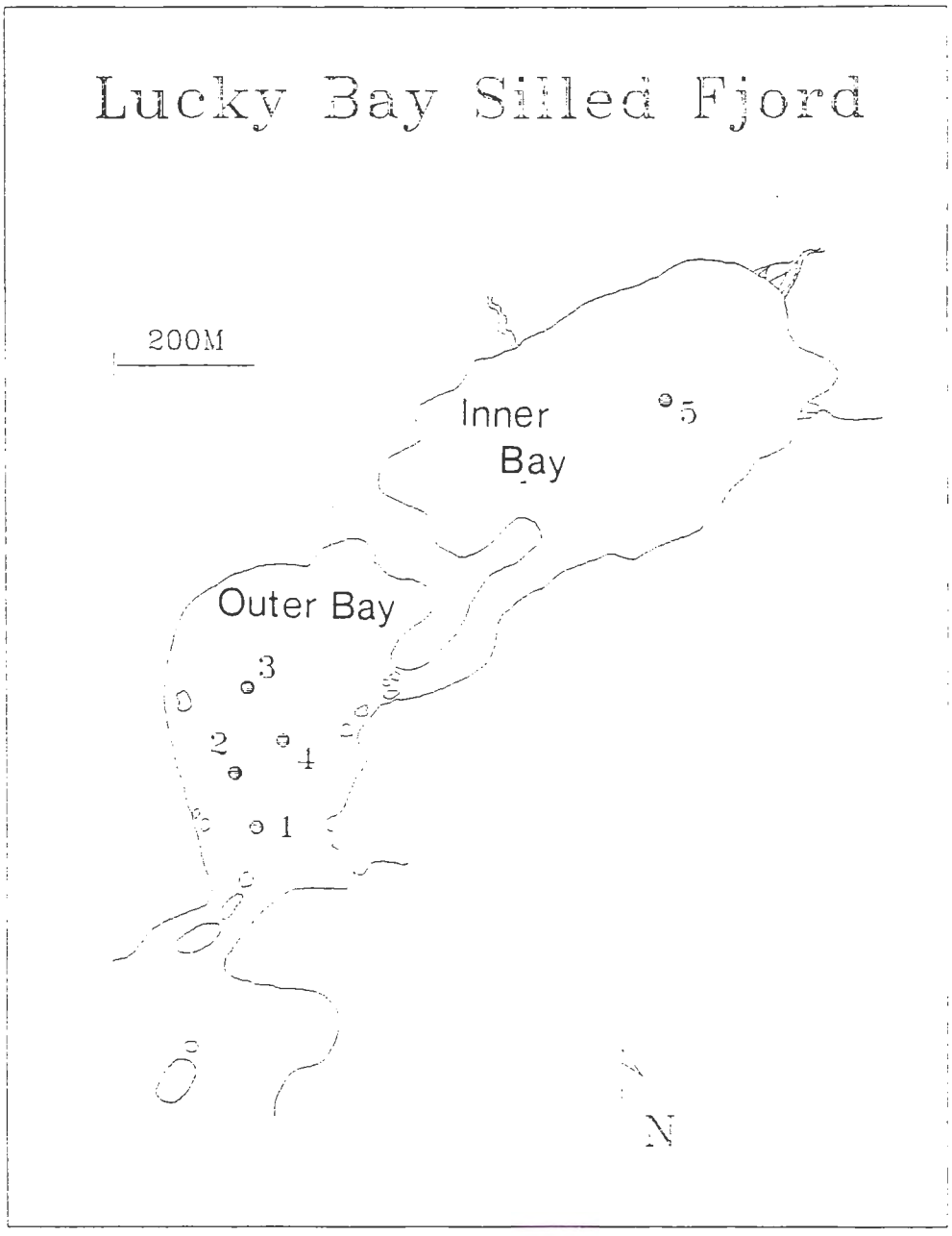


Figure 6.2 (continued) B. Inner and Outer Lucky Bay

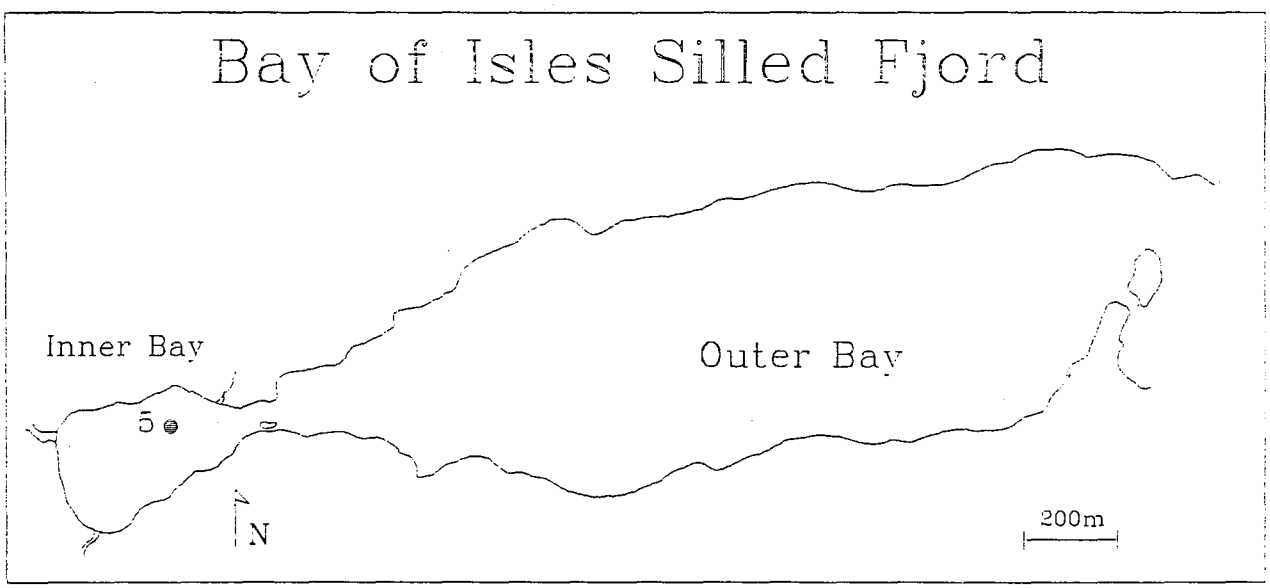


Figure 6.2 (continued) C. Inner and outer Bay of Isles.

density, biomass) for the dominant plant, invertebrate, and fish species. These estimates will be used to indicate the effects of the *Exxon Valdez* oil spill on these communities by comparing density (and other parameters) at oiled vs. control sites.

We sampled within 4 strata representing different habitats: *Nereocystis*, eelgrass, *Laminaria* in island bays, *Laminaria* on island points. Sites within each strata were selected for sampling based on the summer 1989 oil maps and the September "walkathon" data. Areas that were moderately to heavily oiled in both surveys were used as oiled sites. Control sites were selected that were oiled in neither the summer oil survey nor the walkathon. Controls were matched with selected oiled sites, as closely as possible, with regard to aspect, proximity to sources of freshwater input, slope, wave exposure, and water circulation. We randomly selected a matched site if more than one existed.

A total of 3 or 4 oiled sites and 3 or 4 control sites were selected and sampled from each habitat. A complete description of the site selection process is given in Appendix IV. Data from eelgrass and *Laminaria* Bay habitats are presented in this report.

Sampling Within the Eelgrass Habitat

At each eelgrass site, we established three 30 m long transects running parallel to shore. These were placed in the middle of the depth range of eelgrass. Along each transect, we harvested all eelgrass from 4 randomly placed 1/4 m^2 quadrats. The turions (above sediment portions of the plant arising from the rhizome) of the plants were cut approximately 1 cm above the sediment surface. The plants were bagged underwater and returned to the boat. There, the number of turions per quadrat were counted, and all turions in each quadrat weighed. In addition, we noted the number of flowering stalks per quadrat, and counted the number of seeds spathes per stalk.

Large (> 10 cm) motile invertebrates and fish were counted by divers within a 2 m swath along each 30 m long transect.

Densities of infaunal invertebrates were estimated from two 0.1 m^2 suction dredge samples taken at each of the three stations within the eelgrass bed. Similar dredge samples were taken from three additional transects established in each of two strata

(3 to 6 m and 6 to 20 m) that were established independent of the distribution of eelgrass. These transects were placed at random positions within the sampling site and at random depths within the strata.

Sediment samples for grain size and hydrocarbon analysis were taken along each of the 9 transects. No data are available for grain size or hydrocarbon at this time.

We sampled 8 eelgrass sites in 1990. Four oiled sites (Bay of Isles, Herring Bay, Sleepy Bay, and a bay on the eastern side of Naked Island) and four paired control sites (Drier Bay, Lower Herring Bay, Mooselips Bay on Montague Island, and a bay on the north side of Storey Island) were visited.

Sampling Within the *Laminaria/Agarum* Habitat

Three transects were randomly placed within each of two depth strata, 4 to 12 and 12 to 20 m, within the *Laminaria/Agarum* habitat. The percent cover by understory algae was determined in four 1/4 m^2 quadrats placed at random positions along each transect. All algae greater than 10 cm in height were collected from these quadrats and returned to the boat where each individual was weighed and measured.

Infaunal invertebrates associated with pockets of soft sediment along the transects were sampled using a suction dredge. Two randomly placed 1/10 m^2 quadrats were sampled from each transect.

Large (>10 cm) motile invertebrates and fishes were counted in a 2 m swath along each transect. These counts were made prior to the clearing of algae from quadrats.

We sampled six sites within the *Laminaria/Agarum* habitat in 1990. Three oiled sites (Northwest Bay, Herring Bay, and Bay of Isles) and three paired control sites (Cabin Bay, Lower Herring Bay, and Mummy Bay) were visited.

Experiments Evaluating Reproductive Success of *Dermasterias*

We examined the potential for reproductive success in the starfish, *Dermasterias imbricata*, at oiled vs. control sites by collecting stars from these sites and examining

gonad weight, spawning success, percent fertilization, and percent of normal larval development. Animals used to determine gonad index were collected from Herring Bay and Lower Herring Bay. Spawning success was determined for animals collected from 2 oiled sites (Herring Bay and Northwest Bay) and 2 control sites (Lower Herring Bay and Cabin Bay). Fertilization rate and normal embryo development were determined for animals collected from Northwest and Cabin Bay. All animals were taken from the field to the Seward Marine Laboratory for these tests.

In dissecting animals collected from Herring and Lower Herring Bay for their gonads, we noted a relatively high proportion of animals were parasitized by an unidentified barnacle (Order Ascothoracica). In order to examine the possible effects of oil on the level of infection by these parasites, we collected and dissected *Dermasterias* from four additional sites, 2 oiled (Northwest Bay and Bay of Isles) and 2 controls (Cabin Bay and Mummy Bay).

Data Analysis

The form of analysis for most data was a comparison of an oiled *vs.* its paired control site using a t test. In general there were replicate stations sampled within each site, and in some cases there were replicate quadrats sampled within each station. In all cases we used station rather than quadrat means as replicates in our analyses.

The only transformations used were arcsin-square root transformations for percent cover data. For all tests, we assumed that there would be unequal variances among sites and have used a correction to the number of degrees of freedom for the test in evaluating probabilities associated with t statistics.

Results and Discussion

Macroinfauna of Silled Fjords

We focus our discussion of subtidal (11-20 m) benthic macroinfauna on three comparisons among silled fjord habitats: a time-series comparison at an oiled site in Herring Bay (early October 1989 versus late May 1990); a spatial comparison between the oiled Herring Bay site and a control site in Outer Lucky Bay (both

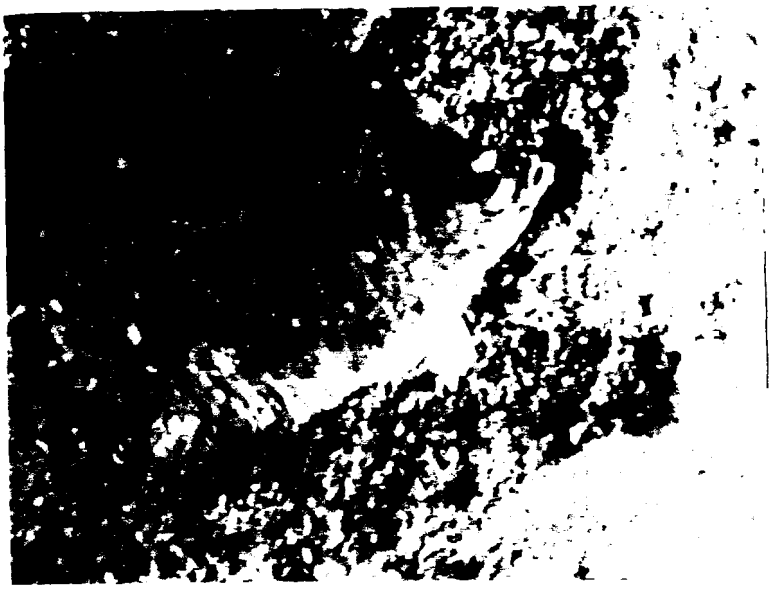
sampled in late May - early June 1990); and a spatial comparison between another oiled site in Inner Bay of Isles and an unoiled control site in Inner Lucky Bay (both sampled in early June 1990) (Fig. 6.2). Additional sampling within these and other silled fjords occurred in September and October 1990; however, data from these samples are not presented here.

Oiled Herring Bay 1989 *vs.* 1990

Sampling within the silled fjord of Herring Bay in October 1989 showed signs of gross disturbance, especially on silty substrate at depths greater than 13 m. This was the area originally referred to as the "Dead Zone". We observed numerous dead animals in deeper portions of this fjord (Fig. 6.3). In one area surveyed (approximately 70 m^2) at Herring Bay, we observed over 40 dead animals on the bottom, including 23 polychaete worms and 11 *Pycnopodia* sea stars. Also encountered were dead fish (Pacific cod), shrimp, clams, and squid. Similar surveys at this site in 1990 revealed significantly fewer dead animals. In video transect surveys conducted in May 1990 we saw only one dead *Pycnopodia* over a 90 m^2 area. In fall 1990 only one dead cod and three dead polychaete worms were observed over an equal 90 m^2 survey area. There were no concentrated pockets of dead organisms as observed in 1989. Similar surveys and searches at seven other fjords in Prince William Sound in spring and fall 1990 also failed to indicate high abundances of dead animals. The die-off of organisms at the Herring Bay fjord in 1989 was presumably the result of oiling or cleanup activities at this site.

In addition to the observed dead organisms in Herring Bay fjord, the substrate had a patchy, cobweb-like layer of the bacteria, *Beggiatoa*. This colorless, sulfur-dependent, hemolithotrophic bacteria is associated with decaying vegetation and low dissolved oxygen. Although no dissolved oxygen measurements were made in 1989, a strong sulfur odor was prevalent as divers sampled. The two stations (total area sampled = 0.5 m^2) that were within this disturbed zone in early October 1989 were compared with three stations (total area sampled = 0.5 m^2) in similar depth and substrate approximately 8 months later in late May 1990 (Fig. 6.2 A).

Although evidence of disturbance was visible in 1989 it was not clearly borne out through sampling. Subsequent analysis of 1989 data showed moderately high values



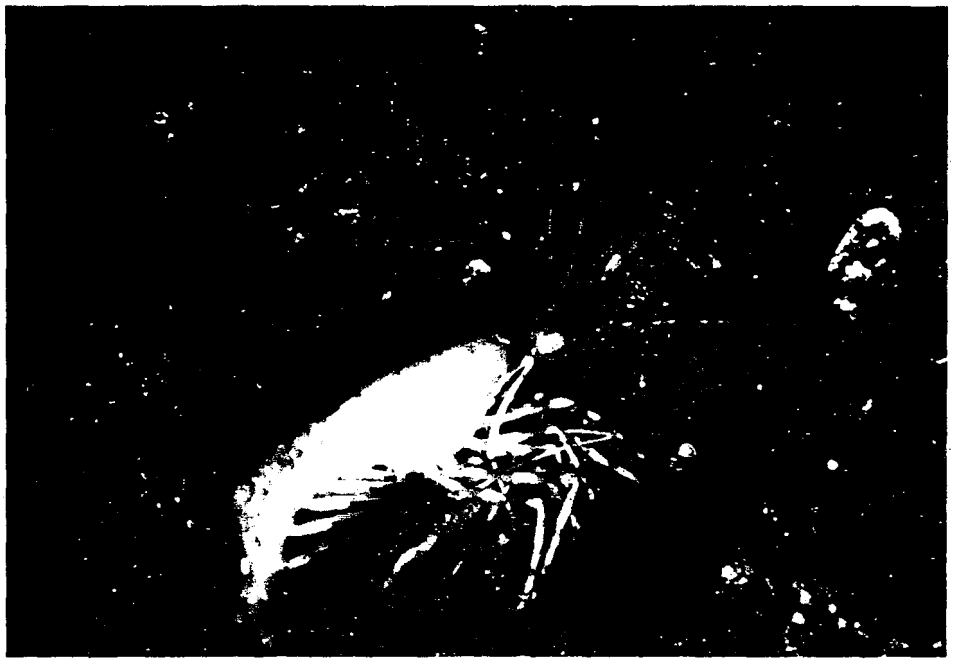


Figure 6.3 Photographs of dead organisms in Herring Bay silled fjord, fall 1989.

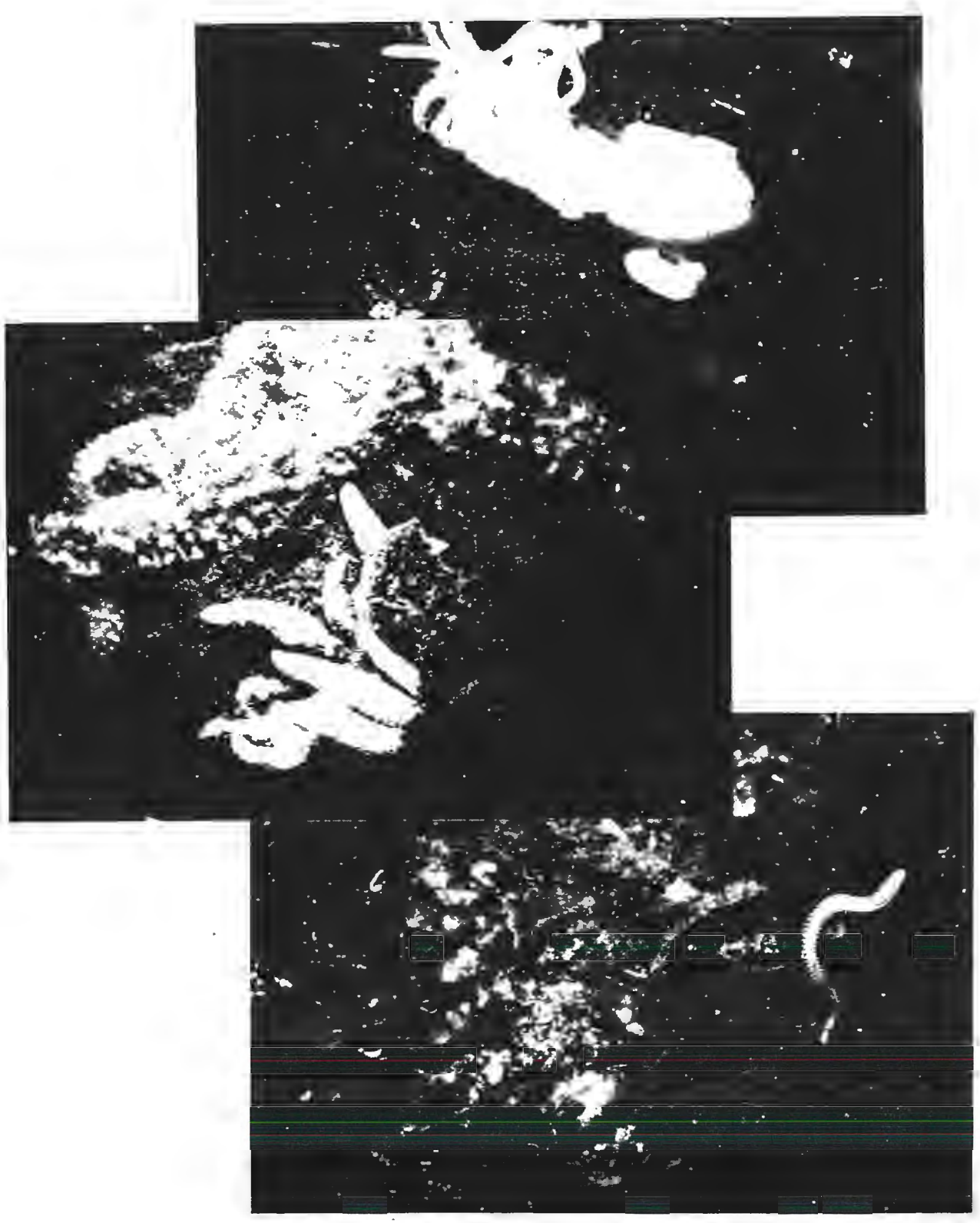


Figure 6.3 (continued)

of species richness (SR), evenness (J'), and size ratio (B/A) and low abundance ratio (A/T), all of which typically indicate an undisturbed environment (Table 6.2). However, signs of disturbance was revealed in the moderately low Shannon diversity index ($H' = 1.7$) and the moderately high Simpson dominance index ($D = 0.39$) (Fig. 6.4). The latter index was mainly attributed to the bivalve *Lucina tenuisculpta*, which had a mean density of 2,544/m^2 and accounted for nearly 61% of the site's mean faunal density (Table 6.3). The bivalve *Mysella tumida* and the polychaetes *Nephtys cornuta* and *Polydora socialis* ranked 2, 3, and 4 in density, respectively, with mean values between 256 and 336 individuals/m^2.

Further evidence of disturbance at this site in 1989 is provided by the presence of stress-resistant taxa or members of stress-resistant groups of taxa (Table 6.3; Fig. 6.5). For example, the bivalve *Lucina tenuisculpta* is in the same order as the stress-tolerant *Thyasira*. Several species of *Thyasira (T. flexuosa, T. sarsi, T. mtokanagai, T. miyadii)* have been reported from organically enriched and polluted substrates (see Table 1 in Pearson and Rosenberg, 1978). The high dominance of *Lucina tenuisculpta* at Herring Bay in 1989 suggests that this deposit-feeding clam not only was able to tolerate the adverse conditions of the environment (oil toxicity and/or low-oxygen stress), but also was able to utilize the additional organic material associated with the dead organisms present at the sediment surface.

Two other examples are provided by the polychaetes *Nephtys cornuta* and *Polydora socialis*. Lizarraga-Partida (1974) reported *Nephtys cornuta* in semi-polluted substrates in Ensenada Bay, Mexico, in areas enriched with organic material derived from sewage-fish waste. Pearson and Rosenberg (1978) give several other examples of *Nephtys (N. incisa, N. hombergi, N. ciliata, N. longosetosa*, and *N. faneiscona)* appearing in organically enriched and polluted areas, often low in dissolved oxygen. Atlas *et al.* (1978) report that *Nepthys longosetosa* is attracted to oil-contaminated sediments.

Pearson and Rosenberg (1978) also report several species of *Polydora* in organically enriched and polluted substrates: *P. ciliata, P. ligni, P. kempi, P. paucibranchiata, P. quadrilobata*, and *P. nuchalis*. Members of this genus are often observed as initial colonizers of the disturbed substrates.

In late May 1990, the benthic community at Herring Bay showed further signs of disturbance. Although there were no apparent dead organisms, as in October 1989,

Table 6.2 Parameters of sampling sites and infaunal invertebrate communities at paired oiled and control silled fjord sites, 1989-90. HB = Herring Bay; OLB = Outer Lucky Bay; IBI = Inner Bay of Isles; ILB = Inner Lucky Bay. Mixed substrate = 50% silt and 50% small cobble and shell material.

Oiled (O) or Control (C):	O	O	O	C	O	C
Sampling Site:	HB	HB	HB	OLB	IBI	ILB
Sampling Period (MM/YY):	10/89	5/90	5/90	6/90	6/90	6/90
Sill Depth, m:	4	4	4	8	3	1
Sampling Depth, m:	14-18	20	20	15-16	11	11
Substrate:	silt	silt	silt	mixed	silt	silt
Bottom Oxygen, mg/L:	-	2.2-5.0	2.2-5.0	7.1-9.3	11.8	0.4
Area Sampled (m^2):	0.5	0.5	0.6	0.6	0.2	0.2
Number of Taxa (T):	27	7	8	116	21	4
Mean Ind./m^2 (A):	4,192	4,262	4,863	4,022	1,403	740
Mean Ind./m^2 (less dominants) (A-):	1648	16	15	2,388	348	30
Mean grams/m^2 (B):	79.44	14.02	19.02	52.04	11.41	1.13
Abund. ratio (A/T/100):	1.6	6.1	6.1	0.3	0.7	1.8
Size Ratio (B/A x 500):	9.5	1.6	2.0	6.5	4.1	0.8
Shannon Diversity (H'):	1.70	0.03	0.03	3.96	2.15	0.85
Simpson Dominance (D):	0.39	0.99	0.99	0.03	0.17	0.46
Richness (SR):	3.12	0.72	0.82	13.86	2.76	0.45
Evenness (J'):	0.52	0.02	0.01	0.83	0.71	0.61

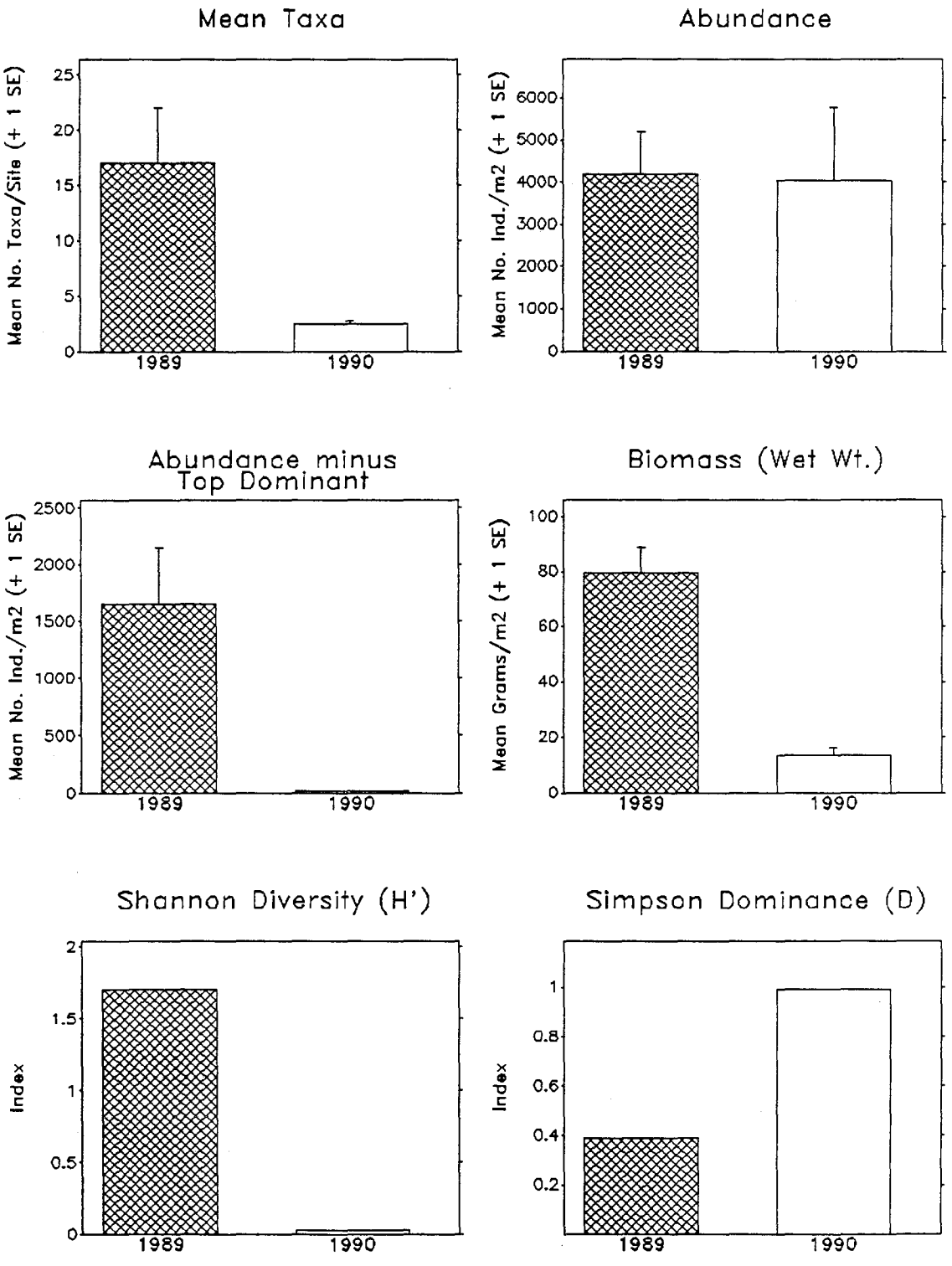


Figure 6.4 Community parameters of infaunal invertebrates at Herring Bay fjord in October 1989 *vs.* May 1990.

Table 6.3 Dominant infaunal invertebrate taxa within silled fjords in Prince William Sound, 1989-90. Sites are presented by comparative pairs.

Sites	Oiled (O)/ Control (C)	Area Sampled (m^2)	Dominant Taxa	Mean Abundance (Ind./m^2)	Cumulative % Abundance
Herring Bay (1989)	O	0.5	*Lucina tenuisculpta* (B)	2,544	60.7
			All Taxa (27)	4,192	100.0
Herring Bay (1990)	O	0.5	*Nepthys cornuta* (P)	4,246	99.6
			All Taxa (7)	4,262	100.0
Herring Bay (1990)	O	0.6	*Nepthys cornuta* (P)	4,848	99.7
			All Taxa (8)	4,863	100.0
Outer Lucky Bay (1990)	C	0.6	*Pholoe minuta* (P)	332	8.3
			Prionospio steenstrupi (P)	320	16.2
			Leucon sp. (C)	301	23.7
			Lumbrineris sp. (P)	273	30.5
			Ophiuroidea (BS)	143	34.0
			Lucina tenuisculpta (B)	141	37.5
			Prionospio sp. (P)	122	40.6
			All Taxa (116)	4,022	100.0
Inner Bay of Isles (1990)	O	0.2	*Nephtys cornuta* (P)	363	25.9
			Gastropoda	350	50.8
			Decapoda larvae	180	63.6
			Lucina tenuisculpta (B)	163	75.3
			All Taxa (21)	1,403	100.0
Inner Lucky Bay (1990)	C	0.2	Decapoda larvae	370	50.0
			Nepthys cornuta (P)	340	95.9
			All Taxa (4)	740	100.0

B = Bivalve
BS = Brittle star
C = Cumacean
P = Polychaete

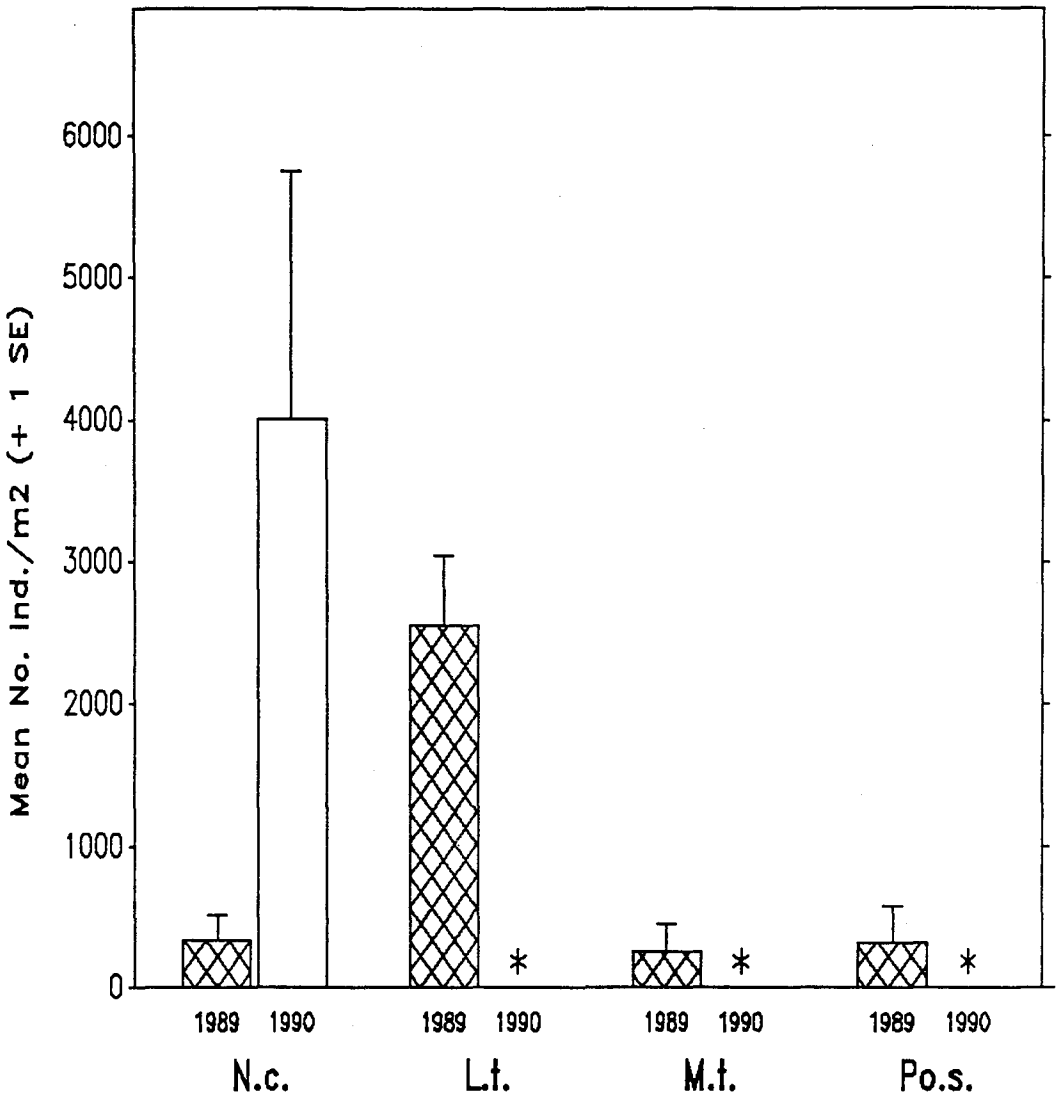


Figure 6.5 Abundances of dominant infaunal taxa at oiled Herring Bay fjord in 1989 *vs.* 1990. N.c.=*Nephtys cornuta*; L.t.=*Lucina tenuisculpta*; M.t.=*Mysella tumida*; Po.s.=*Polydora socialis*. * indicates zero abundance.

the bacteria *Beggiatoa* was still prevalent throughout the area. The consistency of the substrate was a loose mixture of fine silt and detritus. This somewhat flocculant layer approached one meter in depth, deeper than observed in 1989. making it difficult to sample.

The May 1990 samples at Herring Bay revealed greater disturbance as seen through reductions in H', SR, J', A-, B and B/A and elevated D and A/T; the mean faunal density (A) remained essentially the same (Table 6.2; Fig. 6.4). Concurrent low H' and high D and low B/A and high A/T substantiate that major disturbance had occurred. Comparisons of population statistics (T, A, and B), infaunal ratios (A/T and B/A), and diversity (H') and dominance (D) indices are presented in Figures 6.6, 6.7, and 6.8, respectively.

The high value for D (0.99) was attributed to the preponderance of *Nephtys cornuta*. This polychaete dramatically increased in abundance while *Lucina tenuisculpta*, *Polydora socialis*, and *Mysella tumida* were no longer present. We do not know why *Nepthys* increased as *Lucina, Polydora* and *Mysella* disappeared, although possibly *Nephtys* is a better competitor. *Nephtys cornuta* is mainly a predator, but also deposit feeds and thus can utilize the high organic loads associated with the decay of dead organisms. The *Nephtys* sampled were comprised of juveniles and adults (up to 25 mm long). Therefore, it appears that conditions in Herring Bay were altered in such a way to optimize growth and recruitment of this polychaete. *Nephtys* is also relatively motile and thus is probably capable of migrating to sites of disturbance (at least over short distances).

Members of the opportunistic polychaete genus *Polydora*, although able to colonize empty (defaunated) substrates rapidly and in high numbers, are also described as being poor competitors (Grassle and Grassle, 1974). *Polydora socialis* was not found in the Herring Bay samples in 1990, although it was the third most abundant species in 1989.

The shoreline of Herring Bay was heavily oiled following the initial spill. Thus, if oil reached the bottom at approximately 20 m where the present infaunal samples were taken, then its presence could be the cause of the faunal disturbances observed on both sampling occasions. Adverse effects of oil on subtidal benthic communities at similar or deeper depths have been noted in other studies (Cabioch *et al.*, 1978; Sanders *et al.*, 1980; Hyland *et al.* 1989). However, the effects of low oxygen cannot be

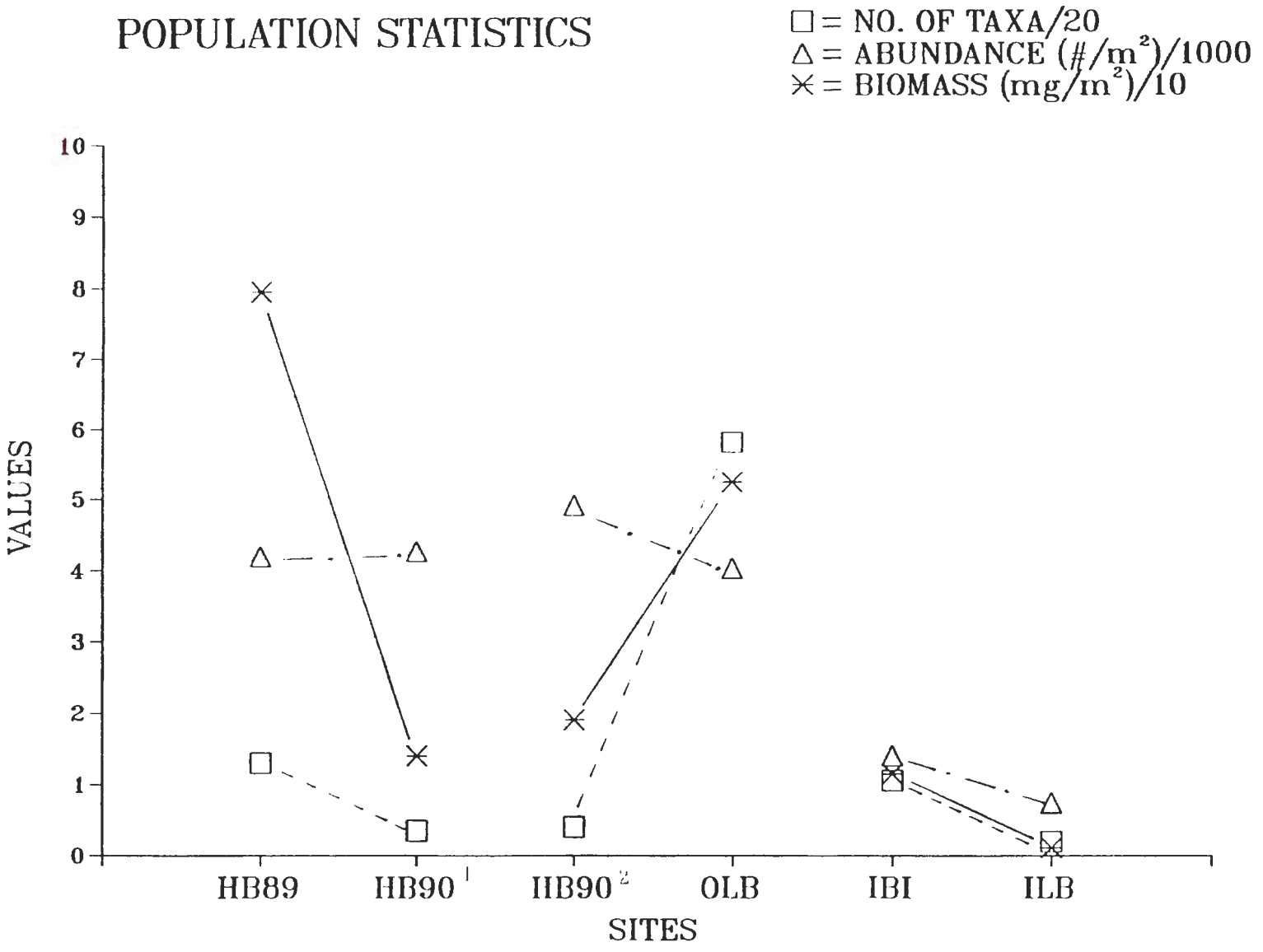


Figure 6.6 Comparison of number of taxa, abundance, and biomass between oiled and control fjord sites. 1 = information based on 0.5 m^2 sampled; 2 = information based on 0.6 m^2 sampled.

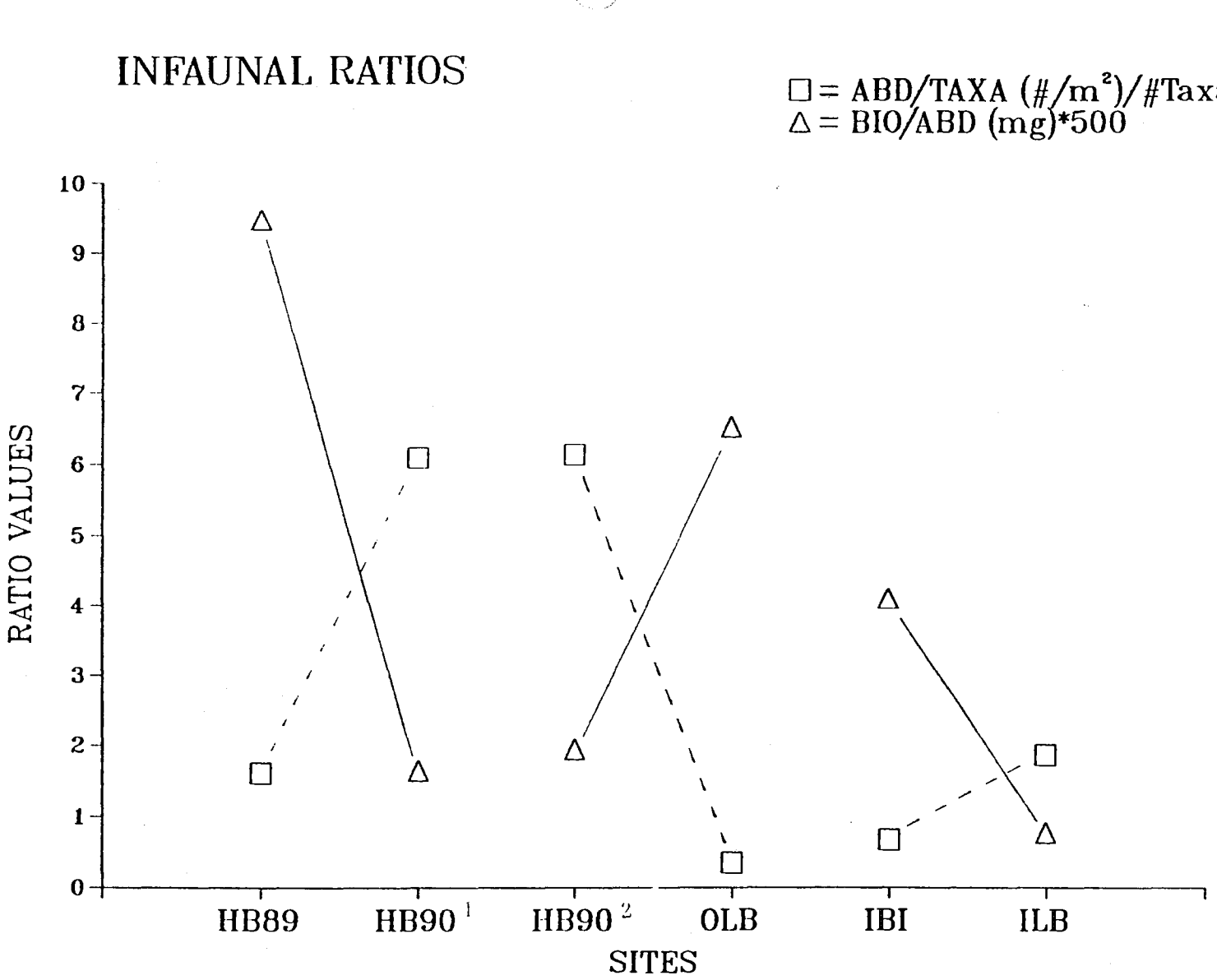


Figure 6.7 Comparison of ratios of abundance/number of taxa (A/T) and biomass/abundance (B/A) between oiled and control fjord sites. 1=information based on 0.5 m^2 sampled; 2=information based on 0.6 m^2 sampled.

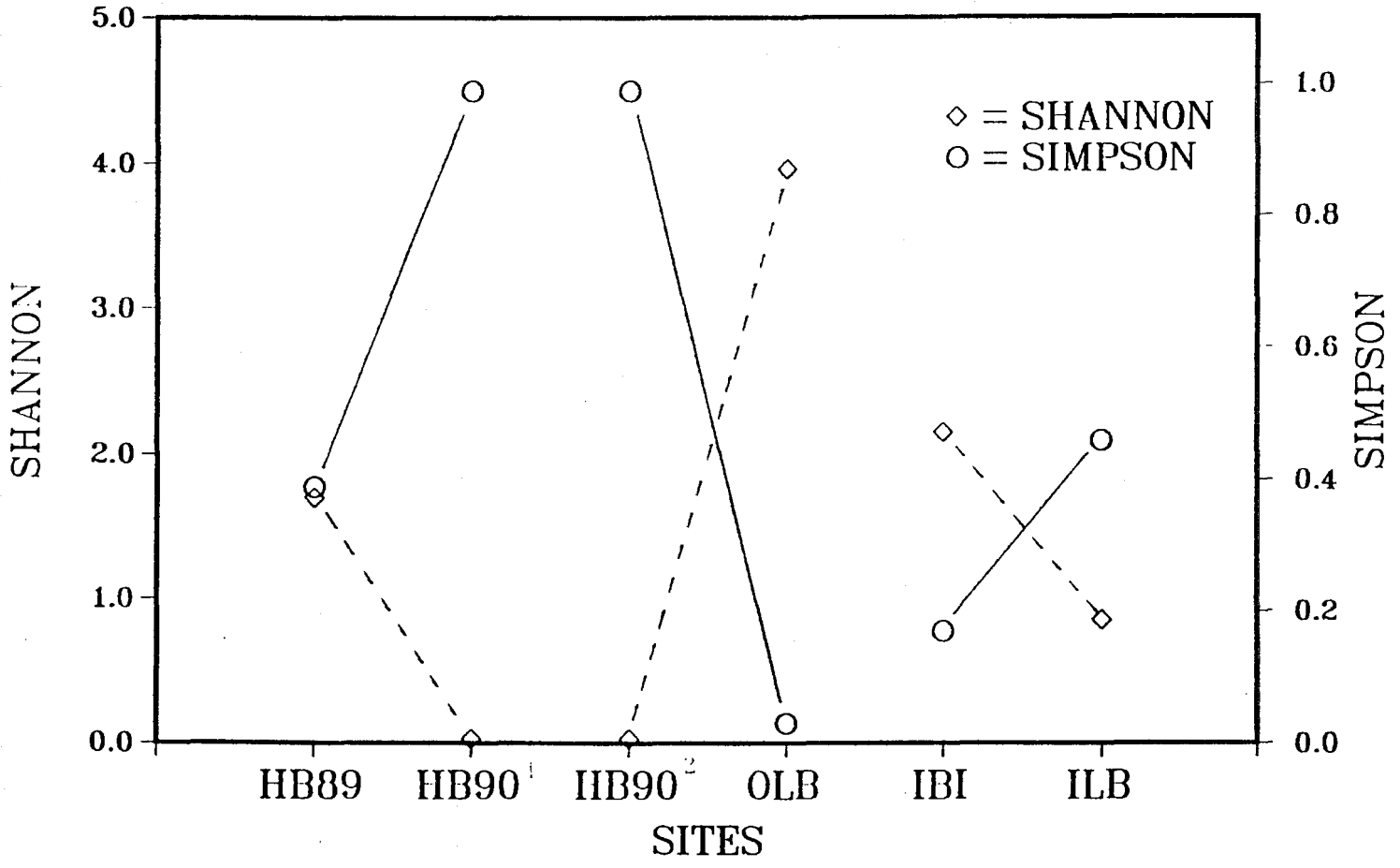


Figure 6.8 Comparison of Shannon diversity (H') and Simpson Dominance (D) indices between oiled and control fjord sites. [1] = information based on 0.5 m^2 sampled; [2] = information based on 0.6 m^2 sampled.

dismissed as a causative factor (see further discussion below). Oxygen levels in bottom water at this site in 1990 (2.2 to 5 mg/L) were low relative to those of an unoiled control site in Outer Lucky Bay (7.1 - 9.3 mg/L). Forthcoming results of sediment hydrocarbon analyses will verify whether or not these offshore sediments were contaminated with oil, and thus are critical in our efforts to determine the sources of disturbance to the impoverished Herring Bay infaunal community.

Statistical comparisons were not made on any of the 1989 versus 1990 Herring Bay data since the number of stations (N) for 1989 and 1990 was only 2 and 3, respectively.

Oiled Herring Bay 1990 *vs* Outer Lucky Bay Control 1990

Comparison of samples collected in 1990 at the heavily oiled Herring Bay site to the unoiled control site in Outer Lucky Bay (total area sampled at each site was 0.6 m^2) revealed striking differences (Tables 6.2-6.3; Figs. 6.6-6.10). Samples from Outer Lucky Bay revealed no disturbance. It contained 15 times more taxa and 159 times higher densities of the non-dominant taxa (A-) than samples from Herring Bay. In Outer Lucky Bay, numbers of individuals were much more evenly distributed among the species present (J' = 0.83 for Outer Lucky Bay vs 0.01 for Herring Bay). Low species equitability at Herring Bay is caused by the overwhelming dominance of *Nephtys cornuta*, which as noted above is a stress-tolerant species. The diversity of fauna at Outer Lucky Bay (H' = 3.96) is over two orders of magnitude higher than at Herring Bay (H' = 0.03); this difference is related both to higher richness (SR) and evenness (J') components in the Outer Lucky Bay samples. Concurrent high B/A and low A/T ratios are further indicators of no disturbance.

Among the 116 distinct taxa at Outer Lucky Bay are at least 16 species of amphipod crustaceans; no amphipods appeared in the Herring Bay samples in 1990 and only one species was present in low abundance in 1989. Amphipods are known as being sensitive to oil toxicity (Cabioch *et al.*, 1978; Sanders *et al.*, 1980; Hyland *et al.*, 1989).

Statistical comparisons were not made on any of the 1990 Herring Bay versus Outer Lucky Bay data since the number of stations (N) for each of these sites was only 3.

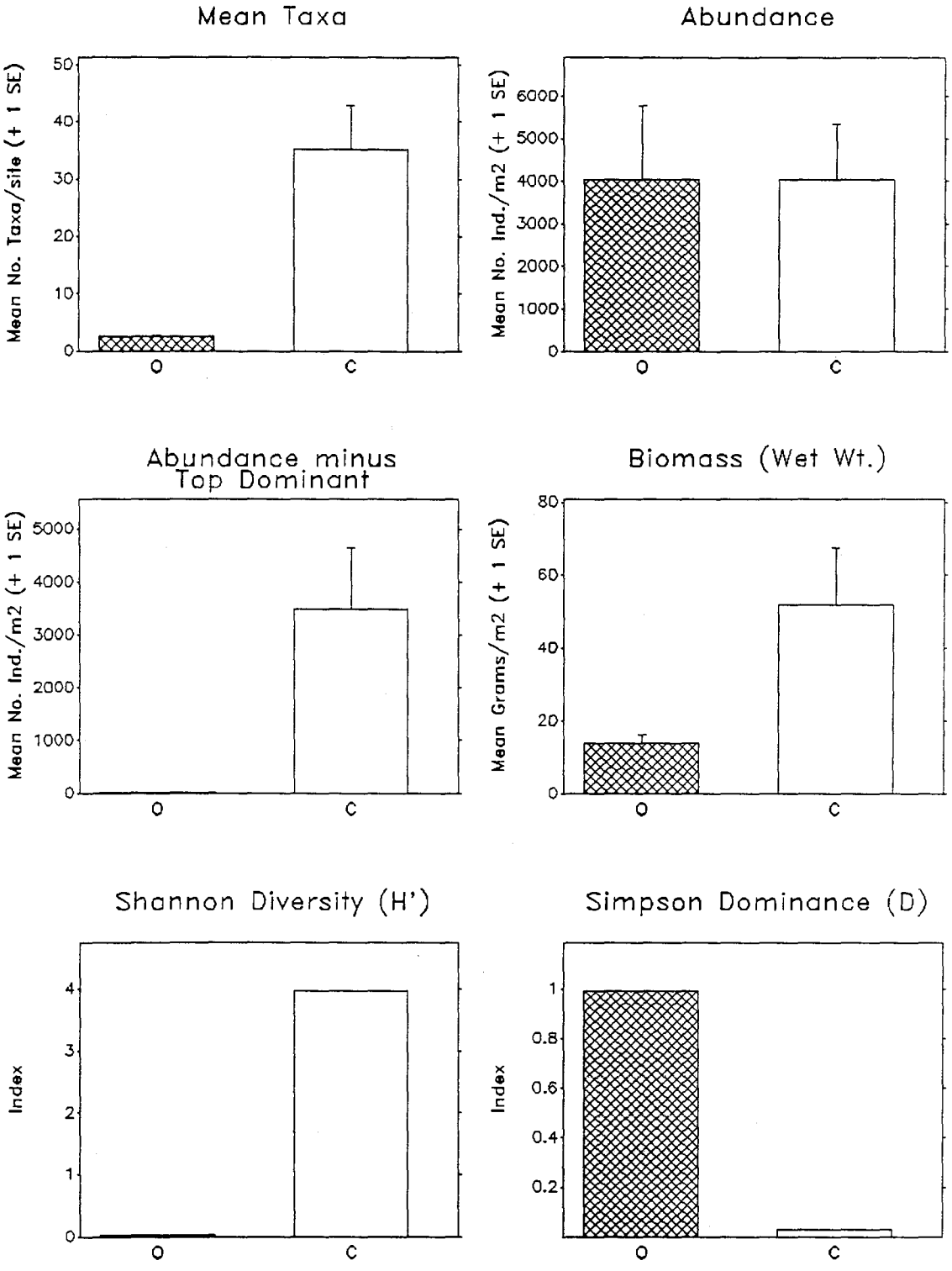


Figure 6.9 **Community parameters of infaunal invertebrates at Herring Bay and Outer Lucky Bay fjords, summer 1990.**

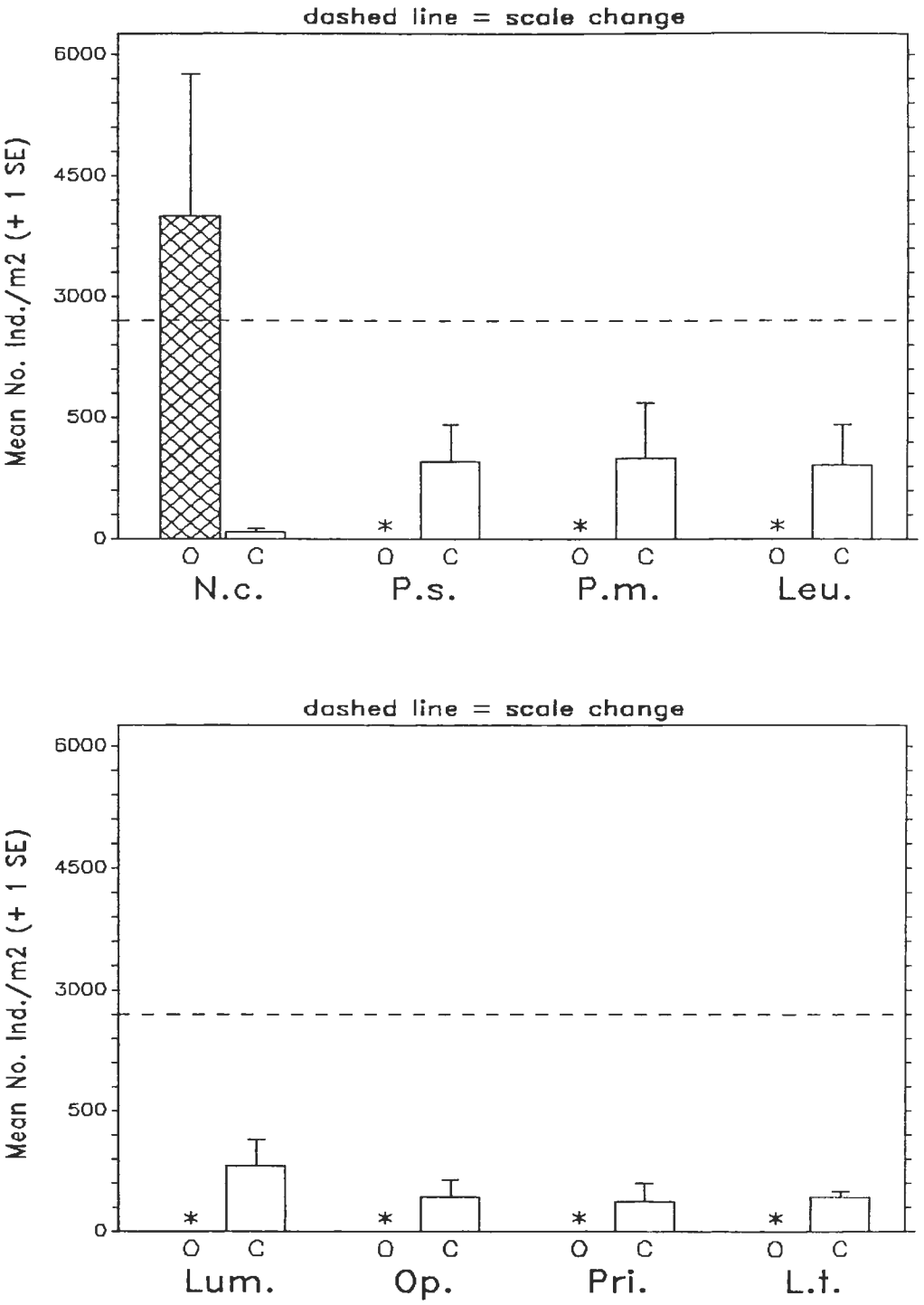


Figure 6.10 Abundances of dominant infaunal taxa at Herring Bay (oiled:0) *vs.* Outer Lucky Bay (control:C) fjords, summer 1990. N.c.=*Nephtys cornuta*; P.s.=*Prionospio steenstrupi*; P.m.=*Pholoe minuta*; Leu.=*Leucon* sp.; Lum.=*Lumbrineris* sp.; Op.=Ophiuroidea; Pri.=*Prionospio* sp.; L.t.=*Lucina tenuisculpta*. * indicates zero abundance.

Outer Lucky Bay is a smaller (approximately 9 times), shallower fjord with a deeper sill (18 m at deepest point and sill depth of 8 m) in comparison to Herring Bay (47 m at deepest point and sill depth of only 4 m). Consequently, there probably is a much greater exchange between bottom water and surface water in Outer Lucky Bay than in Herring Bay. As noted above, oxygen levels in bottom water during the 1990 sampling period were 7.1 - 9.3 mg/L for Outer Lucky Bay and 2.2 - 5 mg/L for Herring Bay. Thus, the low diversity and abundance of benthic fauna in Herring Bay may be caused by, or confounded by, anoxic or hypoxic conditions on the bottom related to poor water exchange and natural cycles of organic enrichment. Also, the absence of the bacteria *Beggiatoa* suggests a more oxygen-rich environment. Greater substrate heterogenity at Outer Lucky Bay presumably also attributed, in part, to the greater diversity there.

Oiled Inner Bay of Isles 1990 *vs.* Inner Lucky Bay Control 1990

Samples from the oiled Inner Bay of Isles site had a lower value for D and greater values for H', J', SR, T, A, A- and B than the unoiled control site in Inner Lucky Bay (Tables 6.2-6.3; Figs. 6.6-6.8, 6.11). Concurrent high B/A and low A/T ratios at Bay of Isles indicated normality. Therefore, the data tends to support unexpected results, i.e., disturbance at the control site and little disturbance at the oiled site. The only faunal similarity between these two sites was that *Nephtys cornuta* and decapod larvae were dominant at both sites (Fig. 6.12). It is important to note that dissolved oxygen levels in bottom waters were 11.8 mg/L for the oiled Inner Bay of Isles site and only 0.4 mg/L for the unoiled Inner Lucky Bay site. These results further demonstrate the importance of natural factors, such as low-oxygen stress, in the structuring of these fjord communities.

Summary: Oil Spill Effects *vs.* Natural Cycles of Organic Enrichment and Low-Oxygen Stress

Our data provide evidence of disturbance to the subtidal benthos of silled fjord habitats in Prince William Sound following the *Exxon Valdez* oil spill. There are a number of possible mechanisms of oil transport to these depths sampled (approximately 11-20 m), including sedimentation of oil sorbed onto settling particles

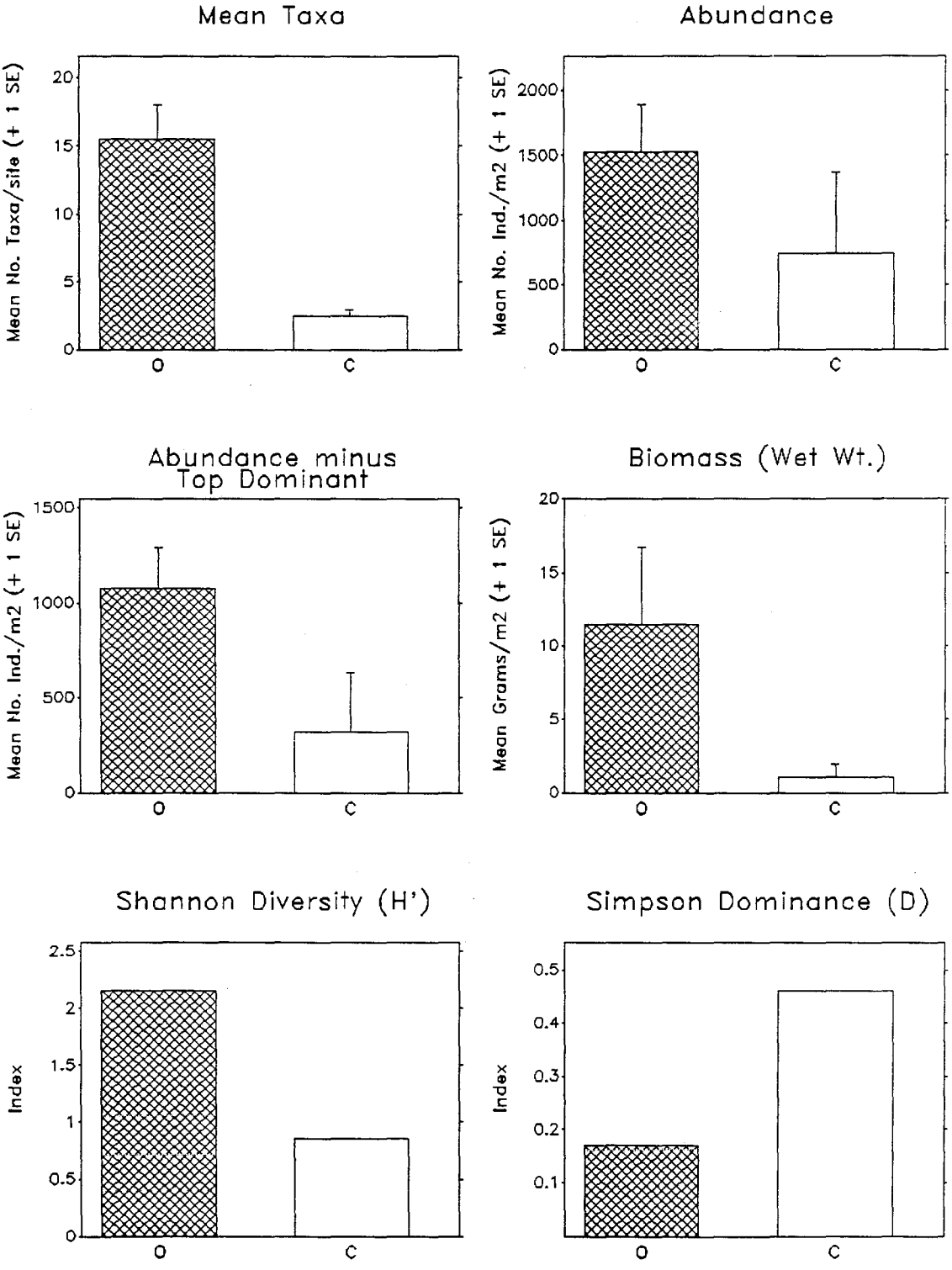


Figure 6.11 Community parameters of infaunal invertebrates at Inner Bay of Isles (oiled:0) *vs.* Inner Lucky Bay (control:C) fjords, summer 1990.

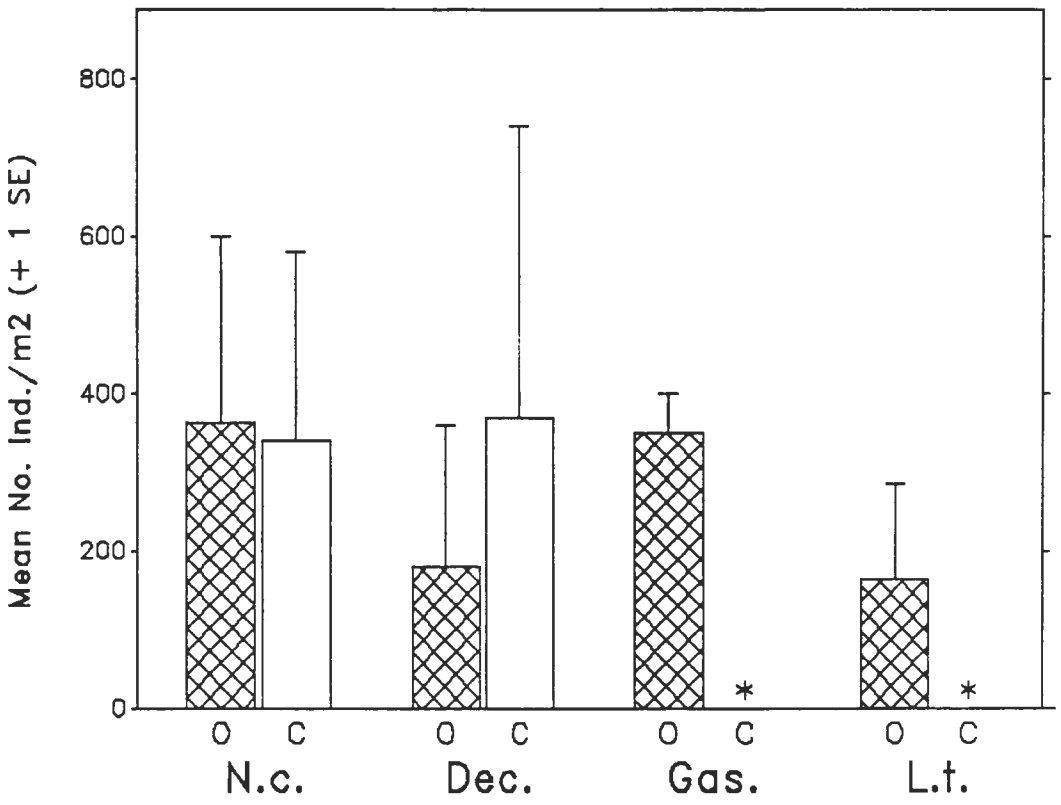


Figure 6.12 Abundance of dominant infaunal taxa at Inner Bay of Isles (oiled:O) *vs.* Inner Lucky Bay (control:C) fjords, summer 1990. N.c. = *Nephtys cornuta*; Dec. = Decapoda larvae; Gas. = Gastropoda; L.t. = *Lucina tenuisculpta*. * indicates zero abundance.

or incorporated into the fecal pellets of planktonic organisms; sinking of heavier oil components; and direct mixing of oil with shallower sediments and subsequent transport to offshore locations by bottom currents (National Research Council, 1985). As noted above, the effects of oil on subtidal benthic communities at similar or deeper depths have been observed in other studies (Cabioch *et al.*, 1978; Sanders *et al.*, 1980; Hyland *et al.*, 1989). Among the possible causes of oil-related effects are chemical toxicity of aromatic derivatives; asphyxiation or entanglement due to direct physical coating; and a variety of reproductive, behavioral, and other sublethal disorders leading ultimately to long-term population changes. In the case of the *Exxon Valdez* spill, there also is the possibility of indirect effects of bioremediation and other clean-up efforts. Such activities were observed, for example, at the heavily-oiled Herring Bay site.

Although the shoreline of Herring Bay was heavily oiled following the initial spill, we do not have evidence at this time as to whether or not oil reached the offshore subtidal depths where disturbances to the infauna were noted. Forthcoming results of sediment hydrocarbon analyses are critical in our efforts to determine the sources of disturbance.

The highly disturbed condition of the infauna at the unoiled, control site in Inner Lucky Bay suggests that other natural factors may be important in structuring these communities. The data suggest that the low diversity and abundances of these fauna could be caused by, or confounded by, anoxic or hypoxic conditions on the seafloor related to poor water exchange and natural cycles of organic enrichment. Similar effects on the benthos of low-oxygen stress and organic enrichment have been seen in Scandinavian and Scottish fjords (review by Pearson, 1980; Mirza and Gray, 1981; Gray *et al.*, 1988) and documented as the "August Effect" in New England estuaries (Rhoads and Germano, 1982).

This coming year, additional benthic samples collected from the Prince William Sound fjords will be analyzed and evaluated in conjunction with pending chemistry data, to provide information critical in distinguishing possible oil impacts from natural sources of change.

Eelgrass Habitats

Eelgrass

The density of eelgrass turions (Fig. 6.13, Table 6.4) was generally higher at control vs oiled sites within Prince William Sound. For all comparisons except for Mooselips Bay *vs.* Sleepy Bay, turions were about twice as dense at oiled vs control sites. However, none of the site comparisons had differences that were significant at the $p < 0.01$ level.

We suspect that the generally lower densities of turions at the oiled sites were the result of the effects of oil or of associated cleanup activities. There is also an indication that differences between oiled and control sites were greater at more protected sites with finer grain sediments. The sites pairs can be ordered from highest to lowest exposure and sediment grain size as Sleepy Bay/Mooselips Bay > Naked Island/Storey Island > Lower Herring Bay/Herring Bay > Drier Bay/Bay of Isles. Differences in turion density were ordered in the same manner.

There were also generally higher densities of flowering turions and flower spathes at the control sites (Fig. 6.13; Tables 6.5, 6.6). However, these differences were generally small except for the Lower Herring Bay/Herring Bay comparison. The eelgrass bed at Herring Bay was several meters deeper than other sites and this may have influenced flower density.

No consistent differences were observed with regard to standing crop (wet weight) of eelgrass at oiled vs control sites Fig. 6.13, Table 6.7).

Epibenthic Invertebrates

The epibenthos within eelgrass beds was dominated by starfish (primarily *Pycnopodia*) and the porcelain crab, *Telmessus*. There were no consistent trends with respect to starfish density (Figure 6.14, Table 6.8) among oiled and control sites. However, starfish other than *Pycnopodia* (primarily *Dermasterias* and *Evasterias*) were significantly more abundant at Lower Herring Bay than at Herring Bay (Fig. 6.14, Table 6.9).

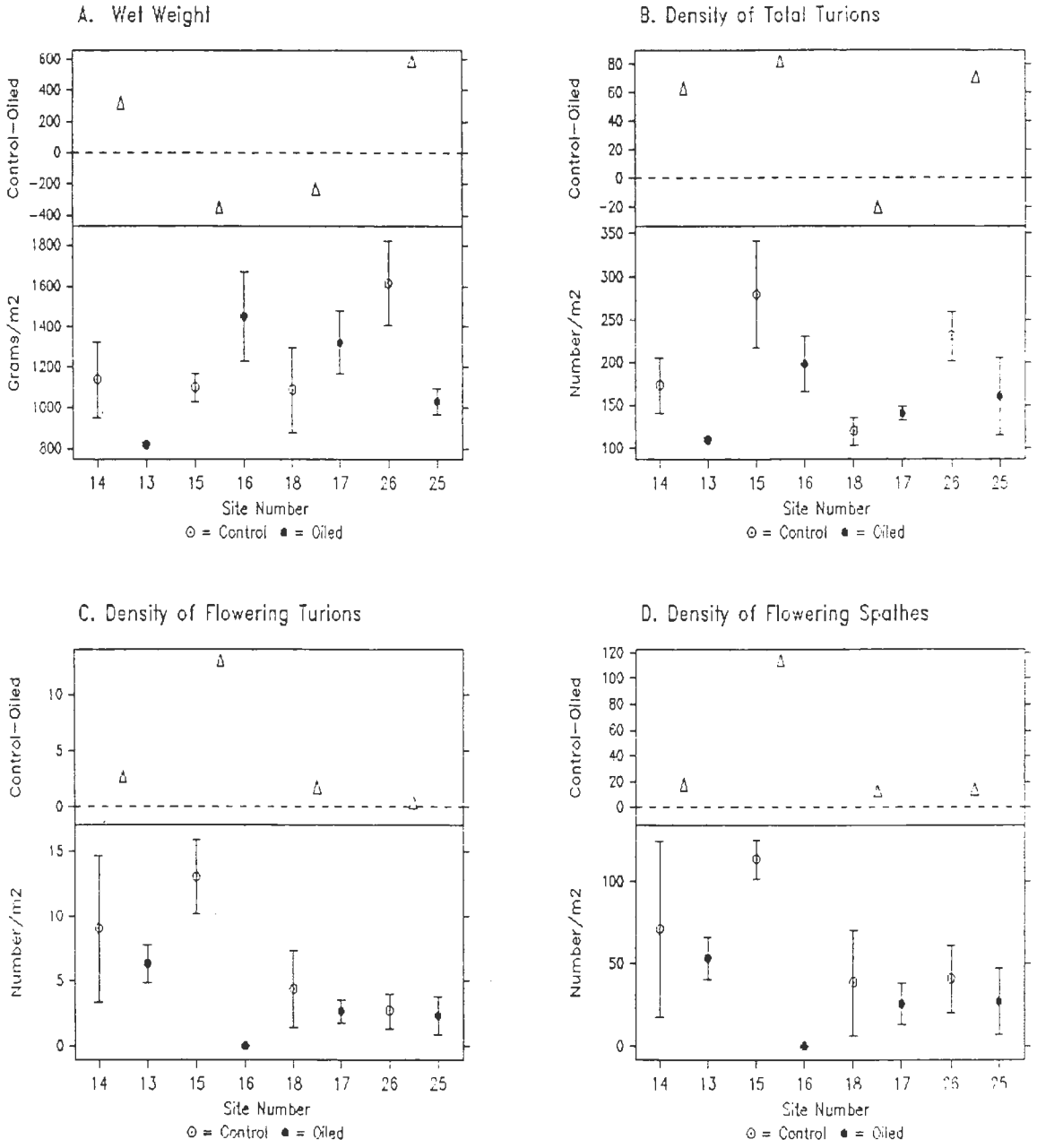


Figure 6.13 Means, plus and minus one standard error, and differences in means between oiled and control eelgrass study sites for A - wet weight of eelgrass, B - Turion density, C - density of flowering turions, D - density of flowering spathes.

Table 6.4 Results of t-tests of differences in density of turions of Zostera marina (no./0.25 m2) at oiled and control sites in eelgrass habitats in 1990.

Control	Site Name	Site #	Mean	SE	t	df	P
Control	Drier Bay	14	43.2	8.1	1.94	2.0	0.19
Oil	Bay of Isles	13	27.5	0.5			
Control	L. Herring Bay	15	69.8	15.5	1.17	3.0	0.33
Oil	Herring Bay	16	49.4	8.0			
Control	Mooselips Bay	18	29.8	3.96	1.18	3.0	0.33
Oil	Sleepy Bay	17	35.1	2.05			
Control	Storey Island	26	57.6	7.3	1.31	3.4	0.27
Oil	Naked Island	25	40.0	11.3			

Table 6.5 Results of t-tests of differences in density (no./0.25 m2) of flowering turions of Zostera marina at oiled and control sites in eelgrass habitats in 1990.

Control	Site Name	Site #	Mean	SE	t	df	P
Control	Drier Bay	14	2.25	1.42			
					0.45	2.3	0.69
Oil	Bay of Isles	13	1.58	0.36			
Control	L. Herring Bay	15	3.25	0.72			
					4.50	2.0	0.05
Oil	Herring Bay	16	0.0	0.0			
Control	Mooselips Bay	18	1.08	0.74			
					0.54	2.4	0.64
Oil	Sleepy Bay	17	0.67	2.22			
Control	Storey Island	26	0.67	0.33			
					0.17	4.0	0.87
Oil	Naked Island	25	0.58	0.36			

Table 6.6 Results of t-tests of differences in density (no./0.25 m2) of flowering spathes of Zostera marina at oiled and control sites in eelgrass habitats in 1990.

Control	Site Name	Site #	Mean	SE	t	df	P
Control	Drier Bay	14	17.7	13.4	0.31	2.2	0.78
Oil	Bay of Isles	13	13.3	3.2			
Control	L. Herring Bay	15	28.3	2.9	9.71	2.0	0.01
Oil	Herring Bay	16	0.0	0.0			
Control	Mooselips Bay	18	9.6	8.0	0.37	2.6	0.74
Oil	Sleepy Bay	17	6.4	3.2			
Control	Storey Island	26	10.20	5.2	0.47	4.0	0.66
Oil	Naked Island	25	6.8	5.1			

Table 6.7 Results of t-tests of differences in standing crop (wet weight in gms./0.25 m2) of Zostera marina at oiled and control sites in eelgrass habitats in 1990.

Control	Site Name	Site #	Mean	SE	t	df	P
Control	Drier Bay	14	284.0	46.62			
					1.69	2.0	0.23
Oil	Bay of Isles	13	205.3	1.4			
Control	L. Herring Bay	15	274.0	17.1			
					1.53	2.4	0.25
Oil	Herring Bay	16	362.7	55.3			
Control	Mooselips Bay	18	271.2	52.7			
					-0.90	3.7	0.42
Oil	Sleepy Bay	17	330.3	39.0			
Control	Storey Island	26	403.3	51.7			
					2.68	2.4	0.09
Oil	Naked Island	25	257.8	16.4			

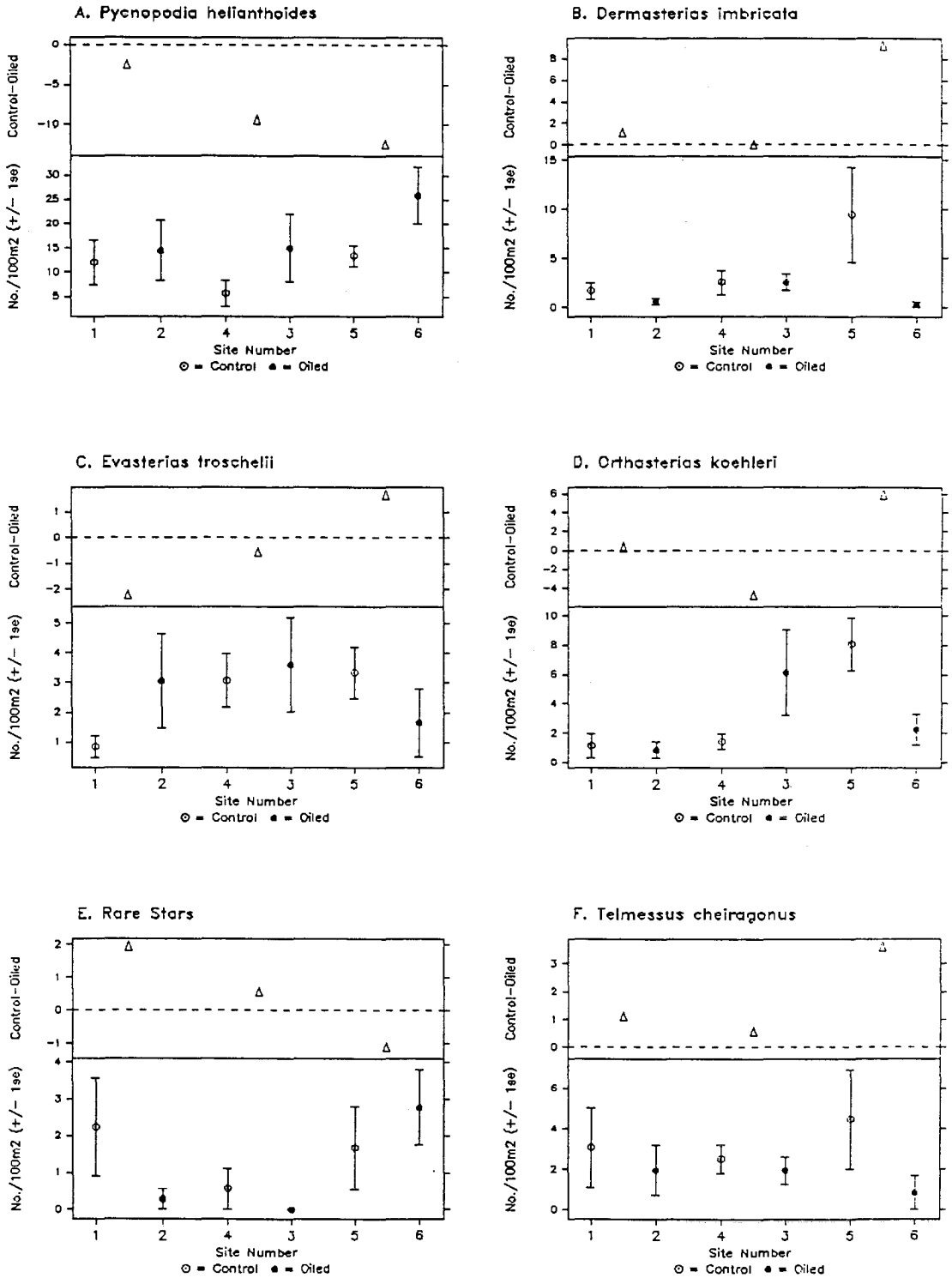


Figure 6.14 Means, plus and minus one standard error, and differences in means between oiled and control eelgrass study sites for starfishes and the crab *Telmessus*.

Table 6.8 Results of t-tests of differences in density (no./m2) of Pycnopodia helianthoides at oiled and control sites in eelgrass habitats in 1990.

Control	Site Name	Site #	Mean	SE	t	df	P
Control	Drier Bay	14	10.56	2.22	1.66	3.7	0.18
Oil	Bay of Isles	13	4.44	2.94			
Control	L. Herring Bay	15	1.67	0.96	1.22	4.0	0.29
Oil	Herring Bay	16	3.33	0.96			
Control	Mooselips Bay	18	6.67	2.55	0.39	3.7	0.72
Oil	Sleepy Bay	17	5.00	3.47			
Control	Storey Island	26	12.22	4.34	0.17	3.4	0.88
Oil	Naked Island	25	13.57	6.83			

Table 6.9 Results of t-tests of differences in density (no./m2) of starfish other than Pycnopodia helianthoides at oiled and control sites in eelgrass habitats in 1990.

Control	Site Name	Site #	Mean	SE	t	df	P
Control	Drier Bay	14	1.67	0.00	2.00	2.00	0.18
Oil	Bay of Isles	13	0.56	0.56			
Control	L. Herring Bay	15	8.33	0.96	4.16	3.9	0.01
Oil	Herring Bay	16	2.22	1.11			
Control	Mooselips Bay	18	0.56	0.56	1.00	3.2	0.39
Oil	Sleepy Bay	17	1.67	0.96			
Control	Storey Island	26	0.00	-	-	-	-
Oil	Naked Island	25	0.00	-			

Telmessus densities were extremely low at all oiled sites and were moderately high ($> 2.5/100\ m^2$) at three of the control sites (Figure 6.14, Table 6.10). None of the site comparisons were significantly different at $P<0.05$, but 2 comparisons differed significantly at the 0.10 probability level. We suspect that these differences were the result of oil. Crustaceans have been shown to be particularly sensitive to crude oil in polar regions (Capuzzo, 1987) and reduced abundances of *Telmessus* may have been a result of direct toxic effects of oil.

Fishes

Over fifteen species of fish were found in sampled eelgrass habitats. For analyses, the less common species were combined into family-level taxa (Table 6.11). Pacific cod young-of-the-year (YOY) were the most abundant species in both oiled and control sites (Table 6.12). If Pacific cod YOY are excepted, the eelgrass habitat includes a variety of benthic species (Table 6.12).

The overall abundance of fishes in oiled vs control site pairs displays a non-significant ($p>0.05$) pattern of higher abundance in oiled study sites (Fig. 6.15, Table 6.13). As might be expected, this pattern is due primarily to a similar trend in the abundance of Pacific cod YOY (Fig. 6.15, Table 6.13). Although the differences between each pair of stations is non-significant, the consistency of the trend is highly suggestive.

The abundance of fishes excepting Pacific cod YOY is very similar between all pairs of oiled/control study sites.

Laminaria/Agarum Habitats

Agarum and *Laminaria* Abundance

The shallow subtidal throughout much of Prince William Sound is dominated by *Agarum cribosum* and/or *Laminaria saccharina*. There were no apparent difference among oiled and control sites with respect to the abundance or percent cover of *Agarum*, *Laminaria* plus *Agarum*, or all algae combined (Figures 6.16, 6.17; Tables 6.14 - 6.18). Densities and percent covers were very comparable at most sites.

Table 6.10 Results of t-tests of differences in density (no./m2) of Telmessus cheiragonus at oiled and control sites in eelgrass habitats in 1990.

Control	Site Name	Site #	Mean	SE	t	df	P
Control	Drier Bay	14	0.56	0.56	1.00	2.00	0.42
Oil	Bay of Isles	13	0.00	0.00			
Control	L. Herring Bay	15	5.00	1.67	2.53	2.4	0.10
Oil	Herring Bay	16	0.56	0.56			
Control	Mooselips Bay	18	2.78	2.78	0.78	2.2	0.51
Oil	Sleepy Bay	17	0.56	0.56			
Control	Storey Island	26	3.33	0.96	3.46	2.0	0.07
Oil	Naked Island	25	0.00	0.00			

Table 6.11 Fish species observed in eelgrass habitats, with species group designation for analyses.

FAMILY	SPECIES	GROUP
GADIDAE	Pacific cod	Young of Year, Juvenile/Adult
HEXIGRAMMIDAE	ling cod	ling cod
	masked greenling	Greenlings
	kelp greenling	Greenlings
	whitespotted greenling	Greenlings
STICHAEIDAE	snake prickleback	Other
	arctic shanny	arctic shanny
	slender eelblenny	Other
PHOLIDAE	crescent gunnel	Gunnels
	penpoint gunnel	Gunnels
	unidentified gunnel	Gunnels
COTTIDAE	red irish lord	Sculpins
	great sculpin	Sculpins
	silverspotted sculpin	Sculpins
	unidentified cottid	Sculpins
AMMODYTIDAE	Pacific sandlance	Other
AULORHYNCHIDAE	tubesnout	Other
SCORPAENIDAE	rockfishes	Other
PLEURONECTIDAE	flatfishes	Other

Table 6.12 Relative abundances of fish species groups in eelgrass habitats; including combined and segregated oiled and control sites.

SPECIES GROUP	COMBINED	OIL	CONTROL
PACIFIC COD YOUNG OF YEAR AND ALL OTHERS			
Pacific cod young of year	.76	.84	.46
All others	.24	.16	.54
n =	983	759	224
ALL SPECIES EXCLUDING PACIFIC COD YOUNG OF YEAR			
Gunnels	.26	.30	.22
Greenlings	.22	.17	.27
Pacific cod Juv. and Adult	.18	.31	.06
Arctic shanny	.14	.12	.17
Sculpins	.05	.04	.07
Lingcod	.04	.04	.04
Others	.10	.02	.18
n =	240	120	120

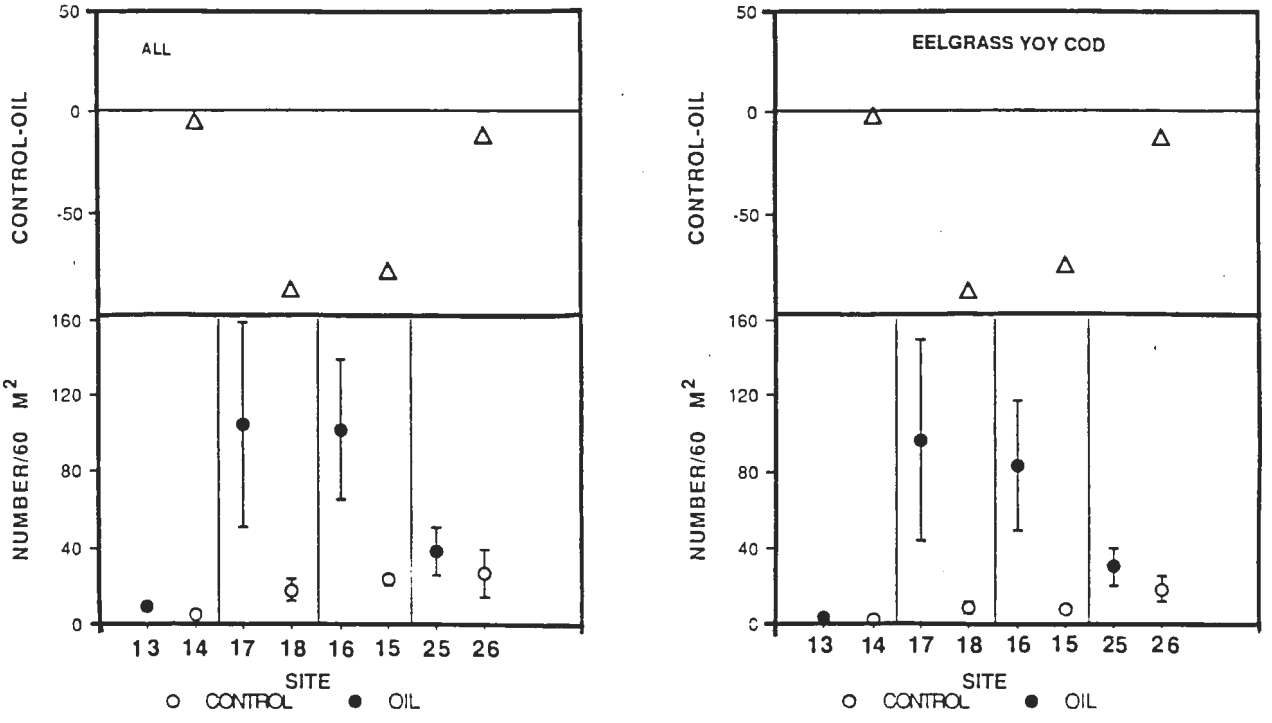


Figure 6.15 Means, plus and minus one standard error, and differences in means between oiled and control eelgrass study sites for all fish and for young of year Pacific cod.

Table 6.13 Results of t-tests in differences in density of all fishes combined and young-of-year (YOY) pacific cod in oiled (O) and control (C) eelgrass study sites in 1990.

OIL/CONROL	SITE #	DENSITY (no/60m^2)	STD.ERR.	t VALUE	P
ALL FISHES					
Control	14	5.3	1.3	2.4	0.07
Oil	13	9.7	1.2		
Control	18	18.0	5.6	1.6	0.18
Oil	17	105.0	53.7		
Control	15	23.7	2.7	2.1	0.10
Oil	16	102.3	36.5		
Control	26	27.3	12.5	0.6	0.56
Oil	25	38.3	12.2		
PACIFIC COD YOUNG OF YEAR					
Control	14	1.7	1.2		
Oil	13	3.0	0.0		
Control	18	8.0	3.0	1.6	0.17
Oil	17	96.7	52.9		
Control	15	7.0	0.6	2.2	0.09
Oil	16	83.0	34.6		
Control	26	18.0	7.0	1.0	0.38
Oil	25	30.0	10.0		

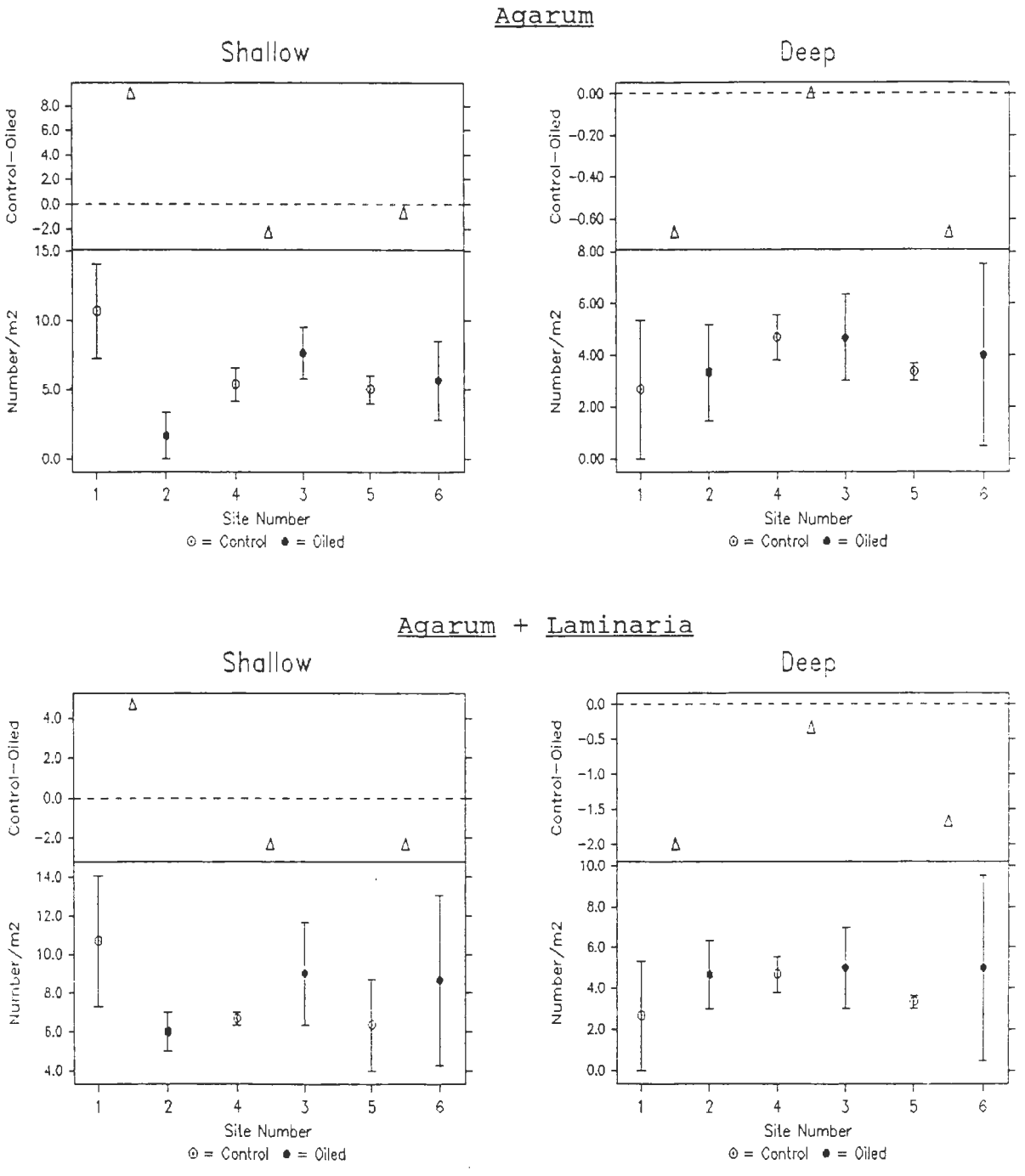


Figure 6.16 Means, plus and minus one standard error, and differences in means between oiled and control *Laminaria/Agarum* study sites for density of *Agarum*, *Agarum* and *Laminaria*, and all algae.

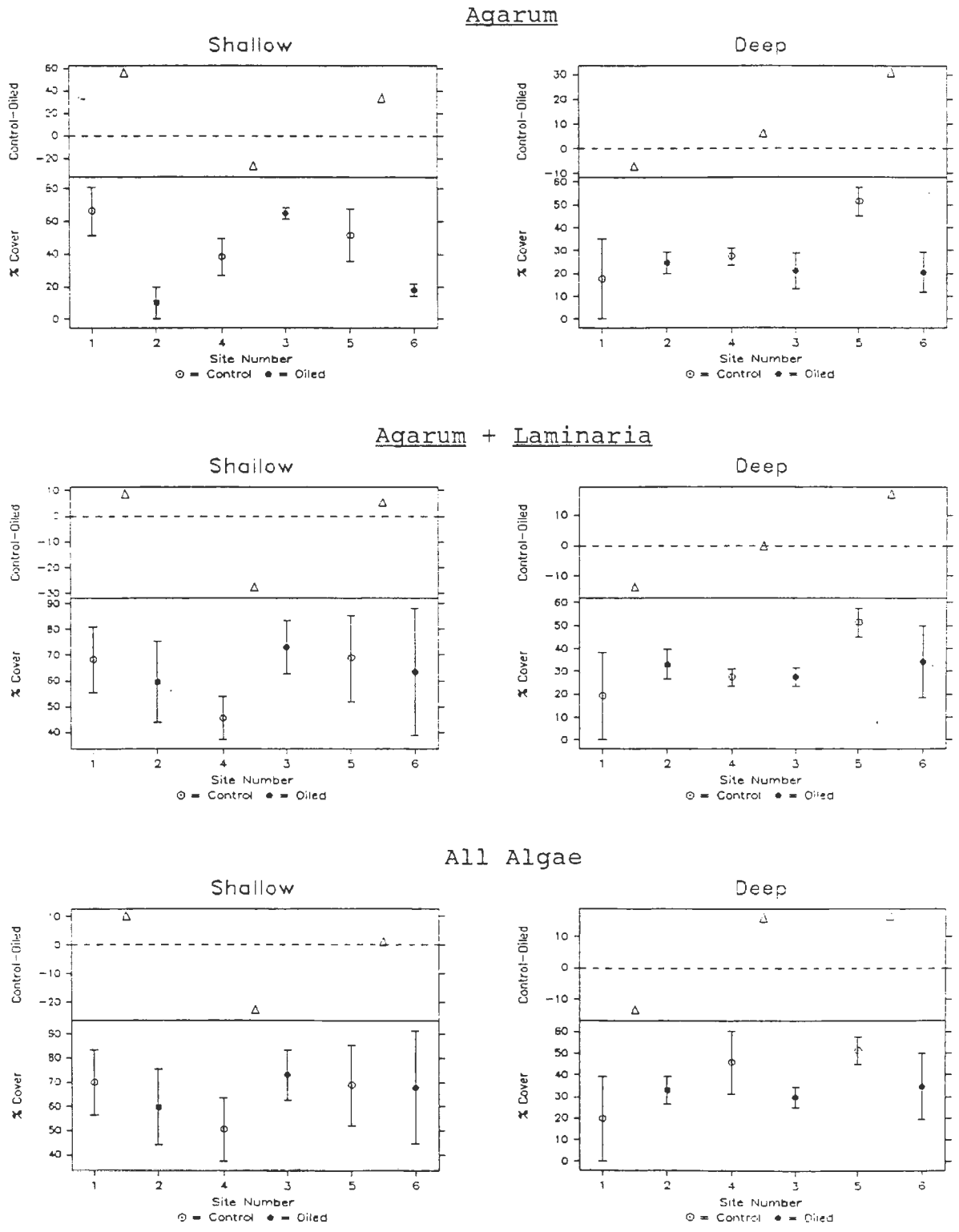


Figure 6.17 Means, plus and minus one standard error, and differences in means between oiled and control *Laminaria/Agarum* study sites for percent cover of *Agarum*, *Agarum* and *Laminaria*, and all algae.

Table 6.14 Results of t-tests of differences in density (no./m2) of Agarum cribrosum at oiled and control sites in Laminaria habitats in 1990.

Depth Strata = 12 to 20 m

Oil/ Control	Site Name	Site #	Mean	SE	t	df	P
Control	Cabin Bay	1	0.67	0.67	0.21	3.6	0.85
Oil	Northwest Bay	2	0.83	0.46			
Control	L. Herring Bay	4	1.17	0.22	0.00	3.0	1.00
Oil	Herring Bay	3	1.17	0.42			
Control	Mummy Bay	5	0.83	0.08	0.19	2.0	0.87
Oil	Bay of Isles	6	1.00	0.88			

Depth Strata = 4 to 12 m

Oil/ Control	Site Name	Site #	Mean	SE	t	df	P
Control	Cabin Bay	1	2.67	0.85	2.39	2.9	0.10
Oil	Northwest Bay	2	0.42	0.42			
Control	L. Herring Bay	4	1.33	0.30	1.06	3.4	0.36
Oil	Herring Bay	3	1.92	0.46			
Control	Mummy Bay	5	1.25	0.25	0.22	2.5	0.84
Oil	Bay of Isles	6	1.42	0.71			

Table 6.15 Results of t-tests of differences in density (no./m2) of Agarum cribrosum and Laminaria saccharina at oiled and control sites in Laminaria habitats in 1990.

Depth Strata = 12 to 20 m

Oil/ Control	Site Name	Site #	Mean	SE	t	df	P
Control	Cabin Bay	1	0.67	0.67	0.64	3.4	0.57
Oil	Northwest Bay	2	1.16	0.42			
Control	L. Herring Bay	4	1.17	0.22	0.15	2.7	0.89
Oil	Herring Bay	3	1.25	0.50			
Control	Mummy Bay	5	0.83	0.08	0.37	2.0	0.74
Oil	Bay of Isles	6	1.25	1.13			

Depth Strata = 4 to 12 m

Oil/ Control	Site Name	Site #	Mean	SE	t	df	P
Control	Cabin Bay	1	2.67	0.85	1.32	2.3	0.30
Oil	Northwest Bay	2	1.50	0.25			
Control	L. Herring Bay	4	1.67	0.08	0.88	2.1	0.47
Oil	Herring Bay	3	2.25	0.66			
Control	Mummy Bay	5	1.58	0.58	0.47	3.1	0.67
Oil	Bay of Isles	6	2.17	1.09			

Table 6.16 Results of t-tests of differences in percent cover of Agarum cribrosum at oiled and control sites in Laminaria habitats in 1990. T-tests were performed on data that were transformed (arcsin square root). Untransformed means and standard errors are tabulated.

Depth Strata = 12 to 20 m

Oil/ Control	Site Name	Site #	Mean	SE	t	df	P
Control	Cabin Bay	1	16.77	14.96	0.45	2.5	0.69
Oil	Northwest Bay	2	23.99	5.53			
Control	L. Herring Bay	4	27.29	3.62	0.72	3.4	0.52
Oil	Herring Bay	3	22.47	5.65			
Control	Mummy Bay	5	44.48	4.58	2.60	3.3	0.07
Oil	Bay of Isles	6	21.61	7.51			

Depth Strata = 4 to 12 m

Oil/ Control	Site Name	Site #	Mean	SE	t	df	P
Control	Cabin Bay	1	58.48	11.16	3.51	3.7	0.03
Oil	Northwest Bay	2	9.98	8.17			
Control	L. Herring Bay	4	33.14	9.78	2.07	2.4	0.15
Oil	Herring Bay	3	54.31	3.02			
Control	Mummy Bay	5	44.88	13.10	1.87	2.3	0.19
Oil	Bay of Isles	6	19.64	3.37			

Table 6.17 Results of t-tests of differences in percent cover of Agarum cribrosum and Laminaria saccharina at oiled and control sites in Laminaria habitats in 1990. T-tests were performed on data that were transformed (arcsin square root). Untransformed means and standard errors are tabulated.

Depth Strata = 12 to 20 m

Oil/ Control	Site Name	Site #	Mean	SE	t	df	P
Control	Cabin Bay	1	18.30	17.02	0.69	2.4	0.55
Oil	Northwest Bay	2	31.20	5.27			
Control	L. Herring Bay	4	27.29	3.62	0.03	3.5	0.97
Oil	Herring Bay	3	27.43	2.47			
Control	Mummy Bay	5	44.48	4.58	0.09	2.5	0.45
Oil	Bay of Isles	6	32.60	12.46			

Depth Strata = 4 to 12 m

Oil/ Control	Site Name	Site #	Mean	SE	t	df	P
Control	Cabin Bay	1	59.69	9.98	0.45	3.8	0.67
Oil	Northwest Bay	2	52.31	12.80			
Control	L. Herring Bay	4	40.99	7.29	1.83	4.0	0.14
Oil	Herring Bay	3	60.80	8.02			
Control	Mummy Bay	5	58.90	13.03	0.16	3.6	0.88
Oil	Bay of Isles	6	55.42	18.24			

Table 6.18 Results of t-tests of differences in percent cover of all algae at oiled and control sites in Laminaria habitats in 1990. T- tests were performed on data that were transformed (arcsin square root). Untransformed means and standard errors are tabulated.

Depth Strata = 12 to 20 m

Oil/ Control	Site Name	Site #	Mean	SE	t	df	P
Control	Cabin Bay	1	19.77	17.95	0.61	2.3	0.60
Oil	Northwest Bay	2	31.20	5.27			
Control	L. Herring Bay	4	41.00	10.16	1.11	2.4	0.36
Oil	Herring Bay	3	29.13	3.09			
Control	Mummy Bay	5	44.48	4.58	0.88	2.6	0.45
Oil	Bay of Isles	6	35.53	11.54			

Depth Strata = 4 to 12 m

Oil/ Control	Site Name	Site #	Mean	SE	t	df	P
Control	Cabin Bay	1	60.99	10.56	0.52	3.9	0.63
Oil	Northwest Bay	2	52.31	12.80			
Control	L. Herring Bay	4	44.10	10.34	1.28	3.8	0.27
Oil	Herring Bay	3	60.80	8.02			
Control	Mummy Bay	5	58.90	13.02	0.06	3.7	0.95
Oil	Bay of Isles	6	60.22	17.05			

The one exception was at Northwest Bay where at shallower depths, *Agarum* abundances were relatively low compared with the control (Cabin Bay) . However, *Agarum* appeared to be replaced by *Laminaria* at this site, and both sites were comparable with respect to density and percent cover when *Agarum* and *Laminaria* were combined.

Epibenthic Invertebrates

The epibenthos in *Agarum/Laminaria* habitats was dominated by a variety of starfish and by *Telmessus*. There were no obvious trends with respect to differences among oiled and control sites for any of the starfish species (Figures 6.18, Tables 6.19 - 6.22). *Telmessus* tended to be more abundant at control vs oiled sites - they were very rare at all oiled sites and were moderately abundant at two of the three control sites (Fig. 6.18, Table 6.23). This is the same trend as observed in eelgrass habitats. While means at oiled vs control sites did not differ significantly at the $P<0.05$ level, the weight of evidence suggests an adverse effect of oil on populations of these crabs.

Fishes

Deep Strata

The fish community observed in deep depth strata of island bay *Laminaria/Agarum* habitats was dominated by arctic shanny and a mixed group of sculpins (Table 6.24, 6.25). In two out of three pairs of oiled/control study sites there was a tendency to higher overall density of fishes in oiled sites, with significantly higher density in one of the three (Table 6.26, Figure 6.19). This pattern was due principally to a similar pattern in small sculpins (Table 6.26, Figure 6.19). The other important species, arctic shanny, showed negligible differences between oiled/control study site pairs (Table 6.26, Figure 6.19).

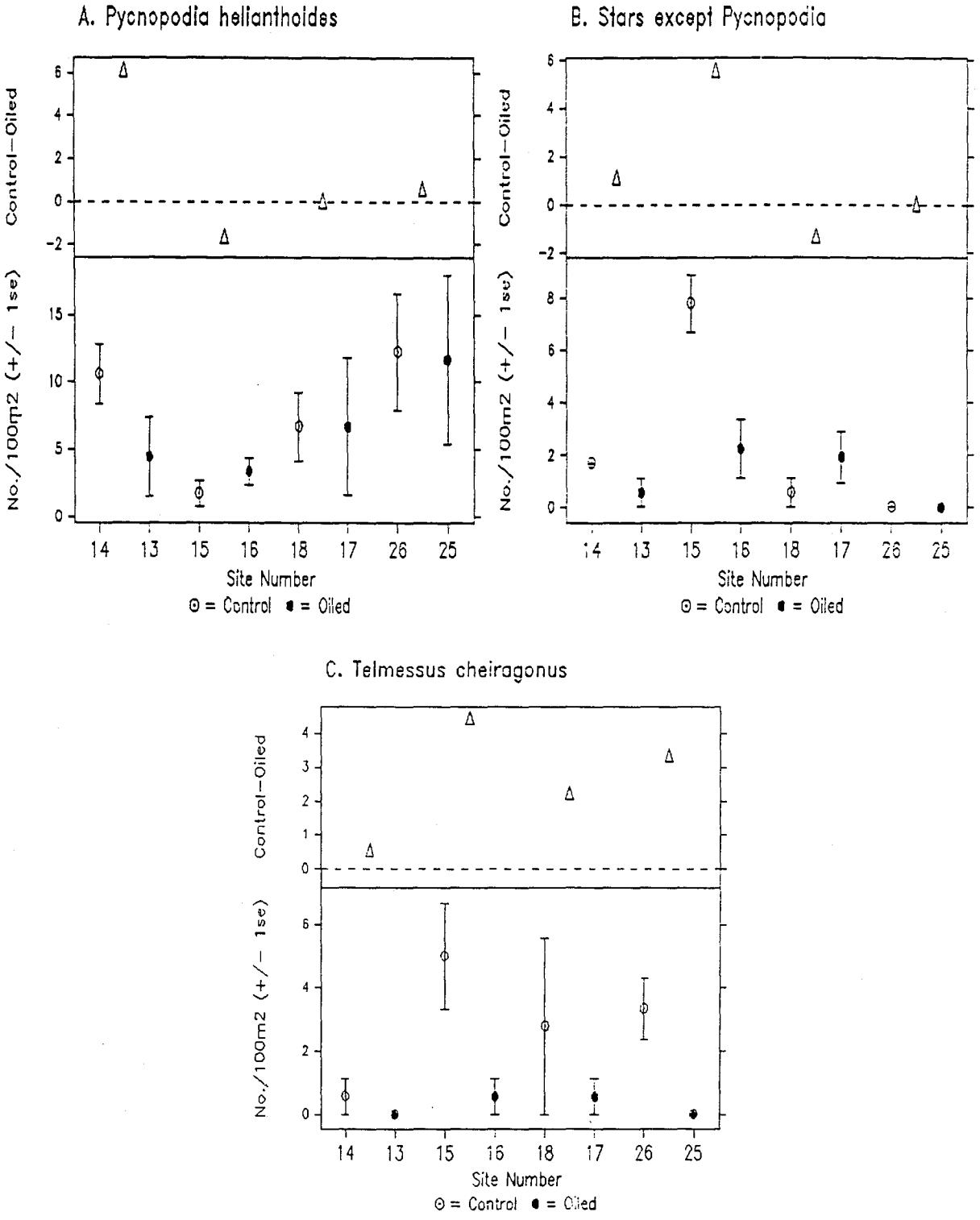


Figure 6.18 Means, plus and minus one standard error, and differences in means between oiled and control *Laminaria/Agarum* study sites for density of *Pycnogodia*, other starfish, and *Telmessus*.

Table 6.19 Results of t-tests of differences in density (no./m^2) of Pycnopodia helianthoides at oiled and control sites in Laminaria habitats in 1990.

Depth Strata = 12 to 20 m

Oil/ Control	Site Name	Site #	Mean	SE	t	df	P
Control	Cabin Bay	1	3.33	1.67	0.25	3.9	0.81
Oil	Northwest Bay	2	2.78	1.47			
Control	L. Herring Bay	4	1.11	0.56	1.49	2.1	0.27
Oil	Herring Bay	3	5.56	2.94			
Control	Mummy Bay	5	9.45	2.22	1.01	2.5	0.40
Oil	Bay of Isles	6	16.11	6.19			

Depth Strata = 4 to 12 m

Oil/ Control	Site Name	Site #	Mean	SE	t	df	P
Control	Cabin Bay	1	20.56	5.30	0.61	3.6	0.58
Oil	Northwest Bay	2	26.11	7.47			
Control	L. Herring Bay	4	10.00	4.19	1.14	2.5	0.35
Oil	Herring Bay	3	24.44	11.95			
Control	Mummy Bay	5	17.22	1.11	2.88	2.1	0.09
Oil	Bay of Isles	6	35.56	6.26			

Table 6.20 Results of t-tests of differences in density (no./m2) of Dermasterias imbricata at oiled and control sites in Laminaria habitats in 1990.

Depth Strata = 12 to 20 m

Oil/ Control	Site Name	Site #	Mean	SE	t	df	P
Control	Cabin Bay	1	0.00	-	-	-	-
Oil	Northwest Bay	2	0.00	-			
Control	L. Herring Bay	4	1.11	0.96	0.00	4.0	1.0
Oil	Herring Bay	3	1.11	0.96			
Control	Mummy Bay	5	2.23	1.47	1.06	2.6	0.38
Oil	Bay of Isles	6	0.56	0.56			

Depth Strata = 4 to 12 m

Oil/ Control	Site Name	Site #	Mean	SE	t	df	P
Control	Cabin Bay	1	3.33	1.93	0.00	4.0	1.0
Oil	Northwest Bay	2	3.33	1.93			
Control	L. Herring Bay	4	3.89	2.22	0.00	2.9	1.0
Oil	Herring Bay	3	3.89	1.11			
Control	Mummy Bay	5	16.67	7.87	?	2.0	0.17
Oil	Bay of Isles	6	0.00	0.00			

Table 6.21 Results of t-tests of differences in density (no./m2) of Evasterias troschelii at oiled and control sites in Laminaria habitats in 1990.

Depth Strata = 12 to 20 m

Oil/ Control	Site Name	Site #	Mean	SE	t	df	P
Control	Cabin Bay	1	0.00	0.00	2.00	2.0	0.18
Oil	Northwest Bay	2	1.13	0.56			
Control	L. Herring Bay	4	2.22	1.11	0.89	2.9	0.43
Oil	Herring Bay	3	1.11	0.56			
Control	Mummy Bay	5	2.22	1.47	1.51	2.0	0.27
Oil	Bay of Isles	6	0.00	0.00			

Depth Strata = 4 to 12 m

Oil/ Control	Site Name	Site #	Mean	SE	t	df	P
Control	Cabin Bay	1	1.67	0.00	1.15	2.0	0.37
Oil	Northwest Bay	2	5.00	2.89			
Control	L. Herring Bay	4	3.89	1.47	0.79	3.3	0.48
Oil	Herring Bay	3	6.11	2.42			
Control	Mummy Bay	5	4.44	0.56	0.55	2.3	0.63
Oil	Bay of Isles	6	3.33	1.92			

Table 6.22 Results of t-tests of differences in density (no./m2) of Orthasterias koehleri at oiled and control sites in Laminaria habitats in 1990.

Depth Strata = 12 to 20 m

Oil/ Control	Site Name	Site #	Mean	SE	t	df	P
Control	Cabin Bay	1	0.56	0.56	1.00	2.0	0.42
Oil	Northwest Bay	2	0.00	0.00			
Control	Cabin Bay	4	1.67	0.96	0.32	3.4	0.77
Oil	Northwest Bay	3	2.22	1.47			
Control	Cabin Bay	5	8.33	2.55	1.57	3.7	0.20
Oil	Northwest Bay	6	3.33	1.92			

Depth Strata = 4 to 12 m

Oil/ Control	Site Name	Site #	Mean	SE	t	df	P
Control	Cabin Bay	1	1.67	1.67	0.00	3.2	1.00
Oil	Northwest Bay	2	1.67	0.96			
Control	Cabin Bay	4	1.11	0.56	1.72	2.0	0.22
Oil	Northwest Bay	3	10.00	5.09			
Control	Cabin Bay	5	7.78	3.09	2.12	2.1	0.16
Oil	Northwest Bay	6	1.11	0.56			

Table 6.23 Results of t-tests of differences in density (no./m2) of Telmessus cheiragonus at oiled and control sites in Laminaria habitats in 1990.

Depth Strata = 12 to 20 m

Oil/ Control	Site Name	Site #	Mean	SE	t	df	P
Control	Cabin Bay	1	0.00	-	-	-	-
Oil	Northwest Bay	2	0.00	-			
Control	L. Herring Bay	4	2.22	0.55	2.12	4.0	0.10
Oil	Herring Bay	3	0.56	0.56			
Control	Mummy Bay	5	1.11	0.56	2.00	2.0	0.18
Oil	Bay of Isles	6	0.00	0.00			

Depth Strata = 4 to 12 m

Oil/ Control	Site Name	Site #	Mean	SE	t	df	P
Control	Cabin Bay	1	6.11	3.09	0.60	3.4	0.58
Oil	Northwest Bay	2	3.89	2.00			
Control	L. Herring Bay	4	2.78	1.47	0.38	2.0	0.74
Oil	Herring Bay	3	3.33	0.00			
Control	Mummy Bay	5	7.78	4.34	1.31	2.6	0.29
Oil	Bay of Isles	6	1.67	1.67			

Table 6.24 Fish species observed in Laminaria/Agarum habitats, with species groupings used in analyses, and occurrence by depth strata (D = deep, S = shallow).

FAMILY	SPECIES	GROUP	DEPTH
GADIDAE	walleye pollock	Other	D
	Pacific Cod	Pacific cod (YOY)	D,S
HEXIGRAMMIDAE	kelp greenling	Greenlings	D,S
	rock greenling	Greenlings	D,S
	Whitespotted greenling	Greenlings	S
	masked greeling	Greenlings	S
COTTIDAE	Cottid, small	Small sculpins	D,S
	grunt sculpin	small sculpins	D,S
	Artedius sp.	Small sculpins	D,S
	pith-head sculpin	Small sculpins	D,S
	Cottid, large	Large sculpins	D,S
	buffalo sculpin	Large sculpins	D
	red Irish lord	Large sculpins	D,S
	yellow Irish lord	Large Sculpins	D
	Irish lord, unident.	Large Sculpins	S
	great sculpin	Large Sculpins	D.S
	sand sculpin	Large Sculpins	D
PHOLIDAE	gunnel, unident.	Gunnels	D,S
	crescent gunnel	Gunnels	D,S
	saddleback gunnel	Gunnels	S
STICHAEIDAE	arctic shanny	arctic shanny	D,S
	slender eelblenny	Other	D,S
	black/rock prickleback	Other	S
	prickleback, unident.	Other	D,S
BATHYMASTERIDAE	searcher	Ronquils	D,S
	northern ronquil	Ronquils	D,S
SCORPAENIDAE	yelloweye rockfish	Other	D
	copper rockfish	Other	S
	dusky rockfish	Other	S
	rockfish, unident.	Other	D,S
PLEURONECTIDAE	flatfish, unident.	Other	D,S
AGONIDAE	poacher, unident.	Other	D,S
ZOARCIDAE	eelpout, unident.	Other	D

Table 6.25 Relative abundance, as proportion of total, of fish species groups in the deep depth strata (9 - 20 m) in combined and segregated oil and control study sites.

SPECIES GROUP	COMBINED	OIL	CONTROL
Small sculpins	.47	.57	.27
arctic shanny	.37	.27	.56
Gunnels	.04	.04	.05
Greenlings	.03	.03	.01
Large sculpins	.02	.03	.01
Searchers	.01	.02	0
Pacific cod (YOY)	.01	0	.02
Other	.04	.04	.06
n =	269	181	88

Table 6.26 Results of t-tests of differences in density of all fishes, small sculpins, and arctic shanny at oiled and control deep depth strata of Laminaria/Agarum Bay study sites in 1990.

CONTROL/ OIL	SITE #	DENSITY ($no/60m^2$)	STD.ERR.	t VALUE	P
ALL FISHES					
Control	1	3.3	0.7	0.15	0.89
Oil	2	3.7	2.2		
Control	4	10.7	0.3	1.79	0.15
Oil	3	26.3	8.7		
Control	6	15.0	3.1	2.75	0.05
Oil	5	30.0	4.5		
SMALL SCULPINS					
Control	1	1.3	0.9	0.50	0.64
Oil	2	2.0	1.0		
Control	4	3.7	1.4	2.10	0.10
Oil	3	18.0	6.6		
Control	6	3.0	1.2	3.17	0.03
ARCTIC SHANNY					
Control	1	1.3	0.9	0.60	0.58
Oil	2	0.7	0.7		
Control	4	5.3	0.9	0.51	0.64
Oil	3	4.3	1.7		
Control	6	9.7	1.4	0.71	0.52
Oil	5	11.3	1.8		

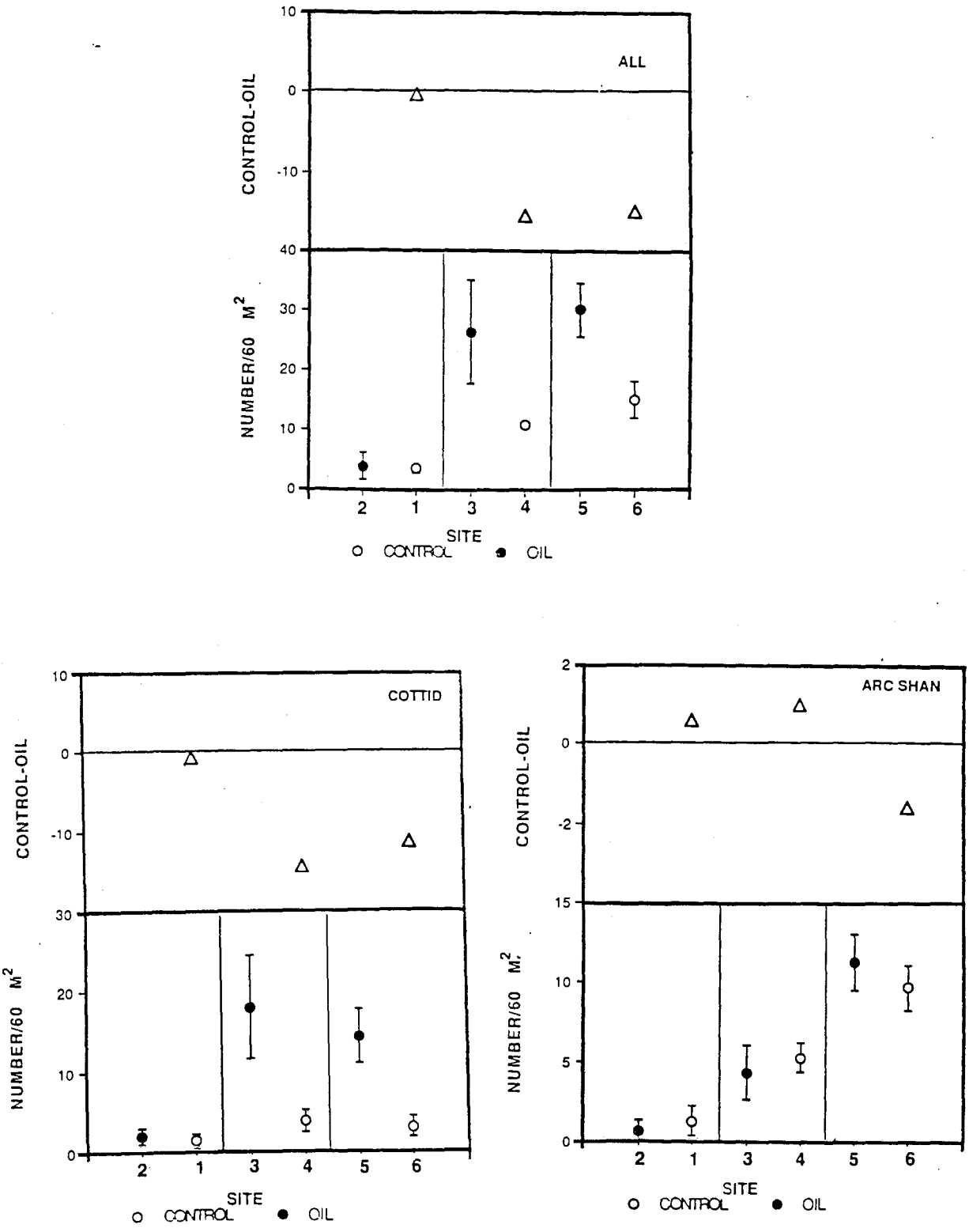


Figure 6.19 Means, plus and minus one standard error, and differences in means between oiled and control *Laminaria/Agarum* study sites for density of all fishes, small sculpins, and arctic shanny in the deep depth stratum.

Shallow Strata

In shallow *Laminaria/Agarum* bays the fish community had a suite of species that was generally similar to those found in deep strata at the same sites (Table 6.24). As in the deeper depths, arctic shanny and a mixed group of cottids were the most important taxa (Table 6.27). Comparisons of oiled/control study site pairs also revealed a pattern similar to the deep depth strata, with two out of the three pairs having significantly higher densities of all fish in the oiled habitat (Table 6.28, Figure 6.20). In this case, the difference is almost entirely due to differences in the abundance of arctic shanny juveniles and adults (Table 6.28, Figure 6.20). In both shallow and deep depth strata, the same pair of stations (5 and 6) had the most pronounced difference between oiled and control habitats.

Reproductive Success in *Dermasterias*

Experiments conducted on *Dermasterias* revealed no substantial differences among animals collected from oiled and control sites with respect to gonad index, spawning success, fertilization rate, or embryonic development (Tables 6.29, 6.30). The fertilization rate of eggs released from animals collected from an oiled site was slightly higher than for those collected from a control, but both showed relatively high rates of fertilization.

One surprising result from our studies of *Dermasterias* was that nearly 30% of the population of animals collected were parasitized by an internal barnacle parasite of the order *Ascothoracia*. This barnacle invaded the gonad tissue of the starfish and parasitized stars had a significantly lower rate of spawning success (Table 6.31)

There was no apparent difference in the infection rate among animals collected from oiled *vs.* control sites (Table 6.32). However, this does not rule out the possibility that the high rate of parasitism noted throughout the Sound was the result of the oil spill. Increased stress in starfish within local populations could have led to increased reproductive success of parasites. The parasites could then disperse larger than normal numbers of larvae that could invade hosts over a broad geographical area.

Table 6.27 Relative abundance, as proportion of total, of fish species groups in the shallow depth strata (0 - 8 m) in combined and segregated oil and control study sites.

SPECIES GROUP	COMBINED	OIL	CONTROL
arctic shanny (juv. and adult)	.49	.63	.31
Small sculpins	.15	.20	.10
arctic shanny	.13	.01	.29
Gunnels	.12	.04	.22
Searchers	.03	.05	.004
Large sculpins	.02	.03	.02
Greenlings	.02	.02	.01
Other	.03	.02	.04
n =	525	294	231

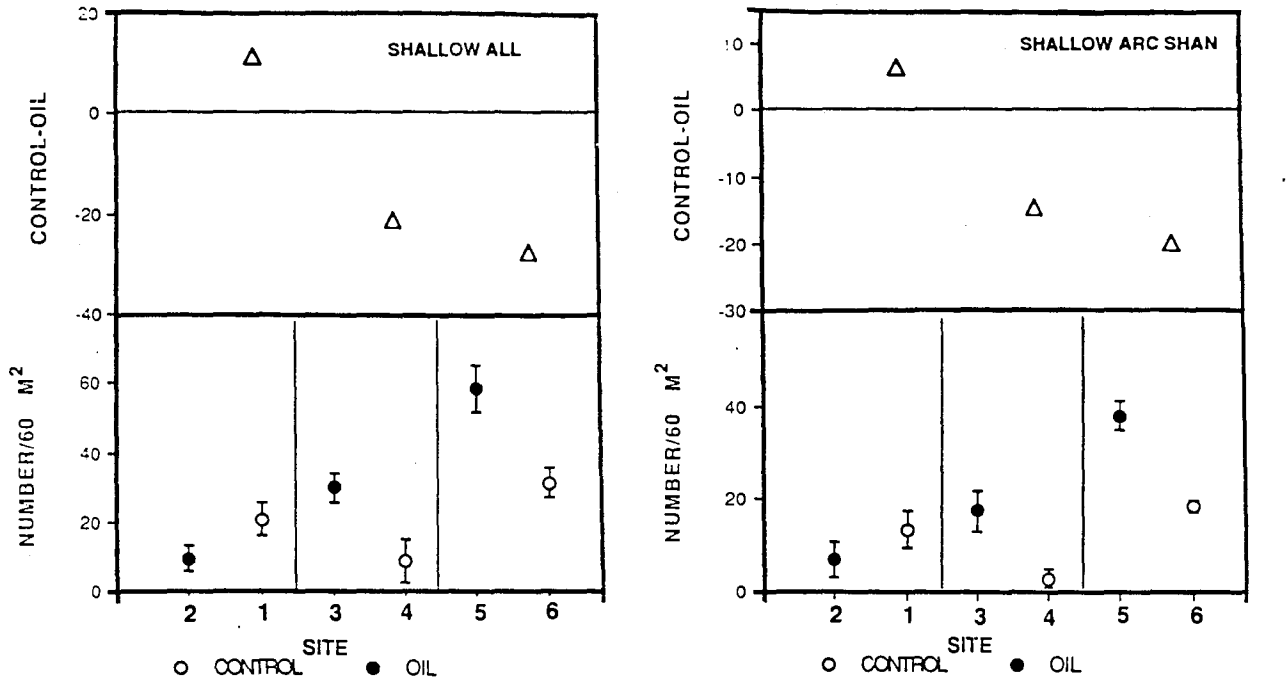


Figure 6.20 Means, plus and minus one standard error, and differences in means between oiled and control *Laminaria/Agarum* study sites for density of all fishes and arctic shanny in the shallow depth stratum.

Table 6.28 Results of t-tests of differences in density of all fishes and arctic shanny at oiled and control shallow depth strata of Laminaria/Agarum Bay study sites in 1990.

CONTROL/ OIL	SITE #	DENSITY (no/60m^2)	STD.ERR	t VALUE	P
ALL FISHES					
Control	1	20.7	4.7	1.86	0.14
Oil	2	9.3	3.9		
Control	4	8.7	6.3	2.81	0.05
Oil	3	30.0	4.2		
Control	6	31.3	4.3	3.39	0.03
Oil	5	58.7	6.8		
ARCTIC SHANNY JUVENILES AND ADULTS					
Control	1	13.3	4.0	1.19	0.30
Oil	2	6.7	3.9		
Control	4	2.7	1.8	2.96	0.04
Oil	3	17.3	4.6		
Control	6	18.3	1.2	5.73	0.004
Oil	5	38.0	3.2		

Table 6.29 Results of t-tests of differences in percent fertilization, percent normal development, and gonad index for Dermasteria imbricata collected from oiled and control sites in Laminaria habitats in 1990.

Control	Site Name	Site #	Mean	SE	t	df	P
Percent Fertilization							
Control	Cabin Bay	1	78.19	1.53	2.32	18.3	0.03
Oil	Northwest Bay	2	82.42	0.99			
Percent Normal Development Larvae							
Control	Cabin Bay	1	58.25	5.43	0.22	19.8	0.83
Oil	Northwest Bay	2	56.63	4.86			
Gonad Index							
Control	L. Herring Bay	4	7.15	0.60	0.33	35.8	0.75
Oil	Herring Bay	3	6.79	0.93			

Table 6.30 Results of Chi-square tests examining differences in spawning success of Dermasterias imbricata collected from oiled and control sites.

Herring (oiled) and Lower Herring Bay (Control)

Oil/control	Y	N	% Spawned
Oiled	18	6	75%
Control	18	6	75%
Total	36	12	75%

x2 = 1.0, df = 1, P = 1.00

Herring (oiled) and Lower Herring Bay (Control)

Oil/control	Y	N	% Spawned
Oiled	29	14	67%
Control	33	13	72%
Total	62	27	70%

x2 = 0.19, df = 1, P = 0.66

Table 6.31 Results of Chi-square tests examining differences in spawning success of Dermasterias imbricata parasitized by barnacles.

Herring and Lower Herring Bay

Number of Rays Parasitized	Y	N	% Spawned
0	48	11	81
1	9	3	75
2	2	3	40
3	0	4	0
4	1	1	50
5	2	5	29
Total	62	27	70%

x2 = 21.2, df = 5, P = 0.0007

Table 6.32 Result of Chi-square tests examining differences in parasitism by barnacles in Dermasterias imbricata collected from oiled and control sites.

Northwest (oiled) and Cabin (Control) Bays

Paratisized

Site	Y	N	%
Oiled	8	18	31%
Control	4	20	17%
Total	12	38	24%

x2 = 1.36, df = 1, P = 0.24

Herring (oiled) and Lower Herring (Control) Bay

Site	Y	N	%
Oiled	12	31	28%
Control	18	28	39%
Total	30	59	34%

x2 = 1.25, df = 1, P = 0,26

Bay of Isles (oiled) and Mummy Bay (Control) Bay

Paratisized

Site	Y	N	%
Oiled	19	34	23%
Control	14	28	33%
Total	24	62	28%

x2 = 1.20, df = 1, P = 0.27

Assessment of Damage

The effects of oil on subtidal habitats in Prince William Sound is being assessed through comparisons of pairs of oiled and control study sites in five habitat types: silled fjords, eelgrass areas, *Laminaria/Agarum* bays, *Laminaria/Agarum* points, and *Nereocystis* areas. Silled fjords were sampled three times to date: Fall 1989, Spring and Fall 1990. Remaining habitats were sampled in Spring 1990 only. This report provides preliminary results from the first three of these habitats in 1990. Results of the silled fjords are based on samples collected in the Fall 1989 and Spring 1990.

In Fall 1989, numerous dead organisms, including highly mobile forms such as squid and fishes, were observed at depths >13 m in an oiled silled fjord (Herring Bay). In Spring 1990 this site was revisited, as were three other similar habitats. Few dead organisms were observed in the Spring survey, suggesting that the mortalities observed in 1989 could have been oil-related, although low oxygen may have been a factor. Examination of the 1990 samples revealed greater disturbance than observed in 1989. Low values for diversity, richness, evenness and biomass, with a corresponding high dominance value reflected gross disturbance.

The high dominance was due to the eruption of the stress-tolerant polychaete *Nephtys cornuta*; its closely related congeneric, *N. longosetosa*, is known to be attracted to oil-contaminated sediments (Atlas, *et al.*, 1978). The effects of oil on subtidal infaunal communities at similar or deeper depths have been observed in other studies (Cabioch *et al.*, 1978; Sanders *et al.*, 1980; Hyland *et al*, 1989). Although the shoreline of Herring Bay was heavily oiled following the initial spill, we do not have complete evidence at this time as to whether or not oil reached the offshore subtidal depths where disturbances to the infauna were noted. Thus, until we obtain and integrate results of hydrocarbon analysis, we are unable to determine the exact sources of disturbance. A highly disturbed infaunal community was also observed at an unoiled control site in Inner Lucky Bay, suggesting that other natural factors also may be important in structuring these communities. The data suggests that the low diversity and abundances of most fauna could be caused by, or confounded by, anoxic or hypoxic conditions on the seafloor related to poor water exchange and natural cycles of organic enrichment.

A more extensive survey of silled fjord habitats was completed in Fall 1990, and will provide additional data to assess the possible role of seasonal anoxia as a cause of disturbance.

In eelgrass habitats, there was a consistent trend to lower density of eelgrass at oiled sites, although individual paired comparisons were not significant. There also was a similar, but weaker, trend to lower density of flowering turions and spathes at oiled sites. Among large epibenthic invertebrates, there were on patterns associated with oiled sites, with the exception of the crab *Telmessus,* which showed depressed densities. No data on infauna are yet available. Fishes tended to be less abundant at the control sites. This difference was due almost entirely to a non-significant, but persistent, trend to higher densities of young-of-the-year Pacific cod at oiled eelgrass sites. Densities of other fishes were similar between oiled and control paired sites.

In *Laminaria/Agarum* bay habitats, there was little difference in density or percent cover of algae, including the dominant *Agarum* and *Laminaria* species. Large epibenthic invertebrates were also similar between oiled and control paired sites; however, the crab *Telmessus* again displayed a consistent trend to lower density in oiled sites. This result is consistent with those of Rice *et al.* (1976) who found crustaceans, as a group, to be more sensitive than gastropods, bivalves, or echinoderms in response to exposure to the water-soluble fractions of Cook Inlet crude oil. However, generalizations about the relative sensitivity of different phylogenetic groups must be viewed with caution, because of a wide range of responses often observed at the individual species level. No data on infauna are yet available. Fishes tended to occur at higher density at oiled sites. In the deep stratum (9-20 m) the trend was due principally to a group of small sculpin species; whereas in the shallow stratum, the pattern was due to significantly higher densities of arctic shanny at two or three oil/control site pairs.

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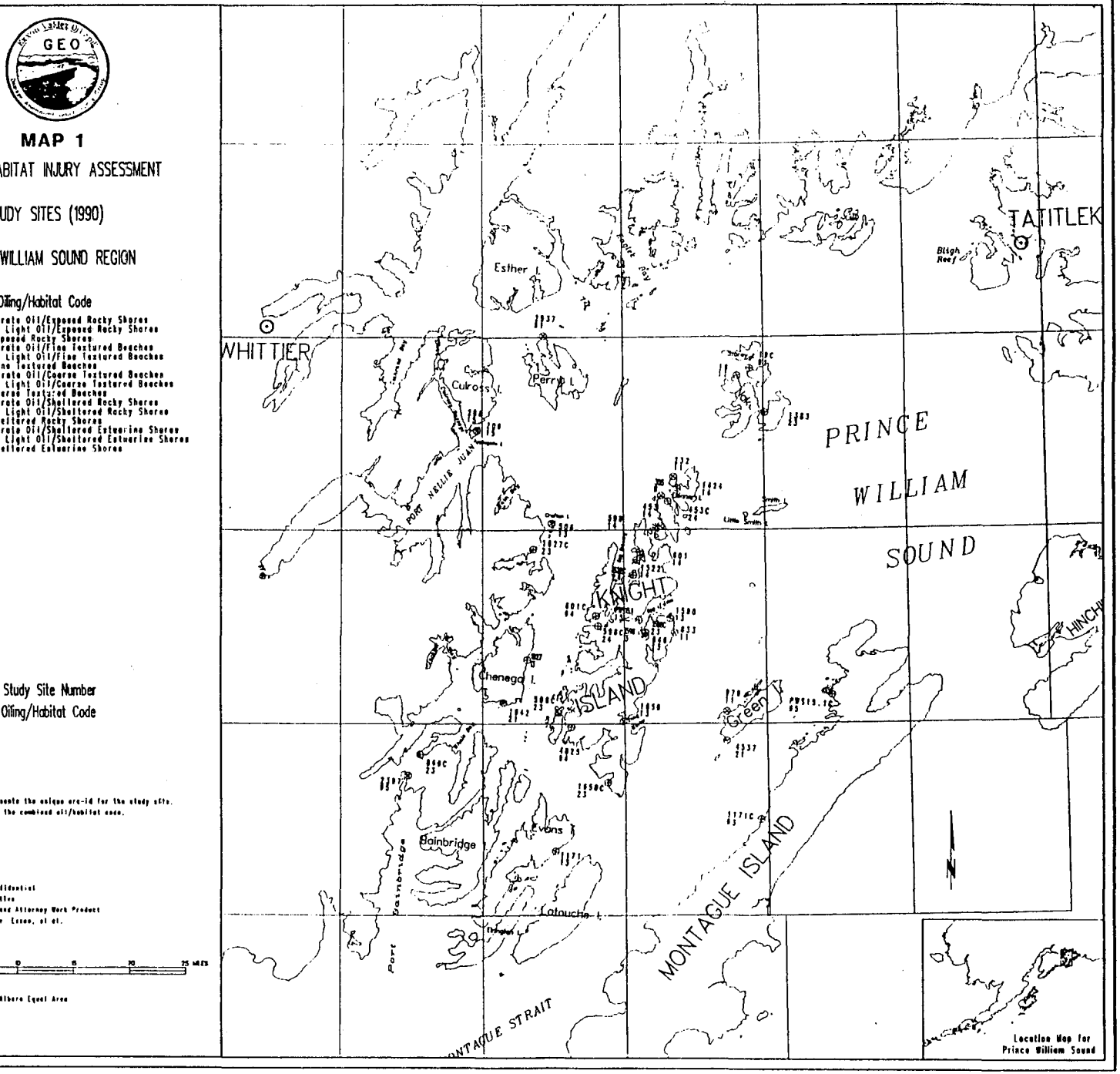
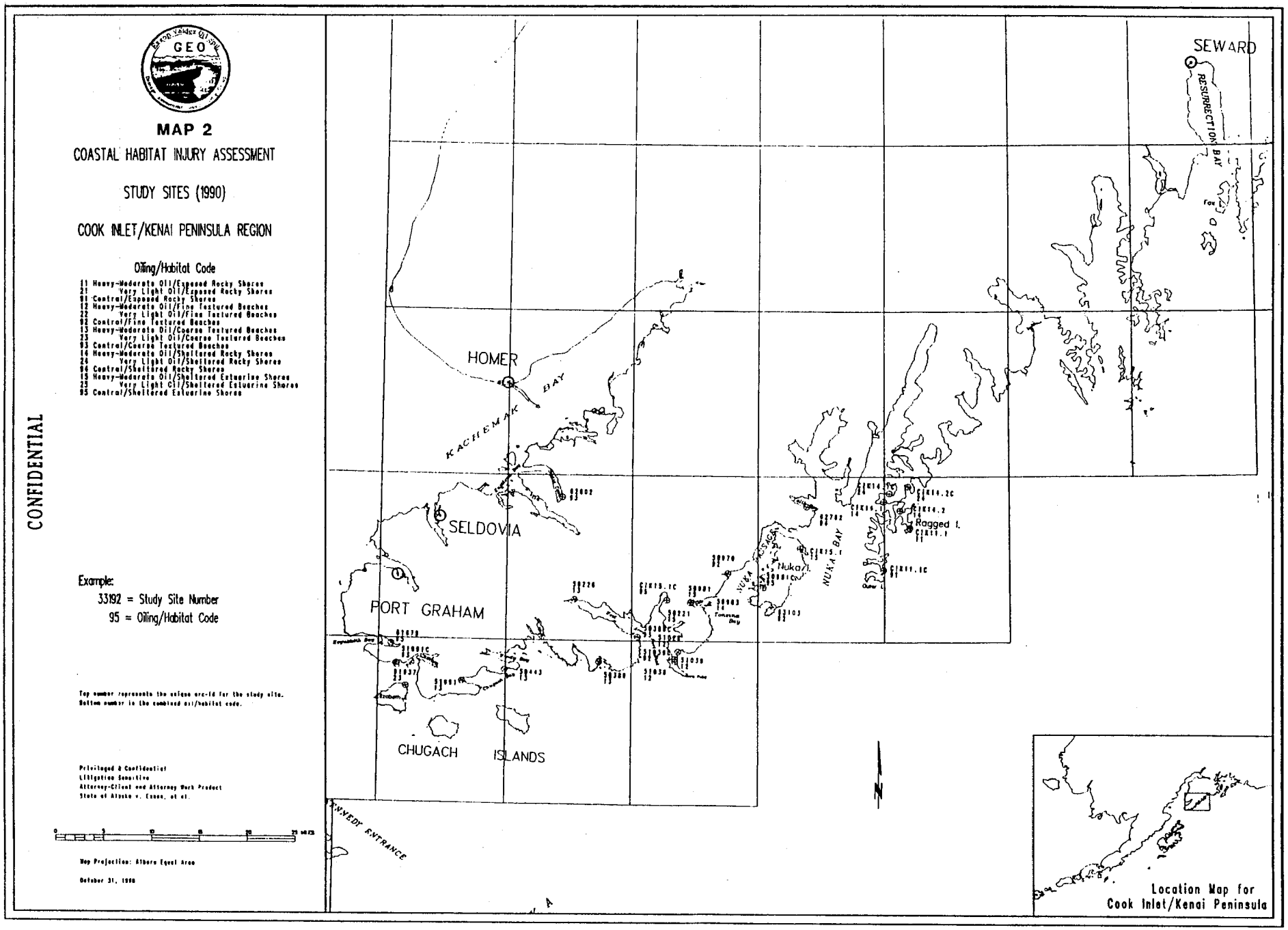
APPENDIX I





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ATTORNEY WORK PRODUCT

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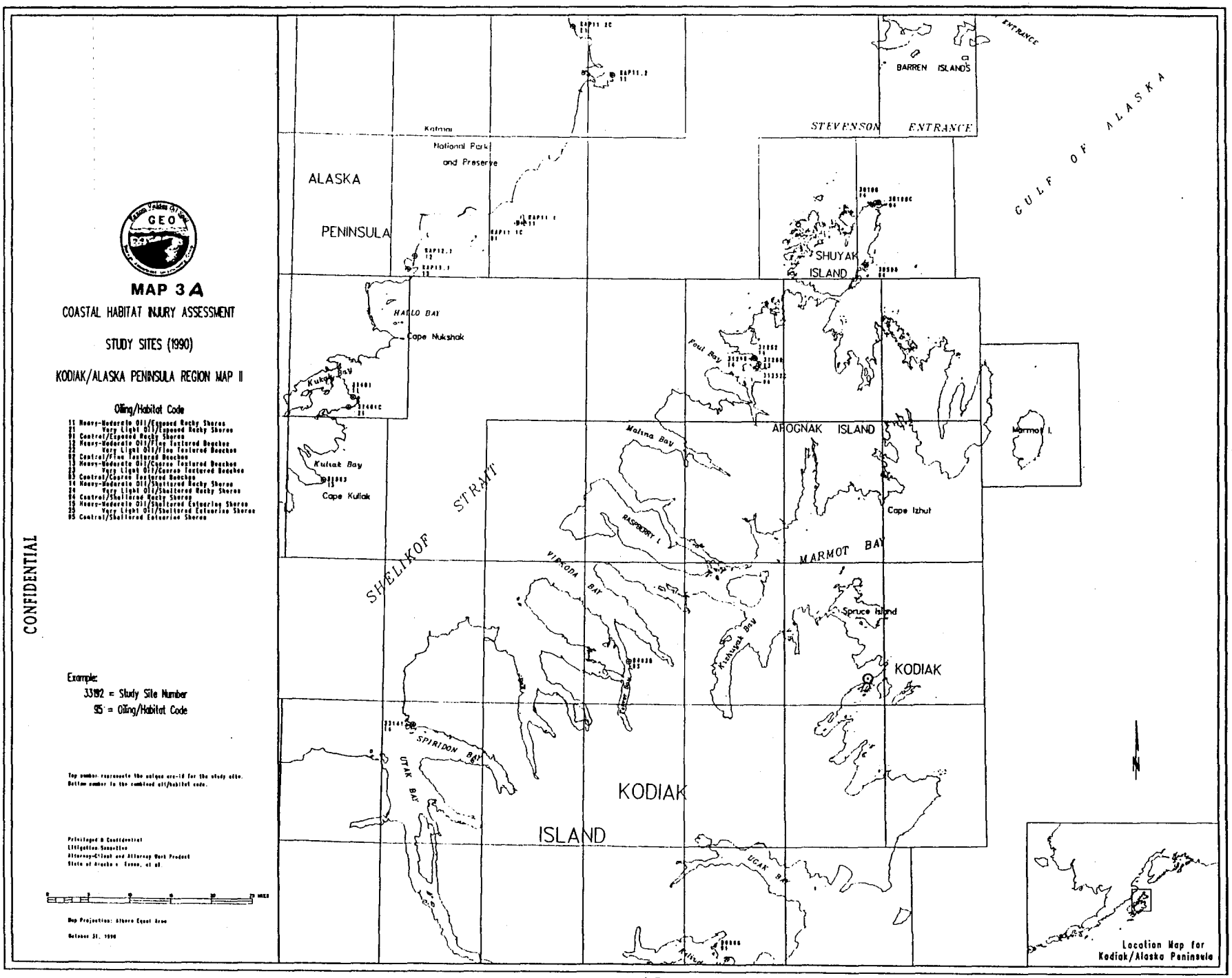

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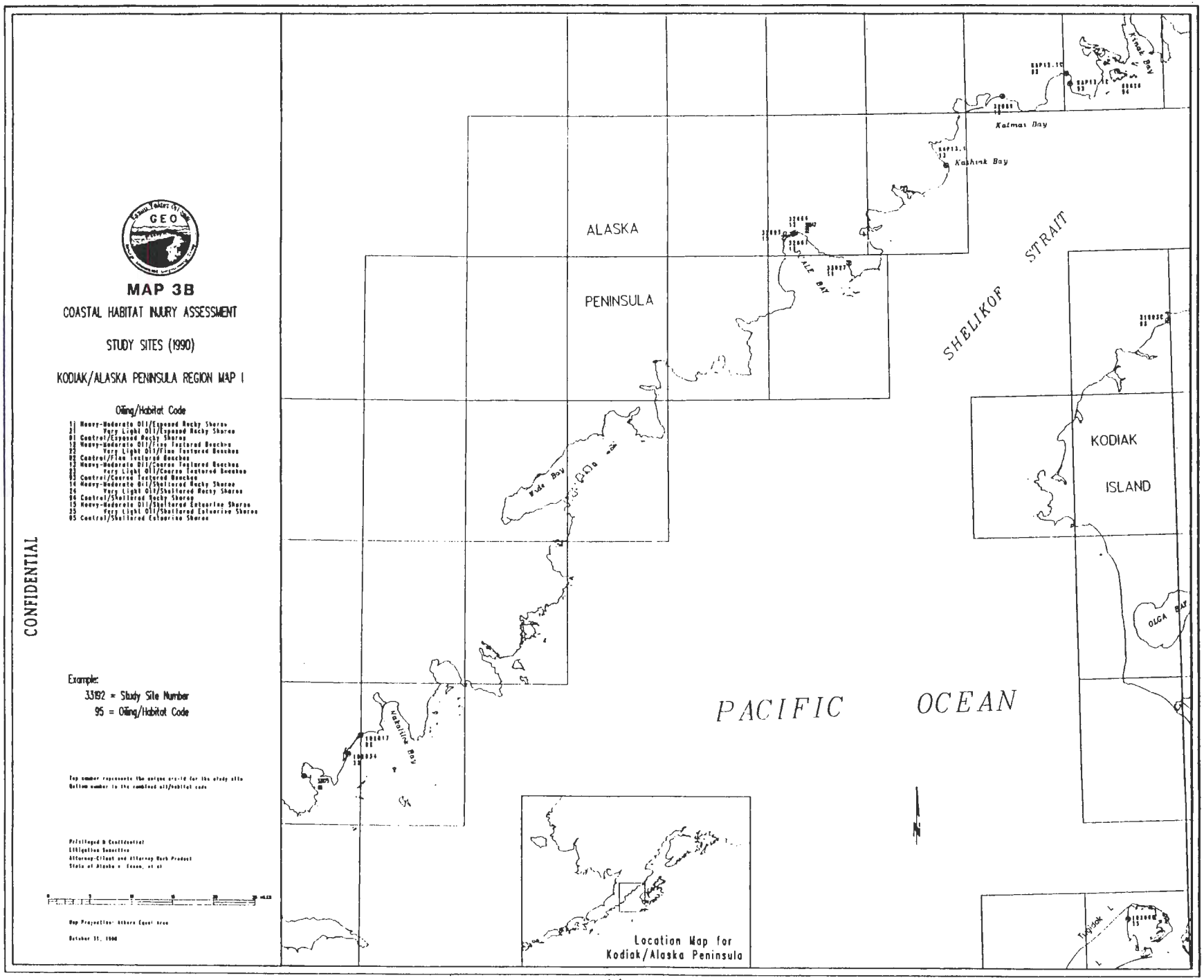

COASTAL HABITAT STUDY SITES, PRINCE WILLIAM SOUND (PWS) REGION**********CONFIDENTIAL**********

SITE #	QUAD	OIL/HAB CODE	LOCATION	LATITUDE LONGITUDE	SEG. #	PAIRED SITE #	LENGTH (M)	ACCESS	MARKER	TREATMENT HISTORY[1]
PWS15.1	SEWARD B-2	15	BAY OF ISLES	60° 23.02'N 147° 42.70'W	KN004	PWS15.1C	143	S,H	PF	DR,BR
PWS15.1C	SEWARD B-1	95	STOCKDALE HARBOR	60° 17.16'N 147° 12.29'W	NONE	PWS15.1	200	S,H	RB,PF	NT
19	SEWARD C-2	11	NAKED ISLAND	60° 42.00'N 147° 26.90'W	NA021	19C	414	S,H	OP,PF	HPHW,LPCW, FR,DR,BR
19C	SEWARD C-2	91	PEAK ISLAND	60° 42.50'N 147° 24.70'W	PK001	19	180	S,H	OP,PF	NT
208/209	SEWARD C-4	15	APPLEGATE ISLAND	60° 37.70'N 148° 08.35'W	AE005	2397	229	S,H	OP,PF, RB,WS	FR,DR,MPHW, LPCW,BR
232	SEWARD C-2	11	NORTHWEST BAY	60° 34.10'N 147° 37.00'W	EL101	1642	217	S,H	OP,RB, PF,WS	HS,HPHW,LPCW, DR,BR
305	SEWARD C-2	11	INGOT ISLAND	60° 32.60'N 147° 38.90'W	IN023	2937	370	S	OP,PF, WS	DR,BR
453	SEWARD C-2	14	DISK ISLAND	60° 30.30'N 147° 39.20'W	DI068	453C	232	S,H	OP,PF, WS	MPHW,HPHW,HS, OB,BR
453C	SEWARD C-2	24	INGOT ISLAND	60° 32.18'N 147° 37.88'W	IN023	453	172	S,H	OP,PF	DR,BR
506	SEWARD C-3	13	CRAFTON ISLAND	60° 30.60'N 147° 56.50'W	CR001	506C	156	S,H	OP,PF, WS	MPHW,LPCW,HS, HPHW,OB,BR

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SITE #	QUAD	OIL/HAB CODE	LOCATION	LATITUDE LONGITUDE	SEG. #	PAIRED SITE #	LENGTH (M)	ACCESS	MARKER	TREATMENT HISTORY[1]
506C	SEWARD B-3	23	SQUIRE ISLAND	60° 15.85'N 147° 55.60'W	SQ003	506	200	S,H	PF,RN	NT
598	SEWARD B-2	14	HERRING BAY	60° 28.20'N 147° 42.40'W	KN118	598C	289	S	NOT MARKED	DR,BR,HPHW, HPCW
598C	SEWARD B-3	24	LOWER HERRING BAY	60° 22.58'N 147° 49.21'W	KN552	598	298	S,H	OP,PF	NT
601	SEWARD B-2	14	HERRING BAY	60° 28.10'N 147° 42.20'W	KN118	601C	518	S,H	OP,PF, WS	DR,BR,HPHW, HPCW
601C	SEWARD B-3	94	LOWER HERRING BAY	60° 23.35'N 147° 49.55'W	KN551	601	300	S,H	OP,PF	NT
833	SEWARD B-2	11	KNIGHT ISLAND	60° 22.15'N 147° 37.18'W	KN212	1642	383	S	OP,PF, WS	LPCW,HPCW, HPHW,FR,BR
846	SEWARD B-2	13	SOUTH ARM, BAY OF ISLES	60° 21.80'N 147° 41.90'W	KN205	846C	436	S,H	OP,PF	FR,DR
846C	SEWARD A-4	23	NORTH ARM, WHALE BAY	60° 12.56'N 148° 17.52'W	NONE	846	320	S,H	OP,RB, PF	NT
979	SEWARD B-2	11	GREEN ISLAND	60° 15.90'N 147° 29.00'W	GR001B	4537	218	S,H	OP,PF, RB,	HPHW,LPCW, HPCW,HS,BR,DR
1171	SEWARD A-3	13	EVANS ISLAND	60° 05.00'N 147° 56.20'W	EV900	1171C	196	S,H	RB,PF	NT
1171C	SEWARD A-2	93	MONTAGUE ISLAND	60° 07.52'N 147° 23.70'W	NONE	1171	150	S,H	OP,PF	NT

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SITE #	QUAD	OIL/HAB CODE	LOCATION	LATITUDE LONGITUDE	SEG. #	PAIRED SITE #	LENGTH (M)	ACCESS	MARKER	TREATMENT HISTORY[1]
1383	SEWARD C-1	23	MCPHERSON BAY	60° 39.10'N 147° 22.69'W	NA006	1580	414	S,H	OP,RB, WS,PF	DR
1424	SEWARD C-2	14	NORTHWEST BAY	60° 33.30'N 147° 36.60'W	EL054	4825	586	S	OP,PF, WS	DR,BR
1522	SEWARD B-2	14	HERRING BAY	60° 27.60'N 147° 42.90'W	KN121	1522C	571	S,H	OP,PF, WS	HPHW,HPCW,OB, DR,BR
1522C	SEWARD B-2	24	HERRING BAY	60° 26.52'N 147° 43.51'W	KN501	1522	300	S,H	OP,PF	DR
1580	SEWARD B-2	13	KNIGHT ISLAND	60° 23.20'N 147° 37.80'W	KN212	1383	317	S	OP,PF	FR,LPCW,HPCW, MPHW,HPHW,HS, BR
1598	SEWARD B-2	13	SOUTH ARM, BAY OF ISLES	60° 21.80'N 147° 42.00'W	KN013	1598C	140	S,H	OP,RB, PF,WS	ND
1598C	SEWARD B-2	23	SOUTH ARM, BAY OF ISLES	60° 22.05'N 147° 41.66'W	KN205	1598	140	S,H	OP,PF	DR
1627	SEWARD B-3	13	CHENEGA ISLAND	60° 19.90'N 148° 00.40'W	CH001	1627C	581	S,H	RB,WS, PF	DR
1627C	SEWARD B-3	23	ESHAMY BAY	60° 28.53'N 147° 59.38'W	EB006	1627	490	S,H	OP,PF	ND
1642	SEWARD B-3	21	CHENEGA ISLAND	60° 16.70'N 148° 04.00'W	CH016	232	580	S	OP,PF, RB,WS	FR,DR,LPCW, MPHW

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SITE #	QUAD	OIL/HAB CODE	LOCATION	LATITUDE LONGITUDE	SEG. #	PAIRED SITE #	LENGTH (M)	ACCESS	MARKER	TREATMENT HISTORY[1]
1650	SEWARD B-3	13	SNUG HARBOR	60° 15.30'N 147° 45.30'W	KN403	1650C	438	S,H	RB	LPCW,HPCW, MPHW,HPHW,HS, BR
1650C	SEWARD A-3	23	LITTLE BAY	60° 10.38'N 147° 47.80'W	KN608	1650	438	S,H	OP,PF	NT
2397	SEWARD D-3	95	PUFFIN COVE	60° 10.80'N 148° 19.50'W	NONE	208/209	579	S,H	OP,PF, WS,RB	NT
2937	SEWARD D-3	91	PERRY ISLAND	60° 45.00'N 147° 58.00'W	PR013	305	556	S	OP,PF	NT
4537	SEWARD A-2	21	GREEN ISLAND	60° 13.60'N 147° 29.20'W	GR301	979	589	S,H	OP,PF, WS	NT
4825	SEWARD A-3	94	DEER COVE	60° 14.60'N 147° 53.50'W	KN578	1424	583	S,H	OP,PF	NT

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COASTAL HABITAT STUDY SITES, COOK INLET/KENAI PENINSULA (CIK) REGION**********CONFIDENTIAL**********

SITE #	QUAD	OIL/HAB CODE	LOCATION	LATITUDE LONGITUDE	SEG. #	PAIRED SITE #	LENGTH (M)	ACCESS	MARKER	TREATMENT HISTORY[2]
CIK11.1	SELDOVIA B-1	11	HOOF POINT, RAGGED ISLAND	59° 25.35'N 150° 17.99'W	PY006	CIK11.1C	135	S	OP	ND
CIK11.1C	SELDOVIA B-2	91	RABBIT ISLAND	59° 21.58'N 150° 22.74'W	PY003	CIK11.1	100	H,S	OP	NT
CIK14.1	SELDOVIA B-2	14	MCARTHUR PASS	59° 27.76'N 150° 22.67'W	MR001	CIK14.1C	200	S,H	PF	NT
CIK14.1C	SELDOVIA B-1	24	UNNAMED COVE, NUKA BAY	59° 28.50'N 150° 21.67'W	NC001	CIK14.1	180	S	PF	NT
CIK14.2	SELDOVIA B-1	14	MORNING COVE	59° 26.95'N 150° 19.75'W	PY008	CIK14.2C	200	S,H	PF	ND
CIK14.2C	SELDOVIA B-1	24	CHANCE COVE	59° 29.11'N 150° 18.31'W	BM001	CIK14.2	270	S	PF	NT
CIK15.1	SELDOVIA B-2	15	UNNAMED BAY, NUKA ISLAND	59° 23.52'N 150° 37.57'W	NK001	CIK15.1C	184	S,H	OP,PF	ND
CIK15.1C	SELDOVIA B-3	95	TAYLOR BAY	59° 18.73'N 151° 01.32'W	PD011A	CIK15.1	150	S,H	OP,PF	NT
50221	SELDOVIA B-3	15	TONSINA BAY	59° 18.60'N 150° 57.20'W	TB003	61679	420	S,H	OP,RB, WS,PF	DR,BR
50226	SELDOVIA B-4	13	WEST ARM, PORT DICK	59° 18.70'N 151° 17.70'W	PD002	62802	444	H,S	OP,PF, WS	DR,BR

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SITE #	QUAD	OIL/HAB CODE	LOCATION	LATITUDE LONGITUDE	SEG. #	PAIRED SITE #	LENGTH (M)	ACCESS	MARKER	TREATMENT HISTORY[2]
50389	SELDOVIA A-4	13	ONE HAUL BAY	59° 13.10'N 151° 13.60'W	NONE	50389C	531	H,S	OP,PF	NT
50389C	SELDOVIA B-3	93	WEST ARM, PORT DICK	59° 15.33'N 151° 06.47'W	PD010	50389	380	H,S	OP,PF	NT
50443	SELDOVIA A-4	13	BADGER COVE	59° 12.30'N 151° 30.10'W	CB001	61937	391	S	OP,RB, PF	DR,BR
50970	SELDOVIA A-3	92	NUKA PASSAGE	59° 21.30'N 150° 50.30'W	NONE	51026	384	S,H	RB,WS	NT
50981	SELDOVIA B-3	15	TONSINA BAY	59° 18.50'N 150° 56.70'W	TB003	50981C	583	S,H	RB,PF, WS	DR,BR
50981C	SELDOVIA B-2	95	BERGER BAY, NUKA ISLAND	59° 19.96'N 150° 44.13'W	NK004A	50981	150	S,H	OP,PF	NT
50983	SELDOVIA B-3	14	TONSINA BAY	59° 18.50'N 150° 55.40'W	TB002B	62762	572	S	OP,PF, WS	DR
51026	SELDOVIA A-3	12	GORE POINT	59° 14.10'N 150° 59.10'W	GP1001	50970	498	H,S	OP,PF, WS	DR
51038	SELDOVIA A-3	12	GORE POINT	59° 13.50'N 150° 59.70'W	GP1002	63103	590	H,S	OP,WS	DR
51039	SELDOVIA A-3	12	GORE POINT	59° 13.20'N 150° 59.80'W	GP1002	51039C	144	H,S	WS	DR
51039C	SELDOVIA A-3	92	GORE POINT	59° 13.38'N 151° 00.34'W	GP1003	51039	144	H,S	OP,RB, PF	ND

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SITE #	QUAD	OIL/HAB CODE	LOCATION	LATITUDE LONGITUDE	SEG. #	PAIRED SITE #	LENGTH (M)	ACCESS	MARKER	TREATMENT HISTORY[2]
51091	SELDOVIA A-5	13	CHUGACH BAY	59° 11.30'N 151° 37.90'W	CB003	51091.C	578	H,S	OP,PF, WS	DR,FR
51091C	SELDOVIA A-5	93	CHROME BAY	59° 12.66'N 151° 49.37'W	CS001	51091	500	H,S	RB	NT
61679	SELDOVIA A-5	95	KOYUKTOLIK BAY	59° 14.60'N 151° 50.50'W	KC904	50221	598	S,H	RB,WS, PF	NT
61937	SELDOVIA A-5	23	ELIZABETH ISLAND	59° 10.60'N 151° 48.00'W	EI001	50443	581	H,S	OP,RB, PF	NT
62762	SELDOVIA B-2	94	YALIK BAY	59° 27.30'N 150° 36.60'W	YB005	50983	448	S	OP,PF, WS	NT
62802	SELDOVIA B-4	93	SADIE COVE	59° 27.90'N 151° 20.30'W	NONE	50226	449	H,S	OP,PF	NT
63103	SELDOVIA B-2	92	UNNAMED BAY, NUKA ISLAND	59° 18.20'N 150° 42.60'W	NK005	51038	448	H,S	RB,FF, WS	NT

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COASTAL HABITAT STUDY SITES, KODIAK/ALASKA PENINSULA (KAP) REGION**********CONFIDENTIAL**********

SITE #	QUAD	OIL/HAB CODE	LOCATION	LATITUDE LONGITUDE	SEG. #	PAIRED SITE #	LENGTH (M)	ACCESS	MARKER	TREATMENT HISTORY[3]
KAP11.1	Afognak C5	11	Kiukpalik Island	58° 35.84'N 153° 33.04'W	K-9 15	KAP11.1C	200	S,H	RB,PF	
KAP11.1C	Afognak C5	91	Kiukpalik Island	58° 35.81'N 153° 34.47'W	K-9 15	KAP11.1	130	S,H	RN,PF	
KAP11.2	Afognak D4	11	Cape Douglas	58° 51.61'N 153° 15.01'W	K-9 10	KAP11.2C	200	S,H	PF,RB	
KAP11.2C	Afognak D5	21	Northwest of Cape Douglas	58° 56.70'N 153° 23.20'W	K-9 06	KAP11.2	200	S,H	RN,PF	
KAP12.1	Afognak C6	12	Kaguyak	58° 32.21'N 153° 55.09'W	K-9 17	KAP12.1C	200	S,H	RB,PF	
KAP12.1C	Mt. Katmai A2	92	Dakavak Bay	58° 03.93'N 154° 39.57'W	K-9 32	KAP12.1	200	S,H		
KAP13.1	Karluk D4	13	Kashvik Bay	57° 54.40'N 155° 04.22'W	K-9 36	KAP13.1C	200	S,H	RB,PF	
KAP13.1C	Mt. Katmai A2	93	Dakavak Bay	58° 02.85'N 154° 38.87'W	K-9 32	KAP13.1	200	S,H	RB,PF	
KAP15.1	Afognak C6	15	Chiniak Lagoon	58° 30.79'N 153° 56.50'W	K-9 17	103098	200	S,H	PF	
30196	Afognak C1&C2	14	Perevalnie Passage	58° 37.90'N 152° 22.00'W	K-1 11	30196C	296	S,H	WS,OP	

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SITE #	QUAD	OIL/HAB CODE	LOCATION	LATITUDE LONGITUDE	SEG. #	PAIRED SITE #	LENGTH (M)	ACCESS	MARKER	TREATMENT HISTORY[3]
30196C	Afognak C1&C2	94	East Perevalnie I.	58° 38.10'N 152° 20.51'W	K-1 11	30196	295	S,H	RN,PF	
30590	Afognak C1&C2	94	Andreon Bay	58° 31.50'N 152° 23.10'W	K-1 01	33141	183	S	OP,RB, WS,PF	
31248	Afognak B3	14	Foul Bay	58° 21.70'N 152° 46.10'W	K-2 04	99826	198	S,H	OP,PF, WS,RB	
31252	Afognak B3	14	Foul Bay	58° 20.53'N 152° 45.67'W	K-2 04	31252C	258	S		
31252C	Afognak B3	94	Foul Bay	58° 20.52'N 152° 45.70'W	K-2 04	31252	200	S	RN,PF	
31288	Afognak B3	13	Foul Bay	58° 21.00'N 152° 45.20'W	K-2 04	94935	165	S	OP,WS	
31461	Mt. Katmai B1	11	Cape Ugyak	58° 17.10'N 154° 07.20'W	K-9 21	31461C	165	S,H	OP,WS	
31461C	Mt. Katmai B1	21	Kaflia Bay	58° 16.07'N 154° 08.00'W	K-9 22	31461	170	S,H	RN,PF	
31893	Mt. Katmai A1	13	Cape Kuliak	58° 08.50'N 154° 12.80'W	K-9 24	31893C	587	S,H	OP	
31893C	Karluk C2	93	Northeast Harbor	57° 37.50'N 154° 20.50'W	K-6 33	31893	200	S,H	RB,PF, WS	
32086	Mt. Katmai A3	12	Katmai Bay	58° 01.70'N 154° 52.20'W	K-9 34	102834	587	S,H	WS	

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SITE #	QUAD	OIL/HAB CODE	LOCATION	LATITUDE LONGITUDE	SEG. #	PAIRED SITE #	LENGTH (M)	ACCESS	MARKER	TREATMENT HISTORY[3]
32847	Karluk D5	22	Puale Bay	57° 47.42'N 155° 34.45'W	K-10 07	32854	332	S,H	WS	
32854	Karluk D5	12	Puale Bay	57° 47.40'N 155° 34.70'W	K-10 07	32847	591	S,H	WS	
32861	Karluk D5	12	Puale Bay	57° 47.30'N 155° 47.30'W	K-10 07	102817	199	S,H	WS	
32895	Karluk D5	15	Puale Bay	57° 47.10'N 155° 36.90'W	K-10 07	33875	374	S,H	WS	
33027	Karluk C4&C5	11	Puale Bay	57° 44.00'N 155° 23.80'W	K-10 07	96665	598	S,H	OP	
33141	Kodiak C6	14	Chief Cove	57° 42.50'N 153° 54.00'W	K-6 19	30590	122	S,H	OP,WS	
33875	Sutwik I. D4	95	Yantarni Bay	56° 50.11'N 157° 11.03'W	K-12 09	32895	276	S,H	RB,WS	
94935	Kodiak D4	93	Uganik Passage	57° 49.60'N 153° 11.20'W	K-6 03	31288	199	S,H	RB,WS	
96665	Kodiak B3	91	Santa Flavia Bay	57° 18.00'N 152° 51.90'W	K-8 12	33027	132	S,H	OP,WS	
99826	Mt. Katmai A2	94	Takli Island	58° 04.20'N 154° 29.50'W	K-9 29	31248	579	S	OP	
102817	Sutwik I. D4	92	North of Yantarni Bay	56° 54.50'N 157° 00.00'W	K-12 07	32861	585	S,H	RB,WS	

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SITE #	QUAD	OIL/HAB CODE	LOCATION	LATITUDE LONGITUDE	SEG. #	PAIRED SITE #	LENGTH (M)	ACCESS	MARKER	TREATMENT HISTORY[3]
102834	Sutwik I. D4	22	North of Yantarni Bay	56° 52.40'N 157° 02.70'W	K-12 07	32086	583	S,H	RB,WS	
103098	Trinity Is. B2&C2	25	Tugidak Island	56° 34.80'N 154° 31.80'W	K-7 16	KAP 15.1	590	S,H	RB,WS	

1. Treatment (clean up) history recorded by Alaska Department of Environmental Conservation for segment number in 1989.

2. Treatment (clean up) history recorded by Alaska Department of Environmental Conservation for segment number in 1989.

3. 1989 treatment (clean up) history for Kodiak & Alaska Peninsula Region not available.

OP=ORANGE PAINT, PF=PINK FLAGGING, WS=WHITE SIGN, RB=REBAR STAKE, RN=ROCK NUT, H=HELICOPTER, S=SKIFF, ND=NOT DETERMINED, FR=FUCUS REMOVAL, DR=DEBRIS REMOVAL, MPHW=MEDIUM PRESSURE HOT WATER, LPCW=LOW PRESSURE COLD WATER, BR=BIOREMEDIATION, NT=NO TREATMENT, HS=HIGH PRESSURE STEAM, HPHW=HIGH PRESSURE HOT WATER, OB=OMNI BOOM, HPCW=HIGH PRESSURE COLD WATER

APPENDIX II

TABLES

APPENDIX TABLE 1. Sites where fish were collected in the field for hydrocarbon analysis. Each sample contained at least 10 g fish. Blank (background) sample jars also were prepared at each site.

Site	Code	Site Pair	Hydrocarbon Samples
506	13	506C	*Oligocottus maculosus* *Xiphister atropurpuresceus* Blank
506C	23	506	*Anoplarchus purpurescens* Blank
305	11	2937	*Anoplarchus purpurescens* *Xiphister atropurpuresceus* Blank
1627	13	1627C	*Anoplarchus purpurescens* *Xiphister atropurpuresceus* Blank
1642	21	232	*Anoplarchus purpurescens* Blank

APPENDIX TABLE 2. Numbers and species of fish collected at oiled and unoiled sites in Prince William Sound, for gill histological analysis. Matched sites are listed as pairs, with the oiled site listed first.

Site	*Anoplarchus purpurescens*	*Oligocottus maculosus*
1522	6	6
1522C		6
598	20	
598C	4	1
979		6
4537		14
506		3
506C	1	6
453	1	3
453C	6	1
305		10
2937		2
19		1
19C		6
1650	5	1
1650C	-	-
1424	6	
4825	-	-
833		9
1642	-	-
846	7	
846C	-	-
601	6	6
601C	-	-
1171	-	-
1171C		10
208/209	-	-
2397		1
1627	-	-
1627C		3

APPENDIX TABLE 3. Average number (No.) of fish per square meter, number of square meters sampled (m^2), and sample size (n) for each strip quadrat at each paired site during the first sampling cycle. The first of each pair is the oiled (treated) site.

Pair	Site Pair	Quadrat 1			2			3			4			5		
		No.	m^2	n	No.	m^2	n	No.	m^2	n	No.	m^2	n	No.	m^2	n
1	1171	0.0	46	6	0.0	57	6	0.0	68	6	0.52	46	5	0.50	10	1
	1171C	0.0	46	6	0.0	68	6	0.54	37	6	0.44	34	4	0.73	11	2
2	1580	0.0	39	6	0.0	54	6	0.02	63	6	0.16	51	5	0.33	15	2
	1383	0.0	51	6	0.01	72	6	1.65	68	6	0.06	48	5	0.0	26	3
3	1424	0.0	15	6	0.35	23	6	0.14	21	6	1.18	11	4	–	–	–
	4825C	0.0	21	6	0.39	18	5	5.00	12	5	5.00	0.4	1	–	–	–
4	1522	0.0	34	5	0.0	30	5	0.0	5	4	–	–	–	–	–	–
	1522C	0.0	10	4	0.0	24	4	0.0	17	4	–	–	–	–	–	–
5	1598	0.0	40	5	0.01	79	5	0.08	53	5	0.72	25	5	0.0	2	1
	1598C	0.0	35	5	0.08	48	5	0.0	7	3	–	–	–	–	–	–
6	1627	0.0	44	6	0.09	53	6	0.08	63	6	0.40	20	4	0.43	7	2
	1627C	0.0	36	6	0.0	40	6	0.07	42	6	0.42	50	6	0.15	13	3
7	232	0.0	7	2	0.0	14	2	0.0	3	1	–	–	–	–	–	–
	1642	0.0	15	6	0.0	26	6	0.72	32	6	2.14	29	5	1.45	11	3
8	1650	0.0	50	6	0.0	60	6	0.05	57	6	0.21	39	4	0.89	28	4
	1650C	0.0	57	6	0.0	51	6	2.76	45	6	4.25	8	3	–	–	–
9	19	0.03	32	6	0.40	45	6	0.75	12	2	–	–	–	–	–	–
	19C	0.38	21	6	0.17	30	6	0.74	47	6	1.80	15	3	–	–	–
10	208/209	0.0	42	4	0.0	59	4	0.0	4	1	–	–	–	–	–	–
	2397	0.0	121	6	0.01	154	6	0.09	82	3	0.0	2	1	–	–	–
11	305	0.0	20	6	0.18	22	6	0.80	30	6	2.0	18	4	–	–	–
	2937	0.0	11	4	0.0	24	3	0.0	16	3	0.0	5	2	–	–	–
12	453	0.0	23	6	0.04	28	6	0.90	23	6	0.0	1	1	–	–	–
	453C	0.0	20	6	0.0	17	6	0.91	23	6	5.57	28	5	15.4	5	1
13	4537	0.0	152	6	0.03	165	6	0.29	133	5	0.29	34	1	–	–	–
	979	0.0	64	6	0.08	78	6	0.39	67	6	0.79	47	5	0.75	8	1
14	506	0.0	8	2	0.0	11	2	0.0	5	1	5.42	12	2	–	–	–
	506C	0.07	27	6	0.88	33	6	9.73	15	5	0.08	2	1	–	–	–
15	598	0.0	31	6	0.0	49	6	0.10	58	6	1.00	3	1	–	–	–
	598C	0.0	16	6	0.0	16	6	0.57	21	6	1.71	7	4	0.0	2	1
16	601	0.0	22	6	0.17	29	6	1.68	28	6	5.33	3	2	–	–	–
	601C	0.0	16	6	0.06	17	6	0.50	14	6	–	–	–	–	–	–
17	833	0.0	6	3	0.0	11	3	0.0	13	2	2.76	17	2	–	–	–
	1642	0.0	15	6	0.0	26	6	0.72	32	6	2.14	29	5	1.45	11	3
18	846	0.0	105	6	0.0	117	6	0.0	53	5	0.0	42	2	0.15	13	1
	846C	0.0	270	6	0.01	190	6	0.10	130	6	0.0	9	1	–	–	–
19	PWS15.1	NOT SAMPLED														
	PWS15.1C	0.0	64	6	0.0	131	6	0.0	52	3	–	–	–	–	–	–

APPENDIX TABLE 4. Average number (No.) of fish per square meter, number of square meters sampled (m^2), and sample size (n) for each strip quadrat at each paired site during the second sampling cycle. The first of each pair is the oiled (treated) site.

Pair	Site Pair	Quadrat 1			2			3			4			5		
		No.	m^2	n	No.	m^2	n	No.	m^2	n	No.	m^2	n	No.	m^2	n
1	1171	0.0	39	6	0.06	85	6	0.24	59	6	0.42	19	3	0.33	6	1
	1171C	0.0	46	6	0.0	58	6	0.0	51	6	0.11	47	5	2.0	2	1
2	1580	0.0	52	6	0.0	69	6	0.02	66	6	0.31	35	4	–	–	–
	1383	0.0	48	6	0.0	59	6	0.90	42	6	0.28	54	5	0.0	26	3
3	1424	0.0	26	6	0.39	14	5	0.30	10	3	–	–	–	–	–	–
	4825C	0.0	10	6	0.06	16	6	2.00	13	5	–	–	–	–	–	–
4	1522	0.0	33	6	0.14	36	6	0.60	18	6	0.0	11	4	0.50	2	1
	1522C	0.0	16	5	0.25	24	5	0.86	22	5	–	–	–	–	–	–
5	1598	0.0	37	5	0.0	72	5	0.17	54	5	1.22	36	5	3.14	14	3
	1598C	0.0	33	5	0.13	47	5	0.33	49	5	0.44	48	5	–	–	–
6	1627	0.0	34	6	0.0	117	6	0.01	175	6	0.12	98	3	–	–	–
	1627C	0.0	46	6	0.0	105	6	0.05	128	5	0.11	93	3	–	–	–
7	232	0.0	8	2	1.17	6	2	1.73	22	2	3.14	7	2	–	–	–
	1642	0.21	24	6	0.33	30	6	0.32	22	5	4.15	20	3	1.5	2	1
8	1650	0.0	51	6	0.0	55	6	0.10	58	6	0.62	47	6	1.0	9	2
	1650C	NOT SAMPLED														
9	19	0.0	26	5	0.0	20	5	0.35	26	3	0.83	6	1	–	–	–
	19C	0.03	31	5	0.64	36	5	0.52	21	3	0.0	9	2	–	–	–
10	208/209	0.0	56	4	0.02	112	4	0.21	148	4	0.05	39	1	–	–	–
	2397	0.07	132	6	0.05	307	6	0.04	201	3	–	–	–	–	–	–
11	305	0.0	23	6	0.0	24	6	0.0	35	5	0.58	38	3	–	–	–
	2937	0.0	9	2	0.25	8	2	0.0	7	1	0.0	5	1	–	–	–
12	453	0.0	22	5	0.24	21	6	0.48	25	6	–	–	–	–	–	–
	453C	0.0	19	6	0.19	21	6	0.43	23	6	2.22	9	3	–	–	–
13	4537	0.20	117	6	0.31	91	5	0.29	14	2	0.68	50	1	–	–	–
	979	0.03	59	6	0.33	67	6	0.35	31	5	0.79	19	2	0.33	9	1
14	506	0.0	18	3	0.0	36	3	0.56	34	2	0.90	21	1	–	–	–
	506C	0.0	24	6	0.50	26	6	4.88	17	6	20.0	3	1	–	–	–
15	598	0.0	23	6	0.0	22	6	1.85	20	6	0.50	4	3	–	–	–
	598C	0.0	18	6	0.24	21	6	3.11	18	5	4.67	3	1	–	–	–
16	601	0.0	21	6	0.21	33	6	3.38	24	5	–	–	–	–	–	–
	601C	0.0	20	6	0.50	14	6	2.54	13	4	6.50	2	1	–	–	–
17	833	0.71	14	3	0.31	13	3	1.0	9	2	–	–	–	–	–	–
	1642	0.21	24	6	0.33	30	6	0.32	22	5	4.15	20	3	1.5	2	1
18	846	0.0	81	6	0.0	122	6	0.26	171	6	1.17	72	4	–	–	–
	846C	0.0	239	6	0.0	223	6	0.23	138	6	1.0	7	3	–	–	–
19	PWS15.1	NOT SAMPLED														
	PWS15.1C	0.0	77	6	0.0	141	6	0.0	38	2	–	–	–	–	–	–

APPENDIX TABLE 5. Dates when fish were sampled in laboratory experiments for gill histological analysis, liver Cytochrome P-450 enzyme assay, and hydrocarbon analysis. Water, fish food and blank (background) samples also were taken for hydrocarbon analysis. The tanks were set up as follows: tank 1, fish from oiled site and rocks from unoiled site; tank 2, fish from unoiled site and rocks from unoiled site; tank 3, fish from unoiled site and rocks from oiled site. A.p. = *Anoplarchus purpurescens*, P.l. = *Pholis laeta*.

Sampling date	Fish and Water Samples Tank 1	Tank 2	Tank 3	Other samples	Elapsed days of study
6/22/90	6 A.p. 6 P.l.		6 A.p. 4 P.l.	1 blank	0
6/23/90	6 A.p. 1 water	6 A.p. 1 water	6 A.p. 1 water		1
6/25/90	6 A.p.	6 A.p.	6 A.p. 1 water		3
6/28/90	6 A.p.	6 A.p.	6 A.p. 1 water	1 blank 1 food	6
7/5/90	6 A.p.	6 A.p.	6 A.p. 1 water		13
7/12/90	6 A.p.	6 A.p.	6 A.p. 2 water	2 blank	20
7/23/90	6 A.p. 2 water	6 A.p. 2 water	6 A.p. 2 water	2 food	31
8/2/90	6 A.p.	6 A.p.	6 A.p. 2 water	2 blank	41
8/16/90	6 A.p.	6 A.p.	6 A.p. 2 water	2 food	55
10/13/90	8 A.p. 6 P.l. 2 water	7 A.p. 2 water	8 A.p. 4 P.l. 2 water	2 blank	113

APPENDIX III

New S.O.P.s

I. Collection of Samples in Laboratory Experiments

A. Fish Samples

1) Wash glass tubing with soap and water, and rinse with methylene chloride (in Teflon squirt bottle). Rinse the end of the tube that will enter the water (always keep this the same end) with methylene chloride and cover with a methylene chloride-rinsed square of aluminum foil. Connect Tygon tubing to the other end of the glass tubing. Always use one set of tubing for the oily tank and the other for clean tanks.

2) Lower glass tubing into water and blow off foil cover. Using the tubing set-up as a siphon, siphon fish into the glass tube, crimp Tygon tubing to hold them there, and release into a bucket. Always use one bucket for fish from the oily tank and others for fish from clean tanks. Do not touch any surface that will contact fish.

3) Repeat step 2 until 6 fish have been collected from each tank. Rinse end of glass tube with methylene chloride and cover with foil after each siphoning. When finished sampling, wash and rinse glass tube as in step 1.

B. Tank Water Samples

1) Wash and rinse glass tubing and cover end with foil as for sampling fish. Place a stopper with a hole in it in the other end of the tubing. Wash and rinse 2 1-L beakers and 2 1-L separatory funnels in the same manner.

2) Lower tubing vertically into water, dislodge foil from end, and let tubing fill with water. Holding finger over hole in stopper, withdraw tubing. Release water into a cleaned beaker. Repeat until at least 900 ml of water have been collected from the tank. Rinse end of tubing and cover with rinsed foil after each insertion into water. Always use one set of equipment for the oily tank and the other for clean tanks. Do not touch any surface that will contact the water.

C. Fish Food Samples

1) Using a glass or metal implement, cleaned with soap and water and rinsed with methylene chloride, place at least 10 g of fish food into an I-Chem jar precleaned to EPA specifications. Do not touch the sample, any surface that will contact the sample, or the lip of the jar.

2) Label and freeze.

D. Blank (Background) Samples

1) Go through all the motions of filling an I-Chem jar with a sample, under the same conditions, without adding a sample. Label and freeze.

II. Initial Processing and Storage of Laboratory Samples

A. Dissecting Fish Tissues

1) Soak cleaned instruments (large and fine forceps, dissecting scissors, Vannas-type micro scissors, dissecting needles) in a beaker of methylene chloride, in a hood. Rinse aluminum foil with methylene chloride and use to line dissecting tray. Rinse micro-centrifuge tubes with HPLC- grade methanol and keep on ice. Keep I-Chem jars, precleaned to EPA specifications, on ice. Fill scintillation vials with fixative (2.5% glutaraldehyde in pH 7.2 Millonig's phosphate buffer), label lids with sample identification number for gill samples, and keep cold. Fill out labels for individual liver and pooled body samples, and place each in a Whirl-Pak bag.

2) Anesthetize fish with MS-222. Do not touch fish or any surface that will contact fish throughout the following operations. Pick up fish with cleaned forceps, place on foil in dissecting tray and measure total length by holding ruler next to, but not touching, fish. Decapitate fish, drop body into I-Chem jar, replace lid and return to ice. Remove the opercula and place head in a scintillation vial with cold fixative. Keep refrigerated. Dissect out liver and place in microcentrifuge tube. Place in appropriate bag with label and keep on ice.

3) Repeat step 2 for one sample of 6 fish from one tank, pooling bodies in one I-Chem jar. Place I-Chem jar in appropriately labeled bag. Freeze liver and body samples.

4) Repeat above steps with remaining fish.

5) After 2-h fixation of head (gill) samples, replace fixative with cold plain buffer for 1 h, replace with fresh buffer for 1 h, replace with fresh buffer again and leave overnight, refrigerated. Replace buffer with cold 30% isopropanol for 30 min, then 70% isopropanol, label and store, refrigerated.

B. Methylene Chloride Extraction of Water Samples

1) Add 900-ml water sample to cleaned separatory funnel (mark 900-ml level on outside of funnel). Using a methylene chloride-rinsed volumetric pipet, add 50 ml pesticide grad methylene chloride. Shake for 2 min (vent gas frequently, very frequently at first, to avoid pressure build up). Let sit 5 min. Decant dense, methylene chloride layer into I-Chem jar. Repeat 2 times using 25 ml methylene chloride, and adding methylene chloride fraction to same jar, each time.

2) Fill out label as for fish samples, place in bag with sample jar and freeze.

III. Embedding and Sectioning of Gill Tissues

A. Embedding

1) Under a dissecting microscope and with micro instruments, dissect out the second gill arch from the left side with as little damage to the gill tissue as possible.

2) In a hood, post-fix, dehydrate and infiltrate tissue with embedding medium, according to the following steps:

Step	Reagent	Time	Temp.
1	70% Isopropanol	15 min	room
2	30% Isopropanol	15 min	room
3	0.1 M Phosphate Buffer	15 min	room
4	0.1 M Phosphate Buffer	15 min	room
5	1% Osmium Tetraoxide in 0.1 M Phosphate Buffer	90 min	room
6	0.1 M Phosphate Buffer	15 min	room
7	0.1 M Phosphate Buffer	15 min	room
8	25% Ethanol	15 min	room
9	50% Ethanol	15 min	room
10	75% Ethanol	15 min	room
11	95% Ethanol	15 min	room
12	100% Ethanol	15 min	room
13	100% Ethanol	30 min	room
14	100% Ethanol	45 min	room
15	100% Propylene Oxide	15 min	room
16	100% Propylene Oxide	30 min	room
17	1:1 Propylene Oxide: Araldite 502 Resin	45 min	room
18	1:3 Propylene Oxide: Araldite 502 Resin	90 min	room
19	Araldite 502 Resin	overnight	4°C

3) Type labels with sample identification numbers and reduce with a copy machine to the smallest legible size. Place labels in embedding molds.

4) In a hood, cover labels in molds with Araldite 502 resin. Place gill arch in resin next to label, so that it lies flat at the edge of the mold with filament tips pointed outward. Carefully cover gill arch with resin, slightly over-filling the mold.

5) Cure filled molds overnight in an oven at 60oC.

6) Remove embedments from molds and store.

B) Sectioning

1) Under a dissecting microscope, score the embedded gill arch with a sharp razor blade along a line perpendicular to as many gill filaments as possible midway along the length of the arch and about one-third of the distance from the tips of the gill filaments to the base of the arch. Sectioning in the plane made when the tissue block is cut along this line should result eventually, at some depth, in cross sections of filaments and approximate cross sections of secondary lamellae on some of the filaments.

2) Secure the scored block in a vise and, using a jeweler's saw, saw along the scored line, perpendicular to the surface of the embedment. Reposition the embedment in

the vise and saw out the tissue on the other three sides, making a block of the embedded tissue and its label.

3) Clean the block, secure in a specimen holder on a microtome, and trim with a sharp razor blade.

4) On an ultramicrotome with a good glass or diamond knife, cut 1-um sections into a water-filled boat attached to the knife. Clean a microscope slide with 70% isopropanol, label, and place a few drops of 10% acetone on the slide. Transfer sections to the liquid on the slide with an eyelash tool and arrange sections with one end affixed to the slide at the edge of the pool of liquid. Let dry for at least 30 min +on a 60oC hot plate.

5) Stain sections on the hot plate for 20-30 s with 0.2% Azur 1 in 0.2 M pH 8.3 borate buffer. Let dry and mount with a coverslip.

6) Observe the slide in a microscope for the desired result, with secondary lamellae cut in cross-section. Adjust the angle and depth of sectioning until at least six filaments with secondary lamellae in cross-section are observed.

APPENDIX IV

Coastal Habitat Assessment Program - Subtidal Habitats
Standard Operating Procedure - 1990

1.0. Definitions

Habitat type - One of the three habitat types defined by the dominant plants present: *Laminaria / Agarum*, *Nereocystis*, or *Zostera*. The *Laminaria / Agarum* habitat is further subdivided in Island Bays and Island Points.

Study site - An area of coastline to be sampled; within each habitat type study sites may be defined as moderate-heavily oiled, unoiled (control).

Site baseline - A line connecting the endpoints of the study site, approximately 200 m long.

Station transect - A line perpendicular to the site baseline extending from the 0 tide depth out to a depth of 20 m, at locations selected randomly within a study site.

Depth strata - Subsets of the site in various depth ranges.

Sampling stations - Randomly selected points in the depth strata on a station transect.

Sampling transect - A line following the contour to the right of a station transect along which subtidal sampling is conducted.

Quadrat - A 0.25 m^2 (0.5 m by 0.5 m) plot randomly located along a sampling transect.

2.0. Preliminary Study Site Selection

Silled Fjords - A total of eight potential study sites were selected: 2 heavily oiled sites (Arm of Herring Bay and the West arm of the Bay of Isles), 2 moderately oiled sites (Disk Lagoon and Marsha Bay), one lightly oiled site (Louis Bay) and 3 control sites (Northwest arm of Lower Herring Bay, Johnson Bay, and Copper Bay), . We choose all sites within the Knight Island group that were similar in geomorphology to the Herring Bay site where we observed a "Dead Zone" in 1989. All had restricted entrances with an apparent sill, based on examination of hydrographic charts. Heavily oiled sites had over 50% of their shoreline classified as moderately to heavily oiled on the map indicating cumulative impact of oiling through July 2, 1989 (the July map) and had at least 10% of the shoreline moderately to

heavily oiled in September, as per the September "walkathon" data (the September map). The control sites had no oil indicated on either map. We anticipated sampling at all sites over a two week period.

Island Points - Island Points were selected according to the following process. All potential sites were marked on a map. We first drew an outside polygon around the island groups, one around Naked, Storey, and Peak Islands and another around the islands from the northern tip of Eleanor Island to the southern tip of Knight Island. (Smith, Little Smith, Green, Montague, and Latouche Islands were considered part of the outer sound group because of the strong influence of oceanic currents). The polygons were drawn such that any two interior points that were separated by less than 4 km were contained within the polygon. Islands less than 1/2 km in the longest dimension were not considered. The verticies of the polygon were considered as points if the angle formed by lines drawn along the shoreline to 1/2 km in either direction from the vertex was less than 60#.

Points were classified as oiled if there was moderate to heavy oil present within 100 m to either side of the point as indicated in both the cumulative oiling map as of July and the September map. There were 11 such oiled points identified.

We selected 3 oiled sampling sites from these 11 sites. The sites were placed into three groups based on location: Four sites in the northwestern quadrant (1,2,3,and 4), four sites in the southeastern (6,7,8, and 9), and three miscellaneous sites (site 5 on the southwestern side of Knight Island and sites 10 and 11 in the northeastern quadrant).

We then examined the September walkathon data in more detail and gave preference to sites with heavy to moderate oil within 100 m to either side of the point. On this basis, Point # 10 was the selected over points 11 and 5 . The remaining sites within each group were ranked using a random process. Final ranking are as follows:

Area	Selected	Alter. 1	Alter. 2	Alter. 3.
NW	4	2	3	1
SE	7	9	6	8
Other	10	11	5	-

Control sites were selected that were not oiled on both the July and September oil map, and that most closely matched the oiled sites with regard to location.

Island Bays - All bays were examined within the island group from Storey Island south to Knight Island. A bay was defined as a body that was longer (distance from the mouth to the uppermost reaches) than the mouth was wide and that had a length greater than or equal to 2 km. Bays were classified as oiled if at least half of their shorelines were moderately to heavily oiled on the July map and at least 20 % was heavily to moderately oiled on the September map. Five potential oiled bays were identified: Northwest Bay, Herring Bay, Horn Bay, Snug Harbor, and Bay of Isles. We selected Herring Bay as one of our sites to be sampled because it is being used as a base for intertidal and subtidal experiments. We then selected two other sites from a simple random sample of the remaining 4. The 2 selected sites were Northwest Bay and Snug Harbor. Alternate sites ,

in order of preference, as chosen through a random process, were Bay of Isles and Horn Bay.

Sites within bays were selected based on the presence of moderate to heavy oil in the September walkathon (along at least 1/2 km of shoreline), and on the existence of previously established NOAA/DEC sampling sites, at which samples were collected for hydrocarbon analysis in 1989. These generally represent shoreline segments of approximately 500 to 1000 m. Actual sites within these segments will be selected based on physical characteristics of substratum type and slope in a reconnaissance cruise in April, 1990.

Control bays were selected that most closely resembled the oiled bays and that were not oiled in both the July and September oil maps. These were as follows:

Oiled Site	Control	Alter. 1
Herring Bay	Lower Herring Bay	Drier Bay
Snug Harbor	Mummy Bay	
Northwest Bay	Cabin Bay	
Bay of Isles	Mummy Bay	
Horn Bay	Mummy Bay	

Eelgrass - Sites where eelgrass is present within the PWS area were identified by Kim Sundberg, Rick Rosenthal, and the NOAA staff of Chuck O'Clair and Stanley Rice. Oiled eelgrass beds were selected that were indicated as moderately to heavily oiled on both July and September oil maps. This resulted in 9 potential sites. One of these (Perry Island) was eliminated from consideration because there was no adequate control. The other 8 were placed into 3 groups: Group 1 are bowls on the eastern side of the islands, with mouths facing North (site # 2 on Naked Island, site # 3 on Latouche Island, and site # 7 in Snug Harbor). Group 2 is in the northwest quadrant of the Knight Island group (#3 on Disk Island and #'s 4 and 5 in Herring Bay). Group 3 are sites within Bay of Isles (8 and 9). Order of preference for sampling of sites within groups was determined based on the presence of DEC/NOAA sampling sites used in 1989. If hydrocarbon samples were taken at all sites within a group, then sites were selected at random. These were as follows:

Area	Selected	Alter. 1	Alter. 2
Bowls	6	7	2
NW	5	3	4
Bay of Isles	9	8	

Control sites were selected that were not oiled on both the July and September oil maps, that were in the same geographic region, and were of similar aspect and exposure. The control site for Herring Bay (#5) was within Lower Herring Bay, for the Latouche Island site was in Sawmill Bay on Evans Island, and for the Bay of Isles site was in Drier Bay.

Nereocystis - Sites where *Nereocystis* is present within the PWS area were identified by Kim Sundberg and Rick Rosenthal. Oiled *Nereocystis* beds were selected that were indicated as moderately to heavily oiled in both July and September oil maps. This resulted in 5 potential sites: Danger Island, Latouche Pt., Green Island, Smith Island, and Little Smith Island. The sites were placed into 3 groups based on location: Group 1 is Danger and Latouche, group 2 is Green Island, and group 3 is Smith and Little Smith. Danger Island and Smith Island sites were randomly selected as priority sites among groups 1 and 3.

Control sites were selected that not oiled on both the July and September oil maps, that were in the same geographic region, and were of similar aspect and exposure. The control site for Danger Island was Pt. Elrington, for Smith Island was Zaikof Pt., and for Smith Island was Pt. Montague.

3.0. Study Site Confirmation and Site Descriptions

Site confirmation - An aerial and ship based survey of all potential study sites was made in April, 1990. Tom Dean, Rick Rosenthal, and Dave Laur flew the Sound, examined each site from the air to insure that habitat types were correctly defined and that control sites resemble oiled sites with regard to geomorphology. Sites accessible by float plane were visited. Some study sites were marked with a pink paint mark on the shore line. Other sites have distinguishing features that allow sites to be identified. Photographs and/or videos were taken of each site. Those sites inaccessible by plane will be visited by Tom Dean and Troy Tirrell aboard a boat.

Sites Selected - The sites selected in the confirmatory survey, and their site codes are given below. The site numbers used here were reassigned after site conformation visits and do not necessarily correspond to numbers assigned to sites during the preliminary selection phase as described in section 2.0.

Silled fjords - Several of the potential silled fjord sampling sites were visited in April 1990. Most were found to be inadequate because they were too large and did not have sills. The only sites found to fit the prescribed characteristics were the previously sampled site in Herring Bay and the western arm of Bay of Isles. An additional control site was located in Port Audrey Cove, Drier Bay. The uppermost part of Lucky Bay may also be an adequate control.

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Site name	Site #	Oil[1] Code	Hab.[2] Code	Lat.	Long.
Cabin Bay	01	9	1		
Northwest Bay	02	1	1		
Herring Bay	03	1	1		
(Visit Herring Bay Silled Fjord)					
Lower Herring	04	9	1		
(Visit Port Audrey and Italian Bay silled fjords)					
Mummy Bay	05	9	1	60-13.48	147-49.07
Bay of Isles	06	1	1	60-22.98	147-42.62
(Visit silled fjord in Bay of Isles)					
Latouche Point	07	1	4		
Procession Rocks	08	9	4		
Zaikof Point	09	9	4		
Montague Point	10	1	4	60-12.71	147-18.32
Naked Island	11	9	4		
Little Smith	12	1	4		
Bay of Isles	13	1	3		
Drier Bay	14	9	3		
Lower Herring	15	~~1~~ 9	3		
Herring Bay	16	~~9~~ 1	3		
Sleepy Bay	17	1	3		
Moose Lips Bay	18	9	3		
Discovery Pt.	19	1	2		
(Revisit Bay of Isles and Mummy Bay)					
Lucky Point	20	9	2		
Outside L.H. Bay	21	~~1~~ 9	2		
(Revisit Lower Herring Bay)					
Outside H Bay	22	~~9~~ 1	2		
(Revisit Herring Bay)					
Ingot Point	23	1	2		
(Revisit Northwest and Cabin Bays)					
Peak Point	24	9	2		
NE Naked Is	25		3		
NE Storey Is	26		3		

[1]Oil Codes 1 = Oiled
9 = Control

[2]Habitat Codes 1 = Laminaria / Agarum - Island Bays
2 = Laminaria / Agarum - Island Points
3 = Eelgrass
4 = Nereocystis

Several sites and their alternatives were not adequate and new sites were selected. For example, the *Nereocystis* sites at Green Island and Pt. Elrington were deleted because of a lack of *Nereocystis* (on Green Island) or because the site was more exposed than the oiled site (Pt. Elrington). New sites were selected based on previously established criteria.

Site Descriptions - A verbal description of each site follows. Photos and or sketches of some sites are attached in appendix A. A video showing sites not photographed also accompany this SOP.

Site # 01 - Cabin Bay - Naked Island. Site is on northern shore of the southern arm, near the point between the northern and southern arms of the bay. A paint mark was placed on a rock above the high tide mark. The substrate is large cobble and boulder. (Note - there is a cobble beach to the east that may be a good collecting spot for clams.)

Site # 02 - Northwest Bay - Eleanor Island. Site is on the eastern shore of the eastern arm, near a stream in the southern most portion of the bay. A paint mark was placed on a rock above the high tide mark. The substrate is large cobble and boulder. (Note - The site may need to be moved toward the mouth of the bay in order to match the Cabin Bay site better).

Site # 03. - Herring Bay - Site is on the western shore, about 2/3 down the bay, just south of a salmon stream. The site is marked by a regulatory stream marker, 2 points south of the stream. The middle of the site is to be in the middle of the run just south of the 3rd point south of the stream. The substrate is rock outcrop with some boulders.

Site # 04. - Lower Herring Bay. Site is on the western shore near the start of the western arm of the Bay. The site is marked with a paint mark just to the south of a waterfall. Our site is to be centered 2 points (about 300 m) to the south of the mark. The substrate is rock outcrop.

Site # 05 - Mummy Bay - Site is on the northwestern shore of Mummy Bay, about 2/3 up the bay. The site is centered in the middle of a plateau that sticks out into the bay, with water falls on either side. The substrate is mostly cobble and boulder. No marks were made.

Site # 06 - Bay of Isles. - Site is on the southern shore of the bay, near the juncture of the western and southern arms. Center of site is on an outcropping of the eastern most of 2 points, just to the east of a small (30 m long) cobble beach. The substrate at the site is rock bluffs with boulder and cobble. No site marks were made.

Site # 07 - Latouche Pt. Site is on the southwestern tip of Latouche Island. Site center is about 100 m to the west of a small hooked tip off this point. No site mark was made.

Site # 08 - Procession Rocks, Bainbridge Island. Site is near the southern most tip of Bainbridge Island, between the island and Procession rocks. No site mark was made. An on site evaluation and determination of the best match for Latouche needs to be made.

Site # 09 - Zaikof Bay, Montague island. Site is on a Pt. on the southern shore of Zaikof Bay, about 2 km from Zaikof Pt. Site center is on the middle of 3 reefs that run

offshore about 200 m southeast of a regulatory stream marker next to a large white rock on the bluff. No site mark was made. Substrate at the site is reef outcrop with large boulders.

Site # 10 - Montague Pt., Montague Island. Site is on the northwest shore of Montague Island near Pt. Montague. The site is directly offshore of a high bluff, just where the bluff falls off the west into a wooded meadow. The site is marked with pink paint on the roots of a spruce tree that appears to be falling from the bluff into the meadow. The site center is 100 m to the west of a razor back reef directly offshore of the mark. Substrate at the site is reef outcrop.

Site # 11 - Dubois Pt., Naked Island. Site is a small southerly projection on the southeast shore of Naked Island. There are 2 small islands off the tip of the point. The site center is on the southern tip of the eastern most island. No site mark was made.

Site # 12 - Little Smith Point. Site is at the southern most tip of Little Smith Island. Site center is a western tip of a small island off the point. No site mark was made.

Site # 13 - Bay of Isles. Site center is 100 m east of a salmon stream, along the southern shore of the western arm of the bay. The substrate is small cobble and silt. No site marks were made.

Site # 14 - Drier Bay (Northeast cove), Site center is 100 m west of the western most of 2 salmon streams along the southern shore of the cove. Substrate is mixed cobble with softer silt sediment. No site marks were made.

Site # 15 - Lower Herring Bay. Site is at the mouth of a salmon stream near the northern extreme of the western arm of the bay. No site mark was made. Site center is the salmon stream. The substrate is cobble with silt.

Site # 16 - Herring Bay. Site is at the mouth of a salmon stream about 2/3 of the way into the bay on the western shore. Rebar that are painted pink mark an ADEC underwater transect. A site marker (flashing) was placed on a fallen tree just to the north of the site. The substrate at the site is cobble with silt.

Site # 17 - Sleepy Bay, Latouche Island. Site is in the southern most part of the bay, at the mouth of a salmon stream. The site center is the mouth of the salmon stream. Rebar that are painted pink mark an ADEC underwater transect. No site marks were made.

Site # 18 - Moose Lips Bay - Montague Island. Site is in a small embayment due east of the Northern tip of Little Green Island. There are 2 salmon streams at this site. The southern most is an active stream. The northern most dead ends in a marsh behind the cobble berm. The site center is marked with a small buoy placed off the northern most stream, about 200 m from shore. The substrate is mostly silt and sand with some cobble.

Site # 19 - Discovery Pt. Site is on the southern entrance to Snug harbor. Site center is on the northern most extension of the point. No site marks were made.

Site # 20 - Lucky Point (What's it called Pt.) The site is at the western side of the mouth of Lucky Bay. The island is identified by 2 spruce trees. The site center is on the northwestern most point on the island. No site marks were made.

Site # 21 - Outside L.H. Bay (Pt Lyman control). Site is centered on the southernmost island off the point. The site center is on the northwestern most point on the island. No site marks were made.

Site # 22 - Outside Herring Bay (Pt. Lyman). Site is on the western most island off the point, with the site center at the western point of the island. No site marks were made.

Site # 23 - Ingot Pt. Site is on the southern most point of Ingot Island. Site center is at the middle of the largest island off the point, just west of three smaller islands. No site marks were made.

Site # 24 - Peak Pt. Site is on the northern most point of Peak Island. Site center is at the center of the long axis of the largest of three island off the point. No site marks were made.

4.0 Sampling in silled fjords

An abbreviated survey of silled fjords will be conducted in May and June, 1990. Four sites will be visited and sampled (Herring Bay, Port Audrey Cove, Lucky Bay, and Bay of Isles). Estimates of density of infaunal invertebrates will be obtained from 6 benthic airlift samples (0.1 m^2) taken by divers at random positions along the 20 m depth contour in each of the 4 bays. A small boat with a fathometer will cruise the bay along three transects. The first will extend from the mouth of the bay, along its axis and to the uppermost reaches. A second will be run perpendicular to the first, and a third will be run that bisects the first two. A buoy will be placed at the first and last sounding of 20 m experienced on the transect. A diver will then go to the bottom and place a 0.1 m^2 frame 3 m from the buoy anchor in a predetermined random compass direction from anchor. The sediment within the frame will be sampled to a depth of 10 cm.

A second diver will characterize the bottom using a video. A 20 m transect will be videoed at each sampling site.

Two sediment samples will be taken at each station: one for hydrocarbon analysis and the other for grain size analysis. Each sample will be collected from within 3 m of the buoy anchor. A sample for hydrocarbons will be collected in a wide mouth jar (precleaned ICHEM) by scraping the top 5 cm from the surface of the substrate until the jar is half to three quarters filled. A sample for grain size analysis will taken in the same manner. In addition, at the second sampling station, a sample of the water 0.5 m above the bottom will be taken for hydrocarbon analysis.

A sample of any dead animals will be made (within the limits of safe diving - <20 meters) at each infaunal sampling station within the bay. An attempt will be made to sample at least 5 worms and 1 starfish (the most abundant dead organisms observed in 1989), and any dead fish observed at each sampling station. The invertebrates will be collected and frozen for hydrocarbon analysis. Fish will be dissected and preserved for histological/hydrocarbon analysis.

Upon return to the ship, lids to the hydrocarbon sample jars will be loosened and some water poured off, leaving a 2 cm headspace in order to prevent breakage upon freezing. A water sample will also be collected and analyzed for salinity.

A temperature, salinity, and dissolved oxygen profile will be made in each bay by lowering a temperature/dissolved oxygen probe into the water at the deepest part of the bay and measuring oxygen and salinity at every 1 m interval.

In the Fall, a more extensive survey will be conducted at these, and possibly 2 other sites. Upon arrival at the study site, a bathymetric survey of the bay will be made using a portable fathometer aboard a small inflatable boat and radar aboard the mother ship. Three survey lines will be run. The first will extend from the mouth of the bay, along its axis and to the uppermost reaches. A second will be run perpendicular to the first, and a third will be run that bisects the first two. Depth measurements will be made every 20 m along each line.

We will characterize the bottom using a diver held video camera. Three video transects will be made: One along the long axis of the bay, one along the short axis and through the deepest part of the bay, and one that bisects the short and long axis. The diver will swim along a compass course.

Sampling of sediments and infauna will be the same as in the Spring survey.

5.0. Stratified sampling in Laminaria habitats in Island Bays and on Island Points

5.1 Station set up

A. Locate the center of the study site. Drop buoys approximately 100 m to either side of the marker. (In cases where the habitat does not extend for 100 m on either side of the center, the distance may be reduced to 50 m). Drop the buoys so that a line connecting them is parallel to the site baseline. Start the skiff approximately 25 m to the right of right buoy and approach the buoy at a constant rate of speed. (Determine left versus right when facing the site from the sea). Record the time required to cover the distance from approximately 30 m from the right buoy to the left buoy. Do not vary the speed of the boat during this operation and maintain a constant distance from shore.

B. Divide the time by 3 (e.g., 7.2/3 = 2.4 Min). Select a random number on the calculator and multiple the two values (e.g., 2.4 X 0.8978 = 2.15 min). Add (Total Time)/3 to the result and 2 (Total Time)/3 to the result. For example, 2.15 min, 2.15+2.4 = 4.55 min, and 4.55+2.4 = 6.95 min are the random starting points at which to start the station transects when measured from the left hand side and traveling at the same speed. Buoys to mark the starting point of each station transect will be dropped at 2.15 min, 4.55 min, and 6.95 min when measured from the left hand side of the site.

C. At each station, a small boat will be driven seaward from nearshore along a course perpendicular to the site baseline, dropping marker buoys at randomly preselected depths in each of the depth strata. The original buoy marking the station transect will be retrieved

after the marker buoy is dropped in the first depth stratum. The protocol for random selection of positions for the buoys is: (1) for each station transect select a random number (proportion) between 0.0 and 1.0, (2) multiply the range of depth in each strata by the proportion. For example, if the random proportion is 0.35, the depth (D) in the two depth strata would be:

2-11 m D = 0.35 x 9 m + 2 m = 5.2 m

11-20 m D = 0.35 x 9 m + 11 m = 16.2 m

All depths should be corrected to mean low low water using output form TIDE.1 software for the region closest to the sampling site.

5.2. Censusing Fish Populations

Two divers swim to the bottom at the deepest of the two marker buoys. Diver # 1 attaches a 30 m fiberglass transect tape to the anchor and swims a 30 m isobathyal sampling transect to the right. The diver visually counts fish, by species, within 2 m of the transect line and within 3 m of the bottom. Non-cryptic specimens of echinoderms and crustaceans larger than 10 cm will also be counted in this 2 m by 30 m band.

Diver # 2 swims along the sampling transect from the buoy anchor recording a 2 m by 30 m video transect pointing the camera down toward the substrate.

After a 2 minute wait 3 m off the end of the transect, the divers swim side by side with the transect line between them each diver counting the number of benthic fishes within a 1 m band on their side of the transect tape. An attempt will be made to count all individuals of length 5 cm or larger.

Following completion of the deepest transect the two divers will move up to the next shallowest marker buoy and repeat the procedure. Identical procedures as described above for the deepest sample transect will be followed at the sample stations in the shallower depth strata.

5.3. Sampling Plant and Epifaunal Invertebrate Populations

Following the fish subsampling at a sampling transect two divers (#'s 3 and 4) swim down to the marker buoy anchor. At randomly preselected locations on the sampling transect, diver #3 places four large (0.25 m^2) quadrat frames, with the upper left hand corner of the frame on the specified random points.

The quadrats are place on the shoreward side of the sampling transect.

The random positions for the upper left corner of the large quadrats are determined by the following protocol. Multiple 7 m by a random number between 0.0 and 1.0 (proportion) to find the point for the upper left hand corner of the quadrat in the 7.5 m segments with the zero end of each section being closest to the marker buoy. The segment length is reduced from 7.5 m to 7 m before multiplying by the random proportion it insure that the resulting quadrats do not extend off the 30 m sampling transect. For example, if

the random proportion is 0.26, the four quadrat locations on the 30 m sample transect would be 1.82, 9.32, 16.82, and 24.32 m.

Diver #3 estimates the amount of algal cover in each of the large quadrats. Diver #3 then clears all macroalgae from each large quadrat, placing the cut pieces in labeled mesh bags. The algae are to be clipped 5 cm above the substrate. Laminarian algae smaller than 5 cm are counted. The smaller algae (including small laminarians, leafy reds, and encrusting forms) are to be collected from 1 quadrat at each sampling transect (see invertebrate sampling procedures below). Diver #4 photographs each quadrat using a camera with a 28 mm lens. Six frames are required in order to photograph the entire 50 by 50 cm area in each quadrat. The sequence of photographs in each quadrat is as reading a book, i.e. starting in the upper left hand corner and moving from left to right, when facing the 30 m end of the transect.

Aboard ship, algal samples are separated by species, counted, patted dry. Each plant is weighed individually and it's weight recorded. The reproductive condition (either with or without evident sori) of each Laminarian species is noted.

Samples of representatives of each canopy species are preserved by placing them in labeled jars with 5% formalin.

5.4. Estimation of Population Parameters for Infaunal Invertebrates

Following completion of the photographic quadrats and algal sampling, two divers (#5 and 6) will go to the sampling transect which still has the 30 m tape and the four large quadrat frames in place. Diver #5 swims the tape until reaching the first quadrat frame and continues to swim until reaching a patch of soft substrate (silt, sand, or small gravel with depth greater than 5 cm.) larger than 0.1 m^2. Diver #5 then vacuums all material within a 0.1 m^2 frame placed in the center of the patch, to a depth of 10 cm, using an airlift sampler. Diver #5 then swims to the second quadrat and repeats the procedure. (Only 2 small quadrats are sampled for benthic infauna at each station transect). Diver #6 collects sediment samples for hydrocarbon and grain size analyses and then roles up the tape and collects the quadrats on the return swim. At one station per site (#2) Diver #6 also collects a water sample from 0.5 above the bottom for hydrocarbon and salinity measurement. On board ship all airlift samples will be preserved in 10% buffered (sea water) formalin.

5.5. Physical measurements

Salinity and temperature will be measured at the middle sampling transect within each depth stratum. Measurements will made at depths of 0.5 m below the surface, 2 m below the surface, and 0.5 m above the bottom using a YSI temperature/salinity meter.

6.0. Stratified sampling in Nereocystis habitat

6.1 Station set up

All *Nereocystis* sampling sites will be marked with a single paint mark on the shore at the center of the site. Set sampling locations as follows: Locate the center marker of the

study site. Drop buoys approximately 100 m to either side of the marker. Drop the buoys so that an imaginary line connecting them is parallel to the site baseline, just offshore of any visible kelp canopy. Start the skiff approximately 25 m to the right of right buoy and approach the buoy at a constant rate of speed. (Determine left versus right when facing the site from the sea). Record the time required to cover the distance from approximately 30 m from the right buoy to the left buoy. Do not vary the speed of the boat during this operation and maintain a constant distance from shore.

Repeat steps B and C as described in 5.1 above to establish 3 stations at each of 2 depth strata.

Divers #1 and 2 enter at the buoy on the outer margin of the kelp canopy and drop to the buoy anchor. They then swim a tape from the buoy on a compass course perpendicular to shore until no more canopy forming kelps (*Nereocystis*) are observed. The distance from the buoy to the inner margin of distribution for kelp canopy species is recorded. The divers then swim back the tape to 1/2 the distance to the buoy and mark the station with a pop float. This process is repeated for each transect station, establishing 3 stations per site in the center of the distribution of *Nereocystis*.

6.2 Sampling fish, plants, and invertebrates

Fish, plants, invertebrates, and sediments are sampled at each station as described for *Laminaria* habitats with the following additions.

Along the three station transects within the center of distribution of *Nereocystis*, divers #2 and 3 will count all *Nereocystis* within a 2 m wide band on either side of the transect, and will measure diameters of the stipes of the first 20 *Nereocystis* encountered on each transect. All plants will be measured at a height of 1 m above the bottom. Divers #5 and 6 will obtain an independent sample of 40 *Nereocystis* (at 1 oiled and 1 control site only), and these plants will be weighed and measured to establish a regression between stipe diameter, length, and weight of the plants. These are to be collected outside of the sampling transects, but within 100 m and at the same depths as the sampling transects.

Also, on each of the 3 sampling transects within the *Nereocystis* canopy, canopy fishes will be counted along a 2m x 30 m band at a depth of 2 m. An attempt will be made to count all fish in a 2 m by 3 m column parallel to the surface. Sampling for fishes will be conducted at least 1 hr. after all other survey work has been completed.

7.0 Stratified sampling in eelgrass habitat

7.1 Station set up

All eelgrass sampling sites will be marked with a single paint mark on the shore at the center of the site. Set sampling locations as follows: Locate the center marker of the study site. Drop buoys approximately 100 m to either side of the marker. Drop the buoys so that an imaginary line connecting them is parallel to the site baseline, just offshore of any visible eelgrass. (Snorkeling may be required to identify the outer margin of the eelgrass bed). Start the skiff approximately 30 m to the right of right buoy and approach the buoy at a constant rate of speed. (Determine left versus right when facing the site from the sea).

Record the time required to cover the distance from approximately 30 m from the right buoy to the left buoy. Do not vary the speed of the boat during this operation and maintain a constant distance from shore.

Repeat steps B and C as described in 5.1 above to establish 3 stations at each of 2 depth strata, 3 to 6 m and 6 to 20 m.

Divers #1 and 2 enter at the buoy on the outer margin of the eelgrass bed and drop to the buoy anchor. They then swim a tape from the buoy on a compass course perpendicular to shore until no more eelgrass is observed. The distance from the buoy to the inner margin of distribution eelgrass is recorded. The divers then swim back the tape to 1/2 the distance to the buoy and mark the station with a pop float. This process is repeated for each transect station, establishing 3 stations per site in the center of the distribution of eelgrass.

7.2 Sampling fish

Within each of the 3 strata on each transect, divers will establish three 30 m long transects running parallel to shore. Divers #1 and 2 enter the water, lay out the tape, and sample fish and large motile invertebrates as described in section 5.2. (Note - A video may be required only on transects within the eelgrass bed).

7.3 Sampling motile invertebrates associated with eelgrass

Motile invertebrates associated with eelgrass will be sampled only along the 3 station transects that lies within the eelgrass zone. A 500 mesh plankton net, with an opening 1 m in diameter, will be towed behind a small boat running at the lowest possible speed. The net will be towed through the eelgrass for a distance of approximately 30 m at each of 3 station transects. The total volume passing through the net will be determined using a flow meter attached to the net. The contents of the net will be placed into a sample jar, preserved with buffered formalin, and labeled.

7.4 Sampling eelgrass

Eelgrass will be sampled only along the sampling transect that lies within the eelgrass zone. Divers #3 and 4 will harvest all eelgrass from each of the 4 quadrats per depth stratum. The turions (blades) of the plants will be cut approximately 1 cm above the sediment surface. The plants will be bagged underwater and returned to the boat. There, the number of turions per quadrat will be counted, and all turions in each quadrat weighed. In addition, we will note the number of flowering stalks per quadrat, and count the number of seeds per stalk in the first 10 seed stalks encountered per quadrat. (Note - no photographs will be required in the eelgrass habitat.

7.5. Sampling infaunal invertebrates

Infaunal invertebrates will be sampled in a 0.1 m^2 airlift sample from each of first two quadrats per station transect. Station transects to be sampled include both those within the eelgrass zone and those outside of the eelgrass. Two sediment samples will be taken to a depth of 5 cm at each sampling transect. One will be used to determine grain size and the other to determine hydrocarbon concentrations.

8.0. Special notes on sample collection for sediments, water, and fish.

8.1. Collecting fishes for food habits, condition factor and hydrocarbon concentration studies.

Following completion of the above sampling, a collection of fishes will be made to assess diets, condition factor, and tissue hydrocarbon levels. An attempt will be made to maximize collection of two species: (1) a commonly occurring benthic feeding species (kelp or whitespotted greenling if possible) and (2) a commonly occurring midwater feeding species (dusky=planktivore or black=piscivore if possible). Twenty-25 individuals of each species are desired from each site.

Fish will be collected at sites at least 50 m from the nearest sampling transect if possible.

Techniques including diver spearing, hook and line fishing, and diver operated hand nets will be used in an attempt to collect fish.

Collected fishes will be measured (fork length) and weighed. Selected tissues and/or organs will be removed and treated as specified in the documents detailing collection and handling of samples for hydrocarbon analyses (State/Federal damage assessment plan, analytical chemistry, collection and handling of samples, August 9, 1989, Auk Bay Lab Attorney Work Product). Tissue samples and organ samples should consist of 1 g per fish for 15 fish. Their stomachs will then be excised and fixed in 10% formalin.

8.2. Collecting sediment and bottom water samples.

Two samples of sediment will be collected at each station transect, 3 m to the right of the buoy anchor. One sample will be used to determine hydrocarbon levels and the other to determine grain size. The following protocol will be followed: Collect sediment by scooping directly into the opened sample container. Scoop to a 5 cm depth to obtain 10-100 g of sediment (equivalently 4 oz. jars will be filled to just below the shoulder). If sediment is not readily available at the point then collect the closest available material, including small rocks and organic material, along the sampling transect avoiding the locations of study quadrats for plant and animal collections. After returning the jar to the surface, loosen the lid and pour off approximately 2 cm of water to allow room for expansion of the sample upon freezing.

A water sample will be taken at a depth of 0.5 m above the surface at the middle sampling transect within each depth stratum.

All sediment and water samples are to be numbered sequentially, tagged, logged, sealed with evidence tape, and frozen.

All samples collected in the procedures described above will be handled and documented as specified in the protocols for sample accountability and chain of custody described in Coastal SOP No. 3.

9.0 Experiments evaluating reproductive success

9.1. Site selection

All experiments will be conducted at 1 oiled and 1 control site. These will be either in the *Laminaria* habitat within island bays or within eelgrass habitat. The oiled sites will be selected at random from the 3 oiled sites used in the stratified sampling program. The control site will be the location matched with that site.

9.2. Design

- Species and collection sites

Experiments will be conducted with 3 invertebrate species (The blue mussel, *Mytilus edulis*, One of two clam species, *Prototheca*, and the starfish *Dermasterias*) and two plant species (a kelp, *Laminaria saccharina* or *Agarum cribrosum*, and eelgrass, *Zostera marina*). Mussels, starfish, and kelp will be collected from sites used in stratified sampling for *Laminaria/Agarum* habitat in island bays. Clams and eelgrass will be collected from eelgrass habitat.

- Invertebrate experiments

Twenty individuals of each species will be collected from each of 3 stations per site. We will collect individuals of approximately equal size. Mussels and clams will be collected from 1 m below mean low low water and starfish from 10 m below mean low low water. All samples will be returned to the University of Alaska Marine Laboratory in Seward for analysis. Samples will be collected from oiled and control sites on the same day and flown immediately to the laboratory.

One randomly selected individual per station (3 per site) will be sampled for hydrocarbons. Six individuals per station (18 per site) will be dissected, their body weight determined, and their gonads weighed. The ovaries from 3 females per station (9 per site) will then be fixed, stained, and sectioned for histological analysis. We will determine the developmental stage and the diameter of 100 oocytes per individual.

The remaining 13 individuals from each station will be spawned into individual containers. The eggs from 3 randomly selected females per station (9 per site) will be fertilized with the pooled sperm of 3 randomly selected males per station. Fertilization will take place in containers with filtered seawater placed in a controlled temperature room held a 10 #C. For starfish, 100 eggs will be sampled after 1 hour and the proportion of fertilized eggs noted, as evidenced by the presence of a fertilization membrane. For both starfish and bivalves, 100 individuals per container will be sampled after 48 hours and the proportion of normal larvae noted. A sample of 10 individuals per container will be preserved for later cytogenetic analysis. The cytogenetic analysis will consist of scoring 10 embryos per individual for chromosomal aberrations or the formation of micronuclei.

- *Laminaria/Agarum* experiments

Ten individuals of approximately equal size will be collected from a depth of 10 m at each station (30 individuals per site). The plants will be returned to the laboratory in Seward where the area of the blade and the area of the sorus will be measured. The plants will then be placed into 1 liter jars with filtered seawater and after 1 hour, the number of

spores released per plant will be determined. Spores from 3 randomly selected plants per station (9 per site) will be used to make inoculation solutions of known spore density. A separate solution will be made for each plant. The solution will be added to petri dishes (one dish per plant) containing a glass slide. The dishes will be placed in an incubator and held a 10° C and 45 $\mu E/m^2/sec$ of light (continuous exposure). After 48 hours, the slides will be removed and 100 spores examined for germination success.

- Eelgrass experiments

Fifty eelgrass seeds will be sieved from the sediment at each of 3 stations per site. Quantitative airlift samples will be used for seed collection, if possible, in order to obtain an estimate of the density of seeds in the sediments. The seeds will be returned to the laboratory (in Seward) and placed into Petri dishes with filtered seawater (10 ppt) and held in a temperature controlled room at 10° C. After 1 week, and at daily intervals for the next two weeks, we will determine the number of seeds germinating. All germinated seedlings will be preserved for possible examination of cytogenetic effects.

- Sampling frequency

All organisms used in these studies will be sampled once in 1990. The exact timing of experiments will depend on the reproductive condition of animals and plants. Animals will be checked at the beginning of the study period until sexually mature individuals are present. Based on existing literature, we anticipate that the invertebrates will be at a reproductive peak in late April or early May, that eelgrass will have its peak seed set in late July, and that *Laminaria/Agarum* will be at its peak in August.

10.0 Experiments evaluating germination success of eelgrass seeds in oiled and unoiled sediments.

10.1. Site selection

All experiments will be conducted using sediments collected from 1 oiled and 1 control site within the eelgrass habitat. The oiled site will be selected at random from the 3 oiled sites used in the stratified sampling program. The control site will be the location matched with that site.

10.2. Design

Twenty-one sediment cores (7 per station) will be collected from each site. These will be immediately transported (on ice) to the laboratory in Seward. Twelve of the sediments cores will be placed undisturbed into petri dishes (one dish per core) and placed into a flowing seawater bath. Nine cores will be sieved and placed into Petri dishes.

Four hundred fifty eelgrass seeds will be collected from a control site. The seeds will be taken to the laboratory and placed into petri dishes containing sediments. Twenty-five seeds will be placed in each of 18 dishes per site. The remaining three petri dishes (containing unsieved sediments) per site will be used as germination controls to evaluate the presence of naturally occurring seeds. The presence of germinating seeds will be noted daily for a period of 21 days. Any germinating seeds will be removed three days after

germination and preserved for possible cytogenetic analysis. There have been no previous attempts to evaluate cytogenetic effects in eelgrass seedlings, so an initial screening of samples will be performed prior to full analysis. At the end of three weeks, a sediment sample will be taken from each dish and preserved for possible hydrocarbon analysis. All sediments in the previously unsieved sediments will be sieved and the number of ungerminated seeds determined.

11.0 Settling experiments

11.1. Site selection

All experiments will be conducted at 3 oiled and 3 control sites. These will be in *Laminaria/Agarum* habitat within island bays. The sites will be the same as those used for the stratified sampling program.

11.2. Design

Nine settling surfaces (tiles) will be placed at a depth of 7 m within each site. The tiles will be attached to rebar driven into the bottom and held in a vertical position, with faces parallel to shore, at a depth approximately 10 cm above the bottom. The rebar are to be laid along a line running parallel to shore with rebar spaced at 2 m intervals or greater. The site will be located in the approximate center of the sampling site. The location of the tiles is marked with a small surface float and with 3 subsurface floats spaced at 10 m intervals. The position of the buoy is triangulated using shoreline features and markings (if necessary) and these features are photographed to facilitate relocation of the site if the surface buoy is lost.

After 3 months, the tiles will be photographed and the number of algal sporelings and large benthic invertebrates will be counted. The tiles will be collected and preserved for latter quantification of the number species, and number of individuals (or percent cover) of each species.

12.0. Agarum growth experiment

12.1. Site selection

All experiments will be conducted at 3 oiled, 3 control sites. These will be in the *Laminaria/Agarum* habitat within island bays. The sites will be the same as those selected in the stratified sampling program.

12.2. Design

At each island bay site, divers #3 and 4 will enter the water on the temporary buoy used to mark site for settling substrates. Diver #3 will tag 30 *Agarum* plants at each station. The plants will be the first 30 plants observed past the 3 m mark along the transect tape that are between 70 and 90 cm in length. The tape is laid from the buoy to the right (facing shore) and runs parallel to shore. If there are other *Agarum* or *Laminaria* of 70 cm or larger within 25 cm of the selected individual, the surrounding plant(s) will be removed (in

order to eliminate potential confounding effects of competition) and additional plants will be selected. Plants will be double tagged by tying numbered surveyors flashing around the stipe of each individual and by placing a numbered tag on a steel spike next to each plant.

Diver #4 will follow Diver # 3 and measure the total length of each tagged plant and will place a small piece of flashing in a hole in the blade of each plant at a distance of 10 cm from the base of the blade.

After the plants are tagged and measured, an additional 30 plants will be collected, measured and weighed to obtain a regression of length vs. weight.

After 2 months, the tagged plants will be collected, weighed, and measured. The distance from the base of the blade to the whole will also be measured. Differences in initial weight (estimated by a length weight regression) and final weight will be used to estimate net production (total growth - tissue lost to sloughing/grazing). The differences in distance of the hole from the base of the blade during initial and final measurements will be used to estimate relative gross production.

13.0 Data Analysis

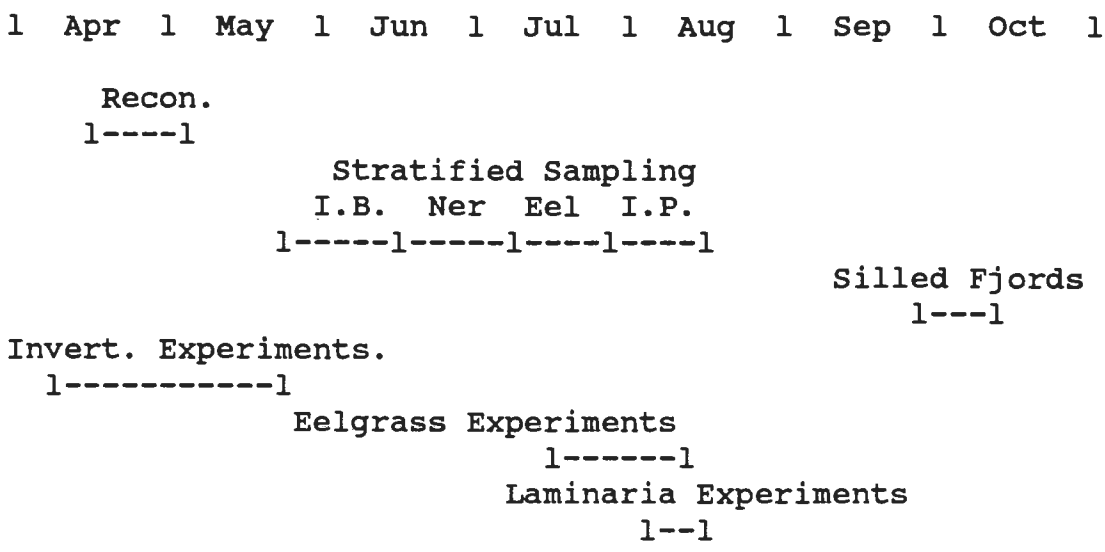
All data will be entered and stored in an "INGRESS" database at the University of Alaska, Fairbanks. Data analysis will be supervised by Dr. Lyman McDonald.

The generic form of analysis for all data gathered will be a comparison of oiled vs control sites using t-tests or nested analyses of variance. In studies where more than one site is sampled, sites will be the primary sampling unit, with various degrees of subsampling within a site. For some experimental studies, there will be no replication of sites, and the primary sampling unit will be stations within sites.

14.0 Schedule

The field schedule for the subtidal studies is given in Figure 1.

Figure 1. Sampling schedule for subtidal studies.



I.B. = Island Bays
Eel = Eelgrass
Ner = Nereocystis
I.P. = Island Points

APPENDIX V

DRAFT

STUDY TO DETERMINE THE ABILITY OF THE BORSTAD ASSOCIATES LTD. COMPACT AIRBORNE SPECTROGRAPHIC IMAGER (CASI) TO DETECT INJURIES TO FUCUS

FINAL REPORT

November 21, 1990

Litigation Sensitive
Attorney Work Product
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SUMMARY

Following the Exxon Valdez oil spill (EVOS), the Alaska Department of Fish and Game (ADF&G) along with other state and federal agencies has undertaken the Coastal Habitat Injury Assessment (CHIA) in the Prince William Sound area. Because of the inherent spatial variability in the coastal zone, there was a requirement to investigate assessment methods based on remote sensing. None of the existing satellite sensors have sufficient spatial resolution to adequately sample the narrow beaches, while aerial photography does not have the spectral resolution or digital capability required. For these reasons, the Borstad Associates Ltd. Compact Airborne Spectrographic Imager (CASI), being a small, flexible state-of-the-art remote sensing instrument, was of considerable interest. In this context, G.A.Borstad Associates Ltd. of Sidney, British Columbia, Canada was contracted to provide professional remote sensing and data analysis services to ADF&G in order to test the utility of CASI for this purpose. The project involved acquisition and analysis of remote sensing data from Borstad Associates' CASI over several oiled and unoiled beach pairs during July and August of 1990. The sites were chosen by ADF&G as representative of sheltered rocky and exposed rocky habitats and are currently being intensively sampled and studied as part of the CHIA.

The purpose of the project was to determine whether oil induced injuries to the marine brown alga Fucus and other marine plants can be accurately detected using the CASI and if so, whether these injuries can be quantified and mapped on shoreines throughout the EVOS area. The objectives were:

1. to determine whether the CASI can detect significant differences between the reflectance spectra of in situ Fucus and other marine plants at oiled and unoiled sites.

2. to determine whether the CASI data can be correlated with quantitative data being collected by the CHIA including estimates of percent cover and biomass of Fucus.

3. to determine the feasibility of using the CASI to detect and quantify oil induced injuries to Fucus and/or other marine plants that exhibit injury at additional CHIA study sites and other shorelines in the Prince William Sound, Kenai Peninsula and Kodiak/Alaska Peninsula regions.

The field work for this project took place on two field visits in late July and early August 1990. On the first visit to Herring Bay, poor weather prevented collection of any useful airborne data, but spectral data was obtained for plant samples taken from 6 experimental beaches. On the second visit, the weather was again poor, but airborne image data was obtained which has been processed successfully even though signal levels were very low and signal to noise ratios were therefore low. The poor weather, extended field time, and poor quality data requiring more manual processing have driven us over budget and this analysis is not as extensive as it might otherwise have been. Delays in obtaining in situ transect data from other CHIA teams has also meant that objective 2 can not be addressed. However, some in situ inspections were conducted by Dr. Larry Deysher of Coastal Resources Associates, California as part of a separate contract. He carried photographs of true-color CASI

images made for sites 1522 and 1522C to the beaches and annotated the photographs while on the sites, thus providing interpretive confirmation. A simple ratio calculation made from the image data for the locations he visited correlates well with his estimates of percent Fucus cover.

Relevant to the objectives above, we present here spectral data to confirm that CASI can distinguish Fucus from other classes of vegetation through differences in their color and pigmentation. Experimental spectral data collected on samples from 1522 and 1522C also suggests that Fucus from oiled sites was brighter overall than the control sites. This agrees with anecdotal reports from CHIA workers at the barge and our own visual observations, that the oiled beaches had younger, brighter yellow plants. Brighter Fucus zones are also seen in the image data, and these have been confirmed to represent bright yellow zones of younger more upright plants with more receptacles and darker areas of predominantly older more recumbent plants exhibiting more of the dark brown stipe.

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ACKNOWLEDGEMENTS

We would like to acknowledge Mr. Fritz Funk, Alaska Fish & Game Commercial Fisheries Division, Juneau who indirectly introduced us to Kim Sundberg of the Habitat Division. Kim was the recipient of this work and organized most of the field logistics. He and Dr. Larry Deysher were very patient in explaining coastal biology to an uninitiated biological oceanographer. Dave Erbe and the pilots of Cordova Air Limited did a great job of flying in difficult weather and in the many tight spots, in spite of the demands we made on them for straight and level lines. Dave and Judy Jesson provided very comfortable living conditions on the Camp David barge and were very helpful. Lynne Armstrong assisted with some aspects of the data analysis and report preparation.

1. INTRODUCTION

Following the Exxon Valdez oil spill (EVOS), the Alaska Department of Fish and Game (ADF&G) along with other state and federal agencies, has undertaken the Coastal Habitat Injury Assessment (CHIA) in the Prince William Sound area. Because of the very great natural variability of the coastal zone, there was a requirement to investigate assessment methods based on imaging sensors which provide greater coverage than spot or transect sampling techniques. Until recently, conventional aerial photography was usually used to extrapolate from in situ data. Photographs have very high spatial resolution (fine detail can be seen), but this detail is difficult to quantify and boundaries must be digitized to calculate areas and summarize variability. With the growing realization of the power of digital analysis methods using Geographic Information Systems, techniques based on photographic film are being replaced by those utilizing digital imagery from airborne or satellite sensors. At present, none of the present civilian satellite sensors have sufficient spatial resolution to adequately sample the narrow beaches (the American LANDSAT Thematic Mapper has a resolution of only 30 m; the French SPOT sensor has 20 m spectral bands and a 10 m pan-chromatic band). The temporal repeat cycle of these sensors (near noon on 2 consecutive days of every 16 days) is also inadequate to catch low tide on a clear weather day. For these reasons, the Borstad Associates Ltd. Compact Airborne Spectrographic Imager (CASI), being a small, flexible state-of-the-art airborne remote sensing instrument capable of being flown on small locally chartered float aircraft, was of considerable interest.

Therefore, G.A.Borstad Associates Ltd. was contracted to provide professional remote sensing and data analysis services to ADF&G in order to test the utility of CASI for determining vegetative cover on beaches in Prince William Sound. The project involved acquisition and analysis of remote sensing data from Borstad Associates' CASI over several oiled and unoiled beach pairs during July and August of 1990. The sites were chosen by ADF&G as representative of sheltered rocky and exposed rocky habitats and are currently being intensively sampled and studied as part of the CHIA.

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The project was of short duration and limited scope, without rigorous replication required for statistically defensible hypothesis testing.

2. METHODS

2.1. THE INSTRUMENT

The Borstad Associates Ltd. Compact Airborne Spectrographic Imager (CASI) is an imaging spectrometer providing non-contact measurements of color in spectral and image form. In its 'spatial' or image mode, the instrument makes digital multispectral images, which can be manipulated and enhanced by computer, enabling multi-spectral classification, simple area calculations and other mathematical operations. In 'spectral' mode, the instrument operates as one or more (up to 39) separate spectrometers, with a 1.8 nm spectral resolution across the range 423 to 946 nm.

The CASI is manufactured commercially by Itres Research Limited of Calgary (Borstad and Hill, 1989; Babey and Anger, 1989) and represents the second generation of Canadian imaging spectrometers which began with the DFO Fluorescence Line Imager (Borstad, 1985). Compared to more conventional mechanical scanners, such imaging spectrometers exhibit increased signal to noise and high radiometric sensitivity because of the longer dwell times possible through 'pushbroom imaging'. Instead of one sensor sweeping across the field of view for each line of the image, pushbroom devices have many sensors operating in parallel and each views the target longer. This considerably increases their signal-to-noise characteristics, and together with 12 bit or 16 bit recording gives them much higher dynamic range than conventional scanners. The imaging spectrometers also offer much increased flexibility in spectral band selection over both scanners and pushbroom imagers since they use diffraction gratings and two dimensional detector arrays in place of optical filters and separate detectors.

CASI uses as its sensing elements a UT104 CCD Frame Transfer Array manufactured by the English Electric Valve Company of Chelmsford, England. The CCD is a two dimensional array of light sensitive elements, on which the charge accumulating on each element is proportional to the integrated light intensity. The charges can be transferred to the neighbouring elements (and therefore by sequential transfers, off the array to a read-out amplifier) by application of external control signals. The CCD array is illuminated so that across-track spatial information falls along its long dimension (512 of the 612 elements are illuminated), while spectral information for each spatial pixel registers across the shorter (288 element) dimension (figure 1). The array provides spectral resolution of 1.8 nm over the range 423 to 946 nm and across-track spatial information in the form of a 512 element push-broom array. The instrument specifications are summarized in Appendix 7.1. More details of the instrument are given by Borstad and Hill, 1989; Borstad et al., 1989; and by Babey and Anger, 1989.

In 'spatial mode' the instrument operates like many other remote sensing imagers and performs high spatial resolution mapping by forming push-broom images in up to 15 spectral bands. However,

unlike more conventional systems in use today, the placement and width of spectral bands are selectable through computer software in steps of 1.8 nm. In 'spectral mode', the device provides one monochromatic image referred to as a Track Recovery Image, as well as spectral data for up to 39 columns of the image. In this mode the instrument can be thought of as operating as a variable number of spectrometers, each with resolution of 1.8 nm. The Track Recovery Image and the spectral data are coregistered and the combination allows precise geo-location of the spectral data with respect to targets on the ground or sea. All operating parameters, such as number, width and spectral position of bands or 'spectrometers' are programmable. The nominal field of view of the instrument across the direction of motion is 35^{o} and is spread across 512 of the 612 spatial pixels of the detector; the unilluminated detectors are used for recording the dark current and electronic offset. A 12-bit A/D converter is used internally and a 16 bit dynamic range is achieved by digitally summing the spectral elements within a band. Data are recorded digitally on compact 8 mm video cassette tape with 1 Gigabyte capacity using an 'EXABYTE'(TM) drive. At maximum recording rates, this represents about 1.5 hours continuous survey operation.

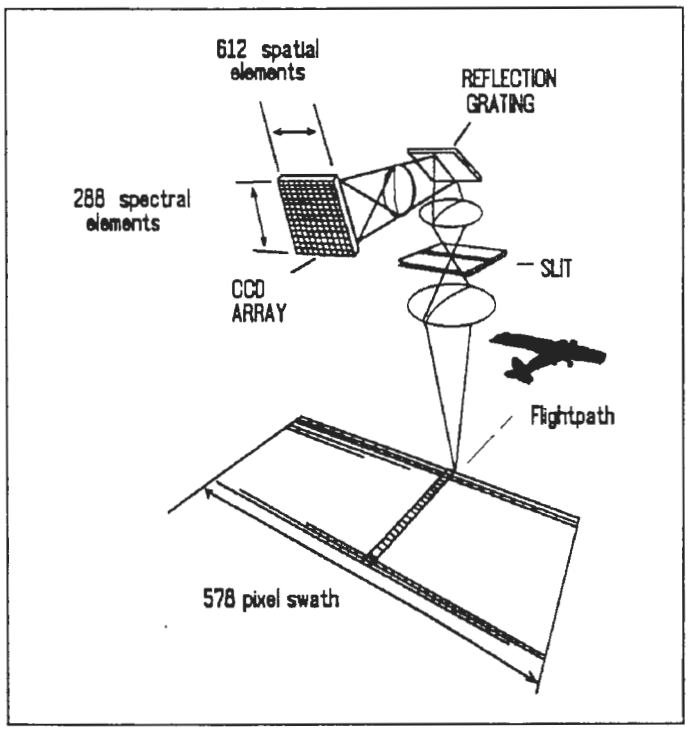

Figure 1. Schematic summary of the Compact Airborne Spectrographic Imager (CASI). CASI uses a 612 wide element single array; a reflection grating and a folded light path. The image is formed across 512 elements and dark and electronic offset data are recorded during each readout.

2.2. FIELD METHODOLOGY

2.2.1. MULTISPECTRAL IMAGING

In the field operations for this project, CASI was used in several different ways. The instrument views its target through a slit, and builds up multi-spectral imagery by moving in the cross slit direction relative to the target. In order to map the beaches from an aircraft, it was mounted conventionally (with the slit across the direction of flight) in the aircraft to look down vertically at the beach from low altitude. This gives the equivalent of a digital air photo, but with much better color resolution. At the speeds and altitudes we were able to achieve during the mission, we were obtaining approximately 0.25 m resolution across track and 1.9 m along track. The cross track resolution is determined by the 1.2 mRadian instantaneous field of view (pixel size will be 12/10000 of the distance from the target to the lens) and the operating altitude. The along track resolution is a function of the aircraft speed (usually 70 to 100 mph, 30 to 45 m/sec) and the integration time, which in turn is chosen so as to maximize signal strength for given spectral band widths and illumination. In these operations integration times were kept short (50 msec) in order to retain as much spatial resolution as possible, with the disadvantage that signal levels were low and signal-to-noise ratios were much lower than would have been if the data was collected under clear sunny conditions. This affected the automatic classification procedures and made manual processing necessary.

The digital nature of the image data makes it easy to enhance, contrast stretch, magnify and make calculations on. This means that much more can often be seen in the digital data than in photographs where the development procedure fixes the enhancement. Therefore we have also provided examples for most sites in a digital .GIF (Graphics Interchange) Format with a public domain display program called CSHOW. While this program does not have full enhancement capabilities, it does give considerably more range than photographs.

It was recognized that because many of the beaches were more or less vertical, an aerial view might give a distorted impression. The CASI was therefore also mounted to look horizontally and was used to view vertical rock faces from the barge, a small open boat and a taxiing aircraft. This worked well from the barge field camp and in the small boat under dead calm conditions, and was reasonably successful in the aircraft, but the vertical motion of the aircraft with the waves distorted the imagery. This distortion could be removed in processing but time did not allow it in this project. An instrument attitude sensor to be added to the Borstad Associates CASI during the winter of 1990 should allow removal of this effect during calibration in the future.

2.2.2. MEASUREMENT OF SPECTRAL SIGNATURES

During the first field mission, the poor weather did not allow flying and a great deal of spectral signature data was collected with the instrument suspended over plant samples, either with the instrument moving along a rail or by passing plant samples under it. Most of this experimental data was collected during periods of heavy rain and variable illumination. Therefore the radiance measurements (uWatts/cm2 sr nm) can not be directly compared. However, a standard Kodak R-27 18% grey reflectance panel was viewed immediately after each target, and the spectral data have been normalized by dividing their upwelling spectrum by that from the grey card and multiplying by the reflectance of the card (specified at 18% +/_ 1%) to form "Reflectance" ratios. This "Reflectance"

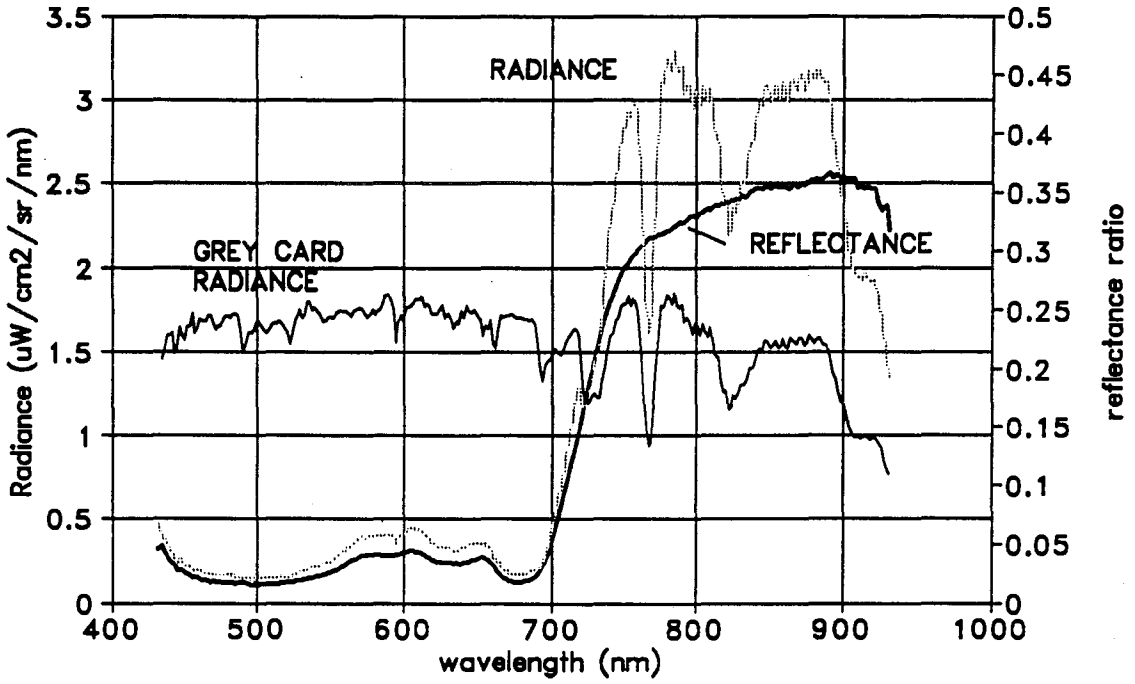

Figure 2. The radiance spectra of Alaskan Fucus measured under heavy overcast and rain, the radiance spectrum of an 18% Kodak grey reference panel, and the Reflectance of Fucus calculated by dividing the Fucus radiance spectrum by that of the grey card.

represents the fraction of the illumination which is returned from the plant material at any wavelength and has the effect of smoothing out the spectra and removing narrow spectral features due to absorption by water or oxygen in the atmosphere, or elements in the sun (figure 2). It is similar to reflectances measured relative to a 100% white panel and comparable to others for trees and vegetation reported in the literature.

The weather was not good during either of the field missions, and the heavy overcast encountered resulted in low signal levels. In spite of the fact that we were operating at or near the lower limit of operating conditions for this type of work, most of the data examined so far are quite usable and show the Fucus well.

The instrument was also hand held over a rocky beach to obtain spectra on one occasion, but it was impossible to hold the instrument still and this produced data which was badly distorted and difficult to interpret. The experiment was also regarded as too risky with an expensive instrument and was not repeated.

2.3. CASI DATA HANDLING

The CASI instrument itself writes data onto 8 mm video tapes in a helical scan digital format. These tapes are then read and the data calibrated by a MS-DOS 386 computer equipped with an Exabyte tape drive similar to the one in the CASI. During the first field session, this calibration computer was brought into the field, so that calibrations and preliminary data analysis could be undertaken at the barge field camp. After calibration, the spectral data was reviewed and representative radiometric spectra extracted for samples of different types of intertidal vegetation from beaches 1522 and 1522C, using Borstad Associates proprietary SPECPLOT2 software.

As described above in section 2.1., in Spectral Mode the CASI generates one monochromatic image of its target. For the spectral signature data collected on the barge, average and standard deviation radiance spectra were calculated from the data from one pass across the plant sample. In all cases, the average spectrum includes at least 30 to 100 individual spectra representing 1 mm spots on the plant material, in a line crossing the sample. This means that both well illuminated and darker spots (between receptacles or stipes) are recorded. Because of the illumination variations described earlier, these radiance spectra have been normalized to Reflectance.

Table 1. Spectral band definitions for Spatial Mode image data collected during airborne operations, August 6 - 9, 1990.

Band 1	478.9 - 500.3
Band 2	538.4 - 561.7
Band 3	598.4 - 614.7
Band 4	616.1 - 632.5
Band 5	646.3 - 661.0
Band 6	665.9 - 680.6
Band 7	750.5 - 759.9

2.4. IMAGE PROCESSING METHODOLOGY

Under normal circumstances, we would take advantage of the digital nature of the CASI data to carry out either unsupervised multi-spectral classification or eigen vector analysis, to separate areas in the imagery having similar spectral characteristics. Following our British Columbia experimental observations on Fucus (which were made under clear skies but not under direct sunlight) , we had also planned to use a 'radiance difference' operator which we use to image chlorophyll fluorescence. This 'FLH' operator calculates the signal in one band above a base line drawn between two adjacent bands. Since the Fucus spectral signature has a peak at 650 nm which is not present in other intertidal vegetation, this seems appropriate. However, the very low illumination under which most of the image data was obtained means that the signal levels are very low. Instrument effects and noise which are usually much lower than the signal and therefore not a problem, have interfered with these classifications. We were able to preprocess examples of the data to 'clean it up', allowing use of these methods, but a good deal of time was lost in trying to do this. Although we have not been able to adequately explore the power of these techniques, time constraints have forced us to use more manual methods.

In order to fully address objective 2, we need to have quantitative Fucus estimates from the other CHIA teams, but as of writing this data has not yet been available. We have calculated areas of vegetative cover on the beaches, but have no comparative data with which to test our methods. Also as explained above, since multispectral methods proved unsatisfactory with the noisy data, we have taken a more manual approach. In order to address the relative amounts of Fucus on the three test beach pairs chosen for this project (1522/1522C, 453/453C, and 601/601C), we have used a manual classification procedure called EXPLORE which is a function in the PCI Inc. EASI/PACE image processing software which we use. In a X-Y plot of band 7 versus band 3 for the entire image, the water, bare rock, terrestrial vegetation and beach vegetation fall out as separate clouds of points. The operator then outlines a cloud and the program identifies which image pixels fall into that cloud. This is done iteratively and the operator can thus confirm that the identified areas agree with his subjective interpretation of a band 3,2,1 true (or other combination) color composite on the video screen.

Once the areas have been outlined as graphics 'bitmaps', a histogram operator counts the pixels, and knowing the pixel size (from altitude, airspeed and integration time) we can calculate the respective areas. This procedure has allowed us to determine the amount of chlorophyll containing vegetation on the beaches, but not to distinguish between Fucus and other vegetation. We have calculated the ratio of vegetation to the total intertidal area for each beach (defined as from the lower edge of the Verrucaria zone to the water line), using the red marker bouys visible in the image to delineate the beach. In some areas where it was difficult or impossible to distinguish the Verrucaria zone, the upper boundary had to be estimated by the operator.

As noted above, we do not have CHIA data with which to compare our calculations. However, some in situ inspections were conducted on September 5 by Dr. Larry Deysher of Coastal Resources

Associates, California as part of a separate contract. We provided Larry with photographs of near true color renditions of the image data for beaches 1522 and 1522C collected on August 7 so that he was able to relate visual observations on the beaches to particular areas in the image data. He made notes directly on the snapshots, and this has allowed us to locate his estimates with respect to the digital data. Digital calculations can therefore be compared directly to these visual estimates.

3. OPERATIONS SUMMARY

June 30, 1990: Work for this project began in Sidney, British Columbia, when on June 30 we obtained spectral and spatial or image mode data of samples of mature Fucus distichus plants obtained washed up on the beach at Deep Cove Bay on the Saanich Peninsula near Victoria, BC.

July 20-22: The field component of the study began when Dr. Gary Borstad and Mr. Randy Kerr travelled to Cordova, Alaska on July 20 with the Borstad Associates Ltd. CASI and a PC 386 computer to be used for data analysis. With the help of Dave Erbe of Cordova Air Ltd. we installed the CASI in a Cordova Air DeHavilland Beaver aircraft, and made one short test flight. Initial attempts to use a small separate aircraft battery failed and the CASI was then run directly off the aircraft battery (through our DC-AC inverters). Purchase of the necessary bits of hardware and fabrication of an ad hoc mount for the Beaver was completed and the field crew transferred to the Camp David barge around 2100 h on July 22. Both spectral and image data were obtained at 6000' (1830 m) altitude during the transit, with the front lens on the system opened to f2 because of the expected low signal late in the day. This caused the imagery to be poorly focused. Spatial resolution at this altitude in image mode was about 2.5 m. The sky was clear for all of this flight, but with low sun angle because of the time of the day. On arrival at the barge, the data was verified using the analysis computer. The data is of good quality but the resolution is low because of the high altitude.

July 23: Fog early in the morning burned off by 0900 and we set up and tested an apparatus in which the CASI instrument was moved along a 'saw horse' type frame over samples to obtain test spectral data. The instrument did not move smoothly and we discovered that it was easier to move the samples under the instrument instead of the other way around. In this way several oiled rocks and rocks encrusted with the black lichen Verrucaria were imaged. Kim Sundberg of ADF&G and Dr. Larry Deysher of Coastal Resources Associates arrived at the camp around noon. Late on the 23rd we took the instrument to beach 1522 and attempted to obtain spectral data in situ on undisturbed plants. This proved difficult (rocks were slippery and the instrument cables were short) as well as dangerous with an expensive instrument and it was not attempted again. The data was of poor quality because it was difficult to hold the instrument steady. During the return to the barge, some CASI image data was obtained by scanning horizontally while the boat moved along the beach. While the signal levels were extremely low, this experiment provided interesting data and showed that it would be possible to look horizontally at vertical rock faces which would be difficult to image from the air. We did

confirm however, that the small movements of the boat with waves distorted the imagery. While these distortions can be removed in post processing, the process is time consuming.

July 24: The clear skies of the previous week had given way to overcast conditions, and the planned aircraft charter for the 24th was cancelled. The CASI was used to acquire both spectral and image data over several types of plant material collected from beaches 1522 and 1522C as shown in Table 2.

These data were verified immediately with the analysis computer, and spectra extracted for germlings and mature plants from both sites. Spectral bands for the instrument 'spatial' or image mode which were setup using spectral data obtained for British Columbia Fucus were checked.

July 25: Weather on the 25th was worse than on the 24th, with rain and heavy overcast. The aircraft charter was cancelled again. We continued to work up the spectral and image data obtained on the barge on the 23rd and 24th. More plant material was gathered in order to obtain good spectral data of Fucus germlings, Neorhodomela and a filamentous blue-green growing in the lower intertidal.

Table 2: Plant material for which spectral and image data was obtained on July 24th.

Beach	Germling	Mature	Mature	Zostera	Young	
Laminaria						
	Fucus	Encrusted	Fucus		Fucus	
1522C upper 1 m	X		X			
1522C lower 1 m	X	X	X	X		
1522 upper 1 m	X		X		X	
1522 lower 1 m	X		X	X	X	X

July 26: Skies early on the 26th were overcast, but fairly light. Because time was now running out, the Beaver aircraft was brought from Cordova, arriving about 0900 h. By the time the installation was complete, the weather had further deteriorated. At the time of take-off (0950), the wind had picked up, skies were heavily overcast and rain had begun. Wind coming over the islands created a good deal of turbulence (referred to by the pilot as 'williwaws'). Since, signal levels were very low and the image data was badly distorted by aircraft movements the flight was aborted. We did try scanning the beach horizontally while taxiing. Three passes of beach 1522 were made at different speeds. The slowest speed gave the least distorted image. While signal levels are very low, these data should provide an interesting perspective for comparison with the vertical view when processed. The video camera imagery from this flight and the taxi experiment also appears useful. Viewed on the television

on the barge, the imagery appeared in normal color. However, viewed on the Mitsubishi computer monitor, the Fucus appeared bright red.

July 27: Weather on the 27th had further deteriorated, with heavy overcast and heavy rain (8 cm in 24 h). Time was spent analyzing spectral data obtained so far and obtaining some horizontal scans of the beach south of the barge. Sundberg and Deysher left for Anchorage in the afternoon.

July 28: Borstad and Kerr left the barge via Cordova, in poor weather late on the 28th. Most of the flight was at altitudes below 100' (30 m). The instrument was taken back to Anchorage where it was used in another operation from the 29th to the 5th. Borstad, Kerr and the analysis computer returned to Sidney, BC on the 28th.

August 6: Borstad returned to Alaska on the 6th and together with David Hill of Borstad Associates met Kim Sundberg and installed the CASI in a DeHavilland Beaver belonging to Rust's Flying Service of Anchorage. A problem with the instrument tape drive, which was encountered in the previous week during another mission, had been partially solved by swapping in a new drive brought up from Sidney, but we were unable to acquire spectral data. This was not a significant problem, since more than enough spectral data was obtained during the previous stay on the barge. More of a problem, was the fact that the instrument was not allowing more than 3 separate files to be written on any one tape before it hung. This was circumvented by writing long files which included the turns at the end of lines. While this allowed acquisition of the data, it made extraction of the data much more time consuming.

The weather at this time in Prince William Sound was still not very good, with overcast skies and light rain on the transit out to Camp David late on the 6th. Some test data was acquired in the vicinity of Portage Pass, which may be of interest for those interested in terrestrial vegetation. We also passed over a Fucus test site on the south west corner of Culross Island but the band configuration on the instrument was not correct for mapping Fucus, and because of a heavy cloud ceiling at 2000' (610 m) the signal levels were very low. These data will not be extracted.

August 7: Skies were overcast but the rain had stopped on the 7th. The Cordova Air Beaver aircraft arrived at 0730. We installed the equipment, took off at 0830 and collected image data on several circuits at altitudes between 700' (215 m) and 1200' (365 m) under heavy overcast, mostly north to south over 1522 and then 1522C. At the short (50 msec) integration times required for maximum spatial resolution using the band configuration defined for this mission, the signals were very low (below 512 counts out of a possible 4096). We changed to 75 msec after several files. For these passes we were operating one long file at first then after we had several good runs, we started breaking files. After 4 files the instrument hung. By this time, rain had begun so we landed and installed CASI to look oblique out of baggage door. Sundberg and Hill went up, Sundberg taking color IR photos (Ektachrome 200 at 1/250th second; at f3.3 ASA 100 - 1/60th to 1/100th)

The aircraft returned to Cordova, and after lunch we went by boat to sites 453 and 453C on Disk Island and Upper passage. We put out 60 cm diameter fluorescent red floats at either end of the sites and at least one buoy on the beach itself marking a transect on the beach for which ADF&G has Fucus counts. On 453C we used about 3 m of red flagging tape strung back and forth from the re-bar at high tide across the Verrucaria zone. Both the bouys and flagging tape can be seen in the imagery. It was cloudy most of the morning and into the afternoon, but there were small breaks to blue sky late in the afternoon.

Table 3. Image data acquired over sites 1522 and 1522C on August 7, 1990.

Site #	Time of overflight (AKDT)
1522	0945, 0948, 0953, 0958, 1003, 1010, 1016
1522C	0955, 0959, 1015, 1012, 1014

August 8: The sky was overcast but fairly light on the morning of the 8th. The Beaver arrived at 0800, we installed and took off at 0900. We flew sites 1522 and 1522C for vertical color and color IR photos, then crossed to site 453C on Disk Island, which we flew several times with CASI collecting one long file. The air was very smooth and there was very little turbulence. The buoys showed up very well from the air and made excellent visual aids for the pilot. They are also very visible in the real time image display of 600 - 700nm bands. After several good passes we stopped CASI and landed to take in the bouys at 453 and 453C. These were taken into the aircraft and we returned to the barge to drop off some of them (they took up too much room in the aircraft) and pick up some more video tapes for CASI. We then flew to site 601C, landed and placed the buoy markers. This site was flown several times at 800'(245 m), 1200' (365 m) and 1800' (550 m) altitude. Site 598C was also imaged both south to north along the beach and east to west across the beach. After leaving Lower Herring Bay, we planned to cross to the Bay of Isles but took the wrong mountain pass and ended up in the west arm of Herring Bay. Image data was therefore obtained along the western shore of this part of the bay before we moved to site 601 and 598 to install buoys removed from 601C. After completing flights over 601 and 598 we crossed to the Bay of Isles and obtained spatial imagery over an oiled marsh at site 15.1. Table 4 summarizes the spatial mode image data obtained on August 8 for numbered CHIA sites (CASI was running most of the time and many beaches in the area were imaged). Only a small amount of data for the test sites has been analyzed and is discussed here.

Table 4. Spatial mode image data obtained over test sites on August 8, 1990

Site #	Times of overflights (AKDT)
453C	0929, 0930, 0932, 0937, 0954
453	0943, 0945, 0948, 0950
601C	1146, 1148, 1151, 1156
598C	1157, 1201
601	1227, 1232, 1237
15.1	1249

4. RESULTS AND DISCUSSION

4.1. SPECTRAL SIGNATURE OF BRITISH COLUMBIA FUCUS

The preparatory work on Fucus collected near Sidney, B.C., confirmed earlier low resolution spectral measurements made with another non-imaging spectro-radiometer during the winter. We found that southern Fucus distichus exhibited apparent peaks in the reflectance spectra near 600 nm and 650 nm which are not found in green plants. Image data collected at this time confirmed that this feature could be imaged to separate Fucus from other plant material. This allowed us to define spectral bands for the airborne component prior to coming to Alaska.

4.2. RADIANCE SPECTRA FROM ALASKAN FUCUS AND OTHER MARINE PLANTS

The Fucus radiance spectra obtained in Alaska were similar but not identical to those obtained in British Columbia. The Alaska Fucus, which appeared very yellow to the eye, exhibited a smaller 600 nm peak and a much higher 650 nm peak in the radiance spectra relative to the BC Fucus which appeared green-brown. This difference was at first thought to relate to a difference in pigmentation. However, the measurements were made under very different conditions (the BC exercise was conducted under indirect illumination from clear blue skies, while all of the Alaskan spectra were obtained under heavy overcast). When spectra from both exercises are normalized to the 18% grey card measured at the same time, the differences are much less obvious. Figure 2 illustrates calculation of the Reflectance ratio.

Figure 3a illustrates average reflectance spectra obtained for 3 marine plants from the Herring Bay intertidal zone. Differences between the green flowering plant Zostera marina, the brown alga Fucus distichus, and the red alga Neorhodomela. are a reflection of differences in the pigmentation of these three very different types of plants. The spectral band definitions used in CASI image mode are also shown. The spectral signature for Laminaria saccharina was also measured, but because it is also a brown alga, it is similar to that of Fucus. It is not shown here for clarity. The reflectance is really the light escaping the plant, and the total absorption by the plant can be represented as the inverse of reflectance (figure 3b). The spectral signature of plants can be divided into 2 regions within the range visible to CASI:

Region I: visible range (425 - 700 nm.) Reflectance is mainly influenced by the absorption of pigments (chlorophyll a,b and c; carotene; lutein; biliproteins; fucoxanthins.

Region II: near infra-red (700 - 925 nm) Reflectance is mainly determined by the size and arrangement of cells. Intercellular air spaces play an important role.

Zostera contains chlorophyll a (absorbing at 435 nm and 675 nm) and chlorophyll b (absorbing at 480 nm and 650 nm), and therefore exhibits an absorption minimum (= reflectance peak) at 550 nm in the green region of the spectrum. The brown algae Fucus and Laminaria both contain chlorophyll a (absorbing at 435 nm and 675 nm), chlorophyll c (absorbing in the 470 to 480 nm region and at 650 nm) and Fucoxanthin absorbing between 480 and 530 nm (Jeffrey, 1980). Because of the stronger absorption by fucoxanthin in the green region, the reflectance maxima is shifted to longer (redder) wavelengths making the plants appear dark orange - red or brown. In Neorhodomela, the biliprotein phycoerythrin absorbs at 495 nm, 545 nm and 565 nm and phycocyanin at 625 nm (Jeffrey, 1980). There are absorption minima at 600 nm and 650 nm and as a result the alga appears dark red. In all three types of plants, there is strong absorption at 675 nm by chlorophyll a, but nearly none above 700 nm. With strong scattering from the plant cellular material itself and the intercellular air spaces in the plant the reflectance is nearly 100% at these wavelengths (although since it is outside of range of the human eye, 420 nm - 680 nm, this light is invisible) (Hofer and Johannsen, 1969). The sharp rise of reflectance spectra in the vicinity of 700 nm is diagnostic of all chlorophyll containing plants and can be used to distinguish living plants from rocks and water.

Plant	Taxon	Chlorophylls	Biliproteins	Carotenoids
Zostera	flowering green plant	a, b		
Fucus + Laminaria	Phaeophyta (brown algae)	a, c		Fucoxanthin
Neorhodomela	Rhodophyta (red alga)	a	Phycoerythrin + Phycocyanin	

As will be discussed in the next section, there are apparent spectral differences between Fucus from oiled and unoiled sites. There is of course also considerable color variation within and among plants of the same species, depending on their growth stage or physiology. There is at present active research into pre-visual detection of plant stress using remote sensing, especially high spectral resolution (see for example, Rock et al., 1986). Figure 4 shows the ratios of reflectance spectra from samples of germling and mature Fucus from beach 1522C. The shape of the top curve suggest that the darker mature plant contains more of all its constituent pigments than the younger material. There are smaller differences between different germlings, again the inference is that all pigments are concerned. The bottom curve has less absorption at 500 nm and relatively more at 670 nm, suggesting that the differences within mature plants (fronds vs stipes) are mostly due to chlorophyll a. This is an important line of investigation since it will bear on the interpretation of the image data. However, it was only begun in this project and has not been followed up for lack of time.

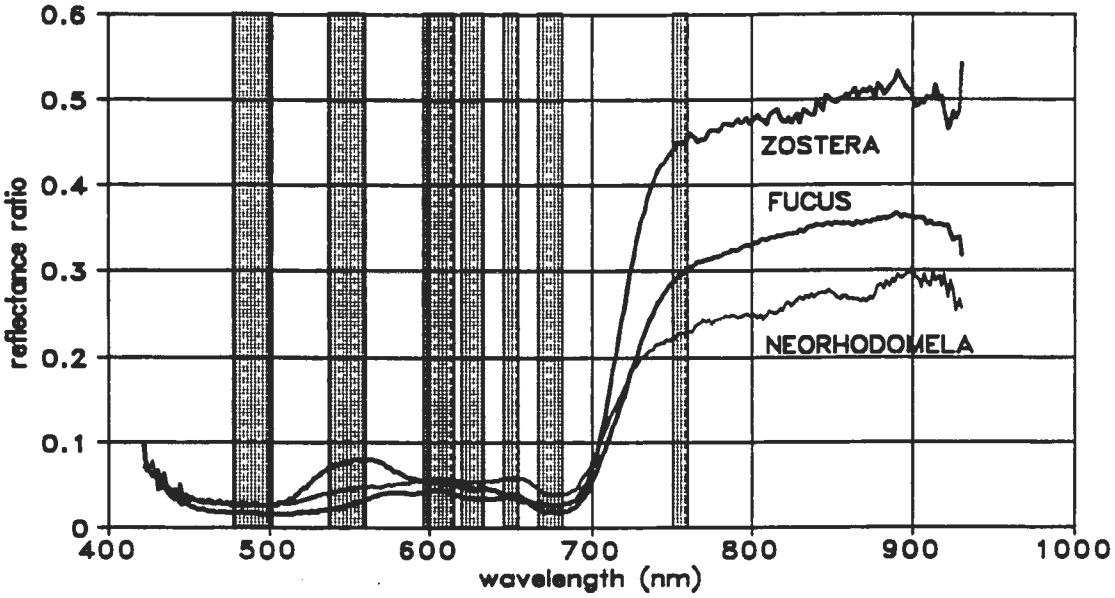

Figure 3a. Reflectance spectra for 3 marine plants collected from the Herring Bay intertidal zone, to show the differences which will allow separation of these plants in imaging mode. Also shown are the spectral band definitions used in CASI image mode. The green flowering plant Zostera marina (heavy line), the brown alga Fucus distichus (mature plants, dashed), and the red alga Neorhodomela sp.(thin line).

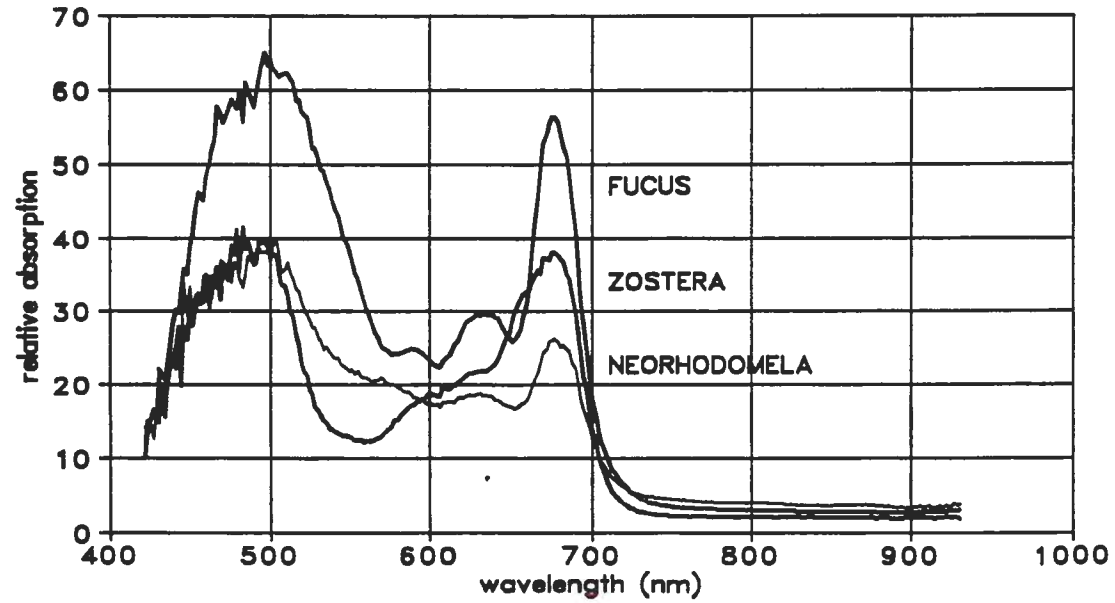

Figure 3b: Relative spectral absorption (calculated as the inverse of reflectance) for the Zostera, Fucus and Neorhodomela in figure 3a.

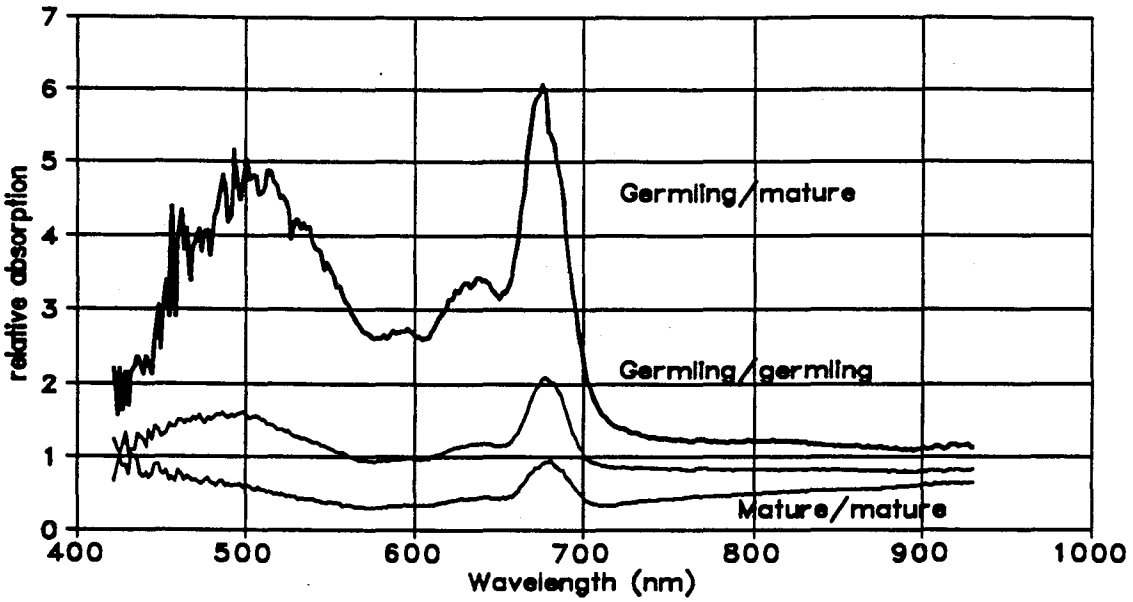

Figure 4. Relative absorption (ratios of reflectance spectra) for mature and germling Fucus from beach 1522c.

4.3. SPECTRAL DIFFERENCES BETWEEN FUCUS FROM OILED AND UNOILED SITES

Figures 5a and b illustrate spectral reflectance data from the exercise comparing samples of mature and germling Fucus from sites 1522 and 1522C. Table 5 compares the spectral reflectance at 600 nm (chosen simply to represent the overall brightness in the visible region where pigments determine reflectance characteristics) and at 800 nm (in the near infra-red where reflectance is related to cellular structure) for all eight combinations of site, position in the intertidal zone and plant age. In the visible part of the spectrum, the germlings were brighter (higher reflectance) than the mature plants at both the oiled and unoiled sites, whether or not they came from the upper or lower part of the intertidal zone. At the oiled site (1522), the germlings from both the upper and lower parts of the zone were nearly twice as bright as those from the unoiled site. However, at 1522 the mature plants from the lower intertidal were bright (similar to the germlings) but those from the upper zone were not. Reflectances for the upper mature plants from 1522 were similar to the mature plants from 1522C. In the non-visual part of the spectrum sampled all but the germlings from the oiled beach were similar. These were much higher than all of the others.

If the interpretation of these algal reflectance spectra can be transferred from the studies of terrestrial vegetation (Hofer and Johannsen, 1969; Rock et al., 1986), then the data imply less pigmentation and a different cellular structure (more air spaces) in the younger plant samples from site 1522 than for the same type of material from the control beach. It also suggests a similar lower pigment content for mature plants from the lower intertidal of 1522, but not in the mature plants.

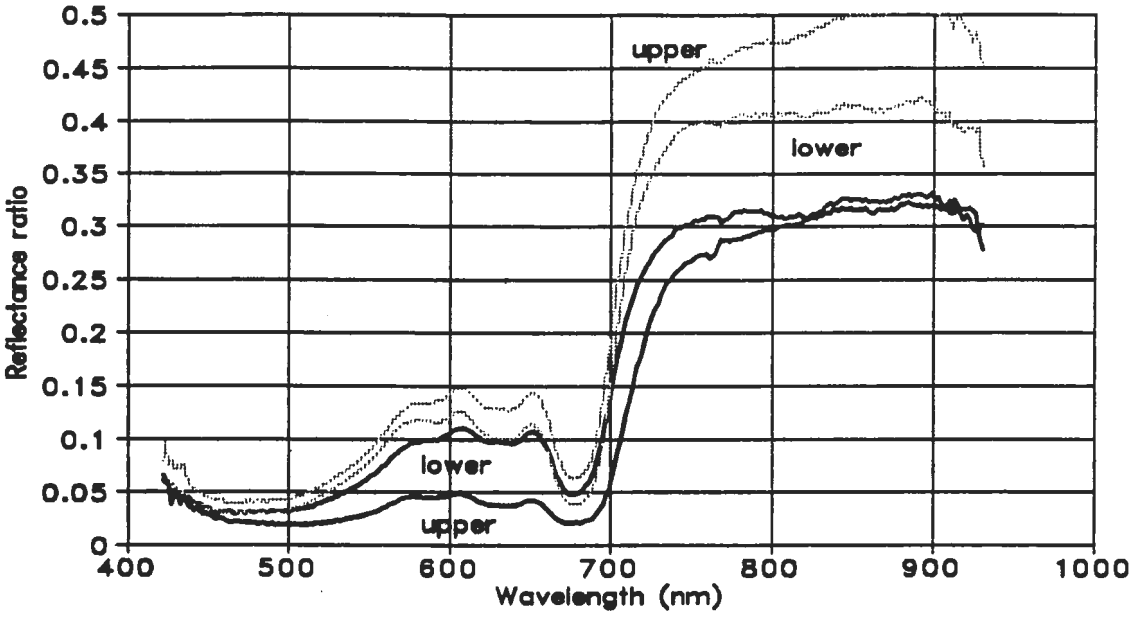

Figure 5a. Reflectance spectra for mature (heavy lines) and germling (stippled lines) Fucus collected from the upper 1 m and lower 1 m of the intertidal zone at site 1522, illustrating spectral differences at an oiled beach.

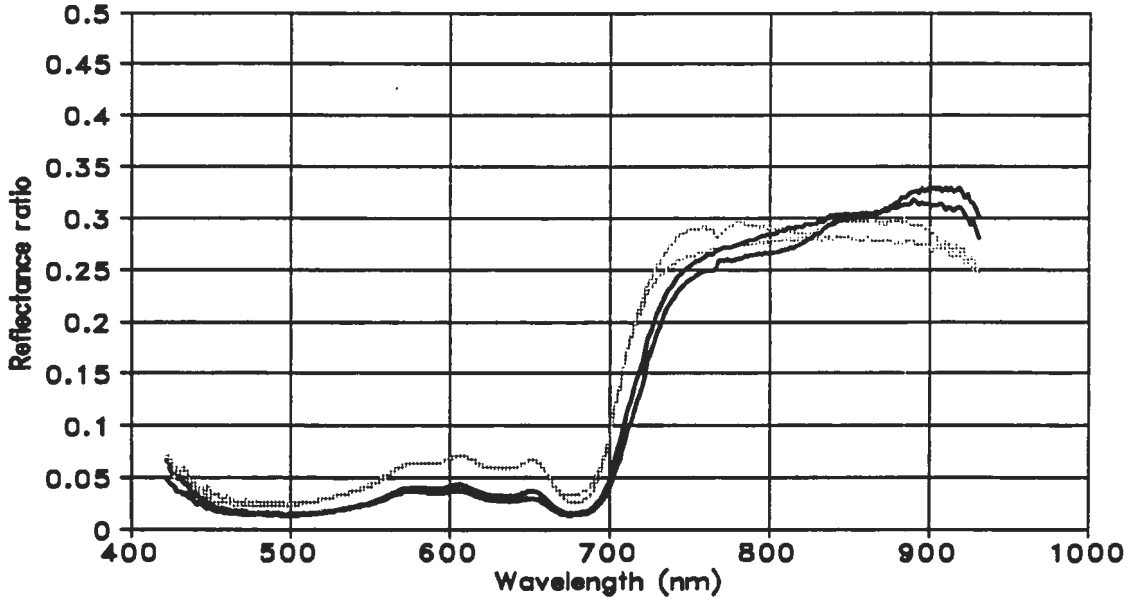

Figure 5b. Reflectance spectra for mature (heavy lines) and germling (stippled lines) Fucus collected from the upper 1 m and lower 1 m of the intertidal zone at site 1522C, illustrating the spectral differences at an unoiled beach.

These preliminary spectral observations should be confirmed with replicated samples from these and other beaches backed up with chemical tissue analysis, and the differences tested statistically. However, the data seem to agree with visual and photographic observations of younger and brighter plants on the oiled beaches reported to us by University of Alaska workers at the barge field camp. In general older plants, or older parts of plants (stipes) are darker than the receptacles and the younger germlings.

Table 5. Comparison of reflectances at 600 nm and 800 nm for Fucus plants from sites 1522 and 1522C.

Site #	Reflectance at 600 nm		Reflectance at 800 nm	
	Germlings	Mature plants	Germlings	Mature plants
1522 (Oiled) upper intertidal	.12	.05	.40	.30
1522 (oiled) lower intertidal	.14	.11	.47	.31
1522C (Unoiled) upper intertidal	.07	.04	.28	.28
1522C (Unoiled) lower intertidal	.07	.04	.29	.27

4.4. IMAGING AND MEASUREMENT OF VEGETATIVE COVER ON BEACHES

4.4.1. TRUE COLOR REPRESENTATIONS

Based on the spectral differences shown in figure 3, we defined 7 spectral bands for data acquisition in the CASI image mode. Imagery of each of the 6 beaches has been calibrated and is shown in figures 6 through 11. The vegetation on the beaches can be easily distinguished in these images where bands 5 (646 - 661 nm), 2 (538 - 561 nm) and 1 (478 - 500 nm) have been displayed on the red, green and blue guns on the video, presenting a more or less true color rendition. The image at right has been linearly enhanced to brighten it, but the color balance has not been altered. The image at the right side has been enhanced using a histogram equalization technique which brightens the image and alters the balance between the three bands. In this case this allows low contrast features below the water line to be distinguished.

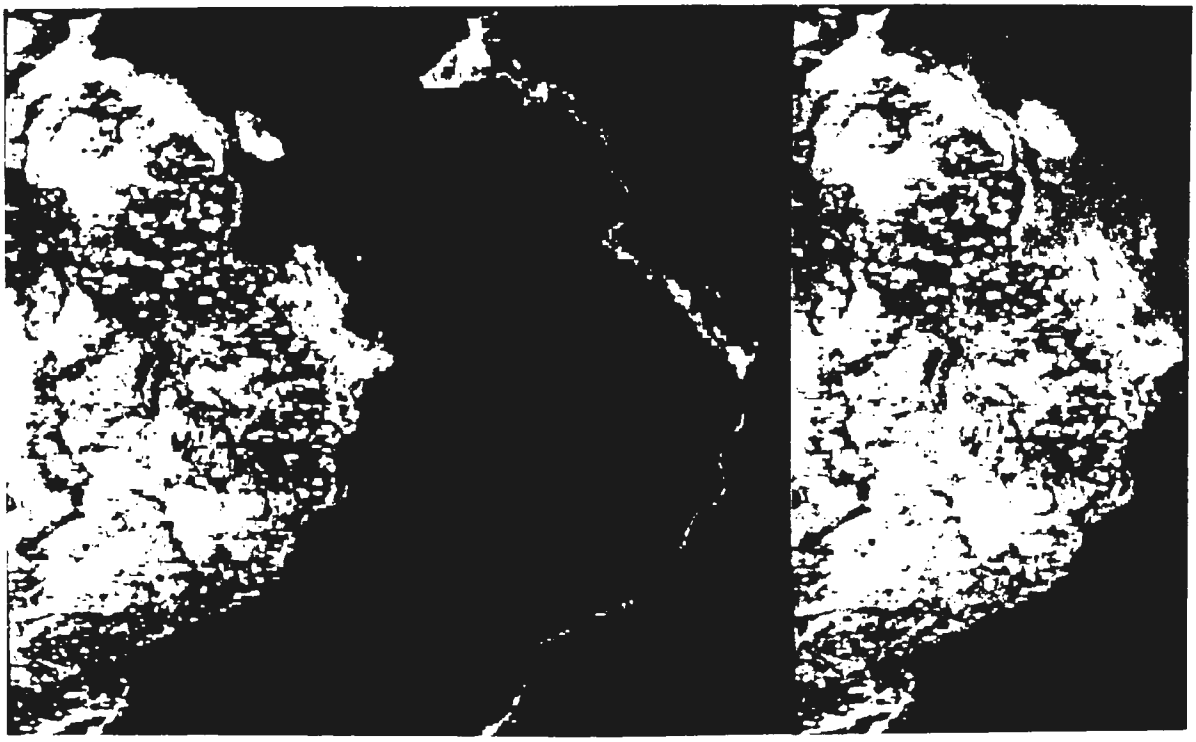

Figure 6. Near true color images of beach 1522C (left), the calculated land, intertidal, vegetative cover and water zonation themes, and an enhanced true color image showing subtidal zonation (right).

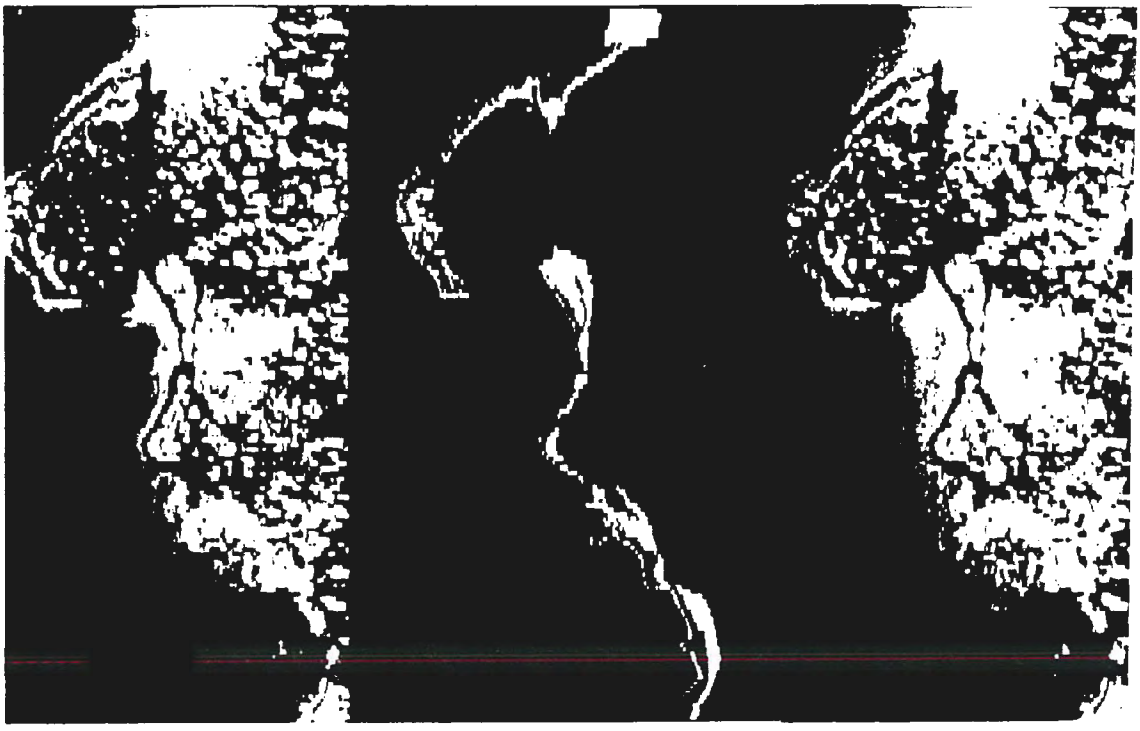

Figure 7. Near true color images of beach 1522 (left), the calculated land, intertidal, vegetative cover and water zonation themes, and an enhanced true color image showing subtidal zonation (right).

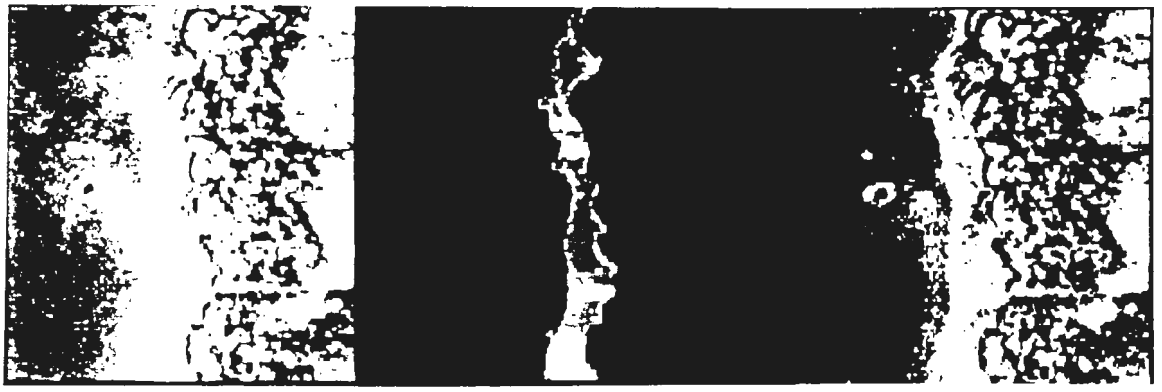

Figure 8. Near true color images of beach 453C(left), the calculated land, intertidal, vegetative cover and water zonation themes, and an enhanced true color image showing subtidal zonation (right).

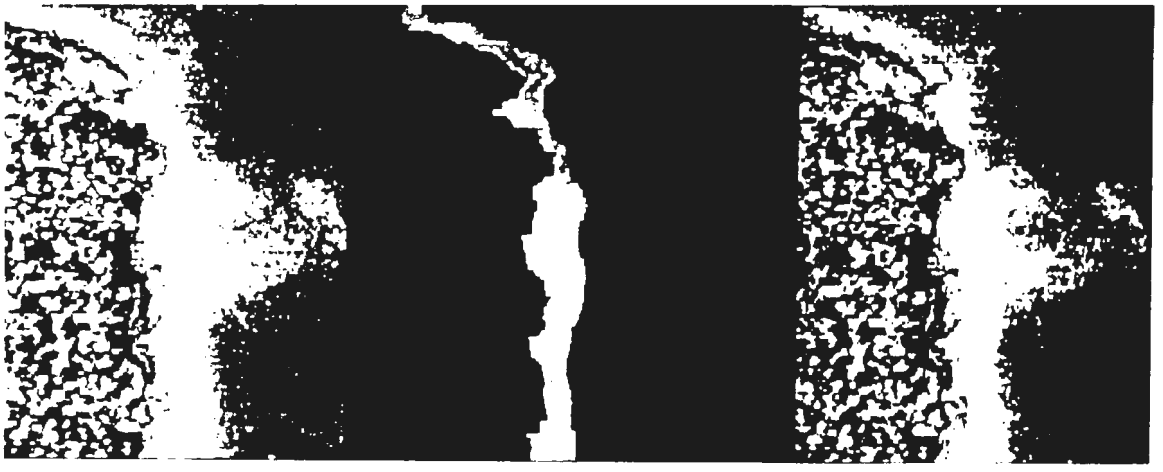

Figure 9. Near true color images of beach 453 (left), the calculated land, intertidal, vegetative cover and water zonation themes, and an enhanced true color image showing subtidal zonation (right).

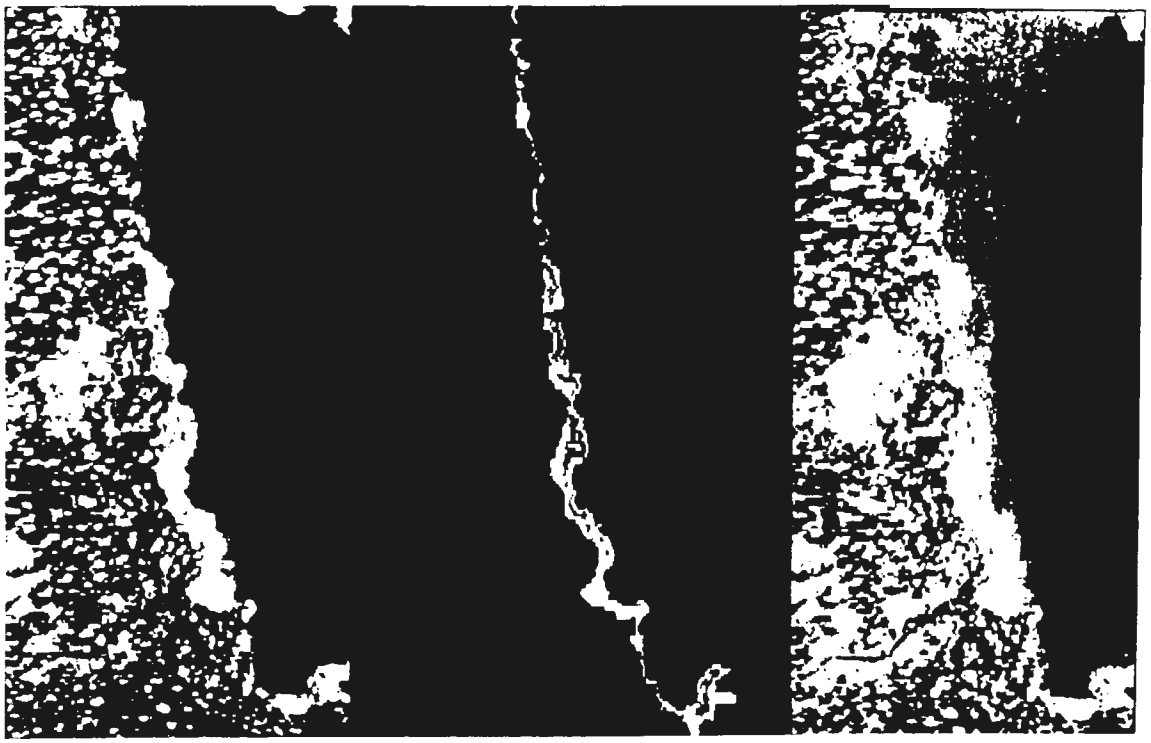

Figure 10. Near true color images of beach 601C(left), the calculated land, intertidal, vegetative cover and water zonation themes, and an enhanced true color image showing subtidal zonation (right).

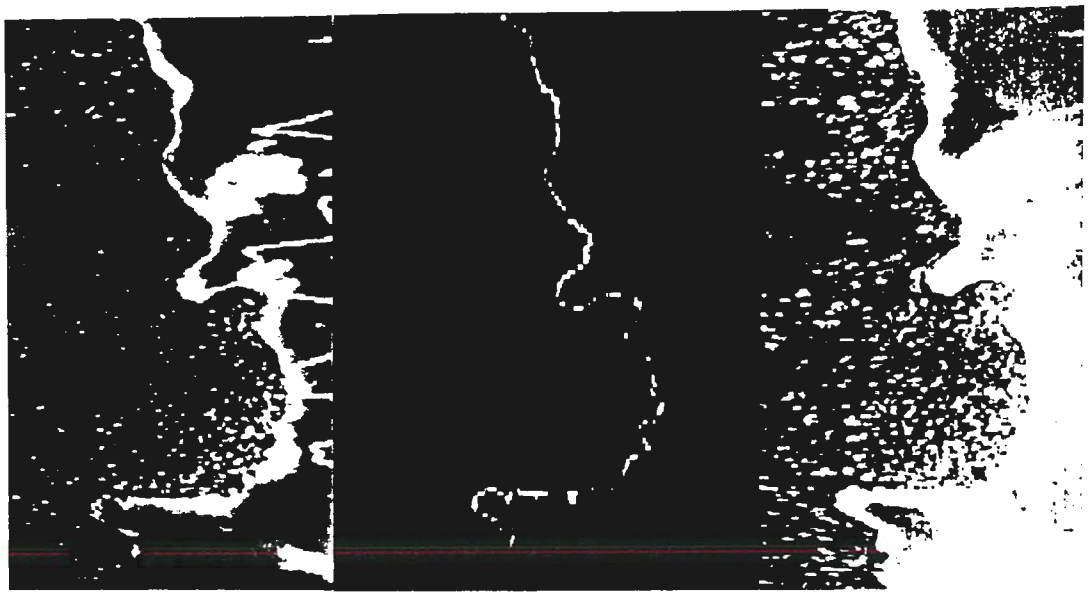

Figure 11. Near true color images of beach 601 (left), the calculated land, intertidal, vegetative cover and water zonation themes, and an enhanced true color image showing subtidal zonation (right). The white line at right is an aircraft tie-down rope waving in front of the camera.

The upper part of the Fucus zone on beach 1522C is distinctly brighter than the lower part (figure 6a), and although this zonation had not been noted previously, it was confirmed by Dr. Larry Deysher during a visit on September 5. He found that the bright yellow zones were predominantly younger more upright plants with more receptacles, while the darker areas were predominantly older more recumbent plants exhibiting more of the dark brown stipe. This agrees with spectral signature work which found that germlings had higher overall reflectance than mature plants (section 4.3 above). However, contrary to our finding that germling samples from the oiled site had higher reflectance, no such extended bright zone is seen on beach 1522 (figure 6b). Only one small bright patch is seen on a small promontory near the bottom of the image. This seems inconsistent, but may mean that the oiled sites had less young material. It was pointed out to us by others at the field camp that the oiled sites generally lacked Fucus in the upper intertidal, perhaps because of the physical scouring of the oil-cleaning process.

There were some difficulties with beach 601. Figure 11 is not exactly centered on the experimental beach. In order to save time on the last flying day of the field mission, no bouys were placed in the water to mark the start and end of the beach. The site is in a tight location for aircraft maneuvering and in reviewing the CASI data later, we discovered that the aircraft turned onto line too late and the data begins part way into the transect. It also happened that the red buoy markers we placed on the beach were later determined to be very near the east end.

It was late in the tidal cycle by the time the data was collected and while it appears from the true color imagery that there is little or no Fucus on this beach, Kim Sundberg informed us after review of the draft report that CHIA data shows 80% cover in the upper part of the intertidal at the transects marked by our bouys. The part of the beach visible in the data was then re-examined closely and it was confirmed that there are very few pixels showing a high band 7 (750 - 760 nm) signal and these are very near the buoy markers. Further, the markers themselves appear to be very close to the water line. Table 4 shows that beach 601 was not imaged until 12:30 AKDT, when the tide had begun to rise.

4.4.2. ATTEMPTS TO MAKE QUANTITATIVE FUCUS-SPECIFIC IMAGERY

4.4.2.1. 'FLH' CALCULATIONS

A Fucus image (figure 12), made from bands 4, 5 and 6 (the radiance in band 5 above a baseline drawn between bands 4 and 5) delineates the Fucus zone well. This is the 'Fluorescence Line Height' algorithm developed by us for studies of phytoplankton chlorophyll fluorescence (Gower, 1978; Borstad et al, 1985), however in this case we are not measuring fluorescence but simply the shape of the reflectance spectrum in the region of 650 nm. With further work, other algorithms could be derived which make more physiological sense. The boundaries of the zone outlined by this 'FLH'

calculation correspond well with subjective interpretation of the Band 3,2,1 true color imagery. This is because reflectance spectra of rocks, Verrucaria, water and terrestrial vegetation do not have a shape which gives a positive value for this calculation. However, the calculation gives a higher signal for the younger, brighter plants than for the darker ones. A histogram of the values shows two peaks which may relate to the relative areas of the bright and dark zones. This should be investigated further. At the time of writing, we have not obtained the CHIA quantitative data, and therefore do not have ground truth with which to check the accuracy of classification.

Figure 12. 'FLH' images calculated as the radiance difference in band 5 above a baseline calculated from bands 4 and 6, for beach 1522 and 1522C, illustrating how it is possible to specifically delineate Fucus.

4.4.2.2. RATIO OF BANDS 7 AND 6

During the final stages of write up, we compared a ratio calculated from band 7 over band 6 for the locations of the visual percent cover estimations made by Dr. Larry Deysher on beaches 1522 and 1522C. Figure 13 illustrates the relationship obtained for the 7 quantitative observations he made (r^2 = .84). As seen in the ratio images in figure 14, there is a good deal of variability, and this relationship is obviously not well described by only 7 points. It will not be specific for Fucus since it only uses two bands describing the chlorophyll reflectance shoulder at 700 nm and will probably also be confounded by the brightness variations of the Fucus itself (younger, brighter vs older, darker zones). However,

we think it does illustrate the potential of these digital image methods. Band 7/6 ratio images have not been calculated for the other beaches.

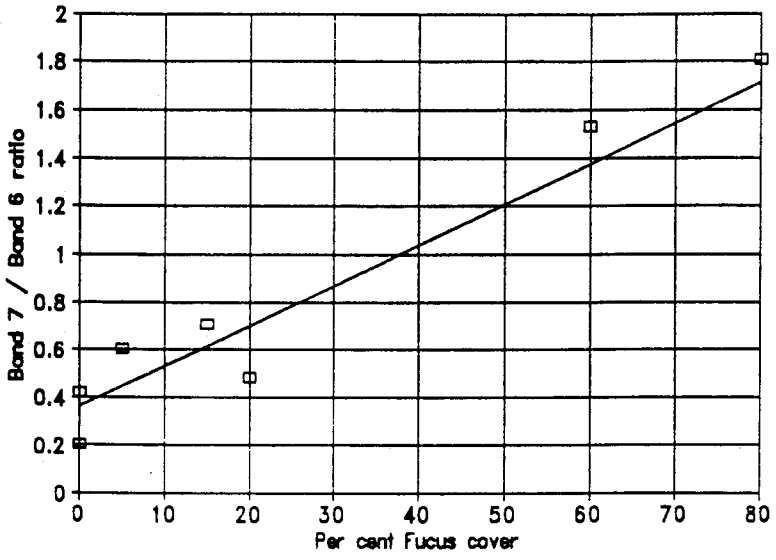

Figure 13. Relationship between band 7 / band 6 ratio calculated from the digital image data and visual estimations of percent Fucus cover made on beaches 1522 and 1522C by Dr. Larry Deysher.

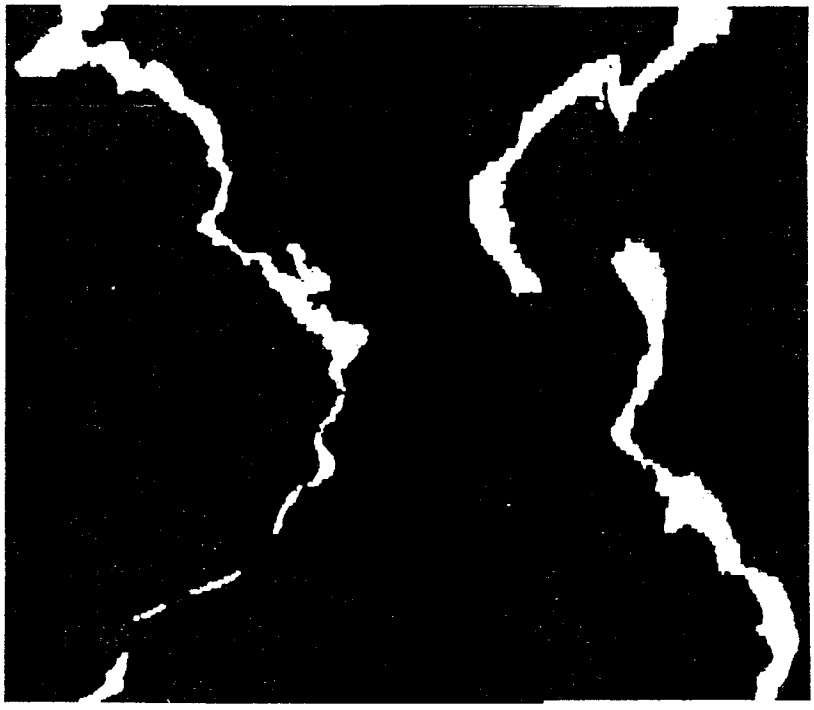

Figure 14. Calculated band 7 / band 6 ratio images for beaches 1522 and 1522C.

4.4.3. ESTIMATES OF VEGETATIVE COVER ON THREE EXPERIMENTAL BEACHES

Relevant to objective 2, "Determine whether CASI data can be correlated with quantitative data being collected by the CHIA teams, including estimates of percent cover and biomass of Fucus.", we have calculated the total vegetative cover on three experimental beach pairs (1522, 453 and 601). As mentioned earlier, the final analysis can not be carried out because the CHIA data was not available at the time of writing.

Figures 6 through 11 demonstrate the great variability in the Prince William Sound shoreline, and the difficulty in using only line transects. Assuming that the beach pairs are well matched, and that the vegetative areas can be accurately calculated from CASI data, it will be possible to compare the total area vegetative coverage on each pair of beaches. Unfortunately, we do yet not have quantitative CHIA data with which to test our results. Also because of the low signal levels, we chose a simple supervised classification method using the relationship between bands 7 and 3 and checked visually against a band 3,2,1 true color composite. This method does not account for differences in percent vegetative cover in any one pixel, but gives the total area covered by at least some vegetation. The lower threshold of what constitutes 'vegetative cover' will be a subjective decision of the operator in this calculation.

Table 6 lists the pixel resolutions, number of pixels counted, and areas calculated for the intertidal zone (water line to bottom of Verrucaria zone) and the vegetated zone for each of 6 beaches. There seems to be a consistently smaller percentage vegetative cover on the oiled beaches than on the controls, however the beaches were not all imaged at exactly the same stage of the tide (601 and 601C were on a rising tide). With only 6 beaches examined statistical testing is not possible.

Table 6. Comparison of vegetative cover on beach pairs 1522, 453 and 601.

Site #	pixel dimensions(m)	area(m2)	Total Intertidal # pixels	area	Bare Intertidal # pixels	area	Vegetative Cover # pixels	area	percent cover
1522	.26*1.79	.47	7405	3391	3744	1714	3661	1677	49%
1522C	.37*1.56	.57	5489	3134	2299	1313	3190	1821	58%
453	.37*1.79	.65	7027	4596	4096	2679	2931	1917	41%
453C	.37*1.79	.65	5234	3423	2048	1339	3186	2084	60%
*601	.37*1.79	.65	1861	1217	1577	1031	284	186	18%
*601C	.37*1.79	.65	4476	4342	3307	3208	1169	1134	26%

* imaged late on a rising tide. Beach and vegetation areas are therefore low. Only part of beach 601 was imaged because the entire beach was not marked and the aircraft turned onto line late.

The images in figures 6 to 11 are depicted in nearly (not exactly) the correct geometry, but area calculations use calculated pixel sizes, which depend on the aircraft altitude, speed and instrument parameters. A potential problem in comparing beaches using photographs or digital imagery obtained from the air is that parts of some beaches are much less horizontal than others and will be undersampled when viewed vertically. It is also obvious that the type of substrate closely controls the vegetative cover, and that the beach pairs do not have identical amounts of substrate. Cobble and gravel beaches are much less likely to have Fucus or other vegetation. It may be possible to estimate the relative proportion of substrate using a combination of CASI digital data and higher spatial resolution photographs, but we have not investigated this.

4.4.4. DETECTABILITY OF SUBTIDAL VEGETATION

The digital nature of the CASI data allows the imagery to be easily enhanced (simply by movement of a mouse on a digitizing tablet), in much the same way that photographs are exposed longer. This enhancement can be carried out on individual channels separately or on the 3 displayed bands together. Figures 6c to 11c illustrate how it is possible to see below the surface of the water, even when the signal levels above the water were already very low. The penetration will depend on the spectral attenuation coefficients of the water, and hence will vary according to wavelength (greater penetration at short wavelengths, almost zero at wavelengths greater than 700 nm). These properties will also vary with seasonal phytoplankton concentration and suspended sediment load, but for these images should be similar from beach to beach. At the time of imaging, the water was clear (no measurements of attenuation were made) and we expect to see 1 to 2 m below the surface in the 605 nm band, and 2 to 3 m in the 490 nm band. Under bright sunny conditions, this penetration should be correspondingly greater. For the three spectral bands used, the interpretation is fairly intuitive since they more or less mimic the human visual response except that band 3 (605 nm in the yellow-orange part of the spectrum) is used on the red video gun. This is on the Fucus reflectance maximum and will make these zones look red in the image. Band 2 (550 nm, green-yellow), at the Zostera reflectance maximum is shown on the green gun, making any Zostera beds look green. The blue video gun has been assigned to the blue-green 490 nm region where both plants have very low reflectance, hence high signal levels in this band probably mean bare rock. A dark blue in the images corresponds to deep water in which the maximum signal is in band 1, with very small signals in the other two.

At site 1522C, there is a small patch of Fucus just off the beach in the northern half of the site near a subtidal rock and near the point at the center of the image. The southern half of the beach, which is not visible from the air does not seem to show subtidal vegetation. This part of the beach is near vertical and probably drops off sharply. At site 1522, there appears to be Fucus below the surface in the lower (southern) half of the image but not around the promontory in the top half of the image.

At site 453C there is one offshore rock with subtidal vegetation between it and the shore line in the top half of the image. A patch of green vegetation (Zostera?) is visible about 1/4 down the scene in the

digital data but does not show up well in these photographs. There are at least 3 subtidal Fucus patches along the top 2/3 of site 453 image, with at least two offshore rocks rising to near the surface.

At site 601C only one small patch near the bottom (west end of the beach) of the image is seen. In spite of the fact that there is very little vegetation visible in the intertidal at site 601, this beach has the most subtidal Fucus visible in these images. This is probably related to the tidal height at the time of imaging. Strong red color is seen all along the top (eastern) half of the enhanced image. The offshore rocks are well covered (best seen in the true-color images at left). Some green (Zostera?) is seen near the end of the promontory in the lower part of the image in the digital data.

4.4.5. DISCUSSION OF THE USE OF CASI DIGITAL IMAGING

Compared to low altitude aerial photography, which has been used for this kind of task in the past, most digital imagery available today is of lower spatial resolution. Unclassified sensors on civilian satellite platforms can currently obtain 10 x 10 m resolution at best, while most airborne systems can not do better than about 3 x 3 m. As explained earlier, cross-track resolution of such sensors (and cameras) is determined by their optics and the flying altitude. Along track resolution is a function of the 'speed' of the instrument (integration time) and the ground speed of the aircraft platform. In our 1990 operations we were able to obtain at best .256 m cross track and 1.56 m along track by flying low and slow. Further decrease in along track pixel size is possible if we reduce the number of spectral bands or go to a slower platform (ie. a helicopter). However, our early experience with helicopters indicates that platform roll and yaw will be greater problems than with fixed wing aircraft.

Aerial photographs do provide high spatial resolution, but have very low spectral resolution, a low dynamic range, and must be re-digitized in order to be used in GIS. We have shown here that useful CASI data can be collected under extremely poor conditions, with a simple installation on locally available aircraft. Further, the data is quickly translated and calibrated from the tape recording medium to computer compatible files. It was therefore possible to carry out a good deal of data review and analysis at the field camp with a simple personal computer system. If full image processing facilities were available in the field, more complete analysis could be carried out. This in turn would allow biologists to take image maps out to the beaches to conduct the ground truthing operations in reverse, rather than collecting in situ data separately and then trying to 'calibrate' the images later in the lab. This will also allow us to better investigate algorithms specific to Fucus and better use of the digital data through multi-spectral classifications. This was begun in this project but limited by the time available.

5. RECOMMENDATIONS

1. This work should be repeated earlier in the year when better weather can be expected, and when the algae are in active growth stages.

2. More time should be budgeted to allow for a longer weather window, and further exploration of the data. Multi-spectral classification has not been addressed and several aspects of the work (interpretation of pigment contents, quantitative calibration etc.) have only just begun.

3. An expanded program should include multiple visits to the sites, or a longer field session. Consideration should be given to temporarily installing an image processor on the field barge during the field program, so that a 'tight loop' can be achieved with the botanist(s) and the remote sensing scientist or technician visiting the sites to confirm and correlate digital calculations with on site visual observations. Much of the interpretation for this project was done well after leaving the field and unsupported by field observations. On site image processing of the data at the field camp (at least in a preliminary sense) will be critical to successful operations aimed at quantitative vegetation estimates. Immediate review will confirm data quality, allow close interaction with biologists providing ground truth and interpretation of image data, and provide everyone participating with a better understanding of the potentials, limitations and requirements of remote sensing data.

4. Digital classification methods should be investigated further, with the goal of obtaining Fucus specific indices. This will require more in situ data identifying areas of different types of plants and close coordination between botanists and the remote sensing scientist.

5. If a simple vegetative cover index is sufficient, then it may be possible to use a shorter integration time in the data acquisition phase and achieve much higher spatial resolution. Repeat flights on the same beach at different resolutions should be carried out to assess affect of spectral and spatial resolution on the omission / comission errors.

6. Further consideration should be given to the geometric problems caused by contorted, non-horizontal beach surfaces. It may for instance be possible to acquire aerial photography at the same time, and develop digital terrain maps of the beaches to which the CASI data could be compared. This would allow better comparison of areas on different beaches, but would significantly increase the costs.

7. Knowledge of the tidal stage at the time of imaging each beach will be critical if comparative area estimates are to be obtained. Tidal stage, which obviously affects the cover estimates, could then be better controlled, and better area estimates to be calculated.

8. All beaches should be marked with red marker bouys at each end of the beach and at least 2 transects on the shore. The marker bouys are essential for the pilot and navigator to find the appropriate section of the beach and ensure that the data has been properly collected.

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7. APPENDICES

7.1. SPECIFICATIONS FOR THE BORSTAD ASSOCIATES LTD. COMPACT AIRBORNE SPECTROGRAPHIC IMAGER

Spectral:	coverage	423 to 946 nm
	number of pixels	288
	resolution	1.8 nm
Spatial:	coverage	35 degrees with one camera
	number of pixels	512
	resolution	1.2 mRadian
Modes	spatial	up to 15 spectral bands formed from spectral pixels, but the number of bands governs integration time. The choice of pixels is under software control. A push-broom image 512 pixels wide is formed in each band.
	spectral	spectra covering 423 to 946 nm with 1.8 nm resolution are recorded from up to 39 different 'look directions' across the swath (but number of look directions governs integration time).
Integration times		50 msec (spatial), 100 msec (spectral) typical (depends on signal level)
Digitization		12 bits on chip
Detector type		one 612 x 288 element CCD array
Optics		Reflective grating with F 4 lens
Data recording		Exabyte (TM) digital recording on 8 mm video cassette tapes, 2 Gigabyte/tape 280Kbit sec-1 recorded
Weight		55 Kg
Size	Camera head	15 x 22 x 22 cm
	Instrument Control Unit	42 x 48 x 24 cm
	Video monitor	35 x 46 x 38 cm
Power		110V 2.5A (28V through DC/AC inverters)

7.2. SPECTRAL SIGNATURES OF VERRUCARIA AND OILED ROCKS

While it was not part of the objectives of this study, some data was obtained for oiled rocks and others covered with the encrusting lichen, Verrucaria. The spectral radiance data shows that the color of the oiled rocks is more variable than those with Verrucaria (about 20% standard deviation compared with about 10%). The spectra from the oiled rocks also exhibited a 'red slope' (lower blue returned radiance and higher red radiance) whereas the lichen spectra were very flat (figure 15). This suggests that the two could perhaps be distinguished in image mode. Image data shows that the two can indeed be recognized when side by side, but it is unclear whether in the presence of illumination and shadow variations caused by variation in aspect, this small difference will be sufficient to distinguish algae and oil in the field, or if it will be possible to define an algorithm which will allow definitive separation. Because of a lack of time, this work was not completed.

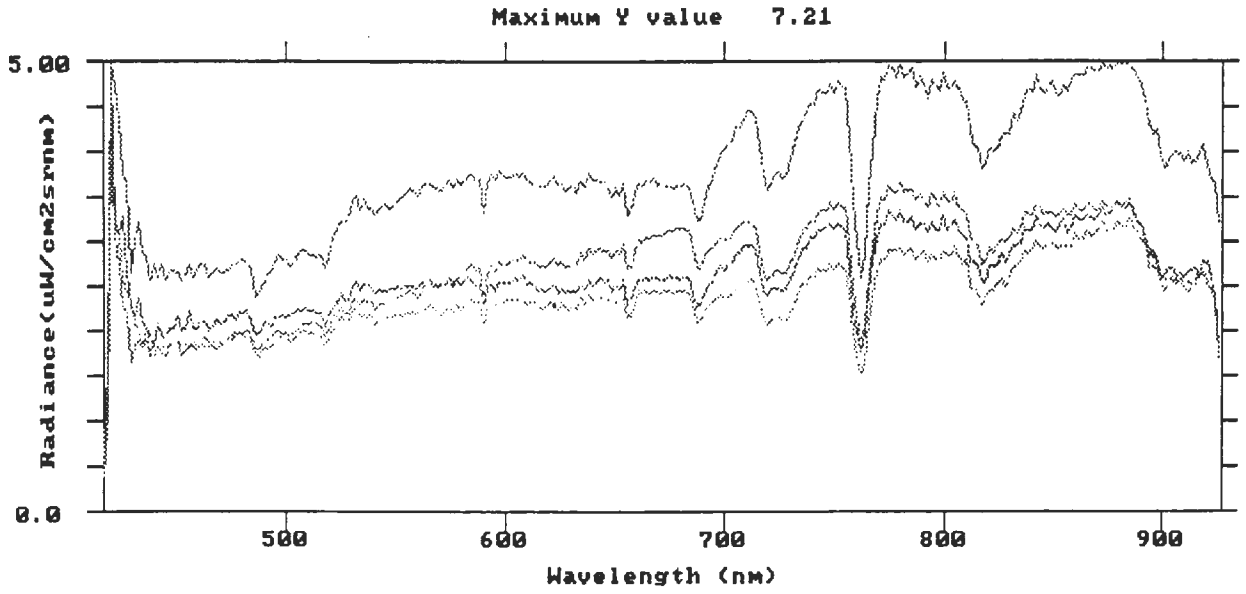

Figure 15. Returned radiance spectra from two oiled rocks (upper curves at 700 nm) and from two rocks with the black lichen Verrucaria found in the spray zone at the extreme upper edge of the intertidal zone.

DRAFT

NATURAL RESOURCE DAMAGE ASSESSMENT

DRAFT STATUS REPORT - NOVEMBER 1990

Petroleum Hydrocarbon-Induced Injury to Subtidal Marine Sediment Resources

Air/Water Study Number 2

Stanley D. Rice and Charles E. O'Clair

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ATTORNEY WORK PRODUCT

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EXECUTIVE SUMMARY

In the first two years following the Exxon Valdez oil spill a total of 2,721 sediment samples were collected from the intertidal and nearshore subtidal regions of Prince William Sound and Gulf of Alaska to as far west as the Shumigan Islands. Gas chromatographic/mass spectrometric analysis is complete for 153 of 393 of those samples collected from 20 locations in Prince William Sound and eight locations outside Prince William Sound in 1989 and submitted to Technical Services Study Number 1 for analysis. Preliminary results indicate that the degree of contamination varied spatially and temporally. Within Prince William Sound subtidal sediments were contaminated by oil at no fewer than 11 sites. Hydrocarbon contamination of sediments had reached a depth of 20 m at at least 7 sites. In or near two heavily contaminated bays petroleum hydrocarbons were detected in sediments at a depth of 100 m.

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 14 ppm total aromatic hydrocarbons, about 50-100 times the concentration of aromatics found in pre-spill, 0-tide level sediments by Karinen and Babcock (Coastal Habitat Study Number 1, 1990 Status Report). Concentrations of total hydrocarbons exceeding 100 µg/g (ppm) 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.

Examination of temporal changes in the contamination of sediments by oil at Sleepy Bay 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. Convincing evidence of this trend awaits analysis of additional sediment samples.

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.

OBJECTIVES

A. Determine occurrence, persistence, and chemical composition of petroleum hydrocarbons in subtidal marine sediments.

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

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

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

INTRODUCTION

Of the approximately 11 million gallons of crude oil released into the marine environment following the grounding of the Exxon Valdez according to the early estimates of Exxon about 18% of the oil had become stranded on the shoreline (Abelson 1989). A proportion of the oil that entered the water (either the original crude oil derived from the spill, oil leaching from contaminated shorelines, and/or oil dispersed into receiving waters via shoreline remediation procedures) was expected to reach subtidal sediments as a result of physical (Boehm et al. 1987) and biological processes. Preliminary analysis using ultra-violet fluorescence spectrophotometry of about 70% of the sediment samples collected under Air/Water Study Number 2 in 1989 indicated that in bays where Exxon Valdez oil heavily contaminated the intertidal region petroleum hydrocarbons may have been transported as deep as 20 m below mean lower low water by mid-July 1989 (Science Applications International Corporation 1989). Here we report preliminary results of the gas chromatographic/mass spectrometric analyses conducted on a subset of those samples as well as on some additional samples collected in spring and fall 1989.

Three projects formerly funded under Air/Water Study Number 4 were included in the present study in 1990. The first was conducted by the Alaska Department of Environmental Conservation and assessed the potential for 'in situ' biodegradation of selected hydrocarbon substrates at various sites within and outside of Prince William Sound. Coupled with data on 'in situ' hydrocarbon concentrations, the microbial data will allow determination of the absolute hydrocarbon removal rates by naturally occurring hydrocarbon-degrading bacteria. The second project conducted by the Environmental Conservation Division of the Northwest Fisheries Center was designed to screen sediments for petroleum hydrocarbons

using ultra-violet fluorescence spectrophotometry and assessed the toxicity of marine sediments using the luminescent marine bacterium Photobacterium phosphoreum to test for aqueous toxicants (Schiewe et al. 1985). The third project titled "Injury to Deep Benthos" was conducted by the University of Alaska and examined the responses of infaunal communities below a depth of 20 m to the oil spill. The present study also coordinated with the subtidal project of Coastal Habitat Study Number 1 in eelgrass habitats. This report covers only those objectives listed above. Results of the three other projects encompassed by Air/Water Study Number 2 will be reported elsewhere.

STUDY METHODOLOGY

Sediments were sampled at 25 sites in Prince William Sound 11 reference sites and 14 contaminated sites; Table 1). Sampling was conducted during three periods (31 May to 9 Jun, 27 June to 23 July and 4-16 September; Table 1). Six sites were the same as those sampled by the subtidal project of Coastal Habitat Study No. 1. Outside Prince William Sound 8 sites were sampled. These sites were distributed such that 6 sites were on the Kenai Peninsula and 2 sites were near Kodiak Island (Table 2). The sites were sampled in July/Aug.

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 depths of 3, 6 and 20 m below MLLW in Jun and September and at 3, 6, 20, 40 and 100 m in June/July. 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. The subtidal project of Coastal Habitat Study Number 1 sampled sediments, infauna and epifauna in the same depth range at six of the Prince William Sound sites sampled by the present study. Samples taken at depths below 20 m were collected a Smith-McIntyre grab. We had attempted to collect sediments with the Haps corer on a number of occasions, but failed to obtain adequate samples each time and were forced to resort to the Smith-McIntyre grab instead. 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 benthos was collected (the deep benthos part of this study is reported sepatately elsewhere), however sediments were not taken from the same grab as the benthos samples because the volume removed for sediment hydrocarbon analysis 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 as to whether the sediments submitted for hydrocarbon analysis were contaminated with petroleum hydrocarbons.

STUDY RESULTS

A total of 2,721 sediment samples 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 two years following the Exxon Valdez oil spill. We have submitted 393 sediment samples 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 in 1989 from the intertidal region and from subtidal depths at selected sites in Prince William Sound and along the Kenai and Alaska Peninsulas (Figures 1 and 2). We used results of ultra-violet fluorescence spectrophotometric analysis performed on 837 of the 1,160 samples collected under Air/Water Study Number 2 in 1989 (SAIC, 1989) to help us choose which samples should be submitted for analysis by GC/MS. Hydrocarbon analysis is complete for 113 samples submitted from Prince William Sound and 40 samples

submitted from the Kenai and Alaska Peninsulas (Tables 3 and 4). Because only a small percentage (5.6%) of the samples collected to date have been analysed our results at this stage are preliminary.

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 (Table 5). In five localities sediments were contaminated at all subtidal depths sampled down to 20 m. Two bays (Northwest and Sleepy Bays) suffered relatively low level contamination at 100 m, although only one of three samples collected at 100 m at Sleepy Bay showed unequivocal hydrocarbon contamination (Table 5).

The highest level of total hydrocarbons (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 5, Figure 3a). Concentrations of total hydrocarbons 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 5, Figure 4a).

Concentrations of total hydrocarbons in benthic sediments decreased rapidly below mean lower low water generally reaching levels below 50 μg/g wet weight at 6 m at most sites in Prince William Sound for which results are currently available (Table 5, Figures 3a, 4a and 5a). There may have been a tendency for total hydrocarbon concentrations to rebound to higher levels between 40 and 100 m at Northwest and Sleepy Bays. This trend was illustrated more clearly when we examined changes in the concentration of dibenzothiophenes (one of the groups of compounds characteristic of petrogenic hydrocarbons) at these two sites (Figures 3b and 5b). However, only one of the three samples analysed from 100 m at Sleepy Bay showed unequivocal contamination by petroleum hydrocarbons. The other two showed questionably positive evidence of contamination. More samples must be analysed from Northwest Bay to conclusively support the trend there. The increasing trend in hydrocarbons between 40 and 100 m may also have occurred at Herring Bay, but the hydrocarbons were apparently not petrogenic (Figure 4).

The analytical results available suggest support for a hypothesis that hydrocarbon contamination tended to move to greater depths over the first seven months following the oil spill. Data from Sleepy Bay best support the hypothesis because the data are most complete from that site for the appropriate time period. The concentration of total hydrocarbons in sediments from 0 m seemed to decrease between May and July (Figure 6). The temporal pattern of increase or decrease in total hydrocarbon concentration at 3, 6 and 20 m was consistent with a trend of movement of oil to greater

bathymetric depths with time except for the apparent decrease in hydrocarbon concentration between May and July at 6 m (Figure 6).

Outside Prince William Sound subtidal sediments showed evidence of contamination by petroleum hydrocarbons at seven of eight sites for which analytical results are currently available (Figures 2 and 7). 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). Statistical analysis of between site differences in hydrocarbon contamination with depth awaits hydrocarbon analysis of additional samples.

STATUS OF INJURY ASSESSMENT

Because results of the GC/MS analyses supplied by Technical Services Study Number 1 are available for only a small fraction of the number of samples we have collected we cannot draw comprehensive conclusions about either the geographical or bathymetric distribution of oil in subtidal sediments inside or outside Prince William Sound. Nor can definitive statements be made on the temporal changes in distribution or the persistence of oil in subtidal sediments in the study area. We can, however, set a lower limit on the number of locations that suffered contamination of subtidal sediments by petroleum hydrocarbons. We can also identify at least one possible temporal trend in hydrocarbon distribution that warrants future examination when more samples have been analysed (see Study Results section). The lack of completed results on the hydrocarbon analysis is the chief limitation to the completion of the objectives of Air/Water Study Number 1 at this time. We have requested of Technical Services Study Number 1 that an additional 795 samples be analysed by GC/MS. When the analyses of these samples is complete we should have enough data to support a reasonably complete story about the temporal and spatial distribution of oil in subtidal sediments in Prince William Sound in 1989 and 1990. However, we will still not have enough data to draw definite conclusions about the distribution of oil in sediments outside Prince William Sound.

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Table 1

Location of sites in Prince William Sound where intertidal and subtidal sediment samples were collected in 1990. Letters in body of table indicate depths sampled: A = 0, 3, 6 and 20 m; B = 0, 3, 6, 20, 40 and 100 m; C = 6 m; D = 0 and 6 m; E = 0, 3, 6, 20 and 40.

Location	North Latitude o ' "	West Longitude o ' "	Depth Code Jun	Jul	
Bay of Isles	60 23 00	147 44 54	A	B	A
Block Island	60 31 49	147 26 36	A	B	A
Chenega Island	60 19 49	148 00 24	A	B	A
Disk Island	60 29 55	147 39 40	A	B	A
Drier Bay	60 19 12	147 44 00	-	B	C
Eshamy Bay	60 26 54	147 58 30	D	-	D
Ewan Bay	60 22 00	148 08 00	D	-	D
Fox Farm	59 58 26	148 10 30	A	B	A
Green Island	60 16 18	147 26 18	A	B	A
Herring Bay	60 25 51	147 47 06	A	B	A
Iktua Bay	60 06 00	147 59 42	D	-	D
Lower Herring Bay	60 24 12	147 47 48	-	B	C
MacLeod Harbor	59 53 43	147 45 48	A	B	A
Moose Lips Bay	60 12 30	147 18 06	-	E	-
NE Knight Island	60 26 21	147 37 39	A	B	A
Northwest Bay	60 33 07	147 34 36	A	B	A
Olsen Bay	60 45 05	146 11 13	A	B	A
Paddy Bay	60 25 00	148 06 00	D	-	D
Port Fidalgo	60 50 12	146 12 35	A	B	A
Rocky Bay	60 20 19	147 07 59	A	B	-
Sleepy Bay	60 04 01	147 50 11	A	B	A
Smith Island	60 31 47	147 20 45	A	B	A
Snug Harbor	60 15 46	147 45 55	A	B	A
West Bay	60 51 53	146 46 31	A	B	A
Zaikof Bay	60 16 53	147 02 19	A	B	-

Table 2

Location of sites outside Prince William Sound where intertidal and subtidal sediment samples were collected in 1990. Letters in body of table represent depths sampled: A = 0, 3, 6, 20, 40 and 100 m below mean lower low water; B = 0, 6, 20, 40 and 100 m; C = 0, 6, 20 and 100 m.

Location	North Latitude o ' "	West Longitude o ' "	Date	Depth Code	Sample Size
Agnes Cove	59 46 00	149 34 24	25 Jul	A	18
Black Bay	59 32 07	150 12 17	26 Jul	B	15
Chugach Bay	59 11 12	151 37 48	25 Jul	A	18
Hallo Bay	58 27 29	154 00 14	3 Aug	A	18
Katmai Bay	57 55 00	155 05 00	3 Aug	A	18
Sunny Cove	59 56 12	149 19 06	24 Jul	A	18
Tonsina Bay	59 18 42	150 55 00	25 Jul	A	18
Windy Bay	59 13 50	151 31 00	5 Aug	C	15
				Total	138

Table 3

Location, date of sampling and depth of collection of samples collected in Prince William Sound for which hydrocarbon analysis is complete. All samples were collected in 1989.

Location	North Latitude o ' "	West Longitude o ' "	Date	Depth Code[1]	Sample Size[2]
Applegate Island	60 37 40	148 08 19	29 Nov	A	1
Bay of Isles	60 22 37	147 42 27	12 Nov	A	1
	60 22 53	147 42 45	13 Nov	B	4
Block Island	60 31 49	147 36 10	10 Nov	A	1
	60 31 49	147 36 26	30 Nov	A	1
Chenega Island	60 19 49	148 00 24	19 Nov	A	1
Fox Farm	59 58 26	148 10 30	1 Jul	C	5
Green Island	60 17 57	147 25 06	16 Nov	B	4
Ingot Island	60 31 41	147 39 09	25 Nov	B	4
Helen Point	60 09 48	147 45 21	18 Nov	A	1
Herring Bay	60 25 51	147 47 06	9 May	D	6
			14 Jul	E	8
			7 Sep	F	4
	59 26 34	147 50 16	20 Nov	B	4
	60 29 20	147 43 07	23 Nov	A	1
	60 26 34	148 47 12	24 Nov	A	1
Lone Island	60 41 48	147 44 48	8 Dec	A	1
NE Knight Island	60 26 21	147 37 44	11 Nov	A	1
Northwest Bay	60 33 07	147 34 36	12 Jul	G	12
			6 Sep	H	3
	60 33 03	147 34 42	26 Nov	B	4
	60 32 37	147 36 09	7 Dec	I	2
Port Fidalgo	60 50 17	146 16 30	3 Dec	A	1
Rua Cove	60 20 55	147 38 27	14 Nov	A	1
Sleepy Bay	60 4 01	147 50 11	5 May	D	6
			16 Jul	J	15
			9 Sep	K	3
			17 Nov	B	4
Smith Island	60 31 48	147 20 49	4 Dec	B	4
Snug Harbor	60 14 23	147 45 07	6 Jul	K	3
	60 14 13	147 43 58	15 Nov	B	4
Two Moon Bay	60 44 00	146 34 24	2 Dec	I	2
				Total	113

1. Letters indicate depths at which completed samples were collected: A = 3 m below mean lower low water; B = 3, 6, 10 and 20 m; C = 0, 3, 6, 20 and 40 m; D = 0 and 6 m; E = 0, 3, 6, 20, 40 and 100 m; F = 0, 3, 6 and 20 m; G = 0, 3, 6, 12, 20, 30, 40 and 100 m; H = 3, 6 and 20 m; I = 3 and 20 m; J = 3, 6, 20, 40 and 100 m; K = 0, 3 and 6 m.
2. Number of samples for which hydrocarbon analysis is complete.

Table 4

Concentration of total hydrocarbons (THC) in samples from 13 sites in Prince William Sound determined to unequivocally contain petrogenic hydrocarbons by Technical Services Study Number 1. Data from two reference (control) sites are included for comparison. Samples from neither site unequivocally contained petrogenic hydrocarbons. All samples were collected in 1989.

Location	Date	Depth (m)	THC µg/g
Reference Sites			
Port Fidalgo	3 Dec	3	15.7
Two Moon Bay	2 Dec	3	17.5
		20	17.6
Oiled Sites			
Bay of Isles	12 Nov	3	43.2
	13 Nov	3	32.8
		6	120.6
		10	29.9
		20	27.2
Block Island	30 Nov	3	111.7
Fox Farm	1 Jul	0	31.3
		3	15.2
		6	14.8
Green Island	16 Nov	3	22.4
		6	36.0
		20	10.2
Herring Bay	9 May	0	102.3[a]
		6	25.1[a]
	14 Jul	0	259.4[b]
		3	75.5[b]
	7 Sep	0	139.8
		3	50.9
	20 Nov	3	46.2
		6	20.3
		10	22.8
		20	25.5
	23 Nov	3	27.2
Ingot Island	25 Nov	6	31.1
		10	35.1
		20	26.4
Northwest Bay	12 Jul	0	507.0
		3	81.7[b]
		6	56.5[b]
		12	34.9
		20	31.5[b]
		100	12.4
	6 Sep	3	116.4

Table 4 (cont.)

Location	Date	Depth (m)	THC µg/g
Northwest Bay	6 Sep	6	133.1
		20	54.1
	26 Nov	10	307.6
	7 Dec	3	31.4
		20	34.4
Rua Cove	14 Nov	3	41.0
Sleepy Bay	5 May	0	21.8[a]
		6	30.3[a]
	16 Jul	3	24.3[a]
		6	13.8[a]
		100	14.9[a]
	9 Sep	3	49.3
		6	37.0
	17 Nov	3	28.9
		6	70.2
		10	41.2
		20	37.2
Smith Island	4 Dec	3	27.1
		10	16.4
Snug Harbor	6 Jul	0	59.9
	15 Nov	3	17.2
		6	14.5
		10	18.5
		20	25.8

a. Mean of three measurements.
b. Mean of two measurements.

Table 5

Location, date of sampling and depth of collection of samples collected outside Prince William Sound for which hydrocarbon analysis is complete. All samples were collected in 1989.

Location	North Latitude o ' "	West Longitude o ' "	Date	Depth Code[1]	Sample Size[2]
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
				Total	40

1. Letters indicate depths at which completed samples were collected: A = 0, 3, 6, 20, 40 and 100 m below mean lower low water; B = 20, 30 m; C = 40, 100 m.
2. Number of samples for which hydrocarbon analysis is complete.

Figure Legends

Figure 1. Distribution of study sites in Prince William Sound sampled in 1990. Numbers with asterisks indicate locations for which hydrocarbon analysis is complete on selected 1989 samples. See Table 1 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. 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. 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. Change in the concentration of hydrocarbons with bathymetric depth at Sleepy Bay. 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 6. Concentration of total hydrocarbons at various bathymetric depths and sample periods at Sleepy Bay. Error bars are one standard error of the mean. Numbers in the key refer to bathymetric depth in meters.

Figure 7. 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.

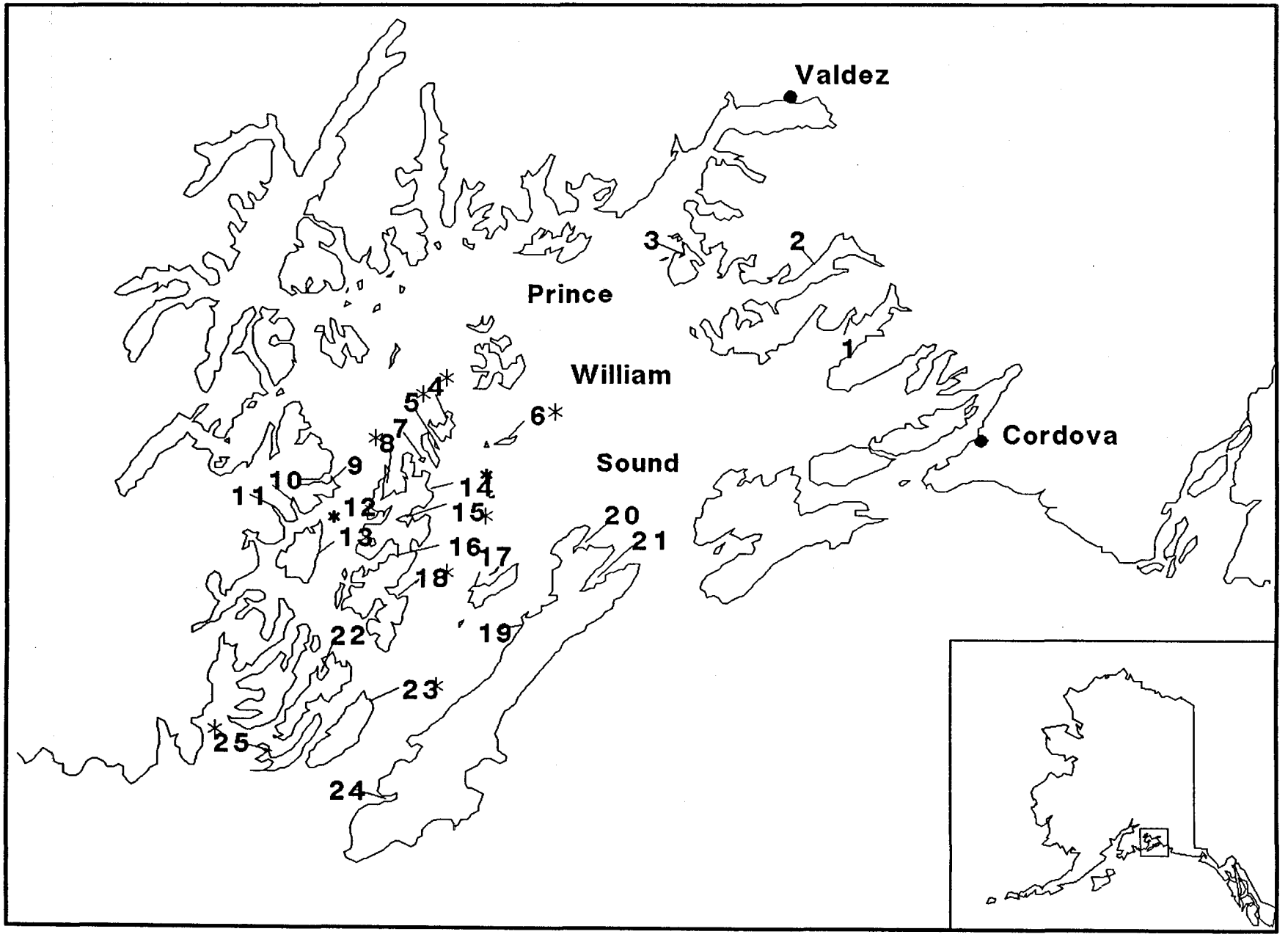

Figure 1

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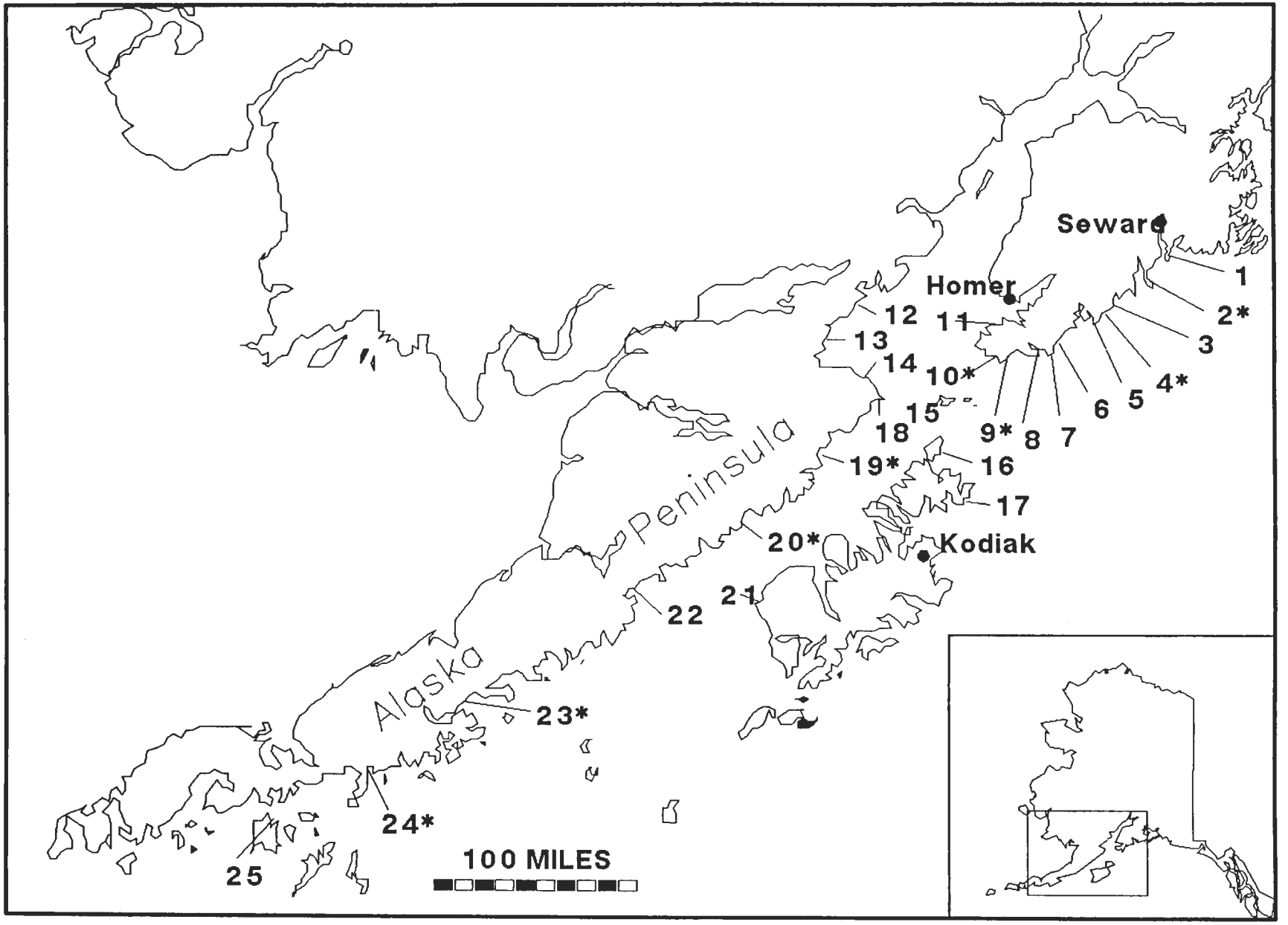

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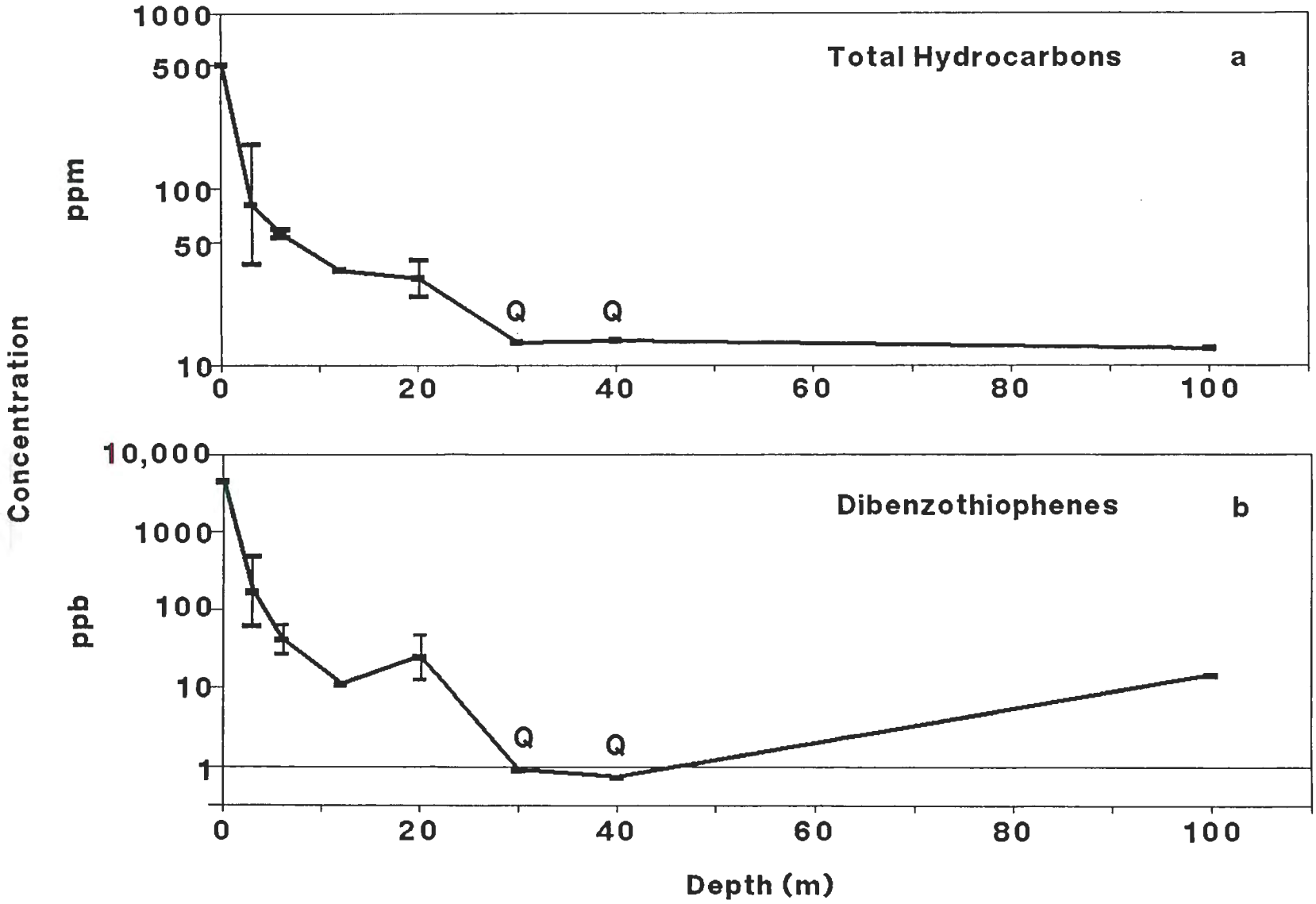
Figure 2



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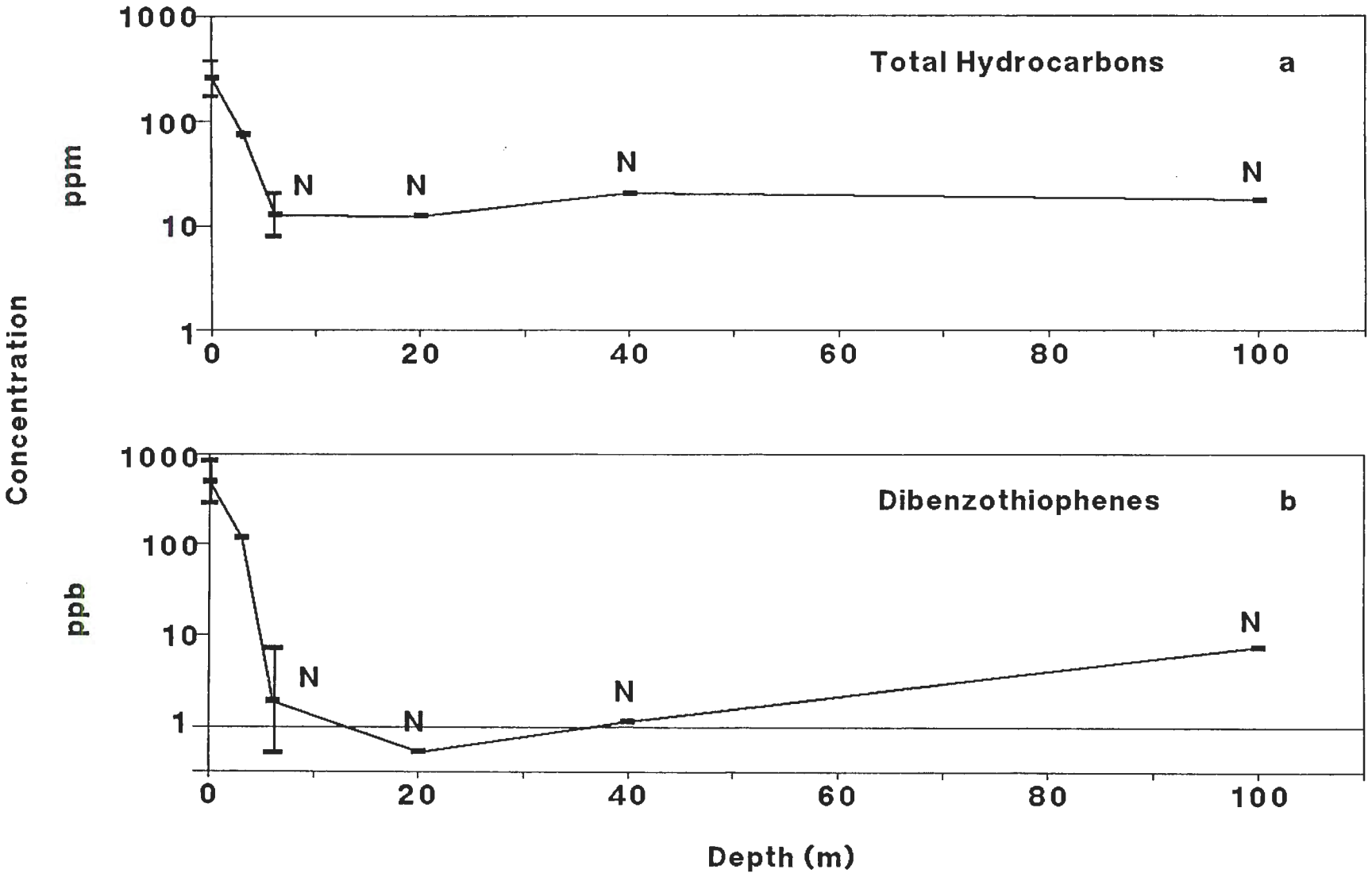

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Figure 4

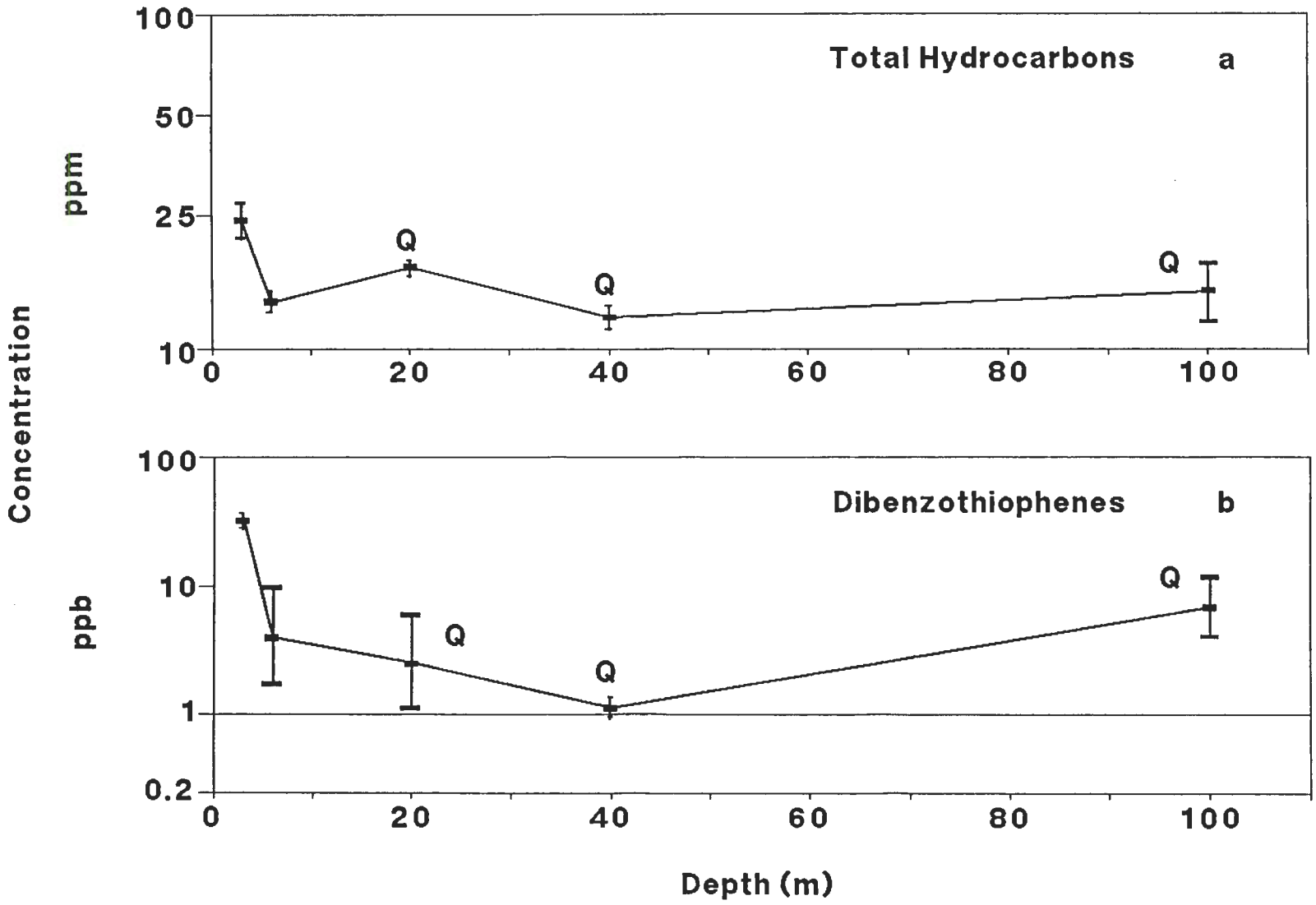

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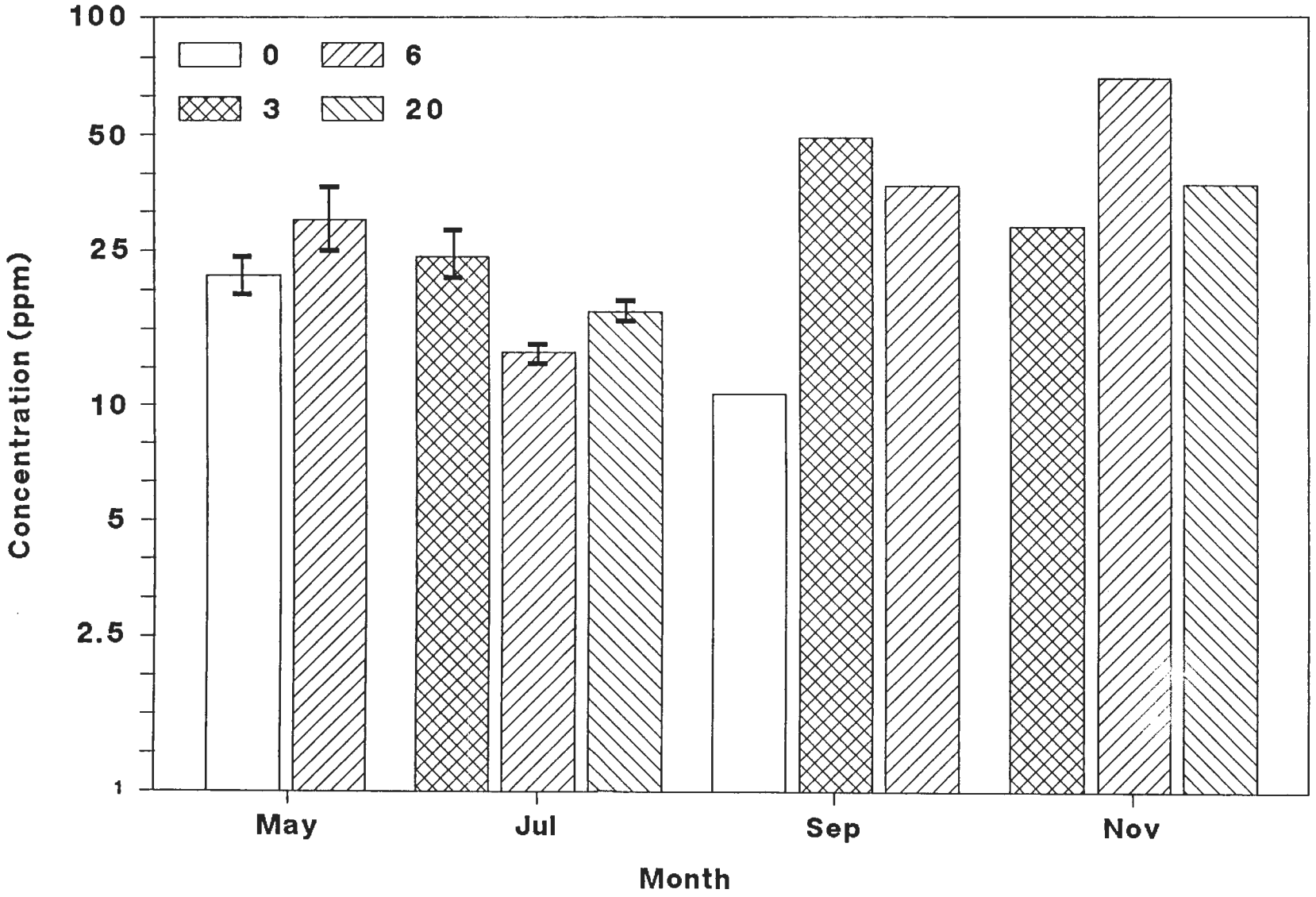
Figure 5



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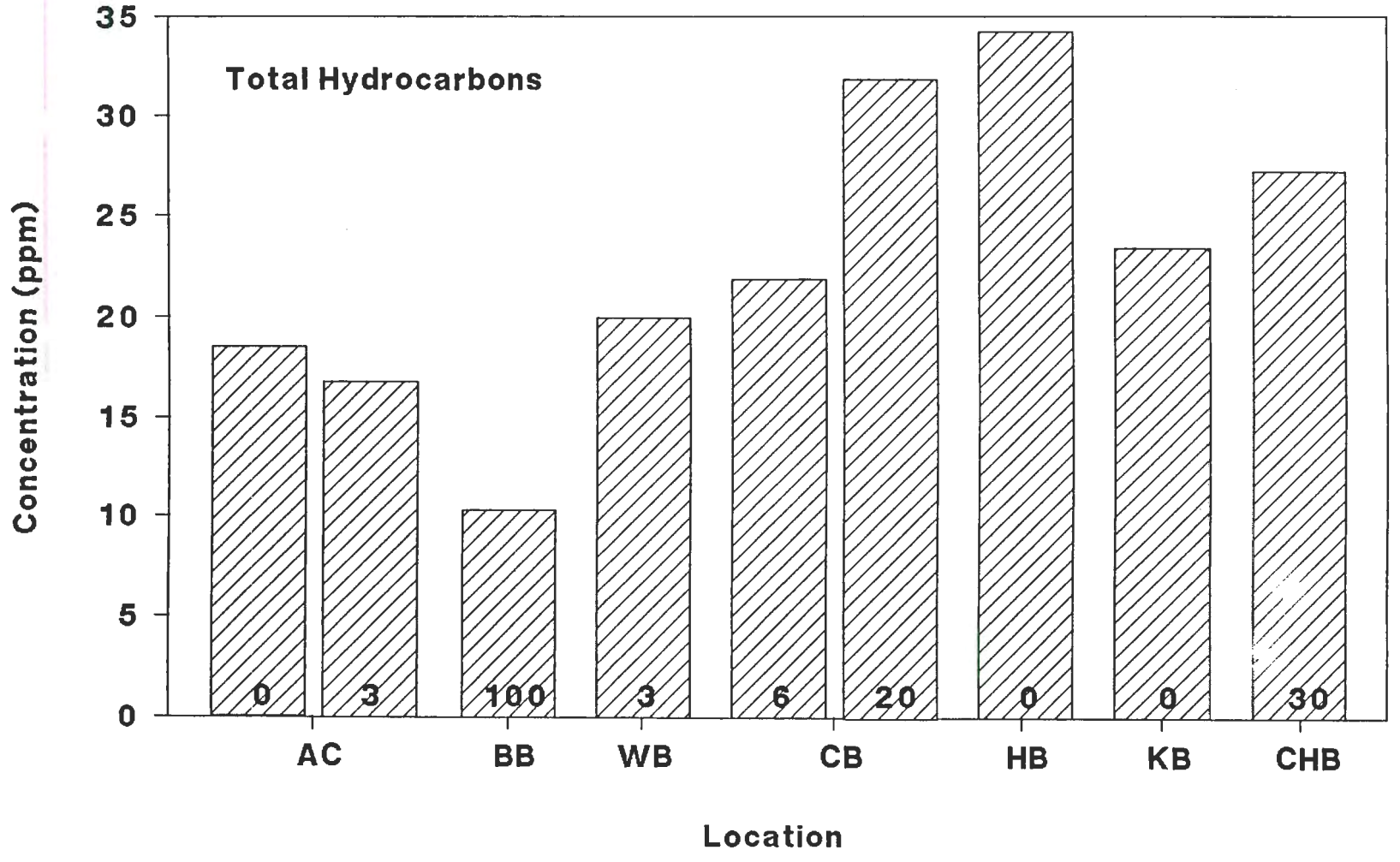

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Figure 7

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INJURY TO DEEP BENTHOS

AIR/WATER NO. 2
PROJECT NUMBER 2109

Project Leader: Howard M. Feder

Status Report 1990

TABLE OF CONTENTS

EXECUTIVE SUMMARY

Benthic samples were collected by van Veen grab from 7 oiled and 7 unoiled (reference) bays in July 1990. Stations were occupied on a transect at 40, 60-100, and > 100m within bays known to contain sea grass (*Zostera*) beds. The stations at six of the bays were below the 20 m stations occupied by the S. Jewett diving group. Samples were screened through nested 1.0 and 0.5 mm mesh screens. At all stations sediment samples for hydrocarbons were collected by NOAA.

The 40 m station samples from the 1.0 mm and some 0.5 mm fractions of six bay sites, 3 oiled (Herring Bay, Disk Island, Snug Harbor) and 3 reference (Zaikof Bay, Rocky Bay, West Bay), were sorted, data entered into the computer, and various analyses completed, including ANOVA of taxon abundance data. The remaining eight bay samples (1.0 and 0.5 mm fractions) at 40 m are either completely analyzed now or will be finished by early February 1991. At this time, data from all 40 m stations will be examined statistically. The deep stations will also be processed and analyzed when the 40 m stations are completed.

The Herring Bay oiled site, based on pooled 1.0 and 0.5 mm samples, had number of taxa (T), abundance (A), and biomass (B) values suggestive of a moderately disturbed area. This disturbance is also suggested by the high A/T ratio and low B/A ratio, conditions indicative of disturbance. The K-dominance curve for this site is of a moderately disturbed site. ANOVA of the abundance data for dominant taxa within this bay, as compared to Zaikof Bay (reference site; also with 1.0 and 0.5 mm samples analyzed) indicated statistically significant differences for 13 dominant taxa between the bays. Most of these taxa were more abundant in Herring Bay, and many juveniles were present. The latter points suggest the presence of opportunistic species.

Interbay comparisons of abundance of dominant taxa within each of the six bays with ANOVA are similar to comparisons between the pooled 1.0 and 0.5 mm samples for Herring and Zaikof Bays. Based on the interbay ANOVA, most of the dominant taxa are significantly more abundant in two of the oiled bays (Herring and Disk Island) than in any of the reference bays. The other oiled bay, Snug Harbor, had only a few taxa with higher abundance values than found at the reference sites.

The three pooled oiled and three pooled unoiled bays compared by ANOVA showed fifteen dominant taxa with significant differences between oiled and unoiled bays. In general, the comparative analysis of pooled bays supports results of the other ANOVAs completed for abundance data. Most of the dominant taxa were more abundant in the oiled bays.

OBJECTIVES

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

B. To determine if changes occurred in the benthos as determined by comparing taxon (primarily determined at the Family level: see Methods) richness and diversity, abundance and biomass, and trophic composition of the 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 how much time is required for the benthos to recover to a 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) that live on and within subtidal sediments represent good in situ monitors for measuring the effects of oil fluxing to the bottom (for example see Cabioch et al. 1978, Kineman et al. 1980, and Sanders et al. 1980. These organisms typically remain close to or at the site of larval settlement and, consequently, represent good monitoring tools. 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 et al. 1980; Kineman et al. 1980; 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. Benthic data collected in polluted waters

elsewhere suggest that changes in species number, abundance, biomass, and diversity can be expected if sizable amounts of oil settle to the bottom. Changes in bottom fauna within the Sound could have serious trophic implications since benthic invertebrates are important food resources for commercially important bottom-feeding species such as pandalid shrimps, crabs, bottomfishes, and sea otters. Further, the 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.

Sampling of subtidal benthic populations should be continued for at least five years to assess the effects of oil on subtidal benthic communities as related to the redistribution of oil-laden sediments from adjacent onshore sites (i.e., the seagrass beds located alongshore in all of the bays sampled). Seagrass beds contain fine, unconsolidated sediments that are moved offshore by wave action and current transport. Oil that initially coated sediments in shallow water may eventually be transported offshore as a result of this redistribution and thus contribute to long-term effects on subtidal benthic fauna. This process 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 and microbiological sampling by other research components in the Air/Water studies suggest that oil is present at some of the subtidal stations along initially oiled seagrass areas.

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, and trophic structure?"

STUDY METHODOLOGY

Sampling

Five replicate samples were collected 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. All stations sampled were at approximate depths of 40, 100, and >100 m on a transect extending below seagrass (Zostera) beds within each of the identified bays. The intertidal and shallow subtidal stations on the transect were sampled for benthic biota by C. O'Clair and S. Jewett, respectively. A total of 44 deep stations x 5 replicates were collected on a single cruise in early July 1990 in conjunction with microbiological and hydrocarbon sampling projects underway from the same ship platform (Table 1). Benthic samples at oiled and reference sites were collected on bottoms that were as physically similar as possible, based on chart data and some preliminary grab samples accomplished before actual sampling occurred. However, insufficient pre-July ship time made it impossible to sample all bays and all stations within the bays prior to the July cruise. Thus, dissimilarities in substrate type were apparent at some of the occupied stations. Additionally, at a few of the sites it was not possible to find a suitable substrate at the deepest stations of the transects. The seven oil-exposed sites sampled for deep benthos were Northwest Bay, Disk Island, Herring Bay, Bay of Isles, Snug Harbor, Chenega Bay and Sleepy Bay. The seven unexposed reference sites sampled for the deep benthos were West Bay, Rocky Bay, Zaikof Bay, MacLeod Harbor, Lower Herring Bay, Drier Bay, and Moose Lips Bay.

The sampling plan outlined above was determined 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 March 7, 1990 (copies sent to Chuck O'Clair/Stan Rice, NOAA and Roy Nowlin, ADF&G), it was agreed that 12 paired bays would be occupied. However, it was possible on the July 1990 cruise, following consultation with the Chief Scientist, to collect samples at one additional pair of bays (i.e., one oiled and one unoiled).

The benthic biological samples were collected with a $0.1m^2$ van Veen grab weighted with 31.7 kg of lead. Five replicate samples were taken. Material from each grab 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 were initially to be washed only 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.

Analysis and Processing of Data

Although the addition of two more bays (i.e., 14 bays rather than the original 12 bays selected at the Seattle meeting) to the analysis will require additional time for sorting and identification, it was decided that information from the extra

Table 1. Station locations, approximate depths, and substrate type from Prince William Sound sites sampled 1-23 July 1990 on the NOAA Ship Davidson. O=Oiled Bay; R=Reference, Unoiled Bay; H=Historical Site (collected in 1989).

Station/Number	Depth (m)	Substrate	Latitude	Longitude
Smith Island (H) SI 001	51-53	Gray Mud, Shell	60° 32.2'	147° 20.6'
Zaikof Bay (R) ZB 001	40-44	Gray, Mud, Shell, Rock	60° 16.5'	147° 05.2'
Zaikof Bay (R) ZB 002	96-101	Gray Mud, Shell	60° 19.1'	147° 00.1'
Zaikof Bay (R) ZB 003	93-103	Gray Mud, Shell	60 19.9	146° 55.4'
Rocky Bay (R) RB 001	39-40	Sandy Mud, Shell, Rock	60° 20.8'	147° 06.6'
Rocky Bay (R) RB 003	65-73	Gray Mud, Shell, Rock	60° 21.5'	147° 03.9'
Rocky Bay (R) RB 002	97-100	Gray Mud, Shell, Rock	60° 21.2'	147° 04.9'
West Bay (R) WB 001	36-41	Black Mud, H2S, Shell	60° 52.3'	147° 47.9'
West Bay (R) WB 002	97-122	Gray Mud	60° 52.5'	146° 50.6'
West Bay (R) WB 003	140-167	Gray Mud	60° 53.3'	146° 50.3'
Herring Bay (O) HB 001	38-60	Brown Mud, Gravel, Rock	60° 26.7'	147° 46.8'
Herring Bay (O) HB 002	87-106	Brown Mud, Gravel, Rock	60° 27.5°	147° 46.3'
Herring Bay (O) HB 003	139-164	Gray Mud, Gravel, Rock	60° 28.3'	147° 45.2'
Disk Island (O) DI 001	43-45	Brown Mud, Gravel, Rock, Shell	60° 30.2'	147° 39.9'
Disk Island (O) DI 002	98-109	Gray Mud, Rock	60° 30.6'	147° 41.1'
Disk Island (O)	No Suitable	Bottom Deeper	-	- -
Block Island (H) BI 001	104-114	Gray Mud, Rock	60° 32.7'	147° 37.9'
Northwest Bay (O) NWB 001	37-42 m	Brown Mud, Org. Deb. Rock, Gravel, Sand	60° 33.3	147° 34.6'
Northwest Bay (O) MWB 002	122-128	Gray Mud, Rock,	60° 34.0'	147° 35.3'
Northwest Bay (O) NWB 003	146-190	Gray-Brown Mud, Rock, Gravel	60° 34.5'	147° 36.4'
Northeast Knight(H) NEK 001	71-105	Gray-brown Mud, Rock, Gravel	60° 26.7'	147° 36.5'
Bay of Isles (O) BOI 001	41-42	Brown-Mud, Sand, Rock, Gravel	60° 22.8'	147° 42.0'
Bay of Isles (O) BOI 002	92-100	Black Mud, H2S	60° 23.8'	147° 41.3'

Table 1. Continued

Station/Number	Depth (m)	Substrate	Latitude	Longitude
Bay of Isles (0) BOI 002	92-100	Black Mud, H2S	60° 23.8'	147° 41.3'
Bay of Isles (0) BOI 003	152-159	Gray Mud	60° 24.9	147° 35.4'
Green Island (H) GI 001	94-103	Gray Mud, Rock, Gravel	60° 18.1'	147° 29.5'
MacLeod Harbor (R) MCH 001	40-41	Black Sand, Shell	59° 53.0'	147° 47.7'
MacLeod Harbor (R) MCH 002	95-102	Black Sand, Shell	59° 54.2'	147° 51.1'
MacLeod Harbor (R) MCH 003	149-153	Black Sand, Shell	59° 55.4'	147° 51.2'
Moose Lips Bay (R) MLB 001	37-41	Brown Mud, Shell, Rock, Gravel	60° 12.5'	147° 22.4'
Moose Lips Bay (R) MLB 002	96-100	Gray Mud, Shell, Rock, Gravel	60° 11.5'	147° 25.7'
Moose Lips Bay (R) MLB 003	No Appropiate Bottom Deeper		-	-
Snug Harbor (O) SH 001	42-44	Black Mud Shell, Rock, Gravel	60° 15.4'	147° 45.0'
Snug Harbor (O) SH 002	102-105	Gray Mud	60° 15.2'	147° 43.2'
Snug Harbor	No Appropiate Bottom Deeper			
Chenega (O) CHN 001	38-51	Brown Mud, Shell	60° 19.8'	148° 00.0'
Chenega (O) CHN 002	76-99	Brown Mud Shell, Rock, Gravel	60° 19.9	148° 00.3'
Chenega (O) CHN 003	163-182	Gray Mud, Rock, Gravel	60° 19.4'	147° 59.9'
Lower Herring B. (R) LHB 001	39-42	Brown Mud, Shell, Wood	60° 23.8'	147° 48.0'
Lower Herring B. (R) LHB 002	95-120	Brown Mud, Rock, Gravel	60° 23.2'	147° 48.6'
Lower Herring B. (R) LHB 003	143-154	Gray Mud	60° 23.0'	147° 48.9'
Drier Bay (R) DB 001	39-40	Brown Mud, Shell, Rock, Gravel	60° 19.7'	147° 45.3'
Drier Bay (R) DB 002	101-105	Gray Mud, Rock, Gravel	60° 19.1'	147° 47.6'
Drier Bay (R) DB 003	144-145	Gray Mud	60° 18.8'	147° 49.2'
Sleepy Bay (O) SLB 001	35-38	Gray Mud, Gravel	60° 04.9'	147° 50.3'
Sleepy Bay (O) SLB 002	103-107	Gray Mud, Rock, Gravel	60° 04.7'	147° 49.4'
Sleepy Bay (O) SLB 003	151-158	Gray Mud, Rock, Gravel	60° 05.1'	147° 49.8'
Fox Farm (H) FF 001	85-97	Black Sand, Shell, Gravel	59° 59.7'	148° 09.8'

sites is too valuable to be unavailable to the analysis. Thus, all inter-bay comparisons will ultimately be made with data from 14 bays. Based on time and monetary constraints, and in consultation with Roy Nowlin of ADF&G, primarily material from the 1.0 mm screened samples are currently being processed. It would be impossible, given the preceding constraints, to complete the 14 bay comparisons by the end of the funding year. However, prior to this decision the 0.5 mm fractions were sorted from Herring and Zaikof Bays, and the preliminary analysis of these data will be included in this report. The 0.5 mm fractions were subsequently sorted from two additional sites, Snug Harbor and Disk Island. Should preliminary analyses of these data be unavailable to this report, they will be submitted separately at the December 1990 meeting in Anchorage. The valuable material in the balance of the 0.5 mm samples will ultimately be sorted and made available to the project during the next fiscal year.

Material from historical stations collected on the R/V Alpha Helix just prior to and after the spill will be processed as time permits using the same procedures described below for samples collected from the 14 bays.

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. 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 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 will ultimately be sorted, it is appropriate to include larger representatives of the meiofauna 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 0.5mm 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.

Based on a conference call in July 1990 with the Peer Review Team on the project, a decision was made to speed up sample processing by identifying organisms to the family or higher taxonomic level. Earlier analyses of samples obtained at some study sites shortly after the Exxon Valdez oil spill indicated that species diversity was typically high. It was estimated that the time necessary to determine taxa to species level for the samples would result in a multifold increase in hours spent in sorting and taxonomic

identification. Nevertheless, generic and specific designations of common species will be included in the raw data sheets and the computer printouts whenever these categories are known. 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 will be calculated (See Appendix A for further details on methodology), and biomass values will be reported in wet weight and carbon weight.

Data will be 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. All calculations of the data will be based on taxonomic determination made to the family or other higher taxonomic levels.

Numerical Analysis

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

A Kruskal-Wallis and a multiple comparison test for significance will be used to test for differences in the total abundance and biomass between the stations sampled in 1992 and in subsequent multiyear data sets. These same tests will be made on the abundance and biomass of selected, dominant taxa at stations between years. The taxa will be chosen from the rank abundance and biomass printouts for each station, and will be those commonly present within bays being compared. However, taxa that are common at stations within unoiled bays, but rare or missing at stations within oiled bays, will also be tested. Other statistical tests (e.g., ANOVA) will be used to examine differences in abundance of dominant taxa between stations at similar depths within unoiled and oiled bays, (1) Herring Bay and Zaikof Bay (1.0 + 0.5 mm fractions), (2) the fourteen individual (1.0 mm fractions only) and (3) the pooled oiled and unoiled bays. Coordination with the

"Coastal Habitat Assessment Program-Subtidal Habitats" of S. Jewett will be accomplished so that similar statistical testing procedures are applied to benthic data for both programs. Statistical procedures applied are included in Appendix B.

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 (is 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 is a relatively recent technique 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 In Press). Distributions of geometric classes of abundance of taxa will also be calculated when data from all stations 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.

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).

Data Analysis Comments

The goal of the data analysis in this study is to determine effects of short and long-term accumulations of petroleum hydrocarbons in sediment, resulting from the Exxon Valdez oil spill, on benthic taxon composition, diversity, abundance, biomass, and trophic composition. The critical aspect of the study is 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 organismal, population, and ecological effects on the benthic infauna. However, to fully interpret most of the "deleterious effects" expected to occur on benthic organisms, a comprehensive and extremely costly program would have to be initiated to compliment this benthic study. However, 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, and (3) examining literature on effects demonstrated by deep benthic organisms subjected to hydrocarbon contamination.

Study 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 2). 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 by data are note 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

The following temporal station at 40 m, data collected June 1989, has taxonomic identifications completed and data entered in the computer. Some data from this station will be compared with a similar station occupied in July 1990. This comparison may not be available to this report:

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.

Table 2. Benthic Stations Occupied in Prince William Sound in 1989 by the Institute of Marine Science, University of Alaska Fairbanks.

Station	Date	Latitude (N)	Longitude (N)	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,
GI-7	6 Oct				rock, dead shell, grabs half full
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,
GI-2	6 Oct				grabs full
GI-1	5 May	60° 14.99'	147° 39.90'	70-90	soft grey mud, dead
GI-1	6 Oct				sponge, rock, grabs 3/4 full
Snug Harbor					
SHX-1	5 May	60° 15.85'	147° 41.87'	53	compact grey mud, rock, grabs full
SH-1	4 Jun	60° 10.4'	147° 42.3'	35	soft grey mud, sand, shell, wood, grabs full
SH-3	11 Apr	60° 15.75'	147° 45.30'	32-36	soft grey mud, wood, shell, grabs full

Table 2. continued.

Station	Date	Latitude (N)	Longitude (W)	Depth (M)	Bottom Type, Grabs Volume
Herring Bay					
HB-1	11 Apr	60^0 28.21'	147^0 44.45'	110-140	compact grey mud, rock,
HB-1	10 May	60^0 28.21'	147^0 44.45'	110-140	grabs full
HB-1	7 Oct				
Main Bay					
MB	3 Oct	60^0 34.02'	147^0 57.0'	610	soft grey mud, full grabs
Montague Sound					
MSX	2 Oct	59^0 58.5'	147^0 48.72'	238	soft grey mud, full grabs
Saw mill Bay					
SB-1	11 Apr	60^0 03.49'	147^0 57.95'	120-175	soft grey mud, many rocks,
SB-1	7 May				grabs 3/4 full
SB-1	2 Oct				
Esther Island					
EI	9 May	60^0 46.24'	147^0 03.34'	378-388	soft grey mud full grabs
EI	3 Oct				
Orca Bay					
OB-1	9 Apr	60^0 34.98'	146^0 02.00'	164-177	soft grey mud, full grabs
OB-1	7 May				
OB-1	10 Oct				
OB-6	8 May	60^0 34.78'	146^0 54.08'	417	soft grey mud, full grabs
OB-6	7 Oct				
Knight Island Passage					
KI-1	10 May	60^0 20.58'	147^0 56.98'	413	soft grey mud and rocks, grabs half full
KI-2	7 Oct	60^0 10.80'	147^0 53.40'	388	soft grey mud, full grabs

Table 2. continued.

Station	Date	Latitude (N)	Longitude (W)	Depth (M)	Bottom Type, Grab Volume
Naked Island					
NI-4	10 Apr	60^0 44.86'	147^0 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	60^0 38.35'	147^0 28.82'	40	soft grey mud, sand, shell, grabs 3/4 full
Bligh Island					
BI	10 Apr	60^0 50.22'	146^0 55.03'	84-97	soft grey mud, gravel,
BI	8 May				shell. rock, grabs full
BI	6 Oct				
Rocky Bay					
RB-2	8 Apr	60^0 21.80'	147^0 01.77'	76-84	soft grey mud, rocks, same
RB-2	7 May				reps with shell and
RB-2	7 Oct				gravel, full grabs
RB-7	8 Apr	60^0 21.48'	147^0 03.92'	65	compact grey mud, full grabs
Hinchinbrook Entrance					
HE-3	7 May	60^0 17.37'	146^0 46.24'	234	soft grey mud, full grabs
Smith Island					
SI-01	5 Jun	60^0 31.39'	147^0 24.12'	43	compact grey mud, sand, shell, grabs half full
Port Valdez					
PVA 040	8 May	61^0 06.30'	146^0 28.70'	223	soft grey mud and wood, full grabs
PVA 050	10 Apr	61^0 06.35'	146^0 35.70'	248	soft grey mud, full grabs

Stations Sorted and Taxa Identified from Material of the July 1990 Cruise

Currently organisms from all replicates (1.0 mm mesh screen samples only) from six stations at the 40 m depth have been identified, and these data are available as ranked abundance and biomass stations (tables are on file at IMS; dominant taxa are used in statistical comparisons). The bays from which these stations were collected were (1) oiled sites: Herring Bay, Disk Island, and Snug Harbor (2) unoiled sites: Zaikof Bay, Rocky Bay, and West Bay. Additional 40 m stations almost completed, or completed at the time of this report, but not entered into the computer are Drier Bay (Reference Site: completed), Lower Herring Bay (Reference Site: completed), Northwest Bay (Oiled Site; in progress), MacLeod Harbor (Reference Site: completed), and Bay of Isles (Oiled Site: completed).

Initially both 0.5 and 1.0 mm samples were being processed. However, as alluded to above, the amount of funds and time available to the project could not support processing of both the fine and coarse fractions. Nevertheless, 0.5 mm samples from the following bays were sorted and identifications of organisms made. Most of these data are not yet entered into the computer. The Herring Bay and Zaikof Bay 0.5 mm samples are available to this report. The number of hours required to rough sort (the time required for processing fine fractions) this material is included after each entry:

Herring Bay - 5 replicate samples completed: 6-30 hrs/replicate to rough sort

Zaikof Bay - 5 replicate samples completed: 4-13 hrs/replicate to rough sort

Disc Island - 5 replicates completed: up to 60+ hrs to rough sort each replicate

Snug Harbor - 5 replicates completed: up to 16 hrs to rough sort each replicate

Based on the high abundance levels of Foraminifera (mostly consisting of shells of dead organisms) and the extensive amount of time required to rough sort such samples (up to 40+ hours per replicate), it was decided to eliminate Foraminifera from the analysis. This problem was especially troublesome during the time when 0.5 mm samples were in the process of analysis (early September). Nevertheless, despite this sorting modification, the rocky and/or shelly consistency of the 40 m samples frequently required up to 30+ hours to rough sort a single replicate of the 1.0 mm fractions.

Number of Taxa, Abundance, Carbon Biomass, Diversity, Evenness, and Taxon Richness at Individual Stations

These univariate measures are tabulated in Table 3 and displayed in Figures 1-3. The ranked abundance and biomass values for each station will be presented in the Annual Report. The printouts showing ranked values for abundance and biomass are on file in Dr. Feder's office.

K-Dominance Curves

The K-Dominance curves for the 1.0 mm fractions for the six bays are presented in Figures 4-9. The K-Dominance curves for the 1.0 + 0.5 mm fractions for Herring Bay (oiled) and Zaikof Bay (reference site) are presented in Figures 10-11.

Statistical Tests

The abundance of dominant taxa (1.0 + 0.5 fractions), and their transformed abundance values at the 40 m stations at Herring Bay and Zaikof Bay are shown in Table 4. Tests of significance (ANOVA), based on the combined 1.0 0.5 mm fractions, between their dominant taxa at Herring Bay (oiled) and Zaikof Bay (unoiled) are completed at the time of this report, and preliminary comments are included below. Tests of significance (ANOVA) of abundance between three oiled and three unoiled bays for the dominant taxa within the 1.0 mm samples are completed at the time of this report but are not tabulated as yet. Some of the results of the analyses may be available to the peer review group for the meeting in December. The 0.5 mm samples for all of the other bays will not be completed and entered into the computer until approximately mid-January. These data will be analyzed at this time. A comparison, via ANOVA, of the abundance of the dominant taxa at the pooled oiled (3 bays) and pooled unoiled bays (3 bays) has been completed but not totally analyzed at the time of this report. A preliminary examination of the ANOVA results for the pooled bays are present in Appendix C.

Status of Injury Assessment

a. The Disk Island oiled site. based on the 1.0 mm mesh samples only, appears healthy as assessed by the number of taxa (T), total abundance (A) and total carbon biomass (B) present. Neither diversity values or the K-dominance plot indicate disturbance. However, high abundance values of some taxa could reflect disturbances at the (Ranked Abundance Table on File in Dr. Feder's office) site. The latter taxa are Nematoda. the polychaete groups Cirratulidae, Maldanidae, Lumbrineridae, Polyodontidae, and Owenidae, the sipunculid group Golfingiidae, and the bivalve group Thyasiridae. A potential disturbance here is also suggested by the relatively high Abundance/Taxa ratio and low Biomass/Abundance ratio.

Table 3. Abundance, biomass, number of taxa, and diversity indices for the 6 bays, 1.0 mm and 0.5 fraction included. Samples collected in Prince William Sound, July 1990.

Station	Abundance	Biomass (gc/m2)	No. of Taxa	Simpson	Shannon	SW Even	Species Richness
DI (1mm) o	2554.00	2.128	63.0	0.06	3.37	0.81	7.90
HB (1mm) o	1904.00	1.504	75.0	0.05	3.43	0.79	9.80
HB (0.5+1mm)	3844.00	1.578	79.0	0.07	3.26	0.75	9.45
SH (1mm) o	666.00	1.241	49.0	0.05	3.30	0.85	7.38
RB (1mm)	2902.00	5.606	78.0	0.08	3.24	0.74	9.66
WB (1mm)	346.00	0.373	36.0	0.06	3.16	0.88	5.99
ZB (1mm)	656.00	2.477	37.0	0.09	2.86	0.79	5.55
ZB (0.5+1mm)	1908.00	2.548	45.0	0.12	2.71	0.71	5.82

Table 4. Abundance as x=square root (x+.5) for dominant taxa at Herring Bay (oil site) and Zaikof Bay (reference site). Means, standard deviations, F ratios with associated levels of significance, and comparisons between bays. Data analyzed include organisms from 1.0 and 0.5 mm screened fractions.

Taxa	Herring Bay (HB) $\bar{X}$	Herring Bay (HB) SD	Zaikof Bay (ZB) $\bar{X}$	Zaikof Bay (ZB) SD	F Ratio	P	Comparison
Nematoda	4.409	3.854	0.766	0.123	8.791	0.009	HB>ZB
Ampharetidae	1.469	1.351	0.766	0.123	2.821	ns	---
Capitellidae	2.267	1.275	1.420	0.653	3.538	1.078	HB>ZB
Cirratulidae	4.101	2.103	1.790	1.153	8.930	1.009	HB>ZB
Lumbrineridae	3.684	1.018	1.816	1.001	17.253	0.001	HB>ZB
Maldanidae	2.466	1.341	0.766	0.123	14.746	0.001	HB>ZB
Nephtyidae	1.187	0.738	3.996	3.437	7.635	0.014	HB<ZB
Owenidae	1.527	0.861	0.788	0.179	8.324	0.011	HB>ZB
Polyodontidae	3.051	1.664	0.766	0.123	18.513	0.001	HB>ZB
Spionidae	1.460	0.721	2.322	1.456	2.620	ns	---
Syllidae	2.540	1.779	0.788	0.179	9.013	0.008	HB>ZB
Bivalvia	3.240	2.310	2.392	2.022	0.716	ns	---
Chaetodermatidae	0.788	0.79	1.639	0.590	17.215	0.001	HB<ZB
Nuculanidae	1.096	0.705	2.149	1.709	8.422	0.010	HB<ZB
Thyasiridae	1.256	0.897	1.086	0.496	0.270	ns	---
Veneridae	0.766	0.123	0.922	0.430	3.184	0.093	HB<ZB
Harpacticoida	1.498	1.396	0.766	0.123	3.013	ns	---
Golfingidae	2.340	1.979	0.766	0.123	7.733	0.013	HB>ZB

High abundance values for some taxa suggest a response by some opportunistic taxa responding to unstable or disturbed conditions (Gray et al, 1988). ANOVA of abundance data for some of the dominant taxa present at the Disk Island site (Ampharetidae, Cirratulidae, Lumbrineridae, and Golfingiidae) as compared to Zaikof Bay (Reference Site) indicate that these taxa are all significantly more abundant in the oiled bay. The latter point should be clarified when the 0.5 mm fraction from this site are completed. A qualitative assessment of three of the 0.5 mm fractions from this site indicate that large numbers of Nematoda, Cirratulidae, Ownidae, and Golfingiidae were present and that an increase in opportunists will be apparent when all 0.5 mm smples at this station are examined.

b. The Herring Bay oiled site, based on the pooled 1.0 and 0.5 mm mesh samples, has TAB values suggestive of a moderate disturbance (Gray et al., 1988). This disturbance is also suggested by the high A/T ratio and the low B/A ratio (also see Gray et al., 1988). Although the diversity and taxon richness values do not reflect a disturbance at the site, the K-dominance curve is that of a moderately disturbed site (see Gray et al., 1988). ANOVA of the abundance data for the dominant taxa within Herring Bay as compared to Zaikof Bay (Reference Site with 1.0 and 0.5 mm fractions pooled) indicate statistically significant differences for 13 taxa between the two bays. Most of the taxa are significantly more abundant in Herring Bay. Additionally, many juveniles of the dominant taxa were added to the Herring Bay abundance value when the 0.5 mm fraction were assessed, (data on file at the Institute of Marine Science, University of Alaska Fairbanks). The latter finding suggests the presence of opportunist species at this site.

c. The Snug Harbor Oil Site, based on the 1.0 mm samples only, may be a disturbed one as suggested by the relatively low TAB and species richness values present. Qualitative examination of the data sheet for the 0.5 mm fractions from this site (raw data on forms not entered into computer; data available at Institute of Marine Science) indicate a few taxa with large numbers of juveniles, although the occurrence of these taxa had a disjunct distribution (i.e., patchy distribution). Thus, the following taxa were common to abundant in the 0.5 mm fraction: Nematoda, the polychaete Families Spionidae, Cirratulidae, and Paraonidae, Diversity values and the K-dominance curve for this site, based on the 1.0 mm data, do not indicate disturbance. Further calculations incorporating the 0.5 mm data might change some of the univariate values.

d. The sediments of the West Bay reference site were black and the odor of H_2S was detectable in fresh samples. Thus, some of the sediment at this site may become anoxic periodically.

The low TAB and species richness values at this site indicate that it is not a particularly healthy environment, although the diversity values and the K-dominance curve do not back up this assertion. It is possible that this site should be dropped as a reference site from the comparison of pooled bay data.

e. Only the 40 m stations from six of the fourteen sites have been processed and entered into the computer to date. Further, as noted previously, data from only two of the 0.5 mm fractions have been processed and entered into the computer at the time of this report. As recorded in the results, additional 40 m stations have been processed, but the data has not been entered into the computer. Thus, little data were available to this report for analysis. It is probable that all 40 m station data (1.0 and 0.5 mm fractions) will be completed and entered into the computer, and available for analysis, in early January 1991. At this time the samples from the deeper stations will be processed and should be available for analysis by late March or early April. A full assessment of the effect of oil on the deep benthos can only be attempted once samples (1.0 and 0.5 mm mesh) from all stations at all depths are examined. Further, the sediment hydrocarbon data at all of the benthic stations should also be available to the analysis. At the time of this report the hydrocarbon data is not available to the benthic project. Thus, no conclusions relative to the entrainment of oil from the shoreline to deep water can be drawn until all benthic biological and sediment hydrocarbon data can be assessed.

f. The July 1990 interbay benthic collections are the first ones available to the project. It is essential that at least one additional set of samples be collected in July 1991 to enable some assessment of temporal changes of taxa within the bays.

g. Interbay comparisons have been made with an ANOVA comparing the abundance of dominant taxa within each of the bays (data tables will be available at the Dec. 3 meeting). In general, the analyses show results similar to the comparison between the 1.0 + 0.5 mm samples of Herring Bay and Zaikof Bay. Thus, most of the dominant taxa are significantly more abundant in two of the oiled bays (Herring Bay and Disk Island) than they are in any of the reference bays. The other oiled bay, Snug Harbor, shows some significantly higher numbers of individuals in only a few taxa.

h. The three pooled oiled and three pooled unoiled bays were examined with 2 x 3 ANOVAs. Fifteen, of the eighteen, dominant taxa examined showed significant differences between oiled vs unoiled sites. In general, these analysis back up the other bay comparisons examined by ANOVA.

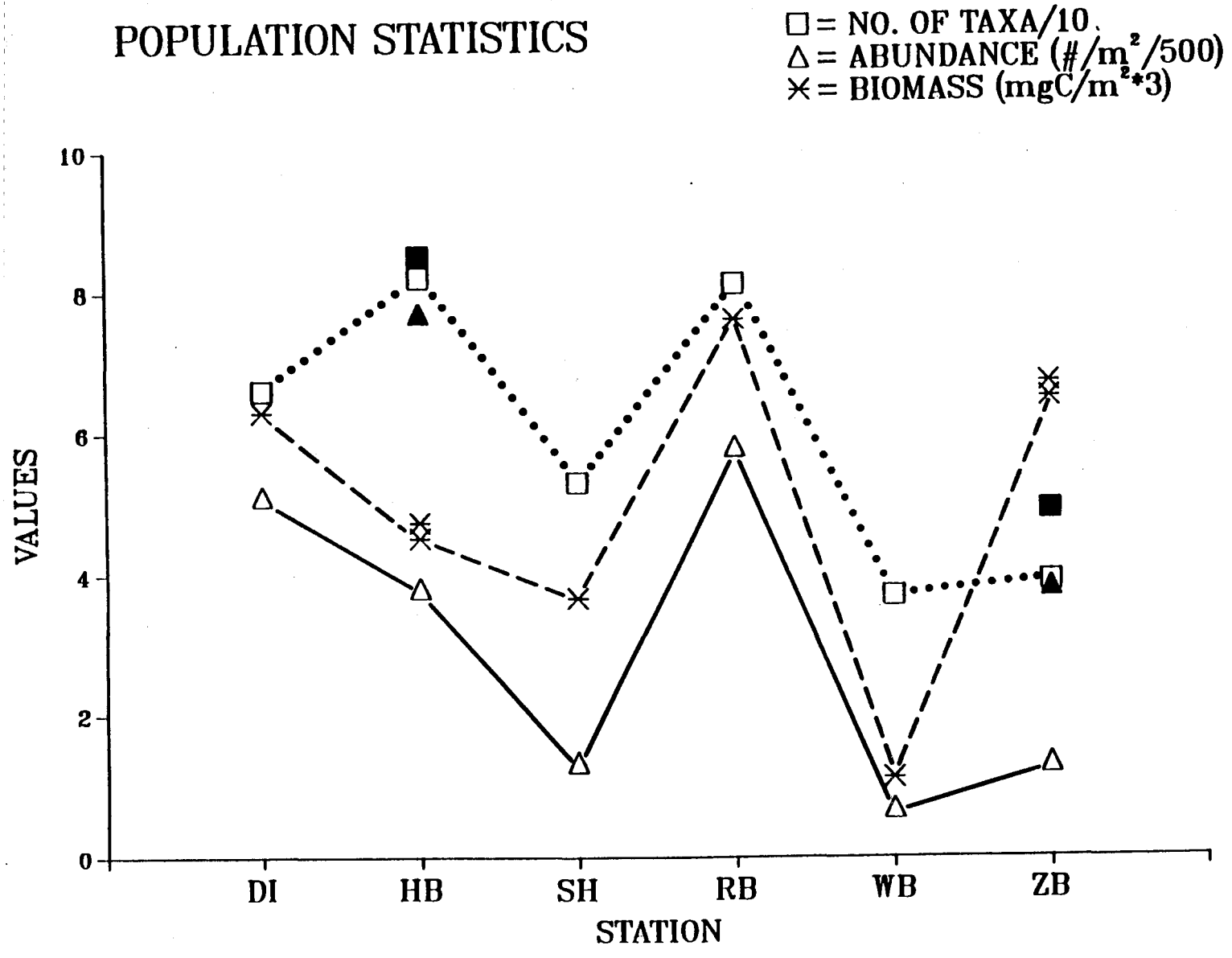

Figure 1. Number of taxa, abundance, and biomass of benthic organisms collected at 3 oiled (Disk Island: DI; Herring Bay: HB; Snug Harbor: SH;) and 3 unoiled bays (Rocky Bay, West Bay, and Zaikof Bay in the Prince William Sound in July 1990. = number of taxa including 1.0 and 0.5 mm fractions from Herring and Zaikof Bays. The upper symbols for the other two categories for the two bays indicate the values for the 1.0 + 0.5 mm samples.

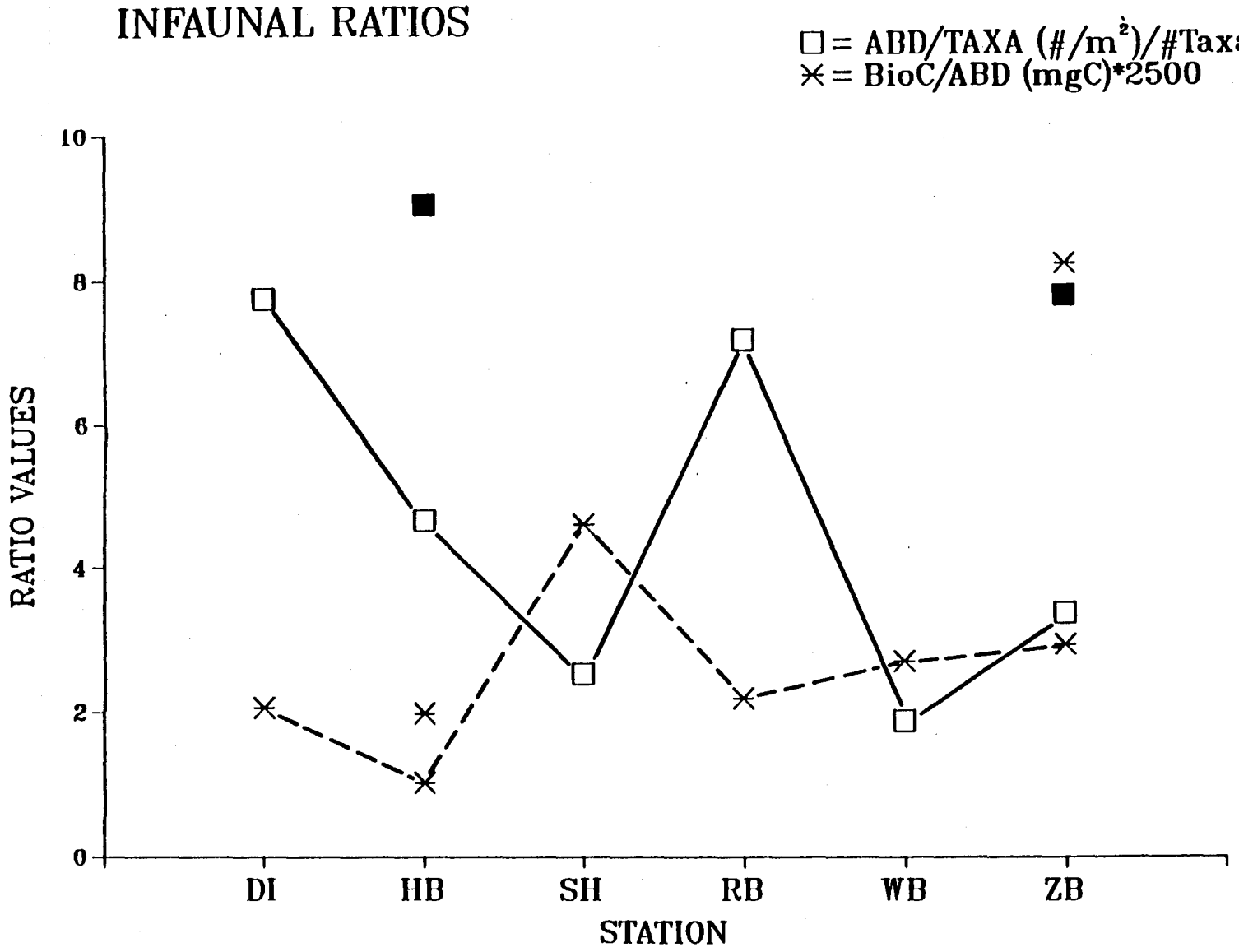

Figure 2. Infaunal ratios (abundance/taxa, wet weight/abundance, carbon weight/abundance) for taxa collected at 3 oiled (DI, HB, SH) and 3 unoiled (RB, WB, ZB) bays in Prince William Sound in July 1990. ■ = abundance/taxa including 1.0 and 0.5 mm samples from Herring Bay and Zaikof Bay. The upper symbols for the other category for these two bays also indicates the value for the 1.0 + 0.5 mm samples.

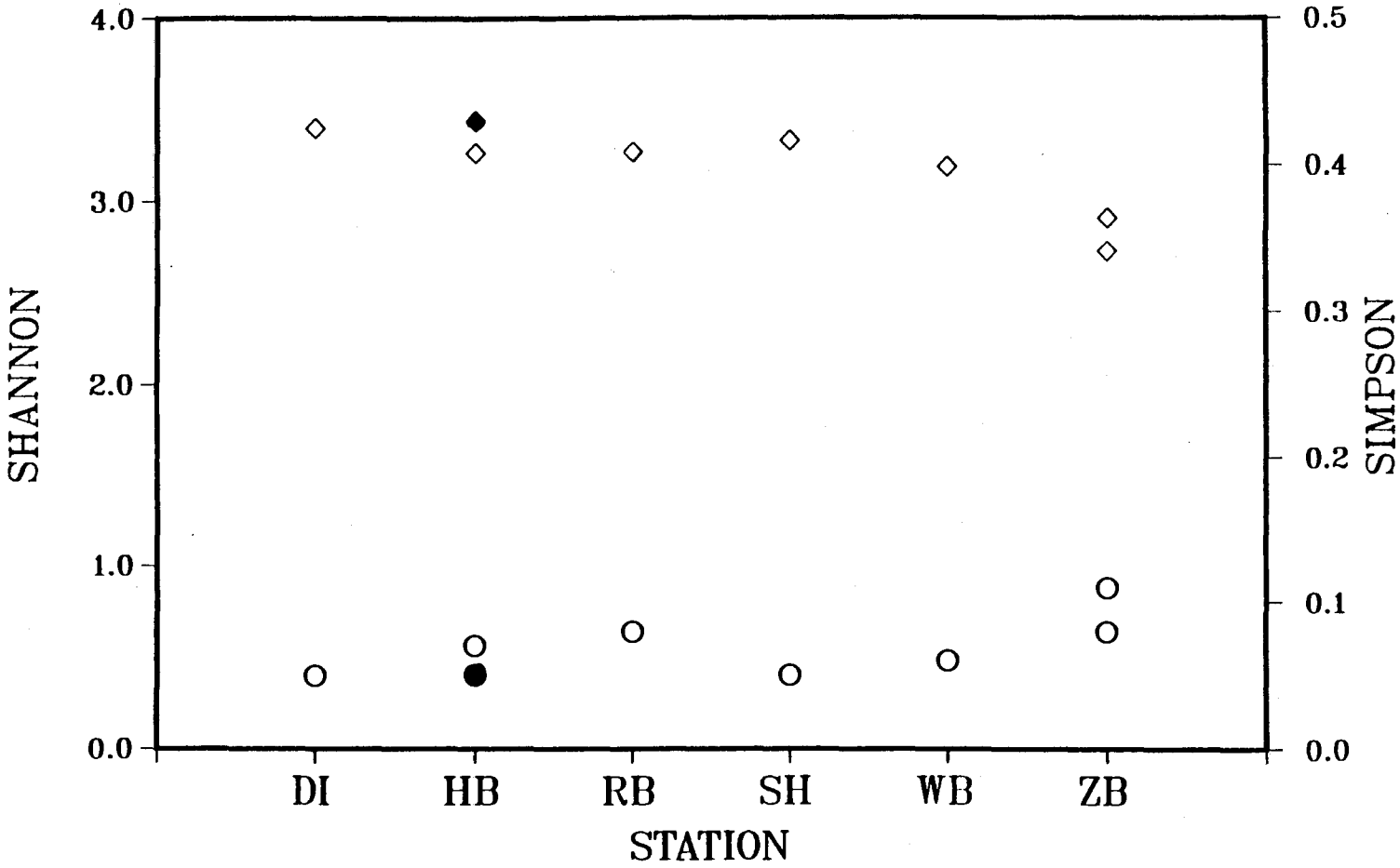

Figure 3. Shannon Diversity (upper symbols ◇ ; ◆ = 1.0 + 0.5 mm samples) and Simpson Dominance (lower circles; ● = 1.0 + 0.5mm samples) for taxa collected at 3 oiled (DI, HB, SH) and 3 unoiled (RB, WB, ZB) bays in Prince William Sound in July 1990.

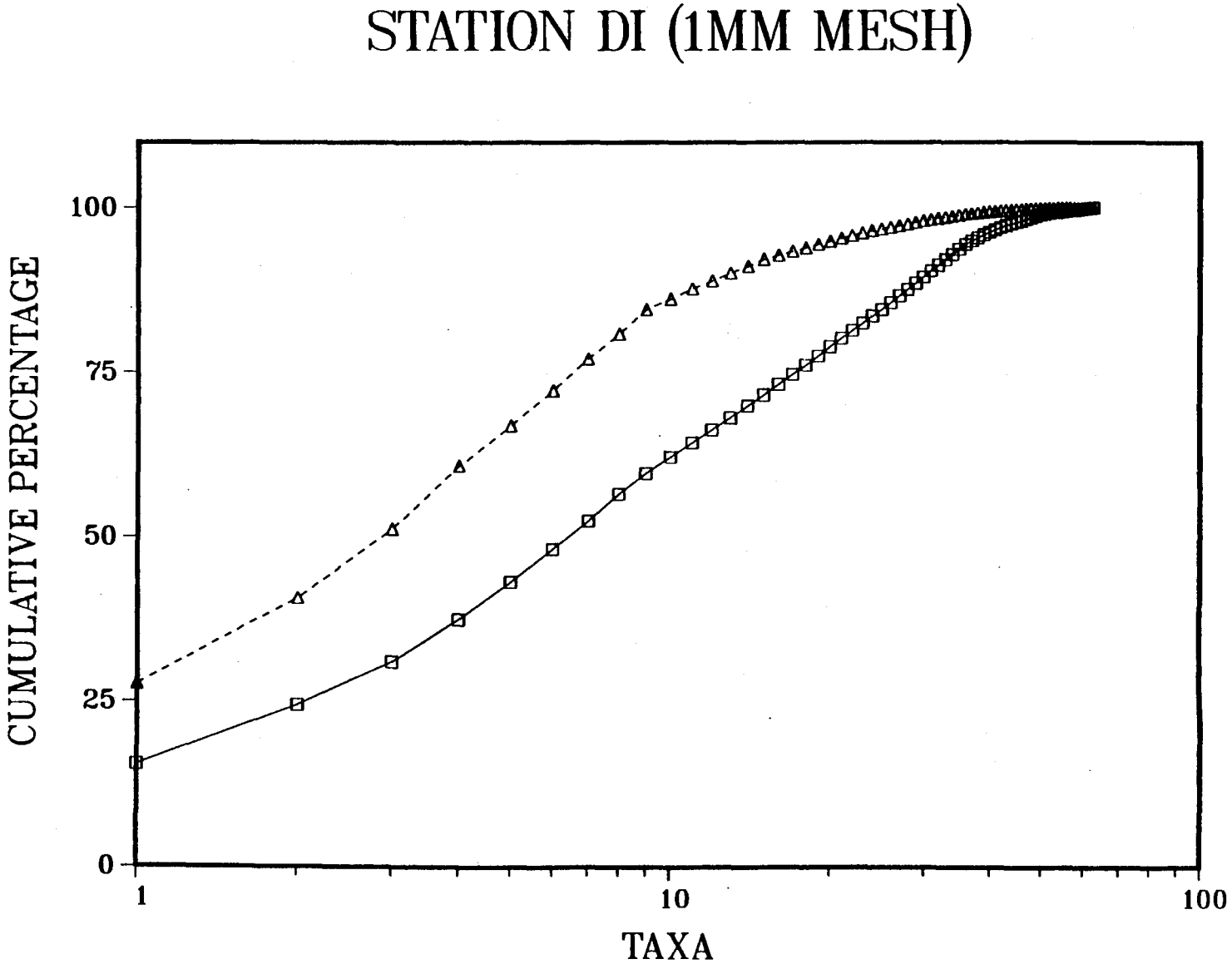

Figure 4. K-dominance curves for taxa within the 1.0 mm samples collected at Disk Island (DI) in July 1990.

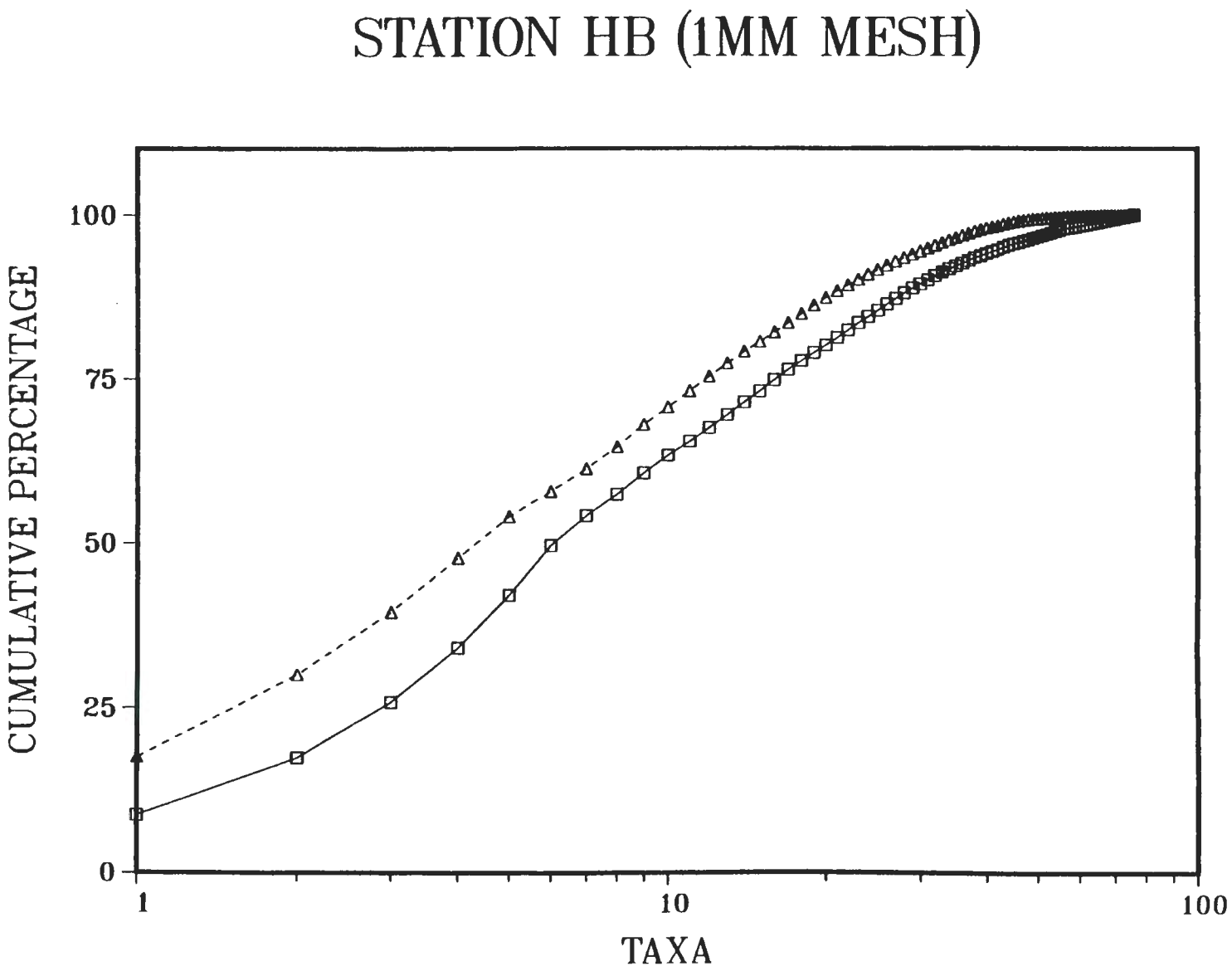

Figure 5. K-dominance curves for taxa within the 1.0 mm samples collected at Herring Bay (HB) July 1990.

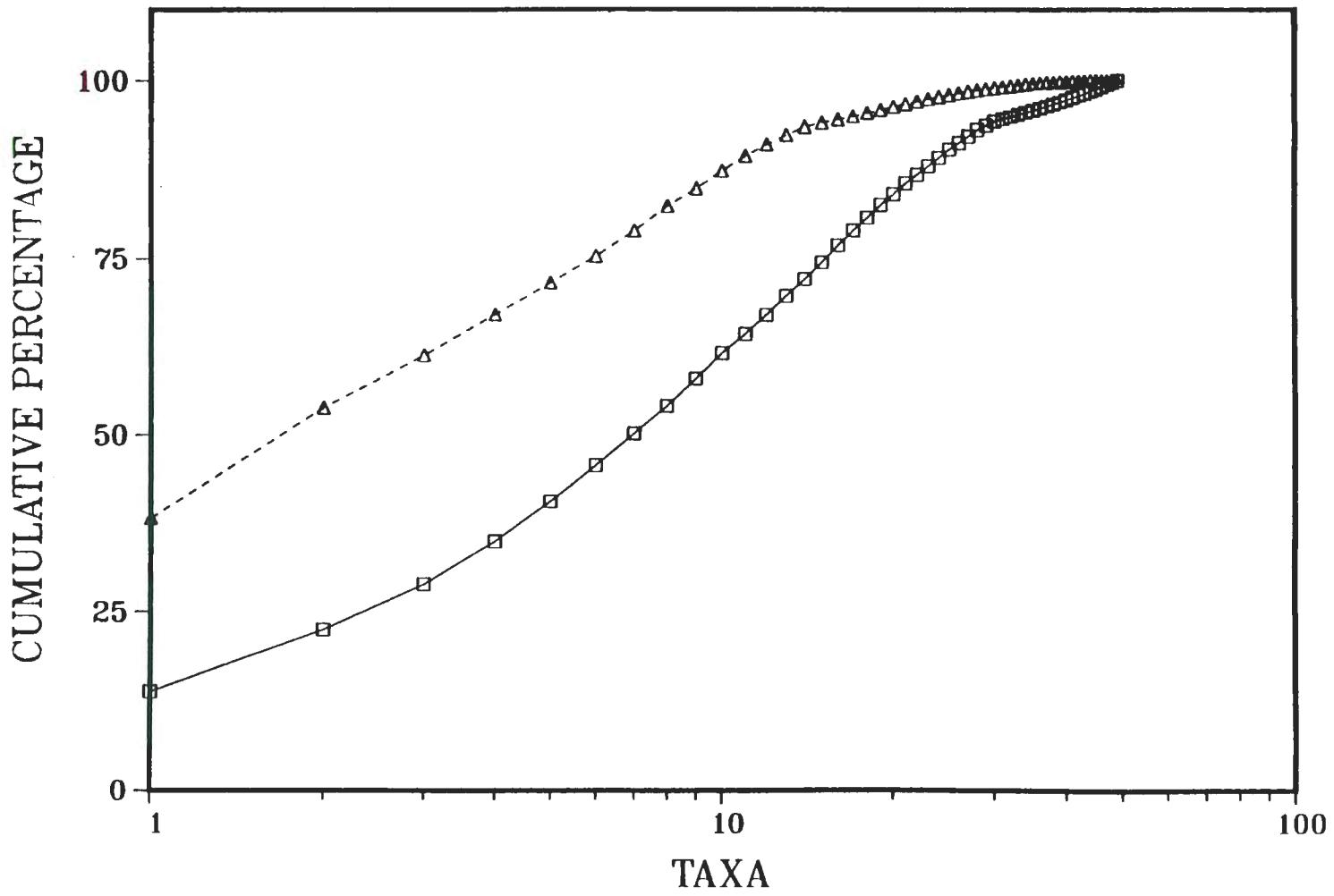

Figure 6. K-dominance curves for taxa within the 1.0 mm samples collected at Snug Harbor (SH) July 1990.

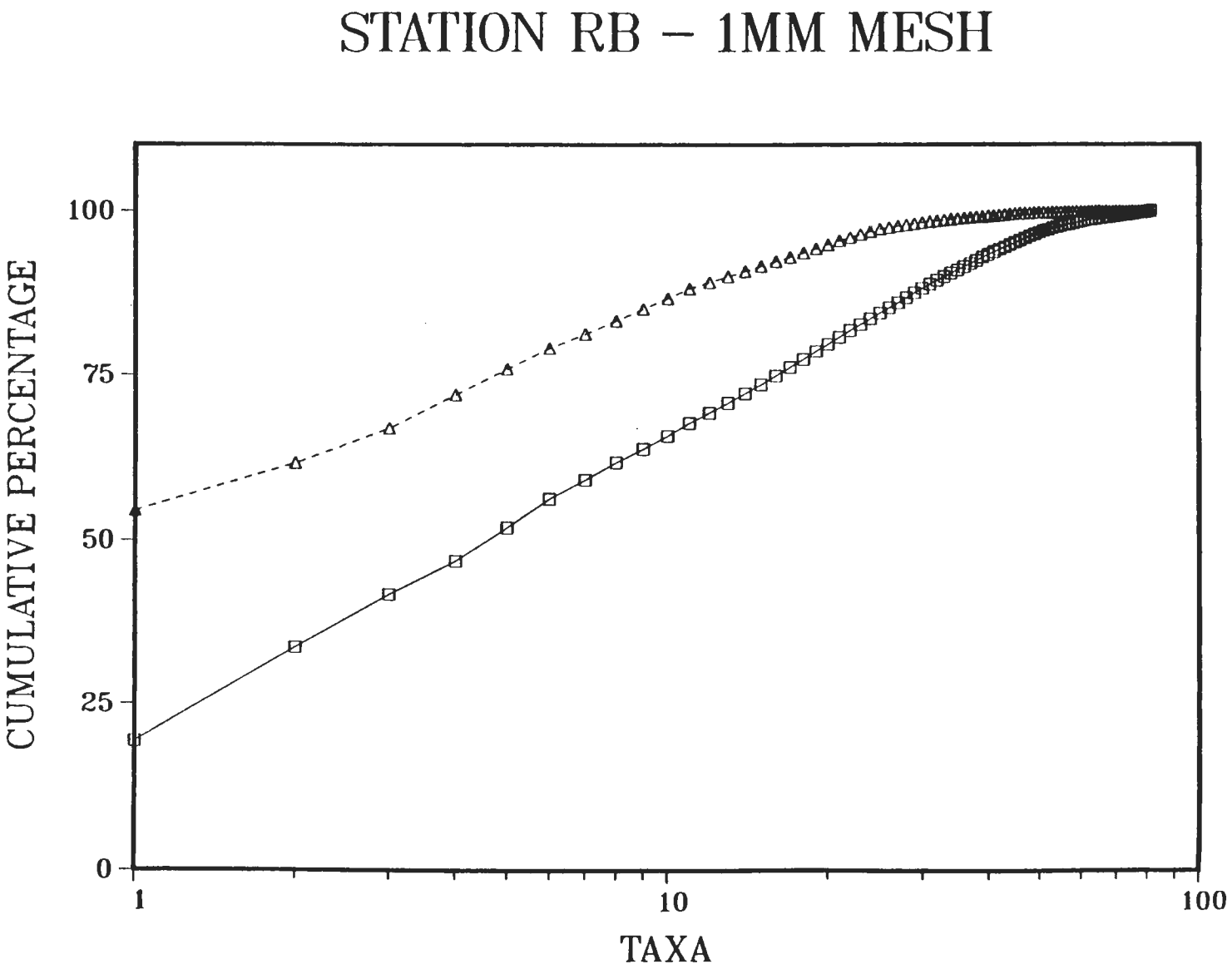

Figure 7. K-dominance curves for taxa within the 1.0 mm samples collected at Rocky Bay (RB) in July 1990.

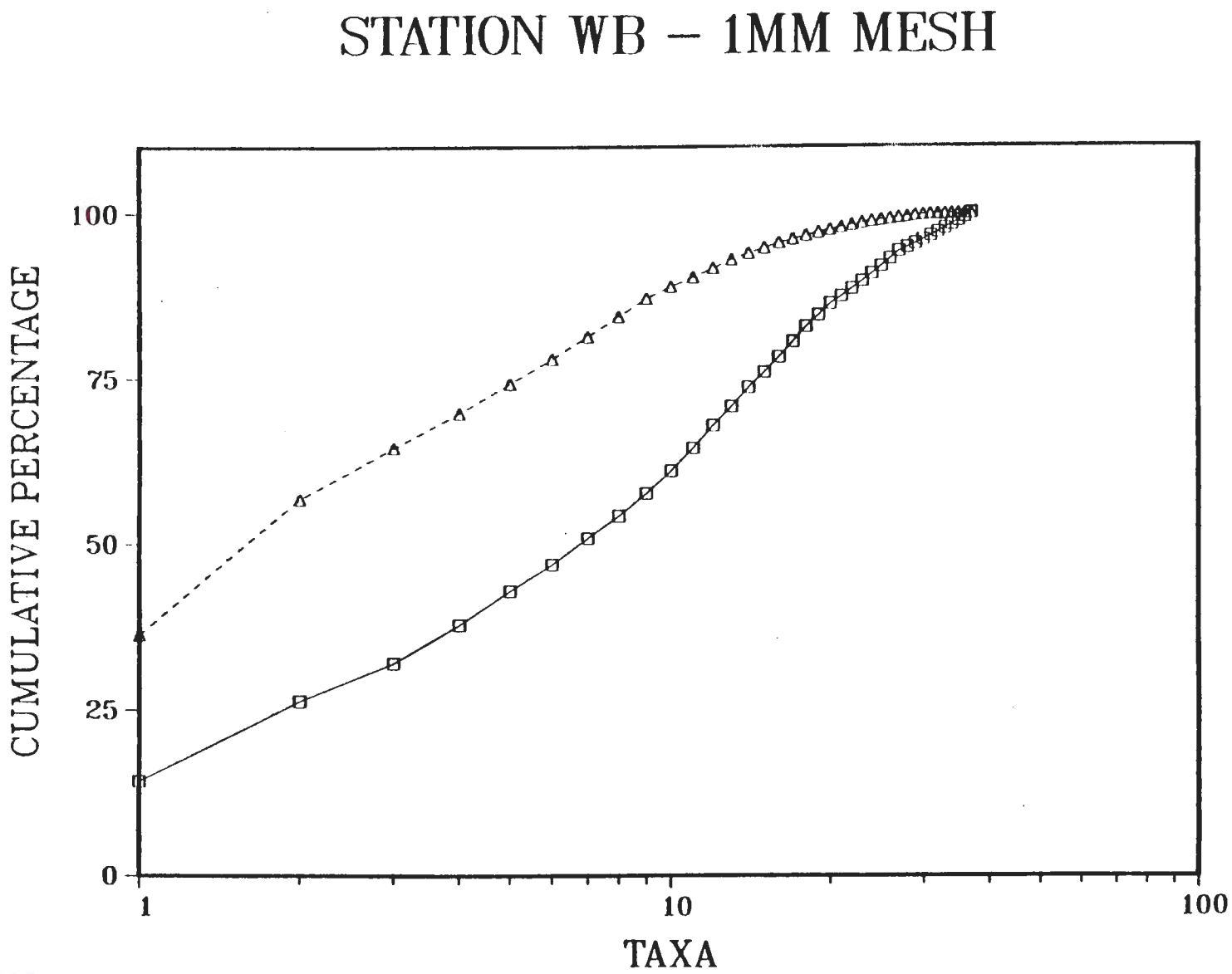

Figure 8. K-dominance curves for taxa within the 1.0 mm samples collected at West Bay (WB) in July 1990.

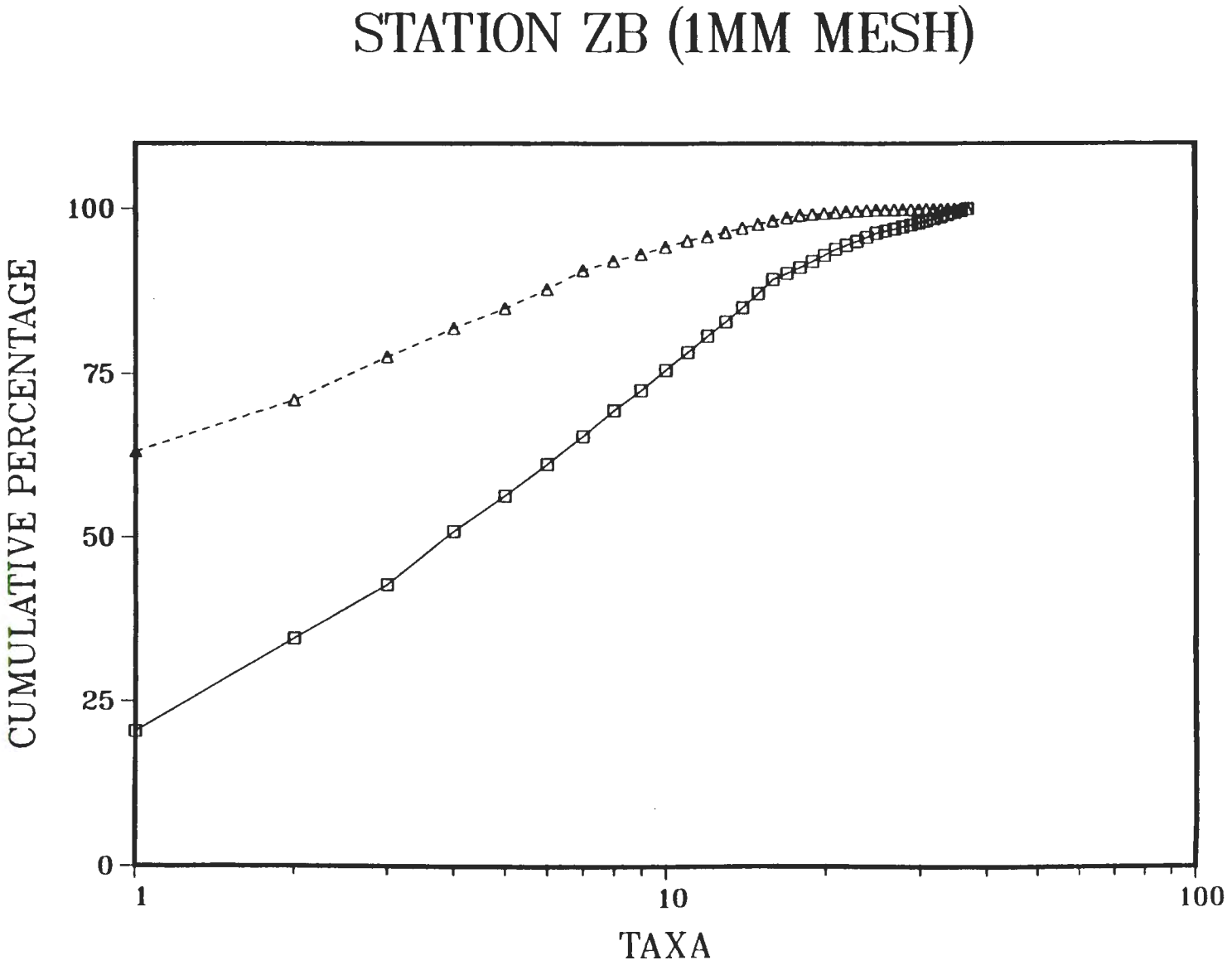

Figure 9. K-dominance curves for taxa within the 1.0 mm samples collected at Zaikof Bay (ZB) in July 1990.

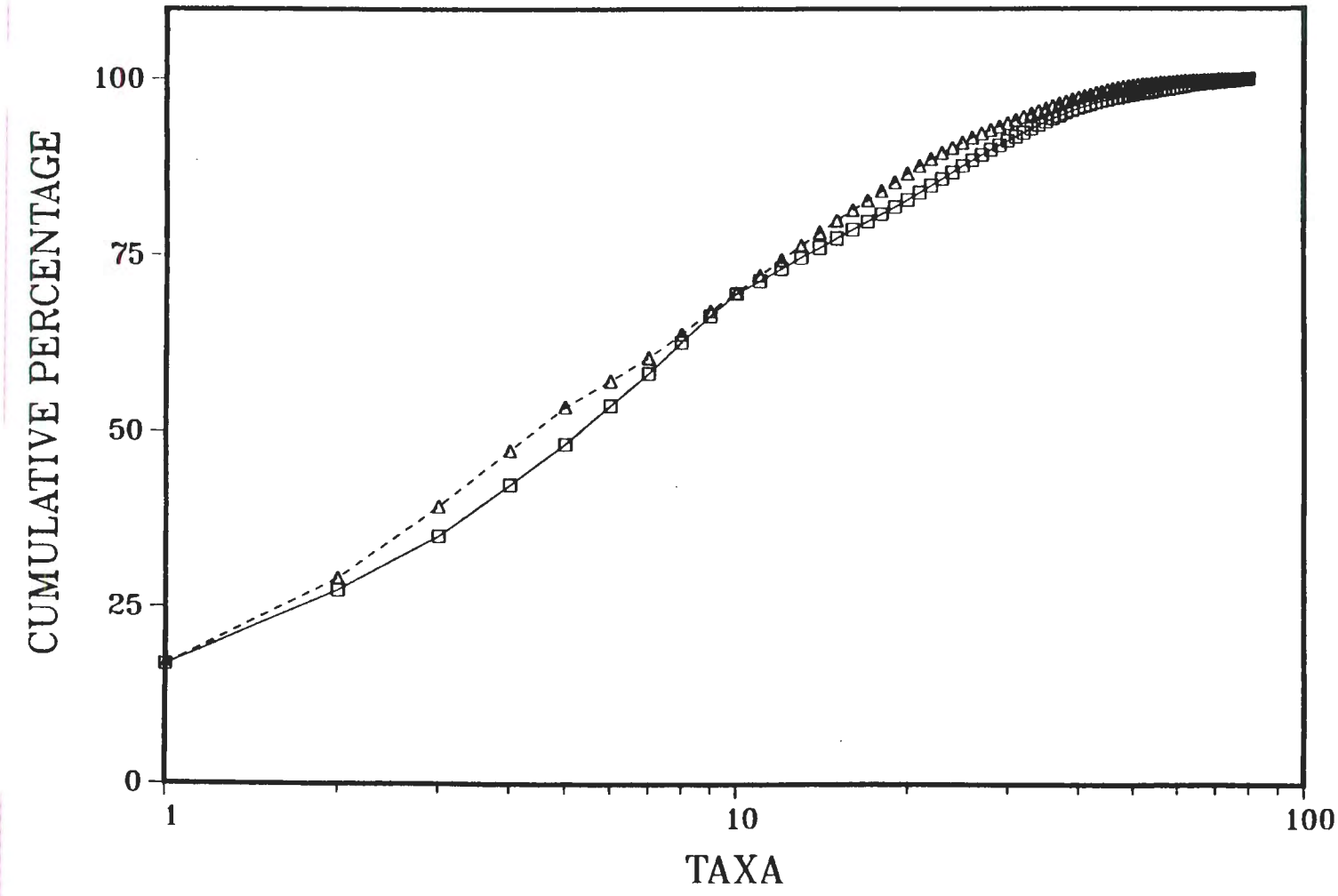

Figure 10. K-dominance curves for taxa within the 1.0 + 0.5 samples collected at Herring Bay (HB) in July 1990.

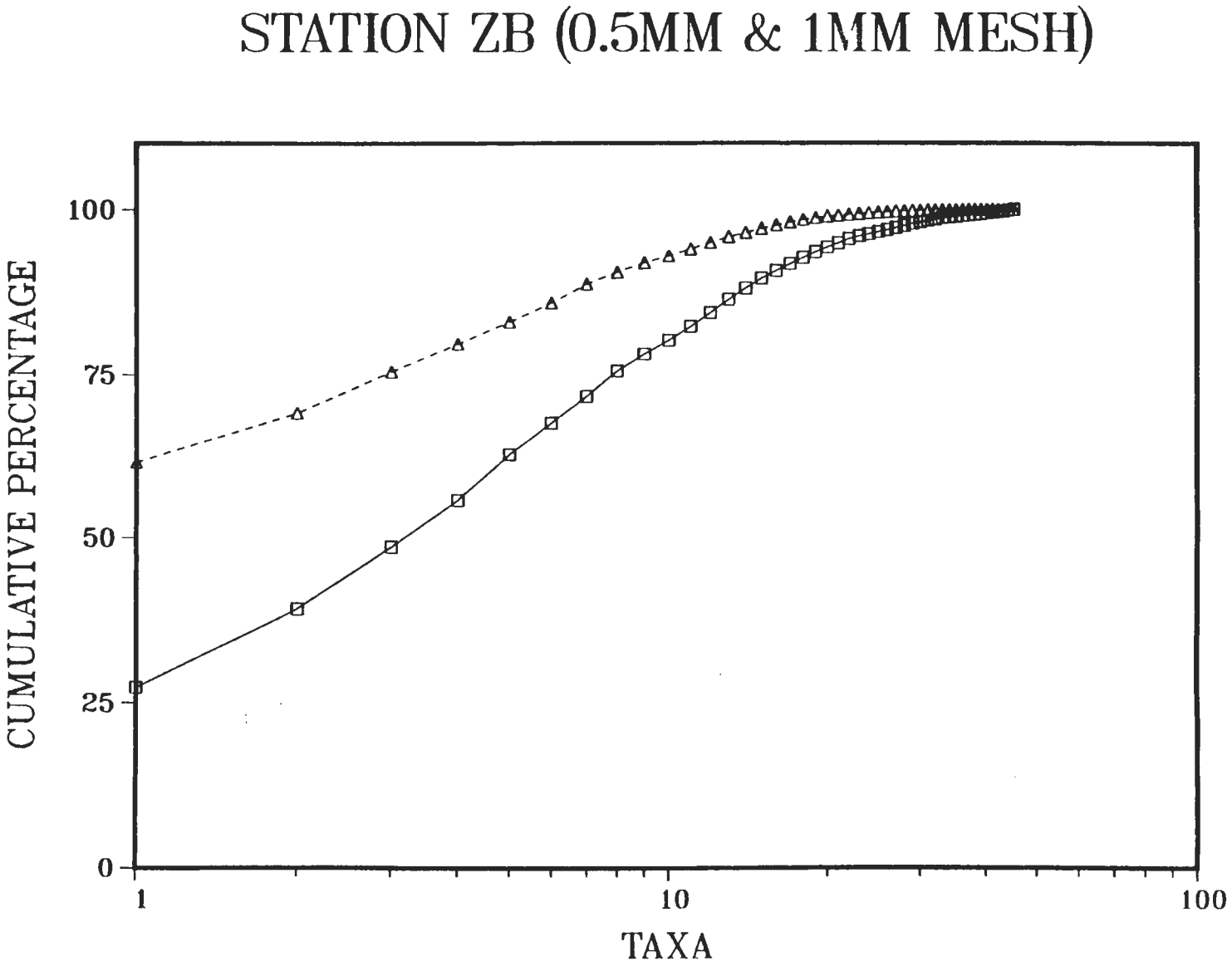

Figure 11. K-dominance curves for taxa within the 1.0 + 0.5 mm samples collected at Zaikof Bay (ZB) in July 1990.

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APPENDIX A
SAMPLE COLLECTION AND ANALYSIS PROTOCOL
SUBTIDAL BIOLOGICAL SAMPLES TO BE ANALYZED FOR INFAUNA

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Questions about the following should be directed to Howard M. Feder.

I. COLLECTION APPARATUS AND METHOD OF SAMPLE HANDLING

Collection Device. Samples are collected with a 0.1 m^2 van Veen grab with bottom penetration facilitated by addition of 31.7 kg of lead. The van Veen grab is available from the Seward Marine Center, University of Alaska. A discussion of the effectiveness of the van Veen grab as a quantitative instrument is included in Feder et al. (1973). The benthic samples will be collected using the same methods recommended by Baltic Sea biologists for examining benthos following a major oil spill by the Tsesis (Kineman et al. 1980) and the methods previously used in Prince William Sound and the Gulf of Alaska by Feder and associates (see Feder and Jewett, 1987, for pertinent references and discussion). Samples are washed on 1.0 mm stainless steel screen and retained organisms removed by means of forceps and placed in sample bags.

Sample Containers. Specimens from each replicate grab sample are placed in a separate Whirlpak bag. A label (Rite-in-the-Rain paper is used) is placed in each bag, and sufficient hexamine-buffered 10% formalin placed in each bag so as to cover the specimen. Each bag is then enclosed in another Whirlpak bag. All replicates from each station are then placed in a large plastic bag which is then sealed. Each bag from each station is placed in a five-gallon container for transportation to Fairbanks (see Chain of Custody protocol included in the Technical Study Plan).

Sample Analysis. In the laboratory all organisms are identified, counted, and weighed after excess moisture is removed with an absorbent towel. Carbon values for all wet weights will be calculated based on conversion values included in Stoker (1978) for the same or closely related species. All data are entered on code sheets and submitted to IMS Data Management for data entry into the computer.

II. DATA ANALYSIS

Multivariate Analysis. Station groups and taxon assemblages for each year and for the combined data collected on all future surveys will be identified using the technique of hierarchical cluster analysis (Field and MacFarlane 1968; Field 1969, 1970, 1971; Day et al 1971). The procedure consists of three steps:

1. Calculation of a measure of similarity between entities to be classified.

2. Sorting through a matrix of similarity coefficients to arrange the entities in a hierarchy or dendrogram.

3. Recognition of station classes or groups within the hierarchy.

The Czekanowski coefficient will be used to calculate similarity matrices for cluster analysis routines. The Czekanowski coefficient is synonymous with the Motyka (Mueller-Dombois and Ellenberg, 1974) and Bray-Curtis (Clifford and Stephenson 1975) coefficients and is defined by

$$C_{cz\,1,2} = \frac{2W}{A+B}$$

$C_{cz1,2}$ =

where A = the sum of the measures of attributes of entity one
B = the sum of the measures of attributes of entity two
C = the sum of the lesser measures of attributes shared by entities one and two

The Czekanowski coefficient emphasizes the effect of dominant species on the classification and is used with a natural logarithm transformation, $Y=\ln(X + 1)$, which reduces the influence of dominant species on the similarity determination. Dendrograms will be constructed from the similarity matrices using a group-average agglomerative hierarchical cluster analysis (Lance and Williams 1966).

Principal coordinate analysis (Gower 1967, 1969) will be used as an aid in interpreting the results of the cluster analysis of the data (Stephenson and Williams 1971, Boesch 1973) and identifying misclassification of stations by cluster analysis. Misclassifications in an agglomerative cluster analysis can occur by the early fusion of two stations and their subsequent incorporation into a group whose stations have a high similarity to only one member of the original pair (Boesch 1973). In principal coordinate analysis an interstation similarity matrix generated can be conceived of as a multidimensional space in which the stations are arranged in such a way that they are separated from one another according to their similarities. An ordination is then performed on the matrix to extract axes from this multidimensional space. The first axis extracted coincides with the longest axis and accounts for the largest amount of variation in the similarity matrix. Subsequent axes account for successively smaller amounts of variation in the data.

Statistical Tests. A Kruskal-Wallis and a multiple comparison test for significance (Kruskal and Wallis 1952, Dunn 1964) will be used to test for differences in the total abundance and biomass between the stations sampled in unoiled control bays and oiled bays in each year and in multiyear data sets. These same tests will be applied to the abundance and biomass of selected taxa between bays in the first year of the study and in multiyear data sets. Differences in abundance and biomass between stations in unoiled and oiled bays will be tested with a two-tailed Wilcoxon signed ranks test for pairwise observations (Siegel 1956) and with ANOVA.

Diversity. Species diversity can be thought of as a measurable attribute of a collection or a natural assemblage of species and consists of two components: the number of species or "species richness" and the relative abundance of each species or "evenness." The two most widely used measures of diversity which include species richness and evenness are the Brillouin (Brillouin 1962) and Shannon (Shannon and Weaver 1963) information measures of diversity (Nybakken1978). There is still to interpret (Sager and Hasler 1969, Hurlbert 1971, Fater 1972, Peet 1974, Pielou 1966a,b). Pielou (1966a,b) has outlined some of the conditions under which these indices are appropriate.

The Shannon function

$$H' = -\Sigma_i p_i \log p_i \text{ where } p_i = \frac{n_i}{N}$$

where n_i = number of individuals in the i^{the} species
N = total number

assumes that a random sample has been taken from an infinitely large population, whereas the Brillouin function

$$H = -\log \frac{N!}{n_1!n...N_s!}$$

is appropriate only in the entire population has been sampled. Thus, if it is desired to estimate the diversity of the fauna at a sampling site, then the Shannon function is appropriate. The Brillouin function is merely a measure of the diversity of five grab samples taken at each site and makes no predictions about the diversity of the benthic community from which the samples were drawn. Nevertheless, the Shannon and Brillouin diversity indices are closely correlated (Feder and Matheke, 1980b), indicating that either index would be acceptable, as both Loya (1972) and Nybakken (1978) have suggested.

The evenness of samples taken at each site will be calculated using the Brillouin measure of evenness,

$$J = H/H_{max'}$$

where H = Brillouin diversity f ction.

$H/H_{max'}$ the maximum possible diversity for a given number of species) occurs if all species are equally common and is calculated as:

$$H_{max'} = \frac{1}{N} \log \frac{N!}{\{[N/s]!\}^{s-r}\{[N/s]+1!\}^{r}}$$

where $[N/s]$ = the integer part of N/s

s = number of species in the censused community

r = $N - s[N/s]$.

Theoretically, the evenness component of the Shannon function can be calculated from the following:

$$J' = \frac{H'}{\log s^*}$$

where H' = Shannon diversity function

s^* = the total number of species in the randomly sampled community.

However, s*, the total number of species in a randomly sampled community, is seldom known for benthic infaunal communities. Therefore, the evennesss component of the Shannon diversity index was not calculated (for a discussion, see Pielou, 1977).

Species richness (margalef, 1958) will be calculated as:

$$SR = \frac{(s-1)}{lnN}$$

where S = the number of species

N = the total number of individuals

The Simpson dominance index (Simpson, 1949; Odum, 1975) will also be calculated:

$$S = \Sigma \frac{n_i(n_i-1)}{N(N-1)}$$

where n_i = number of individuals of species $i_1, i_2, i_3 \ldots i_x$

N = total number of individuals.

Diversity indices will be calculated for station, for each year and for multi-year data sets.

K-Dominance Curves. A technique designed to detect natural, physical, biological, and pollution-induced disturbances on marine benthic communities (Warwick 1986, Warwick et al. 1987) will be applied to the benthic abundance and biomass data. The application of this technique is to detect oil-induced disturbance that might result from the Exxon Valdez oil spill. Warwick (1986) suggested that the distribution of numbers of individuals among species and in distribution of biomass among species in marine benthic communities show a differential response to pollution-induced disturbance. Such a response can be demonstrated by the comparison of K-dominance curves for abundance and biomass. These curves rank species in order of importance on the x-axis (logarithmic scale) with percentage dominance on the y-axis (cumulative scale). Warwick (1986) indicates that in unpolluted communities, the k-dominance curve for biomass lies above that for number of individuals, in moderately polluted communities the two curves more or less coincide, and in grossly polluted communities the numbers curve lies above the biomass curve. However, the method should be used with caution as suggested recently by Beukema (in press). He showed from his data on intertidal sites that some of his shores demonstrated the "polluted" pattern although the sites were not polluted. The intertidal (and probably subtidal shallow areas) area is subjected to a greater range of environmental variable than deeper subtidal habitats, so perhaps the model of Warwick (1986) distinguishes some sites by stress rather than between polluted and unpolluted sites (Gray 1989).

Distribution of Geometric Classes of Abundance of Species (Taxa). The data from selected stations will be used to examine the distribution of the geometric abundance classes of the numbers Iindiv/m^2 of all taxa collected each year and for multiyear data sets. Assessment of the distribution of taxa (method described by Gray and Mirza 1979, with modifications suggested by Gray and Pearson, 1982, and Pearson pers. comm.) in these abundance classes is useful to identify indicator species within a disturbed area or a region with potential for disturbance. The abundance data will be tabulated into Geometric Abundance Classes (= abundance groups) (e.g., Class I: 1 indiv/m^2; Class II: 2-3 indiv/m^2; Class VI: 32-63 indiv/m^2; Class XI: 512-2047 indiv/m^2). The data will mainly be plotted as the number of individuals per taxon on the abscissa and the number of each taxon in each geometric class on the ordinate. As suggested by Dr. T. Pearson (pers. comm.), "number of taxa" rather than "percent of taxa" is a more accurate display of species (taxa) composition within a community when examining geometric classes of abundance and more precisely shows general taxon changes within that community. Species will also be tabulated bysampling year for their abundance in Geometric Classes III-X.

This technique has been used successfully by Gray and Pearson (1982) to detect the effect of organic enrichment on the benthic biota of a Scottish Sea Loch (Pearson 1975). The most important aspect of this monitoring scheme is that an "indicator" group of species can be isolated objectively. Several changes will generally be apparent when disturbance occurs (Gray and Pearson 1982). The "moderately common" taxa, composed of the first species to respond to disturbance by increasing in abundance, can be used as indicators of change (Gray and Pearson 1982). A slightly modified approach to the above technique will be used.

Appendix B. Statistical procedures applied to the benthic abundance data for samples collected in Prince William Sound July 1990.

A. Analysis of Variance.

1. A 2 x 3 analysis of variance (condition x bays) was employed to determine significant differences in the abundance of 18 selected taxa. Differences included those between conditions, non-oiled and oiled, as well as those between sites Rocky Bay, West Bay, Zaikof Bay, Disk Island, Herring Bay and Snug Harbor.

a. Results of the analysis of variance indicated whether significant differences in the abundance of selected taxa existed with respect to condition, i.e., non-oiled versus oiled, regardless of sites. That is, abundance of taxa at Rocky Bay, West Bay and Zaikof Bay were pooled to yield a non-oiled value, while the abundance of taxa at Disk Island, Herring Bay and Snug Harbor were pooled to yield an oiled value. Means and standard deviations were presented for each of the taxa.

b. Results of the analysis of variance indicated whether significant differences existed across, Rocky Bay, West Bay, Zaikof Bay, Disk Island, Herring Bay and Snug Harbor regardless of condition, i.e., non-oiled or oiled. Means and standard deviations were presented for each of the taxa.

c. Results of the analysis of variance indicated whether a significant interaction occurred between conditions, non-oiled versus oiled, and sites, Rocky Bay West Bay, Zaikof Bay, Disk Island, Herring Bay, and Snug Harbor.

d. In order to compare differences between sites, i.e., Rocky Bay, West Bay, Zaikof Bay, Disk Island, Herring Bay and Snug Harbor, univariate F tests were computed for each pair of bays resulting in inter-bay comparisons. Univariate F Tests and the associated probabilities were presented.

2. A 2 x 2 analysis of variance (mesh size x bay) was employed to determine significant differences in the abundance of 18 selected taxa. Differences included those between mesh sizes, 0.5 mm and 1.0 mm, as well as those between different sites, respectively.

a. Results of the analysis of variance indicated whether significant differences existed in the abundance of selected taxa collected by mesh of different sizes, i.e., 0.5 mm and 1.0 mm, regardless of collection site. That is, abundance of taxa at Herring Bay and Zaikof were pooled, and abundance computed for each mesh size. Means and standard deviations were presented.

b. Results of the analysis of variance indicated whether significant differences existed in the abundance of selected taxa at Zaikof Bay and Herring Bay. Taxa collected with mesh sizes of 0.5 mm and 1 mm were pooled for each site. Means and standard deviations were presented.

c. Results of the analysis of variance indicated whether a significant interaction occurred between mesh size, 0.5 mm and 1 mm and sites, Zaikof Bay and Herring Bay.

B. Data Transformation

As discussed by Sokal and Rohlf (1969) and Zar (1974), when data consists of counts, frequently the square root transformation is found to be of value in normalizing the underlying distribution prior to analysis. In view of the fact that statistics employed assume that the scores were normally distributed, a square root transformation, x = square root (x + 0.5), was employed prior to data analysis. This formula is preferred over a square root or zero (Bartlett, 1936).

C. Data Adjustment

Since the statistical analysis required a mean abundance and variance at every site for each taxon, data adjustment was required in those replicate samples in which no abundance was detectable. If the five replicated samples contained no organisms, a single value of 0.5 was substituted for one of the replicates. Appendix Table 1 indicates those sites for each taxon at which an adjustment was performed prior to analysis.

Appendix B.
Table 1. Indicated by a + are those sites for each taxon, listed alphabetically, at which a data adjustment was made.

Taxon	Rocky Bay	West Bay	Zaikof Bay	Disk Island	Herring Bay	Snug Harbor
Ampharetidae			+			
Bivalvia						
Capitellidae						
Chaetodermatidae					+	+
Cirratulidae						
Golfingidae		+	+			
Harpacticoida		+	+			+
Lumbrineridae						
Maldanidae			+			
Nematoda			+			
Nephtyidae						
Nuculanidae						
Owenidae						
Polyodontidae		+	+			
Spionidae						
Syllidae		+	+			
Thyasiridae						
Veneridae					+	

Appendix C. Comparison of 18 documented taxa from pooled oiled (3 Bays) and unoiled Bays (3) by ANOVA for samples collected in Prince William Sound.

Taxon Group	Comparison	F Ratio	Probability	Significant
Capitellidae	oiled<unoiled	8.104	0.009	Yes
Ampharetidae	oiled>unoiled	1.852	0.186	Marg.
Chaetoder-matidae	oiled<unoiled	12.203	0.002	Yes
Cirratulidae	oiled>unoiled	21.723	0.000	Yes
Lumbrineridae	oiled>unoiled	6.818	0.015	Yes
Maldanidae	oiled>unoiled	4.635	0.042	Yes
Nephtyidae	oiled<unoiled	6.974	0.014	Yes
Owenidae	oiled>unoiled	9.508	0.005	Yes
Spionidae	oiled<unoiled	22.107	0.000	Yes
Polyodontidae	oiled>unoiled	14.924	0.001	Yes
Syllidae	oiled>unoiled	4.460	0.045	Yes
Golfingidae	oiled>unoiled	1.469	0.237	NO
Bivalvia	oiled>unoiled	5.018	0.035	Yes
Nuculanidae	oiled<unoiled	7.135	0.013	Yes
Thyasiridae	oiled>unoiled	0.087	0.771	NO
Veneridae	oiled<unoiled	10.522	0.003	Yes
Harpacticoida	oiled>Unoiled	1.569	0.222	NO
Nematoda	oiled>unoiled	9.962	0.004	Yes

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AIR/WATER #3

GEOGRAPHIC AND TEMPORAL DISTRIBUTION OF DISSOLVED AND PARTICULATE PETROLEUM HYDROCARBONS IN THE WATER COLUMN

November 1990

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EXECUTIVE SUMMARY

The goal of the NOAA component of project Air/Water #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 and 1990 by deployment of hydrocarbon free mussels along the oil spill trajectory for one month exposure periods.

1989 Direct Water Analyses:

Chemical analysis of the seawater samples, taken in triplicate at 1 and 5 meter depths at 30 stations shortly after the spill, shows the presence of aromatic hydrocarbons of petroleum origin. Total concentrations range up to about 5 µg/l (ppb) at the most heavily contaminated sites analyzed to date.

1989 Indirect Water Analyses from Caged Mussels:

Both aromatic and aliphatic hydrocarbons of petroleum origin were detected in tissue of caged mussels deployed in 1989. Total hydrocarbon concentrations, up to 100 µg/g wet tissue (ppm), were highest at Smith Island, Herring Bay, 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.

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 proportion of total hydrocarbons and total aromatic hydrocarbons found in the caged mussels indicates 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.

1990 Indirect Water Analyses from Caged Mussels:

Mussel were deployed at 19 stations within the Sound and at 9 outside the Sound during 1990. Stations were added within the Sound to monitor oil re-mobilization in cleanup areas. Outside the Sound, some sites were deleted for logistic reasons; three were added at locations where 1989 preliminary results by Air Water #2 showed some contamination. Chemical analysis of the 321 samples collected in 1990 has just begun.

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.

INTRODUCTION

This study will continue 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. Although the Alaska Department of Environmental Conservation conducted work under this project in 1989 and 1990, this status report will deal only with the NOAA/NMFS portion of the study.

In 1989, hydrocarbon loading was monitored by direct sampling of seawater at 30 sites in Prince William Sound and by exposing hydrocarbon free mussels along the oil spill trajectory. In 1990, mussels were deployed at 19 sites within Prince William Sound and at 9 sites along the Kenai Peninsula, the Alaska Peninsula, and Afognak Island.

METHODOLOGY

In 1989 water samples were collected at 30 sites 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. Five hundred forty NOAA water samples were collected. The majority of samples collected on the first cruise, 31 March- 4 May have been analyzed at Auke Bay Lab under the direction of Technical Services Study #1. 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.

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 and at 18 stations outside the Sound. 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. Three hundred ninety three samples ("caged" mussels, unexposed "base" mussels, native mussels and air blanks) were collected. Analyses of the mussels from the first exposure inside and outside the Sound in 1989, done at Auke Bay Lab, are approximately 95% complete; replicates of 80% of these samples have also been analyzed. About 80% of mussels from the last exposure inside the Sound have been analyzed once.

Mussels were again deployed in 1990 as proposed in Air Water #3 Study Plan 1990. Changes made to the 1990 plan were the addition of two sites coincident with ADEC 's sediment traps offshore of proposed berm relocations in Snug Harbor and Herring Bay (sites 39 and 40 figure 1). Katmai Bay, an impacted site on the Alaska Peninsula, was also added (site 38 figure 2). In response to a peer review comment re the 1990 plan, field replicates were eliminated.

STUDY RESULTS

1989 Water

Total concentrations of aromatic hydrocarbons range up to about 5 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.

1989 Mussels

Inside Prince William Sound

Petroleum hydrocarbons from the Exxon Valdez appear to be present in caged mussels at all deployment sites along the spill trajectory within PWS (table 1). Total hydrocarbon concentrations range up to 100 µg/g wet tissue (ppm). Concentrations were highest in mussels at 1 meter depths at Smith Island, Herring Bay, and Snug Harbor during the first exposure period. Petroleum derived hydrocarbons were lower but detectable at all other stations and depths inside PWS along the spill trajectory. Mussels were verified to be hydrocarbon free prior to deployment, and remained so when deployed at unoiled sites. Replicate measurements of total hydrocarbons, total aromatics, and total dibenzothiophenes are within a factor of about 2 at sites and depths where appreciable hydrocarbons are present in mussel tissues; replicate measurements are less close at other

ites where mussel tissue hydrocarbon burdens approach analytical detection imits.

The relative proportions of polynuclear aromatic hydrocarbon (PAH) compounds in mussel tissue at exposed sites during May 1989 is similar to the relative proportions of these compounds in Exxon Valdez crude oil. The relative proportion of a PAH compound in figure 3 is calculated as the ratio of a C_n- substituted PAH (where $0 \leq n \leq 4$) and the sum of all PAH compounds considered. The pattern of these relative PAH proportions in Exxon Valdez crude oil and in caged mussels deployed for 1 month at 1) Herring Bay, 1 m depth; 2) Sawmill Bay, 5 m depth; and 3) Elrington Passage, 25 m depth; are compared in figure 3. These sites and depths are representative of all sites and depths where petroleum derived hydrocarbons were detected in caged mussels. Although the overall patterns of relative PAH proportions are similar, the relative proportions of unsubstituted and C_1 substituted PAH compounds found in mussel tissue are lower than in the crude oil, probably because of microbial degradation prior to mussel ingestion. The general similarity of these patterns of relative PAH proportions implicates Exxon Valdez crude oil as the source of hydrocarbons found in the caged mussels.

Petroleum hydrocarbons remained detectable at most sites in September, 1989, although at lower concentrations (table 1). At Herring Bay, 1 m depth, total hydrocarbon concentrations decreased from about 100 µg/g wet weight in May 1989 to about 10 µg/g wet weight in September. Decreases of total hydrocarbon concentrations were less pronounced at sites that were ess heavily impacted initially.

Outside Prince William Sound

For sites outside the Sound, only data from the first exposure (mid June-late July) are presently available. Hydrocarbon concentrations in deployed mussels were generally low (table 2). Only at Tonsina Bay and Chignik do mussels appear to have definitely accumulated petroleum hydrocarbons; levels approximate moderately impacted sites in PWS. Again proportions of PAH compounds to total PAHs in tissues at these 2 sites are similar to proportions of those compounds in Exxon Valdez crude. Mussels were not recovered from Hallo Bay, definitely an impacted area.

Hydrocarbon concentrations were highly variable among replicates. 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.

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 (Amoco Cadiz, Fates and effects of the oil spill, Proceedings of the International Symposium, Centre Oceanologique de Bretagne, Brest (France) November 19-22, 1979, p. 564), who commented on a caged mussel deployment study of the Amoco Cadiz oil spill conducted by Wolfe *et at.* (Amoco Cadiz, *op. cit.*, pp. 599-616). 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".

STATUS OF INJURY ASSESSMENT

A. Water Analyses

Final hydrocarbon analysis of all remaining water samples collected for this project are expected to be done by the end of 1990. Data analysis will begin on receipt of these data, and should be completed and a report drafted within the following 6 months.

B. Caged Mussels

Final hydrocarbon analysis of remaining caged mussel tissues deployed in 1989 are expected to be done by the end of 1990. Data analysis will begin on receipt of these data, and should be completed and a report drafted within the following 6 months.

Final hydrocarbon analysis of caged mussel tissues first deployed inside PWS during 1990 are expected to be done by early 1991. Data analysis will begin on receipt of these data, and remaining 1990 deployment mussels to be recommended for analysis will be identified based on these results. It is expected that all 1990 caged mussels to be analyzed will be by June, 1990, and that subsequent data analysis and report preparation will be completed 6 months hence.

ble 1. Hydrocarbon concentrations in mussels caged at 1,5,and 25 meter depths in Prince William Sound in 1989. Hydrocarbon concentrations are ng/g wet tissue weight (ppb). TOT HC = total hydrocarbons= the sum of all aliphatics (including the unresolved complex mixture) and all aromatics. TOT ARO = total aromatics. TOT DIBEN = total dibenzothiophenes. Rep 1 = replicate 1. Blank spaces indicate sample not yet analyzed. "--" indicates no sample. Base samples are unexposed mussels.

		FIRST EXPOSURE (5/1-6/1)					
			1 meter				
	TOT HC		TOT ARO		TOT DIBEN		
SITE	Rep 1	Rep 2	Rep 1	Rep 2	Rep 1	Rep 2	
Herring Bay	70965	102955	4815	5943	1270	1385	
Smith Island	55488	16993	3263	3008	731	686	
Snug Harbor	18380	38347	622	1573	154	347	
Johnson Cove	12564	17718	372	507	64	95	
Elrington Psg	13000	21971	147	449	24	91	
. Wales Psg	10288	10768	316	75	37	18	
Bainbridge Psg	3492	3855	34	102	2	16	
Squire Island	6031	8376	108	154	12	29	
Needle	5756	8109	64	121	0	19	
Outside Bay	6455	8836	162	86	13	10	
Main Bay	5209	8935	81	77	7	19	
Olsen Bay	-737	1896	67	12	0	0	
base 117	3088	5689	70	0	0		
base 124	3937		78		0		
base 129	3164	3961	9	105	0	0	

le 1 (cont)

			5 meters				
SITE	TOT	HC	TOT	ARO		TOT	DIBEN
Herring Bay	44840	38585	4600	1922		1194	502
Smith Island	38038	37454	1118	1480		257	347
Snug Harbor	17400	22542	507	831		122	172
Johnson Cove	15942	11609	279	330		54	58
Elrington Psg	10134	14857	153	238		28	39
P. Wales Psg	7021	5886	138	168		17	20
Bainbridge Psg	2238	2887	129	115		15	8
Squire Island	5474	9718	140	185		15	26
Needle	3403	4960	138	129		14	12
Outside Bay	4935	6175	105	151		8	16
Main Bay	4220	5818	102	141		6	13
Olsen Bay	383	6718	77	89		0	0
			25 meters				
	TOT HC		TOT ARO			TOT DIBEN	
SITE	Rep 1	Rep 2	Rep 1	Rep 2		Rep 1	Rep 2
Herring Bay	6725	12998	437	106		95	7
Smith Island	2037	2543	106	529		9	118
Snug Harbor	7076	7006	228	193		38	38
Johnson Cove	3678	1366	196	67		32	0
Elrington Psg	5453	10745	169	196		28	32
P. Wales Psg	4457	5125	128	119		14	10
Bainbridge Psg	104	4189	128	23		14	0
Squire Island	1565	5779	132	215		13	32
Needle	--	--	--	--		--	--
Outside Bay	--	--	--	--		--	--
Main Bay	2132	4584		187			29
Olsen Bay	-124	1261	102	99		0	0

ble 1 (cont)

LAST EXPOSURE (8/3-9/10)			
		1 meter	
	TOT HC	TOT ARO	TOT DIBEN
SITE	Rep 1	Rep 1	Rep 1
Herring Bay	12435	605	126
Smith Island	--	--	--
Snug Harbor	--	--	--
Johnson Cove			
Elrington Psg			
P. Wales Psg	--	--	--
Bainbridge Psg	-1451	137	10
Squire Island			
Needle			
Outside Bay	--	--	--
Main Bay	-1009	195	11
Olsen Bay	-2233	129	3
		5 meters	
Herring Bay	5097	526	112
Smith Island	--	--	--
Snug Harbor	--	--	--
Johnson Cove			
Elrington Psg	--	--	--
P. Wales Psg			
Bainbridge Psg	-2078	137	9
Squire Island			
Needle	--	--	--
Outside Bay	--	--	--
Main Bay	973	144	12
Olsen Bay	-2657	140	3

Table 1 (cont)

		25 meters	
SITE	TOT HC	TOT ARO	TOT DIBEN
Herring Bay	774	194	16
Smith Island	--	--	--
Snug Harbor	--	--	--
Johnson Cove			
Elrington Psg	--	--	--
P. Wales Psg			
Bainbridge Psg	551	150	7
Squire Island			
Needle			
Outside Bay	--	--	--
Main Bay	-615	133	6
lsen Bay	-2163	121	0

Table 2. Hydrocarbon concentrations in mussels caged at 1,5,and 25 meter depths at Kenai Peninsula, Alaska Peninsula, and Afognak Island sites in 1989. Hydrocarbon concentrations are ng/g wet tissue weight (ppb). TOT HC = total hydrocarbons= the sum of all aliphatics (including the unresolved complex mixture) and all aromatics. TOT ARO = total aromatics. TOT DIBEN = total dibenzothiophenes. Rep 1 = replicate 1. Blank spaces indicate sample not yet analyzed. "--" indicates no sample. Base samples are unexposed mussels.

		FIRST EXPOSURE (6/6-8/22)				
		1 meter				
	TOT HC		TOT ARO		TOT DIBEN	
SITE	Rep 1	Rep 2	Rep 1	Rep 2	Rep 1	Rep 2
Sunny Cove	656	-57	8	100	0	0
Black Bay	1651	751	8	141	0	0
Tonsina Bay	8538	9311	502	12	95	0
W. Port Dick	2121	1033	157	342	6	0
P. Chatham	3199	1499	2	129	0	4
P. Graham	--	--	--	--	--	--
Discoverer Bay	50	773	9	126	0	2
Blue fox B.	832	7348	156	-6	4	0
Raspberry Str.	--	--	--	--	--	--
Kukak Bay						
Hallo Bay	--	--	--	--	--	--
Chignik Bay	14049	18581	73	595	4	92
Balboa Bay	1374	1177	24	183	0	5
Base 159B	-384		82		0	

Table 2 (cont)

				5 meters				
SITE	TOT	HC		TOT	ARO		TOT	DIBEN
Sunny Cove	Rep 1	Rep 2		Rep 1	Rep 2		Rep 1	Rep 2
Black Bay	7219	6030		152	145		6	0
Tonsina Bay	7004	17658		266	174		25	11
W. Port Dick	10961			145			10	
P. Chatham	2983	2838		110	174		5	5
P. Graham	--	--		--	--		--	--
Discoverer Bay	6950	-1214		137	126		5	0
Blue fox B.	5886			119			9	
Raspberry Str.	--	--		--	--		--	--
Kukak Bay	--	--		--	--		--	--
Hallo Bay	--	--		--	--		--	--
Chignik Bay	12312	7964		258	249		28	22
Balboa Bay	5782			191			13	

SITE				25 meters				
	TOT HC			TOT ARO			TOT	DIBEN
SITE	Rep 1	Rep 2		Rep 1	Rep 2			
Sunny Cove	105	-947		104	6		0	0
Black Bay	8314			-2			0	
Tonsina Bay	1922			142			11	
W. Port Dick	6536	-2375		4	158		0	2
P. Chatham	900			175			4	
P. Graham	--	--		--	--		--	--
Discoverer Bay	2083			160			4	
Blue fox B.	2144	1434		143	223		5	2
Raspberry Str.	--	--		--	--		--	--
Kukak Bay	--	--		--	--		--	--
Hallo Bay	--	--		--	--		--	--
Chignik Bay	--	--		--	--		--	--
Balboa Bay	--	--		--	--		--	--

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ATTORNEY WORK PRODUCT

Fig. 1 Air/Water #3 Mussel Deployment Sites in PWS 1990

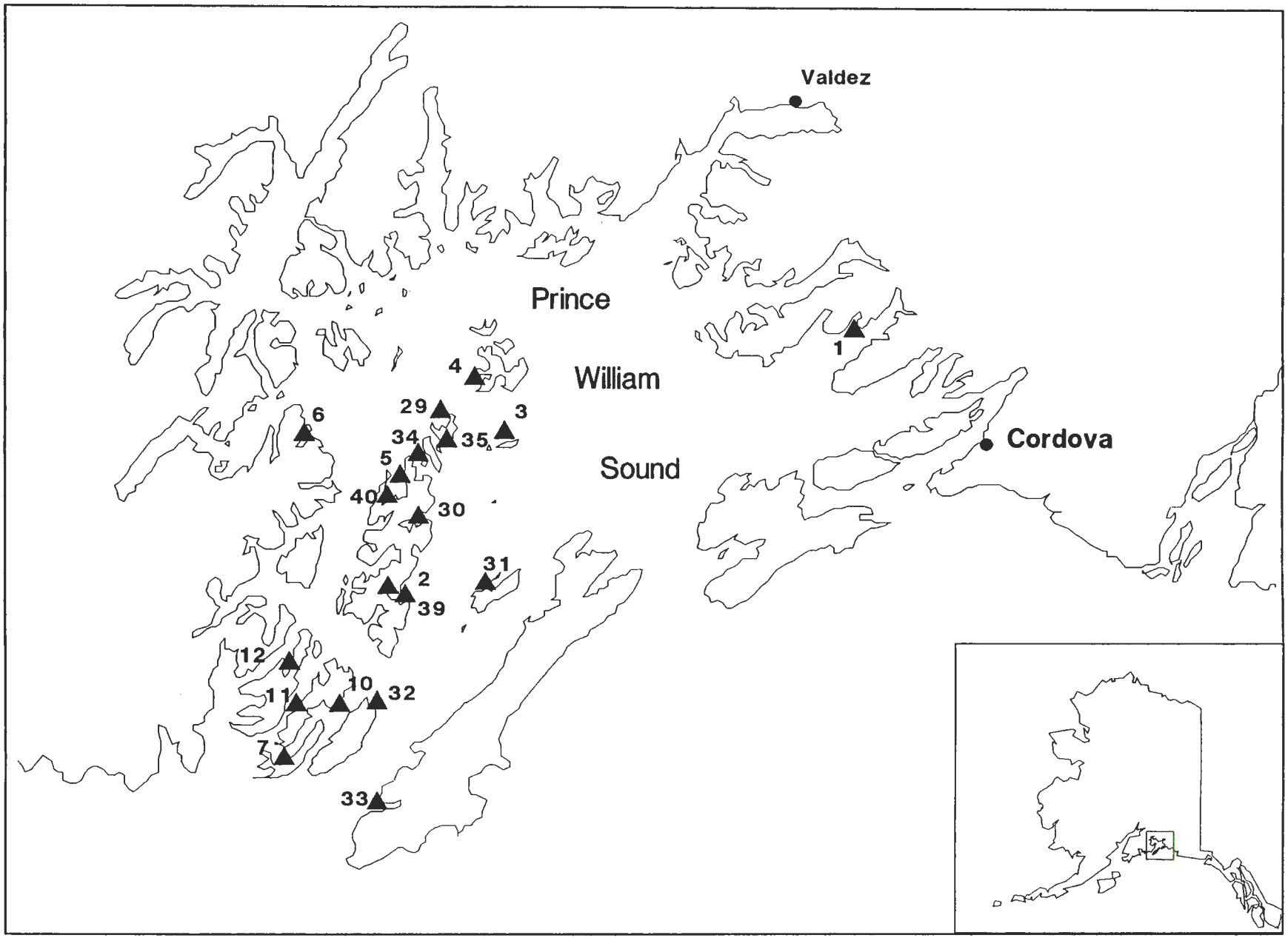


Fig. 2 Air/Water #3 Mussel Deployment Sites Outside PWS 1990

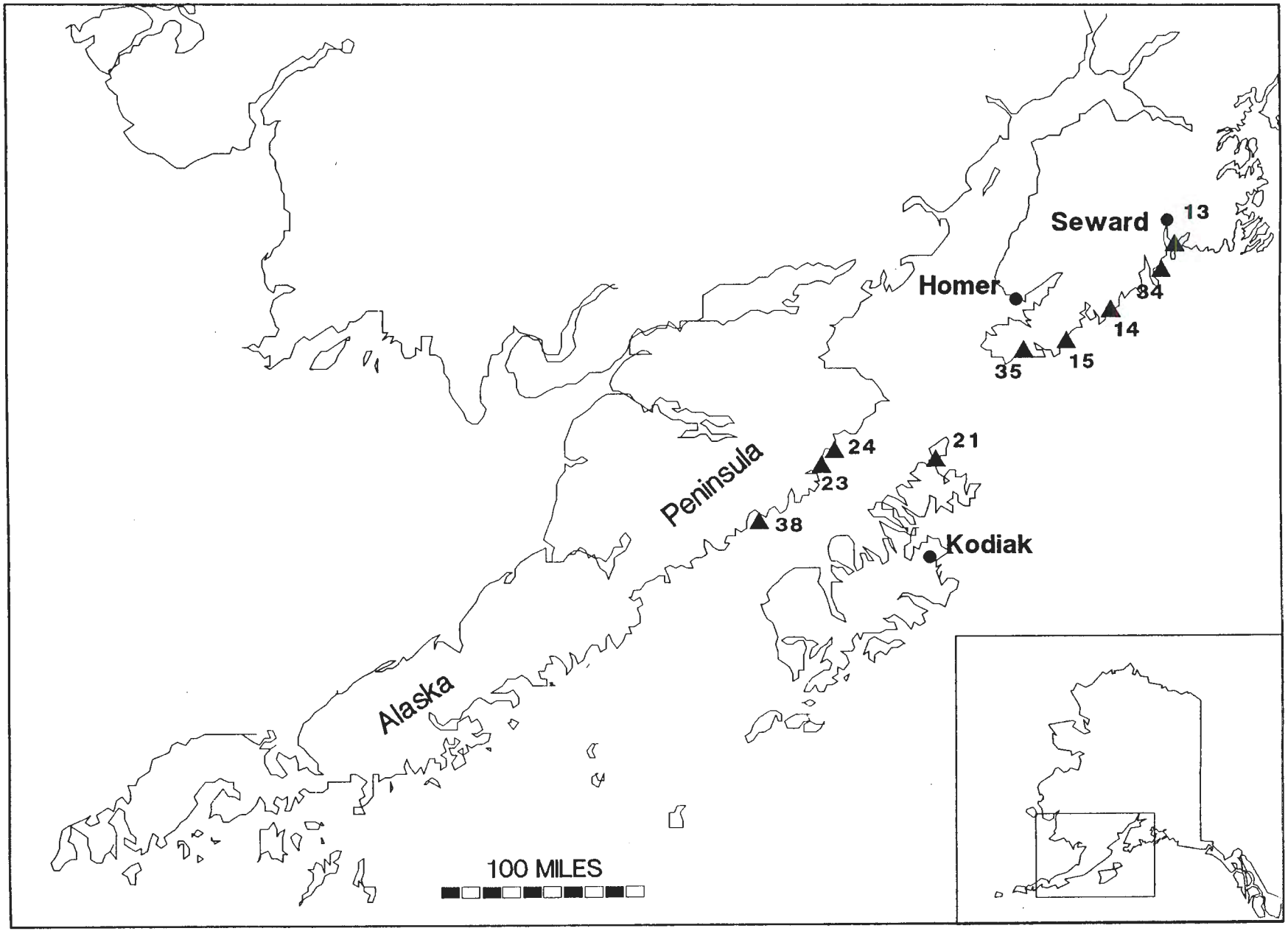
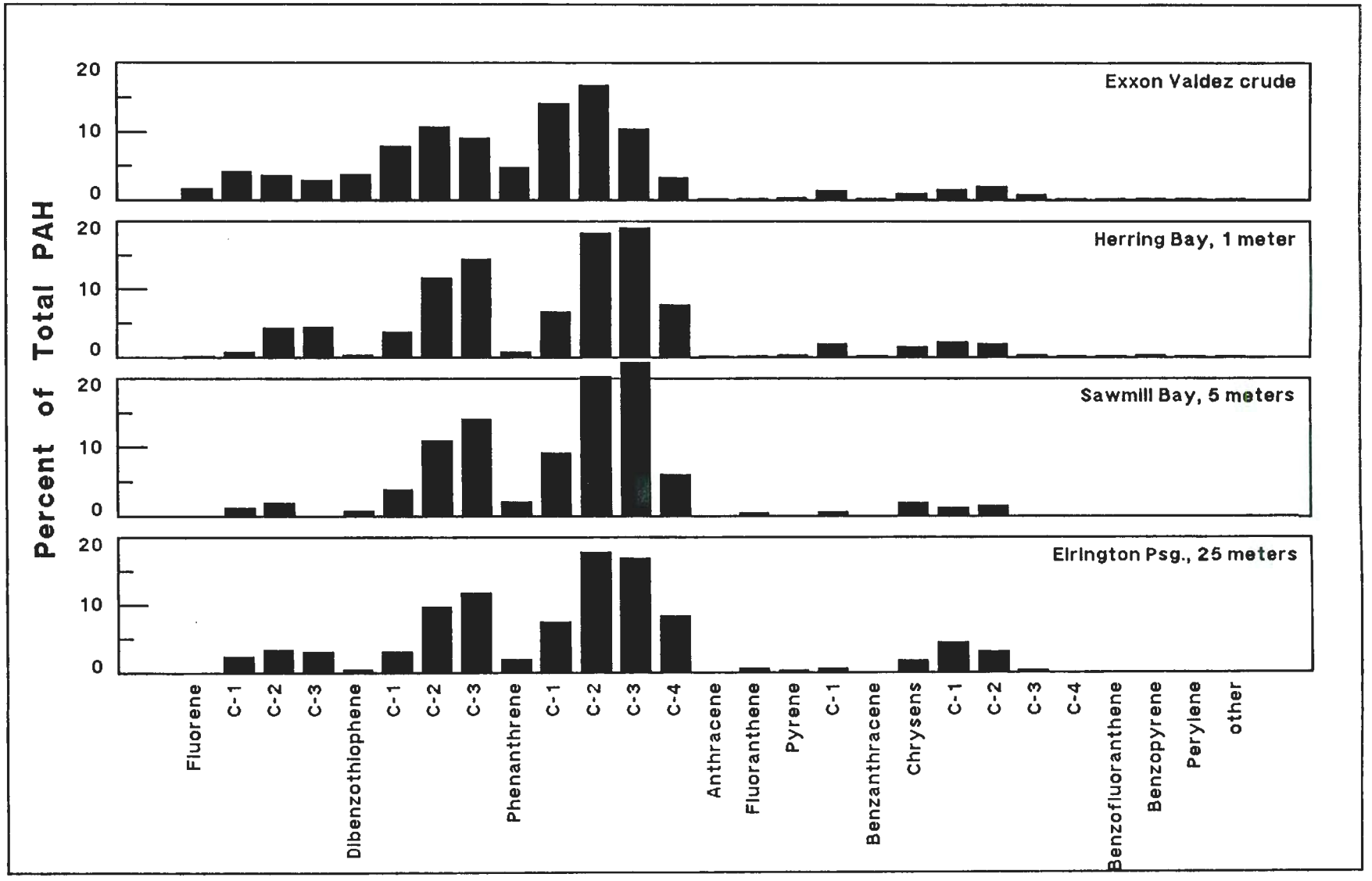


Fig 3. Comparison of proportions of specific polynuclear aromatic hydrocarbons to the sum of all PAH compounds considered



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CERCLA/NRDA STUDIES

Geographic Distribution of Dissolved and Particulate Hydrocarbons in the Water Column

(Subtidal Sediment Traps)

Air and Water Study #3

David Sale, Dr. C. Peter McRoy and Jim Gibeaut
Alaska Department of Environmental Conservation, Anchorage, AK

ATTORNEY WORK PRODUCT

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EXECUTIVE SUMMARY

The Alaska Department of Environmental Conservation, as co-lead agency for this study, has deployed 15 sediment traps in Prince William Sound to monitor nearshore sedimentation in the wake of the EXXON VALDEZ oil spill (Figure 1). The sediment traps consist of a cement base with rebar, on which are clamped three PVC pipes, open at one end to collect sediments settling out of the water column. Five of the traps were deployed in November, 1989, during the ADEC Comprehensive Environmental Monitoring Cruise; the other ten were deployed in August, 1990, in response to interest from the NRDA peer review and management groups in expanding the program.

By capturing particulates from the water column, the sediment traps provide a measure of one component of mobility, suspended load, as storms and treatment expose remaining oil to weathering. These traps are seen potentially linking several damage assessment studies and the response action of berm relocation. The trap sites have been chosen to be in proximity to either NOAA mussel cages (A&W #3), Coastal Habitat intertidal or subtidal sites, or berm relocation sites. Additionally, six of the sediment traps are located on transects used for Air and Water #2 subtidal chemistry and microbiology sampling, providing data relating spatial and temporal trends in benthic sediments in proximity to the traps.

Two retrieval cruises have occurred in 1990 for the original set of five traps. None of the ten new traps deployed in August have been sampled. At the time of retrieval, or before a new trap is deployed, samples are taken of the upper two centimeters of benthic sediments, including any flocculent layer present. These bottom samples can be compared with sediments captured in the trap to see if the chemistry data from the traps can be extrapolated to the surrounding benthos.

Currently, 100 sediment, and sediment and filter, samples have been taken and stored under Chain of Custody. Eighty-five samples have been sent through the ACG to Texas A&M for analysis; fifteen samples remain at the Anchorage OSRC under Custody. No conclusions regarding presence, composition or transport of petroleum hydrocarbons can be drawn, nor relationships to other studies be made, pending analysis of the samples. A sample from the trap in east arm, NW Bay, retrieved in May, 1990, showed a "fingerprint" to North Slope crude oil. Results from the samples taken from the sediment traps and bottom samples from nearby the newly deployed traps should be available by early March.

OBJECTIVES

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

STUDY METHODOLOGY

The five traps placed in the Sound in 1989 were deployed at sites where ADEC divers were sampling subtidal sediments. These sites were part of the twenty three oiled target sites representing a variety of exposures, energy regimes and substrate types sampled in November, 1989. Site selection for the ten new traps deployed in 1990 sought additionally to balance degrees of oiling, environmental sensitivity, and proximity to NOAA mussel cages (monitoring bioaccumulation), Coastal Habitat intertidal and subtidal sites and the treatment technique of berm relocation, in an attempt to draw relationships between these other studies, exposed oil remaining on the shoreline and sedimentation of oiled particles in the nearshore subtidal(Table 1).

Trap Recovery

The sediment traps were 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 descended a permanent buoy line that marks the location of the traps. Once the traps are located, one diver filled four-250 ml. sample jars with sediments from the upper benthic layer (0-2 cm) within a 2 meter radius around the trap base, being careful to include any flocculent material. Once sampling was complete, the second diver capped the cylinders to avoid potential contamination of the traps as they were moved through the water column. The cylinders and base were then raised to the vessel deck.

Once on the deck, the cylinders were removed from the base. New cylinders, precleaned with an alconox wash, rinsed with acetone,and wiped with hexane, were clamped onto rebar stands. Ten pounds of sodium chloride (table salt precombusted to 450 C for 4 hrs) was added to the bottom of the cylinder to act as a brine trap and create a hypersaline condition to reduce degradation of any oil trapped. The trap was lowered over the side of the vessel until the top of the cylinders was just above waterline, where the trap was filled with seawater by filtering through a 0.5mm nylon mesh to exclude organisms and sediments (care is taken to assure no oil or diesel sheen is on the water close to the traps; blanks are taken periodically of the water used). The trap was placed as close to its original position on the bottom as possible. The cylinders are numbered, and the numbers kept in the same geometric position, i.e., #1 points along the transect heading to the beach, with succeeding numbers

clockwise.

Filtration

After the cylinders have been allowed to settle on the deck for one hour, 2/3 of the water volume is decanted, saving 2-3 liters for rinse water. The remaining 1/3 volume is transferred to a clean container along with any suspended sediments; the trap is rinsed with decanted water, and poured into clean container. This volume is filtered through pre-combusted 9mm glassfiber 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.

Experimental and Sediment Trap Design

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 direction and velocity of any currents and the geometry of the trap (aspect ratio and axial symmetry) will determine whether the cylinder itself disrupts the flow field and results in turbulent eddies within and around the trap that will change any naturally occurring sedimentation patterns. The spacing of the traps determines whether the cylinders affect each other. Based on a lack of data regarding local currentss, the traps were designed so that the aspect, symmetry and spacing would be adequate for a variety of conditions. The trapping cylinders are constructed of Schedule 40, high chemical resistance PVC, 6" inside diameter and 48" tall. A baffle of 0.5" square grid, 0.5" deep fits flush with the top of each trap. These cylinders are mounted on a 20" x 20" square base, with rebar extending upward 24" on which to clamp the cylinders.

The presence of the traps in the complex, multidirectional, oscillatory current and wave environment of Prince William Sound makes control of variables difficult. Design considerations follow the Woods Hole report (1989):

1) Use of a cylindrical geometry for axial symmetry promotes trapping efficiency.
2) An 8:1 aspect ratio, shown to be sufficient for current velocities to 20 cm/sec (0.39 knots), minimizes eddies, reduces in-trap flow, and allows for a tranquil layer within the trap. In the sheltered bays where most traps are deployed, currents are probably within this range (Port Fidalgo is an exception).
3) Triangular configuration of the traps on the base and orientation of the base to wave-induced shore-normal currents. Cylinders are spaced at 18" centers to maximize distance between traps and aligned to reduce chances any cylinder would be downstream of another.

4) Leveling of the cylinders after deployment to maintain orientation to currents and the water column.

Statistical replication comes from the installation of three cylinders per trap base, rather than several bases on the same isobath at a site.

STUDY RESULTS

As of this date, only preliminary results are available from the first trap retrieval in May, 1990. This retrieval, in the east arm of NW Bay, Eleanor Isl (TR04), showed "fingerprint" patterns similar to North Slope crude oil, especially in the alkylated phenanthrenes and alkylated dibenzothiophenes . The presence of a sheen on water during filtration of subtidal sediments from NW Bay has been verified by ADEC, NOAA and Coastal Habitat researchers. Samples of the sediments captured in five of the traps, along with bottom samples from the other ten sites have been sent to Texas A&M for analysis. These results should be available around 3/1/91.

STATUS OF INJURY ASSESSMENT

The sediment traps have been capturing sediments settling out of the water column since their placement in November, 1989. Except for the May, 1990 samples from the NW Bay, Eleanor Isl. traps, no chemistry results are available, and thus no conclusions regarding damage to subtidal resources can be inferred.

This study is designed to monitor an aspect of transport and deposition of oil, either settling through adsorption to sediment particles or through accumulation in fecal pellets. The sediment traps provide an important link between release of the oil from the EXXON VALDEZ, and subsequent beaching, and its eventual inclusion in and damage to subtidal systems. Coordination with sediment chemistry, bioaccumulation and toxicity studies is necessary to show the actual damage to subtidal ecosystems.

Knowledge of particle size and actual currents in the bays where traps are located could allow us to predict with more accuracy whether oil is being added to the subtidal by continual erosion of remaining oil and sediments on the shoreline, or whether resuspension of previously deposited sediments dominates.

Bibliography

Woods Hole Oceanographic Institution, 1989, Sediment Trap Technology and Sampling; U.S. Global Ocean Flux Planning Report No. 10, August, 1989.

Table 1 **SEDIMENT TRAP LOCATIONS**

The following list represents sediment trap locations within Prince William Sound matched with nearby Coastal Habitat studies, NOAA Mussel Cages and areas of berm relocation.
(* sites are on A&W #2 subtidal chemistry and microbiology transects)

LOCATION (SITE#)	SEGMENT	COASTAL HABITAT # (SUBTIDAL)	NOAA MUSSEL #	BERM RELOCATION
West Arm, NW Bay Eleanor Isl. (TR05)	EL-52	1424	MS-29	no
East Arm, NW Bay Eleanor Isl. (TR04)*	EL-56	(ST02)	MS-29	no
Snug Harbor, Knight Isl. (TR01) *	KN-408	1650	MS-2	yes
Sleepy Bay, Latouche Isl.(TR02)*	LA-18	(ST17)	MS-32	yes
Port Fidalgo (TR03)*	none	none	none	no
Stockdale Harbor, Montague Isl. (TR06)	none	none	none	no
north Green Isl (TR07)	GR-001B	979	MS-31	no
Johnson Cove, Evans Isl. (TR09)	EV-053	none	MS-10	yes
Bay of Isles, Knight Isl.(TR08)	KN-134	(ST06) 1598	none	no
Herring Bay, Knight Isl. (TR13)	KN-118	(ST03) 1522	MS-5	no
Squire Isl. (TR10)	SQ-005	none	MS-8	no
N. Chenega Isl. (TR11)	CH-010	none	none	no
Eshamy Bay (TR12)	EB-010	none	none	no
Disk Isl. (TR14) *	DI-067	453	MS-34	no
Dubois Pt., Naked Isl. (TR15)	NA-027	(ST11)	none	no

DRAFT 11/07/90

I. DRAFT PRELIMINARY STATUS REPORT

AIR/WATER STUDY NUMBER 6

FATE AND TOXICITY OF SPILLED OIL FROM THE EXXON VALDEZ

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III. EXECUTIVE SUMMARY

This study was designed and undertaken by NOAA 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) 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. A contract has been let to SAIC to carry out toxicity testing of sediment samples taken during the DAVIDSON cruise in July. A draft final report has been received and analysis of the data is underway. Final analysis will require data on hydrocarbon chemistry and grain size of the sediments (expected from A/W2). A second contract has been let to SAIC to determine the extent to which any toxicity present in sediments and interstitial waters may be attributed to polar, oxidation products (as opposed to parent hydrocarbons) in petroleum. Another contract has been let to the Bermuda Biological Station to determine whether polar oxidation products of petroleum can be detected in significant amounts in tissues of Mytilus edulis taken from the spill zone. No results are available from either of these latter efforts; some results should become available by January 1, 1991 (BBS) and March, 1991 (SAIC). A Steering Group has been assembled for the petroleum budget synthesis exercise (objective c above), and a preliminary meeting has been held to identify sources of information and potential approaches to completing the synthesis. A synthesis workshop should be scheduled sometime during spring of 1991 as an important step in 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

This study was designed and undertaken by NOAA 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) 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. Under the direct management of the NOS/OMA Ocean Assessments Division (OAD), the study is very closely coordinated with Air/Water Study Number 2, directed by the NMFS Auke Bay Laboratory, and relies also on collaboration with personnel and resources of the NMFS Northwest Fisheries Science Center.

A review and analysis of the scientific literature relating to the objectives of this project is contained in the detailed study plan.

VI. STUDY METHODOLOGY

Sampling Locations

Most sampling was carried out from the NOAA Ship DAVIDSON between June 25 and August 5, 1990. Samples were taken concurrently from the same stations under Air/Water Study Number 2 for the chemical analyses and sediment grain-size analyses. The strategy was to sample one site per day, in support of three studies: Air/Water Studies 2, 3, and 6. The sites were selected from the list of sites visited in 1989 under A/W Studies 2 and 4, supplemented by a group of unoiled "reference sites" that were added to determine effects on benthic community composition. These sites, selected on the basis of preliminary information on distribution of floating and/or beached oil, encompassed essentially the full geographic range of exposure (i.e., from Bligh Island in PWS to Katmai Bay on the Alaska Peninsula). Positions of the intertidal sampling locations follow (R = reference site; E = exposed site; i = infaunal site):

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	Ri	60° 16.85' N	147° 02.1' W
05	Rocky Bay	Ri	60° 20.3' N	147° 08.2' W
06	West Bay	Ri	60° 51.8' N	146° 46.5' W
07	Herring Bay	Ei	60° 26.54'N	147° 47.13' W
08	Disk Island	Ei	60° 29.8' N	147° 39.7' W
09	Block Island	E	60° 31.7' N	147° 36.4' W
10	Northwest Bay	Ei	60° 33.1' N	147° 34.6' W
11	NE Knight Island	E	60° 26.35' N	147° 37.65' W
12	Bay of Isles	Ei	60° 22.8' N	147° 45.4' W
13	Green Island	E	60° 16.2' N	147° 26.1' W
14	MacLeod Harbor	Ri	59° 53.21' N	147° 45.8' W
15	Mooselips Bay	Ri	60° 17.55' N	147° 18.0' W
16	Snug Harbor	Ei	60° 14.25' N	147° 44.1' W
17	Chenega	Ri	60° 19.85' N	148° 00.45' W
18	Lower Herring Bay	Ri	60° 24.4' N	147° 47.8' W
19	Drier Bay	Ri	60° 19.2' N	147° 44.0' W
20	Sleepy Bay	Ei	60° 03.95' N	147° 50.35' W
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

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). A similar survey was proposed for this year under the combined A/W Studies 2 and 4, mainly on a subset of last year's stations. The toxicity bioassay used in this 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). Results of last year's Microtox survey also showed some correlations with UV fluorescence analyses of the sediment samples.

To ascertain that the Microtox test was responding to Exxon Valdez oil, calibration experiments were conducted this year 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 the Environmental Conservation Division of the Northwest Fisheries Science Center, 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 (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 cruises. 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*. 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. All bioassays were initiated within 10 days of the collection of the samples.

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 are being pursued together through a series of 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 have been 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). Based on the results of these assays,

some samples will be selected for further testing with a 48-hr survival and development assay using bivalve (oyster) larvae and with the Ames mutagenicity test (Ames et al 1975). Anaphase aberrations and sister chromatid exchange will also be determined in the exposed bivalve larvae. Those extracts that elicit significant responses in the foregoing tests of genotoxicity, toxicity and/or mutagenicity will be subjected to chemical fractionation procedures to separate any polar oxidized derivatives from the parent, non-oxidized constituents of petroleum. These fractions will be 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.

If a substantial portion of the toxicity is contributed by the polar fraction, the chemical constituents in those fractions will be identified and quantified in some detail, and related if possible to the original parent compounds in petroleum. Target compounds of the fractionation and analytical scheme will 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*, will be 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. Eight to ten tissue samples, representing a broad range of exposure compositions and concentrations, will be selected from the inventory of samples collected under Projects A/W3 and CH1, and sent to Dr. Kathryn Burns at the Bermuda Biological Station, where they will be extracted and analyzed for parent hydrocarbons and oxidized derivatives. A report will be provided by December 31, 1990, summarizing the results, identifying oxidized products where possible, comparing concentrations of oxidation products with parent hydrocarbons, and assessing both the rationale and technical feasibility of more extensive analysis of oxidation products in bivalves from Prince William Sound.

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. Through her previous collaboration, Dr. Burns has ready access to a proprietary library of specialized mass spectra, developed by Dr. Manfed Ehrhardt of Kiel University in Germany, which focuses on the photochemical oxidation products of petroleum. Dr. Burns is expected to carry out these analyses during the months of November-December 1990.

Depending on the outcomes from the above projects, a limited pursuit of chemical fractionation and characterization may also be undertaken by the Environmental Conservation Division of NMFS Northwest Fisheries Center (perhaps in association with the toxicity testing originally proposed under A/W2). This study will include two major objectives with the following approaches:

1. Determine how the toxicity (if any) of organic extracts from Exxon Valdez oil-contaminated sediments is partitioned between polar and non-polar constituents over the geographic range sampled:by A/W2.

Approach:

- perform Microtox on unfractionated extracts
- perform one-step separation on PAC column to fractionate polar from non-polar constituents
- repeat Microtox on the two separated fractions
- perform simple carcinogenicity/mutagenicity bioassay (eg Ames test, S.O.S. Chromotest) on selected fractions

2. Determine what types of polar constituents are present in these extracts:

Approach:

- perform HPLC/UV on polar (and non-polar) fractions to identify presence of PNA derivatives
- perform detailed GC/MS on selected polar fractions to identify and quantify (major) constituents

The work should focus on (a) determining the geographic extent over which a significant fraction of the observed toxicity or mutagenicity can be ascribed to polar derivatives; and (b) a preliminary characterization of the polar constituents that may be involved. The selection of samples for this chemical fractionation was to be guided by the Microtox and UVF signals.

OBJECTIVE D. Summary budget or "mass balance" for the fate of spilled 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 were proposed initially for analysis and 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

Representatives of the above noted activities, along with other recognized experts on oil weathering and fates, will be consulted for recommendations on appropriate approaches to synthesis, and for their judgments on the suitability and adequacy of existing information for development of the FATES model. Apart from the information on polar constituents described above, no original data were proposed for collection under this project. Timely progress on the FATES budget will depend on the availability of suitable infor-mation from other sources and projects; chemical data, i.e., from TS1, will be of utmost importance to the completion of this project.

Where existing information is found to be deficient, means will be explored for gathering of improved information. The reliability of all estimates will be assessed and qualified in the final analysis.

A small team will be composed to develop the preliminary FATES budget, and to identify needs for more detailed analysis and/or modeling. The interim draft FATES budget will be subjected to review by a larger group of experts. For this purpose, a small workshop was proposed originally to be held during the period November or December, 1990. Following this review of the initial estimates, 2-4 months was to be allotted to refining the estimates and to completing any further modeling necessary to describe the fates of oil up through year 2 (fall of 1990). The "final" report would include estimates for oil fates up to the date of the workshop and projections for any oil remaining in the environment at that time. Efforts will be made to present estimates of oil distribution in a form amenable to comparison with existing information on toxicity to facilitate any subsequent assessments of the potential effects on biological resources.

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

The sensitivity of the Microtox assay to Exxon Valdez oil was evaluated by the EC Division of the NMFS Northwest Fisheries Center, in accord with the A/W2 study plan. The results indicate that the Microtox assay was not sensitive to whole, fresh Prudhoe Bay crude oil, to 11-day old weathered Exxon Valdez oil, or to extensively weathered (1.5 years) Exxon Valdez oil. Toxicity was observed however, from a model mixture of six aromatic

hydrocarbons mixed in the proportions found in Exxon Valdez oil. On the basis of these results, all further ECD-proposed applications of the Microtox test under project A/W6 have been postponed pending results of other, ongoing toxicity bioassays on samples from Prince William Sound.

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

All tests have been completed and a draft report was received from SAIC on October 10, 1990. The draft report was reviewed for completeness relative to the statement of work and proposal, and comments were provided to SAIC on October 22. The final report is expected on November 13, 1990.

Analysis of the toxicity data is incomplete at the time of this report. In the final analysis, the toxicity data will need to be related to the chemical data for selected sediment samples (from Project numbers A/W2 and/or TS1).

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 were collected in Prince William Sound by scientists from SAIC during the first week of September. The interstitial water samples have been extracted; the extracts have undergone preliminary chemical screening by GC/MS, and the extracts have been submitted to Parametrix, Inc (subcontractor to SAIC) for screening of toxicity with Microtox and SOS chromotest. Extraction of sediment samples began in late October. No results are available for this reporting period. A draft report is due from SAIC in May/June 1991.

2. K. Burns contract

A proposal was received from Kathryn Burns on October 5, and following review, a revised (October 12) version of the proposal was submitted to procurement on October 13. The contract was sent to Bermuda Biological Station 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 is expected on the results of the extractions and analyses around the first of January 1991.

3. NWFC/ECD effort

No funds have been transferred for this activity. On the basis of the results of the Microtox calibration study (A/W2), it was decided to delay startup until some results were available from the SAIC components of this project, and then to reconsider the design and potential implementation.of this effort.

OBJECTIVE D. Summary budget or "mass balance" for the fate of spilled oil.

On June 6, 1990, a small Steering Group met 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. The Steering Group convened by D. A. Wolfe, project manager for A/W6, 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), and Dr. Jacqui Michel (Research Planning, Inc.). 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 the Oilfates budget will depend therefore on the availability of suitable information from other sources and projects; eg., chemical data from Technical Services Project No. 1 (TS1), will be of utmost importance to the completion of this project.

The Steering Group 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 steering group meeting.

The strategy outlined in the detailed study plan for A/W6 called for an initial synthesis of information to be presented and discussed at a technical workshop on or around 1 December 1990. This workshop was to serve as a forum for technical review and comment which would guide the completion of the synthesis. Following this workshop review of the initial estimates, 2-4 months would be allotted to refining the estimates and to completing any further modeling necessary to describe the fates of oil up through year 2 (fall of 1990). The "final" report was envisioned to provide estimates for oil distributions and fates at least through October 1990, along with projections for any oil remaining in the environment at that time.

VII. STATUS OF INJURY ASSESSMENT

OBJECTIVE A

When fully analyzed, the results of the toxicity bioassays are expected to document the presence/absence of residual toxicity associated with the Exxon Valdez oil, as measured by the standard bioassays employed in this project. The final report on the bioassays should be complete and the data available for analysis by mid-November, 1990. To test whether the observed toxicity, if any, can be correlated with residual oil constituents in the sediments will require the analysis of selected sediment samples collected and inventoried under project A/W2.

OBJECTIVES B & C

Results from the first tier of toxicity testing (of the whole extracts from interstitial water and sediments) should become available by December 1, 1990. However, no information on the relative contribution of polar constituents to that observed level of toxicity, if any, can be expected until after the extracts have been fractionated and the fractions have been subjected to (the second tier of) toxicity tests. Completion of this phase of

the work can be expected by early January 1991 Detailed biological testing and chemical characteri-zation of the polar constituents, if necessary, should be carried out before April 1, and analysis and interpretaion of results will be completed for the draft report (to be submitted no later than June 1, 1991). This project is self-contained, and does not rely on results from other activities for its conclusions.

Results of the pilot study on polar constituents in mussel tissues (analyses by K. Burns) are expected to be complete and the data available by January 1, 1991. These results will provide insights on the merits of expanding the suite of conventional chemical analyses being conducted on other samples to include polar constituents.

OBJECTIVE D

Although some avenues of coordination and synthesis have been pursued by various members of the Steering Group since the June meeting, it is now clear that the original schedule proposed for the synthesis workshop was overly optimistic. For many of the identified compartments in the budget, analytical data are not yet available to support a comprehensive analysis of the distribution and fates of the oil. The proposed approach for this synthesis needs to be reviewed in light of current developments in the overall NRDA, and the implementation schedule needs to be revised. The general approach proposed previously, however, may still be effective.

Under that approach, a lead individual should 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 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. To the extent practical, these "concensus syntheses" should be the primary

focus of the review workshop. If substantive information remains unincorporated or unconsidered at the time of the workshop, however, it should be presented for separate review and discussion in that forum. Every effort possible should also be made in advance of the workshop to compare and reconcile any independent estimates of inter-compartmental transfers; however these should be scrutinized in detail at the workshop itself. As part of this analysis, the reliability of all estimates will be assessed and qualified. Efforts will also be made to present estimates of oil distribution in a form amenable to comparison with existing information on toxicity to facilitate any subsequent assessments of the potential effects on biological resources.

Depending on the analytical results from samples taken in this project (and others), some or all of the sites should be resampled in 1991 and 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 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.

VIII. CITATIONS

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TABLE ONE: STUDIES AUTHORIZED IN 1989 AND 1990

Study Category	Number	Title	1989	1990
Coastal Habitat	CH1	Comprehensive Assessment	X	X
Air/Water	AW1	Geographical Extent in Water	X	Study Discontinued
	AW2	Injury to Subtidal Sediments	X	X
	AW3	Hydrocarbons in Water	X	X
	AW4	Injury to Deep Water	X	1/
	AW5	Injury to Air	X	Study Discontinued
	AW6	Oil Toxicity		X
Fish/Shellfish	FS1	Salmon Spawning Area Injury	X	X
	FS2	Egg and Preemergent Fry Sampling	X	X
	FS3	Coded-Wire Tagging	X	X
	FS4	Early Marine Salmon Injury	X	X
	FS5	Dolly Varden Injury	X	X
	FS6	Sport Fishery Harvest & Effort	X	Study Discontinued
	FS7	Salmon Spawning Area Injury, Outside PWS	X	X
	FS8	Egg & Preemergent Fry Sampling, Outside PWS	X	X
	FS9	Early Marine Salmon Injury, Outside PWS	X	Study Discontinued

Study Category	Number	Title	1989	1990
	FS10	Dolly Varden & Sockeye Injury, Lower Cook Inlet	X	Study Discontinued
	FS11	Herring Injury	X	X
	FS12	Herring Injury, Outside PWS	X	Study Discontinued
	FS13	Clam Injury	X	X
	FS14	Crab Injury	X	Study Discontinued
	FS15	Spot Shrimp Injury	X	X
	FS16	Injury to Oysters	X	Study Discontinued
	FS17	Rockfish Injury	X	X
	FS18	Trawl Assessment	X	X
	FS19	Larvae Fish Injury	X	Study Discontinued
	FS20	Underwater Observations	X	Study Discontinued
	FS21	Clam Injury, Outside PWS	X	2/
	FS22	Crab Injury, Outside PWS	X	X
	FS23	Rockfish Injury, Outside PWS	X	3/
	FS24	Trawl Assessment, Outside PWS	X	X
	FS25	Scallop Mariculture Injury	X	Study Discontinued
	FS26	Sea Urchin Injury	X	Study Discontinued
	FS27	Sockeye Over-Escapement		X
	FS28	Run Reconstruction		X
	FS29	Life History Modeling		4/
	FS30	Salmon Database Mgmt		X

Study Category	Number	Title	1989	1990
Marine Mammals	MM1	Humpback Whale	X	X
	MM2	Killer Whale	X	X
	MM3	Cetacean Necropsy	X	Study Discontinued
	MM4	Sea Lion	X	X
	MM5	Harbor Seal	X	X
	MM6	Sea Otter Impact	X	X
	MM7	Sea Otter Rehabilitation	X	X
Terrestrial Mammals	TM1	Injury to Sitka Black-Tail Deer	X	X
	TM2	Injury to Black Bear	X	X
	TM3	Injury to River Otter and Mink	X	X
	TM4	Injury to Brown Bear	X	X
	TM5	Injury to Small Mammals	X	Study Discontinued
	TM6	Reproduction of Mink	X	X
Birds	B1	Beached Bird Survey	X	X
	B2	Censuses & Seasonal Distribution	X	X
	B3	Seabird Colony Surveys	X	X
	B4	Bald Eagles	X	X
	B5	Peale's Peregrine Falcons	X	X
	B6	Marbled Murrelets	X	Study Discontinued
	B7	Storm Petrels	X	Study Discontinued
	B8	Black-legged Kittiwakes	X	Study Discontinued

Study Category	Number	Title	1989	1990
	B9	Pigeon Guillemots	X	Study Discontinued
	B10	Glaucous-winged Gulls	X	Study Discontinued
	B11	Sea Ducks	X	X
	B12	Shorebirds	X	Study Discontinued
	B13	Passerines	X	X
	B14	Exposure to North Slope Oil	X	Study Discontinued
Technical Services	TS1	Hydrocarbon Analysis	X	X
	TS2	Histopathology	X	X
	TS3	Mapping	X	X
Archeology	ARCH1	Archeological Resources	5/	X
Restoration	RP1	Restoration Planning	X	X
Economics	ECON1	Commercial Fisheries Losses	X	X
	ECON2	Fishing Industry Costs	X	6/
	ECON3	Bioeconomic Models	X	6/
	ECON4	Public Land Effects	X	X
	ECON5	Recreation Damages	X	X
	ECON6	Subsistence Losses	X	X
	ECON7	Intrinsic Values	X	X
	ECON8	Research Program Effects	X	X
	ECON9	Archeological Damage Quantification	X	X

1/ AW4 Combined with AW2
2/ FS21 Combined with FS 13
3/ FS23 Combined with FS17
4/ FS29 Combined with FS28
5/ Part of Econ. 9
6/ Combined with Econ. 1