SUBTIDAL

NATURAL RESOURCE DAMAGE ASSESSMENT

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Petroleum Hydrocarbon-Induced Injury to Subtidal Marine Sediment Resources

Subtidal Study Number 1

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

EXECUTIVE SUMMARY

In the three years following the Exxon Valdez oil spill a total of 3,127 sediment samples have been collected from the intertidal and nearshore subtidal regions of Prince William Sound and the northeastern Gulf of Alaska under Subtidal Study Number 1 (formerly Air/Water Study Number 2). These sediment samples were collected from 32 locations inside Prince William Sound and eight locations outside the Sound.

Significant problems with the chemical analyses performed by GERG of a subset of the group of samples submitted for analysis in 1990 have severely limited progress on the interpretation of results on the spatial and temporal distribution of petroleum hydrocarbons in subtidal sediments throughout the geographical area of study. Gas chromatographic/mass spectrometric analysis has been completed on 174 of 1,188 (15%) of those samples collected from 32 locations in Prince William Sound and eight locations outside the Sound in 1989-90 and submitted to Technical Services Study Number 1 for analysis. Problems of sample contamination were first detected in the spring of 1991. Considerable effort has been spent to first document the problems and then to determine the source and extent of contamination. Damage to Subtidal Study Number 1 has not been completely determined. The process of sorting out the samples that have been compromised from those that have not is still in progress. No samples collected in 1991 have been submitted for analysis.

Results previously reported by Rice and O'Clair (1990) gave a preliminary view of the spatial and temporal variability in the degree of contamination of benthic sediments by oil. Within Prince William Sound subtidal sediments were contaminated by oil at no fewer than 11 sites in 1989. Subtidal sediments were found to be contaminated by oil at four more sites in June 1990 (no samples collected in 1989 from these sites have been analysed). Hydrocarbon contamination of sediments had reached a depth of 20 m in a minimum of 7 sites in 1989. One more site was found to have contaminated sediments to 20 m in June 1990.

The highest level of total hydrocarbons (defined as the sum of the total aromatic hydrocarbons, total alkanes and unresolved complex mixture) reached 507 μg/g (ppm) wet weight in a sediment sample collected at about 0 m (mean lower low water) at Northwest Bay in July 1989. This sample contained a concentration of total aromatic hydrocarbons (14 ppm) about 50-100 times the concentration of aromatics found in pre-spill, 0-tide level sediments (Rice and O'Clair 1990). The greatest concentration of total hydrocarbons found in sediment samples from June 1990 was 220 μg/g at 0 m at Block Island. Concentrations of total hydrocarbons exceeding 100 μg/g occurred only at Block Island (0 and 6 m) and Northwest Bay (3-20 m) in June 1990.

Examination of temporal changes in the contamination of sediments by oil at Sleepy Bay in 1989 revealed that there may have been a trend for petroleum hydrocarbons to move from the intertidal region to greater depths (3, 6, and 20 m) between May and November 1989. Northwest Bay and Herring Bay showed some tendency toward an increase in contamination of the 6 and 20 m depths between July 1989 and June 1990.

Rice and O'Clair (1990) found that outside Prince William Sound at least 7 sites along the Kenai and Alaska Peninsulas showed contamination of subtidal sediments by hydrocarbons. Petroleum hydrocarbons were detected below a depth of 6 m at three sites. No more sediment samples have been analysed from outside Prince William Sound under Air/Water Study Number 2 or Subtidal Study Number 1.

OBJECTIVES

A. Determine occurrence, persistence, and chemical composition of petroleum hydrocarbons in subtidal marine sediments at selected sites in Prince William Sound and the northeastern Gulf of Alaska.

B. Describe spatial (geographical and bathymetric) and temporal distributions of petroleum hydrocarbons in subtidal sediments.

C. Provide marine sediment data to assist agencies in mass balance calculations on the fate of oil in the marine environment.

D. Relate subtidal oil concentrations to adjacent intertidal concentrations and other studies.

E. Identify potential alternative methods and strategies for restoration of lost use, populations, or habitat where injury is identified.

INTRODUCTION

Initial results from gas chromatographic/mass spectrometric (GC/MS) analyses of subtidal sediments collected in Prince William Sound and the northeastern Gulf of Alaska following the EXXON Valdez oil spill have allowed some preliminary interpretations of the spatial and temporal patterns of distribution of petroleum hydrocarbons within and outside Prince William Sound during 1989 (Rice and O'Clair 1990). Here we attempt to extend the preliminary interpretations of Rice and O'Clair (1990) based on GC/MS analysis

of sediment samples collected in Prince William Sound in June 1990.

In addition to the present project assessing contamination of subtidal sediments by petroleum hydrocarbons, three projects funded under Air/Water Study Number 2 or Coastal Habitat Study Number 1 in 1990 were included in Subtidal Study Number 1 in 1991. All three were conducted by the University of Alaska Fairbanks. The goal of the first project was to assess the potential for 'in situ' biodegradation of selected hydrocarbon substrates at various sites in Prince William Sound. The second project titled "Injury to Deep Benthos" examined the responses to the oil spill of infaunal communities below a depth of 20 m. The third study examined the effects of the oil spill on infaunal communities associated with eelgrass and Laminaria beds in Prince William Sound. This report covers only those objectives listed above. Results of the other three projects encompassed by Subtidal Study Number 1 will be reported elsewhere.

STUDY METHODOLOGY

Sediments were sampled at 21 sites in Prince William Sound (10 reference sites and 11 contaminated sites) in 1991. Sampling was conducted during three periods (27 April to 2 May, 15-25 June and 5-9 September; Table 1).

Three samples each a composite of 8 subsamples collected randomly along a 30 m transect laid parallel to the shoreline were taken at each intertidal site. These samples were collected at low tide or by divers. Intertidal collections were made at a single tidal height in the range of +1 to -1 m relative to mean lower low water (MLLW) depending on the distribution of fine sediments.

Subtidal sediment collections were made at a depth of 6 m below MLLW in April/May and September and at depths of 3, 6, 20, 40 and 100 m in June. Additional sediment samples taken at 140 m at selected locations. Only the 40 and 100 m depths were sampled at West Bay (Table 1). Collections at 3, 6 and 20 m were be made by divers on transects laid along the appropriate isobath and sampled in the same way as described above for the intertidal transects. Samples taken at depths below 20 m were collected with a stainless steel Smith-McIntyre grab. Three grabs were taken at each depth. Four subsamples were removed at randomly selected points within each grab. The subsamples were combined to form one sample per grab. The samples were taken at the same sites as those where benthos was collected (results of the deep benthos project of Subtidal Study Number 1 are reported elsewhere), however sediments were taken from a different grab than were the benthos samples because the volume removed for sediment hydrocarbon analysis would have jeopardized the quality of the benthos samples.

All samples collected by hand (including those removed by hand from the Smith-McIntyre grab) were taken from the surface (top 0-2 cm) of the sediment column. Samples taken by hand in the intertidal region were collected using a stainless steel core tube (2.5 cm inside diameter), spoon or stainless steel scoop. Each subsample was transferred to a sample jar using a spatula or deposited directly from the spoon or scoop. All implements were washed, dried and rinsed with methylene chloride between sampling periods. Sample jars were purchased clean according to EPA specifications. The jars were fitted with precleaned teflon lined caps. Samples were kept cool then frozen within 1-2 hours after collection. Appropriate blanks were collected at each site. Standard operating procedures for sediment sampling were followed. Chain of custody procedures were followed after collection of all samples.

Technical Services Study Number 1 has promulgated a set of provisional criteria for determining the presence of petrogenic hydrocarbons. The criteria comprise the following indices: 1. the pristane/phytane ratio, 2. the Carbon Preference Index (CPI, Farrington and Tripp, 1977), 3. the unresolved complex mixture (UCM) and 4. the number of compounds that are present from the naphthalene, phenanthrene and dibenzothiophene groups of aromatic hydrocarbons. A low value (1-3) for CPI indicates high concentrations of petrogenic hydrocarbons as does a large UCM and a large proportion of possible naphthalene, phenanthrene and dibenzothiophene compounds. A high pristane/phytane ratio generally indicates the absence of petrogenic hydrocarbons. For the purposes of the present report we follow the determination of Technical Services Study Number 1 of the presence or lack of petroleum hydrocarbons in sediments analysed with a gas chromatograph/mass spectrometer.

STUDY RESULTS

A total of 3,127 sediment samples (including 402 in 1991) have been collected in Prince William Sound and along the shores of the Gulf of Alaska from Prince William Sound to the Shumigan Islands in the three years following the Exxon Valdez oil spill. We have submitted 1,188 sediment samples of those collected in 1989 and 1990 for analysis by gas chromatography/mass spectrometry (GC/MS) under the Hydrocarbon Analytical Support Services Study (Technical Services Study Number 1). The samples were collected from the intertidal region and from subtidal depths at selected sites in Prince William Sound in 1989 and 1990 and along the Kenai and Alaska Peninsulas in 1989 (Figures 1 and 2). Hydrocarbon analysis is complete for 134 samples submitted from Prince William Sound and 40 samples submitted from the Kenai and Alaska Peninsulas (Tables 2 and 3). Because only a small percentage (5.6%) of the samples collected to date have been analysed and appear to be free of analytical problems our results at this stage are PRELIMINARY.

In the spring of 1991 major problems apparently resulting from sample contamination were discovered in a subset of samples collected in 1990 and analysed by GERG. A considerable amount of time has been spent to determine the source and extent of the contamination problem. We are not confident that samples analysed after the date when the apparent analytical problem first arose are free of contamination. None of the samples collected in 1991 have been submitted for analysis. Statistical analysis of the data collected under Subtidal Study Number 1 has progressed little because of the paucity of samples for which chemical analysis is complete and uncompromised. Beginning 1 October 1991 a new effort was initiated to determine which groups of samples and which samples within groups are contaminated. This effort is still in progress.

Samples analysed subsequent to our 1990 report (Rice and O'Clair 1990) and discussed herein were collected in June 1990. These included samples from four reference sites (Ewan Bay, Macleod Harbor, Olsen Bay and Paddy Bay) and three oiled sites (Disk Island, Eshamy Bay and the "NOAA" site at Green Island; Table 2) not included in Rice and O'Clair (1990). June 1990 analytical data were available for five of the 18 locations in Prince William Sound for which some hydrocarbon analysis was complete in 1990. No hydrocarbon analyses were available for samples collected outside Prince William Sound in 1990.

The results of the hydrocarbon analyses obtained thus far indicate that subtidal sediments at no fewer that 11 locations showed unequivocal contamination (as judged by Technical Services Study Number 1) by petrogenic hydrocarbons in 1989 (Table 4). Four additional sites were found to have contaminated subtidal sediments in 1990. Sediments were contaminated at all subtidal depths sampled down to 20 m at one of these sites (Disk Is.). Five locations showed a similar pattern of "continuous" contamination down to 20 m in 1989. Hydrocarbon analyses from 1990 were available for only two of those locations; one location (Northwest Bay) continued to show sediment contamination to 20 m in June 1990. Two bays (Northwest and Sleepy Bays) suffered relatively low level contamination at 100 m in 1989. As of this writing no hydrocarbon analyses were available for depths greater than 20 m in 1990 (Table 2).

The greatest concentration of total hydrocarbons (THC, defined as the sum of the total aromatic hydrocarbons, total alkanes and unresolved complex mixture) reached in samples thus far analysed was 507 μg/g wet weight of sediment at about 0 m (mean lower low water) at Northwest Bay in July 1989 (Table 4, Figure 3a). Concentrations of THC exceeding 100 μg/g occurred at 0 m at Herring Bay in May, July and September and at shallow subtidal depths (in the range 3 - 10 m) at three locations (Bay of Isles, Block Island and Northwest Bay) in the fall of 1989 (Table 4, Figure 4a). Incomplete data from June 1990 showed THC concentrations exceeding

100 μg/g at Block Island (0, 6 m) and Northwest Bay (3-20 m). The greatest THC concentration recorded thus far in Prince William Sound in 1990 was about 220 μg/g at 0 m at Block Island (Table 4).

Concentrations of THC in the benthic sediments of contaminated sites decreased rapidly below mean lower low water in 1989 generally reaching levels below 50 μg/g wet weight at 6 m at most sites in Prince William Sound for which results were available (Table 4, Figures 3a and 4a). Two heavily contaminated sites (Northwest Bay and Herring Bay) showed some tendency toward greater contamination of 6 and 20 m depths in June 1990 than in July 1989 (Table 4, Figures 3a and 4a). This tendency received stronger support at Herring Bay where the concentration of dibenzothiophenes (one of the groups of compounds characteristic of petrogenic hydrocarbons) as well as THC appeared elevated at these depths (Figure 4b). The concentration of dibenzothiophenes at Northwest Bay appeared to increase only at 6 m between July 1989 and June 1990 (Figure 3b). Though quite incomplete (eg. the 1990 data consist of one sample at each depth), these results are consistent with those reported by Rice and O'Clair (1990) for Sleepy Bay where there was some evidence indicating a trend of movement of oil to greater bathymetric depths with time between May and September, 1989.

Rice and O'Clair (1990) presented evidence of contamination of subtidal sediments by petroleum hydrocarbons at seven of eight sites outside Prince William Sound for which analytical results were available (Figures 2 and 5). The concentration of total hydrocarbons in the sediments was relatively low never exceeding 35 μg/g wet weight. At most sites hydrocarbon contamination was confined to shallow sediments. However, petroleum hydrocarbons were detected in sediments below a depth of 6 m at Black Bay, Chugach Bay and Chignik Bay with contamination apparently reaching 100 m at Black Bay (Figure 7). No additional hydrocarbon data has become available from outside Prince William Sound since Rice and O'Clair (1990).

STATUS OF INJURY ASSESSMENT

The paucity of completed GC/MS analyses supplied by Technical Services Study Number 1 continues to hamper interpretation of the geographical and bathymetric trends and the temporal changes in the distribution of oil in subtidal sediments in Prince William Sound and the northeastern Gulf of Alaska. Examination of the analytical data received to date has allowed us to set a lower limit on the number of locations that suffered contamination of subtidal sediments by petroleum hydrocarbons and to provide a preliminary sketch of the bathymetric distribution of hydrocarbon contamination at those sites. These estimates of the spatial distribution of petroleum hydrocarbons within the study area are probably conservative and will be updated as more chemical data becomes

available. Analytical data received by us in 1991 provides modest support for the notion that oil moved to greater bathymetric depths at heavily contaminated sites from July 1989 to June 1990.

When hydrocarbon analysis is complete on those sediment samples that we have placed in the analytical queue we can adequately describe the temporal and spatial distribution of oil in subtidal sediments in Prince William Sound in 1989 and 1990 and should have enough data to test statistically the spatial and temporal factors influencing hydrocarbon distribution in the Sound. Additional samples must be analysed before we can adequately interpret the trends in data on the distribution of oil in sediments outside Prince William Sound. We have not submitted further samples for analysis since February 1991 for reasons given below.

In late spring 1991 it was brought to our attention that the analytical results from five sample catalogues analysed under Technical Services Study Number 1 were probably unreliable (Short, unpublished data). These catalogues involved samples analysed in December 1990 including samples collected chiefly after June 1990 although some samples collected in June 1990 were affected. Consequently, we have refrained from including results from these catalogues and from all catalogues analysed subsequent to December 1990 in the present report. We will remain cautious about reporting on data from these catalogues and will hold back samples identified for future hydrocarbon analysis until our confidence in the reliability of the sample analyses performed under Technical Services Study Number 1 is restored.

LITERATURE CITED

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Table 1

Location of sites in Prince William Sound where intertidal and subtidal sediment samples were collected in 1991. A dash indicates that no sampling was conducted during the time period shown.

Location	North Latitude[1] o ' "	West Longitude o ' "	Depths[2] Apr/May	Jun	Sep
Bay of Isles	60 23 00	147 44 54	-	C	-
Block Island	60 31 49	147 36 24	-	B	-
Chenega Island	60 19 49	148 00 24	-	C	-
Disk Island	60 29 55	147 39 40	-	B	-
Drier Bay	60 19 12	147 44 00	-	C	-
Eshamy Bay	60 26 54	147 58 30	A	-	A
Ewan Bay	60 22 00	148 08 00	A	-	A
Fox Farm	59 58 26	148 10 30	A	-	A
Herring Bay	60 25 51	147 47 06	A	C	A
Iktua Bay	60 06 00	147 59 42	A	-	A
Lower Herring Bay	60 24 12	147 47 48	-	C	-
MacLeod Harbor	59 53 43	147 45 48	-	B	-
Moose Lips Bay	60 12 30	147 18 06	-	B	-
Northwest Bay	60 33 07	147 34 36	-	B	-
Olsen Bay	60 45 05	146 11 13	A	B	A
Paddy Bay	60 25 00	148 06 00	A	-	A
Rocky Bay	60 20 19	147 07 59	-	B	-
Sleepy Bay	60 04 01	147 50 11	A	C	A
Snug Harbor	60 15 46	147 45 55	A	B	A
West Bay	60 52 21	146 47 54	-	D	-
Zaikof Bay	60 16 53	147 02 19	-	B	-

1. Latitude/Longitude refers to the "0" m site at each location where "0" is mean lower low water (MLLW).

2. Letters in body of table indicate depths sampled relative to MLLW: A = 0, 6 m; B = 0, 3, 6, 20, 40, 100 m; C = 0, 3, 6, 20, 40, 100, 140 m. D = 40, 100 m.

Table 2
Location, date of sampling, depth of collection and number of sediment samples from Prince William Sound for which hydrocarbon analysis is complete.

Location[1]	North Latitude[2] o ' "	West Longitude o ' "	Date	Depth[3]	Sample Size[4]
Applegate Is.	60 37 40	148 08 19	Nov 89	A	1
Bay of Isles					
Site 86	60 22 37	147 42 27	Nov 89	A	1
Site 90	60 22 53	147 42 45	Nov 89	B	4
NOAA	60 23 00	147 44 54	Jun 90	N	2
Site 90	60 22 53	147 42 45	Jun 90	O	1
Block Is.					
Site 7	60 31 49	147 36 10	Nov 89	A	1
Site 47	60 31 49	147 36 26	Nov 89	A	1
			Jun 90	L	3
Chenega Is.	60 19 49	148 00 24	Nov 89	A	1
Disk Is.	60 29 55	147 39 40	Jun 90	C	4
Eshamy Bay	60 26 56	147 58 26	Jun 90	E	2
Ewan Bay	60 24 10	148 09 24	Jun 90	E	2
Fox Farm	59 58 26	148 10 30	Jul 89	F	5
Green Is.					
Site 22	60 17 57	147 25 06	Nov 89	B	4
			Jun 90	C	4
NOAA	60 16 18	147 26 18	Jun 90	H	1
Herring Bay	60 25 51	147 47 06	May 89	E	6
NOAA			Jul 89	I	8
			Sep 89	C	4
Site 53	59 26 34	147 50 16	Nov 89	B	4
Site 125	60 29 20	147 43 07	Nov 89	A	1
Site 110	60 26 34	148 47 12	Nov 89	A	1
NOAA	60 25 51	147 47 06	Jun 90	C	4

Location	North Latitude	West Longitude	Date	Depth	Sample Size
Herring Bay					
Site 125	60 29 20	147 43 07	Jun 90	C	4
Ingot Is.	60 31 41	147 39 09	Nov 89	B	4
Lone Is.	60 41 48	147 44 48	Dec 89	A	1
MacLeod Hbr.	59 58 28	147 45 24	Jun 90	G	3
NE Knight Is.	60 26 21	147 37 44	Nov 89	A	1
Northwest Bay	60 33 04	147 34 36	Jul 89	J	12
NOAA			Sep 89	D	3
Site 4	60 33 03	147 34 42	Nov 89	O	1
Site 5	60 32 37	147 36 09	Dec 89	K	2
NOAA	60 33 04	147 34 36	Jun 90	C	4
Olsen Bay	60 44 45	146 12 42	May 90	L	3
Paddy Bay	60 25 06	148 05 45	Jun 90	E	2
Port Fidalgo	60 50 17	146 16 30	Dec 89	A	1
Rua Cove	60 20 55	147 38 27	Nov 89	A	1
Sleepy Bay	60 04 01	147 50 11	May 89	E	6
NOAA			Jul 89	M	15
			Sep 89	G	3
Site 43			Nov 89	B	4
Smith Is.	60 31 48	147 20 49	Dec 89	B	4
Snug Harbor					
NOAA	60 15 46	147 46 02	Jul 89	G	3
Site 25	60 14 13	147 43 58	Nov 89	B	4
NOAA	60 15 46	147 46 02	Jun 90	E	2
Site 25	60 14 13	147 43 58	Jun 90	G	3
Two Moon Bay	60 44 00	146 34 24	Dec 89	K	2

1. At locations where both Alaska Department of Environmental Conservation (ADEC) and National Oceanic and Atmospheric Administration (NOAA) sites were established the ADEC sites are numbered. All locations sampled in November or December 1989 are ADEC sites.

2. Latitude/Longitude refers to the "0" m site at each location where "0" is mean lower low water (MLLW).

3. Letters in body of table indicate depths relative to MLLW at which completed samples were collected: A = 3 m; B = 3, 6, 10, 20 m; C = 0, 3, 6, 20 m; D = 3, 6, 20 m; E = 0, 6 m; F = 0, 3, 6, 20, 40 m; G = 0, 3, 6 m; H = 0 m; I = 0, 3, 6, 20, 40, 100 m; J = 0, 3, 6, 12, 20, 30, 40, 100 m; K = 3, 20 m; L = 0, 6, 20 m; M = 3, 6, 20, 40, 100 m; N = 6, 20 m; O = 20 m.

4. Number of samples for which hydrocarbon analysis is complete.

Table 3

Location, date of sampling, depth of collection and number of sediment samples from the northeastern Gulf of Alaska outside Prince William Sound. All samples were collected in 1989.

Location	North Latitude[1] o ' "	West Longitude o ' "	Date	Depth[2]	Sample Size[3]
Agnes Cove	59 46 00	149 34 24	26 Jul	A	6
Black Bay	59 32 07	150 12 17	28 Jul	A	6
Chignik Bay	56 19 36	158 25 06	20 Aug	B	2
Chugach Bay	59 11 12	151 37 48	3 Aug	A	6
Hallo Bay	58 27 29	154 00 14	16 Aug	A	6
Ivanof Bay	55 50 16	159 23 17	21 Aug	C	2
Katmai Bay	57 55 00	155 05 00	17 Aug	A	6
Windy Bay	59 13 50	151 31 00	2 Aug	A	6

1. Latitude/Longitude refers to the "0" m site at each location where "0" is mean lower low water (MLLW).

2. Letters in body of table indicate depths relative to MLLW at which completed samples were collected: A = 0, 3, 6, 20, 40, 100 m; B = 20, 30 m; C = 40, 100 m.

3. Number of samples for which hydrocarbon analysis is complete.

Table 4

Concentration of total hydrocarbons (THC), total aromatic hydrocarbons (TAH) and total alkanes (TAL) in sediment samples from 26 sites in Prince William Sound. Concentrations (expressed in wet weights) are means where n exceeds one. n, sample size; SE, standard error of the mean.

Location[1]	Date	Depth (m)	n	Oil[2]	THC[3] µg/g	SE	TAH ng/g	SE	TAL ng/g	SE
Reference Sites										
Ewan Bay	Jun 90	0	1	N	35.6	-	17.3	-	531.9	-
		6	1	N	29.2	-	19.1	-	569.8	-
MacLeod Hbr.	Jun 90	0	1	N	17.5	-	91.3	-	202.3	-
		3	1	N?	3.1	-	152.7	-	534.1	-
		6	1	N	14.4	-	209.0	-	1005.8	-
Olsen Bay	May 90	0	1	N	4.2	-	12.9	-	140.6	-
		6	1	N	38.9	-	34.8	-	353.6	-
		20	1	N	18.6	-	43.6	-	883.1	-
Paddy Bay	Jun 90	0	1	N?	57.0	-	39.0	-	2299.4	-
		6	1	N?	61.3	-	66.6	-	1614.7	-
Port Fidalgo	Dec 89	3	1	Y?	15.7	-	30.6	-	632.3	-
Two Moon Bay	Dec 89	3	1	Y?	17.5	-	75.8	-	1532.7	-
		20	1	N?	17.6	-	76.2	-	830.2	-

Location	Date	Depth (m)	n	Oil	THC µg/g	SE	TAH ng/g	SE	TAL ng/g	SE
Oiled Sites										
Applegate Is.	Nov 89	3	1	Y	13.8	-	57.7	-	210.3	-
Bay of Isles										
Site 86	Nov 89	3	1	Y	43.2	-	288.2	-	831.1	-
Site 90	Nov 89	3	1	Y	32.8	-	147.1	-	618.3	-
		6	1	Y	120.6	-	311.1	-	3112.4	-
		10	1	Y	29.9	-	65.7	-	680.8	-
		20	1	Y	27.2	-	124.8	-	854.3	-
NOAA	Jun 90	6	1	Y	13.4	-	41.6	-	279.8	-
		20	1	Y	78.5	-	180.2	-	1796.6	-
Site 90	Jun 90	20	1	Y	89.8	-	173.3	-	1104.0	-
Block Is.										
Site 7	Nov 89	3	1	Y?	92.0	-	363.2	-	1754.3	-
Site 47	Nov 89	3	1	Y	111.7	-	310.0	-	2015.0	-
	Jun 90	0	1	Y	219.5	-	803.9	-	5258.6	-
		6	1	Y	111.4	-	462.0	-	1161.4	-
		20	1	Y	47.5	-	112.6	-	587.3	-
Chenega Is.	Nov 89	3	1	N	10.5	-	8.8	-	47.9	-
Disk Is.	Jun 90	0	1	Y	77.2	-	108.9	-	2818.1	-
		3	1	Y	25.9	-	47.6	-	694.8	-

Location	Date	Depth	n	Oil	THC µg/g	SE	TAH ng/g	SE	TAL ng/g	SE
Disk Is.	Jun 90	6	1	Y	14.5	-	36.2	-	425.3	-
		20	1	Y	99.3	-	227.7	-	1490.3	-
Eshamy Bay	Jun 90	0	1	Y	66.4	-	87.4	-	16462.3	-
		6	1	Y	32.1	-	73.4	-	1509.9	-
Fox Farm	Jul 89	0	1	Y	31.2	-	63.4	-	682.9	-
		3	1	Y	15.0	-	327.6	-	1122.1	-
		6	1	Y	14.6	-	120.0	-	611.5	-
		20	1	N	3.6	-	1.0	-	281.7	-
		40	1	N	5.8	-	2.1	-	270.4	-
Green Island	Nov 89	3	1	Y	22.4	-	155.3	-	343.6	-
Site 22		6	1	Y	36.0	-	148.3	-	508.2	-
		10	1	N?	14.5	-	24.5	-	292.1	-
		20	1	Y	10.2	-	91.2	-	355.4	-
Site 22	Jun 90	0	1	Y	19.6	-	110.3	-	534.1	-
		3	1	Y	10.8	-	70.0	-	470.9	-
		6	1	Y	19.0	-	115.7	-	567.6	-
		20	1	Y	13.6	-	66.0	-	312.5	-
NOAA	Jun 90	0	1	Y	16.6	-	91.0	-	792.6	-
Herring Bay	May 89	0	3	Y	111.2	27.7	992.1	221.8	3976.4	1116.6
NOAA		6	3	Y	28.2	9.9	59.4	11.7	701.6	50.6

Location	Date	Depth (m)	n	Oil	THC µg/g	SE	TAH ng/g	SE	TAL ng/g	SE
Herring Bay	Jul 89	0	2	Y	278.3	100.3	1962.5	997.8	5800.5	629.3
NOAA		3	2	Y	75.7	5.5	484.9	14.3	2915.2	367.7
		6	2	Y	14.5	6.5	29.4	28.4	551.2	202.8
		20	1	N	12.6	-	22.3	-	1029.8	-
		40	1	N?	20.7	-	106.8	-	1073.5	-
		100	1	N?	17.6	-	471.5	-	1138.6	-
	Sep 89	0	1	Y	140.0	-	722.6	-	5442.6	-
		3	1	Y	51.0	-	126.4	-	1381.2	-
		6	1	Y?	22.1	-	11.7	-	497.8	-
		20	1	Y?	19.9	-	48.7	-	567.4	-
Site 53	Nov 89	3	1	Y	46.3	-	234.8	-	1457.8	-
		6	1	Y	20.4	-	64.0	-	398.6	-
		10	1	Y	22.9	-	64.7	-	610.4	-
		20	1	Y	25.5	-	125.0	-	666.0	-
Site 125	Nov 89	3	1	Y	27.2	-	95.9	-	491.5	-
Site 110	Nov 89	3	1	Y?	17.1	-	23.3	-	370.4	-
Site 125	Jun 90	0	1	Y	34.4	-	113.1	-	1001.7	-
		3	1	Y	13.9	-	38.7	-	633.8	-
		6	1	Y	24.8	-	62.2	-	539.4	-
		20	1	Y	21.8	-	51.3	-	550.9	-

Location	Date	Depth	n	Oil	THC µg/g	SE	TAH ng/g	SE	TAL ng/g	SE
Herring Bay	Jun 90	0	1	Y	43.7	-	120.2	-	946.3	-
NOAA		3	1	Y	46.3	-	115.6	-	922.4	-
		6	1	Y	31.3	-	142.0	-	577.2	-
		20	1	Y	34.1	-	94.9	-	550.4	-
Ingot Is.	Nov 89	3	1	N	40.7	-	18.2	-	642.5	-
Site 82		6	1	Y	31.1	-	87.0	-	825.1	-
		10	1	Y	35.1	-	108.7	-	458.4	-
		20	1	Y	26.4	-	99.2	-	272.3	-
Lone Is.	Dec 89	3	1	N	14.5	-	51.8	-	128.8	-
NE Knight Is.	Nov 89	3	1	N?	213.1	-	24.5	-	199.1	-
Northwest Bay	Jul 89	0	1	Y	507.5	-	14320.5	-	35696.6	-
NOAA		3	2	Y	108.2	71.2	1421.8	776.8	5505.0	3754.8
		6	2	Y	56.6	3.2	236.9	118.7	2178.2	25.8
		12	1	Y	34.8	-	147.4	-	1550.1	-
		20	2	Y	32.3	7.7	291.6	165.0	1305.0	243.2
		30	1	Y?	13.4	-	86.8	-	866.0	-
		40	1	Y?	13.8	-	96.5	-	611.8	-
		100	1	Y	12.3	-	323.1	-	806.3	-
	Sep 89	0	1	Y	315.2	-	2040.1	-	11622.5	-
		3	1	Y	116.5	-	294.9	-	1888.5	-

Location	Date	Depth (m)	n	Oil	THC µg/g	SE	TAH ng/g	SE	TAL ng/g	SE
Northwest Bay	Sep 89	6	1	Y	133.1	-	584.0	-	2946.8	-
NOAA		20	1	Y	54.0	-	186.8	-	1193.1	-
Site 4	Nov 89	10	1	Y	307.6	-	718.0	-	2879.3	-
Site 5	Dec 89	3	1	Y	31.4	-	175.8	-	888.2	-
		20	1	Y	34.4	-	182.8	-	865.2	-
NOAA	Jun 90	0	1	Y?	38.0	-	152.0	-	1276.4	-
		3	1	Y	132.1	-	577.3	-	1650.2	-
		6	1	Y	124.2	-	362.7	-	1452.8	-
		20	1	Y	173.5	-	250.6	-	1416.0	-
Point Helen	Nov 89	3	1	N?	20.7	-	6.8	-	46.4	-
Rua Cove	Nov 89	3	1	Y	41.0	-	215.6	-	800.0	-
Sleepy Bay	May 89	0	3	Y	22.0	2.1	146.9	12.7	602.3	79.1
NOAA		6	3	Y	31.2	5.0	188.3	36.0	1207.0	278.7
	Jul 89	3	3	Y	24.7	2.9	131.5	17.1	587.5	107.1
		6	3	Y	13.9	0.9	40.6	3.6	239.7	14.7
		20	3	Y?	17.6	0.9	219.1	29.4	525.9	18.9
		40	3	Y?	12.5	1.0	161.2	23.4	427.4	28.4
		100	3	Y?	15.4	3.2	684.8	329.2	907.9	72.7
	Sep 89	0	1	N	10.7	-	1.8	-	260.8	-
		3	1	Y	49.2	-	176.0	-	847.5	-

Location	Date	Depth (m)	n	Oil	THC µg/g	SE	TAH ng/g	SE	TAL ng/g	SE
Sleepy Bay										
NOAA		6	1	Y	36.9	-	114.0	-	471.6	-
Site 43	Nov 89	3	1	Y	28.9	-	157.7	-	484.6	-
		6	1	Y	70.2	-	282.6	-	907.7	-
		10	1	Y	41.2	-	199.0	-	595.4	-
		20	1	Y	37.2	-	256.0	-	683.8	-
Smith Is.	Dec 89	3	1	Y	27.1	-	171.5	-	468.2	-
		6	1	Y?	18.0	-	49.0	-	265.3	-
		10	1	Y	16.4	-	104.6	-	274.6	-
		20	1	N?	8.6	-	33.6	-	176.5	-
Snug Harbor	Jul 89	0	1	Y	59.9	-	571.6	-	1060.0	-
NOAA		3	1	Y	6.2	-	137.6	-	259.8	-
		6	1	Y?	4.2	-	109.6	-	197.4	-
Site 25	Nov 89	3	1	Y	17.2	-	182.5	-	365.1	-
		6	1	Y	14.4	-	186.0	-	362.2	-
		10	1	Y	18.5	-	147.8	-	382.7	-
		20	1	Y	25.8	-	238.6	-	724.1	-
Site 25	Jun 90	0	1	N?	0.3	-	26.6	-	231.3	-
		3	1	N?	2.5	-	53.1	-	158.6	-
		6	1	N	1.9	-	20.5	-	134.2	-

Location	Date	Depth (m)	n	Oil	THC µg/g	SE	TAH ng/g	SE	TAL ng/g	SE
Snug Harbor										
NOAA		0	1	N?	87.7	-	43.2	-	527.8	-
		6	1	N?	72.6	-	111.6	-	560.0	-

1. At locations where both Alaska Department of Environmental Conservation (ADEC) and National Oceanic and Atmospheric Administration (NOAA) sites were established the ADEC sites are numbered. All locations sampled in November or December 1989 are ADEC sites.

2. Presence/absence of oil determined by Technical Services Study Number 1.

3. Includes the unresolved complex mixture (UCM).

Figure Legends

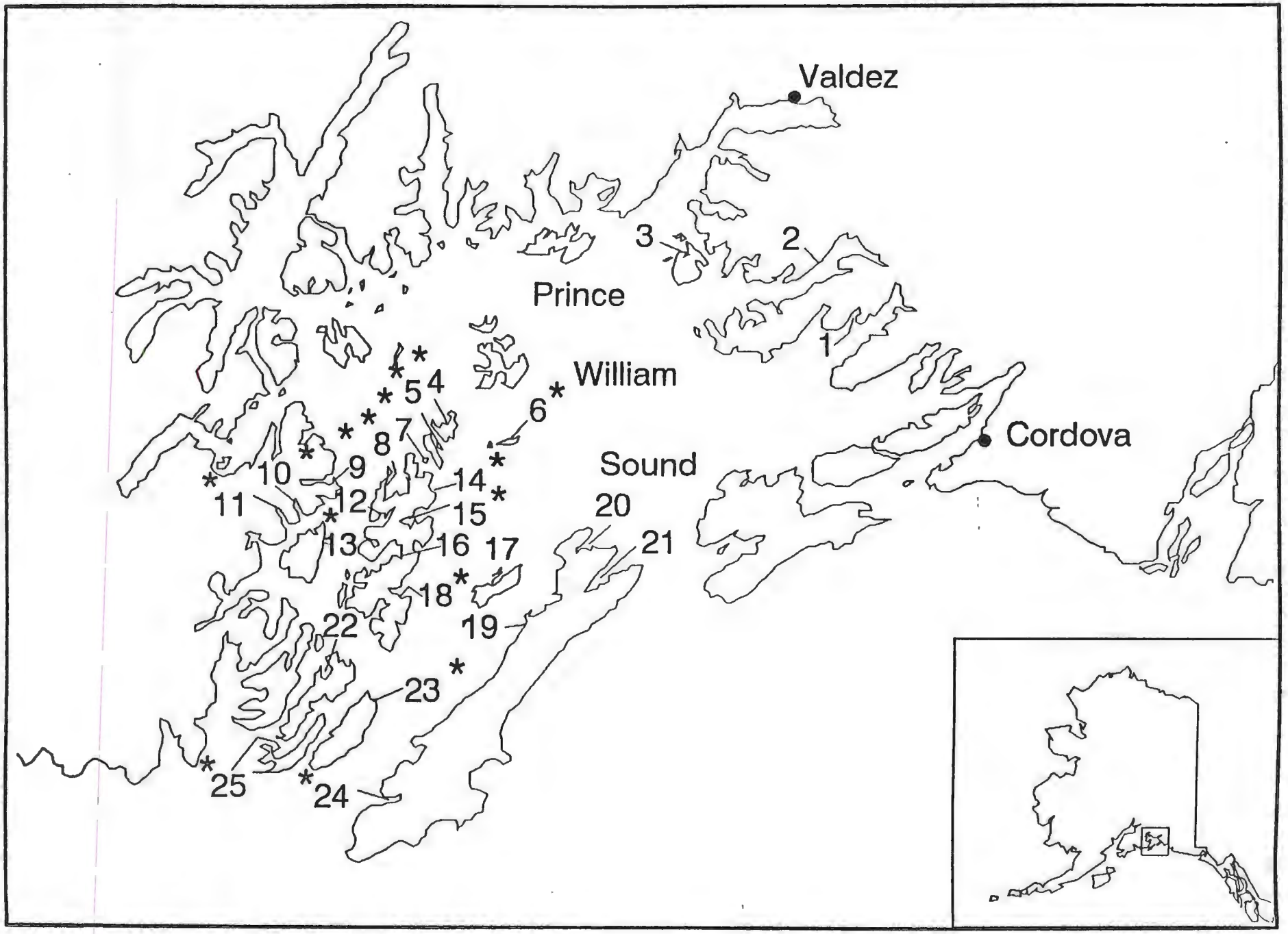
Figure 1. Distribution of study sites in Prince William Sound. Numbers with asterisks indicate locations for which hydrocarbon analysis is complete on selected 1989 and 1990 samples. See Table 1 for a list of the sites sampled in 1991 and for the geographical coordinates of each site. Numbered locations are: 1, Olsen Bay; 2, Port Fidalgo; 3, West Bay; 4, Northwest Bay; 5, Block Island; 6, Smith Island; 7, Disk Island; 8, Herring Bay; 9, Eshamy Bay; 10, Paddy Bay; 11, Ewan Bay; 12, Lower Herring Bay; 13, Chenega Island; 14, NE Knight Island; 15, Bay of Isles; 16, Drier Bay; 17, Green Island; 18, Snug Harbor; 19, Moose Lips Bay; 20, Rocky Bay; 21, Zaikof Bay; 22, Iktua Bay; 23, Sleepy Bay; 24, Macleod Harbor; 25, Fox Farm.

Figure 2. Distribution of study sites outside Prince William Sound. Numbers with asterisks indicate locations for which hydrocarbon analysis is complete on selected 1989 samples. See Table 2 for the geographical coordinates of each site. Numbered locations are: 1, Fox Island; 2, Agnes Cove; 3, Taroka Arm; 4, Black Bay; 5, Mc Arthur Cove; 6, Tonsina Bay; 7, Gore Point; 8, Port Dick; 9, Windy Bay; 10, Chugach Bay; 11, Seldovia Bay; 12, Ursus Cove; 13, Amakdedori Beach; 14, Douglas Beach; 15, Ushagat Island; 16, Andreon Bay; 17, King Cove; 18, Cape Douglas; 19, Hallo Bay; 20, Katmai Bay; 21, Halibut Bay; 22, Wide Bay; 23, Chignik Bay; 24, Ivanof Bay; 25, Zachary Bay.

Figure 3. Change in the concentration of hydrocarbons with bathymetric depth at Northwest Bay in July 1989 and June 1990. a. Change in the concentration of total hydrocarbons with depth. b. Change in the concentration of total dibenzothiophenes with depth. A "Q" indicates that the petrogenic origin of the hydrocarbons is in question. Error bars are one standard error of the mean.

Figure 4. Change in the concentration of hydrocarbons with bathymetric depth at Herring Bay in July 1989 and June 1990. a. Change in the concentration of total hydrocarbons with depth. b. Change in the concentration of total dibenzothiophenes with depth. An "N" indicates that the hydrocarbons in the sample(s) were not of petrogenic origin. Error bars are one standard error of the mean.

Figure 5. Concentration of total hydrocarbons at various depths at seven locations outside Prince William Sound. The figure includes only those samples which were unequivocally contaminated with petroleum hydrocarbons. Site abbreviations are: AC, Agnes Cove; BB, Black Bay; WB, Windy Bay; CB, Chugach Bay; HB, Hallo Bay; KB, Katmai Bay; CHB, Chignik Bay.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Prince
William
Sound
Cordova
1
2
3
4
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6
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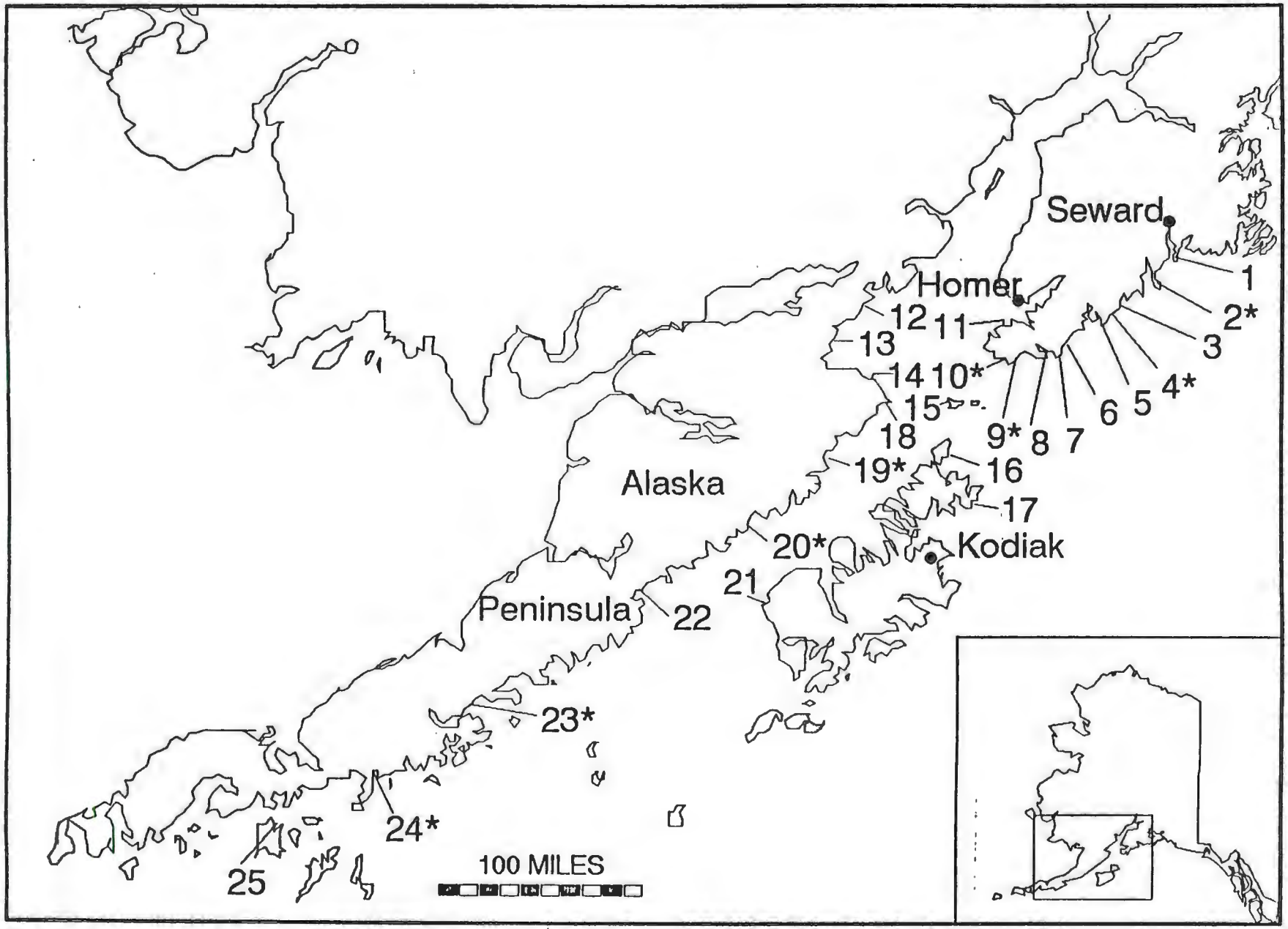
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Alaska
Peninsula
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17
18
19*
20*
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22
23*
24*
25
100 MILES

Figure 2

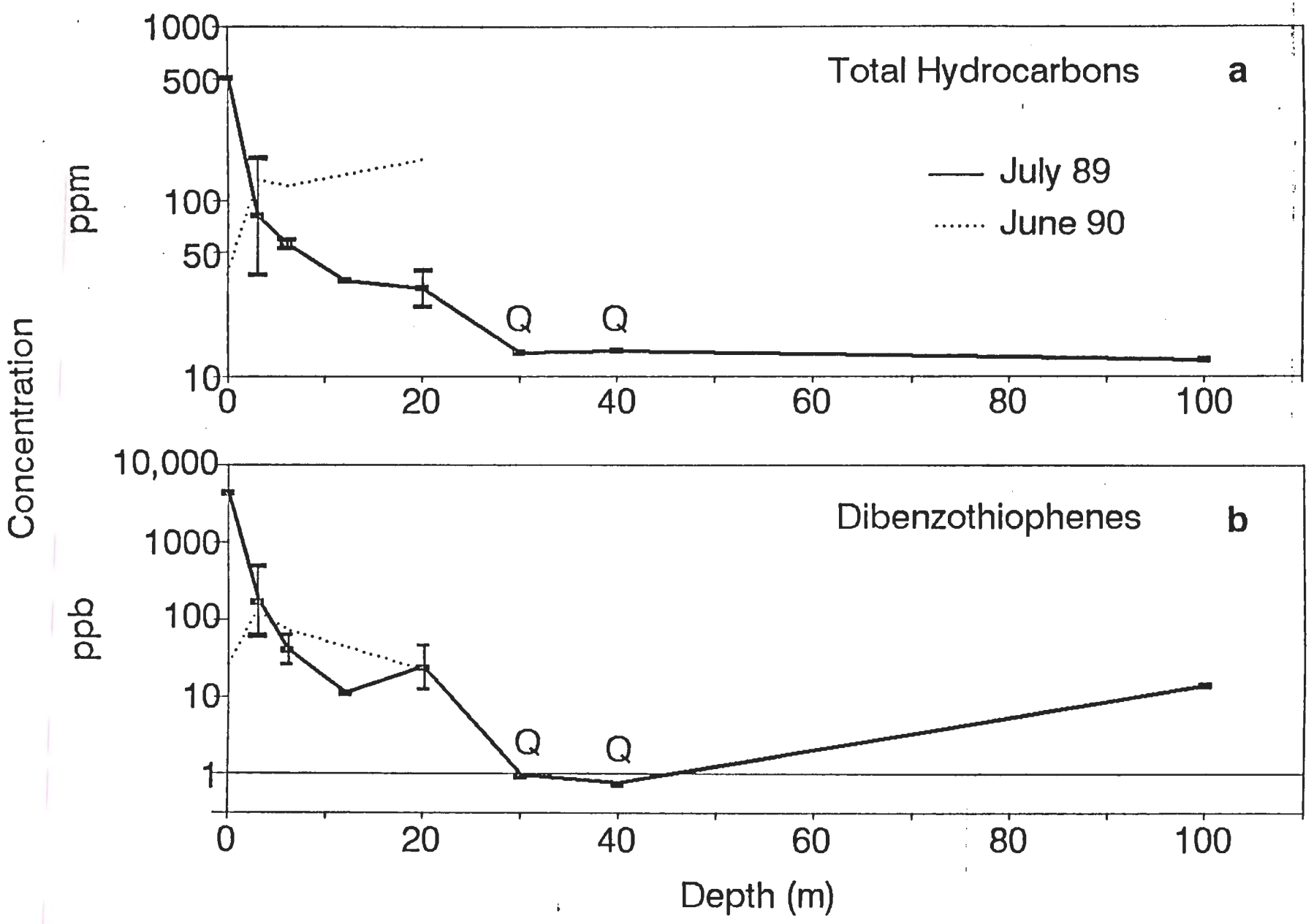
Concentration
ppm
1000
500
100
50
10
Total Hydrocarbons
a
July 89
June 90
Q
Q
0
20
40
60
80
100
ppb
10,000
1000
100
10
1
Dibenzothiophenes
b
Q
Q
0
20
40
60
80
100
Depth (m)

Figure 3

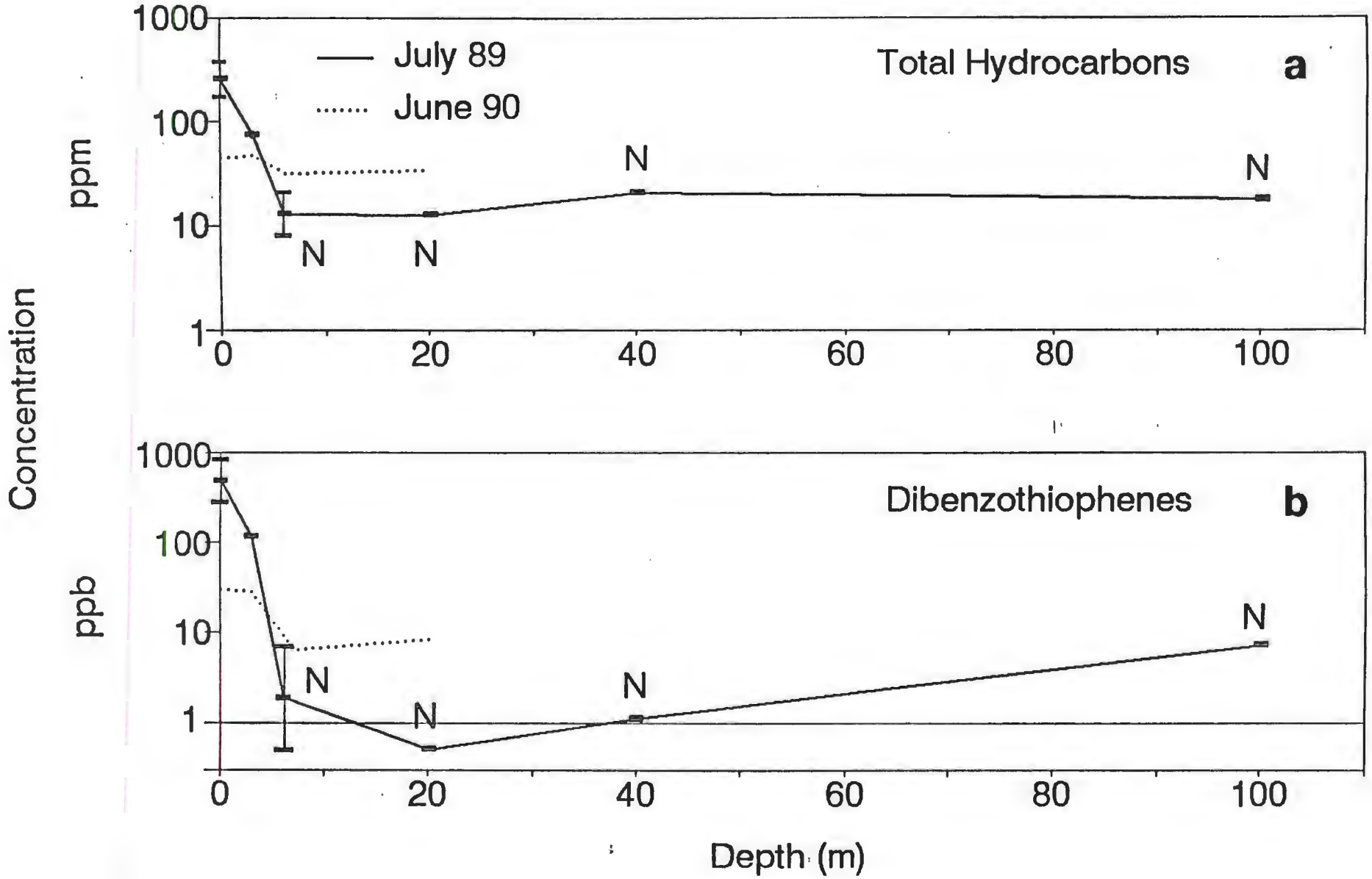
Concentration
ppm
1000
100
10
1
July 89
June 90
Total Hydrocarbons
a
N
N
N
N
0
20
40
60
80
100
ppb
1000
100
10
1
Dibenzothiophenes
b
N
N
N
N
0
20
40
60
80
100
Depth (m)

Figure 4

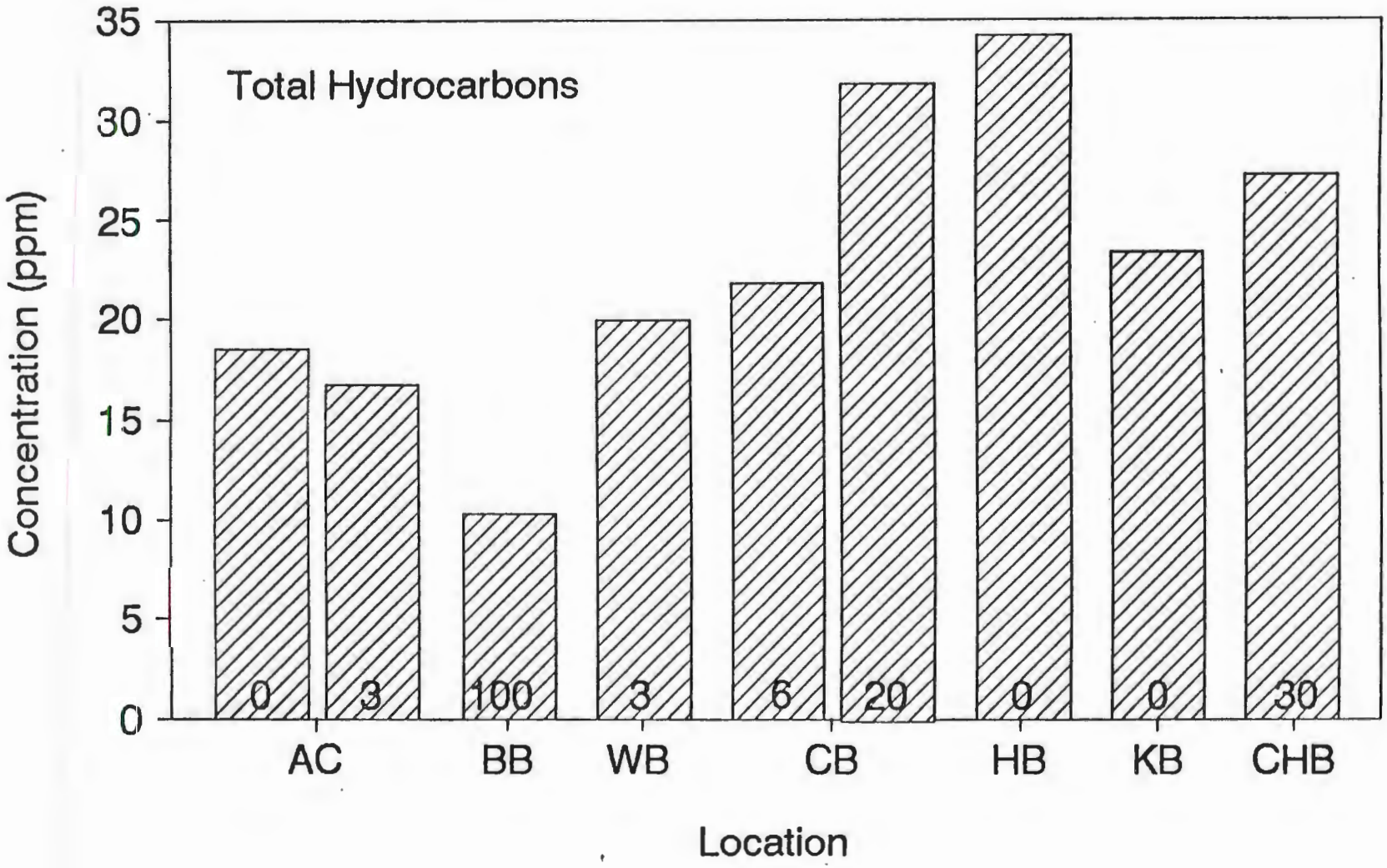
Total Hydrocarbons
Concentration (ppm)
35
30
25
20
15
10
5
0
0
3
100
3
6
20
0
0
30
AC
BB
WB
CB
HB
KB
CHB
Location

Figure 5

INJURY TO DEEP BENTHOS

AIR/WATER NO. 2
PROJECT NUMBER 2109

Status Report 1991

Project Leader: Howard M. Feder

November 1991

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

EXECUTIVE SUMMARY

Benthic infaunal samples were collected by van Veen grab from seven oiled and seven unoiled (reference) sites (bays) in July 1990 (approximately fifteen months after the EVOS) and late June 1991. One station at 40 m in Snug Harbor was occupied in June 1989. Stations were occupied on a transect at 40, 100, and >100 m within sites known to contain sea grass (*Zostera* sp.) beds. Stations at six of the sites were below the shallow stations occupied by the S. Jewett Coastal Habitat diving program. Samples were screened through nested 1.0 and 0.5 mm mesh screens. At all stations sediment samples for hydrocarbons were collected by NOAA on the same ship platform.

The samples collected in 1990 at all depths from the 1.0 and 0.5 mm fractions are analyzed, and the data are included and discussed in this report. Analysis of station samples collected in 1991 are in progress. Analysis of four oiled sites and one unoiled site at 40 m are completed, and the results are briefly presented in this report. The balance of the 1991 samples at all depths will be completed by June 30, 1992.

Although no hydrocarbon data are available as yet, oil was noted in laboratory infaunal samples at six of the oiled sites at 40 m. No oil was observed from samples at other depths. However, assessment of hydrocarbon degrading bacteria by Dr. Joan Braddock for ADEC indicates that significant numbers of these bacteria were found at all depths in 1990 and 1991. Statistical assessment of abundance of taxa at six of the oiled sites in 1990 as compared to unoiled sites showed significantly higher abundance values for many taxon groups considered to be opportunistic at oiled sites (identification of opportunistic taxa based on previous information from Port Valdez, Prince William Sound and literature from other oil-spill and organically enriched sites worldwide). Continued significant increases of opportunistic taxa at two of the oiled sites in 1991 were recorded. Statistical assessment of abundance of infaunal feeding types in 1990 consistently demonstrated significantly higher abundance values for organisms feeding at the benthic boundary layer at oiled sites (sediment-water interface; i.e., surface deposit feeders, suspension feeders, and scavengers). The presence of large numbers of opportunists and organisms feeding at the benthic boundary layer suggest that large amounts of particulate organic carbon (presumably oil, hydrocarbon degrading bacteria, and detrital debris resulting from oil-spill mortality) must have been available at the sediment-water interface. It is suggested by many authors, based on oil-spill related studies elsewhere, that the sequence of events after a catastrophic oil spill is (1) a toxic effect with considerable mortality, (2) an organically enriched period in which opportunist taxa become extremely abundant, and (3) a period in which opportunists decrease in importance and abundance and fauna begin to return to conditions similar to adjacent unoiled areas and/or to benthic assemblages characteristic of relatively undisturbed sites. The high abundance values for fauna, many of which are known opportunists, at most of the oiled sites in July 1990 and late June 1991 suggests that the benthos at these sites had completed its toxic phase and was in the organic enrichment/opportunist phase. However, faunal events at the 40 m station at Snug Harbor between 1989-1991 suggest that this embayment was lagging behind the other oiled sites, and the 1990 data indicated that the site was just moving out of the toxic phase (i.e., there were still significantly lower abundance values in 1990 than recorded there before oil reached the bottom in 1989). A major statistically significant increase in abundance of taxon groups, mainly opportunists such as capitellid polychaetes, was recorded at Snug Harbor in 1991, and this site in 1991 was apparently now in the organically enriched phase. Abundance values at two of the oiled sites in 1991, Bay of Isles and Chenega Bay, significantly decreased. At some oiled sites after the Amoco Cadiz

oil spill, it was determined that the bottom had become eutrophic (too much organic carbon and too many organisms utilizing that carbon resulted in anoxic conditions) and a marked decrease in fauna occurred again. It is possible that these two sites have become eutrophic which would result in a delay in recovery of the sites to an undisturbed state. However, two other oiled sites, Snug Harbor (noted above) and Herring Bay, still demonstrated increasing numbers of opportunists in 1991, which suggests that these sites continue to be organically enriched by oil and associated hydrocarbon degrading bacteria and detritus derived from adjacent intertidal and shallow subtidal sites. Thus, it would be expected that recovery of these two sites might be delayed for a long period of time if oil continues to be present in shallow sediments and is transported to deeper benthic locations.

Assessment of taxon groups that were more abundant at oiled sites has delineated a suite of organisms that can be considered opportunistic. The decreased presence of these taxon groups at oiled sites would then represent a mechanism for determining if a site is recovering from the major disturbance of the EVOS. Additionally, a number of taxon groups that were always more abundant at unoiled sites can be considered organisms sensitive to oil. Continued assessment of these taxon groups should make it possible, in conjunction with assessment of opportunists, to monitor the gradual recovery of the oiled sites. This is an approach that has been successfully used to monitor the recovery of oiled sites after the *Amoco Cadiz* oil spill.

OBJECTIVES

A. To determine if disturbance occurred in the benthos at oiled sites as assessed by comparing taxon (primarily determined at the Family level: see Methods) abundance and biomass, diversity and richness, and trophic composition of benthic biota living on similar substrata at approximately 40, 100, and >100 m below sea grass beds at oiled and unoiled sites.

B. To determine if changes occurred in the benthos as determined by comparing taxon (primarily determined at the Family level: see Methods) abundance and biomass, diversity and richness, and trophic composition of benthic biota living on similar substrata at approximately 40, 100, and >100 m below sea grass beds in oiled and unoiled bays on an annual basis for at least five years.

C. If changes are detected in the faunal components of the benthic system, to determine the time required for the benthos to recover to an undisturbed or relatively stable assemblage of taxa.

D. If changes are detected in the benthic fauna, to examine the relationship between the accumulation and retention of hydrocarbons in sediments and the effect on the benthic biota (this objective will be accomplished in conjunction with the AIR/WATER component assessing hydrocarbon levels in sediments at the sampled stations).

INTRODUCTION

Benthic organisms (both meiofauna and infaunal macrofauna) living on and within subtidal sediments represent good *in situ* monitors for measuring the effects of oil fluxing to the bottom (for examples, see Cabioch *et al.*, 1978; Kineman *et al.*, 1980; and Sanders *et al.*, 1980). These organisms are mostly sedentary or relatively slow moving and typically remain close to or at the site of larval settlement and, consequently, sampling for them on a temporal basis can result in statistically comparable samples (Boesch and Rosenberg, 1981). The marine benthic fauna has been successfully used at various locations throughout the industrial world as a tool to measure effects of pollutants on the bottom (e.g., Pearson, 1975; Cabioch *et al.*, 1978; Pearson and Rosenberg, 1978; Gray and Mirza, 1979; Sanders, 1980; Kineman *et al.*, 1980; Dauvin, 1982; Gray and Pearson, 1982; Warwick, 1986; Warwick *et al.*, 1987; Gray, 1989), and should prove useful for assessing effects of the *Exxon Valdez* oil spill in Prince William Sound.

It was expected that a certain proportion of the oil in the water column (either the original crude oil derived from the *Exxon Valdez* spill, oil leaching from contaminated shorelines, and/or oil dispersed into receiving waters via shoreline remediation procedures) would reach the bottom as a result of physical and biological processes (see reviews in Boesch and Rabalais, 1987, and Kuiper and Van Den Brink, 1987). Benthic data collected in waters elsewhere suggest that changes in taxon number, abundance, biomass, diversity, species richness, and trophic composition is expected if sizable amounts of oil settle to the bottom. Changes in bottom fauna within Prince William Sound could have serious trophic implications since benthic invertebrates are major food resources for commercially important bottom-feeding species such as pandalid shrimps, crabs, bottomfishes, and sea otters. Additionally, utilization of benthic invertebrates from oil-impacted bottoms as food could result in the transfer of hydrocarbons to these higher trophic levels. Further, larvae of most benthic organisms in Prince William Sound move into the water column (in March through June) and are utilized as food by large zooplankters and larval and juvenile stages of pelagic fishes, small salmon fry, and herring. Thus, damage to the benthic system by hydrocarbon contamination could affect feeding interactions of commercially important species both on the bottom and in the water column.

An additional effect observed after the *Amoco Cadiz* oil spill (off the French coast) might also occur within some protected bays of Prince William Sound. It was reported by Bodin (1988) that a potential for eutrophication and eventual anoxia on bottoms subject to deposition of non-toxic petroleum fractions and detritus (e.g., dead and dying organisms resulting from the spill) can occur. The enrichment of the bottom by such carbon sources in bays adjacent to the spill induced a stimulation phase with resulting great increases in bacteria and opportunistic benthic fauna utilizing this new food resource. Oxygen impoverishment ultimately resulted in some bays, and a great decrease in benthic fauna was then observed a second time (i.e., first mortality was observed directly after the spill). Such a process, initiated by organic pollution in general, is described for macrofauna of numerous areas by Pearson and Rosenberg (1978). Anoxic conditions in some bays in Prince William Sound would not only affect survival of benthic macrofauna but also of the commercially-important organisms (e.g., pandalid shrimps and Dungeness, Tanner and king crabs) dependent on macrofauna as a food resource.

Sampling of subtidal benthic populations should be continued for at least five years in order to assess the effects of oil on subtidal benthic communities to determine the levels of redistribution of oil-laden sediments from adjacent onshore sites (e.g., the

seagrass beds located alongshore in all of the bays sampled). Seagrass beds contain fine, unconsolidated sediments that are readily moved offshore by wave action and current transport. Consequently, oil that initially coated sediments in these beds and other shallow shore locations may eventually be transported offshore as a result of this redistribution and contribute to long-term effects on subtidal benthic fauna. This process of oil redistribution from shallow to deep water was observed following the *Amoco Cadiz* crude oil spill of 1978 in the Bay of Morlaix off the Brittany coast of France (Cabioch *et al.*, 1978) and after the *Florida* No. 2 fuel oil spill of 1969 in Buzzards Bay near West Falmouth, Massachusetts (Sanders *et al.*, 1980). Preliminary results of hydrocarbon analyses (unpubl. data) and microbiological sampling (J. Braddock, pers. commun.) suggest that oil was present at most of the deep benthic stations when the 1990 sampling for benthos occurred.

This study addresses the program element AIR/WATER STUDY NUMBER 2 (Injury to Deep Water [>20 meters] Benthic Infaunal Resources from Petroleum Hydrocarbons) for benthic biota located within Prince William Sound. The data from this project will be examined in conjunction with the Air/Water studies addressing petroleum hydrocarbons and microbial populations in sediments at stations sampled for benthic fauna. The basic question to be addressed by Air/Water Study Number 2 is "What are the effects of petroleum hydrocarbons (resulting from the *Exxon Valdez* oil spill) in the overlying water column and within bottom sediments on benthic invertebrate taxon composition, abundance, biomass, diversity, species richness and trophic structure?"

METHODOLOGY

SAMPLING

The sampling plan used in this study was mandated by a peer advisory group during a planning/coordination meeting for *Exxon Valdez* Natural Resource Damage Assessment Projects A/W2, 3, 6, and F/S4, 18, and 24) at the NOAA/NMFS Montlake Laboratory in Seattle on 22-23 February 1990. At this meeting, and as stated in the Technical Study Plan submitted 7 March 1990 (copies sent to Chuck O'Clair/Stan Rice, NOAA, and Roy Nowlin, ADF&G), it was decided that twelve paired bays would be occupied. At a subsequent meeting in Seattle at the Montlake Laboratory in April 1990, it was decided that the common denominator for selection of sites would be that each site must have a sea grass (*Zostera* spp.) bed associated with it. It was assumed that all benthic organisms below the sea grass beds (>40 m) would be affected by detrital input from those beds and that the types of organisms present might therefore have some similarities between sites. Substrate type was to be as similar as possible given the short time available to choose the sites (only to be selected on the cruise). Sites were to be chosen by NOAA personnel on shipboard during the cruise of July 1990. Dr. Doug Wolfe, NOAA scientist, was subsequently chosen to be the Chief Scientist on this cruise. Difficulties were soon apparent in the location of sufficient numbers of oiled and unoiled sites with associated sea grass beds in the vicinity of the oil-damaged regions to be examined. Consequently, the deep benthic region for two of the sites chosen (Chenega Island and Mooselips Bay) were actually not within embayments, but were associated with sea grass beds adjacent to the deep sampling sites. Some problems with the interpretation of the data from one of these sites are addressed in the Discussion of this report. For the purposes of the various comparisons made in this report, bays and sites are terms used interchangeably. It was possible to locate an additional pair of sites to sample on the June 1990 cruise;

consequently, fourteen sites were selected and sampled. The seven oil-exposed sites sampled for deep benthos at 40 and 100 m were Northwest Bay, Disk Island, Herring Bay, Bay of Isles, Snug Harbor, Chenega Bay (Island) and Sleepy Bay. The seven unexposed reference sites sampled for the deep benthos at 40 m and 100 m were West Bay, Rocky Bay, Zaikof Bay, MacLeod Harbor, Lower Herring Bay, Drier Bay, and Mooselips Bay (Fig. 1). At stations >100 m, two oiled (Snug Harbor and Disk Island) stations and one unoiled (Mooselips Bay) station could not be located at this depth. At Rocky Bay the "100 m" and ">100 m" stations are at somewhat lesser depths than required. This was a result of the topographic features of the bottom which precluded sampling the requisite depths while still remaining within the bay. The latter samples were treated as 100 and >100 m stations in all analyses.

Samples were collected in 1990 and 1991 with a 0.1 m^2 van Veen grab at each of three stations within seven bays identified as oil-exposed sites and three stations within seven other bays determined to be uncontaminated reference sites (Table 1, Fig. 1; see Appendix A). All stations sampled were at approximate depths of 40, 100, and >100 m on a transect extending below seagrass (*Zostera* sp.) beds within each of the identified bays[1]. A total of 45 deep stations x 5 replicates were collected on a single cruise in early July 1990 and 42 stations x 5 replicates were collected on a single cruise in late June 1991. Benthic sampling in both years was in conjunction with microbiological and hydrocarbon sampling projects underway from the same ship platform. As noted above, benthic samples were collected on bottoms that were as physically similar as possible based on chart data and preliminary grab samples accomplished before actual sampling occurred. However, insufficient pre-July 1990 ship time made it impossible to sample all sites and all stations within sites prior to the July cruise. Thus, dissimilarities in substrate type are apparent at occupied stations. Sediment analyses at occupied stations are presently unavailable, but a more detailed qualitative assessment of substrate type and other pertinent shipboard observations for the July 1990 cruise related to sediment are included in Table 2.

Benthic biological samples were collected with a 0.1 m^2 van Veen grab weighted with 31.7 kg of lead. Five replicate samples were taken. Material from each grab in 1990-1991 was washed through 1.0 mm and 0.5 mm nested stainless steel screens and preserved in 10% formalin-seawater solution buffered with hexamine. Samples collected in 1989 were washed through a 1.0 mm mesh screen, but based on a conference call with the peer review group for the project, it was decided to add a 0.5 mm screen to the washing procedure. Thus, samples were washed through 1.0 and 0.5 mm screens in 1990 and 1991.

ANALYSIS AND PROCESSING OF DATA

General

Material from historical stations collected on the R/V *Alpha Helix* just prior to and after the spill (Table 3) were processed as time permitted using the same procedures described below for samples collected from the fourteen bays. However, for the 1989 samples, material was only washed through a 1.0 mm screen. For all stations

[1] No appropriate bottom at a depth >100 m was located at Disk Island, Mooselips Bay, and Snug Harbor and samples at this depth were not taken.

Text continued on page 15.

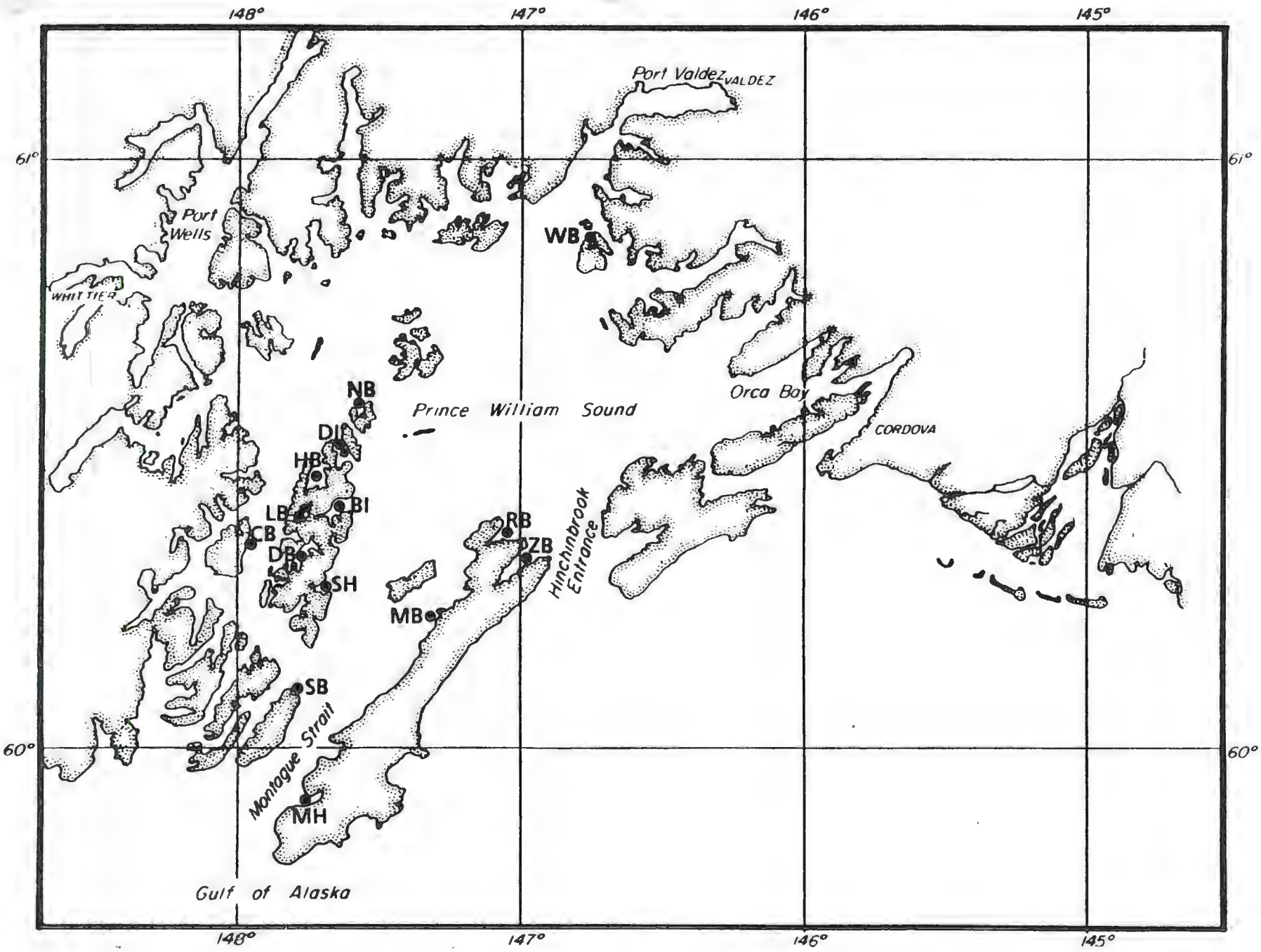

Figure 1. Oiled and unoiled sites sampled for benthos at 40, 100, and >100 m in Prince William Sound in July 1990.

Table 1. Station locations, approximate depths, and substrate type from Prince William Sound sites sampled 1-23 July 1990 on the NOAA ship *Davidson* and 15-25 June 1991 on the *Big Valley*. Substrate type is based on field and laboratory qualitative observations only. O = Oiled bay; R = Reference, Unoiled bay; H = Historical site (collected in 1989).

Station/ Number	Date Sampled	Depth (m)	Substrate	Latitude	Longitude
Smith Island (H) SI 001	1990	51-53	Gray Mud, Shell	60°32.2'	147°20.6'
Zaikof Bay (R) ZB 001	1990, 1991	40-44	Gray Mud, Shell, Rock	60°16.5'	147°05.2'
Zaikof Bay (R) ZB 002	1990, 1991	96-101	Gray Mud, Shell	60°19.1'	147°00.1'
Zaikof Bay (R) ZB 003	1990, 1991	93-103	Gray Mud, Shell	60°19.9'	146°55.4'
Rocky Bay (R) RB 001	1990, 1991	39-40	Sandy Mud, Shell,Rock	60°20.8'	147°06.6'
Rocky Bay (R) RB 003	1990, 1991	65-73	Gray Mud, Shell, Rock	60°21.5'	147°03.9'
Rocky Bay (R) RB 002	1990, 1991	97-100	Gray Mud, Shell, Rock	60°21.2'	147°04.9'
West Bay (R) WB 001	1990, 1991	36-41	Black Mud, H_2S, Shell	60°52.3'	147°47.9'
West Bay (R) WB 002	1990, 1991	97-122	Gray Mud	60°52.5'	146°50.6'
West Bay (R) WB 003	1990, 1991	140-167	Gray Mud	60°53.3'	146°50.3'
Herring Bay (O) HB 001	1990, 1991	38-60	Brown Mud, Gravel, Rock	60°26.7'	147°46.8'
Herring Bay (O) HB 002	1990, 1991	87-106	Brown Mud, Gravel, Rock	60°27.5'	147°46.3'
Herring Bay (O) HB 003	1990, 1991	139-164	Gray Mud, Gravel, Rock	60°28.3'	147°45.2'
Disk Island (O) DI 001	1990, 1991	43-45	Brown Mud, Gravel, Rock, Shell	60°30.2'	147°39.9'
Disk Island (O) DI 002	1990, 1991	98-109	Gray Mud, Rock	60°30.6'	147°41.1'
Disk Island (O)	No appropriate bottom deeper				

Table 1. (cont'd)

Station/ Number	Date Sampled	Depth (m)	Substrate	Latitude	Longitude
Block Island (H) BI 001	1990, 1991	104-114	Gray Mud, Rock	60°32.7'	147°37.9'
Northwest Bay (O) NWB 001	1990, 1991	37-42	Brown Mud, Org. Debris, Rock, Gravel, Sand	60°33.3'	147°34.6'
Northwest Bay (O) NWB 002	1990, 1991	122-128	Gray Mud, Rock	60°34.0'	147°35.3'
Northwest Bay (O) NWB 003	1990, 1991	146-190	Gray-Brown Mud Rock, Gravel	60°34.5'	147°36.4'
Northeast Knight (H) NEK 001	1990	71-105	Gray-brown Mud, Rock, Gravel	60°26.7'	147°36.5'
Bay of Isles (O) BOI 001	1990, 1991	41-42	Brown Mud, Sand, Rock, Gravel	60°22.8'	147°42.0'
Bay of Isles (O) BOI 002	1990, 1991	92-100	Black Mud, H_2S	60°23.8'	147°41.3'
Bay of Isles (O) BOI 002	1990, 1991	92-100	Black Mud, H_2S	60°23.8'	147°41.3'
Bay of Isles (O) BOI 003	1990, 1991	152-159	Gray Mud	60°24.9'	147°35.4'
Green Island (H) GI 001	1990	94-103	Gray Mud, Rock, Gravel	60°18.1'	147°29.5'
MacLeod Harbor (R) MCH 001	1990, 1991	40-41	Black Sand, Shell	59°53.0'	147°47.7'
MacLeod Harbor (R) MCH 002	1990, 1991	95-102	Black Sand, Shell	59°54.2'	147°51.1'
MacLeod Harbor (R) MCH 003	1990, 1991	149-153	Black Sand, Shell	59°55.4'	147°51.2'
Mooselips Bay (R) MLB 001	1990, 1991	37-41	Brown Mud, Shell, Rock, Gravel	60°12.5'	147°22.4'
Mooselips Bay (R) MLB 002	1990, 1991	96-100	Gray Mud, Shell Rock, Gravel	60°11.5'	147°25.7'
Mooselips Bay (R) MLB 003	No appropriate bottom deeper				

Table 1. (cont'd)

Station/ Number	Date Sampled	Depth (m)	Substrate	Latitude	Longitude
Snug Harbor (O) SH 001	1990, 1991	42-44	Black Mud, Shell, Rock, Gravel	60°15.4'	147°45.0'
Snug Harbor (O) SH 002	1990, 1991	102-105	Gray Mud	60°15.2'	147°43.2'
Snug Harbor (O)	No appropriate bottom deeper				
Chenega (O) CHN 001	1990, 1991	38-51	Brown Mud, Shell	60°19.8'	148°00.0'
Chenega (O) CHN 002	1990, 1991	76-99	Brown Mud, Shell, Rock, Gravel	60°19.9'	148°00.3
Chenega (O) CHN 003	1990, 1991	163-182	Gray Mud, Rock, Gravel	60°19.4'	147°59.9'
Lower Herring Bay (R) LHB 001	1990, 1991	39-42	Brown Mud, Shell, Wood	60°23.8'	147°48.0'
Lower Herring Bay (R) LHB 002	1990, 1991	95-120	Brown Mud, Rock, Gravel	60°23.2'	147°48.6'
Lower Herring Bay (R) LHB 003	1990, 1991	143-154	Gray Mud	60°23.0'	147°48.9'
Drier Bay (R) DB 001	1990, 1991	39-40	Brown Mud, Shell, Rock, Gravel	60°19.7'	147°45.3'
Drier Bay (R) DB 002	1990, 1991	101-105	Gray Mud, Rock, Gravel	60°19.1'	147°47.6'
Drier Bay (R) DB 003	1990, 1991	144-145	Gray Mud	60°18.8'	147°49.2'
Sleepy Bay (O) SLB 001	1990, 1991	35-38	Gray Mud, Gravel	60°04.9'	147°50.3'
Sleepy Bay (O) SLB 002	1990, 1991	103-107	Gray Mud, Rock, Gravel	60°04.7'	147°49.4'
Sleepy Bay (O) SLB 003	1990, 1991	151-158	Gray Mud, Rock, Gravel	60°05.1'	147°49.8'
Fox Farm (H) FF 001	1990	85-97	Black Sand, Shell, Gravel	59°59.7'	148°09.8'

Table 2. Substrate type at oiled and unoiled sites from samples collected in Prince William Sound in July 1990 on the R/V *Davidson*. Assessment of substrates are based on field notes only. No quantitative characterization of sediment at sites is presently available.

Site	Depth (m)	Substrate Type	Comments
OILED SITES[1]			
DI	40	Compact, brown mud, gravel, rock and shell	Smelled faintly of H_2S
	100	Soft, grey mud, rock	
	>100	NOT SAMPLED	
NB	40	Compact, brown mud, gravel, sand, rock, and shell	Organic matter present
	100	Compact, grey mud and rock	
	>100	Compact, brown-grey mud, gravel and rock	
BI	40	Compact, brown mud, sand gravel and rock	
	100	Soupy, black mud	Strong H_2S odor
	>100	Light grey mud, few rocks or gravel and soft	
HB	40	Compact, brown mud, gravel and rock	Rocks of varying size
	100	Compact, brown mud and rock	Rocks of varying size
	>100	Compact, grey mud and rock	Rocks and gravel
SB	40	Compact, grey mud, gravel and rock	
	100	Compact, grey mud, gravel, rock and other	Sponge materials in one grab
	>100	Compact, grey mud, rock and gravel	
CB	40	Compact, grey mud, gravel, rock and shell	Mostly rock, gravel, large shell
	100	Compact, brown mud, gravel, rock and shell	Mostly rock and gravel
	>100	Compact, grey mud, rock and gravel	
SH	40	Soft, black mud	Slight H_2S odor; one grab also contained gravel, rock and shell
	100	Soupy, grey mud	
	>100	NOT SAMPLED	

Table 2. (cont'd)

Site	Depth (m)	Substrate Type	Comments
UNOILED SITES[2]			
DB	40	Compact, brown mud, gravel, rock and shell	
	100	Compact, grey mud, rock and gravel	
	>100	Soft, grey mud	Fine grey mud with NO rock or gravel
ZB	40	Soft, grey mud, rock and shell	
	100	Soft, grey mud and shell	
	>100	Compact, grey mud and shell	
RB	40	Compact, black mud, sand, rock and shell	
	100	Compact, grey mud, rock and shell	
	>100	NO INFORMATION	
MH	40	Compact, black sand and shell	Grey/black sand, shells and a few small rocks
	100	Compact, black sand and shell	Bottom was hard, sandy and shelled
	>100	Compact, black sand and shell	Black sand and shells
MB	40	Compact, brown mud, rock, gravel and shell	Abundant shell material
	100	Compact, grey mud, gravel, rock and shell	Large rock, gravel and shell
	>100	NOT SAMPLED	
LB	40	Soupy to compact brown mud, wood, gravel and shell	
	100	Compact, brown mud, rock and gravel	
	>100	Soft, grey mud	No rocks or gravel
WB	40	Compact, black mud and shell	Full of shells, black in color Smelled of H_2S
	100	Grey, very soft mud	
	>100	Soft, grey mud	

[1] DI = Disk Island, NB = Northwest Bay, BI = Bay of Isles, HB = Herring Bay, SB = Sleepy Bay, CB = Chenega Bay, SH = Snug Harbor.

[2] DB = Drier Bay, ZB = Zaikof Bay, RB = Rocky Bay, MH = MacLeod Harbor, MB = Mooselips Bay, LB = Lower Herring Bay, WB = West Bay.

Table 3. Benthic Stations occupied in Prince William Sound in 1989 by the Institute of Marine Science, University of Alaska Fairbanks.

Station	Date	Latitude (N)	Longitude (W)	Depth (M)	Bottom Type, Grab Volume
Cape Fairfield					
CF-2	6 Apr	59°53.03'	148°49.95'	117	Soft grey mud, full grabs
CF-12	6 Apr	59°33.0'	148°50.0'	152	Soft grey mud and sand, full grabs
Green Island					
GI-7	7 Apr	60°11.98'	147°22.01'	29-35	Compact grey mud, sand, rock, dead shell, grabs half full
GI-7	6 Oct				
GI-6	6 Oct	60°12.48'	147°24.60°	77-81	Compact grey mud, sand, rock, dead shell, grabs half full
GI-4	6 Oct	60°13.50'	147°31.20'	55-80	Soft grey mud, rock, shell, grabs 3/4 full
GI-3	6 Oct	60°13.98'	147°34.02'	119-135	Soft grey mud, some rock, grabs full
GI-2	5 May	60°14.53'	147°37.08'	249-263	Soft grey mud, some rock, grabs full
GI-2	6 Oct				
GI-1	5 May	60°14.99'	147°39.90'	70-90	Soft grey mud, dead sponge, rock, grabs 3/4 full
GI-1	6 Oct				
Snug Harbor					
SH-3	11 Apr	60°15.75'	147°45.30'	32-36	Soft grey mud, wood, shell, grabs full
SH-1	4 Jun	60°10.4'	147°42.3'	35	Soft grey mud, sand, shell, wood, grabs full

Table 3. (cont'd)

Station	Date	Latitude (N)	Longitude (W)	Depth (M)	Bottom Type, Grab Volume
Herring Bay					
HB-1	11 Apr	60°28.21'	147°44.45'	110-140	Compact grey mud, rock, grabs full
HB-1	10 May				
HB-1	7 Oct				
Main Bay					
MB	3 Oct	60°34.02'	147°57.0'	610	Soft grey mud, full grabs
Montague Sound					
MSX	2 Oct	59°58.50'	147°48.72'	238	Soft grey mud, full grabs
Saw Mill Bay					
SB-1	11 Apr	60°03.49'	147°57.95'	120-175	Soft grey mud, many rocks, grabs 3/4 full
SB-1	7 May				
SB-1	2 Oct				
Esther Island					
EI	9 May	60°46.24'	147°03.34'	378-388	Soft grey mud, full grabs
EI	3 Oct				
Orca Bay					
OB-1	9 Apr	60°34.98'	146°02.00'	164-177	Soft grey mud, full grabs
OB-1	7 May				
OB-1	10 Oct				
OB-6	8 May	60°34.78'	146°54.08'	417	Soft grey mud, full grabs
OB-6	7 Oct				
Knight Island Passage					
KI-1	10 May	60°20.58'	147°56.98'	413	Soft grey mud and rocks, grabs half full
KI-2	7 Oct	60°10.80'	147°53.40'	388	Soft grey mud, full grabs

Table 3. (cont'd)

Station	Date	Latitude (N)	Longitude (W)	Depth (M)	Bottom Type, Grab Volume
Naked Island					
NI-4	10 Apr	60°44.86'	147°26.02'	84-106	Soft grey mud, shells, sponge, rocks, grabs half full
NI-4	9 May				
NI-4	7 Oct				
Naked Island (Outside Bay)					
NI-01	2 Jun	60°38.35'	147°28.82'	40	Soft grey mud, sand, shell, grabs 3/4 full
Bligh Island					
BI	10 Apr	60°50.22'	146°55.03'	84-97	Soft grey mud, gravel, shell, rock, grabs full
BI	8 May				
BI	6 Oct				
Rocky Bay					
RB-2	8 Apr	60°21.80'	147°01.77'	76-84	Soft grey mud, rocks, same reps with shell and gravel, full grabs
RB-2	7 May				
RB-2	7 Oct				
RB-7	8 Apr	60°21.48'	147°03.92'	65	Compact grey mud, full grabs
Hinchinbrook Entrance					
HE-3	7 May	60°17.37'	146°46.24'	234	Soft grey mud, full grabs
Smith Island					
SI-01	5 Jun	60°31.39'	147°24.12'	43	Compact grey mud, sand, shell, grabs half full
Port Valdez					
PVA 040	8 May	61°06.30'	146°28.70'	223	Soft grey mud and wood, full grabs
PVA 050	10 Apr	61°06.35'	146°35.70'	248	Soft grey mud, full grabs

collected in 1990, the 1.0 mm and 0.5 mm fractions were sorted. The organisms from both fractions were combined prior to analyses. In the next report, organisms from 0.5 mm fractions will also be examined.

In most benthic biological studies, as well as the study reported here, organisms collected by grab and subsequently used in analyses include infaunal macrofauna, slow-moving macrofaunal surface dwellers, and small, sessile epifauna (inclusive of amphipods). Highly motile epifauna such as large gastropods, shrimps, crabs, and sea stars (except the infaunal sea star *Ctenodiscus crispatus*) are not adequately collected by grab and are usually excluded from analyses. The latter types of organisms were deleted in the present study as well. Since 0.5 mm mesh fractions were collected and sorted, it is appropriate to include larger representatives of the meiofauna, many of which are retained quantitatively by this screen. Thus, the following organisms are included in the analyses: nematodes, tardigrades, ostracods. harpacticoid copepods, tanaids and cumaceans. Although Foraminifera were common at some stations, most specimens examined appeared to have been dead at the time of collection. Additionally, the considerable amount of sorting time (up to 60 hours per 0.5 mm replicate) mandated that this group be deleted from the analyses. Thus, Foraminifera are not included in the analyses; however, all samples containing Foraminifera are archived.

Prior to the analysis of the first year of data (for 1990), it was decided by the peer-review group for this project that it would be appropriate to identify organisms to the family or higher taxonomic level (and wherever possible to identify to species level) in order to complete analyses in a reasonably appropriate time at an acceptable cost. The Principal Investigator (H. M. Feder) approved of this suggestion since at least two well-known benthic biologists have utilized the family approach to analysis of benthic data. A quote from one of these paper summarizes the validity of using the Family or higher taxon approach to identification.

> "Although [diversity indices are] normally applied to species, Pielou (1974) has shown that the overall diversity of a community is comprised of hierarchial components, family, genus and species, and thus the concept can be applied to families. Examples of the calculation of hierarchial diversity are provided by Lloyd *et al.* (1968) and Valentine (1973). We [and in my investigation in Prince William Sound] have considered family diversity and evenness as useful for describing trends within our communities and do not imply a direct comparison with species indices."

Generic and specific designations of common species are included in the raw data sheets and the computer printouts whenever these categories are known. In fact, most dominant and many rare taxa were identified to species and/or genus. A recent paper by Warwick (1988) and other papers (Rosenberg, 1972, Heip *et al.*, 1988) indicate that better resolution of multivariate data emerges when taxonomic levels higher than species are used. However, availability of generic and specific names for common organisms on computer printouts still make it possible to examine station data in more detail if any of these taxa are abundant.

All individuals will be counted and weighed. Approximate carbon values for all wet-weights are calculated (see Appendices A and B for further details on methodology), and biomass values are reported in wet weight and carbon weight.

Data are recorded on data sheets, entered on magnetic tape and processed with the VAX computer at the University of Alaska Fairbanks (UAF). Previously written programs at UAF for comparisons of number of taxa and rank abundance and biomass of taxa will be used. A diversity program will also be used to compare stations. Most of the calculations of data are based on taxonomic determination made to the family or other higher taxonomic levels (see Appendix C).

Multivariate Analysis

Once data were available for all stations within the sites, station groups were identified using the technique of hierarchical cluster analysis (see Appendix A for details of this analysis). Principal coordinate analysis is used as an aid in interpretation of cluster analysis and for identifying misclassifications of stations by cluster analysis (see Apppendix A). Use of both multivariate techniques makes it possible to examine similarities (or dissimilarities) between groups of stations and should be useful when comparing oiled vs. unoiled bays.

Univariate Analyses

Various measures of diversity will be calculated and compared qualitatively between stations at similar depths within unoiled and oiled bays. Indices to be calculated are: Shannon Diversity (measures total diversity; weighted in favor of rare taxa), Simpson Dominance (useful for identifying dominance by one or a few taxa at a station), Evenness and Species Richness.

The calculation of K-dominance curves (Warwick, 1986) for abundance and biomass data will be used in an attempt to assess the effect of disturbance by hydrocarbons on benthic organisms in oiled bays. This technique is designed to detect pollution-induced disturbance on marine benthic communities. However, there are problems of interpretation of the output of this technique that must be considered before environmentally-related conclusions can be drawn (Gray, 1989, Beukema, 1988). Distributions of geometric classes of abundance of taxa will also be calculated when data from all stations for 1990 and 1991 at all sites are available. Assessment of the distribution of taxa in these abundance classes is often useful to identify indicator taxa within a disturbed area, and may give clues about a return of a benthic system to a less disturbed condition.

The methodologies, rationale, and problems with the use of univariate measures such as diversity indices, K-dominance curves, and geometric abundance classes to assess pollution-induced disturbance are discussed in Appendix A (also see Bayne *et al.*, 1988). Presumably, univariate techniques will prove most useful when temporal data (i.e., 1990, 1991, etc.) can be compared rather than assessment using a single year of data as presented in this report.

Statistical Analysis

A Kruskal-Wallis and multiple comparison test for significance will ultimately be used to test for differences in benthic abundance and biomass between stations once

multi-year data sets are available. ANOVA will be used to examine differences in abundance and feeding types of dominant taxa between stations at similar depths within unoiled and oiled sites. Taxa will be chosen from rank abundance printouts for each station and will generally be those commonly present within sites being compared. However, taxa common at stations within unoiled bays, but rare or missing at stations within oiled bays, or vice versa, will also be tested. ANOVA will also be used to compare 1990 and 1991 and long-term data when such information is available. Coordination with the "Coastal Habitat Assessment Program - Subtidal Habitats" of S. Jewett will be accomplished whenever possible so that similar statistical testing procedures are applied to benthic data for both programs. Statistical procedures applied are included in Appendix D.

DATA ANALYSIS COMMENTS

The principal goal of the data analysis in this benthic study was to determine effects of petroleum hydrocarbons resulting from the *Exxon Valdez* oil spill on taxon composition, diversity, species richness, abundance, biomass, and trophic composition. The critical aspect of the study was to determine if petroleum contaminants from the *Exxon Valdez* occurred at concentrations which caused deleterious effects on benthic organisms. Because the "deleterious effects" criteria are complex and often require subjective interpretation, a detailed comparison is ultimately required of the hydrocarbon concentrations at which various biochemical, behavioral, physiological, organismal, population, and ecological effects occur. The present study only addresses certain aspects of the ecological effects of the oil spill on the benthic fauna. In order to fully interpret most of the "deleterious effects" expected to occur on benthic organisms, a comprehensive, broad-based program was needed to compliment this benthic study. Such a program was not initiated. Nevertheless, it should be possible to meet some aspects of the goals of the CERCLA process (i.e., documentation of damage to the environment) by 1) integrating the biological results of this project with results of the hydrocarbon and biological studies at shallow subtidal stations in the upper portions of the study area, 2) assessing hydrocarbon levels at all of the deep stations in conjunction with benthic biological data, 3) integrating deep benthic data with microbiological data collected at the same stations, and 4) comparing the results of the Prince William Sound studies with literature on effects demonstrated by deep benthic organisms subjected to hydrocarbon and other organic contamination.

COMMENTS ON METHOD OF PRESENTATION OF DATA FROM THREE DEPTHS AT SITES OCCUPIED IN 1990

The format for the 1990 data presentations for 40, 100, and >100 m are similar. Each depth is presented separately and the categories considered are generally as follows:

1. Composition of individual stations based on higher taxon values.
2. Numerical analysis.
3. K dominance curves.

4. Feeding types.

5. Amphipod composition at stations.

6. Statistical assessment of taxon abundance and biomass.

RESULTS

STATIONS OCCUPIED IN 1989

Stations were occupied in Prince William Sound and the adjacent Gulf of Alaska shelf in April, May, June, and October 1989 (Table 3). All samples were processed on shipboard with 1.0 mm mesh screen. Unsorted samples are currently stored in sealed containers at the Institute of Marine Science, UAF.

Temporal (1989) Stations Processed and Identifications Completed

The following temporal (1989) stations have taxonomic identifications completed, but data are not entered in the computer:

Bligh Island (1 station)
Green Island area (7 stations)
Hinchinbrook Entrance (1 station)
Knight Island Passage (1 station)
Main Bay (1 station)
Naked Island (2 stations)
Orca Bay (2 stations)
Rocky Bay (2 stations)
Sawmill Bay (1 station)
Smith Island (1 station)
Herring Bay (1 station)
Montague Strait (1 station)

The following temporal station at 40 m, data collected June 1989, has taxonomic identifications completed and data entered in the computer. Data from this station is compared in this report with a similar station occupied in July 1990 and late June 1991.

Snug Harbor (1 station)

STATIONS OCCUPIED ON CRUISE OF 1-23 JULY 1990, NOAA SHIP *DAVIDSON*

The location data for stations occupied on the NOAA Ship *Davidson* in July 1990 are summarized in Table 1. All samples were processed on shipboard with nested 1.0 and 0.5 mm screens.

STATIONS OCCUPIED ON CRUISE OF 13-25 JUNE 1991 ON THE *BIG VALLEY*

The location data for stations occupied on the *Big Valley* in June 1991 are summarized in Table 1. All samples were processed on shipboard with nested 1.0 and 0.5 mm screens.

LABORATORY OBSERVATIONS OF OIL IN SAMPLES COLLECTED IN JULY 1990

The following observations are from samples prepared in the laboratory for identification of infaunal invertebrates. Oil droplets were detected in variable numbers of replicates of stations at 40 m for six of the seven oil sites (Table 4). No oil was detected in samples from Sleepy Bay at 40 m. No oil was observed in replicates from sites at 100 m and >100 m.

ASSESSMENT OF 1990 DATA FROM 40, 100 AND >100 M

Faunal Assessment of Data from Stations Sampled July 1990 at 40 m

Composition of Individual Stations Based on Values for Higher Taxa

At stations occupied at oiled and unoiled sites at 40 m, polychaetous annelids were dominant in abundance. Mollusca appeared to be somewhat more abundant at unoiled sites (Figs. 2 and 3). At most oiled sites Nematoda appeared to be more common than at unoiled sites. Statistical assessment of Nematoda abundance is treated in a later section. Little difference could be observed qualitatively for any of the other major groups between oiled and unoiled sites.

The abundance, biomass, number of taxa and diversity[2] of benthic fauna for the fourteen stations sampled at this depth are tabulated in Table 5a. Abundance values at oiled sites varied between 1158-5682 and for unoiled sites 398-8190 indiv./m^2. Carbon biomass at oiled sites varied between 1.27 and 4.28 gC/m^2 and for unoiled sites 0.4-13.75 gC/m^2. Number of taxa at oiled sites varied between 62-103 and at

[2] The number of taxa and diversity are based on identifications to Family level or higher. A list of all taxa to higher taxon levels are included in Appendix C.

Text continued on page 24.

Table 4. Laboratory observations of oil in samples at 40 m at sites in Prince William Sound, July 1990.

Station	Comments
OILED SITES	
Disk Island (40 m)	Oil droplets noted in two replicates of the 1.0 mm fraction and two replicates of the 0.5 mm fraction. Faint odor of H_2S.
Northwest Bay	Oil droplets in one replicate of the 1.0 mm fraction and two replicates of the 0.5 mm fraction. Strong H_2S odor in some replicates.
Bay of Isles	No oil in 1.0 mm fraction. Oil droplets in three replicates of 0.5 mm fraction.
Herring Bay	No notes for 1.0 mm fraction. Oil droplets in 0.5 mm fraction.
Sleepy Bay	No oil observed
Chenega Bay	Oil Droplets in one replicate of 1.0 mm fraction.
Snug Harbor	Oil droplets in two replicates of the 1.0 mm fraction and one replicate of 0.5 mm fraction.
UNOILED SITES	
No oil observed at any site.	

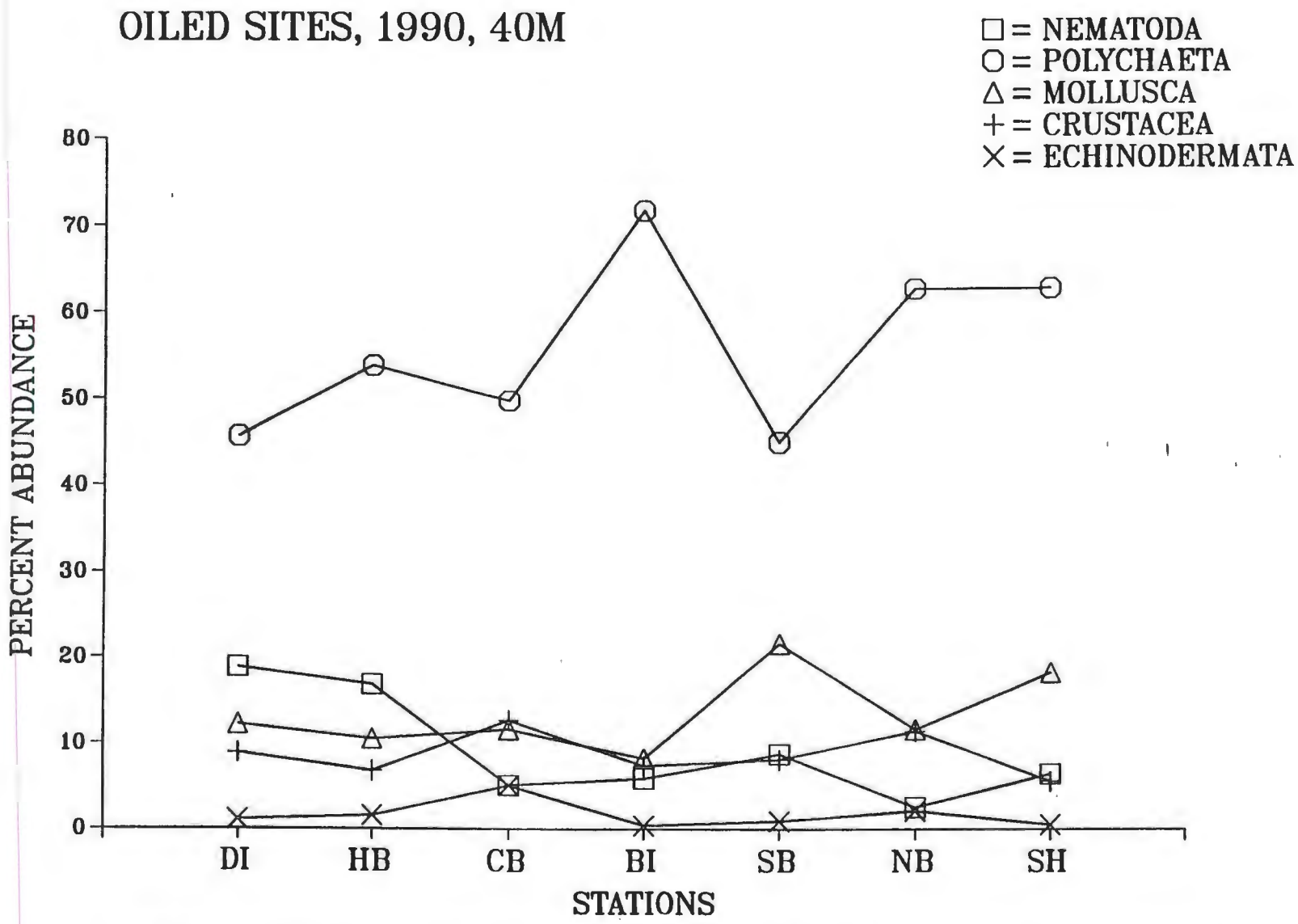

Figure 2. Percent abundance of major higher taxonomic groups at stations at oiled sites for samples collected at 40 m in July 1990.

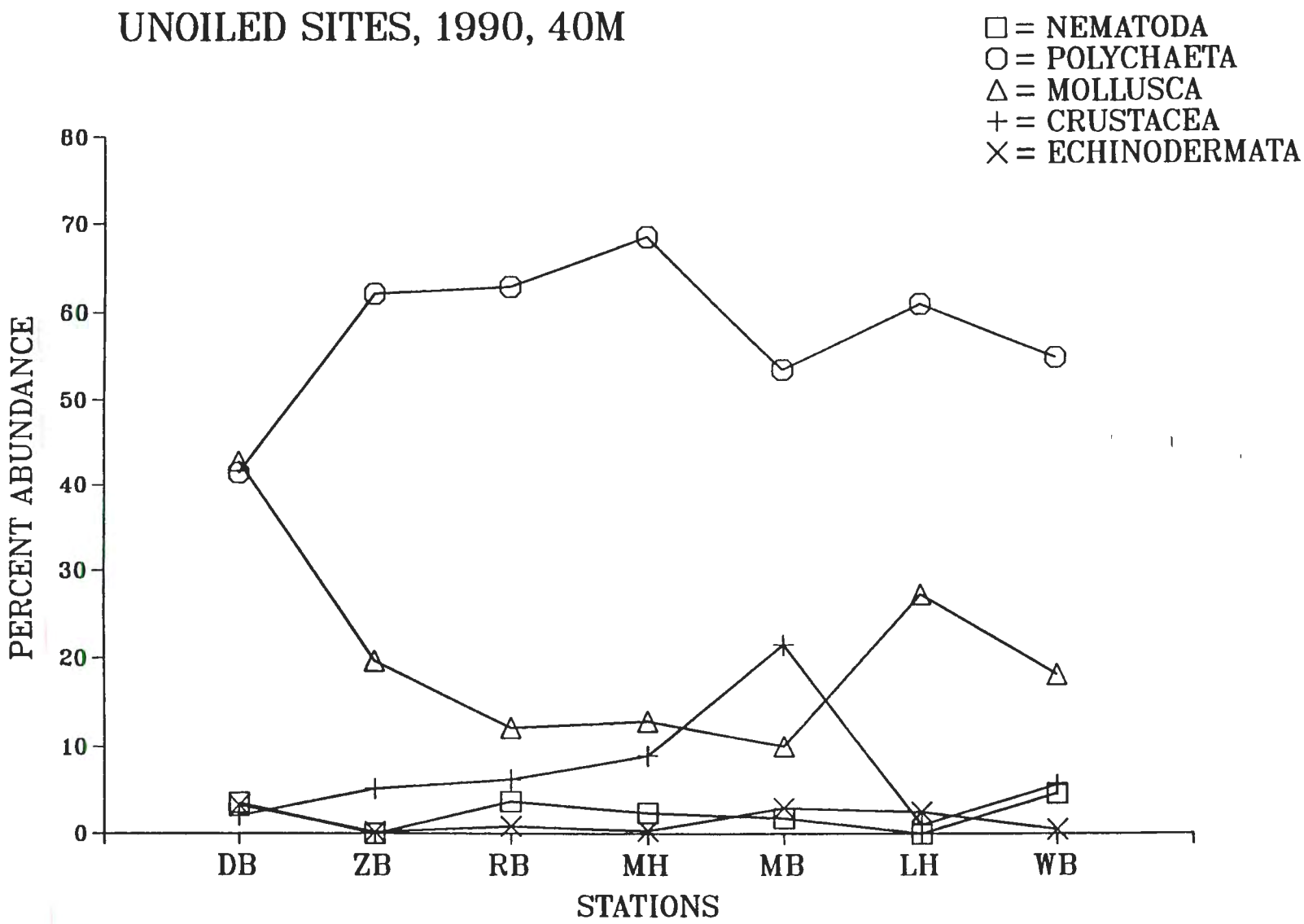

Figure 3. Percent abundance of major higher taxonomic groups at stations at unoiled sites for samples collected at 40 m in July 1990.

Table 5a. The abundance, biomass, number of taxa, and diversity of sites sampled at 40 m in Prince William Sound in July 1990. Fragments and species excluded from cluster analysis ARE excluded in all computations except biomass. The identifications were typically to the Family level whenever possible and to higher levels if necessary.

STATION	ABUNDANCE	WET WEIGHT BIOMASS	CARBON BIOMASS	NUMBER OF TAXA	SIMPSON	SHANNON	SW EVEN	SPECIES RICHNESS
DA901BI1	5682.0	76.254	4.2780	77.0	0.066	3.167	0.729	8.791
DA901CB1	4344.0	82.117	2.2877	103.0	0.042	3.672	0.792	12.177
DA901DB1	1464.0	33.404	1.3864	50.0	0.066	3.079	0.787	6.723
DA901DI1	5132.0	71.013	2.9340	78.0	0.063	3.309	0.760	9.013
DA901HB1	4370.0	42.996	1.7232	89.0	0.065	3.242	0.722	10.498
DA901LH1	398.0	23.607	0.9019	35.0	0.062	3.017	0.849	5.679
DA901MB1	7794.0	139.225	6.5465	114.0	0.068	3.377	0.713	12.610
DA901MH1	8190.0	399.764	13.7541	75.0	0.150	2.817	0.653	8.212
DA901NB1	2960.0	75.871	2.8835	88.0	0.046	3.600	0.804	10.885
DA901RB1	7268.0	166.377	6.1361	99.0	0.086	3.265	0.710	11.022
DA901SB1	5598.0	99.544	3.5792	94.0	0.039	3.606	0.794	10.776
DA901SH1	1158.0	31.475	1.2651	62.0	0.054	3.295	0.798	8.647
DA901WB1	816.0	7.366	0.4046	48.0	0.047	3.309	0.855	7.010
DA901ZB1	1950.0	233.481	13.3370	53.0	0.112	2.779	0.700	6.864

unoiled sites 48-114. Diversity and evenness values were roughly similar at oiled and unoiled stations, but Shannon diversity values were slightly lower and Simpson dominance slightly higher at unoiled stations (also see Figs. 4 and 5). Species richness at oiled stations varied between 8.65-12.18 and at unoiled stations from 6.72-12.61. A similar pattern of diversity, dominance, and species richness values are noted in Table 5b prepared from numbers based on lower taxon values[3].

Species richness (SR) was variable between the oiled and unoiled stations, but SR appeared to be generally higher at the pooled oiled stations (mean SR=10.1) than at the pooled unoiled stations (mean SR=8.3). These values were not statistically significant (T test). However, if one unoiled station, Mooselips Bay, is not included in the analysis[4], the mean SR for unoiled stations is 7.6. The mean SR for oiled stations, 10.1, is then significantly higher (P=0.015) than the SR value of 7.6 for the unoiled stations. The number of taxa at oiled and unoiled stations was also variable, but the number of taxa was typically higher (but not statistically significant) at the oiled stations (mean no. taxa=84.4) than the unoiled stations (mean no. taxa=68). However, if Mooselips Bay is again deleted, the mean value for number of taxa for unoiled stations is then 60, which is significantly lower (P=0.036) than the number of taxa (84) at the oiled stations.

The rank abundance of the dominant fauna collected at all stations occupied are tabulated in Tables 6 and 7. Differences in taxa generally dominating oiled and unoiled stations can be seen in this table (statistical assessment of some of these differences are considered in a later section). For example, the following taxa are typically most abundant at oiled stations: Nematoda, the surface-feeding polychaete groups-Ampharetidae, Cirratulidae; the subsurface deposit feeding polychaete Maldanidae; the surface-feeding sipunculid worm group Golfingiidae; and the surface feeding crustacean group Harpacticoida. Alternatively, the following taxa are most abundant at unoiled stations: the subsurface deposit-feeding polychaete group Owenidae (specifically *Myriochele*); the suspension-feeding bivalve group Lucinidae; the subsurface deposit feeding bivalve groups Nuculidae and Nuculanidae.

Numerical Analysis

A normal cluster analysis of *ln*-transformed abundance data indicated the presence of three station groups and two stations that did not join any group (oiled station at Snug Harbor; unoiled station at Lower Herring Bay) at the 66% of similarity (Fig. 6). Station groups consisted of the following (Table 8): Group I – six oiled sites and one unoiled site; Group II – two unoiled sites; and Group III – three unoiled sites. In a Principal Coordinate analysis (PCA) 100% of the variance in the data is explained by Axes I and II. Station Groups I and III are confirmed by this analysis (Fig. 7). Although the stations of Group II (the unoiled sites, Rocky Bay and MacLeod Harbor) are somewhat separated from Group I stations in the PCA, their close association to Group I is suggested by the fact that the two groups join at a low level of similarity in the cluster analysis. Snug Harbor and Lower Herring Bay are not aligned with any

[3] Mainly consisted of generic and specific names; as stated in Methods, whenever possible identifications were made to lower taxa and sufficient lower taxon identifications were available to make a table of diversity to compare with Table 5a.

[4] An unusual unoiled (control) site that does not appear to be physically similar to the other unoiled sites chosen, especially in terms of exposure and resulting turbulence present; this point will be discussed later. See Appendix E.

Text continued on page 36.

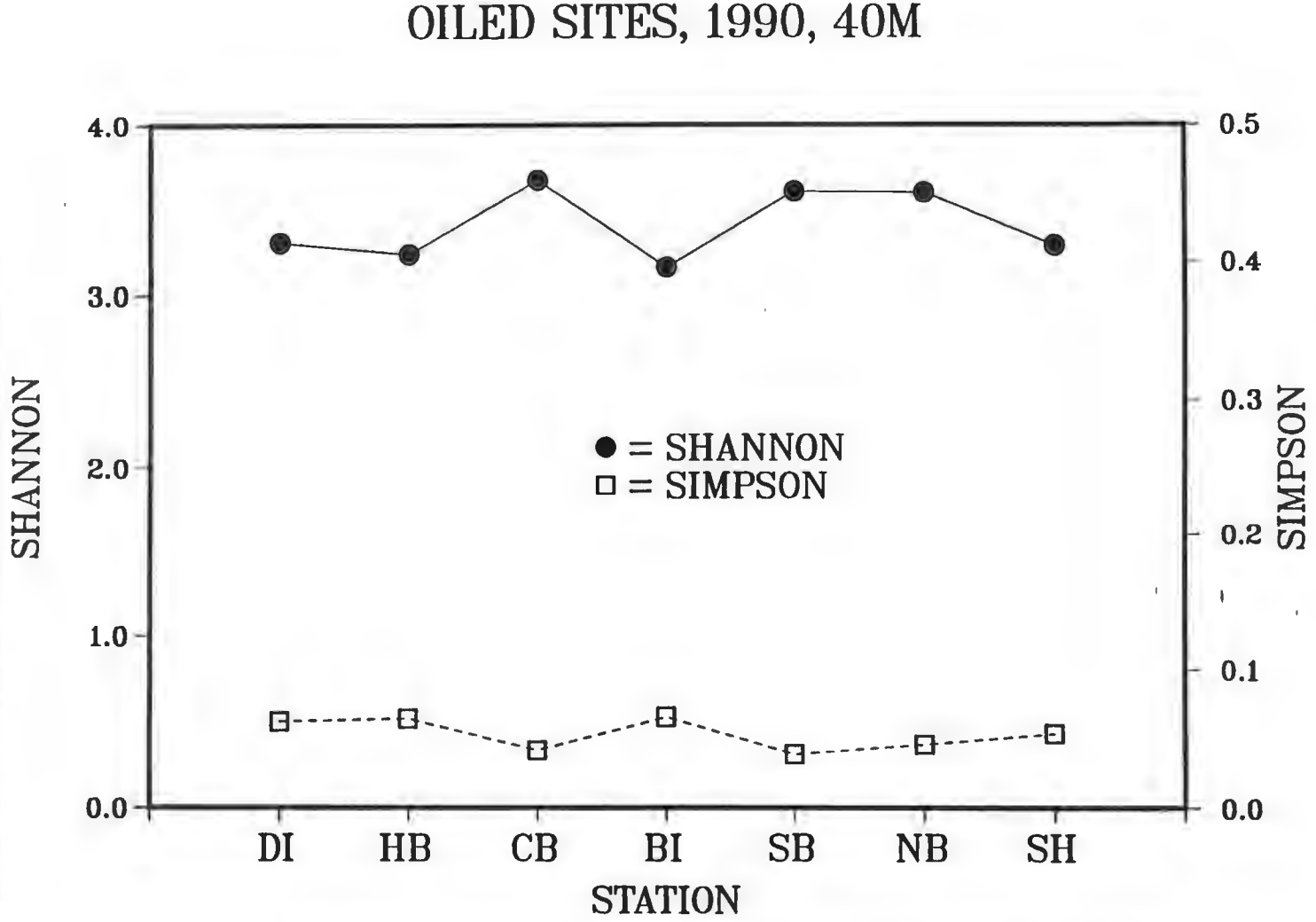

Figure 4. Shannon diversity and Simpson dominance values at stations at 40 m at oiled sites for samples collected July 1990 in Prince William Sound.

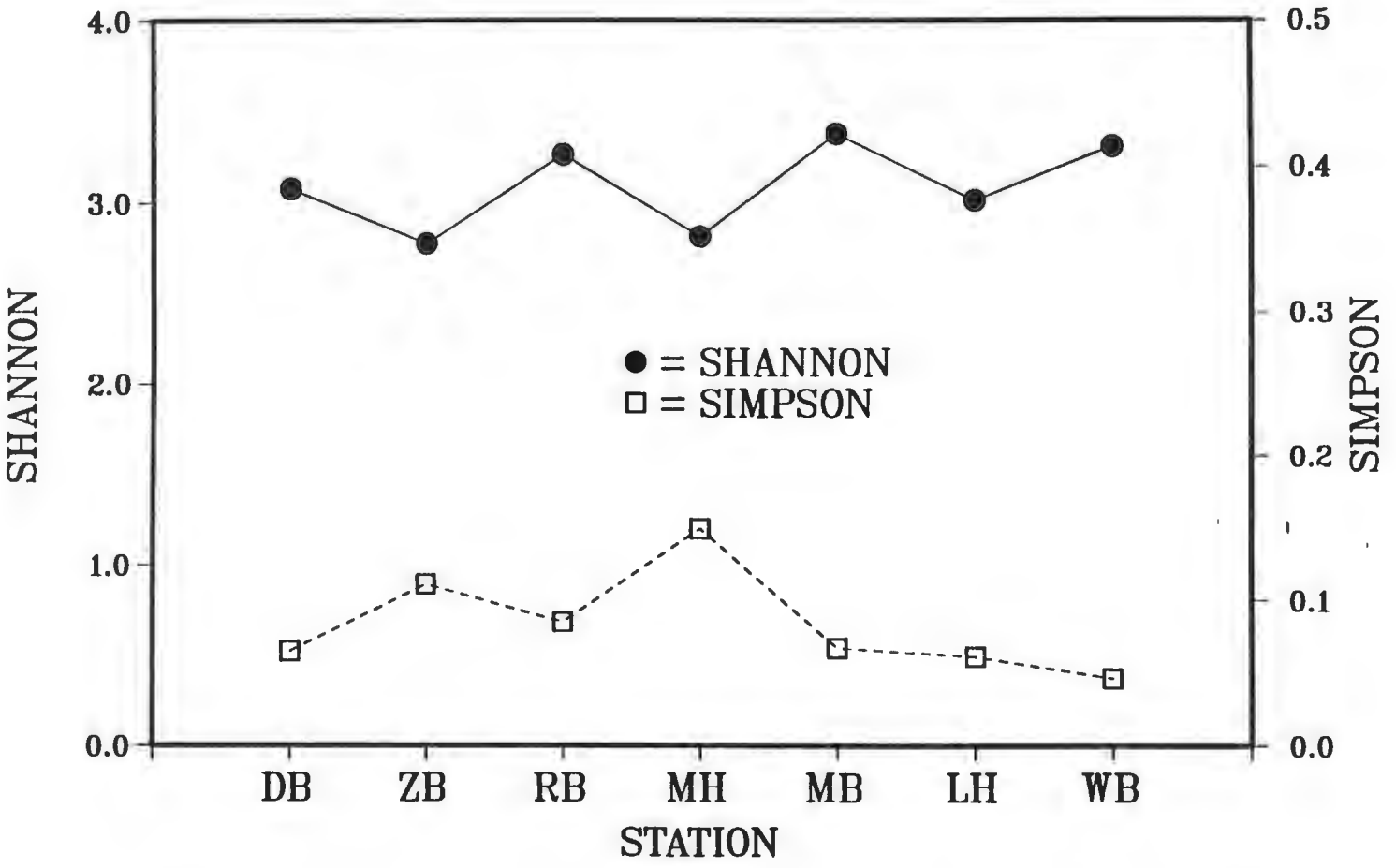

Figure 5. Shannon diversity and Simpson dominance values at stations at 40 m at unoiled sites for samples collected July 1990 in Prince William Sound.

Table 5b. The abundance, biomass, number of taxa, and diversity of sites sampled at 40 m in Prince William Sound in July 1990. Fragments and species excluded from cluster analysis ARE excluded in all computations except biomass. The identifications in most cases were to the generic and specific level. Included in the data analysis shown in this table are organisms that could not be identified to generic and specific levels and were entered at the Family or higher taxonomic level.

STATION	ABUNDANCE	WET WEIGHT BIOMASS	CARBON BIOMASS	NUMBER OF TAXA	SIMPSON	SHANNON	SW EVEN	SPECIES RICHNESS
DA901BI1	5670.0	76.254	4.2780	103.0	0.052	3.449	0.744	11.802
DA901CB1	4254.0	82.117	2.2877	143.0	0.035	3.910	0.788	16.995
DA901DI1	5106.0	126.347	4.4833	126.0	0.059	3.559	0.736	14.640
DA901HB1	4362.0	42.996	1.7231	139.0	0.061	3.499	0.709	16.466
DA901NB1	2950.0	75.871	2.8835	131.0	0.041	3.905	0.801	16.271
DA901SB1	5560.0	99.544	3.5783	133.0	0.037	3.771	0.771	15.307
DA901SH1	1154.0	31.475	1.2650	84.0	0.041	3.596	0.812	11.771
DA901DB1	1462.0	33.404	1.3864	71.0	0.058	3.322	0.779	9.605
DA901LH1	398.0	23.607	0.9019	44.0	0.055	3.182	0.841	7.183
DA901MB1	7760.0	139.225	6.5459	176.0	0.065	3.580	0.692	19.538
DA901MH1	8186.0	399.764	13.7541	128.0	0.146	3.066	0.632	14.095
DA901RB1	7248.0	166.377	6.1382	163.0	0.043	3.787	0.743	18.226
DA901WB1	816.0	7.366	0.4046	67.0	0.042	3.497	0.832	9.844
DA901ZB1	1948.0	233.481	13.3370	73.0	0.077	3.144	0.733	9.506

Table 6. Rank abundance (no. m^{-2}) by family and higher taxa for all stations at 40 m for data collected in Prince William Sound, July 1990.

Station	Condition	Dominant Taxa	Abundance (no. m^{-2})
DI	Oiled	Nematoda	896
		Golfingiidae	446
		Cirratulidae	368
		Bivalvia	274
		Paraonidae	266
		Maldanidae	220
		Lumbrineridae	218
		Owenidae	200
		Thyasiridae	172
		Capitellidae	102
		Sabellidae	86
		Polyodontidae	80
		Harpacticoida	76
		Magelonidae	74
		Spionidae	74
		Ostracoda	70
		Syllidae	62
		Leuconidae	56
		Phoxocephalidae	54
		Gnathiidae	54
		Ophiuroidea	50
		Sigalionidae	46
		Pyrenidae	46
HB	Oiled	Nematoda	646
		Cirratulidae	406
		Bivalvia	296
		Lumbrineridae	280
		Polyodontidae	226
		Paraonidae	212
		Syllidae	176
		Golfingiidae	170
		Maldanidae	144
		Capitellidae	122
		Harpacticoida	70
		Ampharetidae	66
		Sabellidae	60
		Owenidae	50
		Sigalionidae	50
		Ophiuroidea	46
		Polyplacophora	44
		Spionidae	42
		Gnathiidae	40
		Lepidopleuridae	40
		Phoxocephalidae	40
SB	Oiled	Nematoda	466
		Mytilidae	432
		Bivalvia	402
		Cirratulidae	306
		Syllidae	276
		Golfingiidae	242
		Sigalionidae	238
		Polyodontidae	236
		Maldanidae	224
		Capitellidae	190
		Polyplacophora	188
SB (cont'd)		Lumbrineridae	166
		Limidae	148
		Lepidopleuridae	138
		Ascidiacea	120
		Astartidae	94
		Harpacticoida	92
		Spionidae	82
		Ampharetidae	76
		Gnathiidae	76
		Paraonidae	76
		Onuphidae	72
		Ostracoda	64
		Chaetopteridae	50
		Ampeliscidae	48
		Owenidae	48
NB	Oiled	Cirratulidae	436
		Lumbrineridae	212
		Paraonidae	200
		Owenidae	146
		Leuconidae	106
		Maldanidae	106
		Bivalvia	94
		Capitellidae	90
		Syllidae	78
		Nematoda	72
		Lepidopleuridae	72
		Sigalionidae	70
		Thyasiridae	62
		Polyodontidae	62
		Spionidae	60
		Ophiuroidea	60
		Onuphidae	60
		Golfingiidae	54
		Sabellidae	44
		Mytilidae	44
		Nephtyidae	42
		Glyceridae	42
SH	Oiled	Spionidae	144
		Capitellidae	118
		Nuculidae	96
		Nematoda	74
		Paraonidae	66
		Cirratulidae	62
		Maldanidae	54
		Owenidae	46
		Leuconidae	40
		Lucinidae	34
		Nephtyidae	32
		Bivalvia	32
		Polyodontidae	30
		Amphictenidae	26
		Orbiniidae	26
		Lepidopleuridae	22
		Rhynchocoela	18
		Thyasiridae	18

Table 6. (continued)

Station	Condition	Dominant Taxa	Abundance (no. m^{-2})
CB	Oiled	Syllidae	464
		Polyodontidae	372
		Harpacticoida	294
		Spirorbidae	234
		Nematoda	204
		Ophiuroidea	194
		Onuphidae	130
		Ampharetidae	106
		Caecidae	102
		Serpulidae	100
		Bivalvia	92
		Sabellidae	78
		Astartidae	76
		Golfingiidae	70
		Hiatellidae	70
		Thyasiridae	70
		Lepidopleuridae	68
		Sigalionidae	68
BI	Oiled	Paraonidae	858
		Capitellidae	656
		Polyodontidae	500
		Bivalvia	394
		Nematoda	332
		Cirratulidae	292
		Maldanidae	264
		Lumbrineridae	236
		Leuconidae	230
		Sigalionidae	226
		Syllidae	190
		Nephtyidae	140
		Polynoidae	128
		Magelonidae	110
		Phoxocephalidae	104
		Spionidae	82
		Lepidopleuridae	72
		Rhynchocoela	58
		Trichbranchidae	54
DB	Unoiled	Bivalvia	186
		Nephtyidae	178
		Lucinidae	146
		Paraonidae	130
		Nuculidae	112
		Tellinidae	84
		Lumbrineridae	54
		Nematoda	52
		Ophiuroidea	48
		Rhynchocoela	38
		Thyasiridae	38
		Sigalionidae	38
		Capitellidae	34
		Nuculanidae	32
ZB	Unoiled	Nephtyidae	522
		Paraonidae	228
		Bivalvia	178
		Spionidae	136
		Nuculanidae	134
		Pyrenidae	92
		Cirratulidae	78
		Lumbrineridae	74
		Chaetodermatidae	50
		Ostracoda	40
		Leuconidae	40
		Rhynchocoela	40
		Capitellidae	38
		Sigalionidae	32
		Scaphandridae	28
		Hesionidae	22
		Tellinidae	20
		Thyasiridae	18
RB	Unoiled	Spionidae	1742
		Capitellidae	830
		Golfingiidae	498
		Bivalvia	316
		Nematoda	270
		Lumbrineridae	264
		Paraonidae	246
		Maldanidae	210
		Thyasiridae	206
		Owenidae	178
		Magelonidae	154
		Ostracoda	118
		Cirratulidae	112
		Nephtyidae	108
		Nuculidae	104
		Gastropoda	100
		Leuconidae	94
		Lucinidae	88
		Syllidae	86
		Glyceridae	76
		Dentaliidae	68

Table 6. (continued)

Station	Condition	Dominant Taxa	Abundance (no. m^{-2})
MH	Unoiled	Owenidae	2950
		Ostracoda	590
		Bivalvia	508
		Sternaspidae	414
		Paraonidae	408
		Magelonidae	298
		Maldanidae	220
		Nematoda	198
		Thyasiridae	188
		Scaphandridae	184
		Lumbrineridae	184
		Capitellidae	174
		Cirratulidae	174
		Nuculidae	156
		Syllidae	98
		Pyramidellidae	94
		Trichbranchidae	86
		Retusidae	82
		Spionidae	82
		Nannastacidae	72
MB	Unoiled	Cirratulidae	1466
		Owenidae	766
		Ampeliscidae	678
		Bivalvia	456
		Capitellidae	338
		Golfingiidae	316
		Balanidae	312
		Syllidae	262
		Paraonidae	256
		Phoxocephalidae	244
		Ophiuroidea	212
		Polyodontidae	194
		Nematoda	138
		Maldanidae	112
		Lepidopleuridae	104
		Lumbrineridae	96
		Sigalionidae	90
		Nuculanidae	84
		Gnathiidae	82
		Thyasiridae	72
LH	Unoiled	Lucinidae	48
		Orbiniidae	46
		Paraonidae	44
		Capitellidae	26
		Tellinidae	26
		Sigalionidae	22
		Cirratulidae	20
		Nephtyidae	18
		Bivalvia	14
		Nuculidae	12
		Spionidae	12
		Rhynchocoela	12
		Pyrenidae	10
		Ophiuroidea	10
		Hesionidae	8
		Scalibregmidae	8
		Syllidae	8
		Scaphandridae	6
		Polyodontidae	6
		Thyasiridae	6
WB	Unoiled	Capitellidae	84
		Bivalvia	56
		Nephtyidae	52
		Lumbrineridae	52
		Spionidae	50
		Rhynchocoela	42
		Nuculanidae	42
		Sternaspidae	42
		Cirratulidae	40
		Nematoda	38
		Ostracoda	36
		Paraonidae	32
		Scaphandridae	24
		Sigalionidae	22
		Nuculidae	18
		Dentalidae	16
		Lucinidae	14

Table 7. Rank abundance (no. m^{-2}) by genus and species (i.e., with as many of these identifications available; see Methods) for all stations at 40 m for data collected in Prince William Sound, July 1990.

Station	Condition	Dominant Taxa	Abundance (no. m^{-2})
DI	Oiled	Nematoda	896
		Golfingia margaritacea	432
		Cirratulidae	366
		Bivalvia	274
		Myriochele oculata	196
		Lumbrineris spp.	190
		Paraonidae	158
		Thyasiridae	158
		Maldanidae	118
		Capitellidae	90
		Asychis sp.	86
		Harpacticoida	76
		Spionidae	72
		Sabellidae	70
		Ostracoda	70
		Peisidice aspera	62
		Phoxocephalidae	54
		Gnathia sp.	54
		Ophiuroidea	50
		Alia gausapata	46
HB	Oiled	Nematoda	646
		Cirratulidae	402
		Bivalvia	296
		Lumbrineris spp.	272
		Peisidice aspera	214
		Golfingia margaritacea	170
		Maldanidae	128
		Aricidea ramosa	106
		Capitellidae	104
		Harpacticoida	70
		Syllidae	54
		Exogone sp.	52
		Pholoe minuta	50
		Myriochele oculata	48
		Melinna elisabethae	48
		Ophiuroidea	46
		Aricidea sp.	46
		Polyplacophora	44
		Gnathia sp.	40
		Leptochiton rugatus	40
SB	Oiled	Nematoda	466
		Crenella decussata	428
		Bivalvia	402
		Cirratulidae	306
		Pholoe minuta	238
		Peisidice aspera	236
		Golfingia margaritacea	220
		Syllidae	202
		Maldanidae	200
		Capitellidae	188
		Polyplacophora	188
		Lumbrineris spp.	146
		Leptochiton rugatus	138
		Limatula subauriculata	122
		Ascidiacea	120
		Harpacticoida	92
		Paraonidae	66
		Gnathia sp.	66
		Astarte esquimaulti	64
		Ostracoda	64
		Ampharetidae	62
NB	Oiled	Cirratulidae	436
		Lumbrineris spp.	194
		Myriochele oculata	146
		Aricidea ramosa	104
		Leucon sp.	94
		Bivalvia	94
		Capitellidae	90
		Nematoda	72
		Leptochiton rugatus	72
		Pholoe minuta	70
		Paraonidae	70
		Peisidice aspera	62
		Maldanidae	62
		Ophiuroidea	60
		Syllidae	60
		Golfingia margaritacea	54
		Crenella decussata	42
		Glyceridae	42
		Phoxocephalidae	38
		Campylaspis sp.	36
SH	Oiled	Capitellidae	104
		Spionidae	100
		Nucula tenuis	96
		Nematoda	74
		Cirratulidae	62
		Paraonidae	50
		Myriochele oculata	46
		Prionospio sp.	44
		Maldanidae	42
		Leucon sp.	32
		Bivalvia	32
		Peisidice aspera	30
		Lucinidae	26
		Amphictenidae	26
		Leptochiton rugatus	22
		Leitoscoloplos pugettensis	22
		Cossura sp.	18
		Rhynchocoela	18
		Pholoe minuta	18
		Nephtys cornuta	18
CB	Oiled	*Peisidice aspera*	372
		Harpacticoida	294
		Typosyllis armillaris	292
		Nematoda	204
		Ophiuroidea	194
		Syllidae	164
		Spirorbis sp.	152
		Micranellum crebricinctum	98
		Bivalvia	92
		Spirorbidae	82
		Onuphis sp.	80
		Sabellidae	78
		Astarte sp.	72
		Golfingia margaritacea	70
		Hiatella arctica	70
		Leptochiton rugatus	68
		Pholoe minuta	68
		Cirratulidae	64

Table 7. (continued)

Station	Condition	Dominant Taxa	Abundance (no. m^{-2})
BI	Oiled	Not sampled	
DB	Unoiled	Not sampled	
ZB	Unoiled	Not sampled	
RB	Unoiled	Not sampled	
MH	Unoiled	*Myriochele oculata*	2936
		Ostracoda	590
		Bivalvia	508
		Sternaspis scutata	414
		Nematoda	198
		Magelona sp.	190
		Tauberia gracilis	176
		Capitellidae	174
		Cirratulidae	174
		Thyasiridae	158
		Nucula tenuis	156
		Scaphandridae	138
		Maldanidae	136
		Lumbrineris spp.	130
		Paraonidae	118
		Magelona longicornis	108
		Turbonilla sp.	94
		Syllidae	86
		Trichobranchus glacialis	72
MB	Unoiled	Cirratulidae	1466
		Myriochele oculata	756
		Ampelisca agassizi	610
		Bivalvia	456
		Capitellidae	338
		Golfingia margaritacea	310
		Phoxocephalidae	244
		Semibalanus cariosus	238
		Paraonidae	218
		Ophiuroidea	212
		Peisidice aspera	194
		Nematoda	138
		Syllidae	120
		Leptochiton rugatus	102
		Maldanidae	98
		Pholoe minuta	90
		Lumbrineris spp.	84
		Gnathia sp.	82
		Yoldia sp.	68
		Semibalanus balanoides	66
LH	Unoiled	Orbiniidae	46
		Lucina tenuisculpta	40
		Aricidea sp.	40
		Capitellidae	26
		Macoma sp.	26
		Pholoe minuta	22
		Cirratulidae	20
		Bivalvia	14
		Spionidae	12
		Rhynchocoela	12
		Nucula tenuis	12
		Lumbrineris spp.	10
		Ophiuroidea	10
		Alia gausapata	10
		Nephtyidae	10
WB	Unoiled	Capitellidae	84
		Bivalvia	56
		Spionidae	50
		Nephtyidae	50
		Lumbrineris spp.	48
		Rhynchocoela	42
		Sternaspis scutata	42
		Cirratulidae	40
		Nematoda	38
		Ostracoda	36
		Pholoe minuta	22
		Paraonidae	20
		Nuculana fossa	18
		Nucula tenuis	18
		Scaphandridae	16
		Dentalium sp.	18
		Nuculana sp.	14

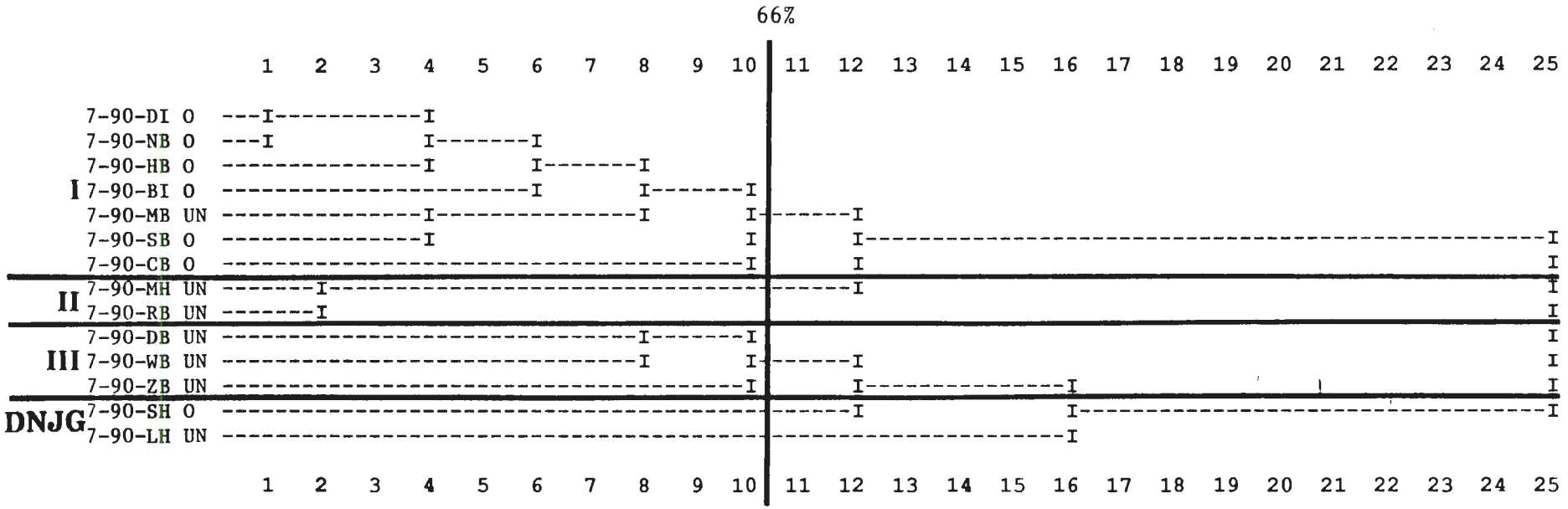

Figure 6. Dendrogram depicting the station groups formed at the 66% level of similarity by a normal cluster analysis on *ln*-transformed abundance data from 40 m in July 1990 at sites within Prince William Sound. Stations appear, for example, as 7-90-DI (date - site of Disk Island). DNJG = did not join a cluster group at 66% level of similarity. O = oiled, UN = unoiled.

Table 8. Station groups based on hierarchical cluster and principal coordinate analyses of the July 1990 Prince William Sound benthic data from 40 m. O = Oiled station; UN = Unoiled station.

Group I Stations	Group II Stations	Group III Stations	Ungrouped Stations
Disk Island (O)	Rocky Bay (UN)	Drier Bay (UN)	Snug Harbor (O)
Bay of Isles (O)	MacLeod Harbor (UN)	West Bay (UN)	Lower Herring Bay (UN)
Northwest Bay (O)		Zaikof Bay (UN)	
Herring Bay (O)			
Sleepy Bay (O)			
Chenega Bay (O)			
Mooselips Bay (UN)			

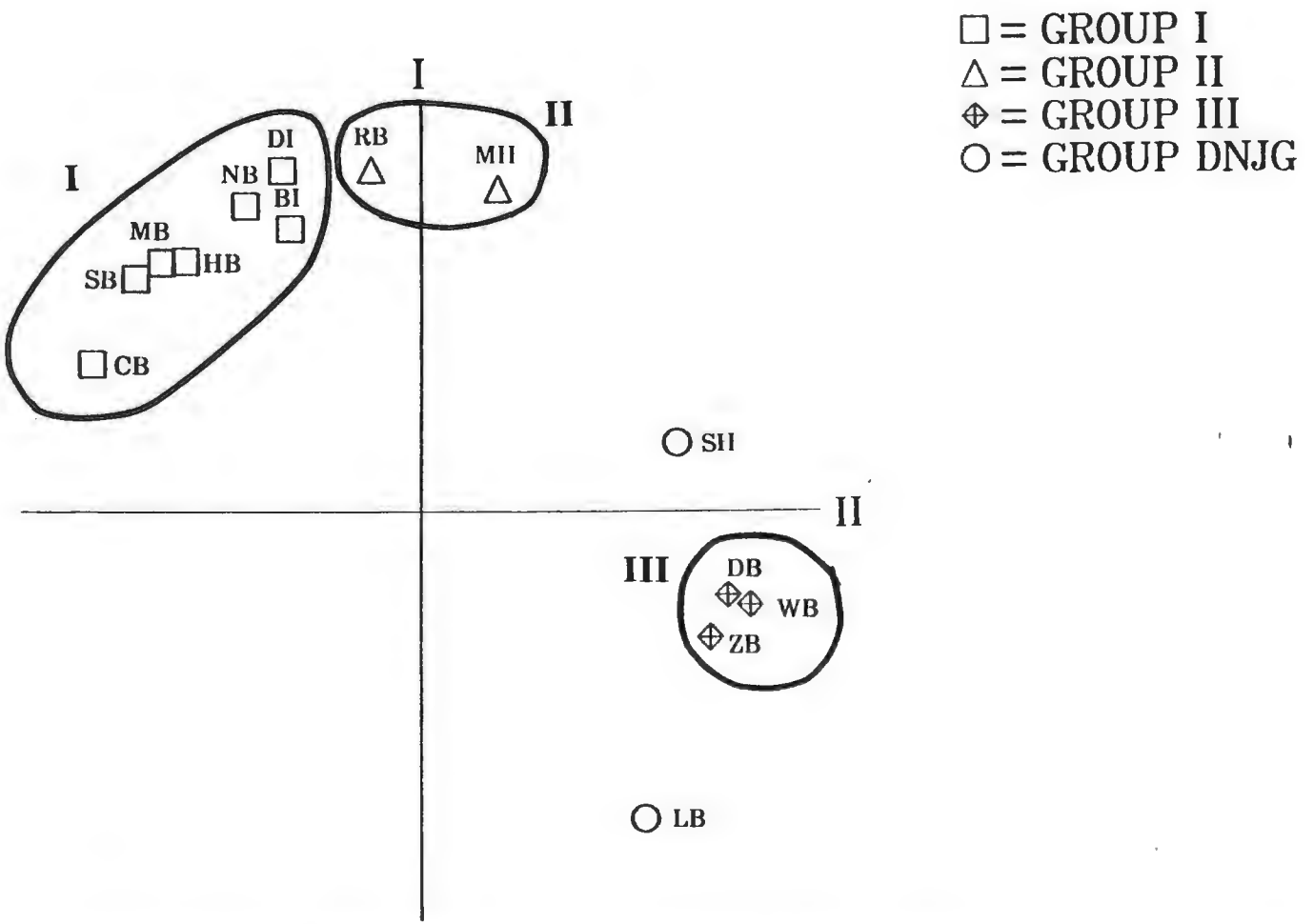

Figure 7. Plot of the first two coordinate axes of a principal coordinate analysis of *ln*-transformed abundance data from 40 m in July 1990 at sites within Prince William Sound.

other groups but that they are most similar to Group III is affirmed in the cluster and PC analyses.

The ranking of dominant taxa in each of the station groups and the percent occurrence of each dominant taxon at stations within the groups (e.g., a value of 100% means that a given taxon occurred at all stations within the groups) is included in Table 9. The differences in taxa dominating at oiled and unoiled stations are apparent in this table. For example, the following groups are most abundant at oiled stations: Nematoda; polychaetes in the Families Ampharetidae, Cirratulidae, Lumbrineridae, Maldanidae, Polyodontidae and Syllidae; the sipunculid group Golfingidae; and the bivalve group Tellinidae. Alternatively, the polychaete Families Nephtyidae, Orbiniidae, Owenidae, Scalibregmidae, Sigalionidae, and Spionidae; and the bivalve Families Nuculidae, Nuculanidae, Lucinidae, and Veneridae are more abundant at unoiled stations. Statistical analyses of abundance of dominant taxa at oiled vs. unoiled sites are presented later in the section on Statistical Assessment of Abundance and Feeding Data.

K-Dominance Curves

The K-Dominance curves for the 14 sites are presented in Figures 8 to 21. The only oiled site for which the curves suggest disturbance is Herring Bay, although the closeness of the curves for Disk Island, Chenega Bay, and Sleepy Bay suggest a mild disturbance. The curves for the other three oiled sites suggest an undisturbed environment. The curves for five of the unoiled sites are far apart suggesting healthy environments, but the curves for Mooselips Bay are suggestive of a disturbed environment.

Feeding Types

The percent of feeding types present at oiled and unoiled sites are presented in Table 10 and Figures 22 and 23. The categories depicted are surface deposit feeders (SDF), subsurface deposit feeders (SSDF), carnivores (CARN), suspension feeders (SF), and others. At the oiled sites SDF are dominant, relative to the other feeding types, at six of the seven stations (the one exception: Snug Harbor). SDF are also common at the unoiled sites, but the other feeding types are almost as important so that a separation of the SDF curve from the other curves is not as obvious as it is for the oiled sites.

Although the abundance of SDF at the pooled 7 oil sites is slightly greater than at the unoiled sites, the difference is not significant (Table 11). However, if a comparison is made between SDF at the six oiled sites (grouped together by cluster analysis) and three unoiled sites (grouped together by cluster analysis, Fig. 6), SDF are highly significantly greater at the oiled sites (Table 12). Likewise, if comparisons between sites chosen by the shallow diving component of the Coastal Habitat study[5] are made, both of the oiled sites have significantly more SDF. The latter comparisons are for Bay of Isles (oiled) vs. Drier Bay (unoiled); Herring Bay (oiled) vs. Lower Herring Bay (unoiled). A comparison presented in last year's Annual Report, Herring Bay (oiled) vs. Zaikof Bay (unoiled), also indicates a significantly ($P=0.037$) higher number of SDF at the oiled site. If SDF and SF are combined (both types of organisms feeding at the benthic boundary layer: McCave, 1974) into a group called Interface Feeders (IF; Josefson, 1985), pooled oil sites have highly significantly more IF than unoiled sites.

Text continued on page 57.

Table 9. Ranking by abundance of dominant taxa within the station groups at 40 m delineated by multivariate analysis of deep benthic data collected in Prince William Sound July 1990. Percent frequency = percentage of stations within the station group.

Dominant Taxa	Abundance (no. m^{-2})	Frequency[1] (%)
Station Group I		
Cirratulidae	477	100
Nematoda	393	100
Bivalvia	287	100
Paraonidae	273	100
Polyodontidae	239	100
Capitellidae	217	100
Syllidae	215	100
Golfingiidae	189	100
Owenidae	184	100
Lumbrineridae	175	100
Maldanidae	160	100
Ampeliscidae	117	100
Sigalionidae	113	100
Mytilidae	93	100
Ophiuroidea	90	100
Harpacticoida	87	100
Phoxocephalidae	74	100
Lepidopleuridae	71	100
Leuconidae	71	100
Thyasiridae	62	100
Spionidae	59	100
Sabellidae	53	100
Polyplacophora	51	71
Nephtyidae	50	100
Ampharetidae	49	100
Onuphidae	48	86
Gnathiidae	41	86
Station Group II		
Owenidae	1564	100
Spionidae	912	100
Capitellidae	502	100
Bivalvia	412	100
Ostracoda	354	100
Paraonidae	327	100
Golfingiidae	250	100
Sternaspidae	237	100
Nematoda	234	100
Magelonidae	226	100
Lumbrineridae	224	100
Maldanidae	215	100
Thyasiridae	197	100

Table 9. (cont'd)

Dominant Taxa	Abundance (no. m^{-2})	Frequency[1] (%)
Cirratulidae	143	100
Nuculidae	130	100
Scaphandridae	118	100
Syllidae	92	100
Gastropoda	84	100
Nephtyidae	82	100
Lucinidae	75	100
Glyceridae	70	100
Rhynchocoela	62	100
Pyramidellidae	58	100
Leuconidae	56	100
Nuculanidae	54	100
Orbiniidae	52	100
Phyllodocidae	50	100
Trichbranchidae	49	100
Dentaliidae	46	100
Ophiuroidea	43	100
Ampharetidae	42	100
Veneridae	41	100
Polynoidae	40	100
Station Group III		
Nephtyidae	251	100
Bivalvia	140	100
Paraonidae	130	100
Nuculanidae	69	100
Spionidae	69	100
Lumbrineridae	60	100
Lucinidae	54	100
Capitellidae	52	100
Cirratulidae	47	100
Nuculidae	47	100
Rhynchocoela	40	100
Tellinidae	36	100
Sigalionidae	31	100
Ostracoda	29	100
Scaphandridae	27	100
Thyasiridae	22	100

[1]The values in this column, for each of the dominant taxa within the station group, are based on the number of stations at which the particular taxon occurs.

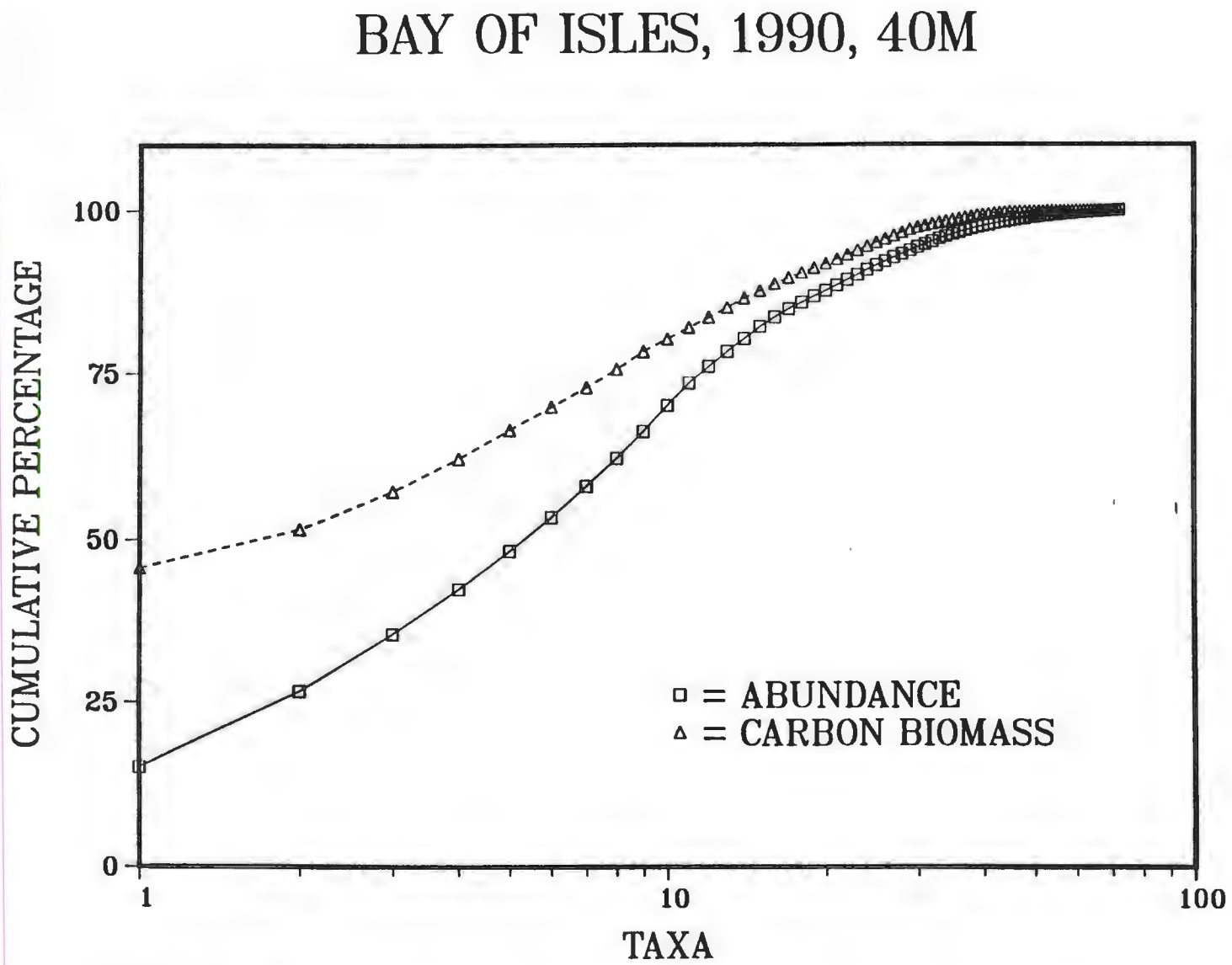

Figure 8. K-dominance curves for benthic organisms collected at 40 m in Bay of Isles in July 1990.

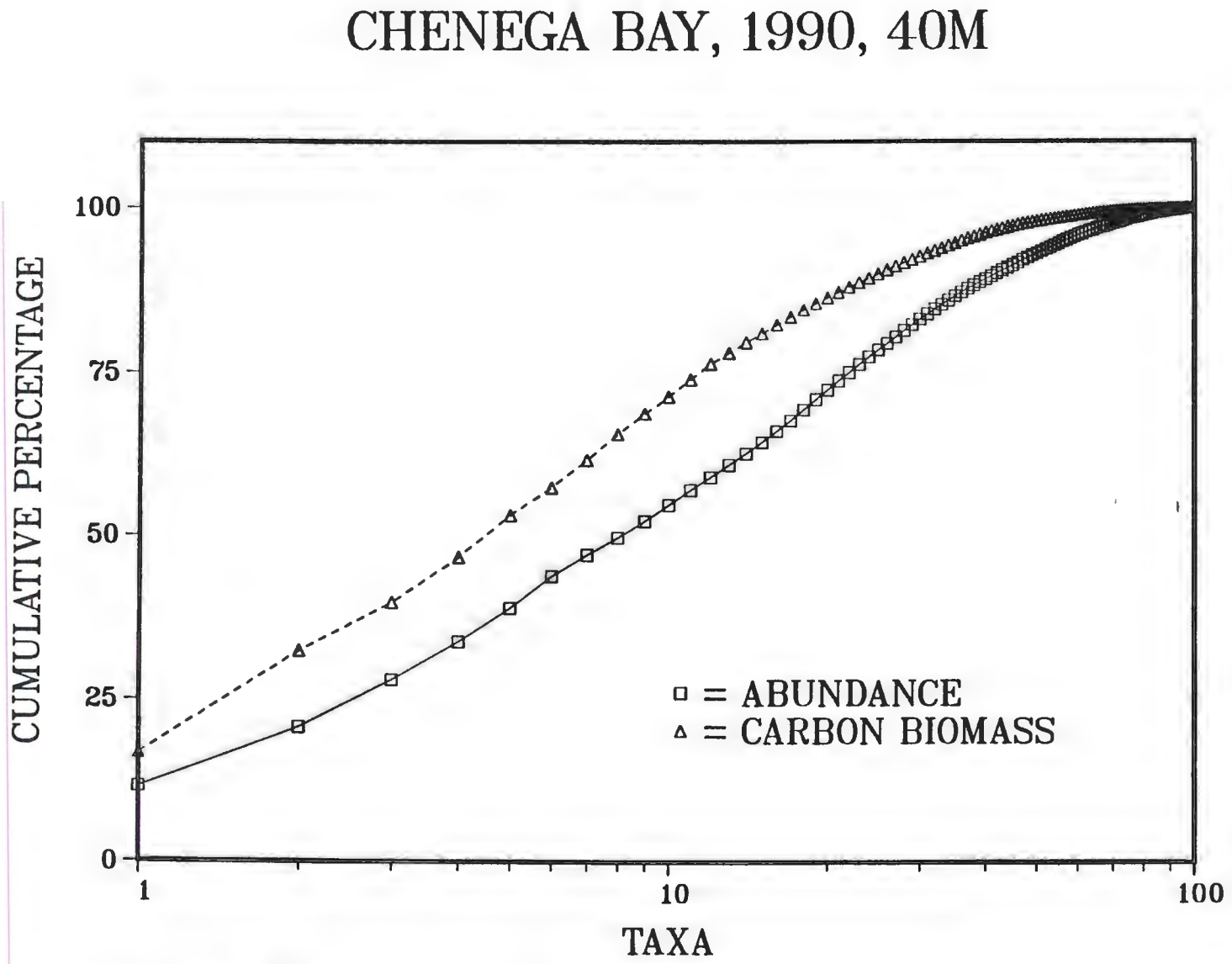

Figure 9. K-dominance curves for benthic organisms collected at 40 m in Chenega Bay in July 1990.

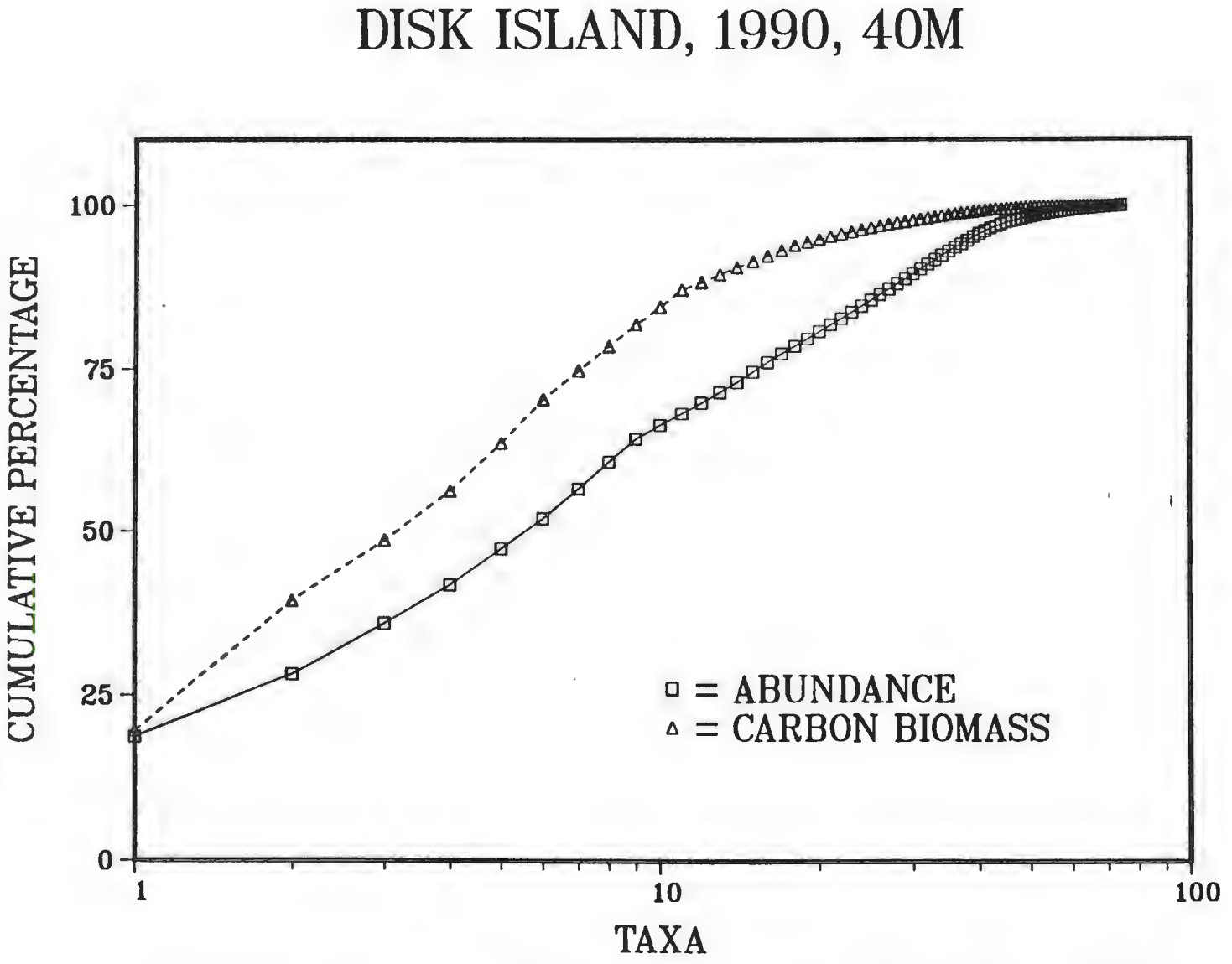

Figure 10. K-dominance curves for benthic organisms collected at 40 m at Disk Island in July 1990.

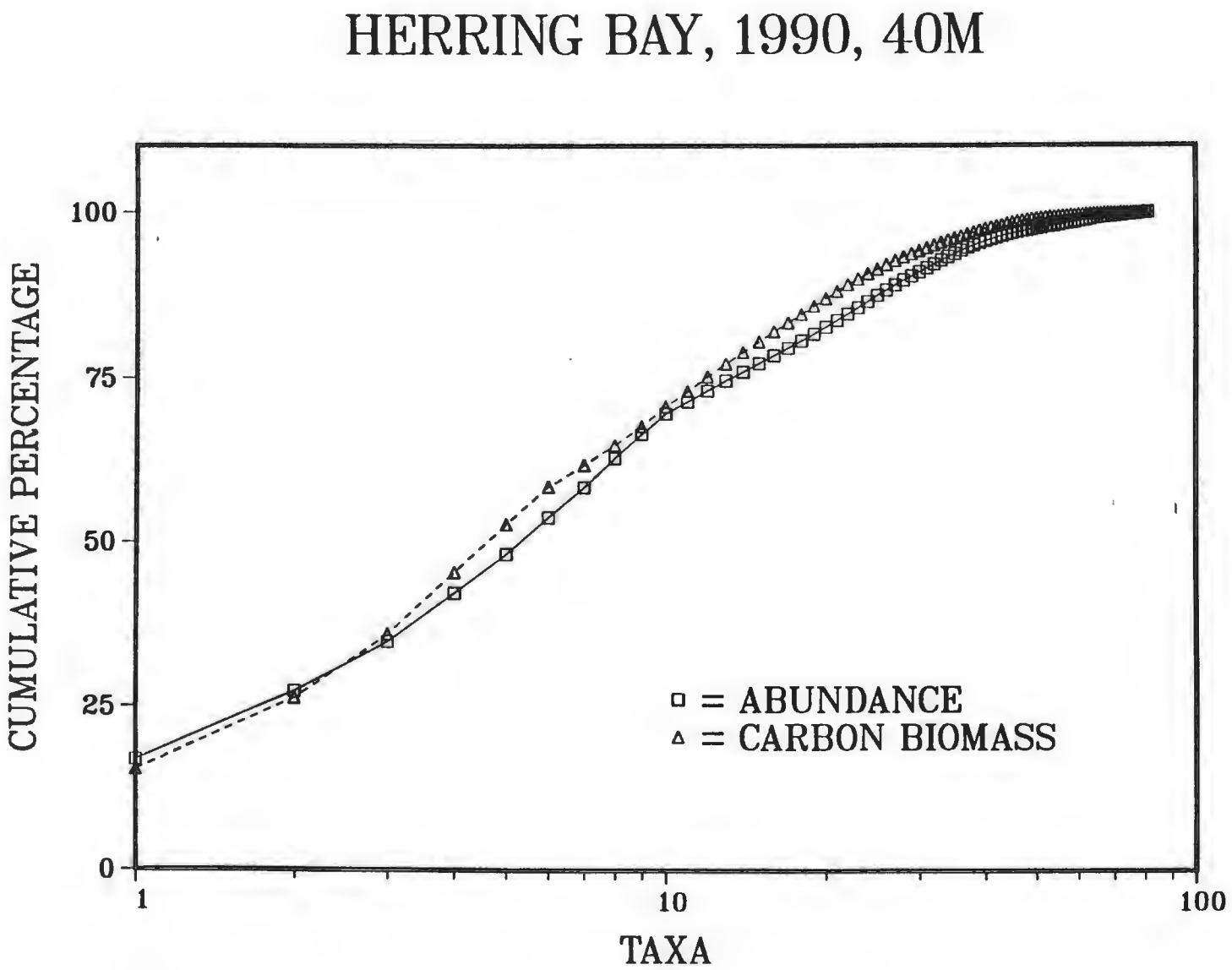

Figure 11. K-dominance curves for benthic organisms collected at 40 m in Herring Bay in July 1990.

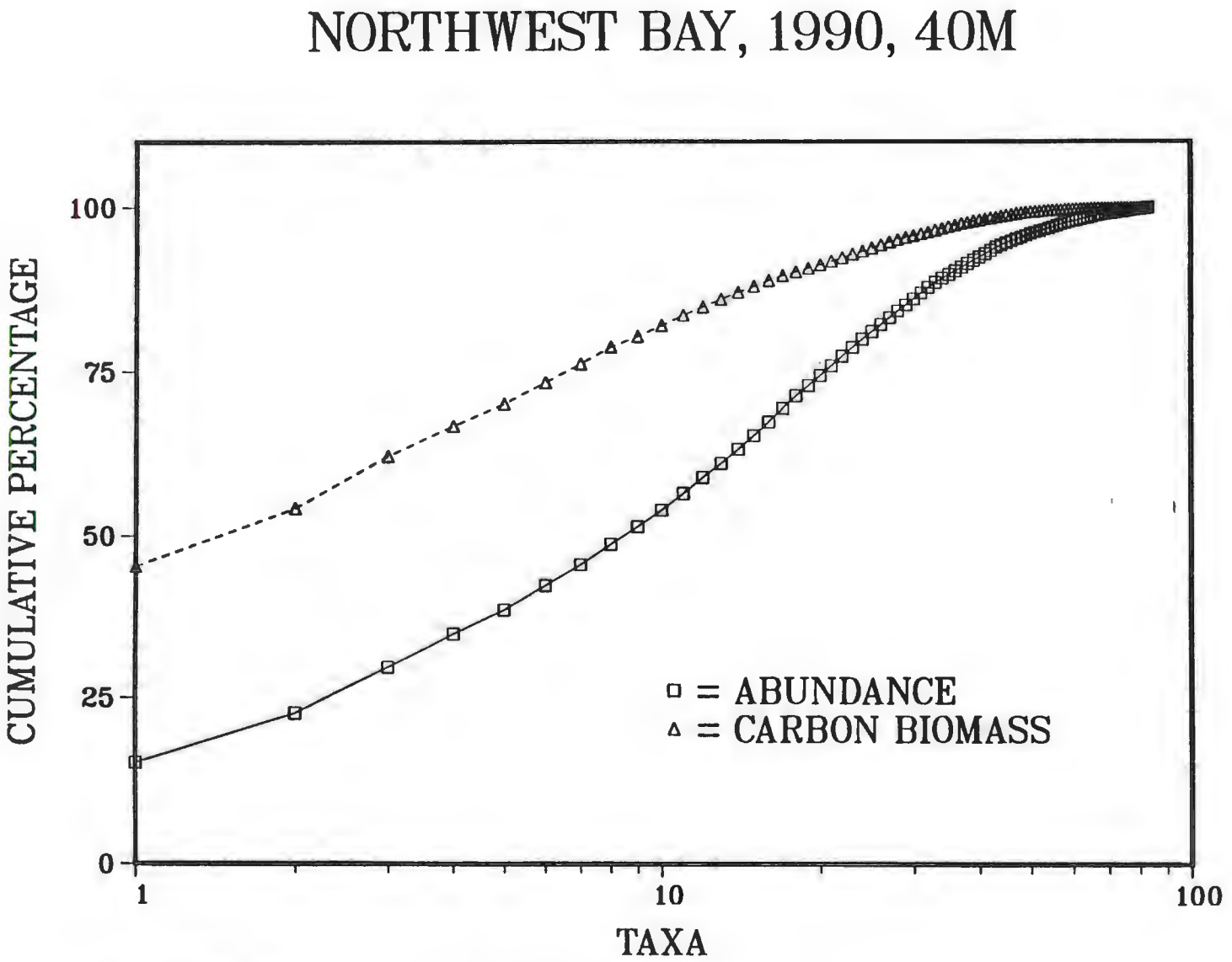

Figure 12. K-dominance curves for benthic organisms collected at 40 m in Northwest Bay in July 1990.

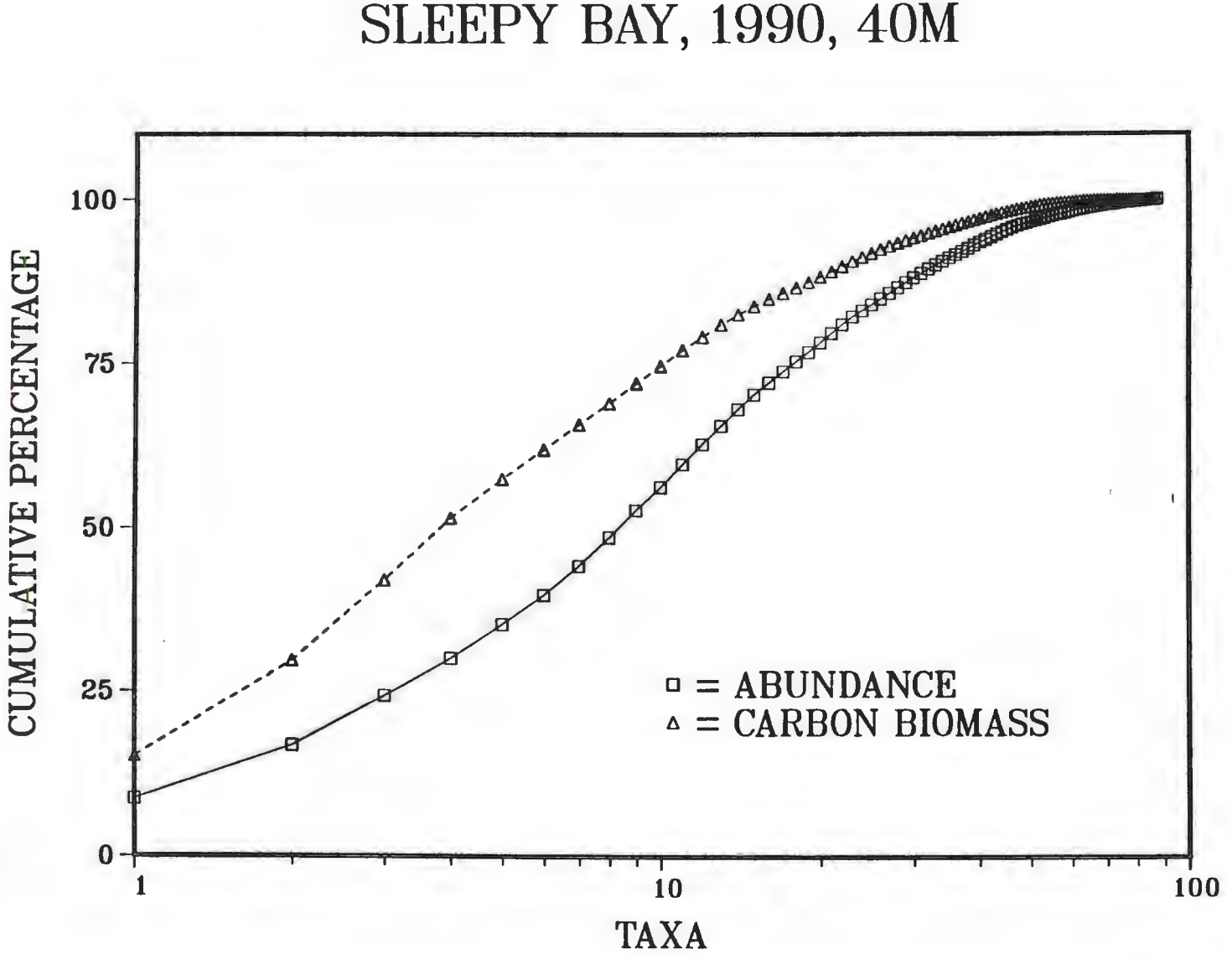

Figure 13. K-dominance curves for benthic organisms collected at 40 m in Sleepy Bay in July 1990.

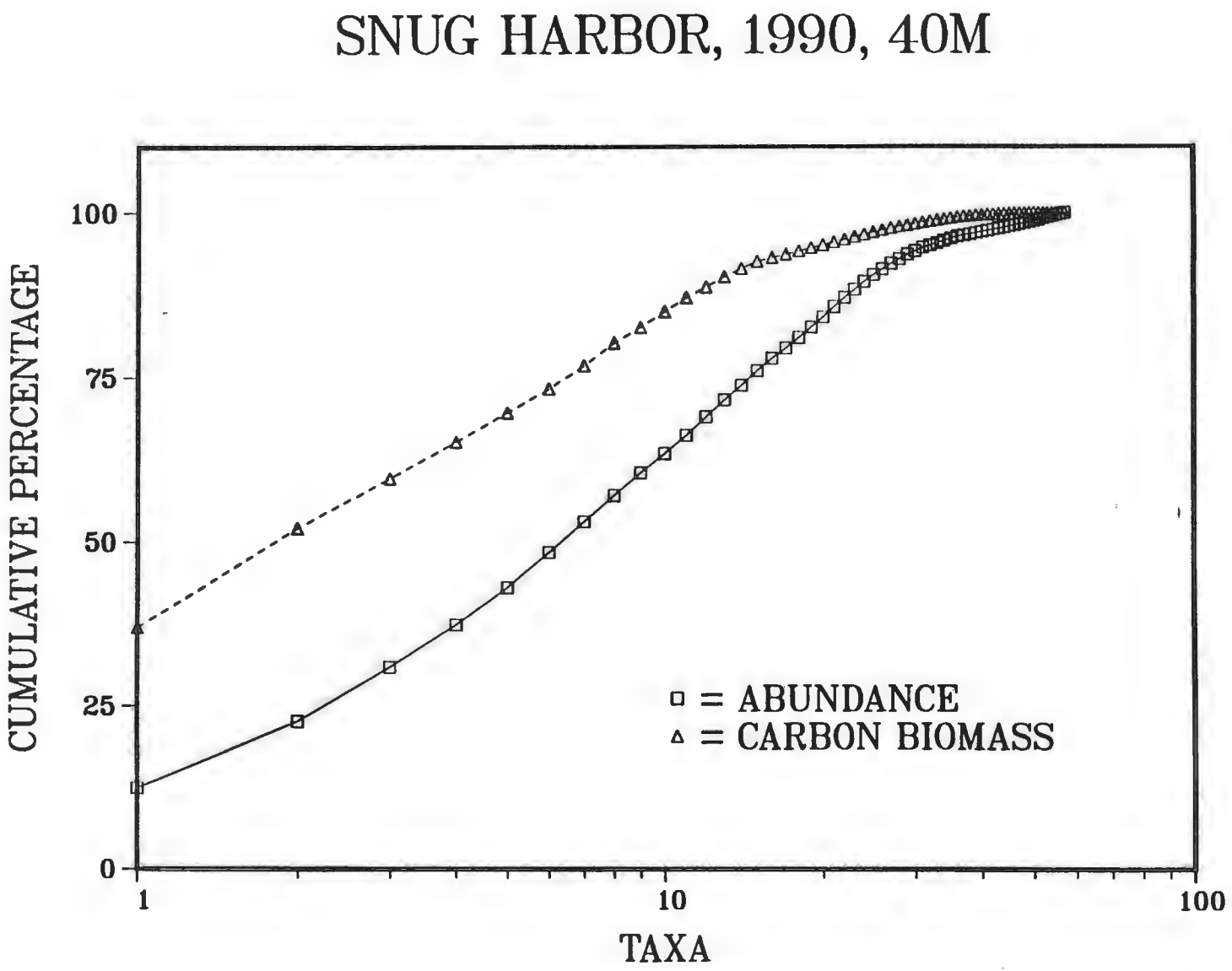

Figure 14. K-dominance curves for benthic organisms collected at 40 m in Snug Harbor in July 1990.

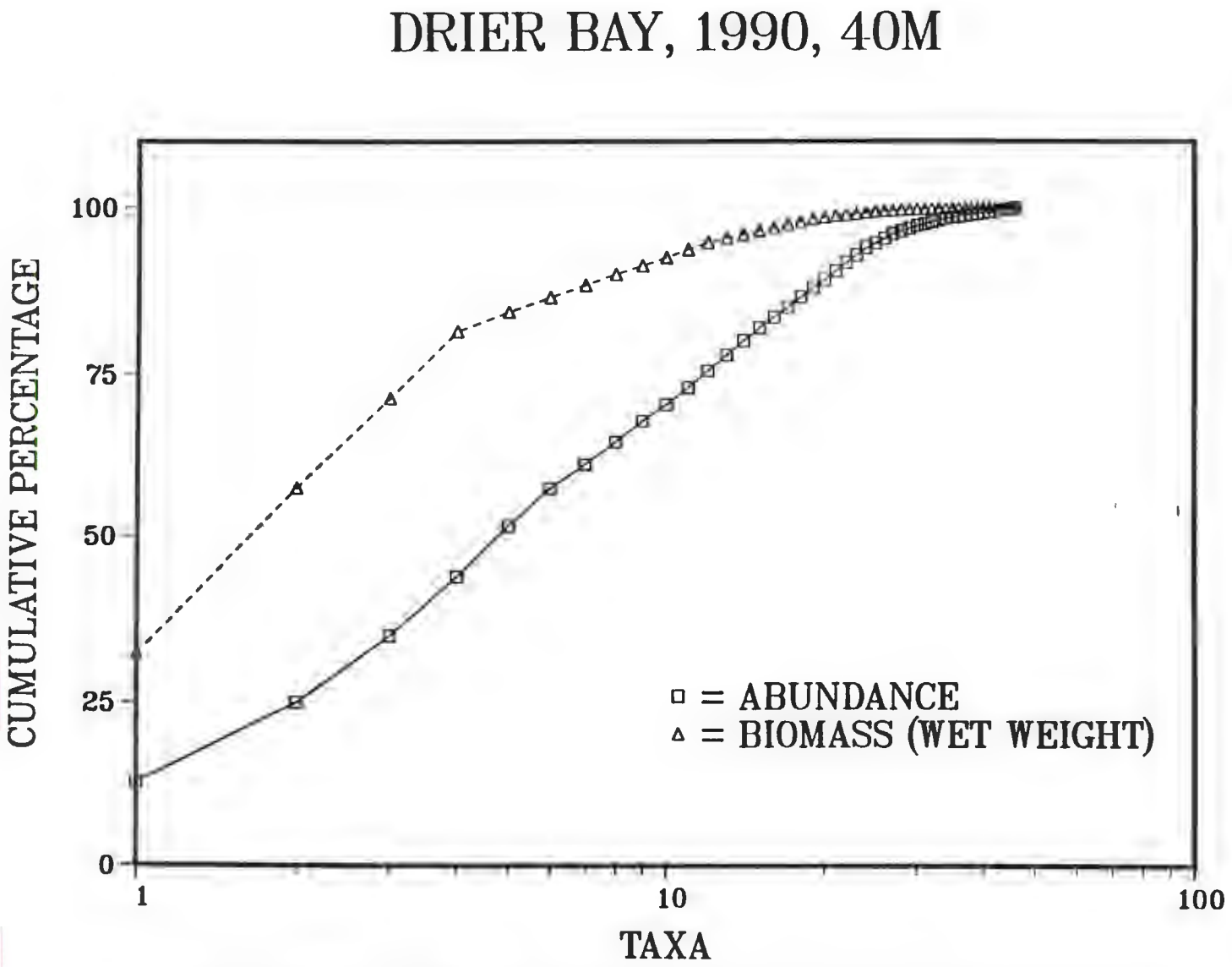

Figure 15. K-dominance curves for benthic organisms collected at 40 m in Drier Bay in July 1990.

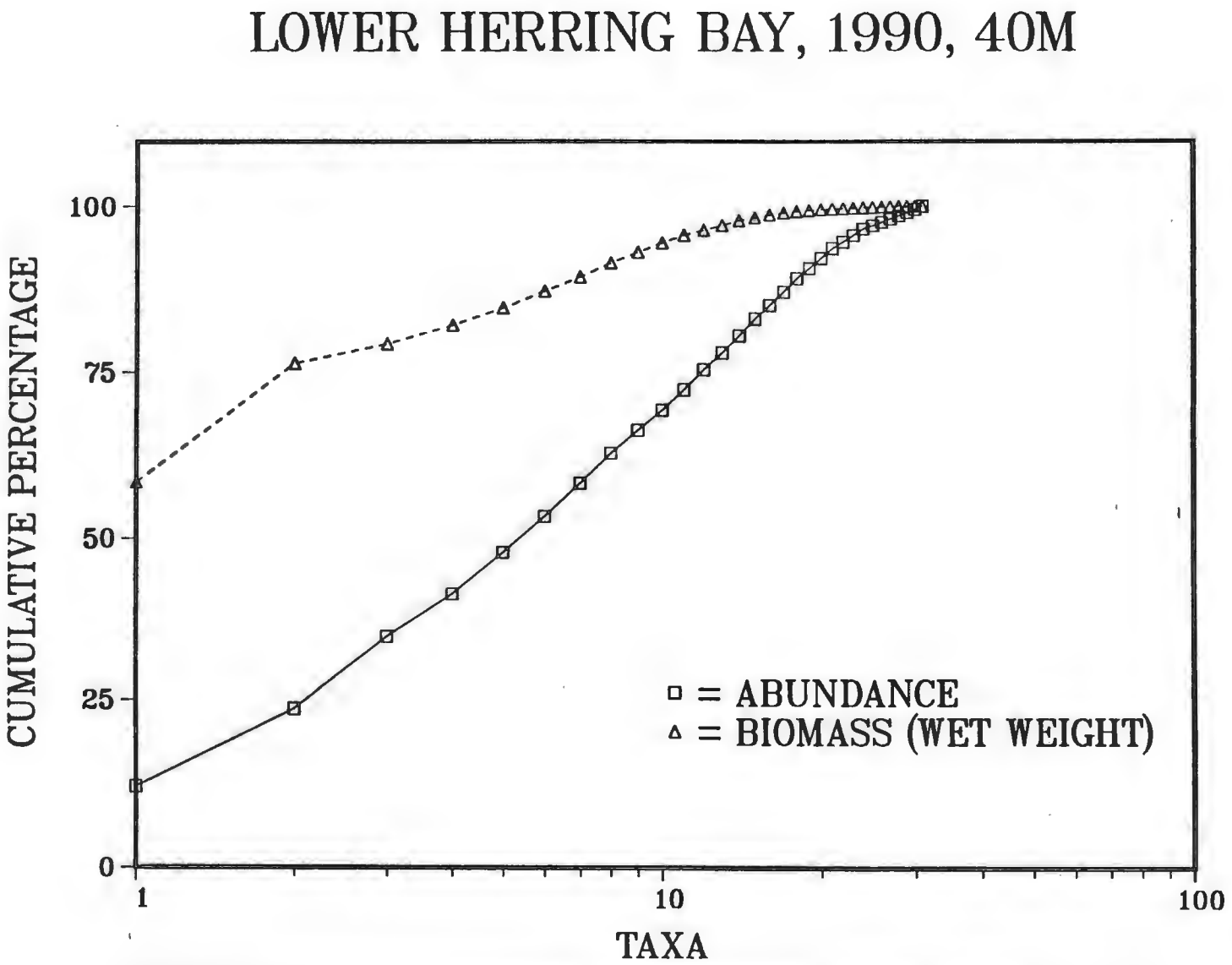

Figure 16. K-dominance curves for benthic organisms collected at 40 m in Lower Herring Bay in July 1990.

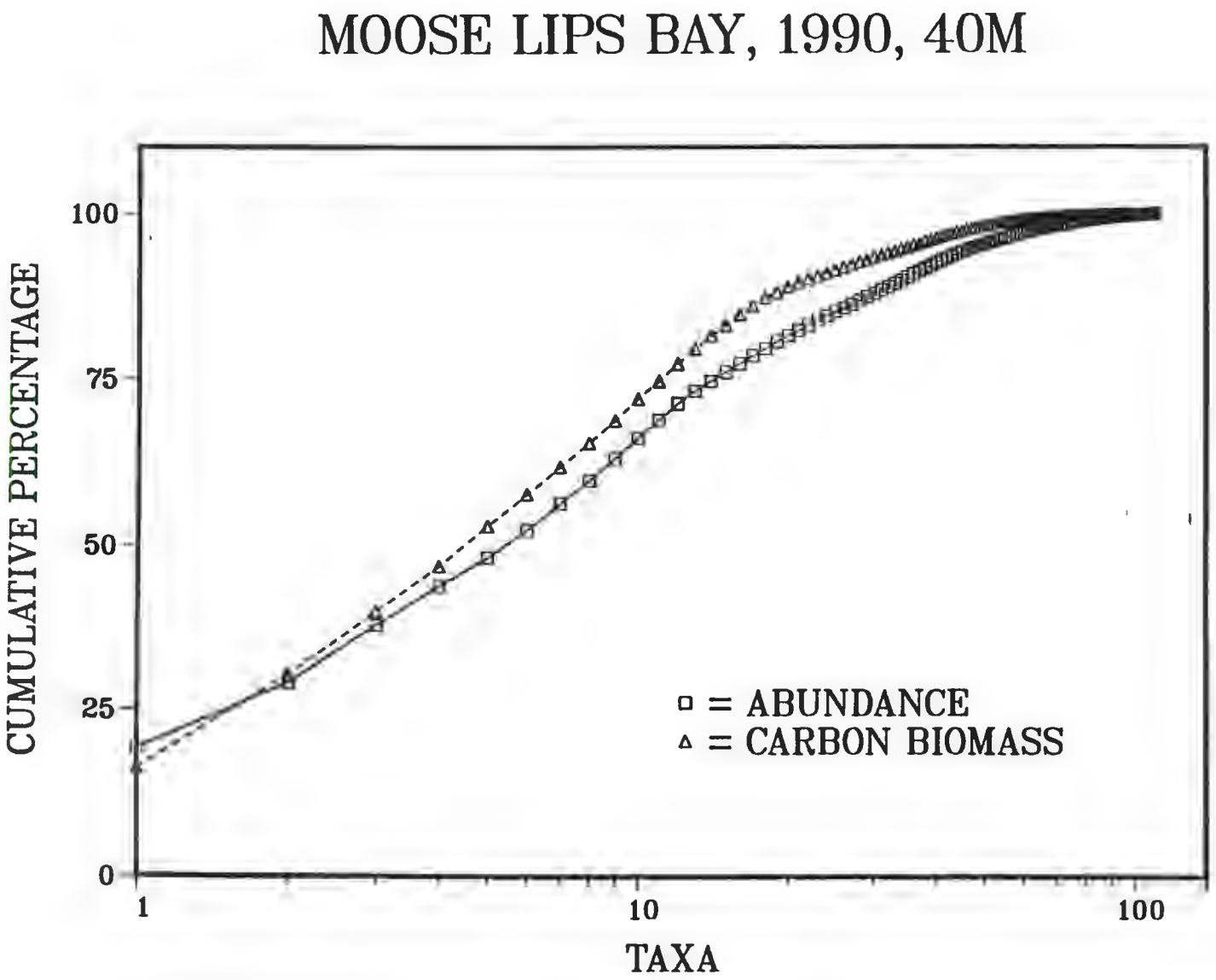

Figure 17. K-dominance curves for benthic organisms collected at 40 m in Mooselips Bay in July 1990.

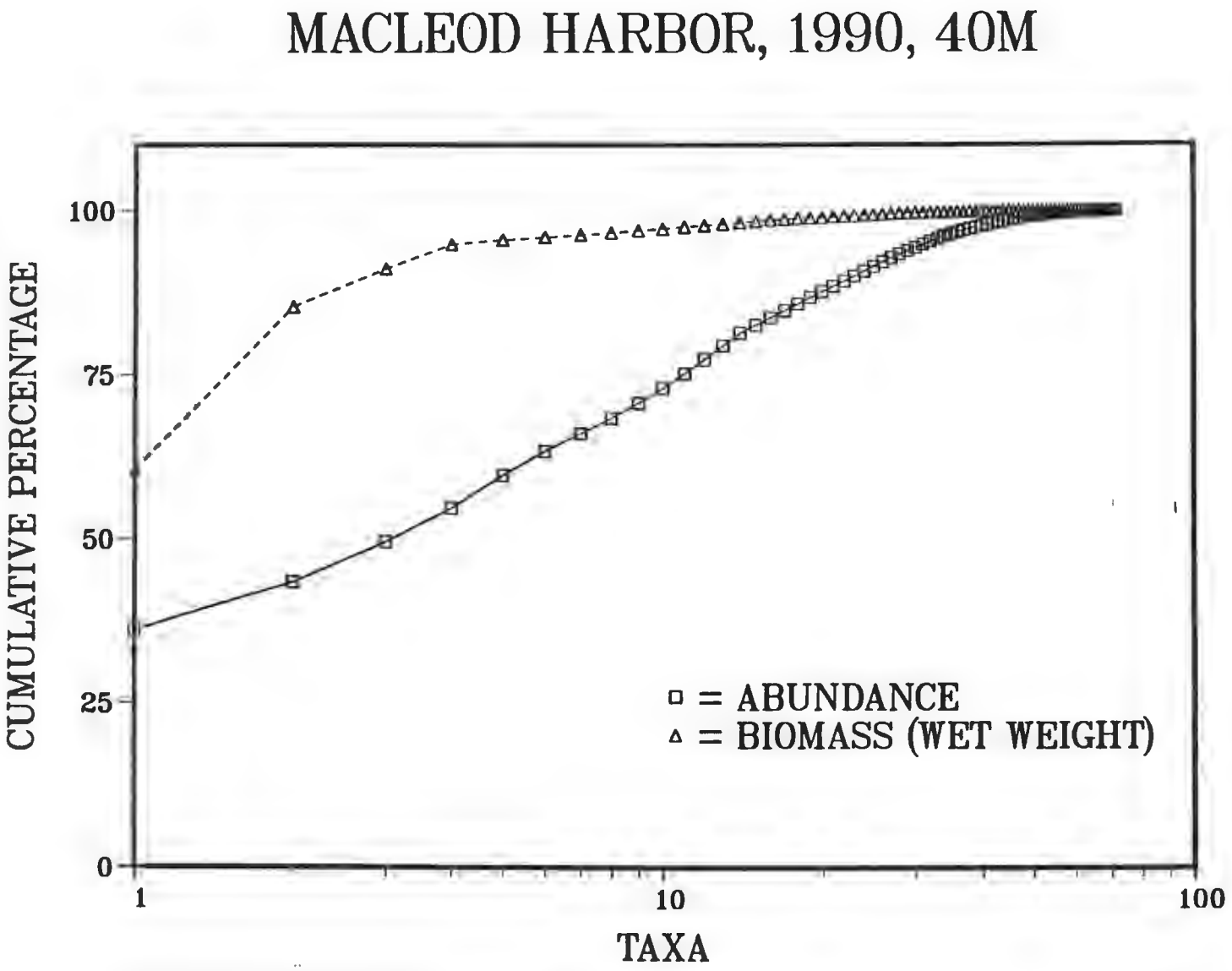

Figure 18. K-dominance curves for benthic organisms collected at 40 m in MacLeod Harbor in July 1990.

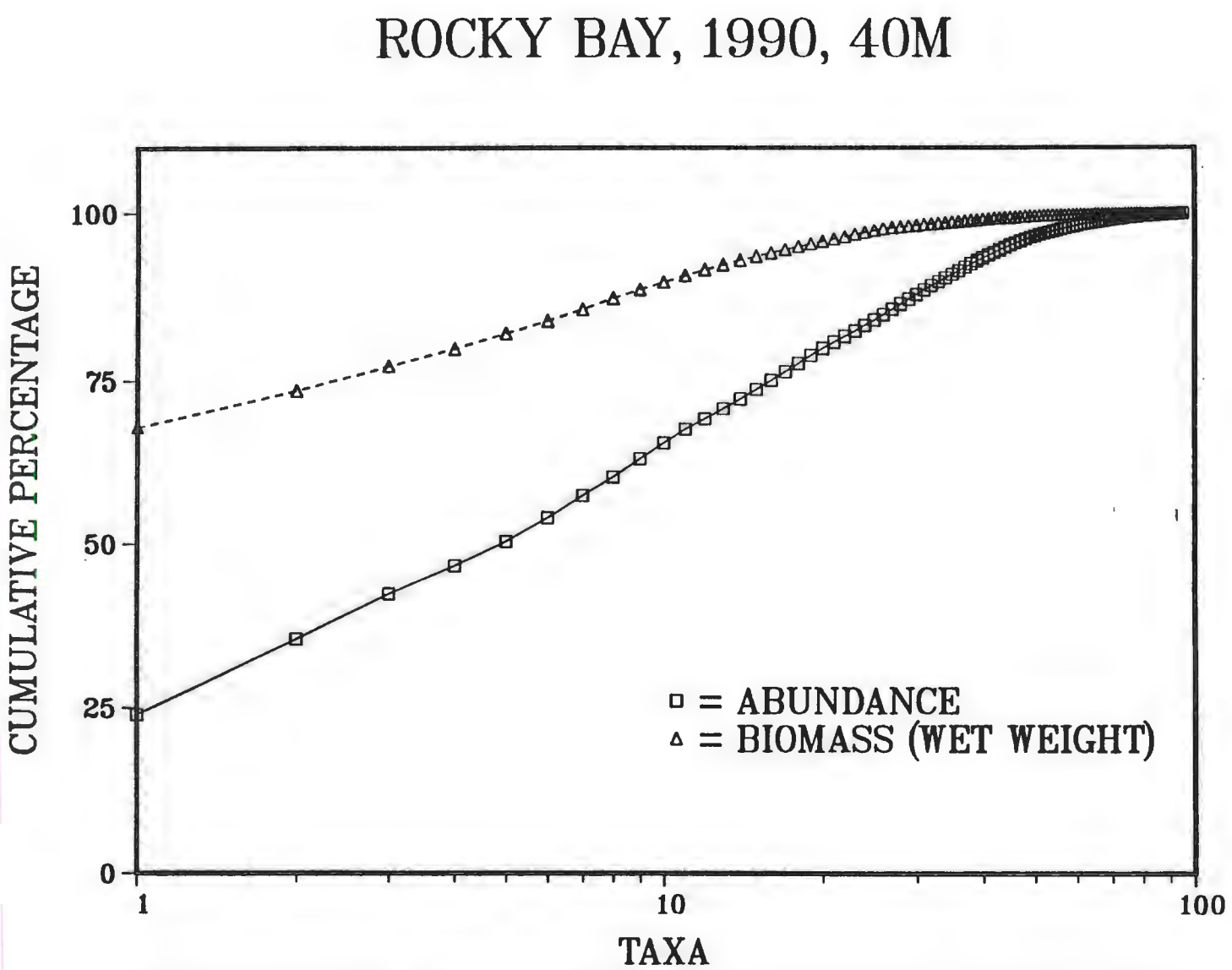

Figure 19. K-dominance curves for benthic organisms collected at 40 m in Rocky Bay in July 1990.

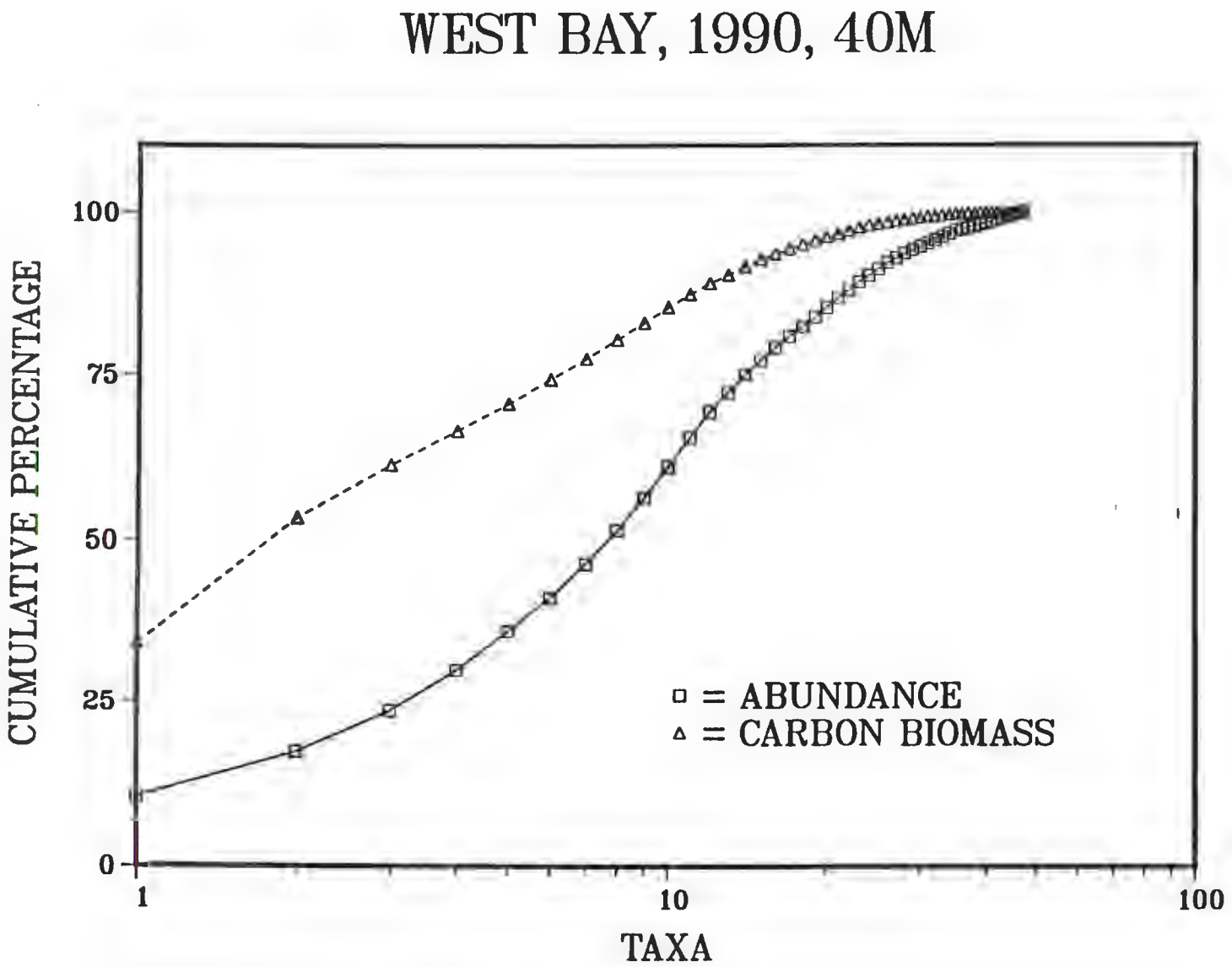

Figure 20. K-dominance curves for benthic organisms collected at 40 m in West Bay in July 1990.

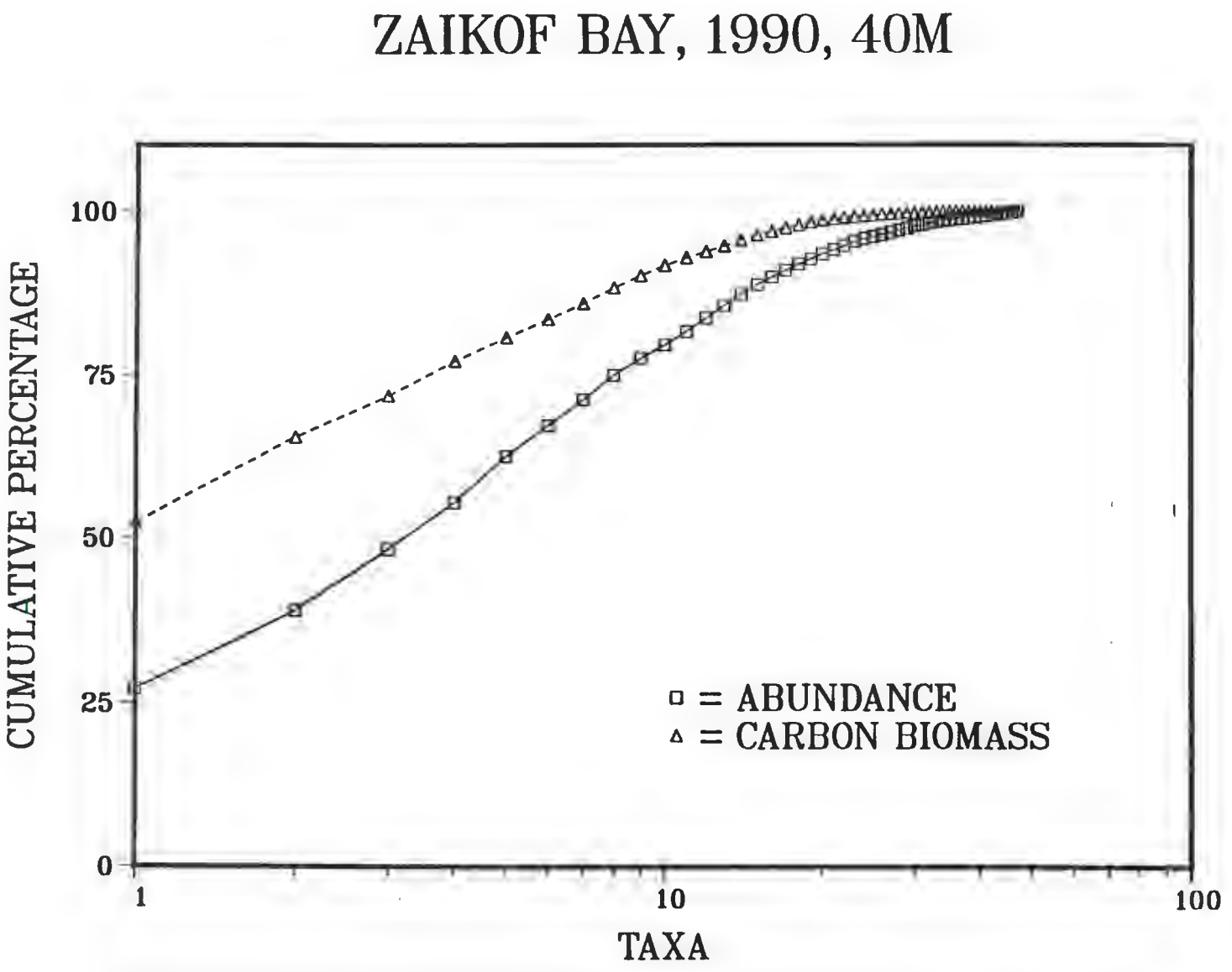

Figure 21. K-dominance curves for benthic organisms collected at 40 m in Zaikof Bay in July 1990.

Table 10. The percent of individuals (by abundance) at each of five feeding categories at 40, 100, and >100 m at sites occupied in Prince William Sound in July 1990.

SITE	DEPTH	SDF	SSDF	CARN	SF	OTHERS
BI	40M	38.53%	21.97%	15.26%	7.36%	16.88%
CB	40M	32.20%	7.84%	17.25%	18.66%	24.06%
DB	40M	27.83%	24.56%	23.04%	16.64%	7.96%
DI	40M	45.32%	13.78%	13.82%	12.58%	14.51%
HB	40M	41.62%	13.01%	16.08%	9.58%	19.72%
LH	40M	29.67%	31.28%	17.84%	12.32%	8.89%
MB	40M	49.60%	14.87%	8.52%	14.53%	12.49%
MH	40M	34.71%	28.88%	9.59%	18.56%	8.27%
NB	40M	45.36%	13.46%	13.49%	12.70%	15.00%
RB	40M	37.73%	23.07%	10.06%	20.62%	8.53%
SB	40M	30.22%	14.56%	13.95%	20.02%	21.26%
SH	40M	31.45%	33.76%	10.82%	13.32%	10.65%
WB	40M	23.94%	32.29%	23.60%	8.94%	11.25%
ZB	40M	28.75%	15.70%	36.64%	10.30%	8.62%
BI	100M	42.88%	8.83%	20.40%	20.48%	7.41%
CB	100M	39.88%	10.76%	13.93%	19.11%	16.32%
DB	100M	27.31%	25.00%	29.15%	9.95%	8.61%
DI	100M	44.11%	20.70%	10.10%	16.51%	8.59%
HB	100M	44.82%	15.42%	15.31%	8.88%	15.57%

Table 10. (cont'd)

LH	100M	44.50%	16.01%	21.24%	9.40%	8.86%
MB	100M	34.64%	23.60%	12.67%	14.56%	14.53%
MH	100M	36.99%	20.09%	11.65%	16.20%	15.08%
NB	100M	41.92%	22.86%	9.71%	17.63%	7.91%
RB	100M	20.41%	27.06%	28.90%	13.67%	9.96%
SB	100M	41.48%	10.01%	15.74%	15.08%	17.70%
SH	100M	35.77%	13.79%	24.07%	9.44%	16.94%
WB	100M	34.57%	20.53%	17.06%	16.38%	11.49%
ZB	100M	33.34%	31.73%	12.10%	12.17%	10.67%
BI	>100M	34.03%	24.78%	18.35%	11.97%	10.89%
CB	>100M	42.78%	9.03%	15.14%	19.51%	13.54%
DB	>100M	23.19%	40.98%	28.81%	3.42%	3.61%
HB	>100M	50.28%	15.55%	10.24%	14.08%	9.86%
LH	>100M	36.60%	13.43%	31.71%	6.48%	11.78%
MH	>100M	34.34%	27.68%	8.80%	19.31%	9.87%
NB	>100M	55.49%	8.34%	10.29%	15.83%	10.06%
RB	>100M	50.54%	15.50%	8.49%	18.35%	7.12%
SB	>100M	41.85%	10.12%	12.36%	23.38%	12.29%
WB	>100M	28.76%	21.60%	24.36%	11.86%	13.44%
ZB	>100M	37.77%	12.80%	11.59%	18.49%	19.36%

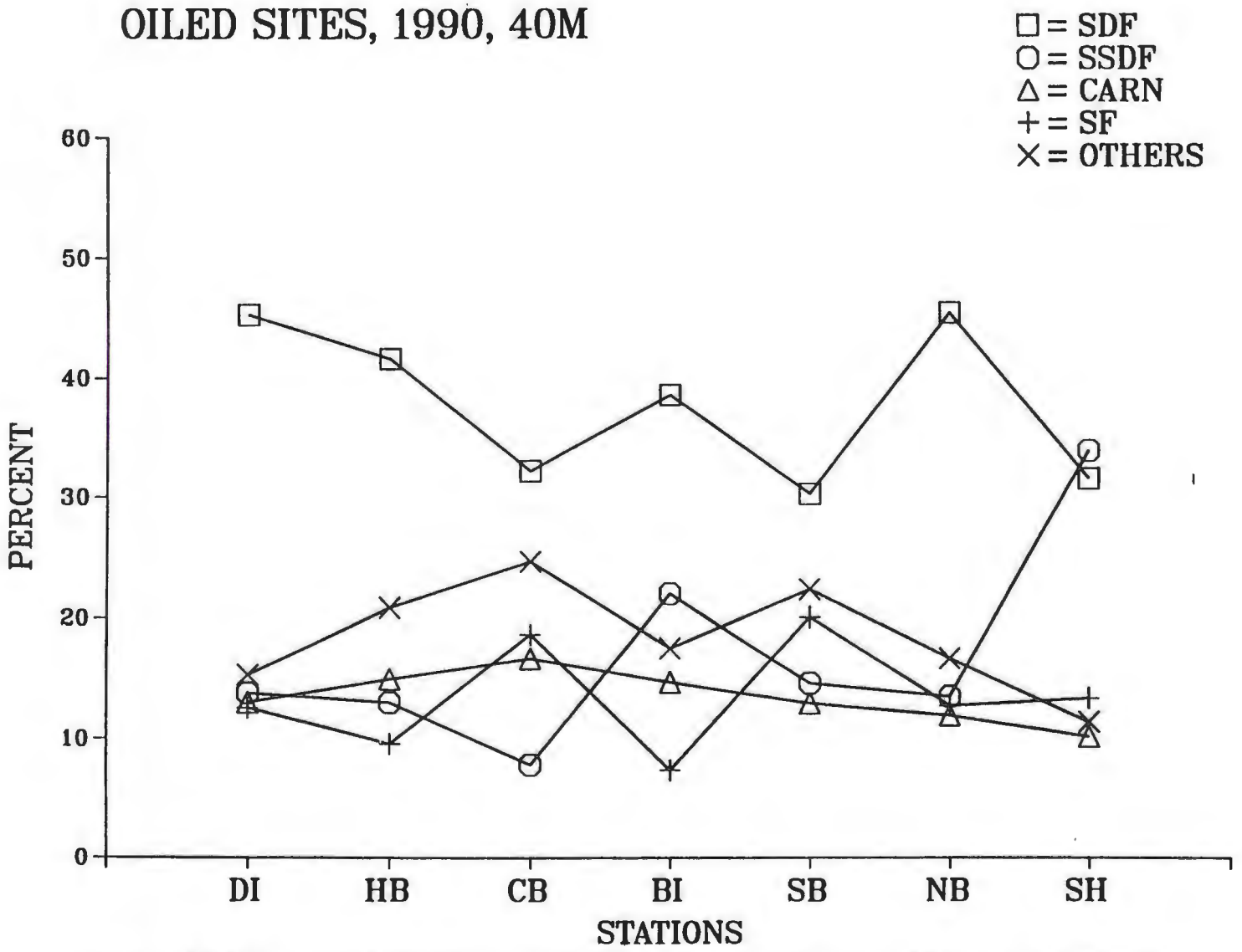

Figure 22. The percent abundance of feeding types at stations at 40 m at oiled sites from data collected July 1990 in Prince William Sound. SDF = surface deposit feeders; SSDF = subsurface deposit feeders; CARN = predators; SF = suspension feeders; Others = scavengers and herbivores.

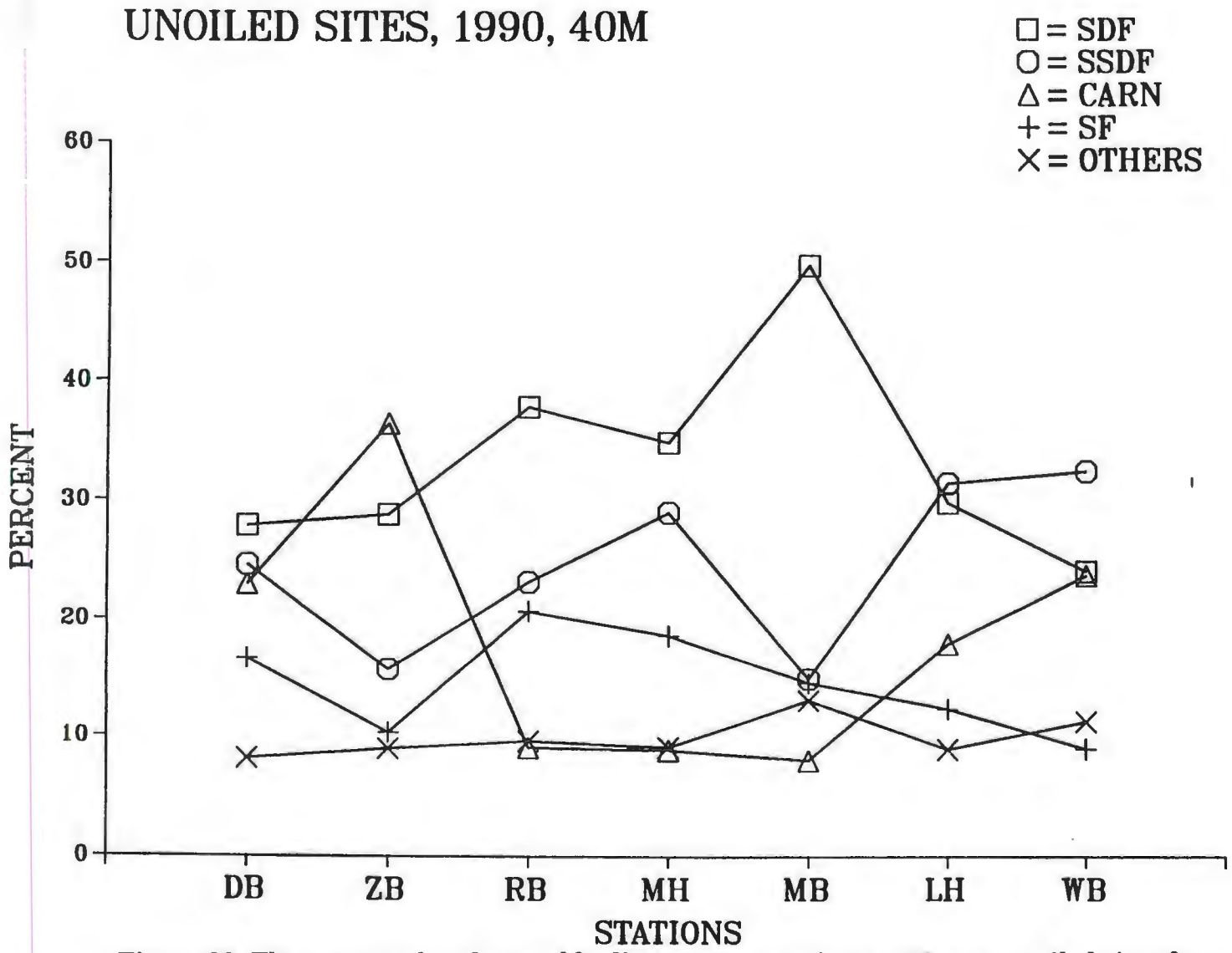

Figure 23. The percent abundance of feeding types at stations at 40 m at unoiled sites from data collected July 1990 in Prince William Sound. SDF = surface deposit feeders; SSDF = subsurface deposit feeders; CARN = predators; SF = suspension feeders; Others = scavengers and herbivores.

Additionally, all other bay comparisons indicate that IF are significantly more abundant at oiled sites. Further, if SDF are combined with another feeding type, "Other" (Scavengers and Herbivores), the abundance of these combined feeding types at the pooled seven oiled sites is significantly greater ($P=0.019$) than at the seven unoiled sites (Table 11). All the significantly more abundant feeding groups noted above are ones to be expected in an area adjacent to an oil-impacted shore: i.e., SDF – utilizing detrital debris resulting from oil mortality; IF – also using detrital debris in water interface; Others – utilizing debris from oil-related mortality in the intertidal and shallow subtidal above.

Amphipod Composition at Stations

Amphipods were generally more common at oiled sites, with the exception of Mooselips Bay[5] (Table 13a). Phoxocephalids and tube-dwelling ampeliscids (both primarily SDF; ampeliscids may also be SF and can thus be termed IF) are the dominant amphipods present (Figs. 24 and 25; Table 13a). All of the oiled sites had ampeliscids present, although in low numbers. However, the very low values for total amphipods at the oiled station within Snug Harbor suggests that this site has not recovered to the same extent as the other oiled sites. Since amphipods (in particular, ampeliscids) are susceptible to toxic oil fractions (Dauvin, 1982; Kineman *et al.*, 1980), the common occurrence of amphipods at most oiled sites indicates that toxic oil components are no longer present, organic material is available as food (as noted in above section on feeding types), and recovery is obviously taking place. Only one unoiled site, Mooselips Bay, had high numbers of ampeliscids. As suggested previously, this site appears different than the other unoiled sites, and its identification as a very dynamic site with considerable exposure to wave turbulence is verified by the large numbers of ampeliscids present. The other unoiled sites either had relatively few or none of these amphipods. Thus, another statistical comparison of abundance values of amphipods at oiled vs. unoiled sites is presented in Table 13b. For the reasons noted above, Snug Harbor (oiled site) and Mooselips Bay (unoiled site) were deleted from the analysis (i.e., they differ markedly from other oiled and unoiled sites, respectively. All amphipod categories are significantly ($P>0.021$) higher at the oiled sites in comparison with the unoiled sites.

Statistical Assessment of Taxon Abundance and Biomass

Tests of significance (ANOVA) for transformed abundance/biomass values (for higher taxa) for the combined 0.5 and 1.0 mm fractions at the 40 m stations at oiled vs unoiled sites are presented in a series of tables (Tables 14 - 22). A variety of comparisons were made, and the results are summarized below.

[5] Since the shallow diving component of the Coastal Habitat Study was not initially tied to the deep benthic program, two of the oiled and unoiled sites that group chose were fortuitously the same ones we had chosen.

Text continued on page 81.

Table 11. Abundance, as square root (abundance +0.5), at 40 m, 100 m, and >100 m of surface deposit feeders (SDF), interface feeders (INT), subsurface deposit feeders (SSDF), others (OTH), and surface deposit feeders + others (SDF + OTH) at oiled bays (Bay of Isles, Chenega Bay, Disk Island, Herring Bay, Northwest Bay, Sleepy Bay and Snug Harbor for 40 m and 100 m; and Bay of Isles, Chenega Bay, Herring Bay, Northwest Bay and Sleepy Bay for 150 m) and unoiled bays (Drier Bay, Lower Herring Bay, MacLeod Harbor, Mooselips Bay, Rocky Bay, West Bay and Zaikof Bay for 40 m and 100 m; and Drier Bay, Lower Herring Bay, MacLeod Harbor, Rocky Bay, West Bay and Zaikof Bay for >100 m). Means, X, standard deviations, SD, and t values with associated levels of significance, p. Values of t preceded by a minus sign indicate decreases in mean abundance for the unoiled bays when compared to the oiled bays. Probability levels of "ns" indicate that the difference in mean values is not significant (p>0.10).

		Oiled Bays		Unoiled Bays			
Depth	Feeding Type	$\bar{X}$	SD	$\bar{X}$	SD	t	p
40 M	SDF	11.517	4.339	10.429	6.741	-0.803	ns
100 M	SDF	11.576	4.286	8.383	3.174	-3.543	0.001
150 M	SDF	1.829	0.403	1.949	0.457	1.023	ns
40 M	INT	13.463	4.860	12.565	7.998	-0.568	ns
100 M	INT	13.528	4.888	9.911	3.689	-3.494	0.001
150 M	INT	14.085	4.764	10.678	7.282	-2.013	0.049
40 M	SSDF	7.292	2.950	8.315	4.576	1.112	ns
100 M	SSDF	6.708	2.427	7.024	2.363	0.551	ns
150 M	SSDF	6.170	1.999	6.348	2.988	0.254	ns
40 M	OTH	7.292	2.950	8.315	4.576	1.112	ns
100 M	OTH	6.708	2.427	7.024	2.363	0.551	ns
150 M	OTH	6.170	1.999	6.348	2.988	0.254	ns
40 M	SDF+OTH	9.889	4.239	7.862	5.765	-2.369	0.019
100 M	SDF+OTH	9.119	4.885	6.630	3.156	-3.580	<0.000
150 M	SDF+OTH	8.861	4.530	6.776	5.139	-2.235	0.027

Table 12. Abundance, as square root (abundance +0.5), at 40 m, 100 m, and >100 m of surface deposit feeders (SDF) and interface feeders (INT) at oiled bays (Bay of Isles, BI, Chenega Bay, CB, Disk Island, DI, Herring Bay, HB< Northwest Bay, NB, and Sleepy Bay, SB) and unoiled bays (Drier Bay, DB, West Bay, WB, and Zaikof Bay, ZB). Means, X, standard deviations, SD, and t values with associated levels of significance, p. Values of t preceded by a minus sign indicate decreases in mean abundance for the unoiled bays when compared to the oiled bays. Probability levels of "ns" indicate that the change in mean values is not significant (p>0.10).

				Oiled Bays		Unoiled Bays			
Depth	Feeding Type	Oiled - Unoiled Bays		$\bar{X}$	SD	$\bar{X}$	SD	t	p
40 M	SDF	BI+CB+DI+ HB+NB+SB	DB+WB+ZB	12.613	3.348	5.831	2.308	-7.034	<0.000
		BI	DB	14.681	2.212	6.148	2.044	-4.148	<0.000
		HB	LHB	11.616	5.695	3.126	1.780	-4.126	<0.001
		HB	ZB	11.616	5.695	7.011	2.871	-2.238	<0.037
100 M	SDF	BI	DB	11.353	3.389	6.456	2.546	-2.409	0.026
		HB	LHB	14.238	3.921	8.959	3.830	-2.598	0.017
		HB	ZB	14.238	3.921	8.439	1.903	-2.853	0.010
150 M	SDF	HB	LHB	15.321	3.797	4.563	0.467	-6.939	<0.000
		HB	ZB	15.321	3.797	14.142	1.842	-0.760	ns
40 M	INT	BI+CB+DI+ HB+NB+SB	DB+WB+ZB	14.690	3.770	7.015	2.723	-7.006	<0.000
		BI	DB	15.979	2.731	7.795	2.413	-3.574	0.002
		HB	LHB	12.924	6.200	3.857	1.709	-3.959	0.001
		HB	ZB	12.924	6.200	8.181	3.302	-2.071	0.052
100 M	INT	BI	DB	13.804	4.061	7.482	3.120	-2.752	0.012
		HB	LHB	15.601	4.198	9.819	4.317	-2.517	0.020
		HB	ZB	15.601	4.198	9.909	1.866	-2.478	0.022
150 M	INT	HB	LHB	17.285	4.513	4.951	0.368	-6.817	<0.000
		HB	ZB	17.285	4.513	17.277	2.012	-0.000	ns

Table 13a. Total abundance of amphipods and abundance of ampeliscid and phoxocephalid amphipods at 40 m for six oiled and six unoiled stations in Prince William Sound in July 1990. Snug Harbor is deleted from the analysis of oiled sites. Mooselips Bay is deleted from the analysis of unoiled sites. See text for explanation. O = Oiled; UN = Unoiled; DI = Disk Island; HB = Herring Bay; CB = Chenega Bay; BI = Bay of Isles; SB = Sleepy Bay; NB = Northwest Bay; SH = Snug Harbor; DB = Drier Bay; ZB = Zaikof Bay; RB = Rocky Bay; MH = MacLeod Harbor; LH = Lower Hering Bay; WB = West Bay.

Sites	Total Amphipods	Ampeliscid Amphipods	Phoxocephalid Amphipods
DI (O)	98	36	54
HB (O)	62	16	40
CB (O)	46	10	12
BI (O)	130	4	104
SB (O)	146	48	26
NB (O)	84	26	38
SH (O)	16	2	10
$\bar{x}$ (minus SH)	79	23	48
DB (UN)	10	0	0
ZB (UN)	16	0	14
RB (UN)	140	30	16
MH (UN)	34	4	18
MB (UN)	1008	678	244
LH (UN)	0	0	0
WB (UN)	0	0	0
$\bar{x}$ (minus MB)	33	6	8
Comparison P value	>oiled site P=0.021	>oiled site P=0.025	>oiled site P=0.006

Table 13b. Total number of amphipods as number = square root (number + 0.5) at oiled sites (minus Snug Harbor) and unoiled sites (minus Mooselips Bay) for total amphipods, total ampeliscid amphipods, and phoxocephalid amphipods. Means, standard deviations, SD, t values, and probability levels. A T value preceded by a minus sign indicates a decrease in total abundance at the unoiled sites when compared to the oiled sites. Probability levels of "ns" indicate that the change in mean values is not significant ($p > 0.10$).

	Oiled Sites		Unoiled Sites			
	$\bar{x}$	SD	$\bar{x}$	SD	t	p
Amphipods	9.562	2.023	4.407	4.158	-2.730	0.021
Ampeliscid amphipods	6.964	4.596	1.745	1.935	-2.640	0.025
Phoxocephalid amphipods	6.476	2.252	2.382	1.841	-3.447	0.006

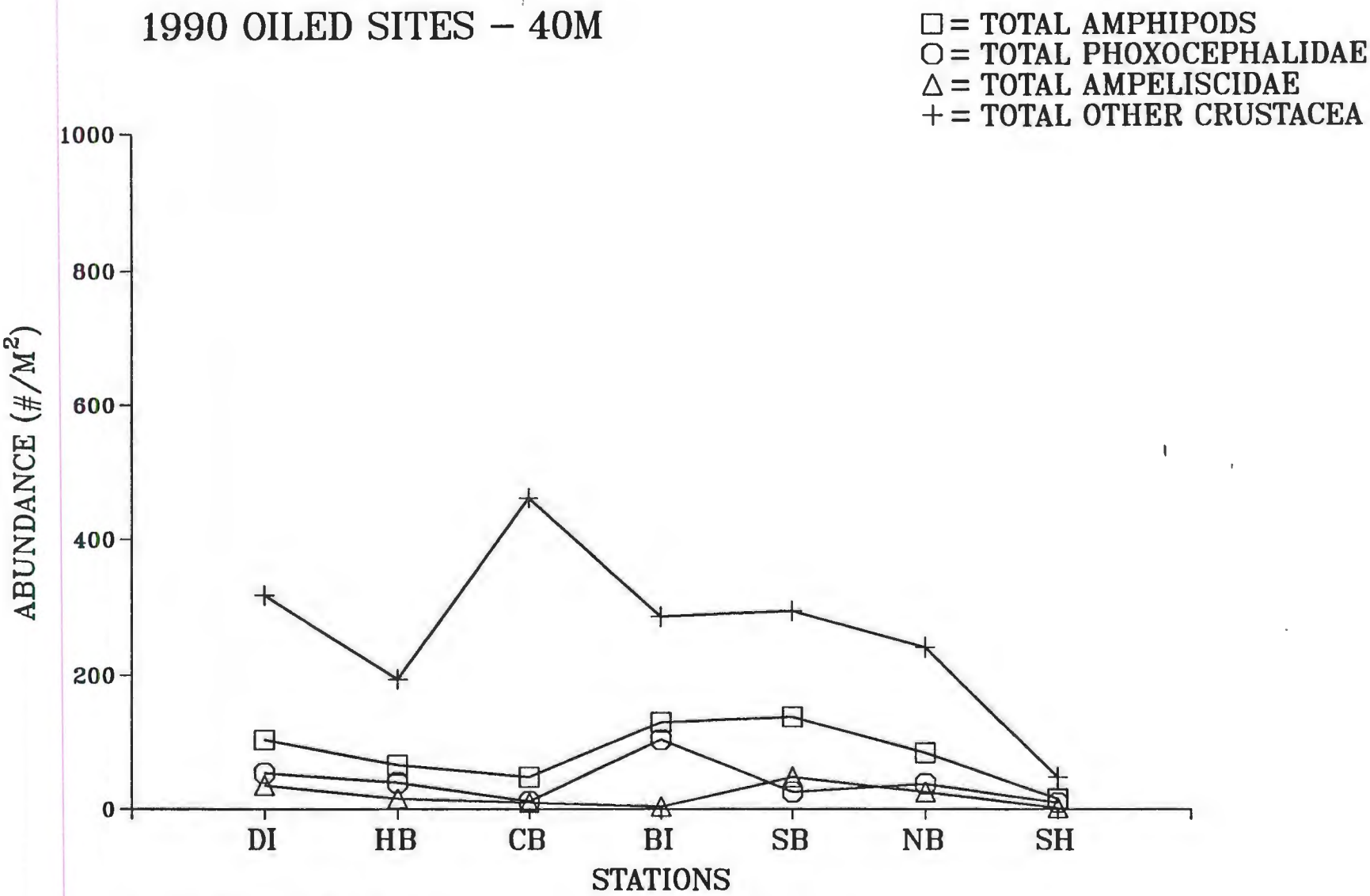

Figure 24. The abundance of total amphipods, phoxocephalid and ampeliscid amphipods, and other crustaceans at oiled stations at 40 m.

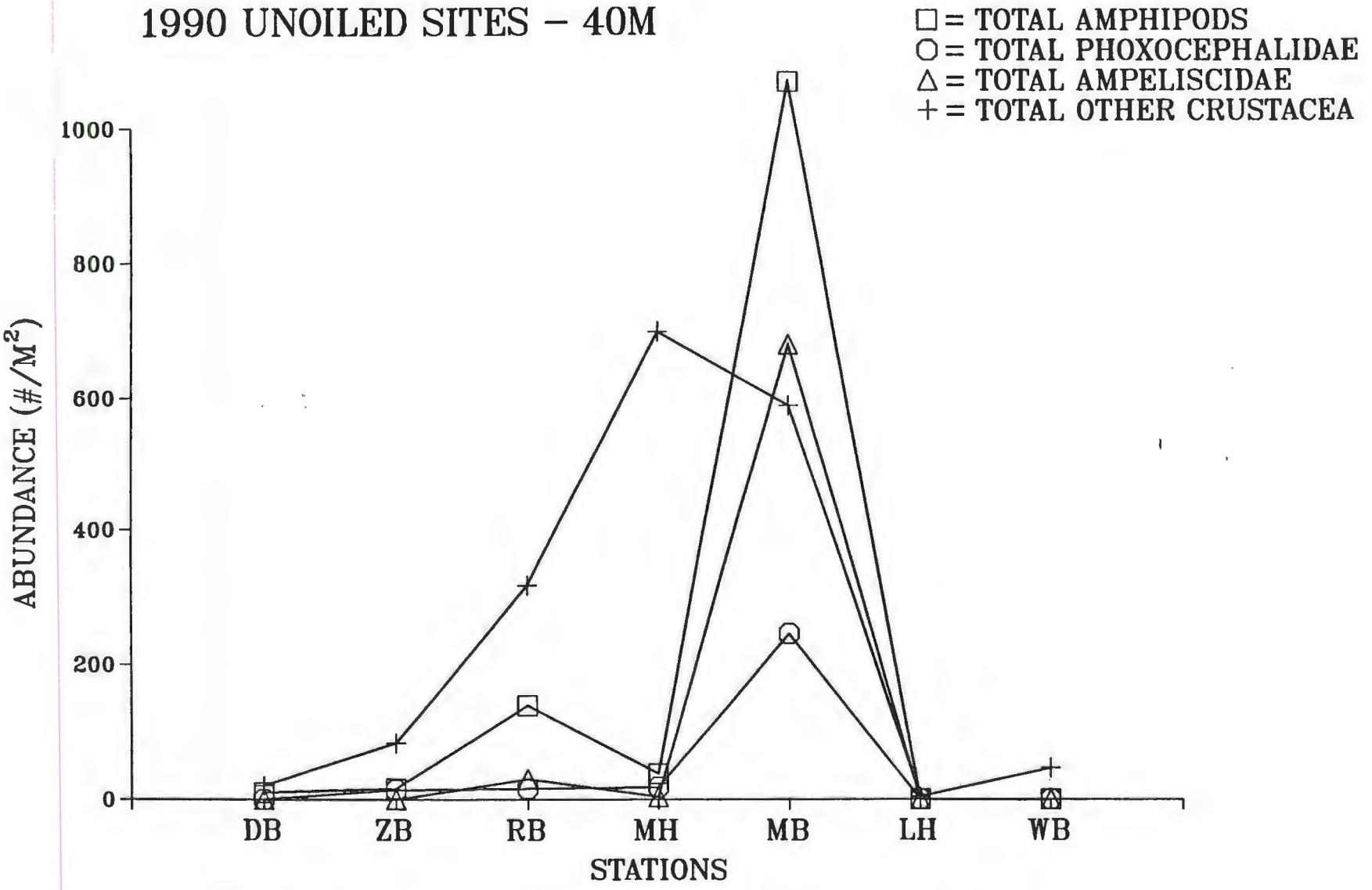

Figure 25. The abundance of total amphipods, phoxocephalid and ampeliscid amphipods, and other crustaceans at unoiled stations at 40 m.

Table 14. Abundance, as square root (abundance +0.5), of each taxon at pooled oiled bays (Bay of Isles, Chenega Bay, Disk Island, Herring Bay, Northwest Bay, Sleepy Bay, and Snug Harbor) and unoiled bays (Drier Bay, Lower Herring Bay, MacLeod Harbor, Mooselips Bay, Rocky Bay, West Bay, and Zaikof Bay). Means, standard deviations, and F-ratios with associated levels of significance, p.

	Oiled Bays of Isles		Unoiled Drier Bay			
Dominant Taxa	$\bar{x}$	SD	$\bar{x}$	SD	t	p
Ampeliscidae	0.914	0.284	0.707	0.000	-0.310	ns
Ampharetidae	1.218	0.520	0.811	0.231	-0.794	ns
Bivalvia	5.837	2.700	3.598	2.774	-1.729	ns
Capitellidae	7.847	2.376	1.886	0.654	-6.108	0.000
Chaetodermatidae	0.914	0.284	0.707	0.000	-0.871	ns
Cirratulidae	5.397	0.846	1.537	0.819	-4.010	0.000
Cossuridae	2.137	0.646	0.811	0.231	-4.160	0.000
Golfingidae	1.500	0.902	0.811	0.231	-0.768	ns
Harpacticoida	1.693	0.890	0.707	0.000	-1.411	ns
Hesionidae	1.223	0.712	1.308	0.858	0.303	ns
Leuconidae	4.723	1.223	1.147	0.480	-6.160	0.000
Lucinidae	0.707	0.000	3.284	2.323	4.632	0.000
Lumbrineridae	4.831	0.978	2.355	0.664	-4.229	0.000
Maldanidae	4.859	2.028	1.500	0.749	-4.566	0.000
Mytilidae	1.681	1.447	0.707	0.000	-1.991	ns
Nematoda	5.093	3.115	2.129	1.208	-1.962	ns
Nephtyidae	3.738	0.809	4.096	1.381	0.423	ns
Nuculanidae	0.985	0.401	1.908	0.275	2.021	0.048
Nuculidae	0.811	0.231	3.109	1.594	3.705	0.000

Table 14. (cont'd)

Ophiuroidea	1.412	0.796	1.857	1.521	0.756	ns
Orbiniidae	0.985	0.401	1.393	0.447	1.094	ns
Owenidae	2.161	0.887	1.497	0.275	-0.768	ns
Paraonidae	9.232	1.154	3.345	1.700	-6.692	0.000
Polynoidae	3.599	0.659	0.914	0.284	-7.797	0.000
Polyodontidae	6.992	1.422	0.985	0.401	-6.639	0.000
Sabellidae	1.839	0.804	0.707	0.000	-2.166	0.035
Scalibregmidae	0.811	0.231	0.707	0.000	-0.330	ns
Scaphandridae	0.985	0.401	1.616	0.928	1.649	ns
Sigalionidae	4.487	1.927	1.942	0.813	-3.561	0.001
Spionidae	2.890	0.661	1.286	1.029	-1.613	ns
Syllidae	4.155	1.672	0.811	0.231	-3.733	0.000
Tellinidae	1.746	0.752	0.811	0.231	-2.697	0.009
Thyasiridae	0.811	0.231	1.833	1.083	1.449	ns
Veneridae	0.707	0.000	0.707	0.000	0.000	ns

Table 15. Biomass, as square root (biomass +0.5), of each taxon at pooled oiled bays (Bay of Isles, Chenega Bay, Disk Island, Herring Bay, Northwest Bay, Sleepy Bay, and Snug Harbor) and unoiled bays (Drier Bay, Lower Herring Bay, MacLeod Harbor, Mooselips Bay, Rocky Bay, West Bay, and Zaikof Bay). Means, standard deviations, and F-ratios with associated levels of significance, p.

	Oiled Bays		Unoiled Bays			
Taxa	$\bar{X}$	SD	$\bar{X}$	SD	F	p
Ampharetidae	1.890	1.242	1.329	0.741	8.346	0.005
Bivalvia	4.226	2.328	4.173	2.793	0.012	ns
Capitellidae	3.712	2.322	3.820	2.812	0.085	ns
Chaetodermatidae	1.061	0.457	1.190	0.640	2.061	ns
Cirratulidae	4.791	2.308	3.827	3.686	7.023	0.010
Cossuridae	1.076	0.707	1.090	0.617	0.013	ns
Golfingidae	3.127	2.315	2.287	2.676	6.137	0.016
Harpacticoida	2.376	1.855	1.000	0.606	27.117	0.000
Hesionidae	0.865	0.356	1.100	0.546	4.482	0.032
Leuconidae	2.142	1.662	1.692	0.975	4.210	0.045
Lucinidae	1.022	0.664	1.991	1.359	21.264	0.000
Lumbrineridae	3.701	1.807	2.977	1.478	10.710	0.002
Maldanidae	3.538	1.801	2.373	1.786	17.571	0.000
Nematoda	5.159	3.561	2.576	1.992	20.604	0.000
Nephtyidae	2.027	1.107	3.165	2.141	12.708	0.001
Nuculanidae	1.189	0.643	2.263	1.075	38.775	0.000
Nuculidae	1.388	1.072	2.278	1.338	14.399	0.000
Ophiuroidea	2.184	1.323	1.919	1.441	1.424	ns

Table 15. (cont'd)

Orbiniidae	1.187	0.710	1.593	0.798	8.305	0.006
Owenidae	2.520	1.526	4.789	5.876	48.066	0.000
Polyodontidae	3.977	2.525	1.338	1.359	59.566	0.000
Scalibregmidae	0.879	0.319	1.090	0.662	3.172	0.080
Scaphandridae	0.929	0.355	2.007	1.208	55.594	0.000
Sigalionidae	2.770	1.774	1.941	0.835	9.414	0.003
Spionidae	2.468	1.321	3.668	4.175	10.192	0.002
Syllidae	3.618	2.360	2.062	1.695	21.116	0.000
Tellinidae	1.437	0.648	1.040	0.559	9.156	0.004
Thyasiridae	2.025	1.382	2.352	1.652	1.500	ns
Veneridae	0.904	0.339	1.214	0.684	13.251	0.001

Table 16. Summary of means, standard deviations, t statistics, t, and associated levels of significance, p, for dominant taxa in comparisons of abundance as square root (abundance +0.5) between the Bay of Isles and Drier Bay. Values of t statistics preceeded by a minus sign indicate decreases in mean abundance for Drier Bay when compared to the Bay of Isles, while other values indicate increases in mean values for Drier Bay when compared to the Bay of Isles. Probability levels of "ns" indicate that the change in mean values is not significant ($p > 0.05$).

	Oiled Bays		Unoiled Bays			
Taxa	$\bar{X}$	SD	$\bar{X}$	SD	F	p
Ampeliscidae	0.718	0.021	0.732	0.071	2.518	ns
Amphictenidae	0.708	0.007	0.716	0.035	1.972	ns
Ascidiacea	0.879	0.477	0.815	0.350	0.735	ns
Astartidae	0.872	0.447	0.713	0.018	4.130	0.047
Capitellidae	0.728	0.032	0.729	0.025	0.013	ns
Cardiidae	0.930	0.428	0.834	0.375	1.086	ns
Chaetopteridae	0.776	0.105	0.722	0.036	8.246	0.006
Cirratulidae	0.728	0.020	0.724	0.034	0.817	ns
Flabelligeridae	0.749	0.131	0.708	0.003	4.823	0.032
Golfingidae	0.769	0.136	0.713	0.012	5.745	0.020
Limidae	0.805	0.237	0.707	0.000	23.846	0.000
Lucinidae	0.716	0.034	0.848	0.325	7.539	0.008
Lumbrineridae	0.776	0.078	0.752	0.054	4.072	0.048
Maldanidae	0.958	0.375	0.760	0.130	14.907	0.000
Mytilidae	0.788	0.214	0.708	0.002	4.680	0.035
Nephtyidae	0.744	0.133	0.766	0.151	0.453	ns
Nuculanidae	0.760	0.118	0.917	0.400	11.857	0.001
Nuculidae	0.725	0.073	0.756	0.112	2.607	ns

Table 16. (cont'd)

Ophiuroidea	0.865	0.171	0.782	0.173	7.574	0.008
Orbiniidae	0.714	0.035	0.717	0.021	0.189	ns
Owenidae	0.720	0.018	0.727	0.030	3.651	0.061
Phyllodocidae	0.738	0.170	0.710	0.006	1.004	ns
Rhynchocoela	0.734	0.076	0.742	0.063	0.280	ns
Sabellidae	0.730	0.074	0.709	0.008	2.633	ns
Tellinidae	0.770	0.313	0.985	0.395	7.066	0.010
Veneridae	0.851	0.447	1.188	0.751	10.849	0.002

Table 17. Summary of means, standard deviations, t statistics, t, and associated levels of significance, p, for dominant taxa in comparisons of biomass as square root (abundance +0.5) between the Bay of Isles and Drier Bay. Values of t statistics preceeded by a minus sign indicate decreases in mean abundance for Drier Bay when compared to the Bay of Isles, while other values indicate increases in mean values for Drier Bay when compared to the Bay of Isles. Probability levels of "ns" indicate that the change in mean values is not significant ($p > 0.05$).

	Oiled Bays of Isles		Unoiled Drier Bay			
Dominant Taxa	$\bar{X}$	SD	$\bar{X}$	SD	t	p
Ampeliscidae	0.707	0.001	0.707	0.000	0.000	ns
Amphictenidae	0.708	0.002	0.765	0.085	3.854	0.000
Ascidiacea	0.711	0.008	0.717	0.023	0.032	ns
Astartidae	1.048	0.721	0.707	0.000	-1.649	ns
Capitellidae	0.758	0.064	0.711	0.003	-2.984	0.004
Cardiidae	0.720	0.029	0.716	0.018	-0.000	ns
Chaetopteridae	0.815	0.131	0.707	0.000	-2.134	0.037
Cirratulidae	0.731	0.016	0.708	0.001	-2.305	0.025
Flabelligeridae	0.951	0.276	0.707	0.000	-4.956	0.000
Golfingidae	0.784	0.162	0.707	0.000	-1.226	ns
Limidae	0.707	0.000	0.707	0.000	0.000	ns
Lucinidae	0.707	0.000	1.075	0.385	2.900	0.005
Lumbrineridae	0.820	0.117	0.732	0.038	-2.704	0.009
Maldanidae	1.552	0.674	0.717	0.012	-6.168	0.000
Mytilidae	0.758	0.073	0.707	0.000	-0.522	ns
Nephtyidae	0.719	0.018	0.732	0.021	0.155	ns
Nuculanidae	0.740	0.056	0.891	0.147	1.253	ns
Nuculidae	0.707	0.000	0.945	0.219	4.691	0.000

Table 17. (cont'd)

Ophiuroidea	0.837	0.186	0.711	0.005	-1.584	ns
Orbiniidae	0.708	0.001	0.711	0.004	0.212	ns
Owenidae	0.715	0.006	0.708	0.000	-0.734	ns
Phyllodocidae	0.710	0.007	0.707	0.000	-0.045	ns
Rhynchocoela	0.827	0.187	0.740	0.050	-2.039	0.046
Sabellidae	0.722	0.014	0.707	0.000	-0.427	ns
Tellinidae	0.707	0.000	1.161	0.428	2.122	0.038
Veneridae	0.707	0.000	0.707	0.000	0.000	ns

Table 18. Summary of means, standard deviations, t statistics, t, and associated levels of significance, p, for dominant taxa in comparisons of abundance as square root (abundance +0.5) between Sleepy Bay and Mooselips Bay. Values of t statistics preceeded by a minus sign indicate decreases in mean abundance for Mooselips Bay when compared to Sleepy Bay, while other values indicate increases in mean values for Mooselips Bay when compared to Sleepy Bay. Probability levels of "ns" indicate that the change in mean values is not significant ($p > 0.05$).

	Oiled Sleepy Bay		Unoiled Mooselips Bay			
Dominant Taxa	$\bar{X}$	SD	$\bar{X}$	SD	t	p
Ampeliscidae	2.166	0.874	7.666	3.452	8.222	0.000
Ampharetidae	2.736	0.876	1.956	0.585	-1.519	ns
Bivalvia	6.122	2.007	6.265	2.927	0.110	ns
Capitellidae	4.250	1.341	5.754	1.222	1.541	ns
Chaetodermatidae	1.799	0.573	1.393	0.447	-1.707	ns
Cirratulidae	5.370	1.681	11.911	2.557	6.795	0.000
Cossuridae	0.707	0.000	0.707	0.000	0.000	ns
Golfingidae	4.808	1.408	5.457	1.703	0.723	ns
Harpacticoida	3.034	0.786	1.268	0.604	-2.527	0.014
Hesionidae	0.707	0.000	0.914	0.284	0.732	ns
Leuconidae	0.707	0.000	2.390	0.994	2.899	0.005
Lucinidae	0.707	0.000	1.089	0.378	0.686	ns
Lumbrineridae	4.029	1.040	3.125	0.645	-1.544	ns
Maldanidae	4.685	1.088	3.338	0.834	-1.831	ns
Mytilidae	6.466	1.539	1.887	0.962	-9.361	0.000
Nematoda	6.616	2.041	3.323	2.019	-2.180	0.033
Nephtyidae	2.013	0.556	2.185	0.400	0.203	ns
Nuculanidae	0.882	0.391	2.795	1.165	4.193	0.000

Table 18. (cont'd)

Nuculidae	1.089	0.378	1.829	0.971	1.193	ns
Ophiuroidea	2.095	0.942	4.486	1.404	4.063	0.000
Orbiniidae	1.983	0.843	0.811	0.231	-3.146	0.003
Owenidae	2.252	0.532	8.533	2.316	7.255	0.000
Paraonidae	2.816	0.462	4.999	1.180	2.481	0.016
Polynoidae	1.121	0.231	1.572	0.534	1.310	ns
Polyodontidae	4.429	2.369	4.161	1.798	-0.295	ns
Sabellidae	1.572	0.534	2.188	0.372	1.179	ns
Scalibregmidae	1.115	0.567	1.588	1.213	1.512	ns
Scaphandridae	0.985	0.401	1.550	0.788	1.477	ns
Sigalionidae	4.680	1.731	2.970	0.921	-2.393	0.020
Spionidae	2.626	1.480	2.193	0.930	-0.446	ns
Syllidae	5.148	1.413	5.102	0.917	-0.055	ns
Tellinidae	1.572	0.534	1.250	0.819	-0.928	ns
Thyasiridae	1.626	0.267	2.730	0.559	1.564	ns
Veneridae	0.811	0.231	0.811	0.231	0.000	ns

Table 19. Summary of means, standard deviations, t statistics, t, and associated levels of significance, p, for dominant taxa in comparisons of biomass as square root (abundance +0.5) between Sleepy Bay and Mooselips Bay. Values of t statistics preceeded by a minus sign indicate decreases in mean abundance for Mooselips Bay when compared to Sleepy Bay, while other values indicate increases in mean values for Mooselips Bay when compared to Sleepy Bay. Probability levels of "ns" indicate that the change in mean values is not significant ($p > 0.05$).

	Oiled Sleepy Bay		Unoiled Mooselips Bay			
Dominant Taxa	$\bar{X}$	SD	$\bar{X}$	SD	t	p
Ampeliscidae	0.713	0.004	0.868	0.125	6.731	0.000
Amphictenidae	0.708	0.001	0.709	0.003	0.089	ns
Ascidiacea	1.765	0.855	1.361	0.749	-2.030	0.047
Astartidae	0.814	0.073	0.735	0.036	-0.382	ns
Capitellidae	0.726	0.008	0.715	0.003	-0.694	ns
Cardiidae	0.992	0.261	1.201	0.641	0.855	ns
Chaetopteridae	0.797	0.067	0.715	0.011	-1.622	ns
Cirratulidae	0.716	0.005	0.798	0.040	8.215	0.000
Flabelligeridae	0.707	0.000	0.709	0.002	0.032	ns
Golfingidae	0.862	0.266	0.725	0.011	-2.206	0.031
Limidae	1.314	0.303	0.708	0.001	-11.493	0.000
Lucinidae	0.707	0.000	0.710	0.003	0.000	ns
Lumbrineridae	0.750	0.025	0.738	0.007	-0.349	ns
Maldanidae	0.945	0.105	0.772	0.018	-1.280	ns
Mytilidae	0.832	0.094	0.710	0.004	-1.241	ns
Nephtyidae	0.711	0.004	0.955	0.358	2.844	0.006
Nuculanidae	0.718	0.025	0.865	0.130	1.215	ns
Nuculidae	0.713	0.009	0.710	0.004	-0.045	ns

Table 19. (cont'd)

Ophiuroidea	1.055	0.202	1.103	0.260	0.599	ns
Orbiniidae	0.709	0.002	0.707	0.000	-0.095	ns
Owenidae	0.719	0.012	0.774	0.027	5.939	0.000
Phyllodocidae	0.912	0.447	0.707	0.000	-2.709	0.009
Rhynchocoela	0.725	0.010	0.710	0.003	-0.374	ns
Sabellidae	0.790	0.182	0.717	0.000	-2.147	0.036
Tellinidae	0.707	0.000	0.925	0.350	1.018	ns
Veneridae	0.710	0.006	0.811	0.231	0.373	ns

Table 20. Summary of means, standard deviations, t statistics, t, and associated levels of significance, p, for dominant taxa in comparisons of abundance as square root (abundance +0.5) between the Herring Bay and Lower Herring Bay. Values of t statistics preceeded by a minus sign indicate decreases in mean abundance for Lower Herring Bay when compared to Herring Bay, while other values indicate increases in mean values for Lower Herring Bay when compared to Herring Bay. Probability levels of "ns" indicate that the change in mean values is not significant ($p > 0.05$).

	Oiled Herring Bay		Unoiled Lower Herring Bay			
Dominant Taxa	$\bar{x}$	SD	$\bar{x}$	SD	t	p
Ampeliscidae	1.250	0.819	0.707	0.000	-0.812	ns
Ampharetidae	2.073	1.871	0.707	0.000	-2.660	0.010
Bivalvia	4.695	3.174	1.242	0.669	-2.666	0.010
Capitellidae	3.168	1.825	1.564	0.904	-1.643	ns
Chaetodermatidae	0.811	0.231	0.811	0.231	0.000	ns
Cirratulidae	5.788	3.082	1.286	1.029	-4.678	0.000
Cossuridae	0.882	0.391	0.914	0.284	0.100	ns
Golfingidae	3.574	2.430	0.707	0.000	-3.195	0.002
Harpacticoida	2.057	2.022	0.707	0.000	-1.931	ns
Hesionidae	0.882	0.391	0.990	0.632	0.382	ns
Leuconidae	1.664	1.291	0.707	0.000	-1.648	ns
Lucinidae	0.811	0.231	1.944	1.379	2.037	0.046
Lumbrineridae	5.183	1.430	1.093	0.617	-6.987	0.000
Maldanidae	3.422	1.996	0.707	0.000	-3.691	0.001
Mytilidae	1.507	0.887	0.707	0.000	-1.635	ns
Nematoda	6.399	5.495	0.707	0.000	-3.768	0.000
Nephtyidae	1.550	1.059	1.497	0.275	-0.063	ns

Table 20. (cont'd)

Nuculanidae	1.427	0.911	0.811	0.231	-1.351	ns
Nuculidae	0.985	0.401	1.165	0.655	0.290	ns
Ophiuroidea	2.019	1.132	1.147	0.480	-1.482	ns
Orbiniidae	0.707	0.000	2.148	0.779	3.869	0.000
Owenidae	2.158	1.027	0.707	0.000	-1.676	ns
Paraonidae	4.587	0.911	1.678	1.614	-3.306	0.002
Polynoidae	1.441	1.015	0.914	0.284	-1.530	ns
Polyodontidae	4.321	2.352	0.940	0.520	-3.738	0.000
Sabellidae	2.110	1.600	0.707	0.000	-2.684	0.010
Scalibregmidae	0.914	0.284	1.089	0.378	0.558	ns
Scaphandridae	0.707	0.000	0.985	0.401	0.728	ns
Sigalionidae	1.875	1.575	1.618	0.321	-0.359	ns
Spionidae	1.996	0.946	1.264	0.359	-0.736	ns
Syllidae	3.515	2.680	0.990	0.632	-2.819	0.007
Tellinidae	1.276	0.583	0.707	0.000	-1.641	ns
Thyasiridae	1.655	1.304	0.985	0.401	-0.949	ns
Veneridae	0.707	0.000	0.707	0.000	0.000	ns

Table 21. Summary of means, standard deviations, t statistics, t, and associated levels of significance, p, for dominant taxa in comparisons of biomass as square root (abundance +0.5) between the Herring Bay and Lower Herring Bay. Values of t statistics preceeded by a minus sign indicate decreases in mean abundance for Lower Herring Bay when compared to Herring Bay, while other values indicate increases in mean values for Lower Herring Bay when compared to Herring Bay. Probability levels of "ns" indicate that the change in mean values is not significant ($p>0.05$).

	Oiled Herring Bay		Unoiled Lower Herring Bay			
Dominant Taxa	$\bar{X}$	SD	$\bar{X}$	SD	t	p
Ampeliscidae	0.732	0.040	0.707	0.000	-1.082	ns
Amphictenidae	0.707	0.000	0.707	0.000	0.000	ns
Ascidiacea	0.794	0.194	0.707	0.000	-0.437	ns
Astartidae	0.711	0.007	0.707	0.000	-0.000	ns
Capitellidae	0.723	0.016	0.732	0.035	0.590	ns
Cardiidae	0.707	0.000	0.707	0.000	0.000	ns
Chaetopteridae	0.792	0.173	0.707	0.000	-1.682	ns
Cirratulidae	0.730	0.027	0.708	0.002	-2.217	0.031
Flabelligeridae	0.707	0.000	0.707	0.000	0.000	ns
Golfingidae	0.712	0.004	0.707	0.000	-0.071	ns
Limidae	0.718	0.016	0.707	0.000	-0.212	ns
Lucinidae	0.707	0.000	1.232	0.637	4.140	0.000
Lumbrineridae	0.803	0.028	0.719	0.026	-2.592	0.012
Maldanidae	0.782	0.085	0.707	0.000	-0.555	ns
Mytilidae	0.749	0.090	0.707	0.000	-0.431	ns
Nephtyidae	0.709	0.002	0.711	0.005	0.032	ns
Nuculanidae	0.887	0.256	0.739	0.071	-1.232	ns

Table 21. (cont'd)

Nuculidae	0.710	0.006	0.749	0.057	0.765	ns
Ophiuroidea	0.796	0.092	0.719	0.013	-0.957	ns
Orbiniidae	0.707	0.000	0.752	0.044	2.521	0.015
Owenidae	0.713	0.005	0.707	0.000	-0.666	ns
Phyllodocidae	0.709	0.001	0.707	0.000	-0.032	ns
Rhynchocoela	0.718	0.020	0.746	0.065	0.649	ns
Sabellidae	0.744	0.076	0.707	0.000	-1.312	ns
Tellinidae	0.720	0.029	0.934	0.213	1.000	ns
Veneridae	0.707	0.000	0.707	0.000	0.000	ns

Table 22. Abundance, as square root (abundance +0.5), of each taxon at oiled bays (Bay of Isles, Chenega Bay, Disk Island, Herring Bay, Northwest Bay and Sleepy Bay) and unoiled bays (Drier Bay, West Bay and Zaikof Bay). Means, standard deviations, and F-ratios with associated levels of significance.

	Oiled Bays		Unoiled Bays			
Taxa	$\bar{x}$	SD	$\bar{x}$	SD	F	p
Ampharetidae	2.020	1.255	0.811	0.214	13.575	0.001
Bivalvia	4.649	2.218	3.154	2.208	4.555	0.039
Capitellidae	3.819	2.396	2.224	0.898	6.153	0.017
Chaetodermatidae	1.103	0.474	1.326	0.808	1.367	ns
Cirratulidae	5.278	1.993	2.059	1.032	34.267	0.000
Cossuridae	1.058	0.654	0.975	0.401	0.205	ns
Golfingidae	3.484	2.304	0.742	0.134	20.972	0.000
Harpacticoida	2.654	1.864	0.707	0.000	16.178	0.000
Hesionidae	0.874	0.375	1.357	0.647	10.106	0.003
Leuconidae	2.240	1.676	1.395	0.723	3.462	0.070
Lucinidae	0.897	0.521	1.816	1.670	7,747	0.008
Lumbrineridae	4.098	1.610	2.387	0.928	14.437	0.000
Maldanidae	3.859	1.579	1.115	0.646	41.431	0.000
Nematoda	5.649	3.550	1.608	0.990	18.516	0.000
Nephtyidae	2.090	1.114	4.236	2.859	13.165	0.001
Nuculanidae	1.234	0.677	2.530	1.053	25.039	0.000
Nuculidae	1.226	0.613	1.930	1.272	6.349	0.016
Ophiuroidea	2.413	1.290	1.218	0.973	9.995	0.003

Table 22. (cont'd)

Orbiniidae	1.156	0.613	1.172	0.527	0.008	ns
Owenidae	2.671	1.462	1.063	0.427	17.214	0.000
Polyodontidae	4.415	2.409	0.800	0.254	33.213	0.000
Scalibregmidae	0.908	0.336	0.742	0.134	3.357	0.074
Scaphandridae	0.927	0.333	1.668	0.642	26.248	0.000
Sigalionidae	3.012	1.780	1.737	0.767	6.972	0.011
Spionidae	2.364	1.023	2.167	1.691	0.236	0.629
Syllidae	4.035	2.270	0.776	0.182	30.457	0.000
Tellinidae	1.541	0.637	0.742	0.134	22.879	0.000
Thyasiridae	2.134	1.443	1.437	0.825	2.990	0.091
Veneridae	0.920	0.354	0.936	0.411	0.019	ns

Comparisons of All (Seven) Pooled Oiled vs. All Pooled (Seven) Unoiled Sites

A. Abundance comparisons

1. Taxonomic groups at significantly higher abundance levels at oiled sites were (p values in Table 14):

Nematoda		Golfingidae	(sipunculid)
Ampharetidae (polychaete)		Harpacticoida	(copepod)
Cirratulidae	"	Leuconidae	(cumacean)
Maldanidae	"	Tellinidae	(bivalve)
Polyodontidae	"		
Sigalionidae	"		
Syllidae	"		
Lumbrineridae	"		

2. Taxonomic groups at significantly higher abundance levels at unoiled sites were (Table 14):

Hesionidae (polychaete)	Lucinidae (bivalve)
Nephtyidae "	Nuculanidae (protobranch bivalve)
Orbiniidae "	Nuculidae "
Owenidae "	Scaphandridae (mainly the gastropod *Cylichna*) (mainly *Myriochele* spp.)
Scalibregmidae (polychaete)	
Spionidae "	

B. Biomass (wet weight) comparisons between pooled oiled (seven) vs. unoiled (seven) sites

1. Taxonomic groups at significantly higher levels at oiled sites

Many of the same taxonomic groups that were significantly higher in abundance (Table 14) were significantly higher in biomass (p values in Table 15). Additional significantly higher groups at $P > 0.03$ are:

Chaetopteridae (polychaete)
Flabelligeridae "
Limidae (bivalve)
Mytilidae (bivalve-*Crenella*)

2. Taxonomic groups at significantly higher levels at unoiled sites (Table 15)

Some of the same taxonomic groups that were significantly higher in abundance (Table 14) were significantly higher in biomass. An additional group ($P = 0.002$) added is Veneridae (bivalve)

Comparisons Between Bays Assessed by the Shallow Diving Component of the Coastal Habitat/Air Water Program (sampled by diving to 20 m; only the six bays included below were sampled jointly by the shallow diving and deep benthic groups)[6]

A. Abundance Comparisons (P values in Tables 16, 18, and 20)

1. Bay of Isles (oiled) vs. Drier Bay (unoiled)

a. Taxonomic groups at significantly higher abundance levels at oiled sites

Many taxonomic groups included here were identified in the 7x7 (oiled vs. unoiled) comparisons (Table 14). Additional taxa resulting from this comparison are:

Capitellidae (polychaete)
Cossuridae "
Paraonidae "
Polynoidae "
Sabellidae "
Sigalionidae "
Syllidae "

b. Taxonomic groups at significantly higher abundance levels at unoiled sites

Similar to the taxa that were most abundant for the pooled (seven) unoiled sites (Table 14)

2. Sleepy Bay (oiled) vs. Mooselips Bay (unoiled)

a. Taxonomic groups at significantly higher abundance levels at oiled sites

Nematoda
Orbiniidae (polychaete)
Sigalionidae "
Harpacticoida (copepod)
Mytilidae (mainly the bivalve *Crenella*)

[6] An additional pair of bays were also sampled jointly by the diving and deep benthic group and are included here: Sleepy Bay (oiled) and Mooselips Bay (unoiled). Generally, Mooselips Bay is excluded as a different type of unoiled site; however, despite this, some striking differences are apparent at the oiled station relative to Mooselips Bay.

b. Taxonomic groups at significantly higher abundance levels at unoiled sites:

Cirratulidae (polychaete)
Owenidae "
Paraonidae "
Leuconidae (cumacean)
Ampeliscidae (tube-dwelling amphipod)
Nuculanidae (protobranch bivalve)
Ophiuroidea

Note: Some taxa that were more abundant at MB are more typical of oiled sites. This suggests that MB is a disturbed or organically enriched site. Despite the unusual aspect of MB as a control site, the oiled site showed significantly more nematodes and harpacticoids, two taxonomic groups always more abundant at the oiled sites.

3. Herring Bay (oiled) vs. Lower Herring Bay

All abundance comparisons were generally similar to those noted for pooled oiled bays and the single interbay comparisons above.

B. Biomass (wet weight) comparisons for the above interbay comparisons are included in Tables 17, 19, and 21.

Abundance Comparisons Between the Two Largest Groups of Oiled and Unoiled Stations as Determined by Cluster and Principal Coordinate Analyses: Oiled Bays–BI, CB, DI, HB, NB, SB; Unoiled Bays–DB, WB, ZB (Figs. 6, 7, and Table 8).

A. Taxonomic groups at significantly higher abundance levels at oiled sites

Most of the same taxonomic groups are significantly more abundant at the grouped oiled sites as observed for the other site comparisons included above. A few additional taxonomic groups were added by this comparison:

Capitellidae (polychaete)
Thyasiridae (bivalve)

B. Taxonomic groups at significantly higher abundance levels at unoiled sites.

Most of the same taxonomic groups are significantly higher in abundance at the unoiled sites as noted for all other abundance comparisons presented above.

Biomass Comparisons Between the Two Largest Groups of Oiled (6) and Unoiled (3) Stations as Determined by Cluster Analysis (see Figs. 6, 7, and Table 8)

Many of the same taxonomic groups, as in the abundance comparisons included above, are significantly different when comparing oiled and unoiled sites (data on file at IMS).

Faunal Assessment of Data from Stations Sampled July 1990 at 100 m

At stations at oiled and unoiled sites, polychaetous annelids were dominant in abundance. As observed for the 40 m stations, Mollusca appeared to be somewhat more abundant at unoiled sites (Figs. 26 and 27). At four of the oiled sites (Disk Island, Herring Bay, Chenega Bay, and Sleepy Bay), Nematodes were more abundant than at unoiled sites. Statistical assessment of Nematoda abundance is treated in a later section. As also observed for the 40 m sites, little difference could be observed qualitatively for any of the other major groups between oiled and unoiled sites. The abundance, biomass, number of taxa and diversity[7] of benthic fauna for the 14 stations sampled at 100 m are tabulated in Table 23a (also see Figs. 28 and 29). Abundance values at oiled stations varied between 1392–1912 and for unoiled sites between 1232-5060 indiv./m^2. Carbon biomass at oiled stations varied between 0.46-17.55 and for unoiled stations 0.37-4.75 gC/m^2. Number of taxa at oiled stations varied between 44-89 and at unoiled stations 47-99. Shannon diversity and evenness values were slightly lower at most of the oiled stations; Simpson dominance was generally somewhat higher at oiled stations in comparison with unoiled stations. Species richness at oiled stations varied between 5.33-9.9 and at unoiled stations from 6.03-12.62. A few changes were noted in some values for number of taxa, dominance, Shannon diversity, evenness, and species richness based on inclusion of lower levels for taxa (mainly generic and specific names; as stated in Methods, whenever possible identifications were made to lower taxa; Table 23b). Sufficient lower taxon identifications were available to make a table of diversity to compare with Table 23a. As expected, the number of taxa increased at all stations. Simpson dominance values, as noted in Table 23b, were similar to those shown in Table 23a with values somewhat higher at oiled stations in comparison with unoiled stations. Thus, as expected, evenness was slightly lower at the oiled stations. Shannon values, as expected, also increased slightly, but values were still somewhat lower at oiled stations.

Species richness (SR), based on Table 23a for higher taxa, was generally similar at oiled and unoiled sites, with the exception of the high values for two unoiled sites – Mooselips Bay and MacLeod Harbor. This is in contrast with SR at 40 m in which the SR there tended to be higher than at the unoiled sites. The number of taxa at oiled and unoiled sites was also variable with no trend apparent. The highest number of taxa were also at the same two unoiled sites – Mooselips Bay and Macleod Harbor. However, two of the oiled sites also had high numbers of taxa – Herring Bay and Sleepy Bay. As might be expected, the great increase in taxa at most stations relative to abundance when genera and species are included (Table 23b) is reflected

[7] The abundance, biomass, number of taxa and diversity are based on identifications to Family level or higher. A list of all taxa to species and higher taxon levels are included in Appendix C.

Text continued on page 91.

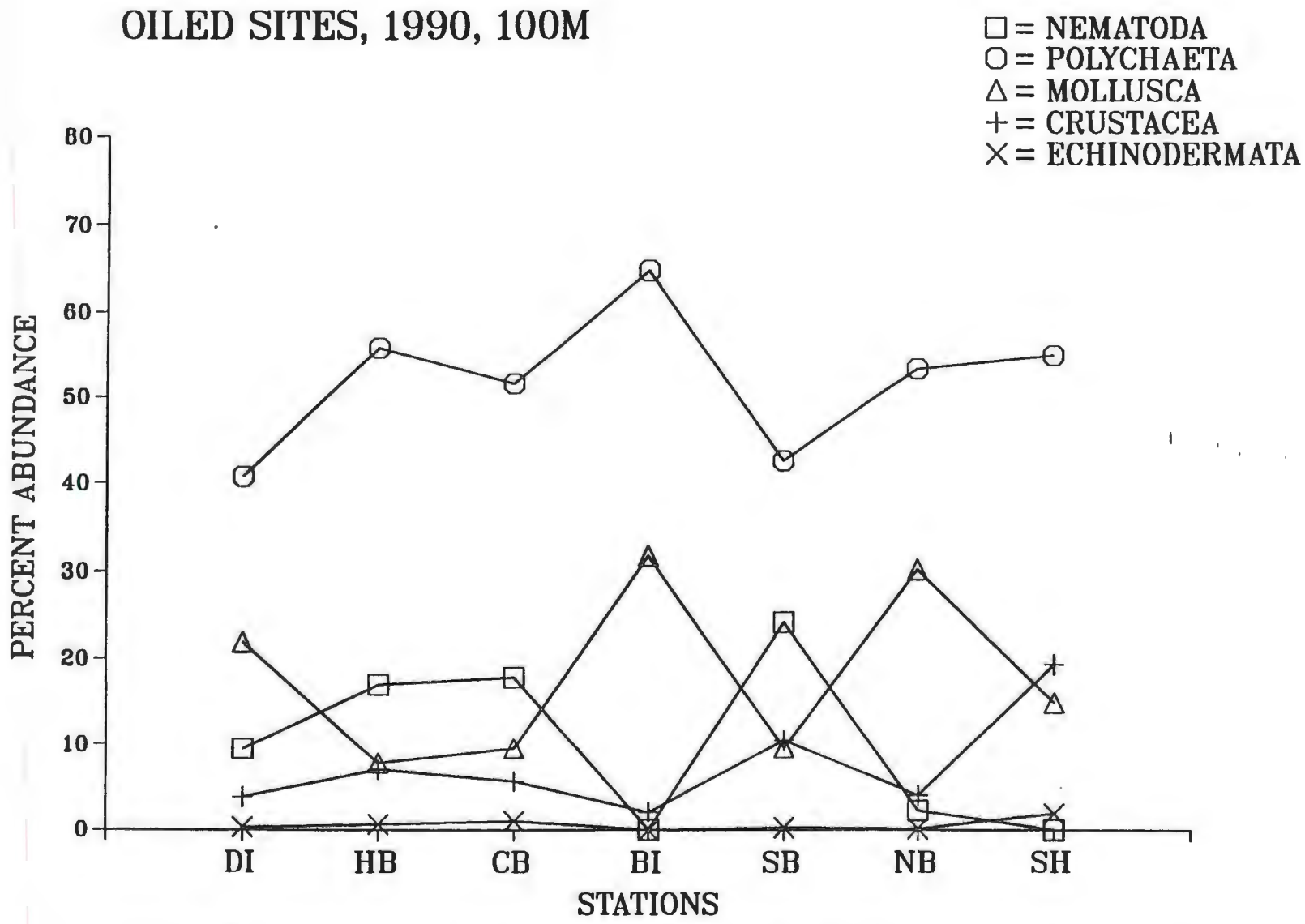

Figure 26. Percent abundance of major higher taxonomic groups at stations at oiled sites for samples collected at 100 m in July 1990.

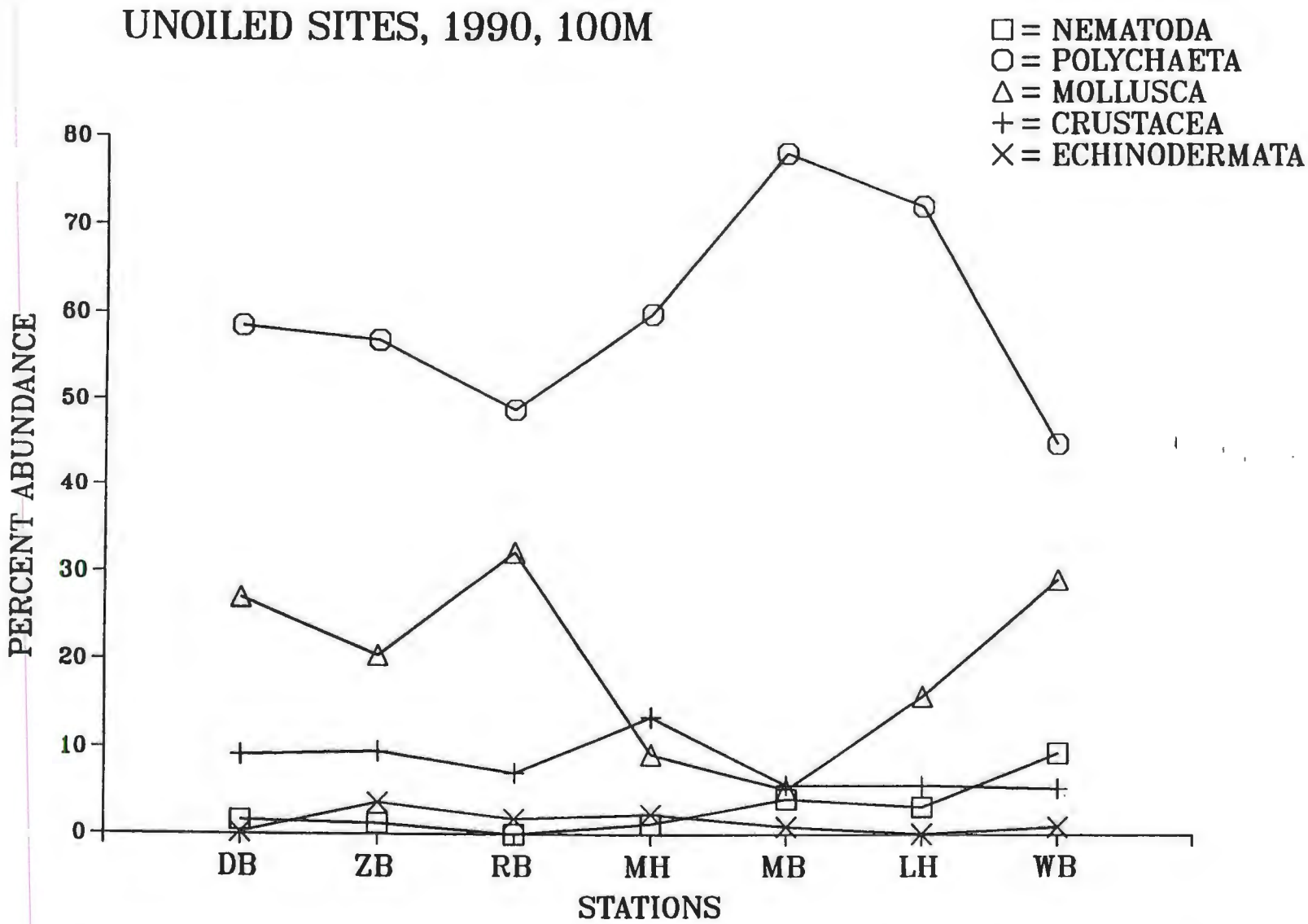

Figure 27. Percent abundance of major higher taxonomic groups at stations at unoiled sites for samples collected at 100 m in July 1990.

Table 23a. The abundance, biomass, number of taxa, and diversity of sites sampled at 100 m in Prince William Sound in July 1990. Fragments and species excluded from cluster analysis ARE excluded in all computation except biomass. The identifications were typically to the Family level whenever possible and to higher levels if necessary.

STATION	ABUNDANCE	WET WEIGHT BIOMASS	CARBON BIOMASS	NUMBER OF TAXA	SIMPSON	SHANNON	SW EVEN	SPECIES RICHNESS
DA901BI2	3208.0	494.262	17.5501	44.0	0.148	2.339	0.618	5.326
DA901CB2	2830.0	8.792	0.4622	72.0	0.064	3.247	0.759	8.933
DA901DB2	1698.0	7.356	0.3710	49.0	0.112	2.698	0.693	6.454
DA901DI2	3646.0	14.460	0.8667	66.0	0.089	2.966	0.708	7.925
DA901HB2	6886.0	31.235	1.6026	80.0	0.126	2.812	0.642	8.939
DA901LH2	2056.0	27.509	1.5750	47.0	0.093	2.723	0.707	6.030
DA901MB2	5060.0	55.471	3.0637	91.0	0.070	3.167	0.702	10.552
DA901MH2	2364.0	23.826	1.1070	99.0	0.055	3.572	0.777	12.616
DA901NB2	2512.0	19.737	1.1670	63.0	0.115	2.830	0.683	7.919
DA901RB2	1232.0	113.988	4.7565	50.0	0.082	2.993	0.765	6.886
DA901SB2	6912.0	65.048	2.2594	89.0	0.091	3.079	0.686	9.954
DA901SH2	1392.0	20.915	1.0393	54.0	0.074	3.006	0.754	7.322
DA901WB2	1770.0	11.633	0.6848	65.0	0.099	2.932	0.702	8.558
DA901ZB2	2208.0	84.589	4.2284	61.0	0.058	3.237	0.788	7.792

Table 23b. The abundance, biomass, number of taxa, and diversity of sites sampled at 100 m in Prince William Sound in July 1990. Fragments and species excluded from cluster analysis ARE excluded in all computations except biomass. The identifications in most cases were to the generic and specific level. Included in the data analysis shown in this table are organisms that could not be identified to generic and specific levels and were entered at the Family or higher taxonomic level.

STATION	ABUNDANCE	WET WEIGHT BIOMASS	CARBON BIOMASS	NUMBER OF TAXA	SIMPSON	SHANNON	SW EVEN	SPECIES RICHNESS
DA901BI2	3208.0	494.262	17.5501	58.0	0.140	2.539	0.625	7.060
DA901CB2	2830.0	8.792	0.4622	93.0	0.059	3.426	0.756	11.575
DA901DI2	3646.0	14.460	0.8667	90.0	0.085	3.106	0.690	10.852
DA901HB2	6886.0	31.235	1.6025	118.0	0.122	3.029	0.635	13.239
DA901NB2	2512.0	19.737	1.1670	86.0	0.106	3.087	0.693	10.857
DA901SB2	6912.0	65.048	2.2594	126.0	0.087	3.281	0.678	14.139
DA901SH2	1392.0	20.915	1.0394	75.0	0.049	3.437	0.796	10.223
DA901DB2	1698.0	7.356	0.3710	68.0	0.084	3.044	0.722	9.009
DA901LH2	2056.0	27.509	1.5750	67.0	0.076	3.035	0.722	8.652
DA901MB2	5040.0	55.471	3.0637	125.0	0.051	3.495	0.724	14.545
DA901MH2	2364.0	23.826	1.1071	149.0	0.046	3.908	0.781	19.052
DA901RB2	1232.0	113.988	4.7565	76.0	0.054	3.472	0.802	10.539
DA901WB2	1770.0	11.633	0.6848	88.0	0.091	3.124	0.698	11.633
DA901ZB2	2208.0	84.589	4.2284	103.0	0.046	3.668	0.791	13.247

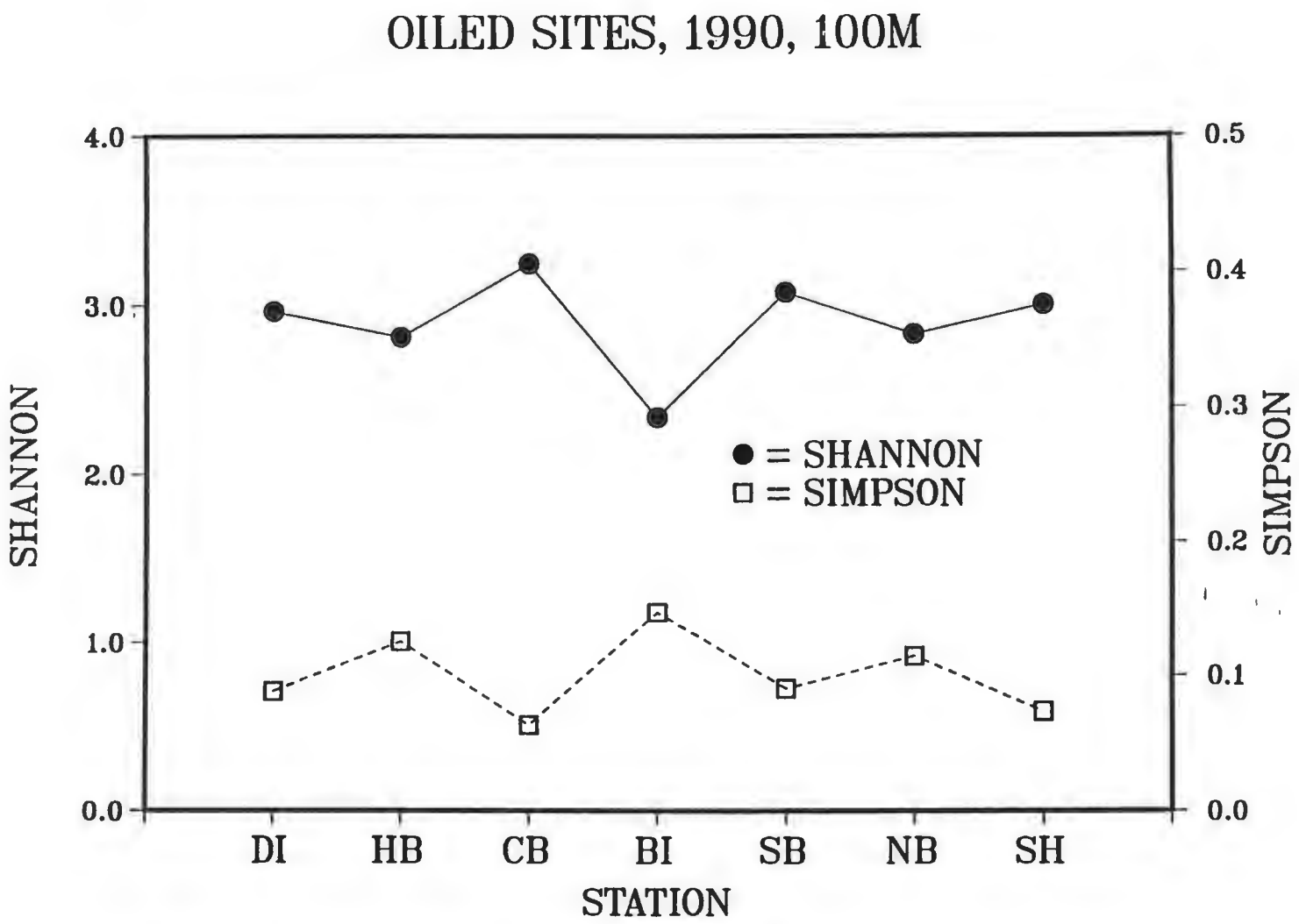

Figure 28. Shannon Diversity and Simpson Dominance values at stations at 100 m at oiled sites for samples collected July 1990 in Prince William Sound.

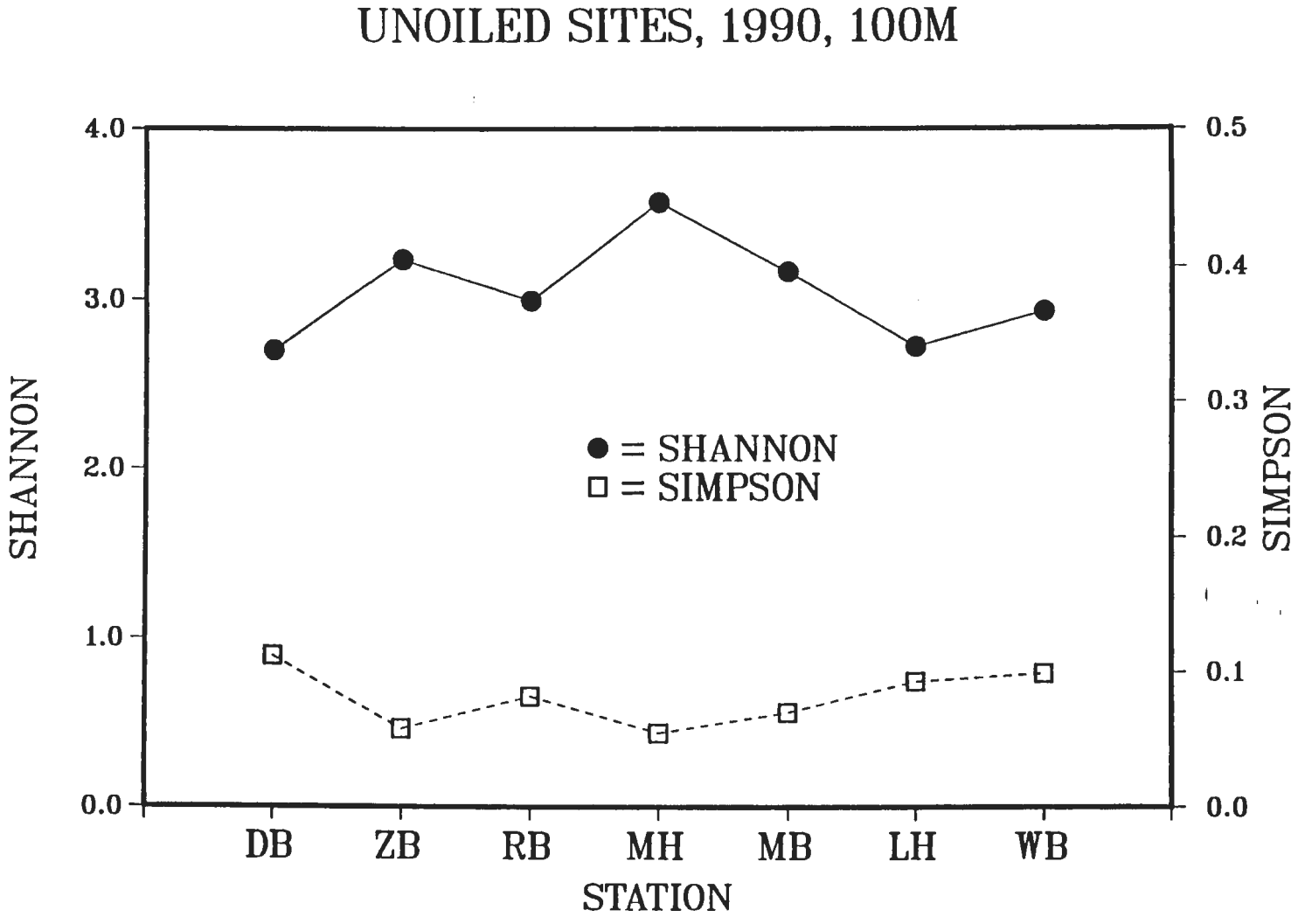

Figure 29. Shannon Diversity and Simpson Dominance values at stations at 100 m at unoiled sites for samples collected July 1990 in Prince William Sound.

by SR increases at all oiled and unoiled stations. However, no trends in SR are discernible.

The rank abundance of the dominant fauna collected at all stations occupied at 100 m are included in Tables 24 and 25. Differences in taxa generally dominating oiled and unoiled stations can be seen in these tables (statistical assessment of some of these differences, for higher taxon levels, are considered in a later section). The ranking differences, as might be expected, are not as clearcut in some respects as observed for stations at 40 m, although some of the same taxa observed at 40 m tend to dominate at oiled and unoiled sites.

Numerical Analysis

A normal cluster of *ln*-transformed data did not clearly differentiate station groups, and, in fact, suggested station groupings at a variety of similarity levels (Fig. 30). However, an assessment of the principal coordinate analysis (PCA) made it relatively clear that there are four station groups and one unoiled station (MacLeod Harbor) not joining any group (Table 26; Fig. 31). In the PCA, most of the variance in the data (74.2%) is explained by PC Axes 1 and 2. Station groupings are not particularly similar to what was determined for stations at 40 m. However, three of the oiled sites (Herring, Sleepy, and Chenega Bays) that were similar at 40 m are also closely allied at 100 m; the unoiled Mooselips Bay showed great affinity with these three oiled bays at 40 and 100 m. Two of the other oiled sites are closely associated in the analyses, Northwest Bay and Disk Island; however, they are also closely associated with an unoiled site at 100 m, West Bay.

The ranking of dominant taxa in each of the station groups and the percent occurrence of each dominant taxon at stations within the groups (e.g., a value of 100% means that a given taxon occurred at all stations within the group) is included in Table 27. The differences in taxa dominating within the station groups can be seen in this table.

K-Dominance Curves

The K-Dominance curves for the 14 stations at 100 m are presented in Figures 32 to 45. The only oiled sites whose curves suggested moderate disturbance (curves cross each other) are Herring Bay, Chenega Bay, and Sleepy Bay. The only curve that suggests a mild disturbance (i.e., curves close together but not crossing each other) at oiled sites was Disk Island. The curves for the other oiled sites suggest an undisturbed environment. The curves for most of the unoiled sites are those expected for an undisturbed environment; however, the closeness of curves at MacLeod Harbor and West Bay suggest a mild disturbance at these sites.

Feeding Types

The percent of feeding types present at oiled and unoiled stations are presented in Figures 46 and 47. The categories depicted are surface deposit feeders (SDF), subsurface deposit feeders (SSDF), carnivores (CARN), suspension feeders (SF), and

Text continued on page 119.

Table 24. Rank abundance (no. m^{-2}) by families and higher taxa for all stations at 100 m for data collected in Prince William Sound, July 1990.

Station	Condition	Dominant Taxa	Abundance (no. m^{-2})
DI	Oiled	Golfingiidae	754
		Bivalvia	512
		Nematoda	344
		Cossuridae	240
		Lumbrineridae	202
		Thyasiridae	160
		Cirratulidae	148
		Paraonidae	140
		Owenidae	134
		Sabellidae	130
		Capitellidae	92
		Nephtyidae	82
		Nuculanidae	64
		Syllidae	54
		Maldanidae	52
		Spionidae	42
HB	Oiled	Nematoda	812
		Golfingiidae	412
		Paraonidae	352
		Bivalvia	334
		Cirratulidae	326
		Lumbrineridae	326
		Syllidae	302
		Maldanidae	232
		Capitellidae	188
		Phoxocephalidae	162
		Spionidae	138
		Sabellidae	134
		Owenidae	94
		Ampharetidae	92
		Harpacticoida	62
		Cossuridae	48
		Terebellidae	48
SLB	Oiled	Nematoda	1664
		Golfingiidae	642
		Sabellidae	528
		Bivalvia	520
		Syllidae	420
		Paraonidae	296
		Owenidae	266
		Cirratulidae	266
		Polyodontidae	210
		Phoxocephalidae	190
		Maldanidae	132
		Lumbrineridae	132
		Gnathiidae	128
		Ampharetidae	116
		Ampeliscidae	106
		Capitellidae	98
		Terebellidae	82
		Sigalionidae	72
		Spionidae	68
		Harpacticoida	68
		Gastropoda	62
		Tanaidacea	58
		Ostracoda	50
		Montacutidae	44
		Nephtyidae	44
		Dentaliidae	42
NWB	Oiled	Bivalvia	708
		Lumbrineridae	276
		Cirratulidae	216
		Golfingiidae	172
		Paraonidae	144
		Sabellidae	120
		Capitellidae	116
		Spionidae	90
		Nematoda	56
		Nephtyidae	52
		Cossuridae	46
		Maldanidae	38
		Dentaliidae	36
		Owenidae	34
		Owenidae	34
		Syllidae	30
		Polyodontidae	30
		Phoxocephalidae	30
SH	Oiled	Nephtyidae	192
		Leuconidae	190
		Paraonidae	162
		Bivalvia	110
		Lumbrineridae	106
		Spionidae	84
		Ostracoda	60
		Cirratulidae	50
		Scaphandridae	42
		Gastropoda	36
		Nuculanidae	34
		Cossuridae	34
		Capitellidae	30
		Hesionidae	28
		Nuculidae	26
		Ophiuroidea	20
CN	Oiled	Nematoda	476
		Sabellidae	284
		Bivalvia	188
		Golfingiidae	186
		Paraonidae	144
		Ampharetidae	132
		Syllidae	126
		Onuphidae	108
		Cirratulidae	92
		Owenidae	90
		Archaeogastropoda	68
		Lumbrineridae	62
		Maldanidae	58
		Spionidae	48
		Capitellidae	44
		Dentaliidae	42
		Astartidae	40
BI	Oiled	Tellinidae	910
		Cirratulidae	498
		Lumbrineridae	472
		Spionidae	312
		Hesionidae	212
		Nephtyidae	202
		Capitellidae	168
		Polynoidae	70
		Paraonidae	58
		Cossuridae	30
		Nuculidae	28
		Bivalvia	28

Table 24. (continued)

Station	Condition	Dominant Taxa	Abundance (no. m^{-2})
BI (cont'd)		Nuculanidae	24
		Thyasiridae	22
		Phyllodocidae	20
		Oedicerotidae	20
DB	Unoiled	Nephtyidae	388
		Bivalvia	310
		Paraonidae	152
		Lumbrineridae	134
		Leuconidae	114
		Orbiniidae	82
		Nuculidae	72
		Cossuridae	64
		Nuculanidae	52
		Spionidae	40
		Cirratulidae	34
		Nematoda	28
ZB	Unoiled	Cirratulidae	278
		Sternaspidae	266
		Lumbrineridae	172
		Nuculanidae	172
		Leuconidae	142
		Thyasiridae	92
		Tellinidae	92
		Amphiuridae	78
		Capitellidae	70
		Nephtyidae	68
		Bivalvia	66
		Spionidae	60
		Ostracoda	52
		Owenidae	50
		Paraonidae	46
		Dentaliidae	42
		Orbiniidae	42
RB	Unoiled	Nephtyidae	256
		Nuculanidae	152
		Tellinidae	94
		Nuculidae	72
		Paraonidae	64
		Thyasiridae	50
		Spionidae	48
		Ostracoda	44
		Lumbrineridae	42
		Leuconidae	40
		Capitellidae	38
		Sternaspidae	36
		Owenidae	36
		Scaphandridae	26
		Gastropoda	24
		Hesionidae	24
		Pyrenidae	24
		Bivalvia	20
		Amphiuridae	20
MCH	Unoiled	Owenidae	424
		Polyodontidae	152
		Syllidae	122
		Maldanidae	118
		Golfingiidae	96
		Capitellidae	90
		Spionidae	90
		Cirratulidae	90
MCH (cont'd)		Bivalvia	86
		Phoxocephalidae	72
		Terebellidae	66
		Mytilidae	64
		Pyrenidae	62
		Gnathiidae	46
		Paraonidae	42
MLB	Unoiled	Capitellidae	666
		Spionidae	588
		Polyodontidae	578
		Cirratulidae	522
		Syllidae	420
		Nematoda	206
		Bivalvia	188
		Phoxocephalidae	160
		Maldanidae	152
		Paraonidae	144
		Sabellaridae	124
		Owenidae	114
		Lumbrineridae	112
		Sabellidae	82
		Laqueidae	62
		Ampharetidae	58
		Terebellidae	50
		Anthozoa	42
		Golfingiidae	42
		Nuculidae	40
		Ophiuroidea	40
LH	Unoiled	Paraonidae	312
		Bivalvia	264
		Nephtyidae	260
		Cirratulidae	246
		Lumbrineridae	238
		Spionidae	142
		Capitellidae	82
		Leuconidae	72
		Nematoda	66
		Orbiniidae	40
		Nuculanidae	40
		Phoxocephalidae	36
		Maldanidae	32
		Gastropoda	28
		Opheliidae	24
		Hesionidae	20
		Rhynchocoela	20
WB	Unoiled	Bivalvia	420
		Lumbrineridae	248
		Nematoda	166
		Nephtyidae	104
		Golfingiidae	84
		Dentaliidae	70
		Owenidae	68
		Paraonidae	66
		Cirratulidae	64
		Sternaspidae	42
		Leuconidae	38
		Capitellidae	34
		Montacutidae	32
		Thyasiridae	32
		Spionidae	30
		Onuphidae	24

Table 25. Rank abundance (no. m^{-2}) by species orgenus[1] for all stations at 100 m for data collected in Prince William Sound, July 1990.

Station	Condition	Dominant Taxa	Abundance (no. m^{-2})
DI	Oiled	*Golfingia margaritacea*	740
		Bivalvia	512
		Nematoda	344
		Cossura sp.	240
		Adontorhina cyclia	160
		Lumbrineris spp.	158
		Cirratulidae	148
		Myriochele oculata	134
		Sabellidae	130
		Paraonidae	116
		Capitellidae	92
		Nuculana sp.	58
		Nephtyidae	54
		Ninoe gemmea	44
		Maldanidae	44
		Spionidae	38
		Dentalium sp.	38
		Exogone sp.	38
		Gastropoda	36
		Ostracoda	34
HB	Oiled	Nematoda	812
		Golfiniga margaritacea	412
		Bivalvia	334
		Cirratulidae	326
		Lumbrineris spp.	256
		Exogone sp.	218
		Maldanidae	190
		Capitellidae	188
		Phoxocephalidae	162
		Paraonidae	158
		Peisidice aspera	138
		Tauberia gracilis	110
		Spionidae	88
		Myriochele oculata	78
		Sabellidae	70
		Harpacticoida	62
		Cossura sp.	48
		Aricidea ramosa	44
		Syllidae	44
		Polydora sp.	42
SB	Oiled	Nematoda	1664
		Golfingia margaritacea	640
		Sabellidae	526
		Bivalvia	520
		Cirratulidae	266
		Exogone sp.	242
		Paraonidae	220
		Peisidice aspera	210
		Myriochele oculata	204
		Phoxocephalidae	190
		Gnathia sp.	128
		Maldanidae	116
		Lumbrineris spp.	110
		Capitellidae	98
		Sphaerosyllis sp.	86
		Ampharetidae	86
		Syllidae	72
		Pholoe minuta	72
		Harpacticoida	68
		Gastropoda	62
SLB (cont'd)		Tanaidacea	58
		Spionidae	52
		Byblis sp.	50
		Owenia fusiformis	50
		Ampelisca sp.	50
		Spinosphaera sp.	50
		Ostracoda	50
NB	Oiled	Bivalvia	708
		Cirratulidae	216
		Lumbrineris spp.	186
		Golfingia margaritacea	172
		Sabellidae	120
		Capitellidae	116
		Tauberia gracilis	70
		Ninoe gemmea	60
		Nematoda	56
		Polydora sp.	50
		Aeidicira antennata	48
		Cossura sp.	46
		Spionidae	40
		Dentalium sp.	36
		Myriochele oculata	34
		Phoxocephalidae	30
		Peisidice aspera	30
		Lumbrineridae	30
SH	Oiled	Nephtyidae	172
		Tauberia gracilis	122
		Bivalvia	110
		Spionidae	84
		Eudorella sp.	78
		Lumbrineris spp.	66
		Eudorella emarginata	64
		Ostracoda	60
		Cirratulidae	50
		Eudorellopsis sp.	46
		Paraonidae	40
		Gastropoda	36
		Cossura sp.	34
		Capitellidae	28
		Nucula tenuis	26
		Scaphandridae	24
		Lumbrineridae	24
		Hesionidae	22
		Nuculana fossa	20
		Ophiuroidea	20
CN	Oiled	Nematoda	476
		Sabellidae	284
		Bivalvia	188
		Golfingia margaritacea	184
		Foraminiferida	156
		Paraonidae	122
		Cirratulidae	92
		Myriochele oculata	86
		Archaeogastropoda	68
		Onuphis sp.	68
		Melinna elisabethae	66
		Ampharetidae	66
		Syllidae	66
		Lumbrineris spp.	60
		Maldanidae	54

Table 25. (continued)

Station	Condition	Dominant Taxa	Abundance (no. m^{-2})
CN (cont'd)		Capitellidae	44
		Exogone sp.	44
		Dentalium sp.	42
		Onuphidae	40
		Spionidae	40
BI	Oiled	*Macoma carlottensis*	910
		Cirratulidae	498
		Lumbrineris spp.	472
		Prionospio cirrifera	222
		Hesionidae	174
		Capitellidae	166
		Nephtyidae	124
		Polynoidae	70
		Paraonidae	58
		Nephtys sp.	40
		Gyptis brevipalpa	38
		Spionidae	36
		Prionospio sp.	34
		Nephtys cornuta	34
		Cossura sp.	30
DB	Unoiled	Bivalvia	310
		Nephtys cornuta	280
		Paraonidae	140
		Lumbrineris spp.	112
		Nephtyidae	98
		Orbiniidae	82
		Nucula tenuis	72
		Leucon sp.	66
		Cossura sp.	64
		Nuculana sp.	42
		Cirratulidae	34
		Polydora sp.	30
		Nematoda	28
		Eudorella sp.	24
		Ninoe gemmea	22
ZB	Unoiled	Cirratulidae	278
		Sternaspis scutata	266
		Lumbrineris spp.	142
		Macoma sp.	88
		Amphiuridae	78
		Capitellidae	70
		Bivalvia	66
		Spionidae	60
		Nuculana sp.	60
		Ostracoda	52
		Adontorhina cyclia	48
		Eudorellopsis sp.	46
		Eudorella emarginata	44
		Dentalium sp.	42
		Orbiniidae	42
		Pholoe minuta	38
		Nuculanidae	36
		Eudorellopsis integra	34
		Thyasiridae	34
		Nephtys sp.	32
		Myriochele oculata	32
		Ninoe gemmea	30
RB	Unoiled	*Nephtys cornuta*	212
		Macoma sp.	84
		Nucula tenuis	72
		Nuculana sp.	66
		Yoldia sp.	56
		Spionidae	48
		Ostracoda	44
		Tauberia gracilis	42
		Capitellidae	38
		Sternaspis scutata	36
		Lumbrineris spp.	32
		Nephtys sp.	32
		Adontorhina cyclia	30
		Myriochele oculata	30
		Gyptis brevipalpa	24
		Eudorella emarginata	24
		Gastropoda	24
		Alia gausapata	24
		Paraonidae	22
		Cylichna alba	20
MH	Unoiled	*Myriochele oculata*	400
		Peisidice aspera	152
		Golfingia margaritacea	96
		Capitellidae	90
		Cirratulidae	90
		Bivalvia	86
		Exogone sp.	76
		Spionidae	72
		Petaloproctus tenuis borealis	68
		Alia gausapata	62
		Phoxocephalidae	56
		Gnathia sp.	46
		Syllidae	38
		Terebellidae	32
		Scaphandridae	30
		Maldanidae	30
		Pista cristata	30
		Magelona sp.	30
MB	Unoiled	*Peisidice aspera*	578
		Cirratulidae	522
		Capitellidae	408
		Exogone sp.	330
		Spionidae	296
		Polydora sp.	286
		Mediomastus sp.	256
		Nematoda	206
		Bivalvia	188
		Phoxocephalidae	160
		Myriochele oculata	114
		Lumbrineris spp.	112
		Maldanidae	92
		Idanthyrsus sp.	86
		Paraonidae	86
		Sabellidae	82
		Laqueus californianus	62
		Ampharetidae	56
		Syllidae	52
		Tauberia gracilis	52
		Golfingia margaritacea	42
		Anthozoa	42
		Ophiuroidea	40

Table 25. (continued)

Station	Condition	Dominant Taxa	Abundance (no. m^{-2})
LH	Unoiled	Bivalvia	264
		Nephtyidae	256
		Cirratulidae	246
		Paraonidae	216
		Lumbrineris spp.	214
		Spionidae	96
		Capitellidae	82
		Nematoda	66
		Tauberia gracilis	54
		Polydora sp.	44
		Orbiniidae	40
		Phoxocephalidae	40
		Eudorellopsis sp.	36
		Maldanidae	30
		Gastropoda	28
		Nuculana fossa	24
		Ninoe gemmea	24
		Opheliidae	24
		Aedicira antennata	22
WB	Unoiled	Bivalvia	420
		Lumbrineris spp.	210
		Nematoda	166
		Golfingia margaritacea	82
		Dentalium sp.	70
		Myriochele oculata	66
		Cirratulidae	64
		Nephtys sp.	62
		Tauberia gracilis	60
		Sternaspis scutata	42
		Ninoe gemmea	38
		Capitellidae	38
		Odontogena borealis	32
		Adontorhina cyclia	32
		Onuphis sp.	22
		Nephtys cornuta	22
		Eudorella emarginata	20

[1] As noted in Methods, generic and specific names were recorded on data sheets whenever possible. Although analyses were accomplished with data at the family or higher taxonomic levels, this table makes available the generic and specific designations recorded on data sheets.

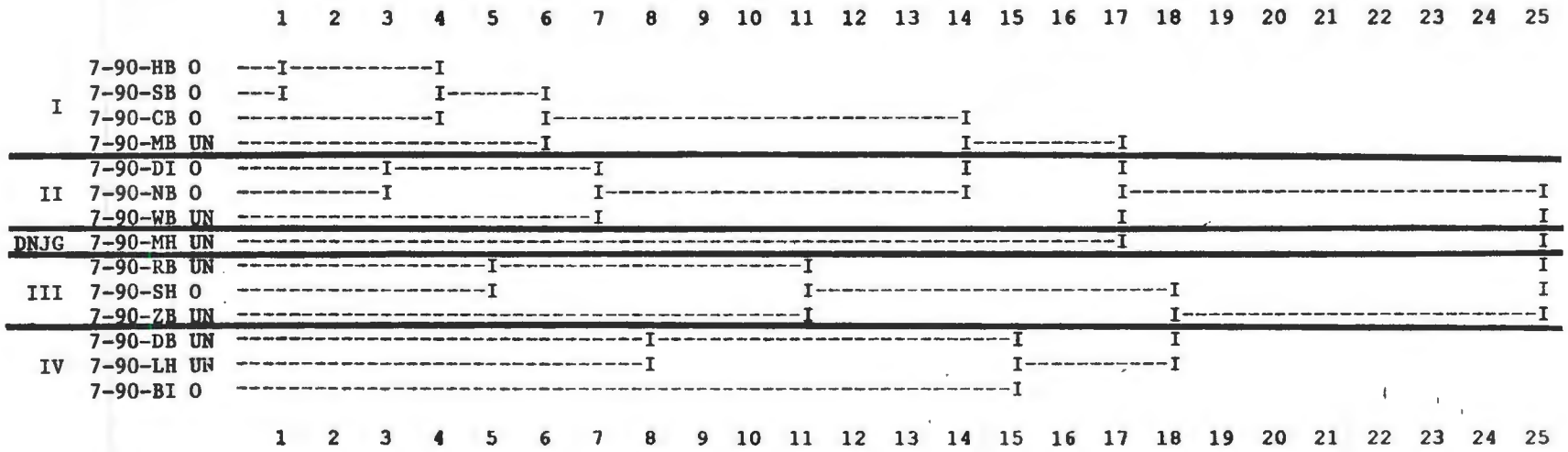

Figure 30. Dendrogram depicting the station groups formed at several levels of similarity by a normal cluster analysis on *ln*-transformed abundance data from 100 m in July 1990 at sites within Prince William Sound. Stations appear, for example, as 7-90-DI (date - site of Disk Island). DNJG = did not join a cluster group. O = oiled, UN = unoiled.

Table 26. Station groups based on hierarchical cluster and principal coordinate analyses of the July 1990 Prince William Sound benthic data from 100 m. O = oiled station, UN = unoiled station.

Group I Stations
Herring Bay (O)
Sleepy Bay (O)
Chenega Bay (O)
Mooselips Bay (UN)
Group II Stations
Disk Island (O)
Northwest Bay (O)
West Bay (UN)
Group III Stations
Rocky Bay (UN)
Snug Harbor (O)
Zaikof Bay (UN)
Group IV
Drier Bay (UN)
Lower Herring Bay (UN)
Bay of Isles (O)
Ungrouped Station
MacLeod Harbor

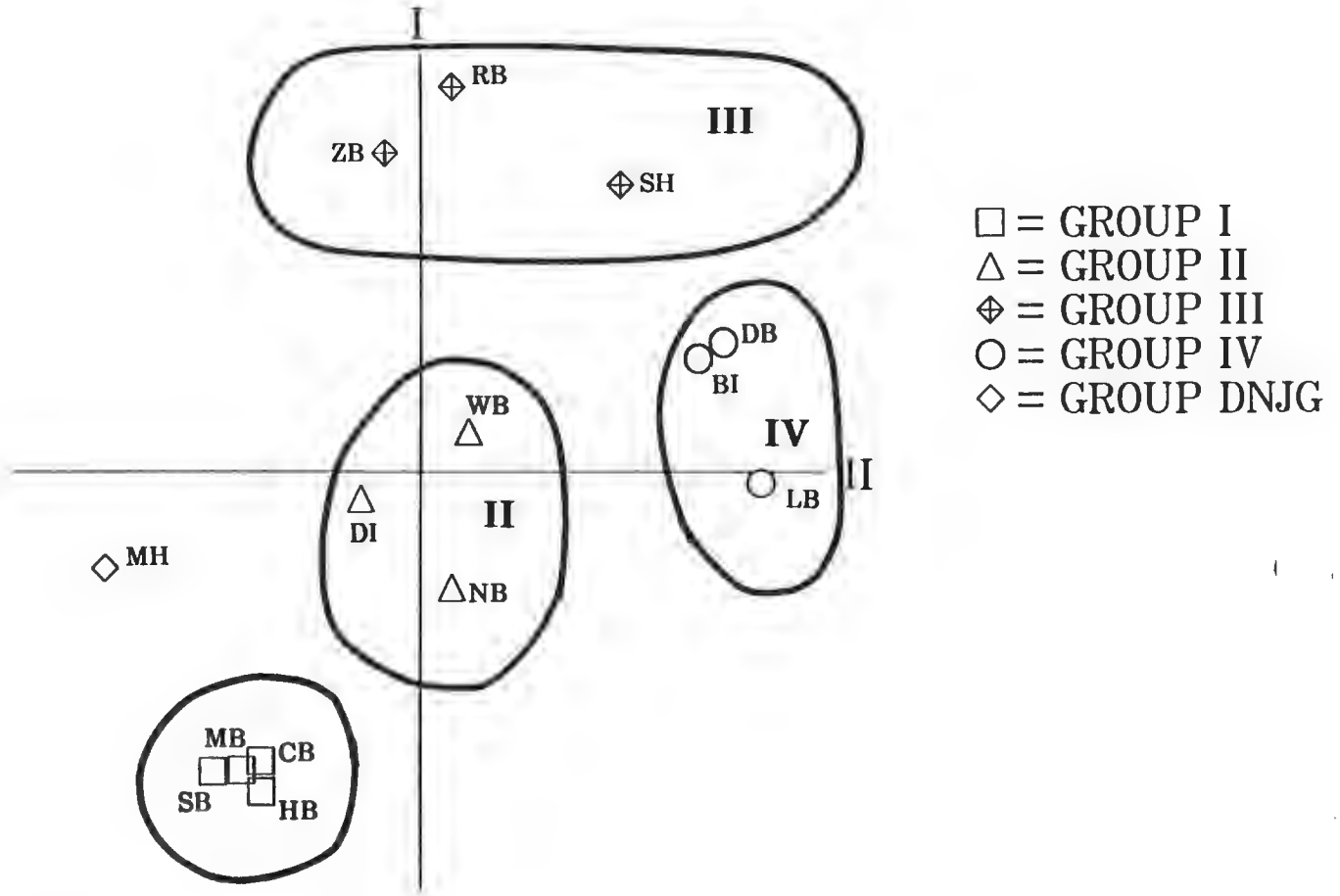

Figure 31. Plot of the first two coordinate axes of a principal coordinate analysis of *ln*-transformed abundance data from 100 m in July 1990 at sites within Prince William Sound.

Table 27. Ranking by abundance of dominant taxa within the station groups at 100 m delineated by multivariate analysis of deep benthic data collected in Prince William Sound July 1990. Percent frequency = percentage of stations within the station group.

Dominant Taxa	Abundance (no. m^{-2})	Frequency[1] (%)
Station Group I		
Nematoda	790	100
Golfingiidae	321	100
Syllidae	317	100
Bivalvia	308	100
Cirratulidae	302	100
Capitellidae	249	100
Sabellidae	247	100
Polyodontidae	241	100
Paraonidae	234	100
Spionidae	210	100
Lumbrineridae	158	100
Maldanidae	144	100
Owenidae	141	100
Phoxocephalidae	134	100
Ampharetidae	96	100
Gnathiidae	58	100
Terebellidae	48	100
Station Group II		
Bivalvia	547	100
Golfingiidae	337	100
Lumbrineridae	242	100
Nematoda	189	100
Cirratulidae	143	100
Paraonidae	117	100
Cossuridae	97	100
Sabellidae	89	100
Capitellidae	81	100
Nephtyidae	79	100
Owenidae	79	100
Thyasiridae	64	67
Spionidae	54	100
Dentaliidae	48	100
Maldanidae	33	100
Syllidae	29	100
Nuculanidae	29	100

Table 27. (cont'd)

Dominant Taxa	Abundance (no. m^{-2})	Frequency[1] (%)
Station Group III		
Nephtyidae	172	100
Leuconidae	124	100
Nuculanidae	119	100
Cirratulidae	113	100
Lumbrineridae	107	100
Sternaspidae	101	67
Paraonidae	91	100
Tellinidae	67	100
Bivalvia	65	100
Spionidae	64	100
Thyasiridae	53	100
Ostracoda	52	100
Capitellidae	46	100
Nuculidae	40	100
Amphiuridae	34	100
Owenidae	31	100
Scaphandridae	29	100
Gastropoda	22	100
Hesionidae	20	100
Orbiniidae	19	100
Station Group IV		
Tellinidae	311	100
Nephtyidae	283	100
Lumbrineridae	281	100
Cirratulidae	259	100
Bivalvia	201	100
Paraonidae	174	100
Spionidae	165	100
Capitellidae	87	100
Hesionidae	81	100
Orbiniidae	43	100
Nuculanidae	39	100
Cossuridae	35	100
Nematoda	32	100
Polynoidae	25	100

Table 27. (cont'd)

Dominant Taxa	Abundance (no. m^{-2})	Frequency[1] (%)
Station Group V		
Owenidae	424	100
Polyodontidae	152	100
Syllidae	122	100
Maldanidae	118	100
Golfingiidae	96	100
Spionidae	90	100
Capitellidae	90	100
Cirratulidae	90	100
Bivalvia	86	100
Phoxocephalidae	72	100
Terebellidae	66	100
Mytilidae	64	100
Pyrenidae	62	100
Gnathiidae	46	100
Paraonidae	42	100
Scaphandridae	32	100
Magelonidae	32	100
Nematoda	28	100

[1]The values in this column, for each of the dominant taxa within the station group, are based on the number of stations at which the particular taxon occurs.

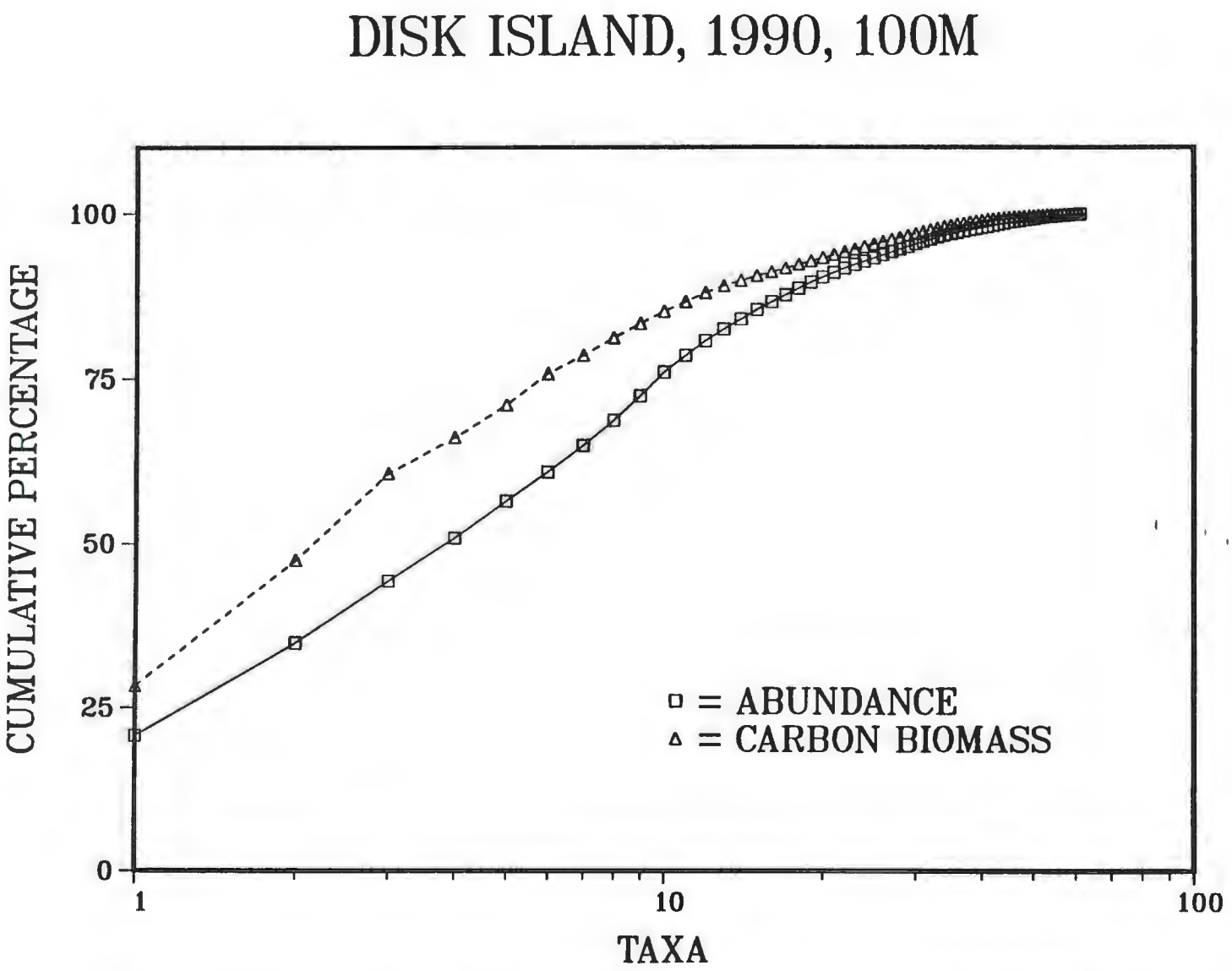

Figure 32. K-dominance curves for benthic organisms collected at 100 m at Disk Island in July 1990.

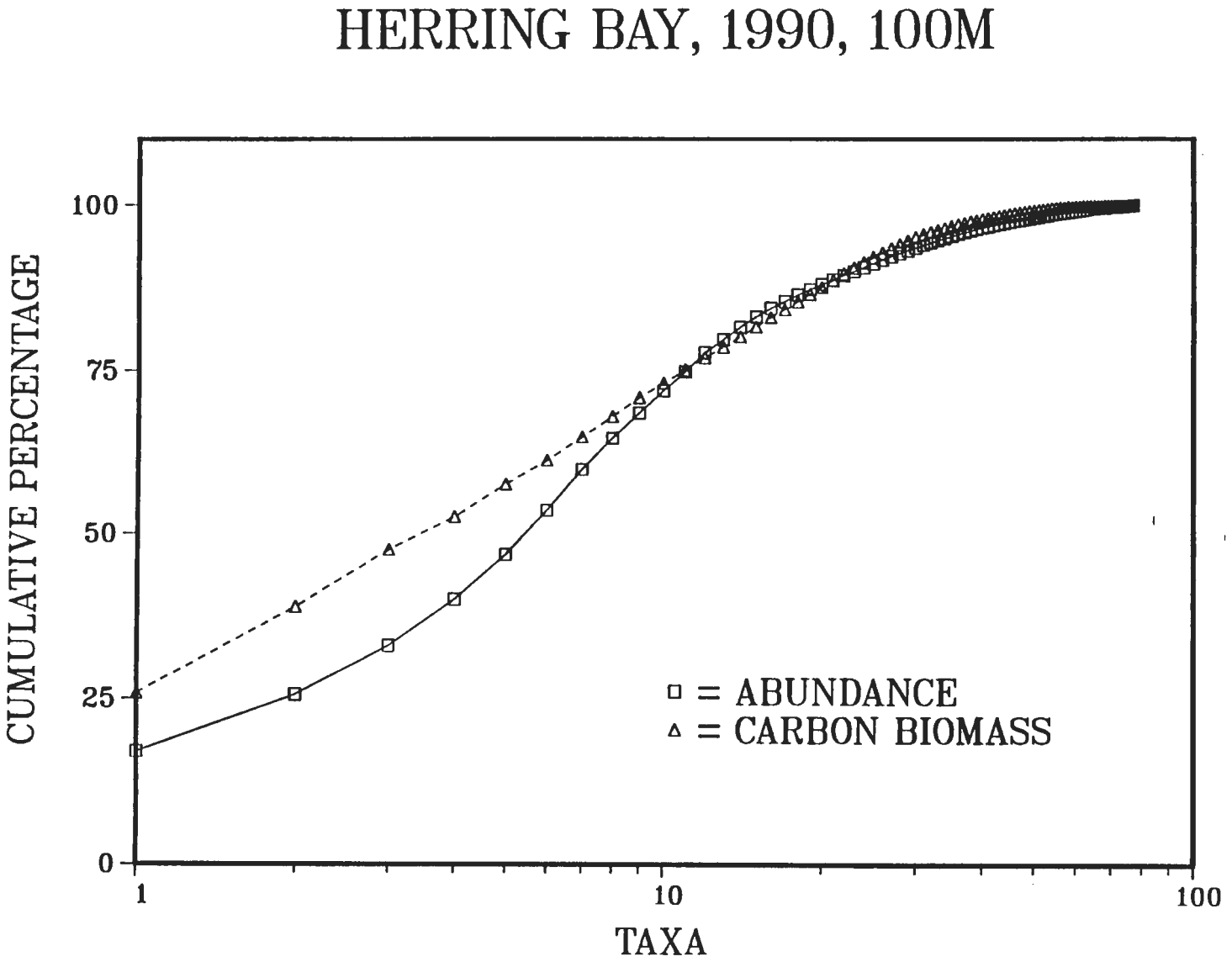

Figure 33. K-dominance curves for benthic organisms collected at 100 m in Herring Bay in July 1990.

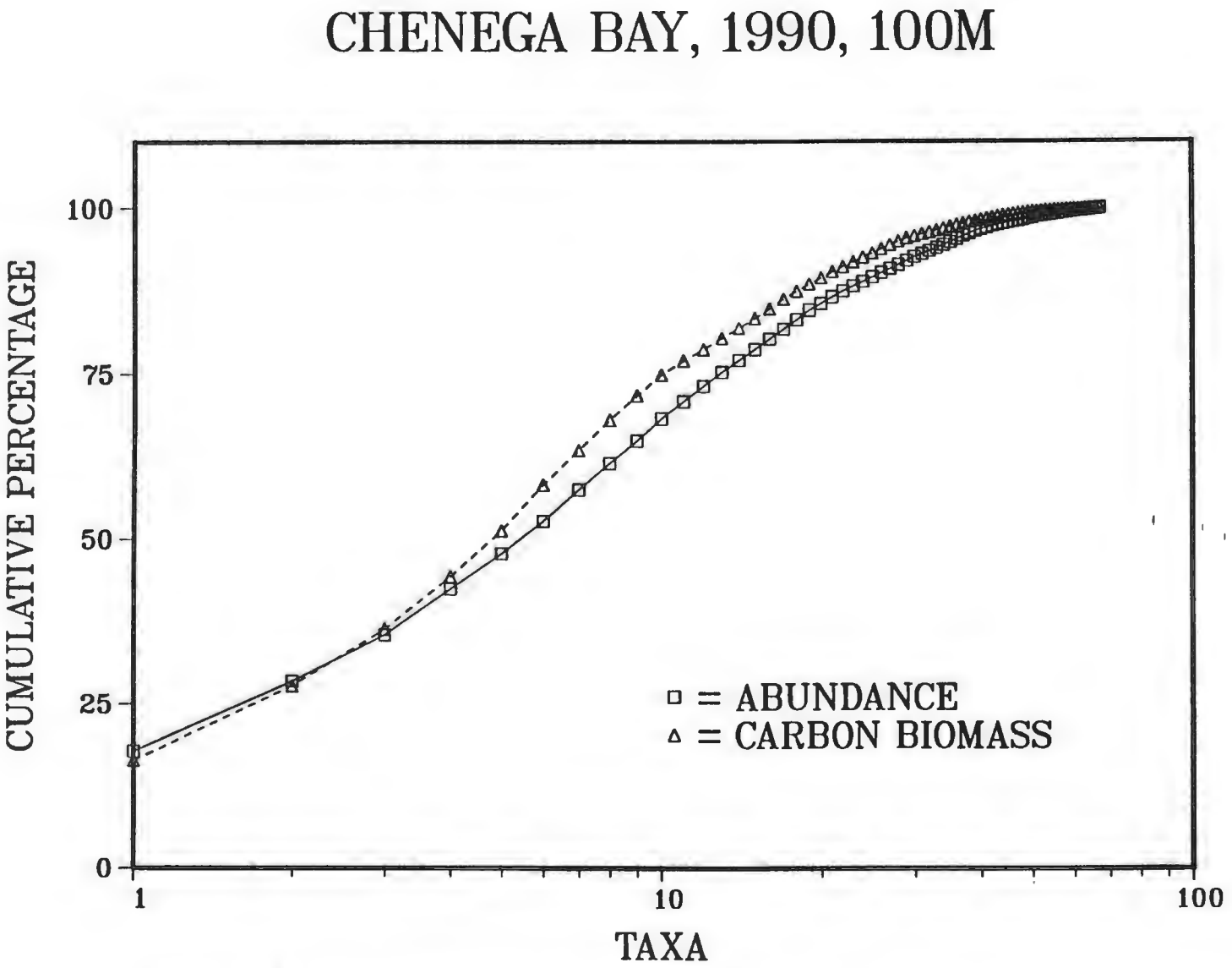

Figure 34. K-dominance curves for benthic organisms collected at 100 m in Chenega Bay in July 1990.

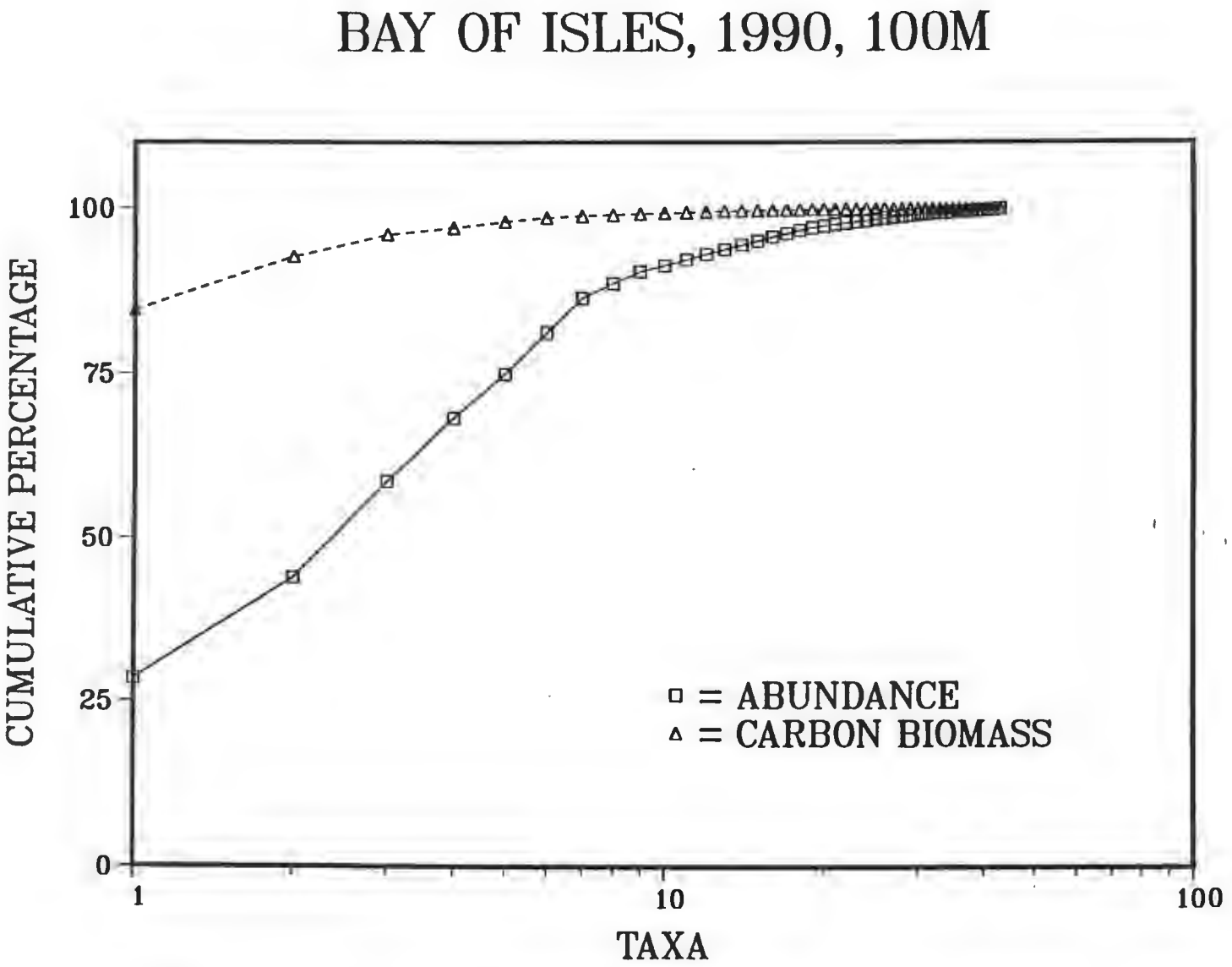

Figure 35. K-dominance curves for benthic organisms collected at 100 m in Bay of Isles in July 1990.

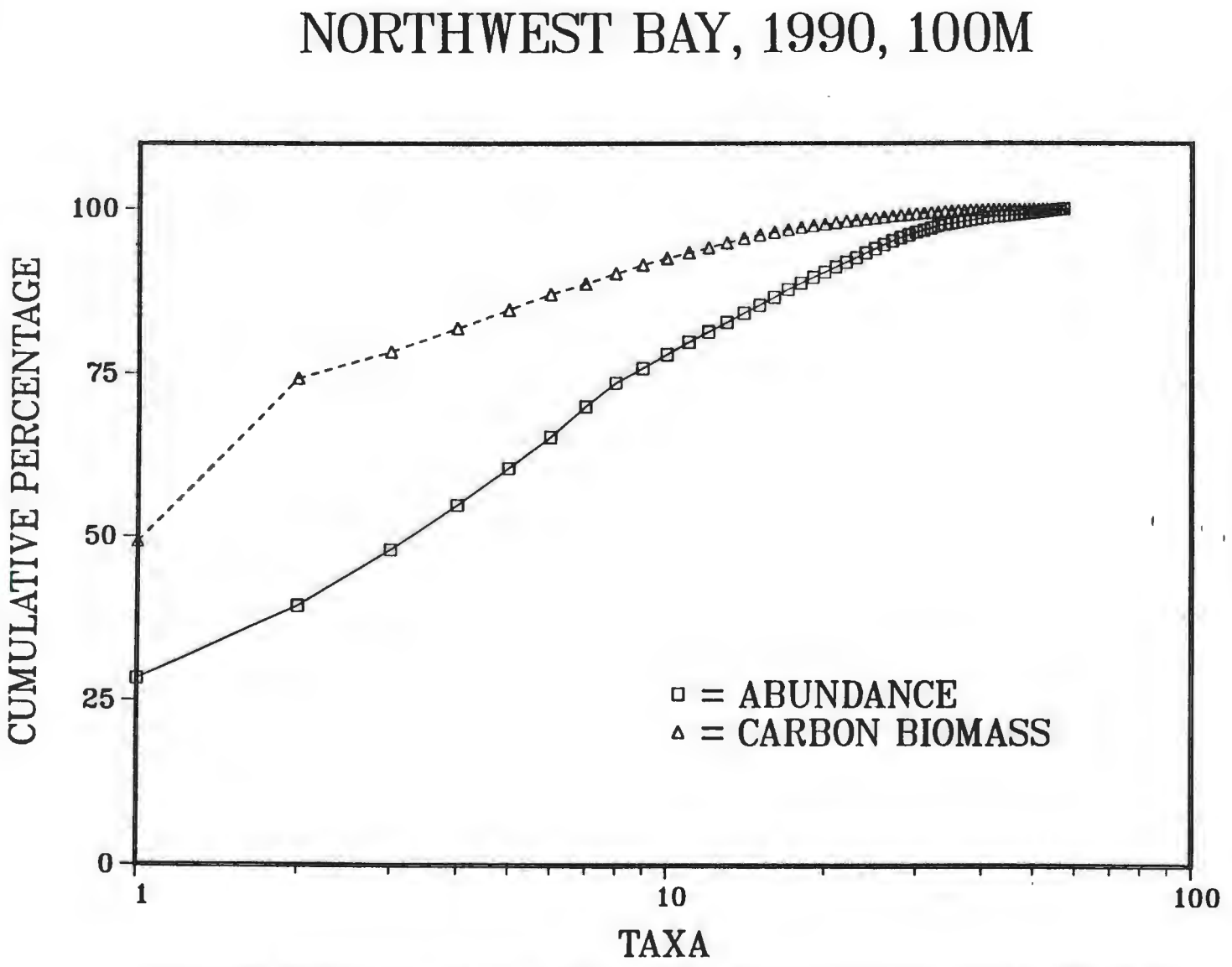

Figure 36. K-dominance curves for benthic organisms collected at 100 m in Northwest Bay in July 1990.

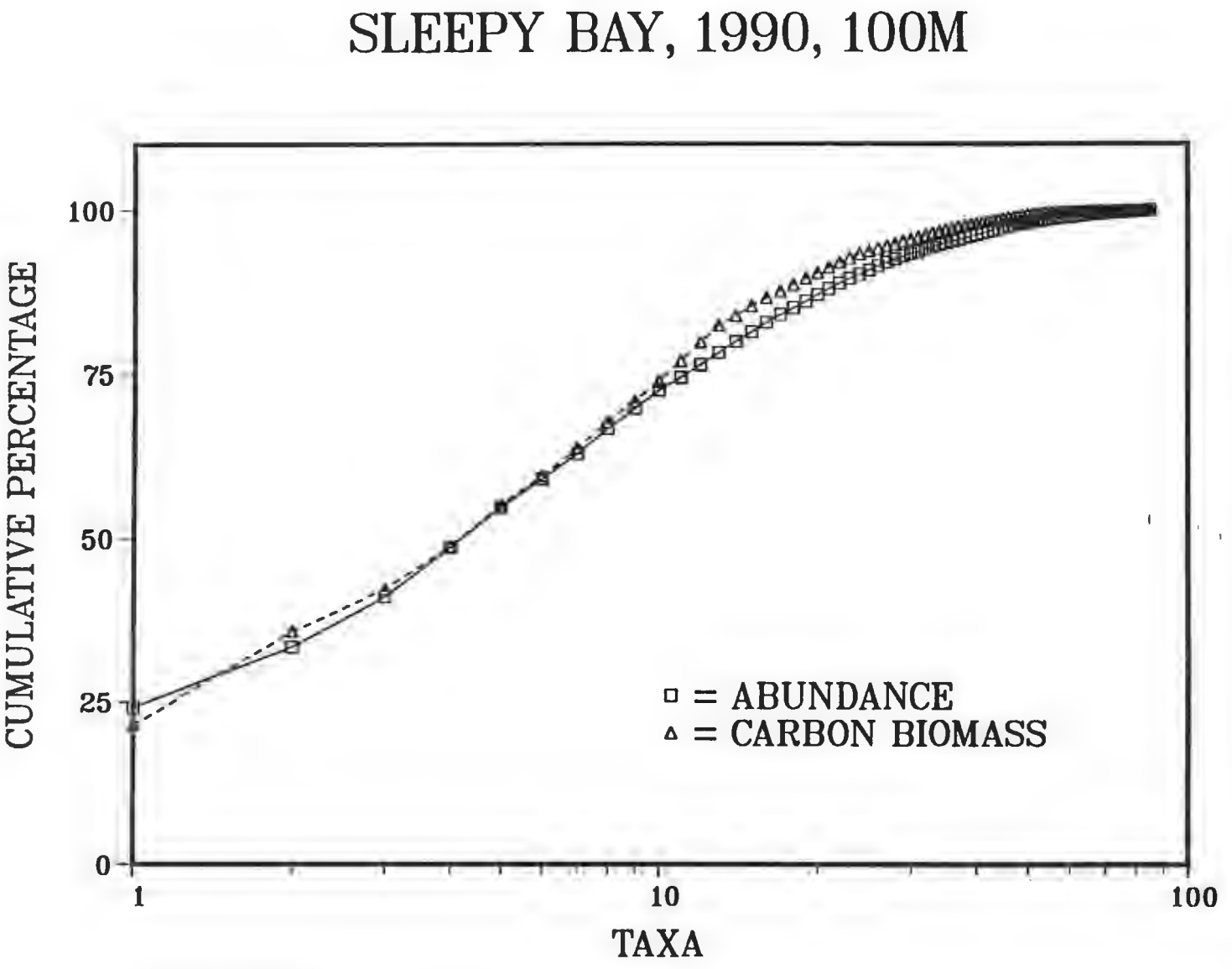

Figure 37. K-dominance curves for benthic organisms collected at 100 m in Sleepy Bay in July 1990.

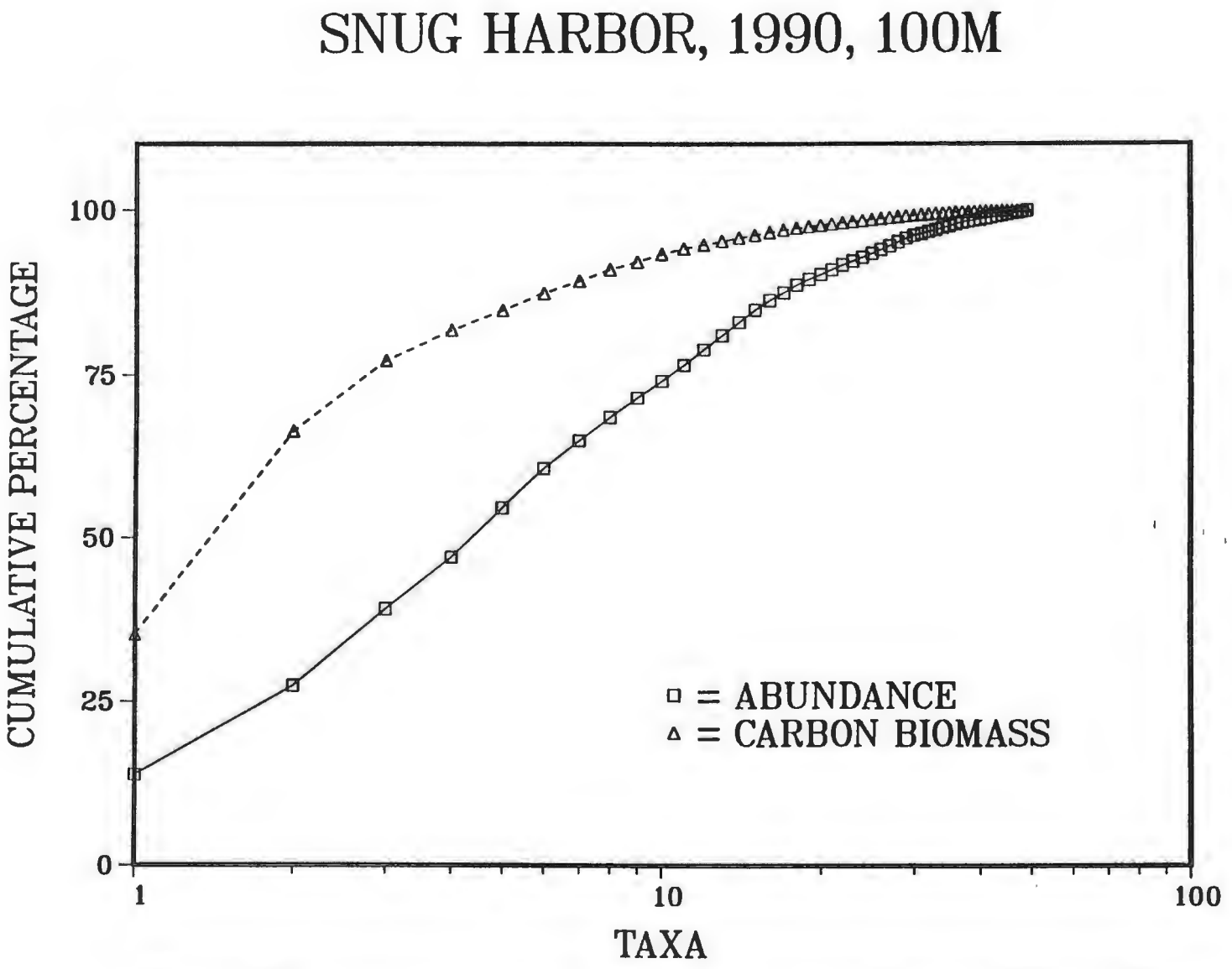

Figure 38. K-dominance curves for benthic organisms collected at 100 m in Snug Harbor in July 1990.

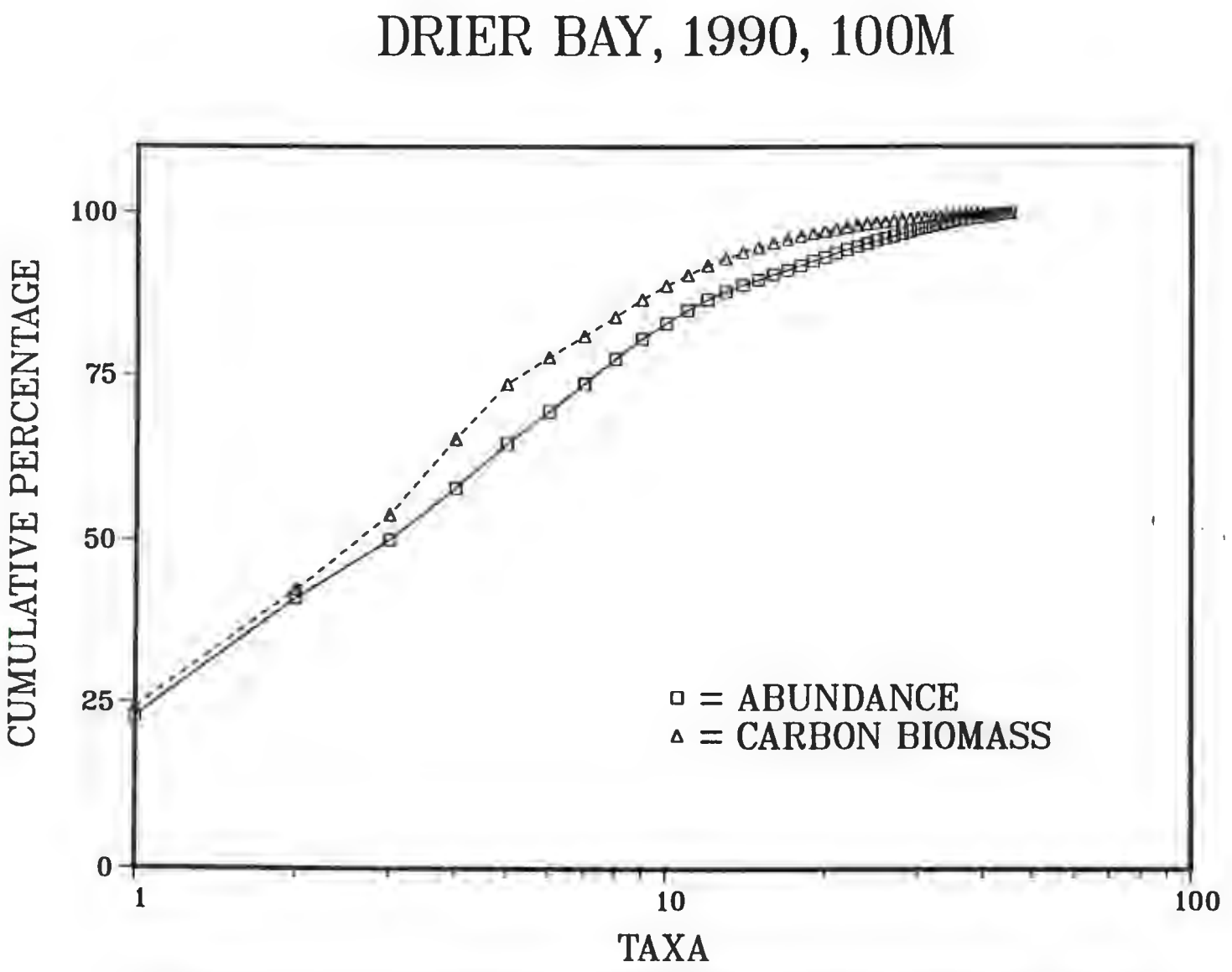

Figure 39. K-dominance curves for benthic organisms collected at 100 m in Drier Bay in July 1990.

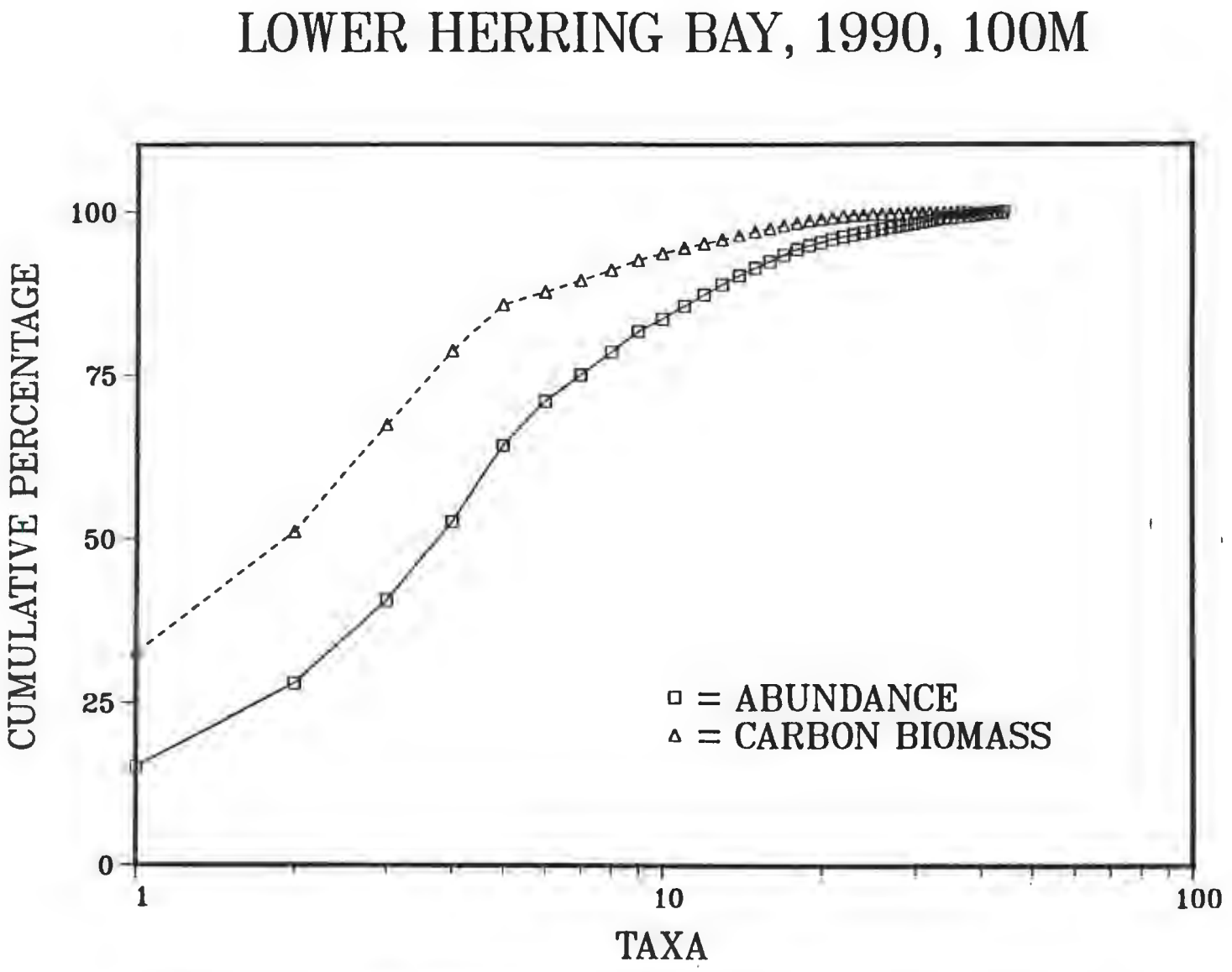

Figure 40. K-dominance curves for benthic organisms collected at 100 m in Lower Herring Bay in July 1990.

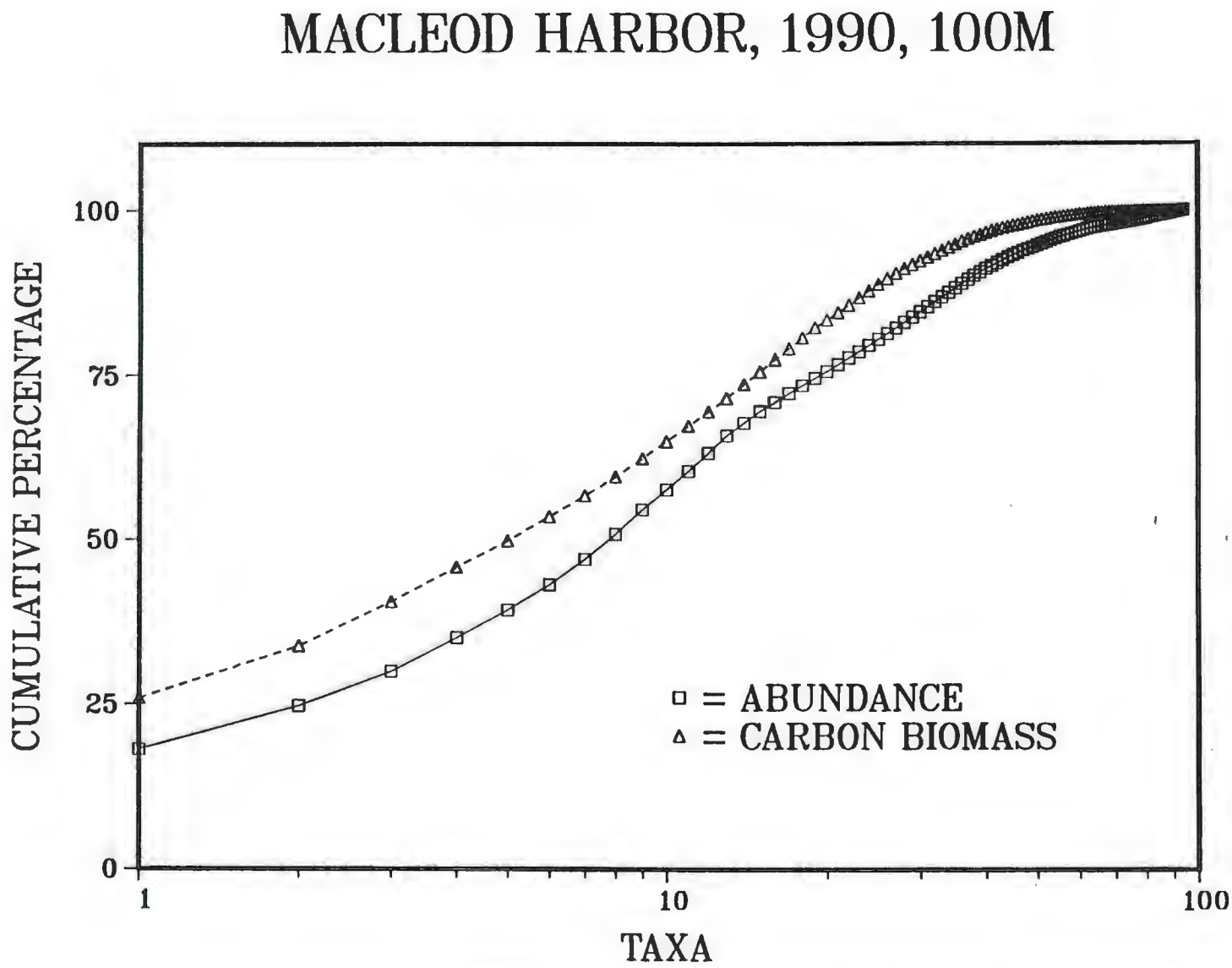

Figure 41. K-dominance curves for benthic organisms collected at 100 m in MacLeod Harbor in July 1990.

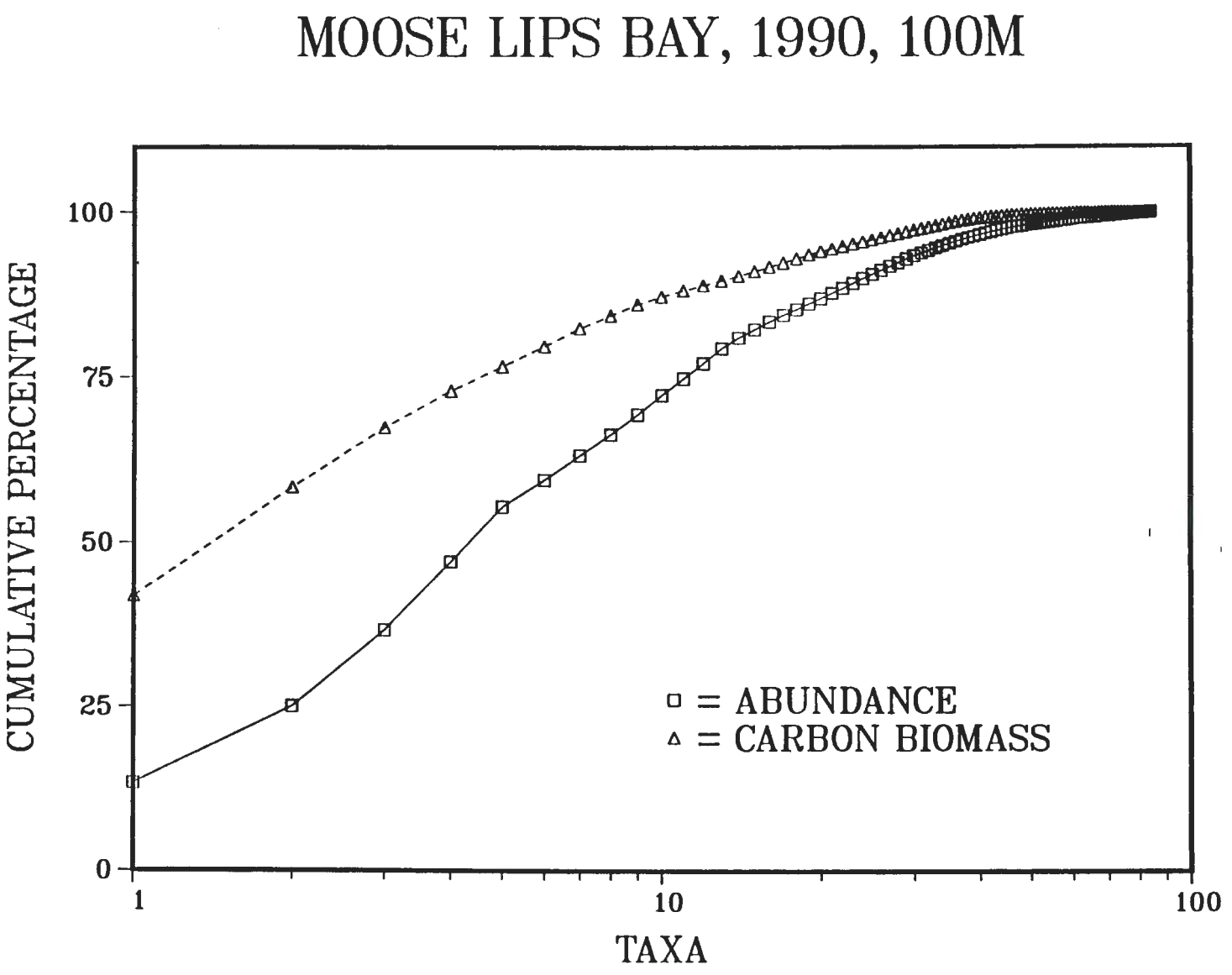

Figure 42. K-dominance curves for benthic organisms collected at 100 m in Mooselips Bay in July 1990.

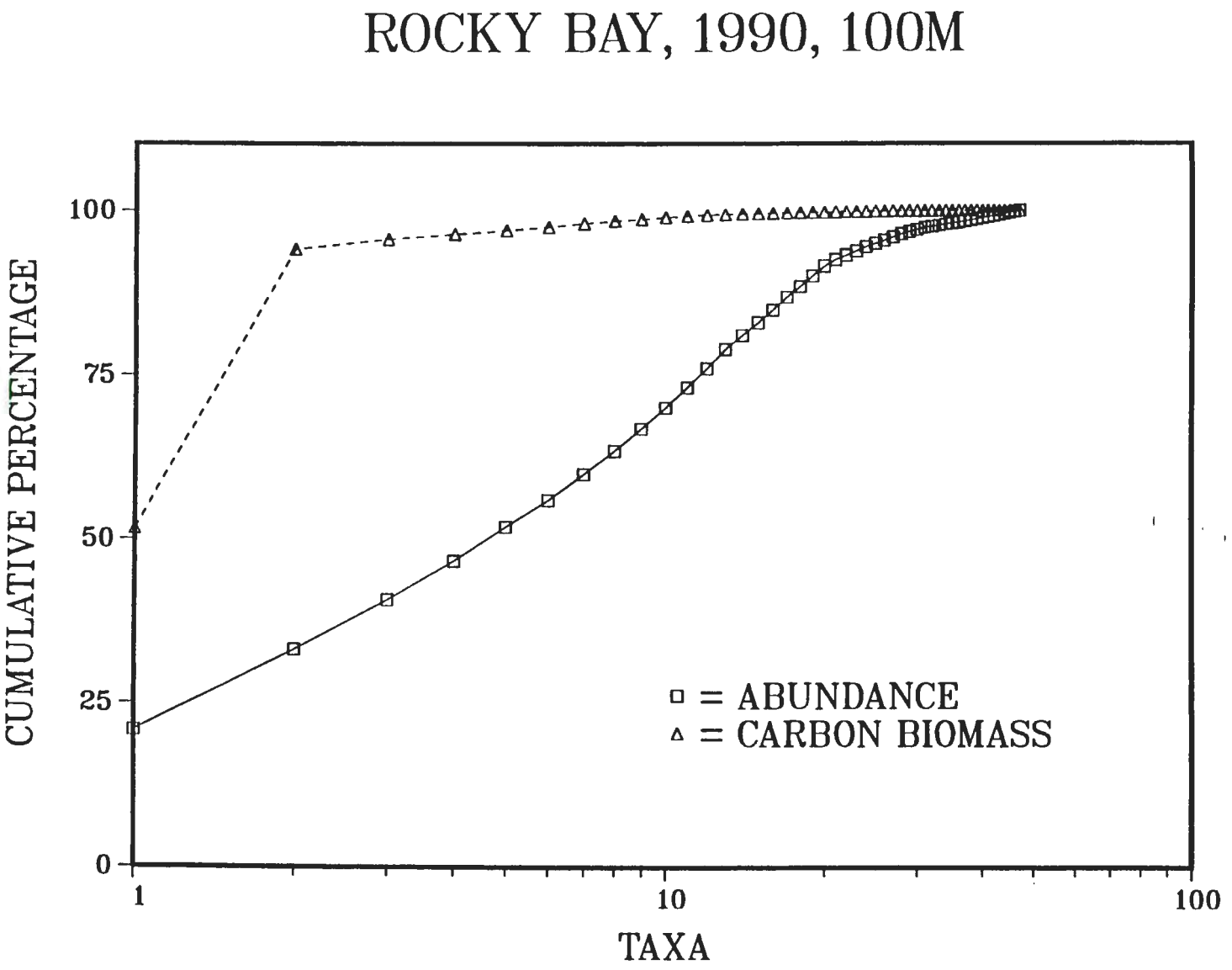

Figure 43. K-dominance curves for benthic organisms collected at 100 m in Rocky Bay in July 1990.

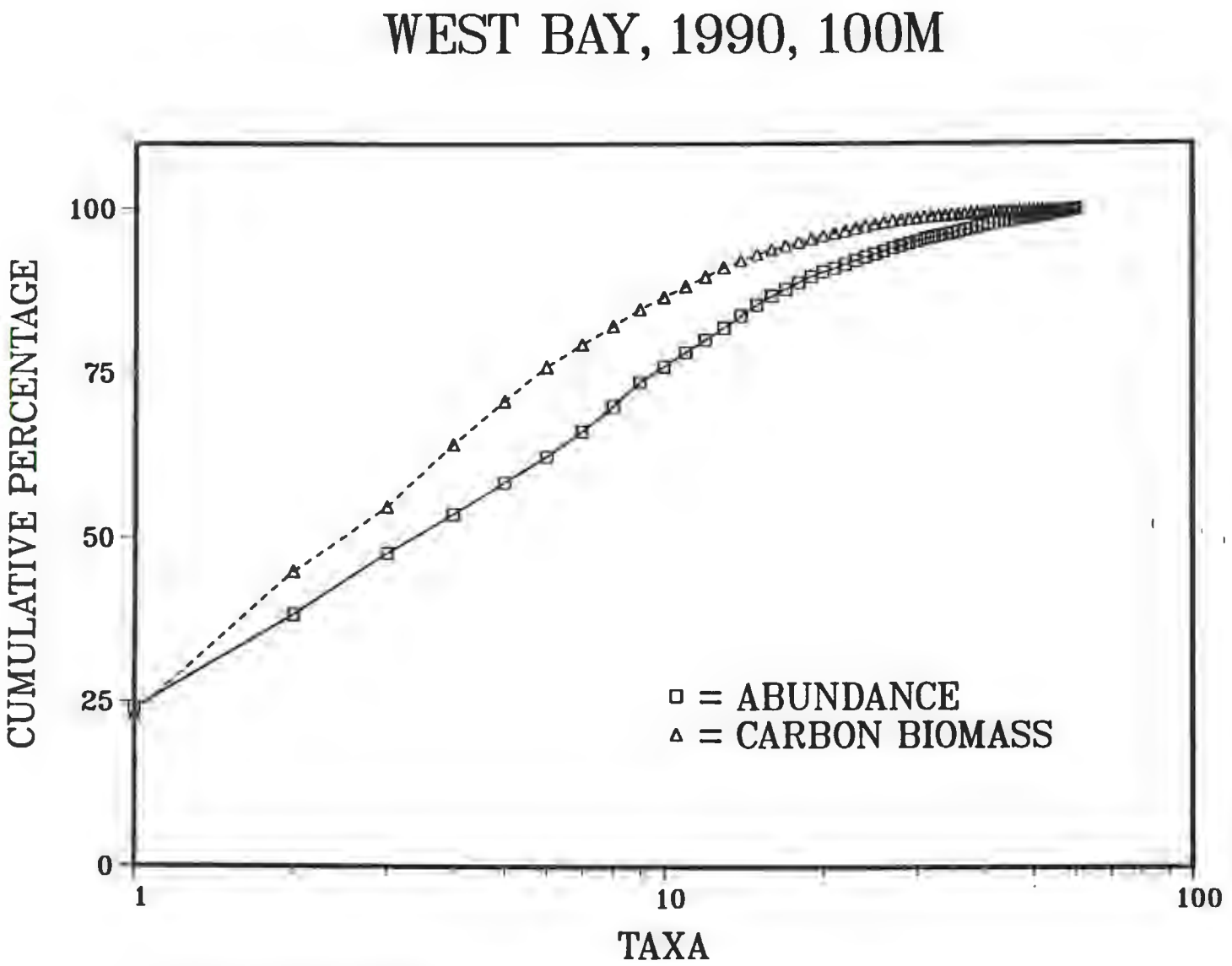

Figure 44. K-dominance curves for benthic organisms collected at 100 m in West Bay in July 1990.

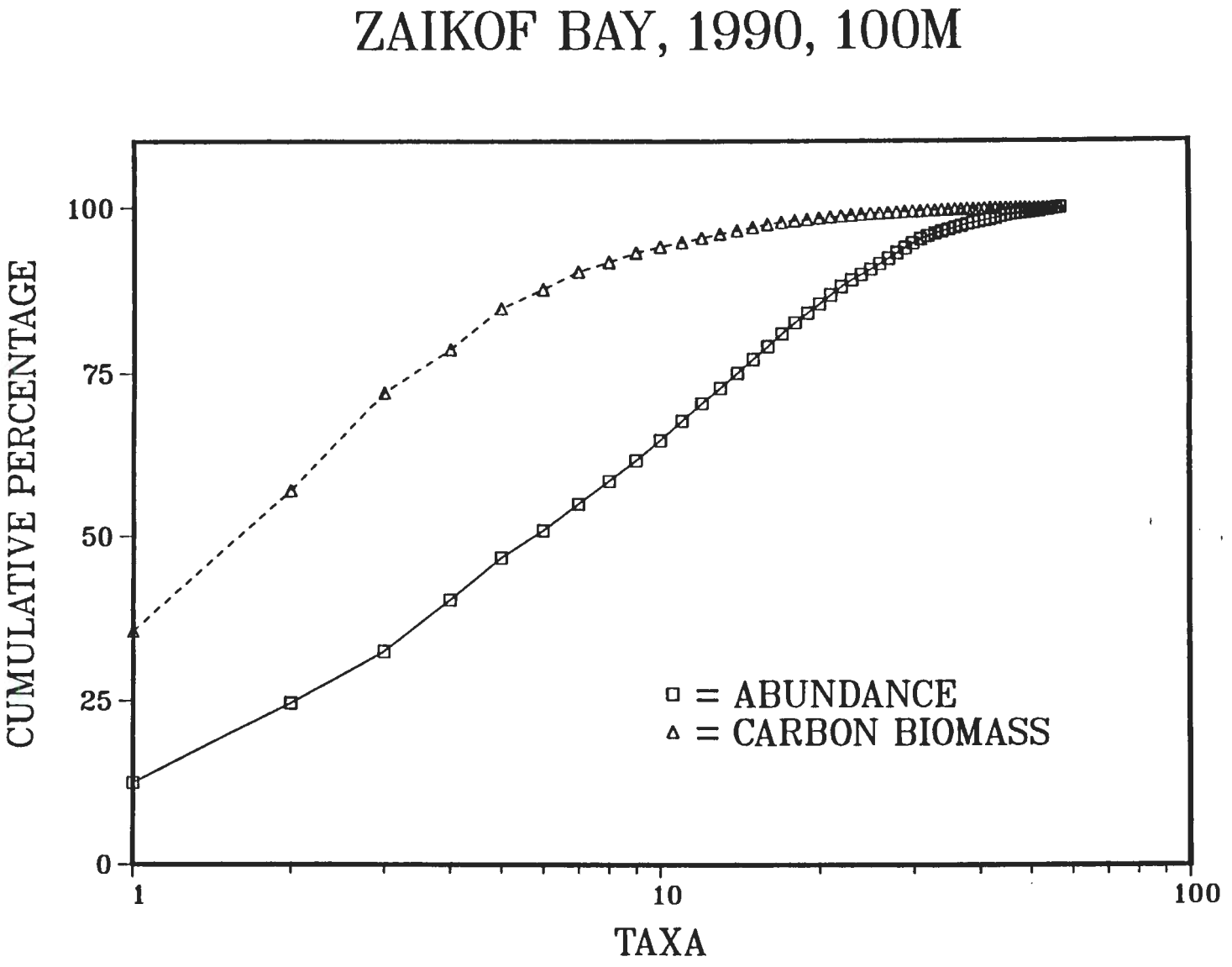

Figure 45. K-dominance curves for benthic organisms collected at 100 m in Zaikof Bay in July 1990.

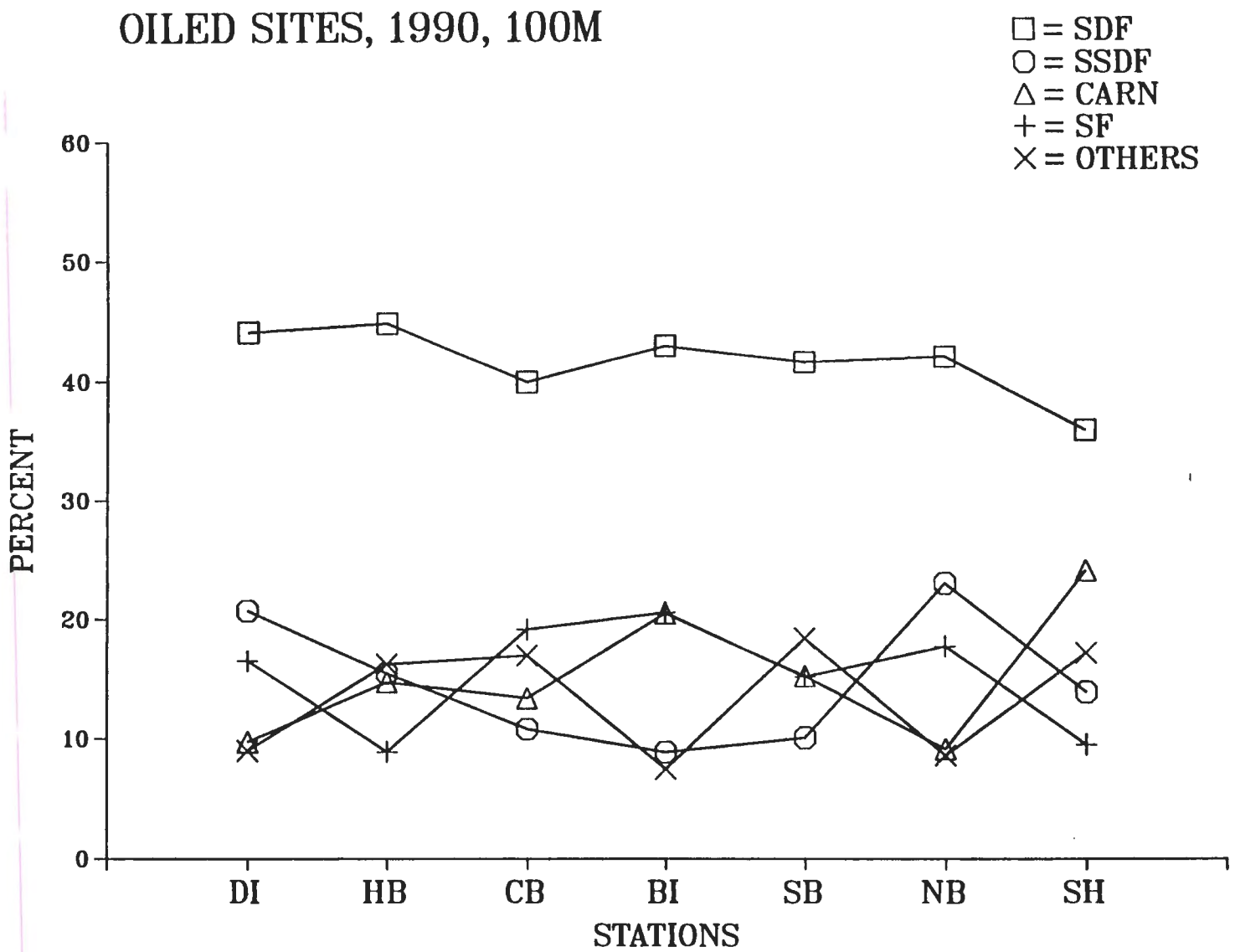

Figure 46. The percent abundance of feeding types at stations at 100 m at oiled sites from data collected July 1990 in Prince William Sound. SDF = surface deposit feeders; SSDF = subsurface deposit feeders; CARN = predators; SF = suspension feeders; Others = scavengers and herbivores.

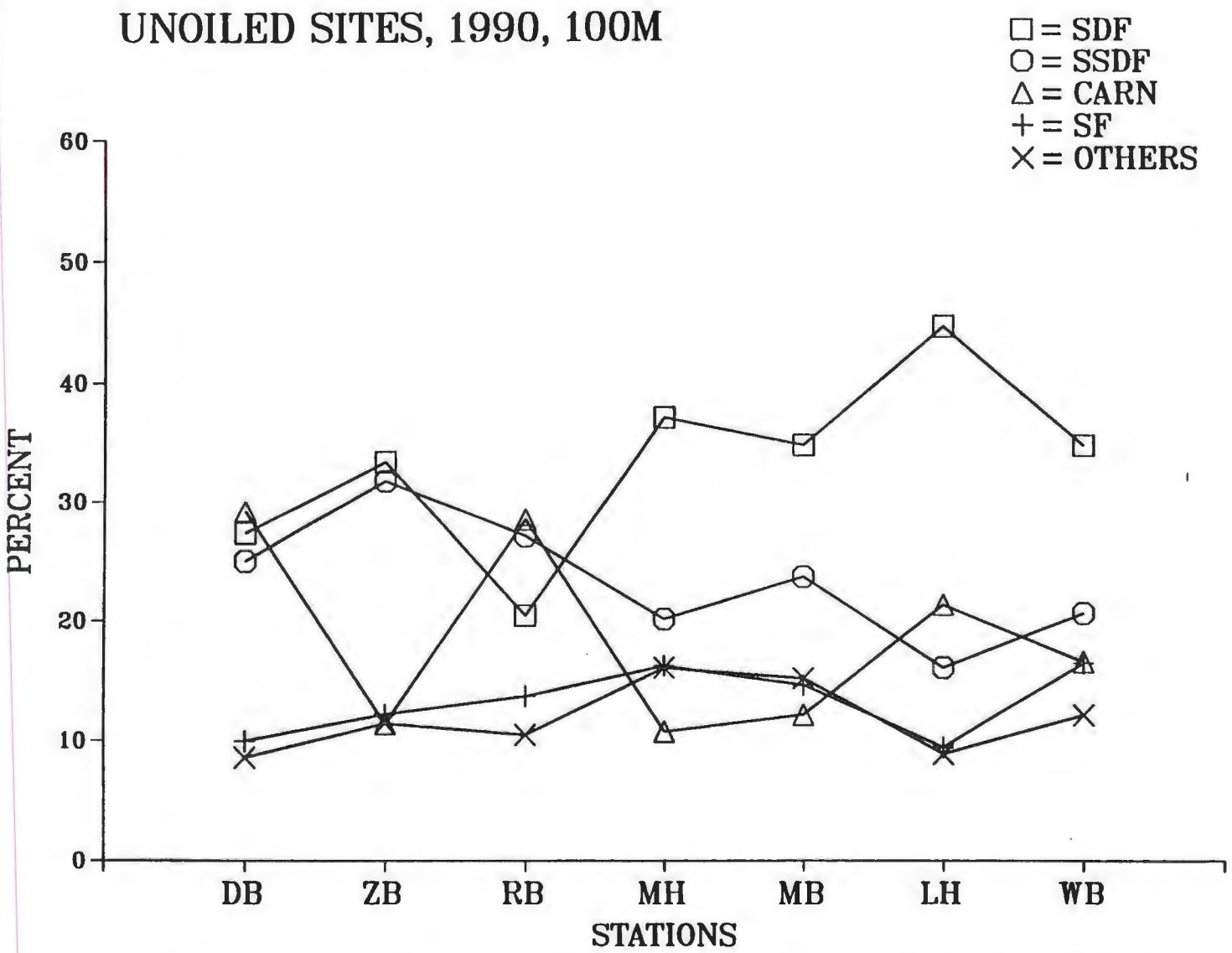

Figure 47. The percent abundance of feeding types at stations at 40 m at unoiled sites from data collected July 1990 in Prince William Sound. SDF = surface deposit feeders; SSDF = subsurface deposit feeders; CARN = predators; SF = suspension feeders; Others = scavengers and herbivores.

others (comprised of scavengers and herbivores). At the oiled sites SDF are dominant, relative to the other feeding types, at all of the stations sampled. SDF are common at the unoiled sites as well, but the other feeding types at most of the sites are almost as important so that a separation of the SDF curve from the other curves is not always as obvious.

The abundance of SDF at the pooled 7 oil sites is significantly greater ($P=0.001$) than at the unoiled sites (Table 11). Likewise, if comparisons between sites chosen by the diving component of the Coastal Habitat study are made, the oiled sites have significantly more individuals that are SDF. The latter comparisons are for Bay of Isles (oiled) vs. Drier Bay (unoiled): $P=0.026$, and Herring Bay (oiled) vs Lower Herring Bay (unoiled): $P=0.017$. A comparison made in last year's Annual Report for 40 m, Herring Bay vs. Zaikof Bay (unoiled) was repeated for the 100 m station. SDF were significantly more abundant ($P=0.010$) in Herring Bay, the oiled site. If the SDF and SF are combined (both feeding at the benthic boundary layer: McCave, 1974) into a group termed Interface Feeders (IF; Josefson, 1985), IF at pooled oil sites are significantly more abundant at oiled sites than at unoiled sites. Similar significantly higher abundance values were found for IF at Bay of Isles (oiled) relative to Drier Bay (unoiled), Herring Bay (oiled) vs. Lower Herring Bay (unoiled) and Herring Bay vs. Zaikof Bay (unoiled). Additionally, if SDF are combined with the feeding type "Other" (scavengers and herbivores), the abundance of these feeding types at the oiled sites is significantly higher ($P=0.000$) than at the unoiled sites.

Presence of Amphipods and other Crustaceans at Stations

At 100 m no particular pattern of abundance could be noted for the amphipods and crustaceans, in general, at oiled vs unoiled sites (Figs. 48 and 49). However, crustacean populations seem to be relative high at most of the oiled sites. Abundance values for crustaceans at unoiled sites also seemed to be variable with no particular pattern apparent.

Statistical Assessment of Taxon Abundance and Biomass

Comparison of all (seven) pooled oiled vs. unoiled sites at 100 m (Tables 28-35)

A. Abundance Comparisons (see Tables 28, 30, 32, 34 for P values)

1. Taxonomic groups at significantly higher abundance levels at sites were:

Nematoda		Sabellidae (polychaete)	
Ampharetidae (polychaete)		Syllidae	"
Cirratulidae	"	Terebellidae	"
Cossuridae	"	Golfingidae (sipunculid)	
Hesionidae		Ampeliscidae (amphipod)	
Lumbrineridae	"	Phoxocephalidae	"
Maldanidae	"	Gnathidae	
Onuphidae	"	Bivalvia (clams)	
Paraonidae	"	Tellinidae	"
		Dentaliidae (scaphopod)	

Text continued on page 138.

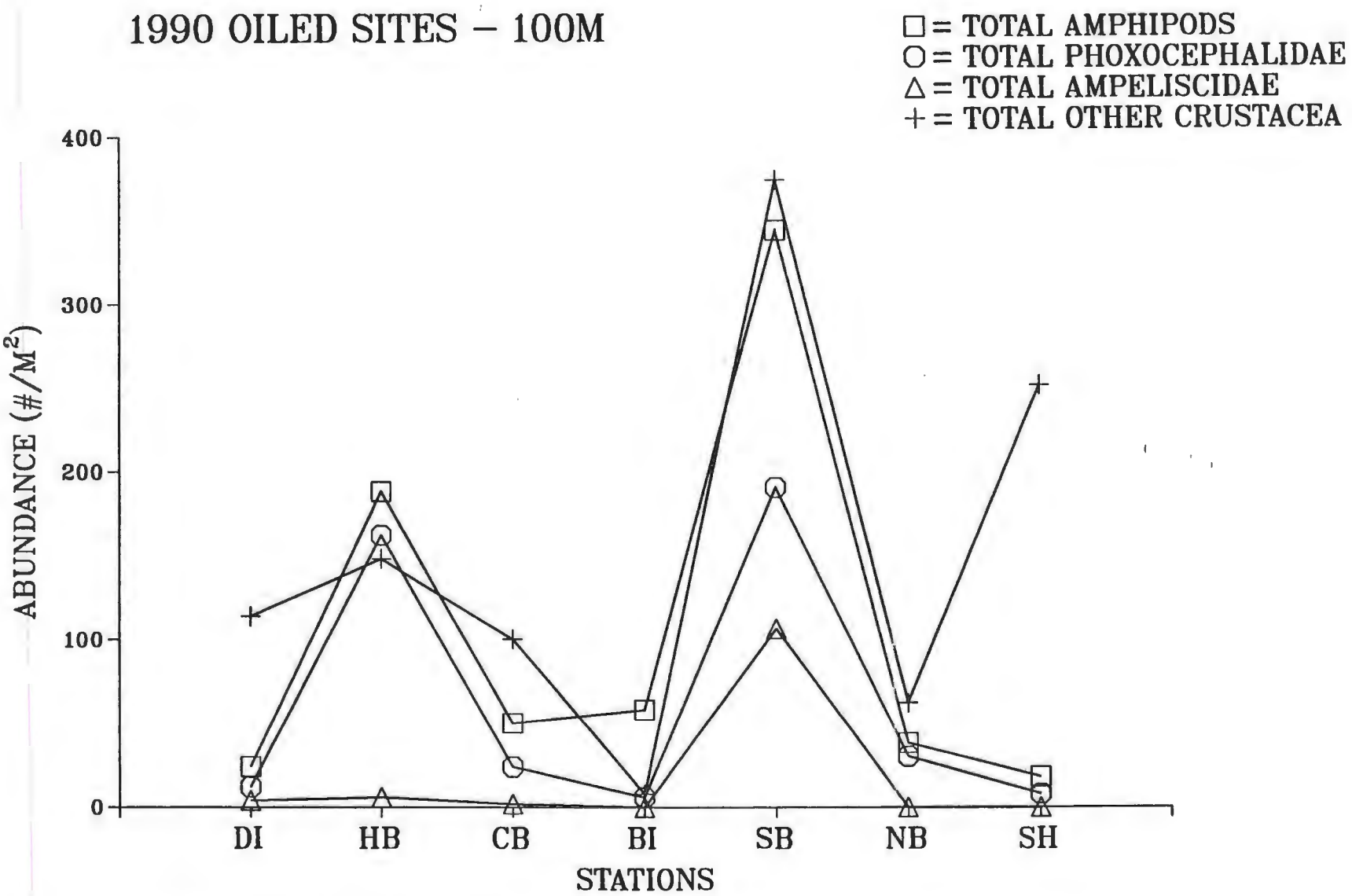

Figure 48. The abundance of total amphipods, phoxocephalid and ampeliscid amphipods, and other crustaceans at oiled stations at 100 m.

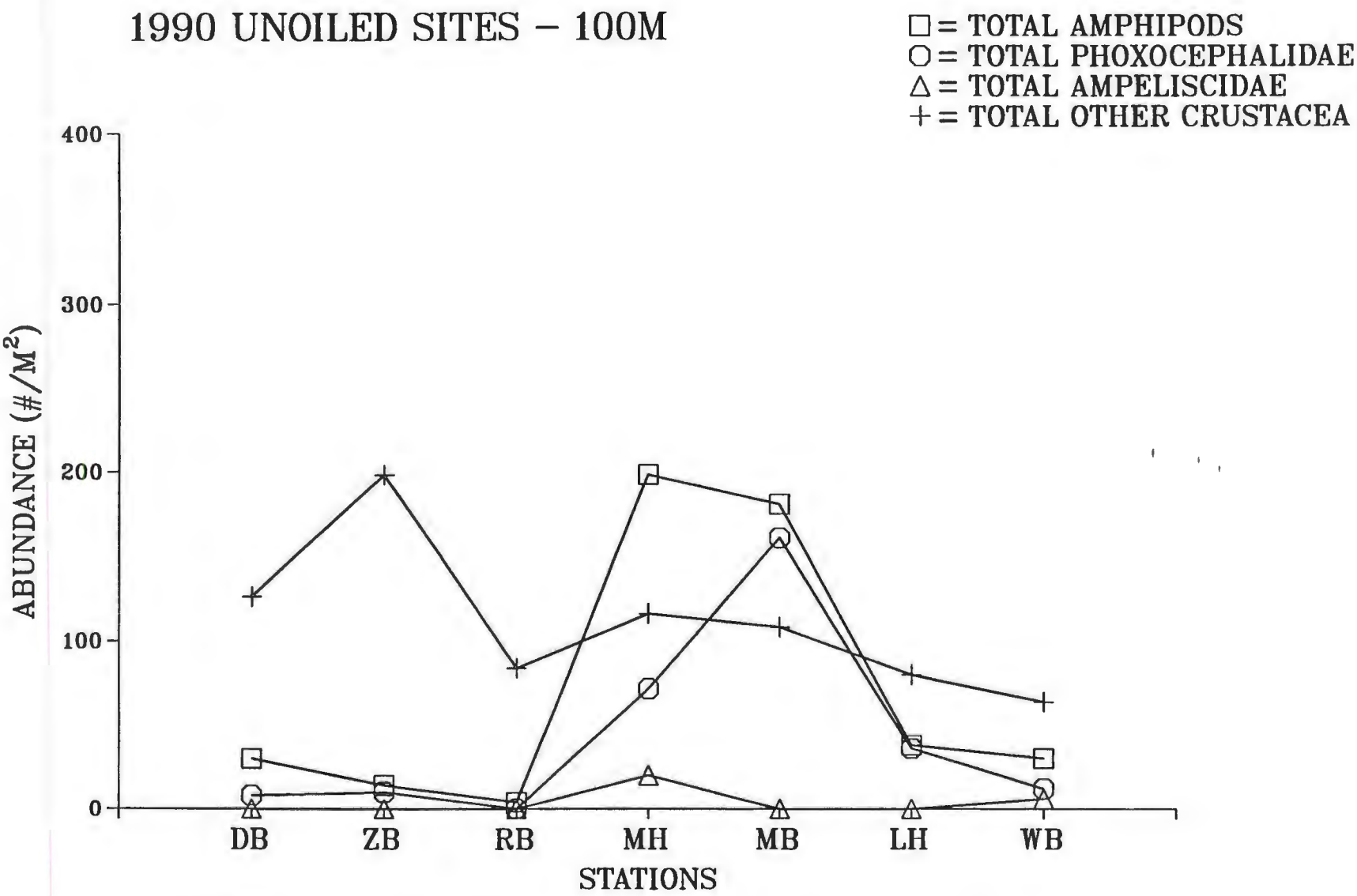

Figure 49. The abundance of total amphipods, phoxocephalid and ampeliscid amphipods, and other crustaceans at unoiled stations at 100 m.

Table 28. Abundance, as square root (abundance +0.5), at 100 meters of each taxa at oiled bays (Bay of Isles, Chenega Bay, Disk Island, Herring Bay, Northwest Bay, Sleepy Bay, and Snug Harbor) and unoiled bays (Drier Bay, Lower Herring Bay, MacLeod Harbor, Mooselips Bay, Rocky Bay, West Bay, and Zaikof Bay). Means, X, standard deviations, SD, and F-ratios with associated levels of significance, p. Values of F preceeded by a minus sign indicate decreases in mean abundance for the unoiled bays when compared to the oiled bays. Probability levels of "ns" indicate that the change in mean values is not significant ($p > 0.10$).

	Oiled Bays		Unoiled Bays			
Taxa	$\bar{X}$	SD	$\bar{X}$	SD	F	p
Ampeliscidae	1.142	0.953	0.854	0.383	-9.560	0.003
Ampharetidae	2.000	1.356	1.224	0.644	-18.868	<0.000
Amphiuridae	0.818	0.301	1.187	0.943	8.191	0.006
Bivalvia	5.294	2.561	4.505	1.756	-13.092	<0.000
Capitellidae	2.985	1.471	3.003	2.412	0.003	ns
Cirratulidae	4.344	2.135	3.638	2.283	-3.912	0.053
Cossuridae	2.024	1.476	1.120	0.699	-41.585	<0.000
Dentaliidae	1.643	0.748	1.339	0.853	-5.001	0.029
Gnathiidae	1.597	1.101	1.129	0.726	-13.024	0.001
Golfingidae	4.583	3.292	1.646	1.256	-75.134	<0.000
Hesionidae	1.603	1.345	1.212	0.422	-16.847	<0.000
Leuconidae	1.644	1.247	2.275	1.193	15.483	<0.000
Lumbrineridae	4.411	1.913	3.377	1.709	-9.240	0.004
Maldanidae	2.300	1.620	1.915	1.385	-2.621	ns
Nematoda	5.031	4.877	2.272	1.697	-20.489	<0.000
Nephtyidae	2.679	1.447	3.586	1.937	19.688	<0.000
Nuculanidae	1.597	0.772	2.197	1.462	11.421	<0.000
Nuculidae	1.383	0.654	1.659	0.942	3.281	0.075
Onuphidae	1.525	0.941	1.214	0.539	-6.504	0.014

Table 28. (cont'd)

Orbiniidae	0.866	0.309	1.605	0.864	48.971	<0.000
Ostracoda	1.625	0.788	-1.377	0.806	-2.640	ns
Owenidae	2.578	1.705	2.495	2.084	-0.076	ns
Paraonidae	3.979	1.811	3.148	1.569	-5.908	0.018
Phoxocephalidae	2.105	1.519	1.795	1.257	-2.926	0.093
Polyodontidae	2.056	1.577	2.159	2.569	0.622	ns
Sabellidae	3.304	2.528	1.256	0.845	-44.142	<0.000
Scaphandridae	1.022	0.542	1.203	0.590	4.189	0.045
Spionidae	2.896	1.822	3.039	2.384	0.142	ns
Sternaspidae	0.863	0.246	1.719	1.591	121.888	<0.000
Syllidae	2.784	2.510	2.013	2.140	-5.201	0.026
Tellinidae	2.127	3.121	1.501	1.159	-7.997	0.006
Terebellidae	2.666	0.690	2.553	0.538	-0.922	ns
Thyasiridae	1.376	1.251	1.488	1.015	0.385	ns

Table 29. Biomass, as square root (biomass +0.5), at 100 meters of each taxa at oiled bays (Bay of Isles, Chenega Bay, Disk Island, Herring Bay, Northwest Bay, Sleepy Bay, and Snug Harbor) and unoiled bays (Drier Bay, Lower Herring Bay, MacLeod Harbor, Mooselips Bay, Rocky Bay, West Bay, and Zaikof Bay). Means, X, standard deviations, SD, and F-ratios with associated levels of significance, p. Values of F preceeded by a minus sign indicate decreases in mean abundance for the unoiled bays when compared to the oiled bays. Probability levels of "ns" indicate that the change in mean values is not significant ($p > 0.10$).

	Oiled Bays		Unoiled Bays			
Taxa	$\bar{X}$	SD	$\bar{X}$	SD	F	p
Ampeliscidae	0.716	0.026	0.709	0.005	-6.474	0.014
Ampharetidae	0.719	0.019	0.720	0.031	0.000	ns
Amphiuridae	0.710	0.010	0.734	0.075	4.663	0.035
Bivalvia	0.724	0.026	0.720	0.030	-0.354	ns
Capitellidae	0.718	0.029	0.716	0.015	-0.154	ns
Cirratulidae	0.722	0.026	0.715	0.009	-2.495	ns
Cossuridae	0.708	0.001	0.707	0.000	-15.512	<0.000
Dentaliidae	0.710	0.007	0.724	0.046	9.386	0.003
Gnathiidae	0.708	0.002	0.708	0.001	3.757	0.058
Golfingidae	0.826	0.370	0.710	0.004	-3.458	0.068
Hesionidae	0.709	0.005	0.708	0.001	-18.154	<0.000
Leuconidae	0.711	0.009	0.714	0.008	5.682	0.021
Lumbrineridae	0.770	0.070	0.749	0.047	-2.750	ns
Maldanidae	0.755	0.102	0.806	0.220	2.234	ns
Nematoda	0.709	0.002	0.708	0.000	-11.029	0.002
Nephtyidae	0.733	0.035	0.825	0.234	11.349	0.001
Nuculanidae	0.873	0.277	0.976	0.698	0.652	ns
Nuculidae	0.715	0.018	0.714	0.010	-0.063	ns

Table 29. (cont'd)

Onuphidae	0.720	0.023	0.770	0.112	8.945	0.004
Orbiniidae	0.708	0.002	0.711	0.007	7.615	0.008
Ostracoda	0.709	0.002	0.708	0.001	-8.808	0.004
Owenidae	0.720	0.019	0.714	0.012	-2.565	ns
Paraonidae	0.716	0.009	0.711	0.005	-9.418	0.003
Phoxocephalidae	0.711	0.005	0.709	0.003	-5.353	0.024
Polyodontidae	0.714	0.011	0.732	0.049	25.996	<0.000
Sabellidae	0.715	0.029	0.708	0.005	-2.302	ns
Scaphandridae	0.714	0.019	0.712	0.011	-0.334	ns
Spionidae	0.716	0.012	0.716	0.020	0.002	ns
Sternaspidae	0.720	0.032	0.786	0.148	16.761	<0.000
Syllidae	0.710	0.005	0.710	0.004	0.343	ns
Tellinidae	1.592	2.055	1.102	0.907	-10.981	0.002
Terebellidae	0.718	0.020	0.724	0.038	0.719	ns
Thyasiridae	0.885	0.684	0.709	0.003	-2.966	0.091

CERCLA/NRDA STUDIES

SUBTIDAL STUDY #3

BIO-AVAILABILITY AND TRANSPORT OF HYDROCARBONS

SUBTIDAL SEDIMENT TRAPS

1992 STUDY PLAN

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EXECUTIVE SUMMARY

The Alaska Department of Environmental Conservation (ADEC) has deployed sediment traps on six occasions in Prince William Sound starting in November 1989 to monitor nearshore sedimentation in the wake of the EXXON VALDEZ oil spill. Two sampling cruises occurred in 1990 for the original set of five traps. Ten additional traps were deployed in August 1990. The fifteen traps were sampled in March and June 1991. Work in 1991 concentrated all fifteen traps at five sites, to allow more site-specific monitoring. The traps were retrieved in March 1991, after winter storms and before the plankton bloom; in June, at the beginning of summer; and in September, before winter storms. The sediment traps were redeployed in September and will be retrieved for the last time in March, 1992.

Currently, 394 sediment, sediment and filter, and blank samples have been taken and stored under Chain of Custody: 283 samples have been sent through the Technical Services Group for hydrocarbon analysis: 12 of these samples were analyzed by NOAA/Auke Bay Lab, and 61 have been analyzed at Texas A&M. Three samples have been analyzed by ADEC Douglas Lab. Ninety seven sediment samples have been sent to UAF Institute of Marine Studies for grain size analysis; 14 sediment cores will be analyzed at University of South Florida for stratigraphy. Core HC chemistry samples will be analyzed by NOAA/Auke Bay Lab. Conclusions regarding presence, composition or transport of petroleum hydrocarbons and relationships to other studies are preliminary and based on analysis of 78 of the 283 chemistry samples.

As of this date, results are available from three retrieval cruises; retrieval of the first trap was in May, 1990; retrieval of traps at five sites occurred in August, 1990; and retrievals at fifteen sites was in March, 1991. The first retrieval, in the east arm of NW Bay, Eleanor Island, showed "fingerprint" patterns similar to North Slope crude oil, especially in the alkylated phenanthrenes and alkylated dibenzothiophenes (ADEC Douglas Lab). Results from August, 1990, show petroleum hydrocarbon patterns in trapped sediments consistent with Exxon Valdez crude oil. Elevated hydrocarbon concentrations are consistently associated with oiled beach locations. The set of samples from March, 1991, shows the continued presence of hydrocarbons in settling particulates at the moderate to heavily oiled study sites, with good replication between sediment traps from the same time and location.

OBJECTIVES

A) To determine if sediments settling out of the water column in nearshore subtidal environments contain adsorbed hydrocarbons.

B) To decipher subtidal oiled sediment transport mechanisms through analysis of benthic sediments, and stratigraphic analysis of bottom cores.

INTRODUCTION

Sedimentation of hydrocarbons into subtidal areas has been shown to be a rapid and quantitatively important pathway in the distribution of spilled oil. Though an accurate and thorough mass balance has not been completed on any major oil spill in a marine environment, estimates of oil sedimenting onto the subtidal vary from 8-10% of Amoco Cadiz oil (Gundlach et al., 1983), to 10-15% of Tsesis oil (Johansson, et. al., 1980) and 7-16% for a controlled ecosystem (Gearing, et. al., 1979). Oil can sink by adsorption to sediments, possibly by electrostatic bonding to fine-grained clay micelles (Bassin and Ichiye, 1977); by turbulent mixing of an oil slick with sand which increases the density of the mixture; and through uptake by zooplankton and subsequent deposition in fecal pellets (Conover, 1971). Once the oil is beached, hydrocarbons may still be mobilised and deposited in subtidal sediments. Oiled beaches act as a reservoir from which hydrocarbons may be removed by erosion and deposited in offshore sediments. Ten percent of oil stranded on beaches after the Baffin Island Oil Spill project (BIOS) was transported offshore(Boehm, et al.,1987). A laboratory study by Bragg, et al (1990) using oiled sediments from PWS shorelines found the formation of a solids-stabilized emulsion of micron-sized mineral sediments, polar components of the oil residue, and seawater on oiled shorelines impeded the adhesion of oil particles to larger rocks. Given that the floc "particles" are lighter than seawater, and the theoretical settling rates of the largest particles, it is assumed that most of the oiled particles removed with the emulsion will be transported great distances before settling and be widely dispersed.

Persistence and mobilization is related to physical processes, such as wave, tide and wind energy. Sediment grain size and oil quantity and composition also contribute (Blount,1978; Gundlach, et al., 1978). Oil eroded from contaminated shorelines and entering the water column may settle in the nearshore subtidal, or be transported into deeper waters before settling depending on particle size and shape (and hence settling velocity), wave energy, tidal current velocities, longshore currents and the effect of salinity gradients, such as salt wedges in estuaries or the location of the turbidity maximum (Gundlach, et al., 1978). Once deposited on the bottom, oiled sediments can continue to be moved by bottom currents, by wave-induced oscillatory currents which may result in resuspension, or be buried deeper into benthic sediments by bioturbation.

Several questions remain regarding subtidal transport of oil in Prince William Sound:

1) Where does the oil eventually reside?
2) What mechanisms account for the dispersal of subtidal oiled sediments? (i.e. what contribution is made by bedload? suspended load?)

EXPERIMENTAL DESIGN

Sediment Traps

The sediment trap design incorporates guidelines developed from previous sediment trap work with open ocean moored traps and laboratory flume studies (Woods Hole, 1989). The original design of the traps was intended to capture sediments in the nearshore subtidal to show presence or absence of adsorbed hydrocarbons, without quantification of flux rates.

Fifteen sediment traps were deployed at seven sites in 1991 - Sleepy Bay, Snug Harbor, Bay of Isles, Disk Island, NW Bay, Eleanor Island, Herring Bay and Olsen Bay in Port Gravina (Fig. 1). At each site, divers placed a suite of traps at 10, 15 and 20 meters below MLLW (Fig. 2), with the exception of Bay of Isles and NW Bay, where steep subtidal slopes limited the traps to 10 and 20 meters.

The trap sites have been matched with Coastal Habitat intertidal and subtidal sites, previous ADEC subtidal sampling, Subtidal #2 sampling, and NOAA mussel cages. Coordination with other studies is a method of extrapolating the results both spatially and temporally. Sediment chemistry data will thus be available over time and from a larger area to add validity to inferences. Hydrocarbon chemistry analysis of sediment cores from around the base of the traps can provide background hydrocarbon levels for each site.

Erosional and Depositional Processes

Knowledge of particle size and settling velocities will aid in the differentiation of bed-load movement versus resuspension (Visher, 1969; Middleton, 1976), delineation of erosional and depositional events (Sundborg, 1956), as well as allowing calculations of trap efficiency. Differentiating between new sediment input to the subtidal and cycling of previously deposited sediments will give a better understanding of localized transport processes. Due to the great distances fine sediment particles can travel before settling out of the water column (in a current flow of 10cm/sec, a 0.06mm silt particle may travel as far as 10 km before settling at 100 m.), coordination with AW #2 deep water sampling is emphasized. Detailed grain size distributions will be developed to assist in determinations of transport processes at the study sites, and allow comparisons with HC chemistry results from the traps and the surrounding benthic surface layers (see Analysis below). Approximations of wave energy, currents and possible shear forces at particular sites will come from hindcasts using the Automated Coastal Engineering System (ACES) model developed by the U.S. Army Engineer Waterways Experiment Station, Coastal Engineering Research Center. The results will provide data regarding wave energy affecting the traps and resultant shear forces that could be expected to operate on sediments at each site and depth.

Sediment Cores

During trap retrievals in June and September 1991, cores were taken of benthic sediments around the base of the sediment traps. These cores can provide data on

background HC chemistry levels at each specific site (Wakeham and Carpenter, 1976; Wakeham and Farrington, 1980), on erosional/depositional histories and on depths of petroleum HC contamination in benthic sediments.

STUDY METHODOLOGY

Retrieval

The sediment traps are recovered and deployed using two divers and the winch on the support vessel. Divers carrying lids for the cylinders and sampling jars and tools in a goodie bag descend along a permanent buoy line that marks the location of the traps. One diver fills two, 250 ml. jars with sediment samples from the upper benthic layer (0-2 cm) in a 1 meter radius around the trap base, each sample a composite from two locations; two other samples are taken along a transect line between the three traps. Once sampling is complete, the second diver caps the cylinders to avoid potential contamination of the traps as they are moved through the water column. A hand-driven core is taken from the bottom around the trap for stratigraphic analysis. The cylinders and base are then raised to the vessel deck. Samples taken by hand are tat uscollected using a stainless steel cup, spoon or trowel. All implements are washed with Alconox, rinsed with acetone and hexane, air dried and wrapped in sterile aluminum foil. Sample jars are purchased clean according to EPA specifications. Samples are kept cool and frozen within 2 hours of collection or filtration.

Core samples: The benthic sediment core samples are collected at the time of retrieval using a stainless steel coring tube precleaned as above. The water is siphoned off after settling, and the sediments are frozen in the tube, following a method of Wakeham and Carpenter (1976). When the sediments are frozen solid, the outside of the core is heated with a propane torch, releasing the still-frozen core into a plastic container and placed in a freezer. The samples are stored under Custody, and shipped to the laboratory with specific instructions on sampling protocols for removing subsamples for HC chemistry.

Measurement of erosion/deposition: In the fine-grained, soft-bottom sediments that characterize most of the trap sites, 1/2 inch rebar is placed in the bottom next to each sediment trap, and 5/8 inch washer is placed over the rebar and set on the top surface of the sediments. The distance from the washer to the top of the rebar is measured and logged. During the trap retrieval in March, the distance from the top of the rebar to the top of the sediment, and to the top of the washer will be noted.

Filtration

After the cylinders have been allowed to settle on the deck for one hour, 2/3 of the water volume is decanted and 2 to 3 liters are saved for rinse water. The remaining seawater and suspended sediment is transferred to a decontaminated stainless steel container. This volume is filtered through pre-combusted 9mm glass-fiber filters in a

Buchner funnel array. The samples and filters are frozen in custody sealed I-Chem sample jars. Bottom samples are also Custody sealed and frozen.

Trap cylinders are precleaned with an Alconox wash, rinsed with acetone, wiped with hexane, and clamped onto the base. One-half pound of sodium chloride (table salt precombusted to 450 C for 4 hrs) is added to the bottom of the cylinder by mixing the salt with deionized water and filtering the solution through a 0.2um filter. This brine solution will act to prevent resuspension of captured sediments (Woods Hole, 1989).

The trap is then lowered over the side of the vessel until the tops of the cylinders are just above the waterline. The cylinders are filled with seawater by filtration through a 0.5mm nylon mesh to exclude organisms and sediments. Precautions are taken to assure no oil or diesel sheen is on the water near the traps; blanks are also taken periodically of the water used. The trap is then placed as close to its original position on the bottom as possible. The cylinders are numbered, and the numbers are kept in the same geometric position, i.e., #1 points toward the transect heading to the beach, with succeeding numbers clockwise.

ANALYSIS

Samples will be analyzed for polynuclear aromatic hydrocarbons (PAH) and total petroleum hydrocarbons (TPH) according to procedures established by Technical Services Study 1.

Particle size analysis will be performed by sieving the sample in a stacked set of Wentworth grade sieves to 62 um. Analysis of the silt-clay fraction will be obtained by pipette analysis. Granulometric analysis will follow the method of Krumbein and Pettijohn (1938), modified by Folk (1980), either by sieve analysis, pipette analysis or a combination, dependent on the nature of the sediments. Analytical precision will be presented as percent coefficient of variation based on replicate analysis.

Sediment cores will be x-rayed; photographed; inspected for composition (by microscope) and grain size (by settling, where appropriate) and sedimentary structures and core logs will be prepared. Subsamples of each core will be taken and sent to Auke Bay Laboratory for analysis of TPH/PAH as above. Subsamples for HC chemistry will be removed with decontaminated implements while the sample is still frozen , and will remain under Chain of Custody during subsequent storage and shipment for analysis.

RESULTS

Elevated levels of petroleum hydrocarbons were consistently found in sediment traps located near heavily oiled beaches. Sediments in traps deployed June 1990 and retrieved August 1990 at 5 locations were chemically analyzed for TPH/PAH in duplicate. Results show petroleum hydrocarbon patterns consistent with Exxon Valdez crude oil at levels that are highest at Sleepy Bay, and lowest at the control site, Port Fidalgo (fig. 3). Elevated petroleum hydrocarbon levels were also clearly present at two

locations in Northwest Bay, and were slightly higher at Snug Harbor than at the control site. These results indicate that petroleum hydrocarbons in trapped sediments are consistently associated with oiled beach locations.

The association of petroleum hydrocarbons in trapped sediments with oiled beach locations persists in sediments deployed August 1990 and retrieved March 1991, although the pattern of hydrocarbons present is substantially altered. Trapped sediments retrieved March 1991 from east Northwest Bay and Sleepy Bay were analyzed in duplicate, and similar sediments from 6 other locations were analyzed singly. The highest levels of petroleum hydrocarbons were found in trapped sediments from east Northwest Bay, Sleepy Bay, and Snug Harbor, while the lowest levels were found at Eshamy Bay, Stockdale Harbor, and Port Fidalgo (fig. 4), indicating clear association of petroleum hydrocarbon levels of trapped sediments with the degree of oil impact of the adjacent beaches. Note, however, that the pattern of aromatic hydrocarbons present in trapped sediments retrieved March 1991 shows consistent and substantial enrichment of chrysenes relative to the other aromatic hydrocarbon classes at each trap location presented in figure B. The reasons for this alteration of the pattern of aromatic hydrocarbons present in these trapped sediments are currently not well understood, but may reflect complex weathering processes.

About 50 sediment samples collected August 1990 adjacent to sediment traps have been analyzed for petroleum hydrocarbons, but these data have not yet been fully evaluated. In general, preliminary results indicate elevated petroleum hydrocarbon levels in sediments at trap sites adjacent to oiled beaches.

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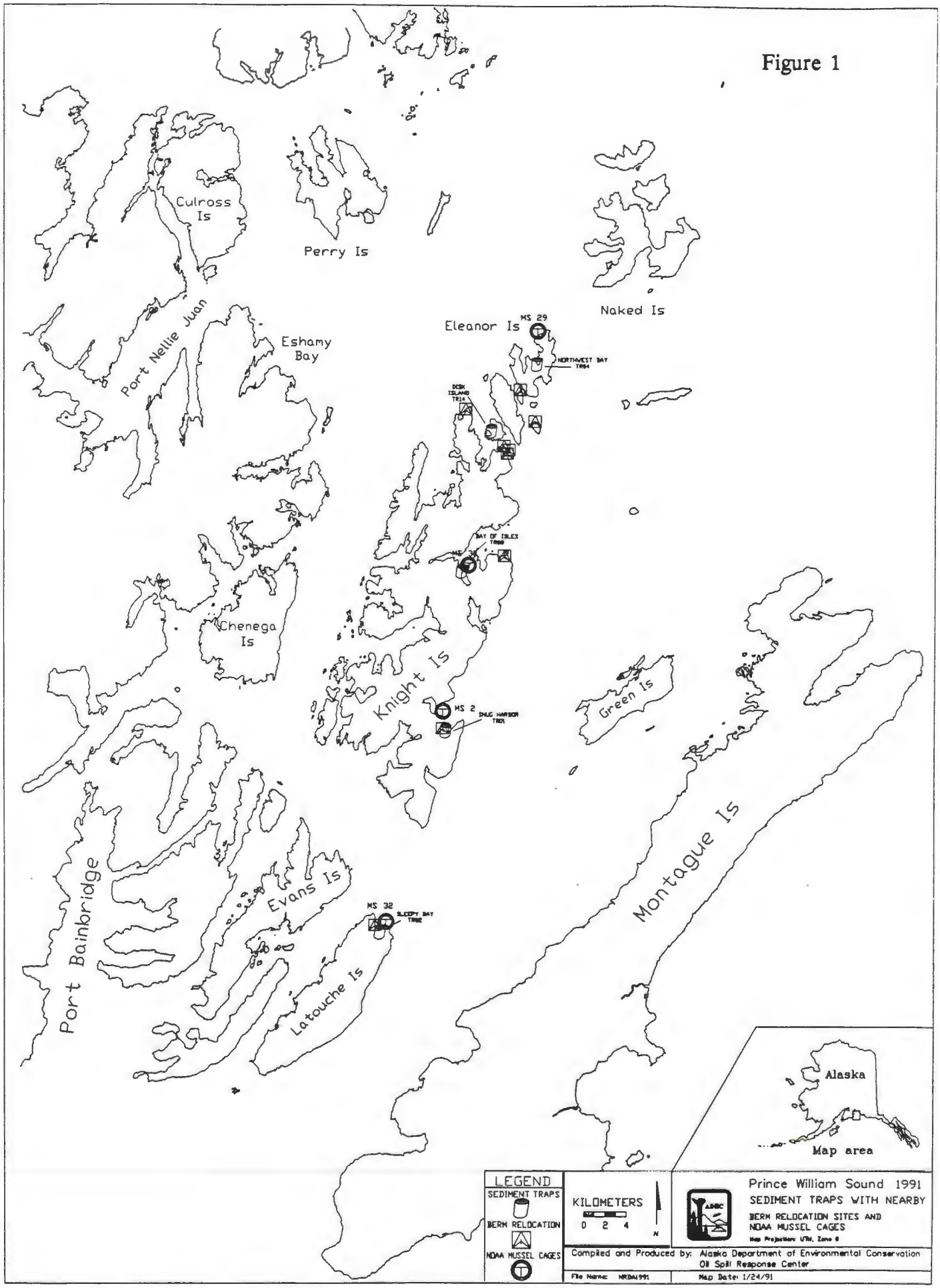
Figure 1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Chenega Is
Knight Is
MS 2
SMUG HARBOR TRI1
Green Is
Montague Is
Port Bainbridge
Evans Is
MS 32
SLEEPY BAY TRI2
Latouche Is
Alaska
Map area
LEGEND
SEDIMENT TRAPS
BERM RELOCATION
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0 2 4
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Prince William Sound 1991
SEDIMENT TRAPS WITH NEARBY
BERM RELOCATION SITES AND
NOAA MUSSEL CAGES
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Oil Spill Response Center
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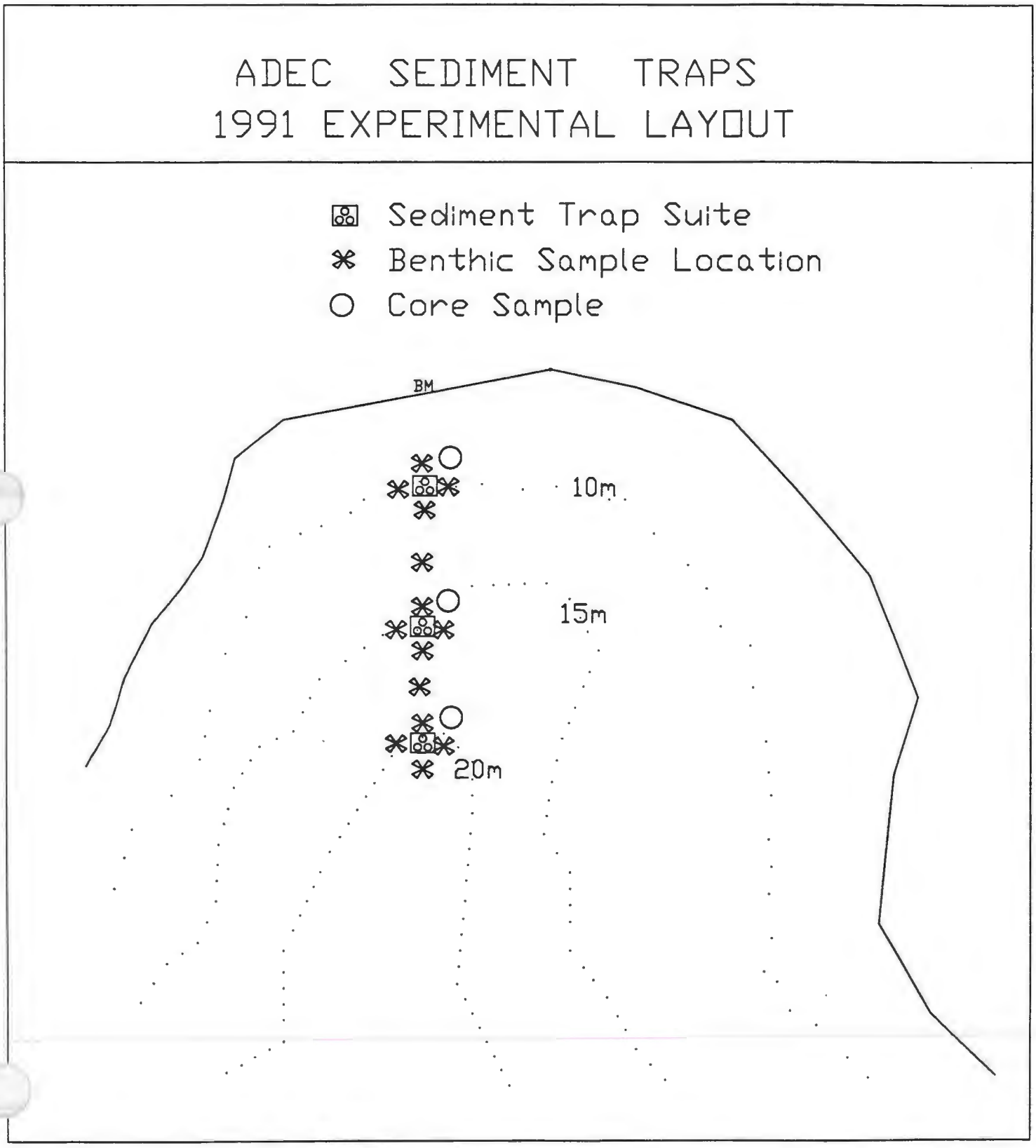
Figure 2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1991 EXPERIMENTAL LAYOUT
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Benthic Sample Location
Core Sample
BM
10m
15m
20m

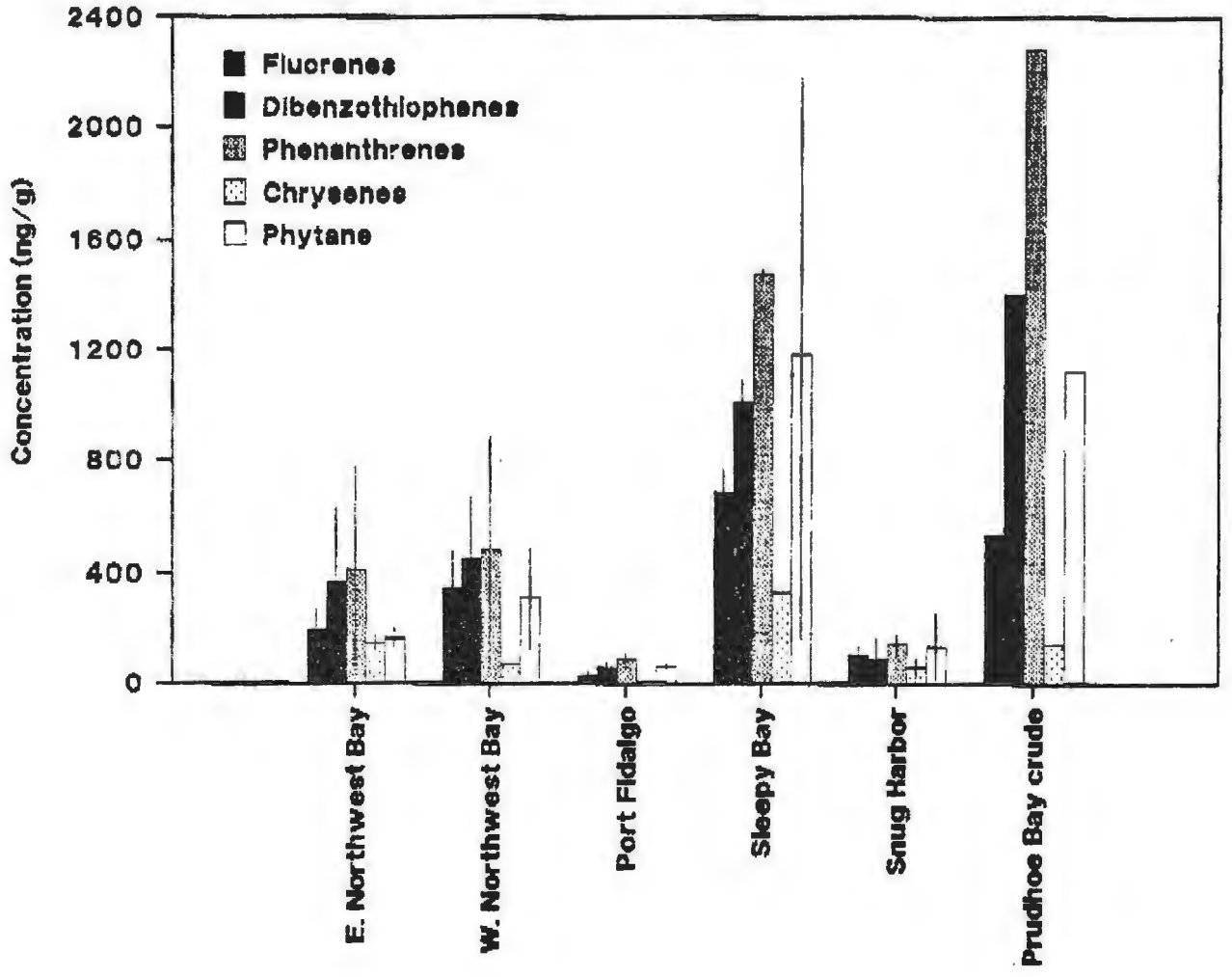

Figure 3. Petroleum hydrocarbons in trapped sediments deployed June 1990 and retrieved August 1990. Fluorenes includes fluorene and carbon substituted fluorenes, and similarly for dibenzothiophenes, phenanthrenes, and chrysenes. Vertical bars indicate the range of the duplicate determinations. Concentrations are ng analyte(s) per g wet sediment (parts per billion). The relative concentrations of these analytes in Prudhoe Bay crude oil sampled from the hold of the Exxon Valdez are presented at the far right.

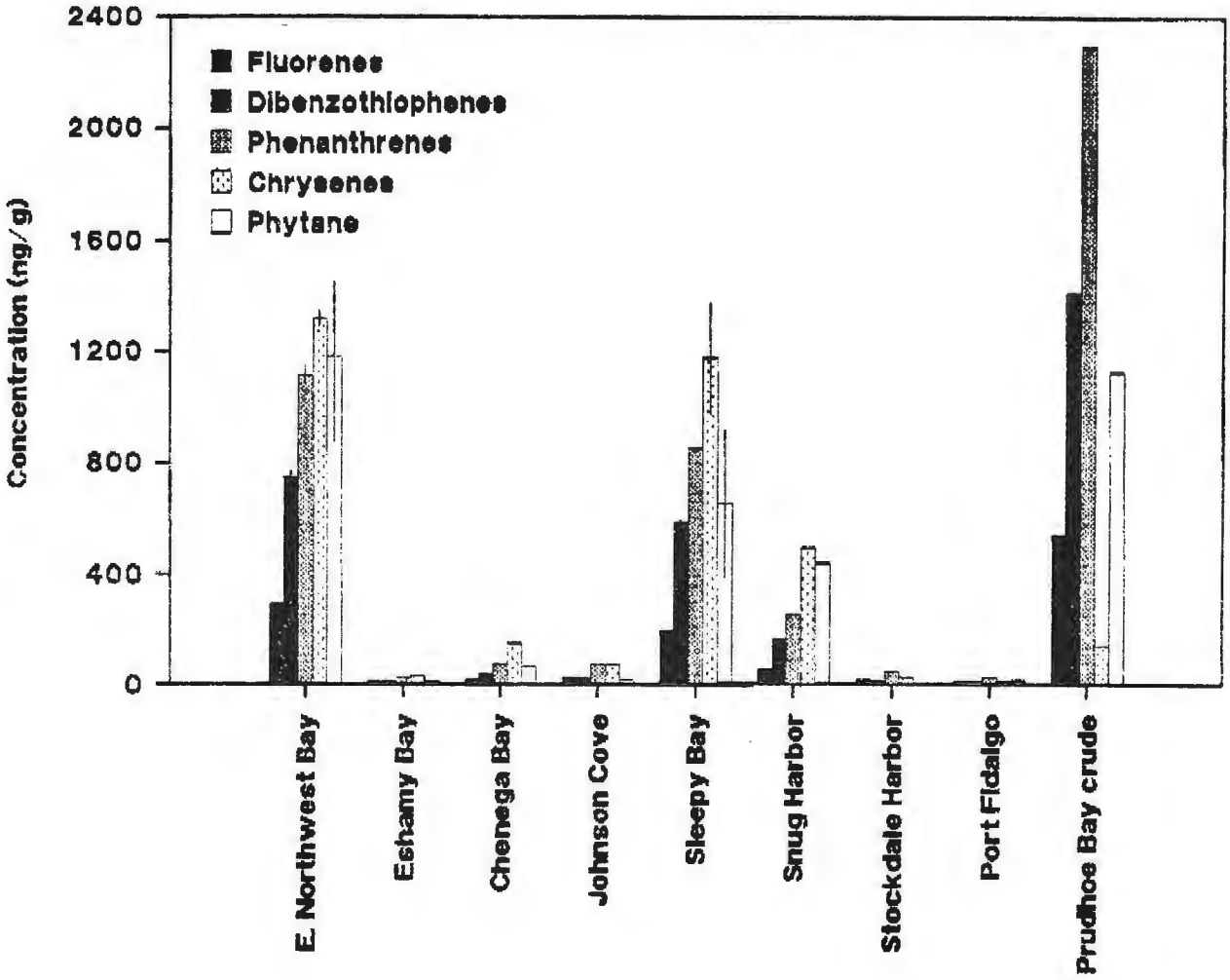

Figure 4. Petroleum hydrocarbons in trapped sediments deployed August 1990 and retrieved March 1991. Fluorenes includes fluorene and carbon substituted fluorenes, and similarly for dibenzothiophenes, phenanthrenes, and chrysenes. Vertical bars indicate the range of duplicate determinations (Northwest Bay and Sleepy Bay only). Concentrations are ng analyte(s) per g wet sediment (parts per billion). The relative concentrations of these analytes in Prudhoe Bay crude oil sampled from the hold of the Exxon Valdez are presented at the far right.

NATURAL RESOURCE DAMAGE ASSESSMENT

DRAFT STATUS REPORT

SUBTIDAL STUDY #3

BIO-AVAILABILITY AND TRANSPORT OF HYDROCARBONS IN THE NEAR SHORE WATER COLUMN

November 1991

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

EXECUTIVE SUMMARY

The goal of the NOAA component of project Subtidal Study #3 is to document petroleum hydrocarbon loading in near shore waters impacted by the *Exxon Valdez* oil spill. In 1989, hydrocarbon loading was monitored by direct sampling of seawater in Prince William Sound (PWS) and in 1989, 1990, and 1991 by deployment of hydrocarbon free mussels along the oil spill trajectory for exposure periods of 1 to several months.

In 1989, chemical analysis of the seawater samples, taken in triplicate at 1 and 5 meter depths, shows the presence of aromatic hydrocarbons of petroleum origin. Total concentrations range up to about 8 μg/l (ppb) at the most heavily contaminated sites shortly after the spill, but decline to below detection limits by 6 weeks following the spill.

Caged mussels are more sensitive indicators of oil in seawater, because they effectively contact larger volumes of seawater. In 1989, both aromatic and aliphatic hydrocarbons of petroleum origin were detected in tissue of caged mussels. Total hydrocarbon concentrations, up to 100 μg/g wet tissue (ppm), were highest at Herring Bay, Smith Island, and Snug Harbor. Petroleum derived hydrocarbons were detected at all other stations and depths inside PWS along the spill trajectory. Outside the Sound, although hydrocarbon concentrations were generally low and highly variable among replicates, mussels exposed at Tonsina Bay and Chignik showed moderate levels of contamination. Oil contamination levels in the caged mussels declined after May 1989 and approached control levels by Fall 1989.

In 1990, oil contamination levels that were significantly above control levels were low and sporadic. Consequently, 1991 mussel deployments were closely coordinated with ADEC sediment trap deployments, after relatively high aromatic hydrocarbon concentrations were detected in sediment trap samplese at oiled sites in 1990.

Hydrocarbon analysis data of 1989 and 1990 caged mussels was examined using principal component analysis (PCA) to evaluate PCA as a method for identifying oil contaminated samples and for summarizing oil contamination levels. Preliminary results of the PCA are consistent with previous interpretations of these hydrocarbon data, and suggest that PCA may be a valuable approach for consistently evaluating and interpreting hydrocarbon analysis data in a variety of matrixes across all NRDA projects.

Our results indicate that biologically available hydrocarbons from the *Exxon Valdez* oil spill were generally pervasive in the upper water column along the spill trajectory inside PWS during the summer of 1989. The results of the PCA indicate that the mussels

accumulated particulate oil from the *Exxon Valdez*, although the mechanism of transport of the oil to the caged mussels is not clear.

In 1991, a total of 99 samples were collected. Chemical analyses have begun on all samples from the May-June deployment, but no data are yet available.

OBJECTIVES

A. Estimate concentrations of petroleum derived hydrocarbons at 1 m and at 5 m depth along the oil spill trajectory within PWS such that the estimate is within 25% of the actual concentrations 95% of the time during the first six weeks of the oil spill.

B. Evaluate trends in ambient water quality using bioaccumulators (*Mytilus trossulus*) as surrogates for chemical measurements. Estimate concentrations of petroleum derived hydrocarbons accumulated by initially hydrocarbon-free bay mussels transplanted for 1 or 2 months to water depths of 1 m, 5 m, and 25 m, along the oil spill trajectory such that the estimate is within 25% of the actual concentrations 95% of the time.

Objective B was broadened in 1991 to estimate concentrations of petroleum derived hydrocarbons accumulated concurrently by transplanted bay mussels (NOAA/NMFS) and sediment traps (ADEC).

C. Synthesize all water and mussel hydrocarbon data in the Technical Services #1 database to provide a comprehensive geographic and temporal picture of trends in petroleum hydrocarbon concentrations in the near shore water column.

INTRODUCTION

This study continues to assess the geographic and temporal distribution of dissolved and particulate hydrocarbons in the water column resulting from the *Exxon Valdez* oil spill. Knowledge of these concentrations will determine whether violations of State of Alaska Water Quality Criteria have occurred, and in addition will allow estimation of the exposure risk of subsurface marine biota to petroleum hydrocarbons. This study extends work begun within one week of the grounding of the *Exxon Valdez* and continued to date under Air/Water #3 and Subtidal Study #3 (in 1991). Although the Alaska Department of Environmental Conservation (ADEC) conducted work under this project in 1989, 1990, and 1991, this status report will discuss ADEC's work only in its relation to that portion of the study conducted by NOAA/NMFS.

In 1989, seawater hydrocarbons were monitored by direct sampling of seawater at 30 sites in Prince William Sound, and by transplanting hydrocarbon free mussels to 28 sites along the oil spill path inside and outside the Sound. In 1990, mussels were deployed at 19 sites within Prince William Sound and at 9 sites along the shoreline of the Kenai Peninsula, the Alaska Peninsula, and Afognak Island. In 1991, mussels were deployed at 10 sites within Prince William Sound in May; in June the number of sites was reduced to 6 and cages were deployed directly adjacent ADEC's sediment traps.

Chemical analysis of first priority water and mussel samples from 1989 and 1990 are either completed or are in progress. Examination of the results indicates that no further chemical analyses of samples collected in 1989 or 1990 will be necessary. These data were evaluated using principal component analysis (PCA) as an initial effort to address objective C of this project. Some of the 1991 mussel samples have been submitted for chemical analysis, but results are not yet available.

METHODS

Water Samples

In 1989 water samples were collected at 30 sites (fig. 1) within the Sound on each of three sampling cruises (31 March - May 10 1989). Triplicate samples were collected at 1 and at 5 meter depths at each station. Details of sampling are contained in Air Water #3 Study Plan 1989. Five hundred thirty three NOAA water samples were collected. No further direct sampling of the near shore water column was done after mid May because hydrocarbon concentrations were likely below detection in practically sized field samples.

Caged Mussel Deployments

Trends in hydrocarbon concentration were studied by analyzing hydrocarbon accumulation by "clean" bay mussels *Mytilus trossulus* transplanted to impacted areas. The use of a bioaccumulator provides a time integrated indication of hydrocarbons available for biotic accumulation from the water column. Mussels were deployed at 1, 5, and 25 meter depths at 12 stations within the Sound (fig. 2) and at 18 stations outside the Sound (fig. 3). There were 4 one month exposure periods at each of 12 sites inside the Sound. At Kenai, Alaska Peninsula, and Afognak sites, there were two exposure periods, varying in length from one to two months depending on logistics. Methods of sample collection and handling are described in detail in Air Water #3 Study Plan 1989. A total of 383 samples ("caged" mussels, unexposed "base" mussels, native mussels and air blanks) were collected.

Mussels were deployed in 1990 as proposed in the Air/Water #3 study plan with the addition of two sites in Snug Harbor and Herring Bay adjacent berm relocation sites and Katmai Bay, an impacted site on the Alaska Peninsula. Inside the Sound there were four exposures, each one month long; outside the Sound there was one exposure at the majority of sites, two exposures at Kukak and Hallo Bays. Collected were 294 samples.

Mussels were deployed in 1991 at 10 sites in May and recovered in June (fig. 1). Subsequent analysis of 1990 caged mussels found no appreciable amounts of hydrocarbons at sites where high amounts were found in 1989, so there were no further caged mussel deployments at these 1989 or 1990 sites. However, sediments collected by ADEC's sediment traps over the winter 1990-91 contained relatively high aromatic concentrations, so caged mussels and sediment traps were deployed concurrently to examine the relationship between hydrocarbons associated with particles settling out of the water column and hydrocarbons biologically available to mussels. Mussels and traps were subjected to two exposure periods, early June-mid September 1991 and mid-September 1991-March 1992, at Northwest Bay, Snug Harbor, Sleepy Bay, Bay of Isles, Disk Island, and Olsen Bay (fig. 1). Mussel cages were attached to a mooring line at 1m and 25m from the surface and at 2m from the bottom. The bottom bag simulates the position of a sediment trap in the water column.

Ninety nine samples were collected in 1991. Chemical analyses have begun on all samples from the May-June deployment, but no data are yet available.

Principal Component Analysis

Hydrocarbon concentrations determined in 1989 and 1990 caged mussel samples were examined using PCA to determine the presence and intensity of oil contamination in these samples. A total of 303 samples, which included samples of deployed caged mussels, mussels prior to deployment, control mussels, and native beach mussels were analyzed by PCA together. The hydrocarbon compounds or classes of compounds listed in table A are considered as independent variables of the PCA, and are assumed to either vary independently and randomly among mussel samples, or alternatively to co-vary among these samples with the intensity of oil contamination. The hydrocarbon concentrations, c, determined in these mussel samples were transformed using $\ln(c + 1)$ because the variance is typically directly proportional with concentration in these samples. The PCA was performed on the transformed data using SYSTAT.

STUDY RESULTS

Water Samples

Thirty six per cent of the total NOAA water samples have been analyzed to date at Auke Bay Lab under the direction of Technical Services Study #1. All three replicates at each depth from the first cruise, March 31-April 4, 1989, and one replicate at each depth from the last cruise, May 2-8, 1989, have been analyzed.

Total concentrations of aromatic hydrocarbons range up to about 8 μg/l (ppb) at the most heavily contaminated stations. Stations with the highest aromatic hydrocarbon concentrations in seawater correspond with areas where heavy oil slicks were present, and these stations were within the spill trajectory. Aliphatic hydrocarbons are generally not present in these samples, indicating that (1) oil was present as a water soluble fraction (WSF) and (2) samples were not contaminated by surface slicks.

Caged Mussels - Principal Component Analysis

The first principal component determined by the PCA is due to oil contamination, and explains 35% of the total variance of the 303 caged mussel samples included in the analysis. The identification of component 1 with crude oil contamination is supported by the observation that the component coefficients a_i are roughly similar for all the hydrocarbon compounds or classes of compounds listed in table 1. These coefficients a_i satisfy

$$z = \sum_{i=1}^{63} a_i k_i$$

for each component in each sample, where k_i is the standardized deviate of the ith log-transformed hydrocarbon concentration in the sample, and z is the value of component 1 in that sample. Equivalently, note that the correlation coefficient of each hydrocarbon compound or class of compounds considered with component 1 is quite high for compounds and classes prevalent in *Exxon Valdez* crude oil (table 1).

The value of component 1, hereafter denoted as the oil component, is consistently low in caged mussel samples deployed at control sites, and is highest at sites heavily impacted by oil (fig. 4). This suggests that PCA may be used to objectively determine the presence of oil in samples. The value of the oil component in caged mussel samples deployed at *a priori* control sites (Olsen Bay, Discovery Bay, Kukak Bay, Port Graham, Sunny Cove; figs. 2 & 3) has an approximately normal distribution (fig. 5). A critical value Z_c can therefore be determined as

$$Z_c = \bar{Z} + t_{n-1,1-\alpha}\, s \sqrt{(n+1)/n}$$

where Z bar and s are the mean value and standard deviation, respectively, of the oil component of the caged mussels deployed at control sites; $t_{n-1,1-\alpha}$ is Student's t; n is the number of control site caged mussel samples; and α is the type I error. The value of the oil component from any caged mussel sample at a non-control site may be compared with this critical value, and if greater than that sample may be accepted as contaminated with oil.

The value of the oil component of caged mussels at sampling locations may be used to examine temporal trends at those locations. Using Herring Bay as an example, the value of the oil component of caged mussels generally decreases with deployment depth, and decreases with time after the oil spill at all depths (fig. 6). In general, the value of the oil component approaches control levels by the Fall of 1989, and is at control levels in 1990 at all sampling locations (fig. 7). Note that, due to the high correlation of oil hydrocarbons with the oil component, examination of any other quantitative measure of oil contamination will lead to similar conclusions.

Oil component values above the critical level were found at all sampling locations inside the Sound save Olsen Bay during the first deployment in 1989, indicating widespread subsurface distribution of particulate oil for at least several weeks following the initial spill. This result corroborates a similar observation of last year's status report for this project, and indicates that biologically available petroleum hydrocarbons were present at that time to depths of several meters.

The concentrations of petroleum derived hydrocarbons found in the caged mussels contrast with the general absence of hydrocarbons found by direct chemical analysis of seawater. This contrast has been noted by Vandermeulen (1979) who commented on a caged mussel deployment study of the *Amoco Cadiz* oil spill conducted by Wolfe *et al.* (1979). One reason for this contrast may relate to the relative amounts of water sampled during direct chemical analysis (900 ml.) and the amount filtered by a mussel in one month (approximately 1000 l). Higher concentrations of hydrocarbons may be expected in mussels because of the higher volume of water they effectively "sample".

Hydrocarbon concentrations were more variable among replicates of caged mussels deployed outside the Sound than those deployed inside. The high variability may be due to holding mussels for long periods prior to deployment and long exposure periods. Mussels deployed outside PWS were generally very stressed, as evidenced by the mean mortality rate of 39% during deployment. In contrast, mussels deployed inside PWS had an overall mean mortality

during deployment of 6%. The high mortality of mussels deployed outside PWS was due to logistic difficulties transporting mussels to deployment sites.

STATUS OF INJURY ASSESSMENT

Water Analyses

A final report for these data is in preparation; no additional data are required. No additional effort in this area is anticipated.

Caged Mussels

Mussels deployed in 1989 and 1990 have been retrieved and analyzed for hydrocarbons. Data analysis and preparation of a final report is currently underway. The last of the mussels deployed in 1991 will be retrieved in March 1992. Final hydrocarbon analysis of remaining caged mussel tissues deployed in 1991 are expected to be done by mid to late 1992. Data analysis will begin on receipt of these data, and should be completed and a final report drafted within the following 6 months. No additional mussel deployments are anticipated for this project, although similar mussel deployments may be parts of restoration projects.

Data Synthesis

Provided the approach outlined herein using PCA is acceptable, this effort will be expanded as a separate NRDA project in OY4 to include analysis of water samples, mussel samples collected for other projects, and sediment samples collected for other projects. This OY4 project is entitled "Mussel Tissue and Sediment Hydrocarbon Data Synthesis". The synthesis will include a data quality assurance component, which will evaluate hydrocarbon analysis data provided by the analytical laboratories in context, to determine whether these data conform with the minimal expectations of the project investigators generating the samples. Expansion of this effort in a separate NRDA project is indicated by the volume and complexity of data to be evaluated and the number of comparisons to be made, all of which were substantially underestimated when initially proposed for the present project.

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Table 1. Oil component coefficients and correlations with hydrocarbon analytes, and relative abundance of hydrocarbon analytes in Exxon Valdez crude oil. The oil component coefficients, a_i, are those of equation 1 in the text. The correlations are correlation coefficients of the hydrocarbon analyte with the oil component. The relative abundance is in per cent total hydrocarbons found in analysis.

COMPOUND	OIL COMPONENT COEFFICIENTS (a_i)	CORRELATION COEFFICIENT	RELATIVE PERCENT	COMPOUND	OIL COMPONENT COEFFICIENTS (a_i)	CORRELATION COEFFICIENT	RELATIVE PERCENT
NAPH	0.007	0.160	5.05	BENZOKFL	-0.007	-0.150	0.00
C1NAPH	0.005	0.120	15.63	BENEPY	0.032	0.708	0.00
C2NAPH	0.029	0.633	23.37	BENAPY	0.008	0.186	0.00
C3NAPH	0.041	0.913	16.67	PERYLENE	0.004	0.095	0.00
C4NAPH	0.019	0.419	6.25	INDENO	0.008	0.186	0.00
BIPHENYL	0.005	0.117	1.40	DIBENZ	0.008	0.167	0.00
ACENTHY	0.002	0.046	0.00	BENZOP	0.007	0.151	0.00
ACENTHE	-0.001	-0.017	0.00	C10ALK	0.004	0.080	8.62
FLUORENE	0.015	0.326	0.62	C11ALK	0.008	0.174	7.15
C1FLUOR	0.028	0.613	1.36	C12ALK	0.022	0.492	7.05
C2FLUOR	0.031	0.693	0.88	C13ALK	0.020	0.450	7.56
C3FLUOR	0.036	0.779	0.26	C14ALK	0.025	0.557	7.02
DITHIO	0.032	0.705	1.39	C15ALK	0.025	0.544	6.51
C1DITHIO	0.043	0.947	2.55	C16ALK	0.032	0.717	5.80
C2DITHIO	0.042	0.936	3.63	C17ALK	0.009	0.191	5.17
C3DITHIO	0.041	0.913	2.78	PRISTANE	0.021	0.461	4.34
PHENANTH	0.038	0.832	1.88	C18ALK	0.034	0.762	4.49
C1PHENAN	0.041	0.907	5.02	PHYTANE	0.035	0.777	2.47
C2PHENAN	0.041	0.919	5.63	C19ALK	0.036	0.807	4.64
C3PHENAN	0.040	0.892	3.61	C20ALK	0.036	0.800	4.05
C4PHENAN	0.038	0.840	0.75	C21ALK	0.027	0.604	3.85
ANTHRA	0.007	0.149	0.00	C22ALK	0.029	0.650	3.87
FLUORANT	0.018	0.393	0.00	C23ALK	0.030	0.664	3.32
PYRENE	0.017	0.369	0.00	C24ALK	0.031	0.683	2.96
C1FLUORA	0.037	0.830	0.27	C25ALK	0.028	0.617	2.43
BENANTH	0.017	0.378	0.00	C26ALK	0.027	0.598	2.31
CHRYSENE	0.036	0.787	0.35	C27ALK	0.027	0.592	2.13
C1CHRYS	0.038	0.842	0.42	C28ALK	0.021	0.464	1.49
C2CHRYS	0.025	0.563	0.23	C29ALK	0.024	0.530	1.47
C3CHRYS	0.031	0.692	0.00	C30ALK	0.019	0.431	0.96
C4CHRYS	0.013	0.299	0.00	UCM	0.019	0.419	0.34
BENZOBFL	0.017	0.368	0.00				

Figure 1. Subtidal #3 water sampling sites in Prince William Sound in 1989.

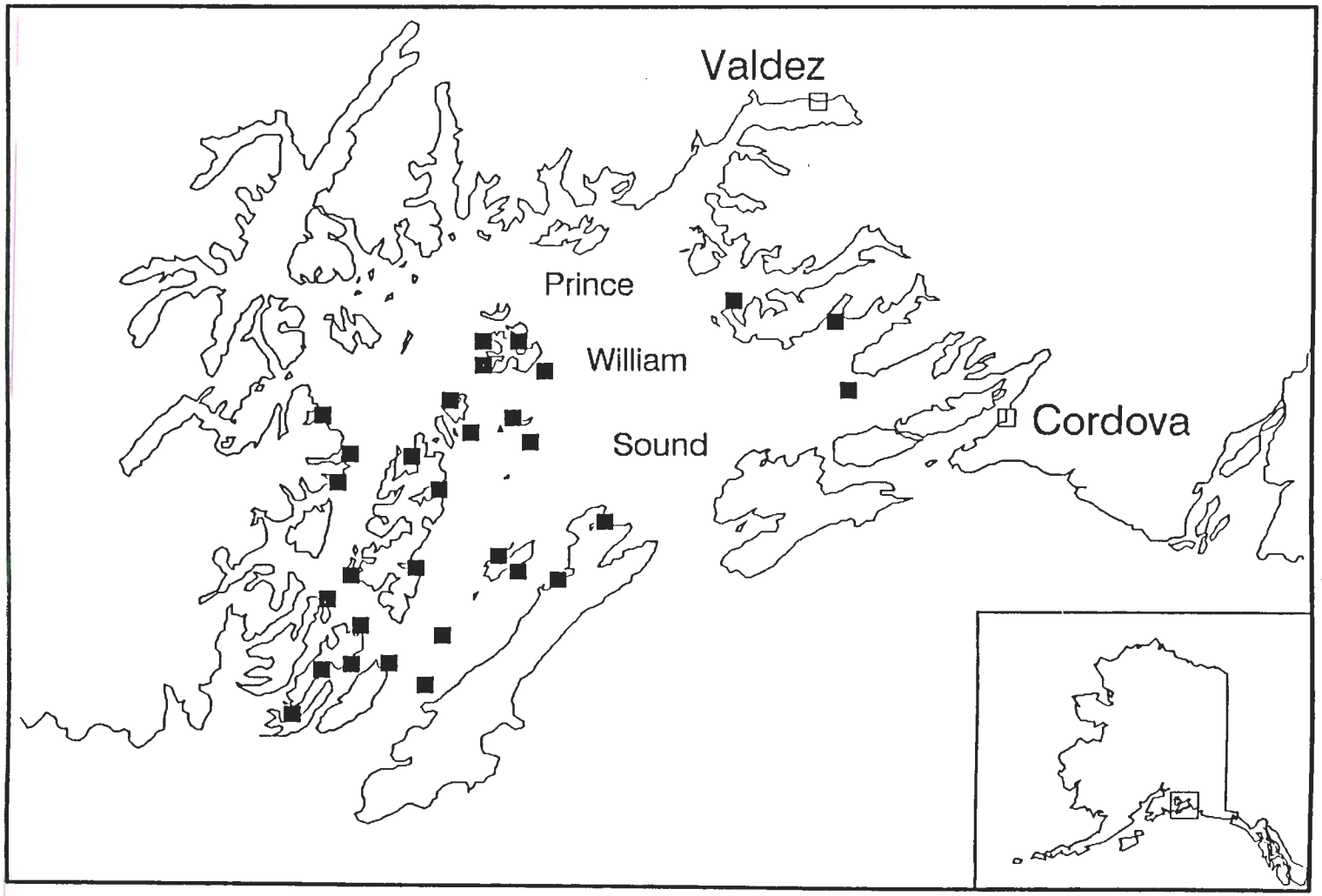


Figure 2. Subtidal #3 caged mussel deployment sites in Prince William Sound in 1989,1990, and 1991.

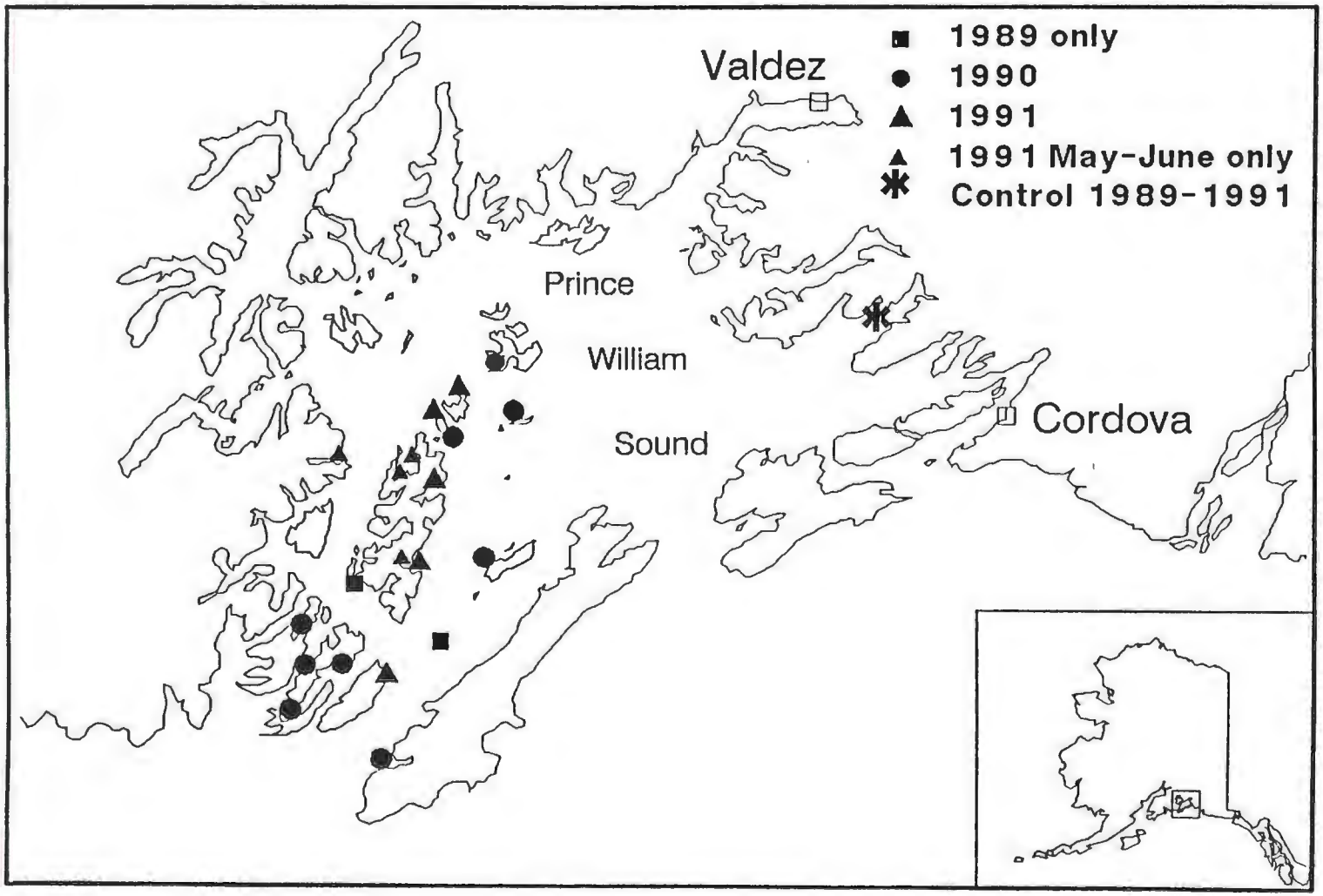


Figure 3. Subtidal #3 caged mussel deployment sites in the Kenai Peninsula, Alaska Peninsula, and Afognak Island areas where samples were collected in 1989 and 1990.

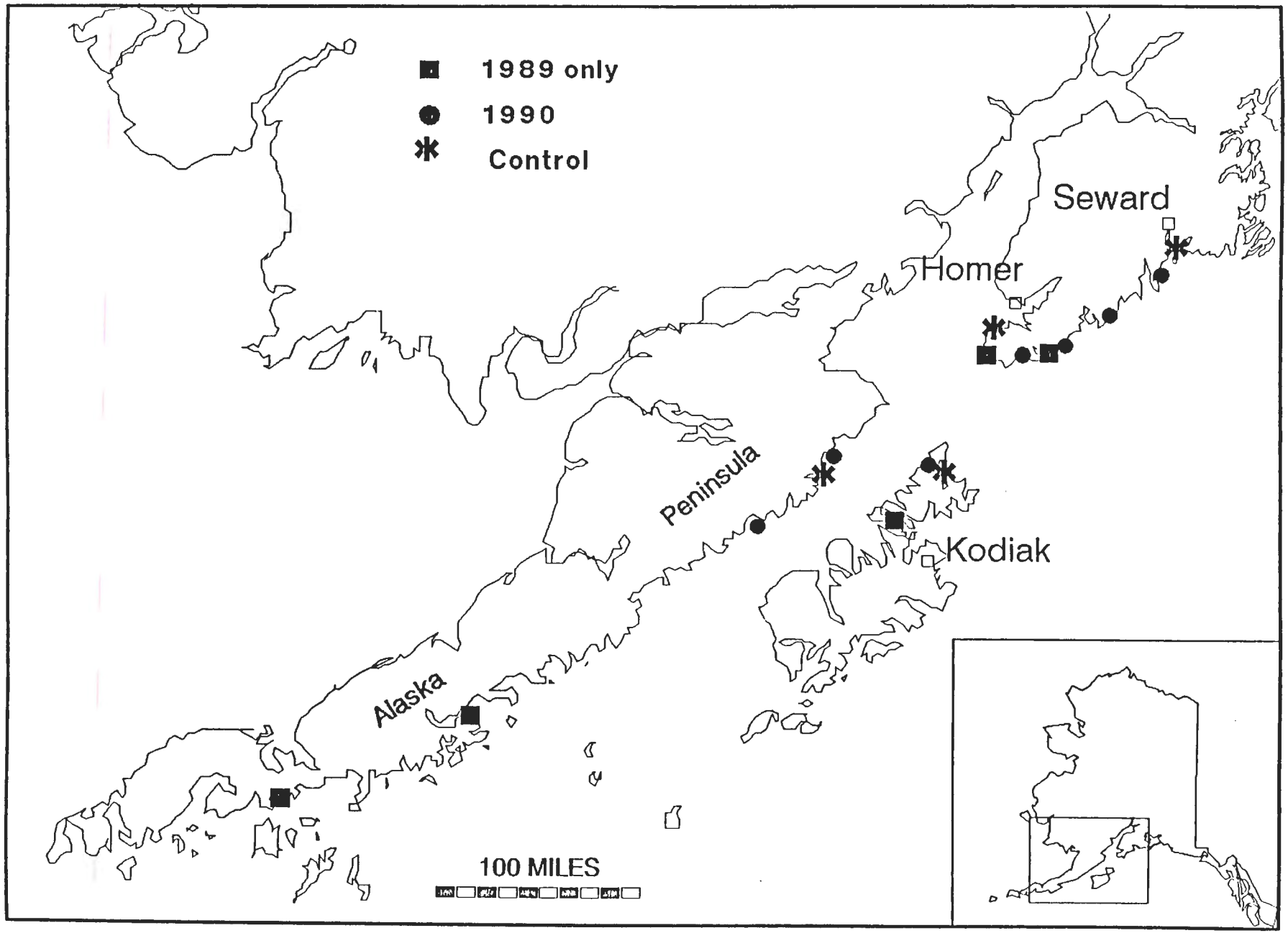


Figure 4. Oil component values vs. deployment site of caged mussels deployed in 1989 and 1990. Plotted at each site are oil component values of all deployment depths and all deployment times for which hydrocarbon analyses are available. Oil component values are related to the logarithm of the amount of oil contamination.

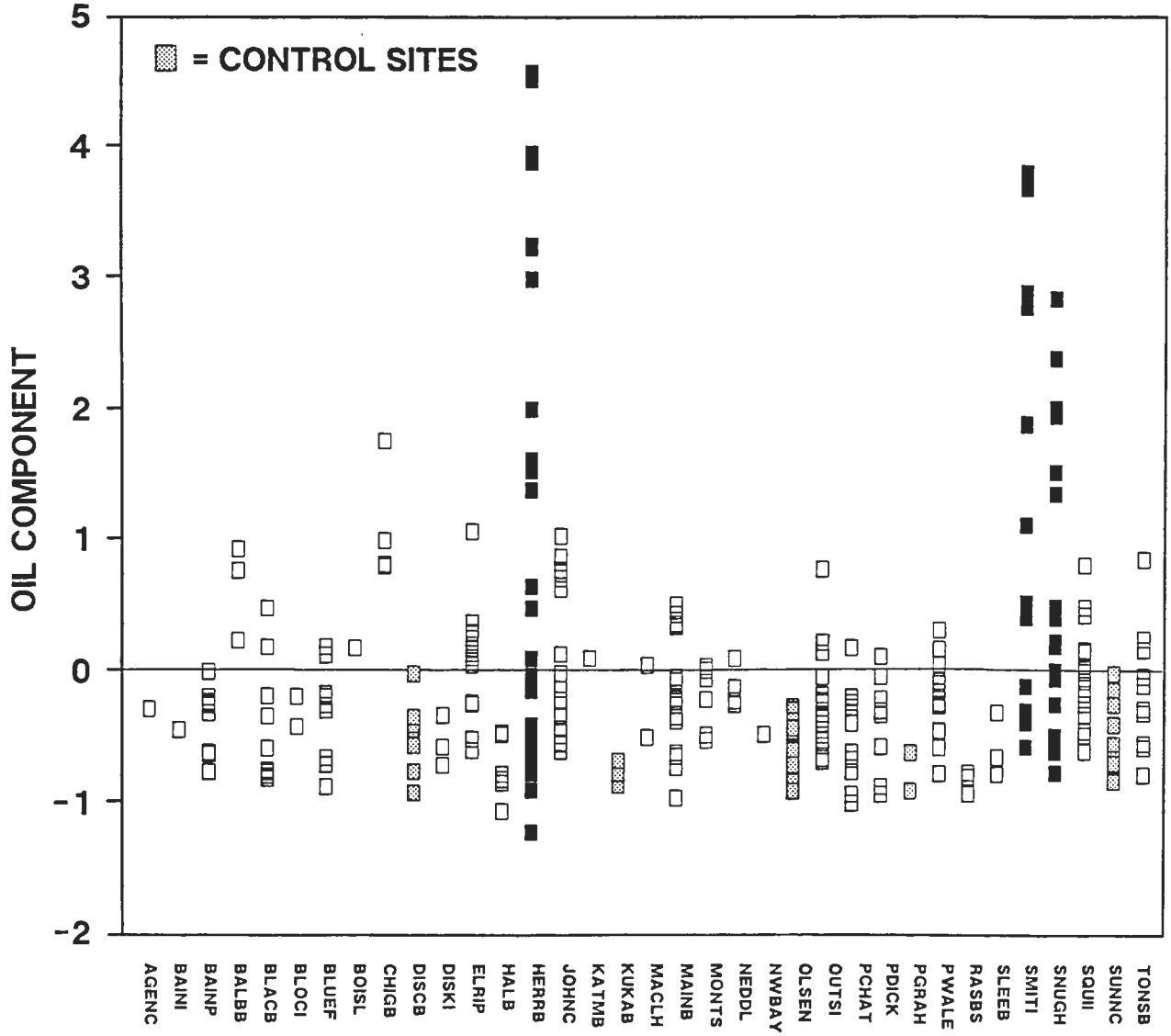


Figure 5. Histogram of oil component value distribution of caged mussels deployed at control sites in 1989 and 1990. Plotted are the number of control site caged mussel samples within one-half standard deviation unit interval of the mean value of component 1 for these control samples.

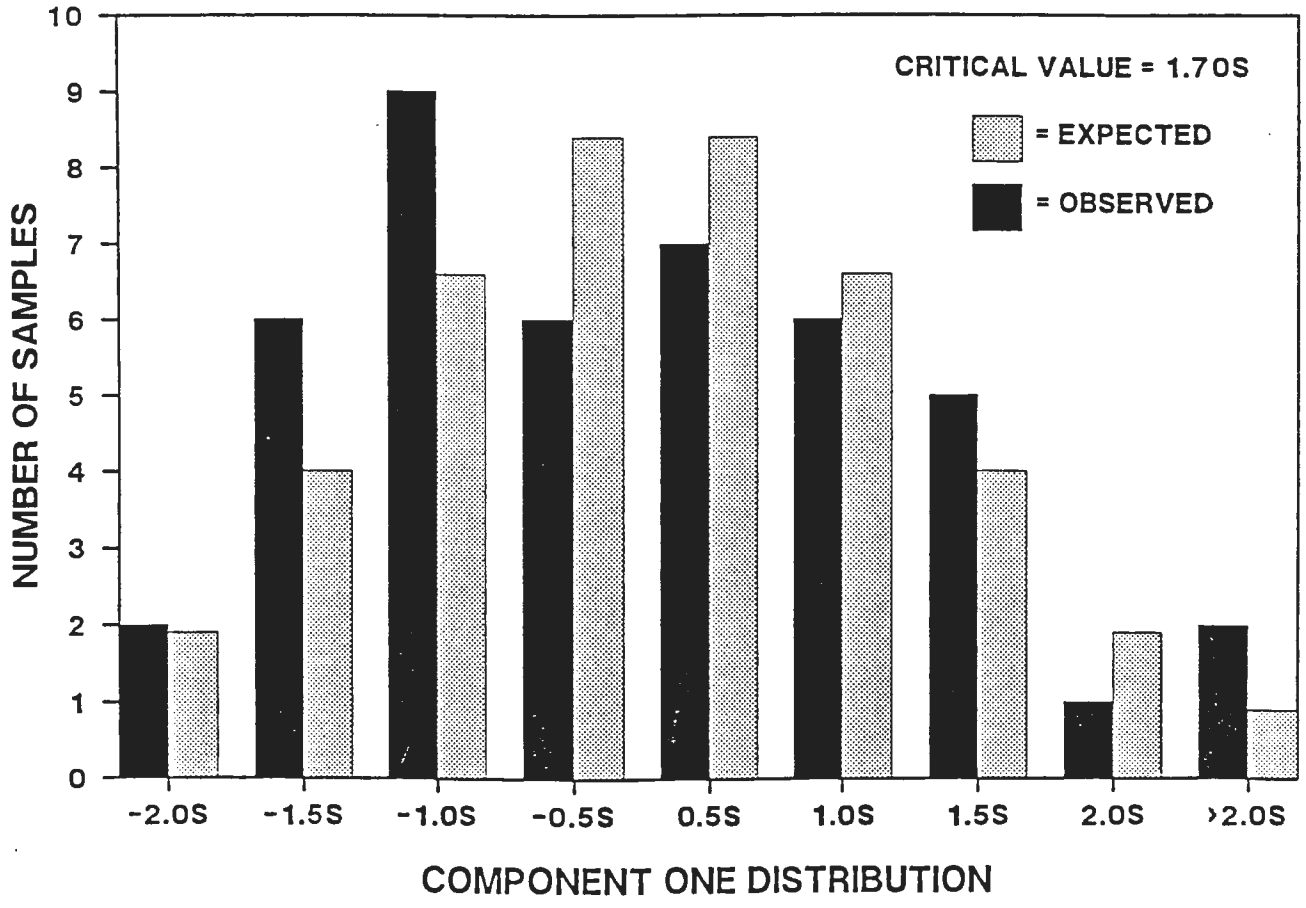


Figure 6. Oil component value of caged mussels deployed at 1 m, 5 m, and 25 m depths at Herring Bay, 1989 and 1990. Dates on the x axis are caged mussel retrieval dates. The shaded area indicates expected values for the oil component of control sites. Oil component values are related to the logarithm of the amount of oil contamination.

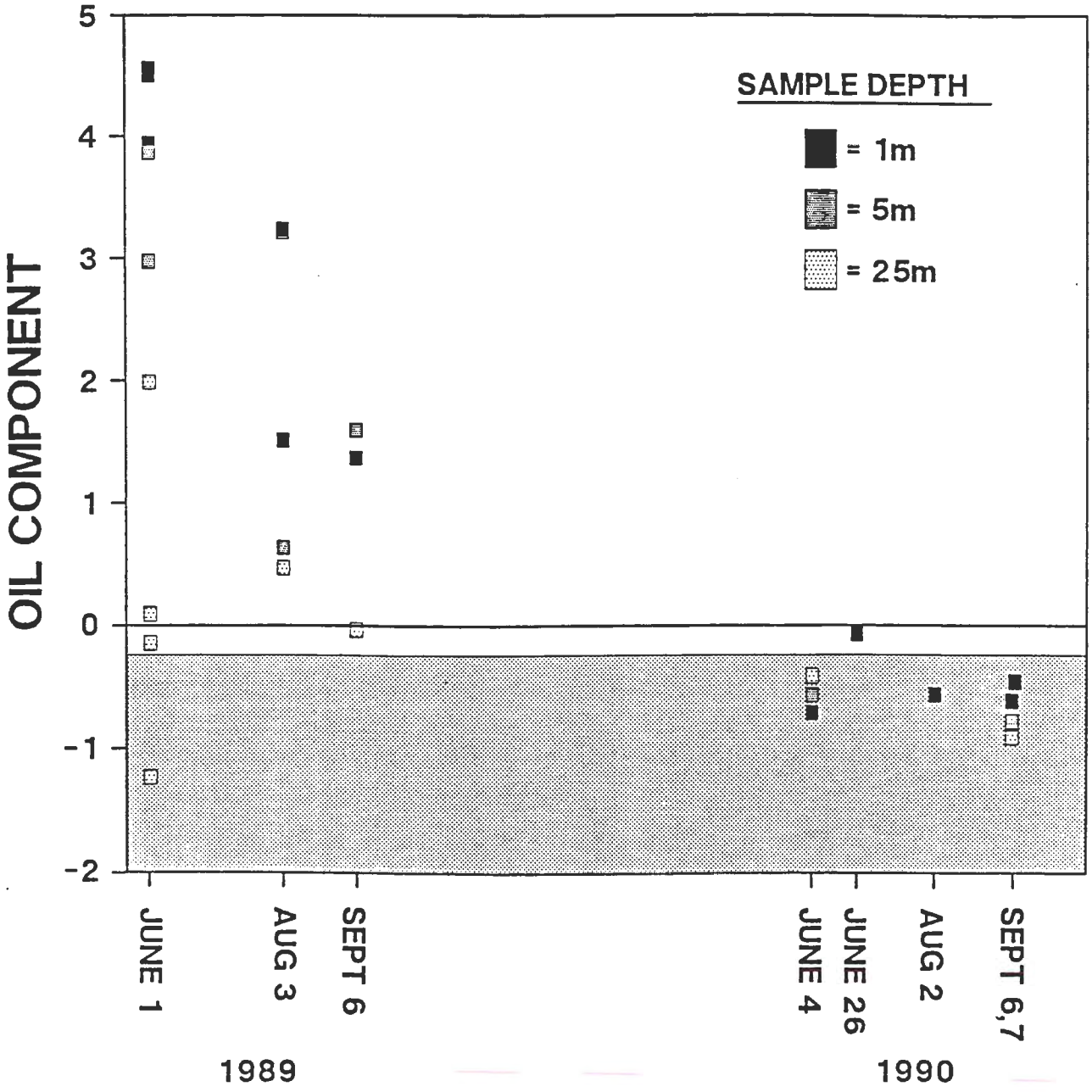
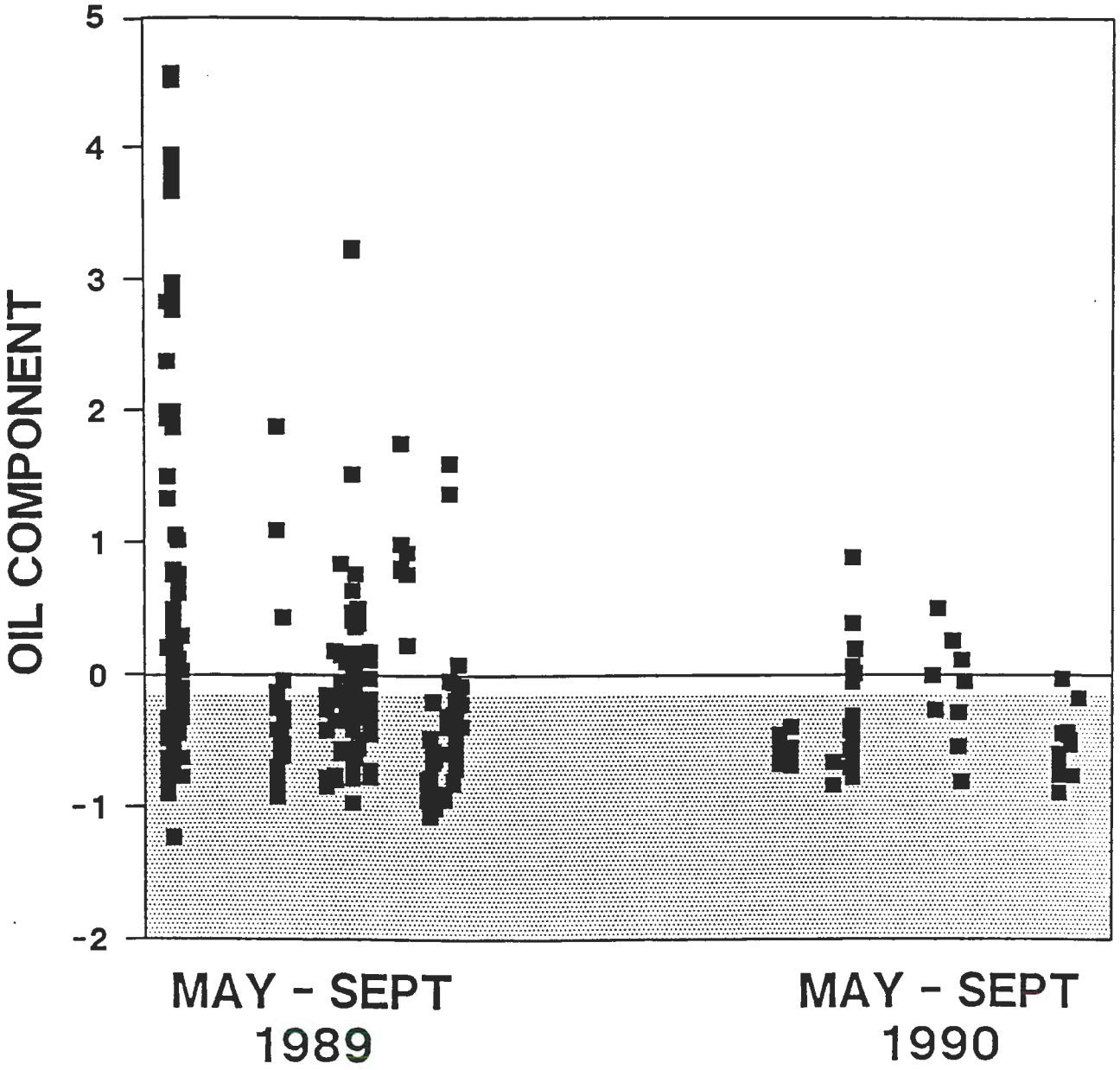


Figure 7. Oil component value vs. exposure time of caged mussels deployed in 1989 and 1990. The oil component value found for all deployed caged mussel samples analyzed is plotted with deployment date. Oil component values are related to the amount of oil contamination.



DRAFT 11/22/91

I. DRAFT PRELIMINARY STATUS REPORT

SUBTIDAL STUDY NUMBER 4

FATE AND TOXICITY OF SPILLED OIL FROM THE EXXON VALDEZ

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III. EXECUTIVE SUMMARY

This study, originally called Air/Water Project number 6, was designed and undertaken by NOAA in 1990 at the request of the NRDA Trustee Council, in response to specific recommendations from the Department of Justice's Peer Reviewers. The study is designed to: a) determine the toxicity of oiled environmental samples, using standard toxicity tests; b) examine the extent to which any observed toxicity may be attributed to oxygenated, polar products in weathered oil (versus the parent hydrocarbons found in fresh crude); and c) promote the synthesis of data and information (generated largely by other projects) on the geographic distribution, weathering, and potential effects of petroleum on living marine resources. This project has involved the collaboration of NMFS scientists from the Environmental Conservation Division of NWFC and the Auke Bay Laboratory of the AFSC, as well as projects carried out under contract to NOAA by SAIC. Toxicity testing has been carried out on sediment samples taken during the cruises of the FAIRWEATHER in 1989, the DAVIDSON in 1990, and THE BIG VALLEY in1991. Petroleum hydrocarbons were analyzed by ultraviolet fluorescence spectroscopy on the samples collected in 1989 and 1990, and the toxicity of sediments from oiled sites was greater than that from unoiled reference sites in both years. Smaller differences between oiled and reference sites were found in 1991. Final analysis and interpretation of the toxicity data will require data on hydrocarbon chemistry and grain size of the sediments (expected from Technical Services Project number 1). These analytical data are now available for 1989 and 1990, but have not yet been analyzed in detail; data for 1991 are not yet available. A contract was let to SAIC to study the extent to which any toxicity present in oiled sediments and interstitial waters may be attributed to polar, oxidation products (as opposed to parent hydrocarbons) in petroleum. Intertidal sediments and interstitial waters were collected from oiled and reference sites in Prince William Sound. Organic extracts of these samples were separated into polar and non-polar fractions, and the fractions were tested for relative toxicity by a number of tests. Results of the initial tiers of toxicity testing indicate that the polar fractions from the most heavily oiled site exhibit genotoxicity at least equal to that associated with the nonpolar fractions, but this toxicity is detectable only at very high concentrations. Further testing and chemical analysis of these fractions are still underway; a draft final report is expected in March 1992. Another contract was let to the Bermuda Biological Station to study whether polar oxidation products of petroleum occur in significant amounts in tissues of Mytilus edulis taken from the spill zone. Extracts of mussel tissues were chemically fractionated into nonpolar and polar

constituents and analyzed by ultraviolet fluorescence spectroscopy. Polar constituents were measured in mussels from oiled sites and from unoiled sites. Polar constituents occurred in tissues of mussels from oiled sites at levels that were proportional to (or less than proportional to) the amounts present in the original parent oil simultaneously accumulated in the tissues. The proportions of polar constituents appeared to increase, however, with molecular weight; that is, 5- and 6-ringed polar PAHs were retained in tissue in approximate proportion to their occurrence in crude oil, 3- to 4-ringed PAHs were retained in lower proportion, and 2-membered rings were retained in the lowest proportion. While some relevant literature and data have been identified and assembled for the petroleum budget synthesis exercise (objective c above), no other progress on this objective has been made since the last report. A synthesis workshop still is recommended as an important step in completing this synthesis task.

IV. OBJECTIVES

A. Document the toxicity of contaminated sediments and related environmental samples to selected marine biota

B. At selected sites, document and quantify the occurrence of oxidized derivatives of EXXON VALDEZ oil

C. Determine the extent to which the observed toxicity of oil-contaminated environmental samples may be attributable to oxidation products of petroleum.

D. Construct a summary budget or "mass balance" summarizing the fate of the spilled oil.

V. INTRODUCTION

The sediment toxicity surveys conducted under this project's objective A were initiated in 1989 under A/W Study 4 as part of the multidisciplinary studies conducted aboard the NOAA vessel FAIRWEATHER. Other portions of this study were designed and undertaken in 1990 by NOAA at the request of the NRDA Trustee Council, partly in response to specific recommendations from the Department of Justice's Peer Reviewers. The

study is designed to: a) demonstrate and quantify the toxicity of oiled environmental samples, using standard toxicity tests; b) determine the extent to which any observed toxicity may be attributed to oxygenated, polar products in weathered oil (versus the parent hydrocarbons found in fresh crude); and c) promote the synthesis of data and information (generated largely by other projects) on the geographic distribution, weathering, and potential effects of petroleum on living marine resources. This study is very closely coordinated with Subtidal Studies Number 1 and 2, directed by the NMFS Auke Bay Laboratory and the Alaska Department of Environmental Conservation, respectively, and relies also on collaboration with personnel of the NMFS Northwest Fisheries Science Center.

A detailed review and analysis of the scientific literature relating to the objectives of this project were presented in the detailed study plan (A/W6) for 1990.

VI. STUDY METHODOLOGY

Sampling Locations

Sediment sampling was carried out from the NOAA Ship FAIRWEATHER between June 29 and August 22, 1989; from the NOAA Ship DAVIDSON between June 25 and August 5, 1990; and from the charter vessel THE BIG VALLEY between June 15-25, 1991. Separate samples were taken concurrently from the same stations under Air/Water Study Number 2 (now Subtidal Study Number 1) for the chemical analyses and sediment grain-size analyses. The strategy was to sample one site per day, in support of three studies: Subtidal Studies 1, 2, and 4 (Air/Water Studies 2, 3, and 6, respectively in 1990). The sites sampled in 1989 were identified in the Preliminary Status Report for A/W Study 4 (1/12/90). The sites visited in 1990 were selected from the list of sites visited in 1989 under A/W Studies 2 and 4, supplemented by a group of "reference sites" that were added specifically to test effects on benthic community composition. The 1990 sites, selected on the basis of preliminary information on distribution of floating and/or beached oil, encompassed essentially the full geographic range of spill exposure (i.e., from Bligh Island in PWS to Katmai Bay on the Alaska Peninsula). The 1991 sampling for toxicity testing was restricted to 7 "exposed" sites and 8 "reference" sites within Prince William Sound. Positions of the intertidal sampling locations for each site sampled in 1990 and 1991 are shown in Table 1.

TABLE 1. NAMES AND LOCATIONS OF SITES SAMPLED FOR TOXICITY TESTING AND CHEMICAL ANALYSIS OF SEDIMENTS IN 1990 AND/OR 1991

1990 Site No.	Name	Nominal Oiling Designation*	Latitude	Longitude	1991 Site No.
01	Olsen Bay	R	60° 44.8' N	146° 13.1' W	---**
02	Port Fidalgo	R	60° 50.20'N	146° 12.58' W	---
03	Smith Island	E	60° 31.8' N	147° 20.8' W	---
04	Zaikof Bay	R	60° 16.85' N	147° 02.1' W	15
05	Rocky Bay	R	60° 20.3' N	147° 08.2' W	14
06	West Bay	R	60° 51.8' N	146° 46.5' W	01
07	Herring Bay	E	60° 26.54'N	147° 47.13' W	05
08	Disk Island	E	60° 29.8' N	147° 39.7' W	03
09	Block Island	E	60° 31.7' N	147° 36.4' W	04
10	Northwest Bay	E	60° 33.1' N	147° 34.6' W	02
11	NE Knight Island	E	60° 26.35' N	147° 37.65' W	---
12	Bay of Isles	E	60° 22.8' N	147° 45.4' W	13
13	Green Island	E	60° 16.2' N	147° 26.1' W	---
14	MacLeod Harbor	R	59° 53.21' N	147° 45.8' W	10
15	Mooselips Bay	R	60° 17.55' N	147° 18.0' W	11
16	Snug Harbor	E	60° 14.25' N	147° 44.1' W	12
17	Chenega	R	60° 19.85' N	148° 00.45' W	07
18	Lower Herring Bay	R	60° 24.4' N	147° 47.8' W	06
19	Drier Bay	R	60° 19.2' N	147° 44.0' W	08
20	Sleepy Bay	E	60° 03.95' N	147° 50.35' W	09
21	Fox Farm	E	59° 58.4' N	148° 10.65' W	---
22	Sunny Cove	R	59° 56.2' N	149° 19.1' W	---
23	Agnes Cove	E	59° 46.05' N	149° 34.55' W	---
24	Black Bay	R	59° 32.61' N	150° 12.78' W	---
25	Chugach Bay	E	59° 11.16' N	151° 37.9' W	---
26	Tonsina Cove	E	59° 19.75' N	150° 54.9' W	---
27	Windy Bay	E	59° 13.85' N	151° 31.0' W	---
28	Hallo Bay	E	58° 27.45' N	154° 00.3' W	---
29	Katmai Bay	E	57° 54.5' N	155° 04.5' W	---

*R = reference site; E = exposed site.

**--- = Not sampled in 1991.

OBJECTIVE A. Toxicity of Oil-Contaminated Sediments

1. Calibration of Microtox test on Exxon Valdez oil and oiled sediments

A survey of surficial sediment toxicity was carried out in 1989 under A/W Study 4, at all stations sampled during June to August 1989 from the NOAA Ship FAIRWEATHER (Leg II). Results of the Microtox survey in 1989 showed some weak correlations with UV fluorescence analyses of the sediment samples, and a similar survey was proposed for 1990 under the combined A/W Studies 2 and 4, on sediments from a subset of the previous year's stations. The toxicity bioassay used in the 1989 effort was the standard Microtox assay, in which a composite of the replicate sediment samples obtained at each depth from each sampling site was analyzed for sediment toxicity based on the inhibition of bioluminescence in Photobacterium phosphoreum (15-min Microtox assay). Organic extracts of the sediments are prepared and assayed for toxicity by the methods of Schiewe et al (1985). The Microtox assay is rapid, simple, inexpensive, and sensitive; and the bioassay results have correlated well in other studies with the results of other standard bioassays that use fish, amphipods or bivalve larvae as test organisms (Chang 1981, Williams et al. 1986, Giesy et al. 1988).

To ascertain that the Microtox test was responding to Exxon Valdez oil, calibration experiments were conducted in 1990 using whole Prudhoe Bay crude oil and weathered Prudhoe Bay crude oil obtained from heavily oiled locations in Prince William Sound. This calibration exercise was carried out by Dr. E. Casillas at the Environmental Conservation Division of the NMFS Northwest Fisheries Science Center in Seattle, WA, to support and improve the interpretation of Microtox test results obtained in 1989 and those proposed for 1990.

2. Toxicity Bioassays with *Ampelisca* and *Crassostrea*

Additional toxicity tests were performed on sediment samples taken at selected sites in 1990 and 1991 (See Table 2 for sites). Two specific tests, both following well-established protocols, were carried out: a sediment elutriate test using larval oysters *Crassostrea gigas*, and a whole sediment test using *Ampelisca abdita* . Oyster larvae are a sensitive test organism that has been used extensively in surveys of sediment toxicity. *Ampelisca* inhabits soft nearshore sediments that are possible sinks for petroleum. Subtidal Ampeliscid amphipods exhibited considerable sensitivity to oil in the aftermath of the AMOCO CADIZ spill (Cabioch et al. 1982). These two

species were selected to provide a direct measure of the toxicity of the residual oil to actual marine species, and to complement and calibrate the Microtox survey, which was to provide the basis for any spatial and temporal comparisons of toxicity.

Detailed methods for both of the proposed tests have been described previously: for the oyster (or mussel) larvae bioassay (Chapman and Morgan 1983; Chapman and Becker 1986); and for the *Ampelisca* test (Long, Buchman et al. 1989; Scott and Redmond 1990).

Sediment samples, representing both the more heavily oiled areas and the reference areas, were collected during the DAVIDSON cruise in 1990. At each of 21 sites, four two-liter samples of surficial sediments (top 5 cm) were collected (one each at the intertidal, 6-meter, 20-meter, and 100-meter depths) for toxicity testing with *Crassostrea* and *Ampelisca*. In 1991, similar suites of samples were collected during the cruise of the charter vessel THE BIG VALLEY at 14 sites within Prince William Sound. In both years, these samples were stored on board ship at 0-4 °C, and offloaded at regular intervals for shipment on ice to the testing laboratory (Parametrix, Inc., as subcontractor to SAIC International) in Seattle. A few of the sediment samples were lost due to breakage during shipment in each year, and some of the samples did not contain enough material to carry out the full suite of bioassay tests. In these cases where sediment volume was limited, the amphipod bioassay was given priority and the oyster larvae bioassay was either deleted or carried out with fewer than the desired number (5) of replicate bioassays. All bioassays in both years were initiated within the prescribed 10 days of the collection of the samples, with the exception of 3 samples (1991) which were initiated on the eleventh day after collection. In 1989 and 1990 replicate sediment samples were taken at the same sites for analysis of hydrocarbons by an ultraviolet fluorescence (UVF) screening method. Samples collected in 1989 were analyzed using excitation and emission wavelengths of 310 and 370 nm, respectively (SAIC 1989), while the 1990 samples were analyzed using either phenanthrene (260/380 nm) or naphthalene (290/335) wavelengths ((Dr. M. Krahn, NMFS/NWFC.ECD). Replicate samples were also analyzed by gas chromatography-mass spectrography (GC-MS, under Subtidal Study No 2, and Technical Services Study No. 1). In 1991, replicate samples were taken only for GC-MS analysis.

OBJECTIVE B. Document and quantify the occurrence of oxidized derivatives of EXXON VALDEZ oil

OBJECTIVE C. Determine toxicity attributable to oxidation products

These two objectives have been pursued together through two separate projects. Under contract to NOAA, samples of interstitial water (2 x 180 liters) and sediments (2 x 20 kg) were collected by SAIC from the intertidal areas at three sites in Prince William Sound: an unoiled reference site at Mooselips Bay (Montague Island), and two oiled beach sites at Bay of Isles and Cluster Fox Cove (both on Knight Island). In addition, subtidal sediment samples (2 x 10 kg) were collected (by NOAA divers) at 6-m depths from Bay of Isles and Northwest Bay (Eleanor Island) and shipped to SAIC for analysis. Interstitial water samples were acidified and extracted with a mixture of dichloromethane and ethyl acetate; and the extracts were concentrated and screened by FID-GC analysis to verify recovery levels for spiked standards and adequacy of blanks. Aliquots of the extracts were transferred to DMSO and submitted to Parametrix, Inc. for initial toxicity screening with the Microtox assay and the SOS Chromotest mutagenicity assay (Quillardet and Hofnung 1985, Quillardet et al 1985). Those extracts that elicited significant responses in the initial tests of toxicity and/or genotoxicity were subjected to chemical fractionation procedures to separate polar constituents and oxidized derivatives from the parent, non-oxidized constituents of petroleum. These fractions were tested for genotoxicity, and mutagenicity using the same suite of assays applied to the whole extracts to determine the importance of the polar derivatives' contribution to the observed toxicity. Based on the results of these assays, some samples were selected for further testing with a 48-hr survival and development assay using bivalve (oyster) larvae. Anaphase aberrations and sister chromatid exchange will also be determined in the exposed bivalve larvae, and and the Ames mutagenicity test (Ames et al 1975) may be used to confirm some of the results with other tests.

Some of the chemical constituents in the polar fractions will be identified and quantified by GC-MS techniques, and related if possible to the original parent compounds in petroleum. Target compounds of the fractionation and analytical scheme include phenolic, carbonyl, quinone, and carboxylic derivatives of polynuclear aromatic hydrocarbons, for example: 9-fluorenone, 9-fluorenone carboxylic acids, phenanthraquinone as potential derivatives of phenanthrene. Analogs related to naphthalene, anthracene, chrysene, benzanthracene, pyrene, and benzpyrene will also be sought specifically, as will oxidation products of dibenzothiophenes. GC-MS data will be analyzed also for other major constituents in associated polar

fractions to provide a general characterization of the polar compounds found in these samples.

In a separate pilot effort, tissues of mussels, *Mytilus edulis*, were analyzed to determine whether polar oxidation products of petroleum can be detected in the tissues of bivalve mollusks exposed to Exxon Valdez oil in Prince William Sound, Alaska, at concentrations sufficient to modify the estimates of exposure and toxicity that would otherwise be based on measurements of parent petroleum hydrocarbons alone. Dr. Kathryn Burns at the Bermuda Biological Station was selected to perform this work, and her report summarizing the results was provided to NOAA in February, 1991. Dr. Burns previously participated as a principal scientist for an international group of experts to test the need for inclusion of oxidized derivatives in estimates of toxic organics in the marine environment. Her results (Burns et al. 1990; Ehrhardt and Burns 1990) confirmed that oxygenated products of petroleum-derived aromatic hydrocarbons were present in Bermuda coastal waters at concentrations higher than the parent compounds and were also present in at significant levels in bivalve tissues and sediments.

Ten tissue samples, representing a broad range of exposure compositions and concentrations, were selected from the inventory of samples collected under Projects A/W3 and CH1, and were shipped frozen to Bermuda, where they were extracted with methylene chloride. Total extractable lipid was determined gravimetrically, and aliquots of each sample were then filtered and cleaned on a small silica gel precolumn to separate contaminating lipids from hydrocarbons and their oxidation products. The primary fraction from this precolumn was concentrated and then fractionated further based on polarity by normal phase HPLC (silica and nucleosil columns in series). Five fractions, F1 to F5 in order of increasing polarity, were collected. A sixth fraction (F6) was taken directly from the precolumn by further elution with methylene chloride. Each of the six fractions from each sample were analyzed by ultraviolet fluorescence at three different combinations of excitation and emission wavelengths: 280/327nm (1- to 3-ringed aromatics and quinone derivatives), 310/360nm (4- to 6-ringed PAHs and N-substituted PAHs), and 380/430nm (oxidation products of 4- to 6-ringed PAHs). At the first two wavelengths, Prudhoe Bay crude oil (PBC) was used as the calibration standard, while hydroxy-pyrene was used at the longer wavelength. The F1 fraction from each sample was analyzed also by gas chromatography (FID).

OBJECTIVE D. Summary Budget or "Mass Balance" for the Fate of Oil.

This task is primarily a synthesis function. Data and information on the distribution and fates of EXXON VALDEZ oil need to be assembled from a number of sources, interpreted in the light of existing information and models, and presented in a way that will support a region-wide assessment of potential effects of the spill.

The following compartments and processes Are proposed for inclusion in the FATES budget. Potential sources of data, historical information, and modeling expertise are also noted:

1.	Floating oil (distribution in Time & Space)	A/W1, Galt OSSM
2.	Evaporation and atmospheric dispersion	A/W5, J. Galt ALOHA
3.	Photoxidation in the atmosphere	
4.	Mousse formation	J. Payne (1983,4)
5.	Beaching of oil & mousse (T&S)	A/W1, CH1, HMRB, DEC
6.	Water column accommodation (T&S)	A/W3, DEC, Payne 1983,4, Spaulding 1983
7.	Photooxidation in water column, in slicks and on beaches	A/W2, A/W3, TS1
8.	Biodegradation in water column	A/W3
9.	Transport to subtidal sediments	A/W2, Payne 1983,4
10.	Biodegradation in sediments	A/W2, A/W6, TS1

Results from the above projects will be assembled as they become available. Representatives of the above noted activities, along with other recognized experts on oil weathering and fates, will then be consulted for recommendations on appropriate approaches to synthesis, and for their judgments on the suitability and adequacy of existing information for development of an overall summary of oil fate. Apart from the information on polar constituents described in this report, no original data are proposed for collection under this project. Timely progress on the development of this oil budget therefore depends on the availability of suitable information from other sources and projects; chemical data, i.e., from TS1 and Subtidal Study no.1, will be of utmost importance to the completion of this project. The reliability of all information used will be assessed and qualified in the final analysis.

Quality Assurance and Control

All samples have been taken with careful adherence to Chain-of-Custody requirements. All of the intertidal and subtidal sediment samples analyzed under this study will be retained in the custody of the laboratories performing the analyses, as called for in the guidelines provided by the Technical Services 1 Analytical Committee. The detailed procedures for collection of intertidal and subtidal sediment samples are given in the proposal for Air/Water Study Number 2.

VI. STUDY RESULTS

OBJECTIVE A. Toxicity of Contaminated Sediments

1. Toxicity Bioassays with *Ampelisca* and *Crassostrea*

A final report on the 1990 toxicity bioassays was received on November 12, 1990 (SAIC 1990). All tests have been completed also on the 1991 samples, and a draft report (SAIC 1991) was received from SAIC on October 7, 1991. The draft report has been reviewed for completeness relative to the statement of work and proposal, and minor comments were provided to SAIC on October 14. Also, an informal data report was received January 4, 1991 on the UVF analyses carried out on the 1990 sediment samples by Dr. M. M. Krahn of the NMFS Northwest Fisheries Science Center's Environmental Conservation Division. Results of GC-MS analyses on replicates of these samples also became available during summer 1991 from Technical Services Study No. 1. No chemical data are available yet for the samples collected in 1991. The available data are summarized and discussed in this section.

1989 Results

Preliminary analyses and interpretations of the UVF, Microtox, and microbial data from 1989 were presented in a Preliminary Status Report from A/W Study 4 (Wolfe 1990). They are summarized briefly here for comparison with the 1990 and 1991 data.

Hydrocarbon concentrations measured by UVF in intertidal sediments ranged from <1 ppm to about 10,000 ppm, while those in intertidal pore water ranged from about 0.01 ppm to over 900 ppm. Sediment toxicity as measured by EC-50's in the Microtox bioassay ranged from less than 0.5 mg/ml (most toxic) to more than 50 mg/ml (least toxic). Most Probable

Numbers (MPN) of hydrocarbon-degrading microorganisms ranged from less than 20 to more than 1 million cells/g dry sediment.

Pearson's rank correlation analysis showed the UVF results for intertidal sediment and porewater to be highly correlated ($n=42$; $r= 0.628$; $p< 0.001$) The UVF data for intertidal sediments were also highly rank correlated with EC-50's (mg/ml) determined by the Microtox bioassay ($n=42$; $r= -0.499$; $p< 0.001$), and with the MPN of hydrocarbon-degrading micro-organisms in the intertidal sediments ($n=41$; $r= 0.603$; $p< 0.001$). UVF response in the intertidal pore water was also highly rank correlated with the MPNs in the pore water ($n=42$; $r= 0.691$; $p< 0.001$).

The UVF data showed a generally decreasing trend in hydrocarbon concentration at all depths except 100m with increasing distance from the spill site. This pattern was strongest for the data from the 3- and 6-meter depths. The Microtox EC-50s of sediment extracts from the 3- to 20-meter depths increased (i.e., were less toxic) as distance from the spill site increased.

Both hydrocarbon concentrations measured by UVF and EC-50s measured by Microtox varied considerably with depth and exhibited quite different patterns with depth at different stations. At each individual depth, however, as well as for all depths at all stations ($n=247$; $r= -0.465$; $p< 0.001$), EC-50 was strongly rank correlated with UV/F values . In the subtidal samples, MPN was not correlated with either UVF values or with EC-50s.

1990 Results

The UVF data ranged from zero to 970 ppm, with the highest values generally in the intertidal zone at oiled sites within Prince William Sound. Three qualitative distributional patterns of UVF signal with depth were tentatively identified: A) moderate to very high intertidal values, probably associated with intertidal oiling, with decreasing values at depth; B) a usually variable maximum value at 20-40 meters, with lower values both intertidally and at greater depth; C) probably unoiled intertidal values, increasing to a maximum at 40-100 meters. In 1989, one additional pattern (D) had been identified, with variable but quite low values at all depths. The three qualitative patterns are illustrated in Figure 1, and the sites exhibiting each pattern are identified in Table 2 in declining order of UVF signal either for 1989 or 1990.

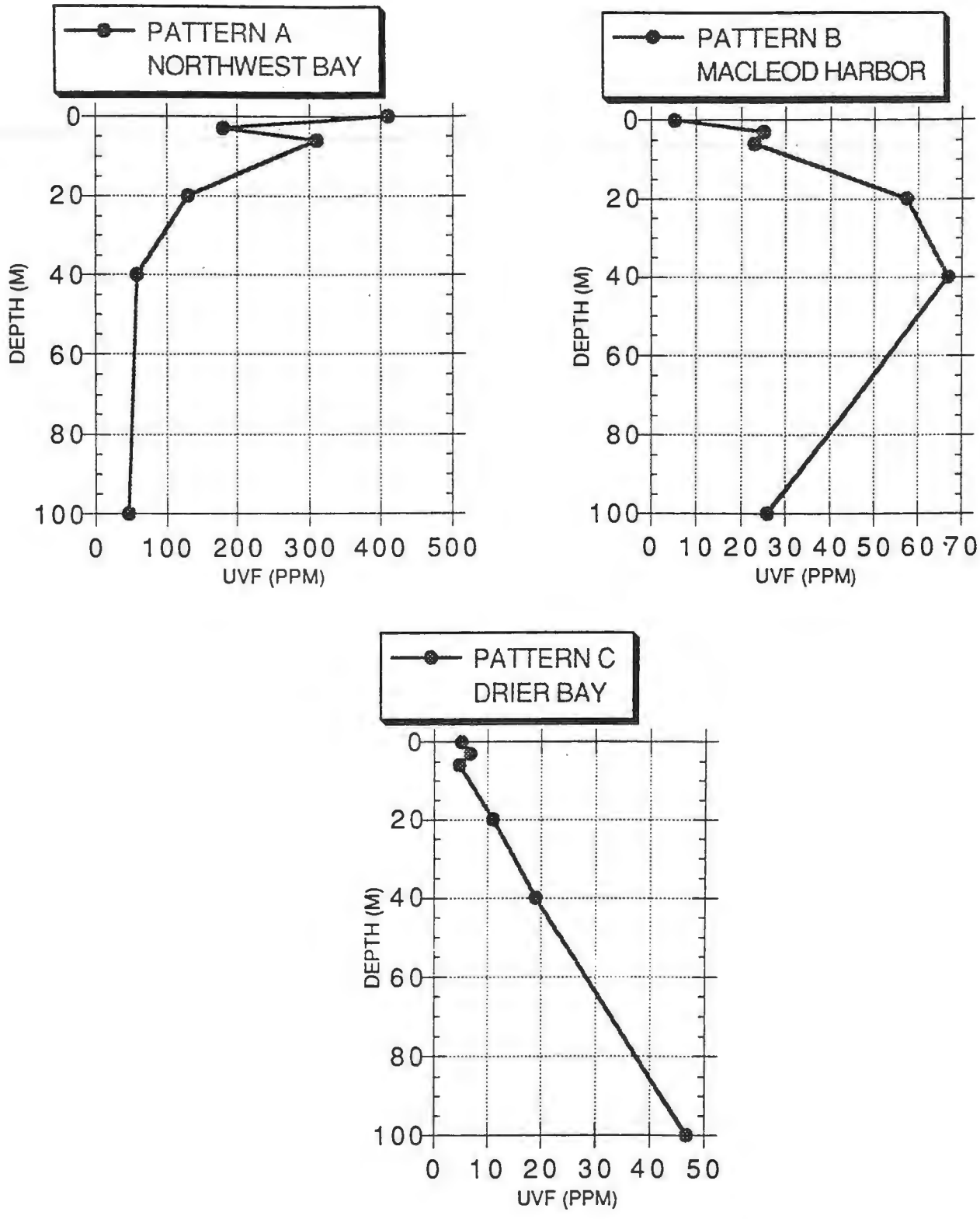

Figure 1. Typical Patterns of Hydrocarbon Disribution with Depth at Prince William Sound Sites, 1990. See Tables 2A and 2B for sites exhibiting these patterns in 1990 and 1989.

Table 2A. Patterns of Hydrocarbon Distribution at Prince William Sound and Gulf of Alaska Sites, 1989-1990, Based on Ultraviolet Fluorescence Analyses (1989 analyses by SAIC; 1990 analyses by NMFS/NWFC/ECD)

Desig-nation	Site Name	Pattern A 1989 Max	Pattern A 1990 Max	Pattern B 1989 Max	Pattern B 1990 Max	Pattern C/D 1989 Max	Pattern C 1990 Max
X*	Herring Bay	289.5	970•				
X*	Northwest Bay	1589	410•				
X*	Snug Harbor	27.42	100•				
X*	Disk Island	9842	53•				
X	NE Knight Island	NS	51•				
R*	Chenega	NS	41•				
X	Fox Farm	51.01	29•				
X*	Bay of Isles	35.31	26•				
X	Smith Island	2420	23•				
Gx	Agnes Cove			48.1	600		
X*	Block Island			NS	210•		
X*	Sleepy Bay			86.73	76.7•		
R*	MacLeod Harbor			NS	67		
Gx	Windy Bay	21.67			62•		
Gx	Tonsina Cove	55.07			37•		
X	Green Island			21.09			79•
R	Olsen Bay					4.99	54
R*	Drier Bay					NS	46.7
R*	Zaikof Bay					NS	33
R*	Rocky Bay					8.8	33
R*	West Bay					NS	30
R*	Mooselips Bay					NS	22
R*	Lower Herring Bay					NS	21
R	Port Fidalgo					NS	20
Gx	Chugach Bay			46.06			20•
Gr	Black Bay					21.07	18•
Gx	Hallo Bay	30.47					17•
Gx	Katmai Bay	37.04					12•
Gr	Sunny Cove			77.4			9.7•

*Sites sampled also in 1991. NS = No sample at this site in 1989. • = Weathered PBCO pattern

Table 2B. Patterns of Hydrocarbon Distribution at Prince William Sound and Gulf of Alaska Sites, for those Sites Sampled only in1989, Based on Ultraviolet Fluorescence Analyses

Designation	Site Name	Pattern A 1989 Max	Pattern A 1990 Max	Pattern B 1989 Max	Pattern B 1990 Max	Pattern C/D 1989 Max	Pattern C 1990 Max
	Mummy Isd	188.6					
	Chignik Bay			19.35			
	McArthur Cove			14.39			
	Taroka Arm			10.9			
	Seldovia Bay			10.03			
	Ivanof Bay					25.51	
	Gore Point					11.64	
	Iktua Bay					11.23	
	Columbia Bay					9.56	
	Eshamy Bay					7.65	
	Cabin Bay					7.34	
	Zachary Bay					5.39	
	Sawmill Bay					4.87	
	Port Dick					4.7	
	Andreon Bay					4.51	
	Shelter Bay					3.82	
	Ushagat Isd					2.64	
	Wide Bay					2.63	
	Cape Douglas					2.55	
	Halibut Bay					1.37	
	Amakdedori Bch					1.31	
	Ursus Cove					1.2	
	King Cove					0.94	
	Douglas Beach					0.84	

Group A consists of sites (mainly within PWS) that received heavy intertidal oiling and that may have received either coincident or resultant subtidal exposure. Group B includes sites that exhibit near minimal UVF values in the intertidal area, but show indications of elevated subtidal oil. Many of the oiled sites along the Kenai Peninsula exhibited this pattern. Pattern C represents stations for which the UVF values and patterns generally suggested lack of significant oiling. With the exception of Chenega (Pattern A) and MacLeod Harbor (pattern B), all of the sites designated *a priori* as reference sites exhibited this pattern, as did most of the 1989 sites located outside of PWS (Table 2A,B). All of the PWS sites that exhibited Pattern A in 1989 did so again in 1990; while those outside of PWS shifted either to Pattern B or C in 1990. Similarly, some sites that exhibited Pattern B in 1989 shifted to pattern C in 1990. These shifts may represent transport of oil from intertidal to subtidal sediments, or simply preferential retention of oil by subtidal sediments. Despite methodological differences between 1989 and 1990, which preclude detailed, quantitative comparisons between years, the UVF data clearly distinguish with only one exception (Chenega) the two sets of sites, exposed and reference, originally selected for toxicity testing. The Chenega site, though known to have been oiled intertidally, was originally considered nonetheless likely to be acceptable as a reference site for effects on subtidal (40-100m) infauna. While the UVF data for that site reinforce that initial conclusion (i.e., the PBCO pattern was not discernible in the 40/100 m samples), the shallower samples from this site did exhibit the PBCO pattern. For purposes of analyzing the toxicity data, therefore, Chenega is most appropriately considered an exposed site. Quantitative GC-MS data are currently being evaluated for all these sites; preliminary examination of the 1990 GC-MS data suggests separation of intertidal samples between the exposed and reference site groups based on data for Sum of Dibenzothiophenes, Sum of Aromatics, and Pristane/Phytane Ratio, whereas data for Unresolved Complex Mixture and Carbon Preference Index suggest differences in subtidal samples as well. More detailed analyses are needed for definitive comparisons.

The results of the 1990 toxicity bioassays with *Ampelisca abdita* and *Crassostrea gigas* are summarized in Tables 4A,B and 5A,B. Mean percent mortalities of test organisms (corrected for controls) were compared for the set of reference sites against the set of exposed sites (the sites are listed in Table 3), using a t-test. The mean percent mortality of amphipods was significantly ($p < 0.05$) greater for the PWS exposed sites only at the zero-meter tidal level, and only when Chenega was considered an exposed site (Table 4A). Many of the zero-meter samples produced amphipod mortalities that were significantly different from control mortalities in the bioassay. The mean percent mortality of amphipods was also greater for the

PWS reference sites at the 20-meter depth, regardless of whether Chenega was considered an exposed site ($p = 0.061$) or a reference site ($p = 0.032$). The control-corrected mortalities were low, however, for these 20-m samples, and none of the amphipod test results with subtidal samples were significantly different from controls. Oyster larvae exhibited significantly ($p < 0.05$) greater mortality at the 20-m and 100-m depths in the exposed sites, if Chenega was treated as a reference site (Table 5B). Chenega deep sediments were non-toxic, however, and the difference between means at reference and exposed sites was not significant ($p > 0.10$) when Chenega was treated as an exposed site.

Amphipod mortality and oyster mortality were not significantly correlated (Pearson rank correlation) either with each other or with UVF signal over the entire suite of PWS sites and depths ($n = 63$). Mortality of test amphipods was significantly correlated ($p<0.01$) with UVF signal, however, at the zero-meter depth alone ($n = 16$). Neither oyster larvae or amphipod mortality were significantly correlated ($p < 0.05$) with UVF at any other depth.

Table 3. Reference and Exposed Sites in Prince William Sound, 1990

PWS SITES USED FOR TOXICITY COMPARISONS-1990

PWS REFERENCE SITES (6)		PWS EXPOSED SITES (10)	
5	ROCKY BAY	7	HERRING BAY
6	WEST BAY	8	DISK ISLAND
14	MACLEOD HARBOR	10	NORTHWEST BAY
15	MOOSELIPS BAY	12	BAY OF ISLES
18	LOWER HERRING BAY	16	SNUG HARBOR
19	DRIER BAY	20	SLEEPY BAY
		9	BLOCK ISLAND
		11	NORTHEAST KNIGHT
		13	GREEN ISLAND
		17*	CHENEGA ISLAND

*Chenega was originally designated a Reference Site

TABLE 4. Mean Percent Mortality of *Ampelisca abdita* tested with Sediments from four different depths at Reference and Exposed Sites in Prince William Sound in 1990.

A. T-TEST: PWS REFERENCE VS EXPOSED SITES
(with Chenega as Reference, N = 7 vs 9)

AMPHIPOD MORTALITY (%), control corrected data

	REFERENCE		EXPOSED		SIGNIFICANCE
DEPTH (M)	MEAN	S.D.	MEAN	S.D.	LEVEL
0	29.4	19.5	51.7	28.2	**0.096**
6	23.7	12.0	18.2	9.0	0.317
20	23.4	8.0	13.6	7.6	0.032
100	14.1	7.3	11.3	4.2	0.368

B. T-TEST: PWS REFERENCE VS EXPOSED SITES
(with Chenega as Exposed, N = 6 vs 10)

AMPHIPOD MORTALITY (%), control corrected data

	REFERENCE		EXPOSED		SIGNIFICANCE
DEPTH (M)	MEAN	S.D.	MEAN	S.D.	LEVEL
0	22.7	9.1	53.5	27.2	**0.019**
6	22.5	12.6	19.5	9.4	0.601
20	23.6	8.9	14.5	7.7	0.061
100	13.0	7.7	12.1	4.7	0.787

TABLE 5. Mean Percent Mortality of Oyster Larvae tested with Sediments from four different depths at Reference and Exposed Sites in Prince William Sound in 1990.

A. T-TEST: PWS REFERENCE VS EXPOSED SITES
(with Chenega as Reference, N = 7 vs 9)

OYSTER LARVAL MORTALITY (%)

	REFERENCE		EXPOSED		SIGNIFICANCE
DEPTH (M)	MEAN	S.D.	MEAN	S.D.	LEVEL
0	47.7	27.9	31.3	12.2	0.134
6	40.5	19.4	48.6	17.8	0.398
20	35.5	8.6	51.2	17.1	**0.044**
100	38.6	19.6	61.0	22.0	**0.064**

B. T-TEST: PWS REFERENCE VS EXPOSED SITES
(with Chenega as Exposed, N = 6 vs 10)

OYSTER LARVAL MORTALITY (%)

	REFERENCE		EXPOSED		SIGNIFICANCE
DEPTH (M)	MEAN	S.D.	MEAN	S.D.	LEVEL
0	48.7	30.5	32.4	12.0	0.147
6	43.3	19.7	46.2	18.5	0.770
20	36.7	8.7	48.9	17.6	0.140
100	40.2	21.4	58.0	22.8	0.171

1991 Results

Toxicity data for *Ampelisca abdita* and *Crassostrea gigas* were obtained for the suite of 14 sites listed in Table 6. The 6 reference sites included Zaikof Bay, which was not tested for toxicity in 1990, and did not include West Bay, which was so tested. The 8 exposed sites (including Chenega) tested in 1991 were all tested in 1990. The 1991 toxicity data were less variable among replicates, for both control and test sediments, than the 1990 data had been. Also, control mortalities were lower in 1991. These differences permitted the detection of lower statistically significant levels of toxicity in 1991 than in 1990. The differences in sensitivity of the test organisms, however, precluded direct comparison of the 1991 and 1990 mortality data. UVF data were not obtained for the 1991 samples, and no chemical data are yet available for comparison with the toxicity data.

Table 6. Reference and Exposed Sites in Prince William Sound, 1990

PWS SITES USED FOR TOXICITY COMPARISONS-1991

PWS REFERENCE SITES (6)	PWS EXPOSED SITES (8)
ROCKY BAY	HERRING BAY
ZAIKOF BAY	DISK ISLAND
MACLEOD HARBOR	NORTHWEST BAY
MOOSELIPS BAY	BAY OF ISLES
LOWER HERRING BAY	SNUG HARBOR
DRIER BAY	SLEEPY BAY
	BLOCK ISLAND
	CHENEGA ISLAND*

*Chenega was originally designated a Reference Site

The condition of test organisms was superior in 1991 to that in 1990, leading to lower mortalities in the controls and greater overall test sensitivity. Mortality of one or both test organisms was statistically greater ($p < 0.05$) than for control organisms with sediments from several sites including samples from some depths at all 8 of the exposed sites, and at 4 of the 6 reference sites (Table 7). Sediments from one of the reference sites (Drier Bay) exhibited significant toxicity to *Ampelisca* at 0m, 6m, and 20m depths. No significant differences between test and control mortalities were noted with any sediments from 100m depths. Significant Ampelisca mortality, however, was noted at either the 6m or the 20m depth for all the exposed sites except Chenega, even though the amphipod mortality was significant for the 0m samples at only three of those sites. This result is consistent with the 1989-1990 shifts in UVF signal from intertidal to shallow subtidal depths noted earlier (Table 2), and could reflect transport of oil (and associated toxicity) from intertidal to subtidal sediments, or simply preferential retention of oil by subtidal sediments.

Table 7. 1991 Sediment Samples which exhibited toxicity to Ampelisca abdita and/or Crassostrea gigas larvae at levels significantly ($p < 0.05$) greater than controls. Sites are listed in order of diminishing intertidal toxicity to amphipods, based on 1990 results. Zaikof Bay was not tested in 1990. Sites marked * were significantly different in 1990 (Table 3A). (A = Ampelisca; C = Crassostrea; - = not significant)

Station	Ref/ Exp	Depth (meters) 0	6	20	100
Northwest Bay*	E	A	A	-	-
Snug Harbor*	E	-	A	A	-
Block Island*	E	-	A,C	C	-
Chenega Island*	E	C	C	-	-
Sleepy Bay*	E	A,C	A	-	-
Disk Island	E	-	-	A	-
Herring Bay	E	A	A	-	-
Bay of Isles	E	C	A	-	-
Lower Herring Bay	R	-	-	-	-
Mooselips Bay	R	A	C	-	-
Drier Bay	R	A,C	A	A	-
Rocky Bay	R	-	C	-	-
MacLeod Harbor	R	C	-	-	-
Zaikof Bay	R	-	-	-	-

There were no significant differences ($p < 0.10$) in 1991 between mean test mortalities of exposed sites and reference sites at any depth for either test organism. As in 1990, test mortalities of oyster larvae were not significantly correlated (Spearman rank) with those of amphipods. Overall, mortalities of both test organisms were lower in 1991 than in 1990, but these differences probably arose mainly from differences in control mortalities between the two years. To gain insight on possible changes between 1990 and 1991, the ratios of mean mortalities of test amphipods at the 8 exposed and 5 reference sites sampled in both years were compared (table 8). Between 1990 and 1991 the exposed/reference ratio decreased from three to one at the zero-meter depth; while rising at the 6m depth from less than one to 1.8, and at the 20m depth from 0.6 to 1.1 (Table 8). This observation is also consistent with the possible transport of oil (and associated toxicity) from intertidal to subtidal sediments, and/or the preferential retention of oil in subtidal sediments. Careful examination of the GC-MS data being generated for this series of sediment samples will be required to confirm this possibility.

Table 8. Comparison of mean percent mortalities of test amphipods (*Ampelisca abdita*) at each of four depths for 8 exposed sites and 5 reference sites (Table 4) sampled and tested in both 1990 and 1991.

	-------Depth (meters)-------			
	0	6	20	100
Reference sites (n = 5):				
1990	19.8	25.9	23.6	10.2
1991	11.8	8.40	4.98	1.12
Exposed sites (N = 8):				
1990	60.1	21.4	13.7	12.4
1991	11.7	14.8	5.58	0.96
Ratio of Exposed/Reference Means:				
1990	3.0	0.83	0.58	1.1
1991	0.99	1.8	1.1	0.86

OBJECTIVE B. Document and Quantify the Occurrence of Oxidized Derivatives of EXXON VALDEZ Oil

OBJECTIVE C. Determine Toxicity Attributable to Oxidation Products

1. SAIC contract

Samples of intertidal sediment and interstitial water were collected in Prince William Sound by scientists from SAIC during the first week of September,1990. The samples were extracted and preliminary chemical screening by GC/FID confirmed the oiled status of the samples from Bay of Isles and Cluster Fox Cove in Herring Bay and the unoiled status of Mooselips Bay. In initial screening tests for toxicity, the extracts from all 3 sites showed significant toxicity with the Microtox test, whereas mortality of mussel larvae was much greater for the two oiled sites than at Mooselips. Genotoxicity (SOS toxichromotest) was elicited by some the extracts from the exposed sites, but not by the samples from Mooselips Bay. Following initial screening, the extracts were separated into polar and nonpolar fractions which were tested separately thereafter. It was learned that the uniformly high toxicity indicated by the Microtox test in exposed and reference samples arose mainly during concentration steps (solvent removal at elevated temperatures) during solvent exchange into DMSO, the solvent used to introduce the samples into the toxicity tests. Solvent removal was thereafter effected by evaporation at room temperature under a stream of ultra-pure nitrogen. In subsequent toxicity tests on the fractions, the Microtox response was consistently higher in samples from the exposed sites than in the Mooselips samples. There was no consistent pattern of toxicity, however, between the polar and nonpolar fractions for any site. Genotoxicity (SOS Toxichromotest) was determined only in samples from Bay of Isles: in the nonpolar aromatic fractions from one water sample and one sediment sample and in the polar fractions from both sediment samples.

2. K. Burns contract

A proposal was received from Dr. Kathryn Burns of the Bermuda Biological Station (BBS) on October 5, 1990 and following review, a revised (October 12) version of the proposal was submitted to NOAA procurement on October 13. The contract was sent to BBS on November 5. Ten samples of Mytilus edulis tissues were selected by Dr. Jeff Short from the inventory collected under A/W3, tissue homogenates were prepared at Auke Bay Laboratory

and shipped to Bermuda on November 1. A report on the results of the extractions and analyses was received on March 5, 1991 (Burns 1991); and the findings are summarized briefly here.

Six of the mussel samples were collected at oiled sites either within PWS (two samples from Bay of Isles, 9/89, and one each from Elrington Island, 8/89, and Sleepy Bay, 8/89) or along the Kenai Peninsula (two samples from Tonsina Bay, 6/89). The other four samples were from unoiled sites (one sample from Olsen Bay in PWS, 6/89, and three from Admiralty Island, near Auke Bay in SE Alaska, 8/89). Frozen tissue homogenates were shipped to Bermuda Biological Station where they were extracted with methylene chloride. Total extractable lipid was determined gravimetrically, and aliquots of each sample were then prefiltered and precleaned on a small silica gel column to separate contaminating lipids from hydrocarbons and their oxidation products. The primary fraction from the precolumn was concentrated and then fractionated further based on polarity by normal phase HPLC (silica and nucleosil columns in series). Five fractions, F1 to F5 in order of increasing polarity, were collected. A sixth fraction (F6) was taken directly from the precolumn by further elution with methylene chloride. Each of the six fractions from each sample were analyzed by ultraviolet fluorescence at three different combinations of excitation and emission wavelengths: 280/327nm (1- to 3-ringed aromatics and quinone derivatives), 310/360nm (4- to 6-ringed PAHs and N-substituted PAHs), and 380/430nm (oxidation products of 4- to 6-ringed PAHs). At the first two wavelengths, Prudhoe Bay crude oil (PBC) was used as the calibration standard, while hydroxy-pyrene was used at the longer wavelength. The F1 fraction from each sample was analyzed also by gas chromatography (FID).

Gas chromatography and UVF showed clear oil contamination in the samples from the six oiled sites, and the nearly complete absence of same in those from the unoiled sites. Figure 2 illustrates the content of PBC (UVF, 310/360nm, summed for F1 and F2) and of polar constituents as hydroxy-pyrene equivalents (UVF, 380/430nm, summed for F4-F6) for the individual samples. The four unoiled sites appear near zero on the abscissa, while the sample from Elrington Island contained the greatest oil concentration. All of the samples, including those from unoiled sites, contained significant quantities of polar constituents that exhibited fluorescence patterns expected from oxidation products of petroleum. The sample of Prudhoe Bay crude oil used as the calibration standard also contained such polar constituents (the proportion of polar to non-polar components in PBC is represented by the dashed line in Figure 2). While these results clearly show the concentration of oxidation products to be related to the levels of petroleum hydrocarbons absorbed, the levels of hydroxy-pyrene

equivalents in oiled mussels appear to be explainable by simple accumulation of the polar constituents in the PBC itself, in direct proportion to the accumulation of non-polar constituents. The Elrington Island sample, with the highest PBC levels, departs from this pattern with a level of polar component lower than what would be expected from the accumulation and retention of PBC.

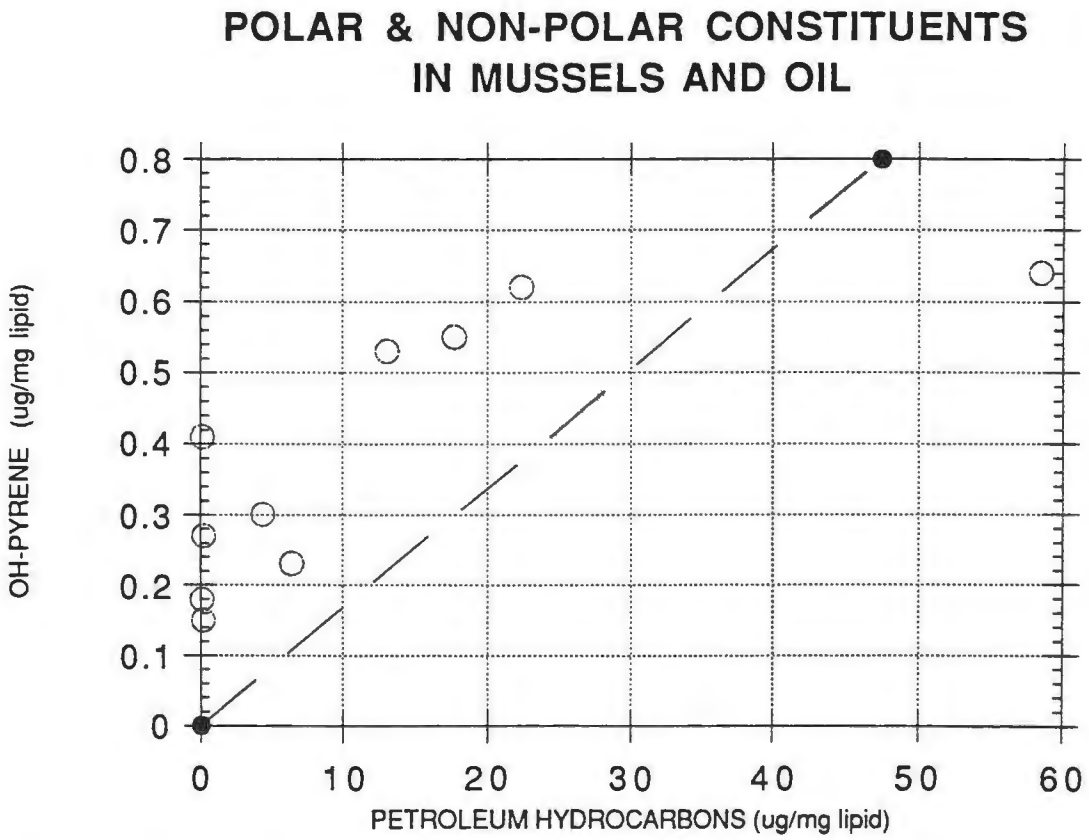

Figure 2. Distribution of non-polar petroleum hydrocarbons and polar organic constituents (as hydroxy-pyrene) in 10 samples of *Mytilus edulis* (open circles) from oiled and unoiled sites in Prince William Sound and elsewhere, measured by ultraviolet fluorescence at selected excitation and emission wavelengths. The line indicates the ratio of polar/non-polar constituents, measured by the same technique, in Prudhoe Bay crude oil.

The present study provided no evidence for the accumulation of polar constituents in excess of those levels present in the original oil itself. It is clear, however, that the UVF technique used here is semi-quantitative at best, and that the polar fractions from oiled and unoiled mussels could be significantly different in actual composition and still give comparable overall estimates of concentration. To provide a more quantitative estimate of the composition and concentration of oxidized PAHs would require GC/MS analysis in the SIM mode to locate specific compounds. Dr. Burns suggests in her report that a promising approach would be to use a gel permeation HPLC step in conjunction with the adsorption HPLC, followed by GC/MS.

OBJECTIVE D. Summary Budget or "Mass Balance" for the Fate of Spilled Oil.

Except for identifying and accumulating relevant literature and reports for this task, no progress has been made since the last report. The status and recommendations are summarized briefly here.

A small steering group met on June 6, 1990, at the NOAA Sand Point Facility in Seattle to identify sources of information potentially pertinent to this synthesis effort, and to discuss strategies for achieving the desired synthesis goals. This group consisted of the following individuals: Dr. Erich Gundlach (ASA/ETECH),, Dr. David Gibbons (U.S. Forest Service), Dr. Carol-Ann Manen (NOAA), Mr. Douglas Redburn (Alaska Dept. Environmental Conservation), Dr. Jacqui Michel (Research Planning, Inc.), and Dr. D. A. Wolfe, NOAA project manager for A/W6. Dr. Michel was unable to attend this first meeting. The meeting was also attended by Dr. Byron Morris and Mr. David Cantillon from the NRDA Management Team and Dr. Jerry Galt from NOAA's Spill Response Team.

This task is primarily a synthesis function. Information on the distribution and fates of EXXON VALDEZ oil needs to be assembled from a number of sources, interpreted in the light of existing information and models, and presented in a way that will support a region-wide assessment of the potential effects of the spill. Timely progress on this synthesis will depend therefore on the availability of suitable information from other sources and projects; notably, chemical data from Technical Services Project No. 1 (TS1), will be of utmost importance to the completion of this project.

The steering group discussions identified the following compartments for initial analysis and inclusion in the Oilfates budget: 1. Water Surface (floating oil), 2. Intertidal Zone (stranded oil), 3. Water Column (dissolved

and accommodated oil), 4. Subtidal Sediments (sunken and settled oil, or oil otherwise transported to bottom sediments), 5. Atmosphere (evaporated oil). The actual masses of oil in these different compartments are quite different, and because of transfers among compartments as the spill was transported through and out of Prince William Sound, the pertinent time and space scales are also quite different. As a result, very different estimation methods have been used (by different people) for the various compartments. The steering group concluded therefore that information for these five compartments would best be synthesized separately, with appropriate effort to reconcile both the separate compartmental estimates as well as any estimates of fluxes between compartments . For each compartment and its associated major fluxes, the steering group identified and discussed important sources of data, historical information, and modeling expertise, and suggested preliminary courses of action, as summarized in the report (June 18, 1990) of the meeting.

The strategy outlined in the detailed study plan for A/W6 called for available information on each of the compartments to be presented and discussed at a technical workshop. This workshop was to serve as a forum for initial technical review and comment which would guide the completion of the synthesis. Following the review of the initial estimates, 2-4 months would be required for refining the estimates and for reconciling differences among different components. This general approach is still viewed as a viable strategy, and with the progress being demonstrated in related projects of the NRDA, it now appears realistic to recommend that these activities be scheduled for 1992, to consider available data relevant to the fates of oil up through fall of 1991. A "final" report is envisioned to provide estimates for oil distributions and fates at least through October 1991, along with projections for any oil remaining in the environment at that time.

VII. STATUS OF INJURY ASSESSMENT

OBJECTIVE A

The toxicity bioassay results suggest that residual toxicity was still associated, in summer 1991, with the Exxon Valdez oil in subtidal sediments at several of the oiled sites. However, the differences between mean toxicities at oiled and unoiled sites were smaller in 1991 than in 1990 (and not significantly different from each other), consistent with the decreasing levels of petroleum hydrocarbons in the sediments (as measured by UVF and GC-MS). The finding of significant toxicity at some of our nominal

reference sites was unexpected; this result emphasizes the need for detailed chemical data at all sites. The final analysis of the existing toxicity data will depend upon the availability of hydrocarbon data (from TS1) for the sediment samples collected and inventoried under project S/T1 on the 1991 cruise of THE BIG VALLEY. It is recommended that a repeat survey of sediment toxicity be conducted in Oil Year 4 to verify further the postulated diminution of toxicity at all sites and to clarify the toxicity status of our nominal reference sites.

OBJECTIVES B & C

Results from the first tiers of toxicity testing (of the polar and nonpolar fractions from interstitial water and sediments) indicated low levels of toxicity in both fractions. Toxicity (Microtox and mussel larvae mortality) was clearly greater in samples from oiled sites than in those from unoiled sites; genotoxicity (SOS toxichromotest) was indicated only in about half of the sample fractions from the most heavily oiled site. There was no disproportionate contribution of toxicity from the polar fractions, however, that would warrant special attention to, or analysis of, the polar oxidized derivatives in addition to the suite of petroleum analytes currently included in analytical protocols. Selected fractions will be tested further with other measures of genotoxicity (anaphase aberrations and sister chromatid exchange in mussel and salmon larvae). Detailed chemical data are also not yet available on the polar fractions used in these tests. When those data become available (expected in December, 1991), the toxicity data will be re-interpreted, to infer which constituents in these weathered oil samples are most likely to be the major sources of toxicity and/or mutagenicity.

Results of the pilot study on polar constituents in mussel tissues (analyses by K. Burns) are complete. These data clearly demonstrated the presence of polar constituents in mussel tissues, including those mussels which had not experienced significant exposure to petroleum. The results suggested that mussels accumulate the polar components of Prudhoe Bay crude oil in direct proportion to their levels in the parent oil, and provided no evidence for disproportionate uptake of either polar petroleum components or oxidized derivatives from other sources and pathways in the oiled environment. Nonetheless, the chemical and toxicological properties of these polar constituents are not well understood, and there remains the possibility that mussels could indeed accumulate small amounts of potentially toxic petroleum oxidation products not present in the parent petroleum. At this time, however, the available data do not demonstrate a clear need for expanding the suite of conventional petroleum analytes to include polar constituents. No further research is proposed in this area.

OBJECTIVE D

Although some avenues of coordination and synthesis have been pursued since the June, 1990 meeting, it is very clear that the schedule originally proposed for this synthesis effort was overly optimistic. For many of the identified compartments in the budget, analytical data are only now becoming available for analysis of the distribution and fates of the oil. The approach proposed for this synthesis needs to be reviewed in light of current developments in the overall NRDA. Although implementation has been postponed, the originally proposed approach is still recommended.

Under that approach, a lead individual would be designated for coordination and completion of the synthesis related to each identified compartment, and presentation of the summary results at the review workshop. Effort should be made to identify all sources of relevant data and information for each of the individual compartments in the Oilfates budget; to examine, explain, and eliminate inconsistencies among data sets; and to encourage and promote development of a single, complete, and accurate consensus synthesis product for each component. The syntheses should include detailed assessments of the quality of the the data and information, including analytical confidence limits, sampling adequacy in time and space, and model reliability. The synthesis for each of the compartments should include best-possible estimates of the rates of transport and transformation processes ongoing within and/or between compartments, including such processes as moussification, photooxidation, biodegradation, evaporation, dissolution, accommodation, chemical weathering/compositional change, "bleeding" of sheen, adsorption-sedimentation, sinking, down-slope transport of oiled sediments, etc. These "concensus syntheses" will provide be the primary substance for the review workshop. Effort will be made in advance of the workshop to identify and compare independent estimates of inter-compartmental transfers and these should be scrutinized in detail, and reconciled if possible, at the workshop.

Depending on the chemical analytical results from the 1991 sediment samples taken in this project and those coordinated with it (S/T1-3, S/T7, and TS1), some or all of the PWS sites should be resampled in subsequent years to document the persistence of the oil, its continued bioavailability to organisms, and the persistence of effects, if any, on benthic and demersal resources. As the spilled oil weathers in the sediments and the levels of petroleum residues and metabolites decrease in the tissues, both the number of sites and frequency of sampling should be decreased. Sampling of sediments and benthos should continue, however, at some of the more

heavily oiled sites until the amounts and compositions of the oil present have degraded to near-background conditions, and the benthos have shown recovery to pre-spill (or otherwise "normal") conditions. This strategy for continued sampling and analysis is essential for: a) estimation of cumulative injury to the resources; b) estimation of natural recovery rates; and c) evaluation of the practicality of restoration alternatives.

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STATE/FEDERAL NATURAL RESOURCE DAMAGE ASSESSMENT
DRAFT PRELIMINARY STATUS REPORT

Project Title:	INJURY TO PRINCE WILLIAM SOUND SPOT SHRIMP
Study ID Number:	Subtidal Study Number 5
Lead Agency:	State of Alaska, ADF&G; Commercial Fisheries Division
Cooperating Agency(ies):	None
Principal Investigator	Charlie Trowbridge
Assisting Personnel:	Linda Brannian, Project Biometrician Wayne Donaldson Dan Urban Ivan Vining Vera Kinzer Dan Coyer
Date Submitted:	November 20, 1991

TABLE OF CONTENTS

LIST OF TABLES

LIST OF TABLES (Continued)

LIST OF FIGURES

LIST OF APPENDICES

EXECUTIVE SUMMARY

Three damage assessment surveys were conducted to sample spot shrimp (*Pandalus platyceros*) in November 1989, March 1990, and November 1990 as Fish/Shellfish study 15. This project continued under its current title and a fourth survey was completed November 14, 1991. The first two surveys (November 1989 and March 1990) sampled spot shrimp during the same egg bearing period and have been compared. This report will be limited to findings from the November 1990 survey and draw comparisons to the November 1989 survey.

Spot shrimp support important commercial, recreation, and subsistence fisheries in Prince William Sound. A significant portion of their habitat was in the direct path of the 1989 Exxon Valdez oil spill. Spot shrimp are a representative species of the deepwater nearshore benthic ecosystem, serving as a food source for a variety of fish and shellfish. Spot shrimp also share aspects of their distribution, life history, or food habits with other economically important shellfish species residing in the oil-affected area (Table 1).

Relative abundance of spot shrimp remained lower in the oiled than unoiled area for a second year even after limited commercial fishing was allowed in the unoiled area during the spring of 1990. Furthermore, catch in number of shrimp per pot was lower in 1990 than 1989 in the oiled area even in the absence of fishing. Percent female in the oiled area remained low at 2% in 1989 and 2.5% in 1990 while the percent in the unoiled area increased from 7.3% to 11.3%. Over three times as many shrimp were non-ovigerous in the oiled (18.9%) than unoiled (5.1%) area in 1989 and twice as many (16.5% versus 8.0%) in 1990. Furthermore, 23% of the spot shrimp and 50% of the pink shrimp (*P. borealis*) collected in 1989 and examined for histological aberrations had gill lesions severe enough to reduce stock production through increased mortality. These gill lesions are indicative of exposure to a toxic substance (D.V. Lightner, University of Arizona, personal communication).

The total number of eggs per female at a given size was less in the oiled area in 1989. No such difference existed in 1990. Shrimp from the oiled area did not have a significantly greater number of females with one or more dead eggs in 1990 as they did in 1989.

Hydrocarbons were not detected in spot shrimp muscle or eggs based on ten samples analyzed from the oiled area and seven samples from the unoiled area out of 193 samples collected. Oil was detected in sediments deeper than 100 m near oiled study sites (Air/Water study 2). Hydrocarbon-degrading bacteria were also detected at depths to 100 m near oiled study sites. Given the low bioconcentration of aromatic hydrocarbons by spot shrimp and their theorized susceptibility to genetic damage as larvae (Sanborn and Malins 1980), an assessment of genetic damage should be conducted targeting the most vulnerable year classes (1987-1989) rather than continue with hydrocarbon analysis.

We recommend that this project continue under restoration as the means of monitoring the natural recovery of the oiled population. It is essential to evaluate the success of harvest strategies used to regulate human use of the resource to allow rebuilding of injured stocks and insure their long term health.

OBJECTIVES

1. Determine the relative abundance by weight and sex of spot shrimp (*Pandalus platyceros*) and the relative abundance by weight of incidentally caught pink shrimp (*P. borealis*) and coonstripe shrimp (*P. hypsinotus*) in oiled and unoiled areas and compare these values to those obtained during surveys in 1989 and 1990.

2. Compare size and age frequencies of spot shrimp (by sex and depth stratum) between sites using various methods of length frequency analysis (mixture model analysis).

3. Analyze fecundity, egg mortality, and other sublethal effects between oiled and unoiled areas over time, and determine whether those effects result in adverse changes in reproductive viability.

4. Analyze tissue and egg samples for presence of hydrocarbons and compare differences between oiled and unoiled sites. Test the hypothesis that the level of hydrocarbons is not related to the level of oil contamination present at a site. The experiment is designed to detect a difference of 1.2 standard deviations in hydrocarbon content with the probability of making a type I or type II error of 0.05 and 0.10, respectively.

5. Document injury to tissues and compare differences between oiled and unoiled sites if warranted by results from tissue hydrocarbon analysis.

6. Provide information on stock status, hydrocarbon concentration and other indicators of stock condition for restoration of damages and management of the spot shrimp resource for subsistence, personal, and commercial user groups.

INTRODUCTION

Spot shrimp support important commercial, recreation, and subsistence fisheries in Prince William Sound (PWS). Their habitat is generally contained within the traditional harvest area of the commercial fishery which includes the area west of a line from Montague Point to Bidarka Point in PWS (Figure 1) and is characterized by numerous, steeply cut glacial fjords and passages. A significant portion of this area was in the direct path of the 1989 Exxon Valdez oil spill (EVOS). Minor populations of spot shrimp outside PWS include Lituya Bay to the east and the outer coast of the Kenai Peninsula to the west. The latter is also in the EVOS affected area.

Spot shrimp are a representative species of the deepwater nearshore benthic ecosystem, serving as a food source for a variety of fish and shellfish. Spot shrimp share aspects of their distribution, life history, or food habits with other economically important shellfish species residing in the oil affected areas (Table 1). Spot shrimp are known to be sensitive to oil contamination in both the

larval and adult phase. The effects of oil on spot shrimp in particular and shrimp in general are well documented (Anderson et al. 1981, Brodersen et al. 1977, Brodersen 1987, Mecklenburg et al. 1977, Sanborn and Malins 1980, Stickle et al. 1987, Vanderhorst et al. 1976). In 1989 the eggs of spot shrimp and other shellfish species (Table 1) hatched immediately before the oil spill and were in the planktonic community as zoea larvae, making them vulnerable to aromatic hydrocarbons. Juvenile spot shrimp from the 1988 and 1987 year classes would have been present at more nearshore locations preferring hard-bottom rock crevices or kelp covered periphery (Barr 1974).

Commercial fishing for spot shrimp was allowed in the unoiled area in the spring (March 15 through April 8) of 1990, the first time since the EVOS. Commercial fishing was allowed in both the unoiled and the oiled areas in the fall (September 10 through October 25) of 1991. The 1990 fishery harvested 13,912 kg of spot shrimp in 59 landings by 23 vessels. Preliminary catch statistics for the fall, 1991 fishery show 7,939.8 kg taken by 15 vessels in 44 landings. The fishery was opened in 1991 in order to assess the status of the stock on an area-wide basis because a large portion of southwestern PWS had been closed since 1988. In addition there was some difficulty in relating survey catch per unit effort (CPUE) to commercial fishery performance.

The November 1991 survey is expected to begin capturing shrimp from the 1989 year class as they join the adult portion of the stock. Of utmost importance to the damage assessment process will be to test the assumption that the 1989 year class (which was in the water column as zoea larvae at the time of the oil spill) will not show a significant difference in recruitment between oiled and unoiled sites.

METHODS

Spot shrimp habitat within PWS was divided into oiled and unoiled strata. Localized spot shrimp distribution in these areas was established by interviewing commercial fishermen. The unoiled strata included the northwestern portion of PWS. Sampling was conducted in Unakwik Inlet, Port Wells (Golden), and Culross Passage (Figure 1). Unakwik Inlet was also chosen as it was the site of previous Alaska Department of Fish and Game (ADF&G) research on abundance and growth of spot shrimp (Kimker 1984, 1985; Kimker and Donaldson 1986, 1987). The oiled strata included central and southwestern Prince William Sound. Sampling was conducted at Green Island, Chenega Island, and Herring Bay. Green Island was also chosen as similar research had been conducted there in 1982 (Kimker 1983).

Each site was stratified into shallow, 35 to 130 m, and deep, 130 to 220 m, strata. Eleven pots comprised a station and were spaced 18 m apart on a longline. During the 1989 survey, pots were spaced 9 m apart then changed to 18 m in 1990 to provide more coverage of the depth range within a stratum. In 1990, at least two stations were fished in each stratum per site for a minimum of 44 pots per site. The exception being Green Island where 33 pots were fished in the shallow stratum only due to lack of success in the deep strata on previous surveys. Up to 66 pots were set at a depth stratum if past experience indicated that the sample size of 500 spot shrimp for length frequency analysis would not

be met. If necessary, pots were redeployed the next day targeting the areas of highest catches from the previous sample until the required sample size per depth stratum and site were captured.

Spot shrimp were sampled using standardized commercial shrimp pot gear measuring 16 x 16 x 36 in (40.6 x 40 .6 x 91.4 cm) with a 2.5 in (6.4 cm) tunnel located 7 in (17.8 cm) into each end. Pots were baited with a 2-quart jar of frozen chopped herring. Longlines of pots were set in late afternoon and retrieved the following morning for an average 18 hour soak time. The survey was conducted aboard the R/V *Montague* from November 5 through 14, 1990.

Pandalid shrimp were sorted by species and weighed. Weights were obtained using an electronic digital scale and recorded to the nearest 2 g. The total weight of spot shrimp was estimated by adding representative weights of shrimp removed for hydrocarbon and histopathology samples to the sample weight. A station's catch was subsampled if it was determined that the number of shrimp greatly exceeded 500. Subsamples were obtained by taking a constant proportion of shrimp from each pot in a station.

In comparing relative abundance between oil and unoiled strata CPUE will be calculated from only those pots set the first day at a depth strata and site combination. Placement of pots redeployed an additional day was based on the first day's catch with the objective of obtaining more spot shrimp to meet the sample size requirements for length frequency analysis. CPUE from these pots are thought not to represent abundance or be comparable with sites where additional fishing was not needed.

A general linear model was fit to the spot shrimp data and the hypothesis of no difference in number or weight caught between oiled and unoiled strata was tested at the α=0.05 level. Because of the potential of significant interaction terms the full model was fit:

$$CPUE_{ijkm} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \gamma_{k(i)} + (\beta\gamma)_{jk(i)} + e_{ijkm}$$

incorporating the grand mean (μ), an oiling effect (α), depth stratum effect (β), sampling site effect nested within oiling strata ($\gamma_{k(i)}$) and all interaction terms. Where interaction terms were significant hypothesis for testing differences between oiled and unoiled strata was done using the least square means (Milliken and Johnson 1984). Difference between years was tested separately for the oiled and unoiled areas using the following model:

$$CPUE_{ijkm} = \mu + \delta_i + \beta_j + (\delta\beta)_{ij} + \gamma_k + e_{ijkm}$$

which included a year effect (δ). The Bonferoni inequality was used to control type I error for planned a-posteriori comparisons of least square means.

Sex, carapace length, and fecundity data were recorded only for spot shrimp. Carapace length was measured from the rear of the right eye socket to the posterior midpoint of the carapace and recorded to the nearest 0.1 mm using a digital electronic caliper. Sex was identified as juvenile, male, transitional or female according to the detailed study plan (Donaldson et al. 1990). Egg

condition, egg color, egg fouling, egg mortality, and the presence of breeding dress if no eggs were present, was recorded for all females. A maximum of 25 ovigerous females at each station for each site was collected to estimate fecundity and egg mortality. Egg samples were processed and the total number of eggs per female estimated according to Kinzer (1991) using the following formula:

$$X = (x'/y') Y$$

where x' is the number eggs in the subsample, y' is the dry weight of the subsample and Y is the total dry weight of the sample.

The percentage of spot shrimp females bearing eggs; the stage of spot shrimp egg development (color and presence or absence of eyes); the percentage of spot shrimp egg fouling and egg mortality; the fecundity by size; and the relative clutch size was determined for each station. Chi-square statistics were used to test for differences in sites and levels in data which involved percentages and proportions. Differences between strata and sites for fecundity and relative size of clutch were tested using analysis of covariance, with carapace length as the covariate. Analyses to determine possible effects of the oil spill on the number of dead eggs per female were conducted using ANOVA on ranked data.

Specimens for histopathology analysis were taken from the catch before it was weighed and processed. Twenty spot shrimp from a single station in each stratum were selected and preserved following recommendations of the Histopathology Technical Group outlined in the detailed study plan (Donaldson et al. 1991). Dr. Donald Lightner of the University of Arizona examined the tissue samples.

To prevent contamination, specimens for hydrocarbon testing were taken from the pot immediately after its removal from the water and before contents were weighed. Three female spot shrimp formed one composite sample of muscle and one composite sample of eggs. Each composite was taken from a different pot. Two replicates of the composite were taken randomly from one station in the stratum and the third replicate came from the other station. All hydrocarbon samples were processed according to the methods outlined in the detailed study plan (Donaldson et al. 1991).

Environmental data were recorded at each site. Water temperature, salinity and dissolved oxygen content were recorded using a Seabird Electronics CTD (model SBE19, serial # 192488-297) at a location near the deepest portion of the second stratum of each site.

RESULTS

Three damage assessment surveys were conducted (November 1989, March 1990, and November 1990) for Fish/Shellfish study 15. This project continued under its current title, Subtidal study 5 and a fourth survey was completed November 14, 1991. The first two surveys (November 1989 and March 1990) sampled spot shrimp during the same egg bearing period. The damage assessment of spot shrimp in the 1990 status report (Donaldson et al. 1990) covered only these two surveys as the

November 1990 survey was underway. This report will limit itself to findings from the November 1990 survey and draw comparisons to the November 1989 survey. Any reference to a 1990 survey implies the November cruise only. The most recent survey was completed November 14, 1991 and data analysis has not begun.

The November 1990 survey sampled spot shrimp at the same six sites as in 1989 (Table 2, Figure 1). Pots per site and depth strata varied from 22 pots per depth stratum at Unakwik to 66 pots in the shallow stratum of Herring Bay.

Relative Abundance

A total of 8,491 spot shrimp weighing 151.3 kg were captured in 440 pots during the November, 1990 survey. There were 209 pots deployed at the unoiled stratum study sites capturing 4,558 spot shrimp weighing 95 kg. Two hundred thirty one pots captured 3,933 spot shrimp weighing 56.3 kg in the oiled stratum study sites.

Spot shrimp biomass was greatest at all sites (Appendix A) except Green Island where pink shrimp was greatest followed by spot shrimp. Coonstripe shrimp was second in biomass at Unakwik, Golden, and Culross Passage (all unoiled sites) with pink shrimp second in biomass at Herring Bay and Chenega (oiled sites). This agrees with our understanding of shrimp distribution in PWS. The pink shrimp population has supported a commercial trawl fishery in the southwest portion of PWS. Average CPUE (biomass per pot) was greater in the oiled area than unoiled for pink shrimp and greater in the unoiled area than oiled for coonstripe shrimp (Table 3).

Average catch per pot (kg) of spot shrimp were very similar between the November 1989 and 1990 surveys (Table 4). No significant difference between years was found using kg per pot for the oiled (least square mean (lsm) = 0.19 (1989), 0.17 (1990); p=0.35) and unoiled (lsm = 0.58 (1989), 0.62 (1990); p=0.48) areas. In contrast, a difference in number of spot shrimp caught (lsm = 15.9 (1989), 11.7 (1990); p=0.048) was found for the oiled sites and a low p-value for the comparison between years for unoiled sites (lsm = 34.6 (1989), 28.3 (1990); p=0.069). One explanation for these results is that there was insufficient recruitment to offset mortality (fishing, natural, oil induced) explaining the possible drop in numbers. Yet those remaining alive increased in weight with the result that biomass did not differ between years. Commercial fishing was allowed only in the unoiled area between the 1989 and 1990 surveys.

The average weight of spot shrimp per pot was consistently lower in the oiled than the unoiled area. In 1989 catch per pot in number of shrimp and weight (kg) was significantly lower in the oiled (lsm(s) = 16.0, 0.19) versus the unoiled (lsm(s)= 34.6, 0.58) strata (p(s)=0.0001). Even though commercial fishing had been allowed in the unoiled area in 1990 relative abundance was again lower in the oiled (lsm(s) = 11.7, 0.19) versus the unoiled (lsm(s) = 28.3, 0.58) strata (p= .0001). Commercial fishing for pot shrimp had been closed since the EVOS.

Size and Sex Composition

In 1990 the average carapace length was 21 mm for juvenile spot shrimp, 29.1 mm for male spot shrimp, and 41.9 mm for female spot shrimp (Table 5). Differences in average carapace length between 1989 and 1990 were 1.5 mm for male shrimp at both oiled and unoiled sites (Table 6). Mean carapace length for female spot shrimp was not significantly different between years at the oiled or unoiled sites.

In 1989 only 2% of the spot shrimp sampled in the oiled area were female as compared to 7.3% in the unoiled. Again in 1990 only 2.5% of those captured were female in the oiled area as compared to 11.3% in the unoiled area. Chi square analysis found that in both 1989 and 1990, oiled sites had a significantly lower proportion of female shrimp ($P<.001$) than unoiled. Furthermore, while the proportion of females increased significantly from 1989 to 1990 ($P<.001$) in unoiled sites, a similar increase was not evident in the oiled sites. This is surprising as a commercial fishery which targets large shrimp (females) was conducted in the unoiled area in the spring of 1990.

Length frequency distributions are formed by recruitment of the smallest year class to our sampling gear, natural mortality of all age classes, and a selective commercial removal of individuals greater than 30 mm. Differences between the 1989 and 1990 length distributions in the oiled area are only a function of natural (or oil induced) mortality and recruitment. In contrast, commercial removal of individuals greater than 30 mm occurred in the unoiled area in the spring of 1990.

Changes in the length frequency distribution (LFD) at Unakwik may in part be due to commercial removal. The LFD in 1990 appears to lack some of the recruit size shrimp (20-27 mm) (Figure 2) seen in 1989. Lack of recruitment is more strongly also suggested by the LFDs at the Golden study site where it appears that the 1990 LFD is just a forward 4 mm projection of the 1989 LFD (Figure 3). This is close to the average annual growth of approximately 10% of the carapace length per year for spot shrimp estimated from tagging (Kimker and Donaldson 1987). Culross Passage LFD may also show the symptoms of selective removal by the commercial fishery. Yet a much larger proportion of its population is less the 28 mm (Figure 4). The estimated LFD for the unoiled population becomes a combination of these sites. Again the primary mode has increased from 28 mm to 32 mm in 1990 creating three distinct modes (Figure 5).

The LFD of spot shrimp at Herring Bay did not change much from 1989 to 1990 (Figure 6). This may indicate that the recruit size year class in 1990 did nearly replace that of 1989 though the mode increased from 25 to 29 mm. The 1990 LFD for Chenega indicates that less recruit size shrimp were present than in 1989 (Figure 7). The LFDs for Green Island (Figure 8) are based on very small sample sizes though appear to be similar. The estimated LFD for the oiled population becomes a combination of these three sites (Figure 9) and represents an aging of the population without commercial removal illustrating lower recruitment than last year and growth of the mode from 25 to 28 mm in 1990.

Fecundity and Related Parameters

Fecundity was estimated for 315 ovigerous females in 1990 (Table 7). Number of eggs per female averaged 2,266 at unoiled sites and 2,217 at oiled sites. Individual fecundity ranged from 417 to 4,273 eggs. In November 1990 all eggs were uneyed, which was similar to the 96% uneyed, observed in 1989.

A comparison of the fecundity relationship, number of eggs versus carapace length, between oiled and unoiled classifications found a significantly greater slope for unoiled than oiled sites in 1989 (Donaldson et al. 1990) indicating a greater number of eggs for females of a size category in the unoiled area. In 1990, no significant difference in fecundity between oiled and unoiled classification was detected (P=0.952). The regression lines for 1990 are:

unoiled $E=-4142.43 + 152.51\ L$, $R^2= 0.36$ $P= 0.0001$
oiled $E=-4078.18 + 152.13\ L$, $R^2= 0.49$ $P= 0.0001$

where E is the total number of eggs and L is the carapace length. When fecundity-length relationships in the oiled stratum were compared between years the 1989 relationship had a significantly lower slope than 1990 (P=0.007). Within the unoiled stratum, slopes for the 1989 and 1990 relationships were not significantly different (P= 0.65). Therefore the female shrimp in oiled sites were of lower fecundity in oiled sites than unoiled in 1989 with this effect disappearing in 1990.

Number of dead eggs per female in 1990 ranged from 0 at Green Island to 804 at Golden (Table 8). No significant difference in the number of dead eggs per female was found in 1989 between oiled and unoiled sites (P= 0.27). In 1990 female shrimp in unoiled sites were found to have significantly more dead eggs per female (P=0.03) than those in oiled sites. When data were pooled across oiling strata, there were significantly more dead eggs per female in 1989 than 1990 (P=0.001). When data were constrained within oiling strata, it was found that in 1989, shrimp had significantly more dead eggs per female than did shrimp in 1990 in both oiled (P=0.007) and unoiled (P=0.008) strata.

In 1989, there was a significantly greater proportion of female shrimp with one or more dead eggs in oiled than unoiled sites (Donaldson et al. 1990). No such difference (P= 0.253) was found in 1990. There was significant variability within oiling strata among sites as a chi square analysis found a significant difference in number of females with dead eggs among unoiled sites (P<0.001), and a low p-value among oiled sites (P=0.069).

Though results of the analyses with dead egg numbers and frequency of occurrence showed no trend of increasing egg mortality in the oiled area, Donaldson et al. (1990) stated that it is possible that spot shrimp clean dead eggs from their brood, or that the dead eggs simply slough off as other eggs mature. Presence of dead eggs may not be a good indicator variable to monitor for oil damage for this reason.

Of shrimp identifiable as female, significant proportions were found to be without eggs during the 1989 and 1990 surveys (Table 9). Chi square analysis

found oiled sites to have a significantly higher proportion of females without eggs than unoiled sites in both 1989 (P=0.002) and 1990 (P=0.009). Females in 1989 were likely to be in breeding dress and though egg extrusion should have occurred it could have been delayed. In contrast in 1990 non-ovigerous females were not in breeding dress and therefore less likely to breed soon. There was no significant difference between the percent of non-ovigerous females in breeding dress between oiled and unoiled strata in 1989 (χ^2 = 0.57, p=0.45) and 1990 (χ^2 = 0.03, p= 0.86). However, the fact that we estimate only 2% of the shrimp population in the oiled area to be female does have serious implications to its population dynamics, especially when coupled with the fact that approximately 15-20% of these females were not carrying eggs. These stocks do not appear to be reproducing at a very high rate.

Documenting Hydrocarbon Contamination

Sixty five samples of spot shrimp eggs and adult muscle were collected for hydrocarbon analysis (Table 10) bringing the collection total to 193 samples. To date seven samples collected in 1989 from unoiled sites and 10 samples from oiled sites have been analyzed with no oil detected. NRDA Air/Water study 2 collected 148 sediment samples at depths greater than 100 m in 1989 and 13 in 1991 (Carol-Ann Manen, National Marine Fisheries Service (NMFS), Auke Bay, Alaska, personal communication). Nineteen of those, all from 1989, have been analyzed and seven were found to contain oil. Location names and depths sampled of those seven listed in the NMFS database include Main Bay (603 m), Esther Is (388 m), Valdez (238 m), Orca Bay (164 m), Green Island (251 m), Herring Bay (137 m) and Bligh Island (102 m) (Figure 10). This confirms that oil has reached the depths that spot shrimp inhabit and was detected near our oiled study sites of Green Island and Herring Bay. Oil found at Main Bay brings into question the appropriateness of the unoiled status of the Culross site.

Another indication of the presence of hydrocarbons at depth is the presence of hydrocarbon-degrading microorganisms in marine sediments. Dr. Joan Braddock, Assistant Professor of Microbiology, University of Alaska Fairbanks participated in the rapid-response assessment program conducted by the National Atmospheric and Oceanographic Administration shortly after the EVOS and later was contracted by the Alaska Department of Environmental Conservation in a follow up program. Included in that program was most-probable-number (MPN) measurements (Brown and Braddock 1990) of oil-degrading microorganisms (cells per g dry sediment) in sediment and water samples throughout the EVOS affected area. Concentrations of oil-degrading microorganisms were significantly greater than control sites only to depths of 20 m in 1989 at sampling locations near our study sites (Northwest Bay, and Disk Island). By 1990, concentrations of oil-degrading microorganisms were detected to 100 m at L. Herring Bay, Disk Island, Green Island, and Chenega Island.

It may not be surprising that spot shrimp muscle and egg samples to date have not been positive for hydrocarbons. Sanborn and Malins (1980) report that in adult shrimp the bioconcentration factors (concentration in the tissue divided by the concentration in the water-soluble fraction) were low in comparison to the values generally observed with fish. Yet Sanborn and Malins (1980) report that early

development stages of shrimp have well developed enzyme systems which may convert polycyclic aromatic hydrocarbons into potentially damaging free radicals and epoxides that can interact with DNA causing irreversible damage. Again this puts the 1989 year class, present as zoea larvae in the plankton community, at risk.

Lack of hydrocarbon detection in spot shrimp tissue or eggs does not indicate lack of damage or contamination. Rather, we may lack the power statistically (17 out of 193 samples processed) or biologically to detect hydrocarbon damage in spot shrimp. Given the damage evidenced by the histopathology study the emphasis should shift to detection of genetic damage through analysis with a flow cytometer or other methods.

Histopathology Observations

An initial histological study of spot shrimp and pink shrimp collected during the November 1989 survey was completed by Dr. Donald Lightner, Associate Professor of the Department of Veterinary Science, University of Arizona (Appendix B). A high priority was given samples 7 through 12 from oiled sites and analysis of samples from the unoiled stratum is underway.

Melanized cuticular lesions were examined histologically and found to be either wounds or classical examples of bacterial shell disease (Appendix A, Table 1 and 3). Occurrence of bacterial shell disease ranged from 0 to 40% averaging 28% for the oiled area. Melanized cuticular lesions were noted only grossly for the unoiled samples and occurred on 40% of the shrimp. The actual rate of shell disease will be lower after the histological examination categorized these lesions as wounds or bacterial shell disease.

Ten shrimp from a site specific sample of 20 were sectioned for histological examination. A severity grade of 0 to 4 was used to assign severity of infection, surface infestation, or disease syndrome severity to each shrimp (Appendix A, Table 5).

Gill lesions on spot shrimp were detected in samples from Chenega and Green Island and were present on shrimp from all sites (Table 11). Lesions ranged from multifocal necrosis, inflammation and melanization of areas in gill lamellae to marked hemoctic congestion and fibrosis of the hemocoel within the primary gill rachis of one or more gill processes. Incidence of gill lesions in spot shrimp ranged from 10% at the shallow stratum to 90% at the deep stratum of the Chenega site averaging 48% for the oiled area. Twenty three percent of those sampled had severity grades greater than 1 with a prognosis for possible production losses and or increases in mortality. Dr. Lightner thought that shrimp with gill lesions of severity grade 4 would not be feeding and therefore would not be attracted into our baited pots.

One sample of pink shrimp was collected at Green Island in November 1989 due to the shortage of spot shrimp. Similar histological examinations found 70% to be afflicted with gill lesions and 50% with a severity grade of 2 indicating increased mortality.

Dr. Lightner did indicate that the high incidence of gill lesions from Chenega and Green Island samples (location was not known to examiner) suggest exposure of these shrimp to some sort of toxic material. Dr. Lightner is currently processing samples from the unoiled stratum. Analysis to test differences in frequency of occurrence between oiled and unoiled strata for shell disease and gill lesions will follow.

Histopathology samples were again collected during the November 1990 survey (Table 12). As in 1989 samples were preserved in 10% buffered formalin which in crustacea causes marked shrinkage and hardening of shrimp tissues and penetrates slowly allowing autolysis in larger shrimp to occur. Therefore examination of 1991 samples which were preserved in Davidson's fixative (preferred) will be given a higher priority.

Environmental Observations

Bottom temperature, salinity, and oxygen were recorded for each site at depths ranging from 135 to 306 m (Table 13). Average temperature varied by less than 0.12°C between oiled and unoiled strata in 1989 and 1990 and salinity by 0.21 ppt. Environmental conditions appear fairly uniform at the depths adult spot shrimp inhabit.

STATUS OF INJURY ASSESSMENT

Abundance data and hydrocarbon and histological samples have been collected to meet all project objectives. The November 1991 survey was successfully completed and results will be included in the next status report.

Relative Abundance

The November 1990 survey gave us the first opportunity to make comparisons of relative abundance between years for the oiled and unoiled areas. In the oiled area, number of shrimp per pot has declined from 1989 to 1990. Abundance continues to be lower in the oiled area than the unoiled area even with continued closure to commercial fishing in the oiled area and a spring 1990 fishery in the unoiled area.

Size and Sex Composition

Sampling needs to continue to assess survival of the 1989 year class in the oiled and unoiled areas. Initial results concerning recruitment through comparing size

and age frequencies of spot shrimp between sites has addressed year classes not vulnerable as plankton but as juveniles in more nearshore environments (1988 and 1987 year classes). Previous work by Barr (1974), with juvenile spot shrimp at Little Port Walter in southeastern Alaska, indicates that juveniles do not join the adult stock until 20 months (1.7 years) after hatching or 25 mm carapace length. Donaldson et al. (1990) found that growth is slower for spot shrimp in Prince William Sound and a period of 2.5 to 3 years is required before they reach a size to recruit into our sampling gear. Spot shrimp hatched at the time of the oil spill should begin to recruit to the adult population between August 1991 and March 1992.

Fecundity and Related Parameters

Results from the observation of dead eggs and fecundity did not strongly indicate damage to eggs in the oiled area. Fecundity data indicates possible differences between oiled and unoiled areas in 1989 disappearing in 1990. Preliminary results show a lower number of eggs per female in the oiled area in 1989. The decline in the number of dead eggs during the ovigerous period suggests that the dead eggs which suffer mechanical damage or were not fertilized may slough off or be removed from the clutch (Donaldson et al. 1990). Therefore the number or frequency of occurrence of dead eggs may not be a good indicator of damage. Fecundity will continue to be compared between the oiled and unoiled areas in 1991.

Documenting Hydrocarbon Contamination

Sample sizes for hydrocarbon muscle tissue analysis have been met for each survey, however the full complement of egg samples was not available at all sites. Sufficient hydrocarbon samples have not been analyzed to document hydrocarbon contamination and draw a conclusion on the presence of hydrocarbons in spot shrimp muscle and eggs. Sufficient evidence does exist to state that oil has reached depths spot shrimp inhabit and is present in the vicinity of our oiled study sites. There is concern that oil and hydrocarbon-degrading bacteria was detected too close to our Culross study site to ensure its unoiled status. We recommend that another unoiled study site be added to the survey. Given spot shrimp's low bioconcentration of aromatic hydrocarbons and their theorized susceptibility to genetic damage as larvae (Sanborn and Malins 1980) we recommend that an assessment of genetic damage be conducted targeting the most vulnerable year classes (1987 - 1989).

Histopathology Observations

Data were collected to address the damage to tissues and compare between oil and unoiled sites. An initial histological study of the 1989 samples has detected

high levels of gill lesions in spot shrimp collected at our oiled study sites. The prognosis for 23% of the shrimp is for increased mortality. Analysis of samples from the unoiled site is underway. We recommend analysis of the 1990 and 1991 samples.

RECOMMENDATIONS TO CONTINUE UNDER RESTORATION

Spot shrimp stocks in PWS injured by the *M/V Exxon Valdez* oil spill are also heavily exploited in commercial, sport, and subsistence fisheries and can most effectively be restored through stock specific management practices designed to reduce exploitation of injured stocks. We recommend that this project continue under restoration to monitor recovery of the oiled population until there is no significant difference as to relative abundance, average recruitment, reproductive health, histopathology, or genetic damage (etc.). We need to continue to collect abundance data in order to regulate human use of the resource to allow rebuilding of injured stocks and insure their long term health. The project should be expanded to include development of a spot shrimp stock recovery management plan to facilitate the long term recovery and health of the spot shrimp population in PWS. This project is also needed to support restoration proposals titled *Spot Shrimp Restoration*, which in addition to restoration through manipulation of human use also evaluates the feasibility of artificial propagation and definition of stock boundaries using genetic techniques; and *Juvenile Spot Shrimp Habitat*, which will add to our understanding of larval distribution (analysis of existing data), habitat requirements of juveniles, and the relationship between juvenile and adult abundance.

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Table 1. Life history comparisons for economically important shellfish in the Exxon Valdez oil spill affected area. [a]

SHELLFISH SPECIES	EGG BEARING PERIOD	MONTHS HATCHING OCCURS	PLANKTONIC LARVAL PERIOD	SETTLEMENT PERIOD	JUVENILE HABITAT	ADULT FOOD[b] PREFERENCE
Spot Shrimp (Pandalus platyceros)	Oct–Mar	March–April	March–August	Late Summer	Inshore and shallower than adults rock crevices, kelp patches,	detritus, worms (annelids and polychaetes)
Pink Shrimp (P. borealis)	Nov–April	March–April	March–August	Late Summer	Inshore and shallower than adults	polychaetes, mysids and other crustaceans (in general, pandalid shrimp feed on detritus, amphipods, euphausids, annelids, and other shrimps).
Tanner Crab (Chionoecetes bairdi)	April–May (11 mons)	March–May	March–August	Late Summer	Inshore and shallower than adults	polychaetes, ophiuroids, fishes, *Nucula tenuis*, [c] bivalves, shrimp, amphipods, crab
Red King Crab (Paralithodes camtschatica)	April–May (11 mons)	March–May	March–August	Mid–June to Late Summer	Inshore and shallower than adults	molluscs, brittle stars, polychaetes, snails sandollars, pelecypods, basketstars, sea urchins
Blue King Crab (P. platypus)	April–May (11 mons)	March–May	March–August	Late Summer	Rock shellhash substrates	molluscs, brittle stars, polychaetes
Brown King Crab (Lithodes aequispina)	Variable year round	Variable year round	Unknown	Unknown	Shallower depths than adults	echinoderms, polychaetes, hydroids, molluscs, amphipoda, decapoda,

[a] Most information is from the literature and applies to the species throughout its range.
[b] Food habits are from the general literature and represent prey items utilized throughout their range.
[c] From study of Tanner crab in Prince William Sound (Feder and Hoberg 1981)

Table 2. Sampling locations for the November, 1990 oil spill impact assessment survey of spot shrimp.

SITE	DEPTH STRATUM[a]	STATION	LATITUDE[b]	LONGITUDE	MINIMUM DEPTH[c]	MAXIMUM DEPTH	SOAK[d]
UNAKWIK	1	A	60°59.91'	147°32.88'	74	120	17
UNAKWIK	1	B	61°00.03'	147°32.65'	61	119	17
UNAKWIK	2	A	60°59.87'	147°33.06'	135	189	17
UNAKWIK	2	B	61°00.09'	147°32.53'	130	174	17
GOLDEN	1	A	60°57.73'	148°01.86'	56	120	42
GOLDEN	1	B	60°57.98'	148°01.33'	46	106	42
GOLDEN	2	A	60°57.89'	148°01.76'	130	176	42
GOLDEN	2	B	60°58.05'	148°01.53'	130	139	42
GOLDEN	2	C	60°58.16'	148°01.43'	130	174	42
CULROSS	1	A	60°39.09'	148°11.41'	56	120	16
CULROSS	1	B	60°38.87'	148°10.87'	57	113	16
CULROSS	1	C	60°36.02'	148°12.18'	65	130	19
CULROSS	1	D	60°36.10'	148°11.51'	87	87	19
CULROSS	1	E	60°36.07'	148°11.88'	83	83	18
CULROSS	2	A	60°36.10'	148°11.50'	130	167	16
CULROSS	2	B	60°36.08'	148°11.52'	139	185	16
CULROSS	2	C	60°36.00'	148°11.77'	130	204	16
CULROSS	2	D	60°35.99'	148°12.04'	130	157	18
CULROSS	2	E	60°36.04'	148°11.65'	135	185	19
HERRING	1	A	60°28.13'	147°45.82'	74	107	19
HERRING	1	B	60°28.30'	147°45.73'	93	120	19
HERRING	1	C	60°28.52'	147°45.59'	109	130	19
HERRING	1	D	60°28.36'	147°45.72'	93	117	18
HERRING	1	E	60°28.79'	147°45.63'	93	102	19
HERRING	1	F	60°27.34'	147°44.34'	93	107	16
HERRING	2	A	60°28.49'	147°45.52'	139	148	19
HERRING	2	B	60°28.33'	147°45.62'	111	139	19
HERRING	2	C	60°28.37'	147°45.37'	139	172	19
HERRING	2	D	60°28.47'	147°45.59'	130	139	18
HERRING	2	E	60°28.59'	147°45.51'	130	167	18
HERRING	2	F	60°28.77'	147°45.52'	135	157	18
CHENEGA	1	A	60°24.79'	147°58.04'	93	130	18
CHENEGA	1	B	60°23.24'	147°58.91'	76	124	18
CHENEGA	1	C	60°24.71'	147°58.45'	67	119	18
CHENEGA	2	A	60°24.66'	147°58.18'	130	159	18
CHENEGA	2	B	60°23.32'	147°58.61'	130	172	18
CHENEGA	2	C	60°23.49'	147°58.41'	130	167	18
GREEN	1	A	60°19.15'	147°29.19'	87	126	18
GREEN	1	B	60°19.18'	147°25.20'	76	111	18
GREEN	1	C	60°19.01'	147°29.57'	65	93	18

[a] 1 = shallow (35 – 130 m); 2 = deep (130 – 220 m)
[b] Latitude and longitude are listed to one–hundredth of a minute.
[c] Depth in meters.
[d] Number of hours.

Table 3. Average weight[a] (kg) per pot of Pandalid shrimp captured during the November, 1990 oil spill impact assessment survey of spot shrimp.

SITE	AVERAGE WEIGHT OF SHRIMP PER POT		
	SPOT	PINK	COONSTRIPE
Unakwik	0.956	0.022	0.101
Golden	0.708	0.047	0.078
Culross	0.111	0.013	0.020
Herring	0.138	0.157	0.039
Chenega	0.400	0.062	0.046
Green[b]	0.024	0.062	0.008
Unoiled	0.592	0.027	0.066
Oiled	0.187	0.094	0.031

[a] Includes only weights from the first day's set at each site; does not include redeployment which targeted depths of known spot shrimp abundance.

[b] Only 33 pots set in the shallow stratum at this site in November 1990.

Table 4. Comparison of average weight[a] (kg) per pot of spot shrimp for the November, 1989 and 1990 oil spill impact assessment surveys of spot shrimp.

SITE		NUMBER OF POTS	TOTAL WEIGHT OF SPOT SHRIMP	AVERAGE WEIGHT OF SPOT SHRIMP
Unakwik	Nov. 89	44	28.087	0.638
	Nov. 90	44	42.056	0.956
Golden	Nov. 89	44	36.622	0.832
	Nov. 90	55	38.918	0.708
Culross	Nov. 89	44	11.535	0.262
	Nov. 90	55	6.120	0.111
Herring	Nov. 89	44	12.296	0.279
	Nov. 90	66	9.104	0.138
Chenega	Nov. 89	44	11.630	0.264
	Nov. 90	66	26.374	0.400
Green[b]	Nov. 89	44	1.654	0.038
	Nov. 90	33	0.798	0.024
Unoiled	Nov. 89	132	76.244	0.578
	Nov. 90	154	87.094	0.591
Oiled	Nov. 89	132	25.580	0.194
	Nov. 90	165	36.276	0.187

[a] Includes only weights from the first day's set at each site; does not inclue redeployment which targeted depths of known spot shrimp abundance.

[b] Only 33 pots set in the shallow stratum at this site in November 1990.

Table 5. Summary of carapace length information by sex for spot shrimp captured during the November, 1990 oil spill impact assessment survey of spot shrimp.

SITE	SEX	NUMBER OF SPOT SHRIMP MEASURED	MEAN CARAPACE LENGTH (mm)	STANDARD DEVIATION
Unakwik	Juvenile	5	22.1	1.6
	Male	1,480	31.7	2.8
	Transitional	0		
	Female	253	41.4	2.1
	Total	1,738		
Golden	Juvenile	2	19.9	<.1
	Male	497	31.4	2.6
	Transitional	0		
	Female	185	43.4	2.3
	Total	684		
Culross	Juvenile	30	20.6	1.4
	Male	884	25.6	4.1
	Transitional	0		
	Female	60	41.2	1.9
	Total	974		
Herring	Juvenile	33	21.4	1.3
	Male	1,892	27.9	3.7
	Transitional	0		
	Female	68	40.6	2.1
	Total	1,993		
Chenega	Juvenile	32	21.3	1.2
	Male	1,759	27.8	3.9
	Transitional	0		
	Female	28	40.6	3.4
	Total	1,819		
Green	Juvenile	1	14.4	0.0
	Male	30	26.8	4.9
	Transitional	0		
	Female	1	41.3	0.0
	Total	32		
Total	Juvenile	103	21.0	1.5
	Male	7,542	29.1	4.1
	Transitional	0		
	Female	595	41.9	2.5
	Total	8,240		

Table 6. Comparison of average size data among oiled and unoiled sites from the November, 1989 and 1990 oil spill impact assessment surveys of spot shrimp.

SEX	NUMBER OF SPOT SHRIMP MEASURED		MEAN CARAPACE LENGTH (mm)		STANDARD DEVIATION	
	1989	1990	1989	1990	1989	1990
UNOILED SITES						
Juvenile	2	37	20.4	20.7	0.05	1.5
Male	2,969	3,861	28.7	30.2	4.1	4.0
Transitional	0	0				
Female	234	498	41.8	42.1	2.5	2.4
Total	3,205	4,396				
OILED SITES						
Juvenile	4	66	20.1	21.2	1.9	1.5
Male	1,822	3,681	26.3	27.8	4.1	3.8
Transitional	0	0				
Female	37	97	40.7	40.6	2.4	2.5
Total	1,863	3,844				
ALL SITES COMBINED						
Juvenile	6	103	20.2	21.0	1.3	1.5
Male	4,791	7,542	27.8	29.1	4.3	4.1
Transitional	0	0				
Female	271	595	41.6	41.9	2.5	2.5
Total	5,068	8,240				

Table 7. Comparison of fecundity samples collected during the November, 1989 and 1990, oil spill impact assessment surveys of spot shrimp.

SITE	YEAR	NUMBER OF SAMPLES	MINIMUM NUMBER OF EGGS PER FEMALE	MAXIMUM NUMBER OF EGGS PER FEMALE	AVERAGE NUMBER OF EGGS PER FEMALE
Unakwik	Nov. 89	91	826	3296	2165
	Nov. 90	98	417	3326	1979
Golden	Nov. 89	43	910	3362	2369
	Nov. 90	85	1416	4273	2527
Culross	Nov. 89	29	1440	3964	2308
	Nov. 90	51	1176	3734	2293
Herring	Nov. 89	19	343	2759	1691
	Nov. 90	54	817	3009	2036
Chenega	Nov. 89	10	1380	2298	1963
	Nov. 90	26	1004	3120	2034
Green	Nov. 89	0			
	Nov. 90	1	2581	2581	2581
Unoiled	Nov. 89	163	826	3964	2280
Unoiled	Nov. 90	234	417	4273	2266
Oiled	Nov. 89	29	343	2759	1827
Oiled	Nov. 90	81	817	3120	2217

Table 8. Comparison of dead egg counts on ovigerous spot shrimp captured during the November, 1989 and 1990, oil spill impact assessment surveys of spot shrimp.

SITE		NUMBER OF OVIGEROUS FEMALES	MAXIMUM DEAD EGGS PER FEMALE	SUM OF DEAD EGGS ALL FEMALES	AVERAGE DEAD EGGS PER FEMALE
Unakwik	Nov. 89	145	40	152	1.05
	Nov. 90	235	7	81	0.34
Golden	Nov. 89	49	71	393	8.02
	Nov. 90	172	32	804	4.67
Culross	Nov. 89	32	58	241	7.53
	Nov. 90	51	4	22	0.43
Herring	Nov. 89	23	40	150	6.52
	Nov. 90	54	10	55	1.02
Chenega	Nov. 89	11	7	15	1.36
	Nov. 90	26	4	7	0.27
Green	Nov. 89	0			
	Nov. 90	1	0	0	0
Unoiled	Nov. 89	226	71	786	3.48
Unoiled	Nov. 90	458	43	907	1.81
Oiled	Nov. 89	34	40	165	4.85
Oiled	Nov. 90	81	14	62	0.43

Table 9. Comparison of the numbers of non–ovigerous female spot shrimp captured during the November, 1989 and 1990 oil spill impact assessment surveys of spot shrimp.

SITE	TOTAL NUMBER AND PERCENT NON–OVIGEROUS FEMALES				NUMBER AND PERCENT OF NON–OVIGEROUS FEMALES IN BREEDING DRESS			
	NUMBER		PERCENT		NUMBER		PERCENT	
	1989	1990	1989	1990	1989	1990	1989	1990
UNAKWIK	7	18	4.7	7.1	7	1	100.0	5.6
GOLDEN	4	13	7.5	7.0	4	1	100.0	7.7
CULROSS	1	9	3.1	15.0	1	0	100.0	0.0
HERRING	6	14	23.1	20.6	4	0	66.7	0.0
CHENEGA	1	2	9.1	7.1	0	0	0.0	0.0
GREEN	0	0	0.0	0.0				
UNOILED	12	40	5.1	8.0	12	2	100.0	5.0
OILED	7	16	18.9	16.5	4	0	57.1	0.0

Table 10. Summary of hydrocarbon samples collected during the November, 1990 oil spill impact assessment survey of spot shrimp.

SITE	DEPTH STRATUM[a]	STATION	POT NUMBER	SAMPLE NUMBER	SAMPLE TYPE	SAMPLE DISPOSITION	RESULTS
UNAKWIK	1	A	11	128601	EGG	TAKEN	UNANALYZED
UNAKWIK	1	A	11	128602	MUSCLE	TAKEN	UNANALYZED
UNAKWIK	1	A	8	128603	EGG	TAKEN	UNANALYZED
UNAKWIK	1	A	8	128604	MUSCLE	TAKEN	UNANALYZED
UNAKWIK	1	B	2	128605	EGG	TAKEN	UNANALYZED
UNAKWIK	1	B	2	128606	MUSCLE	TAKEN	UNANALYZED
UNAKWIK	2	A	5	128607	EGG	TAKEN	UNANALYZED
UNAKWIK	2	A	5	128608	MUSCLE	TAKEN	UNANALYZED
UNAKWIK	2	A	1	128609	EGG	TAKEN	UNANALYZED
UNAKWIK	2	A	1	128610	MUSCLE	TAKEN	UNANALYZED
UNAKWIK	2	B	3	128611	EGG	TAKEN	UNANALYZED
UNAKWIK	2	B	3	128612	MUSCLE	TAKEN	UNANALYZED
UNAKWIK	2	B		128613	FLD.BLK.	TAKEN	UNANALYZED
GOLDEN	1	B	1	128614	EGG	TAKEN	UNANALYZED
GOLDEN	1	B	1	128615	MUSCLE	TAKEN	UNANALYZED
GOLDEN	1	B	3	128616	EGG	TAKEN	UNANALYZED
GOLDEN	1	B	3	128617	MUSCLE	TAKEN	UNANALYZED
GOLDEN	2	A	2	128618	EGG	TAKEN	UNANALYZED
GOLDEN	2	A	2	128619	MUSCLE	TAKEN	UNANALYZED
GOLDEN	2	A	3	128620	EGG	TAKEN	UNANALYZED
GOLDEN	2	A	3	128621	MUSCLE	TAKEN	UNANALYZED
GOLDEN	1	A	1	128622	MUSCLE	TAKEN	UNANALYZED
GOLDEN	1	A	1	128623	EGG	TAKEN	UNANALYZED
GOLDEN	2	B	3	128624	EGG	TAKEN	UNANALYZED
GOLDEN	2	B	3	128625	MUSCLE	TAKEN	UNANALYZED
GOLDEN	2	B		128626	FLD.BLK.	TAKEN	UNANALYZED
GOLDEN				128627	OIL[b]	TAKEN	UNANALYZED
CULROSS	1	A	11	128628	EGG	TAKEN	UNANALYZED
CULROSS	2	A	10	128630	EGG	TAKEN	UNANALYZED
CULROSS	1	A	11	128629	MUSCLE	TAKEN	UNANALYZED
CULROSS	2	B	5	128632	MUSCLE	TAKEN	UNANALYZED
CULROSS	2	C	7	128633	EGG	TAKEN	UNANALYZED
CULROSS	2	C	7	128634	MUSCLE	TAKEN	UNANALYZED
CULROSS	2	A	10	128631	MUSCLE	TAKEN	UNANALYZED
CULROSS	1	B	1	128636	MUSCLE	TAKEN	UNANALYZED
CULROSS	1	B	10	128637	MUSCLE	TAKEN	UNANALYZED
CULROSS	1	B	10	128638	EGG	TAKEN	UNANALYZED
CULROSS	1	B		128639	FLD.BLK.	TAKEN	UNANALYZED
CULROSS	1	B	1	128635	EGG	TAKEN	UNANALYZED
HERRING	1	B	10	128640	EGG	TAKEN	UNANALYZED
HERRING	1	C	7	128642	MUSCLE	TAKEN	UNANALYZED
HERRING	1	B	10	128641	MUSCLE	TAKEN	UNANALYZED
HERRING	2	A	4	128644	EGG	TAKEN	UNANALYZED
HERRING	2	A	4	128645	MUSCLE	TAKEN	UNANALYZED
HERRING	2	A	3	128646	EGG	TAKEN	UNANALYZED
HERRING	2	A	3	128647	MUSCLE	TAKEN	UNANALYZED
HERRING	1	B	2	128648	MUSCLE	TAKEN	UNANALYZED
HERRING	1	B		128649	FLD.BLK.	TAKEN	UNANALYZED
HERRING	2	B	10	128643	MUSCLE	TAKEN	UNANALYZED
CHENEGA	1	B	2	128650	EGG	TAKEN	UNANALYZED
CHENEGA	1	B	1	128802	EGG	TAKEN	UNANALYZED
CHENEGA	1	B	1	128803	MUSCLE	TAKEN	UNANALYZED
CHENEGA	1	A	4	128804	EGG	TAKEN	UNANALYZED
CHENEGA	1	A	4	128805	MUSCLE	TAKEN	UNANALYZED
CHENEGA	2	B	2	128806	EGG	TAKEN	UNANALYZED

–CONTINUED–

Table 10. (page 2 of 2)

SITE	DEPTH STRATUM	STATION	POT NUMBER	SAMPLE NUMBER	SAMPLE TYPE	SAMPLE DISOSTITION	RESULTS
CHENEGA	2	B	2	128807	MUSCLE	TAKEN	UNANALYZED
CHENEGA	2	C	3	128808	EGG	TAKEN	UNANALYZED
CHENEGA	2	C	3	128809	MUSCLE	TAKEN	UNANALYZED
CHENEGA	2	C	8	128810	EGG	TAKEN	UNANALYZED
CHENEGA	2	C	8	128811	MUSCLE	TAKEN	UNANALYZED
CHENEGA	2	C		128812	FLD.BLK.	TAKEN	UNANALYZED
CHENEGA	1	B	2	128801	MUSCLE	TAKEN	UNANALYZED
GREEN	1	A	11	128814	MUSCLE	TAKEN	UNANALYZED
GREEN	1	B	6,10	128815	MUSCLE	TAKEN	UNANALYZED
GREEN	1	B		128816	FLD.BLK.	TAKEN	UNANALYZED
GREEN	1	A	10	128813	MUSCLE	TAKEN	UNANALYZED

[a] 1 = shallow (35 – 130 m); 2 = deep (130–220 m)
[b] Petroleum type substance collected from groundline.

Table 11. Observations of gill lesions in spot shrimp and pink shrimp collected during the November 1989 oil spill impact assessment survey in Prince William Sound.

SHRIMP SPECIES	SITE	DEPTH [a] STRATUM	SEVERITY GRADING	NUMBER BY GRADE	PERCENT INFLICTED	PERCENT >1 SEVERITY
Spot	Herring Bay	Shallow	0	8	20	0
			1	2		
Spot	Chenega	Shallow	0	9	10	0
			1	1		
Spot	Chenega	Deep	0	1	90	60
			1	3		
			2	3		
			3	3		
Spot	Green Is.	Shallow	0	3	70	30
			1	4		
			2	2		
			3	1		
Pink	Green Is.	Deep	0	3	70	50
			1	2		
			2	5		
Spot	Total		0	21	48	23
			1	10		
			2	5		
			3	4		

[a] Shallow = 35–130 m; Deep = 130–220 m.

Table 12. Summary of histopathological samples collected during the November, 1990 oil spill impact assessment survey of spot shrimp.

SITE	DEPTH STRATUM[a]	STATION	POT NUMBER	SAMPLE NUMBER	SAMPLE DISPOSITION	RESULTS
UNAKWIK	2	B	ALL	128701	TAKEN	UNANALYZED
UNAKWIK	1	B	ALL	128702	TAKEN	UNANALYZED
GOLDEN	2	A	ALL	128703	TAKEN	UNANALYZED
CULROSS	1	B	ALL	128704	TAKEN	UNANALYZED
CULROSS	2	D	ALL	128705	TAKEN	UNANALYZED
HERRING	1	A	ALL	128706	TAKEN	UNANALYZED
HERRING	2	C	ALL	128707	TAKEN	UNANALYZED
CHENEGA	2	A	ALL	128708	TAKEN	UNANALYZED
CHENEGA	1	B	ALL	128709	TAKEN	UNANALYZED
GREEN	1	A	ALL	128710	TAKEN	UNANALYZED

[a] 1 = shallow (35 – 130 m); 2 = deep (130 – 220 m)

Table 13. Summary of environmental data for November, 1989 and 1990, oil spill impact assessment surveys of spot shrimp.

SITE	YEAR	BOTTOM TEMPERATURE (Celcius)	BOTTOM SALINITY (ppt)	BOTTOM OXYGEN (ml/l)	DEPTH (meters)
Unakwik	Nov. 89	5.52	32.81	2.63	200
	Nov. 90	5.99	32.30	3.11	199
Golden	Nov. 89	5.63	32.83	2.46	223
	Nov. 90	5.87	32.26	3.20	197
Culross	Nov. 89	5.51	32.13	2.36	135
	Nov. 90	5.56	32.87	2.17	306
Herring	Nov. 89	5.43	32.86	2.61	232
	Nov. 90	5.76	32.39	3.31	184
Chenega	Nov. 89	5.43	32.81	2.73	200
	Nov. 90	5.66	32.66	2.69	283
Green	Nov. 89	5.50	32.72	2.67	201
	Nov. 90	5.66	32.43	3.11	201
Unoiled	Nov. 89	5.55	32.59	2.48	186
	Nov. 90	5.81	32.48	2.83	234
Oiled	Nov. 89	5.45	32.80	2.67	211
	Nov. 90	5.69	32.49	3.04	223

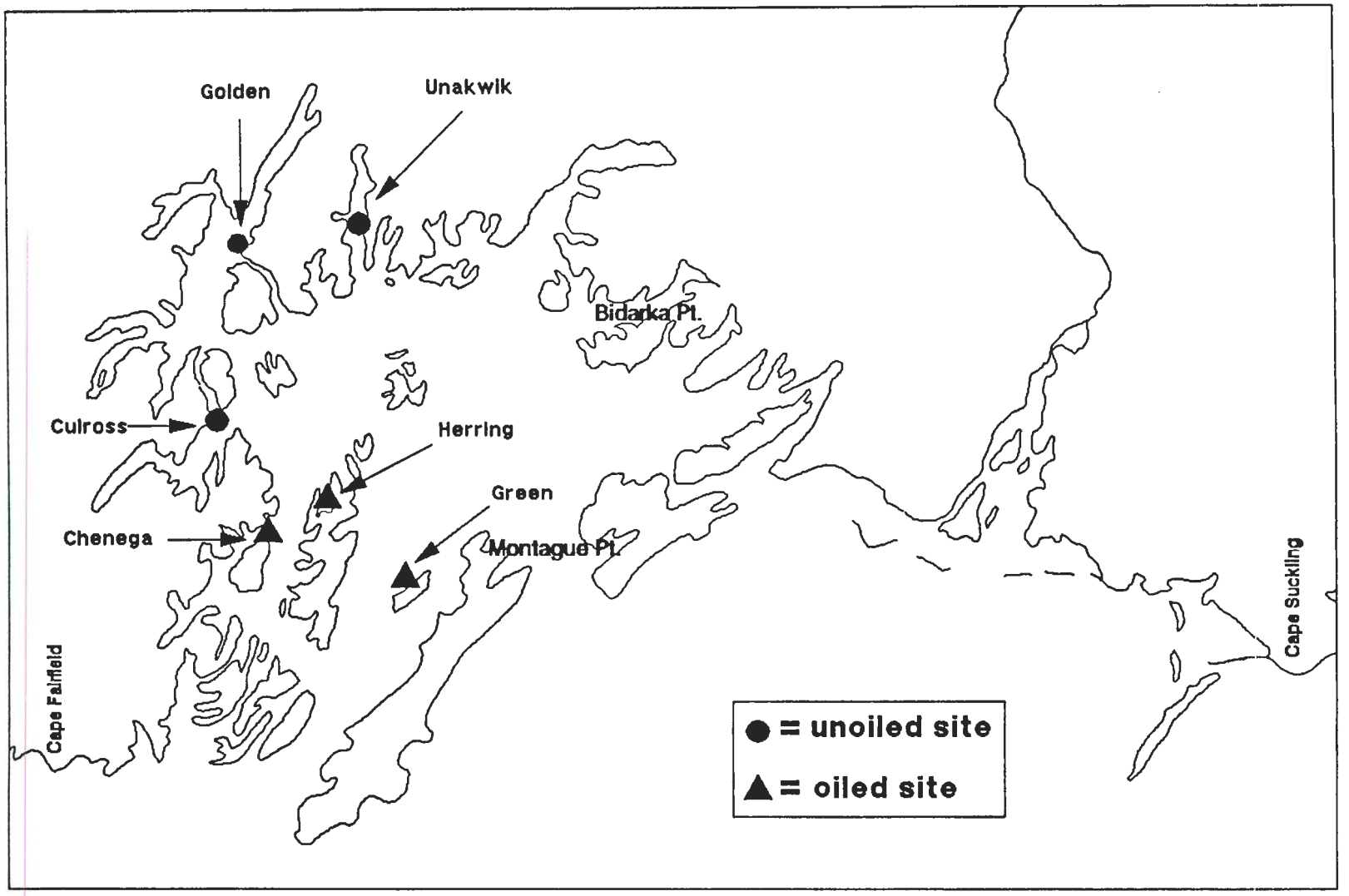

Figure 1. Spot shrimp sampling locations in Prince William Sound for Subtidal Study 5.

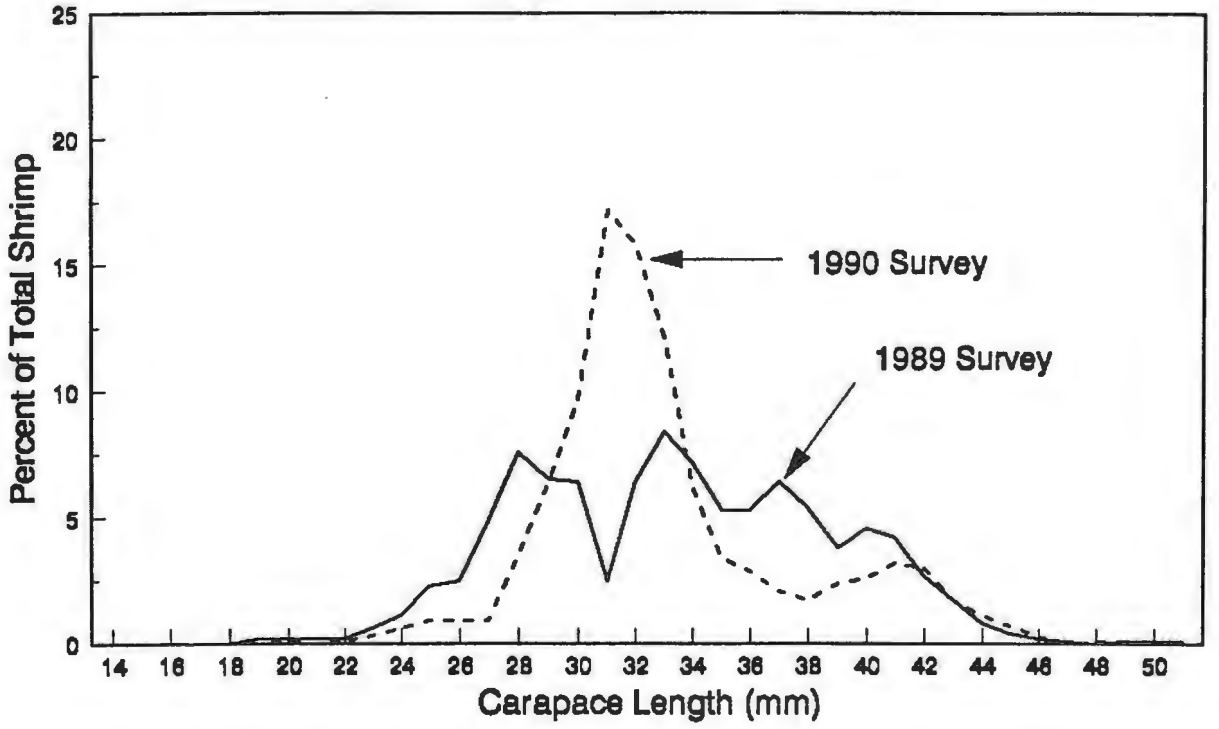

Figure 2. Comparison of spot shrimp length frequencies between the November 1989 (n = 926) and November 1990 (n = 1738) surveys in Unakwik Inlet.

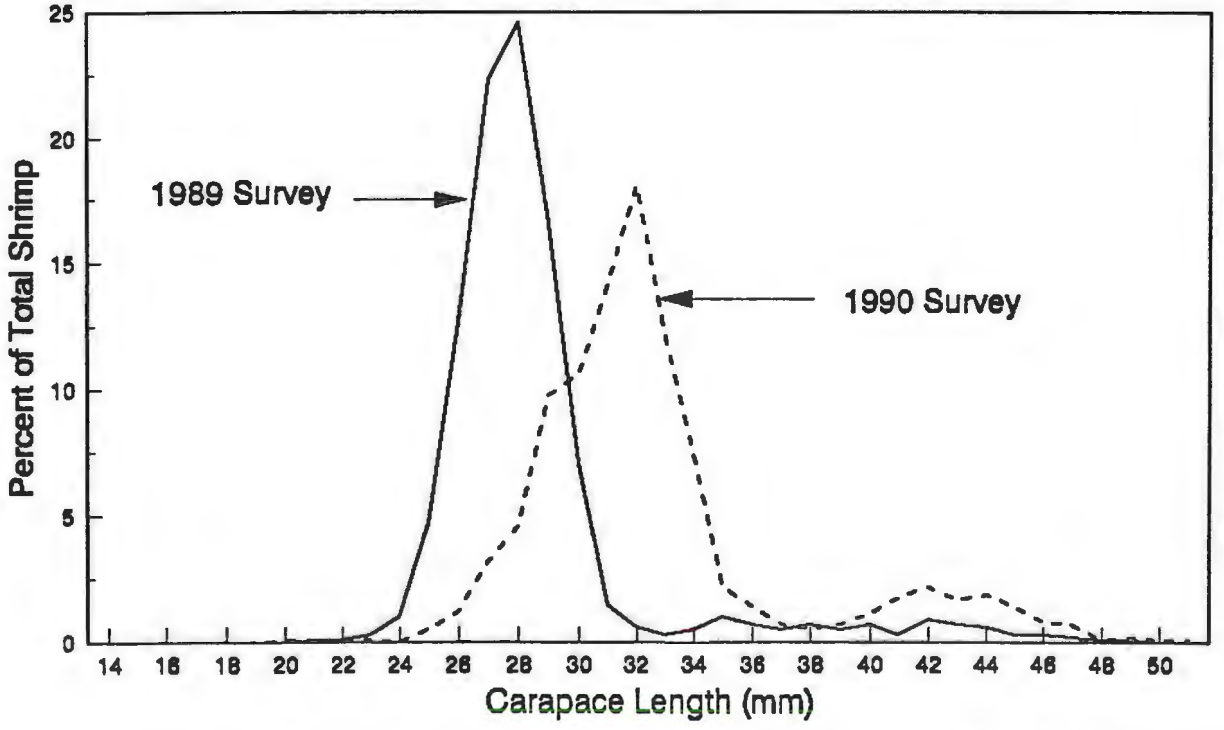

Figure 3. Comparison of spot shrimp length frequencies between the November 1989 (n = 1470) and November 1990 (n = 1684) surveys at Golden.

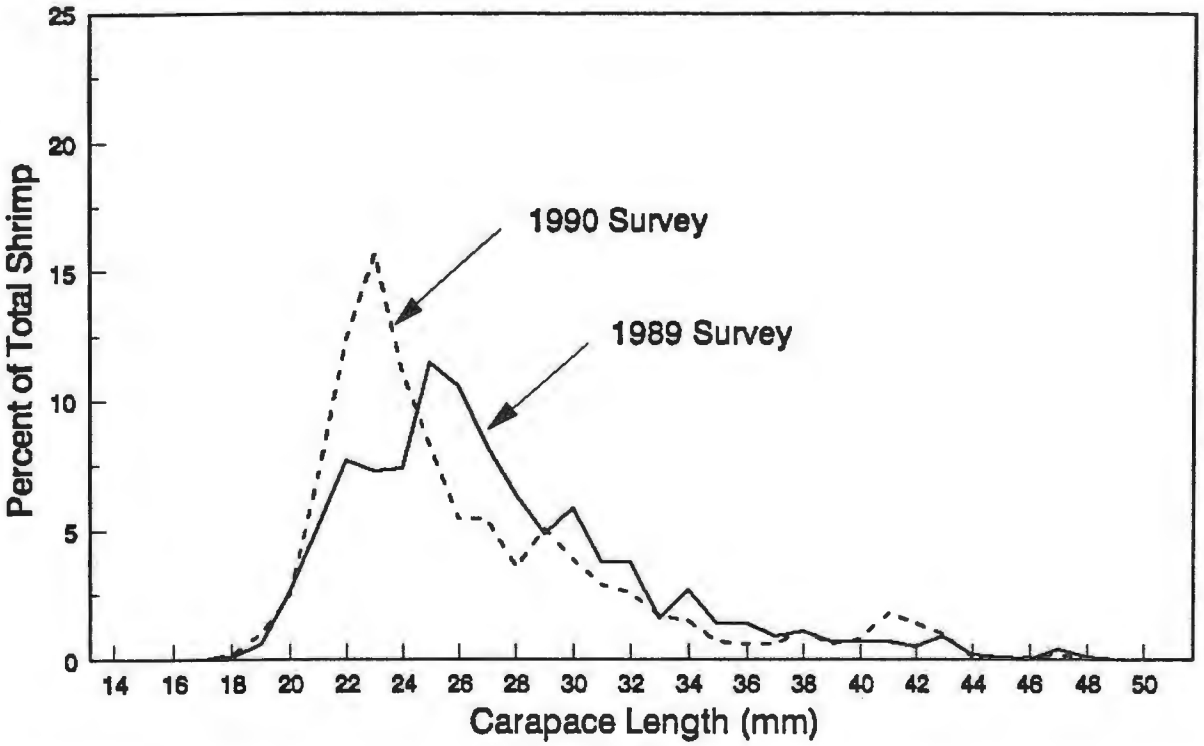

Figure 4. Comparison of spot shrimp length frequencies between the November 1989 (n = 809) and November 1990 (n = 974) surveys of Culross Passage.

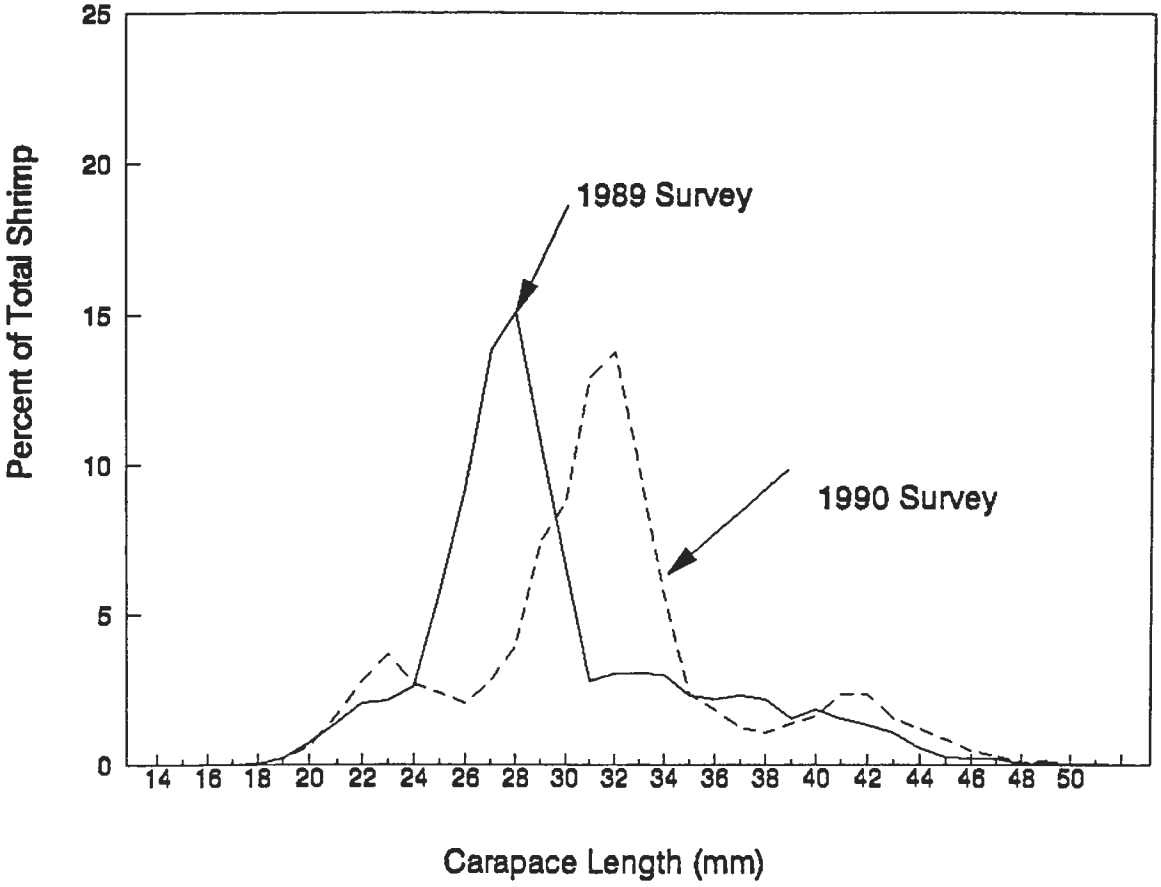

Figure 5. Comparison of spot shrimp length frequencies between the November 1989 and 1990 surveys in the unoiled area of PWS

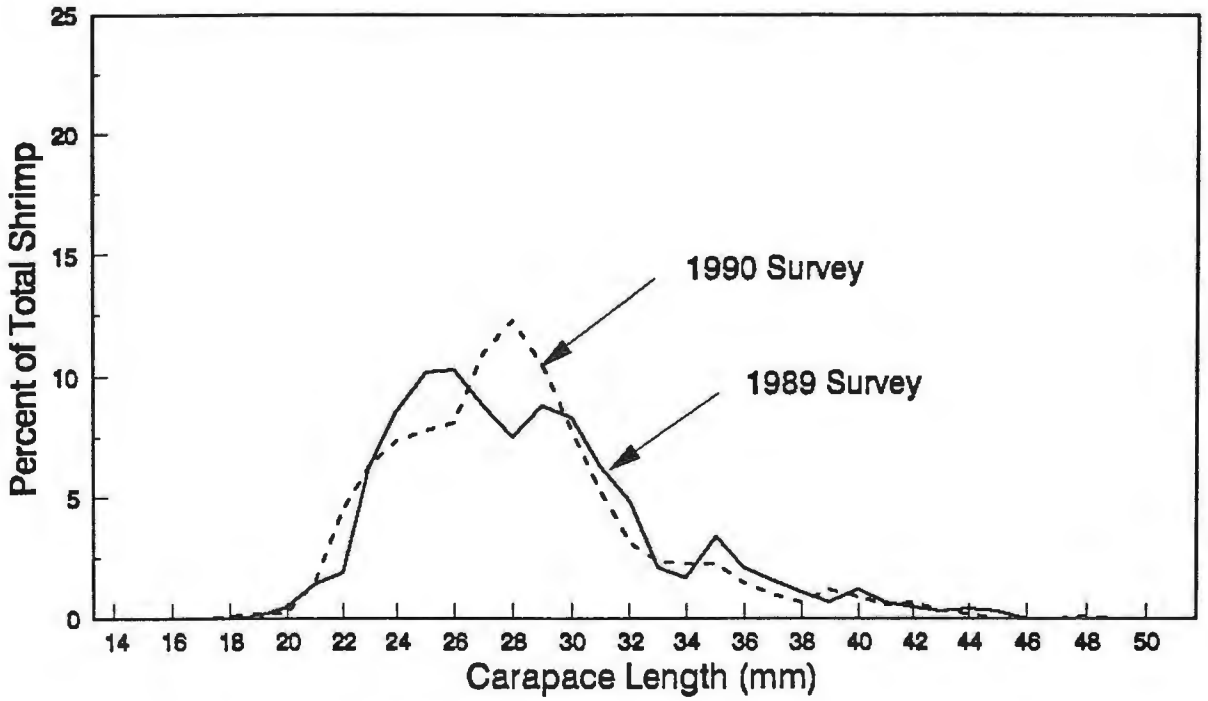

Figure 6. Comparison of spot shrimp length frequencies between the November 1989 (n = 746) and November 1990 (n = 1993) surveys in Herring Bay.

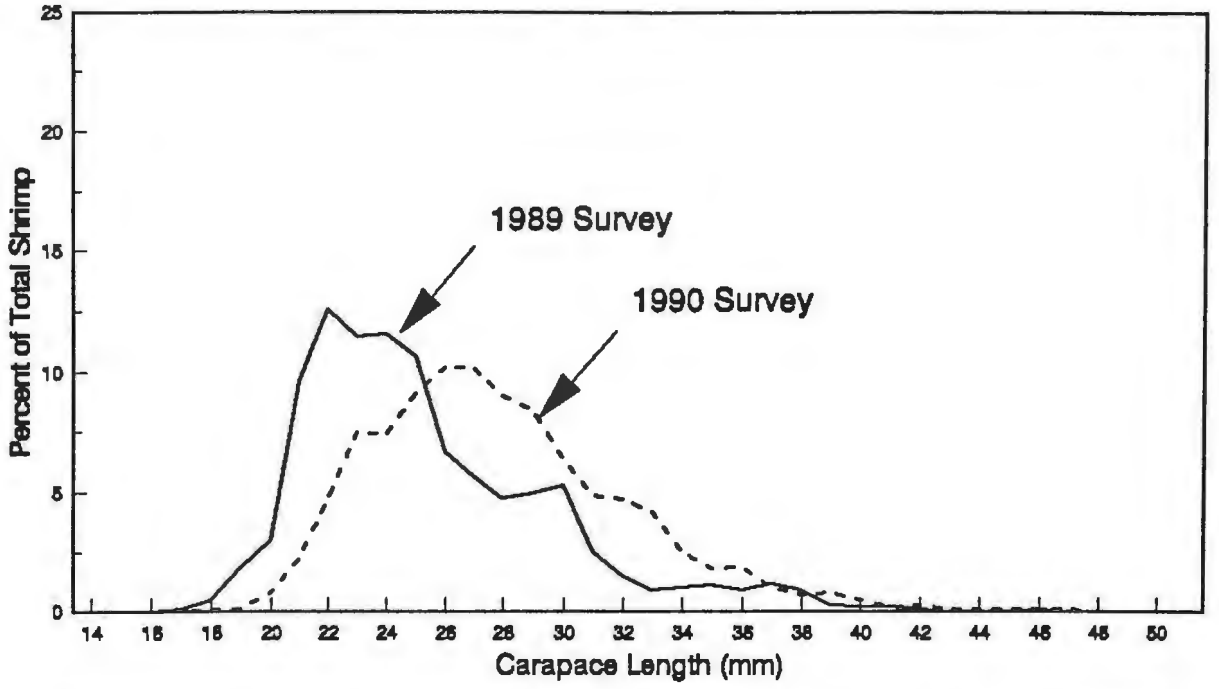

Figure 7. Comparison of spot shrimp length frequencies between the November 1989 (n = 983) and November 1990 (n = 1819) surveys at Chenega Island.

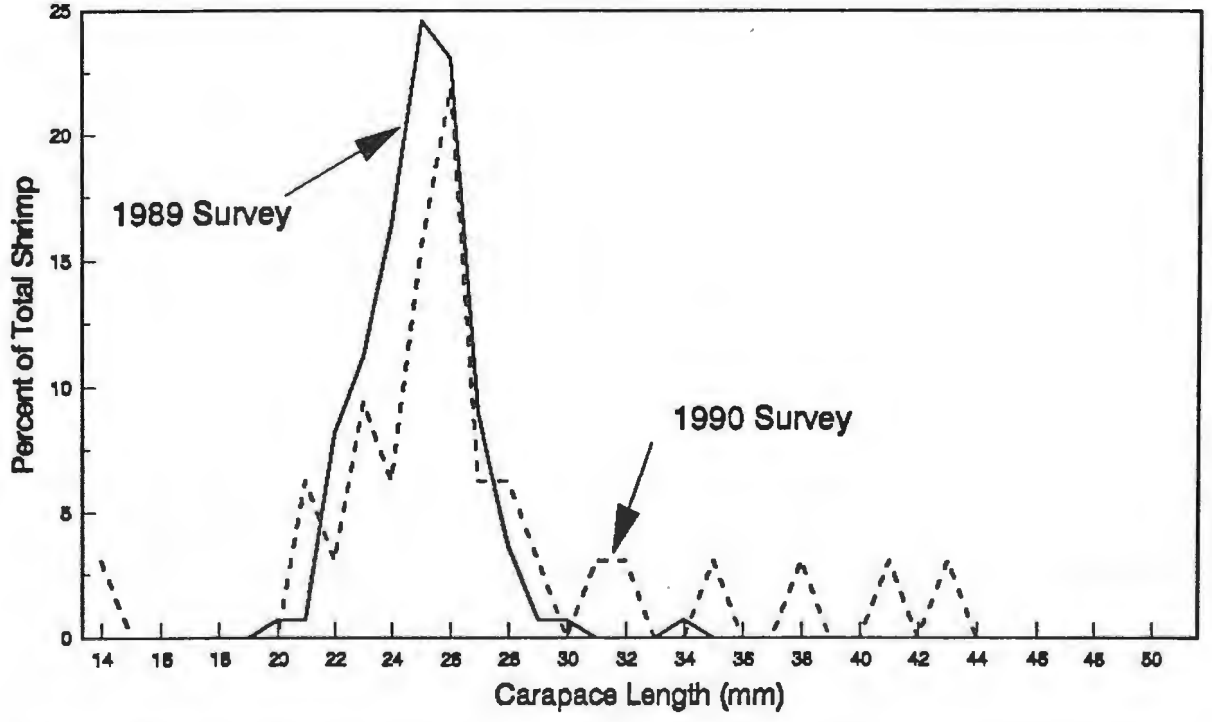

Figure 8. Comparison of spot shrimp length frequencies between the November 1989 (n = 134) and November 1990 (n = 32) surveys at Green Island.

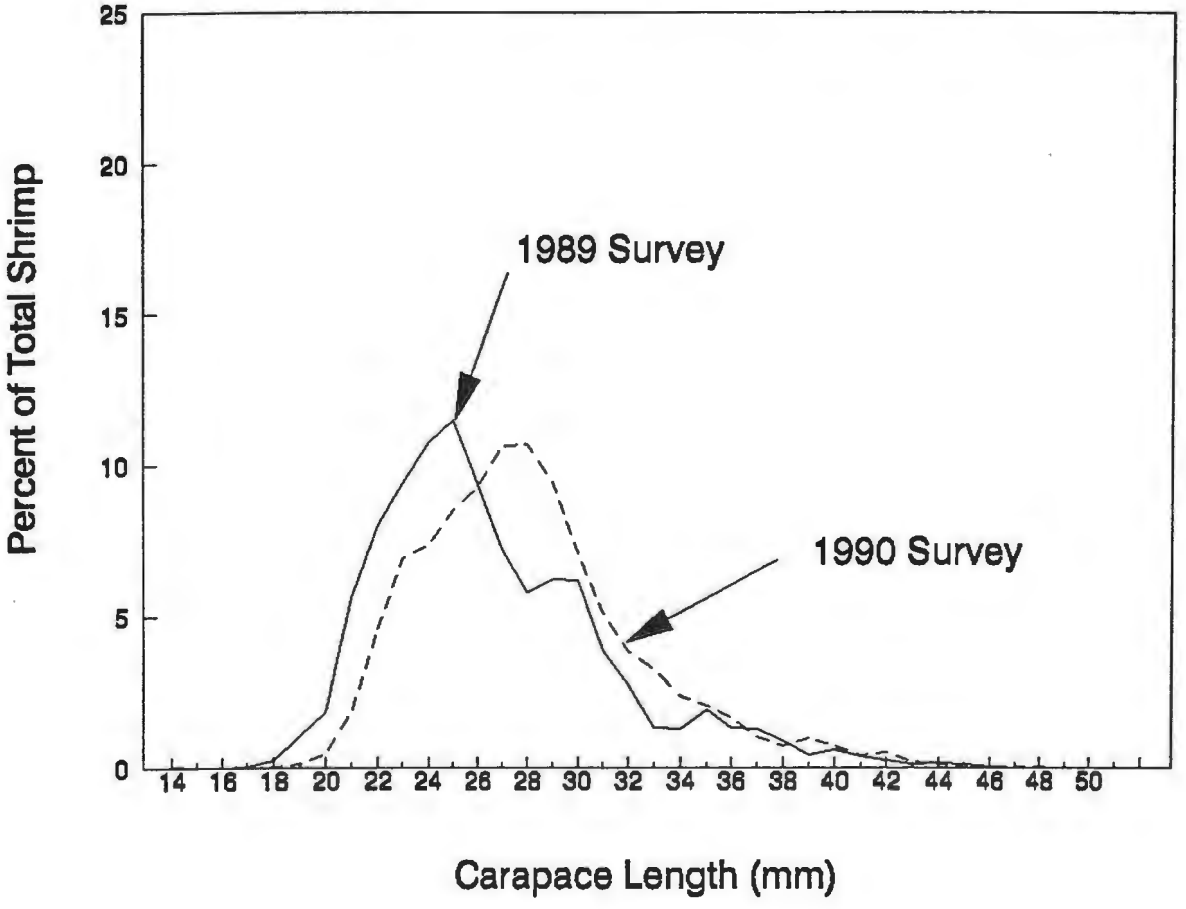

Figure 9. Comparison of spot shrimp length frequencies between the November 1989 and 1990 surveys in the oiled area of PWS.

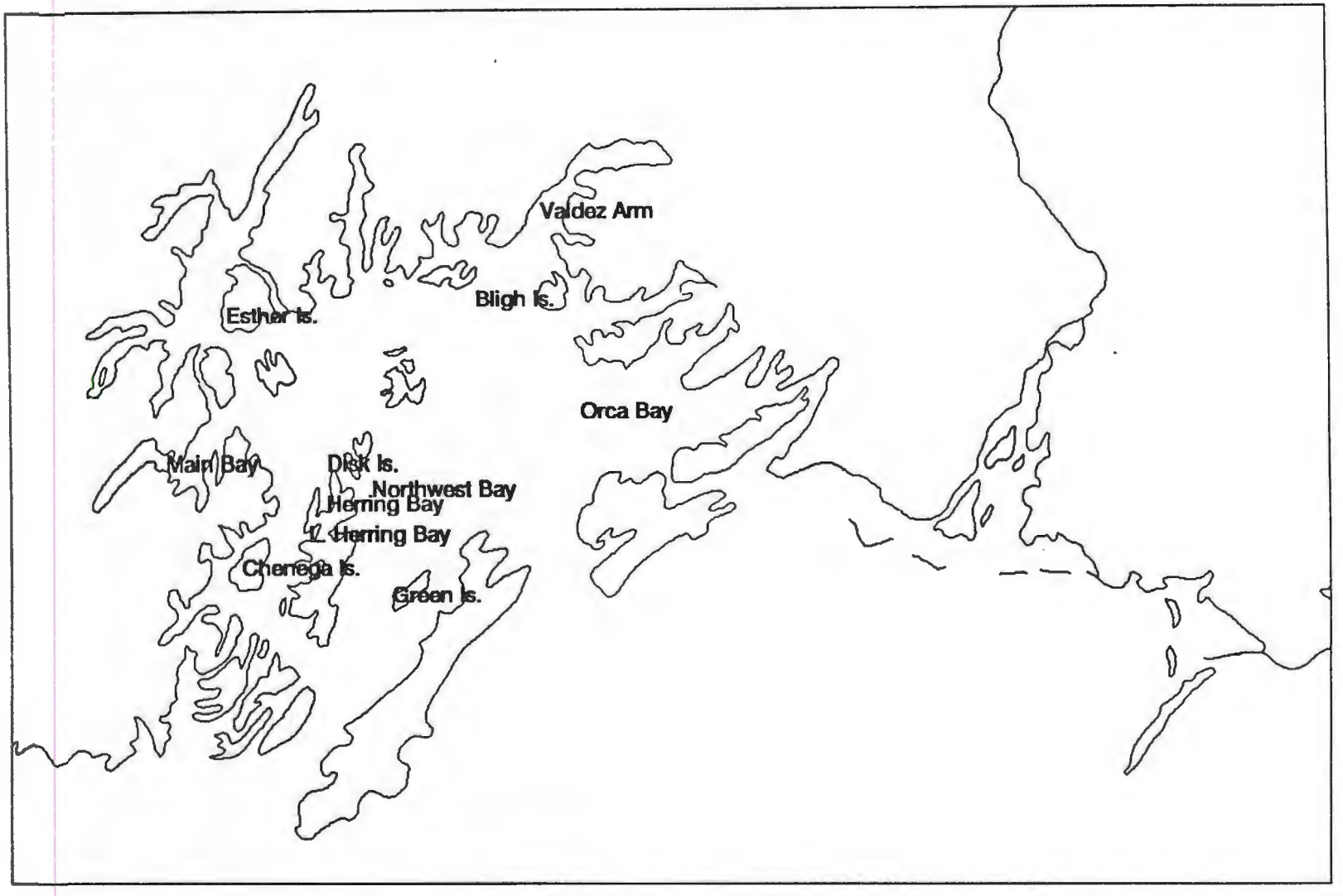

Figure 10. Locations with evidence of hydrocarbons at depths spot shrimp inhabit.
Names represent approximate location only.

Appendix A. Weight (kg) by species of Pandalid shrimp captured during the November 1990 oil spil impact assessment survey of spot shrimp. Weights are summarized by site.

SITE	DEPTH STRATUM[a]	STATION	POT NUMBER	SHRIMP WEIGHT (kg) SPOT	PINK	COONSTRIPE	TOTAL
UNAKWIK	1	A	1	2.260	0.000	0.170	2.430
UNAKWIK	1	A	2	2.460	0.000	0.062	2.522
UNAKWIK	1	A	3	2.210	0.000	0.022	2.232
UNAKWIK	1	A	4	0.940	0.000	0.038	0.978
UNAKWIK	1	A	5	1.170	0.000	0.000	1.170
UNAKWIK	1	A	6	0.680	0.000	0.058	0.738
UNAKWIK	1	A	7	1.070	0.004	0.220	1.294
UNAKWIK	1	A	8	1.475	0.010	0.130	1.615
UNAKWIK	1	A	9	1.770	0.002	0.088	1.860
UNAKWIK	1	A	10	1.410	0.000	0.138	1.548
UNAKWIK	1	A	11	1.155	0.008	0.094	1.257
UNAKWIK	1	A	1	1.018	0.000	0.046	1.064
UNAKWIK	1	B	2	2.931	0.000	0.010	2.941
UNAKWIK	1	B	3	2.286	0.000	0.016	2.302
UNAKWIK	1	B	4	1.722	0.000	0.018	1.740
UNAKWIK	1	B	5	2.064	0.000	0.032	2.096
UNAKWIK	1	B	6	2.100	0.000	0.034	2.134
UNAKWIK	1	B	7	1.410	0.000	0.030	1.440
UNAKWIK	1	B	8	1.520	0.000	0.118	1.638
UNAKWIK	1	B	9	0.280	0.006	0.034	0.320
UNAKWIK	1	B	10	1.380	0.000	0.110	1.490
UNAKWIK	1	B	11	0.870	0.000	0.210	1.080
UNAKWIK	2	A	1	1.335	0.016	0.138	1.489
UNAKWIK	2	A	2	0.380	0.016	0.090	0.486
UNAKWIK	2	A	3	0.450	0.020	0.116	0.586
UNAKWIK	2	A	4	0.190	0.024	0.108	0.322
UNAKWIK	2	A	5	1.225	0.012	0.260	1.497
UNAKWIK	2	A	6	0.318	0.010	0.030	0.358
UNAKWIK	2	A	7	0.062	0.048	0.090	0.200
UNAKWIK	2	A	8	0.206	0.060	0.040	0.306
UNAKWIK	2	A	9	0.160	0.044	0.316	0.520
UNAKWIK	2	A	10	0.374	0.108	0.116	0.598
UNAKWIK	2	A	11	0.204	0.038	0.174	0.416
UNAKWIK	2	B	1	0.000	0.000	0.000	0.000
UNAKWIK	2	B	2	0.000	0.000	0.000	0.000
UNAKWIK	2	B	3	1.315	0.118	0.228	1.661
UNAKWIK	2	B	4	0.066	0.132	0.086	0.284
UNAKWIK	2	B	5	0.380	0.166	0.410	0.956
UNAKWIK	2	B	6	0.000	0.000	0.000	0.000
UNAKWIK	2	B	7	0.000	0.000	0.000	0.000
UNAKWIK	2	B	8	0.440	0.046	0.036	0.522
UNAKWIK	2	B	9	0.090	0.036	0.136	0.262
UNAKWIK	2	B	10	0.580	0.032	0.350	0.962
UNAKWIK	2	B	11	0.100	0.032	0.038	0.170
TOTALS			44	42.056	0.988	4.440	47.484
AVERAGE WEIGHT/POT (kg)				0.956	0.022	0.101	1.079
SD				0.811	0.039	0.098	0.795

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SITE	DEPTH STRATUM	STATION	POT NUMBER	SHRIMP WEIGHT (kg) SPOT	PINK	COONSTRIPE	TOTAL
GOLDEN	1	A	1	0.785	0.000	0.070	0.855
GOLDEN	1	A	2	0.270	0.000	0.100	0.370
GOLDEN	1	A	3	1.580	0.000	0.036	1.616
GOLDEN	1	A	4	0.700	0.004	0.048	0.752
GOLDEN	1	A	5	0.180	0.000	0.022	0.202
GOLDEN	1	A	6	0.270	0.000	0.026	0.296
GOLDEN	1	A	7	0.740	0.004	0.040	0.784
GOLDEN	1	A	8	1.500	0.002	0.072	1.574
GOLDEN	1	A	9	1.250	0.008	0.078	1.336
GOLDEN	1	A	10	0.540	0.002	0.068	0.610
GOLDEN	1	A	11	0.710	0.000	0.172	0.882
GOLDEN	1	B	1	1.107	0.000	0.000	1.107
GOLDEN	1	B	2	1.092	0.000	0.000	1.092
GOLDEN	1	B	3	1.027	0.000	0.012	1.039
GOLDEN	1	B	4	1.612	0.000	0.014	1.626
GOLDEN	1	B	5	0.580	0.000	0.038	0.618
GOLDEN	1	B	6	0.390	0.000	0.060	0.450
GOLDEN	1	B	7	2.030	0.000	0.000	2.030
GOLDEN	1	B	8	1.370	0.000	0.134	1.504
GOLDEN	1	B	9	2.300	0.002	0.080	2.382
GOLDEN	1	B	10	0.000	0.000	0.000	0.000
GOLDEN	1	B	11	0.860	0.000	0.060	0.920
GOLDEN	2	A	1	1.010	0.000	0.090	1.100
GOLDEN	2	A	2	1.025	0.040	0.082	1.147
GOLDEN	2	A	3	2.055	0.026	0.030	2.111
GOLDEN	2	A	4	0.090	0.100	0.006	0.196
GOLDEN	2	A	5	0.090	0.076	0.056	0.222
GOLDEN	2	A	6	0.160	0.078	0.024	0.262
GOLDEN	2	A	7	0.100	0.240	0.066	0.406
GOLDEN	2	A	8	0.040	0.078	0.024	0.142
GOLDEN	2	A	9	0.200	0.102	0.070	0.372
GOLDEN	2	A	10	0.150	0.104	0.028	0.282
GOLDEN	2	A	11	0.210	0.054	0.240	0.504
GOLDEN	2	B	1	0.610	0.006	0.220	0.836
GOLDEN	2	B	2	0.380	0.094	0.070	0.544
GOLDEN	2	B	3	1.695	0.000	0.120	1.815
GOLDEN	2	B	4	0.640	0.124	0.092	0.856
GOLDEN	2	B	5	0.400	0.044	0.024	0.468
GOLDEN	2	B	6	0.540	0.070	0.074	0.684
GOLDEN	2	B	7	0.780	0.032	0.096	0.908
GOLDEN	2	B	8	0.430	0.028	0.082	0.540
GOLDEN	2	B	9	0.440	0.076	0.078	0.594
GOLDEN	2	B	10	0.910	0.034	0.298	1.242
GOLDEN	2	B	11	0.380	0.072	0.162	0.614
GOLDEN	2	C	1	0.540	0.022	0.270	0.832
GOLDEN	2	C	2	1.100	0.118	0.136	1.354
GOLDEN	2	C	3	0.760	0.108	0.030	0.898
GOLDEN	2	C	4	0.820	0.028	0.088	0.936
GOLDEN	2	C	5	0.920	0.096	0.108	1.124
GOLDEN	2	C	6	0.540	0.066	0.188	0.794

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SITE	DEPTH STRATUM	STATION	POT NUMBER	SHRIMP WEIGHT (kg) SPOT	PINK	COONSTRIPE	TOTAL
GOLDEN	2	C	7	0.330	0.112	0.090	0.532
GOLDEN	2	C	8	0.090	0.170	0.014	0.274
GOLDEN	2	C	9	0.130	0.090	0.042	0.262
GOLDEN	2	C	10	0.060	0.128	0.012	0.200
GOLDEN	2	C	11	0.400	0.160	0.124	0.684
TOTALS			55	38.918	2.598	4.264	45.780
AVERAGE WEIGHT/POT (kg)				0.708	0.047	0.078	0.832
SD				0.558	0.055	0.068	0.538

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SITE	DEPTH STRATUM	STATION	POT NUMBER	SHRIMP WEIGHT (kg) SPOT	PINK	COONSTRIPE	TOTAL
CULROSS	1	A	1	0.040	0.000	0.000	0.040
CULROSS	1	A	2	0.084	0.000	0.036	0.120
CULROSS	1	A	3	0.068	0.000	0.138	0.206
CULROSS	1	A	4	0.142	0.000	0.122	0.264
CULROSS	1	A	5	0.104	0.000	0.036	0.140
CULROSS	1	A	6	0.058	0.000	0.014	0.072
CULROSS	1	A	7	0.000	0.000	0.010	0.010
CULROSS	1	A	8	0.040	0.000	0.016	0.056
CULROSS	1	A	9	0.000	0.000	0.000	0.000
CULROSS	1	A	10	0.018	0.000	0.000	0.018
CULROSS	1	A	11	0.291	0.000	0.054	0.345
CULROSS	1	B	1	0.203	0.000	0.000	0.203
CULROSS	1	B	2	0.112	0.000	0.000	0.112
CULROSS	1	B	3	0.040	0.000	0.000	0.040
CULROSS	1	B	4	0.324	0.000	0.006	0.330
CULROSS	1	B	5	0.128	0.000	0.006	0.134
CULROSS	1	B	6	0.124	0.000	0.000	0.124
CULROSS	1	B	7	0.066	0.000	0.010	0.076
CULROSS	1	B	8	0.172	0.000	0.028	0.200
CULROSS	1	B	9	0.040	0.002	0.004	0.046
CULROSS	1	B	10	0.197	0.004	0.012	0.213
CULROSS	1	B	11	0.092	0.002	0.010	0.104
CULROSS	1	C	1	0.000	0.000	0.000	0.000
CULROSS	1	C	2	0.000	0.000	0.000	0.000
CULROSS	1	C	3	0.000	0.000	0.000	0.000
CULROSS	1	C	4	0.078	0.000	0.004	0.082
CULROSS	1	C	5	0.000	0.000	0.010	0.010
CULROSS	1	C	6	0.214	0.000	0.026	0.240
CULROSS	1	C	7	0.230	0.002	0.000	0.232
CULROSS	1	C	8	0.744	0.024	0.054	0.822
CULROSS	1	C	9	0.212	0.010	0.042	0.264
CULROSS	1	C	10	0.208	0.010	0.032	0.250
CULROSS	1	C	11	0.096	0.012	0.068	0.176
CULROSS	1	D	1	0.158	0.000	0.000	0.158
CULROSS	1	D	2	0.062	0.000	0.014	0.076
CULROSS	1	D	3	0.000	0.000	0.000	0.000
CULROSS	1	D	4	0.000	0.000	0.002	0.002
CULROSS	1	D	5	0.090	0.000	0.004	0.094
CULROSS	1	D	6	0.078	0.002	0.010	0.090
CULROSS	1	D	7	0.374	0.006	0.012	0.392
CULROSS	1	D	8	0.416	0.024	0.010	0.450
CULROSS	1	D	9	0.062	0.028	0.008	0.098
CULROSS	1	D	10	0.090	0.010	0.008	0.108
CULROSS	1	D	11	0.482	0.008	0.006	0.496
CULROSS	1	E	1	0.010	0.000	0.002	0.012
CULROSS	1	E	2	0.122	0.000	0.024	0.146
CULROSS	1	E	3	0.034	0.000	0.000	0.034
CULROSS	1	E	4	0.186	0.002	0.010	0.198
CULROSS	1	E	5	0.148	0.000	0.000	0.148

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SITE	DEPTH STRATUM	STATION	POT NUMBER	SHRIMP WEIGHT (kg) SPOT	PINK	COONSTRIPE	TOTAL
CULROSS	1	E	6	0.016	0.000	0.000	0.016
CULROSS	1	E	7	0.102	0.006	0.006	0.114
CULROSS	1	E	8	0.012	0.004	0.004	0.020
CULROSS	1	E	9	0.000	0.000	0.000	0.000
CULROSS	1	E	10	0.052	0.006	0.030	0.088
CULROSS	1	E	11	0.078	0.008	0.014	0.100
CULROSS	2	A	1	0.014	0.000	0.004	0.018
CULROSS	2	A	2	0.216	0.002	0.004	0.222
CULROSS	2	A	3	0.094	0.026	0.000	0.120
CULROSS	2	A	4	0.080	0.010	0.000	0.090
CULROSS	2	A	5	0.114	0.008	0.030	0.152
CULROSS	2	A	6	0.198	0.000	0.026	0.224
CULROSS	2	A	7	0.368	0.024	0.012	0.404
CULROSS	2	A	8	0.036	0.000	0.026	0.062
CULROSS	2	A	9	0.124	0.008	0.002	0.134
CULROSS	2	A	10	0.351	0.052	0.032	0.435
CULROSS	2	A	11	0.386	0.060	0.056	0.502
CULROSS	2	B	1	0.006	0.000	0.002	0.008
CULROSS	2	B	2	0.124	0.004	0.016	0.144
CULROSS	2	B	3	0.114	0.002	0.002	0.118
CULROSS	2	B	4	0.196	0.012	0.010	0.218
CULROSS	2	B	5	0.158	0.198	0.026	0.382
CULROSS	2	B	6	0.098	0.118	0.032	0.248
CULROSS	2	B	7	0.110	0.014	0.052	0.176
CULROSS	2	B	8	0.008	0.008	0.020	0.036
CULROSS	2	B	9	0.008	0.006	0.022	0.036
CULROSS	2	B	10	0.000	0.022	0.010	0.032
CULROSS	2	B	11	0.044	0.010	0.020	0.074
CULROSS	2	C	1	0.022	0.004	0.004	0.030
CULROSS	2	C	2	0.006	0.016	0.012	0.034
CULROSS	2	C	3	0.000	0.000	0.010	0.010
CULROSS	2	C	4	0.000	0.018	0.024	0.042
CULROSS	2	C	5	0.000	0.000	0.000	0.000
CULROSS	2	C	6	0.018	0.000	0.000	0.018
CULROSS	2	C	7	0.463	0.118	0.062	0.643
CULROSS	2	C	8	0.000	0.060	0.000	0.060
CULROSS	2	C	9	0.016	0.006	0.000	0.022
CULROSS	2	C	10	0.260	0.002	0.000	0.262
CULROSS	2	C	11	0.150	0.006	0.014	0.170
CULROSS	2	D	1	0.130	0.018	0.034	0.182
CULROSS	2	D	2	0.186	0.008	0.000	0.194
CULROSS	2	D	3	0.106	0.004	0.006	0.116
CULROSS	2	D	4	0.190	0.002	0.036	0.228
CULROSS	2	D	5	0.090	0.030	0.062	0.182
CULROSS	2	D	6	0.270	0.034	0.080	0.384
CULROSS	2	D	7	0.362	0.112	0.034	0.508
CULROSS	2	D	8	0.078	0.076	0.058	0.212

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SITE	DEPTH STRATUM	STATION	POT NUMBER	SHRIMP WEIGHT (kg) SPOT	PINK	COONSTRIPE	TOTAL
CULROSS	2	D	9	0.252	0.100	0.094	0.446
CULROSS	2	D	10	0.132	0.132	0.038	0.302
CULROSS	2	D	11	0.174	0.002	0.014	0.190
CULROSS	2	E	1	0.176	0.002	0.014	0.192
CULROSS	2	E	2	0.228	0.010	0.030	0.268
CULROSS	2	E	3	0.210	0.024	0.028	0.262
CULROSS	2	E	4	0.060	0.008	0.024	0.092
CULROSS	2	E	5	0.202	0.010	0.002	0.214
CULROSS	2	E	6	0.244	0.028	0.068	0.340
CULROSS	2	E	7	0.046	0.010	0.056	0.112
CULROSS	2	E	8	0.018	0.006	0.054	0.078
CULROSS	2	E	9	0.088	0.020	0.056	0.164
CULROSS	2	E	10	0.154	0.032	0.032	0.218
CULROSS	2	E	11	0.124	0.000	0.014	0.138
TOTALS			110	13.999	1.652	2.266	17.917
AVERAGE WEIGHT/POT (kg)				0.127	0.015	0.021	0.163
SD				0.126	0.032	0.026	0.149

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SITE	DEPTH STRATUM	STATION	POT NUMBER	SHRIMP WEIGHT (kg) SPOT	PINK	COONSTRIPE	TOTAL
HERRING	1	A	1	0.060	0.000	0.000	0.060
HERRING	1	A	2	0.000	0.002	0.008	0.010
HERRING	1	A	3	0.000	0.002	0.006	0.008
HERRING	1	A	4	0.000	0.002	0.000	0.002
HERRING	1	A	5	0.000	0.002	0.004	0.006
HERRING	1	A	6	0.000	0.004	0.016	0.020
HERRING	1	A	7	0.000	0.010	0.016	0.026
HERRING	1	A	8	0.000	0.006	0.012	0.018
HERRING	1	A	9	0.000	0.000	0.010	0.010
HERRING	1	A	10	0.012	0.068	0.044	0.124
HERRING	1	A	11	0.008	0.004	0.052	0.064
HERRING	1	B	1	0.090	0.140	0.072	0.302
HERRING	1	B	2	0.372	0.134	0.040	0.546
HERRING	1	B	3	0.076	0.054	0.010	0.140
HERRING	1	B	4	0.308	0.098	0.166	0.572
HERRING	1	B	5	0.296	0.104	0.058	0.458
HERRING	1	B	6	0.074	0.090	0.004	0.168
HERRING	1	B	7	0.166	0.056	0.134	0.356
HERRING	1	B	8	0.050	0.212	0.058	0.320
HERRING	1	B	9	0.210	0.228	0.268	0.706
HERRING	1	B	10	0.172	0.358	0.032	0.562
HERRING	1	B	11	0.126	0.236	0.074	0.436
HERRING	1	C	1	0.434	0.074	0.074	0.582
HERRING	1	C	2	0.030	0.020	0.024	0.074
HERRING	1	C	3	0.014	0.058	0.054	0.126
HERRING	1	C	4	0.000	0.010	0.004	0.014
HERRING	1	C	5	0.000	0.006	0.022	0.028
HERRING	1	C	6	0.000	0.044	0.050	0.094
HERRING	1	C	7	0.168	0.152	0.010	0.330
HERRING	1	C	8	0.132	0.240	0.112	0.484
HERRING	1	C	9	0.370	0.226	0.158	0.754
HERRING	1	C	10	0.124	0.220	0.050	0.394
HERRING	1	C	11	0.220	0.042	0.046	0.308
HERRING	1	D	1	0.060	0.000	0.030	0.090
HERRING	1	D	2	0.360	0.100	0.110	0.570
HERRING	1	D	3	0.020	0.004	0.000	0.024
HERRING	1	D	4	0.310	0.060	0.130	0.500
HERRING	1	D	5	0.140	0.030	0.130	0.300
HERRING	1	D	6	0.160	0.100	0.110	0.370
HERRING	1	D	7	0.080	0.030	0.010	0.120
HERRING	1	D	8	0.490	0.090	0.130	0.710
HERRING	1	D	9	0.450	0.040	0.250	0.740
HERRING	1	D	10	0.140	0.100	0.170	0.410
HERRING	1	D	11	0.000	0.010	0.040	0.050
HERRING	1	E	1	0.650	0.020	0.120	0.790
HERRING	1	E	2	0.700	0.010	0.020	0.730
HERRING	1	E	3	1.050	0.020	0.040	1.110
HERRING	1	E	4	0.870	0.004	0.010	0.884

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SITE	DEPTH STRATUM	STATION	POT NUMBER	SHRIMP WEIGHT (kg) SPOT	PINK	COONSTRIPE	TOTAL
HERRING	1	E	5	0.820	0.002	0.010	0.832
HERRING	1	E	6	1.290	0.030	0.170	1.490
HERRING	1	E	7	0.020	0.008	0.030	0.058
HERRING	1	E	8	0.110	0.010	0.030	0.150
HERRING	1	E	9	0.920	0.010	0.050	0.980
HERRING	1	E	10	1.150	0.020	0.100	1.270
HERRING	1	E	11	1.320	0.040	0.110	1.470
HERRING	1	F	1	0.000	0.000	0.000	0.000
HERRING	1	F	2	0.000	0.006	0.000	0.006
HERRING	1	F	3	0.000	0.030	0.050	0.080
HERRING	1	F	4	0.060	0.000	0.040	0.100
HERRING	1	F	5	0.010	0.010	0.020	0.040
HERRING	1	F	6	0.000	0.006	0.005	0.011
HERRING	1	F	7	0.000	0.004	0.020	0.024
HERRING	1	F	8	0.000	0.000	0.000	0.000
HERRING	1	F	9	0.000	0.000	0.006	0.006
HERRING	1	F	10	0.020	0.010	0.030	0.060
HERRING	1	F	11	0.000	0.010	0.000	0.010
HERRING	2	A	1	0.124	0.084	0.024	0.232
HERRING	2	A	2	0.230	0.094	0.058	0.382
HERRING	2	A	3	0.855	0.078	0.048	0.981
HERRING	2	A	4	0.713	0.060	0.102	0.875
HERRING	2	A	5	0.634	0.100	0.036	0.770
HERRING	2	A	6	0.090	0.078	0.020	0.188
HERRING	2	A	7	0.144	0.278	0.034	0.456
HERRING	2	A	8	0.074	0.030	0.012	0.116
HERRING	2	A	9	0.224	0.162	0.018	0.404
HERRING	2	A	10	0.270	0.168	0.014	0.452
HERRING	2	A	11	0.492	0.138	0.032	0.662
HERRING	2	B	1	0.036	0.160	0.000	0.196
HERRING	2	B	2	0.000	0.150	0.000	0.150
HERRING	2	B	3	0.032	0.396	0.016	0.444
HERRING	2	B	4	0.010	0.044	0.000	0.054
HERRING	2	B	5	0.000	0.254	0.006	0.260
HERRING	2	B	6	0.068	0.330	0.006	0.404
HERRING	2	B	7	0.014	0.386	0.006	0.406
HERRING	2	B	8	0.022	0.622	0.028	0.672
HERRING	2	B	9	0.048	0.432	0.012	0.492
HERRING	2	B	10	0.446	0.410	0.160	1.016
HERRING	2	b	11	0.050	0.670	0.008	0.728
HERRING	2	C	1	0.268	0.082	0.000	0.350
HERRING	2	C	2	0.198	0.114	0.010	0.322
HERRING	2	C	3	0.144	0.130	0.022	0.296
HERRING	2	C	4	0.062	0.186	0.030	0.278
HERRING	2	C	5	0.040	0.348	0.018	0.406
HERRING	2	C	6	0.156	0.314	0.000	0.470
HERRING	2	C	7	0.040	0.238	0.000	0.278
HERRING	2	C	8	0.048	0.170	0.000	0.218

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SITE	DEPTH STRATUM	STATION	POT NUMBER	SHRIMP WEIGHT (kg) SPOT	PINK	COONSTRIPE	TOTAL
HERRING	2	C	9	0.020	0.368	0.000	0.388
HERRING	2	C	10	0.020	0.186	0.000	0.206
HERRING	2	C	11	0.020	0.148	0.000	0.168
HERRING	2	D	1	0.050	0.120	0.060	0.230
HERRING	2	D	2	0.060	0.160	0.000	0.220
HERRING	2	D	3	0.130	0.130	0.040	0.300
HERRING	2	D	4	0.110	0.190	0.060	0.360
HERRING	2	D	5	0.070	0.160	0.010	0.240
HERRING	2	D	6	0.090	0.090	0.020	0.200
HERRING	2	D	7	0.120	0.120	0.040	0.280
HERRING	2	D	8	0.690	0.140	0.040	0.870
HERRING	2	D	9	0.360	0.030	0.020	0.410
HERRING	2	D	10	0.190	0.190	0.040	0.420
HERRING	2	D	11	0.290	0.230	0.020	0.540
HERRING	2	E	1	0.190	0.270	0.170	0.630
HERRING	2	E	2	0.090	0.220	0.050	0.360
HERRING	2	E	3	0.150	0.270	0.070	0.490
HERRING	2	E	4	0.032	0.270	0.004	0.306
HERRING	2	E	5	0.360	0.100	0.040	0.500
HERRING	2	E	6	0.110	0.170	0.030	0.310
HERRING	2	E	7	0.250	0.190	0.030	0.470
HERRING	2	E	8	0.090	0.150	0.130	0.370
HERRING	2	E	9	0.260	0.120	0.030	0.410
HERRING	2	E	10	0.840	0.080	0.100	1.020
HERRING	2	E	11	0.300	0.110	0.040	0.450
HERRING	2	F	1	0.130	0.020	0.010	0.160
HERRING	2	F	2	0.010	0.050	0.010	0.070
HERRING	2	F	3	0.400	0.070	0.010	0.480
HERRING	2	F	4	0.720	0.190	0.060	0.970
HERRING	2	F	5	0.000	0.140	0.050	0.190
HERRING	2	F	6	0.620	0.320	0.080	1.020
HERRING	2	F	7	0.000	0.000	0.000	0.000
HERRING	2	F	8	0.850	0.430	0.050	1.330
HERRING	2	F	9	0.700	0.280	0.070	1.050
HERRING	2	F	10	0.470	0.110	0.020	0.600
HERRING	2	F	11	0.110	0.120	0.000	0.230
TOTALS			132	29.146	16.364	5.783	51.293
AVERAGE WEIGHT/POT (kg)				0.221	0.124	0.044	0.389
SD				0.292	0.128	0.051	0.336

–continued–

SITE	DEPTH STRATUM	STATION	POT NUMBER	SHRIMP WEIGHT (kg) SPOT	PINK	COONSTRIPE	TOTAL
CHENEGA	1	A	1	0.430	0.004	0.080	0.514
CHENEGA	1	A	2	0.410	0.000	0.030	0.440
CHENEGA	1	A	3	1.530	0.000	0.080	1.610
CHENEGA	1	A	4	0.385	0.000	0.030	0.415
CHENEGA	1	A	5	0.670	0.004	0.020	0.694
CHENEGA	1	A	6	0.450	0.010	0.080	0.540
CHENEGA	1	A	7	0.160	0.000	0.010	0.170
CHENEGA	1	A	8	0.170	0.010	0.040	0.220
CHENEGA	1	A	9	0.060	0.030	0.070	0.160
CHENEGA	1	A	10	0.090	0.050	0.030	0.170
CHENEGA	1	A	11	0.200	0.030	0.070	0.300
CHENEGA	1	B	1	1.325	0.000	0.420	1.745
CHENEGA	1	B	2	1.615	0.000	0.060	1.675
CHENEGA	1	B	3	0.040	0.000	0.002	0.042
CHENEGA	1	B	4	0.520	0.002	0.050	0.572
CHENEGA	1	B	5	0.590	0.000	0.040	0.630
CHENEGA	1	B	6	0.320	0.006	0.070	0.396
CHENEGA	1	B	7	0.430	0.010	0.120	0.560
CHENEGA	1	B	8	0.410	0.000	0.020	0.430
CHENEGA	1	B	9	0.490	0.060	0.040	0.590
CHENEGA	1	B	10	0.410	0.040	0.060	0.510
CHENEGA	1	B	11	0.860	0.008	0.130	0.998
CHENEGA	1	C	1	0.310	0.000	0.020	0.330
CHENEGA	1	C	2	0.520	0.000	0.000	0.520
CHENEGA	1	C	3	0.290	0.000	0.000	0.290
CHENEGA	1	C	4	0.020	0.020	0.010	0.050
CHENEGA	1	C	5	0.220	0.010	0.220	0.450
CHENEGA	1	C	6	0.170	0.020	0.140	0.330
CHENEGA	1	C	7	0.310	0.000	0.050	0.360
CHENEGA	1	C	8	0.030	0.010	0.010	0.050
CHENEGA	1	C	9	0.200	0.060	0.030	0.290
CHENEGA	1	C	10	0.470	0.070	0.020	0.560
CHENEGA	1	C	11	0.020	0.020	0.090	0.130
CHENEGA	2	A	1	0.076	0.000	0.000	0.076
CHENEGA	2	A	2	0.488	0.030	0.036	0.554
CHENEGA	2	A	3	0.120	0.030	0.010	0.160
CHENEGA	2	A	4	0.350	0.100	0.090	0.540
CHENEGA	2	A	5	0.150	0.050	0.020	0.220
CHENEGA	2	A	6	0.240	0.010	0.010	0.260
CHENEGA	2	A	7	0.880	0.030	0.002	0.912
CHENEGA	2	A	8	0.770	0.030	0.030	0.830
CHENEGA	2	A	9	0.180	0.070	0.010	0.260
CHENEGA	2	A	10	0.380	0.010	0.130	0.520
CHENEGA	2	A	11	0.500	0.002	0.070	0.572
CHENEGA	2	B	1	0.230	0.070	0.020	0.320
CHENEGA	2	B	2	0.145	0.120	0.030	0.295
CHENEGA	2	B	3	0.550	0.150	0.090	0.790
CHENEGA	2	B	4	0.220	0.640	0.030	0.890

–continued–

SITE	DEPTH STRATUM	STATION	POT NUMBER	SHRIMP WEIGHT (kg) SPOT	PINK	COONSTRIPE	TOTAL
CHENEGA	2	B	5	0.500	0.460	0.070	1.030
CHENEGA	2	B	6	0.350	0.180	0.040	0.570
CHENEGA	2	B	7	0.430	0.380	0.010	0.820
CHENEGA	2	B	8	0.110	0.110	0.040	0.260
CHENEGA	2	B	9	0.310	0.050	0.030	0.390
CHENEGA	2	B	10	0.360	0.150	0.020	0.530
CHENEGA	2	B	11	0.630	0.090	0.030	0.750
CHENEGA	2	C	1	0.590	0.010	0.020	0.620
CHENEGA	2	C	2	0.290	0.060	0.004	0.354
CHENEGA	2	C	3	0.805	0.010	0.010	0.825
CHENEGA	2	C	4	0.530	0.050	0.010	0.590
CHENEGA	2	C	5	0.210	0.030	0.010	0.250
CHENEGA	2	C	6	0.050	0.020	0.000	0.070
CHENEGA	2	C	7	0.550	0.210	0.010	0.770
CHENEGA	2	C	8	0.535	0.160	0.010	0.705
CHENEGA	2	C	9	0.240	0.140	0.020	0.400
CHENEGA	2	C	10	0.200	0.040	0.010	0.250
CHENEGA	2	C	11	0.280	0.100	0.000	0.380
TOTALS			66	26.374	4.066	3.064	33.504
AVERAGE WEIGHT/POT (kg)				0.400	0.062	0.046	0.508
SD				0.318	0.110	0.062	0.353

–continued–

SITE	DEPTH STRATUM	STATION	POT NUMBER	SHRIMP WEIGHT (kg) SPOT	PINK	COONSTRIPE	TOTAL
GREEN	1	A	1	0.000	0.000	0.000	0.000
GREEN	1	A	2	0.000	0.010	0.000	0.010
GREEN	1	A	3	0.012	0.082	0.034	0.128
GREEN	1	A	4	0.000	0.000	0.002	0.002
GREEN	1	A	5	0.012	0.038	0.024	0.074
GREEN	1	A	6	0.000	0.240	0.008	0.248
GREEN	1	A	7	0.100	0.098	0.004	0.202
GREEN	1	A	8	0.050	0.112	0.022	0.184
GREEN	1	A	9	0.060	0.108	0.006	0.174
GREEN	1	A	10	0.135	0.068	0.000	0.203
GREEN	1	A	11	0.135	0.070	0.000	0.205
GREEN	1	B	1	0.008	0.008	0.008	0.024
GREEN	1	B	2	0.000	0.044	0.012	0.056
GREEN	1	B	3	0.000	0.014	0.004	0.018
GREEN	1	B	4	0.006	0.022	0.020	0.048
GREEN	1	B	5	0.000	0.024	0.000	0.024
GREEN	1	B	6	0.020	0.120	0.008	0.148
GREEN	1	B	7	0.000	0.000	0.000	0.000
GREEN	1	B	8	0.000	0.000	0.000	0.000
GREEN	1	B	9	0.000	0.020	0.000	0.020
GREEN	1	B	10	0.040	0.144	0.000	0.184
GREEN	1	B	11	0.020	0.006	0.002	0.028
GREEN	1	C	1	0.002	0.002	0.004	0.008
GREEN	1	C	2	0.012	0.026	0.014	0.052
GREEN	1	C	3	0.000	0.008	0.026	0.034
GREEN	1	C	4	0.042	0.022	0.022	0.086
GREEN	1	C	5	0.044	0.168	0.024	0.236
GREEN	1	C	6	0.000	0.074	0.000	0.074
GREEN	1	C	7	0.016	0.054	0.006	0.076
GREEN	1	C	8	0.048	0.192	0.012	0.252
GREEN	1	C	9	0.000	0.024	0.000	0.024
GREEN	1	C	10	0.000	0.042	0.000	0.042
GREEN	1	C	11	0.036	0.196	0.006	0.238
TOTALS			33	0.798	2.036	0.268	3.102
AVERAGE WEIGHT/POT (kg)				0.024	0.062	0.008	0.094
SD				0.037	0.066	0.010	0.087

[a] 1 = shallow (35 – 130 m); 2 = deep (130 – 220 m)

APPENDIX B

HISTOLOGICAL STUDY

OF SPOT SHRIMP AND PINK SHRIMP

Department of Veterinary Science

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October 16, 1991

Dr. Joe Sullivan
Department of Fish and Game
333 Raspberry Road
Anchorage, AK 99518-1599

Dear Dr. Sullivan: Re: UAZ Case 91-52

We have completed initial histological study of the high priority subsamples of specimens of Pandalus platyceros and P. borealis that were received here on May 6, 1991. As we discussed by telephone on October 14th, this letter will constitute a status report on the samples processed and examined to date, and not a final report. The preliminary histological work done to date encompassed only the preparation and examination of "routine" sections stained with H&E. Special stains will be required on sections of a few of the specimens to determine the nature of some unique lesions/parasites with which we have no prior experience. Likewise, photomicrographs that have been taken to date (that illustrate "normal" shrimp tissue and the lesions or parasites encountered) have not yet been processed.

Also as we discussed on October 14th, the samples processed to date were preserved in 10% buffered formalin rather than Davidson's fixative, or other fixatives containing acetic acid (AFA, Bouin's, etc.), which in my experience are far more suitable for the juvenile and adult stages of decapod crustacea than is formalin. Formalin in crustacea causes marked shrinkage and hardening of shrimp tissues, it penetrates slowly, and, therefore, in larger shrimp autolysis results even when special care is taken to insure proper fixation. Furthermore, because the specimens had been preserved with formalin, decalcification in Davidson's fixative (a procedure we have found to result in less tissue damage and subsequent processing difficulties than when formic acid is used) was necessary before histological processing could be initiated.

Rita Redman, our histotechnologist, documented her observations and photographed subsamples of 5 representative shrimp from each sample group as she unpacked the specimens for histological processing. Her observations made while unpacking the samples and during subsequent processing of the shrimp are given in Tables 1 and 2. Table 3 provides a description of the lesions and other characteristics of the 5 shrimp shown in each of the accompanying transparencies (sent by Federal Express earlier today). According to her notes in Table 2 (and to the

obvious incisions in the cuticles of the abdomens and gnathothoracies of many of the shrimp in the photographs) the abdominal cuticles of the larger shrimp were opened to enhance fixative penetration. The cuticles of many of the smaller shrimp were not opened, except in samples 91-52K (NSS011) and 91-52L (NSS012) which were opened.

Our histological observations are summarized in Table 4. Histological examination of the sections prepared from each shrimp processed showed uniform problems with fixation. In general, the tissues of these shrimp were hard, brittle and difficult to section. Autolysis of some organs and tissues (especially the ventral nerve cord and ganglia, the central region of the hepatopancreas, and the anterior midgut) was uniformly present in the majority of the specimens. Such autolytic changes, however, did not preclude histological detection of several lesions, epicommensal, and parasitic microorganisms. In tissues or organs in which we found at least some histopathology (as indicated by necrosis and inflammation), the presence of inflammatory cells clearly distinguished the tissue changes observed in such lesions from autolytic changes.

Also present on nearly every specimen of all sample groups processed were low to moderate numbers of a presumed loricate protozoan. These were commonly present on the cuticle in recessed or highly folded areas such as on the gill lamellae where they originate from the primary gill rachus, and on the cuticle of various appendages. While we have not attempted to classify this protozoan, we presume (based on its morphology) that it may be a species of Lagenophrys. When abundant in foci on the gills, these protozoans evoked a slight to moderate inflammatory response as indicated by the presence of hemocyte congestion of the parasitized lamellae.

On the gills of only the P. borealis specimens, a metazoan epicommensal was present. As no significant host response accompanied this organism, it appeared to have little direct adverse effects on the affected shrimp.

One specimen of P. borealis showed a remarkably heavy systemic infection by an amoeboid protozoan. This organism occurred singly as multinucleated syncytia throughout the hemocoel and loose connective tissues of the affected individual. Equally remarkable was the absence of a host inflammatory response to the parasite and the presence of ingested material in the stomach of the affected shrimp. (By comparison, penaeid shrimp with severe parasitism normally do not feed).

Melanized cuticular lesions that were noted grossly (Tables 1 and 3) were also sectioned and examined histologically. These lesions were found by histology, in some examples, to be either wounds or classical examples of bacterial shell disease. Their nature and prevalence in the samples is indicated in Tables 1, 3 and 4.

A number of specimens in many of the samples showed the presence of reserve cells with dense eosinophilic cytoplasms and pyknotic and/or karyorrhectic nuclei. Such cells were most often present in the subcuticular connective tissues and in the heart of these shrimp among the heart muscle fibers (the LC cells listed in Table 4). Because no inflammatory response accompanied their presence, even when abundant, I doubt that they are pathologic.

Also of interest was the total absence of males in the samples of P. platyceros. We noted no males during our unpacking and gross examination of these shrimp, nor were any detected by histology. All the P. platyceros appeared to be functional females. In contrast, the P. borealis sample contained a number of hermaphrodites which possessed both testis containing mature sperm and ovaries with developing ova. One individual had both mature testis and ovaries and recently set brooding eggs on its pleopods.

I hope that our preliminary observations and report will be helpful to you. While we provided only with code numbers to identify the specimens and not with information as to where sample sets were collected, the higher prevalence of gill lesions in samples 91-52J (NSS010), 91-52K (NSS011), and 91-52L (NSS012) suggest exposure of these shrimp to some sort of toxic material.

A separate invoice, with time and effort figures tabulated, will follow with a copy of this report via regular mail.

Best regards,

Donald V. Lightner

Donald V. Lightner, Ph.D.
Associate Professor

Department of Veterinary Science

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October 18, 1991

Dr. Joe Sullivan
Department of Fish and Game
333 Raspberry Road
Anchorage, AK 99518-1599

Dear Dr. Sullivan:

While proof-reading the UAZ Case 91-52 report dated October 16, 1991, I noticed two typographical errors. These are:

1. Page 3, paragraph 3: Second sentence should read "While we were provided..."

2. Page 3, paragraph 3, line 5: should read "...in samples 91-52J (NSS010)..."

Dr. Lightner is out of town and unavailable to sign a corrected page 3. Therefore, I am providing you with this addendum.

Sincerely,

Leslie S. Jones
Secretary

Witnessed: Thomas A. Bell, Assistant Staff Scientist

Table 1. Summary of Alaskan Shrimp Examination.

Bottle	Bottle No.	Total No. Shrimp Received	Females/eggs	Normal	Shell Disease Melanization	WEIGHT Smallest	WEIGHT Average	WEIGHT Largest	Total Shrimp sectioned
G	NSS 007	20	1	12	8	5.94 g.	13.10 g.	25.15 g.	10
H	NSS 008	20	0	14	6	5.05 g.	12.96 g.	23.55 g.	10
I	NSS 009	20	0	13	7	5.88 g.	9.38 g.	15.95 g.	10
J	NSS 010	20	0	13	7	4.92 g.	8.13 g.	12.91 g.	10
K	NSS 011	20	0	15	5	6.63 g.	8.98 g.	11.48 g.	10
L	NSS 012	19	6	19	0	1.59 g.	2.75 g.	7.85 g.	10

91-52; Oct. 16, 1991; Page 4

Table 2. Shrimp received from Alaska

1. Were post-fixed in Davidson's Fixative for 48 hours prior to dissection to decalcify the cuticle.

2. Were counted, weighed, and grossly examined for lesions.

3. Were photographed using a representative group of @ 5 shrimp for each sample group.

4. Under 12 grams did not have the cuticles opened nor was there evidence of any injection points. The exceptions were bottles K (NSS 011) and L (NSS 012). These animals were all under 12 grams but the cuticles were opened and the tails had been cut.

5. Over 12 grams had the carapaces opened and cut along the length of the tail. No injection points were noted.

6. All seemed to have full stomachs and the regurgitated contents were evident in the mouth region and around the gills. Some of the photographs show this.

Table 3. Summary of Photographs. Discussion of shrimp clockwise starting center middle or upper right side.

NSS 007 - 5 shrimp

1. Female carrying eggs

2. Has melanized shell disease lesions fringing the ventral edge of the pleural plates of the 4th and 5th abdominal segments.

3. Appears normal.

4. Has a large melanized lesion on the dorsal aspect of the 3rd abdominal segment - probably a contusion from a tail flip response.

5. Same as shrimp number 4.

NSS 008 - 5 shrimp

1. Broken rostrum possibly due to handling. Has multifocal shell disease lesions on ventral and dorsal aspects of the 6th abdominal segment.

2. Has a single focal shell disease lesion on the medial lateral aspect of the anterior posterior junction of the 2nd and 3rd abdominal segments.

3. Appears normal.

4. Appears normal.

5. Has small multifocal melanized shell disease lesions on the lateral aspect of the 3rd abdominal segment, on the trailing edge of the 4th abdominal segment, and on the pleopods.

NSS 009 - 4 shrimp

1. Appears normal.

2. Appears normal.

3. Has a single focal melanized lesion on the rostrum.

4. Broken rostrum. Has a melanized shell disease lesion on the dorsal lateral aspect of the 1st abdominal segment.

NSS 010 - 5 shrimp

1. Has multiple petechial melanized shell disease lesions on abdominal segments and appendages.
2. Appears normal.
3. Appears normal.
4. Same as shrimp number 1.
5. Appears normal.

NSS 011 - 5 shrimp

1. Has a prominent melanized lesion on the base coxa of the 3rd pereiopod and a focal melanized lesion on the lateral aspect of the 4th abdominal segment.
2. Has a melanized shell disease lesion on the lateral aspect of the 4th abdominal segment.
3. Broken rostrum (processing).
4. Appears normal.
5. Has a focal melanized shell disease lesion on the right inner uropod dorsal surface.

NSS 012 - 5 shrimp

1. Appears normal.
2. Appears normal.
3. Appears normal.
4. Appears normal.
5. Appears normal.

Table 4. Summary of histological observations on samples of Pandalus platyceros and P. borealis from the OSIAR Shellfish Project (UAZ case study number 91-52).

UAZ ID No.	Alaska ID No.	No.Sex/ Stage	Gills No. with/G Lagen.	Infm. Les.	Cuticle SD/Wounds	Heart	Detailed explanation
52G/1-10	NSS007	7F@G1-2 3F>G2 1 berried[2]	6@G0 2@G1 2@G2	8@G0 2@G1MFHEN[1]	1[3] / 1	4 w/>G2LC	[1]MFHEN affect lamellae only. [2]Embryos developing normally, but pre-eye pigment formation. [3]SD appears to be classic bacterial SD, not fungal; the second shrimp's lesion appears to be a normally resolving wound. Other: HPs of all 10 contain G2-3 lipid storage in R-cells, good B-cell activity. HP with low grade gregarine parasitism of some proximal tubules.
52H/1-10	NSS008	5F@G1-2 5F@G2-3	2@G0 3@G1 4@G2 1@G2-3	N/S	N/S	2 w/G1LC 1 w/G3LC	Other: H/3 shows algae cells in SC and epidermis of a branchiostegate gill process (a dinoflagellate?); algae cells are single and in clusters; no host inflammatory response. HP of H/1 shows possible low grade chlamydia/rickettsia infection. HPs of all 10 contain G2-3 lipid storage in R-cells, good B-cell activity.
52I/1-10	NS009	9F@G1 1F@G3	1@G0 6@G1 3@G2	1@G1FHCong	2[4]	2w/G1LC 2w/G2LC	[4]Melz SD lesions; bacterial, not fungal; cuticle eroded and perforated in some lesions; host inflammatory response resolving each lesion sectioned. Other: HPs of all 10 contain G2-3 lipid storage in R-cells, good B-cell activity.
52J/1-10	NS010	4F<G1-2 6F@G2	1@G0 3@G1 6@G2	1 normal 3@G1[5] 3@G2[5] 3@G3[5]	1? 1?[6]	1w/G1LC	[5]Gill lesions in group are significant. Lesions range from multifocal necrosis, inflammation and melanization of areas in gill lamellae to marked hemocytic congestion and fibrosis of the hemocoel within the primary gill rachis of one or more gill processes in some of the specimens. [6]SD lesion looks more like a resolving puncture wound than bacterial SD. Other: HPs of all 10 contain G1-3 lipid storage in R-cells, good B-cells, and most stomaches contain ingested food.

STATE/FEDERAL RESOURCE DAMAGE ASSESSMENT
DATA SUMMARY REPORT

Project Title:	Injury to Demersal Rockfish and Shallow Reef Habitats in Prince William Sound
Study ID Number:	Subtidal Study Number 6 (Fish/Shellfish Study Number 17)
Lead Agency:	State of Alaska, ADF&G; Sport Fish Division
Principal Investigator:	Andrew Hoffmann, Fishery Biologist
Assisting Personnel:	Kelly Hepler, Fishery Biologist Patricia Hansen, Biometrician
Date Submitted:	November 20, 1991

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

EXECUTIVE SUMMARY

Populations of demersal rockfish and shallow reef habitats in Prince William Sound were studied in 1991 as a continuation of a study to assess potential injury due to the Exxon Valdez oil spill (EVOS). Injury in the form of continued exposure to hydrocarbons and sublethal effects of exposure was evaluated. Sampling was also done to determine potential routes of exposure. The sublethal effects were measured by determining the presence or absence of sublethal levels of hydrocarbons and histopathological evidence in rockfish that inhabit reefs located in oiled and control sites in the study area. Samples of bile, liver, kidney, and muscle were collected from ten fish per sampling location for hydrocarbon analysis. Samples of liver, spleen, kidney, heart, and gill were collected from 30 fish per site for histopathological examination. Routes of potential exposure were evaluated by collection of sediment samples, benthic invertebrates, and stomach contents for hydrocarbon analysis. All fish caught were measured to the nearest millimeter and both sagittal otoliths were obtained for aging.

Oil spilled from the Exxon Valdez was the probable cause of death for demersal rockfish killed in Prince William Sound immediately after the spill. Of the 20 dead rockfish brought to the collection centers in Valdez and Cordova from sites of reported fish kills, tissues of 15 were too decomposed for accurate analysis. The remaining five were sampled and crude oil was found to be the cause of death. Of these 20 rockfish, all were demersal rockfish, 18 of which were yelloweye rockfish. Results of the 1989 studies showed that at least eleven fish of thirty-six examined from treatment sites had been exposed to oil within the two weeks prior to collection, no fish in control sites were exposed to oil. These two pieces of information prompted the 1990 and 1991 studies to sample rockfish for continued exposure to sublethal levels of hydrocarbons and to try and determine potential routes of exposure. Results from the histopathologic evaluation of livers collected from rockfish in PWS in 1990 indicated that rockfish were exposed to toxic agents. There were differences between control and treatment sites in PWS in two of the four liver lesion scores (liver lipidosis and liver sinusoidal fibrosis), however, no differences in lesion scores were seen between sites on the outer Kenai Peninsula. Results of hydrocarbon analysis for 1990 and 1991 and histopathological analysis for 1991 are still pending.

Recommendations for restoration efforts for rockfish are directed toward support of the new restoration science study proposal for rockfish entitled "Development of a Restoration Plan for Groundfish Stocks Affected by the Exxon Valdez Oil Spill". The goal of this new project is to develop a restoration plan that modifies human use to facilitate the enhancement of groundfish resources impacted by EVOS. Initial objectives of this study are to: 1) identify the species of concern, 2) describe the biological characteristics of the species or stocks of concern, 3) identify and define stocks to be enhanced through modification of human use, and 4) describe the current and past patterns of human use of the groundfish resource.

OBJECTIVES

1. Determine the presence or absence of hydrocarbons in demersal rockfish, stomach contents, benthic suspension feeders, and sediments from two control and two oiled sites in Prince William Sound.

2. Determine the physiological effects resulting from oil contamination through histopathological examination of six organs, and mixed function oxidase enzyme system activity in liver tissue.

3. Determine the feasibility of using microstructure of otoliths from juvenile rockfish to evaluate depressed growth as a result of oil contamination.

4. Identify potential alternative methods and strategies for restoration of lost use, populations, or habitat where injury is identified (to be accomplished upon completion of the project).

INTRODUCTION

This report constitutes a status summary of activities related to evaluating the effects of the Exxon Valdez oil spill on demersal rockfish and shallow reef habitats in Prince William Sound (PWS) following the third year of study. Studies during 1989, the year of the spill, documented lethal and sub-lethal effects. Lethal effects were shown by dead fish collected immediately after the spill which exhibited pathological evidence characteristic of exposure to hydrocarbons; and sub-lethal effects were documented by elevated levels of hydrocarbons in the bile of fish collected six weeks after the spill (Hepler et al 1989). Studies during the second year were expanded to better document sub-lethal effects through histopathological evaluation and to determine the route of contamination by looking at stomach contents, sediments, and sessile invertebrates (Hepler et al 1990). Results are not completed yet regarding the 1990 hydrocarbon analysis, however results from the histopathological evaluations show significant sub-lethal effects of rockfish in PWS present a year after the spill. Samples collected during 1991 will be able to document if these sub-lethal effects are persistent into the third year. Additional analyses are still pending from 1989 and 1990 hydrocarbon samples. As the results of these analyses become available a better picture of the extent and persistence of sub-lethal effects as well effects in the shallow reef habitats will emerge.

METHODS

Site Selection

The locations visited in PWS during 1991 were the same as those visited in 1990. No samples were collected along the lower Kenai Peninsula in 1991 based on recommendations of the peer reviewers. The locations of the study sites are identified in Table 1 and Figure 1. The initial criteria for choosing sample sites were based on: (1) accessibility to boat and diving operations (depth surrounding the reefs 20 fathoms or less); (2) documented exposure or lack of exposure of surface waters to oil; (3) location of reported kills and/or sublethal contamination of demersal rockfish; (4) occurrence of sampling by other oil spill assessment studies relative to this study; and (5) previous study site during 1989 or during a study of shallow water fish assemblages conducted by Rosenthal in 1980.

Sampling techniques

Four sites (two oiled and two control) in PWS were sampled in 1991. Tissues from three of the most common species of demersal rockfish, yelloweye *Sebastes rubberimus*, quillback *S. maliger*, and copper *S. caurinus*, were collected for histopathological evaluation. Rockfish tissues, stomach contents, unconsolidated benthic sediments, and sessile suspension feeders were collected at each study site for analysis of hydrocarbons. Sampling was conducted during late July through early September, the period of peak abundance described by Rosenthal (1980).

The rockfish were collected using either rod and reel jigging techniques or by SCUBA divers using spears depending upon the water conditions and relative success of each technique. Baited and unbaited lures were lowered to the substrate and raised enough to allow for adequate jigging action. When a fish was on the line it was retrieved slowly to allow the air bladder to equilibrate in order to reduce the occurrence of stomach extrusion and regurgitation of its contents. When hook and line techniques did not yield results, divers tried to collect additional rockfish using spears. Thirty adult demersal rockfish were collected at each sample site. This sample size was increased from the 15 collected in 1990 to 30 in 1991 in order to increase the power of the statistical tests. Species identification of adult rockfish was verified using the methods of Kramer and O'Connell (1988) and Hart (1973).

Juvenile rockfish were collected using the same capture techniques as used for adult rockfish. A goal of fifty juvenile demersal rockfish was set for each site, this was determined (Zar 1984) given estimated proportions of otoliths with and without stress checks of 0.6 and 0.2, where $\alpha = 0.05$. Species identification of juveniles was accomplished using methods of Matarese et al. (1989).

Table 1. Locations of study sites sampled in Prince William Sound, 1991.

Study Sites	Latitude / Longitude	
Control Sites		
Gravina Rocks	60° 39' 25"N	146° 16' 36"W
Zaikof Point	60° 18' 07"N	146° 54' 25"W
Oiled Sites		
Herring Bay	60° 27' 00"N	147° 45' 00"W
Danger Island	59° 55' 30"N	148° 04' 12"W

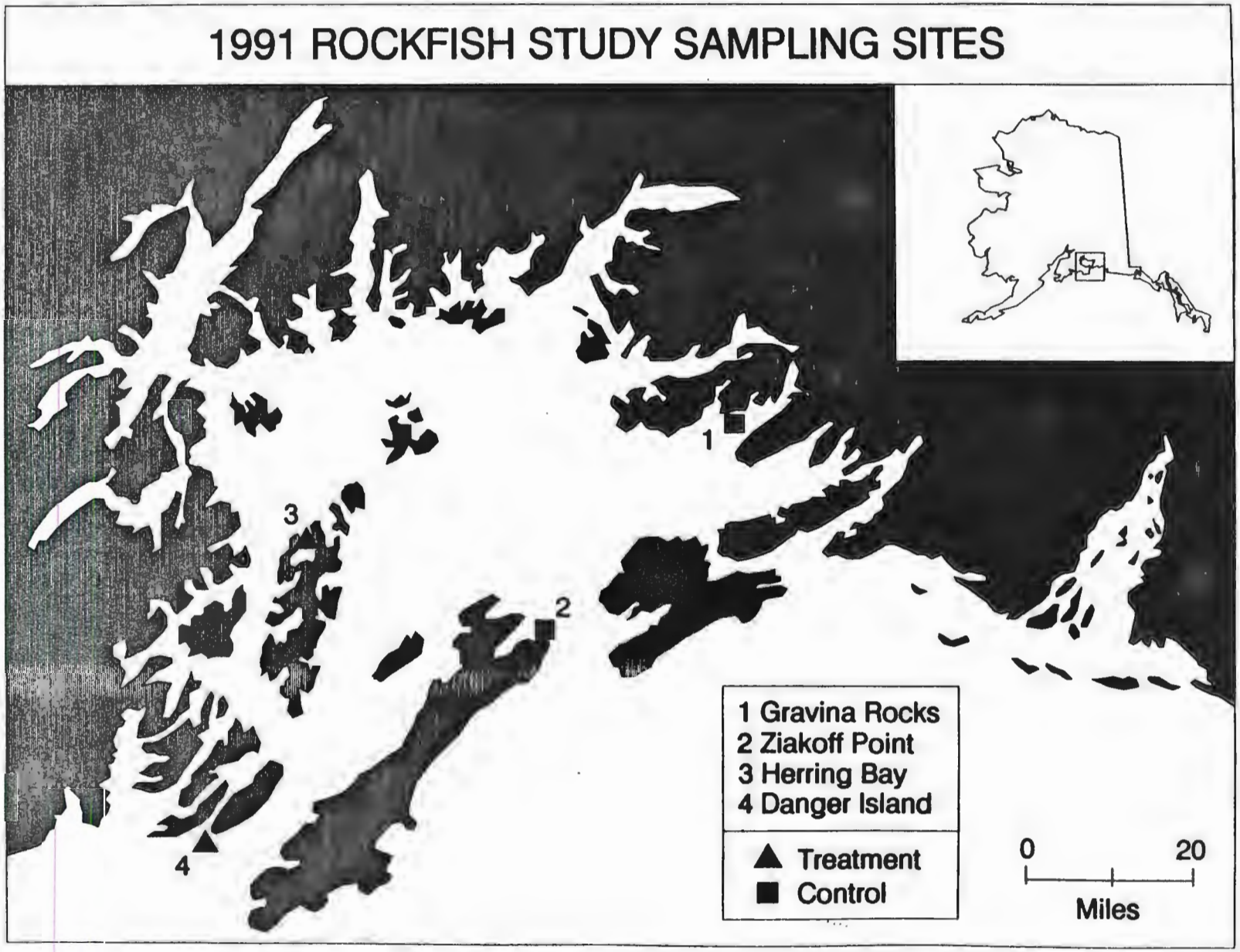

Figure 1. Map of study area and sampling locations.

Nine sediment samples (Rice personal communication 1990) were collected for hydrocarbon analysis at each study site by SCUBA divers. Divers filled hydrocarbon-free glass jars with water just below the surface, and took grab samples of sediment within the reef area where fish and invertebrates were collected. At the surface the sediments were allowed to settle and excess water was decanted before freezing. Each sample consisted of at least ten milliliters taken from the top 2 cm of the substrate.

Three samples of sessile filter feeders (Rice, 1990) were collected from each reef by SCUBA divers for hydrocarbon analysis. These samples were collected using an air-lift sampler designed by Chess (1978 and Chess personal communication 1990). The air-lift sampler uses suction to collect organisms and deposits them into a container. Substrates were swept using the air-lift sampler and samples were brought to the surface where they were sorted with utensils rinsed in methylene chloride. Species of interest were put in hydrocarbon-free jars and frozen. The sessile invertebrates selected were those species that were common to a majority of the reefs. These included tubeworms *Crucigera sp.*, bryazoans, primarily *Microporina boralis*, compound ascisians, anemones *Metridium sp.* and muscles *Musculus sp.*.

All samples collected were handled in the prescribed chain-of-custody procedures as outlined by National Marine Fisheries Service (NMFS) Auke Bay Laboratory. Upon sampling, jars were sealed with evidence tape, signed and dated by the sampler, labeled with location, date, species, and tissue type, and given a project identification number. Jars were placed in a box and sealed with evidence tape. Chain-of-Custody records were completed at the time of sampling and transported with the samples.

There were two deviations from the operational plan: 1) during the peer review of the operational plan it was recommended that the locations along the outer Kenai Peninsula be dropped and that logistics be combined with the sub-tidal project #2; and 2) sample size of rockfish for histopathological examination was doubled in order to improve the power of the statistical tests.

Hydrocarbon Procedures:

Samples for hydrocarbon analysis were collected in accordance to procedures established by the NMFS, Analytical Chemistry Group (Manen 1989) as presented in the Natural Resource Damage Assessment sample collection training sessions held in May of 1990. The following procedures were used for each sample: (1) hands and sampling gear were washed with soap and water; (2) dissection tools were rinsed in methylene chloride; (3) samples of each tissue or sediment were individually stored in certified hydrocarbon-free sampling jars; and (4) samples were frozen immediately. Samples were not touched nor was there any contact with any petrochemical product (e.g. plastic). These samples were transferred to the NMFS

Auke Bay Laboratory for analysis following prescribed chain-of-custody procedures (Manen 1989).

Ten of the thirty rockfish collected were used for hydrocarbon analysis (Rice 1990). Bile samples were collected first by removing the whole gall bladder and emptying the bile into 0.5 oz. amber sampling jars. Approximately ten grams each of liver, muscle, and gonad tissue and stomach contents were then collected from each rockfish. All tissue samples were collected from freshly killed fish and each tissue type was stored in separate 4 oz. sampling jars and frozen immediately.

The proportion of oiled sites containing contaminated samples will be compared to the proportion of control sites with contaminated samples using a two-sampled Z-test from Zar (1984):

$$Z = \frac{\hat{p}_c - \hat{p}_t}{\sqrt{\bar{p}\bar{q}\left(\frac{1}{N_c} + \frac{1}{N_t}\right)}}$$

where:

P_c = proportion of control samples contaminated

P_t = proportion in oiled samples contaminated

N_c = number of control samples

N_t = number of oiled samples

and:

$$\bar{p} = \frac{(N_c\hat{p}_c + N_t\hat{p}_t)}{(N_t + N_c)}$$

This formulation of the test is robust, especially for small sample sizes.

Histopathology Procedures:

Tissue samples were taken from all 30 rockfish collected at each reef for histopathological analysis and processed under the guidelines outlined by the Histopathology Technical Group (Meyers 1989). One centimeter sections of tissue were removed from the following organs: liver, spleen, anterior kidney, heart, and gills. Samples were stored in 10% buffered formalin and transferred to the University of California Davis, School of Veterinarian Medicine (UCD) for examination. All tissues are to be examined for

histological evidence of exposure to hydrocarbons. In addition, the mixed function oxidase enzyme system (MFO) activity in the liver tissue will be measured. Samples of embryos were taken from all ripe females that were captured. Embryos were also preserved in 10% buffered formalin and sent to UCD for examination.

Rockfish tissue samples collected in 1990 were examined for histopathological abnormalities at UCD. In the final summary report from UCD, lesions were scored as none (0), mild (1), moderate (2), or severe (3) in relation to other similar lesions. A general linear models procedure showed that scores exhibited homogeneity of variance and were appropriate for analysis of variance. An ANOVA with a nested treatment arrangement was used to test for the effects of oil. The model used was:

$$\text{score} = \mu + \text{treatment} + \text{site(treatment)} + \text{fish(site,treatment)}$$

Age, Length, Weight Procedures:

All fish caught were measured (fork length), weighed, and following dissection, both sagittal otoliths were removed and stored dry in coin envelopes labeled with location, species, date, length, weight, and a project identification number. Length and weight were used to calculate a relative condition factor for all rockfish captured. Otoliths from adult rockfish will be aged using the break and burn procedures described by Chilton and Bemish (1982) and the Pacific coast groundfish aging technicians (1984), and viewed under transmitted light with a compound microscope at 400X magnification.

Age composition and mean length-at-age will be calculated for each species of rockfish. Letting p_h equal the estimated proportion of age group h in the population:

$$V(\hat{p}_h) = \frac{\hat{p}_h(1-\hat{p}_h)}{n-1}$$

where n is the sample size.

LeCren's relative condition factor (Kn) (Anderson and Gutreuter 1983) was calculated for each adult and juvenile rockfish where:

$$K_n = \frac{Weight}{a\ Length^b}$$

The value of a and b are the intercept and slope respectively of the regression of the log of the weight on the log of length. The mean condition factor for adult rockfish for each site was calculated and differences between control and oiled groups was compared using a t-test.

Juvenile otoliths will be prepared for examination following methods outlined by Boehlert and Yoklavich (1987). Otoliths will be examined with a scanning electron microscope. Presence and location of hyaline zones comprising annuli, daily growth increments, and checks resulting from physiological factors including a reduction in growth will be examined. The feasibility of distinguishing differences in the type of hyaline zones will be explored by measuring the width of growth zones deposited over consecutive periods of time (days and years). Where physiological checks are clearly discernible from annuli, the presence of checks will be determined with respect to annuli. Checks deposited within the growth zone of the previous year will be noted. The proportion of otoliths containing checks within this growth zone will be compared between control and oiled groups.

Proportion of otoliths containing checks between the last two annuli will be compared between control and oiled groups using the Z-test from Zar (1984) described previously.

STUDY RESULTS

Objectives 1 and 2

Tables 2 and 3 summarize the number and species of rockfish collected for hydrocarbon analysis and histopathological evaluation. Table 4 is a summary of the embryo samples collected for histopathological evaluation. Table 5 is a summary of the sessile invertebrates collected for hydrocarbon analysis. All samples have been sent to the appropriate labs for analysis or evaluation. No results have been received to date for the 1991 data.

Results of the histopathologic examination for tissues collected in 1990 were received in July of 1991. A copy of the results provided by the pathologists at UCD is provided in Appendix A. Statistical analysis was done comparing the occurrence of specific lesions between control and treatment sites. Four of the nine histopathologic lesions were tested, liver lipidosis, liver sinusoidal fibrosis, liver karyomegaly, and kidney macrophage aggregates. These lesions were selected because they were the most likely to be caused by exposure to toxins (personal communication Joe Sullivan 1991). Liver glycogen depletion was not tested because this can be caused by a wide range of stress conditions and is highly variable in normal populations. Hepatic single cell necrosis and kidney vacuolar degeneration could be indicators of a wide range of toxic or pathogenic conditions and were considered too general for analysis. Liver and spleen macrophage aggregates were not examined because they would be expected to show the same trend as the kidney macrophage.

Figure 2 shows that there were differences in the lesion scores between control and treatment sites in PWS for two of the four selected lesions. No differences in lesion scores were seen between sites on the outer Kenai Peninsula. Results of the statistical analysis of histopathological evaluation are presented in Table 6. Figure 2 shows the comparison of the mean and 95% confidence intervals for control sites verses oiled sites in PWS. Figure 3 shows the mean and 95% confidence levels of the lesion scores by site in PWS.

Comparisons of the LeCren's condition factors by species between oiled and control sites showed no significant differences (t-test, all P-values >0.23).

Objective 3

Determining the feasibility of using otolith microstructure to evaluate depressed growth as a result of oil contamination is still in progress. Otoliths from approximately 120 juvenile rockfish have been collected during 1990-91. Capture success in 1991 was marginal for juveniles, ranging from 0 to 5 fish per site.

Table 2. Summary of rockfish collected for tissue samples* for hydrocarbon analysis in 1991.

	Species			
	Yelloweye	Quillback	Copper	Total
Control Sites				
Gravina	1	3	6	10
Zaikof	0	10	0	10
Sub-total	1	13	6	20
Oiled Sites				
Herring Bay	7	3	0	10
Danger Island	2	2	6	10
Sub-total	9	5	6	20
Total	10	18	12	40

*Tissues samples included: liver, bile, muscle, gonad, and stomach contents.

Table 3. Summary of rockfish collected for tissue samples* for histopathological examination in 1991.

	Species			
	Yelloweye	Quillback	Copper	Total
Control Sites				
Gravina	7	13	10	30
Zaikof	2	15	0	17
Sub-total	9	28	10	47
Oiled Sites				
Herring Bay	18	12	0	30
Danger Island	12	8	10	30
Sub-total	30	20	10	60
Total	39	48	20	107

*Tissues samples included: liver, spleen, kidney, heart, and gill.

Table 4. Summary of rockfish embryo samples collected for histopathological examination in 1991.

	Species			
	Yelloweye	Quillback	Copper	Total
Control Sites				
Gravina	0	5	3	8
Zaikof	0	0	0	0
Sub-total	0	5	3	8
Oiled Sites				
Herring Bay	0	1	0	1
Danger Island	2	0	3	5
Sub-total	2	1	3	6
Total	2	6	6	14

Table 5. Summary of sessile invertebrates collected for hydrocarbon analysis in 1991.

	Species					
	Crucigera	Microporina	Ascidian	Musculus	Dendrobenia	Total
Control Sites						
Gravina	1	1	0	1	0	3
Zaikof	0	2	0	0	1	3
Sub-total	1	3	0	1	1	6
Oiled Sites						
Herring Bay	1	2	0	0	0	3
Danger Island	0	2	1	0	0	3
Sub-total	1	4	1	0	0	6
Total	2	7	1	1	1	12

Table 6. Summary of statistical analysis for determining differences between control and treatment sites for results from histopathological examination of 1990 tissue samples.

	Liver Lipidosis	Liver Fibrosis	Liver Karyomegaly	Kidney Macrophage
Prince William Sound	different (P=0.0016)	different (P=0.0118)	not different (P=0.5851)	not different (P=0.5834)
Lower Kenai Peninsula	not different (P=0.7197)	not different (P=0.1488)	not different (P=0.5254)	not different (P=0.5117)

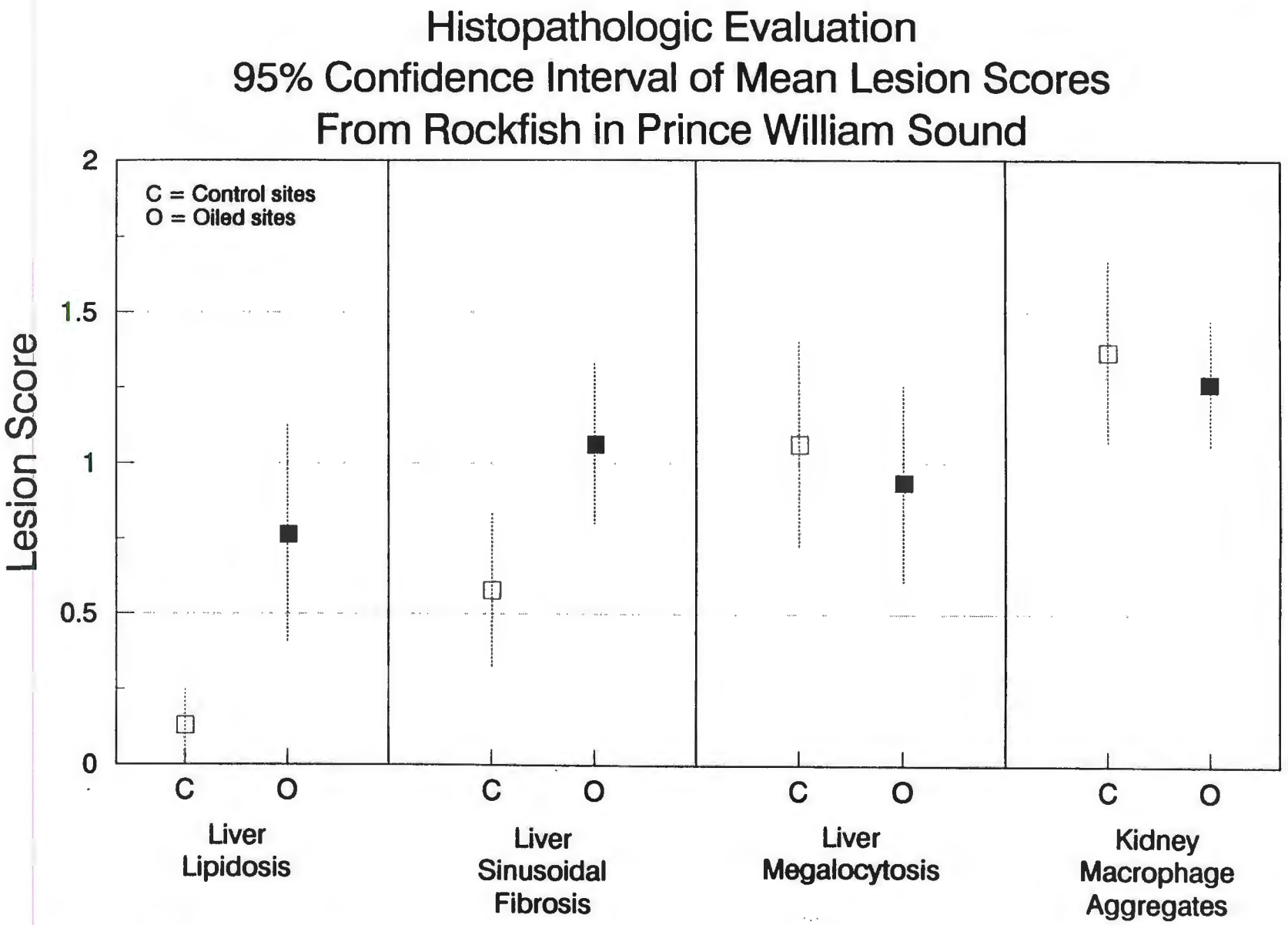

Figure 2. 95% confidence intervals of mean lesion scores for rockfish from Prince William Sound.

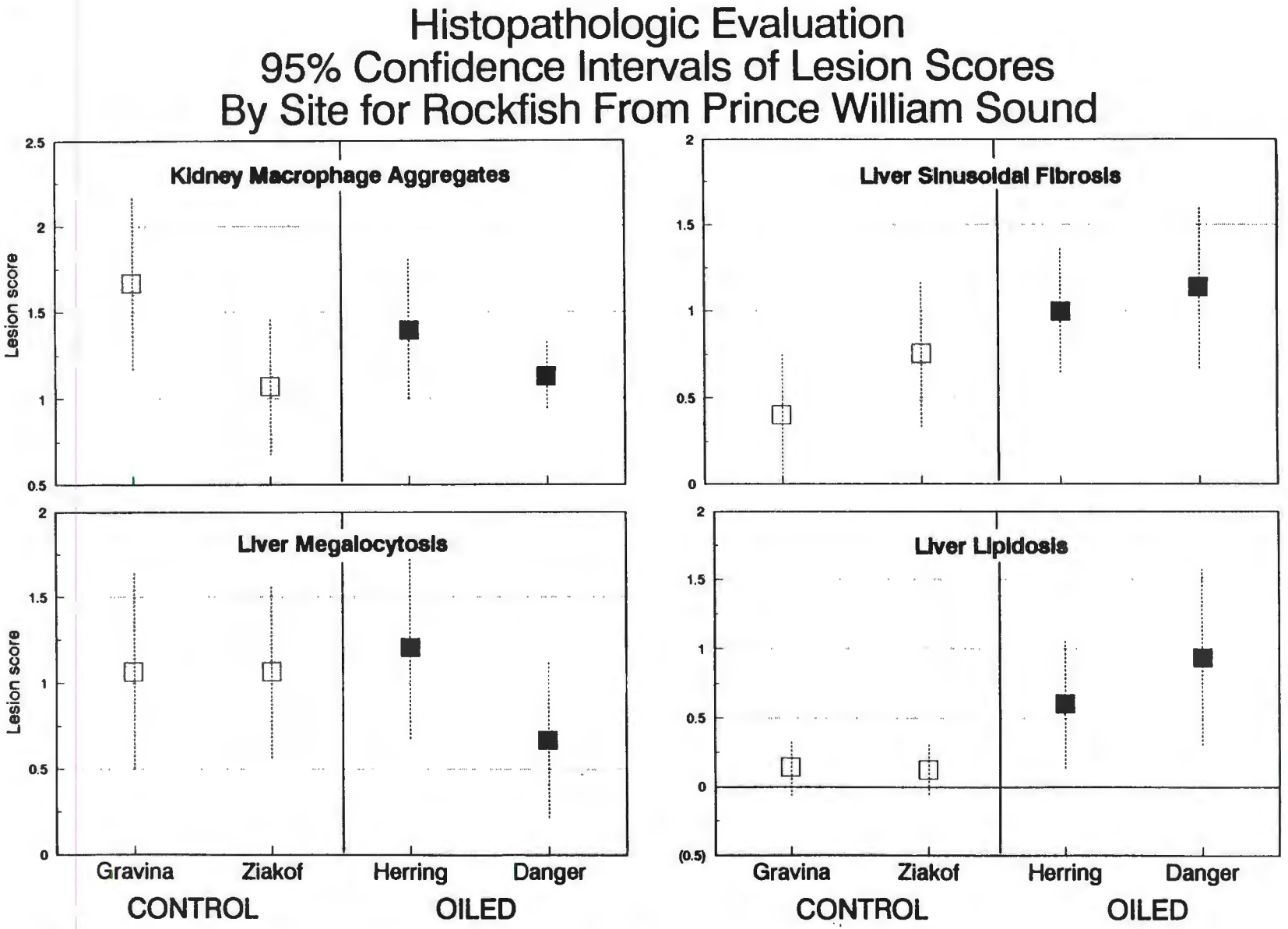

Figure 3. 95% confidence intervals of lesion scores by site for rockfish from Prince William Sound.

Objective 4

We are not recommending that Subtidal Study #6 (Fish/Shellfish Study #17) evolve into a monitoring project under restoration. Instead the Division of Sport Fish and Commercial Fisheries of Alaska Department of Fish and Game, strongly support a new restoration science study proposal for rockfish entitled "Development of a Restoration Plan for Groundfish Stocks Affected by the Exxon Valdez Oil Spill". This project is described in the Status of Injury Assessment and Recommendations section.

STATUS OF INJURY ASSESSMENT AND RECOMMENDATIONS

Results from the histopathologic evaluation of livers collected from rockfish in PWS in 1990 indicated that rockfish were exposed to toxic agents. There were differences in the liver lesion scores between control and treatment sites for two (liver lipidosis and liver sinusoidal fibrosis) of the four selected lesions in PWS, however, no differences in lesion scores were seen between sites on the outer Kenai Peninsula. Exposure to a toxin may cause liver lipidosis by several metabolic mechanisms which do not affect the uptake of fatty acids by liver cells, but do prevent the ultimate release of lipoproteins. Thus fats and fat metabolites accumulate in the liver cells. Sinusoidal fibrosis is a classic sign of chronic inflammation. This would indicate that exposure to a foreign substance or body has occurred over a significant period of time. Fibrosis without other signs of inflammation, such as macrophage and other mononuclear cell infiltration or tissue destruction, would indicate that chronic inflammation had occurred at one time but was no longer present. While none of these signs are pathognomonic for exposure to hydrocarbons, they are collectively indicative of a continuing exposure to some kind of toxin in the aquatic environment. Since they occur more frequently in fish from oiled than from unoiled areas, the presumption is that the toxins to which they are still being exposed are hydrocarbons.

The absence of differences in lesion scores between oiled and control sites along the Kenai Peninsula could be indicative of the difficulty in finding areas totally unaffected by the spill. Although control sites were selected where no oil was observed on the surface, sub-surface movements could have contaminated these areas.

This study is extremely dependant upon analysis of samples by outside laboratories, thus results and discussion is limited to what information has been received to date from these laboratories. The following is a summary of the status of the analysis of these samples collected over the life of this project, of particular urgency is the listing of analysis not yet received from contracted laboratories. We recommend that completion of the outstanding analyses be expedited.

1989 Samples

Tissue samples from 206 rockfish were collected for hydrocarbon analysis at thirty sites in PWS and along the lower Kenai Peninsula during four sampling trips in 1989. Tissue types collected were: gall bladder (bile), stomach, pyloric caeca, liver, and muscle. These were all sent to NMFS Auke Bay Laboratory for analysis. The analysis strategy established by NMFS was to analyze the bile first, then if positive results were obtained, analyze the other tissues (Manen personal communication 1989). Analysis was conducted on the bile samples from only one of the four trips (49 fish), the bile samples collected during the other three trips were

not usable because of improper sample collection or preservation methods. Positive results for hydrocarbon were found in eleven of 36 treatment sites, however, no subsequent tissue analyses were done.

The analyses that remain to be completed for these 1989 samples include the analysis for hydrocarbons on other tissues of fish with positive bile results. In addition analysis for MFOs in the frozen liver tissues in storage at NMFS Auke Bay Laboratory could also be done. The MFO analysis would probably be more worthwhile than analysis of other tissues for hydrocarbons.

1990 Samples

Tissues from 121 rockfish were collected and sent to the UCD for histopathological evaluation and MFO analysis. The results of the histopathologic evaluation were received in July of 1991. MFO analyses were not done because samples were transferred to alcohol after receipt at UCD, which was not compatible with MFO analysis (Marty personal communication 1991). Tissues from 79 of these fish were also sent to NMFS Auke Bay Laboratory for hydrocarbon analysis. In addition stomach contents, sediment and invertebrate samples were also collected and sent to NMFS Auke Bay Laboratory for hydrocarbon analysis. No results have been received from NMFS Auke Bay Laboratory for these samples to date.

Analyses still pending for samples collected during the 1990 field season include the following:

1) Hydrocarbon analysis on fish tissue samples (at a minimum bile samples).

2) Hydrocarbon analysis on stomach contents, sediments and invertebrate samples (see note following this list).

3) MFO analysis could be done at Woods Hole on liver tissue samples currently at NMFS Auke Bay Laboratory. This could compensate for the samples that could not be run at UCD.

4) Examination of the 13 embryo samples sent to UCD in 1990 could be examined for developmental abnormalities.

Note: Prior to the second year of field work there was a major change to the rockfish projects objectives and subsequent field work as a result of peer reviewers input. These changes were intended to help determine the mode of contamination by examining the rockfish's food (stomach contents) and surroundings (sediment and sessile invertebrates) for hydrocarbon contamination. These changes required the inclusion of SCUBA diving to collect these samples which increased both the projects cost and risk to the field personnel. To date none of the samples collected as a result of these changes have been analyzed. Unless these samples are analyzed these changes and added risk and cost were pointless.

1991 Samples

Tissue samples from 107 rockfish from four sites in PWS were sent to UCD for histopathological evaluation. Portions or slides of the liver from each fish were sent from UCD to Woods Hole for MFO analysis. Frozen tissue samples from 40 of the same fish were sent to NMFS Auke Bay Laboratory for hydrocarbon analysis. In addition, stomach content, sediment and invertebrate samples were also sent to NMFS Auke Bay Laboratory for hydrocarbon analysis.

Restoration Recommendations

As stated in the results we are not recommending that Subtidal Study #6 (Fish/Shellfish Study #17) evolve into a monitoring project under restoration. Instead the Division of Sport Fish and Commercial Fisheries of Alaska Department of Fish and Game, strongly support a new restoration science study proposal for rockfish entitled "Development of a Restoration Plan for Groundfish Stocks Affected by the Exxon Valdez Oil Spill". The goal of this project is to develop a restoration plan that modifies human use to facilitate the enhancement of groundfish resources impacted by EVOS. Initial objectives of this study are to: 1) identify the species of concern, 2) describe the biological characteristics of the species or stocks of concern, 3) identify and define stocks to be enhanced through modification of human use, and 4) describe the current and past patterns of human use of the groundfish resource.

A restoration plan with clearly defined objectives is needed to enhance groundfish impacted by the EVOS. Present and future rockfish populations supporting human use suffered direct and indirect impacts. Documented direct impacts include mortality of demersal rockfish, and sublethal effects of spawning age adults. In addition there was potential for damage to larval and pre-recruit rockfish as well as lingcod, however no studies were initiated to document this. Indirect impacts include the substantial increase in commercial fishing mortality during 1990 and 1991 seasons. Fishermen who normally utilize short-lived pelagic species, such as salmon and herring, shifted to long-lived demersal rockfish as a primary or secondary income source. This shift in fishing effort increased both targeted harvest and bycatch of rockfish (Bechtol personal communication 1991).

Rockfish are important components of the marine ecosystem, serving as important prey for other fishes and marine mammals. Additionally, many species of rockfish in the EVOS affected area are at the northern or western limit of their ranges, and these populations represent an important genetic resource for the entire species. The documented direct and indirect effects of oil, combined with EVOS related increases in fishing mortality, threaten rockfish and lingcod populations. The slow recovery rates characteristic of these fishes necessitate the prompt formulation of a restoration plan to assure their future. A complete version of this restoration science proposal for groundfish is found in Appendix B.

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APPENDIX A

Histopathology Report
from
University of California, Davis
School of Veterinary Medicine

This appendix contains only those sections pertinent to the rockfish analysis. The sections detailing analyses for other species has been excluded, therefore page numbers are not consecutive.

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July 3, 1991

Dr. Joseph R. Sullivan
Alaska Dept. of Fish & Game
333 Raspberry Road
Anchorage, Alaska 99518

Dear Dr. Sullivan,

As required by Histopathology Contract IHP-91-033, I have enclosed the second quarterly report on progress of histopathological analysis of fish specimens by Dr. David Hinton's laboratory. I have summarized the tissues received, tissues processed, and hours spent working on the contract (by person, date, and duty). Reports on completed histopathologic examinations include: 1) 1990 pink salmon eggs and larvae (170 "damage assessment" and 60 "response" samples); 2) 1990 pink salmon adults (520 fish, 1960 tissues); and 3) 1990 rockfish adults (121 fish, 603 tissues). A bill for these tissues is enclosed. In addition, we have included preliminary histopathology reports on the 1989 and 1990 adult herring (we are not submitting a bill for herring histopathology with this report). Our emphasis has been to read as many slides as possible; final statistical and graphical analysis, as well as detailed literature search and discussion, will be delayed until later reports.

We trimmed, embedded, cut, and sent sections of 22 chitons and 11 clams to Dr. Albert Sparks in Seattle, Washington. We will continue to process the remaining clams during the next quarter.

Based on the priority list we received by phone conversation, effort during the next quarter will focus on reading and summarizing adult herring tissues from 1990. The two pathologists in charge of the slide reading (Drs. Gary Marty and Mark Okihiro) are preparing for the Board Certification Examination in Veterinary Pathology to be given on September 25 and 26, 1991; therefore, activity on the Alaska Histopathology contract will be slow during the third quarter, but will be considerably increased during the fourth quarter. Because the board examination is scheduled at the same time as the next quarterly report, we would like to ask that the due date for the third quarterly report be extended until October 20, 1991. If you have any questions on progress of the histopathologic analysis, please contact me.

Sincerely,

Gary D. Marty

Gary D. Marty, DVM

encl.

IX. Histopathological summary - 1990 rockfish adults

PATHOLOGISTS - Mark Okihiro, DVM; David Hanes, Ph.D.

METHODS - Tissues were received, logged in, random numbers were generated, and sections were cut and examined the same as for the 1990 pink salmon adults, except large gills (not included with the pink salmon) were decalcified before sectioning. The data sheets used for scoring a variety of potential lesions in each rockfish tissue are given in Appendix 9. Statistical analysis was the same as for the 1990 pink salmon adults. Results from all species of rockfish

were combined from each site for analysis. A general linear models procedure showed that scores for glycogen depletion, hepatic megalocytosis, and hepatic sinusoidal fibrosis exhibited equality of variance, and hence, were appropriate for ANOVA.

RESULTS - Lesion scores for each fish are listed in Appendix 10. Table 7 summarizes means and standard deviations of major lesions.

I. Quillback Rockfish - 41 quillback rockfish were examined

A. Liver

1. Megalocytosis: The most striking feature of quillback rockfish livers was megalocytosis. Affected hepatocytes had marked nuclear and moderate cellular enlargement. Enlarged nuclei varied from 2 to 10X normal size, and cytomegalic hepatocytes varied from 2 to 6X normal size. Some megalocytes were multinucleated (up to five nuclei per cell) and enlarged nuclei were often elongate and/or irregular. Nucleoli were usually single and prominent, but some nuclei had two or three nucleoli. There was pseudoinclusion formation in some karyomegalic nuclei.

In severely affected livers, there was often disruption of the normal tubular architecture by enlarged, irregular hepatocytes. Sinusoids were compressed and some foci of tubules appeared to lack nuclei (assumed to be due to enlargement of hepatocytes such that nuclei were out of the plane of section). Enlarged hepatocytes often contained moderate to large amounts of coarsely granular, light brown pigment which was similar to that seen in macrophages. Severe megalocytosis was usually associated with increased numbers of macrophage aggregates and scattered individually necrotic hepatocytes (apoptosis).

Interestingly, there also appeared to be some microcytic hepatocytes in livers that had severe megalocytosis. In a separate study in our laboratory, we are in the process of confirming differences in hepatocyte and nuclear size with morphometry of rockfish caught off the California coast.

Differences in mean megalocyte scores among sites were not significant (ANOVA, P = 0.13).

Comment: Megalocytosis in mammals (primarily horses) is usually associated with pyrrolizidine alkaloid toxicity, but we have occasionally seen this lesion in medaka (Oryzias latipes, a small aquarium fish) in both controls and in medaka exposed to diethylnitrosamine (DEN). Severe megalocytosis was also observed by Dr. Ron Hedrick's laboratory (Department of Medicine, School of Veterinary Medicine, University of California, Davis) in a group of pond-raised striped bass. Although the cause was never precisely determined, algal toxins were suspected. In any event, megalocytosis in quillback rockfish was a very significant lesion

and we are in the process of trying to determine if it has been previously associated with crude oil toxicity in fish. Myers et al. (1990, Science of the Total Environment 94:33-50) describes megalocytosis in toxically altered livers of flatfishes from polluted habitats in Puget Sound, Washington. This lesion has been recommended by the S.E.T.A.C committee as a biomarker of exposure.

2. Sinusoidal fibrosis: An uncommon, but striking hepatic lesion, was sinusoidal fibrosis. Distribution tended to be patchy, with affected sinusoids lined by variable amounts of fibrillar collagen (confirmed using Masson's Trichrome stain). In some areas, sinusoidal fibrosis was continuous with the connective tissue of large veins. Differences in mean fibrosis scores among sites were not significant (ANOVA, P = 0.099).

3. Necrosis:
   a. Coagulation necrosis: not observed

   b. Single cell necrosis: Individual hepatocyte necrosis or apoptosis was a common, but usually mild finding.

4. Inflammation:
   a. Macrophage aggregates: Macrophage aggregates were a common finding in quillback livers. Macrophages in these aggregates were usually vacuolated (possibly due to fat accumulation in phagolysosomes) and filled with granular brown pigment (either hemosiderin or lipofuscin).

      Comment: Macrophage aggregates were probably an indicator of previous hepatocyte degeneration and necrosis (i.e., macrophages phagocytized dead hepatocytes). The scoring scheme for macrophage aggregates in the liver was slightly different than for the spleen and kidney because the liver is not a normal terminal site for macrophages. Macrophage aggregates are being used by the EPA and NMFS as indicators of pollutant stress.

   b. Lymphocytic aggregates: Small clusters of lymphocytes were occasionally present in the liver.

5. Hepatocyte storage disorders
   a. Glycogen depletion: Glycogen depletion was a common finding and characterized by loss of cell volume and increased cytoplasmic basophilia. Differences in mean glycogen depletion scores among sites were highly significant (ANOVA, P = 0.0001). Separating the means with Tukey's studentized range test demonstrated that rockfish from Zaikof/Schooner and Pony cove (minimal depletion, probably unexposed) had significantly different glycogen scores than did rockfish

from Danger Island and Granite Island (marked depletion, most likely to have been exposed); no other site comparisons were significant at the $\alpha = 0.05$ level. Multiple comparisons with Student's t-test yielded the same results (Table 8).

Comment: Both glycogen depletion and lipidosis are nonspecific lesions; however, toxically altered liver will show these changes.

b. Lipidosis (hepatocellular fatty change): Mild lipidosis was seen in a few fish.

c. Eosinophilic bodies: Some hepatocytes contained refractile, eosinophilic, intracytoplasmic droplets that may represent large lysosomes.

6. Bile duct hyperplasia: not observed

7. Parasitism: There was minimal evidence of parasitism. Small numbers of Ichthyophonus, nematodes, and trematodes were found in a few fish.

B. Kidney

1. Renal tubular degeneration and necrosis: Another common finding was vacuolar degeneration and necrosis of individual or small clusters of tubular epithelial cells. This was associated with the influx of individual macrophages into the tubular epithelium and the presence of small amounts of necrotic debris in some tubules.

Comment: Renal tubular necrosis certainly could be related to xenobiotic exposure.

2. Glomerulonephritis: One of the most consistent renal lesions in quillback rockfish was the presence of generalized membranous glomerulonephritis. Affected glomeruli had mild to severe thickening of basement membranes by pale eosinophilic, acellular material. In some glomeruli, there also appeared to be mild to moderate proliferation of mesangial cells (podocytes) and mild dilation of Bowman's capsule. This lesion was, in some fish, associated with large amounts of protein droplets in proximal tubular epithelial cells.

Comment: Membranous glomerulonephritis is a chronic renal disease which is usually associated with the deposition of immune complexes or anti-glomerular antibodies on the glomerular basement membranes. The lesion must be differentiated from amyloidosis. We do not know if the lesions could be related to oil exposure, but it seems unlikely.

3. Inflammation
   a. Macrophage aggregates: Many kidneys were massively infiltrated by macrophage aggregates.

      Comment: The degree and number of macrophage aggregates probably reflects the amount of degeneration and necrosis of renal tubular epithelial cells.

   b. Lymphoid aggregates: were occasionally seen

C. Spleen
   1. Inflammation:
      a. Macrophage aggregates: Macrophage aggregates were a consistent finding. Some fish had large numbers of large aggregates that replaced a considerable volume of splenic parenchyma.

      b. Lymphoid aggregates: were occasionally seen

   2. Periarteriolar sheath hyperplasia: Periarteriolar sheaths often appeared hyperplastic and prominent. In some, there appeared to be the deposition in the sheaths of a hyaline material similar to that seen in glomeruli. The amount of pigmentation (with brown-black pigment assumed to represent melanin) was highly variable.

D. Gonads: The only significant finding in a few fish was the presence of small numbers of macrophage aggregates and a few lymphoid follicles.

E. Gills
   1. Inflammation: The majority of fish had multifocal infiltrates of lymphocytes, and in some fish, the infiltrates were very dense and large.

   2. Hyperplasias: The most consistent finding was mild mucous cell hyperplasia with affected fish having individual or small clusters of mucous cells scattered over the lamellae. Quantification of mucous cell numbers require special staining (e.g. Periodic Acid-Schiff), not provided for in the original contract.

II. Other rockfish species
   A. Yelloweye rockfish: 26 yelloweye rockfish were examined. The most prominent lesions was sinusoidal fibrosis, with 22/25 livers examined having at least mild fibrosis. In some, the fibrosis was diffuse and severe. In contrast, there was minimal evidence of megalocytosis with only 7/25 livers showing only mild (1+) megalocytosis. Lipidosis was another fairly common liver lesion.

   B. China rockfish: 20 China rockfish were examined. There were minimal

liver lesions in these fish, but macrophage aggregates were common in both the kidney and spleen.

C. Copper rockfish: 19 copper rockfish were examined. These fish had minimal lesions in the liver. Vacuolar degeneration was fairly common in the kidney, as were macrophage aggregates in the spleen.

D. Tiger rockfish: 7 tiger rockfish were examined. Liver lesions were mild, but macrophage aggregates in the spleen were common.

E. Silvergrey rockfish: 5 silvergrey rockfish were examined. 1/5 fish had moderate megalocytosis and sinusoidal fibrosis in the liver.

F. Yellowtail rockfish: 2 yellowtail rockfish were examined. Both had mild lesions in all organs.

G. Splitnose rockfish: 1 splitnose rockfish was examined. The most prominent lesion was moderate sinusoidal fibrosis in the liver.

Final Comments: A total of 121 rockfish (41 quillback, 26 yelloweye, 20 china, 19 copper, 7 tiger, 5 silvergrey, 2 yellowtail, and 1 splitnose) were examined. The most severe lesions were observed in the quillback rockfish, but all rockfish species had similar lesions in liver, kidney, and spleen. Evidence of both parasitism and infectious disease was minimal and we believe that the lesions probably do represent exposure to some hepatotoxic and nephrotoxic agent. Based on the analysis of glycogen depletion scores, we speculate that Danger Island and Granite Island are exposed sites and Pony Cove and Zaikof/Schooner are clean sites. Future recommendation are as follows:

1) Sampling be concentrated on Quillback and Yelloweye rockfish

2) Equal numbers of males and females be sampled

3) Similar sized (age) fish be sampled

4) Sampled tissues to include: liver, kidney, spleen, and gill
[Gonads could be eliminated from analysis because lesions were minimal, and confounding problems such as stage of gonad maturation and seasonal cycling, unknown to the pathologists, likely cloud the detection of lesions.]

X. Preliminary Histopathological summary - 1989 herring adults

PATHOLOGISTS - Mark Okihiro, DVM; David Hanes, Ph.D.

METHODS - Tissues were received, logged in, random numbers were generated, and

liver lesions in these fish, but macrophage aggregates were common in both the kidney and spleen.

C. Copper rockfish: 19 copper rockfish were examined. These fish had minimal lesions in the liver. Vacuolar degeneration was fairly common in the kidney, as were macrophage aggregates in the spleen.

D. Tiger rockfish: 7 tiger rockfish were examined. Liver lesions were mild, but macrophage aggregates in the spleen were common.

E. Silvergrey rockfish: 5 silvergrey rockfish were examined. 1/5 fish had moderate megalocytosis and sinusoidal fibrosis in the liver.

F. Yellowtail rockfish: 2 yellowtail rockfish were examined. Both had mild lesions in all organs.

G. Splitnose rockfish: 1 splitnose rockfish was examined. The most prominent lesion was moderate sinusoidal fibrosis in the liver.

Final Comments: A total of 121 rockfish (41 quillback, 26 yelloweye, 20 china, 19 copper, 7 tiger, 5 silvergrey, 2 yellowtail, and 1 splitnose) were examined. The most severe lesions were observed in the quillback rockfish, but all rockfish species had similar lesions in liver, kidney, and spleen. Evidence of both parasitism and infectious disease was minimal and we believe that the lesions probably do represent exposure to some hepatotoxic and nephrotoxic agent. Based on the analysis of glycogen depletion scores, we speculate that Danger Island and Granite Island are exposed sites and Pony Cove and Zaikof/Schooner are clean sites. Future recommendation are as follows:

1) Sampling be concentrated on Quillback and Yelloweye rockfish

2) Equal numbers of males and females be sampled

3) Similar sized (age) fish be sampled

4) Sampled tissues to include: liver, kidney, spleen, and gill
[Gonads could be eliminated from analysis because lesions were minimal, and confounding problems such as stage of gonad maturation and seasonal cycling, unknown to the pathologists, likely cloud the detection of lesions.]

Table 7. Summary of rockfish histopathologic data (Mean ± Standard Deviation) from selected lesions.

	LIVER						KIDNEY
Site (N)	GLY	LIP	MA	SCN	MEG	FIB	MA
Danger Island (15)	2.2±1.0	0.9±1.2	1.0±0.4	0.1±0.4	0.7±0.8	1.1±0.8	1.1±0.4
Day Harbor (15)	1.2±1.2	0.6±0.9	0.9±0.5	0.1±0.3	0.6±0.6	1.1±0.6	1.0±0.6
Granite Is (15)	2.1±1.1	0.4±0.9	0.9±0.3	0.1±0.3	0.7±0.9	0.9±0.6	1.4±0.6
Gravina Rks (15)	1.0±1.1	0.1±0.4	1.6±1.1	0.1±0.4	1.1±1.0	0.4±0.6	1.7±0.9
Herring Bay (15)	1.6±1.1	0.6±0.8	1.6±1.1	0.4±0.6	1.2±0.9	1.0±0.7	1.4±0.7
Mourning Cove (15)	1.1±1.3	0.5±1.0	0.8±0.6	0.1±0.3	0.7±0.8	0.8±0.6	1.0±0.4
Pony Cove (15)	0.5±0.7	0.3±0.7	0.9±0.3	0.1±0.3	0.4±0.5	0.6±0.9	1.6±0.7
Schooner (16)	0.6±1.0	0.1±0.3	0.6±0.5	0.2±0.4	1.1±0.9	0.8±0.8	1.1±0.7

Key to Table symbols:

LIVER GLY = Liver glycogen depletion; 0 = none, 1 = mild, 2 = moderate, 3 = severe depletion
LIVER LIP = Liver Lipidosis; 0 = none, 1 = mild, 2 = moderate, 3 = severe
LIVER MA = Liver macrophage aggregates
LIVER SCN = Hepatocytic single cell necrosis
LIVER MEG = Hepatocytic karyomegaly
LIVER FIB = Sinusoidal fibrosis in liver
KIDNEY MA = Kidney macrophage aggregates

Table 8. Comparisons of glycogen depletion score by site (t-test, $\alpha = 0.05$). Significant comparisons are indicated by site number.

Site	1	2	3	4	5	6	7	8
1							1/7	1/8
2								
3							3/7	3/8
4								
5								
6								
7	1/7		3/7					
8	1/8		3/8					

Appendix 9. Lesion recording sheets for rockfish adults.

FISH Species: _____ Number: _____

. GILL (Present / Absent)

. Cartilage dysplasia:

1. primary cartilage	NP	MILD/MOD/SEV	F/MF/MF-DIF/DIF
2. side branches	NP	MILD/MOD/SEV	F/MF/MF-DIF/DIF
3. fusion	NP	MILD/MOD/SEV	F/MF/MF-DIF/DIF
4. nodules	NP	MILD/MOD/SEV	F/MF/MF-DIF/DIF

. Parasitism:

1. flukes	NP	MILD/MOD/SEV	F/MF/MF-DIF/DIF
2. other:	NP	MILD/MOD/SEV	F/MF/MF-DIF/DIF

. Inflammation:

1. granulomatous	NP	MILD/MOD/SEV	F/MF/MF-DIF/DIF
2. syn. giant cells	NP	MILD/MOD/SEV	F/MF/MF-DIF/DIF
3. macrophage agg.	NP	MILD/MOD/SEV	F/MF/MF-DIF/DIF
4. EGL's	NP	MILD/MOD/SEV	F/MF/MF-DIF/DIF
5. neutrophilic	NP	MILD/MOD/SEV	F/MF/MF-DIF/DIF
6. lymphocytic	NP	MILD/MOD/SEV	F/MF/MF-DIF/DIF

) cular lesions:

aneurysms	NP	MILD/MOD/SEV	F/MF/MF-DIF/DIF
2. thrombi	NP	MILD/MOD/SEV	F/MF/MF-DIF/DIF
3. vasculitis	NP	MILD/MOD/SEV	F/MF/MF-DIF/DIF

. Hyperplasia:

1. squamous cell	NP	MILD/MOD/SEV	F/MF/MF-DIF/DIF
2. mucous cell	NP	MILD/MOD/SEV	F/MF/MF-DIF/DIF
3. chloride cell	NP	MILD/MOD/SEV	F/MF/MF-DIF/DIF

F. BM thickening NP MILD/MOD/SEV F/MF/MF-DIF/DIF

G. Fibrosis NP MILD/MOD/SEV F/MF/MF-DIF/DIF

H. Autolysis NP MILD/MOD/SEV

2. LIVER (Present / Absent)

A. Hepatocyte storage defects:			
1. glycogen depletion	NP	MILD/MOD/SEV	F/MF/MF-DIF/DIF
2. lipidosis	NP	MILD/MOD/SEV	F/MF/MF-DIF/DIF
B. Parasitism:			
1. flukes	NP	MILD/MOD/SEV	F/MF/MF-DIF/DIF
2. other:	NP	MILD/MOD/SEV	F/MF/MF-DIF/DIF
C. Inflammation:			
1. macrophage agg.	NP	MILD/MOD/SEV	F/MF/MF-DIF/DIF
2. lymphocytic	NP	MILD/MOD/SEV	F/MF/MF-DIF/DIF
3. other:	NP	MILD/MOD/SEV	F/MF/MF-DIF/DIF
D. Necrosis	NP	MILD/MOD/SEV	F/MF/MF-DIF/DIF
E. Degeneration	NP	MILD/MOD/SEV	F/MF/MF-DIF/DIF
F. Preneoplasia & neoplasia:			
1. foci or areas	NP	MILD/MOD/SEV	F/MF/MF-DIF/DIF
2. nodules	NP	MILD/MOD/SEV	F/MF/MF-DIF/DIF
3. tumors	NP	MILD/MOD/SEV	F/MF/MF-DIF/DIF
G. Megalocytes	NP	MILD/MOD/SEV	F/MF/MF-DIF/DIF
H. cells	NP	MILD/MOD/SEV	F/MF/MF-DIF/DIF
I. Sinusoidal fibrosis	NP	MILD/MOD/SEV	F/MF/MF-DIF/DIF
J. Autolysis	NP	MILD/MOD/SEV	

3. Kidney (Present / Absent)

A. Inflammation:			
1. macrophage agg.	NP	MILD/MOD/SEV	F/MF/MF-DIF/DIF
2. granulomatous	NP	MILD/MOD/SEV	F/MF/MF-DIF/DIF
3. lymphocytic	NP	MILD/MOD/SEV	F/MF/MF-DIF/DIF
B. Tubules:			
1. vacuolar degen.	NP	MILD/MOD/SEV	F/MF/MF-DIF/DIF
2. necrosis	NP	MILD/MOD/SEV	F/MF/MF-DIF/DIF
3. protein droplets	NP	MILD/MOD/SEV	F/MF/MF-DIF/DIF
4. lumenal debris/cal.	NP	MILD/MOD/SEV	F/MF/MF-DIF/DIF
C. Glomeruli:			
1. BM thickening	NP	MILD/MOD/SEV	F/MF/MF-DIF/DIF
2. hypercellularity	NP	MILD/MOD/SEV	F/MF/MF-DIF/DIF
3. dil. Bowman's cap.	NP	MILD/MOD/SEV	F/MF/MF-DIF/DIF
D. Parasitism:	NP	MILD/MOD/SEV	F/MF/MF-DIF/DIF
E. Autolysis	NP	MILD/MOD/SEV	

Number _____

. Spleen (Present / Absent)

. ammation
1. macrophage agg. NP MILD/MOD/SEV F/MF/MF-DIF/DIF
2. lymphoid agg. NP MILD/MOD/SEV F/MF/MF-DIF/DIF
3. other: NP MILD/MOD/SEV F/MF/MF-DIF/DIF

. Periarteriolar sheath hyperplasia NP MILD/MOD/SEV F/MF/MF-DIF/DIF

. Autolysis NP MILD/MOD/SEV

. Testis (Present / Absent)

. Inflammation:
1. macrophage agg. NP MILD/MOD/SEV F/MF/MF-DIF/DIF
2. granulomatous NP MILD/MOD/SEV F/MF/MF-DIF/DIF
3. lymphocytic NP MILD/MOD/SEV F/MF/MF-DIF/DIF
4. other: NP MILD/MOD/SEV F/MF/MF-DIF/DIF

. Spermatogonia:
1. necrosis NP MILD/MOD/SEV F/MF/MF-DIF/DIF
2. degeneration NP MILD/MOD/SEV F/MF/MF-DIF/DIF

. m production NONE / FEW / MODERATE / ABUNDANT

. Autolysis NP MILD/MOD/SEV

. Ovary (Present / Absent)

. Inflammation:
1. macrophage agg. NP MILD/MOD/SEV F/MF/MF-DIF/DIF
2. granulomatous NP MILD/MOD/SEV F/MF/MF-DIF/DIF
3. lymphocytic NP MILD/MOD/SEV F/MF/MF-DIF/DIF
4. other: NP MILD/MOD/SEV F/MF/MF-DIF/DIF

. Oocyte atresia NP MILD/MOD/SEV F/MF/MF-DIF/DIF

. Oocyte numbers FEW / MODERATE / ABUNDANT

. Autolysis NP MILD/MOD/SEV

Reviewer's Initials: _____
Date: _____

COMMENTS:

Appendix 10. Summary of major histopatholgic lesions in 1990 rockfish.

Key to Table symbols:

Hinton Processing # = Random number generated by Dr. Hinton's Laboratory
Sex = M for Male or F for Female
LIVER GLY = Liver glycogen depletion; 0 = none, 1 = mild, 2 = moderate, 3 = severe depletion
LIVER LIP = Liver Lipidosis; 0 = none, 1 = mild, 2 = moderate, 3 = severe
LIVER MA = Liver macrophage aggregates
LIVER SCN = Hepatocytic single cell necrosis
LIVER MEG = Hepatocytic karyomegaly
LIVER FIB = Sinusoidal fibrosis in liver
KIDNEY MA = Kidney macrophage aggregates
KIDNEY VD = Vacuolar degeneration of kidney tubular epithelium
SPLEEN MA = Macrophage aggregates in the spleen
SITE = Sample collection site

Processing Code	Liver						Kidney		Spleen		Hinton Fish #	ADF&G Jar #	Place	Species
	GLY	LIP	MA	SCN	MEG	FIB	MA	VD	MA	SITE				
CH 55	3	0	1	1	1	1	2	1	2	1	35	UCD 220,221	Danger Island	China Rockfish
CH 371	3	0	1	0	1	1	1	0	2	1	36	UCD 222,223	Danger Island	China Rockfish
CH 445	2	0	1	0	1	1	1	1	1	1	37	UCD 224,225	Danger Island	China Rockfish
QB 348	2	0	2	0	3	2	1	1	1	1	41	UCD 232,233	Danger Island	Quillback Rockfish
QB 103	2	0	1	0	0	0	2	1	2	1	45	UCD 240,241	Danger Island	Quillback Rockfish
YE 368	2	0	1	0	1	1	1	1	1	1	31	UCD 212,213	Danger Island	Yelloweye Rockfish
YE 85	3	1	1	0	1	1	1	0	2	1	32	UCD 214,215	Danger Island	Yelloweye Rockfish
YE 37	2	3	0	0	0	1	1	0	1	1	33	UCD 216,217	Danger Island	Yelloweye Rockfish
YE 185	3	3	1	0	0	3	1	1	2	1	34	UCD 218,219	Danger Island	Yelloweye Rockfish
YE 52	3	2	1	0	1	1	1	1	1	1	38	UCD 226,227	Danger Island	Yelloweye Rockfish
YE 488	2	2	1	0	0	2	1	1	1	1	40	UCD 230,231	Danger Island	Yelloweye Rockfish
YE 230	3	1	1	0	0	2	1	1	1	1	43	UCD 236,237	Danger Island	Yelloweye Rockfish
YE 360	0	0	1	0	0	1	1	1	2	1	44	UCD 238,239	Danger Island	Yelloweye Rockfish
YT 400	0	0	1	0	0	0	1	0	1	1	39	UCD 228,229	Danger Island	Yelloutail
YT 290	3	2	1	1	1	0	1	0	1	1	42	UCD 234,235	Danger Island	Yelloutail
CO 49	0	0	2	0	1	1	2	2	3	2	93	UCD 537,538	Day Harbor	Copper Rockfish
CO 412	1	1	1	0	0	1	1	1	1	2	94	UCD 539,540	Day Harbor	Copper Rockfish
CO 186	3	1	1	0	1	1	1	2	2	2	96	UCD 543,544	Day Harbor	Copper Rockfish
CO 71	0	0	1	0	1	0	1	2	3	2	99	UCD 549,550	Day Harbor	Copper Rockfish
CO 166	3	3	1	0	1	1	1	0	1	2	102	UCD 605,606	Day Harbor	Copper Rockfish
QB 346	0	0	0	0	0	0	0	0	1	2	95	UCD 541,542	Day Harbor	Quillback Rockfish
QB 113	1	0	0	0	0	1	1	1	2	2	100	UCD 601,602	Day Harbor	Quillback Rockfish
QB 60	0	0	1	0	1	1	1	1	1	2	101	UCD 603,604	Day Harbor	Quillback Rockfish
QB 344	1	0	0	0	1	1	1	1	1	2	103	UCD 607,608	Day Harbor	Quillback Rockfish
QB 126	1	0	1	0	1	1	1	1	2	2	104	UCD 609,610	Day Harbor	Quillback Rockfish
QB 130	2	2	1	1	2	2	0	2	1	2	105	UCD 611,612	Day Harbor	Quillback Rockfish
YE 149	.	.	.	.	.	.	1	0	1	2	91	UCD 533,534	Day Harbor	Yelloweye Rockfish
YE 279	0	0	1	0	0	1	1	0	1	2	92	UCD 535,536	Day Harbor	Yelloweye Rockfish
YE 182	2	1	1	0	0	2	2	0	1	2	97	UCD 545,546	Day Harbor	Yelloweye Rockfish
YE 68	3	1	1	0	0	2	.	.	1	2	98	UCD 547,548	Day Harbor	Yelloweye Rockfish

Processing Code	Liver GLY	Liver LIP	Liver MA	Liver SCN	Liver MEG	Liver FIB	Kidney MA	Kidney VD	Spleen MA	Spleen SITE	Hinton Fish #	ADF&G Jar #	Place	Species
CH 45	1	0	1	0	0	1	2	2	2	3	61	UCD 332,333	Granite Island	China Rockfish
CH 278	1	0	1	0	0	1	1	1	2	3	62	UCD 334,335	Granite Island	China Rockfish
CH 108	3	1	1	0	1	1	2	0	3	3	63	UCD 336,337	Granite Island	China Rockfish
CH 233	3	0	1	0	0	1	1	2	1	3	65	UCD 340,341	Granite Island	China Rockfish
CH 7	0	0	1	0	0	0	1	0	2	3	67	UCD 344,345	Granite Island	China Rockfish
CH 58	3	0	1	0	1	2	1	0	1	3	72	UCD 405,406	Granite Island	China Rockfish
CH 447	3	0	1	0	1	0	1	0	3	3	73	UCD 407,408	Granite Island	China Rockfish
CH 355	2	0	1	0	0	1	2	1	3	3	74	UCD 409,410	Granite Island	China Rockfish
CH 131	3	0	1	1	0	1	2	3	2	3	75	UCD 411,412	Granite Island	China Rockfish
CO 123	0	0	1	0	0	0	1	2	2	3	70	UCD 350,401	Granite Island	Copper Rockfish
CO 178	3	3	1	0	0	1	3	3	3	3	71	UCD 402,403,404	Granite Island	Copper Rockfish
QB 97	3	0	1	0	2	1	1	2	1	3	68	UCD 346,347	Granite Island	Quillback Rockfish
QB 430	2	0	0	0	3	2	1	0	2	3	69	UCD 348,349	Granite Island	Quillback Rockfish
TI 473	2	0	1	0	1	0	1	2	2	3	64	UCD 338,339	Granite Island	Tiger Rockfish
TI 162	2	2	1	0	1	1	1	1	2	3	66	UCD 342,343	Granite Island	Tiger Rockfish
CH 324	2	0	1	0	2	0	2	1	2	4	6	UCD 111,112	Gravina Rocks	China Rockfish
CO 264	0	0	3	0	1	0	3	1	3	4	1	UCD 101,102	Gravina Rocks	Copper Rockfish
CO 266	0	0	1	0	1	0	2	2	3	4	15	UCD 130,131	Gravina Rocks	Copper Rockfish
QB 470	1	0	1	0	1	0	1	1	1	4	2	UCD 103,104	Gravina Rocks	Quillback Rockfish
QB 338	2	0	2	0	2	1	2	1	2	4	3	UCD 105,106	Gravina Rocks	Quillback Rockfish
QB 151	0	0	3	0	1	1	2	1	3	4	7	UCD 113,114	Gravina Rocks	Quillback Rockfish
QB 112	1	0	3	0	3	0	3	1	3	4	12	UCD 124,125	Gravina Rocks	Quillback Rockfish
QB 448	2	0	3	1	3	0	3	2	2	4	13	UCD 126,127	Gravina Rocks	Quillback Rockfish
QB 483	1	0	3	1	1	0	2	0	2	4	14	UCD 128,129	Gravina Rocks	Quillback Rockfish
YE 194	3	1	1	0	0	1	1	2	1	4	4	UCD 107,108	Gravina Rocks	Yelloweye Rockfish
YE 258	0	0	0	0	0	0	1	1	1	4	5	UCD 109,110	Gravina Rocks	Yelloweye Rockfish
YE 23	3	1	1	0	1	2	1	1	1	4	8	UCD 115,116,117	Gravina Rocks	Yelloweye Rockfish
YE 62	0	0	1	0	0	0	1	1	1	4	9	UCD 118,119	Gravina Rocks	Yelloweye Rockfish
YE 75	0	0	0	0	0	0	0	2	1	4	10	UCD 120,121	Gravina Rocks	Yelloweye Rockfish
YE 22	0	0	1	0	0	1	1	1	2	4	11	UCD 122,123	Gravina Rocks	Yelloweye Rockfish
CO 404	0	0	0	0	0	0	1	1	2	5	26	UCD 202,203	Herring Bay	Copper Rockfish
QB 138	2	1	3	0	2	1	2	2	3	5	20	UCD 140,141	Herring Bay	Quillback Rockfish
QB 411	3	0	3	0	2	1	2	1	2	5	21	UCD 142,143	Herring Bay	Quillback Rockfish
QB 248	3	0	1	0	1	1	1	0	2	5	23	UCD 146,147	Herring Bay	Quillback Rockfish
QB 501	1	0	1	1	3	0	3	2	3	5	27	UCD 204,205	Herring Bay	Quillback Rockfish
QB 9	0	0	3	0	2	1	3	2	3	5	28	UCD 206,207	Herring Bay	Quillback Rockfish
QB 452	1	0	3	1	2	1	1	1	3	5	30	UCD 210,211	Herring Bay	Quillback Rockfish
SG 259	1	1	1	0	1	0	1	1	1	5	16	UCD 132,133	Herring Bay	Silvergrey Rockfish
SG 201	1	1	1	1	2	2	1	1	1	5	25	UCD 150,201	Herring Bay	Silvergrey Rockfish
SG 409	0	0	0	0	1	1	1	1	1	5	29	UCD 208,209	Herring Bay	Silvergrey Rockfish
YE 210	2	1	1	0	0	1	1	1	1	5	17	UCD 134,135	Herring Bay	Yelloweye Rockfish
YE 99	3	0	2	1	1	1	1	1	3	5	18	UCD 136,137	Herring Bay	Yelloweye Rockfish
YE 499	3	1	3	2	1	1	1	1	3	5	19	UCD 138,139	Herring Bay	Yelloweye Rockfish
YE 38	2	3	1	0	0	2	1	1	1	5	22	UCD 144,145	Herring Bay	Yelloweye Rockfish
YE 449	2	1	1	0	0	2	1	2	1	5	24	UCD 148,149	Herring Bay	Yelloweye Rockfish

Processing Code	Liver GLY	Liver LIP	Liver MA	Liver SCH	Liver MEG	Liver FIB	Kidney MA	Kidney VD	Spleen MA	Spleen SITE	Hinton Fish #	ADF&G Jar #	Place	Species
CH 379	2	0	0	0	1	0	1	1	1	6	46	UCD 301,302	Morning Cove	China Rockfish
CO 302	2	3	1	0	0	1	1	0	3	6	50	UCD 309,310	Morning Cove	Copper Rockfish
CO 294	0	0	1	0	0	1	1	1	2	6	52	UCD 313,314	Morning Cove	Copper Rockfish
CO 242	0	0	1	0	0	0	2	0	3	6	54	UCD 317,318,331	Morning Cove	Copper Rockfish
CO 216	0	0	1	0	1	1	1	2	3	6	55	UCD 319,320	Morning Cove	Copper Rockfish
CO 327	2	0	1	0	0	0	1	2	2	6	58	UCD 325,326	Morning Cove	Copper Rockfish
CO 255	0	0	1	0	0	1	1	1	3	6	60	UCD 329,330	Morning Cove	Copper Rockfish
QB 271	3	2	1	0	0	1	1	0	2	6	47	UCD 303,304	Morning Cove	Quillback Rockfish
QB 386	2	2	1	0	2	2	.	.	.	6	49	UCD 307,308	Morning Cove	Quillback Rockfish
QB 4	0	0	0	0	1	0	1	1	.	6	53	UCD 315,316	Morning Cove	Quillback Rockfish
QB 424	3	0	2	0	2	1	1	0	1	6	56	UCD 321,322	Morning Cove	Quillback Rockfish
QB 142	3	1	0	1	2	1	0	0	1	6	57	UCD 323,324	Morning Cove	Quillback Rockfish
SG 381	0	0	0	0	1	1	1	3	1	6	48	UCD 305,306	Morning Cove	Silvergrey Rockfish
TI 443	0	0	1	0	0	1	1	1	2	6	51	UCD 311,312	Morning Cove	Tiger Rockfish
TI 301	0	0	1	0	0	1	1	1	3	6	59	UCD 327,328	Morning Cove	Tiger Rockfish
CH 345	0	0	1	0	1	1	3	2	3	7	76	UCD 501,502	Pony Cove	China Rockfish
CH 183	0	0	1	0	0	0	1	0	2	7	77	UCD 503,504	Pony Cove	China Rockfish
CH 117	1	0	1	0	0	0	2	2	3	7	85	UCD 519,520	Pony Cove	China Rockfish
CH 154	0	0	1	0	0	0	1	0	2	7	86	UCD 521,522	Pony Cove	China Rockfish
CH 370	0	0	1	0	0	0	3	2	3	7	89	UCD 527,528	Pony Cove	China Rockfish
CO 490	0	0	0	0	1	0	1	1	1	7	78	UCD 505,506	Pony Cove	Copper Rockfish
CO 491	0	0	1	0	0	0	1	1	2	7	87	UCD 523,524	Pony Cove	Copper Rockfish
CO 487	0	0	1	0	0	0	1	2	2	7	90	UCD 529,530	Pony Cove	Copper Rockfish
QB 238	0	0	1	0	1	0	1	1	1	7	88	UCD 525,526	Pony Cove	Quillback Rockfish
SG 298	0	0	1	0	1	1	2	2	1	7	80	UCD 509,510	Pony Cove	Silvergrey Rockfish
TI 3	1	2	1	0	1	0	2	1	3	7	79	UCD 507,508	Pony Cove	Tiger Rockfish
TI 42	0	0	1	0	0	1	2	1	3	7	83	UCD 515,516	Pony Cove	Tiger Rockfish
TI 464	2	1	1	0	0	1	1	2	3	7	84	UCD 517,518	Pony Cove	Tiger Rockfish
YE 378	1	0	1	0	1	2	1	1	3	7	81	UCD 511,512	Pony Cove	Yelloweye Rockfish
YE 101	2	2	1	1	0	3	2	1	2	7	82	UCD 513,514	Pony Cove	Yelloweye Rockfish
CH 27	2	0	1	0	0	0	1	0	1	8	113	UCD 627,628	Zaikof/Schooner	China Rockfish
QB 77	1	0	1	0	2	1	1	2	1	8	107	UCD 615,616	Zaikof/Schooner	Quillback Rockfish
QB 321	3	1	1	1	2	2	1	1	1	8	108	UCD 617,618	Zaikof/Schooner	Quillback Rockfish
QB 46	0	0	1	0	3	0	3	1	3	8	109	UCD 619,620	Zaikof/Schooner	Quillback Rockfish
QB 481	0	0	1	0	1	0	2	0	1	8	110	UCD 621,622	Zaikof/Schooner	Quillback Rockfish
QB 359	0	0	0	1	1	1	1	1	2	8	111	UCD 623,624	Zaikof/Schooner	Quillback Rockfish
QB 389	0	0	0	0	1	0	1	2	1	8	116	UCD 633,634	Zaikof/Schooner	Quillback Rockfish
QB 209	0	0	0	0	0	0	0	0	1	8	117	UCD 635,636	Zaikof/Schooner	Quillback Rockfish
QB 440	0	0	0	0	1	0	0	1	1	8	118	UCD 637,638	Zaikof/Schooner	Quillback Rockfish
QB 43	2	0	1	0	2	1	1	0	2	8	119	UCD 639,640	Zaikof/Schooner	Quillback Rockfish
QB 261	2	1	1	0	2	2	1	1	1	8	120	UCD 641,642	Zaikof/Schooner	Quillback Rockfish
QB 455	0	0	0	1	1	1	.	.	1	8	126	UCD 703,704	Zaikof/Schooner	Quillback Rockfish
QB 283	0	0	0	0	1	1	1	1	1	8	127	UCD 705,706	Zaikof/Schooner	Quillback Rockfish
QB 405	0	0	0	0	0	0	1	2	1	8	128	UCD 707,708	Zaikof/Schooner	Quillback Rockfish
SN 367	0	0	1	0	0	2	1	1	1	8	112	UCD 625,626	Zaikof/Schooner	Splitnose Rockfish
YE 257	0	0	1	0	0	1	1	0	2	8	106	UCD 613,614	Zaikof/Schooner	Yelloweye Rockfish

APPENDIX B

Restoration Science Study Proposal

Development of a Restoration Plan for Groundfish Stocks Affected by the Exxon Valdez Oil Spill

LITIGATION SENSITIVE / ATTORNEY WORK PRODUCT

RESTORATION SCIENCE STUDY PROPOSAL

A. Study name:

Development of a Restoration Plan for Groundfish Stocks Affected by the Exxon Valdez Oil Spill.

B. Injured species to be addressed:

Rockfishes (Sebastes and Sebastolobus spp.) and lingcod (Ophiodon elongatus). Adult demersal rockfishes suffered direct lethal and sublethal injuries from the Exxon Valdez oil spill (EVOS). Larval and pre-recruit rockfishes, as well as lingcod, probably sustained injury, although no studies were initiated to document this. In addition, the directed harvest and bycatch of rockfishes increased dramatically in 1990 and 1991 as fishing effort was redirected from salmon and herring to groundfish.

C. Principal investigators and biometricians:

William Bechtol, Biologist, Alaska Dept. of Fish and Game
Andrew Hoffmann, Biologist, Alaska Dept. of Fish and Game
Lisa Seeb, Statewide Geneticist, Alaska Dept. of Fish and Game
Patricia Hansen, Biometrician, Alaska Dept. of Fish and Game
Ivan Vining, Biometrician, Alaska Dept. of Fish and Game

D. Project objectives:

The goal of this project is to develop a restoration plan that modifies human use for the enhancement of groundfish resources in the area impacted by the EVOS. Initial objectives of this study are to:

1. Identify the species of concern. Over 20 species of rockfish are present in the study area; the species of concern will be identified by collecting species composition data from the sport and commercial fisheries.

2. Describe the biological characteristics of the species or stocks of concern. The commercial and sport fisheries will be sampled to describe age and size composition, natural mortality rates, growth rates, general seasonal movements, relative stock abundance, and relative recruitment.

3. Identify and define stocks to be enhanced through modification of human use. Genetic studies will document population structure and gene flow within and outside of the study area. These data can be used to predict how rapidly damaged or overfished populations can be restored from adult migration or larval drift from outside the

affected area. Tagging studies will be initiated, based on results of genetic and port sampling, to describe stock movements and potential recruitment sources in order to refine stock definition.

4. Describe the current and past patterns of human use of the groundfish resource. This will be done by summarizing current and historical harvest data by date, area, and fishery.

E. Project methods, including technical feasibility of the study:

Species, sex, size, and age data will be collected from commercial landings at ports processing rockfishes and lingcod from the EVOS affected area. Sampling and logistics will be coordinated with the ongoing sport harvest sampling (Assessment of Southcentral Alaska Sport Groundfish Harvest).

Observers will be placed on board a sample of commercial fishing vessels to quantify the magnitude and species composition of the discard.

Natural mortality rates will be estimated using: 1) age composition of unexploited or lightly exploited rockfish and lingcod stocks obtained by sampling from a chartered vessel, and 2) empirical relationships based on related biological characteristics.

Genetic structure and gene flow will be estimated using allozyme electrophoresis and restriction fragment length polymorphism (RFLP) of mitochondrial DNA (mtDNA). Rockfish and lingcod specimens will be collected from five sites within the EVOS affected area through coordination with commercial and sport fishery sampling described above. Rockfishes will be sampled from the three primary management groupings used by state and federal regulatory agencies: demersal shelf, pelagic shelf, and slope.

Biological data from current and historical landings (fish ticket system) will be analyzed over time and area to describe temporal and spatial patterns in human use of the groundfish resource.

The direction, magnitude, and timing of seasonal movements will be described through tagging. Results of port sampling and genetic analysis will be used to define the scope and objectives of tagging. Shallow dwelling rockfishes and lingcod will be tagged with external anchor tags, while demersal rockfishes will be marked with detachable hook tags. Tags will be recovered through port sampling programs and through voluntary returns. A reward and lottery program will

be used to encourage voluntary returns.

All of the methods described are common, reliable tools that have been or are currently applied successfully to rockfishes and lingcod. The integrated approach to sampling and data-sharing reduces cost and fosters development of a balanced, practical restoration plan.

F. Duration of the project (number of seasons needed to fulfill project objectives):

A minimum of five years are required to collect data and formulate a workable restoration plan. Port sampling, estimation of natural mortality, and genetic analyses will begin immediately. Within two years, results from these studies will guide formulation of tagging objectives and refinement of furthur sampling. Tagging studies will require two to three years. Analysis and formulation of a management plan will require one year.

G. Estimated cost (per year if more than one year):

Year 1: $255,000; Year 2: $180,000; Year 3: $235,000; Year 4: $195,000; Year 5: $195,000.

H. Restoration activity or endpoint to be addressed:

A restoration plan will be developed to enhance and protect injured stocks and provide for sustained human uses including recreational, commercial, and subsistence fishing.

I. Relation to science information needs identified by RPWG:

This proposal addresses several general science information needs identified by the RPWG. First, it will "improve our understanding of the long-range mechanisms limiting populations..." With population concerns present before the spill, and additional concerns imposed by the lethal and sub-lethal effects of the spill, there is a greater need to understand the population dynamics of these species and identify restoration options. Second, data will be collected from "beyond Prince William Sound and throughout (the) affected area." Third, rockfishes and lingcod can be considered appropriate indicators to "monitor ecosystem recovery" and "determine the level of restoration necessary."

J. Importance of initiating project in 1992:

A restoration plan with clearly defined objectives is needed to enhance groundfishes impacted by the EVOS. Rockfish populations supporting present and future fisheries suffered direct and indirect impacts. Documented <u>direct</u> impacts

included mortality of adult demersal rockfishes, and sublethal effects on spawning age adults. In addition, potential damage to larval and pre-recruit rockfishes and lingcod was acknowledged although no larval or pre-recruit studies were conducted. Indirect impacts included a substantial increase in fishing mortality during the 1990 and 1991 seasons. Fishermen who normally utilized short-lived pelagic species, such as salmon and herring, shifted to long-lived demersal rockfishes as a primary or secondary income source. This shift in fishing effort increased both targeted harvest and bycatch of rockfishes.

Rockfishes and lingcod are important links in the marine ecosystem, serving as important prey for other fishes and marine mammals. Rockfishes live long, grow slowly, have low larval-to-recruit survival rates, are often territorial, and require from 7 to 17 years to reach sexual maturity. Because many rockfishes in the EVOS affected area are at northern or western range limits, these populations represent an important genetic resource. Lingcod are also territorial and have highly variable recruitment. For both rockfishes and lingcod, recovery from overfishing or other perturbations can take decades, particularly if recruit and pre-recruit age classes are affected.

The documented direct and indirect effects of oil, combined with EVOS related increases in fishing mortality, threaten rockfish and lingcod populations. The slow recovery rates characteristic of these fishes necessitate the prompt formulation of a restoration plan to assure their future.

K. Link to other NRDA damage assessment or restoration studies:

The NRDA Fish/Shellfish Study #17 (now Sub-tidal Study #6), Injury to Rockfish and Shallow Reef Habitat in Prince William Sound, documented lethal and sub-lethal effects on rockfishes in oiled areas of Prince William Sound.

A proposed restoration study, DNA Breakages of Fish and Shellfish Populations in Prince William Sound, will provide the technical service of flow cytometry on groundfish samples. This technique can be used to monitor the purging of damaged DNA from EVOS affected populations.

DRAFT

OIL SPILL PROGRESS REPORT

GROUNDFISH TRAWL ASSESSMENT INSIDE AND OUTSIDE PRINCE WILLIAM SOUND

Subtidal Number 7

Assessment of Oil Spill Impacts on Fishery Resources:

Measurement of Hydrocarbons and Their Metabolites, and Their Effects, in Important Species

by

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EXECUTIVE SUMMARY

Studies were conducted in 1991to continue to assess damage to fisheries resources related to the *EXXON VALDEZ* oil spill (EVOS). These studies were designed to help determine the degree of exposure of biota to petroleum derived compounds, specifically aromatic hydrocarbons, and assess possible effects on various species resulting from such exposure. From February to June, 1991, samples of fish were collected from 29 sites in Prince William Sound and the Shelikof Straits. Over 550 bile and 500 liver samples were obtained from 5 species of fish from pelagic and benthic habitats. To date, over 400 of the bile samples have been analyzed for the presence of fluorescent aromatic compounds (FACs), and about 90 of the liver samples have been analyzed for aryl hydrocarbon hydroxylase (AHH) activity, which is known to be increased after exposure of fish to chemical contaminants. These assays (biliary FACs and hepatic AHH) were used to determine degree of exposure of fish to aromatic compounds. Changes in hepatic AHH activities can also indicate a physiological change as a consequence of exposure. Results from measurements of levels of FACs in bile suggest that some fish (flathead sole and pollock) continued to be exposed to petroleum derived compounds at sites inside Prince William Sound. Generally, however, concentrations of FACs in bile of all fish species were lower in 1991 than in 1990. Based on the results of the bile FAC and hepatic AHH analyses, samples were chosen for analyses of histopathological changes and reproductive dysfunction. The histopathological analyses are in progress. Assessment of reproduction has thus far comprised analysis for plasma estradiol concentration, gonadosomatic index (GSI) and ovarian maturation stage in about 80 yellowfin sole from sites in Prince William Sound and approximately 300 pollock from 8 sites within Prince William Sound and 10 sites outside PWS. The data obtained thus far do not indicate a substantial impact on measured reproductive processes in these 2 species.

OBJECTIVES

A. To sample selected fish species (e.g. pollock, yellowfin sole, rock sole, flathead sole, Pacific cod) from several sites inside and outside Prince William Sound, with emphasis on sites inside Prince William Sound. Site selection is primarily based on data from the last two years of sampling and analyses. Representative sediment samples are also taken from each benthic sampling site for subsequent chemical analysis.

B. To estimate the exposure to petroleum hydrocarbons by measuring levels of hydrocarbon metabolites in bile of the above species from oiled and nonoiled habitats such to detect significant differences in bile concentrations with $\alpha = 0.05$. Additionally, stomach contents of fish showing high levels of hydrocarbon metabolites in bile will be analyzed for hydrocarbons, such to detect significant differences in concentrations with $\alpha = 0.05$.

C. To estimate the induction of hepatic aryl hydrocarbon hydroxylase activity or increased levels of cytochrome P-450IA in the above species from oiled and nonoiled habitats such to detect statistical differences in levels of effects with $\alpha = 0.05$.

D. To estimate the prevalence of pathological conditions in the above species from oiled and nonoiled habitats such to detect statistical differences in levels of effects with $\alpha = 0.05$.

E. To estimate the levels of plasma estradiol, the degree of ovarian maturation, and fecundity in adult females of two of the above species (yellowfin sole and pollock) from oiled and nonoiled habitats such to detect statistically significant differences with $\alpha = 0.05$.

F. To estimate temporal changes in the parameters described in Objectives B&C, by comparing data obtained in 1991 to data obtained in 1989 and 1990. In order to assess either recovery or increased damage of habitats from the oil spill, trends in these parameters must be statistically significant at $\alpha = 0.05$.

G. Using the above data, as appropriate, construct simulation models similar to those of Schaaf et. al. (1987) for important Alaskan fish species for use in estimating oil spill impacts on fishery resources. These models will incorporate pre-spill information from the fisheries literature on mortality and fecundity together with information on reproductive impairment, pathological conditions, and biochemical effects in fish exposed to petroleum hydrocarbons as a result of the spill.

INTRODUCTION

Because petroleum and its components can cause severe damage to fishery resources, and because our studies in 1990 showed evidence of continuing exposure to petroleum-derived compounds in fish from several sites, monitoring of the nearshore fisheries resources of Prince William Sound was carried out in 1991. The monitoring included measurement of petroleum exposure and short-term effects, as was done in the summer and fall of 1989 and the summer of 1990, and encompassed a selected assessment of long-term biological effects, including

measurements of reproductive dysfunction and histopathological lesions of liver, gill,and gonad, as was done in the summer of 1990 (Varanasi et al., 1990, 1991). However, the scope of the continued studies was reduced substantially compared to studies done in 1989 and 1990, in that the primary study area was limited to Prince William Sound, and fewer species were examined. This narrowing of focus reflects our findings of the previous two years, and is aimed at continuing only those portions of the study which are most likely to assist in documentation of injury or damage. An expansion to this study included the measurement of petroleum exposure and possible effects in pollock from Prince William Sound and the Shelikof Strait. The rationale for this is described below.

Certain petroleum components [e.g. aromatic hydrocarbons (AHs)] can cause reproductive toxicity and teratogenicity in rodents (Shum et al., 1979; Gulyas and Mattison 1979, Mattison and Nightingale, 1980). Similarly, reproductive impairment has been noted in benthic fish residing in contaminated areas of San Francisco Bay (Spies and Rice, 1988) and southern California (Cross and Hose, 1988). Moreover, English sole from areas of Puget Sound having high sediment concentrations of AHs showed inhibited ovarian maturation (Johnson et al., 1988), and fish from these areas that did mature often failed to spawn after hormonal treatment to induce spawning (Casillas et al., 1991). In general, reproductive impairment (including reduced plasma levels of the sex steroid, estradiol) was found in English sole which showed evidence of exposure to aromatic compounds. Moreover, our laboratory studies have shown that plasma levels of estradiol are reduced in gravid female English sole exposed to chemical contaminants extracted from urban sediments (Stein et al., 1991), and, more importantly, our preliminary laboratory studies have also shown that exposure to Prudhoe Bay crude oil reduced plasma levels of estradiol in gravid female rock sole. In view of our findings of the

last two years, the continued assessment of possible reproductive dysfunction in animals from impacted areas will be very important in determining biological damage to living marine resources as a result of the oil spill. Levels of plasma estradiol, GSI and ovarian maturation have been determined in selected species. Combined with our measurements of petroleum exposure (e.g. metabolites in bile and enzyme activities in liver), these studies enable us to estimate the degree of reproductive dysfunction which may be occurring in oil-exposed fish.

Exposure of animals to crude oil can also result in changes at the tissue and cellular levels (National Academy of Sciences, 1985). Examples of such changes after exposure of fish to oil-contaminated sediments include liver hypertrophy and fatty liver in winter flounder (Payne et al., 1988) and the occurrence of hepatocellular lipid vacuolization in English sole (McCain et al., 1978). Certain AHs (e.g., benzo[a]pyrene) are known carcinogens in rodents and fish (Lutz, 1979; Bailey et al., 1989), and studies with several bottomfish species show that, of the xenobiotic chemicals in sediments, AHs are most strongly associated with high prevalences of liver lesions, including neoplasms (Myers et al., 1987; Varanasi et al., 1987; Baumann, 1989). Generally, histopathological lesions of the types noted above do not become manifest until at least several months after exposure. However, by the summer of 1991, fish in and around oil impacted sites have potentially been exposed to petroleum components for more than two years. Moreover, there are some published data which suggest that histopathological changes have occurred in some fish species as a result of exposure to oil spilled from the EXXON Valdez (Khan et al., 1990). Accordingly, assessment of histopathological effects in selected species is strongly warranted.

Finally, preliminary studies conducted by our Division in the spring of 1990

(independent of the damage assessment process) suggested that pollock were being exposed to petroleum both inside and outside Prince William Sound. Because of the enormous commercial importance of the pollock fishery, assessment of exposure and possible associated biological effects in pollock, both inside and outside Prince William Sound was carried out.

Briefly, measurement of exposure to oil and oil components in the biota of Prince William Sound and other areas affected by the oil spill was continued, by determining levels of hydrocarbon metabolites in bile and by measuring hepatic AHH activities. Additionally, a range of biological effects, especially indicators of reproductive dysfunction and histopathological effects was evaluated. Only by employing such a broad spectrum of state-of-the art chemical, biochemical and biological methods can analytical data be obtained to document the degree of exposure and resultant biological effects of petroleum hydrocarbons on economically and ecologically important fish species.

METHODOLOGY

(see Appendix A for details)

A. *Collection areas*

Sampling activities were conducted from February 12 to June 1991 at 28 sites both inside and outside of Prince William Sound (see the site maps, Figure 1).

B. *Sampling procedures*

Sample collection methods were outlined in the 1991 Study Plan . Fish were collected at depths ranging from 2 to 60 meters using bottom trawls, long-line gear, gill nets, and beach seines. This equipment was deployed from launches and skiffs carried aboard the NOAA vessel *MILLER FREEMAN* at the sites sampled in the Bering Sea and Shelikof Strait, the Research vessel *PANDALUS* and the charter vessel *BIG VALLEY* in Prince William Sound.. All fish used for sample collections were kept alive in a flowthrough seawater system at ambient water temperature until necropsied.

C. *Laboratory Analyses*

1. Bile Metabolite Assay

Assay methods generally were the same as outlined in the Study Plan . Briefly, bile samples were analyzed at wavelengths appropriate for detection of naphthalene (NPH) and phenanthrene (PHN) and their metabolites. These aromatic hydrocarbons (and especially their alkylated derivatives) are predominant components of the aromatic fraction of crude oil. Hereafter, analyses done at these wavelengths are referred to as $FACS_{NPH}$ and $FACS_{PHN}$, respectively. In addition, bile samples this year were analyzed for total biliary protein by the method of Lowry et al. (1951), and $FACS_{NPH}$ and $FACS_{PHN}$ were normalized against the total biliary protein, as this type of normalization (within a single species) can account for some physiological variability in bile concentration, as described in Collier and Varanasi (1990).

2. Liver Aryl Hydrocarbon Hydroxylase (AHH) Assay

Analytical methods used for measuring hepatic AHH activity were as outlined in the 1991 Study Plan

3. Histopathology

Histopathological procedures followed were as described in the 1991 Study Plan .

4. Reproductive Indicators

Assessment of reproductive activity was done as described in the 1991 Study Plan .

D. *Quality Assurance and Control Plans*

All quality assurance and control plans for bile analytes, and AHH analysis, followed the procedures outlined in the 1989 Study Plan (p. 28).

E. *Data Analysis*

Statistical tests and analytical procedures for preliminary statistical analyses were as described in the 1991 Study Plan

RESULTS

A. *Fish and Tissue Sample Collection*

Samples were collected from over 550 individual animals representing 5 fish species (Table 1). Totals of 508 liver samples, 553 bile samples, 44 samples of stomach contents and 314 plasma samples were collected at the 29 sites sampled. In addition, histological samples of liver, gonad, and gill were taken from all fish.

B. *Laboratory Analysis*

The samples which have been analyzed thus far were selected to represent sites with a range of potential oil exposure. In order to insure adequate numbers for statistical analyses, samples were generally analyzed only from sites where six

or more individuals were collected.

1. Bile analysis

Figure 2 shows mean levels of $FACs_{PHN}$ in bile from yellowfin sole sampled from Alaskan waters prior to the EVOS, and in addition includes results from analyses of bile from salmon and halibut collected after the spill, but from an area (Angoon) believed to be unaffected by the EVOS. Figure 2 was taken from the 1989 Progress Report for this Project. As stated in that report, the data presented in Figure 2 are only intended for use as general reference values.

This year, 409 individual bile samples, from 5 fish species from 25 sites, have been analyzed. Each of these samples was analyzed at both PHN and NPH wavelengths, and, similar to what we found in 1989 and 1990, there was a very strong correlation between $FACs_{PHN}$ and $FACs_{NPH}$. Accordingly, in this preliminary report we will present primarily the $FACs_{PHN}$ data. We report the bile data both per g bile ($FACs_{PHN}$) and normalized against total biliary protein ($FACs_{PHN/PROTEIN}$), as described in the methods section above.

The mean $FACs_{PHN/PROTEIN}$ values in bile of flathead sole, Pacific cod, rock sole, and yellowfin sole collected in 1991 are shown in Figures 3-6. In general, there is excellent agreement between the protein normalized bile data and the uncorrected bile values, so we will discuss only the protein normalized results here. Only for flathead sole (Figure 3) were levels of $FACs_{PHN/PROTEIN}$ in bile were significantly different (higher) at Fox Farm and Snug Harbor than those found for the lowest site (Olsen Bay). In contrast, there were no significant site differences found in levels of $FACs_{PHN/PROTEIN}$ in bile of yellowfin sole, Pacific cod and rock sole (Figures 4-6).

A statistical analysis was conducted of the bile metabolite data from several sites for 1990 and 1991, as well as for the one site (Snug Harbor) from which fish were collected in all three years (1989-1991), and the results are shown in Figures 7-10. For fish collected in 1991, rock sole from Snug Harbor and Sleepy Bay and yellowfin sole from Snug Harbor had levels of $FACS_{PHN/PROTEIN}$ in bile which were significantly different (lower) than the 1990 levels (Figures 8 and 9). In addition, flathead sole collected in 1991 from Snug Harbor and Sleepy Bay had levels of $FACS_{PHN}$ in bile that were significantly different (lower) than the 1990 levels (Figure 7). Levels of $FACS_{PHN/PROTEIN}$ in bile of flathead sole and rock sole collected in 1989 and yellowfin sole collected in 1989 and 1990 from Snug Harbor were significantly different (higher) than the 1991 levels (Figure 8).

In 1991, pollock were collected from 19 sites within Prince William Sound (PWS) and from the Shelikof Strait for Fish/Shellfish Study 24; a previous collection in 1990 was conducted under the oil spill response effort. Again, we will discuss only the protein normalized results. The mean $FACS_{PHN/PROTEIN}$ values in bile of the pollock collected, both inside and outside PWS, in 1991 are shown in Figure 11. Pollock at four sites (Figure 11), all within PWS, had levels of $FACS_{PHN/PROTEIN}$ in bile that were significantly different (higher) than those found for pollock from Uganik Island (lowest site) or Eastend Transect (site from an area unimpacted by oil). A statistical analysis was conducted of the bile metabolite data for pollock collected from sites sampled in both 1990 and 1991; the results are shown in Figures 12. For pollock collected in 1991, levels of $FACS_{PHN/PROTEIN}$ in bile were significantly different (lower) than the 1990 levels at Mummy Island/Bay, Naked Island and Point Bazil.

Recent studies in our laboratory (Appendix B) carried out as a complement

to our oil spill studies have provided further evidence that the FACs measured in bile of fish after the EVOS are in fact derived from petroleum compounds. This publication should be consulted for further details.

2. Measurement of hepatic AHH activity

Thus far, hepatic AHH activity has been measured in 90 samples from three species of fish collected at 3 sites. Further analyses are ongoing. The initial round of analyses emphasized comparisons between a known oil-impacted site (Snug Harbor) and a site where little or no oil impact has been noted (Olsen Bay, near Port Gravina). Analyses thus far have also emphasized flatfish species, which were shown in the previous two years of studies under F/S 24 have continuing exposure to oil at oil-impacted sites both inside and outside Prince William Sound. Our results are presented in Figures 13-15. Briefly, AHH activity continues to be induced in flatfish sampled from Snug Harbor, presumably as a result of the EVOS. Both rock sole and flathead sole had significantly increased hepatic AHH activities at Snug Harbor compared to fish captured at Olsen Bay, and compared to the previous year's data, neither species showed any decrease in AHH activity from 1990 to 1991, in fish sampled from Snug Harbor. However, there was a significant decrease in hepatic AHH activity between 1990 and 1991 in rock sole sampled from Sleepy Bay. There were not enough flathead sole collected from Sleepy Bay in 1991 to allow a reasonable statistical analysis of the results. Analyses of hepatic AHH activity are continuing in rock sole and flathead sole collected during 1991. Initial analyses of hepatic AHH activity in yellowfin sole have shown that there was an apparent depression of this enzyme in females, most probably related to their gonadal maturity, as AHH activity is generally depressed in female fish as they near spawning. Analyses of AHH activity in male yellofin sole, however, showed

apparent induction of hepatic AHH activity in fish from Snug Harbor. However, analyses of AHH activities in male from Olsen Bay are not yet completed, thus a statistical comparison of 1991 data cannot be done at this time. Overall, these results suggest that induction of hepatic AHH activity is continuing in flatfish from at least one site in Prince William Sound, as an apparent result of the EVOS.

3. Indicators of reproductive success

Adult female yellowfin sole and adult female pollock were examined for evidence of petroleum-associated reproductive dysfunction. Several indicators of reproductive development were measured, including concentrations of estradiol in plasma, gonadosomatic index (GSI), and ovarian maturation stage (determined grossly). For yellowfin sole, plasma estradiol concentrations and GSI have been determined in 90 fish captured at three sites. For pollock, plasma estradiol concentrations and GSI have been measured in 227 fish from 18 sites, 10 within Prince William Sound and 8 in the Shelikof Straits.

Analysis of yellowfin sole samples indicate that neither plasma estradiol concentrations nor GSI appear to be depressed in fish from Snug Harbor, the site at which fish had highest concentrations of metabolites in bile (Fig. 16 a,b). Moreover, plasma estradiol concentrations show little relationship with levels of NPH or PHN metabolites in bile (Fig. 17 a,b). GSI is negatively correlated with concentrations of both NPH and PHN metabolites in bile, and appears to be depressed in fish with highest concentrations of metabolites in bile (i.e. > 25000 mg/g bile protein for NPH and > 3000 ng/g bile protein for PHN) (Fig. 17 c,d). However, the fish with highest concentrations of bile metabolites were somewhat smaller than other animals (278 ± 28 mm vs 311 ± 46 mm), and possibly too young to be sexually mature. Results of multiple regression analysis (Table 2) indicate

that when fish size is taken into account, the relationship between GSI and bile metabolites is no longer statistically significant, suggesting that depressed GSI was not necessarily related to petroleum exposure.

Analysis of pollock samples indicates that both GSI and plasma estradiol concentrations tended to be lower in pollock sampled from within Prince William Sound than in those from the Shelikof Strait (Fig. 18 a,b). However, these differences in reproductive status did not appear to be due to current levels of oil exposure because there was no correlation between concentrations of NPH or PHN metabolites in bile and either plasma estradiol or GSI (Fig. 19 a-d). It is possible that past exposure could account for reduced plasma estradiol concentrations and GSI in fish from sites that were originally heavily oiled such as Naked Island and Mummy Bay. However, an alternative explanation for the observed differences in reproductive development between fish from inside and outside Prince William Sound is that spawning occurs slightly earlier in the Prince William Sound fish. From visual examination of ovaries it appeared that a higher proportion of females was spawning within Prince William Sound than outside (24% vs 2%). There also appeared to be a higher number of regressed females at sites within Prince William Sound (31% vs 15%), but animals judged to be immature based on visual examination of the ovaries may actually be spawned out, since the two stages can be difficult to distinguish without microscopic examination of the ovaries. Pollock ovaries are now being examined histologically for accurate assessment of their developmental stage. If histological examination confirms that a substantial proportion of pollock from within Prince William Sound had just finished spawning, this could account for low GSI and plasma estradiol concentrations in fish from these sites, rather than any effect of oil exposure.

4. Histopathology

Histopathological analyses of tissues are currently in progress.

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Table 1. Species and number of fish collected for F/S 24 aboard the NOAA Ships *MILLER FREEMAN* , the Research Vessel *PANDALUS* and the charter vessel *BIG VALLEY* from February to June, 1991.

Species common name	Scientific name	# collected
flathead sole	*Hippoglossoides elassodon*	62
yellowfin sole	*Limanda aspera*	145
rock sole	*Lepidopsetta bilineata*	61
Pacific cod	*Gadus macrocephalus*	24
pollock	*Theragra chalcogramma*	300
TOTAL		589

Table 2. Relationship between day of capture, length, condition factor, and concentrations of fluorescent aromatic compounds in bile measured at phenanthrene (PHN) and napthalene (NPH) wavelengths (mg/g bile protein) and gonadosomatic index (GSI) and plasma estradiol concentrations in female yellowfin sole from Prince William Sound.

					Independent Variable				
Dependent Variable	df	Significance of Regression	RS	Snug Harbor	length	Day of Capture	Condition Factor	PHN	NPH
GSI	88	p=0.0001	0.52	ns	(+) p=0.0001	(+) p=0.0003	ns	ns	ns
Estradiol	88	p=0.0001	0.29	ns	(+) p=0.0056	(+) p=0.0006	ns	ns	ns

ns = not significant

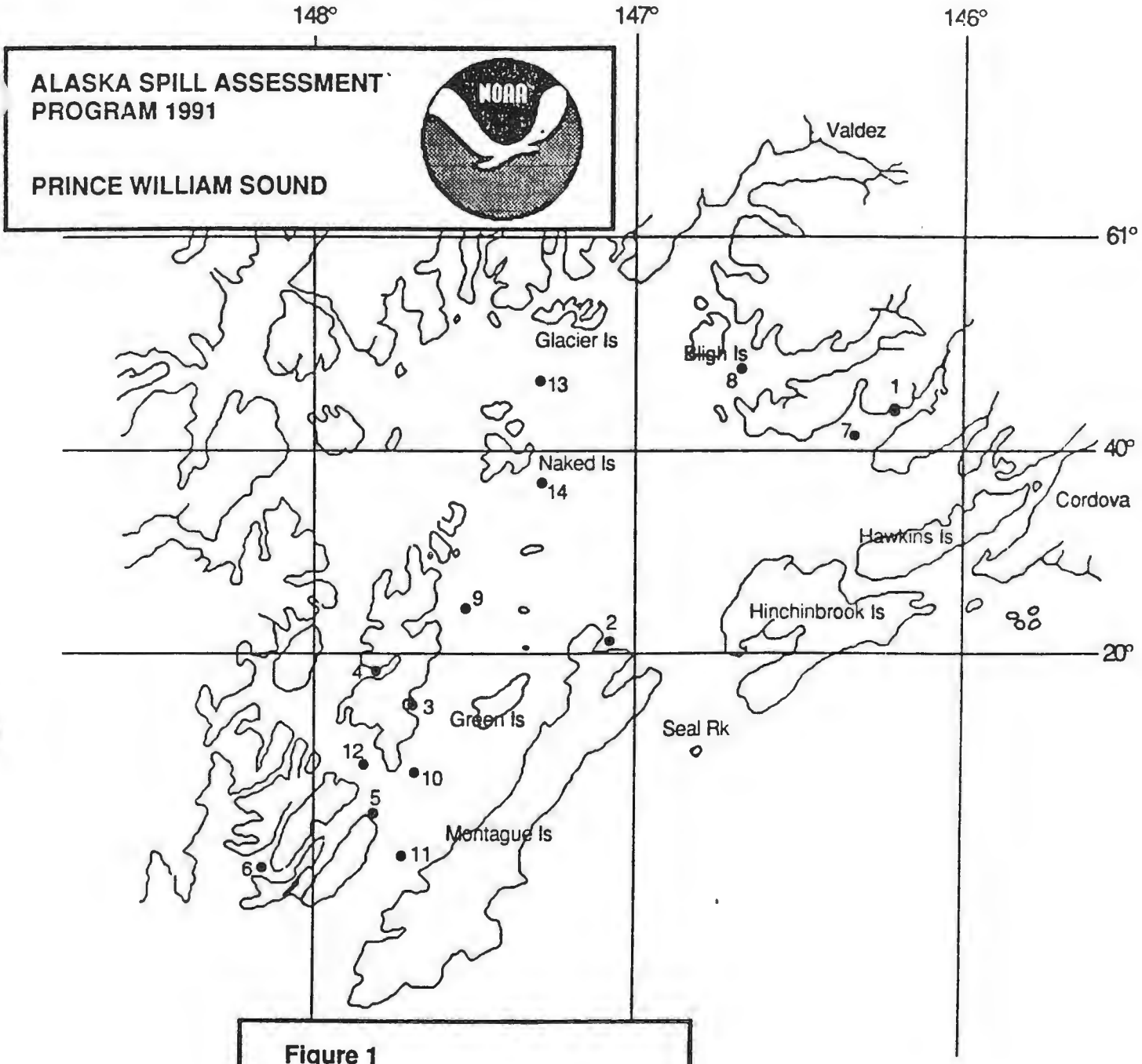


Figure 1

Sites - Prince William Sound

1 Olsen Bay	8 Port Fidalgo
2 Rocky Bay	9 Bay of Isles
3 Snug Harbor	10 Hogan Bay
4 Drier Bay	11 Point Bazil
5 Sleepy Bay	12 Mummy Bay
6 Squirrel Bay	13 Naked Island North
7 Port Gravina	14 Naked Island East

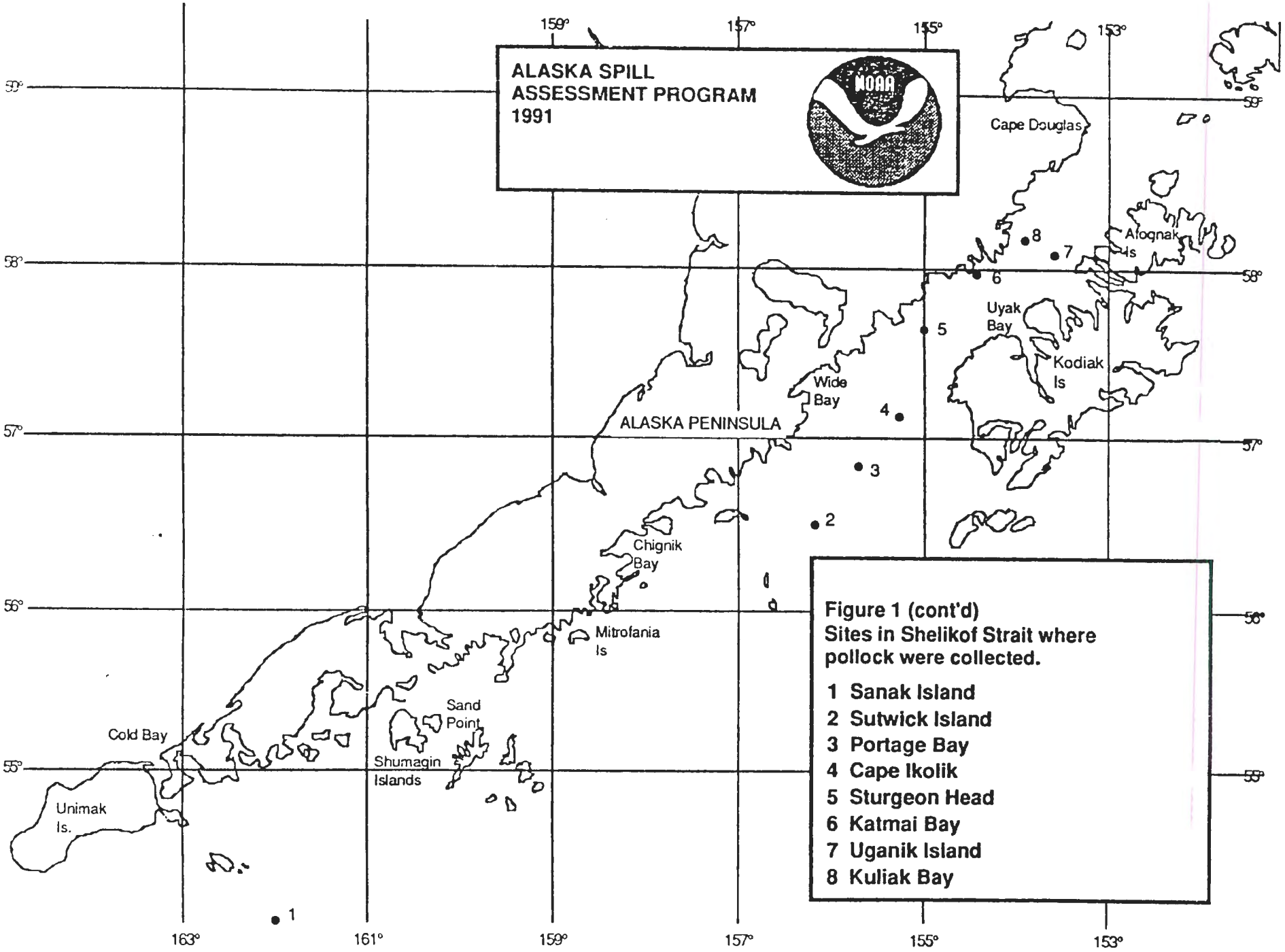


Figure 1 (cont'd)
Sites in Shelikof Strait where pollock were collected.

1 Sanak Island
2 Sutwick Island
3 Portage Bay
4 Cape Ikolik
5 Sturgeon Head
6 Katmai Bay
7 Uganik Island
8 Kuliak Bay

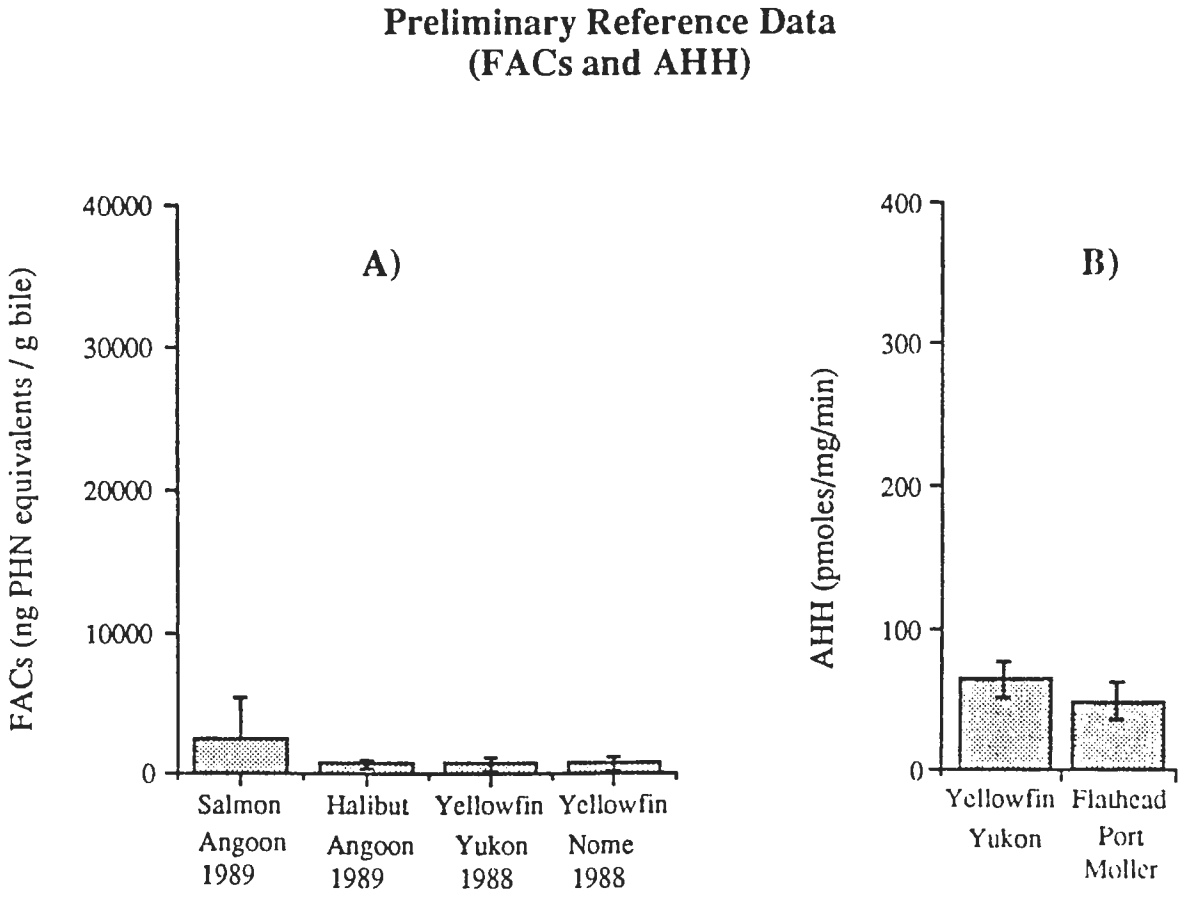


Figure 2. A) Average levels (± standard deviation) of fluorescent aromatic compounds determined at phenanthrene wavelengths ($FACs_{PHN}$) in bile of three species of fish collected in 1989 from locations not affected by the EXXON VALDEZ oil spill, or prior to the spill (from 1989 Progress Report for F/S 24).

B) Average hepatic AHH activities (± SE) in yellowfin sole and flathead sole collected during 1988. Data for yellowfin sole from National Benthic Surveillance Project, Cycle V; data for flathead sole from Collier and Varanasi, 1987.

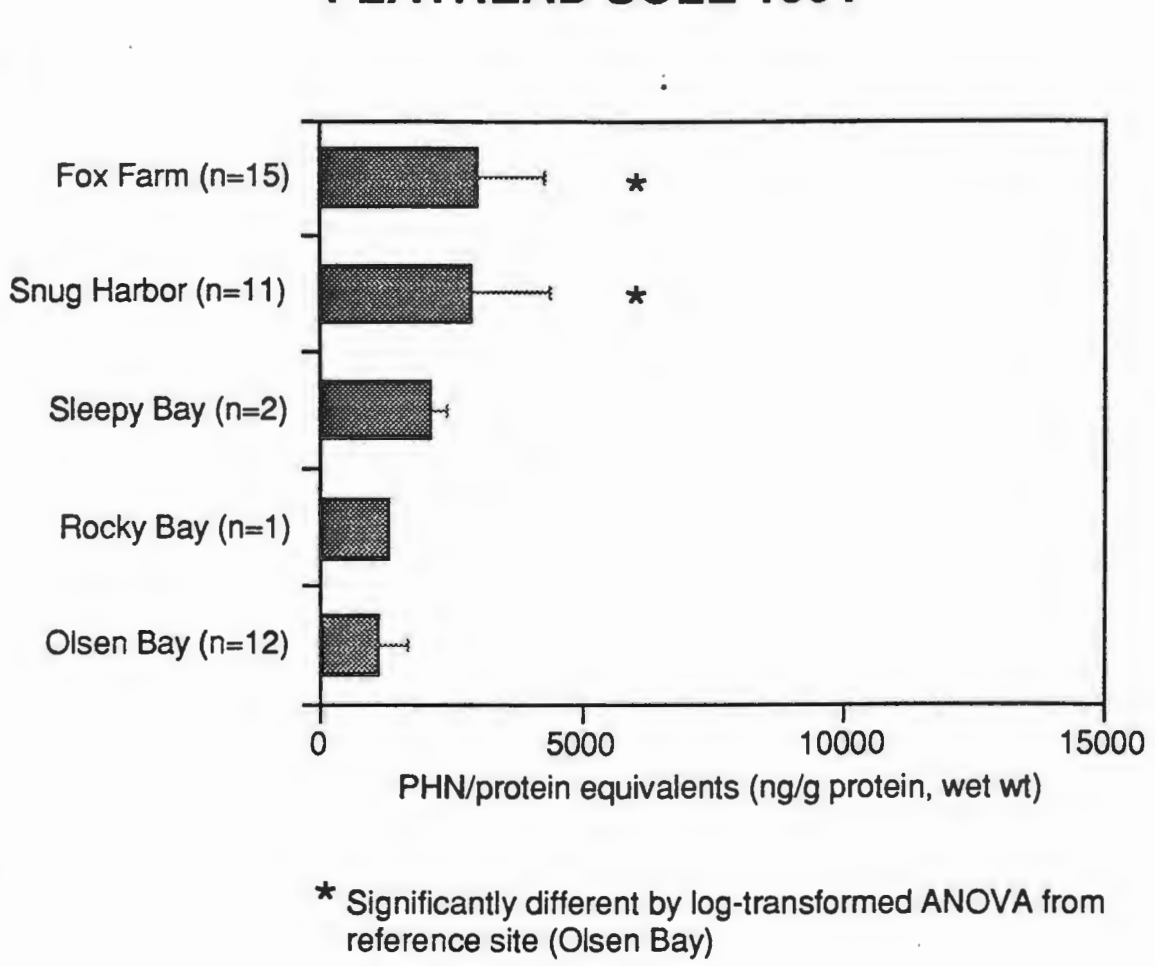


Figure 3. Average levels (± SD) of FACs PHN in bile of flathead sole collected in 1991.

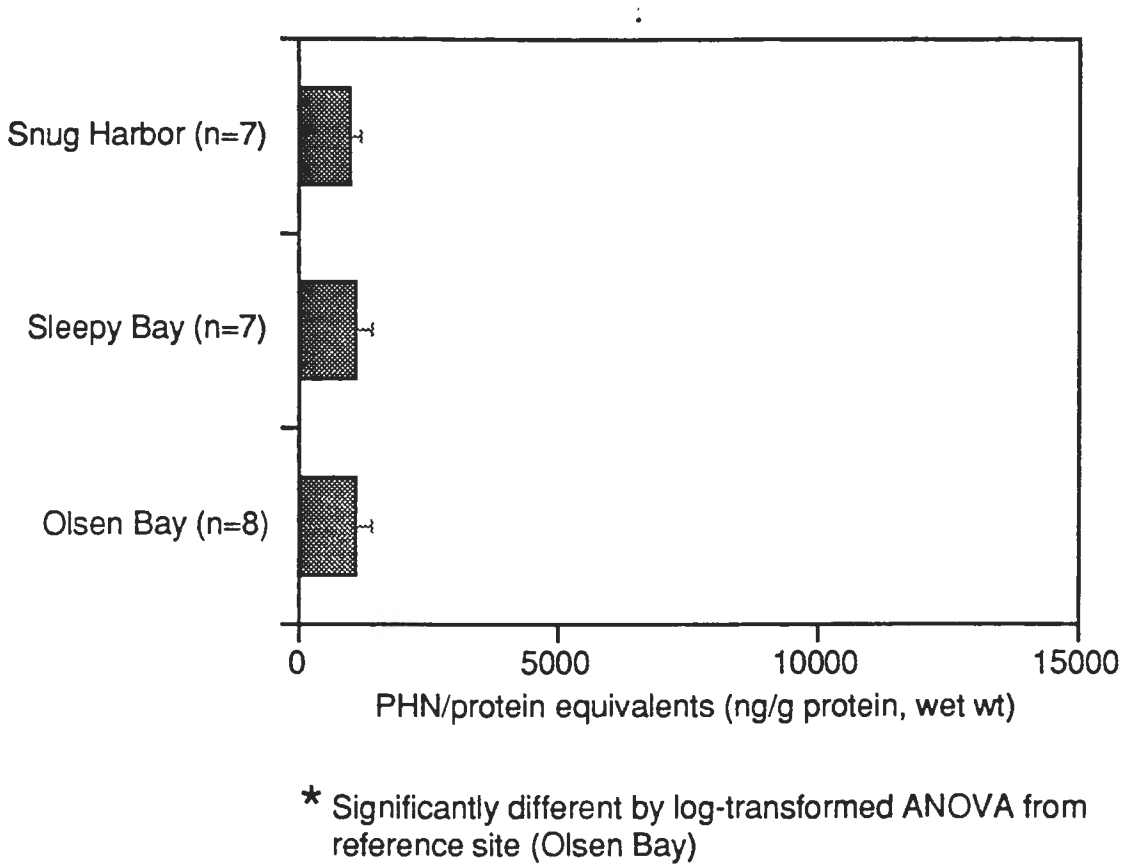


Figure 4. Average levels (± SD) of FACs PHN in bile of Pacific cod collected in 1991.

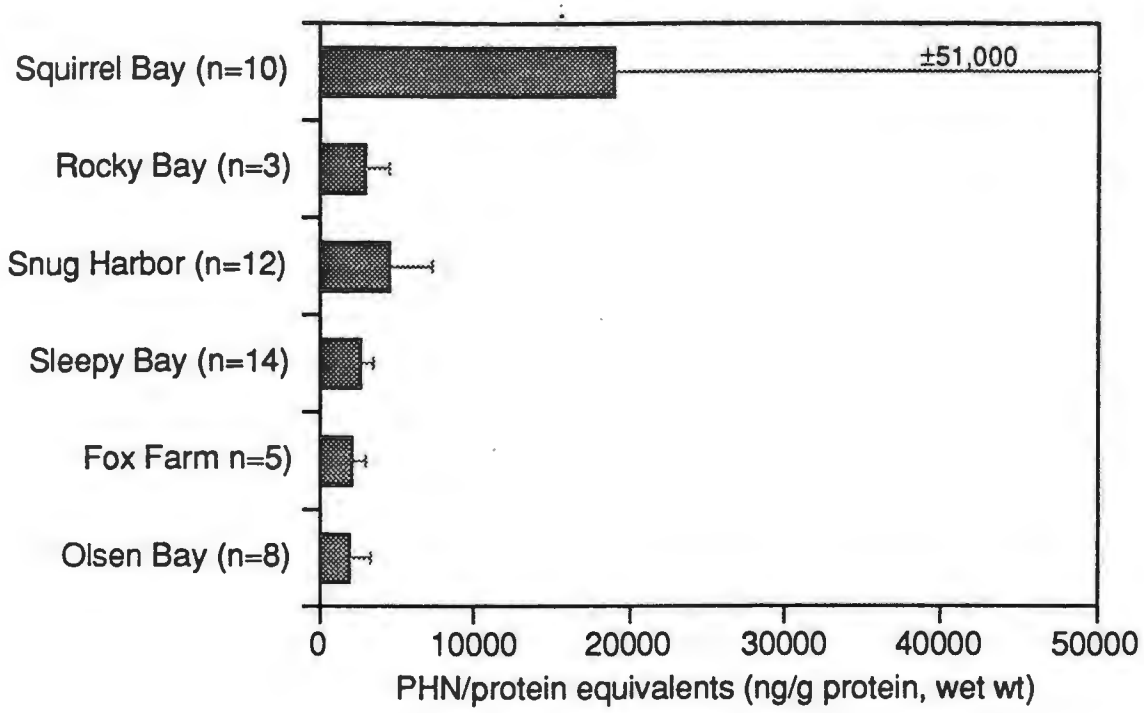


Figure 5. Average levels (± SD) of FACs PHN in bile of rock sole collected in 1991.

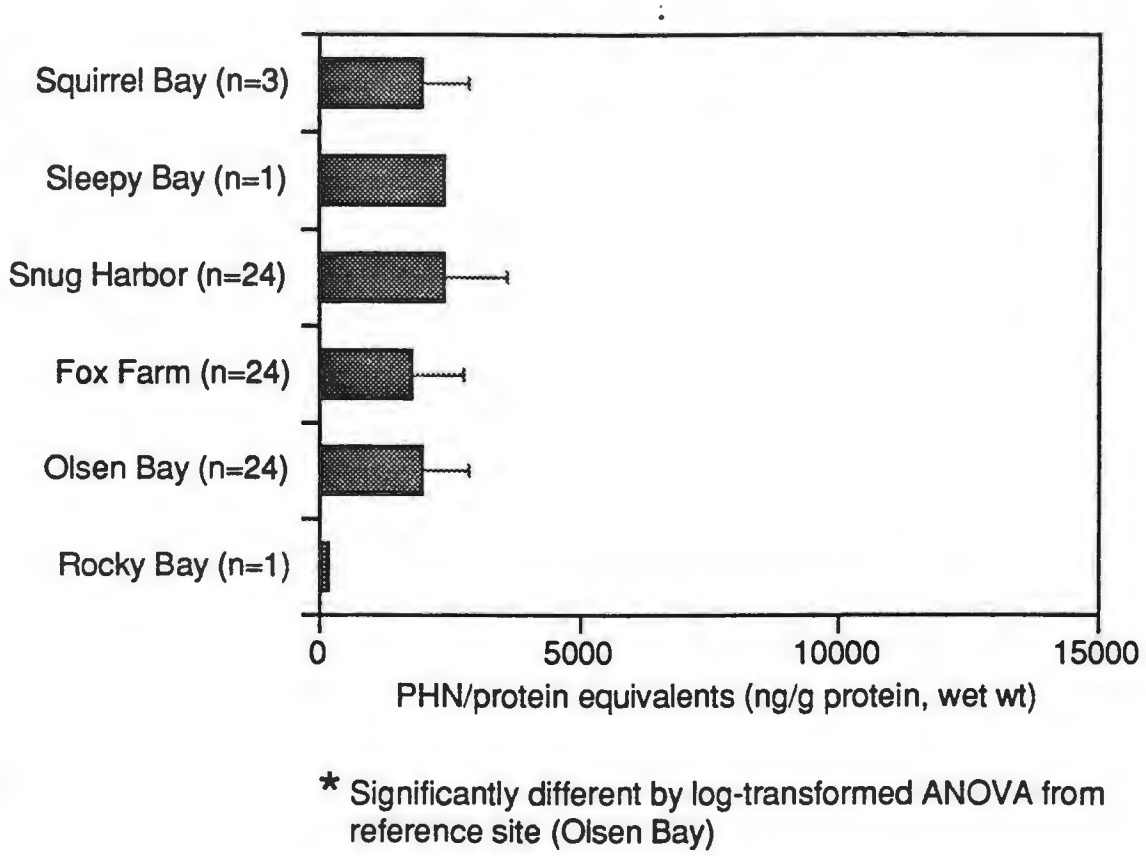
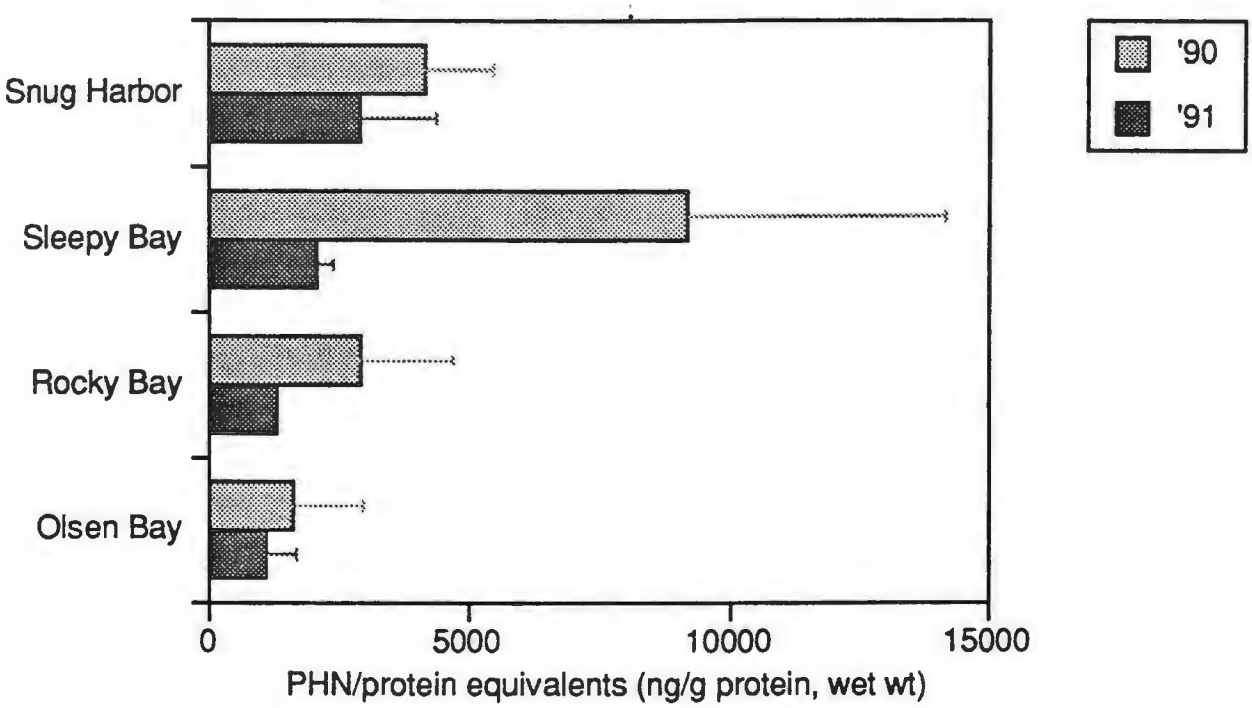


Figure 6. Average levels (± SD) of FACs PHN in bile of yellowfin sole collected in 1991.

FLATHEAD SOLE 1990-1991



* Significantly different by log-transformed ANOVA ('90 vs'91)

Figure 7. Average levels (± SD) of FACs PHN in bile of flathead sole collected in 1991 compared to levels for 1990.

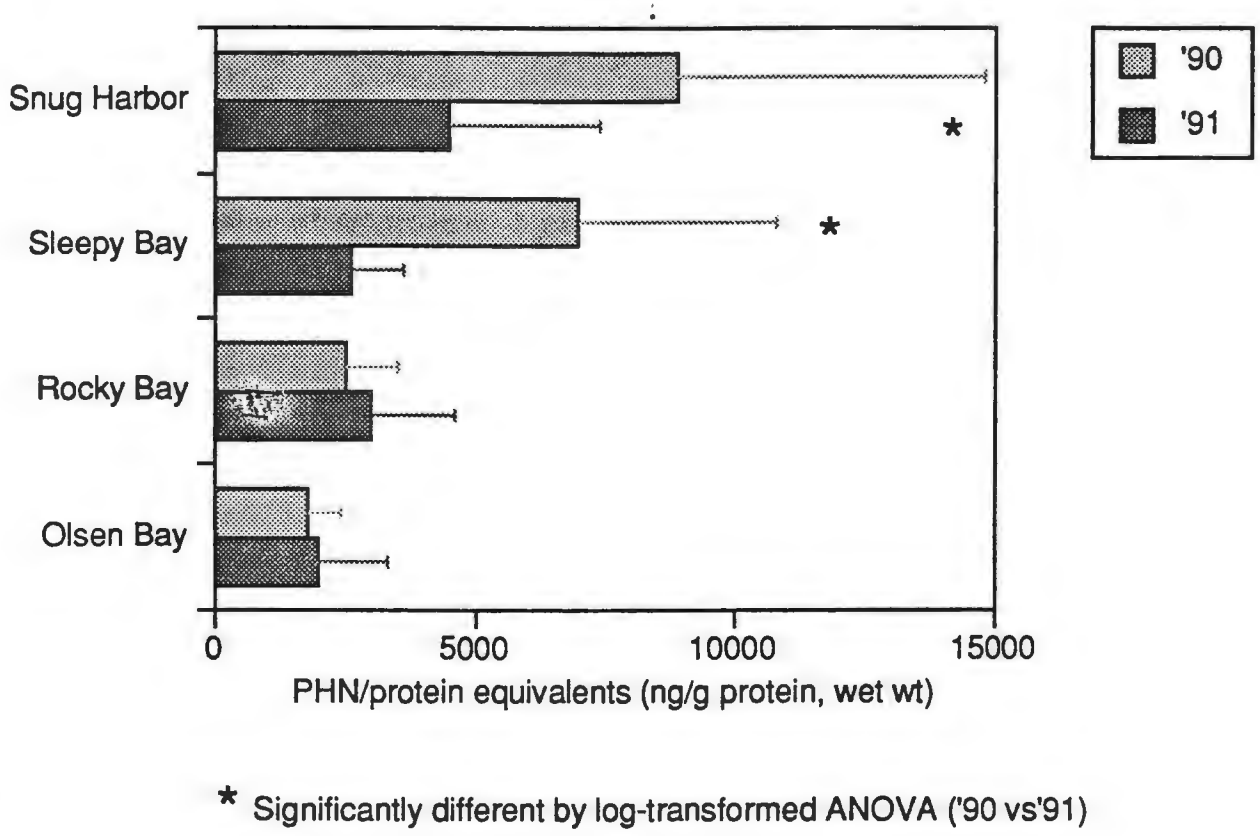


Figure 8. Average levels (± SD) of FACs PHN in bile of rock sole collected in 1991 compared to levels for 1990.

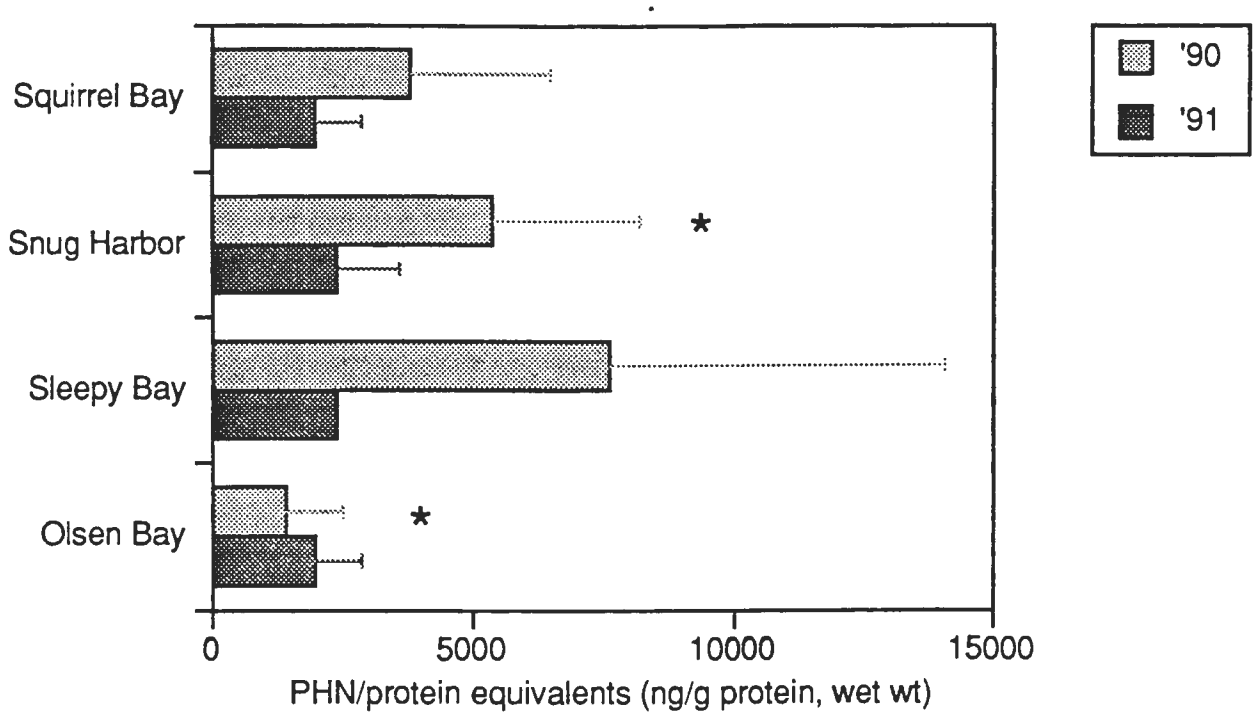


Figure 9. Average levels (± SD) of FACs PHN in bile of yellowfin sole collected in 1991 compared to levels for 1990.

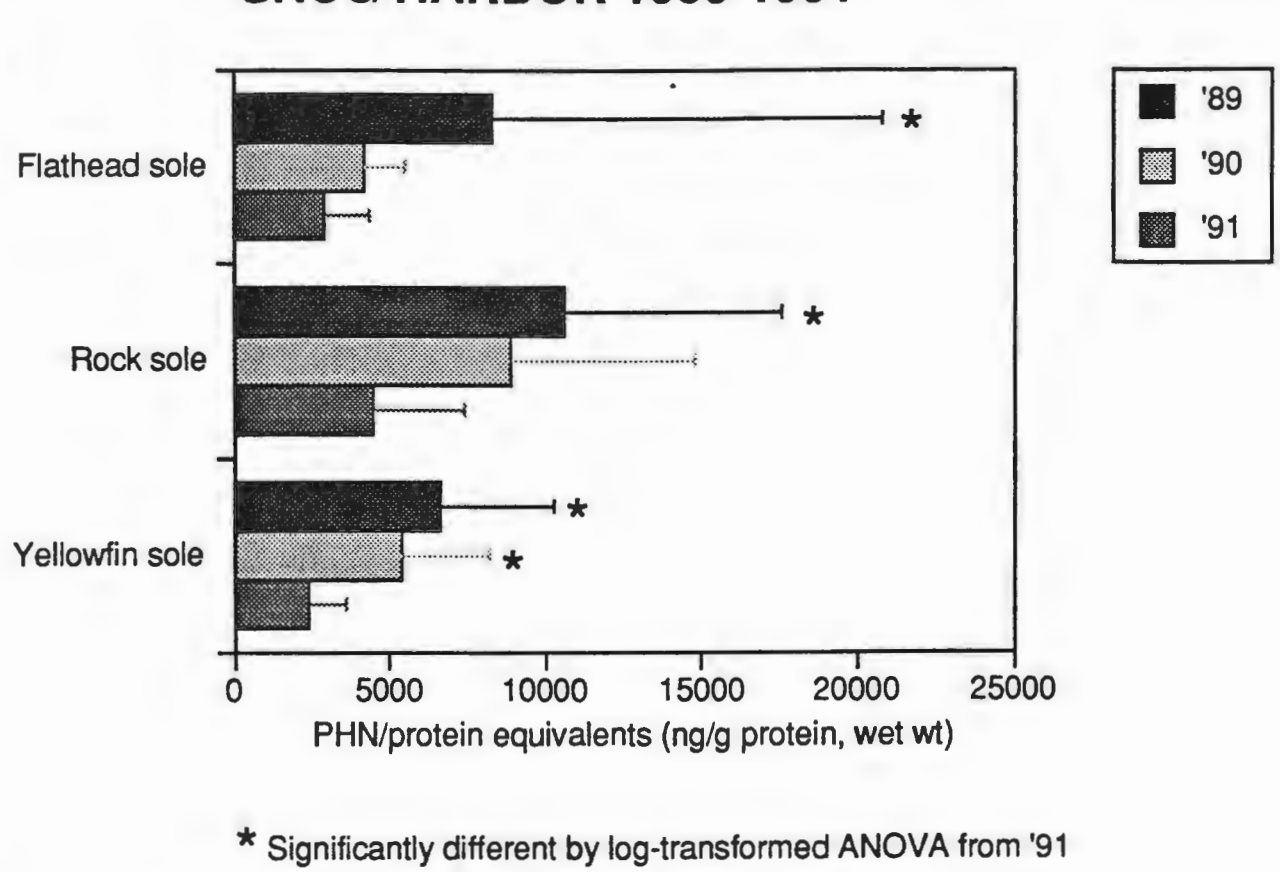
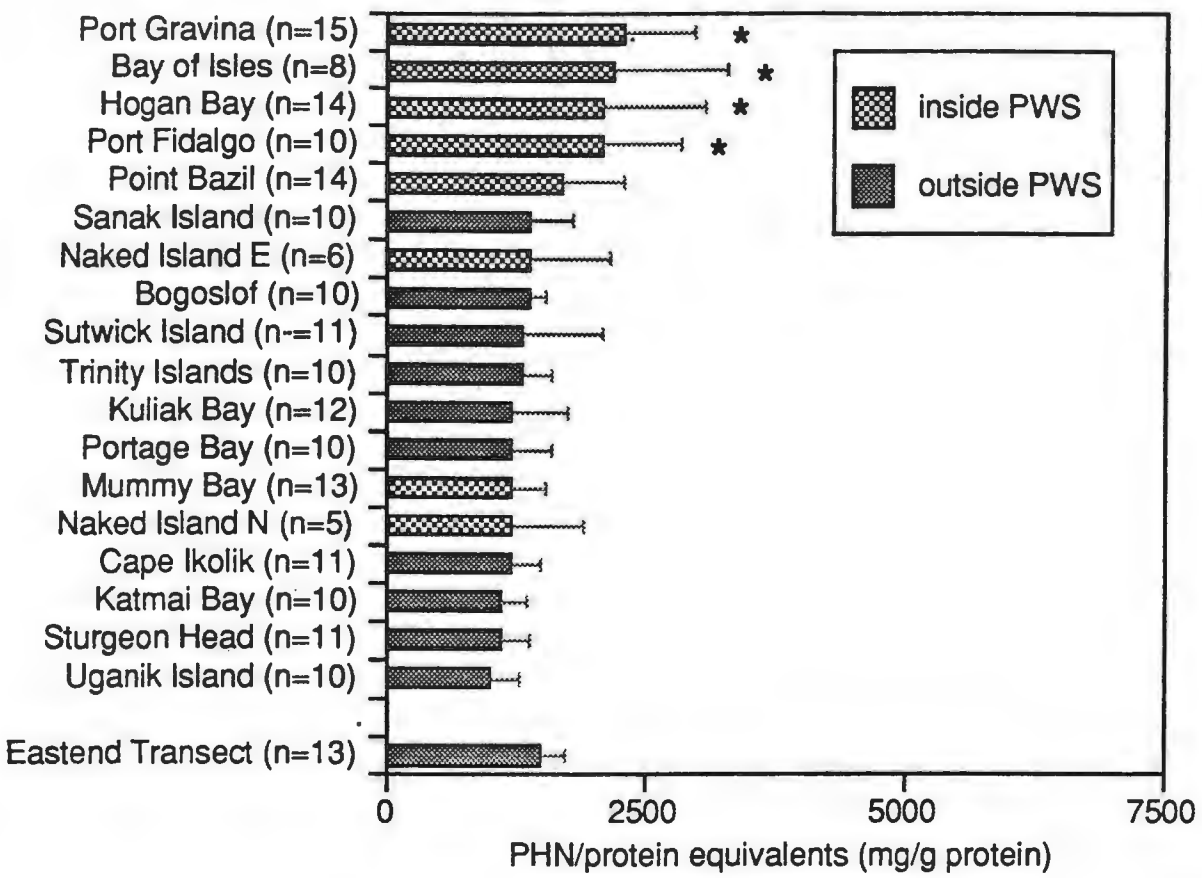


Figure 10. Average levels (± SD) of FACs PHN in bile of flathead sole, rock sole and yellowfin sole collected at Snuf Harbor in 1989, 1990 and 1991.



* Significantly different by log-transformed ANOVA from Uganik Island (lowest site) or Eastend Transect (unimpacted site)

Figure 11. The mean FACsPHN values in bile of pollock collected in 1991 in Prince William Sound and the Shelikof Strait.

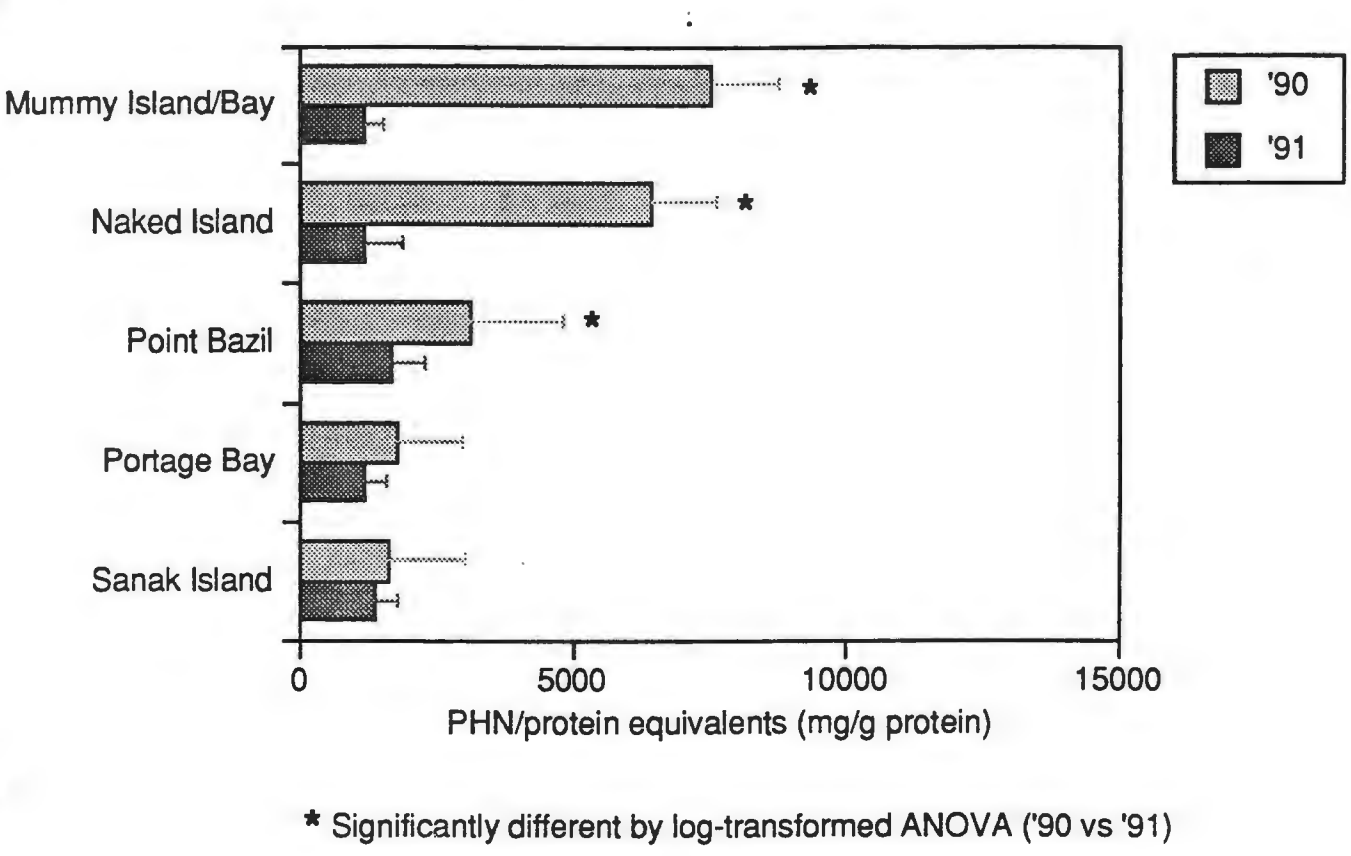


Figure 12. The mean FACsPHN values in bile of pollock collected in 1990 and 1991.

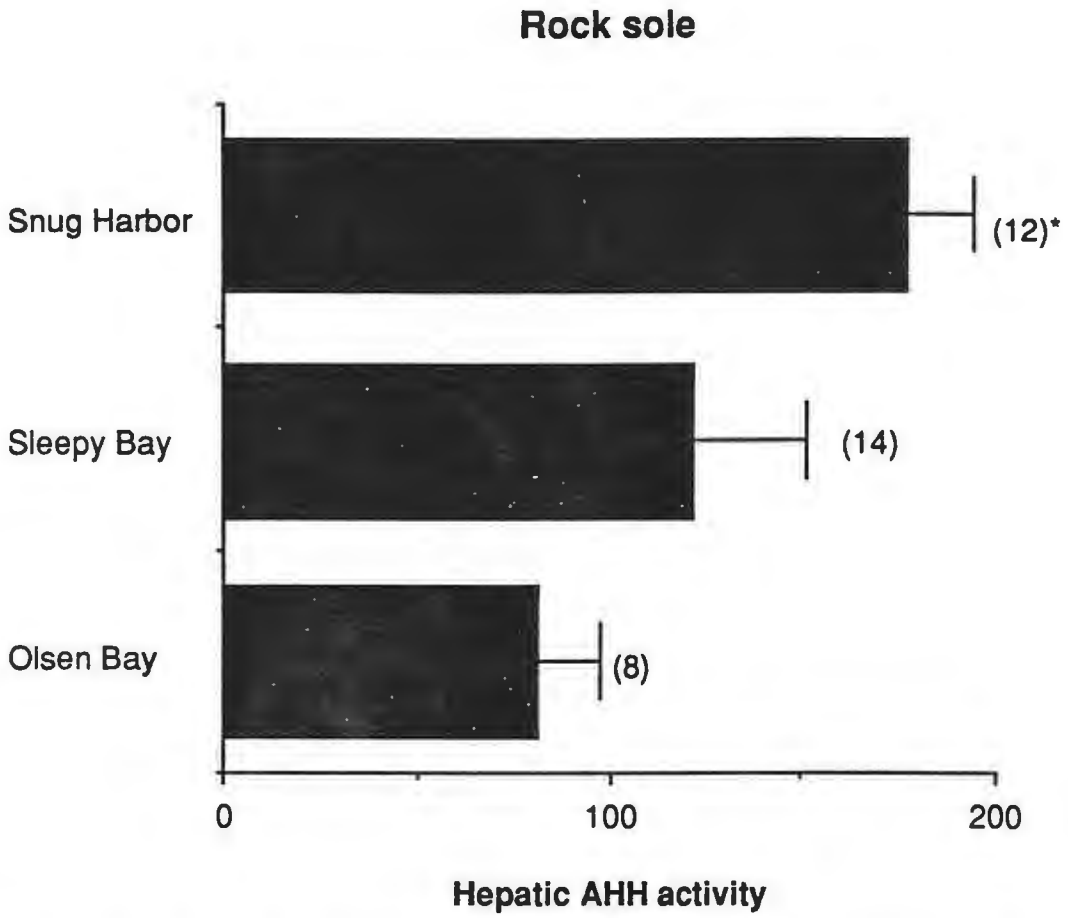


Figure 13. Average aryl hydrocarbon hydroxylase (AHH) activities (± SE) in liver of rock sole collected in 1991. Parenthetical numbers indicate sample size. * = significantly different ($p < 0.05$) from values for fish from Olsen Day, as determined by ANOVA of log transformed data.

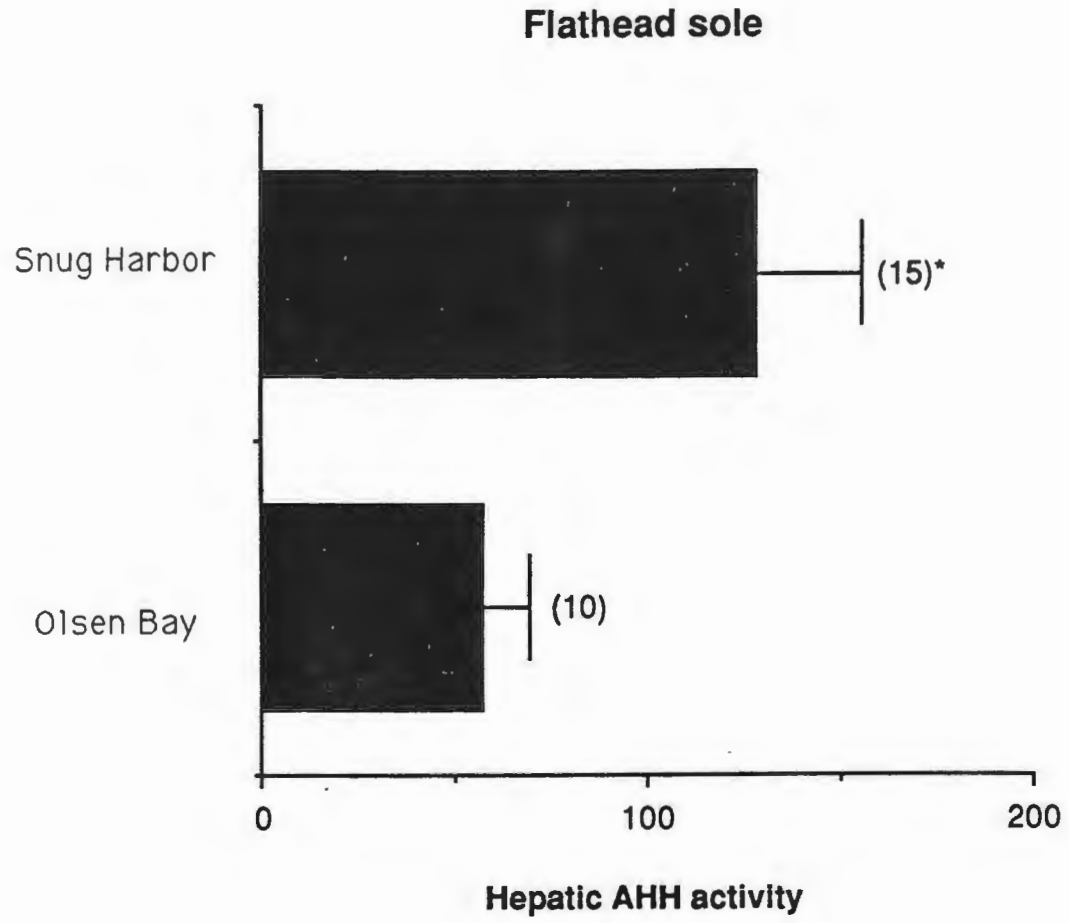


Figure 14. Average aryl hydrocarbon hydroxylase (AHH) activities (± SE) in liver of flathead sole collected in 1991. Parenthetical numbers indicate sample size. * = significantly different ($p < 0.05$) from values for fish from Olsen Day, as determined by ANOVA of log transformed data.

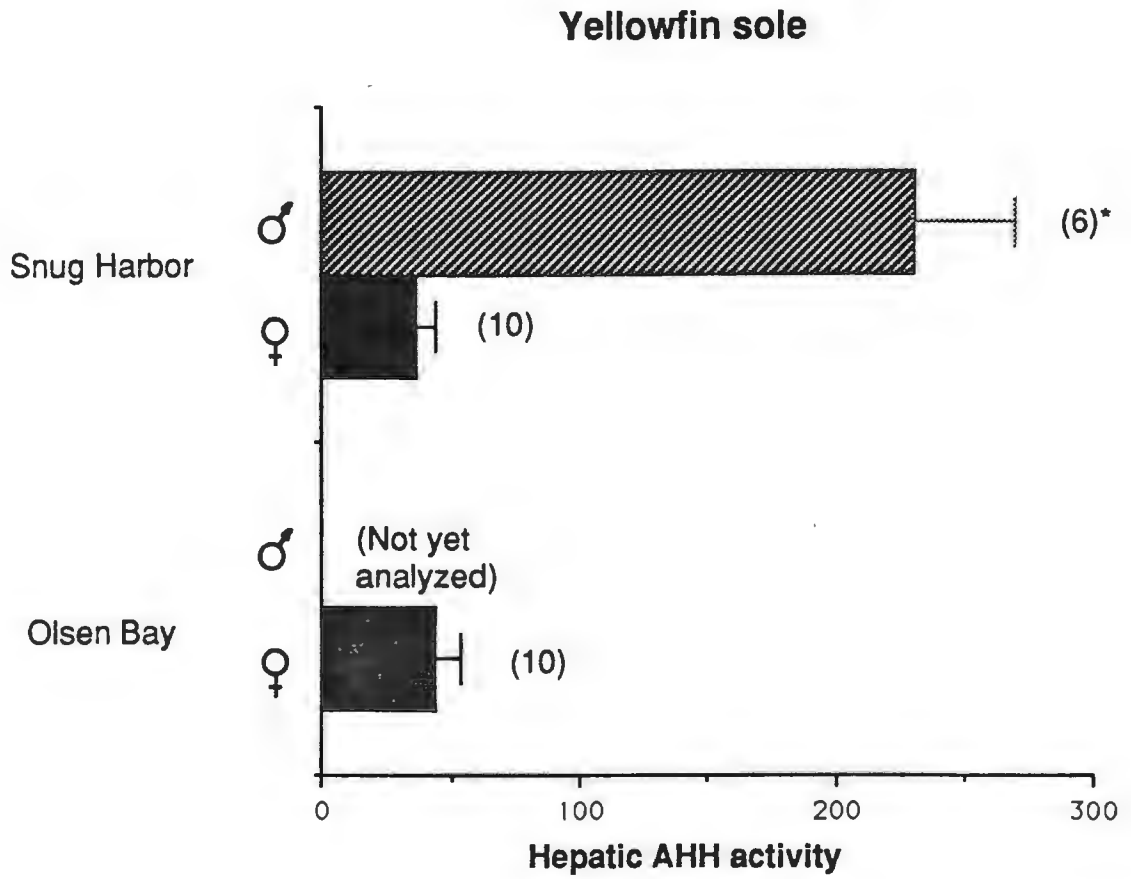


Figure 15. Average aryl hydrocarbon hydroxylase (AHH) activities (± SE) in liver of flathead sole collected in 1991. Parenthetical numbers indicate sample size. * = significantly different ($p < 0.05$) from values for female yellowfin sole from Snug Harbor, as determined by ANOVA of log transformed data.

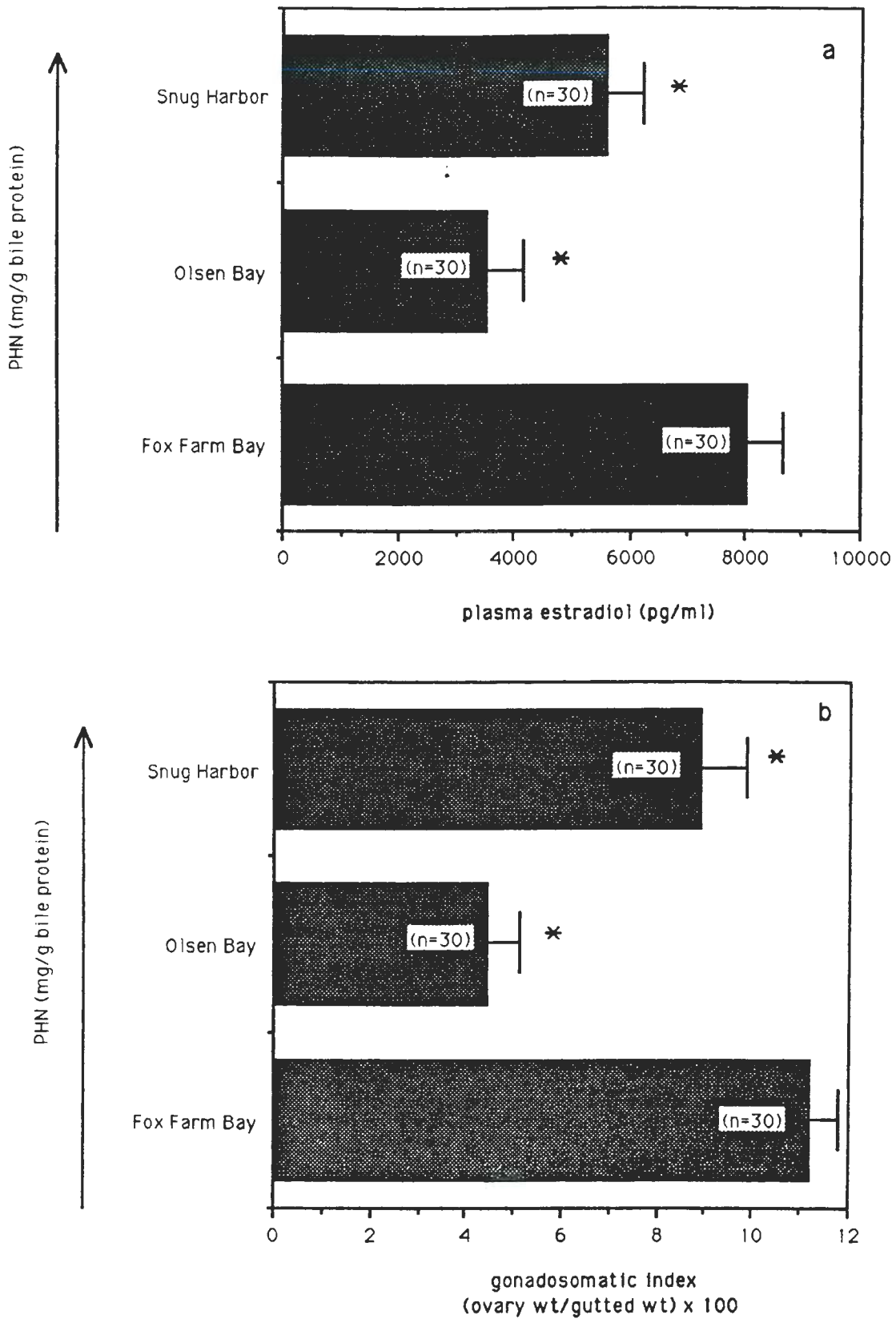


Figure 16. (a) Mean gonadosomatic indices (GSI) and (b) plasma estradiol concentrations (pg/ml) in yellowfin sole from from three sites in Prince William Sound. Snug Harbor was heavily oiled in the 1988 spill, while Olsen Bay and Fox Farm Bay were minimally impacted. Sites are arranged from in order of increasing oil exposure, as indicated by mean concentration of phenanthrene metabolites in bile. Asterisk (*) indicates site mean is significantly different from Fox Farm (ANOVA, $p < 0.05$), the site with lowest concentration of PHN metabolites in bile.

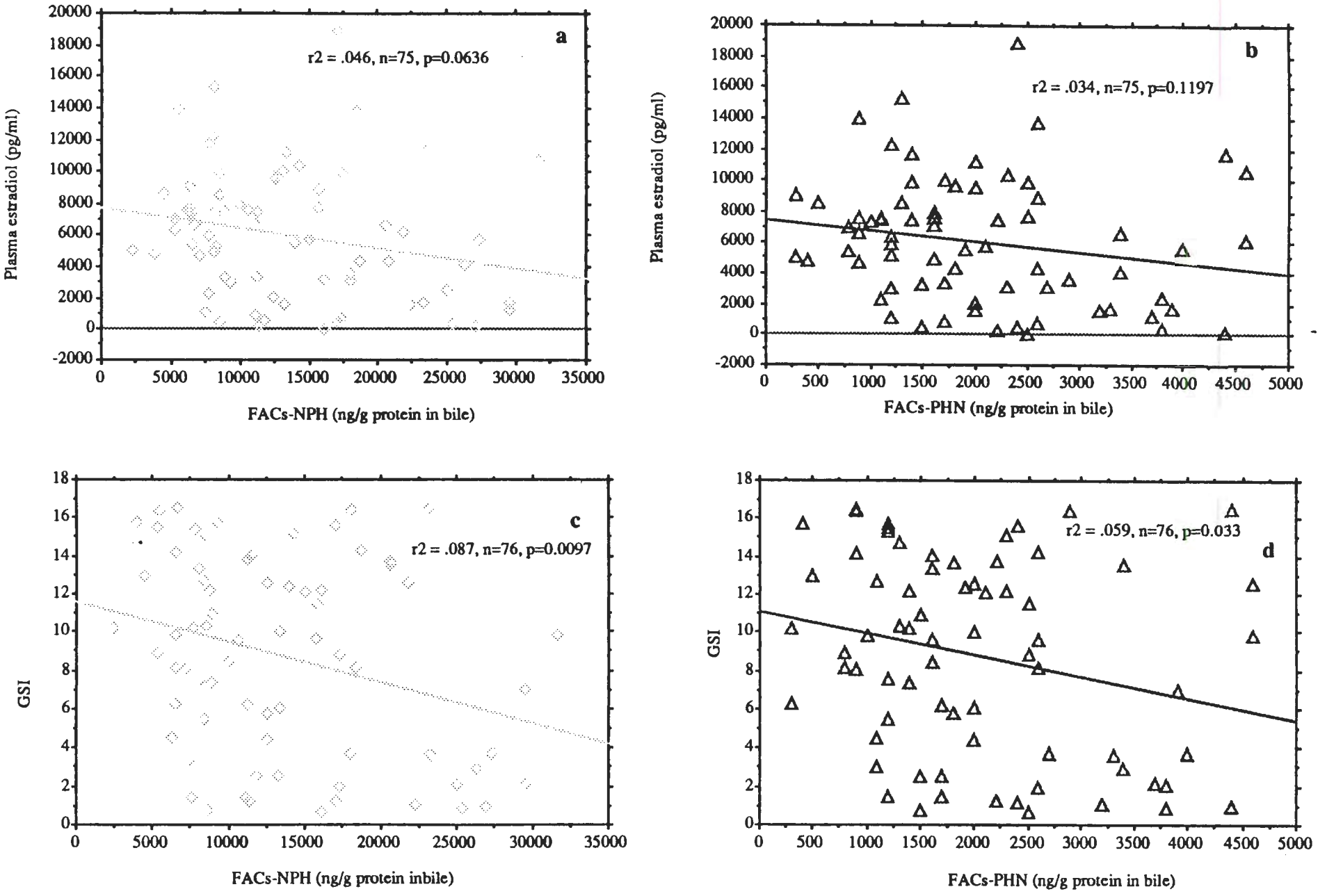


Figure 17. Relationships (linear regression $p < 0.05$) between (a) gonadosomatic index in yellowfin sole and concentration of phenanthrene (PHN) metabolites in bile (mg/g bile protein); (b) gonadosomatic index and concentration of napthalene (NPH) metabolites in bile (mg/g bile protein); (c) plasma estradiol levels (pg/ml) and concentrations of PHN metabolites in bile; and (d) plasma estradiol levels and concentrations of NPH metabolites in bile.

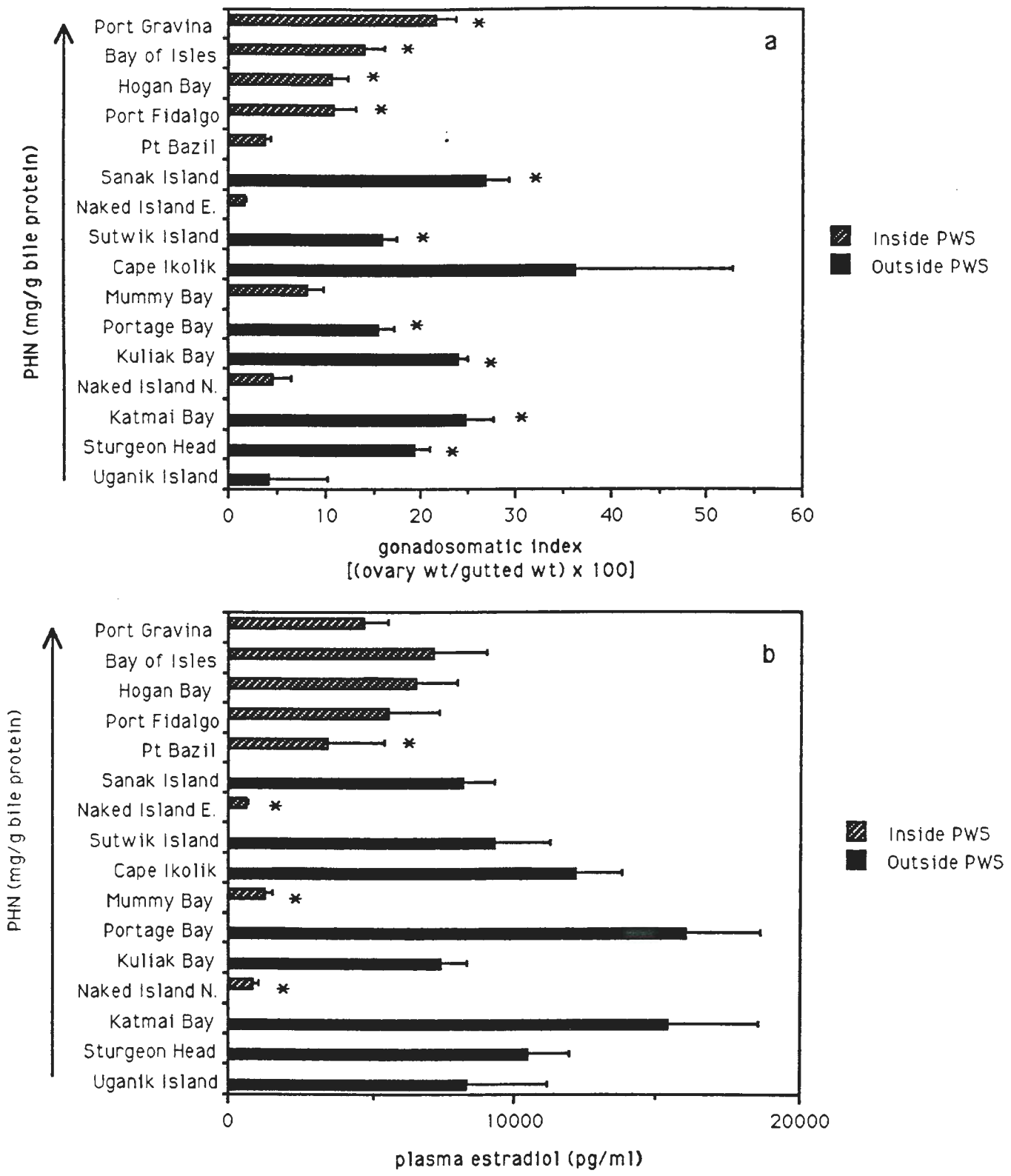


Figure 18. (a) Mean gonadosomatic indices (GSI) and (b) plasma estradiol concentrations (pg/ml) in pollock from eight sites within Prince William Sound and eight sites outside of Prince William Sound. Sites are arranged in order of increasing oil exposure, as indicated by mean concentration of PHN metabolites in bile. Mean plamsa estradiol concentration was significantly lower in pollock from four sites within Prince William Sound (Pt. Bazil, Naked Island East, Mummy Bay, and Naked Island North). GSI was not depressed at any of the sites within Prince William Sound in comparison to the Uganik reference site, and were significantly higher at four sites within Prince William Sound (Port Gravina, Bay of Isles, Hogan Bay, and Goose Island) compared to the Uganik reference site.

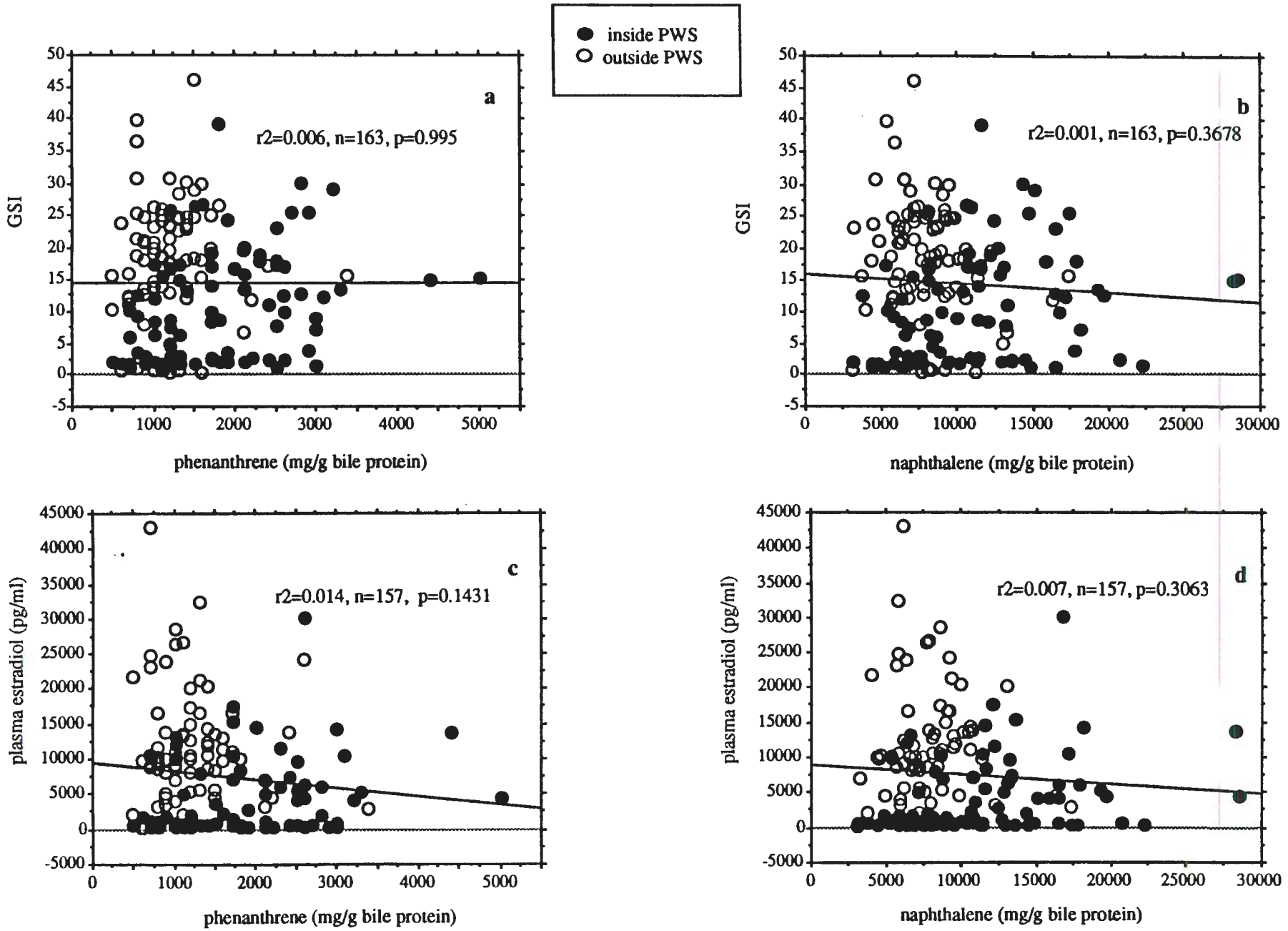


Figure 19. Relationships (linear regression $p < 0.05$) between (a) gonadosomatic indies in pollock and concentrations of PHN metabolites in bile (mg/g bile protein); (b) gonadosomatic indies and concentrations of naphthalene (NPH) metabolites in bile (mg/g bile protein); (c) plasma estradiol levels (pg/ml) and concentrations of PHN metabolites in bile; and (d) plasma estradiol levels and concentrations of NPH metabolites in bile.

APPENDIX A

(excerpts from 1991 Study Plan)

METHODS

A. General Strategy and Approach

Samples of benthic fish (yellowfin sole, rock sole, flathead sole, and to a lesser extent, Pacific cod) will be collected from five sites during 1991, from mid-May to mid-June. Sites proposed for sampling are Olsen Bay, Rocky Bay, Snug Harbor, Sleepy Bay, and Squirrel Bay. As feasible, the sample locations will be coordinated with Air/Water Study #2. The selection of species is based primarily on results obtained in 1990 and 1989 under Fish/Shellfish Study 24, and to a lesser extent, Fish/Shellfish Study 18. Surficial sediment samples for establishing levels of petroleum hydrocarbon residues will be collected at these sites, with analyses projected to be done under Air/Water Study 2. Pollock will be collected in March, 1991, at several sites inside Prince William Sound and in the Shelikof Strait. Because of the schooling nature of this species, and because we will be largely dependent on assistance from other federal and state groups for use of sampling platforms, sites cannot be predetermined, but efforts will be made to sample sites representing a spatial gradient away from the spill's occurrence and path.

Petroleum exposure of fish will primarily be assessed by measuring: (a) concentrations of metabolites of aromatic petroleum compounds in bile, and (b) AHH activities in liver. These types of measurements are necessary because petroleum hydrocarbons in fish are rapidly metabolized to compounds that are not detectable by routine chemical analyses. AHH activity in fish is due primarily to a single cytochrome P-450, apparently cytochrome P-450IA (Varanasi et al., 1986, Buhler and Williams 1989). Measurement of hepatic AHH activity will provide a very sensitive indicator of contaminant exposure of sampled animals (Collier and Varanasi, 1987; Collier and Varanasi, 1991). Moreover, the induction of AHH

activity indicates not only that contaminant exposure has occurred, but also that biological changes have occurred as a result of the exposure. In addition to measuring AHH activity, cytochrome P-450IA will be directly quantified in selected liver or tissue samples by an immunochemical method recently developed at the University of Bergen (Collier et al., 1989; Goksøyr, 1991). Direct quantification of cytochrome P-450IA1 has the advantage that this method can be used on archived samples and samples frozen at non-cryogenic temperatures (> -80° C), thus allowing for future comparisons to be made between data collected in this Damage Assessment Program and data from other sample collection programs, if samples from the other programs are subjected to the same immunochemical quantification techniques.

Other biological effects in fish will be estimated by examining selected species for pathological conditions and by assessing reproductive impairment in suitably mature female fish. Pathological conditions will include grossly visible abnormalities (e.g., fin erosion) and other lesions diagnosed by histological procedures (e.g., gill necrosis, liver cell necrosis). Reproductive capacity will be estimated by examining the developmental stages of ovaries and by measuring plasma levels of certain reproductive hormones (Johnson et al., 1988), in addition to measuring fecundity (Cross and Hose, 1988). The two primary species for assessing reproductive impairment are yellowfin sole and pollock. It is anticipated that, during the respective sampling periods (May/June and March), these two species will be at an appropriate stage in their reproductive cycle for such assessments to be done. Concurrent with these studies, we are conducting laboratory studies to determine the effects of known doses of oil and oil components on reproductive processes in these or related species.

Samples of sediment, and selected stomach contents of fish (from fish whose bile had evidence of oil exposure) will be analyzed (sediment under Air/Water Study 2) for hydrocarbons by recently developed, scientifically sound and cost-effective analytical procedures involving high-performance liquid chromatography, gas chromatography and mass spectroscopy (Krahn et al., 1988).

Environmental damage will be assessed using statistical and simulation models, which will be developed as part of these proposed studies, as well as from other investigations with related fish species. The bile and tissue chemistry data will be used to establish relationships between biological damage and estimated exposures to petroleum hydrocarbons.

B. Sampling Methods

Sampling activities will be conducted at several sites in Prince William Sound, including nonoiled sites in Rocky Bay (60°20.2'N, 147°08.1'W) and Olsen Bay (60°43.8'N, 146°13.2'W), and petroleum-exposed sites in Snug Harbor (60°14.5'N, 147°43.1'W), Sleepy Bay (60°04.1'N, 147°50.6'W), and Squirrel Bay/Fox Farm (60°00.4'N, 148°08.9'W). Sample collection will be performed from a charter vessel for the three flatfish species and cod, at water depths of approximately 0 to 100 meters. At each site, sediment samples will be collected with a box corer, VanVeen or Smith-McIntyre grab. Sediments will be stored at - 20° C. The coordinates and depths of each station will be recorded. For pollock, samples will be collected from a NOAA vessel (R/V MILLER FREEMAN) at the sites outside Prince William Sound, and from an Alaska Department of Fish and Game vessel (R/V PANDALUS) at sites inside Prince William Sound.

Fish will be collected with a bottom trawl, long-line gear, or midwater trawl. Bottom trawls will be performed with an otter trawl (7.5 m opening, 10.8 m total length, 3.8 cm-mesh in the body of the net, and 0.64 cm-mesh in the liner of the cod end). Tows will be of 5 to 15 minutes duration. In order to reduce contamination of the catch by free oil, trawling will avoid areas of surface films or slicks. If a net is fouled by subsurface or bottom oil, it will be replaced (or cleaned, if possible) and a new area for trawling will be selected. Other fish sampling gear appropriate to the species and conditions will also be deployed. Individuals of selected target fish species will be sorted and examined for externally visible lesions; up to 30 fish of selected species will be measured, weighed, and necropsied; and tissue samples will be excised and preserved in fixative for histopathological examination or frozen for chemical analyses.

C. Laboratory Analyses

1. Bile Metabolite Assay (analyses done under Technical Services-1)

Samples of bile will be injected directly into a liquid chromatograph and a gradient elution conducted using a Perkin-Elmer HC-ODS with a gradient of 100% water (containing 5µL acetic acid/L) to 100% methanol (Krahn et al., 1984, 1986a, b, c). Two fluorescence detectors are used in series. The excitation/emission wavelengths of one detector are set to 290/335 nm, where metabolites of naphthalene (NPH) fluoresce. Excitation/emission wavelengths of the other detector are set to 260/380 nm, where metabolites of phenanthrene (PHN) fluoresce. The total integrated area for each detector is then converted (normalized) to units of either NPH or PHN that would be necessary to give that integrated area.

2. Liver Aryl Hydrocarbon Hydroxylase (AHH) Activity and Cytochrome P-450IA1 Analysis

Hepatic microsomes are prepared essentially as described by Collier et al. (1986) and microsomal protein is measured by the method of Lowry et al. (1951), using bovine serum albumin as the standard. AHH activity is assayed by a modification of the method of Van Cantfort et al. (1977) as described by Collier et al. (1986), using 14C-labeled benzo[a]pyrene as the primary substrate. All enzyme assays will be run under conditions in which the reaction rates are in the linear range for both time and protein. Cytochrome P-450IA1 will be measured by an ELISA utilizing rabbit antibodies to cytochrome P-450c isolated from Atlantic cod (Goksøyr,1991).

3. Histopathology

Histopathological procedures to be followed are described in the report from the Histopathology Technical Group for Oil Spill Assessment Studies in Prince William Sound, Alaska. Briefly, the procedures will involve the following: (a) tissues preserved in the field will be routinely embedded in paraffin and sectioned at five microns (Preece, 1972); and (b) paraffin sections will be routinely stained with Mayer's hematoxylin and eosin, and for further characterization of specific lesions, additional sections will be stained using standard special staining methods (Thompson, 1966; Preece, 1972; and Armed forces Institute of Pathology, 1968). All slides will be examined microscopically without knowledge of where the fish were captured. Hepatic lesions will be classified according to the previously described diagnostic criteria of Myers et al. (1987). Ovarian lesions will be classified as described in Johnson et al. (1988).

4. Reproductive Indicators

Reproductive activity will be assessed by examining the ovaries of the sampled fish

histologically to determine their developmental stage, and for the presence of ovarian lesions that would be indicative of oocyte resorption (Johnson et al., 1988). Other parameters associated with reproductive activity will also be measured, including fecundity (Bagenal and Braum, 1971), plasma vitellogenin (Gamst and Try, 1980; DeVlaming et al., 1984) and estradiol (Sower and Schreck, 1982) levels, and gonadosomatic index (ovary wt/gutted body wt x 100). Relationships between ovarian maturation, fecundity, plasma estradiol, plasma vitellogenin, and petroleum hydrocarbon exposure will then be evaluated.

D. Quality Assurance and Control Plans

1. Bile Analytes

Quality assurance procedures for bile analyses will include NPH and PHN calibration standards and the calibration standard will be analyzed after every 6 samples and the RSD will be reported. In addition, one blank sample and one reference material (control material) will be analyzed daily. The concentrations of analytes should be within 2 SD of the established concentrations in control material. Replicate analyses will be performed on 10% of the samples, if a sufficient amount exists.

2. AHH Activity and Cytochrome P-450IA1

Quality assurance procedures for AHH measurements include duplicate zero-time and boiled enzyme blanks for each set of assays. Each sample will be run in duplicate and those samples showing > 20% absolute difference between duplicates and >10 units (pmoles benzo[a]pyrene metabolized/mg microsomal protein/minute) difference between duplicates will be repeated. ELISAs for cytochrome P-450IA1 will be run in triplicate, and if the resulting coefficient of variation (CV) is > 10%, the outlying replicate will be omitted from the calculations. If the CV still exceeds 10%, the analysis of that sample will be repeated.

3. Histopathology

Pathologists on this project will use consistent, standard diagnostic criteria to be strictly adhered to by those who will also be examining slides in this project. These criteria will be established using color photographs of external lesions and standard reference slides containing tissues with the major lesion types expected in the study. Unusual or atypical lesions will be referred to specialists for confirmation. The

accuracy of the histopathologic diagnosis also will be assured by consulting with and sending sections of tissues with representative lesion types to the Registry of Tumors in Lower Animals, National Museum of Natural History at the Smithsonian Institution in Washington, D.C.

4. Reproductive Indicators

Quality assurance for the measurement of plasma estradiol and vitellogenin include analysis of standards to confirm linearity and calibrate the assays. Blank analyses will be conducted to eliminate matrix effects. Analyses of pooled plasma from vitellogenic female English sole and winter flounder containing known levels of estradiol and vitellogenin will also be done. Duplicate analyses of each sample to evaluate performance of the assays will also be conducted. These quality checks are run daily with each set of samples. Fecundity measurements will be done in triplicate on each individual.

DATA ANALYSIS

A. Statistical Tests

The relative concentrations of contaminants in sediment and fish tissues at the study sites will be compared statistically using the Kruskal-Wallis test (ANOVA by ranks; see Sokal and Rohlf, 1981; Zar, 1984). Where significant differences among chemical concentrations are found, the α-value will be understood to be < 0.05. To determine whether the prevalence of histopathological effects noted in each of the fish species is statistically uniform among the sites, the G test for heterogeneity (Sokal and Rohlf, 1981) will be performed.

B. Analytical Methods

Where possible, non-parametric statistical tests will be employed to avoid assumptions that the data are normally distributed. Non-parametric tests give highly reliable results. The principal non-parametric tests that will be used are Spearman rank correlation, which has about 91% of the power of product-moment correlation when the parametric assumptions are met (Zar, 1984), and the heterogeneity-G statistic. Spearman rank correlation will be used for estimating uptake and metabolism of petroleum hydrocarbons from oiled and non-oiled habitats when an independent measure of contamination (e.g., levels of AHs in sediment) is available.

The heterogeneity-G statistic (Sokal and Rohlf, 1981) will be used to study prevalence of pathological conditions at oiled and non-oiled habitats. In addition, logistic regression (appropriate where the outcome variable is binomial) will be used to model the prevalences of pathological conditions in relation to contamination.

The Kruskal-Wallis test (a non-parametric form of ANOVA) will be used for supporting statistical analyses of variation in sediment PAH levels at sites sampled. If the null hypothesis of no differences among sites is rejected at $\alpha = 0.05$, a non-parametric multiple comparison test (Dunn, 1964; Hollander and Wolfe, 1973; Zar, 1984) will be used to determine differences between sites at $\alpha = 0.05$. Principal components analysis and LOWESS (Chambers et al., 1983) will also be employed for this purpose; both are methods of exploratory data analysis rather than inferential statistical methods.

Cohen (1977) will be used for computations of statistical power.

APPENDIX B

(Preprint "Mass Spectrometric Analysis of Aromatic Compounds in Bile of Fish Sampled After *EXXON VALDEZ* Oil Spill"

by M.M. Krahn, D.G. Burrows, G.M. Ylitalo, D.W. Brown, C.A. Wigren, T.C. Collier, S.-L. Chan and U. Varanasi)

MASS SPECTROMETRIC ANALYSIS FOR AROMATIC COMPOUNDS IN BILE OF FISH SAMPLED AFTER THE *EXXON VALDEZ* OIL SPILL

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ENVIRONMENTAL SCIENCE & TECHNOLOGY (IN PRESS—8/8/91)

Abstract

After the *Exxon Valdez* oil spill, the exposure of marine organisms to petroleum had to be determined. Gas chromatography/mass spectrometry was used to identify metabolites of aromatic compounds (ACs), such as alkylated naphthols, phenanthrols, and dibenzothiophenols, in the hydrolyzed bile of 5 salmon (*Oncorhynchus gorbuscha*) and 4 pollock (*Theragra chalcogramma*) captured in Prince William Sound several months after the oil spill. These metabolites were not found in control fish sampled from areas not impacted by the oil. The metabolites were identified by comparison to those from the hydrolyzed bile of a halibut (*Hippoglassus stenolepis)* which had been injected with weathered PBCO. The dibenzothiophenols are proposed as promising marker compounds for identifying the exposure of fish to certain crude oils. In addition, a high-performance liquid chromatographic method to screen bile for metabolites of ACs was validated for use in estimating the exposure of fish to petroleum.

Introduction

Following the spill of 11 million gallons of Prudhoe Bay crude oil (PBCO) from the *Exxon Valdez* into Prince William Sound (PWS), Alaska, in March 1989, analyses to determine oil exposure in the biota along the path of the spill were essential. The degree of exposure of marine organisms to oil is often assessed by measuring their body burden of petroleum-related aromatic compounds (ACs), because ACs are potentially harmful to the animals (1). However, fish and marine mammals extensively metabolize most ACs in their livers and then the metabolites are excreted, predominantly into bile (2-6). A rapid screening method for bile, which determines the metabolites as fluorescent ACs (FACs), has proven useful in estimating the exposure of fish and marine mammals to petroleum (7,8). But this screening method is limited to providing relative concentrations of FACs in bile; individual metabolites are not identified and quantitated. Accordingly, detailed chemical analyses are needed to determine the concentrations of individual metabolites of petroleum-related ACs in selected bile samples and, thus, to support the results of the semiquantitative bile screening method showing exposure of marine organisms to spilled oil.

The specific individual metabolites that result from the uptake and metabolic conversion of petroleum ACs in fish have not been well-characterized. Two previous studies identified only a few individual AC metabolites in the livers (2) or bile (3) of fish that had been exposed to No. 2 fuel oil, a distillate fraction of petroleum that contains only a portion of the ACs found in crude oil. In a more detailed study, several individual metabolites were identified by gas chromatography/mass spectrometry (GC/MS) in the bile of fish captured from urban sites (9,10). However, urban sediments generally contain high proportions of products from the combustion of fossil fuels, such as

unsubstituted, 4- to 6-ring ACs. In contrast, PBCO contains a variety of 1- to 3-ring alkylated ACs, as well as the alkylated dibenzothiophenes typical of the North Slope crude oils (11-14). But because spilled oil is degraded in time by physical, chemical and microbial processes, the aromatic fraction of the weathered oil will be dominated by those ACs (e. g., highly alkylated naphthalenes, phenanthrenes and dibenzothiophenes) that are most resistant to weathering (15,16). Therefore, metabolites of these resistant ACs should be found in bile of fish exposed to weathered crude oil; in this paper, we report the results of our efforts to characterize such compounds.

Initially, the products resulting from the metabolism of PBCO by fish were identified by GC/MS in the enzymatically hydrolyzed bile of a halibut (*Hippoglossus stenolepis)* and a Dolly Varden (*Salvelinus malma*) injected with weathered PBCO. Subsequently, many of these metabolites were determined in the hydrolyzed bile of 5 pink salmon (*Oncorhynchus gorbuscha*) and 4 pollock (*Theragra chalcogramma*) collected in PWS several months after the oil spill. Finally, the bile screening method was validated by demonstrating a strong statistical correlation between concentrations of FACs determined by screening and the sums of metabolite concentrations determined by GC/MS in oil-exposed fish.

Experimental

Chemicals. A sample of PBCO was obtained from the oil remaining in the hold of the *Exxon Valdez*, and the weathered PBCO sample was collected from PWS 11 days after the spill occurred. 2,6-Dimethyl-3-naphthol, 6-methyl-2-naphthalenemethanol and *trans*-3,4-dihydroxy-3,4-dihydro-2,6-dimethylnaphthalene were prepared in our laboratories (17).

Reference Standard. A GC/MS standard, containing reference compounds dissolved in methanol, was prepared (listed in order of GC elution, ng/μL): 2,6-dibromophenol (surrogate standard, 7.38), hexamethylbenzene (GC internal standard, 7.56), 1-naphthol (15.66), 2-hydroxybiphenyl (15.72), 6-methyl-2-naphthalenemethanol (2.76), 4-methyl-1-naphthol (15.42), 2,6-dimethyl-3-naphthol (2.82), *trans*-3,4-dihydroxy-3,4-dihydro-2,6-dimethylnaphthalene (3.36), 9-fluorenol (15.54), phenanthrene-d10 (HPLC internal standard, 6.00), 9-phenanthrol (15.06), 9-anthracenemethanol (14.76) and 1-pyrenol (13.80).

Injection of fish with PBCO. Halibut (*Hippoglossus stenolepis)* were captured by long line from a relatively uncontaminated site (reference site) in PWS (Figure 1). The halibut were injected, into the dorsal anterior musculature, with (a) 1.0 μL/g fish wt of a 1:1 (v:v) mixture of weathered PBCO dissolved in a carrier or (b) with 0.5 μL/g fish wt of the carrier alone. The carrier was a 1:1 (v:v) mixture of acetone and Emulphor. The fish were maintained in a flow-through seawater aquaria and were not fed after injection. After 48 hr, the fish were killed by severing the spinal column; bile was then removed from the gall bladders and frozen. The above experiment was repeated using Dolly Varden char (*Salvelinus malma*) captured by gill net from a site in PWS not impacted by oil (Figure 1). Bile samples were selected for this study from the following fish: an oil-injected halibut (wt = 2500 g); a carrier-injected halibut (5400 g); an oil-injected Dolly Varden (368 g) and a carrier-injected Dolly Varden (378 g).

Field collection of fish. Adult pink salmon (*Oncorhynchus gorbuscha*) were captured in PWS (Figure 1) by hook and line at an oiled site (Chenega Bay) about 5 months post spill and by gill net from a site relatively unimpacted by oil

(Columbia Bay) about 4 months post spill. Adult pollock (*Theragra chalcogramma*) were captured by trawl approximately 1 year after the spill from areas that had been oiled (Mummy Island and Goose Island) in PWS (Figure 1) and from a relatively uncontaminated site, Seymour Canal, in southeast Alaska. Bile was collected from the gall bladders and the samples were frozen. Fish from the field were then selected for this study based on a number of criteria. FAC concentrations determined by bile screening were available for approximately 300 salmon and 200 pollock collected within a year of the spill. Because we intended to confirm the results of bile screening by analyzing for individual bile metabolites by GC/MS, we selected bile samples with a range of FAC concentrations. The minimum amount of bile that was suitable for both screening and GC/MS analysis was 125 μL.

Hydrolysis of bile and extraction of metabolites. A modification of the enzymatic hydrolysis and extraction method of Varanasi, Nishimoto, Reichert and Eberhart (18) was used. Each bile sample (100 μL) was treated with 2000 units β–glucuronidase (containing 25 units of arylsulfatase activity) in 0.4 M acetate buffer (1 mL; pH 5). A surrogate standard, 2,6-dibromophenol (100 μL; 24.6 ng/μL), was added. The samples were incubated in a warm water bath at 40°C for 2 hours and then extracted with methylene chloride (1 mL) and methanol (100 μL). After extracting 2 additional times with methylene chloride (1 mL each), the extracts were combined and sodium sulfate (0.1 g) was added. The combined extracts were transferred to concentrator tubes and the solvent was evaporated to 1 mL. Phenanthrene-d10 (HPLC internal standard; 50 μL, 60 ng/μL) was added and the solvent was further evaporated to 400 μL under a stream of nitrogen gas.

HPLC cleanup of bile extracts. A modification of an HPLC cleanup technique (19,20) was used to separate the metabolites from any biogenic material in the extracts from bile hydrolysis. The procedure was modified by slowing the solvent flow rate to 5 mL/min and by calibrating to collect a fraction (containing the metabolites) from the beginning of the biphenyl elution to the end of 1-pyrenol elution (approximately 19-30 min). A 250-µL portion of the 400-µL extract from hydrolysis was injected onto the HPLC column, the fraction was collected, and the solvent was evaporated to 1 mL. Methanol (0.500 mL) was added, the solvent was evaporated to 1 mL and HMB (GC internal standard; 30 µL, 25.2 ng/µL) was added; evaporation was continued under a stream of nitrogen gas until the volume was reduced to 30 µL.

GC/MS analysis of bile extracts. The extracts from hydrolyzed bile were analyzed by GC/MS as described earlier (9), except that the GC/MS system now included a 5970 Hewlett-Packard mass selective detector (MSD), a 59940A Hewlett-Packard HP-UX Chemstation data system, a 5890 Hewlett-Packard GC and a 7673B autosampler. The mass spectrometer was scanned using a sequenced selected ion monitoring (SSIM) descriptor at approximately 1 scan/sec. The GC run time was divided into segments in which different sets of ions were scanned (see Table I; also, Table A-I in the microfilm edition has a complete list of the ions scanned in each segment). For full scan spectra, the mass spectrometer was scanned from 45 to 450 amu at approximately 1 scan/sec.

Identification and quantitation of metabolites. Full-scan GC/MS analyses were conducted on bile from the oil-injected halibut. Numerous metabolites of ACs were tentatively identified by comparing retention times and

mass spectra for each metabolite to (a) reference standards or (b) mass spectra from the mass spectral library. This library includes spectra of metabolites from a previous study in which fish were injected with unsubstituted ACs (10). In addition, identification of some metabolites, particularly those of the C_2-C_3 alkylated ACs, was based on the molecular ion and on fragmentation patterns. Subsequently, SSIM was used to increase sensitivity in analyzing for metabolites of ACs in the bile of the halibut and the other fish species studied. Several major ions in the spectrum of each metabolite were selected for scanning in the SSIM mode (Table I). [Full scan and SSIM mass spectra of a C_2 dibenzothiophenol are compared in the microfilm edition (Figure A-1).]

The concentrations of the metabolites were calculated using single-point response factors and were corrected for the recovery of the surrogate standard. When no commercially available reference standard was available, metabolites were quantitated using a GC/MS response factor for an isomer (i.e., the C_1 naphthol isomers were quantitated by the response factor for 4-methyl-1-naphthol, the C_2-C_3 naphthols by 2,6-dimethyl-3-naphthol, the fluorenols by 9-fluorenol, the phenanthrols by 9-phenanthrol, the fluoranthenols/pyrenols by 1-pyrenol). In addition, when no isomer or reference standard was available, the concentrations of certain metabolites were calculated using a GC/MS response factor of 1 (i.e., dibenzofuranols, dibenzothiophenols and dibenz[a]anthracenols/chrysenols). As a result, the concentrations determined for many of the metabolites were semiquantitative.

The accuracy of quantitation of certain metabolites may also be limited by their dehydration in the GC. For example, phenanthrene is preferentially metabolized to a dihydrodiol in many fish (21,22). However, the dihydrodiol

dehydrates in the GC resulting in two phenols (10). Although derivatization of dihydrodiols to trimethylsilyl ethers prevents dehydration during the GC analyses and thus should allow quantitation of the dihydrodiol itself, the molecular ion of the derivative is small and the identification of the metabolite is difficult (9).

Matrix spikes. To determine recovery efficiencies, reference standards were added to bile from a halibut from a reference site (matrix spike; n=3); hydrolysis, extraction, HPLC cleanup and GC/MS analyses were then conducted as described above. Reference standards (ng) were: 1-naphthol (1044); 2-hydroxybiphenyl (1048); 6-methyl-2-naphthalenemethanol (184); 4-methyl-1-naphthol (1028); 2,6-dimethyl-3-naphthol (188); *trans*-3,4-dihydroxy-3,4-dihydro-2,6-dimethylnaphthalene (224); 9-fluorenol (1036); 9-anthracenemethanol (984); and 1-pyrenol (920).

HPLC/fluorescence screening of bile. A previously reported HPLC method (23) was used with fluorescence detection at excitation/emission wavelengths where 2-ring (290 nm/335 nm; naphthalene standard), 3-ring (260 nm/380 nm; phenanthrene standard) or 4- and 5-ring [380/430 nm; benzo[a]pyrene (BaP) standard] ACs fluoresce to quantitate total fluorescent ACs metabolites in (naive) bile.

Statistical analyses. The relationship between concentrations of bile metabolites determined by GC/MS and concentrations measured by screening of the same bile samples was evaluated by correlation (24). The concentrations obtained by each method were first log transformed to improve homogeneity in the variances.

Results

Chromatograms of Prudhoe Bay crude oil. GC/MS chromatograms of the aromatic fraction of PBCO and weathered PBCO are shown in Figure 2. PBCO contained large proportions of highly alkylated ACs with 1-3 rings (e.g., benzenes, naphthalenes and phenanthrenes; Figure 2A). After only 11 days of weathering, the composition of the crude oil had changed dramatically; major losses of low molecular weight ACs, particularly the 1- and 2-ring ACs, had occurred (Figure 2B). Less severe losses were noted for the alkylated phenanthrenes and alkylated dibenzothiophenes in the weathered PBCO.

Identification and quantitation of metabolites in hydrolyzed bile. Mass chromatograms, such as those shown for the molecular ions of alkylated phenanthrols (Figure 3A and 3B) and dibenzothiophenols (Figure 3C and 3D), were graphed for each homologous series of metabolites in each fish. Then, mass spectra (Figure 4) and relative retention times of metabolites identified in the oil-exposed halibut were used to establish the identity of the corresponding metabolites in the oil-injected Dolly Varden and in the environmentally-exposed fish sampled from PWS. As many of the metabolite as feasible were identified and quantitated in the bile from three of the fish: 158 in the oil-injected halibut; 119 in salmon-A; and 54 in the pollock-A. Alkylated naphthols, fluorenols, phenanthrols, dibenzofuranols and dibenzothiophenols comprised the majority; smaller numbers of alkylated fluoranthenols/pyrenols and benz[a]anthracenols/ chrysenols were also identified (see Tables II-III for selected metabolites and Table A-II in the microfilm edition for a complete list).

In the remainder of the fish, including the oil-injected Dolly Varden, a limited number of bile metabolites were quantitated to illustrate intra- and interspecies

differences in relative proportions and concentrations of the metabolites. The 22 metabolites selected were: C_2 and C_3 naphthalenes (n=2); C_1, C_2 and C_3 dibenzothiophenols (n=11); C_1, C_2 and C_3 phenanthrols (n=9). Concentrations of these metabolites are given for oil- and carrier-injected halibut, for salmon and for the method blank in Table II and for oil- and carrier-injected Dolly Varden and for pollock in Table III. Bile samples from the reference fish and the carrier-injected fish were analyzed for all metabolites found in the oil-injected halibut. The salmon and pollock collected from reference sites, as well as the carrier-injected halibut and Dolly Varden, contained either low or undetectable concentrations of these metabolites (Tables II-III; detection limits for the other metabolites were similar to those shown). An exception was the C_1 phenanthrol (400 ng/g) found in the bile from the reference pollock (Table III); the source of this contaminant is unknown.

Both phenols and alcohols are formed from the metabolism of certain ACs (3,17,25). However, the structure of these isomeric hydroxy metabolites cannot be assigned solely from their GC/MS spectra; either reference standards are needed for comparison or the phenols must first be separated from the alcohols based on their acidity. Thus, we have arbitrarily identified most of these hydroxy metabolites as phenols (Tables II, III, A-II), although a number of alcohols may have been formed.

Identification of dibenzothiophenols. The dibenzothiophenols, important metabolites because they can serve as marker compounds in bile for detecting the exposure of fish to certain crude oils (see Discussion), have not been reported previously. The homologous series of C_0-C_3 dibenzothiophenols were identified in the bile of the oil-injected halibut from their molecular ions

(mass/charge = 200, 214, 228, 242) and their fragmentation patterns (Table I and Figure 4). The dibenzothiophenols were also identified in the field-exposed fish. Nine C_2 dibenzothiophenols isomers were present in bile of the oil-injected halibut (Figure 3C) and from 4-9 isomers in the other species, e. g., 9 isomers were present in salmon-A (Figure 3D). However, the occurrence and relative proportions of these isomeric dibenzothiophenols were different among the individual fish (Figure 5; see Discussion).

Recoveries in matrix spikes. Percent recoveries (mean ± standard deviation) of the reference standards in the spiked matrices (n = 3) were as follows: 1-naphthol (110±10); 2-hydroxybiphenyl (110±6); 6-methyl-2-naphthalenemethanol (110±29); 4-methyl-1-naphthol (79±11); 2,6-dimethyl-3-naphthol (86±5); trans-3,4-dihydroxy-3,4-dihydro-2,6-dimethylnaphthalene (140±38); 9-fluorenol (120±12); 9-anthracenemethanol (120±21); 1-pyrenol (92±11); and 2,6-dibromophenol (surrogate standard; 79±6). In general, the polar phenols were more difficult to extract and to recover from chromatographic procedures than were the alcohols. Because recoveries of the analytes (above) were corrected for the surrogate standard (a phenol), the recoveries of the alcohols tended to be high.

Comparisons of screening and GC/MS methods. Bile samples from all the fish in the study were analyzed by the screening method of Krahn, Rhodes, Myers, Moore, MacLeod and Malins (26); the FAC equivalents at phenanthrene (260/380 nm) and naphthalene (290/335 nm) wavelengths are reported in Table IV. The highest concentrations of FACs in bile were found in the halibut injected with PBCO. The salmon had the next highest levels, the oil-injected Dolly Varden and pollock had intermediate concentrations, and the reference

fish had the lowest levels of all the fish. BaP equivalents were determined for the salmon: salmon-A (2300 ng/g, wet wt); salmon-B (1300 ng/g); salmon-C (1100 ng/g); salmon-D (830 ng/g); and salmon-E (870 ng/g).

To make a comparison between the semiquantitative results obtained with the bile screening method and the quantitative results from the detailed GC/MS method, the metabolites in Tables II-III were divided into two groups by their fluorescence characteristics—those that fluoresce at 260/380 nm and those at 290/335 nm. Although no dibenzothiophenol standards were available, these compounds are presumed to fluoresce at 290/335 nm because dibenzothiophene fluoresces at this wavelength pair and phenolic metabolites usually fluoresce at wavelengths close to those of the parent compound. Therefore, the concentrations of the naphthols and dibenzothiophenols were summed to represent metabolites fluorescing at naphthalene wavelengths. Similarly, the phenanthrols were summed for phenanthrene wavelengths (Tables II-III). The total phenanthrene equivalents (analyzed by bile screening; Table IV) were highly correlated with the summed concentrations (GC/MS) of those metabolites that fluoresce at phenanthrene wavelengths (260/380 nm; Figure 6A). A similar correlation was found for metabolites that fluoresce at naphthalene wavelengths (290/335 nm; Figure 6B).

Comparisons of metabolite profiles in salmon and pollock. For the fish in this study, each metabolite concentration (from Tables II-III) was divided by (normalized to) the concentration of the most abundant metabolite. The resulting normalized values were then plotted for each metabolite to provide a visual representation of the metabolite profiles (Figure 5). The profiles of the

various pollock are similar, while those of the salmon are somewhat similar to each other, but dissimilar to the pollock profiles.

Discussion

The environmental exposure of the feral salmon and pollock in this study to weathered crude oil was supported by results from the analysis of their hydrolyzed bile showing high proportions of metabolites of those petroleum-related ACs that resist the weathering processes. In particular, homologous series of alkylated naphthols, phenanthrols, and dibenzothiophenols were found by GC/MS to be abundant in these fish captured in PWS from 5-12 months after the spill. The homologous series of dibenzothiophenols have not been reported previously. We propose that these metabolites can serve as marker compounds to indicate the exposure of fish to particular crude oils. For example, PBCO and other North Slope crude oils contain relatively high proportions of dibenzothiophenes, unlike most other Alaskan (e.g., Cook Inlet crude) and continental U.S. crude oils (11-13). Therefore, identification of high concentrations of homologous dibenzothiophenols in the bile of the pollock and salmon suggests a North Slope crude oil as the source of exposure. In future spills of high-sulfur crude oils, the presence of dibenzothiophenols in bile of fish can be used to help establish the source of exposure.

The results of this study have validated bile screening as a method for estimating petroleum exposure in fish. A statistical analysis of concentrations of metabolites of ACs in bile, determined both by bile screening (Table IV) and by GC/MS (Tables II-III), revealed highly significant correlations (Figure 6). These results agree with the results for bile samples analyzed by similar methods in our earlier study of fish exposed to urban pollution (10). Furthermore, we

suggest the ratios of phenanthrene or naphthalene equivalents to benzo[a]pyrene (BaP) equivalents from bile screening can be used to differentiate the source of the AC contamination. The concentrations of 4- and 5-ring ACs that fluoresce at BaP wavelengths are much lower in crude oil than in urban sediments that have pyrogenic inputs. As a result, the naphthalene/BaP ratios should be higher in fish exposed to crude oil. For example, the ratios of naphthalene to BaP equivalents from the salmon (n=5) in this study (mean ± standard deviation = 1300 ± 600) were higher than similar ratios in English sole (n = 38) from a site in Puget Sound known to be contaminated with creosote (mean ± standard deviation = 110±62) (26,27). Therefore, these results strongly support use of the bile screening method in oil spill situations to test large numbers of bile samples for exposure to petroleum. Then, concentrations of individual metabolites in selected samples can be confirmed by GC/MS. This approach should ensure that exposure data will be available in a cost-effective and timely manner.

In the current study, the suite of petroleum-related metabolites that were identified in the oil-injected halibut and the Dolly Varden were also found in the environmentally exposed salmon and pollock, although the proportions of the metabolites varied (Figure 5). When different fish species are exposed to a particular AC (e. g., benzo[a]pyrene), the efficiency of metabolism and the types and proportions of metabolites produced may differ significantly, i.e., interspecies differences (6). Even when individual fish of the same species are exposed to an AC, the rates of bioconversion and excretion and, thus, the proportions of each metabolite may vary, i.e., intraspecies differences (6). In fact, we observed these variations in our field samples (Figure 5): the pollock, as a group, had relatively similar profiles, while those of the salmon were somewhat

similar to each other, but dissimilar to the profiles of the pollock. In addition to species differences, exposure to oil with different degrees of weathering could account for some differences in the observed metabolite profiles.

Metabolites of ACs are rapidly excreted into bile for elimination. As a result, bile is considered a short-term indicator of contamination. For example, Collier and Varanasi (28) showed that 6 weeks after fish were injected with an extract from a contaminated urban sediment, concentrations of fluorescent ACs in their bile were reduced to about 15% of the highest measured concentration. Therefore, the presence of petroleum-related metabolites in the bile of the salmon and pollock captured from PWS (Tables II-III) is evidence of relatively recent exposure of these fish to crude oil.

Because analyses of the bile of fish from these two commercially important species provided evidence of their exposure to crude oil, other studies were conducted to analyze samples of edible tissue from selected salmon and pollock for petroleum-related contaminants (8,29). The study of the salmon showed that, even for the salmon containing the highest levels of FACs in bile (8), concentrations of parent ACs in the flesh were low (i.e., the sums of concentrations of about 300 ACs quantitated by GC/MS were generally <50 ng/g). The edible tissue of pollock also contained low or undetectable concentrations of ACs (29). These results from field-exposed fish are supported by laboratory studies that have shown that ACs are efficiently metabolized in liver and the metabolites are excreted via bile. As a result, the accumulation of ACs or their metabolites in edible tissue is generally low or below detection limits (30-32).

In summary, numerous metabolites of ACs have been determined by GC/MS in the hydrolyzed bile of 5 salmon and 4 pollock captured in PWS several months after PBCO was spilled from the *Exxon Valdez*. High proportions of alkylated phenanthrols, dibenzothiophenols and other metabolic products of ACs that tend to persist after crude oil weathers, were present in bile of the experimentally injected fish and field-exposed fish, but not in the carrier-injected or reference fish. Therefore, identification of these metabolites in bile provided evidence that the fish had been exposed to crude oil. In addition, alkylated dibenzothiophenols were suggested as promising marker compounds for identifying the source of crude oil. Furthermore, the bile screening method was validated for use in determining exposure of fish to petroleum, because concentrations determined by the screening method were highly correlated with those determined by GC/MS. In future studies, an assessment of exposure of fish from PWS and the Gulf of Alaska to the spilled oil will be obtained by conducting bile screening analyses on a large number of fish and confirming metabolite concentrations by GC/MS analysis of bile from selected fish. To complete the evaluation, sediments and stomach contents will also be analyzed for ACs.

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Table I: Ions of metabolites of aromatic compounds scanned by sequenced selected ion monitoring (SSIM)†.

Compound	Molecular ion (M^+)	Ion 2	Ion 3	Ion 4	Ion 5	Segment	Scan start time (min)
2,6-Dibromophenol	250	252				1	10
Hexamethylbenzene	147					1	10
Phenanthrene-d10	188	184				1,2	10
C_2 Naphthols	172	157	143	129		1,2	10
C_3 Naphthols	186	171	143	129		1,2	10
C_1 Dibenzothiophenols	214	185	184	165		3,4	31
C_2 Dibenzothiophenols	228	213	185	184	165	4	36
C_3 Dibenzothiophenols	242	227	185	184	179	4,5	36
C_1 Phenanthrols	208	179	178	165		4	36
C_2 Phenanthrols	222	193	179	178	165	4,5	36
C_3 Phenanthrols	236	193	192	178		5,6	41

† For the naphthols, dibenzothiophenols and phenanthrols, the fragment ions are identified as follows: $M^+ - 15$ = —CH_3; $M^+ - 29$ = —CHO/—C_2H_5; $M^+ - 30$ = —CH_2O/—C_2H_6; $M^+ - 43$ = —C_2H_3O/—C_3H_7; $M^+ - 44$ = —C_2H_4O/—C_3H_8; and $M^+ - 57$ = —C_3H_5O/—C_4H_9. A table of the other ions scanned and the start time for each segment is available in the microfilm edition.

TABL Relative retention times (RRT) based on phenanthrene d10 d10) and concentrations by SSIM GC/MS of selected metabolites of naphthalenes, dibenzothiophenes and phenanthrenes in bile of (a) halibut injected with Prudhoe Bay crude oil and with carrier, (b) salmon captured in Prince William Sound 5 months after oil was spilled into the Sound, and (c) the method blank.

		Concentration (ng/g, wet wt)								
	RRT (PHNd10)	oil-inj HALIBUT	carrier-inj. HALIBUT	SALMON-A	SALMON-B	SALMON-C	SALMON-D	SALMON-E	reference SALMON	METHOD BLANK
Naphthols										
C_2										
	0.913	66	<7	310	87	1,400	75	71	10	< 10
C_3										
	1.065	1,600	<7	960	210	2,600	470	300	10	< 10
Dibenzothiophenols										
C_1										
	1.367	17,000	55	6,900	810	4,900	1,800	2,800	56	< 12
	1.385	5,900	<30	2,600	870	3,500	940	890	33	< 12
	1.391	2,600	48	5,100	690	12,000	1,900	1,800	39	< 12
C_2										
	1.465	14,000	73	11,000	2,000	6,000	3,700	4,400	<29	< 12
	1.471	9,800	45	10,000	1,900	6,400	3,700	4,100	<29	< 12
	1.475	11,000	44	12,000	2,000	7,700	5,100	4,300	49	< 12
	1.492	21,000	<30	15,000	2,700	8,300	5,400	5,700	<29	< 12
	1.509	9,100	<30	11,000	1,900	8,500	4,100	4,500	<29	< 12
C_3										
	1.546	9,000	<30	9,100	1,800	3,200	3,500	3,900	<29	< 12
	1.561	4,200	<30	5,800	3,300	10,000	4,300	3,300	<29	< 12
	1.565	4,600	<30	12,000	2,700	7,300	6,600	6,100	<29	< 12
Sum of metabolites which fluoresce at 290/335 nm •		**110,000**	**360**	**102,000**	**21,000**	**82,000**	**42,000**	**42,000**	**300**	**80**

Continued on next page

TABLE II. (cont'd.)

	RRT (PHNd10)	Concentration (ng/g, wet wt) oil-inj HALIBUT	carrier-inj HALIBUT	SALMON-A	SALMON-B	SALMON-C	SALMON-D	SALMON-E	reference SALMON	METHOD BLANK
Phenanthrols										
C_1										
	1.409	14,000	52	10,000	1,300	18,000	2,800	3,800	140	< 27
	1.432	1,200	<12	1,400	150	1,600	580	610	<11	< 17
	1.451	3,100	<12	1,800	290	2,800	1,000	780	<11	< 17
C_2										
	1.512	3,200	22	3,700	490	2,000	1,300	1,300	18	< 17
	1.519	2,700	<12	4,900	830	4,300	1,900	1,900	44	< 17
	1.529	1,900	<12	4,200	480	2,200	1,200	1,200	17	< 17
	1.541	6,600	<12	3,200	660	2,800	1,800	690	<11	< 17
C_3										
	1.622	1,400	<12	3,300	640	1,400	1,600	1,600	<11	< 17
	1.626	3,200	<12	3,600	580	1,200	1,700	1,900	<11	< 17
Sum of metabolites which fluoresce at 260/380 nm *		37,000	120	36,000	5,400	36,000	14,000	14,000	250	80
Recovery (%) of 2,4-dibromophenol		100	94	100	92	99	110	99	84	120

* When a concentration is below the limit of quantitation (LOQ; preceded by "<"), an amount equal to1/2 the LOQ is included in the sum of metabolites.

TABLE: Relative retention times (RRT) based on phenanthrene d10 (PHN d10) and concentrations by SSIM GC/MS of selected metabolites of naphthalenes, dibenzothiophenes and phenanthrenes in bile of (a) Dolly Varden injected with Prudhoe Bay crude oil and with carrier and (b) pollock captured in Prince William Sound 1 year after oil was spilled into the Sound.

		Concentration (ng/g, wet wt)							
	RRT (PHNd10)	oil-injected D. VARDEN	carrier-inj. D. VARDEN	POLLOCK-A	POLLOCK-B	POLLOCK-C	replicate 1 POLLOCK-D	replicate 2 POLLOCK-D	reference POLLOCK
Naphthols									
C_2									
	0.913	<14	<12	<0.8	<3	<0.8	<12	<15	<1
C_3									
	1.065	53	<12	<0.8	<3	<0.8	<12	<15	<1
Dibenzothiophenols									
C_1									
	1.367	150	<15	220	150	170	58	87	<3
	1.385	350	<15	<2	<12	<2	15	<18	<3
	1.391	290	<15	<2	<12	<2	<14	<18	<3
C_2									
	1.465	250	<15	480	170	330	110	160	<3
	1.471	160	<15	250	90	130	48	53	<3
	1.475	290	<15	<2	<12	<2	28	63	<3
	1.492	160	<15	1,100	210	560	150	200	<3
	1.509	270	<15	< 2	<12	< 2	37	45	<3
C_3									
	1.546	74	<15	350	120	110	33	38	34
	1.561	80	<15	170	69	140	21	23	<3
	1.565	96	<15	<2	<12	<2	38	38	<3
Sum of metabolites which fluoresce at 290/335 nm*		**2,200**	**90**	**2,600**	**880**	**1,400**	**560**	**740**	**50**

Continued on next page

TABLE [illegible]ntinued.

RRT (PHNd10)	oil-injected D. VARDEN	carrier-inj. D. VARDEN	POLLOCK-A	POLLOCK-B	POLLOCK-C	replicate 1 POLLOCK-D	replicate 2 POLLOCK-D	reference POLLOCK
	Concentration (ng/g, wet wt)							
Phenanthrols								
C_1								
1.409	370	<33	1,100	180	970	280	300	400
1.432	93	<20	<1	<5	<1	<19	<25	<2
1.451	580	<20	<1	<5	<1	<19	<25	<2
C_2								
1.512	<23	<20	200	49	81	94	110	<2
1.519	270	<20	290	67	160	140	130	<2
1.529	91	<20	330	30	71	50	50	<2
1.541	560	<20	<1	<5	<1	<19	<25	<2
C_3								
1.622	<23	<20	190	55	69	79	25	<2
1.626	<23	<20	<1	<5	<1	<19	<25	<2
Sum of metabolites which fluoresce at 260/380 nm*	**2,000**	**100**	**2,100**	**400**	**1,400**	**680**	**670**	**410**
Recovery (%) of 2,4-dibromophenol	100	110	110	98	110	110	89	93

* When a concentration is below the limit of quantitation (LOQ; preceded by "<"), an amount equal to1/2 the LOQ is included in the sum of metabolites.

TABLE IV: Equivalents of fluorescent aromatic compounds in bile determined by the HPLC screening method described by Krahn et al. (26).

	Equivalents of fluorescent aromatic compounds HPLC bile screening (ng/g wet wt)	
	(260/380 nm)	(290/335 nm)
SAMPLE	phenanthrene wavelengths	naphthalene wavelengths
Oil-injected halibut	1,300,000	3,800,000
Solvent-injected halibut	4,000	30,000
Oil-injected Dolly Varden	150,000	470,000
Solvent-injected Dolly Varden	6,700	40,000
Pollock-A	90,000	340,000
Pollock-B	44,000	270,000
Pollock-C	68,000	280,000
Pollock-D (n=2)	66,000	340,000
Pollock reference	5,000	36,000
Salmon-A	370,000	1,800,000
Salmon-B	240,000	990,000
Salmon-C	380,000	2,600,000
Salmon-D	190,000	780,000
Salmon-E	380,000	1,700,000
Salmon reference	5,000	44,000

Figure Captions

1. Chart of Prince William Sound showing the sites of capture for salmon, pollock and fish (halibut and Dolly Varden) injected with weathered Prudhoe Bay crude oil. The relatively uncontaminated site (reference site) from which pollock were captured, Seymour Canal, is located in Southeastern Alaska near Ketchikan (not shown).

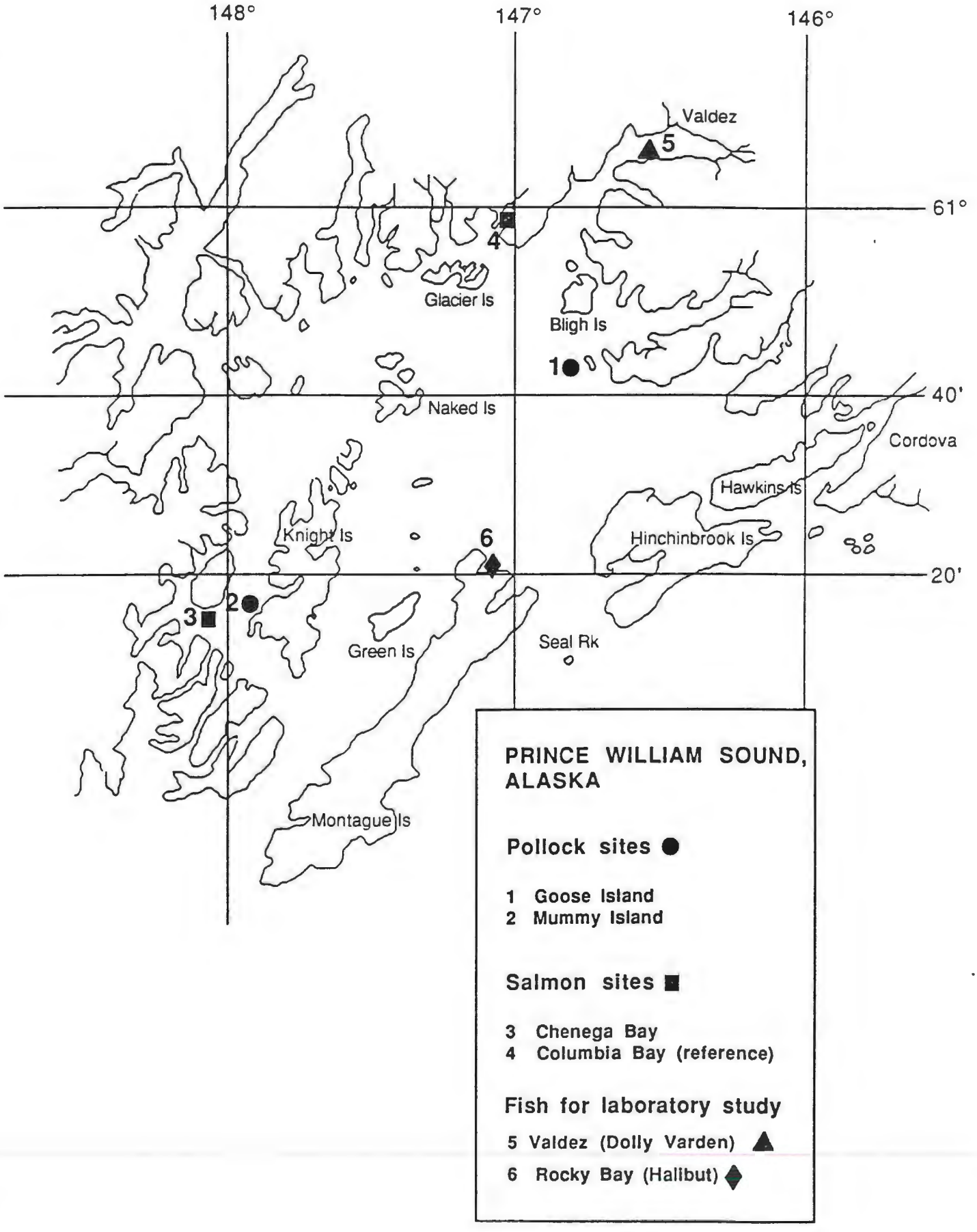
2. Full-scan GC/MS chromatogram of Prudhoe Bay crude oil (A) taken from the *Exxon Valdez* and (B) collected from a beach in Prince William Sound 11 days after the spill. Abbreviations: BENZ = benzene; NPH = naphthalene; DBF = dibenzofuran; PHN = phenanthrene; DBT = dibenzothiophene; FLA = fluoranthene; PYR = pyrene; CHR = chrysene.

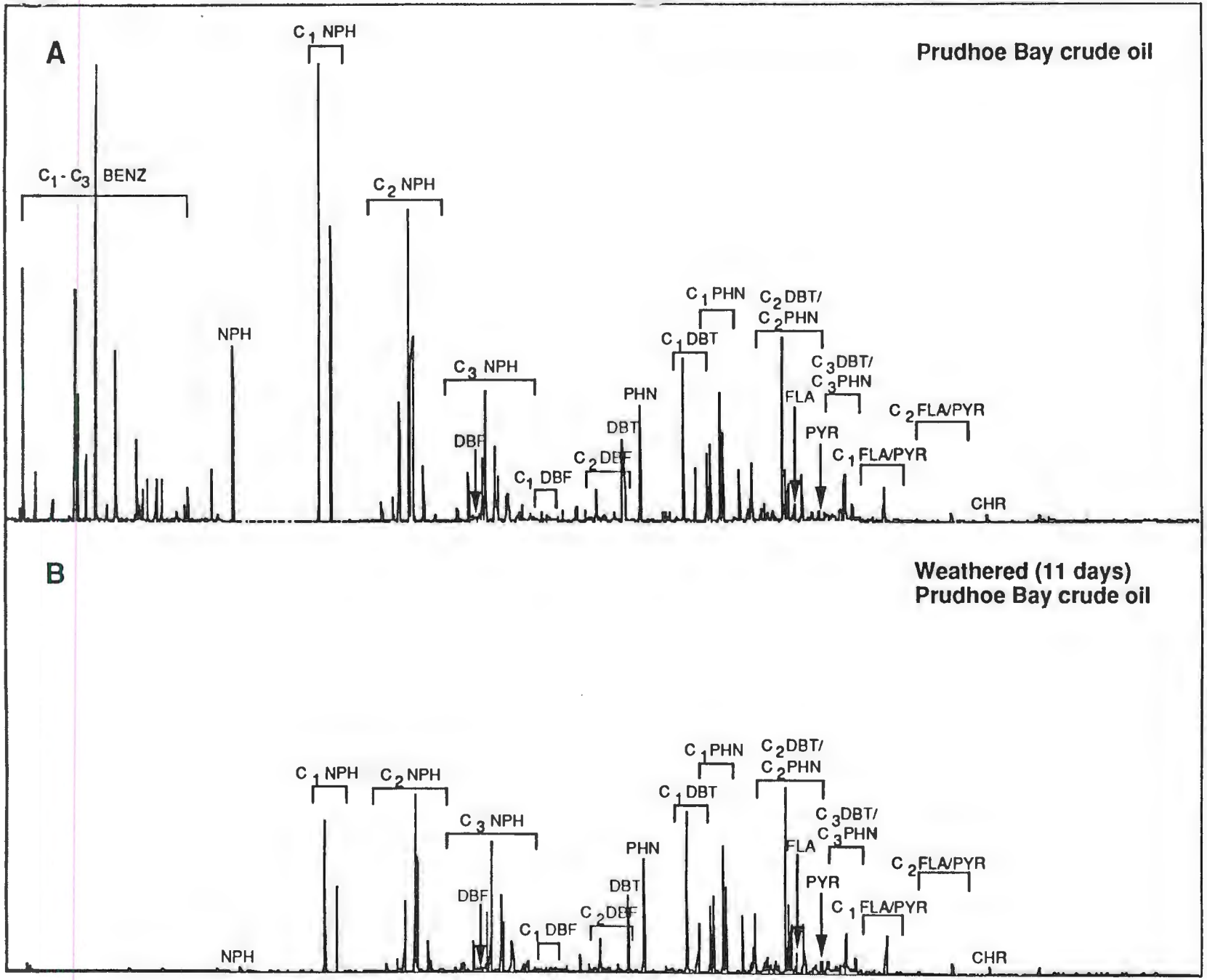
3. Mass chromatograms of the C_1-C_3 phenanthrols (mass/ charge = 208, 222, 236) from the bile of (A) the oil-injected halibut and (B) salmon-A; and C_1-C_3 dibenzothiophenols (mass/ charge = 214, 228, 242) from the bile of (C) the oil-injected halibut and (D) salmon-A.

4. Sequenced selected ion monitoring (SSIM) mass spectra of bile metabolites: C_3 naphthol [Relative retention time (RRT) based on phenanthrene d10 = 1.065] in (A) salmon-A and (B) the oil-injected halibut; C_1 phenanthrol (RRT = 1.409) in (C) salmon-A and (D) the oil-injected halibut; C_2 dibenzothiophenol (RRT = 1.450) in (E) pollock-A and (F) the oil-injected halibut; C_3 dibenzothiophenol (RRT = 1.546) in (G) pollock-A and (H) the oil-injected halibut.

5. Profiles of selected metabolites in the bile of salmon, pollock and oil-injected halibut and Dolly Varden (see Tables II-III for RRT). Concentrations are normalized to the metabolite with the highest concentration. Abbreviations: DBT-OH = dibenzothiophenol; PHN-OH = phenanthrol.

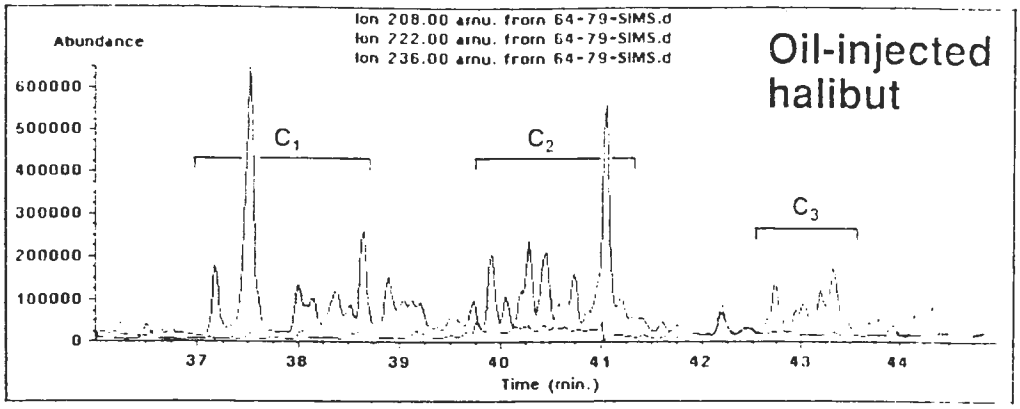
6. Correlation of fluorescent ACs in bile by screening and sums of individual metabolites of ACs determined by GC/MS for compounds fluorescing at (A) phenanthrene wavelengths and (B) naphthalene wavelengths.


148°
147°
146°
61°
40'
20'
Valdez
5
4
Glacier Is
Bligh Is
1
Naked Is
Cordova
Hawkins Is
Knight Is
6
Hinchinbrook Is
2
3
Green Is
Seal Rk
Montague Is
PRINCE WILLIAM SOUND, ALASKA
Pollock sites
1 Goose Island
2 Mummy Island
Salmon sites
3 Chenega Bay
4 Columbia Bay (reference)
Fish for laboratory study
5 Valdez (Dolly Varden)
6 Rocky Bay (Halibut)

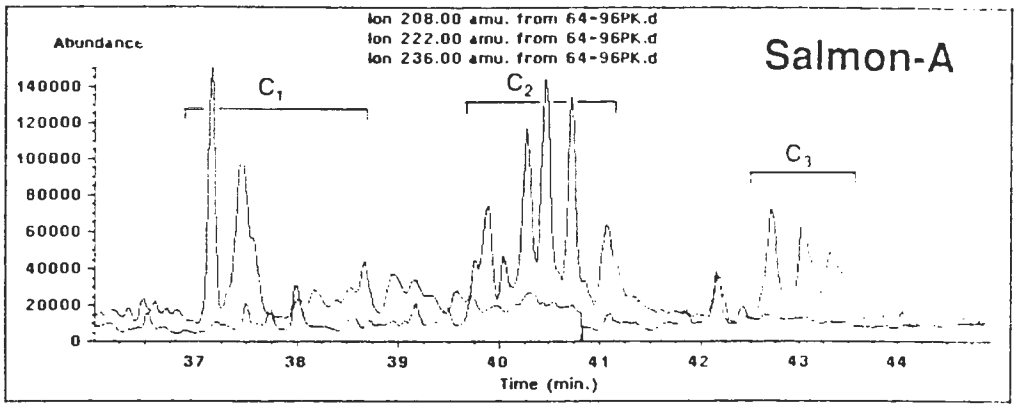

A
Prudhoe Bay crude oil
C1 NPH
C1 - C3 BENZ
C2 NPH
NPH
C3 NPH
C1 PHN
C2 DBT/
C2 PHN
C1 DBT
C3 DBT/
C3 PHN
PHN
FLA
C2 FLA/PYR
DBT
PYR
DBF
C2 DBF
C1 FLA/PYR
C1 DBF
CHR
B
Weathered (11 days)
Prudhoe Bay crude oil
C1 NPH
C2 NPH
C1 PHN
C2 DBT/
C2 PHN
C1 DBT
C3 NPH
C3 DBT/
C3 PHN
PHN
FLA
C2 FLA/PYR
DBT
PYR
DBF
C2 DBF
C1 FLA/PYR
C1 DBF
NPH
CHR

Phenanthrols

A

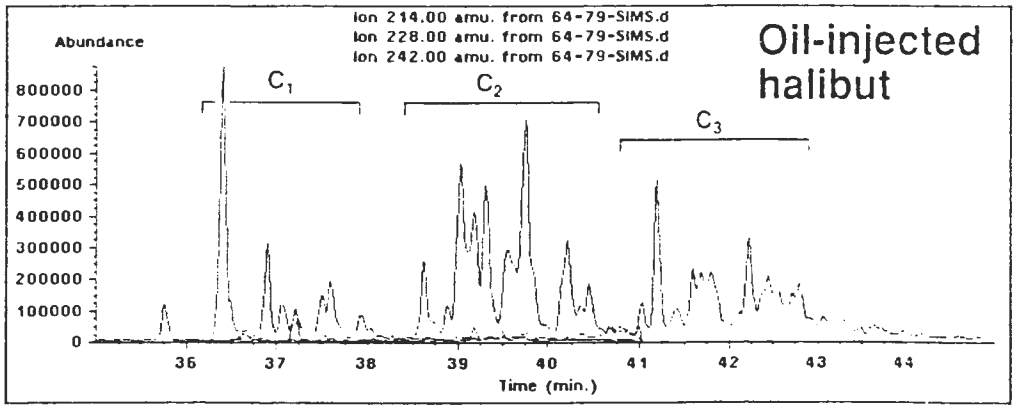


B

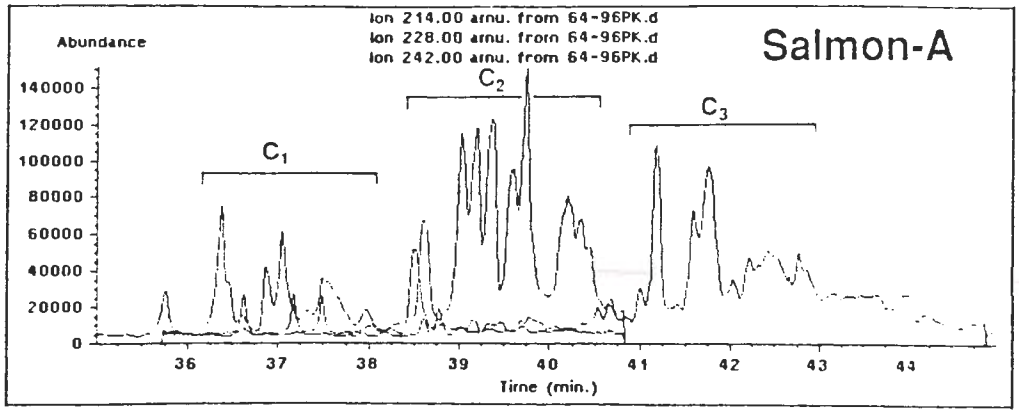


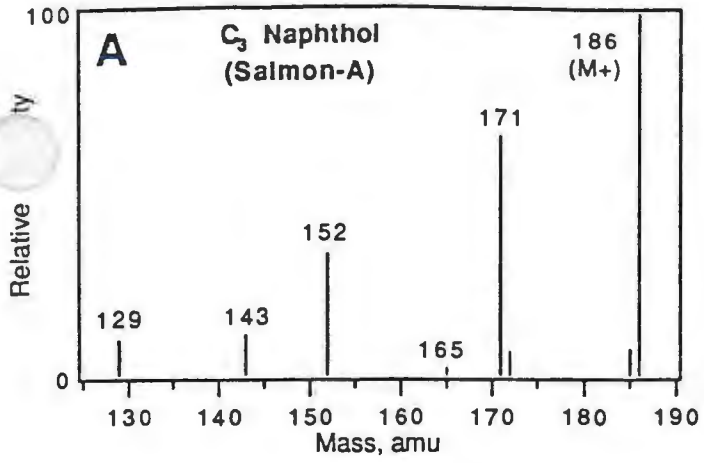
Dibenzothiophenols

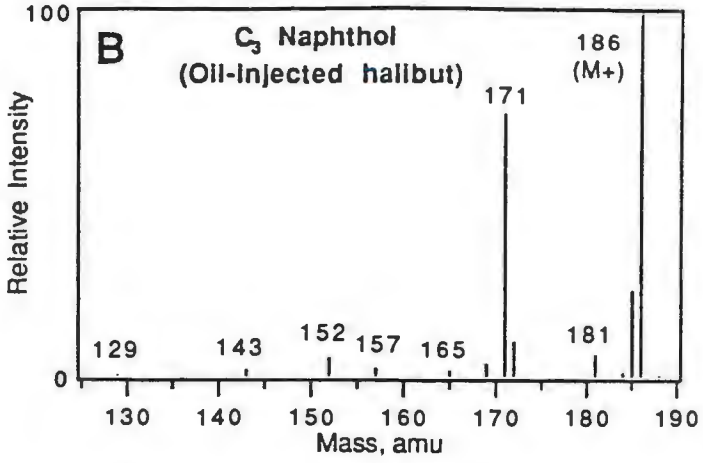
C

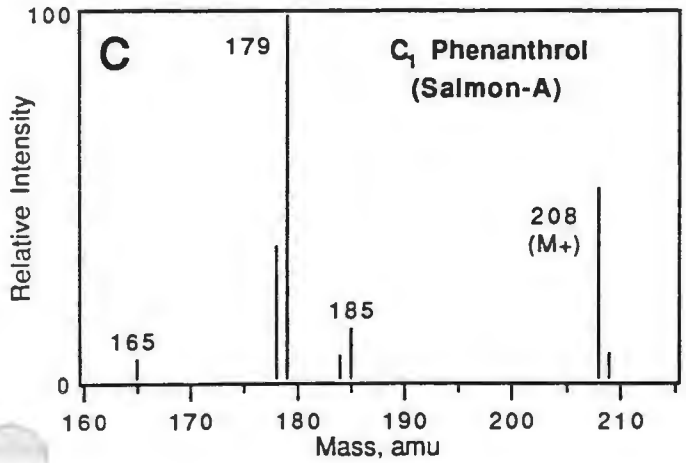


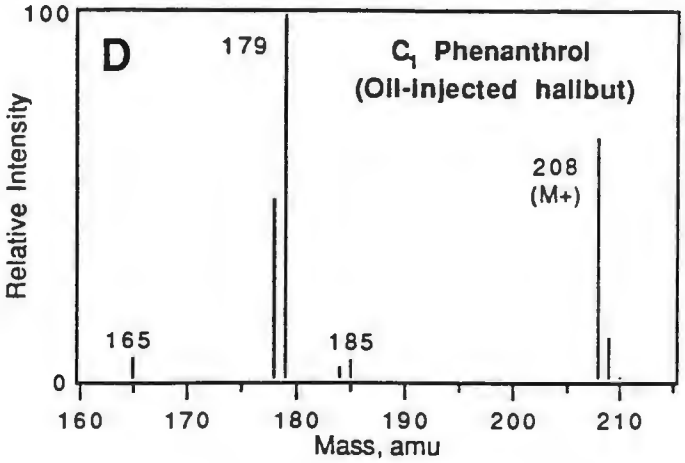
D

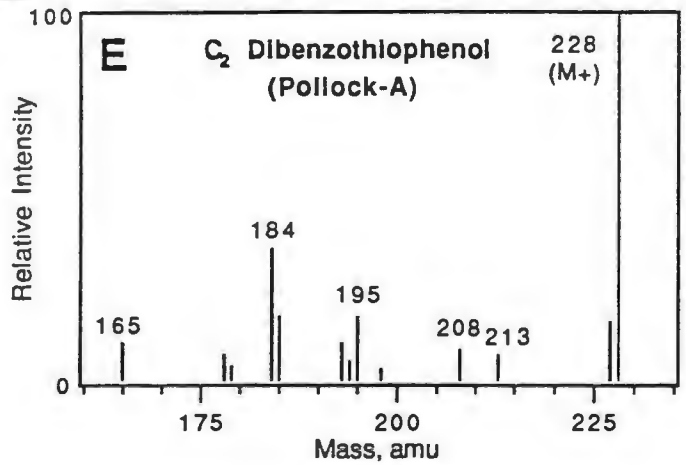


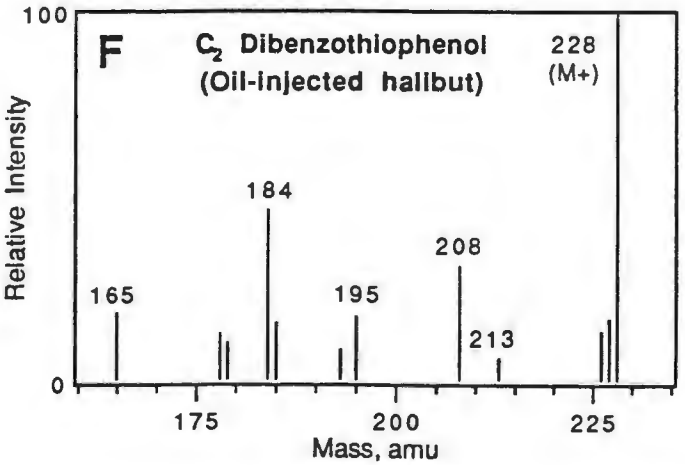

A
C3 Naphthol
(Salmon-A)
186
(M+)
171
152
129
143
165
100
0
Relative
130 140 150 160 170 180 190
Mass, amu

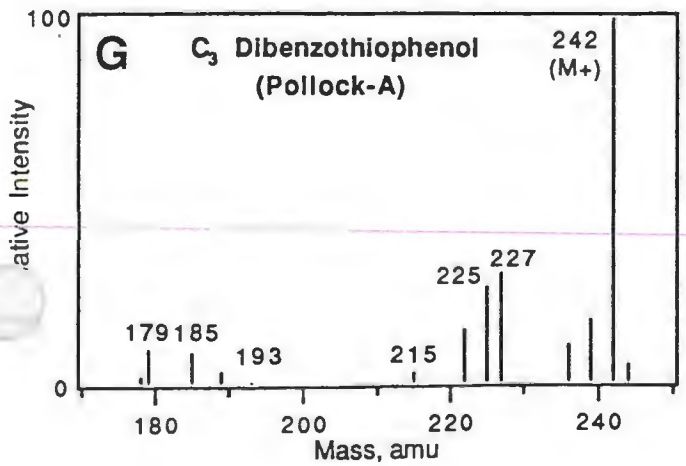

B
C3 Naphthol
(Oil-injected halibut)
186
(M+)
171
129
143
152
157
165
181
100
0
Relative Intensity
130 140 150 160 170 180 190
Mass, amu

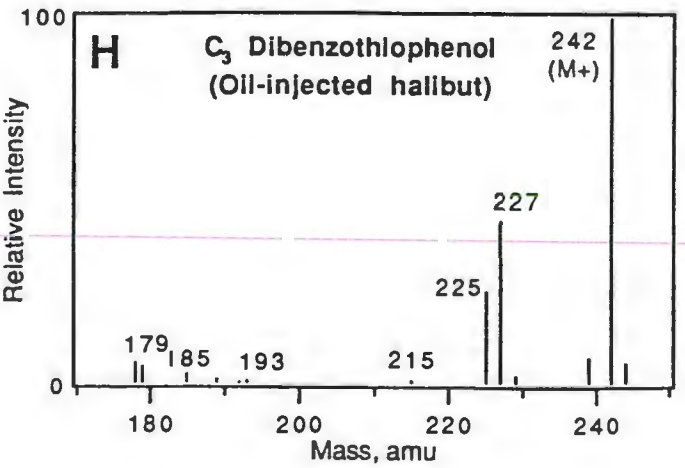

C
C1 Phenanthrol
(Salmon-A)
179
208
(M+)
185
165
100
0
Relative Intensity
160 170 180 190 200 210
Mass, amu

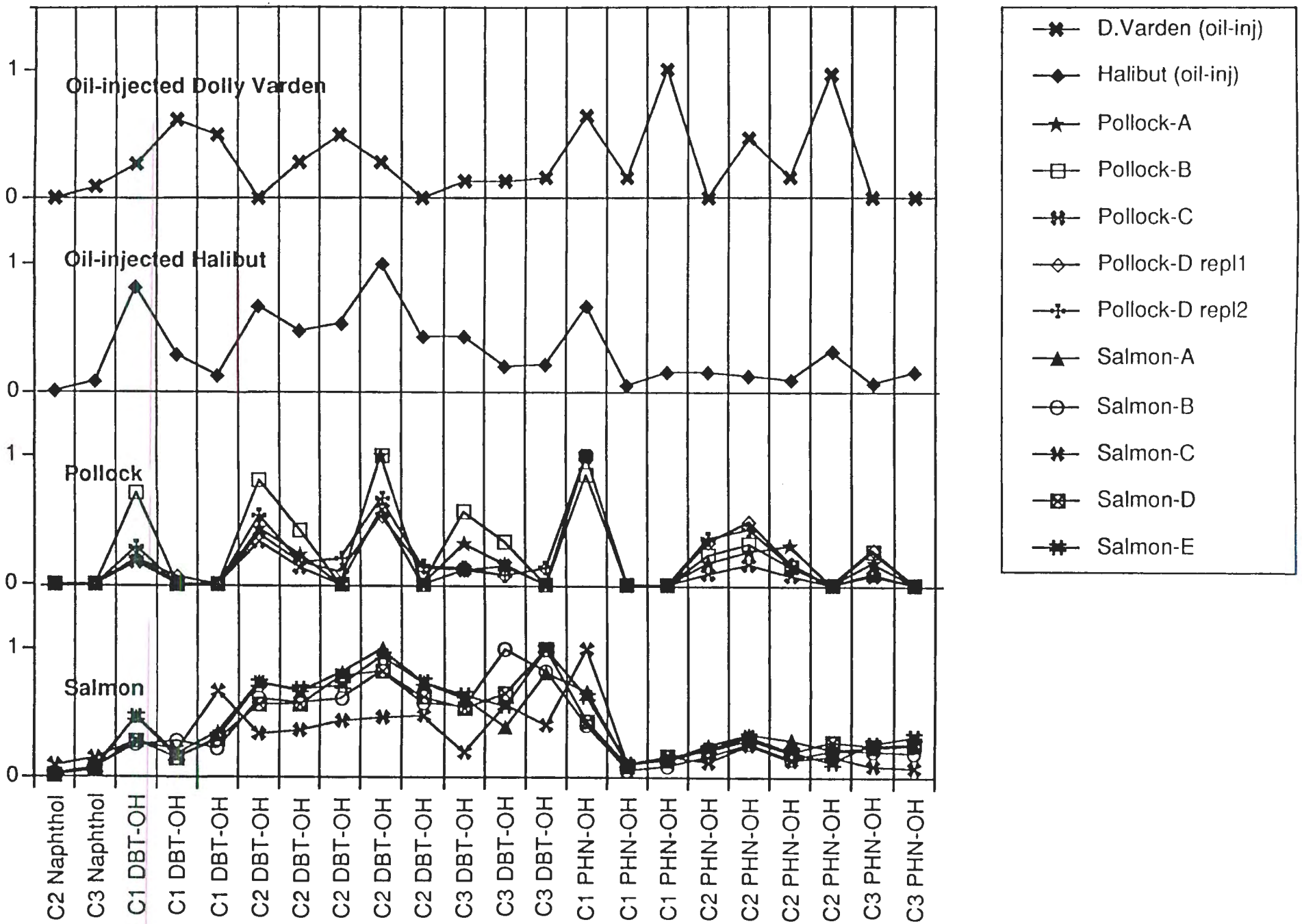

D
C1 Phenanthrol
(Oil-injected halibut)
179
208
(M+)
165
185
100
0
Relative Intensity
160 170 180 190 200 210
Mass, amu

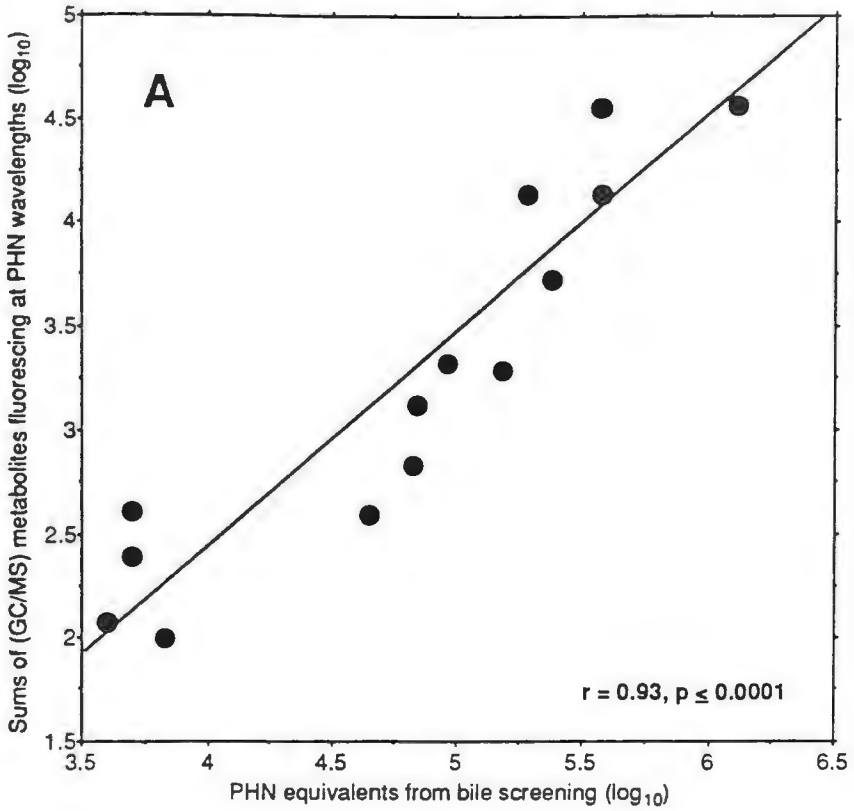

E
C2 Dibenzothiophenol
(Pollock-A)
228
(M+)
184
195
165
208
213
100
0
Relative Intensity
175 200 225
Mass, amu

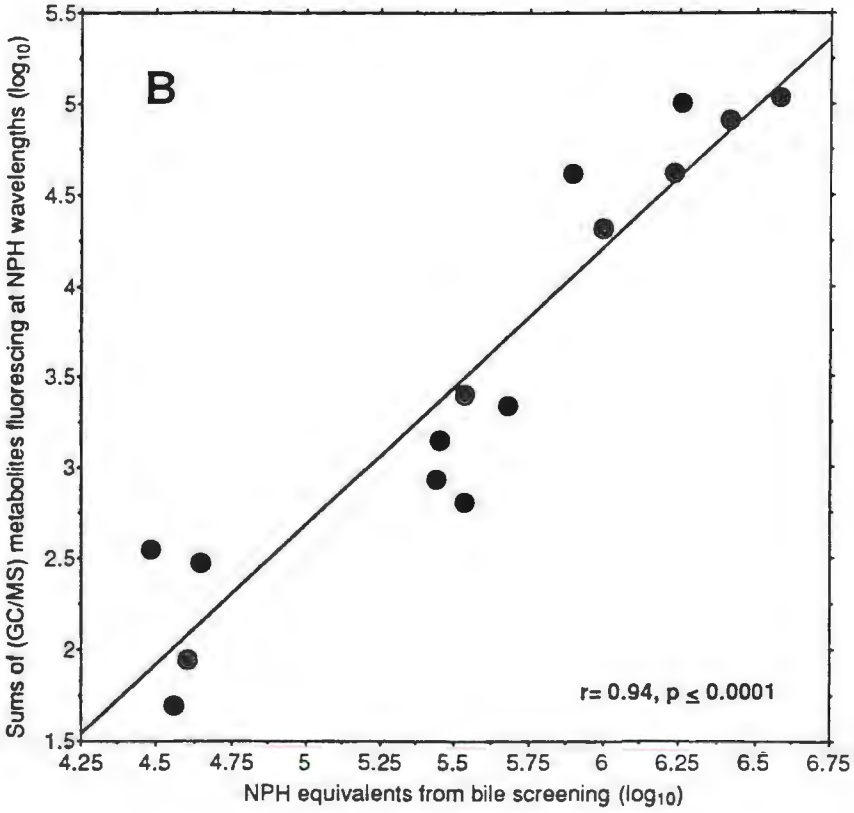

F
C2 Dibenzothiophenol
(Oil-injected halibut)
228
(M+)
184
208
165
195
213
100
0
Relative Intensity
175 200 225
Mass, amu


G
C3 Dibenzothiophenol
(Pollock-A)
242
(M+)
225
227
179
185
193
215
100
0
ative Intensity
180 200 220 240
Mass, amu


H
C3 Dibenzothiophenol
(Oil-injected halibut)
242
(M+)
227
225
179
185
193
215
100
0
Relative Intensity
180 200 220 240
Mass, amu


Oil-injected Dolly Varden
Oil-injected Halibut
Pollock
Salmon
1
0
C2 Naphthol
C3 Naphthol
C1 DBT-OH
C1 DBT-OH
C1 DBT-OH
C2 DBT-OH
C2 DBT-OH
C2 DBT-OH
C2 DBT-OH
C2 DBT-OH
C3 DBT-OH
C3 DBT-OH
C3 DBT-OH
C1 PHN-OH
C1 PHN-OH
C1 PHN-OH
C2 PHN-OH
C2 PHN-OH
C2 PHN-OH
C2 PHN-OH
C3 PHN-OH
C3 PHN-OH
D.Varden (oil-inj)
Halibut (oil-inj)
Pollock-A
Pollock-B
Pollock-C
Pollock-D repl1
Pollock-D repl2
Salmon-A
Salmon-B
Salmon-C
Salmon-D
Salmon-E


A
Sums of (GC/MS) metabolites fluorescing at PHN wavelengths (log_{10})
PHN equivalents from bile screening (log_{10})
r = 0.93, p ≤ 0.0001
5
4.5
4
3.5
3
2.5
2
1.5
3.5
4
4.5
5
5.5
6
6.5


B
Sums of (GC/MS) metabolites fluorescing at NPH wavelengths (log_{10})
NPH equivalents from bile screening (log_{10})
r= 0.94, p ≤ 0.0001
5.5
5
4.5
4
3.5
3
2.5
2
1.5
4.25
4.5
4.75
5
5.25
5.5
5.75
6
6.25
6.5
6.75

SUPPLEMENTARY MATERIAL FOR THE MICROFILM EDITION
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Table A-I. Sequenced selected ion monitoring (SSIM) descriptors for metabolite ions from aromatic compounds. The dwell time is the length of time each ion is scanned.

Segment	Scan start time (min)	Ions scanned (amu)	Dwell time (msec)
1	10	115, 129, 141, 143, 144, 147, 152, 157, 158, 165, 170, 171, 172, 182, 184, 186, 188, 190, 250, 252	40
2	26	129, 143, 144, 152, 157, 165, 169, 171, 172, 181, 182, 184, 185, 186, 188, 190, 198, 200, 211, 212	40
3	31	152, 165, 169, 178, 181, 182, 184, 185, 194, 195, 196, 198, 200, 209, 210, 211, 212, 214, 224, 226	40
4	36	165, 178, 179, 184, 185, 193, 194, 195, 198, 208, 209, 210, 213, 214, 222, 224, 226, 227, 228, 242	40
5	41	178, 179, 185, 189, 192, 193, 202, 215, 218, 222, 225, 227, 229, 232, 236, 239, 242, 244, 256, 258	40
6	45	179, 189, 192, 202, 215, 218, 223, 225, 228, 229, 232, 236, 239, 243, 244, 246, 256, 258, 272	45

TABLE A-II: Concentrations, determined by SSIM GC/MS, of metabolites of aromatic compounds in bile of a halibut injected with Prudhoe Bay crude oil and fish captured in Prince William Sound 5 months (salmon) and 1 year (pollock) after oil was spilled into the Sound.

					Concentration (ng/g, wet wt)		
	RT (min)	RRT (HMB)	RRT (PHNd10)	COMPOUND	oil-injected HALIBUT	SALMON-A	POLLOCK-A
Naphthols							
C_0							
	19.008	1.101	0.714	1-Naphthol	73	120	<2
	19.166	1.117	0.721	2-Naphthol	<3	440	<2
C_1							
	20.397	1.181	0.766	C_1 Naphthol	130	<6	<2
	20.532	1.189	0.771	C_1 Naphthol	71	<6	<2
	22.162	1.283	0.832	C_1 Naphthol	130	300	<2
	22.571	1.307	0.847	4-Methyl-1-naphthol	250	120	340
	22.853	1.332	0.860	C_1 Naphthol	<9	520	<2
C_2							
	23.756	1.376	0.892	C_2 Naphthol	170	340	<0.8
	24.166	1.399	0.907	6-methyl-2-naphthalene methanol	790	2,400	32
	24.321	1.408	0.913	C_2 Naphthol	66	310	<0.8
	24.864	1.440	0.933	C_2 Naphthol	370	<6	30
	25.062	1.451	0.941	C_2 Naphthol	300	<6	<0.8
	25.225	1.461	0.947	2,6-dimethyl-3-naphthol	150	47	8
	25.392	1.470	0.953	2,6-dimethylnaphthalene dihydrodiol	1,200	<96	<10
	25.558	1.480	0.959	C_2 Naphthol	220	490	15
	25.892	1.499	0.972	C_2 Naphthalene dihydrodiol	810	<96	<10
	26.337	1.525	0.989	C_2 Naphthalene dihydrodiol	790	<96	<10
	28.578	1.666	1.076	C_2 Naphthoquinone	<7	510	<10
C_3							
	25.039	1.450	0.940	C_2 Naphthalene methanol	390	510	<0.8
	27.029	1.576	1.017	C_3 Naphthol	<3	500	<0.8
	27.228	1.577	1.022	C_3 Naphthol	120	620	<0.8
	27.455	1.601	1.033	C_3 Naphthol	<3	550	32
	27.622	1.600	1.037	C_3 Naphthol	660	310	<0.8
	27.840	1.612	1.045	C_3 Naphthol	1,100	770	53
	27.985	1.631	1.053	C_3 Naphthol	<3	220	<0.8
	28.212	1.634	1.059	C_3 Naphthol	470	570	<0.8

† Some of the metabolites identified as C_1, C_2 and C_3 dibenzofuranols could also be C_2, C_3 and C_4 biphenylols/acenaphthenols.

TABLE A-II. continued.

RT (min)	RRT (HMB)	RRT (PHNd10)	COMPOUND	Concentration (ng/g, wet wt) oil-injected HALIBUT	SALMON-A	POLLOCK-A
Naphthols, continued.						
28.231	1.631	1.059	C2 Naphthalene methanol	<8	<18	210
28.377	1.643	1.065	C3 Naphthol	1,600	960	<0.8
28.891	1.673	1.085	C3 Naphthol	450	880	<0.8
Biphenylols						
C0						
19.140	1.108	0.719	2-Biphenylol	37	220	<1
24.644	1.437	0.928	Biphenylol	<5	250	<1
Fluorenols						
C0						
25.686	1.487	0.964	9-Fluorenol	94	260	<1
30.042	1.751	1.131	Fluorenol	<5	1,600	<1
30.564	1.770	1.147	Fluorenol	2,000	850	110
C1						
32.341	1.873	1.214	Fluorene methanol	2,600	1,000	<2
32.723	1.895	1.228	C1 Fluorenol	400	700	<1
32.828	1.901	1.232	C1 Fluorenol	2,600	1,800	<1
32.997	1.911	1.239	C1 Fluorenol	470	<11	<1
33.121	1.918	1.243	C1 Fluorenol	4,000	4,600	170
33.364	1.932	1.252	C1 Fluorenol	1,900	1,900	<1
33.629	1.947	1.262	C1 Fluorenol	2,700	<11	95
33.822	1.959	1.270	C1 Fluorenol	540	<11	<1
33.920	1.964	1.273	C1 Fluorenol	920	<11	150
C2						
33.666	1.950	1.264	C2 Fluorenol	580	<11	51
34.095	1.974	1.280	C2 Fluorenol	270	<11	<1
34.796	2.015	1.306	C2 Fluorenol	350	<11	<1
35.276	2.043	1.324	C2 Fluorenol	1,500	2,400	<1
35.539	2.058	1.334	C2 Fluorenol	2,800	4,100	<1
35.864	2.077	1.346	C2 Fluorenol	5,000	5,400	120
36.067	2.089	1.354	C2 Fluorenol	7,100	5,700	130
36.182	2.095	1.358	C2 Fluorenol	4,100	3,700	180
36.328	2.104	1.364	C2 Fluorenol	940	1,000	<1
36.472	2.112	1.369	C2 Fluorenol	1,400	1,400	<1

† Some of the metabolites identified as C1, C2 and C3 dibenzofuranols could also be C2, C3 and C4 biphenylols/acenaphthenols.

TABLE A-II, continued.

RT (min)	RRT (HMB)	RRT (PHNd10)	COMPOUND	Concentration (ng/g, wet wt) oil-injected HALIBUT	SALMON-A	POLLOCK-A
Fluorenols, continued.						
36.827	2.147	1.386	C_2 Fluorenol	<5	1,100	<1
C_3						
37.313	2.161	1.401	C_3 Fluorenol	670	<11	<1
37.382	2.165	1.403	C_3 Fluorenol	900	<11	<1
37.758	2.186	1.417	C_3 Fluorenol	810	<11	<1
37.896	2.194	1.423	C_3 Fluorenol	1,100	2,200	74
38.073	2.205	1.429	C_3 Fluorenol	3,600	5,200	170
38.267	2.216	1.437	C_3 Fluorenol	2,300	2,700	210
38.519	2.231	1.446	C_3 Fluorenol	2,800	3,600	150
38.660	2.239	1.451	C_3 Fluorenol	1,400	<11	<1
38.959	2.256	1.463	C_3 Fluorenol	1,700	4,500	97
39.133	2.266	1.469	C_3 Fluorenol	4,700	5,400	<1
39.562	2.291	1.485	C_3 Fluorenol	1,400	2,300	<1
Dibenzofuranols†						
C_0						
27.121	1.571	1.018	Dibenzofuranol	2,100	910	<2
29.147	1.688	1.094	Dibenzofuranol	930	<19	<2
C_1						
29.365	1.712	1.105	C_1 Dibenzofuranol	<7	2,400	<2
29.689	1.719	1.115	C_1 Dibenzofuranol	1,200	2,800	<2
29.861	1.729	1.121	C_1 Dibenzofuranol	2,800	4,300	<2
30.080	1.742	1.129	C_1 Dibenzofuranol	3,900	2,600	180
31.009	1.796	1.164	C_1 Dibenzofuranol	1,900	<23	<2
31.513	1.825	1.183	C_1 Dibenzofuranol	2,100	2,300	<2
31.659	1.833	1.188	C_1 Dibenzofuranol	2,200	<23	<2
C_2						
30.251	1.752	1.136	C_2 Dibenzofuranol	720	<23	<2
31.310	1.813	1.175	C_2 Dibenzofuranol	810	2,300	<2
31.792	1.841	1.193	C_2 Dibenzofuranol	790	1,200	<2
32.131	1.861	1.206	C_2 Dibenzofuranol	2,300	3,700	260
33.059	1.914	1.241	C_2 Dibenzofuranol	1,700	2,300	<2
33.201	1.923	1.246	C_2 Dibenzofuranol	6,200	4,700	150
33.390	1.934	1.253	C_2 Dibenzofuranol	3,200	6,000	380

† Some of the metabolites identified as C_1, C_2 and C_3 dibenzofuranols could also be C_2, C_3 and C_4 biphenylols/acenaphthenols.

TABLE A-II. continued.

RT (min)	RRT (HMB)	RRT (PHNd10)	COMPOUND	Concentration (ng/g, wet wt) oil-injected HALIBUT	SALMON-A	POLLOCK-A
Dibenzofuranols†, continued.						
33.908	1.964	1.273	C_2 Dibenzofuranol	3,900	3,700	120
34.067	1.973	1.279	C_2 Dibenzofuranol	1,300	3,000	<2
34.216	1.981	1.284	C_2 Dibenzofuranol	4,600	6,500	150
34.395	1.992	1.291	C_2 Dibenzofuranol	3,500	<23	<2
34.567	2.002	1.298	C_2 Dibenzofuranol	4,000	6,300	240
C_3						
34.911	2.022	1.311	C_3 Dibenzofuranol	2,300	<23	230
35.227	2.040	1.322	C_3 Dibenzofuranol	960	<23	64
35.744	2.070	1.342	C_3 Dibenzofuranol	2,000	<23	<2
35.856	2.076	1.346	C_3 Dibenzofuranol	1,800	<23	<2
36.107	2.091	1.355	C_3 Dibenzofuranol	3,200	8,100	<2
36.233	2.098	1.360	C_3 Dibenzofuranol	1,200	<23	<2
36.729	2.127	1.379	C_3 Dibenzofuranol	5,700	<23	<2
36.859	2.134	1.384	C_3 Dibenzofuranol	2,000	<23	<2
37.192	2.154	1.396	C_3 Dibenzofuranol	2,200	<23	<2
Dibenzothiophenols						
C_0						
29.497	1.720	1.110	Dibenzothiophenol	<7	2,000	52
30.864	1.783	1.158	Dibenzothiophenol	<7	<23	230
C_1						
36.403	2.108	1.367	C_1 Dibenzothiophenol	17,000	6,900	220
36.895	2.136	1.385	C_1 Dibenzothiophenol	5,900	2,600	<2
37.061	2.146	1.391	C_1 Dibenzothiophenol	2,600	5,100	<2
37.971	2.214	1.429	C_1 Dibenzothiophenol	<7	1,500	<2
C_2						
37.202	2.154	1.397	C_2 Dibenzothiophenol	2,000	2,500	<2
38.616	2.236	1.450	C_2 Dibenzothiophenol	5,600	8,000	440
39.016	2.259	1.465	C_2 Dibenzothiophenol	14,000	11,000	480
39.177	2.269	1.471	C_2 Dibenzothiophenol	9,800	10,000	250
39.302	2.276	1.475	C_2 Dibenzothiophenol	11,000	12,000	<2
39.546	2.290	1.485	C_2 Dibenzothiophenol	9,600	9,400	<2
39.741	2.301	1.492	C_2 Dibenzothiophenol	21,000	15,000	1,100
40.207	2.328	1.509	C_2 Dibenzothiophenol	9,100	11,000	< 2

† Some of the metabolites identified as C_1, C_2 and C_3 dibenzofuranols could also be C_2, C_3 and C_4 biphenylols/acenaphthenols.

TABLE A-II. continued.

RT (min)	RRT (HMB)	RRT (PHNd10)	COMPOUND	Concentration (ng/g, wet wt) oil-injected HALIBUT	SALMON-A	POLLOCK-A
Dibenzothiophenols, continued.						
40.444	2.342	1.518	C_2 Dibenzothiophenol	4,800	4,900	<2
C_3						
40.680	2.356	1.527	C_3 Dibenzothiophenol	940	<23	<2
41.028	2.376	1.540	C_3 Dibenzothiophenol	2,200	2,300	<2
41.189	2.385	1.546	C_3 Dibenzothiophenol	9,000	9,100	350
41.415	2.398	1.555	C_3 Dibenzothiophenol	3,100	1,600	<2
41.588	2.408	1.561	C_3 Dibenzothiophenol	4,200	5,800	170
41.681	2.414	1.565	C_3 Dibenzothiophenol	4,600	12,000	<2
41.794	2.420	1.569	C_3 Dibenzothiophenol	6,300	<23	470
42.209	2.444	1.585	C_3 Dibenzothiophenol	8,700	4,200	<2
42.436	2.457	1.593	C_3 Dibenzothiophenol	6,400	4,400	<2
42.544	2.464	1.597	C_3 Dibenzothiophenol	4,300	5,000	<2
42.714	2.473	1.603	C_3 Dibenzothiophenol	2,400	1,200	<2
42.792	2.478	1.606	C_3 Dibenzothiophenol	5,300	3,800	<2
42.856	2.498	1.613	C_3 Dibenzothiophenol	<7	4,600	<2
Phenanthrols						
C_0						
35.218	2.039	1.322	9-Phenanthrol	170	<9	<1
35.594	2.061	1.336	Phenanthrol	1,000	1200	<1
35.816	2.074	1.345	Phenanthrol	1,300	<9	<1
36.552	2.117	1.372	Phenanthrol	1,900	<9	<1
C_1						
37.522	2.173	1.409	Phenanthrene methanol	14,000	10,000	1,100
38.000	2.200	1.427	C_1 Phenanthrol	2,200	<9	<1
38.143	2.209	1.432	C_1 Phenanthrol	1,200	1,400	<1
38.366	2.222	1.440	Phenanthrene methanol	3,400	<14	<2
38.641	2.238	1.451	C_1 Phenanthrol	3,100	1,800	<1
C_2						
39.906	2.311	1.498	C_2 Phenanthrol	2,400	2,400	280
40.053	2.319	1.504	C_2 Phenanthrol	1,000	1,200	<1
40.282	2.333	1.512	C_2 Phenanthrol	3,200	3,700	200
40.451	2.342	1.519	C_2 Phenanthrol	2,700	4,900	290
40.737	2.359	1.529	C_2 Phenanthrol	1,900	4,200	330

† Some of the metabolites identified as C_1, C_2 and C_3 dibenzofuranols could also be C_2, C_3 and C_4 biphenylols/acenaphthenols.

TABLE A-II, continued.

RT (min)	RRT (HMB)	RRT (PHNd10)	COMPOUND	Concentration (ng/g, wet wt) oil-injected HALIBUT	SALMON-A	POLLOCK-A
nanthrols, continued.						
41.045	2.377	1.541	C_2 Phenanthrol	6,600	3,200	<1
C_3						
42.711	2.490	1.608	C_3 Phenanthrol	<4	3,400	270
43.195	2.501	1.622	C_3 Phenanthrol	1,400	3,300	190
43.326	2.509	1.626	C_3 Phenanthrol	3,200	3,600	<1
Fluoranthenols/Pyrenols						
C_0						
41.606	2.426	1.566	Fluoranthenol/pyrenol	<2	1,000	<0.4
42.325	2.467	1.593	Fluoranthenol/pyrenol	<2	1,200	<0.4
43.369	2.511	1.628	1-pyrenol	730	<4	<0.4
43.611	2.542	1.642	Fluoranthenol/pyrenol	<2	760	<0.4
C_1						
44.554	2.580	1.673	C_1 Fluoranthenol/pyrenol	130	<9	<0.4
44.865	2.598	1.684	C_1 Fluoranthenol/pyrenol	200	<9	<0.4
44.999	2.606	1.689	C_1 Fluoranthenol/pyrenol	170	390	<0.4
45.170	2.616	1.696	C_1 Fluoranthenol/pyrenol	260	410	9
45.633	2.642	1.713	C_1 Fluoranthenol/pyrenol	210	<9	<0.4
45.744	2.649	1.717	C_1 Fluoranthenol/pyrenol	190	3,600	<0.4
46.173	2.674	1.733	C_1 Fluoranthenol/pyrenol	560	<9	3
46.359	2.685	1.740	C_1 Fluoranthenol/pyrenol	440	1,700	8
C_2						
46.737	2.706	1.755	C_2 Fluoranthenol/pyrenol	170	<9	<0.4
47.264	2.737	1.774	C_2 Fluoranthenol/pyrenol	200	2,100	31
47.355	2.742	1.778	C_2 Fluoranthenol/pyrenol	230	<9	<0.4
47.507	2.751	1.783	C_2 Fluoranthenol/pyrenol	110	890	<0.4
47.724	2.764	1.792	C_2 Fluoranthenol/pyrenol	190	<9	<0.4
47.869	2.772	1.797	C_2 Fluoranthenol/pyrenol	520	1,100	<0.4
48.294	2.797	1.813	C_2 Fluoranthenol/pyrenol	330	<9	<0.4
48.965	2.835	1.838	C_2 Fluoranthenol/pyrenol	350	390	<0.4
Benz[a]anthracenols/chrysenols						
C_0						
45.883	2.657	1.722	Benz[a]anthracenol/chrysenol	1,500	<23	<2
46.294	2.681	1.738	Benz[a]anthracenol/chrysenol	1,700	<23	<2
46.391	2.705	1.746	Benz[a]anthracenol/chrysenol	<7	3,800	<2

† Some of the metabolites identified as C_1, C_2 and C_3 dibenzofuranols could also be C_2, C_3 and C_4 biphenylols/acenaphthenols.

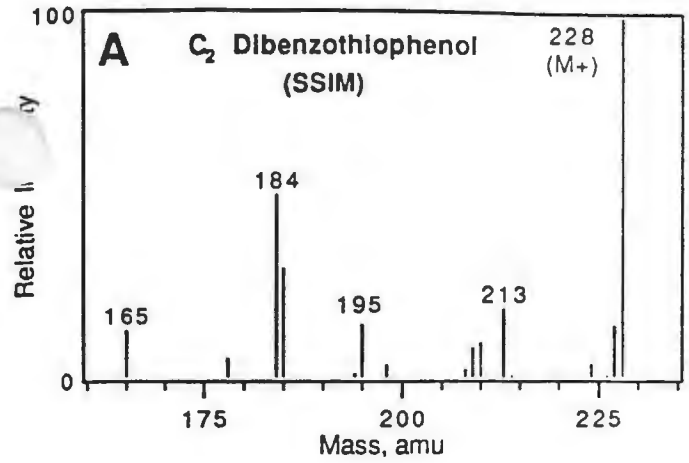
TABLE A-II, continued.

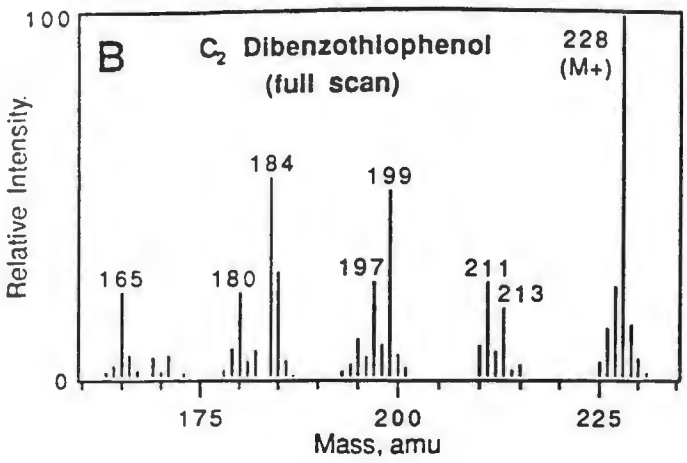
RT (min)	RRT (HMB)	RRT (PHNd10)	COMPOUND	Concentration (ng/g, wet wt) oil-injected HALIBUT	SALMON-A	POLLOCK-A
Benz[a]anthracenols/chrysenols, continued.						
46.731	2.706	1.754	Benz[a]anthracenol/chrysenol	1,600	1,800	<2
46.781	2.727	1.761	Benz[a]anthracenol/chrysenol	<7	3,000	<2
C_1						
47.173	2.732	1.771	C_1 Benz[a]anthracenol/chrysenol	1,000	790	<2
47.596	2.756	1.787	C_1 Benz[a]anthracenol/chrysenol	2,600	<23	<2
48.081	2.784	1.805	C_1 Benz[a]anthracenol/chrysenol	1,000	<23	<2
48.641	2.817	1.826	C_1 Benz[a]anthracenol/chrysenol	3,400	<23	<2
49.095	2.843	1.843	C_1 Benz[a]anthracenol/chrysenol	1,300	6,100	47
51.434	2.978	1.931	C_1 Benz[a]anthracenol/chrysenol	6,500	<23	200
51.772	2.998	1.944	C_1 Benz[a]anthracenol/chrysenol	610	6,300	<2
C_2						
49.428	2.862	1.856	C_2 Benz[a]anthracenol/chrysenol	1,600	<23	<2
50.327	2.914	1.889	C_2 Benz[a]anthracenol/chrysenol	1,600	<23	<2
50.782	2.941	1.906	C_2 Benz[a]anthracenol/chrysenol	1,400	<23	<2
53.347	3.089	2.003	C_2 Benz[a]anthracenol/chrysenol	480	<23	20
53.450	3.095	2.007	C_2 Benz[a]anthracenol/chrysenol	870	<23	<2
53.740	3.112	2.017	C_2 Benz[a]anthracenol/chrysenol	400	1,900	<2

† Some of the metabolites identified as C_1, C_2 and C_3 dibenzofuranols could also be C_2, C_3 and C_4 biphenylols/acenaphthenols.

Figure Captions-Microfilm

A-1. Comparison of (A) a SSIM mass spectrum and (B) a full scan mass spectrum of a C_2 dibenzothiophenol metabolite (RRT = 1.465) from the bile of the oil-injected halibut.


A
C_2 Dibenzothiophenol
(SSIM)
228
(M+)
184
165
195
213
100
0
175
200
225
Mass, amu


B
C_2 Dibenzothiophenol
(full scan)
228
(M+)
184
199
165
180
197
211
213
Relative Intensity
100
0
175
200
225
Mass, amu