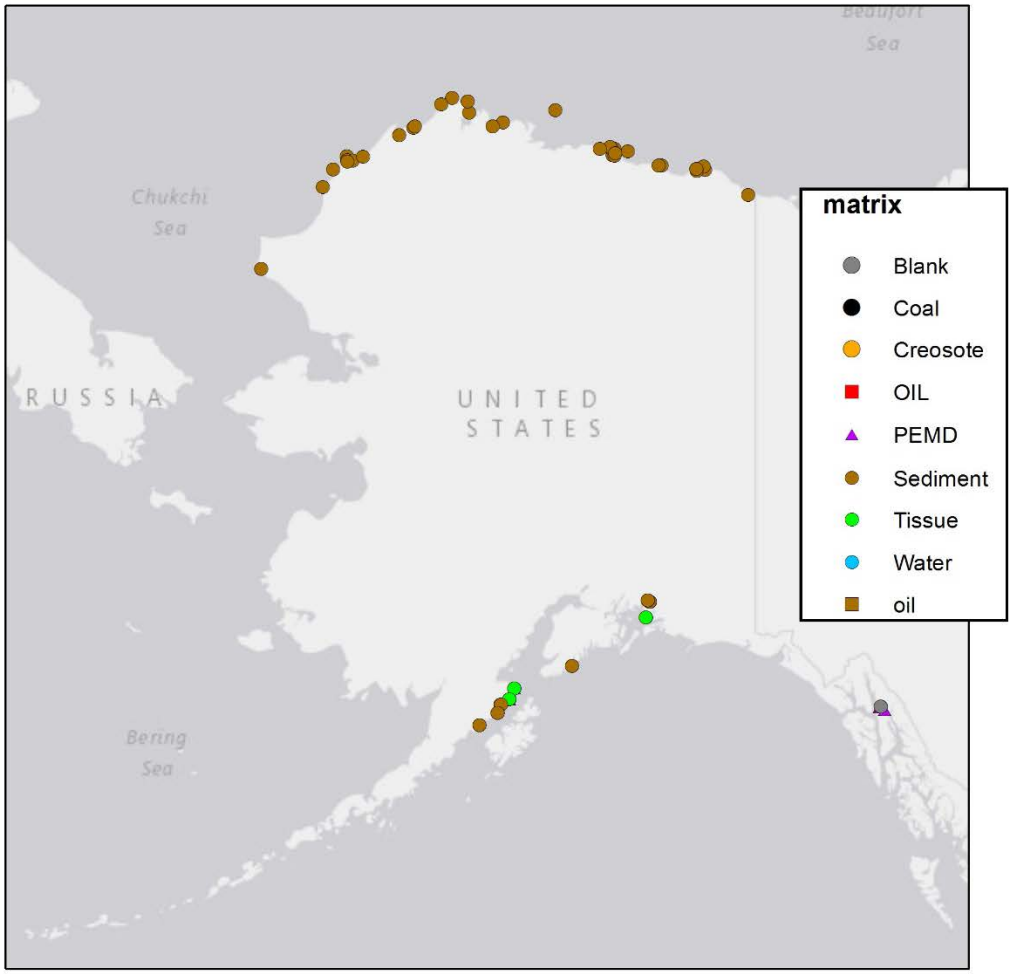


Map illustrates environmental samples only

2012

		Matrix					
Year	Project	Sediment	Tissue	Water	PEMD	Blank	Other
2012	Bioremediation/PWS	92					
	Creosote Thesis				191		4
	DWH/LSU	1					
	Fluoresce				10		2
	Lingering oil-GOA	17	2		33	3	
	LTEMP	12	9			1	3
	Peat						1

2012

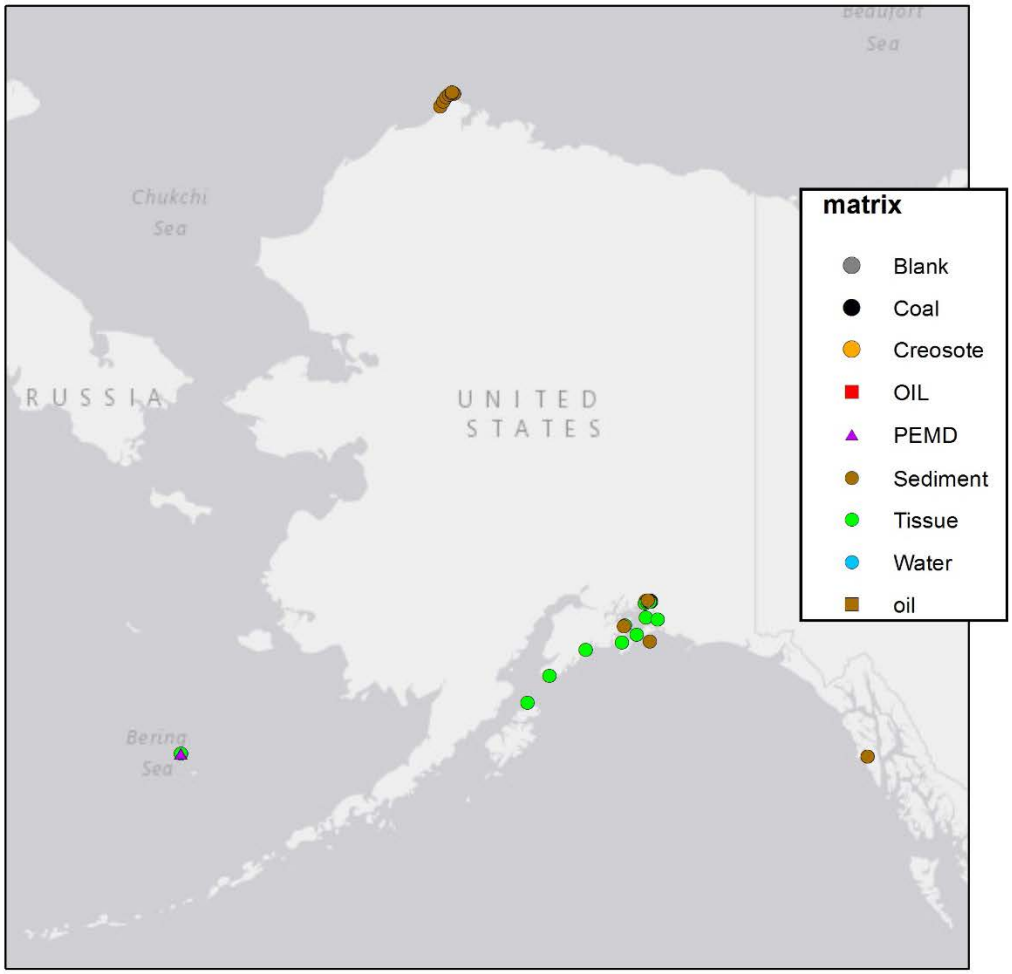


Map illustrates environmental samples only

2013

Year	Project	Matrix					
		Sediment	Tissue	Water	PEMD	Oil	Other
2013	ADFG Birds		5		18		
	Arctic	24					
	EVO Barge					7	
	EVOS Gulf Watch	10					
	LTEMP	13	30			2	3
	PWS Shrimp	20	228				
	Steep Creek				7		

2013

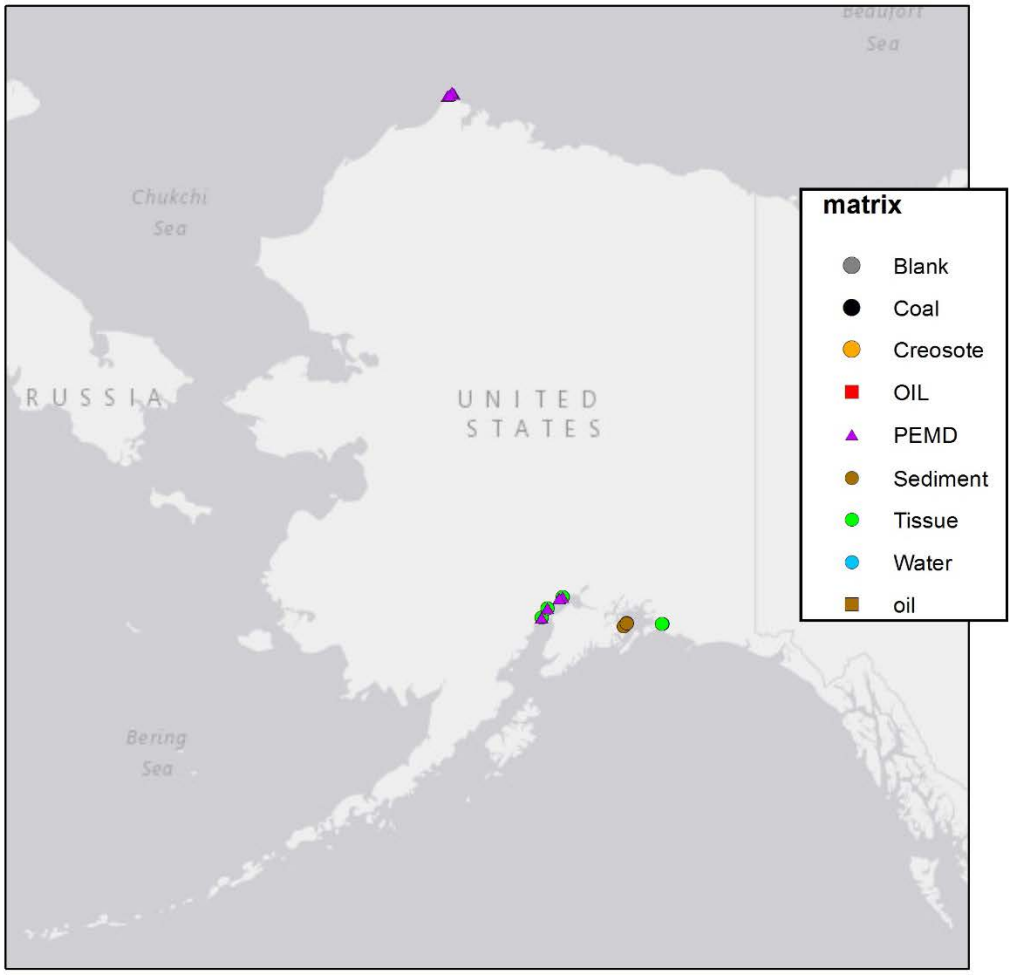


Map illustrates environmental samples only

2014

Year	Project	Matrix Sediment	Tissue	Water	PEMD	Oil	Other
2014	ADFG Birds	9	4		54		
	Arctic				19		
	Cordova		6				
	Lingering Oil	9					

2014



Map illustrates environmental samples only

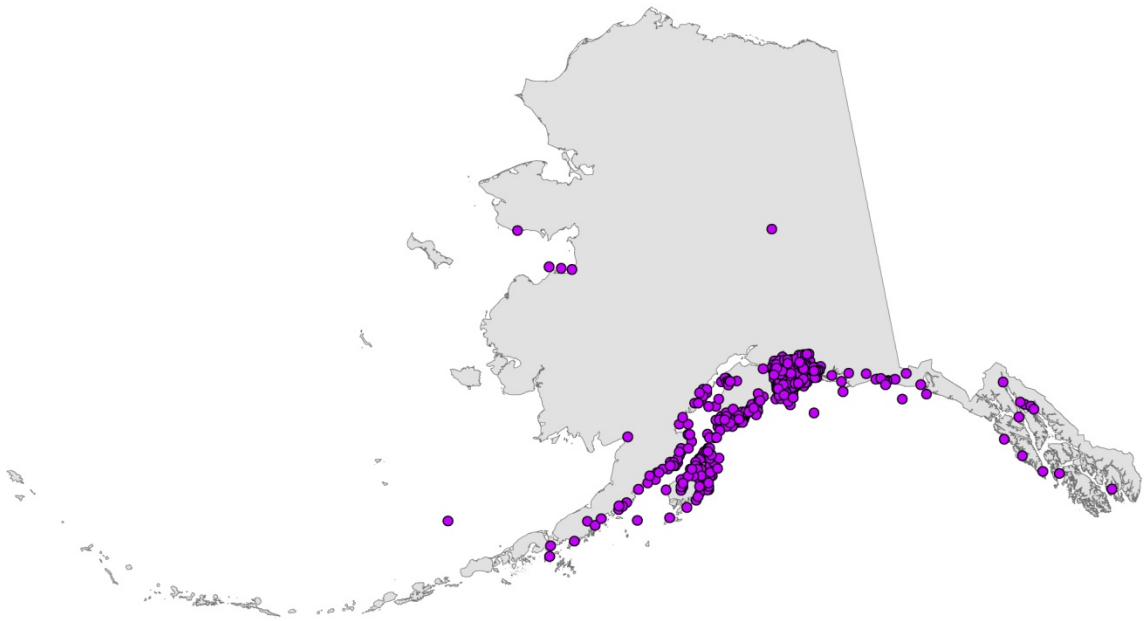
2015

Year	Project	Matrix: Sediment	Tissue	Water	PEMD	Oil	Creosote
2015	Cordova		6			1	1
	SCAT 2015	79			24		

2015

Map illustrates environmental samples only

Locations identified in table Locations.



Database Structure

Overview

This database contains sample collection information (table Sample) and analytical hydrocarbon analyses of those samples in tables Alkane, PAH, and Biomarkers together with quality control codes for each analyte (tables QC code Alkane and QC code PAH). Other supporting tables are present to describe the content of the database. For example, table ANALYTE describes each analyte abbreviation and indicates reporting units (ng/g wet weight for sediments and tissue, ng/L for water, or ng/device for PEMDs and SPMDs). In the text that follows, key variables are described first, then an alphabetical listing of major data tables (Alkane, Biomarker, PAH, QC code Alkane, QC code PAH, and Sample), and a section describing supporting tables, and a description of queries.

Key Linking Variables

Three key variables are required in this database structure, sample identification number (SIN), replicate (Rep), and quality control batch (QCbatch). SINs uniquely identify each sample, Rep further identifies analytical results, particularly where one or more replicate measures were completed on a single sample, and QCbatch uniquely identifies each group ('string') of samples processed together in the laboratory. SINs link sample information (table SAMPLE) to analytical information (tables Alkane, PAH, and Biomarkers). SIN, Rep, and QCbatch are required to link information in tables Alkane, PAH, QC code Alkane and QC code PAH. QCbatch is required to identify which quality control samples are associated with specific sample analyses; particularly for quality assurance samples which have negative SINs.

SIN

Sample Identification Numbers uniquely identify each sample. They are used to link sample and analytical information. For samples, SINs are positive integers and are assigned before sample collection using Chain of Custody documentation and procedures. Beginning in 2010, SIN structure was changed to incorporate year as the first four digits of the number; the remaining four digits are sequentially assigned by laboratory management in the Chain of Custody logbook. See section "Content" in this document for specific information on SINs present in the database.

Negative SINs represent quality assurance samples completed with each group ("string") of samples analyzed in the laboratory. Method blanks (MBLK), spiked blanks (SBLK), and aliphatic reference standards (NMFSALI) are assigned unique negative SINs (see table below). Aromatic reference standards (NISTARO) are assigned either -600 or -601. Quality assurance samples for precision and accuracy are assigned various negative SINs because composition changes as old standards are depleted and new standards are prepared (AREF and BREF in table below).

Rep

Rep is sample replicate number and is determined by the analytical laboratory. Most samples were analyzed once (Rep = 1). A minority were analyzed a second time (or more)

for quality assurance purposes. In addition to this procedure, replicates >29 identify spiked reference material or spiked blanks in analytical tables (Alkane and PAH).

QCbatch

Unique identifiers supplied by the analytical laboratory to track groups ('strings') of samples that were analyzed together. The numeric portion of these alpha-numeric identifiers (e.g., R031108) codes day, and year (mm, dd, yy) of analysis. The initial alphabetical character was originally specific to the analyst but no longer has meaning.

Abbreviation	Type of Standard	SIN
AREF	Reference sample for precision and or accuracy, replicate 1	-555, -580, -581, -720, -721,-877, -878, -879
BREF	Reference sample for precision and or accuracy, replicate 2	-555, -580, -581, -720, -721,-877, -878, -879
MBLK	Method blank	-900
NISTARO	National Marine Fisheries Service (Auke Bay Laboratories) calibration standard using National Institute of Standards and Technology Standard Reference Material (SRM) 1491	-600,-601
NMFSALI	National Marine Fisheries Service (Auke Bay Laboratories) Aliphatic Reference Standard	-600
SBLK	Spiked blank	-901

Detailed reference information

SIN	Standard, or blank
-500	NIST calibration standards (all)
-550	New PAH REF QCSed or QC-SED-1 (GERG)
-555	New PAH QCSed SRM 1944
-556	QCSed SRM 1941b
-557	QCOil SRM 1582
-558	QCOil ANS: EVO 05/19/1989 (hold oil)
-559	QCTis SRM 2977

-580	LDPE Sblk = AREF or BREF
-581	SPMD ref
-582	SPM ref (suspended particulate material, from filters)
-600	NMFS calibration standards
-601	LDPE calibration standard
-602	New NMFS PAH calibration standards
-605	Biomarker standard
-700	NP QCbatches
-720	REF QCwater Aro
-721	REF QCwater Ali
-870	NP QCbatches
-871	NP QCbatches
-872	Old Tis Ref
-873	QCTis
-874	QCTis
-875	QCTis
-876	QCTis
-877	Ref QCTis
-878	New PAH ref QCTis SRM 1974a
-879	New PAH ref QCTis SRM 1974b
-880	QCTis SRM 2974a
-900	MBLK (method blank)
-901	SBLK (spiked blank), sediment or tissue
-902	SBLK (spiked blank)

Common Fields

Analysis Type

There are five specific processing methods, PEMD, sediment, SPMD, tissue, and water. Each matrix (table Sample) has specific processing requirements and MDLs matched to one of these methods. For example, agarose processing is the same as water. Blanks are processed as water, sediment, or tissue. Coal, creosote, peat, and oil are processed as sediment and PARTICLT are generally also processed as sediment.

Catno

Alphanumeric identifier used to track groups of samples released by the database manager to an analytical laboratory for analysis.

Comment

Comments as needed

DryWt, WetWt

Sample wet and dry weights in grams (except for water, SPMD, and PEMD samples).

LAB

Identifies the laboratory where the analysis was performed. The Auke Bay Laboratory, Juneau, AK (ABL) is currently the only laboratory processing hydrocarbon samples for this database (as of 1992). Some of the earlier samples were processed by the Geochemical and Environmental Research Group, Texas A&M University, College Station, Texas (GERG). A few tissue samples were processed by the Northwest Environmental Conservation Division (NECD) in 1990 (n = 94).

LabSam

Provides additional detail for quality control sample type or repeats SINs for samples. See the description of negative SINs in section “key variables” for further detail.

Proportion

This field is used for passive samplers (PEMDs and SPMDs) only and is the proportion (0 to 1) of passive sampler analyzed.

QCbatch

Unique identifiers supplied by the analytical laboratory to track groups ('strings') of samples that were analyzed together. The numeric portion of these alpha-numeric identifiers (e.g., R031108) codes day, and year (mm, dd, yy) of analysis. Beginning in 2010, QCbatch structure was changed to yyyymmdd, where yyyy = year, mm = month, dd = day.

Rep

Rep is sample replicate number and is determined by the analytical laboratory. See section "key variables" for further detail.

SIN

Sample Identification Numbers uniquely identify each sample. They are used to link analytical information with sample information and other hydrocarbon analyses. See section "key variables" for further detail.

Type

Indicates sample type: quality control samples are indicated by QC, all others by 0 or blank.

Units

Sample reporting units, such as ng/g wet weight

Vol

Sample volumes in milliliters are recorded for water samples (only).

Table Alkane

Table ALKANE contains analytical alkane results. These data can be linked to sample via sample identification numbers (SIN). Below are descriptions of variables present in the table; common variables (preceding) are not repeated.

C12d26, C16d34, C20d42, C24d50, and C30d64

Deuterated alkane surrogates used to determine recovery; n-dodecane-d_{26} (C12d26), n-hexadecane-d_{34} (C16d34), n-eicosane-d_{42} (C20d42), n-tetracosane-d_{50} (C24d50), and triacontane-d_{50} (C30d64).

C9ALK, C10ALK, … C36ALK

Concentrations (ng/g wet weight) of saturated aliphatic hydrocarbons C9- through C36-alkanes, and the isoprenoids pristane and phytane. See table ANALTYE for further descriptive detail.

TotAlkanes

Total alkane concentration (ng/g wet weight) of all alkanes, including both uncalibrated and calibrated compounds.

UCM

Concentration of the unresolved complex mixture (ng/g wet weight).

Table Biomarkers

Table Biomarkers contains analytical biomarker results (triterpanes, hopanes, steranes). These data can be linked to sample via sample identification numbers (SIN). Below are descriptions of variables present in the table; common variables (preceding) are not repeated.

d-C20, d2-C27

Internal standards

Norpristane, Pristane, Phytane

Concentrations (ng/g wet weight) of isoprenoids. See table biomarker abbreviations for more detail.

TR23, TR24, …TR29b

Concentrations (ng/g wet weight) of terpanes. See table biomarker abbreviations for more detail.

Ts, Tm, … H35R

Concentrations (ng/g wet weight) of hopanes. See table biomarker abbreviations for more detail.

S22, DIA27S, … C29R

Concentrations (ng/g wet weight) of steranes. See table biomarker abbreviations for more detail.

Table Gravimetric

Table Gravimetric contains gravimetric measures of oil mass in sediment samples.

Pit#

The pit number is equivalent to investigator number. These refer to specific locations in a beach, chosen randomly.

Pit_mass

Total mass from an excavated pit (kg).

Sample mass

Mass of sample collected for analysis (kg).

Mass extracted

This is the mass of the sample extracted for oil. The mass extracted is either the sample mass or a subset of sample mass (g).

Tare

Tare weight of a pan or flask (g).

Gross mass

The gross mass includes sediment and oil and the tare weight (g).

Type

Sample type. Some records are identified as subsets.

Oil_mass

Mass of oil in grams.

Oil/(o+sed)

This is milligrams oil per gram of (sediment + oil)

Oil/sed

This is milligrams of oil per gram sediment. The oil mass was removed from the denominator.

Pit_oil

Mass of oil in the pit (g)

Table PAH

Table PAH contains analytical polynuclear aromatic hydrocarbon results. These data can be linked to sample via sample identification numbers (SIN). Below are descriptions of variables present in the table; common variables (preceding) are not repeated.

NaphD8, Acend10, Phend10, Anthra10, Banth12, Chryd12, Benad12, Peryd12

Deuterated aromatic surrogates used to determine recovery; naphthalene-d_8 (NaphD8), acenaphthene-d_{10} (Acend10), phenanthrene-d_{10} (Phend10), anthracene-d_{10} (Anthra10), benzo(a)anthracene-d_{12} (Banth12), chrysene-d_{12} (Chryd12), benzo(a)pyrene-d_{12} (Benad12), and perylene-d_{12} (Peryd12).

Naph, … Benzop

Concentrations (ng/g wet weight) of polynuclear aromatic hydrocarbons naphthalene through benzo(ghi)perylene. See table ANALTYE for further descriptive detail.

Table QC code Alkane

The purpose of table QC code Alkane is to report quality control (QC) information associated with each analyte. These data can be linked to alkane concentrations (table Alkane) by SIN, (QCBatch), and Rep. See the common variable section for additional detail.

C9ALK, C10ALK, … C36ALK

QC data for saturated aliphatic hydrocarbons C9- through C36-alkanes, and the isoprenoids pristane and phytane. See table QCcodes for further descriptive detail.

Table QC code PAH

The purpose of table QC code PAH is to report quality control (QC) information associated with each analyte. These data can be linked to PAH concentrations (table PAH) by SIN, (QCBatch), and Rep. See the common variable section for additional detail.

Naph, … Benzop

QC data for polynuclear aromatic hydrocarbons naphthalene through benzo(ghi)perylene. See table QCcodes for further descriptive detail.

Table SAMPLE

Much of the information contained in table SAMPLE can be cross referenced with the original Chain of Custody forms submitted by investigators when samples are delivered to the laboratory for analysis (and before samples are analyzed).

SIN

Sample Identification Number uniquely identifies each sample. It is used to link sample information with all hydrocarbon analyses. Values are positive integers and are assigned before sample collection using Chain of Custody documentation and procedures. Beginning in 2010, SIN structure was changed to incorporate year as the first four digits of the number; the remaining four digits are sequentially assigned in the Chain of Custody logbook. See section "Content" in this document for specific information on SINs present in the database.

Depth

Optional sample depth in meters, measured from mean lower low water (MLLW) as measured or estimated by the sample collector. Depths are positive below MLLW and negative above.

Project

Abbreviated names for projects present in the database and additional detail is given in table "Project Names." Further information regarding projects supported by the *Exxon Valdez* Oil Spill Trustee Council (EVOSTC) can be obtained using the projectCode field and linking to the EVOSTC website search engine (http://www.evostc.state.ak.us/Projects/SearchStart.cfm).

FundingSource

Identifies source of project funding; the most common, as of 2010, is the *Exxon Valdez* Oil Spill Trustee Council (EVOSTC). See table FundingSource for additional detail.

ProjectCode

Provides the project number assigned by the funding agency (required for EVOSTC projects). For further project detail, use projectCode with the EVOSTC website search engine (http://www.evostc.state.ak.us/Projects/SearchStart.cfm).

Invest

Alphanumeric identifier assigned to the sample by field personnel or the principal investigator.

CollectMethod

Method used to collect the sample. See table Collection Methods for further detail.

Matrix

Required identification of sample type. Valid sample types (as of 2013) are agarose, blank, coal, creosote, oil, suspended particulate matter (PARTICLT), peat, PEMD, sediment, SPMD, tissue, rinse, and water.

PARTICLT. Data are expressed as ng/L, as are water data. To accomplish this, "wet weight" fields are actually liters, not grams. However, for clarity in the database, volume is recorded (in milliliters) and weights are not recorded, thus mass remains consistently grams throughout the database and volume is consistently expressed in ml.

Rinse. Some samples represent rinsate from apparatus, collected for QA purposes. Actual volumes rinsed and collected are unknown, hence volume is not recorded in the database and mass is reported as 1.

See table Matrices for further detail.

SubMatrix

Specific additional information about matrix [such as WHOLE (whole specimen), egg, stomach, tarball and asphalt].

Species

Species used for tissue samples. See table species for further explanation.

DateCollected

Date sample was collected (required).

Year

Year sample was collected (required)

Location

General sample collection area (required); see table LOCATION for abbreviations. Location refers to general areas, not specific latitudes and longitudes. All locations in Herring Bay, for example, are recorded as HERRB; latitudes and longitudes of specific sites vary. See field INVEST for more detailed site information and fields LAT and LONG for exact locations.

Sampler

Required last name of individual responsible for the collecting, handling, and security of field samples.

Agency

Required organization responsible for sample collection.

LAT

Latitude in decimal degrees. Required for all environmental samples (optional for blanks and generally not needed for experimental samples).

LONG

Longitude in decimal degrees. Required for all environmental samples (optional for blanks and generally not needed for experimental samples).

SampleType

Required field identifies the type of sample: ENV = environmental, EXP = experimental, blank = blank sample for quality assurance purposes, spike = spiked blank, OTHR = other type of sample (e.g., samples collected to test municipal water supply).

QCERROR

Identifier of reliability of the analytical results for individual samples. GOOD = no problems with the analytical data, BIAS = probable problems with the analytical data (Short, Jackson et al. 1996). Biased analytical data (alkanes and PAHs) are in separate tables. Field QCERROR is not currently in use.

Comment

The comment field is provided for other notes as needed.

Table SARA

Table SARA (saturates, aromatics, resins, and aliphatics) contains analyses completed with thin-layer chromatography for these classes.

SARAstring

Sample string name (specific to SARA samples).

pSaturate

Proportion of saturates

pAro

Proportion of aromatics

pResin

Proportion of resins

pAsphalt

Proportion of asphaltenes

AnalysisConcentration

Analysis concentration (optional)

Database Support Structure

Table ANALYTE

Names and brief descriptions of all hydrocarbon analytes reported in data tables.

Table Collection Methods

Description and abbreviations of methods used to collect samples

Table FundingSource

Describes FundingSource abbreviations.

Table Location

Location abbreviations (location) and descriptions with approximate latitudes and longitudes. The description column contains some abbreviations; Cr = Creek, B = Bay, I = Island, L = Lake, R = River, Pa = Peninsula. A comment field is included for quality control purposes.

Table Matrices

Provides descriptions of each matrix type. Matrices identify sample type. Valid sample types (as of 2010) are agarose, blank, coal, creosote, oil, suspended particulate matter (PARTICLT), low-density polyethylene membrane devices (PEMDs), sediment, semipermiable membrane devices (SPMDs), tissue, and water. One PEMD device is defined as a low-density polyethylene plastic strip 5 × 50 cm.

Table MDL

Method detection limits by analyte. Method detection limits have been estimated several times, hence each estimation is dated, and are laboratory dependent. Tabled values are ng/g wet weight; sample masses used for the estimates are g wet weight.

Table QCcodes

Description of QC codes associated with each analyte measurement. See tables QC code Alkane and QC Code PAH for data; these can be linked to Alkane and PAH data tables via SIN and Rep.

Table REPLIC

This table explains sample replicate number. Replicates >29 identify spiked reference material or spiked blanks.

Table Species

Provides species descriptions and abbreviations used for field species in table Sample.

Table Biomarker abbreviations

Purpose: detailed description of biomarker abbreviations and names.

Carbons

Number of carbon atoms in molecule

Class

Isoprenoid, terpane, hopane, or sterane

Abbreviation

Abbreviation used in table biomarkers and elsewhere in this database

Biomarker

Complete chemical name of each compound

Alternative name

Alternative chemical name for each compound

Target Ions

Characteristic ions to examine in the selected ion monitoring mode

Table Project Names

Purpose: this table contains the project identification information received from PI, often EVOSTC project numbers.

Project

Abbreviated project name

ExtendedProjectName

Project name

OldProjectName

Provides tracking information for projects that were originally recorded differently than at present (2013). For example, the few project names that were numerical were changed to text. These numbers are preserved in field projectCode in table Sample.

LSTNAME

Last name of investigator

Comment

FRSTNAME

First name of investigator

AGENCYN

Agency name

OFFICE

Office location, such as Auke Bay Laboratory

ADD1

Address line 1

ADD2

Address line 2

CITY, STATE, ZIPS

PHONE1, FAX

Investigator phone number and FAX

TITLE, YR

Complete project title and project year

Table Inventory

Purpose: track physical sample locations in hydrocarbon freezer.

SIN

Sample identification number

Recommendation

Recommends sample disposal or retention. This cannot be accomplished until the lawyers are ready to release samples.

Year

Sample year

Box

Box number; location where sample is stored. Structure is yyyy-nnn, where yyyy is sample year and nnn is sequential box number within year.

Comment

Comments as necessary, such as sample condition

References

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APPENDIX E: SOP - *EXXON VALDEZ* LINGERING OIL MONITORING

Standard Operating Procedures for
Excavating Random Pits, Collecting Oil, and Conducting Gravimetric Sampling
for *Exxon Valdez* Lingering Oil

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January 2017

U.S. Department of Commerce | National Oceanic and Atmospheric Administration
National Marine Fisheries Service | Alaska Fisheries Science Center

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Introduction

This document is a standard operating procedure (SOP) for the lingering oil project (16120114-S) funded through the Gulf Watch Alaska long-term monitoring program and the *Exxon Valdez* Oil Spill Trustee Council (EVOSTC). The goal of this project is to provide the EVOSTC with long term assessments of persistent *Exxon Valdez* oil in PWS, descriptions of its chemical characteristics, and initiate a routine, long-term monitoring program that will resample the same sites every five years over the next 20 years.

Background

The release of 10.8 million gallons of Alaska North Slope crude oil from the *Exxon Valdez* on 24 March 1989, PWS, Alaska, resulted in the largest oil spill in U.S. history. The *Exxon Valdez* oil spill (EVOS) resulted in the oiling of more shoreline than any recorded spill. Massive efforts were needed to survey the oiled shoreline and document the extent and degree of contamination. Several major such efforts were undertaken by Shoreline Cleanup Assessment Teams (SCAT) during 1989, 1990, and 1991. After the termination of the SCAT surveys, other surveys of the shoreline were carried out during the 1990s by Exxon and ADEC. In September 1989, NOAA established eighteen permanent survey sites to determine the amount of lingering oil on the shoreline over time. Four surveys were carried out in 1989, six in 1990, two in 1991, and one each in 1992, 1994, and 1997 (Irvine et al. 1999; Irvine et al. 2006). Irvine et al. (2006) monitored the persistence of oil mousse at five sites along the Alaska Peninsula shoreline in Shelikof Strait in 1994, 1999, and 2005. During the 2000s detailed lingering oil surveys carried out by National Marine Fisheries Service (NMFS) Auke Bay Laboratories in 2001 and 2003 and Research Planning Inc. (RPI) surveys conducted in 2007 and 2008.

Despite unprecedented clean-up efforts and decades of natural processes, oil persists on some beaches (Short et al. 2006; Short et al. 2007). Within the umbrella of the Gulf Watch Alaska long term monitoring program we have developed a lingering *Exxon Valdez* Oil (EVO) monitoring plan for PWS (PWS). The design and methodology is consistent with previous lingering oil surveys but slightly modified and focused for manageable long term monitoring. Determining the end point where EVO is no longer detectable is ultimately the goal of this monitoring effort.

Goals and Objectives

The goals of this project are to provide the EVOS Trustee Council with a long-term monitoring protocol for the assessment of persistent *Exxon Valdez* oil in PWS:

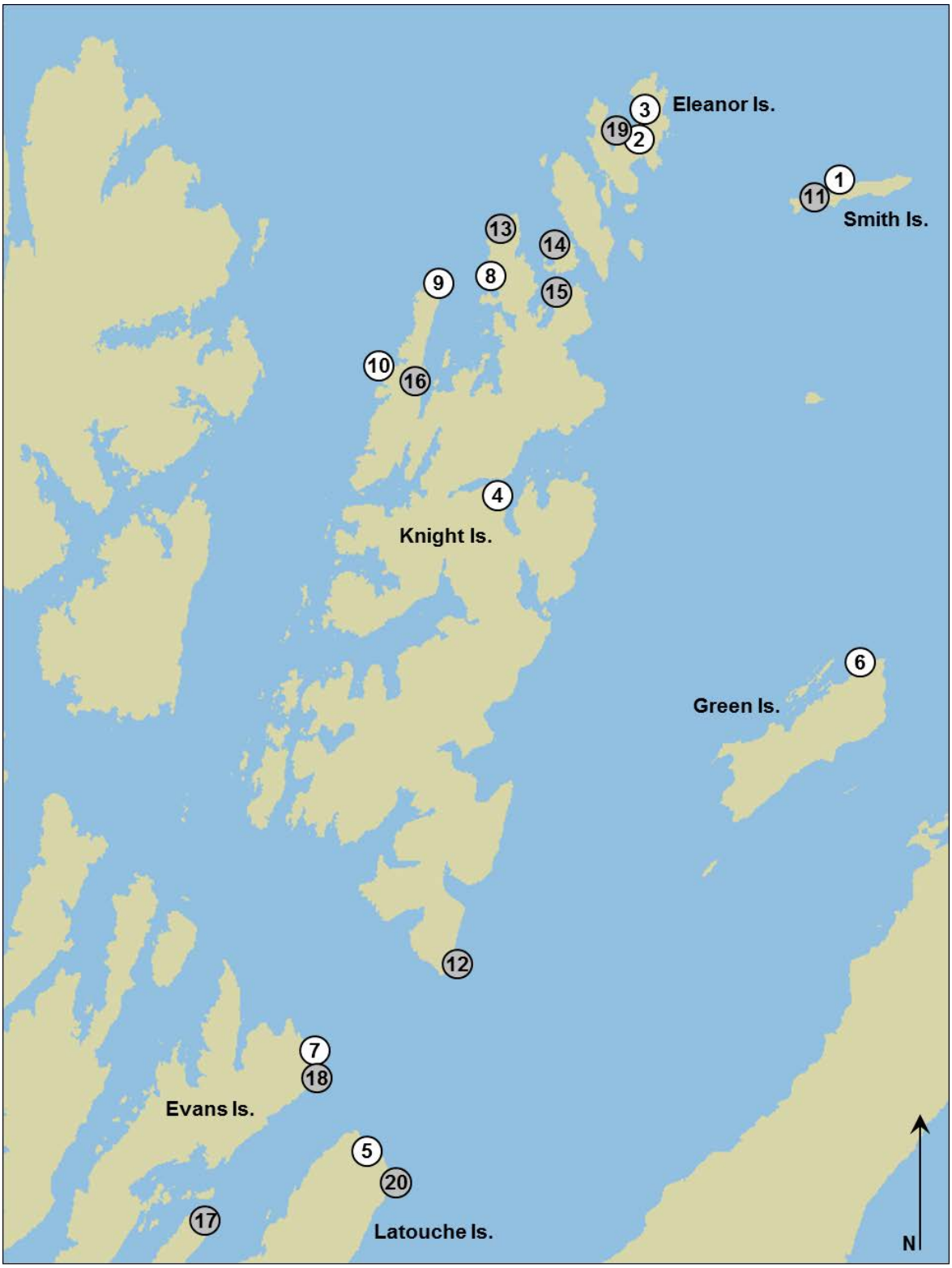
1. *Assessment of oil persistence* – Conducting surveys to understand the quantity and natural degradation of oil over time in PWS beaches.
2. *Chemistry of oil* - fingerprinting, weathering, and long-term sourcing of oil: polynuclear aromatic hydrocarbons (PAH), alkanes, and biomarker composition will be measured in sediment samples. Biomarkers (triterpanes, hopanes, and steranes) will provide definitive long-term source oil identification

3. *Bioavailability of oil* - Understanding exposure levels to EVO for key prey species near lingering oil will help make linkages to species recovering at higher trophic levels (e.g. cytochrome P4501 levels in sea otters and sea ducks).

Sampling Design

The sampling protocol is designed to be simple and relatively inexpensive so that future assessments of oil persistence and chemical composition are cost-effective. In general our strategy was to revisit and survey about 10 beaches that had the highest probability of encountering lingering oil in 2015 and survey them using a stratified random sampling (SRS) design. We recommend these sites become the established long term monitoring sites for lingering oil and be resampled every 5 years over the next 20 years (see the figure on the next page).

Site Selection - Prioritization of beaches selected for monitoring lingering subsurface oil (SSO) is summarized in Table 1. Factors considered for prioritization were based on: initial oiling, shore types prone to oil retention (Michel and Hayes 1993; Hayes and Michel 1998; Hayes et al. 2010; Michel et al. 2010), past oil surveys to aid our understanding of loss rates (NOAA ORR 1989-1992; Gibeaut and Piper, 1993; NOAA ABL 2001-05; Research Planning, Inc. 2007-08), most recently observed oil in heaviest categories (HOR and MOR), and a high probability of oil persistence (Michel and Nixon model, RPI).



Map of prioritized sites for monitoring lingering oil in western PWS. Alternate sites are gray icons, #11-20.

Table 1. Prioritized sites considered for monitoring lingering oil on beaches in PWS. Priority was given to sites with heavy subsurface oil (SSO) surveyed in most recent years, a variety of shore types prone to oil retention, and a high probability of oil persisting. Prioritization of alternative sites have been included (shaded in gray) if needed. Sites actually sampled in 2015 were EL056C, EL058B, EV039A, GR103B, KN0114A, KN0300A-2, KN0506A, LA018A-1, and SM006B.

	Location Name	Shore Segment	Initial oiling/cleanup	Remediation	Oil Survey Excavation History	Shore type prone to persistent oil	Michel Model Heavy SSO
1	Smith Is.	SM006B	Heavy oil 1990-1993	Boufadel 2005-12	1989-92[1], 2001[3], 2008[4]	armored	>30%
2	Northwest Bay, Eleanor Is.	EL056C	Medium oil 1990-1993	Boufadel 2005-12	2001[3], 2007[4]	rubble accumulations	>30%
3	Northwest Bay, Eleanor Is.	EL058B	Heavy oil1989 only	Boufadel 2005-12	2001[3], 2005[3]	breakwater	>30%
4	Bay of Isles, Knight Is.	KN0136A	Heavy oil 1989 only		1993[2], 2003[3], 2008[3]	lagoon, peat	5-15%
5	Sleepy Bay, Latouche Is.	LA018A-1	Heavy oil1990-1993		1989-92[1], 2001[3], 2005[3]	rubble, slope	5-15%
6	Green Is.	GR103B	Heavy oil 1990-1993		2001[3], 2005[3], 2007[4]	armored, slope	1-5%
7	N. Evans Is.	EV039A	Heavy oil 1990-1993	PES-51® 1997	1993[2], 2005[3]	edge effect	1-5%
8	Herring Bay, Knight Is.	KN0114A	Heavy oil1990-1993		2003[3]	breakwater	>30%
9	Herring Pt., Knight Is.	KN0300A-2	Medium oil 1990-1993		1993[2], 2005[3]	breakwater	1-5%
10	Herring Pt., Knight Is.	KN0506A	Heavy oil 1990-1993		2001[3], 2005[3]	edge effect	0-1%
11	Smith Is.	SM006C-1	Heavy oil 1990-1993	Boufadel 2005-12	2001[3]	armored	>30%
12	Pt Helen, Knight Island	KN0405A-1	Heavy oil1990-1993		2001[3], 2008[4]	armored	5-15%
13	NW Pt. of Knight Island	KN0109A	Medium oil1990-1993	Boufadel 2005-12	2003[3], 2007[4]	breakwater	5-15%
14	Disk Is.	DI067A	Medium oil 1990-1993		1993[2], 2003[3]	rubble accumulations	5-15%
15	Louis Bay, Knight Island	KN0107	Heavy oil1990-1993		2003[3], 2008[4]	rubble accumulations	1-5%
16	Herring Bay, Knight Is.	KN0132D	Medium oil 1990-1993		2001[3], 2005[3]	rubble accumulations	1-5%
17	Elrington Is.	ER020B	Heavy oil 1990-1993		1993[2], 2001[3], 2005[3]	edge effect	1-5%
18	Northwest Bay, Eleanor Is.	EL056A	Heavy oil 1990-1993		2001[3], 2005[3]	rubble accumulations	1-5%
19	N. Evans Is.	EV037A	Medium oil 1990-1993	PES-51® 1997	2001[3]	edge effect	1-5%
20	N. Latouche Is.	LA015E	Heavy oil 1990-1993	Boufadel 2011	1989-92[1], 2001[3]	armored	1-5%

Note for oil survey excavation history: 1. NOAA ORR surveys; 2. Gibeaut surveys; 3. NOAA ABL surveys; 4. Michel surveys.

Field Work

The intent of this section is to provide step by step guidance in order to carry out each aspect of the field monitoring effort for a lingering EVO survey in PWS. Included are visual aids, materials lists, and examples of suggested data sheets.

General Requirements

Lingering oil monitoring surveys will require a vessel charter during one 11-12 day cruise that coincides with spring/summer low tidal windows (zero tide height or lower; usually May, June, or July). A Chief scientist will lead a survey crew (~4-7) to carry out all aspects of the SOPs detailed in this document. We recommend a vessel no smaller than 58 feet with a skiff to ferry survey crews to and from beaches daily.

PEMD Deployment and Retrieval

Goal: PEMD Deployment and Retrieval

Determine short-term bioavailability of oil.

- Pre-select a beach for deployment. This will be the first site of the survey and the last beach sampled for lingering oil.
- Place suitable anchors that will withstand tidal and weather energy.
- Place 10 devices; randomly located in the productive intertidal zones (MVD 3-4), 5 at each tide level.
- Record GPS locations.
- Deployment blank - carry 1 unopened trip blank to the site during deployment.
- On last day, retrieve PEMDs using retrieval kits and standard protocol before digging sediment pits.
- Retrieval blank – open a field blank at retrieval; expose for 1 minute, re-bag, and freeze.
- Trip blank – unopened device that makes the round trip to the field and back to the lab.

Introduction

At the beginning of a survey, 10 low-density polyethylene membrane devices (PEMDs) need to be deployed on one monitoring site, preferably a heavily oiled beach. This monitoring site should not be surveyed until the end of the cruise (i.e. last survey site). This will allow the devices to sample dissolved PAH over the 9-10 period of the survey.

The general strategy is to pre-pack PEMDs and associated field gear into kits for deployment before the trip begins to assure laboratory grade quality control. Once on site, determine where the devices are to be located, place suitable anchors that will withstand tidal and weather energy, and then deploy/pick-up PEMDs using kits.

Preparation of PEMDs

PEMDs are assembled in the laboratory and placed in the freezer awaiting shipment to deployment in the field. To reduce any potential contamination issues in storage, PEMDs should not be assembled too far in advance of deployment but a week or two will be adequate. When the PEMDs are ready to be shipped out they will be placed in a cooler or 5 gallon bucket w/screw-top lid, with blue ice and sealed tight with duct tape. PEMDs can be put in a freezer on the charter vessel. If they are not placed in a vessel freezer then the cooler or bucket should not be opened until they are ready to be deployed on the beach.

PEMD Anatomy and Assembly:

PEMDs were custom designed at NMFS Auke Bay Laboratories in Juneau, Alaska. See figures at end of this section for illustration of the device and its parts. For details on preparation and analyses of the PEMDs see Auke Bay Laboratories Standard Operating Procedures for the Analysis of Petroleum Hydrocarbons in Seawater, Sediments, Tissue, and Passive Samplers at the Auke Bay Laboratory (Appendix C)

PEMD Array and Deployment

The PEMDs will be anchored in the intertidal using expansion bolts for boulders and bedrock or duck bill anchors for softer substrate.

A. **Ten** PEMDs will be randomly placed on an oiled site in the biologically rich intertidal zone [meter vertical drops (MVDs) 3-4]. Samplers will be randomly placed in the general MVDs so each zone has replicates (e.g. 5 PEMDs in MVD 3 and 5 PEMDs in MVD 4).
B. **Ten** PEMDs will be randomly placed on a control site in the biologically rich intertidal zone (MVDs 3-4). The control site should be nearby the oil site and preferably in a rocky, cliffy substrate to minimize any potential nearby subsurface oil contamination. Samplers will be randomly placed in the general MVDs so each zone has replicates (e.g. 5 PEMDs in MVD 3 and 5 PEMDs in MVD 4).
C. **Three** PEMDs will be used as blanks:
    1. 1 deployment blank (on site - open 1 min., re-bag, label, and freeze).
    2. 1 retrieval blank (on site - open 1 min., re-bag, label, and freeze).
    3. 1 trip blank (not opened; label as so).

A total of 23 PEMDs are needed for the survey. Fill out field log book with necessary info such as location, anchoring type, GPS waypoint, and SIN (see example at end of this section).

Safe Practices while handling PEMDs

PEMDs sample both air and water and tiny unwanted quantities can swamp the target signal! For example, gassy hands from an outboard, cigarette smoking, or eating smoked foods will contaminate samplers, even through gloved hands. Petroleum products have a way of migrating from hands/clothing to the PEMDs. Clean bags are acceptable storage for PEMDs, tools, and gear.

Anchoring

We have successfully used several techniques for anchoring PEMDs in intertidal areas. These include duckbill anchors for soft substrate and expansion bolts drilled into boulders and bedrock. Zip ties are used as connectors.

A. Put together a deployment kit in 5 gallon bucket w/screw-top and make sure all hardware is hydrocarbon free. Only open bucket when necessary avoiding outboard engines or EVO tainted field gear.
B. For large boulders or bedrock drill a hole (1/4"diam.) into the rock and pound in an expansion bolt. See figure section 4.2.5.
C. For mixed substrate (cobble, pebble) pound in 1 duck bill anchor and use available large cobble to weigh down anchor cable. See figure section 4.2.5.
D. For soft substrate pound in 2 duck bill anchors on either side of the PEMD and attach to the quick links using 2 heavy duty, UV resistant zip ties. Be sure to cut zip tie tails off. See figure section 4.2.5.
E. Attach PEMD - Do not open a PEMD until you are ready to attach it to an anchor. Put on clean disposable gloves. Tear through the two heat-sealed Ziploc bags and remove the aluminum foil. Use a minimum of two zip ties to secure PEMD to anchor system. Redundancy is highly recommended to ensure the device can be retrieved later.

PEMD Retrieval

Retrieval is essentially the reverse of deployment. You will need to prepare a retrieval kit, assign SINs to PEMDs and remove hardware used to anchor the PEMDs.

Retrieval Kits

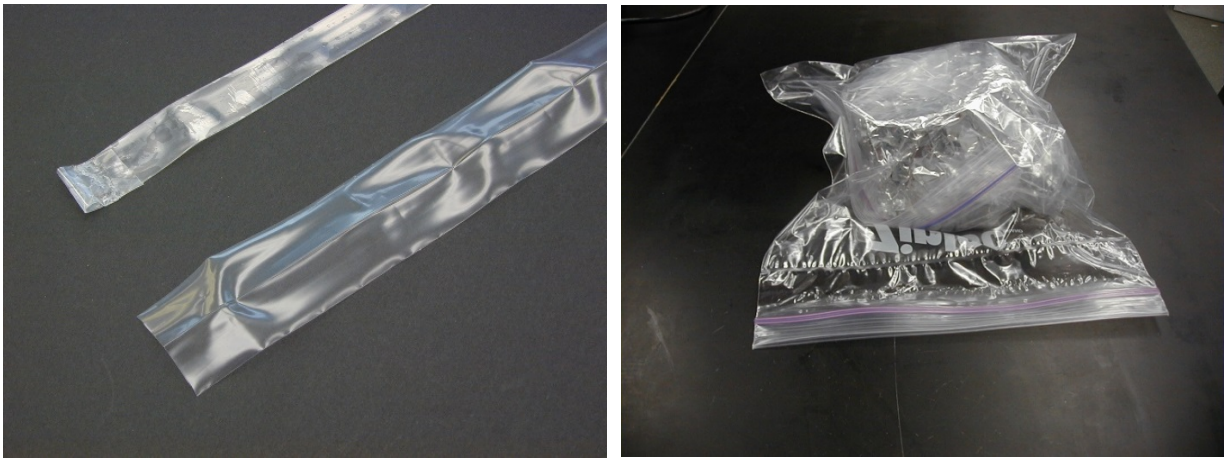
A. Pre-cut aluminum foil sheets (2 per device)
B. Place cut foil into Ziploc bags. How many per bag.
C. Arrange collection gear to optimize efficiency and minimize time; put on clean disposable gloves. Retrieve the PEMD; this may require tools, such as wire cutters, wrenches, etc.
D. Be sure to swirl sediment out of the canister with the water it has been in.
E. Fold the shackle to the canister and place the PEMD at the center of an aluminum sheet; fold to cover completely. Repeat with a second sheet; starting at the opposite side.

F. Place PEMD double wrapped in foil in a Ziploc bag. Place this in a second Ziploc bag.
G. Add a label and seal (site, date, survey grid location, SIN). Make labels out of paper; "Rite in the rain" all weather paper with pre-printed information. Keep labels clean in a small Ziploc bag and only use a pencil to write information on them. Place labels outside of the inner PEMD bag and inside the second bag.
H. Fill out COC form.
I. Put this package in a garbage bag inside a bucket.
J. Field blank - open PEMD and expose to air about 1 minute, then re-bag with label inside.
K. Freeze PEMDs as soon as possible.
L. Remove anchoring hardware.

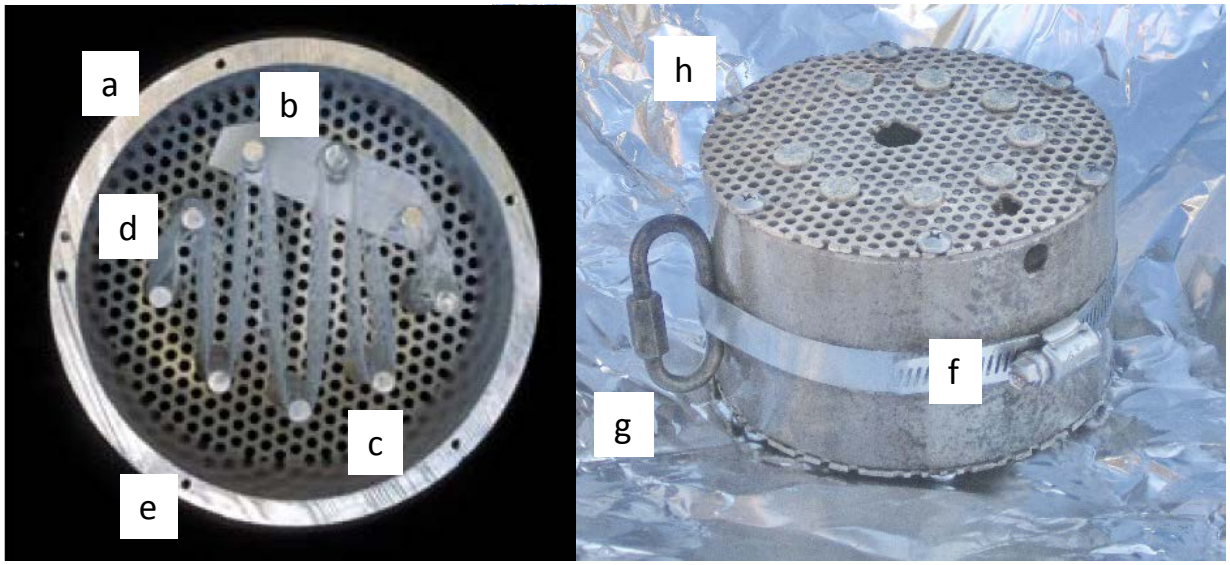
Data Recording

A complete record of deployment and collection should be kept in a field note book. Each PEMD should have the following information recorded: site, date deployed and retrieved, MVD and/or survey grid address (e.g. A1; column A and MVD1), substrate and anchoring system, GPS waypoints, and SINs upon retrieval. See example notebook filled out at the end of this section. We recommend using the digital cameras to take pictures of the log book pages as backup. Complete chain of custody (COC) forms which are required by Auke Bay Laboratories for record keeping and processing. See example COC form filled out at end of this document.

PEMD Figures



Example of LDPE- tubing with looped end; tubing split open. PEMD double wrapped in foil and double ziplock bags which are heat sealed.



Anatomy of PEMD: a) aluminum housing, b) prongs affixed to wire mesh, c) wire mesh, d) LDPE strip looped on prong, e) holes for screws/mesh, f) hose clamps, g) D-ring or carabiner, h) stainless screws.

Example of kit. Five gallon buckets with screw-top lids. Buy new ones and keep the insides clean.

Duck Bill Anchor, driving rod; stainless steel bold Hanger - Metolius, Bolt hole fits 3/8" (10 mm) bolts or 1/2" full-sleeve bolts, Strength: radial 25 kN (5620 lbf) and expansion bolt.

PEMDs anchored to various substrates. Top photos are anchored with duck bills, bottom are expansion bolts. Both anchoring systems use zip ties to attached PEMD.

PEMD Materials list

Item #	**PEMD Hardware and Assembly**
1	Aluminum housing (1/4" thick; 4.5" outside diameter; 1/device)
2	Perforated aluminum end plates (~1 mm thick with 3 mm holes spaced 4.8 mm apart
3	Prongs (shaped from 5 mm aluminum nails)
4	Prong plate (same as perforated end plate plus holes for prongs)
5	Screws
6	D ring or shackle
7	LDPE tubes
8	Foil
9	Ziploc bags
10	
11	

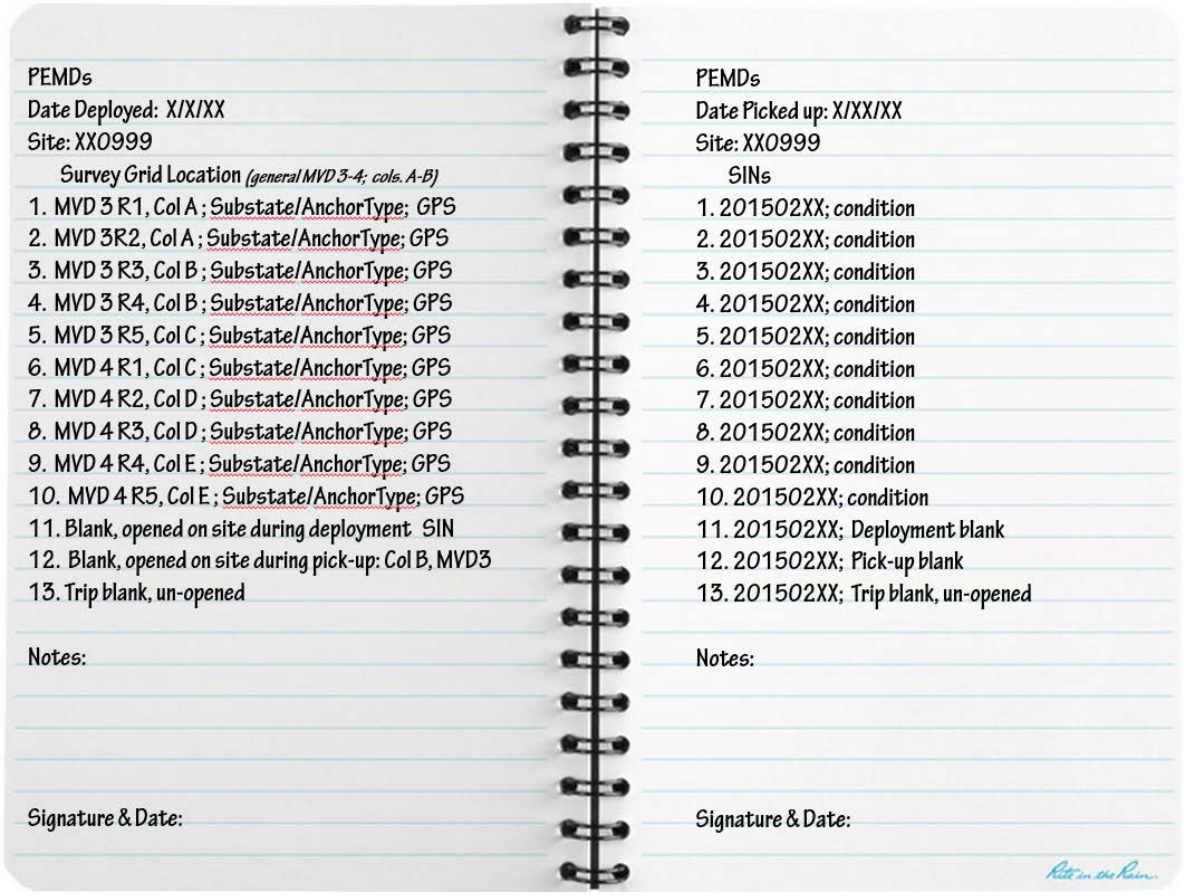
Item #	**PEMD deployment Kit**
1	PEMDs prepared from the lab in 5 gallon bucket w/ lid
2	Data sheets, pen or pencils, clip board
3	Disposable gloves
4	Garbage bags
5	Blue ice (hard shell)
6	GPS
7	
8	
9	

Item #	**PEMD Anchoring Kit**
1	Duck bill anchors, driver, mallet
2	Electric Hammer drill (not gasoline); charged battery & backup
3	Expansion bolts, bolt hangers, washers, nuts
4	Wrench
5	Zip ties (extra large to medium size)
6	Knife, wire cutters (cut zip tie tails off)
7	
8	
9	

Item #	**PEMD Retrieval Kit**
1	5 gallon bucket
2	COC, pen or pencils, clip board
3	Foil kits, chem gloves
4	Ziploc bags

5	Knife, wire cutters (cut zip ties off)
6	Garbage bag for disposing of used hardware
7	Blue ice (hard shell)
8	Labels – inside ziploc bag

PEMD Field Data Records



Field notebook showing the type of notes that should be taken during deployment and retrieval of PEMDs. We recommend using the digital cameras to take pictures of the log book pages as backup.

2015 PWS Lingering Oil

Sample Type: PEMD
Site: KN0114 A
Date: M/Day/YR
Grid Address: ~ MVD 3; rep 1 of 5
SIN: 20150201

2015 PWS Lingering Oil

Sample Type:
Site:
Date:
Grid Address:
SIN:

2015 PWS Lingering Oil

Sample Type:
Site:
Date:
Grid Address:
SIN:

2015 PWS Lingering Oil

Sample Type:
Site:
Date:
Grid Address:
SIN:

2015 PWS Lingering Oil

Sample Type:
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2015 PWS Lingering Oil

Sample Type:
Site:
Date:
Grid Address:
SIN:

2015 PWS Lingering Oil

Sample Type:
Site:
Date:
Grid Address:
SIN:

Example of tags to be filled out when PEMDs are retrieved and placed inside Ziploc bag.

Establishing A Site Survey Grid

Goal: Establish Site Survey Grid

Determine the presence, distribution, and relative amount of oil remaining on selected beach segments.

- Find starting point of monitoring site.
- Establish stratified random sampling grid.
- Divide each 100-m beach segment into 5 columns, each 20 m wide.
- Partition the sampling grid columns into rectangular blocks by 1 m vertical tidal elevation intervals, resulting in 25 blocks, and 50 random pit locations.
- Once grid has been established, record GPS waypoints of starting point at the top of each column and any oiled pits that are discovered. Record a track line around the perimeter of the site.

Recommendation: dedicate some labor to mussel collection while the grid is being established. Mussels must be collected before disturbance to determine natural exposure levels.

Introduction

A survey grid must first be established on a monitoring site before we can determine the presence, distribution, and relative amount of oil remaining on a beach segment. This survey grid enables us to calculate site area and to conduct sampling using a stratified random sampling design to estimate the amount of lingering subsurface oil.

All sites should be sampled on the early morning tide, the larger of the two daily low tides in a diurnal cycle. If needed the site could be finished on the second tide of the day but this is not recommended. The crew should arrive at the site on a falling tide around 30-45 minutes before zero tide. Adjust this as you see fit given the number of surveyors you have, their efficiency, and difficulty of the beach. You don't want to arrive on site when there is over a meter of vertical drop before zero tide because this could increase the error in establishing the mean high water level (MHWL).

A survey crew of three people will work together and establish the site grid. Two team members will use the survey equipment to measure and mark the grid columns (A-E) and the Meter Vertical Drops (MVD 1-5/column). See examples at end of this section. The third person will fill out the survey data sheet, as survey block distances are found by the other two members, and generate coordinates for the two random pits within each grid block.

Establishing Survey Grid on Monitoring Site

A. Find and mark the starting point on the beach. This is the left end of the beach segment when looking at the beach from the water and all columns are to the right of that start point.

B. At the start point, lay a vertical meter tape from the top of the beach down to the water's edge (e.g. 0 m at top of beach and xx m to bottom). Keep tape perpendicular to the top of the beach.

C. On the vertical tape, lay a horizontal meter tape across the whole site (0 to X m) near upper third of the intertidal zone. Keep the tape as true to horizontal as possible. Mark every 20 m, the width of each column so the survey crew knows where MVDs need to be established for each of the 5 columns in the site grid (A-E).

D. At each of the 20 m marks lay vertical meter tapes (A-E; 5 survey tapes) perpendicular to the top of the beach. Estimate where MHWL is and then the survey crew will adjust each as they establish the grid. You now have your 5 columns established. You now need to find the MVDs for each column. See figure section 4.3.3.

E. Find top of column A at Mean High Water Level (MHWL).
   1. MHWL for PWS is 16 ft. or 5 m.
   2. Set up laser level on tripod near the upper third of the intertidal zone close to the beginning of site.
   3. Walk stadia rod with receiver down to water's edge and extend to until the receiver is in line (pings) with the Topcon.
   4. Adjust stadia rod receiver up or down ("+ X cm" or "– X cm" from 5 meter MHWL to 0 tide line) based on time at tide correction from the tide tables listed in 5 min increments. This is your vertical height for MHWL ().
   5. Now move laser level with tripod up or down the beach until it pings stadia receiver. The laser level is now leveled to MHWL.
   6. Find MHWL at top of beach by moving stadia rod/receiver to start point of site, turn upside down so receiver is near ground and find location where it pings laser level. This is start of Column A, MHWL (see example at end of this section). Adjust vertical survey tape so zero meters is on MHWL mark. Mark this location with a tent stake (color code stakes; one color for grid points and another for random pits). Use this technique if you need to relocate the laser level and tripod due to contour on the beach and loss of line of site.

F. Find 1 MVD on Column A.
   1. Standing at Column A MHWL marker, set up the stadia rod and receiver to 1 m height.
   2. Walk down the survey tape with the stadia rod until the 1 MVD is found with the receiver pinging. Record this <u>horizontal tape distance</u> (round to nearest 0.5 m). See figure of filled out data sheet at end of this section.
   3. Place a tent stake at the base of the pinged stadia rod.

G. Find MVD 2, 3, 4, and 5 of Column A
   1. Repeat steps F.1 to F.3, above, but increasing the height of the receiver on the stadia rod to reflect the vertical drop. That is for MVD 2, 2 meters, MVD 3, 3 meters and so on until the bottom of the grid at 5 meters (bottom of

MVD 5).

H. Find top of Column B and MVDs (1-5).

1. Move to next vertical meter tape 20 m to your right (note - your right or left is always referenced as you are facing the beach, looking toward the top of the beach).
2. Find top of Column by moving stadia rod/receiver to start point of site, turn upside down so receiver is near ground and find location where it pings laser level. This is start of the Column and MHWL (see example at end of this section). Adjust vertical survey tape so zero meters is on MHWL mark. Mark this location with a tent stake.
3. Repeat steps F and G.

I. Find top of Columns C-E and MVDs (1-5) in the same fashion as above.

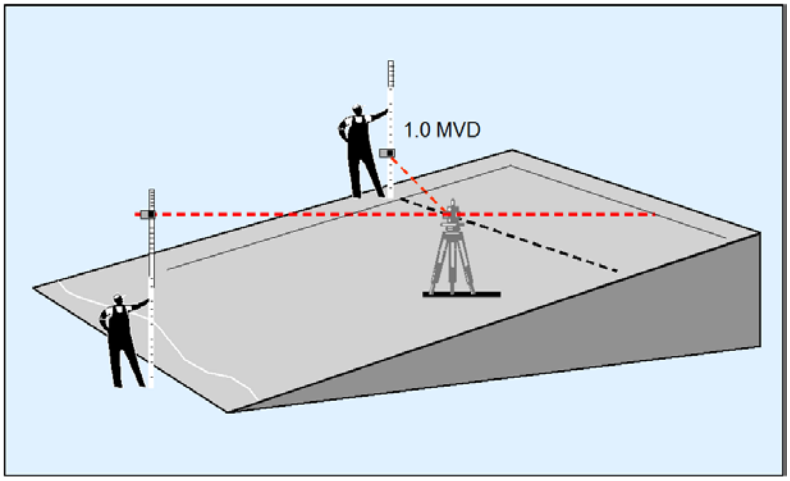
J. Calculate Random pit locations.

1. Once a block and its dimension has been established the random pit locations can be calculated so diggers can begin.
2. Using the survey data sheet, calculate the coordinates (horizontal and vertical tape distances) for the random pits within each block.
    i. The horizontal distance is calculated by multiplying the width of the block minus 0.5 meters (since each pit is 0.5 meters wide) to make sure all random pits are within the block by a uniformly generated random number between 0 and 1.
    ii. The vertical distance is calculated by multiplying the height of the block minus 0.5 meters by a uniformly generated random number between 0 and 1.
3. Transfer the random pit coordinates to the random pit tags. See figure in section 4.3.5.
4. Deploy random pit tags to their coordinates and mark with a tent stake so diggers know where to excavate. It may be helpful to add the tape distance of the top of the MVD within which the pits are to be located to ease location of the pits.
5. If a subsequently chosen pit overlaps a previously selected pit, regenerate the random number until there are no overlapping pit locations (i.e. the pits within a block are sampled without replacement).

K. Record GPS waypoints of grid. The minimum requirement is a waypoint of the starting point of the grid at MHWL (e.g. Column A, 0 MVD). Walk perimeter of site recording GPS trackline.

1. Additional waypoints that should be recorded are the top of each column (A-E).
2. If time permits the top of each MVD (Cols A-E; MVD 1-5).
3. Record every random pit that has oil. SINs will be associated with these coordinates.

Survey Grid Figures



Eshamy, PWS	Tide Height	
mvd	(ft)	(m)
0.0	16.0	5.0
0.5	14.8	4.5
1.0	13.1	4.0
1.5	11.5	3.5
2.0	9.8	3.0
2.5	8.2	2.5
3.0	6.6	2.0
3.5	4.9	1.5
4.0	3.3	1.0
4.5	1.6	0.5
5.0	0.0	0.0
5.5	-1.6	-0.5
6.0	-3.3	-1.0
MHHW (cm)	m	ft
368	3.68	12.07408

Survey team establishing site sampling grid; reference table for tidal range in PWS.

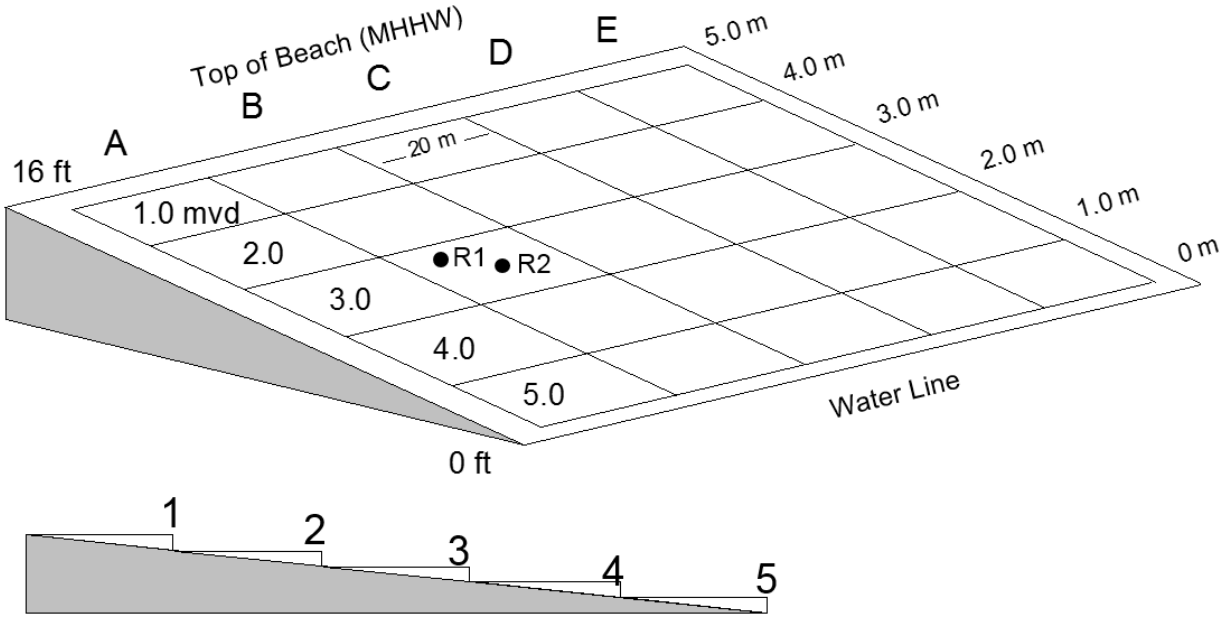
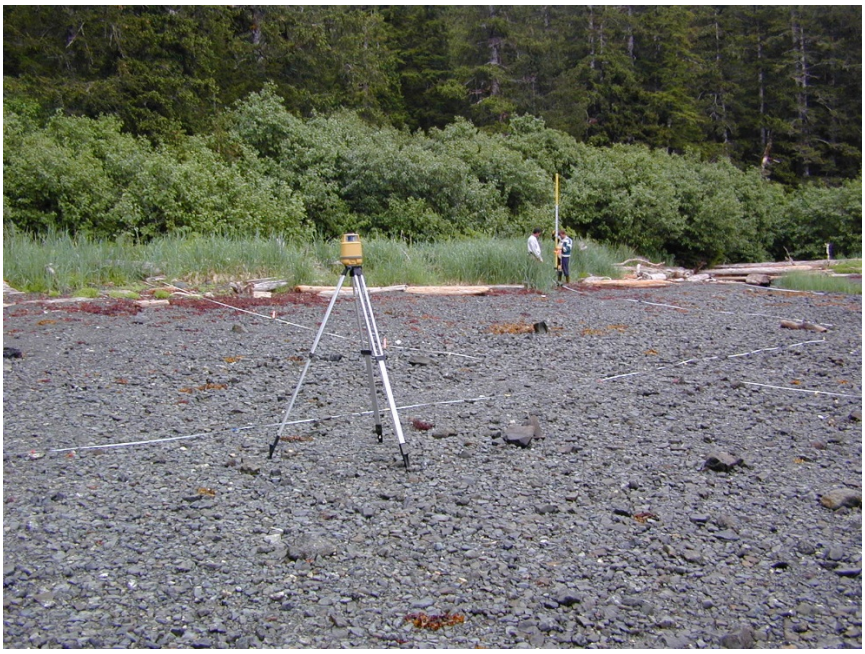


Diagram of survey grid for a 100 m beach segment showing: 10 m columns (A-E), MVDs (1-5), and random pits (R1, R2) within column B, MVD 3.



Laser level in foreground set at MHWL. Finding top of column in background with stadia rod/receiver.

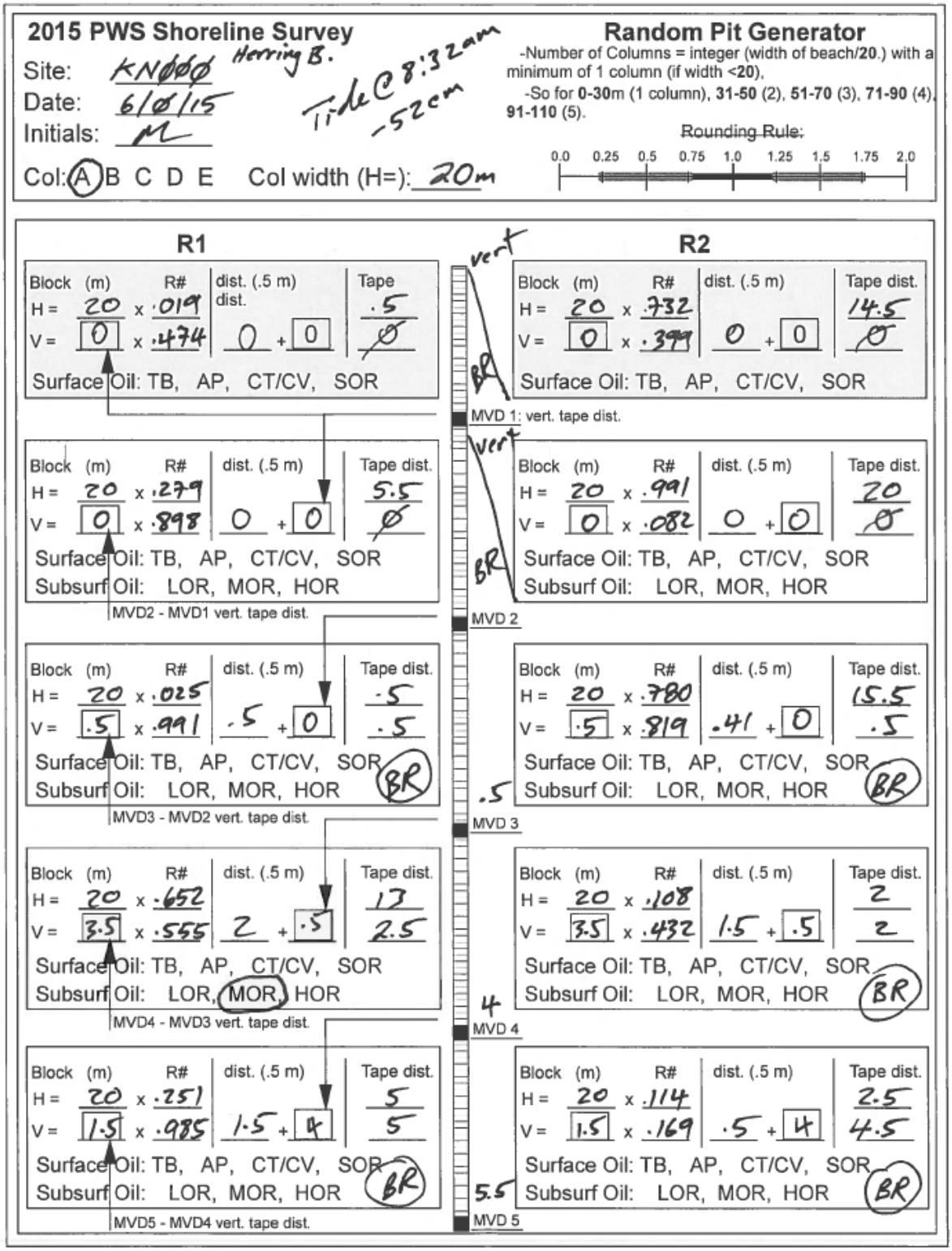
Finding Meter Vertical Drops (MVDs) – note the diggers anxiously awaiting the location of the first pit to dig.

Finding Mean High Water Line using stadia rod and reciever.

Survey Grid Materials List

Item #	**Description** (or type in subject)
1	Topcon 360 deg. self-leveling laser with receiver & batteries
2	Tripod for laser level
3	Stadia rod (5 m)
4	Colored HD plastic tent stakes (9”) in duffle bag in two colors
5	Calculator (has rand# generator, as back-up)
6	Survey Data sheet, pens or pencils
7	Random Pit tags
8	100 m survey tapes (6)
9	Tide Tables for MHWL correction (5 min increments; cm)

Survey Data Sheet filled out and pit tags.



Use portable scanner to archive a backup of filled out forms.

A1.0 R1 V=_____ H=_____	A1.0 R2 V=_____ H=_____
A2.0 R1 V=_____ H=_____	A2.0 R2 V=_____ H=_____
A3.0 R1 V=_____ H=_____	A3.0 R2 V=_____ H=_____
A4.0 R1 V=_____ H=_____	A4.0 R2 V=_____ H=_____
A5.0 R1 V=_____ H=_____	A5.0 R2 V=_____ H=_____

Example of random pit tags for column A.

Random Pit Excavation

Goal: Random Pit Excavation

Discover presence or absence of buried oil, describe, sample, and estimate oil mass.

- Pits will be excavated to a surface area of 0.25 m^2 (0.5 × 0.5 m) and a maximum depth is 0.5 m
- Carefully remove overburden and pile to one side of pit
- Carefully dig contents of pit and pile sediment in singular pile
- If oil is encountered stop and collect sample for GCMS and conduct gravimetric sampling
- When sampling is done, back-fill pit with remaining material
- Sample the lowest elevation quadrats as they become available on a falling tide.
- If no oil is encountered in random pits and time permitting, try to locate previously surveyed oil patches and collect an oil sample.

Introduction

Care must be taken to follow procedure while excavating random pits in anticipation of encountering oil and ultimately calculating the volume of oil via gravimetric sampling.

Excavating a pit

A. Find tent stake with random pit coordinates and double check that the location is correct.
B. If you encounter an immovable boulder (locate the rock bar and try harder) or bedrock (stop) let the person with the random pit data sheet know so they can mark it as not excavated.
C. Establish the 0.5 m x 0.5 m perimeter of your random pit quadrat. Marking 50 cm on your shovel handle can be helpful (e.g. electrical tape).
D. Remove overburden by using hands, prybar, or shovel and pile carefully to one side of pit. Overburden is considered the top surface layer of boulders and cobble. This is not part of the pit weight if gravimetric samples are collected.
E. Start digging out content of pit down to a depth of 0.5 m carefully making one pile to the side of the pit. As you are digging look for oil seeping along the edges of your hole, on the undersides of cobble or boulders, and smell for oil. See example at end of this section.
F. If oil is encountered stop digging and alert rest of team you have oil and then help team with additional sampling (e.g. pit evaluation, GC samples, and gravimetric sampling).
G. Backfill pit with excavated material. If the pit was oiled try to top off the pit with surrounding clean material.
H. If you had an oiled pit, thoroughly clean off your shovel with brush and soap before going to the next pit. This will ensure no oil or sheen from your shovel is introducing visual oil into the next pit.

I. Pick up tent stake and move on to next pit.
J. We recommended that priority should be given to the lowest random pits across the site to avoid losing them to the incoming tide.

Evaluating oiled pits

Preferably one member of the team is designated oiled pit evaluator. This person will need to understand the various oiling classifications, collect oil chemistry sampling (see next section), and oversee gravimetric sampling (see section on gravimetric sampling). They will be responsible for thoroughly filling out the oiled pit data sheet (see example data sheet filled out at end of this section).

A. Once an oiled pit has been identified, initiate gravimetric sampling observing pit digger finishing the pit by placing material in buckets to be weighted.
B. Collect a sample of the oil for GCMS analysis from dug out pit. Look for oiled mussel or clams in the pit and collect them if enough are present for a GCMS sample.
C. Before the pit is backfilled, fill out the oiled pit evaluation data sheet.
    1. Decide what the oil classification of the pit is (HOR, MOR, LOR or OF; see photos at the end of this section.
    2. Note depth of pit and what depth the oil starts and ends. The top of the pit is considered 0 cm and the bottom of the pit 50 cm. There may be just one band of oil in the pit or multiple bands. Oil may be a layer near the surface or at the bottom of the pit going deeper than our sampling effort. Note these bands and different substrate on the pit diagram sheet.
    3. Note if there is water in the pit and how much (e.g. 10 cm in bottom of pit). If there is water in the pit note the color of the oil sheen floating on the surface (brown, rainbow, silver, none).
    4. Note what kind of sediment is in the pit. See classifications on data sheet. Substrate classifications follow standard Wentworth scale for boulder, cobble, pebble, granule, sand, and mud. A slash between substrate codes indicates surface sediments vs subsurface sediments. The first code represents the most common substrate followed by the second most common and so forth.
    5. Under notes confirm samples that were taken in the pit, photos, etc.
    6. Make sure to record the GPS coordinate of the pit along with the pit location (i.e. A 4, R1)
B. If no random pits hit oil in the sampling grid, review documentation on where oil patches were found previously on the site. Do some quick exploratory digging at these locations and see if you can find oil. If oil is found take a sample in 2 oz I-chem jar, record GPS waypoint, and photo.

Oiling Classifications

Surface Oil Types

Code	Type	Definition
AP	Asphalt Pavement	Hevily oiled beach seds held cohesively together
MS	Mousse/pooled oil	Any oil/water emulsion with thickness of >1cm
TB	Tar Balls	Small, distinct oil deposits lying on top of beach surface; possibly binding debris but typically not sediments
SOR	Surface Oil Residue	Significantly oil coated beach sediments in the top 5cm; sediments don't form a cohesive layer; may be described as heavy or light
CV	Cover	Oil more than 1 mm to 1 cm thick
CT	Coat	Oil more than 0.1mm to less than or equal to 1mm thick; can be easily scratched off with fingernail
ST	Stain	Oil less than or equal to 0.1mm thick; cannot be easily scratched off with fingernail
FL	Film or Sheen	Transparent or translucent film or sheen
DB	Oiled Debris	Any oiled debris or cleanup material stranded on a shore

Surface Oil Distribution Classes

Code	Type	Definition
C	Continuous	Area or band with 91% to 100% oil coverage
B	Broken	Area or band with 51% to 90% oil coverage
P	Patchy	Area or band with 11% to 50% oil coverage
S	Splash	Area or band with 1% to 10% oil coverage
T	Trace	Area or band with less than 1% oil coverage

Subsurface Oil Types

Code	Type	Definition
OP	Oil Pore	Pore spaces are completely filled with oil resulting in oil oozing out of sediments - water can't penetrate OP zone
HOR	Heavy Oil Residue	Pore spaces partially filled with oil residue but not generally flowing out of sediments
MOR	Medium Oil Residue	Heavily coated sediments; pore sapces are not filled with oil; pore spaces may be filled with water
LOR	Light Oil Residue	Sediments lightly coated with oil
OF	Oil Film	Continuous layer of sheen or film on sediments; water may bead on sediments
TR	Trace	Discontinuous film; spots of oil on sediments; an odor or tackiness with no visible evidence of oil

Surface Oiling Classifications

Subsurface Oil:

Light Oil Residue (LOR)

Moderate Oil Residue (MOR)

Heavy Oil Residue (HOR)

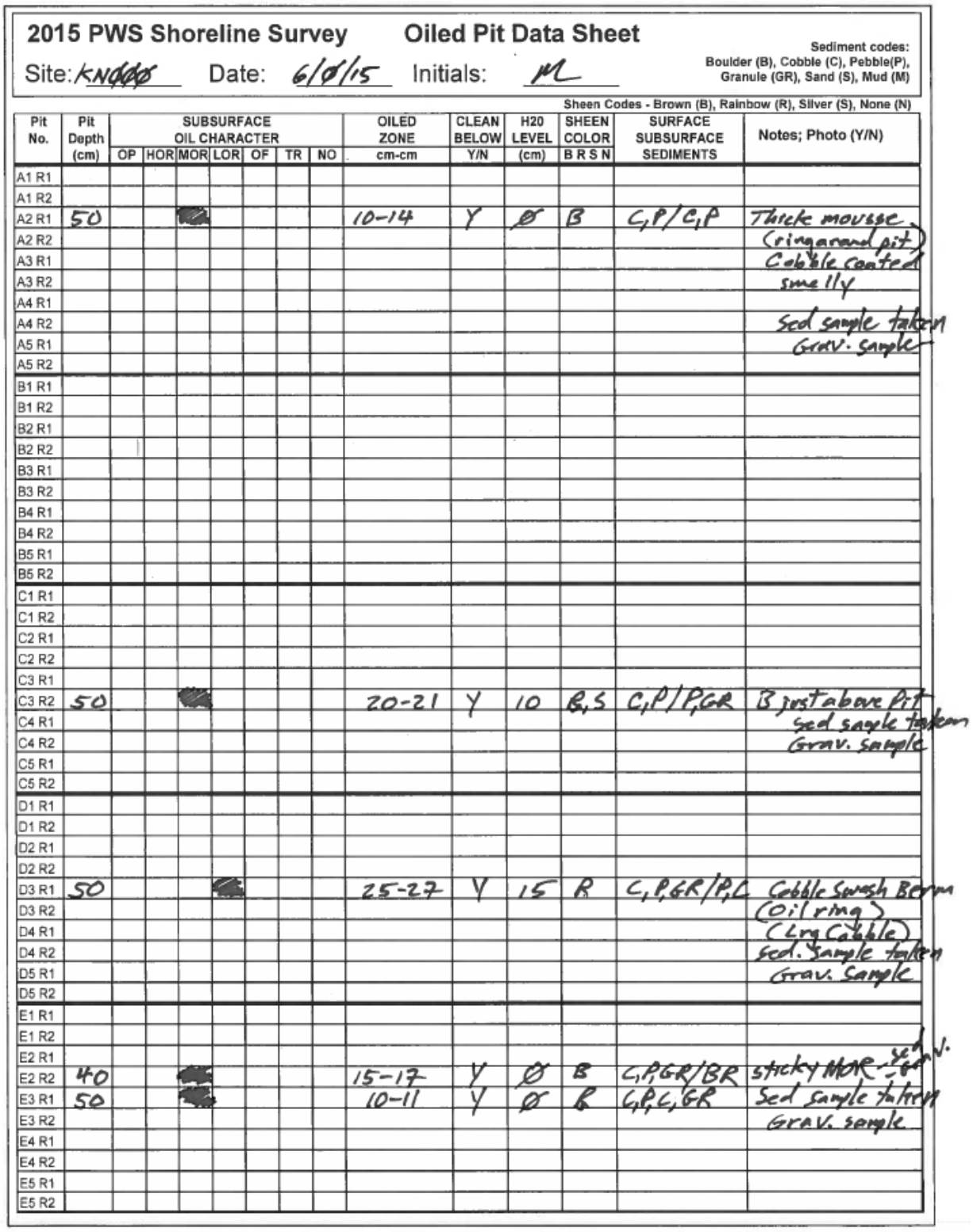
Pit Excavation Figures

Examples of various substrates and pit digging. Veneers of rounded cobble or large boulders, mixed angular cobble and granules. Top pit showing nice singular pile of pit contents before backfilled. Last two photos examples of HOR pits.

Pit Excavation Materials List

Item #	**Description** (or type in subject)
1	Shovel
2	pry bar
3	0.25 m measure for visual on pit dimensions
4	Gloves
5	Meter stick for evaluating pit
6	Trowels for evaluating pit
7	Oiled pit data sheet
8	
9	

Oiled Pit Evaluation Field Data Sheet filled out



2015 PWS Shoreline Survey — Oiled Pit Data Sheet

Site: KNDDD Date: 6/0/15 Initials: ML

Sediment codes: Boulder (B), Cobble (C), Pebble(P), Granule (GR), Sand (S), Mud (M)

Sheen Codes - Brown (B), Rainbow (R), Silver (S), None (N)

Pit No.	Pit Depth (cm)	SUBSURFACE OIL CHARACTER OP	HOR	MOR	LOR	OF	TR	NO	OILED ZONE cm-cm	CLEAN BELOW Y/N	H20 LEVEL (cm)	SHEEN COLOR B R S N	SURFACE SUBSURFACE SEDIMENTS	Notes; Photo (Y/N)
A1 R1														
A1 R2														
A2 R1	50			■					10-14	Y	Ø	B	C,P/C,P	Thick mousse (ring around pit) Cobble coated smelly
A2 R2														
A3 R1														
A3 R2														
A4 R1														
A4 R2														Sed sample taken Grav. Sample
A5 R1														
A5 R2														
B1 R1														
B1 R2														
B2 R1														
B2 R2														
B3 R1														
B3 R2														
B4 R1														
B4 R2														
B5 R1														
B5 R2														
C1 R1														
C1 R2														
C2 R1														
C2 R2														
C3 R1														
C3 R2	50			■					20-21	Y	10	B,S	C,P/P,GR	B just above Pit Sed sample taken Grav. sample
C4 R1														
C4 R2														
C5 R1														
C5 R2														
D1 R1														
D1 R2														
D2 R1														
D2 R2														
D3 R1	50				■				25-27	Y	15	R	C,P,GR/P,C	Cobble Swash Berm (Oil ring) (Lrg Cobble) Sed. Sample taken Grav. Sample
D3 R2														
D4 R1														
D4 R2														
D5 R1														
D5 R2														
E1 R1														
E1 R2														
E2 R1														
E2 R2	40			■					15-17	Y	Ø	B	C,P,GR/BR	sticky MOR - Sed sample N.
E3 R1	50			■					10-11	Y	Ø	B	C,P,C,GR	Sed sample taken Grav. sample
E3 R2														
E4 R1														
E4 R2														
E5 R1														
E5 R2														

Use portable scanner to archive a backup of filled out forms.

Collecting Oil Chemistry Samples

Goal: Oil Samples for Gas Chromatography

Determine oil source and weathering state.

- Collect an oil sample from every oiled pit for analysis
- Collect samples with hydrocarbon-free spoons and gloved hands
- Place in hydrocarbon-free glass jars (2 oz).
- Leave room for expansion (about 10%); pour off excess water
- Record GPS coordinates of pit location, record data on COC with assigned SIN
- Also record SIN, date, quadrat, beach on jar label
- Collect jars in a cooler or bucket with blue ice
- Freeze as soon as possible

Introduction

The purpose of this section is to give guidance on how to collect sediment samples in the field destined for hydrocarbon analyses. The steps below outline the general process. It is recommended that you contact the lab beforehand that will be doing your analyses and ask them for their preferences in materials and shipping.

After ABL has received hydrocarbon samples they will be inventoried, securely archived in a cold freezer, and Chain Of Custody (COC) data added to their statewide database. If the samples are to be analyzed, analyses will be by fluorescence for presence/absence information or by GC/MS for detailed composition and assessment of weathering.

Sediment Chemistry Sampling

A. Make a cooler (small or medium size) with all the items you will need to collect your samples. Keep the lid closed on site.
B. **Do not collect sample until your surroundings are free from possible sources of contamination: exhaust from combustible engines, cigarette smoke, or even smoked food such as jerky or fish.**
C. Put your disposable lab gloves on first.
D. Fill a 2 oz. HC-free jar with oiled sediment using a HC-free stainless steel spoon. The finest grain sediments you can find with oil are optimal (silt, mud, sand, granules). Sediments have high water content so do not fill to top or the jar will burst when frozen. Pour out any excess water.
E. Label sample with SIN# from COC form, collection date, site name or segment #. Lids should also have SIN# and date. Record a GPS waypoint with Longitude and Latitude for each sample.
F. Fill out Chain of Custody form.
G. Freeze sample as soon as possible and keep frozen until shipping.

Mussel Chemistry Sampling

A. Follow similar steps for collecting sediment samples.
B. Collect the largest mussels you can find.
C. For mussels ~10-15mm, fill a 4 oz. HC-free jar with mussels. Do not collect mussels smaller than 10mm because too many mussels will have to be collected to get enough tissue for analysis.
D. For mussels 15-25mm, collect 25-30 individuals and wrap in foil square. Wrap in foil square again.
E. Place in Ziploc bag with sample tag so it is readable from outside the bag.
F. Fill out COC form.
G. Freeze sample as soon as possible and keep frozen until shipping.

Chemistry Sampling Figure

Example of I-Chem jar (4 oz.) and spoon with oiled sediments.

Chemistry Sampling Materials List

Item #	Description
1	I-Chem Jars (sediment 2 oz. or 4 oz.); certified hydrocarbon free
2	foil squares in Ziploc bags for large mussels
3	Stainless steel spoons; hydrocarbon free (rinsed with $MeCl_2$; sealed with clean foil)
4	Disposable lab gloves
5	Electrical tape to secure lids
6	Labeling tape for jar; permanent marker (Extra fine tip Sharpie)

7	Chain of Custody forms
8	Small cooler for collecting/shipping (not hazardous shipping)
9	Hard shell blue ice
10	Gallon Ziploc bags; packing material (if needed)

Chemistry Sampling Field Data Sheets

Note on oiled pit data sheet all samples collected for GCMS analysis in the appropriate pit address. Notes can also be expanded onto the back side of this data sheet.

Gravimetric Sampling

Goal: Oiled Pit Gravimetric Sample Collection

Determine an estimate of the volume of subsurface oil at a monitoring site.

- Collect a gravimetric sample from every oiled pit
- Transfer pit contents to 5 gallon buckets for weighing
- Fill out gravimetric data sheet
- Empty bucket contents into homogenizing tote
- Thoroughly mix pit contents in homogenizing tote
- Collect subsample of homogenate in specimen jug
- Gravimetric sample should have tag inside, label outside, taped lid, and COC form filled out.

Introduction

Approximately 100 gravimetric samples of subsurface oil will be collected from this monitoring survey if enough oil is encountered. We will determine the volume of subsurface oil remaining on each monitoring site by taking a gravimetric sample from every random oiled pit. Remember to take a GCMS sediment sample from each oiled pit before gravimetric sampling begins.

Oiled Pit Preparation

A. Excavate 0.25m^2 quadrat (0.5 m × 0.5 m) taking care to place all oiled material in 5 gallon buckets. Exclude larger rocks like cobble (larger than 2.5" or 64 mm in diameter but make sure to transfer any oil from these rocks (i.e. use a spatula, putty knife etc. to scrape the oiled material from the rock surface) to buckets. Do not remove peripheral boulders but quantitatively transfer any oil to the subsample.

B. Weigh each 5 gal bucket before dumping in large tote for homogenization. Be sure to tare the bucket wt. on the 100 Lb. spring scale provided. See example at end of this section. Use the calculation table on the Gravimetric Data Sheet to arrive at a Total Pit Wt.
C. Note on the gravimetric data sheet the type and location of oil as it is encountered. Sketch a pit profile and make any notes concerning the sampling (see example of notes at end of this section).
D. Drain off water to reduce transfer to subsample. Also keep tote or buckets covered if rain is occurring.

Subsample of Oiled Pit Homogenate

A. Before collection of the subsample, the material in the tote or buckets must be thoroughly mixed using shovels or trowels. Breakup any clumps of oil or sediment and agitate until uniformly distributed (See example at end of this section).
B. Sub-sample no greater than 10 kg (~4.5 Lbs.) should be placed in the 1 gal plastic buckets. Record on the gravimetric data sheet the Total Subsample Wt. Place a SIN# tag (see example at end of this section) in the bucket and mark on the outside of the bucket site name, pit #, and SIN#. Cover pit up with remaining homogenate material.
C. Tape the lids and store on boat at ambient temperature in fish boxes. Fill out COC Form.

Cleaning/Prep

A. After all sampling is complete on a site dispense some Joy detergent into a bucket and scrub the tools and equipment, starting with the cleanest tools and work to the dirtiest. Discard wash water often to ensure thorough cleaning. Thoroughly rinse equipment before reuse. If buckets/totes are too dirty to clean replace with new one on the vessel for the next site.

Gravimetric Sampling Figures

Weighing 5 gallon bucket with spring scale.

Tote with oiled pit contents being homogenized with a shovel.

Gravimetric Materials List

Item #	**Description** (or type in subject)
1	5 gallon buckets
2	Homogenizing totes
3	Spring scale
4	Trowels
5	Soap
6	Brushes
7	COC form, pen or pencils
8	Specimen jugs, tags, electrical tape for lid; sharpie for outside label
9	Gravimetric data sheet

Gravimetric Data Sheet filled out and Sample Tag

2015 PWS Gravimetric Samples SIN# 20150501

Site: KNOOO Herr. B. Name: M Date: 6/0/15

Pit Calculations **Pit #** C3R2

Bucket #	B wt (kg)	B+sed wt (kg)	Sed wt (kg)
B1	1	25	24
B2	1	20	19
B3	1	25	24
B4	1	15	14
B5	1	10	9
B6			
B7			
B8			
B9			
B10			
B11			
B12			
B13			

Total Pit Wt. 90kg

Tot. Subsample Wt. Calc. In. Lab.

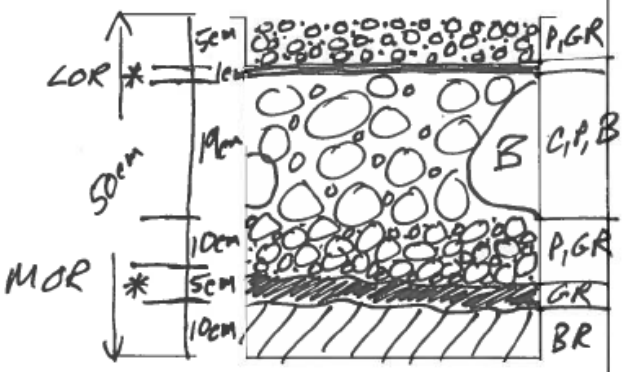
Pit Notes:

Type of Oil: MOR

- majority of oil was sequestered on top of BR @ bottom of pit.
- < 1cm thin layer of LOR towards surface
- oil found under lrg. B.
- No oiled clams
- No surface oil

Sediment codes:
Boulder (B), Cobble (C), Pebble(P), Granule (GR), Sand (S), Mud (M)

Pit Profile



Use portable scanner to archive a backup of filled out forms.

2015 PWS Gravimetric Subsample		2015 PWS Gravimetric Subsample	
Site:	KN0114A	Site:	
Date:	M/Day/YR	Date:	
Grid Address:	D4R2 M0R	Grid Address:	
SIN:	20150201	SIN:	

2015 PWS Gravimetric Subsample		2015 PWS Gravimetric Subsample	
Site:		Site:	
Date:		Date:	
Grid Address:		Grid Address:	
SIN:		SIN:	

2015 PWS Gravimetric Subsample		2015 PWS Gravimetric Subsample	
Site:		Site:	
Date:		Date:	
Grid Address:		Grid Address:	
SIN:		SIN:	

2015 PWS Gravimetric Subsample		2015 PWS Gravimetric Subsample	
Site:		Site:	
Date:		Date:	
Grid Address:		Grid Address:	
SIN:		SIN:	

2015 PWS Gravimetric Subsample		2015 PWS Gravimetric Subsample	
Site:		Site:	
Date:		Date:	
Grid Address:		Grid Address:	
SIN:		SIN:	

Example tags for gravimetric subsample jug. These should be printed on plastic paper.

Site Photo Documentation

Goal: Photo Documentation

Document monitoring survey and discovery of oil.

- Take photo of starting point of survey grid at a minimum; if possible take photo of start of each column
- Take photo of every oiled pit
- Take along shore shot across survey grid and site
- Take photos and videos showing methods – surveying, digging pits, gravimetric, GCMS samples
- Take photo of site from just off shore so it can be found in the future
- Back up image files daily

Introduction

Photo documentation of a monitoring site can be invaluable over the years. These days photo documentation is made easy with digital cameras that are small, water and shock proof, and produce high resolution images and video at a reasonable price. These cameras are designed for shooting in low light, under water, have vibration reduction, auto focus, auto exposures, and will even geo-tag each photo. There is no excuse for not taking photos and they are surprisingly useful later.

Photo Subjects and documentation

The following is a prioritized list of photo subjects that should be taken while at a site:

<u>Still photos</u>

- General offshore shot of site
- Start of site and column A
- All oiled pits; if no oiled pits then representative pits showing substrates
- All columns (A-E) – start at top
- Along shore shot from column A across site; from column E looking back across site
- Methods examples – surveying, digging pits, taking chemistry samples, gravimetric samples, etc.
- All MVD block starts – upper left hand corner where stake is placed.

<u>Videos</u> – mainly for methods purposes and outreach (is there a story to be told)

- Surveying
- Digging Pits; oiled pits, sheen, etc.
- Taking chemistry samples
- Gravimetric sampling
- Crew arriving & departing on site

- Filling out data sheets

Digital Photo Management

- Transfer files daily from camera to your computer
- Set up a file directory so you can transfer image files from your camera to computer daily. Here is a suggested listing:
    - Site Photo Name
        - Site photos
        - Pit photos
        - Oiled pit photos
        - Misc. photos
        - Video clips
- Time permitting go into each folder and re-name image files with standardized nomenclature that describes content of image (e.g. SM006C_Start-ColA.jpg; SM006C_D4R2_MORpit). Photo management software allows you to re-name in batch jobs which can save time.
- Back up image files on external hard drive

Camera tips

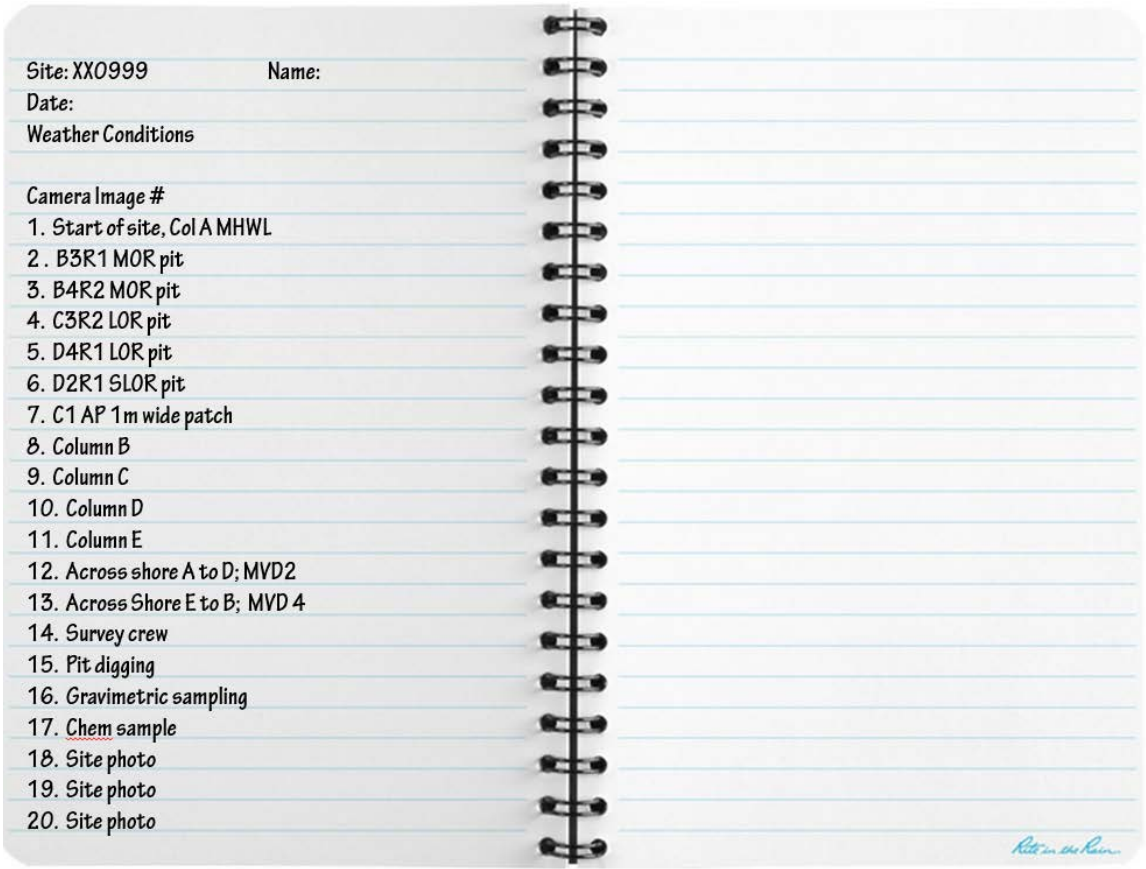
- Set camera to take phots and video at the highest resolution. Anything less is a waste of the technology. Just make sure you have enough memory cards.
- Check and charge battery every day
- Check time/date on camera daily (e.g. take picture and then view with info displayed on LCD). Adjust manually if needed.
- After turning on camera make sure the geo-tag feature is on and satellite icon is showing
- Auto focus and exposure are recommended. Pre-programmed settings for specific situations like “sunny day at beach” are not recommended.
- Back-up & clear off memory card every day

Photo Documentation Materials List

Item #	**Description** (or type in subject)
1	Pocket digital camera, water/shock proof, geotagging (e.g. Nikon Coolpix AW130, 16 MP or comparable brand model)
2	Memory card (e.g. SanDisk 32 GB Ultra SD card or similar model)
3	Power strip for charging
4	Photo management software (e.g. ACDSee)
5	
6	
7	

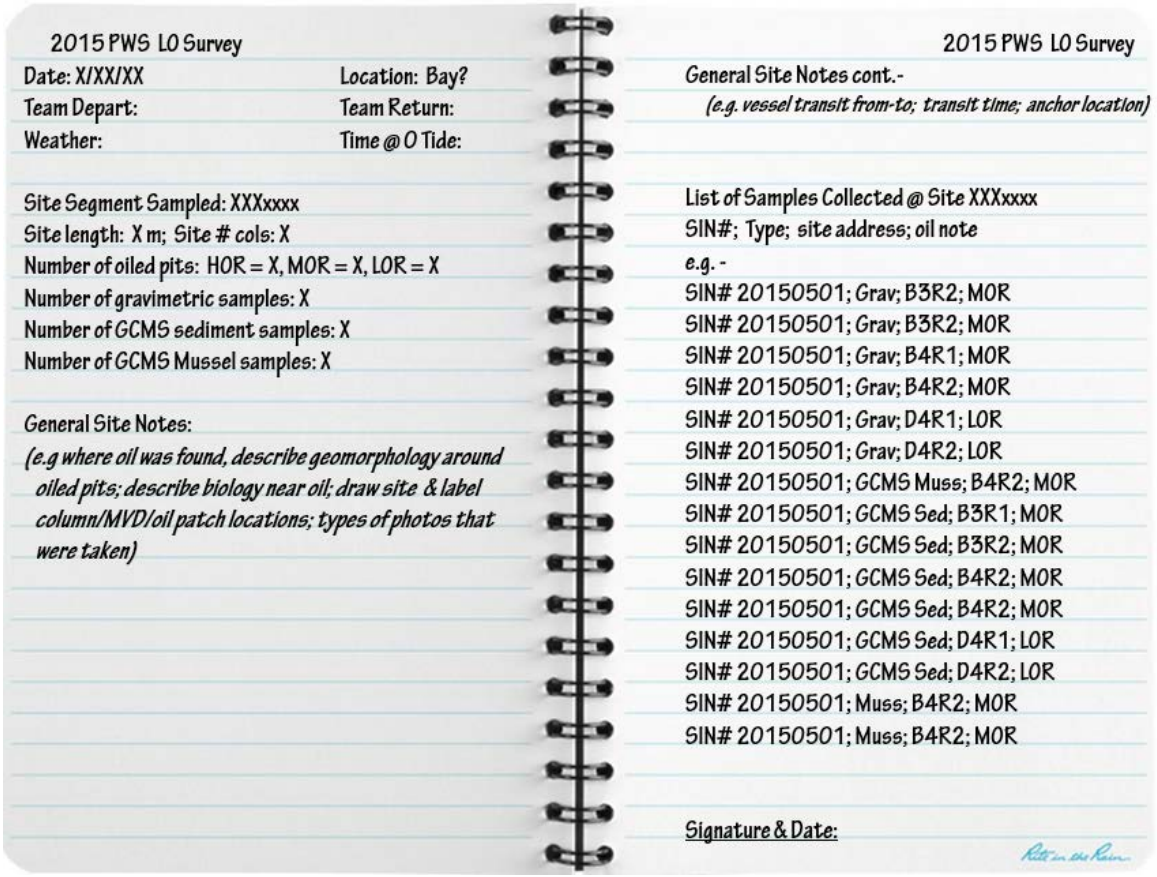
Photo Documentation Field Data Sheets

Be sure to note in field log book photo number on camera associated with each oiled pit. The filenames can then be labeled correctly later. We recommend using the digital cameras to take pictures of the log book pages as backup.



Overall Site Documentation and Field Notes

Lead Scientist in the field should keep field notes daily on activities, sampling effort, site summaries, etc. We recommend using the digital cameras to take pictures of the log book pages as backup. See example below:



2015 PWS LO Survey

Date: X/XX/XX Location: Bay?

Team Depart: Team Return:

Weather: Time @ 0 Tide:

Site Segment Sampled: XXXxxxx

Site length: X m; Site # cols: X

Number of oiled pits: HOR = X, MOR = X, LOR = X

Number of gravimetric samples: X

Number of GCMS sediment samples: X

Number of GCMS Mussel samples: X

General Site Notes:

(e.g where oil was found, describe geomorphology around oiled pits; describe biology near oil; draw site & label column/MVD/oil patch locations; types of photos that were taken)

2015 PWS LO Survey

General Site Notes cont.-

(e.g. vessel transit from-to; transit time; anchor location)

List of Samples Collected @ Site XXXxxxx

SIN#; Type; site address; oil note

e.g. -

SIN# 20150501; Grav; B3R2; MOR

SIN# 20150501; Grav; B3R2; MOR

SIN# 20150501; Grav; B4R1; MOR

SIN# 20150501; Grav; B4R2; MOR

SIN# 20150501; Grav; D4R1; LOR

SIN# 20150501; Grav; D4R2; LOR

SIN# 20150501; GCMS Muss; B4R2; MOR

SIN# 20150501; GCMS Sed; B3R1; MOR

SIN# 20150501; GCMS Sed; B3R2; MOR

SIN# 20150501; GCMS Sed; B4R2; MOR

SIN# 20150501; GCMS Sed; B4R2; MOR

SIN# 20150501; GCMS Sed; D4R1; LOR

SIN# 20150501; GCMS Sed; D4R2; LOR

SIN# 20150501; Muss; B4R2; MOR

SIN# 20150501; Muss; B4R2; MOR

Signature & Date:

Handling of Samples, Chain of Custody, and Shipping

Handling of Samples

PEMDs - PEMDs in buckets or coolers can be shipped via Alaska Air Freight. Indicate that the buckets are to be kept frozen. General delivery is ok under these conditions but it is not that much more to ship priority (overnight is significantly more). If freezers aren't working or are not available, then ship priority (or AA Gold Streak).

Oil Chemistry Samples - Care should be taken to make sure the glass jars are packed so they do not bang against one another and break during shipping. Using the same box from the manufacturer will help since these already have separate compartments for each jar. Pack as much "pre-frozen" blue ice as possible in a cooler with the samples. Place the "Original COC form" WITH SIGNATURES of "collector" and "relinquished by" in the cooler with the samples. ABL will not accept samples unless the proper COC forms with signatures accompany the samples. Be sure to keep a copy of the COC form for your records. Samples need to stay frozen until they arrive at ABL. Please contact ABL staff prior to shipping to ensure samples are picked up promptly and not allowed to thaw. This is especially important if the samples are due to arrive over the weekend. Alaska Air Cargo or Alaska Gold Streak are the ONLY reliable means to ship samples to Juneau. Have the carrier label your sample "Keep Frozen".

Chain of Custody (COC)

Completion of chain-of-custody forms is required. They provide the information necessary for tracking samples from collection through the laboratory to the database. Without this information a sample is worthless. Chain of custody forms are a permanent record. The originals are filed by chemistry staff and scanned versions are maintained on the network. Separate chain-of-custody forms have been provided for each matrix (mussels, sediment, gravimetric, and PEMD). An extra COC is also available if needed. Use portable scanner to archive a backup of filled out forms.

Filling out chain-of-custody forms

A. **Assigned Sample #**. The chain of custody form contains a list of sample identification numbers (see yellow highlights on example of COC sheet at end of this section). These are assigned by chemistry staff BEFORE collection. SINs are unique and never overlap with any other project. Remember that the SIN must also be written on the sample container; inclusion of location and date is often also helpful.
B. **Collector's sample code**. A place for unique, project-specific collection information. This information can be highly variable, e.g., "Selendang Ayu, STBD HFO settling tank," or "PV-7M-SPM Station 7," or "Redoubt 3." This information must be meaningful to the investigator and, because it becomes a permanent part of the database, sufficiently detailed that others can later understand and

use the information. Suggested for this project are position codes, e.g., "A3R2" where A = column, 3 = vertical drop (meters), and R2 = random pit number 2. Explain any coding used on each chain-of-custody form; the preceding example could be explained once as a comment or in the margins, so that code meaning is not lost.

C. **Date Collected**. Use the mm/dd/yyyy format, e.g., 06/18/2015.
D. **Matrix & species, organ**. Valid matrices include sediment, tissue, PEMDs, oil; most of these will be present in this project. "Oil" is reserved for samples that will be analyzed as pure oil without the presence of sediment and will probably not be used for these collections. Put "mussel" in this field as appropriate.
E. **Location collected**. Use beach segment for this project, e.g., SM006B, EL058B, KN0109A. These specific segment names match other information in the hydrocarbon database. Naming consistency is important for long-term database usefulness.
F. **Latitude** and **longitude**. These numbers provide geographic locations. Please use decimal degrees; include as many decimals as provided by the GPS receiver (typically 4 to 6). West longitude (all PWS samples) is expressed as a negative number, thus a sample collected in northwest bay might be 60.55067, −147.57933.
G. **Collection method**. Provide information about how the sample was collected. Mussels are typically collected by hand, thus the entry is "hand." Sediment will likely be collected by shovel. Use stylized names for database consistency (hand, spoon, shovel, core). Leave this field blank for PEMDs.
H. **Comments**. Add any other necessary additional information here.
I. **Chain of Custody**. This is the most important part of the form requiring signatures and proof samples were in custody by someone at all times. This form must be signed for relinquishing and shipped with the samples (this is why it is printed on plastic paper). The person on the receiving end of the shipment must find the COC form and sign it. It's a good idea if possible to make a copy or scan the COC form before it is shipped off.

Filling out electronic data submission form

Chain-of-custody data must be supplied in coded form so it can be added to the hydrocarbon database. Pre-populated tables can be acquired for PEMD, mussel, sediment, and gravimetric samples. There are separate tabs for each matrix (sediment, mussel, PEMD, gravimetric, extra). Some fields needed for the database have been filled in (or left blank) and hidden (don't worry about these).

A. **SIN**s are filled in. Fill in each corresponding record as appropriate.
B. **Depth**. Leave blank unless intertidal elevations are determined for the specific sample. Note that any elevation (meters) above mean lower low water is expressed as negative.

C. Field "*invest*" (investigator number) corresponds to "collector's sample code" in the chain-of-custody.
D. **Collection method**; use stylized names for database consistency (hand, spoon, shovel, core). Leave this field blank for PEMDs. The drop-down arrow can be used as a reminder for names in this field and several others.
E. **Matrix**. This corresponds to the matrix & species field in the chain-of-custody.
F. **Submatrix**. Use for mussels; the expected entry is "whole."
G. **Species**. Corresponds to the matrix & species field in the chain-of-custody. The expected entry for mussels is "MUSS." Fields matrix, submatrix, and species have been pre-populated as appropriate.
H. **Date collected**. Use mm/dd/yyyy format.
I. **Year** has been pre-populated (hidden).
J. **Location**. Use beach segment for this project, e.g., SM006B, EL058B, KN0109A. These specific segment names match other information in the hydrocarbon database. Naming consistency is important for long-term database usefulness.
K. **Sampler**. This records who had primary responsibility for collecting the samples. That person signs and dates the chain-of-custody form before it is turned over to chemistry staff.
L. **Latitude** and **longitude**. Use decimal degrees. Express west longitude as a negative number.
M. **Sample type**. This field distinguishes environmental (ENV) and experimental (EXP) samples. It also records blanks. For the purpose of this project, blanks are generally expected only for PEMDs. All other samples should be ENV.
N. **Comment**. Record any additional comments here.

Shipping

Shipping Address: Auke Bay Laboratories
c/o ABL staff name and phone number
17109 Point Lena Loop Road
Juneau, AK 99801

Handling of Samples Materials List

Item #	**Description** (or type in subject)
1	Signed COC form; make copy/scan before shipping
2	Coolers (20 L) or 5 gal. bucket with lid
3	Lots of hard shell blue ice to fill completely fill cooler
4	Duct tape
5	Labels for each cooler/bucket; indicate which one has COC form
6	80 lb. wet loc fish boxes can be used for gravimetric samples
7	
8	
9	

COC Form Filled Out filled out

2015 PWS SCAT SURVEYS — **OILED SEDIMENT**

2015 — **Chain of Custody Form** — Page 1 of ___

Project Name — **Auke Bay Laboratory Hydrocarbon Assessment** — **Serial # 20154**

Assigned Sample #	Collector's Sample Code	Date Collected	Matrix & species, organ	Location Collected	Latitude	Longitude	Collection Method	Comments
20150401	D3R2	6/x/15	SED	KN0136B	60.xxxx	147.xxxx	SP00	MOR; P, GR
20150402	A4R1	↓	↓	↓	↓	↓	↓	MOR; P, GR smells
20150403	B3 R1							LOR; S
20150404	C2 R1							LOR; P, S
20150405	C2R2							LOR; GR (lightOR)
20150406	E4R1							LOR; P, S
20150407	E4R2	6/xx/15	SED	SM006C	60.xxxx	147.xxxx	SP00	MOR; GR sticky
20150408	C4R1	↓	↓	↓	↓	↓	↓	MOR; P, GR
20150409	B3R1							MOR; S
20150410	E3R1							LOR; GR, S
20150411	E4R2							LOR; GR, S
20150412								
20150413								
20150414								
20150415								

Chain of Custody:
Collected by — Mandy Lindeberg (print name(s)/agency) — Signature — 6/x/15 (Signature date)
comments: all samples labeled & secure for shipping

Relinquished by — Signature — 6/xx/15 (Date) — Cordova, AK (Place)
comments: all samples frozen

Received by — Beaker Tilak — Signature — 6/xx/15 (Date) — Juneau, AK (Place)
comments: all samples arrived frozen & intact.

For information call
Larry Holland
NMFS/Auke Bay Lab
17109 Pt. Lena Loop Rd
Juneau, AK 99801
(907) 789-6083
larry.holland@noaa.gov

Use portable scanner to archive a backup of filled out forms.

References

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