Exxon Valdez Oil Spill
Restoration Project Final Report

Mussel Bed Restoration and Monitoring

Restoration Project 95090
Final Report

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Study history: The persistence of *Exxon Valdez* crude oil underlying dense mussel (*Mytilus trossulus*) beds on unconsolidated sediment in Prince William Sound and along the Gulf of Alaska began to cause concern in the spring of 1991. Contamination was confirmed in surveys by Auke Bay Laboratory (National Marine Fisheries Service) and National Park Service personnel. This project was funded from 1992 through 1997 under Trustee Council studies R103, 93036, 94090, 95090, 96090, and 97090.

Abstract: Many mussel beds in the spill area, particularly those on soft sediment, were not cleaned immediately after the *Exxon Valdez* oil spill in 1989. Consequently these beds had high concentrations of contaminated sediments and tissues. Surveys documented the geographic extent (primarily Prince William Sound and the Kenai Peninsula) and intensity of oiling. Hydrocarbon concentrations declined naturally from 1992-1995 in some, but not all beds. Distribution of oil in sediments was related to tidal elevation, depth, and grain size. Oil concentration in mussels correlated with that in sediment. Mussel condition was adversely affected by oil; prevalence of digestive gland metaplasia, brown cells, and hemocytic infiltrates in gonads increased, and storage cell abundance decreased. However, some physiological responses (byssal thread production, condition index, feeding rate, or glycogen content) in mussels contaminated 3-4 years were not correlated with oil concentration. Bed restoration caused immediate reductions in oil concentration in surface sediment, but these sediments were later partially recontaminated by remaining oil. Restoration efficacy was less evident in mussels; concentration reductions were significant in less than half the beds by the end of study. Mussel densities declined in one-half of the beds after restoration, but density declines were similar in untreated reference beds.

Key words: mussel bed, *Exxon Valdez* oil, petroleum hydrocarbon, restoration, monitoring, persistence, distribution, physiology, histology, *Mytilus trossulus*

Project data: *Description of data* - Hydrocarbon data are available in the State/Federal trustee council hydrocarbon database 1989-1995 (EVTHD). All other data are archived in spreadsheets; Graphics files are in Freelance and AutoCAD formats. Text files are in WordPerfect 6.1 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 E-mail pat.harris@noaa.gov. *Availability* - Copies of all data and related text files are available on CDROM for the cost of duplication.

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EXECUTIVE SUMMARY

Large quantities of crude oil spilled from the TV *Exxon Valdez* in 1989 were deposited intertidally on beaches in Prince William Sound, the Kenai and Alaska Peninsulas, and the Kodiak Archipelago. Soon after the spill, a cleanup effort was launched to remove oil stranded in intertidal areas, which involved extensive use of hydraulic treatment methods. Approximately one-third of all shoreline segments in western Prince William Sound were washed with high-pressure hot water; much of the marine life so treated perished. Because aggressive cleanup was often devastating, some of the mussel beds located on soft sediments were not cleaned. Expectation at the time of this decision was that concentrations of oil under these beds would decline fairly rapidly as the oil weathered, and that it was better to preserve the integrity of the beds and underlying sediments than to destroy them.

By 1991 it was obvious that substantial quantities of petroleum hydrocarbons from the *Exxon Valdez* oil spill remained trapped in soft sediments underlying untreated dense mussel beds. Contaminated mussels were a potential source of chronic hydrocarbon exposure for vertebrate predators. Surveys were initiated to establish the geographic extent and intensity of oiling, and to monitor annual hydrocarbon changes in selected mussel beds (1992-1995).

Distribution of oil

The geographic distribution of mussel beds with significant oil contamination after 1991 included most areas originally impacted by the spill in Prince William Sound and along the Kenai Peninsula, but most of the contaminated mussel beds in PWS were located within the Knight Island group, an area particularly impacted by the *Exxon Valdez* oil spill. Oil also persisted in one bed on the AP (Cape Nukshak) through at least 1993. Concentrations of total petroleum hydrocarbons in sediments ranged from less than 100 μg/g in unoiled reference beds to 62,000 μg/g wet weight in 1992. Polynuclear aromatic hydrocarbon concentrations greater than 8 μg/g dry weight were observed in some mussels (1992), and were among the highest concentrations measured in tissue of any species after the spill.

Not only was oil distributed unevenly on a broad geographic scale, distribution was also uneven within untreated mussel beds in Prince William Sound. Distribution of oil in sediments was correlated with several factors, including sediment elevation, depth, and grain size. (Elevation and grain size were also correlated.) Oil from trapped sediments continued to recontaminate mussels as it dispersed from sediments into water surrounding the mussels. Patterns of oil distribution and composition in mussels and sediment within each bed suggested that *Exxon Valdez* oil dispersed from sediments in particulate form into surrounding water, and was accumulated by nearby mussels. On the average, oil in these sediments was moderately weathered in 1992. Oil in mussels was more weathered than in sediments, consistent with increased exposure to surrounding water. Chronic, long-term contamination of mussels may have adversely impacted other species dependent on them for food or habitat.

Time-dependent changes in oil concentration and composition

Hydrocarbon concentrations declined with time in some, but not all mussels and sediments. Significant natural reductions in hydrocarbon concentration were observed in roughly half of the beds surveyed. Concentrations should reach background levels within three decades of the spill in these beds. However, storm events can significantly rework sediments and expose buried oil. Such a disturbance was documented after the winter of 1992-93 at Eleanor Island. Storm disturbances and paucity of information on the volume and composition of oil buried deeper than 2 cm limits the predictability of oil persistence.

Composition of PAH was generally consistent with weathered *Exxon Valdez* oil indicating that *Exxon Valdez* oil was the source of contamination. The source of oil in bed sediments and mussels was confirmed with a model designed to determine if PAH composition was consistent with that in weathered EVO. The degree of weathering increased in some samples, but decreased in others, possibly because storm activity re-exposed fresh oil. Condition of the oil ranged from unweathered to highly weathered, and relatively unweathered oil was detected in each year for which we have compositional analyses (through 1995).

Physiological and histological consequences of exposure to oil

The physiological condition of mussels chronically exposed to residual *Exxon Valdez* oil in Prince William Sound was surveyed in 1992 and 1993. Originally there was little interest in determining damage to the ubiquitous mussels, until it was feared that continued high tissue concentrations could impact higher vertebrate predators through oil accumulation, or through a loss in prey population because of chronic oiling. At some of the oiled beaches, mussels were collected from beds overlying oiled sediments, and also from relatively uncontaminated adjacent bedrock. Mussels were also collected from beaches that had not been impacted by the oil spill. Polynuclear aromatic hydrocarbon concentrations in mussel tissue, as well as stress-related physiological responses (byssal thread production, condition index, clearance rate, and glycogen content), were determined for each group of mussels. Total polynuclear aromatic hydrocarbon concentrations in tissue ranged from 0-6 ppm, with the low values coming from control sites, and the highest concentrations from sites that were oiled following the spill. No significant differences were noted in byssal thread production, condition index, feeding rate, or glycogen content between oiled sample sites and control sites. The lack of physiological response was surprising because mussels in this study were chronically exposed to petroleum hydrocarbons for 3 to 4 years, and none of the measured physiological responses appeared to be impacted by that exposure. Lack of physiological response to oil could not be explained by postulating that natural variability between sample sites masked response, because differences were also absent within sites where uncontaminated and contaminated mussels coexisted. The lack of response may be explained by development of tolerance to PAH, but a subsequent study has found that long-term oil exposure reduces the ability of mussels to survive in air.

Although physiological response to chronic oil exposure was not detected, there were significant histological changes in mussel tissue. Oiled mussels were collected for histopathological examination from relatively dense mussel beds overlying contaminated sand

and gravel. Additional mussels were collected from uncontaminated control sites or unoiled bedrock adjacent to oiled beds. Examination was limited to a single collection trip in June 1993, except mussels for one control site (Barnes Cove) were collected in June 1992. Collection sites were categorized according to polynuclear aromatic hydrocarbon concentration in mussel tissue and location history as control, low, medium, or high oil. Mussel condition was reduced in oiled beds, as demonstrated by significantly increased prevalence of digestive gland metaplasia, increased prevalence of brown cells, decreased abundance of storage cells, and increases in hemocytic infiltrates in gonads. Holocrine activity in kidneys was also elevated by oil exposure, but not significantly. Prevalence of trematode infections was significantly elevated in oiled beds, but prevalence of two other infections, rickettsial organisms, and ectocommensal ciliates, was significantly reduced in oiled beds. Similar cell and tissue changes have been routinely reported in other molluscs from polluted environments. We concluded that mussels in beds overlying oil-contaminated soft sediment were negatively impacted by exposure to oil, and had not fully recovered from the *Exxon Valdez* spill by mid 1993.

Mussel-bed Restoration

Need for restoration of contaminated mussel beds became obvious because of the persistence of *Exxon Valdez* oil under identified mussel beds, accumulation of petroleum hydrocarbons by mussels, demonstrably negative oil impacts on mussel tissue, and concern that chronic, long-term contamination of mussels may have been adversely impacting marine vertebrates dependent on them for food.

Restoration efforts were first investigated with two small-scale, minimally intrusive experimental methods. These methods, designed to reduce persistently high hydrocarbon concentrations without decreasing mussel density, were tested in several mussel beds oiled by the *Exxon Valdez* spill in Prince William Sound. Linear strips (0.3 m wide) of mussels and attached sediments were removed to increase natural flushing of the beds. Total hydrocarbon concentrations in sediments were not significantly reduced by stripping and adult mussels from the surrounding bed recolonized exposed areas within three months, thus preventing further hydrocarbon flushing. We also transplanted several small patches (0.25 $\times$ 0.50 m) of mussels from two oiled beds onto nearby clean sediments. Transplanted mussels depurated hydrocarbons quickly; within three months total polynuclear aromatic hydrocarbon loads were about 10% of pre-transplant levels, but concentrations in undisturbed mussels remained about the same. However, mortality of transplanted mussels was high, possibly due to placement of mussels into suboptimal habitat or senescence of mature mussels. Neither technique fulfilled both criteria (bed-wide reduction of hydrocarbon concentrations and mussel survival) for successful restoration. These preliminary experimental manipulations suggested more aggressive mussel bed manipulation would be necessary to accelerate the rate of hydrocarbon loss from them, but the cost of treatment might be increased mussel mortality.

After considering the results of experimental restoration methods, we began manual restoration of nine mussel beds still contaminated with *Exxon Valdez* oil in July and early August 1994. Oiled mussels were moved from bed surfaces, the exposed surface sediment was replaced with clean sediment, then the mussels were placed onto the clean sediment. Hydrocarbon

concentrations in surface sediments were generally quickly lowered by restoration activity. Hydrocarbon concentrations in deeper, underlying sediment also generally declined, but declines were not always significant, and there was some evidence within the two-year study period that surface sediments reacquired oil from deeper sediment. Restoration efficacy was less evident in mussels. Hydrocarbon concentrations declined in mussels, but reductions were never significant in the first year, and concentrations were reduced significantly in less than half of the beds by the end of study. However, concentrations in mussel tissue declined below the estimated background concentration (0.09 μg/g) in 6 of 9 beds at the end of study.

On the average, restoration efforts were partially successful in reducing hydrocarbon concentrations in sediments more rapidly than natural processes, but effects on mussels was less clear. High inter-bed variability in hydrocarbon concentrations and concentration changes, the small number of oiled, unmanipulated reference beds, and infrequent sampling of reference beds hampered comparisons between manipulated and reference beds. Thus, the best evidence we can offer for accelerated hydrocarbon reductions as a consequence of restoration activity are site-specific differences between predicted and actual rates. Hydrocarbon concentrations in the surface sediment in half the restored beds declined more rapidly than predicted by natural rates of decline. However, the separation of concentration declines in restored beds from the regional declines evident in unmanipulated, oiled reference beds was not always possible. Post-restoration hydrocarbon loss rates from mussels were consistently more rapid than predicted in the three beds where natural loss rates were previously modeled.

The restoration methods employed in this study probably caused some mussel mortality, but changes in mussel density following restoration activity could not be statistically distinguished from regional declines in density. In preliminary mussel relocation experiments, handling mortality was high, possibly due to relocation of mussels into marginal habitat (Harris et al. Chapter 2). Although we expected some mortality as a resultant of restoration activity, mortality was far less than the catastrophic mortality caused by the cleaning methods employed in 1989 and 1990 to remove *Exxon Valdez* oil from shorelines. Although we have a less than satisfactory understanding of mussel mortality caused by our restoration activity, there was no evidence of mass mortality.

Recommendations

We recommend continued survey efforts, roughly every 3 years, to monitor oil concentrations in remaining contaminated sediments and mussels. These surveys would provide valuable service to the public, and could provide closure on the subject of persistent contamination as various beds reach background hydrocarbon levels. Surveys would also provide a scientifically valuable time series record, useful for decision making in future spills.

Mussel beds are a valuable natural resource that should be cleaned if extensive areas are contaminated with oil, as happened in PWS after the EVO spill. Mussel beds are too valuable to leave uncleaned because they are capable of trapping oil for long periods of time, but they are too fragile to allow destructive cleaning processes such as hot water washes. We recommend that mussels be manually removed from contaminated soft substrates and placed in floating pens

where they can depurate oil and survive while intrusive cleaning procedures are applied on shore. Cleanup procedures might involve backhoes or other appropriate equipment, as used in some cases to clean beaches oiled with EVO. Alternatively, after mussel removal, the substrate could be hot-water washed, chemically treated, or fertilized to promote bacterial degradation. Treatments designed to clean the relocated mussels should also be considered; possibly oil adherent to mussel shells could be removed with a brief d-limonene wash in air while shells are closed followed by appropriate seawater rinsing. Once cleaning processes are 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.

Perspective

Crude oil will persist in protected sediments for a long time, and is a source of chronic contamination for overlying mussel beds. Furthermore, contaminated mussels are a potential source of contamination for species that prey upon them, including vertebrates (e.g., otters, birds, and man), and species that utilize mussel beds as habitat. The weathering of trapped oil is highly variable, and some protected reservoirs remained relatively unweathered through 1995. Highly weathered oil was also observed, but weathering does not necessarily decrease the toxicity of remaining oil. Rather, weathered oil can be considerably more toxic per unit mass than unweathered oil because the most toxic compounds are also the most refractory, as clearly demonstrated in associated teleost egg studies. Only when these highly toxic compounds - including phenanthrenes and chrysenes - are gone will the toxicity of residual oil decline. Hydrocarbon concentrations declined over time under natural conditions, but three decades or more may elapse before concentrations reach background levels in some beds.

The experience of this study and the many other cleanup efforts associated with the *Exxon Valdez* oil spill in PWS, coupled with observation of long-term (decadal) persistence of oil in certain areas, suggests that it is impractical for humans to ever thoroughly restore intertidal habitat to pristine conditions after a major oil spill. Cleanup efforts will be resource and labor intensive, and likely only partially successful. Our technique may have some utility for beach cleanup, but the questions such as 'how clean is clean enough?' will always be problematic. Thus, the same ambiguities faced in the *Exxon Valdez* case will likely confront future spill-response decision makers.

INTRODUCTION

The main body of this report is subdivided into six chapters, each of which represents a complete study. Some of these studies have been published or submitted for publication; previously unpublished chapters will also be submitted for peer-reviewed publication. Here we introduce the topics of each chapter; salient findings are presented in the executive summary. Introductions specific to each study, with appropriate references to previous literature, are included with each chapter.

The six studies described in this report present 1) geographic extent and intensity of mussel bed contamination by *Exxon Valdez* crude oil[1], 2) distribution of polynuclear aromatic hydrocarbons in sediments and overlying mussels three years after the spill[2], 3) experimental manipulation of oil-contaminated mussel beds to determine the feasibility of oil removal, 4) removal of oil-contaminated sediments from mussel beds in 1994 at five locations in Prince William Sound, 5) a physiological survey of mussel condition (byssal thread production, condition index, clearance rate, and glycogen content) in Prince William Sound prior to bed restoration[1], and 6) a survey of histological condition of mussel tissue from contaminated and uncontaminated sites in Prince William Sound.

OBJECTIVES

Objectives as proposed in the 1996 detailed study plan

1. Conduct chemical analyses of samples collected in 1995.
2. Prepare a report synthesizing and summarizing all research and activities conducted under the 4-year history of this project.

Previous objectives have been:

3. Establish the geographic extent and intensity of oiling in contaminated mussel beds in Prince William Sound and the Gulf of Alaska.
4. Determine within-bed distribution of crude oil in sediments underlying contaminated mussel beds.
5. Test minimally intrusive methods (stripping and patch removal) of decreasing the amount of *Exxon Valdez* oil underlying oiled mussel beds.
6. Test for physiological and biological differences between chronically exposed mussels and clean mussels.
7. Manually restore selected oiled mussel beds with relatively high levels of contamination.

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[1]Published or in press.

[2]Chapter 1 now spans more time, includes predictive modeling, and will be republished.

Chapter 1: Persistence of Oiling in Mussel Beds after the *Exxon Valdez* Oil Spill

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ABSTRACT

Exxon Valdez oil was not removed from dense, intertidal mussel (*Mytilus trossulus)* beds in Prince William Sound (PWS) and along the Kenai and Alaska Peninsulas to protect these communities from the negative effects of post-spill cleanup efforts. Although the general assumption was that natural processes would rapidly reduce hydrocarbon concentrations, substantial quantities of oil remained trapped in these beds 3 years after the spill, and contaminated mussel beds were a potential source of chronic hydrocarbon exposure for vertebrate and invertebrate predators. Our objective was to establish the geographic extent and intensity of oiling, and to monitor annual hydrocarbon changes in selected mussel beds. Beds with significant contamination after 1991 included most previously oiled areas in PWS, particularly within the Knight Island group, and the Kenai Peninsula. Yearly mean concentrations of total petroleum hydrocarbons (TPH) in sediments ranged from less than 60 µg/g in known reference beds to 62,258 µg/g wet weight, or approximately 0 to 523 µg/g dry weight total polynuclear aromatic hydrocarbons (TPAH). Mean TPAH concentrations >8 µg/g dry weight were observed in some mussels. Hydrocarbon concentrations declined significantly with time in some, but not all mussels and sediments, and should reach background levels within three decades of the spill in most beds. In the last year of study, mean hydrocarbon concentration was greater than 2 times background concentration in sediments from 27 of 34 sites, and in mussels from 18 of 31 sites. Composition of PAH was consistent with weathered *Exxon Valdez* oil; the degree of weathering increased in some samples, but decreased in others, possibly because storm activity exposed fresh oil. Because natural degradation and removal of oil from contaminated mussel beds has been slow, the knowledge of oil distribution and quantity gained in this study may be used to guide further cleanup activity.

INTRODUCTION

Large quantities of crude oil spilled from the TV *Exxon Valdez* in 1989 were deposited intertidally on beaches in Prince William Sound (PWS) and along the Gulf of Alaska (GOA). Soon after the spill, a cleanup effort was launched to remove oil stranded in intertidal areas, which involved extensive use of hydraulic treatment methods on some beaches. Approximately one-third of all shoreline segments in western PWS were washed with high-pressure hot water; much of the marine life so treated perished (Mearns 1996; Houghton et al. 1996). Because aggressive cleanup was often devastating, most dense, oiled mussel (*Mytilus trossulus)* beds on

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finer, unconsolidated substrates were not cleaned (by request of the *Exxon Valdez* oil spill Interagency Shoreline Cleanup Committee). These important communities provide food and habitat for other organisms and physically stabilize intertidal areas. The general assumption was that natural processes would clean the beds in a reasonable time.

Natural rates of hydrocarbon loss were slower than anticipated, and substantial quantities of *Exxon Valdez* oil (EVO) remained trapped in sediments underlying dense mussel beds in 1991 (Babcock et al. 1994). These contaminated mussels were a potential source of chronic hydrocarbon exposure for consumers and other species dependent on them, including humans. Studies suggested consumption of oiled mussels by vertebrate predators had negative impacts (Duffy et al. 1996; Sharp et al. 1996), although this has been contested by others (Stubblefield et al. 1995; Hartung 1995).

As a first step in assessing the magnitude of the problem, our primary objective was to establish the geographic extent and intensity of oiling in contaminated mussel beds in PWS and along the GOA. A secondary objective was to monitor annual hydrocarbon changes in mussels and sediments of selected beds.

To determine the extent of mussel bed contamination by EVO and estimate natural hydrocarbon loss rates, extensive surveys were conducted in PWS and along the Kenai Peninsula (KP) and Alaska Peninsula (AP) (Figure 1.1). Hereafter, we refer to KP and AP together as GOA (Gulf of Alaska). Sampling was based on prior knowledge of oiling and was non-random. Initial samples were collected only where significant oiling was observed by the 1) Alaska Department of Environmental Conservation (Shoreline Assessment records), 2) Alaska Department of Fish and Game (S. M. Patten, unpublished data), and 3) U. S. Fish and Wildlife Service (Sharp et al. 1996). Coupled with historical data, our research describes the geographic extent of significant mussel and sediment contamination. However, because sampling was intentionally not random, we could not estimate the percentage of significantly contaminated beds from the universe of all beds. 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 (see Chapter 2 for a study of within-bed variability of hydrocarbon distribution). Samples were collected from 98 beds in 1992 through 1995, but only 24 beds were sampled frequently enough to estimate loss rates. These baseline data allowed estimation of natural recovery rates and provided information for decisions on human-assisted recovery in selected areas.

We also investigated the mechanism of oil transfer from sediment to mussel by comparing TPAH / total hydrocarbon ratios. Equal ratios in the two matrices should indicate that mussels acquired particulate oil when filter feeding. Alternatively, the ratio should be greater in mussels than in sediment if mussels were exposed to hydrocarbons in solution because alkanes are much less soluble than PAH.

METHODS

Site selection

To determine the geographic extent of significantly oiled mussel beds, and quantify oil concentrations in sediments and mussels, beds previously identified as contaminated were sampled (Alaska Department of Environmental Conservation; S. M. Patten, unpublished data; Sharp et al. 1996) (Figure 1.1). Primary criteria for site selection were the presence of moderately to densely packed mussel beds on relatively fine sediments (i.e., <1 cm diameter), and detection of crude oil by visual or olfactory means. For comparison, samples were also collected from two reference sites with little or no EVO contamination (Figure 1.1) (Short and Babcock 1996). Surveys and sampling of sediments and mussels were conducted in PWS by personnel from the Auke Bay Laboratory, the Alaska Department of Fish and Game, and the U. S. Fish and Wildlife Service. Personnel from the National Park Service and the National Biological Service sampled the GOA. A total of 98 beds were sampled. Each bed is identified in this report by the *Exxon Valdez* Interagency Shoreline Cleanup Committee segment number plus a unique suffix. (See Appendix 1 for bed latitudes and longitudes.)

Identified sites were first sampled in 1992. Sites with measurable oil were generally resampled to determine inter-annual change in hydrocarbon concentration (Table 1.1). Nine sites that had oil in 1992 were dropped from further study in following years because no visual or olfactory evidence of oil remained. A few previously unknown sites were added in 1993 and 1995 after contamination was discovered. Sites along the GOA were sampled in 1992, 1993, and 1995.

Table 1.1 Number of mussel beds sampled by year, from 1992 through 1995 in Prince William Sound (PWS) and along the Gulf of Alaska (GOA).

	Number sampled	
Year	PWS	GOA
1992	65	13
1993	32[1]	16[1]
1994	29[2]	0
1995	24[3]	10

[1]Includes five previously unidentified sites.
[2]Includes six previously unidentified sites.
[3]Includes four previously unidentified sites.

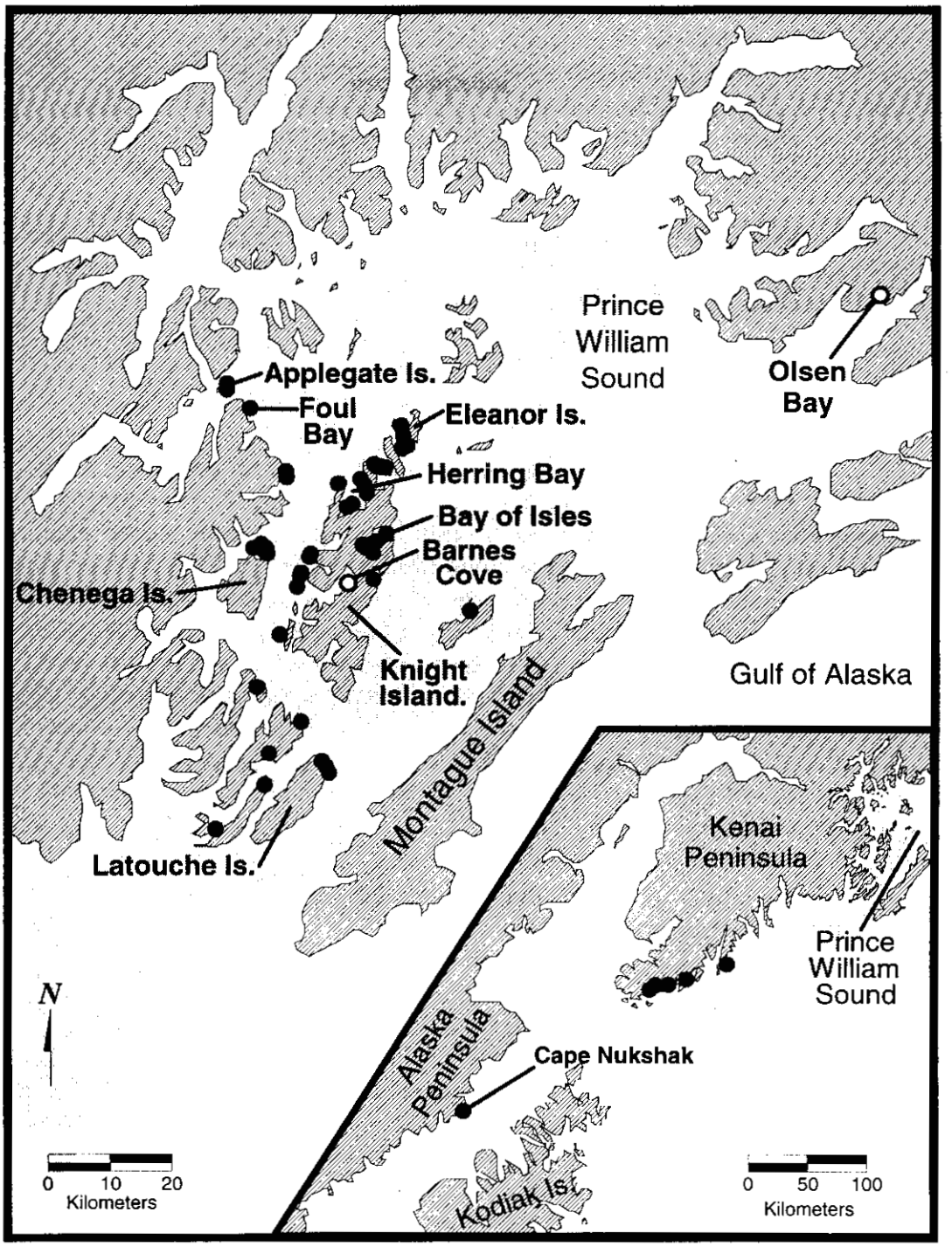

Figure 1.1. Location of mussel beds sampled for petroleum hydrocarbons in Prince William Sound and along the Gulf of Alaska. The shaded area indicates the extent of floating *Exxon Valdez* oil. All beds were located in the spill impact area except the Olsen Bay reference site. Reference sites are indicated by open circles; solid circles indicate contaminated sites.

In PWS, mussel bed size ranged from approximately 20 m^2 (a small bed on Disk Island) to 700 m^2 (the large bed on the tombolo adjacent to Eleanor Island). Density of mussels ranged from thinly interspersed (288 mussels/m^2 at Aguliak Island) to multiple layers (5,000 mussels/m^2 at Eleanor Island). Most beds were situated on mixed sand and gravel substrates, and the mussels were usually relatively evenly dispersed throughout the sampling area. However, the presence of large cobbles and boulders created heterogeneity in many beds.

In the GOA, mussel bed size ranged from approximately 20 to 800 m^2. The largest bed was at Pikes Point in the west arm of Port Dick. Mussel density was not measured in GOA beds. The substrate along the GOA ranged from mud to boulders and bedrock.

Sampling Procedures

A transect line parallel to the waterline (as topography allowed), was established through the middle of each mussel bed or the obviously oiled portion of the bed using modified methods of Karinen et al. (1993) and Babcock et al. (1994). 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). 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. Air blanks were collected for quality control purposes at most sites. Some variation of this basic sampling method occurred at the GOA sites, and at PWS sites sampled for other studies (see Chapters 2-5). All samples were cooled immediately and frozen within 2-4 h. Data from samples collected from several beds in PWS after they were manually cleaned in 1994 (Babcock et al. Chapter 4) were not included in the analysis.

Chemical analysis

All sediment samples were analyzed by an ultraviolet fluorescence (UVF) 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 EVO was used to estimate total petroleum hydrocarbon (TPH) concentration. Mean TPH concentration is reported in μg/g wet weight; $n = 3$ unless otherwise noted. The method detection limit (MDL) for TPH was 50 μg/g.

All mussels from the GOA were analyzed by gas chromatography/mass spectroscopy (GC/MS), but mussels from PWS were analyzed only when TPH concentration in underlying sediments was significant. A subset (69 of 776 samples) of sediments with elevated TPH were selected for GC/MS analysis to confirm polynuclear aromatic hydrocarbon (PAH) composition.

Samples were analyzed by GC/MS at the National Marine Fisheries Service, Auke Bay Laboratory (Short et al. 1996a). Experimentally determined MDL depended on sample weights, and generally were 1 ppb in tissue, and < 2 ppb in sediment. Concentrations of individual 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. Total PAH (TPAH) concentrations were calculated by summing concentrations of individual PAH except perylene. Perylene was excluded because there are natural biological it is produced by biogenic sources. (See Appendix 2 for a listing of PAH identified by GC/MS.) Relative PAH concentrations were calculated as the ratio of PAH concentration to the TPAH concentration.

The UVF estimates were reliable predictors of TPAH content in sediment (Figure 1.2). Correlation between TPH concentration and TPAH concentration was good [$r^2 = 0.68$, $P < 0.001$, $F_o/F_{(1,70,0.95)} = 38$ (see following paragraph for explanation of F_o/F_{crit})].

Data analysis

To predict the time when concentrations in sediments and mussels would reach background levels, concentrations were regressed against time. Regressions were limited to oiled beds with data spanning 3 or more years to ensure that short-term variation in TPH concentration did not yield spurious predictions. Models considered for time series data were ladder of powers (x-transformations from linear through $-1/x^3$), $y = ae^{bx}$, and power. No single model fit all data adequately. The predictive usefulness of regression models was judged by correlation, probability, and F_o/F_{crit}, where F_o = observed F-ratio and $F_{crit} = F(v_m, v_r, 1 - \alpha)$, 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 $F_o/F_{crit} \geq 4$ were considered to be useful (Draper and Smith 1981). Sediment regressions were based on TPH, and mussel regressions were based on TPAH. Times when concentrations in sediments and mussels would reach background concentrations were estimated from these models. For sediments, background concentrations at the two reference sites (Barnes Cove and Olsen Bay) were essentially synonymous with MDL - only one background value (52 ug/g) exceeded MDL. Background TPAH concentration in tissue, 0.09 µg/g, was estimated empirically from the entire mussel data set. Below this concentration it became difficult to discern the EVO signal in PAH composition fingerprints. This estimated background concentration for mussels was the same as mean reference concentration in mussels at Barnes Cove and Olsen Bay (0.09 ± 0.03 ug/g, n =16).

Oil weathering in mussels was estimated in two ways, and 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 in weathered EVO. 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

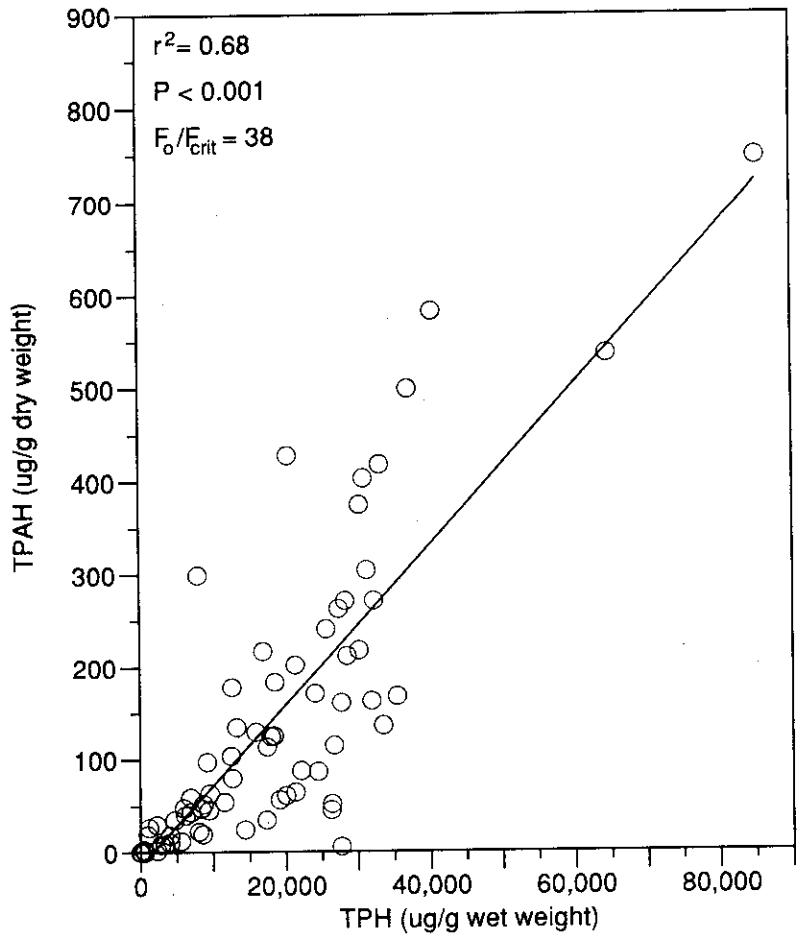

Figure 1.2. Correlation of total polynuclear aromatic hydrocarbon (TPAH) concentration and total hydrocarbon concentration (measured by gas chromatography/mass spectroscopy) with total petroleum hydrocarbons (TPH) in sediments (measured by ultraviolet fluorescence). Sediments were collected in Prince William Sound and the Kenai and Alaska Peninsulas in 1992 and 1993.

calculate an index of weathering (w) that summarizes exposure history (Appendix 2). [$W = 0$ in unweathered samples and increases with weathering. For all environmental samples recorded in the Natural Resource Damage Assessment database (Short et al. 1996b), w ranges up to 11.3 for sediment and 9.9 for mussels. For this paper we use the following definitions: unweathered ($w = 0$), slightly weathered ($0 < w \leq 2$), moderately weathered ($2 < w \leq 8$), and highly weathered ($w > 8$).] 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 EVO (Short and Heintz 1997). However, w could only be estimated when 14 of the more persistent PAHs were present in the sample, and was calculable for only 27% of the data. Weathering was also estimated by regressing percent phenanthrenes (sum phenanthrenes / TPAH) against time; typically percentages of phenanthrenes increase prominently and percentages of naphthalenes decrease as EVO weathers (Short and Heintz 1997). To avoid samples where oil was no longer detectable, records where sum phenanthrenes was less than MDL were excluded. There were too few GC/MS data to adequately estimate weathering in sediments.

To determine if concentrations in sediments and mussels were significantly elevated at each site sampled in the last year of study (1995), we compared the lower 95% confidence bound of sample means to twice the respective estimated background concentrations.

Total PAH to total hydrocarbon ratios were calculated for sediment and mussel samples as a means of investigating the mechanism of oil transfer from sediment to mussel. Ratios were calculated for all sediment and mussel GC/MS data. Sample location was not considered in these comparisons, but data were restricted to concentrations that were > 2 times the background concentration (i.e., $TPH_{sediment} > 100$ µg/g, and $TPAH_{mussel} > 0.18$ µg/g). Ratio distributions were inspected for each matrix, and normality was tested with the D'Agostino D-test. Ratio data were arc-sin transformed before single-factor analysis of variance to compare values in sediment and mussels.

RESULTS

Sediments and mussels from some visibly oiled intertidal areas in PWS and the GOA remained contaminated through 1995. Contaminated sediments were visibly oiled and odorous, and the oil was easily detected. Oil sheens on pools of water were often visible without any manual disturbance. Composition of PAH in mussels and sediments was consistent with the composition of EVO (Short and Heintz 1997) (Appendices 5-6). Of 738 mussel samples, w was estimable in 196, and EVO was identified in 186 of these ($2.6 \leq w \leq 9.2$; moderately to highly weathered) (Short and Heintz 1997). Of 72 sediment samples, w was estimable in 42, and EVO was identified in 39 of these ($0 \leq w \leq 10.7$; unweathered to highly weathered) (Short and Heintz 1997).

Geographic extent and intensity of oil in sediment and mussels

In PWS, annual mean TPH concentrations in sediment were greater than 7,000 µg/g wet weight in 35 of 83 mussel beds in one or more observation years (Figure 1.3). The highest mean

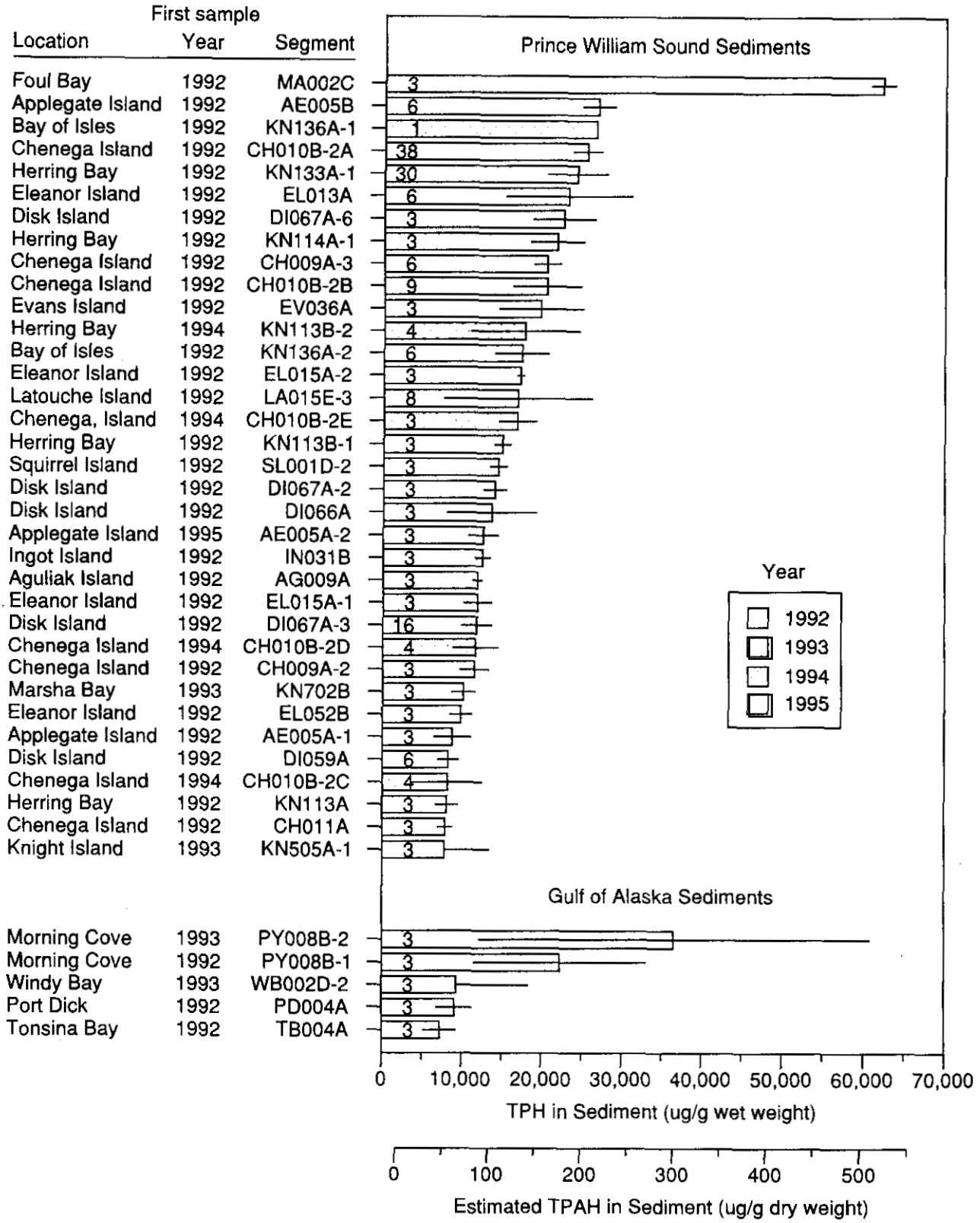

Figure 1.3. Annual mean concentrations of total petroleum hydrocarbons (TPH) in mussel bed sediments from 1992 through 1995 where concentration exceeded 7,000 µg/g wet weight. Only the highest annual mean (±SE) is reported for each site, and the corresponding observation year is indicated. The number of observations is indicated on each bar.

TPH concentrations occurred in 1992, and ranged up to 62,258 μg/g at Foul Bay. Highly contaminated sediments (5,000 to 7,000 μg/g) were observed in 7 other PWS mussel beds, and moderately contaminated sediments (1,000 to 5,000 μg/g) were documented in another 13 beds. The highest mean concentrations in sediments were generally observed in 1992 (24 of 28 samples; 7 additional sites were not sampled in 1992) (Figure 1.3). However, the highest concentrations at a site did not always occur in the first year of observation, demonstrating the persistence and patchiness of contamination and possibly the mobility of subsurface oil. See Appendix 3 for complete time-series concentrations at each site.

Along the GOA, the annual mean TPH concentration in sediment in at least one observation year was greater than 7,000 μg/g wet weight in 5 of 18 mussel beds (Figure 1.3). The highest annual mean TPH concentrations occurred in 1993, and ranged up to 36,538 μg/g in Morning Cove. Moderately contaminated sediments (1,000 to 5,000 μg/g) were documented in 10 additional mussel beds along the GOA. The only oiled mussel bed identified on the AP was at Cape Nukshak (4,639 μg/g). Although the highest mean concentrations in sediments in the GOA were generally observed in 1993 (3 of 5 samples), 2 of these sites were not sampled until 1993 (Figure 1.3). See Appendix 3 for complete time-series concentrations at each site.

Annual mean TPAH concentration in mussels from 21 beds in PWS and 6 beds along the GOA was greater than 1.0 μg/g dry weight in at least one observation year (Figure 1.4). In PWS, the highest mean TPAH concentrations ranged up to 8.1 μg/g at Foul Bay, and concentrations greater than 3.0 μg/g were observed at 5 additional sites. The highest mean concentrations in PWS mussels were generally observed in 1992 (14 of 16 samples; 5 additional sites were not sampled in 1992) (Figure 1.4). Along the GOA, concentrations ranged up to 5.0 μg/g at Morning Cove in 1995, and half the highest mean concentrations in mussels were in 1995, demonstrating persistent contamination over the study period. See Appendix 4 for complete time-series concentrations at each site.

The relationship between TPAH and alkane concentrations in sediments and mussels were roughly equal in both matrices, but the TPAH / total hydrocarbon ratio was strongly skewed in mussels. Arc-sin transformed mean ratios did not differ significantly between sediments and mussels ($P = 0.663$). Distribution of the TPAH / total hydrocarbon ratio in sediments was approximately normal; mean = 2.3% ± 0.2, median = 2.1%, skew = 0.9, kurtosis = 1.2, range 0.2 to 6.4%. However, the ratio was strongly skewed in mussels; mean = 5.0% ± 0.7, median = 1.0%, skew = 4.6, kurtosis = 21.8, range 0.1 to 100%.

Changes in petroleum hydrocarbon content of sediments and mussels

Concentration of TPH in sediment declined significantly without human intervention in some, but not all mussel beds (Table 1.2 and Figure 1.5). Under the time restrictions imposed (observations spanning 3 or more years at accepted sites), TPH concentration at 24 sites was regressed to determine natural rates of hydrocarbon decline. Declines of TPH in sediment were significant at 18 of these sites ($P \leq 0.042$), but scatter was high in some cases ($0.12 \leq r^2 \leq 0.91$) (At sites where declines were not significant, correlation was generally poor $0.08 \leq r^2 \leq 0.39$). Although significant increases in TPH concentration were not observed, there was no evidence of

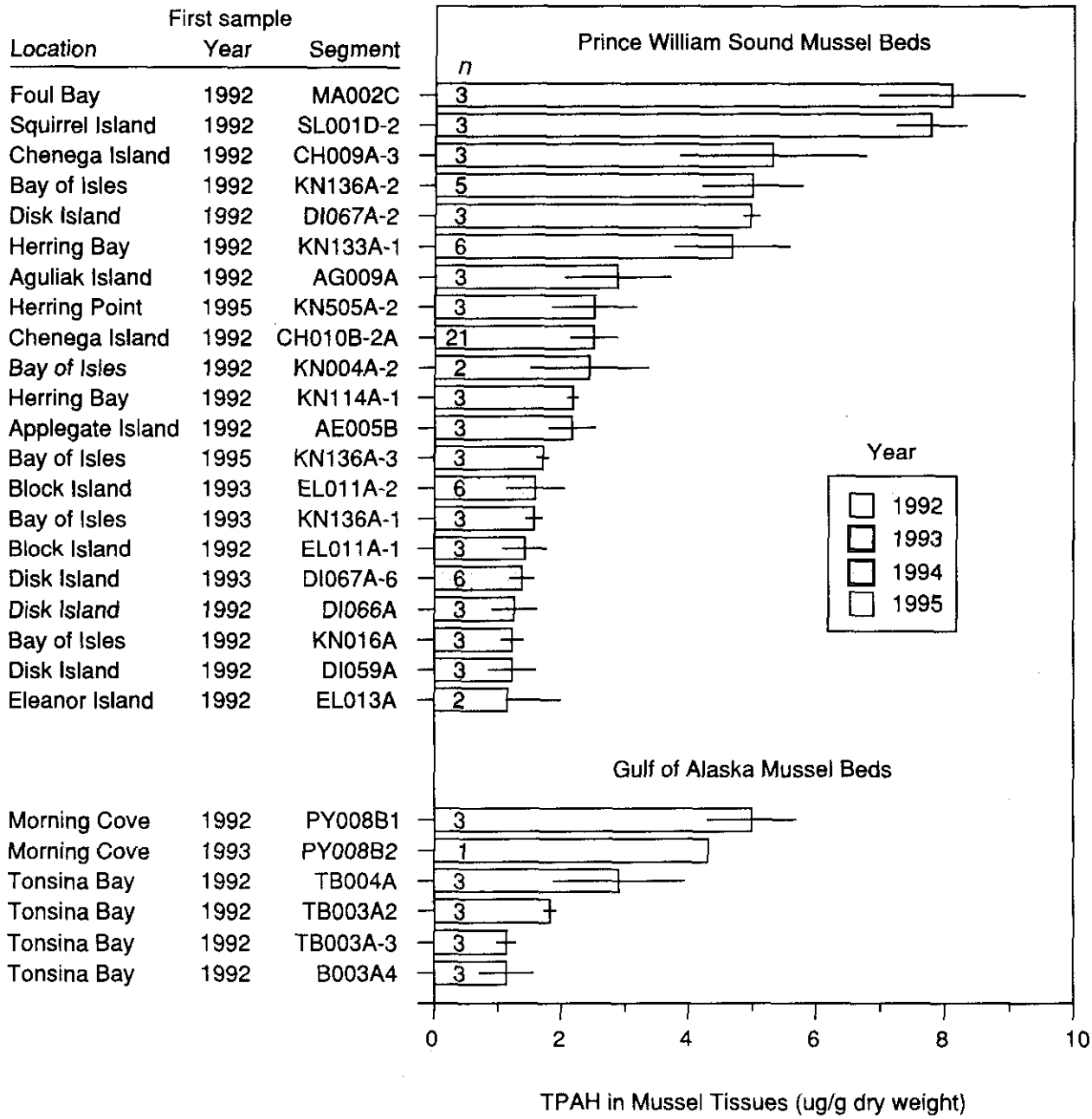

Figure 1.4. Annual mean concentrations of total polynuclear aromatic hydrocarbons (TPAH) in mussels from 1992 through 1995 where concentration exceeded 1.0 μg/g dry weight. Only the highest annual mean (±SE) is reported for each site, and the corresponding observation year is indicated. The number of observations is indicated on each bar. Beaches are identified by name and segment number.

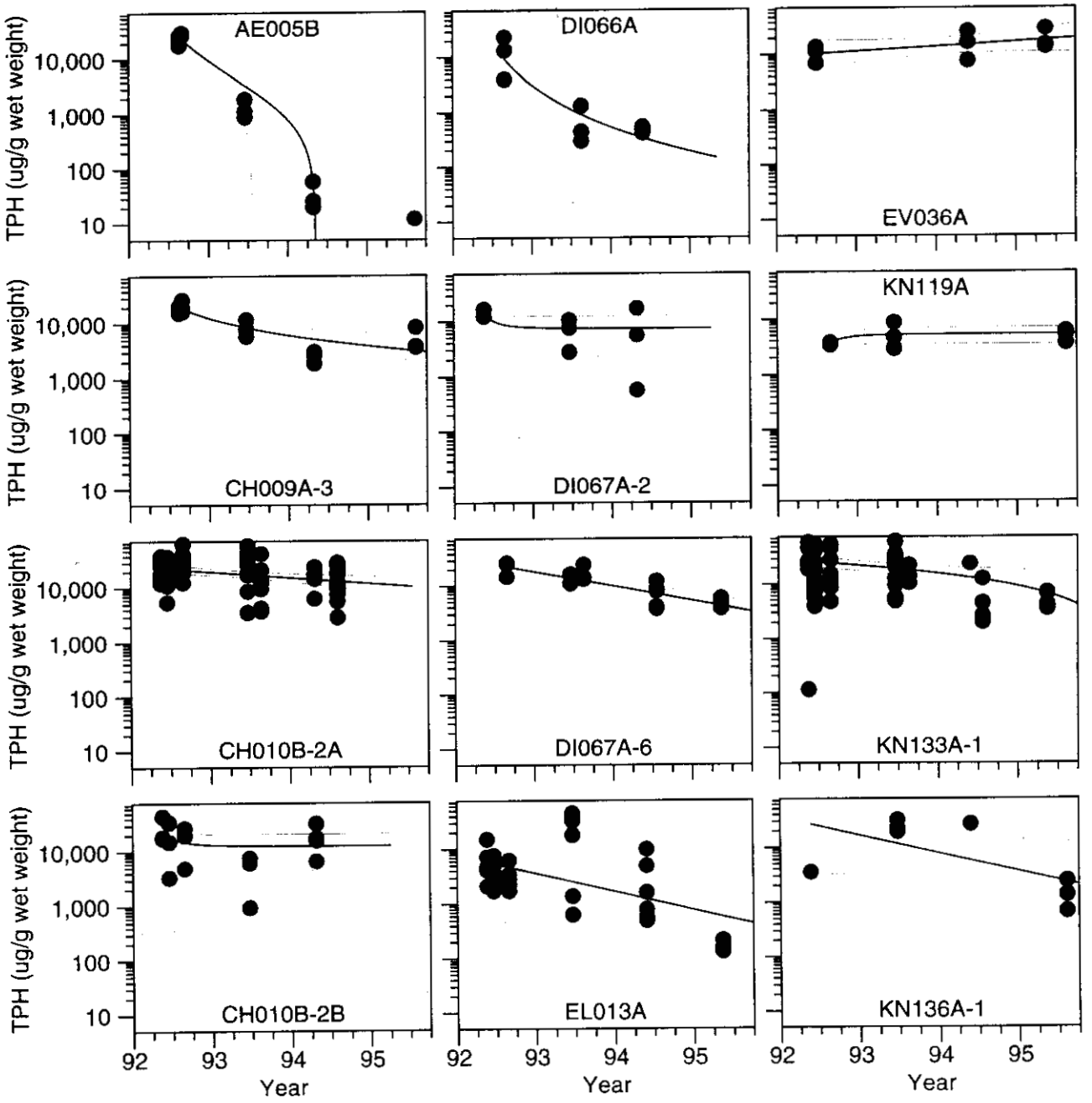

Figure 1.5. Total petroleum hydrocarbon (TPH) concentrations in sediments at sites sampled in 3 or more years, regression fits (bold lines), and 95% confidence bands (thin lines). Graphs are labeled with beach segment numbers; see Table 1.2 for regression models and site names. Estimated total polynuclear aromatic hydrocarbon (TPAH) concentrations are also displayed. Graph continues on next page.

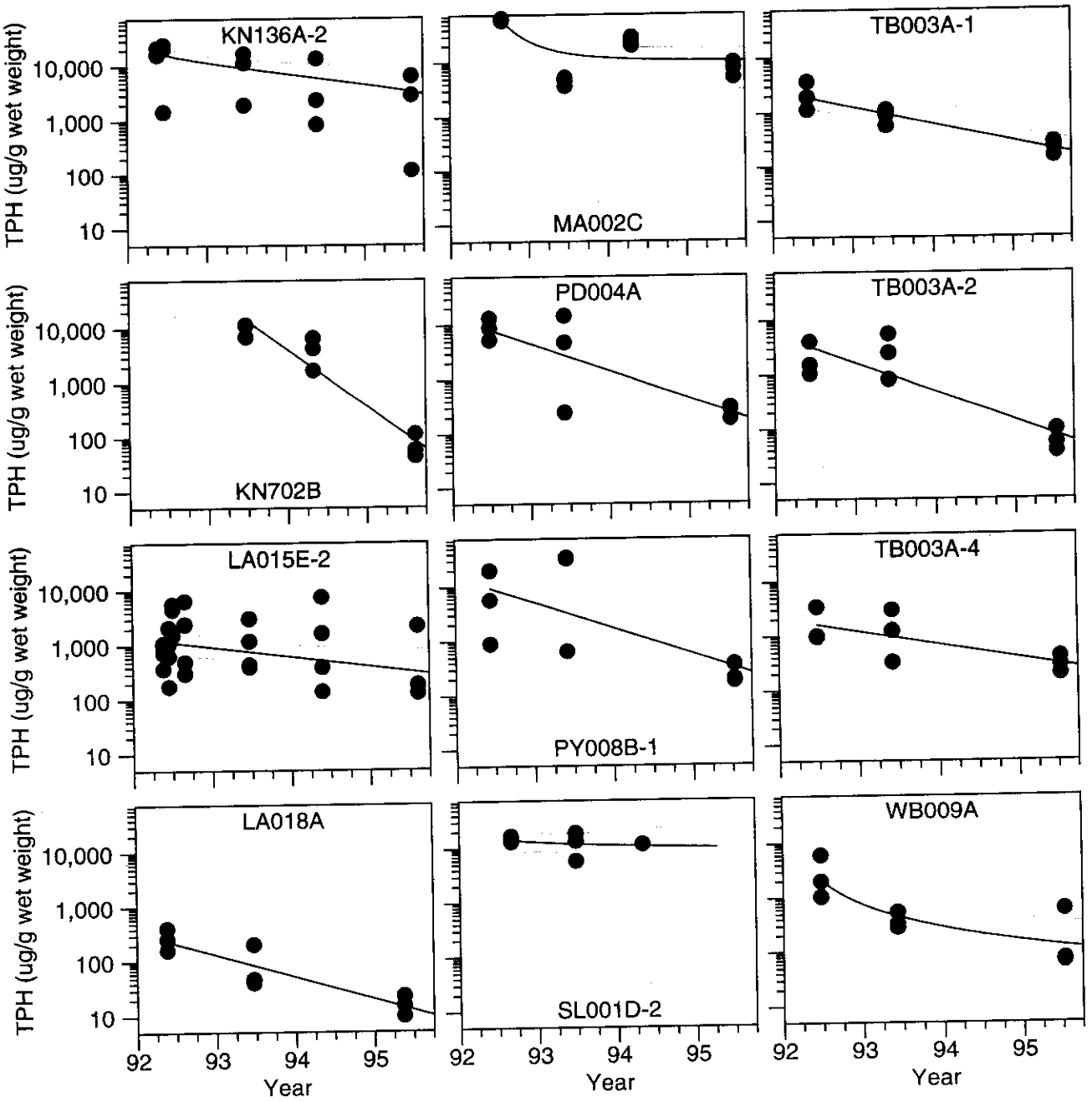

Figure 1.5, continued.

Table 1.2. Predicted dates when total petroleum hydrocarbon concentration in sediment will reach background level. Regressions were limited to beaches where data were collected in 3 or more years. Beaches are identified by name and segment number Observations ranged (obs. range) from 1992 through 1995; n = number of observations, r^2 is squared correlation coefficient, P is probability of significant regression, $F_o/F_{crit} \geq 4$ where P is printed in bold type. Predicted background date was estimated using the best-fit model (see text); estimates were not valid where concentration (and slope) increased. Background concentration was estimated empirically from reference sites as 50 μg/g; ①) lower 95% confidence band was greater than background concentration during the observation period.

Name	Segment	Obs. range	n	model	slope	r^2	P	Predicted background date
Applegate Island, e	AE005B	92 -95	15	$-1/x^2$	-1.19×10^4	0.91	**<0.001**	04/30/94
Bay of Isles	KN136A-1	92 -95	8	exponential	-7.60×10^{-1}	0.39	0.100	08/19/2000
Bay of Isles	KN136A-2	92 -95	15	log	-6.62×10^3	0.47	0.005	11/13/97
Chenega Island, n	CH009A-3	92 -95	15	$-1/x$	-1.33×10^4	0.83	**<0.001**	09/07/2012
Chenega Island, n	CH010B-2A	92 -94	77	exponential	-2.59×10^{-1}	0.12	0.002	03/01/2016
Chenega Island, n	CH010B-2B	92 -94	16	$-1/x^3$	-6.85×10^2	0.17	0.114	remains > back①
Disk Island, w	DI066A	92 -94	9	power	-2.56×10^0	0.81	**0.001**	01/14/97
Disk Island, nw	DI067A-2	92 -94	9	$-1/x^3$	-3.64×10^2	0.31	0.118	remains > back①
Disk Island, nw	DI067A-6	92 -95	18	exponential	-6.26×10^{-1}	0.70	**<0.001**	06/09/2002
Eleanor Island, sw	EL013A	92 -95	33	exponential	-7.68×10^{-1}	0.27	0.002	07/27/98
Evans Isl., Bishop's Rk.	EV036A	92 -95	9	exponential	2.03×10^{-1}	0.27	0.156	positive slope①
Foul Bay	MA002C	92 -95	12	$-1/x^3$	-1.50×10^4	0.86	**<0.001**	remains > back①
Latouche Island, ne	LA015E-2	92 -95	26	exponential	-4.15×10^{-1}	0.18	0.032	01/19/2000
Herring Bay, e	KN119A	92 -95	9	$-1/x^3$	4.87×10^2	0.19	0.243	positive slope①
Herring Bay, s. islet	KN133A-1	92 -95	57	linear	-6.69×10^3	0.13	0.006	04/30/96
Marsha Bay	KN702B	93 -95	9	exponential	-2.40×10^0	0.92	**<0.001**	11/03/95
Morning Cove, Pye Islands	PY008B-1	92 -95	9	exponential	-1.10×10^0	0.47	0.042	03/20/97
Port Dick, Mars Cove	PD004A	92 -95	9	exponential	-1.16×10^0	0.68	0.007	11/10/96
Sleepy Bay	LA018A	92 -95	9	exponential	-9.39×10^{-1}	0.83	**0.001**	01/16/94
Squirrel Island, e	SL001D-2	92 -94	7	power	-2.24×10^{-1}	0.09	0.508	remains > back①
Tonsina Bay, w	TB003A-1	92 -95	9	exponential	-7.12×10^{-1}	0.87	**<0.001**	07/08/97
Tonsina Bay, w	TB003A-2	92 -95	9	exponential	-1.22×10^0	0.78	**0.002**	11/11/95
Tonsina Bay, Otter B	TB003A-4	92 -95	9	exponential	-5.55×10^{-1}	0.54	0.023	09/25/98
Windy Bay, Oystercatcher Isl.	WB009A	92 -95	9	power	-1.40×10^0	0.70	0.005	11/28/98

hydrocarbon loss in sediment from 2 beds (i.e., where the lower 95% confidence band was above background and the slope was positive - see Table 1.2). During the observation period, estimated lower confidence bands at 13 sites remained above twice the background concentration (2·50 = 100 μg/g), dropped below 50 μg/g at 9 sites, and dropped below 100 μg/g at the 2 remaining sites (Figure 1.5). Predicted dates by which TPH concentration in sediment would decline to background concentration (50 μg/g) ranged from late 1993 to 2016 (i.e., roughly 5 to 32 years after the EVO spill).

The frequency of sediments containing high oil concentrations declined with time in PWS, corroborating regression predictions of oil decline. The number of beds with sediment concentration greater than 1,000 μg/g was 40 (of 54) in 1992, and 14 (of 24) in 1995 [data collected after restoration activity began (see Chapter 4) were not included in these comparisons]. Concentration declines were similarly obvious along the GOA.

In mussels, TPAH concentration declined significantly with time in 9 of 23 beds ($P \leq 0.043$; $0.08 \leq r^2 \leq 0.94$), but did not return to background in 10 beds (Table 1.3 and Figure 1.6). Considering only data with significant regressions, predicted dates by which TPAH concentration in tissue would decline to background concentration (0.09 μg/g) ranged from mid 1992 to mid 1994 (i.e., 3 to 5 years after the EVO spill). However, TPAH concentrations in mussels remained significantly above background at 10 sites, and substantive TPAH concentrations remained in at least 2 additional sites (Foul Bay and Tonsina Bay), but their distribution was patchy. There were no significant increases in concentration ($P_{regression} \geq 0.116$), and there was no evidence of hydrocarbon loss in mussels from at least 4 beds (i.e., cases where the lower 95% confidence band was above background and the slope was positive - see Table 1.3). Three of the beds with significant remaining TPAH were subsequently restored (Babcock et al. Chapter 4).

Relationship between sediment and mussel contamination

Predicted changes in hydrocarbon concentration in sediments and mussels were generally similar (compare Figures 1.5 and 1.6). Although there were some discrepancies, there were no cases where concentration increased significantly in one matrix and decreased significantly in the other matrix. Some minor discrepancies might be explained by differences in the time spanned by the data: e.g., sediment data were collected from 1992-1995 at DI067A-6, but mussel data ranged from 1993-1995. The most obvious model discrepancy was in Herring Bay (KN133A-1), where TPH concentration in sediment declined significantly ($P = 0.006$), but TPAH in mussels appeared to increase ($P = 0.154$). Scatter in both matrices at KN133A-1 was high, and we infer hydrocarbon concentrations were patchy. Additional data surveys, proposed for 1999, may resolve some of these differences.

For all sites combined, TPAH concentration in mussels was significantly related to TPH concentration in sediments ($P < 0.001$, $F_o/F_{crit} = 14$), but scatter was very high ($r^2 = 0.31$) (Figure 1.7). The most extreme point observed, where TPH > 60,000 μg/g, appeared to be a leverage point, but the regression was also significant and positive without this point ($P < 0.001$, $F_o/F_{crit} = 7$). There were generally too few data pairs for adequate regression statistics at individual beds generally $n = 3$; maximum $n = 6$).

Table 1.3. Predicted dates when total polynuclear aromatic hydrocarbon (TPAH) concentration in mussels will reach background level. Regressions were limited to beaches where data were collected in 3 or more years. Beaches are identified by name and segment number. Observations ranged (obs. range) from 1992 through 1995; *n* = number of observations, r^2 is squared correlation coefficient, *P* is probability of significant regression, $F_o/F_{crit} \geq 4$ where *P* is printed in bold type. Predicted background date was estimated using the best-fit model; estimates were not valid where concentration (and slope) increased. Background concentration (back) was estimated empirically from the entire mussel tissue data set as 0.09 µg/g; ①) lower 95% confidence band was greater than background concentration, ②) lower 95% confidence band overlaped background concentration, ③) confidence bands bracketed background concentration, and ④) lower 95% confidence band overlapped background concentration in early observations, but not at all times.

Name	Segment	Obs. range	*n*	model	slope	r^2	*P*	Predicted background date
Applegate Island, E	AE005B	92 - 95	11	$-1/x^3$	-6.28×10^2	0.92	**<0.001**	12/17/93
Bay of Isles	KN136A-1	92 - 95	7	$-1/x^3$	-3.01×10^3	0.82	0.005	remains > back①
Bay of Isles	KN136A-2	93 - 95	14	$-1/x^2$	-5.02×10^2	0.69	**<0.001**	remains > back①
Chenega Island, N	CH009A-3	92 - 95	10	$-1/x^2$	-2.45×10^3	0.81	**<0.001**	06/14/94
Chenega Island, N	CH010B-2A	92 - 94	49	$-1/x^{0.5}$	-1.07×10^3	0.08	0.047	remains > back①
Chenega Island, N	CH010B-2B	92 - 94	9	$-1/x^3$	-1.14×10^1	0.16	0.282	remains > back①
Disk Island	DI066A	92 - 94	8	exponential	-2.24×10^0	0.94	**<0.001**	10/03/93
Disk Island, NW	DI067A-6	93 - 95	17	$-1/x^3$	1.20×10^3	0.07	0.291	positive slope①
Eleanor Island, S	EL013A	92 - 95	13	exponential	2.77×10^{-1}	0.15	0.189	positive slope①
Evans Island	EV036A	92 - 95	12	$-1/x^3$	-6.04×10^0	0.07	0.399	remains > back①
Foul Bay	MA002C	92 - 95	12	$-1/x^1$	-5.04×10^3	0.62	0.002	remains > back②
Herring Bay, E	KN119A	92 - 95	9	exponential	-3.68×10^0	0.96	**<0.001**	03/28/93
Herring Bay, S. islet	KN133A-1	92 - 95	35	linear	5.22×10^2	0.06	0.154	positive slope①
Latouche Island, NE	LA015E-2	92 - 95	15	linear	5.15×10^1	0.03	0.552	positive slope②
Marsha Bay, islet	KN702B	93 - 95	8	exponential	-3.47×10^0	0.52	0.045	01/11/94
Morning Cove	PY008B1	92 - 95	9	exponential	1.32×10^{-1}	0.17	0.272	positive slope①
Mars Cove	PD004A	92 - 95	8	linear	8.05×10^1	0.05	0.601	positive slope②
Sleepy Bay	LA018A	92 - 95	8	$-1/x^3$	6.12×10^0	0.19	0.285	06/29/92③
Squirrel Island, E	SL001D-2	92 - 94	9	$-1/x^3$	-9.80×10^2	0.80	**0.001**	remains > back①
Tonsina Bay, W	TB003A-1	92 - 95	9	linear	8.87×10^1	0.14	0.330	positive slope④
Tonsina Bay, W	TB003A-2	92 - 95	9	$-1/x^3$	-9.45×10^1	0.39	0.073	remains > back②
Tonsina Bay, Otter B	TB003A-4	92 - 95	9	linear	2.25×10^2	0.32	0.116	positive slope④
Windy Bay	WB009A	92 - 95	8	$-1/x^{0.5}$	-1.40×10^2	0.40	0.090	03/31/93

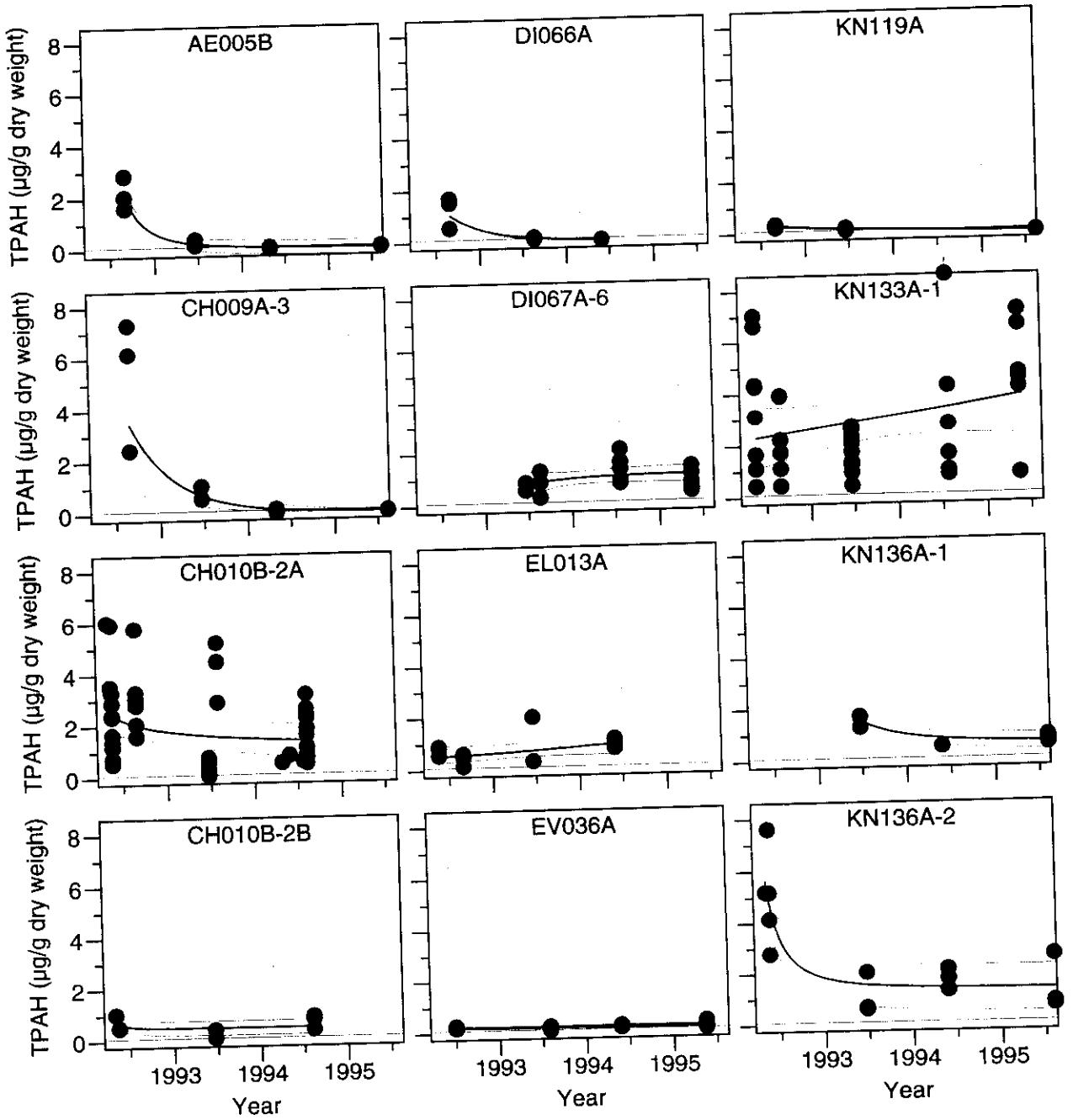

Figure 1.6. Total polynuclear aromatic hydrocarbon (TPAH) concentrations in mussels at sites sampled in 3 or more years, regression fits (bold lines), and 95% confidence bands (thin lines). Graphs are labeled with beach segment numbers; see Table 1.3 for regression models. Figure continues on next page.

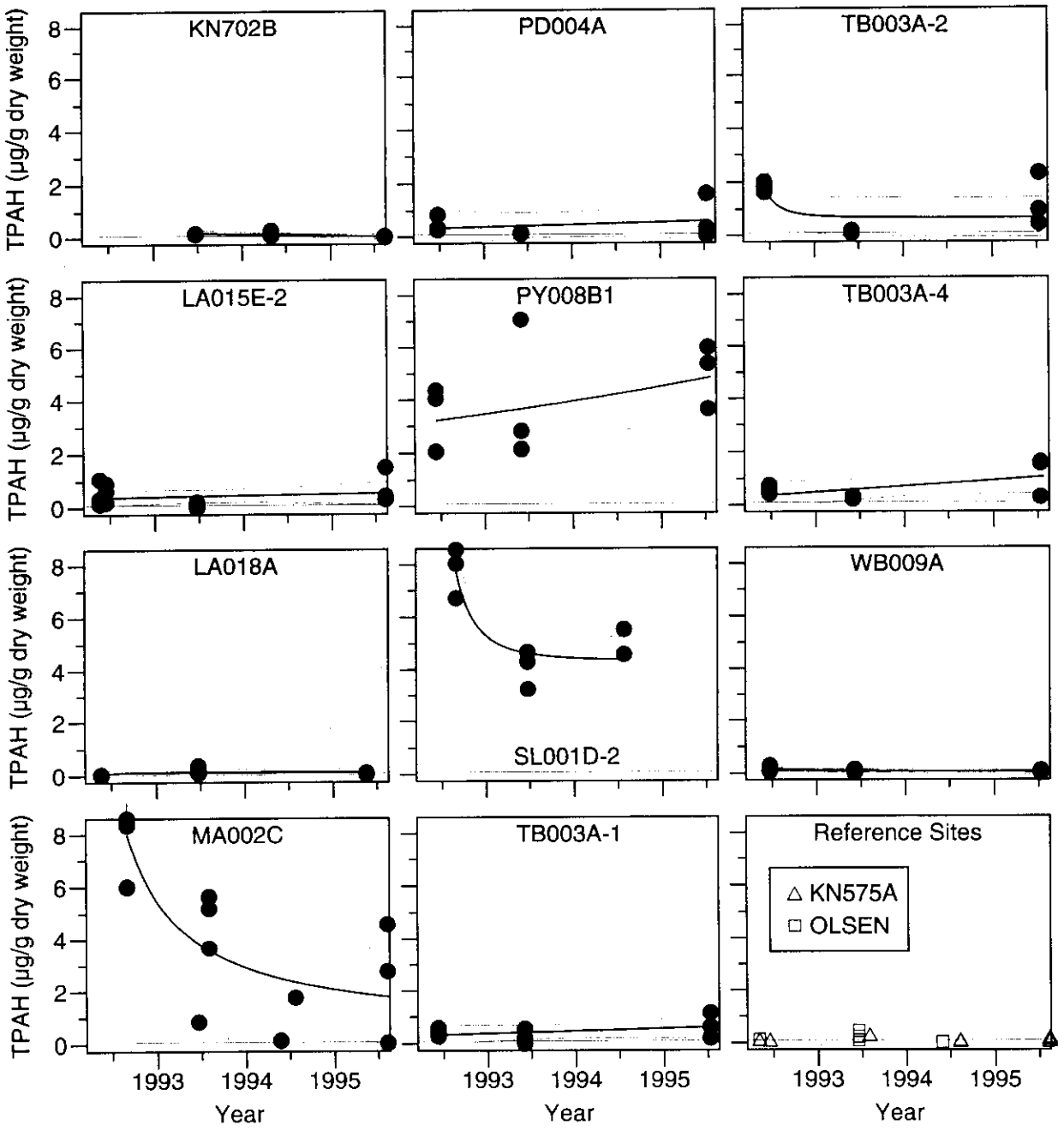

Figure 1.6 continued.

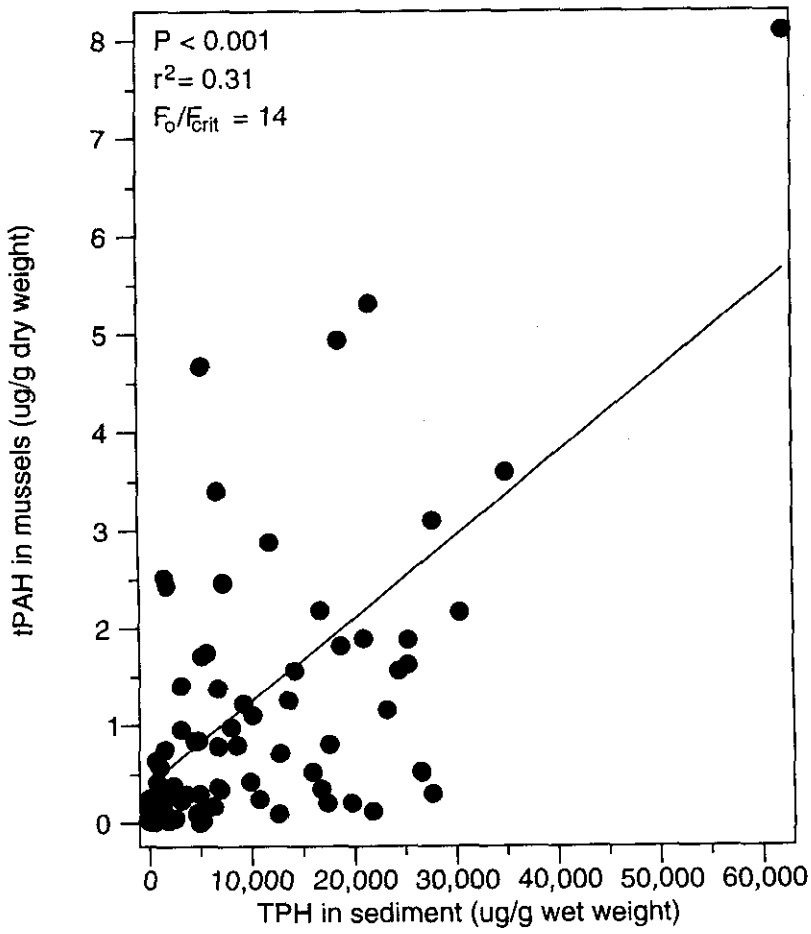

Figure 1.7. Relationship of total polynuclear aromatic hydrocarbon (TPAH) concentration in mussels and total petroleum hydrocarbon (TPH) concentration in sediments. All Prince William Sound and Gulf of Alaska sites with paired TPAH and TPH data are included.

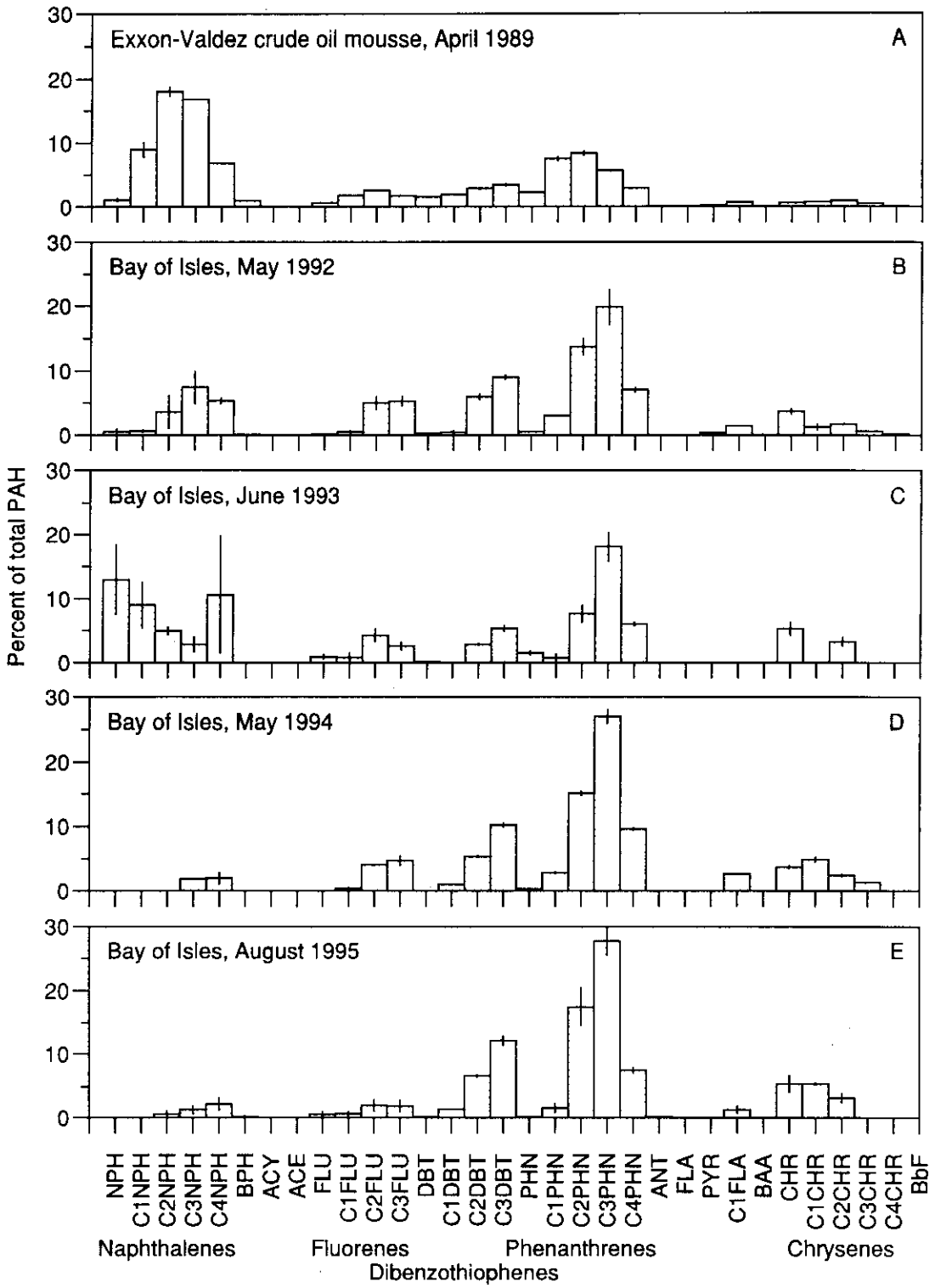

Figure 1.8. Composition of polynuclear aromatic hydrocarbons in mussels from a representative bed, Bay of Isles (KN136A-2), compared to that of partially weathered *Exxon Valdez* oil.

PAH composition and weathering in sediments and mussels.

Composition of PAH in mussels and sediments was consistent with that of weathered EVO (Figure 1.8 and Appendices 2.3 and 2.4). Napthalenes through chrysenes were generally present; occasionally heavier compounds, such as benzo(e)pyrene, were also detected. Fewer naphthalenes were present than would be expected in fresh oil, and there was a tendency toward phenanthrene dominance. Composition of alkylated compounds in homologous PAH families was characteristic of typical weathering (Short and Heintz 1997). However, weathering of PAH was generally slow or negligible over the 1992-1995 observation period, and re-exposure of less weathered oil was observed in some cases. Regressions of *w* in mussels versus time were possible at only 6 of 26 sites, and only two of these regressions were significant ($0.003 \leq P \leq 0.044$). Both significant slopes were positive, suggesting increased weathering; correlation was moderate to strong ($0.59 \leq r^2 \leq 0.91$). Percent phenanthrene regressions were estimable for 20 of 35 sites; increased weathering was significant at 5 sites, but decreased weathering was significant at 2 sites. Inspection of composition graphs corroborated the latter observation; oil composition appeared to be fresher in 1995 at two Tonsina Bay sites than it had in preceding years.

Effects of storm activity on mussel bed structure, hydrocarbon concentration, and composition.

Vigorous winter storm activity over the winter of 1992-1993 removed approximately 40% of the mussel layer from the Eleanor Island (EL013A) bed. This bed, which was located on a tombolo on the southwest side of the island, originally had the highest density of mussels in our study (>5,000 individuals/m^2). Oil sheens were observed on the entire upper portion of the bed in June 1993, and underlying sediments were visibly redistributed. After the storm, TPH concentrations in surface sediments (23,206 μg/g in 1993) were significantly greater than those in 1992 (3,098 μg/g) ($P = 0.031$) (Figure 1.5), and the oil appeared to be less weathered in both matrices. By 1994, the TPH concentration in surface sediments had returned to about the 1992 level (3,056 μg/g), and in 1995 a further reduction was evident (169 μg/g) (Figure 1.5). Mussel hydrocarbon concentrations also tended to increase after the storm, but pre- and post-storm differences were not significant (Figure 1.6). By 1995, the last year of the study, approximately 95% of the mussels were gone.

Concentrations in sediments and mussels at the end of study (1995)

Hydrocarbon concentrations in sediments and mussels at the majority of sites sampled in the last year of study were greater than background concentrations, suggesting continued contamination. Mean TPH concentration sediments from 27 of 34 sites (79%) in the last year of study was >100 μg/g (2 times background concentration), and was significantly greater than background concentration in 14 of these samples (41%). Mean TPAH concentration in mussels from 18 of 31 sites (58%) in the last year of study was >1.8 μg/g (2 times background concentration) and was significantly greater than background concentration in 6 of these samples (15%). Sites manually restored were not included in this end-of-study analysis (see Chapter 4.)

In the last year of study, three- and four-ring compounds were present in the majority of mussels where TPAH concentration was greater than background. Phenanthrenes were detected in 100 of 107 samples (93%), and chrysenes were detected in 93 of 107 samples (87%), demonstrating their persistence in the environment. Phenanthrenes accounted for 40% ± 2 of the TPAH in 1995 samples where TPAH was greater than background, and chrysenes accounted for 11% ± 1 of TPAH in these samples.

DISCUSSION

The original assumption that natural weathering would cause residual *Exxon Valdez* oil concentrations in sediments underlying all mussel beds to decline rapidly was incorrect. Although cleanup of sensitive mussel beds was avoided in 1989 and 1990 to protect these beds, significant long-term contamination persisted, raising concerns for mussel health and reproduction, and entry of petroleum hydrocarbons into species that prey on mussels. Our surveys document high concentrations of oil 3 to 6 years after the *Exxon Valdez* spill, and these surveys were used to recommend and guide post-spill cleanup efforts in 1994.

The hydrocarbon concentrations observed in this survey greatly exceeded background and historical levels. Concentrations of EVO in mussels and sediments were among the highest observed in any study a year or more after the spill. In sediments, a TPH concentration greater than 62,000 µg/g wet weight was found at one site, and TPAH concentrations greater than 8 µg/g dry weight were documented in mussels. In contrast, concentrations in sediments of reference beds were less than 60 µg/g TPH and less than 0.5 µg/g TPAH in mussels, both near MDL . Historically (1977 through 1980), sediments and mussels collected from established stations along the shipping lane through PWS, and collected in 1989 from these and additional stations in the EVO impact area before landfall of the oil, indicated little or no presence of contaminating petroleum hydrocarbons (Karinen et al. 1993; Babcock et al. 1996; Short and Babcock 1996).

The geographic distribution of mussel beds with significant oil contamination after 1991 included most areas originally impacted by the spill in PWS and along the KP. Most of the contaminated mussel beds in PWS were located within the Knight Island group, an area particularly impacted by the EVO spill. Most of the oiled beds in the GOA were along the southwestern portion of the KP, but oil also persisted in at least one bed on the AP (Cape Nukshak). The primary source of PAH was EVO (Short and Heintz 1997), but other sources were also apparent in some cases. Pyrolytic sources of PAH were evident in some mussels collected in 1993 along the KP, e.g., in Morning Cove. However, only EVO had been evident in samples collected in 1992 along the KP.

The volume of stranded oil may be the principal reason why most long-term contamination was observed within PWS rather than along the GOA. A large volume of oil (approximately 41% of the total spilled) was stranded in PWS. Less oil was stranded over a larger area outside PWS - about 5% of the total volume along the KP and 2% along the AP (Wolfe et al. 1994).

Variability of oil contamination within beds was likely a primary source of observed variability in hydrocarbon concentration, but inclusion of samples from many sources may have been a confounding factor. To develop as complete an understanding as possible of oil distribution, intensity of oiling, and natural rate of oil loss, samples from other studies were included in our data set, and collections were made by many people. Acceptance of all data may have increased measurement variability, but was justified by gains in temporal and geographic coverage. The pooled sampling strategy was designed to characterize average concentration in each bed, but observed hydrocarbon concentrations were still highly variable, both in sediments and mussels. We suggest three possible sources of variability: 1) the inherent horizontal and vertical variability of oil distribution within beds (Michel and Hayes 1993a,b; Harris et al. 1996; Chapter 2), 2) inclusion of samples from other studies, where samples were not pooled to reduce variability and variability in sampling protocol, possible because numerous personnel from several agencies were involved, and 3) minor variation in place of collection when beds were resampled over time.

The most reasonable source of oil in mussels appears to be from oil trapped in sediment. However, the high variability between TPAH concentration in mussels and sediments suggests the linkage between them is indirect. Similar conclusions were reached by Boehm et al. (1996), and Harris et al. (1996; Chapter 2) who found that correlation of TPAH concentration in mussels and sediment at specific sampling points was low. All three studies are in agreement that TPAH concentrations in sediment were much greater than in mussels. Although the range of mean TPAH concentrations observed in mussels in 1993 were very similar between our study and that by Boehm et al. (1996) (0.03-4.8 μg/g and 0.02-4.0 μg/g, respectively) we observed much higher mean TPAH concentrations in sediments (0.0-301 μg/g) than Boehm et al. (1996) (0.02 - 26 μg/g). Discrepancies in TPAH concentration in sediment may have resulted from differences in sampling procedures or locations, and other uncontrolled factors.

Transfer of hydrocarbon contamination from underlying sediment to mussels is likely mediated by water. Oil in particulate form may enter surrounding water from contaminated sediment and later be consumed by filter-feeding mussels. Similarity of TPAH / total hydrocarbon ratios in sediment and mussels support this mechanism as the primary route of mussel contamination. Boehm et al. (1996) also suggest ingestion of small droplets or tarry particles may explain the hydrocarbon transfer. However, unlike in sediments, where the distribution of the TPAH / total hydrocarbon ratio was normal, the distribution was highly skewed in mussels, and ranged up to 100% TPAH. The observed skew could not be explained simply by differences in method detection limits, or by removing samples where TPAH < 2 times background concentration. This suggests that mussels may also have been exposed to PAH in solution, but this route of exposure was likely secondary.

Serious long-term contamination of sediments and mussels was evident, but there were indications that natural environmental processes were reducing hydrocarbon concentrations, and that much of the remaining oil will gradually dissipate without human intervention. Evidence of decreasing oil concentration was based on visual and olfactory observation, regression predictions, and on concentration data from sites sampled too infrequently for regression analysis. Some sites were dropped from the survey because little or no oil remained. Significant natural reductions in hydrocarbon concentration were observed in approximately one-half of the

beds, and concentrations should reach background levels within three decades of the spill in most beds. However, rates of hydrocarbon loss among beach sediments were quite variable, and in a minority of cases there was no evidence of loss. Beaches that lost oil most slowly were either sheltered or were armored by cobble or boulders, thus reducing wave-induced sediment movement and remobilization of buried oil.

Dense layers of mussels apparently also protected underlying sediment from the weathering processes that naturally degrade EVO. The lack of weathering was indicated by observation of low oil viscosity, aromatic odors, and visible slicks on site, and by similarity of PAH composition to that in moderately weathered EVO. Weathering proved to be difficult to estimate; few mussels samples (27%) had all PAH required for rigorous first-order kinetic loss rate modeling (Short and Heintz 1997) and results were not clear. Consequently, the change in percent phenanthrenes was used as a less rigorous estimation of weathering. Again results were equivocal; increased weathering was apparent at some sites, but decreased weathering was also observed.

In some cases, storm activity had obvious and dramatic impacts on bed structure and sediment movement, and likely influenced hydrocarbon concentrations. For example, about 40% of the mussels from the Eleanor Island tombolo (EL013A) disappeared after vigorous storm activity in the winter of 1992-93, and the underlying sediments were redistributed. Apparently this disruption remobilized EVO trapped in sediment, and accounted for the marked increases in TPAH concentrations in surface sediments observed in 1993. Disturbances of this nature and lack of information on volume and composition of oil buried deeper than 2 cm for all survey beds limit the usefulness of the regression models used to predict long-term oil concentrations.

Unlike surveys reported by Boehm et al. (1996), we were unable to estimate the percentage of significantly contaminated mussel beds in PWS and the GOA from our data set because our sample locations were not chosen randomly. Thus we can neither support nor refute the conclusion of Boehm et al. (1996) that the percentage of mussels associated with residual oil in PWS was less than 3% in 1993. We do concur, however, that the percentage of significantly oiled mussels is decreasing with time but find that it may take three decades or more before PAH concentrations in all mussel bed sediments reach background levels.

Although the quantity of residual *Exxon Valdez* oil is decreasing in the environment, there is now strong evidence (Carls et al. 1999 ; Heintz et al. 1999) that weathering does not decrease the toxicity of EVO as suggested by Boehm et al. (1996). Rather, weathered EVO is actually considerably more toxic per unit mass than unweathered oil because the most toxic compounds are also the most refractory. This has been clearly demonstrated in controlled laboratory trials with Pacific herring eggs (Carls et al. 1999) and pink salmon eggs (Heintz et al. 1999). Toxicity of PAH increases both with ring number and alkylation (Rice 1977; Black et al. 1983; Rice et al. 1985). Phenanthrenes and chrysenes, which appear to be much more toxic than naphthalenes (Carls et al. 1999), frequently accounted for a significant fraction of the PAH in mussel tissues in 1995. Thus, remaining contaminated mussels may continue to pose a significant toxic threat to the health of predatory species. We caution that the generally short duration toxicity tests for predatory species reported by Boehm et al. (1996) (5 d maximum for mammals) may not adequately predict response to the long-term PAH exposure predators may experience in PWS.

Studies still in progress have not excluded residual oil contamination as a factor inhibiting recovery sea otter populations in western PWS (Holland-Bartels et al. 1998). Several predatory species, including Barrow's Goldeneyes, Harlequin ducks, river otters, and sea otters may still be encountering oil in areas impacted by the spill because mixed-function oxidase (MFO) induction is significantly greater in tissues of animals captured in oiled areas than in those from reference areas (Holland-Bartels et al. 1998). [Cytochrome P-450 dependent MFOs are a group of enzymes involved in the metabolism of PAH and other xenobiotics (Jimenez and Stegeman 1990; Britvic' et al. 1993), thus induction signals exposure. EVO as the source of induction is consistent with the observations of Holland-Bartels et al. (1996)].

Although oil-contaminated mussel beds were broadly distributed throughout the spill-impact region, and the intensity of contamination was high, the total area of contamination has become rather small. This raises a question. Should we be concerned about small physical areas? If there were disproportionate consumption by top predators, including humans, there would be obvious concern. Disproportionate consumption is possible because areas with high mussel density may be more attractive to predators than areas with less density, and may generally be more sheltered from wave activity, further increasing attractiveness. Because contamination has not been completely eliminated by 6 years of natural weathering, some beds will likely continue to be sources of oil for a rather long time. Residual oil may continue to affect the biota, even though we may not fully understand these effects. Although Stubblefield et al. (1995) and Hartung (1995) conclude that long-term sublethal toxic effects of *Exxon Valdez* oil on wildlife are unlikely, we contend that proof that measurably contaminated food does not affect predators is very difficult in a highly variable natural ecosystem. Thus, we recommend that contaminated beds should be monitored until EVO concentrations in mussels and underlying sediments are at background levels, and that predators that utilize these resources be similarly monitored.

CONCLUSIONS

Sediments under some dense mussel beds remained contaminated with petroleum hydrocarbons long after the *Exxon Valdez* oil spill. In the last year of study (1995), mean TPH concentration in sediments from 27 of 34 sites was >100 µg/g (2 times background concentration), and exceeded 1,000 µg/g wet weight in 14 beds. Over the period of study (1992-1995), mean concentrations of TPH ranged from < 60 µg/g in reference beds to 62,258 µg/g in the most contaminated bed.

In mussels, TPAH concentrations declined significantly with time in 9 of 23 beds, but remained significantly above background levels in 10 beds. There was no evidence of hydrocarbon loss in mussels from at least 4 beds. In the last year of study, mean TPAH concentration in mussels from 18 of 31 sites was greater than 2 times background concentration, and exceeded 1 µg/g dry weight in 9 beds. Total TPAH concentration in mussels ranged up to 8.1 µg/g in 1992.

Composition of PAH in mussels and sediments was consistent with *Exxon Valdez* oil as the source. Secondary, pyrolytic sources of PAH were occasionally encountered.

The geographic distribution of beds with significant contamination included most previously oiled areas in PWS, particularly within the Knight Island group, and the Kenai Peninsula. Long-term contamination was worse in PWS than along the GOA.

Significant natural reductions in hydrocarbon concentration were observed in approximately one-half of the beds; concentrations in sediment may reach background levels within three decades of the spill in these beds. Storm disturbances and lack of information on volume and composition of oil buried deeper than 2 cm limit the predictability of residual contamination levels.

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Chapter 2: Within-Bed Distribution of *Exxon Valdez* Crude Oil in Prince William Sound Blue Mussels and Underlying Sediments.

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ABSTRACT

The distributions of moderately weathered *Exxon Valdez* crude oil in sediments and mussels (*Mytilus trossulus*) were examined in three Prince William Sound mussel beds in 1992. Distribution of oil in sediments within each bed was uneven and was related to several factors, including tidal elevation, sediment depth, and grain size; elevation and grain size were also correlated. Variation in total petroleum hydrocarbon concentration in sediment ranged up to two orders of magnitude within a few meters. The distribution of total polynuclear aromatic hydrocarbon (PAH) concentrations in mussels overlying the sediments was also uneven, but these concentrations were consistently less than in sediments. Because mussels depurate hydrocarbons rapidly if not chronically exposed, we infer that mussels in contaminated beds likely acquired oil from surrounding sediment. Total PAH concentrations in mussels were correlated with those in underlying sediment, but correlation was sometimes poor, suggesting mussels acquired hydrocarbons from an area larger than that immediately covered by them. The ratio of PAH to total hydrocarbons in mussels was the same as in sediment, consistent with accumulation of whole oil rather than dissolved PAH. Mussel density (number of individuals per m^2) was not correlated with differences in hydrocarbon concentration.

INTRODUCTION

Concern focused on blue mussel (*Mytilus trossulus*[3]) beds impacted by the *Exxon Valdez* oil spill when damage assessment studies suggested links between oiled mussels and negative impacts on several predator species (Duffy et al.1996; Sharp et al. 1996). Most oiled mussel beds were not cleaned intensively in 1989 and 1990 by request of the *Exxon Valdez* Oil Spill Interagency Shoreline Cleanup Committee because mussels are such an important food source in intertidal communities. However, natural rates of hydrocarbon loss were slow; oil persisted longer and at much higher concentrations than expected, especially in beds on finer sediments. In 1991, more than two years after the spill, the presence of *Exxon Valdez* oil (EVO) in mussels and underlying sediments was confirmed in 13 mussel beds (Babcock et al. 1994), and concentrations in these beds were among the highest measured in Prince William Sound (PWS) that year. Total polynuclear aromatic hydrocarbon (PAH) concentrations ranged as high as 10 ± 3 μg/g dry weight in blue mussels and as high as 489 ± 32 μg/g dry weight in sediments (Babcock et al. 1994). These persistent high concentrations, and the importance of mussel beds

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[3]*Mytilus trossulus* Gould, 1850 (formerly *M. edulis* Linnaeus, 1758) (McDonald and Koehn 1988).

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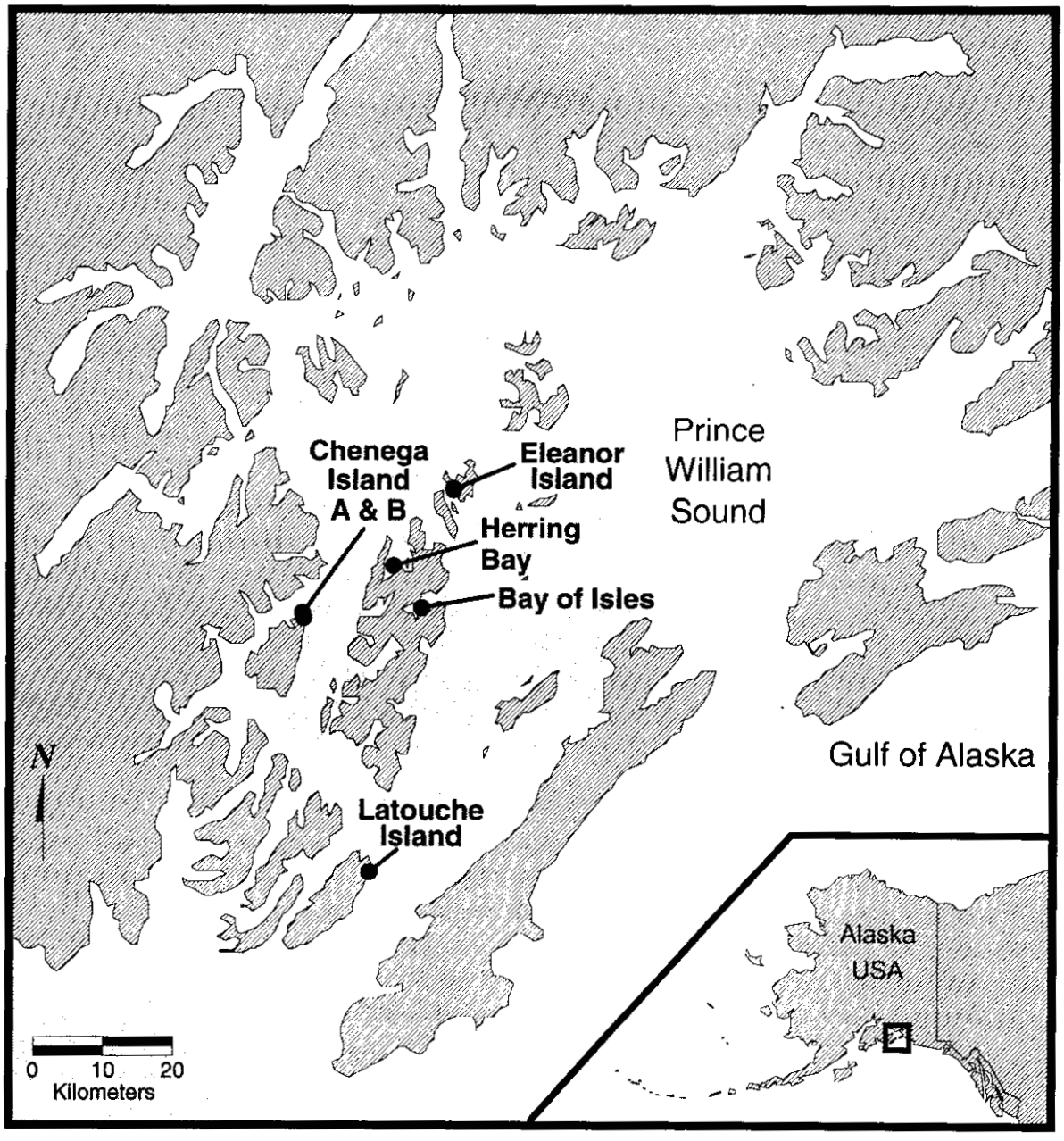

Figure 2.1. Location of oiled mussel beds sampled in Prince William Sound.

as feeding areas for many species, gave oiled mussel beds an importance disproportionate to their relatively small size and numbers.

This study was initiated in 1992 to assess hydrocarbon distributions in mussel bed sediments, and the relationship of this distribution to hydrocarbon uptake by mussels. Although sampling in 1991 (Babcock et al. 1994) had not addressed within-bed variability of oiling, it was apparent that oil distributions in underlying sediments was very uneven. An understanding of these uneven distributions was needed to adequately monitor oil remaining in the beds, and to design restoration studies.

METHODS

Site selection

Three sampling sites were identified through Alaska Department of Environmental Conservation Shoreline Patrol Assessment records, information from other Natural Resource Damage Assessment principal investigators, examination of hydrocarbon data from mussel beds sampled in 1991, and site visits early in 1992. Mussel beds were selected at Chenega Island A (CH010B-2A), Eleanor Island (EL013A), and Herring Bay (KN133A-1) (Figure 2.1). (Lables in parentheses are the *Exxon Valdez* Interagency Shoreline Cleanup Committee segment numbers plus a unique suffix.) These sites were selected for treatment because they were among the largest, most dense, and most contaminated beds identified by the 1991 study (Babcock et al. 1994; Chapter 1).

Study beds were on gently (<5%) sloping beaches and ranged in area from approximately 50-700 m^2 (Table 2.1). The tidal range occupied by the beds was 0.1-1.7 m above mean lower low water, but the tidal range occupied by each bed was at most 0.9 m. In the lower portions of those beds, oil-contaminated water remained close to the surface of the bed even at the lowest tides; on the upper transects subsurface water was below sampling depth. Sediments were gravel/granules over sand/silt, often with interspersed cobbles. Mussel shell length ranged from 30 to 45 mm and mean density was 1,924 ± 279, 4,917 ± 222, and 1,947 ± 272 mussels per m^2 at Chenega Island A, Eleanor Island, and Herring Bay, respectively. Recently settled and young mussels (<35 mm) were not present at any of the sites.

Sampling

Samples were collected in a grid laid out over the bed in May 1992 (Figure 2.2). Two or three transects were placed parallel to the water line in each bed. Exact location of transects depended on bed size and topography; they ranged from 2.6-8.25 m in length and were placed 2-7 m from adjacent transects (Table 2.1). Upper transects were located furthest from the water. Elevation at each transect was calculated from beach-slope estimates. Five 0.25 × 0.25 m quadrats were located along each transect at predetermined intervals except when bed shape limited a transect length. See figure 2.2 for quadrat intervals at Chenega Island A and Herring

Bay: except for the two endpoint positions (-5 and 3.25), intervals at Eleanor Island were the same as those in the middle transect at Chenega Island A.

At each quadrat, mussel densities were estimated and hydrocarbon samples were collected. Mussel density was estimated by counting mussels in one-half of the quadrat area. Samples collected for hydrocarbon analysis included mussels, byssal sediment, and surface sediment (0-2 cm deep). Each mussel sample consisted of 20 individuals, collected uniformly throughout each quadrat. The byssal sediments consisted of sediment originally attached to mussels by byssal threads. Surface sediment samples were composites of 8-10 subsamples collected throughout each quadrat (approximately 200 g total). Sampling instruments and sample 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.) Samples were frozen within 2-3 hours after collection. At Eleanor Island, coarseness of the sediment often made collection of separate byssal mat samples difficult, so byssal mat and surface sediments were generally combined. These combined samples were later dropped from analysis. Byssal and surface sediment samples from the Chenega Island bed were dry sieved to estimate percentages of silt plus clay (particles <62 μm) on a dry weight basis (Krumbein and Pettijohn 1988).

Table 2.1. Mussel bed areas, slopes, and transect positions. Each transect was placed parallel to the water line; total lengths ranged from 2.6 to 8.25 meters. Perpendicular spacing between transects (y) along beach surfaces was variable, as were corresponding tidal elevations (elev), reported with respect to mean lower low water.

Bed location	Area (m^2)	slope (%)	transect number	y (m)	elev (m)	length (m)
Chenega Island A	50	4.4	1	-2.0	1.50	6.50
			2	0.0	1.59	8.00
			3	3.0	1.73	5.00
Eleanor Island	700	3.7	1	-3.5	0.96	8.25
			2	3.5	1.23	8.25
Herring Bay	100	1.7	1	-3.0	0.12	2.60
			2	0.0	0.17	6.00
			3	5.0	0.25	4.00

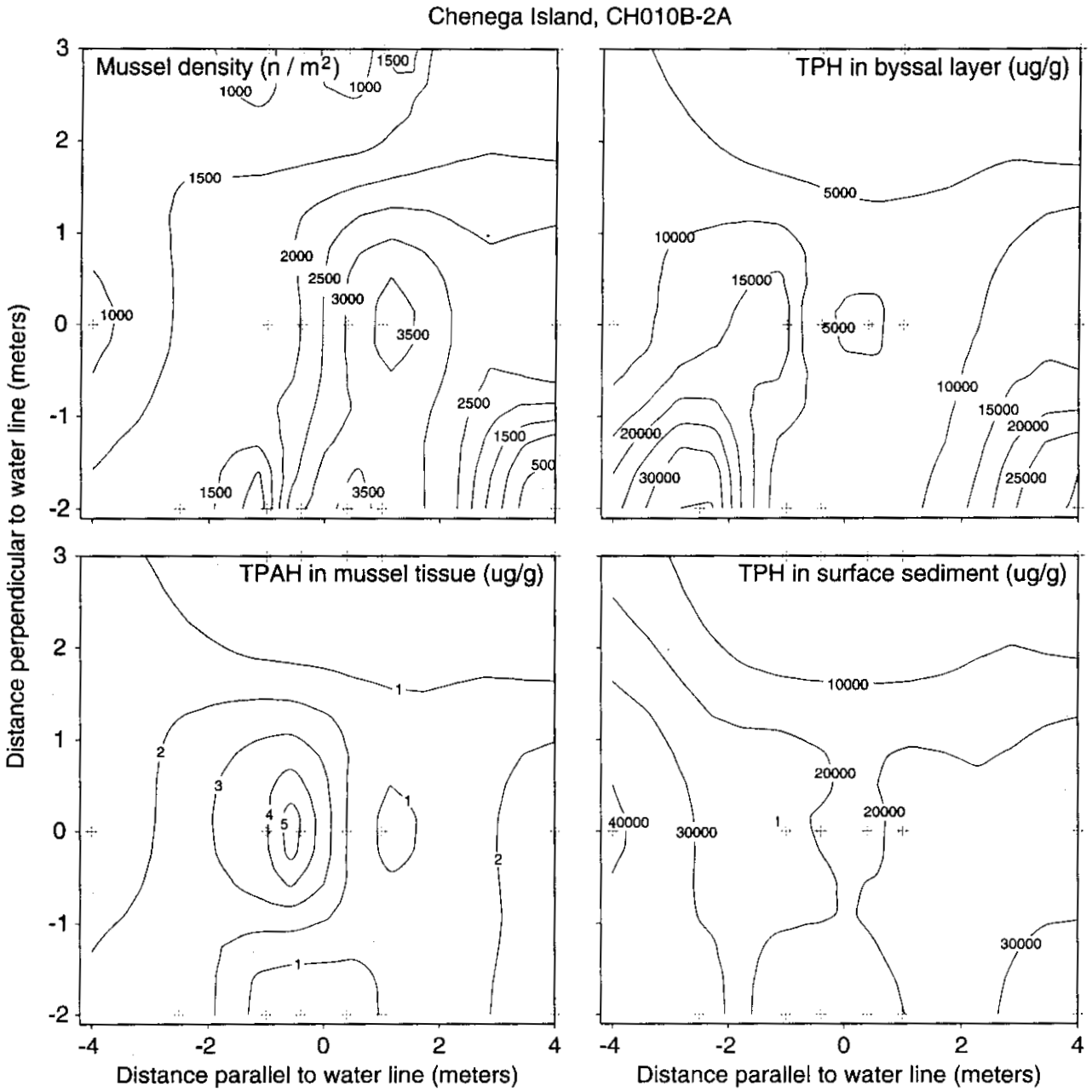

Figure 2.2. Approximate spatial distributions of mussel density, total polynuclear aromatic hydrocarbon (TPAH) concentration in mussel tissue, and total petroleum hydrocarbon (TPH) in byssal and surface sediment in the Chenega Island (a) and Herring Bay (b) mussel beds. Circles with cross-hairs (⊕) indicate quadrat positions. Figure continues on next page.

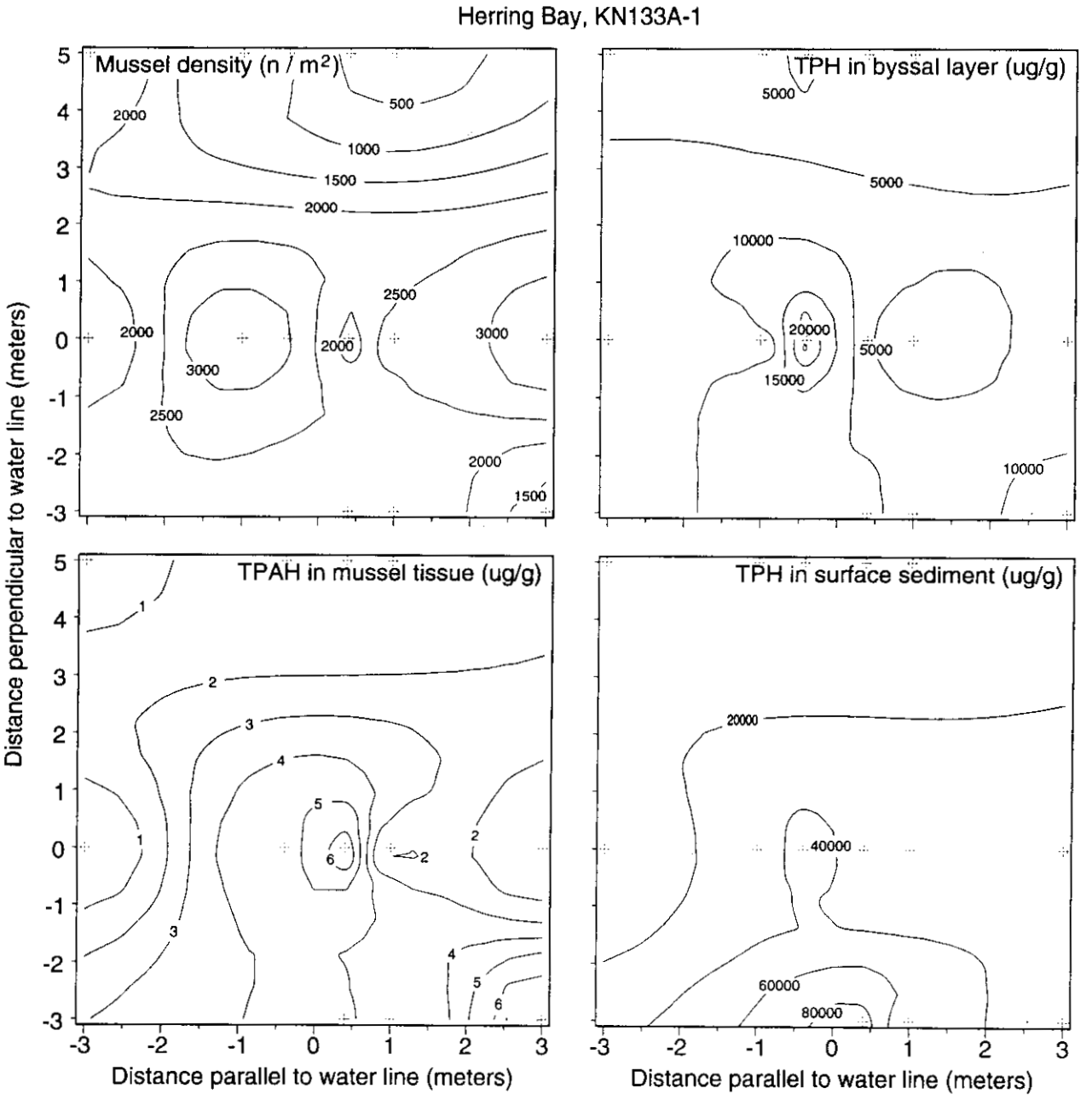

Figure 2.2, continued.

Chemical analyses

Two methods, ultraviolet fluorescence (UVF) fast screening and gas chromatography/mass spectroscopy (GC/MS), were used to quantify petroleum hydrocarbon concentrations. Although GC/MS analysis provides a more accurate measure of petroleum hydrocarbon content, a quicker, less expensive ultraviolet fluorescence (UVF) fast screening technique adapted from Krahn et al. (1993) was routinely used to estimate total petroleum hydrocarbons (TPH) (aromatic plus aliphatic compounds) in sediments (μg/g wet weight). Concentrations of TPH in each sample were quantified by comparing its fluorescence at 380 nm (maximal phenanthrene output) to the fluorescence of unweathered EVO. Little error was anticipated comparing an unweathered standard with possibly weathered samples because phenanthrene is relatively less susceptible to weathering than most other aromatics. A subset of sediment samples was selected for confirmatory GC/MS analysis.

Analysis of mussel samples and selected sediment samples by GC/MS was performed at the National Marine Fisheries Service, Auke Bay Laboratory (Short et al. 1996a). After addition of six internal standards, samples were extracted with dichloromethane. Isolation and purification of calibrated and uncalibrated compounds was completed by silica gel/alumina column chromatography followed by size-exclusion high-pressure liquid chromatography (HPLC) and fractionation. Extracts of PAH were separated and analyzed by gas chromatography equipped with a mass selective detector. Calibrated PAH were identified by retention time and two mass fragment ions characteristic of each PAH and quantified using a five point calibration curve. Uncalibrated PAH homologs (which included alkyl-substituted isomers of naphthalene, fluorene, dibenzothiophene, phenanthrene, and chrysene) were identified by retention time and the presence of a single characteristic mass fragment ion. Uncalibrated PAH were quantified by using calibration curves of their respective parent homologs. Experimentally determined method detection limits (MDL) depended on sample weights, and generally were 1 ppb in tissue and <2 ppb in sediment. Concentrations below MDL were treated as 0. 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 about 20%, depending on the PAH. Total PAH (TPAH) concentration is reported in μg/g dry weight, and represents the sum of all measured aromatic hydrocarbons except perylene because perylene is produced contemporaneously by biological sources in PWS (Appendix 2).

Data analyses

Contour maps were developed for Chenega Island and Herring Bay sites to portray physical location, mussel density, and hydrocarbon distributions. (The sampling frequency was insufficient to justify mapping at Eleanor Island.) Although these spatial distributions provide an generalized overview of conditions in mussel beds, they can be misleading, hence we relied on regression analyses for definitive results.

Relationships among observed factors were explored with regression techniques. To determine possible correlation among variables, all data (distance along transect, elevation,

hydrocarbon concentrations in various matrices, and grain size) from each quadrat in each bed were regressed against each other (univariate regressions). Models considered were ladder of powers (x-transformations from x^3 through $-1/x^3$), $y = ae^{bx}$, and $y = ab^x$. Although *P*-values were always significant ($P \leq 0.05$) where $r^2 \geq 0.5$, a more conservative test, F_o/F_{crit}, where F_o = the observed F-ratio and F_{crit} = the critical F-ratio ($F_{(1,vr,0.95)}$, where vr = residual degrees of freedom), was also applied to judge the usefulness of the regression (Draper and Smith 1981). Significant relationships determined by regression were compared among sites with analysis of covariance (ANCOVA). Stepwise regression was used to determine if TPAH concentration in mussels was related to a combination of other factors.

Hydrocarbon TPH concentrations in sediments were further compared among transects and between depths within quadrats using two-way split-plot analysis of variance (ANOVA). Transects were considered blocks and depth was the treatment. Depth included two groups, byssal and surface sediment. Separate analyses were completed for each site. Concentrations were log-transformed before analysis. Log conversion influenced results, but did not change overall interpretation; differences at low concentrations were more frequently significant with the transformation, and differences at high concentrations were more frequently significant without transformation.

Split-plot analyses were expanded to include estimated TPAH concentration in all three matrices - mussel tissue, byssal sediment, and surface sediment. Standard curves relating TPAH to TPH concentrations in surface sediment and in byssal sediment were developed from samples analyzed with both UVF and GC/MS methods and applied to remaining TPH concentrations in sediment to estimate equivalent TPAH concentrations. Data included in the standard curves included observations by Babcock et al. (Chapter 1; Chapter 4), plus our data. Because the slope was significantly different for byssal data, as tested with analysis of covariance ($P < 0.001$), separate standard curves were used to estimate TPAH concentration in byssal and surface sediment layers ($TPAH_{byssal} = -26.614 + 0.025 \cdot TPH_{byssal}$, $r^2 = 0.89$, $n = 16$; $TPAH_{sediment} = -15.872 + 0.009 \cdot TPH_{sediment}$, $r^2 = 0.73$, $n = 103$). Concentrations of TPAH were estimated only for samples where TPAH was not measured directly.

Petroleum hydrocarbon fingerprinting and weathering

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 in weathered EVO. 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 (Appendix 2). [$W = 0$ in unweathered samples and increases with weathering. For all environmental samples recorded in the Natural Resource Damage Assessment database (Short et al. 1996b), w ranges up to 11.3 for sediment and 9.9 for mussels. For this paper we use the following definitions: unweathered ($w = 0$), slightly weathered ($0 < w \leq 2$), moderately weathered ($2 < w \leq 8$), and highly weathered ($w > 8$).] 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 EVO (Short and Heintz 1997). The

model could not be applied to samples where one or more critical PAH concentrations were below MDL.

Weathering and ratios of TPAH to total hydrocarbons were compared among sites and sample types with two-factor ANOVA (site × sample type). The number of sediment samples confirmed by GC/MS was relatively low, thus there were insufficient data to support the more complex split-plot ANOVA design used for TPH and TPAH. The ratio of TPAH to total hydrocarbons was calculated as follows: TPAH / (TPAH + $\sum$alkanes), where $\sum$alkanes = the sum of all alkanes including the unresolved complex mixture. Because PAH are more soluble than alkanes, increased ratios of TPAH to total hydrocarbons in mussels would indicate uptake of dissolved hydrocarbons. Conversely, similar ratios in sediment and in mussels would indicate uptake of whole oil particles. Ratios were arc-sin transformed before analysis (interpretation of the statistical analysis was the same with untransformed data).

RESULTS

Spatial distributions

The distribution of mussel densities and oil in mussels and sediment varied spatially at each site (Figure 2.2). Density and oil concentration gradients were apparent at Chenega Island and Herring Bay. (The sampling frequency was insufficient to justify mapping at Eleanor Island.) There appeared to be some consistency between hydrocarbon concentrations in mussel tissue, in byssal sediment, and in surface sediment in Chenega Island and Herring Bay sites, but mussel density was apparently unrelated to hydrocarbon distribution. Hydrocarbon distributions at Chenega Island and Herring Bay were unique to each specific bed. Consistent between Chenega Island and Herring Bay beds, concentrations tended to be lower furthest from the water line. However, at Eleanor Island hydrocarbon concentrations in the upper transect were higher than in the lower transect. The ensuing analyses quantify these generalized relationships.

Relationships between variables

At Chenega Island, there was significant correlation between several variables (i.e., where $F_o/F_{crit} \geq 4$, $P \leq 0.01$; Table 2.2). TPAH concentration in mussels was significantly correlated with TPH concentration in byssal and surface sediment ($0.67 \leq r^2 \leq 0.72$). TPH concentration in byssal sediment was also correlated significantly with TPH concentration in surface sediment, grain size in byssal sediment, and elevation ($0.80 \leq r^2 \leq 0.88$). Also significantly correlated with elevation were TPH in surface sediment ($r^2 = 0.65$) and grain size in byssal sediment ($r^2 = 0.92$). Grain size in byssal sediment was significantly correlated with that in surface sediment ($r^2 = 0.84$). Correlation among grain size and all variables except distance parallel to the water line was ≥ 0.62 ($P \leq 0.02$), but F_o/F_{crit} was <4 for these additional relationships. Because grain size was significantly related to elevation, it was not possible to definitively separate the importance of these two factors.

Table 2.2. Relationships among variables at Chenega Island: X is distance parallel to water line, ELEV is elevation, TPHB is total petroleum hydrocarbon in byssal sediment, TPHS is total petroleum hydrocarbon concentration in surface layer, TPAH is total polynuclear aromatic hydrocarbon concentration in mussel tissue, BGRA is percent grain <62 μm in byssal sediment, SGRA is percent grain <62 μm in surface sediment, and DENS is mussel density. Asterisks in the r^2 portion of the table indicate that $F_{observed} / F_{crit} \geq 4$, where where $F_{observed}$ is regression mean square divided by residual mean square, and $F_{crit} = F_{(1,vr,0.95)}$, vr = residual degrees of freedom. Best fit models were as marked: a) exponential, b) power, c) x^3, d) x^2, e) linear, f) $x^{0.5}$, g) ln(x), h) $-1/x^{0.5}$, i) $-1/x$, j) $-1/x^2$, k) $-1/x^3$.

r^2

	ELEV	TPHB	TPHS	TPAH	BGRA	SGRA	DENS
X	0.01	0.04	0.20	0.06	0.07	0.11	0.06
ELEV		0.80*	0.65*	0.48	0.92*	0.62	0.12
TPHB			0.81*	0.72*	0.88*	0.72	0.05
TPHS				0.67*	0.72	0.70	0.15
TPAH					0.66	0.71	0.07
BGRA						0.84*	0.04
SGRA							0.10

Probability

	ELEV	TPHB	TPHS	TPAH	BGRA	SGRA	DENS
X	0.643^{g}	0.478^{b}	0.073^{j}	0.359^{b}	0.523^{k}	0.417^{k}	0.324^{i}
ELEV		0.000^{a}	0.000^{a}	0.002^{a}	0.000^{h}	0.020^{c}	0.170^{c}
TPHB			0.000^{f}	0.000^{b}	0.001^{b}	0.008^{g}	0.420^{g}
TPHS				0.000^{a}	0.008^{f}	0.009^{a}	0.124^{g}
TPAH					0.014^{i}	0.009^{h}	0.293^{i}
BGRA						0.001^{k}	0.643^{j}
SGRA							0.447^{b}

Number of observations

	ELEV	TPHB	TPHS	TPAH	BGRA	SGRA	DENS
X	17	15	17	17	8	8	17
ELEV		15	17	17	8	8	17
TPHB			15	15	8	8	15
TPHS				17	8	8	17
TPAH					8	8	17
BGRA						8	8
SGRA							8

Table 2.3. Relationships among variables at Eleanor Island: X is distance parallel to water line, ELEV is elevation, TPHB is total petroleum hydrocarbon in byssal sediment, TPHS is total petroleum hydrocarbon concentration in surface layer, TPAH is total polynuclear aromatic hydrocarbon concentration in mussel tissue, BGRA is percent grain <62 μm in byssal sediment, SGRA is percent grain <62 μm in surface sediment, and DENS is mussel density. Note: $F_{observed} / F_{crit} \geq 4$, where where $F_{observed}$ is regression mean square divided by residual mean square, and $F_{crit} = F_{(1,vr,0.95)}$, vr = residual degrees of freedom was < 4 in every case. Best fit models were as marked: a) exponential, b) power, c) x^3, d) x^2, e) linear, f) $x^{0.5}$, g) ln(x), h) $-1/x^{0.5}$, i) $-1/x$, j) $-1/x^2$, k) $-1/x^3$.

r^2

	ELEV	TPHB	TPHS	TPAH	DENS
X	0.02	0.16	0.34	0.16	0.24
ELEV		0.66	0.30	0.05	0.28
TPHB			0.28		0.36
TPHS					0.40
TPAH					0.03

Probability

	ELEV	TPHB	TPHS	TPAH	DENS
X	0.657[k]	0.254[a]	0.059[a]	0.599[k]	0.102[d]
ELEV		0.004[c]	0.080[b]	0.775[a]	0.074[e]
TPHB			0.117[b]		0.151[c]
TPHS					0.127[f]
TPAH					0.820[c]

Number of observations

	ELEV	TPHB	TPHS	TPAH	DENS
X	16	10	11	4	12
ELEV		10	11	4	12
TPHB			10		7
TPHS					7
TPAH					4
DENS					

Table 2.4. Relationships among variables in Herring Bay: X is distance parallel to water line, ELEV is elevation, TPHB is total petroleum hydrocarbon in byssal sediment, TPHS is total petroleum hydrocarbon concentration in surface layer, TPAH is total polynuclear aromatic hydrocarbon concentration in mussel tissue, BGRA is percent grain <62 µm in byssal sediment, SGRA is percent grain <62 µm in surface sediment, and DENS is mussel density. Note: $F_{observed} / F_{crit} \geq 4$, where where $F_{observed}$ is regression mean square divided by residual mean square, and $F_{crit} = F_{(1,vr,0.95)}$, vr = residual degrees of freedom was < 4 in every case. Best fit models were as marked: a) exponential, b) power, c) x^3, d) x^2, e) linear, f) $x^{0.5}$, g) ln(x), h) $-1/x^{0.5}$, i) $-1/x$, j) $-1/x^2$, k) $-1/x^3$.

r^2

	ELEV	TPHB	TPHS	TPAH	DENS
X	0.18	0.02	0.30	0.35	0.02
ELEV		0.19	0.53	0.38	0.36
TPHB			0.43	0.25	0.20
TPHS				0.56	0.33
TPAH					0.04

Probability

	ELEV	TPHB	TPHS	TPAH	DENS
X	0.131[c]	0.657[b]	0.044[b]	0.042[b]	0.669[b]
ELEV		0.118[c]	0.003[i]	0.033[b]	0.022[c]
TPHB			0.010[e]	0.095[f]	0.111[b]
TPHS				0.005[b]	0.032[f]
TPAH					0.547[a]

Number of observations

	ELEV	TPHB	TPHS	TPAH	DENS
X	14	14	14	12	14
ELEV		14	14	12	14
TPHB			14	12	14
TPHS				12	14
TPAH					12

Fewer significant correlations among variables were observed at Eleanor Island and in Herring Bay, and F/Fcrit $\leq$ 3.0 at these sites (Table 2.3-2.4). Concentration of TPH in byssal sediment was correlated with elevation ($r^2 = 0.66$, $P \approx 0.004$) at Eleanor Island. Correlation where r^2 was >0.5 in Herring Bay was limited to a correlation between TPAH concentration in mussels and TPH concentration in sediment ($r^2 = 0.56$), and between TPH concentration in sediment and elevation ($r^2 = 0.53$) ($P \leq 0.003$). The sampling frequency at Herring Bay, and particularly at Eleanor Island, was lower than at Chenega Island (Tables 2.2-2.4).

Notably, mussel density did not correlate with any other variable at any site ($0.03 \leq r^2 \leq 0.40$, $0.022 \leq P \leq 0.820$, $F_o/F_{crit} \leq 1.4$), and distance along transects (parallel to the water line) did not correlate with any other variable ($0.02 \leq r^2 \leq 0.35$, $0.042 \leq P \leq 0.669$), $F/F_{crit} \leq 1.1$).

Mean hydrocarbon concentrations (by transect) were consistently least in mussel tissue, intermediate in byssal sediment, and highest in surface sediment at all three sites (Figure 2.3). Mean TPAH concentrations in mussels were consistently less than in byssal sediment and surface sediment; these differences were significant in 4 of 8 transects ($0.001 < P \leq 0.039$). Concentration of TPAH in mussels tended to center about 1 µg/g, and did not fluctuate as much as TPAH concentration in byssal and surface sediment. Where differences were significant ($0.007 \leq P \leq 0.026$), concentration in byssal sediment was less than in surface sediment (middle and upper Chenega Island A transects). [When tested without log transformation, TPH concentration also differed significantly between byssal and surface sediments in the lower and middle transects at Herring Bay ($0.001 \leq P \leq 0.024$).]

TPAH composition

Composition of PAH in byssal and surface sediment and in mussel tissue collected more than three years after the spill was characteristic of *Exxon Valdez* oil. Of 14 confirmatory GC/MS byssal samples, 10 met model criteria; of these, 50% were identified by the Short & Heintz (1997) model as significant for *Exxon Valdez* oil. Of 15 confirmatory GC/MS surface sediments, 10 met model criteria; of these, 67% were identified as significant for *Exxon Valdez* oil. In mussels, 33 of 47 samples met model criteria; of these, 42% were identified as significant for *Exxon Valdez* oil. Estimated weathering factor w ranged from 0.1 to 12.0, 0.7 to 10.5, and 2.6 to 8.9 in byssal sediment, surface sediment, and mussels, respectively, thus samples ranged from slightly to highly weathered. Weathering did not differ significantly among beds ($P = 0.119$) or sample type ($P = 0.536$); mean w was 5.5 ± 0.4 (moderately weathered). Differences among weathering in byssal sediment, surface sediment, and mussels were not significant ($P \approx 0.300$). Ratios of TPAH to total hydrocarbons did not differ significantly among beds ($P = 0.254$) or among sample types ($P = 0.936$). Percent TPAH of total hydrocarbons averaged $1.0 \pm 0.1\%$, about the same as the percentage ($0.8 \pm 0.1\%$) in unweathered *Exxon Valdez* crude oil.

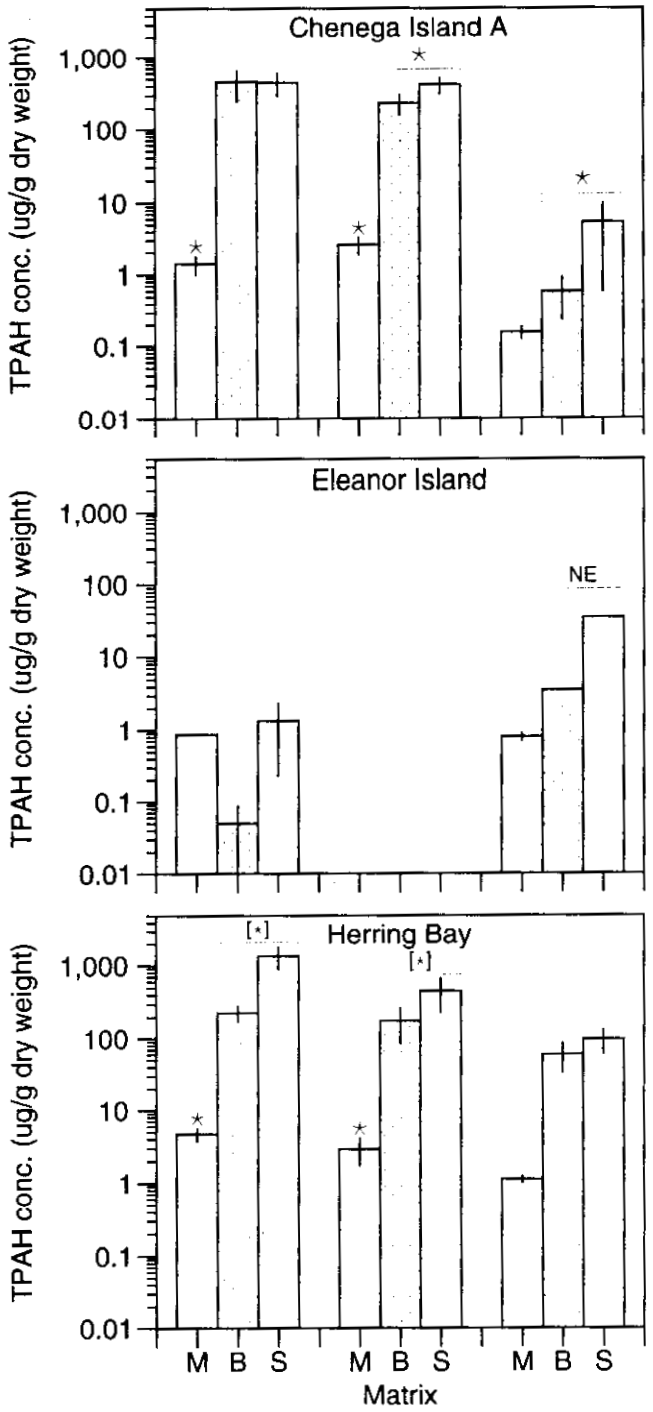

Figure 2.3. Total polynuclear aromatic hydrocarbon (TPAH) concentrations in three matrices: mussels (M), byssal sediment (B), and surface sediment (S) from three mussel beds in Prince William Sound, May 1992. All data in this figure were either converted to TPAH or had direct TPAH measurements (see methods). Asterisks over mussel bars indicate that concentration differed significantly from that in byssal or surface sediment: one comparison was not estimable (NE). Asterisks between byssal and surface sediment bars indicate that these concentrations differed significantly. Asterisks in brackets indicate significant differences when analysis was completed without log transformation; all other asterisks report the results of log-transformed TPAH analysis.

DISCUSSION

Considerable variation in the spatial distribution of petroleum hydrocarbons in the mussels and sediments in each of mussel beds overlying soft sediment in PWS was apparent in our data. For example TPH concentrations high as 30,000-40,000 μg/g wet weight in sediment were found within a few meters of those 2 orders of magnitude less. Concentrations were generally correlated with tidal elevation, but reasons for this correlation are not clear. Possibly the interaction of beach substrate, oil, and tides at the time of contamination influenced later hydrocarbon distributions. Grain size may also have influenced concentration distributions; high percentages of silt and clay in soils are usually associated with increased oil retention by providing more surface area for oil adsorption than coarser sediments (Wade and Quinn 1980). Although we observed a correlation between grain size and TPH concentration, grain size was also correlated with other factors, including tidal elevation, thus confounding the results. Mussel density did not correlate with any other factors, thus we infer that mussel density did not influence hydrocarbon distributions in underlying sediment (within the limits of this study), and conversely that concentrations in sediment were not sufficiently high to preclude mussel survival. We do not know how mussel density, water currents, and other factors interacted at the time of oiling, nor do we know how stable the observed hydrocarbon distributions are over time. Water currents and subsurface hydrology probably also influence hydrocarbon distributions both temporally and spatially, but our data do not address this issue.

Hydrocarbon distribution in mussels was also uneven within a mussel bed, and related to sediment oiling. Mussels likely acquired hydrocarbons from surrounding sediment, indicated by correlation between concentration in mussels and that in byssal and surface sediment, the generally lower TPAH concentrations in mussels than in sediments, and the similar weathering condition of oil in mussels and sediment. However, the sometimes weak correlation between TPAH in mussels and in sediment suggests that mussels may accumulate hydrocarbons over a broader area than that covered by any sample-sized group of individuals. Oil in mussels could not have been retained since the *Exxon Valdez* oil spill, because mussels depurate hydrocarbons if not chronically exposed (Lee et al. 1973; Clark and Finley 1975) and mussels on nearby bedrock, which would have received similar initial oiling, were no longer contaminated in 1992 (Harris 1996; Thomas et al. 1998; Chapter 5). Therefore, contamination measured in 1992 must have resulted from continued exposure. If mussels had accumulated oil directly from the sediment under them, correlation would have been more precise between mussel and sediment contamination at specific quadrats. The more general distribution pattern in each bed of oil in mussels and in sediments implies that hydrocarbons were washed out of the sediment, dispersed locally into the water, and were taken up by the mussels from the water. Because the ratio of PAH to total hydrocarbons in mussels was the same as in sediment, mussels likely accumulated whole oil not dissolved PAH.

CONCLUSIONS

Exxon Valdez oil remained trapped in mussel beds 3 years after the spill. On the average, the oil was moderately weathered. Weathering was similar at all three beds in mussels and sediments.

Distribution of EVO in sediments under mussel beds were uneven. Hydrocarbon concentrations were correlated with tidal elevation and grain size, but grain size and elevation were also correlated thus confounding further interpretation.

Hydrocarbon concentrations in mussels were correlated with those in byssal and surface sediments, but concentrations in mussels were generally lower than those in sediment. Because other studies have demonstrated that mussels depurate hydrocarbons in clean water and will not remain contaminated without chronic exposure, we infer that mussels in contaminated beds likely acquired oil from surrounding sediment.

Correlation between hydrocarbons in mussels and underlying sediment was not always good, suggesting that hydrocarbons were accumulated over a broader area than that covered by any sample-sized group of individuals. Hydrocarbons are likely washed out of sediment, dispersed locally into surrounding water, and accumulated by mussels from the water. Mussels apparently accumulated whole oil, not dissolved PAH, because the ratio of PAH to total hydrocarbons in mussels was the same as in sediment.

Mussel density was not correlated with hydrocarbon concentration.

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LITERATURE CITED

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Chapter 3: Manipulation of oiled mussel beds to accelerate depuration of hydrocarbons

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ABSTRACT

Two experimental manipulations to reduce persistently high hydrocarbon concentrations without decreasing mussel density were tested in several mussel beds oiled by the *Exxon Valdez* spill in Prince William Sound. In each of three beds, a linear strip (0.3 m wide) of mussels and attached sediments was removed to increase natural flushing of the beds. Total hydrocarbon concentrations in sediments and mussels were not significantly reduced by stripping, and adult mussels from the surrounding bed recolonized exposed areas within three months, thus preventing the possibility of further hydrocarbon flushing. We also transplanted several small patches (0.25 × 0.50 m) of mussels from two oiled beds onto nearby clean sediments. Transplanted mussels depurated hydrocarbons quickly; within three months total polynuclear aromatic hydrocarbon loads were about 10% of pre-transplant concentrations, but concentrations in undisturbed mussels remained about the same. However, mortality of transplanted mussels was high, possibly due to placement of mussels in suboptimal habitat or senescence of mature mussels. Neither experimental technique fulfilled both criteria for successful restoration (reduction of hydrocarbon concentration and mussel survival). These preliminary experimental manipulations suggest more aggressive mussel bed manipulation will be necessary to accelerate the rate of hydrocarbon loss from them, but the cost of treatment might be increased mussel mortality.

INTRODUCTION

Many blue mussel (*Mytilus trossulus*) beds impacted by the *Exxon Valdez* oil spill (EVOS) of March 24, 1989 were not cleaned because the cleaning would likely have destroyed them, thus eliminating an important food source for many invertebrates, birds and mammals. Expectation was that natural processes would soon clean the beds, and thus eliminate a possible oil-hydrocarbon uptake pathway to predators. In 1991, however, substantial amounts of polynuclear aromatic hydrocarbons (PAH) from *Exxon Valdez* oil (EVO) still remained in mussels (up to 10.3 μg/g dry weight total PAH) and in sediments underlying mussel beds (up to 489.1 μg/g dry weight total PAH) (Babcock et al. 1994). Oil trapped in sediment is the most likely source for long-term contamination of mussel tissue (Harris et al. 1996; Chapter 2). Persistent, high concentrations of hydrocarbons in mussels were identified as a possible source of contamination for several consumer species (Duffy et al. 1996; Sharp et al. 1996) and for human subsistence users. Annual monitoring was initiated by the EVOS trustees to track hydrocarbon losses from many beds, to determine if active restoration and cleaning would be needed in subsequent years to accelerate natural restoration processes.

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Potential restoration of oiled mussel beds posed a significant and controversial management problem. Hot water treatment might have reduced hydrocarbon concentrations in underlying sediments, but would have resulted in the probable loss of the mussels and the loss of major intertidal habitat. Our goal was to reduce petroleum hydrocarbon concentrations to original background levels without destroying mussels and further impacting predators dependent on them.

Two types of mussel bed manipulations designed to enhance hydrocarbon depuration in mussels without lowering mussel densities were studied. The first method involved removal of 0.3 m-wide strips of mussels perpendicular to the water line at each of 3 beds in an effort to reduce oil concentrations in sediments and mussels by increasing water circulation throughout the beds. Nearby unmanipulated oiled beds served as references. Our objectives for this portion of the study were to determine 1) if petroleum hydrocarbon concentrations in sediment and mussels were reduced within strips and throughout each bed, 2) if manipulation would establish a concentration gradient throughout each bed, and 3) to observe the effect of manipulation on mussel survival and bed integrity. Our second method was to transplant small patches of contaminated mussels to nearby clean sediments, thus removing them from the source of chronic contamination and opening the original bed to increased flushing. Objectives for the second method were to observe transplant effects in both old and new locations on 1) hydrocarbon concentrations in sediment and mussels, and 2) on mussel density. Evaluating the feasibility and effectiveness of these techniques is critical in planning future mussel bed restoration given the slow natural rates of hydrocarbon loss.

METHODS

Site selection

Potential sites were identified through Alaska Department of Environmental Conservation (ADEC) Shoreline Patrol Assessment records, information from other principal investigators, and examination of petroleum hydrocarbon data from oiled mussel beds sampled in 1991 (Babcock et al. 1994). Three beds were selected for experimental treatment, and three similar beds were selected as references. Each bed is identified in this report by the *Exxon Valdez* Interagency Shoreline Cleanup Committee segment number plus a unique suffix.

Chenega Island A (CH010B-2A), Herring Bay (KN133A-1), and Eleanor Island (EL013A) mussel beds (Figure 3.1) were selected for treatment because they were among the largest, most dense, and most contaminated beds identified by the 1991 study (Babcock et al. 1994; Chapter 1). Bed sediments were gravel/granules underlain with sand/silt; the absence of large cobbles or boulders allowed easy removal of strips of mussels and attached sediments by shovel. Selected bed areas ranged from 50-700 m^2 on gently sloping beaches (gradient < 5%). Tidal range occupied by the beds was 0.1-1.7 m above mean lower low water. Mussel shell length ranged from 30-45 mm. Detailed initial bed conditions have been previously reported (Harris et al. 1996; Chapter 2).

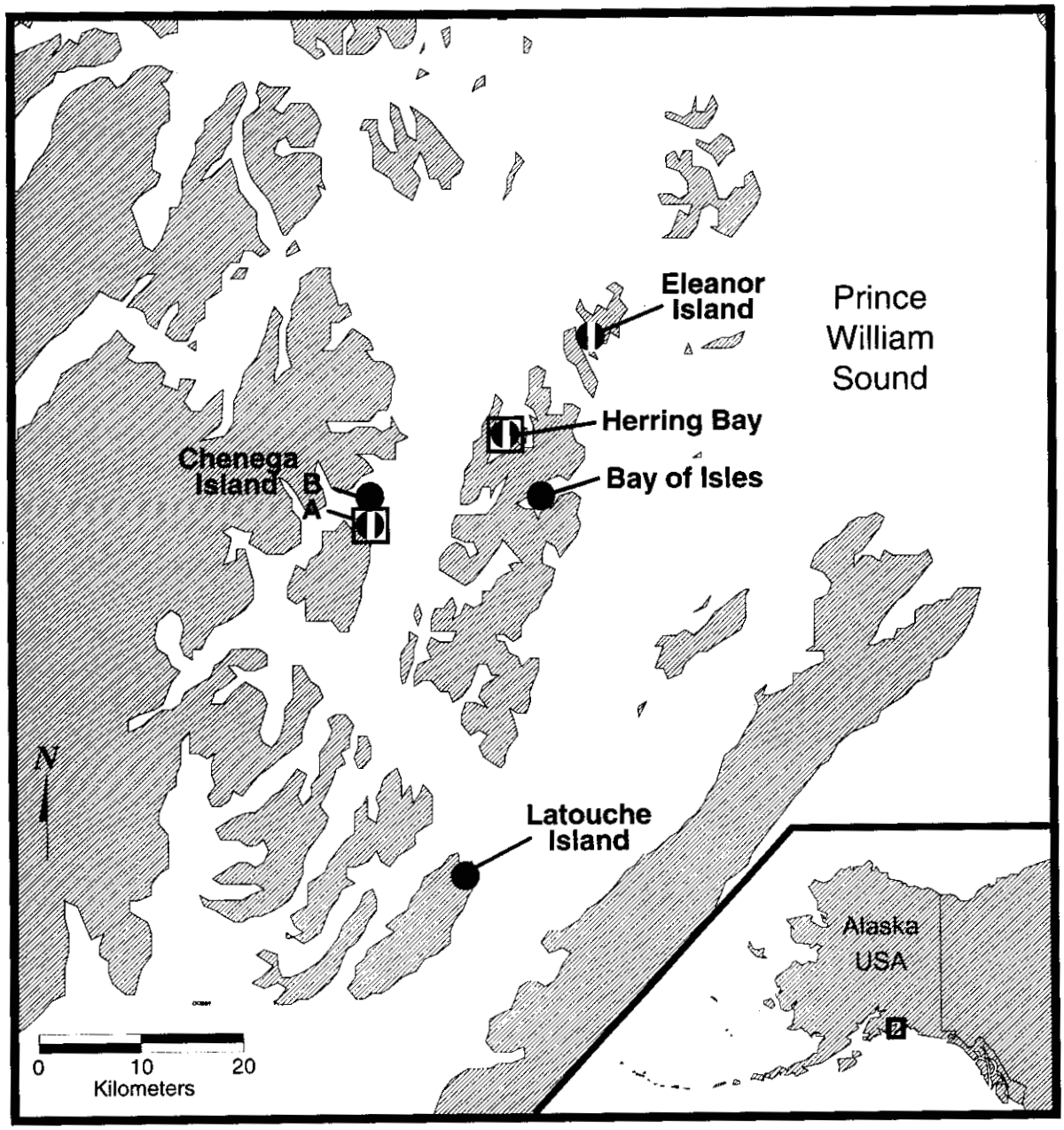

Figure 3.1. Experimentally manipulated and reference mussel beds in Prince William Sound. All beds were contaminated with *Exxon Valdez* crude oil. Circles with stripes represent beds where a strip of mussels and attached sediments was removed, full circles indicate reference beds, and squares mark beds where transplantation occurred.

Three beds similar to treated beds, Bay of Isles (KN136A-2), Chenega Island B (CH010B-2B), and Latouche Island (LA015E-2), were selected as untreated reference beds (Figure 3.1). Surface sediment at all three reference beds was gravel/granules, but the underlying layer was different at each site - peat at Bay of Isles, sand/silt at Chenega Island B, and bedrock at Latouche Island. Reference bed areas ranged from 9 m^2 to 180 m^2 and slopes ranged from 0.7-11.2% (Table 3.1). Tidal range occupied by the beds was about 0.1 to 1.8 m above mean lower low water. Mussel shell length ranged from 30-45 mm.

The Herring Bay and Chenega Island A beds were chosen for small scale mussel patch transplanting in 1993, after completion of the strip removal experiment (Figure 3.1). Locations of recipient patches were constrained by the requirement that these areas have low hydrocarbon concentrations, be located near donor beds, and capable of supporting mussels. Because hydrocarbon concentrations varied with elevation, we were forced to locate recipient areas either upslope (at Herring Bay) or downslope (at Chenega Island B) of donor patches. Recipient patches were at the limit of the tidal range occupied by local mussels in Herring Bay and were in

Table 3.1. Mussel bed areas, slopes, and transect positions. Each transect was placed parallel to the water line; total lengths ranged from 2.6 to 8.25 meters. Strips were positioned perpendicularly to the transects and passed through the zero point of each transect at a given site. Strips extended from -3.8 to +4.0 m at Chenega Island A, -4.7 to +4.4 m at Eleanor Island, and -3.4 to +7.0 m at Herring Bay. Perpendicular spacing between transects (y) along beach surfaces was variable, as were corresponding tidal elevations (elev), reported with respect to mean lower low water.

						Distance parallel to water line		
Bed location	Area (m^2)	slope (%)	transect number	y (m)	elev (m)	min (m)	max (m)	length (m)
Chenega Island A	50	4.4	1	-2.0	1.50	-2.50	4.00	6.50
			2	0.0	1.59	-4.00	4.00	8.00
			3	3.0	1.73	-1.00	4.00	5.00
Eleanor Island	700	3.7	1	-3.5	0.96	-5.00	3.25	8.25
			2	3.5	1.23	-5.00	3.25	8.25
Herring Bay	100	1.7	1	-3.0	0.12	0.40	3.00	2.60
			2	0.0	0.17	-3.00	3.00	6.00
			3	5.0	0.25	-3.00	1.00	4.00
Chenega Island B	8.9	11.2						
Bay of Isles	180	0.7						
Latouche Island	110	<1						

an area more exposed to wave action than the donor beds. Recipient sediments at both sites lacked the fine biogenic material (contributed by a dense layer of mussels and associated fauna) characteristic of the donor beds. Thus our choice of recipient locations optimized hydrocarbon differences, but may have been suboptimal for mussel survival.

Strip Removal Experiment

Mussels and surface sediments (0-2 cm depth) were sampled in the six beds in May 1992, before strips of mussels were removed at the three treatment sites and reported by Harris et al. (1996; Chapter 2). Hydrocarbon concentrations in sediments and mussels collected after removal were compared with initial concentrations to evaluate effectiveness of the manipulation. Hydrocarbon concentrations in reference beds were monitored to detect any general changes not related to strip removal. Mussel densities before removal were compared to those after removal, both in the bed as a whole and in the strip to evaluate the effect on the integrity of the beds and to see how quickly the strip would be reoccupied. The positions of individually tagged mussels along strip margins were monitored to indicate movement toward or away from the strips.

Fixed transects and/or sample points were established for each bed. For manipulated beds, 2-3 transects were located parallel to the shoreline and bisected by the area to stripped. The location of these transects depended on bed size and topography; transects generally extended 2-5 m horizontally and were spaced 0.1 m vertically from adjacent transects (Table 3.1). At each sample time, 0.25 m $\times$ 0.25 m quadrats were systematically adjusted about each fixed point in the vertical direction to avoid previously sampled spots. (All locations sampled were positioned within 0.25 m of the fixed points). Similarly, 3-4 fixed sample points were placed in each reference bed, but no transects were established in these beds.

In each quadrat, separate samples of mussels, shallow sediment (0-2 cm), and deep sediment (4-6 cm) were collected for hydrocarbon analysis. Each mussel sample consisted of 20 individuals to provide at least 10 g of tissue for hydrocarbon analysis. Surface sediments were collected below the byssal layer. Approximately 200 g sediment samples were collected with hydrocarbon-free stainless steel spoons. All hydrocarbon samples were placed in hydrocarbon-free sampling jars, chilled, and frozen within 2-3 h. Mussel density was estimated for all six beds by counting mussels in one-half of each quadrat before hydrocarbon samples were collected.

After initial sampling, a 0.3-m wide strip of mussels and the sediments attached to the byssal threads (to a depth of approximately 1 cm), was removed along the central axis of each bed at right angles to, and bisecting or passing through the 0 point of the sample transects (Table 3.1). At the Chenega Island A and Herring Bay beds, the strip extended completely through the mussel bed; at the large Eleanor Island bed, the strip extended from the seaward edge of the bed to 0.5 m above the upper sample transect. The area of mussels removed was small in proportion to bed size (4.7%, 0.4%, and 3.1% for Chenega Island A, Eleanor Island, and Herring Bay, respectively). Individual mussels were tagged with colored 0.3 cm $\times$ 0.7 cm tags at 0.5 m intervals along the margins of each strip, and their distances along and from the center of and along the strip were recorded.

Sediment samples were collected and mussel density was estimated 1, 3, and 13 months after strip removal. Positions of the individually tagged mussels were determined at 1 and 3 months. Photographs documented the movement of adult mussels onto strips. Stability of strip margins and sedimentation in the strip were also recorded photographically.

Patch transplant experiment

Mussels were transplanted from the most contaminated parts of the two selected mussel beds (Chenega Island B and Herring Bay) to cleaner sediments adjacent to the beds. Hydrocarbon concentrations in mussels and sediments in both the spot from which they had been transplanted (donor patch) and in the new spot (recipient patch) were monitored 2 and 13-14 months after relocation and compared with pre-treatment concentrations. Bed-wide changes in hydrocarbon concentrations and mussel densities in the donor beds provided perspective on changes not related to the transplanting.

Before transplantation, mussels previously present in recipient patches and the immediate area were removed. Mussel densities were not measured in recipient locations before transplantation, but were visibly lower than in the donor beds. Mussels and attached sediments were transplanted from four 0.25 × 0.5 m patches in the oiliest parts of the Chenega Island A bed and from three areas in the Herring Bay bed to the same sized recipient patches. Mussel densities were estimated by counting one-half of the mussels in each plot. A seine net was placed over recipient areas to keep the mussels in place until they could reattach. Transplanted mussels were very oily so we rinsed them with several buckets of seawater. Netting was removed after one tide cycle because the mussels appeared to be sufficiently attached to underlying sediment. Mussel densities were observed 2, 13, and 23 months after relocation.

Chemical Analysis

Two methods, ultraviolet fluorescence (UVF) fast screening and gas chromatography/mass spectroscopy (GC/MS), were used to quantify petroleum hydrocarbon concentrations. Although GC/MS analysis provides a more accurate measure of petroleum hydrocarbon content, a quicker, less expensive ultraviolet fluorescence (UVF) fast screening technique adapted from Krahn et al. (1993) was routinely used to estimate total petroleum hydrocarbons (TPH) (aromatic plus aliphatic compounds) in sediments (µg/g wet weight). Concentrations of TPH in each sample were quantified by comparing its fluorescence at 380 nm (maximal phenanthrene output) to the fluorescence of unweathered EVO. Little error was anticipated comparing an unweathered standard with possibly weathered samples because phenanthrene is relatively less susceptible to weathering than most other aromatics. A subset of sediment samples was selected for confirmatory GC/MS analysis.

Analysis of mussel samples and selected sediment samples by GC/MS was performed at the National Marine Fisheries Service, Auke Bay Laboratory (Short et al. 1996a). After addition of six internal standards, samples were extracted with dichloromethane. Isolation and purification of calibrated and uncalibrated compounds was completed by silica gel/alumina

column chromatography followed by size-exclusion high-pressure liquid chromatography (HPLC) and fractionation. Extracts of PAH were separated and analyzed by gas chromatography equipped with a mass selective detector. Calibrated PAH were identified by retention time and two mass fragment ions characteristic of each PAH and quantified using a five point calibration curve. Uncalibrated PAH homologs (which included alkyl-substituted isomers of naphthalene, fluorene, dibenzothiophene, phenanthrene, and chrysene) were identified by retention time and the presence of a single characteristic mass fragment ion. Uncalibrated PAH were quantified by using calibration curves of their respective parent homologs. Experimentally determined method detection limits (MDL) depended on sample weights, and generally were 1 ppb in tissue and < 2 ppb in sediment. Concentrations below MDL were treated as 0. 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 about 20%, depending on the PAH. Total PAH (TPAH) concentration is reported in μg/g dry weight, and represents the sum of all measured aromatic hydrocarbons except perylene because perylene is produced contemporaneously by biological sources in PWS.

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 in weathered EVO. The model, which was successfully validated by comparison with thousands of samples from the study area, uses experimentally determined loss-rate constants for 14 PAHs to calculate an index of weathering (w) that summarizes exposure history (Appendix 2). [$W = 0$ in unweathered samples and increases with weathering. For all environmental samples recorded in the Natural Resource Damage Assessment database (Short et al. 1996b), w ranges up to 11.3 for sediment and 9.9 for mussels. For this paper we use the following definitions: unweathered ($w = 0$), slightly weathered ($0 < w \leq 2$), moderately weathered ($2 < w \leq 8$), and highly weathered ($w > 8$).] 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 EVO (Short and Heintz 1997).

Data Analyses

Analysis of covariance (ANCOVA) was used to determine if TPAH in mussels or TPH in surface and deep sediment was correlated with time and if so, whether rates of change differed among treated and reference beds. Hydrocarbon concentrations were combined by transect at treated sites because initial observations indicated concentration varied significantly as a function of elevation (Harris et al. Chapter 2). Because concentrations varied significantly among some transects [based on two-way ANOVA (transect × day)], further data reduction at treated sites was not advisable. Concentrations were log-transformed and combined by bed for reference beds. Hydrocarbon concentrations that varied significantly with time at treated sites in first round of ANCOVA were compared to significant changes at reference sites, although non-significant reference slopes were included in comparisons when none were significant. Surface and deep sediment data were combined in a single analysis; TPAH concentration in mussels was analyzed separately. (Conclusions were the same when TPH concentrations in surface and deep sediment

were analyzed separately). Follow-up ANOVAs ([depth] × site × transect × day) compared differences over time for specific transects at each site.

Because any disturbance effects were most likely to be detected in surface sediment from the open strips, we also examined TPH concentration in these sediments in a restricted ANCOVA analysis. In this restricted data set, concentration was measured once at each point and time, thus there was no replication. As before, concentrations were log-transformed, and time was the covariate. A summary analysis, where all data within a given trench were combined by date, was similarly completed; this analysis had the advantage of replication, but the possible disadvantage of elevation-related differences in initial concentrations from various levels within trenches.

As a final cross-check, ANCOVA was used to determine if stripping caused changes in hydrocarbon concentration gradients in manipulated beds. To account for differences in initial concentrations, residual concentrations were computed for each transect: $C_{ijt} - \overline{C}_{\cdot jt}$, where C_{ijt} = concentration at the ith (x) and j^{th} (y) grid-point and t^{th} time, and $\overline{C}_{\cdot jt}$ = mean concentration in the jth transect at the tth time. These residual concentrations were computed by transect because initial concentration was correlated with elevation (Harris et al. Chapter 2). Residual concentrations for all beds and all sample days were analyzed with distance from the strip as the covariate. (The square root of distance was used in sediment analyses because initial modeling suggested fits were better with this transformation.) Expected indications of stripping effectiveness would be establishment of a significant relationship between residual concentration and distance from the strip and an increase in residual slopes ($\mu g \cdot g^{-1} \cdot m^{-1}$) with time. Accordingly, *a priori* comparisons were included in the ANCOVA to determine if the initial slope at a given site differed from subsequent slopes. Reference sites could not be included in the ANCOVA because they were not sampled on a grid system. Likewise, sediment samples collected along the strip but outside the standard grid at Eleanor Island were also excluded from analysis because it was not possible to compute residual concentrations for these points.

Hydrocarbon concentration and mussel density data from the transplant study were analyzed with 3-way ANOVA (site × treatment × time), where treatments were the donor bed, donor patch, and recipient patch. Each data set (concentration in sediment, concentration in mussels, and mussel density) was analyzed separately. Because variance was proportional to the means, concentration data were log-transformed before analysis. Means were compared with *a priori* multiple comparisons where the overall model was significant. Where $P \leq 0.05$ for this, and all other statistical tests, we report results as "significant."

RESULTS

Source of hydrocarbon contamination

Mussels and sediments in experimental and reference beds were contaminated with *Exxon Valdez* crude oil. Of the 101 mussel samples from the strip experiment, 30 had sufficient PAH for analysis with Short & Heintz (1997) model, and 25 of these contained EVO; the model could not be applied to remaining samples because 1 or more critical PAH concentrations were

below MDL. Similarly, of the 62 samples from the transplant experiment, 19 of 19 testable samples contained EVO. Estimated weathering factor *w* in mussels ranged from 2.6 to 8.9 (moderately to highly weathered). Of 54 confirmatory GC/MS sediment samples from the strip experiment, 17 of 20 testable samples contained EVO. Estimated weathering factor *w* ranged from 0.0 to 5.2 in sediment samples (unweathered to moderately weathered).

Major uncontrolled environmental influences

Vigorous winter storm activity sometime in the winter of 1992-1993 removed approximately 40% of the mussel layer from the Eleanor Island bed. This 700 m^2 bed was located on a tombolo on the southwest side of the island. About one half of the mussels were removed from the sample area by wave action and bare sediment was exposed throughout the central portion of the area. This exposure of sediment was apparently not related to strip placement because the same pattern of mussel removal was repeated across the much larger bed area outside of our study boundaries (Figure 3.2). (The study area covered about 13% of the total bed.) Oil sheens were observed on the entire upper portion of the bed in June 1993, and underlying sediments were visibly redistributed.

Strip removal experiment

Mussel density was fairly stable in most beds, but generally increased in stripped areas and did not differ significantly between strips and beds at the end of study (Figure 3.3). Mean mussel density ranged from 884 ± 88 mussels/m^2 at Bay of Isles to 5,856 ± 680 mussels/m^2 at Latouche Island. Mussel density was significantly higher after 1 month in the Chenega Island A and Latouche Island beds ($P = 0.049$ and $P = 0.009$, respectively), but endpoint density was not significantly different than initial density in 5 of 6 beds ($0.130 \leq P \leq 0.900$). Density was significantly lower after 3 months at Eleanor Island ($P = 0.050$), and was much lower at the end of study, primarily because of storm activity in the preceding winter ($P < 0.001$) (Figure 3.2). Mussel density tended to increase in each strip, but the increase was statistically significant only in the Eleanor Island strip ($P = 0.006$). However, endpoint densities in strips were not significantly different from endpoint densities in their respective beds ($0.259 \leq P \leq 0.723$). Mussel density in the Eleanor Island strip was low at the end of study as a result of storm activity.

Mussel density increased in strips as individuals adjacent to these open areas moved into them. Mussels had partially colonized strip areas 1-3 months after treatment, evident from the increasing irregularity of strip edges. Movement onto the strip was most apparent at Eleanor Island, where mussels two or three layers deep along the strip margins sloughed into the strip in clumps. Data on movement of tagged mussels were available for all three beds only in June (1 month after tagging), when 91% of the tags were found. Approximately 33% of the 90 mussels tagged in May moved more than 0.1 m along the strip axis or at right angles to the strip. At Chenega Island A and Herring Bay, the direction of movement was generally up slope and away from the strip; at Eleanor Island, movement was up slope and toward the strip.

Figure 3.2. Eleanor Island mussel bed 1992-1993. A) Appearance of strip immediately after manipulation. B) Aerial overview of bed 1 month after manipulation; arrows mark strip at intersections of upper (left) and lower (right) transects. C) Strip 1 month after manipulation; note mussel encroachment. D) Bed condition in 1993 after storm activity: parallel white lines indicate transect positions, and the perpendicular white line indicates the strip position. The repeating scallop-shaped bare areas were covered with mussels in 1992, including the experimental area.

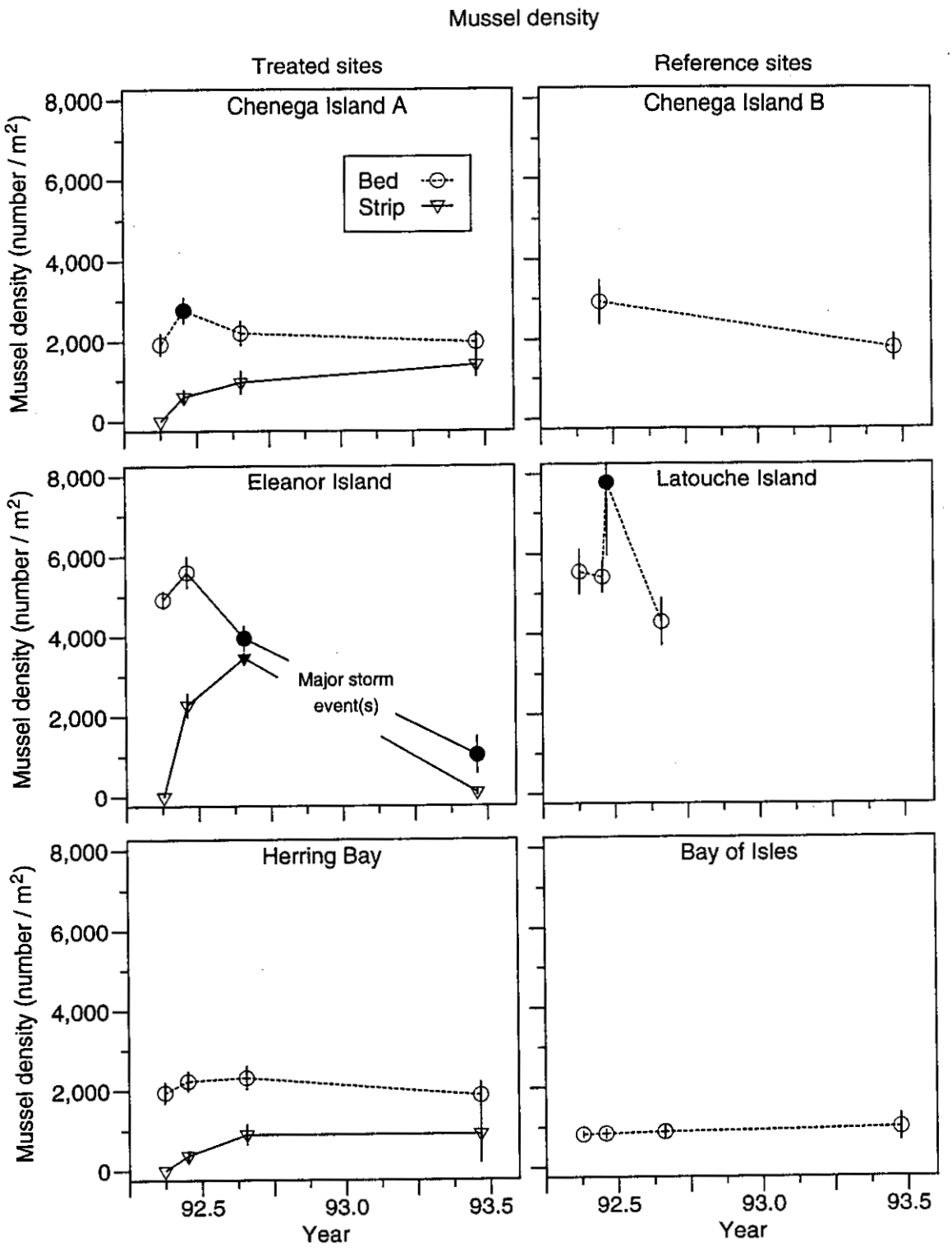

Figure 3.3. Mean mussel densities (±SE) in strips and beds at treated and reference sites as functions of time. Solid symbols indicate significant differences with respect to initial bed or strip conditions.

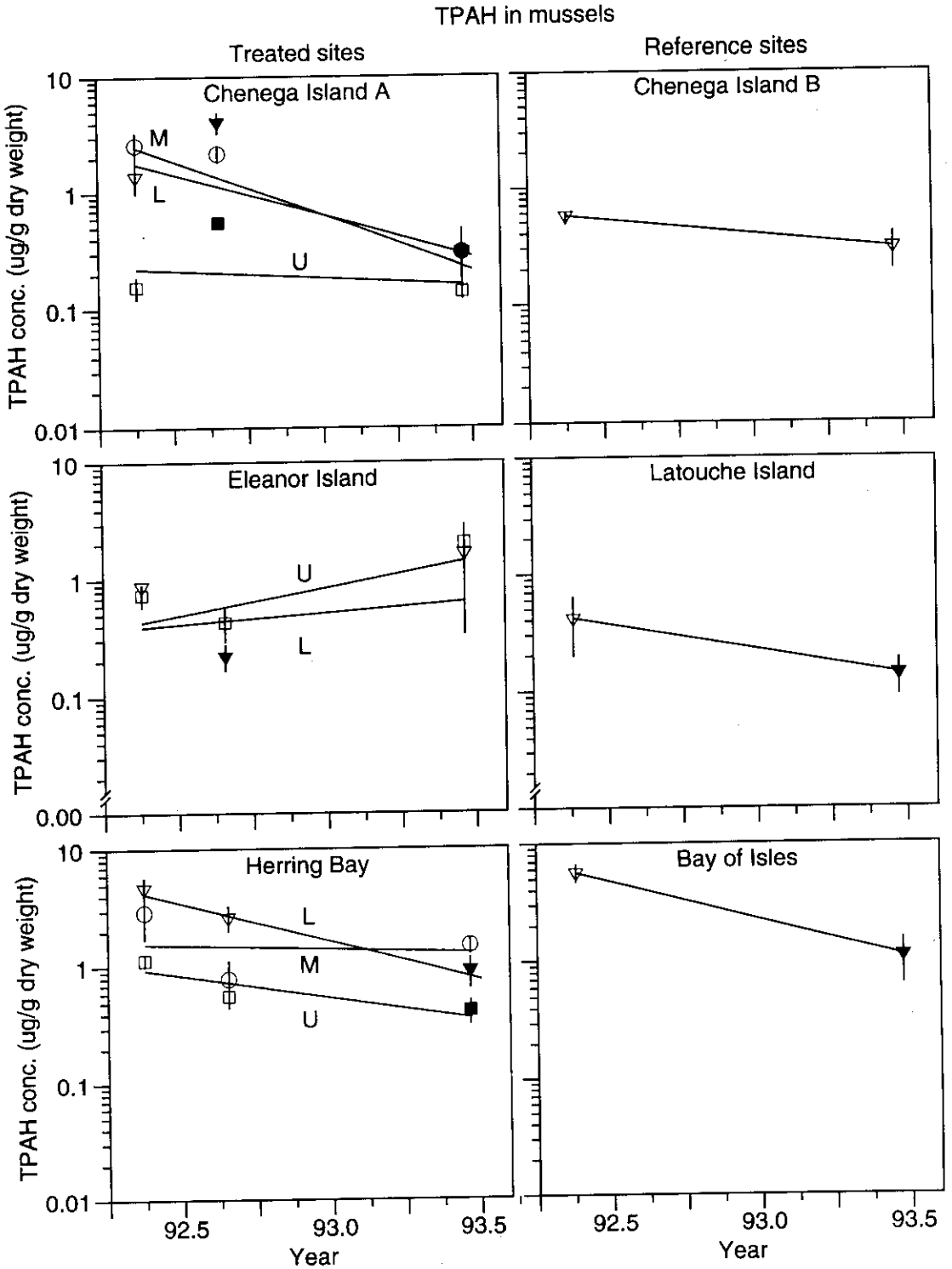

Figure 3.4. Mean total polynuclear aromatic hydrocarbon (TPAH) concentration (±SE) in mussels at treated and reference sites. Data were collected along lower (L), middle (M), or upper (U) transects at treated sites. Bold lines indicate significant regressions. Solid symbols indicate significant differences with respect to initial conditions in each transect or reference bed.

Sediments in strips did not erode, but rather, there was an indication of sediment deposition in the Eleanor Island strip. We found a second layer of mussels 5 cm deep under surficial mussels and sediments in the strip in August 1992.

Total PAH concentration in mussels did not decline more rapidly in treated beds than in reference beds (Figure 3.4). Total PAH concentration declined significantly over time in the lower and middle transects at Chenega Island A, the lower transect at Herring Bay, at Bay of Isles, and at Latouche Island ($0.001 < P \leq 0.050$). Concentration change was not significant in the remaining 6 cases ($0.106 \leq P \leq 0.749$). Concentration declines in treated beds were not significantly different from those in reference beds ($0.165 \leq P \leq 0.904$), and slope ranges overlapped (-2.1 ± 0.5 to 0.4 ± 0.6 $\mu g \cdot g^{-1} \cdot year^{-1}$ in treated beds and -1.6 ± 0.6 to -0.7 ± 0.6 $\mu g \cdot g^{-1} \cdot year^{-1}$ in reference beds). Mean concentrations of TPAH in treated and reference beds were similar across time (1.4 ± 0.2 µg/g and 1.2 ± 0.3 µg/g, respectively). Thus, there was no evidence that hydrocarbon loss from mussel tissue was accelerated by treatment.

Concentration of TPH in surface sediment did not decline significantly in treated beds (Figure 3.5). Similar to trends in mussel tissue at Eleanor Island, hydrocarbon concentration in surface sediment tended to increase in this bed, and this increase was significant in the lower transect ($P = 0.001$). Concentration at other treated sites generally tended to decrease (in 5 of 6 cases), but changes were not significant ($0.385 \leq P \leq 0.962$). The rate of concentration loss was greatest in the Chenega Island B reference bed (-1.5 ± 0.7 $\mu g \cdot g^{-1} \cdot year^{-1}$), the only bed with a significant concentration decline ($P = 0.046$). Rates of hydrocarbon loss in treated beds were consistently less than those at Chenega Island B. Therefore, there was no evidence that hydrocarbon loss from surface sediment was accelerated by treatment.

Trends in TPH concentration in surface sediment of strips were similar to those in surface sediment throughout transects in each bed, and did not provide evidence that hydrocarbon loss was accelerated by experimental manipulation (Figure 3.6). (Sediment in strips was examined in a restricted analysis because we anticipated manipulation effects would be most discernable in the disturbed sediment.) Concentration of TPH in surface sediment from strips correlated with negatively with time in 3 cases and positively in 3 cases where $r^2 > 0.5$. However, regressions were significant in only 1 of 9 cases [concentration increased over time at one Eleanor Island point ($P = 0.023$)], and time was not significant overall ($P = 0.316$). When concentrations in surface sediment from all positions in strips were combined in a summary analysis, there was no relationship to time evident at any site ($0.567 \leq P \leq 0.888$).

Changes in TPH concentration in deep sediment were equivocal (Figure 3.7). Concentration declined significantly in the lower Chenega Island A and Eleanor Island transects ($0.001 < P \leq 0.011$), and this decline was generally more rapid than in deep sediment at Latouche Island and Bay of Isles ($0.001 < P \leq 0.090$). Concentration did not decline significantly in the two reference beds ($0.228 \leq P \leq 0.726$). The rates of concentration decline in deep sediment at Chenega Island A and Eleanor Island were not significantly different than the significant rates of decline in surface sediment at Chenega Island B ($0.248 \leq P \leq 0.308$). Concentration increased significantly over time in the upper Chenega Island A bed ($P < 0.001$). The rate of concentration change in treated beds (-2.95 ± 1.1 to 7.9 ± 1.3 $\mu g \cdot g^{-1} \cdot year^{-1}$) overlapped that in reference beds (-0.4 ± 1.0 to 1.4 ± 1.2 $\mu g \cdot g^{-1} \cdot year^{-1}$). Both increasing and decreasing trends in concentration

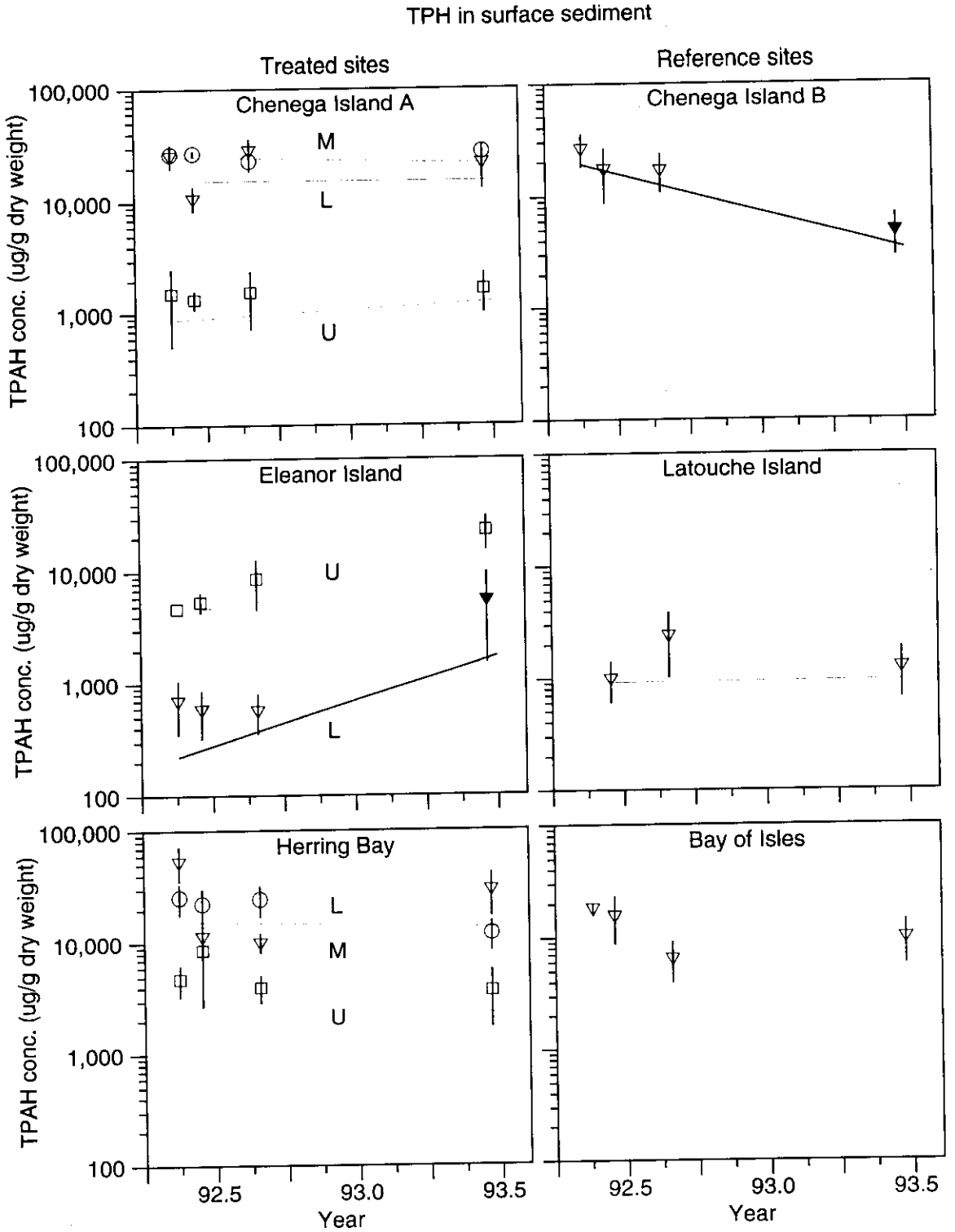

Figure 3.5. Mean total petroleum hydrocarbon (TPH) concentration (±SE) in surface sediment at treated and reference sites. Data were collected along lower (L), middle (M), or upper (U) transects at treated sites. Bold lines indicate significant regressions. Solid symbols indicate significant differences with respect to initial conditions in each transect or reference bed.

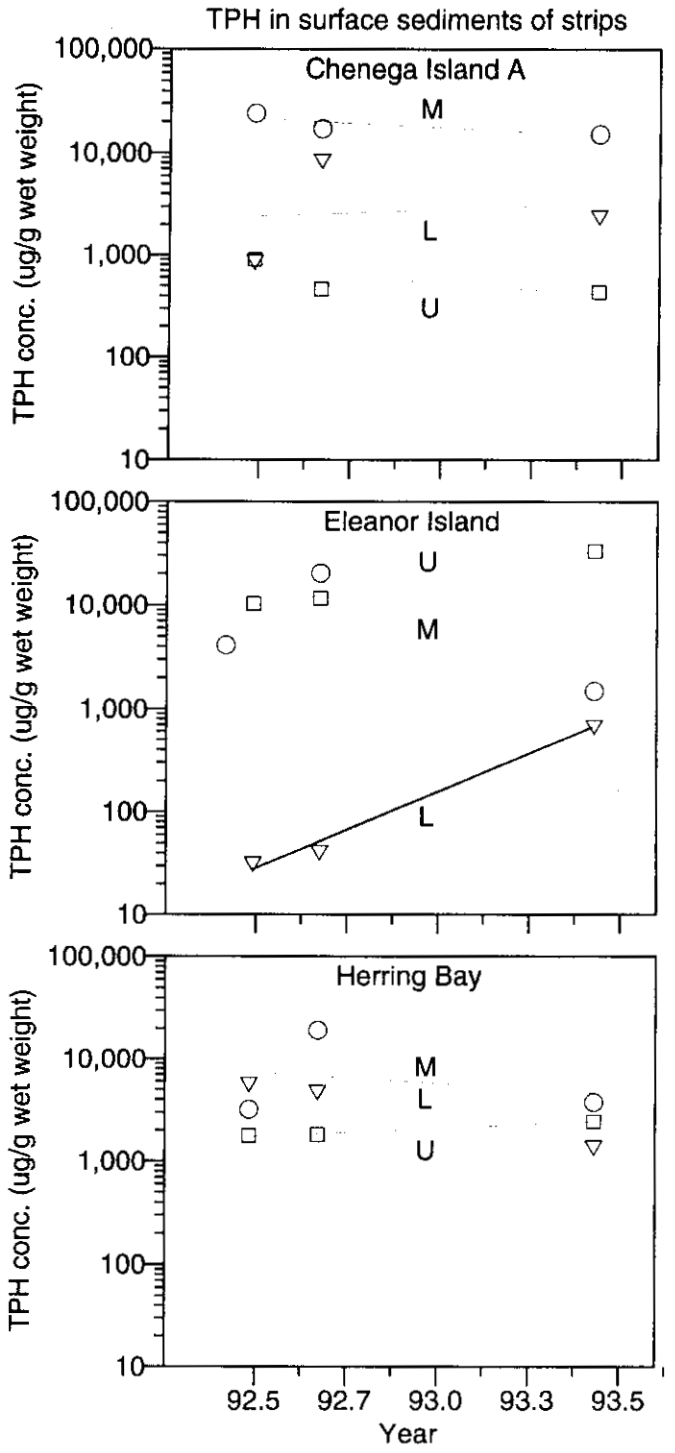

Figure 3.6. Total petroleum hydrocarbon (TPH) concentration in surface sediment from strips. Data were collected from lower (L), middle (M), or upper (U) points. Bold lines indicate significant regressions.

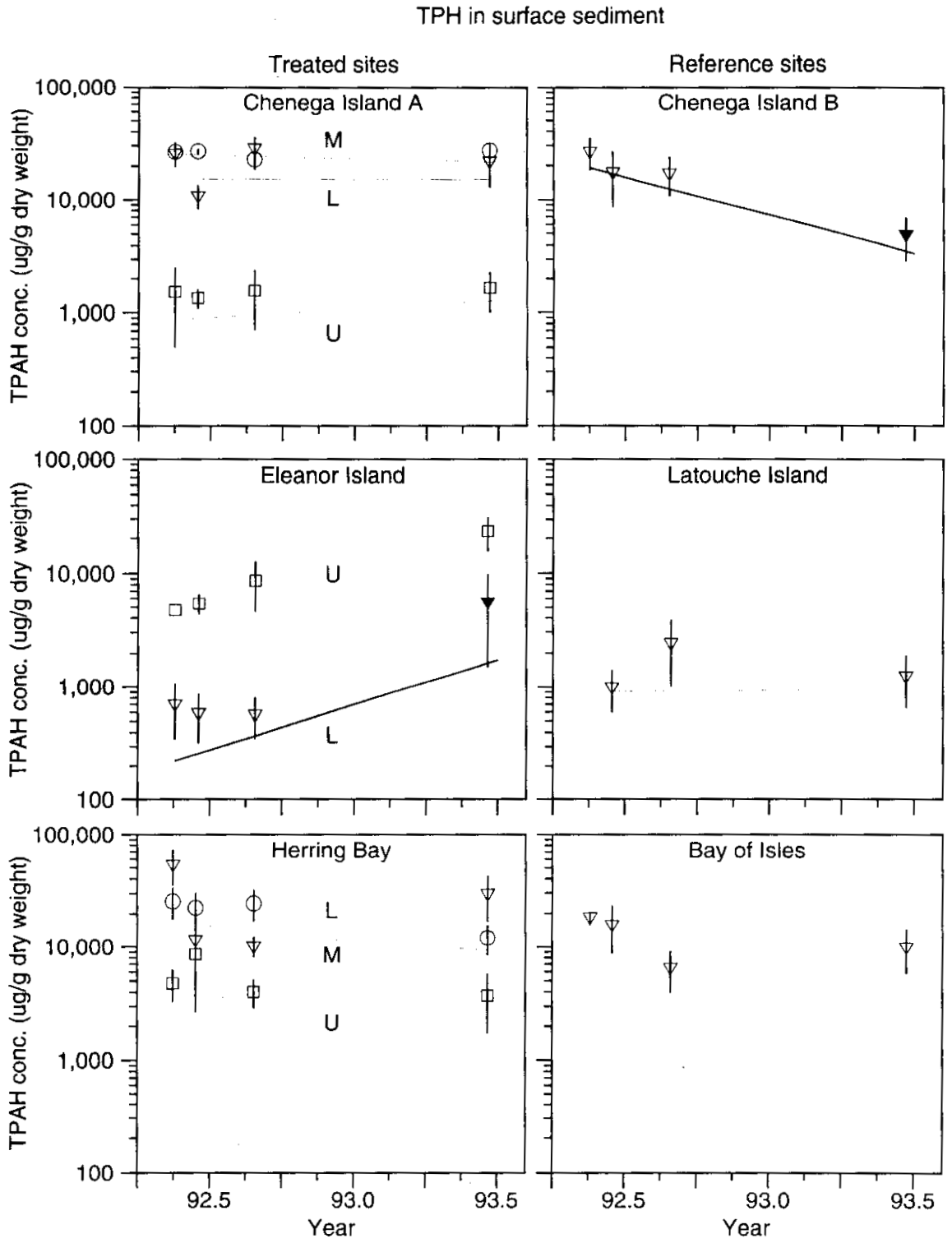

Figure 3.7. Mean total petroleum hydrocarbon (TPH) concentration (±SE) in deep sediment at treated and reference sites. Data were collected along lower (L), middle (M), or upper (U) transects at treated sites. Bold lines indicate significant regressions. Solid symbols indicate significant differences with respect to initial conditions in each transect or reference bed.

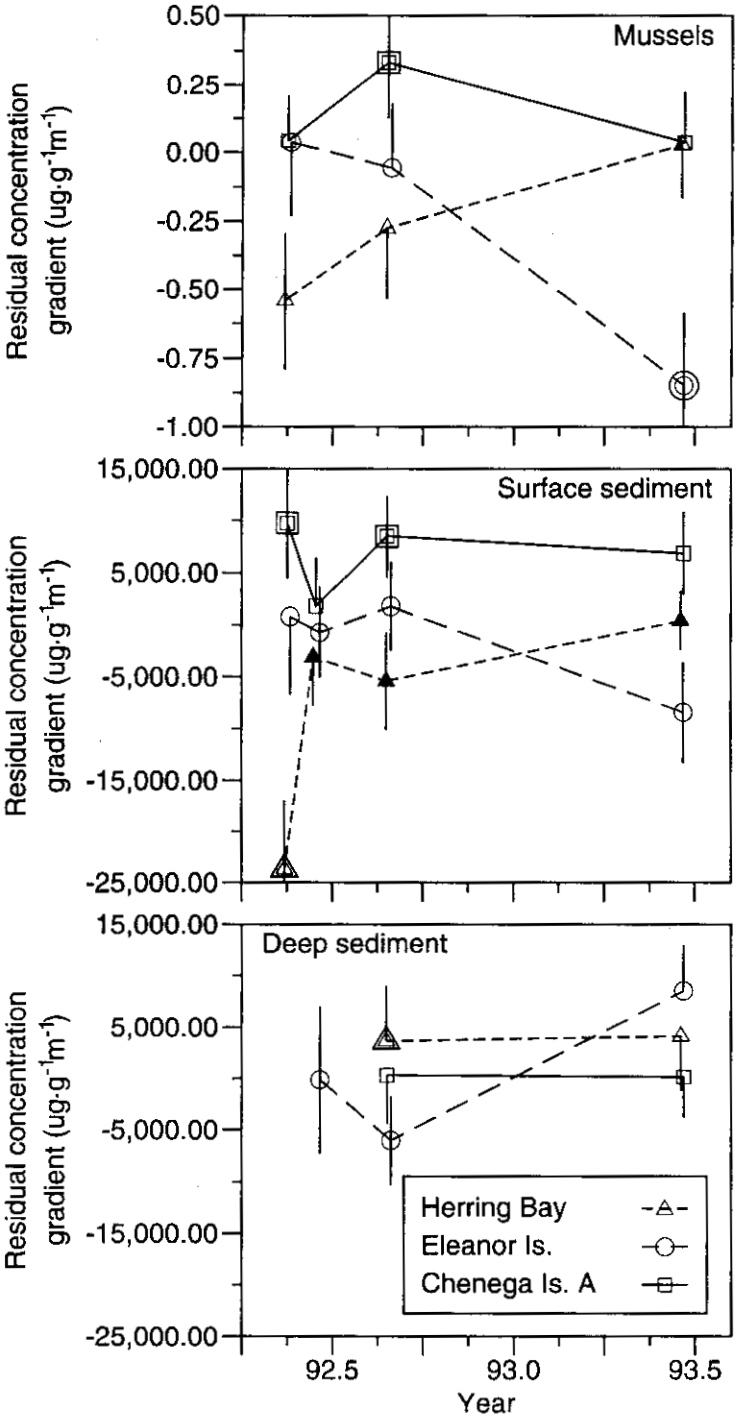

Figure 3.8. Changes in concentration gradients (as estimated by regression where the dependent variable was residual concentration and the independent variable was distance from the strip - see methods) (±SE) as functions of time. Positive gradients indicated residual concentration increased with distance from strip. Solid symbols indicate significant differences with respect to initial conditions in bed. Regressions with significant slopes are indicated by concentric symbols.

were observed at two of three manipulated sites. Thus, there was no clear evidence that hydrocarbon loss from deep sediment was accelerated by treatment.

Changes in residual concentration gradients in mussels and bed sediments as a result of manipulation were equivocal (Figure 3.8). Changes in residual concentration gradient (or slope) in mussels over time were not significant at Chenega Island A or Herring Bay ($0.076 \leq P \leq 0.981$). The slope in mussels decreased significantly at Eleanor Island ($P = 0.021$), a change was opposite that predicted by manipulation effectiveness. [The trend toward increased slope in mussels at Herring Bay and approached significance at the endpoint ($P = 0.076$).] Changes in slope in surface sediment over time were not significant at Chenega Island A or Eleanor Island ($0.261 \leq P \leq 0.911$), but the slope increased significantly at Herring Bay ($0.001 \leq P \leq 0.028$). Changes in slope in deep sediment were not observed ($0.310 \leq P \leq 0.976$). Accordingly, there was no unambiguous evidence that hydrocarbon concentration gradients in mussels and sediments were altered by treatment.

Transplant experiment

Mussel densities in the recipient patches and donor beds declined over the observation period, but increased in donor patches (Figure 3.9). In the Herring Bay bed density declined significantly in recipient patches, and only 5% of the transplanted mussels were present 13 months after relocation ($P < 0.001$). No mussels were found immediately outside the recipient patches, so the low densities likely indicated mussel mortality. However, mussel densities in the undisturbed portions of the Herring Bay bed also declined significantly ($P < 0.001$), and mussel density in the recipient area was not significantly different from that in the bed 23 months after relocation ($P = 0.355$). Density increases in the Herring Bay donor patches were significant after 13 months ($P = 0.047$), and by the end of the study there were no visible differences between donor patches and the remainder of the bed, and density differences were not significant ($P = 0.689$). At Chenega Island A, the density in the recipient patch was stable for the first two months after transplanting, but declined significantly by 11 months ($P = 0.002$) and no mussels remained after 23 months. Mussel densities in undisturbed portions of the donor bed at Chenega Island A did not decline significantly in the first 14 months of study ($P = 0.446$). At Chenega Island A, donor patch densities were visibly less than bed density 14 months after mussel removal, but density was not enumerated, and further observation was not possible because the bed was disturbed by restoration activity (Babcock et al. Chapter 4).

Total PAH concentrations in mussels transplanted to clean recipient patches decreased over time, but concentrations in mussels that migrated into the donor patch and those in the bed tended to increase (Figure 3.9). At Herring Bay, concentrations were significantly lower 2 and 13 months after relocation than initially ($0.031 < P \leq 0.034$). Total PAH concentrations in mussels that repopulated the donor patch and those in the bed did not change significantly ($0.335 \leq P \leq 0.732$). Although concentration dropped significantly within recipient patches but not in the bed, after 13 months concentration differences in recipient patches were only marginally different from those in the bed ($P = 0.07$). Trends were similar at Chenega Island A; concentration changes were generally not significant ($0.147 \leq P \leq 0.558$), except for a significant increase in mussels in the donor bed ($P = 0.011$).

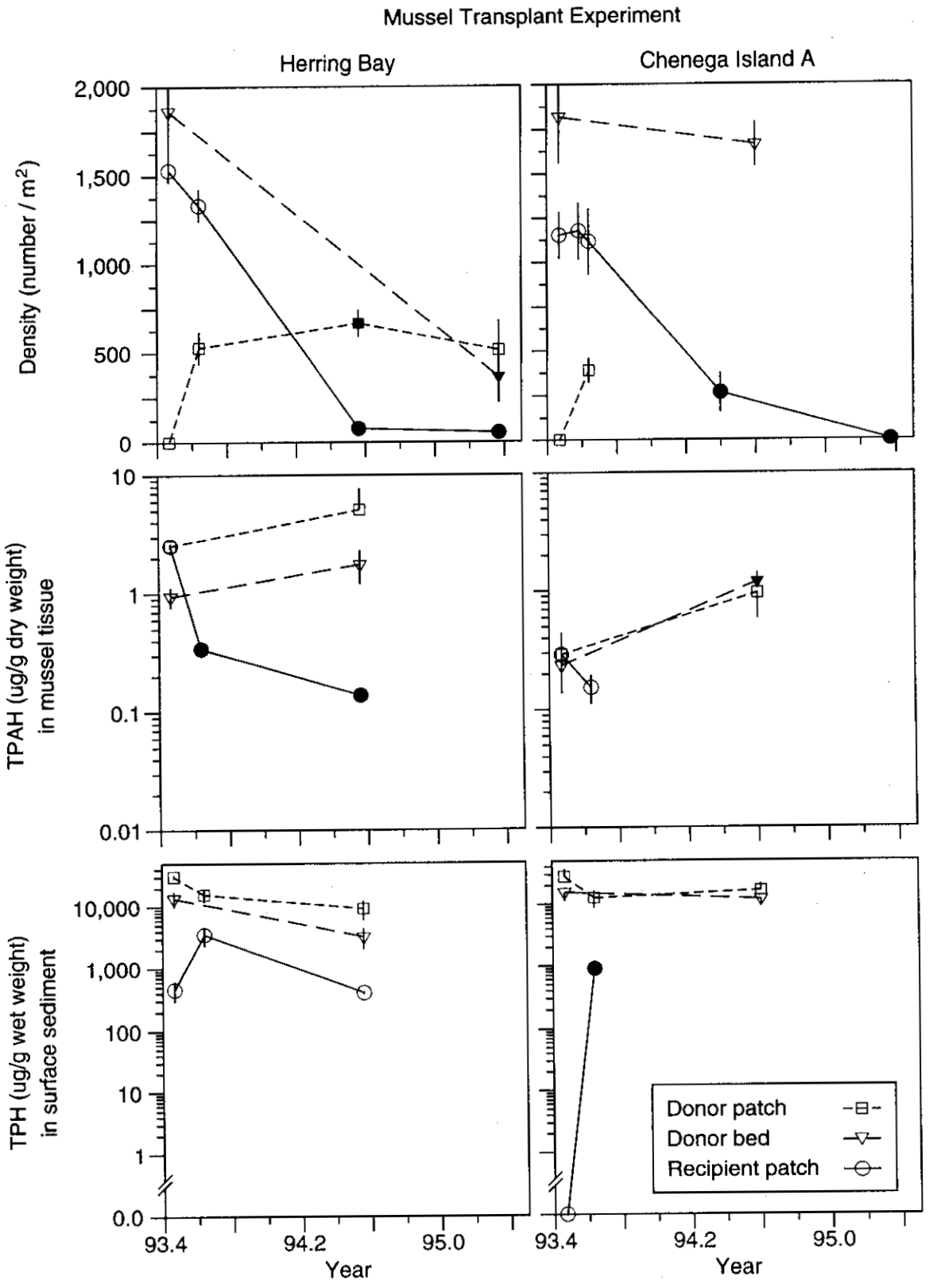

Figure 3.9. Time-dependent changes in mussel density, total polynuclear aromatic hydrocarbon (TPAH) concentration in mussels, and total petroleum hydrocarbon (TPH) concentration in surface sediment in donor beds, donor patches, and recipient patches of the mussel transplant experiment. Plotted data are means ± SE.

Concentrations of TPH in the sediment of recipient patches increased rapidly after mussels were transplanted onto them, but declined to pre-transplant levels after 13 months (Figure 3.9). Hydrocarbon concentrations at Herring Bay increased by approximately an order of magnitude in the first 2 months, but this increase was not significant ($P = 0.192$). The increase in hydrocarbon concentration in Chenega Island A recipient patch at 2 months was more pronounced and significant ($P < 0.001$). Declines in TPH concentration in the sediment of donor beds and patches were not significant ($0.186 \leq P \leq 0.738$). Concentrations of TPH in recipient and donor patches did not differ significantly from those in the bed after 13 months ($0.250 \leq P \leq 0.601$).

DISCUSSION

We explored two low-impact mechanical methods to reduce hydrocarbon concentrations in mussel beds contaminated with *Exxon Valdez* oil without destroying the mussel community. One method involved removal of 0.3 m-wide linear strips of mussels and attached sediments, and other method involved relocation of small (0.125 m^2) patches of mussels so that oil in the donor bed was more susceptible to weathering processes and relocated mussels could survive and depurate hydrocarbons. Proportionately few mussels (<5%) were removed by either method. The resultant bare strips and patches proved ineffective in reducing bed-wide hydrocarbon concentrations. The small sizes of bare areas allowed rapid recolonization by mussels from the beds, thus extended hydrocarbon flushing was prevented. Transplanted mussels depurated hydrocarbons quickly, but there was high mortality. The suboptimal habitat where transplanted mussels were placed may have been the principal reason these mussels died.

Strip removal did not cause consistent or significant reductions in oil concentration in the uncovered sediments, and were there no significant bed-wide reductions of hydrocarbons in either sediments or mussels. Heavy sheens were observed when rising tides flooded the stripped beds and donor patches immediately after mussel removal. Sheens, very visible 1 and 3 months after removal at the Chenega Island A and Herring Bay beds, were probably from the disturbed areas. Although some of this remobilized oil was undoubtedly transported away from the study beds, some was stranded in beds with the outgoing tides, and some was probably retained by mussel feeding activity. Other studies have demonstrated that mussels can retain oil in a bed when they filter particulate oil from the water and discharge it in feces and psuedofeces back into the sediments (Ganning et al. 1983; Harris 1993, unpublished data). Significant unaided hydrocarbon loss was observed in reference mussels and sediments, and loss rates in treated beds did not exceed these rates. The significant increases in hydrocarbon concentration in sediment at Eleanor Island and similar trends in mussels may have been caused by the major winter storm event(s) that reshaped this bed and reworked sediments. The significant loss of hydrocarbons from deep sediment at Eleanor Island may have also been influenced by this mechanical reorganization as the more contaminated deep sediment mixed with less contaminated surface sediment. There was weak evidence that a hydrocarbon gradient may have developed in surface sediment at Herring Bay as the result of strip removal, but there was no evidence for a similar gradient at Chenega Island A. Given the lack of significant hydrocarbon change in strip sediments at Herring Bay, the observed gradient most likely developed by chance, and was not a result of the strip. A hydrocarbon gradient also developed at Eleanor Island, but was opposite to

the predicted gradient, and was likely influenced by storm activity. Thus, we conclude that bed-wide changes in hydrocarbon concentrations were not related to strip removal.

Exchange between hydrocarbons in sediment and mussels was evident in our data. Mussels can both introduce petroleum hydrocarbons into bed sediment and obtain them from sediment. Sediment in recipient patches clearly acquired oil from transplanted mussels. Although the previously discussed food elimination mechanism probably contributed to this increase in hydrocarbon concentration in recipient sediment, oil adherent to mussel shells may have been a primary contributing factor. Increased hydrocarbon concentration in recipient sediment was transitory at Herring Bay, and probably also at Chenega Island A, although endpoint measurements were not completed before the bed restoration activities reported by Babcock et al. (Chapter 4) commenced.

Hydrocarbons were probably not retained for long periods in recipient sediment (and mussels) because these patches were well drained, relatively exposed to wave activity, small in area, and became uncovered as mussel density declined, thus the hydrocarbons present were more susceptible to natural flushing than corresponding hydrocarbons in donor beds. That mussels can obtain hydrocarbons from sediment was suggested by increased concentration within the first two months of sediment disturbance at Chenega Island A, but similar increases were not observed in the other beds, probably because the sampling frequency was too low. The natural disturbance at Eleanor Island was likely responsible for increased endpoint hydrocarbon concentrations in mussels at that site. Whether disturbances were natural or caused by humans, visual and chemical evidence suggests that the increased volume of mobile oil will translate into increased hydrocarbon body burdens in nearby mussels. Transient increases in TPAH concentration in mussels located near mechanically disturbed oily sediment have been more clearly documented by Babcock et al. (Chapter 4); these transient increases generally persisted for only a few days before concentrations fell back to pre-disturbance levels, but hydrocarbon concentrations in underlying sediments were also intentionally reduced in that study.

Exposed substrate area may be an important factor for bed restoration. The absence of significant concentration reduction in donor patches suggests that strips must be larger than 0.5 × 0.25 m to have an effect. The narrow strip width we used (0.3m) was similarly ineffective and allowed reestablishment of the mussel layer within two months which again insulated the sediments from tidal flushing. A wider strip presumably would have remained open longer and been more likely to reduce hydrocarbon concentrations, but extensive manipulation may increase the possibility of bed destabilization.

Bed exposure and time of strip removal were probably important factors in the quick recolonization of strips. In a study on the recolonization of artificially created gaps in *Mytilus californianus,* Suchanek and Duggins (unpublished results in Gosling 1992) found that rate of gap closure was fastest durring the summer months and in *M. edulis* beds, gap closure was faster at moderately exposed sites than at more exposed sites. Because of rapid recolonization, the transplant study was likely unaffected by the earlier strip removal study.

Accumulation of hydrocarbons by mussels likely represents an integration of sediment concentration from an area larger than that occupied by specific mussels because correlation

between concentration in mussels and concentration in immediately underlying sediment was poor [$r^2 = 0.15$, but $P < 0.001$ ($n = 95$)]. Similarly noisy relationships between concentrations in mussels and sediment were reported by Harris et al. (1996; Chapter 2) and Babcock et al. (Chapter 1) ($r^2 = 0.42$, and $r^2 = 0.31$, respectively). Thus we infer that the minimum sediment area responsible for mussel contamination is larger than the area occupied by the mussels. Constraints on the maximum size of the mussel uptake area are suggested by our observations, because mussels transplanted approximately 10-25 m from contaminated beds depurated hydrocarbons quickly. Thus the area involved in uptake is likely to be less than 10 m but greater than 25 cm (quadrat size). Typically lower hydrocarbon concentrations in mussels attached to relatively clean bedrock near oiled beds than in mussels from those beds was additional evidence of localized contamination (Harris 1996; Thomas et al. 1998; Chapter 5). However, local topography, distribution of petroleum hydrocarbons within sediments, and water circulation patterns are also likely to be important controlling factors.

The suboptimal habitat that transplanted mussels were placed into may have been the principal reason why these mussels died. Environmental conditions in recipient patches, including wave energy, patch size, and tidal height likely reduced mussel survival. Transplants were taken from the middle of donor beds, where the surrounding bed would have created a more favorable environment for mussel survival than the recipient patch, even though the donor patch was oiled. Temperatures, wave action, and humidity would have been moderated in the well established bed (Gosling 1992) but not in small recipient patches, which were devoid of fine biogenic sediment, and subjected to more dessication because of their more exposed positions on the beach. Low pre-experimental mussel density in recipient locations was evidence that these areas were marginal mussel habitat. Thus, we suggest suboptimal habitat conditions probably had a strong influence on mussel survival.

Other factors contributing to mussel mortality in recipient patches may have included a general regional decline in mussel populations during the study period, and possibly age class senescence. Significant density declines during the same period in donor beds and several other beds in Herring Bay (Highsmith et al. 1996) suggest that the mortality was not due solely to transplantation. One possibility is that transplanted mussels were old; mean shell lengths in the two donor beds were about 40 mm (large for PWS[4]) and animals less than 30 mm were rare. A similarly dramatic decline in a single-aged bed of large mussels occurred at the AFK Hatchery over the winter of 1995-96 (David Fundak, AFK Hatchery, Prince William Sound Aquaculture Association, personal communication). High mortality, especially related to poor post-spawning condition in older mussels, has been reported for some *Mytilus* populations (Worrall and Widdows 1984, Emmett et al. 1987). Transplantation could have been a contributing factor to the mortality of pre-senescent mussels. General regional declines in mussel populations continued over the winter of 1994-1995 (Babcock et al. Chapter 4).

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[4]The Nearshore Vertebrate Predator project found that the frequency of mussels with shell lengths greater than 40 mm in 57 quadrats in the Knight Island area in 1996 was less than 1%. Preliminary aging of Herring Bay mussels indicated that shells over 40 mm had at least 7 annuli (*Exxon Valdez* Restoration Project 95025, unpublished data).

The physical integrity of mussel beds was not compromised by removing strips or patches of mussels, as demonstrated by the lack of sediment erosion and the essentially constant mussel densities in stripped beds. Declines in mussel density in donor beds (1993-1995) apparently reflected regional declines in mussel density (as previously discussed), and did not indicate that patch disturbances adversely influenced bed integrity. Bed-wide mussel densities at Chenega Island A and Herring Bay remained stable during the strip removal study, and open strips were filled with adult mussels within 2 months either by directed movement or sloughing. The oil mobilized by mechanical disturbance of sediments apparently did not discourage adults from moving onto the strips and patches. The strip at Eleanor Island apparently accumulated sediment within the first 3 months of study because we found recently buried mussels in strip sediment. The previously discussed dramatic erosion of was not related to strip removal. Thus, we conclude that treated beds quickly reverted to their original condition and were not significantly damaged by mechanical manipulation.

CONCLUSION

Removal of a narrow (0.3 m) strip or several disjunct small patches of mussels and attached sediments from oiled mussel beds was ineffective in reducing bed-wide hydrocarbon concentrations. The small size of exposed areas allowed rapid recolonization by mussels from the beds thereby preventing extended hydrocarbon flushing from the strips. The stability of hydrocarbon concentrations in Herring Bay and Chenega Island A sediment indicates that the beds will need more than minor manipulation if to accelerate the rates of hydrocarbon loss.

Transplanted mussels depurated hydrocarbons quickly on clean sediment, but the high mortality associated with relocation argues against relocation of older mussels to suboptimal habitat during restoration efforts aimed at lowering hydrocarbon concentrations in mussel tissue, hence exposure of predators and human subsistence users. Future transplanting efforts should carefully consider the age of mussels to be transplanted and the characteristics of recipient areas.

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Chapter 4: Restoration of Oiled Mussel Beds in Prince William Sound, Alaska

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ABSTRACT

Manual restoration of mussel beds contaminated by *Exxon Valdez* oil was partially successful in reducing hydrocarbon concentrations in mussels and sediments more rapidly than would have occurred in the absence of restoration. Nine mussel beds that remained significantly contaminated were cleaned five years after the spill to decrease oil concentrations in mussels, thus reducing exposure potential for higher-order consumers. We manually removed mussels, replaced contaminated surface sediment, and allowed the original mussels to reattach on clean sediment. Mortality potentially caused by restoration activity was indistinguishable from regional declines in mussel density. Total petroleum hydrocarbon concentrations were initially reduced in surface sediment, but there was evidence that these sediments reacquired oil from deeper sediment and surrounding areas because all oil could not be removed. Declines in petroleum hydrocarbon concentrations in mussels were not always significant, and it was generally not possible to separate observed concentration declines from the regional declines evident in unmanipulated oiled reference beds. However, hydrocarbon concentrations in mussels and sediment (in three of three, and three of six restored beds, respectively) declined below projected estimates of what concentrations would have been in the absence of restoration activity. One year later, hydrocarbon concentrations in mussels were likely significantly greater than the estimated background concentration in only one bed. The concept of removing mussels before cleaning is probably valid, but other methods of cleaning sediment (physical or chemical) would likely be more rapid and effective than manual replacement of contaminated sediment.

INTRODUCTION

Many blue mussel (*Mytilus trossulus*) beds impacted by the *Exxon Valdez* oil spill (EVOS) of March 24, 1989 were not cleaned by request of the EVOS Interagency Shoreline Cleanup Committee because mussels were an important food source and a physically stabilizing element in intertidal areas. Decision-makers assumed that natural processes would cause hydrocarbon concentrations in these beds would decline to background levels in reasonable time. However, substantial amounts of *Exxon Valdez* oil (EVO) still remained in mussels and sediments underlying mussel beds in 1991 (Babcock 1991; Babcock et al. 1996; 1994; Chapter 1). Persistent, high concentrations of hydrocarbons in mussels were identified as a possible source of contamination for several consumer species (Duffy et al. 1996; Sharp et al. 1996) and could potentially impact human subsistence users.

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Minimally intrusive methods to reduce hydrocarbon concentrations in mussel beds were previously examined in pilot projects. Strips of mussels and sediments 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 (Bauer et al. 1992; Harris et al. Chapter 3). These methods accelerated the rate of hydrocarbon loss in transplanted mussels, but not from donor or manipulated beds (Harris et al. Chapter 3). Overall, hydrocarbon concentrations in the manipulated beds, as well as in many unmanipulated oiled beds, remained high in 1993 (Babcock et al. 1994; 1996) indicating that restoration with more intrusive methods would be needed if oiled mussel beds were to return to prespill concentrations more rapidly than possible in unaided beds.

Hydrocarbon concentrations in mussels from oiled beds could be expected to decrease if the mussels were no longer exposed to oiled sediments. Options for removing this oil were chemical, mechanical, and manual. Chemical and mechanical methods were rejected as being unproved or potentially too destructive. Therefore, in 1994, we restored selected mussel beds by manually replacing oiled sediments with clean sediments, and closely monitored hydrocarbon concentrations and mussel densities.

Our goal was to determine if manual restoration could effectively and practically accelerate the loss of petroleum hydrocarbons from mussels and sediment. Because preceding research indicated that EVO hydrocarbon concentrations in surface sediment and mussels generally declined over time (Babcock et al. Chapter 1 - others?), 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) reduce concentrations of petroleum hydrocarbons in mussels and sediments in selected oiled mussel beds in Prince William Sound (PWS) by manually removing oiled sediments, 2) monitor petroleum hydrocarbon concentrations in restored mussels and underlying sediments, 3) compare concentration changes in restored beds to those in unrestored oiled reference beds and to time-series data collected before restoration began, and 4) determine the impact of our restoration activity on mussel populations.

METHODS

Site Selection

Nine oiled mussel beds at five sites in PWS were selected for cleaning; 6[5] of these beds were monitored in years prior to restoration (1992 and/or 1993) (Babcock et al. Chapter 1.) (Figure 4.1). Primary criteria for selection were high total petroleum hydrocarbon (TPH) concentrations (>5,000 µg/g wet weight) in sediments underlying fairly dense mussel beds. Total polynuclear aromatic hydrocarbon (TPAH) concentrations in mussels in candidate beds ranged from 0.20 to 8.30 µg/g dry weight. During sampling in April and May 1994, we confirmed the

[5]Samples were collected near each of the remaining 3 beds in years prior to restoration, and these data were used to approximate pre-restoration conditions.

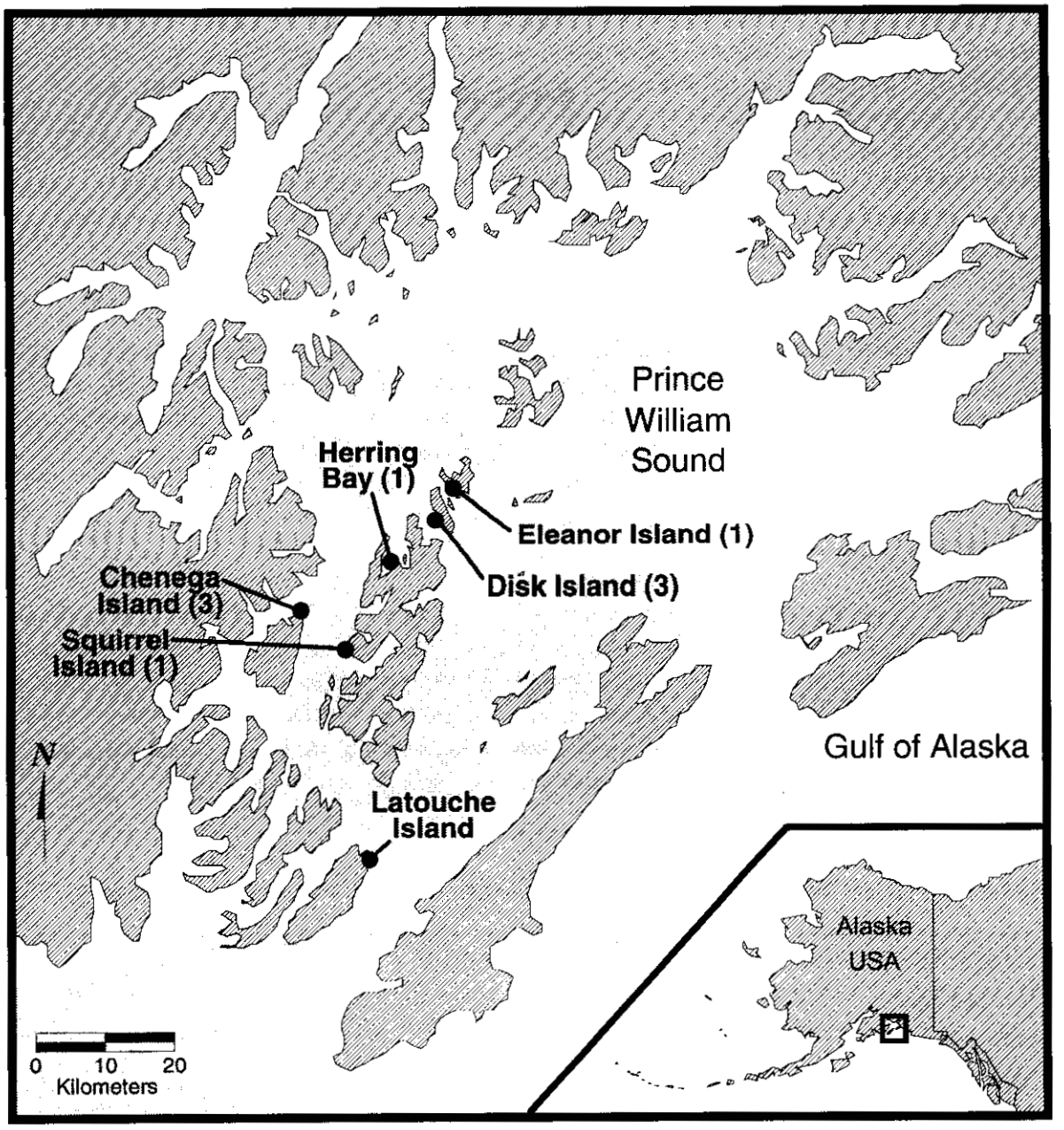

Figure 4.1. Locations of restored mussel beds in Prince William Sound. Numbers in parentheses indicate the number of beds at each site that were restored. Shaded area indicates the *Exxon Valdez* oil spill impact area.

presence of high TPH concentrations in sediments (2,000 to 18,000 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. Several beds that were among the most contaminated in 1992 and 1993 (e.g. Foul Bay and an islet in Herring Bay) were not cleaned because the sites were not physically suitable; additionally, several small beds at selected sites were monitored but not cleaned to compare hydrocarbon concentration and bed integrity with those in cleaned beds. Selected beds ranged in size from 9 to 62 m^2 (Table 4.1).

Reference mussels beds were selected at Chenega Island (CH010B-2D), Eleanor Island (EL011A-2D), and Disk Island (DI067A-2C). Sizes of reference beds, 11 to 24 m^2, overlapped restored bed sizes. Except for collection of mussel and sediment samples and measurement of densities, reference beds were not disturbed and remained unrestored.

Table 4.1. Area, mean depth, volume, and weight (metric tons, MT) of excavated sediments in Prince William Sound mussel beds restored in 1994. Bed number includes beach segment numbers assigned by the *Exxon Valdez* Interagency Shoreline Cleanup Committee followed by alphanumeric characters to specify mussel beds within the segment. Where present, the sub-bed designator indicates that a bed was subdivided during restoration; sub-bed data were recombined for final analysis. Date indicates the day restoration activity began in 1994.

Location	Bed number	sub-bed	Date	Area (m^2)	Mean Depth (m)	Volume (m^3)	Weight (MT)
Eleanor Island	EL011A-2	B	Aug 6	13.58	0.11	1.49	2.63
Eleanor Island	EL011A-2	C	Aug 6	13.95	0.10	1.44	2.53
Chenega Island	CH010B-2A	L	Aug 8	19.70	0.11	2.23	3.92
Chenega Island	CH010B-2A	R	Aug 9	19.71	0.11	2.11	3.71
Chenega Island	CH010B-2B	-	Aug 10	8.93	0.10	0.86	1.52
Chenega Island	CH010B-2C	-	Aug 8	9.07	0.11	0.95	1.68
Disk Island	DI067A-1	-	Jul 20	18.60	0.10	1.86	3.27
Disk Island	DI067A-2A	L	Jul 22	26.88	0.09	2.28	4.02
Disk Island	DI067A-2A	R	Jul 21	35.04	0.07	2.45	4.32
Disk Island	DI067A-2B	-	Jul 23	16.12	0.06	0.89	1.56
Herring Bay	KN113B	-	Jul 24	9.24	0.09	0.85	1.50
Squirrel Island	SL001D-2	-	Jul 25	17.18	0.09	1.60	2.81
Total				208.00		19.01	33.47

Restoration

Bed restoration involved removal of mussels, replacement of oily sediment with clean sediment (hereafter described as donor sediment), and the repositioning of mussels as evenly as possible in original bed areas. Areas within beds to be restored were staked, measured, and mapped (Appendix 1.3). The Eleanor Island bed (EL011A-2) and the two largest beds (CH010B-2, and DI067A-2) were subdivided so that designated areas could be excavated during one low tide window (approximately 5 hours). Subdivided beds were recombined for analysis. Mussels and sediments attached to byssal threads were carefully removed with shovels or trowels, transported in 20-L buckets, and spread out on sorbent pads placed intertidally near each bed. To maximize stability when mussels were replaced, care was taken to avoid severing byssal threads to other mussels and substrate. Oiled sediments were removed to depths of 12 cm, dependent on depth of oil penetration and substrate characteristics (Table 4.1).

Mussels and donor sediments were placed in beds during the low tide following excavation. Donor sediments were obtained within 100 m of excavated sediments and tended to have coarser grain[6]. Concentrations of TPH in donor sediment were generally less than MDL and were always less than 200 μg/g (Figure 4.2). In some beds where oiled sediment could not all be removed, we anticipated 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 after one high tide cycle.

Oiled sediments were dispersed on sorbent pads located at least 25 m from restored beds in the mid to high intertidal zones. After 1-2 tidal cycles, sorbent pads were removed, and dispersed sediments were raked to expose new surfaces to tidal washing. Sediment was not discarded in biologically productive areas. Paths to sediment dispersal, mussel clean up, and donor sediment areas avoided unrestored areas of the beds to reduce impact on the beds. A total of 19 m^3 sediment weighing an estimated 33 metric tons was removed and dispersed (Table 4.1). Subsequent high tides flushed oil from the surface of the excavation, from removed sediments, and from mussels. Sorbent boom and pads were placed in the lower intertidal zone to adsorb oil as it washed from mussels and oily sediments. Oiled sorbent material was bagged and removed from each site.

Cleaning and restoration was completed in late July and early August (Table 4.1) by an 11 person crew from Chenega Village under the direction of the Alaska Department of Environmental Conservation (6 people per cruise). Skiffs were used to transport replacement sediments at the Eleanor Island site where there were no suitable sediments within practical walking distance.

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[6]Grain size was estimated visually, but was not measured.

Sampling

Sediment samples were collected before, during, and after restoration to form a TPH concentration time series. In April or May 1994, surface (0-2 cm) and deep (4-14 cm) sediments were sampled from 4 random locations within most[7] candidate beds to quantify TPH concentrations in sediments, delineate areas that should be cleaned, determine excavation depths, and provide a baseline to evaluate effectiveness of the restoration process. Additional samples were collected immediately before excavation in some beds. Samples were collected from the surface of remaining deep sediment immediately after oiled surface sediment was removed. Donor sediments were resampled after placement in the beds. In general, triplicate, pooled, sediment samples were randomly collected from 8 to 10 spots throughout sample areas (Babcock et al. 1994), except that the April and May samples were not pooled so concentration variation within each bed could be more accurately delineated. (Collection spoons and 118 ml glass storage jars were hydrocarbon-free. Equipment used for hydrocarbon sampling was prewashed with soap and hot water, rinsed, dried, and rinsed with dicholoromethane or certified as hydrocarbon-free by the manufacturer.) All samples were immediately cooled and frozen within 2-4 h. Surface and deep sediments were periodically resampled through June 1996.

Mussels were also collected from each bed for hydrocarbon analysis, and mussel population densities were estimated. Triplicate pooled samples of 15-20 mussels were collected from 6-8 spots in sample areas and placed in 3 hydrocarbon-free jars, except only 1 pooled sample was collected where mussel densities were very low. Samples were immediately cooled, and frozen within 2-4 h. Mussels were sampled for hydrocarbons during restoration and through 1995. Mussel densities were estimated by counting live mussels in 2 quarters of a 0.25×0.25 m quadrat at 6 randomly chosen locations in restored and unmanipulated reference beds to provide an estimate of mussel density. Mussel densities were estimated before and during restoration, and periodically through June 1996.

Hydrocarbon analysis

Sediment samples were analyzed by an ultraviolet fluorescence (UVF) 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 the maximum phenanthrene output. A standard curve based on the amount of phenanthrene in EVO was used to estimate total petroleum hydrocarbon (TPH) concentration. Mean TPH concentration is reported in μg/g wet weight. The empirically estimated method detection limit (MDL) for TPH was about 50 μg/g.

Hydrocarbon concentrations in all mussels and some sediment samples were determined by gas chromatography/mass spectroscopy (GC/MS). A small subset of sediments with elevated TPH were selected for GC/MS analysis to confirm polynuclear aromatic hydrocarbon (PAH)

[7]Sediments were not sampled at DI067A-2B before treatment because samples from a larger, adjacent bed (DI067A-2) were considered to adequately describe pre-treatment conditions.

composition. Samples were analyzed at the National Marine Fisheries Service, Auke Bay Laboratory (Short et al. 1996). Experimentally determined MDL depended on sample weights, and generally were 1 ppb in tissue, and < 2 ppb 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. Total PAH (TPAH) concentrations were calculated by summing concentrations of individual PAH except perylene. Perylene was excluded because it is produced contemporaneously by biogenic sources. (See Appendix 2 for a listing of PAH identified by GC/MS.) Relative PAH concentrations were calculated as the ratio of PAH concentration to the TPAH concentration.

Determination of Petroleum Source

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 in weathered EVO. The model, which was successfully validated by comparison with thousands of samples from the study area, uses experimentally determined loss-rate constants for 14 PAHs to calculate an index of weathering (w) that summarizes exposure history (Appendix 2). [$W = 0$ in unweathered samples and increases with weathering. For all environmental samples recorded in the Natural Resource Damage Assessment database (Short et al. 1996), w ranges up to 11.3 for sediment and 9.9 for mussels. For this paper we use the following definitions: unweathered ($w = 0$), slightly weathered ($0 < w \leq 2$), moderately weathered ($2 < w \leq 8$), and highly weathered ($w > 8$).] 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 EVO (Short and Heintz 1997).

Data Analysis

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 developed by Babcock et al. (Chapter 1).

Pre- and post-restoration TPH concentrations in sediment were compared using three-factor ANOVA (site, depth, and group, where factor "group" represented day, but distinguished among pre- and post-restoration samples collected on the same day). Concentrations were log-transformed before analysis. For each site, post-restoration TPH concentrations in sediment were compared to the initial pre-restoration concentration (observed in April or May 1994) using *a priori* multiple comparisons except no deep samples were collected before restoration in bed KN113B. A single composite pre-restoration sediment sample was collected for beds DI067A-2 and DI067A-2B. Earliest data included in the analysis were collected in 1994. Data from the

three oiled reference beds were included in the analysis; there were sufficient data to compare changes in TPH concentration in surface sediments in two of these beds.

Pre- and post-restoration TPAH concentrations in mussels were compared using three-factor ANOVA (substrate, site, and group, where factor "group" represented day, but distinguished among pre- and post-restoration samples collected on the same day or combined 2 days for the initial sample when restoration took more than 1 day). Concentrations were translated +0.01 units and log transformed before analysis. For each site, post-restoration TPAH concentrations were compared to those in initial samples using *a priori* multiple comparisons. Instances where initial mussel samples were collected after restoration began are noted in the results. Earliest data included in the analysis were collected in 1994. Data from the three oiled reference beds were included in the analysis; there were sufficient data to compare changes in TPAH concentration in mussels in two of these beds.

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 and regressions (1992-1993; Babcock et al. Chapter 1). Post-restoration data were considered to be significantly different than predicted concentrations if means ± SE were outside 95% confidence bands of the regressions. Seven of nine restored beds were sampled before restoration, but CH010B-2C and DI067A-1 were not. (The bed originally designated as DI067A-2 was arbitrarily subdivided into DI067A-2 and DI067A-2B at the beginning of this study, thus 1992-1993 pre-restoration data were applicable to both beds). Due to insufficient data there were no predictions for sediments in 3 restored beds, and none for mussels in 6 beds. Because pre-restoration data were insufficient for model predictions in every bed, the previously described ANOVA models were expanded to include 1992-1993 data and allow extended comparisons.

To determine if mussel density varied as a function of time, post-restoration densities were compared to initial densities using two-factor ANOVA (site and day). For each site, post-restoration densities were compared to density in the first samples collected in 1994 using *a priori* multiple comparisons. Data collected prior to 1994 were not included in the analysis. Data from the three unmanipulated, oiled reference beds were included in the analysis, but there were sufficient data to compare changes in mussel density over time in only two of these beds (EL011A-2D and DI067A-2C). Because regressions of density against time did not always adequately describe the data for each site, regression analysis was not used.

To compare mussel density observed in years prior to this study (Babcock et al. Chapter 1) with the initial 1994 densities, constraints on the previous ANOVA were relaxed to include all data (1992-1996). For each site, pre-restoration densities were compared to density in the first samples collected in 1994 using *a priori* multiple comparisons.

To determine if restoration activity affected mussel density, change in mussel density in restored beds was compared to change in two unmanipulated, oiled reference beds (EL011A-2D and DI067A-2C). For each site, densities were divided by initial 1994 densities. Normalized reference densities were combined into a single group, and observation times were regrouped for

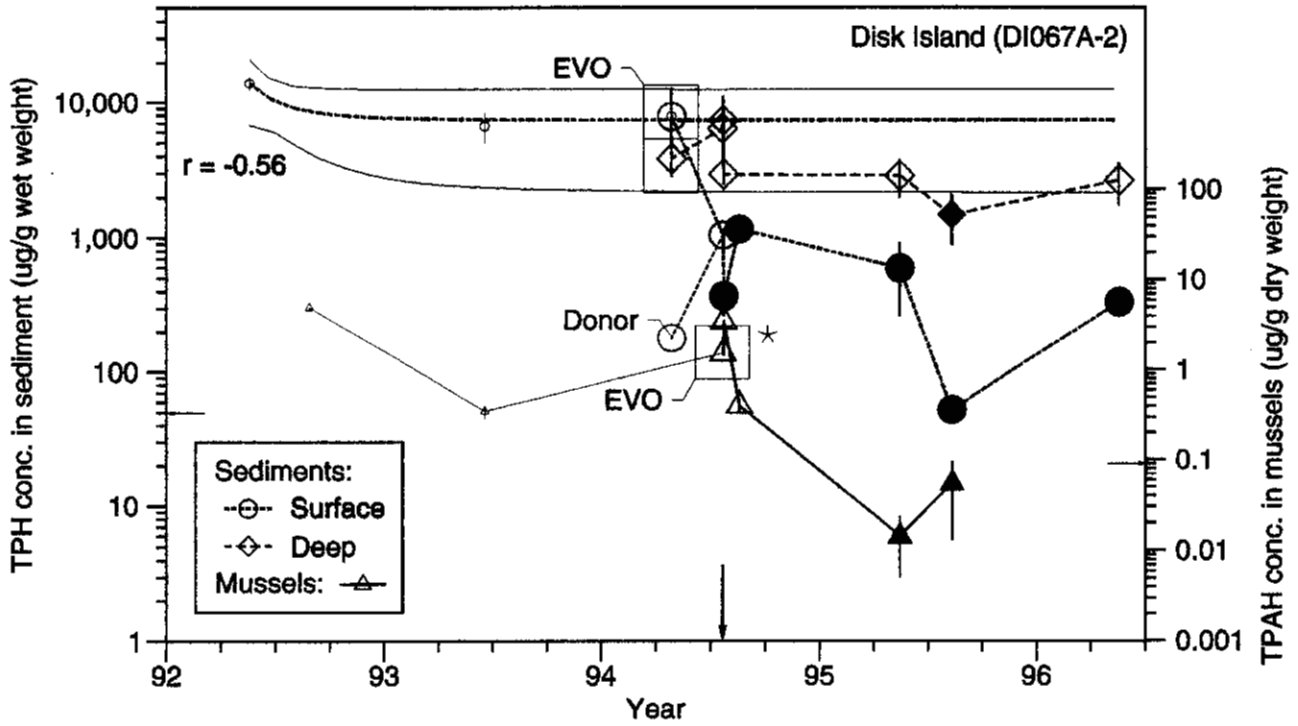

Figure 4.2. Mean concentrations of total petroleum hydrocarbons (TPH) in sediment and total polynuclear aromatic hydrocarbons (TPAH) in mussels from mussel beds in Prince William Sound restored in 1994. Vertical arrows indicate times restoration began. Horizontal arrows indicate estimated background concentration (Babcock et al. Chapter 1) for sediments (left) and mussels (right). Boxes indicate which samples served as references for statistical analysis. The presence of *Exxon Valdez* oil (EVO) in initial 1994 samples so labeled was confirmed (Short and Heintz 1997). Concentrations of TPH in undisturbed donor sediments are labeled "donor". Solid symbols indicate significant differences from initial 1994 concentrations ($P \leq 0.05$). Small symbols and regressions are from Babcock et al. (Chapter 1 in this report) and indicate conditions prior to restoration. The thin lines bounding regressions are 95% confidence bands. The first post-restoration mussel sample collected at DI067A-2 (single asterisk) served as the statistical reference because earlier samples were not collected. This figure continues on next four pages.

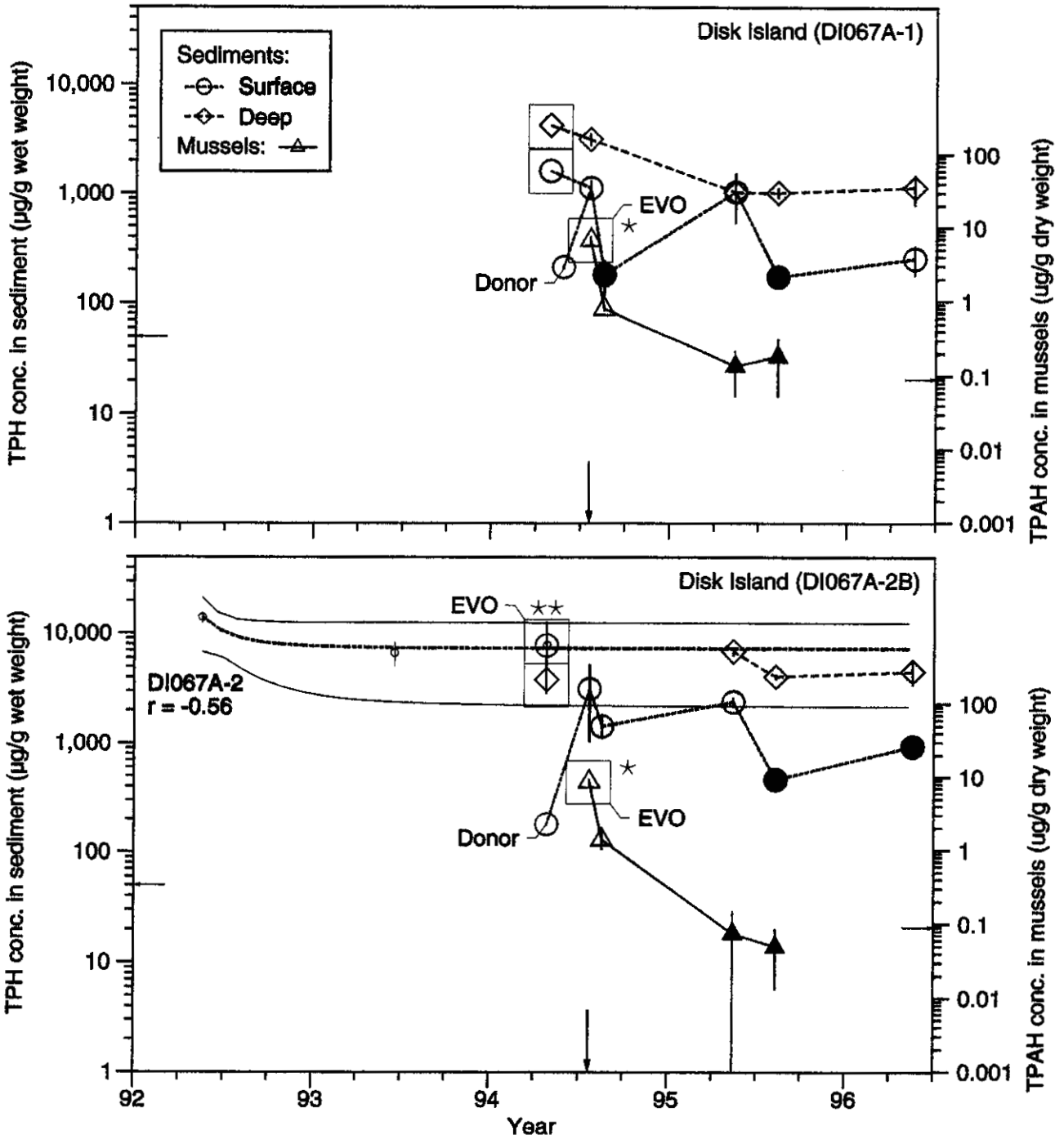

Figure 4.2, continued (DI067A-1 and DI067A-2B). The initial 1994 sediment samples marked with a double asterisk represent a composite DI067A-2B and DI067A-2 sample. The first post-restoration mussel samples collected (single asterisks) served as statistical references because earlier samples were not collected.

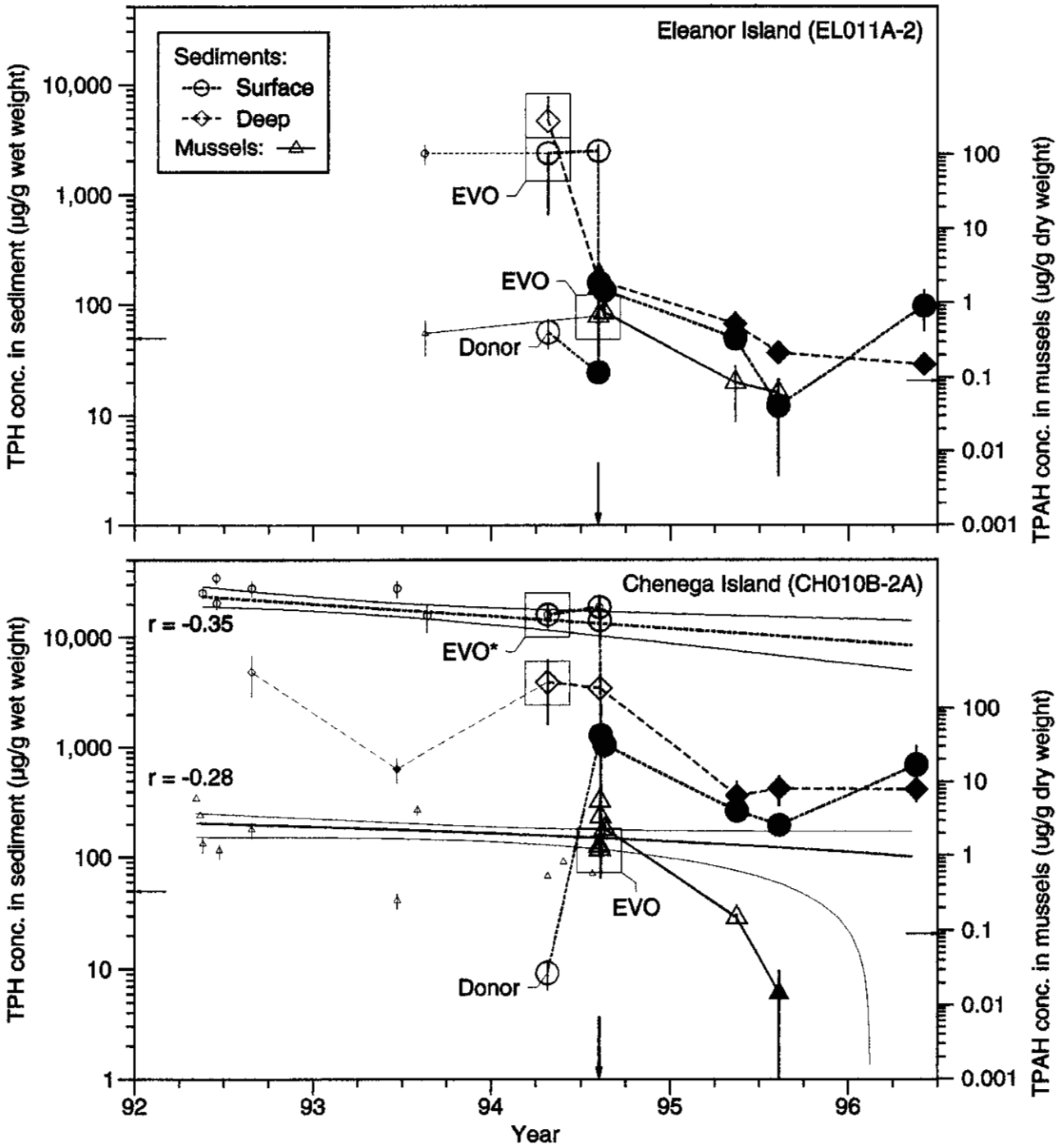

Figure 4.2, continued (EL011A-2 and CH010B-2A). Asterisk: EVO was confirmed in a sediment sample collected 5 d after the initial sample.

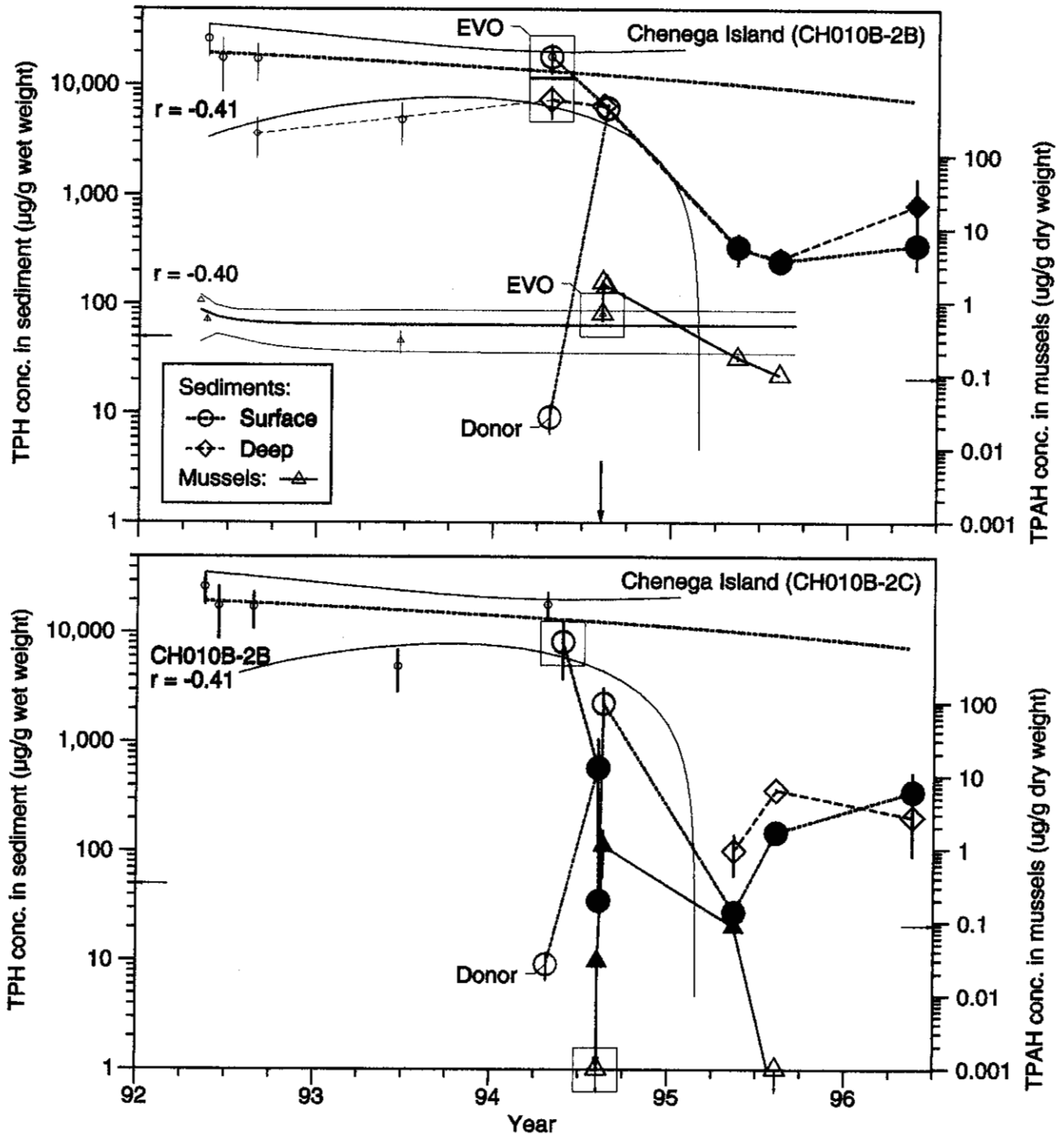

Figure 4.2, continued (CH010B-2B and CH010B-2C).

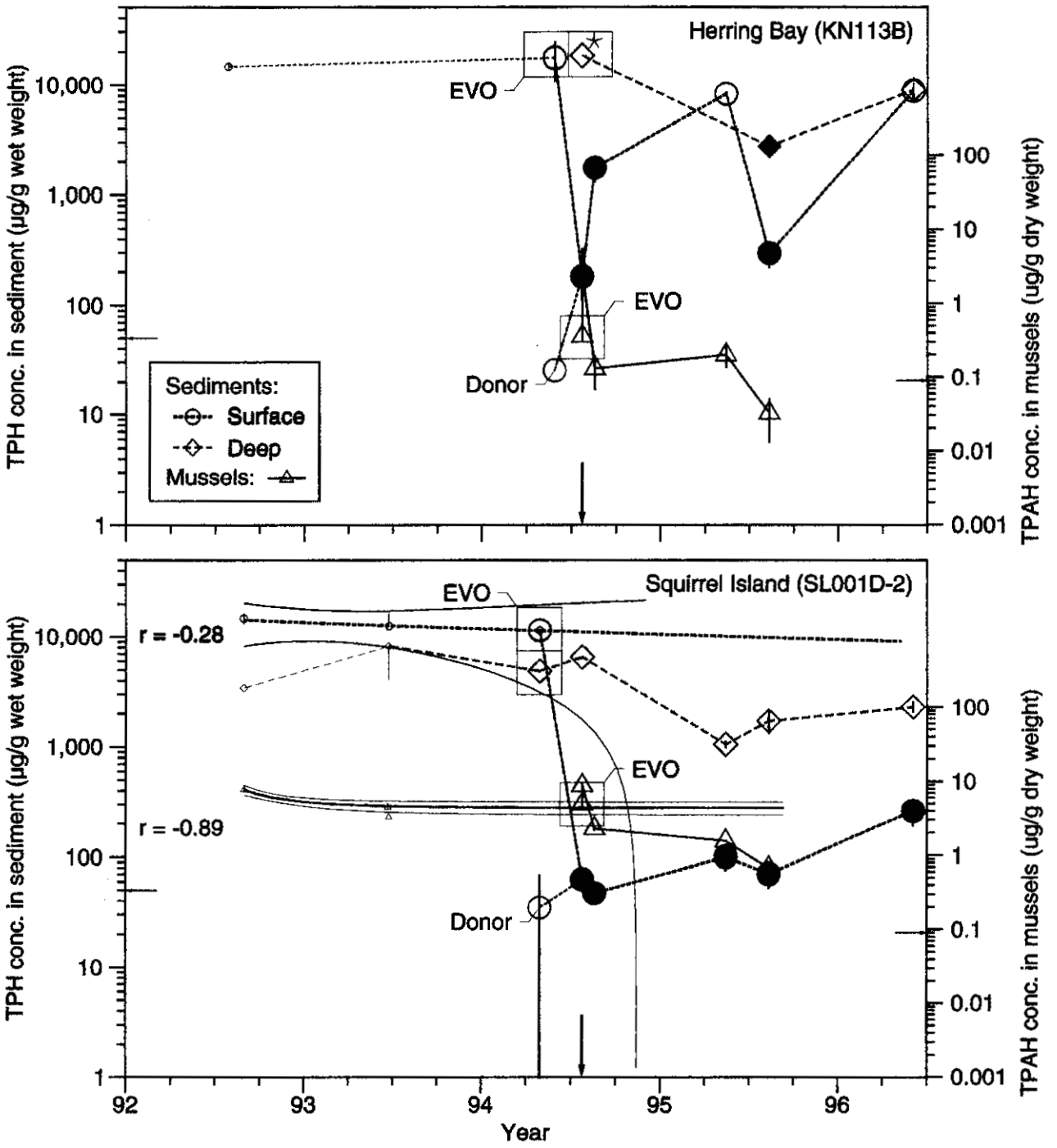

Figure 4.2, continued (KN113B and SL001D-2). The first post-restoration deep sediment sample collected (single asterisk) served the statistical reference because earlier samples were not collected.

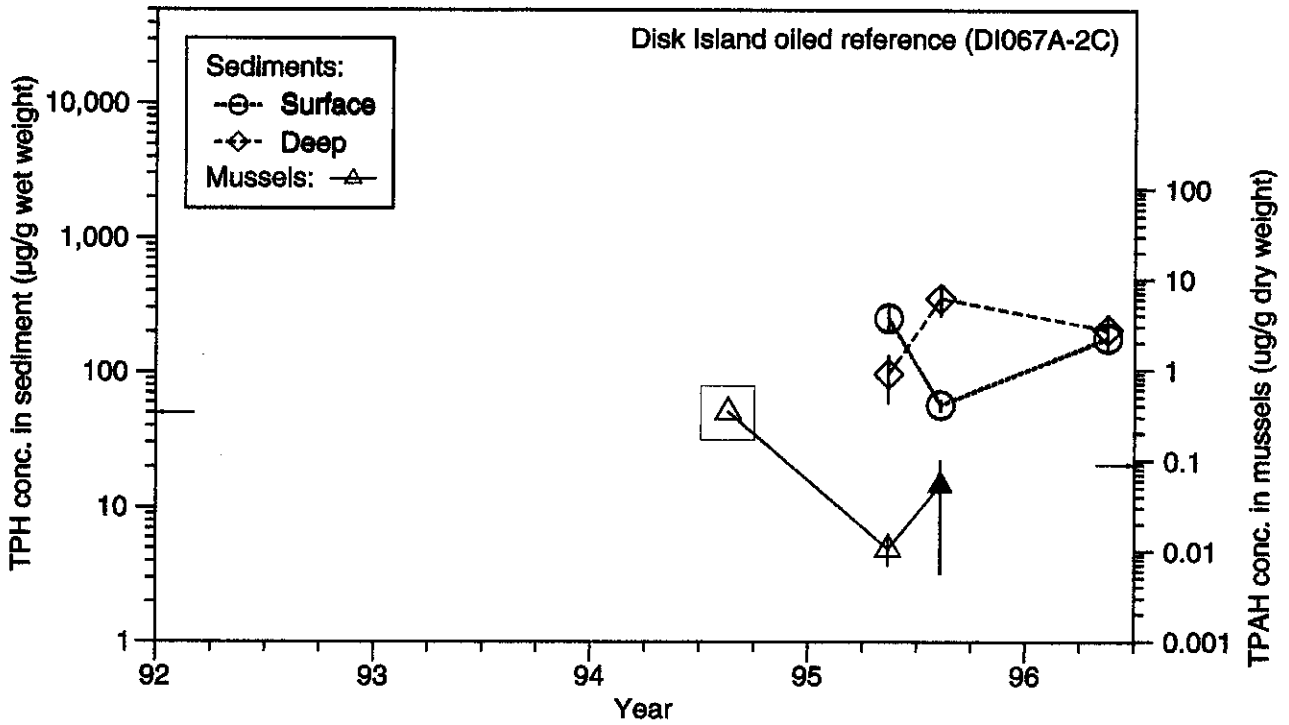

Figure 4.3. Mean concentrations of total petroleum hydrocarbons (TPH) in sediment and total petroleum aromatic hydrocarbons (TPAH) in mussels from unmanipulated, oiled reference mussel beds in Prince William Sound. Horizontal arrows indicate estimated background concentration (Babcock et al. Chapter 1) for sediments (left) and mussels (right). Boxes indicate which samples served as references for statistical analysis. Solid symbols indicate significant differences from reference concentrations ($P \leq 0.05$). This figure continues on the next page.

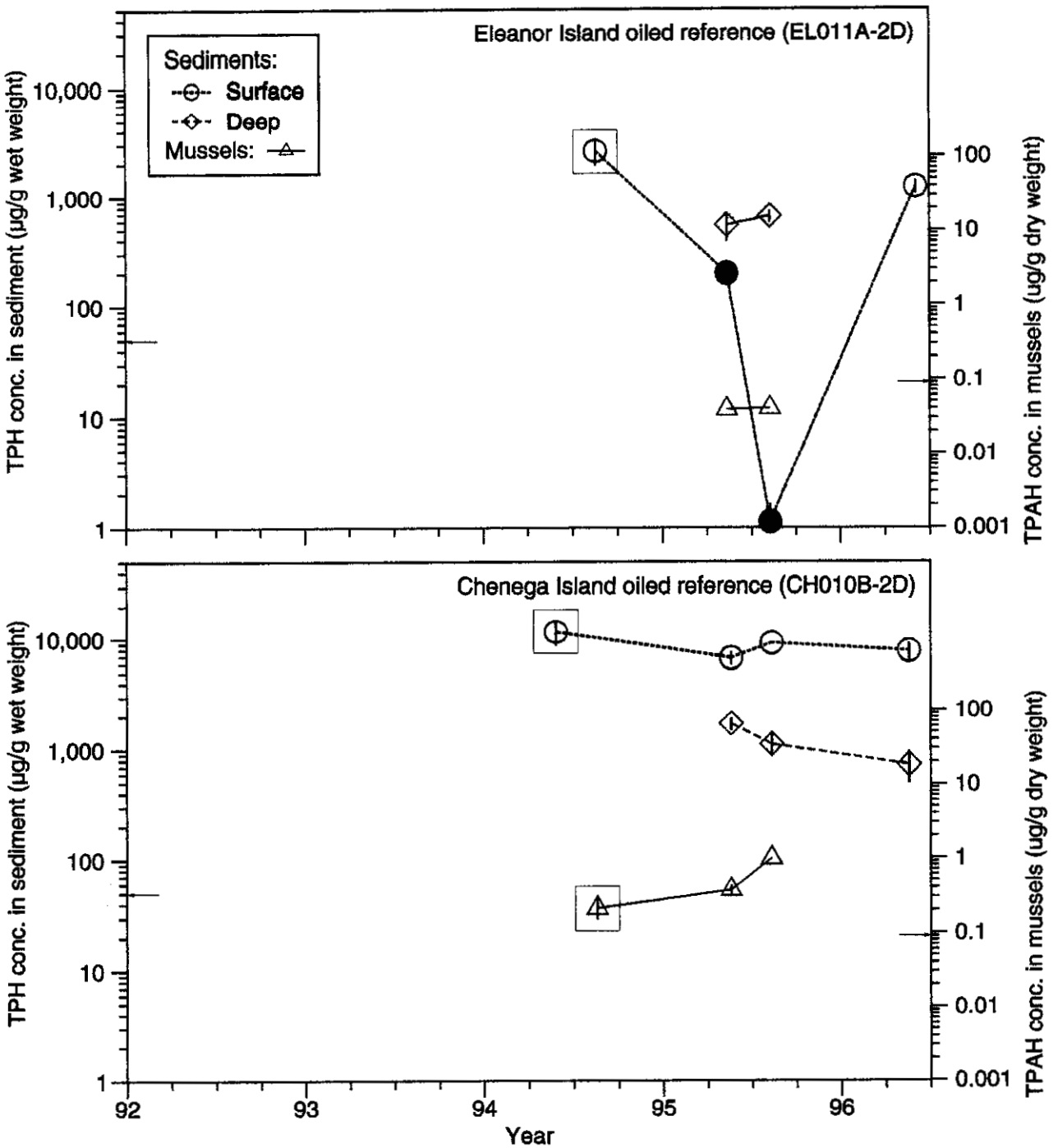

Figure 4.3, continued.

records (DI067A-1, DI067A-2, KN113B, and SL001D-2), there was a tendency for concentration to decline to an asymptote; post-restoration concentrations were occasionally significantly less than initial concentrations in these beds ($0.003 \leq P \leq 0.813$). Concentrations of TPH in deep sediment were less variable than in surface sediment and generally exceeded those in surface sediment. In 7 of 9 beds, TPH concentrations in deep sediment exceeded twice background concentration ($2 \cdot 50 = 100$ µg/g) at the end of study, usually by a wide margin. The EL011A-2 bed was a major exception; TPH concentration in August 1995 and 1996 were less than background concentration.

The pre-restoration TPH concentrations in deep sediment in three restored beds prior to 1994 were generally not significantly different from initial pre-restoration concentrations in 1994 ($0.011 \leq P \leq 0.767$) (Figure 4.2). The one exception occurred in 1993 in bed CH010B-2A, where the concentration was significantly less than the initial 1994 concentration, and similar to post-restoration concentrations. Concentrations of TPH in deep sediment were significantly greater than background concentration in all unmanipulated, oiled reference beds.

Polynuclear aromatic hydrocarbon concentrations in mussels

Hydrocarbon concentrations in mussels first tended to increase for a short time after restoration activity and then declined (Figure 4.2). Total PAH concentrations in mussels were consistently greater within one day after restoration than before restoration for every bed with a complete data record (6 cases), but this increase was significant only at CH010B-2C. (Bed CH010B-2C was an unusual case; both initial and final concentrations were zero.) Total PAH concentrations declined after the initial increase, but significant reductions were not observed until 1995. Concentration reductions were significant in 4 beds ($0.001 < P \leq 0.021$), but not in the remaining 5 beds ($0.117 \leq P \leq 1.000$). However, mean endpoint concentrations were less than the estimated background concentration (0.09 µg/g) in 6 beds, including 3 beds where concentration reductions were not statistically significant. Total PAH concentrations in mussels likely remained significantly greater than the background concentration in only one bed, SL001D-2, based on generalized variance estimates applied to some endpoint samples collected without replication.

Total PAH concentrations in mussels remained approximately constant in one oiled, unmanipulated reference bed, but declined in another reference bed from 1994 to 1995 (Figure 4.3). In the CH010B-2D bed, TPAH concentrations in 1995 were not significantly different than in 1994, and remained significantly above the estimated background concentration. In the DI067A-2C bed, TPAH concentration declined below background concentration in 1995, and the endpoint concentration was significantly less than that in 1994 ($P = 0.022$). Total PAH concentrations in mussels (observed only in 1995) in a third unmanipulated, oiled reference bed were below background concentration.

Natural hydrocarbon loss from mussels was modeled for three restored beds (Babcock et al. Chapter 1), and the post-restoration loss in all three of these beds was more rapid than predicted (Figure 4.2). Total PAH concentrations declined below predicted concentrations in beds CH010B-2A, CH010B-2B, and SL001D-2 by 1995 ($P \leq 0.05$). Pre-restoration

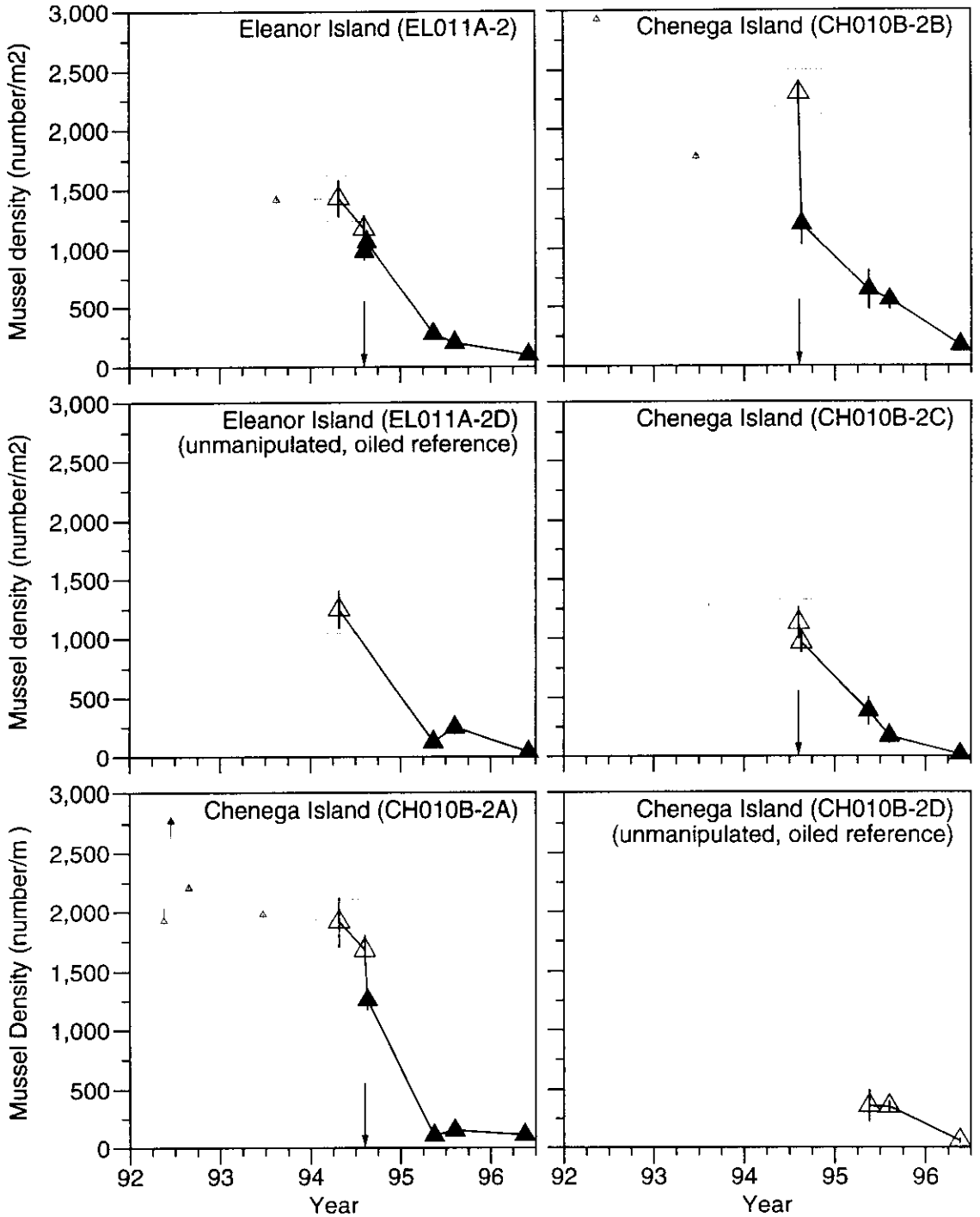

Figure 4.4. Mean mussel density in restored and unmanipulated, oiled reference mussel beds in Prince William Sound. Vertical arrows indicate times restoration began. Boxes indicate which samples served as references for statistical analysis. Solid symbols indicate significant differences from reference densities ($P \leq 0.05$). Small symbols and thin lines are from Babcock et al. (Chapter 1 in this report) and indicate conditions prior to restoration. This figure continues on the next page.

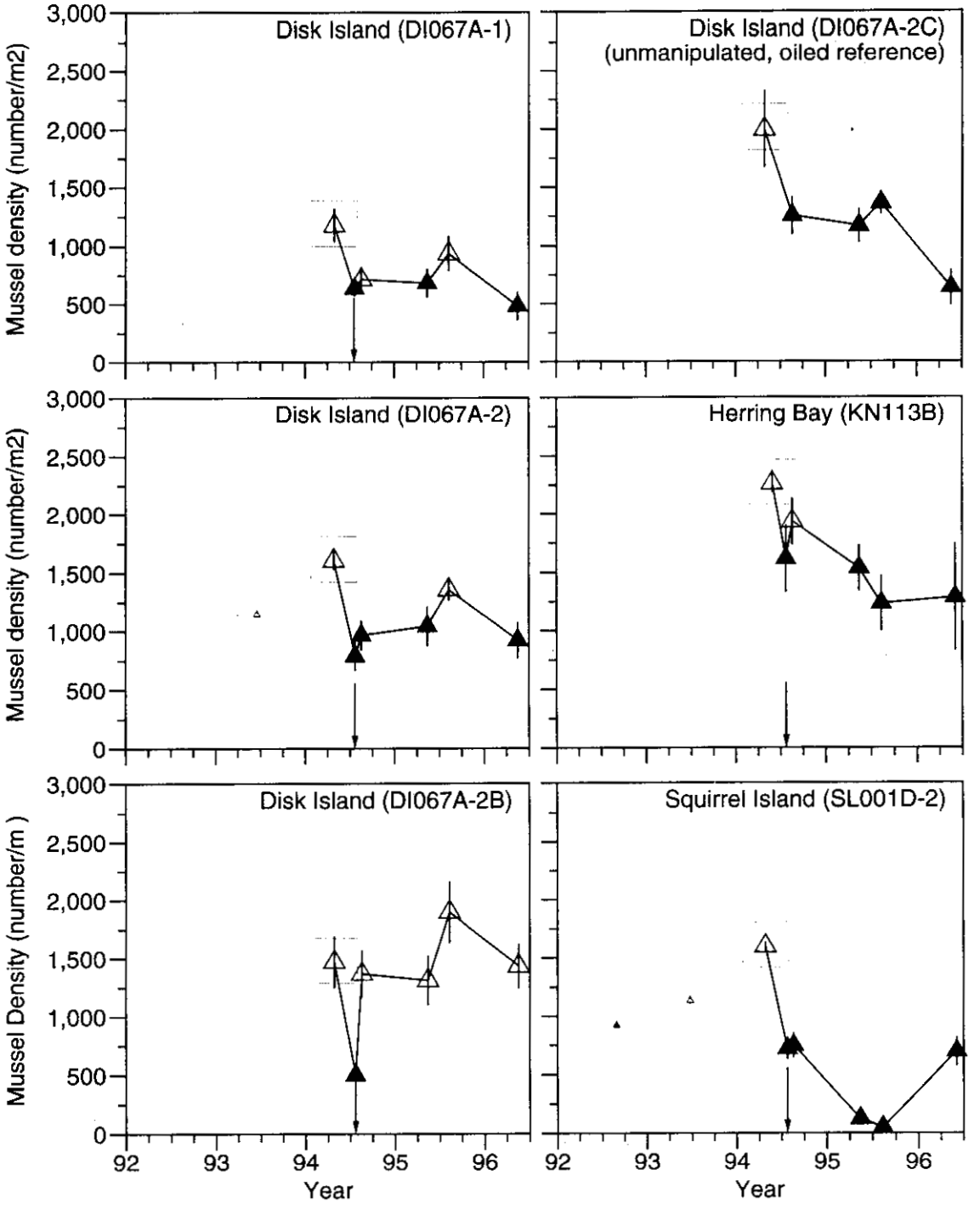

Figure 4.4, continued.

concentration data were also collected in 1992 and/or 1993 for two other beds; TPAH concentrations in mussels were significantly less than previously observed in the DI067A-2 bed ($0.001 < P \leq 0.016$), but were not significantly depressed in the other bed (EL011A-2, $0.554 \leq P \leq 0.556$).

Mussel Density

Mussel density generally declined from 1994 to 1996, both in restored and unmanipulated, oiled reference beds (Figure 4.4). Density declined significantly after restoration in all but one restored bed. In the exceptional bed (DI067A-2B), density was significantly depressed immediately after restoration ($P < 0.001$), but quickly rebounded to pre-restoration levels. At several sites, density declines were roughly asymptotic, and at one site (SL001D-2) density increased significantly after declining to a minimum in 1995 ($0.004 \leq P \leq 0.010$). Densities in the two testable, unmanipulated, oiled reference beds also declined significantly ($0.001 < P \leq 0.019$).

Mussel densities in years prior to restoration were generally not significantly different than initial 1994 densities (Figure 4.4). There were two exceptions; density was significantly higher than the initial 1994 density in one 1992 observation at CH010B-2A ($P < 0.001$), and significantly low in 1992 at SL001D-2 ($P = 0.034$).

There were no consistent differences in mussel density change between restored and unmanipulated, oiled reference beds. At times, normalized densities in four restored beds were significantly less than those in reference beds (13.6% of observations, $0.001 < P \leq 0.016$), but normalized densities in five other restored beds were significantly greater than in reference beds (29.6% of observations, $0.001 < P \leq 0.035$). Normalized densities were not significantly different between restored and reference beds for 57% of the observations,. In the SL001D-2 bed, normalized density was significantly lower than in reference beds in 1995 but significantly high in 1996.

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. Furthermore, although there were significant declines in mussel density after restoration, these declines were consistent with regional declines in mussel density, and may not have been caused by the restoration the methods employed. Hydrocarbon concentrations in surface sediments were generally quickly lowered by restoration activity. Restoration efficacy was less evident in mussels; hydrocarbon concentrations declined in mussels, but reductions were never significant in the first year of study, and concentrations were reduced significantly in less than half of the beds by the end of study. However, post-restoration hydrocarbon loss rates from mussels were consistently more rapid than predicted in the three beds where natural loss rates were previously modeled. Hydrocarbon concentrations in deeper, underlying sediment also generally declined, but declines were not always significant, and there was some evidence within

the two-year study period that surface sediments reacquired oil from deeper sediment. Thus the long-term consequences of manual restoration have not been resolved. However, it may never be possible to fully resolve the long-term consequences of restoration because of high regional variability in oil concentration, persistence, and change.

Restoration efficacy was most evident in replaced surface sediment, where TPH concentrations were rapidly and significantly reduced in mussel beds in 1994, but these short-term reductions did not always assure long-term improvement. Although reductions in TPH concentration were observed, donor sediment was contaminated with hydrocarbons as a result of restoration activity, and TPH concentrations measured after placement were generally much higher than observed before human disturbance. Sources for this immediate recontamination included oil from deep sediment, oil sources surrounding excavated areas, and oil associated with mussels placed onto donor sediment. Despite these increases in hydrocarbon concentration in donor sediment, the net result was an immediate reduction in TPH concentration in restored beds. Reduction in TPH concentration as a result of restoration activity was generally corroborated by comparison to the pre-study concentration predictions made by Babcock et al. (Chapter 1) for six sites. However, concentrations of TPH in surface sediment did not always decline to background, and by 1996 had significantly increased from minimum observed concentrations in 4 of 9 beds. Sources of long-term recontamination included oil that remained below the excavation depth and likely other nearby oil. For example, an area up slope from the Herring Bay bed, which was not cleaned because of rock armoring, was a likely source of recontamination.

Although oil in sediment below the excavation depth could potentially recontaminate surface sediment, TPH concentrations in deep sediment were generally reduced by restoration activity. However, after restoration, TPH concentrations in deep sediment generally exceeded those in surface sediment, suggesting that deep sediments are protected oil reservoirs. Because the distribution of oil within a bed is not homogeneous or static (Harris et al. 1996), we anticipated that overlying sediment would slowly become recontaminated, and that concentrations in deep sediment would decline as oil was redistributed by hydraulic processes throughout the larger sediment mass. As a result of general reductions in hydrocarbon concentrations in sediment, we anticipated substantive reductions in the amount of EVO available to mussels.

Efficacy of restoration was least evident in mussels. Although concentrations declined in mussels, reductions were never significant in the first year of study, and concentrations were reduced significantly in less than half of the beds by the end of study. It was difficult to distinguish the significant reductions in TPAH concentration in mussels observed in 1995 from natural reductions in tissue concentration, such as occurred at one reference site (DI067A-2C). A much wider range of mussel beds in PWS were surveyed by Babcock et al. (Chapter 1) for TPAH in mussel tissue. The regional trend was a generalized decline in TPAH concentration, but Babcock et al. (Chapter 1) reported declines were significant in only 10 of 23 beds (as modeled by regression), and there was no evidence of TPAH loss in mussels from at least 4 beds. Thus, it is difficult to draw conclusions from comparison of changes in restored beds to generalized regional changes. The best evidence we have for accelerated reduction in TPAH concentration in mussels as a result of restoration activity was from a limited number of sites (three) where

sufficient pre-study data were available to model concentration changes before restoration (Babcock et al. Chapter 1) and post-restoration concentrations fell below predicted values.

The restoration methods employed in this study probably caused some mussel mortality, but changes in mussel density following restoration activity could not be statistically distinguished from regional declines in density. In preliminary mussel relocation experiments, handling mortality was high, although this mortality may have been partially due to relocation of mussels into marginal habitat (Harris et al. Chapter 2). Thus we expected mortality 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. (Chapter 5) did not find inhibited thread production in chronically exposed mussels. Mussel densities in manipulated beds declined after restoration, but so did densities in unmanipulated reference beds. There was additional evidence of regional declines in mussel density in three additional unmanipulated, oiled beds (KN119A, KN133A-1, and KN702B; Babcock et al. unpublished data). Reasons for regional declines in mussel density 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 PWS were washed with high-pressure hot water, and much of the marine life so treated perished (Mearns 1996; Houghton et al. 1996). Although we have a less than satisfactory understanding of mussel mortality caused by our restoration activity, there was no evidence of mass mortality.

Hydrocarbon concentrations were reduced by manual restoration activities, but the success of restoration activity was limited by two factors - recontamination and practicality. The evidence that reductions in hydrocarbon concentrations in surface sediment may have been relatively short term, that concentration reductions in sediments and mussels were not always distinguishable from unaided concentration declines, and the high labor and time costs ($350,000 and approximately 620 person-hours) required to restore a relatively small area [208 m^2 out of 1,750 km of oiled shoreline (Wolfe et al. 1994)] forces us to conclude that manual methods are not recommendable as primary cleanup techniques for large spills. The principal reason our method was not more effective was that it was not possible to remove all the oil. Underlying and surrounding oil, therefore, continued to contaminate mussels, and also resulted in recontamination of donor sediments.

Mussel beds are a valuable natural resource that should be cleaned if extensive areas are contaminated with oil, as happened in PWS after the EVO spill. Mussel beds are too valuable to leave uncleaned because they are capable of trapping oil for long periods of time, but they are too fragile to allow destructive cleaning processes such as hot water washes. We recommend that mussels be manually removed from contaminated soft substrates and placed in floating pens where they can depurate oil and survive while intrusive cleaning procedures are applied on shore. Cleanup procedures might involve backhoes or other appropriate equipment, as used in some

cases to clean beaches oiled with EVO (Merns 1996). Alternatively, after mussel removal, the substrate could be hot-water washed, chemically treated, or fertilized to promote bacterial degradation. Treatments designed to clean the relocated mussels should also be considered; possibly oil adherent to mussel shells could be removed with a brief d-limonene wash in air while shells are closed followed by appropriate seawater rinsing. 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 PWS, coupled with observation of long-term (decadal) persistence of oil in certain areas[8], suggests restoration of intertidal habitat to pristine conditions after a major oil spill may generally be impractical. Similarly, Mearns (1996) concluded that perhaps just 4 to 19% of stranded EVO oil was removed by treatment and cleanup operations (1989-1991). Our technique may have some utility for beach cleanup, but the questions such as 'how clean is clean enough?' will always be problematic.

CONCLUSIONS

Manual restoration of mussel beds contaminated by *Exxon Valdez* oil was partially successful in reducing hydrocarbon concentrations in mussels and sediments more rapidly than would have occurred in the absence of restoration. However, donor surface sediments were contaminated by remaining oil as they were put in place and covered by mussels, and there was evidence of continued long-term recontamination of surface sediments.

Not all oil could be removed by manual efforts. Unknown amounts of oil remained in sediment below excavation depths and in surrounding areas. This remaining oil was the most likely source of surface-sediment recontamination.

Hydrocarbon concentrations in the surface sediment in half the restored beds declined more rapidly than predicted by natural rates of decline. However, separation of concentration declines in restored beds from the regional declines evident in unmanipulated, oiled reference beds was not always possible.

Hydrocarbon concentrations declined in mussels, but reductions were never significant in the first year of study, and concentrations were reduced significantly in less than half of the beds by the end of study. However, post-restoration hydrocarbon loss rates from mussels were consistently more rapid than predicted in the three beds where natural loss rates were previously modeled.

Mussel mortality potentially caused by restoration activity was indistinguishable from regional declines in mussel density.

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[8]Areas where oil was persistent included mussel beds overlying soft sediment (Babcock et al. Chapter 1), stream deltas (Murphy et al. submitted), armored high-intertidal areas, such as at Sleepy Bay (Brodersen 1998; Munson et al. 1997), and high intertidal lagoons, such as in Bay of Isles (Babcock et al. Chapter 1).

The concept of removing mussels before cleaning is probably valid, but other methods of cleaning sediment (physical or chemical) might be more rapid and effective than manual replacement of contaminated sediment.

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Chapter 5: Lack of Physiological Responses to Hydrocarbon Accumulation by *Mytilus trossulus* After 3 to 4 Years Chronic Exposure to Spilled *Exxon Valdez* Crude Oil in Prince William Sound

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ABSTRACT

Mussels (*Mytilus trossulus*) were sampled in 1992 and 1993 from beaches in Prince William Sound that had been oiled by the *Exxon Valdez* oil spill of March 1989. At some of the oiled beaches, mussels were collected from beds overlying oiled sediments and from bedrock adjacent to these beds. Mussels were also collected from beaches within the Sound that had not been impacted by the oil spill. Polynuclear aromatic hydrocarbon (PAH) concentrations in mussel tissue and physiological responses (byssal thread production, condition index, clearance rate, and glycogen content) were determined for each group of mussels. Total PAH concentrations in mussel tissue ranged from 0 to 6 μg/g, and were significantly greater (P = 0.001) in mussels from oiled beds than those from reference beds. No significant differences were noted in byssal thread production, condition index, clearance rate, or glycogen content between oiled sample sites and reference sites The lack of physiological response was surprising because mussels in this study were chronically exposed to PAH for 3 to 4 years, and none of the physiological responses measured appeared to be affected by that exposure. The lack of physiological response suggests that chronically exposed mussels may develop a physiological tolerance to PAH, but we recognize that these measures may not have been sensitive enough to discriminate response from background noise.

INTRODUCTION

The grounding of the T/V *Exxon Valdez* in Prince William Sound (PWS) on March 24, 1989, resulted in 42 million liters of crude oil being spilled, and the subsequent oiling of hundreds of kilometers of PWS shoreline. Mussels (*Mytilus trossulus*), which occupy the upper intertidal zone in PWS, received the brunt of exposure to oil after the spill. By fall of 1992, an estimated 2% of the spilled oil remained on the beaches, most in a highly weathered condition (Wolfe et al. 1994). Some mussel beds had been excluded from cleaning in 1989 and 1990 to protect the prey species (mussels) for several vertebrate predators. Consequently, the highest concentrations of oil in sediments were found underlying some mussel beds, and because of the natural armoring by overlying mussels, much of this oil was relatively un-weathered (Babcock et al. 1996; Harris et al. 1996). The reservoir of oil under the mussels led to the highest polynuclear aromatic hydrocarbon (PAH) concentrations found in mussel tissue (up to 8 μg PAH /g tissue) (Harris et al. 1996).

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In the present study, we examine the physiological impacts on mussels after 3 to 4 years of chronic exposure to oil accumulated from contaminated underlying sediments. Measuring oil in mussels has been used extensively in PWS to assess oiling levels for many different damage assessment studies, but had not been used as part of the assessment of damage to mussel beds. There was little interest in determining damage to the ubiquitous mussels until it was feared that continued high tissue concentrations could impact higher vertebrate predators through oil consumption, or through a loss in prey population because of chronic oiling. This study attempts to assess the physiological state of oiled and unoiled (reference) mussels collected 3 to 4 years after the spill as part of an assessment of the impact of oil on mussels themselves.

Mussels have been widely used as both chemical and biomonitors. The filter feeding behavior of mussels facilitates the accumulation of contaminants such as polychlorinated biphenyls, trace metals, and PAH, which can then reflect contaminant levels at the place of exposure. Mussels are especially useful as biomonitors when there are multiple or unknown pollutants, and the net effect on them may be additive. For this reason, there is extensive literature on the use of mussels in biomonitoring programs (Borchardt 1988; Widdows and Johnson 1988; Nelson 1990; Smaal et al. 1991). Several different physiological responses of *Mytilus* to environmental pollutants have been suggested as a means to monitor the degree of stress on the organism induced by the pollutant. Widdows et al. (1987) and Stickle et al. (1985) measured a reduction of scope for growth following long-term exposure to oil in the laboratory, and Widdows et al. (1981, 1996) to PAH in field exposures. A reduction in feeding rate (clearance rate) in oil-exposed mussels (3 to 4 week exposure required to impact feeding) has also been documented (Widdows et al. 1981, 1987, 1996; Stickle et al. 1985). Byssal thread production has been used as an indicator of stress resulting from petroleum exposure. Reduced byssal thread production has been reported for juvenile and adult mussels (Martella 1974; Linden 1977; Carr and Reish 1978). Stekoll et al. (1980) reported a decrease in carbohydrate content of *Macoma balthica* exposed to hydrocarbons. Condition index has also been shown to be impacted in mussels by hydrocarbon exposure (Bayne and Worrall 1980). Although these physiological responses have shown a positive correlation to exposure to xenobiotics, most of the studies have involved acute laboratory exposures or relatively short-term field exposures to multiple pollutants, as occurs in contaminated harbors. These parameters have been shown to be effective in measuring physiological impacts of stress, and we used them to assess impacts from 3 to 4 years of chronic oiling of mussels in PWS following the *Exxon Valdez* oil spill.

Our general objective was to measure physiological impacts in chronically oiled mussels from PWS, 3 to 4 years after the initial oiling. Specifically, our objectives were to 1) measure PAH concentrations in mussel tissue from oiled and reference sites, and 2) measure several different physiological parameters in mussels collected from oiled and reference sites. Byssal thread production, condition indices, feeding rates, and tissue glycogen were used to assess physiological impacts of long-term oiling. Measurement of byssal thread production and feeding rates can be measured practically only in the laboratory under controlled and similar conditions; thus mussels were collected from all sites in one day and returned to the Auke Bay Laboratory for a series of measurements under controlled conditions.

We recognized that differences in physiological response could be caused by subtle differences in environment at the sites, such as currents, food availability, weather exposure, and

many others. For this reason, our experimental design contained both inter-bed comparisons, where reference and oiled sites were geographically distinct, and intra-bed comparisons, where mussels were collected from beds overlying oiled sediment and from hard substrates a few meters from these oiled beds. Hydrocarbon concentrations were documented for each collection.

METHODS

Byssal thread production rate

To determine if byssal thread production was correlated with exposure to *Exxon Valdez* oil, mussels were collected from PWS in 1992 for separate inter-bed and intra-bed experiments. To test inter-bed differences 36 mussels (38 to 40 mm long) were collected from sediment on May 3, at each of nine widely separated mussel beds in PWS, including oiled sites at Bay of Isles (KN136A), Chenega Island [CH010B-2A (sub-site 1) and CH010B-2B (sub-site 2)], Eleanor Island (EL013A), Herring Bay (KN133A), and Latouche Island (LA015E), and reference sites at Barnes Cove (KN575A), Bligh Island, and Olsen Bay (Figure 5.1). Except where noted, reference to Chenega Island in succeeding text indicates sub-site 2. Nine additional groups of 36 mussels were collected from Chenega Island and Herring Bay on June 14, to test for intra-bed differences in byssal thread production. These mussels were collected from three 25 × 50 cm quadrats on oiled substrate and from adjacent bedrock. During collection, byssal threads were cut to reduce injury to the mussels. The mussels were placed in nylon net bags and covered with wet *Fucus* spp. in an insulated ice chest with added ice packs. Mussels were flown to the Auke Bay Laboratory and transferred to experimental apparatus with flowing seawater within 24 hours of collection; 1 additional 10-gram sample from each group was frozen for hydrocarbon analysis. Additional tissue samples were collected for PAH analysis from Chenega Island and Herring Bay samples over the course of study to provide a description of hydrocarbon depuration over time.

The experimental apparatus consisted of glass plates placed on edge in plastic racks. Six mussels from the same bed were glued to each plate with epoxy, and racks were oriented parallel to water flow in long, rectangular fiberglass tanks. Water flowing in one end of the tank readily passed between the plates and out the other end. There were six plates of mussels from each bed, divided among three replicate tanks, for a total of 36 mussels from each bed. Mussels from each bed were present in each tank in equal numbers.

Byssal thread production rates were determined by counting threads attached to the glass. Threads were cut and their attachments were scraped from the glass 48 hours before each count. Initial count frequency was every 2 d. Eight such counts were made over 24 d for each mussel in the inter-bed test, and nine counts per mussel over 38 days in the intra-bed test. Mortality was low, 4.6% and 3.7%, respectively.

Byssal thread production was regressed against log total polynuclear aromatic hydrocarbon (TPAH) concentration as measured in mussel tissue at the time of collection. Inter-bed and intra-bed regressions were analyzed separately because the experiments were not conducted concurrently, and it was likely that uncontrolled factors influenced production rate between experiments. Uncontrolled factors also apparently caused production to vary with time,

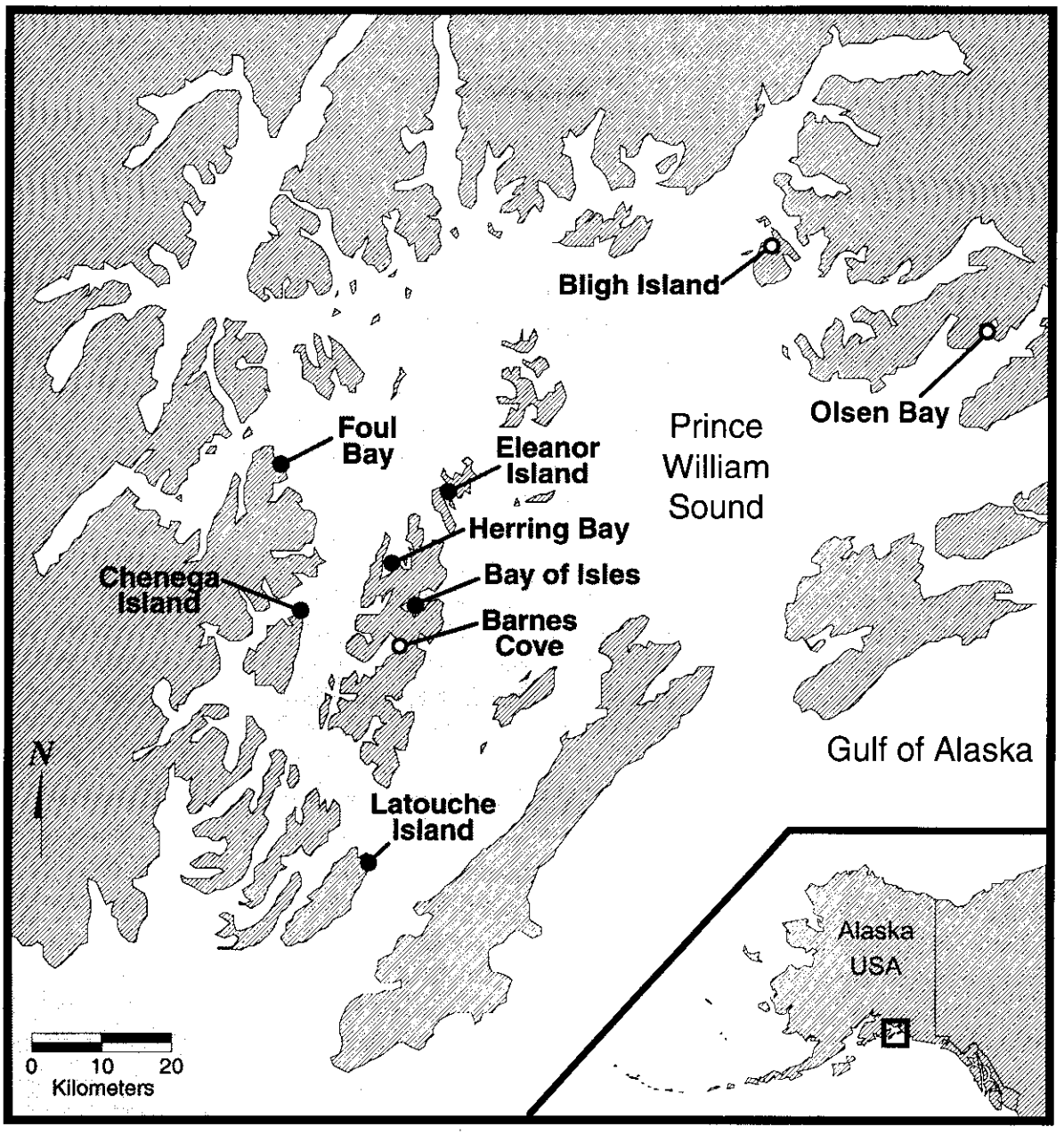

Figure 5.1. Map of Prince William Sound showing sources of all mussels collected for this study. Shaded area indicates extent of oiling during the *Exxon Valdez* oil spill. Reference sites are indicated by open circles; solid circles indicate contaminated sites.

thus independent regressions were applied at each observation time. Each intra-bed site was examined separately, but because intra-bed tests were concurrent, combined regressions were also tested.

Hydrocarbon Analysis

Hydrocarbon concentrations in mussels were determined by gas chromatography/mass spectroscopy (GC/MS) at the National Marine Fisheries Service, Auke Bay Laboratory (Short et al. 1996a). Tissue was obtained from sufficient numbers of mussels to provide 10 g tissue per mussel group; 1-3 such samples were assayed for each mussel group. Experimentally determined MDL depended on sample weights, and generally were 1 ng/g. 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. Total PAH concentrations were calculated by summing concentrations of individual PAH. (See Appendix 2 for a listing of PAH identified by GC/MS.) Background TPAH concentration in mussel tissue, 0.09 μg/g, was adopted from Babcock et al. (Chapter 1 in this report). Relative PAH concentrations were calculated as the ratio of PAH concentration to the TPAH concentration.

Total PAH concentrations in mussels from inter-bed reference and oiled sites were examined with single factor ANOVA, where site classification was the factor. Data were log-transformed prior to analysis to stabilize variance, because variance increased markedly with mean concentration. Depuration of hydrocarbons from two oiled sites (Herring Bay and Chenega Island) was modeled with least-squares regression techniques. Models considered were ladder of powers (x-transformations from linear through $-1/x^3$) and $y = ae^{bx}$. Because TPAH concentrations in relatively few mussels were analyzed as a function of time, initially observed TPAH concentrations provided the measure of site contamination (inter-bed tests) or mussel contamination (intra-bed tests) in analyses relating biological parameters (e.g., byssal thread production rate) and TPAH.

Determination of the source oil and estimation of oil weathering in mussel tissue was accomplished by application of a model developed by Short and Heintz (1977). A weathering parameter, w, was regressed over time, where w represents the extent of first-order kinetic losses of PAH from petroleum. The weathering parameter summarizes the exposure history of the sample; $w = 0$ in unweathered samples, and increases with weathering (Appendix 2). [For all environmental samples recorded in the Natural Resource Damage Assessment database (Short et al. 1996b), w ranges up to 11.3 for sediment and 9.9 for mussels. For this paper we use the following definitions: unweathered ($w = 0$), slightly weathered ($0 < w \leq 2$), moderately weathered ($2 < w \leq 8$), and highly weathered ($w > 8$).] Significant model fits were interpreted as positive identification of *Exxon Valdez* oil as the source of PAH.

Feeding rate

Mussels were collected on August 1, 1993, from two sub-sites at each of two highly oiled beaches [Chenega Island and Foul Bay (MA002C)]) in PWS. At each site, one group of mussels was collected from a bed overlying soft sediment and the other from nearby bedrock. Mussels collected from the bedrock had significantly less oil in their tissues than those collected from oil-contaminated sediments ($P < 0.001$). A fifth group of mussels was collected from a non-oiled beach (Barnes Cove) in PWS. Mussels were collected in the morning at low tide and transported to Auke Bay as before, arriving that afternoon. Upon arrival, the animals were selected by size, 34 to 36 mm, and placed in the experimental chambers for overnight acclimation. Feeding tests began the next morning, approximately 24 hours after the mussels were collected. An additional 20 mussels were sampled from each group for glycogen determination.

Experimental chambers were PVC cylinders, 9 cm long × 38 mm inside diameter, fitted with screw-on end caps. Each cap was provided with a fitting to permit connection to plastic tubing for water inflow and outflow. A rectangle of perforated PVC was placed in each chamber to provide a level surface to which the animals could attach. Ninety-six such chambers were arranged side by side and submersed in an ambient temperature seawater bath. Mussels were loaded into all but 16 of these chambers, with their incurrent siphons facing the incoming water. The sixteen empty chambers were distributed among chambers that contained animals, and served as references for feeding-rate determinations. Animals were permitted 12 hours of acclimation in the chambers before feeding rate was determined; $n = 16$ for each group of mussels. Flow rates were 60 ± 1 ml/min; estimated 95% water replacement time in each chamber was 5 min.

Phaeodactylum tricornutum was used as the food source for this test because it lends itself to fluorimetric determination of cell numbers (Bayne et al. 1977). A pure culture of this diatom was grown in the laboratory using a culture (clone designation CCMP630) obtained from the Center for Culture of Marine Phytoplankton, West Boothbay Harbor, Maine, USA.

Before we tested the PWS mussels, Auke Bay mussels of similar size were used to determine the cell density and water flow rates that would permit the mussels to remove 40 to 45% of the cells from water passing through the chambers. On the morning of the test, *Phaeodactylum* was added to sand-filtered seawater in a 3 × 3 × 1.5 m fiberglass tank, and filtered seawater added to give the desired density of cells. Water was pumped from this tank to a head tank, from which it flowed by gravity through flow meters and into the test chambers. All test chambers were submersed in an ambient seawater bath to control temperature (8.6 ± 0.2°C).

Following the 12-hour acclimation, water flow was switched from ambient seawater to water containing *Phaeodactylum* (mean range 18,654 to 22,431 cells/ml). Water samples were collected at 3-hour intervals from the effluent line from each chamber over the next 12 hours. Flow rate was determined for each chamber when water samples were obtained. Samples were analyzed fluorimetrically (excitation wavelength at 430 nm and emission wavelength 650 nm) to determine cell density (Lorenzen 1966). Calibration of fluorescence intensity was by microscopic count. At the end of the test period, all mussels from each group were collected for analysis of PAH concentration in tissue.

Feeding rate was determined from the rate at which mussels cleared incoming water of diatoms, according to the method of Bayne et al. (1977). Rates were regressed against TPAH in mussel tissue.

Glycogen content

Twenty mussels were sampled from each group of mussels collected for the feeding-rate study. These mussels were shipped live, as previously described, from Auke Bay to the University of Southern Mississippi for glycogen analysis. All samples arrived at Mississippi within 4 days of collection in PWS.

Mussel tissues were removed from shells and homogenized in approximately 10 volumes of ice-cold, distilled water using a motor-driven homogenizer. A sonic dismembrator was used to drive out air bubbles and break down cell membranes. The protocol for glycogen determination was based on the phenol-sulfuric acid method of Dubois et al. (1956). Fifty µl of the tissue homogenate were added to 50 µl of 6 N KOH and mixed by vortexing. The samples were then heated at 50°C for one hour, then cooled on ice. We added 4.5 µl of 2% sodium sulfite and 200 µl of 100% ethanol to precipitate the glycogen. After the samples had incubated on ice for 1 hour, they were centrifuged at 10,000 × g for 5 min at 4°C. The supernatants were discarded and the pellets washed with 200 µl of ice-cold 75% ethanol. After the pellets had dried in a lyophilizer for 10 min, they were dissolved in 200 µl of distilled water. For colorimetric development, 200 µl of 5% phenol and 1 ml of sulfuric acid were added. The glycogen content of each sample was determined by comparing the optical density of the sample with those of standards prepared from *Mytilus* glycogen at 490 nm (Sigma Chemical Co.).

Mean glycogen concentration of each mussel was calculated as total glycogen (µg) per tissue dry weight (mg). Glycogen concentrations were regressed against log TPAH concentration.

Condition index

Condition indices were calculated for mussels analyzed for PAH. (Indices were not calculated for mussels maintained in the laboratory.) Tissues from 10 mussels from each sample were weighed, and a conversion factor from wet to dry weight found by drying an aliquot of homogenized tissue to stable dry weight. Shell volumes were determined by weighing the fine silica sand required to fill one valve, applying a previously determined weight-to-volume standard curve for that sand, and multiplying by two. The condition index (CI) used was dry weight of tissue in grams times 100 divided by shell volume in milliliters (Higgins 1938). Condition indices were regressed against log TPAH concentration. Initially, 1992 inter-bed and intra-bed data were regressed separately, and intra-bed regressions were independent by site. However, because condition was determined only for mussels obtained directly from the field, and because the ranges of 1992 inter- and intra-bed condition indices closely overlapped

(differences were not significant, as tested with 1-way ANOVA: $P = 0.900$), all 1992 condition data were combined for the final analysis. The 1993 condition indices were regressed separately because values were significantly different ($P < 0.001$) between years.

RESULTS

Tissue hydrocarbons

Total PAH concentrations in mussel tissue ranged from 0.01 to 6.0 μg/g. In 1992 inter-bed tests, TPAH concentrations were significantly greater in oiled mussels (2.6 ± 1.0 μg/g) than in reference mussels (0.08 ± 0.02 μg/g) ($P = 0.001$) (Figure 5.2). In 1992 intra-bed tests, TPAH concentrations were significantly greater in oiled mussels (1.9 ± 0.4 μg/g) than in the Olsen Bay reference mussels (0.02 μg/g) (P = 0.001) (Figure 5.3).

In 1993, TPAH concentrations in mussels from highly contaminated portions of oiled beds ranged from 4.2 ± 0.7 to 4.8 ± 0.6 μg/g (Chenega Island and Foul Bay, respectively), and were significantly greater than concentrations in the Barnes Cove reference bed (0.20 ± 0.04 μg/g, $P < 0.001$) (Figure 5.4). Total PAH concentrations in mussels from less contaminated portions of Chenega Island and Foul Bay beds were not significantly different from those in the reference bed ($P \geq 0.816$).

Mussels maintained in the laboratory for experimentation depurated hydrocarbons over the course of study, as demonstrated by concentration declines in mussels from Herring Bay and Chenega Island (Figure 5.5). Declines in TPAH concentration with time were significant in Chenega Island mussels ($P = 0.003$, exponential model), and approached significance in Herring Bay mussels ($P = 0.073$, linear model).

Composition of PAH in mussels from oiled beds was commensurate with weathered *Exxon Valdez* oil as the source. In 1992 inter-bed comparisons, oil weathering model fits were significant at the time of collection in 4 of 6 oiled beds, but there was no evidence of *Exxon Valdez* oil in the three reference beds. Naphthalenes, fluorenes, dibenzothiophenes, phenanthrenes and chrysenes were present in samples from the remaining two oiled beds in ratios suggestive of weathered oil. In 1992 intra-bed mussels, weathering model fits were significant for every sample from oiled sites. Indicative of weathered oil, w in all 1992 samples ranged from 5.1 to 8.6 (moderately to highly weathered). In 1993, weathering model fits were significant for the highly contaminated portion of the Chenega Island bed; $w = 4.7$ (moderately weathered). Although composition in the highly contaminated portion of the Foul Bay bed was similar to that in the Chenega bed, and w was similar (4.5), weathering model fits were not significant. The PAH composition in other 1993 samples was not consistent with composition of *Exxon Valdez* oil.

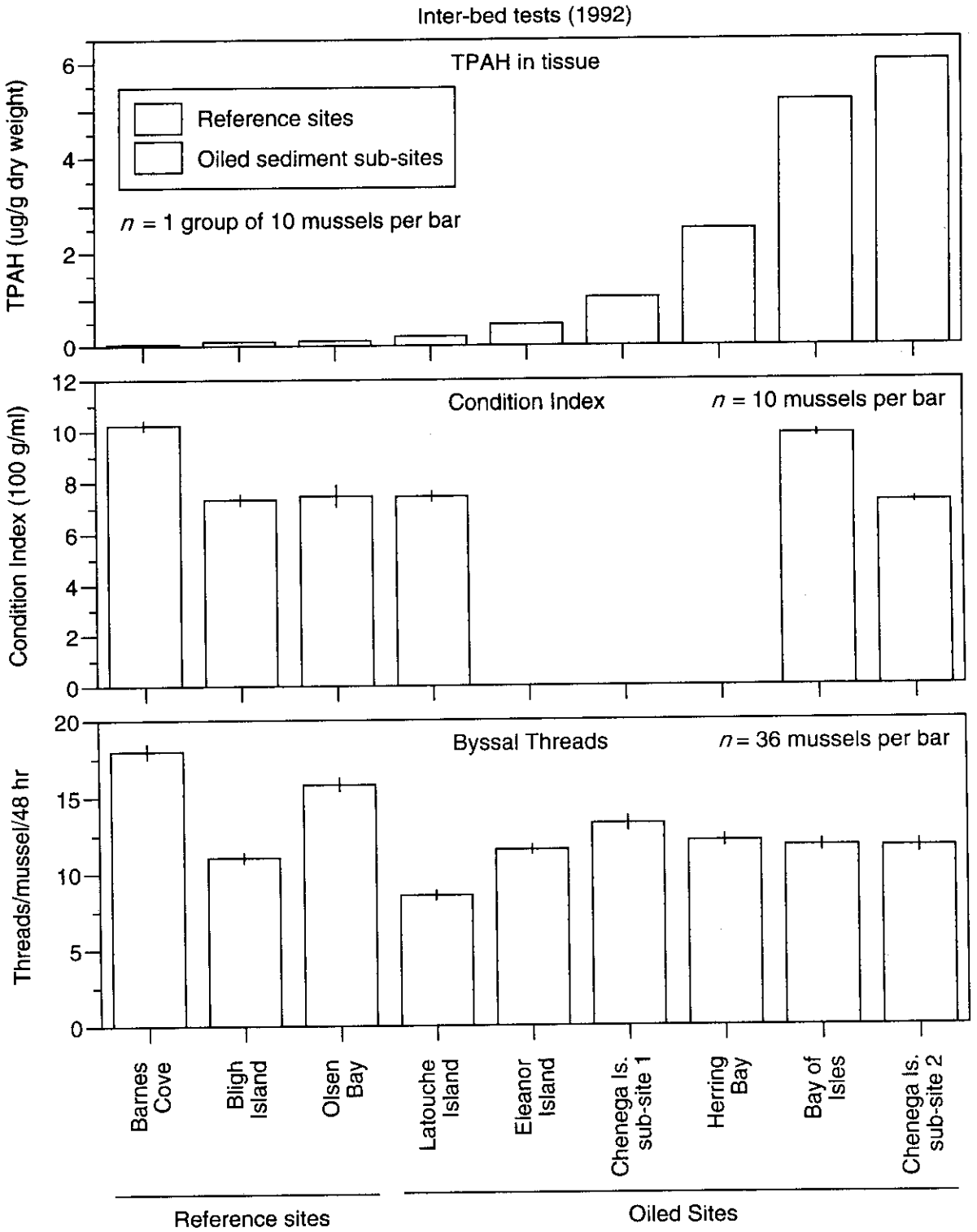

Figure 5.2. Total polynuclear aromatic hydrocarbon (TPAH) concentrations in mussel tissue, and mean (± SE) condition index and byssal thread production of mussels collected in 1992 from oiled and reference beaches in Prince William Sound. Byssal thread values are averages of 36 mussels with 8 observations each.

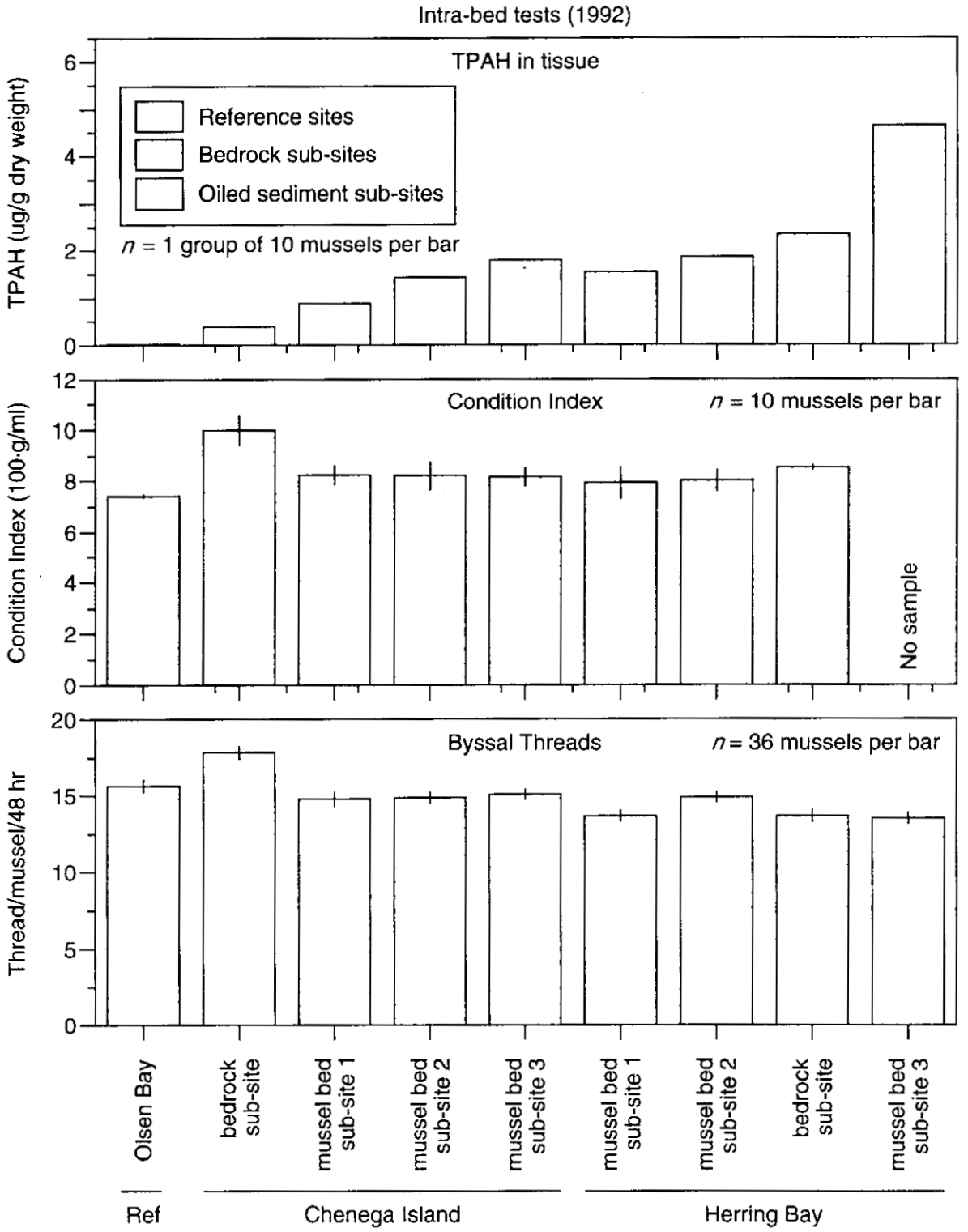

Figure 5.3. Total polynuclear aromatic hydrocarbon (TPAH) concentrations in mussel tissue, and mean (± SE) condition index and byssal thread production of mussels collected in 1992 from bedrock, oiled mussel beds, and a reference (Ref) bed in Prince William Sound. Byssal thread values are the mean of 36 mussels with 9 observations each.

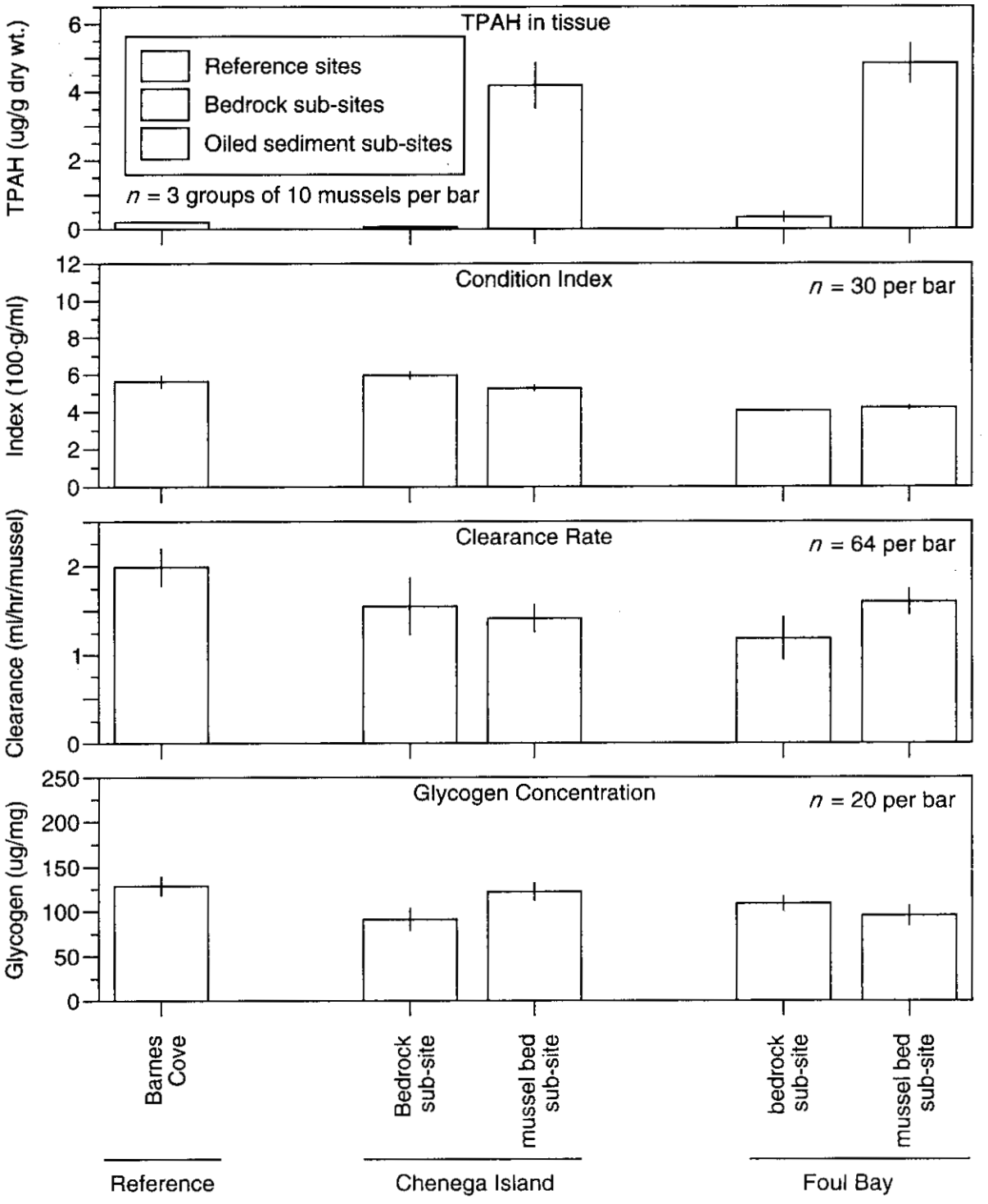

Figure 5.4. Total polynuclear aromatic hydrocarbon (TPAH) concentrations in mussel tissue, and mean (± SE) condition index, clearance rate, and glycogen concentration in mussels collected in 1993 from bedrock, oiled mussel beds, and a reference bed in Prince William Sound. Bedrock samples were not collected from the reference beach.

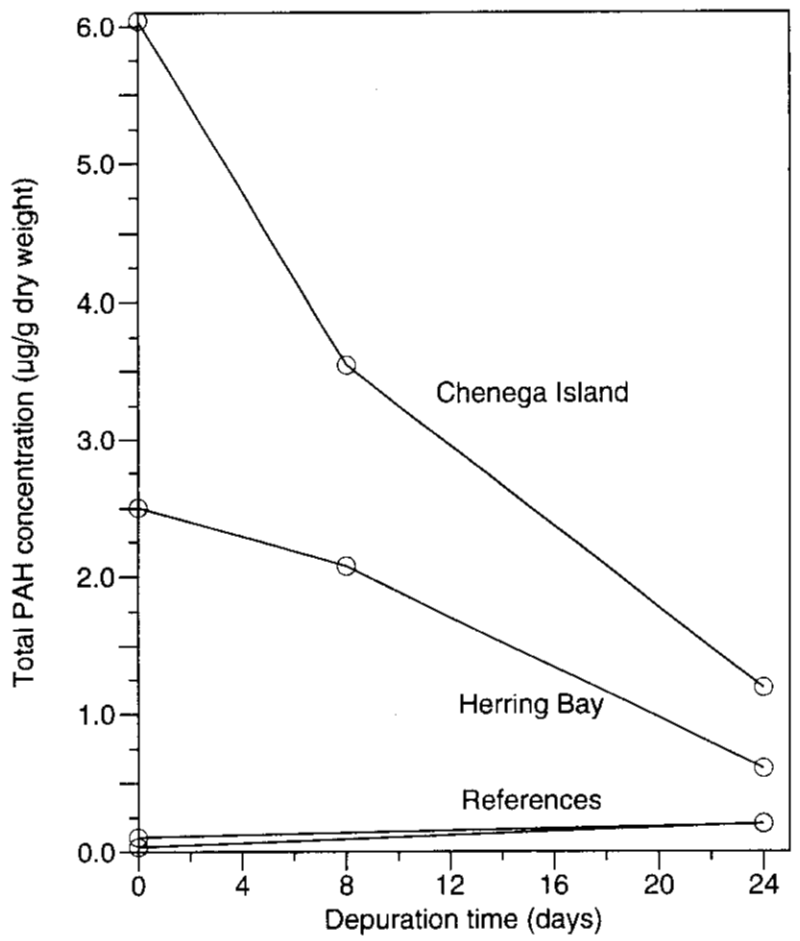

Figure 5.5. Total polynuclear aromatic hydrocarbon (PAH) concentration as a function of depuration time for mussels collected from two oiled sites (Chenega Island and Herring Bay) and two reference sites (Barnes Cove and Olsen Bay).

1992 Inter-bed Comparisons

In the inter-bed tests of 1992 (Fig. 5.2), mussel byssal thread production did not correlate with TPAH ($r^2 = 0.04$). The highest production rates (18 and 16 threads/mussel/48 h) were in mussels from non-oiled reference beaches in Olsen Bay and Barnes Cove. These mussels were among those with the lowest tissue hydrocarbon concentrations. However, the lowest byssal production rates were from a reference bed on Bligh Island (11 threads/mussel/48 h) and by mussels from one of the oiled beds on Latouche Island (9 threads/mussel/48 h). Concentrations of PAH in mussels from the oiled Latouche Island site were lower than in those from any other oiled beds (TPAH, 0.2 vs. 0.46 - 6.0 μg/g), scarcely above concentrations in control beds (0.04 - 0.11μg/g). Mussels with the highest tissue hydrocarbon concentrations produced byssal threads at an intermediate rate.

The condition index of inter-bed mussels in 1992 was not correlated to TPAH concentrations ($r^2 = 0.00$) (Fig. 5.2). The highest condition indices, 10 and 9.9, were from Barnes Cove reference site and the oiled Bay of Isles site, respectively. No relationship or trend was evident with respect to TPAH and condition index.

1992 Intra-site Comparisons

There were no significant trends in either byssal thread production (Chenega Island, $r^2 = 0.07$; Herring Bay, $r^2 = 0.01$) or condition index (Chenega Island, $r^2 = 0.18$; Herring Bay, $r^2 = 0.03$) relative to TPAH concentrations (Fig. 5.3). Variability in byssal thread production was extremely high, even for individual mussels. Production rate in individual mussels ranged from 0-32 threads per 48 h recording period. There was no correlation between condition index and TPAH for combined 1992 data ($r^2 = 0.00$).

1993 Intra-site Comparisons

Physiological responses and TPAH concentration were also not correlated in 1993 (Figure 5.4). Condition index ($r^2 = 0.07$), clearance rate ($r^2 = 0.00$), and glycogen concentration ($r^2 = 0.00$) did not correlate with TPAH. Clearance rates were higher and glycogen content was lower in the less-oiled mussels at Chenega Island, but these relationships were reversed at Foul Bay.

DISCUSSION

Mussels collected from oiled sites had significant PAH concentrations in their tissues, but impacts on their physiology were not significant, and no trends were evident. This was surprising because these mussels were subjected to chronic pollution from underlying oil-contaminated sediments for 3 to 4 years. It did not matter whether oil-exposed mussels were compared to references from separate sites, or were compared to reference mussels from bedrock substrates at the oiled sites. Physiological response was simply not related to oil exposure.

The lack of physiological impacts in the oiled mussels was unexpected because many studies have shown correlations exist between tissue accumulation of pollutants and physiological response (Linden 1977; Carr and Reish 1978; Stekoll et al. 1980; Widdows et al. 1981, 1987, 1996; Stickle et al. 1985). However, most of these studies have involved acute, or relatively short-term exposure to pollutants. This is the first study to seek a relationship between accumulation of PAH and physiological response in mussel populations exposed for 3 to 4 years in their natural environment to contamination from a catastrophic oil spill. No prior study monitored physiological responses of mussels exposed in their natural environment to the consequences of several years of chronic xenobiotic contamination.

The PAH concentrations should have been sufficient to cause physiological impacts. The highest measured concentrations in mussel tissue in this study were 6 μg/g dry weight. The PAH was composed of naphthalene (two rings) to larger, multi-ringed compounds through the chrysenes, and the composition was typical of the distribution of aromatic hydrocarbons from *Exxon Valdez* crude oil in biological tissue (Short and Babcock 1996). These multi-ring and alkyl-substituted PAH are more toxic than mono- and di-aromatic compounds (Rice et al. 1977; Black et al. 1983). Recent studies determined that weathered *Exxon Valdez* oil was more toxic to developing Pacific herring ($\overline{w} = 1.2$) and pink salmon embryos ($w > 4.9$) than the unweathered oil because of the persistence of the more toxic alkyl-substituted and multi-ring PAH in the environment (Carls et al. in press; Heintz et al. in press). Biologically effective concentrations in these studies, 0.4 to 1.0 ppb (ng/g) [or 0.0004 to 0.001 ug/g], were roughly 10^4 times smaller than the highest ineffective concentrations observed in this study. Furthermore, mussel exposures extended 3 to 4 years, possibly the entire lifetime of some animals. The oil in underlying sediments ranged from unweathered to highly weathered; $0 \leq w \leq 8.6$ with a mean of 3.1. The armoring of soft sediments by the mussels trapped oil for several years (Babcock et al. 1996; Harris et al. 1996). This trapped oil was obviously bio-available; TPAH concentrations in mussel tissue were greater than in mussels from unoiled areas, and typically were about 1% of the concentrations in sediment (Harris et al. 1996). For many species, the concentration and the length of exposure would have been lethal; for mussels, it was apparently not toxic enough to measurably affect the physiological variables examined.

It is not readily evident why short-term exposures to oil, as reported in the literature, have induced physiological responses in mussels, whereas in this study, exposures of 3 to 4 years had no discernable effect on the physiological responses monitored. Ecological differences between the sites at which mussels were collected in PWS might explain the lack of differences in physiological response. Possibly local environmental factors such as temperature, water currents, and food availability could obscure physiological differences in the inter-bed tests, but environmental factors are expected to play only a minor role in the intra-bed studies.

Factors not controlled in this experiment were likely important, but we can only speculate about what these factors were, and how influential they were in our experiment. Gonad development and spawning are expected to have significant physiological and energetic impacts on individual mussels. Mussels in PWS have a protracted spawning period, from late February into August (Keiser 1978). Virtually all of the mussels in these tests were spawned out, but they had undoubtedly spawned over a wide time range, some recently, and some much earlier. Different times of peak food availability and of peak spawning activity produce different

schedules of glycogen accumulation and loss in mussels from different areas (Lowe et al. 1982; Emmett et al. 1987). Although all the tested mussels were from PWS, differing ecological factors between mussel beds could have influenced average spawning time and food availability. This may explain why oil exposure did not impact glycogen content and condition index in mussels between sites; however, reproductive condition and food availability cannot completely explain lack of correlation with oil exposure and response within sites.

Rates of byssal thread production and of filtration should have been less directly affected by reproductive state and availability of food than condition index and glycogen storage, although all are affected by general health. A variety of complicating factors may be involved. For instance, Glaus (1968), Allen et al. (1976), and Lee et al. (1989) indicate that mussels produce more byssal threads in stronger water flows. Although all the PWS byssal thread production tests were conducted under constant laboratory conditions, it is possible the environment from which the mussels were removed could have a lasting effect on byssal thread production. Animals accustomed to battering wave action might respond differently in the laboratory than those accustomed to calmer, more protected waters. Even so, this argument does not explain the results from the intra-bed test, where wave action was similar for both groups of mussels.

Tolerance to PAH concentrations in tissue may best explain the lack of physiological effects in these mussels, but two other studies suggest that long-term exposure does have adverse consequences. When animals are first exposed to a stressful environment, their physiological response will typically overshoot and then adjust to a new steady-state level (Prosser 1958). The animals used in this study were exposed for 3 to 4 years, perhaps their entire life span, to relatively high concentrations of toxic PAH. Even with this chronic exposure and a significant PAH burden in their tissue, these mussels survived. Toxic hydrocarbons were found in body tissues, but these mussels had physiologically accommodated to these conditions during the years of exposure. These populations may have a resistance or tolerance, either innate or acquired, to elevated tissue PAH. However, in a histological investigation of mussels from PWS in 1993, Morado et al. (Chapter 6) concluded that the tissues of these animals were still affected by residual oil 4 years after the spill, and a subsequent study found that similar long-term exposure to oil reduced the ability of mussels to survive in air (Thomas et al. 1998).

The lack of physiological response after long-term exposure to spilled crude oil casts some doubt on the utility of mussels as biomonitors, but not as chemical monitors. The accumulation of pollutants, PAH in tissue in this instance, permits quantification of contamination of the environment. Determination of physiological or biological response is a measure of animal health or condition, and a measure of stress imposed by the pollutant. Correlation of PAH in mussel tissues with oil content in underlying sediments indicates that mussels are appropriate chemical monitors for oil in the environment. However, lack of any correlation between tissue PAH and physiological response suggests mussels are not suitable as long-term biomonitors, although they have been shown to be sensitive monitors in short-term, relatively acute exposures.

In a subsequent study, PWS mussels collected from EVOS impacted beaches in 1996 (7 years after the spill) were tested for their ability to survive in air. Unlike the mussels used in the

current study, there was a strong correlation between TPAH concentration in tissue and physiological response (Thomas et al. submitted). When exposed to air, mussels with high TPAH concentrations survived significantly less time than references. The added stress (air exposure) to the stress of PAH in tissues resulted in a difference in survival times in oil-exposed and reference mussels. While more work is required if mussels are to be used as biomonitors of chronic pollution, it is obvious that care must be taken in the selection of physiological responses to be observed.

In summary, mussels in PWS accumulated significant PAH concentrations from oil trapped in underlying sediments. These mussels were exposed to petroleum hydrocarbons for 3 to 4 years, possibly for their entire life. Yet their physiology, measured by byssal thread production, condition index, feeding rate, and glycogen content, was not impacted. Mussels exposed long-term to oil appear to develop a physiological tolerance to PAH, at least with respect to the measured parameters. We recognize, however, that these particular measures may not have been sensitive enough to discriminate oil-related responses from background noise.

CONCLUSIONS

Mussel tissues accumulated PAH from oil-contaminated soft sediment underlying beds contaminated by the *Exxon Valdez* oil spill.

The physiological response of mussels, measured by byssal thread production, condition index, feeding rate, and glycogen content, was not affected by exposure to oil.

Lack of physiological response to oil could not be explained by postulating that natural variability between sample sites masked response, because differences were also absent within sites where uncontaminated and contaminated mussels coexisted in close proximity.

Mussels exposed long-term to residual *Exxon Valdez* oil may have developed a physiological tolerance to the oil; alternatively, these measures were not sensitive to distinguish response to oil from background noise.

Mussels are useful as chemical monitors, but may be less useful as biomonitors. Many investigators have used mussels for both purposes, but because physiological tolerance may be developed, caution should be exercised when using them as long-term biomonitors.

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Chapter 6: Histopathological Observation of Bay Mussels, *Mytilus trossulus*, Exposed to *Exxon Valdez* Crude Oil in Prince William Sound

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ABSTRACT

The condition of mussels *(Mytilus trossulus)* chronically exposed to residual oil from the 1989 *Exxon Valdez* oil spill in Prince William Sound was examined histologically. Oiled mussels were obtained in 1993 from relatively dense mussel beds overlying contaminated sand and gravel and adjacent bedrock at three locations. Additional mussels were collected from one uncontaminated reference location in 1993 and another in 1992. Collection sites were categorized according to polynuclear aromatic hydrocarbon concentration in mussel tissue and site history as reference, low, medium, or high oil. Mussel condition was reduced in oiled beds, as demonstrated by significantly increased prevalence of digestive gland metaplasia and brown cells, decreased abundance of storage cells, and increased abundance of hemocytic infiltrates in gonads. Holocrine activity in kidneys may also have been elevated by oil exposure, but results were only marginally significant. Prevalence of trematode infections was significantly elevated in oiled beds, but prevalences of two other infections, rickettsia-like organisms and ectocommensal ciliates, were significantly reduced in oiled beds. Similar cell and tissue changes have been routinely reported in other molluscs from polluted environments. Despite the limited scope of the study we conclude that mussels in beds overlying oil-contaminated soft sediment were negatively impacted by exposure to oil, and had not fully recovered from the *Exxon Valdez* spill by 1993.

INTRODUCTION

On March, 24, 1989, the supertanker *Exxon Valdez* ran aground on Bligh Reef in Prince William Sound (PWS), Alaska, spilling nearly 42 million liters of Alaska North Slope crude oil. In the weeks that followed, oil from the spill was directed by prevailing wind and surface currents southwest past Knight Island, PWS, and into the Gulf of Alaska. The oil continued southwest in the Gulf of Alaska, contaminating beaches along the Kenai Peninsula, Alaska Peninsula, and the Kodiak Archipelago (Morris and Loughlin 1994; Wolfe et al. 1994). Overall, about 1,750 km of coastline was contaminated by the oil (Wolfe et al. 1994). Industry-supported shoreline cleanup efforts were launched in 1989 and completed in 1990.

Most oiled mussel beds were not intensively cleaned in 1989 and 1990 to avoid destruction of these ecologically important organisms. Although expectation was that oil would

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be removed by natural weathering processes, concentrations of oil trapped under some mussel beds overlying soft sediments remained high, and little weathering occurred. For example, in contaminated mussel beds in 1992, mean total polynuclear aromatic hydrocarbon (TPAH) concentrations ranged up to 523 μg/g dry weight in sediments, and 8.1 μg/g dry weight in mussels (Babcock et al. in prep.). These persistently high concentrations, and the importance of mussels as food and habitat structure for many other species, conferred disproportionately high importance to oiled mussel beds with respect to their relatively small size and number.

Like other crude oils, Alaska North Slope crude oil is composed of a wide variety of hydrocarbons possessing marked ranges in volatility (Clark and Brown 1977). Because of this variance in composition and properties, the effects of crude oil on exposed organisms can vary considerably (Rice et al. 1977). Not only do acute and chronic effects vary because of the life histories of exposed organisms, but the various components of oil may interact antagonistically, additively, or synergistically to produce a wide range of effects in exposed organisms (Sparks 1985).

This limited study was initiated to compare the condition of mussels *(Mytilus trossulus)* chronically exposed to residual oil from the *Exxon Valdez* oil spill in Prince William Sound with the condition of control mussels from non-oiled locations. Oiled mussels were collected from relatively dense mussel beds overlying unconsolidated sand, gravel, and bedrock for histological and hydrocarbon analyses. Normal and pathological features were recorded to compare the general condition of mussels from oiled and non-oiled sites. Although we encountered a large number of cell and tissue changes, only the predominant observations are described and their prevalences presented. They include ctenidia epithelium hypertrophy, digestive gland metaplasia, changes in abundance of brown and storage cells, the presence of hemocytic infiltrates in gonads, holocrine activity and cytoplasmic bleeding of kidney epithelium, rickettsia-like infections, ciliate infection and infestation, and trematode infection. To relate mussel condition to oil exposure, collection sites were categorized into four oil-contamination groups ranging from reference to high oil, based on hydrocarbon concentration in mussel tissue, substrate type, and site history.

METHODS

At least 30 mussels were randomly collected from the sediments and adjacent bedrock at three oiled sites and the sediment at one reference location in Prince William Sound (Figure 6.1) during low tide, June 16 to 21, 1993. Mussels from Barnes Cove, another reference site, were collected on June 16, 1992. The mussels (25-40 mm shell length) from each site were placed in labeled bags and transported to a processing station where they were shucked and placed in polypropylene jars containing 10% formalin buffered with sodium acetate. At the same time, additional triplicate mussel samples were collected and frozen in hydrocarbon-free glass containers for chemical analysis. All samples were transported to the Auke Bay Laboratory in Juneau, Alaska.

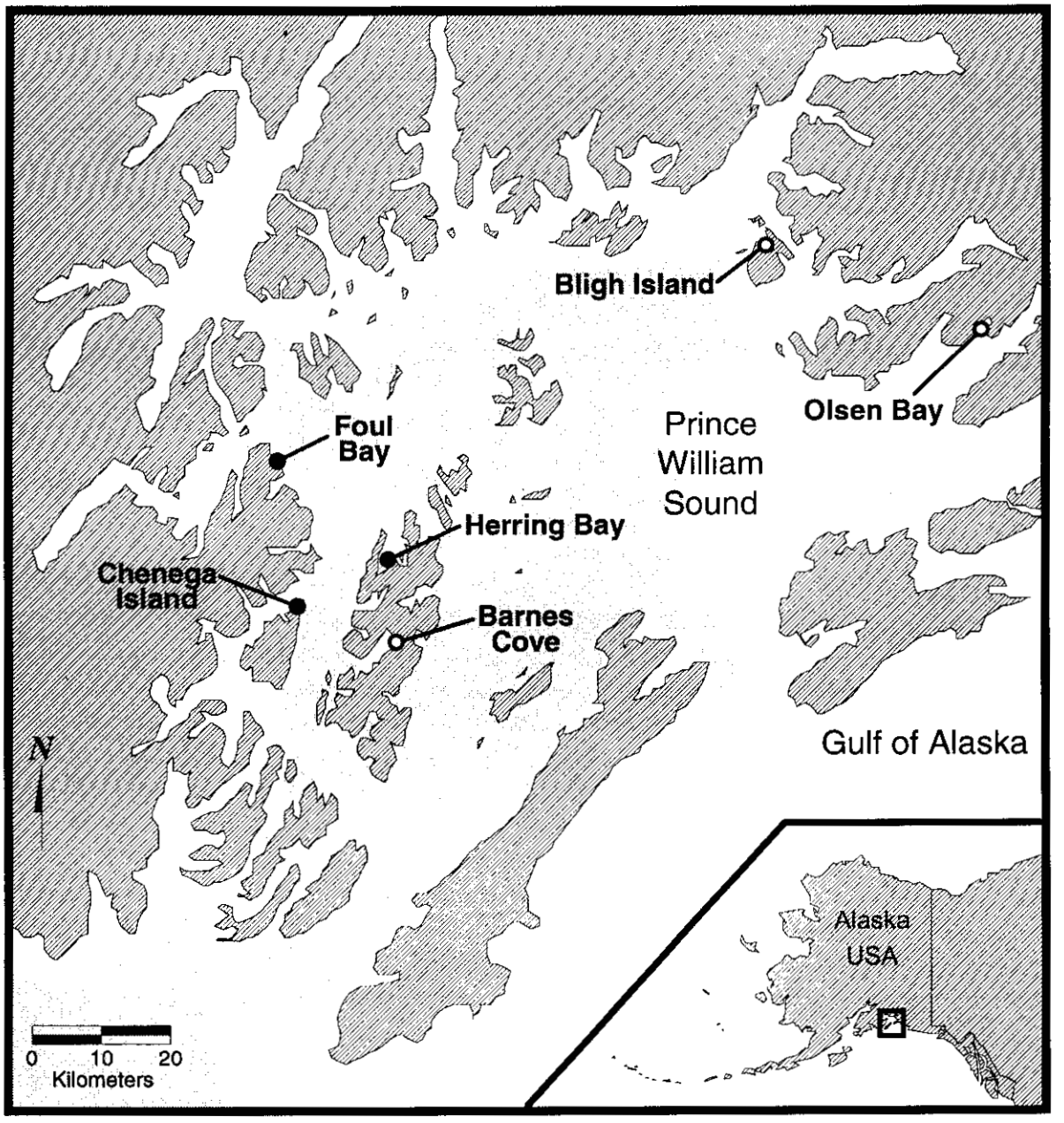

Figure 6.1. Location of mussel collections from oiled (Chenega Island, Herring Bay, and Foul Bay) and reference (Olsen Bay and Barnes Cove) mussel beds, Prince William Sound, Alaska.

Mussel samples were analyzed for petroleum hydrocarbons by gas chromatography/mass spectrometry as described by Short et al. (1996). Mussels sampled contemporaneously with histopathological samples and those collected one year earlier at each site were included in the hydrocarbon analysis. The five geographic sample locations were subdivided into eight sites (e.g., there were two sites at Herring Bay - bedrock and mussel bed). Including the reference group, sites were partitioned into four oil-contamination levels based on hydrocarbon concentration and site history (Table 6.1). Mean TPAH concentration is reported as µg/g dry tissue weight, excluding perylene which is produced by biogenic sources.

Table 6.1. Mean concentration of total polynuclear aromatic hydrocarbons (TPAH µg/g dry weight) in mussels collected from sediment and bedrock contaminated by the *Exxon Valdez* oil spill and reference beds. Segment number refers to the code assigned to a shoreline section within the oil-impacted area by the interagency Shoreline Cleanup Assessment Team (Babcock et al. 1996; Babcock and Short 1996). Collection sites were categorized into four oil-contamination groups ranging from reference to high oil, based on hydrocarbon concentration in mussel tissue, substrate type, and site history. However, only in high-oil samples were TPAH concentrations significantly greater than in reference samples: $^{***}P \leq 0.001$, $^{**}P \leq 0.01$.

		Bed /	Mussels		
Location	Segment #	Bedrock	N	Mean ± SE	(Range)
High oil					
Foul Bay	MA002C	Bed	7	5.66*** ± 1.12	(0.84 - 9.91)
Herring Bay	KN133A-1	Bed	24	2.37*** ± 0.36	(0.43 - 7.06)
Chenega Island	CH010B-2	Bed	31	2.04** ± 0.34	(0.05 - 6.04)
Medium oil					
Herring Bay	KN133A-1	Bedrock	6	0.67 ± 0.23	(0.16 - 1.54)
Low oil					
Foul Bay	MA002C	Bedrock	4	0.26 ± 0.14	(0.02 - 0.54)
Chenega Island	CH010B-2	Bedrock	9	0.17 ± 0.06	(0.01 - 0.55)
Reference					
Barnes Cove	KN575A	Bed	5	0.03 ± 0.01	(0.02 - 0.05)
Olsen Bay	None	Bed	5	0.17 ± 0.07	(0.02 - 0.43)

Coded site numbers were placed on each sample jar before forwarding to Seattle, Washington, for histological processing. Upon arrival, the mussels were dehydrated through a graded ethanol series and cleared in xylene substitute. Each individual mussel was then sectioned transversely to ensure eventual inspection of all major organ systems before embedding in paraffin. A random number was then placed on each embedded specimen thereby creating a double-blind study. Tissue sections were cut at 3μm, stained with hematoxylin and eosin (H&E), and read. Giemsa, Brown and Hopp's gram, Mallory's trichrome, periodic acid Schiff, iron hematoxylin, and nuclear feulgen stains were applied to additional sections as needed.

Histopathological observations were recorded for all major organs via an in-house coding system, and subsequently entered into a relational database. Each data record required an entry for organ, tissue, and cell affected, lesion description, etiology, distribution, and severity. After microscopical analysis, the origin of the mussels was revealed by the collectors. This information plus mean TPAH concentration were also entered into the database. A total of 236 mussels were examined (n = 63, 59, 29, and 85 for reference, low-, mid-, and high-oil groups, respectively); seven mussels were lost during processing and were not included in these counts.

The database generated for this study was queried to determine the prevalence, distribution, and severity of remarkable lesions and parasites for the reference and oil-contaminated groups . Because of the small sample size, simple Chi-square (χ^2) analysis was used to test the null hypothesis that lesion/parasite prevalence was independent of oil contamination.

RESULTS AND DISCUSSION

Petroleum Hydrocarbon Exposure

Mean TPAH concentrations in mussels ranged from 0.03 to 5.66 μg/g; concentrations at highly oiled sites were significantly greater than at unoiled sites ($0.001 < P \leq 0.006$) (Table 6.1). Mean TPAH concentration in all high oil sites was $\geq 2.0 \pm 0.3$ μg/g with mussels from Foul Bay sediment having the highest mean TPAH concentration, 5.66 ± 1.12 μg/g. Medium oil contamination was represented by mussels on bedrock in Herring Bay where bed and bedrock sampling sites were unusually close, approximately 0.5 m apart. The unusual proximity of the two sampling sites at Herring Bay likely created a condition in which bedrock mussels could have been exposed to resuspended oiled from the adjacent bed following or during major weather disturbances. Mussels collected from bedrock on Chenega Island and in Foul Bay were more than 5 m from adjacent oiled beds, and were categorized as low oil. Although mean TPAH concentration at the medium and low oil sites did not differ significantly from those at reference sites, the low oil sites were heavily oiled in 1989, and adjacent soft sediments remained contaminated. Because mussels were only sampled twice for hydrocarbons, they possibly could have been exposed periodically by remobilized oil at medium- and low-oil sites without our detection. In contrast, reference sites were never oiled (Babcock and Short 1996; Babcock et al. 1996; Short and Babcock 1996).

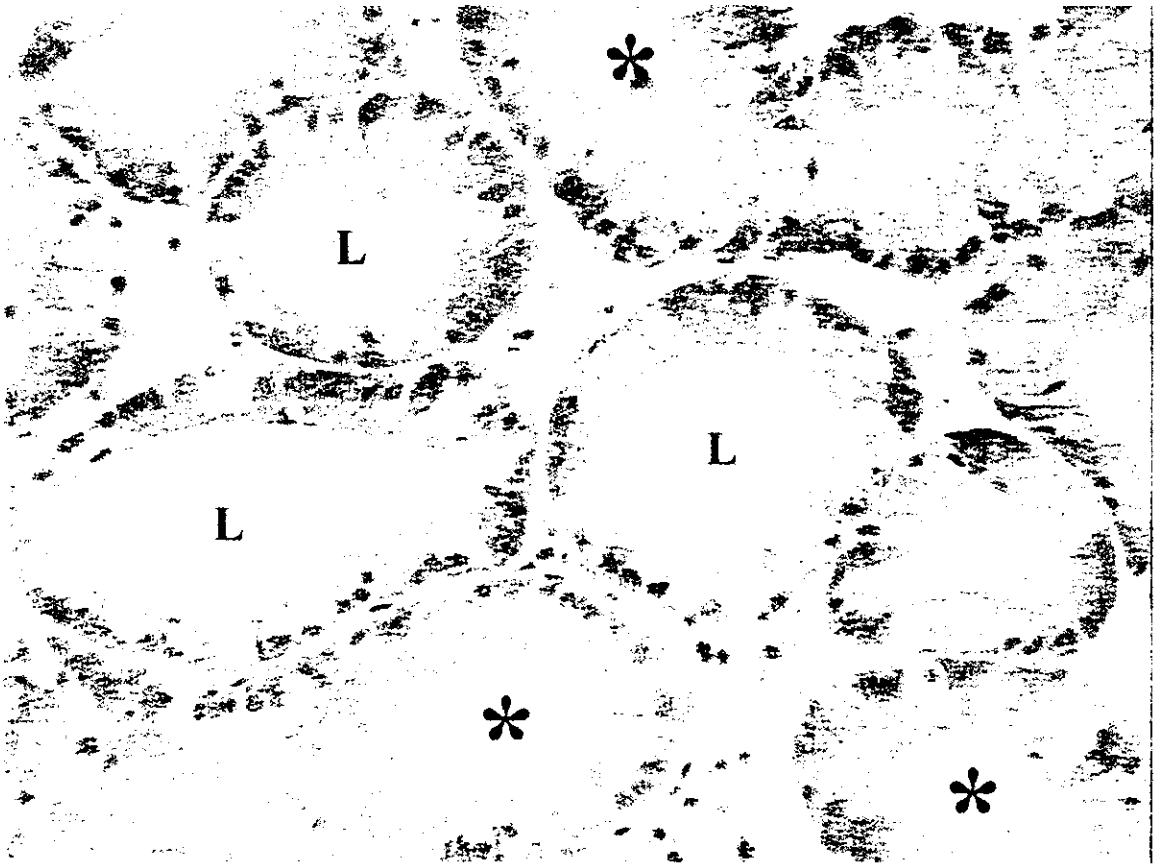

Figure 6.2. Metaplasia of digestive gland epithelium. L - lumina of digestive gland tubules showing metaplasia; * - digestive gland tubules showing normal epithelia.

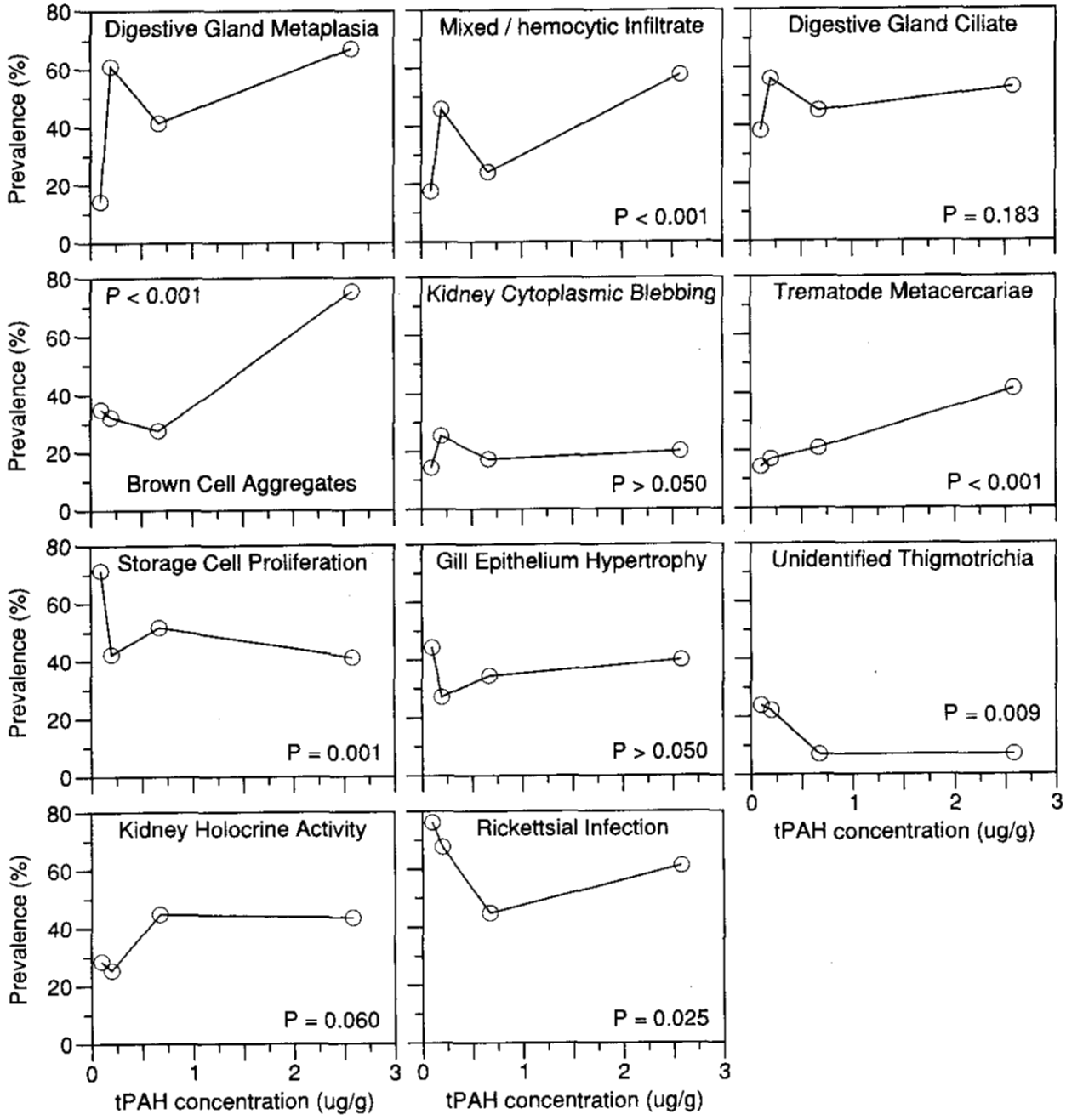

Figure 6.3. Lesion/anomaly prevalences in mussels (*Mytilus trossulus*) as functions of total polynuclear aromatic hydrocarbon concentration (TPAH) in tissue. Prevalence is the percentage of mussels with each lesion or infection. The level of significance (*P*) for each lesion/anomaly is based on the Chi-square test.

Lesions, Prevalences, and Trends

Digestive gland metaplasia (Fig. 6.2) is a reversible change of one mature cell type to another. The change may be small or extreme, but the epithelium will revert to its normal state once the stimulus is removed. Digestive gland epithelium is composed of three cell types (basophilic, holocrine, and embryonic cells) that can vary in height from columnar to low cuboidal during a normal diurnal period (Langton 1975), in response to starvation (Thompson et al. 1974), or upon exposure to an environmental stressor (Sunila 1986, 1987; Lowe 1988) or parasitism (Sparks 1962). The implication of digestive gland metaplasia is that although it is a normal diurnal response in bivalves as well as many other molluscs, it is also a general response to stress. If the stressor is not removed, the observed cell change could proceed to a more severe but still reversible form (columnar to squamous cell metaplasia) or ultimately to an irreversible change, such as necrosis. Because the digestive gland is the primary organ for nutrient storage and metabolism, marked changes in organ structure and function could reflect the overall condition of affected mussels.

Prevalence of digestive gland metaplasia (Figure 6.3) was elevated by oil contamination ($P < 0.001$). Digestive gland metaplasia was 2.5 to 4 times more prevalent in mussels from oiled sites than in the reference group, but this condition was observed in all mussel groups (Figure 6.2). Because all mussels were collected at low tide when mussels were not feeding, the data suggest that mussels from oiled sites were exposed to an additional environmental stressor that was not presented to reference animals.

Brown or serous cells have been reported from numerous bivalves and are considered a third type of hemocyte of separate cell lineage (Cheng 1981). The functions of brown cells are essentially unknown, but their increased presence has been associated with increased excretory activity (White 1942) but especially parasitism (Mackin 1951; Stein and Mackin 1955; Cheng and Burton 1965, 1966). Studies have shown the brown cell abundance increases with increased levels of internal parasitism. They apparently possess phagocytic properties (Takatsuki 1934) and are frequently observed in diapedesis (Morado et al. 1984).

Brown cell prevalence was elevated by oil contamination (Figure 6.3, $P < 0.001$). Approximately three times as many mussels from highly oiled sites were observed with brown cells than in reference, low-, and medium-oiled sites. Prevalences of brown cell aggregates were not markedly different in mussels from reference, low-, and medium-oiled sites. Brown cell aggregates were observed in the connective tissues of all organs, especially the ctenidia (Figure 6.4) and the digestive tract epithelia. Because internal parasites were not abundant in any study group, the marked increase in brown cell prevalence in highly oiled mussels likely was caused by hydrocarbon exposure.

Many organisms possess the ability to store nutrients within well-characterized storage cells. *M. edulis* is known to possess two types of storage cells (Lowe et al. 1982), and *M. trossulus* is probably equally capable. Nutrient storage cells of mussels are eosinophilic and occur throughout connective tissues of the mussel. Their purpose is to provide an extra source

Figure 6.4. Brown cell aggregates within the hemal spaces (BC) and epithelia (*) of the ctenidia.

of nutrients for gamete production (Lowe and Pipe 1987) or, as in crustaceans, during extended periods of starvation.

Storage cell (Figure 6.3) abundance was reduced by oil contamination ($P = 0.001$). A smaller percentage of mussels possessed storage cells at the three oiled sites than at reference sites (Figures 6.5 and 6.6). Storage cell prevalence did not differ significantly among the three oiled sites. These data suggest that mussels from reference sites were in better condition than mussels from the oiled sites, regardless of the level of oil contamination.

Holocrine excretion is the most vigorous of three types of excretion identified in bivalves. The kidneys of bivalves are simple folded tubules which possess extensive epithelial infolds that produce crypts or pockets. The epithelium is simple, columnar, with a microvillus apical border. Mussels are essentially osmoconformers, although their kidneys are able to regulate ion concentrations in the formed urine. Indeed, mussel kidneys are often the site of heavy metal sequestration and excretion (Coombs and George 1978; Robinson and Ryan 1986). Holocrine excretion is characterized by the loss of the supranuclear portion of the cell. In extreme instances, the cell may be lost altogether, leaving behind a naked basement membrane.

Prevalence of kidney holocrine activity in medium and highly oiled mussels was almost twice that in reference and low-oil groups, but the difference was marginally significant (Figure 6.3, $P = 0.06$). The levels of kidney holocrine activity were similar for the reference and low-oiled groups, and for the medium-oiled and highly oiled sites. The cause of increased activity in the medium and high groups is unknown, but may be related to the generally poorer condition of the mussels as indicated by increased prevalence of brown cells and digestive gland metaplasia.

Mixed hemocytic infiltrates are generalized host responses that can be observed in all mussel organs and tissues. In this study, most hemocytic infiltrates were associated with gonad resorption, which is a common post-spawning event. During this process of detritus removal, large numbers of hemocytes can be observed both within the ovarian follicle and testicular lobe, although infiltrates tend to be more remarkable in males than females.

Hemocytic infiltrates were more prevalent in oiled mussels than in reference mussels (Figure 6.3, $P < 0.001$). The prevalence of hemocytic infiltrates in the ovary and testes was not significantly different although the most remarkable microscopical observations were more frequent in testes. Infiltrates of the testes were marked by large numbers of actively phagocytosing hemocytes within the testicular lobe and the frequent occurrence of giant cells (Figure 6.7). The importance of hemocytic infiltrates within the gonads is uncertain; it is possible that because of the general poorer condition of oiled mussels, spawning was incomplete or may have occurred earlier in the season than in reference mussels.

There were no remarkable changes in kidney epithelium cytoplasmic blebbing or gill epithelial hypertrophy with either reduced or increased oil contamination levels ($P > 0.05$). The values for each of these observations varied considerably for each site.

Figure 6.5. Storage cells (SC) occupy much of the interstitial connective tissue space in well-conditioned mussels. TE - testes.

Figure 6.6. The interstitial connective tissue of stressed mussels is depleted of storage cells. TE - testes; HEP - hepatopancreas.

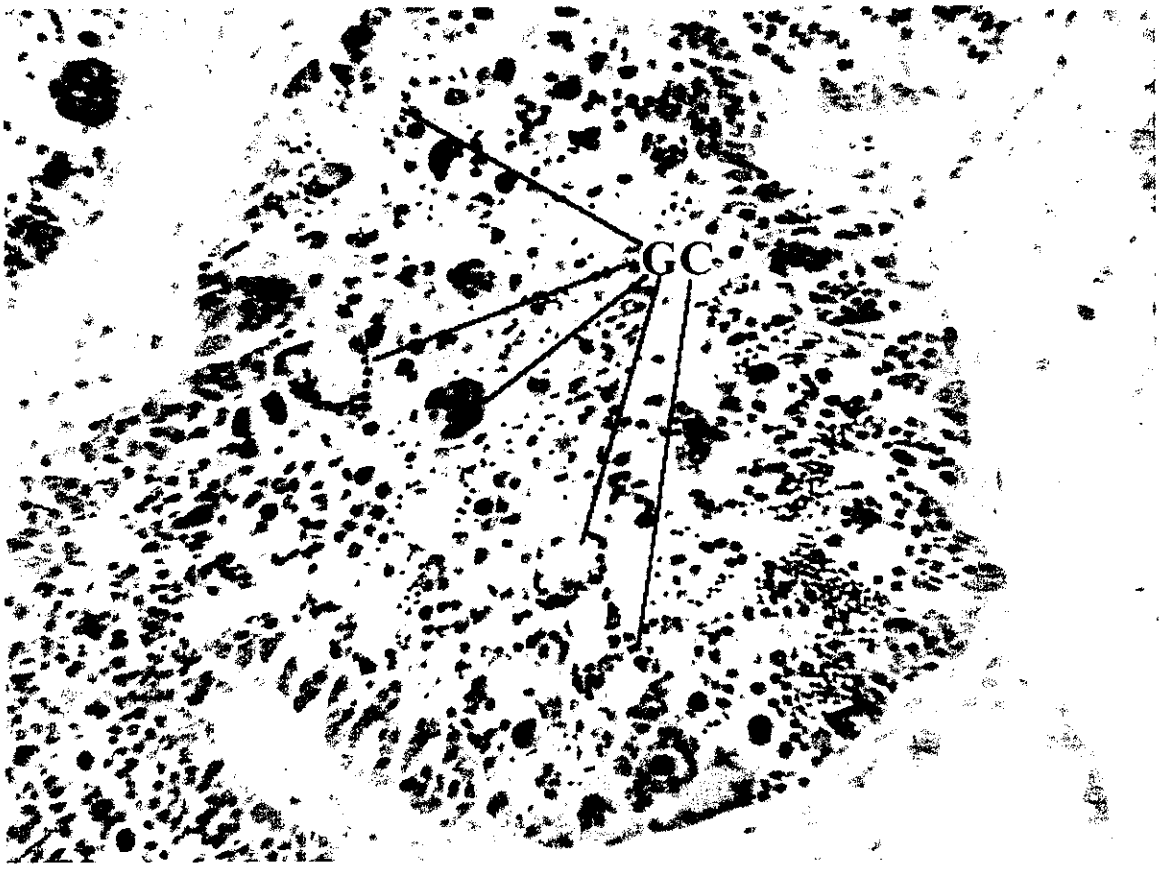

Figure 6.7. An invasive inflammatory reaction of the testes. Note the presence of giant cells (GC).

A cause and effect relationship between lesion prevalence and hydrocarbon exposure of bivalves in the field and laboratory has been suggested by several studies. In the field, reported anomalies include necrosis and vacuolization of the digestive diverticula of *Mya truncata* and *M. calcarea* respectively (Neff et al. 1987), and atrophy of the basophilic cells of the digestive diverticula of *Mytilus trossolus* (Lowe and Clarke 1989). In the field, various tissue alterations were reported following the 1978 *Amoco Cadiz* tanker oil spill; they include digestive diverticula and gonadal atrophy in two species of oysters and sarcomas in cockles (Neff and Haensly 1982; Auffret and Poder 1986; Berthou et al. 1987). Accumulations of lipid and lysosomal granules were reported in digestive diverticula of mussels (*Mytilus edulis*) and cockles (*Cerastoderma edule*) (Wolfe et al. 1981). Atrophy of the gonad epithelium of oysters and lost reproductive capability occurred two years after the spill, and total destruction of the digestive diverticulum was noted seven years post spill (Berthou et al. 1987).

Parasite Prevalences and Trends

Rickettsia and rickettsia-like organisms are obligate intracellular parasites that have been reported in many species of molluscs, as well as other invertebrates (Sparks 1985) and vertebrates. They typically reside within the cytoplasm, but some are known to infect the cell nucleus (Elston 1986). Occasionally, they have been strongly associated with mortality in molluscs (Gulka et al. 1983; Sparks 1985; Bower et al. 1994). *Rickettsia* possesses a short reproductive cycle in which a dissemination or infective stage must exist in the environment for a variable amount of time. Currently, the relationship between viability of the rickettsial infective stage and the environment is unknown and requires further study.

Infections of rickettsia-like organisms were more prevalent in reference mussels than in oiled mussels, and depended on oil contamination (Figure 6.3, $P = 0.025$). Rickettsial organisms in *Mytilus trossulus* infected the columnar epithelium of the ctenidia, causing marked hypertrophy (Figure 6.8). Cell lysis was not observed, and infections were not of sufficient severity to cause marked impairment of ctenidia function.

A review of the literature indicates that ciliate infections of bivalve mussels, especially those of the digestive gland, are rare; external infestations are more common. Entry of the parasitic ciliate into infected mussels likely occurred via the digestive tract, but some parasitic ciliates have demonstrated an ability to penetrate host tissues. The present parasitic ciliate was typically observed within holocrine cells of the digestive gland and occurred singly within an infected cell (Figure 6.9). Even in moderate infections, little or no host cell changes were observed. Thus the effects of the parasitic ciliate on mussel health are uncertain. The general morphology of the ciliate suggested that it was a scuticociliate, but due to the nature of this study, few details of the ciliate were discernable. As a result, the parasitic ciliate can not be identified with certainty. The unidentified parasitic ciliate frequently infected the digestive gland of mussels from all sites, thus, prevalence was not significantly related to oil ($P = 0.183$).

Ectocommensal ciliates are common epibionts of aquatic surfaces. Most are nonpathogenic, but some are capable of producing disease and even mortality (Morado and

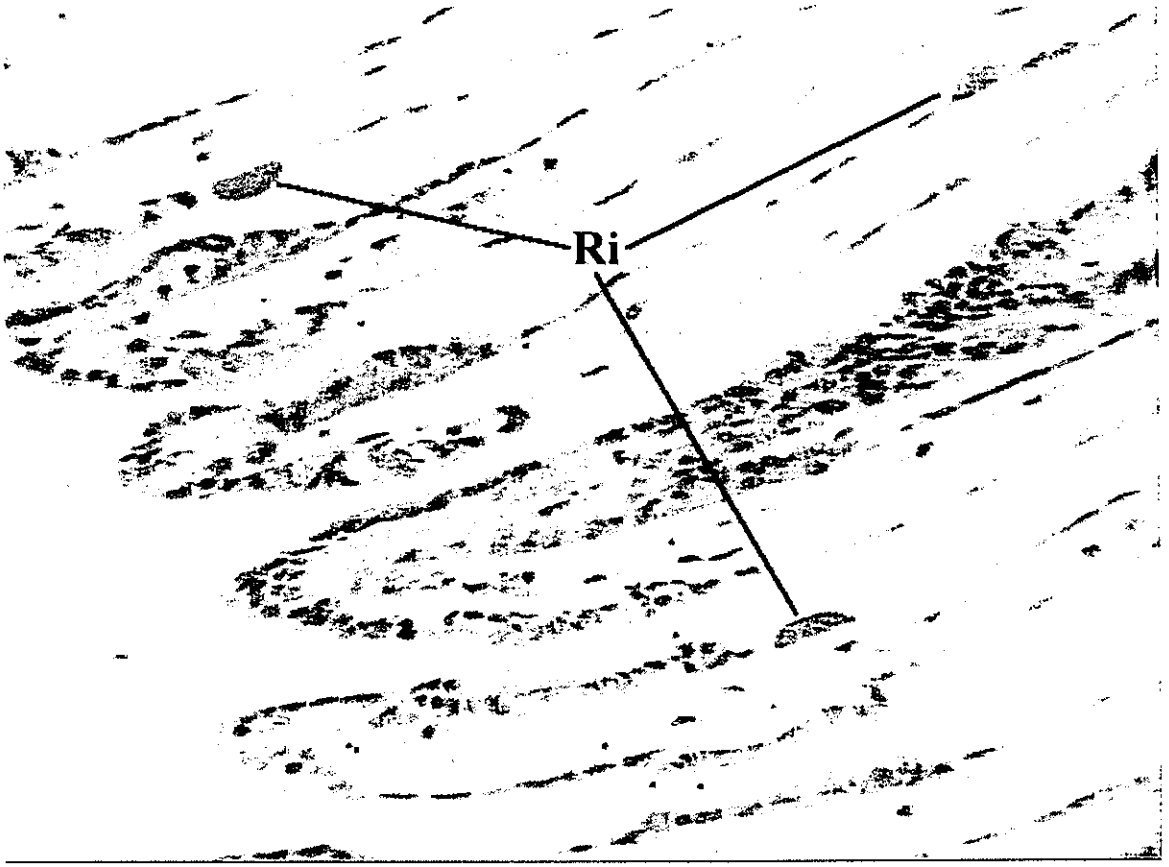

Figure 6.8. Hypertrophied squamous cells of the ctenidia infected by a rickettsia-like (Ri) organism.

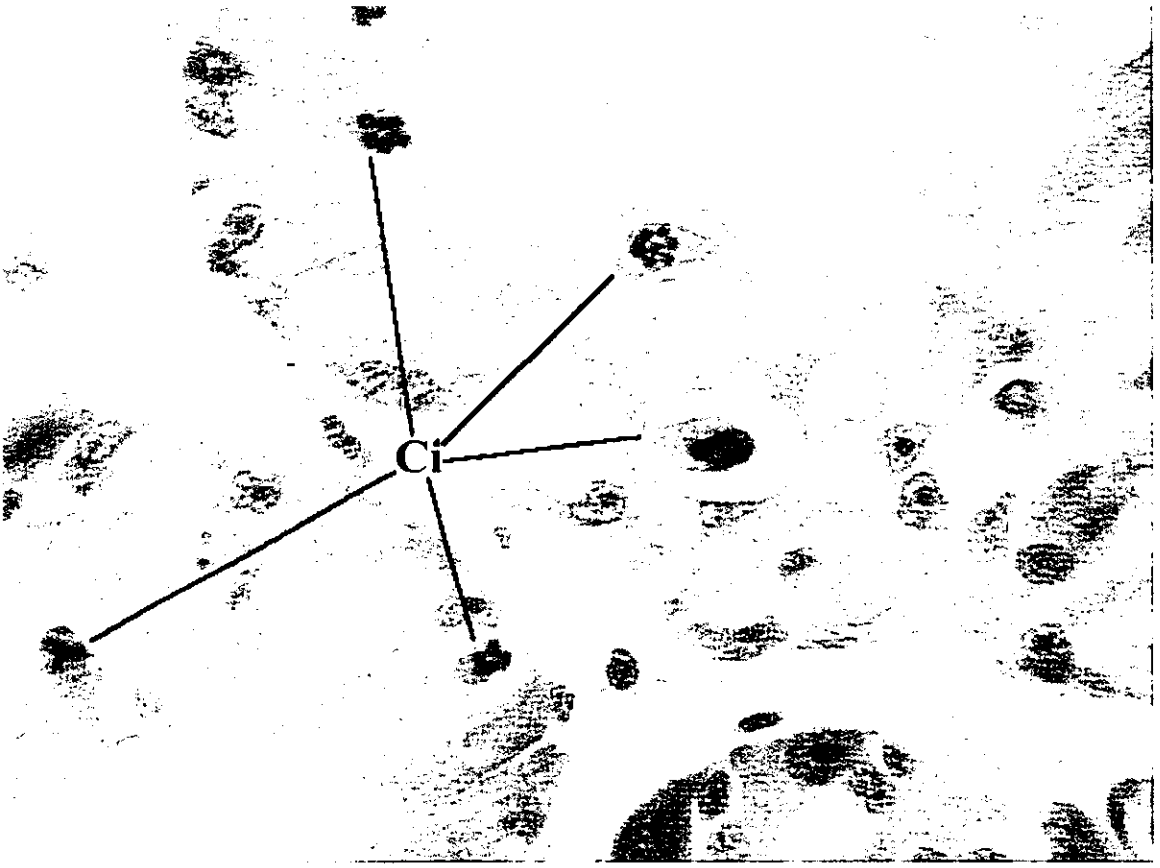

Figure 6.9. An unidentified parasitic ciliate (Ci) in the epithelium of the digestive gland.

Small 1995). Molluscs are hosts to a large variety of ciliates, both sessile and motile. Several studies have demonstrated that ciliate abundance and species composition are good indicators of pollution and stress of the host (reviewed by Khan and Thulin 1991). Thigmotrichs are frequent and common epibionts of molluscs, but peritrichs, suctorians, pleurostomes, astomes, and apostomes can be found over the body surface of bivalves. Ciliates have a short reproductive cycle, but environmental effects on thigmotrich life history are not understood. Thus, drawing any inferences about the relationship between ciliate colonization and oil contamination is difficult. Our results, however, do suggest that the conditions at the medium and high oiled sites did not favor thigmotrich ciliate colonization. Indeed, infestation of thigmotrich ciliates[9] and oil contamination were inversely related ($P = 0.009$). Thigmotrichs were generally found on the ctenidia and were more frequent on mussels from reference and low oiled sites than on mussels from medium and high oiled sites (Figure 6.10).

Digenetic trematodes are common parasites of bivalve molluscs which can function as both definitive and intermediate hosts of digenetic trematodes. Both adult and larval digenetic trematodes of different species can be encountered within a given bivalve species. In light infections, little or no effects on the host are typical. However, the fitness of heavily infected hosts can be severely compromised, causing marked failure in reproduction and even mortality.

Prevalence of larval digenetic trematodes were similar for the reference, low, and medium oiled sites, but rose sharply in the highly oiled group (Figures 6.3 and 6.11). Thus, larval trematode prevalence depended on oil contamination ($P < 0.001$). Infections by juvenile trematodes in examined *M. trossulus* were light and typically marked by light to moderate hemocytic infiltrates and encapsulations of varying thickness.

Several previous studies have suggested a link between parasitism and pollution in aquatic organisms (see Kahn and Thulin 1991) while others have indicated that such proposed links are not clear (Möller 1986). For example, parasitic diseases in oysters (*Ostrea edulis*) and larval trematodes in mussels were not related to the *Amoco Cadiz* spill (Berthou et al. 1987; Auffret 1988), but parasitism by ciliates and other protistans increased when mussels or oysters were exposed to pollutants (Barszcz et al. 1978; Yevich and Barszcz 1983; Sunila 1987; Winstead and Couch 1988). Ciliate, nematode, and trematode prevalences were apparently elevated in *M. edulis* exposed to multiple pollutants (Yevich and Barszcz 1983), but trematode and nematode infection intensity appeared to be unrelated to water quality in a study by Sunila (1987). The need clearly exists for further host-parasite research in molluscs as a monitoring tool for pollutant stress.

At least two hypotheses have been proposed to explain the host/parasite relationship in polluted environments. One that is frequently proposed is that pollution has a negative effect on the host's immune system; thus, the host is more susceptible to infection. The other leading opinion is that polluted environments are less favorable to short-lived, but highly sensitive parasite larval infective stages. Both hypotheses deserve consideration, but neither hypothesis is

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[9]Because this study was histological in nature, the ciliates could not be identified to species; however, their morphology was suggestive of thigmotrichs.

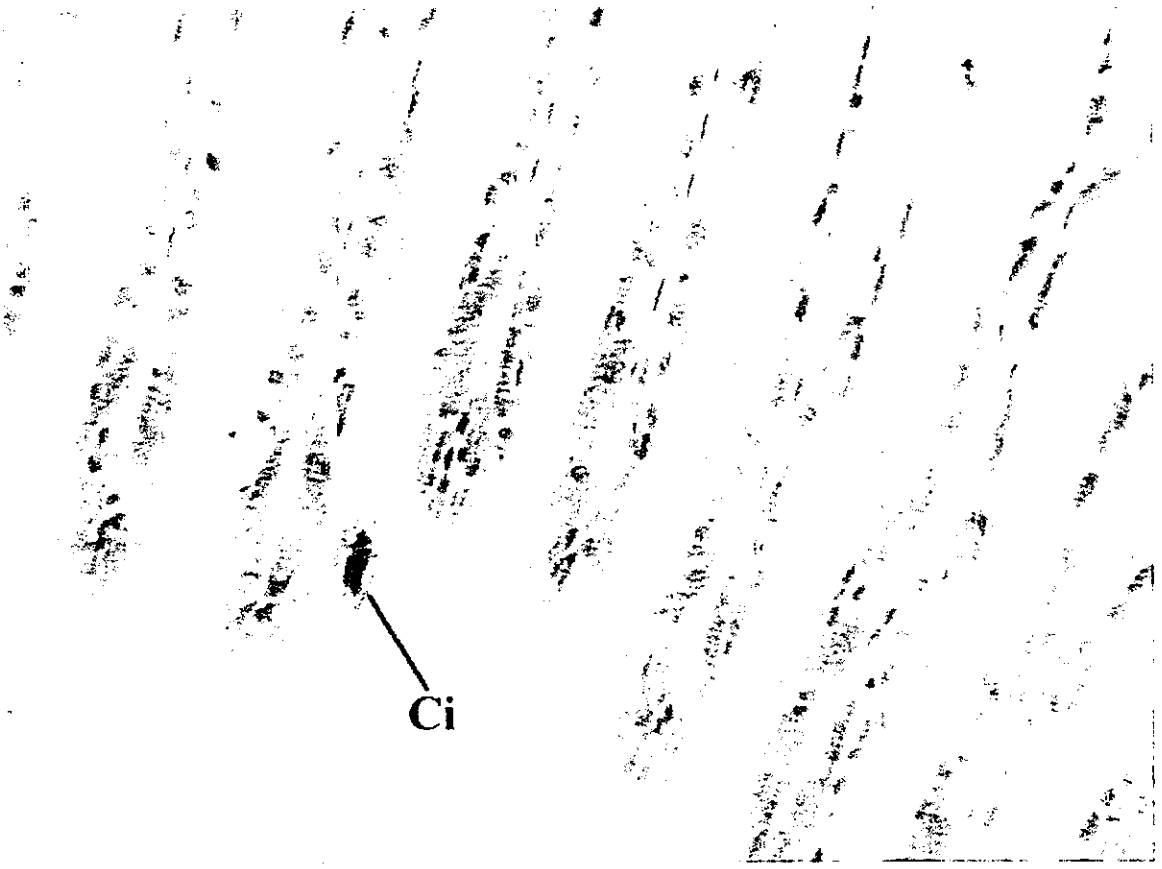

Figure 6.10. A thigmotrich ciliate (Ci) on the surface of the ciliated epithelium of the ctenidia.

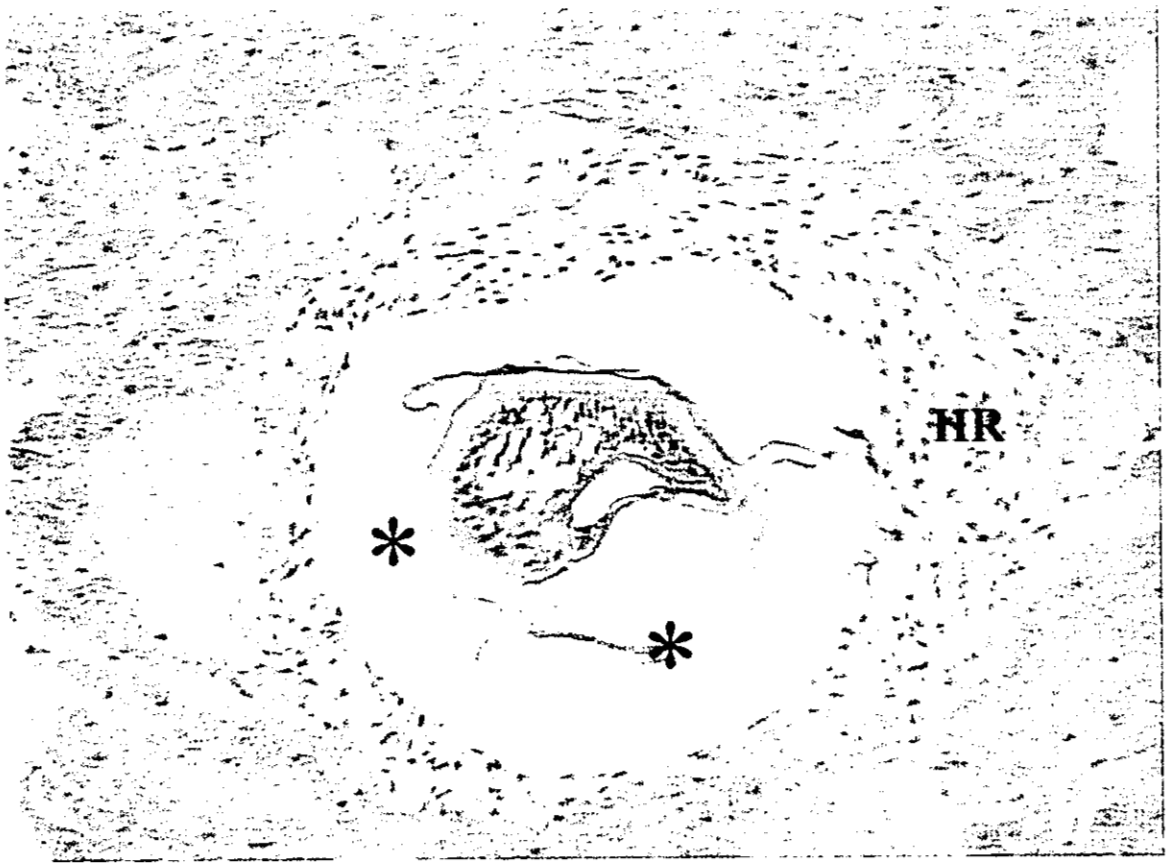

Figure 6.11. Trematode metacercaria in the foot of a mussel; space around parasite shows amount of shrinkage as a result of fixation. Note the host response (HR) that surrounds the parasite. * - cyst of metacercaria.

likely to fully explain the host/parasite/environment relationship because of several reasons: 1) variations in infective stage survival in response to a particular perturbation (physical, chemical or biological); 2) a poor understanding of parasite and host dynamics in the aquatic environment; 3) the environmental impact on host immunocompetency has been examined to a limited extent, but unequivocal data are not available that identify the point at which the host is severely compromised; 4) the dynamic nature of the environment and our poor understanding of its processes; and 5) the spatial and temporal limitations of most field research. Clearly, the dynamics of the host/parasite/environment relationship requires further study.

CONCLUSIONS

Mussel condition was reduced in oiled beds, as demonstrated by significantly increased prevalence of digestive gland metaplasia, increased prevalence of brown cells, decreased abundance of storage cells, and increased hemocytic infiltrates. Holocrine activity in kidneys may also have been elevated by oil exposure, but results were only marginally significant.

Prevalence of trematode infections was significantly elevated in oiled beds, but prevalence of two other infections, rickettsia-like organisms and ectocommensal ciliates, was significantly reduced in oiled beds.

The results of this limited study suggest that mussels in beds overlying oil-contaminated soft sediment in Prince William Sound were negatively impacted by exposure to oil 4 years after the *Exxon Valdez* spill.

Changes in cells and tissues similar to those in our study have been routinely reported in other molluscs from polluted environments (Barszcz et al. 1978; Yevich and Barszcz 1983; Sunila 1987; Winstead and Couch 1988). Indeed, much effort has been directed toward the identification of a highly improbable, single biological indicator for monitoring pollution or stress, the most notable being neoplasia, hemocyte proliferation, hypoplasia, hyperplasia, lipofucsin accumulation, or metaplasia. The objective of this study was to determine the general condition of the target species by recording both the prevalence and distribution of normal and abnormal conditions. We believed that a pluralistic approach would permit a better assessment of mussel "fitness" even though the study was limited in scope.

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APPENDICES

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Appendix 1.1. Locations of mussel beds sampled along the Gulf of Alaska. Segment# is the *Exxon Valdez* Interagency Shoreline Cleanup Committee segment number plus a unique suffix. Latitude and longitude are reported in degrees (deg), minutes (min), and seconds (sec).

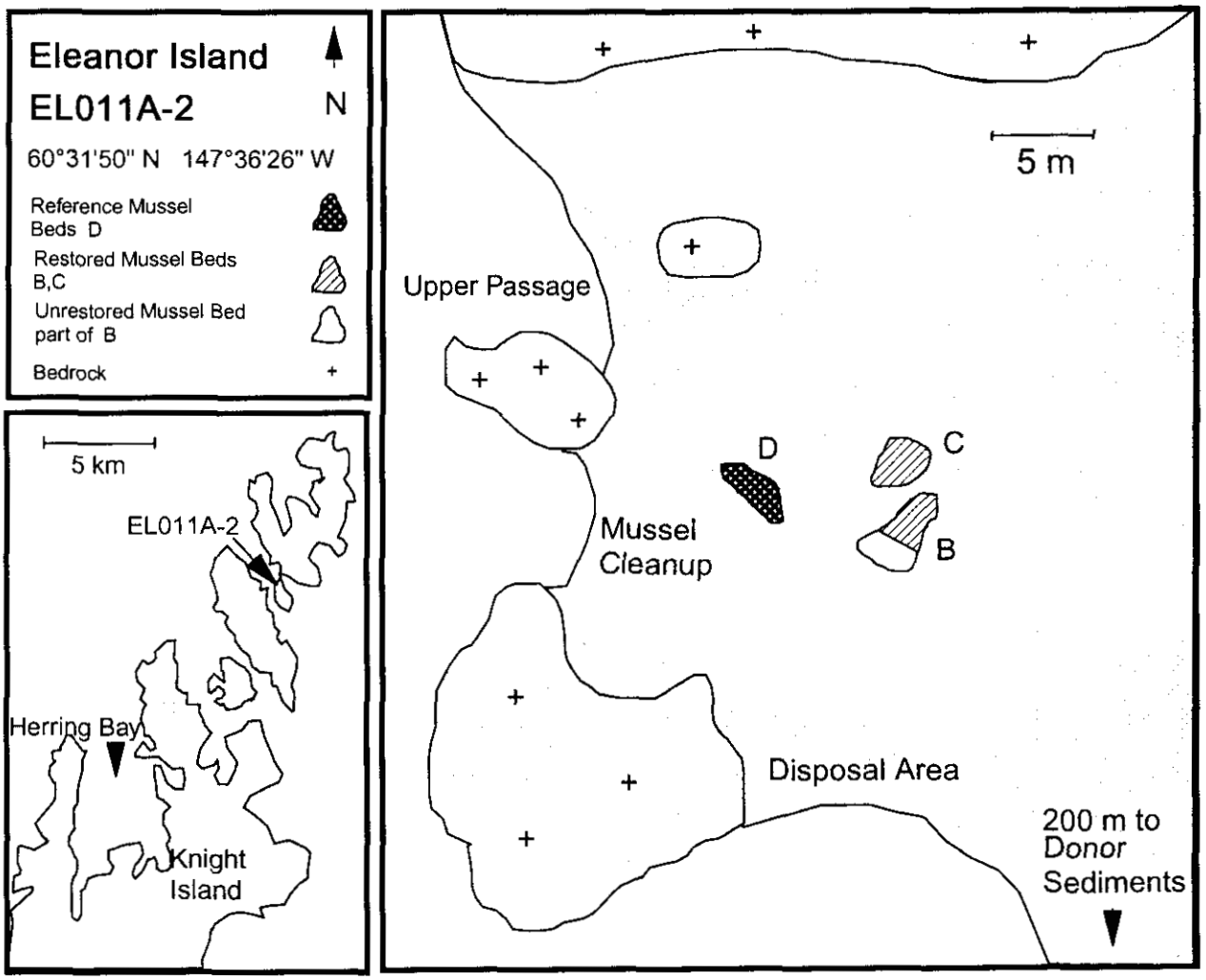
		Latitude			Longitude		
Site	**Segment#**	**deg**	**min**	**sec**	**deg**	**min**	**sec**
Cape Nukshak	CN002A	58	23	31	153	58	47
Port Dick, Mars Cove	PD004A	59	16	42	151	10	41
Port Dick, Pikes Point	PD010A	59	15	39	151	7	24
Morning Cove, Pye Islands	PY008B-1	59	26	46	150	19	25
Morning Cove, Pye Islands	PY008B-2	59	26	59	150	19	49
Rocky Bay, Point	RB001A	59	12	38	151	18	37
Rocky Bay	RB005B	59	14	30	151	28	15
Tonsina Bay, Camp B	TB002A	59	18	29	150	55	44
Tonsina Bay, w	TB003A-1	59	18	33	150	57	6
Tonsina Bay, w	TB003A-2	59	18	33	150	57	2
Tonsina Bay, s.w.	TB003A-3	59	18	39	150	56	58
Tonsina Bay, Otter B	TB003A-4	59	18	22	150	56	35
Tonsina Bay, s Otter B	TB003A-5	59	18	28	150	56	43
Tonsina Bay, Grim B	TB004A	59	18	9	150	56	35
Windy Bay	WB002B	59	14	4	151	31	27
Windy Bay, n	WB002D-1	59	13	43	151	32	19
Windy Bay, n	WB002D-2	59	13	44	151	32	16
Windy Bay, Oystercatcher Is.	WB009A	59	13	31	151	30	46

Appendix 1.2. Locations of mussel beds sampled in Prince William Sound. Segment# is the *Exxon Valdez* Interagency Shoreline Cleanup Committee segment number plus a unique suffix. Latitude and longitude are reported in degrees (deg), minutes (min), and seconds (sec).

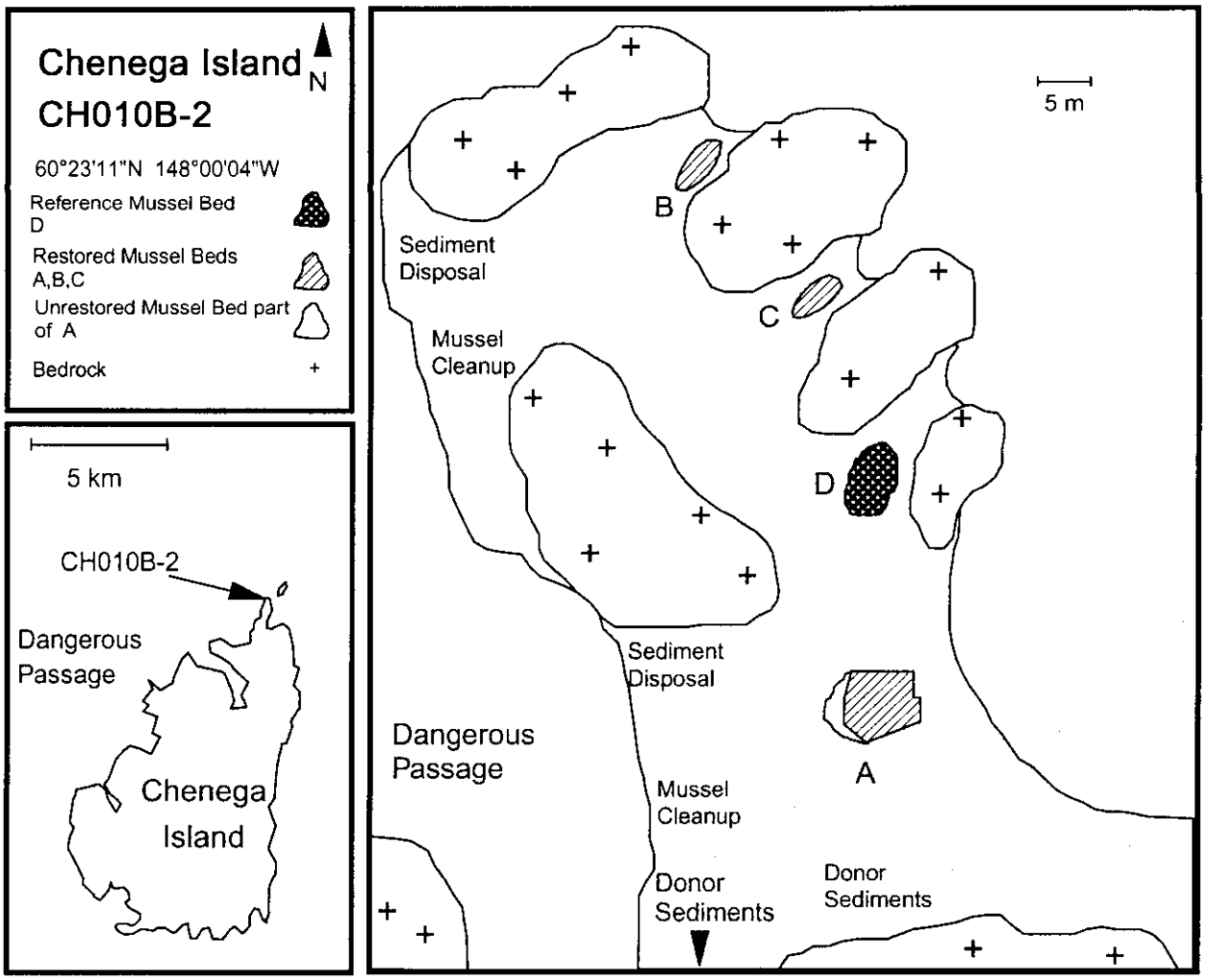
		Latitude			Longitude		
Site	**Segment#**	**deg**	**min**	**sec**	**deg**	**min**	**sec**
Applegate Island, e	AE005A-1	60	37	22	148	8	19
Applegate Island, e	AE005A-2	60	37	17	148	8	6
Applegate Island, e	AE005B	60	37	27	148	8	8
Aguliak Island, n	AG001A	60	22	4	147	52	37
Aguliak Island, s	AG009	60	21	45	147	52	34
Chenega Island, n	CH009A-1	60	22	43	147	59	36
Chenega Island, n	CH009A-2	60	22	40	147	59	40
Chenega Island, n	CH009A-3	60	22	44	147	59	41
Chenega Island, n	CH010B-2A	60	23	14	148	0	9
Chenega Island, n	CH010B-2B	60	23	14	148	0	9
Chenega Island, n	CH010B-2C	60	23	14	148	0	9
Chenega Island, n	CH010B-2D	60	23	14	148	0	9
Chenega, Isl., n	CH010B-2E	60	23	14	148	0	9
Chenega Island, n	CH011A	60	23	29	147	59	32
Crafton Island	CR004A	60	29	37	147	57	3
Crafton Island	CR005A	60	29	57	147	56	51
Disk Island, n	DI059A	60	30	2	147	38	20
Disk Island, w	DI066A	60	29	40	147	39	56
Disk Island, nw	DI067A-1	60	29	52	147	39	24
Disk Island, nw	DI067A-2	60	29	52	147	39	30
Disk Island	DI067A-2B	60	29	52	147	39	30
Disk Island, nw	DI067A-2C	60	29	59	147	39	30
Disk Island, nw	DI067A-3	60	29	52	147	39	30
Disk Island, nw	DI067A-4	60	29	52	147	39	30
Disk Island, nw	DI067A-5	60	29	52	147	39	30
Disk Island, nw	DI067A-6	60	29	52	147	39	30
Disk Island, nw	DI067A-7	60	29	52	147	39	30
Block Island, nw	EL011A-1	60	31	48	147	36	24
Block Island, nw	EL011A-2	60	31	48	147	36	26
Block Island, sw	EL011A-2D	60	31	48	147	36	26
Eleanor Island, sw	EL013A	60	32	6	147	36	50
Eleanor Island, sw	EL015A-1	60	31	58	147	36	31
Eleanor Island, sw	EL015A-2	60	31	58	147	36	31

Site	Segment#	Latitude deg	Latitude min	Latitude sec	Longitude deg	Longitude min	Longitude sec
Eleanor Island, sw	EL015A-3	60	31	53	147	36	18
Block Island, ne	EL015A-4	60	31	59	147	36	31
Eleanor Island, NW Bay	EL052A-1	60	33	4	147	35	54
Eleanor Island, NW Bay	EL052A-2	60	33	4	147	35	54
Eleanor Island, NW Bay	EL052B	60	32	37	147	36	13
Eleanor Island, NW Bay	EL054A	60	35	9	147	36	48
Elrington Island, Fox Farm	ER007A	59	58	15	148	8	32
Elrington Island, n	ER020B	60	2	12	147	59	45
Evans Isl., Bishop's Rk.	EV036A	60	6	14	147	53	16
Crab Bay, Evans Island	EV500A	60	4	20	147	59	48
Fleming Island	FL004A	60	10	24	148	2	11
Green Island	GR008A	60	16	59	147	24	54
Ingot Island, sw	IN031B	60	29	52	147	38	14
Bay of Isles	KN004A-1	60	22	58	147	42	38
Bay of Isles, w	KN005A	60	23	6	147	42	54
Bay of Isles, Islet	KN016A	60	23	18	147	41	6
Knight Island, n	KN103A	60	29	49	147	41	45
Herring Bay, e	KN113A	60	29	7	147	43	4
Herring Bay, e	KN113B	60	29	12	147	43	19
Herring Bay, e	KN114A-1	60	29	5	147	43	38
Herring Bay, e	KN114A-2	60	29	3	147	43	28
Herring Bay, e	KN115A	60	28	28	147	42	35
Herring Bay, e	KN119A	60	28	2	147	41	41
Herring Bay, e	KN120A	60	27	32	147	41	52
Herring Bay, e Islet	KN121A	60	28	4	147	42	42
Herring Bay, s Islet	KN133A-1	60	22	48	147	42	44
Herring Bay, s Islet	KN133A-2	60	26	46	147	45	32
Bay of Isles	KN136A-1	60	22	48	147	42	30
Bay of Isles	KN136A-2	60	22	48	147	42	30
Bay of Isles	KN136A-3	60	22	48	147	42	30
Herring Bay, se Islet	KN144B	60	26	30	147	44	12
Bay of Isles, w	KN203A	60	23	12	147	43	18
Bay of Isles, South Arm	KN205B	60	18	31	147	45	43
Bay of Isles, se	KN207B	60	23	18	147	38	28
Herring Point	KN500B	60	28	25	147	47	29
Knight Island, w	KN505A-1	60	27	0	147	49	0
Knight Island, w	KN505A-2	60	26	36	147	49	11

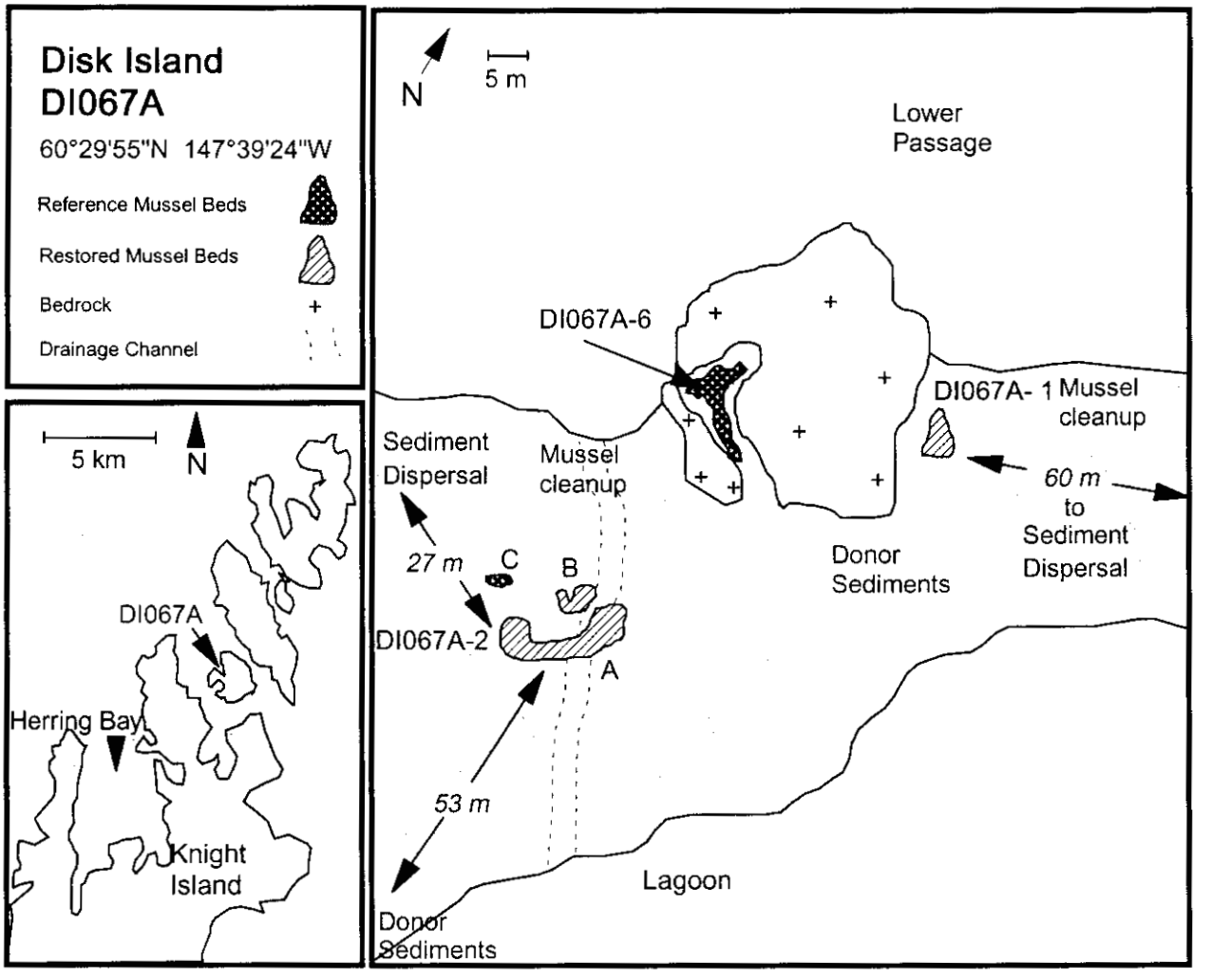
Site	Segment#	Latitude deg	Latitude min	Latitude sec	Longitude deg	Longitude min	Longitude sec
Barnes Cove	KN575A	60	18	31	147	45	43
Marsha Bay	KN702B	60	19	32	147	40	14
Latouche Island, ne	LA015E-2	60	3	45	147	48	57
Latouche Island, ne	LA015E-3	60	3	45	147	48	57
Sleepy Bay	LA018A	60	3	58	147	50	7
Foul Bay	MA002C	60	34	57	148	3	33
New Year Island	NY001	60	19	6	147	55	0
Olsen Bay	Olsen	60	44	30	146	11	53
Squirrel Island, e	SL001D-1	60	19	55	147	53	40
Squirrel Island, e	SL001D-2	60	19	57	147	53	54
Squirrel Island, Islet	SQ004B	60	14	54	147	58	0



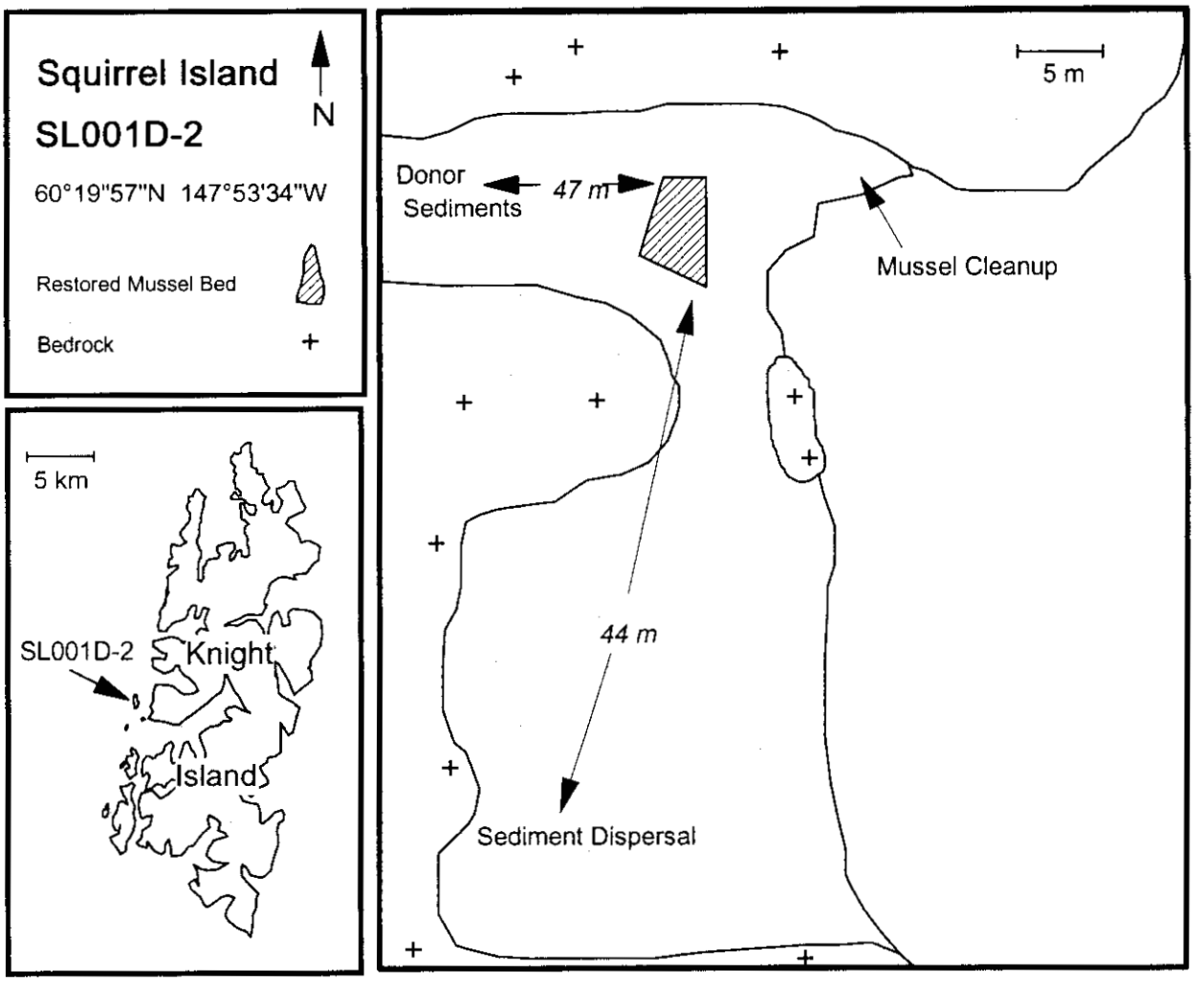
Appendix 1.3. The mussel beds at Eleanor Island (EL011A-2) were restored August 6, 1994. Oiled sediments were removed to a depth of 10-11 cm over a total area of 13.58 m^2. The volume of sediments removed was 1.49 m^3 and the weight was 2.63 metric tons. Clean sediments were then placed in the excavation and mussels that were originally in the bed were replaced on the clean sediments. Scale bar in the largest figure applies only to the restored beds, other distances are approximate or as noted. Figure continues on the next four pages.



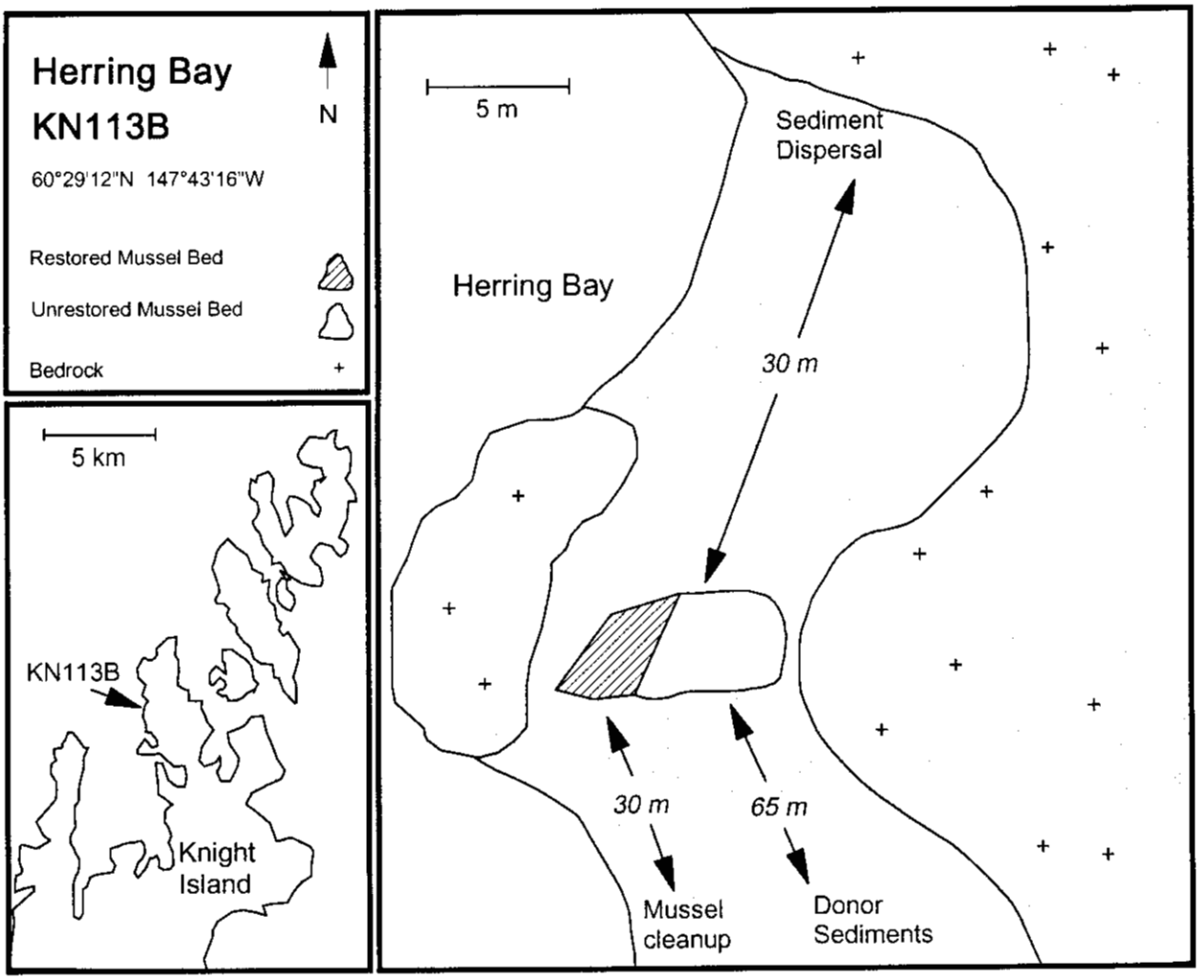
Appendix 1.3, continued. The mussel beds at Chenega Island (CH010B-2A) were restored August 6, 1994. Oiled sediments were removed to a depth of 10-11 cm over a total area of 57.41 m^2. The volume of sediments removed was 6.15 m^3 and the weight was 10.82 metric tons. Clean sediments were then placed in the excavation and mussels that were originally in the bed were replaced on the clean sediments. Scale bar in the largest figure applies only to the restored beds, other distances are approximate or as noted.



Appendix 1.3, continued. The mussel beds at Disk Island (DI067A) were restored July 20-23, 1994. Oiled sediments were removed to a depth of 6-10 cm over a total area of 96.64 m^2. The volume of sediments removed was 7.48 m^3 and the weight was 13.17 metric tons. Clean sediments were then placed in the excavation and mussels that were originally in the bed were replaced on the clean sediments. Scale bar in the largest figure applies only to the restored beds, other distances are approximate or as noted.



Appendix 1.3, continued. The mussel bed at Squirrel Island (DI067A)was restored July 25, 1994. Oiled sediment was removed to a depth of 9 cm over an area of 17.18 m^2. The volume of sediment removed was 1.60 m^3 and the weight was 2.81 metric tons. Clean sediment was then placed in the excavation and mussels that were originally in the bed were replaced on the clean sediments. Scale bar in the largest figure applies only to the restored bed, other distances are approximate or as noted.



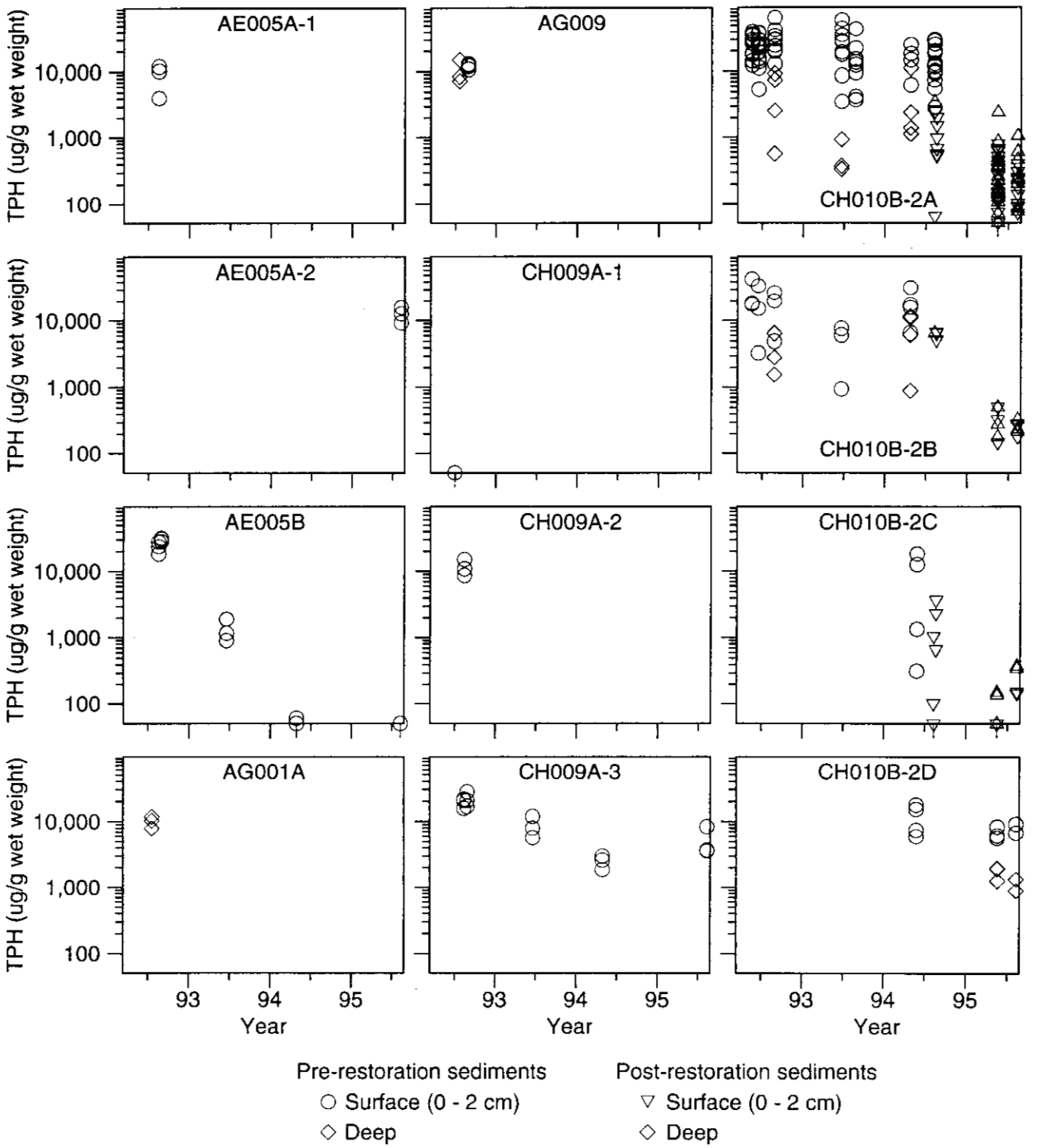
Appendix 1.3, continued. The mussel bed in Herring Bay (KN113B) was restored July 24, 1994. Oiled sediment was removed to a depth of 9 cm over an area of 9.24 m^2. The volume of sediments removed was 0.85 m^3 and the weight was 1.50 metric tons. Clean sediment was then placed in the excavation and mussels that were originally in the bed were replaced on the clean sediments. Scale bar in the largest figure applies only to the restored bed, other distances are approximate or as noted.

Appendix 2. Polynuclear aromatic hydrocarbons (PAH) identified by gas chromatography, confirmed by mass spectrometry, and their abbreviations. Hydrocarbons marked with an asterisk were used when estimating weathering with the first-order kinetic model developed by Short and Heintz (1997)[1].

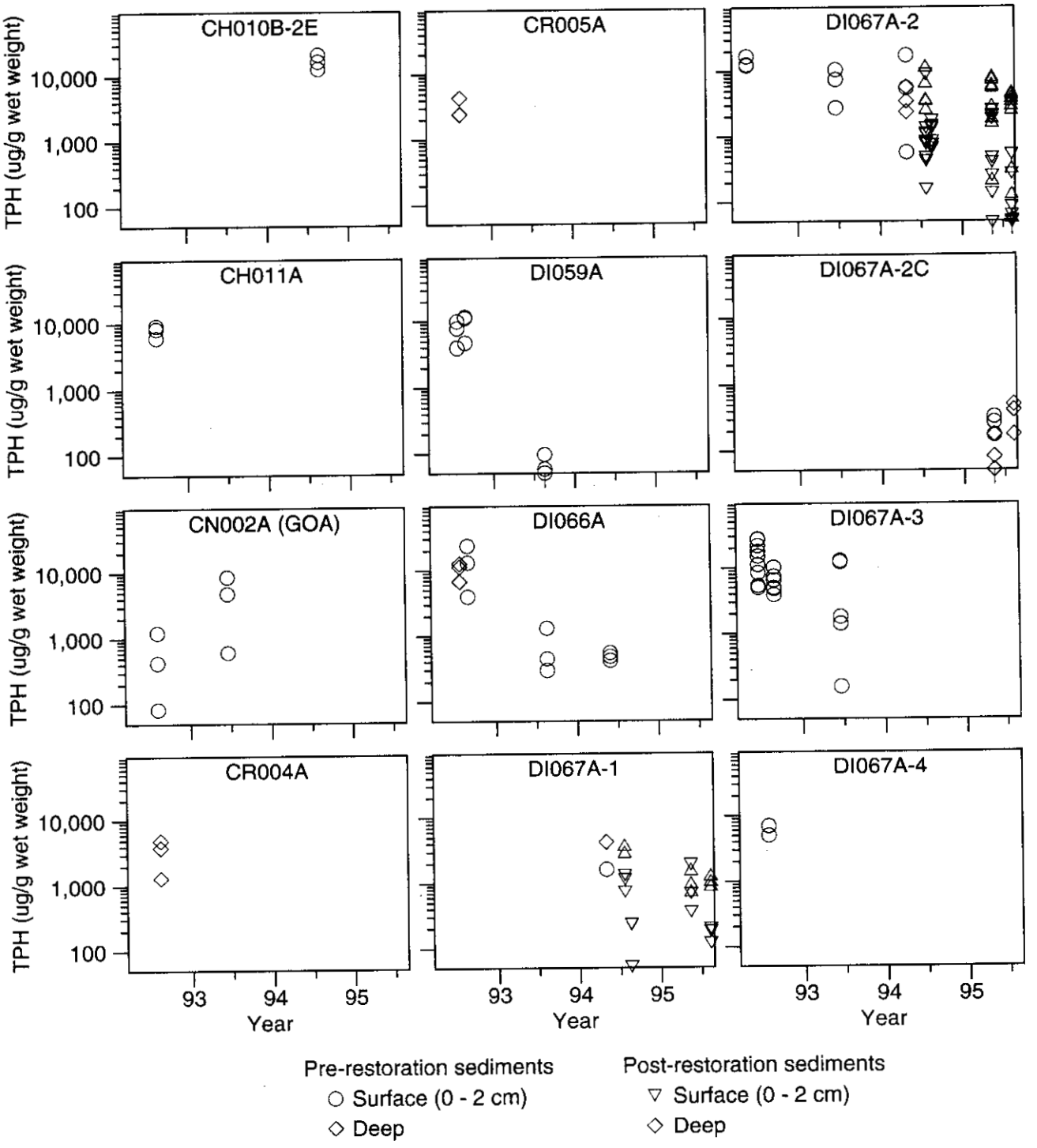
Abbreviation	PAH
NPH	naphthalene
C1NPH	C-1 naphthalenes
C2NPH	C-2 naphthalenes
C3NPH*	C-3 naphthalenes
C4NPH*	C-4 naphthalenes
BPH	biphenyl
ACY	acenaphthylene
ACE	acenaphthene
FLU	fluorene
C1FLU	C-1 fluorenes
C2FLU*	C-2 fluorenes
C3FLU*	C-3 fluorenes
DBT	dibenzothiophene
C1DBT*	C-1 dibenzothiophenes
C2DBT*	C-2 dibenzothiophenes
C3DBT*	C-3 dibenzothiophenes
PHN	phenanthrene
C1PHN*	C-1 phenanthrenes/anthracenes
C2PHN*	C-2 phenanthrenes/anthracenes
C3PHN*	C-3 phenanthrenes/anthracenes
C4PHN*	C-4 phenanthrenes/anthracenes
ANT	anthracene
FLA	fluoranthene
PYR	pyrene

Abbreviation	PAH
C1FLA	C-1 fluoranthenes/pyrenes
BAA	benz-a-anthracene
CHR*	chrysene
C1CHR*	C-1 chrysenes
C2CHR*	C-2 chrysenes
C3CHR	C-3 chrysenes
C4CHR	C-4 chrysenes
BbF	benzo-b-fluoranthene
BkF	benzo-k-fluoranthene
BEP	benzo-e-pyrene
BAP	benzo-a-pyrene
PER	perylene
IDP	indeno-123-cd-pyrene
DBA	dibenzo-a,h-anthracene
BZP	benzo-g,h,i-perylene

[1]Short, J. W. and R. A. Heintz. 1997. Identification of *Exxon Valdez* oil in sediments and tissues from Prince William Sound and the Norwestern Gulf of Alaska based on a PAH weathering model. Environ. Sci. Technol. 31:2375-2384.



Appendix 3. Total petroleum hydrocarbon (TPH) concentrations in surface and deep sediments, 1992-1995, including pre- and post-restoration data at manually restored sites. Each bed is identified by the *Exxon Valdez* Interagency Shoreline Cleanup Committee segment number plus a unique suffix. Figure continues on next eight pages.



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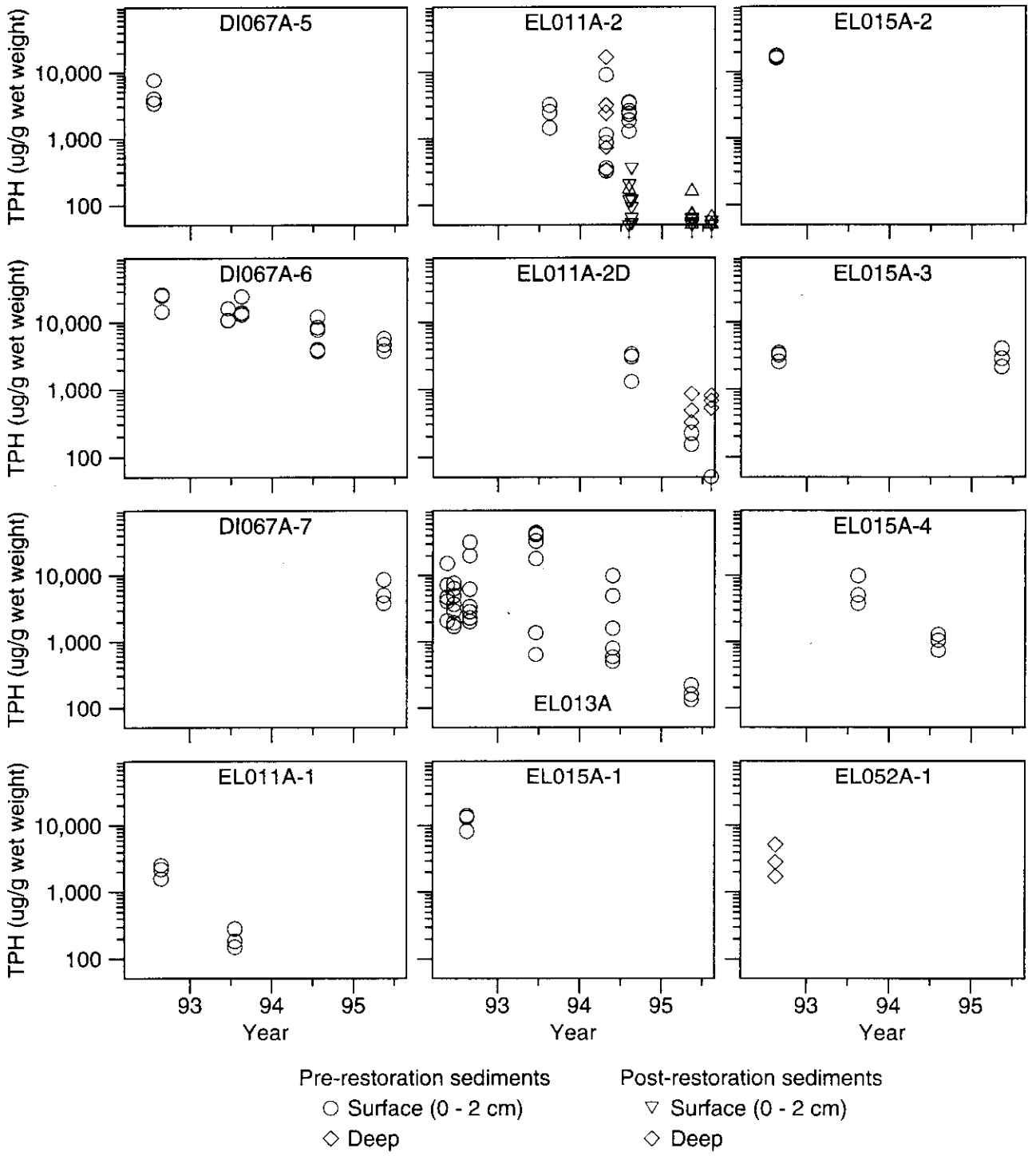

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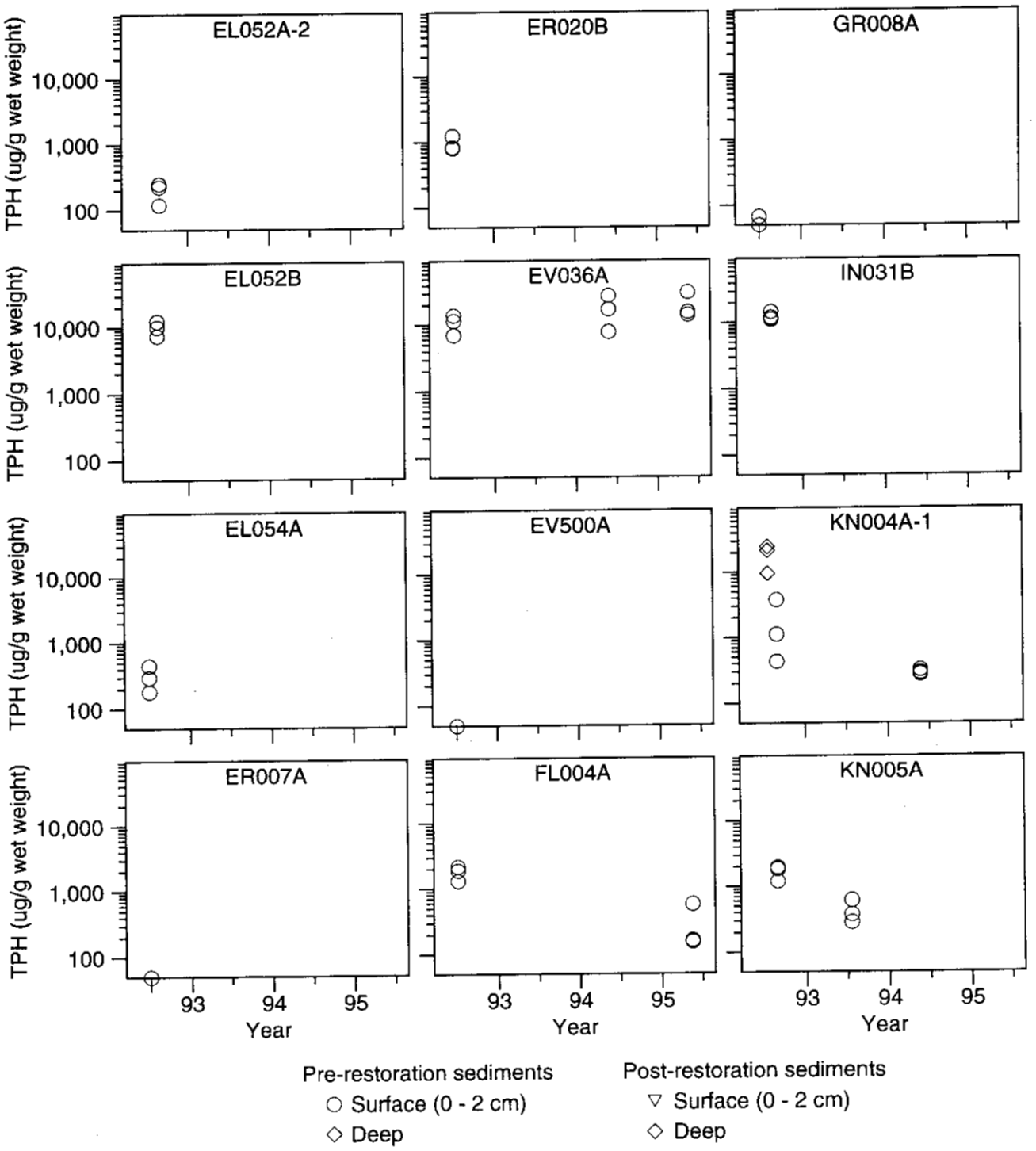

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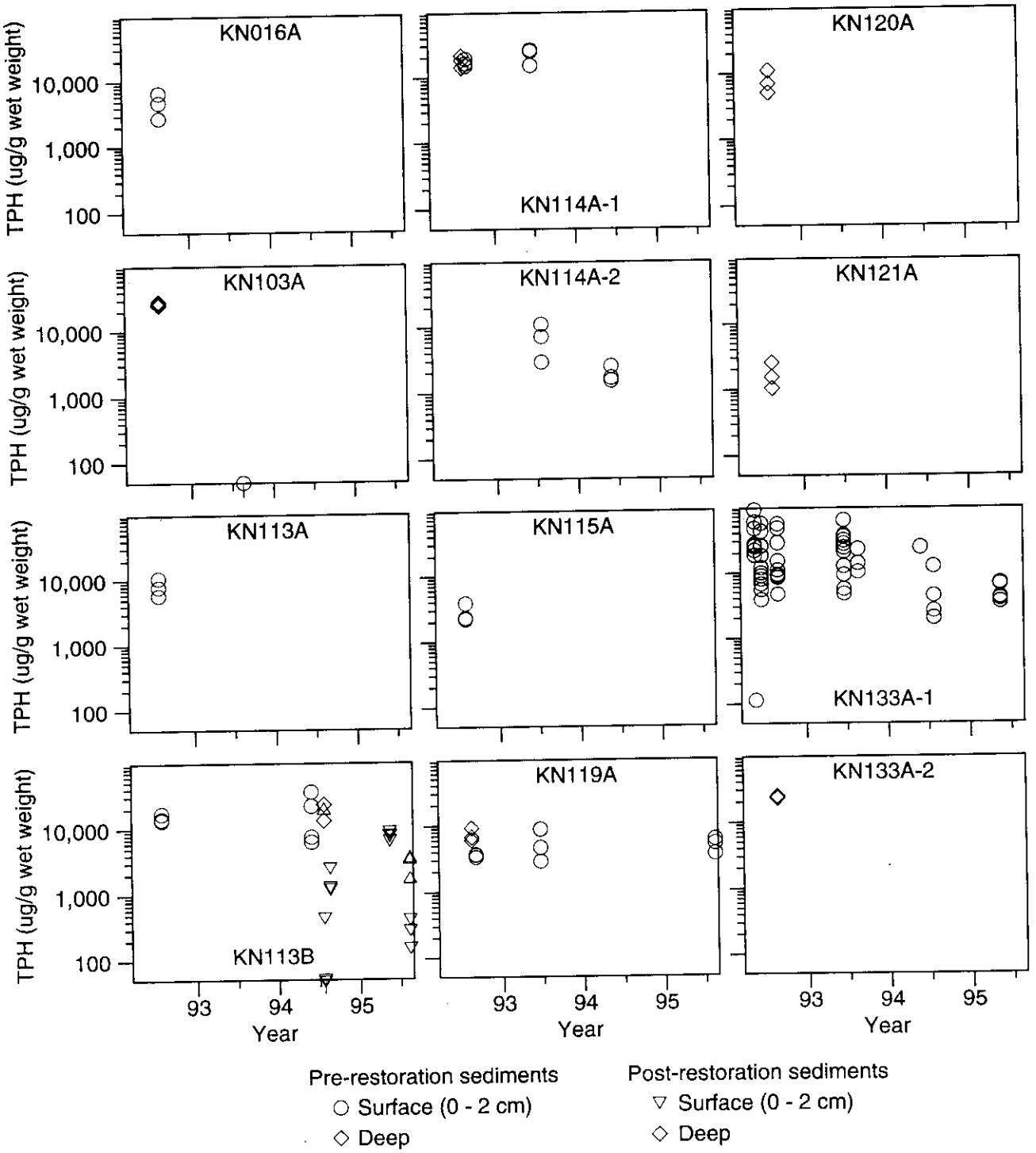

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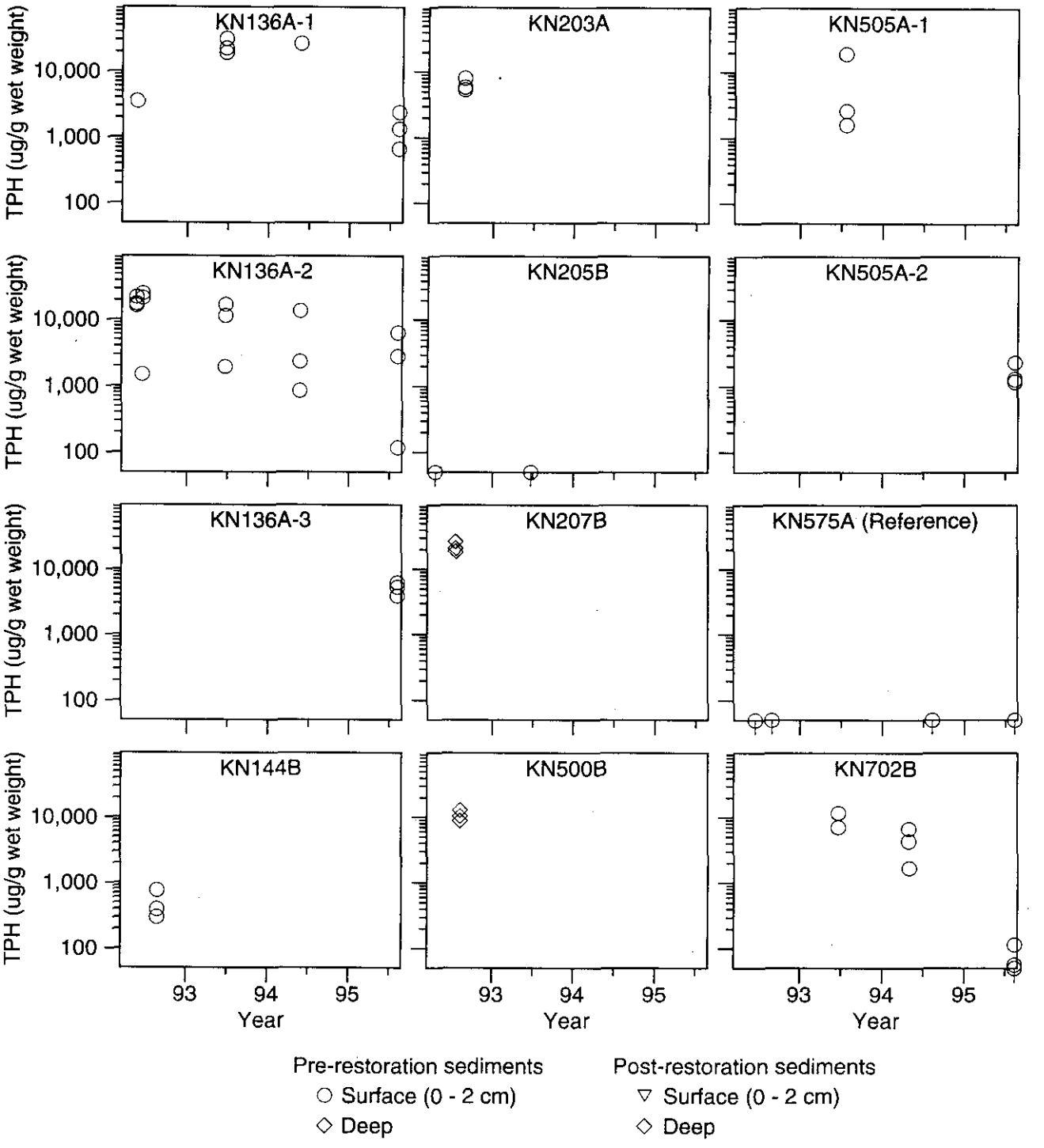

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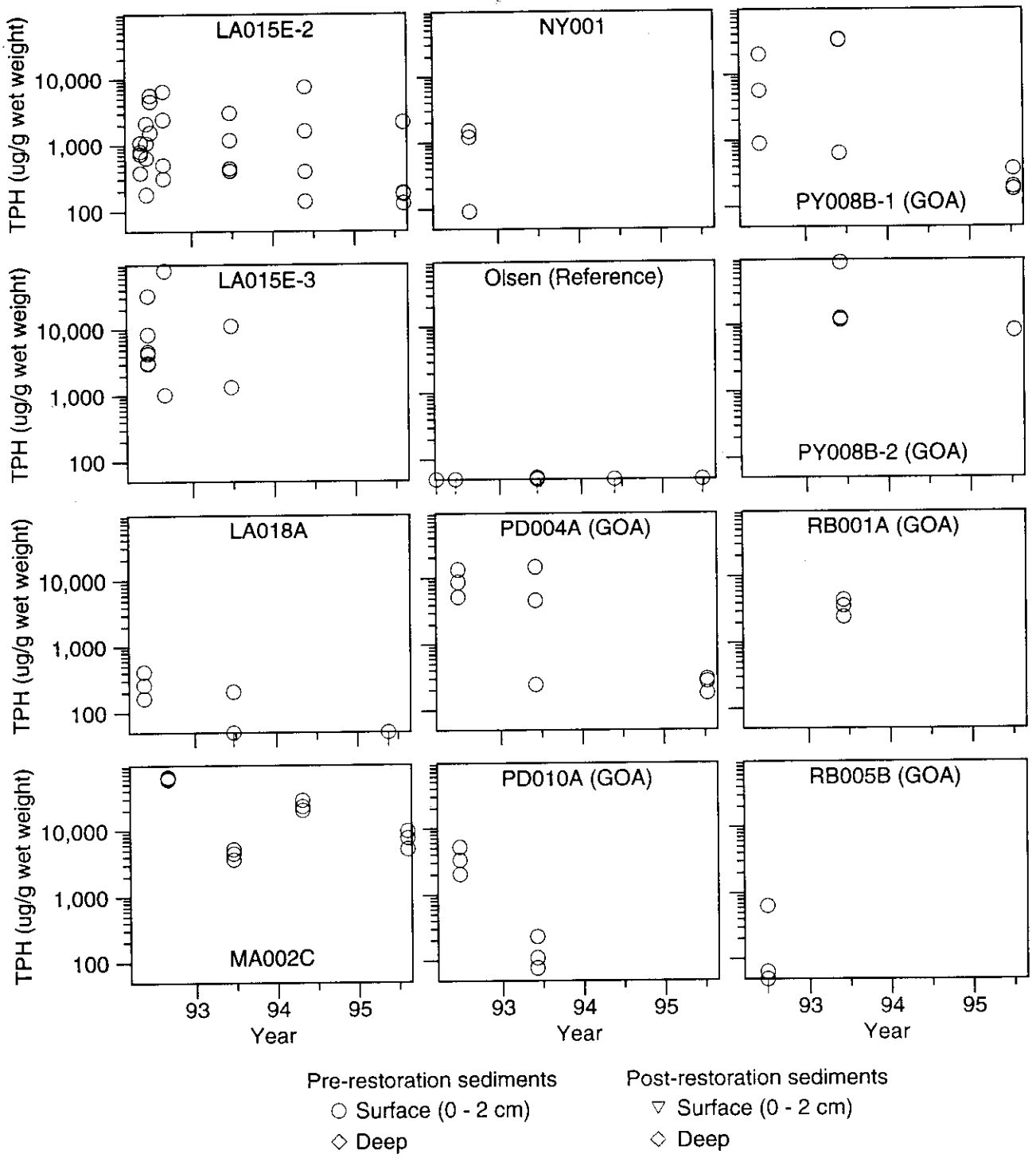

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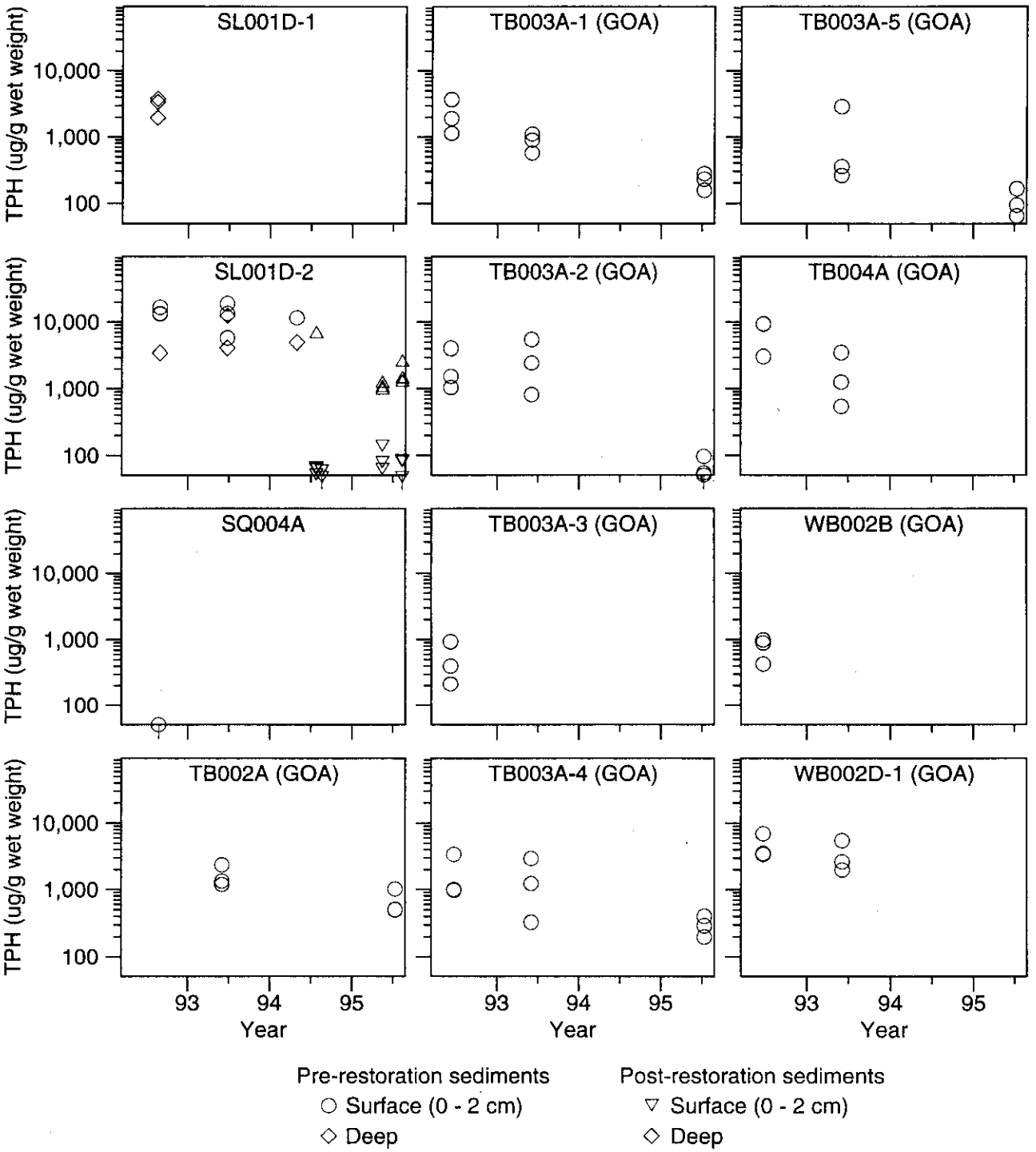

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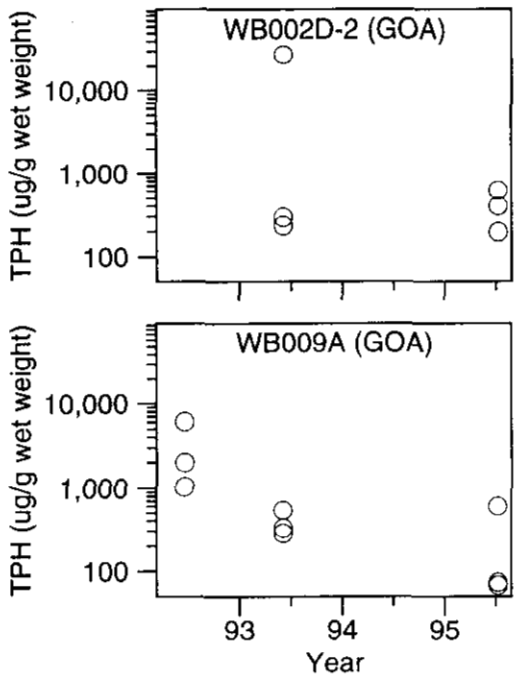
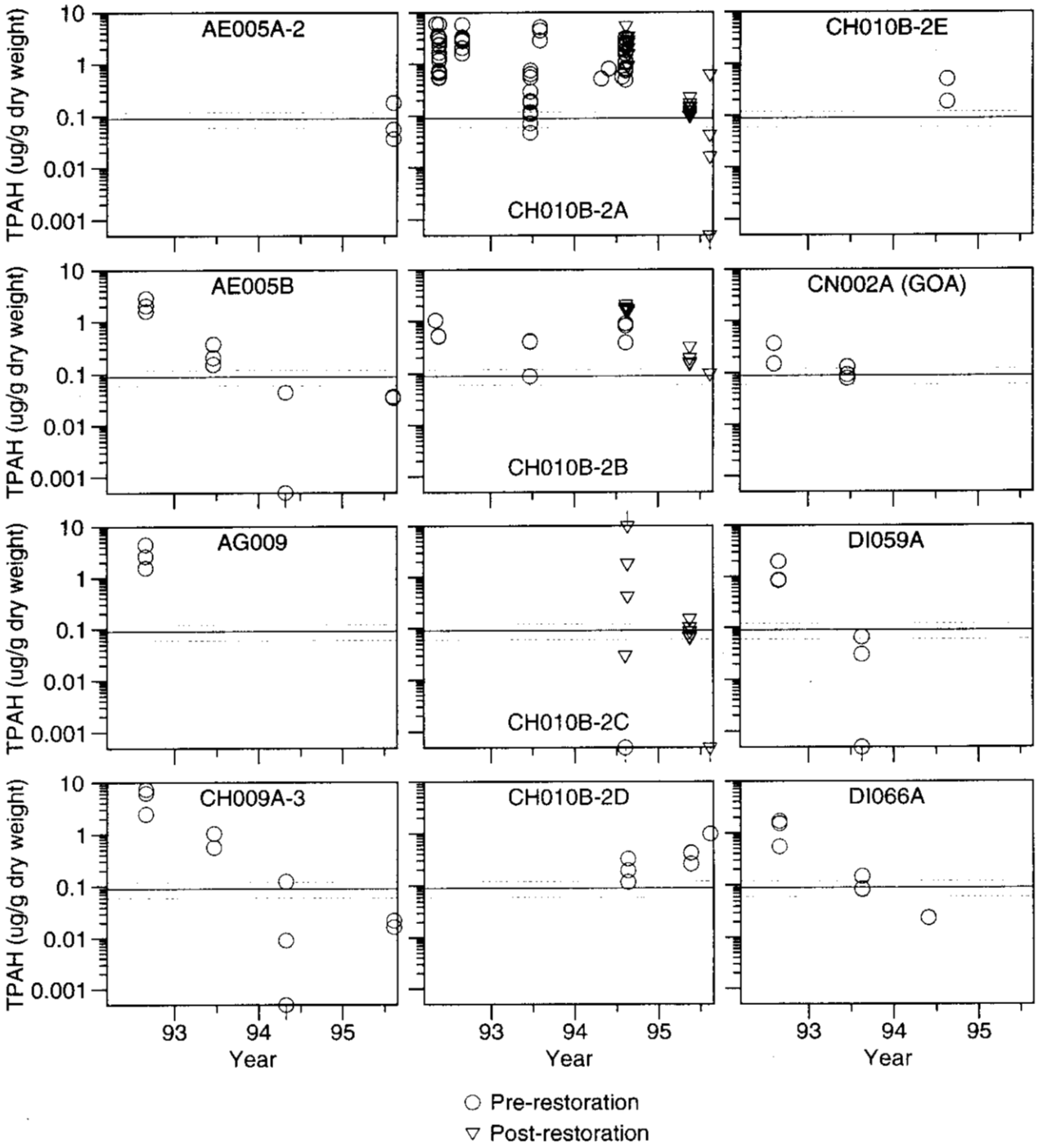


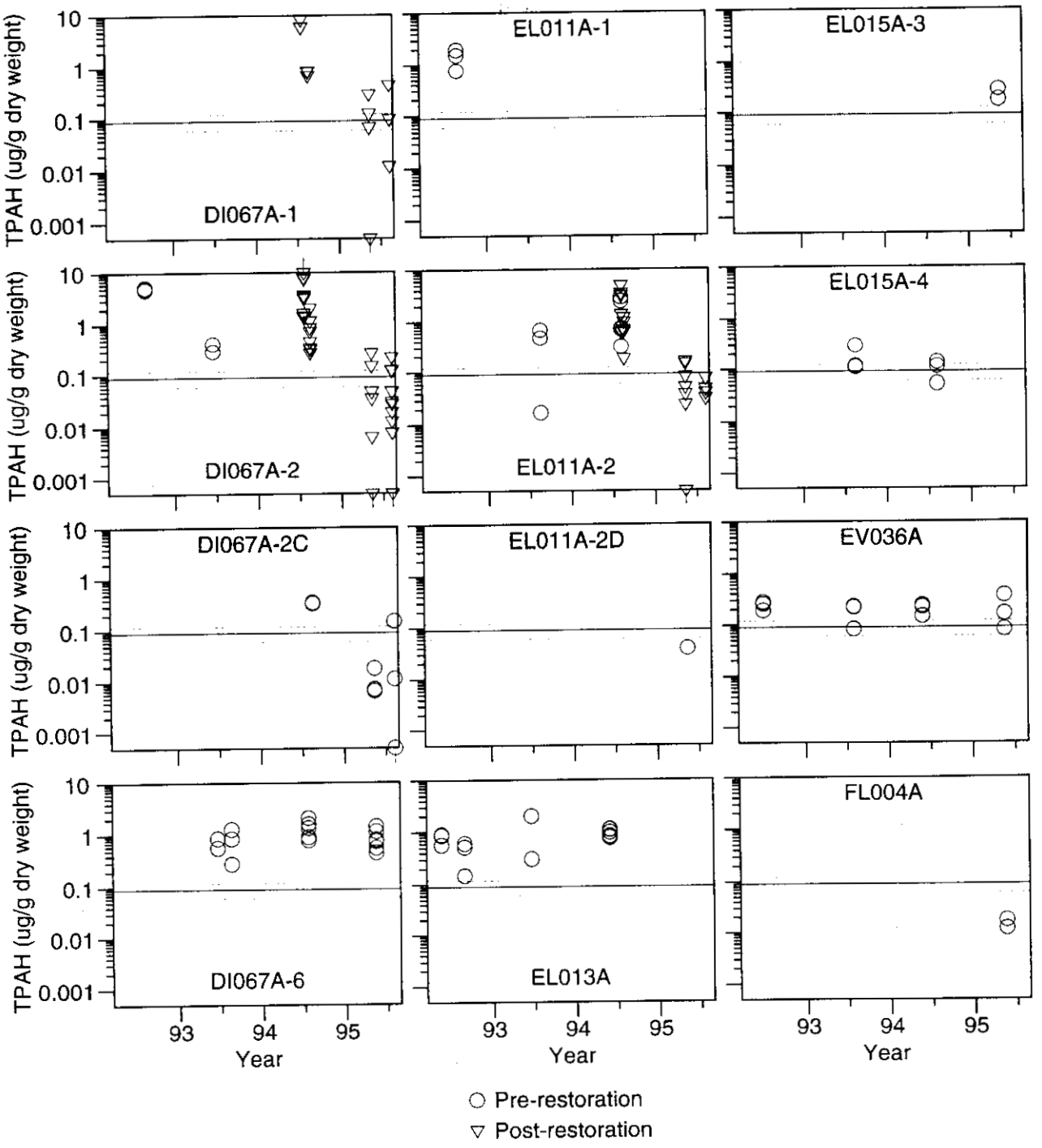
Pre-restoration sediments
○ Surface (0 - 2 cm)
◇ Deep

Post-restoration sediments
▽ Surface (0 - 2 cm)
◇ Deep

Appendix 3, continued.



Appendix 4. Total polynuclear aromatic hydrocarbon (PAH) concentrations in mussels, 1992-1995, including pre- and post-restoration data at manually restored sites. Estimated background concentration, 0.09 ± 0.03 µg/g, is indicated (horizontal lines). Figure continues on next five pages.



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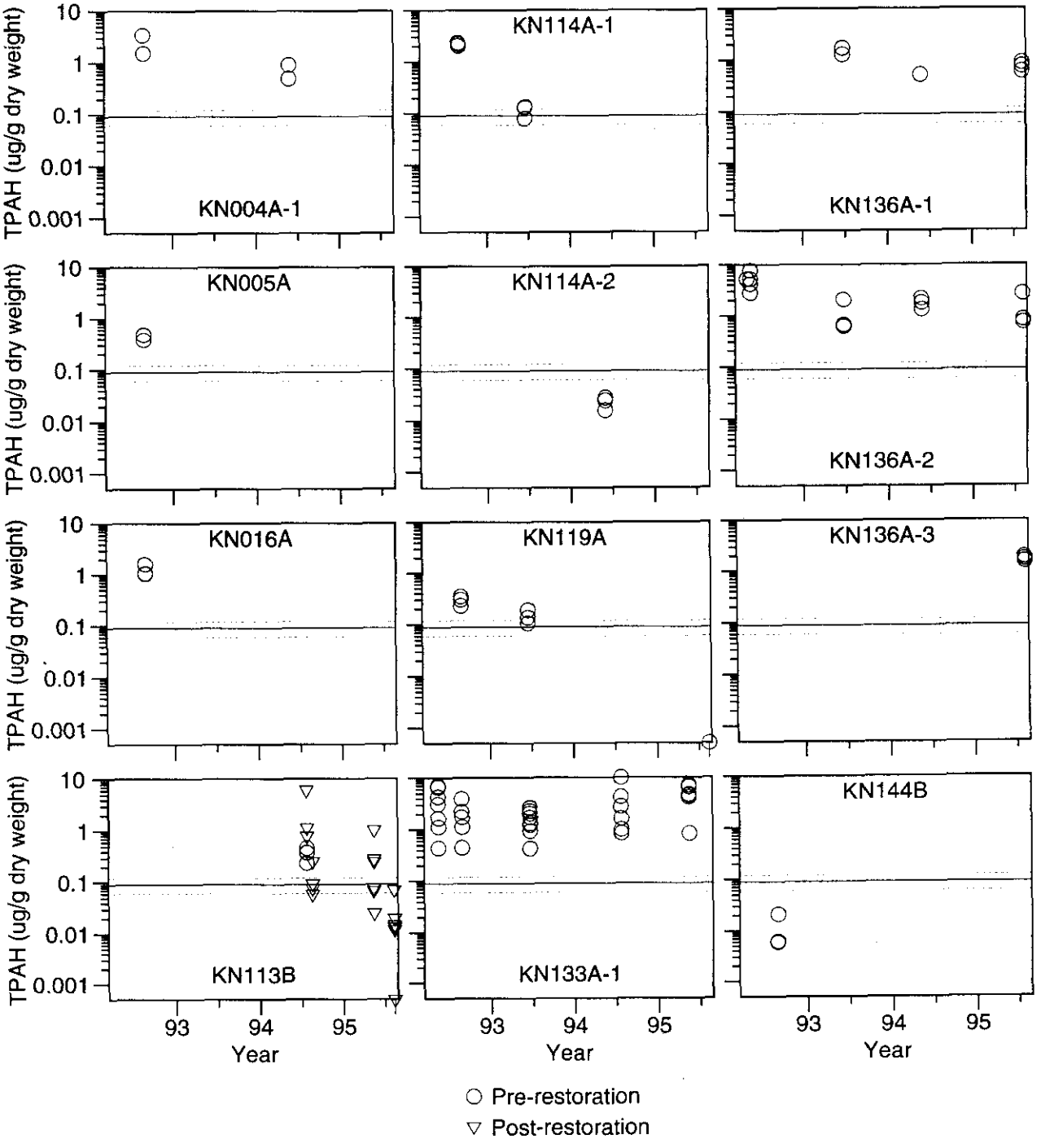

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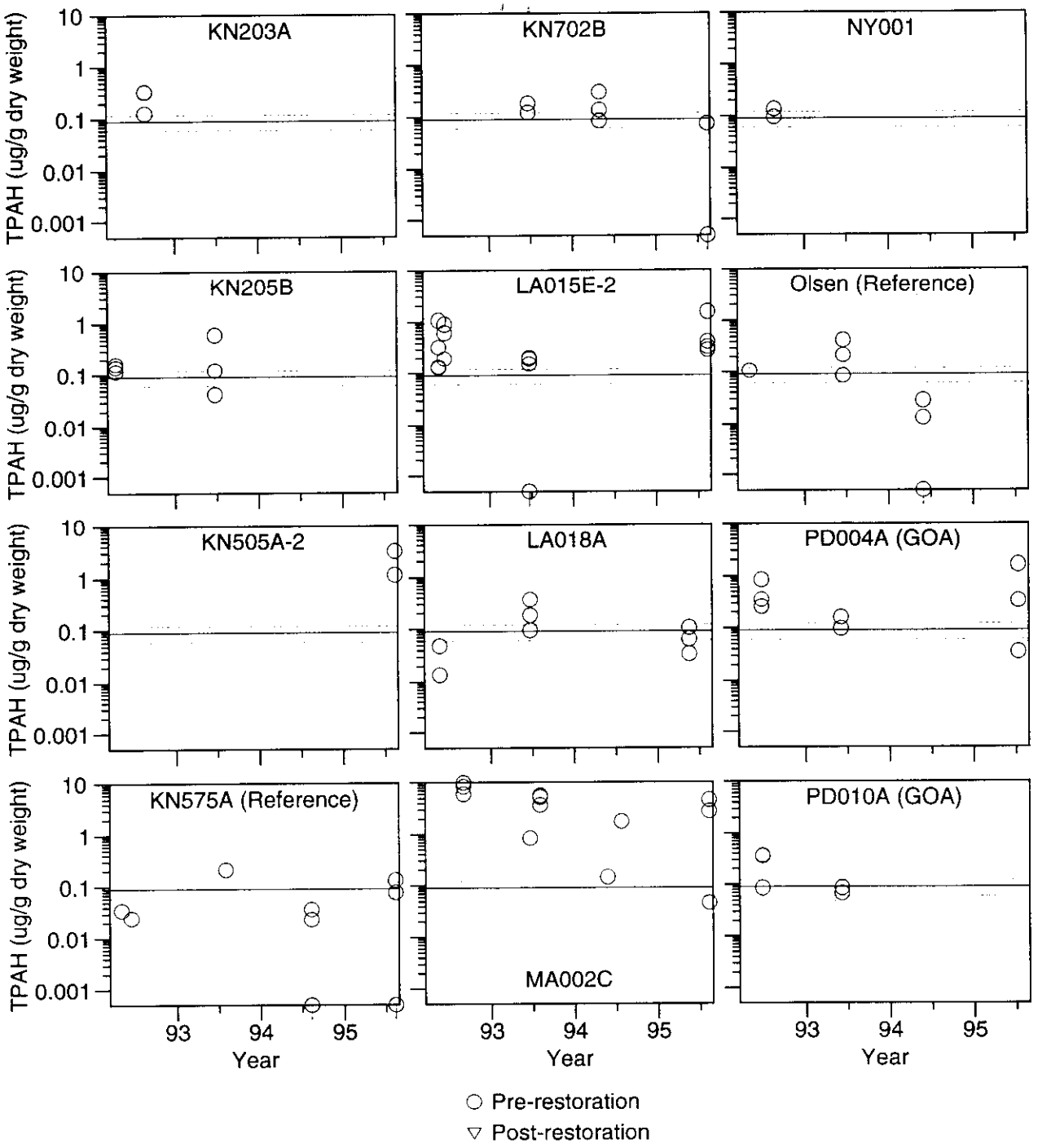
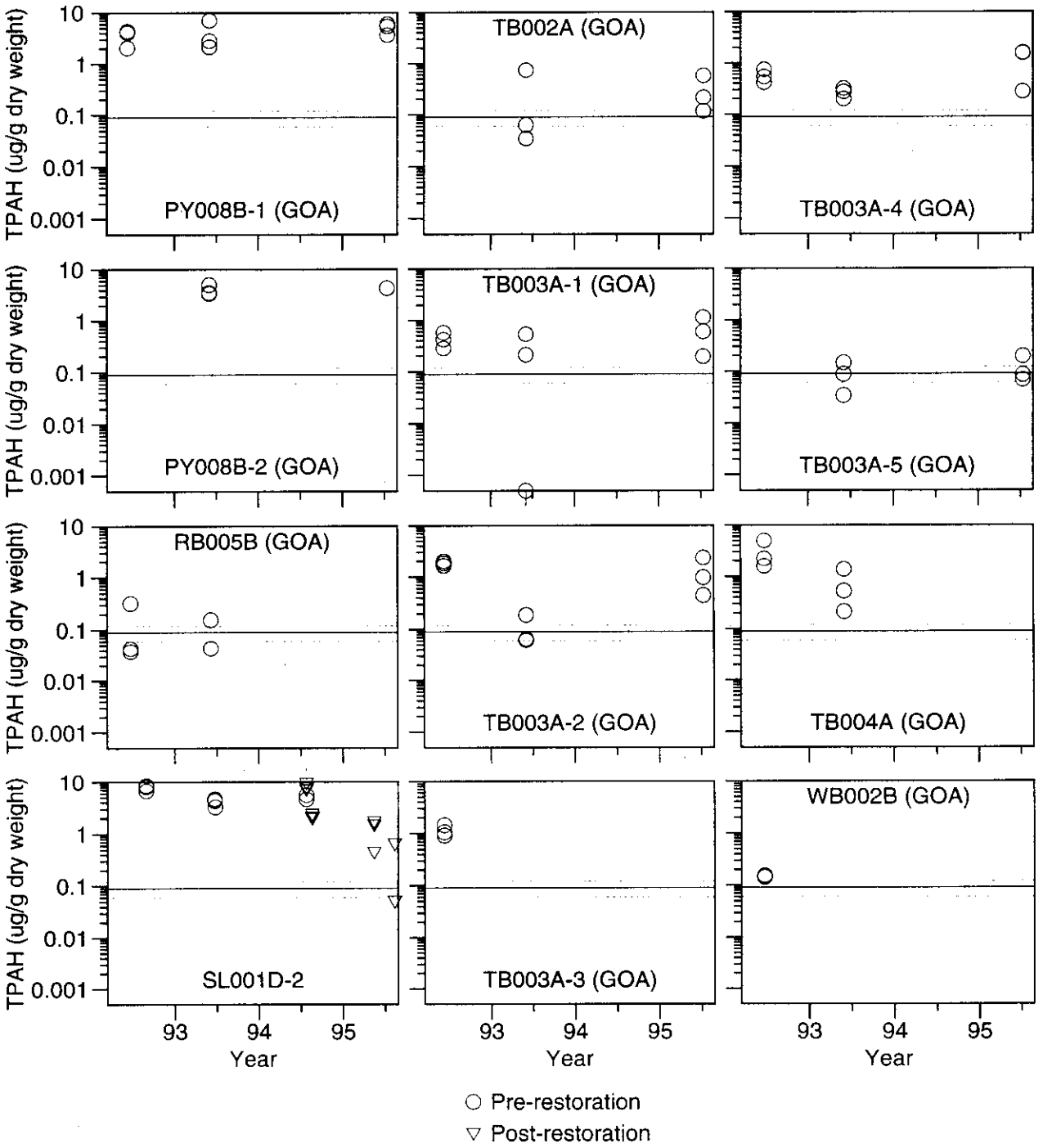
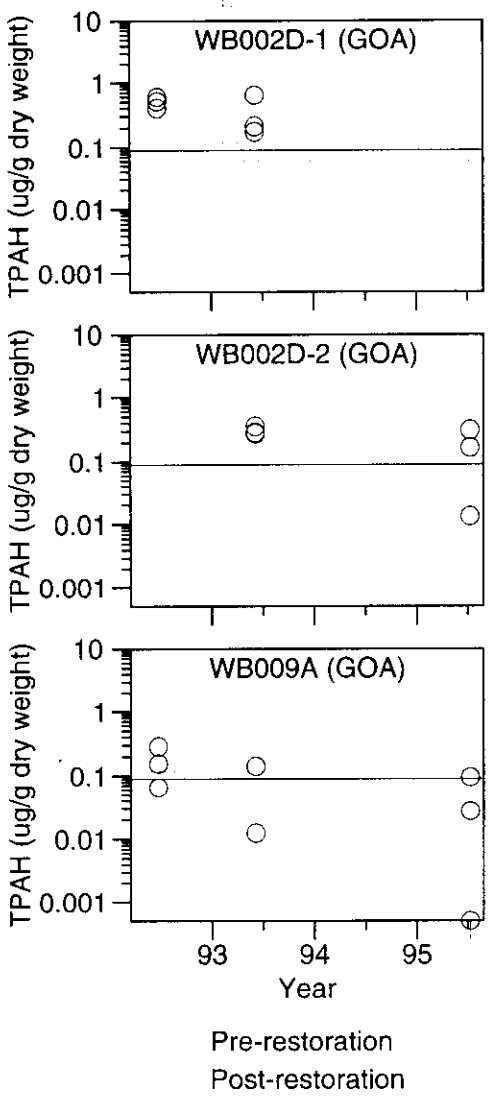


Appendix 4, continued.



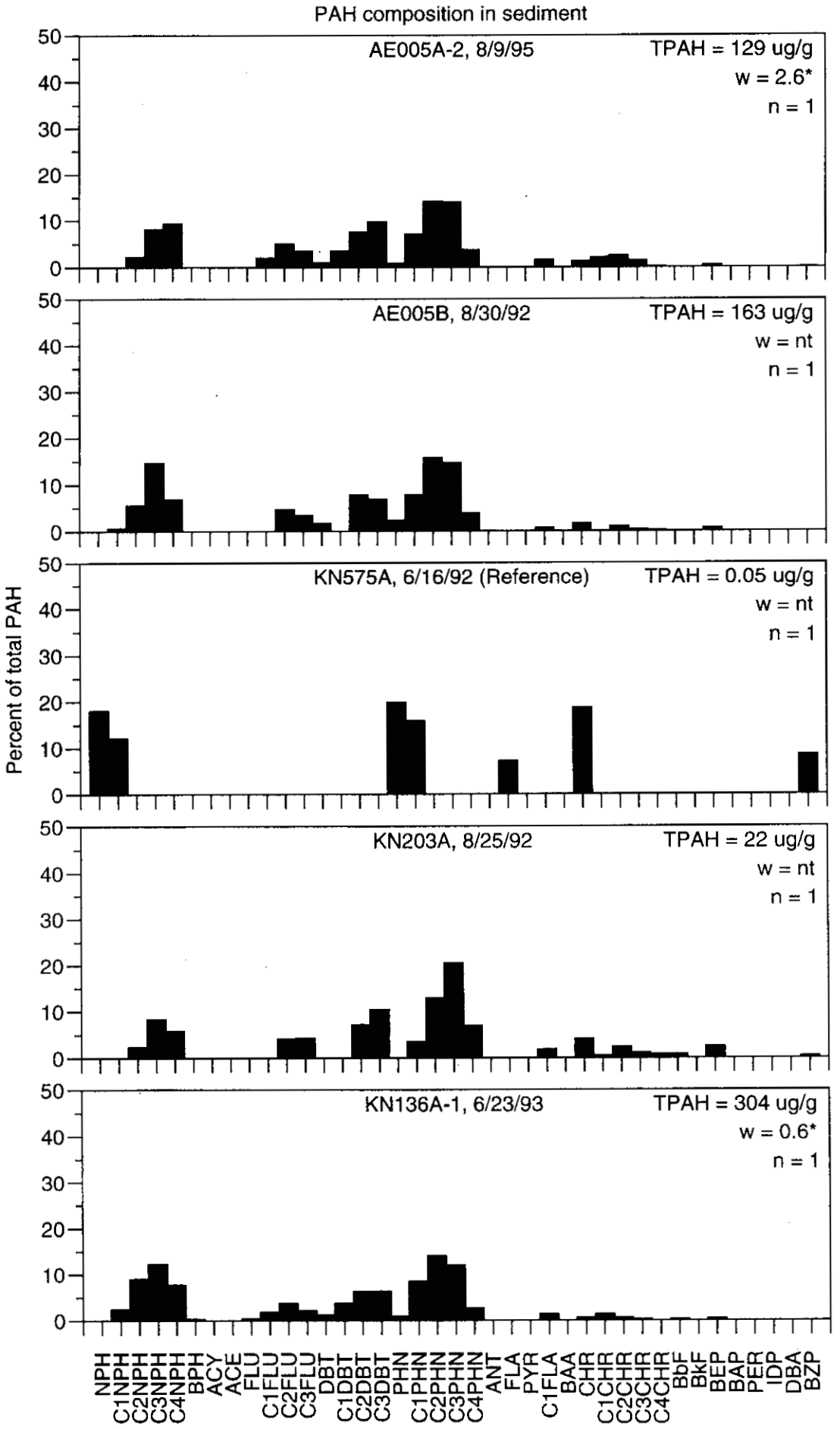
Appendix 4, continued.

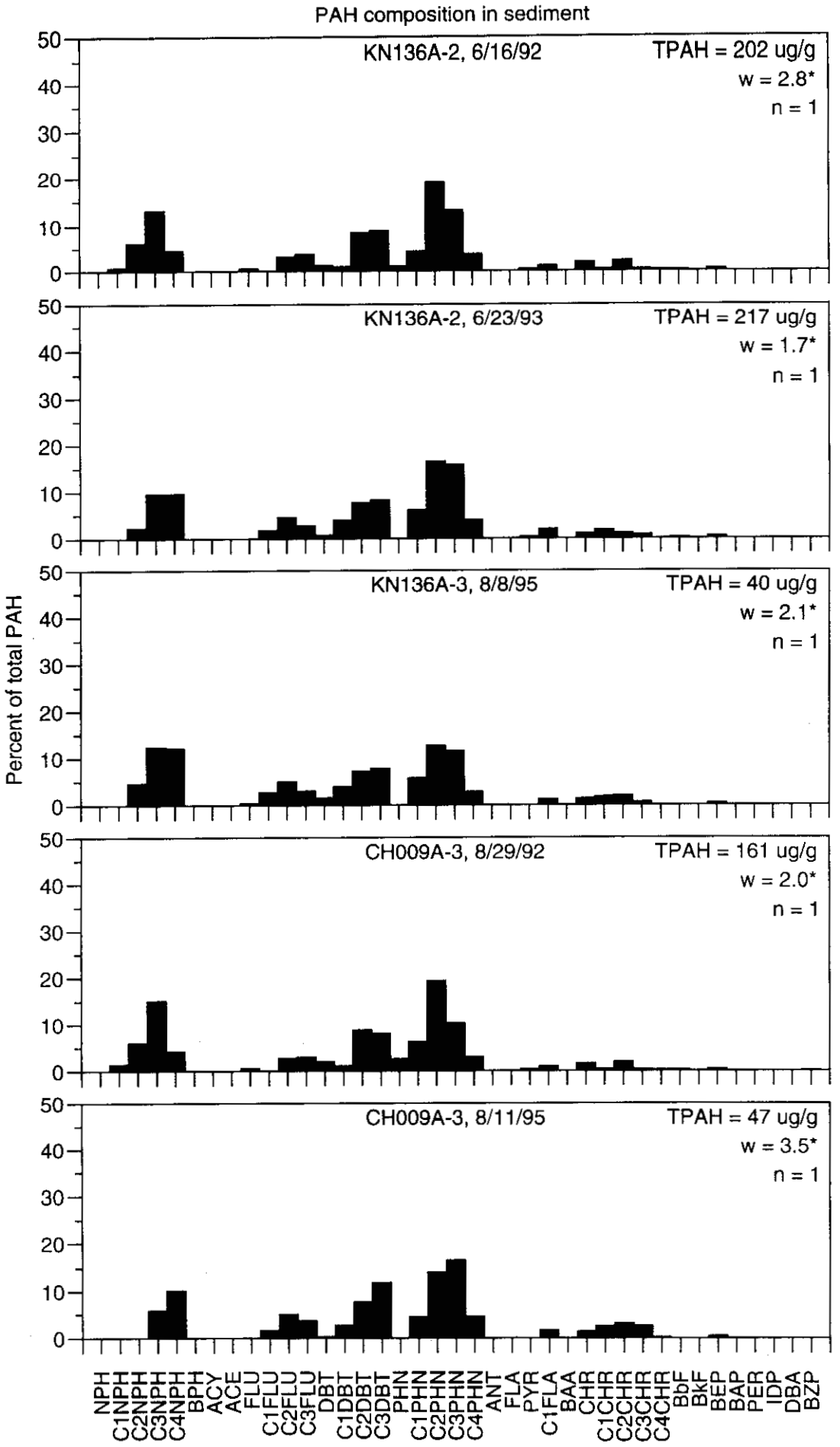


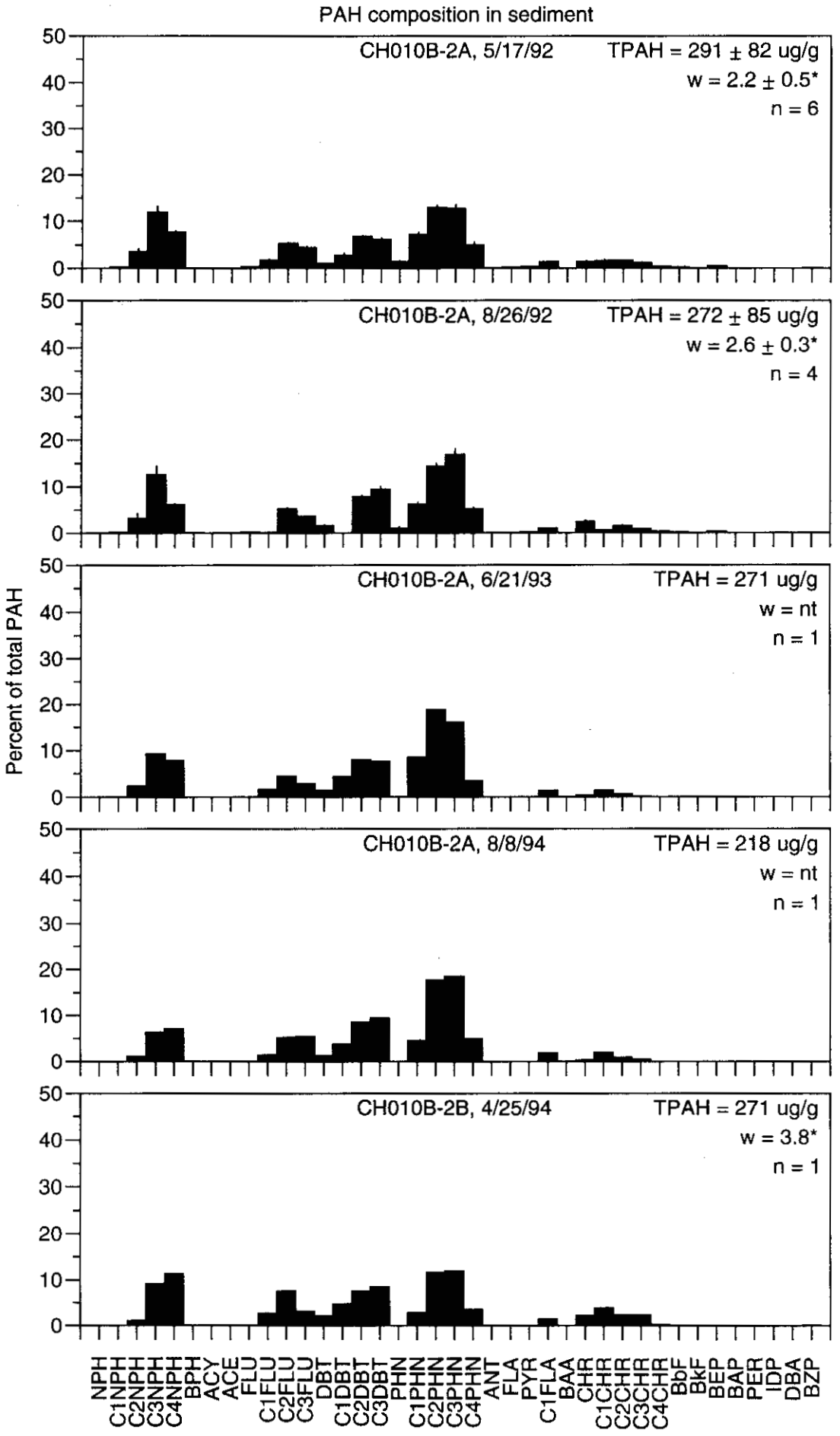
Appendix 4, continued.

Appendix 5 *(at right)*. Polynuclear aromatic hydrocarbon (PAH) composition in surface sediments. Each bed is identified by the *Exxon Valdez* Interagency Shoreline Cleanup Committee segment number plus a unique suffix. Mean (±SE) total PAH (TPAH) concentrations and estimated weathering (w), and sample size (n) are indicated for each data set; asterisks indicate *Exxon Valdez* oil was verified as the source of contamination by the Short and Heintz (1997) oil-weathering model: nt = not estimable, ns = not significant (source was not *Exxon Valdez* oil). Data are from Chapter 1 in this report. Figure continues on the next 11 pages.

Short, J. W. and R. A. Heintz. 1997. Identification of *Exxon Valdez* oil in sediments and tissues from Prince William Sound and the Norwestern Gulf of Alaska based on a PAH weathering model. Environ. Sci. Technol. 31:2375-2384.


PAH composition in sediment
Percent of total PAH
AE005A-2, 8/9/95
TPAH = 129 ug/g
w = 2.6*
n = 1
AE005B, 8/30/92
TPAH = 163 ug/g
w = nt
n = 1
KN575A, 6/16/92 (Reference)
TPAH = 0.05 ug/g
w = nt
n = 1
KN203A, 8/25/92
TPAH = 22 ug/g
w = nt
n = 1
KN136A-1, 6/23/93
TPAH = 304 ug/g
w = 0.6*
n = 1
0
10
20
30
40
50
NPH
C1NPH
C2NPH
C3NPH
C4NPH
BPH
ACY
ACE
FLU
C1FLU
C2FLU
C3FLU
DBT
C1DBT
C2DBT
C3DBT
PHN
C1PHN
C2PHN
C3PHN
C4PHN
ANT
FLA
PYR
C1FLA
BAA
CHR
C1CHR
C2CHR
C3CHR
C4CHR
BbF
BkF
BEP
BAP
PER
IDP
DBA
BZP


PAH composition in sediment
Percent of total PAH
KN136A-2, 6/16/92
TPAH = 202 ug/g
w = 2.8*
n = 1
KN136A-2, 6/23/93
TPAH = 217 ug/g
w = 1.7*
n = 1
KN136A-3, 8/8/95
TPAH = 40 ug/g
w = 2.1*
n = 1
CH009A-3, 8/29/92
TPAH = 161 ug/g
w = 2.0*
n = 1
CH009A-3, 8/11/95
TPAH = 47 ug/g
w = 3.5*
n = 1
0
10
20
30
40
50
NPH
C1NPH
C2NPH
C3NPH
C4NPH
BPH
ACY
ACE
FLU
C1FLU
C2FLU
C3FLU
DBT
C1DBT
C2DBT
C3DBT
PHN
C1PHN
C2PHN
C3PHN
C4PHN
ANT
FLA
PYR
C1FLA
BAA
CHR
C1CHR
C2CHR
C3CHR
C4CHR
BbF
BkF
BEP
BAP
PER
IDP
DBA
BZP


PAH composition in sediment
Percent of total PAH
CH010B-2A, 5/17/92
TPAH = 291 ± 82 ug/g
w = 2.2 ± 0.5*
n = 6
CH010B-2A, 8/26/92
TPAH = 272 ± 85 ug/g
w = 2.6 ± 0.3*
n = 4
CH010B-2A, 6/21/93
TPAH = 271 ug/g
w = nt
n = 1
CH010B-2A, 8/8/94
TPAH = 218 ug/g
w = nt
n = 1
CH010B-2B, 4/25/94
TPAH = 271 ug/g
w = 3.8*
n = 1
0
10
20
30
40
50
NPH
C1NPH
C2NPH
C3NPH
C4NPH
BPH
ACY
ACE
FLU
C1FLU
C2FLU
C3FLU
DBT
C1DBT
C2DBT
C3DBT
PHN
C1PHN
C2PHN
C3PHN
C4PHN
ANT
FLA
PYR
C1FLA
BAA
CHR
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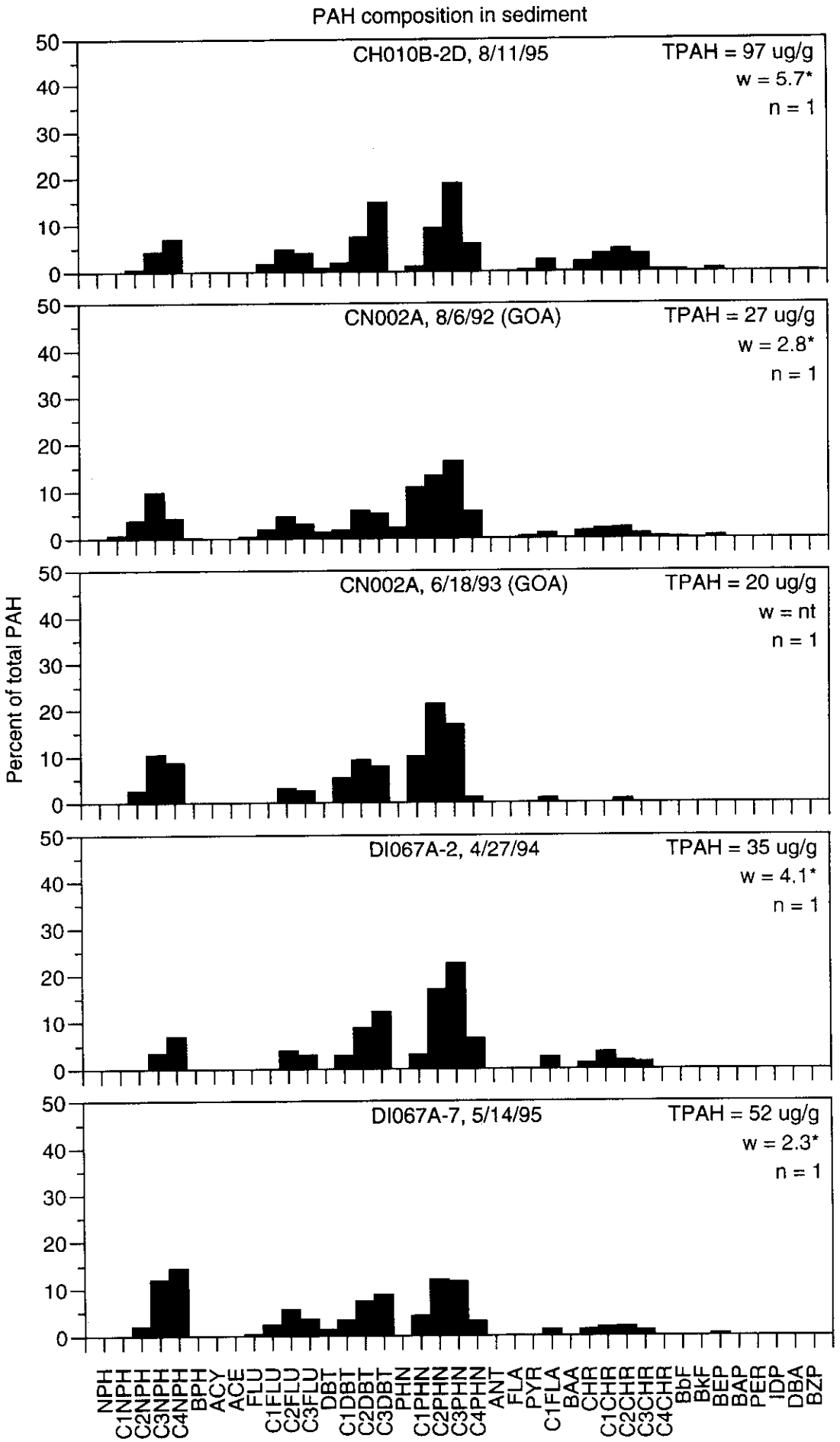
BbF
BkF
BEP
BAP
PER
IDP
DBA
BZP


PAH composition in sediment
Percent of total PAH
CH010B-2D, 8/11/95
TPAH = 97 ug/g
w = 5.7*
n = 1
CN002A, 8/6/92 (GOA)
TPAH = 27 ug/g
w = 2.8*
n = 1
CN002A, 6/18/93 (GOA)
TPAH = 20 ug/g
w = nt
n = 1
DI067A-2, 4/27/94
TPAH = 35 ug/g
w = 4.1*
n = 1
DI067A-7, 5/14/95
TPAH = 52 ug/g
w = 2.3*
n = 1
0
10
20
30
40
50
NPH
C1NPH
C2NPH
C3NPH
C4NPH
BPH
ACY
ACE
FLU
C1FLU
C2FLU
C3FLU
DBT
C1DBT
C2DBT
C3DBT
PHN
C1PHN
C2PHN
C3PHN
C4PHN
ANT
FLA
PYR
C1FLA
BAA
CHR
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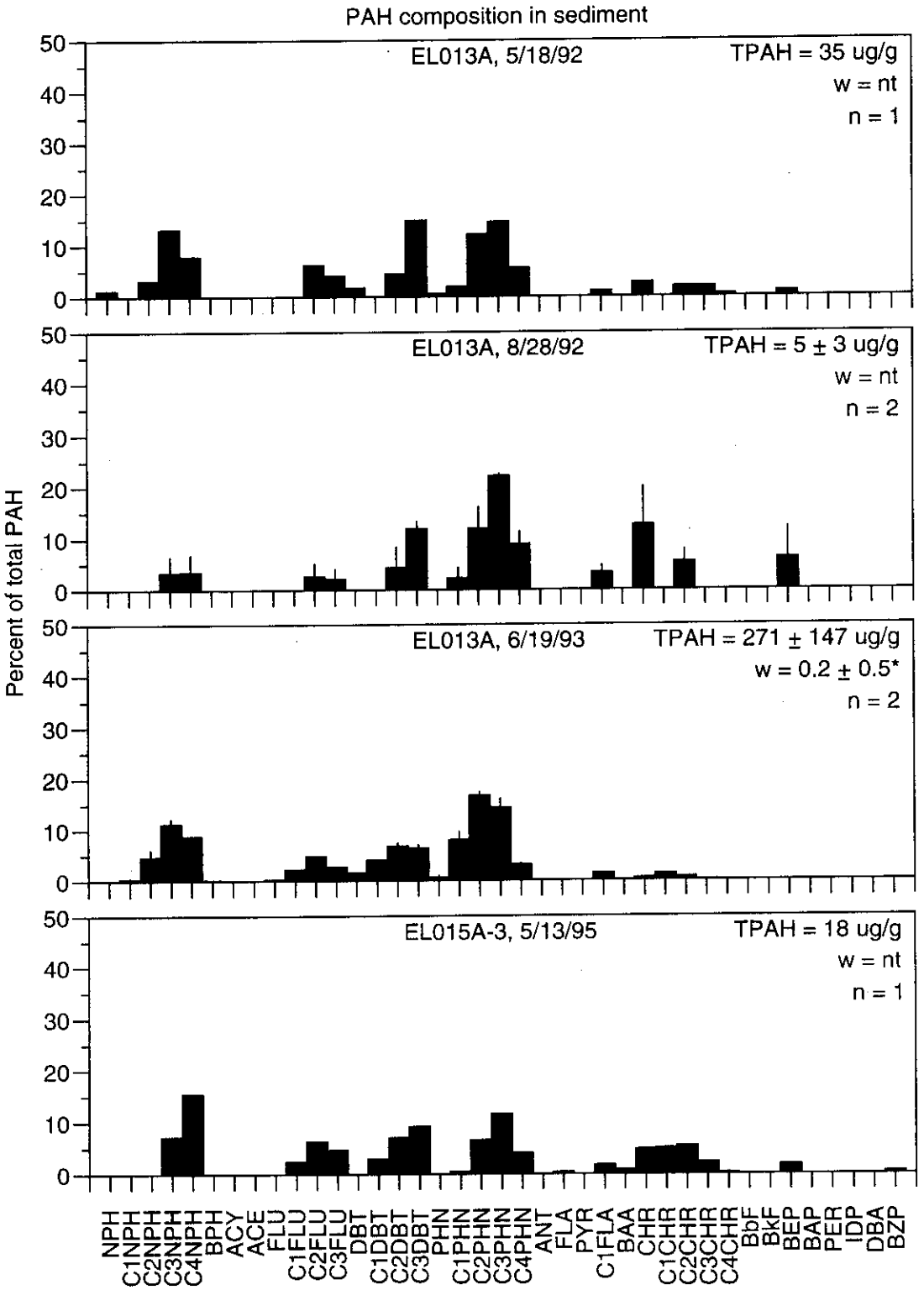
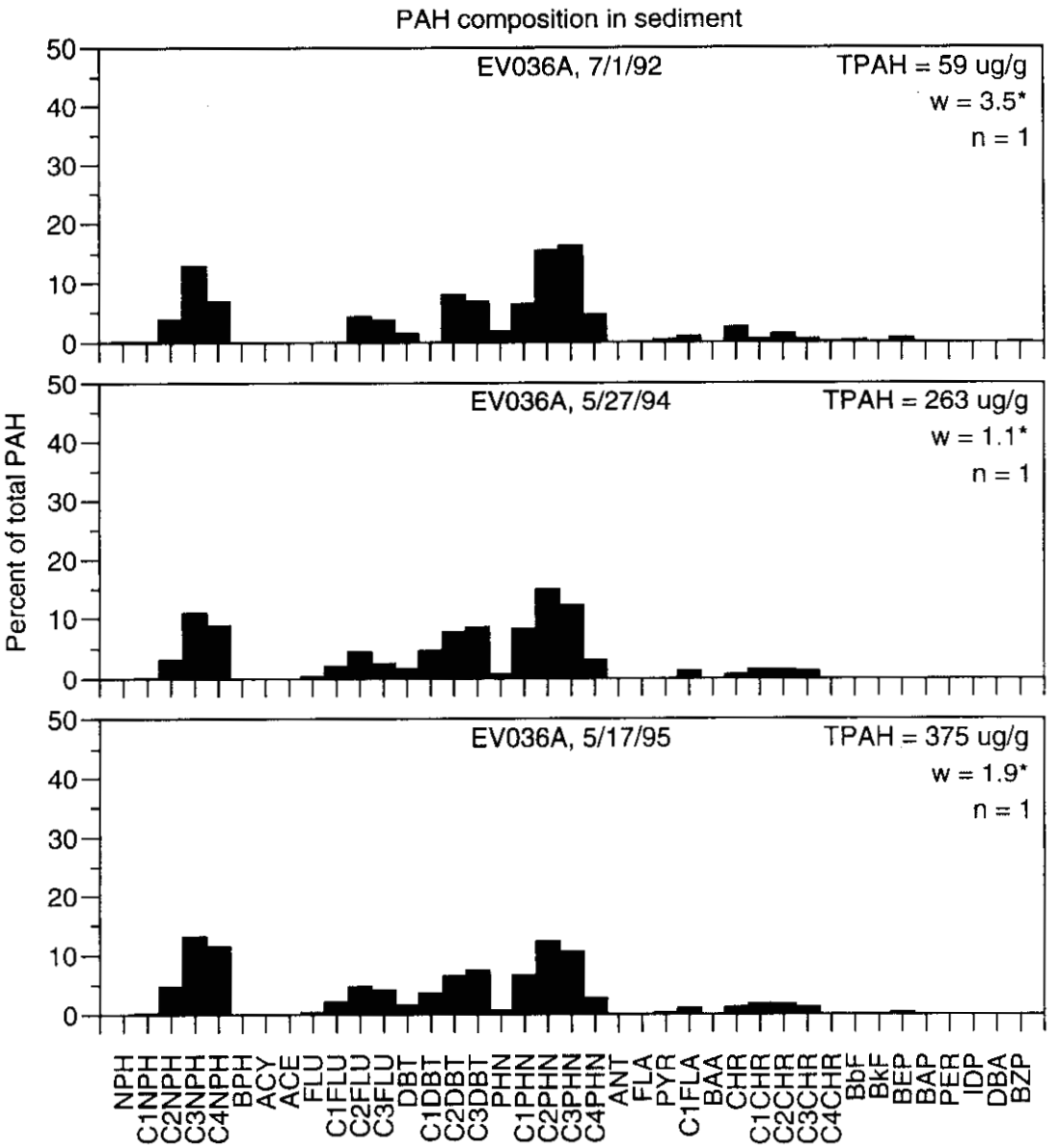
BkF
BEP
BAP
PER
IDP
DBA
BZP


PAH composition in sediment
Percent of total PAH
EL013A, 5/18/92
TPAH = 35 ug/g
w = nt
n = 1
EL013A, 8/28/92
TPAH = 5 ± 3 ug/g
w = nt
n = 2
EL013A, 6/19/93
TPAH = 271 ± 147 ug/g
w = 0.2 ± 0.5*
n = 2
EL015A-3, 5/13/95
TPAH = 18 ug/g
w = nt
n = 1
0
10
20
30
40
50
NPH
C1NPH
C2NPH
C3NPH
C4NPH
BPH
ACY
ACE
FLU
C1FLU
C2FLU
C3FLU
DBT
C1DBT
C2DBT
C3DBT
PHN
C1PHN
C2PHN
C3PHN
C4PHN
ANT
FLA
PYR
C1FLA
BAA
CHR
C1CHR
C2CHR
C3CHR
C4CHR
BbF
BkF
BEP
BAP
PER
IDP
DBA
BZP


PAH composition in sediment
EV036A, 7/1/92
TPAH = 59 ug/g
w = 3.5*
n = 1
EV036A, 5/27/94
TPAH = 263 ug/g
w = 1.1*
n = 1
EV036A, 5/17/95
TPAH = 375 ug/g
w = 1.9*
n = 1
Percent of total PAH
0
10
20
30
40
50
NPH
C1NPH
C2NPH
C3NPH
C4NPH
BPH
ACY
ACE
FLU
C1FLU
C2FLU
C3FLU
DBT
C1DBT
C2DBT
C3DBT
PHN
C1PHN
C2PHN
C3PHN
C4PHN
ANT
FLA
PYR
C1FLA
BAA
CHR
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C4CHR
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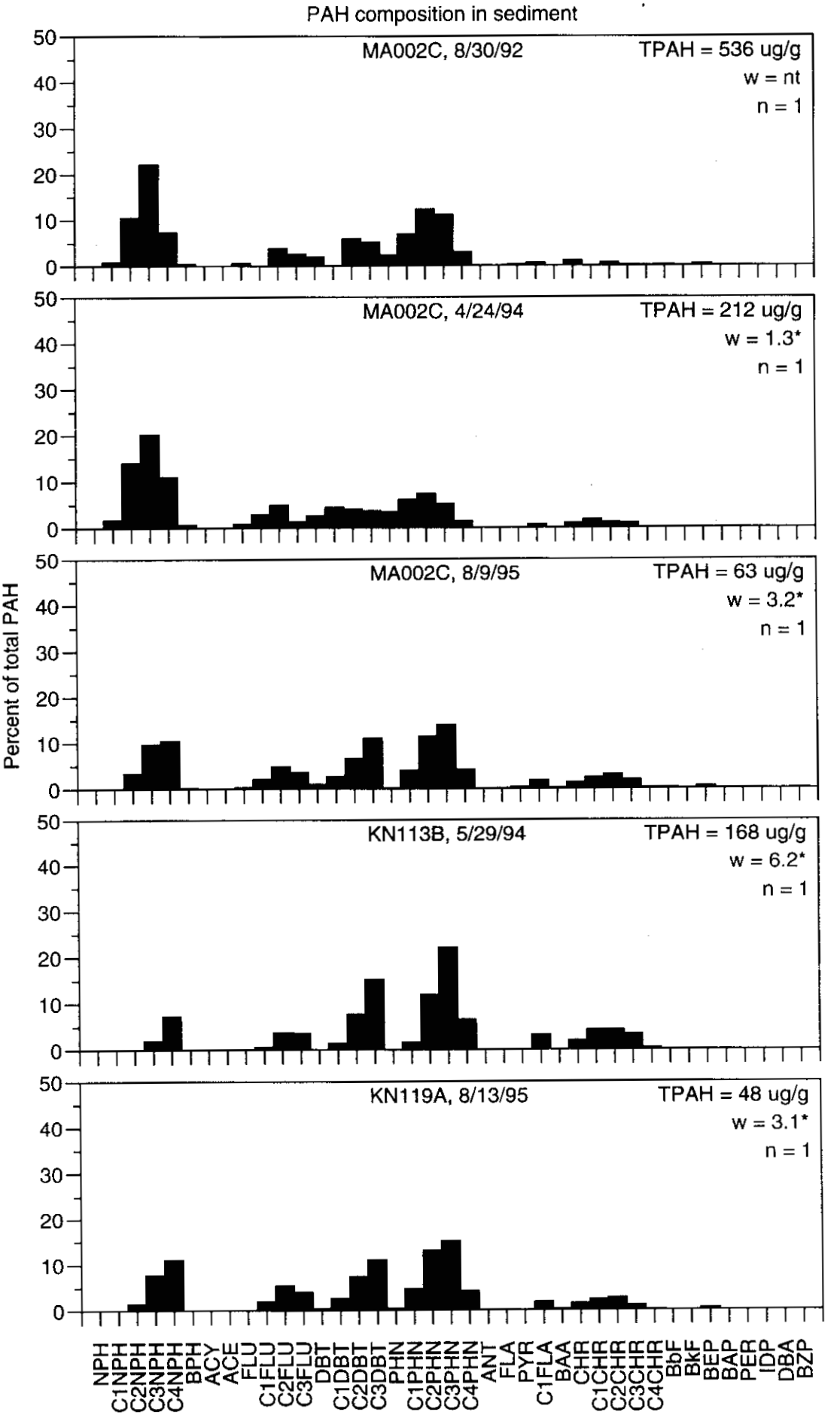
BkF
BEP
BAP
PER
IDP
DBA
BZP


PAH composition in sediment
Percent of total PAH
MA002C, 8/30/92
TPAH = 536 ug/g
w = nt
n = 1
MA002C, 4/24/94
TPAH = 212 ug/g
w = 1.3*
n = 1
MA002C, 8/9/95
TPAH = 63 ug/g
w = 3.2*
n = 1
KN113B, 5/29/94
TPAH = 168 ug/g
w = 6.2*
n = 1
KN119A, 8/13/95
TPAH = 48 ug/g
w = 3.1*
n = 1
0
10
20
30
40
50
NPH
C1NPH
C2NPH
C3NPH
C4NPH
BPH
ACY
ACE
FLU
C1FLU
C2FLU
C3FLU
DBT
C1DBT
C2DBT
C3DBT
PHN
C1PHN
C2PHN
C3PHN
C4PHN
ANT
FLA
PYR
C1FLA
BAA
CHR
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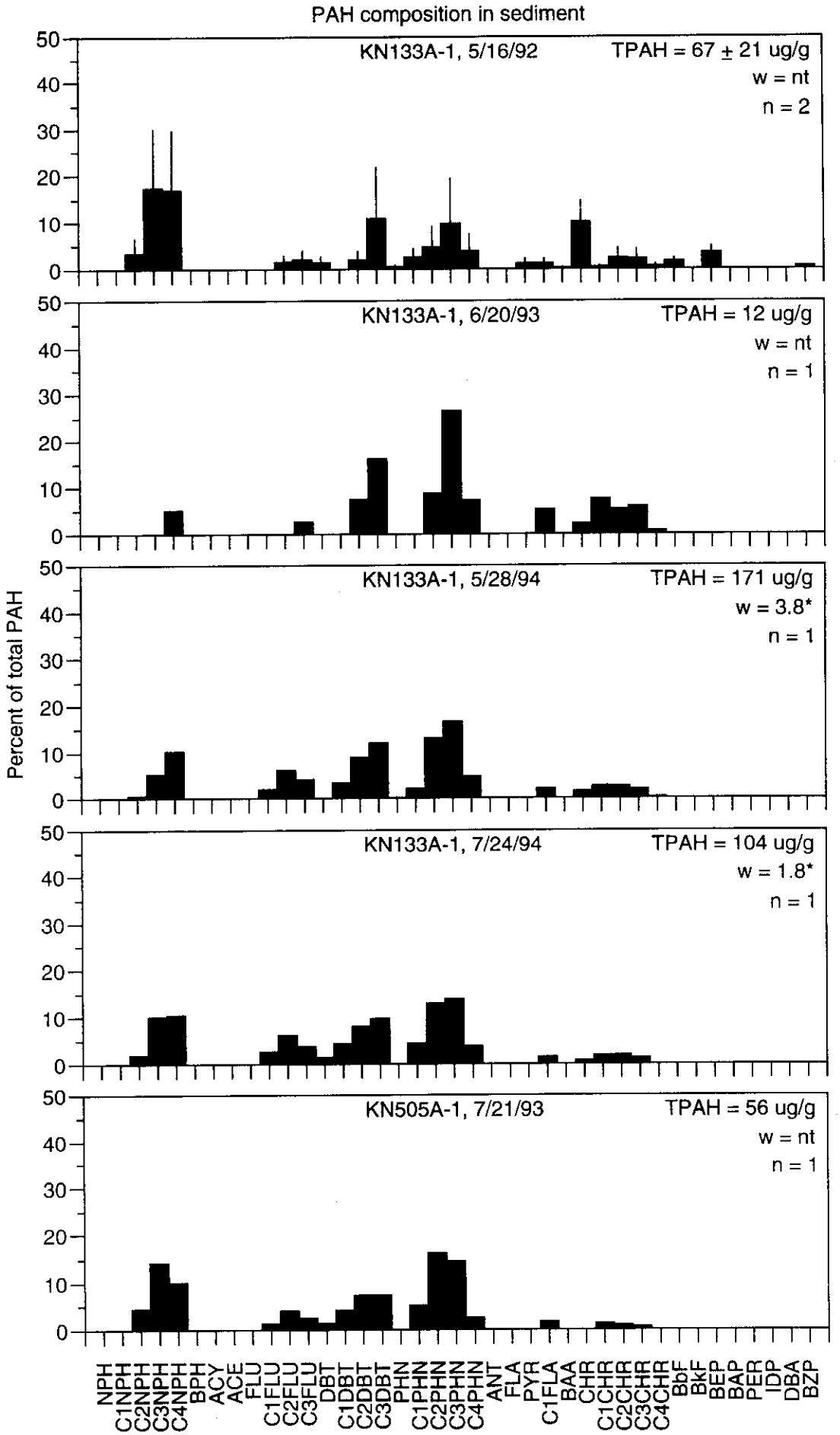
C4CHR
BbF
BkF
BEP
BAP
PER
IDP
DBA
BZP


PAH composition in sediment
Percent of total PAH
KN133A-1, 5/16/92
TPAH = 67 ± 21 ug/g
w = nt
n = 2
KN133A-1, 6/20/93
TPAH = 12 ug/g
w = nt
n = 1
KN133A-1, 5/28/94
TPAH = 171 ug/g
w = 3.8*
n = 1
KN133A-1, 7/24/94
TPAH = 104 ug/g
w = 1.8*
n = 1
KN505A-1, 7/21/93
TPAH = 56 ug/g
w = nt
n = 1
0
10
20
30
40
50
NPH
C1NPH
C2NPH
C3NPH
C4NPH
BPH
ACY
ACE
FLU
C1FLU
C2FLU
C3FLU
DBT
C1DBT
C2DBT
C3DBT
PHN
C1PHN
C2PHN
C3PHN
C4PHN
ANT
FLA
PYR
C1FLA
BAA
CHR
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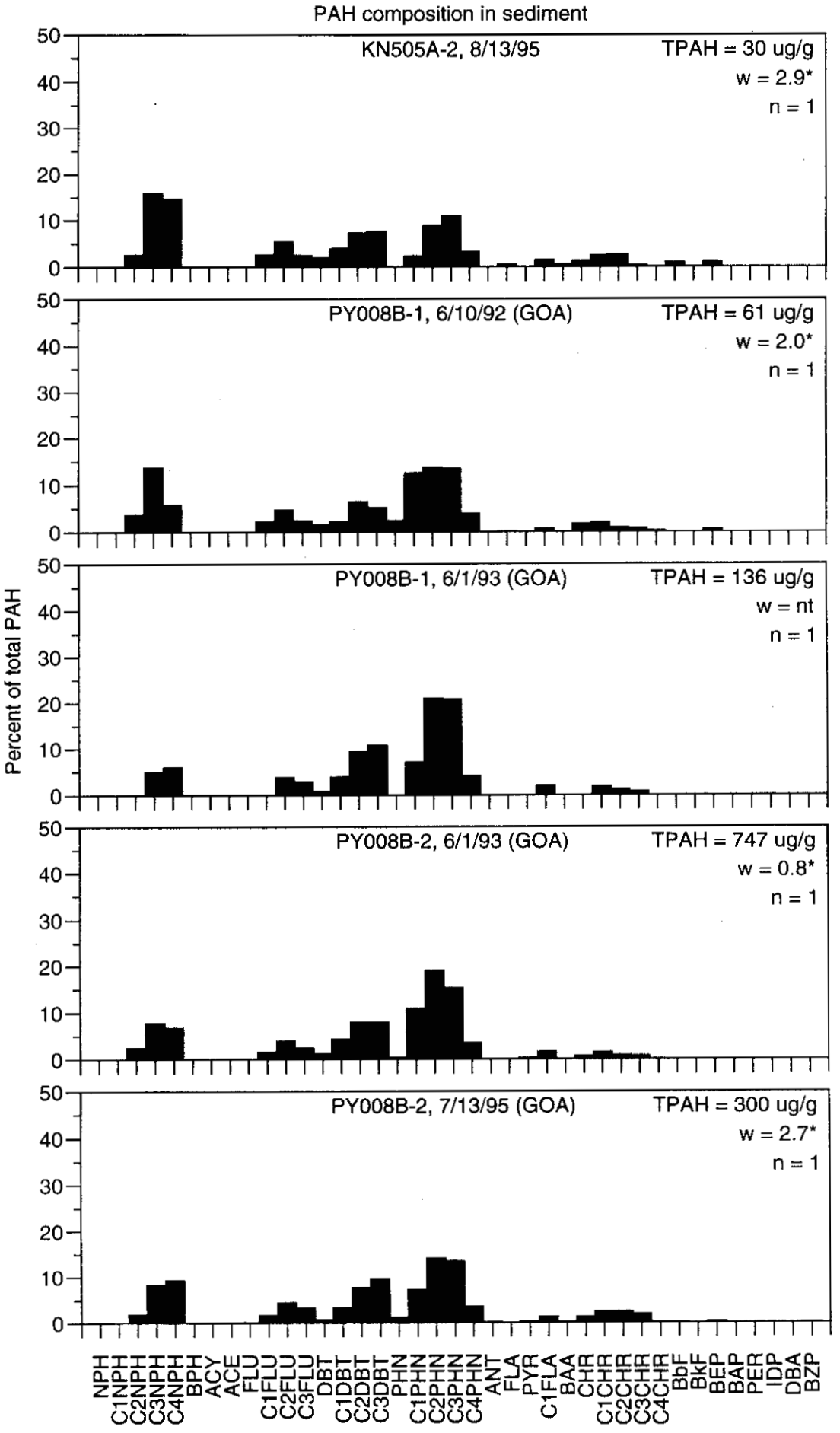
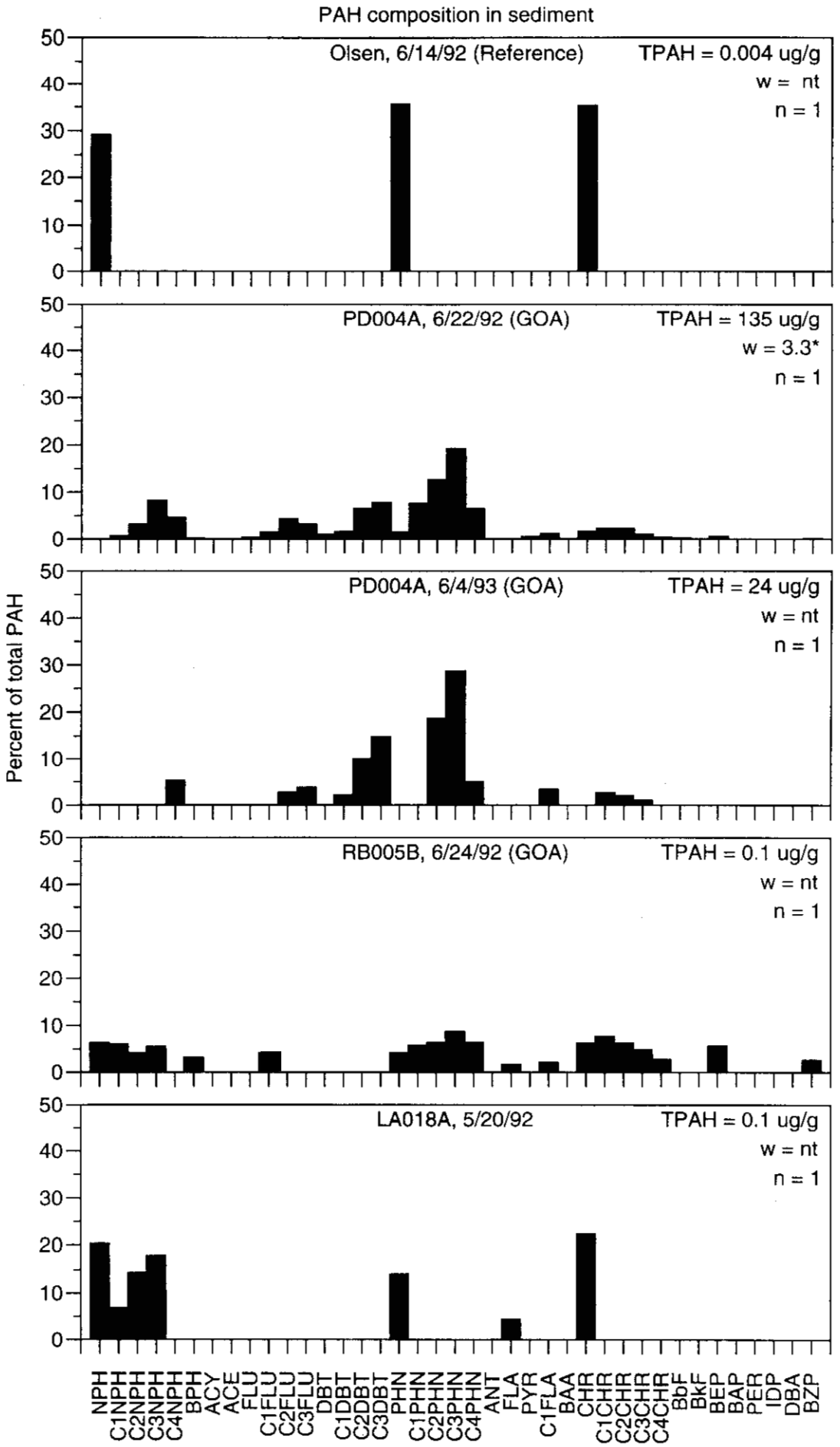
C4CHR
BbF
BkF
BEP
BAP
PER
IDP
DBA
BZP


PAH composition in sediment
Percent of total PAH
KN505A-2, 8/13/95
TPAH = 30 ug/g
w = 2.9*
n = 1
PY008B-1, 6/10/92 (GOA)
TPAH = 61 ug/g
w = 2.0*
n = 1
PY008B-1, 6/1/93 (GOA)
TPAH = 136 ug/g
w = nt
n = 1
PY008B-2, 6/1/93 (GOA)
TPAH = 747 ug/g
w = 0.8*
n = 1
PY008B-2, 7/13/95 (GOA)
TPAH = 300 ug/g
w = 2.7*
n = 1
0
10
20
30
40
50
NPH
C1NPH
C2NPH
C3NPH
C4NPH
BPH
ACY
ACE
FLU
C1FLU
C2FLU
C3FLU
DBT
C1DBT
C2DBT
C3DBT
PHN
C1PHN
C2PHN
C3PHN
C4PHN
ANT
FLA
PYR
C1FLA
BAA
CHR
C1CHR
C2CHR
C3CHR
C4CHR
BbF
BkF
BEP
BAP
PER
IDP
DBA
BZP


PAH composition in sediment
Percent of total PAH
Olsen, 6/14/92 (Reference)
TPAH = 0.004 ug/g
w = nt
n = 1
PD004A, 6/22/92 (GOA)
TPAH = 135 ug/g
w = 3.3*
n = 1
PD004A, 6/4/93 (GOA)
TPAH = 24 ug/g
w = nt
n = 1
RB005B, 6/24/92 (GOA)
TPAH = 0.1 ug/g
w = nt
n = 1
LA018A, 5/20/92
TPAH = 0.1 ug/g
w = nt
n = 1
0
10
20
30
40
50
NPH
C1NPH
C2NPH
C3NPH
C4NPH
BPH
ACY
ACE
FLU
C1FLU
C2FLU
C3FLU
DBT
C1DBT
C2DBT
C3DBT
PHN
C1PHN
C2PHN
C3PHN
C4PHN
ANT
FLA
PYR
C1FLA
BAA
CHR
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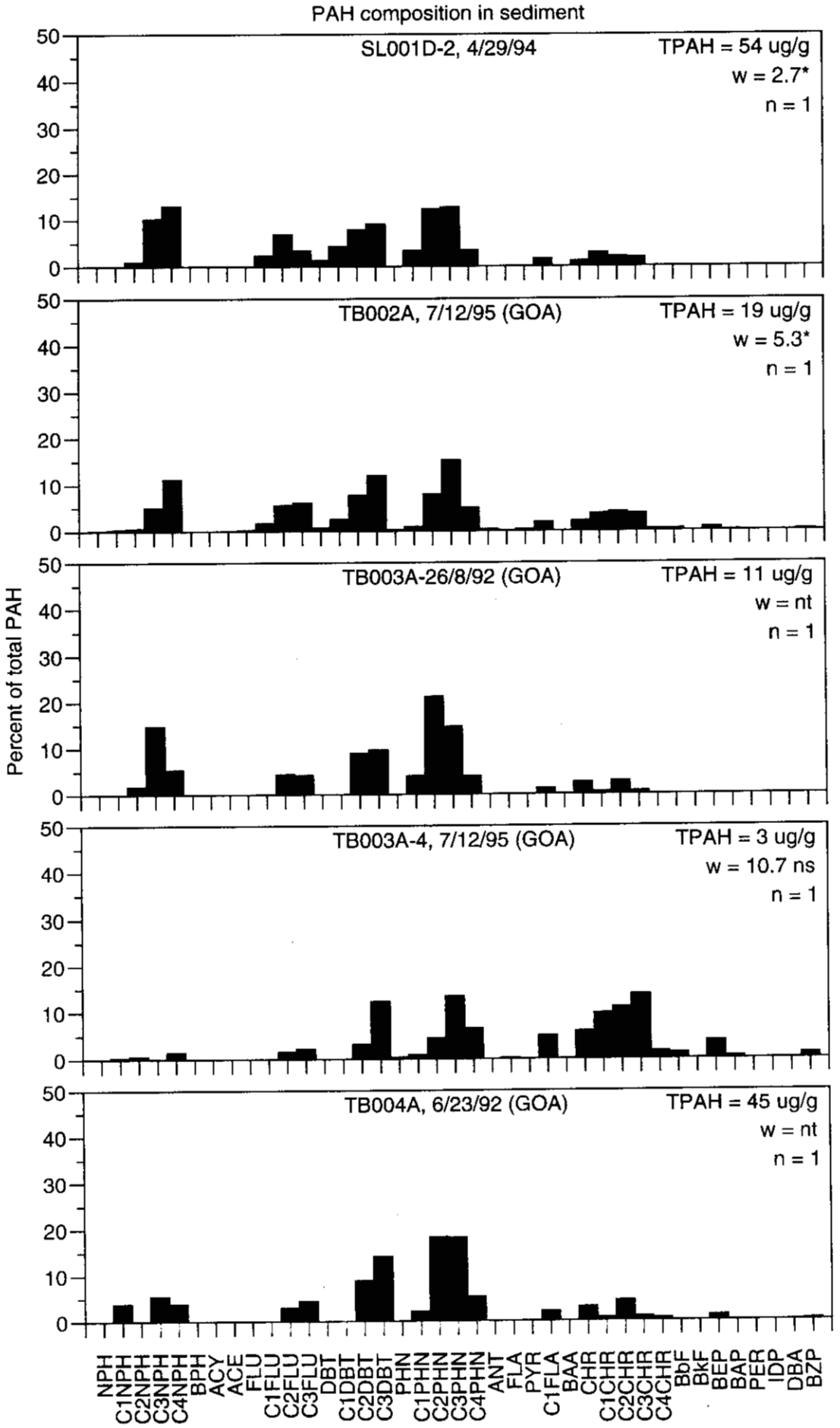
C2CHR
C3CHR
C4CHR
BbF
BkF
BEP
BAP
PER
IDP
DBA
BZP


PAH composition in sediment
Percent of total PAH
SL001D-2, 4/29/94
TPAH = 54 ug/g
w = 2.7*
n = 1
TB002A, 7/12/95 (GOA)
TPAH = 19 ug/g
w = 5.3*
n = 1
TB003A-26/8/92 (GOA)
TPAH = 11 ug/g
w = nt
n = 1
TB003A-4, 7/12/95 (GOA)
TPAH = 3 ug/g
w = 10.7 ns
n = 1
TB004A, 6/23/92 (GOA)
TPAH = 45 ug/g
w = nt
n = 1
0
10
20
30
40
50
NPH
C1NPH
C2NPH
C3NPH
C4NPH
BPH
ACY
ACE
FLU
C1FLU
C2FLU
C3FLU
DBT
C1DBT
C2DBT
C3DBT
PHN
C1PHN
C2PHN
C3PHN
C4PHN
ANT
FLA
PYR
C1FLA
BAA
CHR
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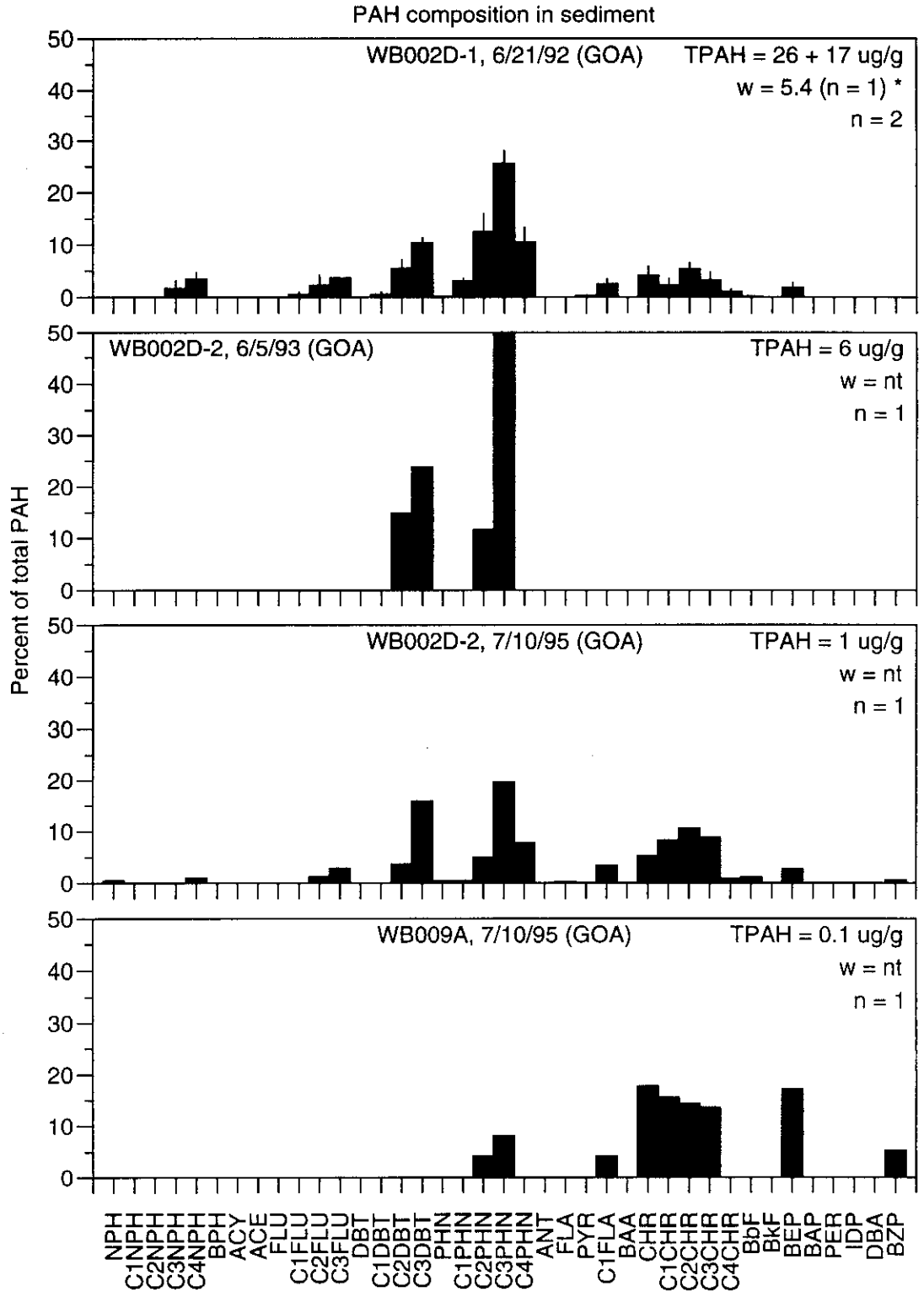
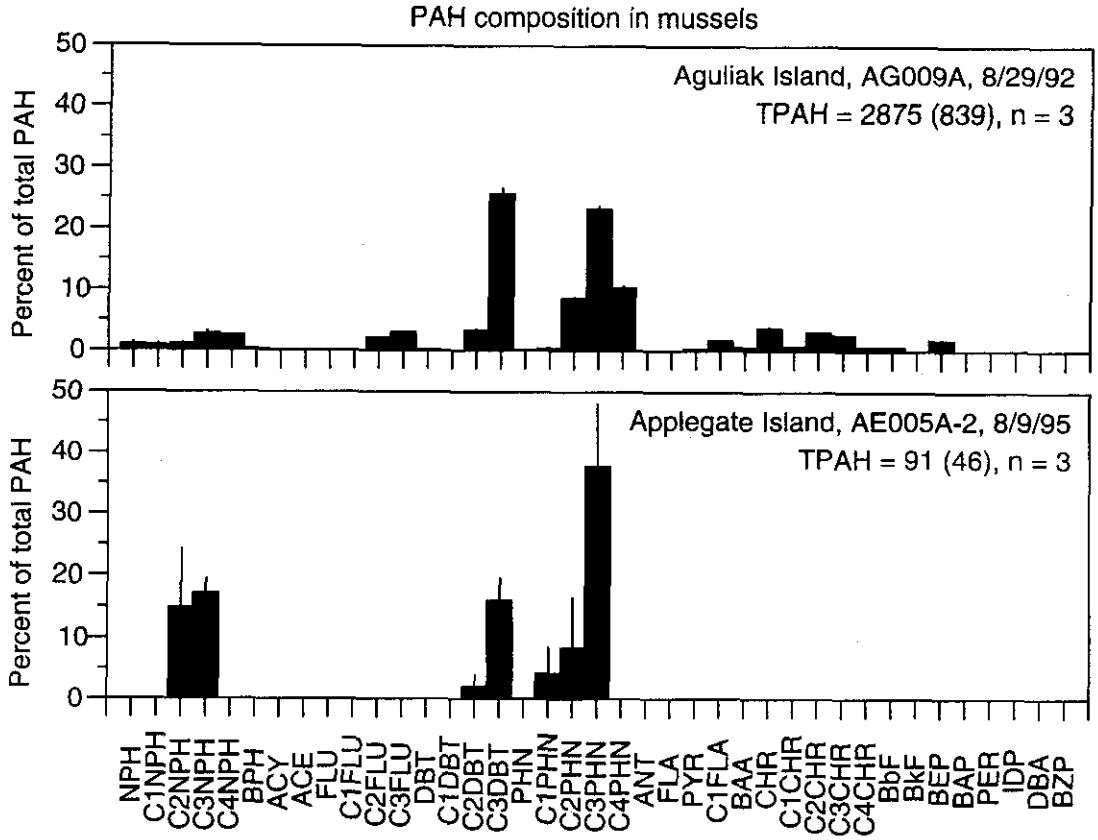
C3CHR
C4CHR
BbF
BkF
BEP
BAP
PER
IDP
DBA
BZP


PAH composition in sediment
Percent of total PAH
WB002D-1, 6/21/92 (GOA)
TPAH = 26 + 17 ug/g
w = 5.4 (n = 1) *
n = 2
WB002D-2, 6/5/93 (GOA)
TPAH = 6 ug/g
w = nt
n = 1
WB002D-2, 7/10/95 (GOA)
TPAH = 1 ug/g
w = nt
n = 1
WB009A, 7/10/95 (GOA)
TPAH = 0.1 ug/g
w = nt
n = 1
0
10
20
30
40
50
NPH
C1NPH
C2NPH
C3NPH
C4NPH
BPH
ACY
ACE
FLU
C1FLU
C2FLU
C3FLU
DBT
C1DBT
C2DBT
C3DBT
PHN
C1PHN
C2PHN
C3PHN
C4PHN
ANT
FLA
PYR
C1FLA
BAA
CHR
C1CHR
C2CHR
C3CHR
C4CHR
BbF
BkF
BEP
BAP
PER
IDP
DBA
BZP

Appendix 6 *(at right)*. Polynuclear aromatic hydrocarbon (PAH) composition in sediments. Each bed is identified by the *Exxon Valdez* Interagency Shoreline Cleanup Committee segment number plus a unique suffix. Mean total PAH (TPAH) concentrations (±SE) and sample sizes are reported for each data set. Mean weathering (w) (±SE) is reported where estimable; ns = not significant (source was not *Exxon Valdez* oil), n = number of estimable sets, and asterisks indicate *Exxon Valdez* oil was verified as the source of contamination in one or more samples (Short and Heintz 1997). Data are from Chapter 1 in this report. Figure continues on the next 44 pages.

Short, J. W. and R. A. Heintz. 1997. Identification of *Exxon Valdez* oil in sediments and tissues from Prince William Sound and the Norwestern Gulf of Alaska based on a PAH weathering model. Environ. Sci. Technol. 31:2375-2384.


PAH composition in mussels
Aguliak Island, AG009A, 8/29/92
TPAH = 2875 (839), n = 3
Applegate Island, AE005A-2, 8/9/95
TPAH = 91 (46), n = 3
Percent of total PAH
50
40
30
20
10
0
NPH
C1NPH
C2NPH
C3NPH
C4NPH
BPH
ACY
ACE
FLU
C1FLU
C2FLU
C3FLU
DBT
C1DBT
C2DBT
C3DBT
PHN
C1PHN
C2PHN
C3PHN
C4PHN
ANT
FLA
PYR
C1FLA
BAA
CHR
C1CHR
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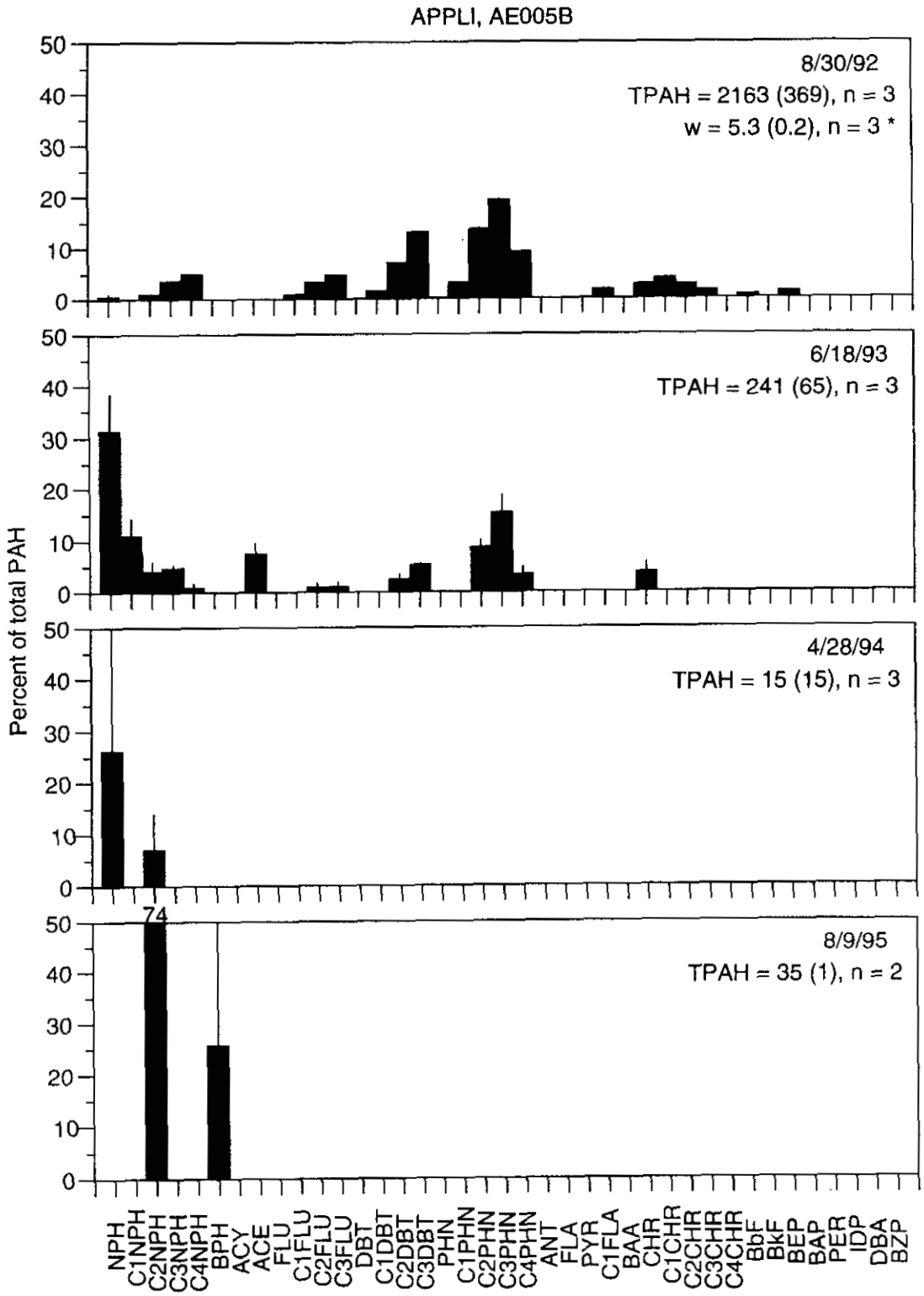
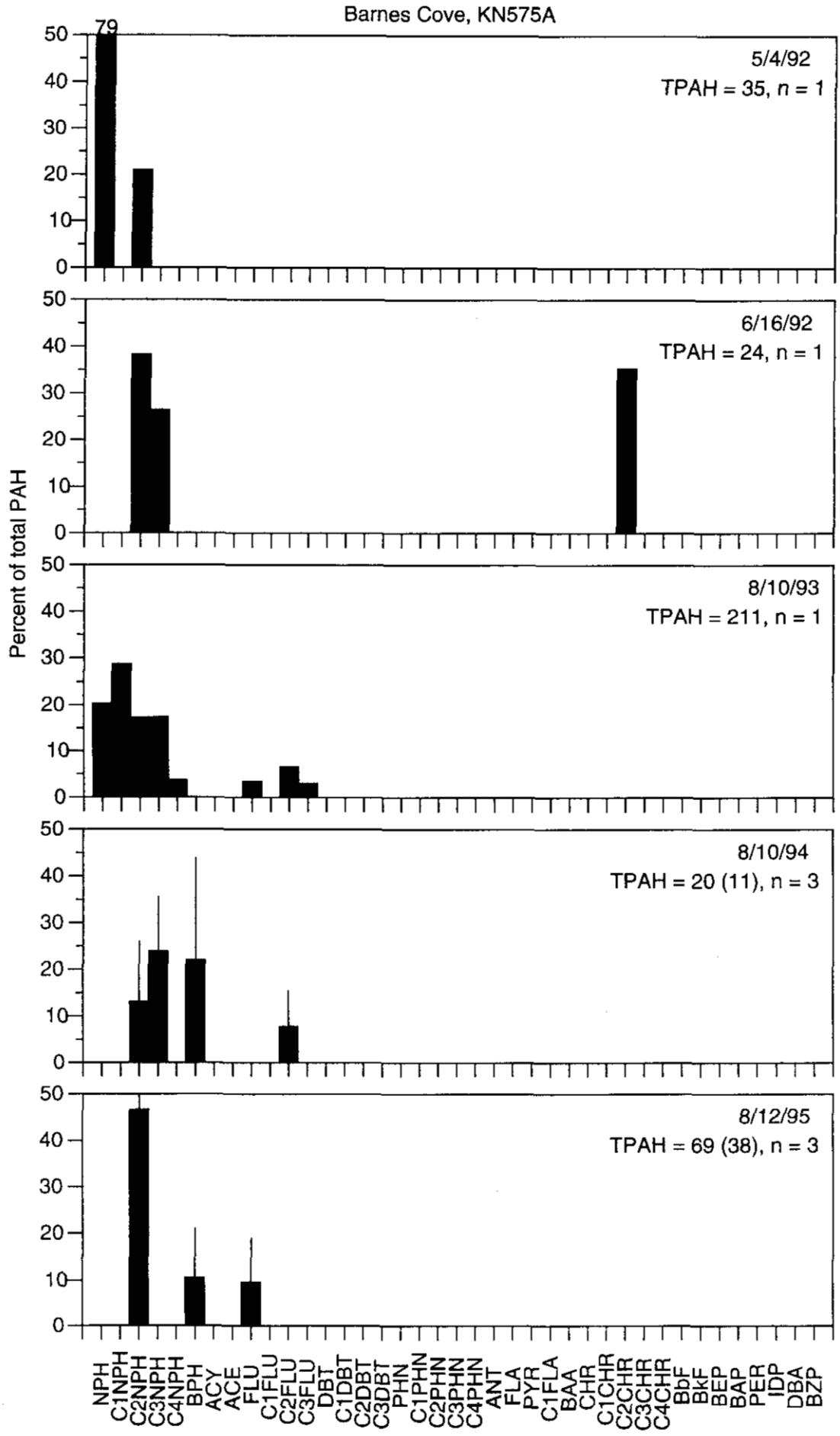
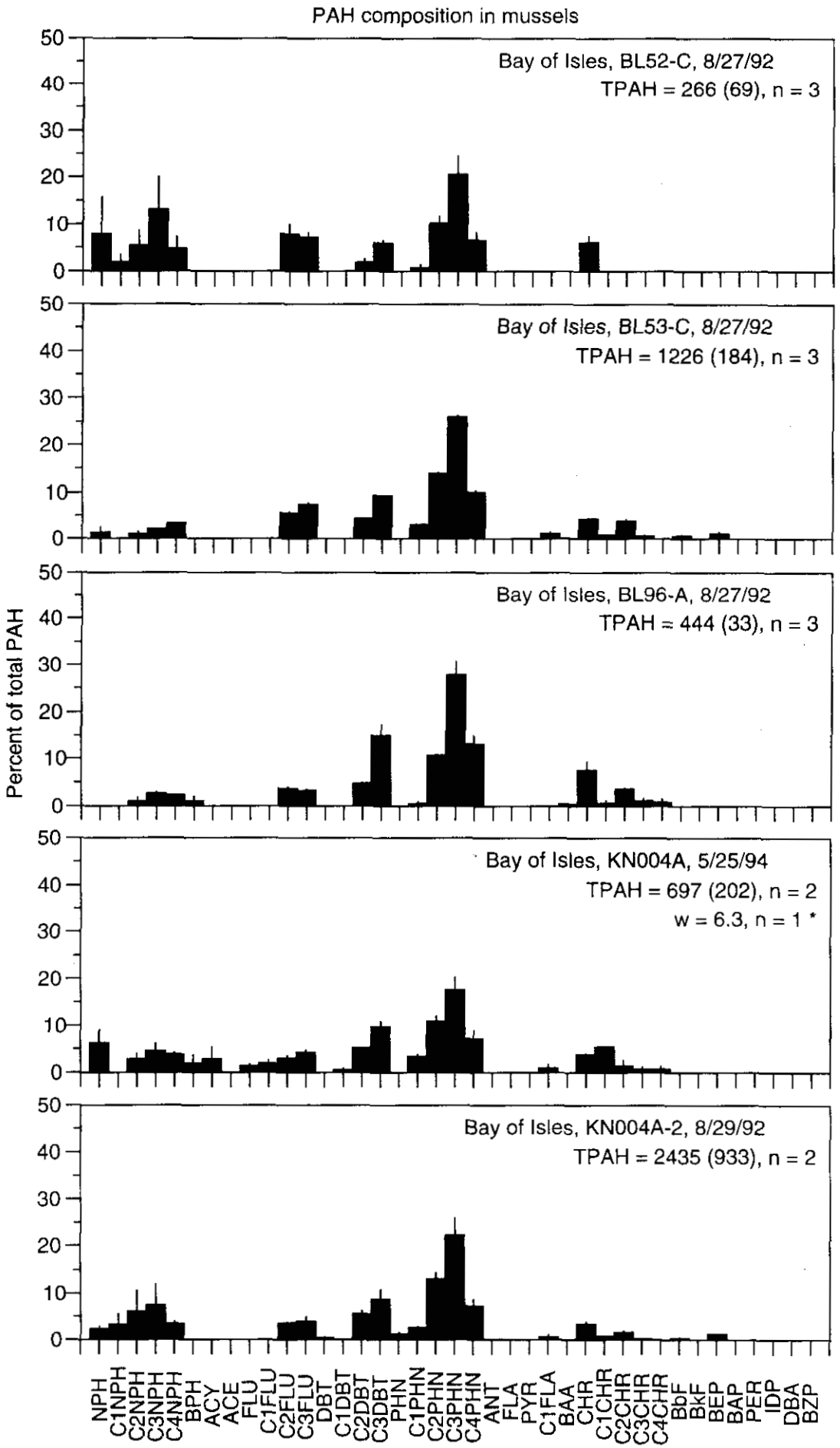
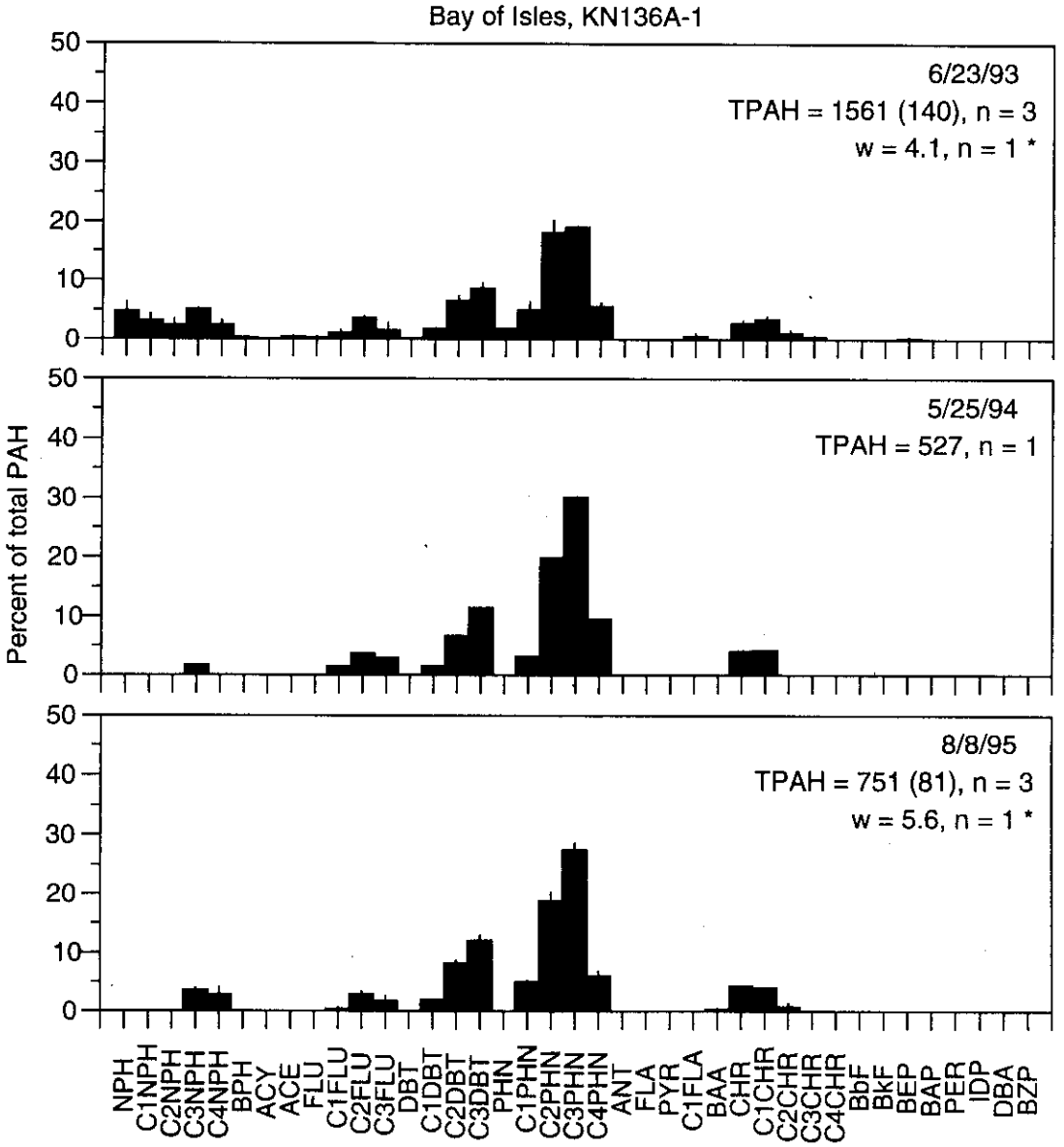
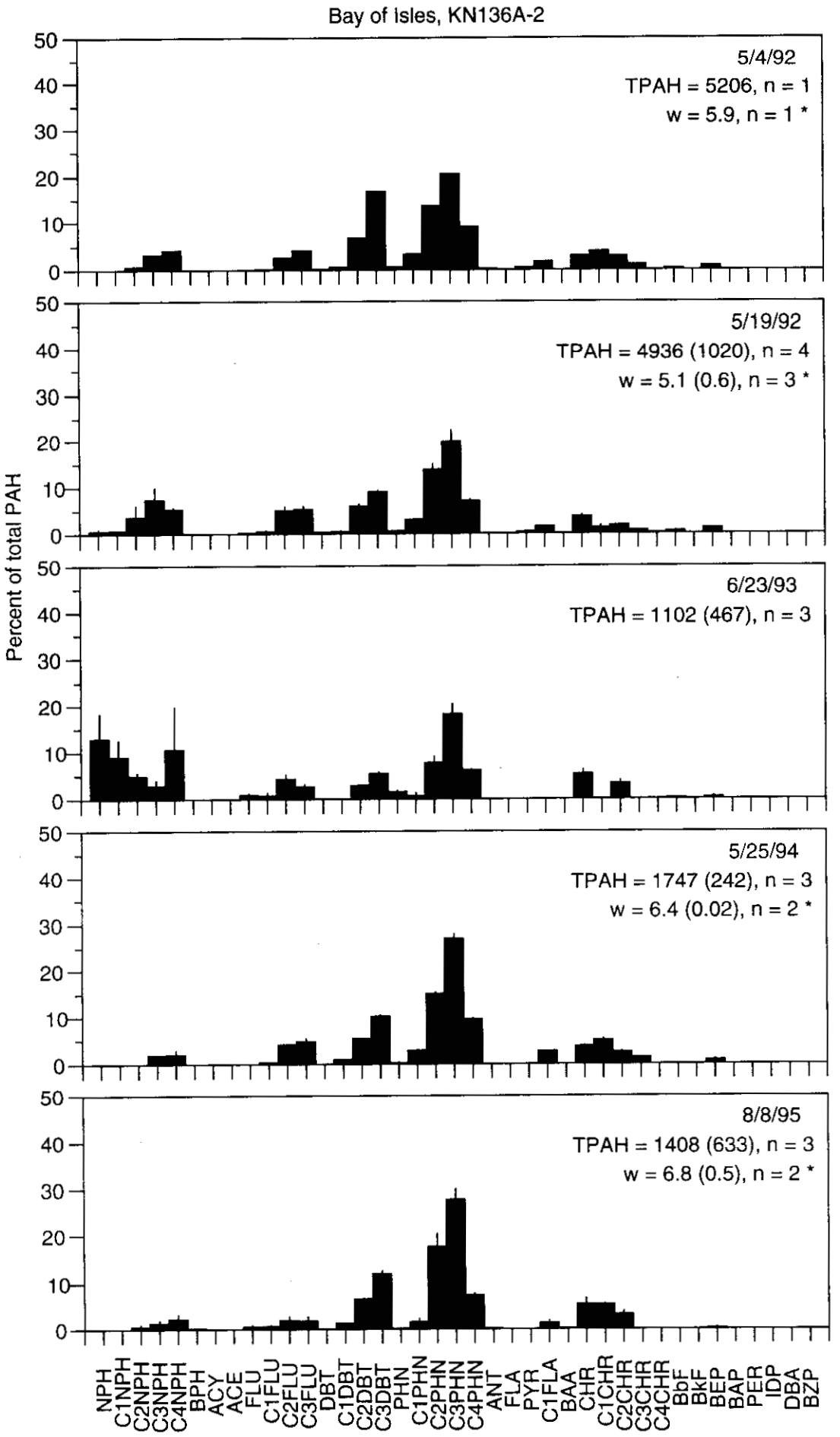
C4CHR
BbF
BkF
BEP
BAP
PER
IDP
DBA
BZP

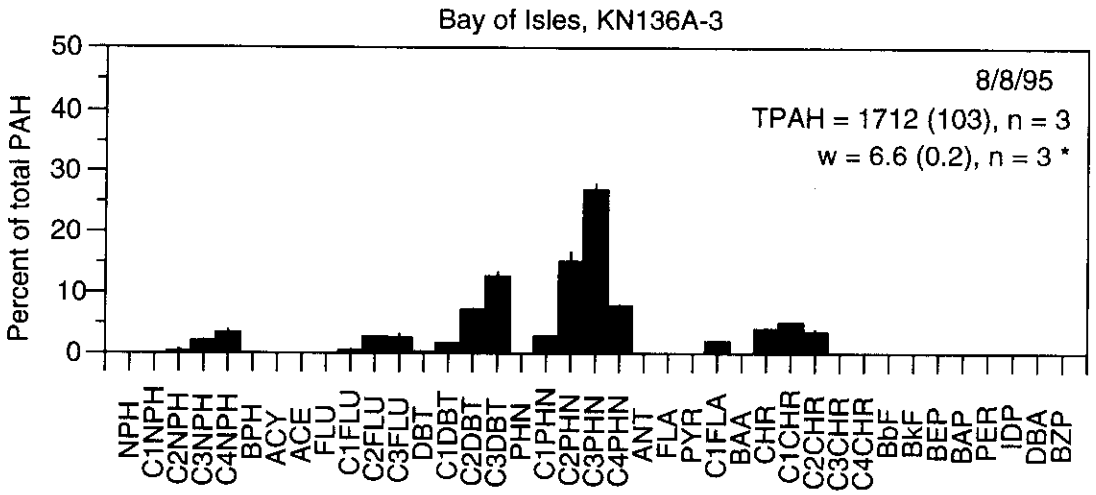

APPLI, AE005B
Percent of total PAH
8/30/92
TPAH = 2163 (369), n = 3
w = 5.3 (0.2), n = 3 *
6/18/93
TPAH = 241 (65), n = 3
4/28/94
TPAH = 15 (15), n = 3
8/9/95
TPAH = 35 (1), n = 2
74
NPH
C1NPH
C2NPH
C3NPH
C4NPH
BPH
ACY
ACE
FLU
C1FLU
C2FLU
C3FLU
DBT
C1DBT
C2DBT
C3DBT
PHN
C1PHN
C2PHN
C3PHN
C4PHN
ANT
FLA
PYR
C1FLA
BAA
CHR
C1CHR
C2CHR
C3CHR
C4CHR
BbF
BkF
BEP
BAP
PER
IDP
DBA
BZP

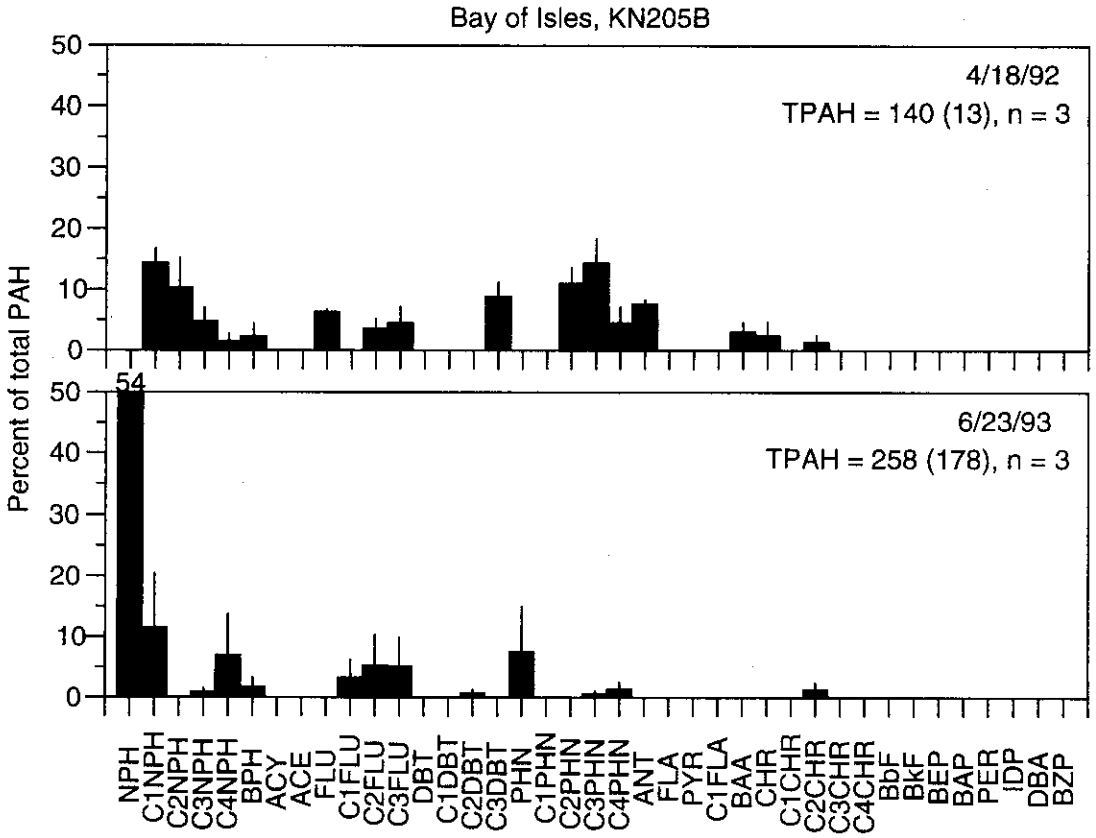

Barnes Cove, KN575A
Percent of total PAH
79
5/4/92
TPAH = 35, n = 1
6/16/92
TPAH = 24, n = 1
8/10/93
TPAH = 211, n = 1
8/10/94
TPAH = 20 (11), n = 3
8/12/95
TPAH = 69 (38), n = 3
0
10
20
30
40
50
NPH
C1NPH
C2NPH
C3NPH
C4NPH
BPH
ACY
ACE
FLU
C1FLU
C2FLU
C3FLU
DBT
C1DBT
C2DBT
C3DBT
PHN
C1PHN
C2PHN
C3PHN
C4PHN
ANT
FLA
PYR
C1FLA
BAA
CHR
C1CHR
C2CHR
C3CHR
C4CHR
BbF
BkF
BEP
BAP
PER
IDP
DBA
BZP

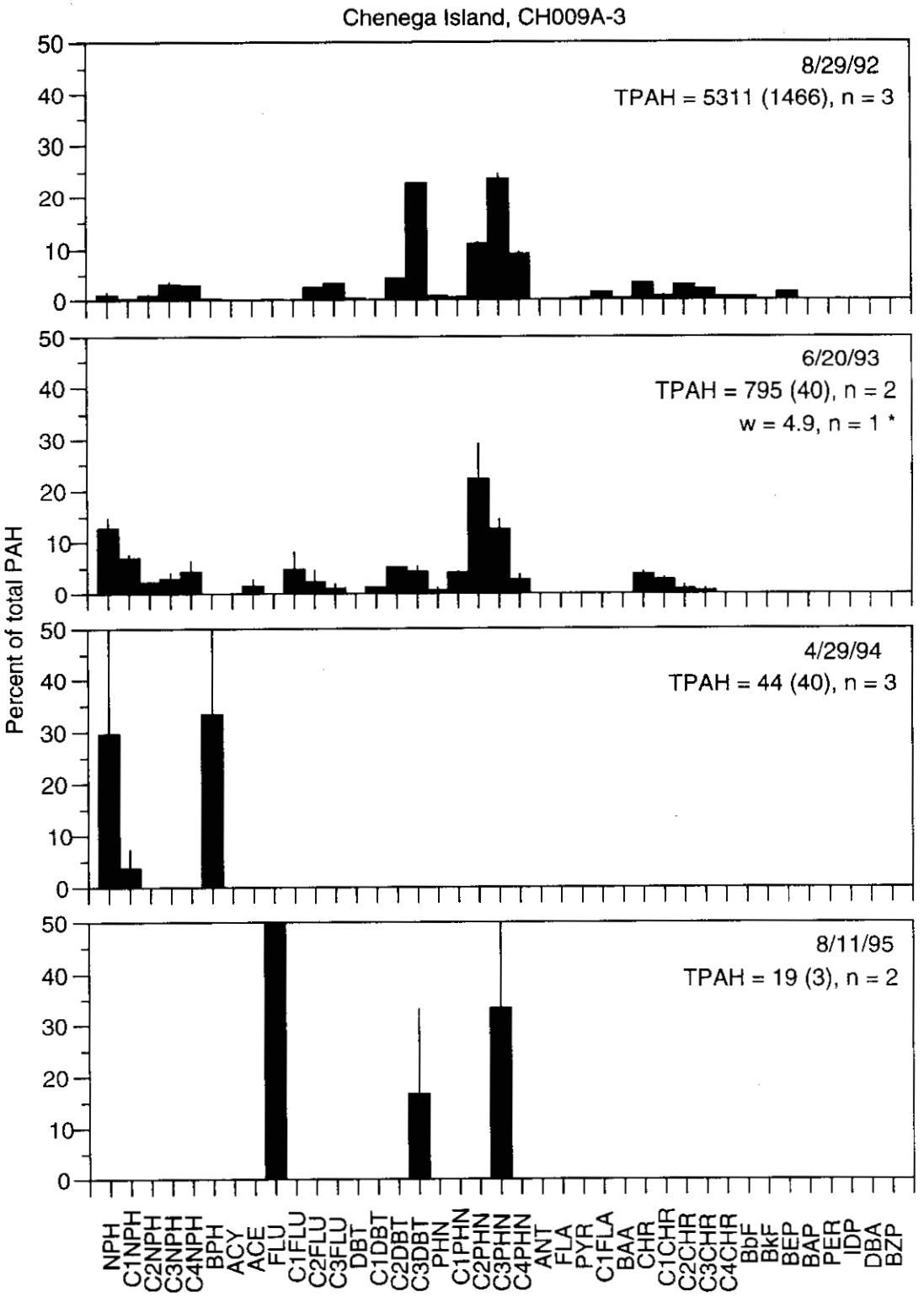

PAH composition in mussels
Percent of total PAH
Bay of Isles, BL52-C, 8/27/92
TPAH = 266 (69), n = 3
Bay of Isles, BL53-C, 8/27/92
TPAH = 1226 (184), n = 3
Bay of Isles, BL96-A, 8/27/92
TPAH = 444 (33), n = 3
Bay of Isles, KN004A, 5/25/94
TPAH = 697 (202), n = 2
w = 6.3, n = 1 *
Bay of Isles, KN004A-2, 8/29/92
TPAH = 2435 (933), n = 2
0
10
20
30
40
50
NPH
C1NPH
C2NPH
C3NPH
C4NPH
BPH
ACY
ACE
FLU
C1FLU
C2FLU
C3FLU
DBT
C1DBT
C2DBT
C3DBT
PHN
C1PHN
C2PHN
C3PHN
C4PHN
ANT
FLA
PYR
C1FLA
BAA
CHR
C1CHR
C2CHR
C3CHR
C4CHR
BbF
BkF
BEP
BAP
PER
IDP
DBA
BZP

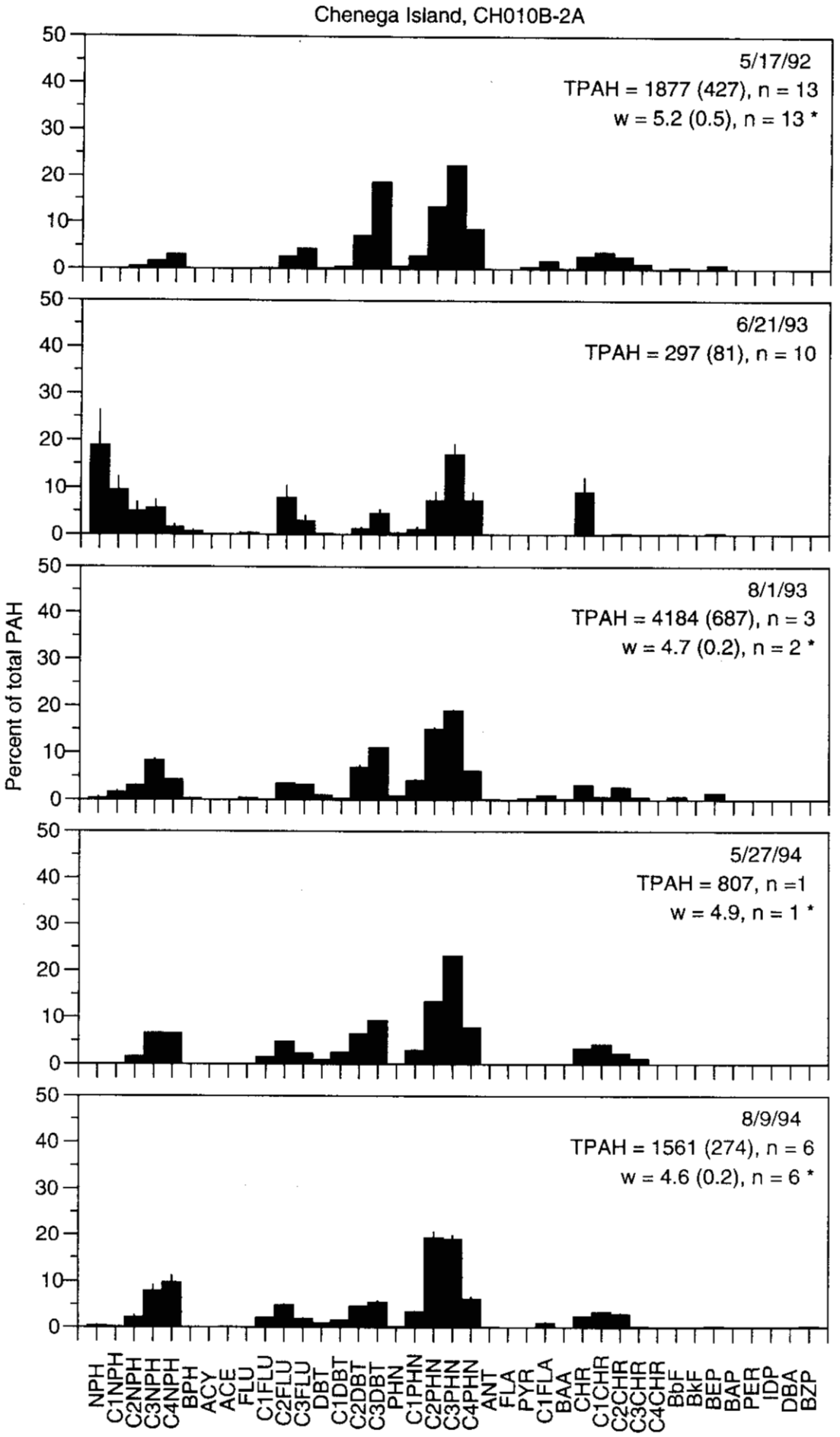

Bay of Isles, KN136A-1
Percent of total PAH
6/23/93
TPAH = 1561 (140), n = 3
w = 4.1, n = 1 *
5/25/94
TPAH = 527, n = 1
8/8/95
TPAH = 751 (81), n = 3
w = 5.6, n = 1 *
0
10
20
30
40
50
NPH
C1NPH
C2NPH
C3NPH
C4NPH
BPH
ACY
ACE
FLU
C1FLU
C2FLU
C3FLU
DBT
C1DBT
C2DBT
C3DBT
PHN
C1PHN
C2PHN
C3PHN
C4PHN
ANT
FLA
PYR
C1FLA
BAA
CHR
C1CHR
C2CHR
C3CHR
C4CHR
BbF
BkF
BEP
BAP
PER
IDP
DBA
BZP

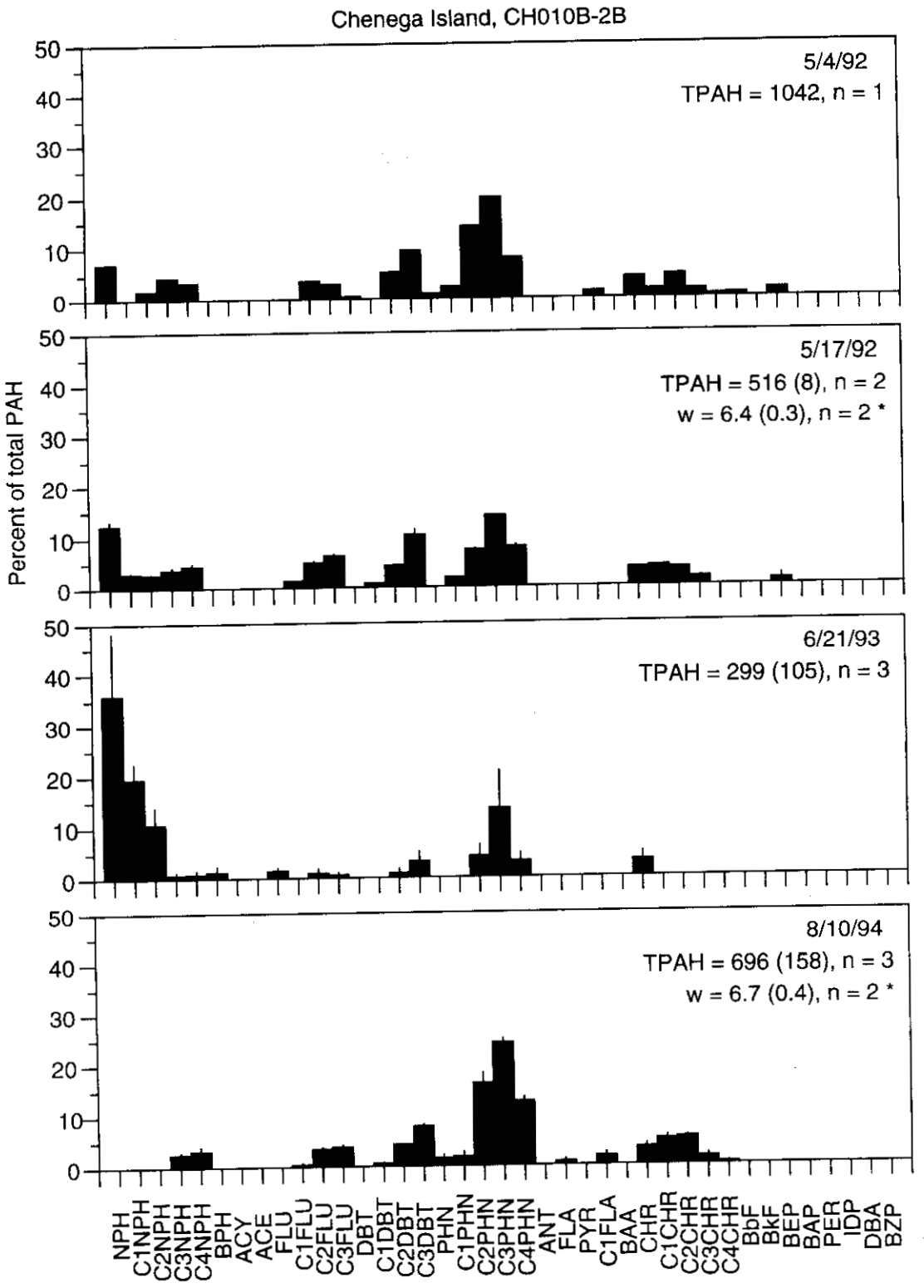

Bay of Isles, KN136A-2
Percent of total PAH
5/4/92
TPAH = 5206, n = 1
w = 5.9, n = 1 *
5/19/92
TPAH = 4936 (1020), n = 4
w = 5.1 (0.6), n = 3 *
6/23/93
TPAH = 1102 (467), n = 3
5/25/94
TPAH = 1747 (242), n = 3
w = 6.4 (0.02), n = 2 *
8/8/95
TPAH = 1408 (633), n = 3
w = 6.8 (0.5), n = 2 *
0
10
20
30
40
50
NPH
C1NPH
C2NPH
C3NPH
C4NPH
BPH
ACY
ACE
FLU
C1FLU
C2FLU
C3FLU
DBT
C1DBT
C2DBT
C3DBT
PHN
C1PHN
C2PHN
C3PHN
C4PHN
ANT
FLA
PYR
C1FLA
BAA
CHR
C1CHR
C2CHR
C3CHR
C4CHR
BbF
BkF
BEP
BAP
PER
IDP
DBA
BZP

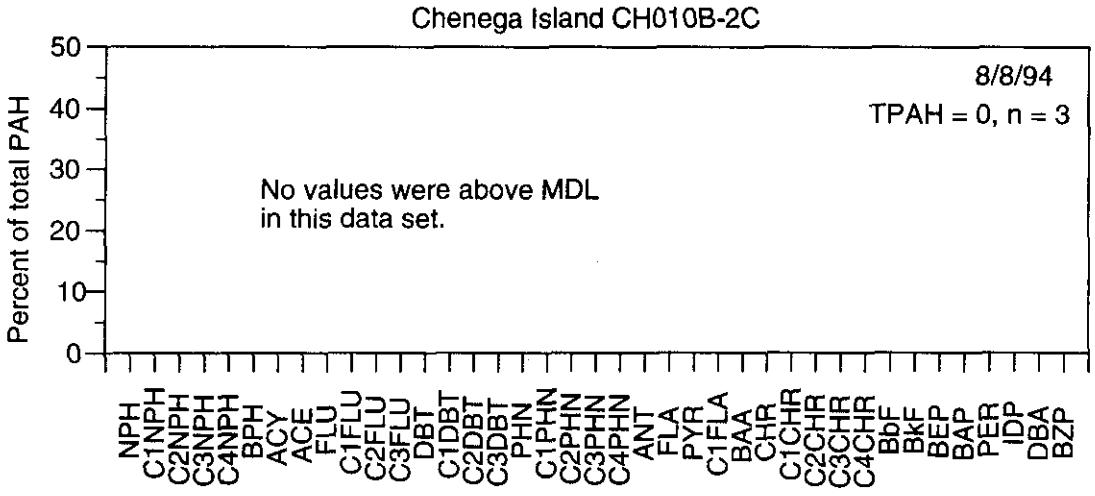

Bay of Isles, KN136A-3
8/8/95
TPAH = 1712 (103), n = 3
w = 6.6 (0.2), n = 3 *
Percent of total PAH
0
10
20
30
40
50
NPH
C1NPH
C2NPH
C3NPH
C4NPH
BPH
ACY
ACE
FLU
C1FLU
C2FLU
C3FLU
DBT
C1DBT
C2DBT
C3DBT
PHN
C1PHN
C2PHN
C3PHN
C4PHN
ANT
FLA
PYR
C1FLA
BAA
CHR
C1CHR
C2CHR
C3CHR
C4CHR
BbF
BkF
BEP
BAP
PER
IDP
DBA
BZP

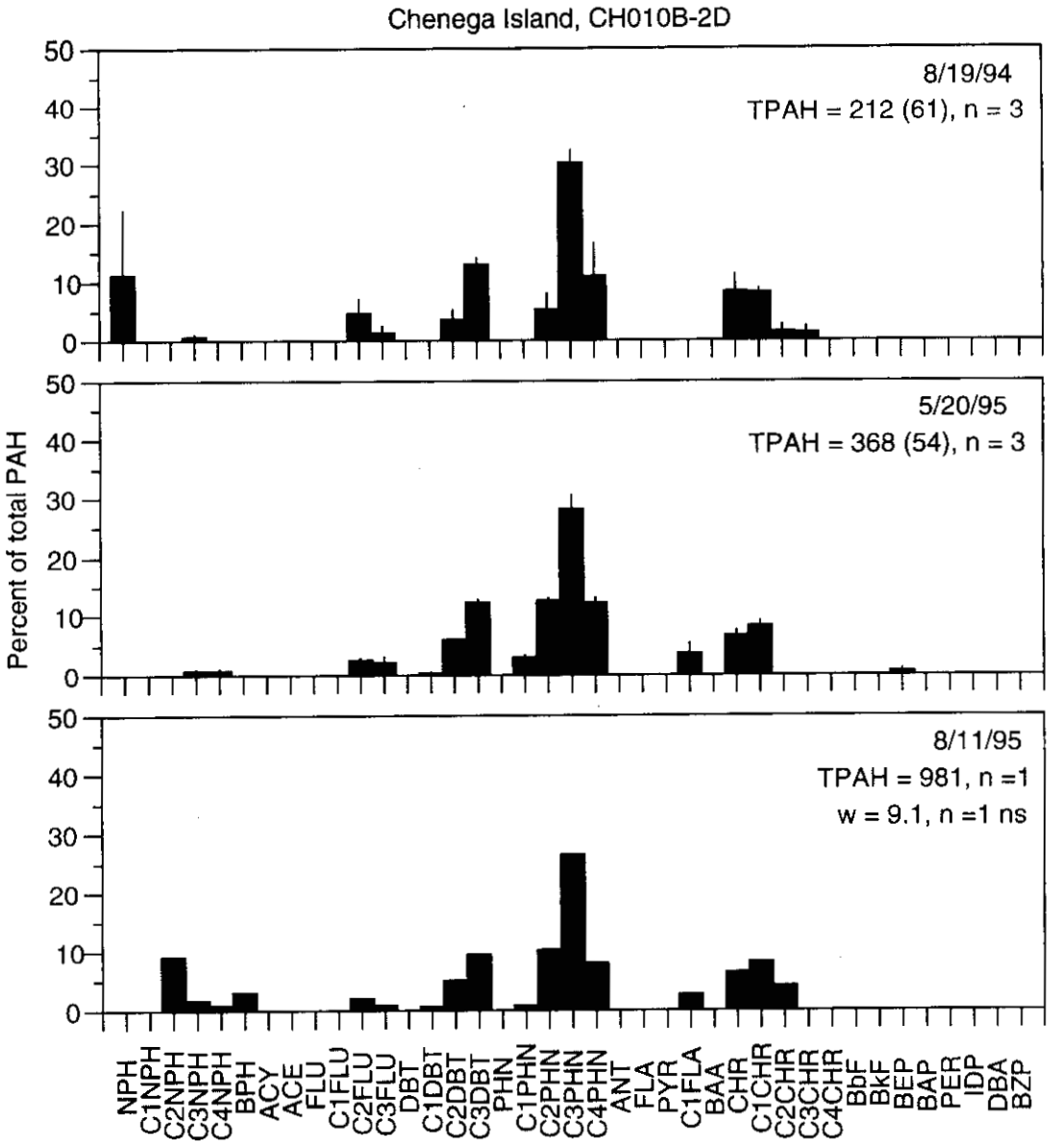

Bay of Isles, KN205B
4/18/92
TPAH = 140 (13), n = 3
6/23/93
TPAH = 258 (178), n = 3
54
Percent of total PAH
0
10
20
30
40
50
NPH
C1NPH
C2NPH
C3NPH
C4NPH
BPH
ACY
ACE
FLU
C1FLU
C2FLU
C3FLU
DBT
C1DBT
C2DBT
C3DBT
PHN
C1PHN
C2PHN
C3PHN
C4PHN
ANT
FLA
PYR
C1FLA
BAA
CHR
C1CHR
C2CHR
C3CHR
C4CHR
BbF
BkF
BEP
BAP
PER
IDP
DBA
BZP

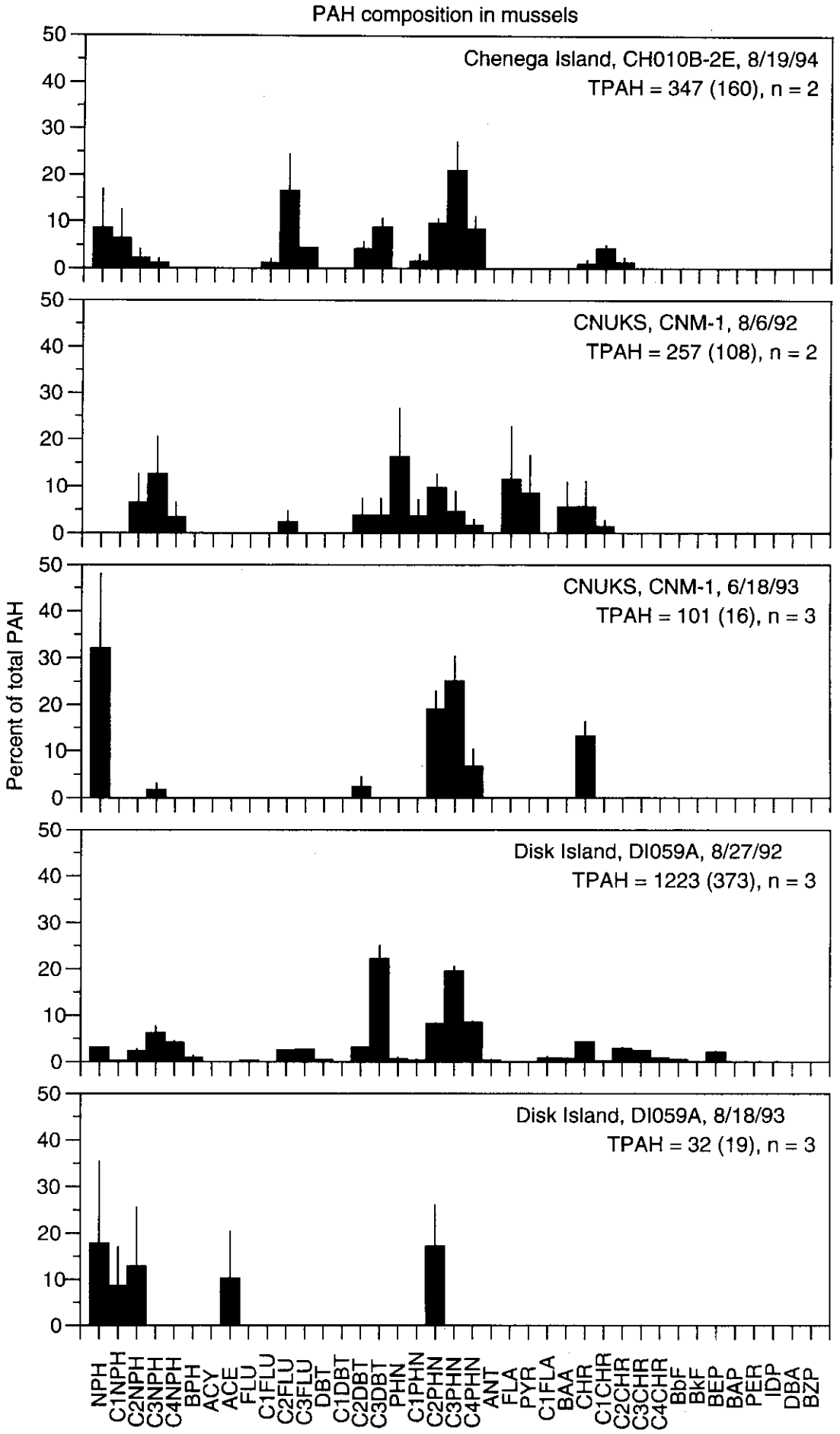

Chenega Island, CH009A-3
Percent of total PAH
8/29/92
TPAH = 5311 (1466), n = 3
6/20/93
TPAH = 795 (40), n = 2
w = 4.9, n = 1 *
4/29/94
TPAH = 44 (40), n = 3
8/11/95
TPAH = 19 (3), n = 2
0
10
20
30
40
50
NPH
C1NPH
C2NPH
C3NPH
C4NPH
BPH
ACY
ACE
FLU
C1FLU
C2FLU
C3FLU
DBT
C1DBT
C2DBT
C3DBT
PHN
C1PHN
C2PHN
C3PHN
C4PHN
ANT
FLA
PYR
C1FLA
BAA
CHR
C1CHR
C2CHR
C3CHR
C4CHR
BbF
BkF
BEP
BAP
PER
IDP
DBA
BZP

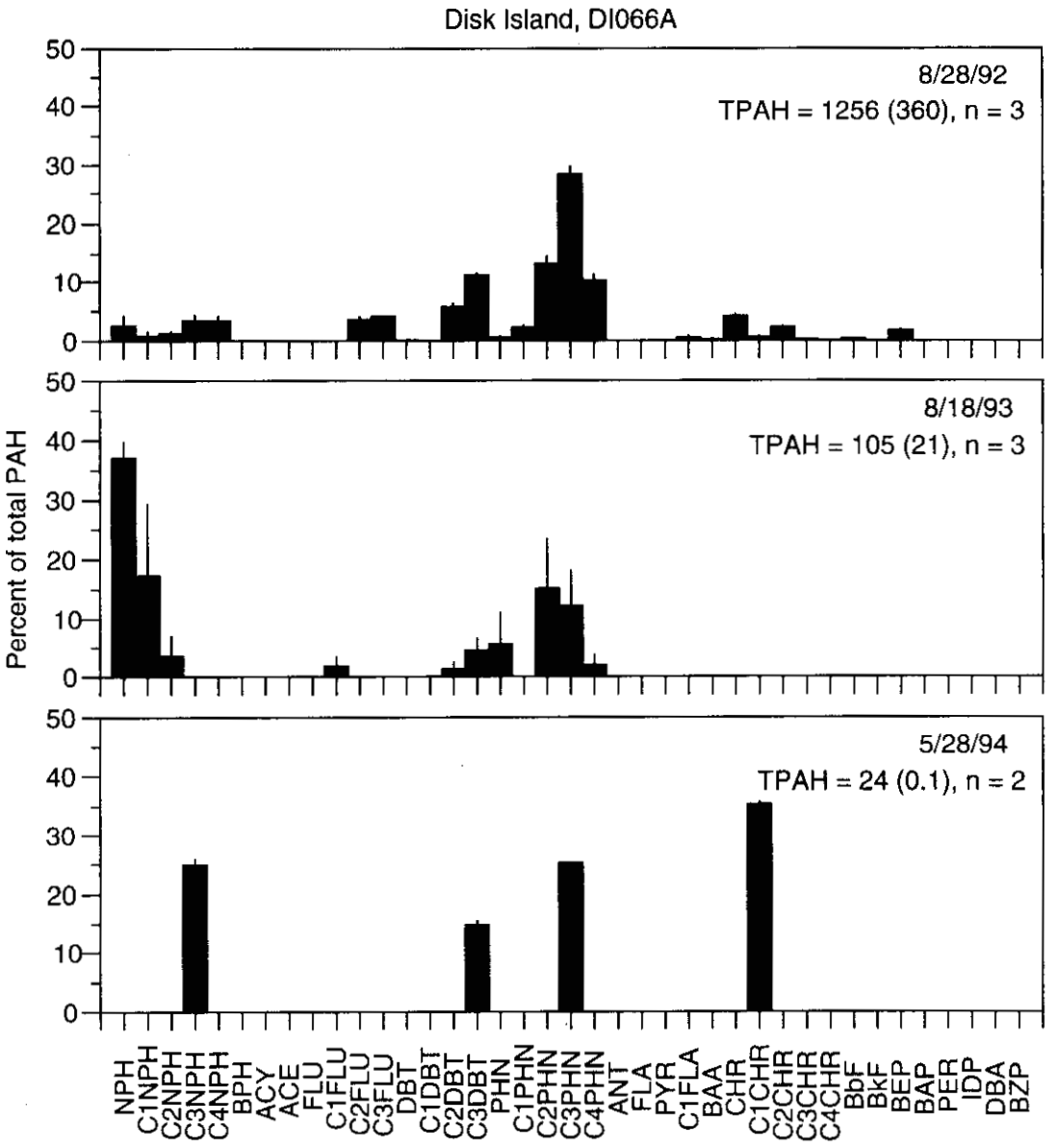

Chenega Island, CH010B-2A
Percent of total PAH
5/17/92
TPAH = 1877 (427), n = 13
w = 5.2 (0.5), n = 13 *
6/21/93
TPAH = 297 (81), n = 10
8/1/93
TPAH = 4184 (687), n = 3
w = 4.7 (0.2), n = 2 *
5/27/94
TPAH = 807, n =1
w = 4.9, n = 1 *
8/9/94
TPAH = 1561 (274), n = 6
w = 4.6 (0.2), n = 6 *
NPH
C1NPH
C2NPH
C3NPH
C4NPH
BPH
ACY
ACE
FLU
C1FLU
C2FLU
C3FLU
DBT
C1DBT
C2DBT
C3DBT
PHN
C1PHN
C2PHN
C3PHN
C4PHN
ANT
FLA
PYR
C1FLA
BAA
CHR
C1CHR
C2CHR
C3CHR
C4CHR
BbF
BkF
BEP
BAP
PER
IDP
DBA
BZP

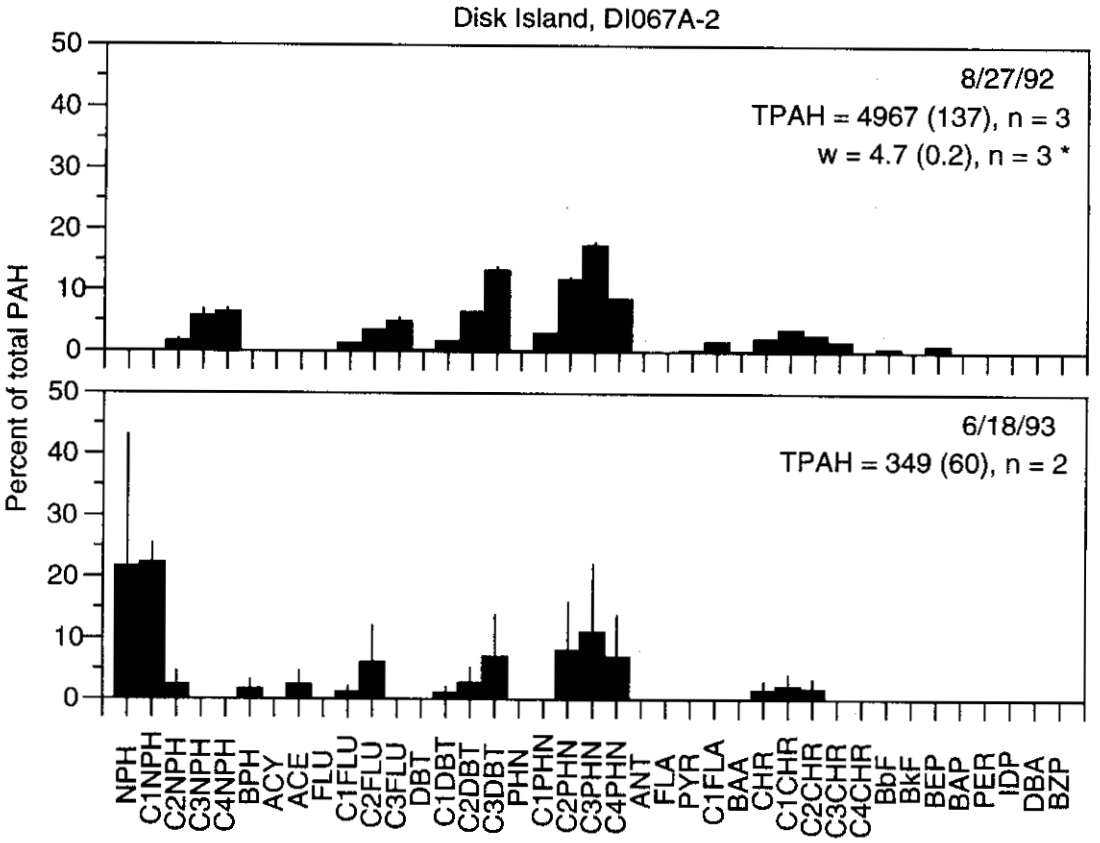

Chenega Island, CH010B-2B
Percent of total PAH
5/4/92
TPAH = 1042, n = 1
5/17/92
TPAH = 516 (8), n = 2
w = 6.4 (0.3), n = 2 *
6/21/93
TPAH = 299 (105), n = 3
8/10/94
TPAH = 696 (158), n = 3
w = 6.7 (0.4), n = 2 *
NPH
C1NPH
C2NPH
C3NPH
C4NPH
BPH
ACY
ACE
FLU
C1FLU
C2FLU
C3FLU
DBT
C1DBT
C2DBT
C3DBT
PHN
C1PHN
C2PHN
C3PHN
C4PHN
ANT
FLA
PYR
C1FLA
BAA
CHR
C1CHR
C2CHR
C3CHR
C4CHR
BbF
BkF
BEP
BAP
PER
IDP
DBA
BZP

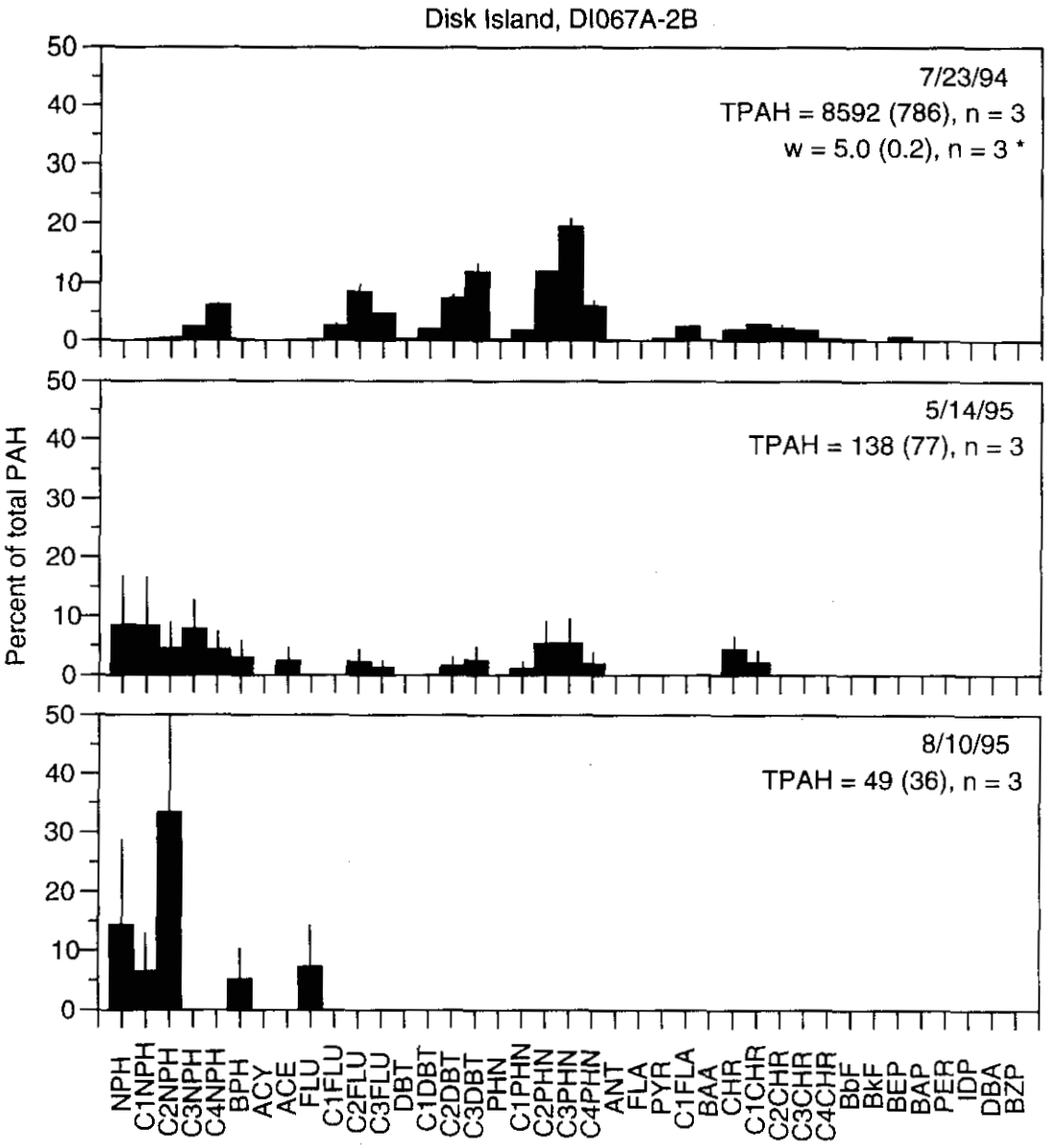

Chenega Island CH010B-2C
8/8/94
TPAH = 0, n = 3
No values were above MDL
in this data set.
Percent of total PAH
50
40
30
20
10
0
NPH
C1NPH
C2NPH
C3NPH
C4NPH
BPH
ACY
ACE
FLU
C1FLU
C2FLU
C3FLU
DBT
C1DBT
C2DBT
C3DBT
PHN
C1PHN
C2PHN
C3PHN
C4PHN
ANT
FLA
PYR
C1FLA
BAA
CHR
C1CHR
C2CHR
C3CHR
C4CHR
BbF
BkF
BEP
BAP
PER
IDP
DBA
BZP

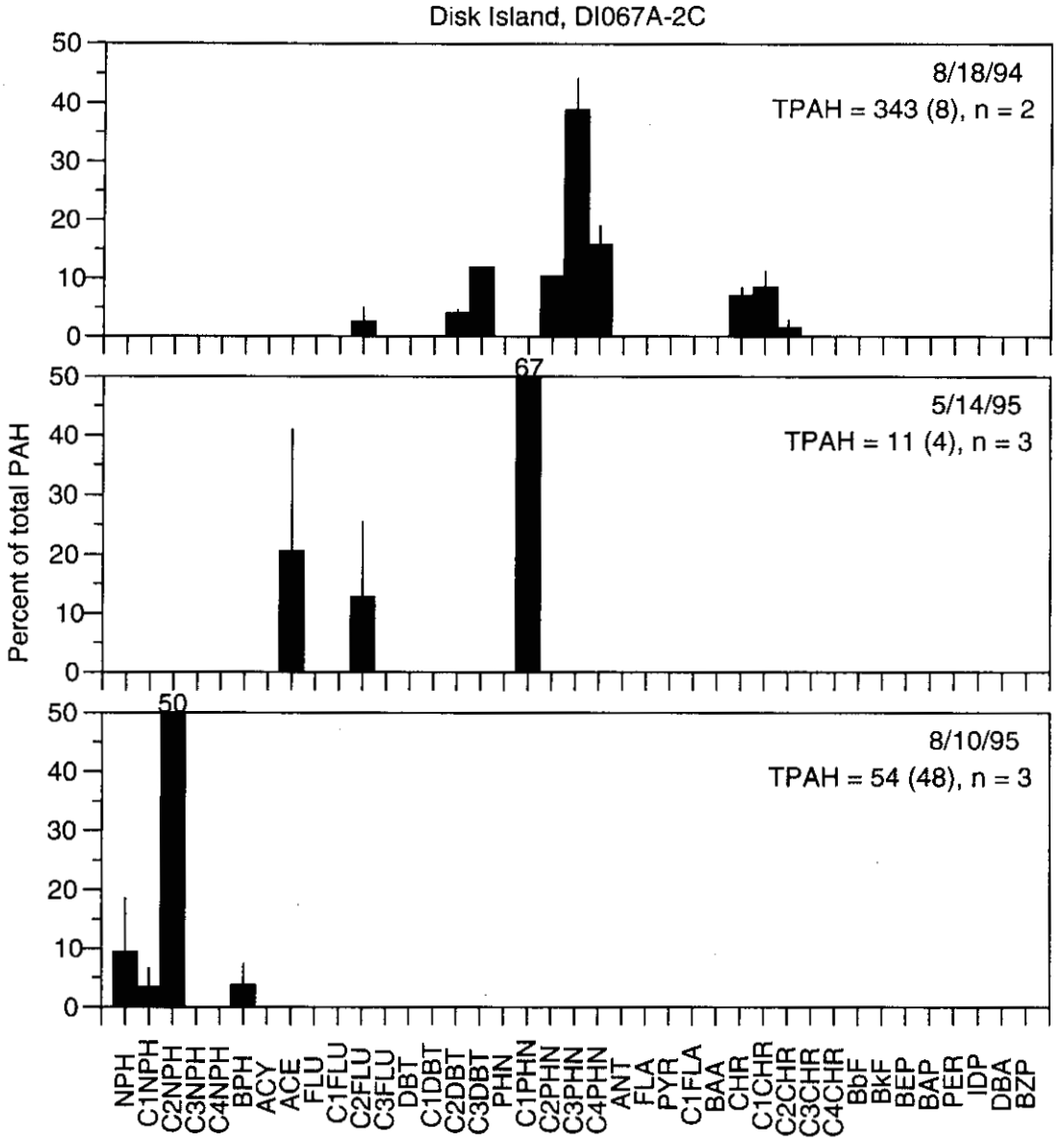

Chenega Island, CH010B-2D
Percent of total PAH
8/19/94
TPAH = 212 (61), n = 3
5/20/95
TPAH = 368 (54), n = 3
8/11/95
TPAH = 981, n =1
w = 9.1, n =1 ns
0
10
20
30
40
50
NPH
C1NPH
C2NPH
C3NPH
C4NPH
BPH
ACY
ACE
FLU
C1FLU
C2FLU
C3FLU
DBT
C1DBT
C2DBT
C3DBT
PHN
C1PHN
C2PHN
C3PHN
C4PHN
ANT
FLA
PYR
C1FLA
BAA
CHR
C1CHR
C2CHR
C3CHR
C4CHR
BbF
BkF
BEP
BAP
PER
IDP
DBA
BZP

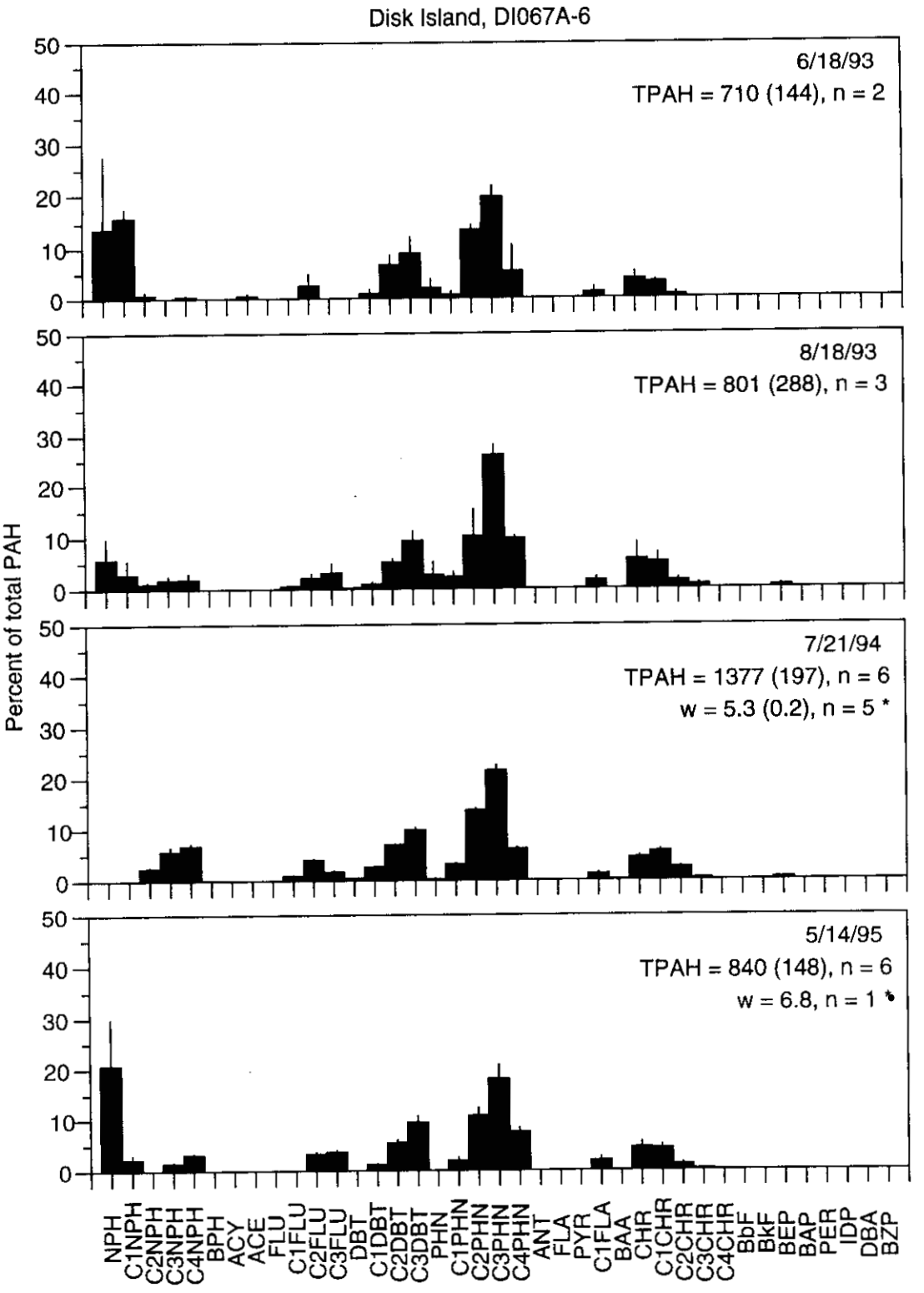

PAH composition in mussels
Chenega Island, CH010B-2E, 8/19/94
TPAH = 347 (160), n = 2
CNUKS, CNM-1, 8/6/92
TPAH = 257 (108), n = 2
CNUKS, CNM-1, 6/18/93
TPAH = 101 (16), n = 3
Disk Island, DI059A, 8/27/92
TPAH = 1223 (373), n = 3
Disk Island, DI059A, 8/18/93
TPAH = 32 (19), n = 3
Percent of total PAH
0
10
20
30
40
50
NPH
C1NPH
C2NPH
C3NPH
C4NPH
BPH
ACY
ACE
FLU
C1FLU
C2FLU
C3FLU
DBT
C1DBT
C2DBT
C3DBT
PHN
C1PHN
C2PHN
C3PHN
C4PHN
ANT
FLA
PYR
C1FLA
BAA
CHR
C1CHR
C2CHR
C3CHR
C4CHR
BbF
BkF
BEP
BAP
PER
IDP
DBA
BZP

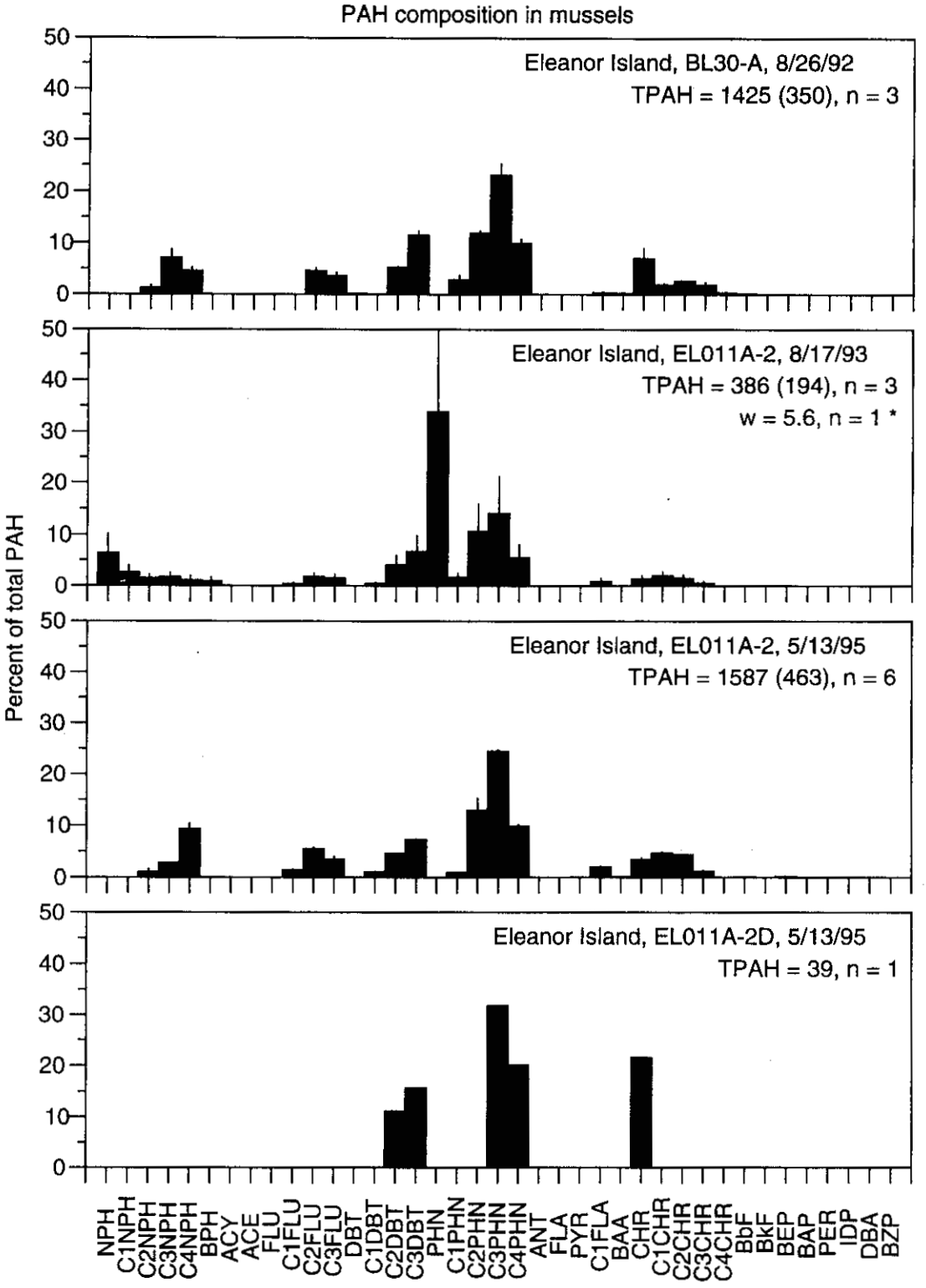

Disk Island, DI066A
8/28/92
TPAH = 1256 (360), n = 3
8/18/93
TPAH = 105 (21), n = 3
5/28/94
TPAH = 24 (0.1), n = 2
Percent of total PAH
0
10
20
30
40
50
NPH
C1NPH
C2NPH
C3NPH
C4NPH
BPH
ACY
ACE
FLU
C1FLU
C2FLU
C3FLU
DBT
C1DBT
C2DBT
C3DBT
PHN
C1PHN
C2PHN
C3PHN
C4PHN
ANT
FLA
PYR
C1FLA
BAA
CHR
C1CHR
C2CHR
C3CHR
C4CHR
BbF
BkF
BEP
BAP
PER
IDP
DBA
BZP

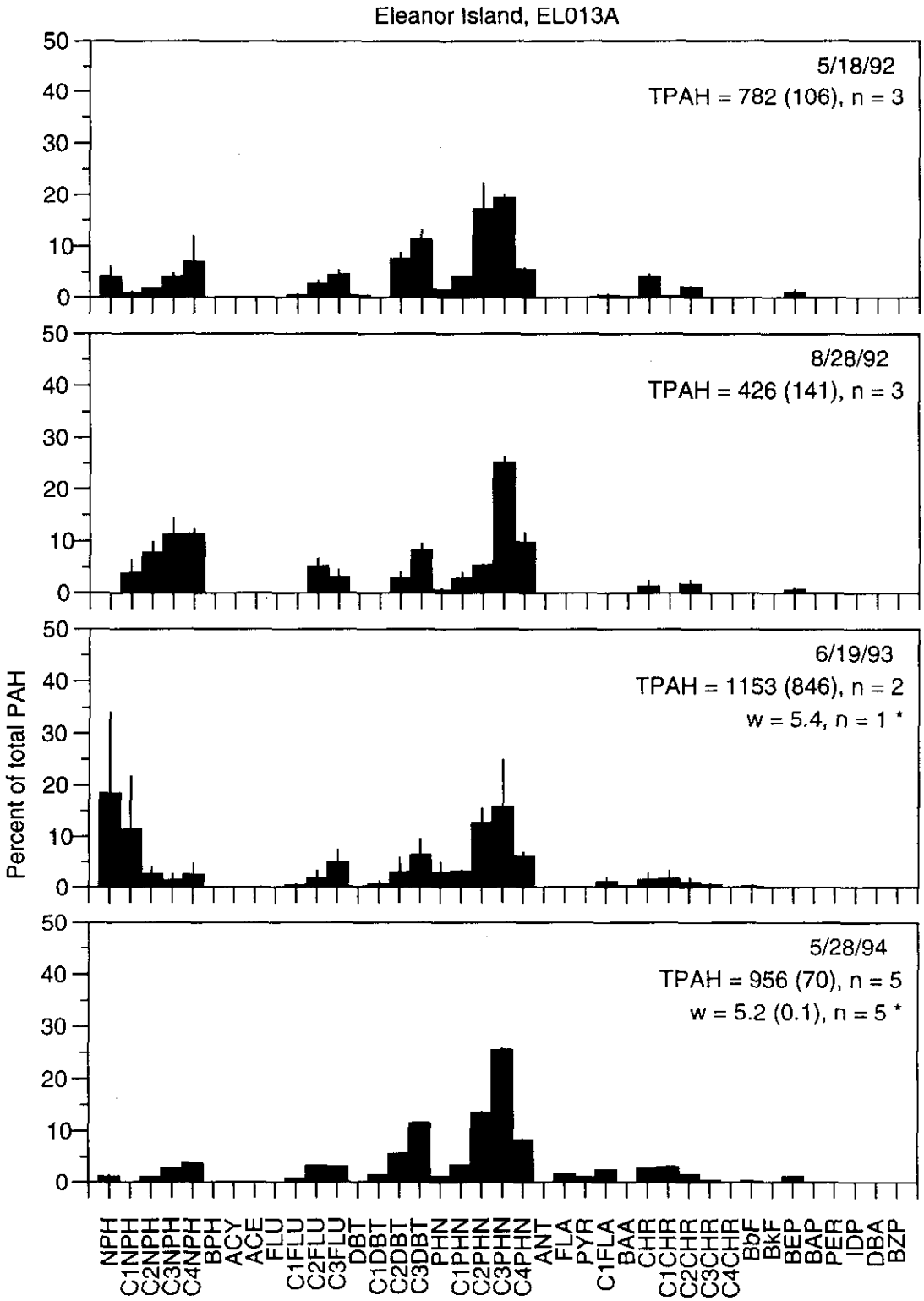

Disk Island, DI067A-2
8/27/92
TPAH = 4967 (137), n = 3
w = 4.7 (0.2), n = 3 *
6/18/93
TPAH = 349 (60), n = 2
Percent of total PAH
0
10
20
30
40
50
NPH
C1NPH
C2NPH
C3NPH
C4NPH
BPH
ACY
ACE
FLU
C1FLU
C2FLU
C3FLU
DBT
C1DBT
C2DBT
C3DBT
PHN
C1PHN
C2PHN
C3PHN
C4PHN
ANT
FLA
PYR
C1FLA
BAA
CHR
C1CHR
C2CHR
C3CHR
C4CHR
BbF
BkF
BEP
BAP
PER
IDP
DBA
BZP


Disk Island, DI067A-2B
Percent of total PAH
7/23/94
TPAH = 8592 (786), n = 3
w = 5.0 (0.2), n = 3 *
5/14/95
TPAH = 138 (77), n = 3
8/10/95
TPAH = 49 (36), n = 3
NPH
C1NPH
C2NPH
C3NPH
C4NPH
BPH
ACY
ACE
FLU
C1FLU
C2FLU
C3FLU
DBT
C1DBT
C2DBT
C3DBT
PHN
C1PHN
C2PHN
C3PHN
C4PHN
ANT
FLA
PYR
C1FLA
BAA
CHR
C1CHR
C2CHR
C3CHR
C4CHR
BbF
BkF
BEP
BAP
PER
IDP
DBA
BZP

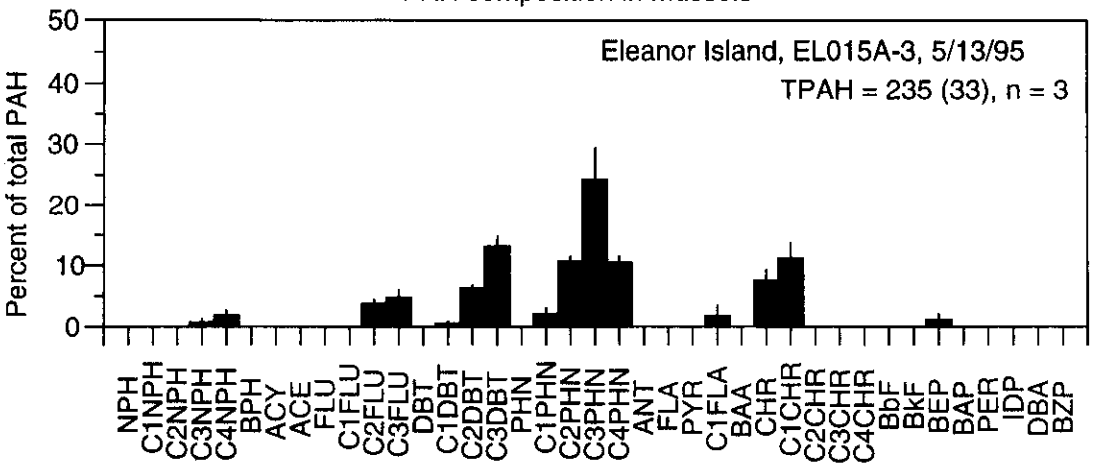

Disk Island, DI067A-2C
Percent of total PAH
8/18/94
TPAH = 343 (8), n = 2
5/14/95
TPAH = 11 (4), n = 3
67
8/10/95
TPAH = 54 (48), n = 3
50
NPH
C1NPH
C2NPH
C3NPH
C4NPH
BPH
ACY
ACE
FLU
C1FLU
C2FLU
C3FLU
DBT
C1DBT
C2DBT
C3DBT
PHN
C1PHN
C2PHN
C3PHN
C4PHN
ANT
FLA
PYR
C1FLA
BAA
CHR
C1CHR
C2CHR
C3CHR
C4CHR
BbF
BkF
BEP
BAP
PER
IDP
DBA
BZP

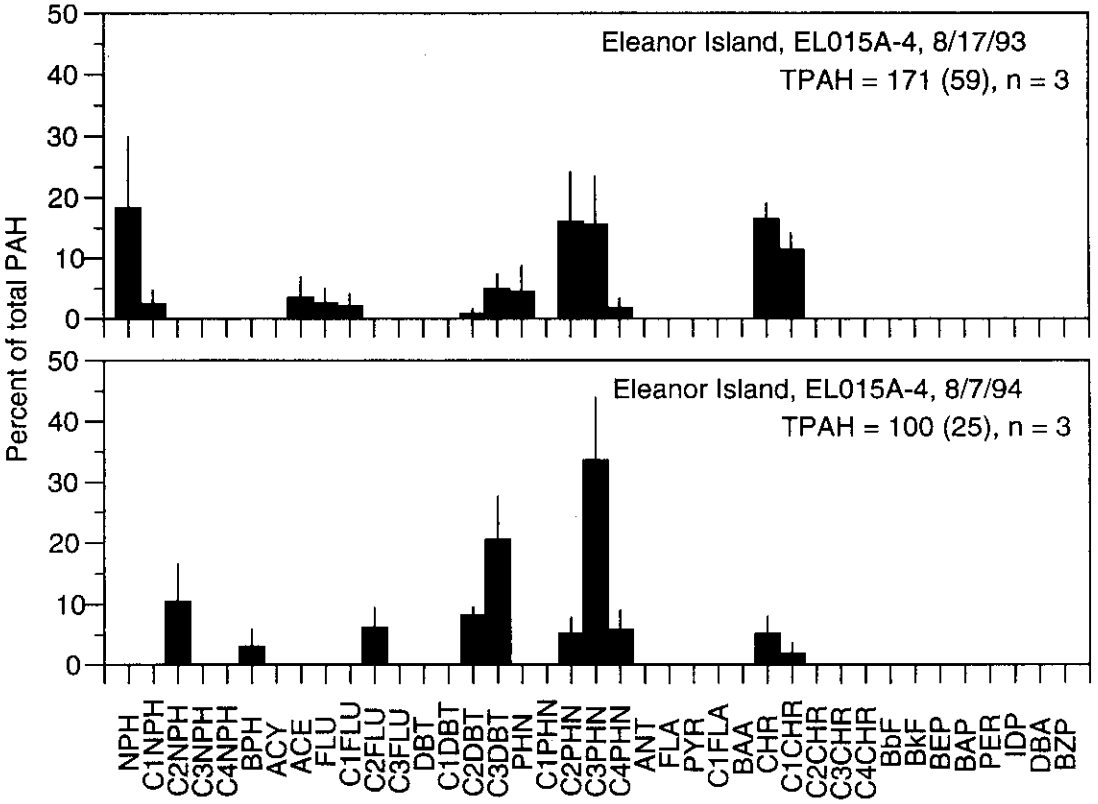

Disk Island, DI067A-6
Percent of total PAH
6/18/93
TPAH = 710 (144), n = 2
8/18/93
TPAH = 801 (288), n = 3
7/21/94
TPAH = 1377 (197), n = 6
w = 5.3 (0.2), n = 5 *
5/14/95
TPAH = 840 (148), n = 6
w = 6.8, n = 1 *
0
10
20
30
40
50
NPH
C1NPH
C2NPH
C3NPH
C4NPH
BPH
ACY
ACE
FLU
C1FLU
C2FLU
C3FLU
DBT
C1DBT
C2DBT
C3DBT
PHN
C1PHN
C2PHN
C3PHN
C4PHN
ANT
FLA
PYR
C1FLA
BAA
CHR
C1CHR
C2CHR
C3CHR
C4CHR
BbF
BkF
BEP
BAP
PER
IDP
DBA
BZP

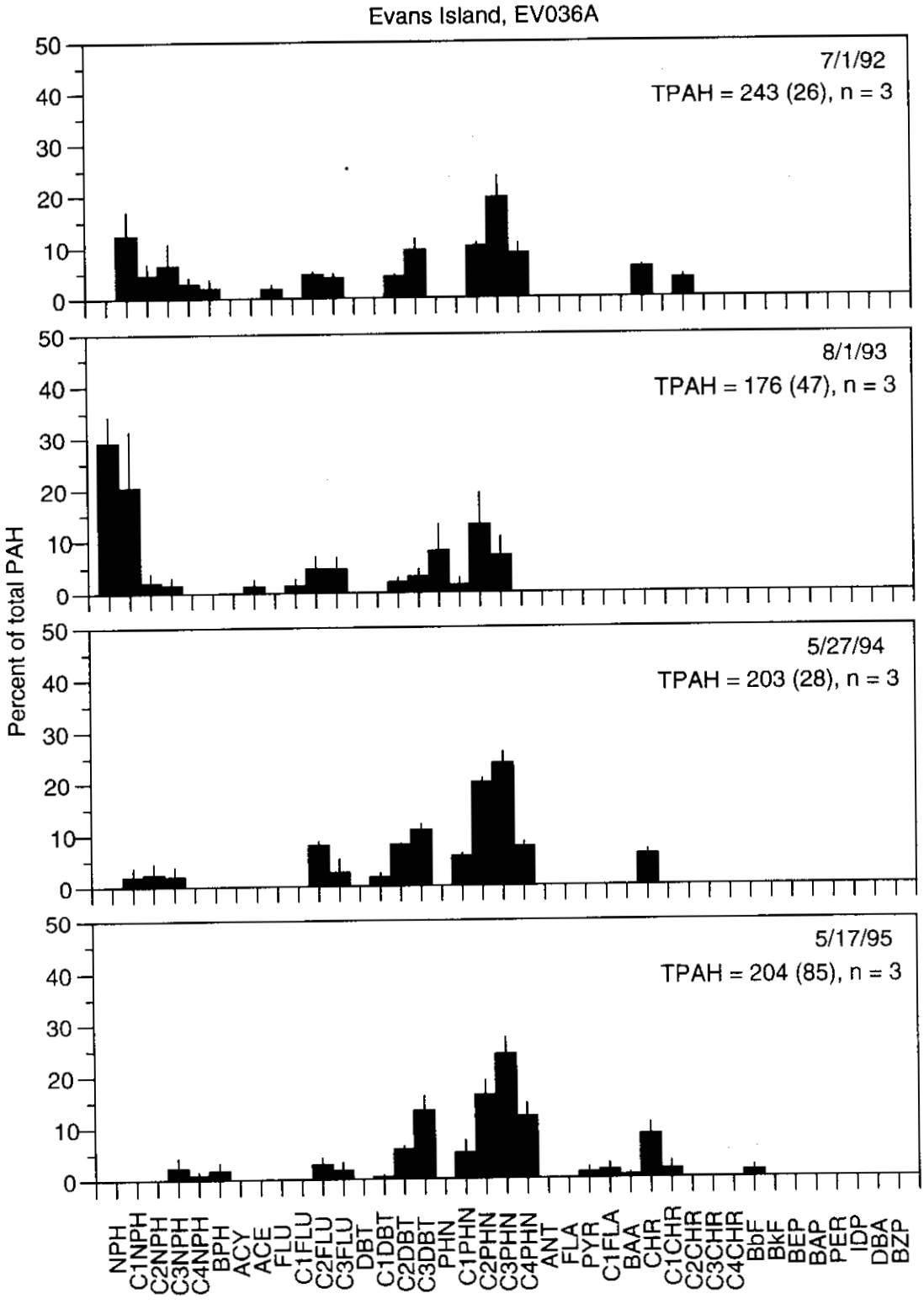

PAH composition in mussels
Eleanor Island, BL30-A, 8/26/92
TPAH = 1425 (350), n = 3
Eleanor Island, EL011A-2, 8/17/93
TPAH = 386 (194), n = 3
w = 5.6, n = 1 *
Eleanor Island, EL011A-2, 5/13/95
TPAH = 1587 (463), n = 6
Eleanor Island, EL011A-2D, 5/13/95
TPAH = 39, n = 1
Percent of total PAH
0
10
20
30
40
50
NPH
C1NPH
C2NPH
C3NPH
C4NPH
BPH
ACY
ACE
FLU
C1FLU
C2FLU
C3FLU
DBT
C1DBT
C2DBT
C3DBT
PHN
C1PHN
C2PHN
C3PHN
C4PHN
ANT
FLA
PYR
C1FLA
BAA
CHR
C1CHR
C2CHR
C3CHR
C4CHR
BbF
BkF
BEP
BAP
PER
IDP
DBA
BZP

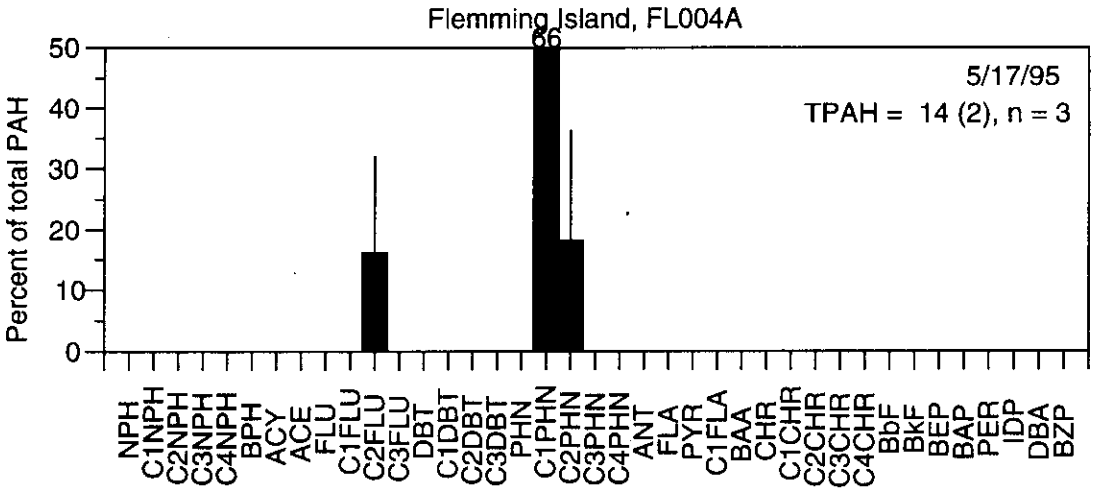

Eleanor Island, EL013A
Percent of total PAH
5/18/92
TPAH = 782 (106), n = 3
8/28/92
TPAH = 426 (141), n = 3
6/19/93
TPAH = 1153 (846), n = 2
w = 5.4, n = 1 *
5/28/94
TPAH = 956 (70), n = 5
w = 5.2 (0.1), n = 5 *
0
10
20
30
40
50
NPH
C1NPH
C2NPH
C3NPH
C4NPH
BPH
ACY
ACE
FLU
C1FLU
C2FLU
C3FLU
DBT
C1DBT
C2DBT
C3DBT
PHN
C1PHN
C2PHN
C3PHN
C4PHN
ANT
FLA
PYR
C1FLA
BAA
CHR
C1CHR
C2CHR
C3CHR
C4CHR
BbF
BkF
BEP
BAP
PER
IDP
DBA
BZP

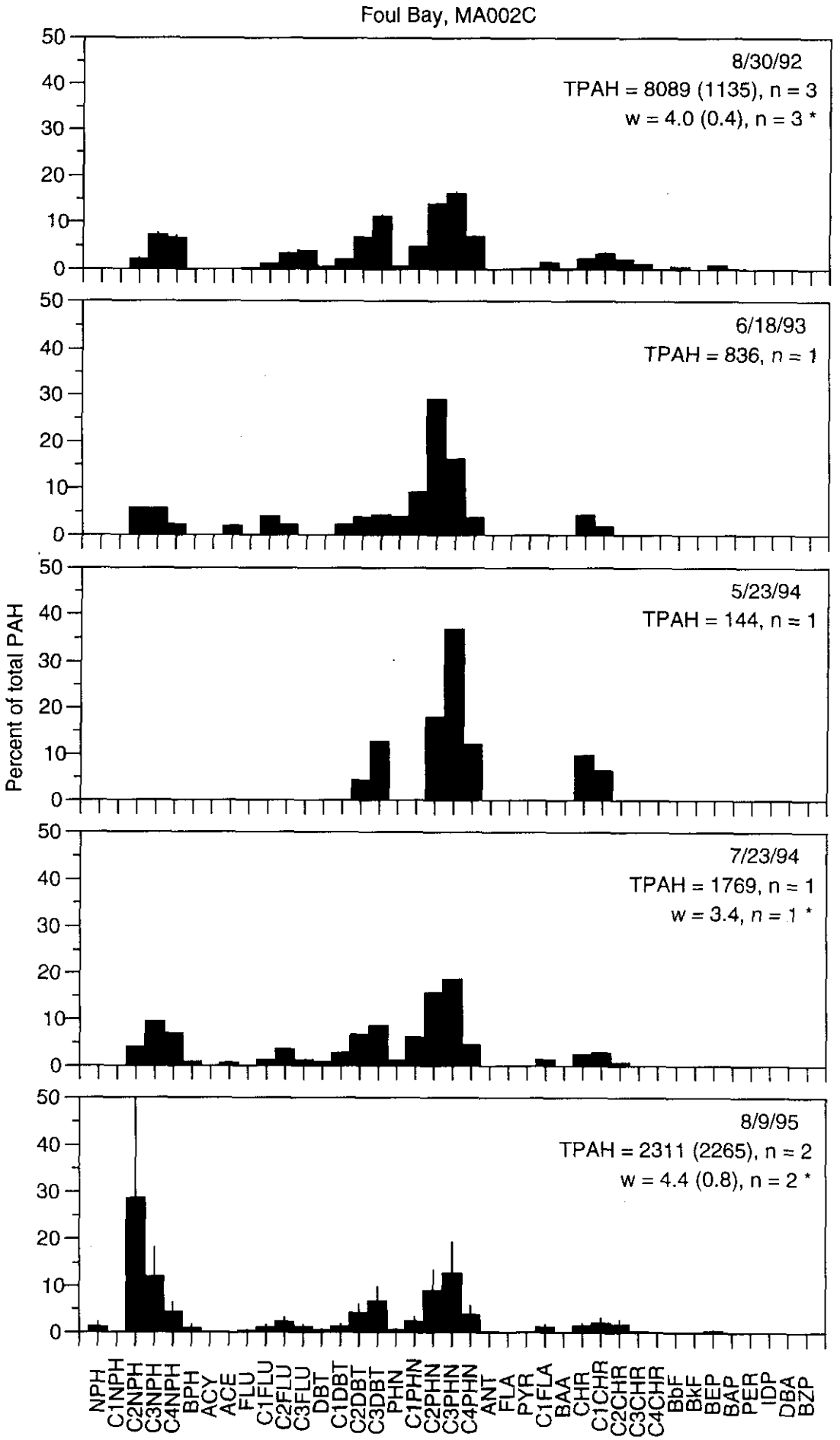
PAH composition in mussels

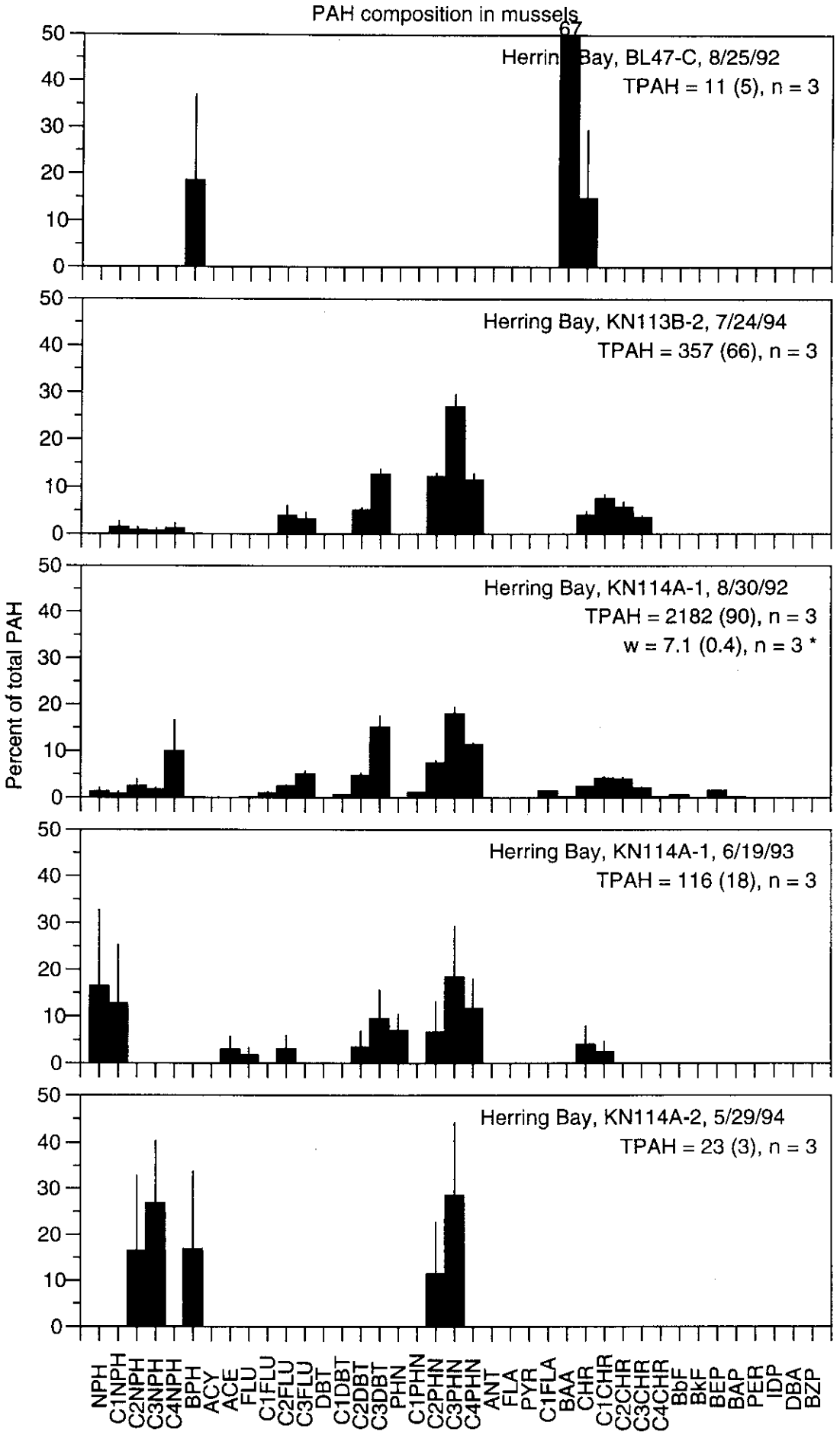


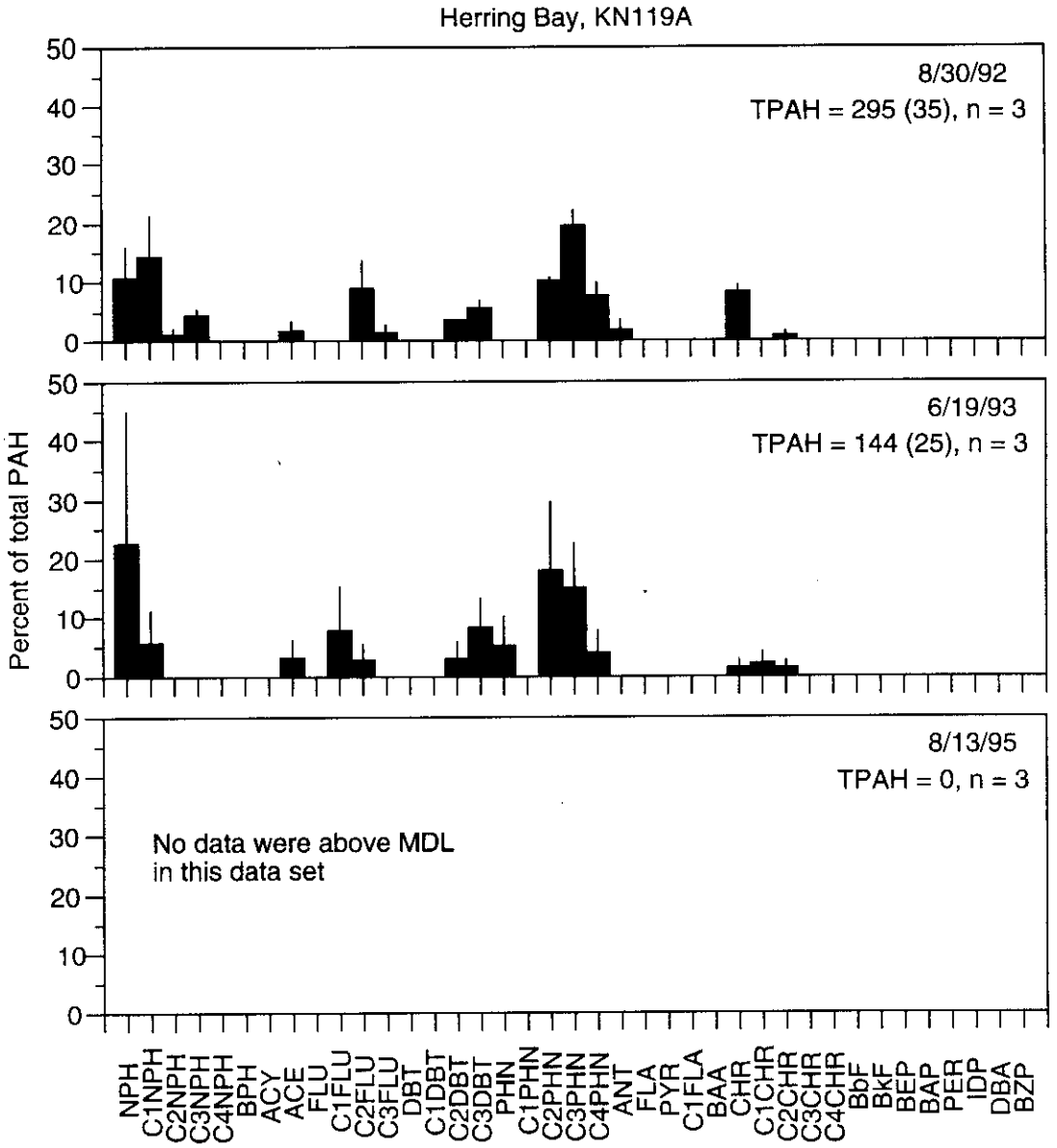


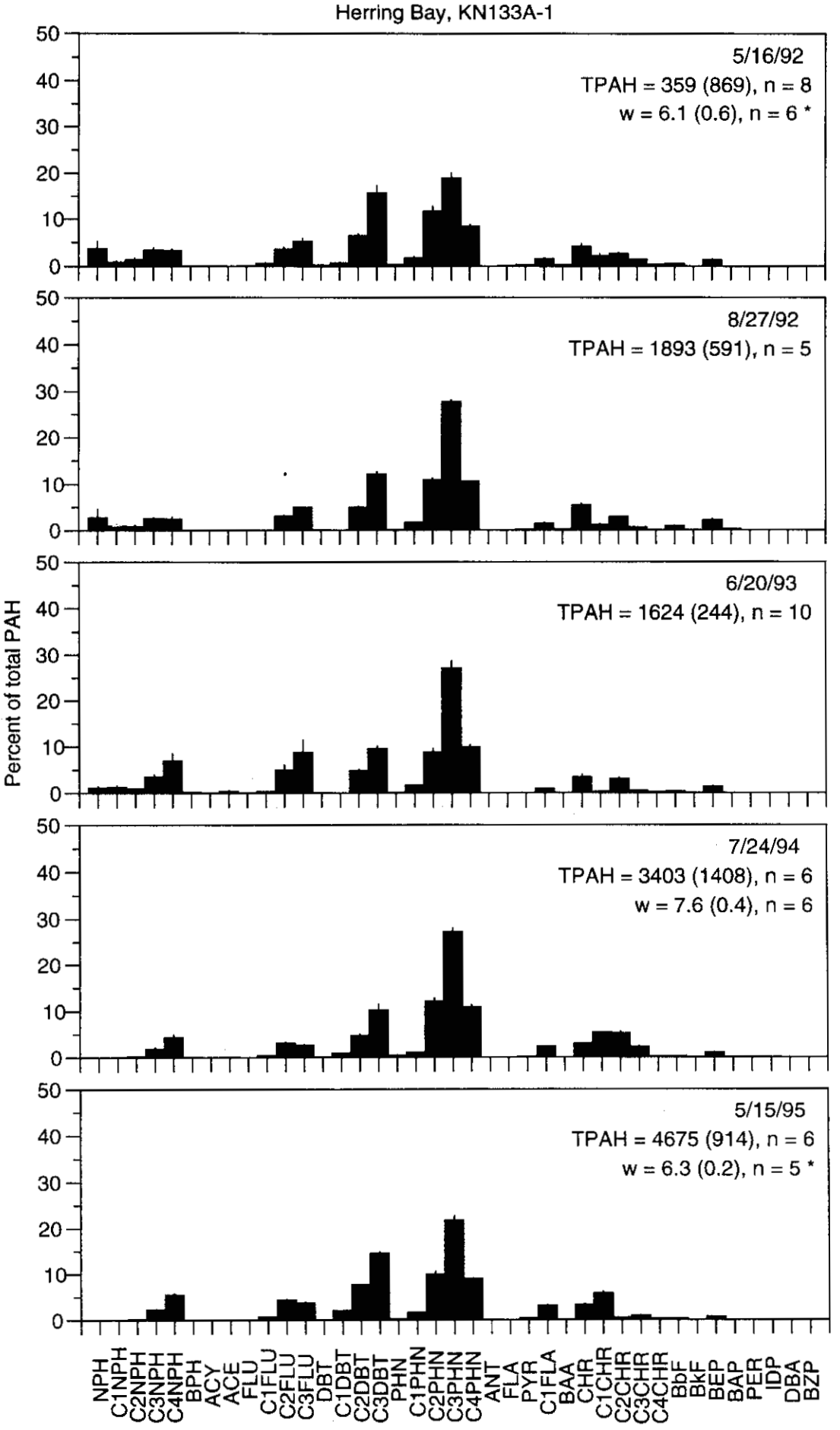

Evans Island, EV036A
Percent of total PAH
7/1/92
TPAH = 243 (26), n = 3
8/1/93
TPAH = 176 (47), n = 3
5/27/94
TPAH = 203 (28), n = 3
5/17/95
TPAH = 204 (85), n = 3
0
10
20
30
40
50
NPH
C1NPH
C2NPH
C3NPH
C4NPH
BPH
ACY
ACE
FLU
C1FLU
C2FLU
C3FLU
DBT
C1DBT
C2DBT
C3DBT
PHN
C1PHN
C2PHN
C3PHN
C4PHN
ANT
FLA
PYR
C1FLA
BAA
CHR
C1CHR
C2CHR
C3CHR
C4CHR
BbF
BkF
BEP
BAP
PER
IDP
DBA
BZP

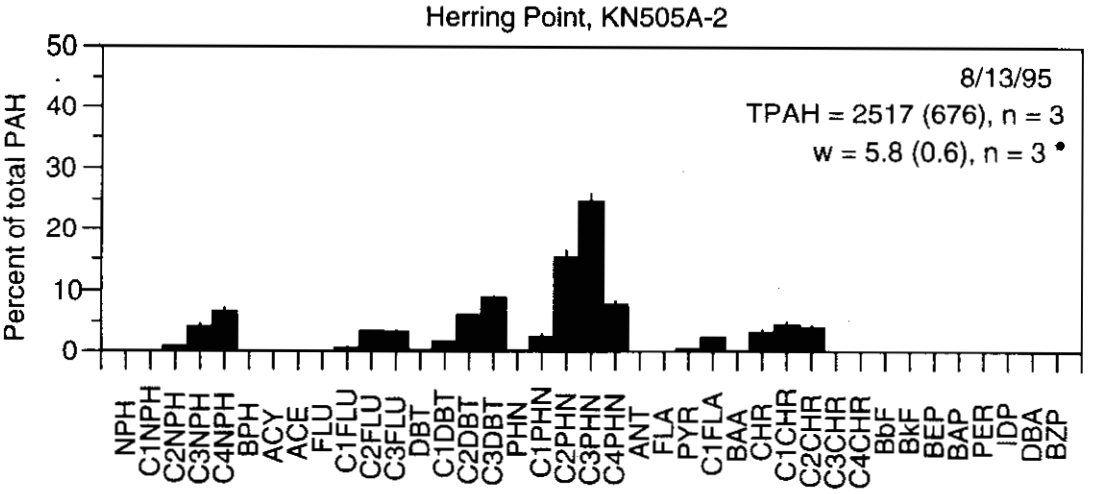

Flemming Island, FL004A
5/17/95
TPAH = 14 (2), n = 3
Percent of total PAH
50
40
30
20
10
0
66
NPH
C1NPH
C2NPH
C3NPH
C4NPH
BPH
ACY
ACE
FLU
C1FLU
C2FLU
C3FLU
DBT
C1DBT
C2DBT
C3DBT
PHN
C1PHN
C2PHN
C3PHN
C4PHN
ANT
FLA
PYR
C1FLA
BAA
CHR
C1CHR
C2CHR
C3CHR
C4CHR
BbF
BkF
BEP
BAP
PER
IDP
DBA
BZP

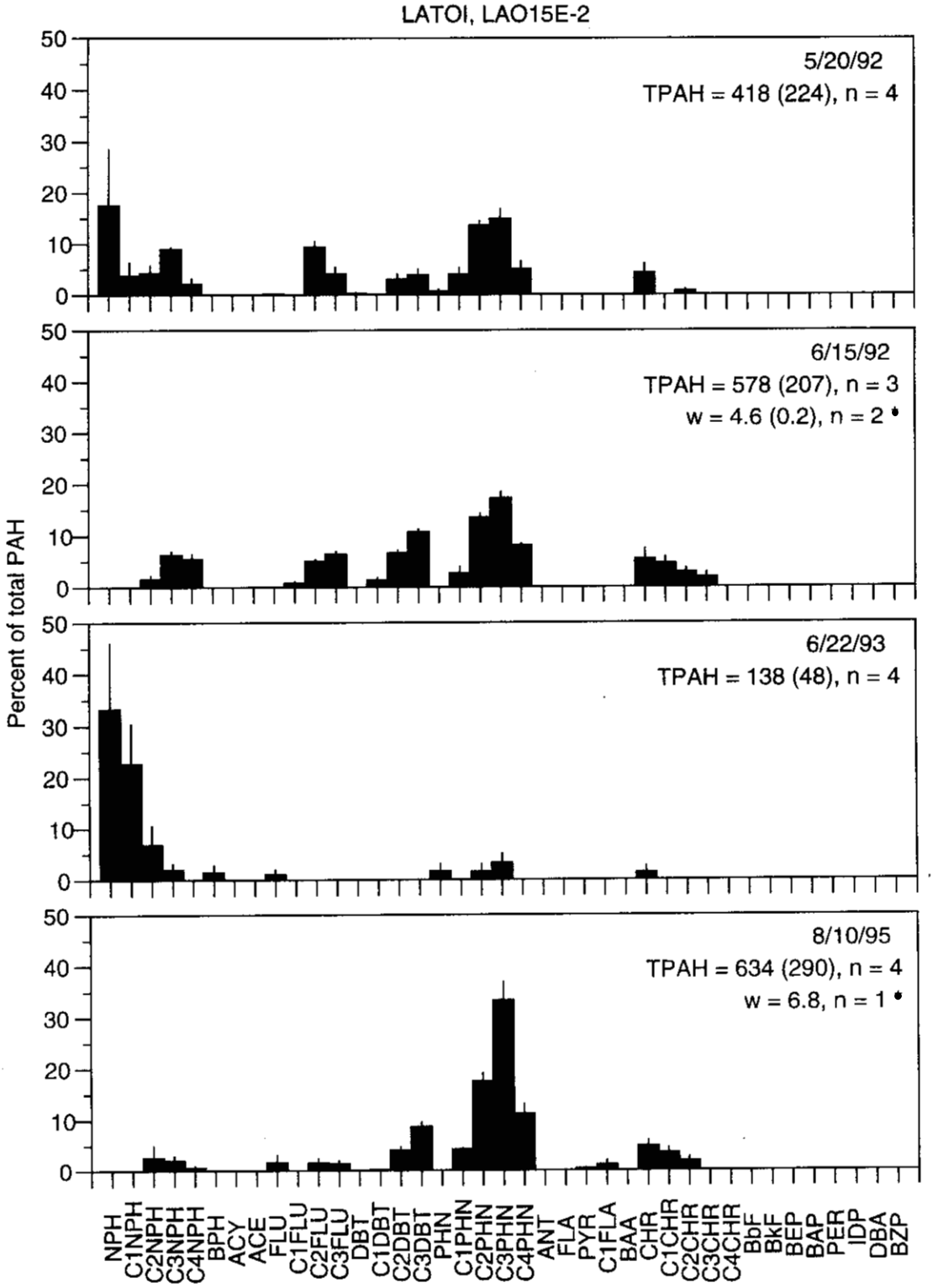

Foul Bay, MA002C
Percent of total PAH
8/30/92
TPAH = 8089 (1135), n = 3
w = 4.0 (0.4), n = 3 *
6/18/93
TPAH = 836, n = 1
5/23/94
TPAH = 144, n = 1
7/23/94
TPAH = 1769, n = 1
w = 3.4, n = 1 *
8/9/95
TPAH = 2311 (2265), n = 2
w = 4.4 (0.8), n = 2 *
0
10
20
30
40
50
NPH
C1NPH
C2NPH
C3NPH
C4NPH
BPH
ACY
ACE
FLU
C1FLU
C2FLU
C3FLU
DBT
C1DBT
C2DBT
C3DBT
PHN
C1PHN
C2PHN
C3PHN
C4PHN
ANT
FLA
PYR
C1FLA
BAA
CHR
C1CHR
C2CHR
C3CHR
C4CHR
BbF
BkF
BEP
BAP
PER
IDP
DBA
BZP

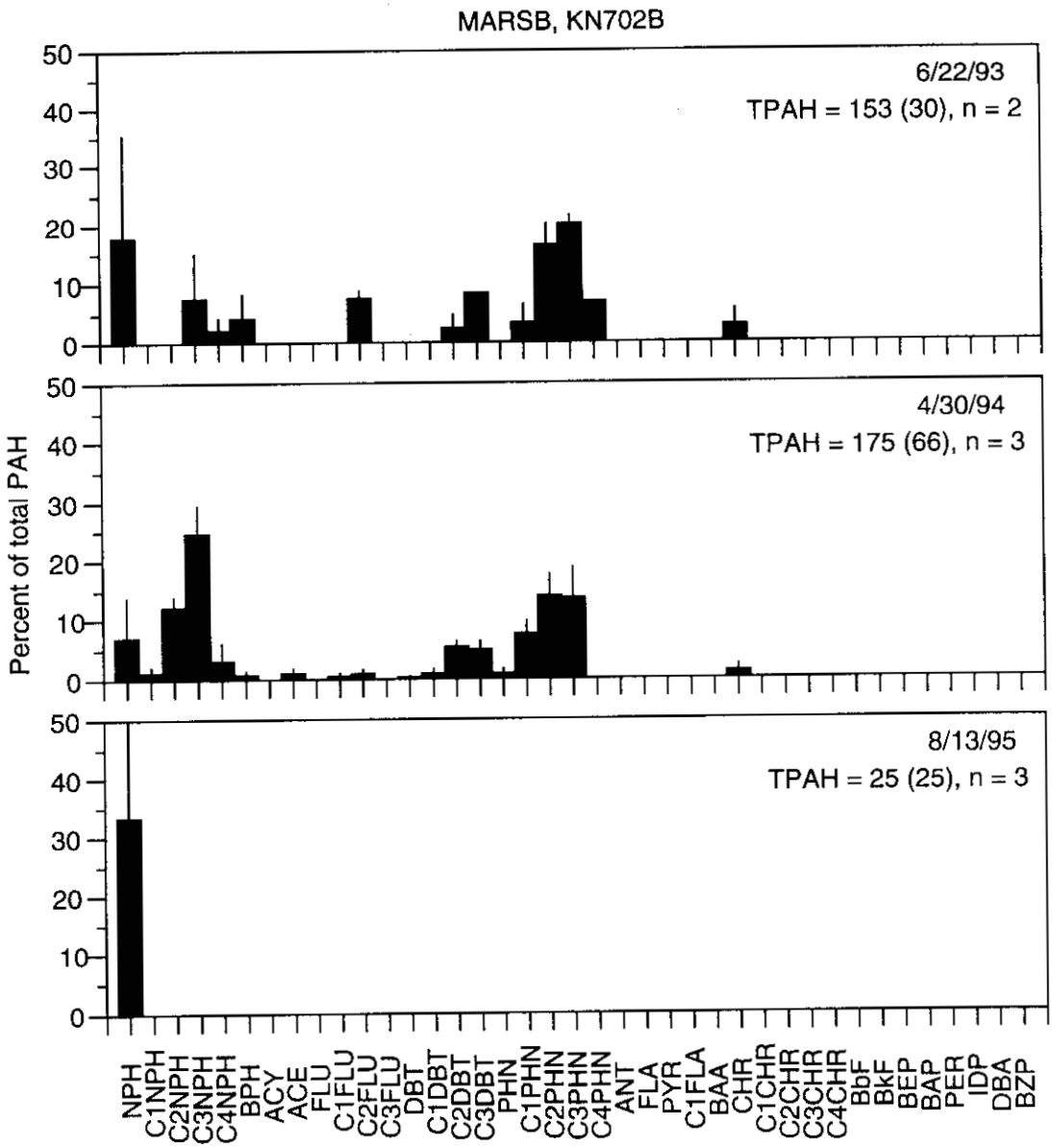

PAH composition in mussels
Percent of total PAH
Herring Bay, BL47-C, 8/25/92
TPAH = 11 (5), n = 3
67
Herring Bay, KN113B-2, 7/24/94
TPAH = 357 (66), n = 3
Herring Bay, KN114A-1, 8/30/92
TPAH = 2182 (90), n = 3
w = 7.1 (0.4), n = 3 *
Herring Bay, KN114A-1, 6/19/93
TPAH = 116 (18), n = 3
Herring Bay, KN114A-2, 5/29/94
TPAH = 23 (3), n = 3
0
10
20
30
40
50
NPH
C1NPH
C2NPH
C3NPH
C4NPH
BPH
ACY
ACE
FLU
C1FLU
C2FLU
C3FLU
DBT
C1DBT
C2DBT
C3DBT
PHN
C1PHN
C2PHN
C3PHN
C4PHN
ANT
FLA
PYR
C1FLA
BAA
CHR
C1CHR
C2CHR
C3CHR
C4CHR
BbF
BkF
BEP
BAP
PER
IDP
DBA
BZP

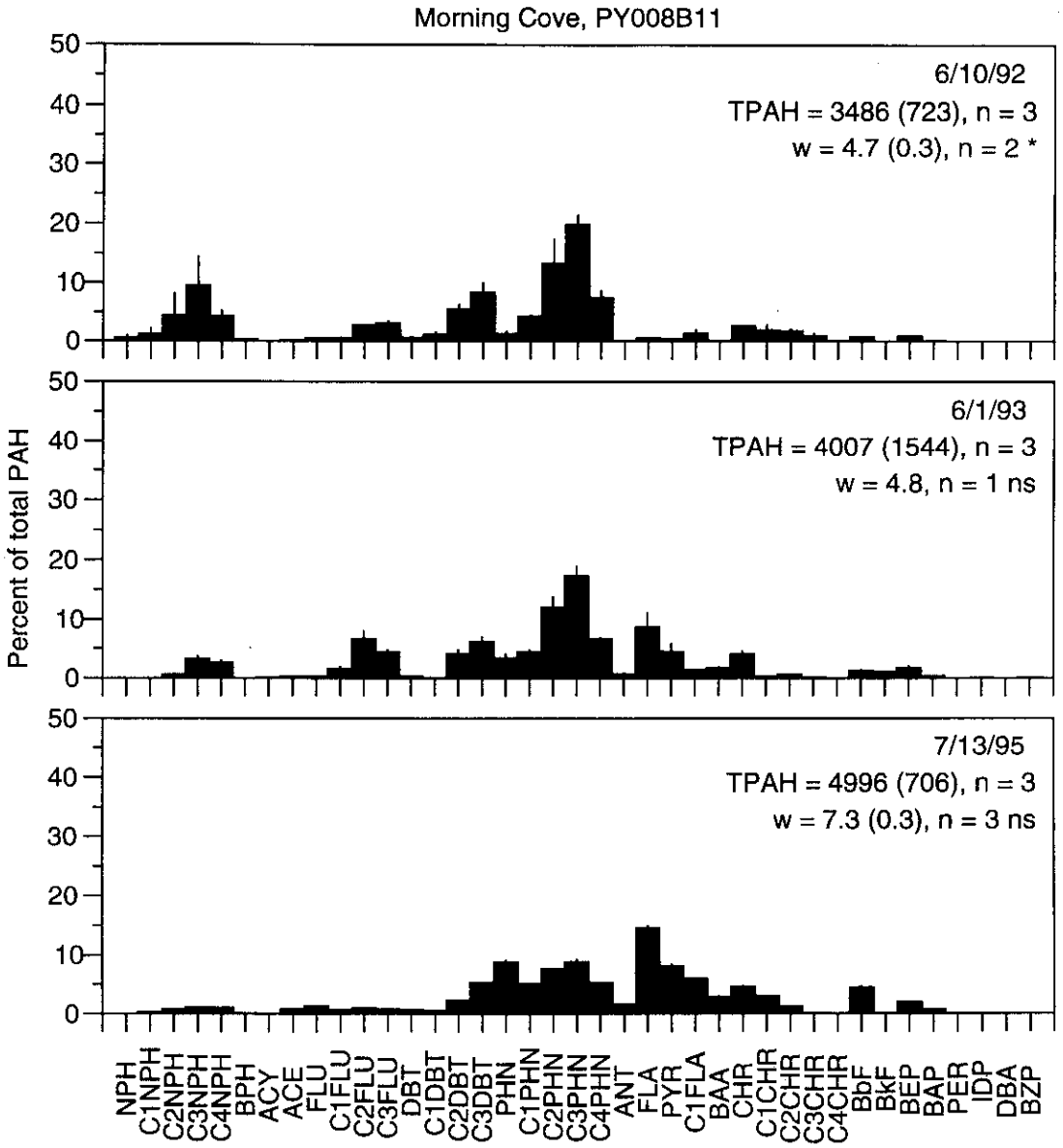

Herring Bay, KN119A
Percent of total PAH
8/30/92
TPAH = 295 (35), n = 3
6/19/93
TPAH = 144 (25), n = 3
8/13/95
TPAH = 0, n = 3
No data were above MDL
in this data set
NPH
C1NPH
C2NPH
C3NPH
C4NPH
BPH
ACY
ACE
FLU
C1FLU
C2FLU
C3FLU
DBT
C1DBT
C2DBT
C3DBT
PHN
C1PHN
C2PHN
C3PHN
C4PHN
ANT
FLA
PYR
C1FLA
BAA
CHR
C1CHR
C2CHR
C3CHR
C4CHR
BbF
BkF
BEP
BAP
PER
IDP
DBA
BZP

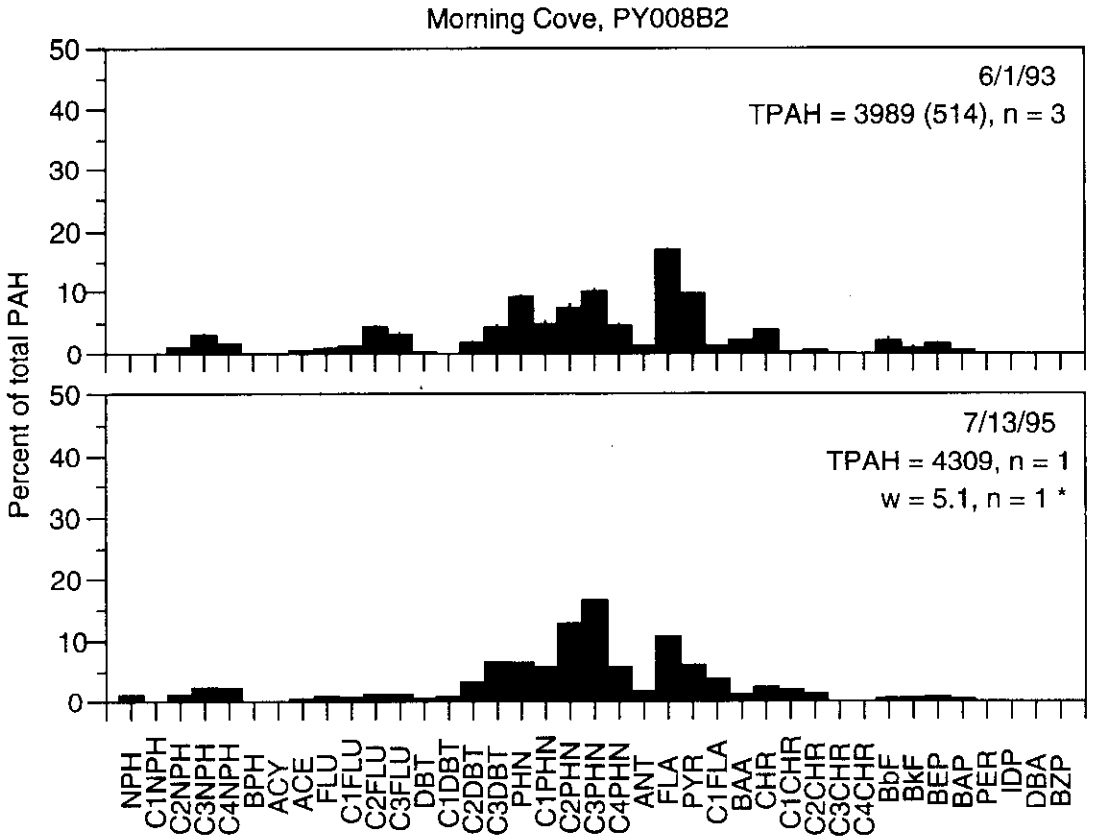

Herring Bay, KN133A-1
Percent of total PAH
5/16/92
TPAH = 359 (869), n = 8
w = 6.1 (0.6), n = 6 *
8/27/92
TPAH = 1893 (591), n = 5
6/20/93
TPAH = 1624 (244), n = 10
7/24/94
TPAH = 3403 (1408), n = 6
w = 7.6 (0.4), n = 6
5/15/95
TPAH = 4675 (914), n = 6
w = 6.3 (0.2), n = 5 *
0
10
20
30
40
50
NPH
C1NPH
C2NPH
C3NPH
C4NPH
BPH
ACY
ACE
FLU
C1FLU
C2FLU
C3FLU
DBT
C1DBT
C2DBT
C3DBT
PHN
C1PHN
C2PHN
C3PHN
C4PHN
ANT
FLA
PYR
C1FLA
BAA
CHR
C1CHR
C2CHR
C3CHR
C4CHR
BbF
BkF
BEP
BAP
PER
IDP
DBA
BZP

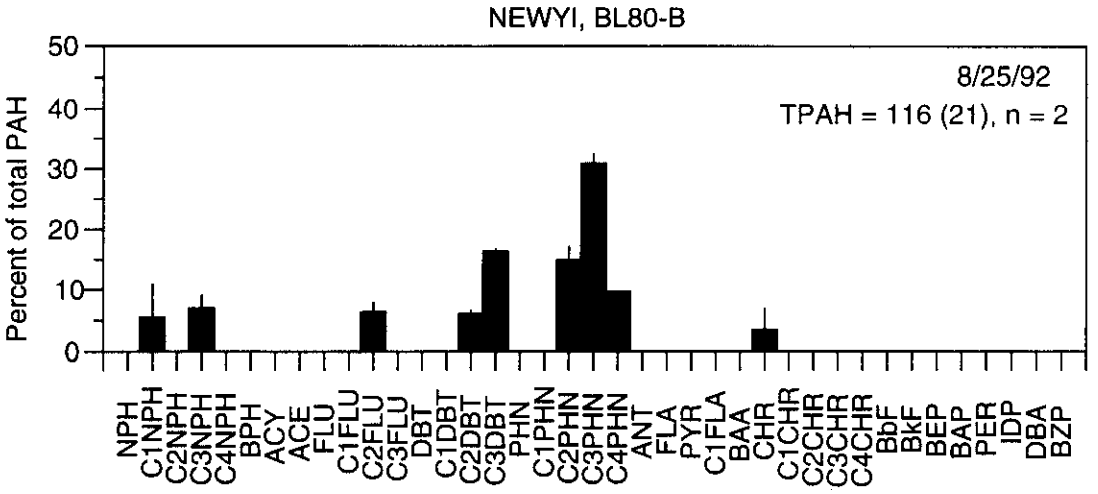

Herring Point, KN505A-2
8/13/95
TPAH = 2517 (676), n = 3
w = 5.8 (0.6), n = 3
Percent of total PAH
50
40
30
20
10
0
NPH
C1NPH
C2NPH
C3NPH
C4NPH
BPH
ACY
ACE
FLU
C1FLU
C2FLU
C3FLU
DBT
C1DBT
C2DBT
C3DBT
PHN
C1PHN
C2PHN
C3PHN
C4PHN
ANT
FLA
PYR
C1FLA
BAA
CHR
C1CHR
C2CHR
C3CHR
C4CHR
BbF
BkF
BEP
BAP
PER
IDP
DBA
BZP

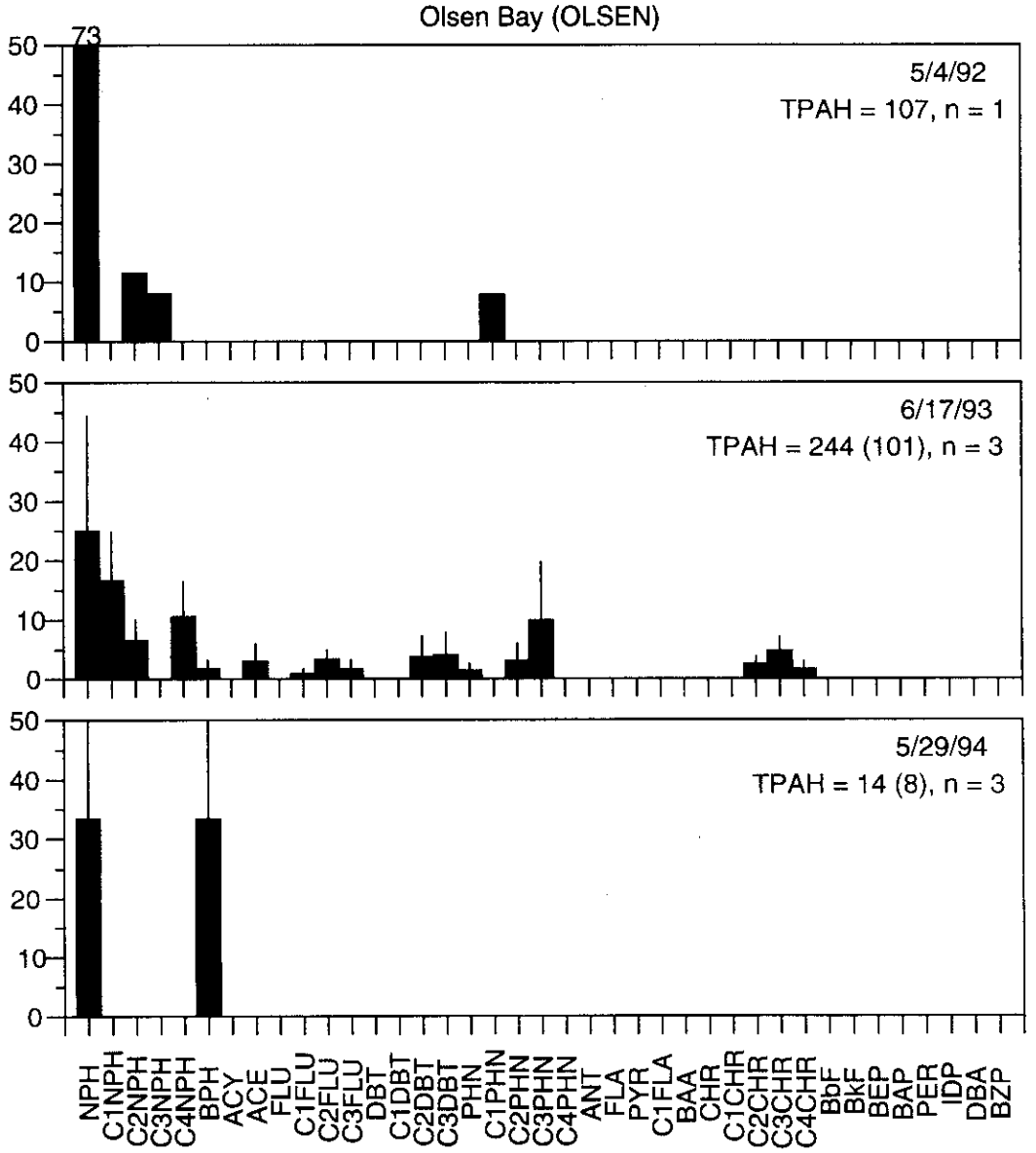

LATOI, LAO15E-2
Percent of total PAH
5/20/92
TPAH = 418 (224), n = 4
6/15/92
TPAH = 578 (207), n = 3
w = 4.6 (0.2), n = 2 *
6/22/93
TPAH = 138 (48), n = 4
8/10/95
TPAH = 634 (290), n = 4
w = 6.8, n = 1 *
NPH
C1NPH
C2NPH
C3NPH
C4NPH
BPH
ACY
ACE
FLU
C1FLU
C2FLU
C3FLU
DBT
C1DBT
C2DBT
C3DBT
PHN
C1PHN
C2PHN
C3PHN
C4PHN
ANT
FLA
PYR
C1FLA
BAA
CHR
C1CHR
C2CHR
C3CHR
C4CHR
BbF
BkF
BEP
BAP
PER
IDP
DBA
BZP

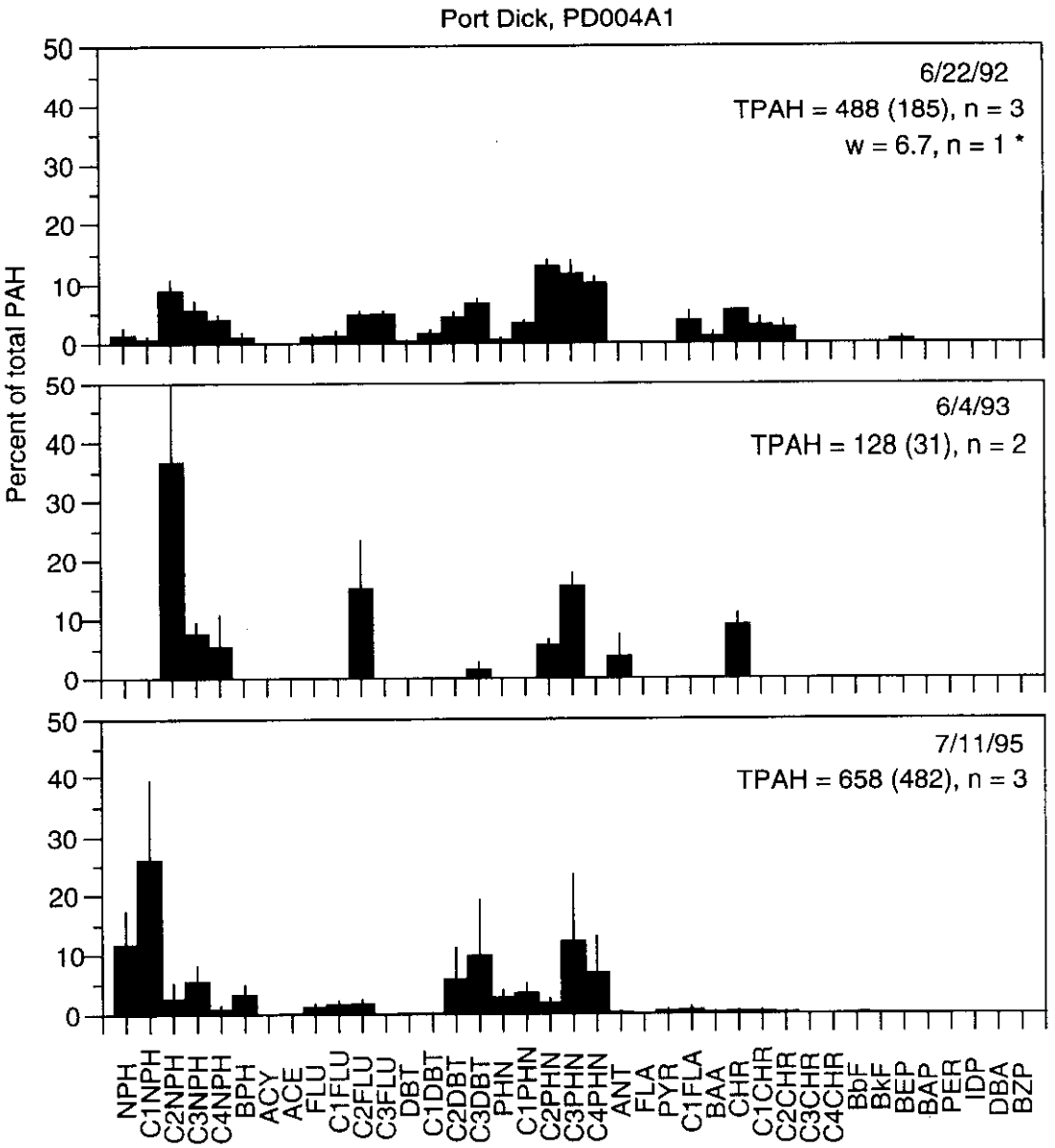

MARSB, KN702B
6/22/93
TPAH = 153 (30), n = 2
4/30/94
TPAH = 175 (66), n = 3
8/13/95
TPAH = 25 (25), n = 3
Percent of total PAH
0
10
20
30
40
50
NPH
C1NPH
C2NPH
C3NPH
C4NPH
BPH
ACY
ACE
FLU
C1FLU
C2FLU
C3FLU
DBT
C1DBT
C2DBT
C3DBT
PHN
C1PHN
C2PHN
C3PHN
C4PHN
ANT
FLA
PYR
C1FLA
BAA
CHR
C1CHR
C2CHR
C3CHR
C4CHR
BbF
BkF
BEP
BAP
PER
IDP
DBA
BZP

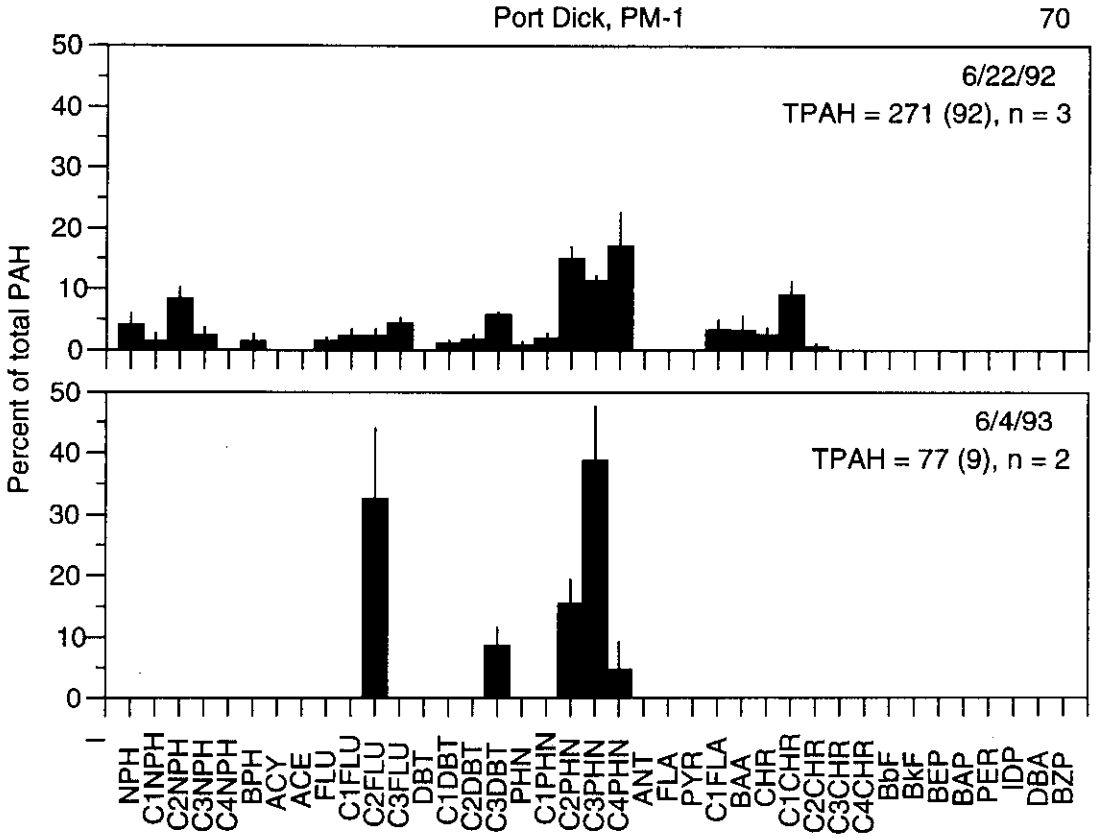

Morning Cove, PY008B11
Percent of total PAH
6/10/92
TPAH = 3486 (723), n = 3
w = 4.7 (0.3), n = 2 *
6/1/93
TPAH = 4007 (1544), n = 3
w = 4.8, n = 1 ns
7/13/95
TPAH = 4996 (706), n = 3
w = 7.3 (0.3), n = 3 ns
NPH
C1NPH
C2NPH
C3NPH
C4NPH
BPH
ACY
ACE
FLU
C1FLU
C2FLU
C3FLU
DBT
C1DBT
C2DBT
C3DBT
PHN
C1PHN
C2PHN
C3PHN
C4PHN
ANT
FLA
PYR
C1FLA
BAA
CHR
C1CHR
C2CHR
C3CHR
C4CHR
BbF
BkF
BEP
BAP
PER
IDP
DBA
BZP

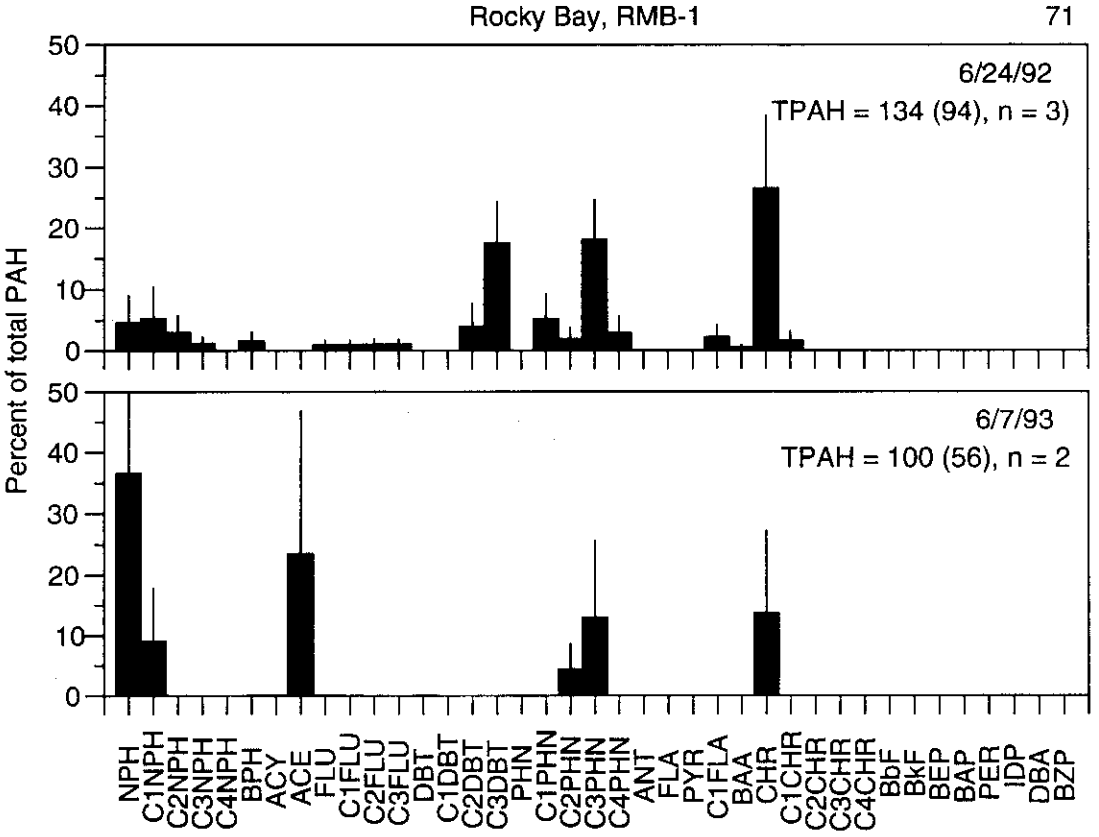

Morning Cove, PY008B2
Percent of total PAH
6/1/93
TPAH = 3989 (514), n = 3
7/13/95
TPAH = 4309, n = 1
w = 5.1, n = 1 *
0
10
20
30
40
50
NPH
C1NPH
C2NPH
C3NPH
C4NPH
BPH
ACY
ACE
FLU
C1FLU
C2FLU
C3FLU
DBT
C1DBT
C2DBT
C3DBT
PHN
C1PHN
C2PHN
C3PHN
C4PHN
ANT
FLA
PYR
C1FLA
BAA
CHR
C1CHR
C2CHR
C3CHR
C4CHR
BbF
BkF
BEP
BAP
PER
IDP
DBA
BZP

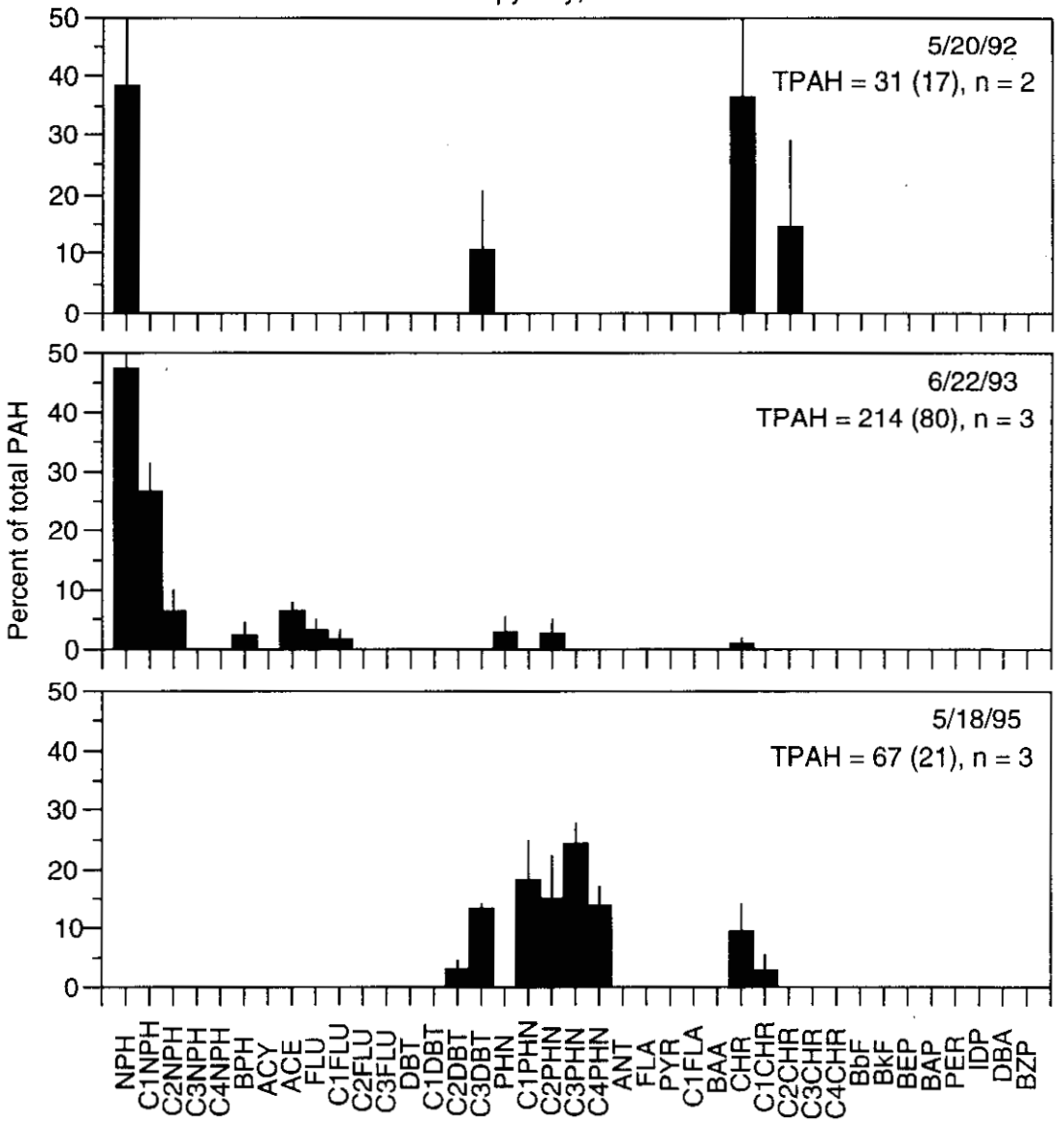

NEWYI, BL80-B
Percent of total PAH
8/25/92
TPAH = 116 (21), n = 2
NPH C1NPH C2NPH C3NPH C4NPH BPH ACY ACE FLU C1FLU C2FLU C3FLU DBT C1DBT C2DBT C3DBT PHN C1PHN C2PHN C3PHN C4PHN ANT FLA PYR C1FLA BAA CHR C1CHR C2CHR C3CHR C4CHR BbF BkF BEP BAP PER IDP DBA BZP

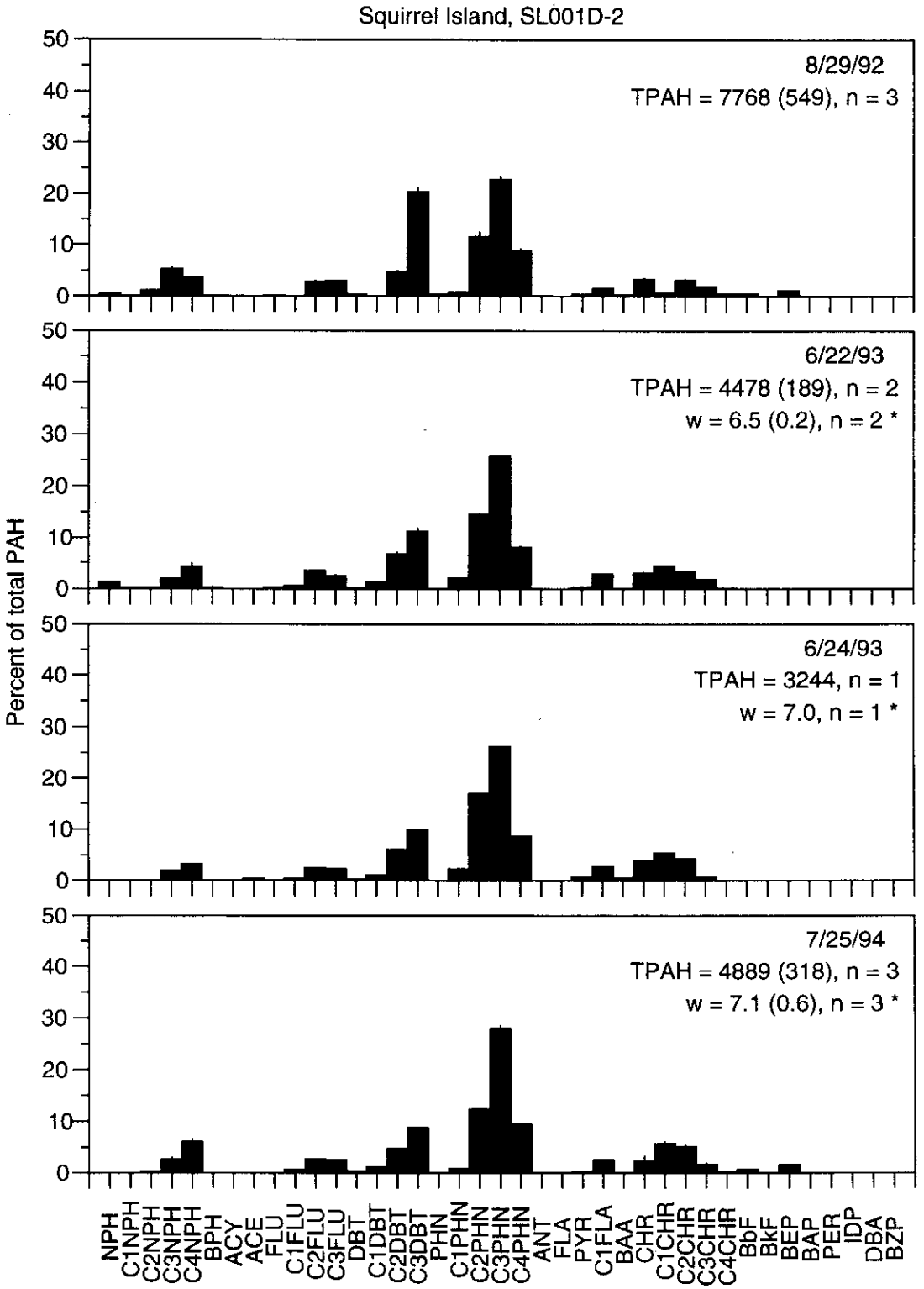

Olsen Bay (OLSEN)
73
5/4/92
TPAH = 107, n = 1
6/17/93
TPAH = 244 (101), n = 3
5/29/94
TPAH = 14 (8), n = 3
NPH C1NPH C2NPH C3NPH C4NPH BPH ACY ACE FLU C1FLU C2FLU C3FLU DBT C1DBT C2DBT C3DBT PHN C1PHN C2PHN C3PHN C4PHN ANT FLA PYR C1FLA BAA CHR C1CHR C2CHR C3CHR C4CHR BbF BkF BEP BAP PER IDP DBA BZP

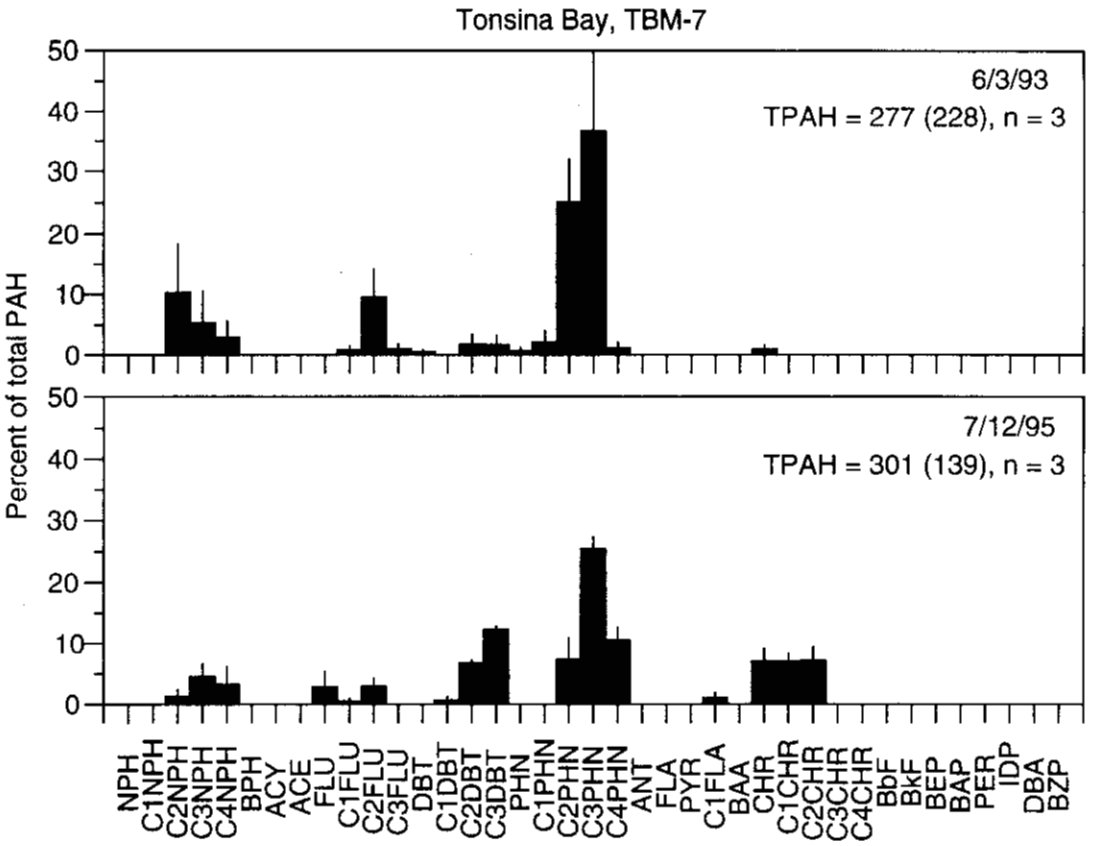

Port Dick, PD004A1
Percent of total PAH
6/22/92
TPAH = 488 (185), n = 3
w = 6.7, n = 1 *
6/4/93
TPAH = 128 (31), n = 2
7/11/95
TPAH = 658 (482), n = 3
NPH
C1NPH
C2NPH
C3NPH
C4NPH
BPH
ACY
ACE
FLU
C1FLU
C2FLU
C3FLU
DBT
C1DBT
C2DBT
C3DBT
PHN
C1PHN
C2PHN
C3PHN
C4PHN
ANT
FLA
PYR
C1FLA
BAA
CHR
C1CHR
C2CHR
C3CHR
C4CHR
BbF
BkF
BEP
BAP
PER
IDP
DBA
BZP

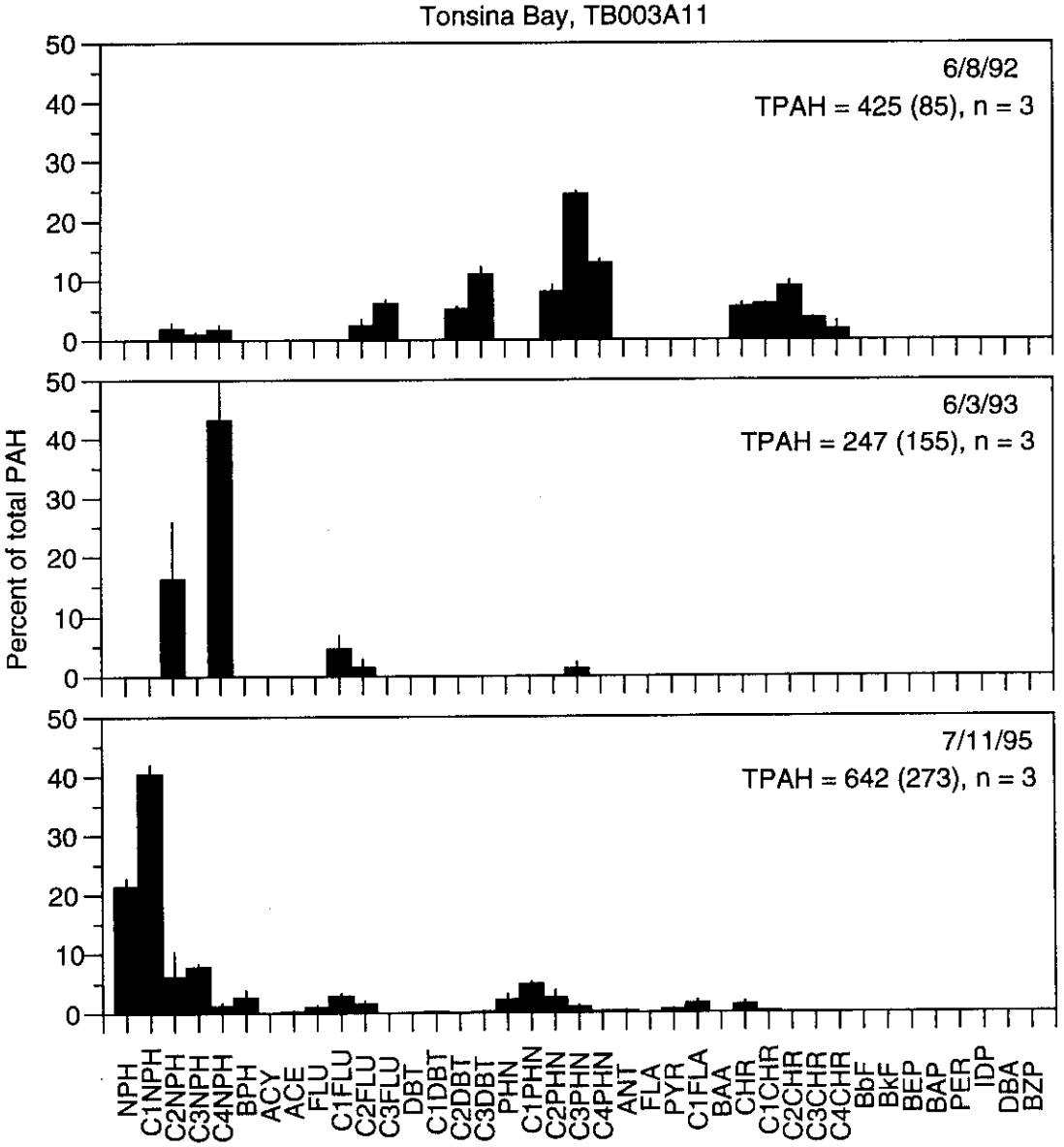

Port Dick, PM-1
70
6/22/92
TPAH = 271 (92), n = 3
6/4/93
TPAH = 77 (9), n = 2
Percent of total PAH
0
10
20
30
40
50
NPH
C1NPH
C2NPH
C3NPH
C4NPH
BPH
ACY
ACE
FLU
C1FLU
C2FLU
C3FLU
DBT
C1DBT
C2DBT
C3DBT
PHN
C1PHN
C2PHN
C3PHN
C4PHN
ANT
FLA
PYR
C1FLA
BAA
CHR
C1CHR
C2CHR
C3CHR
C4CHR
BbF
BkF
BEP
BAP
PER
IDP
DBA
BZP

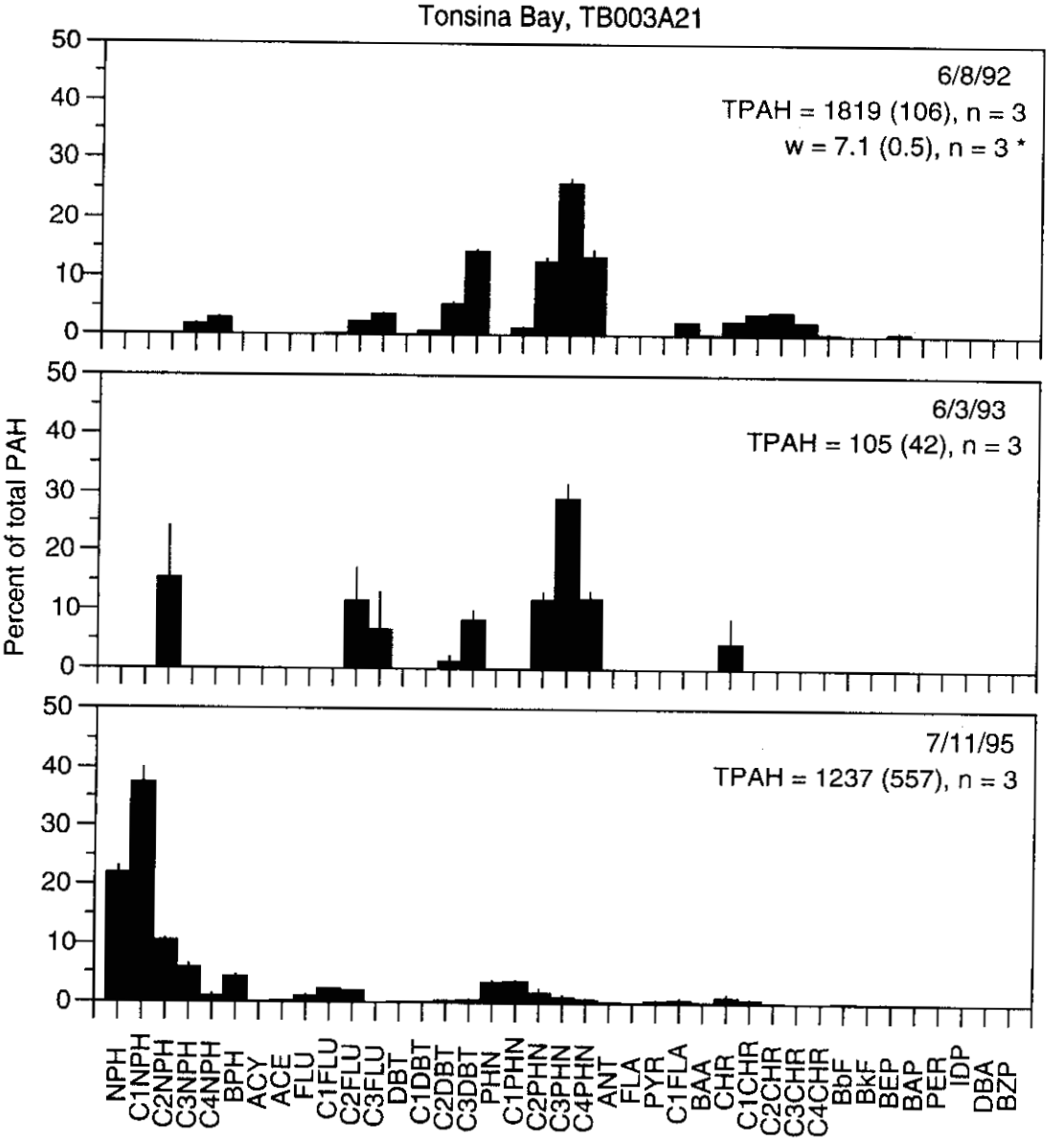

Rocky Bay, RMB-1
71
6/24/92
TPAH = 134 (94), n = 3)
6/7/93
TPAH = 100 (56), n = 2
Percent of total PAH
0
10
20
30
40
50
NPH
C1NPH
C2NPH
C3NPH
C4NPH
BPH
ACY
ACE
FLU
C1FLU
C2FLU
C3FLU
DBT
C1DBT
C2DBT
C3DBT
PHN
C1PHN
C2PHN
C3PHN
C4PHN
ANT
FLA
PYR
C1FLA
BAA
CHR
C1CHR
C2CHR
C3CHR
C4CHR
BbF
BkF
BEP
BAP
PER
IDP
DBA
BZP

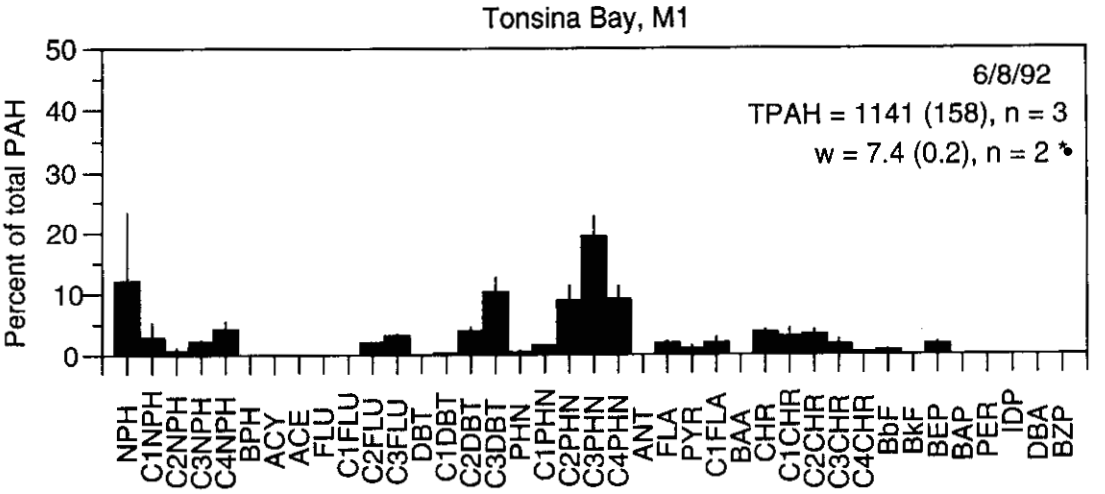

Percent of total PAH
5/20/92
TPAH = 31 (17), n = 2
6/22/93
TPAH = 214 (80), n = 3
5/18/95
TPAH = 67 (21), n = 3
0
10
20
30
40
50
NPH
C1NPH
C2NPH
C3NPH
C4NPH
BPH
ACY
ACE
FLU
C1FLU
C2FLU
C3FLU
DBT
C1DBT
C2DBT
C3DBT
PHN
C1PHN
C2PHN
C3PHN
C4PHN
ANT
FLA
PYR
C1FLA
BAA
CHR
C1CHR
C2CHR
C3CHR
C4CHR
BbF
BkF
BEP
BAP
PER
IDP
DBA
BZP

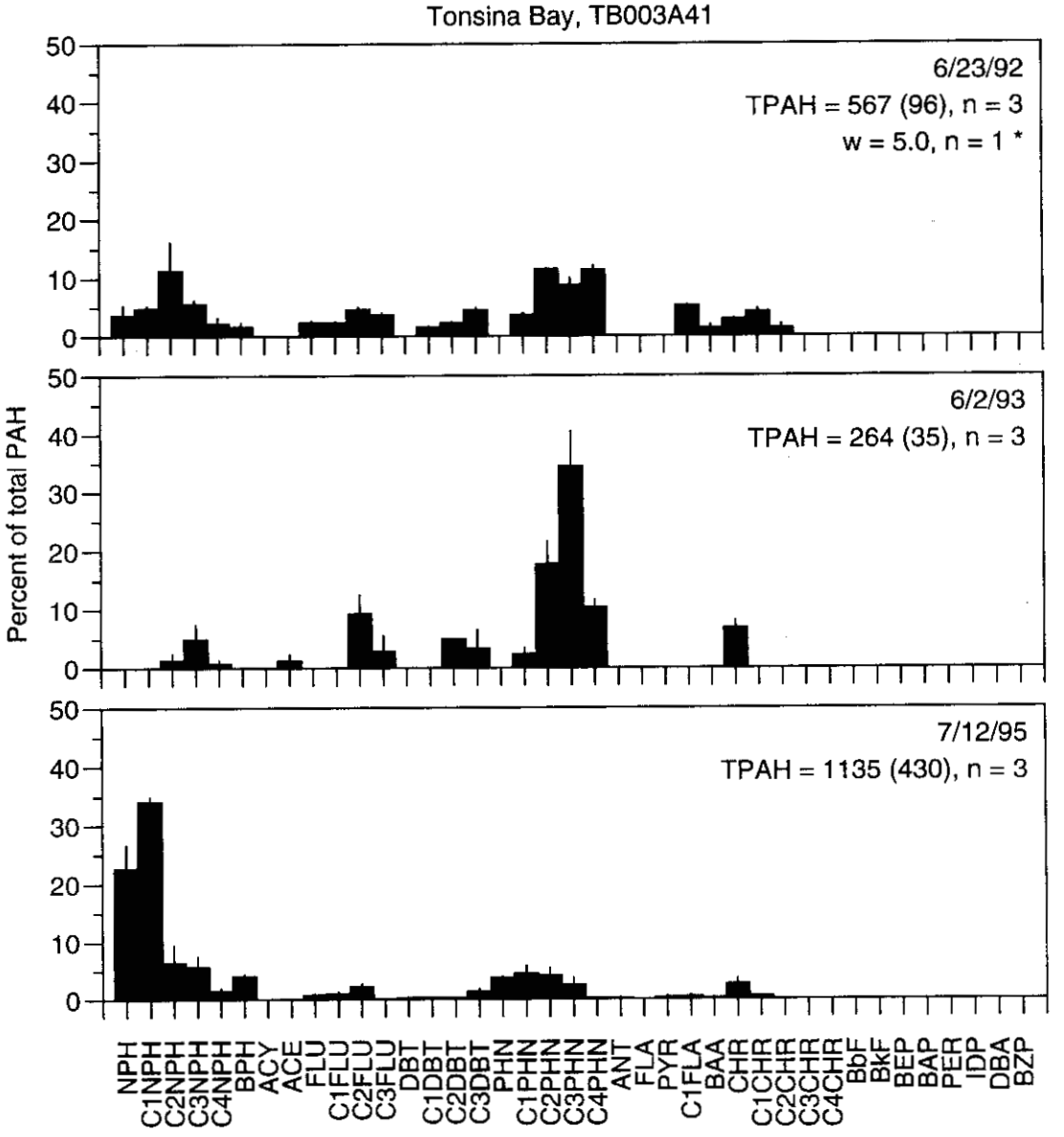

Squirrel Island, SL001D-2
Percent of total PAH
8/29/92
TPAH = 7768 (549), n = 3
6/22/93
TPAH = 4478 (189), n = 2
w = 6.5 (0.2), n = 2 *
6/24/93
TPAH = 3244, n = 1
w = 7.0, n = 1 *
7/25/94
TPAH = 4889 (318), n = 3
w = 7.1 (0.6), n = 3 *
0
10
20
30
40
50
NPH
C1NPH
C2NPH
C3NPH
C4NPH
BPH
ACY
ACE
FLU
C1FLU
C2FLU
C3FLU
DBT
C1DBT
C2DBT
C3DBT
PHN
C1PHN
C2PHN
C3PHN
C4PHN
ANT
FLA
PYR
C1FLA
BAA
CHR
C1CHR
C2CHR
C3CHR
C4CHR
BbF
BkF
BEP
BAP
PER
IDP
DBA
BZP

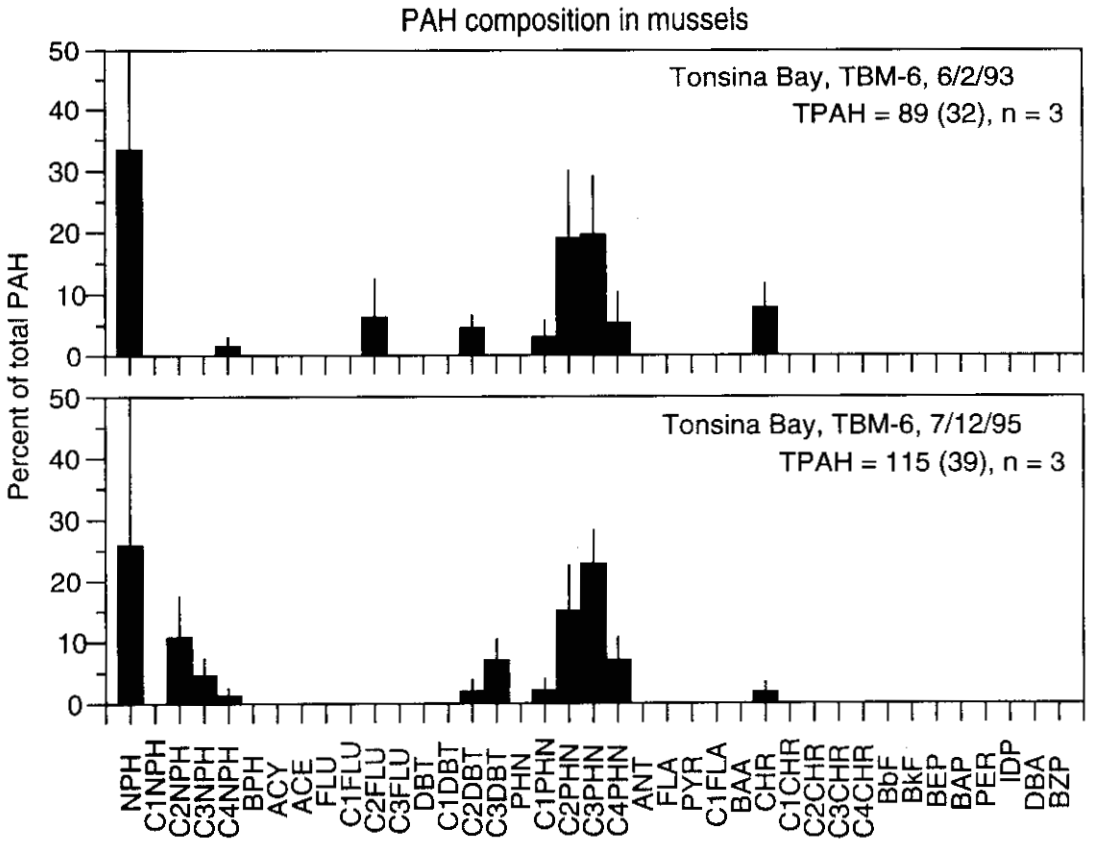

Tonsina Bay, TBM-7
6/3/93
TPAH = 277 (228), n = 3
7/12/95
TPAH = 301 (139), n = 3
Percent of total PAH
0
10
20
30
40
50
NPH
C1NPH
C2NPH
C3NPH
C4NPH
BPH
ACY
ACE
FLU
C1FLU
C2FLU
C3FLU
DBT
C1DBT
C2DBT
C3DBT
PHN
C1PHN
C2PHN
C3PHN
C4PHN
ANT
FLA
PYR
C1FLA
BAA
CHR
C1CHR
C2CHR
C3CHR
C4CHR
BbF
BkF
BEP
BAP
PER
IDP
DBA
BZP

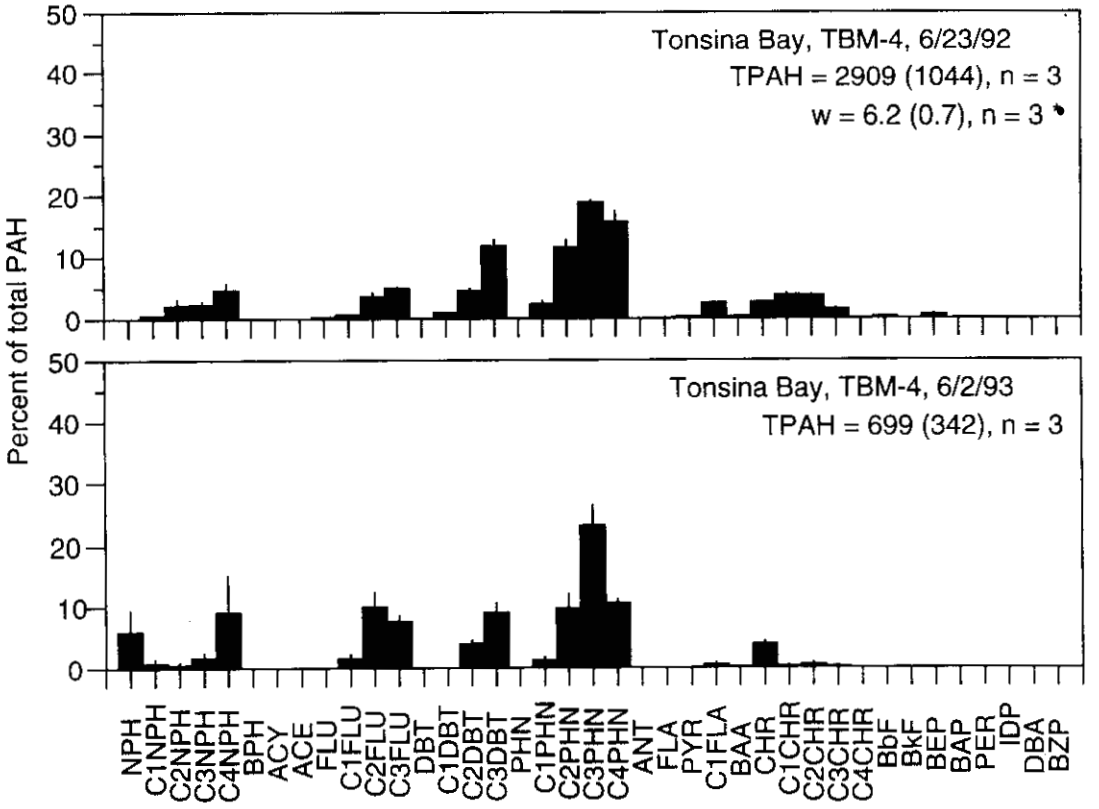

Tonsina Bay, TB003A11
Percent of total PAH
6/8/92
TPAH = 425 (85), n = 3
6/3/93
TPAH = 247 (155), n = 3
7/11/95
TPAH = 642 (273), n = 3
0
10
20
30
40
50
NPH
C1NPH
C2NPH
C3NPH
C4NPH
BPH
ACY
ACE
FLU
C1FLU
C2FLU
C3FLU
DBT
C1DBT
C2DBT
C3DBT
PHN
C1PHN
C2PHN
C3PHN
C4PHN
ANT
FLA
PYR
C1FLA
BAA
CHR
C1CHR
C2CHR
C3CHR
C4CHR
BbF
BkF
BEP
BAP
PER
IDP
DBA
BZP

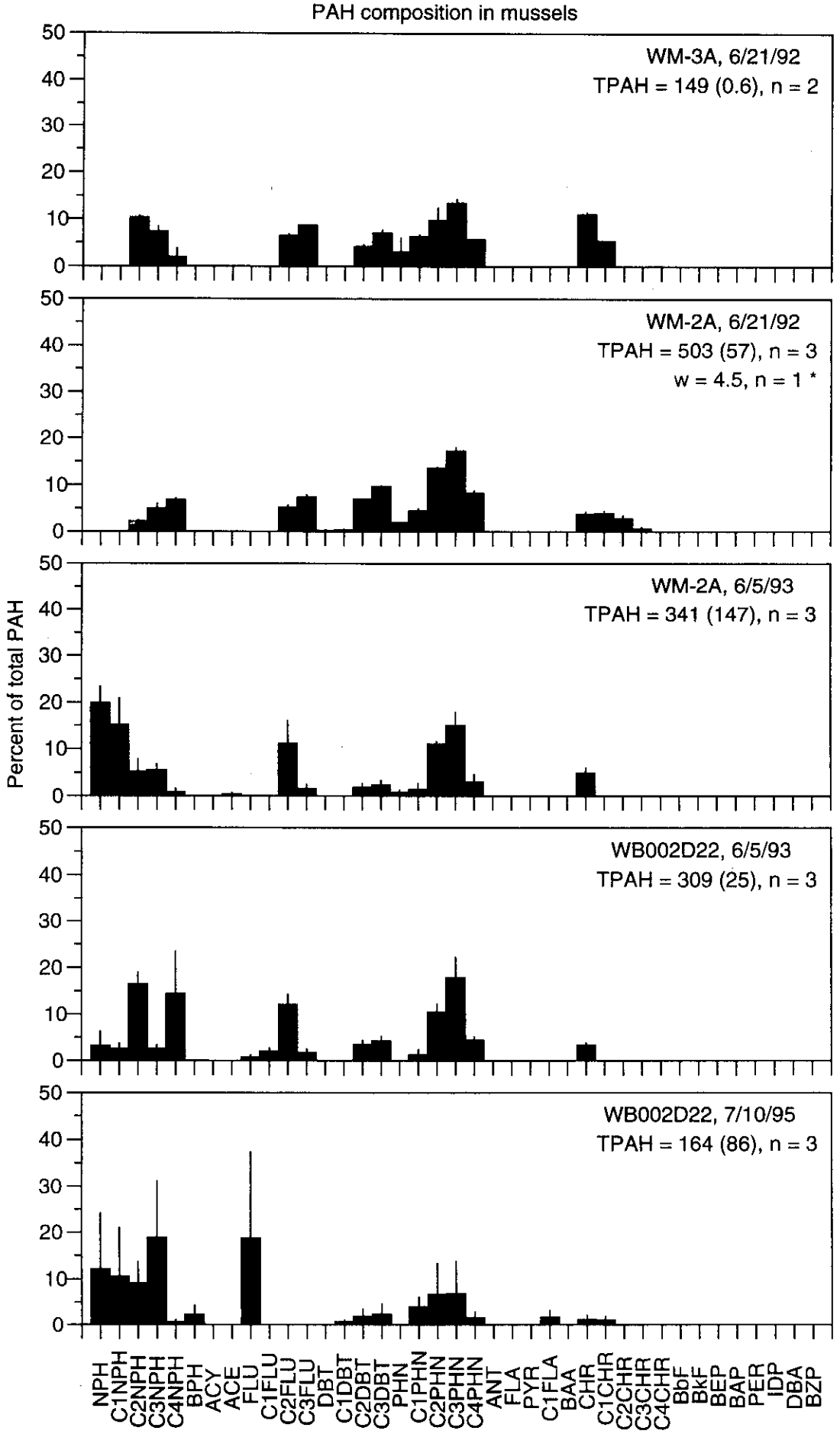

Tonsina Bay, TB003A21
Percent of total PAH
6/8/92
TPAH = 1819 (106), n = 3
w = 7.1 (0.5), n = 3 *
6/3/93
TPAH = 105 (42), n = 3
7/11/95
TPAH = 1237 (557), n = 3
NPH
C1NPH
C2NPH
C3NPH
C4NPH
BPH
ACY
ACE
FLU
C1FLU
C2FLU
C3FLU
DBT
C1DBT
C2DBT
C3DBT
PHN
C1PHN
C2PHN
C3PHN
C4PHN
ANT
FLA
PYR
C1FLA
BAA
CHR
C1CHR
C2CHR
C3CHR
C4CHR
BbF
BkF
BEP
BAP
PER
IDP
DBA
BZP


Tonsina Bay, M1
6/8/92
TPAH = 1141 (158), n = 3
w = 7.4 (0.2), n = 2 *
Percent of total PAH
0
10
20
30
40
50
NPH
C1NPH
C2NPH
C3NPH
C4NPH
BPH
ACY
ACE
FLU
C1FLU
C2FLU
C3FLU
DBT
C1DBT
C2DBT
C3DBT
PHN
C1PHN
C2PHN
C3PHN
C4PHN
ANT
FLA
PYR
C1FLA
BAA
CHR
C1CHR
C2CHR
C3CHR
C4CHR
BbF
BkF
BEP
BAP
PER
IDP
DBA
BZP


Tonsina Bay, TB003A41
6/23/92
TPAH = 567 (96), n = 3
w = 5.0, n = 1 *
6/2/93
TPAH = 264 (35), n = 3
7/12/95
TPAH = 1135 (430), n = 3
Percent of total PAH
0
10
20
30
40
50
NPH
C1NPH
C2NPH
C3NPH
C4NPH
BPH
ACY
ACE
FLU
C1FLU
C2FLU
C3FLU
DBT
C1DBT
C2DBT
C3DBT
PHN
C1PHN
C2PHN
C3PHN
C4PHN
ANT
FLA
PYR
C1FLA
BAA
CHR
C1CHR
C2CHR
C3CHR
C4CHR
BbF
BkF
BEP
BAP
PER
IDP
DBA
BZP


PAH composition in mussels
Tonsina Bay, TBM-6, 6/2/93
TPAH = 89 (32), n = 3
Tonsina Bay, TBM-6, 7/12/95
TPAH = 115 (39), n = 3
Percent of total PAH
0
10
20
30
40
50
NPH
C1NPH
C2NPH
C3NPH
C4NPH
BPH
ACY
ACE
FLU
C1FLU
C2FLU
C3FLU
DBT
C1DBT
C2DBT
C3DBT
PHN
C1PHN
C2PHN
C3PHN
C4PHN
ANT
FLA
PYR
C1FLA
BAA
CHR
C1CHR
C2CHR
C3CHR
C4CHR
BbF
BkF
BEP
BAP
PER
IDP
DBA
BZP


Tonsina Bay, TBM-4, 6/23/92
TPAH = 2909 (1044), n = 3
w = 6.2 (0.7), n = 3
Tonsina Bay, TBM-4, 6/2/93
TPAH = 699 (342), n = 3
Percent of total PAH
0
10
20
30
40
50
NPH
C1NPH
C2NPH
C3NPH
C4NPH
BPH
ACY
ACE
FLU
C1FLU
C2FLU
C3FLU
DBT
C1DBT
C2DBT
C3DBT
PHN
C1PHN
C2PHN
C3PHN
C4PHN
ANT
FLA
PYR
C1FLA
BAA
CHR
C1CHR
C2CHR
C3CHR
C4CHR
BbF
BkF
BEP
BAP
PER
IDP
DBA
BZP


PAH composition in mussels
Percent of total PAH
WM-3A, 6/21/92
TPAH = 149 (0.6), n = 2
WM-2A, 6/21/92
TPAH = 503 (57), n = 3
w = 4.5, n = 1 *
WM-2A, 6/5/93
TPAH = 341 (147), n = 3
WB002D22, 6/5/93
TPAH = 309 (25), n = 3
WB002D22, 7/10/95
TPAH = 164 (86), n = 3
0
10
20
30
40
50
NPH
C1NPH
C2NPH
C3NPH
C4NPH
BPH
ACY
ACE
FLU
C1FLU
C2FLU
C3FLU
DBT
C1DBT
C2DBT
C3DBT
PHN
C1PHN
C2PHN
C3PHN
C4PHN
ANT
FLA
PYR
C1FLA
BAA
CHR
C1CHR
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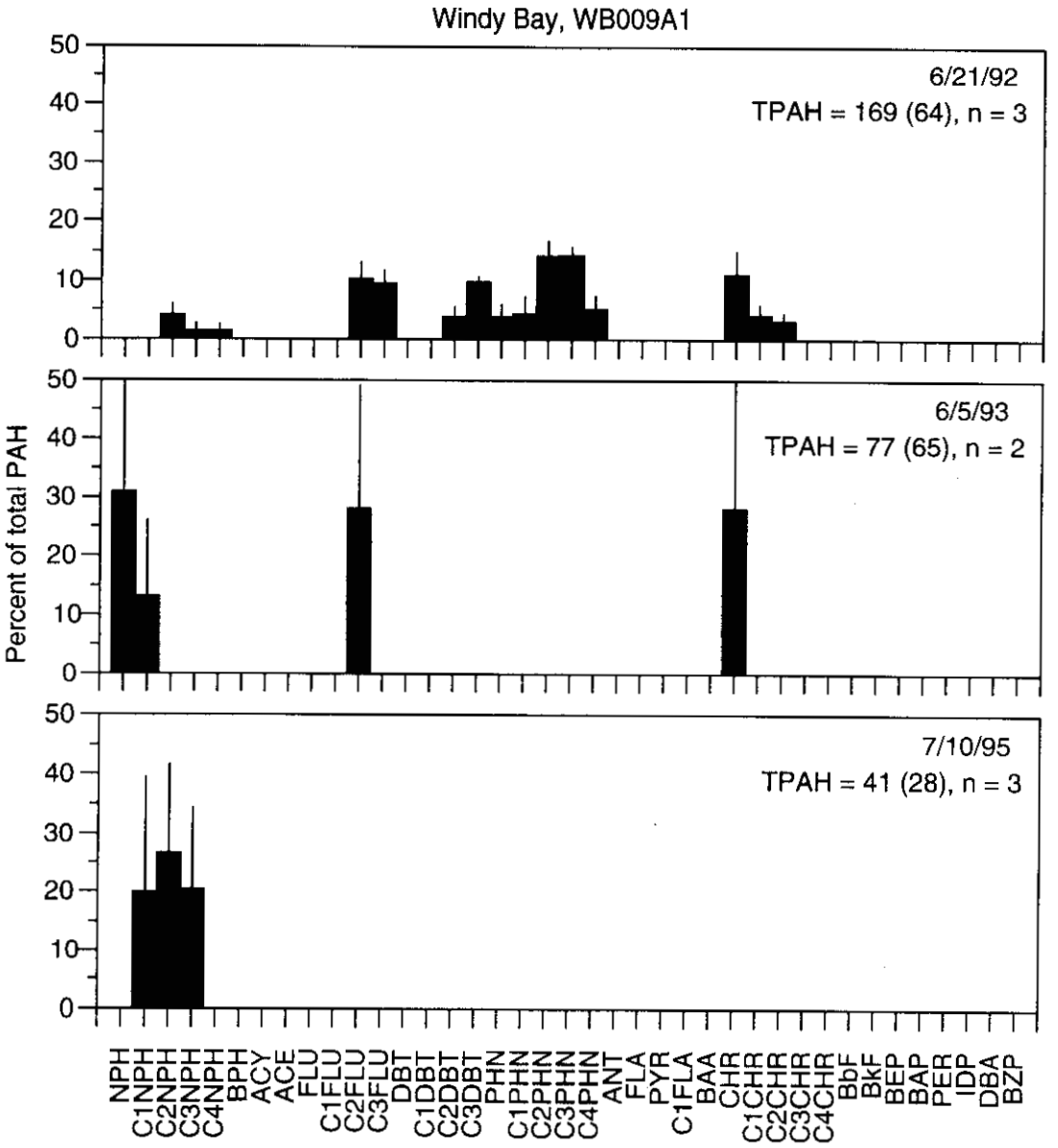
BbF
BkF
BEP
BAP
PER
IDP
DBA
BZP


Windy Bay, WB009A1
Percent of total PAH
6/21/92
TPAH = 169 (64), n = 3
6/5/93
TPAH = 77 (65), n = 2
7/10/95
TPAH = 41 (28), n = 3
0
10
20
30
40
50
NPH
C1NPH
C2NPH
C3NPH
C4NPH
BPH
ACY
ACE
FLU
C1FLU
C2FLU
C3FLU
DBT
C1DBT
C2DBT
C3DBT
PHN
C1PHN
C2PHN
C3PHN
C4PHN
ANT
FLA
PYR
C1FLA
BAA
CHR
C1CHR
C2CHR
C3CHR
C4CHR
BbF
BkF
BEP
BAP
PER
IDP
DBA
BZP