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Meiofaunal Recolonization Experiment with Oiled Sediments

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Abstract.—To gain insight into long-term studies of the effects of the *Exxon Valdez* oil spill on the meiobenthos, a colonization experiment was initiated in 1990. Unweathered *Exxon Valdez* crude oil was mixed with azoic sediment to prepare low (26 ± 3 $\mu g\ g^{-1}$ total aromatics) and high (210 ± 12 $\mu g\ g^{-1}$ total aromatics) treatments of oiled sediments. The resulting mixtures, and azoic sediment without oil as a control, were added to triplicate colonization trays and buried flush with the surface of two beaches near mean low water in Herring Bay, Prince William Sound, Alaska. Hydrocarbon concentrations tended to decline with time. Trays and ambient sediments were sampled by coring and harpacticoid copepod species enumerated on days 0, 1, 2, and 28. Harpacticoids (more than 40 species) were mostly phytal associates from surrounding eelgrass beds. Colonization was rapid (approaching ambient levels in 1–2 d), especially in control and low-oil treatments. High-oil treatments exhibited significantly reduced densities of total harpacticoids and of two rapidly colonizing species (*Halectinosoma* sp. and *Mesochra pygmaea*) especially on days 1 and 2. Two slower colonizers (*Paralaophonte perplexa* and *Amphiascus minutus*) were unaffected by oil additions. Detrended correspondence analysis identified oil effects on the harpacticoid assemblage among the treatment-date collections. Ambient samples from all dates segregated from experimentals and were tightly clustered. Day 1 and 2 low- and high-oil treatments clustered together as did control, low-, and high-oil sediments from day 28. Control day 1 and 2 collections were intermediate. Thus, results suggest that an oil effect on migration and colonization was detectable, but for fewer than 28 d.

The grounding of the *Exxon Valdez* on Bligh Reef in Prince William Sound, Alaska, on 24 March 1989 caused a 44 million-L spill of crude oil that oiled over 1,600 km of shoreline (Maki 1991). The exposed area was largely pristine before this spill; the only sources of hydrocarbons previously present in Prince William Sound were those from natural processes plus some localized anthropogenic hydrocarbons associated with boating (Karinen et al. 1993). Aromatic hydrocarbons, except possibly perylene and occasionally phenanthrene, did not occur naturally in Prince William Sound sediments (Karinen et al. 1993).

Adverse effects of hydrocarbons on the meiobenthos have been demonstrated (see Coull and Chandler 1992). Field studies following oil spills (Wormald 1976; Giere 1979; Elmgren et al. 1983; Bodin 1988, 1991), sample collection in contaminated sediments (Heip et al. 1988) and experimental additions of hydrocarbons to mesocosms (Frithsen et al. 1985; Stacey and Marcotte 1987; Warwick et al. 1988) have all been employed, and show that meiofaunal density declines may occur during exposure to the toxic fractions of polycyclic aromatic hydrocarbons (PAH). Laboratory studies (harpacticoid copepods have been investigated most frequently) demonstrate that the water-soluble fraction of PAH can prompt severe mortality of meiofauna (Kontogiannis and Barnett 1973; Barnett and Kontogiannis 1975) and that PAH can reduce reproductive output (Ustach 1979; Stacey and Marcotte 1987; Misitano and Schiewe 1990).

Nevertheless, many studies have failed to demonstrate a negative impact of PAH on meiofauna, especially when those studies use sediment-bound crude oil. Nematodes from natural hydrocarbon seeps show increases in densities (Montagna et al. 1987), and experimental oiling has been shown to increase or have no influence on meiofaunal densities in experimental additions of oil in the field (Fleeger and Chandler 1983; DeLaune et al. 1984; Smith et al. 1984; Spies et al. 1988; Feder et al.

1990). Density increases are usually assumed to be related to increased microbial food resources in oiled sediments (Montagna et al. 1987). Colonization by meiofauna, and especially by harpacticoid copepods, into oiled sediments has been shown to be rapid (on the order of days), especially at low concentrations of PAH or other pollutants (Alongi et al. 1983; Decker and Fleeger 1984; Palmer et al. 1988; Spies et al. 1988).

Changes in abundance following an oil spill may have several confounding causes; increases or decreases in mortality, reproduction, and migration/recruitment are all possible and may occur simultaneously. The colonizing and dispersing abilities of meiofauna have only recently been recognized, but appear to be surprisingly strong, especially in seagrass beds (Palmer 1988; Walters 1991; Kurdziel and Bell 1992). Our study was designed to determine the effect of unweathered *Exxon Valdez* crude oil on migration and colonization by meiofauna at the site of the spill. Faunal responses were examined in colonization experiments with and without the application of crude oil. Because many harpacticoids (typically the second most abundant meiofaunal group) are especially rapid colonizers with relatively good swimming abilities (Palmer 1988) and because they are important contributors to the diet of a number of Alaskan fishes (Cordell 1986; McCall 1992), harpacticoid species composition was examined in detail.

Methods

Approximately 333 L of sediment were collected in Auke Nu Cove (58°22′48″N, 134°41′39″W) in Auke Bay, Alaska, on 1 March 1990 from the surface of an eelgrass bed and placed in 19-L buckets. Buckets were subjected to three freeze-thaw cycles over 1 month. During the first thaw, sediment was washed through 2- and 0.36-mm sieves to remove large particles and macrofauna. Excess water was decanted throughout the freeze-thaw cycles, reducing sediment volume to 227 L. Examination revealed that the meiofauna had been killed, but not completely decomposed, by this process.

Sediment and unweathered *Exxon Valdez* crude oil were combined in a cement mixer. The interior of the mixer was washed with hot water and soap and rinsed thoroughly before use. Processing was sequential, starting with controls, so that lightly oiled sediments were not contaminated by preceding heavily oiled samples. The sediment was divided randomly into three equal treatment groups (control, low-, and high-oil). Forty liters of sediment were tumbled in the mixer for 15 min; then *Exxon Valdez* crude oil was added (except controls). The total mixture was tumbled an additional 1.5 h and then stored in 20-L buckets with snap-top lids. For the low-oil treatment, 380 ml of oil were added to yield 0.5% oil, and 1,250 ml of oil were added to yield 1.7% for the high-oil treatment. Processing was completed on 5 April 1990; buckets were stored at ambient air temperatures. The contents of each storage bucket were turned once (17 or 18 April) to retard the onset of anoxia.

Two 12-m transects were positioned intertidally along the upper margins of eelgrass beds in Herring Bay, Prince William Sound, in a lightly oiled (HBL) and in a heavily oiled cove (HBH). Oil ratings were based on Alaska Department of Environmental Conservation shoreline data. Nine 28 × 33 × 14 cm (width × length × height) trays were located at 1.5-m intervals along each transect. Tray walls were straight, and the bottoms and sides perforated with 3-mm holes drilled at 8-mm intervals to allow water circulation and drainage. Treatment positions along the transects were randomly assigned within three consecutive 4-m blocks. After addition of sediment, trays were buried flush with the natural substrate. Implements used to distribute sediment and collect samples were separated by treatment to avoid cross-contamination and were initially hydrocarbon free.

The transect at the high-oil site (HBH: 60°28′0″N, 147°41′12″W) was positioned in the soft substrate of a cobble-gravel beach along the upper margin of an eelgrass bed at approximately −0.6 m (with reference to mean low water) on 25 April 1990. Tray elevations were slightly lower on the 0-m end of the transect (−0.7 m) than at the 12-m end (−0.5 m) to accommodate variations in the substrate. Oil remaining from the *Exxon Valdez* spill was obviously present and may have entered trays.

Experimental sediment was added to each tray before burial. Approximately 1 cm of washed gravel, obtained from a stream near the transect, was added to the bottom of each tray to ensure that a sufficient quantity of sediment was available. Gravel was collected well above the maximum tide line (so it was not contaminated with oil), placed in experimental trays, and washed in a stream to eliminate fine sediment and organic debris. The HBH transect was exposed to air 20 times (1.8% of total time) during the entire 89-d study; emergence times ranged from 32 to 162 min.

Meiofauna were subsampled from each tray (at low tide) with hand-held piston corers (modified 60-ml plastic syringes, 2.66 cm in diameter); the upper 4 cm was retained. Corers were separated by

treatment to avoid cross-contamination. Cores were collected in triplicate from each treatment tray on days 0, 1, 2, 28, and 89. Core positions were determined randomly on an x–y grid without replacement through day 2 and was completely random thereafter. Core placement was at least 2 cm from the tray margin to avoid possible edge effects. Additionally, eight ambient sediment samples were collected from haphazardly selected sites along the tidal level between treatment trays at each sampling period to determine the colonizing source pool of harpacticoids. All faunal samples were preserved in 10% formalin shortly after collection. Additional cores for faunal and hydrocarbon analysis were collected on day 89 (faunal cores were not analyzed).

Hydrocarbon samples were collected with a 3-cm diameter, chrome-plated brass tube; a spoon was slipped down beside the corer to cap it off at 4 to 6 cm. All equipment used for hydrocarbon sampling was prewashed with soap and hot water, rinsed, dried, and rinsed with acetone followed by dichloromethane. Corers were separated by treatment to avoid cross-contamination. Two samples were initially collected from each treatment tray before any mixing with ambient sediments. On days 2, 28, and 89, one sample was collected from each tray, plus three from randomly selected ambient sediment locations among trays. Core positions were chosen randomly along with meiofauna cores to avoid conflict between sample types. Hydrocarbon samples were placed directly into hydrocarbon-free glass jars with teflon lids and frozen as soon as possible after collection.

Hydrocarbon concentrations were determined by gas chromatography followed by mass spectrometry for aromatic fractions or by flame ionization detection for aliphatics. Samples were analyzed at the Geochemical and Environmental Research Group at Texas A & M University and reported via the PWSOIL database.[1] Hydrocarbon samples were filtered by deuterated recovery and method detection limits, and questionable samples (3% of total) were excluded using a procedure developed by Short and Heintz (Alaska Fisheries Center, unpublished data). Data filtration eliminated the initial low-and high-oil treatment observations at the HBH site (12 samples), but because these observations were replicates of those collected at the HBL transect, corresponding data were repeated as HBH values.

[1]Available from C. A. Manen, National Ocean Service, Office of Ocean Resource Conservation and Assessment, Damage Assessment Center, 1305 East-West Highway, Silver Spring, Maryland 20910.

Polycyclic aromatic hydrocarbons are not normally present at measurable concentrations intertidally in Prince William Sound (except perylene and occasionally phenanthrene). Because there are natural sources of alkanes in the sound (Karinen et al. 1993), PAH were chosen as the primary measure of hydrocarbons in this study. Concentration changes in various subcomponents of PAH (e.g., naphthalenes, fluorenes, dibenzothiophenes, phenanthrenes, and chrysenes) were similar to changes in PAH, thus the more generalized PAH measurement was used to summarize the data. Some alkane data, including the unresolved complex mixture (UCM), are also presented.

Hydrocarbon concentrations were compared between sites for each treatment with analysis of variance (ANOVA) procedures. Because variance was proportional to the means, data were log transformed before analysis. To avoid pseudoreplication, multiple observations within single replicate pans were averaged before other calculations; this situation occurred on day 0 only. Tests were considered significant when $P \leq 0.05$.

Seawater temperature and salinity were measured with a self-contained sensing device located at an elevation of approximately −0.1 m near the HBL transect. Particle size analysis was performed on ambient and experimental sediments collected on all sampling dates. In the laboratory, sediment was dried and sieved through 2-mm and 63-μm screens. Sand and gravel were treated by the methods of the Geochemical and Environmental Research Group (GERG) (1989) and silt and clay were measured by pipette analysis (Folk 1974). Total organic carbon (TOC) content was measured by combustion in an induction furnace. Carbon dioxide production was quantified after removal of sulfur oxides and water vapor and conversion of CO to CO_2 (GERG 1989).

In the laboratory, meiofauna passing through a 0.5-mm sieve but retained on a 63-μm mesh sieve was examined. A sucrose flotation/centrifugation technique (Fleeger 1979) was used to extract harpacticoids. Samples were suspended in a dense sucrose solution (700 g sugar in 500 ml water) and centrifuged at 350 rpm. The supernatant was rinsed through a 63-μm sieve. The process was repeated at least twice; selected sediment pellets resulting from the centrifugation were examined for sorting efficiency. The technique was estimated to exceed 95% efficiency for nematodes and 98% for copepods in recently published studies of Alaskan meiofauna from similar habitats (Fleeger et al. 1989; Fleeger and Shirley 1990). The concentrated meiofauna was preserved in 5% buffered formalin and stained with

rose bengal to aid sorting. All organisms were identified to major taxon under a stereo-dissection microscope and enumerated using ruled trays; harpacticoids were removed into separate vials. When high densities of meiofauna were present, predominant taxa (mainly nematodes) were subsampled using the technique of Sherman et al. (1984). Adult and larger copepodite harpacticoids were identified to sex (if mature) and species from collections on days 1, 2, and 28 from HBH.

Day 0 collections revealed that many harpacticoids were not completely decomposed through the freeze-thaw cycles. Subsequent collections contained harpacticoids similar in appearance to those from day 0: broken or missing setae and legs, and exoskeletons broken and covered with detritus. The presence or absence of the caudal setae was used to score all individuals as "live" or "dead" at the time of collection. Concurrent and unrelated work on harpacticoids using freeze-killed specimens in a laboratory flume showed that the caudal setae of dead harpacticoids is easily broken (J. W. Fleeger, personal observation).

Changes in abundance were analyzed using a randomized block design with Statistical Analysis System (SAS) generalized linear model (GLM) programming. A two-way ANOVA using oil treatments (control, low-, and high-oil) and dates (days 1, 2, and 28) was used to identify oil-related effects on colonization of total harpacticoids and abundant copepod species. Tukey's Studentized Range Test was used as an a posteriori test to compare means among treatments. Detrended correspondence analysis (as recommended by Gauch 1982) was used as a method of ordination to investigate patterns in harpacticoid community structure using the DECORANA computer program (Hill 1979). This ordination describes spatial or temporal patterns in communities by using shared species compositions. A multidimensional coordinate system is created that locates various entities (in this case collections for each treatment and date) and describes their relationship to each other. The mean density (across all replicates for a treatment-date combination) of the 40 most abundant species was used for this multivariate technique.

Results

Water temperature and salinity fluctuated with tidal cycle, but means remained near 8.0°C and 29.0 ‰ over the first 28 days at HBL. Mean temperatures increased gradually from day 46 to 89 (8.9 to 16.2°C), while salinities declined (28.2 to 20.8‰).

TABLE 1.—Particle-size composition of experimental and ambient sediments, measured initially and on day 89. Initial samples were collected on days 0, 1, or 2. HBL = lightly oiled cove in Herring Bay; HBH = heavily oiled cove in Herring Bay.

Particle	Initial Mean (%) (SE)	Day 89 Mean (%) (SE)
All experimental sediments		
Sand	36.8 (1.4)	40.7 (3.2)
Silt	59.6 (1.5)	57.3 (3.3)
Clay	3.6 (0.2)	2.0 (0.2)
Ambient sediments at HBL		
Sand	6.5 (1.3)	66.2 (21.7)
Silt	89.4 (3.5)	26.5 (22.5)
Clay	4.1 (2.2)	7.3 (2.2)
Ambient sediments at HBH		
Sand	2.6 (0.8)	19.0 (13.1)
Silt	94.1 (1.5)	38.2 (26.1)
Clay	3.4 (0.7)	42.9 (20.1)

Air temperatures ranged from 2.7 to 14.0°C, but may have been influenced by evaporative cooling. Particle-size distributions for all experimental sediments were similar at both transect locations and across all treatments ($0.392 \leq P \leq 0.987$; Table 1). Silt was the dominant size range in the experimental sediments. There was evidence (significant slopes for sand and clay) that particle sizes changed slightly over the course of the experiment in ambient sediments: the percentages of sand and clay increased, while silt percentages decreased (Table 1). Sediment changes in oil-treated sediments were minimal.

Hydrocarbon Concentrations in Sediments

Hydrocarbon concentrations in experimental sediments (measured by TOC, PAH, and alkanes at HBL) correlated well with the percent oil added ($0.81 \leq r^2 \leq 0.94$, Figure 1). Initial PAH concentrations in the azoic sediments ranged from 4.6 $\mu g\ g^{-1}$ (control) to 210 $\mu g\ g^{-1}$ (high oil) (Table 2 and Figure 2). Initial PAH concentrations in ambient sediments, measured on days 2 and 3, were 0.15 ± 0.06 and 0.27 ± 0.03 $\mu g\ g^{-1}$ at HBL and HBH, respectively. The PAH composition in control sediments was unlike that of *Exxon Valdez* crude oil; the naphthalene component was relatively high and there was evidence of low-temperature combustion products. Benzo[*b*]fluoranthene and heavier compounds were present in control sediments, and concentrations of increasingly methyl-substituted compounds tended to decline with respect to their unsubstituted homologues. Concentrations in ambi-

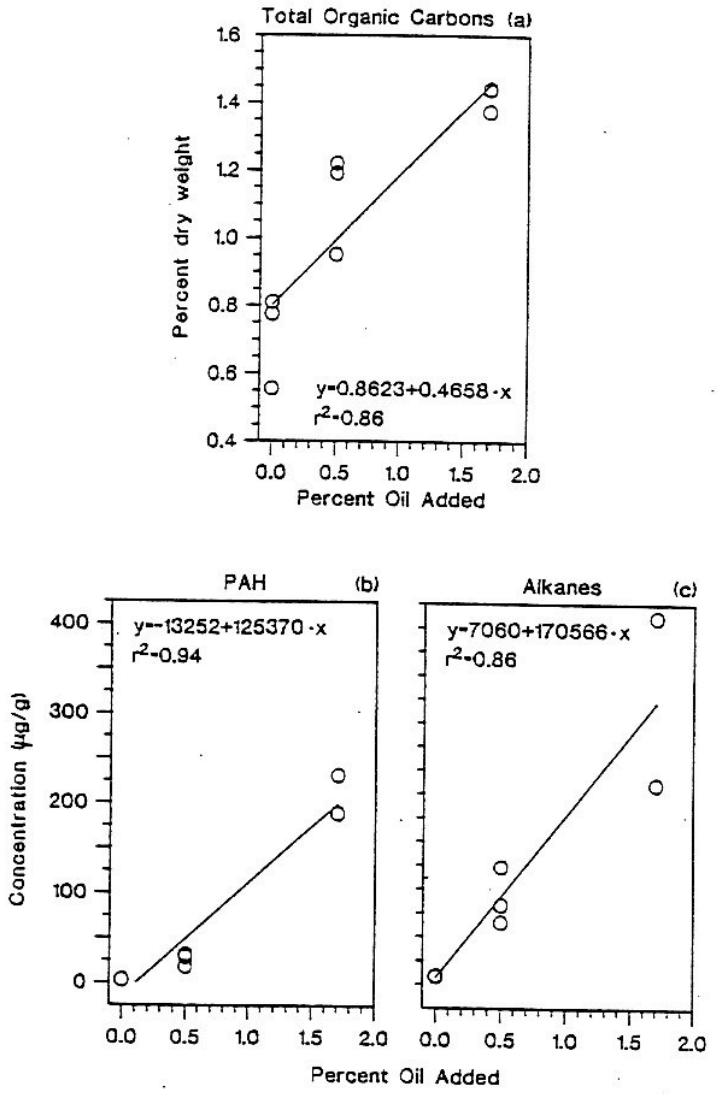

FIGURE 1.—Hydrocarbon concentration on the first day plotted against percentage of oil added. Fitted lines are linear: $y = a + b \cdot x$, where x = percent oil added. (a) Total organic carbons ($N = 18$). (b) Sum aromatics ($N = 17$). (c) Alkane concentrations, excluding the unresolved complex mixture ($N = 18$).

ent sediments were low and essentially constant; PAH composition was characteristic of weathered *Exxon Valdez* crude oil. In oil-treated sediments, PAH composition matched that of *Exxon Valdez* crude oil.

Results of ANOVAs suggest that concentrations of PAH and alkane hydrocarbons in experimental sediments depended primarily on oil treatment and time ($P < 0.001$). At the HBL site, concentrations in oil treatments tended to decline over the first 89 d (Figure 2). At the HBH site, concentrations tended to remain constant or rise slightly during the first 28 d, but declined by day 89 (Figure 2). Although hydrocarbon concentrations declined during the experiment, day 89 PAH and alkane concentrations remained significantly greater than control concentrations at HBL ($0.025 \leq P \leq 0.075$) and HBH ($P \leq 0.028$).

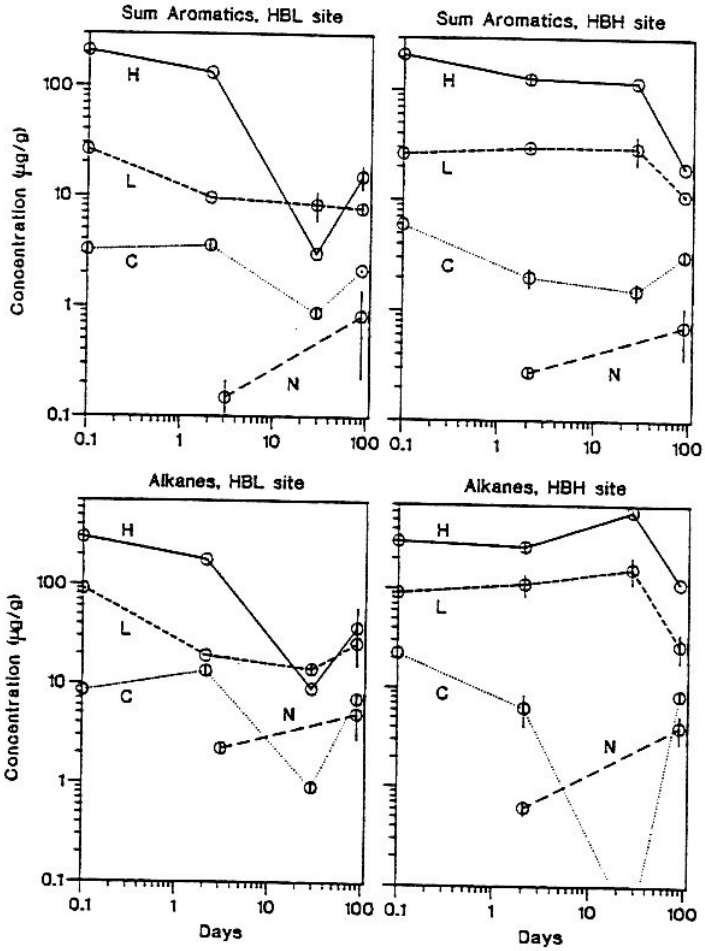

FIGURE 2.—Mean polycyclic aromatic hydrocarbon and alkane concentrations at both transect sites over time for each treatment: H = high oil; L = low oil; C = control; N = natural; HBL = lightly oiled cove at Herring Bay; HBH = heavily oiled cove at Herring Bay. Time data were adjusted upward 0.1 d to allow log transformation. Alkane concentrations in control sediments at HBH were below detection limits on day 28. Error bars are ± SE.

TABLE 2.—Initital hydrocarbon concentrations (µg/g) in treated and control sediments in Herring Bay. PAH = polycyclic aromatic hydrocarbons; UCM = unresolved complex mixture.

	Control	Low oil	High oil
Analyte	Mean (SE)	Mean (SE)	Mean (SE)
PAH	4.6 (0.6)	26 (3)	210 (12)
Alkanes	15.3 (4.6)	90 (11)	298 (51)
UCM	42.8 (5.7)	286 (23)	5,123 (15)
Sum	62.7	402	5,631

Harpacticoid Collections

All collections were dominated by harpacticoids with strongly prehensile first legs and a fusiform prehensile body shape, especially in the families Diosaccidae and Laophonitidae. Hicks and Coull (1983) and Bell et al. (1987) associate these characteristics with a phytal life style, suggesting that most of the more than 40 sediment-dwelling species cataloged in the present study were associated with

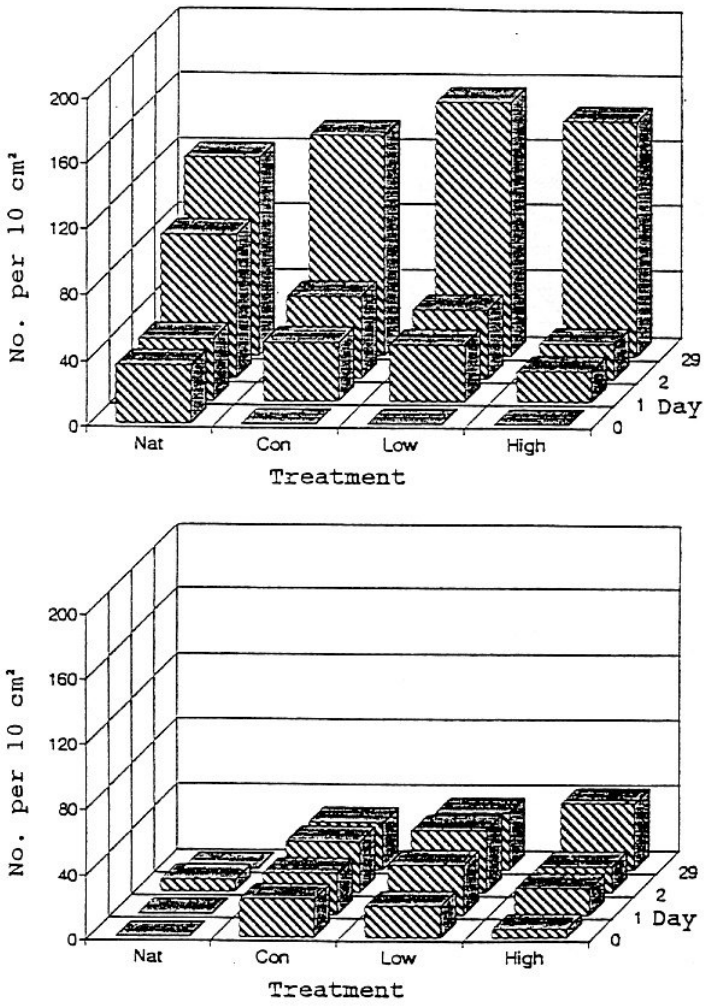

FIGURE 3.—Total copepod density (mean number individuals/10 cm^2) over the time course of the experiment. Upper figure represents the number of copepods scored as living; lower figure, the number of copepods scored as dead at the time of collection. Nat = ambient sediments; Con = control azoic sediments; Low = azoic sediment with low-oil additions; High = azoic sediments with high-oil additions.

surrounding eelgrass beds. Colonists were diverse, and all of the predominant species from the ambient sediments were collected in colonization trays (Table 3). Diversity, measured either as species richness (number of species) or as the Shannon-Wiener index, and relative abundance patterns did not change appreciably over time or differ greatly between ambient and experimental sediments (Table 3). Total harpacticoid densities in ambient sediments increased throughout the experimental period (Figure 3). Differential colonizing abilities were evident among the harpacticoid species (Figures 4 and 5). Among the more abundant species, *Halectinosoma*. sp. and *Mesochra pygmaea* were good colonists (higher densities and relative abundances were observed after 1 or 2 d in the experimental trays, regardless of oil dose, compared to ambient sediments), while *Paralaophonte perplexa* and *Amphiascus minutus* were poor colonists (lower densities and relative abundances in trays compared to ambient sediments).

Two-way ANOVA was conducted in a random-

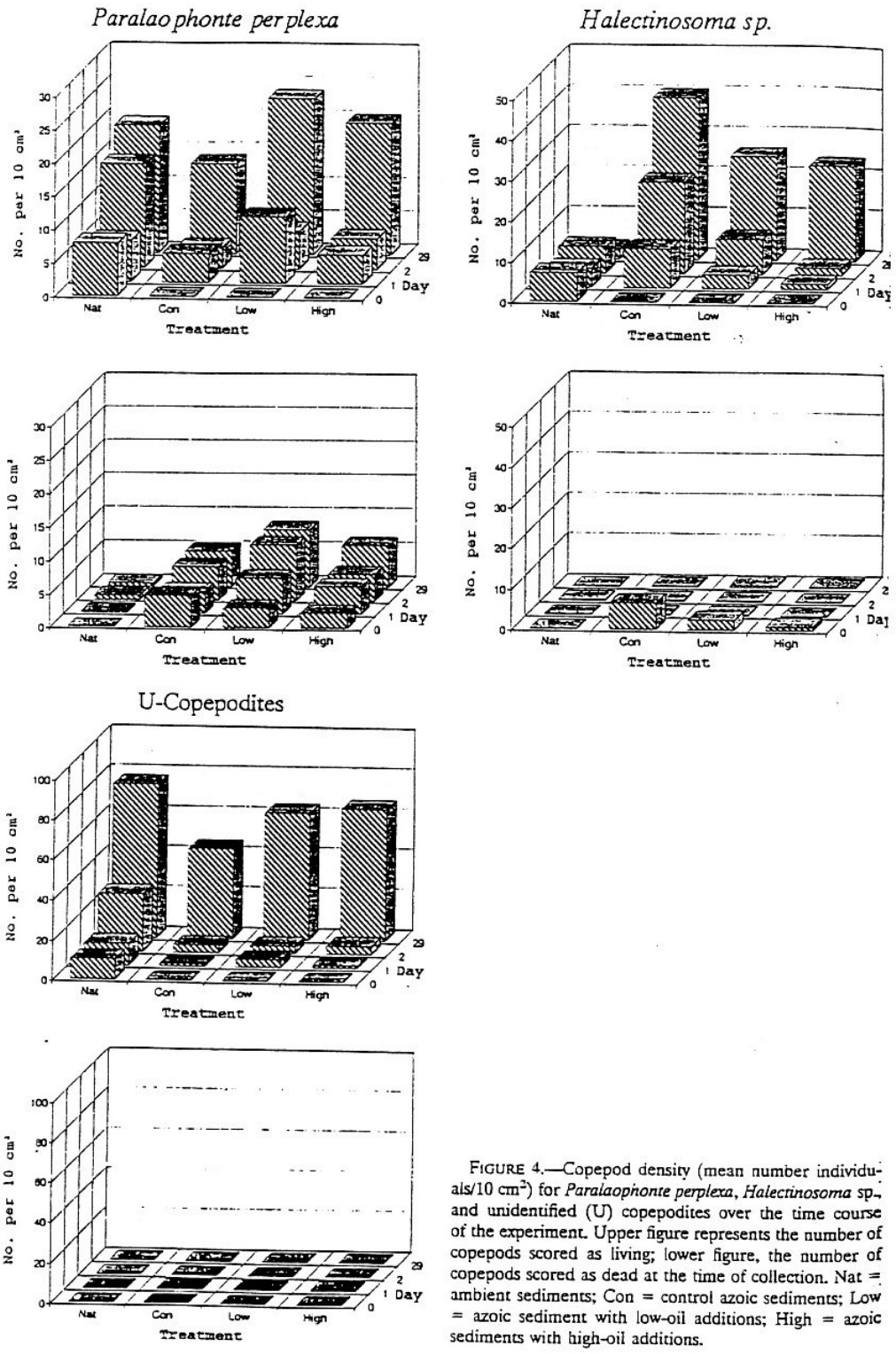

FIGURE 4.—Copepod density (mean number individuals/10 cm^2) for *Paralaophonte perplexa*, *Halectinosoma* sp., and unidentified (U) copepodites over the time course of the experiment. Upper figure represents the number of copepods scored as living; lower figure, the number of copepods scored as dead at the time of collection. Nat = ambient sediments; Con = control azoic sediments; Low = azoic sediment with low-oil additions; High = azoic sediments with high-oil additions.

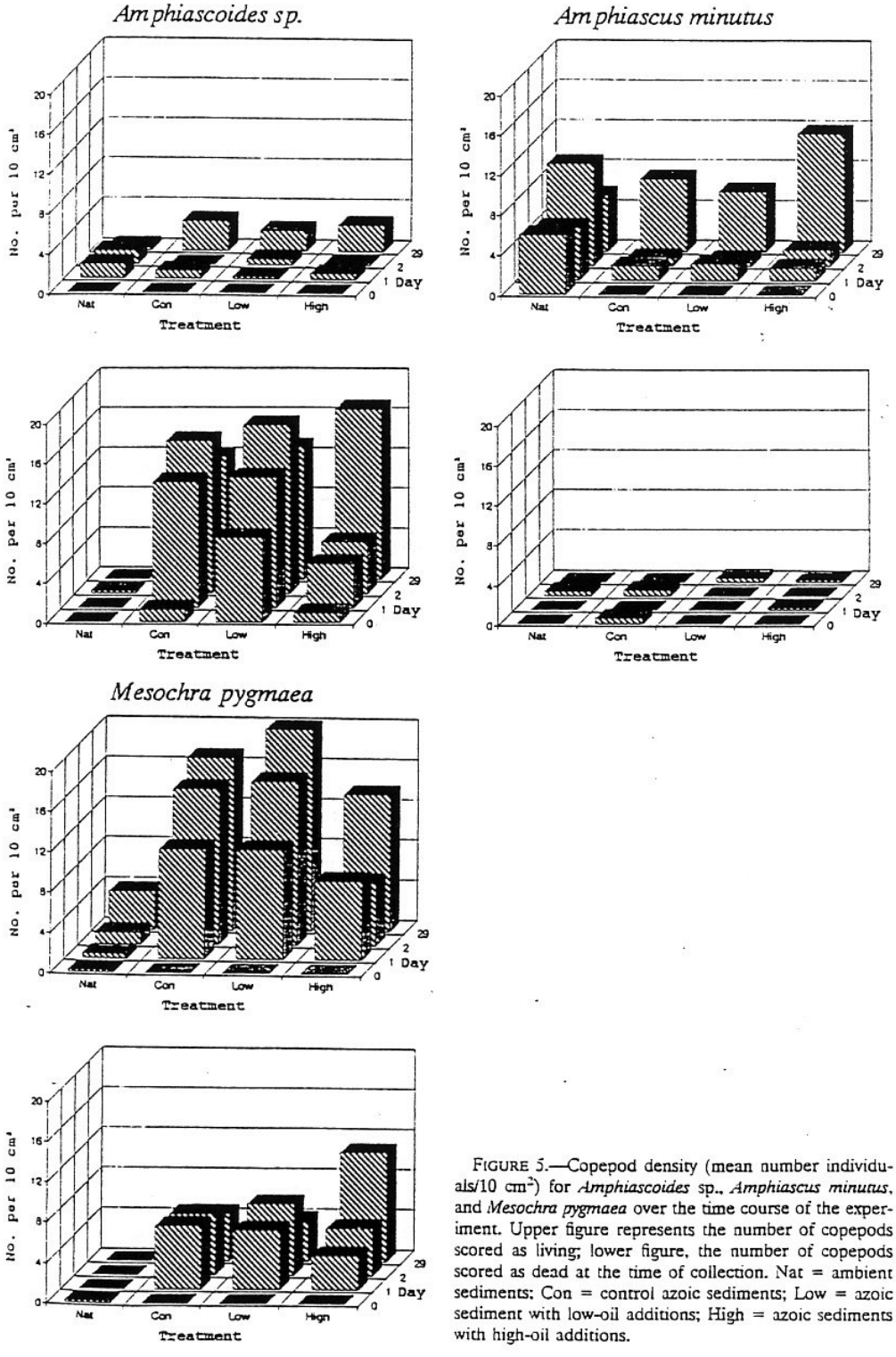

FIGURE 5.—Copepod density (mean number individuals/10 cm^2) for *Amphiascoides* sp., *Amphiascus minutus*, and *Mesochra pygmaea* over the time course of the experiment. Upper figure represents the number of copepods scored as living; lower figure, the number of copepods scored as dead at the time of collection. Nat = ambient sediments; Con = control azoic sediments; Low = azoic sediment with low-oil additions; High = azoic sediments with high-oil additions.

TABLE 3.—Relative abundances (%) and species diversity patterns for abundant harpacticoid species. Percent of the total represents the cumulative percentage represented by the abundant species and the unidentifiable (U) copepodites in a given date-treatment combination. Densities are found in Figures 3 through 8. Richness is the total number of species. H' is the Shannon-Wiener diversity index value.

Species or statistic	Ambient	Control	Low	High
	Day 1			
Mesochra pygmaea	1.2	29.8	31.0	41.9
Paralaophonte perplexa	16.0	11.6	28.1	22.6
Halectinosoma sp.	12.4	26.5	9.8	6.4
Amphiascus minutus	13.6	3.9	4.4	8.6
U copepodites	29.6	3.8	11.5	7.5
% of the total	72.8	75.6	84.8	87.0
Richness	16	20	19	10
H'	2.2	1.9	1.9	1.6
	Day 2			
Mesochra pygmaea	1.3	31.3	39.1	28.7
Paralaophonte perplexa	17.9	6.1	13.5	21.3
Halectinosoma sp.	7.5	45.5	20.8	8.3
Amphiascus minutus	11.7	1.6	1.4	7.4
U copepodites	32.7	9.2	9.7	19.4
% of the total	71.1	93.7	84.5	85.1
Richness	15	14	17	14
H'	2.4	1.7	1.9	2.1
	Day 28			
Mesochra pygmaea	3.2	12.7	12.9	9.5
Paralaophonte perplexa	16.0	10.1	15.1	13.8
Halectinosoma sp.	2.0	29.8	16.4	16.2
Amphiascus minutus	5.0	5.3	4.2	8.3
U copepodites	63.4	33.2	40.6	45.6
% of the total	89.6	91.1	89.2	93.4
Richness	15	14	20	13
H'	1.9	1.9	2.8	2.0

ized block design (tray replication was blocked) to test for date and treatment effects on harpacticoid abundance (Table 4). Analysis of variance treatments were collection day (1, 2, or 28) and oil treatment (control, low-, or high-oil). Tests were performed on the total live harpacticoids, unidentified copepodites, and the four most abundant species. Collection-day effects were significant in all taxa, probably reflecting a true increase in abundances of ambient harpacticoids during the springtime experimental period (Table 4). An oil effect (especially at the high-oil dosage) was observed in total copepods, and in *Halectinosoma* sp. and *M. pygmaea*. For total copepods and *M. pygmaea*, Tukey's Studentized Range Test indicated that densities in control and low-oiled sediments did not differ, but that densities in high-oiled sediments were significantly lower than in other treatments throughout the experiment. For *Halectinosoma* sp., means were significantly different in all experimental treatments; densities in control sediments were

TABLE 4.—Analysis of variance summary: probabilities of greater F-values for a randomized block design for total meiofauna and abundant harpacticoid species. Day refers to collection date 0, 2, or 28. Treatment refers to oil dose: control, low, or high.

Species or group	Block	Treatment	Day	Treatment × day
Total harpacticoids	0.1146	0.0040	0.0001	0.4440
Mesochra pygmaea	0.0011	0.0024	0.0074	0.7214
Paralaophonte perplexa	0.5433	0.1408	0.0001	0.8093
Halectinosoma sp.	0.3592	0.0001	0.0001	0.5222
Amphiascus minutus	0.0002	0.0681	0.0001	0.2087

significantly higher than in low-oiled treatments, and, in turn, densities in low-oiled were significantly higher than in high-oiled treatments. No treatment effects were observed in two other harpacticoids, *P. perplexa* and *A. minutus*.

Harpacticoids scored as "dead" at the time of collection were variable in density, but were found in all treatments and in all collections through day 28. Dead harpacticoids were always rare in ambient collections. Species-level identifications of day 0 collections reveal that the relict assemblage (resident in sediment rendered azoic) was generally similar to that found in HBH, and most species were shared in common. Most of the dead harpacticoids collected on subsequent dates also appeared to be relict individuals that decomposed slowly at the cool temperatures found in Prince William Sound. For example, "dead" *Amphiascoides* sp. was abundant in samples from azoic sediments on day 0, contributing about 25% of all dead specimens. Live *Amphiascoides* sp. was always rare in ambient collections and in experimental trays (Figure 5). Unidentifiable copepodites were virtually absent from all day 0 collections. Densities of unidentifiable copepodites increased with time in the ambient sediments and in the experimental trays (Figure 4). This colonization event was notable because no increase in the numbers of dead copepodites was observed in the experimental treatments, suggesting that there was little or no oil-induced mortality. *Halectinosoma* sp. was present in very low abundance in day 0 relict collections, but colonized azoic sediments rapidly. After day 0, the number of *Halectinosoma* sp. scored as dead were always low regardless of oil treatment, again suggesting low levels of oil-induced mortality (Figure 5).

Harpacticoid Community Ordinations

Detrended correspondence analysis was conducted to determine if an oiling effect influenced

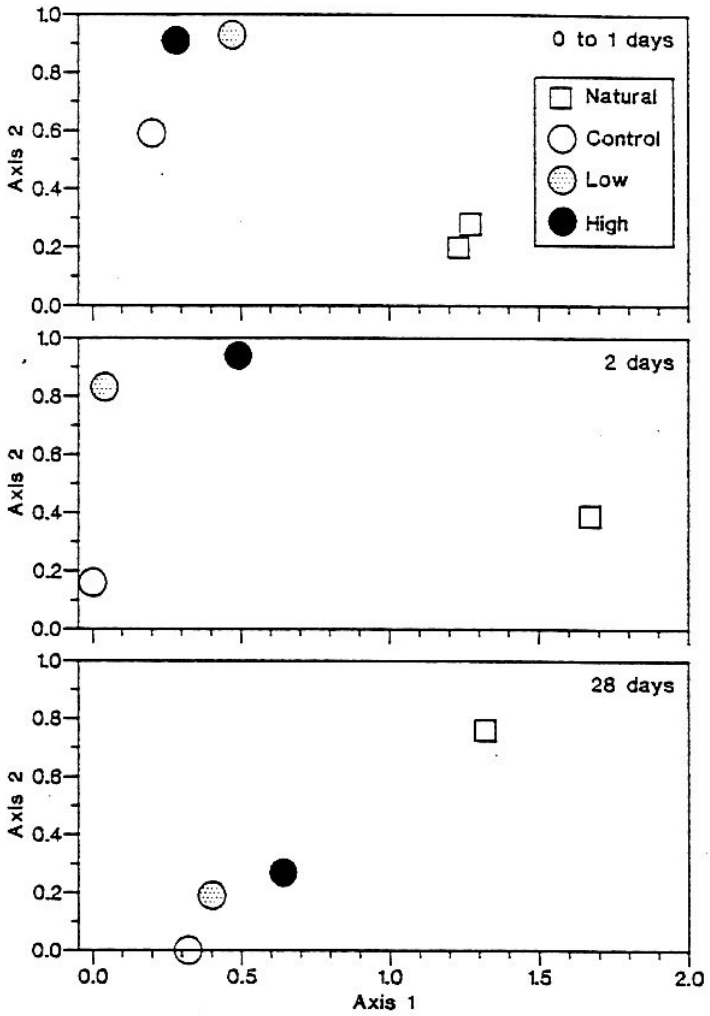

FIGURE 6.—Detrended correspondence analysis of the copepod assemblage in azoic and ambient sediments on various collection dates.

the copepod assemblage (Figure 6). Two axes comprised more than 70% of the variance. Axis 1 contained relatively high levels of variation, with values approaching 2 SDs overall (a variation of 4 SDs indicates complete faunal turnover). All ambient samples separated from experimentals on axis 1 and clustered near each other, indicating that experimental trays could be distinguished from the surrounding sediments in species composition on all collections. Other collections were separated on axis 2 with only 1 SD of faunal turnover. Days 1 and 2 low- and high-oiled treatments clustered together as did control, low-, and high-oiled sediments from day 28. Control day 1 collections were intermediate. Results therefore indicate that an oiling effect was present on days 1 and 2, but no difference between control and oiled sediments was apparent by day 28.

Discussion

Unweathered *Exxon Valdez* crude oil delayed, but did not preclude, colonization by meiofauna into azoic sediment in Prince William Sound. The effect on colonization was short lived, and copepod spe-

cies densities and copepod species composition in oiled sediments and controls became indistinguishable from 2 to 28 days post-oiling. Two of eight abundant species showed reduced rates of recolonization. Aromatic hydrocarbon concentrations in oiled sediments tended to decline with time (Figure 2). Hydrocarbon-related effects on densities were found only in the most rapidly colonizing species (*Halectinosoma* sp. and *M. pygmaea*). Slower colonists (*P. perplexa* and *A. minutus*) may not have reached the trays in large numbers until oil concentrations diminished or until the effects of hydrocarbon additions were mitigated in other ways (see below).

The concentrations of *Exxon Valdez* crude oil used in this experiment were environmentally meaningful. Based on our observations, the maximum PAH concentration in the high-oil treatment (246 $\mu g\ g^{-1}$) was well within the range of PAH concentrations observed in Prince William Sound sediments after the *Exxon Valdez* oil spill (0–482 $\mu g\ g^{-1}$). Very low levels of PAH were observed in the sediments ambient to recolonization chambers. The initial levels of PAH were higher in control sediments (relict sediments from Auke Nu Cove rendered azoic) but were much less than levels in the low-oil treatment (Table 2). The most likely source of contamination to relict sediments was ship activity around the collection location.

Although hydrocarbon concentrations tended to decrease with time, effects on harpacticoid copepods may have been further mitigated by an even modest accumulation of uncontaminated ambient sediments into the colonization trays. Harpacticoids in muddy Alaskan sediments have a very shallow depth profile, with most living in the flocculent layer 2 mm in sediment depth (J. W. Fleeger and T. C. Shirley, personal observation). Hydrodynamic effects that may cause retention of flocculent sediments are minimized when trays are buried flush into the sediment (Snelgrove et al. 1992), as were ours, and grain size was unchanged over time (Table 1). Nevertheless, additions of uncontaminated flocculent sediments, which may be prone to adhere to oil-enriched sediments, could have provided a refuge from PAH for harpacticoids.

Four previous studies examined the effects of crude or seep oil on the colonization of meiofauna, and one, Alongi et al. (1983), used Prudhoe Bay crude oil. Three studies were unable to identify negative effects of oil on density, but found that abundances increased with low levels of oil addition (Alongi et al. 1983; Spies et al. 1988) or that oil increased colonization potential (Palmer et al. 1988). The fourth study, Decker and Fleeger (1984), found that oil retarded colonization rates (for fewer than 20 d in nematodes and polychaetes) but that oil had positive effects on density after 30 d for one species of harpacticoid copepod. Prudhoe Bay crude oil was also used in a controlled spill by Feder et al. (1990), who found either no effect or increases in abundance of harpacticoid copepods in Port Valdez, Alaska. Generally, studies that use crude oil in field additions (and thus as sediment-bound PAH) reveal weak evidence for mortality in meiofauna (Fleeger and Chandler 1983; DeLaune et al. 1984; Smith et al. 1984; Feder et al. 1990), whereas studies that use fuel oil in the water-soluble form identify stronger effects (Frithsen et al. 1985; Stacey and Marcotte 1987). Even so, Stacey and Marcotte (1987) found an increase in density of one harpacticoid species in a mesocosm experiment. Spies et al. (1988) compared seep oil with additions of organic detritus in a colonization study and concluded that both, in low concentrations, had similar effects on meiofaunal abundance. Thus it appears that the sediment-bound phase of many crude oils, including that of *Exxon Valdez* crude oil, is not particularly toxic and that changes in abundance may be related to increases in organic matter. A similar effect was noted by Stickle et al. (1990), who concluded that pink shrimp *Pandalus borealis* exposed to the water-soluble fraction of oil were far more vulnerable to oil effects on their survival and bioenergetics than shrimp exposed to oiled sediment.

Our harpacticoid species data further suggest that oil-induced mortality of colonists was minimal. Species-level comparisons between the colonizing source pool and the relict copepods (dead but not decomposed) retained in azoic sediments were made. Most of the obviously dead harpacticoids found in azoic sediments over the 28-d experimental period appeared to be relict individuals, rather than harpacticoids that may have colonized and then died from exposure to oil. *Amphiascoides* sp. was abundant in relict sediments on day 0 and contributed about 25% of all dead specimens, but it was always infrequent in ambient collections. Few live specimens of *Amphiascoides* sp. were recovered from colonization trays. Unidentifiable copepodites and *Halectinosoma* sp. were rare on day 0 in the azoic sediments but colonized rapidly without mortality.

Meiofauna have only recently been recognized as active colonizers (Palmer 1988). Sediment-dwelling meiofauna associated with seagrass beds, like those in our study, are especially mobile, displaying at-

tributes of active emergence into the water column, rapid resettlement onto sediment and grass blades, and dispersal (Bell et al. 1989; Walters 1991; Kurdziel and Bell 1992). Copepods are especially active, moving back and forth from sediment to grass blades (Walters 1991; Webb and Parsons 1992). As such, seagrass meiofauna may serve as important model experimental subjects because their short-term migration rates are high and are more likely to be altered by an ephemeral environment perturbation.

In a few selected taxa, the presence of oil slowed, but certainly did not halt, meiofaunal migration and colonization. Although harpacticoids colonize sediments from the water column (Chandler and Fleeger 1983), hydrodynamic models suggest that they have only limited abilities to select locations at settlement (Palmer 1988). Nevertheless, harpacticoids occur disproportionately at sites that are favorable to them in the field and laboratory (Kern and Taghon 1986; Decho and Fleeger 1988; Fegley 1988; Kern 1990). Sun (1993) studied harpacticoid settlement and postsettlement movement in a flume and found that postsettlement behavior of harpacticoids is instrumental in determining local distributions. His work suggests that harpacticoids cannot select settlement locations but probably move through the benthic boundary layer to select nearby sites. Alternatively, harpacticoids may be stimulated to leave an unfavorable site either by swimming up into the flow or by allowing themselves to be carried away from the sediment surface. During colonization, *Halectinosoma* sp. and *M. pygmaea* probably entered all trays, regardless of oil treatment, but a disproportionate number probably emigrated from the high-oil treatment trays.

The influence of hydrocarbons on fish feeding is poorly known; however, PAH do not appear to interfere with chemoreception in most fishes, and fishes do not generally avoid PAH in the field (Klaprat et al. 1992). Therefore, fishes and invertebrates may feed on contaminated harpacticoids. Contact of meiofauna with oiled sediments and subsequent emigration from these sediments into the water column may provide a mechanism for introducing oil into the diets of other organisms, such as juvenile pink and chum salmon. In associated field studies, we found that juvenile pink salmon *Oncorhynchus gorbuscha* and chum salmon *O. keta* were contaminated with hydrocarbons from the *Exxon Valdez* oil spill and that ingestion was a likely route of contamination (Carls et al. 1996, this volume). Perhaps harpacticoids contaminated with *Exxon Valdez* hydrocarbons partially mediated transfer of hydrocarbons from sediments to fish.

Changes in abundance of meiofauna following oiling in the field can be rapid. Fleeger and Chandler (1983) found increases in density within 5 d, a time much shorter than that in which these metazoans can increase by reproductive activity (Hicks and Coull 1983). Our data suggest that because colonization readily occurred in oiled Prince William Sound sediments, any change in meiofaunal density associated with the *Exxon Valdez* spill is likely to be the result of changes in mortality or reproductive success rather than changes in migration rates. Furthermore, our experimental design allowed the identification of short-term effects, an opportunity not usually afforded investigators using relatively long intervals between sampling (Spies et al. 1988). Our results suggest that recolonization of an area that suffers high mortality after an oil spill around seagrass beds should occur quickly as long as a source pool of meiofauna remains available, especially given the long-distance dispersal capabilities of seagrass meiofauna (Kurdziel and Bell 1992).

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