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Marine Environmental Research, Vol. 40, No. 3, pp. 289 318, 1995
Elsevier Science Ltd
Printed in Great Britain

0141-1136(94)00146-4

Experimental Investigation of the Effects of Polynuclear Aromatic Hydrocarbons on an Estuarine Sediment Food Web

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(Received 4 April 1994; revised version received 28 September 1994; accepted 5 October 1994)

ABSTRACT

The influence of polynuclear aromatic hydrocarbons (PAH) on a benthic estuarine sedimentary salt-marsh food web was examined using a microcosm system to simulate natural conditions. Microcosms were dosed with sublethal concentrations of PAH-contaminated sediment collected from a produced-water site at Pass Fourchon, Louisiana, USA (final PAH concentrations ranged from 0.3 to 3 mg PAH/kg dry sediment). Bacterial activity, physiological condition and abundance were not influenced by PAH, but microalgal activity and physiological condition were. Grazing by meiobenthic copepods on benthic microalgae was not significantly influenced by PAH concentration, nor was the physiological condition of copepods, as determined by their lipid-storage material. Meiofaunal community composition was influenced by PAH, as nematodes became disproportionately abundant, and the nauplius/copepod ratio increased in High-PAH treatments. Overall, however, sublethal effects of PAH were not pronounced at the concentrations examined. Considering that coastal Louisiana has been exposed to chronic contamination by petroleum hydrocarbons for decades, it is suggested that the sedimentary microbial/meiofaunal community may have adapted to elevated PAH concentrations.

INTRODUCTION

A large body of literature exists on the impact of petroleum hydrocarbons on marine organisms and ecosystems. Many studies have established that

high levels of petroleum hydrocarbons are toxic to a variety of teleosts and invertebrates (Neff & Anderson, 1981), alter physiological performance (Capuzzo, 1987), reduce fecundity (Cowles, 1983*a*) and influence the composition of entire communities (Spies, 1987). Further, petroleum hydrocarbons can impair the fidelity of DNA synthesis (inducing mutations), and can disrupt cellular metabolism (National Research Council, 1985). The primary mechanisms by which petroleum hydrocarbons are deleterious to benthic organisms include oxygen stress (from organic enrichment), and direct toxic or carcinogenic effects on organisms (Connell & Miller, 1984). Seldom, however, are studies of biological effects able to predict subtle, long-term ecological effects, especially with regard to functional interactions among organisms and between trophic levels (Elmgren *et al.*, 1980).

The analysis of estuarine food webs is a compelling approach for detecting ecologically significant, sublethal impacts of contaminants. Laboratory studies indicate that a negative impact on feeding (Cowles, 1983*a*; Cowles, 1983*b*; Farke *et al.*, 1985) and energy reserves (Wang & Stickle, 1988) are among the first responses that result from exposure to petroleum hydrocarbons, even at low (sublethal) concentrations. As a result, it is reasonable to expect that the influence of pollutants should be manifested in the food web (Connell & Miller, 1984).

Most studies of hydrocarbon contamination fall into a few major categories (Coull & Chandler, 1992):

1. Field studies involve post-hoc monitoring of acute (e.g. oil-spills, Elmgren *et al.*, 1983) or chronic (e.g. production sites, Nance, 1991) contamination of a habitat or ecosystem. By their nature, such studies tend to be descriptive, rather than experimental, and environmental impacts are frequently determined from mortality (i.e. reduced abundance) and changes in community structure. Because these are 'natural' experiments, there are a number of variables over which the investigator has little or no control. Thus, interpretation of data is usually limited to correlative analysis, and it is difficult to determine specific causes for changes in faunal abundance or diversity.
2. Laboratory studies involve the exposure of organisms to well-defined hydrocarbon toxicants under carefully controlled conditions (e.g. McElroy, 1990), and clearly delineate the potential toxicity of contaminants. Unfortunately, it is frequently difficult to predict environmental responses to toxicants from these laboratory studies because they cannot take into account a variety of complex environmental interactions (Cairns & Pratt, 1989).

3. Microcosm/mesocosm experiments represent a compromise between field and laboratory studies in that considerable control over experimental conditions and toxicant dosages can be maintained while major features of natural communities are simulated (e.g. Kuiper *et al.*, 1984). While they have yielded valuable information, microcosm studies frequently suffer from a lack of adequate replication and/or proper controls due to logistical constraints (e.g. Farke *et al.*, 1985), and difficulty in relating effects back to the natural environment (Catallo, 1993).

Estuarine salt marshes are critical marine habitats because of their high productivity and importance as nursery grounds for many commercially important species. A report by the National Research Council (1985) identifies salt marshes as susceptible to chronic and/or catastrophic inputs of petroleum hydrocarbons. Fine particles (silts and clays) that are characteristic of salt-marsh sediments act as a sink for hydrophobic contaminants, such as petroleum hydrocarbons (Catallo, 1993; Connell & Miller, 1984; Hassett *et al.*, 1980; Means *et al.*, 1980; Wijayaratne & Means, 1984). These contaminated particles are likely to accumulate in salt marshes because the low-energy environment promotes the deposition and retention of fine-grained sediment (Little, 1987). The organic content of estuarine sediments is high in estuaries as well, and hydrophobic compounds bind very strongly to organic matter, acting as another 'trap' (Di Toro *et al.*, 1991; Means *et al.*, 1980). Once impacted, salt-marsh sediments may remain contaminated for years because of low rates of tidal flushing and slow biodegradation of complex hydrocarbons (Kennish, 1992), and slow desorption into overlying water (Means & McMillin, 1993). Particles at the sediment–water interface may be enriched with polycyclic aromatic hydrocarbons (PAH) by a factor of 10–100 relative to concentrations in bulk sediments or suspended particles (Kirso *et al.*, 1990; Baker *et al.*, 1991). This observation suggests that fluxes of PAH to sediments may be higher than previously believed, and that organisms living or feeding at the sediment–water interface, such as meiofauna and microorganisms, may be exposed to particularly high concentrations of contaminants.

We report here a microcosm study of the impacts of PAH on a benthic food web within a Louisiana salt marsh. Our primary focus involves the meiofaunal (metazoans that pass through a 1 mm sieve and are retained on a 0.042 mm sieve; Higgins & Thiel, 1988) component of the benthos, with emphasis on their trophic relationships with microorganisms (as food). Our approach is novel in that the impact of PAH was determined not merely by monitoring the abundance of various groups of organisms,

but by determining their physiological responses and trophic interactions (i.e. grazing rates). The microcosms represented minimally disturbed, natural assemblages of benthic organisms and the sediment in which they live.

MATERIALS AND METHODS

General design

Microcosm experiments were performed using a 4 × 5 × 7 factorial design, with four wet tables (as blocks), five PAH concentrations, and seven exposure times as factors. Sediment microcosms were treated with three concentrations of PAH (Low, Medium, and High) and compared to two types of control sediments (total of five treatment levels). In one control (C1), no sediment was added to microcosms; in the second control (C2) 'uncontaminated' sediment was added to microcosms. Four replicate microcosms (one from each wet table) of each of the five treatment levels (a total of 20 microcosms) were harvested at each of seven time intervals (0, 1, 3, 7, 14, 21, and 28 days).

Microcosms were constructed of 15.2 cm i.d. PVC pipe with windows covered with Nitex mesh (62 μm) to allow exchange of water. At low tide on 28 May 1991, 140 microcosms of exposed unvegetated sediment were collected with minimal disturbance by hand from mud flats surrounded by *Spartina alterniflora* marshes and transported to the Louisiana Universities Marine Consortium (LUMCON) facility at Cocodrie, LA, USA. Thirty-five microcosms were randomly assigned to each of four wet tables. During the experiment, wet tables were irrigated with water pumped directly from the salt marsh near LUMCON. Water levels were maintained at the top of the Nitex mesh to allow water to percolate through microcosms. Each wet table received approximately 500 l/h, which represented six complete turnovers of water. Because water was pumped directly from the marsh, microcosms were maintained under essentially ambient temperature and salinity conditions (5–15 ppt salinity; 20–30°C). Microcosms were illuminated on a 12 h:12 h light:dark cycle with banks of fluorescent lights positioned approximately 30 cm above microcosms.

Dosing of microcosms

Two sediments, one a contaminated sediment collected near a Pass Fourchon produced-water (contaminated water from offshore petroleum production activity) discharge site (Rabalais *et al.*, 1991; Means & McMillin, 1993) and the other collected from a relatively clean reference area in Lake

Champagne (Means & McMillin, 1993) were used for dosing microcosms with PAH-contaminated sediments. Both sediments were sieved through a 2 mm sieve and then homogenized on a roller mill for one week. High, Medium, and Low doses of sediment for the microcosms were then prepared by combining appropriate masses of wet contaminated and uncontaminated sediment together and mixing them on a roller mill for two days. PAH concentrations in the three sediment doses were approximately as follows: High = 27 mg PAH/kg dry sediment (undiluted Pass Fourchon sediment); Medium = 5.4 mg PAH/kg; Low = 1.02 mg PAH/kg. Control sediment from Lake Champagne contained approximately 0.3 mg PAH/kg. Aliquots of sediment sufficient to create a 1 mm thick layer of sediment on the surface of each of the experimental microcosms were then weighed and placed in individual plastic bags. Microcosms were dosed by pouring the sediment suspension into the water overlying the sediment in each microcosm with slight swirling and agitation of the microcosms to distribute the sediment evenly over the surface. Sediment treatments were administered on the evening of 29 May 1991, and 'Day 0' was defined as the morning of 30 May 1991.

Assays and analyses

The following cores were collected from each microcosm: six 4.9 cm^2 cores (four for meiofauna grazing and community composition, one for lipid stores in meiofauna, one for hydrocarbon assay); four 2.3 cm^2 cores (two for microalgal activity, two for bacterial activity); and two 5 cm^2 syringe cores (12 mm i.d.: one for chlorophyll (chl) *a*, one for bacterial direct counts). For cores used in radiotracer assays, radioisotopes were applied just under the sediment surface by injecting isotope with a glass 50 μl syringe (Hamilton) through a silicon-sealed port on the side of the core (Carman *et al.*, 1989).

Bacteria

Bacterial activity was measured by administering ^{14}C-acetate into sediment cores and following the label into bacterial membrane lipids (phospholipids) and lipid storage products (poly-β-hydroxybutyrate, PHB; Findlay & White, 1987). Cores were injected with 33.4 kBq [1,2]-^{14}C-acetate (specific activity 4.0 GBq/mmol) and incubated in the dark (to prevent recycling of respired $^{14}CO_2$) for 5 h. Water overlying the sediment was discarded, and the top 1 cm of sediment was extruded into a glass 50 ml centrifuge tube containing 25 ml of modified Bligh–Dyer Solution (White *et al.*, 1979). Bulk lipids were extracted and then fractionated into neutral, phospho-, and glycolipids (which contain PHB) following the procedure

of Guckert *et al.* (1985) and assayed for radioactivity. For controls, one core from each microcosm was injected with ^{14}C-acetate and then immediately harvested as described above.

Bacterial abundance in the top 1 cm of sediment was determined from acridine orange direct counts (AODC; Carman, 1993). This procedure included separation of bacteria from sediments by blending sediments in 0.01% sodium pyrophosphate. The resulting supernatant was stained with 0.04% acridine orange for 2 min, and bacteria were enumerated following Hobbie *et al.* (1977).

Microalgae

Cores were injected with 133.2 kBq (specific activity 1.85 GBq/mmol) ^{14}C-bicarbonate and incubated in the light (60 μEi) for 5 h. Control cores were incubated in the dark. At the end of the incubation, water overlying the sediment was discarded and the top 1 cm of sediment collected into a Whirl-pak bag, which was frozen in liquid nitrogen and stored in a -80°C freezer. Samples were processed using the general methods described for bacterial activity, i.e. bulk lipids were extracted, fractionated, and assayed for radioactivity. In addition, ^{14}C in non-lipid fractions (sediments and aqueous phase of lipid extraction) was measured so that total microalgal incorporation of ^{14}C could be determined.

Microalgal abundance was estimated from chl *a* concentration (corrected for phaeopigments) in the top 1 cm. Photosynthetic pigments were extracted from sediment in dimethylsulfoxide:acetone (1:1) and assayed with a Turner Model-10 fluorometer (APHA, 1992).

Meiofaunal grazing

Grazing by two meiobenthic copepod species, *Coullana* sp. (formerly referred to as *Scottolana canadensis* in publications from Louisiana) and *Pseudostenhelia wellsi* (Coull and Fleeger), on benthic microalgae were assessed using ^{14}C-bicarbonate as a tracer (Carman, 1990). Cores were incubated with 370 kBq of ^{14}C-bicarbonate and incubated for 5 h in the light (same conditions as microalgae assay above). Control cores were incubated in the dark. Water overlying the core and the top 1 cm of sediment were collected and preserved in 3% glutaraldehyde. The samples were stained with Rose Bengal as a sorting aid and adult female copepods were separated from the sediment under a dissecting microscope. Copepods were digested in tissue solubilizer and assayed for radioactivity.

Uptake of ^{14}C by copepods was corrected for uptake in dark controls, and grazing rates were expressed as the percentage of the sedimentary algal assemblage consumed by copepods over a 5 h period. This value was calculated by dividing uptake of ^{14}C by grazing copepods by the total

uptake of ^{14}C-bicarbonate by microalgae (from microalgal activity measurements).

Meiofaunal physiological condition

Neutral-lipid-storage products of *Coullana* sp. and *Pseudostenhelia wellsi* adult ovigerous females were examined using Nile Red, a hydrophobic fluorophore that binds specifically to neutral lipids (Carman *et al.*, 1991). A 4.9 cm^2 core was collected and the top 1 cm of sediment extruded into a Whirl-pak bag and preserved in liquid nitrogen until analysis. Samples were thawed and copepods were sorted and stained in Nile Red solution for 30 min. Copepods were then placed on a depression slide and viewed under epifluorescent illumination using a custom-made filter that optimized the enhancement of lipid deposits in animals (525 nm excitation, 589 nm emission, 545 nm dichroic mirror). Images of individual copepods were captured and saved for future analysis with image-analysis software (Carman *et al.*, 1991). Data were expressed as lipid area (μm^2) in the urosome.

Meiofaunal community composition

After the targeted copepods were removed from cores for grazing studies, the remaining meiofauna were quantified to determine community structure. Copepods were identified to species and other fauna to major taxa.

PAH analysis

A 4.9 cm^2 core of sediment was removed from each microcosm and the top 1 cm of sediment was extruded and collected in a glass screw-cap vial. Sediment was refrigerated (4°C) until extraction for PAH analysis. Sediments were homogenized by gentle mechanical stirring, and a subsample (approximately 0.5 g) was removed for moisture content determination. These subsamples were dried to a constant weight in a 105°C oven. The remaining sediment wet weight was determined by quantitatively transferring the sediment from the glass vial to a tared 150 ml beaker and reweighing to the nearest 0.001 g. Dichloromethane was added to each beaker, then an aliquot of deuterated surrogate standards, and finally 30 g of sodium sulfate was mixed in thoroughly using a stainless steel spatula. The beakers were sonicated for 10 min in an ice-cooled bath, and the solvent decanted through sodium sulfate. The sonicating extraction procedure was repeated three times. The resulting extract was concentrated as described previously (Means & McMillin, 1993) and sulfur removed by activated copper.

Extracts were analyzed by GC/MS using a Hewlett-Packard 5890 GC interfaced with an H-P 5970B mass selective detector operated in the

selected-ion-monitoring mode for enhanced sensitivity of the target analytes, and utilizing the temperature program described elsewhere (Means & McMillin (1993) for GC separation on a 0.25 μm film of OB-5 (30 m $\times$ 0.25 mm i.d.) fused silicon column. Alkylated PAH standards were purchased from Chiron Laboratories AS, Norway. A final standard, containing all parent PAH compounds (Ultra Sci., US-106), alkylated PAH, and deuterated surrogate standards, was prepared at 5 ppm by dilution with dichloromethane and was analyzed with hexamethylbenzene (HMB) as an internal standard. A five-point calibration curve for each compound was determined prior to analysis and daily calibration of one standard as well as daily tuning of the mass spectrometer with PFTBA were performed to ensure accuracy of analyses. All analyses were performed in the blind and decoded after final quantification.

STATISTICAL ANALYSIS

Data were analyzed with SAS statistical software as a three-factor analysis of variance (ANOVA) using the general linear models procedure. All variables that involved ratios were converted to proportions and subjected to a $\log_{10}(n + 1)$ transformation. All other data were $\log_{10}$ transformed. Normal–probability plots were examined and data were subjected to Levene's test of homogeneity of variance to determine if data were consistent with the assumptions of ANOVA. Significant ($p < 0.05$) main effects were further examined using Student–Newman–Keuls *a posteriori* comparisons. When a significant PAH effect was detected, ANOVAs were performed on individual days. To determine the sensitivity of tests, a power analysis (the probability of rejecting the null hypothesis when it is false and the alternative hypothesis is correct; Sokal & Rohlf, 1981) was performed for each ANOVA. Coefficients of variation (standard deviation expressed as a percentage of the mean) were calculated to compare variability among treatments and variables (Sokal & Rohlf, 1981).

RESULTS

Results are presented in two sections, the first considers influence of PAH (and PAH $\times$ Time interaction), and the second changes over time (system effects). Main effects of PAH, blocks (wet tables) and PAH $\times$ Time, as well as power for individual tests, and *a posteriori* comparisons are presented in Table 1. ANOVA results indicated no significant influence of blocks (wet tables) for any of the variables. With only one exception

TABLE 1
Summary of ANOVA Results from Three-Way ANOVAs

Variable	*PAH*[a]	*PAH* × *Time*[b]	*Block*[c]	*Power*[d]	A posteriori *comparisons*[e]				
Coullana sp. lipid	0.3625	0.5180	0.6586	0.074	M^a	H^a	$C2^a$	L^a	$C1^a$
P. wellsi lipid[f]	0.5578	0.4070	0.9291	0.050	L^a	M^a	$C1^a$	H^a	$C2^a$
C. canadensis graze	0.7486	0.6751	0.9933	0.050	$C2^a$	L^a	$C1^a$	M^a	H^a
P. wellsi graze[g]	0.2867	0.9251	0.2363	0.085	L^a	$C1^a$	$C2^a$	H^a	M^a
Algal Phospholipid	0.0015	0.9836	0.9575	0.896	H^a	M^b	$C1^b$	L^b	$C2^b$
Algal Neutral lipid	0.2253	0.9924	0.8111	0.453	H^a	M^a	$C2^a$	$C1^a$	L^a
Algal Phosph/Neut[h]	0.0110	0.0654	0.5778	0.377	H^a	L^b	$C1^b$	M^b	$C2^b$
Chlorophyll *a*	0.2128	0.8783	0.6544	0.161	H^a	$C2^a$	L^a	M^a	$C1^a$
Bacterial Phospholipid	0.6495	0.0240	0.9825	0.240	M^a	$C2^a$	H^a	L^a	$C1^a$
Bacterial Phosph/Glyco	0.5039	0.5133	0.1513	0.050	$C1^a$	L^a	M^a	$C2^a$	H^a
Bacterial abundance	0.9134	0.8021	0.5742	0.050	M^a	$C1^a$	L^a	H^a	M^a
Copepods	0.6255	0.0672	0.2432	0.050	L^a	M^a	$C1^a$	$C2^a$	H^a
Nematodes	0.0120	0.9818	0.1405	0.465	H^a	L^a	M^a	$C2^a$	$C1^a$
Nauplii[h]	0.0142	0.1547	0.5856	0.369	H^a	L^{ab}	$C1^{ab}$	M^b	$C2^b$
Total Meiofauna	0.0905	0.6705	0.5492	0.299	H^a	L^a	M^a	$C1^a$	$C2^a$
Nematode/Copepod	0.040	0.9849	0.0509	0.579	H^a	M^{ab}	L^{ab}	$C2^{ab}$	$C1^b$
Nauplius/Copepod	0.0021	0.4460	0.7366	0.832	H^a	L^b	$C1^b$	$C2^b$	M^b
PAH[h]	0.0001	0.0884	0.9906	1.000	H^a	L^b	M^c	$C2^d$	$C1^d$

[a] *p*-values for main effect of PAH treatment.
[b] *p*-values for PAH x Time interaction term.
[c] *p*-values for blocks (wet tables).
[d] Power of test at alpha = 0.05.
[e] Summary of Student–Newman–Keuls test for PAH treatments: C1 is the control microcosm to which no sediments were added; C2 is the control microcosm to which uncontaminated sediments were added; Low, Medium, and High are microcosms to which sediment with various levels of PAH contamination were added (see text for further details). For each variable, PAH treatments that do not share a common superscript letter differ significantly ($p < 0.05$).
[f] Includes data from Days 0–7 only.
[g] Includes data from Days 0–14 only.
[h] Failed Levene's test for homogeneity of variance.

(bacterial synthesis of phospholipids), PAH × Time interactions were also not significant. Variability was examined by tabulating coefficients of variation for each PAH–Time combination for each variable (Table 2).

Sedimentary PAH

Total PAH concentrations in the two types of control microcosms ranged from 0.210 to 0.445 mg PAH/kg (Fig. 1), and did not differ significantly from each other or change significantly in concentration over time. The

TABLE 2

Coefficients of Variation (CVs). Values in Boxes Represent CVs for Each Day and PAH-Treatment Combination. Values at the Bottom of Boxes (X) Represent the Average CV Value for Each Treatment. Values to the Right of Each Box (X) Represent the Average CV Value for Each Day. 'C1' is the Control Microcosm to Which no Sediments were Added. 'C2' is the Control Microcosm to which Uncontaminated Sediments were Added. 'Low', 'Medium' and 'High' are Microcosms to which Sediment with Various Levels of PAH Contamination were Added (see Text for Further Details). Values for *Pseudostenhe-Pseudostenhelia* and *Coullana* Lipids are Based on Three Replicates. All other Values are Based on Four Replicates

Pseudostenhelia grazing

Day	Treatment C1	C2	L	M	H	X
0	22	12	49	26	36	29
1	33	27	46	42	36	37
3	51	50	36	47	39	44
7	71	26	40	21	41	40
14	59	58	59	38	46	52
21	*	*	*	*	*	
28	*	*	*	*	*	
X	34	25	33	25	28	

Coullana grazing

Day	Treatment C1	C2	L	M	H	X
0	53	27	64	24	51	44
1	56	48	35	65	72	55
3	98	36	25	41	69	54
7	59	61	77	40	107	69
14	20	38	44	*	49	30
21	53	69	44	66	35	53
28	27	77	69	88	46	61
X	52	51	51	46	61	

Pseudostenhelia lipids

Day	Treatment C1	C2	L	M	H	X
0	55	17	39	50	10	34
1	74	60	33	66	58	58
3	111	83	49	49	51	68
7	50	45	26	22	89	46
14	*	*	*	*	*	
21	*	*	*	*	*	
28	*	*	*	*	*	
X	41	29	21	27	30	

Coullana lipids

Day	Treatment C1	C2	L	M	H	X
0	76	49	62	78	63	65
1	77	126	79	149	112	109
3	122	100	107	87	149	113
7	135	137	135	92	82	116
14	56	57	84	63	44	61
21	134	76	60	54	93	83
28	80	158	108	117	119	117
X	97	100	91	91	94	

Algal phospholipids

Day	C1	C2	L	M	H	X
0	45	21	49	23	38	35
1	38	33	50	20	63	41
3	57	29	39	33	35	39
7	48	21	55	22	12	32
14	18	34	51	56	47	41
21	55	48	66	20	51	48
28	57	54	66	79	43	60
X	45	34	54	36	41	

Algal neutral lipids

Day	C1	C2	L	M	H	X
0	43	32	41	25	18	32
1	48	38	62	18	71	47
3	60	31	47	44	72	51
7	52	17	58	17	21	33
14	33	20	27	25	20	25
21	49	60	55	53	40	51
28	57	78	89	47	61	66
X	48	39	54	33	43	

Algal phospholipid/neutral lipid

Day	C1	C2	L	M	H	X
0	10	19	18	15	33	19
1	24	12	22	10	14	17
3	16	20	14	22	86	32
7	19	6	9	13	24	14
14	26	27	40	31	36	34
21	18	15	18	44	58	31
28	20	38	43	33	23	32
X	19	22	22	25	39	

Chlorophyll a

Day	C1	C2	L	M	H	X
0	21	34	32	47	11	28
1	45	16	9	36	13	24
3	18	8	18	23	15	16
7	41	38	28	31	31	33
14	56	31	51	40	46	45
21	62	51	66	46	45	54
28	31	89	45	40	69	55
X	39	38	35	37	33	

Bacterial phospholipid

Day	C1	C2	L	M	H	X
0	18	17	30	17	17	20
1	11	25	22	22	51	26
3	13	11	15	33	18	18
7	5	13	12	22	14	13
14	32	29	18	17	15	22
21	14	29	19	22	23	21
28	13	22	22	3	9	14
X	15	21	20	19	21	

Bacterial phospholipid/glycolipi

Day	C1	C2	L	M	H	X
0	29	28	14	8	74	30
1	96	13	23	20	21	35
3	22	79	97	90	11	60
7	26	16	16	54	72	37
14	5	33	28	26	26	24
21	21	24	40	24	33	28
28	17	21	18	12	37	21
X	31	31	34	33	39	

Bacterial direct counts

Day	C1	C2	L	M	H	X
0	28	66	19	49	56	43
1	*	*	*	*	*	
3	*	*	*	*	*	
7	*	*	*	*	*	
14	12	21	18	24	9	17
21	*	*	*	*	*	
28	38	24	29	51	24	33
X	11	16	9	18	13	

Copepods

Day	C1	C2	L	M	H	X
0	25	37	25	39	55	36
1	39	19	60	66	67	50
3	38	15	5	17	26	20
7	12	26	20	18	53	25
14	29	32	27	36	79	40
21	19	28	49	45	36	35
28	20	18	35	26	58	31
X	26	25	32	35	53	

Nematodes

Day	C1	C2	L	M	H	X
0	66	84	44	67	114	75
1	58	45	43	70	88	61
3	18	61	56	28	69	46
7	22	69	33	64	73	52
14	55	35	49	60	47	49
21	108	73	100	70	82	87
28	104	82	76	53	45	72
X	61	64	58	59	74	

Nauplii

Day	C1	C2	L	M	H	X
0	34	60	31	74	55	51
1	99	33	49	47	68	59
3	62	56	64	44	39	53
7	19	63	24	58	59	44
14	88	114	140	92	30	93
21	71	155	113	91	73	101
28	24	81	65	145	69	77
X	57	80	69	79	56	

Total meiofauna

Day	C1	C2	L	M	H	X
0	33	57	10	60	74	47
1	71	17	43	59	37	45
3	35	17	38	24	28	28
7	6	43	17	25	30	24
14	48	26	52	36	29	38
21	69	65	76	51	59	64
28	61	38	47	43	37	45
X	46	38	40	43	42	

Nematode/Copepod

Day	C1	C2	L	M	H	X
0	60	55	54	61	59	58
1	26	46	63	67	147	70
3	38	52	58	31	81	52
7	21	52	24	65	97	52
14	35	47	75	79	118	71
21	98	55	64	35	88	68
28	85	95	55	26	76	68
X	52	57	58	52	95	

Nauplii/Copepod

Day	C1	C2	L	M	H	X
0	24	27	58	68	35	42
1	68	37	27	52	105	58
3	45	70	66	48	56	57
7	27	45	32	41	40	37
14	69	84	132	80	45	82
21	67	153	111	66	71	94
28	27	69	82	151	36	73
X	47	69	73	72	56	

High-PAH treatments resulted in an average total PAH concentration in the top 1 cm of sediment of 2.870 mg PAH/kg at Day 0. High-PAH treatments remained relatively constant through Day 7, then decreased to a concentration of approximately 1.000 mg PAH/kg by Day 28. The decrease over time could be due to a number of processes including desorption into overlying water (Means & McMillin, 1993), microbial metabolism, and burial below the 1 cm horizon due to bioturbation.

PAH within control and contaminated sediments were dominated by two-and three-ring alkylated aromatic hydrocarbons and heterocyclic aromatic hydrocarbons associated with produced-water discharges (Table 3). The heavy four-and five-ring aromatics typically associated with

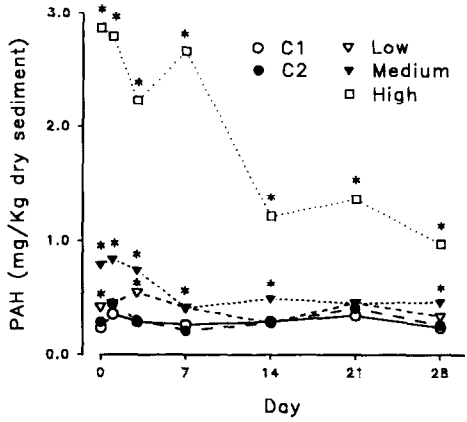

Fig. 1. Total PAH concentration in microcosm sediments. 'C1' is the control microcosm to which no sediments were added. 'C2' is the control microcosm to which uncontaminated sediments were added. 'Low', 'Medium', and 'High' are microcosms to which sediment with various levels of PAH contamination were added (see text for further details). Values are averages of four replicates. *, Treatment differed significantly ($p < 0.05$) from controls (average of C1 and C2) for that day.

combustion sources represented a relatively low proportion of the aromatic fraction. These latter compounds are the ones typically associated with some of the more negative environmental effects of the PAH class such as mutagenicity and carcinogenicity. There is much less known about the toxicological impacts of the alkylated and heterocyclic fractions of the total PAH.

An examination of the depletion rates of the individual aromatic hydrocarbons from Day 0 through Day 28 revealed that the relative ratios of the different isomeric groups remained relatively constant throughout the course of the experiment (analysis not shown). However, as would be expected, the more soluble isomer groups such as naphthalene and methyl naphthalenes were depleted at rates greater than the less soluble compound groups such as dimethyl phenanthrenes. The losses observed are probably due primarily to solubilization but may also be the result of volatilization and/or microbial degradation of labile components of the aromatic fractions.

Bacteria

Bacterial incorporation of ^{14}C-acetate into phospholipids (Fig. 2(A)) and bacterial physiological condition (as indicated from the ratio of incorporation of ^{14}C-acetate into phospholipids and PHB; Fig. 2(B)) were not significantly influenced by PAH additions to microcosms.

TABLE 3
Concentrations of Individual PAH From Day 0 Microcosms.

Analyte	*C1*	*C2*	*Low*	*Medium*	*High*
Napthalene	5.2(0.5)	7.6(1.3)	8.6(2.4)	7.2(1.1)	10.6(3.5)
2-MN	3.0(0.2)	4.2(1.0)	5.2(1.1)	5.7(1.3)	14.2(8.1)
1-MN	2.8(0.3)	3.5(1.2)	5.0(1.6)	4.9(1.5)	16.3(10.3)
2-EN	1.9(0.1)	0.8(0.8)	2.6(1.8)	5.8(1.6)	24.0(16.9)
2,6/2,7-DMN	13.8(3.8)	14.5(2.1)	20.8(5.0)	32.3(14.2)	123.8(85.5)
1,3/1,7-DMN	2.4(0.3)	3.1(0.7)	7.5(1.2)	16.0(11.7)	108.8(82.1)
1,6-DMN	2.2(0.2)	2.7(0.7)	4.1(0.8)	6.8(2.8)	30.5(20.6)
1,4/2,3-DMN	1.9(1.9)	3.1(2.1)	5.5(2.1)	16.7(8.8)	89.0(70.0)
1,5-DMN	0.0(0.0)	1.0(1.1)	2.0(1.3)	0.0(0.0)	4.8(8.2)
Acenaphthylene	1.3(0.1)	2.0(1.0)	2.1(1.7)	1.3(0.4)	1.6(1.0)
1,2-DMN	0.6(0.4)	1.0(0.2)	2.1(0.6)	3.6(1.9)	17.5(10.9)
Acenaphthene	1.1(0.2)	1.8(0.7)	2.5(0.6)	2.9(0.7)	10.8(8.3)
1,6,7-TMN	0.5(0.5)	2.1(0.8)	5.9(1.4)	19.5(8.5)	166.5(112.9)
Fluroene	2.5(0.3)	3.4(0.7)	4.7(0.8)	6.6(2.4)	25.5(14.3)
Dibenzothiophene	1.3(0.2)	1.4(0.3)	3.6(2.2)	5.3(2.5)	24.8(16.9)
Phenanthrene	7.8(0.6)	8.6(2.7)	14.0(4.7)	21.5(5.7)	67.8(36.4)
Anthracene	3.0(0.1)	4.8(1.4)	4.9(1.1)	5.9(1.6)	15.5(6.7)
4-MDBT	3.2(0.5)	3.6(2.2)	5.1(3.0)	16.6(7.0)	71.3(40.0)
2/3-MDBT	0.7(1.2)	3.1(3.5)	7.8(3.8)	10.8(4.7)	37.3(20.8)
3-MP	3.9(0.3)	5.3(1.4)	14.0(3.0)	34.3(15.3)	166.0(95.5)
2-MP	1.5(0.4)	2.9(0.8)	6.2(1.8)	15.8(6.9)	75.5(43.3)
4/9-MP	3.3(0.6)	4.5(1.1)	12.8(3.1)	34.0(15.9)	158.5(88.0)
1-MP	2.5(0.3)	4.1(1.1)	8.9(2.4)	22.0(10.4)	100.3(57.9)
3,6-DMP	2.9(0.5)	4.1(1.0)	9.8(2.4)	22.8(9.8)	87.3(42.3)
2,6-DMP	1.8(1.0)	3.1(0.8)	7.7(1.4)	20.8(9.8)	82.5(39.2)
2,7-DMP	1.9(1.1)	3.4(0.9)	6.5(1.0)	17.2(7.7)	65.3(31.8)
1,2-DMDBT	1.0(1.0)	0.7(1.2)	0.0(0.0)	0.0(0.0)	4.5(7.8)
3,9-DMP	3.6(3.6)	9.2(2.2)	27.3(5.4)	78.8(36.6)	307.5(146.5)
1,6/2,5/2,9-DMP	4.4(0.3)	6.5(1.8)	17.8(3.6)	46.8(22.3)	202.5(103.0)
1,7-DMP	2.8(0.3)	3.7(0.7)	8.4(1.7)	22.5(9.9)	88.0(41.8)
1,9/4,9-DMP	12.8(1.9)	9.9(3.2)	16.0(1.7)	41.3(16.9)	135.0(61.0)
1,5-DMP	4.4(0.6)	3.4(1.4)	2.2(2.2)	2.1(1.3)	0.4(0.6)
Fluoranthene	24.8(3.9)	31.0(11.0)	30.5(5.9)	47.3(11.7)	76.0(32.7)
1,8-DMP	0.7(0.7)	1.7(0.5)	3.6(0.7)	9.3(4.0)	27.9(19.5)
1,2-DMP	1.8(0.5)	1.7(0.6)	3.0(0.7)	5.0(2.1)	10.2(4.0)
9,10-DMP	0.8(0.2)	1.2(0.4)	1.2(0.3)	1.9(0.5)	6.4(3.3)
Pyrene	22.3(4.0)	40.3(10.2)	33.0(6.6)	45.8(11.9)	66.3(21.3)
1,2,8-TMP	1.2(0.3)	0.0(0.0)	2.6(2.4)	14.0(6.8)	54.5(42.9)
Benzanthracene	6.6(0.4)	12.8(4.6)	12.5(2.6)	15.0(2.7)	49.5(28.4)
Chrysene	10.0(1.1)	20.5(7.7)	20.3(4.3)	32.8(8.9)	113.8(61.9)
Benzo(b)fluor	12.0(1.6)	19.5(9.3)	22.3(10.6)	22.5(9.0)	35.3(14.0)
Benzo(k)fluor	0.0(0.0)	0.0(0.0)	0.0(0.0)	0.0(0.0)	0.0(0.0)
Benzo(a)pyrene	6.9(0.4)	12.0(4.4)	11.7(3.3)	15.0(6.0)	28.5(11.4)
Indenopyrene	23.3(3.7)	11.1(3.0)	20.2(13.1)	39.0(15.2)	33.5(20.6)

TABLE 3. — *contd*

Analyte	*C1*	*C2*	*Low*	*Medium*	*High*
Dibenzanthracene	4.7(1.0)	1.8(1.3)	2.9(1.9)	1.2(1.4)	6.1(4.6)
Benzoperylene	21.8(2.0)	2.9(3.1)	3.4(3.4)	0.0(0.0)	30.8(31.0)
Total parent PAH	154.3(4.0)	181.5(56.8)	197.0(38.6)	269.3(69.3)	595.8(260.2)
Total alkylated PA	84.5(10.2)	107.5(22.9)	221.3(44.3)	526.8(234.9)	2275.8(1277.2)
Total PAH	238.6(12.2)	289.0(78.6)	418.4(77.3)	795.7(277.1)	2871.8(1533.9)

Units are 10^{-3} mg PAH/kg dry sediment. Values are means (one standard deviation) of four replicates. Treatments C1, C2, Low, Medium, and High are described in Table 1. Analyte codes are as follows: MN = methyl naphthalene; EN = ethyl naphthalene; DMN = dimethyl naphthalene; TMN = trimethyl naphthalene; MDBT = methyl dibenzothiophene; MP = methyl phenanthrene; DMP = dimethyl phenanthrene; TMP = trimethyl phenanthrene; TMP = trimethyl phenanthrene.

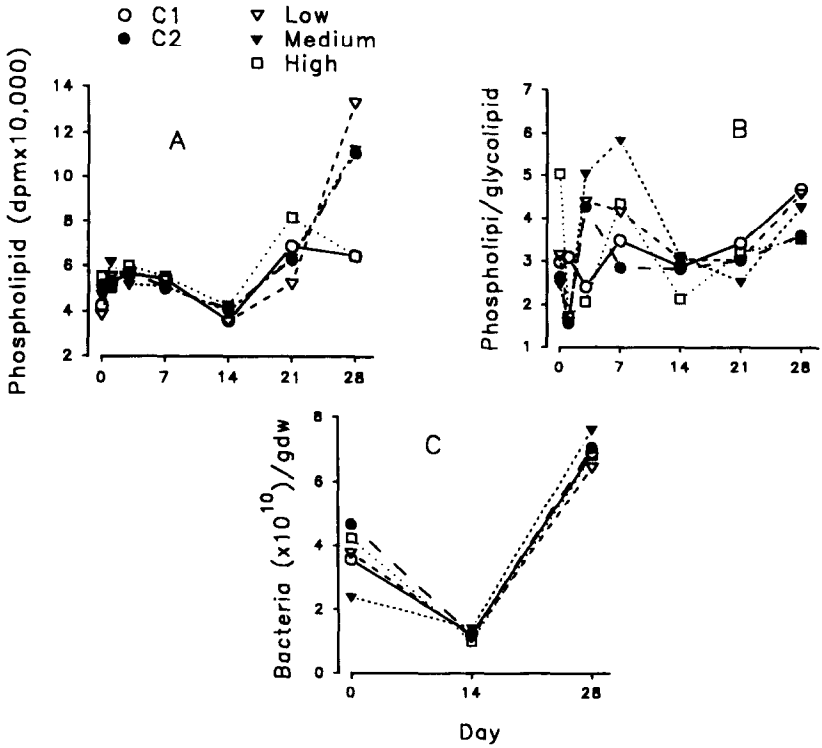

Fig. 2. Measurements of bacterial activity and abundance. (A) Synthesis of phospholipids from ^{14}C-acetate; (B) bacterial physiological condition based on the ratio of ^{14}C-acetate incorporation into phospholipids and glycolipids (which contain PHB); (C) abundance of sedimentary bacteria based on acridine-orange direct counts. See Fig. 1 for further details.

Because of the time required to perform bacterial direct counts, we initially examined bacterial abundance for Days 0, 14, and 28 (Fig. 2(C)). No significant influence of PAH on abundance was detected, and thus we did not perform direct counts on the remaining samples.

Microalgal activity

Incorporation of ^{14}C-bicarbonate into microalgal phospholipid was significantly influenced by PAH concentration (Fig. 3(A)), and was highest in the High-PAH treatments. Microalgal synthesis of neutral (storage) lipids from incorporation of ^{14}C-bicarbonate was not significantly influenced by PAH concentration (Fig. 3(B)). The physiological condition of algae, as

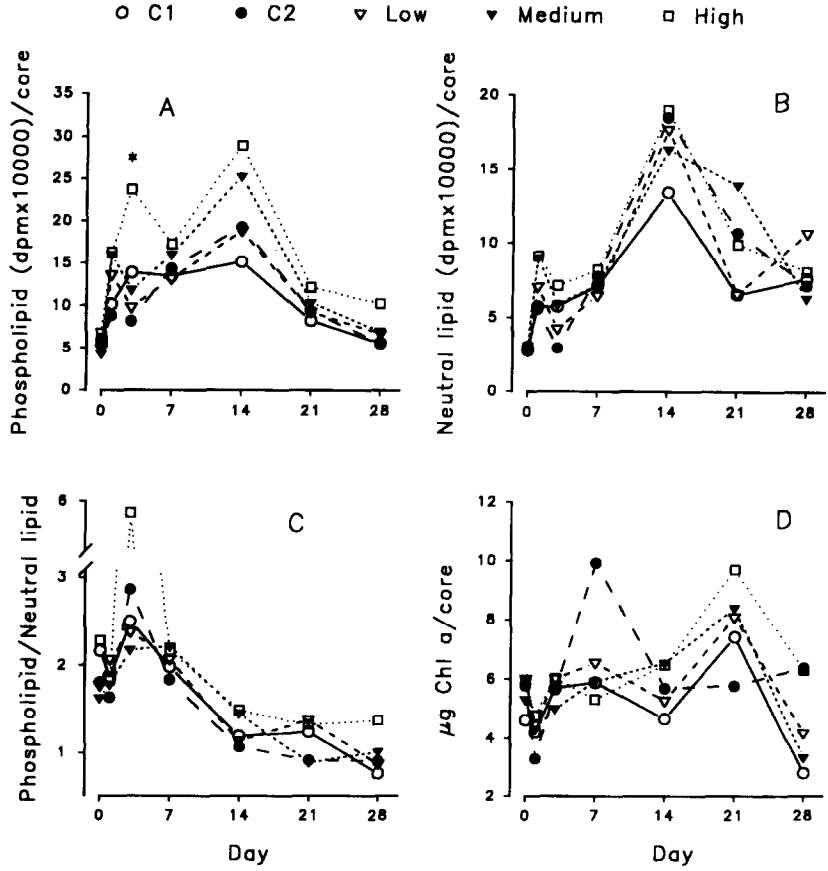

Fig. 3. Measurements of algal activity and biomass. (A) Synthesis of phospholipids from ^{14}C-bicarbonate; (B) synthesis of storage (neutral) lipids from ^{14}C-bicarbonate; (C) physiological condition based on the ratio of ^{14}C-bicarbonate incorporation into phospholipids and neutral lipids; (D) chl *a* concentration expressed as µg chl *a* in the top 1 cm of core with 2.3 cm^2 surface area. See Fig. 1 for further details.

determined from the phospholipid/neutral-lipid ratio of incorporation of ^{14}C-bicarbonate, was significantly influenced by PAH concentration (Fig. 3(C)) and was highest in the High-PAH treatments. The ratio remained significant (p = 0.0171) even after removal of Day 3 data, which yielded anomalously high values for the High-PAH treatment. Higher values of the phospholipid/neutral-lipid ratio represent relatively greater allocation of carbon to membrane synthesis, and thus may indicate relatively greater growth (i.e. cell division). Microalgal biomass (as chl *a*) was not significantly influenced by PAH concentration (Fig. 3(D)), but overall was highest in High-PAH treatments, which is consistent with enhanced microalgal growth.

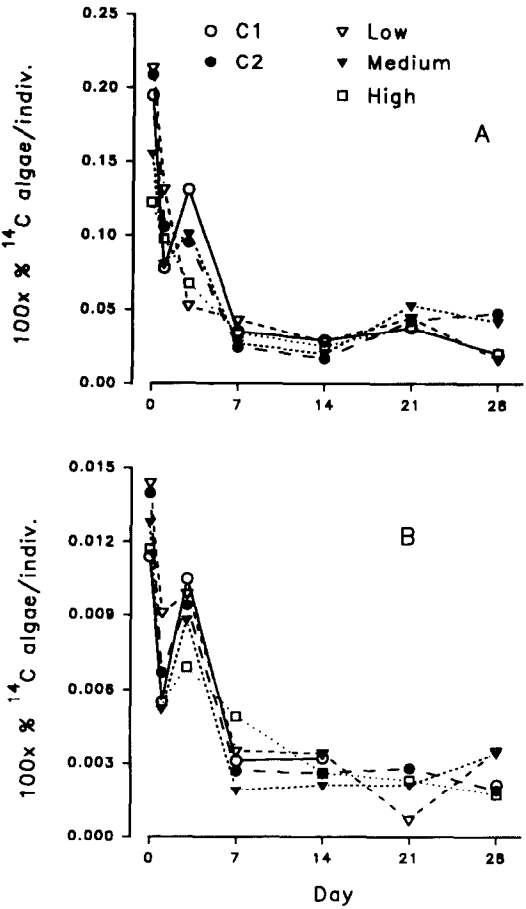

Fig. 4. Grazing by copepods on sedimentary microalgae. Data are expressed as the percentage of the algal standing stock consumed by each copepod over a 5 h period. (A) *Coullana* sp.; (B) *Pseudostenhelia wellsi*. See Fig. 1 for further details.

Copepod grazing and physiological condition

PAH levels did not significantly influence grazing by *Coullana* sp. (Fig. 4(A)) or *Pseudostenhelia wellsi* (Fig. 4(B)). PAH concentration also had no significant influence on lipid-storage material for either *Coullana* sp. (Fig. 5(A)) or *Pseudostenhelia wellsi* (Fig. 5(B)). Only Days 0–7 were included in the ANOVA for *P. wellsi* as we were unable to recover sufficient numbers of ovigerous females for statistical analysis from Day 14 on.

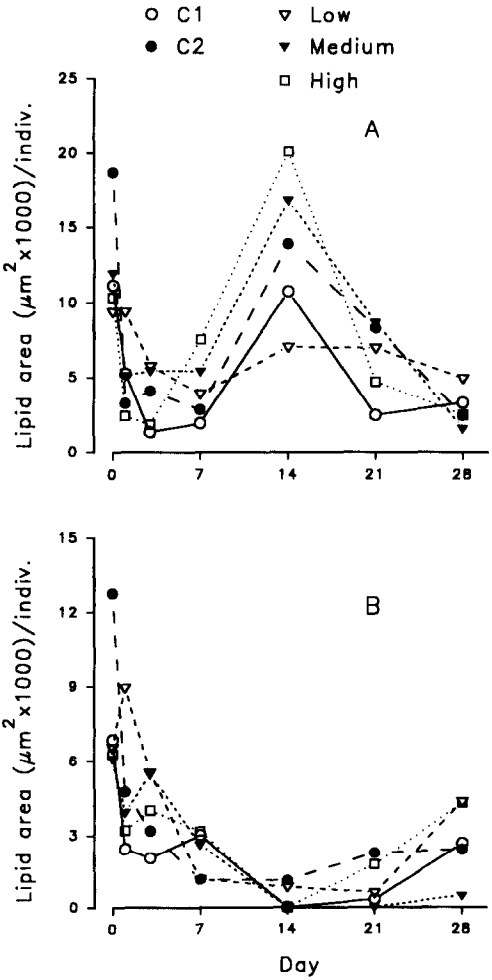

Fig. 5. Lipid-storage material in the urosome of ovigerous copepod females as detected with Nile Red fluorescence. Data are expressed as the urosomal area (μm^2) occupied by lipid droplets. Bars are the averages of copepods from three cores. (A) *Coullana* sp.; (B) *Pseudostenhelia wellsi*. Relatively few *P. wellsi* ovigerous females were found from Day 14 on, and thus several values are either missing, or based on fewer than three replicates. See Fig. 1 for further details.

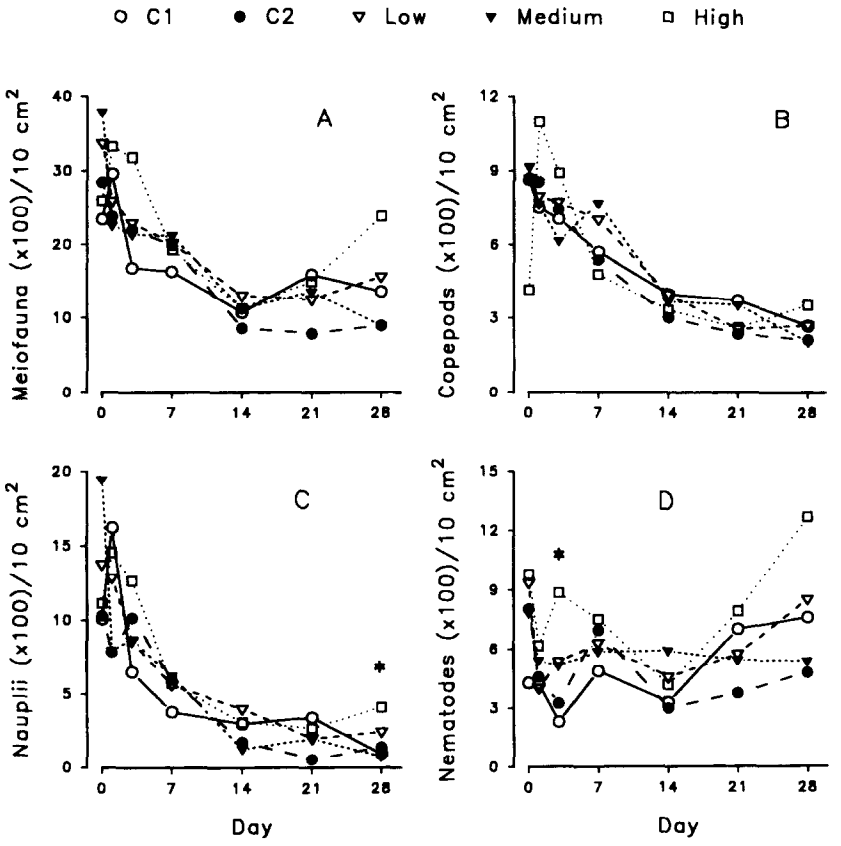

Fig. 6. Abundances of major meiofaunal groups. (A) Total meiofauna; (B) total copepods (includes adults and juveniles); (C) copepod nauplii; (D) nematodes. See Fig. 1 for further details.

Meiofaunal community composition

Total initial (Day 0) average meiofaunal abundance ranged from 2100 to 3400 individuals 10 cm^{-2}. The meiofauna community was numerically dominated by copepods (and nauplii) and nematodes. Several other taxa, such as chironomids, ostracods, polychaetes, and oligochaetes made up the remainder of the community. When considering the entire data set, PAH concentration did not significantly influence total meiofaunal abundance (Fig. 6(A)). However, removal of Day 0 data from the analysis resulted in a significant ($p = 0.0392$) reduction of total meiofauna in High-PAH treatments. Copepod abundance was also not significantly influenced by PAH concentration (Fig. 6(B)). Copepod nauplii were significantly influenced by PAH and were most abundant in the High-PAH

treatments (Fig. 6(C)). When considering individual days, the effect of PAH on nauplii was significant only on Day 28.

The nauplius/copepod ('copepod' includes copepodites and adults) ratio was significantly influenced by PAH concentration, and was significantly higher in High-PAH microcosms than in all other treatment levels (Fig. 7(A)). The effect of PAH remained significant ($p = 0.012$) when data from Day 0, which yielded anomalously high ratios in High-PAH treatments, were removed from the analysis.

Nematode abundance was significantly influenced by PAH concentration (Fig. 6(D)), but *a posteriori* comparisons did not reveal any significant differences among PAH treatment levels (Table 1). Such a result is possible because the Student–Newman–Keuls *a posteriori* comparison is

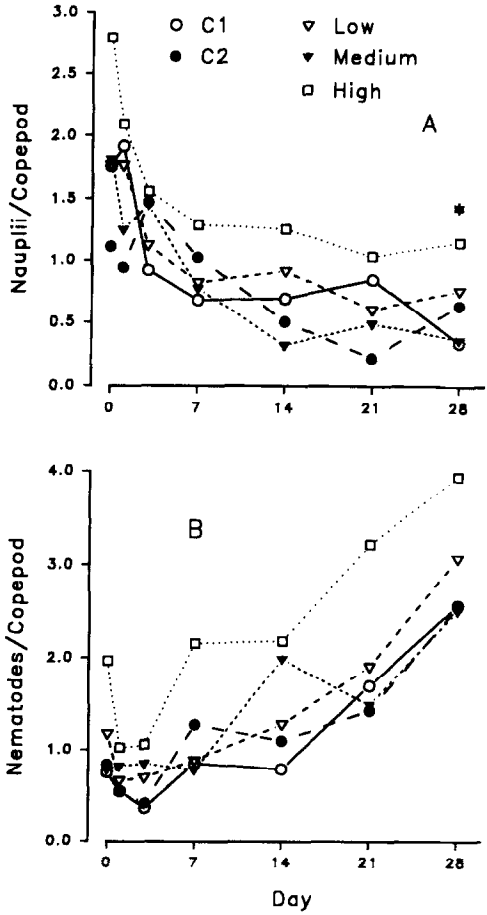

Fig. 7. Relative abundances of meiofuanal groups. (A) The nauplius/copepod ratio of abundance; (B) the nematode/copepod ratio of abundance. See Fig. 1 for further details.

relatively conservative (Underwood, 1981). Nevertheless, average nematode abundance was highest in High-PAH treatments and lowest in controls. Thus, if one accepts that the influence of PAH was significant, it is reasonable to conclude that nematode abundance in High-PAH treatments was significantly higher than in control sediments.

The nematode/copepod ratio was significantly influenced by PAH concentration (Fig. 7(D)). *A posteriori* comparisons revealed that the nematode/copepod ratio was significantly higher in High-PAH treatments than in controls.

EFFECTS OF THE SYSTEM

With the exception of nematode abundance, the main effect of Time was significant for all of the variables examined (Table 4). This observation suggests that containment of sediment in microcosms significantly altered the microbial and meiofaunal communities.

TABLE 4

Summary of ANOVA *p*-Values for the Influence of 'Time', and *a posteriori* Comparisons (Student–Newman–Keuls test) of differences among Days.

Variable	p-*Value*	A posteriori *comparisons*						
Coullana sp. lipid	0.0001	14^{a}	0^{a}	1^{ab}	21^{ab}	3^{b}	7^{b}	28^{b}
P. wellsi lipid[a]	0.0002	0^{a}	1^{a}	3^{a}	7^{b}			
Coullana sp. graze	0.0001	0^{a}	1^{b}	3^{b}	21^{c}	7^{cd}	28^{d}	14^{d}
P. wellsi graze[b]	0.001	0^{a}	3^{b}	1^{c}	7^{d}	14^{d}		
Algal Phospholipid	0.0001	14^{a}	7^{b}	3^{bc}	1^{bc}	21^{c}	28^{d}	0^{d}
Algal Neutral lipid	0.0001	14^{a}	21^{b}	7^{bc}	1^{bc}	28^{bc}	3^{c}	0^{d}
Algal Phosph/Neut	0.0001	3^{a}	7^{b}	0^{b}	1^{b}	14^{c}	21^{c}	28^{c}
Chlorophyll *a*	0.0002	21^{a}	7^{a}	3^{ab}	0^{ab}	14^{ab}	1^{b}	28^{b}
Bacterial Phospholipid	0.0001	28^{a}	21^{b}	3^{bc}	7^{c}	1^{c}	0^{c}	14^{d}
Bacterial Phosph/Glyco	0.0001	28^{a}	7^{ab}	0^{b}	21^{b}	3^{b}	14^{b}	1^{c}
Bacterial abundance	0.0001	28^{a}	0^{b}	14^{c}				
Copepods	0.0001	1^{a}	3^{a}	0^{a}	7^{a}	14^{b}	21^{bc}	28^{c}
Nematodes	0.2963	0^{a}	7^{a}	28^{a}	21^{a}	1^{a}	3^{a}	14^{a}
Nauplii	0.0001	0^{a}	1^{a}	3^{a}	7^{a}	14^{b}	28^{b}	21^{b}
Total Meiofauna	0.0001	0^{a}	1^{a}	3^{a}	7^{a}	28^{b}	21^{b}	14^{b}
Nematode/Copepod	0.0001	28^{a}	21^{ab}	14^{b}	7^{bc}	0^{bc}	3^{c}	1^{c}
Nauplius/Copepod	0.0001	0^{a}	1^{ab}	3^{ab}	7^{bc}	14^{cd}	28^{cd}	21^{d}
PAH	0.0001	1^{a}	3^{ab}	0^{abc}	21^{bcd}	7^{bcd}	14^{cd}	28^{d}

[a] Includes only data from Days 0–7. [b] Includes only data from Days 0–14. For each variable, days that do not share a common superscript letter differ significantly ($p < 0.05$).

Bacteria

Bacterial incorporation of ^{14}C-acetate into phospholipids did not change significantly until Day 14, then increased at Days 21 and 28 (Fig. 2(A)). Day 28 values were significantly greater than those for all other days. Bacterial abundance differed significantly among all three days examined (Fig. 2(C)). Abundance was highest on Day 28, which was consistent with the observation that phospholipid synthesis (growth) was also highest on Day 28. Although bacterial physiological condition (phospholipid/PHB) differed significantly among days, it was difficult to detect any meaningful pattern (Fig. 2(B)).

Microalgae

^{14}C-bicarbonate in phospholipids fluctuated over time, and was lowest at the beginning and end of the experiment (Day 0 and Day 28; Fig. 3(A)). There was a trend for increased synthesis of neutral lipids up to Day 14, followed by a decrease at Days 21 and 28 (Fig. 3(B0). Microalgal physiological condition (phospholipid/neutral lipid) remained relatively constant until Day 7, then decreased significantly from Day 14–28 (Fig. 3(C)). There was significant variation in chl *a* among days, but there was no clear trend over time (Fig. 3(D)).

Copepod grazing and physiological condition

A general decline in grazing rate over the first seven days was observed for both *Coullana* sp. and *Pseudostenhelia wellsi* (Fig. 4). Lipid-storage material also declined over the first week in both *Coullana* and *P. wellsi*. Lipids increased dramatically in *Coullana* on Day 14, then decreased again on Day 21 (Fig. 5(A)). In contrast, lipids in *P. wellsi* were at a minimum on Day 14 and increased slightly thereafter (Fig. 5(B)). It should be noted, however, that very few ovigerous females remained in microcosms after Day 7, and thus data from Days 14–28 are based on only a few observations.

Meiofaunal community

From Day 0–28, total meiofaunal abundance decreased by approximately a factor of two (Fig. 6(A)). This decrease resulted primarily from reductions in copepod (Fig. 6(B)) and nauplius (Fig. 6(C)) abundance. Nematode abundance, however, did not change significantly over time (Fig. 6(D)). Indeed, nematode abundance increased in four of the five PAH treatments

from Day 14–28, with the greatest increase occurring in the High-PAH treatment.

When considering abundances of the two dominant copepod species, the decrease in abundance over time was less obvious. For example, *Coullana* sp. abundance actually increased (though nonsignificantly) in control microcosms over the duration of the experiment (44.5 ± 15.6 (mean 1 s.d.; Day 0) versus 71.0 ± 35.0 (Day 28) individuals 10 cm^{-2}), while *Coullana* in High-PAH microcosms generally declined over time (120.5 ± 100.2 (Day 0) versus 42.0 ± 27.6 (Day 28) individuals 10 cm^{-2}). *P. wellsi* abundance, on the other hand, declined steadily over time in control microcosms (335 ± 105 (Day 0) versus 121.5 ± 51.0 (Day 28) individuals 10 cm^{-2}) but remained remarkably constant in High-PAH microcosms (155.5 ± 65.6 (Day 9) versus 120.0 ± 172.0 (Day 28) individuals 10 cm^{-2}).

The nauplius/copepod ratio declined significantly over time (Fig. 7(A)). This pattern generally contrasted with the influence of PAH, where the nauplius/copepod ratio was highest in High-PAH treatments. The nematode/copepod ratio increased significantly over time (Fig. 7(B)). The influence of time was similar to the influence of PAH, where the nematode/copepod ratio was significantly higher in High-PAH treatments.

DISCUSSION

The design of the microcosm experiment allowed for distinctions between effects of PAH and containment. The significant influence of time indicated that containment effects directly influenced the sedimentary community, with the effect becoming most obvious at Day 14. These containment effects may have been partially from hypoxia in microcosms. Although we did not measure O_2 in microcosm water or sediments, we suspect that the flow of water through the Nitex mesh covering the windows on microcosms was insufficient to adequately oxygenate the microcosms.

A number of variables were also significantly influenced by PAH concentration. The influence of PAH varied among the different microbial and meiofaunal components of the sedimentary community. In all cases where significant ($p < 0.05$) effects of PAH were observed, it was High-PAH treatments that differed significantly from other treatments (Table 1). In no cases did Medium-PAH, Low-PAH, or controls differ significantly from each other. Overall, the High-PAH treatments yielded extreme (maximum or minimum) values in 13 out of the 17 tests performed (Table 1).

While it is recognized that micro-organisms play a critical role in the breakdown of hydrocarbons, the impact of hydrocarbons on the metabolic condition of a natural microbial assemblage is poorly understood (Bartha & Atlas, 1987). Previous studies have shown that petroleum hydrocarbons may enhance the abundance (Bunch, 1987) or alter the activity (Bauer & Capone, 1985) of sedimentary bacteria. In the present study, neither bacterial abundance nor activity were significantly influenced by sedimentary PAH concentration. We chose the ^{14}C-acetate assay of bacterial activity because it has been used as a general indicator of disturbance to marine benthic bacterial communities (Dobbs *et al.*, 1989; Thistle *et al.*, 1984; Findlay & White, 1987). However, we observed no significant influence of PAH on phospholipid synthesis or the phospholipid/PHB ratio of incorporation of ^{14}C-acetate. Thus, we conclude that PAH concentration neither stimulated nor inhibited overall bacterial growth or physiological condition. It is possible, however, that these broad measures of bacterial activity and abundance did not detect significant changes in bacterial community structure.

There was evidence that PAH concentration influenced sedimentary microalgae. Elevated incorporation of ^{14}C-bicarbonate into both phospholipid and neutral lipid and elevated phospholipid/neutral-lipid ratio (Guckert *et al.*, 1992) in the High-PAH treatments suggested that algal growth was stimulated in the High-PAH treatment. Chl *a*, a general indicator of algal biomass, was higher (although not significantly) in High-PAH treatments. Previous studies also have shown that microalgal growth may be stimulated by relatively low additions of petroleum hydrocarbons (Kuiper *et al.*, 1984; Chan & Chiu, 1985).

Changes in the metabolic status of sedimentary microalgae could be perceived by meiofauna as a change in resource availability. Microalgae are an important food resource for many meiofaunal species (Fleeger & Decho, 1987), and co-occurring meiofaunal species may be selective in the types of food that they ingest (Carman & Thistle, 1985). A previous laboratory experiment showed that consumption of algae by *Tigriopus brevicornis* (a harpacticoid copepod) decreased when exposed to aromatic hydrocarbons (Coull & Chandler, 1992). Nevertheless, we detected no significant effects of PAH on copepod grazing in the species studied here. Further, PAH concentration did not influence storage lipids within copepods, which is consistent with the observation that grazing was not affected. The lack of PAH-related reduction in storage lipids also indicated no significant direct stress from petroleum hydrocarbons on copepods, as has been observed on other crustaceans (Wang & Stickle, 1988; Fraser, 1989).

P. wellsi and *Coullana* sp. are predominant species in Louisiana estuaries (Chandler & Fleeger, 1987), and have been the subject of previous

ecological studies. *Coullana nauplii* are photopositive, and adults are photonegative. Juveniles swim into the water column, and *Coullana* is generally a good colonist (Sun & Fleeger, 1994). *Coullana* adults live in a U-shaped burrow (Chandler & Fleeger, 1987), where they can feed on planktonic algae via filter feeding (Decho, 1986). *P. wellsi*, in contrast is an obligate sediment dweller that resides in tubes (Chandler & Fleeger, 1984) and is a poor colonist (Sun & Fleeger, 1994). Little is known about its feeding habits, but it appears to feed on the inner walls of its tubes (Chandler & Fleeger, 1987). Because of their different behavior and feeding habits, *P. wellsi* and *Coullana* probably do not compete for food, but one might expect *P. wellsi* to be more susceptible to sediment-bound contaminants. Again, however, we saw no evidence to indicate this is the case. For example, *P. wellsi* abundance remained constant in High-PAH treatments, while *Coullana* abundance declined.

Studies of the effect of pollution on meiofauna often conclude that harpacticoids as a group are more sensitive than nematodes to a variety of substances including PAH, and stressful environmental conditions such as low oxygen (Coull & Chandler, 1992; Hicks & Coull, 1983). Several types of studies generally support this conclusion, including field studies following oil spills (Elmgren *et al.*, 1983), experimental additions of oil to sediment plots (McLachlan & Harty, 1979) and sampling programs in sediments known to be polluted (Heip *et al.*, 1988). In addition, mesocosm studies, in which hydrocarbons are experimentally added to sediments or the water column, frequently show striking effects on harpacticoid copepods (Stacey & Marcotte, 1987; Warwick *et al.*, 1988).

Not all studies, however, show a negative impact by hydrocarbons on meiofaunal communities. Meiofauna in sediments of natural hydrocarbon seeps are either unaffected by oil or show increases in densities (Montagna *et al.*, 1987), and experimentally oiled sediments sometimes show increases (or no change) in harpacticoids and nematodes (DeLaune *et al.*, 1984; Smith *et al.*, 1984; Spies *et al.*, 1988). Such increases are usually presumed to be related to increased microbial food resources in oiled sediments (Montagna *et al.*, 1987).

In the present study, total copepod abundance was not significantly influenced by PAH concentration, but there was a highly significant influence of PAH on the nauplius/copepod ratio. The high nauplius/copepod ratios were in part the result of higher abundance of nauplii in High-PAH treatments. Antia *et al.* (1985) found that pesticides can adversely influence development of copepods, and Kennish (1992) noted that PAH can inhibit ecdysis in crustaceans, which in turn would preclude molting and normal development. A negative influence on copepod development could explain the relative and absolute increase in

nauplii in our experiment. If PAH had the influence of stunting growth by inhibiting molting, but did not influence egg production by adult females, nauplii would be unable to develop into copepodites and their absolute and relative abundance would increase. This increase in abundance of nauplii could persist until developmental problems significantly impacted the abundance of adult females and their production of more nauplii. Subsequent toxicity tests have revealed that the Port Fourchon sediment used in this study does not result in any significant mortality over 96 h in adult females of *P. wellsi* or *Coullana* sp. (Lotufo & Fleeger, unpublished). However, toxicity tests with these sediments have indicated significant reduction in nauplii production by another copepod (*Cletocamptus deitersi*). Thus, further study may be required to properly interpret the biological mechanism behind the significant influence of PAH on the nauplius/copepod ratio observed in our study. While the above discussion is concerned with influences at the taxon level, we have also detected significant effects of PAH on various life-history parameters of individual copepod species (Carman & Todaro, in prep.).

Nematode abundance was highest in High-PAH treatments, which resulted in a significant influence of PAH on the nematode/copepod (N/C) ratio. The N/C ratio was originally proposed by as a field indicator of pollution (Raffaelli & Mason, 1981), with higher N/C ratios expected under polluted conditions. Although the index is controversial (Coull & Chandler, 1992) the N/C ratios observed in our study are consistent with the general theory behind the index: Nematodes as a group tend to cope better than copepods with stressful conditions, and harpacticoids are more likely than nematodes to experience mortality from the toxic fractions of PAH (Barnett & Kontogiannis, 1975; see review by Coull & Chandler, 1992).

Previous Louisiana field studies have indicated that meiofuanal community structure is relatively insensitive to petroleum hydrocarbons (Fleeger & Chandler, 1983; DeLaune *et al.*, 1984; Smith *et al.*, 1984). This apparent insensitivity may in part be a consequence of the rapid recolonization rates of which meiofauna are capable, and that have been observed in previous studies of experimentally oiled sediments (Decker & Fleeger, 1984; Spies *et al.*, 1988; Warwick *et al.*, 1988). Such immigration in the field could easily have swamped community changes that we observed here.

PAH concentrations in the range 1–20 mg PAH/kg dry sediment have been associated with noticeable impacts on benthic communities (e.g. Steinhauer & Boehm, 1992; Misitano & Schiewe, 1990). Our highest PAH doses were approximately 3 mg PAH/kg in the top 1 cm of sediment.

Because contaminated material was added to the sediment surface, however, the effective PAH concentration in the top few millimetres of sediment where meiofauna and microbes are most abundant was between 3 and 27 mg PAH/kg. Some mixing occurred, as tubes, burrows, and other surface topography could be observed on all microcosms after sediment was added. At these concentrations we detected significant influences of PAH on microalgal activity and meiofaunal community composition. No significant impact on bacteria or meiofaunal grazing were detected. Overall the microcosm benthic systems were much more resilient to acute perturbations than we had expected. There are several possible explanations for these observations. First, the influence of microcosms *per se* (i.e. containment artifacts) could have overwhelmed influences from the addition of PAH. The power of statistical tests for the effect of PAH was generally low (Table 1). Thus, changes in the experimental design and/or improved conditions for microcosms might result in the detection of significant PAH effects on bacteria and meiofaunal grazing. Second, the marsh sediments had a high organic content (approximately 2·5%), and higher levels of contaminant may be required to elicit toxic effects in sediments that are high in organic material (Di Toro *et al.*, 1991). Finally, it is possible that the salt-marsh community has evolved a significant measure of resistance to PAH (and other contaminants). This hypothesis has been proposed to explain variability in responses of sedimentary microorganisms to petroleum hydrocarbons (Baker & Griffiths, 1984). It has been demonstrated previously (Klerks & Levinton, 1989) that a freshwater oligochaete could adapt within one to four generations to elevated cadmium concentrations. Battaglia *et al.* (1980) showed that the harpacticoid copepod *Tisbe holothuriae* could adapt quickly to oil stress. The entire coastal area of Louisiana has been exposed to petroleum production activities for decades (Fang, 1990). We cautiously speculate that the salt-marsh community that we are studying has evolved a resistance to elevated levels of petroleum hydrocarbons.

ACKNOWLEDGEMENTS

The authors would like to thank the following undergraduate and graduate students who assisted with this project: J. Colhier, C. Garrott, J. Gruner, F. Hernandez, B. Hughes, K. Lacour, G. Lotufu, M. McCall, L. Mirande, and B. Sun. A. Todaro assisted with meiofauna sorting and identification. Image-analysis was performed using the facilities of the Life Science Microscopy Facility at LSU, with technical assistance from B. Demler and J. Lynn. E. Chesney and P. Sammarco graciously provided access to

facilities at the LUMCON facility. B. Moser and M. Holly provided statistical advice. The research was supported by a grant (No. 14-35-0001-30470) from the US Department of the Interior, Minerals Management Service, Gulf of Mexico Region, Outer Continental Shelf Office.

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