*Exxon Valdez* Oil Spill
Restoration Project Final Report

# Monitoring of Oiled Mussel Beds in Prince William Sound and the Gulf of Alaska

Restoration Project 00090
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Mark G. Carls and Patricia M. Harris

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National Marine Fisheries Service
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October 2005

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**Study history:** After the 1989 *Exxon Valdez* oil spill, many researchers began measuring hydrocarbon concentrations in mussels, sediment, and other matrices in Prince William Sound to assess resource damage. The specific focus of this project was dense aggregations of intertidal mussels (*Mytilus trossulus*) within the slick trajectory. Oil accumulated in these filter-feeders; total polynuclear aromatic hydrocarbon concentrations were high in 1989 and remained relatively high for several years in mussel beds on unconsolidated sediment. The persistence of oil in these beds in Prince William Sound and along the Gulf of Alaska caused concern, and beginning in 1992 heavily oiled beds were systematically surveyed for oil content in mussel tissue and underlying sediment. Projections from earlier data (through 1995) were that oil would persist for up to three decades, thus funding for study was extended through 1999. This report synthesizes all available natural resource damage assessment data to develop a coherent picture of spatial and temporal oil distributions in mussels and sediment.

**Abstract:** *Exxon Valdez* oil trapped in intertidal sediment in Prince William Sound degraded slowly, was biologically available for at least a decade, and was toxic for at least 9 years. Habitat condition controlled the biological availability of oil. Exposure duration was short where mussels were only exposed to oil in water (months) and long (6 to 10 years) at locations where oil remained in sediment. Limited evidence suggests some oiling extended outside previously reported slick boundaries; polynuclear aromatic hydrocarbon (PAH) sources were verified with three independent petrogenic composition models. After an initial peak, oil concentrations typically declined in sediment and mussels, although distribution was non-uniform and variability was often high. Oil may persist in some intertidal sediment for >50 years, but declines in mussel tissue suggest it became less available to surface organisms and this community is recovering. Attempts to manually accelerate hydrocarbon loss from mussel beds were equivocal, demonstrating that removal of oil from intertidal sediment is difficult. Exposure to residual oil may explain why some vertebrate populations (pigeon guillemots, *Cepphus columba*, and sea otters, *Enhydra lutris*) that forage in the most heavily impacted areas have not yet fully recovered from the spill.

**Key words:** mussels; *Mytilus trossulus*; Prince William Sound; Gulf of Alaska; *Exxon Valdez*; petroleum hydrocarbons; polynuclear aromatic hydrocarbons; PAH; oil spills; persistence; monitoring, recovery

**Project data:** *Description of data* - Hydrocarbon data are available in the State/Federal trustee council hydrocarbon database 1989-2004 (EVTHD). All other data are archived in spreadsheets; graphics files are in AutoCAD formats. Text files are in Word format. *Custodian* - Contact Patricia M. Harris, NOAA/NMFS, Auke Bay Laboratory, 11305 Glacier Highway, Juneau, AK 99801 (work phone: (907) 789-6022, fax: (907) 789-6094, or email pat.harris@noaa.gov. *Availability* - Copies of the report are available on CDROM for the cost of duplication.

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# Table of Contents

## List of Tables



## List of Figures

## Executive Summary

The long-term exposure of mussels (*Mytilus trossulus*) to *Exxon Valdez* oil, the oiling of the intertidal habitat they occupy, and the long term implications for vertebrate predators that utilize this community are inextricably linked. Intertidal oil was retained longer than expected in the aftermath of the spill, thus oil was biologically available for long periods at toxic levels. The objectives of this study were to monitor, describe, and compare hydrocarbon concentrations and distributions in sediment, mussels and other invertebrates that utilize intertidal habitat. A synoptic perspective of the oil impact was developed over space and through time by analyzing all pertinent Natural Resource Damage Assessment data. Although the hydrocarbon data collected by industry were not available to us, application of oil-identification techniques employed by industry (Bence and Burns 1995; Page et al. 2005) allowed a balanced analysis of government data.

Following the *Exxon Valdez* oil spill, mussel beds on sediment were recognized as vulnerable, yet valuable habitat and were protected from cleanup activity (Babcock et al. 1996). Mussels physically stabilize sediment, provide community structure in intertidal areas, and are preyed on by a variety of organisms. Although there were no reports of catastrophic mussel loss due to oiling (e.g., Gilfillian et al. 1995), aggressive shoreline cleanup procedures such as hot-water washes and mechanical displacement of colonized substrate were destructive (Mearns 1996), thus mussel beds were not cleaned. Not anticipated were the slow natural rates of hydrocarbon loss from this habitat, placing the mussel community and consumer species dependent on it at risk of chronic hydrocarbon exposure (Babcock et al. 1996; Carls et al. 2001). In retrospect, the length of impact is not surprising, rather it is consistent with other spill experience.

Oil trapped in coarse-grained gravel sediment on intertidal beaches degraded slowly, remained toxic, and was often protected from removal by cobble-boulder armoring (this report and Hayes and Michel 1999; Peterson et al. 2003). Protected subsurface oil reservoirs, therefore, remained a chronic source of exposure in intertidal habitat. In a study that focused on problem areas (heavily oiled mussel beds), estimates of oil persistence in Prince William Sound sediment ranged from 5 to 30 years (Carls et al. 2001). These results are corroborated and extended by a synthesis of 17 independent studies and include 1909 mussel samples and 1505 sediment samples (Chapter 1). Hydrocarbons in mussel tissue provided evidence that persistent oil was biologically available for at least a decade. Examination of polynuclear aromatic hydrocarbon (PAH) concentrations and composition in Prince William Sound sediment and the experimentally determined relationship between these factors and toxicity allowed estimates of the toxicity of remaining oil. These observations are consistent with other spill data (e.g., Blumer et al. 1973; Gilfillan and Vandermeulen 1978; Reddy et al. 2002). In a review of seven well-studied oil spills, Teal and Howarth (1984) conclude that oil effects can persist for at least 6-12 years in sediment.

Evidence that hydrocarbon levels consistently declined after the Exxon spill demonstrate that natural recovery occurred. Recovery was more rapid in mussels than in sediment (Chapter 2). Total PAH concentrations in mussels frequently reached background levels by 1999. In contrast, oil remained in the sediment of 17 of 22 heavily oiled beds and might persist > 50 years in some of these, yet declines in mean total PAH concentrations also provide evidence of natural recovery. Because investigators often focused on oiled areas after the spill instead of collecting samples at random, these results best describe events in heavily impacted areas and likely underestimate true natural recovery rates. Nevertheless, a randomly-designed study has

demonstrated that *Exxon Valdez* oil remains the largest reservoir of biologically available PAH in Prince William Sound (Short et al. 2004).

Attempts to manually accelerate hydrocarbon loss from mussel beds were equivocal. In our experiment, contaminated surface sediment in 9 beds was replaced (33 metric tons) with clean sediment and the original mussels were returned (Chapter 3). Hydrocarbon concentrations in mussels and sediment were monitored for 5 years. Increased short-term oil loss was apparent, but long-term (5 year) improvement was equivocal and difficult to distinguish from natural losses. At the end of study, total PAH concentrations in replaced sediment were elevated in one third of restored beds, indicating recontamination from underlying or surrounding sediment. Oil concentrations in mussels typically fell to background levels in both restored and oiled reference beds. Post-restoration mussel population fluctuations were indistinguishable from regional changes. Our results suggest mussel relocation is feasible but demonstrate that removal of oil from intertidal sediment is difficult.

The need for a comprehensive approach to oil identification became obvious as synoptic mussel and sediment data were analyzed. This resulted in formulation of novel non-parametric pyrogenic and petrogenic (oil) identification models. Model results were compared to traditional ratio discrimination methods and two independently published petrogenic models. To provide a balanced, unbiased approach, all models were combined into a single output that identified each sample on a continuum ranging from highly pyrogenic to highly petrogenic. This approach was used when synthesizing Prince William Sound sediment and mussel data (Chapter 1).

Habitat condition is the key factor controlling biological availability of oil to the intertidal community. Persistence of oil in mussels was short (months) on shorelines not coated by oil and on beaches where mussels were only exposed to dissolved or particulate oil in water (Carls et al. 2002). Persistence of oil in mussels was longer (up to 10 years or more) on beaches where oil remained in sediment (Babcock et al. 1996; Carls et al. 2001; Carls et al. 2004; Chapter 1).

The persistence of toxic oil in intertidal sediment, mussel beds, and associated fauna may explain the decadal exposure of predators to hydrocarbons such as pigeon guillemots (*Cepphus columba*) and sea otters (*Enhydra lutris*) and the failure of these populations to recover in oiled areas. Biochemical indicators of exposure to PAH persisted in sea otters and guillemots inhabiting oiled areas for 13 years (through 2002) before declining to background levels in 2004 (Bodkin et al. 2002; Golet et al. 2002; James Bodkin, personal communication). Populations of these predators in oiled areas failed to recover, in contrast to populations in reference areas, and continued oil exposure may be causal (Bodkin et al. 2002; Golet et al. 2002). Both species forage in nearshore environments; exposure to oiled sediment during feeding disturbance and consumption of oil-exposed fauna is likely the principal route of exposure for these predatory species. We do not know the relative contributions of oil in mussels or other prey species or oil in sediment to predator exposure but recognize that consumption of oiled mussels is likely not the sole route of exposure because concentrations in mussels generally declined to background levels before exposure ceased in predators. The data synthesis included in this report demonstrates persistence of toxic levels of *Exxon Valdez* oil in some intertidal sediment of Prince William Sound, thus supporting and corroborating pigeon guillemot and sea otter observations.

# Introduction

This report is subdivided into four chapters: 1) synthesis of mussel and sediment contamination, 2) an explicit extension of mussel and sediment data through 1999 as funded by the *Exxon Valdez* Oil Spill Trustee Council for this study, 3) results of manual mussel bed restoration efforts with observations extended through 1999, and 4) comparison of petroleum hydrocarbon detection models.

Chapter 1 synthesizes all available natural resource damage assessment data to develop a coherent picture of spatial and temporal oil distributions in mussels and sediment and predicts where oil concentrations remain toxic. A large data set was analyzed, >1900 mussel samples and >1500 sediment samples, assembled from 17 independent Natural Resource Damage Assessment studies. In addition to reporting total polynuclear aromatic hydrocarbon (PAH) concentrations, petrogenic and pyrogenic detection models were applied to determine if PAH compositions were consistent with *Exxon Valdez* oil. The workings of these models are detailed in chapter 4. The toxic potential of PAH in sediment was estimated by comparing observed concentrations and PAH composition to the lowest known concentrations known to cause significant damage to sensitive organisms (developing fish embryos). Results demonstrate that oil levels were highest within the slick trajectory, that PAH concentrations in mussels and sediment dropped with time, that long term (decadal) exposure occurred where oil was retained by sediment, and that some oil with toxic potential remained in sediment for at least a decade. These results provide a plausible explanation for long-term vertebrate exposure to oil and lack of population recovery in oiled areas.

The focus of chapter 2 is exclusively on the data explicitly funded for this report. Results extend the previous publication (Carls et al. 2001) from 1995 through 1999. More than half the sediment in these previously heavily oiled mussel beds (64%) was likely toxic in 1999. In contrast, TPAH concentrations in mussels in 1999 were frequently indistinguishable from background levels (79%).

Manual mussel bed restoration, chapter 3, has now been published (Carls and Harris 2004) and is complete through 1999. Restoration efforts accelerated short-term oil loss, but long-term (5 year) improvement was equivocal and difficult to distinguish from natural losses. By 1999, oil concentrations in mussels were typically at baseline levels in restored and oiled reference beds but concentrations in replaced sediment were elevated in one third of restored beds, indicating recontamination from underlying or surrounding sediment.

The final chapter, 4, presents a detailed comparison among three independently written oil-detection models, a pyrogenic model and pyrogenic PAH ratios, and petrogenic PAH ratios. Two of the petrogenic models have been published, one written by industry researchers (Bence and Burns 1995) and one by government researchers (Short and Heintz 1997). The third model is novel, reported here for the first time. PAH ratios have been traditionally used to discriminate among hydrocarbon sources. We conclude that used in combination, oil detection, avoidance of false positives, and source discrimination is improved beyond that afforded by any single model. The combined-model approach was used in chapter 1 to estimate the distribution of *Exxon Valdez* oil and simultaneously assesses each sample for pyrogenic and petrogenic content.

## Objectives

Objectives as proposed in the 2000 detailed study plan

1. Measure hydrocarbon concentrations in mussels and underlying sediments and mussel densities [populations] in beds that were restored in 1994 to evaluate degree of recontamination and to assess mussel bed health. Similar measures will be taken in uncleaned control beds for comparison.

2. Measure the hydrocarbon concentrations in mussels and underlying sediments and mussel densities in untreated mussel beds that remained contaminated with Exxon Valdez oil in 1995. Similar measures will be taken in uncleaned control beds for comparison.

3. Measure the hydrocarbon concentrations in selected invertebrate fauna associated with both categories of mussel beds. We will target prey species of vertebrate species still not fully recovered from the spill (harlequin duck, pigeon guillemot, sea otter, and black oystercatcher) and vertebrate species that may be prey of impacted species (nearshore forage fish).

## References


Babcock, M. M., Irvine, G. V., Harris, P. M., Cusik, J. A., & Rice, S. D. 1996. Persistence of oiling in mussel beds three and four years after the *Exxon Valdez* oil spill. *American Fisheries Society Symposium* 18:286-297.

Bence AE, Burns WA. 1995. Fingerprinting hydrocarbons in the biological resources of the Exxon Valdez spill area. Pages 84-140 *in* P.G. Wells, J.N. Butler, and J.S. Hughes (eds.), *Exxon Valdez* oil spill: fate and effects in Alaskan Waters, ASTM STP 1219, American Society for Testing and Materials, Philadelphia.

Blumer M, Ehrhardt M, Jones JH. 1973. The environmental fate of stranded crude oi. Deep-Sea Research 20:239-259.

Bodkin, J. L., Ballachey, B. E., Dean, T. A., Fukuyama, A. K., Jewett, S. C., McDonald, L., Monson, D. H., O=Clair, C. E., VanBlaricom, G. R. (2002). Sea otter population status and the process of recovery from the 1989 *Exxon Valdez* oil spill. *Marine Ecology Progress Series 241*, 237-253.

Carls, M.G., M.M. Babcock, P.M. Harris, G.V. Irvine, J.A. Cusick, and S.D. Rice. 2001. Persistence of Oiling in Mussel Beds after the *Exxon Valdez* Oil Spill. Marine Environmental Research 51:167-190.

Carls, M.G., G.D. Marty, J.E. Hose. 2002. Synthesis of the toxicological impacts of the *Exxon Valdez* oil spill on Pacific herring (*Clupea pallasi*) in Prince William Sound, Alaska, U.S.A. Can. J. Fish. Aquat. Sci. 59:1-20.

Carls, M.G. and P.M. Harris. 2004. Restoration of Oiled Mussel Beds in Prince William Sound, Alaska. Mar. Environ. Res. 57:359-376.

Gilfillan ES, Vandermeulen JH. 1978. Alterations in growth and physiology of soft-shell clams, *Mya arenaria*, chronically oiled with Bunker C from Chedabucto Bay, Nova Scotia, 1970-76. J. Fish. Res. Board Can. 35:630-636.

Gilfillan, E.S., D.S. Page, E.J. Harner, and P.D. Boehm. 1995. Shoreline ecology program for Prince William Sound, Alaska, following the *Exxon Valdez* oil spill: part 3 – biology. Pages 398-443 *in* P.G. Wells, J.N. Butler, and J.S. Hughes (eds.), *Exxon Valdez* oil spill:

fate and effects in Alaskan Waters, ASTM STP 1219, American Society for Testing and Materials, Philadelphia.

Golet, G. H., Seiser, P. E., McGuire, A. D., Roby, D. D., Fisher, J. B., Kuletz, K. J., Irons, D. B., Dean, T. A., Jewett, S. C., & Newman, S. H. (2002). Long-term direct and indirect effects of the *Exxon Valdez* oil spill on pigeon guillemots in Prince William Sound, Alaska. *Marine Ecology Progress Series, 241*, 287-304.

Hayes MO, Michel J. 1999. Factors determining the long-term persistence of *Exxon Valdez* oil in gravel beaches. Mar Pollut Bull 38:92-101.

Mearns, A. J. (1996). *Exxon Valdez* shoreline treatment and operations: implications for response, assessment, monitoring, and research. *American Fisheries Society Symposium, 18*, 309-328.

Peterson CH, Rice SD, Short JW, Esler D, Bodkin JL, Ballachey BE, Irons DB. 2003. Long-term ecosystem response to the *Exxon Valdez* oil spill. Science 302:2082-2086.

Reddy CM, Eglinton TI, Hounshell A, White HK, Xu L, Gaines RB, Frysinger GS. 2002. The West Falmouth oil spill after thirty years: the persistence of petroleum hydrocarbons in marsh sediments. Enviorn Sci Technol 36:4754-4760.

Short J., and R. Heintz. 1997. Identification of *Exxon Valdez* oil in sediments and tissues of PWS. Environ. Sci. Technol. 31:2375-2384.

Short JW, Lindeberg MR, Harris PM, Maselko JM, Pella JJ, Rice SD. 2004. Estimate of oil persisting on the beaches of Prince William Sound 12 years after the Exxon Valdez oil spill. Environ Sci Technol 38:19-25.

Teal JM, Howarth RW. 1984. Oil spill studies: a review of ecological effects. Environmental Management 8:27-44.

# Chapter 1

# Intertidal *Exxon Valdez* oil retention, bioavailability, and toxicity, 1989-2000 in Prince William Sound, Alaska

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## Abstract

*Exxon Valdez* oil stranded in intertidal sediment of Prince William Sound persisted at toxic levels in parts of the spill zone for at least 9 years. Hydrocarbon concentrations in mussels (*Mytilus trossulus*) and sediments from 17 studies were re-examined (3414 samples) to determine trends. *Exxon Valdez* oil accumulated in mussels for at least a decade in locations where oil was retained by sediment, providing evidence of long-term bioavailability. Occurrence of contaminated mussels declined with time: e.g., mean total polynuclear aromatic hydrocarbon (TPAH) concentrations were > 200 ng/g at 14 of 19 locations after 1991 but only at 3 of 13 locations in 1999. TPAH concentrations in sediment likely dropped to background levels (100 ng/g) by 1999 at 10 of 24 oiled locations. In samples with pyrogenic characteristics, TPAH concentrations were generally consistent with background concentrations, regardless of time or place. Our conclusion, that oil with toxic potential remained in intertidal sediment for more than a decade, is consistent with observation of long-term oil retention and toxicity after other major oil spills. Chronic exposure to residual oil may explain why some vertebrate communities have not yet fully recovered from the Exxon spill in the most heavily impacted areas.

## Introduction

The supertanker *Exxon Valdez* grounded on Bligh Reef in northeastern Prince William Sound (PWS), Alaska, on March 24, 1989 (Fig. 1.1). The resultant 42 million liter oil spill was the largest in US history and was ultimately distributed over approximately 28500 $km^{-2}$ (Gundlach et al. 1990). The slick moved southwesterly across PWS, surrounding the Naked Island group (Naked Island, Peak Island, and Storey Island), the Knight Island archipelago, and southwest islands before entering the Gulf of Alaska. The slick did not reach the northern and eastern mainland in PWS, nor Hinchinbrook Island and those to the east, and did not reach some areas on the western mainland (Gundlach et al. 1990). About 40% of the spilled oil was beached in PWS (Wolfe et al. 1996) and there was visible oiling on 1160 km of shoreline (Gundlach et al. 1990). Spill impacts on mammals, birds, fish, and intertidal communities were immediate, often devastating, and affected sensitive species for many years (e.g., see review by Peterson 2001). Efforts to clean beaches were intensive in 1989-1990 but only about 10% of the oil was removed (Mearns 1996).

Remaining *Exxon Valdez* oil (EVO) in PWS beaches had the potential to cause long-term chronic damage over the past decade. Evidence of long-term toxicity has accumulated for pink salmon (*Onchorhynchus gorbuscha*), sea otters (*Enhydra lutris)*, harlequin ducks (*Histrionicus histrionicus*), and pigeon guillemots (*Cepphus columba*). Pink salmon embryo mortalities were elevated in oiled streams through 1993 and again in 1997 (Bue et al. 1998). Remaining EVO has been implicated as the proximate source of PAH currently inhibiting recovery of sea bird and sea otter populations (Esler et al. 2000; Bodkin et al. 2002; Golet et al. 2002). These species have

very different life histories, but all have a common linkage with the oiled intertidal zone; pink salmon embryos incubate in upper zone for up to 7 months, and otters and birds feed in the lower zone. Elevated mixed function oxidase enzyme activity in each of these species provides evidence of oil exposure (Wiedmer et al. 1996; Trust et al. 2000; Bodkin et al. 2002; Golet et al. 2002). An extensive random survey of beaches impacted by EVO confirmed that about 55600 kg of oil remained in PWS 12 y after the spill, on or in about 11.3 hectares of beach (Short et al. 2004), further evidence that species dependent on intertidal habitat may be exposed to lingering oil.

In this paper we re-examine previously analyzed mussel (*Mytilus trossulus*) and sediment data collected by 17 studies to determine how long the oil persisted in the environment, how long it was biologically available, and how long it remained toxic. These data, collected between 1989 and 2000, were reviewed to describe the spatial and temporal extent of the EVO in intertidal areas of PWS and to estimate the potential for chronic toxicity ($n$ = 3414). Intertidal sediment is examined to determine where oil collected and was retained and the potential for prolonged exposure. PAH in native mussels are examined for evidence of biological availability of oil and the length of chronic exposure. Hydrocarbon analysis is restricted to polynuclear aromatic hydrocarbons (PAH) because 1) they are considered to represent the most toxic fraction of crude oil (e.g., Anderson et al. 1974; Hutchinson et al. 1980; Black et al. 1983; Page et al. 2002a), 2) sources of contamination can be determined by analysis of PAH composition, and 3) oil weathering can be estimated from PAH composition (e.g., Short and Heintz 1997). Hydrocarbon sources, pyrogenic and petrogenic, were distinguished with the aid of a combined model that included both industry and NRDA inputs (Carls, Chapter 4).

The toxic potential of oil in PWS sediment was estimated by comparing results to published minimum toxic PAH concentrations constrained by weathering. Maximum weathering known to elicit toxicity was obtained from a laboratory study (Heintz et al. 1999). Minimum toxic PAH concentrations in sediment were obtained from several independent experiments (Couillard 2002; Heintz et al. 2002; Page et al. 2002; Carls et al. 2005). The maximum weathering value we interpret as potentially toxic, $w$ = 4.9 (Short and Heintz 1997), is an index notation that describes PAH dominated by phenanthrenes (48%) with relatively few naphthalenes (20%) compared to unweathered EVO. Previous research indicates that the toxicity of weathered oil increase per unit mass (of remaining oil) as the less toxic PAH are lost (e.g., Carls et al. 1999; Heintz et al. 1999) but not known is how weathered *Exxon Valdez* crude oil must be before toxicity declines per unit mass, thus our estimator of toxicity may be conservative.

Fundamental to understanding when intertidal areas have recovered is recognition of when hydrocarbon loads return to normal. There are several ways to assess this, comparison of observed PAH concentrations to estimated background concentrations, determination of PAH sources, tracking of time-dependent changes, and observation until concentrations begin fluctuating around some undefined, possibly site-specific minimum value. Each of these methods is pursued. Previous background estimates in mussels range from 1 to 100 ng/g dry weight and <10 ng/g to 100 ng/g dry weight in sediment (Boehm et al. 1995; Short and Babcock 1996; Carls et al. 2001).

## Methods

Mussel and sediment data collected by many researchers and archived in the Natural Resource Damage Assessment (NRDA) database (Short et al. 1996a) were used to determine the spatial and temporal extent of oiling. Data were limited to intertidal areas and did not include experimental manipulation (e.g., where intentional oiling occurred). Vertical distribution of

samples within beach sediment were ignored in these analyses because this information is not recorded in the database, but the vast majority represent surface sediment (at least 91% were 0-2 cm deep). Sample elevation, i.e., meters above mean lower low water (MLLW), was also ignored (except that samples outside the intertidal zone were excluded). Included were 1909 mussel samples and 1505 sediment samples, all analyzed by gas chromatography / mass spectroscopy (Short et al. 1996b). Total PAH (TPAH) is defined as the sum of all 39 PAH routinely analyzed (naphthalene through benzo(a)pyrene; Short et al. 1996b). All PAH were examined for the presence of pyrogenic signatures. For all remaining analyses, PAH concentrations less than method detection limits (MDL) were treated as zero.

Spatial oil distribution was inspected by mapping color- and size-coded concentrations by year. Each individual observation was mapped so that the range of concentrations was displayed for each individual collection site. Concentrations were grouped into logarithmically increasing classes. For the purposes of this paper, 'site' is defined as each uniquely sampled latitude-longitude combination, and captures small-scale features (meters). However, these meter-scale differences were not discernable at mapped scales.

Temporal changes in TPAH concentrations in mussels and sediment were inspected for all locations where observation began in 1989 and included two or more subsequent samples. Locations represent larger sampling areas than sites(up to a 5 to 10 km radius; Table 1.1). For example, Herring Point data were combined with Herring Bay data and the entire Naked Island group was analyzed as a single unit. Time-series mussel data were analyzed for 29 locations; 20 of these were within or very near the slick trajectory. Time-series sediment data were analyzed for 30 locations; 22 of these were within or very near the slick trajectory. Concentration change within the first 180 d was categorized only when two or more samples were present in this period.

The source of hydrocarbons in sediments and mussels was inferred using an algorithm that summarizes three independent oil recognition models and two pyrogenic recognition models (Carls, Chapter 4). All models rely on analysis of polynuclear aromatic hydrocarbon (PAH) composition (Bence and Burns 1995; Short and Heintz 1997; Page et al. 2005; Carls in prep.). Each of the oil recognition models had two outputs, a generic recognition of petroleum and specific identification of Alaska North Slope (ANS) crude oil (*Exxon Valdez* oil); the combined score ranged from 0 to 6. The Bence and Burns (1995) model was most likely to identify oil and the Short and Heintz model (1997) was the most conservative. Results of a pyrogenic index used by Page et al. (2005) and a non-parametric pyrogenic model by Carls (Chapter 4) were partitioned to yield a score of 0 to 6 by progressively increasing threshold values. The Carls (in prep.) pyrogenic model was more likely to identify pyrogenic sources than the ratio analysis technique. Pyrogenic scores were subtracted from petrogenic scores to yield a summary score, −6 to 6. Scores <0 were defined as pyrogenic; scores >2 were defined as oiled. Score 0 was indeterminate; scores 1 and 2 were considered ambiguous for statistical purposes but were included on petrogenic maps.

Weathering was determined with the first-order loss-rate model of Short and Heintz (1997). Definitions used here are "unweathered" ($w = 0$), "slightly weathered" ($0 < w \leq 2$), "moderately weathered" ($2 < w \leq 8$), and "highly weathered" ($w > 8$).

To determine change in PAH composition over time, weathering and percent phenanthrenes were regressed against time (linear models). Regression of percent phenanthrenes is an alternative assessment of weathering because percent phenanthrenes increase as the more volatile PAH are lost. To ensure that regressions were meaningful, we adopted the approach suggested by Draper and Smith (1981) that the F-ratio of a regression ($F_o$) should exceed the usual significance ratio ($F_c$) by a multiple of at least 4 times.

The toxic potential of PAH in sediment was estimated by comparing observed concentrations and weathering condition in sediment samples to the lowest concentrations known to cause significant damage to organisms (Table 1.2). Weathering (*w*) was considered in these calculations because it alters oil composition, thereby increasing toxicity per unit mass (Carls et al. 1999; Heintz et al. 1999). The value of *w* above which oil becomes too weathered to be toxic is currently unknown. The highest *w* with known toxicity is at least 4.9 (Heintz et al. 1999); conservatively, samples where $w > 4.9$ were considered nontoxic. Estimates of the lowest toxic concentrations of Alaska North Slope crude oil on sediment range from 270 ng/g TPAH (Couillard 2002) to 4600 ng/g (Heintz et al. 2002; Table 1.2). The total number of oiled samples ($N_{Total}$) was the number of sediment samples from oiled sites where TPAH concentration > 100 ng/g (background concentration). The number of oiled sediments ($N_{oiled}$) where TPAH ≥each toxic threshold concentration (Table 1.2) and $w \leq 4.9$ were independently determined. Percentages of oiled sediments with toxic potential ($100 \bullet N_{oiled}/N_{Total}$) are presented as means (±SE) of the four estimates and ranges. Sample collection criteria changed about 1993, resulting in greater emphasis on heavily oiled sites, thus regression analysis was not applied to this data set.

# Results

## Pyrogenic PAH

Pyrogenic hydrocarbons were identified in 38 to 39 percent of all mussel and sediment samples. Geometric mean pyrogenic TPAH concentrations from all locations were consistent with background concentrations (5.3 ng/g dry weight in mussels and 45.1 ng/g dry weight in sediment). Few pyrogenic TPAH concentrations exceeded 200 ng/g (12 to 13%); just 5% exceeded 400 ng/g (representing 2% of all samples). Pyrogenic TPAH concentrations in sediment did not differ significantly between oiled (42 ng/g, n = 427) and reference areas (55 ng/g, n = 144, P = 0.107). Occasional large pyrogenic outliers were present in sediment (up to 45000 ng/g). Sediment with pyrogenic TPAH concentrations >500 ng/g were generally confined to Knight Island within the slick; exceptions were Long Bay and McClure Bay (where oil was also detected), and Wells Passage (Fig 1.2). Pyrogenic TPAH concentrations in mussels were significantly higher in oiled areas (12.1 ng/g, n = 460) than reference areas (1.3 ng/g, n = 285, P < 0.001; log-transformed data). Occasional large pyrogenic outliers were present in mussels (up to 4000 ng/g); these were usually located inside slick boundaries (Fig 1.3). Sediment and mussels with the strongest pyrogenic characteristics (combined code <-4) were proportionately more frequent outside the spill zone. Mean pyrogenic TPAH concentrations in mussels and sediment were much smaller than corresponding petrogenic TPAH concentrations (P < 0.001; Table 1.3).

## Background PAH

Reference sediment, collected between 1989 and 1993, contained pyrogenic, indeterminate, and petrogenic hydrocarbons (geometric mean 101 ng/g, n = 255). The hydrocarbon source was unclear in more than half of these samples (56%); 20% were pyrogenic, and 24% were petrogenic (n = 255). Ignoring time, geometric mean background concentrations were the same in pyrogenic and indeterminate samples (55 ng/g dry weight) and significantly less than in petrogenic reference samples (730 ng/g). However, TPAH concentrations in reference sediment consistently declined with time (P < 0.001; Fig. 1.4). Pyrogenic concentration declines were significant; petrogenic and indeterminate declines were not. Oil was observed in only 1 reference sediment in 1991 and none in ensuing years, though sampling frequency in these years was low.

Reference mussels, collected between 1989 and 1993, contained pyrogenic, indeterminate, and petrogenic hydrocarbons (geometric mean 2.6 ng/g dry weight, n = 328). The most common PAH source was pyrogenic (87%), though MDL-adjusted TPAH concentration was zero in 60% of these. Petrogenic PAH was detected in only 2% of the reference mussels. Reference TPAH concentrations did not vary significantly with time (P = 0.860). Geometric mean background concentrations in pyrogenic samples were significantly lower (1.3 ng/g dry weight) than in petrogenic and indeterminate reference samples (312 and 104 ng/g, respectively).

**Oil contamination in mussels**

*Spatial distribution of TPAH in mussels*

High petrogenic TPAH concentrations were frequent in mussels within slick boundaries in 1989 (Fig. 1.5). In 1989, TPAH concentrations ≥10000 ng/g in mussels were commonly encountered in the Naked Island group, the Knight Island archipelago and southwestern islands (Bainbridge, Evans, Elrington, and Latouche Islands). These high concentrations were also observed in the outer portion of Rocky Bay on Montague Island and on the northern shore of Port Nellie Juan westward of Culross Passage. Samples, which were collected throughout the Sound in 1989-1990, were limited primarily to the Knight Island group in ensuing years.

*Source of petrogenic hydrocarbons in mussels*

The presence of EVO in mussels was simultaneously indicated by all three ANS-specific oil identification models at 26 of 52 locations (50%) within the slick (e.g., Fig. 1.6). Oil in mussels was identified by any two models at 60% of these locations. Individual oil identification model estimates ranged from 50 to 67% for EVO in mussels within slick boundaries.

*Temporal TPAH concentration change in mussels*

TPAH concentrations in mussels within the slick trajectory increased rapidly to a peak in 1989 and then declined, or were high when first observed and then declined (at 17 of 17 repeatedly sampled locations) (e.g., Fig 1.6). The pattern of increasing, then declining hydrocarbon concentrations in mussels typically occurred within a 180 d period, was evident at different concentration scales (e.g., 0-140000 ng/g at Sleepy Bay, 0-7000 ng/g at Naked Island, and 0-2000 in Culross Passage). Secondary concentration peaks were sometimes present in 1990, possibly as a result of continued large-scale clean-up activities.

Remaining oil was biologically available for many years after the spill but the frequency of occurrence and average TPAH concentrations declined with time (e.g., Fig. 1.7). Mean TPAH concentrations in mussels were > 200 ng/g at 14 of 19 locations after 1991, at 8 of 17 locations after 1994, and at 3 of 13 locations in 1999, demonstrating that recovery was occurring. Biological availability was patchy: e.g., the difference in maximum and minimum TPAH concentrations was > 500 ng/g at 127 of 375 specific location-time combinations where more than one sample was collected. In general, proportionately fewer mussels contained petrogenic PAH as time increased, consistent with recovery.

*Distribution of hydrocarbons in mussels outside slick boundaries*

Low-level changes in mean TPAH concentrations in mussels exceeded 250 ng/g at 6 of 26 locations outside slick boundaries. (Only 10% of TPAH concentrations in mussels from all reference locations were >250 ng/g.) These fluctuations typically involved pyrogenic hydrocarbons and EVO was generally not present. However, concentration changes at Siwash Bay (1.8 km from the slick) may have been directly related to the spill (Fig. 1.6).

**Oil contamination in sediment**

*Spatial distribution of TPAH in sediment*

High petrogenic TPAH concentrations in sediment were frequent within slick boundaries in 1989 (Fig. 1.8). TPAH concentrations > 100000 ng/g in 1989 were common in the Knight Island and extending southwest to Bainbridge, Evans, Elrington, and Latouche Islands, but not in the Naked Island group. Maximum concentrations were consistently lower in the Naked Island group, on Montague Island, and in Culross Passage. After 1991 most sediment samples were collected in western PWS.

*Source of petrogenic hydrocarbons in sediment*

The presence of EVO in sediment was simultaneously indicated by all three ANS-specific oil identification models at 33 of 57 locations (58%) within the slick (e.g., Fig. 1.9). Oil in sediment was identified by any two models at 61% of these locations. Individual oil identification model estimates ranged from 58 to 70% for EVO in sediment within slick boundaries.

*Temporal TPAH concentration change in sediment*

TPAH concentrations in sediment within the slick boundary typically either increased to a peak in 1989 and then declined, or were high when first observed and then declined (at 11 of 15 repeatedly sampled locations). Increased TPAH due to EVO was plausible at 3 additional locations in the slick area and EVO was positively identified on more than one occasion at one of these (Naked Island group). Concentrations typically peaked <180 d after the spill. The pattern of increasing, then declining hydrocarbon concentrations in sediment in 1989 was evident at different concentration scales (e.g., 0-74000 ng/g at Sleepy Bay, 0-9000 ng/g at Herring Bay) (Fig. 1.9).

Declines in TPAH concentrations in sediment typically required several years (e.g., Fig. 1.7). Concentrations higher than those observed in 1989 were frequently encountered in the mid to late 1990s as research was focused on the most contaminated areas (Brodersen et al. 1999; Carls et al. 2001; Carls et al. 2004a; Carls and Harris in prep). Mean TPAH concentrations were > 400 ng/g at 17 of 26 locations after 1991, at 13 of 19 locations after 1994, and at 2 of 3 locations after 1998. Oil distribution was patchy: e.g., the difference in maximum and minimum TPAH concentrations was > 500 ng/g at 121 of 385 specific location-time combinations where more than one sediment sample was collected. TPAH concentrations > 100,000 ng/g were encountered in nearly every year of study, including 2000. TPAH concentrations likely returned to background levels at 29-42% of locations a decade after the spill ($n$ = 24). Complete loss of EVO from sediment by 1999 was unlikely at 46% of these locations. No trends were evident in the proportion of sediments that contained petrogenic PAH, also suggesting recovery is incomplete.

*Distribution of hydrocarbons in sediment outside slick boundaries*

Low-level changes in mean TPAH concentrations in sediment exceeded 250 ng/g at 7 of 16 locations outside slick boundaries. (Only 10% of non-petrogenic (combined score <1) TPAH concentrations in sediment from all reference locations were >250 ng/g.) These fluctuations typically involved pyrogenic hydrocarbons. However, the steep concentration increases (to >1000 ng/g) and declines at Long Bay and McClure Bay were patterns typical of oiled areas and EVO was demonstrable (combined score = 6) at the highest concentrations (Fig. 1.9). Long Bay

samples were about 4 km from the nearest observation of oil (in Culross Passage); McClure Bay samples were collected about 8 km outside the slick. Elevated mean TPAH concentrations at Constantine Harbor were petrogenic but these concentrations were nearly constant, suggesting the source may not be EVO (Fig. 1.9). There were too few data at Simpson Bay and Port Valdez for interpretation.

*Weathering and toxic potential of oil in sediment*

Oil in sediment slowly weathered in the decade after the *Exxon Valdez* spill although in each year of study, weathering ranged from unweathered or slightly weathered to highly weathered (Fig. 1.10). Mean *w* increased from 2.6 in 1989 to 6.5 in 1998, the last year for which data were estimable ($r^2 = 0.04$, $P < 0.001$, $F_o/F_c = 4.8$). Percent phenanthrenes increased from 22% in 1989 to 32% in 2000 ($r^2 = 0.03$, $P < 0.001$, $F_o/F_c = 10.0$). Scatter was very high for both measures, thus correlation was very poor, but the linear predictions are likely useful predictors of the general trend toward increased weathering ($F_o/F_c > 4$).

Sediment with toxic potential was evident throughout the decade after the *Exxon Valdez* spill (Fig. 1.10a). Between 1989 and 1992, sediment with potential toxicity ranged from 4% to 46% of oiled sediment; the lowest estimate was in 1990. When the research focus shifted primarily to description of localized hot-spots, the percentage of samples with estimated toxic potential jumped to 80-81% (1993-1994). Occurrence of sediment with toxic potential declined in the late 1990s as TPAH concentrations declined in these localized hot-spots. After 1991, all four estimates of minimum toxicity levels (Table 1.2) provided identical potential toxicity estimates for sampled PWS sediment. Sediments identified as potentially toxic occurred primarily within the main slick trajectory and included samples in the Naked Island group, the Knight Island archipelago, and southwest through Latouche, Elrington, Evans, and Bainbridge Islands (Fig. 1.8).

**Comparison of TPAH in mussels and sediment**

Parallelism in hydrocarbon concentration change in mussels and sediment and consistent identification of EVO as the source of hydrocarbons in both matrices demonstrates that both the habitat and the biological community were exposed to oil at various locations. Accumulation and loss patterns within the slick boundary were highly similar in mussels and sediment at 11 of 14 repeatedly sampled oiled locations. Outside the slick boundary, simultaneous change in TPAH concentration in mussels and sediment was not observed.

Oil in dissolved in the water column or in particulate or colloidal form was more transportable and apparently affected a larger area both inside and outside slick boundaries than did oil transported as a slick on the water surface. For example, TPAH concentration peaks in 1989 were evident in mussels but not in sediment at 3 locations within the slick boundary (Bligh Island, the Naked Island group, and Rocky Bay) and at 1 location outside the boundary (Siwash Bay). These differences in oil transportation profoundly influenced how contaminated intertidal habitat became and how long oil was retained. Specifically, little oil was retained in sediment at locations not coated by slicks; conversely, high oil concentrations persisted for long periods where coating occurred.

Long-term mussel contamination occurred only where substantial amounts of oil were trapped in sediment. After 1989, EVO was identified by two or more ANS-specific models in mussels at 17 locations where oil was retained in sediment (Table 1.4a). EVO was identifiable in mussels from some of these locations through 1999, the last year of sampling (Table 1.4a). EVO was not identifiable after 1989 in any mussel tissues from 6 locations where oil was not retained in sediment (Table 1.4b). Intermediate were 5 locations with retention of some EVO but after

1989, EVO could not be verified in mussels at these locations (Table 1.4c). For completeness, note three unusual sites, all located outside the slick boundary (Table 1.4d). Hydrocarbons in sediment at Constantine Harbor and Simpson Bay may not be EVO and EVO could not be verified in mussels at these locations after 1989.

## Discussion

Intertidal hydrocarbon data collected from PWS indicate that 1) the principal source of hydrocarbon contamination was EVO throughout the 1990s, 2) oil was distributed by primarily by water to locations within, and possibly to a limited extent, locations outside the slick trajectory, 3) distribution of EVO in sediment was heterogeneous and high concentrations persisted in some oil-coated locations for more than a decade, 4) retention of oil in sediment was the key factor controlling biological availability of oil, 5) EVO remained toxic for a decade after the spill and may explain why some vertebrate communities have not recovered from the spill, and 6) habitat recovery and the potential for recovery is improving as oil concentrations decline and weathering increases.

### Hydrocarbon sources

EVO was the unambiguous main source of aromatic hydrocarbons detected in intertidal sediment and mussel tissue in PWS and has remained so for more than a decade. In 1989, TPAH concentrations (frequently verified as EVO by multiple oil-identification models) rose rapidly after the spill in sediment and mussels, peaked (often at very high levels), then declined, providing strong evidence that the primary source of contamination was the *Exxon Valdez* spill. The petrogenic EVO signal overlaid a low-level pyrogenic hydrocarbon background signature. The vessel activity may have contributed to the observed increase in pyrogenic concentrations at the time of the spill. Possible alternative petrogenic hydrocarbon sources were limited to isolated hot-spots (Constantine Harbor and Simpson Bay) and were infrequent throughout the spill zone. The magnitude of the 1989 Exxon spill far exceeded the magnitude of any other concurrent hydrocarbon sources, thus evidence that EVO was the dominant source of aromatic hydrocarbons in intertidal areas of PWS in the 1990s is not surprising.

Alternative sources of hydrocarbons in PWS suggested by some (Page et al. 1996; Wooley 2002) neither explain the post-*Exxon Valdez* hydrocarbon record nor diminish the importance of this spill. Alternative sources of hydrocarbons include natural background hydrocarbons and others from prior human activity and spills, present in subtidal sediment (Page et al. 1999; Wooley 2002; Page et al. 2002b). While useful as a historical record, trace subtidal hydrocarbon deposits are in a different habitat than the intertidal zone where long-term EVO effects occurred. One major subtidal background hydrocarbon source is apparently coal (Van Kooten et al. 2002), not seep oil as hypothesized by Page et al. (1996), explaining why these hydrocarbons are deposited subtidally, are chemically invariant, and are not biologically available.

Hydrocarbon contamination due to prior human activity, such as those associated with fur trade, mining, military activity, and fishing (Wooley 2002) affect <0.2% of PWS shoreline (Boehm et al. 2004) compared to >14% coverage by EVO. This historical oil has had decades to decline and weather for the same reasons that EVO concentrations are now declining and weathering. Limited distribution and long residence time in the environment explain why historical PAH are biologically available only within very small areas. The same logic holds true for Monterey oil spilled from storage tanks in 1964 by the Great Alaska Earthquake. Not only had this oil weathered to non-biologically available tar by the time of the *Exxon Valdez* spill (Kvenvolden et al. 1995), but Monterey tar was encountered less frequently than EVO residues

in the 1990s (Kvenvolden et al. 1995) and accounted for <10% of the surface oil discovered 12 years after the Exxon spill (Short et al. 2004). Monterey tar was generally located near the high tide line, probably because only those tarballs small enough to soften during periods of high insolation adhered to rock (Kvenvolden et al. 1995; Short et al. 2004). Unlike intertidal EVO, no subsurface Monterey oil was discovered (Short et al. 2004). Observed declines in the occurrence and concentration of EVO in the decade after the spill may roughly describe the fate of Monterey oil in previous decades.

*Exxon Valdez* oil remains the largest source of oil in PWS (Short et al. 2004). It was repeatedly identified by compositional analysis in both tissue and sediment from 1989 to 2000 and is still present in intertidal sediment, leaving little doubt concerning the current source of intertidal contamination.

**Oil transportation**

Surface-oriented, dissolved, and particulate EVO was transported by water to locations within the slick trajectory and possibly to some locations outside reported slick boundaries. Visual observation of slick movement and chemical analysis of water within the spill zone demonstrated that whole and dissolved oil was distributed by water (Gundlach et al. 1990; Neff and Stubblefield 1995; Short and Harris 1996). A recent drogue study has demonstrated water exchange between many areas of PWS (S. Vaughan, Prince William Sound Science Center, personal communication), suggesting EVO transport by water to remote areas was plausible, particularly in dissolved or particulate form. Detection of oil over areas larger than those with visible slicks or sheens has been reported by others (Boehm et al. 1980), consistent with our observation that water transported dissolved or particulate oil in addition to surface-oriented oil slicks and that subsurface contamination was distributed over a larger area.

**Long-term oil distribution**

That significant amounts of oil remain scattered in PWS sediment, thus providing a contaminant reservoir for species utilizing intertidal habitat, is suggested by more than a decade of hydrocarbon analyses. Detection of extremely high TPAH concentrations ($>10^5$ ng/g) in sediment through 2000 suggests that significant amounts of oil remain in PWS, and this inference is corroborated by a recent random survey of remaining oil (Short et al. 2004). Non-random site selection was primarily responsible for the detection of very high TPAH concentrations in PWS sediment beginning about 1992 for the purpose of determining oil loss rates from problem areas (e.g., Babcock et al. 1998; Carls et al. 2001; Brodersen et al. 1999; Carls et al. 2004; Carls et al. in prep). Consequently, these studies do not provide information regarding the total extent of contaminated sediment, rather simply demonstrate that high quantities of oil were present at some locations in the decade after the spill. To address this deficiency, a random survey of previously heavily oiled beaches was completed in 2001.

PWS beaches randomly sampled for the presence of oil in 2001 provide strong evidence that significant quantities of oil remain in the spill area (Short et al. 2004). Sampling was limited to beaches that were originally moderately to heavily oiled, about 117 km. Oil detection was visual; concentrations in the 'light oil residue' category were relatively high ($6000 \leq$ TPAH $\leq$ 66000 ng/g, $n = 5$; Jeff Short, personal communication). EVO in PWS sediment was detected at 53 of 91 locations (58%) by random survey and discovered non-randomly on 25 additional beaches (Short et al. 2004), corroborating our observation that oil-contaminated sediment persisted through the 1990s. Subsurface oil was encountered more frequently than surface oil, was usually liquid, and was moderately to highly weathered ($w$ ranged from 1.4-8.2). The frequency of oil encounter increased from +4.8 m MLLW to +1.8 m MLLW (the lowest zone

sampled). Limited opportunistic sampling suggested oil was also present below 1.8 m MLLW (Short et al. 2004), thus demonstrating contamination of a zone of high biological productivity and consistent with our results (the mussel zone in PWS is typically located 1.3-2.0 m MLLW). The extensive distribution of residual oil with slight to high weathering and relatively high TPAH concentrations indicates considerable potential for negative biological impacts.

**Biological availability of oil**

Habitat condition is the key factor controlling biological availability of oil to the intertidal community. Oil in mussels persisted for a short time (months) on shorelines not coated by oil and where mussels were primarily exposed to dissolved or particulate oil in water shortly after the spill. Conversely, persistence of oil in mussels was long (up to 10 years or more) on beaches where oil was retained by intertidal sediment.

The pattern of hydrocarbon accumulation and decline in PWS mussels in the past decade not only clearly implicates EVO as the source of biological contamination but also demonstrates that other possible hydrocarbon sources are generally not biologically important in intertidal areas within the spill zone. The sudden concurrent increase in tissue burdens in 1989 at multiple locations is best explained by a single hydrocarbon source: EVO. This inference was confirmed by PAH composition analysis at many locations. Steady declines in TPAH concentration in mussel tissue and the declining frequency of contaminated specimens is consistent with lingering but dwindling intertidal reservoirs of EVO. Declines in PAH body burdens to background levels demonstrate that the biological availability of other possible petroleum hydrocarbon sources is negligible throughout most of the spill area.

Slow, protracted transfer of EVO from sediment to mussels is evident. In contrast to the high uptake rates evident where conditions favor transfer (e.g., bioconcentration in mussels exposed to oiled water is about $2 \times 10^5$; Livingstone 1991), bioconcentration of TPAH from sediment was <1 between 1992 and 1999. Exposure of mussels to oil in PWS was mediated by water (Carls et al. 2001) and dilution by water in unconfined beach environments likely influences apparent bioconcentration from sediment. The heterogeneous oil distribution in sediment and non-uniform dilution by water probably also influence bioavailability. The same slow, partial transfer of PAH from oiled rock to organism via water is also evident in controlled laboratory tests (Carls, unpublished data) and may partially explain the wide latitude in reported toxic thresholds of oil in sediment (Table 1.2).

Sediment disturbance is probably a major factor controlling the oil release rate. This inference is consistent with the observation by Hayes and Michele (1999) that beach morphology influences oil retention and the observation of sudden increases in biological availability when sediment was disturbed during mussel bed restoration (Carls et al. 2004). Increased biological availability as a result of disturbance is also evident in the time series record; secondary TPAH increases were frequent in 1990, the second year of wide-spread intense oil cleanup efforts in PWS. We also suspect that bioturbation increases the hydrocarbon transfer rate, as well as increasing the probability of exposure. For example, evidence of prolonged exposure of sea otters to oil (> 10 years) may be linked to excavation of contaminated sediment during foraging activity (Bodkin et al. 2004).

**Toxicity of remaining oil**

Our analysis of hydrocarbon data suggests that EVO remaining in PWS sediment was potentially toxic in the decade after the spill. On the average, weathering was slow and predicted weathering remained within known toxic limits ($w \leq 4.9$) until 1998. Slow PAH weathering in PWS is consistent with data from other spills, e.g., the *Amoco Cadiz*, the BIOS experiment, and

the West Falmouth spill (Mille et al. 1998; Prince et al. 2002; Reddy et al. 2002). The overall decline in the percentage of sediment samples with known toxic potential in PWS was probably generally more rapid than evident from our data because researchers focused on highly contaminated areas for continued study beginning about 1992. Nonetheless, this continuous time-series clearly suggests that sediment with toxic potential was present within PWS sediment for a decade after the spill.

Conflicting conclusions that suggest oil toxicity in PWS sediment declined rapidly after the spill are derived from acute toxicity bioassays (Boehm et al. 1995; Page et al. 2002a) and do not consider chronic sublethal effects. Measures of lethal responses in adult animals, such as the amphipod assays completed by Page et al. (2002a), are usually less sensitive measures of toxicity than sublethal measures, particularly in early life stages (Moore and Dwyer 1974; Weis and Weis 1989; Di Toro et al. 2000). To fully evaluate the toxic potential of contaminated sediment, evaluation of multiple taxa is frequently recommended (Chapman 1984; Bat and Raffaelli 1998; Mattiessen et al. 1998; Pedersen et al. 1998). Chapman (1984) specifically identified sublethal chromosomal damage in rainbow trout (*Salmo gairdneri*) as more sensitive measure than several acute mortality tests, including an amphipod assay (*Eogammarus confervicolus*). The absence of acute sediment toxicity does not demonstrate the absence of benthic degradation (Swartz et al. 1985). Sublethal toxicity due to remaining EVO has negatively affected individuals and populations, as demonstrated in the next paragraph.

Direct observation has demonstrated that oil in sediment remained toxic for years after the *Exxon Valdez* spill, consistent with our conclusion that toxic oil remains in PWS. Contaminated prey and disturbance of oiled substrate during feeding activities may explain the failure of sea otter, harlequin duck, and pigeon guillemot populations to recover in oiled areas (Esler et al. 2000; Bodkin et al. 2002; Golet et al. 2002). Biochemical indicators of PAH exposure in these species are associated with the geographical distribution of the remaining oil. Long-term oil toxicity was also evident in intertidal prey organisms. Mussel condition in oiled beds was poorer than in reference beds in 1993 and oiled mussels were less tolerant of aerial emersion in 1996 (Morado et al. 1998; Thomas et al. 1999). Survival and growth of clams *(Protothaca staminea*) were reduced by residual oil 5-6 years after the spill and oxidative and xenobiotic stress due to PAH exposure was clearly evident in other bivalves (*Mya arenaria* and mussels) from a heavily oiled location in PWS 11 yr after the spill (Fukuyama et al. 2000; Downs et al. 2002). Mortality in sensitive pink salmon embryos was reported in oiled streams through 1993 and again in 1997. These embryos were exposed to dissolved oil (confirmed by elevated cytochrome P4501A levels at oiled sites; Wiedmer et al. 1996) as a result of groundwater flow through adjacent contaminated sediment and drainage into redds (Carls et al. 2003). Collectively these studies demonstrate that biota in PWS were exposed to and adversely affected by toxic EVO for more than a decade.

The observation that remaining EVO persisted in intertidal sediment and was toxic in the decade after the spill is consistent with other spill experience. Blumer et al. (1973) indicated that the half life of oil spilled at West Falmouth was years and that toxic, substituted triaromatic and higher ring number aromatics are degraded slowly. PAH from the barge *Florida* (West Falmouth, Massachusetts) oil spill persisted 30 years in marsh sediment and will likely persist indefinitely (Reddy et al. 2002). Hydrocarbons from the *Florida* induced cytochrome P4501A in mummichogs (*Fundulus heteroclitus*) 8-20 years later, signaling continued biological availability and incomplete habitat recovery (Stegeman 1978; Teal et al. 1992). The fiddler crab (*Uca pugnax*) population remained depressed after the *Florida* spill for more than 7 yr and was correlated with oil retention (Krebs and Burns 1977; Teal and Howarth 1984). Newly settled crabs were more affected by residual oil than adults and the long-term inhibition of recruitment

may have been caused by exposure to oil in interstitial water during sensitive molt periods (Krebs and Burns 1977). Estimated residence time for *Metula* oil in the Strait of Magellan was 15-30 yr in low energy sand and gravel beaches and >100 yr in sheltered tidal flats and marshes (Gundlach et al. 1981). Weathered Bunker C oil was detected in Chedabucto Bay, Nova Scotia, 20 y after the *Arrow* spill (Vandermeulen and Sing 1994). After 6-7 yr, clams (*Mya arenaria*) from an oiled location in Chedabucto Bay remained stressed and species diversity was uniformly lower at oiled locations than at control locations (Gilfillan and Vandermeulen 1978; Thomas 1978). *Mya arenaria* growth was less in those collected 9 years after the *Arrow* spill than in clams collected from the same location shortly after the spill (MacDonald and Thomas 1982). Elapsed time between the *Tampico Maru* diesel fuel spill and reappearance of many invertebrates in an affected cove usually greatly exceeded larval lifespans; species numbers continued to rise for at least 11 yr after the spill (North 1973). The Bay of Morlaix was strongly impacted by the 1978 *Amoco Cadiz* oil spill and recovered slowly (Ghertsos et al. 2000). Reconstitution of the *Abra alba* subtidal community in the Bay of Morlaix required > 10 yr; here the limiting factor apparently was the capacity of *Ampelisca* amphipods to reproduce, disperse, and recolonize (Dauvin 1998). Recovery from the Torrey Canyon spill took 10-15 yr through a series of damped oscillations (Hawkins et al. 1993). In a review of seven well-studied oil spills, Teal and Howarth (1984) conclude that oil effects can persist for at least 6-12 years in sediment. This prediction appears to be on target for the EVO, where habitat recovery was progressing but was not complete a decade after the spill.

**Habitat recovery**

Although available data demonstrates a decade of negative impacts in PWS, they also clearly indicate trends toward recovery. Oil concentrations have consistently declined, rapidly within the first year, then more slowly. Recovery in mussels, pink salmon (Carls et al. 2004b) sea birds, and otters has preceded that in sediment, suggesting that remaining EVO residues are becoming increasingly buffered from the biological community. On the average, remaining oil has become more weathered, and beyond a not-yet clearly defined threshold, toxicity should decrease. Our estimate that the percentage of locations where TPAH levels declined to background levels (as measured by GC/MS) is 29-42% (measured by GC/MS), more rapid than visual estimates completed 12 years after the spill (14% loss, Short et al. 2004). Nonetheless, Short et al. (2004) agree that the volume of remaining oil declined considerably since 1992. Remaining oil in the first decade after the spill presented a series of long-term problems for PWS biota, but unmistakable signs of recovery are also evident. Thus, in the next decade we expect continued recovery, as oil weathering continues and progressively less oil is biologically available.

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## References

Anderson, J.W., J.M. Neff, B.A. Cox, H.E. Tatem, and G.M. Hightower. 1974. Characteristics of dispersions and water-soluble extracts of crude and refined oils and their toxicity to estuarine crustaceans and fish. Mar. Biol. 27:75-88.

Atlas, R.M. 1981. Microbial degradation of petroleum hydrocarbons: an environmental perspective. Microbiol. Rev. 45:180-209.

Babcock, M. M., P. M. Harris, M. G. Carls, C. C. Brodersen, and S. D. Rice. 1998. Mussel bed restoration and monitoring. *Exxon Valdez* Oil Spill Restoration Project 95090 Final Report. National Oceanic and Atmospheric Administration, National Marine Fisheries Service, Auke Bay Laboratory, Juneau, AK. 154 p.

Barron, M.G., M..G. Carls, J.W. Short, and S.D. Rice. 2003. Photoenhanced Toxicity of Aqueous Phase and Chemically-Dispersed Weathered Alaska North Slope Crude Oil to Pacific Herring Eggs and Larvae. Environ. Toxicol. Chem. 22:650-660.

Bat L, Raffaelli D. 1998. Sediment toxicity testing: a bioassay approach using the amphipod *Corophium volutator* and the polychaete *Arenicola marina*. J. Exp. Mar. Biol. Ecol. 226:217-239.

Black J.A., W.J. Birge, A.G. Westerman, and P.C. Francis. 1983. Comparative aquatic toxicology of aromatic hydrocarbons. Fund. Appl. Toxicol. 3:353-358.

Blumer M, Ehrhardt M, Jones JH. 1973. The environmental fate of stranded crude oi. Deep-Sea Research 20:239-259.

Bodin P. 1991. Perturbations in the reproduction cycle of some harpacticoid copepod species further to the *Amoco Cadiz* oil-spill. Hydrobiologia 209:245-257.

Bodkin, J. L., Ballachey, B. E., Dean, T. A., Fukuyama, A. K., Jewett, S. C., McDonald, L., Monson, D. H., O'Clair, C. E., VanBlairicom, G. R. (2002). Sea otter population status and the process of recovery from the 1989 *Exxon Valdez* oil spill. *Marine Ecology Progress Series* 241, 237-253.

Bodkin, JL; Esslinger, GG; Monson, DH. 2004. Foraging depths of sea otters and implications to coastal marine communities. Marine Mammal Science 20: 305-321.

Boehm PD, Barak J, Fiest D, Elskhus A. 1980. The analytical chemistry of *Mytilus edulis*, *Macoma balthica*, sediment trap and surface sediment samples. Pages 219-274 in Kineman JJ, Elmgren R, and Hansson S (eds.), The *Tsesis* oil spill. US Department of Commerce, NOAA, WA DC.

Boehm PD, Page DS, Gilfillan ES, Stubblefield WA, Harner EJ. 1995. Shoreline ecology program for Prince William Sound, Alaska, following the Exxon Valdez oil spill: part 2 – chemistry and toxicology. Pages 347-397 *in* P.G. Wells, J.N. Butler, and J.S. Hughes (eds.), *Exxon Valdez* oil spill: fate and effects in Alaskan Waters, ASTM STP 1219, American Society for Testing and Materials, Philadelphia.

Boehm PD, Page DS, Brown JS, Neff JM, Burns WA. 2004. Polycyclic aromatic hydrocarbon levels in mussels from Prince William Sound, Alaska, USA, document the return to baseline conditions. Environ Toxicol Chem 23:2916-2929.

Brodersen, C., J. Short, L. Holland, M. Carls, M. Larsen, and S. Rice. 1999. Evaluation of oil removal from beaches 8 years after the *Exxon Valdez* oil spill. Proceedings of the Twentysecond Arctic and Marine Oilspill Program (AMOP) Technical Seminar, Environment Canada, Ottawa, Ontario. p p. 325-336.

Bue BG, Sharr S, Moffitt SD, Craig A (1996) Effects of the *Exxon Valdez* oil spill on pink salmon embryos and preemergent fry. Am Fish Soc Symp 18:619-627

Bue BG, Sharr S, Seeb JE (1998) Evidence of damage to pink salmon populations inhabiting Prince William Sound, Alaska, two generations after the *Exxon Valdez* oil spill. Trans Am Fish Soc 127:35-43

Calfee RD, Little EE, Cleveland L, Barron MG. 1999. Photoenhanced toxicity of a weathered oil to *Ceriodaphnia dubia* reproduction. *Environ. Sci. Poll. Res.* 6:207-212.

Carls, M. G., A. C. Wertheimer, J. W. Short, R. M. Smolowitz, and J. J. Stegeman. 1996. Contamination of juvenile pink and chum salmon by hydrocarbons in Prince William Sound after the *Exxon Valdez* oil spill. Am. Fish Soc. Symp. 18:593-607.

Carls, M.G., S.D. Rice, and J.E. Hose. 1999. Sensitivity of fish embryos to weathered crude oil: Part 1. Low level exposure during incubation causes malformations, genetic damage and mortality in larval Pacific herring (*Clupea pallasi*). Env. Toxicol. Chem. 18:481-493.

Carls, M.G., M.M. Babcock, P.M. Harris, G.V. Irvine, J.A. Cusick, and S.D. Rice. 2001. Persistence of Oiling in Mussel Beds after the *Exxon Valdez* Oil Spill. Marine Environmental Research 51:167-190.

Carls, M.G., Thomas, R.E. Rice, S.D. 2003. Mechanism for transport of oil-contaminated water into pink salmon redds. Mar. Ecol. Prog. Ser. 248:245-255.

Carls, M.G., P.M. Harris, and S.D. Rice. 2004a. Restoration of Oiled Mussel Beds in Prince William Sound, Alaska. Mar. Environ. Res. 57:359-376.

Carls, M.G., G.D. Marty, and S.D. Rice. 2004b. Pink salmon spawning habitat is recovering a decade after the *Exxon Valdez* oil spill. Trans Am Fish Soc 133:832-842.

Carls MG, Heintz RA, Marty GD, Rice SD. 2005. Cytochrome P4501A induction in oil-exposed pink salmon *Oncorhynchus gorbuscha* embryos predicts reduced survival potential. Mar Ecol Prog Ser 301:253-265.

Carls, M.G. In prep. Identification of marine hydrocarbon sources: a novel non-parametric approach. *Chapter 4 in this report.*

Carls, MG Harris PM, Irvine GV. In prep. Retention and loss of intertidal *Exxon Valdez* oil, 1992-1999. *Chapter 2 in this report.*

Chapman PM. 1984. Sediment bioassay tests provide toxicity data necessary for assessment and regulation. Abstract. In 11th Annual aquatic toxicity workshop, Nov. 13-15, 1984; Richmond, BC, Canada pp 178-197.

Couillard CM. 2002. A microscale test to measure petroleum oil toxicity to mummichog embryos. Environ Toxicol 17:195-202.

Dauvin JC. 1998. The fine sand Abra alba community of the Bay of Morlaix twenty years after the *Amoco Cadiz* oil spill. Mar Pollut Bul 36:669-676.

Di Toro DM, McGrath JA, Hansen DJ. 2000. Technical basis for narcotic chemicals and polycyclic aromatic hydrocarbon criteria. I. Water and tissue. Environ Toxicol Chem 19:1951-1970.

Downs CA, Shigenaka G, Fauth JE, Robinson CE, Huang A. 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.

Esler, D., Schmutz, J. A., Jarvis, R. L., & Mulcahy, D. M. (2000). Winter survival of adult female harlequin ducks in relation to history of contamination by the *Exxon Valdez* oil spill. *Journal of Wildlife Management*, 64, 839-847.

Floodgate, G.D. 1984. The fate of petroleum in marine ecosystems. Pages 355-396 *in* R.M. Atlas (ed), Petroleum microbiology. Macmillan Publishing Company, New York.

Fukuyama AK, Shigenaka G, Hoff RZ. 2000. Effects of residual *Exxon Valdez* oil on intertidal protothaca staminea: mortality, growth, and bioaccumulation of hydrocarbons in transplanted clams. Mar Pollut Bul. 40:1042-1050

Ghertsos K, Luczak C, Dewarumez J-M, Dauvin J-C. 2000. Influence of spatial scales of observation on temporal change in diversity and trophic structure of fine-sand communities from the English Channel and southern North Sea. ICES J Mar Sci 57:1481-1487.

Gibbs, C.F., and S.J. Davis. 1976. The rate of microbial degradation of oil in a beach gravel column. Microbial Ecology 3:55-64.

Gilfillan ES, Vandermeulen JH. 1978. Alterations in growth and physiology of soft-shell clams, *Mya arenaria*, chronically oiled with Bunker C from Chedabucto Bay, Nova Scotia, 1970-76. J. Fish. Res. Board Can. 35:630-636.

Golet, G. H., Seiser, P. E., McGuire, A. D., Roby, D. D., Fisher, J. B., Kuletz, K. J., Irons, D. B., Dean, T. A., Jewett, S. C., & Newman, S. H. (2002). Long-term direct and indirect effects of the *Exxon Valdez* oil spill on pigeon guillemots in Prince William Sound, Alaska. *Marine Ecology Progress Series* 241, 287-304.

Gundlach ER, Berne S, D'Ozouville L., Topinka JA. 1981. Shoreline oil two years after *Amoco Cadiz*: new complications from *Tanio*. Pages 525-540 in Proceedings of the 1981 Oil spill conference. American Petroleum Institute Publ. No. 4334.

Gundlach, E.R., J. Bauer, R. Bayliss, S. Provant, and M. Kendziorek. 1990. Response to the *Exxon Valdez* oil spill by the Alaska Department of Environmental Conservation. Pages 471-478 *in* M.L. Spaulding, and M. Reed (eds.), Oil spills management and legislative implications: proceedings of the conference, May 15-18, 1990, Newport, Rhode Island, USA. American Society of Civil Engineers, NY.

Hawkins WE, Walker WW, Overstreet RM, Lytle JS, Lytle TF. 1990. Carcinogenic effects of some polycyclic aromatic hydrocarbons on the Japanese medaka and guppy in waterborne exposures. The Science of the Total Environment 94:155-167.

Hawkins SJ, Proud SV, Spence SK, Southward AJ. 1993. From the individual to the community and beyond: water quality, stress indicators and key species in coastal ecosystems. Pp. 35-62 in Sutcliffe DW (ed). Water quality and stress indicators in marine and freshwater ecosystems: linking levels of organisation (individuals, populations, communities)., Freshwater Biological Association, Ambleside (UK).

Hayes, M. O. & Michel, J. (1999). Factors determining the long-term persistence of *Exxon Valdez* oil in gravel beaches. *Marine Pollution Bullletin*, *38*, 92-101.

Heintz R, Short JW, Rice SD (1999) Sensitivity of fish embryos to weathered crude oil: Part II. Increased mortality of pink salmon (*Oncorhynchus gorbuscha*) embryos incubating downstream from weathered *Exxon Valdez* crude oil. Env Toxicol Chem 18:494-503.

Heintz, R.A., S.D. Rice, A.C. Wertheimer, R.F. Bradshaw, F.P. Thrower, J.E. Joyce, and J.W. Short. 2000. Delayed effects on growth and marine survival of pink salmon *Onchorhynchus gorbuscha* after exposure to crude oil during embryonic development. Mar. Ecol. Progr. Ser. 208:205-216.

Helle JH, Williamson RS, Bailey JE (1964) Intertidal ecology and life history of pink salmon at Olsen Creek, Prince William Sound, Alaska. U.S. Fish Wild Serv Spec Sci Rep Fish 483

Houghton JP, Lees DC, Driskell WB, Lindstrom SC, Mearns AJ. 1996. Recovery of Prince William Sound intertidal epibiota from *Exxon Valdez* oiling and shoreline treatments, 1989-1992.

Hutchinson, T.C., J.A. Hellebust, D. Tam, D. Mackay, R.A. Mascarenhas, and W.Y. Shiu. 1980. The correlation of the toxicity to algae of hydrocarbons and halogenated hydrocarbons with their physical-chemical properties. Pages 577-586 in Afghan, B.K. and D. Mackay (eds.), Hydrocarbons and halogenated hydrocarbons in the aquatic environment. Plenum Press, New York.

Jewett, S.C., Dean, T. A., Woodin, B. R., Hoberg, M. K., & Stegeman, J. J. 2002. Exposure to hydrocarbons 10 years after the *Exxon Valdez* oil spill: evidence from cytochrome P4501A expression and biliary FACs in nearshore demersal fishes. *Marine Environmental Research* 54, 21-48.

Karinen JF, Babcock MM, Brown DW, MacLeod WD Jr., Ramos LS, Short JW. 1993. Hydrocarbons in intertidal sediments and mussels from Prince William Sound, Alaska,

1977-1980: characterization and probable sources. NOAA Technical Memorandum NMFS-AFSC-9. US Department of Commerce, National Oceanic and Atmospheric Administration, National Marine Fisheries Service, Alaska Fisheries Science Center.
Kelso D. D., and M. Kendziorek. 1991. Alaska's response to the *Exxon Valdez* oil spill, Part 1. Environ. Sci. Technol. 25:16-23.
Krebs CT, Burns KA. 1978. Long-term effects of an oil spill on populations of the salt-marsh crab *Uca pugnax*. J. Fish. Res. Board Can. 35:648-649.
Kvenvolden KA, Carlson PR, Threlkeld CN, Warden A. 1993. Possible connection between 2 Alaskan catastrophes occurring 25 yr apart (1964 and 1989). Geology 21:813-816.
Kvenvolden KA, Hostettler FD, Carlson PR, Rapp JB, Threlkeld CN, Warden A. 1995. Ubiquitous tar balls with a California-source signature on the shorlines of Prince William Sound, Alaska. Environ Sci Technol 29:2684-2694.
Lee RF, Takahaski M, Beers JR, Thomas WH, Siebert DLR, Koeller P, Green DR. 1977. Controlled ecosystems: their use in the study of the effects of petroleum hydrocarbons on plankton. Pages 323-342 in Vernberg FJ, Calabrese A, Thurberg FP, and Vernberg WB (eds.), Physiological responses of marine biota to pollutants.
Lee RF, Takahaski M, Beers JR. 1978. Short term effects of oil on plankton in controlled ecosystems. Pages 635-650 in Proceedings of the Conference on Assessment of Ecological Impacts of Oil Spills. 14-17 June 1978, Keystone, CO. American Institute of Biological Sciences.
Linden O, Elmgren R, Boehm P. 1979. The Tsesis oil spill: its impact on the coastal ecosystem of the Baltic Sea. Ambio 8:244-253.
Livingstone DR. 1991. Organic xenobiotic metabolism in marine invertebrates. In Gilles R., ed., Advances in Comparative and Environmental Physiology. Springer-Verlag, New York, NY USA, pp 46-162.
MacDonald BA; Thomas MLH. 1982. Growth reduction in the soft-shell clam *Mya Arenaria* from heavily oiled Lagoon in Chedabucto Bay, Nova Scotia. Mar Environ Res 6:145-156.
Marty, G.D., J.E. Hose, M.D. McGurk, and E.D. Brown. 1997. Histopathology and cytogenetic evaluation of Pacific herring larvae exposed to petroleum hydrocarbons in the laboratory or in Prince William Sound, Alaska, after the *Exxon Valdez* oil spill. Can. J. Fish. Aquat. Sci. 54:1846-1857.
Mattiessen P, Bifield S, Jarrett F, Kirby MF, Law RJ, McMinn WR, Sheahan DA, Thain JE, Whale GF. 1998. An assessment of sediment toxicity in the river Tyne estuary, UK by means of bioassay. Mar. Environ. Res. 45:1-15.
Mearns, AJ. 1996. *Exxon Valdez* shoreline treatment and operations: implications for response, assessment, monitoring, and research. Am Fish Soc 18:309-328.
Middaugh, D.P., P.J. Chapman, and M.E. Shelton. 1996. Responses of embryonic and larval inland silversides, *Menidia beryllina*, to a water-soluble fraction formed during biodegradation of artificially weathered Alaska North Slope crude oil. Arch. Environ. Contam. Toxicol. 31:410-419.
Middaugh, D.P., M.E. Shelton, C.L. McKenney, Jr., G. Cherr, P.J. Chapman, and L.A. Courtney. 1998. Preliminary observations on responses of embryonic and larval Pacific herring, *Clupea pallasi*, to neutral fraction biodegradation products of weathered Alaska North Slope crude oil. Arch. Environ. Contam. Toxicol. 34:188-196.
Mille G, Munoz D, Jacquot F, Rivet L, Bertrand J.-C. 1998. The *Amoco Cadiz* oil spill: evolution of petroleum hydrocarbons in the Ile Grande salt marshes (Brittany) after a 13-year period. Estuarine, Coastal and Shelf Science 47:547-559.

Moore SF, Dwyer RL. 1974. Effects of oil on marine organisms: a critical assessment of published data. Water Res 8:819-827.

Morado JF, Mooney LL, Babcock MM, Harris PM. 1998. Histopathological observation of bay mussels, *Mytilus trossulus*, exposed to *Exxon Valdez* crude oil in Prince William Sound. Pages 130-153 in Babcock, M. M., P. M. Harris, M. G. Carls, C. C. Brodersen, and S. D. Rice. 1998. Mussel bed restoration and monitoring. *Exxon Valdez* Oil Spill Restoration Project 95090 Final Report. National Oceanic and Atmospheric Administration, National Marine Fisheries Service, Auke Bay Laboratory, Juneau, AK.

Neff, J.M. 1979. Polycyclic aromatic hydrocarbons in the aquatic environment. Sources, fates, and biological effects. Applied Science Publishers LTD, London.

Neff, J.M., and W.A. Stubblefield. 1995. Chemical and toxicological evaluation of water quality following the *Exxon Valdez* oil spill. Pages 141-177 *in* P.G. Wells, J.N. Butler, and J.S. Hughes (eds.), *Exxon Valdez* oil spill: fate and effects in Alaskan Waters, ASTM STP 1219, American Society for Testing and Materials, Philadelphia.

Neff, J.M., S. Ostanzeski, W. Gardiner, and I. Stejskal. 2000. Effects of weathering on the toxicity of three offshore Australian crude oils and a diesel fuel to marine animals. Environ. Toxicol. Chem. 19:1809-1821.

North WJ. 1973. Background papers for a workshop on inputs, fates, and effects of petroleum in the marine environment. Vol. 2. Physical, chemical and biological fates, chronic, acute and health effects. National Acadamy of Sciences

O'Clair C.E., J.W. Short, and S.D. Rice. 1996. Contamination of intertidal and subtidal sediments by oil from the *Exxon Valdez* in Prince William Sound. Am. Fish. Soc. Symp. 18:61-93.

Ott FS, Harris RP, O'Hara SCM. 1978. Acute and sublethal toxicity of naphthalene and three methylated derivatives to the estuarine copepod, *Eurytemora affinis*. Mar. Environ. Res. 1:49-58.

Page, D.S., P.D. Boehm, G.S. Douglas, and A.E. Bence. 1995. Identification of hydrocarbon sources in the benthic sediments of Prince William Sound and the Gulf of Alaska following the *Exxon Valdez* oil spill. Pages 41-83 *in* P.G. Wells,, J.N. Butler, and J.S. Hughes (eds.), *Exxon Valdez* oil spill: fate and effects in Alaskan Waters, ASTM STP 1219, American Society for Testing and Materials, Philadelphia.

Page DS, Boehm PD, Douglas GS, Bence AE, Burns WA, Mankiewicz PJ. 1996. The natural petroleum hydrocarbon background in subtidal sediments of Prince William Sound, Alaska, USA. Environmental Toxicol Chem 15:1266-1281.

Page DS, Boehm PD, Douglas GS, Bence AE, Burns WA, Mankiewicz PJ. 1999. Pyrogenic polycylic aromatic hydrocarbons in sediments record past human activity: a case study in Prince William Sound, Alaska. Mar Pollut Bul 38:247-260.

Page DS, Boehm PD, Stubblefield WA, Parker KR, Gilfillan ES, Neff JM, Maki AW. 2002a. Hydrocarbon composition and toxicity of sediments following the *Exxon Valdez* oil spill in Prince William Sound, Alaska, USA. Environ Toxicol Chem 21:1438-1450.

Page DS, Boehm PD, Brown JS, Bence AE, Burns WA, Douglas GS. 2002b. Historical analysis as a tool in identifying PAH sources in Prince William Soiund, Alaska sediments. Abstract. SETAC 23rd annual meeting in North America, November 16-20, Salt Lake City, Utah, USA.

Page DS, Boehm PD, Brown JS, Neff JM, Burns WA, Bence AE. 2005 Mussels document loss of bioavailable polycyclic aromatic hydrocarbons and the return to baseline conditions for oiled shorelines in Prince William Sound, Alaska. Mar. Environ. Res. 60:422-436.

Pearson, W.H., D.L. Woodruff, S.L. Kiesser, G.W. Fellingham, and R.A. Elston. 1985. Oil effects on spawning behavior and reproduction in Pacific herring *(Clupea harengus pallasi*). Final report to the American Petroleum Institute, Environmental Affairs Department, Washington, D.C.

Pedersen F, Bjoernestad E, Andersen HV, Kjolholt J, Poll C. 1998. Characterization of sediments from Copenhagen Harbour by use of biotests. Water Sci Technol 37:233-240.

Pelletier MC, Burgess RM, Ho KT, Kuhn A, McKinney RA, Ryba SA. 1997. Phototoxicity of individual polycyclic aromatic hydrocarbons and petroleum to marine invertebrate larvae and juveniles. *Environ. Toxicol. Chem*. 16:2190-2199.

Peterson, CH. 2001. The "*Exxon Valdez*" oil spill in Alaska: acute, indirect, and chronic effects on the ecosystem. Advances in Marine Biology 39:1-103.

Peterson CH, McDonald LL, Green RH, Erickson WP. 2002. The joint consequences of multiple components of statistical sampling designs. Mar Ecol Prog Ser 231:309-314.

Prince RC, Bragg JR. 1997. Shoreline bioremediation following the *Exxon Valdez* oil spill in Alaska. Bioremediation Journal 1:97-104.

Prince RC, Owens EH, Sergy GA. 2002. Weathering of an Arctic oil spill over 20 years: the BIOS experiment revisited. Mar Pollut Bul 44:1236-1242.

Pritchard PH, Mueller JG, Rogers JC, Kremer FV, Glaser JA. 1992. Oil spill bioremediation: experiences, lessons and results from the *Exxon Valdez* oil spill in Alaska. Biodegradation 3:315-335.

Reddy CM, Eglinton TI, Hounshell A, White HK, Xu L, Gaines RB, Frysinger GS. 2002. The West Falmouth oil spill after thirty years: the persistence of petroleum hydrocarbons in marsh sediments. Enviorn Sci Technol 36:4754-4760.

Rice, S.D., J.W. Short, and J.F. Karinen. 1977. A review of comparative oil toxicity and comparative animal sensitivity. Pp. 78-94 *in* D.A. Wolfe (ed.), Fate and Effects of Petroleum Hydrocarbons in Marine Organisms and Ecosystems. Pergamon Press, New York.

Seiser, P. E., Duffy, L. K., McGuire, A. D., Roby, D. D., Golet, G. H., & Litzow, M. A. (2000). Comparison of pigeon guillemot, *Cepphus columba*, blood parameters from oiled and unoiled areas of Alaska eight years after the *Exxon Valdez* oil spill. *Marine Pollution Bulletin,* 40, 152-164.

Shelton, M.E., P.J., Chapman, S.S. Foss, and W.S. Fisher. 1999. Degradation of weathered oil by mixed marine bacteria and the toxicity of accumulated water-soluble material to two marine crustacea. Arch. Environ. Contam. Toxicol. 36:13-20.

Short, J.W. and M.M. Babcock. 1996. Prespill and postspill concentrations of hydrocarbons in mussels and sediments in Prince William Sound. Am Fish Soc Symp 18:149-166.

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. Am. Fish. Soc. Symp. 18:17-28.

Short, J.W., R.A. Heintz, B.D. Nelson, J.M. Maselko, M.F. Kendziorek, M.G. Carls, and S. Korn. 1996a. *Exxon Valdez* oil spill of 1989: State/Federal Trustee Council hydrocarbon database 1989-1995. National Oceanic and Atmospheric Administration, National Marine Fisheries Service, Auke Bay, AK.

Short, J.W., T.J. Jackson, M.L. Larsen, and T.L. Wade. 1996b. Analytical methods used for the analyses of hydrocarbons in crude oil, tissues, sediments, and seawater collected for the natural resource damage assessment of the *Exxon Valdez* oil spill. Am Fish Soc Symp 18:140-148.

Short J., and R. Heintz. 1997. Identification of *Exxon Valdez* oil in sediments and tissues of PWS. Environ. Sci. Technol. 31:2375-2384.

Short JW, Kvenvolden KA, Carlson PR, Hostettler FD, Rosenbauer RJ, Wright BA. 1999. Natural hydrocarbon background in benthic sediments of Prince William Sound, Alaska: oil vs coal. Environ. Sci. Technol. 33:34-42.

Short JW, Lindeberg MR, Harris PM, Maselko JM, Pella JJ, Rice SD. 2004. Estimate of oil persisting on the beaches of Prince William Sound 12 years after the *Exxon Valdez* oil spill. Environ Sci Technol 38:19-25.

Stegeman JJ. 1978. Influence of environmental contamination on cytochrome P-450 mixed-function oxygenases in fish: implications for recovery in the Wild Harbor marsh. J. Fish. Res. Board Can. 35:668-674.

Swartz RC, DeBen WA, Jones JKP, Lamberson JO, Cole FA. 1985. Phoxocephalid amphipod bioassay for marine sediment toxicity. In Cardwell RD, Purdy R, Bahner RC (eds.), Aquatic toxicology and hazard assessment: seventh symposium, ASTM STP 854. American Society for Testing and Materials, Philadelphia. PA. pp 284-307.

Teal JM, Howarth RW. 1984. Oil spill studies: a review of ecological effects. Environmental Management 8:27-44.

Teal JM, Farrington JW, Burns KA, Stegman JJ, Tripp BW, Woodin B, Phinney C. 1992. The West Falmouth oil spill after twenty years: fate of fuel oil compounds and effects on animals. Mar Pollut Bul 24:607-614.

Thomas, R.E., P.M. Harris, and S.D. Rice. 1999. Survival in air of *Mytilus trossulus* following long term exposure to spilled *Exxon Valdez* crude oil in Prince William Sound. Comp. Biochem. Physiol. Part C 122:147-152.

Tilseth S, Solberg TS, Westrheim K. 1984. Sublethal effects of the water-soluble fraction of Ekofisk crude oil on the early larval stages of Cod (*Gadus morhua* L.). Mar. Environ. Res. 11:1-16.

Trust, K. A., Esler, D., Woodin, B. R., & Stegeman, J. J. (2000). Cytochrome P450 1A induction in sea ducks inhabiting nearshore areas of Prince William Sound, Alaska. *Marine Pollution Bulletin*, 40, 397-403.

Vandermeulen J; Penrose WR. 1978. Absence of aryl hydrocarbon hydroxylase (AHH) in three marine bivalves. J Fish Res Bd Can 35:643-647.

Vandermeulen JH; Sing JG. 1994. Arrow oil spill, 1970-90: persistence of 20-yr weathered Bunker C fuel oil. Can J Fish Aquat Sci 51:84-855.

Van Kooten GK, Short JW, Kolak JJ. 2002. Low-maturity Kulthieth formation coal: a possible source of polycyclic aromatic hydrocarbons in benthis sediment of the northern Gulf of Alaska. Environ Forensics 3:227-241.

Velleman PF, Hoaglin DC. 1981. Applications, basics and computing of exploratory data analysis. Duxbury Press, Boston, Massachusetts.

Weis JS, Weis P. 1989. Effects of environmental pollutants on early fish development. Aquat Sci 1:45-73.

White, P.A., S. Robitaille, and J.B. Rasmussen. 1999. Heritable reproductive effects of benzo[a]pyrene on the fathead minnow (*Pimephales promelas*). Environ. Toxicol. Chem. 18:1843-1847.

Wolfe D.A., M.J. Hameedi, J.A. Galt, G. Watabayashi, J. Short, C. O'Clair, S. Rice, J. Michel, J.R. Payne, J. Braddock, S. Hanna, and D. Sale. 1994. The fate of the oil spilled from the *Exxon Valdez*. Environ. Sci. Technol. 28:561A-8A.

Wooley C. 2002. The myth of the "pristine environment:" past human impacts in Prince William Sound and the northern Gulf of Alaska. Spill Science and Technology Bulletin 7:89-104.

**Table 1.1.** Sample locations. Map numbers identify geographic placement (see Fig. 1). Time series data were examined for each of these locations.

| Map Number | Location |
|---|---|
| 23 | Barnes Cove |
| 21 | Bay of Isles |
| 30 | Bligh Island |
| 28 | Constantine Harbor |
| 4 | Crab Bay |
| 11 | Culross Passage |
| 19 | Disk Island |
| 17 | Eleanor Island |
| 1 | Elrington Island |
| 9 | Eshamy Bay |
| 5 | Evans Island (northern end) |
| 7 | Ewan Bay |
| 26 | Green Island |
| 20 | Herring Bay and Herring Point |
| 2 | Latouche Island, except Sleepy Bay |
| 12 | Long Bay |
| 22 | Marsha Bay |
| 10 | McClure Bay |
| 25 | Mummy Bay |
| 15 | Naked Island group |
| 16 | Northwest Bay |
| 29 | Olsen Bay |
| 8 | Paddy Bay |
| 14 | Perry Island |
| 6 | Prince of Wales Passage |
| 27 | Rocky Bay and Rocky Point |
| 32 | Simpson Bay |
| 31 | Siwash Bay |
| 3 | Sleepy Bay |
| 18 | Smith Island |
| 24 | Snug Harbor |
| 13 | Wells Passage |

**Table 1.2.** Estimated toxic threshold concentrations of oil on sediment.

| Species | TPAH | Source |
|---|---|---|
| mummichog (*Fundulus heteroclitus*) | 270 | Couillard 2002[a] |
| pink salmon (*Oncorhynchus gorbusha*) | 540 | Carls et al. in review |
| mixed intertidal species | 2600 | Page et al. 2002 |
| pink salmon (*Oncorhynchus gorbusha*) | 4600 | Heintz et al. 2002 |

[a] Reported value was 12.7 µg/g whole oil; TPAH was 2.1% of the total oil mass in this experiment.

**Table 1.3.** Comparison of total polynuclear aromatic hydrocarbon (TPAH) concentrations in samples identified by modeling as pyrogenic (score < 0) or petrogenic (score > 2). Mussel and sediment samples were compared separately with single factor ANOVA; concentrations were log-transformed for analysis.

| | n | geoMean | min | max | P |
|---|---|---|---|---|---|
| mussels | | | | | |
| pyrogenic | 745 | 5.3 | 0.0 | 4007 | <0.001 |
| petrogenic | 812 | 1570.4 | 41.0 | 266226 | |
| sediment | | | | | |
| pyrogenic | 571 | 45.1 | 0.0 | 45279 | <0.001 |
| petrogenic | 697 | 1810.5 | 6.1 | 6515536 | |

**Table 1.4** (*at right*). Relationship between oiled sediment and contaminated mussels after 1989 at locations oiled in 1989. Column abbreviations: n = total number of sediment samples, n' = number of sediment samples with identifiable *Exxon Valdez* oil (EVO) by two or more oil-fingerprint models, m = total number of mussel samples, m' = number of mussel samples with EVO identified by two or more models. Superscripts: * EVO presence was verifed in mussel tissue in the last year sampled, [r]locations outside the slick boundary, [p]pyrogenic signal apparent in sample with highest TPAH concentration, [e]highly weathered EVO probable but verified only by the oil fingerprint model (Bence and Burns 1995).

| Location | Sediment: Mean TPAH (ng/g dry) | Sediment: n | Sediment: n' | Sediment: % with EVO | Mussels: Mean TPAH (ng/g dry) | Mussels: m | Mussels: m' | Mussels: % with EVO | Last year sampled |
|---|---|---|---|---|---|---|---|---|---|
| a. Locations where EVO was identified in sediment and mussels (after 1989) | | | | | | | | | |
| Green Island | 146 | 22 | 4 | 18 | 687 | 10 | 2 | 20 | 1991 |
| Bainbridge Point | 650 | 3 | 1 | 33 | 102 | 9 | 1 | 11 | 1991 * |
| Northwest Bay | 803 | 17 | 5 | 29 | 311 | 9 | 3 | 33 | 1991 * |
| Snug Harbor | 1890 | 49 | 9 | 18 | 158 | 25 | 3 | 12 | 1991 |
| Prince of Wales Passage | 3211 | 17 | 4 | 24 | 207 | 12 | 2 | 17 | 1995 |
| Elrington Island | 3267 | 28 | 6 | 21 | 700 | 20 | 2 | 10 | 1997 |
| Disk Island | 6847 | 14 | 4 | 29 | 942 | 133 | 12 | 9 | 1999 * |
| Herring Bay | 14675 | 157 | 32 | 20 | 1028 | 173 | 17 | 10 | 1999 * |
| Bay of Isles | 17620 | 70 | 23 | 33 | 1506 | 87 | 17 | 20 | 1999 * |
| Sleepy Bay | 17669 | 95 | 26 | 27 | 2680 | 78 | 14 | 18 | 1998 * |
| Evans Island | 28803 | 41 | 11 | 27 | 242 | 35 | 2 | 6 | 1999 |
| Squirrel Island | 52936 | 3 | 2 | 67 | 3532 | 26 | 8 | 31 | 1999 |
| Chenega Island | 78893 | 99 | 14 | 14 | 890 | 178 | 15 | 8 | 1999 * |
| Eleanor Island | 166153 | 24 | 6 | 25 | 810 | 92 | 10 | 11 | 1999 |
| Applegate Island | 194696 | 2 | 2 | 100 | 467 | 19 | 1 | 5 | 1999 |
| Foul Bay | 310661 | 4 | 4 | 100 | 2505 | 27 | 5 | 19 | 1999 |
| Latouche Island | 2503409 | 11 | 5 | 45 | 857 | 16 | 2 | 13 | 1999 |
| b. Locations where ANS was not detected in either sediment or mussels (after 1989) | | | | | | | | | |
| Siwash Bay[r] | 18 | 11 | 0 | 0 | 70 | 12 | 0 | 0 | 1991 |
| Perry Island | 19 | 9 | 0 | 0 | 22 | 8 | 0 | 0 | 1991 |
| Bligh Island | 21 | 13 | 0 | 0 | 39 | 12 | 0 | 0 | 1991 |
| Culross Passage | 75 | 10 | 0 | 0 | 52 | 4 | 0 | 0 | 1990 |
| Eshamy Bay | 61 | 10 | 0 | 0 | 53 | 3 | 0 | 0 | 1991 |
| Ewan Bay[r] | 378 | 9 | 0 | 0 | 0 | 3 | 0 | 0 | 1990 |
| c. Locations where TPAH was identified in sediment but not in mussels (after 1989) | | | | | | | | | |
| Wilson Bay | 120 | 9 | 1 | 11 | 37 | 1 | 0 | 0 | 1990 |
| Rocky Bay & Point | 276 | 15 | 1 | 7 | 69 | 17 | 0 | 0 | 1991 |
| McClure Bay[r] | 371 | 11 | 2 | 18 | 60 | 5 | 0 | 0 | 1990 |
| Naked Island | 236 | 52 | 3 | 6 | 156[p] | 35 | 0 | 0 | 1999 |
| Long Bay[r] | 381 | 11 | 1 | 9 | 41 | 4 | 0 | 0 | 1990 |
| d. Unusual locations (after 1989) | | | | | | | | | |
| Constantine Harbor[r] | 589 | 7 | 3 | 43 | 9 | 12 | 0 | 0 | 1990 |
| Simpson Bay[r] | 2508 | 9 | 1 | 11 | 114 | 2 | 0 | 0 | 1990 |
| Olsen Bay[r] | 43 | 32 | 0 | 0 | 86 | 33 | 1 | 3 | 1999 |

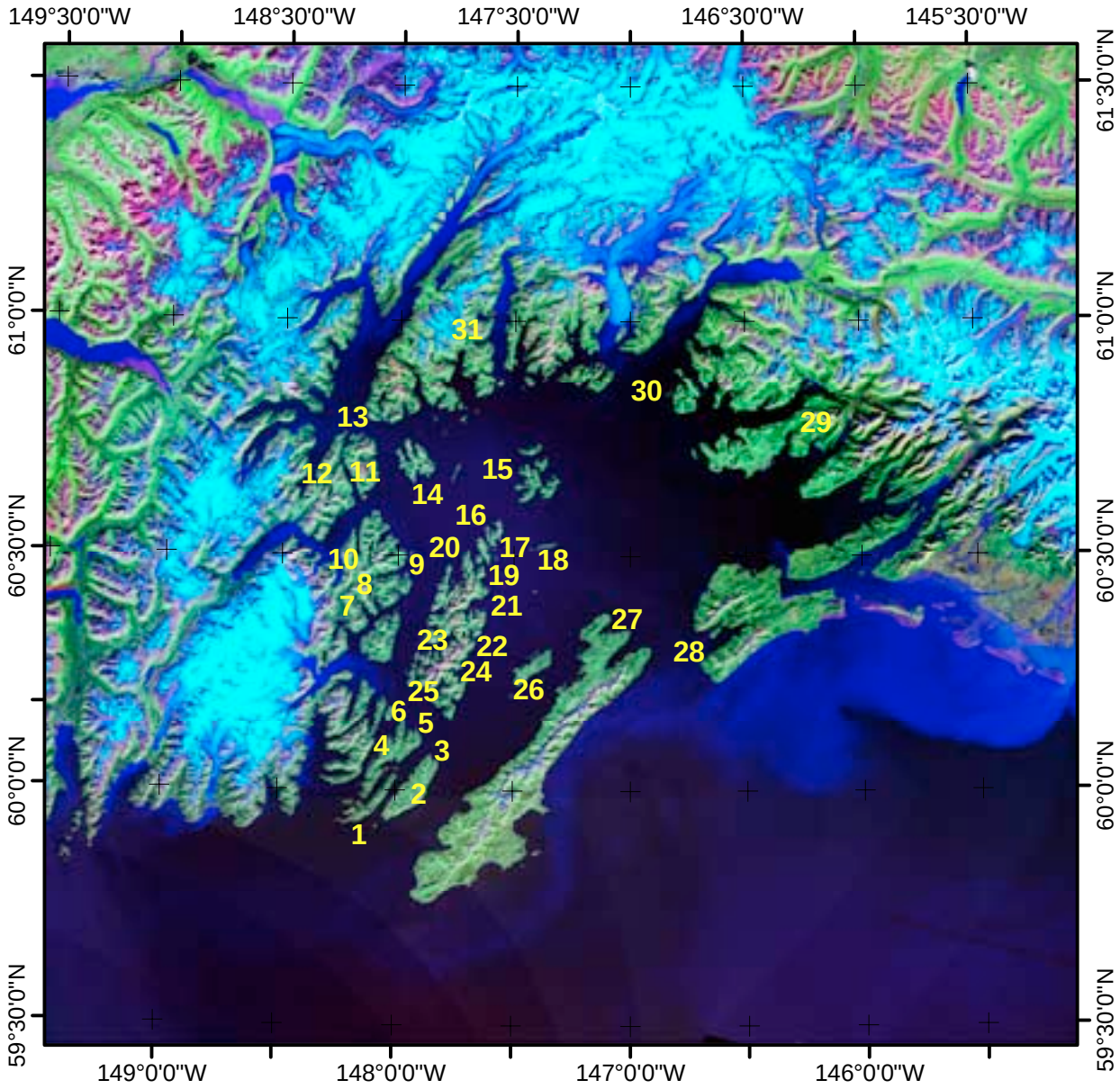


**Fig. 1.1.** Prince William Sound and major study locations; see Table 1.1 for explanation of location numbers.

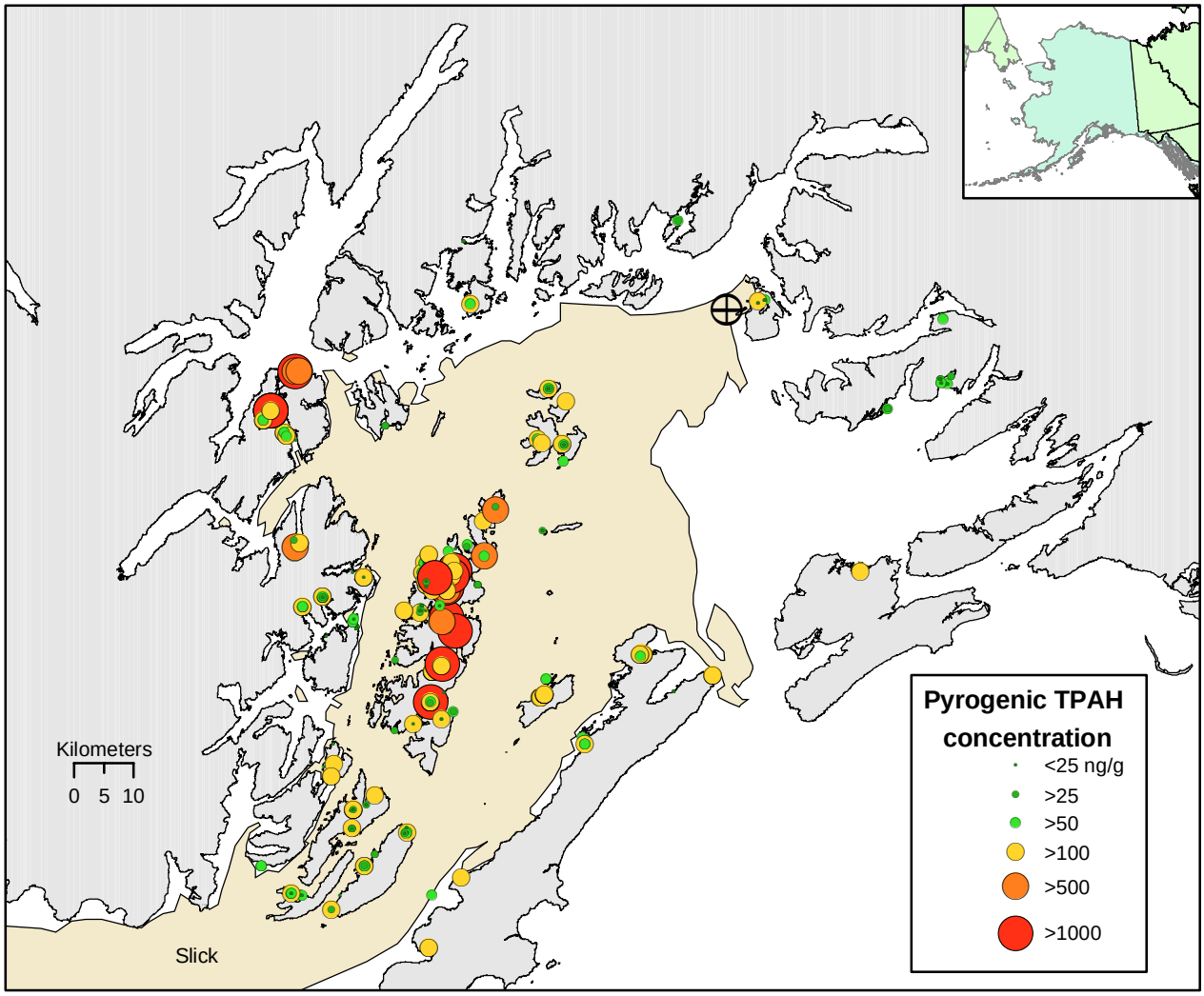


**Fig. 1.2.** Distribution of pyrogenic hydrocarbons in sediment (1989 to 2000). All individual observations are included, each color-coded and sized according to concentration. The slick trajectory outlines the known extent of the *Exxon Valdez* oil slick (Gundlach et al. 1990).

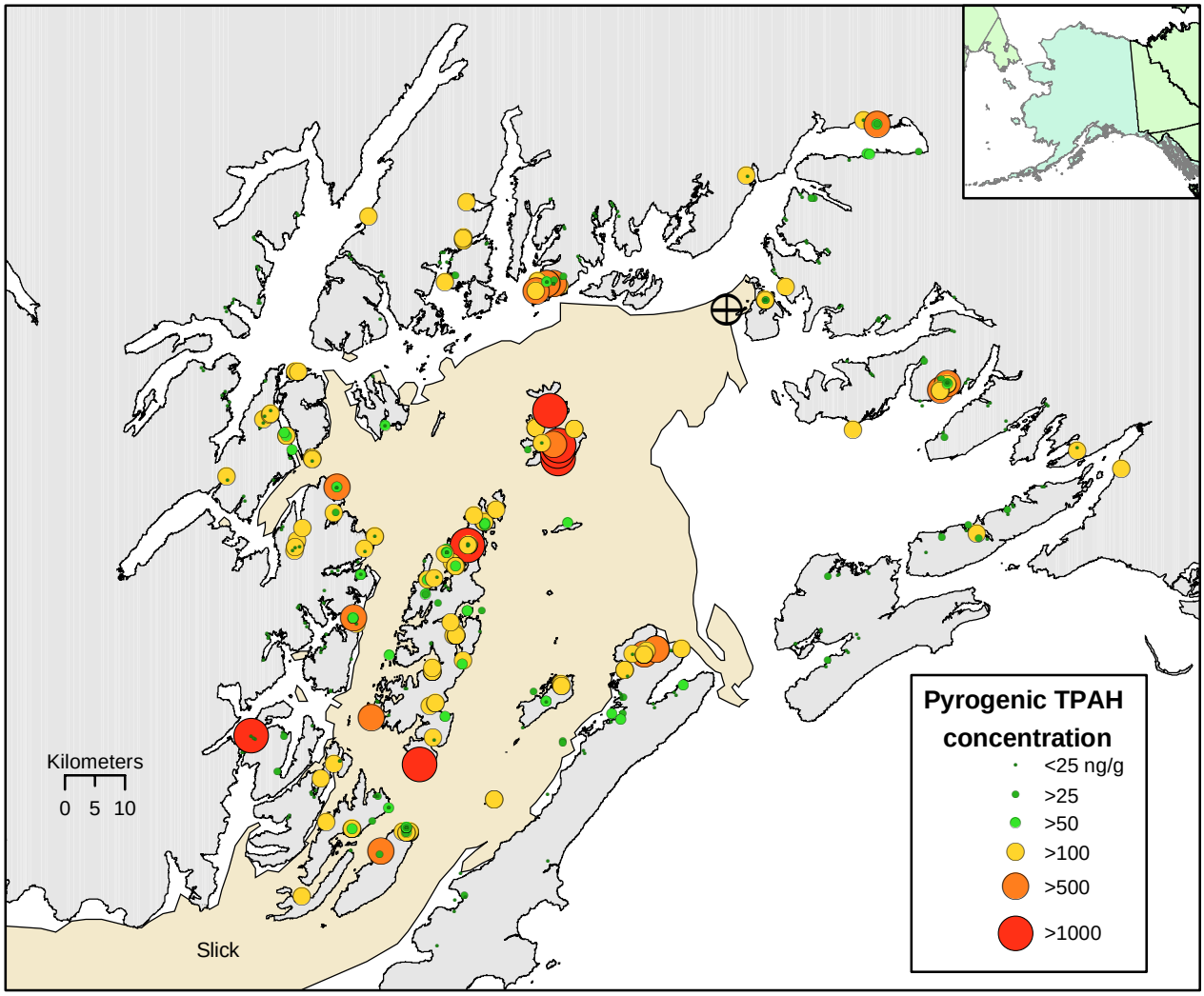


**Fig. 1.3.** Distribution of pyrogenic hydrocarbons in mussels (1989 to 1999). All individual observations are included, each color-coded and sized according to concentration. The slick trajectory outlines the known extent of the *Exxon Valdez* oil slick (Gundlach et al. 1990).

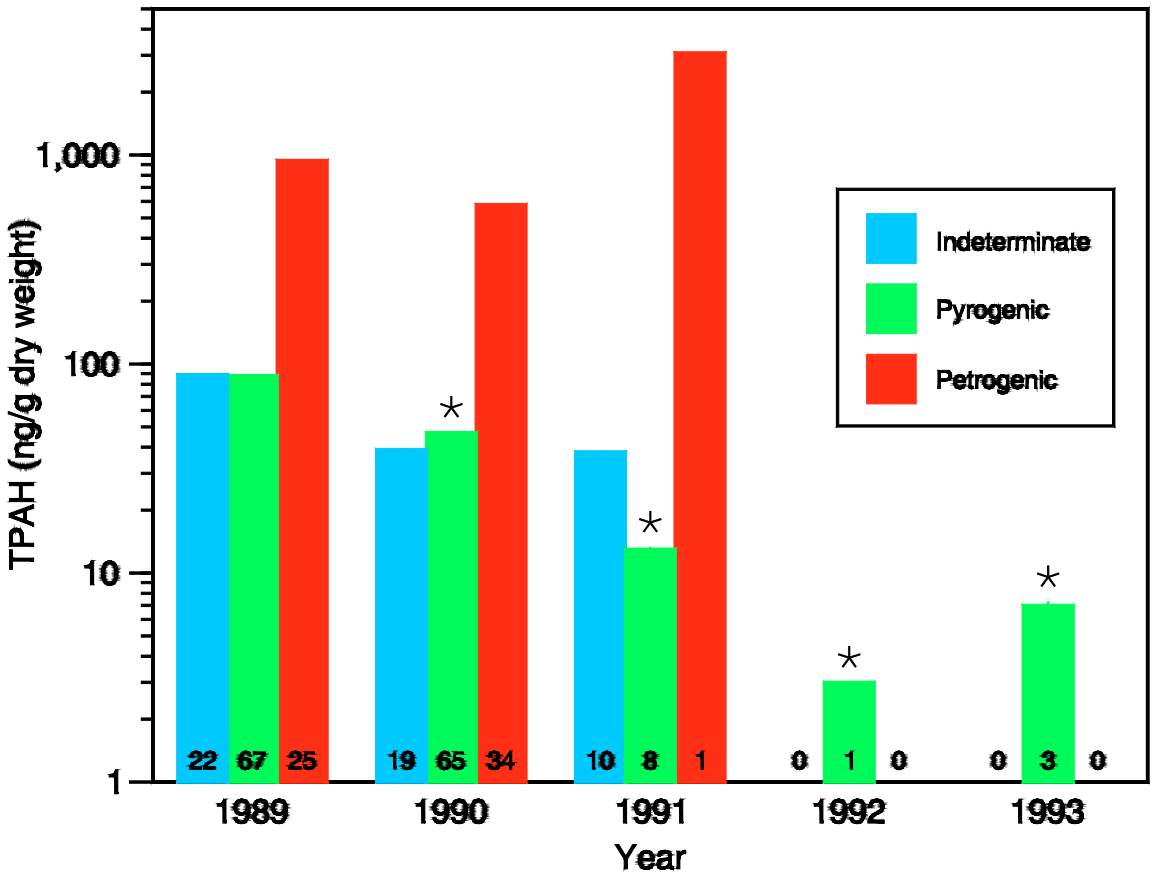


Fig. 1.4. Total polynuclear aromatic hydrocarbon (TPAH) concentration changes in reference sediment over time.

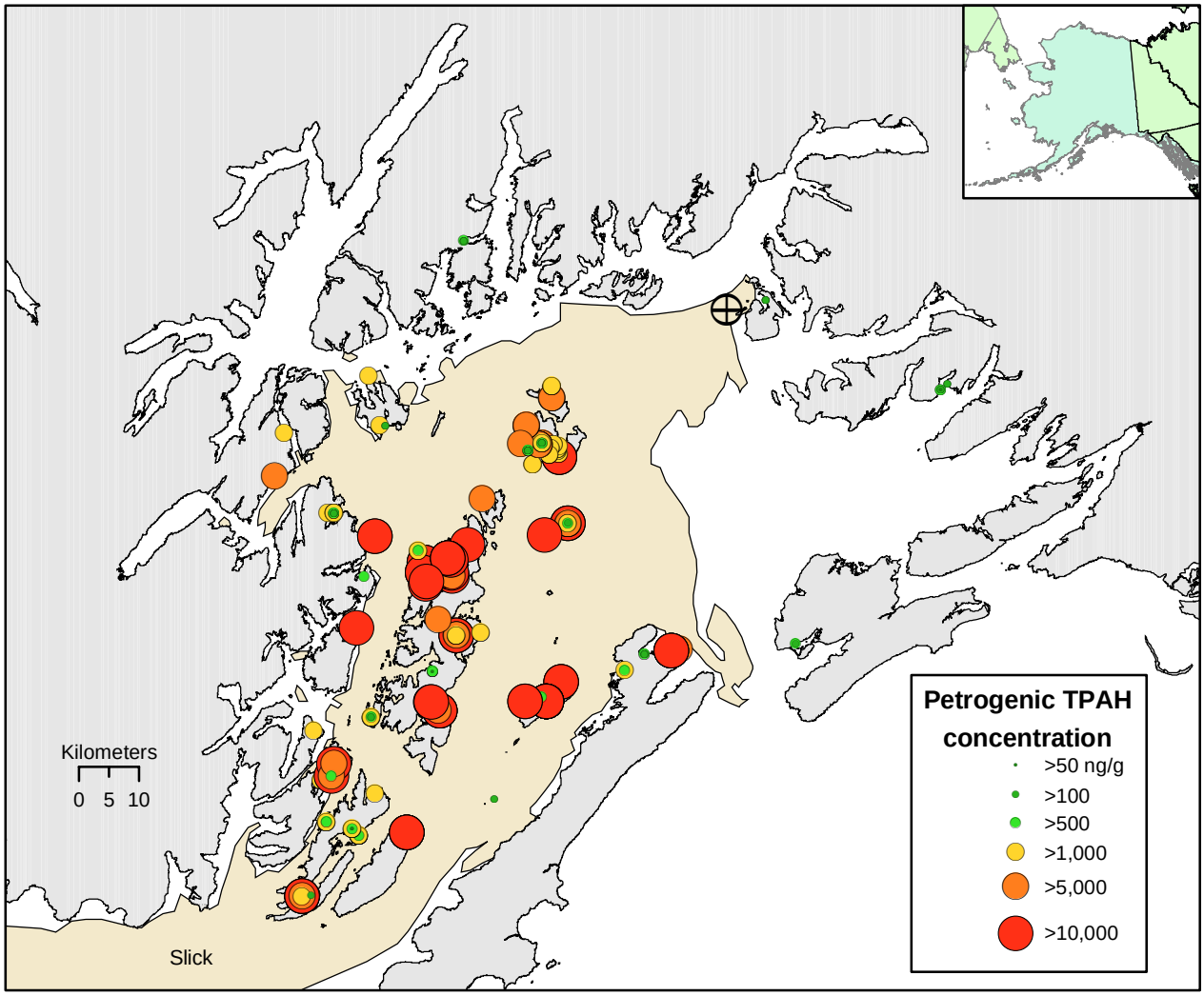


**Fig. 1.5.** Distribution of petrogenic TPAH concentrations in mussel tissue in 1989. All individual observations are included, each color-coded and sized according to concentration. The slick trajectory outlines the known extent of the *Exxon Valdez* oil slick (Gundlach et al. 1990). *See Appendix 1 for distribution in other years*.

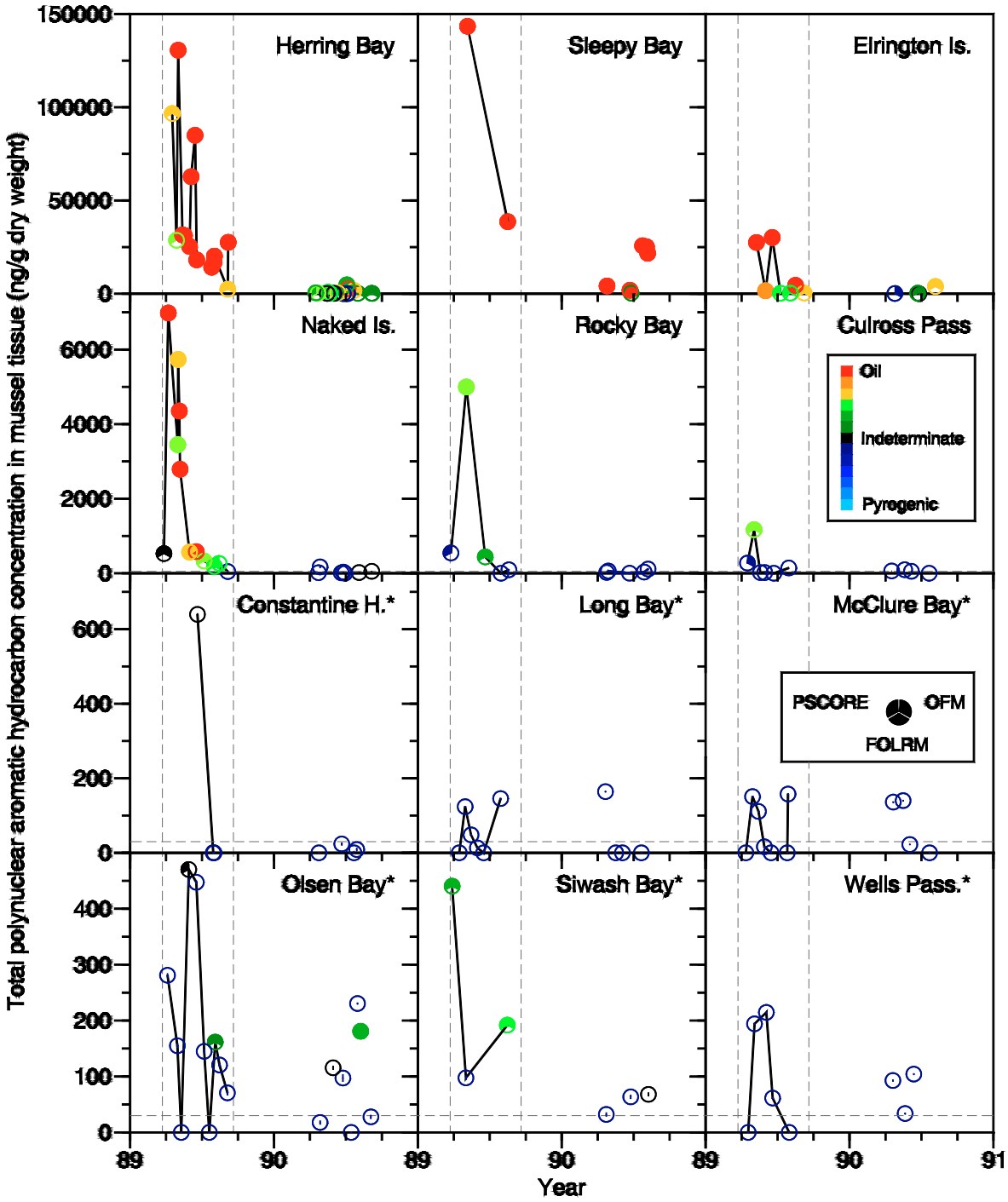


**Fig. 1.6.** Example mean (±SE) total polynuclear aromatic hydrocarbon (TPAH) concentrations in mussels. Locations outside slick boundaries are marked with asterisks. Symbol fill indicates that *Exxon Valdez* oil was identified by one or more models: a non-parametric model ($PSCORE_{ANS}$; Carls submitted), an oil-fingerprint model ($OFM_{ANS}$; Bence and Burns 1995), and a first-order loss-rate model ($FORLM_{ANS}$; Short and Heintz 1997). Color is based on average assessment of oiling by the combined models, including two pyrogenic models. Background concentration for mussels, about 30 ng/g (Boehm et al. 2004) is indicated with horizontal dashed lines for reference. The time of the spill and 180 d later are indicated with vertical dashed lines. See Appendix 3 for more time series detail.

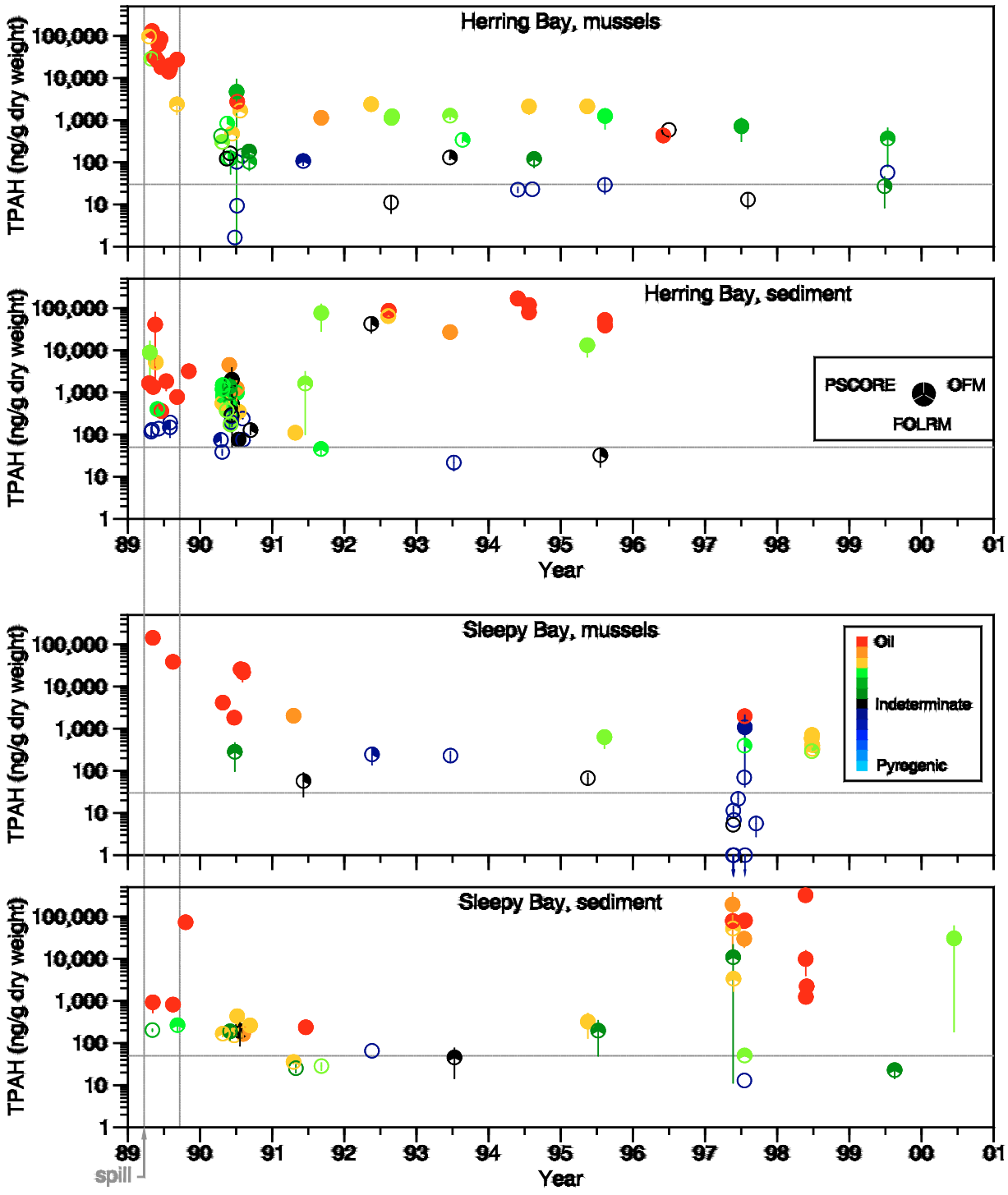


**Fig. 1.7.** Mean (±SE) total polynuclear aromatic hydrocarbon (TPAH) concentrations in mussels and sediment at example oiled locations. Symbol fill indicates that *Exxon Valdez* oil was identified by one or more oil composition models: a non-parametric model ($PSCORE_{ANS}$; Carls submitted), an oil-fingerprint model ($OFM_{ANS}$; Bence and Burns 1995), and a first-order loss-rate model ($FORLM_{ANS}$; Short and Heintz 1997). Color is based on average assessment of oiling by the combined models, including two pyrogenic models. Background concentrations for mussels (about 30 ng/g, Boehm et al. 2004) and sediment (50 ng/g) are indicated with horizontal dashed lines for reference. Time of the spill and 180 d later are indicated with vertical dashed lines. See Appendix 3 for more time series.

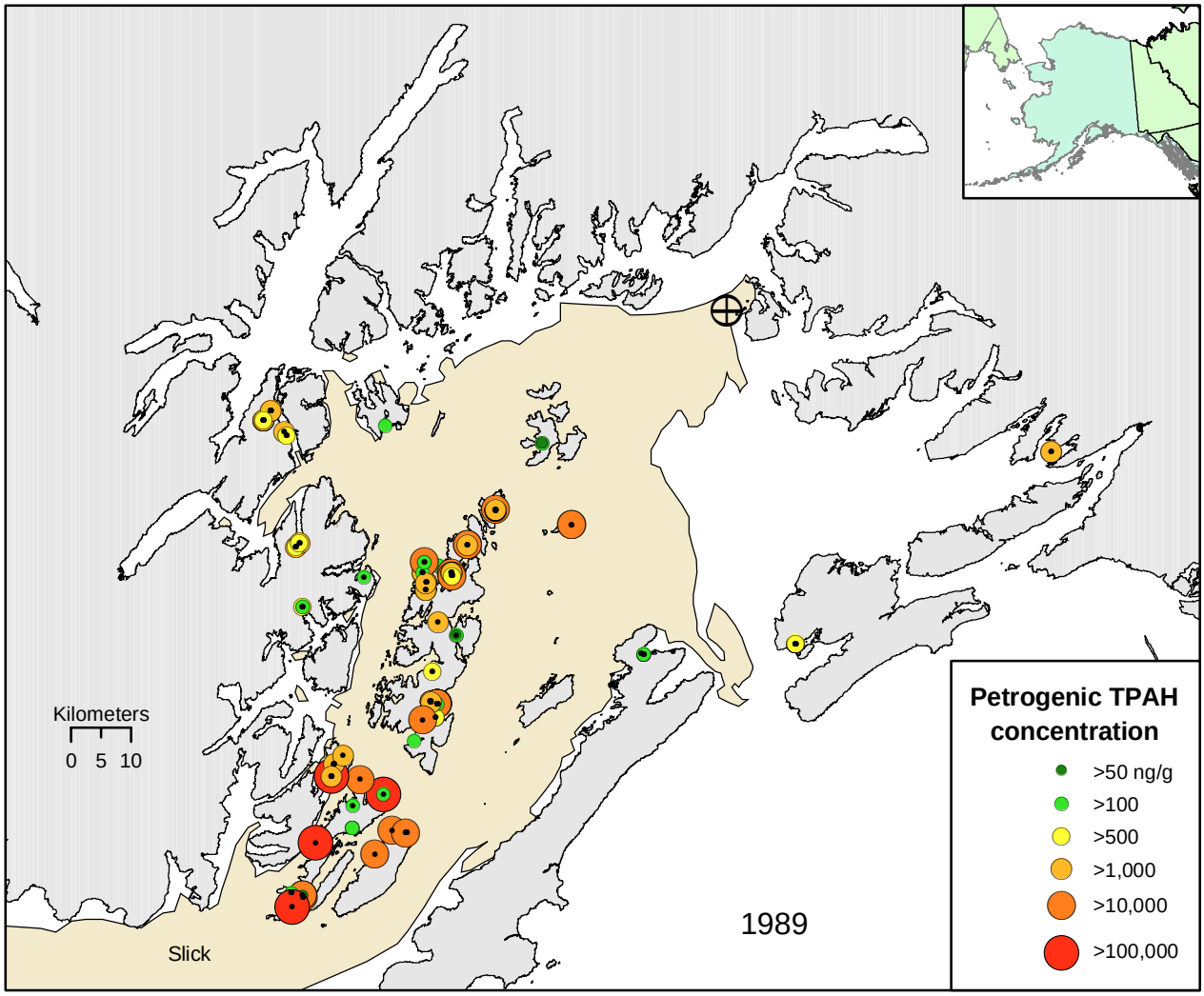


**Fig. 1.8.** Geographic distribution of petrogenic TPAH concentrations in sediment in 1989 and 1995 where concentration >50 ng/g dry weight. The last year with comprehensive sediment sampling (within the spill zone) was 1995. Samples with toxic potential are marked with black dots. *See Appendix 2 for distribution in other years*

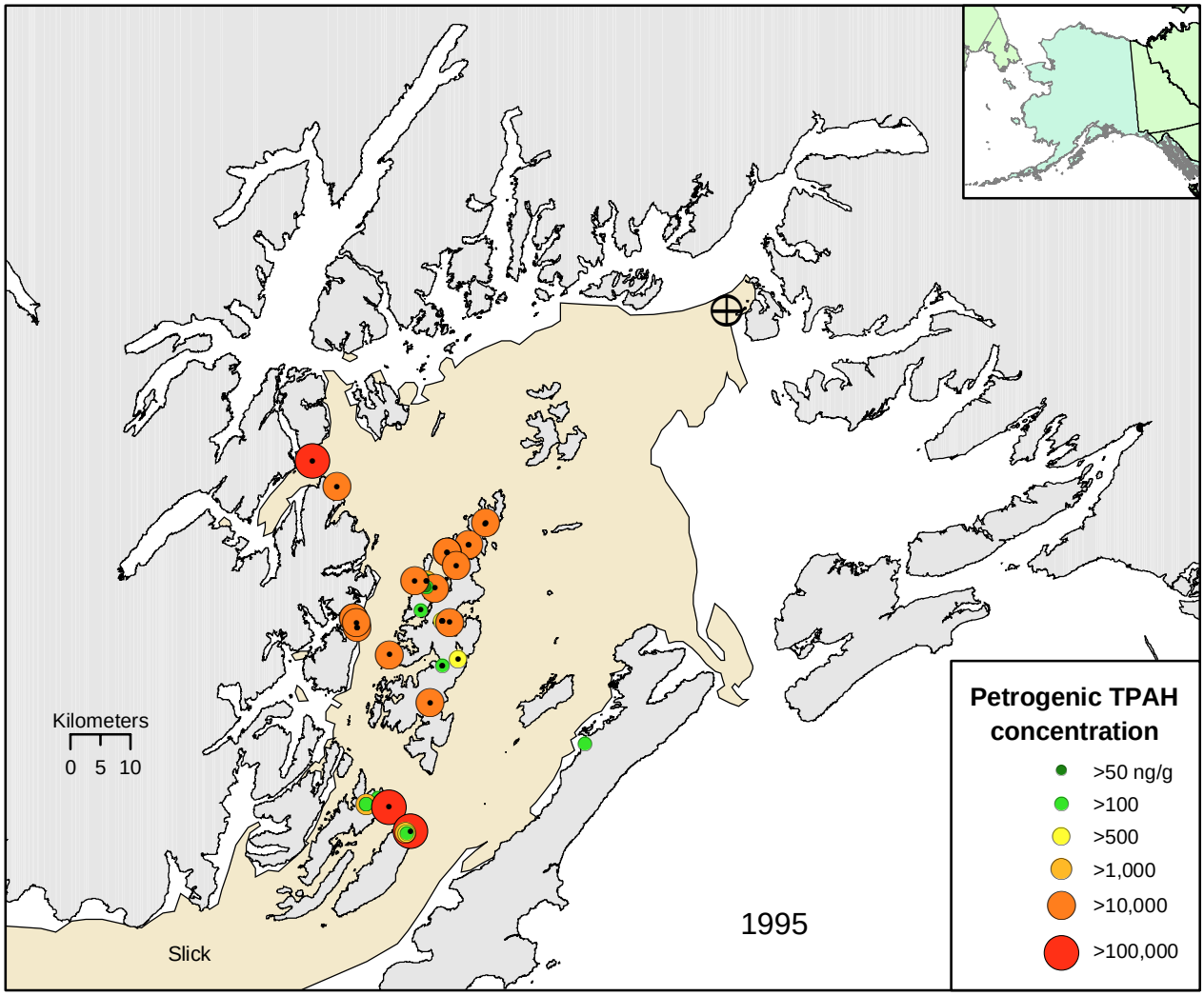


*Fig. 1.8 continued.* Petrogenic TPAH in sediment, 1995.

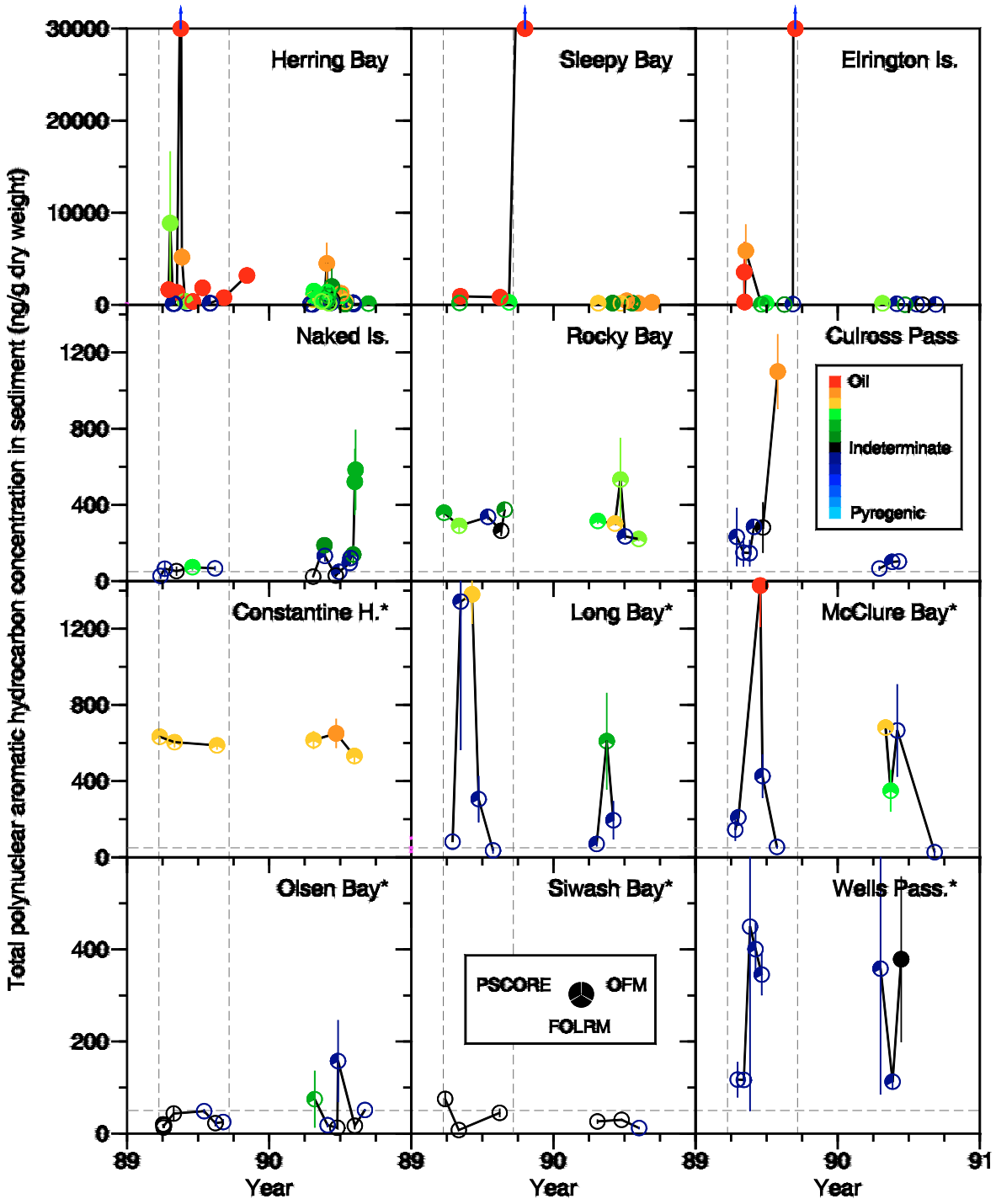


**Fig. 1.9.** Example mean (±SE) total polynuclear aromatic hydrocarbon (TPAH) concentrations in sediment. Locations outside slick boundaries are marked with asterisks. Off-scale TPAH concentrations are indicated with arrows. Symbol fill indicates that *Exxon Valdez* oil was identified by one or more models: a non-parametric model ($PSCORE_{ANS}$; Carls submitted), an oil-fingerprint model ($OFM_{ANS}$; Bence and Burns 1995), and a first-order loss-rate model ($FORLM_{ANS}$; Short and Heintz 1997). Color is based on average assessment of oiling by the combined models, including two pyrogenic models. Background concentration for sediment, about 50 ng/g, is indicated with horizontal dashed lines for reference. The time of the spill and 180 d later are indicated with vertical dashed lines. See Appendix 3 for more time series detail.

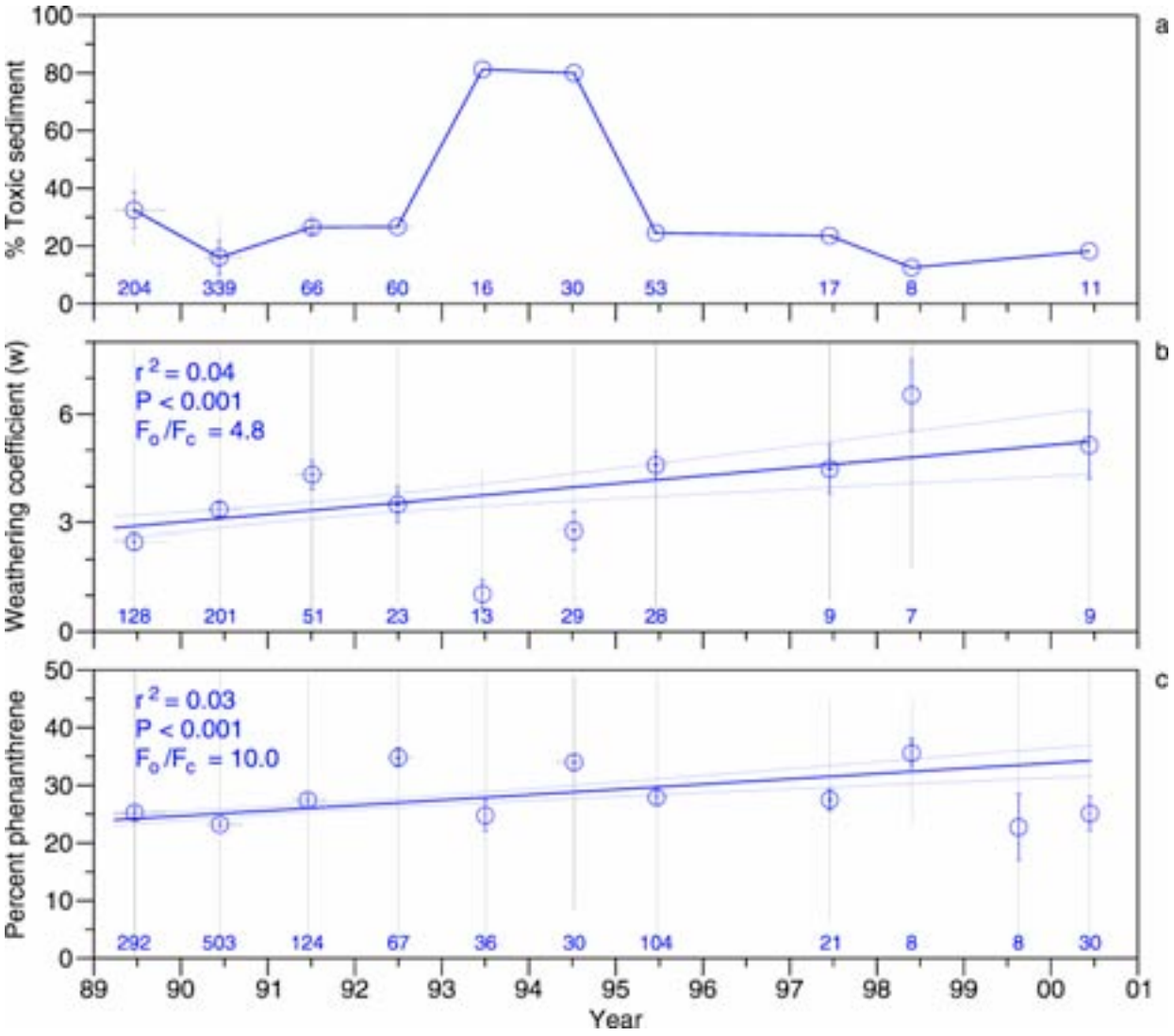


**Fig. 1.10.** Mean percent toxic sediment, mean weathering coefficient *w* (unitless), and mean percent phenanthrenes as functions of time. Data were grouped by observation year: horizontal lines indicate time range for each mean. Thin vertical lines are ranges; thicker, bounded vertical lines are ±SE. Numbers near the bottom of each panel are the total number of sediments examined for potential toxicity (a) or the number of observations contributing to corresponding mean values (b-c). Correlation ($r^2$), P, and $F_{observed}$ / $F_{critical}$ ratios ($F_o/F_c$) were as indicated (linear models).

# Chapter 2

# Retention and loss of intertidal *Exxon Valdez* oil 1992-1999

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**Abstract**

Petroleum hydrocarbon concentrations typically declined from 1992-1999 in surface sediment and mussels of beds heavily oiled by the *Exxon Valdez* spill. Total polynuclear aromatic hydrocarbon (TPAH) concentrations in mussels frequently reached background levels by 1999. In contrast, sediment in most study beds (17 of 22) remained contaminated in 1999; toxic oil might persist until mid century in some beds. Limited observations suggest the impact of this oil reservoir was roughly the same in burrowing species like clams (Prototeca and Saxidomus) as in mussels. Contaminated habitat and prey may explain the continued evidence of bird, sea otter, and fish exposure to oil noted in other studies. We conclude that habitat recovery is in progress but may not be complete for many years.

*keywords*: mussels; *Mytilus trossulus*; Prince William Sound; Gulf of Alaska; *Exxon Valdez*; petroleum hydrocarbons; oil spills; monitoring, recovery

## Introduction

Natural loss of *Exxon Valdez* oil from intertidal sediment and mussels in Prince William Sound, Alaska, was slower than anticipated, thus substantial quantities remained after the spill (Babcock et al. 1994). Because aggressive post-spill cleanup methods, particularly high-pressure hot water washing, often devastated intertidal biota (Mearns, 1996; Houghton et al., 1996), most dense, oiled mussel (*Mytilus trossulus)* beds on finer, unconsolidated substrates were not cleaned. The general assumption was that natural processes would clean the beds fairly quickly, but this was not the case, even on high-energy beaches. Rather, oil that penetrated into armored beaches was protected from rapid removal (Hayes and Michel 1999) and weathering. In retrospect long-term oil retention is not surprising; oil from other spills also persisted in intertidal areas for long periods (e.g., Dow 1978; Buikema and Cairns 1984; Teal and Howarth 1984), sometimes indefinitely (e.g. Gundlach et al. 1981; Reddy et al. 2002).

Contaminated bivalves and disturbance of oiled substrate during feeding activities may explain the failure of some vertebrate communities to recover from the *Exxon Valdez* spill. The intertidal zone is highly productive (e.g., Leigh et al. 1987) and intertidal organisms, including mussels and clams (*Prototeca* and *Saxidomus*) provide food for higher-order consumers, including fish, birds, otters, and humans. Petroleum hydrocarbons in mussels and clams have been identified as a possible source of contamination for several predator species (Duffy et al. 1996; Sharp et al. 1996), and there is continuing evidence of bird (*Cepphus columba*, *Histrionicus histrionicus*, and *Bucephala islandica*), sea otter (*Enhydra lutris*), and fish (*Hexagrammos octogrannnus*) exposure to oil (Seiser et al. 2000; Esler et al. 2000; Trust et al. 2000; Bodkin et al. 2002; Golet et al. 2002; Jewett et al. 2002).

Our primary objective was to continue monitoring oil loss from mussel tissue and sediment of previously oiled mussel beds in Prince William Sound (PWS), extending the published time series (1992-1995; Carls et al. 2001) through 1999. A second objective was to

determine hydrocarbon concentrations in infauna, i.e., animals in direct contact with oiled sediment, and compare them to concentrations in mussels. The latter objective is important because data presented herein suggests that the bioavailability of oil to sessile surface dwellers (e.g., mussels) has declined even in areas with continued sediment contamination. We hypothesized that fauna that disturb or live in direct contact with oiled sediment may accumulate greater hydrocarbon loads than mussels and we discuss the possibility that a portion of this oil remained toxic through 1999.

# Methods

## Site selection and description

Heavily oiled mussel beds in PWS and the Gulf of Alaska (GOA) were selected for study plus two reference sites with little or no oil contamination as previously described by Carls et al. (2001). In brief, primary criteria for site selection were the presence of moderately to densely packed mussels (288-5000 mussels $m^{-2}$) on relatively fine sediments (i.e., <1 cm diameter), and detection of crude oil by visual or olfactory means. Although this research describes the geographic extent of significant mussel and sediment contamination, sampling was not random, thus the percentage of significantly contaminated beds from the universe of all beds could not be estimated. Our conclusions were further constrained to description of the most oiled portions of beds, and do not detail within-bed variability as a function of elevation or other factors because sample transects were located medially through the most oiled portions of these beds, parallel to the shoreline. A total of 98 beds were sampled in the original series; observation of the 23 worst-case beds was extended to 1999. Sampling also continued from two reference sites with little or no oil contamination (Fig. 2.1). One of these reference sites, Olsen Bay, is located about 47 km from the reported extent of the *Exxon Valdez* oil slick (Gundlach et al. 1990). The other reference site, Barnes Cove, is located on Knight Island, within slick extents.

## Sampling Procedures

A transect was placed parallel to the waterline through the middle of each mussel bed (as topography allowed) or the obviously oiled portion of the bed using modified methods of Karinen et al. (1993) and Babcock et al. (1996). The length of the transect line, usually 30 m, varied according to bed size and topography and ranged from 10 m at one Disk Island site to 50 m at Foul Bay. Triplicate, pooled subsamples of surface sediment (59 ml minimum) were randomly collected from the upper 2 cm at 8 to 10 spots within 1 m of the transect line in PWS. Collection spoons and glass storage jars were hydrocarbon-free. (Equipment used for hydrocarbon sampling was prewashed with soap and hot water, rinsed, dried, and rinsed with dichloromethane or certified as hydrocarbon-free by the manufacturer.) Triplicate, pooled samples of 20 to 25 mussels were similarly collected; mussel length ranged from 25 to 40 cm. (Sampling procedures differed slightly in the GOA; samples were pooled within three sample zones parallel to the transects. Distances between the transect line and upper and lower zones ranged up to 2 m. These procedural differences were minor and did not require special analysis.) Air blanks were collected for quality control purposes at most sites prior to 1999. All samples were cooled immediately, frozen within 2-4 h, and stored at -20°C until analyzed. Data from beds manually cleaned in 1994 (Babcock et al., 1998; Carls et al. 2004) were included in this analysis.

## Chemical analysis

To maximize the number of samples analyzed and minimize processing time, oil in most sediments was only analyzed with an ultraviolet fluorescence fast-screening technique adapted

from Krahn et al. (1991; 1993). Sediments were extracted twice with methylene chloride. Extracts were separated with a high-performance liquid chromatograph, and quantified with a fluorescence detector (260 nm excitation, 380 nm emission). Emission output was centered at maximum phenanthrene output. A standard curve based on the amount of phenanthrene in *Exxon Valdez* oil was used to estimate total petroleum hydrocarbon (THC) concentration. Mean THC concentration is reported in μg/g wet weight. The method detection limit for THC was 1.7 μg/g, considerably less than the previously stated limit (50 μg/g, Carls et. al. 2001).

All mussels from the GOA were analyzed by gas chromatography/mass spectroscopy, but mussels from PWS were analyzed only when THC concentration in underlying sediments was substantial. A subset (98 of 972) of sediments with elevated THC were also selected for analysis by gas chromatography (Short et al., 1996a) to confirm polynuclear aromatic hydrocarbon (PAH) composition. Experimentally determined method detection limits depended on sample weights, and generally were 1 ng/g in tissue, and < 2 ng/g in sediment. Concentrations of individual PAH below method detection limits were treated as zero. Total PAH (TPAH) concentrations in tissue are reported in ng/g dry weight; wet to dry weight ratios were measured by dehydrating 1 g wet samples for ≥24 h at 60°C and weighing the remaining mass. The accuracy of the hydrocarbon analyses was about ±15% based on comparison with National Institute of Standards and Technology values, and precision expressed as coefficient of variation was usually less than approximately 20%, depending on the PAH. TPAH concentrations were calculated by summing concentrations of individual PAH. Relative PAH concentrations were calculated as the ratio of PAH concentration to TPAH concentration.

**Data analysis**

The source of oil in bed sediments and mussels was confirmed with a model developed by Short and Heintz (1997) designed to determine if PAH composition was consistent with that of weathered *Exxon Valdez* oil. The model, which was successfully validated by comparison with thousands of samples from the study area, uses experimentally determined first-order loss-rate constants for 14 PAHs to calculate an index of weathering ($w$) that summarizes exposure history. Bootstrapped error distributions from experimental and environmental samples provided the basis for testing the null hypothesis that the composition of PAH in a sample was consistent with that of weathered *Exxon Valdez* oil (Short and Heintz, 1997). Definition used are: unweathered ($w = 0$), slightly weathered ($0 < w \leq 2$), moderately weathered ($2 < w \leq 8$), and highly weathered ($w > 8$) (Carls et al. 2001).

Background concentrations in sediment and mussels were estimated from non-oiled and slightly oiled reference locations. Background THC concentrations in sediment were estimated from two reference sites that had returned to normal by 1992, Olsen Bay and Barnes Cove (Fig. 2.1). Mean THC was 4.7 μg/g wet weight (range 0-53 μg/g) and was < 20 μg/g in 97% of the samples ($n = 31$). Thus, we consider the THC background concentration in sediment to be 20 μg/g wet weight. TPAH concentrations in mussels collected outside the slick trajectory (Natural Resource Damage Assessment database; Short et al. 1996b; Chapter 1) were ≤ 50 ng/g dry weight in 80% of the samples ($n = 104$); we accepted this as background TPAH in mussels.

Exponential regression analysis was used to determine if hydrocarbon concentrations had reached background levels by the end of study or to predict when they might. Current regression estimates were compared to previous (1992-1995) estimates (Carls et al. 2001). Regressions were limited to beds with data spanning three or more years to ensure that short-term variation in concentration did not yield spurious predictions. The predictive usefulness of regressions was judged by correlation, probability, and $F_o/F_{crit}$, where $F_o$ = observed F-ratio and $F_{crit} = F(v_m, v_r, 1 - \alpha)$, where $v_m$ = regression degrees of freedom, $v_r$ = residual degrees of freedom, and $\alpha = 0.05$.

The $F_o/F_{crit}$ criterion is designed to determine how useful the regression is, as distinct from significant, and is the more conservative measure of importance. Outcomes where $F_o/F_{crit} \geq 4$ are considered useful by Draper and Smith (1981); in this paper concentration declines were considered significant only where $F_o/F_{crit} \geq 2$ and $P \leq 0.01$.

Hydrocarbon concentrations in sediments and mussels in the last year of study (1999) were considered significantly elevated if the lower 95% confidence band of the regression was >100 ng/g TPAH in mussels (twice the background level) or > 40 μg/g THC in sediment (twice background) and endpoint concentrations were greater than these values.

To compare relative TPAH accumulation in mussels, other surface organisms, and clams, we assumed that the contemporary source of TPAH is contaminated sediment and normalized all TPAH concentrations in tissue to those in sediment. These bioaccumulation data were analyzed with one-way analysis of variance. Because the distributions of neither bioaccumulation nor arcsin-transformed bioaccumulation were normal, an empirical F-distribution obtained by randomly permuting the group variable (1000 iterations) was used to estimate the P-value. Reported results are based on the arcsin-transformed variable (Snedecor and Cochran 1980); conclusions were unchanged without transformation.

## Results

*Exxon Valdez* oil was identified as the source of contamination in sediments and mussels from oiled beds through 1999. Contaminated sediments were visibly oiled and odorous, particularly between 1992 and 1995, and oil sheens on pools of water were visible without any manual disturbance. Of 26 sediment samples analyzed by GC/MS in 1999 (from oiled PWS beds only), *w* was estimable in 24, and *Exxon Valdez* oil was confirmed in 22 of these ($0.9 \leq w \leq 8.4$; slightly to highly weathered; $w \leq 4.9$ in 14 of 22 samples). In prior years (1992-1995), *Exxon Valdez* oil was verified in 85-100% of sediment from oiled PWS beds with sufficient PAH for modeling. Of 39 mussel samples in 1999 (from oiled PWS beds only), *w* was estimable in just 3, but *Exxon Valdez* oil was identified in each ($4.0 \leq w \leq 7.9$; moderately weathered). *Exxon Valdez* oil was previously identified (1992-1995) in 93-100% of mussels from oiled sites where there was sufficient PAH for modeling. *Exxon Valdez* oil was not identified in any sediments or mussels from the two reference beds between 1992 and 1999.

### Temporal changes in petroleum hydrocarbon content of sediments and mussels

Hydrocarbon concentrations declined in sediment from all oiled beds monitored from 1992-1999 (Fig 2.2). THC concentration declines were significant in 10 of 22 beds. Trends were downward in all except one remaining case (DI067A-2C). Time series data began in 1995 in the exceptional bed, thus earlier concentration declines were likely missed. (Similarly, THC concentration declines were significant in sediment from 6 of 10 additional beds monitored only until 1994 or 1995 and trends were consistently downward; Carls et al. 2001.) By 1999, modeled THC concentrations in sediment dropped to twice background concentration in just 5 of 22 beds (21%), indicating the majority remained contaminated. Estimated times (beyond mid 1999) THC to drop below 40 μg/g ranged from 0.5 to 47 years (2000-2046); the mean estimate was 2013. Projected confidence bands suggest a more rapid decline, 0.5-16 years (2000-2015). These estimates are reasonably consistent with predictions based on 1992-1995 data (1993-2016; Carls et al. 2001). When compared to 1992-1995 estimates (Carls et al. 2001), predicted hydrocarbon concentration declines in sediment were faster in 7 of 14 beds, slower in 6, and about the same in 1.

Hydrocarbon concentrations in mussels from most oiled beds declined from 1992-1999 (Fig. 2.2). Declines were significant in 11 of 19 beds. Trends were downward in all except two

remaining cases (DI067A-6 and CH010B-2D). Corresponding THC concentrations in sediment in these two beds remained very high (5116 to 6075 μg/g) in 1999 and TPAH concentrations in mussel tissue remained above background (127-1393 ng/g). By 1999, modeled TPAH concentrations in mussels dropped to twice background concentration in 16 of 19 beds (84%). Modeled TPAH concentrations in mussels show no decline in only 1 bed (DI067A-6) and TPAH concentration was > 1000 ng/g in 1999. Significant contamination also remains in mussels at KN136A-1, and KN136A-2 (TPAH > 475 ng/g) and CH010B-2D, although modeled concentration is merging with background in the latter bed. TPAH concentrations in mussel tissue tended to decline more rapidly than previously predicted (Carls et al. 2001); predicted declines were faster in 10 of 13 beds, slower in 1 and about the same in 2.

**Changes due to manual restoration**

Differences in hydrocarbon loss between manually restored beds (Carls et al. 2004) and unmanipulated oiled beds were not clearly evident. Seven of 24 oiled PWS mussel beds included in this data set were experimentally restored (see Fig. 2.2). Fewer TPAH concentrations in mussel tissue fell below twice background levels in unmanipulated oiled beds (8 of 12) than in restored beds (7 of 7) but proportions were about the same for sediment: 1 of 4 reached twice background levels in restored bed sediment and 4 of 13 in unmanipulated beds.

**Relationship between sediment and mussel contamination**

Bioavailability of petroleum hydrocarbons to mussels consistently declined even though oil often clearly remained in underlying sediment. In 12 of 17 cases TPAH concentrations in mussels declined to background levels where THC concentrations in sediment remained above background (e.g., KN133A-1; Fig. 2.2). Sediment in the five beds where mussels remained contaminated through 1999 (CH010B-2D, DI067A-6, KN136A-1, KN136A-2, and MA002C) consistently retained highly contaminated sediment (range 357-6129 μg/g, mean 4202 μg/g in 1999).

**Hydrocarbons in Gulf of Alaska mussels**

Hydrocarbon concentrations in mussels from all Gulf of Alaska beds declined from 1992-1999 but remained above background levels in 2 of 6 beds (Fig. 2.3). Modeled declines were significant in only 2 of 6 beds, but directly measured TPAH concentrations in 1999 were less than twice background in 4 beds. Estimated times (beyond mid 1999) for remaining beds to reach background ranged from 16 to 22 years (2015-2021), but projected confidence bands suggest a more rapid decline, 11 years (2010). When compared to 1992-1995 estimates, predicted hydrocarbon concentration declines in sediment were faster in 3 of 3 beds; decline to background was not predictable for any of these beds in earlier years.

**Hydrocarbons in other fauna**

Bioaccumulation of TPAH in mussels (0.04 ± 0.01, $n = 52$), other surface dwellers (0.01 ± 0.003, $n = 15$), and clams (0.08 ± 0.05, $n = 6$) from sediment were statistically indistinguishable (P = 0.069). Other surface dwellers included drills (*Nucella* spp), hermit crab (*Pagurus* spp), high coxcomb prickleback (*Anoplarchus purpurescens*), limpets ( ), periwinkles (*Littorina* spp), rough mantled sea slug (*Onchidoris bilamellata*), shore crab (*Hemigrapsis* spp), and whelks (*Lirabuccinum* spp). Clams were butter clams (*Saxidomus giganteous*), and little neck clams (*Prototheca staminea*). The small bioaccumulation in all organisms (maximum < 0.4 in all cases) indicates that not all oil in sediment is immediately biologically available and is consistent with the slow, protracted release of biologically available oil, mediated by water, and

is evident in our primary data set (e.g., Fig. 2.2). Analysis of a considerably larger species data set is needed to adequately explore the relationship between species, habitat utilization, and hydrocarbon uptake.

## Discussion

Hydrocarbons from the *Exxon Valdez* oil spill in sediment and mussels typically declined from 1992 to 1999 at heavily oiled sites. Models predict that remaining oil should be gone from surface sediment by mid century (2046) and typically sooner (2013). TPAH concentrations in mussels frequently reached background levels by 1999 but remained elevated at some sites (21%). Oil loss rates in mussels from the Gulf of Alaska were similar to those in PWS although continued contamination was evident in 33% of these beds at the end of study. The data herein indicate intertidal habitat is in the process of recovering but that biologically meaningful concentrations of oil with slight-moderate weathering remain in some areas.

Evidence that oil remains in intertidal sediment was corroborated by a random survey in 2001. Unlike our study, where efforts were focused on worst-case mussel beds, Short et al. (2004) randomly sampled about 117 km of PWS beach that were originally moderately to heavily oiled. *Exxon Valdez* oil was discovered randomly in sediment at 53 of 91 sites and non-randomly on 25 additional beaches (86%), corroborating our observation that heavily-contaminated oil patches persisted through the 1990s. Subsurface oil was encountered more frequently than surface oil, was usually liquid, and was moderately to highly weathered (*w* ranged from 1.4-8.2). The frequency of oil encounter increased from +4.8 m MLLW to +1.8 m MLLW (the lowest zone sampled). Limited opportunistic sampling suggested oil was also present below 1.8 m MLLW (Short et al. 2004), thus demonstrating contamination of a zone of high biological productivity and consistent with our results (the mussel zone in PWS is typically located 1.3-2.0 m MLLW). The extensive distribution of residual oil with slight to high weathering and relatively high TPAH concentrations indicates considerable potential for negative biological impacts. Random detection of subsurface oil agrees with our earlier hypothesis that buried oil serves as a reservoir and can recontaminate surface sediment through storm-driven erosion or tidally driven hydraulic processes (Carls et al. 2001; Carls et al. 2004). Results of the random survey also corroborate the observation that subsurface oil is difficult to remove and will likely persist longer than surface oil in the intertidal environment (Carls and Harris 2004).

Remaining oil has negatively affected organisms that inhabit or disturb contaminated sediment for many years. Based on the calculations of Carls et al. (in prep), 54% of all sediment analyzed by GC/MS in 1999 had toxic potential. This is an overestimate of the true percentage of sediment with toxic potential throughout PWS because samples analyzed by GC/MS in this study were selected nonrandomly to identify source oil. In a larger survey, 12% of surface sediment samples were potentially toxic in 1998 (Carls et al. in prep). The same authors reviewed published evidence that oil in sediment remained toxic and conclude that biota were negatively affected by persistent toxic oil for at least 4-10 yr after the spill, including pink salmon embryos (Bue et al. 1998; Craig et al. 2002.), mussels (Morado et al. 1998; Thomas et al. 1999), infauna (Fukuyama et al. 2000; Downs et al. 2002) and vertebrate predators (Seiser et al. 2000; Esler et al. 2000; Trust et al. 2000; Bodkin et al. 2002; Golet et al. 2002; Jewett et al. 2002). In their review, Teal and Howarth (1984) conclude that oil effects can persist for at least 6-12 years, and the *Exxon Valdez* experience is consistent with this time frame.

Results of this study indicate that mussels and other intertidal biota in PWS and the GOA have been exposed to oil for more than a decade after the *Exxon Valdez* spill and suggest recovery is in progress but is not yet complete. Considerable amounts of relatively unweathered

oil remain in sediment at some locations and will likely persist for many years. Some remaining oil is likely toxic may continue to adversely affect the intertidal community, including vertebrate predators.

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## References

Babcock, M.M., G.V. Irvine, P.M. Harris, J.A. Cusick, and S.D. Rice. 1966. Persistence of oiling in mussel beds three and four years after the *Exxon Valdez* oil spill. Am Fish Soc Symp 18:286-297.

Babcock, M. M., P. M. Harris, M. G. Carls, C. C. Brodersen, and S. D. Rice. 1998. Mussel bed restoration and monitoring. *Exxon Valdez* Oil Spill Restoration Project 95090 Final Report. National Oceanic and Atmospheric Administration, National Marine Fisheries Service, Auke Bay Laboratory, Juneau, AK. 154 p.

Bodkin, J. L., Ballachey, B. E., Dean, T. A., Fukuyama, A. K., Jewett, S. C., McDonald, L., Monson, D. H., O'Clair, C. E., VanBlairicom, G. R. (2002). Sea otter population status and the process of recovery from the 1989 *Exxon Valdez* oil spill. *Marine Ecology Progress Series* 241, 237-253.

Bue BG, Sharr S, Seeb JE (1998) Evidence of damage to pink salmon populations inhabiting Prince William Sound, Alaska, two generations after the *Exxon Valdez* oil spill. Trans Am Fish Soc 127:35-43

Buikema AL Jr, Cairns J Jr. 1984. Recovery and restoration of rocky shores, sandy beaches, tidal flats, and shallow subtidal bottoms impacted by oil spills. Pages 7-36 in Cairns J Jr, Buikema AL Jr (eds.), Restoration of habitats impacted by oil spills. Butterworth Publishers. Boston, MA. USA.

Carls, M.G., S.D. Rice, and J.E. Hose. 1999. Sensitivity of fish embryos to weathered crude oil: Part 1. Low level exposure during incubation causes malformations, genetic damage and mortality in larval Pacific herring (*Clupea pallasi*). Env. Toxicol. Chem. 18:481-493.

Carls, M.G., M.M. Babcock, P.M. Harris, G.V. Irvine, J.A. Cusick, and S.D. Rice. 2001. Persistence of Oiling in Mussel Beds after the *Exxon Valdez* Oil Spill. Marine Environmental Research 51:167-190.

Carls, M.G., P.M. Harris, and S.D. Rice. 2004. Restoration of Oiled Mussel Beds in Prince William Sound, Alaska. Mar. Environ. Res. 57:359-376.

Carls MG, Harris PM, Rice SD. In prep. Intertidal *Exxon Valdez* oil retention, bioavailability, and toxicity, 1989-2000 in Prince William Sound, Alaska. Mar. Ecol. Prog. Ser. or Mar. Environ. Res.

Craig AK, Willette M, Evans DG, Bue BG. 2002. Injury to pink salmon embryos in Prince William Sound – Field monitoring. Restoration Report 98191A-1 *Exxon Valdez* Oil Spill Final Report. Alaska Department of Fish and Game, Anchorage

Couillard CM. 2002. A microscale test to measure petroleum oil toxicity to mummichog embryos. Environ Toxicol 17:195-202.

Dow RL. 1978. Size-selective mortalities of clams in an oil spill site. Mar. Pollut. Bul. 9:45-48.

Downs CA, Shigenaka G, Fauth JE, Robinson CE, Huang A. 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.

Draper, N., & Smith, H. 1981. Applied regression analysis, second edition. New York, NY: John Wiley & Sons, Inc.

Duffy, L. K., R. T. Bowyer, J. W. Testa and J. B. Faro. 1996. Acute phase proteins and cytokines in Alaska mammals as markers of chronic exposure to environmental pollutants. Am Fish Soc Symp 18:809-813.

Esler, D., Schmutz, J. A., Jarvis, R. L., & Mulcahy, D. M. (2000). Winter survival of adult female harlequin ducks in relation to history of contamination by the *Exxon Valdez* oil spill. *Journal of Wildlife Management*, 64, 839-847.

Fukuyama AK, Shigenaka G, Hoff RZ. 2000. Effects of residual *Exxon Valdez* oil on intertidal protothaca staminea: mortality, growth, and bioaccumulation of hydrocarbons in transplanted clams. Mar Pollut Bul. 40:1042-1050

Golet, G. H., Seiser, P. E., McGuire, A. D., Roby, D. D., Fisher, J. B., Kuletz, K. J., Irons, D. B., Dean, T. A., Jewett, S. C., & Newman, S. H. (2002). Long-term direct and indirect effects of the *Exxon Valdez* oil spill on pigeon guillemots in Prince William Sound, Alaska. *Marine Ecology Progress Series* 241, 287-304.

Gundlach ER, Berne S, D'Ozouville L., Topinka JA. 1981. Shoreline oil two years after *Amoco Cadiz*: new complications from *Tanio*. Pages 525-540 in Proceedings of the 1981 Oil spill conference. American Petroleum Institute Publ. No. 4334.

Gundlach, E.R., J. Bauer, R. Bayliss, S. Provant, and M. Kendziorek. 1990. Response to the *Exxon Valdez* oil spill by the Alaska Department of Environmental Conservation. Pages 471-478 *in* M.L. Spaulding, and M. Reed (eds.), Oil spills management and legislative implications: proceedings of the conference, May 15-18, 1990, Newport, Rhode Island, USA. American Society of Civil Engineers, NY.

Heintz R, Short JW, Rice SD (1999) Sensitivity of fish embryos to weathered crude oil: Part II. Increased mortality of pink salmon (*Oncorhynchus gorbuscha*) embryos incubating downstream from weathered *Exxon Valdez* crude oil. Env Toxicol Chem 18:494-503

Houghton JP, Lees DC, Driskell WB, Lindstrom SC, Mearns AJ. 1996. Recovery of Prince William Sound intertidal epibiota from *Exxon Valdez* oiling and shoreline treatments, 1989-1992.

Jewett, S.C., Dean, T. A., Woodin, B. R., Hoberg, M. K., & Stegeman, J. J. 2002. Exposure to hydrocarbons 10 years after the *Exxon Valdez* oil spill: evidence from cytochrome P4501A expression and biliary FACs in nearshore demersal fishes. *Marine Environmental Research* 54, 21-48.

Karinen JF, Babcock MM, Brown DW, MacLeod WD Jr., Ramos LS, Short JW. 1993. Hydrocarbons in intertidal sediments and mussels from Prince William Sound, Alaska, 1977-1980: characterization and probable sources. NOAA Technical Memorandum NMFS-AFSC-9. US Department of Commerce, National Oceanic and Atmospheric Administration, National Marine Fisheries Service, Alaska Fisheries Science Center.

Krahn M. M., G. M. Ylitalo, J. Joss, and S.-L. Chan. 1991. Rapid, semi-quantitative screening of sediments for aromatic compounds using sonic extraction and HPLC/fluorescence analysis. Mar. Environ. Res. 31:175-196.

Krahn, M. M., G. M. Ylitalo, J. Buzitis, S.-L. Chan, U. Varanasi, T. L. Wade, T. J. Jackson, J. M. Brooks, D. A. Wolfe, and C.-A. Manen. 1993. Comparison of high-performance liquid chromatography/fluorescence screening and gas chromatography/mass

spectrometry analysis for aromatic compounds in sediments sampled after the *Exxon Valdez* oil spill. Environ. Sci. Technol. 27:699-708.

Leigh EG Jr, Paine RT, Quinn JF, Suchanek TH. 1987. Wave energy and intertidal productivity. Proc. Natl Acad Sci 84:1314-1318.

Mearns, AJ. 1996. *Exxon Valdez* shoreline treatment and operations: implications for response, assessment, monitoring, and research. Am Fish Soc 18:309-328.

Morado JF, Mooney LL, Babcock MM, Harris PM. 1998. Histopathological observation of bay mussels, *Mytilus trossulus*, exposed to *Exxon Valdez* crude oil in Prince William Sound. Pages 130-153 in Babcock, M. M., P. M. Harris, M. G. Carls, C. C. Brodersen, and S. D. Rice. 1998. Mussel bed restoration and monitoring. *Exxon Valdez* Oil Spill Restoration Project 95090 Final Report. National Oceanic and Atmospheric Administration, National Marine Fisheries Service, Auke Bay Laboratory, Juneau, AK.

Reddy CM, Eglinton TI, Hounshell A, White HK, Xu L, Gaines RB, Frysinger GS. 2002. The West Falmouth oil spill after thirty years: the persistence of petroleum hydrocarbons in marsh sediments. Enviorn Sci Technol 36:4754-4760.

Seiser, P. E., Duffy, L. K., McGuire, A. D., Roby, D. D., Golet, G. H., & Litzow, M. A. (2000). Comparison of pigeon guillemot, *Cepphus columba*, blood parameters from oiled and unoiled areas of Alaska eight years after the *Exxon Valdez* oil spill. *Marine Pollution Bulletin,* 40, 152-164.

Sharp, B. and M. Cody. 1996. Effects of the *Exxon Valdez* oil spill on the black oystercatcher. Am Fish Soc Symp 18:748-758.

Short, J.W., T.J. Jackson, M.L. Larsen, and T.L. Wade. 1996a. Analytical methods used for the analyses of hydrocarbons in crude oil, tissues, sediments, and seawater collected for the natural resource damage assessment of the *Exxon Valdez* oil spill. Am Fish Soc Symp 18:140-148.

Short, J.W., R.A. Heintz, B.D. Nelson, J.M. Maselko, M.F. Kendziorek, M.G. Carls, and S. Korn. 1996b. *Exxon Valdez* oil spill of 1989: State/Federal Trustee Council hydrocarbon database 1989-1995. National Oceanic and Atmospheric Administration, National Marine Fisheries Service, Auke Bay, AK.

Short J., and R. Heintz. 1997. Identification of *Exxon Valdez* oil in sediments and tissues of PWS. Environ. Sci. Technol. 31:2375-2384.

Short JW, Lindeberg MR, Harris PM, Maselko J, Rice SD. 2002. Vertical oil distribution within the intertidal zone 12 years after the Exxon Valdez oil spill in Prince William Sound Alaska. Pages 57-72 in Proceedings of the Twenty-fifth Arctic and marine oilspill program (AMOP) technical seminar. June 11-13, 2002. Calgary (Alberta) Canada.

Teal JM, Howarth RW. 1984. Oil spill studies: a review of ecological effects. Environmental Management 8:27-44.

Thomas, R.E., P.M. Harris, and S.D. Rice. 1999. Survival in air of *Mytilus trossulus* following long term exposure to spilled *Exxon Valdez* crude oil in Prince William Sound. Comp. Biochem. Physiol. Part C 122:147-152.

Trust, K. A., Esler, D., Woodin, B. R., & Stegeman, J. J. 2000. Cytochrome P450 1A induction in sea ducks inhabiting nearshore areas of Prince William Sound, Alaska. *Marine Pollution Bulletin*, 40, 397-403.

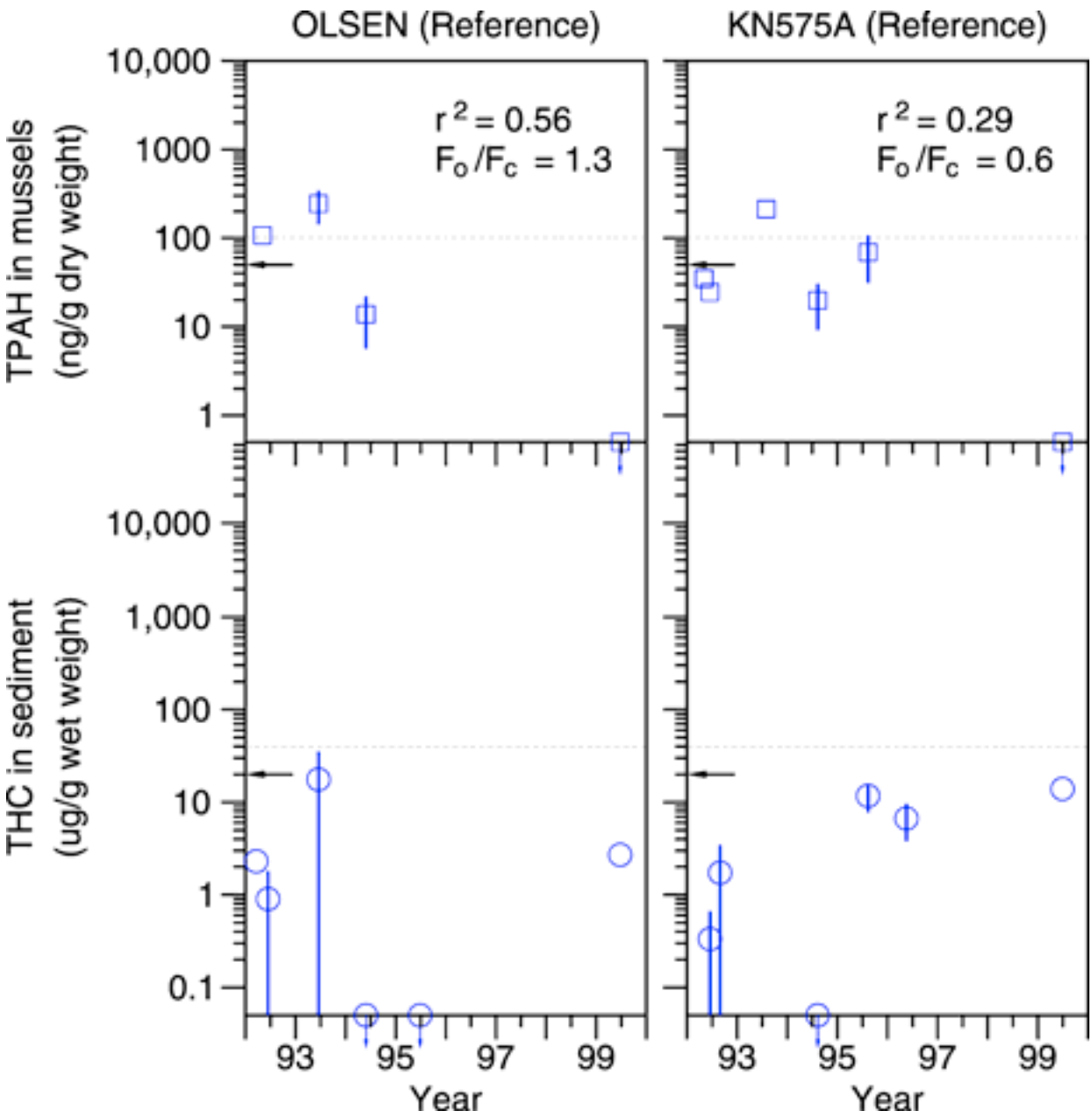



**Fig. 2.1.** Mean (±SE) total polynuclear aromatic hydrocarbon (TPAH) concentrations in mussel tissue and total petroleum hydrocarbons (THC) in underlying sediment, 1992-1999 at two reference sites. Olsen Bay is located about 47 km outside the reported *Exxon Valdez* slick trajectory and Barnes Cove is located within the trajectory. There was evidence of contamination at both of these locations 1-2 years after the spill (Carls et al. in prep.; Chapter 1). Horizontal arrows indicate estimated background concentrations; horizontal lines are drawn at twice background concentration.

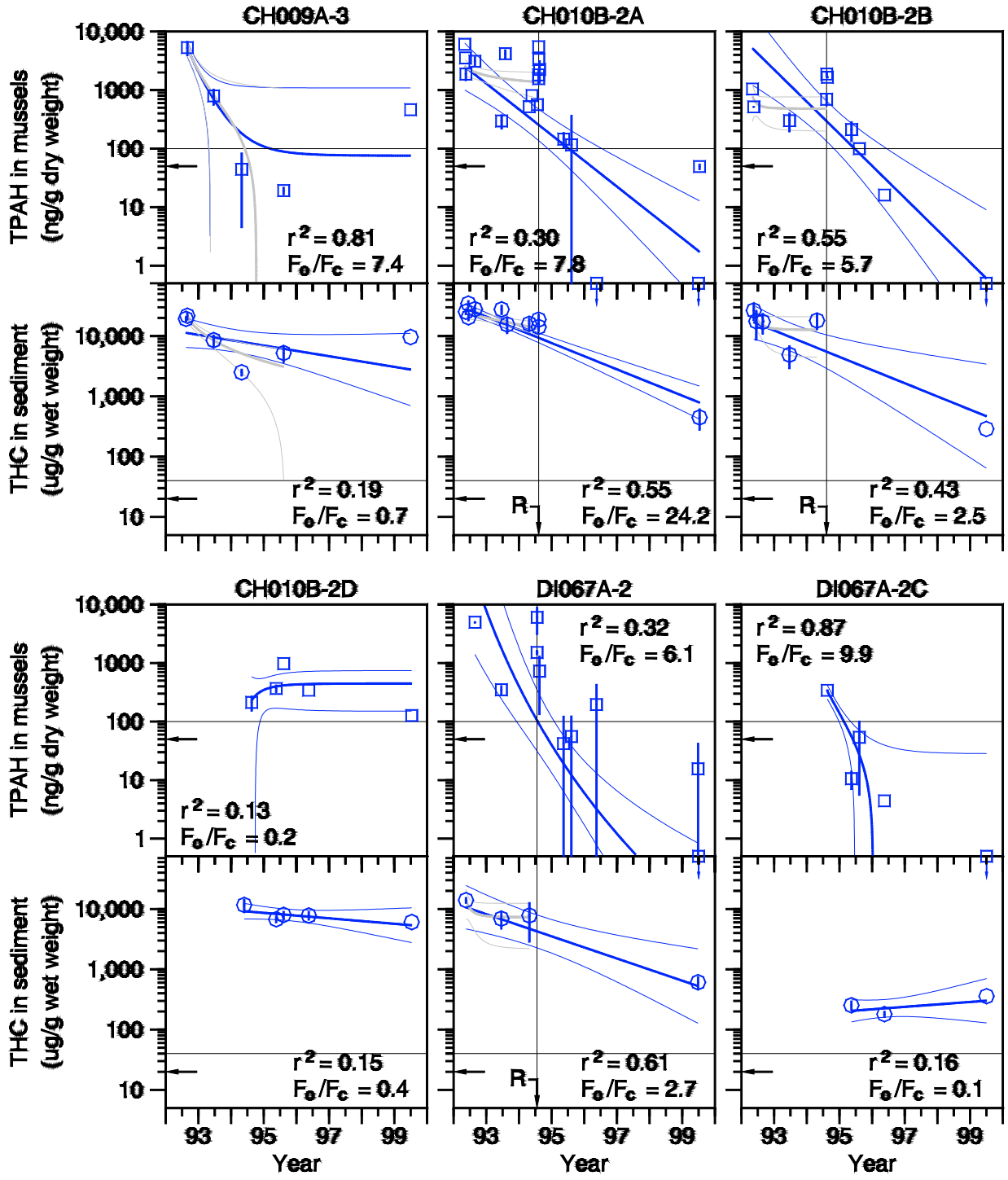



**Fig. 2.2.** Mean (±SE) total polynuclear aromatic hydrocarbon (TPAH) concentrations in mussel tissue and total petroleum hydrocarbons (THC) in underlying sediment, 1992-1999. Bold lines are regression fits, and thin lines are 95% confidence bands. Gray-colored regression fits are previously published estimates (1992-1995; Carls et al. 2001). Horizontal arrows indicate estimated background concentrations; horizontal lines are drawn at twice background concentration. Time of experimental manual restoration (vertical lines at arrow "R"; Babcock et al. 1998; Carls et al. 2004) is indicated where appropriate.

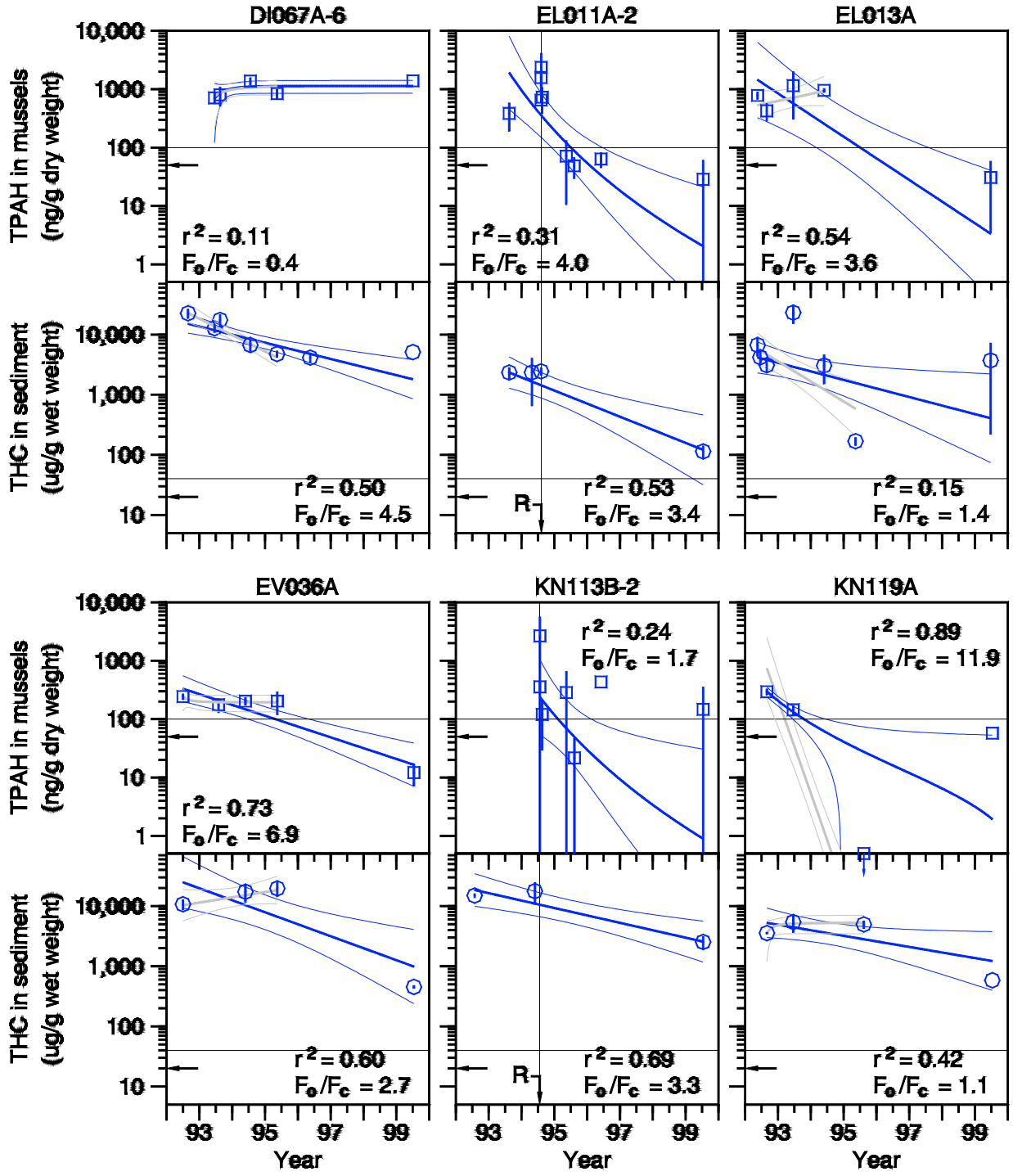
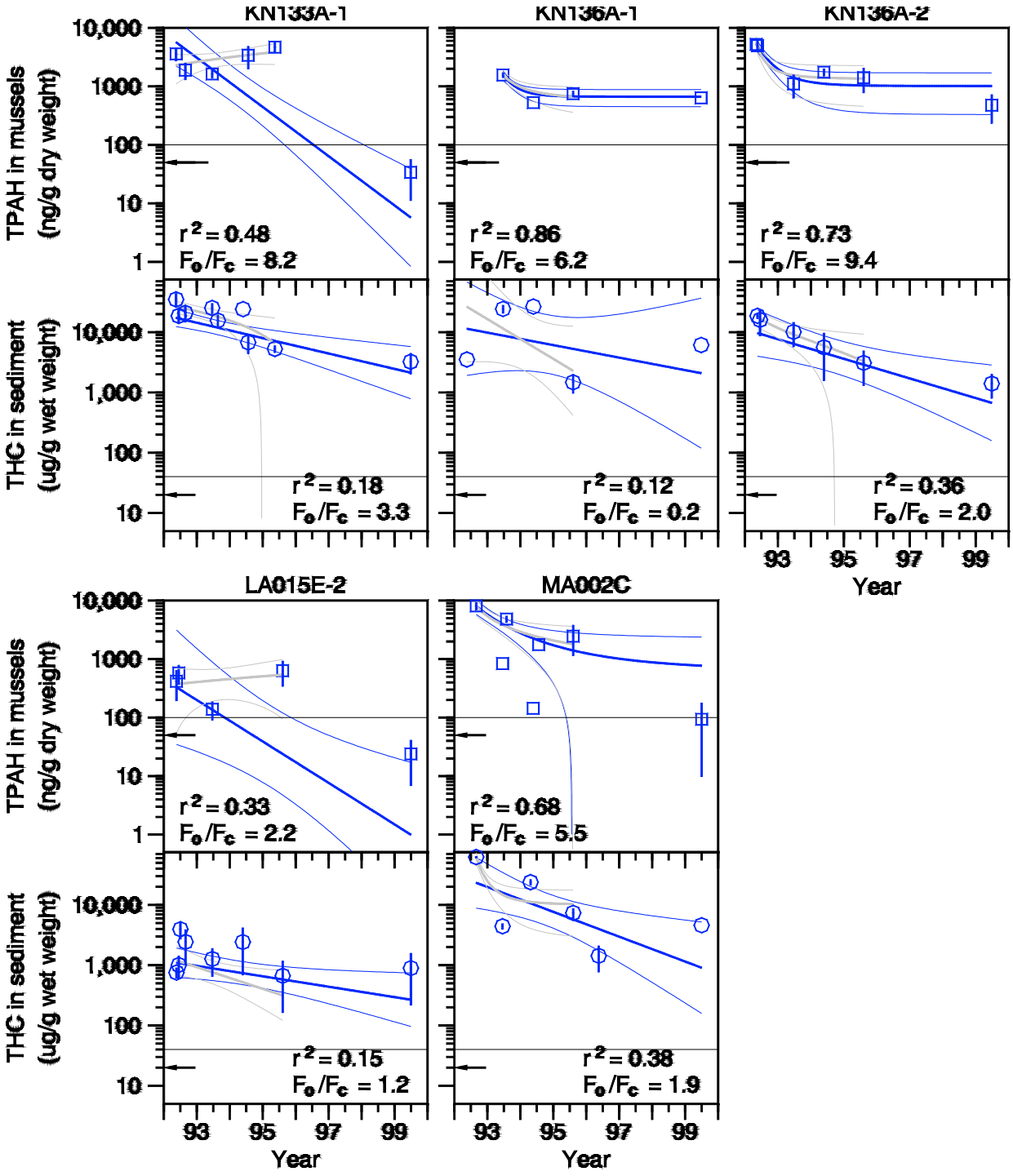



*Fig. 2.2, continued.*

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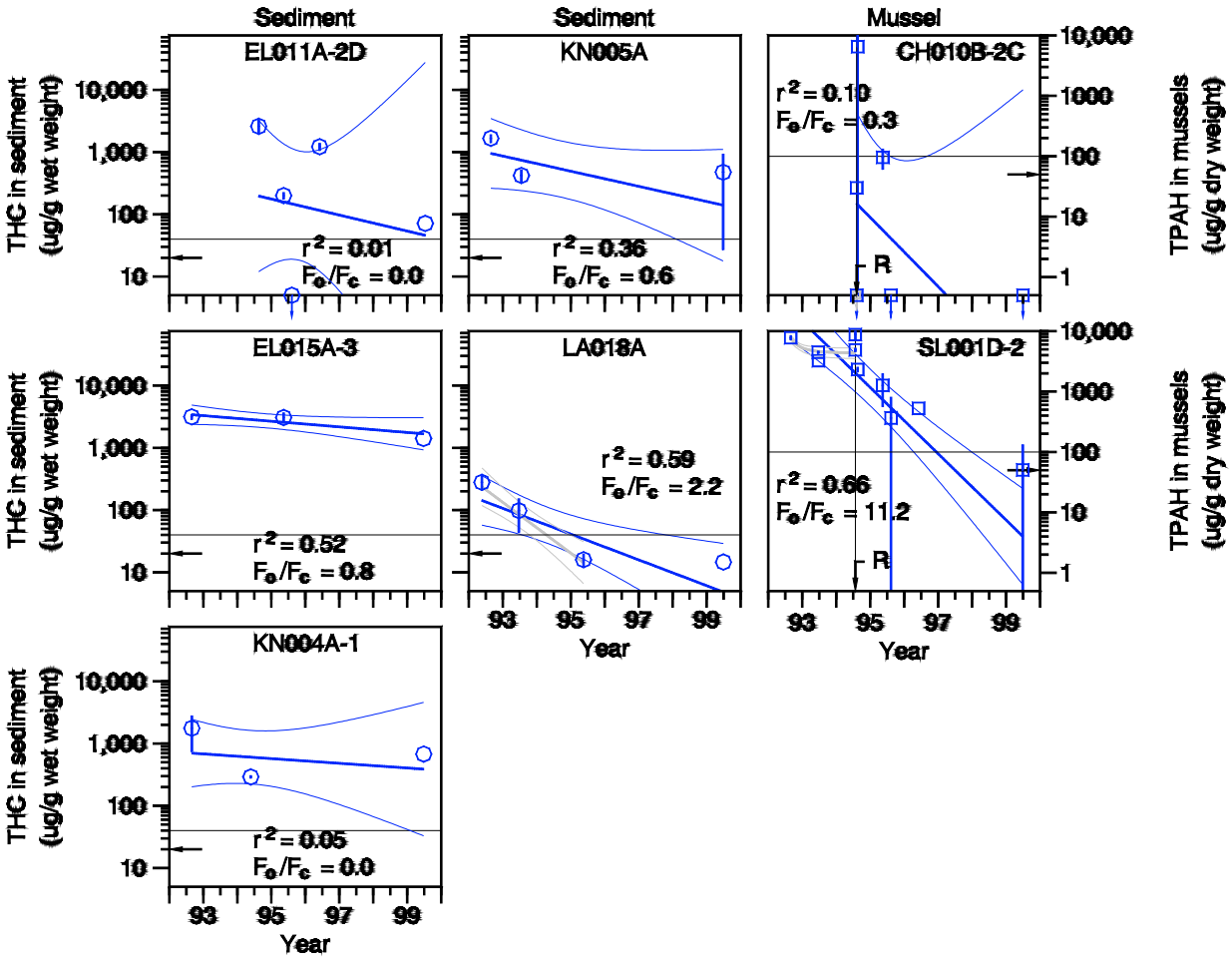


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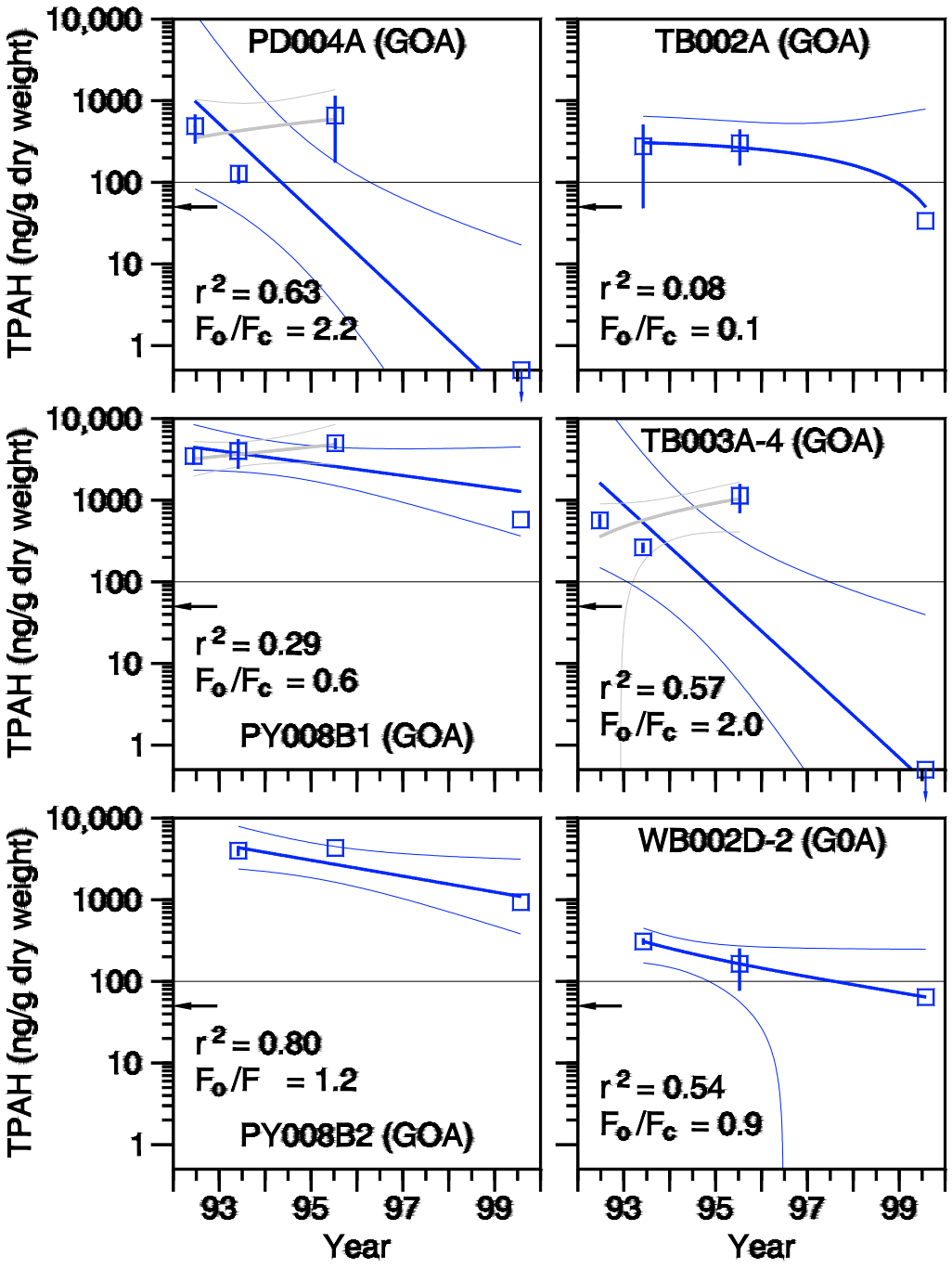


**Fig. 2.3**. Mean (±SE) total polynuclear aromatic hydrocarbon (TPAH) concentrations in mussels from the Gulf of Alaska (GOA), 1992-1999. Bold lines are regression fits, and thin lines are 95% confidence bands. Gray-colored regression fits are previously published estimates (1992-1995; Carls et al. 2001). Horizontal arrows indicate estimated background concentrations; horizontal lines are drawn at twice background concentration.

# Chapter 3

# Restoration of Oiled Mussel Beds in Prince William Sound, Alaska

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**Abstract**

Natural loss of hydrocarbons was often low from mussel (*Mytilus trossulus*) beds (which were typically not cleaned after the *Exxon Valdez* oil spill), thus this habitat remained a long-term source of oil. Consequently, experimental restoration of nine contaminated beds was attempted in 1994; mussels were removed, contaminated surface sediment was replaced (33 metric tons), and original mussels were returned. Hydrocarbon concentrations and mussel populations were monitored for 5 years thereafter. Post-restoration mussel population fluctuations were indistinguishable from regional changes. Increased short-term oil loss was apparent, but long-term (5 year) improvement was equivocal and difficult to distinguish from natural losses. By 1999, oil concentrations in mussels were typically at baseline levels in restored and oiled reference beds; concentrations in replaced sediment were elevated in one third of restored beds, indicating recontamination from underlying or surrounding sediment. Our results suggest mussel relocation is feasible but suggest oil might more effectively be removed from sediment mechanically or chemically than manually.

*Keywords:* mussels; *Mytilus trossulus*; Prince William Sound; *Exxon Valdez*; petroleum hydrocarbons; oil spills; monitoring; restoration

## Introduction

Roughly 1900 km of shoreline were oiled in Prince William Sound, Alaska, and along the Kenai and Alaska Peninsulas by the *Exxon Valdez* oil spill (estimates vary from 786 to 5221 km; Gundlach, Bauer, Bayliss, Provant, & Kendziorek 1990; Exxon Corp. 1992; Michel and Hayes 1991; Wolfe et al. 1994). Of this, about 900 km of shoreline were moderately to heavily oiled (Michel and Hayes 1991). About 42 million liters of crude oil were spilled, the largest in U.S. history. On the third day after the spill (March 26, 1989), a gale dispersed the slick beyond any hope of containment (450 $km^2$; Kelso & Kendziorek, 1991). Floating oil began to pass into the Gulf of Alaska on March 30 (Wolfe et al., 1994), and by June 20 was distributed over approximately 28,500 $km^2$ (Gundlach et al. 1990). About 40% of the spilled oil stranded on beaches in Prince William Sound (Spies, Rice, Wolfe, & Wright, 1996).

Many dense aggregations (beds) of mussels (*Mytilus trossulus*) impacted by the *Exxon Valdez* oil spill were not cleaned to avoid damaging this potential food source and physically stabilizing element in intertidal areas, but natural rates of hydrocarbon loss from these beds were surprisingly slow (Carls, Babcock, Harris, Irvine, Cusick, & Rice, 2001). Persistent concentrations of hydrocarbons in mussels were identified as a possible source of contamination for several predator species (Duffy, Bowyer, Testa, & Faro, 1996; Sharp, Cody, & Turner, 1996), and there is continuing evidence of bird, sea otter, and fish exposure to oil (Esler, Schmutz, Jarvis, & Mulcahy, 2000; Trust, Esler, Woodin, & Stegeman, 2000; Bodkin et al. 2002; Golet et al. 2002; Jewett, Dean, Woodin, Hoberg, & Stegeman, 2002).

Because of oil persistence in mussel beds, minimally intrusive methods designed to

reduce hydrocarbon concentrations were examined in pilot projects (Babcock, Harris, Carls, Brodersen, & Rice, 1998). Strips of mussels and sediment attached to byssal threads were removed from several oiled mussel beds to facilitate tidal flushing of oil, and patches of mussels were transplanted from oiled beds to clean substrates. These methods accelerated the rate of hydrocarbon loss in transplanted mussels, but not from those in donor or manipulated beds, and hydrocarbon concentrations in many oiled beds remained high in 1993, necessitating more intrusive cleaning methods.

In 1994, we attempted to restore nine selected mussel beds by manually replacing oiled sediments (33 metric tons, t) with clean sediments. Hydrocarbon concentrations and mussel populations were monitored for 5 years after experimental restoration, and data collected in years prior to restoration were used to distinguish hydrocarbon loss due to restoration from loss due to natural environmental processes. Chemical and mechanical oil-removal methods were considered but rejected as being unproven or potentially too destructive (e.g., Mearns, 1996), thus restoration was completed manually.

Our goal was to determine if manual restoration could effectively and practically accelerate the loss of petroleum hydrocarbons from contaminated mussels and sediment without destroying the mussel population. Previous research indicated that *Exxon Valdez* oil concentrations in surface sediment and mussels generally declined over time (Boehm, Page, Gilfillan, Stubblefield, & Harner, 1995; Neff, Owens, Stoker, & McCormick, 1995; Murphy, Heintz, Short, Larsen, & Rice, 1999; Carls et al. 2001), so the critical issue concerning the efficacy of our restoration activity was whether concentrations in restored beds declined more rapidly than natural rates of decline. Our specific objectives were to 1) monitor change in petroleum hydrocarbon concentrations in mussels and sediments underlying restored beds and compare to changes in unrestored oiled reference beds and to time-series data collected before restoration began, and 2) determine the impact of restoration activity on mussel populations.

## Methods

### Site selection

Nine oiled mussel beds at five locations in Prince William Sound were selected for cleaning. Hydrocarbons in six of these beds were monitored in years prior to restoration and samples collected near the remaining three beds (within 25 m; Carls et al. 2001) were used to approximate pre-restoration conditions (Fig. 3.1). Primary criteria for selection were high total petroleum hydrocarbon (THC) concentrations (>5000 μg/g wet weight) in sediments underlying fairly dense mussel beds (1400-2260 mussels $m^{-2}$). Total polynuclear aromatic hydrocarbon (TPAH) concentrations in mussels in candidate beds ranged from 0.20-8.30 μg/g dry weight. In April and May 1994, we confirmed the presence of high THC concentrations in sediments (2000-18000 μg/g) and identified potential nearby replacement sediments. Other selection factors were (1) accessibility, (2) presence of underlying substrate that could be excavated and handled manually, (3) a nearby source of suitable clean sediments, and (4) a suitable area for dispersal of oiled sediments. Bed area ranged from 9 to 62 $m^2$ (Table 3.1). Three additional oiled reference beds (11-24 $m^2$, located near oiled Eleanor Island, Chenega Island, and Disk Island beds) were monitored but not cleaned. The source of oil was confirmed as *Exxon Valdez* oil in all 12 beds (Short & Heintz, 1997).

### Manual restoration

To restore beds, mussels were removed and rinsed with seawater, oily sediment (4-12 cm deep) was replaced with clean sediment (hereafter described as donor sediment), and the mussels were repositioned as evenly as possible in original bed areas. Excavation was completed during

a single low tide at most beds (8.9-18.6 $m^2$) except two low tides were required for the three largest beds (27.5-61.9 $m^2$). Mussels were removed with shovels or trowels, transported in 20-L buckets, and spread out on sorbent pads placed intertidally near each bed. Care was taken to avoid severing byssal thread connections to other mussels and substrate. Oiled sediment was removed to mean depths of about 9 cm and dispersed on sorbent pads in the mid- to high intertidal zone at least 25 m from restored beds; $3.3 \times 10^4$ kg of oiled sediment were replaced. However, oil remained in sediment below excavation depths in all beds. Subsequent high tides flushed oil from the excavated surfaces, dispersed sediments, and mussels and this displaced oil was collected by sorbent booms and pads placed in the lower intertidal zone.

Mussels and donor sediments were placed in beds during the low tide following excavation. Donor sediments were obtained within 100 m of excavated sediments; THC concentrations in these sediments were always < 200 μg/g (Fig. 3.2). We hypothesized that clean sediment placed on top of contaminated sediment would substantially reduce exposure of mussels to hydrocarbons. Restored beds were left slightly higher than the uncleaned parts of the bed to allow for settling. Mussels reattached to other mussels and the donor substrate within one high tide cycle.

**Hydrocarbon monitoring**

Sediment and mussels were sampled for hydrocarbons before and during restoration and through 1999. Surface (0-2 cm) and deep (4-14 cm) sediments were sampled from four random locations within candidate beds in April or May 1994 to quantify THC concentrations, delineate areas that should be cleaned, guide excavation depths, and provide baseline data. Surface and deep sediment and mussels were sampled intermittently after sediment replacement. In general, triplicate pooled sediment samples were randomly collected from 8 to 10 spots throughout sample areas. Triplicate samples of 15-20 mussels were similarly collected, but only one pooled sample was collected where mussel populations were low. Glass storage jars and sediment collection spoons were certified as hydrocarbon-free by the manufacturer or cleaned with dicholoromethane before use. Samples were kept cool, frozen within 2-4 h, and stored at -20°C until analyzed.

THC concentrations in sediment were determined by an ultraviolet fluorescence fast-screening technique adapted from Krahn, Ylitalo, Joss & Chan (1991) and Krahn et al. (1993). This method is relatively fast and inexpensive compared to analysis by gas chromatography/mass spectroscopy (GC/MS), thus optimizing the number of samples processed. Sediments were extracted twice with dicholoromethane. Extracts were separated with a high-performance liquid chromatograph, and quantified with a fluorescence detector (260 nm excitation, 380 nm emission). Emission output was centered at the maximum phenanthrene output. A standard curve based on the amount of phenanthrene in *Exxon Valdez* oil was used to estimate THC concentration. Mean THC concentration is reported in μg/g wet weight. The empirically estimated method detection limits (MDL) for THC was 1.7 μg/g.

Hydrocarbon concentrations in all mussels and some sediment samples were determined by GC/MS (Short, Jackson, Larson, & Wade, 1996). A small subset of sediments with elevated THC was selected for GC/MS analysis to confirm polynuclear aromatic hydrocarbon (PAH) composition. Experimentally determined MDLs depended on sample weights, and generally were 1 ng/g in tissue, and < 2 ng/g in sediment. Concentrations of PAH below MDL were treated as zero. Tissue concentrations are reported in μg/g dry weight; wet to dry weight ratios were measured by dehydrating 1 g wet samples for 24 h at 60°C and weighing the remaining mass. The accuracy of the hydrocarbon analyses was about ±15% based on comparison with National Institute of Standards and Technology values, and precision expressed as coefficient of

variation was usually less than approximately 20%, depending on the PAH. TPAH concentrations were calculated by summing concentrations of individual PAH. Relative PAH concentrations were calculated as the ratio of PAH to TPAH.

The previously published relationship between THC and TPAH concentrations in sediment (Carls et al. 2001) is the basis for approximation of TPAH concentration in Fig. 3.2. Previous background concentration estimates in sediment (50 µg THC /g wet weight) and in mussels (0.09 µg TPAH / g dry weight) aided data interpretation (Carls et al. 2001).

**Statistical analysis of hydrocarbon data**

To evaluate the efficacy of restoration activities, hydrocarbon concentrations in sediments and mussels before and after bed restoration were compared two ways. The first method was an analysis of variance (ANOVA) approach that involved only data collected specifically for this study. The second method compared concentrations observed during and after restoration to long-term, site-specific concentration predictions (Carls et al. 2001).

Pre- and post-restoration hydrocarbon concentrations in sediment and mussels were compared using three-factor ANOVA (site, depth, and day). Concentrations were log-transformed before analysis. For each site, post-restoration hydrocarbon concentrations in sediment were compared to initial concentrations with multiple comparisons. The Bonferroni inequality ($\alpha$ divided by the number of comparisons) was applied to ensure the probability of incorrect rejection was no less than 0.95 for all comparisons. Earliest data included in the analysis were collected in 1994.

To compare post-restoration sediment and mussel data to previously estimated concentration predictions, hydrocarbon data collected during and after bed restoration were combined with previously collected data (Carls et al. 2001). Pre-restoration data (only) were regressed (exponential model) to predict hydrocarbon concentrations without restoration. Post-restoration data were considered to be significantly different than predicted concentrations if means $\pm$ SE were outside 95% confidence bands of the regressions. For each bed, the number of means significantly lower than predicted (between the time of restoration through 1995) were counted and divided by the total number of means.

To determine if oil from surrounding sediment recontaminated surface sediment, each of the following were regressed against excavation area: 1) percent oil remaining in 1999 (of initial 1994 quantities), 2) percent change in oiling between 1995 (minimum observed concentrations) and 1999, and 3) slope (concentration over time) between 1995 and 1999. The hypothesis was that if oil migrated laterally then small beds would be more readily recontaminated than large beds.

**Mussel population**

Population density was estimated by counting live mussels in two quarters of a 0.25 $\times$ 0.25 m quadrat at six randomly chosen locations in each bed. Data collection typically coincided with collection of hydrocarbon samples and extended from 1992 or 1993 to 1999. Post-restoration changes in mussel populations were compared to initial (1994) populations using two-factor ANOVA (site and day).

# Results

## THC in surface sediment

THC concentrations in replaced surface sediment were abruptly lower than in original sediment, often declined through the following year (1995), but were typically above background levels in 1999. In 4 of 6 beds, mean post-restoration THC concentrations (through 1995) were less than predicted in ≥ 50% of the means, demonstrating that sediment replacement had an immediate effect (Table 3.2). For at least one observation in 1995, THC concentrations were significantly less than initial concentrations in all restored beds; a similar significant minimum in 1995 was observed in one reference bed. However, THC concentrations in surface sediment did not continue to decline in restored beds and were roughly constant from 1995-1999 (6 of 9 beds, $0.00 \le r^2 \le 0.33$, $0.043 \le P \le 0.863$). Concentration declined significantly in only one bed ($r^2 = 0.81$, $P < 0.001$) and there was evidence of increases in the remaining two beds ($0.25 \le r^2 \le 0.58$, $0.004 \le P \le 0.021$). See Fig. 3.2 for example concentration changes in the smallest, largest, and median-sized restored beds and each reference bed.

At the end of the study, THC concentrations in surface sediment generally remained well above background levels in restored and oiled reference beds. Mean THC concentrations in 1999 ranged from 36 to 2534 μg/g in surface sediment and was > 100 μg/g (twice background concentration) in 8 of 9 restored beds. Similarly, THC concentrations in the surface sediment of two oiled reference beds remained well above background concentrations (358-6075 μg/g) in 1999 but were <100 μg/g in the third reference bed. Correlation ($r^2$) between excavation area and percent oil remaining in 1999, percent change in oiling between 1995 and 1999, and slope (concentration over time) between 1995 and 1999 was < 0.03.

## THC in deep sediment

Hydrocarbon concentrations in the deep sediment of restored beds tended to decline after restoration, but although short-term losses (1995-1996) were significant in three beds, concentrations were significantly lower in only one bed at the end of study (1999). THC concentrations in deep sediment of six beds generally exceeded concentrations in surface sediment (see Fig. 3.2 for examples). Mean THC concentrations (141-10407 μg/g) in deep sediment of all beds were > 100 μg/g (twice background concentration) at the end of study, usually by a wide margin. Concentrations in one bed fell to 29 μg/g in 1996 but were well above background concentrations in 1999 (186 μg/g). THC concentrations also tended to decline in the deep sediment of oiled reference beds but in 1999, mean concentrations (228-647 μg/g) were significantly greater than background concentration (samples were not collected from the third reference bed in 1999).

## TPAH in mussel tissue

TPAH concentrations in mussels tended to increase for a short time after restoration activity and then declined. Initial TPAH concentration increases were generally not significant but were noted in 6 of 9 beds. Significant concentration reductions were not observed until 1995, a year after restoration. By 1999, mean TPAH concentrations in mussels (0.00-0.38 μg/g) were less than the estimated background concentration (0.09 μg/g) in six restored beds, and greater than 0.2 μg/g in only one bed (samples were not collected from one bed where concentration was <0.09 μg/g in 1995). Mean TPAH concentrations in 1999 in oiled reference beds were less than twice background concentration in all cases. See Fig. 3.3 for example concentration changes in the smallest, largest, and median-sized restored beds and each reference bed.

Mean post-restoration TPAH concentrations in mussels (through 1995) was less than

predicted in ≥ 50% of the means in just 1 of 3 beds but the initial increase in sequestered TPAH likely masked improvement (Table 3.2). In 1995, all mean concentrations were less than model predictions in all three beds, suggesting restoration may have reduced biologically available hydrocarbon levels. By 1999, however, TPAH concentrations in mussels had generally declined to background levels throughout Prince William Sound (unpublished data), including those in the oiled reference beds associated with this study.

**Mussel population**

There were no consistent differences in mussel population densities between restored and unmanipulated, oiled reference beds. Populations generally declined from 1994 to 1996 then stabilized or increased to 1999, both in restored and reference beds. (See Fig. 3.4 for examples of changes in the smallest, largest, and median-sized restored beds and each reference bed.). Populations consistently declined after restoration; declines were significant in four of nine restored beds. Similar declines were observed in all three reference beds, but none of these were significant. Peak populations were observed in 1998 and were the highest recorded since 1992 in four beds (3 oiled, 1 reference). This peak was apparently due to an unusually high proportion of small mussels; populations in 1999 were consistently smaller. Final (1999) population densities were about the same or higher than initial densities in three restored beds but lower in the remaining six. The final population was slightly lower in the only reference bed observed through 1999.

## Discussion

Manual restoration of mussel beds contaminated by *Exxon Valdez* oil was partially successful in reducing hydrocarbon concentrations more rapidly than would have occurred in the absence of restoration and did not cause substantial mortality. Rapid short-term reductions of THC concentrations in surface sediment were sometimes reversed by increasing concentrations in ensuing years. Concentration declines in deep sediment were not always significant. Oil from underlying or surrounding sediment may have recontaminated surface sediments. A year after restoration, hydrocarbon loads in mussels were consistently less than predicted in the three beds where natural loss rates were modeled, suggesting sediment replacement effectively reduced tissue burdens. However, mussels frequently accumulated oil mobilized by sediment disturbance, thus immediate hydrocarbon declines in mussels were not observed. TPAH concentrations in mussels typically reached background levels in ensuing years in both restored and oiled reference beds, thus little was gained by laborious efforts. Hydrocarbons in oiled reference beds also either declined or remained near background levels. Long-term improvement resultant from manual restoration was equivocal and difficult to distinguish from natural losses because of regional variability in oil concentration and persistence, and reversal of short-term improvement.

Restoration efficacy was most evident in replaced surface sediment, where THC concentrations were abruptly reduced, but these short-term reductions did not always ensure long-term improvement. Although reductions in THC concentration were observed, donor sediment was subsequently contaminated with hydrocarbons by restoration activity, and THC concentrations measured after placement were generally much higher in donor sediment than observed before disturbance. Probable sources for this immediate recontamination included oil from deeper sediment (including sediment underlying donor areas), oil surrounding excavated areas, and oil associated with mussels placed onto donor sediment. Despite the increases in hydrocarbon concentration in donor sediment, the net result was an immediate reduction in THC concentration in restored beds. Reduction in THC concentration as a result of restoration activity

was corroborated by comparison to the pre-study concentration predictions in 4 of 6 beds but in 1999 concentration was > 100 μg/g in 8 of 9 restored beds.

Sources of long-term recontamination of surface sediment included oil that remained below the excavation depth and possibly other nearby oil. Although we cannot definitively determine where the additional oil came from, we suspect the most proximate source – underlying sediment – was generally the principal source. An exception to this may have been a portion of the Herring Bay bed, cleaned to bedrock and later observed with contaminated surface sediment, suggesting possible downslope oil migration and entrapment behind a large bedrock outcropping. The lack of relationship between excavation area and percent oil remaining in restored beds suggests oil migration from surrounding areas was generally not the major source because small areas would potentially be more readily recontaminated by lateral movement. However, this analysis does not account for other unmeasured variables, such as distribution of oil outside restored areas and drainage patterns, hence lateral oil movement cannot be eliminated as a plausible source of recontamination.

Recontamination of surface sediment clearly illustrates the dynamic nature of intertidal systems. Oil slicks and sheens emanating from oil deposits as tide water interacts with oiled sediment have been repeatedly observed over many years by our group. This study clearly demonstrates that natural processes transport oil within the intertidal system. The potential for tidally-driven transport of oil constituents dissolved in water was demonstrated near intertidal streams (Carls et al. 2003) and both field and laboratory studies demonstrate that PAH move from oil into water (e.g., Neff and Stubblefield 1995; Short and Harris 1996; Marty et al. 1997). Harris, Rice, Babcock, & Brodersen (1996) reported that oil distributions within mussel beds were not static. Dynamic movement of oil and oil constituents within intertidal sediment likely increases the potential for and rate of oil weathering and the ultimate cleansing of sediment but also increases the potential for exposure of organisms living in this habitat.

Efficacy of restoration was least evident in the mussels. Although concentrations eventually declined in the mussels, reductions were never significant in the first year of study, and concentration declines were indistinguishable from general regional trends in oiled reference beds. TPAH concentration declines from mussels in the first year were generally not more rapid than predicted, rather the anticipated immediate abrupt hydrocarbon declines in mussels were masked by accumulation of oil remobilized by sediment disturbance in most (67%) of the beds.

To place the hydrocarbon loads in the mussels examined in this study in a broader context, we offer a brief comparison to TPAH concentrations collected near our study beds in 1989 and TPAH concentrations in mussels from many other locations throughout the continental USA (National Status and Trends, NST, mussel watch 'extended PAH suite,' 1986 to 1997). In 1989 the mean TPAH concentrations in PWS mussels in the vicinity of our study areas was 29 (range 0 to 131 μg/g, n = 37; Short, Heintz, Nelson, Maselko, Kendziorek, Carls, & Korn 1996). The mean TPAH concentration in NST mussels was 2.1 μg/g (range 0 to 43 μg/g, n = 248). The mean TPAH concentration in mussels in 1994 (the first year of our study), 1.8 μg/g (range 0 to 10.7 μg/g, n = 121), was about the same as in NST mussels, but considerably lower than shortly after the *Exxon Valdez* spill. However, relatively more high-molecular weight PAH (anthracene and above) were present in NST mussels (68% of TPAH) than in mussels from PWS (14%; $P < 0.001$). PAH composition in NST mussels suggests the presence of combustion products, unlike the situation in our samples. However, PAH levels were not elevated in all NST mussels; concentrations were ≤ 0.09 μg/g (the background concentration estimate for PWS mussels) in 19% of them, indicating that some were clean according to the same standards applied to PWS mussels.

The restoration methods employed in this study probably caused some mussel mortality

immediately after manipulation, but short-term changes in mussel population (1994-1996) could not be distinguished from regional declines in population, and longer term fluctuations were quite similar. The high mortality evident in earlier mussel relocation experiments may have been partially due to relocation of mussels into marginal habitat (Harris, Babcock, & Rice, 1998). We expected some mortality would be caused by shell breakage and other physical stresses during mussel transfer. However, reduced ability to produce byssal threads and remain attached to suitable substrate as a result of the short-duration rise in concentration observed after restoration activity was not likely: Thomas et al. (1999) did not find inhibited thread production in mussels chronically exposed to *Exxon Valdez* oil. Mussel populations in manipulated beds declined after restoration, but so did populations in unmanipulated reference beds. There was additional evidence of regional declines in mussel populations in 6 of 10 additional unmanipulated oiled beds (unpublished data). Reasons for regional declines in mussel population are unknown. Natural senescence of aging year classes and pathogens have been suggested as possibilities, but there is no supporting evidence. Storm activity did not appear to be causal; all restored beds were relatively protected from waves.

Mussel mortality resultant from our restoration activity was almost certainly far less than that caused by cleaning methods employed in 1989 and 1990 to remove *Exxon Valdez* oil from shorelines. In particular, about one-third of all shoreline segments in western Prince William Sound were washed with high-pressure hot water, and much of the marine life so treated perished (Mearns, 1996; Houghton, Lees, Driskell, Lindstrom, & Mearns, 1996). Although we have a less than satisfactory understanding of mussel mortality caused by our restoration activity, there was no evidence of mass mortality.

One way to reduce or preclude the oiling of shoreline is application of chemical dispersants to oil in open water before landfall, a decision that involves tradeoffs and uncertainty. Protection of shoreline habitat with dispersants puts open water habitat at increased risk to the toxic effects of oil, so the potential risks and benefits must be carefully considered. In particular, which organisms will be damaged in each environment with or without application of dispersant? How sensitive are they to oil and dispersed oil effects? How significant may damage be to each population? What is the recovery potential of each population? How long will oil persist at damaging levels in each environment? What are the secondary effects on predators and other organisms that later use contaminated habitat? Can increased short-term open-water population and habitat damage be traded for potential long-term intertidal damage? Consideration of physical factors is also important. Will application of chemical dispersants be effective given the type of oil and dispersants available and environmental conditions such as temperature, mixing energy, and salinity? Because volatile components are lost as spilled oil spreads and oil-in-water emulsions begin to form, reducing the effectiveness of dispersants, the amount of time available to answer these difficult questions may be limited to a few days at most. Contingency planning will help but each spill is likely to present a unique and complex situation for which there may be no one answer. The goal of dispersant application or any other remedial action should be to provide net environmental benefit.

At one time mussel beds were considered to be responsible for retention of oil in underlying sediment (Babcock, Irvine, Harris, Cusick, & Rice, 1996) but after a decade of measurement and observation that major declines in mussel populations apparently did not accelerate loss of oil from these sediments, we concur with Hayes & Michel (1999) that geomorphic beach structure is the principal reason oil has been retained in these areas. Subsurface oil was detected in 2002 by random survey at elevations (1.8-4.8 m above mean lower low water) generally above the mussel zone (typically 1.3-2.0 m above mean lower low water) at 53 of 91 formerly oiled sites (Short, Lindeberg, Harris, Maselko, & Rice, 2002),

demonstrating that mussels were not responsible for trapping this oil in these beaches.

Regardless of why oil persists in soft intertidal sediment, mussel beds and associated fauna are utilized by predators, and feeding activity may explain the decadal exposure of predators to hydrocarbons [e.g. pigeon guillemots (*Cepphus columba*), harlequin ducks (*Histrionicus histrionicus*), Barrow's goldeneyes (*Bucephala islandica*), sea otters (*Enhydra lutris*), masked greenling (*Hexagrammos octogrammus*), and crescent gunnel (*Pholis laeta*); (Trust et al. 2000; Bodkin et al. 2002; Golet et al. 2002; Jewett et al. 2002)]. TPAH concentrations in mussel tissue have typically reached or fallen below estimated baseline concentrations (this study and other unpublished data), thus mussels are not likely the current route of predator exposure. Conversely, there are clear indications that proximal biota (e.g. mussels) accumulate hydrocarbons when oiled sediment is disturbed. We suspect that direct exposure to oiled sediment during feeding disturbance, consumption of infauna which are directly exposed to oiled sediment, or other predator contact with sediment is likely the principal route of exposure for predatory species.

Hydrocarbon concentrations were reduced by manual restoration in the short-term, but long-term success was limited by recontamination and practicality. Evidence that replaced surface sediment became contaminated, that concentration reductions in sediments and mussels were not always distinguishable from unaided concentration declines, and the effort required to restore a relatively small area (208 $m^2$ representing about 43 m of 900 km of moderately to heavily oiled shoreline) forces us to conclude that manual methods are not recommendable as primary cleanup techniques for large spills if the specific goal is to accelerate hydrocarbon loss in mussels or similar biological resources. However, we recognize that manual cleanup efforts may remain an important remedial procedure in future spills, particularly where large numbers of people are available. In the immediate aftermath of a spill, where many people are motivated by a desire to restore resources for which they have collective ownership, manual labor may be effective.

Alternative cleanup procedures, after manual removal of mussels, might include more aggressive mechanical, chemical, or bioremediation techniques. Removal of mussels would protect a fraction of this community, although the process is undoubtedly highly disruptive. Treatments designed to clean the relocated mussels should also be considered; while relocated, some adherent oil might be removed by rinsing with low-toxicity detergents or other appropriate products. Once the cleaning process is completed, mussels could be returned to the site where they would reattach, stabilize the habitat, and provide both a haven for other invertebrates and prey for predators. However, on a broader scale, the experience of this study and the many other cleanup efforts associated with the *Exxon Valdez* oil spill in Prince William Sound, coupled with observation of long-term (decadal) persistence of oil in intertidal habitat, suggests that human restoration of intertidal habitat after a major oil spill may generally be impractical. Similarly, Mearns (1996) concluded that perhaps just 4-19% of stranded *Exxon Valdez* oil was removed by treatment and cleanup operations (1989-1991) despite intensive effort.

Mussel beds are a valuable natural resource that should be cleaned if extensive areas are contaminated with oil, as happened in Prince William Sound after the *Exxon Valdez* oil spill. They are too valuable as habitat and prey to leave uncleaned but are too fragile to allow destructive cleaning processes such as hot water washes. We suggest that mussels should be protected from aggressive oil-removal treatment, but recognize that any relocation approach is apt to seriously disrupt the community (including other species that depend on this resource for habitat and prey). We are hopeful that removal of significant proportions of oil can accelerate recovery, but given the record of the *Exxon Valdez* oil spill and others (e.g. *Amoco Cadiz*, *Arrow*), we are skeptical that intervention will ever remove sufficient oil gently enough to avoid

short- and long-term community damage.

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## References

Babcock, M. M., Irvine, G., Harris, P. M., Cusick, J., & Rice, S. D. (1996). Persistence of oiling in mussel beds three and four years after the *Exxon Valdez* oil spill. *American Fisheries Society Symposium*, *18*, 286-297.

Babcock, M. M., Harris, P. M., Carls, M. G., Brodersen, C. C., & Rice, S. D. (1998). *Mussel bed restoration and monitoring. Exxon Valdez Oil Spill Restoration Project 95090 Final report*. Juneau, AK: National Oceanic and Atmospheric Administration, National Marine Fisheries Service, Auke Bay Laboratory, 11305 Glacier Hwy., Juneau, AK 99801.

Bodkin, J. L., Ballachey, B. E., Dean, T. A., Fukuyama, A. K., Jewett, S. C., McDonald, L., Monson, D. H., O'Clair, C. E., VanBlaricom, G. R. (2002). Sea otter population status and the process of recovery from the 1989 *Exxon Valdez* oil spill. *Marine Ecology Progress Series 241*, 237-253.

Boehm, P. D., Page, D. S. Gilfillan, E. S., Stubblefield, W. A., & Harner, E. J. (1995). Shoreline ecology program for Prince William Sound, Alaska, following the *Exxon Valdez* spill: part 2−chemistry and toxicology. In P. G. Wells, J. N. Butler, & J. S. Hughes, *Exxon Valdez oil spill: fate and effects in Alaskan waters* (pp. 347-397, ASTM STP 1219). Philadelphia, PA: American Society for Testing and Materials.

Carls, M. G., Babcock, M. M., Harris, P. M., Irvine, G. V., Cusick, J. A., & Rice, S. D. (2001). Persistence of oiling in mussel beds after the *Exxon Valdez* oil spill. *Marine Environmental Research, 51*, 167-190.

Carls, M. G., Thomas, R. E., & Rice, S. D. (2003). Mechanism for transport of oil-contaminated water into pink salmon redds. *Marine Ecology Progress Series 248*, 245-255.

Duffy, L. K., Bowyer, R. T., Testa, J. W., & Faro, F. B. (1996). Acute phase proteins and cytokines in Alaska mammals as markers of chronic exposure to environmental pollutants. *American Fisheries Society Symposium*, *18*, 809-813.

Esler, D., Schmutz, J. A., Jarvis, R. L., & Mulcahy, D. M. (2000). Winter survival of adult female harlequin ducks in relation to history of contamination by the *Exxon Valdez* oil spill. *Journal of Wildlife Management*, *64*, 839-847.

Exxon Corporation 1992. Three years after. Exxon Corp., Houston, TX.

Golet, G. H., Seiser, P. E., McGuire, A. D., Roby, D. D., Fisher, J. B., Kuletz, K. J., Irons, D. B., Dean, T. A., Jewett, S. C., & Newman, S. H. (2002). Long-term direct and indirect effects of the *Exxon Valdez* oil spill on pigeon guillemots in Prince William Sound, Alaska. *Marine Ecology Progress Series, 241*, 287-304.

Gundlach, E. R., Bauer, J., Bayliss, R., Provant, S., & Kendziorek, M. (1990). Response to the

*Exxon Valdez* oil spill by the Alaska Department of Environmental Conservation. Pages In M. L. Spaulding, & M. Reed, *Oil spills management and legislative implications: proceedings of the conference, May 15-18, 1990*, (pp. 471-478). Newport, RI: American Society of Civil Engineers.

Harris, P. M., Rice, S. D., Babcock, M. M., & Brodersen, C. C. (1996). Within bed distribution of *Exxon Valdez* crude oil in Prince William Sound mussels and underlying sediments. *American Fisheries Society Symposium*, *18*, 298-308.

Harris P. M., Babcock, M. M., & Rice, S. D. (1998). Manipulation of oiled mussel beds to accelerate depuration of petroleum hydrocarbons. In M. M. Babcock, P. M. Harris, M. G. Carls, C. C. Brodersen, & S. D. Rice, *Mussel bed restoration and monitoring, Exxon Valdez Oil Spill Restoration Project 95090 Final Report* (pp. 58-82). Juneau, AK: National Oceanic and Atmospheric Administration, National Marine Fisheries Service, Auke Bay Laboratory, 11305 Glacier Hwy., Juneau, AK 99801.

Hayes, M. O. & Michel, J. (1999). Factors determining the long-term persistence of *Exxon Valdez* oil in gravel beaches. *Marine Pollution Bullletin*, *38*, 92-101.

Houghton, J. P., Lees, D. C., Driskell, W. B., Lindstrom, S. C., & Mearns, A. J. 1996. Recovery of Prince William Sound intertidal epibiota from *Exxon Valdez* oiling and shoreline treatments, 1989 through 1992. *American Fisheries Society Symposium, 18*, 379-411.

Jewett, S.C., Dean, T. A., Woodin, B. R., Hoberg, M. K., & Stegeman, J. J. 2002. Exposure to hydrocarbons 10 years after the *Exxon Valdez* oil spill: evidence from cytochrome P4501A expression and biliary FACs in nearshore demersal fishes. *Marine Environmental Research 54*, 21-48.

Kelso D. D., & Kendziorek, M. (1991). Alaska's response to the *Exxon Valdez* oil spill, Part 1. *Environmental Science and Technology*, *25*, 16-23.

Krahn M. M., Ylitalo, G. M., Joss, J., & Chan, S.-L. (1991). Rapid, semi-quantitative screening of sediments for aromatic compounds using sonic extraction and HPLC/fluorescence analysis. *Marine Environmental Research, 31*, 175-196.

Krahn, M. M., Ylitalo, G. M., Buzitis, J, Chan, S. -L., Varanasi, U., Wade, T. L., Jackson, T. J., Brooks, J. M., Wolfe, D. A., & Manen, C. -A. (1993). Comparison of high-performance liquid chromatography/fluorescence screening and gas chromatography/mass spectrometry analysis for aromatic compounds in sediments sampled after the *Exxon Valdez* oil spill. *Environmental Science and Technology*, *27*, 699-708.

Marty, G. D., Short, J. W., Dambach, D. M., Willits, N. H., Heintz, R. A., Rice, S. D., Stegeman, J. J., & Hinton, D. E. (1997). Ascites, premature emergence, increased gonadal cell apoptosis, and cytochrome P4501A induction in pink salmon larvae continuously exposed to oil-contaminated gravel during development. *Canadian Journal of Zoology, 75,* 989-1007.

Mearns, A. J. (1996). *Exxon Valdez* shoreline treatment and operations: implications for response, assessment, monitoring, and research. *American Fisheries Society Symposium, 18*, 309-328.

Michel J., & Hayes, M. O. (1993). Evaluation of the condition of Prince William Sound shorelines following the *Exxon Valdez* oil spill and subsequent shoreline treatment. Volume I: summary of results – geomorphological shoreline monitoring survey of the *Exxon Valdez* spill site, Prince William Sound, Alaska, September 1989 – August 1992. NOAA (National Oceanic and Atmospheric Administration) Technical Memorandum NOS (National Ocean Service) ORCA 73, Seattle, WA 98115.

Murphy, M. L., Heintz, R. A., Short, J. W., Larsen, M. L, & Rice, S. D. (1999). Recovery of pink salmon spawning areas after the *Exxon Valdez* Oil Spill. *Transactions of the American Fisheries Society*,

*128*, 909-918.

Neff, J. M., Owens, E. H., Stoker, S. W., & McCormick, D. M. (1995). Shoreline oiling conditions in Prince William Sound following the *Exxon Valdez* oil spill. In P. G. Wells, J. N. Butler, & J. S. Hughes, *Exxon Valdez oil spill: fate and effects in Alaskan waters* (pp. 312-346, ASTM STP 1219). Philadelphia, PA: American Society for Testing and Materials.

Neff, J. M., & Stubblefield, W. A. (1995). Chemical and toxicological evaluation of water quality following the *Exxon Valdez* oil spill. In P. G. Wells, J. N. Butler, & J. S. Hughes, *Exxon Valdez oil spill: fate and effects in Alaskan waters* (pp. 141-177, ASTM STP 1219). Philadelphia, PA: American Society for Testing and Materials.

Sharp, B., Cody, M., & Turner, R. (1996). Effects of the *Exxon Valdez* oil spill on the black oystercatcher. *American Fisheries Society Symposium*, *18*, 748-758.

Short, J. W., & Harris, P. M. (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.

Short, J. W. & Heintz, R. A. (1997). Identifications of *Exxon Valdez* oil in sediments and tissues from Prince William Sound and the norwestern Gulf of Alaska based on a PAH weathering model. *Environmental Science and Technology*, *31*, 2375-2384.

Short, J. W., Jackson, T. J., Larson, J. L., & Wade, T. L. (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.

Short, J.W., R.A. Heintz, B.D. Nelson, J.M. Maselko, M.F. Kendziorek, M.G. Carls, and S. Korn. 1996. *Exxon Valdez* oil spill of 1989: State/Federal Trustee Council hydrocarbon database 1989-1995. National Oceanic and Atmospheric Administration, National Marine Fisheries Service, Auke Bay Laboratory, 11305 Glacier Hwy., Juneau, AK 99801.

Short, J. W., Lindeberg, M. R., Harris, P. M., Maselko, J., & Rice, S. D. (2002). Vertical oil distribution within the intertidal zone 12 years after the *Exxon Valdez* oil spill in Prince William Sound Alaska. In *Proceedings of the Twenty-fifth Arctic and marine oilspill program (AMOP) technical seminar, June 11-13, 2002* (pp. 57-72). Calgary, Alberta, Canada: Environment Canada

Spies R. B., Rice, S. D., Wolfe, D. A., & Wright, B. A. (1996). Effects of the *Exxon Valdez* oil spill on the Alaskan coastal environment. *American Fisheries Society Symposium*, *18*, 1-16.

Thomas, R. E., Brodersen, C., Carls, M. G., Babcock, M. M., & Rice, S. D. (1999). Lack of physiological responses to hydrocarbon accumulation by *Mytilus trossulus* after 3–4 years chronic exposure to spilled *Exxon Valdez* crude oil in Prince William Sound. *Comparative Biochemistry and Physiology* C, *122*, 153-163.

Trust, K. A., Esler, D., Woodin, B. R., & Stegeman, J. J. (2000). Cytochrome P450 1A induction in sea ducks inhabiting nearshore areas of Prince William Sound, Alaska. *Marine Pollution Bulletin*, *40*, 397-403.

Wolfe, D. A., Hameedi, M. J., Galt, J. A., Watabayashi, G., Short, J., O'Clair, C., Rice, S., Michel, J., Payne, J. R., Braddock, J., Hanna, S. & Sale, D. (1994). The fate of the oil spilled from the *Exxon Valdez*. *Environmental Science and Technology*, *28*, 561A-568A.

**Table 3.1.** Area, mean excavation depth, and approximate distance parallel to the water in mussel beds restored in 1994. Some of the Herring Bay bed was excavated to bedrock; oiled sediment remained below excavation depth in all beds.

| Location | Bed Name | Area ($m^2$) | Excavation depth (cm) | Parallel Distance (m) |
|---|---|---|---|---|
| Restored beds: | | | | |
| Eleanor Island | EL011A-2 | 27.5 | 10 | 4 |
| Chenega Island | CH010B-2A | 39.4 | 11 | 6 |
| Chenega Island | CH010B-2B | 8.9 | 10 | 3 |
| Chenega Island | CH010B-2C | 9.1 | 11 | 2 |
| Disk Island | DI067A-1 | 18.6 | 10 | 3 |
| Disk Island | DI067A-2A | 61.9 | 8 | 14 |
| Disk Island | DI067A-2B | 16.1 | 6 | 4 |
| Herring Bay | KN113B | 9.2 | 9 | 3 |
| Squirrel Island | SL001D-2 | 17.2 | 9 | 3 |
| Oiled Reference beds: | | | | |
| Chenega Island | CH010B-2D | 7.8 | | 4 |
| Eleanor Island | EL011A-2D | 4.8 | | 2 |
| Disk Island | DI067A-2C | 22.2 | | 4 |

**Table 3.2.** Numbers and percentages of mean hydrocarbon concentrations in sediment and mussels less than concentrations predicted by pre-restoration data (exponential models); Σ*n* is the total number of means between restoration and 1996. Estimates were limited to data collected prior to January 1, 1996.

| | sediment | | | mussels | | |
|---|---|---|---|---|---|---|
| | **∑n** | **n** | **%** | **∑n** | **n** | **%** |
| CH010B-2A | 4 | 4 | 100 | 5 | 2 | 40 |
| CH010B-2B | 3 | 2 | 67 | 4 | 0 | 0 |
| CH010B-2C | 5 | 4 | 80 | – | – | – |
| DI067A-2 | 5 | 2 | 40 | – | – | – |
| DI067A-2B | 4 | 0 | 0 | – | – | – |
| SLD001D-2 | 4 | 4 | 100 | 4 | 3 | 75 |

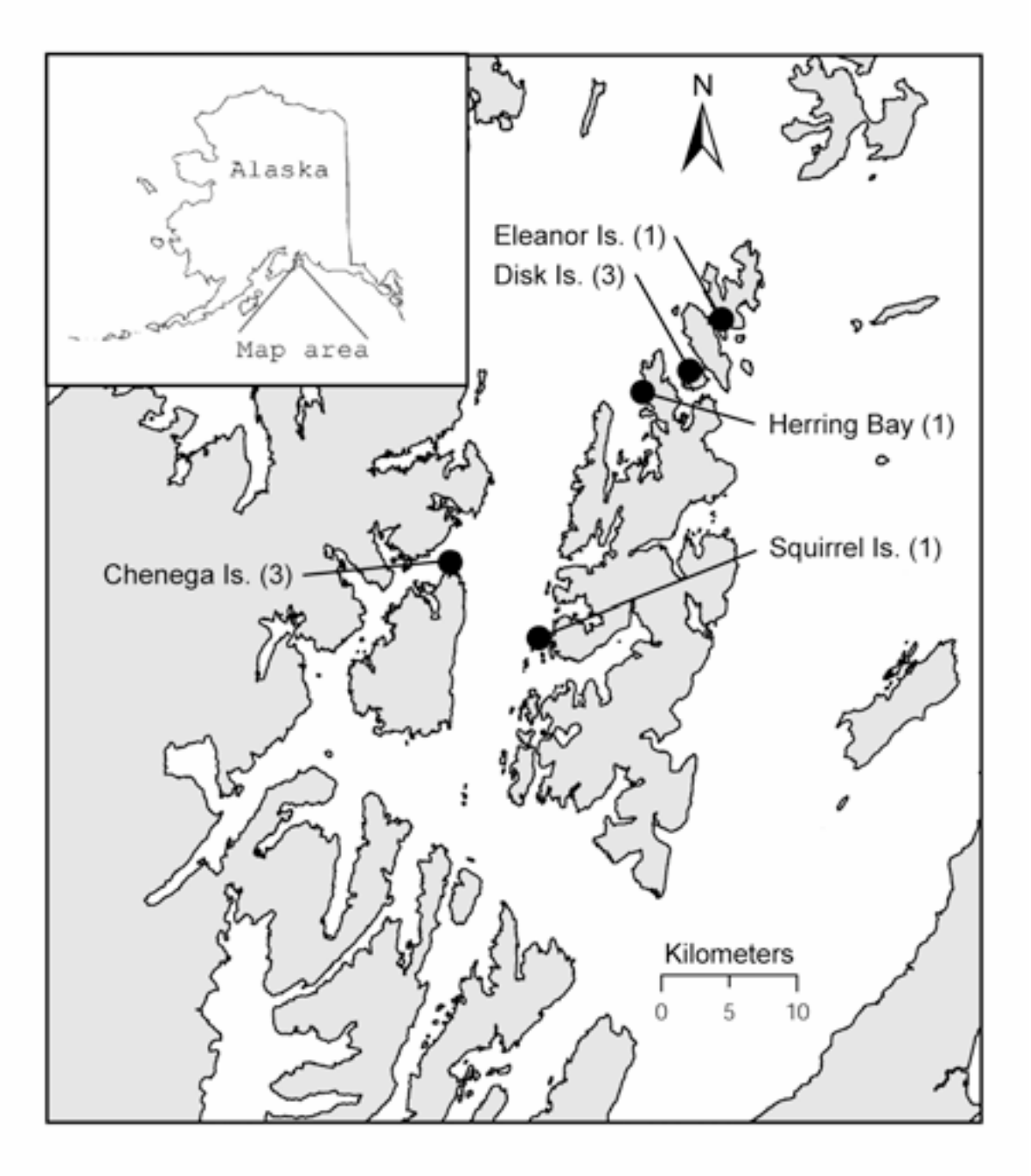


**Fig. 3.1.** Locations of restored mussel beds in Prince William Sound. Numbers in parentheses indicate the number of beds that were restored at each site.

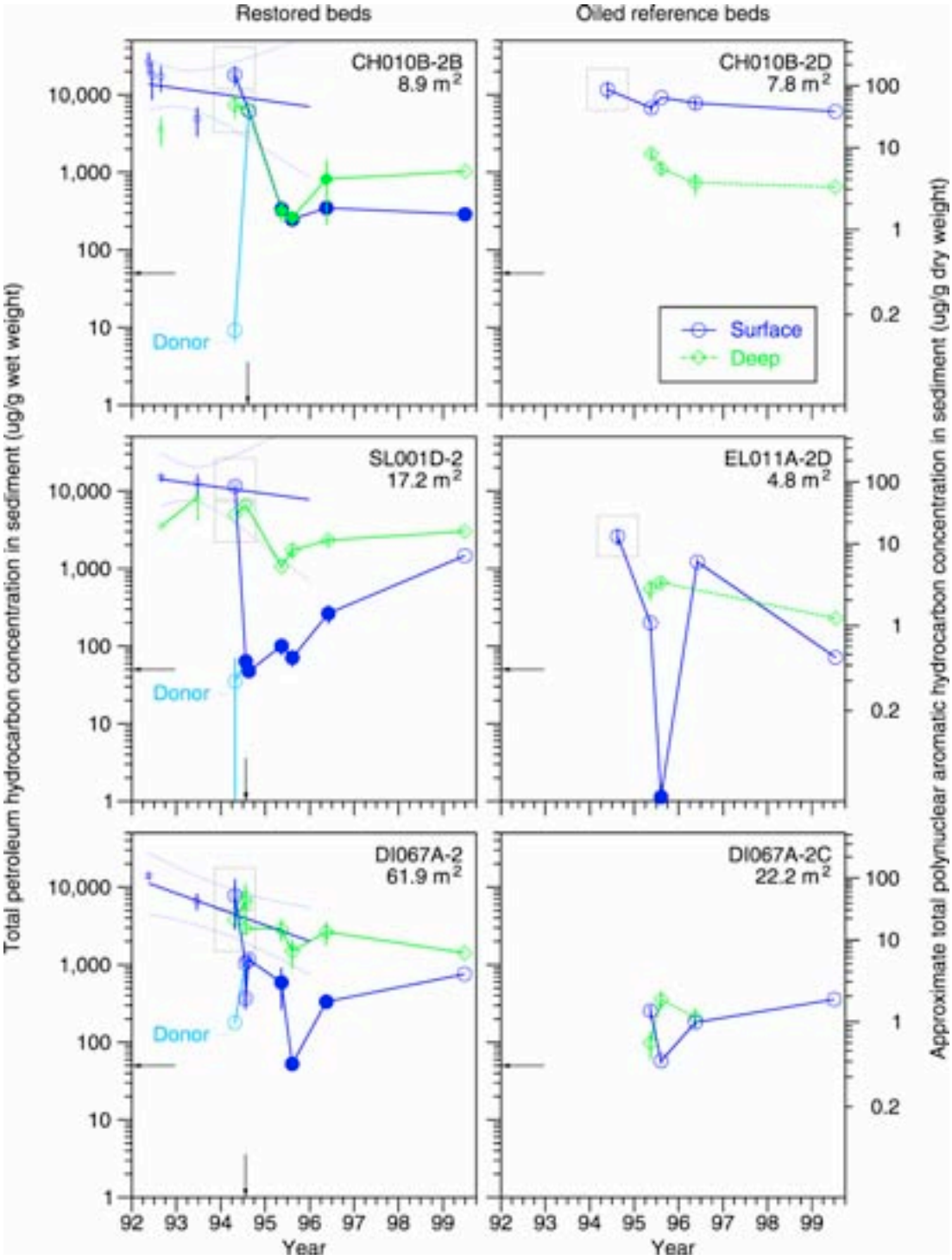


**Fig. 3.2.** Mean total petroleum hydrocarbon (THC) concentrations in surface (0-2 cm) and deep (4-14 cm) sediment from example restored and oiled reference beds,1992-1999. Concentrations of THC in undisturbed donor sediments are labeled "donor." Boxes indicate which samples served as references for statistical analyses. Solid symbols indicate significant differences from initial concentrations. Small symbols and regressions (with 95% confidence bands) indicate conditions and predictions prior to restoration (Carls, Babcock, Harris, Irvine, Cusick, & Rice, 2001; exponential models). Vertical arrows indicate times restoration began. Horizontal arrows indicate estimated background concentration (Carls, Babcock, Harris, Irvine, Cusick, & Rice, 2001). Total polynuclear aromatic hydrocarbon concentrations are approximations based on correspondence between TPAH and THC in dual-analyzed samples.

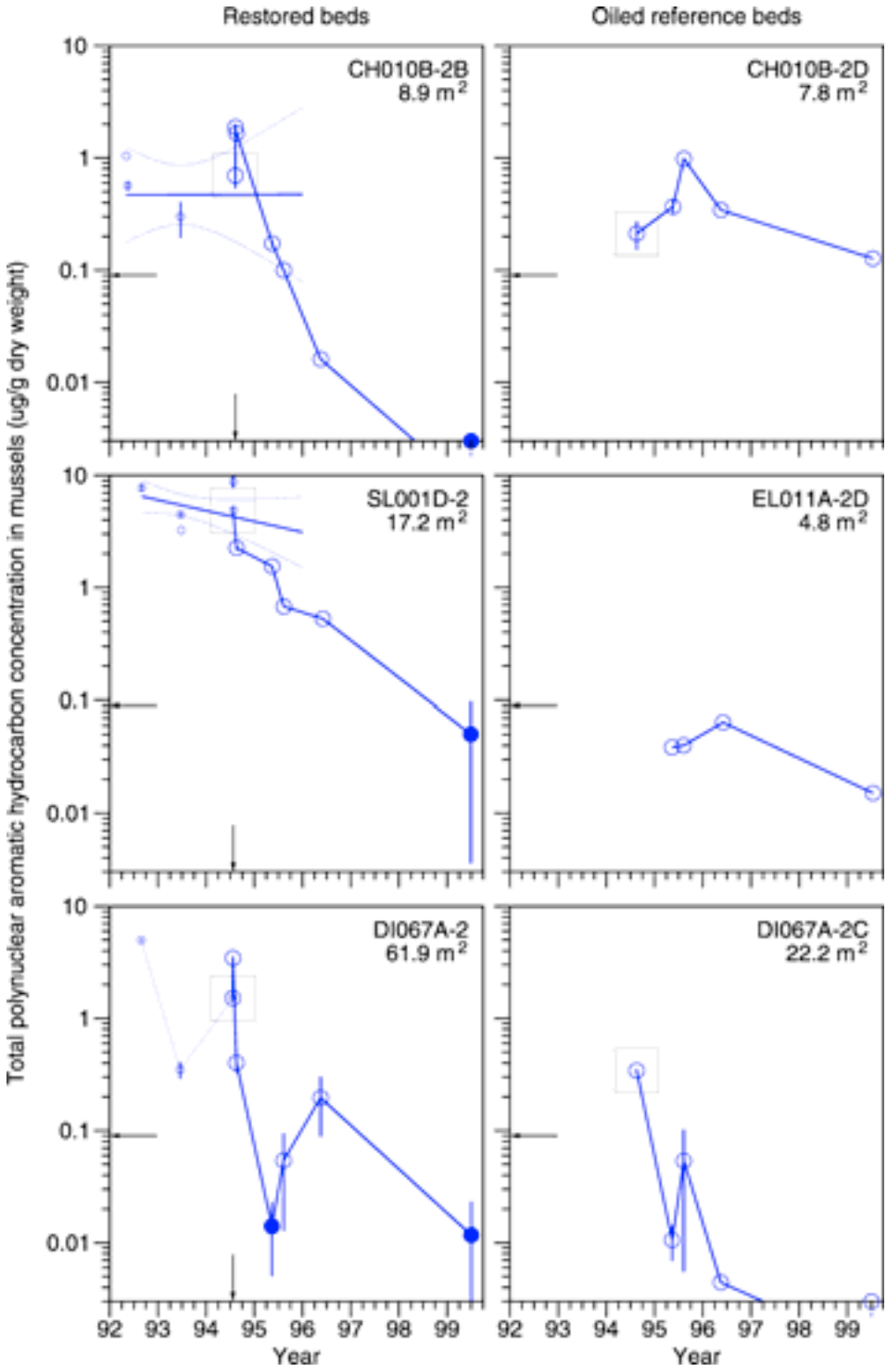


**Fig. 3.3.** Mean total polynuclear aromatic hydrocarbon (TPAH) concentrations in mussel tissue from example restored and oiled reference beds, 1992-1999. Boxes indicate which samples served as references for statistical analyses. Solid symbols indicate significant differences from initial concentrations. Concentrations projected below y-axes were < MDL. Small symbols and regressions (with 95% confidence bands) indicate conditions and predictions prior to restoration (Carls, Babcock, Harris, Irvine, Cusick, & Rice, 2001; exponential models). Vertical arrows indicate times restoration began. Horizontal arrows indicate estimated background concentration (Carls, Babcock, Harris, Irvine, Cusick, & Rice, 2001).

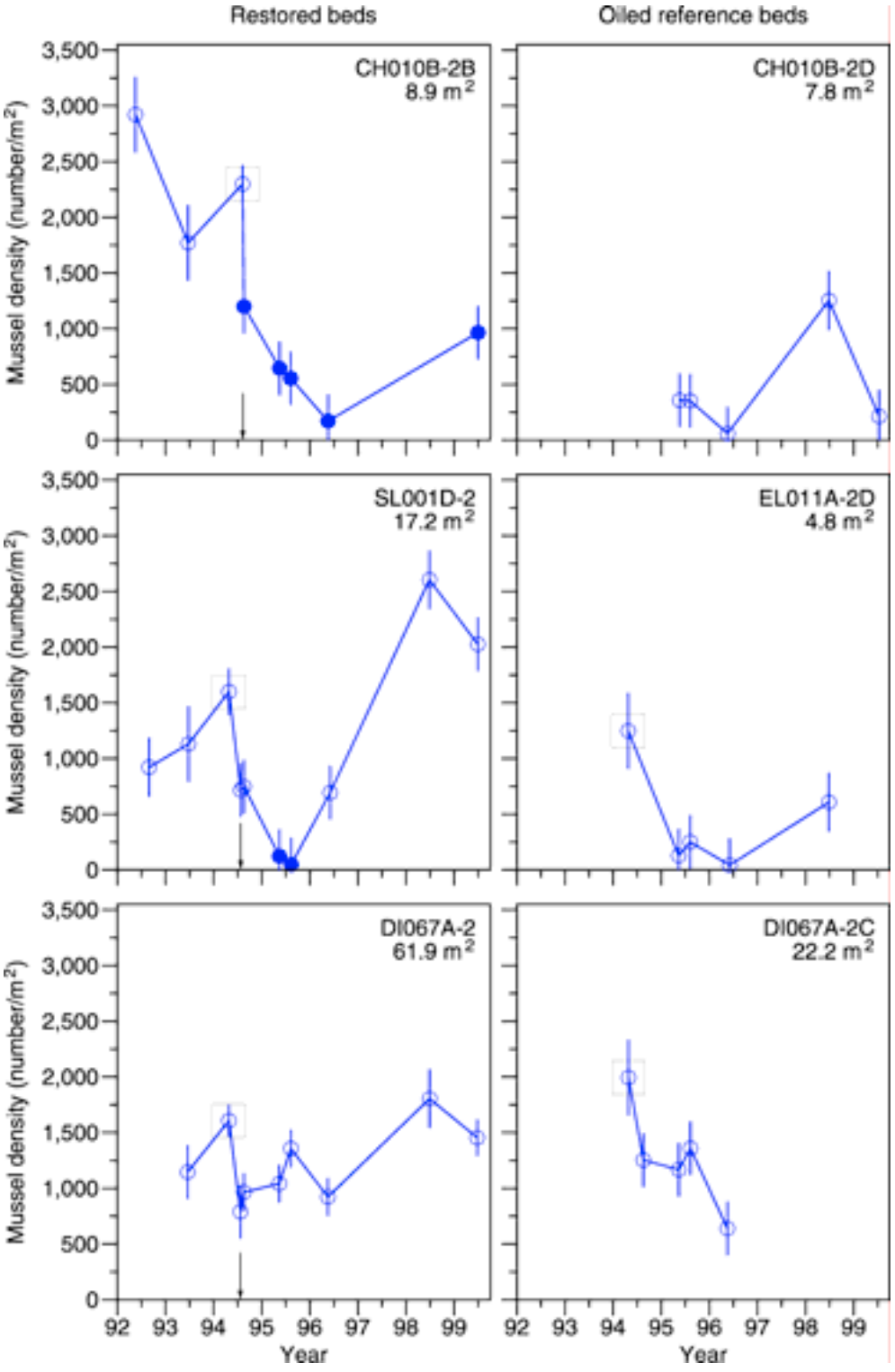


**Fig. 3.4.** Mean mussel population density in example restored and unmanipulated oiled reference mussel beds in Prince William Sound. Vertical arrows indicate times restoration began. Boxes indicate which samples served as references for statistical analysis. Solid symbols indicate significant differences from reference populations ($P \leq 0.05$).

# Chapter 4

# Identification of marine hydrocarbon sources: a novel non-parametric approach

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**Abstract**

Novel non-parametric models developed herein discriminated between oiled and non-oiled or pyrogenic and oiled sources better than traditionally used diagnostic ratios and performed similarly to two previously published oil identification models. These methods were compared using experimental and environmental hydrocarbon data sets (sediment, mussels, water, and fish) collected after the *Exxon Valdez* oil spill and among a variety of sources. Several non-parametric models were investigated, one designed to detect petroleum in general, one specific to Alaska North Slope crude oil (ANS), and one designed to detect pyrogenic PAH. These ideas are intended as guidance; non-parametric models can easily be adapted to fit the specific needs of a variety of petrogenic and pyrogenic sources. Oil identification was clearly difficult where composition was modified by physical or biological processes; model results differed most in these cases, suggesting that a multiple model approach to source discrimination may be particularly useful where data interpretation is contentious. The ultimate goal is to provide an informed, holistic assessment of hydrocarbon sources in the environment and non-parametric modeling adds an independent tool to the assessment procedure.

## Introduction

About 1.3 million metric tons of petroleum from natural seeps, oil spills and other human activities enter the world's oceans each year (1). Determining natural and human sources of hydrocarbon contamination in the environment is necessary to understand why they are present, who (or what) might be responsible, and what the biological and ecological implications of their presence are. The presence of polynuclear aromatic hydrocarbons (PAHs) in water, sediment, and tissue samples provides clues regarding contaminant sources, although identification is complicated by composition changes as the oil is altered by physical, chemical, and biological processes. Not only can PAHs be used for source identification, they are likely primarily responsible the toxicity of petroleum, adding to the incentive to quantify them (2-4).

Because the PAH present in petroleum and other oil products are relatively resistant to degradation and persist in the environment, they are frequently measured and used to determine hydrocarbon sources. Numerous diagnostic PAH ratios have been used to discriminate among sources (reviewed by Wang and Fingas; 5). Frequently these ratios are examined to discriminate between petrogenic sources and others, such as pyrogenic sources. More complex, alternative approaches were used to identify hydrocarbon sources after the *Exxon Valdez* oil spill, the largest spill in a sub-Arctic ecosystem.

Two models designed to identify source oil were developed to distinguish *Exxon Valdez* oil, an Alaska North Slope crude oil (ANS), from other hydrocarbons (6-7). Both models rely on analysis of PAH composition and both were intended primarily for situations where whole oil was present, such as oil on intertidal sediment. The oil-fingerprinting method (OFM; 6) relies on

a series of decisions for identification, including the presence or absence of specific PAH and values of PAH ratios. The first-order loss-rate model (FORLM; 7) relies on first-order loss rate constants for 14 environmentally persistent PAH and bootstrapped error distributions to determine if composition is consistent with ANS. The FOLRM also provides an index of weathering (*w*). The results provided by these models are not identical and rather than arbitrarily choosing one or the other, an independent perspective could provide additional useful information.

The purpose of this study is to present an alternative, non-parametric approach to source identification. This model is distinct from other approaches because it relies on scoring, not specific concentrations. The preliminary goal was to develop a model that identifies petroleum hydrocarbons in a broad range of matrices, e.g., water, tissue, and sediment, over a broad concentration range, and general enough to reasonably test for the presence or absence of oil in all samples. The proposed identification approach employs a simple scoring scheme and is a method that can be readily adapted for other types of oil. Two versions were developed to describe petrogenic PAH. The non-specific version ($PSCORE_{oil}$) is simply based on the presence or absence of homologous PAH families and their constituents. Scoring in the specific version ($PSCORE_{ANS}$), is based on the observation that fewer unsubstituted parent compounds (X0) are present in ANS than alkyl-substituted compounds (X*n*) in each of five homologous families (naphthalenes, fluorenes, dibenzothiophenes, phenanthrenes, and chrysenes). This relationship is generally true in unweathered ANS (except for C3-fluorenes, C4-phenanthrenes, and C4-chrysenes) and remains (or becomes) true as the oil weathers. The same relationship (X0 < X*n)* is evident in Kuroshima Bunker oil, Montreal Bunker oil, and Katalla crude oil (8).

Experimental data were first examined with all models to verify and compare performance. Scores that distinguished oil in the non-parametric models were determined empirically. The OFM and FORLM model outputs were each subdivided into non-specific oil detection and ANS-specific detection portions to compare to the analogous non-parametric model results. The source-discrimination ability of all models and ratios were then evaluated in environmental data. Demonstrating the flexibility of the proposed scoring approach, a pyrogenic model was similarly developed based on the observation that unsubstituted parent homolog concentrations are typically much greater than alkyl-substituted concentrations.

## Methods

Post-spill PAH data from Prince William Sound, Alaska, were obtained from the Natural Resource Damage Assessment database (9), as analyzed by gas chromatography / mass spectroscopy according to the methods of Short et al. (10). Included were 1907 mussel samples, 1505 sediment samples, 236 water samples, and 290 fish samples, combined across 19 studies. Experimental data included water, sediment, and fish tissue, assembled across 7 studies. *Exxon Valdez* and ANS oil samples were collected in conjunction with the spill and experimental studies. Analyzed were 39 PAH: naphthalenes (N0 to N4), biphenyl (BPH), acenaphthylene (ACY), acenaphthene (ACE), fluorenes (F0 to F3), dibenzothiophenes (D0 to D3), phenanthrenes (P0 to P4), anthracene (ANT), fluoranthene (FLA), pyrene (PYR), C1-fluoranthenes/pyrenes (C1FLA), benzo(a)anthracene (BaA), chrysenes (C0 to C4), benzo(b)fluoranthene (BbF), benzo(k)fluoranthene (BkF), benzo(e)pyrene (BeP), benzo(a)pyrene (BaP), perylene (PER), indeno(123-cd)pyrene (IDP), dibenzo(a,h)anthracene (DBA), and benzo(ghi)perylene (BZP). Concentrations less than method detection limits (MDL)

were treated as zero except where specifically noted. Total PAH (TPAH) is the sum of these PAH.

The non-specific $PSCORE_{oil}$ model was developed to explore the possibility that samples might contain petroleum hydrocarbons. Possible scores ranged from 0 to 5, determined as follows. For a maximum of 2.5 points, the score was increased by 0.5 for each of five homologous families present in ANS (N, F, D, P, and C) where the TPAH concentration was > 0. An additional 0.5 was added for each family if more than one N, F, or D homolog was present, or if more than two P or C homologs were present. This latter scoring was based on the presence of homologs in weathered *Exxon Valdez* oil: three N, F, and D homologs were often present, but the proportion of the least substituted homologs was very low. At least three P homologs were well represented in the profile and four C homologs were present. However, detection of chrysenes was often relatively poor because concentrations were usually less than in other homologs, hence the score criterion was for two or more. Determination of successful oil identification by PSCORE and accurate rejection of samples without oil was accomplished empirically with experimental data to determine the score that accomplished both objectives.

The ANS-specific version of PSCORE relied on the observation that X0 < X*n* in ANS, where X represents each of the previously identified homologs (N0 to N4, F0 to F3, D0 to D3, P0 to P4, and C0 to C1). The score was incremented each time the relationship was true, e.g., when N0 < N*i* the score was incremented by 1/*n*, where *n* = the number of homologs (excluding X0) Division by *n* allowed equal weighting of each homologous family. Chrysenes were restricted to comparison of C0 and C1 because more substituted chrysenes were not always detected. Scores ranged from 0 to 5 and were compared directly to PAH diagnostic ratio results. To relate model results to the OFM and FORLM, an empirically determined value was used to indicate the presence or absence of oil as explained above.

The non-parametric pyrogenic detection model was based on the observation that X0 >> X*n* in PAH from pyrogenic sources. Weighed scores within homologs were assigned as follows: if X0 > 10 * X*n* then *s* = 1/*n*, if X0 > 5 * X*n* then *s* = 0.5/*n*, if X0 > 2.5 * X*n* then *s* = 0.25/*n*, if X0 > X*n* then *s* = 0.1/*n* where s = subscore and *n* = the number of homologs considered in the model (excluding X0). Homologs scored were N1 to N4, F1 to F3, D1 to D3, P1 to P4, and C1. The pyrogenic score was the sum of *s*.

The FORLM required the presence of 14 environmentally persistent PAH (N3, N4, F2, F3, D1 to D3, P1 to P4, and C0 to C2) to yield an estimate of *w* and a probability estimate that ANS (or an alternative source, Constantine Harbor) was (or was not) present. Bootstrapped error distributions from experimental and environmental samples provided the basis for testing the null hypothesis that the composition of PAH in a sample was consistent with that of weathered ANS (7). Two model outputs are presented in this paper, the number (or percentage) of estimable samples (the 14 required PAH were present and interpreted as non-specific detection of petrogenic oil) and the number of source-specific ANS detections.

The OFM was implemented as suggested by Bence and Burns (6) for Natural Resource Damage Assessment samples. Detection of ANS required the presence of alkylated-N, D2, and C2. Results were classified as provisional diesel or WSF if C2 was absent. Although the model uses C2/P2 and D2/P2 ratios to further distinguish among possible sources, the authors indicated that all crudes and crude-diesel mixtures identified before these latter tests be classified as ANS in Natural Resource Damage Assessment data (6, 9). Non-specific detection of petroleum hydrocarbons was interpreted as petrogenic detection ($OFM_{oil}$). Specific identification of ANS is described as $OFM_{ANS}$.

Diagnostic PAH ratios considered for comparison to model results included several that increase when the source is petrogenic and several that increase when the source is pyrogenic. Petrogenic models include C/BaA (11), methyl-P/P (e.g., 12), P/ANT (13), the fossil fuel index [Σ(N0..N4) + Σ(D0..D3) + 0.5 * (P0 + P1) + Σ(P2..P4) ) / TPAH] (14), and low molecular weight / high molecular weight PAH [LMW/HMW = (P + ANT + PYR + FLA) / (BaA + C + BbF + BkF + BaP + BeP + PER + IDP + DBA + BZP] (11). Pyrogenic models include the pyrogenic fraction [ACE + ACY + ANT + FLA + PYR + BaA + C0 + BbF + BkF + BeP + BaP + IDP + DBA + BZP) / TPAH] (15), FLA/PYR (13), percent perylene, pyrogenic index [(ACN + ACE + ANT + FLU + PYR + BaA + BbF + BkF + BeP + BaP + PER + IDP + DBA + DBZ) / (Σ(N0..N4) + Σ(F0..F3) + Σ(D0..D3) + Σ(P0..P4) + Σ(C0..C3)] (5), (FLA + PYR) / (P2 + P3) (16), (FLA + PYR) / Σ(P1..P4) (17), and Σ(C1..C4) / C0 (18).

**Model performance in controlled laboratory samples**

PAH composition in water, sediment, and fish tissue exposed to ANS and corresponding controls were drawn from experimental studies completed between 1991 and 2001 (Table 4.1). No chemically dispersed oil samples were included in the data set. Pure ANS samples were also analyzed; these were collected from Prince William Sound and from oil stockpiled for study. Water (saltwater or saltwater-freshwater mixtures) was contaminated by passage through oiled-rock columns (e.g., 2, 19) or by direct oil-water mixing (20). Fish tissues were Pacific herring (*Clupea pallasi*) muscle, ovaries, eggs and larvae, and pink salmon (*Oncorhynchus gorbuscha*) eggs, alevins, whole fry, fry carcasses (sans head and viscera), and fry viscera. All samples were classified either as oiled or control. Model performance in control samples provided a measure of false positives. Performance in oiled groups indicated how successful each model was in identifying the presence of known oil contamination.

**Model performance in environmental samples**

PAH composition in water, sediment, fish, and mussels (*Mytilus trossulus*) from Prince William Sound after the *Exxon Valdez* oil spill were examined. The working hypothesis was that petroleum was most likely present in environmental samples where TPAH concentrations were high and that very low TPAH concentrations indicated no oil. Pink salmon and Pacific herring accounted for most of the fish (76% and 11%, respectively); other species included black prickleback (*Xiphister atropurpureus*), coho salmon (*O. kisutch*), dusky rockfish (*Sebastes ciliatus*), Pacific cod (*Gadus macrocephalus*), tidepool sculpin (*Oligocottus maculosus*), white spotted greenling (*Hexagrammos stelleri*), pricklebacks, and flatfish. Tissues representative of whole fish were analyzed; not included were gonad, gamete, larvae, and gut content samples.

The ability of each model to identify oil in environmental samples was evaluated by logistic regression. Percentages of samples where oil or ANS were detected were calculated over discrete, logarithmically increasing TPAH concentration intervals. In each interval and for each model, percentages of samples with oil were calculated by dividing the number of oiled samples detected by the total number of samples in that interval.

**Diagnostic PAH ratios**

The previously introduced diagnostic ratios were computed for environmental mussel and sediment samples. To provide a model-free summary of trends as a function of TPAH concentration, mean ratios were computed within increasing TPAH concentration groups (0.25 intervals in log space), where TPAH ranged from 0 to about $10^6$ or $10^7$ ng/g dry weight, depending on sample type. Means were smoothed to describe sample trends (4253H; 21).

Initial analysis demonstrated loss of detail for pyrogenic ratios when data were corrected by MDL, thus results of all pyrogenic ratios and models are based on raw data. (All other results are based on MDL-corrected data.)

A summary of the independently determined oil model results was developed to analyze and visualize the relationship between diagnostic ratios and degree of oiling. The combined petrogenic score ($S_e$) included non-specific model results (0 to 3) and ANS-specific results (4 to 6). For example, $S_e = 3$ when all non-specific results were significant and no specific results were significant; $S_e = 5$ for any two significant ANS-specific model results, regardless of non-specific results. This combined score was interpreted as ranging from no oil present (0) through oil highly likely (6). Diagnostic ratios and model scores were analyzed with single-factor ANOVA using these groups; ratios and scores were log-transformed to control variance and permutation was used to assign overall probabilities because distributions were not normal. Model $PSCORE_{ANS}$ was examined in parallel with petrogenic ratios; grouping was similarly determined except to be independent it did not include model $PSCORE_{ANS}$. Means were compared with pairwise contrasts; the Bonferroni inequality ($\alpha$ divided by the number of comparisons) was applied to ensure the probability of incorrect rejection was no more than 0.05 for all comparisons.

The weathering index generated by the FORLM provided a systematic way of exploring the relationship between diagnostic ratios (or scores) and weathering. Ratios (and scores) were regressed against *w* to determine if a relationship existed.

A combined summary score ($S_T$) that included assessment of two pyrogenic and all petrogenic model outcomes provided an unbiased summary of dominant source characteristics. Two pyrogenic scores, $S_y$ and $S_z$ corresponding to (FLA + PYR) / $\Sigma$(P1..P4) and the non-parametric pyrogenic model, ranged from 0 to 3. Pyrogenic scores = 1 for values that included no more than 10% of samples also identified as petrogenic ($S_e > 2$), 2 for 5% and 3 for 1% petrogenic inclusion. $S_T = S_e - (S_y + S_z)$ and ranged from -6 to +6. Values <0 were progressively more likely to be pyrogenic; those >0 were interpreted exactly as $S_e$.

# Results

## Model and ratio performance in controlled laboratory samples

Oil was rarely suggested by any of the models in control samples (Table 4.1a). Scores that distinguished between oiled and control samples were >3.5 for $PSCORE_{oil}$ and >3.0 for $PSCORE_{ANS}$. Some of the false positive control sediment samples identified by $PSCORE_{oil}$ and $OFM_{oil}$ were possibly contaminated: TPAH concentration in a minority of control sediments was > 50 ng/g (3 of 24) and these 3 unusual samples were collected consecutively from a single experiment. Likewise, the TPAH concentration in the single false-positive control tissue identified by both PSCORE and $OFM_{oil}$ was clearly a high outlier. The incidence of false positives in ANS-specific models was 0 to 2%.

All models readily detected oil in experimental samples where composition was about the same as in ANS; detection was more difficult in samples where PAH composition was modified by physical or biological processes (Table 4.1b; Appendix 6.1). All models detected 100% oil in pure ANS samples. Oil was readily detected in sediment; non-specific models detected oil in 76 to 100% of oiled sediment samples and detection was about the same by ANS-specific models. Oil was detected less frequently in water and fish tissue (3 to 88% by non-specific models). The generalized oil-detection models detected more oil in samples where PAH composition was

modified physically or biologically than their ANS-specific counterparts. The FORLM was consistently the most conservative model.

Petrogenic ratios were usually greater in experimentally oiled samples than in control samples; the reverse was true for pyrogenic ratios (Appendix 6.2). Petrogenic ratios in experimentally oiled samples were typically significantly higher than in control samples ($0.001 < P \leq 0.041$). Exceptions were that fossil fuel index values did not differ significantly between oiled and control water ($P = 0.593$) and the LMW/HMW PAH ratio was greater in control sediment. Pyrogenic ratios in experimentally oiled samples were typically significantly lower than in control samples ($0.001 < P \leq 0.031$) except percent perylene was greater in oiled tissue.

**Model and ratio performance in environmental samples**

Detection of oil in water, sediment, fish, and mussels by each model generally increased sigmoidally with TPAH concentration (Fig. 4.1). In general, detection of oil by the models increased from 0% at low TPAH concentrations to 50 to 100% at high TPAH concentrations in every matrix (water, sediment, fish, and mussels; Fig. 4.1). Correlation between TPAH and percent oil detection was strong in water ($0.86 \leq r \leq 0.96$), sediment ($0.87 \leq r \leq 0.96$), and mussels ($0.85 \leq r \leq 0.87$) and moderate in fish ($0.56 \leq r \leq 0.66$) with the exception that ANS was typically not detected by the FORLM in water from Prince William Sound. Detection by non-specific models ($PSCORE_{oil}$ and $OFM_{oil}$) was consistently greater than by ANS-specific models. The FORLM was consistently the most conservative model.

Petrogenic diagnostic ratios (those where values increased with the likelihood of oil) were partially successful in describing environmental samples from Prince William Sound. All of these ratios generally increased with TPAH concentration in mussel and sediment samples (Fig. 4.2). However, relatively few estimates were possible for some ratios (e.g., C0/BaA, P0/ANT) because divisor concentrations were frequently zero. Scatter tended to be high for some ratios (e.g., LMW/HMW and FFPI). Paradoxically, upward trends with increasing TPAH reversed in some cases (P1/P0, P0/ANT, and LMW/HMW in sediment). In comparison to the diagnostic ratios, model scores ($PSCORE_{ANS}$) increased in sigmoid fashion as TPAH concentration increased, clearly describing the increasing probability that ANS was present in mussel and sediment samples. Diagnostic petrogenic ratios typically increased significantly as the probability of oil increased ($0.001 < P_{ANOVA} \leq 0.002$, Appendix 6.3); classification levels are illustrated in color in Fig. 4.2).

The non-parametric petrogenic model ($PSCORE_{ANS}$) provided the best overall discrimination between non-oiled and oiled samples; 93% of oiled mussel and sediment samples were above the concentration exceeded by just 1% of the samples least likely to contain oil (Table 4.2). The ratio C0/BaA also provided good discrimination between non-oiled and oiled mussel samples but this ratio was estimable in a minority of samples (9%).

Pyrogenic hydrocarbons were present in Prince William Sound mussels and sediment at low concentrations. Values of each ratio designed to detect pyrogenic sources generally peaked around 100 ng/g dry weight in mussels and near 10 ng/g in sediment (Fig. 4.3). Scatter was often high. Unlike the case for petrogenic sources, where MDL correction reduced the likelihood of false positives without hampering detection, MDL trimming interfered with pyrogenic detection. For example, percent estimable samples were reduced by as much as 47%; up to 96% of the pyrogenic ratio estimates were zero in MDL-corrected data. For this reason, all pyrogenic analyses were based on data without MDL correction. Even under these optimal detection conditions, the pyrogenic signal typically faded away as TPAH concentration declined below about 100 ng/g (mussels) or 10 ng/g (sediment). Diagnostic pyrogenic ratios decreased

significantly as the probability of oil increased ($P_{ANOVA} < 0.001$, Appendix 6.4); classification levels are illustrated in color in Fig. 4.3).

The non-parametric pyrogenic model provided the best overall discrimination between non-oiled and oiled samples; 67 and 81% of non-oiled mussel and sediment samples (respectively) were above the concentration exceeded by just 1% of the samples most likely to contain oil (the upper three groups determined by combined model analysis; Table 4.3). The ratio (FLA + PYR) / (Σ(P1..P4) also provided reasonable discrimination between samples with pyrogenic and oiled signatures. Although 3 other ratios (the pyrogenic fraction, %PER, and the pyrogenic index) discriminated between pyrogenic and oiled samples in mussels, performance was relatively poor in sediment samples (Table 4.3b).

Few samples had both high pyrogenic and high petrogenic scores. Total PAH concentration in samples with elevated pyrogenic scores (e.g., >1.5) was typically low (median = 75 ng/g in sediment, 136 ng/g in mussels) and typically high in samples with elevated petrogenic scores ($PSCORE_{ANS} > 3$; median = 955 ng/g in sediment, 1713 ng/g in mussels). Overlap between elevated petrogenic and pyrogenic scores was low; TPAH < 200 ng/g in 82% of sediment where pyrogenic scores >1.5 and TPAH > 200 ng/g in 83% of sediment where the petrogenic score > 3. Similarly, TPAH < 200 ng/g in 72% of mussels where pyrogenic scores >1.5 and TPAH > 200 ng/g in 97% of mussels where the petrogenic score > 3.

Diagnostic ratios were generally poorly correlated with weathering (*w*, as determined by the FORLM). This suggests that weathering processes did not play a major role in the patterns evident in Figs. 4.3 and 4.4: $r^2 > 0.25$ only in the pyrogenic fraction (0.31) and FFPI (0.26) in sediment and the pyrogenic fraction (0.33), LMW/HMW (0.40), and P0/ANT (0.30) in mussels.

## Discussion

The novel non-parametric model source identification method presented in this paper typically discriminated among samples better than traditional diagnostic ratios and provided similar, complimentary results to two previously published source identification models. These non-parametric modeling ideas are intended as general guidance; the approach can easily be tailored to fit the specific needs of other petrogenic or pyrogenic sources. The ability to easily match model scores to theoretical composition is a significant advantage and the output reflects the probability that a given source is present. Interpretation is easily accomplished; scores can be interpreted similarly to diagnostic ratios (e.g., Fig. 4.2) or, by choosing a suitable threshold, individual samples can be classified (e.g., oiled or non-oiled, Fig. 4.1). By varying the scoring rules, less and more rigorous models are possible, as demonstrated by non-specific and ANS-specific versions of PSCORE. Detection of oil by the non-specific version was about the same as the non-specific version of the OFM and was more sensitive than the ANS-specific models. All non-specific models were more likely to detect oil in matrices where composition was altered by physical or biological processes than their corresponding ANS-specific counterparts. The ANS-specific models were more conservative and less likely to erroneously suggest oil in samples where it was not present.

Relatively few petrogenic diagnostic ratios tested discriminated between *Exxon Valdez* oil and other sources (11-14, 18). Just 3 of 12 petrogenic ratios adequately discriminated between non-oiled and oiled mussel and sediment samples where "adequate" is defined as detection of ≥80% of the oiled samples and that no more than 10% of non-oiled samples were above the threshold value. Diagnostic ratios were frequently not estimable, e.g., < 10% of the non-oiled group could be estimated in mussels for any ratio because the divisor was zero. The

variable success of diagnostic ratios has been noted previously and varies among different environmental situations (22). In sharp contrast to the diagnostic ratios, non-parametric score discrimination between non-oiled and oiled samples was good, 93% detection with just 1% of the non-oiled samples falsely labeled as oiled.

Only one of the pyrogenic diagnostic ratios adequately discriminated between *Exxon Valdez* oil and the pyrogenic background in Prince William Sound (5, 13, 15-17). Where no more than 10% of oiled samples were above the threshold value, 79 to 80% of the non-oiled mussel and sediment samples were identified as pyrogenic by the ratio (FLA + PYR) / Σ(P1..P4). No other ratios passed this test.

Score discrimination by the non-parametric pyrogenic model in sediment was considerably better than by diagnostic ratios, 81% detection with just 1% of the oiled samples falsely labeled as pyrogenic. Non-parametric model discrimination in mussels was similar to the (FLA + PYR) / Σ(P1..P4) ratio. Although the values used to parameterize the pyrogenic model were arbitrary, discrimination was highly similar in trials with other coefficients, all requiring that X0 >> X*n* (data not illustrated). However, discrimination declined as the requirement that X0 be much greater than X*n* was relaxed and discrimination was poorer in a trial that simply required that X0 > X*n* (80% detection with 10% false positives in sediment). Discrimination was inadequate for a non-parametric pyrogenic model that required X0 > X1 > X2 > … X*n* (22% detection with 10% false positives; data not illustrated).

Detection failure at low concentrations and the masking of pyrogenic signals by oil at higher concentrations are the most likely reasons that pyrogenic hydrocarbon concentrations clustered between about 10 and 100 ng/g. *Exxon Valdez* oil apparently masked the pyrogenic signature at TPAH concentrations >100 ng/g, an inference consistent with Page et al. (17). Detection, hence ability to determine sources, consistently faded away for ratios and models as TPAH concentrations approached zero, even when raw data were examined.

All models provided strong evidence of oil in Prince William Sound sediment and mussels and weaker evidence of oil in matrices where physical or biological processes caused changes in PAH composition. Division of model outputs into non-specific and ANS-specific components provided an advantage; non-specific models were more likely to adequately detect petroleum in tissues where the PAH composition was significantly modified by differential uptake or metabolic activity (e.g., water and fish tissue). The non-specific models also had distinctly lower detection thresholds than the ANS-specific models. The FORLM (7) was consistently the most conservative of all models, including the non-specific version as defined in this paper. The non-specific FORLM only requires that all 14 persistent petroleum PAH be present (> MDL); even this simple requirement was conservative. Oil was detected in difficult matrices by the non-specific version of the non-parametric model because of its flexibility. In water and fish tissue the four homologous PAH families likely to be present were naphthalenes through phenanthrenes; chrysenes were often absent, even though present in the source oil. In weathered oil, sediment, and mussels, the four families most likely present were fluorenes through chrysenes; naphthalenes were absent in substantively weathered samples. Thus, the empirically determined decision point for acceptance or rejection of the presence of oil by $PSCORE_{oil}$ was consistent with expected hydrocarbon distributions in a variety of matrices and weathering situations.

Detection of oil differed among the models, suggesting one reasonable approach is to consider all models simultaneously when interpreting data. This may provide a more level assessment than is possible with any single approach and may be particularly useful in situations where interpretation is contentious (as was the case after the *Exxon Valdez* oil spill). The

combined score demonstrated in this paper was greatest when all three ANS-specific models detected oil and zero where oil was never detected. The veracity of this approach was evident when diagnostic ratios were classified by the combined model score Appendix 6.3 and 6.4); no patterns would have emerged if ratio and combined model information were unrelated. Furthermore, the mean combined model score increased sigmoidally with TPAH concentration just as for each individual model (data not illustrated), consistent with the hypothesis that the presence of oil in the environmental samples was increasingly likely as concentration increased.

The combined model approach provides insight for a wide range of hydrocarbon sources, including several petrogenic sources, one pyrogenic source (creosote), and one mixed source (soot; Appendix 6.5). Non-specific models are most able to detect xenobiotic hydrocarbons at low concentrations but generally discriminate poorly among petrogenic and pyrogenic sources. The $OFM_{ANS}$ and $PSCORE_{ANS}$ are more discriminatory and good detectors of petrogenic sources in general. $PSCORE_{ANS}$ tends to be a better discriminator than $OFM_{ANS}$, particularly when distinguishing between petrogenic and pyrogenic sources. The $FORLM_{ANS}$ is the best discriminator but generally requires higher total PAH concentrations to function. Creosote merits further attention as data become available; the OFM and $PSCORE_{oil}$ identified pure samples as oiled, placing $S_e$ in conflict with $S_y + S_z$. Conflicts of this magnitude were rare in PWS (<0.3% in 3412 samples). Possibly the unusual creosote result occurred because creosote is generally blended with other products before use. The summary scores provide an easily computed first look at hydrocarbon sources in a variety of samples and individual model outputs help with interpretive detail. Further discrimination among sources can likely be improved by specifically tuning each model.

The novel non-parametric modeling approaches described here discriminated between oiled and non-oiled or pyrogenic and oiled sources better than traditionally used diagnostic ratios and performed similarly to two previously published models. Such non-parametric models can easily be written in spreadsheets and tailored to recognize specific PAH sources. Combined analysis of PAH composition by multiple models provides more information upon which to base difficult detection and oil-source discrimination decisions than is possible with any single model. In particular, the multiple-model approach may be particularly useful in situations where interpretation is contentious (as was the case after the *Exxon Valdez* oil spill). Oil identification was clearly difficult where composition was modified by physical or biological processes. Other factors, such as proximity to known sources of contamination, currents, and timing, should not be overlooked as important additional clues regarding hydrocarbon sources. Other compounds, e.g., alkanes and hydrocarbon biomarkers (e.g., oleanane), provide additional source clues (e.g., 29). The ultimate goal is to provide an informed, holistic assessment of hydrocarbon sources in the environment. The non-parametric modeling approach adds another useful tool to the assessment procedure.

## References


(1) NRC. Oil in the Sea III: inputs, fates, and effects. National Research Council (U.S.), Committee on Oil in the Sea: Inputs Fates, and Effects. National Academy of Sciences, Washington, DC. 2003, 280 pp.

(2) Heintz, R.; Short, J. W.; Rice, S. D. Sensitivity of fish embryos to weathered crude oil: Part II. Increased mortality of pink salmon (*Oncorhynchus gorbuscha*) embryos incubating downstream from weathered *Exxon Valdez* crude oil. Env. Toxicol. Chem.

1999, 18, 494-503.
(3) Sundberg, H.; Ishaq, R.; Akerman, G.; Tjarnlund, U.; Zebuhr, Y.; Linderoth, M.; Broman, D.; Balk, L. A bio-effect directed fractionation study for toxicological and chemical characterization of organic compounds in bottom sediment. Toxicological Sciences 2005, 84, 63-72.
(4) Incardona, J. P.; Carls, M. G.; Teraoka, H.; Sloan, C. A.; Collier, T. K.; Scholz, N. L. Aryl hydrocarbon receptor-independent toxicity of weathered crude oil during fish development. Environmental Health Perspectives in press.
(5) Wang, Z.; Fingas, M. F. Development of oil hydrocarbon fingerprinting and identification techniques. Mar. Pollut. Bull. 2003, 47, 423-452.
(6) Bence, A. E.; Burns, W. A. Fingerprinting hydrocarbons in the biological resources of the Exxon Valdez spill area. Pages 84-140 *in* P.G. Wells, J.N. Butler, and J.S. Hughes (eds.), *Exxon Valdez* oil spill: fate and effects in Alaskan Waters, ASTM STP 1219, American Society for Testing and Materials, Philadelphia, 1995.
(7) Short, J.; Heintz, R. Identification of *Exxon Valdez* oil in sediments and tissues of PWS. Environ. Sci. Technol. 1997, 31, 2375-2384.
(8) Short, J. W. Oil identification based on a goodness-of-fit metric applied to hydrocarbon analysis results. Environ Forensics 2002, 3, 349-355.
(9) Short, J. W.; Heintz, R. A.; Nelson, B. D.; Maselko, J. M.; Kendziorek, M. F.; Carls, M. G.; Korn, S. *Exxon Valdez* oil spill of 1989: State/Federal Trustee Council hydrocarbon database 1989-1995. National Oceanic and Atmospheric Administration, National Marine Fisheries Service, Auke Bay, AK. 1996.
(10) Short, J. W.; Jackson, T. J.; Larsen, M. L.; Wade, T. L. Analytical methods used for the analyses of hydrocarbons in crude oil, tissues, sediments, and seawater collected for the natural resource damage assessment of the *Exxon Valdez* oil spill. Am. Fish. Soc. Symp. 1996, 18, 140-148.
(11) Socolo, H. H.; Garrigues, P. H.; Ewald, M. Origin of polycyclic aromatic hydrocarbons (PAHs) in coastal marine sediments: case studies in Cotonou (Benin) and Aquitaine (France) areas. Mar. Pollut. Bull. 2000, 40, 387-396.
(12) Zakaria, M. P.; Takada, H.; Tsutsumi, S.; Ohno, K.; Yamada, J.; Kouno, E.; Kumata, H. Distribution of polycyclic aromatic hydrocarbons (PAHs) in rivers and estuaries in Malaysia: a widespread input of petrogenic PAHs. Envion. Sci. Technol. 2002, 36, 1907-1918.
(13) Doong, R.; Lin, Y. Characterization and distribution of polycyclic aromatic hydrocarbon contaminations in surface sediment and water from Gao-ping River, Taiwan. Wat. Res. 2004, 38, 1733-1744.
(14) Boehm, P. D.; Farrington, J. W. Aspects of the polycyclic aromatic hydrocarbon geochemistry of recent sediments in the Georges bank region. Environ. Sci. Technol. 1984, 18, 840-845.
(15) Boehm, P. D.; Page, D. S.; Brown, J. S.; Neff, J. M.; Burns, W. A. Polycyclic aromatic hydrocarbon levels in mussels from Prince William Sound, Alaska, USA, document the return to baseline conditions. Environ. Toxicol. Chem. 2004, 23, 2916-2929.
(16) Savinov, V. M.; Savinov, T. N.; Matishov, G. G.; Dahle, S.; Næs, K. Polycyclic aromatic hydrocarbons (PAHs) and organochlorines (OCs) in bottom sediments of the Guba Pechenga, Barents Sea, Russia. Sci. Total Environ. 2003, 306, 39-56.
(17) Page, D. S.; Boehm, P. D.; Brown, J. S.; Neff, J. M.; Burns, W. A.; Bence, A. E. Mussels document loss of bioavailable polycyclic aromatic hydrocarbons and the return

to baseline conditions for oiled shorelines in Prince William Sound, Alaska. Mar. Environ. Res. 2005, 60, 422-436.
(18) Neff, J. M.; Stout, S. A.; Gunster, D. G. Ecological risk assessment of polycyclic aromatic hydrocarbons in sediments: identifying sources and ecological hazard. Integrated Environmental Assesssment and Management 2005, 1, 22-33.
(19) Carls, M. G.; Rice, S. D.; Hose, J. E. Sensitivity of fish embryos to weathered crude oil: Part 1. Low level exposure during incubation causes malformations and genetic damage in larval Pacific herring (*Clupea pallasi*). Environ. Toxicol. Chem. 1999, 18, 481-493.
(20) Barron, M. G.; Carls, M. G.; Short, J. W.; Rice, S. D. Photoenhanced Toxicity of Aqueous Phase and Chemically-Dispersed Weathered Alaska North Slope Crude Oil to Pacific Herring Eggs and Larvae. Environ. Toxicol. Chem. 2003, 22, 650-660.
(21) Velleman, P. F.; Hoaglin, D. C. Applications, basics and computing of exploratory data analysis. Duxbury Press, Boston, Massachusetts. 1981.
(22) Stout, S. A; Magar, V. S.; Uhler, R. M.; Ickes, J.; Abbott, J.; Brenner, R. Characterization of naturally-occurring and anthropogenic PAHs in urban sediments – Wycoff/Eagle Harbor superfund site. Environ. Forensics 2001, 2, 287-300.
(23) Colavecchia, M. V.; Backus, S. M.; Hodson, P. V.; Parrott, J. L. Toxicity of oil sands to early life stages of fathead minnows (Pimephales promelas). Environ. Toxicol. Chem. 2004, 23, 1709-1718.
(24) Lindeberg, M. R. unpublished data.
(25) Wang, Z.; Fingas, M.; Landrian, L.; Sigouin, L. Source identification and tracking of an unknown spilled oil from Quebec (1998) by unique biomarkers and diagnostic ratios of "source-specific marker" compounds. In: Proceedings of the Twenty second Arctic and Marine Oil Spill Program (AMOP) Technical Seminar 1999. Ottawa, ON, Environment Canada.
(26) Carls, M. G. unpublished data
(27) Neff, J. M. Bioaccumulation in marine organisms. Effect of contaminants from oil well produced water. Elsevier, Boston. 2002, 452 pp.
(29) Short, J. W.; Lindeberg, M. R.; Harris, P. M.; Maselko, J. M.; Pella, J. J.; Rice, S. D. Estimate of oil persisting on the beaches of Prince William Sound 12 years after the *Exxon Valdez* oil spill. Environ. Sci. Technol. 2004, 38, 19-25.

**Table 4.1.** Detection of petroleum hydrocarbons in (a) laboratory controls and (b) samples experimentally oiled with Alaska North Slope crude oil (ANS) by three models, a PAH composition score model (PSCORE), a PAH oil-fingerprint model (OFM), and a PAH first-order loss-rate model (FOLRM). Matrices examined included pure oil, water, sediment and fish tissue (Pacific herring and pink salmon). Mean (±SE) total polynuclear aromatic hydrocarbon (TPAH) concentrations are listed for each matrix (µg/L in water, ng/g dry weight in other matrices). Percent oil detected by PSCORE is reported for score > 3.5; percent detected by pS2 is where score > 3.0. The divisor for all percentage calculations is $n_{total}$, except for percent overlap. The number of samples consistent with Constantine Harbor PAH (Const) or Katalla oil (Kat) were divided by the number of samples with ANS to determine percent overlap (FORLM model only).

a. Laboratory controls

| | TPAH | | | PSCORE | | OFM | | FOLRM | |
|---|---|---|---|---|---|---|---|---|---|
| **Matrix** | **mean** | **se** | **$n_{total}$** | **% oil** | **% ANS** | **% oil** | **% ANS** | **% oil** | **% ANS** |
| water | 0.04 | 0.01 | 27 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| sediment | 27.6 | 13 | 24 | 8.3 | 0.0 | 29.2 | 0.0 | 0.0 | 0.0 |
| fish tissue | 118.4 | 31 | 60 | 1.7 | 1.7 | 1.7 | 0.0 | 0.0 | 0.0 |

b. Experimentally oiled with ANS

| | TPAH | | | PSCORE | | OFM | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Matrix** | **TPAH** | **se** | **$n_{total}$** | **% oil** | **% ANS** | **% oil** | **% ANS** | **% oil** | **% ANS** |
| pure oil | 8283364 | 2414037 | 11 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 |
| water | 12 | 3 | 104 | 88.5 | 57.7 | 94.2 | 27.9 | 27.9 | 23.1 |
| sediment | 22806 | 5527 | 95 | 93.7 | 93.7 | 100.0 | 97.9 | 75.8 | 75.8 |
| fish tissue | 26595 | 3389 | 174 | 88.5 | 13.2 | 73.0 | 6.3 | 3.4 | 2.9 |

**Table 4.2.** Petrogenic ratio or model thresholds in mussels (a) and sediment (b); FFPI = fossil fuel index. Non-oiled samples are those where combined petrogenic model score = 0, i.e., no evidence of petroleum (pS2 results were not included in the combined score when testing thresholds for this model). Oiled samples are defined as those where the combined petrogenic model score > 2, i.e., reasonable to strong evidence of oil. Three potential threshold (thresh) levels were examined as possible, those where 1, 5, or 10% of the non-oiled samples exceeded the threshold value. Corresponding oiled percentages above these thresholds is evidence of how well (or poorly) the ratio or model can identify samples with petrogenic PAH.

a) Mussels

| | | Non-oiled samples | | | Oiled samples | | |
|---|---|---|---|---|---|---|---|
| ratio or model | thresh | n Total | n > thresh | percent | n Total | n > thresh | percent |
| P1/P0 | 0.85 | 84 | 8 | 10 | 416 | 392 | 94.2 |
| | 2.05 | 84 | 4 | 5 | 416 | 351 | 84.4 |
| | 6.35 | 84 | 1 | 1 | 416 | 164 | 39.4 |
| P/ANT | 1.30 | 5 | 1 | 20 | 60 | 48 | 80.0 |
| | 1.40 | 5 | 0 | 0 | 60 | 46 | 76.7 |
| FFPI | 0.99 | 549 | 123 | 22 | 838 | 0 | 0.0 |
| C0/BaA | 1.31 | 14 | 2 | 14 | 149 | 143 | 96.0 |
| | 1.36 | 14 | 1 | 7 | 149 | 143 | 96.0 |
| | 1.37 | 14 | 0 | 0 | 149 | 143 | 96.0 |
| LMW/HMW | 0.83 | 82 | 8 | 10 | 735 | 108 | 14.7 |
| | 1.73 | 82 | 4 | 5 | 735 | 22 | 3.0 |
| | 4.51 | 82 | 1 | 1 | 735 | 0 | 0.0 |
| Σ(C1..C4) / C0 | 1.83 | 57 | 6 | 10 | 725 | 535 | 73.8 |
| | 4.03 | 57 | 3 | 5 | 725 | 193 | 26.6 |
| | 7.07 | 57 | 1 | 2 | 725 | 22 | 3.0 |
| $PSCORE_{ANS}$ | 1.17 | 880 | 87 | 10 | 783 | 775 | 99.0 |
| | 1.58 | 880 | 45 | 5 | 783 | 766 | 97.8 |
| | 2.17 | 880 | 8 | 1 | 783 | 724 | 92.5 |

b) Sediment

| | | Non-oiled samples | | | Oiled samples | | |
|---|---|---|---|---|---|---|---|
| ratio or model | thresh | n Total | n > thresh | percent | n Total | n > thresh | percent |
| P1/P0 | 1.88 | 400 | 40 | 10 | 614 | 388 | 63.2 |
| | 2.26 | 400 | 20 | 5 | 614 | 353 | 57.5 |
| | 2.86 | 400 | 4 | 1 | 614 | 289 | 47.1 |
| P/ANT | 17.01 | 4 | 1 | 25 | 87 | 23 | 26.4 |
| | 17.02 | 4 | 0 | 0 | 87 | 23 | 26.4 |
| FFPI | 0.97 | 573 | 57 | 10 | 719 | 0 | 0.0 |
| C0/BaA | 4.70 | 107 | 11 | 10 | 363 | 252 | 69.4 |
| | 8.80 | 107 | 5 | 5 | 363 | 174 | 47.9 |
| | 27.50 | 107 | 1 | 1 | 363 | 23 | 6.3 |
| LMW/HMW | 3.19 | 443 | 44 | 10 | 706 | 96 | 13.6 |
| | 6.05 | 443 | 22 | 5 | 706 | 60 | 8.5 |
| | 17.79 | 443 | 4 | 1 | 706 | 27 | 3.8 |
| Σ(C1..C4) / C0 | 0.93 | 246 | 25 | 10 | 684 | 620 | 90.6 |
| | 3.82 | 246 | 12 | 5 | 684 | 376 | 55.0 |
| | 8.43 | 246 | 2 | 1 | 684 | 31 | 4.5 |
| $PSCORE_{ANS}$ | 1.42 | 602 | 54 | 9 | 656 | 649 | 98.9 |
| | 1.75 | 602 | 28 | 5 | 656 | 645 | 98.3 |
| | 2.42 | 602 | 5 | 1 | 656 | 610 | 93.0 |

**Table 4.3.** Pyrogenic ratio or model thresholds in mussels (a) and sediment (b). Non-oiled samples are those where combined petrogenic model score = 0, i.e., no evidence of petroleum. Oiled samples are defined as those where the combined petrogenic model score > 2, i.e., reasonable to strong evidence of oil. Three potential threshold (thresh) levels were examined, those where 1, 5, or 10% of the oiled samples exceeded the threshold value. Corresponding non-oiled percentages above these thresholds is evidence of how well (or poorly) the ratio or model can identify samples with pyrogenic PAH.

a) Mussels

| | | Non-oiled samples | | | Oiled samples | | |
|---|---|---|---|---|---|---|---|
| ratio or model | thresh | n Total | n > thresh | percent | n Total | n > thresh | percent |
| Non-parametric Pyrogenic Model | 1.27 | 569 | 471 | 82.8 | 1093 | 111 | 10 |
| | 1.70 | 569 | 428 | 75.2 | 1093 | 54 | 5 |
| | 2.13 | 569 | 380 | 66.8 | 1093 | 11 | 1 |
| FLA / PYR | 1.63 | 362 | 40 | 11.0 | 656 | 66 | 10 |
| | 2.16 | 362 | 21 | 5.8 | 656 | 33 | 5 |
| | 3.26 | 362 | 5 | 1.4 | 656 | 7 | 1 |
| (FLA + PYR) / Σ(P1..P4) | 0.11 | 437 | 344 | 78.7 | 1089 | 110 | 10 |
| | 0.21 | 437 | 341 | 78.0 | 1089 | 54 | 5 |
| | 0.89 | 437 | 299 | 68.4 | 1089 | 11 | 1 |
| (FLA + PYR) / (P2 + P3) | 0.15 | 85 | 16 | 18.8 | 1084 | 107 | 10 |
| | 0.29 | 85 | 13 | 15.3 | 1084 | 54 | 5 |
| | 2.00 | 85 | 4 | 4.7 | 1084 | 11 | 1 |
| Pyrogenic fraction | 0.10 | 566 | 366 | 64.7 | 1093 | 109 | 10 |
| | 0.14 | 566 | 341 | 60.2 | 1093 | 55 | 5 |
| | 0.21 | 566 | 237 | 41.9 | 1093 | 11 | 1 |
| Percent Perylene | 0.21 | 566 | 332 | 58.7 | 1093 | 109 | 10 |
| | 0.63 | 566 | 289 | 51.1 | 1093 | 55 | 5 |
| | 3.46 | 566 | 88 | 15.5 | 1093 | 11 | 1 |
| Pyrogenic Index | 0.07 | 565 | 390 | 69.0 | 1093 | 109 | 10 |
| | 0.11 | 565 | 360 | 63.7 | 1093 | 55 | 5 |
| | 0.24 | 565 | 266 | 47.1 | 1093 | 11 | 1 |

b) Sediment

| | | Non-oiled samples | | | Oiled samples | | |
|---|---|---|---|---|---|---|---|
| ratio or model | thresh | n Total | n > thresh | percent | N Total | n > thresh | percent |
| Non-parametric Pyrogenic Model | 1.11 | 298 | 257 | 86.2 | 933 | 93 | 10 |
| | 1.50 | 298 | 247 | 82.9 | 933 | 46 | 5 |
| | 1.93 | 298 | 242 | 81.2 | 933 | 9 | 1 |
| FLA / PYR | 1.36 | 250 | 47 | 18.8 | 830 | 83 | 10 |
| | 1.56 | 250 | 28 | 11.2 | 830 | 42 | 5 |
| | 2.04 | 250 | 13 | 5.2 | 830 | 8 | 1 |
| (FLA + PYR) / Σ(P1..P4) | 0.37 | 260 | 229 | 88.1 | 933 | 93 | 10 |
| | 0.62 | 260 | 212 | 81.5 | 933 | 47 | 5 |
| | 1.63 | 260 | 151 | 58.1 | 933 | 9 | 1 |
| (FLA + PYR) / (P2 + P3) | 0.91 | 47 | 17 | 36.2 | 933 | 93 | 10 |
| | 1.45 | 47 | 14 | 29.8 | 933 | 47 | 5 |
| | 2.94 | 47 | 12 | 25.5 | 933 | 9 | 1 |
| Pyrogenic Fraction | 0.24 | 297 | 98 | 33.0 | 933 | 93 | 10 |
| | 0.32 | 297 | 72 | 24.2 | 933 | 47 | 5 |
| | 0.50 | 297 | 27 | 9.1 | 933 | 9 | 1 |
| Percent Perylene | 13.11 | 297 | 91 | 30.6 | 933 | 93 | 10 |
| | 23.79 | 297 | 59 | 19.9 | 933 | 47 | 5 |
| | 44.50 | 297 | 18 | 6.1 | 933 | 9 | 1 |
| Pyrogenic Index | 0.52 | 296 | 116 | 39.2 | 933 | 93 | 10 |
| | 0.92 | 296 | 80 | 27.0 | 933 | 47 | 5 |
| | 1.75 | 296 | 28 | 9.5 | 933 | 9 | 1 |

**Fig. 4.1.** Detection of oil in water, fish tissue, sediment, and mussels from Prince William Sound by non-specific models $PSCORE_{oil}$ (P1), and the $OFM_{oil}$ (B1), and ANS-specific models $PSCORE_{ANS}$ (P2), $OFM_{ANS}$ (B2) and the $FORLM_{ANS}$ (F). Not illustrated are the non-specific $FORLM_{oil}$ results, which were highly similar to the $FORLM_{ANS}$ results. Plotted curves are logistic regressions.

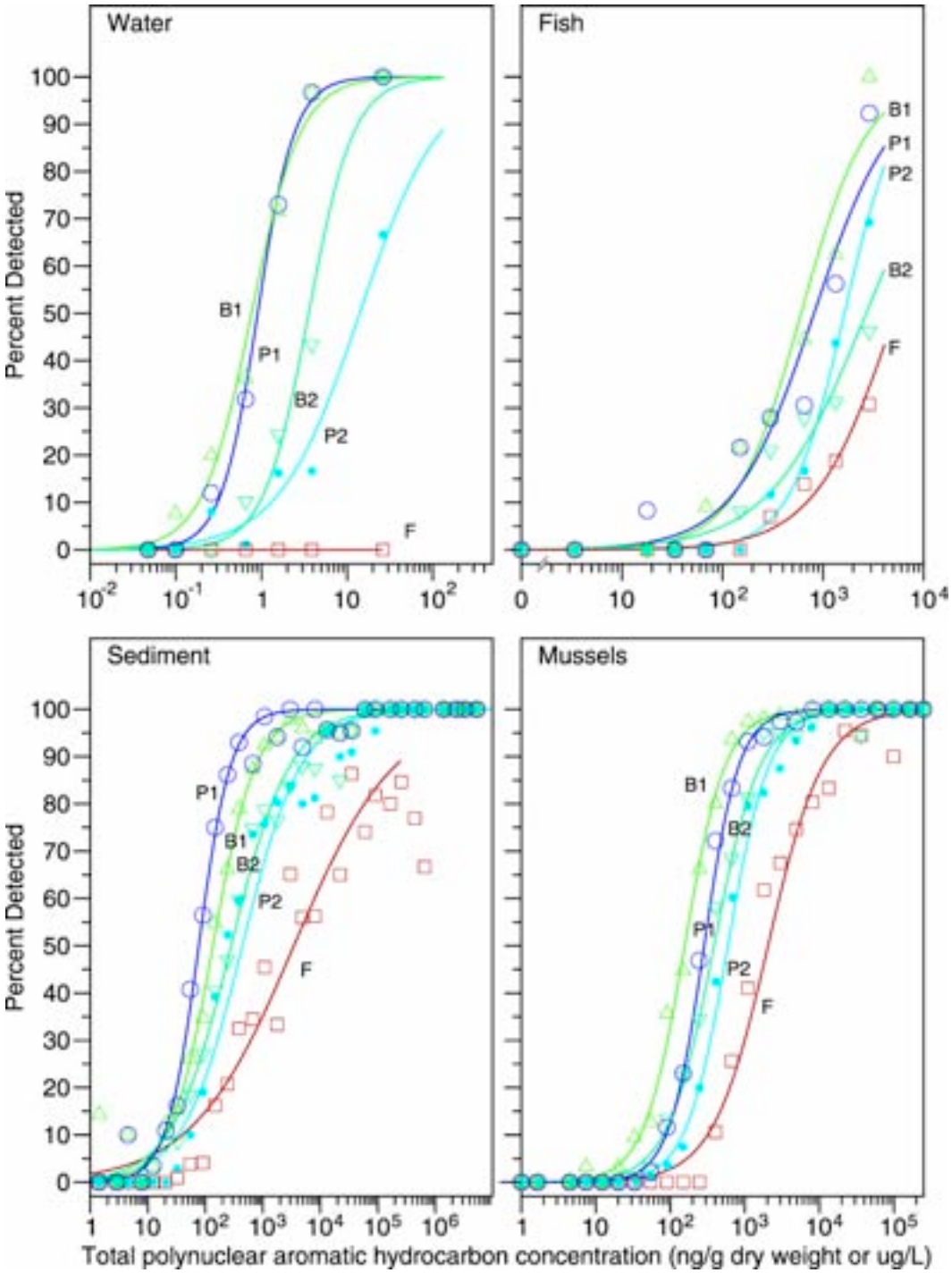


**Fig. 4.2.** Relationship between TPAH and petrogenic ratios and scores in mussel and sediment samples collected in Prince William Sound after the *Exxon Valdez* oil spill. Curves are smoothed diagnostic mean ratios, grouped by increasing TPAH concentration (0.25 intervals in log space). Oil classification (see color key) is based on combined model results. Horizontal lines are 10% threshold levels.

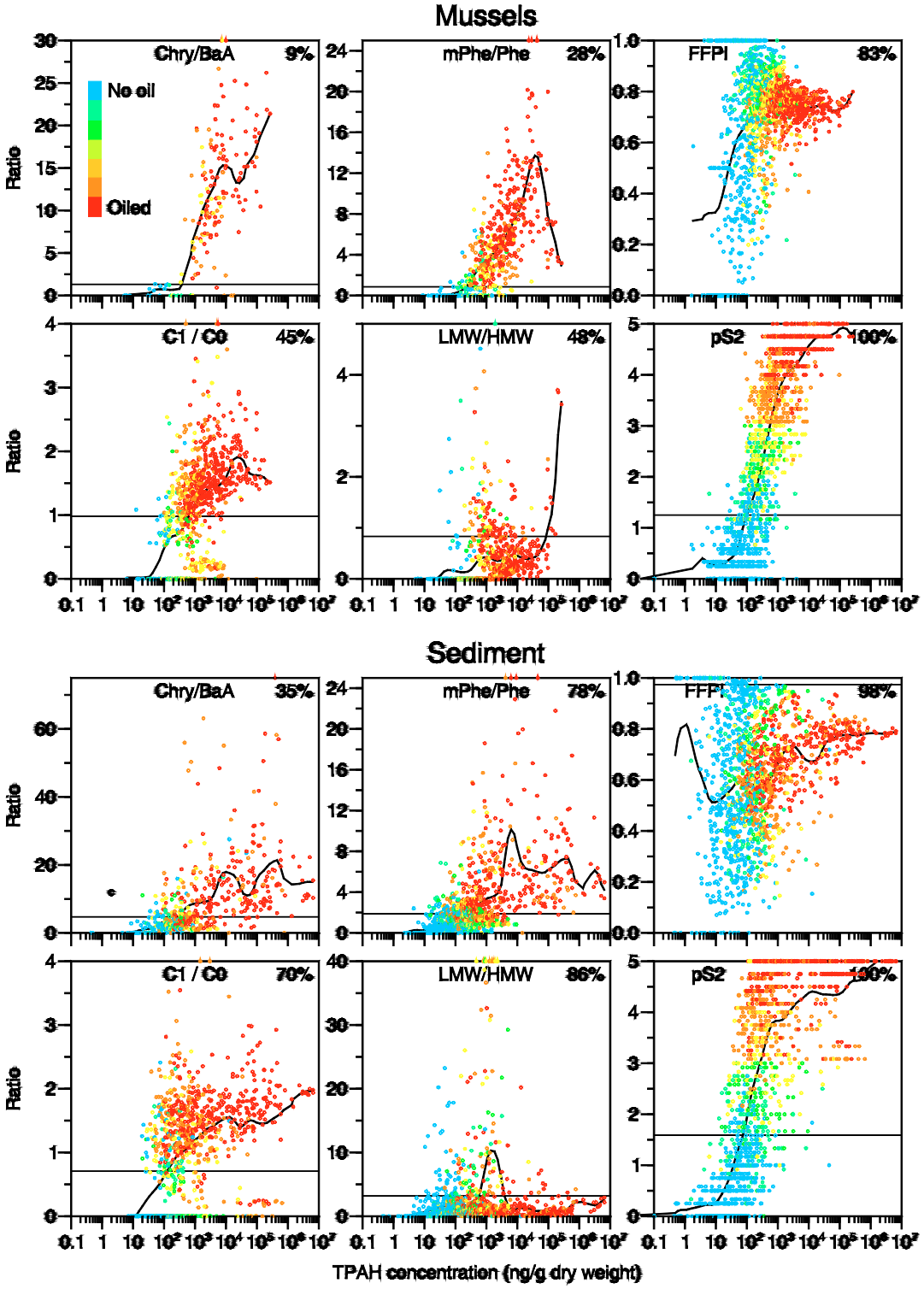
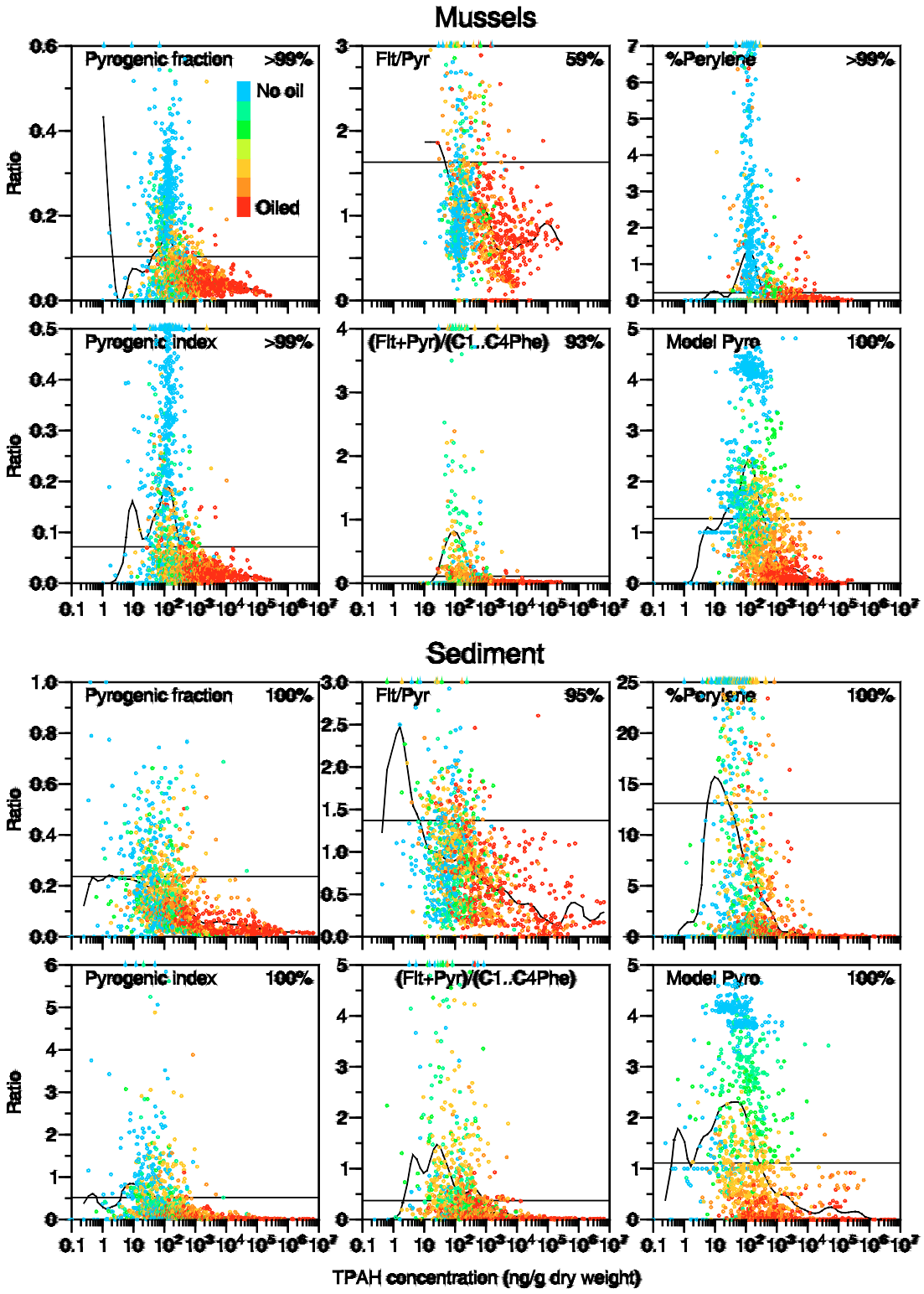


**Fig. 4.3.** Relationship between TPAH and pyrogenic ratios and scores in mussel and sediment samples collected in Prince William Sound after the *Exxon Valdez* oil spill. Curves are smoothed diagnostic mean ratios, grouped by increasing TPAH concentration (0.25 intervals in log space). Oil classification (see color key) is based on combined model results. Horizontal lines are 10% threshold levels.



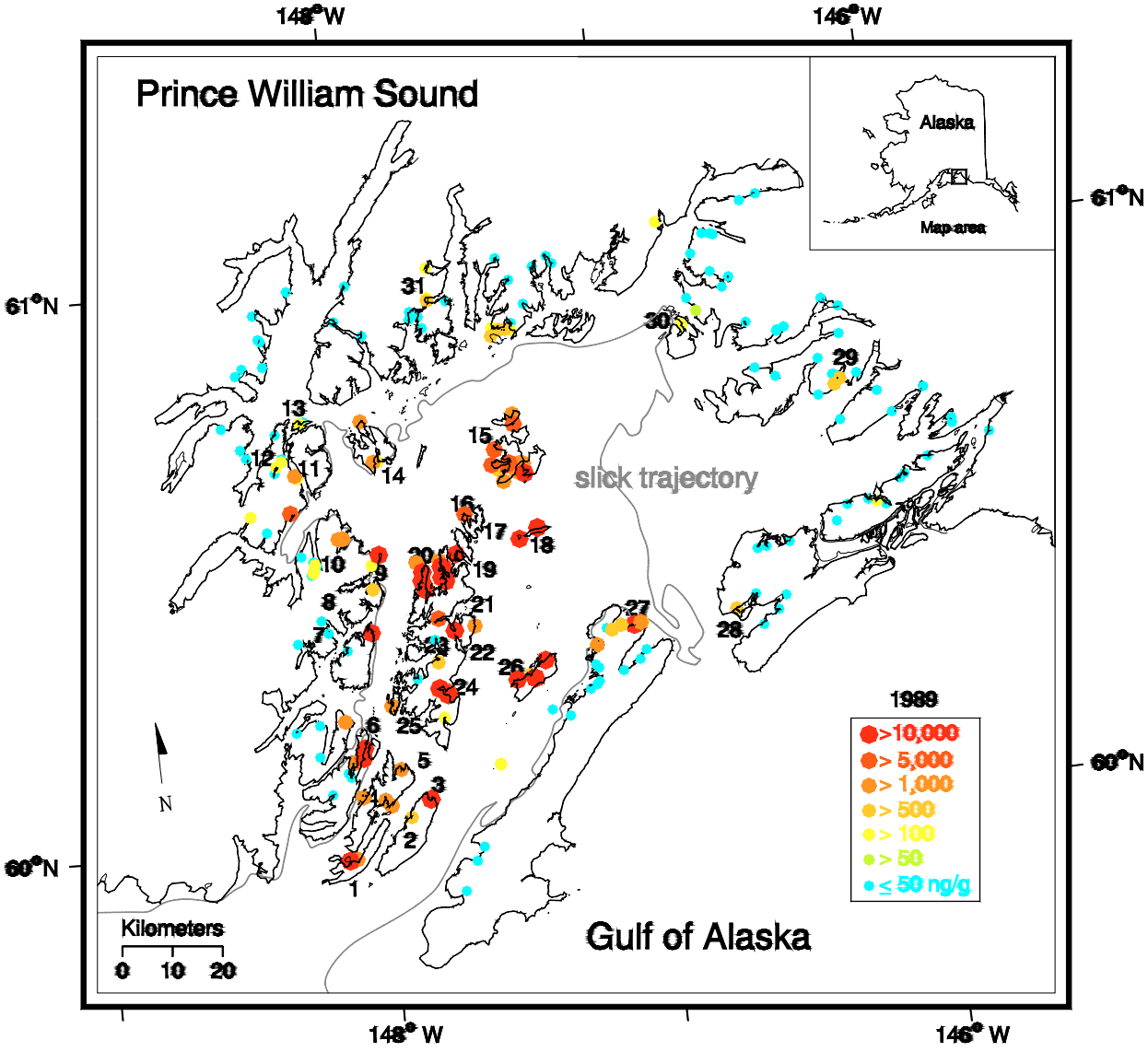
# Appendix 1

Geographic distribution of hydrocarbons in mussels

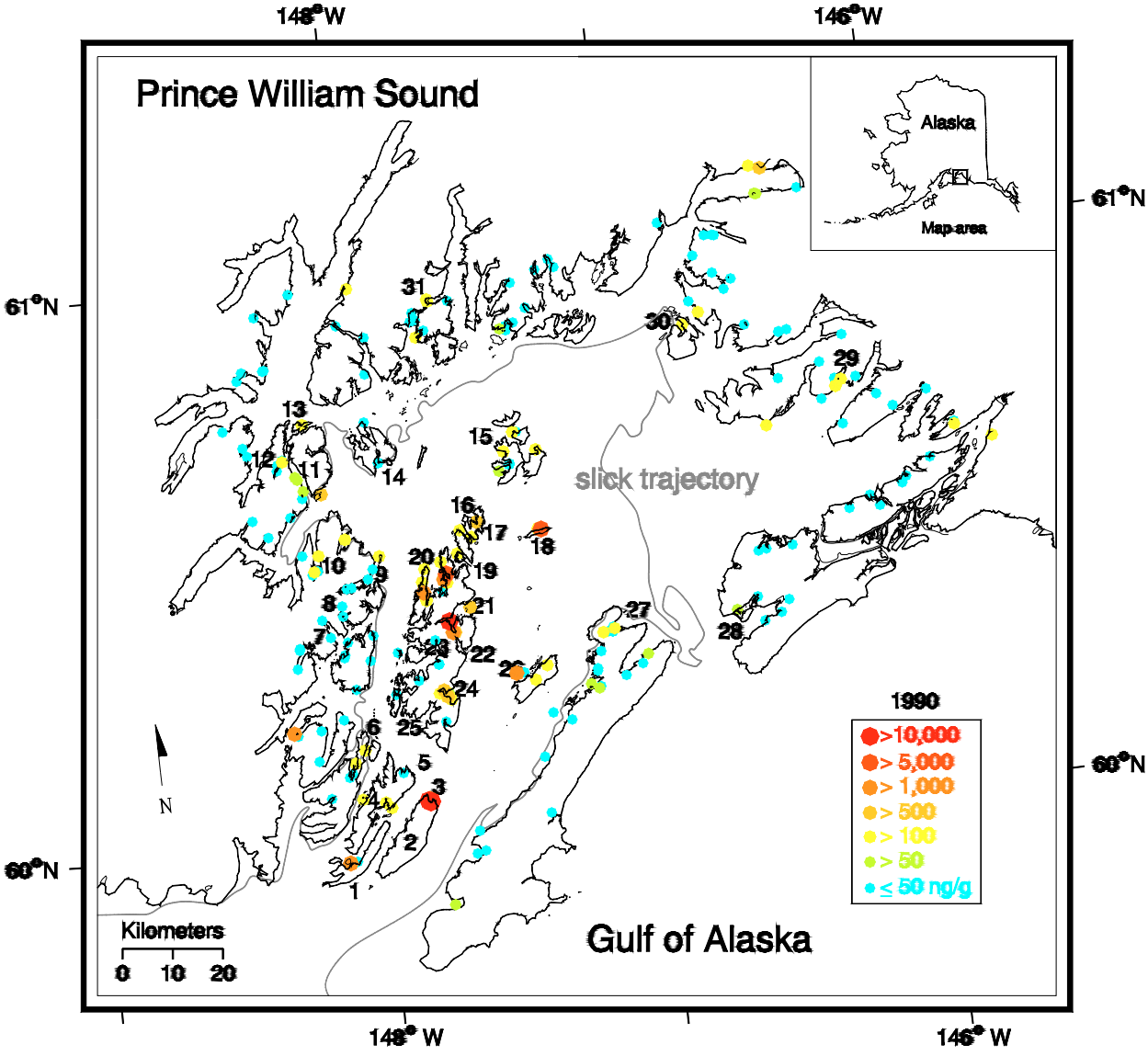
Geographic distribution of TPAH concentrations in mussel tissue. All individual observations are included, each color-coded and sized according to concentration. The slick trajectory outlines the known extent of the *Exxon Valdez* oil slick (Gundlach et al. 1990). Location numbers are listed below.

| **Location** | **Map Number** |
|---|---|
| Elrington Island | 1 |
| Latouche Island, except Sleepy Bay | 2 |
| Sleepy Bay | 3 |
| Crab Bay | 4 |
| Evans Island (northern end) | 5 |
| Prince of Wales Passage | 6 |
| Ewan Bay | 7 |
| Paddy Bay | 8 |
| Eshamy Bay | 9 |
| McClure Bay | 10 |
| Culross Passage | 11 |
| Long Bay | 12 |
| Wells Passage | 13 |
| Perry Island | 14 |
| Naked Island group | 15 |
| Northwest Bay | 16 |
| Eleanor Island | 17 |
| Smith Island | 18 |
| Disk Island | 19 |
| Herring Bay and Herring Point | 20 |
| Bay of Isles | 21 |
| Marsha Bay | 22 |
| Barnes Cove | 23 |
| Snug Harbor | 24 |
| Mummy Bay | 25 |
| Green Island | 26 |
| Rocky Bay and Rocky Point | 27 |
| Constantine Harbor | 28 |
| Olsen Bay | 29 |
| Bligh Island | 30 |
| Siwash Bay | 31 |
| Simpson Bay | 32 |

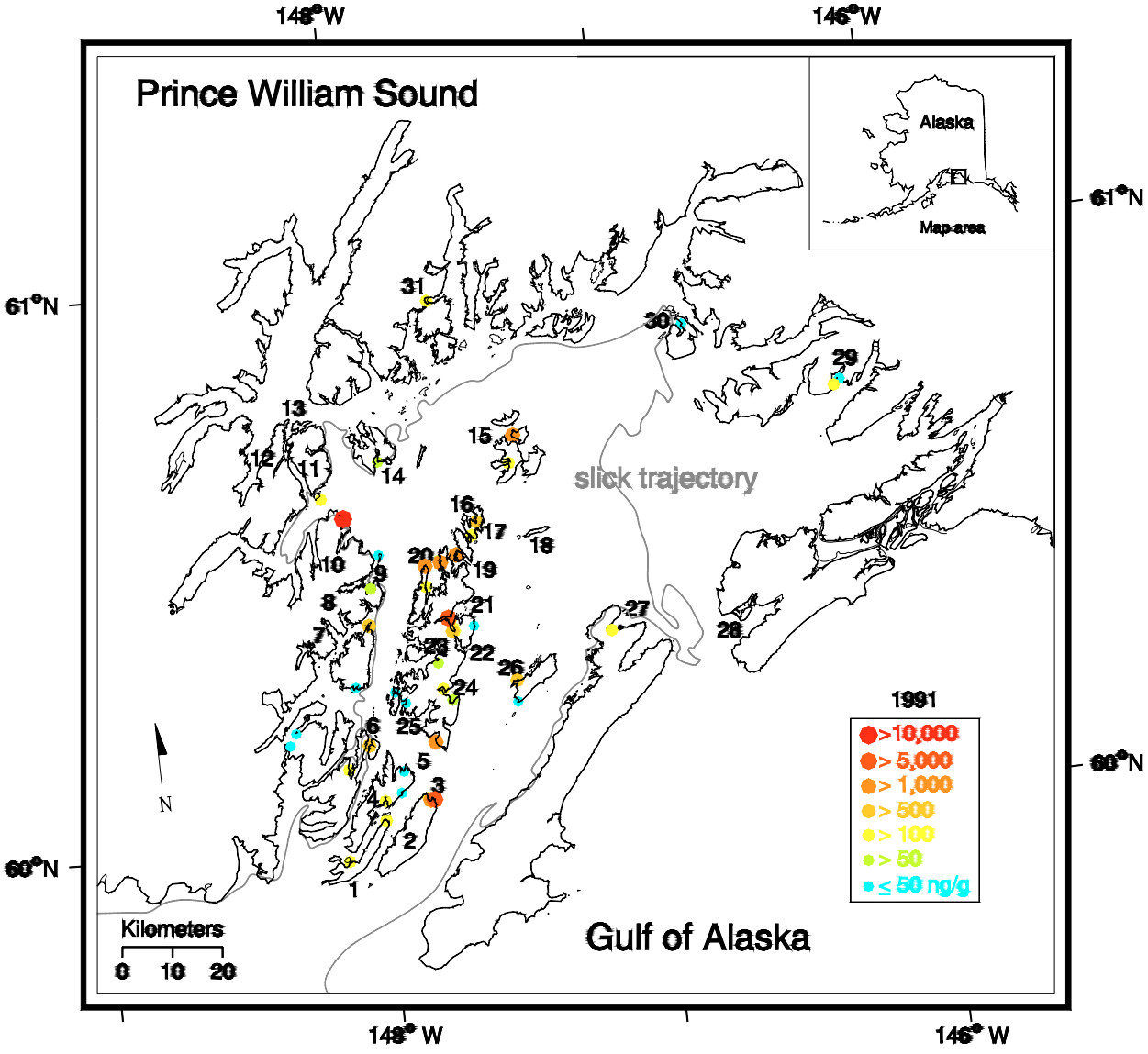
*Appendix 1 continued (mussels).*



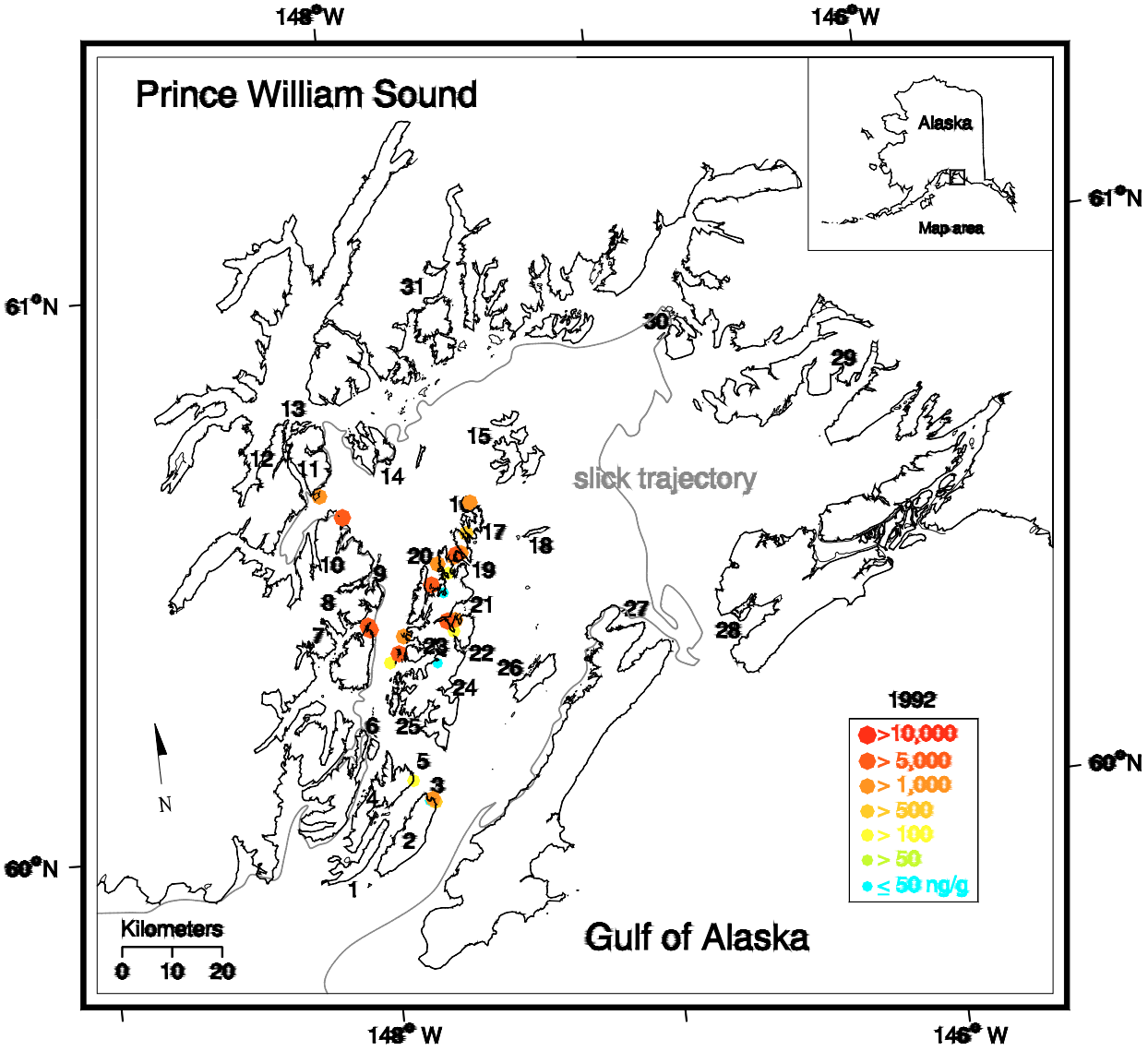
*Appendix 1 continued (mussels).*



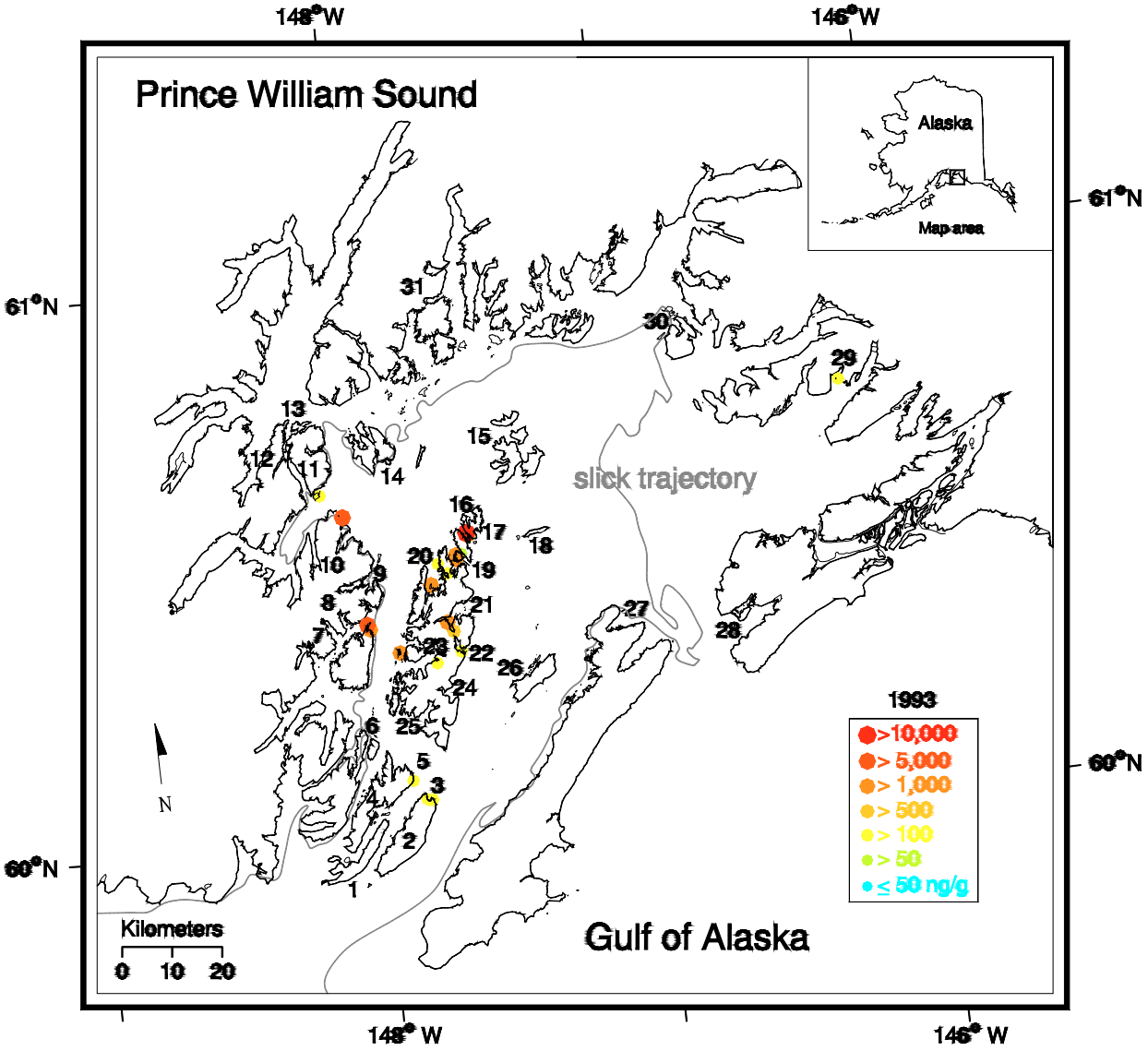
*Appendix 1 continued (mussels).*



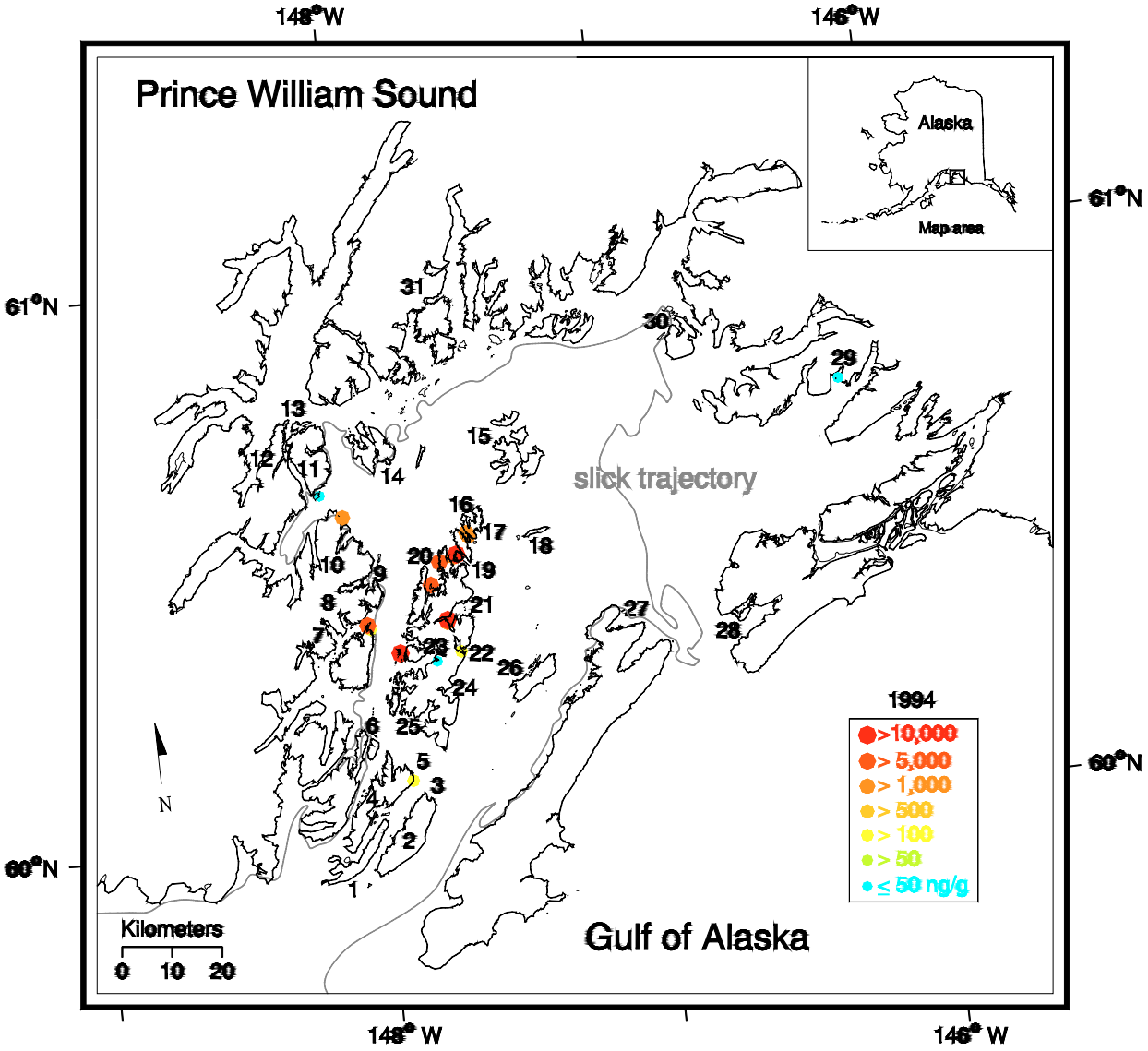
*Appendix 1 continued (mussels).*



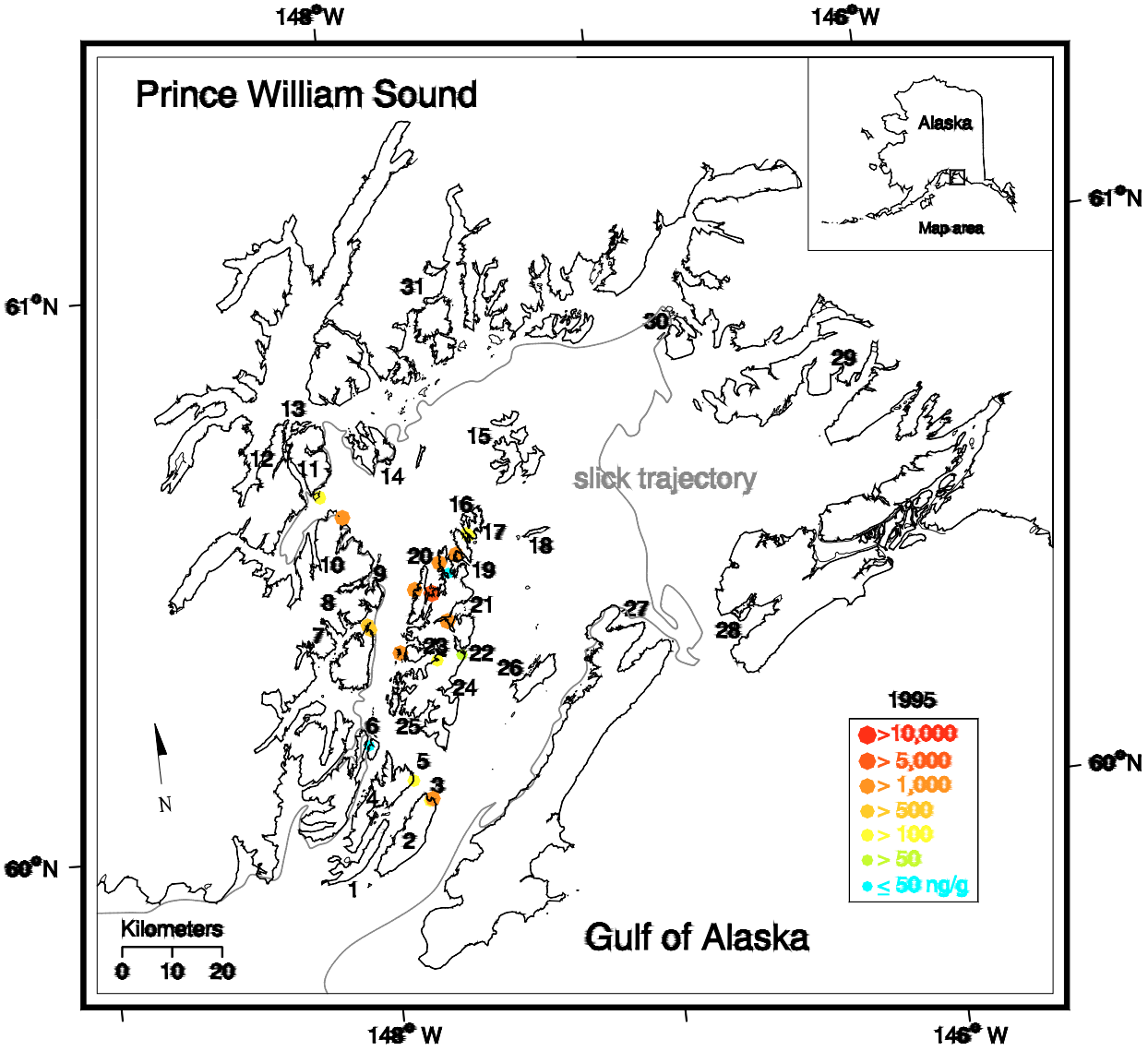
*Appendix 1 continued (mussels).*



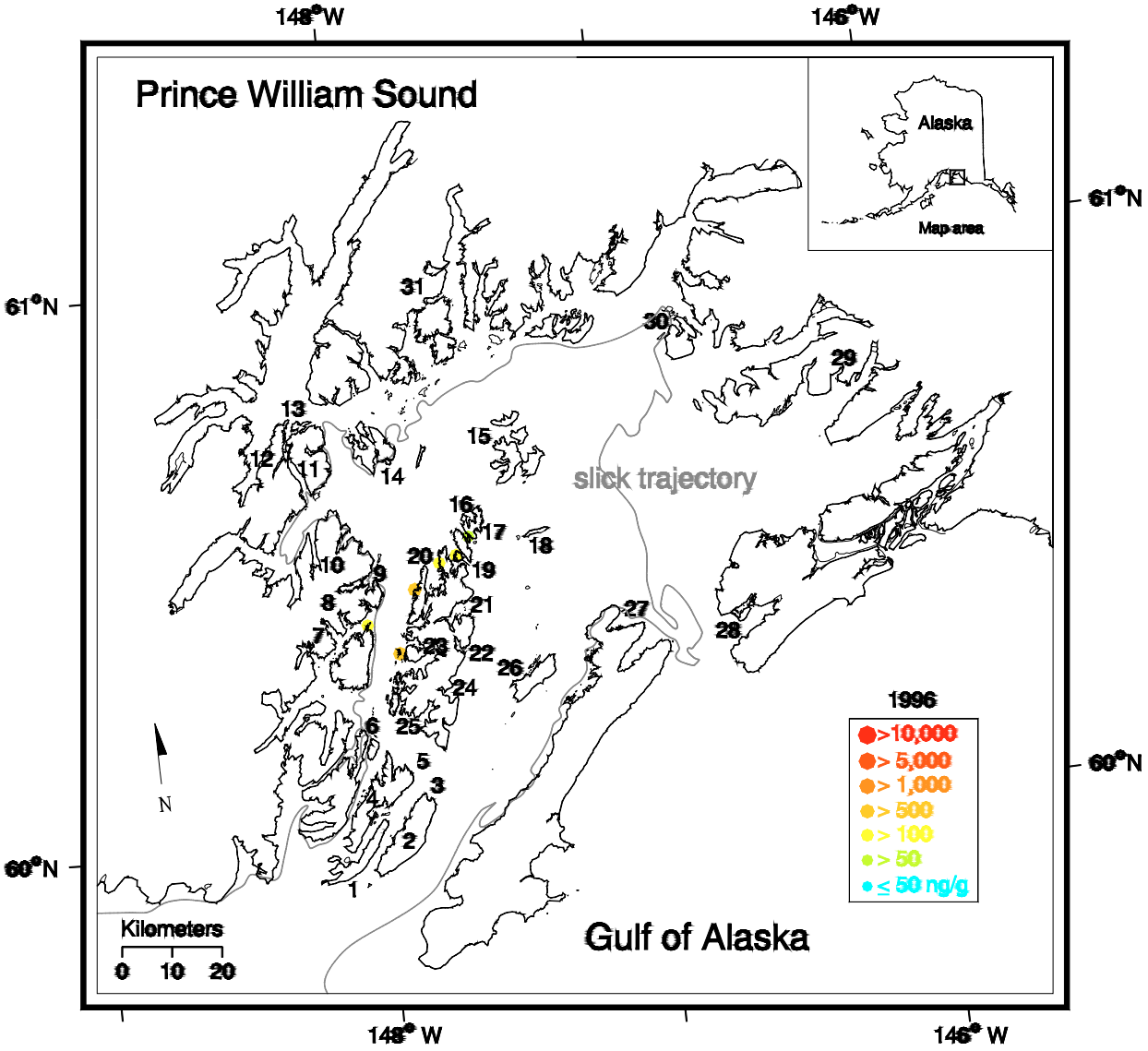
*Appendix 1 continued (mussels).*



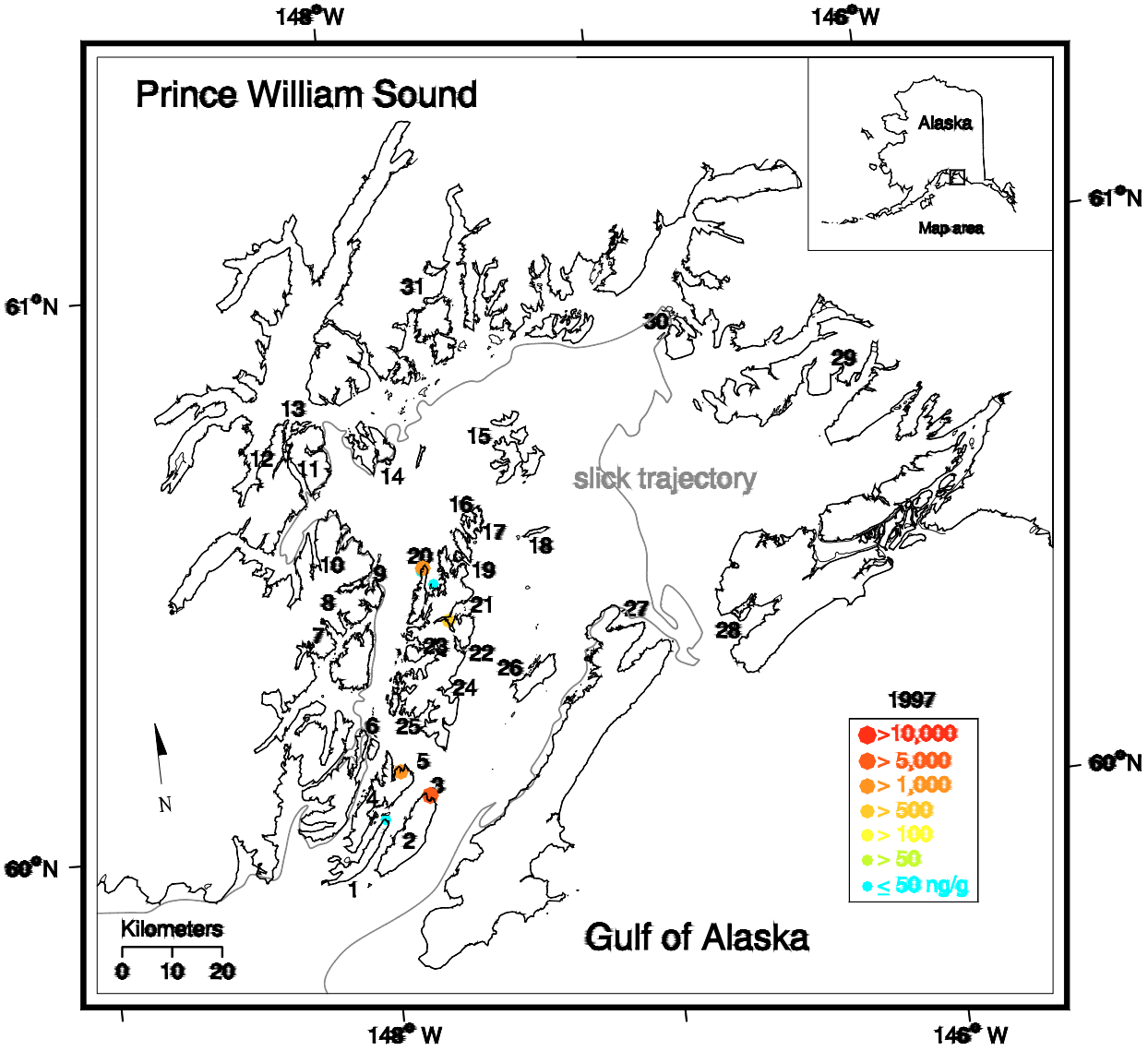
*Appendix 1 continued (mussels).*



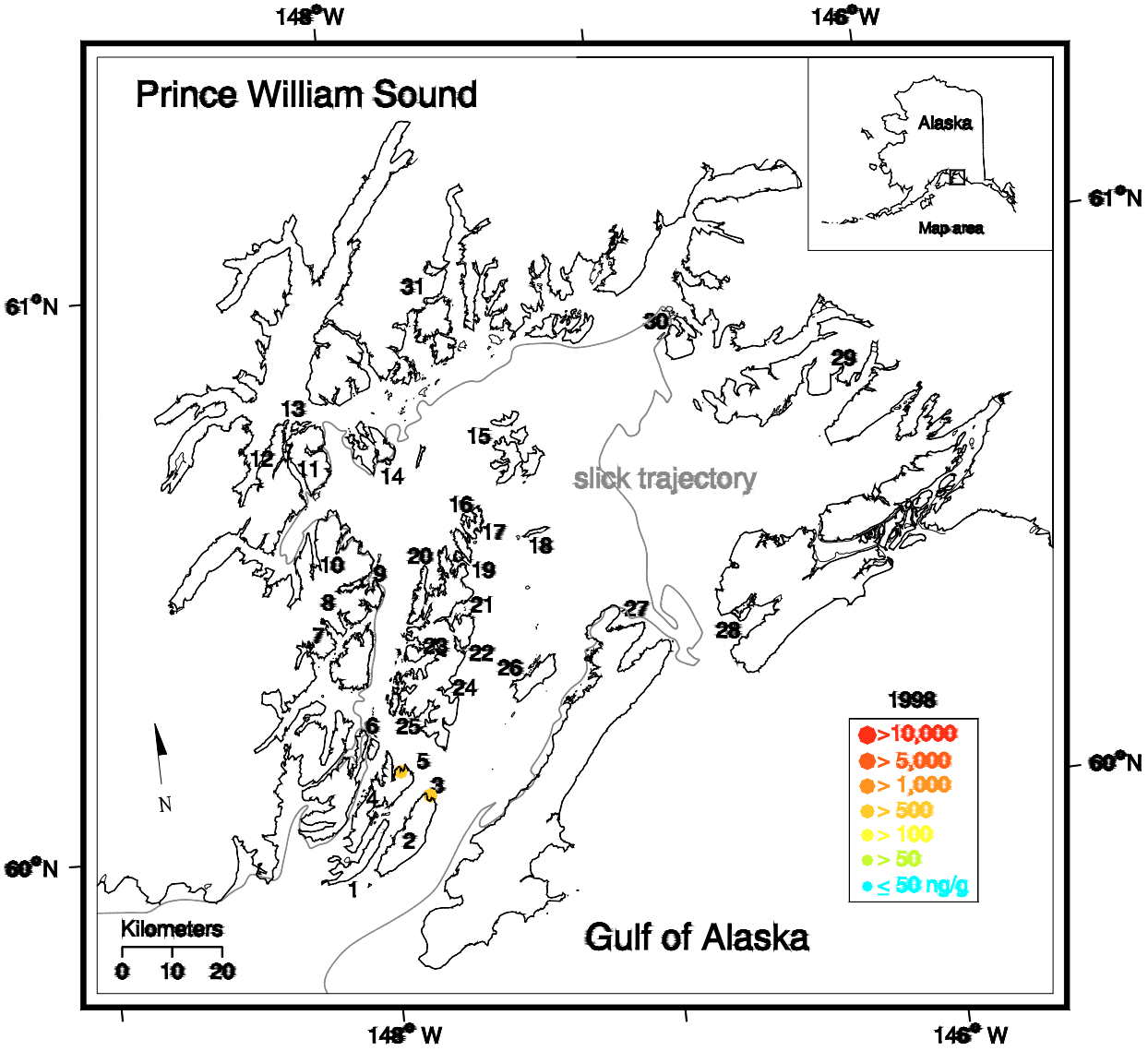
*Appendix 1 continued (mussels).*



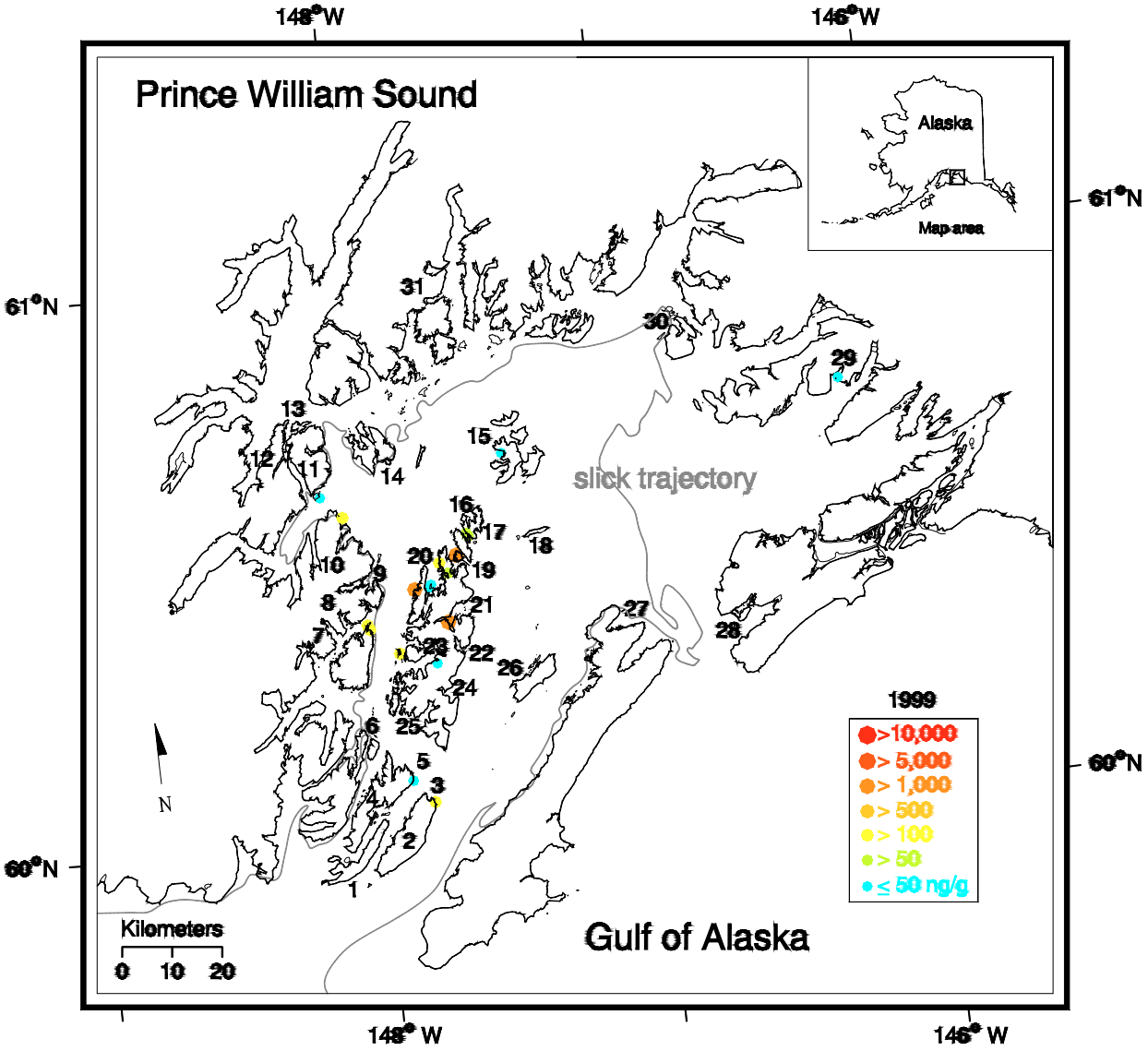
*Appendix 1 continued (mussels).*



*Appendix 1 continued (mussels).*



*Appendix 1 continued (mussels).*



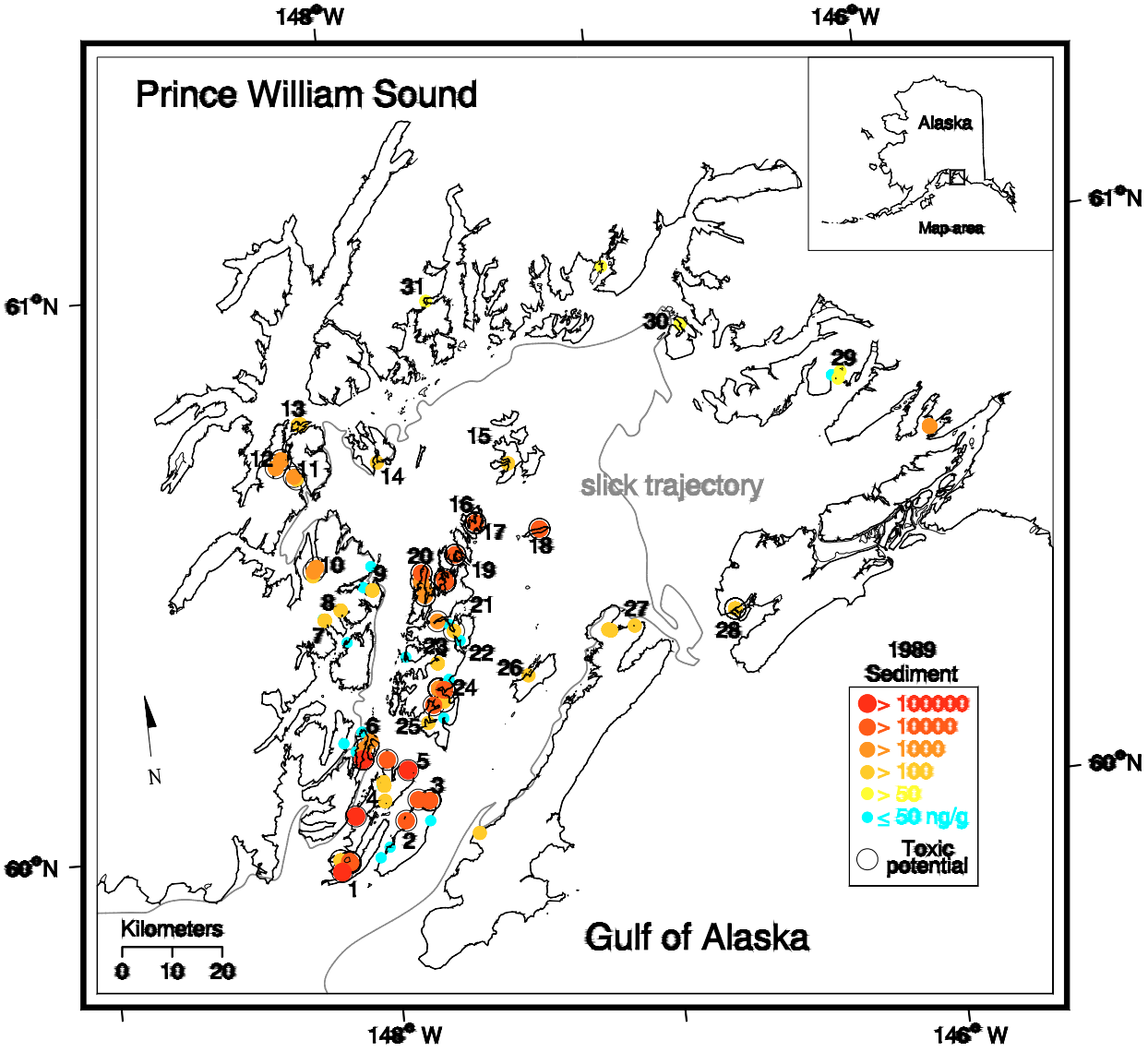
# Appendix 2

Geographic distribution of hydrocarbons in sediment

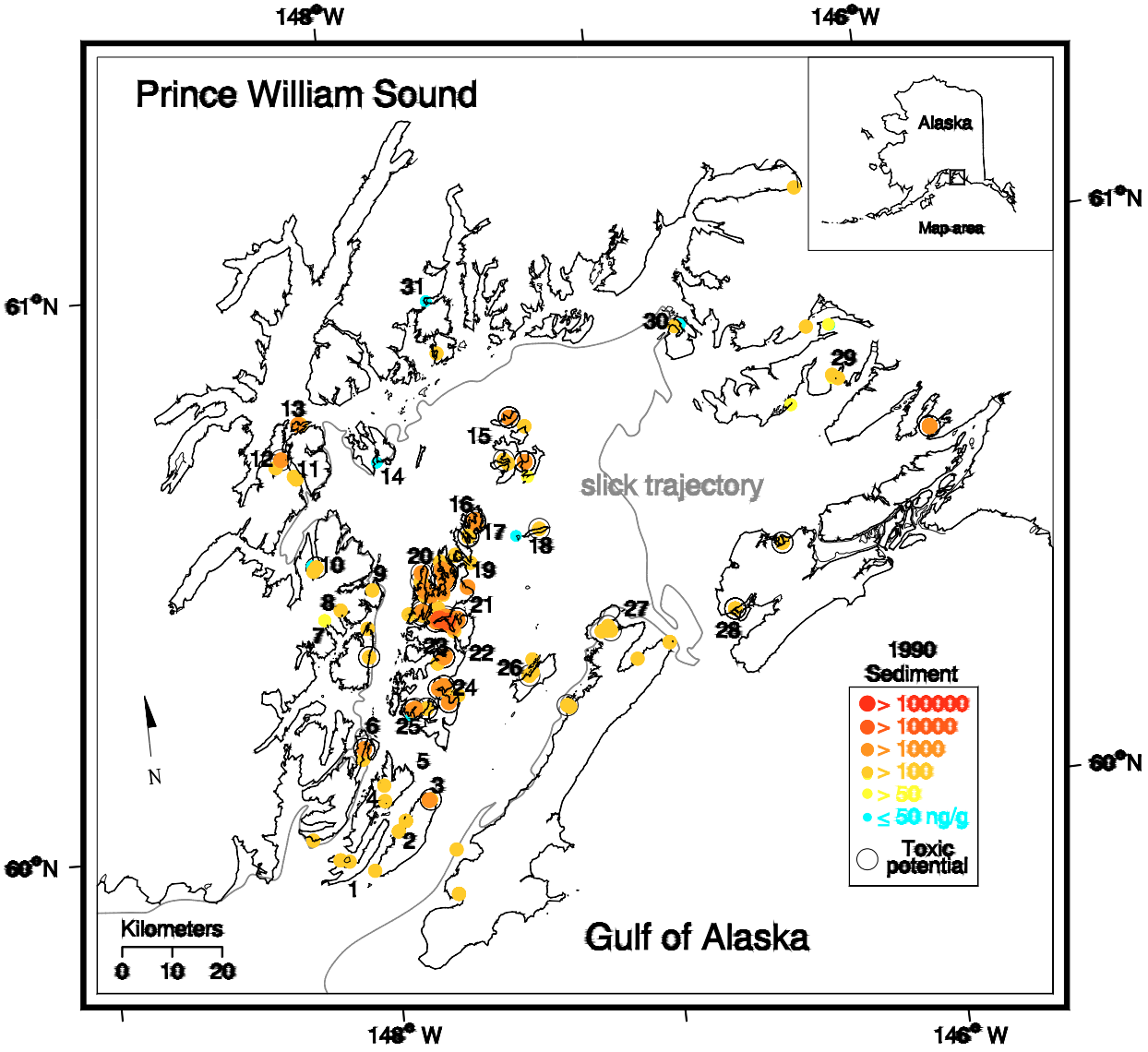
Geographic distribution of TPAH concentrations in sediment. All individual observations are included, each color-coded and sized according to concentration. Samples with estimated toxic potential are outlined with black circles. Location numbers are listed below. No sediment samples were analyzed by GC/MS in 1996.

| Location | Map Number |
|---|---|
| Elrington Island | 1 |
| Latouche Island, except Sleepy Bay | 2 |
| Sleepy Bay | 3 |
| Crab Bay | 4 |
| Evans Island (northern end) | 5 |
| Prince of Wales Passage | 6 |
| Ewan Bay | 7 |
| Paddy Bay | 8 |
| Eshamy Bay | 9 |
| McClure Bay | 10 |
| Culross Passage | 11 |
| Long Bay | 12 |
| Wells Passage | 13 |
| Perry Island | 14 |
| Naked Island group | 15 |
| Northwest Bay | 16 |
| Eleanor Island | 17 |
| Smith Island | 18 |
| Disk Island | 19 |
| Herring Bay and Herring Point | 20 |
| Bay of Isles | 21 |
| Marsha Bay | 22 |
| Barnes Cove | 23 |
| Snug Harbor | 24 |
| Mummy Bay | 25 |
| Green Island | 26 |
| Rocky Bay and Rocky Point | 27 |
| Constantine Harbor | 28 |
| Olsen Bay | 29 |
| Bligh Island | 30 |
| Siwash Bay | 31 |
| Simpson Bay | 32 |

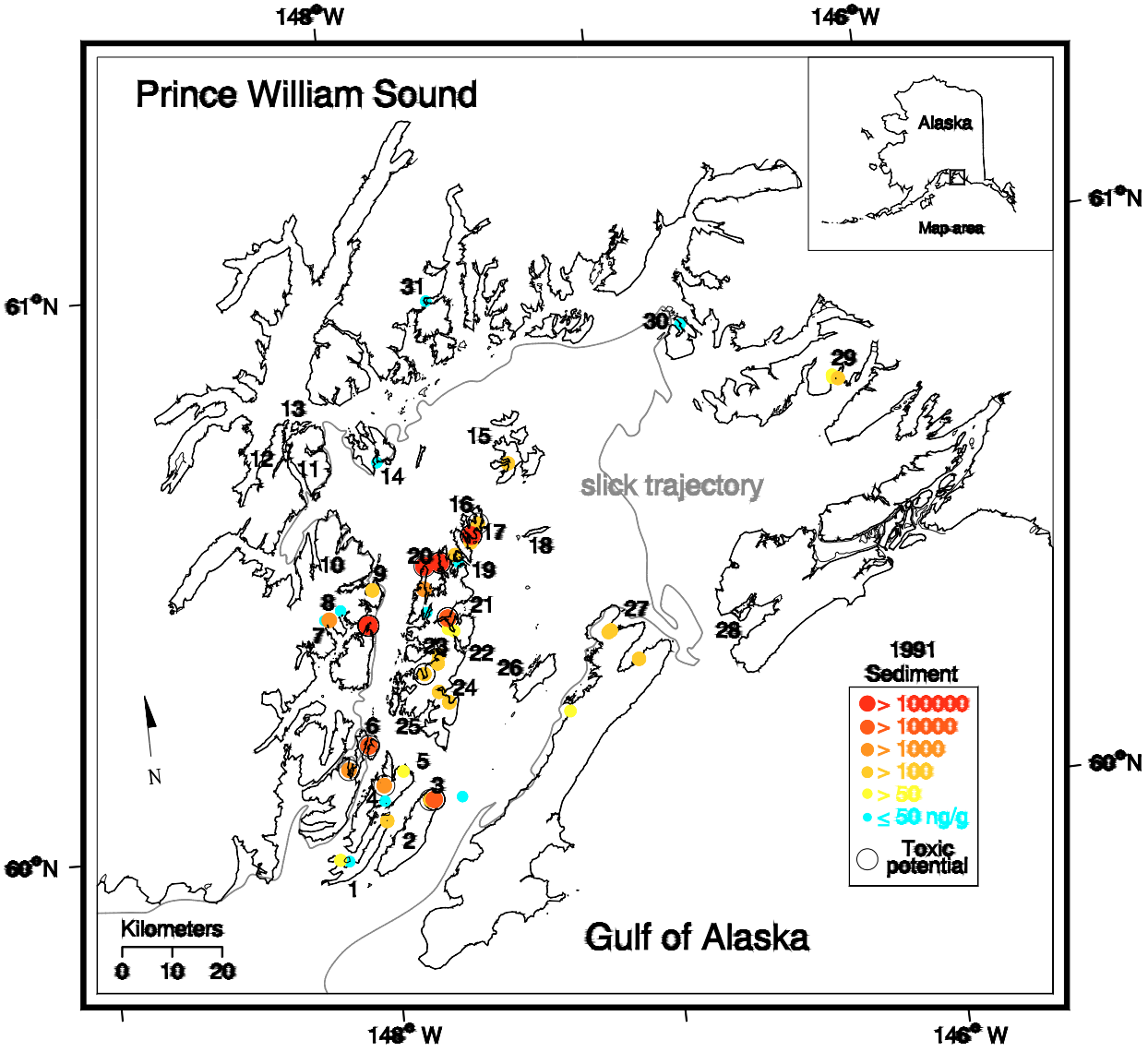
*Appendix 2 continued (sediment).*



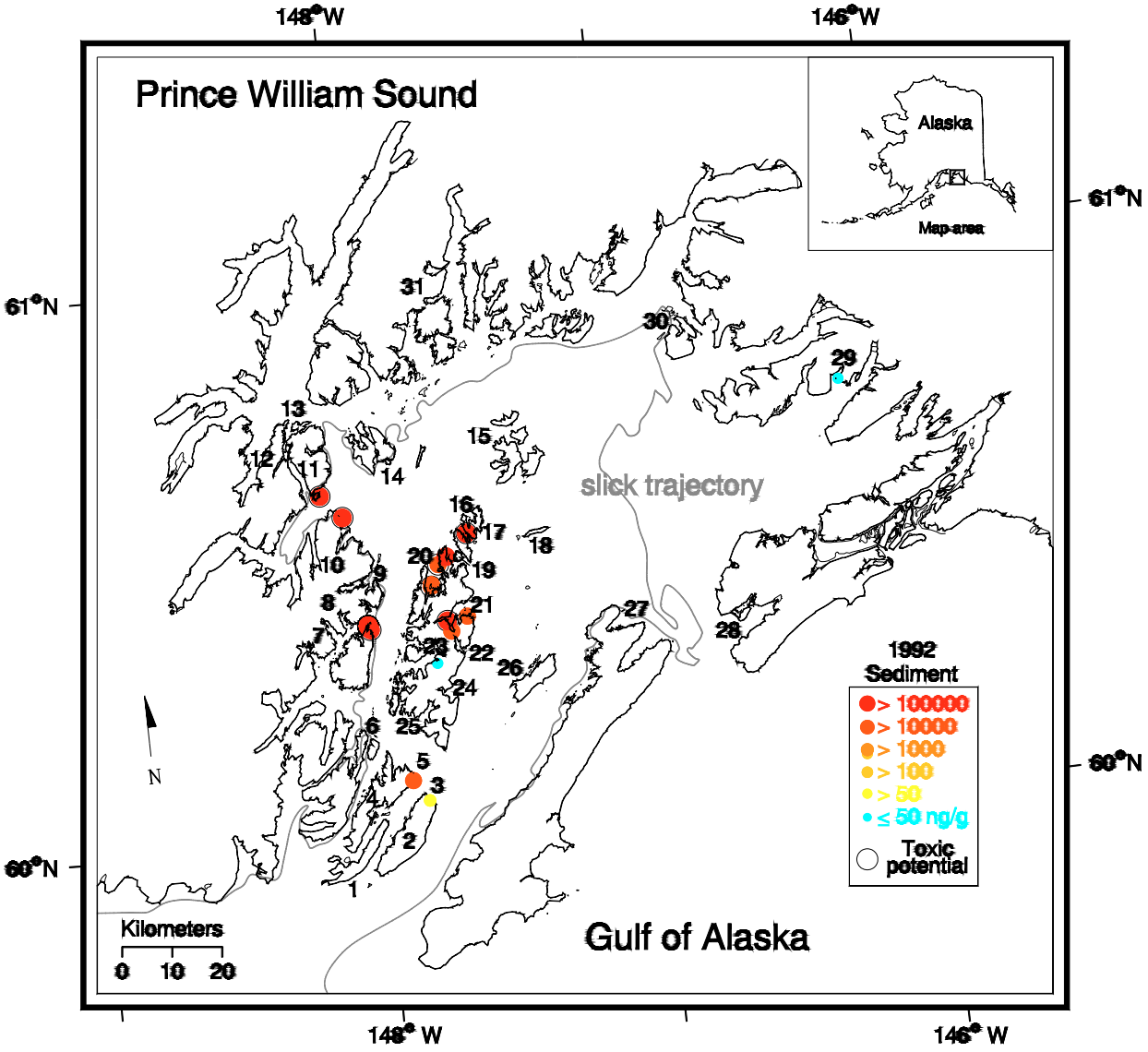
*Appendix 2 continued (sediment).*



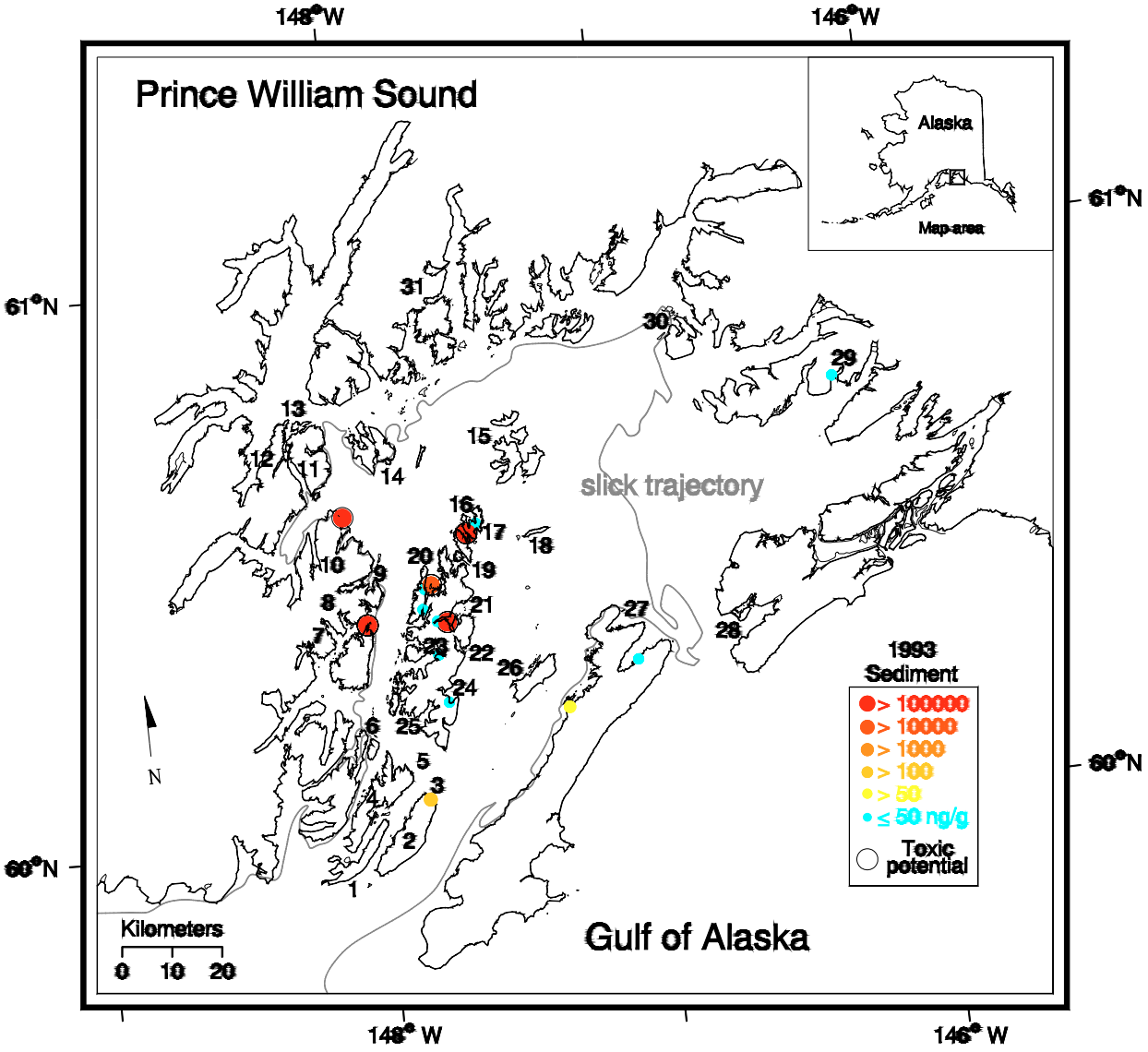
*Appendix 2 continued (sediment).*



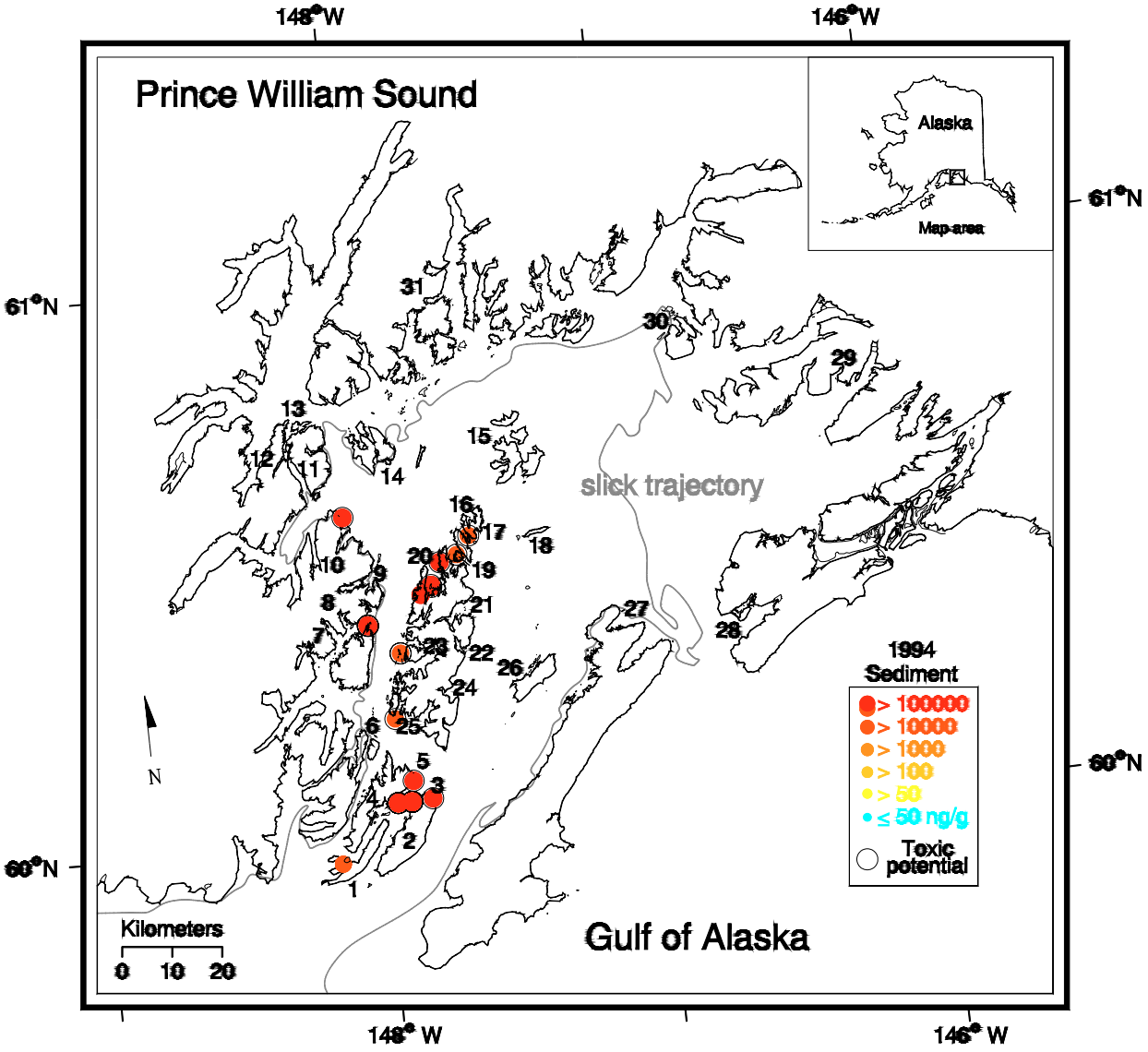
*Appendix 2 continued (sediment).*



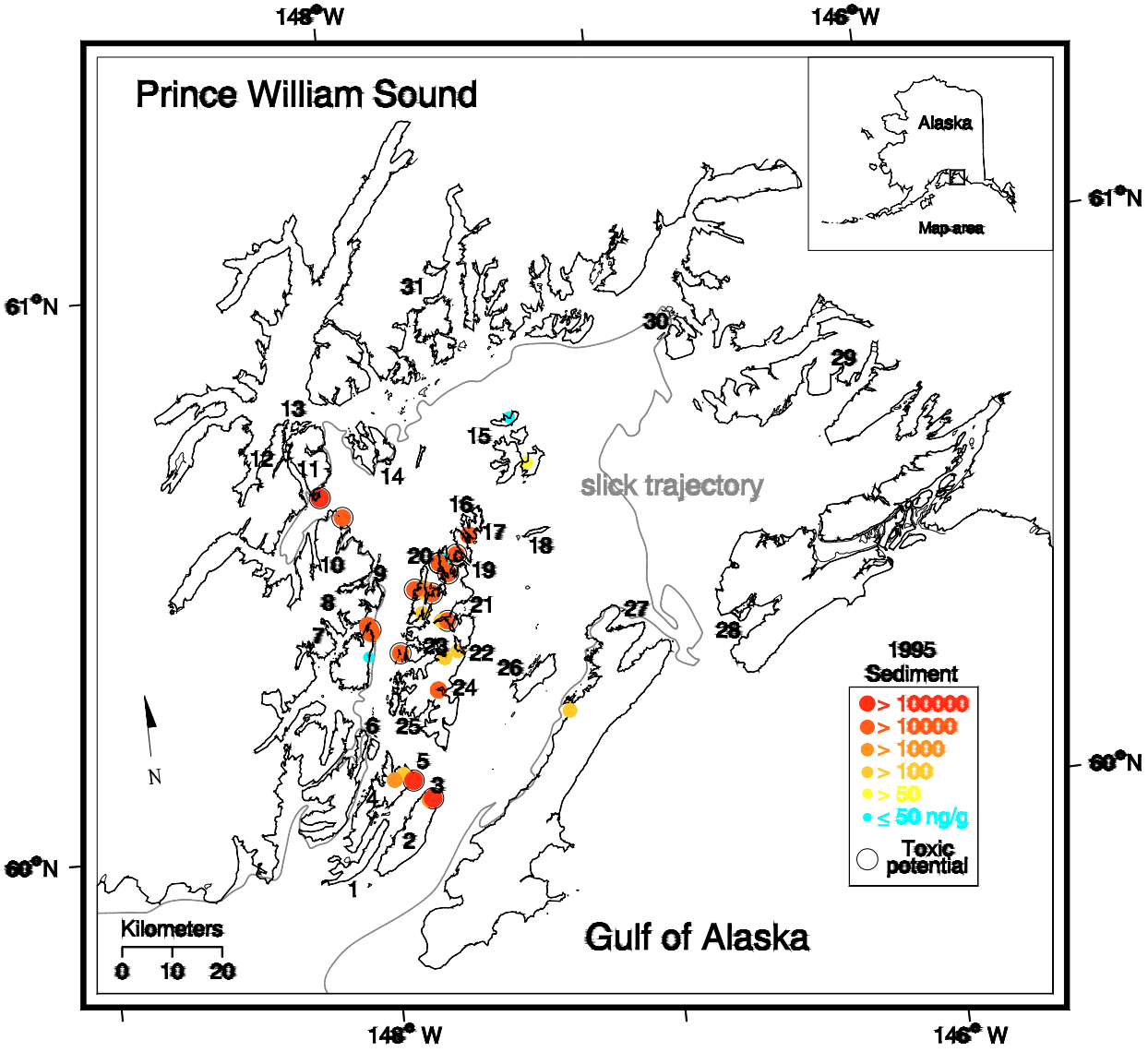
*Appendix 2 continued (sediment).*



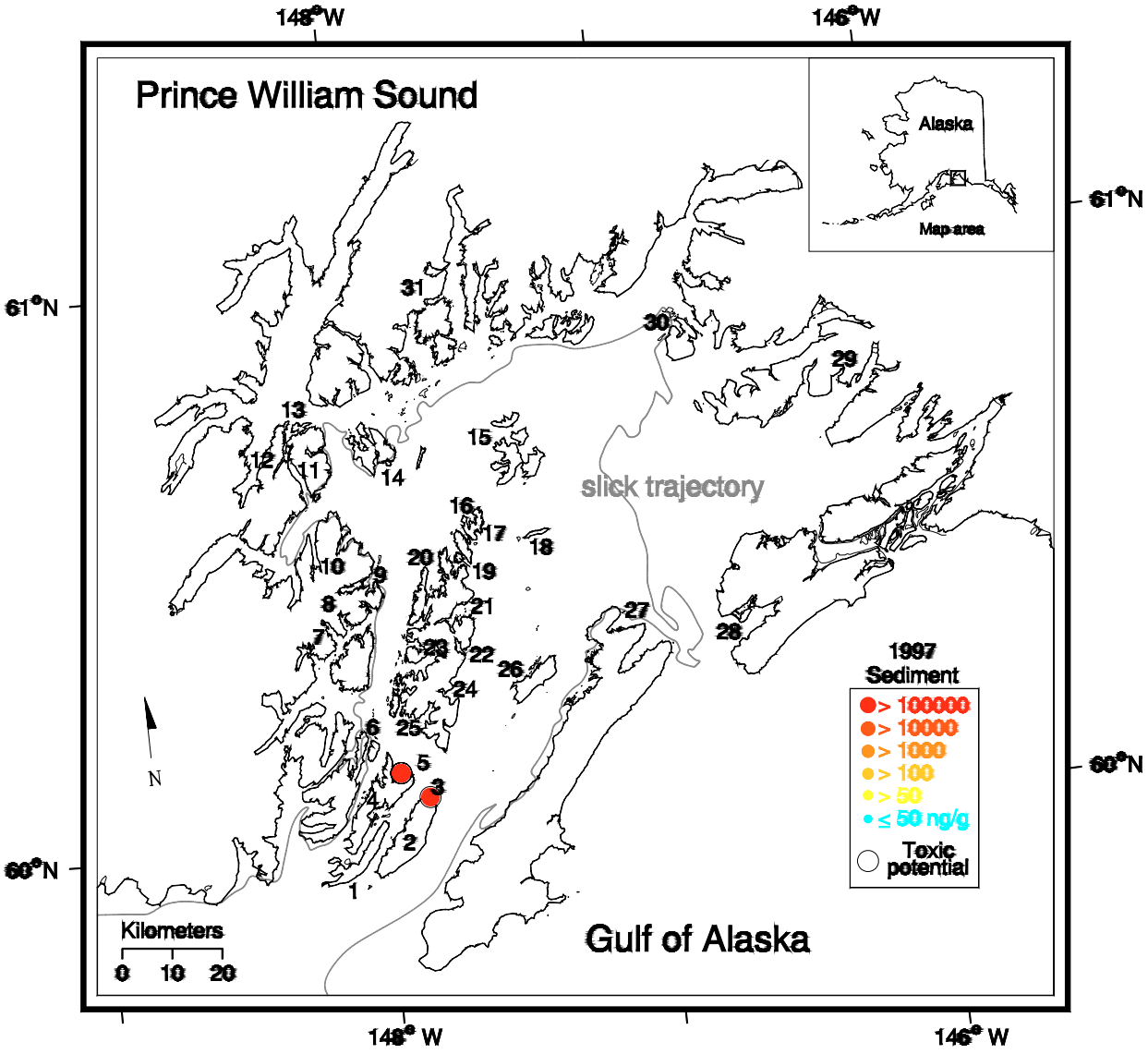
*Appendix 2 continued (sediment).*



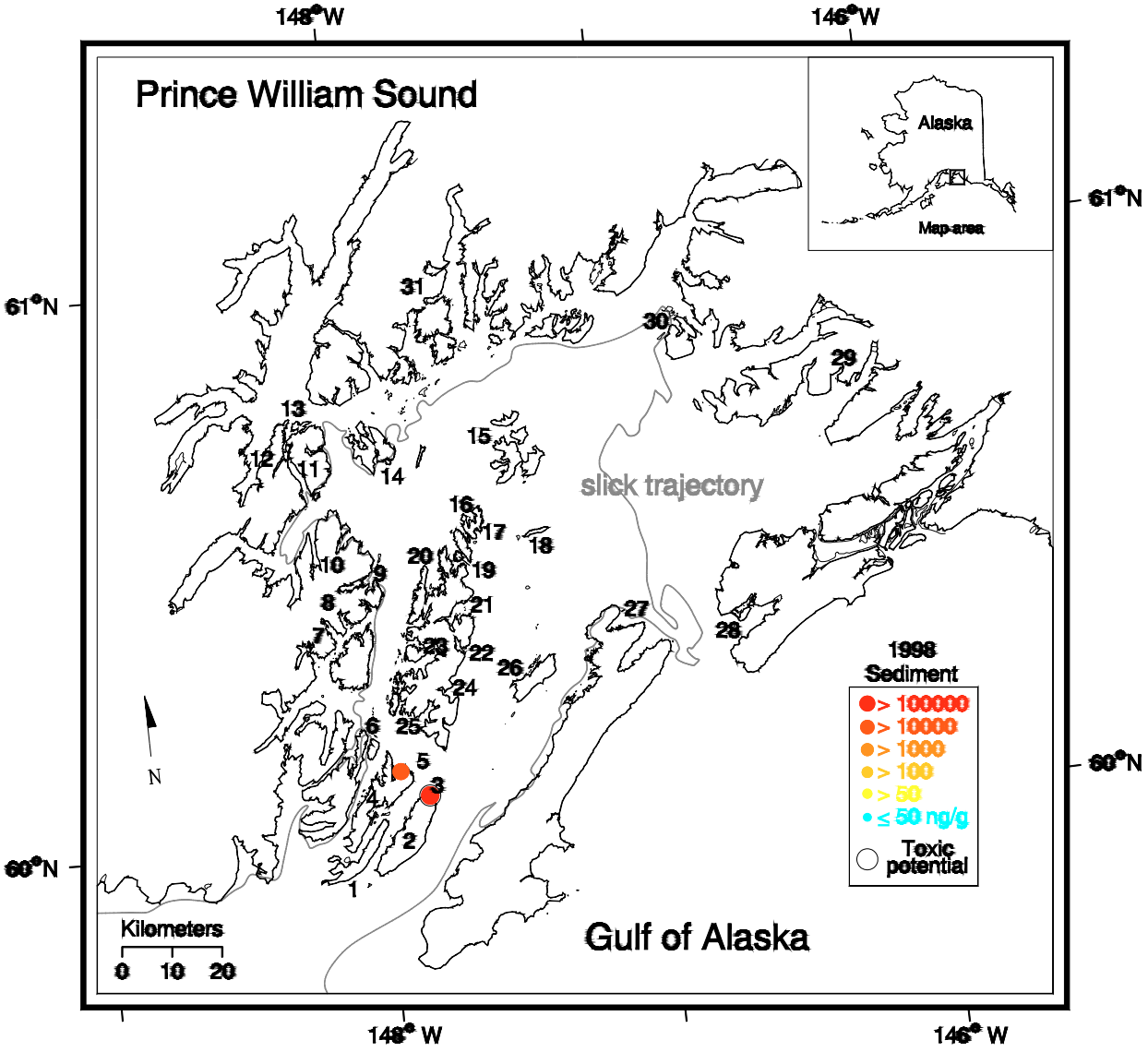
*Appendix 2 continued (sediment).*



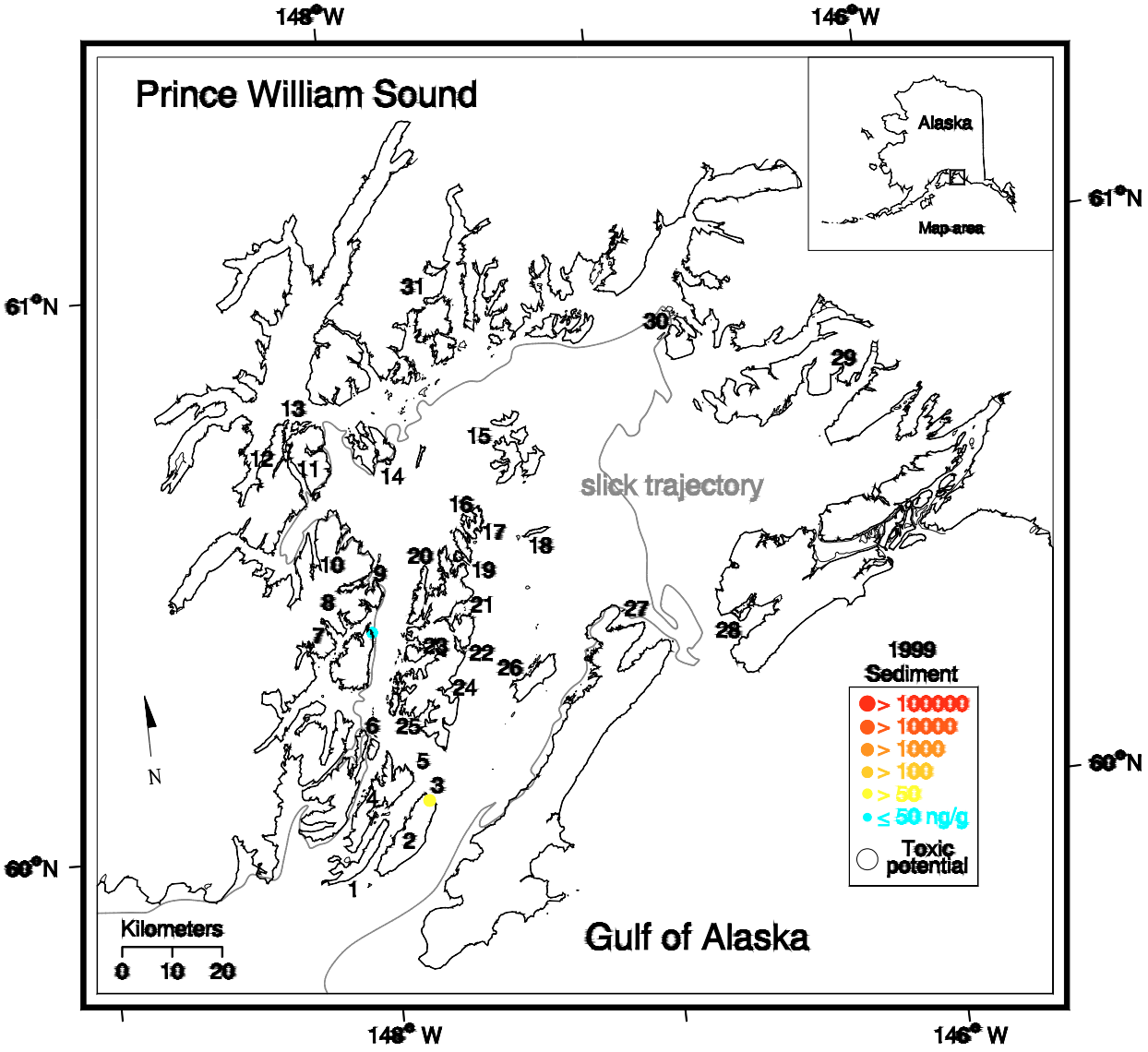
*Appendix 2 continued (sediment). (No sediment were analyzed by GC/MS in 1996.*



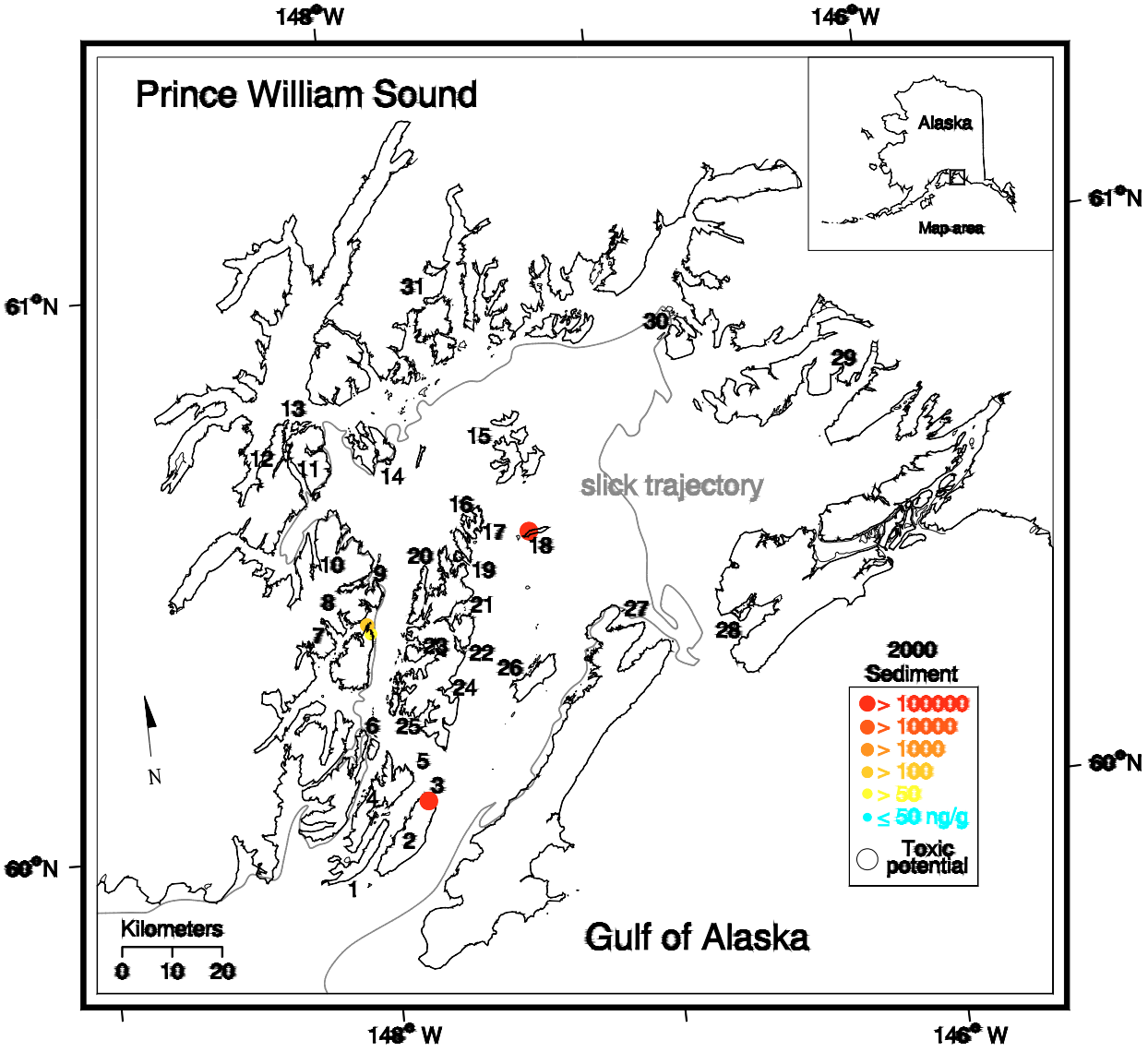
*Appendix 2 continued (sediment).*



*Appendix 2 continued (sediment).*



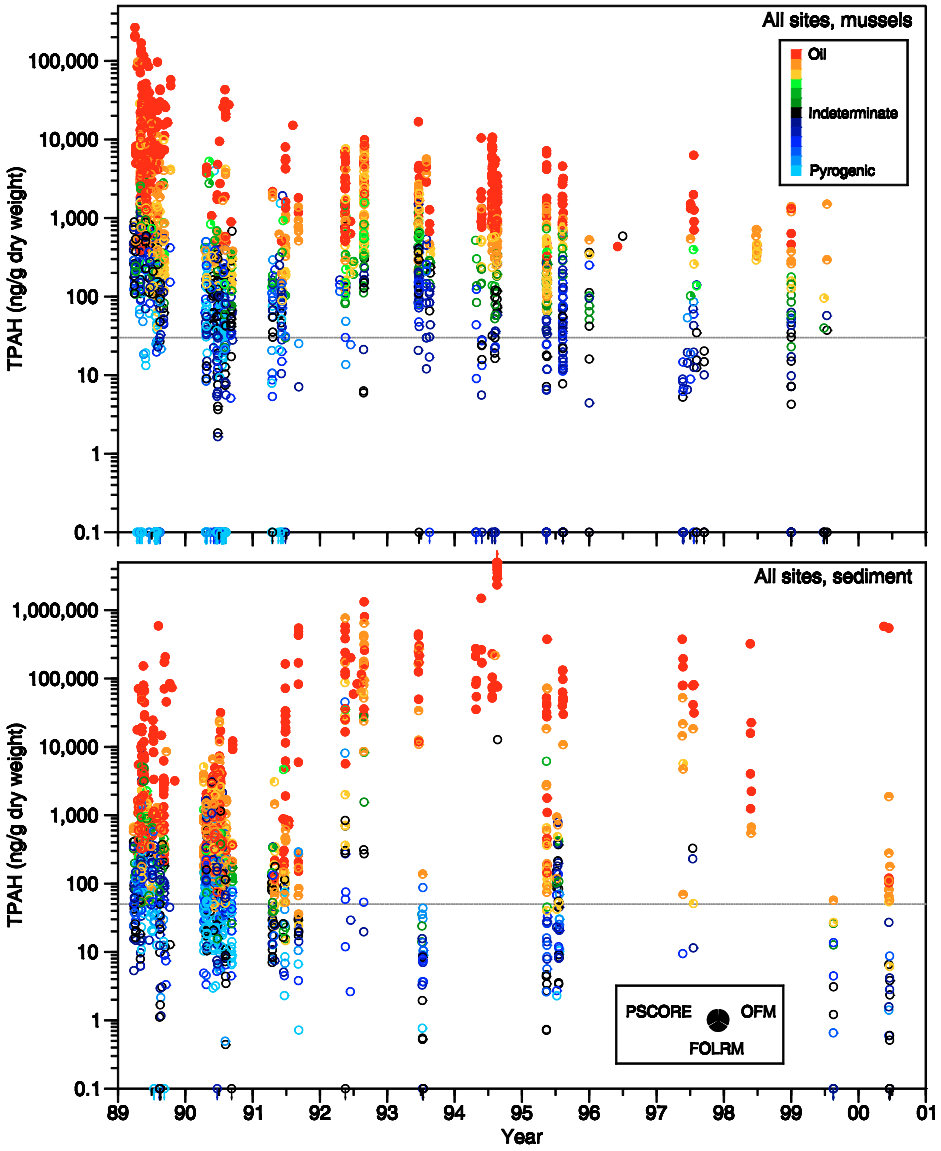
*Appendix 2 continued (sediment).*

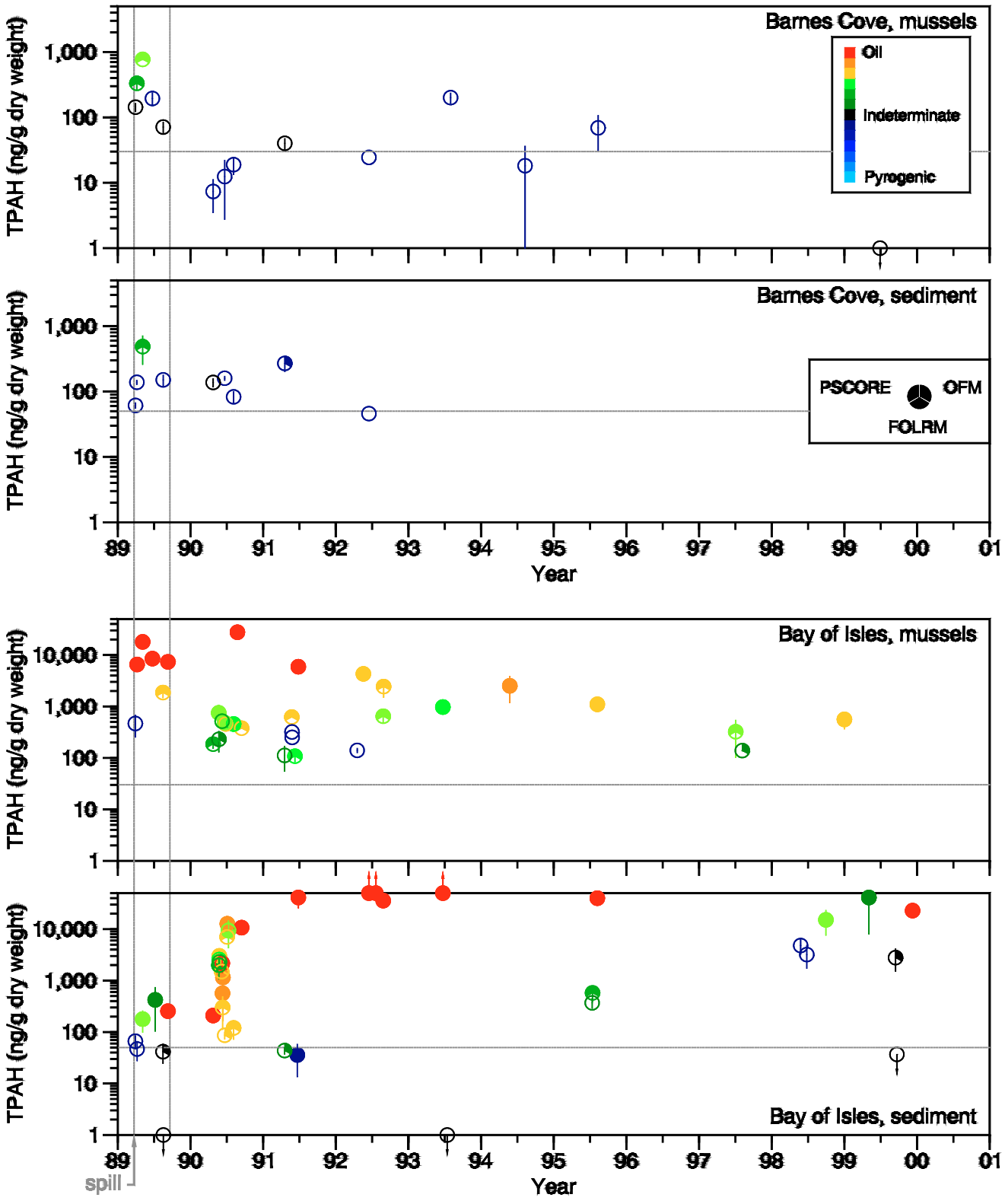


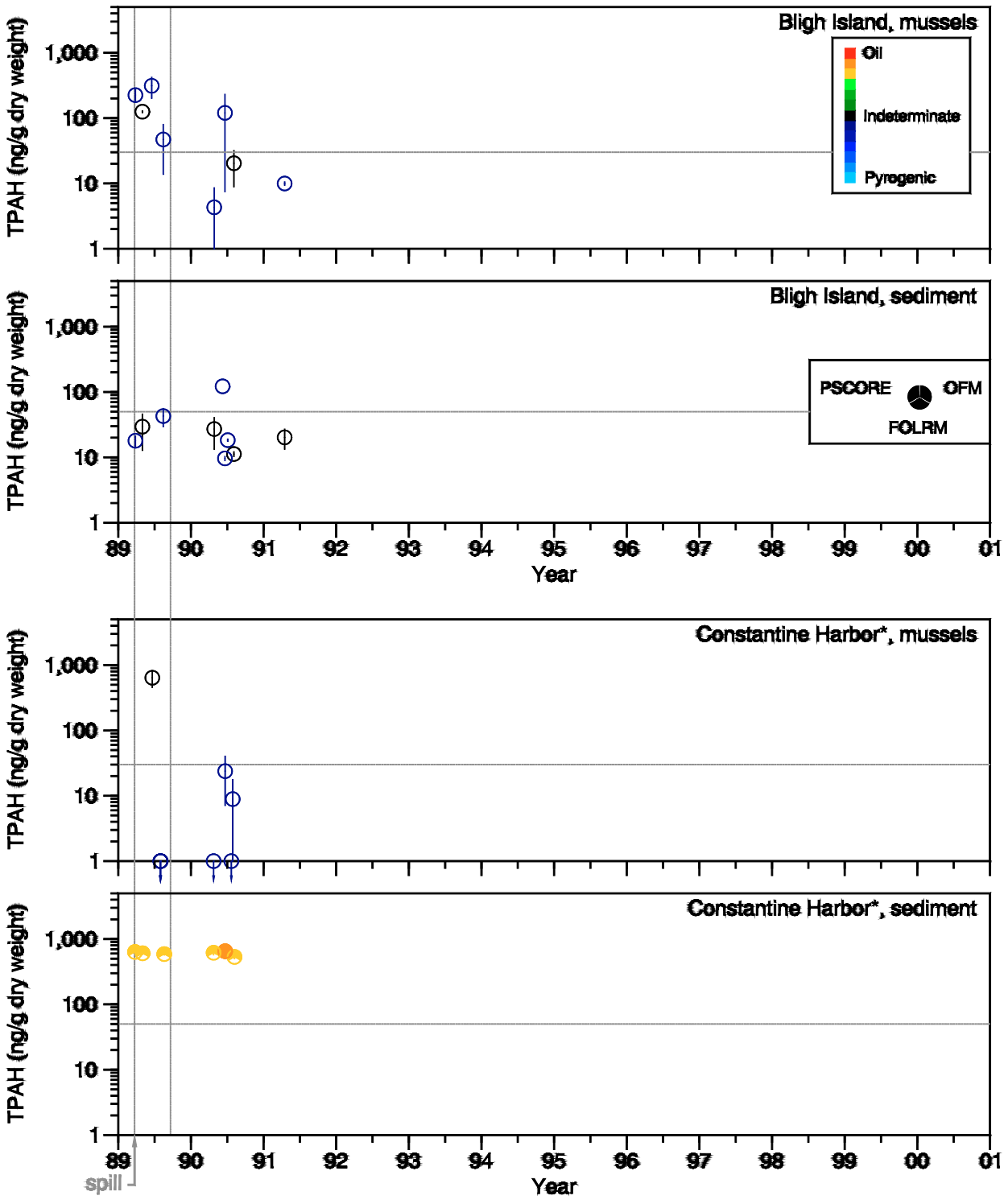
# Appendix 3

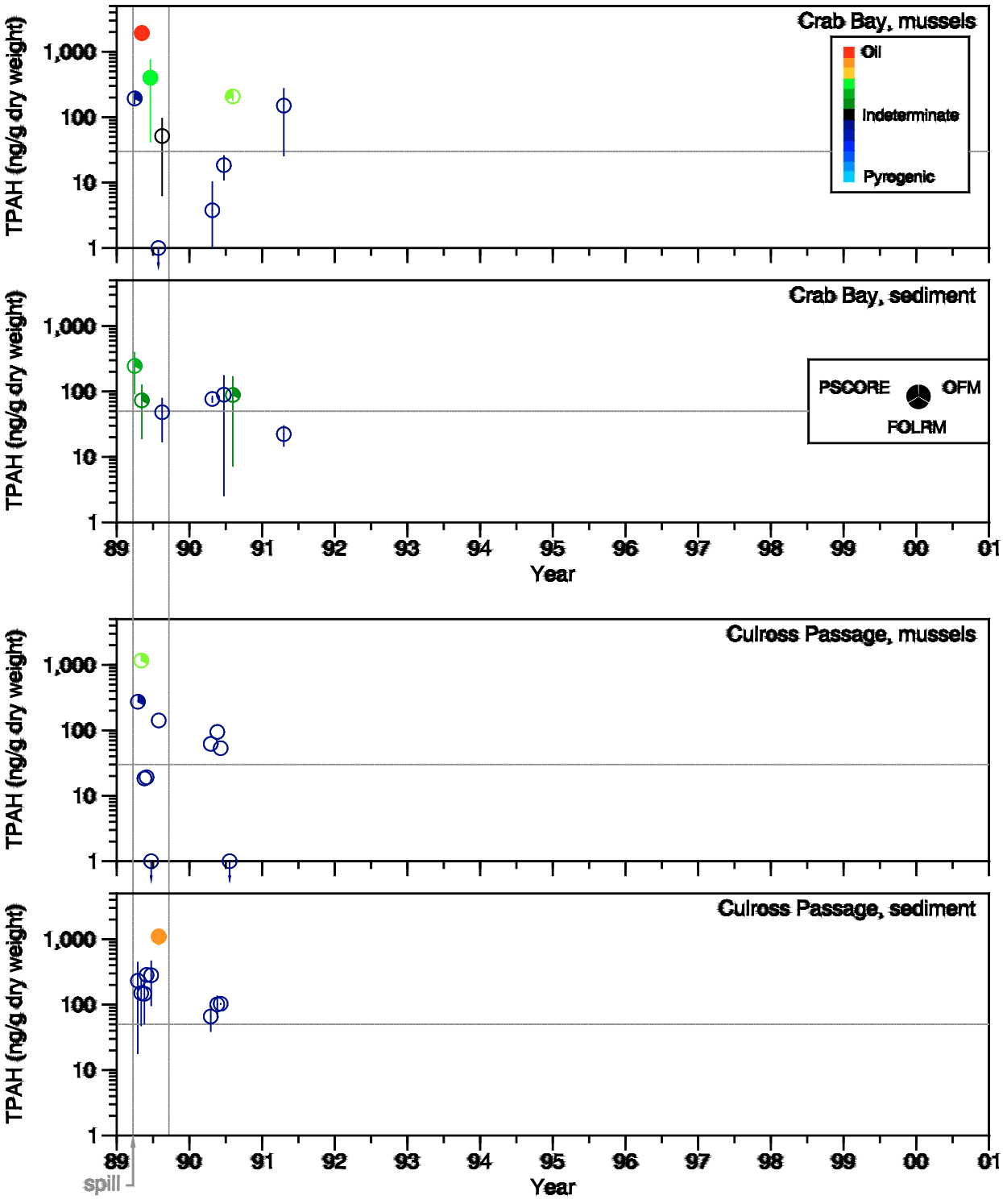
Time series TPAH concentrations in mussels and sediment
in Prince William Sound

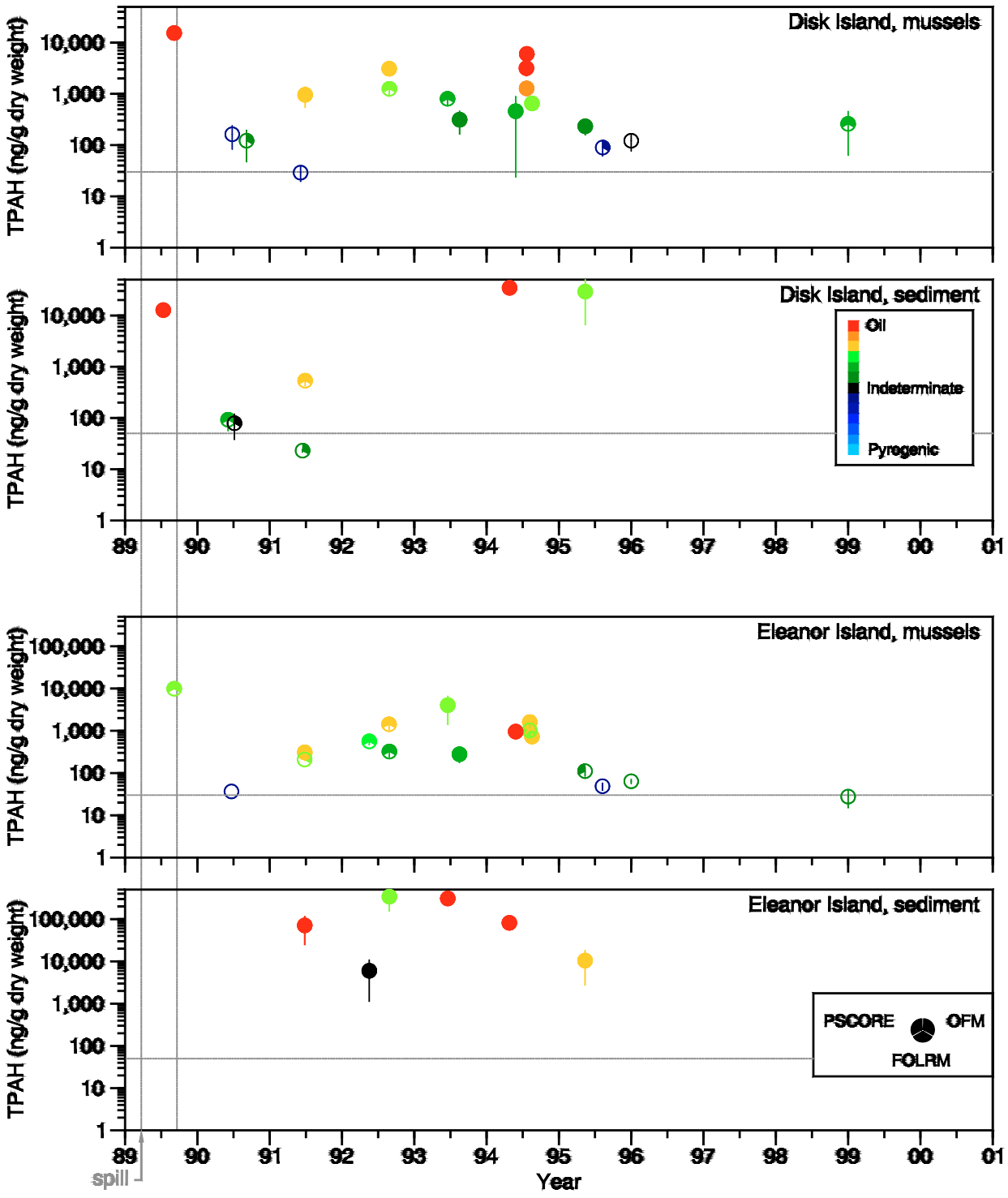
Mean (±SE) total polynuclear aromatic hydrocarbon (TPAH) concentrations in mussels and sediment at oiled locations in Prince William Sound. All locations with two or more years of data beginning in 1989 are illustrated. Symbol fill indicates that *Exxon Valdez* oil was identified by one or more oil composition models: $PSCORE_{ANS}$; Carls submitted (Chapter 4)], an oil-fingerprint model ($OFM_{ANS}$; Bence and Burns 1995), and a first-order loss-rate model ($FORLM_{ANS}$ Short and Heintz 1997). Symbol colors are based on mean combined scores petrogenic and pyrogenic scores (Chapter 4). Approximate background concentrations for mussels (30 ng/g) and sediment (50 ng/g) are indicated with horizontal dashed lines for reference. Time of the spill and 180 d later are indicated with vertical dashed lines. *Below is a scatter plot that includes all data. Site-specific figures begin on the next page.*

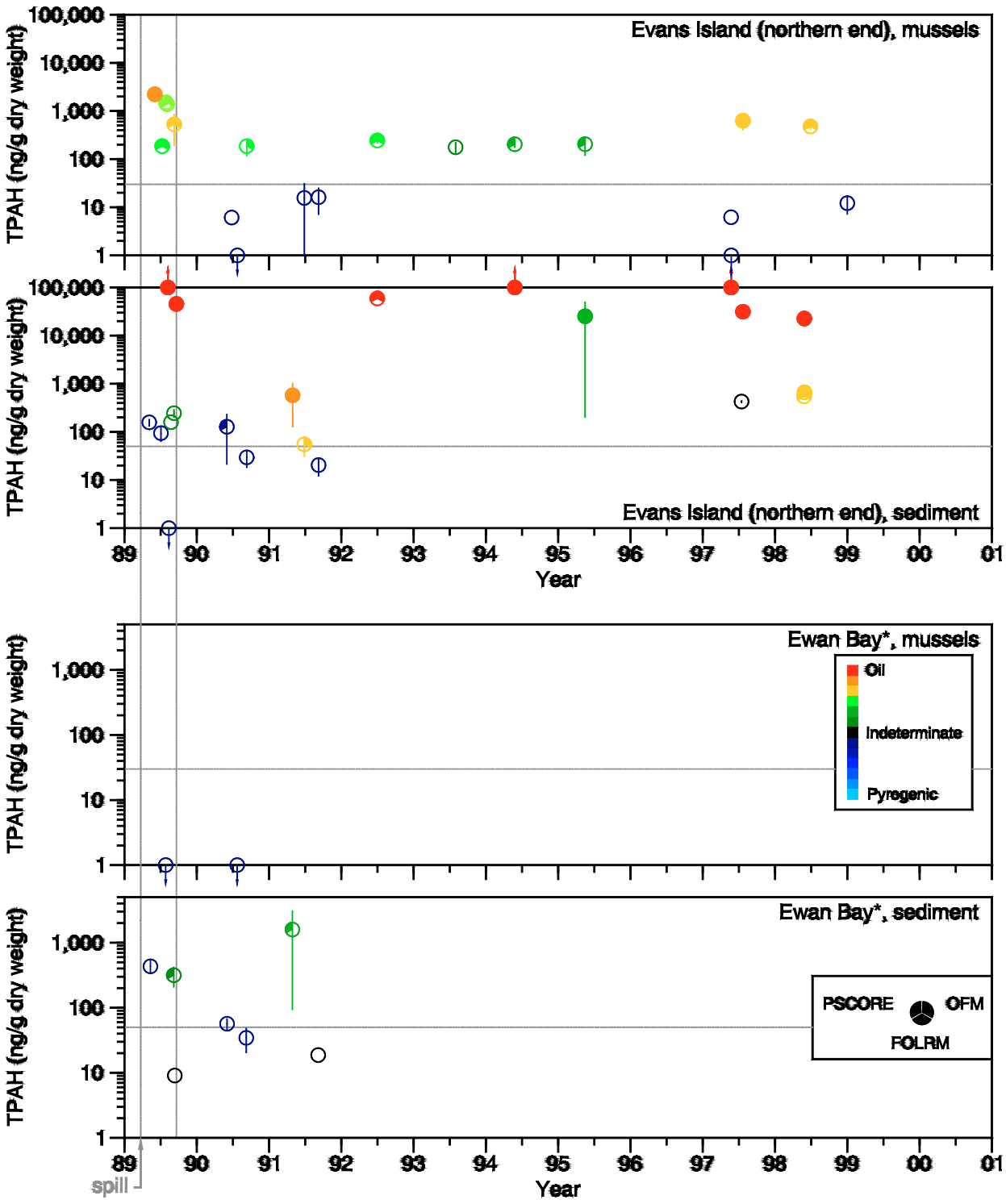


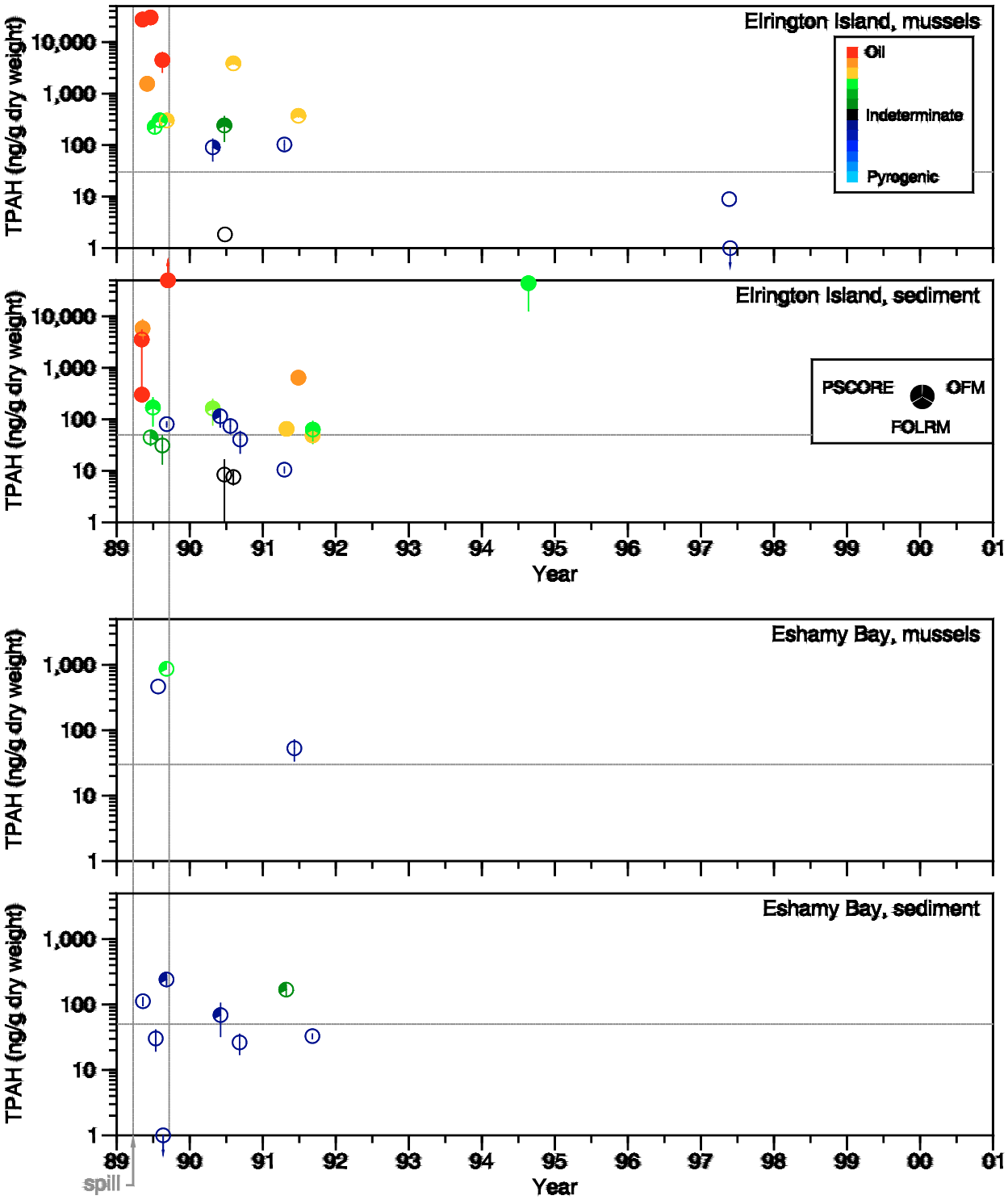

Barnes Cove, mussels
Oil
Indeterminate
Pyrogenic
TPAH (ng/g dry weight)
1,000
100
10
1
Barnes Cove, sediment
PSCORE
OFM
FOLRM
89
90
91
92
93
94
95
96
97
98
99
00
01
Year
Bay of Isles, mussels
10,000
Bay of Isles, sediment
spill

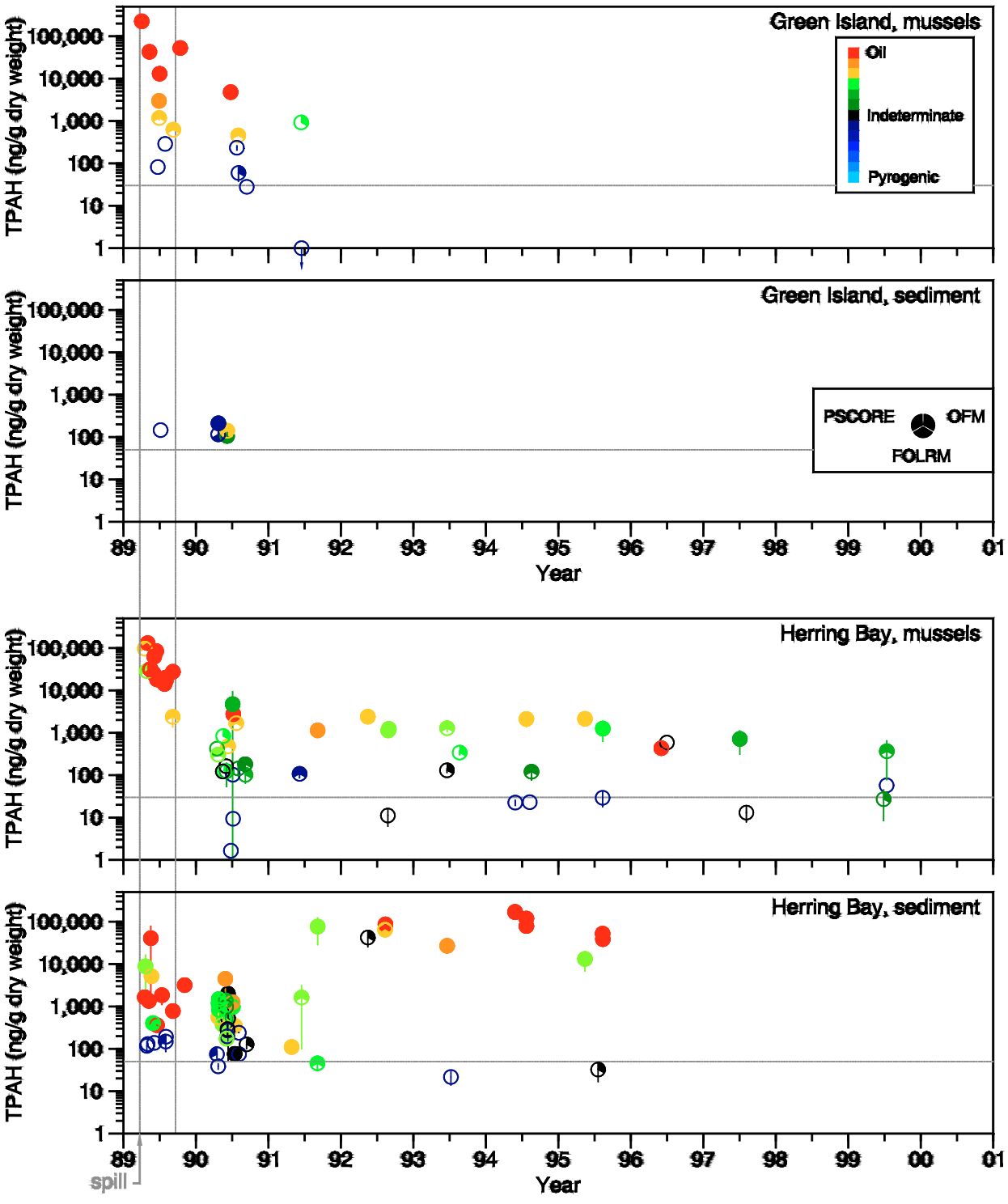

Bligh Island, mussels
Oil
Indeterminate
Pyrogenic
Bligh Island, sediment
PSCORE
OFM
FOLRM
Constantine Harbor*, mussels
Constantine Harbor*, sediment
TPAH (ng/g dry weight)
1,000
100
10
1
89
90
91
92
93
94
95
96
97
98
99
00
01
Year
spill

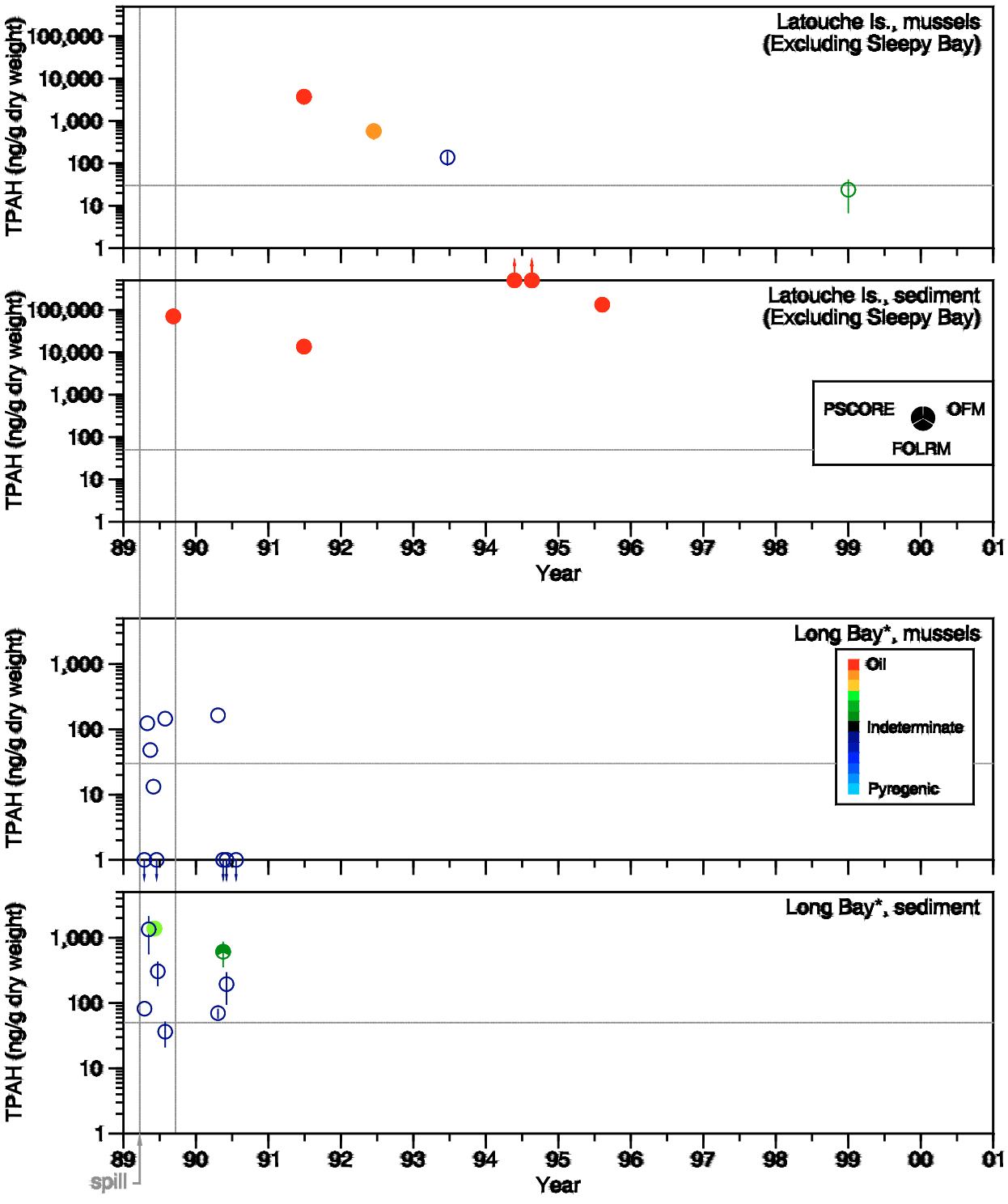

Crab Bay, mussels
Oil
Indeterminate
Pyrogenic
Crab Bay, sediment
PSCORE
OFM
FOLRM
Culross Passage, mussels
Culross Passage, sediment
TPAH (ng/g dry weight)
1,000
100
10
1
89
90
91
92
93
94
95
96
97
98
99
00
01
Year
spill

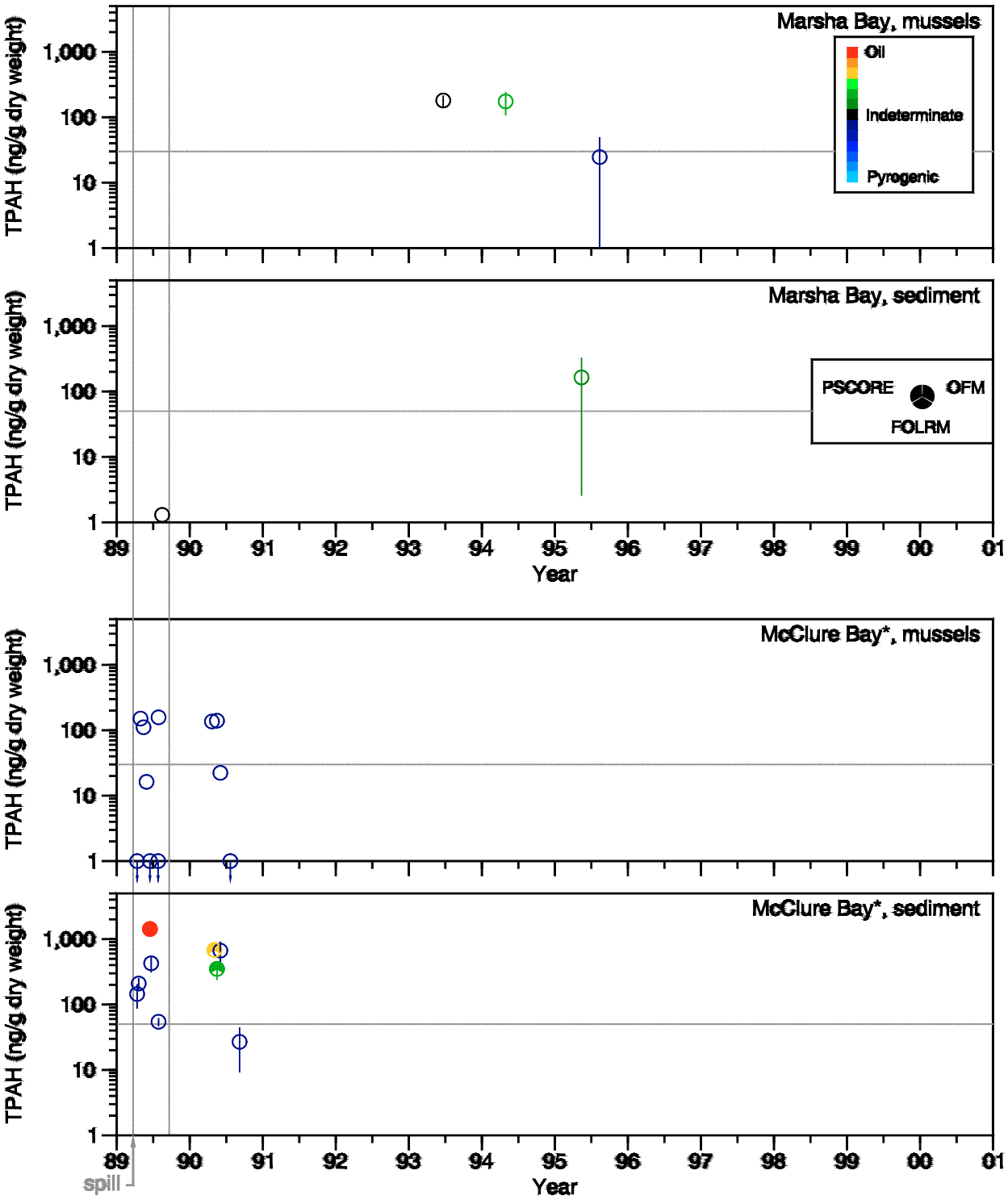

Disk Island, mussels
Disk Island, sediment
Eleanor Island, mussels
Eleanor Island, sediment
TPAH (ng/g dry weight)
1
10
100
1,000
10,000
100,000
Oil
Indeterminate
Pyrogenic
PSCORE
OFM
FOLRM
89
90
91
92
93
94
95
96
97
98
99
00
01
spill
Year

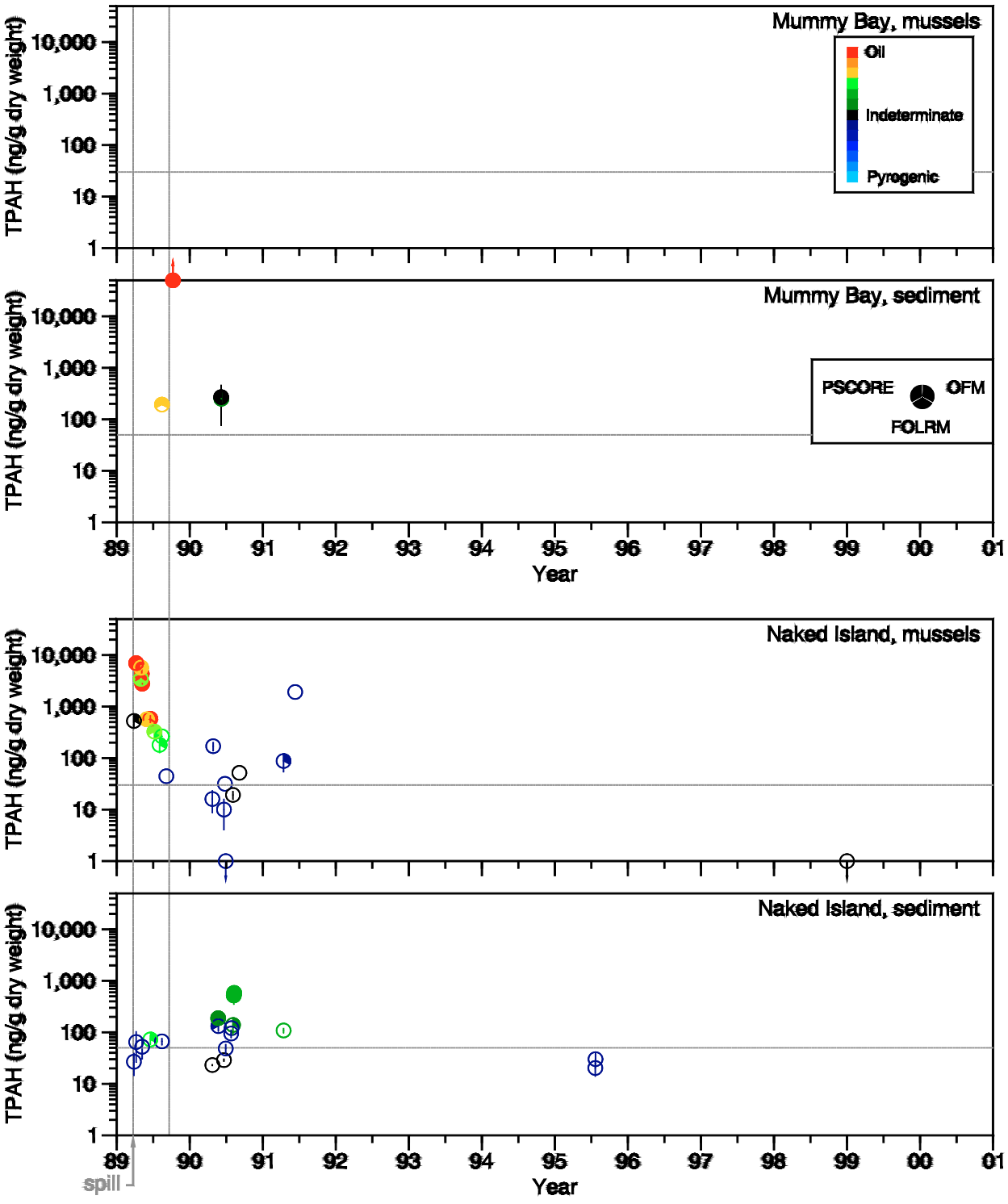

Evans Island (northern end), mussels
Evans Island (northern end), sediment
Ewan Bay*, mussels
Ewan Bay*, sediment
TPAH (ng/g dry weight)
100,000
10,000
1,000
100
10
1
89
90
91
92
93
94
95
96
97
98
99
00
01
Year
Oil
Indeterminate
Pyrogenic
PSCORE
OFM
FOLRM
spill

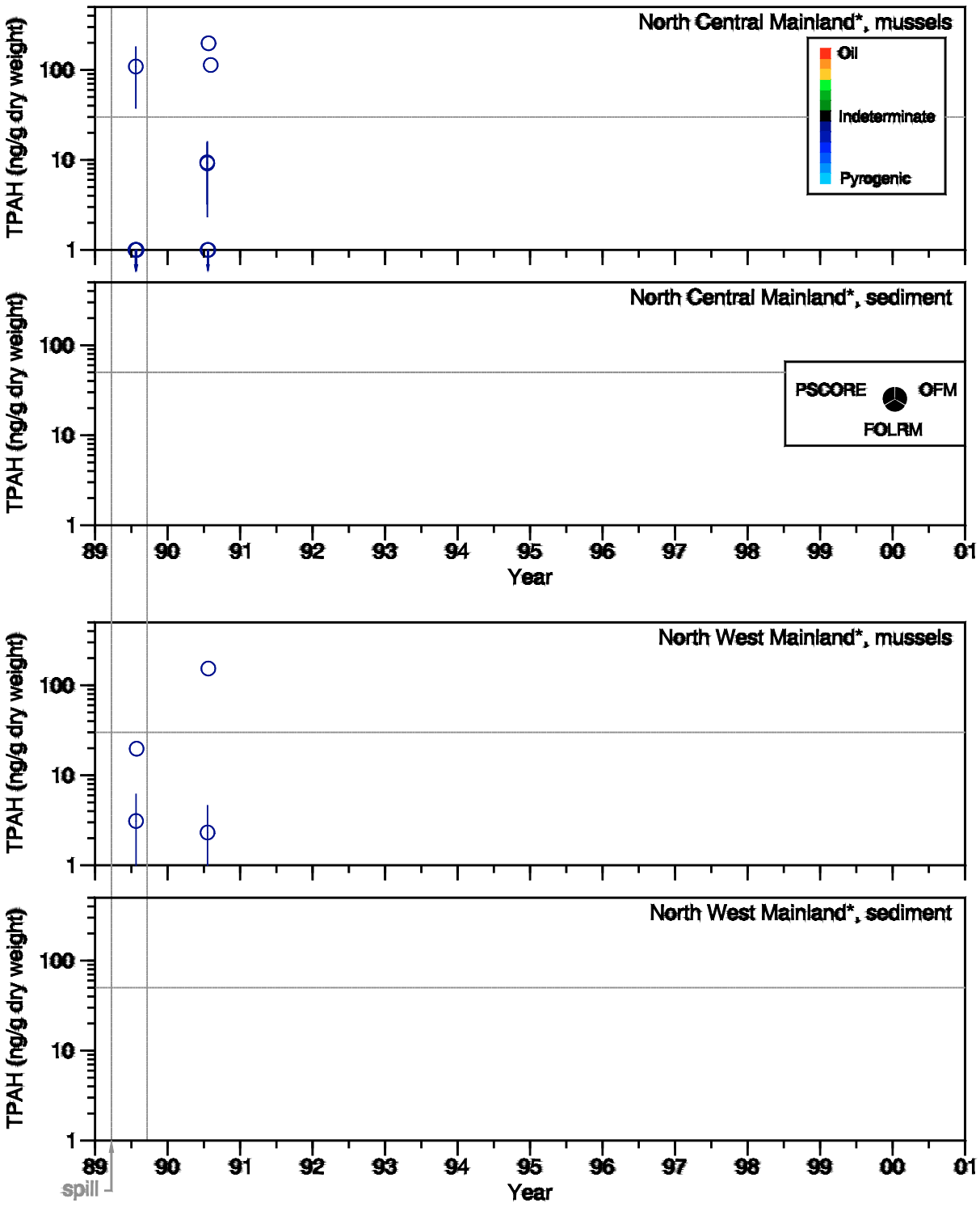

Elrington Island, mussels
Oil
Indeterminate
Pyrogenic
Elrington Island, sediment
PSCORE
OFM
FOLRM
Eshamy Bay, mussels
Eshamy Bay, sediment
TPAH (ng/g dry weight)
10,000
1,000
100
10
1
89
90
91
92
93
94
95
96
97
98
99
00
01
Year
spill

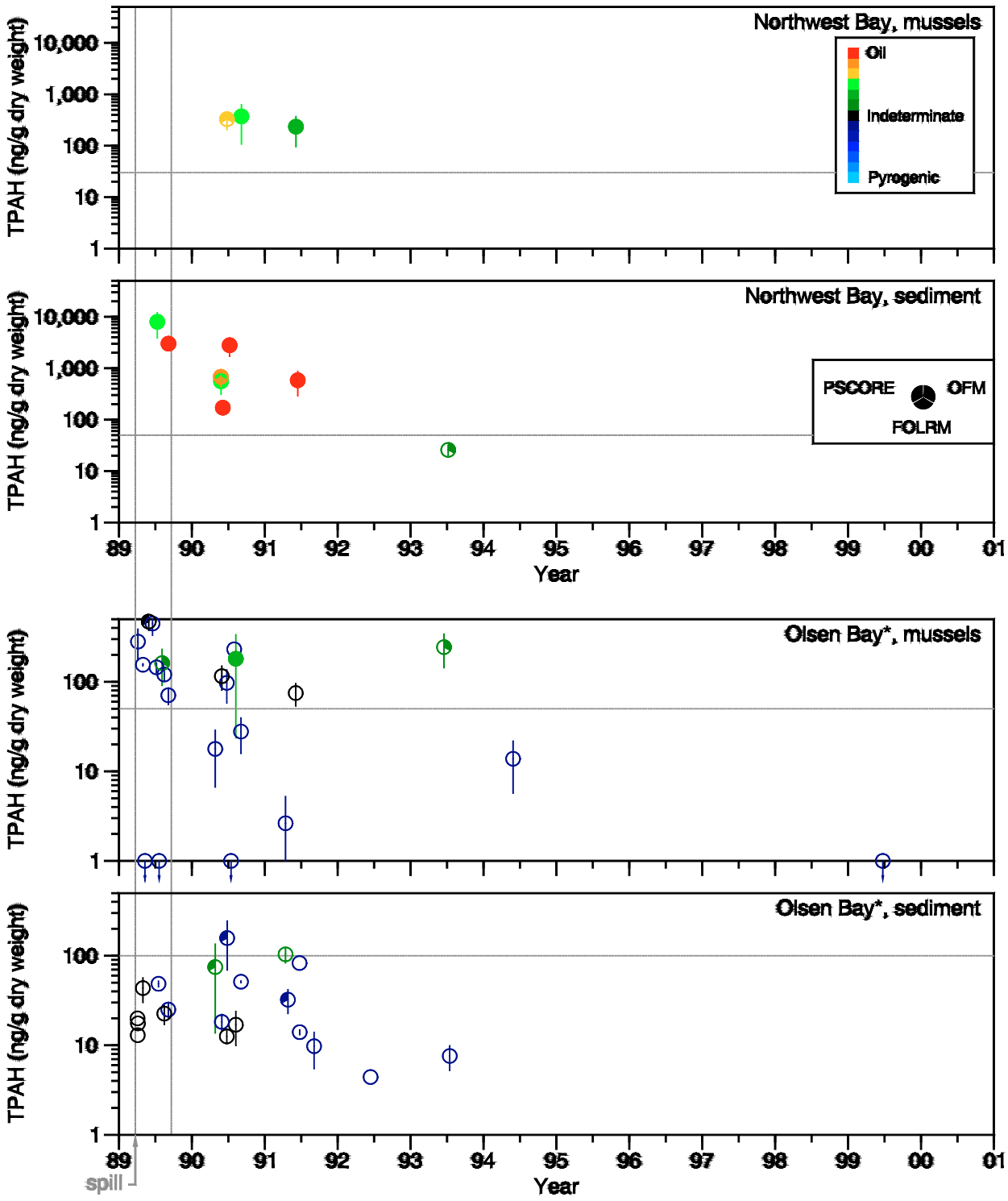

Green Island, mussels
Oil
Indeterminate
Pyrogenic
Green Island, sediment
PSCORE
OFM
FOLRM
Herring Bay, mussels
Herring Bay, sediment
TPAH (ng/g dry weight)
100,000
10,000
1,000
100
10
1
89
90
91
92
93
94
95
96
97
98
99
00
01
Year
spill

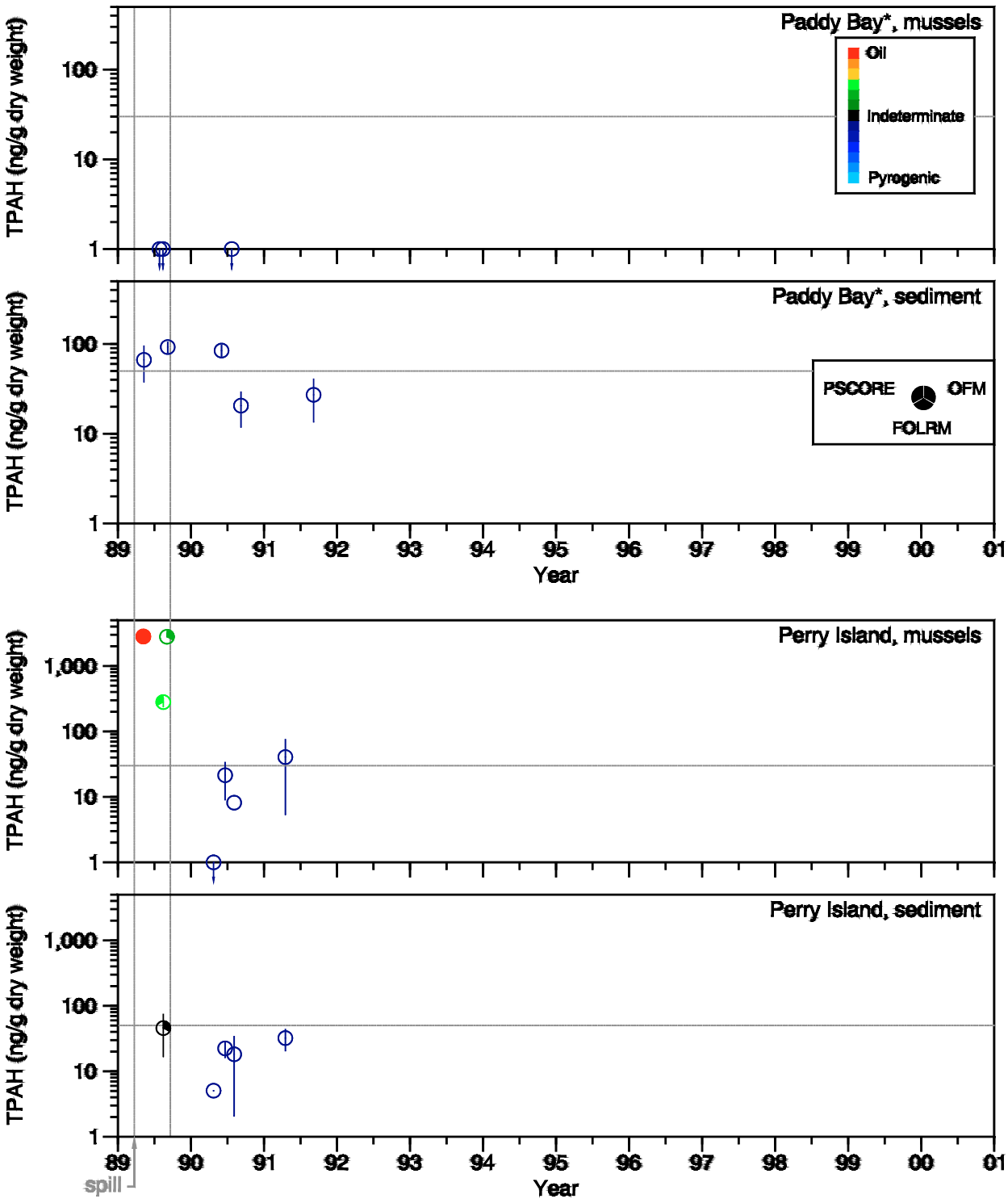

Latouche Is., mussels
(Excluding Sleepy Bay)
Latouche Is., sediment
(Excluding Sleepy Bay)
PSCORE
OFM
FOLRM
Long Bay*, mussels
Oil
Indeterminate
Pyrogenic
Long Bay*, sediment
TPAH (ng/g dry weight)
100,000
10,000
1,000
100
10
1
89
90
91
92
93
94
95
96
97
98
99
00
01
Year
spill

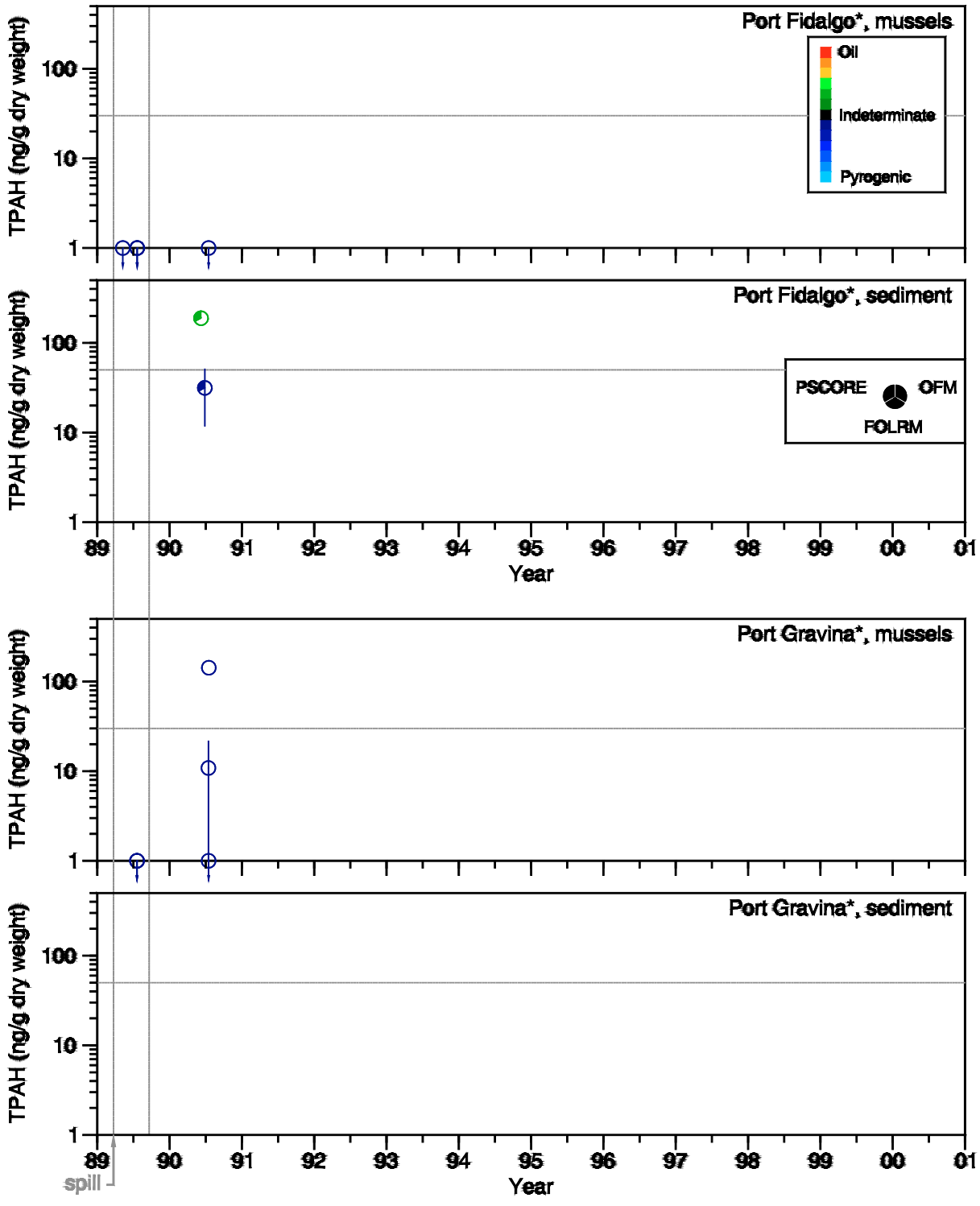

Marsha Bay, mussels
Oil
Indeterminate
Pyrogenic
Marsha Bay, sediment
PSCORE
OFM
FOLRM
McClure Bay*, mussels
McClure Bay*, sediment
TPAH (ng/g dry weight)
1,000
100
10
1
89
90
91
92
93
94
95
96
97
98
99
00
01
Year
spill

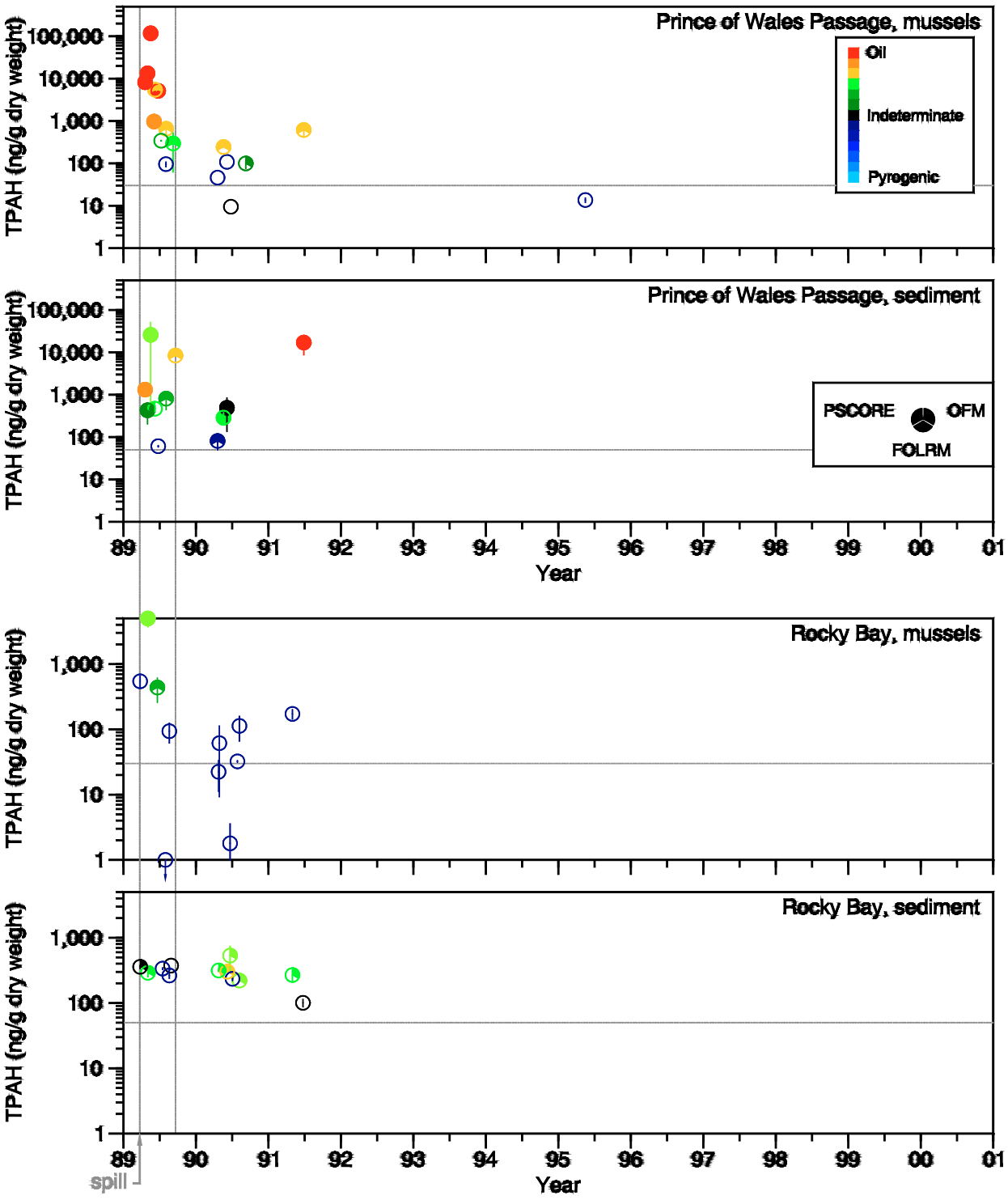

Mummy Bay, mussels
Oil
Indeterminate
Pyrogenic
Mummy Bay, sediment
PSCORE
OFM
FOLRM
Naked Island, mussels
Naked Island, sediment
TPAH (ng/g dry weight)
10,000
1,000
100
10
1
89
90
91
92
93
94
95
96
97
98
99
00
01
Year
spill

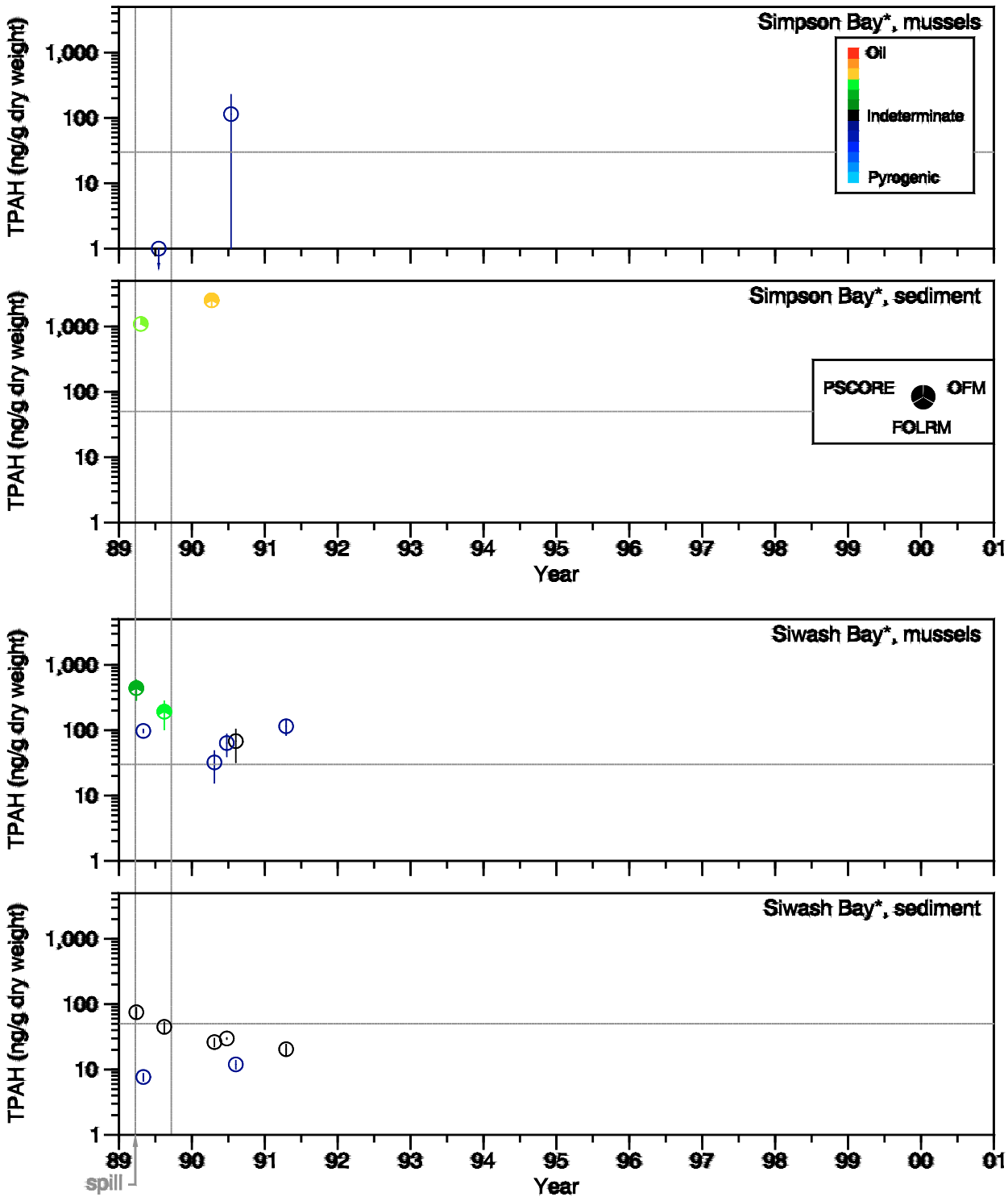

North Central Mainland*, mussels
Oil
Indeterminate
Pyrogenic
TPAH (ng/g dry weight)
100
10
1
North Central Mainland*, sediment
PSCORE
OFM
FOLRM
89
90
91
92
93
94
95
96
97
98
99
00
01
Year
North West Mainland*, mussels
North West Mainland*, sediment
spill

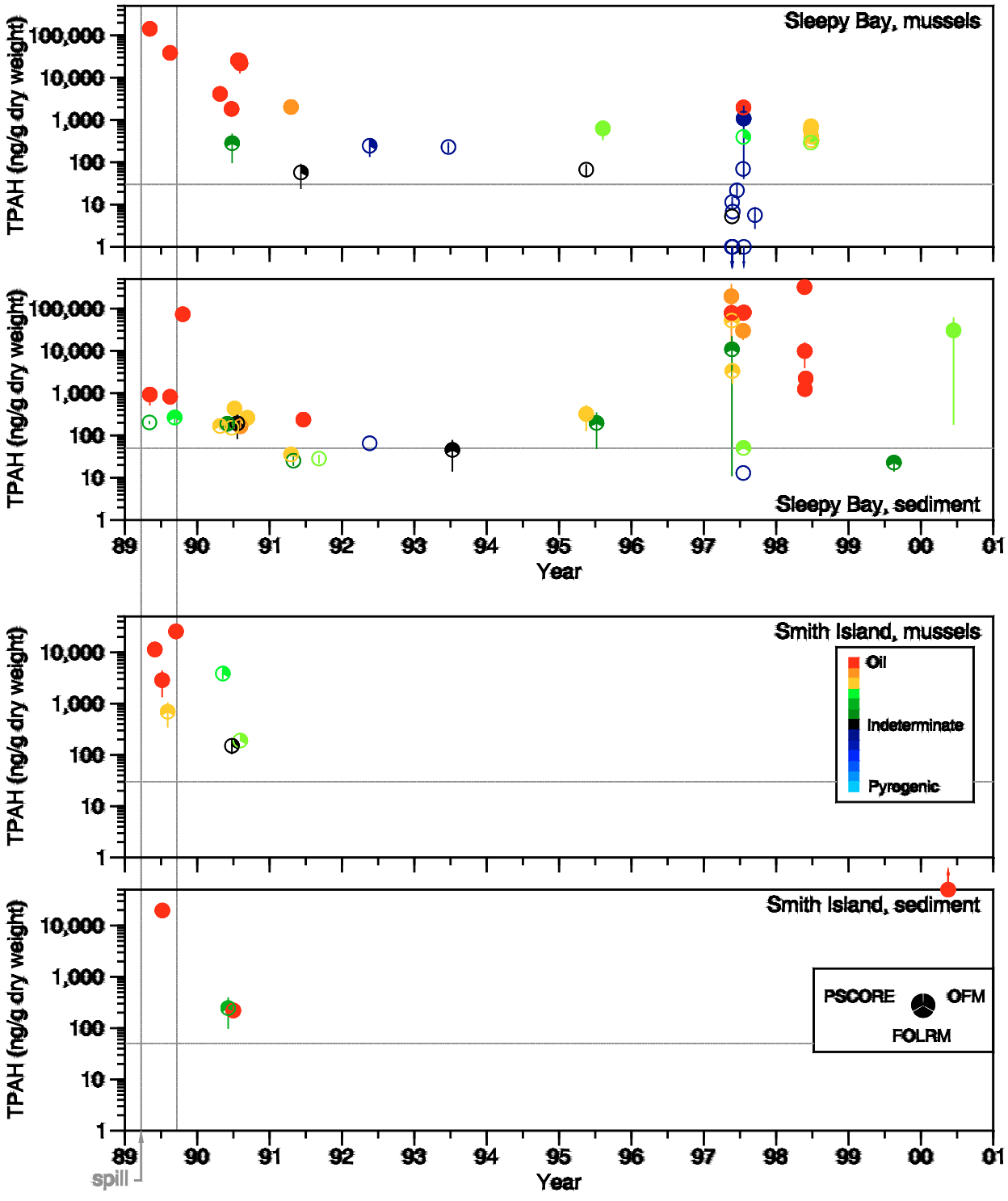

Northwest Bay, mussels
Oil
Indeterminate
Pyrogenic
TPAH (ng/g dry weight)
10,000
1,000
100
10
1
Northwest Bay, sediment
PSCORE
OFM
FOLRM
89
90
91
92
93
94
95
96
97
98
99
00
01
Year
Olsen Bay*, mussels
Olsen Bay*, sediment
spill

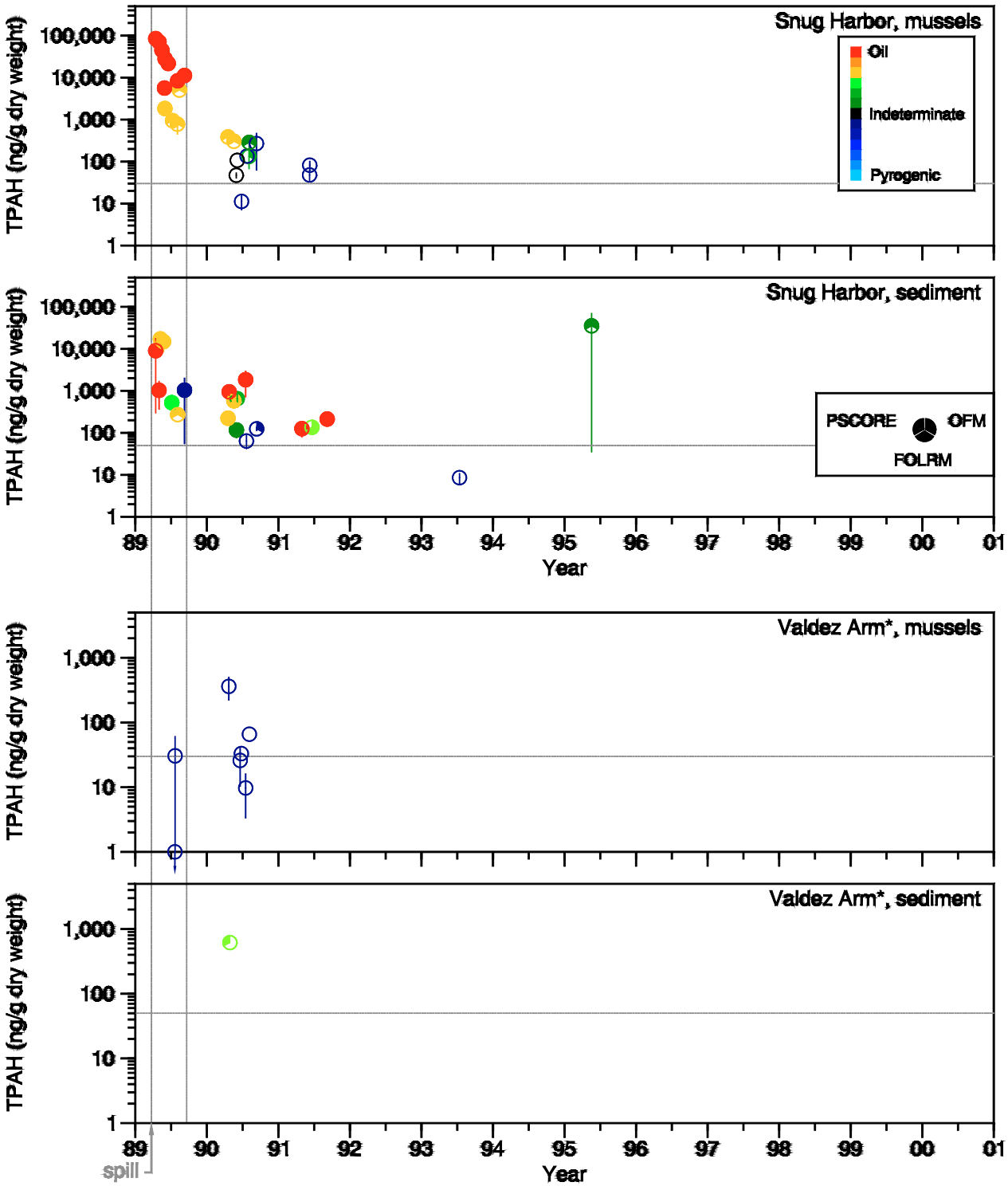

Paddy Bay*, mussels
Oil
Indeterminate
Pyrogenic
TPAH (ng/g dry weight)
100
10
1
Paddy Bay*, sediment
PSCORE
OFM
FOLRM
89
90
91
92
93
94
95
96
97
98
99
00
01
Year
Perry Island, mussels
1,000
Perry Island, sediment
spill

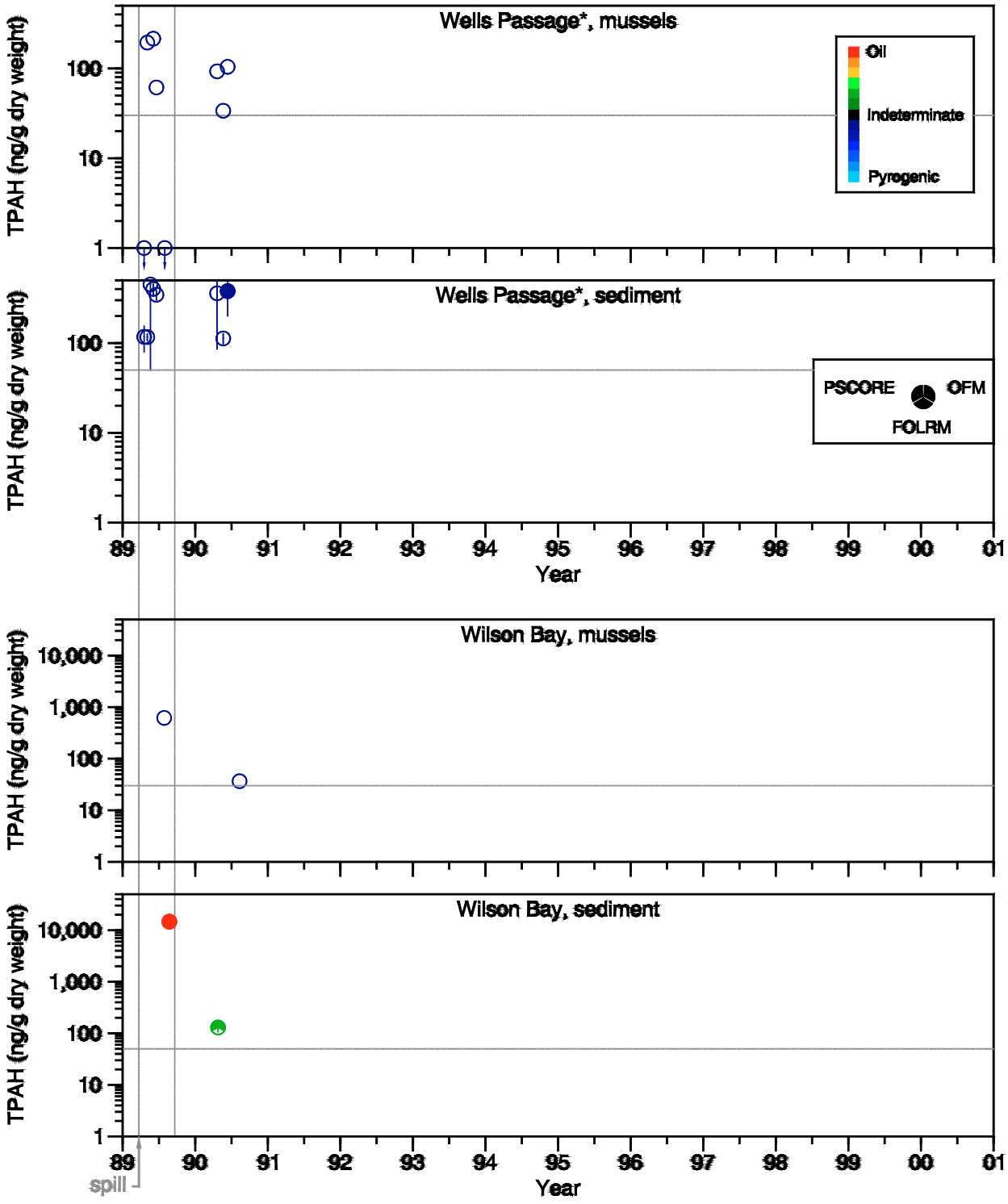

Port Fidalgo*, mussels
Oil
Indeterminate
Pyrogenic
TPAH (ng/g dry weight)
100
10
1
Port Fidalgo*, sediment
PSCORE
OFM
FOLRM
89
90
91
92
93
94
95
96
97
98
99
00
01
Year
Port Gravina*, mussels
Port Gravina*, sediment
spill


Prince of Wales Passage, mussels
Oil
Indeterminate
Pyrogenic
TPAH (ng/g dry weight)
100,000
10,000
1,000
100
10
1
Prince of Wales Passage, sediment
PSCORE
OFM
FOLRM
89
90
91
92
93
94
95
96
97
98
99
00
01
Year
Rocky Bay, mussels
Rocky Bay, sediment
spill


Simpson Bay*, mussels
Oil
Indeterminate
Pyrogenic
Simpson Bay*, sediment
PSCORE
OFM
FOLRM
Siwash Bay*, mussels
Siwash Bay*, sediment
TPAH (ng/g dry weight)
1,000
100
10
1
89
90
91
92
93
94
95
96
97
98
99
00
01
Year
spill


Sleepy Bay, mussels
Sleepy Bay, sediment
Smith Island, mussels
Smith Island, sediment
TPAH (ng/g dry weight)
100,000
10,000
1,000
100
10
1
89
90
91
92
93
94
95
96
97
98
99
00
01
Year
Oil
Indeterminate
Pyrogenic
PSCORE
OFM
FOLRM
spill

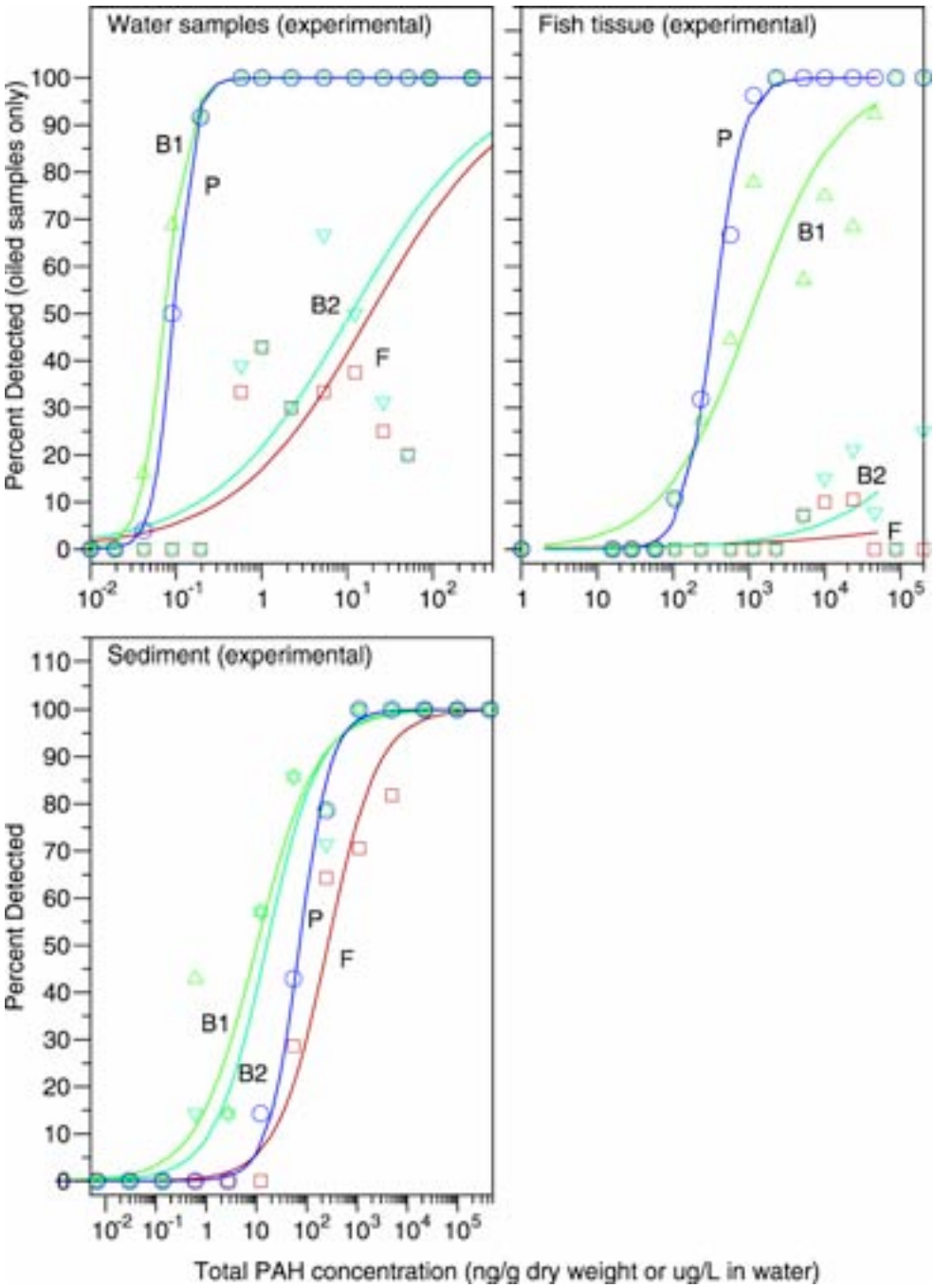

Snug Harbor, mussels
Oil
Indeterminate
Pyrogenic
Snug Harbor, sediment
PSCORE
OFM
FOLRM
Valdez Arm*, mussels
Valdez Arm*, sediment
TPAH (ng/g dry weight)
100,000
10,000
1,000
100
10
1
89
90
91
92
93
94
95
96
97
98
99
00
01
Year
spill


Wells Passage*, mussels
Oil
Indeterminate
Pyrogenic
TPAH (ng/g dry weight)
100
10
1
Wells Passage*, sediment
PSCORE
OFM
FOLRM
TPAH (ng/g dry weight)
100
10
1
89
90
91
92
93
94
95
96
97
98
99
00
01
Year
Wilson Bay, mussels
TPAH (ng/g dry weight)
10,000
1,000
100
10
1
Wilson Bay, sediment
TPAH (ng/g dry weight)
10,000
1,000
100
10
1
89
90
91
92
93
94
95
96
97
98
99
00
01
spill
Year

# Appendix 4

Additional petrogenic and pyrogenic modeling results

**Appendix 4.1.** Detection of oil in water and fish tissue experimentally exposed to Alaska North Slope crude oil by non-specific models PSCORE (P), and the $OFM_{oil}$ (B1), and ANS-specific models the $OFM_{ANS}$ (B2) and the $FORLM_{ANS}$ (F). Not illustrated are the non-specific $FORLM_{oil}$ results, which were highly similar to the $FORLM_{ANS}$ results. Plotted curves are logistic regressions. *See Fig. 4.3 for similar results in environmental matrices.*



**Appendix 4.2.** Diagnostic PAH ratios in experimental samples. A) Petrogenic ratios were: 1) methyl-P/P, 3) P/ANT, 6) fossil fuel index, 7) c/BaA, 8) low molecular weight / high molecular weight PAH, and 16) C1/C0. B) Pyrogenic ratios were 2) FLA/PYR, 4) (FLA + PYR)/ Σ(P1..P4), 5) (FLA + PYR) / (P2 + P3), 9) pyrogenic fraction, 10) percent perylene, and 11) pyrogenic index. P are probabilities, determined by ANOVA (single factor, oiled versus control for each matrix). ND = no estimable data or analysis not possible.

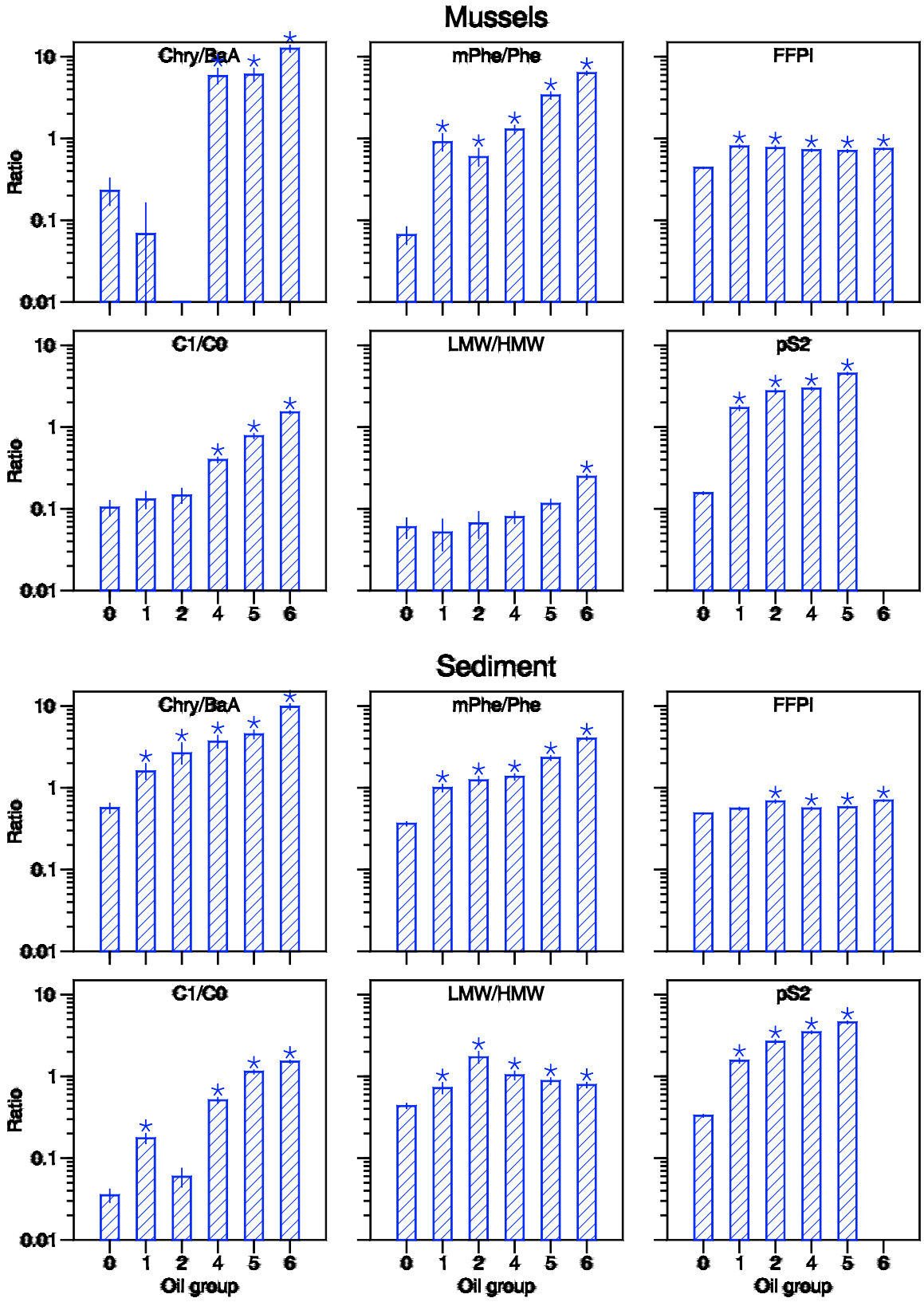
A. Petrogenic ratios

| Matrix | | r1 | r3 | r6 | r7 | r8 | r16 |
|---|---|---|---|---|---|---|---|
| **Experimentally oiled samples** | | | | | | | |
| oil | *mean* | 2.98 | 112.16 | 0.87 | 17.00 | 3.80 | 1.45 |
| | *se* | 0.07 | 41.30 | 0.01 | 4.28 | 0.25 | 0.05 |
| | *n* | 11 | 9 | 11 | 9 | 11 | 11 |
| water | *mean* | 2.46 | 69.47 | 0.79 | 13.20 | 31.51 | 0.82 |
| | *se* | 0.19 | 15.05 | 0.01 | 1.13 | 5.23 | 0.05 |
| | *n* | 86 | 56 | 104 | 24 | 89 | 88 |
| sediment | *mean* | 8.31 | 54.49 | 0.78 | 14.45 | 1.51 | 1.97 |
| | *se* | 3.05 | 18.88 | 0.01 | 1.34 | 0.17 | 0.07 |
| | *n* | 63 | 23 | 95 | 70 | 88 | 81 |
| tissue | *mean* | 1.01 | 49.68 | 0.86 | 0.00 | 47.65 | 0.23 |
| | *se* | 0.07 | 2.49 | 0.01 | 0.00 | 6.67 | 0.07 |
| | *n* | 229 | 58 | 338 | 1 | 93 | 87 |
| **Control samples** | | | | | | | |
| water | *mean* | 0.26 | ND | 0.79 | 0.00 | 0.38 | ND |
| | *se* | 0.16 | | 0.03 | 0.00 | 0.13 | |
| | *n* | 5 | | 26 | 7 | 12 | |
| sediment | *mean* | 0.27 | ND | 0.45 | 2.70 | 4.35 | 0.00 |
| | *se* | 0.09 | | 0.07 | 1.22 | 0.84 | 0.00 |
| | *n* | 13 | | 22 | 9 | 16 | 6 |
| tissue | *mean* | 0.54 | 1.28 | 0.71 | ND | 1.55 | 0.35 |
| | *se* | 0.15 | 0.00 | 0.03 | | 0.00 | 0.00 |
| | *n* | 21 | 1 | 59 | | 1 | 1 |
| **P** | | | | | | | |
| water | | 0.000 | ND | 0.593 | 0.000 | 0.000 | ND |
| sediment | | 0.000 | ND | 0.000 | 0.000 | 0.001 | 0.000 |
| tissue | | 0.041 | ND | 0.000 | ND | ND | ND |

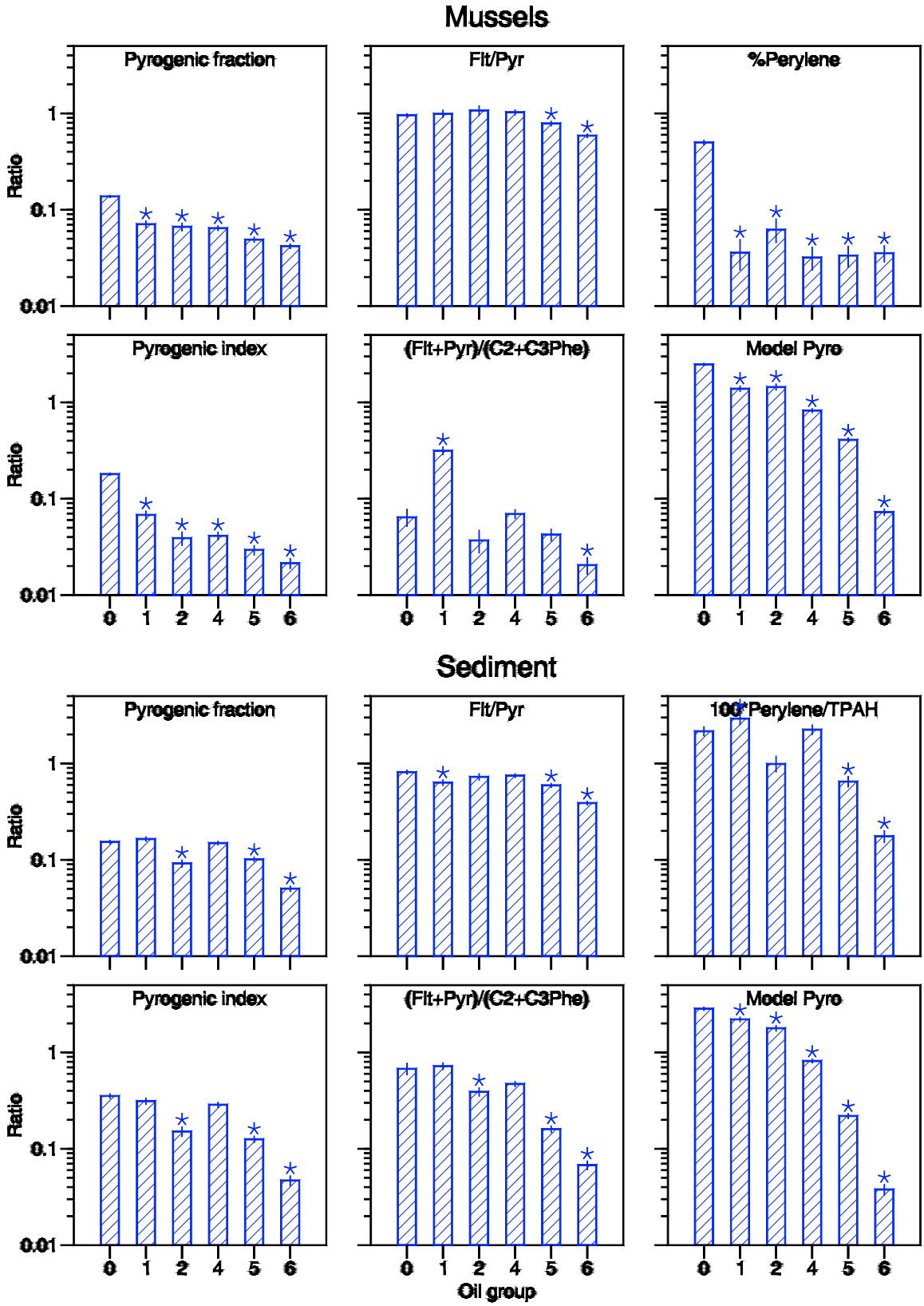
B. Pyrogenic ratios

| Matrix | | r2 | r4 | r5 | r9 | r10 | r11 |
|---|---|---|---|---|---|---|---|
| **Experimentally oiled samples** | | | | | | | |
| oil | *mean* | 0.537 | 0.008 | 0.013 | 0.009 | 0.005 | 0.005 |
| | *se* | 0.120 | 0.001 | 0.002 | 0.001 | 0.004 | 0.001 |
| | *n* | 10 | 11 | 11 | 11 | 11 | 11 |
| water | *mean* | 0.606 | 0.042 | 0.067 | 0.048 | 0.070 | 0.047 |
| | *se* | 0.050 | 0.006 | 0.009 | 0.006 | 0.014 | 0.007 |
| | *n* | 98 | 104 | 104 | 104 | 104 | 104 |
| sediment | *mean* | 0.402 | 0.013 | 0.020 | 0.031 | 0.012 | 0.022 |
| | *se* | 0.045 | 0.002 | 0.004 | 0.004 | 0.004 | 0.004 |
| | *n* | 90 | 95 | 95 | 95 | 95 | 95 |
| tissue | *mean* | 0.875 | 0.271 | 0.285 | 0.031 | 0.260 | 0.041 |
| | *se* | 0.062 | 0.146 | 0.089 | 0.002 | 0.191 | 0.008 |
| | *n* | 119 | 293 | 272 | 339 | 339 | 339 |
| **Control samples** | | | | | | | |
| water | *mean* | 2.195 | 0.438 | 0.947 | 0.181 | 0.576 | 0.260 |
| | *se* | 0.542 | 0.077 | 0.202 | 0.020 | 0.105 | 0.035 |
| | *n* | 24 | 24 | 24 | 27 | 27 | 27 |
| sediment | *mean* | 1.991 | 1.298 | 2.559 | 0.431 | 0.399 | 0.759 |
| | *se* | 0.215 | 0.311 | 0.794 | 0.062 | 0.152 | 0.193 |
| | *n* | 23 | 19 | 19 | 24 | 24 | 22 |
| tissue | *mean* | 1.789 | 0.274 | 1.199 | 0.117 | 0.212 | 0.167 |
| | *se* | 0.243 | 0.056 | 0.369 | 0.007 | 0.089 | 0.028 |
| | *n* | 18 | 47 | 23 | 60 | 60 | 60 |
| **P** | | | | | | | |
| water | | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| sediment | | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| tissue | | 0.000 | 0.995 | 0.005 | 0.000 | 0.917 | 0.000 |

**Appendix 4.3.** Correspondence between combined oil model assessment (oil group) and mean diagnostic petrogenic ratios. Significant differences between the non-oiled group (0) and others are indicated with asterisks (single-factor ANOVA).



**Appendix 4.4.** Correspondence between combined oil model assessment (oil group) and mean diagnostic pryogenic ratios. Significant differences between the non-oiled group (0) and others are indicated with asterisks (single-factor ANOVA).



**Appendix 4.5.** Comparison of model results in samples containing various oils, pyrogenic hydrocarbons, and other sources. Alaska North Slope crude oil is abbreviated as ANS; WAF is water-accommodated fraction, ANS dispersed is a chemically dispersed WAF (Corexit 9527). Matrices sampled include sediment, water, and low density polyethylene passive sampler devices (PEMDs); question marks identify inferred but unknown matrices. [a]Burns et al. (1997) (fresh to heavily weathered), [b]Barron et al. (2003), [c]Short et al. (2005), [d]Carls (unpublished data), [e]Short et al. 1996, [f]Neff (2002), [g]Springman (personal communication). Pyrogenic estimates were scored significant when values exceeded the 1% threshold (Table 3, main text). The alternative summary score includes all six petrogenic model outputs; the petrogenic summary does not include $FORLM_{oil.}$ The combined pyrogenic and petrogenic summary score[1] includes all models, range -6 to 6; negative values indicate pyrogenic dominance, positive values indicate petrogenic dominance. As with other summary scores, the greater the magnitude, the more likely the identified characteristic.

| Matrix | ANS[a] sediment | ANS[b] WAF water | ANS[b] dispersed water | Diesel[c] fuel PEMD | Katalla[d] seep oil PEMD | Constantine[e] Background sediment | Monterey[e] oil pure | Selendang[d] bunker oil PEMD | Creosote[a,f,g] water, pure | Soot[a] & dust particulate |
|---|---|---|---|---|---|---|---|---|---|---|
| n | 5 | 7 | 8 | 12 | 8 | 16 | 11 | 26 | 3 | 3 |
| **TPAH (ppb)** | ng/g | ug/L | ug/L | ng/g | ng/g | ng/g | ng/g | ng/g | | ng/g |
| min | 3705 | 8.5 | 2.3 | 154 | 581 | 494 | 11800 | 228 | 447 ug/l | 249 |
| max | 401000 | 286 | 469 | 35500 | 17700 | 726 | 1420000 | 463000 | 303000 ng/g | 70000 |
| **Percent scored as petrogenic (non-specific models)** | | | | | | | | | | |
| $FORLM_{oil}$ | 100 | 100 | 88 | 42 | 63 | 94 | 100 | 58 | 0 | 33 |
| $OFM_{oil}$ | 100 | 100 | 100 | 100 | 75 | 100 | 100 | 100 | 100 | 100 |
| $PSCORE_{oil}$ | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| **Percent scored as ANS** | | | | | | | | | | |
| $FORLM_{ANS}$ | 100 | 100 | 75 | 17 | 0 | 6 | 45 | 50 | 0 | 0 |
| $OFM_{ANS}$ | 100 | 100 | 88 | 58 | 63 | 100 | 100 | 58 | 67 | 100 |
| $PSCORE_{ANS}$ | 100 | 100 | 38 | 42 | 100 | 88 | 100 | 96 | 0 | 0 |
| **Percent scored as pyrogenic** | | | | | | | | | | |
| Pyrogenic model | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100 | 0 |
| (fla+pyr)/Σ(p1..p4) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 67 | 67 |
| **Summary scores** | | | | | | | | | | |
| Petrogenic ($S_e$) | 6.0 | 6.0 | 4.9 (2 to 6) | 3.8 (2 to 6) | 4.6 (4 to 5) | 4.9 (4 to 6) | 5.5 (5 to 6) | 5.0 (2 to 6) | 3.3 (2 to 4) | 4.0 |
| Pyro- & petrogenic ($S_T$) | 6.0 | 6.0 | 4.9 (2 to 6) | 3.8 (2 to 6) | 4.6 (4 to 5) | 4.9 (4 to 6) | 5.5 (5 to 6) | 5.0 (2 to 6) | -2.3 (-3 to -2) | 1.3 (1 to 2) |

[1] Pyrogenic & petrogenic summary score = Alternative petrogenic score minus 3 * combined pyrogenic score. The factor 3 is included to scale pyrogenic scores (range 0 to 2) equally with petrogenic scores (range 0 to 6).

**References**

Burns, W. A.; Mankiewicz, P. J.; Bence, A. E.; Page, D.S.; Parker, K.R. Environ. Toxicol. Chem. 1997, 16, 1119-1131.

Barron, M. G.; Carls, M. G.; Short, J. W.; Rice, S. D. Photoenhanced Toxicity of Aqueous Phase and Chemically-Dispersed Weathered Alaska North Slope Crude Oil to Pacific Herring Eggs and Larvae. Environ. Toxicol. Chem. 2003, 22, 650-660.

Neff, J. M. Bioaccumulation in marine organisms. Effect of contaminants from oil well produced water. Elsevier, Boston. 2002, 452 pp.

Short, J. W.; Nelson, B. D.; Heintz, R. A.; Maselko, J. M.; Kendziorek, M.; Carls, M. G.; Korn, S. Mussel tissue and sediment hydrocarbon data synthesis, 1989 - 1995. *Exxon Valdez* Oil Spill State/Federal Natural Resource Damage Assessment Final Report, 1996, Subtidal Study Number 8, 132 p.

Short, J. W.; Rice, S. D.; Lindeberg, M. R.; Carls, M. G. Use of polyethylene membrane devices for monitoring diesel oil contamination on and within beaches. Proceedings of the 31st Annual Aquatic Toxicity Workshop, Charlestown, Prince Edward Island, Canada, Canadian Department of Fisheries and Oceans, 2005.

Springman K. Personal communication. Center for Health and the Environment, University of California, Davis.