Exxon Valdez Oil Spill
Restoration Project Final Report

Population Recovery Status of Littleneck Clams in Prince William Sound: An Unexpected Turn of Events…

Restoration Project 070829
Final Report

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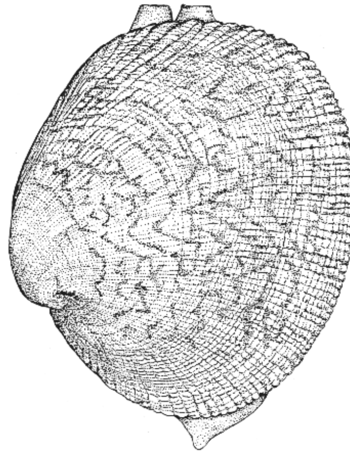

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Study History: Project 070829 has its origins in a long-term intertidal monitoring program initiated by Exxon in 1989, and continued and expanded by NOAA beginning in 1990. As part of that monitoring program, comprehensive surveys, including biological, geomorphological, and chemical assessments, were conducted to quantify the impacts of both the oil spill and subsequent cleanup activities on intertidal communities residing on beaches in Prince William Sound were conducted from 1990 to 2000. Following 2000, experimental studies were launched in Prince William Sound and Kachemak Bay to test hypotheses originating from the monitoring data. Many of these studies continue today.

The NOAA monitoring program included focused studies on trends in specific organisms. Among these was *Leukoma staminea*,[1] the native littleneck clam. Littleneck clams were judged to be of particular importance and interest because they are important prey items for a number of species; are harvested recreationally, commercially, and for subsistence; and reside in the intertidal zone where both oiling and cleanup activities occurred. Initial impacts from the spill and oil recovery activities on clam populations were evident, and the NOAA monitoring documented measurable effects for a number of years after the peak of oiling and beach treatment.

In 2000, the final year of the NOAA monitoring program, the native littleneck clam abundances at oiled/washed sites had essentially converged with abundances at unoiled reference sites. However, based on the characteristics of the observed recovery in other intertidal communities, we recognized that "convergence" is not necessarily equivalent to "recovery," and that "stability" in populations must also be factored in as part of the recovery calculus.

In the EVOSTC 2006 Injured Resources and Services Update, clam studies from several different sources were reviewed and synthesized. Based on the sum of results, clams were judged to be recovering, but not yet recovered. Presumably because of this status, the EVOSTC funded the current study, which is the subject of this report. This study revisited clam monitoring sites in PWS to determine current status of littleneck clams in the context of previous long-term intertidal monitoring results, as well as those from surveys of other clam species.

Abstract: Between 1990 and 2000, NOAA's Emergency Response Division conducted long-term monitoring in Prince William Sound to evaluate the effects of the *Exxon Valdez* oil spill and cleanup activities on intertidal communities. Recovery trajectories in littleneck clams, *Leukoma staminea*, differed from those of other infaunal organisms; namely, the clams exhibited a classic recovery trend where abundance at impacted sites increased steadily relative to control sites over a nine-year period. When monitoring ended in 2000, littleneck clam abundances at control and

[1] Recent revisions to bivalve taxonomy have changed the scientific name of this species from *Protothaca staminea* to *Leukoma staminea*, which we will use in this report.

impacted sites had essentially converged; however, there was no evidence that the populations had stabilized. Thus, they were not considered fully recovered at that time.

For the present project, we returned to NOAA study sites in the summer of 2007 to update the status of the littleneck clam populations. Clam-sampling plots were excavated by hand, and clams were retained for size and age analysis, and for determination of hydrocarbon tissue burdens. Specifically, clam tissues were analyzed for aromatic hydrocarbon concentrations.

This field sampling effort unexpectedly found that there was a significant and consistent decline in littleneck clam abundance at all sites sampled within Prince William Sound compared to the field sampling efforts conducted in prior years. This ubiquitous population decline complicates the assessment of recovery from the spill, and demonstrates the limitations of recovery metrics, including the population stability tests we have applied for the last ten years. Although the widespread decline in littleneck clam abundance does not appear to be linked to the spill, understanding its etiology is both relevant and critical to understanding the process, and even the definition of long-term recovery from this and other spills.

Key Words: *Exxon Valdez* oil spill; *Leukoma* (*Protothaca*) *staminea*; littleneck clams; Prince William Sound, Alaska; polynuclear aromatic hydrocarbons; PAH; recovery.

Project Data:

Description of data – data originated from clams excavated from beaches located in Prince William Sound. Length measurements were collected in the field, aging was performed in laboratories in Edmonds WA, and Ventura CA. Tissues were extracted and processed for chemical analysis at Louisiana State University, Baton Rouge LA.

Format – All data were entered as MS Excel spreadsheets.

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TABLE OF CONTENTS

Table of Contents iii
List of Figures v
List of Tables vi
Executive Summary vii
Introduction 1
Objectives 5
Methods 6
    Recovery status 6
    Bioavailability of lingering oil 7
Results 9
    Population Trends 9
    Tissue Chemistry 9
    Sediment Chemistry 11
Discussion 12
Conclusions 28
Acknowledgments 30
Literature Cited 30
Acronyms and Abbreviations 34
Appendix A. Potential Causes for the Widespread Decline in Littleneck Clam Populations 36
    Predation 36
    Sea temperature 37
    Disease and Parasitic Infection 37
    Contaminant Exposure 39
    Ocean acidification 39
Appendix B. Field Sampling Protocols for the Determination of Hydrocarbon Concentrations within Intertidal Sediments and Littleneck Clam Tissues 41
    Overview 41
    Goals and Assumptions 42
        NOAA Constraints 42
        Goals 42
    Questions of Interest 42
    Collection Procedures 42
        Primary Sampler 42
        Equipment 43
        Tissues 44
        Sediments 44

TABLE OF CONTENTS
(Continued)

Rationale 44
Handling Procedures 45
Rationale 45
Shipping Procedures 46
Rationale 46
Extraction and Analytic Procedures 47
Disposition of the Clam Samples 49
Extraction and Cleanup 50
GC/FID and SIM GC/MS 50
Quality Control and Assurance 51
Grain Size 51
Carbon Content 51
Analytical Strategy 51
Data Reporting 51
Additional Considerations 52
Field checklist 52
Beach Arrival 52
Departing Beach 53
Post-Sampling 53
Packing 53
Airport Freight Counter 54
Equipment List 54
Estimated Sample Count & Cost Estimate 55
Chain of Custody 55

References 57
Appendix C. Analytical Chemistry Methods 59
Extraction Methodologies 59
Sediment Extraction Methodology 59
Tissue Extraction Methodology 60
Instrumental Analysis (Gas Chromatography/Mass Spectrometry) 61
GC Operation 61
Mass Spectrometer Operation 61
Quantitative Analysis 61
Surrogate Corrections 62
Report Generation 62

Appendix D. Sediment and Tissue Chemistry Results for the July 2007 PWS Survey 63

LIST OF FIGURES

Figure 1. Map of PWS region showing the locations of the intertidal monitoring sites..................8

Figure 2. Temporal trends in *Leukoma staminea* populations at unoiled sites and at oiled/washed sites monitored by NOAA OR&R between 1990 and 2007: a) average abundance; b) ratio of populations at the two groups of sites; and c) the statistical significance of linear trends shown in (b) within 6-year time intervals.........10

Figure 3. *L. staminea* population trends from 1990 through 2007 at four sites that traditionally supported high clam abundance..12

Figure 4. Oil sheen observed during clam excavation at the Block Island site on: a) 1 July 1997and b) 13 July 2007 14

Figure 5. Total infaunal populations at unoiled sites and at oiled/washed sites monitored by NOAA OR&R between 1990 and 2000: a) time series of average abundance with 90% confidence intervals; b) temporal trend in relative populations between the two groups of sites; and c) the statistical significance of linear trends shown in (b) within 6-year time intervals...15

Figure 6. Littleneck clam populations at unoiled sites and at oiled/washed sites monitored by NOAA OR&R between 1990 and 2000: a) time series of average abundance; b) temporal trend in relative populations between the two groups of sites; and c) the statistical significance of linear trends shown in (b) within 6-year time intervals 17

Figure 7. Time series of the abundance of *L. staminea* older than one-year within sediments excavated from PWS long-term intertidal monitoring sites during surveys conducted from 1991 through 2007 (Table 4): a) at individual sites; and b) average within treatment categories..19

Figure 8. Molluscan and *L. staminea* population trends at NOAA's Lower Herring Bay experimental site 23

Figure 9. Time series of the estimated standing stock of *L. staminea* within the lower Cook Inlet as tabulated in Table 6 for: a) legal-sized clams; and b) sublegal-sized clams 26

Figure 10. Stock estimates of a) population density and b) biomass for legal-sized specimens (≥38 mm) from four bivalve species at Camano Island State Park, Washington 28

Figure 11. Example of a completed sample chain-of-custody form used by the NMFS/NOAA Auke Bay Laboratory 56

LIST OF TABLES

Table 1. Oiling category and location of NOAA's ten long-term intertidal monitoring sites that were revisited as part of EVOSTC Project 070829 7

Table 2. Total target aromatic hydrocarbons (TTAH) (ng/mg-dry) within L. staminea tissue samples collected in 2007 11

Table 3. TTAH (ng/mg-dry) within selected sediment samples collected in 2007 13

Table 4. Density (m-2) of L. staminea older than one-year within sediments excavated from PWS long-term intertidal monitoring sites during surveys conducted from 1991 through 2007 18

Table 5. Average density (0.0045 m2)-1 and relative abundance (%) of L. staminea and Saxidomus gigantea within sediments excavated from PWS long-term intertidal monitoring sites during surveys conducted from 1990 through 1996, and in 2002 (Source: Lees and Driskell, 2007) 23

Table 6. Estimated littleneck clam populations (reported as numbers of clams) at selected locations within the Lower Cook Inlet, Alaska. Data source: Szarzi et al (in preparation). 25

Table 7. Target hydrocarbon analytes and the associated recommended standards cross-referenced in Table 8. Calibrated analytes are identified by boldface type. 48

Table 8. Recommended laboratory standards for hydrocarbon analytes listed in Table 7 49

Table 9. Recommended sample weights, final pre-injection volumes (PIVs), and injection volumes to optimize method detection limits (MDLs) for hydrocarbon analysis 50

Table 10. Recommended field sampling equipment, quantities, and suppliers for hydrocarbon samples 55

Table 11. Alkane concentrations (ng/mg-dry) within sediments excavated from PWS long-term intertidal monitoring sites during the 2007 field survey 64

Table 12. Aromatic hydrocarbon concentrations (ng/mg-dry) within sediments excavated from PWS long-term intertidal monitoring sites during the 2007 field survey 67

Table 13. Alkane concentrations (ng/mg-dry) within clam tissue collected at PWS long-term intertidal monitoring sites during the 2007 field survey 71

Table 14. Aromatic hydrocarbon concentrations (ng/mg-dry) within clam tissue collected at PWS long-term intertidal monitoring sites during the 2007 field survey 74

Executive Summary

Between 1990 and 2000, NOAA's Emergency Response Division conducted long-term monitoring in Prince William Sound to evaluate the effects of the *Exxon Valdez* oil spill and cleanup activities on intertidal communities. Consistent patterns of impact and recovery were observed for a wide range of intertidal taxa, wherein populations abruptly recolonized impacted sites within a few years of the spill, and then displayed comparative stability in subsequent years (Coats et al., 1999; Skalski et al., 2001). However, the recovery trajectory for littleneck clams, *Leukoma staminea* (formerly *Protothaca staminea*), followed a more-traditional recovery trend. Instead of an abrupt recolonization, littleneck clams exhibited a slow, but steadily increasing abundance at impacted sites relative to control sites throughout the last nine years of the monitoring period, well after populations of most other taxa had stabilized (Shigenaka et al., 1999). When regular sampling ended in 2000, abundances of littleneck clams at control and impacted sites had converged, there was no evidence that they had stabilized, and so the clams at surveyed locations could not be considered fully recovered.

In 2007, the *Exxon Valdez* Oil Spill Trustee Council funded this current study to update the status of littleneck clam recovery based on the original NOAA monitoring sites and protocols. In July 2007, sampling was conducted at the core monitoring sites from the 1990-2000 monitoring effort. Original sampling methods were augmented to improve comparability with other, ongoing Trustee Council research projects. Six 0.25-m^2 lower intertidal quadrats were excavated, and clams were retained for determination of size and age, tissue metrics, and PAH bioavailability from lingering oil. Five clam-gun cores (10.5 cm x 15 cm) were also randomly collected along the same transect. Sediments collected by the hand-held corers were sieved through a 1-mm screen, and all clams were identified and measured for assessment of recruitment and settlement.

The most unexpected result from the 2007 field assessment was the observation that littleneck clam abundance had markedly declined at all sites sampled within the Sound, irrespective of oiling or cleanup history. For example, analysis of abundance trends at four sites with differing oiling and treatment histories showed uniformly similar drops in littleneck clam abundances. That is, all four sites had previously supported robust clam populations, but yielded significantly fewer clams in 2007. The order-of-magnitude reduction in populations at these sites was significant (p<0.05) compared to the spatial variability among the sites at a given time, and compared to interannual fluctuations in mean populations prior to 2001.

Contemporaneous declines in littleneck clam abundance have also been noted in other areas of Alaska, including the Kenai Fjords region and the along the Alaska Peninsula (Bodkin and Dean, 2008). The decline appears to have been restricted to littleneck clams, as analysis of other molluscan taxa did not show a similar pattern. A previous Trustee Council clam survey (Lees and Driskell, 2007) that was carried out in 2002 across a broader swath of the spill-affected area did not document decreases in littleneck numbers relative to prior NOAA studies, ostensibly narrowing the decline's occurrence to sometime between 2002 and 2007.

Analysis of currently archived infaunal samples from a separate NOAA experiment within Lower Herring Bay may permit further refinement as to when littleneck clam populations were affected. Analyses of 2007 and 2008 samples from the NOAA experimental site have yet to be completed. However, if we assume that this location reflects Sound-wide trends in *Leukoma*

staminea populations, the decline occurred between 2006 and 2007 because the Lower Herring Bay clam populations were stable through at least 2006.

Regardless of when the order-of-magnitude decline in *L. staminea* populations occurred, it renders one of the original goals of the current project somewhat moot; namely, testing the hypotheses whether the linear trend seen in relative populations at impacted and control sites prior to 2001, continued into 2007, or alternatively, whether the populations stabilized at some point after 2000. In the original NOAA monitoring effort within the Sound, abundance trends for *L. staminea* reflected a linear pattern of recovery in which numbers of clams at oiled/washed sites steadily increased, eventually converging with the numbers of clams encountered at unoiled sites. By 2000, clam abundances were nearly equal at both impacted and unoiled sites. Unquestioningly applying the statistical hypothesis test for linear trends in relative abundance suggests that the populations had "stabilized" by 2007. This determination is based on the lack of evidence (p=0.15) for a linear trend in the time window that contains 2007 data, whereas windows spanning the 1992 to 2000 time frame exhibit a significant trend ($p \leq 0.03$).

Obviously, however, the paucity of littleneck clams at all sites sampled in the Sound during 2007 connotes anything but stability. Even though relative populations in 2007 were consistent with data collected in 2000, namely similar abundance levels at unoiled and impacted sites, the near absence of littleneck clams throughout the Sound in 2007 means that the data contain almost no real information about potential population differences that may have resulted from habitat differences, such as exposure to lingering oil, armoring, or overall grain-size distribution. Specifically, the 2007 data lend little or no insight into how the populations would have differed had there been more clams overall. As a result, little can be legitimately inferred about population stability from the absence of a significant temporal trend in relative abundance in the time-window containing the most-recent data. The absence of a valid stability assessment leaves us unable to declare littleneck clams recovered, and somewhat moot, even though the statistical hypothesis tests for relative trends would suggest otherwise.

Although the recent decline in littleneck clam populations does not appear to be linked to the spill, understanding its etiology is both relevant, and in fact, critical to understanding the process of long-term recovery from the spill. In the face of the diminishing environmental signal from the spill, and a dynamic environmental baseline influenced by climate change and other large-scale influences, our ability to discern and measure perturbations resulting from the spill will be increasingly challenged.

Introduction

The 2007 invitation for proposals by the *Exxon Valdez* Oil Spill Trustee Council (EVOSTC) states the following:

> In general, the Council seeks proposals that measure the exposure to and effects of recovering or not recovered resources to lingering oil. Additionally, the Council is interested in the recovery process of resources that may not be currently exposed to lingering oil but are still not recovered. Finally, the Council is interested in funding work that directly addresses restoration of human services which are still not considered recovered…
>
> …In order to determine if lingering oil is still impacting intertidal communities, the Council seeks proposals that include an ecological risk assessment of the invertebrate infaunal community. Projects should aid in the Council's determination of future restoration strategies, including monitoring or physical removal of the oil. Additionally, these studies should evaluate the exposure and effects of oil on deep-burrowing invertebrates, because much of the unweathered, more toxic lingering oil remains below the low water line.

Concerning the recovery status of clams, the EVOSTC 2006 Injured Resources and Services Update states:

> *Injury* Clams are widely distributed throughout the oil spill area. They can be found in a variety of substrates and are most abundant in the lower intertidal and subtidal zones. Clams are important prey for various fish and wildlife resources including sea otters, some sea birds, sea ducks and others.
>
> … In 1990 and 1991, growth of littleneck clams at oiled sites was less than at reference sites, and growth rate was directly proportional to hydrocarbon concentrations. Additionally, mortality was higher and growth rates lower in clams transplanted from oiled areas to clean areas, 5 -7 years after the spill.
>
> Clean-up technologies were detrimental to clam populations and included hot water, high pressure washing, manual and mechanical scrubbing and physical removal of oiled sediments. Hot water washing caused thermal stress, oil dispersal into the water column, animal displacement and burial, and the transportation of fine grain sediment from the upper intertidal into the lower intertidal zone. Early assessments reported that clean-up activities resulted in reductions in clam abundance and distribution on treated (oiled-but-treated) beaches up to three years after the spill.
>
> *Recovery Objective* Clams will have recovered when population and productivity measures (such as size and distribution) at oiled sites are comparable to populations and productivity measures at unoiled sites, taking into account geographic differences.
>
> *Recovery Status* Studies have indicated that abundances of some species of clams were lower on treated beaches through 1996. Densities of littleneck and butter clams were depressed through 1997 on cleaned mixed-sedimentary shores where fine sediments had been washed down the beach during pressured water treatments.
>
> As part of an investigation of sea otter populations conducted from 1996-1998, researchers compared clam densities between oiled sites on Knight Island and unoiled sites on Montague Island. They reported an increase in mean size of littlenecks and butter clams at Knight Island, where numbers of sea otters, a major predator of clams were

significantly reduced. Absolute densities of littlenecks and butter clams were not different between oiled and unoiled sites; however, oiled sites had fewer juvenile clams and lower numbers of other clam species. In 2002, differences in species richness, diversity and abundance of several species were still measurable between cleaned (oiled and treated) and untreated (oiled but untreated) beaches. Moreover, as of 2005, several wildlife species that use the intertidal zone and feed on clams (e.g., harlequin ducks and black oystercatchers) are still being exposed to oil. These resources are included on the injured resources list and although the exact route of oil has not been established for these birds, it is likely they are ingesting oil with their prey.

> Some overlap occurs between areas where lingering oil and populations of littleneck and butter clams co-exist. Given the burrowing behavior of these animals, it is likely they would be exposed to oil as they dig into the subsurface sediments known to contain oil. In fact, it has been demonstrated that littleneck clams exposed for a year to the surface layer of contaminated sediments did not accumulate oil, but if the clams were buried in sediments mixed with oil, accumulation did occur.

> Clam populations found on oiled but untreated beaches have likely recovered from the effects of the spill. However, several factors continue to impact clam populations on oiled and treated beaches: Abundances and distribution differences are still measurable between cleaned and untreated sites; Lingering oil occurs in habitats with clams, and exposure of clams to oil could result in upper trophic level predators eating contaminated prey; Other species on the injured resources list are still being exposed to oil and are known to forage on clams. Based on all of the evidence summarized above, clams continue to be recovering, but are not yet fully recovered from the effects of the oil spill.

The study described in this report was designed to be responsive and directly relevant to these EVOSTC observations and entreaties. Littleneck clam abundances at beaches monitored continuously between 1990 and 2000 within Prince William Sound (PWS) were to be compared with 2007 abundance levels to determine the clam recovery status using stability and convergence metrics. In addition, we intended to characterize polynuclear aromatic hydrocarbon (PAH) exposure and biological availability. These were to provide key pieces of information for considering the need for restoration strategies for a resource of substantial importance to other recovering wildlife resources in the spill-affected region, as well as to Alaskan subsistence and recreational fishing communities.

The native, common, or Pacific littleneck clam, *Leukoma staminea* (formerly *Protothaca staminea*),[2] is a regular inhabitant of the lower intertidal zone on well-sheltered Pacific coast beaches and estuaries where mud or sand is present. It ranges from Cape San Lucas in Baja, California north to the Aleutian Islands in Alaska (Chew and Ma, 1987). Its wide geographic distribution and its relatively accessible habitat in the intertidal zone has made *L. staminea* an important commercial and recreational shellfish species. In Washington State, for example, where sheltered bays and the enclosed waters of Puget Sound provide favorable habitat and growing conditions, the littleneck clam is a mainstay of the commercial clam harvest. Between 1990 and 1995, commercial landings averaged 90 t (99.2 U.S. tons), with an annual value of about $480,000 (NMFS, 1997).

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[2] This taxonomic change is recent, and was formalized in the most current edition of Light and Carlton (2007).

In PWS, *L. staminea* is frequently encountered on gravel beaches, and the clam is a regular part of the subsistence diet for native villagers residing in the region. According to Stratton and Chisum (1986), in the 1960s hardshell clams were a wintertime staple for residents of the village of Chenega; up to 136 kg (300 lbs) per household, and a mean of 46 kg (102 lbs) were harvested. While butter clams (*Saxidomus gigantea*) constituted the majority of the harvest, littleneck clams were also popular. Stratton and Chisum found in a subsequent survey in 1986 that clams remained a desired and harvested subsistence resource, but respondents noted that more effort was required than in the 1960s due to predatory competition from expanding sea otter populations.

The *Exxon Valdez* oil spill affected many beaches with resident populations of littleneck clams. Because of its widespread occurrence in the intertidal environment of PWS, *L. staminea* has been a key taxon in the infaunal community monitored by NOAA in its long-term monitoring of impacts and recovery from the spill. The littleneck clam is also of interest due to its role as a recreational and subsistence resource and important prey item for other organisms frequenting the intertidal zone, including oyster drills, moon snails, sea stars, octopi, rock crabs and fishes (Chew and Ma 1987). Wildlife predators, as noted previously, include sea otters, ducks, and other birds (Schink et al., 1983; Cheney and Mumford, 1986).

A number of researchers have described oil-related effects in *L. staminea*. Anderson et al. (1979) exposed *L. staminea* to sediments contaminated with 1237 parts per million (ppm) of weathered Prudhoe Bay crude oil for 54 days, and found a survival rate of 85 percent. This compared to a survival rate of only 17 percent in the pointed macoma clam (*Macoma inquinata*). In the same experiment, Augenfeld et al. (1980) also described effects of the 54-day exposure to oiled sediments on the condition index based on the ratio of dry tissue weight to shell size, and on the content of 17 amino acids of the exposed clams. Condition indices declined in both species when exposed to oil, but the relative decrease was greater in *M. inquinata* than in *L. staminea*. The latter also showed a proportionally smaller decrease in levels of free amino acids when exposed to oil. The researchers attributed the differences in survival, condition index ratios, and amino acid content to the difference in feeding habit and presumed difference in exposure to hydrocarbons; namely, *L. staminea* is a filter feeder, while *M. inquinata* is primarily a deposit feeder.

Pearson et al. (1981) discussed sublethal behavioral changes in *L. staminea* due to oil exposure that affected their susceptibility to predation by Dungeness crab (*Cancer magister*). They found that crabs ate more clams from oiled sand than from clean sand, and attributed this result to the fact that the clams did not burrow as deeply or as quickly in oiled sand as compared to clean sediments.

The initial impacts of the spill were most pronounced in the middle to upper intertidal portions of PWS beaches, because the oil tended to strand at those elevations and shoreline cleanup was concentrated there. Littleneck clams, which generally reside below the substrate surface at lower elevations in the intertidal zone, were more likely to avoid the initial habitat disruption from oil exposure and intrusive cleanup techniques. Nevertheless, acute impacts to littleneck clams were noted anecdotally throughout the first year following the spill. Houghton et al. (1996), for example, observed “dead or moribund” *L. staminea* on the surface of an oiled beach at the head of the west arm of Northwest Bay in April 1989. After washing of this beach took place in June of 1989, substantially reduced clam densities were found, as well as additional deceased

specimens and evidence that populations had been buried under layers of washed and displaced beach material. These observations suggested immediate and substantial impacts to *L. staminea* from both oil exposure alone, as well as the hydraulic washing technique extensively utilized in the first year of the spill response.

There is also evidence that these acute impacts may have been followed by longer-term injuries to clam populations. A recent study sponsored by the EVOSTC (Lees and Driskell, 2007) concluded that densities of longer-lived clams remained depressed at oil and cleanup-affected sites in PWS through 2002. The authors suggested that reduced clam densities decreased the capability and capacity of the impacted areas to support larger predators, such as sea otters, and linked ongoing impacts to disruptions in the protective armoring layers of beaches.

Between 1990 and 2000, NOAA's Emergency Response Division conducted a long-term monitoring program within PWS to evaluate the effects of the spill and cleanup activities on intertidal communities. The results and details of this effort have been discussed at length elsewhere (see, for example, Houghton et. al., 1996; Coats et al., 1999, 2003; and Skalski et al., 2001). Although most major intertidal assemblages, as well as many individual taxa, exhibited consistent, long-term population trends that were indicative of impact and recovery, the recovery trajectory exhibited by littleneck clam populations at the surveyed beaches was different (Shigenaka et al., 1999; Fukuyama et al., 2000). In contrast to other intertidal resources, where impacted populations stabilized with respect to reference populations after only a few years, littleneck clams exhibited a steadily increasing abundance at impacted sites relative to control sites throughout most of the 11-year sampling period. When monitoring ended in 2000, although population abundances at control and impacted sites had essentially converged, there was no evidence that the populations at the impacted sites had stabilized. For that reason, littleneck clams were considered to be a recovering resource, but not yet recovered.

For this EVOSTC study, we revisited the original PWS intertidal-survey locations during the summer of 2007 to update our assessment of the recovery status of littleneck clams, and to determine the nature of recovery trends after 2000. Did impacted clam populations continue to increase after 2000, eventually surpassing populations at control sites? Or, did abundance level off or decline relative to control sites? Simply put: What is the current status of littleneck clam populations at a set of monitored sites in PWS, 18 years after the nation's largest oil spill and cleanup?

Given the clam population trends that were observed through the year 2000, our pre-survey expectation was that clam populations at impacted sites would have demonstrated full recovery through stabilization of relative abundances at both impacted and control sites. Namely, we hypothesized that, after recovery, overall clam populations may have fluctuated in unison from year-to-year at impacted and control sites, but their relative proportion to one another would remain relatively stable over time. Statistical tests for temporal parallelism between impacted and control populations (Skalski and Robson, 1992) were successfully applied to the original monitoring data as an alternative metric for evaluating recovery (Coats et al., 1999, Skalski et al., 2001). The rationale was based on the expectation that trends in abundance at impacted and control sites would begin to mirror each other once the adverse influence of spill and cleanup effects diminished. Under this paradigm of recovery, convergence of absolute abundances does not necessarily need to occur because population differences may have arisen in response to

inevitable differences in individual site conditions that reflect the inherent spatial variability in the natural environment over the scale of the monitoring.

In 2007, therefore, our expectation was that we would be able to fully evaluate whether parallelism had been attained for littleneck clam populations at the survey sites. However, as the field team worked its way around PWS during the July 2007 sampling survey, it became increasingly apparent that abundances of littleneck clams were substantially lower than they had been during all nine of the eleven years previously surveyed as part of the NOAA monitoring program. Furthermore, in contrast to our original findings from the analysis of the 1990 through 2000 database, there was no apparent link between these population declines and the oiling or cleanup history of individual sites. Instead, the decline was observed at all sites regardless of impact from the spill. As the field effort progressed, we began to realize that the original objective of this project, to evaluate recovery status based on comparison of conditions at impacted and reference sites, would be difficult or impossible to achieve. Furthermore, the observed Sound-wide decline in littleneck clam abundance raised a number of questions well outside of the original study scope. When did the decline in littleneck clams occur? How geographically widespread was the decline? What was the cause of the decline?

Objectives

Our original objectives for the study were to provide relevant, meaningful scientific information that would contribute to discussions about recovery status of native littleneck clams and the biological availability of lingering oil. This information was intended for use by the EVOSTC in their consideration of the recovery status of the native littleneck clam, which is an important intertidal resource, and the need for restoration or remediation as it pertained to these clams. To these ends, we proposed to test the following hypotheses:

- Using recovery metrics from the 1990-2000 NOAA monitoring program, littleneck clam populations at surveyed sites have attained recovery endpoints in 2007.
- No significant differences in chemistry (tissue levels or distribution of hydrocarbons) can be discerned between clams collected at oiled and unoiled sites in PWS.

We would meet our stated objectives and test the hypotheses through the following discrete activities:

- Measure current abundance levels of littleneck clams at the same 10 sites within PWS that were surveyed between 1990 and 2000, and determine if current conditions reflect a "recovered" or "recovering" status;
- Measure sediment and tissue hydrocarbon levels at oiled and unoiled locations in PWS, and infer the significance of the measured levels to the health and recovery of clams.

The scope of the project was designed to address the primary objectives listed above. However, we also collected and archived additional samples and measurements to permit us to explore other questions contingent upon further identification of scientific rationale and budget. For example, we collected clam-core samples for determining sediment grain-size distributions, and enumerated other bivalve species at the project sites. In addition, because other EVOSTC clam researchers (Lees and Driskell, 2007) had postulated a link between clam populations and the

presence of physical "armoring" on beaches, we developed, tested, and employed a rapid estimation method for armoring at the sites we surveyed. If there appears to be a future rationale for investigating additional questions, we can proceed with processing and analysis.

As indicated above, the paucity of littleneck clams encountered in the field in 2007 has complicated attainment of this study's original objectives, insofar as requiring additional context and interpretation of the hypothesis-testing results presented in the following sections of this report. In addition, given the recent assessment of littleneck clam stocks, as reflected in the sampling we conducted in PWS in 2007, other questions related to the assessment of recovery from the spill naturally have arisen and will be discussed in this report. These include:

- What are the implications for assessments of recovery for a given event or disturbance when an apparently unrelated influence dramatically affects resident populations in the study area to a degree that meets or exceeds that of the original disturbance?
- Does the practice of comparing impacted site conditions to reference site conditions remain valid when an apparently unrelated influence confounds the metrics used for recovery assessment?

Methods

The overall objectives for this study are to provide scientific information that will contribute to discussions about the recovery status of littleneck clams, the biological availability of lingering oil to clams in PWS, and the need for restoration or remediation as it pertains to littleneck clams. The project was designed to focus on two aspects of clam status at the surveyed sites in PWS: abundance and chemistry. Additional measurements and samples collected during site visits may potentially provide supporting data to facilitate interpretation, although analyses of these samples were not included in the project budget.

Recovery status

In order to evaluate the current recovery status of *L. staminea* at long-term intertidal monitoring sites, the original field-survey procedures that were used throughout the 1990-2000 NOAA monitoring program were repeated, with several additions to improve comparability with methods and results from other EVOSTC projects. The ten original sampling sites (Table 1, Figure 1) were accessed by vessel in July 2007. At each site, the previously surveyed lower-intertidal transect was relocated. When necessary, the transect's elevation was adjusted to better match the 0-m MLLW target elevation.

Two methods were used to sample clam populations. Hand-held corers were used to primarily collect smaller-sized clams, especially recently-settled individuals. The clam-gun corer was 10.7 cm in diameter, 15 cm long, and sampled a surface area of 0.009 m^2. It was used to collect five replicate samples at each site. The corer samples were sieved in the field through a 1.0-mm mesh sieve and the residue remaining on the sieve was preserved in buffered 10% formalin. After initial preservation, the residue was transferred to 70% ethanol containing Rose Bengal stain. Biological samples were sorted under a binocular dissecting microscope. Venerid clams collected by corer were measured with a Vernier caliper, and ages were estimated by counting annular rings on the shell.

Table 1. Oiling category and location of NOAA's ten long-term intertidal monitoring sites that were revisited as part of EVOSTC Project 070829

Oiling Category	Site	Coordinates	
		Latitude	Longitude
Unoiled	Bainbridge Bight	60°06'59"N	148°14'48"W
	Sheep Bay	60°41'06"N	145°56'22"W
	Outside Bay	60°38'17"N	147°27'02"W
Oiled/ Untreated	Snug Harbor	60°15'43"N	147°45'57"W
	Mussel Beach	60°32'10"N	147°36'56"W
	Herring Bay	60°27'25"N	147°42'30"W
Oiled/ Washed	Block Island	60°31'48"N	147°36'24"W
	Northwest Bay West Arm	60°32'38"N	147°36'09"W
	Shelter Bay	60°07'06"N	147°57'24"W
	Sleepy Bay	60°03'56"N	147°50'08"W

Because the area sampled by the hand-held corers was too small to adequately recover larger-sized clams, a second collection method was also employed. Sediments within a 0.5 m quadrat were excavated and sieved through large screens (10-mm mesh).

Quadrat sampling was previously performed at these sites during the 1991, 1992, 1994, 1996, 1998, 2000 and 2007 NOAA monitoring surveys. Generally, four quadrats were randomly chosen along the transect line. However, in 2007, a total of six quadrats were sampled at each site,[3] with the exception of Herring Bay, where ten quadrats were sampled. All clams found within the quadrats were placed into plastic bags for subsequent length measurement. Clam excavations were located to avoid areas sampled by the corer, and where destructive sampling had been previously conducted. For the 2007 quadrat excavations, the numbers of littleneck clams were tallied in the field. Those clam enumerations were analyzed against the existing 1990-2000 monitoring data to determine *L. staminea* population trends using methods described in Coats et al (1999, 2003) and Skalski et al. (1992, 2001).

Bioavailability of lingering oil

Lingering oil bioavailability was evaluated by analyzing sediments and clam tissues collected from oiled and unoiled beaches. Chemical analysis was performed with gas chromatography (GC)/flame ionization detector (FID) with confirmation by mass spectrometry (MS). To maximize the potential for successful chemistry sample collection, analysis, and interpretation, a project-specific set of chemical and biological intertidal-sampling protocols was developed by Payne Environmental Consulting, Inc. (Appendix B). These protocols reflect decades of experience and insight into the environmental chemistry of PWS and Alaskan coastal waters. They build upon the many lessons learned from the NOAA monitoring program, as well as that of other organizations, such as the Prince William Sound Regional Citizens Advisory Council.

[3] The number of quadrats was increased from the originally-planned four to facilitate comparison with data generated in EVOSTC Project 070750, Database Development for Long-Term Monitoring of Nearshore Resources.

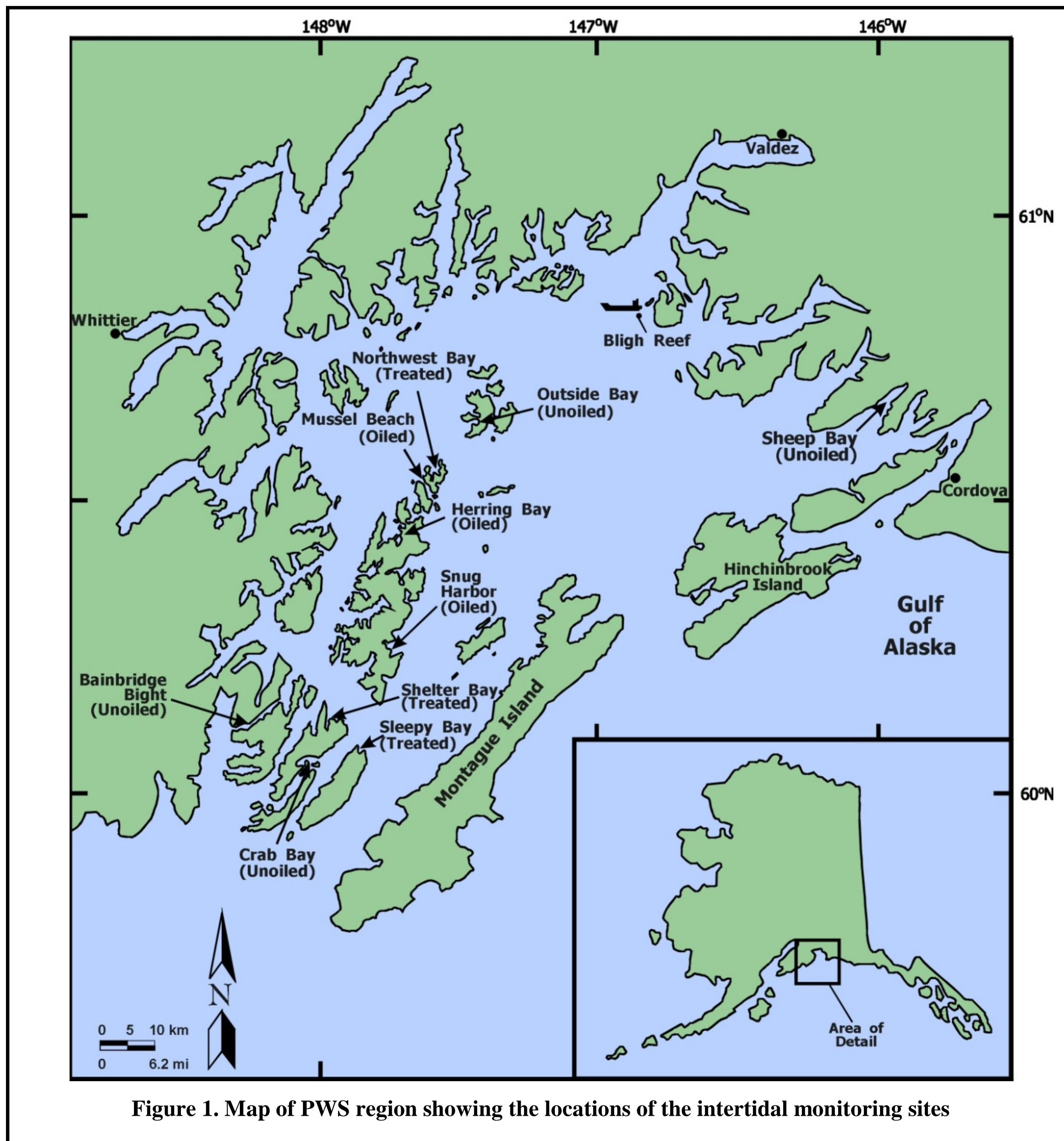

Figure 1. Map of PWS region showing the locations of the intertidal monitoring sites

The original objective of the chemistry sampling was not only to quantify the bioavailability of residual PAH contamination in PWS, but also to investigate links to sediment PAH contamination at a given site. The field sampling protocols were designed to allow assessment of the relationship between tissue and sediment contamination within samples collected at each site. However, complete chemical analysis of all tissue and sediment samples collected for the project, initially estimated to be 40 tissue samples and 80 sediment samples, would have been prohibitively expensive. To reduce overall chemistry costs, a staged approach to sample analysis

was employed where tissue samples were used to screen sites with elevated levels of PAH contamination.[4] If tissue concentrations were found to be elevated at a site, then the associated sediment samples would be analyzed to quantify their relationship to hydrocarbon exposure. Detailed analytical methodologies for the chemical assays were prepared by the NOAA chemistry support team at Louisiana State University as part of this study (Appendix C).

Results

The results of this project provide insight into at least three aspects of the current status of littleneck clam populations in PWS:

- The linear recovery trajectory that had characterized the relationship between clams at unoiled, and oiled/washed sites in the sampling area between 1990 and 2000 was not maintained through 2007 (Figure 2).
- Tissue hydrocarbon levels in sampled native littleneck clams in 2007 were uniformly low at all sites, regardless of oiling history.
- Assessment of clam recovery was affected by significant population declines at all sites sampled within PWS. The observed order-of-magnitude declines were apparently unrelated to oiling or treatment history

Population Trends

Unexpected declines in *L. staminea* abundances were observed at all sites across PWS in 2007. A comparison of clam-abundance trajectories at four, historically populous sites with differing oiling and treatment histories illustrates the absence of any strong relationship between the observed decline and impacts from the spill (Figure 3). All four of these sites had previously supported robust populations of clams, yet all yielded significantly reduced numbers in 2007. Specifically, an order-of-magnitude lower abundance was recorded at the sites in 2007, and the decline was significant ($p<0.05$) compared to the spatial differences in populations at the sites during any given year, and compared to interannual (temporal) fluctuations in the mean populations at all four sites prior to 2001.

Tissue Chemistry

The paucity of clams at the PWS survey sites during 2007 stymied the full implementation of chemistry sampling and analysis protocols that were developed for this project (Appendix B), particularly with respect to using tissue concentrations as screening criteria to prioritize sediment samples for subsequent analysis. Of the ten sites surveyed, only six yielded sufficient tissue mass to permit chemical analysis (Table 2, Appendix D-Table 13, Appendix D-Table 14).

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[4] Previous experience with residual *Exxon Valdez* contamination during NOAA long-term monitoring showed that tissues were a more reliable matrix for determining the presence and availability of PAHs at given site, primarily because sediment PAHs were highly variable and because lipophilic hydrocarbons tend to be preferentially retained in bivalve tissues.

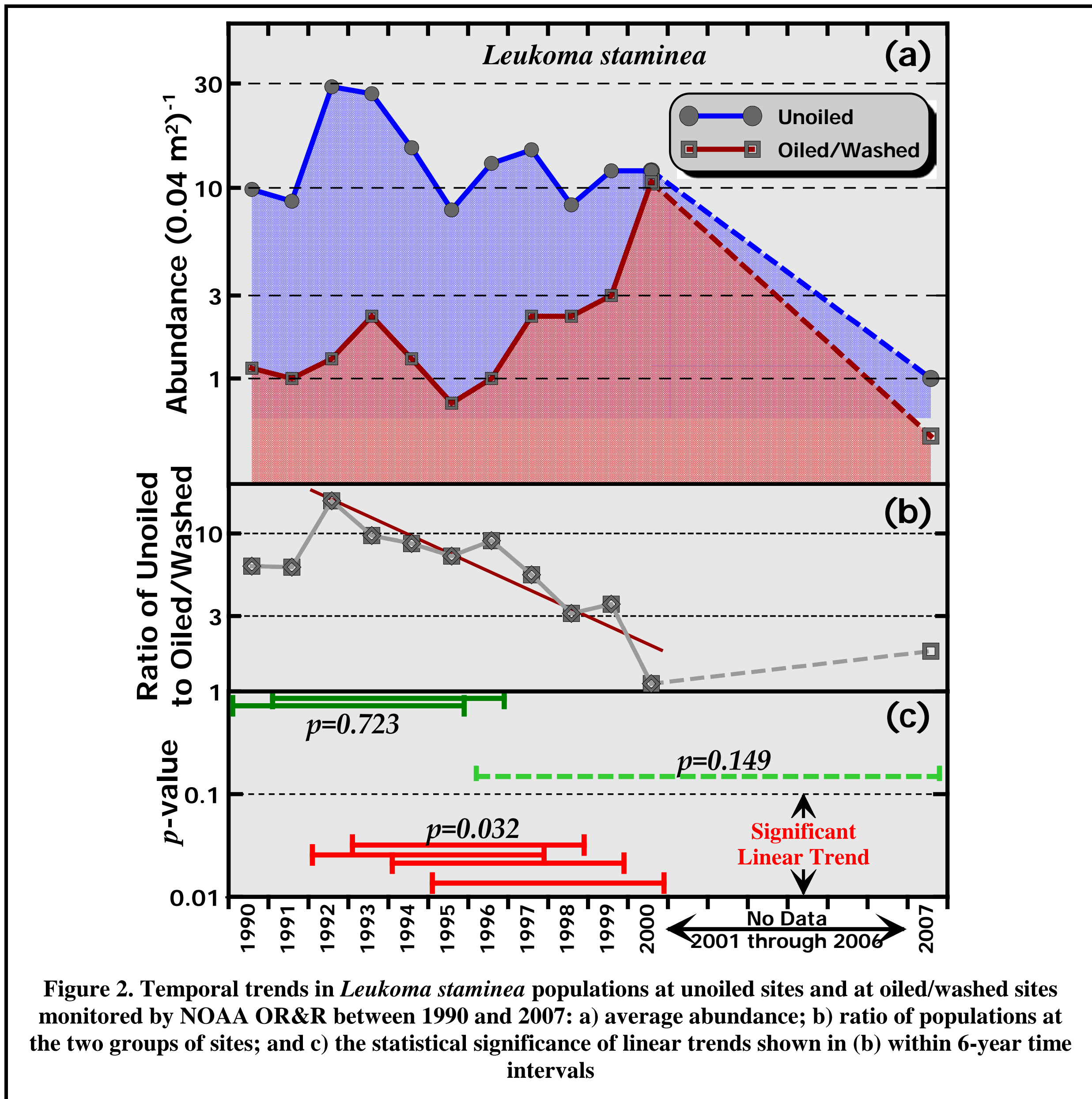

Figure 2. Temporal trends in *Leukoma staminea* populations at unoiled sites and at oiled/washed sites monitored by NOAA OR&R between 1990 and 2007: a) average abundance; b) ratio of populations at the two groups of sites; and c) the statistical significance of linear trends shown in (b) within 6-year time intervals

At three sites, absolutely no live littleneck clams were found, and at a fourth, only a single native littleneck was collected. The six remaining sites where sufficient clam tissue mass was collected were equally distributed across the three original categories of unoiled, oiled, and oiled/washed sites, namely, two results were in each site category.

Tissue concentrations of total target aromatic hydrocarbons were uniformly low in the samples where tissue volume permitted analysis and attainment of reasonable detection limits. The 2007 values were among the lowest measured in our PWS chemistry monitoring to date and may reflect levels approaching background for the region.

Table 2. Total target aromatic hydrocarbons (TTAH) (ng/mg-dry) within *L. staminea* tissue samples collected in 2007

Category	Site	TTAH
Unoiled	Bainbridge Bight	0.03
	Sheep Bay	0.02
	Outside Bay	(no clams)[5]
Oiled/ Untreated	Snug Harbor	0.04
	Mussel Beach	0.03
	Herring Bay	(no clams)5
Oiled/ Washed	Block Island	0.02
	Northwest Bay West Arm	0.03
	Shelter Bay	(no clams)5
	Sleepy Bay	(1 clam)5

Viewed temporally, the 2007 results reflected a one-to-two orders of magnitude decline in tissue contamination from the first analyses conducted to determine PAH tissue burdens in clams collected during the 1991 survey of the NOAA monitoring program. At Block Island, which was one of the more persistently contaminated PWS sites, tissue burdens in littleneck clams steadily declined from approximately 6.4 ng/mg-dry (ppm) in 1991, to 0.02 ng/mg-dry in 2007.

Sediment Chemistry

Sediment chemistry results also showed uniformly low levels of aromatic hydrocarbons at all sites, with no total exceeding 0.026 ng/mg-dry and over half of the results in the 0.011-0.014 ng/mg-dry range (Table 3, Appendix D-Table 12). There was no statistically significant difference (p>0.47) in average PAH concentrations measured in surface sediments compared to those found at the bottom of the clam excavation quadrats. In the chemistry sampling strategy developed for this project (Appendix B), only those sediment samples associated with tissue samples having elevated hydrocarbon concentrations were to be analyzed.

Although the field team did not encounter significant areas of residual oiling at the designated sampling sites for this project, we are well aware that such pockets remain in PWS. For example, anecdotal accounts from other project field parties encountered in 2007 noted areas within Northwest Bay where oil was visually apparent, and preliminary reports from EVOSTC researchers Boufadel et al. (2007) documented the continued and unanticipated presence of lingering oil. It is, however, notable that sites where oil had previously been consistently found in the NOAA monitoring program prior to 2000 (Figure 4a), such as the Block Island site, now show only traces of visible (Figure 4b) and measurable oiling.

[5] Tissue mass was insufficient for analysis.

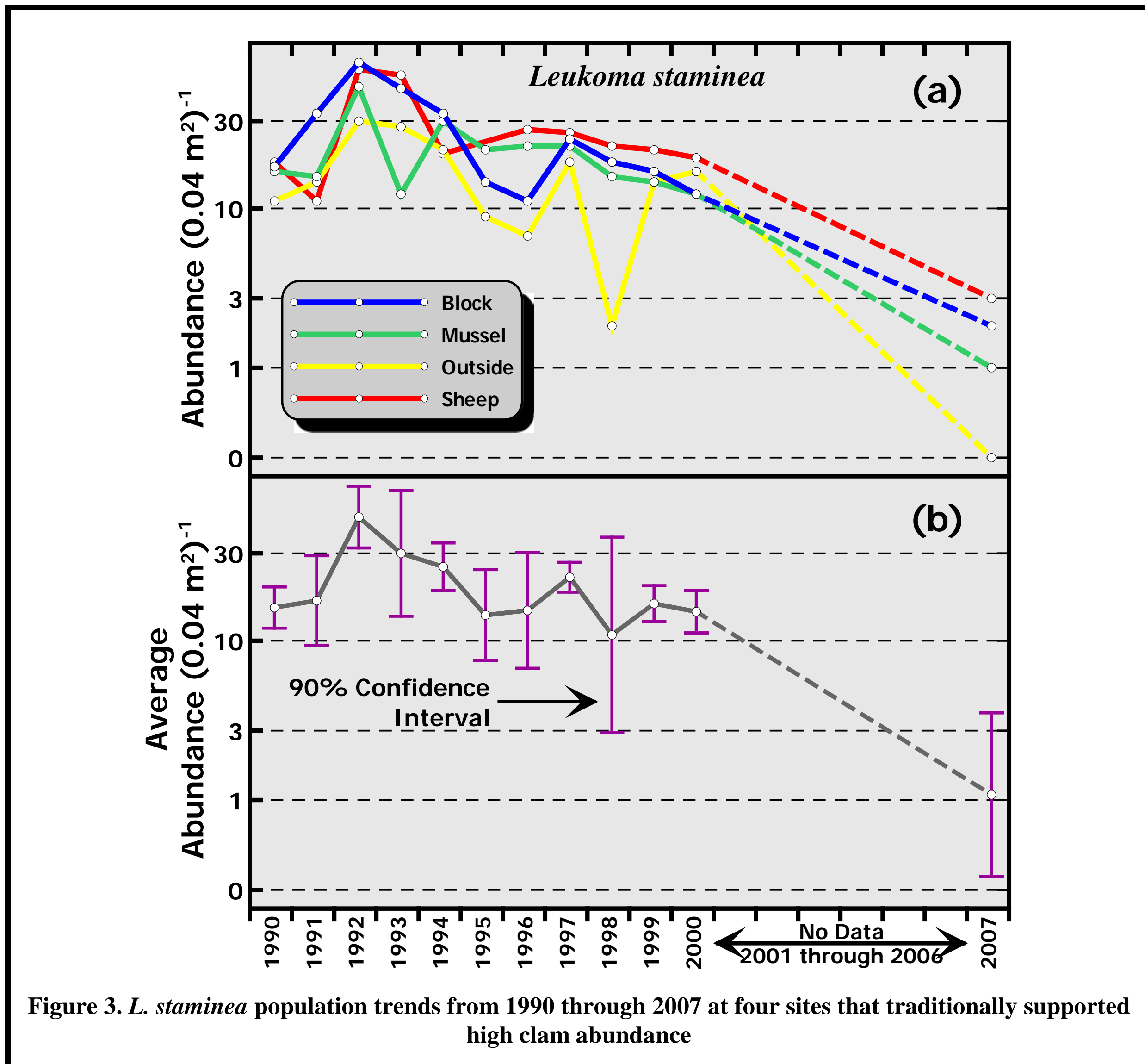

Figure 3. ***L. staminea*** **population trends from 1990 through 2007 at four sites that traditionally supported high clam abundance**

Discussion

Between 1990 and 2000, the NOAA monitoring program documented a consistent pattern of recovery across a wide suite of intertidal assemblages (Coats et al., 1999). Between 1990 and 1993, abundance, number of species and other population parameters exhibited abrupt increases at impacted sites relative to controls. Following these sudden, post-disturbance recolonizations, interannual fluctuations in populations at oiled sites and at oiled/washed sites, began tracking those at unoiled reference sites. Skalski and Robson (1992) developed tests for parallelism to apply to impact assessments in accident scenarios. We applied a series of parallelism tests to PWS intertidal population data to infer when impacted populations began to stabilize and track the population fluctuations at reference sites. Using parallelism to identify when impacted populations stabilize provides an alternative means of addressing the complex subject of recovery within PWS (Skalski et al., 2001). Examining population trends at impacted sites

Table 3. TTAH (ng/mg-dry) within selected sediment samples collected in 2007

Site	Quadrat	Depth	TTAH
Mussel Beach	1	Surface	0.012
Mussel Beach	1	Bottom	0.013
Mussel Beach	4	Surface	0.011
Mussel Beach	4	Bottom	0.012
Block Island	1	Bottom	0.026
Block Island	4	Bottom	0.011
Bainbridge Bight	2	Surface	0.012
Bainbridge Bight	2	Bottom	0.015
Bainbridge Bight	6	Surface	0.014
Bainbridge Bight	6	Bottom	0.017
Sheep Bay	1	Surface	0.014
Sheep Bay	1	Bottom	0.012
Sheep Bay	4	Surface	0.012
Sheep Bay	4	Bottom	0.010
Northwest Bay	1	Surface	0.021
Northwest Bay	1	Bottom	0.016
Northwest Bay	4	Surface	0.015
Northwest Bay	4	Bottom	0.018

relative to reference sites over time provides a unique approach to evaluating recovery that departs from traditional approaches which rely on a test for no difference in absolute abundance at impacted and control sites within a given year.

The time history of total infaunal abundance illustrates the distinctive pattern of abrupt recolonization that was seen in a wide variety of intertidal populations that were impacted by the spill and cleanup within PWS (Figure 5). It also illustrates the advantages of examining temporal trends in relative abundance when substantial within-year variability is present among the samples collected at sites within each treatment group (Figure 5a). Within-year variability is reflected in the 90% confidence intervals whose span approaches the amplitude of the recolonization event that occurred between 1991 and 1992.

As such, the variability may be too great for meaningful detection and interpretation of the reduced spill-induced population levels in 1990 and 1991, as compared to population levels after impacted populations stabilized (*cf.* the red line in Figure 5a). In contrast, analysis of long-term trends in relative populations provides a robust method of identifying transient impacts and subsequent recovery from an accident such as an oil spill (Figure 5b). Tests for parallelism within moving, six-year time windows rely solely on detecting a linear, temporal trend (Figure 5c), and are unaffected by high within-year variability that may be largely due to naturally occurring differences in habitat among the sites used in the average.

Moreover, we cannot assume conditions at the reference and impacted sites were the same prior to the spill, so convergence in absolute abundance is not necessarily a valid test that the community has recovered to “pre-spill” conditions. In fact, systematic environmental differences between control and impacted sites are expected for reasons unrelated to oiling or shoreline cleanup. Over the course of monitoring in Alaska, we found that the inherent variability of the PWS environment has represented one of the major challenges to tracking the signal of

Figure 4. Oil sheen observed during clam excavation at the Block Island site on: a) 1 July 1997and b) 13 July 2007

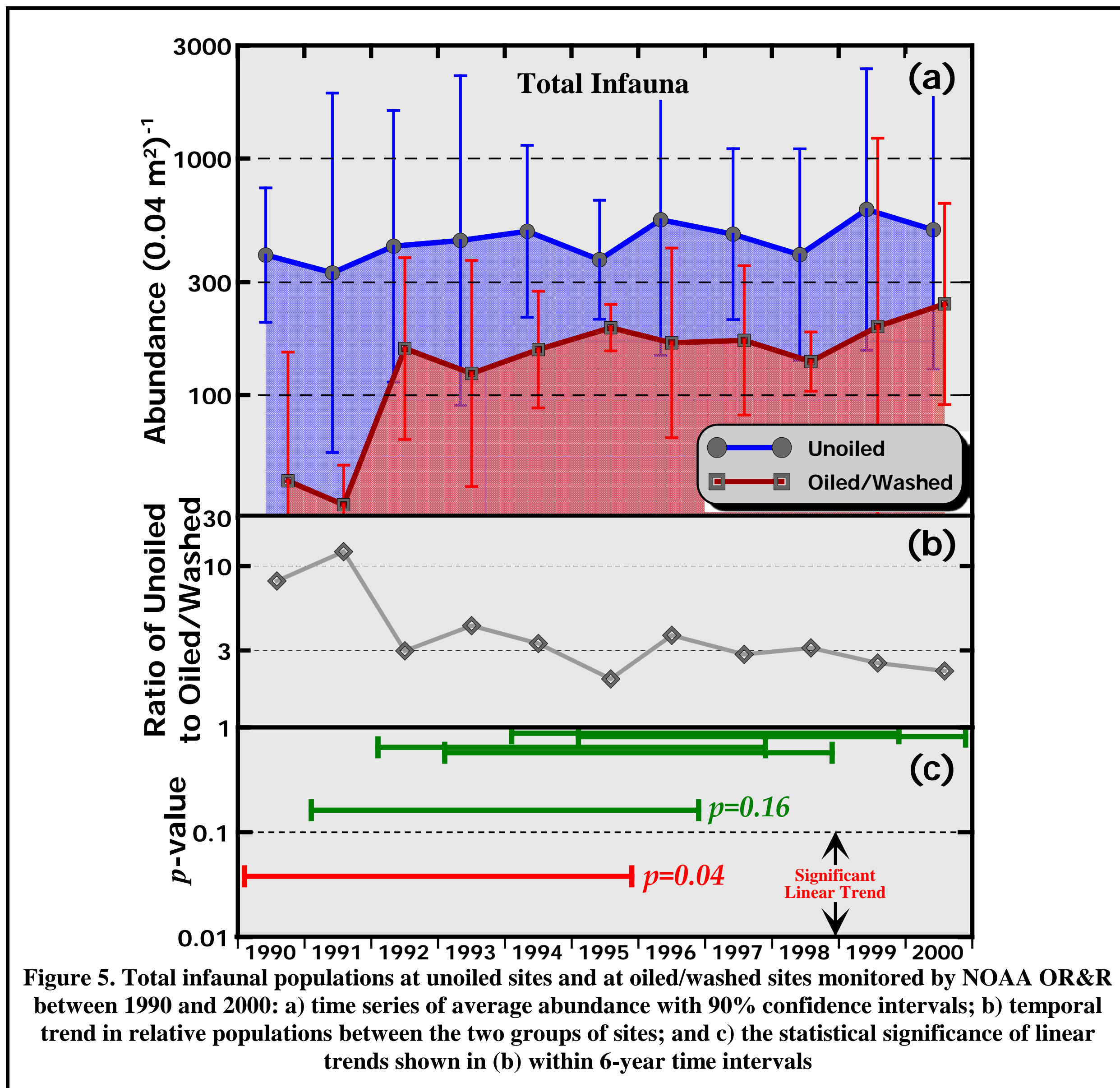

Figure 5. Total infaunal populations at unoiled sites and at oiled/washed sites monitored by NOAA OR&R between 1990 and 2000: a) time series of average abundance with 90% confidence intervals; b) temporal trend in relative populations between the two groups of sites; and c) the statistical significance of linear trends shown in (b) within 6-year time intervals

disturbance over a longer time period. For any given group of nominally similar sites, it is possible that some combination of orientation, circulation, fetch, microclimate, or other external factors would shift abundance, number of species, or any other community property we choose to monitor. Because these differences cannot be randomized, recovery assessments based on direct comparison of measurements at control and impacted sites cannot be statistically supported. Parallelism assumes that while absolute values may not be directly comparable for the purposes of recovery determination, trends over time may be used to determine when communities or conditions at sites are responding to larger environmental influences in similar ways. Thus, we use tests for parallel trends over time as an indicator of one aspect of recovery, namely, stability.

Parallelism tests successfully resolved the abrupt infaunal recolonization of oiled/washed sites that occurred between 1991 and 1992 (Figure 5). This recovery event is evident as a marked increase in the mean abundance at impacted sites, that did not also occur at control sites (Figure 5a), indicating that the change did not occur in response to regional changes in ambient environmental conditions. Also, the abrupt population increase was large enough to generate a significant linear trend (p=0.04; Figure 5c) in relative abundance (Figure 5b) within the first 6-year time window. The second 6-year test window also exhibited a lower p-value (0.16) than subsequent test windows because it encompassed one year (1991) of impacted abundance. In contrast, stabilization of infaunal abundance at oiled/washed sites between 1992 and 2000 is reflected by much higher p-values ($\geq$0.57) that were computed within the four remaining six-year windows.

The temporal trajectory of abundance for most impacted intertidal assemblages was astoundingly similar to that shown in Figure 5a. The repopulation event was evident throughout the intertidal zone at both the oiled sites, and at oiled/washed sites. Widely disparate intertidal assemblages, including infauna, algae, and epifaunal invertebrates, began recolonizing at about the same time (1990-1991) and over a similar duration (one-to-two years). Overall, these data suggest that PWS intertidal populations had experienced a substantial amount of recovery from the effects of the 1989 oil spill and cleanup by 1993. During this recolonization, populations increased, on average, by a factor of eight - an amount that was large enough to be detected by statistical tests for parallelism. For most major intertidal assemblages, there was a statistically significant departure from parallelism within the initial 6-year window, indicating that substantial repopulation had occurred at impacted sites during this time perios. In contrast, time windows that spanned subsequent years showed a high-degree of parallelism indicating that the populations at impacted sites had stabilized, and begun to more-closely track fluctuations in populations at the control sites. These analyses demonstrated that if intertidal populations continued to recover after 1993, that any associated increase in abundance was extremely subtle, and was completely dwarfed by the magnitude of the initial recolonization event.

The abundance trajectory observed for native littleneck clams (Figure 6) markedly departs from that of nearly all other intertidal taxa. Specifically, *L. staminea* populations did not undergo an initial depression in numbers followed by a burst of recruitment at impacted sites a few years after the spill. Littleneck clam populations also did not reflect the initial lack of parallelism with reference sites described above, and shown in Figure 5. In fact, clams at the oiled/washed sites showed a remarkably similar pattern of absolute abundance to clams at unoiled reference sites from the very beginning of the monitoring effort (Figure 6a). It is only when the *relative* abundances are examined (Figure 6b), that a subtle long-term convergence in populations becomes readily apparent. Excluding the abrupt recolonization event that most intertidal taxa underwent from 1990-1993, the results of the statistical tests for parallelism in clam populations (Figure 6c) are opposite those of most other taxa (Figure 5c); namely, parallelism only occurs in the earliest two time windows.

In the case of impacted clam populations, it is probable that the lower clam abundances at oiled/washed sites compared to reference sites were a consequence of the spill. Impacts to *L. staminea* populations at oiled/washed sites were documented by Shigenaka et al. (1999), who concluded that oil and shoreline cleanup activities resulted in adverse impacts that were reflected by abundance levels and hydrocarbon tissue burdens. Although *L. staminea* do not appear to

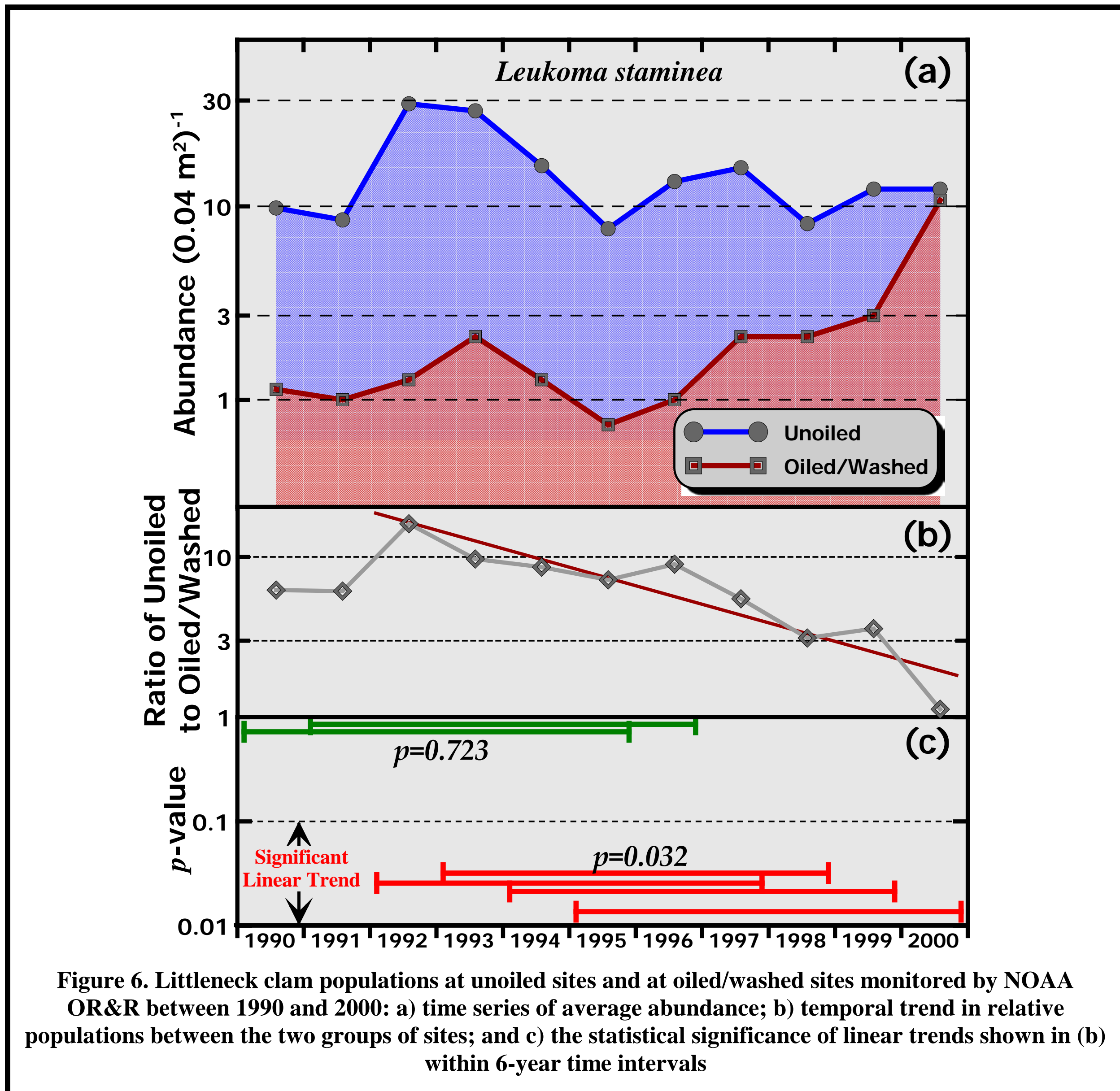

Figure 6. Littleneck clam populations at unoiled sites and at oiled/washed sites monitored by NOAA OR&R between 1990 and 2000: a) time series of average abundance; b) temporal trend in relative populations between the two groups of sites; and c) the statistical significance of linear trends shown in (b) within 6-year time intervals

have experienced the abrupt recolonization event that many other intertidal taxa were subject to, their gradual road to recovery began around the same time (1991-1992). Thereafter, the convergence in clam abundances at unoiled sites and at oiled/washed sites was unexpectedly consistent and steady to the end of the monitoring program in 2000. By 2000, absolute clam abundance levels at impact sites had increased to those of the reference sites, although there was no evidence that they had stabilized ($p \leq 0.032$). It is conceivable that impacted populations continued to increase after 2000 because the sites that happened to be included in the oiled/washed group, provided a more amenable clam environment than the reference sites. The intent of the 2007 sampling was to revisit the PWS clam sites to determine whether the resident littleneck populations at impacted sites had stabilized.

Beyond the population-based parallelism analyses, other NOAA monitoring data suggested that clam populations within PWS were impacted by the spill. For example, tissue burdens showed large declines in aromatic hydrocarbon concentrations between 1990 and 1992. Statistically significant differences between tissue hydrocarbon burdens at oiled sites and unoiled sites remained until at least 1997 (Shigenaka et al., 1999). By 2000, the abundance and chemistry data suggested that littleneck clams were en route to recovery, and it was against this background that the 2007 sampling and analysis took place. Prior to the current project and its field sampling, we hypothesized that *L. staminea* populations would have stabilized at all sites. Instead, a surprisingly steep decline in littleneck clam abundance was apparent at all the PWS monitoring sites, regardless of oiling or cleanup history. This population decline was apparent in both juvenile clams collected from the clam corers (Figure 2, Figure 3), as well as larger, mature clams collected from the excavations (Table 4, Figure 7). Recent reductions in littleneck clam populations have also been noted in other Alaskan regions outside of PWS (Dean, 2008).

The magnitude of the unanticipated decline in littleneck clam abundance that was observed in the 2007 field assessment was particularly apparent at four sites that had previously supported robust populations of clams (Figure 3). These four sites had differing oiling and treatment histories,[6] yet all exhibited an order-of-magnitude lower abundance in 2007. The decline was significant (p<0.05) compared to the spatial variability among the sites at a given time, and compared to interannual (temporal) fluctuations in the mean populations at all four sites prior to 2001.

Table 4. Density (m^{-2}) of *L. staminea* older than one-year within sediments excavated from PWS long-term intertidal monitoring sites during surveys conducted from 1991 through 2007

Category	Site	Year					
		1991	**1992**	**1994**	**1998**	**2000**	**2007**
Unoiled	Bainbridge	N/A[7]	N/A	N/A	N/A	11	0.7
	Crab Bay	N/A	62	69	55	81	N/A
	Outside Bay	94	58	33	74	87	0.0
	Sheep Bay	177	235	338	102	N/A	13.3
Oiled/ Untreated	Block Island	112	420	337	N/A	194	18.7
	Snug Harbor	46	45	58	30	49	2
	Mussel Beach	95	176	263	31	84	10
	Herring Bay	30	15	27	97	13	0.4
Oiled/ Washed	Sleepy Bay	0	2	N/A	N/A	N/A	0.7
	Shelter Bay	10	10	N/A	43	0.0	0.0
	Northwest Bay West Arm	0	10	41	48	159	3.3
Average[8]	**Unoiled**	**98.3**	**80.3**	**85.7**	**74.6**	**42.9**	**1.5**
	Oiled/Untreated	**48.5**	**61.8**	**92.9**	**44.9**	**57.1**	**4.0**
	Oiled/Washed	**0.9**	**5.6**	**26.0**	**41.0**	**8.4**	**0.8**

[6] Outside Bay and Sheep Bay were unoiled reference sites, Block Island and Mussel Beach were documented as oiled.
[7] Not sampled
[8] After conversion by $\log_{10}(x+0.5)$

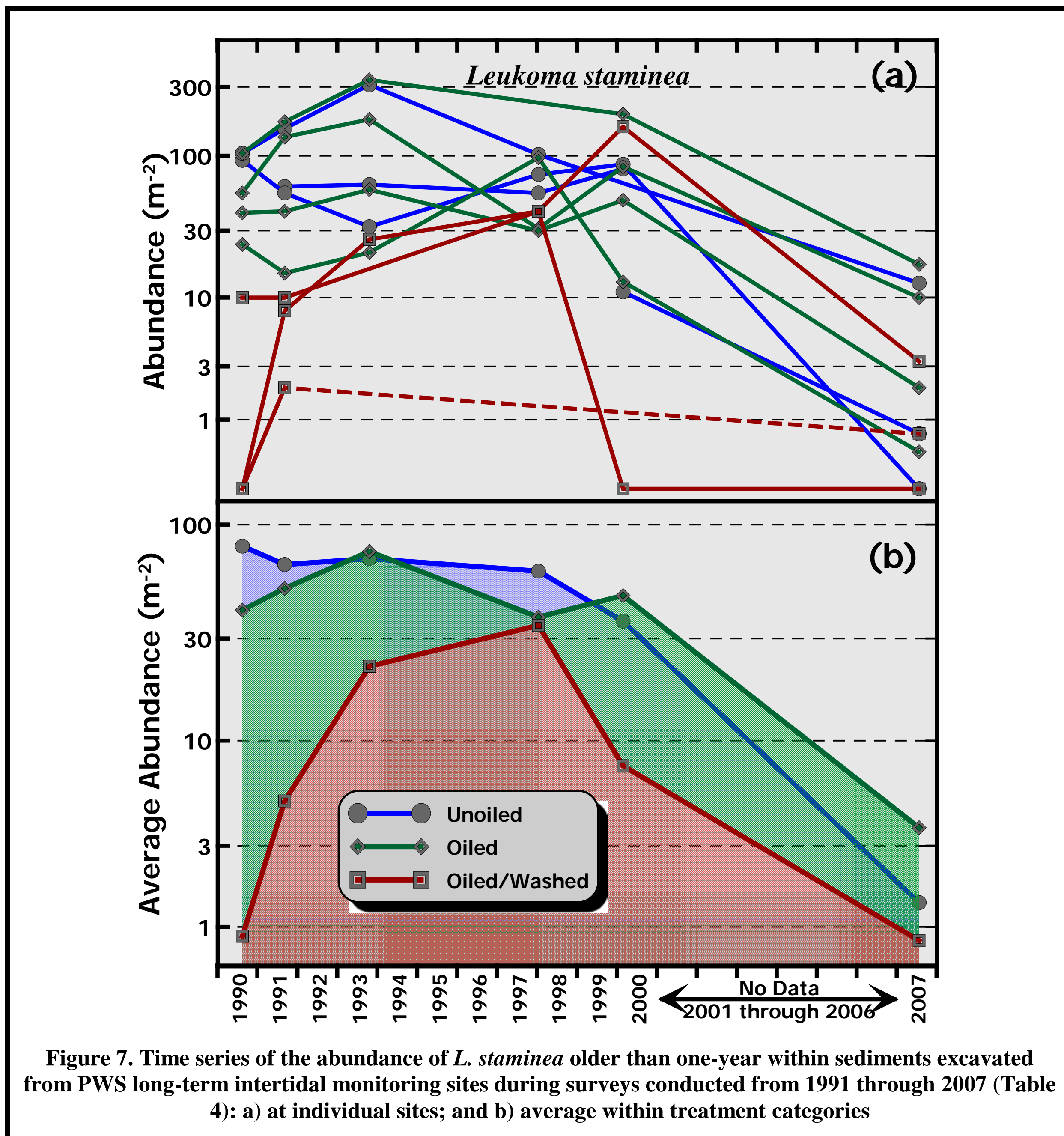

Figure 7. Time series of the abundance of *L. staminea* older than one-year within sediments excavated from PWS long-term intertidal monitoring sites during surveys conducted from 1991 through 2007 (Table 4): a) at individual sites; and b) average within treatment categories

The order-of-magnitude decline in *L. staminea* populations renders one of the original goals of this study moot. Namely, testing the whether the linear trend seen in relative populations at impacted and control sites had continued into 2007; or alternatively, whether the populations stabilized at some point after 2000. In the original 1990-2000 NOAA monitoring effort within PWS, abundance trends for *L. staminea* reflected a linear pattern of recovery in which numbers of clams at oiled/washed sites steadily converged toward numbers of clams at unoiled sites. In 2000, clam abundances were nearly equal at the two site categories. This is portrayed in Figure 6b as convergence of the abundance ratio toward unity.

However, blindly applying parallelism tests to the full dataset leads to the erroneous conclusion that the littleneck clam populations had "stabilized" by 2007. This is based on the lack of evidence (p=0.15) for a linear trend in the time window that contains 2007 data (Figure 2). The unexpected steep decline in littleneck clam abundance observed at all sites sampled in PWS during 2007 does not connote stability. It follows that because stability is reasonably factored into most assessments of recovery, we cannot call native littleneck clam populations "recovered" at the sites we have monitored in PWS.

Under the parallelism recovery metric, trends in impacted and reference populations can become parallel and track one another at different absolute abundance levels. If the parallelism test is applied in a strict and non-contextual way to the available data for clams in PWS, one might incorrectly infer that the populations had "stabilized" sometime within the last time window. That is, the trend in the ratio between unoiled and washed sites no longer exhibits a statistically significant slope (anti-parallel) that had been apparent in all of the time windows between 1992 and 2000. This interpretation is inappropriate though because we cannot realistically characterize a population that has virtually disappeared at all sites to be "stable." If we were to embrace this benchmark, then the dodo (*Raphus cucullatus*) population on Mauritius could just as reasonably be described as "stable."[9] Unfortunately, the near-absence of littleneck clams throughout PWS during 2007 renders spill-recovery assessments for these organisms indeterminate, regardless of whether recovery is assessed through the parallelism metric, or through convergence in absolute abundance at impact and control sites.

This is a manifestation of the "zero-truncation problem" (Beals, 1984) that uniquely plagues the statistical analysis of abundance data. Namely, once a species disappears at one or more sites, we no longer have valid insight into how favorable the environment at that location (e.g., oiled/washed sites in 2007) is for that species relative to other locations (e.g., unoiled sites in 2007). In other words, if the recently observed, widespread decline in the clam population had not occurred, it is conceivable that clam populations could have continued to increase at oiled/washed sites relative to unoiled sites in 2001 and beyond.

This scenario could have occurred because the pre-spill habitat at the impacted sites may have been inherently more favorable, namely, have supported higher clam carrying capacities compared to the unoiled sites. At some point in the future, however, abundance levels would likely have begun to track the unoiled populations, albeit, at a higher average abundance. At this point, the impacted populations would be considered stabilized and no longer recovering in response to spill and cleanup impacts. As stated previously, this departs from the traditional "convergence" measure of recovery, which would have deemed the clam populations recovered in 2000 because the clam abundance at impacted sites had converged ("recovered") to the same absolute levels as the unoiled control sites.

Note that stability in impacted populations does not necessarily indicate that they have fully "recovered" to pre-spill conditions, because the environment at the treated sites may have been permanently damaged. However, without access to pre-spill data we could never know this. For this reason, NOAA began manipulative experiments to investigate whether aggressive oil cleanup methods could, in their own fashion, be responsible for causing long-term damage to

[9] The dodo has been extinct for at least 300 years.

intertidal sedimentary habitats. Preliminary analysis of available data from the ongoing manipulative experiment in Lower Herring Bay suggests that it is in fact possible to produce lasting modifications to the habitat,.and combined with data from the NOAA monitoring program, that these alterations can affect the infaunal community for decades (Shigenaka et al., 2008). Relatively permanent changes to the sedimentary habitat that were caused by invasive hot-water washes could explain the 9-year, post-1991 differences in (stable) total infaunal abundances between unoiled and washed sites (Figure 5), even if pre-spill habitats in the two treatment groups were comparatively similar.

Under the alternative scenario, continued annual sampling beyond 2000 may have found that the impacted and control littleneck clam populations stabilized (became parallel) within the time window beginning in 2000. Had that been the case, the two approaches to recovery assessment, namely stability/parallelism tests and tests for convergence in absolute abundance at impact and control sites, would have had identical outcomes: littleneck clam populations within PWS recovered from the spill in 2000. In the absence of data between 2001 and 2006, this is the conclusion we would have reached if the 2007 sampling showed similar populations at the two categories of sites. This, in fact, was the observed circumstance; however, the populations at all sites were close to zero. As a result, the 2007 data convey little or no information about the relative condition of the habitats at that time, due to the zero truncation problem discussed above.

Our inability to gain further insight into clam recovery using the 2007 data, either with tests for convergence or parallelism, was not a consequence of the fact that the populations declined everywhere; it was because abundances declined so much that there was little useful information left in the remaining data to discern differences in clam habitat within the PWS. Every other conceivable test for clam recovery is rendered moot as well. In the convergence test, for example, we cannot say with confidence that the one data point in 2000 reflects clam populations at the two categories that are truly the same and that absolute abundances had converged. A few more years of data with populations at both categories that were near 2000 levels would have increased our confidence. Even one more data point in 2007 would have helped. In fact, the 2007 data did have similar populations at all three categories, but they obviously were not comparable to the 2000 levels.

Does this mean that there is any less confidence in the convergence of absolute populations in PWS in 2000? No, the widespread decline to near-zero abundance in 2007 simply demonstrates that some regional, or larger scale event made it impossible to discern any Sound-wide differences in habitat, regardless of whether the differences resulted from the spill, or from inherent pre-spill differences between the sites. The amplitude of the littleneck clam decline between 2000 and 2007, which was generally more than an order of magnitude in size, was considerably larger than oil-spill impacts alone, which caused an initial two-fold depression in clam populations after the spill (Coats et al., 1999), and was as at least as large as the impacts incurred from invasive cleanup, where an initial ten-fold depression was observed. Whatever its origin or cause, this event had a greater impact on the native littleneck clam populations within PWS than both the spill and cleanup efforts.

Although the recent decline in littleneck clam abundance may not be linked to the spill, understanding its etiology remains both relevant, and in fact, critical to understanding the process of long-term recovery from the spill. Determining whether conditions at impacted sites are comparable to unimpacted sites becomes far more difficult when conditions at both types of sites

are overwhelmed by the influence of a large-scale driver such as seen with the littleneck clam decline. Continued monitoring no longer yields meaningful results when an adverse, or salutary influence obscures what may be an increasingly faint signal from a disturbance of interest.

If we believe that we may be entering a period of accelerated environmental change globally, then we should expect to encounter an increasing number of situations like the one for littleneck clam populations within PWS; namely, where large-scale shifts in conditions overwhelm our ability to measure those resulting from smaller-scale disturbances, whether they are of natural or human origin. PWS has always represented an especially challenging environment to describe and monitor. From the perspective of the spill, the dynamics of what we consider to be "background" have included recovery from the 1964 earthquake and the major changes to nearshore areas that were wrought from the uplift associated with that event alone; a particularly cold winter that immediately preceded the spill and may have impacted conditions in the intertidal habitats; and range expansion of the sea otter, which affects abundance and distributions of many of the same organisms we monitor for spill impacts. The careful selection and use of reference sites and statistical approaches help to normalize results and isolate impacts of interest, but if the dynamics of the background exceed those of the perturbation of interest, then the monitoring challenge becomes daunting.

In the course of investigating the population dynamics of PWS clams, we have collected both anecdotal and more scientifically rigorous evidence that the spatial scale of the large population decline in native littleneck clams extended well beyond the study area of PWS, and potentially even beyond Alaskan waters. Although our EVOSTC project did not anticipate and was not designed to investigate the decline in *L. staminea* abundances at our study sites in PWS, as the trend became apparent we sought more information on its geographic scope and potential causes. We have proposed studies to formally investigate both of these aspects, and have already garnered compelling information related to geographic scope.

While in the field in 2007, we discussed the apparent decline in littleneck clam abundance with other intertidal researchers, and learned, anecdotally, that it was observed in other areas in Alaska, outside of PWS, such as the Kenai Peninsula (Bodkin and Dean, 2008). This led us to ask: are there other sources of data that might reflect the putative trend we observed? Does the trend reflect declines in other, or perhaps all, infauna? If the trend is confirmed in the coastal areas of PWS and adjacent shorelines, how far does it extend?

Examination of results for other infaunal bivalve taxa from NOAA's monitoring program and the current data did not show similar patterns of a contemporaneous population decline. For example, butter clams (*Saxidomus gigantea*), which frequently co-occur with native littleneck clams on PWS beaches, decreased only slightly between 2000 and 2007. We also observed evidence of successful recruitment in butter clams in the 2007 sampling, which was not the case for littleneck clams.

An independent clam survey was conducted in 2002 by Lees and Driskell (2007) under EVOSTC auspices. This survey spanned a broader swath of the spill-affected area. Data from this survey inidicate that littleneck numbers in 2002 were comparable to prior data collected throughout NOAA's monitoring program (Table 5). Comparison of mean abundances during two periods, 1990-1996 and 2002, showed few significant changes in clam abundances between the two time frames. These data narrow the potential window of occurrence for the clam population decline to sometime between 2002 and 2007.

Table 5. Average density (0.0045 m^2)$^{-1}$ and relative abundance (%) of *L. staminea* and *Saxidomus gigantea* within sediments excavated from PWS long-term intertidal monitoring sites during surveys conducted from 1990 through 1996, and in 2002 (Source: Lees and Driskell, 2007)

Species	Category	Average Abundance		Relative Abundance	
		1990-96	2002	1990-96	2002
Leukoma staminea	Unoiled	16.9 ± 4.7	—	13.2 ± 1.5	—
	Oiled/Untreated	15.0 ± 2.2	11.8 ± 3.5	19.8 ± 1.9	27.4 ± 8.3
	Oiled/Treated	1.4 ± 0.2	7.0 ± 2.8	7.3 ± 1.5	5.3 ± 2.2
Saxidomus gigantea	Unoiled	1.1 ± 0.4	—	1.4 ± 0.5	—
	Oiled/Untreated	1.8 ± 0.4	1.7 ± 0.4	1.7 ± 0.3	4.0 ± 0.9
	Oiled/Treated	0.08 ± 0.06	0.6 ± 0.2	0.4 ± 0.3	0.5 ± 0.1

Analysis of archived infaunal samples from an ongoing NOAA manipulative experiment at the Lower Herring Bay monitoring sites within PWS may permit further refinement as to when littleneck clam populations declined. Although analysis of 2007 and 2008 samples has yet to be completed, data through 2006 indicates that littleneck clam populations at the site were stable through that year, and generally tracked the greater molluscan population, suggesting that the decline in *L. staminea* populations occurred between 2006 and 2007 (Figure 8).

One of the longest-term littleneck-clam monitoring efforts in Alaska has taken place under the auspices of the Alaska Department of Fish and Game (ADF&G). Since 1992, the ADF&G has monitored littleneck-clam stocks in Kachemak Bay, Cook Inlet, where the commercial and non-commercial harvest is concentrated (Gustafson 1995; Gustafson and Bechtol, 2000; Szarzi et al., in preparation). The annual harvest rate in most sampling locations is estimated to be less than 10 percent of the standing stock of legal littleneck clams, namely, those with shell sizes greater than 38.1 mm. ADF&G estimates abundance, biomass, age, and size of legal-sized hardshell clams. The sublegal clam abundance is assumed to be underestimated because smaller clams tend to be overlooked by field samplers.

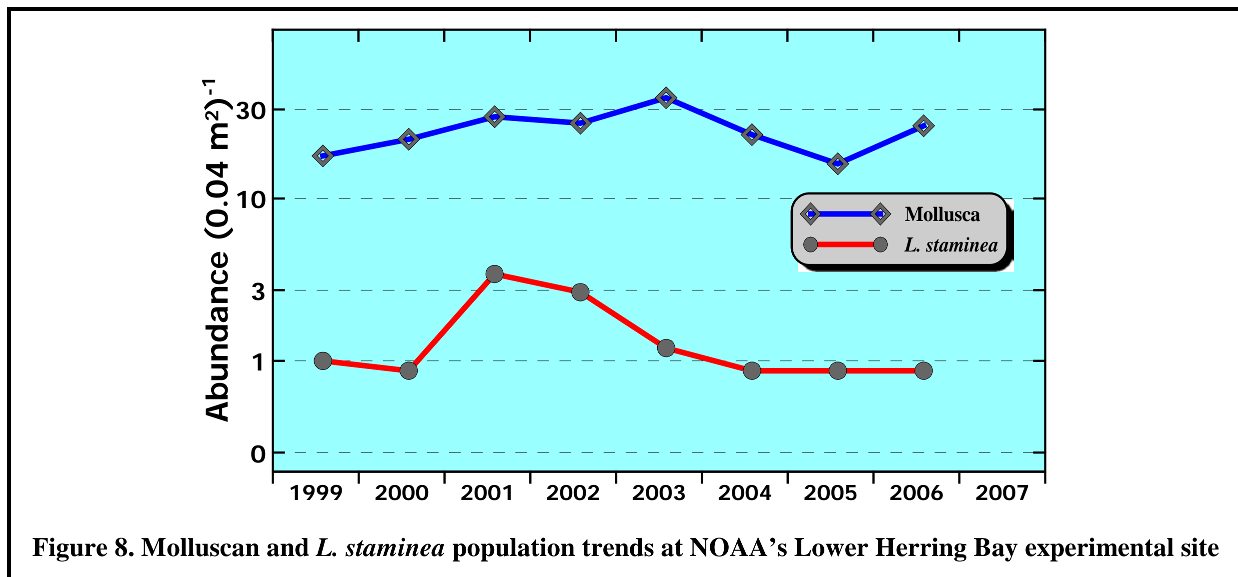

Figure 8. Molluscan and *L. staminea* population trends at NOAA's Lower Herring Bay experimental site

In the ADF&G stock assessments within Kachemak Bay, a three-stage systematic sampling approach was used to estimate abundance. The entire shoreline of each study beach was subdivided into sample sites measuring 200 meters in length and parallel to the shoreline. A selection of sites was randomly chosen within each beach. Transects running perpendicular to the waterline were systematically chosen within the selected sites. Along each transect, 0.25-m^2 quadrats were systematically chosen. The first quadrat was randomly located at a tidal elevation of around 2 m MLLW. The remaining quadrats were placed at every 20 cm to 50 cm change in elevation from the random starting point. Each quadrat was excavated to a depth of 15-20 cm and all littleneck clams were removed and characterized as legal or sublegal-sized. Length composition was estimated from all littleneck clams taken from each study beach, and a subsample of littleneck clams was aged.

The standardized assessment results from the ADF&G surveys in the Kachemak Bay region exhibited declines in littleneck clam abundance at four of the five monitoring sites (Table 6, Figure 9). However, sublegal-sized populations at Chugachik Island exhibited an opposing trend within steadily increasing abundance between 2002 and 2005 (bottom frame of Figure 9b). Moreover, the recent declines observed at most other sites were small compared to uncertainty in the population estimates as represented by the 95% confidence intervals (Table 6, Figure 9). Only the decline in the Grewingk River to Mallard Bay sampling area appears to be adequately resolved (*c.f.*, the confidence intervals and the decline in Figure 9), but that was due to more reliable population estimates (smaller confidence intervals), and not because the decline was larger. Population decreases within Kachemak Bay are thought to have resulted from environmental factors causing direct mortality; however, local depletions exist and may be the result of variable recruitment, environmental factors and/or harvest (Szarzi, 2008).

While abundances of legal-sized clams at locations like China Poot Upper Island, Grewingk River to Mallard Bay, and Jakolof Bay exhibited a consistent factor-of two decline in 2005 and 2006, the amplitude of the population decrease was much smaller than the order-of-magnitude decline observed in PWS by our survey team during the 2007 survey. If the yet-to-be-processed data from 2007 ADF&G Clam Survey in Kachemak Bay exhibit a population decline of the magnitude seen in PWS, the interannual population fluctuations seen in Figure 9 will seem insignificant, and it will add further support for the 2006-2007 time frame of the event. Regardless of the timing and amplitude, the recent population decline that was observed at some sites in Kachemak Bay offers further insight into geographic scope of the littleneck decline.

As additional insight to the geographic scope of the decline, farther to the south, a noticeable decline in native littleneck clam abundance in 2006 was noted by First Nation harvesters in British Columbia, a decline that was subsequently confirmed by Canadian Government fisheries officials (Dunham et al., 2007). In 2006, surveys were conducted in the Broughton Archipelago region of British Columbia, and based on the results of that assessment it was concluded:

> …by comparing past exploratory clam surveys to recent ones done by the authors and Pacificus Biolgical Services, there is evidence to suggest that littleneck clam stocks may have experienced some sort of decline since 1991 at the Burdwood Group, Deep Harbour, Alder Island, Carriden Bay, and Claydon Bay. Since these sites are spread throughout the Broughton Archipelago region, the littleneck clam stock decline might be widespread and not a local phenomenon.

Table 6. Estimated littleneck clam populations (reported as numbers of clams) at selected locations within the Lower Cook Inlet, Alaska. Data source: Szarzi et al (in preparation).

Location	Size	Year	Abundance	Standard Error	95% Confidence Interval Lower	95% Confidence Interval Upper	Relative Precision
China Poot Lower Island	Legal	1999	404,897	113,553	182,334	627,460	55.0
China Poot Lower Island	Legal	2000	437,617	114,305	213,579	661,655	51.2
China Poot Lower Island	Legal	2001	433,868	87,034	263,282	604,454	39.3
China Poot Lower Island	Legal	2002	418,834	143,367	137,835	699,833	67.1
China Poot Lower Island	Legal	2003	428,183	73,711	283,709	572,656	33.7
China Poot Lower Island	Legal	2005	356,810	63,799	231,763	481,856	35.0
China Poot Lower Island	Sublegal	1999	269,517	75,609	121,324	417,710	55.0
China Poot Lower Island	Sublegal	2000	188,458	39,444	111,148	265,768	41.0
China Poot Lower Island	Sublegal	2001	285,322	118,580	52,906	517,739	81.5
China Poot Lower Island	Sublegal	2002	265,093	51,226	164,690	365,495	37.9
China Poot Lower Island	Sublegal	2003	308,576	49,004	212,529	404,624	31.1
China Poot Lower Island	Sublegal	2005	246,319	23,883	199,507	293,130	19.0
China Poot Upper Island	Legal	2000	4,369,020	1,277,268	1,865,575	6,872,464	57.3
China Poot Upper Island	Legal	2002	2,971,239	463,388	2,062,998	3,879,479	30.6
China Poot Upper Island	Legal	2005	2,372,527	280,684	1,822,387	2,922,668	23.2
China Poot Upper Island	Sub-legal	2000	3,902,289	882,250	2,173,080	5,631,499	44.3
China Poot Upper Island	Sub-legal	2002	4,257,567	522,831	3,232,819	5,282,316	24.1
China Poot Upper Island	Sub-legal	2005	3,527,140	324,375	2,891,365	4,162,916	18.0
Grewingk River to Mallard Bay	Legal	2004	371,798	65,883	242,668	500,928	34.7
Grewingk River to Mallard Bay	Legal	2006	188,965	43,493	103,718	274,212	45.1
Grewingk River to Mallard Bay	Sub-legal	2004	1,844,682	252,269	1,350,236	2,339,128	26.8
Grewingk River to Mallard Bay	Sub-legal	2006	911,718	127,989	660,860	1,162,576	27.5
Jakolof	Legal	2003	278,094	84,156	113,150	443,039	59.3
Jakolof	Legal	2005	120,883	32,940	56,321	185,445	53.4
Jakolof	Sub-legal	2003	208,856	117,611	21,662	439,374	110.4
Jakolof	Sub-legal	2005	110,557	41,189	29,657	191,117	73.1
Chugachik Island	Legal	1999	348,467	92,299	167,561	529,374	51.91
Chugachik Island	Legal	2000	194,899	59,389	78,497	311,302	59.72
Chugachik Island	Legal	2001	171,511	56,113	61,530	281,493	64.12
Chugachik Island	Legal	2002	124,910	37,189	52,020	197,800	58.35
Chugachik Island	Legal	2003	225,799	55,679	116,667	334,931	48.33
Chugachik Island	Legal	2004	137,822	39,895	59,627	216,016	56.74
Chugachik Island	Legal	2005	126,883	31,831	64,495	189,271	49.17
Chugachik Island	Legal	2006	245,016	55,767	135,713	354,319	44.61
Chugachik Island	Sublegal	1999	266,795	72,976	123,763	409,828	53.61
Chugachik Island	Sublegal	2000	144,782	40,083	66,219	223,346	54.26
Chugachik Island	Sublegal	2001	205,813	41,035	125,385	286,242	39.08
Chugachik Island	Sublegal	2002	144,127	42,397	61,029	227,226	57.66
Chugachik Island	Sublegal	2003	216,191	42,658	132,581	299,800	38.67
Chugachik Island	Sublegal	2004	296,061	58,343	181,709	410,413	38.62
Chugachik Island	Sublegal	2005	415,652	69,503	279,426	551,878	32.77
Chugachik Island	Sublegal	2006	349,278	54,632	242,199	456,357	30.66

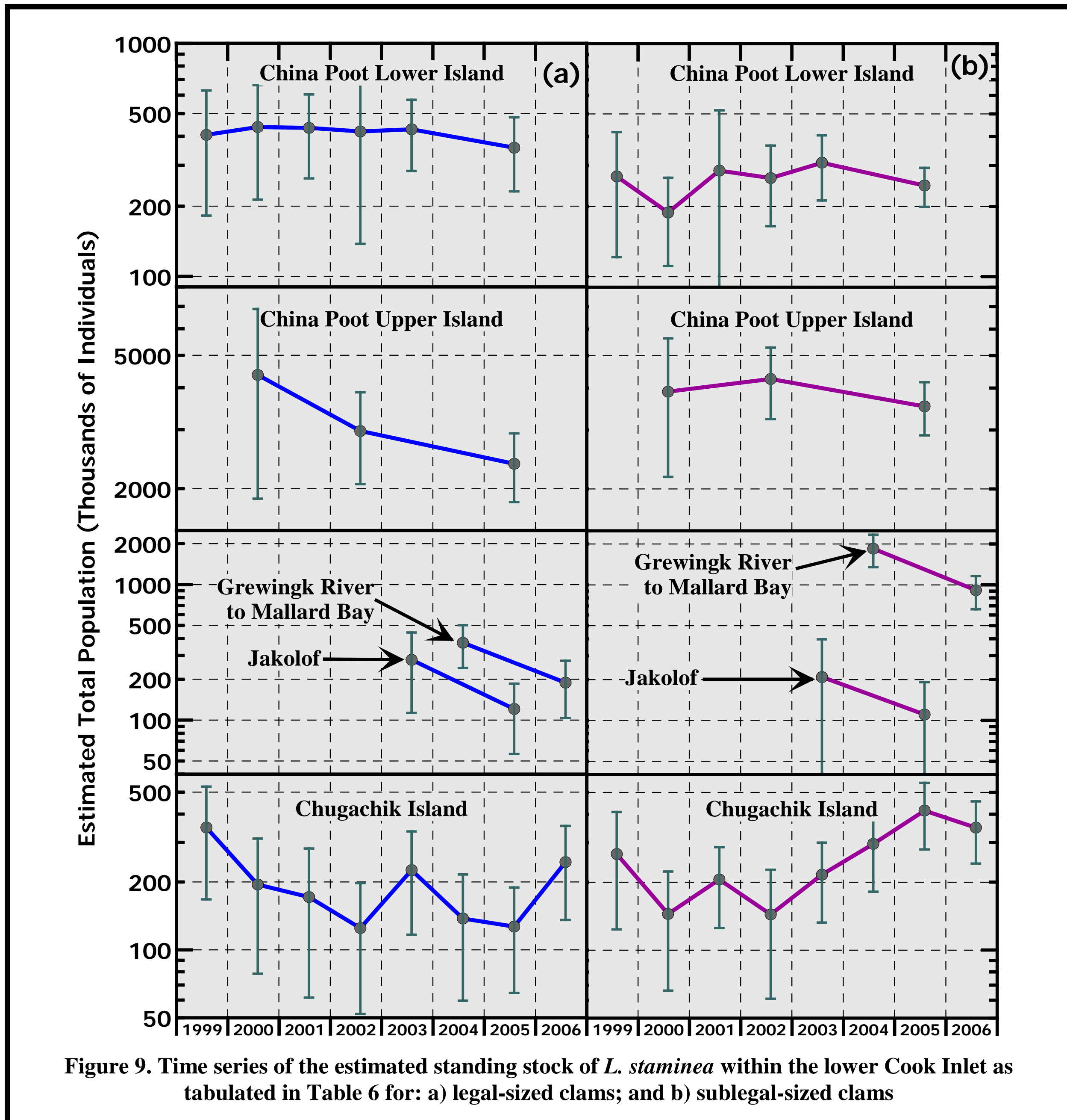

Figure 9. Time series of the estimated standing stock of *L. staminea* within the lower Cook Inlet as tabulated in Table 6 for: a) legal-sized clams; and b) sublegal-sized clams

> …Our results support reports that littleneck clam populations are low. Most of the littleneck clams examined were healthy, so a disease outbreak is not likely the reason why stocks are depressed. More focused research is needed on the impacts of harvesting and other human activities on littleneck clam populations.

Even farther to the south, shellfish resource managers in Washington State routinely monitor the status of clam stocks, particularly in areas popular with recreational and tribal harvesters. Natural

stock harvest rates are estimated using previously established rates. For Manila littleneck clams (*Tapes philippinarum*), the harvest rate was 33 percent by weight for legal-sized clams (≥38 mm); for legal-sized native littleneck clams, the harvest rate was 25 percent by weight.

In addition to estimates of native and Manila littleneck clam populations, estimates for butter clams (*Saxidomus gigantea*), cockles (*Clinocardium nuttallii*), horse clams (*Tresus* sp.), and varnish clams (*Nuttallia obscurata*) were included. However, the survey methodology was designed to estimate native littleneck and Manila abundance, and as a result, estimates for these other species are likely to be less precise.

Surveys by the Washington Department of Fish and Wildlife (WDF&W) documented recent downward trends in native littleneck clam stocks that were monitored in Puget Sound (Whitney, 2008). In northern Puget Sound these trends were summarized in an October 2007 communication from WDF&W to tribal fishery managers:

> Native littleneck populations continue to decline and butter clam abundance continues to increase on many beaches in the Port Susan and Saratoga Pass areas. Two of the sharpest declines of native littleneck abundance are on beaches that have been closed to recreational harvest since 2001, Camano Island State Park and Kayak Point County Park.

The recent decline in native littleneck clam populations at Camano Island State Park in northern Puget Sound is particularly noteworthy when compared against population trends in other bivalves (Figure 10). The shellfish manager for northern Puget Sound noted in 2007:

> Camano Island State Park has been closed to recreational harvest since a 30-day season, June 1-30 in 2002. Since the closure, native littlenecks have continued to decline while butter clams have increased dramatically. During the 2002 season an estimated 7,965 harvesters took a total of 9,032 pounds of native littlenecks, 37,881 pounds of butter clams, 1,146 pounds of cockles, and 2,563 pounds of horse clams.
>
> On the 2007 survey we found only 27 native littlenecks in the 209 samples dug. In those same 209 samples we found 785 butter clams, 31 cockles, and 128 horse clams. We did find evidence of native littleneck recruitment; five of the 27 natives found were less than 20 mm.

A steady decline in the native littleneck clam populations at Camano Island State Park began after 1999 (Figure 10). By 2007, only 2.6% of the 1999 population remained, and biomass was only 4.2% of the biomass that the site had supported for most of the seven years prior to 2000. During the same period, butter clam abundance increased by 45% and biomass increased by 73%. The order-of-magnitude decline in littleneck clam populations at Camano Island State Park between 2000 and 2007 was comparable to that observed in PWS. However, the decline spanned a seven-year period whereas the PWS decline appears to have been more abrupt.

Although littleneck clams have been reported to range as far south as Baja California, population assessments in Oregon and California are anecdotal in nature. There are scattered reports of declines or disappearance of littleneck clams from areas in Oregon and California where they had been historically harvested, but it is unclear whether these occurrences are attributable to overharvesting, gross changes in beach substrate type caused by shifts in river bed, or other causes.

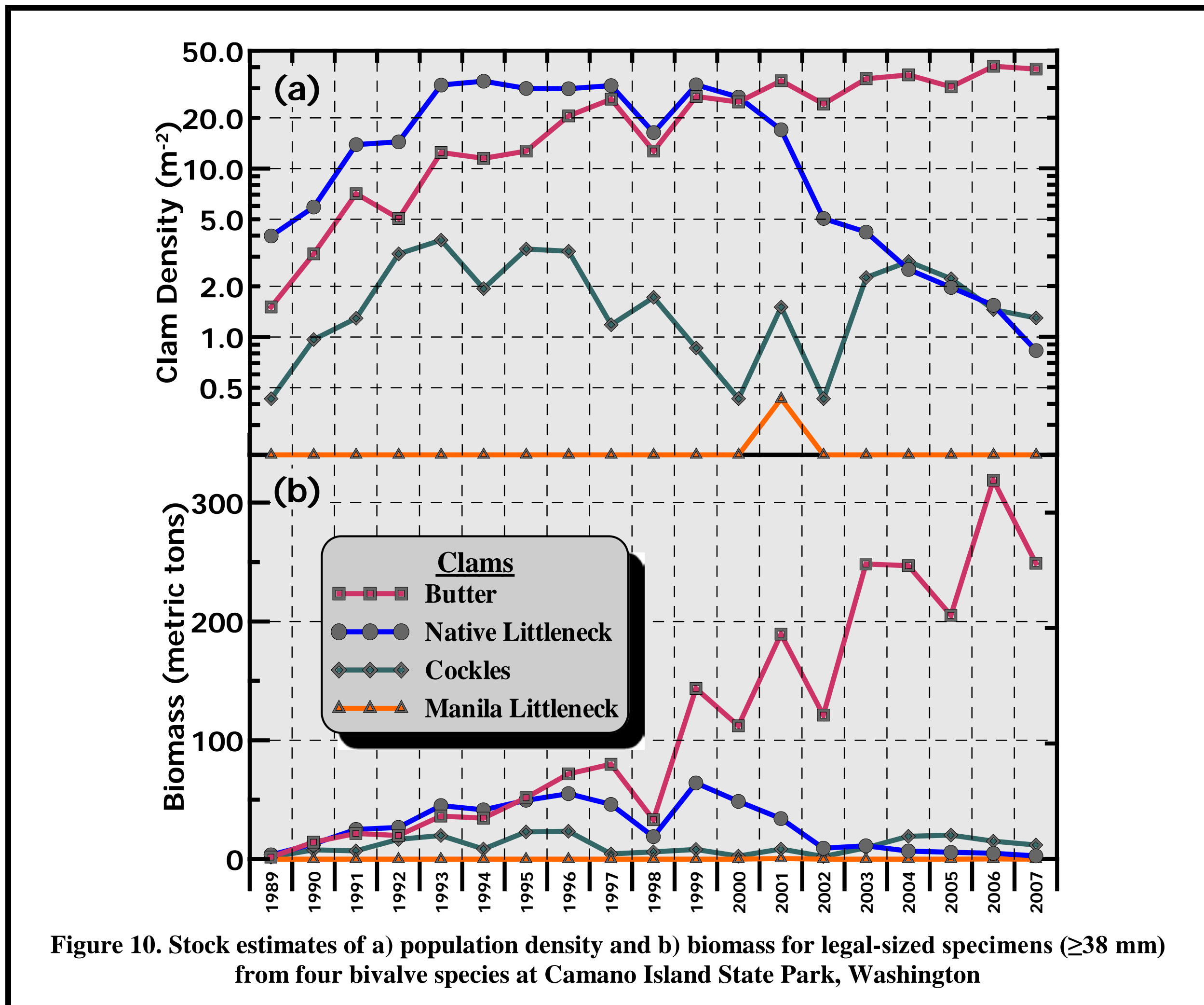

Figure 10. Stock estimates of a) population density and b) biomass for legal-sized specimens (≥38 mm) from four bivalve species at Camano Island State Park, Washington

Conclusions

In the years since the *Exxon Valdez* oil spill, it has become evident that a broad array of changes may be affecting many different parts of the Alaskan marine ecosystem. Causes for these changes are sometimes apparent, such as the role of global warming in the recent listing of polar bears as a threatened species under the Endangered Species Act. In other cases, such as the crash in Steller sea lion populations, the causal or contributing factors have proved to be far more complex and difficult to identify.

In our original study plan for this EVOSTC project, we obviously focused on one cause for potential population differences at the littleneck clam study sites in PWS: the *Exxon Valdez* oil spill. Monitoring and assessment results from other parts of Alaska, the coast of British Columbia, and Puget Sound show trends for native littleneck clams that are similar to those we found in PWS. Sometime after 2000, littleneck clam stocks declined appreciably relative to abundance levels observed in the immediately preceding decades. The geographic scope of this occurrence suggests some as yet unidentified, large-scale cause, strengthening the notion that the conditions observed in PWS during our 2007 survey were unrelated to the spill.

Potential factors that may have been involved in the apparent decline in littleneck clam populations include: predation, interannual fluctuations in sea-surface temperature, disease, contaminants, and ocean acidification. Based on 1) recent well-publicized developments in the commercial shellfish industry along the Pacific coast, and 2) ongoing research recently described at a workshop at the Friday Harbor Laboratories of the University of Washington, we believe that there are two leading candidates for the cause of the decline: disease and ocean acidification. A bacteria, *Vibrio tubiashii,* has been identified as a pathogen significantly affecting oyster larvae, while ocean acidification could affect the viability of earlier life stages of bivalves. However, the definitive cause of the decline can only be addressed with a more directed research effort consisting of several components. Resource agencies overseeing the affected areas, as well as entities with broader geographic purview, such as the North Pacific Research Board, could materially contribute to the various study components.

Our objectives for this study were to provide scientific information concerning the recovery status of native littleneck clams and the biological availability of lingering oil within PWS. This information was to be used by the EVOSTC in its determination of the recovery status of this important intertidal resource, and the need for restoration or remediation of injured littleneck clam populations.

We successfully completed field sampling for the project in July 2007. During the field survey, however, it became apparent that numbers of clams found at the designated survey sites were far lower than had been encountered during previous cycles of annual monitoring performed from 1990 to 2000. This resulted in an unexpected situation where quantitative metrics for evaluating population recovery were rendered untenable because the 2007 populations were too small to provide meaningful insight into differences in clam habitat between the impacted and control sites. For example, blindly applying parallelism tests for determining the stability in relative populations indicates that littleneck clam populations at impacted sites stabilized sometime between 2000 and 2007, after a decade-long period of steady recolonization of oiled and washed beaches. In reality, however, the population status in 2007 simply reflected the influence of a wider-spread impact that reduced abundances at all sites regardless of oiling and treatment history.

This universality not only confounded the recovery metrics, but also suggested that the steep decline in clam abundance was not attributable to the spill. This conclusion was supported by the absence of notable tissue or sediment hydrocarbon concentrations from clams and sites sampled, particularly at previously oiled sites. The subsequent finding that littleneck clam numbers had similarly declined at other distant locations along the Pacific coast provides another strong piece of supporting evidence that a cause unrelated to the spill is currently affecting *L. staminea*.

Although the 2007 data showed similar littleneck clam population levels at sampling sites within all three oiling/treatment categories, the populations encountered were so minimal as to be of little use in drawing valid conclusions about the clam's recovery status. This unanticipated and dramatic decline is a critical aspect of the data that is not incorporated into recovery metrics that are based on trends in relative populations (temporal stability), or convergence in abundance at impacted and reference sites. While absolute abundances at unoiled and oiled/washed sites appear to have converged in 2000, the years leading up to and including that convergence point were characterized by statistically significant trends in relative populations. Consequently,

population data from subsequent years would be needed to evaluate whether impacted and control populations had indeed converged and stabilized in 2000.

The 2007 field survey did not provide that insight and instead identified a widespread decline to near-zero abundance caused by some regional or larger-scale event. This event made it impossible to discern any spill-related influences that were previously apparent as Sound-wide differences in population levels at impacted and reference sites. Moreover, the widespread decline in clam populations between 2000 and 2007 was as at least as large as the ten-fold population decrease that resulted from the use of invasive spill cleanup methods, such as high-pressure hot-water washing of beaches. The recent widespread population decline was considerably larger than the two-fold initial difference in populations that was identified at sites impacted by oiling alone in the NOAA monitoring program. Whatever its origin or cause, this event has had a far greater impact on the native littleneck clam populations within PWS than the spill.

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Literature Cited

Anderson, J.W., J.M. Augenfeld, E.A. Crecelius, and R.G. Riley. 1979. Research to determine the accumulation of organic constituents and heavy metals from petroleum-impacted sediments by marine detritivores of the Alaskan outer continental shelf. In: Environmental assessment of the Alaskan continental shelf, annual reports of principal investigators for the year ending March 1979. Boulder, CO: NOAA/ERL, Outer Continental Shelf Environmental Assessment Program, pp. 172-234.

Augenfeld, J.M., J.W. Anderson, D.L. Woodruff, and J.L. Webster. 1980. Effects of Prudhoe Bay crude oil-contaminated sediments on *Protothaca staminea* (Mollusca: Pelecypoda): Hydrocarbon content, condition index, free amino acid level. Marine Environ. Res. 4:135-143.

Beals, E.W. 1984. Bray-Curtis ordination: an effective strategy for analysis of multivariate ecological data. Advances in Ecological Research 14:1-55.

Bodkin, J.L., B.E. Ballachey, G.G. Esslinger, K.A. Kloecker, D.H.Monson, and H.A. Coletti. 2007. Perspectives on an invading predator—sea otters in Glacier Bay. *in* J.F. Piatt and S.M. Gende, eds., Proceedings of the Fourth Glacier Bay Science Symposium, October 26–28, 2004: U.S. Geological Survey Scientific Investigations Report 2007-5047, p. 133-136.

Bodkin, J.L. and T.A. Dean. 2008. Personal communication with G. Shigenaka on 11 July 2007 during field sampling within Prince William Sound, Alaska.

Boufadel , M. Venosa, A., Wrenn, B. 2007. Factors Responsible for Limiting the Degradation Rate of Exxon Valdez Oil in Prince William Sound Beaches. EVOSTC Project 070836.

Bourne, N. 1983. Clam predation by scoter ducks in the Strait of Georgia, British Columbia. Journal of Shellfish Research 3(1):84.

Bower, S.M. 2007. Synopsis of infectious diseases and parasites of commercially exploited shellfish: *Perkinsus* of clams and cockles. URL: http://www-sci.pac.dfompo.gc.ca/shelldis/pages/perkincc_e.htm

Burn, D.M. 2005. Status of sea otter stocks in Alaska. Presentation at Sea Otter Oil Spill Response Training Program, 12 March 2005, Vancouver BC.

Burreson, E.M., N.A. Stokes, and C.S. Friedman. 2000. Increased virulence in an introduced pathogen: *Haplosporidium nelsoni* (MSX) in the eastern oyster *Crassostrea virginica*. Journal of Aquatic Animal Health 12(1):1-8.

Cheney, D.P. and T.F. Mumford. 1986. Shellfish and Seaweed Harvests of Puget Sound. Seattle, WA: University of Washington Press, 164 pp.

Chew, K.K. and A.P. Ma. 1987. Species profiles: Life histories and environmental requirements of coastal fishes and invertebrates (Pacific Northwest)—common littleneck clam. U.S. Fish and Wildlife Service Biological Report 82(11.78). U.S. Army Corps of Engineers, TR EL-82-4. 22 pp.

Coats, D.A., E. Imamura, A.K. Fukuyama, J.R. Skalski, S. Kimura, and J. Steinbeck. 1999. Monitoring of biological recovery of Prince William Sound intertidal sites impacted by the *Exxon Valdez* oil spill—1997 biological monitoring survey. NOAA Technical Memorandum NOS OR&R 1. 73 pp. + appendices.

Coats, D.A., E. Imamura, A.K. Fukuyama, J.R. Skalski, S. Kimura, and J. Steinbeck. 2003. Sampling effort in assessments of oil-spill impacts to intertidal organisms. NOAA Technical Memorandum NOS OR&R 12.

Dean, T.A., J.L. Bodkin, H. A. Coletti, and K.A. Kloecker. 2008. Nearshore Data Management and Monitoring. Draft final report for EVOSTC Project 060750. Report to the Exxon Valdez Oil Spill Trustee Council, Anchorage.

Dean, T.A. 2008. Personal communication with G. Shigenaka on 12 May 2008.

Department of Fish & Oceans (Canada). 1999. Native littleneck clam. DFO Science Stock Status Report C6-02 (1999), 4 pp.

Desser, S.S. and S.M. Bower. 1997. The distribution, prevalence, and morphologlical features of the cystic stage of an apicomplexan parasite of native littleneck clams (*Protothaca staminea*) in British Columbia. J. Parasitol. 83(4):642-646.

Downs, C.A., G. Shigenaka, J.E. Fauth, C.E. Robinson, and A. Huang. 2002. Cellular-physiological assessment of bivalves after chronic exposure to spilled *Exxon Valdez* crude oil using a novel molecular diagnostic biotechnology. Environ. Sci. Technol. 36: 2987-2993.

Downs, C.A. 2005. Cellular diagnostics and its application to aquatic and marine toxicology. In: Techniques in Aquatic Toxicology, vol. 2: G. Ostrander (ed.). CRC Press, Inc. Boca Raton, Florida.

Dunham, J.S., B. Koke, G.E. Gillespie, and G. Meyer. 2007. An exploratory survey for littleneck clams (Protothaca staminea) in the Broughton Archipelago, British Columbia—2006. Canadian Manuscript Report of Fisheries and Aquatic Sciences 2787.: Fisheries and Oceans Canada, Science Branch, Pacific Region, Nanaimo, B.C. 33 pp.

Elston, R.A., C.F. Dungan, T.R. Meyers, and K.S. Reece. 2003. *Perkinsus* sp. infection risk for Manila clams, *Venerupis philippinarum* (A. Adams and Reeve, 1850) on the Pacific coast of North and Central America. Journal of Shellfisheries Research 22(3):661-665.

Flye-Sainte-Marie, J., S. Pouvreau, C. Paillard, and F. Jean. 2007. Impact of brown ring disease on the energy budget of the Manila clam *Ruditapes philippinarum*. Journal of Experimental Marine Biology and Ecology 349(2):378-389.

Fukuyama A.K., G. Shigenaka, and R.Z. Hoff. 2000. Effects of residual *Exxon Valdez* oil on intertidal *Protothaca staminea*: mortality, growth, and bioaccumulation of hydrocarbons in transplanted clams. Mar Poll Bull 40:1042-1050.

Gustafson R. 1995. Kachemak Bay littleneck clam assessments, 1990-1994. Alaska Department of Fish and Game, Commercial Fisheries Management and Development, Regional Information Report No. 2A95-19, Anchorage.

Gustafson, R. and W. Bechtol. 2000. Kachemak Bay littleneck clam assessments, 1996-1997. Alaska Department of Fish and Game, Division of Commercial Fisheries, Regional Information Report No. 2A01-09, Anchorage.

Hada, H.S., P.A. West, J.V. Lee, J. Stemmler, and R.R. Colwell. 1984. *Vibrio tubiashii* sp. nov., a pathogen of bivalve mollusks. International Journal of Systematic Bateriology 34:1-4.

Haugan, P.M. 2004. Possible effects of ocean acidification. Reports in Meteorology and Oceanography, University of Bergen, Report No. 2-2004, 10 pp.

Houghton, J.P., R.H. Gilmour, D.C. Lees, P.J. Hague, W.B. Driskell, and S.C. Lindstrom. 1996. Evaluation of the condition of Prince William Sound shorelines following the *Exxon Valdez* oil spill and subsequent shoreline treatment. Volume I: 1994 biological monitoring survey. NOAA Technical Memorandum NOS ORCA 91, 145 pp. + appendices.

Lees, D.C. and W.B. Driskell. 2007. Assessment of bivalve recovery on treated mixed-soft beaches in Prince William Sound. Final Report, Project 040574, *Exxon Valdez* Oil Spill Trustee Council, 120 pp.

Light, S.F. and J.T. Carlton (ed.). 2007. The Light and Smith Manual: Intertidal Invertebrates from Central California to Oregon. Berkeley: University of California Press. 1001 pp.

Marshall, W.L., S.M. Bower, and G.R. Meyer. 2003. A comparison of the parasite and symbiont fauna of cohabiting native (*Protothaca staminea*) and introduced (*Venerupis philippinarum* and *Nuttallia obscurata*) clams in British Columbia. Journal of Shellfish Research 22(1):185-192.

National Marine Fisheries Service (NMFS). 1997. Fisheries of the United States, 1996. Website address http://remora.ssp.nmfs.gov/fus/fus96/index.html. Current Fishery Statistics No. 9600.

Orr, J.C., V.J. Fabry, O. Aumont, L. Bopp, S.C. Doney, R.A. Feely, A. Gnanadesikan, N. Gruber, A. Ishida, F. Joos, R.M. Key, K. Lindsay, E. Maier-Reimer, R. Matear, P. Monfray,

A. Mouchet, R.G. Najjar, G-K Plattner, K.B. Rodgers, C.L. Sabine, J.L. Sarmiento, R. Schlitzer, R.D. Slater, I.J. Totterdell, M-F Weirig, Y. Yamanaka, and A. Yool. 2005. Anthropogenic ocean acidification over the twenty-first century and its impact on calcifying organisms. Nature 437:681-686.

Park, K-I, T.T.T. Ngo, S-D Choi, M. Cho, and K-S Choi. 2006. Occurrence *of Perkinsus olseni* in the Venus clam *Protothaca jedoensis* in Korean waters. Journal of Invertebrate Pathology 93(2):81-87.

Pearson, W.H., D.L. Woodruff, P.C. Sugarman, and B.L. Olla. 1981. Effects of oiled sediment on predation on the littleneck clam, *Protothaca staminea*, by the Dungeness crab, *Cancer magister*. Estuarine, Coastal and Shelf Science 13:445-454.

Peterson C.H. 2001. The "*Exxon Valdez*" oil spill in Alaska: acute, indirect and chronic effects on the ecosystem. Adv Mar Biol 39:1-103.

Peterson C.H., S.D. Rice, J.W. Short, D. Esler, J.L. Bodkin, B.E. Ballachey, and D.B. Irons. 2003. Long-term ecosystem response to the *Exxon Valdez* oil spill. Science 302:2082-2086.

Roesijadi, G. 1980. Influence of copper on the clam *Protothaca staminea*: Effects on gills and occurrence of copper-binding proteins. Biol. Bull. 158(2):233-247.

Roberts, P.O., C.B. Henry Jr., G. Shigenaka, and A.K. Fukuyama. 1999. Weathered petroleum "bioavailability" to intertidal bivalve species after the T/V *Exxon Valdez* incident. 1999 Oil Spill Conference Proc., Seattle, WA. pp. 1002-1005.

Schink, T.D., K.A. McGraw, and K.K. Chew. 1983. Pacific Coast Clam Fisheries. Washington Sea Grant Technical Report HG-30, University of Washington, Seattle, Washington.

Shigenaka G., Coats D.A., Fukuyama AK, and P.O. Roberts. 1999. Effects and trends in littleneck clams (*Protothaca staminea*) impacted by the *Exxon Valdez* oil spill. Paper ID #326, Proc of the 1999 International Oil Spill Conference, Seattle, American Petroleum Institute, Washington DC, pp 349-356.

Shigenaka, G., Coats, D.A., and A.K. Fukuyama. 2008. Population Recovery Status of Littleneck Clams (*Leukoma staminea*) in Prince William Sound: An Unexpected Turn of Events. Alaska Marine Symposium, January 20-23, 2008. Hotel Captain Cook, Anchorage, Alaska. Book of Abstracts for Oral Presentations and Posters.

An Unexpected Turn of EventsEffects and trends in littleneck clams (*Protothaca staminea*) impacted by the *Exxon Valdez* oil spill. Paper ID #326, Proc of the 1999 International Oil Spill Conference, Seattle, American Petroleum Institute, Washington DC, p 349-356

Skalski, J.R. and D.S. Robson. 1992. Techniques for Wildlife Investigations. Academic Press. San Diego, CA. 237 pp.

Skalski, J.R., D.A. Coats, and A.K. Fukuyama. 2001. Criteria for Oil Spill Recovery: A Case Study of the Intertidal Community of Prince William Sound, Alaska, following the Exxon Valdez Oil Spill. Environmental Management, Vol. 28, No. 1, pp 9-18.

Stratton, L. and E.B. Chisum. 1986. Resource use patterns in Chenega, western Prince William Sound: Chenega in the 1960s and Chenega Bay 1984-1986. Technical Paper No. 139. Anchorage: Alaska Department of Fish and Game, Division of Subsistence, 161 pp.

Szarzi, N. J. 2008. Personal Communication with G. Shigenaka.

Szarzi, N. J., R. L. Gustafson and P. A. Hansen. In preparation. Hardshell clam population characteristics at selected locations in Kachemak Bay 1999-2006. Alaska Department of Fish and Game, Fishery Data Series, Anchorage.

Szarzi, N. J., R. L. Gustafson and P. A. Hansen. In preparation. Hardshell clam population characteristics at selected locations in Kachemak Bay 1999-2006. Alaska Department of Fish and Game, Fishery Data Series, Anchorage.

Takesue, R.K. and A. van Geen. 2004. Mg/Ca, Sr/Ca, and stable isotopes in modern and Holocene Protothaca staminea sheels from a northern Califronia coastal upwelling region. Geochimica et Cosmochimica Acta 68(19):3845-3861.

Tubiash, H.S., P.E. Chanley, and E. Leifson. 1965. Bacillary necrosis, a disease of larval and juvenile bivalve mollusks. I. Etiology and epizootiology. J. Bacteriol. 90:1036-1044.

Wang, V. 2008. "Oyster larvae dying off at alarming rates." Radio feature produced for KUOW Seattle, aired on 18 August 2008. http://kuow.org/program.php?id=15600.

Weinberg, J.R. 2005. Bathymetric shift in the distribution of Atlantic surfclams: response to warmer ocean temperature. ICES Journal of Marine Science 62:1444-1453.

Weiss, K.R. 2008. A warning from the sea. Los Angeles Times, 13 July 2008. http://www.latimes.com/news/science/environment/la-me-oysters13-2008jul13,0,4701553.story.

Whitney, J. 2008. Personal communication with G. Sheginaka on 2 May 2008.

Acronyms and Abbreviations

ADF&G	Alaska Department of Fish and Game
ANOVA	analysis of variance
BACI	before-after-control-impacted assessment
COC	chain of custody
DCM	dichloromethane
DES	Department of Environmental Studies (Louisiana State University)
EPA	United States Environmental Protection Agency
EVOSTC	*Exxon Valdez* Oil Spill Trustee Council
FID	flame ionization detector
GC/MS	gas chromatography/mass spectrometry
LSU	Louisiana State University
MDL	method detection limit
MLLW	mean lower low water
MRS	Marine Research Specialists (Ventura, CA)
NMFS	National Marine Fisheries Service (NOAA)
NOAA	National Oceanic and Atmospheric Administration
NRDA	natural resource damage assessment
OR&R	Office of Response & Restoration (NOAA)
PAH	polynuclear aromatic hydrocarbon
PWS	Prince William Sound
QA/QC	quality assurance/quality control
SHC	saturated hydrocarbons (alkanes)
SIM	selected ion monitoring
SOP	standard operating procedure
SRM	standard reference material
TOC	total organic carbon
TPAH	total polynuclear aromatic hydrocarbons
TSA	transportation security administration
TTAH	total target aromatic hydrocarbons
WDF&W	Washington Department of Fish and Wildlife

Appendix A. Potential Causes for the Widespread Decline in Littleneck Clam Populations

This appendix explores some factors that may have been involved in the large-scale decline of littleneck clam populations. They include: predation, interannual fluctuations in sea-surface temperature, disease, contaminants, and ocean acidification.

Predation

Leukoma staminea are a preferred prey item for vertebrate and invertebrate predators in PWS and elsewhere along its Pacific range. Chew and Ma (1987) recited a lengthy list of consumers, including moon snails, other gastropods, sea stars, crabs, octopi, and fishes, The latter nip off clam siphons protruding above the substrate. Scoters are major avian consumers of littleneck clams in British Columbia. Bourne (1983) estimated that 200 wintering scoters could forage as much as 14.5 metric tons of clams from two beaches during a six-month stay.

Sea otters (*Enhydra lutris*) similarly designated as major consumer of clams in PWS. With their prodigious food intake, sea otters have the potential to rapidly change the population of prey items in a localized area, or over an entire ecosystem. Bodkin et al. (2007) studied the impact of sea otters as an invading predator within Glacier Bay, Alaska, an area where otters sustained an annual population growth rate of 50% between 1998 and 2004. During the same period, otter populations within Southeast Alaska only grew by 3% each year. Prior to the invasion, the density and size of intertidal clams, including littleneck clams, within Glacier Bay was approximately two- and three-times larger, respectively, than clams in areas that had supported sea otter populations for longer periods, up to twenty years. Identified impacts from the sea otter's range expansion include declines in the abundance and size of benthic invertebrates, and increases in the diversity and complexity of nearshore ecosystems.

The trend of sea otter abundance in Prince William Sound, however, does not reflect a significant increase in range or numbers. The population in western PWS showed an initial modest 4% increase between 1993 and 2000, but has since leveled off at around 2,600 animals. For all of the southcentral Alaskan stock, which includes PWS, the total number of otter has remained relatively stable over the last decade, ranging between 14,000 and 15,000 animals (Burn, 2005).

Interestingly, recent surveys in Alaskan waters by EVOSTC researchers (Dean et al., 2008) have suggested a shift in the favored components of sea otter diets from clams to mussels. The reader is referred to project reports from PJ 070750 for more detailed data analysis of this development.

It is possible that another one or several of the many known littleneck clam predators in PWS has increased in numbers to the point that clam abundances are being driven downward. Our own observations while sampling in the field confirm the potential for sea stars (especially *Pycnopodia helianthoides*) or possibly moon snails (*Cryptonatica* spp. and *Polinices* spp.) to be locally important littleneck clam predators. However, few data are available to support a quantitative assessment of links between clams and these other predators.

Ascribing the recent widespread decline in littleneck clam populations to predation pressures is made more complex by the fact that the native littleneck clams seem to be the only clam species to have been impacted. Thus, if a predator was responsible for the population decline, it must have been preferentially targeting this particular bivalve to the exclusion of all others.

Sea temperature

Much has been published on the warming of the planet and the profound changes manifested in marine communities of the world's oceans. Roemmich and McGowan (1995), for example, determined that between 1951 and the mid-1990s, the biomass of macrozooplankton in waters off southern California decreased by 80 percent. During the same period, the surface layer warmed, by more than 1.5°C in some places, and the temperature difference across the thermocline increased. This increased stratification reduced the vertical cycling of inorganic nutrients that fuel new biological production.

More relevant to the observed declines in native littleneck clams we have reported here is a potential link between distribution and health of Atlantic surfclams (*Spisula solidissima solidissima*) and sea water temperature (Weinberg, 2005). Increased mortality in shallow water and a shift to deeper water habitat in surfclams along the U.S. Atlantic coasts were attributed to unusually warm water over the continental shelf.

However, an increase in sea temperature alone would not be expected to significantly affect the *L. staminea* population as a whole because the species is capable of tolerating a wide range in seawater temperatures, as demonstrated by its geographic range, which extends from Mexico to subarctic Alaska. Temperatures across this geographic extent range from 1.2 to 26.7°C (Takesue and van Geen, 2004). However, indirect impacts from warming seas, for example to food supply or the distribution of predators, may be causing the littleneck clam population to decrease along its entire Pacific range.

More subtle consequences of sea temperature increases may include potential shifts in metabolism affecting shell growth and other physiological processes. Takesue and van Geen (2004) studied the ratios of Mg/Ca and Sr/Ca in modern and Holocene shells of *L. staminea*, noting that Mg/Ca ratios in particular had been previously identified as a "paleotemperature proxy." That is, there is evidence that changes in ambient seawater temperature are reflected in changes in the Mg/Ca ratios in the shells of littleneck clams. Takesue and van Geen, however, found that *L. staminea* shells were not very good geochemical archives, primarily because the clams apparently halt growth under stressful conditions, such as those resulting from episodic events and shifts in the immediate environment.

Disease and Parasitic Infection

Another obvious potential cause of the observed littleneck clam population decline is infectious disease or parasitic infection. However, there have been no reports or indications of increased incidence of disease in littleneck clams collected in Alaska or adjacent regions. Native littleneck clams on the Pacific coast of North America have, to date, been relatively pathogen-free. Chew and Ma (1987) cited studies finding tetraphyllidean cestodes and larval *Echeneibothrium* sp. tapeworms in littleneck clams, but found no evidence of resultant epidemic disease from the presence of parasites. Desser and Bower (1997) found that 70 percent of littleneck clams sampled in Sooke Basin, British Columbia, contained the cystic stage of an apicomplexan. This single-celled spore-forming parasite that possesses a unique organelle called an apical complex. It causes the occurrence of cysts in several different tissues, but especially in the kidney and connective tissue surrounding the intestine. The authors surmised that the cysts probably represented a life stage of a heteroxenous coccidian cycle, with a littleneck clam predator as the

likely definitive host. However, despite parasitic loads that the authors termed “heavy,” there was no observed sign of associated pathology.

Marshall et al. (2003) examined the parasitic and symbiotic associations of three clam species from the same locations in British Columbia waters, including *L. staminea*, and found that each of the three species had a different assemblage of parasitic and symbiotic fauna. This suggests parasitic infection may be capable of impacting only the littleneck clam populations, while leaving the co-occurring clam species, such as butter clams, unharmed. Although researchers have shown that littleneck clams are subject to parasitism, and while related bivalves in other parts of the world are susceptible to bacterial and viral diseases, there is no direct evidence that *L. staminea* along the northern Pacific coast of North America have been exposed to pathogens or affected by parasites.

Nevertheless, disease may be a cause for concern as demonstrated by the recent (2008) impact of *Vibrio tubiashii* (a bacteria) on the oyster culture industry of the U.S. Pacific coast. *V. tubiashii* was identified nearly 50 years ago as a disease organism in juvenile bivalves (Tubiash et al, 1965), and has been subsequently isolated and described in detail (e.g., Hada et al., 1984). For reasons not yet understood, *V. tubiashii* has emerged as a significant problem on the west coast of the U.S., where it currently threatens the entire oyster-culture industry. The bacteria fatally infect larval and juvenile oysters, sometimes decimating entire year classes of cultured oysters. In 2008, the Los Angeles Times (Weiss, 2008) and a National Public Radio affiliate in Seattle (Wang, 2008) published articles on the dire situation faced by oyster growers and consumers, directly attributed to an outbreak of *V. tubiashii* in hatcheries and possibly in wild populations as well. The underlying cause for the increase in the bacterial population remains unknown, although scientists quoted in the articles speculated that oceanic warming or the growth of offshore “dead zones” may be responsible.

While we have no direct evidence that a *Vibrio*-type pathogen is affecting littleneck clams, few juveniles or new recruits were observed in the field in PWS in 2007, or during informal surveys of littleneck clam beds in the San Juan Islands of Washington State in 2008.[10] The paucity of younger clams is consistent with a disease vector that targets early life stages, much like *Vibrio tubiashii*.

In other bivalve species, parasitic disease is problematic. *Perkinsus*, for example, is a genus of protozoan parasite that infects clams, oysters, and cockles in many parts of the world (Bower, 2007). *Perkinsus* manifests itself as milky white cysts in different tissues of the infected bivalve (gills appear to be the main target in clams). Heavy infection results in an aggregation of cysts into lesions that interfere with respiration, reproduction, and other physiological processes. In some cases, mass mortalities and declines in commercial harvest of affected bivalves have been attributed to *Perkinsus* infection. There are no known methods of control or prevention. Fortunately, *Perkinsus* has not been reported to occur on the Pacific coast of the Americas; however, the potential exists, as its occurrence is well-known in some of the same species that reside in Asia and Europe. Park et al. (2006) reported the first occurrence of *Perkinsus olseni* in

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[10] It should be noted, however, that Washington Department of Fish and Wildlife surveys in 2007 found evidence of littleneck recruitment, even at a site where steady declines in legal-sized clams had been documented.

the Korean Venus clam, *Protothaca jedoensis*. Elston et al. (2003) reported finding a high degree of infection in juvenile Manila clams (*Venerupis philippinarum*) from Korea that were originally intended for import into Mexico for rapid growth, then to the United States for further rearing or direct marketing. The *Perkinsus* infection within these clams was found to have caused "significant" tissue damage to the juveniles. This incident illustrates the significant potential for accidental infection of unimpacted clam resources.

Complicating the issue of accidental introduction of a pathogenic agent into a previously unaffected bivalve population are reports of increased virulence of parasitic organisms affecting species within the same genus but on different coasts. Burreson et al. (2000) described the extensive mortality in eastern oysters (*Crassostrea virginica*) along the mid-Atlantic U.S. coast that was caused by the protistan parasite *Haplosporidium nelsoni*. This same parasite was shown to occur in the Pacific coast *Crassostrea gigas*, but with a far less virulent effect. The origin of the *H. nelsoni* infection on the Atlantic coast was shown to have originated from Pacific coast or Asian stocks of *C. gigas* that were imported to the east coast.

Another pathogenic condition in clams, brown ring disease, is caused by a bacterium, *Vibrio tapetis*. Infections have been endemic to European Atlantic stocks of the Manila clam, *V. philippinarum*, although it was also reported in Korean stocks of the same species in 2006. Brown ring disease is so-named because large-scale mortality in infected clams is accompanied by a characteristic brown deposit on the inner surface of the valves (Flye-Sainte-Marie et al., 2007). Death results from disruption of a number of processes, including calcification, hemocyte characteristics and behavior, degeneration of metabolic activity, and immune system function.

Contaminant Exposure

Roesijadi (1980) studied the effects of copper on littleneck clams, and found that over the course of a 30-day exposure, concentrations of 39 ppb and 82 ppb were highly toxic and resulted in respective lethalities of 86% and 97%. In comparison, concentrations of 7 and 18 ppb caused only slightly decreased survival relative to a control concentration of 0.35 ppb. The apparent locus of action was gill tissues, with indications that disruption of cellular ionic regulation was the predominant mechanism for the observed toxic response in the clams.

Of course, there is no evidence of elevated copper exposure to littleneck clams in PWS or other Alaskan coastal locations where the population declines have been noted. However, this documented susceptibility to metallic concentrations in seawater might be considered in the context of other changes that are occurring in the ocean, such as shifts in oceanic pH, that could affect the availability and the toxicity of metals that would otherwise be innocuous.

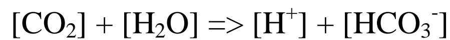
Ocean acidification

Recent reports, for example, by Orr et al. (2005) have shown that rising atmospheric carbon dioxide (CO_2) levels have resulted in measurable increases in the acidity of the oceans. Today's surface ocean is saturated with respect to carbonate (CO_3^{2-}), but acidification threatens this condition, and these changes have major biological implications.

Acidification occurs when CO_2 dissolves in seawater and there is a reaction between the H_2O, CO_2 , and dissolved CO_3^{2-} molecules, which form bicarbonate (HCO_3^-) (Haugan, 2004):

$$[CO_2] + [H_2O] + CO_3^{2-} \Rightarrow 2[HCO_3^-]$$

As bicarbonate increases, carbonate is depleted and more of the CO_2 entering seawater remains as dissolved CO_2 although some additional bicarbonate will be produced by the direct interactions between water and carbon dioxide molecules.



$[CO_2] + [H_2O] => [H^+] + [HCO_3^-]$

The overall effect of dissolving more CO_2 in seawater is that the concentrations of H^+, H_2CO_3 (carbonic acid), and HCO_3^- increase as the concentration of CO_3^{2-} decreases. As a result, the solution becomes more acidic, namely, pH decreases because of the increase in free hydrogen ions associated with carbonic acid. It is the depletion in the availability of dissolved carbonate within ocean waters that is considered to be most consequential for living organisms, but particularly for organisms with carbonate shells, such as *L. staminea*.

Orr et al. used 13 different models of the ocean carbon cycle to project conditions through the 21st century assuming the current levels of carbon emission is sustained. They found that by mid-century, undersaturated conditions of calcium carbonate will appear in surface waters. By the turn of the century, these conditions would occur in the subarctic North Pacific. The biological and ecological implications are potentially profound because many marine organisms make shells or supporting plates out of calcium carbonate in a process called calcification. As water becomes more acidic, the calcification process is inhibited and the growth and/or survival of certain organisms adversely impacted. Orr et al. exposed live pteropods to the predicted level of carbonate undersaturation during a two-day shipboard dissolution experiment, and found their aragonite shells showed notable dissolution of this naturally occurring, stable form of carbonate.

Haugan (2004) synthesized what is known about potential biological impacts of ocean acidification. Beyond the direct issues of impaired carbonate metabolism for exoskeletons and shells, he also discussed the adverse effects of excess CO_2 and acidification, including altered acid-base regulation in body fluids, impaired osmoregulation and respiration, altered utilization of nutrients in algae, changes in ionic distribution of metals, impacts to more sensitive life stages such as eggs and larvae, decreases in carbonate transport to lower depths of the ocean, competitive shifts to species able to benefit from direct utilization of CO_2. Based on these studies, the potential for biological change from ocean acidification is potentially significant, and without further investigation, cannot be ruled out as a possible cause for the observed declines in littleneck clam populations.

Appendix B. Field Sampling Protocols for the Determination of Hydrocarbon Concentrations within Intertidal Sediments and Littleneck Clam Tissues

Prepared by
James R. Payne and William B. Driskell

Overview

In July, 2007, NOAA will oversee a field sampling program to study the current status of littleneck clam populations (*Leukoma staminea*) in Prince William Sound, Alaska. The intent of this work is to discern lingering impacts from the *Exxon Valdez* oil spill to an important intertidal infaunal component. One part of this assessment will be laboratory analysis of clams from the surveyed sites to determine tissue burdens of petroleum hydrocarbons and sediment concentrations in beaches where the clams reside. Because of the inherent difficulties associated with properly and effectively sampling to portray the conditions of exposure and uptake, NOAA intends to structure the field chemistry collections and the laboratory analyses in an integrated and well-reasoned way to maximize the potential for both analytical and interpretive success.

This document offers specific guidance for field sampling and handling littleneck clams and associated sediments for subsequent analysis of saturated and polynuclear aromatic hydrocarbons (SHC and PAH, respectively) remaining from the 1989 *Exxon Valdez* oil spill. It also contains general guidance on laboratory procedures for the analytical facility, acknowledging that any lab will have their own SOPs. A list of specific analytes to be quantified is included.

In order to evaluate the current recovery status of clams at sites monitored over the long-term, the clam sampling procedures from the 1990-2000 NOAA program will be used. At each site, the previously surveyed lower intertidal transect, which is approximately 30 m in length, will be reoccupied. Four randomly located 0.25 m^2 quadrats will be excavated, taking care to not sample areas excavated in the past, and sieved on site. Minimum screen retention size is approximately 10 mm, and all recovered clams (with unbroken shells) will be collected for chemical analyses. In addition, five clam-gun cores will be collected randomly along the same transect using a modified hand-held cylindrical clam gun. This corer, 10.5 cm in diameter by 15 cm in length, samples an area of 0.009 m^2. Sediments collected by the clam guns will be sieved through 1.0 and 0.5 mm screens and all clams encountered will be reserved for biological analysis.

In general, encountering a large clam biomass is not expected at these sites (Lees and Driskell, 2007). Adequate tissue mass for hydrocarbon analyses (50 g desired) may not always be achievable. Hence, any excavated clams will require special handling to avoid cross-contaminating their tissues with oil in the sediments. After tissue chemistry analyses, all excavated clam shells should be returned from the lab for use in size and age analysis.

Beach sediments will also be collected at each site for determination of various sediment parameters and hydrocarbon concentrations.

Goals and Assumptions

NOAA Constraints

- Fixed sample locations (n=10) and limited sample sizes.
- Minimize number of samples to be analyzed in the laboratory to control analytical costs.
- Field personnel do not include personnel from the analytical facility.

Goals

- Assess levels of oiling in clams and sediments
- Assess correlations between clam and sediment hydrocarbon concentrations
- Assess clam morphometrics

Questions of Interest

- What are the current ambient concentrations of aromatic hydrocarbons in littleneck clams and in sediments from the selected group of sites within PWS?
- Do discernible differences exist among the three oiling and treatment categories (i.e., unoiled, oiled & not washed, oiled & washed)?
- Does there appear to be a link between the hydrocarbon distributions in clams and those in beach sediments from the same site?
- What are the ecological implications of ambient environmental concentrations of hydrocarbons measured in this project?

Collection Procedures

We recommend non-composited, replicate-associated tissue and sediment sampling to avoid confounding the results by the patchy nature of the residual oil. That is, we would like each tissue sample to have its own sediment samples. We anticipate very few positive hits of oiled tissue and are trying to maximize the few details available for each sample rather than having to deal with site-averaged data (composites) in evaluating the exposure pathways. This approach could imply a lot of sampling, but most sediment samples will not need to be analyzed (see Analytic Strategy below).

Primary Sampler

Sampling appropriately for environmental oil requires a combination of diligence and awareness. The task is not difficult, but the devil is in the details. Primarily, one needs to be aware of the potential sources of cross-contamination and then diligently avoid them while procuring the sample of interest.

We suggest that one person on the field team be the Primary Sampler (aka "Beach Boss") with responsibilities for obtaining, handling, and recording all samples to be tested for hydrocarbons. Functionally, an excavation team would consist of two persons: a digger and a sample handler/collector. The sample handler can be any diligent Nitrile-gloved crew member capable of collecting and bagging the sieved clams. However, only the Primary Sampler would collect

sediment samples; overseeing their handling, post-processing, tracking and shipping. This structure would ensure sediments were collected and handled in a consistent manner.

Equipment

- Clean digging equipment (shovels, trowels)
- Padded gloves for digging
- Sediment samplers – spoons, wooden tongue depressors, or trowels.
- Nitrile gloves (large and extra-large) for sample collection/handling
- Sample jars (certified pre-cleaned I-Chem jars with Teflon®-lined lids)
- Aluminum foil and freezer-storage, zip-lock bags.
- Coolers
- Gel ice packs
- Labels
- Camera

Each collection person should wear disposable Nitrile gloves before directly touching any sample. When present, EVOS residue oils are often visually apparent to the diggers. The oil appears at depth, as a thick dark ooze, a rainbow sheen on interstitial water, or an unmistakable sharp oil odor in the excavation pit. If there is a possibility of cross-contaminating samples (an oiled sample followed by a clean sample), gloves should be replaced and digging equipment cleaned between samplings.

Cleaning involves scrubbing shovels, trowels, and sieves with Alconox detergent or dish soap, followed by rinsing in local seawater at a location upstream, upwind, and well away from support vessel and skiffs. Outboards can be significant hydrocarbon sources, creating local slicks of fuel and oil from their coolant water and combustion products from their exhaust. ATVs and portable generators have similar issues but are not expected to be used on this trip. We have even encountered local citizens burning campfires (or trash) on the beach. Avoid sampling near or down-wind of any such activities.

The Ziploc® collection bags should be double labeled using a permanent marker on the outside of the bag, with a duplicate paper label placed inside the bag. The outside labels alone are insufficient because they tend to rub off with subsequent handling. Jars are labeled with permanent markers on an external adhesive paper label or label tape. If possible, pre-label the jars based on expected site number, transect location, pit number, etc. After labeling is completed, tape over jar labels with clear shipping tape to avoid either losing the label or having the information compromised, for example when a wet paper label disintegrates a frozen sample thaws or is abraded. We suggest pre-taping the jars before the field effort, for example, on the boat before arriving on station, so problems associated with trying to place tape on wet jars are avoided. The inner paper labels for the bags are typically preprinted in the office with all but the date. Adhesive jar labels can be preprinted in the same manner.

Photograph each excavation and a panorama of the site, and sketch the layout of the transects and excavations. If appropriate, photograph the clams grouped in the sieve for population photometrics. Weighing the whole sample on a portable field scale may also be prudent prior to sending samples to the lab.

Tissues

Tissues are hand collected as whole clam specimens, gathered in either a sample jar or wrapped in fresh aluminum foil and sealed in a Ziploc® bag. Roughly 50 grams of wet tissue are needed for full analysis, but 10 g is the minimum. Exclude individuals with broken shells from the samples earmarked for tissue-hydrocarbon analysis, and bag them separately as a biological specimens, making a record to track the split samples from the excavation. If there is no sheen in the pit's interstitial water, specimens can be optionally rinsed free of sediments within the pit itself.

Any crew member touching the specimens must have uncontaminated hands. This means each digger who reaches into the pit to remove large rocks or is picking out clams from the sieve must be freshly Nitrile-gloved prior to collecting the clam specimens for hydrocarbon analysis. The risk of contamination is small but the data are too expensive to be compromised by the lack of 25 cents worth of precaution, or the inconvenience of gloves.

Sediments

Using a clean utensil and sample jar, collect approximately 100 g to 200 g, which equates to about two fistfuls of scrapped surface detrital or sedimentary material, from the <u>top 1 cm only</u>. Obtain this surficial material from the 0.25 m^2 quadrat before excavation. Collect a second sample from the walls of the hole at the depth of the clams (< 10 cm). Taking slightly more than two heaping tablespoon from each of the four walls should be adequate. Focus on the finer materials, avoid large gravels.

Be sure to keep both surficial and at-depth sediment samples separately identified with their corresponding tissue sample (a single composited clam sample from the quadrat excavation). At this point in the post-spill timeline, any residual oil is quite patchy. One quad/tissue sample may be oily while the next is clean.

An additional sediment sample for particle grain size and TOC should be collected from the wall of the excavation, again at clam depth. Pre-cleaned I-Chem® glass jars should be used for the TOC samples, but a quart-size bag is adequate for particle grain size. Exclude gravels greater than 2 cm and live macro-organisms that would bias the ambient TOC determinations.

Field blanks for sediments are generally provided by the analytical laboratory. Typically approximately 600 g of fine kiln-fired sand are shipped along with lab-supplied sample jars prior to field activities. This assumes that a laboratory will have been selected before the field effort. If that is not the case, see if you can get someone at the NOAA Montlake facility to prepare the field blanks for the survey. Once in the field, the kilned-fired sand can be "transferred" from its original container with a clean spoon or wooden tongue depressor, and placed into the sample jar at or near a sampling transect at each station. Ideally, a sediment field blank should be collected at each site, but to minimize analytical costs, we suggest this project transfer field blanks at the beginning, middle, and end of the field sampling program, for example, at the 1st, 5th, and 10th sites.

Rationale

Avoiding contamination is the primary goal in collecting samples for chemical analyses. Fortunately, this is not a difficult task for heavily oiled samples, but it can be an issue with

cleaner samples, or samples collected at unoiled reference sites. Contamination occurs by contact. If the collector permits only clean surfaces to touch the sample, the chance of contamination is minimized. Aerial sources are also a concern; avoid exposure to smoke or engine exhaust.

The only additional concern for clam tissues pertains to sampling individuals with broken shells. Since a broken shell may introduce oil from surrounding sediments that would not necessarily appear in the tissues from uptake, the injured individual should be bagged separately and not used for estimating tissue hydrocarbon burdens.

Collecting sediments for this project is slightly more complicated than collecting tissues. If clams are found to be contaminated, it remains uncertain whether the source was from surface or subsurface contaminants. Although a small subset of earlier NOAA data on clam tissue burdens did not seem to correlate with subsurface oiling, it seems prudent to collect sediments from both depth levels.

Handling Procedures

Once in sealed containers, there is little danger of contamination. Fortunately, we have never encountered a circumstance in which a sample would need to be re-exposed to the open environment after initial collection. The living specimens should be kept cool prior to freezer storage. It may be prudent to take an ice chest to the beach to keep the samples shaded and cool as they accumulate. Once handling, labeling, and record keeping is complete, the samples should be stored frozen until shipment.

As mentioned above, we find it convenient to pre-label bags and jars, and pre-print labels with blank dates prior to deploying to the beach. This minimizes handling the samples, reduces labeling errors, and serves as a physical "checklist" of samples collected, in other words, don't return to the boat with empty jars.

We also find it convenient to keep the jars in their cardboard shipping boxes to avoid glass-to-glass contact. Unless, severe inclement weather prevails, we recommend taking the shipping box to the beach to minimize breakage. While stored in the box, we also mark the lid tops with a sampling code to help select the appropriate pre-labeled jar. An alternative to schlepping the cardboard box is using pre-formed bubble-wrap sleeves; jars can then be carried in a cooler with less concern for contact breakage. The use of a field chain-of-custody (COC) form designed for tracking hydrocarbon samples, such as that used by NOAA's Auke Bay Laboratory, is recommended (Figure 11).

Rationale

Intertidal species are remarkable tolerant of thermal swings but do not survive high heat. A cooler on the beach, with ice, if needed, serves to organize and insure sample integrity from thermal and physical abuse. For these reasons, ice-chest storage is superior to other approaches such as a backpack collection. Freezing upon return to the vessel provides adequate preservation. Sediments are less finicky than tissue samples, but should also be preserved frozen to halt microbial degradation.

Shipping Procedures

Because the frozen samples must arrive promptly at the analytical lab, priority shipment via air freight is the only realistic option. U.S. Transportation Security Administration (TSA) rules now dictate that samples being shipped must be consigned by a photo-id-toting “Known Shipper” and must be inspected prior to shipment. This system works only in Anchorage and Cordova for shipments leaving the state.

When shipping from Valdez, the shipment must be carried or checked as baggage to Anchorage because TSA does not inspect and hence, does not accept out-of-state shipments. After the cooler is recovered from baggage claim in Anchorage, it has to be carried to the Alaska Airlines Cargo office adjacent to the Airport driveway ramp located in the office on north side of building. Freight clerks will want to see inside the cooler to inspect the items and the coolant, which cannot consist of non-contained ice because of leakage issues. Depending on the destination and thermal bulk of the samples, a few to several gel ice packs may be needed. At times, caught without sufficient gel ice, we have used frozen drinking-water bottles.

There are consequences to thermal degradation. From the NOAA hot tarmac incident, it appeared that total PAH (TPAH) values were impacted through increased microbial degradation although the PAH signal was still recognizable. Vocally reinforce that the shipment must be kept frozen, and apply several stickers stating the same to the outside of the containers. The freight clerks should also bind the cooler prior to shipping; so watch that this actually occurs. On one occasion, samples shipped from Cordova arrived at Juneau in an unsealed cooler, and it was purely fortuitous that they arrived at all, much less in an uncompromised condition. Shipping via the Gold Streak service with Alaska Airlines theoretically ensures the package will not be held for later flights. Otherwise, apply a “Must Ship” sticker. Label the cooler’s destination with a permanent marker. We have had coolers appear on the luggage belt in Seattle without their adhesive shipping label, so we now ask clerks to bind their shipping label down.

We also suggest having the destination contact person pick up the shipment rather than risk an extended ride on an ambient-temperature delivery truck. Also, give the receiving laboratory a heads up by telephone and later follow up to confirm the receipt and condition of the shipment. We have had a failed-to-be-notified recipient retrieve their refrigerated samples sitting behind the San Diego airport delivery desk “because we don’t have a refrigerator.” If possible, try to avoid shipping samples on Friday or Saturday (or just before three-day weekends) to minimize the chance that samples will be held up in transit under suboptimal circumstances.

Rationale

As long as the samples are kept frozen, they can travel anywhere and for any length of time. Unlike the U.S. EPA, NOAA doesn’t specify holding times, namely, how long samples can be held frozen before extraction and analyses, and consistent with this policy, we have not seen any compromise in mussel samples archived frozen for years. Note that there is a minor effect on particle grain size as freezing fractures fine particulates, shifting the distribution to a yet finer fraction. We have seen up to 5% change in fine sediments from PWS as result of freezing. Although refrigeration is preferred, it is not always practical and we consider freezing of grain-size samples a minor, non-critical compromise.

Extraction and Analytic Procedures

Polycyclic aromatic hydrocarbon (PAH) and saturated hydrocarbon (SHC) analytes measured in this study should match those reported in the past (Table 7), and should include specific internal and surrogate standards (Table 8).

The inclusion of this comprehensive list of alkyl-substituted polycyclic aromatic hydrocarbons in addition to the n-alkanes plus pristane and phytane is important for oil-source characterization, and for assessing the state of biological weathering. Most state-of-the-art analytical facilities involved in natural resource damage assessment (NRDA) programs, at a minimum, routinely measure these analytes using selected ion monitoring (SIM) gas chromatography/mass spectrometry (GC/MS), and several also include the alkylated naphthodibenzothiophenes. Inclusion of stable biomarkers (the triterpane, C_{30}-17α(H),21β(H)-hopane, oleanane, 18α(H)-22,29,30-trisnorhopane, 17α(H)-22,29,30-trisnorhopane, and C26 + C27 triaromatic steroids) can also help differentiate among Alaskan north-slope crude oil, Katalla and Yakataga seep oils, and other potential PAH sources (Boehm et al., 2001). As highly stable and source-definitive biomarkers, we would strongly prefer their inclusion in the program but since similar data have not been collected in past NOAA/OR&Rstudies, there would be no previous tissue analyte data against which to make comparisons.

The rationale for including alkylated PAH homologues, beyond just the standard EPA Priority Pollutant PAHs, for oil characterization actually dates back to a pre-EVOS era. During investigations of Prudhoe Bay crude-oil weathering in the mid 1980's, Payne et al. (1984) and Payne and McNabb (1984) reported that the parent PAH components in most crude oils and refined petroleum products were dominated by alkyl-substituted PAH homologues containing one or more methyl (C1), ethyl (C2), and various other C3 and C4 combinations of alkyl groups attached to the aromatic rings. In 1989, during the *Exxon Valdez* oil spill event, Sauer and Boehm were persistent in testing and only selecting laboratories willing to adapt the more sensitive methodology for Exxon-funded studies. Later at the 1991 International Oil Spill Conference, Sauer and Boehm (1991) presented a detailed discussion of the shortcomings of the then-current EPA methods that focused on the 16 parent PAH components constituting the EPA priority-pollutant list for characterizing crude oils and environmental samples associated with oil-pollution events. They demonstrated that many of the EPA Priority Pollutant PAH, and especially the four and five ring compounds, were not present at high concentrations in most crude oils, and that focusing on just those analytes was more appropriate for characterizing hazardous waste sites and industrial process streams. At that time, Sauer and Boehm proposed the utilization of more appropriate SIM GC/MS methods for characterizing oil and oil-contaminated materials, and since then, these methods have been universally applied in oil fingerprinting and source characterization for NRDA efforts following nearly every major oil pollution event in U.S. coastal waters.

Numerous refinements and improvements to the methods have been published (Sauer and Boehm 1995; KLI 1995, Boehm et al. 1997; Short and Harris 1996; Short et al. 1996; Stout et al. 2001, 2002), and the SIM GC/MS approach has been used since 1993 by the Prince William Sound Regional Citizens' Advisory Council in the 1990 Oil Pollution Act-mandated Long Term Environmental Monitoring Program from Port Valdez to Kodiak. Eventually, even the U.S. EPA recognized the importance of including the alkylated PAH in oil-spill research, and the SIM GC/MS approach for quantifying alkyl-substituted PAH has been promulgated by EPA as the

Table 7. Target hydrocarbon analytes and the associated recommended standards cross-referenced in Table 8. Calibrated analytes are identified by boldface type.

	Standard			Standard	
PAH Analyte	Internal	Surrogate	Alkane Analyte	Internal	Surrogate
Naphthalene	A	1	**n-Decane**	B	7
C1-Naphthalene	A	1	**n-Undecane**	B	7
C2-Naphthalene	A	2	**n-Dodecane**	B	7
C3-Naphthalene	A	2	**n-Tridecane**	B	7
C4-Naphthalene	A	2	**n-Tetradecane**	B	8
Biphenyl	A	2	**n-Pentadecane**	B	8
Acenaphthylene	A	2	**n-Hexadecane**	B	8
Acenaphthene	A	2	**n-Heptadecane**	B	8
Fluorene	A	2	**Pristane**	B	8
C1-Fluorenes	A	2	**n-Octadecane**	B	9
C2-Fluorenes	A	2	**Phytane**	B	9
C3-Fluorenes	A	2	**n-Nonadecane**	B	9
Dibenzothiophene	A	3	**n-Eicosane**	B	9
C1-Dibenzothiophene	A	3	**n-Heneicosane**	B	9
C2-Dibenzothiophene	A	3	**n-Docosane**	B	10
C3-Dibenzothiophene	A	3	**n-Tricosane**	B	10
C4-Dibenzothiophene	A	3	**n-Tetracosane**	B	10
Anthracene	A	3	**n-Pentacosane**	B	10
Phenanthrene	A	3	**n-Hexacosane**	B	10
C1-Phenanthrene/Anthracene	A	3	**n-Heptacosane**	B	10
C2-Phenanthrene/Anthracene	A	3	**n-Octacosane**	B	10
C3-Phenanthrene/Anthracene	A	3	**n-Nonacosane**	B	11
C4-Phenanthrene/Anthracene	A	3	**n-Triacontane**	B	11
Fluoranthene	A	3	**n-Hentriacontane**	B	11
Pyrene	A	3	**n-Dotriacontane**	B	11
C1-Fluoranthene/Pyrene	A	3	**n-Tritriacontane**	B	11
C2-Fluoranthene/Pyrene	A	3	**n-Tetratriacontane**	B	11
C3-Fluoranthene/Pyrene	A	3	Total n-Alkanes		
C4-Fluoranthene/Pyrene	A	3			
Benzo(a)Anthracene	A	4			
Chrysene	A	4			
C1-Chrysenes	A	4			
C2-Chrysenes	A	4			
C3-Chrysenes	A	4			
C4-Chrysenes	A	4			
Benzo(b)fluoranthene	A	5			
Benzo(k)fluoranthene	A	5			
Benzo(e)pyrene	A	5			
Benzo(a)pyrene	A	5			
Perylene	A	6			
Indeno(1,2,3-cd)pyrene	A	5			
Dibenzo(a,h)anthracene	A	5			
Benzo(g,h,i)perylene	A	5			
Total PAH					

method that should be utilized for chemical analysis of oil composition in assessing the effectiveness of bioremediation and dispersant countermeasures in response to oil spills (Federal Register, 2003). Most recently, Douglas et al. (2004), reported on methods to further reduce the

Table 8. Recommended laboratory standards for hydrocarbon analytes listed in Table 7

Type	ID	Standard
Internal	A	hexamethylbenzene
Internal	B	dodecylcyclohexane
Surrogate	1	naphthalene-d8
Surrogate	2	acenaphthene-d10
Surrogate	3	phenanthrene-d10
Surrogate	4	chrysene-d12
Surrogate	5	benzo[a]pyrene-d12
Surrogate	6	perylene-d12
Surrogate	7	dodecane-d26
Surrogate	8	hexadecane-d34
Surrogate	9	eicosane-d42
Surrogate	10	tetracosane-d50
Surrogate	11	triacontane-d62

method detection limits routinely achieved by SIM GC/MS. Their recommendations for achieving these lower detection limits, including larger sample sizes, better chromatographic cleanup and removal of lipids and other interfering materials, and smaller final-extract sample volumes should be utilized in this program whenever possible.

Disposition of the Clam Samples

There are two trains of thought regarding clam tissues and shells. First, all shells need to be returned intact for shell morphometrics. Secondly, since we are suggesting an increase in sample size to 50 g of wet weight tissue to optimize the method detection limit, it is likely at impoverished sites, that all clams will be needed in reach the desired tissue mass for chemical analysis.

One approach would say that samples from sites where the clam tissue mass exceeds the desired 50 g tissue wet weight, that is, not including shells, the excess clams could be collected as a split sample for shell morphometric analysis alone, thereby skipping the trip to the chemistry laboratory. A less problematic approach is simply for all clams to go to the laboratory and all shells returned.

Following the later plan, all clams collected for each sample should be shucked and the combined sample wet weight recorded before homogenization. These data can also be used in analyses of biomass distributions. If the homogenized wet tissue weight is more than 50 grams, the excess tissue can be archived in a frozen state, or it can used immediately in additional replicate or matrix-spike analyses if desired.

The analytical lab will save and return intact shells for morphometrics. The shell samples should retain their original metadata including individual site and sample number information. Field photographs can be used as backup for cross-checking counts, sizes and sample identity.

Since all clams will be shucked and composited, this paragraph is no longer germane but perhaps of interest in similar sampling schemes. If the lab was not going to process the entire sample, we would want to ensure that the analyzed sample is representative of the entire field sample. The laboratory technician should make random selections from the available specimens and not just

shuck the largest individuals for the 50-g composite. Clams have methods of actively selecting the particle size of their food, which may vary with clam size. A bias can then arise if particle size co-varies with a particular oil source, for example, a micro-droplet of fresh oil suspended in leptopel, a suspended organic material, as opposed to weathered oil adsorbed onto sediment particles.

Extraction and Cleanup

Numerous perturbations to analytical procedures have been published (see references cited above), and most analytical laboratories involved in NRDA studies have standard operating procedures (SOPs) specific to their facilities, homogenization/extraction equipment, and cleanup methods depending on whether silica gel and alumina column chromatography or size exclusion high performance liquid chromatography (HPLC) methods are applied. As such, it would be presumptuous to proscribe exactly which methods and procedures should be used.

However, it is not out of the scope of the project to suggest specific sample weights and final extract volumes that can be utilized to improve method detection limits (Douglas et al., 2004). As such, to obtain the maximum amount of usable analytical information, the chemistry laboratory should attempt to utilize the sample sizes and concentrate the sample extracts to the pre-injection volumes shown in Table 9.

Table 9. Recommended sample weights, final pre-injection volumes (PIVs), and injection volumes to optimize method detection limits (MDLs) for hydrocarbon analysis

Parameter	**Tissues**	**Sediments**
Sample wet weight (g)	50	100-200
Final pre-injection volume (µL)	100-200	50-100
Injection volume (µL)	2	2

When concentrating samples below 1 mL to a pre-injection volume of 50-200 µL, special care must be taken to prevent unacceptable losses of lighter hydrocarbons to volatilization, in particular the naphthalenes and aliphatic hydrocarbons below n-C12. The sample extracts should be concentrated under a gentle steam of nitrogen in a 1.5-mL vial or conical GC without heating, and great care must be taken to avoid taking the sample to dryness.

GC/FID and SIM GC/MS

Again, all analytical laboratories routinely involved in NRDA studies will have specific SOPs covering instrument operating conditions, calibration procedures, data reduction algorithms, etc., so specific methods will not be proscribed within this guidance document. However, the concentrated extracts should generally be analyzed for the target analytes in Table 7 by capillary gas chromatography with flame ionization detection (GC/FID, EPA Method 8015M) for SHCs, and capillary gas chromatography with mass spectrometry (GC/MS, EPA Method 8270) for PAHs, both of which are operated in the SIM mode (Federal Register 2003, or equivalent).

Quality Control and Assurance

Standard quality control procedures should include the analysis of standard reference materials (SRM), matrix spikes, matrix spike duplicates, and method blanks with each analytical batch of 15 field samples. The laboratory procedural blank or method blank is prepared internally. The standard reference materials should include a control oil that is preferably Alaskan north-slope crude oil. A matrix of tissue or sediment should be analyzed as appropriate. The spike and duplicate spike samples should be spiked with either Alaskan north-slope crude oil or the analytes listed in shown in Table 7 at two-to-three times their MDL. The National Institute of Standards and Technology standard reference materials should consist of either SRM 1491 or SRM 1944 for sediments and SRM 1974b for tissues.

Grain Size

Most organic geochemistry laboratories have SOPs for particle grain-size determinations, but if these are not available, the grainsize analysis methods employed should be a combination of sieving and pipette methods based on the procedures given by Folk (1974).

Carbon Content

Analytical measurements of total organic and total carbon should be determined on oven dried and pulverized sediment samples using a Dohrmann DC-85A TOC catalytic combustion furnace, or its equivalent. The carbon dioxide produced is passed through an acidified liquid sparger that scrubs out entrained water vapor and corrosive species. It is then passed through two scrubbers consisting of copper and tin, and is then measured by linearized non-dispersive infrared detection. Quantification is achieved by comparison with results from a calibration curve based on potassium acid phthalate. Total organic carbon (TOC) and total carbon should be determined on samples treated with and without 10% HCl in methanol. Total inorganic carbon is calculated as the difference between total carbon and TOC.

Analytical Strategy

Ideally, all tissue and sediment samples would be analyzed, but for cost-reduction purposes, sediments can be selective analyzed based on two conditions. Sediments would be analyzed only if their corresponding tissues show significant oil signatures. However, if oiled sediments were observed in field excavations, then those samples would be included in first-round analyses. Second-round analyses would comprise only those sediment samples not previously run, but were post-hoc associated with oiled clam tissues.

Data Reporting

The lab should report all values on a dry sample weight basis, including analytes detected below MDL. With diligent integration and confirming SIM secondary ion patterns, forensic pattern recognition can provide extended confidence from expected co-occurring analytes and result in

quantifications below MDLs. For perspective purposes, analyte-specific, sample-weight-adjusted MDLs should be provided as detailed in the procedures for computing MDLs.[11]

Assessing data quality is equally important to the data itself when interpreting the relationships among the sample results. The dataset should include all surrogate-recovery information and laboratory Quality Control results, including those for method blanks, matrix spikes, matrix spike duplicates, and the results of SRM analyses. The lab should also provide GC/FID profiles and quantify the unresolved complex mixture.

All data should be received as an electronic copy. The objective is to be able to extract the data for further processing. Excel spreadsheets are universally accessible. A relational database format may be specified but most laboratories will have their own standard presentations.

Additional Considerations

Non-analyzed samples should be archived frozen at the laboratory. They may be needed as the analytic details emerge.

Mussels should be collected if they are available in the vicinity of a given field site. If the clams display an oil signal, the mussels will help interpret whether the source is in the water column, suggesting an offsite contamination source as opposed to local sediments. As the standard of oil monitoring, they would also help understand the magnitude of any contamination issue.

Beach armoring is suggested as being highly important to recovery of PWS intertidal clams (Lees and Driskell, 2007). There are no tested methods to assess beach armoring but well-lit photographs of pit wall profiles showing surface and adjacent subsurface sediments would at least document the conditions.

Field checklist

Beach Arrival

- Don't step off the skiff in water deeper than the vertical height of your boots.
- Locate and deploy the transect WITHOUT treading on the downslope side of the transect, thereby avoiding disturbance or contamination of the replicate samples.
- Locate and mark the quadrat and corer sites on the downslope side of the transect.
- Setup a soapy wash bucket at water's edge where excavation teams should scour and pre-clean their shovels and trowels, finishing with a rinse in sea water.
- The Primary Sampler should gear-up with sample containers, clean utensils and gloves, and should quickly gather the surface sediment samples before they are disturbed.

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[11] www.setonresourcecenter.com/CFR/40CFR/P136_008.htm

- Excavation teams should gear-up with clean shovels, sieves, sample containers, and gloves.
- The team sample handler should collect the sieved clams in foil, take photographs, and weigh and bag the sample.
- The Primary Sampler should gear-up for subsurface sediment sampling as each excavation is completed. Collect roughly 200 g total of sediment in a jar at clam depth from the four walls for oil analysis. Collect another jar sample for TOC analysis and collect a larger, quart-bag sample for grainsize analysis. Photograph the walls and surface profile of the pit for documenting surface armoring.
- The Excavation team should clean and exchange collection gear as needed between replicates.
- Repeat the last three steps until all replicates have been sampled.
- Conduct the following activities in parallel with the above: setup a cleaning station, take hand cores, conduct record keeping, collect site photographs, prepare a site sketch, transport finished samples.

Departing Beach

- The Primary Sampler should confirm that all sediment and tissue samples are accounted for and placed in the cooler.
- The excavation crews should gather and clean the gear, personal equipment, and personnel as required.
- Don't walk out to the skiff in water deeper than the vertical height of your boots.

Post-Sampling

- Consult the handling and shipping checklist.
- Confirm that all samples have been accounted for.
- Complete any missing label details.
- Add the inner duplicate paper label to the Ziploc® bags.
- Fill out COCs and shipping list.
- Store the samples in the freezer.

Packing

- Pack cooler(s) in a site-organized manner to expedite laboratory check-in.
- Add sufficient ice packs.
- Stabilize contents with stuffing materials.
- Top the contents with a properly signed COC sealed within a Ziploc® bag.
- Label the cooler with its destination in permanent marker
- Temporarily secure cooler tops with tape to avoid accidental spillage.

Airport Freight Counter

- Identify yourself as a "known shipper." NOAA is listed under US Government in the database of accounts. For example, the Auke Bay Lab is identified as "US/AukeBay."
- Provide a shipping receipt correctly filled-out with addresses, contacts and billing numbers as an example for the clerk.
- Burn incense and sacrifice a chicken.

Equipment List

Table 10 provides a list of suggested vendors and part numbers for some of the scientific sampling supplies and containers.

- 5 gallon bags for hand corer samples
- 4 qt/gallon bags for excavations
- 96 eight oz (250 mL) bottles or jars for oil sediments (2 samples/quadrat x 4 quadrats/site x 10 sites = 80 bottles plus extras for field blanks, breakage, unusual samples, etc.)
- 96 four oz (125 mL) bottles/jars for TOC (2 samples/quadrat x 4 quadrats/site x 10 sites = 80 bottles plus extras as above)
- 4 qt bags for grainsize analysis
- Spare containers
- Shovels
- Trowels
- Spoons or tongue depressors
- Nitrile gloves (large and extra large)
- Sieves
- 5 gal wash bucket
- Dawn soap detergent or Alconox
- Scour brush
- Cooler
- Ice packs (as required)
- Sand Blank (as required obtain a kiln-fired sample from analytical lab 500 g)
- Tape
- Quadrats and Markers
- Forms and site data
- Sharpies
- Camera
- Hand-held clam Cores
- Biomass Scale

Table 10. Recommended field sampling equipment, quantities, and suppliers for hydrocarbon samples

Item	Anticipated Quantity	Catalog Number		Notes
		Fisher	VWR	
Nitrile Gloves	2 packs of 100 4 packs of 100	19-050-221D 19-050-221E	PH2Y1842 PH271813	Large Extra Large
I-Chem 200 series 8 oz bottles	8 cases of 12	05-719-61	IR220-0250	Clear glass with Teflon-lined lids for SHC/PAH sediment samples
I-Chem 200 series 4 oz bottles	4 cases of 24	05-719-55	IR220-0125	Clear glass with Teflon-lined lids for sediment TOC analyses
Alconox powder detergent	1 four lb carton	04-322-4	21835-032	One gallon-sized carton, good for equipment decontamination
Tongue Depressors	1 case (12 boxes of 100)	01-346	62505-007 (2 packs of 500)	Disposable & clean sediment sampling scoops

- GPS

Estimated Sample Count & Cost Estimate

- 10 sites
- 5 sediment cores
- 4 excavations
- 2 sediment depths (surface, subsurface)
- 1 grainsize analysis
- 90 biological samples to overlap with sediment chemistry samples
- 40 tissue oil samples from quadrats only x $500 = $20K
- 80 sediment hydrocarbon samples x $500 x 25% positive hits= <$10K for 20 hits.
- 40 grainsize analysis samples x $100 = $4K
- 40 TOC sediment samples x $100 = $4K
- 3 sediment blanks x $500 = $1.5K
- Plus extras
- Disposable Supply List
- Sharpies
- Labels
- Ziploc® quart freezer bags
- Ziploc® gallon freezer bags

Chain of Custody

Figure 11 shows an example of a COC that can be used for hydrocarbon analysis. It shows the front and back of a completed form from the NOAA Auke Bay Laboratory. That laboratory racks samples using laboratory ID sample numbers generated off this form, for example, “18001-01” for the first sample. We generally fill this out by hand, photograph a copy and send the original sealed in Ziploc® bag placed inside the sealed cooler. These are not perfectly rigorous COC protocols but certainly adequate for tracking.

Auke Bay Laboratory Hydrocarbon Assessment Chain of Custody Form

2007_ _ _

Project LTEMP

Page 1 of 1

Serial # 18001

Assigned Sample #	Collector's Sample code	Date Collected	Matrix & Species, organ, etc	Location Collected	Latitude	Longitude	Collection Method	Comments
01	GOC-B-07-1-1	5 Apr 07	Mytilus	Gold Creek	61'7.474	146'29.655	Hand	
02	-2							
03	-3							
04	AMT-B-07-1-1			Alyeska Marine Term	61' 5.446	146'24.380		
05	-2							
06	-3							
07	AMT-S-07-1-1		SEDIMENT		61'5.406	146'23.681	Grab	73.4 m
08	-2				61' 5.403	146'23.671		70.1 m
09	-3				61' 5.403	146'23.672		72.6 m
10	AMT-PGS-07-1-1				same as seds			Grain Size
11	-2							
12	-3							
13	Rinse 1- Amt		WATER					pre- AMT
14	GOC-S-07-1-1		SEDIMENT	Gold Creek	61' 7.444	146'29.580	Grab	31.6 m
15	-2				61' 7.442	146'29.598		26.4 m

Continue list on back of page -->

Chain of Custody

Collected by: William Driskell [signature] 6 Apr 07

Relinquished by: signature — date / time — place

Received by: signature — date / time — place

For information contact: Bonita Nelson
NMFS Auke Bay Lab bonita.nelson@noaa.gov

Assigned Sample #	Collector's Sample code	Date Collected	Matrix & Species, organ, etc	Location Collected	Latitude	Longitude	Collection Method	Comments
16	GOC-S-07-1-3	5 Apr 07	sediment	Gold Creek	61' 7.441	146'29.582	Grab	28.9 m
17	GOC-PGS-07-1-1				same as seds			
18	-2							
19	-3							
20	GOC Rinse2		water					pre - GOC
21	GOC Rinse 3							post - GOC
22	Sand blank		sand					
23	KNH-B-07-1-1	6 Apr 07	Mytilus	Knowles Head	60'41.692	146'35.790	Hand	site moved from previous location.
24	-2							
25	-3							
26	SHB-B-07-1-1			Sheep Bay	60'38.796	145'59.724		
27	-2							
28	-3							
29								
30								
31								
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Figure 11. Example of a completed sample chain-of-custody form used by the NMFS/NOAA Auke Bay Laboratory

References

Boehm, P.D., G.S. Douglas, W.A. Burns, P. J. Mankiewicz, D.S. Page, and A.E. Burns. 1997. Application of petroleum hydrocarbon chemical fingerprinting and allocation techniques after the Exxon Valdez oil spill. Marine Pollution Bulletin 34, 599-613.

Boehm, P.D., D.S. Page, W.A. Burns, A.E. Bence, P.J. Mankiewicz, and J.S. Brown. 2001. Resolving the origin of the petrogenic hydrocarbon background in Prince William Sound, Alaska. Environmental Science and Technology 35: 471-479.

Douglas, G.S., W.A. Burns, A. E. Bence, D.S. Page, and P. Boehm. 2004. Optimizing detection limits for the analysis of petroleum hydrocarbons in complex environmental samples. Environ. Sci. Technol. 38:3958-3964.

Environmental Protection Agency (EPA). 1986. SW-846 Manual for Waste Testing. EPA, Washington, D.C., Vols. 1B and 1C.

Federal Register 2003, 40 CFR - Chapter I -Environmental Protection Agency. Part 300-- National Oil And Hazardous Substances Pollution Contingency Plan, Appendix C to Part 300 -- Swirling Flask Dispersant Effectiveness Test, Revised Standard Dispersant Toxicity Test, and Bioremediation Agent Effectiveness Test, Para. 4.6.3 to 4.6.5, Chemical analysis of oil composition; in Electronic Code of Federal Regulations, May 28, 2003. (http://www.access.gpo.gov/nara/cfr/cfrhtml_00/Title_40/40cfr300_00.html).

Folk, R.L. 1974. Petrology of Sedimentary Rocks. Hemphill Publ. Co. Austin, Texas.

KLI. (Kinnetic Laboratories, Inc.) 1995. Prince William Sound RCAC Long-Term Environmental Monitoring Program Annual Monitoring Report – 1994. Prepared for the Prince William Sound Regional Citizens' Advisory Council, Anchorage AK. 151 pp plus Appendices.

Lees, D.C. and W.B. Driskell. 2007. Assessment of Bivalve Recovery on Treated Mixed-Soft Beaches In Prince William Sound. Prepared for EVOS Trustees, Restoration Project 040574. 120 pp.

Payne, J.R. and G.D. McNabb, Jr. 1984. Weathering of petroleum in the marine environment, Marine Technology Society Journal, 18(3), 24-42.

Payne, J.R., B.E. Kirstein, G.D. McNabb, Jr., J.L. Lambach, R. Redding, R.E. Jordan, W. Hom, C. de Oliveira, G.S. Smith, D.M. Baxter, and R. Geagel. 1984. Multivariate analysis of petroleum weathering in the marine environment - subarctic. Volume I, Technical Results; Volume II, Appendices. In: Final Reports of Principal Investigators, Vol. 21 and 22. February 1984, U.S. Department of Commerce, National Oceanic and Atmospheric Administration, Ocean Assessment Division, Juneau, Alaska. 690 pp. Volume 21 NTIS Accession Number PB85-215796; Volume 22 NTIS Accession Number PB85-215739.

Sauer, T. and P. Boehm. 1991. The use of defensible analytical chemical measurements for oil spill natural resource damage assessment. Proceedings of the 1991 Oil Spill Conference, American Petroleum Institute, Washington, D.C., pp 363-369 (1991).

Sauer, T. C. and P.D. Boehm. 1995. Hydrocarbon Chemistry Analytical Methods for Oil Spill Assessments. Marine Spill Response Corporation, Washington, D.C. MSRC Technical Report Series 95-032, 114 p.

Short, J.W. and P.M. Harris. 1996. Chemical sampling and analysis of petroleum hydrocarbons in near-surface seawater of Prince William Sound after the Exxon Valdez oil spill. American Fisheries Society Symposium 18: 17-28 (1996).

Short, J.W., T.J. Jackson, M.L. Larsen, and T.L. Wade. 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 (1996).

Stout, S.A., A.D. Uhler, and K.J. McCarthy. 2001. A Strategy and Methodology for Defensibly Correlating Spilled Oil to Source Candidates. Env. Forensics 2: 87-98.

Stout, S.A., A.D. Uhler, K.J. McCarthy, and S. Emsbo-Mattingly. 2002. Chemical Fingerprinting of Hydrocarbons. In: Introduction to Environmental Forensics, (B. Murphy and R. Morrison, Eds.), Academic Press, New York, p. 135-260.

Appendix C. Analytical Chemistry Methods

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The following procedures detail the GC/MS analytical support provided for sediment and littleneck clam (*Leukoma staminea*) tissue samples collected during the NOAA field sampling program of Prince William Sound, Alaska, in July of 2007. The extraction procedures were different for each of the sample matrices; however, the instrumental analysis and report generation procedures were the same, regardless of matrix. The internal standard mixture referred to in each extraction procedure was n-eicosane-d_{42}, n-triacontane-d_{62}, naphthalene-d_8, anthracene-d_{10}, and benzo(a)pyrene-d_{12} (at a concentration of 10 ng/uL). The surrogate standard mixture, at a concentration of 20 ng/uL, referred to in each extraction procedure was n-hexadecane-d_{34} and n-octacosane-d_{58} for the alkane components, and phenanthrene-d_{10} and perylene-d_{12} for the PAH components. A matrix spike mixture included n-octadecane (alkane) and pyrene (PAH). Good laboratory practices were utilized for each of the extraction procedures.

Extraction Methodologies

Sediment Extraction Methodology

The selected sediment samples were homogenized by vigorous mixing, and then were sub-sampled for analysis. If the samples were frozen prior to sample extraction, they were first transferred to a refrigerator to thaw prior to extraction. For each sediment sample, 30 g of material was accurately weighed to the nearest 0.01g, and was placed into a pre-cleaned 500-mL beaker. Granular, anhydrous sodium sulfate was added and mixed into the sample until a "dry" sand-like matrix formed. One milliliter of surrogate standard was spiked directly onto the sample and then saturated immediately with 100-mL of high purity dichloromethane (DCM), followed by approximately one minute of stirring with a spatula. The solvent level was marked to monitor any changes in the solvent volume. The beaker was then covered with two layers of aluminum foil and placed in a slightly warm, ultrasonic bath for 15 minutes. The warm solvent and vigorous sonication aided in enhancing extraction efficiency by ensuring intimate contact of the sample with the solvent. At the end of the first extraction series, the extract was allowed to settle for about one hour.

For highly contaminated samples, no further extraction was required and the extract was filtered through anhydrous, sodium sulfate and into a pre-cleaned jar. One milliliter of the extract was then transferred with a clean graduated, gas-tight syringe into an autosampler vial. If a dilution of the extract was necessary, the volume of extract appropriate to achieve the correct dilution factor was added to the vial with a graduated, gas-tight syringe. Internal standard was added, and the vial was capped and made ready for analysis.

For samples containing low to trace contamination, the extract was poured through granular, anhydrous sodium sulfate into a rotary evaporation flask. The extraction procedure was repeated two more times, each time with a fresh portion of DCM added to cover the sediment/soil sample

in the beaker. During the last two extraction series, the sonication time was 15 minutes. Subsequent extracts were combined in a rotary evaporation flask and reduced to a final volume of 1-mL by a combination of rotary evaporation and "blowdown" under a gentle stream of purified nitrogen. The nitrogen blowdown was achieved by transferring the rotary evaporated extract with a disposable pipette into a graduated tube. The sample was then further concentrated to 1-mL. The 1-mL of extract was then transferred with a clean graduated, gas-tight syringe into a 2-mL autosampler vial. Internal standard was added, and the vial was capped and ready for analysis.

If the sample results were to be calculated based on dry weight, a portion of the sediment/soil sample was prepared for drying in an oven overnight. Five to ten grams of sample were weighed in a tared aluminum weigh boat. The weigh boat with the sample was placed in an oven set for 105°C overnight. The sample was removed and allowed to cool in a desiccator before determining the final, oven-dried weight of the sample. Percent dry weight was then calculated.

Tissue Extraction Methodology

Individual clams in each sample were thoroughly rinsed with distilled water (including the shell and the tissue) to remove any material not associated with the tissue itself. Since the clam samples were frozen prior to tissue extraction, the samples were transferred to a refrigerator until defrosted. All samples were shucked and the combined sample weight was recorded before homogenization. The organisms were then homogenized using a tissuemizer and stored in pre-cleaned jars. Approximately 5-10 grams of the homogenized tissue was removed from the sample and placed into a pre-cleaned 50-mL beaker. The weight of the homogenized tissue to be extracted was determined by the actual number of clam specimens collected. In some cases, specimens collected from the same sampling site may have required compositing to achieve a higher extraction volume. Tissue composites in excess of minimum required extraction mass of 10 grams of homogenized tissue, were used for replicate analyses or matrix-spiking with the matrix standard. Previous research at LSU has indicated no significant differences in the analyte recovery between the digestion and non-digestion methods; therefore, no digestion was performed.

Granular anhydrous sodium sulfate was added to the tissue in quantities of 15 g to 25 g, depending upon the amount of water within the tissues, or until a paste consistency was obtained. The sample was spiked with surrogate standard and then 35-mL of DCM was added to the paste. The beaker was covered with two layers of aluminum foil and sonicated for 15 minutes. After sonication, the solvent extract was filtered through additional anhydrous sodium sulfate and pre-cleaned glass wool into a round bottom flask. The entire extraction procedure was repeated two more times with fresh aliquots of DCM.

To concentrate the solvent extract, the sample was rotary evaporated to approximately a 2-mL final volume in DCM. The sample was then split: 1-mL for lipid analysis; and 1-mL for GC/MS analysis. The GC/MS sample was solvent-exchanged from DCM to hexane by adding 40-mL of hexane to the 1-mL GC/MS fraction of the extract. The sample was concentrated again by rotary evaporation and nitrogen blowdown to 2-mL in hexane. The sample was fractionated on an alumina/silica gel column by placing the 2-mL hexane aliquot on the aluminum/silica gel column, which was then rinsed with high purity hexane. The flow of hexane was stopped prior to exposing the silica gel to air. This fraction contained the normal alkanes. The alumina/silica gel

column was then rinsed with 50% DCM and 50% hexane. The solvents were allowed to elute completely. This fraction contained the PAHs. The alkane and PAH fractions were combined and concentrated to 0.l-mL under a gentle stream of nitrogen and stored until GC/MS analysis.

For the determination of dry lipid weight, the 1-mL lipid sub-sample was filtered through a clean, 0.1 micron filter into a clean, pre-weighed scintillation vial. The scintillation vial was then loosely covered and the solvent allowed to evaporate. The dry lipid weight was recorded and the final lipid weight calculated and reported.

Instrumental Analysis (Gas Chromatography/Mass Spectrometry)

The following GC/MS instrumental analysis procedure was the same for each of the matrices listed above.

GC Operation

All GC/MS analyses used an Agilent 7890A GC system configured with a 5% diphenyl/95% dimethyl polysiloxane high resolution capillary column (30 meter, 0.25 mm ID, 0.25 micron film) directly interfaced to an Agilent 5975 inert XL MS detector system. An Agilent 7638B series Auto Injector was used for sample introduction into the GC/MS system. The GC flow rates were optimized to provide a required degree of separation, particularly n-C_{17} and pristane should have been near baseline-resolved, and n-C_{18} and phytane should have been baseline-resolved. The injection temperature was set at 250°C and only high-temperature, low thermal-bleed septa were used in the GC inlet. The GC was operated in the temperature program mode with an initial column temperature of 55°C for 3 minutes then increased to 280°C at a rate of 5°C/minute and held for 3 minutes. The oven was then heated from 280°C to 300°C at a rate of 1.5°C/min and held at 300°C for two minutes. Total run time was 66.33 minutes per sample. The interface to the MS was maintained at 280°C. Ultra High Purity Helium was the carrier gas for the GC/MS system.

Mass Spectrometer Operation

The mass spectrometer was operated in the Selective Ion Monitoring (SIM) mode to maximize the detection of several trace target constituents unique to crude oil. The instrument was operated such that the selected ions for each acquisition window were scanned at a rate greater than 1.4 scans/sec with a dwell time of 60 milli-seconds. At the start of each analysis period, or every twelve hours, the MS was tuned to perfluorotributylamine, an internal instrument standard. Laboratory reference standards such as a reference oil and a continuing calibration standard were also analyzed prior to the analysis of the unknown sample extracts. This standard operating procedure ensured quality assurance/quality control of the instrument conditions prior to sample analysis.

Quantitative Analysis

Spectral data were processed by Chemstation™ software using a customized data analysis method developed by DES. The analysis method was run on each sample and results in raw integration data that was transferred to a spreadsheet program for quantitative analysis. Each macro printout contained the extracted ion chromatography data in addition to raw integration

data, which was exported to an Excel ™ spreadsheet for quantitative analysis. Each macro printout was carefully reviewed and reintegrated as required.

Analyte concentrations were calculated based on the internal standard method. Therefore, the internal standard mixture was spiked into the sample extracts just prior to analysis.

The concentration of specific target oil analytes was determined by a 5-point calibration and internal standard method. Standards containing parent (non-alkylated) hydrocarbons were used in the calibration curve. Alkylated homologues were quantified using the response factor of the parent, and were therefore only semiquantitative. This was the standard procedure since alkylated standards were not available.

Surrogate Corrections

Recovery of all trace level samples was estimated using the surrogate standard mix of phenanthrene-d_{10}, perylene-d_{12}, n-hexadecane-d_{34}, and n-octacosane-d_{58}, (at a concentration of 20 ng/uL). Sediment samples were never corrected for recovery, but a 70%-120% surrogate recovery acceptance criteria was applied. Tissue samples were corrected for recovery using the surrogate standard mix and similar surrogate recovery acceptance criteria (70%-120%).

Report Generation

Spectral data were processed by Agilent Chemstation™ software using a customized data processing method and macro developed by LSU/DES. Each data file was reviewed and re-integrated as needed after the initial processing. The macro printed a specified set of chromatograms that were used for qualitative comparison. The data processing method created a custom report containing the raw integration data, which were then exported to a spreadsheet for quantitative analysis.

The concentrations of specific target alkanes and PAHs were determined by response factors calculated from commercially available internal and calibration standards. The internal standards for this project were naphthalene-d_8, anthracene-d_{10}, benzo(a)pyrene-d_{12}, n-eicosane-d_{42} and n-triacontane-d_{62}. The calibration standards were prepared at five different concentrations (5-point calibration curve) and contained saturate alkanes in the nC_{10} through nC_{35} range and each parent aromatic hydrocarbon. The calibration curve results in response factors were used to calculate the individual analyte concentrations in the samples. It is important to note that the alkylated homologues in the extracted samples were quantified by response factors generated by the unalkylated parent (e.g. the response factor generated for naphthalene (C-0) was used to calculate the C-l through C-4 naphthalene homologues). Therefore, the results of the quantified alkylated homologues were semi-quantitative only since alkylated homologue standards were not available. Recovery and extraction efficiency of all trace level samples were estimated using the surrogate standard mix. Acceptable surrogate recoveries were in the range of 70%-120%.

LSU reports all tissue data values on a dry sample weight basis including analytes detected below the maximum detection limit, data values for the QA/QC procedures, and surrogate recovery information. Other data to be included are the GC/MS total ion chromatograms and any other ion chromatograms decided on by NOAA and LSU.

Appendix D. Sediment and Tissue Chemistry Results for the July 2007 PWS Survey

Table 11. Alkane concentrations (ng/mg-dry) within sediments excavated from PWS long-term intertidal monitoring sites during the 2007 field survey

Laboratory ID	**2N7226-27**	**2N7226-30**	**2N7226-31**	**2N7226-34**	**2N7226-39**	**2N7226-42**	**2N7226-43**	**2N7226-46**
Field ID	**SS01**	**SS04**	**SB01**	**SB04**	**SS05**	**SS08**	**SB05**	**SB08**
Site ID	**Sheep Q1**	**Sheep Q4**	**Sheep Q1**	**Sheep Q4**	**NWB Q1**	**NWB Q4**	**NWB Q1**	**NWB Q4**
Depth Level	**Surface**	**Surface**	**Sub-Surface**	**Sub-Surface**	**Surface**	**Surface**	**Sub-Surface**	**Sub-Surface**
Moisture (%)	**42**	**35**	**29**	**33**	**31**	**37**	**29**	**21**
nC-10 Decane	ND	ND	ND	ND	ND	ND	ND	ND
nC11 Undecane	ND	ND	ND	ND	ND	ND	ND	ND
nC12 Dodecane	ND	ND	ND	ND	ND	ND	ND	ND
nC13 Tridecane	ND	ND	ND	ND	ND	ND	ND	ND
nC14 Tetradecane	ND	ND	ND	ND	ND	ND	ND	ND
nC15 Pentadecane	ND	ND	ND	ND	ND	ND	ND	0.0001
nC16 Hexadecane	ND	ND	0.0003	0.0002	ND	0.0005	ND	ND
nC17 Heptadecane	0.0003	0.0004	0.0005	0.0004	0.0003	ND	0.0002	0.0002
Pristane	0.0013	0.0013	0.0019	0.0017	0.0014	0.0010	0.0011	0.0013
nC18 Octadecane	0.0002	0.0003	0.0002	0.0002	0.0000	0.0002	0.0001	0.0002
Phytane	0.0011	0.0011	0.0013	0.0014	0.0011	0.0012	0.0006	0.0009
nC19 Nonadecane	0.0011	0.0019	0.0011	0.0011	0.0015	0.0010	0.0008	0.0009
nC20 Eicosane	0.0010	0.0010	0.0004	0.0005	0.0010	0.0007	0.0006	0.0005
nC21 Heneicosane	0.0012	0.0009	0.0007	0.0004	0.0012	0.0009	0.0010	0.0008
nC22 Docosane	0.0011	0.0011	0.0008	0.0007	0.0011	0.0005	0.0013	0.0009
nC23 Tricosane	0.0008	0.0009	0.0009	0.0004	0.0009	0.0005	0.0006	0.0005
nC24 Tetracosane	0.0011	0.0011	0.0009	0.0007	0.0006	0.0007	0.0005	0.0006
nC25 Pentacosane	0.0012	0.0009	0.0006	0.0005	0.0004	0.0015	0.0012	0.0010
nC26 Hexacosane	0.0013	0.0008	0.0009	0.0011	0.0011	0.0008	0.0007	0.0007
nC27 Heptacosane	0.0010	0.0013	0.0004	0.0010	0.0010	0.0006	0.0008	0.0006
nC28 Octacosane	0.0014	0.0012	0.0010	0.0005	0.0006	0.0008	0.0006	0.0007
nC29 Nonacosane	0.0011	0.0011	0.0010	0.0004	0.0003	0.0005	0.0006	0.0007
nC30 Triacontane	0.0006	0.0007	0.0005	0.0002	0.0002	0.0012	0.0010	0.0005
nC31 Hentriacontane	0.0005	0.0004	0.0004	0.0001	0.0002	0.0005	0.0009	0.0008
nC32 Dotriacontane	0.0005	0.0003	0.0007	0.0002	0.0003	0.0005	0.0004	0.0003
nC33 Tritriacontane	0.0003	0.0004	0.0002	0.0002	0.0002	0.0004	ND	ND
nC34 Tetratriacontane	0.0001	ND	0.0001	0.0001	ND	ND	ND	ND
nC35 Pentatriacontane	0.0001	ND	ND	0.0001	ND	ND	0.0002	ND
TOTAL ALKANES	**0.0175**	**0.0173**	**0.0150**	**0.0123**	**0.0136**	**0.0141**	**0.0129**	**0.0123**

Table 11. Alkane concentrations (ng/mg-dry) within sediments excavated from PWS long-term intertidal monitoring sites during the 2007 field survey (continued)

Laboratory ID	2N7226-46MS	2N7226-46MSD	2N7226-53	2N7226-56	2N7226-62	2N7226-65	2N7226-66
Field ID	**SB08**	**SB08**	**SS13**	**SS16**	**SB13**	**SB16**	**SB17**
Site ID	**NWB Q4**	**NWB Q4**	**Mussel Q1**	**Mussel Q4**	**Mussel Q1**	**Mussel Q4**	**Block Q1**
Depth Level	**Matrix Spike**	**Matrix Spike Duplicate**	**Surface**	**Surface**	**Sub-Surface**	**Sub-Surface**	**Sub-Surface**
Moisture (%)	**21**	**21**	**27**	**20**	**19**	**19**	**37**
nC-10 Decane	ND	ND	ND	ND	0.0006	ND	ND
nC11 Undecane	ND	ND	ND	ND	ND	ND	ND
nC12 Dodecane	ND	ND	ND	ND	ND	ND	ND
nC13 Tridecane	ND	ND	ND	ND	ND	ND	ND
nC14 Tetradecane	ND	ND	ND	ND	ND	ND	ND
nC15 Pentadecane	0.0002	ND	ND	ND	ND	0.0007	0.0001
nC16 Hexadecane	ND	0.0002	ND	0.0003	ND	ND	0.0002
nC17 Heptadecane	ND	0.0003	0.0002	0.0002	0.0002	0.0002	0.0006
Pristane	0.0011	0.0011	0.0010	0.0018	0.0016	0.0018	0.0020
nC18 Octadecane	0.3336	0.3622	0.0002	0.0003	0.0002	0.0002	0.0005
Phytane	0.0009	0.0008	0.0015	0.0016	0.0019	0.0014	0.0020
nC19 Nonadecane	0.0008	0.0007	0.0006	0.0015	0.0013	0.0010	0.0005
nC20 Eicosane	0.0006	0.0007	0.0006	0.0006	0.0005	0.0006	0.0005
nC21 Heneicosane	0.0005	0.0004	0.0005	0.0007	0.0005	0.0004	0.0005
nC22 Docosane	0.0007	0.0008	0.0013	0.0003	0.0002	0.0002	0.0003
nC23 Tricosane	0.0005	0.0005	0.0012	0.0005	0.0004	0.0004	0.0009
nC24 Tetracosane	0.0004	0.0002	0.0013	0.0005	0.0004	0.0004	0.0010
nC25 Pentacosane	0.0009	0.0008	0.0011	0.0011	0.0008	0.0002	0.0016
nC26 Hexacosane	0.0006	0.0006	0.0009	0.0012	0.0014	0.0008	0.0018
nC27 Heptacosane	0.0003	0.0004	0.0007	0.0015	0.0016	0.0018	0.0028
nC28 Octacosane	0.0006	0.0005	0.0005	0.0018	0.0020	0.0021	0.0020
nC29 Nonacosane	0.0005	0.0003	0.0004	0.0016	0.0012	0.0014	0.0020
nC30 Triacontane	0.0006	0.0005	0.0004	0.0014	0.0011	0.0012	0.0011
nC31 Hentriacontane	0.0006	0.0005	0.0003	0.0018	0.0013	0.0010	0.0013
nC32 Dotriacontane	0.0001	0.0002	0.0006	0.0011	0.0011	0.0009	0.0009
nC33 Tritriacontane	ND	0.0001	0.0003	ND	ND	ND	0.0010
nC34 Tetratriacontane	0.0001	ND	ND	ND	ND	0.0003	0.0001
nC35 Pentatriacontane	ND	ND	ND	0.0006	ND	ND	0.0001
TOTAL ALKANES	**0.3436**	**0.3718**	**0.0134**	**0.0202**	**0.0183**	**0.0172**	**0.0237**

Table 11. Alkane concentrations (ng/mg-dry) within sediments excavated from PWS long-term intertidal monitoring sites during the 2007 field survey (continued)

Laboratory ID	2N7226-69	2N7226-70	2N7226-73	2N7226-75	2N7226-78
Field ID	**SB20**	**SS21**	**SS24**	**SB21**	**SB24**
Site ID	**Block Q4**	**Bainbridge Q2**	**Bainbridge Q6**	**Bainbridge Q2**	**Bainbridge Q6**
Depth Level	**Sub-Surface**	**Surface**	**Surface**	**Sub-Surface**	**Sub-Surface**
Moisture (%)	**29**	**26**	**34**	**32**	**28**
nC-10 Decane	ND	ND	ND	ND	ND
nC11 Undecane	ND	ND	ND	ND	ND
nC12 Dodecane	ND	ND	ND	ND	ND
nC13 Tridecane	ND	ND	ND	ND	ND
nC14 Tetradecane	ND	ND	ND	ND	ND
nC15 Pentadecane	ND	ND	ND	ND	ND
nC16 Hexadecane	ND	0.0003	ND	ND	0.0004
nC17 Heptadecane	0.0002	0.0002	0.0003	0.0004	0.0002
Pristane	0.0018	0.0019	0.0016	0.0015	0.0015
nC18 Octadecane	0.0002	0.0004	0.0004	0.0002	0.0003
Phytane	0.0015	0.0021	0.0019	0.0017	0.0017
nC19 Nonadecane	0.0001	0.0014	0.0008	0.0006	0.0014
nC20 Eicosane	0.0001	0.0009	0.0004	0.0010	0.0007
nC21 Heneicosane	0.0004	0.0014	0.0006	0.0009	0.0007
nC22 Docosane	0.0001	0.0003	0.0004	0.0011	0.0009
nC23 Tricosane	0.0010	0.0014	0.0006	0.0010	0.0009
nC24 Tetracosane	0.0003	0.0001	0.0007	0.0010	0.0019
nC25 Pentacosane	0.0004	0.0002	0.0010	0.0013	0.0015
nC26 Hexacosane	0.0023	0.0004	0.0022	0.0015	0.0027
nC27 Heptacosane	0.0016	0.0019	0.0018	0.0016	0.0017
nC28 Octacosane	0.0011	0.0015	0.0015	0.0014	0.0010
nC29 Nonacosane	0.0008	0.0012	0.0012	0.0011	0.0009
nC30 Triacontane	0.0005	0.0009	0.0007	0.0009	0.0006
nC31 Hentriacontane	0.0002	0.0003	0.0004	ND	0.0004
nC32 Dotriacontane	0.0002	ND	ND	0.0008	0.0002
nC33 Tritriacontane	0.0002	0.0002	0.0008	ND	ND
nC34 Tetratriacontane	0.0003	ND	0.0006	0.0006	ND
nC35 Pentatriacontane	ND	0.0001	ND	0.0005	ND
TOTAL ALKANES	**0.0130**	**0.0171**	**0.0178**	**0.0190**	**0.0196**

Table 12. Aromatic hydrocarbon concentrations (ng/mg-dry) within sediments excavated from PWS long-term intertidal monitoring sites during the 2007 field survey

Laboratory ID	**2N7226-27**	**2N7226-30**	**2N7226-31**	**2N7226-34**	**2N7226-39**
Field ID	**SS01**	**SS04**	**SB01**	**SB04**	**SS05**
Site ID	**Sheep Q1**	**Sheep Q4**	**Sheep Q1**	**Sheep Q4**	**NWB Q1**
Depth Level	**Surface**	**Surface**	**Sub-Surface**	**Sub-Surface**	**Surface**
Moisture (%)	**42**	**35**	**29**	**33**	**31**
Naphthalene	ND	ND	ND	ND	ND
C-1 Naphthalene	ND	ND	ND	ND	ND
C-2 Naphthalene	ND	ND	ND	ND	ND
C-3 Naphthalene	ND	0.0010	ND	ND	ND
C-4 Naphthalene	0.0009	0.0013	0.0008	ND	0.0012
Fluorene	ND	ND	ND	ND	ND
C-1 Fluorene	ND	ND	ND	ND	ND
C-2 Fluorene	0.0011	0.0012	ND	ND	0.0014
C-3 Fluorene	0.0014	0.0009	0.0008	0.0008	0.0011
Dibenzothiophene	ND	ND	ND	ND	ND
C-1 Dibenzothiophene	ND	ND	ND	ND	ND
C-2 Dibenzothiophene	0.0009	ND	0.0013	ND	0.0009
C-3 Dibenzothiophene	0.0007	0.0008	0.0008	ND	0.0010
Phenanthrene	ND	ND	ND	ND	ND
C-1 Phenanthrene	ND	ND	ND	ND	ND
C-2 Phenanthrene	ND	ND	0.0012	ND	ND
C-3 Phenanthrene	0.0011	0.0006	0.0011	0.0012	0.0015
C-4 Phenanthrene	0.0010	0.0008	ND	0.0009	0.0012
Anthracene	ND	ND	ND	ND	ND
Fluoranthene	ND	ND	ND	ND	ND
Pyrene	ND	ND	ND	ND	ND
C-1 Pyrene	ND	ND	ND	ND	ND
C-2 Pyrene	ND	0.0006	0.0008	ND	0.0015
C-3 Pyrene	0.0005	0.0008	0.0009	0.0007	0.0018
C-4 Pyrene	0.0007	0.0005	ND	0.0008	0.0014
Napthobenzothiophene	ND	ND	ND	ND	ND
C-1 Napthobenzothiophene	ND	ND	ND	ND	ND
C-2 Napthobenzothiophene	0.0005	ND	0.0012	ND	0.0002
C-3 Napthobenzothiophene	0.0003	0.0009	0.0007	0.0015	0.0012
Benzo (a) Anthracene	ND	ND	ND	ND	0.0001
Chrysene	ND	ND	ND	ND	0.0001
C-1 Chrysene	ND	ND	ND	ND	ND
C-2 Chrysene	0.0004	ND	0.0006	ND	0.0008
C-3 Chrysene	0.0009	0.0011	0.0005	0.0008	0.0013
C-4 Chrysene	0.0002	0.0005	0.0005	0.0011	0.0018
Benzo (b) Fluoranthene	ND	0.0004	ND	ND	ND
Benzo (k) Fluoranthene	ND	ND	ND	ND	0.0011
Benzo (e) Pyrene	0.0014	ND	0.0004	0.0005	ND
Benzo (a) Pyrene	0.0008	ND	ND	0.0008	ND
Perylene	ND	ND	0.0003	ND	ND
Indeno (1,2,3 - cd) Pyrene	ND	0.0003	ND	ND	ND
Dibenzo (a,h) Anthracene	0.0009	ND	0.0004	ND	0.0009
Benzo (g,h,i) Perylene	ND	0.0003	ND	0.0009	ND
TOTAL PAH	**0.0137**	**0.0120**	**0.0123**	**0.0100**	**0.0205**

Table 12. Aromatic hydrocarbon concentrations (ng/mg-dry) within sediments excavated from PWS long-term intertidal monitoring sites during the 2007 field survey (continued)

Laboratory ID	2N7226-42	2N7226-43	2N7226-46	2N7226-46MS	2N7226-46MSD
Field ID	**SS08**	**SB05**	**SB08**	**SB08**	**SB08**
Site ID	**NWB Q4**	**NWB Q1**	**NWB Q4**	**NWB Q4**	**NWB Q4**
Depth Level	**Surface**	**Sub-Surface**	**Sub-Surface**	**Matrix Spike**	**Spike Duplicate**
Moisture (%)	**37**	**29**	**21**	**21**	**21**
Naphthalene	ND	ND	ND	ND	ND
C-1 Naphthalene	ND	ND	ND	ND	ND
C-2 Naphthalene	ND	ND	ND	ND	ND
C-3 Naphthalene	ND	ND	ND	ND	ND
C-4 Naphthalene	ND	ND	ND	ND	ND
Fluorene	ND	ND	ND	ND	ND
C-1 Fluorene	0.0005	ND	ND	ND	ND
C-2 Fluorene	ND	0.0008	0.0009	0.0005	ND
C-3 Fluorene	ND	0.0011	0.0015	0.0011	0.0013
Dibenzothiophene	ND	ND	ND	ND	0.0001
C-1 Dibenzothiophene	ND	ND	ND	ND	ND
C-2 Dibenzothiophene	0.0015	0.0006	0.0012	0.0010	0.0009
C-3 Dibenzothiophene	0.0016	0.0014	0.0013	0.0009	0.0012
Phenanthrene	ND	ND	ND	ND	ND
C-1 Phenanthrene	ND	ND	ND	ND	ND
C-2 Phenanthrene	0.0005	0.0009	0.0013	ND	0.0002
C-3 Phenanthrene	0.0012	0.0015	0.0014	0.0015	0.0013
C-4 Phenanthrene	0.0010	0.0012	0.0012	0.0014	0.0012
Anthracene	ND	ND	ND	ND	ND
Fluoranthene	ND	ND	ND	ND	ND
Pyrene	ND	ND	0.0001	0.3717	0.3402
C-1 Pyrene	ND	ND	0.0001	ND	ND
C-2 Pyrene	0.0004	ND	0.0009	0.0012	ND
C-3 Pyrene	0.0011	0.0013	0.0013	0.0014	0.0015
C-4 Pyrene	0.0010	0.0018	0.0011	0.0013	0.0010
Napthobenzothiophene	ND	ND	ND	ND	ND
C-1 Napthobenzothiophene	ND	ND	ND	ND	ND
C-2 Napthobenzothiophene	0.0014	ND	0.0005	0.0006	0.0004
C-3 Napthobenzothiophene	0.0018	0.0015	0.0016	0.0011	0.0013
Benzo (a) Anthracene	ND	ND	ND	ND	ND
Chrysene	ND	ND	ND	ND	ND
C-1 Chrysene	ND	ND	ND	ND	ND
C-2 Chrysene	ND	0.0008	0.0009	ND	0.0004
C-3 Chrysene	0.0008	0.0010	0.0011	0.0012	0.0015
C-4 Chrysene	0.0006	0.0014	0.0013	0.0010	0.0013
Benzo (b) Fluoranthene	ND	ND	ND	ND	ND
Benzo (k) Fluoranthene	ND	ND	ND	ND	ND
Benzo (e) Pyrene	ND	0.0001	0.0001	ND	0.0002
Benzo (a) Pyrene	0.0007	0.0004	ND	0.0003	0.0001
Perylene	ND	ND	ND	ND	ND
Indeno (1,2,3 - cd) Pyrene	0.0008	0.0005	0.0005	ND	0.0001
Dibenzo (a,h) Anthracene	0.0005	ND	ND	0.0002	0.0001
Benzo (g,h,i) Perylene	ND	ND	ND	ND	ND
TOTAL PAH	**0.0154**	**0.0164**	**0.0183**	**0.3864**	**0.3543**

Table 12. Aromatic hydrocarbon concentrations (ng/mg-dry) within sediments excavated from PWS long-term intertidal monitoring sites during the 2007 field survey (continued)

Laboratory ID	2N7226-53	2N7226-56	2N7226-62	2N7226-65	2N7226-66
Field ID	SS13	SS16	SB13	SB16	SB17
Site ID	Mussel Q1	Mussel Q4	Mussel Q1	Mussel Q4	Block Q1
Depth Level	Surface	Surface	Sub-Surface	Sub-Surface	Sub-Surface
Moisture (%)	27	20	19	19	37
Naphthalene	ND	ND	ND	ND	ND
C-1 Naphthalene	ND	ND	ND	ND	ND
C-2 Naphthalene	ND	ND	ND	ND	ND
C-3 Naphthalene	ND	0.0002	0.0007	0.0014	0.0012
C-4 Naphthalene	0.0004	ND	0.0006	0.0013	0.0009
Fluorene	ND	ND	ND	ND	ND
C-1 Fluorene	ND	ND	ND	ND	ND
C-2 Fluorene	0.0012	0.0005	0.0008	ND	0.0009
C-3 Fluorene	0.0014	0.0007	0.0003	ND	0.0016
Dibenzothiophene	ND	ND	ND	ND	ND
C-1 Dibenzothiophene	ND	ND	ND	ND	ND
C-2 Dibenzothiophene	0.0005	ND	ND	0.0004	0.0021
C-3 Dibenzothiophene	0.0002	0.0004	0.0003	0.0013	0.0019
Phenanthrene	ND	ND	ND	ND	ND
C-1 Phenanthrene	ND	ND	ND	ND	ND
C-2 Phenanthrene	ND	0.0002	0.0002	0.0005	0.0011
C-3 Phenanthrene	0.0008	0.0009	0.0009	0.0010	0.0012
C-4 Phenanthrene	0.0003	0.0014	0.0015	ND	0.0015
Anthracene	ND	ND	ND	ND	ND
Fluoranthene	ND	ND	ND	ND	ND
Pyrene	ND	ND	ND	ND	ND
C-1 Pyrene	ND	ND	ND	ND	ND
C-2 Pyrene	ND	0.0002	0.0002	ND	0.0019
C-3 Pyrene	0.0005	0.0006	0.0010	0.0005	0.0015
C-4 Pyrene	0.0008	0.0011	0.0015	0.0012	0.0007
Napthobenzothiophene	ND	ND	ND	ND	ND
C-1 Napthobenzothiophene	ND	ND	ND	ND	ND
C-2 Napthobenzothiophene	0.0015	0.0009	ND	0.0005	0.0019
C-3 Napthobenzothiophene	0.0010	0.0008	0.0012	0.0016	0.0016
Benzo (a) Anthracene	ND	ND	ND	ND	ND
Chrysene	ND	ND	ND	ND	ND
C-1 Chrysene	ND	ND	ND	ND	ND
C-2 Chrysene	ND	ND	0.0005	ND	ND
C-3 Chrysene	0.0010	ND	0.0009	0.0005	0.0014
C-4 Chrysene	0.0005	0.0014	0.0011	0.0005	0.0015
Benzo (b) Fluoranthene	ND	ND	ND	ND	ND
Benzo (k) Fluoranthene	0.0008	ND	0.0004	ND	ND
Benzo (e) Pyrene	0.0003	0.0002	ND	ND	0.0011
Benzo (a) Pyrene	ND	0.0011	0.0005	0.0006	0.0012
Perylene	ND	0.0005	ND	0.0004	ND
Indeno (1,2,3 - cd) Pyrene	0.0005	0.0001	0.0005	0.0002	ND
Dibenzo (a,h) Anthracene	ND	ND	ND	ND	0.0004
Benzo (g,h,i) Perylene	ND	ND	ND	ND	ND
TOTAL PAH	**0.0116**	**0.0113**	**0.0131**	**0.0119**	**0.0257**

Table 12. Aromatic hydrocarbon concentrations (ng/mg-dry) within sediments excavated from PWS long-term intertidal monitoring sites during the 2007 field survey (continued)

Laboratory ID	2N7226-69	2N7226-70	2N7226-73	2N7226-75	2N7226-78
Field ID	SB20	SS21	SS24	SB21	SB24
Site ID	Block Q4	Bainbridge Q2	Bainbridge Q6	Bainbridge Q2	Bainbridge Q6
Depth Level	Sub-Surface	Surface	Surface	Sub-Surface	Sub-Surface
Moisture (%)	29	26	34	32	28
Naphthalene	ND	ND	ND	ND	ND
C-1 Naphthalene	ND	ND	ND	ND	ND
C-2 Naphthalene	ND	ND	ND	ND	0.0006
C-3 Naphthalene	0.0001	0.0005	0.0015	ND	0.0010
C-4 Naphthalene	0.0003	0.0008	ND	0.0010	0.0013
Fluorene	ND	ND	ND	ND	ND
C-1 Fluorene	ND	ND	ND	ND	ND
C-2 Fluorene	0.0002	0.0005	0.0002	0.0003	0.0012
C-3 Fluorene	0.0006	0.0006	0.0006	0.0008	0.0015
Dibenzothiophene	ND	ND	ND	ND	ND
C-1 Dibenzothiophene	ND	ND	ND	ND	ND
C-2 Dibenzothiophene	0.0009	ND	ND	ND	0.0011
C-3 Dibenzothiophene	0.0001	0.0015	ND	ND	ND
Phenanthrene	ND	ND	0.0021	0.0013	0.0002
C-1 Phenanthrene	ND	ND	ND	ND	ND
C-2 Phenanthrene	ND	ND	0.0010	0.0004	ND
C-3 Phenanthrene	0.0015	0.0011	0.0014	0.0014	0.0011
C-4 Phenanthrene	0.0021	0.0001	0.0015	0.0018	0.0013
Anthracene	ND	ND	ND	ND	ND
Fluoranthene	ND	ND	ND	ND	ND
Pyrene	ND	ND	ND	ND	ND
C-1 Pyrene	ND	0.0002	ND	ND	0.0002
C-2 Pyrene	0.0007	ND	0.0009	ND	0.0008
C-3 Pyrene	0.0006	0.0009	0.0011	0.0015	0.0014
C-4 Pyrene	0.0011	0.0012	ND	0.0011	ND
Napthobenzothiophene	ND	ND	ND	ND	ND
C-1 Napthobenzothiophene	ND	ND	ND	0.0002	ND
C-2 Napthobenzothiophene	0.0006	0.0010	ND	ND	0.0009
C-3 Napthobenzothiophene	0.0003	0.0011	ND	0.0019	0.0013
Benzo (a) Anthracene	ND	ND	ND	ND	ND
Chrysene	ND	ND	ND	ND	0.0001
C-1 Chrysene	ND	ND	ND	0.0004	ND
C-2 Chrysene	0.0002	ND	0.0002	ND	0.0008
C-3 Chrysene	0.0005	0.0008	0.0009	0.0011	0.0006
C-4 Chrysene	0.0008	0.0006	0.0013	0.0009	0.0010
Benzo (b) Fluoranthene	ND	ND	ND	ND	ND
Benzo (k) Fluoranthene	0.0000	0.0002	ND	ND	0.0001
Benzo (e) Pyrene	0.0002	ND	0.0002	0.0003	ND
Benzo (a) Pyrene	0.0000	0.0002	0.0005	0.0005	0.0002
Perylene	ND	ND	0.0004	ND	ND
Indeno (1,2,3 - cd) Pyrene	0.0002	ND	0.0001	0.0001	0.0006
Dibenzo (a,h) Anthracene	0.0001	0.0002	ND	ND	ND
Benzo (g,h,i) Perylene	0.0001	ND	ND	0.0002	ND
TOTAL PAH	**0.0112**	**0.0115**	**0.0139**	**0.0152**	**0.0173**

Table 13. Alkane concentrations (ng/mg-dry) within clam tissue collected at PWS long-term intertidal monitoring sites during the 2007 field survey

Laboratory ID	2N7226-15	2N7226-16	2N7226-19	2N7226-17	2N7226-18	2N7226-20	2N7226-21	2N7226-05
Field ID	**TC17**	**TC 18**	**TC 20A**	**TC 19**	**TC 20**	**TC 41**	**TC 24**	**TC 04A**
Site ID	**Block Q1**	**Block Q2**	**Block Q5 &Q6**	**Block Q3**	**Block Q4**	**Bainbridge**	**Bainbridge**	**Sheep Q5**
Solids (%)	**14.6**	**13.7**	**15.1**	**15.7**	**14.9**	**16.1**	**16.3**	**15.8**
nC-10 Decane	ND	ND	ND	ND	ND	ND	ND	ND
nC11 Undecane	ND	ND	0.0003	ND	0.0002	ND	ND	ND
nC12 Dodecane	ND	0.0003	ND	ND	ND	ND	ND	ND
nC13 Tridecane	ND	ND	ND	0.0010	ND	ND	ND	0.0003
nC14 Tetradecane	0.0003	0.0003	ND	0.0009	0.0010	0.0010	ND	0.0002
nC15 Pentadecane	0.0008	0.0010	0.0010	ND	ND	0.0003	ND	0.0003
nC16 Hexadecane	0.0003	0.0003	0.0006	0.0006	0.0007	0.0016	ND	ND
nC17 Heptadecane	ND	ND	0.0001	0.0001	0.0003	ND	0.0002	0.0004
Pristane	0.0017	0.0006	0.0008	0.0011	0.0023	0.0007	0.0019	0.0009
nC18 Octadecane	ND	ND	0.0003	0.0003	0.0002	0.0002	0.0001	0.0002
Phytane	0.0010	0.0011	0.0012	0.0011	0.0012	0.0019	0.0011	0.0019
nC19 Nonadecane	0.0007	0.0007	0.0006	0.0013	0.0015	0.0032	0.0018	0.0004
nC20 Eicosane	0.0002	0.0002	0.0003	0.0007	0.0008	0.0010	0.0006	0.0027
nC21 Heneicosane	0.0002	0.0002	0.0014	0.0031	0.0033	0.0023	0.0014	0.0031
nC22 Docosane	0.0004	0.0004	0.0001	0.0003	0.0003	0.0005	0.0003	0.0014
nC23 Tricosane	0.0010	0.0010	0.0004	0.0009	0.0009	0.0005	0.0007	0.0002
nC24 Tetracosane	0.0014	0.0014	0.0007	0.0016	0.0002	0.0005	0.0003	0.0002
nC25 Pentacosane	0.0029	0.0028	0.0002	0.0003	0.0004	0.0006	0.0004	0.0005
nC26 Hexacosane	0.0029	0.0029	0.0021	0.0004	0.0005	0.0010	0.0006	0.0005
nC27 Heptacosane	0.0029	0.0028	0.0025	0.0005	0.0005	0.0003	0.0017	0.0007
nC28 Octacosane	0.0023	0.0023	0.0016	0.0004	0.0004	0.0009	0.0006	0.0006
nC29 Nonacosane	0.0019	0.0019	0.0015	0.0003	0.0003	0.0011	0.0007	0.0008
nC30 Triacontane	0.0010	0.0001	0.0007	0.0002	0.0002	0.0007	0.0005	0.0006
nC31 Hentriacontane	0.0008	0.0008	0.0011	0.0002	0.0002	0.0006	0.0004	0.0008
nC32 Dotriacontane	0.0006	0.0005	0.0007	0.0016	0.0002	0.0002	0.0002	0.0006
nC33 Tritriacontane	0.0003	0.0007	0.0011	0.0023	0.0003	0.0003	0.0002	0.0006
nC34 Tetratriacontane	0.0014	0.0012	0.0007	0.0019	0.0002	0.0007	0.0005	0.0002
nC35 Pentatriacontane	0.0018	0.0021	0.0005	0.0010	0.0008	0.0003	0.0002	0.0001
TOTAL ALKANES	**0.0267**	**0.0255**	**0.0208**	**0.0224**	**0.0169**	**0.0204**	**0.0141**	**0.0182**

Table 13. Alkane concentrations (ng/mg-dry) within clam tissue collected at PWS long-term intertidal monitoring sites during the 2007 field survey (continued)

Laboratory ID	2N7226-05MS	2N7226-05MSD	2N7226-01	2N7226-02	2N7226-03	2N7226-09	2N7226-07
Field ID	**TC 04A**	**TC 04A**	**TC 01**	**TC 02**	**TC 03**	**TC 08b**	**TC 07**
Site ID	**Sheep Q5**	**Sheep Q5**	**Sheep Q1**	**Sheep Q2**	**Sheep Q3**	**NW Bay**	**NW Bay**
QA/QC	**Matrix Spike**	**Matrix Spike Duplicate**	**Tissue**	**Tissue**	**Tissue**	**Tissue**	**Tissue**
Solids (%)	**15.8**	**15.8**	**17.0**	**15.2**	**16.3**	**14.5**	**15.9**
nC-10 Decane	ND	ND	ND	ND	0.0006	ND	0.0004
nC11 Undecane	ND	ND	ND	ND	ND	ND	0.0005
nC12 Dodecane	ND	0.0006	ND	ND	ND	ND	0.0004
nC13 Tridecane	ND	ND	0.0005	0.0006	0.0005	0.0004	0.0008
nC14 Tetradecane	ND	ND	0.0003	ND	0.0003	ND	0.0006
nC15 Pentadecane	0.0004	ND	0.0005	0.0008	0.0005	0.0007	0.0013
nC16 Hexadecane	0.0016	0.0016	0.0006	0.0003	0.0012	ND	0.0009
nC17 Heptadecane	0.0004	0.0003	0.0001	0.0002	0.0002	0.0002	0.0003
Pristane	0.0012	0.0019	0.0008	0.0013	0.0006	0.0008	0.0015
nC18 Octadecane	0.3646	0.3258	0.0003	0.0004	0.0002	0.0002	0.0002
Phytane	0.0013	0.0015	0.0011	0.0011	0.0009	0.0010	0.0010
nC19 Nonadecane	0.0005	0.0008	0.0006	0.0016	0.0013	0.0010	0.0018
nC20 Eicosane	0.0002	0.0005	0.0005	0.0006	0.0005	0.0002	0.0005
nC21 Heneicosane	0.0008	0.0008	0.0005	0.0007	0.0005	0.0003	0.0006
nC22 Docosane	0.0001	0.0002	0.0003	0.0003	0.0002	0.0002	0.0004
nC23 Tricosane	0.0001	0.0004	0.0004	0.0005	0.0004	0.0001	0.0006
nC24 Tetracosane	0.0001	0.0001	0.0004	0.0005	0.0004	0.0001	0.0002
nC25 Pentacosane	0.0001	0.0002	0.0008	0.0011	0.0008	0.0002	0.0004
nC26 Hexacosane	0.0002	0.0003	0.0009	0.0012	0.0009	0.0003	0.0007
nC27 Heptacosane	0.0003	0.0006	0.0012	0.0015	0.0011	0.0009	0.0017
nC28 Octacosane	0.0002	0.0002	0.0011	0.0013	0.0010	0.0003	0.0006
nC29 Nonacosane	0.0002	0.0003	0.0013	0.0016	0.0012	0.0004	0.0007
nC30 Triacontane	0.0002	0.0002	0.0011	0.0014	0.0011	0.0002	0.0004
nC31 Hentriacontane	0.0003	0.0002	0.0014	0.0018	0.0013	0.0003	0.0005
nC32 Dotriacontane	0.0003	0.0008	0.0002	0.0021	0.0015	0.0002	0.0003
nC33 Tritriacontane	0.0002	0.0011	0.0015	0.0019	0.0016	0.0001	0.0001
nC34 Tetratriacontane	0.0001	0.0002	0.0014	0.0019	0.0014	0.0001	0.0002
nC35 Pentatriacontane	0.0001	0.0002	0.0007	0.0009	0.0009	0.0001	0.0003
TOTAL ALKANES	**0.3733**	**0.3388**	**0.0184**	**0.0253**	**0.0210**	**0.0085**	**0.0178**

Table 13. Alkane concentrations (ng/mg-dry) within clam tissue collected at PWS long-term intertidal monitoring sites during the 2007 field survey (continued)

Laboratory ID	**2N7226-12**	**2N7226-12MS**	**2N7226-12MSD**	**2N7226-13**	**2N7226-14**	**2N7226-24**	**2N7226-25**
Field ID	**TC 15**	**TC 15**	**TC 15**	**TC 16**	**TC 16A**	**TC 35**	**TC 36**
Site ID	**Mussel Q3**	**Mussel Q3**	**Mussel Q3**	**Mussel Q4**	**Mussel Q5 & Q6**	**Snug**	**Snug**
QA/QC	**Tissue**	**Matrix Spike**	**Spike Duplicate**	**Tissue**	**Tissue**	**Tissue**	**Tissue**
Solids (%)	**16.3**	**16.3**	**16.3**	**14.8**	**16.7**	**13.9**	**15.1**
nC-10 Decane	ND	ND	ND	ND	0.0006	0.0007	ND
nC11 Undecane	ND	0.0005	ND	ND	ND	ND	0.0008
nC12 Dodecane	ND	0.0005	ND	0.0014	0.0006	ND	0.0008
nC13 Tridecane	0.0004	ND	ND	ND	ND	0.0005	0.0014
nC14 Tetradecane	ND	ND	0.0003	0.0011	0.0005	0.0006	0.0014
nC15 Pentadecane	0.0006	0.0007	0.0008	ND	ND	ND	ND
nC16 Hexadecane	0.0004	0.0022	0.0007	ND	ND	ND	0.0014
nC17 Heptadecane	0.0001	0.0004	ND	0.0002	0.0006	ND	ND
Pristane	0.0005	0.0014	0.0024	0.0017	0.0022	0.0012	0.0009
nC18 Octadecane	0.0002	0.3258	0.3056	0.0004	0.0004	0.0002	0.0006
Phytane	0.0016	0.0022	0.0020	0.0023	0.0016	0.0015	0.0027
nC19 Nonadecane	0.0008	0.0007	0.0016	0.0014	0.0008	0.0006	0.0024
nC20 Eicosane	0.0002	0.0002	0.0005	0.0009	0.0004	0.0020	0.0007
nC21 Heneicosane	0.0004	0.0003	0.0007	0.0024	0.0011	0.0020	0.0007
nC22 Docosane	0.0002	0.0009	0.0002	0.0003	0.0014	0.0011	0.0039
nC23 Tricosane	0.0002	0.0003	0.0010	0.0014	0.0006	0.0025	0.0009
nC24 Tetracosane	0.0003	0.0011	0.0024	0.0001	0.0007	0.0010	0.0029
nC25 Pentacosane	0.0008	0.0002	0.0005	0.0002	0.0010	0.0014	0.0005
nC26 Hexacosane	0.0015	0.0003	0.0007	0.0004	0.0017	0.0018	0.0007
nC27 Heptacosane	0.0013	0.0007	0.0023	0.0004	0.0018	0.0006	0.0022
nC28 Octacosane	0.0010	0.0003	0.0007	0.0003	0.0015	0.0016	0.0006
nC29 Nonacosane	0.0011	0.0003	0.0006	0.0003	0.0015	0.0016	0.0006
nC30 Triacontane	0.0015	0.0002	0.0004	0.0002	0.0007	0.0009	0.0003
nC31 Hentriacontane	0.0014	0.0002	0.0005	0.0002	0.0010	0.0012	0.0005
nC32 Dotriacontane	0.0010	0.0019	0.0001	ND	ND	0.0008	0.0003
nC33 Tritriacontane	0.0006	0.0008	0.0002	0.0002	0.0008	0.0022	0.0009
nC34 Tetratriacontane	0.0003	0.0014	0.0004	0.0003	0.0016	0.0014	0.0006
nC35 Pentatriacontane	0.0004	0.0007	0.0003	ND	ND	0.0005	0.0020
TOTAL ALKANES	**0.0168**	**0.3445**	**0.3249**	**0.0161**	**0.0230**	**0.0278**	**0.0306**

Table 14. Aromatic hydrocarbon concentrations (ng/mg-dry) within clam tissue collected at PWS long-term intertidal monitoring sites during the 2007 field survey

Laboratory ID	**2N7226-15**	**2N7226-16**	**2N7226-19**	**2N7226-17**	**2N7226-18**
Field ID	**TC17**	**TC 18**	**TC 20A**	**TC 19**	**TC 20**
Site ID	**Block Q1**	**Block Q2**	**Block Q5 &Q6**	**Block Q3**	**Block Q4**
Solids (%)	**14.6**	**13.7**	**15.1**	**15.7**	**14.9**
Naphthalene	ND	ND	0.0013	ND	ND
C-1 Naphthalene	0.0021	0.0015	0.0012	0.0030	0.0028
C-2 Naphthalene	0.0016	0.0004	0.0003	0.0022	0.0008
C-3 Naphthalene	0.0016	0.0002	0.0002	0.0020	0.0004
C-4 Naphthalene	0.0026	0.0001	0.0002	0.0016	ND
Fluorene	ND	ND	0.0003	0.0005	ND
C-1 Fluorene	0.0004	0.0003	0.0006	0.0011	0.0022
C-2 Fluorene	0.0012	0.0002	0.0015	<MDL	0.0051
C-3 Fluorene	0.0008	ND	0.0002	0.0003	0.0005
Dibenzothiophene	0.0002	0.0001	0.0001	0.0002	ND
C-1 Dibenzothiophene	0.0019	ND	0.0001	0.0002	0.0005
C-2 Dibenzothiophene	0.0005	ND	ND	0.0003	ND
C-3 Dibenzothiophene	0.0002	ND	ND	ND	ND
Phenanthrene	0.0021	ND	ND	0.0019	ND
C-1 Phenanthrene	0.0009	0.0002	0.0024	0.0001	0.0082
C-2 Phenanthrene	0.0005	0.0001	0.0001	0.0007	0.0004
C-3 Phenanthrene	0.0002	ND	ND	0.0036	ND
C-4 Phenanthrene	0.0007	ND	0.0001	0.0007	0.0004
Anthracene	0.0001	0.0021	0.0012	0.0002	0.0004
Fluoranthene	0.0003	0.0003	0.0002	<MDL	0.0004
Pyrene	0.0002	0.0002	0.0001	0.0002	0.0002
C-1 Pyrene	0.0007	ND	ND	ND	ND
C-2 Pyrene	ND	ND	ND	ND	ND
C-3 Pyrene	ND	ND	ND	ND	ND
C-4 Pyrene	ND	ND	ND	ND	ND
Napthobenzothiophene	ND	ND	ND	ND	ND
C-1 Napthobenzothiophene	ND	ND	ND	ND	ND
C-2 Napthobenzothiophene	ND	ND	ND	ND	ND
C-3 Napthobenzothiophene	ND	ND	ND	ND	ND
Benzo (a) Anthracene	0.0002	0.0002	0.0002	0.0004	0.0004
Chrysene	<MDL	0.0002	0.0002	0.0001	0.0004
C-1 Chrysene	ND	ND	ND	ND	ND
C-2 Chrysene	0.0002	0.0002	0.0003	0.0000	0.0007
C-3 Chrysene	ND	ND	ND	0.0000	0.0002
C-4 Chrysene	ND	ND	ND	ND	ND
Benzo (b) Fluoranthene	<MDL	ND	ND	ND	ND
Benzo (k) Fluoranthene	0.0002	0.0002	ND	ND	ND
Benzo (e) Pyrene	<MDL	0.0007	0.0007	0.0001	0.0016
Benzo (a) Pyrene	0.0006	0.0007	0.0007	0.0011	0.0017
Perylene	0.0002	0.0003	0.0002	0.0004	0.0005
Indeno (1,2,3 - cd) Pyrene	<MDL	ND	ND	0.0001	ND
Dibenzo (a,h) Anthracene	ND	ND	ND	ND	ND
Benzo (g,h,i) Perylene	ND	ND	ND	ND	ND
TOTAL PAH	**0.0204**	**0.0083**	**0.0126**	**0.0208**	**0.0279**

Table 14. Aromatic hydrocarbon concentrations (ng/mg-dry) within clam tissue collected at PWS long-term intertidal monitoring sites during the 2007 field survey (continued)

Laboratory ID	2N7226-20	2N7226-21	2N7226-05	2N7226-05MS	2N7226-05MSD
Field ID	**TC 41**	**TC 24**	**TC 04A**	**TC 04A**	**TC 04A**
Site ID	**Bainbridge**	**Bainbridge**	**Sheep Q5**	**Sheep Q5**	**Sheep Q5**
QA/QC	**Tissue**	**Tissue**	**Tissue**	**Matrix Spike**	**Spike Duplicate**
Solids (%)	**16.1**	**16.3**	**15.8**	**15.8**	**15.8**
Naphthalene	0.0002	0.0015	0.0002	ND	ND
C-1 Naphthalene	0.0005	0.0037	0.0018	0.0002	0.0005
C-2 Naphthalene	0.0006	0.0045	0.0013	0.0002	0.0003
C-3 Naphthalene	0.0006	0.0044	0.0011	0.0001	0.0003
C-4 Naphthalene	0.0004	0.0033	ND	ND	ND
Fluorene	0.0001	ND	0.0002	<MDL	0.0005
C-1 Fluorene	0.0003	0.0031	ND	ND	ND
C-2 Fluorene	ND	0.0016	ND	ND	ND
C-3 Fluorene	<MDL	0.0001	<MDL	<MDL	ND
Dibenzothiophene	<MDL	0.0001	<MDL	<MDL	0.0002
C-1 Dibenzothiophene	ND	ND	ND	ND	ND
C-2 Dibenzothiophene	ND	ND	ND	ND	ND
C-3 Dibenzothiophene	ND	ND	ND	ND	ND
Phenanthrene	0.0007	0.0061	0.0013	0.0002	0.0031
C-1 Phenanthrene	0.0002	0.0015	0.0018	0.0001	0.0022
C-2 Phenanthrene	0.0002	0.0009	0.0003	<MDL	0.0005
C-3 Phenanthrene	0.0009	0.0084	0.0012	0.0001	0.0015
C-4 Phenanthrene	<MDL	<MDL	ND	<MDL	ND
Anthracene	0.0007	0.0002	0.0014	<MDL	0.0034
Fluoranthene	0.0001	0.0003	0.0001	ND	0.0004
Pyrene	0.0001	0.0005	0.0001	0.3717	0.3402
C-1 Pyrene	ND	ND	0.0001	<MDL	0.0002
C-2 Pyrene	ND	ND	ND	ND	ND
C-3 Pyrene	ND	ND	ND	ND	ND
C-4 Pyrene	ND	ND	ND	ND	ND
Napthobenzothiophene	ND	ND	ND	ND	ND
C-1 Napthobenzothiophene	ND	ND	ND	ND	ND
C-2 Napthobenzothiophene	ND	ND	ND	ND	ND
C-3 Napthobenzothiophene	ND	ND	ND	ND	ND
Benzo (a) Anthracene	0.0001	0.0006	0.0003	<MDL	0.0005
Chrysene	<MDL	0.0001	<MDL	<MDL	0.0001
C-1 Chrysene	ND	ND	ND	ND	ND
C-2 Chrysene	<MDL	<MDL	<MDL	<MDL	<MDL
C-3 Chrysene	ND	ND	ND	ND	ND
C-4 Chrysene	ND	ND	ND	ND	ND
Benzo (b) Fluoranthene	ND	ND	ND	ND	ND
Benzo (k) Fluoranthene	ND	ND	ND	ND	ND
Benzo (e) Pyrene	<MDL	0.0001	0.0001	<MDL	0.0002
Benzo (a) Pyrene	0.0003	0.0019	0.0010	0.0002	0.0020
Perylene	0.0002	0.0011	0.0005	<MDL	0.0008
Indeno (1,2,3 - cd) Pyrene	<MDL	0.0002	0.0003	<MDL	0.0002
Dibenzo (a,h) Anthracene	ND	ND	ND	ND	ND
Benzo (g,h,i) Perylene	ND	ND	ND	ND	ND
TOTAL PAH	**0.0062**	**0.0443**	**0.0131**	**0.3730**	**0.3571**

Table 14. Aromatic hydrocarbon concentrations (ng/mg-dry) within clam tissue collected at PWS long-term intertidal monitoring sites during the 2007 field survey (continued)

Laboratory ID	**2N7226-01**	**2N7226-02**	**2N7226-03**	**2N7226-09**	**2N7226-07**
Field ID	**TC 01**	**TC 02**	**TC 03**	**TC 08b**	**TC 07**
Site ID	**Sheep Q1**	**Sheep Q2**	**Sheep Q3**	**NW Bay**	**NW Bay**
Moisture (%)	**17.0**	**15.2**	**16.3**	**14.5**	**15.9**
Naphthalene	0.0003	ND	ND	0.0016	ND
C-1 Naphthalene	0.0003	0.0038	0.0027	0.0027	0.0054
C-2 Naphthalene	0.0002	0.0029	0.0019	0.0025	0.0055
C-3 Naphthalene	0.0002	0.0033	0.0017	0.0024	0.0055
C-4 Naphthalene	ND	ND	ND	0.0019	0.0040
Fluorene	0.0004	0.0005	0.0003	0.0006	ND
C-1 Fluorene	0.0040	0.0011	0.0011	ND	ND
C-2 Fluorene	0.0027	0.0015	0.0011	ND	ND
C-3 Fluorene	0.0022	0.0031	0.0022	ND	ND
Dibenzothiophene	ND	ND	ND	0.0003	0.0007
C-1 Dibenzothiophene	ND	ND	ND	ND	ND
C-2 Dibenzothiophene	ND	ND	ND	ND	ND
C-3 Dibenzothiophene	ND	ND	ND	ND	ND
Phenanthrene	0.0021	0.0029	0.0020	0.0025	0.0049
C-1 Phenanthrene	0.0028	0.0035	0.0025	0.0016	0.0026
C-2 Phenanthrene	0.0007	0.0010	0.0006	0.0006	0.0011
C-3 Phenanthrene	0.0018	ND	ND	0.0008	0.0014
C-4 Phenanthrene	0.0003	ND	ND	ND	ND
Anthracene	0.0023	0.0031	0.0022	0.0026	0.0051
Fluoranthene	0.0003	0.0004	0.0003	0.0003	ND
Pyrene	0.0002	0.0003	0.0003	0.0002	0.0005
C-1 Pyrene	0.0001	0.0002	0.0002	0.0001	0.0003
C-2 Pyrene	ND	ND	ND	ND	ND
C-3 Pyrene	ND	ND	ND	ND	ND
C-4 Pyrene	ND	ND	ND	ND	ND
Napthobenzothiophene	ND	ND	ND	ND	ND
C-1 Napthobenzothiophene	ND	ND	ND	ND	ND
C-2 Napthobenzothiophene	ND	ND	ND	ND	ND
C-3 Napthobenzothiophene	ND	ND	ND	ND	ND
Benzo (a) Anthracene	0.0006	0.0008	0.0006	0.0003	0.0006
Chrysene	0.0001	0.0001	0.0001	0.0000	0.0001
C-1 Chrysene	ND	ND	ND	ND	ND
C-2 Chrysene	<MDL	<MDL	<MDL	<MDL	<MDL
C-3 Chrysene	ND	ND	ND	ND	ND
C-4 Chrysene	ND	ND	ND	ND	ND
Benzo (b) Fluoranthene	ND	ND	ND	ND	ND
Benzo (k) Fluoranthene	0.0001	0.0002	0.0001	<MDL	0.0001
Benzo (e) Pyrene	0.0001	0.0001	0.0004	0.0001	0.0005
Benzo (a) Pyrene	0.0016	0.0021	0.0015	0.0009	0.0019
Perylene	0.0008	0.0010	0.0007	0.0005	0.0009
Indeno (1,2,3 - cd) Pyrene	0.0002	0.0002	0.0002	0.0002	0.0004
Dibenzo (a,h) Anthracene	ND	ND	ND	ND	ND
Benzo (g,h,i) Perylene	ND	ND	ND	ND	ND
TOTAL PAH	**0.0242**	**0.0320**	**0.0227**	**0.0228**	**0.0415**

Table 14. Aromatic hydrocarbon concentrations (ng/mg-dry) within clam tissue collected at PWS long-term intertidal monitoring sites during the 2007 field survey (continued)

Laboratory ID	**2N7226-12**	**2N7226-12MS**	**2N7226-12MSD**	**2N7226-13**	**2N7226-14**
Field ID	**TC 15**	**TC 15**	**TC 15**	**TC 16**	**TC 16A**
Site ID	**Mussel Q3**	**Mussel Q3**	**Mussel Q3**	**Mussel Q4**	**Mussel Q5 & Q6**
QA/QC	**Tissue**	**Matrix Spike**	**Spike Duplicate**	**Tissue**	**Tissue**
Solids (%)	**16.3**	**16.3**	**16.3**	**14.8**	**16.7**
Naphthalene	0.0022	0.0001	0.0019	0.0009	<MDL
C-1 Naphthalene	0.0024	0.0009	0.0020	0.0010	0.0042
C-2 Naphthalene	0.0019	0.0017	0.0021	0.0007	0.0025
C-3 Naphthalene	0.0016	0.0016	0.0023	0.0005	0.0025
C-4 Naphthalene	0.0013	ND	0.0017	ND	<MDL
Fluorene	0.0004	0.0005	0.0006	0.0001	0.0004
C-1 Fluorene	0.0003	ND	ND	<MDL	<MDL
C-2 Fluorene	<MDL	ND	0.0001	<MDL	<MDL
C-3 Fluorene	<MDL	<MDL	0.0018	0.0006	<MDL
Dibenzothiophene	0.0002	0.0008	0.0008	0.0039	0.0016
C-1 Dibenzothiophene	ND	ND	ND	ND	ND
C-2 Dibenzothiophene	ND	ND	ND	ND	ND
C-3 Dibenzothiophene	ND	ND	ND	ND	ND
Phenanthrene	0.0019	0.0030	ND	0.0005	0.0021
C-1 Phenanthrene	0.0021	0.0045	0.0036	0.0014	0.0005
C-2 Phenanthrene	0.0004	0.0008	0.0010	0.0002	0.0010
C-3 Phenanthrene	0.0008	0.0019	0.0031	0.0011	0.0004
C-4 Phenanthrene	0.0001	0.0001	ND	0.0001	<MDL
Anthracene	0.0020	0.0032	0.0032	0.0006	0.0024
Fluoranthene	0.0003	0.0005	0.0005	0.0001	0.0002
Pyrene	0.0002	0.3412	0.3248	<MDL	0.0002
C-1 Pyrene	0.0001	0.0002	0.0004	ND	ND
C-2 Pyrene	ND	ND	ND	ND	ND
C-3 Pyrene	ND	ND	ND	ND	ND
C-4 Pyrene	ND	ND	ND	ND	ND
Napthobenzothiophene	ND	ND	ND	ND	ND
C-1 Napthobenzothiophene	ND	ND	ND	ND	ND
C-2 Napthobenzothiophene	ND	ND	ND	ND	ND
C-3 Napthobenzothiophene	ND	ND	ND	ND	ND
Benzo (a) Anthracene	0.0002	0.0003	0.0004	0.0001	0.0005
Chrysene	<MDL	0.0001	0.0002	<MDL	<MDL
C-1 Chrysene	ND	ND	ND	ND	ND
C-2 Chrysene	<MDL	<MDL	<MDL	<MDL	<MDL
C-3 Chrysene	ND	ND	ND	ND	ND
C-4 Chrysene	ND	ND	ND	ND	ND
Benzo (b) Fluoranthene	ND	ND	ND	ND	ND
Benzo (k) Fluoranthene	0.0002	0.0001	0.0001	<MDL	ND
Benzo (e) Pyrene	0.0001	0.0002	0.0002	<MDL	<MDL
Benzo (a) Pyrene	0.0007	0.0011	0.0012	0.0001	0.0005
Perylene	0.0003	0.0005	0.0005	0.0001	0.0006
Indeno (1,2,3 - cd) Pyrene	0.0002	0.0001	0.0003	<MDL	0.0001
Dibenzo (a,h) Anthracene	ND	ND	ND	ND	ND
Benzo (g,h,i) Perylene	ND	ND	ND	ND	ND
TOTAL PAH	**0.0199**	**0.3635**	**0.3527**	**0.0123**	**0.0430**

Table 14. Aromatic hydrocarbon concentrations (ng/mg-dry) within clam tissue collected at PWS long-term intertidal monitoring sites during the 2007 field survey (continued)

Laboratory ID	2N7226-24	2N7226-25
Field ID	**TC 35**	**TC 36**
Site ID	**Snug**	**Snug**
Solids (%)	**13.9**	**15.1**
Naphthalene	0.0014	<MDL
C-1 Naphthalene	0.0018	0.0075
C-2 Naphthalene	0.0017	0.0066
C-3 Naphthalene	0.0011	0.0063
C-4 Naphthalene	0.0010	0.0033
Fluorene	0.0004	0.0015
C-1 Fluorene	<MDL	0.0052
C-2 Fluorene	<MDL	0.0043
C-3 Fluorene	<MDL	0.0037
Dibenzothiophene	0.0001	0.0006
C-1 Dibenzothiophene	0.0013	<MDL
C-2 Dibenzothiophene	ND	ND
C-3 Dibenzothiophene	ND	ND
Phenanthrene	0.0013	0.0059
C-1 Phenanthrene	0.0006	0.0023
C-2 Phenanthrene	0.0004	0.0016
C-3 Phenanthrene	0.0004	0.0019
C-4 Phenanthrene	ND	ND
Anthracene	0.0015	0.0062
Fluoranthene	0.0002	ND
Pyrene	0.0002	0.0007
C-1 Pyrene	0.0001	0.0006
C-2 Pyrene	ND	ND
C-3 Pyrene	ND	ND
C-4 Pyrene	ND	ND
Napthobenzothiophene	ND	ND
C-1 Napthobenzothiophene	ND	ND
C-2 Napthobenzothiophene	ND	ND
C-3 Napthobenzothiophene	ND	ND
Benzo (a) Anthracene	0.0002	0.0007
Chrysene	<MDL	0.0001
C-1 Chrysene	ND	ND
C-2 Chrysene	<MDL	0.0008
C-3 Chrysene	ND	ND
C-4 Chrysene	ND	ND
Benzo (b) Fluoranthene	ND	ND
Benzo (k) Fluoranthene	<MDL	0.0001
Benzo (e) Pyrene	0.0001	0.0003
Benzo (a) Pyrene	0.0005	0.0021
Perylene	0.0002	0.0008
Indeno (1,2,3 - cd) Pyrene	0.0001	0.0005
Dibenzo (a,h) Anthracene	ND	ND
Benzo (g,h,i) Perylene	ND	ND
TOTAL PAH	**0.0146**	**0.0635**