

University Research Initiative

Experimental Investigation of the Effects of Aromatic Hydrocarbons on a Sediment Food Web



U.S. Department of the Interior
Minerals Management Service
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ABSTRACT

The influence of polynuclear aromatic hydrocarbons (PAH) on a sedimentary salt-marsh food web was examined using microcosm and laboratory experiments that simulated natural conditions. Microcosms were dosed with concentrations of PAH-contaminated sediment collected from a produced water site at Pass Fourchon, LA. Bacterial activity and abundance were not influenced by PAH, but microalgal activity and physiological condition were. Grazing by 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, sublethal effects of PAH were not pronounced at the concentrations (0.3 to 3.0 ppm) we examined. Considering that coastal Louisiana has been exposed to chronic contamination by petroleum hydrocarbons for decades, we suggest that the meiofaunal/microbial community in these sediments may have adapted to elevated to PAH concentrations.

Fish-predation studies indicated that juvenile spot (Leiostomus xanthurus) could not detect PAH-contaminated sediments, and continued to feed normally when exposed to them. PAH contamination did not decrease the number of feeding strikes or sediment processing time. If anything, spot-predation was increased in PAH-contaminated sediments, possibly in response to emergence of chironomids from the sediment. This lack of ability to discriminate between contaminated and uncontaminated sediments could have serious implications in terms of bioaccumulation of PAH (or other contaminants) by these bottom-feeding fish.

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LIST OF ABBREVIATIONS

AODC	acridine orange direct counts
Bq	Bequerel
dpm	disintegrations per minute
GC	gas chromatograph
h	hour
LSU	Louisiana State University
LSC	liquid scintillation counter
LUMCON	Louisiana Universities Marine Consortium
MMS	Minerals Management Service
MS	mass spectrometer
nm	nanometer
PAH	polynuclear aromatic hydrocarbons
PHB	poly- β -hydroxybutyrate
ppb	parts per billion
ppm	parts per million
QA	quality assessment
QC	quality control
μ E	micro Einstein

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Chapter 1. Introduction

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

Most studies of hydrocarbon contamination fall into a few major categories (Coull and 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. Environmental impacts are frequently determined from mortality (i.e., reduced abundance or species diversity) and changes in community structure. Because these are "natural" experiments, a number of random variables are involved 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). These studies 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 and Pratt, 1989). (3) Microcosm/mesocosm experiments have been proposed as a compromise whereby the investigator maintains considerable control over experimental conditions and toxicant dosages while attempting to simulate the major features of natural communities (e.g., Kuiper et al. 1984). While they have yielded valuable information, these studies frequently suffer from a lack of adequate replication and/or proper controls due to logistical constraints (e.g., Farke et al., 1985).

We report here a microcosm approach to study the impacts of polycyclic aromatic hydrocarbons (PAH) on benthic food webs. Our

primary focus involves the meiofaunal component of the benthos, with emphasis on their trophic relationships with microorganisms (food) and juvenile fish (predators). Our approach is novel for several reasons. First, the impact of contaminants was determined not merely by monitoring the abundance of various groups of organisms, but by determining their physiological responses, as well as trophic interactions (*i.e.*, grazing rates). Second, the microcosms represented minimally disturbed, natural assemblages of benthic organisms and the sediment in which they live. Finally, the system was well replicated and included a complete set of controls, which allowed rigorous hypothesis testing. Adequate replication was possible because of the small size and high abundance of microbes and meiofauna. This allowed us to use microcosms that are relatively small, and which could be readily collected and transported to laboratory facilities in large numbers.

Meiofauna are a diverse and abundant group of small invertebrate metazoans (defined as those animals that pass through a 1-mm sieve and are retained on a 0.042-mm sieve; Higgins and Thiel, 1988). Among the meiofauna, nematodes and harpacticoid copepods typically rank first and second in abundance, respectively (Coull, 1988). Studies of the effect of pollution on meiofauna often conclude that harpacticoids are more sensitive than nematodes to a variety of substances including PAH, and stressful environmental conditions such as low oxygen (Coull and Chandler, 1992; Hicks and 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 and Harty, 1979) and sampling programs in sediments known to be polluted (Heip *et al.*, 1988). In addition, mesocosm studies, in which hydrocarbons were experimentally added to sediments or the water column, frequently show striking effects on harpacticoid copepods (Stacey and 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).

Estuarine salt marshes are critical marine habitat because of their high productivity and importance as nursery grounds for many commercially important species. Any perturbation of salt-marsh food webs would have the potential to impact this high productivity. Further, a report by the National Research Council (1985) identifies salt marshes as susceptible to chronic and/or

catastrophic inputs of petroleum hydrocarbons. This susceptibility is the result of the physical and geochemical character of salt marshes. From a geochemical perspective, the fine particles (silts and clays) that are characteristic of salt-marsh sediments act as a sink for hydrophobic contaminants, such as petroleum hydrocarbons (Connell and Miller, 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. Recent evidence suggests that hydrophobic compounds bind very strongly to organic matter, acting as another "trap" (Di Toro et al., 1991). Once impacted, salt-marsh sediments can remain contaminated for years because of low rates of tidal flushing and slow biodegradation of complex hydrocarbons (Kennish, 1992). Two recent studies (Kirso et al., 1990; Baker et al., 1991) found that particles at the sediment-water interface were enriched with PAH by a factor of 10-100 relative to concentrations in bulk-sediments or suspended particles. This observation suggests that (1) fluxes of PAH to sediments may be higher than previously believed, and (2) organisms living or feeding at the sediment-water interface, such as meiofauna and juvenile fish, may be exposed to particularly high concentrations of contaminants.

We feel that the analysis of estuarine food webs is a compelling approach for detecting ecologically significant, sublethal impacts of contaminants. Feeding (grazing or predation) is the primary mechanism of biomass transfer across trophic levels, and measurements of success or failure in feeding should allow an ecologically meaningful interpretation of biological impacts of contaminants. Laboratory studies indicate that a negative impact on feeding is one of the first responses that result from exposure to toxicants, even at low (sublethal) concentrations (Cowles 1983a, 1983b; Farke et al., 1985). As a result, it is reasonable to expect that the influence of pollutants should be manifested in the food web (Connell and Miller, 1984). Managers and decision makers need this kind of functional information to build predictive models of contaminant effects, especially in sensitive areas such as salt marshes.

Microbes (microalgae and bacteria) are at the base of this food web, and impacts on microorganisms can reverberate across all trophic levels. While it is recognized that microorganisms play a critical role in the breakdown of hydrocarbons, the impact of hydrocarbons on the metabolic condition of a natural assemblage of microbes is poorly understood (Bartha and Atlas, 1987). Changes in the abundance or species composition of microorganisms could lead to altered food availability for meiofauna, the second link in this web. Microalgae in particular appear to be important food resources for many meiofaunal species (Fleeger and Decho, 1987).

PAH may also have a negative effect on the energy reserves of meiofauna. This effect could result from reduced food availability, reduced grazing activity, and/or direct toxic effects on meiofauna. Neutral lipids, used by a variety of aquatic organisms as carbon and energy reserves (Benson et al., 1973), become depleted when animals are exposed to stressful conditions in general, and petroleum hydrocarbons in particular (Fraser, 1989). Reduced levels of lipid-storage products in meiobenthic invertebrates would result in reduced fitness of individuals, and reduce their calorific value to predators. Examination of lipid-storage material in meiofauna compliment the measurements of grazing rates.

The third link in this chain is predation by juvenile fish on the meiofauna and small macrofauna of estuaries. Spot, Leiostomus xanthurus (family sciaenidae), is an abundant and ecologically important estuarine-dependent fish species. Spot is widely distributed in Louisiana, often in areas with PAH contamination (Means, personal observation), and ranks as high as second in abundance among estuarine species in the spring (Day et al., 1973; Baltz et al., 1993). Post-larval and juvenile spot experience frequent and intimate contact with sediment during feeding because spot takes hundreds to thousands of bites from the sediment daily, strains and retains small animals and clears rejected sediment and detritus through its mouth and gill openings (Chao and Musick, 1977; Billheimer and Coull, 1988; Archambault and Feller, 1991). Spot's principal food during the early life history stages consists of meiofauna, primarily copepods, nematodes, small chironomids, and annelids, until it reaches more than 80 mm in length (McCall and Fleeger, 1993; Feller et al., 1990; Stickney et al., 1975; Smith and Coull, 1987). Often thousands of meiofauna can be found in the gut contents of a single fish (McCall, 1992; Coull, 1990).

Bottom-feeding fishes like spot are exposed to contaminants such as PAH by several routes; through water, contact with sediments, and ingestion of contaminated prey (McElroy and Sisson, 1989; Rubinstein et al., 1984). The effects of such exposure have been studied in many species, and fishes have the ability to quickly metabolize PAH (Kennish, 1992; Varanasi et al., 1989). Unfortunately, metabolites of PAH have been frequently shown to be more toxic and carcinogenic than their parent compounds (Kennish, 1992). Spot and other sciaenids have become important model systems for the study of impacts of PAH. Croaker (Micropogonias undulatus) growth rates are slower in areas that are more heavily contaminated with PAH (Burke et al., 1993), and spot has been shown to suffer acute toxicity as well as lesions, weight loss, alterations in pancreas and liver function, and reduced efficiency of macrophages in the presence of sediments contaminated with PAH (Hargis et al., 1984; Weeks and Warinner, 1984; Roberts et al., 1989; Huggett et al., 1992). Inducible enzymes involved with PAH metabolism have frequently

been studied in spot, and several biomarkers of PAH contamination have been successfully identified (Roberts and Sved, 1987; Sved et al., 1992; Goksoyr and Husoy, 1992; Huggett et al., 1992).

Aquatic contaminants (and adverse environmental conditions such as hypoxia, Pihl et al., 1991; Pavela et al., 1983) have been shown to modify fish activity, especially through avoidance behavior (Klaprat et al., 1992; Brown et al., 1982). Some pollutants, e.g. metals, interfere with chemoreception, but fishes do not necessarily avoid PAH (Klaprat et al., 1992). Nevertheless, Berge et al. (1983) showed that PAH alters swimming activity in the sand goby, and Purdy (1989) found that PAH contamination in seawater reduced or stopped feeding by coho salmon. Generally, however, PAH effects on feeding are poorly known, and have not been studied with sediment-bound PAH in bottom-feeding fishes. Feeding effects may be heightened due to the potential for contamination and the need in spot for continuous feeding (Archambault and Feller, 1991). The purpose of this portion of our study was to determine if sediment-bound PAH alters the behavior or feeding intensity of spot. Our working hypothesis is that spot will tend to avoid PAH-contaminated sediment when possible. If spot is unable to discriminate and avoid PAH, increased contamination may result.

Experimental work was divided into two components. In the microcosm experiment responses of, and interactions between microbes and meiofauna were studied. In the second component, interactions between meiofauna and fish predators (spot) were studied (Chapters 2-4). The microcosm experiment is described first, followed by the fish-meiofauna experiments (Chapters 5-7).

Chapter 2. Materials and Methods, Microcosm Experiment

2.1 General Design

The effects of anthropogenic inputs on biological systems are typically examined by quantifying changes in the abundance and/or species composition of benthic organisms in field-monitoring programs. Our approach was to examine the mechanisms by which benthic communities may be altered, especially as it relates to the food web, and further to determine if PAH impacts the benthos in sublethal ways that may not be reflected by commonly used indices of community structure such as composition, diversity, and ordination techniques.

The impact of PAH on the sediment food web was determined from a variety of measurements on the microbial and meiofaunal components of the communities. Microalgal and bacterial abundance were followed using Chlorophyll a (Chl a) and direct counts, respectively. Microalgal and bacterial activities and physiological conditions were determined from uptake and partitioning of ¹⁴C substrates (¹⁴CO₂ and ¹⁴C-acetate, respectively). Meiofaunal grazing on microalgae was determined using ¹⁴CO₂ radiotracer studies. Copepod physiological condition was determined by measuring neutral-lipid deposits in individual animals. Finally, meiofaunal community composition was monitored.

We used a microcosm approach to studying the influence of PAH on the microbial and meiofaunal components of sediment food webs that provided an environmentally realistic evaluation of ecologically meaningful impacts. We used intact, relatively undisturbed microcosms of natural communities of sedimentary microorganisms and meiofauna. The microcosms were a manageable size (15.2 cm i.d. cylinders), which made collection, transport, and maintenance of microcosms relatively straightforward. We were able to use small microcosms because of the small size but high abundance of the organisms we studied. Thus, we were able to bring a natural community into the lab and maintain it under controlled conditions. Microcosms were exposed to a range of environmentally realistic doses of sediment that were contaminated with PAH from a produced-water outfall near Pass Fourchon (Rabalais et al., 1991). Thus, we had the advantage of working with a natural community while maintaining control over the level of contaminant loading within each microcosm. Further, each microcosm represented an independent statistical replicate, and the system contained a complete set of control microcosms. As a result, we had a well-replicated, balanced statistical design that allowed for rigorous testing of hypotheses.

Microcosm experiments were performed using a 4 x 7 x 5 factorial design, with 'wet tables' (as blocks), 'PAH

concentration', and 'exposure time' 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). Four replicate microcosms (one from each wet table) of each of the five treatment levels (20 total microcosms) were harvested at each of seven dates (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- μ m) to allow exchange of water (Fig. 2.1). At low tide on 28 May, 1991, 140 microcosms of exposed marsh sediment were collected by hand with minimal disturbance and transported to the LUMCON facility at Cocodrie, LA. The depth of sediment in each microcosm was approximately 15 cm. Each microcosm was fitted with a base to facilitate transfer of cores from field to lab. Thirty-five microcosms were randomly assigned to each of 4 wet tables (Fig. 2.2).

Wet tables were constructed of plywood and sealed with fiberglass resin. Wet tables were rinsed with running marsh water for 1 week prior to the initiation of the experiment. During the experiment, wet tables were irrigated with water pumped directly from the salt marsh near LUMCON. Each wet table received approximately 500 liters of water per hour, which represent six complete turnovers of water per hr. Because water was pumped directly from the marsh, microcosms were maintained under essentially ambient temperature and salinity conditions.

Microcosms were illuminated with banks of fluorescent lights. Seven ballasts, each accommodating two 48" fluorescent lights, were positioned over each wet table such that one ballast was positioned approximately 30 cm above each row of five microcosms. A 12 h:12 h light:dark cycle was maintained using automatic timers.

2.2 Dosing of Microcosms

In each wet table, seven randomly selected microcosms were each treated with "high", "medium", or "low" doses of PAH-contaminated sediment by adding a known volume of contaminated sediment (see below) sufficient to cover the entire surface of the microcosm with a 1-mm-thick layer of sediment. The same volume of "uncontaminated" sediment was added to an additional 7 microcosms per table; these microcosms served as an "application control", *i.e.*, a control for the process of adding contaminants to microcosms. Finally, 7 microcosms per table served as unmanipulated controls (no sediment added).

Two sediments, one a "contaminated" sediment collected near a Pass Fourchon produced-water discharge site (Rabalais *et al.*, 1991; their Fig. 5.2) and the other a "control" sediment collected from a relatively clean area in Lake Champagne (Fig.

Table 2.1 Example PAH data set. Samples collected in the vicinity of produced-water discharges in Pass Fourchon Louisiana. See Rabalais et al. (1991) for station locations. "FFPI" stands for Fossil Fuel Pollution Index (Boehm and Farrington, 1984).

Analyte	PF--400	NORTH				SOUTH
	ppb	600 ppb	800 ppb	900 ppb	1000 ppb	600 ppb
Naphthalene	31	nd	37	tr	5.1	tr
2-Methylnaph.	trc	nd	68	nd	8.4	trc
1-Methylnaph.	20	41	84	tr	6.6	trc
2-Ethylnaph.	nd	nd	43	nd	nd	nd
1-Ethylnaph.	nd	nd	nd	nd	nd	nd
2,6/2,7-Dimethylnaph.	140	300	300	tr	trc	trc
1,3/1,7-DMN	140	370	230	nd	6.2	trc
1,6-DMN	nd	tr	120	nd	trc	nd
1,4/2,3-DMN	93	200	110	nd	trc	tr
1,5-DMN	32	72	46	nd	nd	nd
1,2-DMN/2-IPN	nd	trc	trc	nd	nd	nd
2-IPN	nd	trc	nd	nd	nd	nd
1,8-DMN.	nd	nd	nd	nd	nd	nd
C1-Naphthalenes, total	20	41	150	tr	15	tr
C2-Naphthalenes, total	410	940	850	tr	6.2	tr
C3-Naphthalenes	3,100	6,400	3,200	nd	33	trc
C4-Naphthalenes	5,300	5,000	920	nd	tr	nd
C5-Naphthalenes	1,800	tr	1,100	nd	tr	nd
Acenaphthylene	nd	nd	nd	nd	nd	nd
Acenaphthene	nd	nd	nd	nd	trc	nd
Fluorene	47	77	77	tr	nd	nd
C1-Fluorenes	250	500	410	nd	tr	tr
C2-Fluorenes	500	1600	630	nd	tr	tr
C3-Fluorenes	890	1400	720	nd	36	160
Dibenzothiophene	tr	27	31	tr	nd	nd
4-MDBT	120	270	150	trc	trc	15
2/3-MDBT	32	120	65	trc	nd	nd
1-MDBT	30	72	25	nd	nd	nd
C1-Dibenzothiophenes, total	180	460	240	tr	tr	15
C2-Dibenzothiophenes, est.	1,000	1,400	670	trc	7.0	150
C3-Dibenzothiophenes, est.	1,200	1,500	860	nd	11	330
Phenanthrene	nd	nd	180	10	18	14
3-MP	230	540	320	tr	7.8	tr
2-MP	35	120	270	tr	6.0	tr
4/9-MP	240	570	360	nd	7.7	tr
1-MP	150	310	260	nd	tr	tr
3,6-DMP	330	490	270	trc	11	49
3,5-DMP	nd	nd	nd	nd	nd	nd
2,6-DMP	150	310	180	tr	4.9	tr
2,7-DMP	170	250	110	tr	5.4	37
3,9-DMP	610	1,078	530	trc	15	74
1,6/2,5/2,9-DMP	480	800	400	tr	12	65
1,7-DMP	160	320	160	tr	4.7	tr
1,9/4,9-DMP	190	270	150	nd	tr	31
1,5-DMP	nd	nd	nd	nd	nd	nd
1,8-DMP	78	120	75	nd	tr	18
1,2-DMP	51	98	47	nd	tr	nd
9,10-DMP	nd	nd	36	nd	nd	tr
C1-Phenanthrenes, total	660	1,500	1,200	tr	22	tr
C2-Phenanthrenes, total	2,200	3,700	2,000	tr	53	270
C2-Phenanthrenes, est.	2,300	3,900	2,000	trc	60	300
C3-Phenanthrenes, est.	2,400	3,400	1,800	tr	44	610

Table 2.1 Example PAH data set (continued). Samples collected in the vicinity of produced-water discharges in Pass Fourchon Louisiana. See Rabalais et al. (1991) for station locations. "FFPI" stands for Fossil Fuel Pollution Index (Boehm and Farrington, 1984).

Analyte	PF--400 ppb	NORTH				SOUTH
		600 ppb	800 ppb	900 ppb	1000 ppb	600 ppb
Anthracene	nd	nd	27	3.5	3.7	12
Fluoranthene	180	190	110	23	54	110
Pyrene	150	190	110	20	56	110
Benzantracene	74	94	46	10	13	38
Chrysene	230	340	140	11	19	70
Benzo(b)fluoranthene	nd	nd	nd	8.0	15	nd
Benzo(k)fluoranthene	nd	nd	nd	6.0	8.2	nd
Benzo(a)pyrene	nd	nd	nd	7.4	tr	nd
Indeno(1,2,3-cd)pyrene	nd	nd	nd	nd	nd	nd
Dibenzo(a,h)anthracene	nd	nd	nd	nd	nd	nd
Benzo(g,h,i)perylene	nd	nd	nd	nd	nd	nd
Total Pyrogenic PAH	710	920	760	99	190	350
Total Petrogenic PAH	20,010	28,041	14,750	tr	234	1,565
Total PAH	21,000	29,000	16,000	99	430	1,900
FFPI	0.92	0.92	0.90	0.05	0.52	0.80
Saturated Hydrocarbons						
Resolved	32,000	57,000	68,000	480	2,100	3,600
Unresolved	458,000	523,000	302,000	1,720	2,200	106,400
Total	490,000	580,000	370,000	2,200	4,300	110,000
MDL (ng/g)	19	24	21	2.5	3.6	9.9

MDL: Minimum Detection Limit

nd: Not detected

tr: Trace; one ion present, but below detection limits

trc: Trace confirmed; two ions present, but below detection limits

n/a: not analyzed

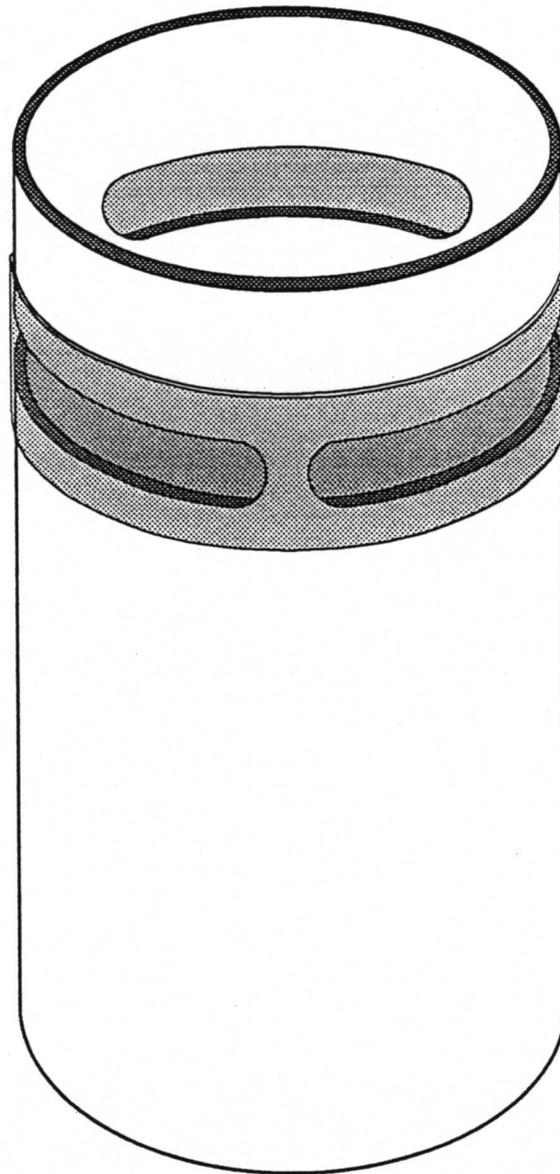


Figure 2.1 Microcosm used in the experiment. Inside diameter is 15.2 cm. Shaded areas represent 62- μm Nitex mesh covering windows that allow water percolation through the microcosm.

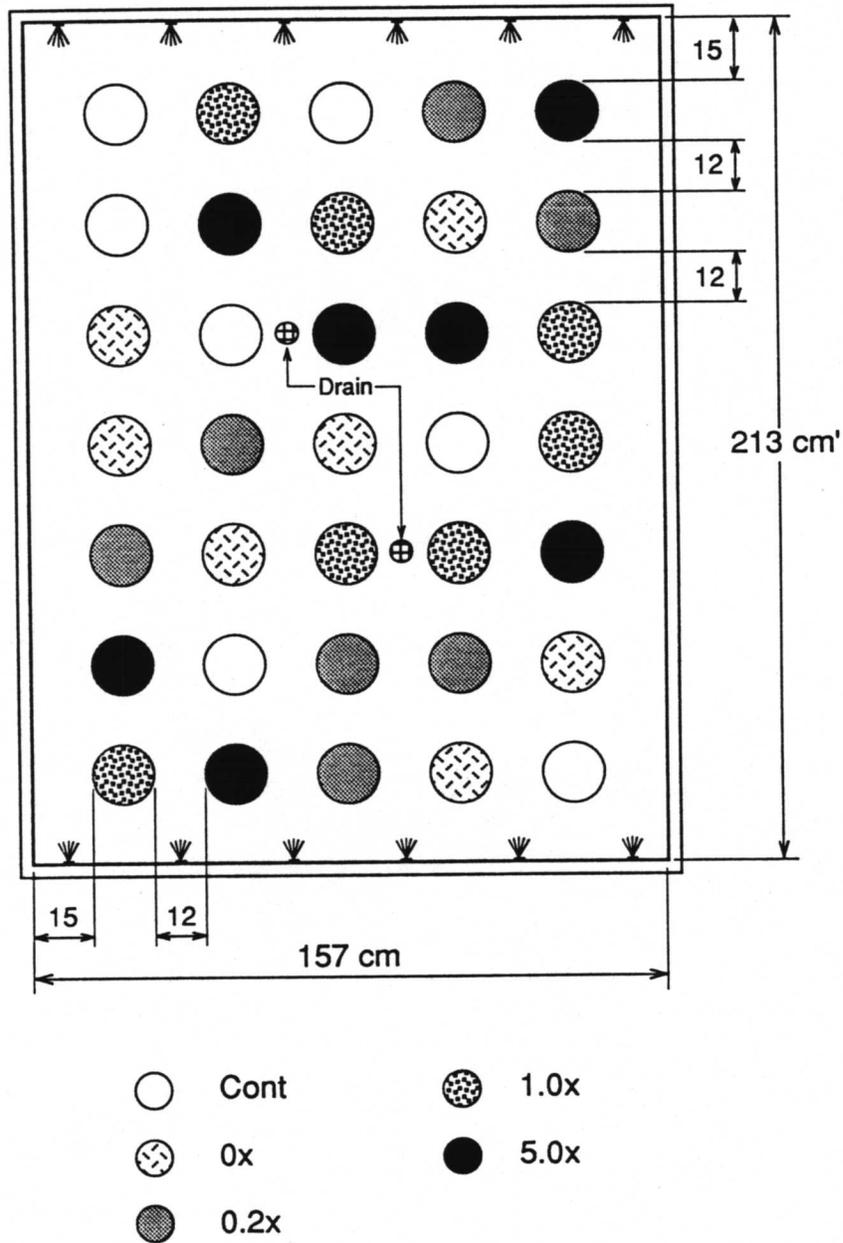


Figure 2.2 Wet-table schematic. Top view of one wet table with microcosms in place. Distribution of microcosms within that table was randomized. Water was introduced via six jets at either end of the table. Circles represent microcosms, and different shadings represent different PAH treatments (see text).

used as a means of dosing microcosms with PAH-contaminated sediments. The two sediments were extensively characterized in previous studies for contaminant load (Table 2.1; Means and McMillin, 1993). Bulk contaminated sediments were collected near the "400N" site described by Rabalais *et al.* (1991), and PAH concentration in this bulk sample was determined to be 27 ppm. This site was actively discharging at the time of sampling at a rate of approximately 25000 barrels per day. The site has been actively discharging for many years. Percent organic carbon in the contaminated and control samples were 2.1% and 3.3%, respectively; textures were 77.5% sand, 19.4% silt, and 3.1% clay (contaminated), and 78.6% sand, 18.1% silt, and 3.3% clay (control). Both sediments were collected in early May and refrigerated for two weeks. Contaminated and control sediments were passed through a 2-mm, stainless-steel sieve to remove plant debris, shell hash, and large particles. Contaminated (27 ppm) and control (0.3 ppm) sediments were homogenized for one week on a roller mill, then mixed to generate the nominal doses of "high" (27 ppm), "medium" (5.4 ppm), and "low" (1.08 ppm). The mixed sediments were homogenized for two days. The mixed sediment was weighed into plastic bags, refrigerated and transported to LUMCON for dosing of microcosms. The amount of sediment was sufficient to create a 1-mm-thick layer of fresh sediment on the surface of each of the experimental microcosms. Dosing of the microcosms consisted of pouring the sediment suspension into the water overlying the sediment in each microcosm, allowing the sediment to bed for two hours with slight swirling and agitation of the microcosms to distribute the sediment evenly over the surface. After the suspended sediment had settled out, seawater flow into the microcosms was restored.

2.3 Assays

Assays to determine microbial activity and abundance, and meiofaunal-grazing activity, abundance, community composition and nutritional status were performed at 0, 1, 3, 7, 14, and 28 days. PAH treatments were administered on the evening of May 29, 1991, and "Day 0" was defined as the morning of May 30. At each sampling date, one microcosm was chosen from each of the four wet tables (based on a previously determined randomization), yielding four replicates (note that each microcosm was an independent replicate) of each of the five treatments. The following cores were collected from each microcosm (see Fig. 2.3): six, 4.9-cm² cores (four for meiofauna grazing and community composition, one for lipid stores in meiofauna, one for hydrocarbon assay); four, 2.3-cm² cores (two for microalgal activity, two for bacterial activity); and two, 3-cc syringe cores (one for Chl *a*, one for bacterial direct counts). For those cores used in radiotracer studies, radioisotopes were applied just under the sediment surface by injecting isotope with a microliter syringe through a

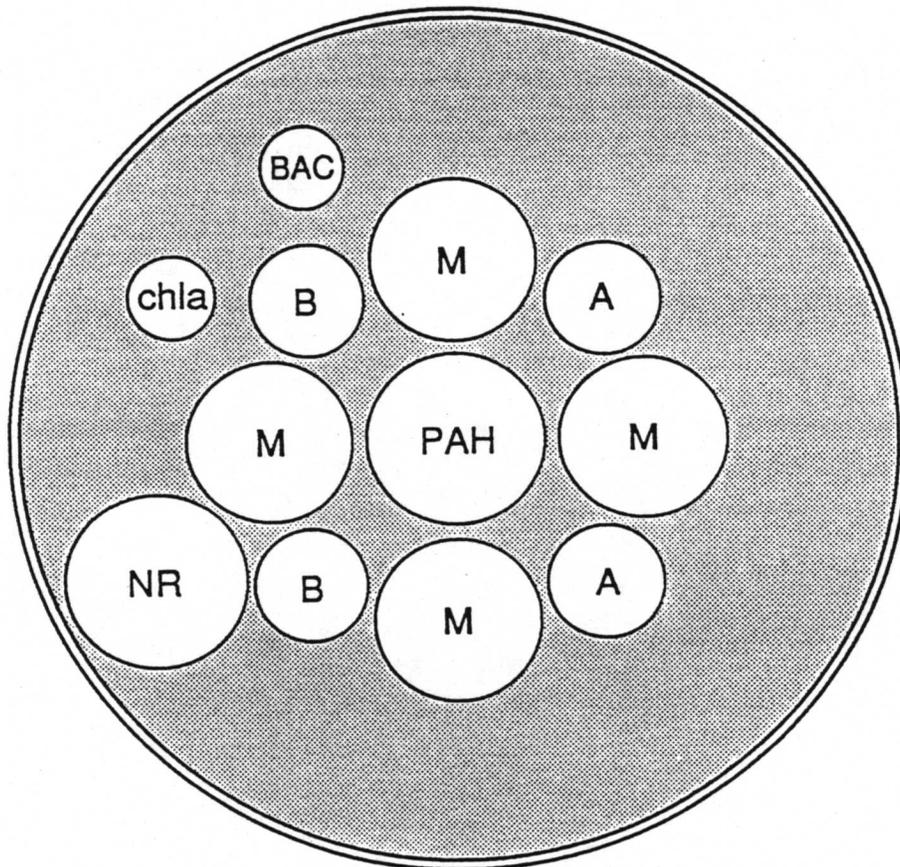


Figure 2.3 Schematic of core samples taken from a microcosm. "M" = core for measurement of meiofauna grazing and community composition; "B" = core for measurement of bacterial activity; "A" = core for measurement of microalgal activity; "NR" = core for measurement of copepod lipid-storage products with the Nile-red assay. "chla" = core for measurement of chlorophyll a; "BAC" = core for measurement of bacterial abundance; "PAH" = core for measurement of PAH content.

silicon-sealed port on the side of the core (Carman *et al.*, 1989). In all cases, the top 1 cm of sediment was collected for analysis. Procedural details of each assay are provided below.

2.3.1 Bacterial Activity

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 and White, 1987). The phospholipid/PHB ratio of incorporation is an index of the metabolic condition of the bacterial assemblage (Findlay and White, 1987). Synthesis of membrane lipids is an indication of bacterial growth, and thus a relatively high (low) phospholipid/PHB ratio of incorporation indicates a relatively high (low) rate of bacterial growth. This technique is independent of isotope specific activity and thus does not require that *in situ* acetate concentrations be known. This is so because the measurement of interest is the relative, not the absolute incorporation of ^{14}C -acetate into phospholipids and PHB.

Cores were injected with 33.4 KBq [1,2]- ^{14}C -acetate (specific activity approx. 4.0 GBq/mmol) and incubated in the dark (to prevent recycling of respired $^{14}\text{CO}_2$) for 5 hr. 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). Radioactivity in each lipid fraction was measured with a liquid scintillation counter (LSC). One core from each microcosm was also used as a killed control. These controls were injected with ^{14}C -acetate and then immediately harvested as described above.

2.3.2. Microalgal Activity

The impact of PAH on benthic microalgae was examined by determining incorporation of ^{14}C -bicarbonate. Cores were injected with 133.2 KBq (specific activity 1.85 GBq/mmol) ^{14}C -bicarbonate and incubated in the light (60 μEi) for five hours. The five-hour incubation was used so that microalgal incorporation of ^{14}C could be related to meiofaunal uptake of ^{14}C -labeled microalgae in grazing experiments. 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. The Whirl-pak bag was placed in a 15 x 60 mm Petri dish, which was taped shut then frozen in liquid nitrogen. These samples were returned to the laboratory at LSU on dry ice 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 radioassayed. In addition, ^{14}C in non-lipid fractions (sediments

and aqueous phase of lipid extraction) were measured so that total microalgal incorporation of ^{14}C could be determined.

2.3.3 Bacterial and Microalgal Abundance

Bacterial abundance in the top 1 cm of sediment was determined from acridine orange direct counts (AODC; Carman, 1993). Microalgal abundance was estimated from Chl a concentration in the top 1 cm. Photosynthetic pigments were extracted from sediment in 90% acetone and assayed with a Turner Model-10 fluorometer (Greenberg et al., 1992).

2.3.4 Meiofaunal Grazing

Grazing by two meiobenthic copepod (Coullana canadensis (Willey) and Pseudostenhalia wellsi (Coull and Fleeger)) species 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). At the end of the incubation period, the 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 meiofauna were separated from the sediment under a dissecting microscope. As a means of standardizing our analysis, we measured grazing only in adult females. Copepods were digested in tissue solubilizer and radioassayed using a LSC. Controls consisted of similarly processed cores that had been incubated in the dark. Estimates of grazing rates were corrected with values from dark controls.

A similar experiment was performed using ^{14}C -acetate as tracer for grazing by copepods on heterotrophic bacteria (Carman et al., 1989). Cores were injected with 91.6 KBq ^{14}C -acetate and incubated in the dark to prevent recycling of respired $^{14}\text{CO}_2$. Controls were injected with ^{14}C -acetate, then immediately preserved in 3% glutaraldehyde.

Clasping pairs of Coullana canadensis were used to examine the assumption that uptake of radioactivity by copepods was from grazing on ^{14}C -labeled microorganisms (Carman, 1990). Briefly, clasping pairs (adult males clasping juvenile females as a precopulatory strategy) were removed from samples used in grazing studies, and males and females were teased apart and radioassayed separately. If radioactivity was taken up by copepods because of grazing on ^{14}C -labeled microbes, the expectation was that relatively little ^{14}C should be recovered in males, since they do not feed while clasping to females.

Grazing rates were expressed as the percentage of the sedimentary algal assemblage consumed by copepods over a five-hour 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).

2.3.5 Meiofaunal Physiological Condition

Neutral-lipid-storage products of Coullana canadensis and Pseudostenhalia 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-cm² core was collected and the top 1 cm of sediment extruded into a Whirl-pak bag. The bag was placed in a 60 x 15 mm Petri dish, which was sealed with tape and preserved in liquid nitrogen until analysis.

For analysis, individual samples were removed from liquid nitrogen and thawed. Meiofauna were sorted and stained in Nile red solution for 30 min then placed on a depression slide and viewed under epifluorescent illumination using a custom-made filter that optimizes the enhancement of lipid deposits in animals (525 nm excitation, 589 nm emission, 545 nm dichroic mirror). Images of individual organisms 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 copepod urosome.

2.3.6 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 (*e.g.*, nematodes, ostracods, polychaetes, etc.).

2.3.7 PAH Concentration

PAH were extracted from sediment samples as follows. A 4.9 cm² core of sediment was removed from each microcosm, and the top 1 cm of sediment extruded and collected in a glass screw-cap vial. Sediment was refrigerated (4°C) until extraction of PAH. For extraction, sediments were transferred to a tared beaker. A sub-sample of ~0.5 g was removed for dry-weight determination and the beaker reweighed to obtain the wet weight of the sample. 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 mins 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 and McMillin, 1993) and sulfur removed by activated copper.

Extracts were analyzed by GC/MS operated in the selected-ion-monitoring mode for enhanced sensitivity of the target analytes, utilizing the same temperature program as previously described (Means and McMillin, 1993) for the GC. The standard mixture was diluted to 1 ppm for calibration of the instrument. Injections into the GC were made by autosampler, and hexamethylbenzene was added to each extract to monitor instrument performance. Because of the new analytical methodology which we have developed and employed, we are able to make several independent estimates of concentrations for several positional isomers of the same compound (i.e. 10 dimethyl naphthalenes) having similar sorption constants and aqueous solubility.

A Hewlett-Packard 5890 Gas Chromatograph directly interfaced to a Hewlett-Packard 5970B Mass Selective Detector was operated using the parameters presented in Table 2.2. A series of linear temperature ramps was necessary for the maximal separation of isomers of alkylated PAH's and for separation of parent PAH compounds from alkylated PAH's with interfering ions, while keeping analysis time (60 min) and band-broadening to a minimum.

Table 2.3 lists the target analytes and deuterated reference standards, their abbreviations, and the mass fragments used for quantitative evaluation ("primary ion") and for confirmation of identity ("confirming ion"). Alkylated PAH standards were purchased from Chiron Laboratories A.S. (Norway). The standards represent the two isomers of methyl-naphthalene, all 10 of the dimethylnaphthalene isomers, isopropylnaphthalene and 1,6,7-trimethylnaphthalene for the class "C3" (three substituted carbon groups) naphthalenes, all four possible isomers of methyl-dibenzothiophene, 1,2-dimethyldibenzothiophene for the class "C2" dibenzothiophenes, five of five possible methylphenanthrenes, 16 of 25 isomers of dimethyl phenanthrene, and 1,2,8-trimethylphenanthrene for the class of "C3" phenanthrenes.

The chromatographic-analysis method was developed by first determining the retention order and mass spectra of all of the individual alkylated PAH isomers, as listed in Table 2.3, then analyzing a mixture of all the analytes together, with adjustment to the temperature ramps to achieve maximum resolution. "Optimal resolution" is a trade off between increased separation and band-broadening that occurs with increased time spent in the column. After what was considered the best possible resolution was achieved, some of the individual mixtures had to be reanalyzed to confirm that retention orders had not changed. This is also necessary when the GC column is replaced, but is not required for all individual components because of the identification power (specificity) of the mass spectrometer. Spectra of the individual isomers that were obtained initially can be used to distinguish some of the isomers from each other in the mixed standard.

Table 2.2 Gas Chromatography/Mass Spectrometry Instrument Parameters.

Heated Zones

Splitless Injection Port Temperature: 235 °C.
Purge Time: 0.5 min.
GC/MS Transfer line temperature: 280 °C

Gas Chromatography Column

Column: DB-5, 30 m, 0.25 µm film thickness,
0.25 mm I.D. J&W Scientific, Inc.
Carrier Gas: Helium
Linear velocity: 40 cm s⁻¹ (butane injection at 100 °C)

Gas Chromatograph Temperature Program

Initial Column Temperature: 50 °C
Time at Initial Temperature: 3 min.
Temperature Ramp: #1: 6 °C min⁻¹ to 120 °C
 #2: 3 °C min⁻¹ to 190 °C
 #3: 12 °C min⁻¹ to 280 °C
Time at Final Temperature: 14.5 min.

Mass Spectrometer Data Acquisition

Scan Range: 45-450 amu.
Scan Rate: 1.06 scans s⁻¹

Table 2.3 Target Analytes

Analyte	Abbreviation	Primary Ion	Confirming ion
Naphthalene	Naphthalene	128	129
2-Methylnaphthalene	2-MN	142	141
1-Methylnaphthalene	1-MN	142	141
2-Ethylnaphthalene	2-EN	156	141
1-Ethylnaphthalene	1-EN	156	141
2,6/2,7-Dimethylnaphthalene	2,6/2,7-DMN	156	141
1,3/1,7-Dimethylnaphthalene	1,3/1,7-DMN	156	141
1,6-Dimethylnaphthalene	1,6-DMN	156	141
1,4/2,3-Dimethylnaphthalene	1,4/2,3-DMN	156	141
1,5-Dimethylnaphthalene	1,5-DMN	156	141
Acenaphthylene	Acenaphthylene	152	153
1,2-Dimethylnaphthalene	1,2-DMN	156	141
2-Isopropylnaphthalene	2-IPN	170	155
1,8-Dimethylnaphthalene	1,8-DMN	156	141
Acenaphthene	Acenaphthene	153	154
Fluorene	Fluorene	166	165
Dibenzothiophene	Dibenzothiophene	184	185
Phenanthrene	Phenanthrene	178	179
Anthracene	Anthracene	178	179
4-Methyldibenzothiophene	4-MDBT	198	197
2/3-Methyldibenzothiophene	2/3-MDBT	198	197
1-Methyldibenzothiophene	1-MDBT	198	197
3-Methylphenanthrene	3-MP	192	191
2-Methylphenanthrene	2-MP	192	191
4/9-Methylphenanthrene	4/9-MP	192	191
1-Methylphenanthrene	1-MP	192	191
4,5-Dimethylphenanthrene	4,5-DMP	206	191
3,6-Dimethylphenanthrene	3,6-DMP	206	191
3,5-Dimethylphenanthrene	3,5-DMP	206	191
2,6-Dimethylphenanthrene	2,6-DMP	206	191
2,7-Dimethylphenanthrene	2,7-DMP	206	191
3,9-Dimethylphenanthrene	3,9-DMP	206	191
1,6/2,5/2,9-Dimethylphenanthrene	1,6/2,5/2,9-DMP	206	191
1,7-Dimethylphenanthrene	1,7-DMP	206	191
1,9/4,9-Dimethylphenanthrene	1,9/4,9-DMP	206	191
Fluoranthene	Fluoranthene	202	101
1,5-Dimethylphenanthrene	1,5-DMP	206	191
1,8-Dimethylphenanthrene	1,8-DMP	206	191
1,2-Dimethylphenanthrene	1,2-DMP	206	191
9,10-Dimethylphenanthrene	9,10-DMP	206	191
Pyrene	Pyrene	202	101
Benzo(a)anthracene	Benzo(a)anthracene	228	226
Chrysene	Chrysene	228	226
Benzo(b)fluoranthene	Benzo(b)fluor	252	253
Benzo(k)fluoranthene	Benzo(k)fluor	252	253
Benzo(a)pyrene	Benzo(a)pyrene	252	253
Indeno(1,2,3-cd)pyrene	Indenopyrene	276	278
Dibenz(a,h)anthracene	Dibenzanthracene	278	276
Benzo(g,h,i)perylene	Benzoperylene	276	278
Deuterated Internal/Surrogate Standards			
d8-Naphthalene	d8-Naph	136	
d10-Acenaphthene	d10-Ace	164	
d10-Phenanthrene	d10-Phen	188	
d12-Chrysene	d12-Chrys	240	
d12-Perylene	d12-Peryl	264	

Retention order of the alkylated PAH isomers was determined by preparing 10 ppm dilutions in hexane containing one of each type of isomer, i.e., a methylnaphthalene, a dimethylnaphthalene, a methylphenanthrene, a dimethyl-phenanthrene, etc. 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. HMB was also coinjected into the GC/MS with each sample aliquot to monitor performance of the instrument and injection technique, but was not used in the calculations of analyte final concentrations. Quantifications were made using the internal standard (I.S.) method based on the corresponding deuterated surrogate standard for each analyte.

Chromatographic data for parent and alkylated PAH were obtained from 20 daily calibration analyses of the standard mixture. In addition to the analytes listed, C4- and C5-naphthalenes, C3-dibenzothiophenes, and C3-phenanthrenes are estimated using relative response factors generated from similar class analytes represented by the standard mixture. For example, the C3-dibenzothiophenes were estimated using the relative response factor for C2-dibenzothiophenes, 1,2-dimethyldibenzothiophene. In this way, we were able to quantify additional compound classes without the considerable expense of these additional standards. C1-C3-fluorenes were estimated using the response factor from the parent compound.

Standard QA/QC procedures were followed, including analysis of duplicate and spiked samples, and daily tuning and calibration of the GC/MS system.

2.4 Statistical Analysis

Data were initially analyzed to determine if any significant blocking effects could be detected. None were, and thus we analyzed the data as a completely randomized, two-factor (Days and PAH dosage) ANOVA. This approach serves to increase the overall power of the ANOVA.

Data were tested for normality and homogeneity of variance (Bartlett's test), and appropriate transformations of the data were made when necessary. A posteriori comparisons were made using the Student-Newman-Keuls test.

Chapter 3. Results, Microcosm Experiment

ANOVA results for the various tests are summarized in Table 3.1. A posteriori comparisons are summarized in Table 3.2.

3.1 Bacterial Activity

Bacterial incorporation of ^{14}C -acetate into phospholipids (Fig. 3.1) was not significantly influenced by PAH additions to microcosms ($p = 0.136$). Incorporation of ^{14}C -acetate into phospholipids was reasonably constant through day 14, then increased at Days 21 and 28. These increases resulted in a significant influence of time on the incorporation of ^{14}C -acetate into phospholipids ($p < 0.001$). Day-28 values were significantly greater than those for all other days (Table 3.1), and Day 21 values were significantly greater than values for Days 0 through 14.

Bacterial physiological condition (Fig. 3.2) (as indicated from the ratio of incorporation of ^{14}C -acetate into phospholipids and PHB) was also not significantly influenced by PAH concentration at any time during the experiment. Again, there was an influence of time, with the phospholipid/PHB ratio at Day 1 being significantly higher than at all other days (Table 3.1). Day-3 ratios were also significantly higher than Day-28 ratios.

3.2 Microalgal Activity

Microalgal synthesis of phospholipid from ^{14}C -bicarbonate was significantly influenced by both time ($p < 0.001$) and PAH concentration (Fig. 3.3; $p < 0.001$). Incorporation of ^{14}C -bicarbonate into phospholipids was highest in the high-PAH treatments. ^{14}C -bicarbonate in phospholipids fluctuated over time, and was lowest at the beginning and end of the experiment (Day 0 and Day 28), and highest at the midpoint (Day 14).

Microalgal synthesis of neutral (storage) lipids, as determined from incorporation of ^{14}C -bicarbonate, was not influenced by PAH concentration (Fig. 3.4; $p = 0.210$). There was, however, a significant influence of time ($p < 0.001$), with a trend for increased synthesis of neutral lipids up to Day 14, followed by a slight decrease at Days 21 and 28.

The physiological condition of algae, as determined from the ratio of incorporation of ^{14}C -bicarbonate into phospholipids and neutral lipids, was significantly influenced by PAH concentration (Fig. 3.5; $p = 0.020$). Phospholipid/neutral-lipid ratios were significantly higher in high-PAH treatments than in medium-PAH or zero-PAH controls. 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). The influence of time was also significant ($p < 0.001$). The phospholipid/neutral-lipid ratio remained relatively constant through Day 7, then decreased significantly from Day 14 through Day 28.

3.2 Bacterial and Microalgal Abundance

Because of the large amount of time required to perform bacterial direct counts, we initially examined bacterial abundance for Days 0, 14, and 28 (Fig. 3.6). Although there were significant differences among Days ($p < 0.001$), no significant influence of PAH on abundance was detected ($p = 0.895$), and thus we did not perform direct counts on the remaining samples.

Microalgal biomass, as determined from sedimentary chlorophyll a was not significantly influenced by PAH concentration (Fig. 3.7; $p = 0.210$). There was significant variation among days ($p < 0.001$), but there was no clear trend over time. Chlorophyll a concentration at Days 21 and 7 were both significantly greater than at Days 28 and 1.

3.3 Meiofaunal Grazing

Grazing by Coullana canadensis (Fig. 3.8) and Pseudostenhalia wellsi (Fig. 3.9) followed the same general trend. PAH levels did not significantly influence grazing ($p = 0.525$ for C. canadensis, $p = 0.213$ for P. wellsi), but there was a significant reduction in grazing rate over time ($p < 0.001$ for both species). Grazing rates on Day 0 were higher than on all other days. Grazing rates on Day 7 were significantly less on those on Days 1 or 3, and remained relatively low for the remainder of the study.

Uptake of ^{14}C from ^{14}C -bicarbonate by male C. canadensis of clasping pairs was significantly less than by females (Fig. 3.10; $p < 0.001$; $n = 5$); only $7.3\% \pm 2.3\%$ (1 s.d.) of ^{14}C in pairs was present in males. In contrast, from cores that had been incubated with ^{14}C -acetate, $47.9\% \pm 19.2\%$ of ^{14}C in clasping pairs was recovered in males, which was not significantly different from the amount of radioactivity recovered in females (Fig. 3.10; $p = 0.679$; $n = 5$).

3.5 Meiofaunal Physiological Condition

PAH concentration had no significant influence on lipid-storage material for either Coullana canadensis (Fig. 3.11; $p = 0.268$) or Pseudostenhalia wellsi (Fig. 3.12; $p = 0.527$). Only Days 0 through 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. It should be noted that sorting of animals from sediments used for Nile-red analysis was

Bacterial synthesis of phospholipids

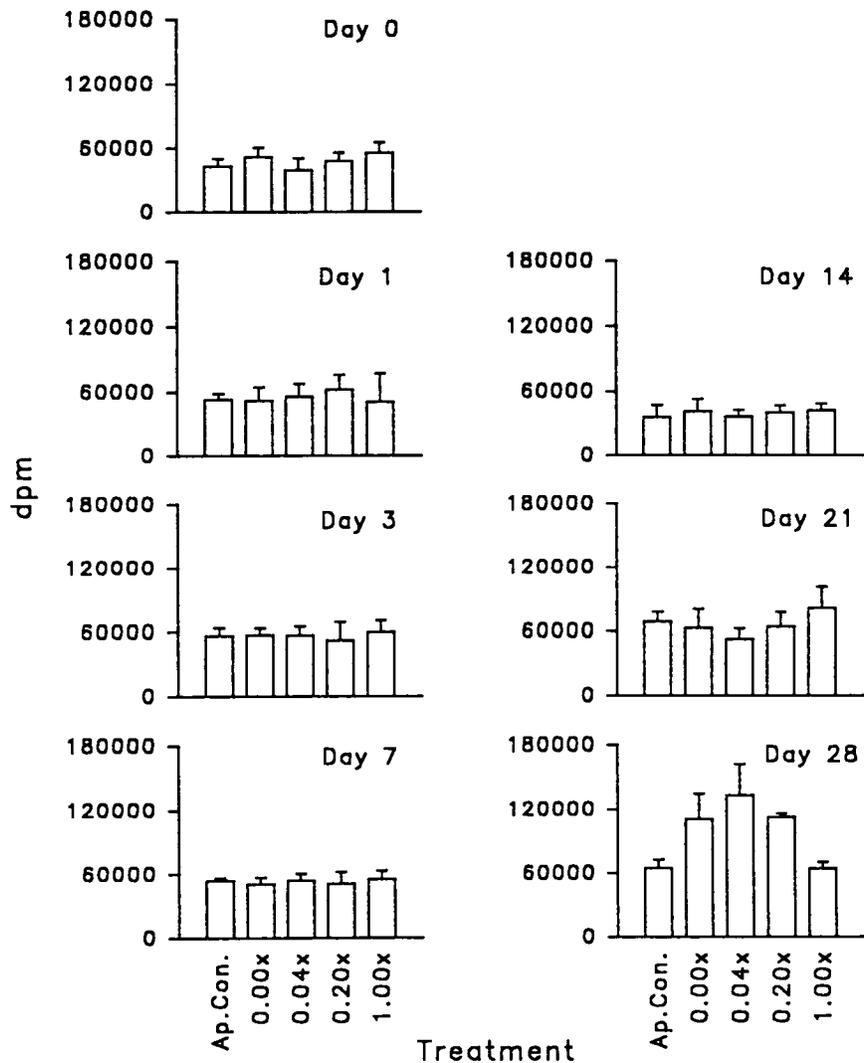


Figure 3.1 Bacterial synthesis of phospholipids. Results from each day (Day 0 through Day 28) are shown separately in each of the seven graphs. Y-axis is radioactivity (dpm) recovered in the phospholipid fraction of sediments incubated with ^{14}C -acetate. The x-axis depicts various treatment levels: 1.00x = addition of undiluted Pass Fourchon sediment; 0.20x and 0.04x = addition of Pass Fourchon sediment diluted with uncontaminated sediment (see text); 0.00x = addition of uncontaminated sediment; Ap.Con. = "Application Control", in which no sediment was added to the microcosm. Bars are mean ($n = 4$), ± 1 s.d.

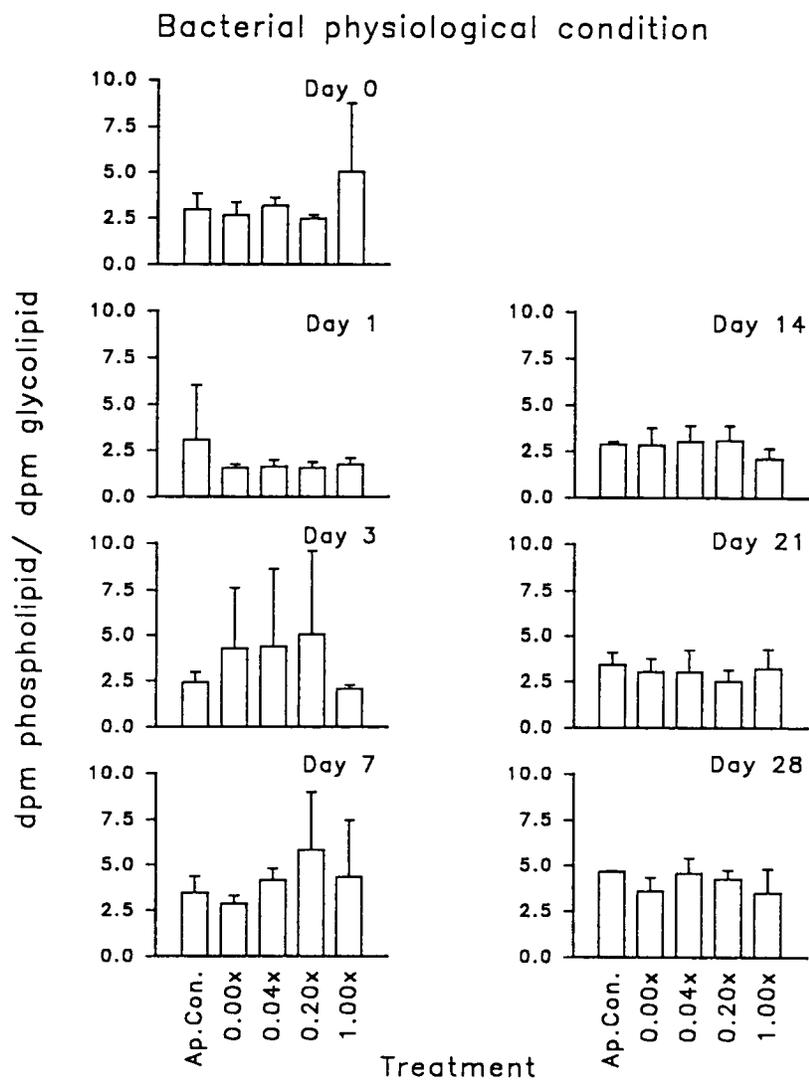


Figure 3.2 Bacterial physiological condition. Physiological condition was based on the ratio of ^{14}C -acetate incorporation into phospholipids and glycolipids (which contain PHB). See Figure 3.1 for further details.

Algal synthesis of phospholipids

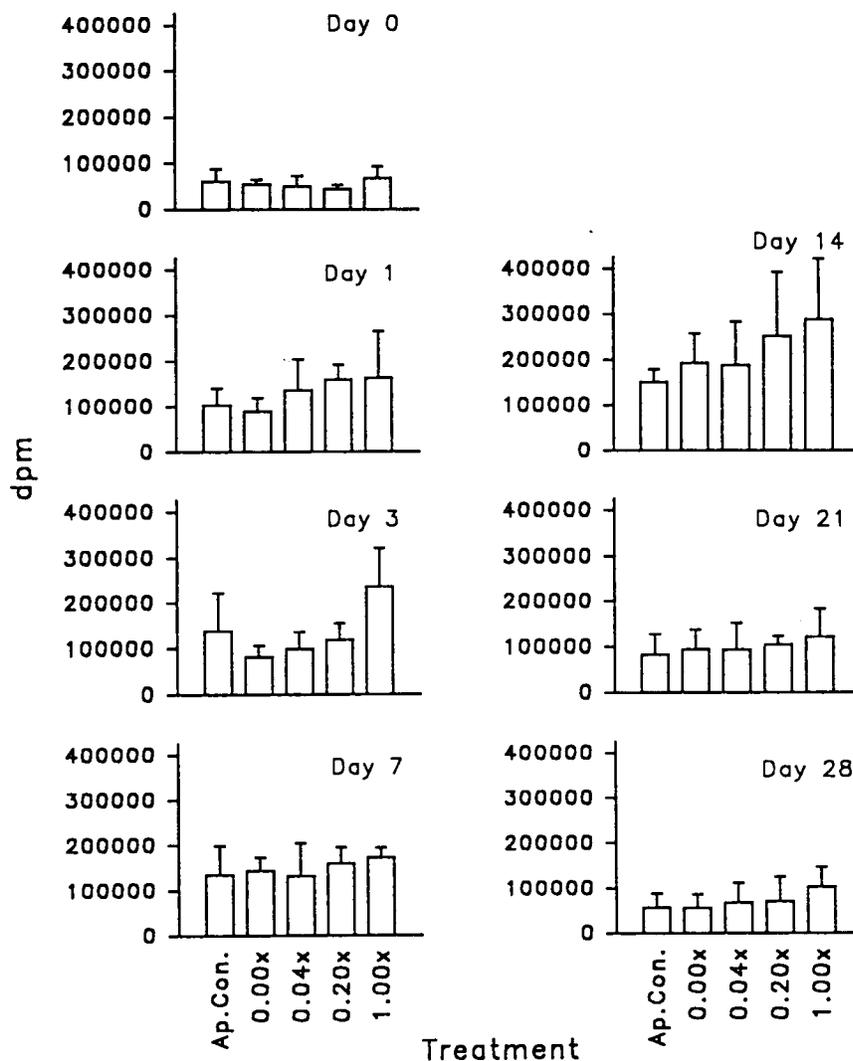


Figure 3.3 Algal synthesis of phospholipids. Values determined from photosynthetic fixation of ^{14}C -bicarbonate. See Figure 3.1 for further details.

Algal synthesis of storage lipids

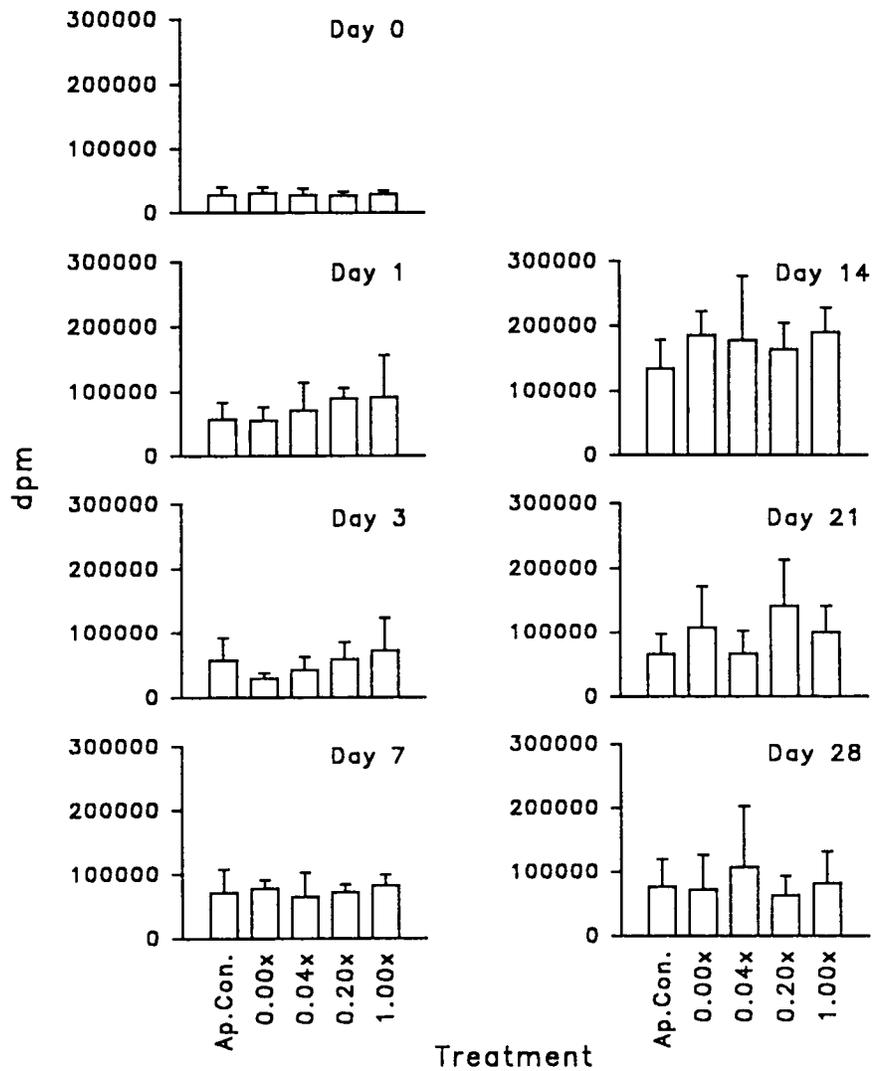


Figure 3.4 Algal synthesis of storage lipids. Values determined from photosynthetic fixation of ^{14}C -bicarbonate into neutral lipid. See Figure 3.1 for further details.

Algal physiological condition

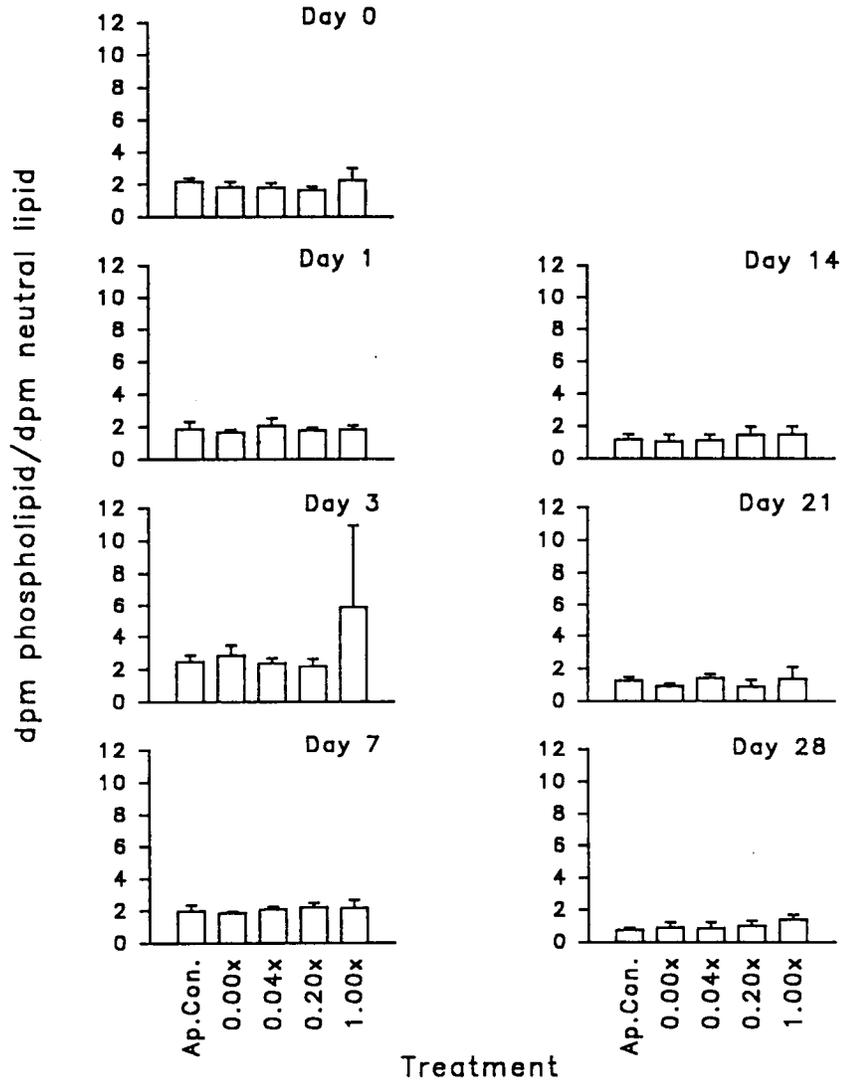


Figure 3.5 Algal physiological condition. Condition determined from the ratio of ^{14}C -bicarbonate incorporation into phospholipids and neutral lipids. See Figure 3.1 for further details.

Table 3.1 Summary of ANOVA results, microcosm experiment. 'PAH' and 'Day' are main effects, and 'PAH*Day' is the interaction term. Columns 'Max' and 'Min' designate the PAH treatments with the highest and lowest average values observed for that particular test: 'High' = high PAH, "Med" = medium PAH, "Low" = low PAH, "Cont" = control (no addition or application control; see text for details).

Measurement	p-value				
	PAH	Day	PAH*Day	Max	Min
<u>C.canadensis</u> lipid	0.117	< 0.001	0.220	Cont	Cont
<u>P.wellsi</u> lipid	0.527	< 0.001	0.315	Low	Cont
<u>C.canadensis</u> graze	0.526	< 0.001	0.772	Cont	High
<u>P.wellsi</u> graze [#]	0.327	< 0.001	0.918	Low	High
Algal Phospholipid	<0.001	< 0.001	0.981	High	Low
Algal Neutral lipid	0.210	< 0.001	0.992	High	Low
Algal Phosph/Neut	0.020	< 0.001	0.607	High	Cont
Chlorophyl <u>a</u>	0.210	< 0.001	0.871	High	Cont
Bacterial Phospholipid	0.136	< 0.001	<0.001	Med	Cont
Bacterial Phosph/Glyco	0.598	< 0.001	0.440	Cont	High
Bacterial abundance	0.895	< 0.001	0.701	Cont	Med
Copepods	0.719	< 0.001	0.194	Low	High
Nematodes	0.042	0.282	0.989	High	Cont
Nauplii	0.070	< 0.001	0.824	High	Cont
Total Meiofauna	0.100	< 0.001	0.806	High	Cont
Nematode/Copepod	0.017	< 0.001	0.989	High	Cont
Nauplius/Copepod	0.003	< 0.001	0.689	High	Cont

[#] Includes data from Days 0-14 only.

Table 3.2 A posteriori tests, microcosm experiment. Treatment levels connected by a solid line are not significantly different (Student-Neuman-Keuls a posteriori tests; experiment-wide p-value < 0.05).

Bacterial Physiological Condition

Day						
28	7	0	21	3	14	1
_____			_____			_____

Bacterial Phospholipids

Day						
28	21	3	1	7	0	14
_____	_____	_____				_____

Bacterial abundance

Day		
28	0	14
_____	_____	_____

Microalgal Physiological Condition

Day						
3	7	0	1	14	21	28
_____	_____			_____		_____

PAH					
High		Low		Ap.Con.	Med. Zero
_____	_____			_____	

Microalgal Phospholipids

Day						
14	7	3	1	21	28	0
_____	_____			_____		_____

PAH					
High		Medium		Ap.Con.	Low Zero
_____	_____			_____	

Microalgal Storage Lipids

Day						
14	21	7	1	28	3	0
_____	_____			_____		_____

Table 3.2 Results of Student-Neuman-Keuls a posteriori tests (continued). Treatment levels connected by a solid line are not significantly different (experiment-wide p-value < 0.05).

Chlorophyll a

Day
21 7 14 3 0 28 1

Coullana canadensis Lipid Reserves

Day
14 0 21 1 7 3 28

Pseudostenhalia wellsi Lipid Reserves

Day
0 1 3 7

Coullana canadensis Grazing

Day
0 1 3 21 28 7 14

Pseudostenhalia wellsi Grazing

Day
0 3 1 28 7 21 14

PAH Concentration in Sediments

Day
1 3 0 21 7 14 28

PAH
High Medium Low Zero Ap.Con.

Total Meiofauna

Day
0 1 3 7 28 21 14

Table 3.2 Results of Student-Neuman-Keuls a posteriori tests (continued). Treatment levels connected by a solid line are not significantly different (experiment-wide p-value < 0.05).

Total Copepods

Day
1 3 0 7 14 21 28

Total Nematodes

PAH
High Low Medium Zero Ap.Con.

Nauplii

Day
0 1 3 7 14 28 21

Nematode/Copepod Ratio

Days
28 21 14 7 0 3 1

PAH
High Medium Low Zero Ap.Con.

Nauplius/Copepod Ratio

Day
0 1 3 7 14 28 21

PAH concentration

High Low Ap.Con. Medium Zero

Table 3.2 Results of Student-Neuman-Keuls a posteriori tests (continued). Treatment levels connected by a solid line are not significantly different (experiment-wide p-value < 0.05).

<u>PAH concentration*</u>				
High	Low	Ap.Con.	Zero	Medium
_____			_____	

* Results of ANOVA after Day-0 data removed

not 100% efficient, as Rose Bengal was not used as a sorting aid. Thus, the absence of values for P. wellsi from Day 14 on, is a reflection of the reduced number of gravid females, but it should not be taken to mean that no P. wellsi were present. Indeed, P. wellsi was found in most samples from Day 14 on in the grazing experiment (which were sorted with aid of Rose Bengal).

Lipid-storage material was highest at Day 0, then generally declined over time in C. canadensis. The major exception to this trend was an increase in lipids at Day 14. Storage lipids in P. wellsi at Day 0 were significantly greater than at Days 1 through 7 (Table 3.1).

3.6 Meiofaunal Community Composition

Total initial (Day 0) average meiofaunal abundance ranged from 2100 to 3400 individuals 10 cm². 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. PAH concentration did not significantly influence total meiofaunal abundance (Fig. 3.13; $p = 0.100$). There was a statistically significant trend toward a reduction in total meiofaunal abundance over time (Table 3.1; $p < 0.001$). From Day 0 to Day 28, meiofaunal abundance decreased by approximately a factor of two.

Copepod abundance was also not significantly influenced by PAH concentration (Fig 3.14; $p = 0.878$), but decreased significantly ($p < 0.001$) over time following the same general pattern as total meiofauna. From Day 0 to Day 28, copepod abundance decreased by approximately a factor of 2.8.

When considering abundances of the two dominant copepod species, the decrease in abundance over time was less obvious. For example, C. canadensis abundance actually increased (though nonsignificantly) in control microcosms over the duration of the experiment, while C. canadensis in high-PAH microcosms generally declined over time (Fig. 3.15). P. wellsi abundance, on the other hand, declined steadily over time in control microcosms, but remained remarkably constant in high-PAH microcosms (Fig. 3.16).

The influence of PAH on the abundance of copepod nauplii was marginally significant ($p = 0.070$), but nauplii were reduced by a factor of over 10 over the course of the experiment ($p < 0.001$; Fig. 3.17).

The nauplius:copepod ("copepod" includes copepodites and adults) ratio was significantly influenced by both PAH concentration ($p = 0.003$) and time ($p < 0.001$; Fig. 3.18). There

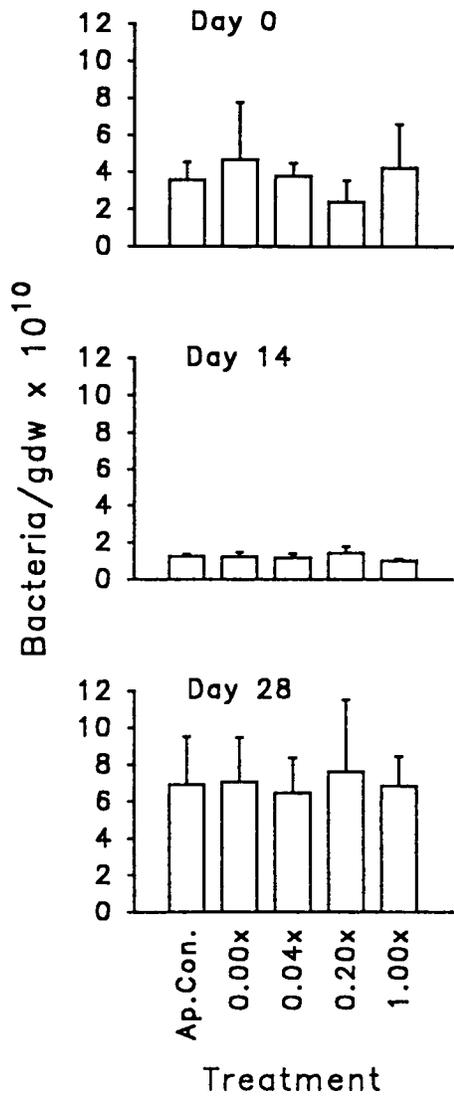


Figure 3.6 Abundance of sedimentary bacteria. "gdw" = gram dry weight. See Figure 3.1 for further details.

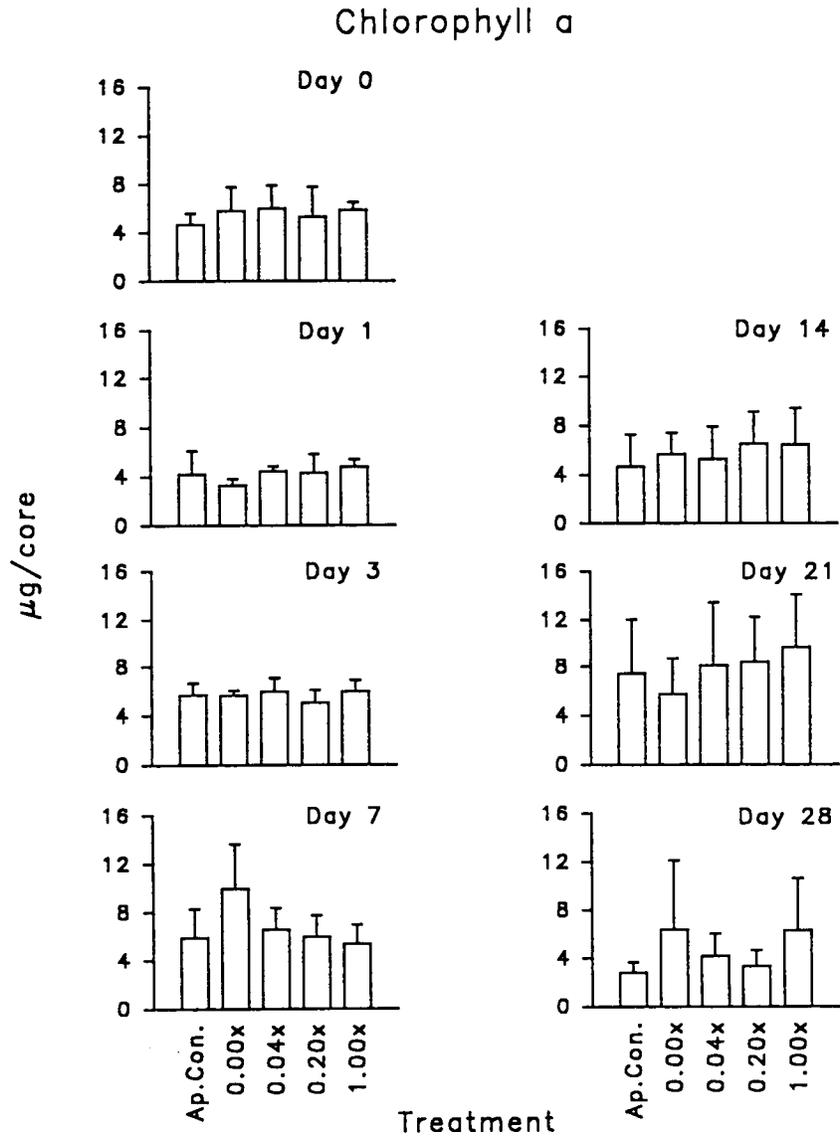


Figure 3.7 Chlorophyll a concentration. Data are μg Chl a in the top 1 cm of core with 2.3 cm^2 surface area. See Figure 3.1 for further details.

Coullana canadensis

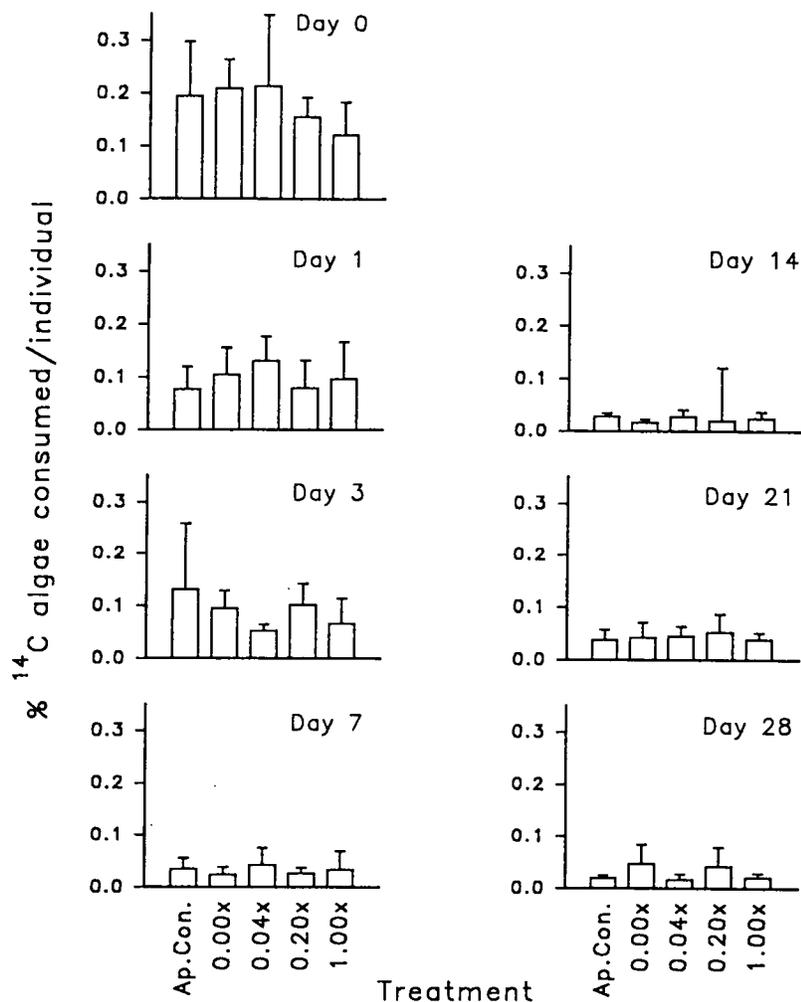


Figure 3.8 Grazing by *Coullana canadensis*. Data are expressed as the percentage of the microalgal standing stock consumed by each copepod over a five-hour period. See Figure 3.1 for further details.

Pseudostenhalia wellsi

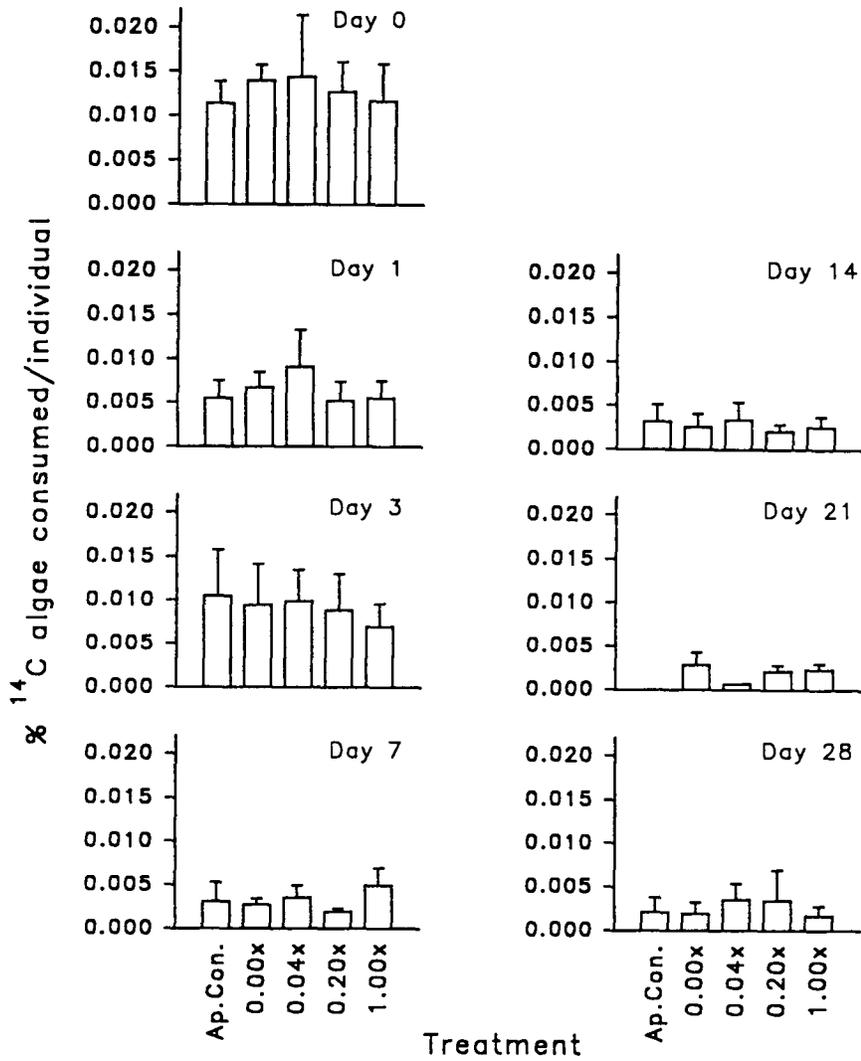
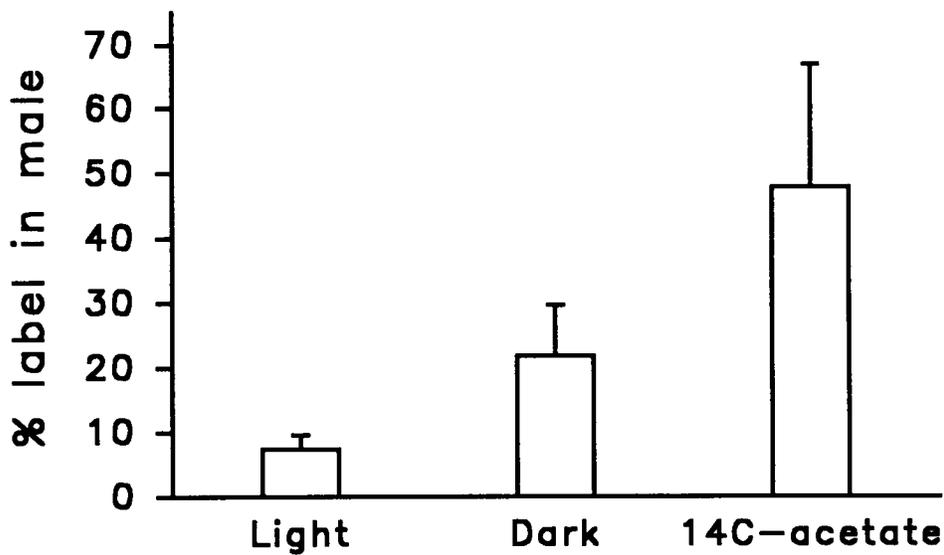
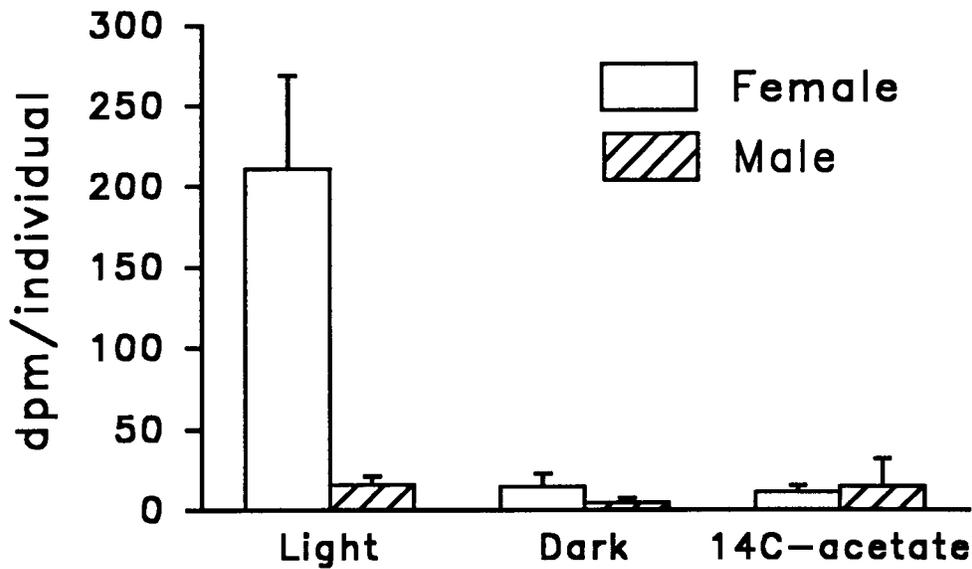


Figure 3.9 Grazing by *Pseudostenhalia wellsi*. Data are expressed as the percentage of the microalgal standing stock consumed by each copepod over a five-hour period. See Figure 3.1 for further details.



Treatment

Figure 3.10 ^{14}C in clasping pairs of *Coullana canadensis*. "Light" = copepods from cores injected with ^{14}C -bicarbonate and incubated in the light. "Dark" = copepods from cores injected with ^{14}C -bicarbonate and incubated in the dark. " ^{14}C -acetate" = copepods from cores injected with ^{14}C -acetate. See text for further details on grazing studies. Top panel is radioactivity per individual for both males and females from clasping pairs. The bottom panel is the percentage of radioactivity in the clasping pair that was recovered in the male.

Coullana canadensis

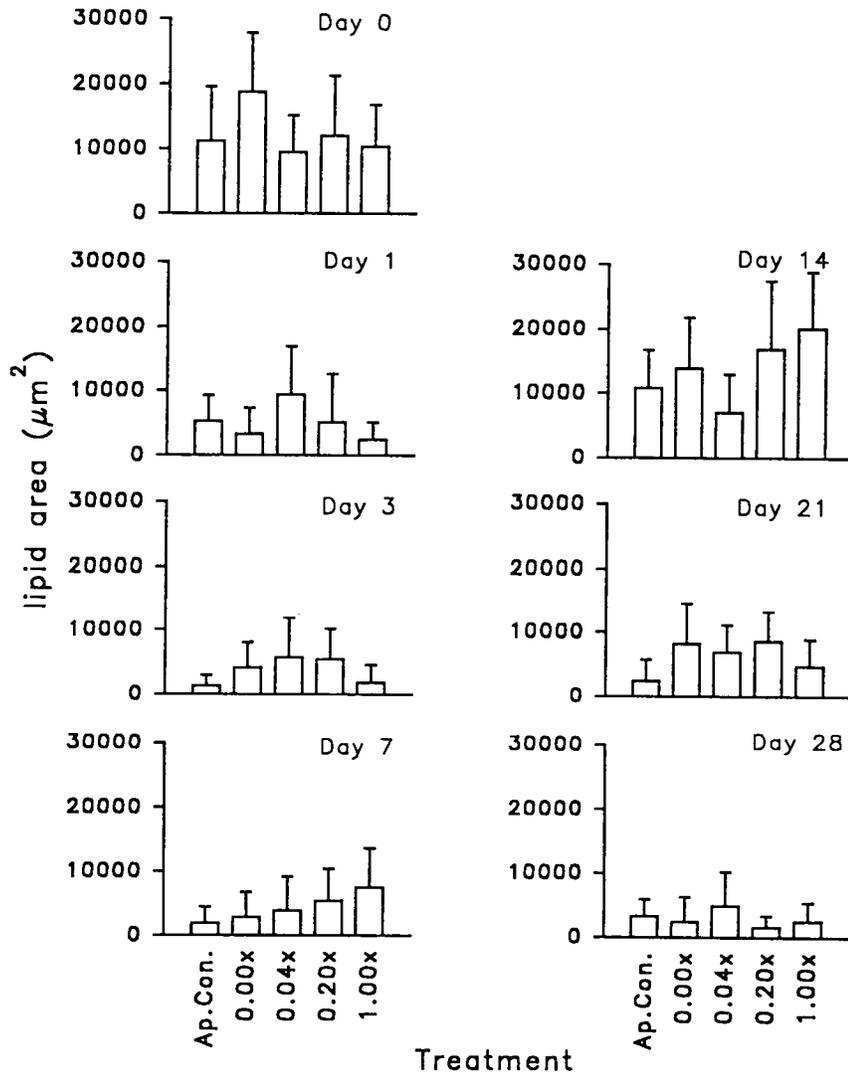


Figure 3.11 Lipid-storage material in *Coullana canadensis*. Lipid-storage material measured in ovigerous females using Nile-red fluorescence. Data are expressed as the urosomal area (μm^2) occupied by lipid droplets. Bars are the averages of copepods from three cores. See Figure 3.1 for further details.

Pseudostenhalia wellsi

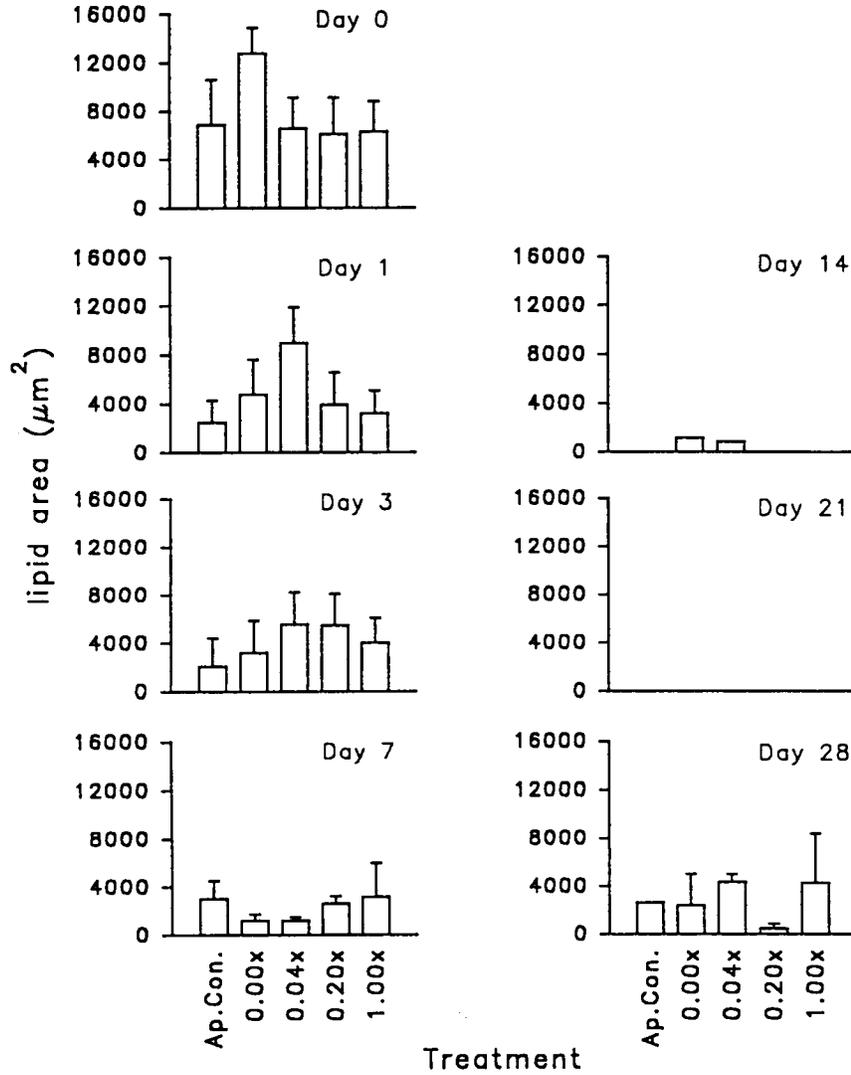


Figure 3.12 Lipid-storage material in *Pseudostenhalia wellsi*. Lipid-storage material measured in ovigerous females using Nile-red fluorescence. Relatively few ovigerous females were found from Day 14 on, and thus several values are either missing, or based on fewer than three replicates. See Figure 3.11 for further details.

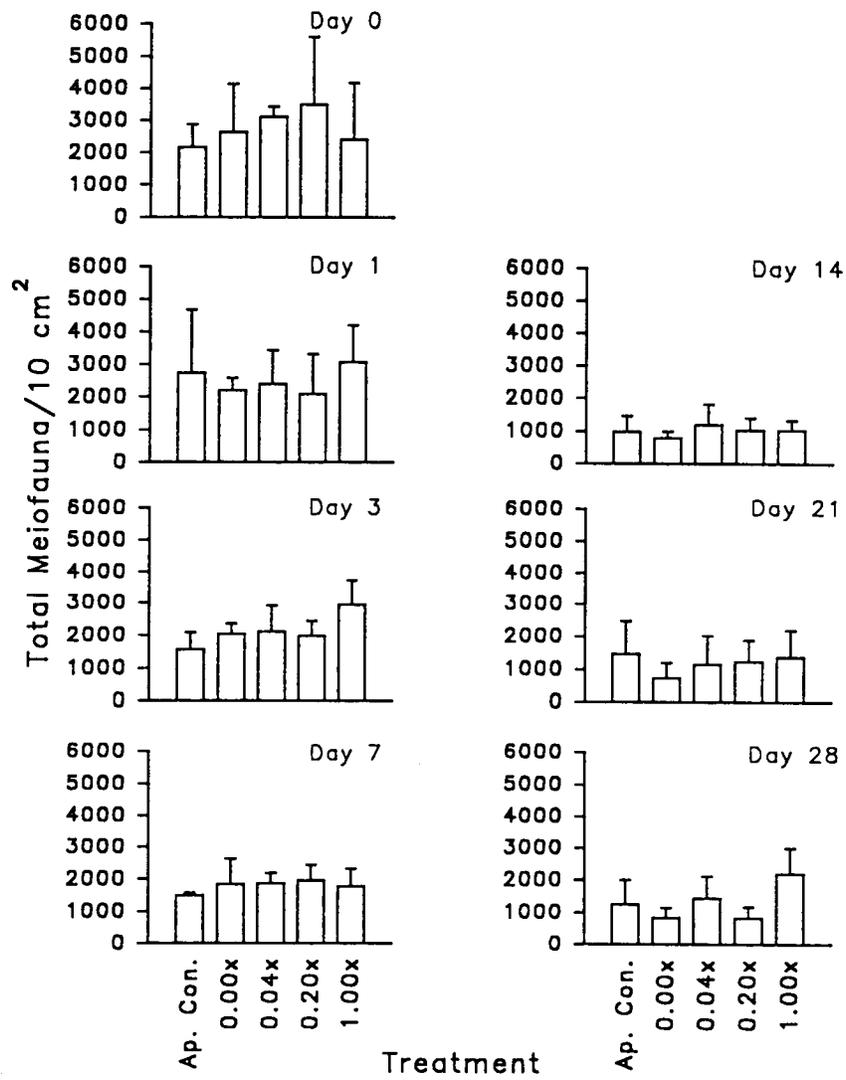


Figure 3.13 Total meiofaunal abundance. See Figure 3.1 for further details.

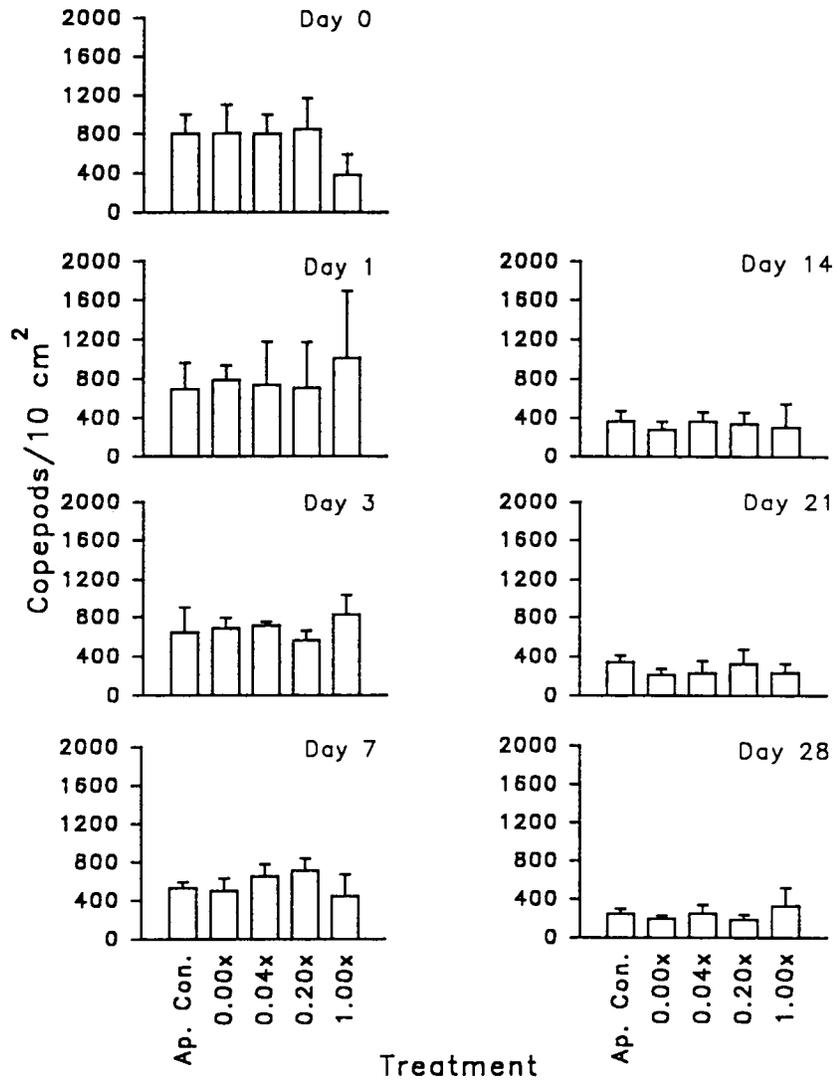


Figure 3.14 Total copepod abundance. Values include adults and copepodites (nauplii not included). See Figure 3.1 for further details.

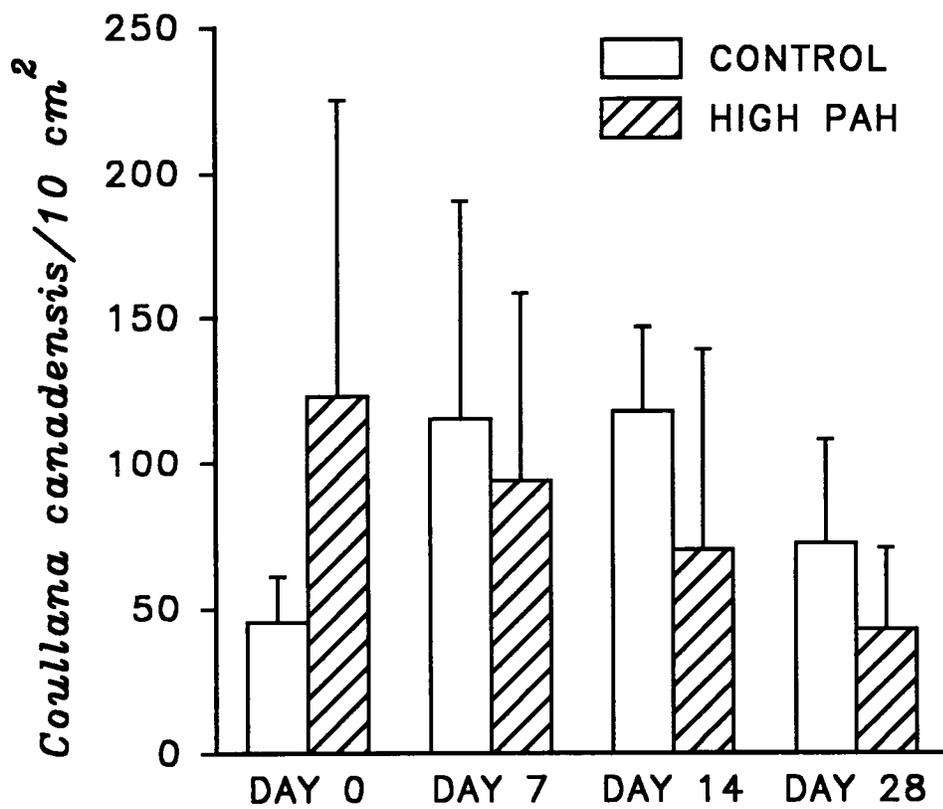


Figure 3.15 Abundance of *Coullana canadensis*. Data are from high-PAH and control microcosms (no PAH added) at the weekly sampling dates. Values include adults and copepodites (nauplii not included). Bars are means \pm 1 s.d. (n = 4).

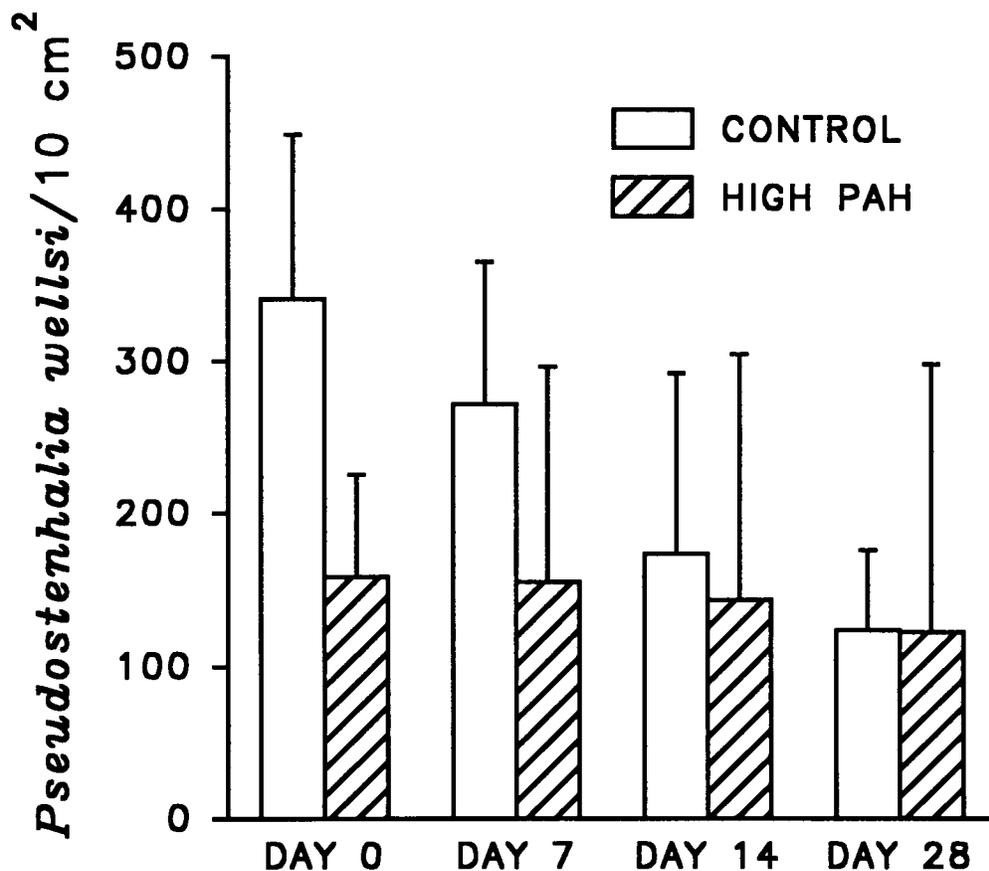


Figure 3.16 Abundance of *Pseudostenhalia wellsi*. Data are from high-PAH and control cores (no PAH added) at the weekly sampling dates. Values include adults and copepodites (nauplii not included). Bars are means \pm 1 s.d. (n = 4).

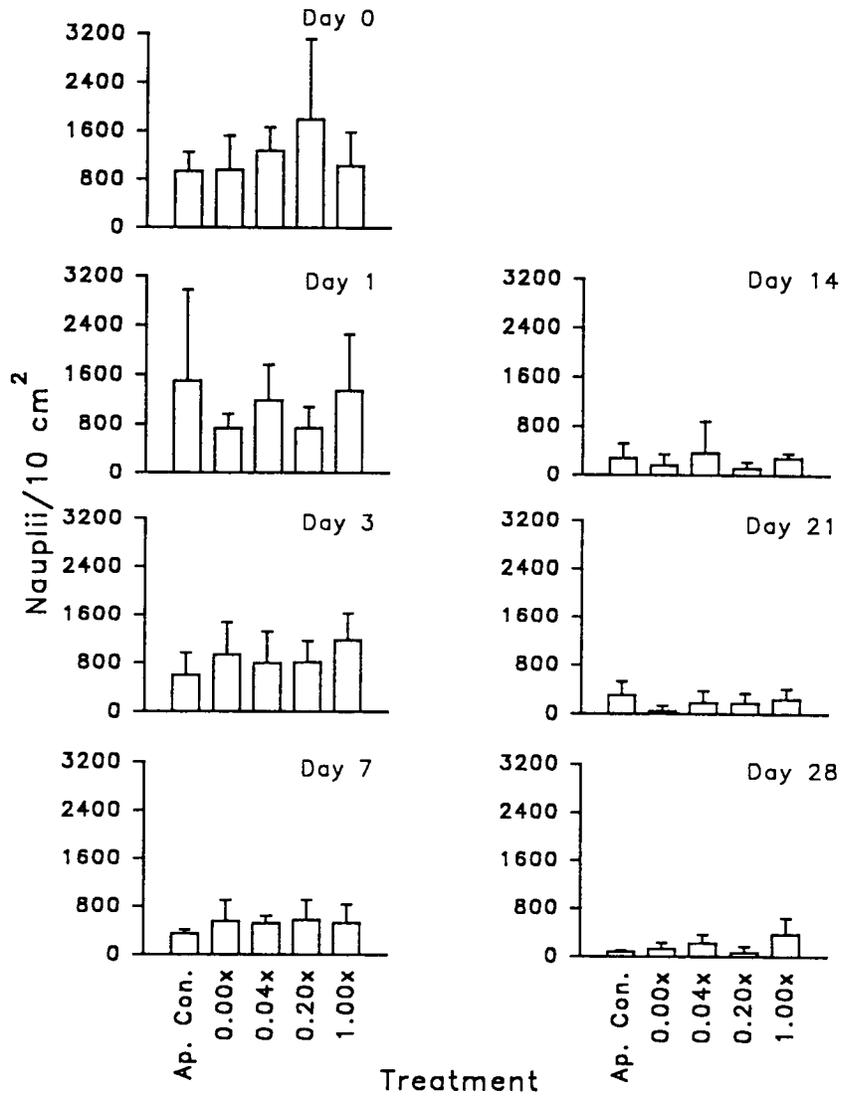


Figure 3.17 Total abundance of copepod nauplii. See Figure 3.1 for further details.

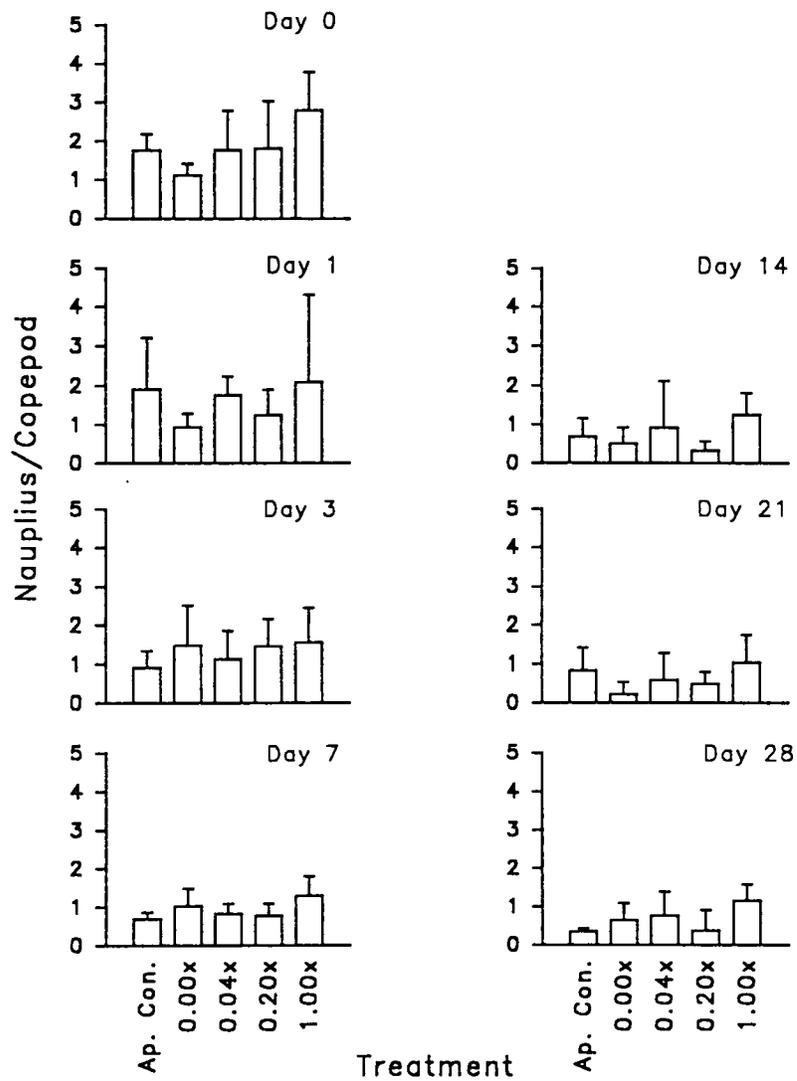


Figure 3.18 The nauplius/copepod ratio of abundance. See Figure 3.1 for further details.

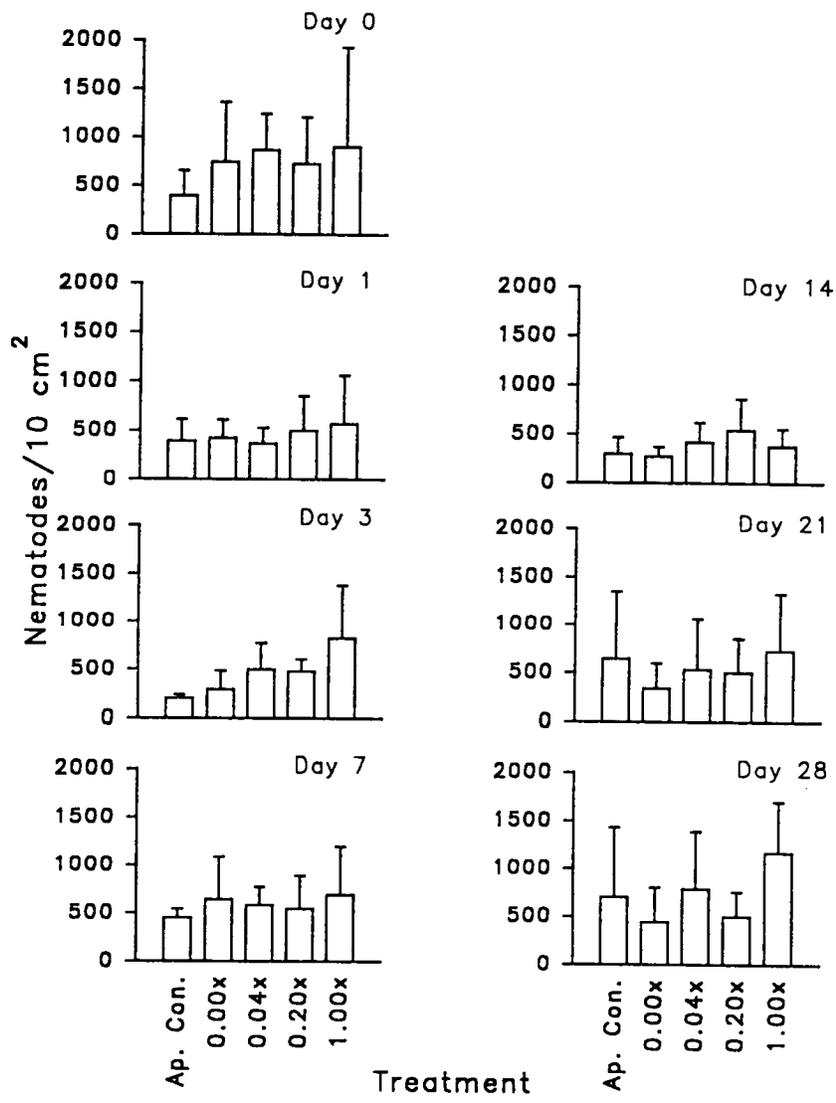


Figure 3.19 Total abundance of nematodes. See Figure 3.1 for further details.

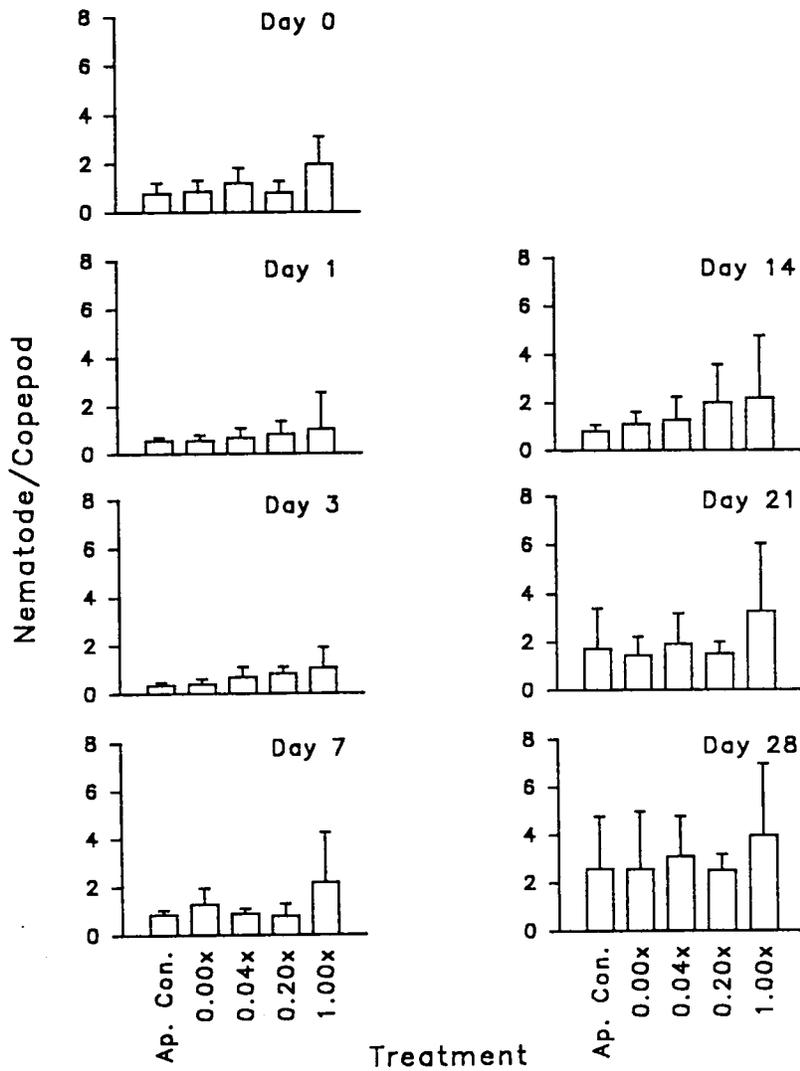


Figure 3.20 The nematode/copepod ratio of abundance. See Figure 3.1 for further details.

was no significant interaction between Days and PAH treatment ($p = 0.689$). The effect of PAH remained significant ($p = 0.012$) when data from Day 0 were removed from the analysis. The nauplius:copepod ratio was significantly higher in high-PAH microcosms than in all other treatment levels. The temporal trend was one of a decreasing nauplius:copepod ratio over time; Day-0 through Day-3 ratios were significantly higher than Day-14 through Day-28 ratios (Table 3.2).

Nematode abundance was significantly influenced by PAH concentration ($p = 0.042$) but did not vary significantly over time ($p = 0.282$; Fig. 3.19). Although the overall effect of PAH concentration on nematode abundance was significant, a posteriori comparisons did not reveal any significant differences among PAH-treatment levels (Table 3.2). Such a result is possible because the Student-Newman-Keuls a posteriori comparison is 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 ratio of nematode abundance to copepod abundance was significantly influenced by PAH concentration ($p = 0.017$) and time ($p < 0.001$; Fig. 3.20). A posteriori comparisons revealed that the nematode/copepod ratio was significantly higher in high-PAH treatments than in controls, and was significantly higher during the last two weeks of the experiment than at earlier sampling periods (Table 3.2).

3.7 Sedimentary PAH

PAH concentrations in the two types of control microcosms ranged from 210 to 445 ppb (Fig. 3.21), and did not differ significantly from each other or change significantly in concentration over time. The "high" dose treatments resulted in an average PAH concentrations of 2870 ppb at Day 0. As shown in Table 3.3, the desired gradient of PAH concentrations in microcosms was generally achieved. High-dose treatments remained relatively constant through Day 7, then decreased to a concentration of approximately 1000 ppb by Day 28.

Raw data from the GC/MS analyses, including concentration of individual PAH, are presented in Appendix I.

PAH in sediment

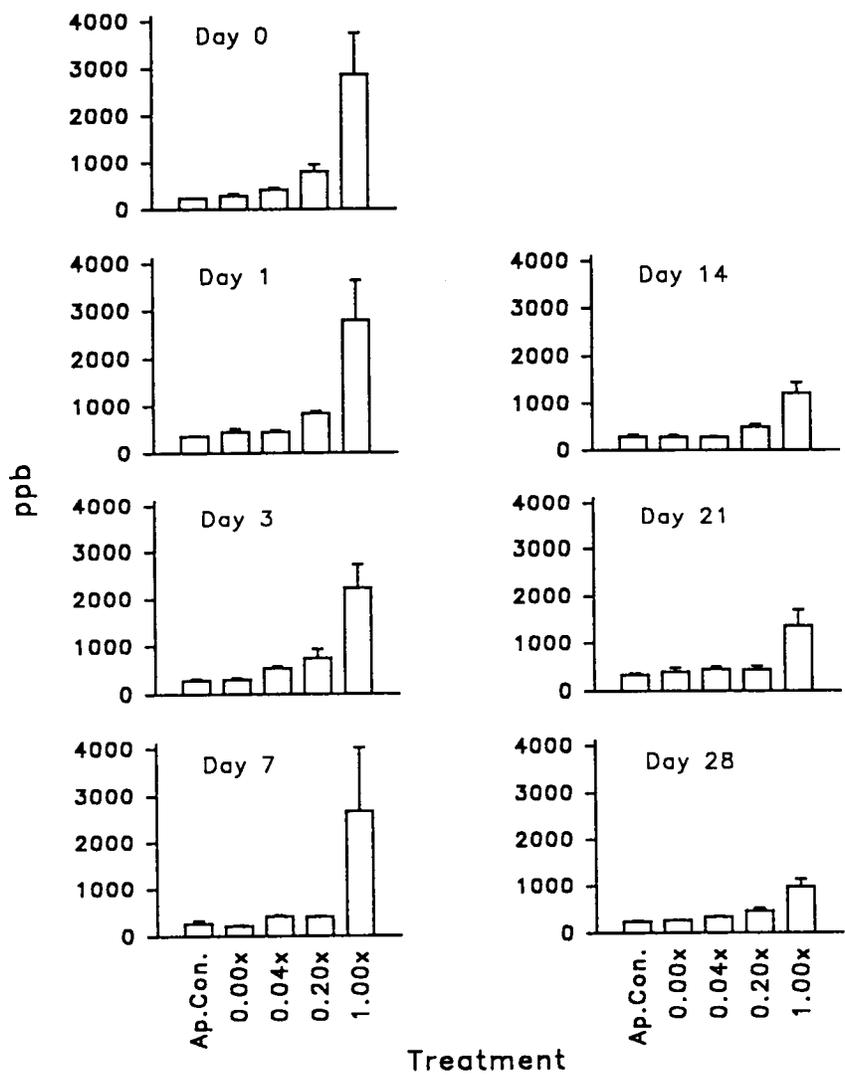


Figure 3.21 Total PAH concentration in microcosm sediments. These values represent PAH concentrations in the top 1 cm of microcosm sediment after dosing (see Material and Methods). Data are expressed ppb (dry weight). See Figure 3.1 for further details.

Table 3.3 Gradient of PAH concentrations. Values are for Day-0 microcosm sediments.

Treatment	ΣPAH (ppb)	Fraction of 1.00x treatment	Fraction of 1.00x after subtraction of ΣPAH in control microcosm
No addition	239	0.08	-
Ap. Cont.	289	0.10	0.01
0.04x	419	0.15	0.07
0.20x	796	0.28	0.21
1.00x	2872	1.00	1.00

Chapter 4. Discussion, Microcosm Experiment

We observed a significant influence of time on most of the parameters measured. The general trend was that data from early time points differed significantly from later time points, with the effect becoming most obvious at Day 14. The significant influence of time suggests that "containment" effects may have been associated with the microcosms. These containment effects may have been from hypoxia in microcosms. Although we did not measure O₂ in microcosm water or sediments, we suspect that the flow of water through the Nitex mesh covering the windows on microcosms was insufficient to keep the microcosms adequately oxygenated. In future studies, we will modify our design to achieve better oxygenation. In spite of these microcosm effects, the statistical design allowed us to factor out the influence of time and explicitly test for the influence of PAH concentration on the sedimentary food web.

The influence of PAH varied among the different microbial and meiofaunal components of the sedimentary food web. For example, PAH had no significant influence on copepod grazing, lipid-storage products, bacterial activity, or bacterial biomass. PAH concentration did significantly influence various aspects of microalgal activity and meiofaunal-community composition. In all five cases where significant ($p < 0.05$) effects of PAH were observed, the average value for high-PAH treatments was an extreme, *i.e.*, a maximum or a minimum value (Table 3.1). Overall, the high-PAH treatments yielded extreme values in 13 out of the 17 tests performed (Table 3.1).

Previous studies have shown that petroleum hydrocarbons may enhance the abundance (Bunch, 1987) or alter the activity (Bauer and Capone, 1985) of sedimentary bacteria. Data from direct counts of bacteria did not indicate a significant influence of PAH on bacterial abundance. The assay that we used to monitor bacterial activity, the relative incorporation of ¹⁴C-acetate into bacterial storage products (PHB) and phospholipids has been used as a general indicator of disturbance to marine benthic bacterial communities (Dobbs *et al.*, 1989; Thistle *et al.*, 1984; Findlay and White, 1987). Stimulation of bacterial growth should lead to relatively higher synthesis of phospholipids (for membrane synthesis), while conditions that are limiting to bacterial growth should result in relatively greater synthesis of storage material (PHB). We observed no significant influence of PAH on the phospholipid/PHB ratio of incorporation of ¹⁴C-acetate. Thus, we conclude that PAH concentration neither stimulated nor inhibited overall bacterial growth or physiological condition.

There was evidence that PAH concentration influenced sedimentary microalgae. Incorporation of ¹⁴C-bicarbonate into both phospholipid and neutral lipid (storage material of

eukaryotic algae such as diatoms) was highest in the high-PAH treatments, although the effect was significant only for phospholipids. Using the same rationale as that used in interpreting phospholipid/PHB ratio in bacteria, the relative incorporation of ^{14}C -bicarbonate into algal phospholipids and storage products (neutral lipids) can be used as an index of the physiological condition of eukaryotic algae (Guckert *et al.*, 1992). We observed that the phospholipid/neutral-lipid ratio of incorporation of ^{14}C -bicarbonate was highest in the high-PAH treatments, suggesting that algal growth was stimulated in the high-PAH treatment. This conclusion is supported by the observation that Chl a, a general indicator of algal biomass, was higher (though not significantly) in high-PAH treatments. These observations are generally consistent with previous studies that have shown that microalgal growth may be stimulated by relatively low additions of petroleum hydrocarbons (Kuiper *et al.*, 1984; Chan and Chiu, 1985).

Changes in the metabolic status of sedimentary microalgae could be perceived by benthic fauna as a qualitative, as well as quantitative change in resource availability. This impact is potentially significant as there is evidence that co-occurring meiofaunal species are selective in the types of food that they ingest (Carman and Thistle, 1985). Overall, however, there was little evidence for a direct effect of PAH on feeding by adult copepods. Neither grazing nor lipid-storage products were significantly influenced by PAH concentration in adult females of either Coullana canadensis or Pseudostenhalia wellsi. There are few data with which we can compare our results, as our study is the first to determine the influence of PAH (or any other contaminant) on grazing activity under near-natural conditions. A laboratory experiment, however, showed that consumption of algae by Tigriopus brevicornis (a harpacticoid copepod) decreased when exposed to aromatic hydrocarbons (Coull and Chandler, 1992).

Total copepod abundance was also not influenced by PAH, but there was a highly significant influence of PAH on the nauplius/copepod ratio, with the high-PAH treatments having a significantly higher ratio than all other PAH treatments. The high nauplius/copepod ratios were apparently at least partially the result of higher abundance of nauplii in high-PAH treatments (the PAH influence was marginally significant; $p < 0.070$; Table 3.1). 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. First, if copepods experience mortality as the result of their inability to molt, the abundance of more advanced stages of development (such as copepodites and adults) would decrease at higher rate than the

abundance of earlier developmental stages, and thus the nauplius/copepod ratio would increase. Second, 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 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 Pseudostenhalia wells or Coullana canadensis (Lotufo and Fleeger, in preparation). It should also be noted however, that toxicity tests with these sediments have indicated that 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.

Nematode abundance was significantly influenced by PAH concentration, with highest abundances of nematodes occurring in high-PAH treatments. This increase in nematode abundance in high-PAH treatments, combined with a (nonsignificant) decrease in copepod abundance in high-PAH treatments, resulted in a significant influence of PAH on the nematode/copepod (N/C) ratio. The N/C ratio was originally proposed by Raffaelli and Mason (1981) as a field indicator of pollution, with higher N/C ratios expected under polluted conditions. As noted by Coull and Chandler (1992) in their recent review, this index of pollution is controversial, and has fallen in and out of favor since its introduction. Currently, it is recommended that only interstitial copepods be included in the N/C ratio, limiting the applicability of this index to sandy habitats. We are unaware of previous attempts to apply this index to microcosm studies such as ours. Nevertheless, PAH concentration significantly influenced the N/C ratio, and resulted in N/C ratios that are consistent with the general theory behind the index. That is, 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 and Kontogiannis, 1975; see review by Coull and Chandler, 1992).

Colonization studies of oiled sediment (Decker and Fleeger, 1984; Spies et al., 1988) reveal rapid recolonization especially in low concentrations of PAH or other pollutants. Previous Louisiana field studies have indicated that meiofaunal community structure is relatively insensitive to petroleum hydrocarbons (Fleeger and Chandler, 1983; DeLaune et al., 1984; Smith et al., 1984), and thus our a priori prediction was that additions of PAH-contaminated sediment would not alter meiofaunal community structure. An important difference between field studies and

microcosm experiments may have lead to the different results that we observed in our experiment. Specifically, colonization and oiling experiments have been quite limited in the area of sediment influenced (usually < 1 m² of sediment surface), while large areas (> 1 km²) of sediment bottom are usually affected by an oil spill or by chronic, low-level hydrocarbon contamination. The result could be that colonization into experimental sediments may be rapid if the colonizing pool in unaffected sediments is large. Certainly, harpacticoid copepods are known to be good colonizers (Palmer, 1988) with frequent movements into the overlying water column. Thus, changes in density associated with mortality or reproduction in oiled sediments or colonization trays may be confounded due to colonization events. Several studies (e.g., Fleeger and Chandler, 1983; Warwick *et al.*, 1988) have found increases due to harpacticoid species that might invade in large numbers. Such immigration in the field could easily have swamped community changes that we observed here. The microcosm experiment presented here prevents colonization by meiofauna from unaffected sediments, which may dilute the impact of petroleum hydrocarbons on the resident meiofaunal community.

The goal of our study was to examine the impact of PAH at sublethal concentrations. Our review of the literature indicates that PAH concentrations in the range of 1 to 20 ppm (dry weight) of sediment have been associated with noticeable impacts on benthic communities (e.g., Steinhauer and Boehm, 1992, Misitano and Schiewe, 1990). Even at our highest PAH doses (approximately 3 ppm) we detected only a modest, nonsignificant impact on the bacteria or meiofaunal grazing on microbes. There several possible explanations for these observations. One possibility is that benthic systems are much more robust to such acute perturbations than we expected. Further, the marsh sediments used in our study have a high organic content. Organic material complexes with hydrophobic contaminants such as PAH, making them unavailable to benthic organisms. Thus, higher levels of contaminant are required to elicit toxic effects in sediments that are high in organic material (Di Toro *et al.*, 1991). Another possibility is that the community that we are working with 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 and Griffiths, 1984). Further, Klerks and Levinton (1989) demonstrated that a freshwater oligochaete could quickly adapt (within 1-4 generations) to elevated cadmium concentrations. Battaglia *et al.* (1980) have shown that the harpacticoid copepod *Tisbe holothuriae* can 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.

Chapter 5. Materials and Methods, Fish-Predation Experiment

In March and April, 1992 and 1993, juvenile spot were collected from the western end of Bay Champagne, Louisiana at a site about 5 km from the produced-water discharge site at Port Fourchon used as our source of PAH (the same site as the source for sediment for the application controls in the microcosm experiment). Spot between 35 and 60 mm standard length were retained, and transported to our laboratory at LSU. Spot were held in a large wooden water table for approximately one week before the start of an experiment. Sediments containing meiofauna were placed into the water table in flat trays daily to provide food. Tubifex worms were irregularly added to sediments to supplement diet. Fish were maintained in artificial sea water at 22‰, the approximate salinity of Bay Champagne when collected. Twenty four hours before the beginning of each feeding trial, several spot were placed in a separate aquarium without food supplements.

Feeding trials were conducted using sediment collected near Cocodrie, Louisiana, and rendered azoic using the methods of Chandler (1986). The resulting sediment is free of metazoans and has a texture conducive to spot feeding and to meiofauna extraction (McCall and Fleeger, 1993). Contaminated sediment, containing a known amount of PAH, was taken from an access canal near Pass Fourchon, Louisiana with two produced-water outfall stations. This PAH-contaminated sediment (21 ppm total PAH) was mixed with azoic Cocodrie sediment to create intermediate levels of PAH, and meiofauna of known densities were added.

Meiofauna for feeding trials were collected at various times from sediments near Cocodrie, LA in a mudflat near the LUMCON Marine Education Center. During low tide, the upper 1-2 cm of exposed sediment was concentrated in a plastic dust pan and placed into large plastic buckets. These buckets were immediately transported to LUMCON and washed through a 250 and 125 μm sieves. The material retained on the 125 μm sieve was rinsed into a bucket containing seawater, and later extracted by centrifugation in a dense sucrose solution following Fleeger (1979). Extracted meiofauna were combined in a beaker of 15‰ artificial sea water. This concentrated pool of meiofauna, with most of the detritus removed, was divided into aliquots using an eight-chambered sample splitter after Jensen (1982). Each of the eight aliquots was then split into eight aliquots.

Azoic sediments were added to plastic Petri dishes to a depth of 1 cm. Dishes were placed on the bottom of an aquarium, and a plastic tube was positioned over each dish. Aliquots of meiofauna were poured into each tube and allowed to settle for 30 min. The tubes were removed with as little sediment displacement as possible. Displaced sediment was allowed to settle for an additional 15 min before a single spot was placed in the test

chamber, and observed until feeding began. All feeding trials were filmed in a quiet room with constant light. Video-recording of the feeding bout was initiated with the first feeding strike at the sediment in one of the dishes. Each feeding trial was observed and taped using an 8 mm video-camcorder and later viewed with a SVHS video-cassette recorder. Filming continued for 30 min or until the water became too murky to record. Such abbreviated trials were abandoned and not analyzed. Fish from successful trials were immediately removed from the aquarium and measured for standard length (SL). The complete gut of each spot was removed and placed in 10% buffered formalin with Rose Bengal for later gut-content analysis.

Fish behaviors were observed and quantified from video tapes. The frequency of behaviors over the 30 min was recorded and statistically analyzed. Behaviors included a feeding strike, defined as fish taking a bite of sediment and/or detritus into mouth and processing it in buccal cavity. A strike was completed when rejected material and sediment was expelled either from the mouth or as a stream through the gill openings. Processing time was also measured for selected feeding strikes. A stop watch was used while viewing the tapes in real time, and the time taken from the bite to the expulsion was recorded. A maximum of 50 strikes was observed for each fish; fewer were sometimes used due to an inability to observe each strike. Other behaviors commonly observed, but not quantified, included searching (swimming slowly over the sediment with head inclined toward bottom without taking strikes), escape (swimming around perimeter of aquarium very close to the glass) and resting or hiding (quietly sitting on the bottom or on sediment).

Three separate fish-predation experiments were conducted. The first ("Frequency Experiment") was designed to determine how PAH effects spot feeding intensity and behavior. A spot was placed in an aquarium with dishes containing meiofauna and sediment with a single PAH concentration. Three different concentrations of PAH were used in separate aquaria. The second experiment ("Preference Experiment") was designed to determine if spot has a preference for feeding in sediments with or without PAH within the same aquarium. Such preference experiments yield important biological information, but are problematic to analyze because of the lack of independence (Peterson and Renaud, 1989). The third experiment ("Selection Experiment") tested the ability of spot to detect and exploit high-density patches of meiofauna as has been shown by McCall and Fleeger (1993) in sediments contaminated with PAH. Spot has been shown to feed preferentially on high-density patches of meiofauna, but the effect of PAH on this important behavior is unknown. Details of experimental design are given below.

5.1 Frequency Experiment

The Frequency Experiment was conducted in the spring of 1992. Three levels of PAH contamination were used. A control consisted of azoic Cocodrie sediment with no PAH added. A low-PAH concentration, putatively 4.2 ppm PAH, was created by mixing azoic Cocodrie and PAH-contaminated (21 ppm) Port Fourchon sediment at a rate of eight parts Cocodrie to two parts contaminated sediment. A high-PAH concentration, putatively 16.8 ppm, was also prepared by mixing Cocodrie sediment, two parts, with eight parts contaminated sediment. Prepared sediment of a single PAH concentration was added to eight trays, placed in an aquarium, and one aliquot of meiofauna added to each. The sequence of trials among PAH treatments was not randomized; nine replicate spot were first video-taped in control aquaria, followed by nine spot in low-PAH aquaria and seven spot in high-PAH aquaria. Meiofauna were collected on different dates for each PAH-treatment group. Meiofauna are notoriously variable in space and time (Fleeger and Decho, 1987), and selected aliquots were therefore preserved in 10% buffered formalin with Rose Bengal to quantify major taxon composition and estimate density used in the different feeding trials. Aliquots of meiofauna and spot gut contents were enumerated under a stereo-dissection microscope. Feeding strikes were enumerated and processing time measured from the video tapes.

The influence of PAH contamination on the number of feeding strikes and processing time was analyzed by ANOVA. Gut-content samples were analyzed by two-way ANOVA, comparing the number of prey in predominant taxa (nematodes, copepods and chironomids) with the level of PAH contamination. Shorigin Selectivity index, defined as $(sel) = \log_{10} (r_i/p_i)$, where r_i is the proportion of food item i in the diet (as determined from gut contents) and p_i is the proportion available as food (determined from aliquots of meiofauna added to sediment), was used to evaluate prey selectivity. A negative value indicates avoidance of a food type and positive values are associated with selection. The value ranges from -1 to +1.

5.2 Preference Experiment

The Preference Experiment was conducted in the spring of 1993. A contaminated sediment was prepared by mixing two parts azoic Cocodrie sediment with eight parts PAH-contaminated sediment, resulting in a putative concentration of 16.8 ppm. Four dishes, two with control and two with PAH-contaminated sediment, were added to an aquarium in random order, a single aliquot of meiofauna was added, and spot feeding was recorded. This procedure, lacking independence, was repeated until a total of seven replicates were recorded. All feeding trials for this experiment were conducted from a single collection of meiofauna,

and aliquots of meiofauna and fish gut contents were not enumerated. The number of feeding strikes in each dish was determined from video tapes, and summed by treatment group. Processing time, for the two treatments, was also measured from the video tapes. Because of the lack of independence in such preference experiments, no statistical tests were performed.

5.3 Selection Experiment

The Selection Experiment was conducted in the spring of 1993. Two levels of PAH contamination were used, one a control (no PAH) and one prepared by mixing two parts Cocodrie sediment with eight parts contaminated sediment resulting in a putative concentration of 16.8 ppm. Prepared sediment of a single PAH concentration was added to six dishes, and placed in an aquarium. Dishes were randomly assigned one of three densities of meiofauna. The zero (control) dishes received no addition of meiofauna. Low-density dishes received one aliquot of meiofauna, and high-density dishes received three aliquots of meiofauna. Azoic detritus was added to the zero-meiofauna dishes after McCall and Fleeger (1993), and spot feeding activity recorded as above. This process was repeated until seven spots were recorded in control and an additional seven spots were recorded in the PAH-contaminated aquaria. The sequence of trials among PAH treatments was randomized. All feeding trials for this experiment were conducted from one collection of meiofauna, and aliquots of meiofauna and spot gut contents were not enumerated. Spot feeding strikes in each dish were counted and summed for each meiofauna density. Spot processing time was also measured. A split-plot ANOVA was conducted to determine if the number of feeding strikes differed among treatments (PAH) and meiofaunal density.

Chapter 6. Results, Fish-Predation Experiment

6.1 Frequency Experiment

Six of the nine fish exposed to control (uncontaminated) sediments fed. All fish that fed took a minimum of five min to acclimate to the aquarium before feeding. Those that did not feed made no strikes at the sediment and stayed in one area of the aquarium for the entire time. Four of the nine spot tested on sediments contaminated at 2.5 ppm fed. Four spot displayed escape behavior and one search behavior. All seven spot tested on the highly contaminated (9.5 ppm) sediment fed. Most began feeding within three min after introduction. Only one fish displayed stress and escape behavior, but began feeding after seven min.

The replicate trials for the three treatments (control, low, and high PAH) were conducted using three separate collections of meiofauna. Aliquot counts (8 of 64 were counted) indicate large differences in the relative abundance of major taxa with each collection (Table 6.1). Although nematodes were always the most abundant taxon, their numbers were highest in collections used in control and lowest in high-PAH trials. Copepods were most abundant in collections used in the high-PAH treatment. Chironomids were much higher (2-3X) in abundance in collections used in PAH-contaminated trials compared to collections used for control trials.

Gut contents indicated significant differences in the numbers and types of prey consumed among the experimental trials (Fig. 6.1). A two-way ANOVA did not detect a significant effect of PAH ($p = 0.32$) on the number of prey consumed, however consumption of different taxa differed in different trials ($p = 0.03$). There was a significant PAH x taxon interaction ($p = 0.01$) as well. The interaction term strongly suggests that the diet of spot differed among the experimental trials. Nematodes, copepods and chironomids were ingested by spot in roughly equal numbers in control trials, but chironomids were overwhelmingly numerically dominant in gut contents of spot feeding in PAH-contaminated sediments (Fig. 6.1). The Shorigin Selectivity Index (Table 6.1) also reflected the change in diet among feeding trials. Chironomids were positively selected for in all cases, however very strong selection (+1.4 chironomids in the low PAH and +1.6 for the high PAH) occurred in PAH-contaminated sediments. Copepods were selected for in control trials, but negative selectivity values occurred for copepods in PAH-contaminated sediments. Nematodes were always selected against (negative selectivity values).

The mean number of feeding strikes per 30 min ranged from 119.8 ± 60.8 (mean \pm 1 standard deviation) in control to $199.7 (\pm 60.7)$ in the high PAH trials (Fig. 6.2). Low PAH was

Frequency Experiment

Gut contents

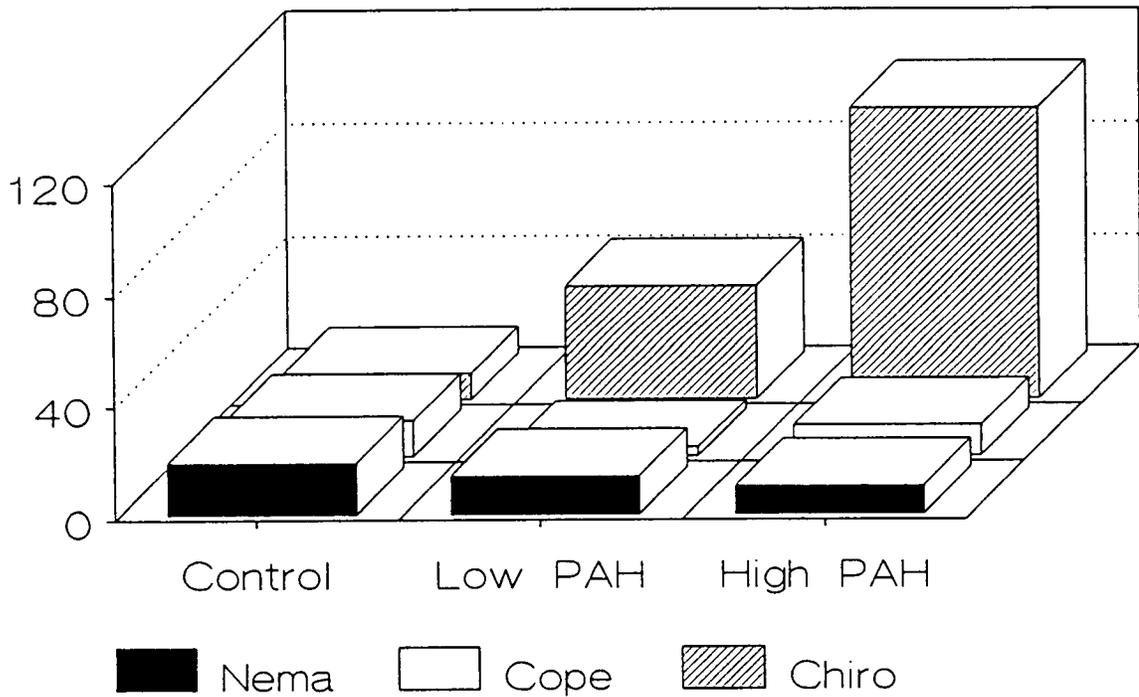


Figure 6.1 Prey taxa in spot guts. Data are mean number of predominant prey taxa in the gut contents of spot used in the Frequency Experiment. Nema refers to nematodes, cope to copepods and chiro to chironomids.

Frequency Experiment

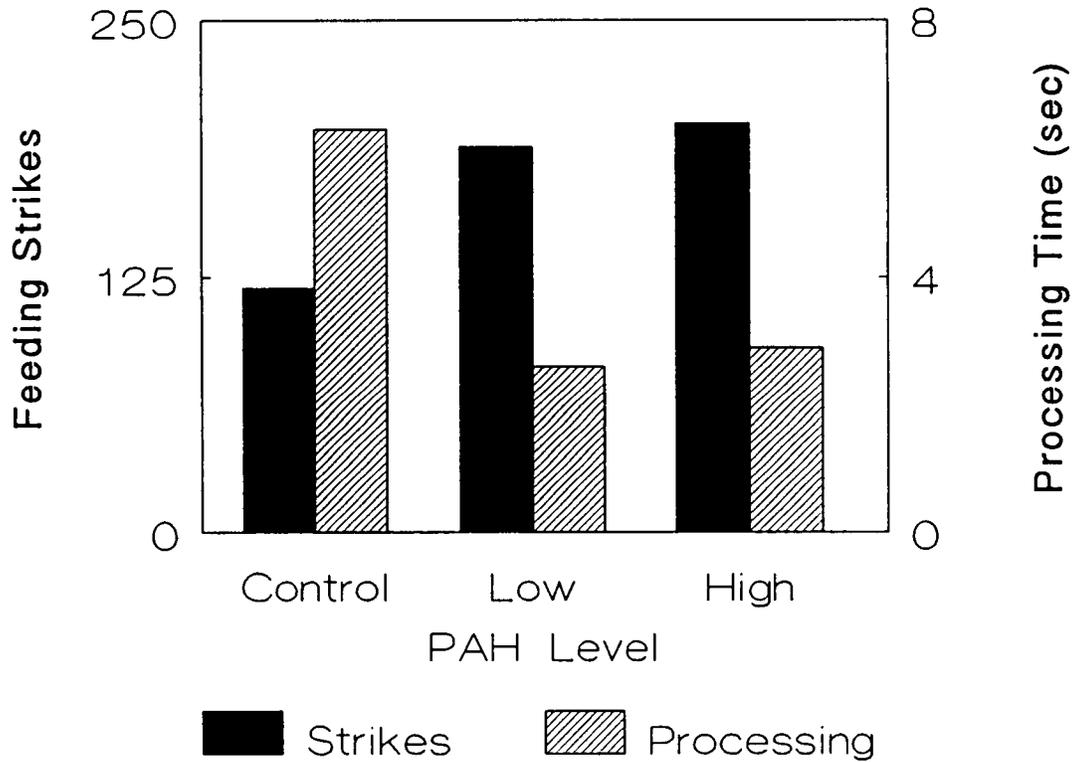


Figure 6.2 Feeding and processing by spot. Data are mean number of feeding strikes and processing time of spot used in the Frequency Experiment.

Table 6.1 Meiofauna abundance, Frequency Experiment. Data are aliquot counts of abundant meiofauna used in the Frequency Experiment. All feeding trials for a given treatment were performed from a single meiofauna collection, referred to as control (for the control trials), low and high PAH, (for the low and high PAH trials respectively). Selectivity index (see text) calculated by the Shorigin method. s.d. is standard deviation.

Chironomids			
	Control	Low PAH	High PAH
Rep 1	33	214	128
Rep 2	38	149	80
Rep 3	38	165	73
Rep 4	33	128	54
Rep 5	54	195	60
Rep 6	40	88	102
Rep 7	38	148	105
Rep 8	67	151	72
Mean (s.d.)	42.6 (11.)	154.8 (36.2)	84.3 (23.6)
Selectivity	+1.0	+1.4	+1.6
Nematodes			
	Control	Low PAH	High PAH
Rep 1	3587	4452	3899
Rep 2	5630	4449	2260
Rep 3	3110	3830	2720
Rep 4	4055	3341	1527
Rep 5	6062	4693	1978
Rep 6	3491	3120	2350
Rep 7	4188	2858	2485
Rep 8	5053	3991	1663
Mean (s.d.)	4397.0 (1001)	3841.8 (635)	2360.3 (693)
Selectivity	-0.21	-0.5	-0.9
Copepods			
	Control	Low PAH	High PAH
Rep 1	685	750	1677
Rep 2	1080	646	953
Rep 3	644	790	1053
Rep 4	1008	608	772
Rep 5	1271	731	965
Rep 6	760	498	1106
Rep 7	683	477	1252
Rep 8	1030	479	847
Mean (s.d.)	895.1 (217)	622.4 (119)	1078.1 (266)
Selectivity	+0.4	-0.4	-0.5

intermediate with 188.5 ± 80.5 strikes. ANOVA revealed no statistical difference among feeding strike means ($p = 0.25$) in different trials. ANOVA ($p = 0.01$) suggests that processing time did, however, differ among experimental trials. Processing time was longest in controls ($6.3 \text{ sec} \pm 2.3$) and about equal in low ($2.6 \text{ sec} \pm 0.8$) and high PAH ($2.9 \text{ sec} \pm 2.3$) sediments (Fig 6.2). Differences in the nature of feeding strikes were noticeable from visual examination of the video tapes. Spot in control sediments took large, deep bites of sediment as described by Billheimer and Coull (1988), while those feeding in PAH-contaminated sediments took small, shallow bites as if they were "picking" at the sediment surface. Spot passes rejected sediment out through the gill rakers, or expels it from the mouth. Spot feeding in control sediments appeared to expel much more rejected sediment than those feeding in PAH-contaminated sediments.

6.2 Preference Experiment

The Selection and Preference Experiments were conducted in 1993 after examination of the results of the Frequency Experiment (conducted in 1992). All spot tested in selection and the preference experiments fed. The Preference Experiment was designed to determine if spot avoid PAH-contaminated sediment if non-contaminated sediment is readily available, i.e. within the same aquarium. The number of feeding strikes into contaminated and uncontaminated sediment in the same aquarium was always very similar, suggesting that no change in behavior occurred and that spot did not avoid PAH (Table 6.2). The mean number of feeding strikes was 143.7 in control sediments compared to 168.1 in PAH-contaminated sediments. Processing times were almost identical in sediments with and without PAH; 3.03 ± 2.7 ($n = 82$) in control and 2.99 ± 3.7 ($n = 82$) sec in PAH-contaminated sediments.

6.3 Selection Experiment

The Selection Experiment was designed to determine if PAH alters spot's ability to locate and exploit high densities of meiofauna. Generally, spot took slightly more feeding strikes in PAH-enriched sediment (Fig. 6.3), although a split-plot ANOVA conducted to determine if the number of feeding strikes differed among treatments (and meiofaunal density) revealed no difference between control and PAH treatments (Table 6.3). The number of feeding strikes was, however, significantly related to the density of meiofauna regardless of PAH contamination. Significantly more feeding strikes occurred in the high-density patches, especially in PAH-enriched sediment compared to sediment without meiofauna (Tukey's Studentized test, Table 6.3). Feeding strikes in low-density meiofaunal treatments were not significantly different from zero-meiofauna treatments. Processing time did not differ between control ($1.5 \text{ sec} \pm 0.37$) and PAH-enriched sediments, $1.3 \text{ sec} \pm 0.36$. Overall, the presence of PAH did not effect spot's ability to locate and exploit high-density of patches of meiofauna.

Selection Experiment

Feeding Strikes

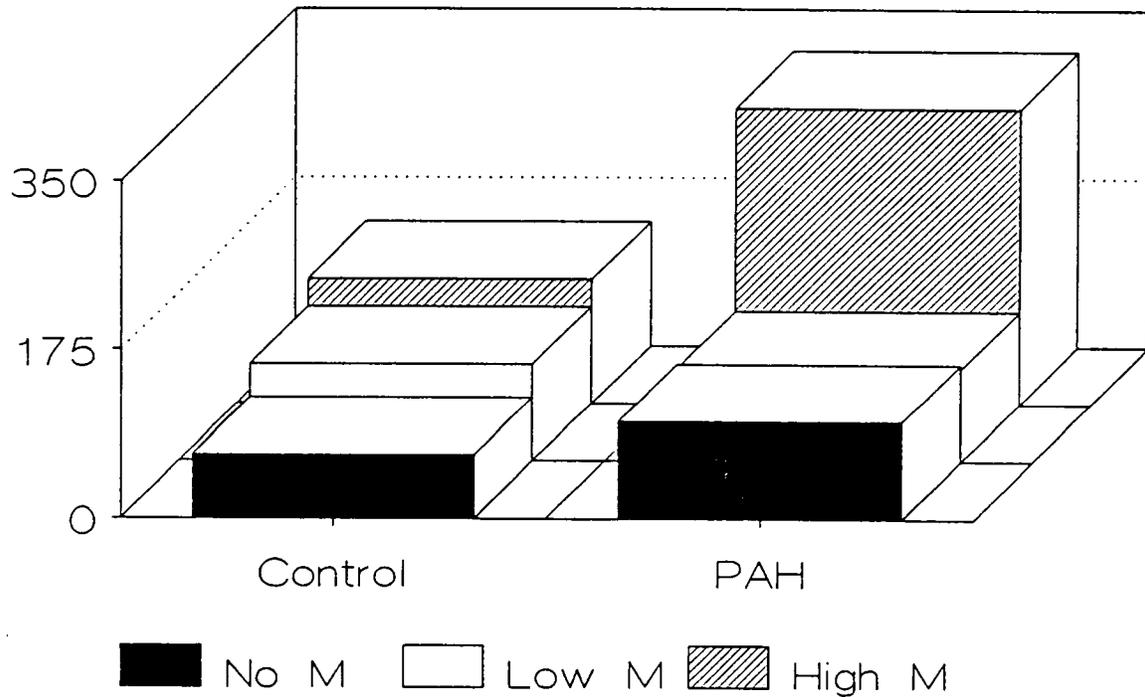


Figure 6.3 Feeding strikes on meiofauna. Data are mean number of feeding strikes in sediments containing different densities of meiofauna and at different levels of PAH contamination. "No M" is the zero meiofauna treatment, "Low M" represents an addition of one aliquot of meiofauna and "High M" represents an addition of three aliquots of meiofauna.

Table 6.2 Feeding strikes, raw data. Raw data for the number of feeding strikes in the Preference Experiment. Counts for each replicate spot are given.

REPLICATION #	CONTROL	PAH
1	231	195
2	101	183
3	111	132
4	269	144
5	110	96
6	45	211
7	139	216
MEAN	143.7	168.1
STANDARD DEVIATION	72.8	41.7

Table 6.3 ANOVA, feeding strikes. Split-plot ANOVA analyzing the number of feeding strikes in the Selection Experiment. TRT refers to control and PAH enrichment and DENSITY to the abundance of meiofauna (none, low, high). Raw data were $\log x + 1$ transformed. Tukey's Studentized Range test revealed no difference between control and PAH enrichment but significantly more strikes in high, compared to zero, meiofauna density.

Source	DF	Type I SS	Mean Square	F Value	PR > F
TRT	1	1.51	1.51	2.28	0.1439
TRT (REP)	12	35.57	2.96	4.48	0.0009
DENSITY	2	6.14	3.07	4.64	0.0198
TRT*DENSITY	2	0.05	0.02	0.04	0.9632

Chapter 7. Discussion, Fish-Predation Experiment

We found no indication that juvenile spot avoids feeding in sediments with the environmentally meaningful levels of PAH contamination used in our experiments. PAH-contamination did not decrease the number of feeding strikes or sediment processing time (the time that sediment was held in the mouth while capturing meiofauna). Nor was the ability of juvenile spot to locate and exploit high-density patches of meiofauna impaired by PAH contamination. This ability is critical to feeding in spot, as spot continuously ingests large numbers of meiofauna (McCall and Fleeger, 1993). In fact and surprisingly, our results indicate that spot may even increase feeding intensity in PAH-contaminated sediments. In all three predation experiments, the number of feeding strikes tended to increase and processing time decrease in PAH-contaminated sediments indicating increased feeding activity. This trend was not statistically significant (except for processing time in the Frequency Experiment), but was consistently observed.

Our data suggest that any increased feeding activity in PAH-contaminated sediments is likely the result of PAH-induced changes in prey behavior rather than effects on spot. There are numerous reports of burrowing avoidance or lethargy in invertebrates exposed to contaminated sediments, especially in oligochaetes and chironomids (Keilty *et al.*, 1988a; 1988b; White and Keith, 1988). Chironomids were the preferred prey of spot in our experiments. Perhaps chironomids in our experiments were slow to burrow or avoided burrowing when added to PAH-contaminated sediments. Alternatively, chironomid escape responses to predators may have been retarded if activity rates were reduced. Spot is an opportunistic feeder that takes advantage of moribund prey lying on the sediment surface (Pihl *et al.*, 1991). Furthermore, fishes are known to readily ingest prey that have modified their behavior under the influence of a contaminant. For example, Farr (1978) found that gulf killifish ingest proportionately more grass shrimp (than other prey types) when prey were exposed to parathion. Exposed grass shrimp were less able to avoid capture by predators. Therefore it is possible that spot feeding activity in contaminated sediments may increase, leading to increased levels of exposure either from contaminated prey or by increased contact with contaminated sediment.

The results of the Frequency Experiment are ambiguous due to the confounding influence of variability in meiofauna densities associated with our non-random sequence of experimental trials. Chironomids were 2-3 x more abundant in aliquots used to add meiofauna to the PAH-contaminated sediment compared to those used in control trials (Table 6.1). The resulting consumption of prey in these trials was even more skewed (Fig. 6.1). Chironomids were ingested in significantly higher numbers from PAH-

contaminated sediments. Thus observable changes in feeding behavior may have been a result of the increase in prey density associated with a functional response by the predator. Chironomids typically live in the sediment in a head down posture, and are a preferred fish food (Hayes et al., 1992). Because more chironomids were present in PAH-contaminated sediment, spot may have developed a search image for this larger prey and ingested them in even larger numbers. Decreased processing times and the shallow bites associated with spot feeding in PAH-contaminated sediments may be a reflection of this change in behavior. Additional experiments are needed to separate the effect of PAH on prey burrowing avoidance from changes in predator-prey interactions associated with spot's functional response.

Very little is known about the method by which spot locates its prey. McCall and Fleeger (1993) showed that spot tests various sediments with feeding strikes, and will increase feeding activities in high-density patches of prey only after processing these feeding strikes. This suggests that chemoreception may not be important in prey location and that gustatory responses are probably used. Moreover, PAH does not appear to interfere with chemoreception in most fishes, and fishes do not generally avoid PAH in the field (Klaprat et al., 1992). Our data suggest that spot will continue active feeding in PAH-contaminated sediments.

Although the levels of exposure to PAH observed in our experiments probably did not alter spot feeding intensity or behavior, they may be harmful. Weeks and Warinner (1984) found that similar PAH exposure caused changes in macrophage function, and Hargis et al. (1984) and Roberts et al. (1989) identified various physiological modifications (leading to acute mortality) caused by exposure to sediments contaminated with relatively low levels of PAH. To determine if spot under our experimental conditions was exposed to PAH in amounts that elicit a physiological response, EROD (a biomarker in the cytochrome P450 family, see Roberts and Sved, 1987 and Sved et al., 1992) was measured in spot gut and oral tissue after 1, 3 and 6 days of exposure to sediments collected from Port Fourchon (analyses conducted by Dr. Gary Winston, Department of Biochemistry, LSU, Winston and Fleeger, in preparation). High levels of EROD were detected, suggesting that this level of exposure triggers metabolic breakdown of the PAH. Exposure through contact with contaminated sediments is a chief mode of exposure in spot (Rubinstein et al., 1984), that, our data suggest, is not reduced due to a behavioral avoidance response. We did not seek to determine at what level of exposure, if any, feeding will stop or avoidance occur, but certainly levels of contamination in other areas (such as the Elizabeth River in Virginia, Hargis et al., 1984; Huggett et al., 1992) can be much higher than the 21 ppm total PAH used in our study. Clearly the absence of avoidance behavior increases the likelihood of significant and damaging levels of bioaccumulation in spot.

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Appendix I

Concentrations of individual PAH in each of the replicate samples from the microcosm experiment are provided in the following pages. At the top of each column of data is an alpha-numeric heading such as "0-1A". The first number refers to the day the sample was taken (Day 0, 1, 3, 7, 14, 21, or 28); the second number refers to the PAH treatment: 1 = application control; 2 = zero PAH; 3 = low PAH; 4 = medium PAH; and 5 = high PAH. The letter (A, B, C, or D) refers the replicate within a treatment.

ANALYTE	1-1A	1-1B	1-1C	1-1D	1-2A	1-2B	1-2C	1-2D	1-3A	1-3B	1-3C	1-3D	1-4A	1-4B	1-4C	1-4D	1-5A	1-5B	1-5C	1-5D	1-5E	1-5F
Moisture, %	68%	68%	64%	67%	67%	71%	70%	70%	70%	69%	71%	71%	69%	59%	56%	56%	64%	65%	68%	59%	64%	65%
Naphthalene	6.5	6.7	4.3	6.3	6.1	1.6	9.8	8.9	6.3	6.4	7.8	7.4	5.8	4.8	NA	NA	7.6	7.5	6.4	7.0	7.6	7.5
2-MN	5.0	4.2	2.8	4.7	4.4	7.2	7.7	4.8	4.9	4.6	4	5.3	4.6	5.0	NA	NA	11	7.6	6.1	12	11	7.6
1-MN	3.1	4.4	3.3	3.3	5.0	7.9	9.0	3.7	4.6	3.5	3.1	3.8	7.2	5.2	NA	NA	14	9.6	6.9	10	14	9.6
2-EN	3.5	ND	2.7	2.4	2.5	ND	ND	ND	5.5	ND	ND	3.8	ND	8.1	NA	NA	25	14	11	8.9	25	14
1-EN	NA	ND	NA																			
2,6/2,7-DMN	22	12	6.7	23	19	49	25	17	32	18	21	29	24	42	NA	NA	120	70	66	80	120	70
1,3/1,7-DMN	3.6	3.3	2.8	4.1	3.2	4.6	3.5	4.3	9.5	5.2	5.5	8.4	11	30	NA	NA	110	60	48	67	110	60
1,6-DMN	3.3	2.4	2.2	3.3	2.8	6.6	3.6	3.7	5.3	3.9	4.2	5.6	5.4	8.8	NA	NA	31	17	16	21	31	17
1,4/2,3-DMN	ND	3.5	2.4	4.9	4.5	1.3	ND	6.2	7.7	4.4	5.2	1.4	3.1	2.7	NA	NA	100	39	46	42	100	39
1,5-DMN	5.6	ND	3.8	ND	ND	ND	ND	ND	NA	NA	ND	16	ND	23	ND	16						
Acenaphthylene	1.5	1.5	1.6	1.5	1.3	1.9	1.7	1.2	3.2	1.0	1.2	0.95	1.6	0.63	1.2	1.3	3.4	2.9	2.3	2.4	3.4	2.9
1,2-DMN	1.8	1.1	0.56	1.5	ND	ND	ND	ND	0.60	ND	ND	1.8	ND	4.9	NA	NA	16	13	8.3	8.3	16	13
Acenaphthene	2.0	2.2	1.4	1.8	1.8	2.4	3.3	3.0	2.7	2.0	2.2	2.0	4.3	4.7	8.4	ND	17	9.8	6.1	9.4	17	9.8
1,6,7-TMN	1.9	1.3	2.2	2.3	ND	3.9	4.3	ND	17	3.7	6.8	5.9	1.3	3.1	NA	NA	160	100	53	96	160	100
Fluorene	3.7	3.8	3.0	3.4	2.8	6.8	5.0	5.1	6.1	3.8	3.7	5.1	5.4	9.5	2.3	3.1	19	14	19	14	19	14
Phenanthrene	2.3	2.8	1.6	2	1.8	3.2	2.1	3.0	3.3	3.4	2.4	2.6	4.5	5.8	8.7	5.3	30	15	10	6.8	30	15
Dibenzothiophene	1.4	1.4	1.0	1.2	9.3	1.9	1.6	1.3	1.7	1.2	1.3	1.4	1.5	2.3	2.8	1.9	280	56	36	44	280	56
Anthracene	4.8	4.5	4.2	4.4	3.3	7.4	5.6	5.4	7.1	5.6	5.2	5.3	5.5	5.7	6.4	5.6	27	14	8.5	12	27	14
4-MDBT	6.1	4.3	3.7	6.5	2.9	9.4	6.6	5.4	9.9	7.2	7.0	9.5	1.3	2.4	3.1	2.0	93	58	37	60	93	58
2/3-MDBT	2.2	ND	3.7	2.1	ND	4.9	ND	9.7	4.6	4.9	4.6	4.4	1.1	1.2	1.5	1.1	46	34	19	33	46	34
3-MP	7.0	6.2	5.0	6.4	4.0	12	8.8	8.1	18	11	11	15	2.8	5.0	69	45	230	130	84	140	230	130
2-MP	3.1	3.0	2.7	2.7	2.1	5.7	4.4	3.9	6.5	4.9	4.6	5.9	1.3	2.0	3.1	1.9	130	60	37	68	130	60
4/9-MP	6.2	5.1	4.5	5.4	3.3	11	7.4	6.8	16	11	9.8	1.4	2.8	4.9	7.0	4.7	210	120	82	140	210	120
1-MP	4.7	4.5	4.0	4.3	2.9	9.7	6.3	5.1	11	7.8	7.6	9.6	1.8	3.1	4.4	2.9	140	78	52	82	140	78
3,6-DMP	4.9	4.3	4.1	4.1	3.0	9.2	7.2	5.9	11	7.2	7.4	10	2.1	3.0	3.9	3.1	100	74	45	87	100	74
3,5-DMP	NA																					
2,6-DMP	4.0	3.5	2.7	3.4	2.2	7.7	5.3	4.0	9.6	6.0	6.7	9.0	1.8	2.7	3.6	2.7	97	70	49	76	97	70
2,7-DMP	4.0	3.3	3.6	3.2	2.8	7.6	6.3	5.3	8.1	6.0	5.9	8.7	1.9	2.1	2.8	2.3	76	56	34	60	76	56
1,2-DMDBT	3.0	ND	ND	2.8	ND	ND	ND	ND	3.7	6.1	ND	3.8	ND	10	9.6	8.1	23	24	3.5	9.4	23	24
3,9-DMP	12	11	9.7	10	6.9	2.3	1.7	1.5	3.5	2.4	2.3	3.2	7.2	11.0	130	110	350	260	160	300	350	260
1,6/2,5/2,9-DMP	8.4	6.4	6.4	7.2	4.9	16	12	8.6	22	16	15	19	5.0	6.6	57	77	260	190	120	190	260	190
1,7-DMP	3.8	3.9	4.0	3.2	3.5	8.7	7.4	5.7	10	8.8	6.8	10	2.3	3.1	ND	31	100	73	45	77	100	73
1,9/4,9-DMP	13	13	6.7	4.2	1.3	4.1	2.4	1.9	2.8	1.7	2.1	2.3	4.0	5.0	59	60	150	120	74	73	150	120
1,5-DMP	3.2	3.8	1.6	3.0	4.2	1.4	8.4	7.4	5.4	4.4	5.5	7.1	ND									
Fluoranthene	37	37	30	31	23	69	57	43	52	35	36	41	37	32	29	32	580	63	36	76	580	63
1,8-DMP	1.9	1.7	1.5	1.5	1.3	4.9	3.6	2.0	4.5	3.1	3.6	3.9	9.2	1.1	2.2	1.2	35	28	17	33	35	28
1,2-DMP	1.5	1.8	1.3	1.4	1.2	3.9	3.2	2.3	2.9	2.3	2.8	3.4	5.6	5.8	6.3	1.8	15	8.7	2.1	21	15	8.7
9,10-DMP	0.76	0.80	1.1	1.2	0.80	1.9	2.0	1.0	0.93	1.9	1.1	1.3	2.2	1.1	3.7	1.8	4.8	5.1	2.6	10	4.8	5.1
Pyrene	36	40	32	29	23	72	60	51	49	38	33	39	44	30	28	34	360	58	34	71	360	58
1,2,8-TMP	1.3	0.90	1.5	1.4	1.2	0.97	1.2	1.1	2.1	2.8	2.0	3.7	5.8	1.6	1.7	1.4	39	35	19	46	39	35
Benzo(a)anthracene	11	15	15	13	6.9	15	13	13	17	11	12	12	9.4	18	16	16	110	38	23	31	110	38
Chrysene	15	19	30	17	11	28	19	15	34	23	19	20	22	40	51	32	450	91	55	78	450	91
Benzo(b)fluor	15	18	19	17	11	30	26	27	33	19	19	19	17	19	16	17	340	32	20	92	340	32
Benzo(k)fluor	ND																					
Benzo(a)pyrene	12	14	12	13	9.5	12	10	10	19	7.9	14	7.8	14	12	10	12	86	20	12	NA	86	20
Indenopyrene	31	25	28	30	29	19	18	15	16	11	11	9.0	2.1	1.4	8.9	9.7	9.4	2.1	1.1	ND	9.4	2.1
Dibenzanthracene	5.7	1.9	6.6	5.4	5.5	6.3	ND	8.0	4.3	3.1	3.2	2.9	4.0	3.4	3.1	2.6	18	6.7	3.5	ND	18	6.7
Benzo(ghi)perylene	57	24	47	39	54	43	25	13	ND	ND	ND	ND	7.2	13	3.0	9.0	80	21	7.0	11	80	21
Total Parent PAH	255	229	246	226	199	351	272	235	270	182	183	188	263	236	241	219	2545	475	285	400	2545	475
Total Alkylated PAH	141	110	93	123	102	284	184	156	300	197	195	269	452	727	644	562	2689	1776	1144	1882	2689	1776
Total PAH	395	339	339	349	301	635	456	391	570	379	378	457	735	962	885	781	5233	2251	1429	2281	5233	2251
MEANS																						
Moisture, %	67%						70%					70%									59%	64%
Total Parent PAH	239						264					206									244	926
Total Alkylated PAH	117						181					240									596	1873
Total PAH	356						445					446									841	2799
Ratio Alk/Par.	0.49						0.69					1.17									2.44	2.02

ANALYTE	3-1A	3-1B	3-1C	3-1D	3-2A	3-2B	3-2C	3-2D	3-3A	3-3B	3-3C	3-3D	3-4A	3-4B	3-4C	3-4D	3-5A	3-5B	3-5C	3-5D
Moisture, %	63%	62%	64%	64%	61%	64%	60%	62%	65%	64%	61%	62%	64%	62%	63%	64%	64%	66%	66%	64%
Naphthalene	3.5	1.4	4.3	5.5	3.0	3.9	13	7.1	8.2	4.8	5.9	7.1	7.5	4.0	5.2	6.2	8.5	6.9	5.3	7.2
2-MN	4.0	4.9	4.6	4.6	3.8	4.2	15	4.7	8.2	5.4	4.9	4.9	5.9	2.4	5.2	5.8	9.0	7.1	7.8	14
1-MN	4.1	3.4	3.2	4.7	2.4	3.3	8.1	2.6	5.5	3.8	5.4	4.3	4.6	2.2	5.9	5.1	11	8.7	8.7	17
2-EN	ND																			
1-EN	ND																			
2,6,7-DMN	12	11	12	13	11	ND	ND	11	20	20	21	19	24	14	26	39	110	68	82	170
1,3,7,7-DMN	2.1	1.9	3.4	3.8	1.8	2.9	22	3.4	8.6	8.6	7.4	7.4	11	3.8	7.4	26	71	36	55	150
1,6-DMN	1.8	1.6	2.3	4.6	2.1	3.9	8.3	3.1	4.2	4.0	5.7	3.6	6.7	2.0	3.1	10	21	13	17	44
1,4,2,3-DMN	1.5	1.7	1.4	ND	2.6	ND	ND	5.7	5.0	5.2	8.0	5.3	6.5	5.0	9.5	29	74	28	55	140
1,5-DMN	ND	1.6	1.0	ND	1.5	5.5	ND	ND	3.7	ND	ND	ND	4.2	ND						
Acenaphthylene	1.2	1.2	1.6	2.1	1.7	2.4	1.7	1.1	1.5	1.6	1.8	1.6	3.7	1.3	1.9	1.3	TR	1.2	TR	TR
1,2-DMN	ND	1.4	ND	ND	ND	2.2	ND	ND	2.2	2.6	3.2	ND	2.8	ND	1.9	4.7	16	7.2	11	30
Acenaphthene	1.3	1.7	1.9	3.7	1.3	1.8	ND	0.91	3.3	2.9	2.0	1.5	3.2	ND	2.6	4.3	7.0	5.8	3.9	16
1,6,7-TMN	2.3	2.2	2.3	ND	1.7	1.8	ND	4.8	14	21	28	15	25	3.0	17	27	90	72	69	160
Fluorene	3.0	2.8	3.0	3.4	3.0	3.7	4.3	3.4	4.4	4.8	6.9	5.4	5.1	2.9	6.1	11	?	15	8.7	36
Dibenzothioephene	1.9	1.5	2.1	2.3	1.6	2.0	2.2	1.7	2.6	2.9	2.3	2.6	2.2	2.0	4.3	7.3	15	10	6.1	24
Phenanthrene	10	17	25	19	21	21	15	12	18	19	12	12	16	9.8	17	26	79	63	25	85
Anthracene	4.2	3.9	5.6	6.5	4.4	4.0	4.0	7.0	6.7	5.7	4.4	4.5	6.5	3.6	6.1	8.3	41	13	7.7	TR
4-MDBT	3.5	ND	5.5	5.2	3.8	4.2	5.4	3.6	7.8	9.5	13	8.3	15	6.8	13	30	54	37	26	120
2/3-MDBT	ND	ND	4.9	1.0	ND	7.5	ND	7.5	4.9	4.8	9.5	6.9	11	5.1	8.2	16	27	18	14	57
3-MP	6.5	6.7	8.1	8.6	6.2	6.9	7.7	5.8	15	20	27	17	27	17	25	62	110	77	57	250
2-MP	3.0	3.5	3.8	4.4	2.5	3.3	4.4	3.2	6.8	6.3	11	5.4	8.7	4.6	10	25	54	35	23	110
4/9-MP	6.0	6.4	7.2	8.4	6.3	6.3	8.6	6.5	14	21	28	18	29	12	27	64	120	77	58	250
1-MP	3.7	3.4	4.1	5.0	3.5	4.2	5.1	4.2	7.5	13	16	11	16	7.9	18	39	76	49	37	160
3,6-DMP	5.7	4.3	5.0	7.6	5.6	4.1	5.8	6.8	15	12	17	11	19	8.6	21	41	75	51	36	220
3,5-DMP	ND																			
2,6-DMP	3.0	2.9	4.2	4.6	3.4	2.8	4.7	3.4	7.7	11	14	8.8	15	7.9	18	38	69	46	34	92
2,7-DMP	2.6	2.9	3.4	3.8	2.7	3.0	4.5	3.0	6.7	8.5	11	7.3	13	6.6	16	31	56	39	28	12
1,2-DMDBT	ND	1.0	ND	2.5	2.3	1.9	3.6	ND	TR	11	19	15	11	TR						
3,9-DMP	9.1	11	12	10	8.7	9.6	13	8.9	27	39	55	33	58	28	67	150	260	170	130	470
1,6,2,5,2,9-DMP	6.1	6.5	7.6	7.3	6.2	6.0	10	6.5	20	29	41	25	44	19	47	110	190	130	96	210
1,7-DMP	1.9	1.9	3.1	2.9	1.8	2.1	3.4	1.8	6.6	9.2	14	8.0	15	8.2	19	42	75	47	38	ND
1,9,4,9-DMP	5.7	5.1	6.3	5.7	3.7	4.4	6.4	5.2	10	11	16	11	17	25	43	76	120	86	61	3.4
1,5-DMP	ND	1.6	ND	ND	4.9	5.5	ND	1.7	ND	ND	ND									
Fluoranthene	32	29	34	52	25	27	38	25	44	36	30	26	40	28	52	50	62	85	40	86
1,8-DMP	2.2	1.4	2.0	2.4	1.1	ND	2.9	1.4	3.3	5.4	7.0	5.2	7.0	4.2	9.7	18	31	20	16	9.3
1,2-DMP	2.1	1.8	2.6	2.4	1.7	1.4	1.8	1.6	3.2	3.0	5.8	5.8	4.8	4.9	4.7	9.5	15	7.3	8.0	9.0
9,10-DMP	0.89	1.1	0.78	1.3	0.67	0.97	1.9	1.6	1.7	1.6	2.2	1.5	2.6	3.2	5.3	4.7	7.7	5.6	3.8	13
Pyrene	38	35	41	55	29	33	49	36	53	42	37	33	48	31	52	57	66	76	46	86
1,2,8-TMP	2.0	3.1	2.6	2.8	2.3	4.2	2.3	2.4	8.8	11	18	9.0	18	2.1	5.1	11	17	12	8.9	27
Benzantracene	10	10	13	21	9.7	11	15	10	15	15	13	11	17	8.9	18	20	54	35	20	45
Chrysene	14	17	22	26	20	15	23	21	27	27	23	19	29	15	37	46	190	80	46	230
Benzo(b)fluor	20	21	28	40	21	23	39	23	33	27	23	18	27	16	30	30	49	44	26	56
Benzo(k)fluor	ND																			
Benzo(a)pyrene	NA	7.2	14	15	25	22	14	31												
Indenopyrene	ND	TR	4.2	6.9	3.8	TR	9.6	22	23	19	32	9.3	8.0	6.2	13	11	15	16	10	18
Dibenzanthracene	TR	TR	TR	5.2	TR	TR	14	19	16	10	15	16	8.9	0.69	3.2	2.2	6.7	5.7	4.0	12
Benzo(a)anthracene	TR	TR	TR	TR	TR	TR	14	19	16	10	15	16	8.9	0.69	3.2	2.2	6.7	5.7	4.0	12
Total Parent PAH	139	154	199	258	156	156	253	218	297	253	241	205	258	144	272	307	634	495	271	751
Total Alkylated PAH	92	93	108	118	88	87	149	101	237	290	398	254	419	202	439	933	1793	1182	1005	2773
Total PAH	231	246	307	376	244	243	402	319	534	543	639	459	677	347	711	1240	2428	1677	1276	3524

MEANS	63%
Moisture, %	62%
Total Parent PAH	196
Total Alkylated PAH	103
Total PAH	290
Ratio Alk/Par.	0.55

ANALYTE	7-1A	7-1B	7-1C	7-1D	7-2A	7-2B	7-2C	7-2D	7-3A	7-3B	7-3C	7-3D	7-4A	7-4B	7-4C	7-4D	7-5A	7-5B	7-5C	7-5D
Moisture, %	70%	68%	68%	67%	67%	65%	64%	67%	63%	65%	65%	63%	62%	63%	66%	62%	65%	60%	64%	67%
Naphthalene	4.9	3.9	3.3	4.4	3.2	3.7	4.3	5.3	2.9	3.6	7.7	6.1	4.1	3.4	NA	4.0	4.6	9.0	5.1	5.8
2-MN	5.9	3.6	3.3	3.0	2.0	2.4	2.5	4.2	2.2	3.4	5.7	4.1	3.7	3.4	NA	4.0	7.5	38	8.9	5.9
1-MN	2.7	3.0	2.4	2.3	3.1	2.8	3.6	2.1	1.7	2.3	5.1	2.7	2.2	2.2	NA	3.1	4.5	34	6.2	7.8
2-EN	ND	ND	ND	1.7	ND	NA	1.4	4.5	44	7.1	6.1									
1-EN	ND	NA	1.4	4.5	44	7.1	6.1													
2,6,2,7-DMN	8.0	ND	ND	10	6.0	ND	ND	7.0	11	10	24	16	9.7	20	NA	17	44	330	73	61
1,3,7,7-DMN	4.1	2.1	1.9	2.3	1.7	3.2	2.0	2.4	3.3	4.3	4.5	3.4	3.4	7.4	NA	4.4	32	330	60	25
1,6-DMN	3.4	1.5	1.8	1.7	1.8	1.6	2.0	2.2	1.5	2.1	3.1	2.1	2.4	3.5	NA	2.0	13	83	19	10
1,4,2,3-DMN	2.1	0.71	1.3	1.5	1.8	1.6	1.8	ND	1.9	1.7	3.1	2.1	2.4	4.9	NA	3.2	22	190	37	17
1,5-DMN	ND	3.2	ND	2.0	2.4	NA	1.7	13	88	19	12									
Acenaphthylene	1.2	1.3	1.4	1.7	1.8	1.3	1.5	1.5	1.6	1.5	2.8	2.7	2.8	2.0	NA	1.8	5.0	ND	4.9	3.2
1,2-DMN	ND	0.82	1.2	ND	ND	ND	ND	ND	ND	1.6	ND	0.99	1.7	NA	1.8	4.5	35	7.8	10	10
Acenaphthene	1.5	1.6	1.6	1.4	0.86	3.0	1.8	1.8	1.3	1.5	4.3	ND	2.3	1.7	NA	2.1	6.3	10	7.5	3.1
1,6,7-TMN	5.0	0.78	3.3	1.3	1.6	3.0	ND	3.0	3.2	9.8	2.0	ND	4.2	1.2	NA	11	100	570	160	41
Fluorene	4.4	2.8	2.5	3.3	2.5	2.5	3.9	3.7	2.9	3.2	5.2	5.6	3.2	3.2	NA	3.2	12	61	17	5.6
Dibenzothiophene	5.8	1.2	1.9	1.5	1.3	1.5	1.5	1.7	1.6	2.1	3.3	1.6	2.1	2.8	NA	2.2	8.3	47	15	4.4
Phenanthrene	19	3.4	3.3	5.2	3.4	3.2	3.5	9.4	8.7	9.7	18	6.7	9.8	9.8	NA	9.7	27	180	38	21
Anthracene	3.8	4.2	3.6	4.8	3.5	3.9	5.0	4.7	4.0	4.8	5.0	2.8	3.6	4.6	NA	3.4	13	43	11	6.0
4-MDBT	8.3	4.2	4.0	4.4	2.7	3.3	3.9	4.8	4.6	6.1	6.2	4.6	5.4	8.6	NA	6.7	44	220	59	13
2/3-MDBT	4.5	4.2	2.8	3.3	2.6	2.4	2.5	2.2	2.1	4.9	3.0	3.6	3.7	5.3	NA	3.5	23	110	27	11
3-MP	9.4	6.2	5.9	6.1	4.9	5.3	4.4	7.3	8.9	13	7.7	7.4	10	17	NA	13	81	450	130	29
2-MP	8.6	3.1	2.2	3.6	2.0	3.0	2.8	2.9	3.0	4.7	3.5	3.8	3.8	3.8	NA	3.8	33	200	49	10
4/9-MP	10	5.8	5.7	5.1	4.7	4.5	4.1	8.9	8.2	12	8.5	6.7	11	17	NA	14	78	390	120	27
1-MP	6.4	2.6	8.6	4.4	2.5	7.3	8.2	3.9	6.9	8.1	4.5	4.6	6.1	1.0	NA	8.1	47	230	1.3	15
3,6-DMP	9.0	5.8	3.8	4.7	3.0	3.4	3.1	7.5	5.2	7.7	4.3	6.3	11	11	NA	8.5	41	240	57	25
3,5-DMP	ND	NA	ND	ND	NA	ND	ND													
2,6-DMP	4.2	2.9	2.7	2.6	2.6	2.7	2.1	4.0	4.3	6.3	7.5	3.8	5.9	8.9	NA	7.1	37	150	52	15
2,7-DMP	3.3	2.7	2.5	2.2	2.5	2.3	2.4	3.3	4.1	5.1	7.5	3.3	5.5	7.5	NA	6.0	30	110	40	12
1,2-DMDBT	ND	ND	ND	1.0	0.54	ND	ND	ND	ND	0.53	ND	ND	1.9	1.6	NA	1.5	8.3	22	3.6	4.2
3,9-DMP	10	8.1	7.8	7.1	6.6	7.3	6.2	11	15	23	14	12	23	34	NA	26	130	520	190	57
1,6/2,5/2,9-DMP	7.2	6.0	5.5	5.5	5.8	5.2	4.9	8.8	12	18	10	9.2	18	26	NA	20	90	360	120	38
1,7-DMP	2.4	1.9	1.8	1.6	1.8	1.5	1.8	2.6	3.7	5.7	3.9	3.1	5.8	8.6	NA	6.3	34	140	47	14
1,8-DMP	4.0	3.3	3.9	4.1	3.7	4.5	3.5	4.2	5.1	6.0	5.1	5.0	8.4	10	NA	8.1	33	120	45	16
1,5-DMP	ND	NA	ND	ND	140	ND	ND													
Fluoranthene	27	26	23	22	29	22	21	35	27	38	34	23	26	26	NA	24	34	100	42	54
1,8-DMP	1.5	0.80	0.74	1.3	1.3	1.8	1.3	1.7	2.1	2.9	2.3	1.7	3.0	3.8	NA	9.4	14	53	20	6.9
1,2-DMP	4.1	1.9	1.7	1.9	2.4	1.7	ND	2.2	2.1	4.0	2.4	2.9	2.9	3.7	NA	2.7	10	22	16	6.1
9,10-DMP	1.7	1.2	0.77	0.84	0.93	0.91	0.90	1.2	1.1	1.7	1.5	1.3	1.3	1.2	NA	0.88	4.1	ND	6.3	0.74
Pyrene	27	29	26	25	29	26	25	40	34	42	40	30	30	31	NA	28	32	84	37	45
1,2,8-TMP	3.1	3.3	1.7	4.4	3.9	4.1	ND	2.0	4.8	5.7	3.1	3.4	6.9	13	NA	12	59	96	34	19
Benzanthracene	160	8.7	8.2	11	13	8.1	11	10	8.1	15	10	8.2	9.0	10	NA	10	40	110	38	18
Chrysene	42	15	14	29	18	13	25	18	16	22	16	12	18	21	NA	17	43	110	40	36
Benzo(b)fluor	26	16	16	23	18	16	21	25	18	22	28	23	20	17	NA	17	ND	ND	ND	ND
Benzo(k)fluor	ND	NA	ND	ND	ND	ND	ND													
Benzo(a)pyrene	NA																			
Indenopyrene	TR	ND	TR																	
Dibenzanthracene	TR	ND	TR																	
Benzo(p)ylene	TR	ND	TR																	
Total Parent PAH	323	113	105	132	124	104	125	176	250	285	324	232	222	183	NA	197	327	1316	400	287
Total Alkylated PAH	129	77	77	88	70	76	64	99	118	173	146	113	165	251	NA	201	1045	5345	1421	522
Total PAH	452	190	182	220	194	181	189	276	368	458	470	345	386	434	NA	399	1372	6661	1821	809
MEANS																				
Moisture, %	68%							66%				64%				63%				64%
Total Parent PAH	168							132				273				201				582
Total Alkylated PAH	93							77				137				206				2083
Total PAH	261							210				410				406				2666
Ratio Alk./Par.	0.55							0.59				0.50				1.02				3.58

ANALYTE	14-1A	14-1B	14-1C	14-1D	14-2A	14-2B	14-2C	14-2D	14-3A	14-3B	14-3C	14-3D	14-4A	14-4B	14-4C	14-4D	14-5A	14-5B	14-5C	14-5D
Moisture, %	63%	66%	65%	60%	61%	64%	77%	70%	63%	66%	65%	66%	63%	66%	62%	71%	73%	72%	72%	70%
Naphthalene	5.1	5.2	4.7	6.0	4.7	4.5	8.0	3.5	4.6	3.9	4.5	4.6	3.8	3.5	4.2	5.0	8.6	5.9	5.3	7.2
2-MN	2.8	4.1	4.2	5.9	4.4	3.3	5.6	3.8	4.2	3.4	4.4	3.1	4.4	3.7	3.2	6.0	9.4	8.8	6.2	8.0
1-MN	3.3	2.4	3.7	3.6	3.9	4.3	5.5	2.7	2.3	2.1	3.3	5.1	3.7	2.7	5.5	4.1	6.5	5.4	3.8	5.2
2-EN	ND	2.6	ND	ND	ND	ND	2.6	ND	ND	ND	2.5	7.4	3.7	2.3						
1-EN	ND	6.3	ND	2.1																
2,6/2,7-DMN	23	20	28	24	8.0	ND	33	ND	19	11	19	11	ND	ND	3.0	49	89	120	75	120
1,3/1,7-DMN	1.7	3.1	4.0	2.4	3.3	2.0	4.7	3.0	3.0	1.9	3.0	3.7	7.7	5.9	5.1	6.6	43	12	12	27
1,6-DMN	ND	2.0	2.7	ND	2.4	1.8	3.5	2.1	1.6	1.8	1.8	1.5	3.8	3.5	3.7	3.4	15	5.0	6.5	11
1,4/2,3-DMN	ND	ND	ND	ND	ND	ND	4.0	1.8	ND	1.1	ND	ND	4.5	4.8	3.0	4.9	28	10	8.7	18
1,5-DMN	ND	ND	ND	ND	ND	ND	2.3	1.4	1.5	1.6	ND	2.9	2.1	2.4	2.3	15	4.6	4.6	12	4.0
Acenaphthylene	1.5	4.1	2.1	3.4	1.8	1.1	3.1	1.6	2.0	2.5	2.4	2.7	1.9	2.1	2.2	2.9	4.2	3.2	3.2	4.0
1,2-DMN	ND	0.62	ND	ND	ND	ND	1.3	1.3	ND	1.9	6.1	4.0	2.3	4.8						
Acenaphthene	ND	1.6	2.8	ND	1.4	1.4	3.0	1.5	1.2	1.7	ND	ND	2.5	1.9	2.3	3.2	7.9	3.0	3.5	5.7
1,6,7-TMN	ND	3.9	1.9	ND	3.2	3.3	7.6	2.6	5.3	2.5	3.6	4.8	15	10	12	19	120	19	20	83
Fluorene	2.8	5.2	3.1	4.5	3.3	3.2	4.5	3.0	3.2	2.0	3.0	3.0	4.5	2.9	3.8	4.2	15	6.0	7.5	11
Dibenzothiophene	ND	1.8	2.5	1.8	1.8	1.6	2.7	1.8	1.8	1.5	1.8	1.9	3.4	2.9	2.1	3.6	13	5.2	5.3	8.4
Phenanthrene	6.4	2.1	1.7	1.1	8.6	1.3	1.3	9.0	1.1	1.0	8.8	1.1	12	9.0	1.1	12	36	19	19	26
Anthracene	2.9	2.5	5.0	4.9	4.1	7.0	5.0	4.4	3.4	4.2	3.4	3.3	5.3	3.9	4.4	5.5	12	7.3	7.3	8.9
4-MDBT	2.8	3.4	4.2	2.5	3.7	2.9	5.2	5.9	4.1	4.1	4.1	3.6	10	9.3	6.1	12	49	15	18	28
2/3-MDBT	4.2	2.3	ND	4.7	2.1	1.6	1.7	1.7	1.8	0.92	ND	3.2	4.8	4.3	3.3	6.2	22	7.2	7.7	13
3-MP	4.3	7.3	6.9	8.1	6.8	5.4	9.5	6.4	7.6	6.1	9.7	7.5	20	20	13	23	100	28	34	52
2-MP	2.1	4.0	3.5	4.1	2.4	3.0	5.4	2.4	3.1	2.6	2.9	4.6	5.7	4.3	3.3	5.5	28	7.7	9.8	12
4/9-MP	5.1	5.8	7.0	8.4	5.5	5.1	7.9	6.2	6.3	5.9	7.6	6.5	19	20	12	23	92	26	33	52
1-MP	2.7	3.6	4.7	4.4	3.2	2.3	4.0	2.9	3.9	3.4	5.3	3.5	10	12	6.4	14	56	15	19	31
3,6-DMP	2.8	4.4	6.3	4.4	4.2	3.2	4.0	2.9	3.9	3.4	5.3	3.5	10	12	6.4	14	56	15	19	31
3,5-DMP	ND																			
2,6-DMP	1.8	2.7	3.5	3.4	2.9	2.5	3.8	3.0	3.4	3.2	4.1	3.6	10	12	6.7	13	44	14	16	26
2,7-DMP	2.4	2.6	2.8	2.7	3.1	1.9	3.6	2.6	3.0	2.9	3.9	3.4	8.5	9.6	5.4	11	33	13	13	21
1,2-DMDBT	ND	0.99	ND	ND	ND	2.0	2.6	1.9	3.2	5.4	3.5	2.9	4.3							
3,9-DMP	5.8	8.0	9.1	8.8	9.4	6.7	12	9.2	10	9.9	15	10	39	46	23	53	170	55	59	97
1,6/2,5/2,9-DMP	5.2	7.4	7.6	7.6	6.1	9.9	7.2	8.7	8.1	12	8.0	26	34	18	41	110	36	39	64	64
1,7-DMP	1.6	2.8	2.0	2.2	2.1	1.5	3.0	2.0	2.7	2.6	4.0	2.7	9.1	11	5.5	12	39	13	13	24
1,9/4,9-DMP	3.9	5.1	4.3	4.6	5.3	3.7	8.8	3.7	5.6	4.5	7.9	4.5	11	13	7.8	16	38	16	15.0	24
1,5-DMP	ND	1.6	ND	ND	ND	ND														
Fluoranthene	17	40	38	28	26	26	41	29	30	36	26	56	26	27	28	32	40	47	30	33
1,8-DMP	ND	1.2	2.1	1.1	1.5	0.99	3.4	1.2	2.2	2.4	2.3	2.2	4.5	5.4	3.4	6.1	16	6.2	8.0	9.7
1,2-DMP	2.2	2.2	2.8	ND	1.7	0.95	4.5	2.4	2.3	2.4	2.4	2.5	3.9	4.4	3.2	4.7	10	6.4	4.0	8.1
9,10-DMP	1.1	1.3	1.4	1.5	1.3	0.98	2.4	1.4	1.3	0.91	1.8	1.4	1.3	1.5	1.1	1.3	ND	2.5	6.6	5.2
Pyrene	2.1	4.2	4.1	3.4	3.3	3.0	4.6	3.1	3.5	3.6	3.4	5.4	28	30	35	40	37	51	33	32
1,2,8-TMP	ND	3.8	4.3	ND	2.5	1.4	2.3	2.7	2.6	1.8	4.0	2.2	7.6	16	7.3	22	45	32	25	48
Benzanthracene	9.2	19	21	11	9.7	9.3	17	9.5	9.7	14	10	9.7	13	13	10	15	39	23	22	33
Chrysene	17	28	35	25	25	22	40	28	28	29	28	23	27	15	17	26	36	31	28	55
Benzo(b)fluor	ND																			
Benzo(k)fluor	NA																			
Benzo(a)pyrene	ND	19	15	20	7.3	5.0	7.5	7.7	5.4	12	5.4	5.1	6.5	6.5	12	30	65	52	38	45
Indenopyrene	ND																			
Dibenzanthracene	ND																			
Benzoperylene	5.0	ND	20	20	8.2	6.6	13	9.2	ND	ND	ND	7.2	5.5	4.6	4.9	5.7	6.8	5.2	6.4	8.4
Total Parent PAH	98	237	236	166	154	154	244	160	157	178	147	198	180	203	200	278	513	408	339	473
Total Alkylated PAH	79	103	117	105	90	65	161	87	114	93	128	105	251	280	177	385	1275	507	486	863
Total PAH	176	340	353	290	245	218	405	247	270	271	275	302	431	483	377	664	1788	915	825	1336
MEANS																				
Moisture, %	63%						68%					65%				65%				72%
Total Parent PAH	189						178					170				215				433
Total Alkylated PAH	101						101					110				274				783
Total PAH	290						279					280				489				1216
Ratio Alk./Par.	0.53						0.57					0.65				1.27				1.81

ANALYTE	21-1A	21-1B	21-1C	21-1D	21-2A	21-2B	21-2C	21-2D	21-3A	21-3B	21-3C	21-3D	21-4A	21-4B	21-4C	21-4D	21-5A	21-5B	21-5C	21-5D
	74%	70%	64%	64%	64%	64%	64%	64%	64%	58%	61%	62%	63%	63%	64%	65%	66%	66%	65%	62%
Moisture, %	5.8	3.9	4.6	4.6	6.1	5.0	7.8	4.4	4.4	3.9	3.8	5.7	4.6	4.4	5.4	3.6	5.6	4.1	5.4	5.2
Naphthalene	4.4	3.8	4.7	2.8	3.1	4.2	3.7	4.9	4.4	3.1	3.5	4.0	3.4	3.9	4.6	3.8	9.0	4.1	6.4	6.8
2-MN	3.0	2.5	2.5	3.5	3.6	2.8	5.4	4.9	2.6	2.0	3.6	4.0	2.3	2.7	4.8	2.9	4.2	2.5	3.9	4.5
1-MN	ND	1.5	ND	ND	1.3	5.3	1.8	2.8	ND											
2-EN	ND	2.0	2.0	ND																
1-EN	ND	2.0	2.0	ND																
2,6/2,7-DMN	4.8	ND	64	32	56	30	56	74	28	82	32	33	82							
1,3/1,7-DMN	3.1	2.3	2.8	2.9	2.3	2.9	4.2	3.8	3.4	2.6	2.7	3.4	3.7	4.2	4.9	2.7	3.5	6.4	14	22
1,6-DMN	2.4	2.1	2.8	2.0	2.4	2.9	2.0	3.0	3.5	2.2	2.6	2.9	2.7	2.6	4.0	2.2	9.7	3.2	6.2	9.4
1,4/2,3-DMN	1.9	1.8	ND	ND	2.2	1.8	2.3	ND	5.0	1.8	3.1	2.1	2.5	2.8	2.1	2.1	2.5	4.5	11	16
1,5-DMN	2.0	1.5	ND	ND	1.5	ND	1.8	2.2	ND	ND	1.9	1.9	1.6	2.4	3.1	1.5	5.0	2.0	4.8	4.8
Acenaphthylene	ND	2.1	2.7	ND	1.8	1.2	ND	ND	1.2	5.2	2.2	3.0	2.0							
1,2-DMN	2.1	2.1	ND	1.3	2.4	1.6	1.8	2.1	5.2	2.3	1.5	2.0	1.9	2.1	ND	1.5	6.6	2.2	6.5	4.8
Acenaphthene	4.7	3.0	3.1	ND	ND	2.9	ND	6.4	5.4	3.7	4.5	5.8	5.4	6.1	9.4	3.2	11.0	10	37	56
1,6,7-TMN	4.3	5.0	3.0	1.8	2.4	2.1	2.4	4.2	4.0	3.3	2.6	4.2	3.5	3.2	6.0	3.6	2.8	4.0	6.8	8.8
Fluorene	1.9	2.1	1.4	1.7	1.8	1.5	1.7	2.3	2.6	1.9	1.7	2.3	2.5	2.9	3.1	2.0	1.2	3.1	6.6	9
Dibenzothiophene	9.4	14	9.5	6.9	12	9.5	11	14	25	14	9.3	11	8.8	13	12	8.7	5.8	11	24	24
Phenanthrene	5.1	4.1	4.3	3.4	3.8	4.6	4.4	4.4	5.7	4.9	4.6	5.5	7.1	8.6	7.4	5.3	9.3	31	44	44
Anthracene	2.8	3.0	3.7	1.0	1.8	1.4	ND	4.3	5.1	2.6	2.2	2.6	3.4	4.4	3.2	1.8	2.1	3.6	13	19
4-MDBT	7.4	6.6	4.2	6.5	5.3	6.4	7.4	7.1	13	8.9	7.5	9.6	13	15	16	8.0	10.0	17	58	86
3-MP	2.7	3.2	2.6	2.3	3.0	2.5	2.5	3.4	5.2	5.3	2.7	4.1	3.0	3.8	6.2	2.0	2.4	4.2	16	16
2-MP	6.8	6.3	5.6	5.5	6.0	4.9	6.0	6.9	11	8.1	6.5	8.9	13	16	15	7.4	9.8	18	62	91
4/9-MP	6.3	4.9	3.0	3.6	3.6	3.0	3.9	5.1	7.2	4.8	3.7	5.2	7.9	9.5	8.6	4.2	5.7	10	37	51
1-MP	6.8	3.9	4.8	3.3	4.7	3.1	6.2	7.1	6.6	7.6	7.8	6.5	9.8	12	9.6	4.8	6.1	11	65	92
3,6-DMP	ND																			
3,5-DMP	2.8	2.6	2.4	2.5	2.1	2.2	3.1	4.1	5.2	2.8	3.6	5.2	7.1	9.0	7.0	3.9	4.9	8.3	38	52
2,6-DMP	3.0	2.9	2.6	2.5	2.2	2.2	3.0	3.9	4.9	3.2	3.5	4.7	6.4	8.0	7.4	3.3	4.0	7.2	31	41
2,7-DMP	ND	2.1	ND	ND	ND	1.8	2.7	2.3	0.81	5.9	1.9	2.2	8.3							
1,2-DMDBT	9.5	9.1	7.9	7.6	7.9	6.9	8.0	12	20	12	12	17	28	34	29	13	19.0	34	150	200
3,9-DMP	8.1	7.2	5.6	6.2	5.8	5.0	5.8	9.2	14	10	10	14	20	26	23	8.8	14.0	20	88	160
1,6/2,5/2,9-DMP	1.9	1.8	2.2	1.6	1.6	1.7	1.7	3.4	4.5	2.1	2.7	4.0	6.2	7.8	6.9	2.6	4.5	7.4	35	49
1,7-DMP	5.4	4.2	4.4	3.4	3.5	4.7	2.5	4.7	8.3	6.0	4.3	6.1	10	12	16	4.8	4.4	11	36	50
1,9/4,9-DMP	ND	2.5	ND																	
1,5-DMP	25	27	24	22	32	22	29	45	45	68	33	41	22	39	36	18	49	22	66	60
Fluoranthene	1.1	0.81	1.8	0.91	0.87	ND	1.1	2.4	2.8	1.7	1.8	2.3	3.3	4.2	4.6	1.7	1.9	3.4	15	22
1,8-DMP	2.0	1.6	ND	1.4	1.7	1.5	1.5	3.0	3.4	2.1	1.9	3.0	2.6	2.6	7.9	1.5	1.2	3.1	11	14
1,2-DMP	1.0	1.4	1.4	1.1	1.0	1.0	1.4	2.8	0.69	1.1	1.4	1.3	1.9	1.2	2.2	2.0	5.0	0.83	5.9	3.6
9,10-DMP	29	31	35	32	42	31	38	58	61	57	39	46	28	46	48	22	45	26	61	55
Pyrene	6.4	7.9	1.8	1.6	1.4	2.0	1.7	2.3	5.0	5.2	2.8	6.1	7.6	12	12	3.5	5.5	6.5	26	68
1,2,8-TMP	12	12	19	13	14	12	13	24	28	14	9.2	11	12	19	14	9.0	32	13	48	23
Benzanthracene	19	24	22	14	17	13	13	47	27	28	1.5	24	32	38	27	19	120	32	110	100
Chrysene	20	20	55	29	46	42	41	56	64	31	22	23	29	32	30	25	41	28	80	43
Benzo(b)fluor	NA	9.0	8.3	13	12	5.9	5.2	8.0	26	22										
Benzo(k)fluor	8.9	13	9.1	14	12	16	8.2	25	27	18	15	16	8.3	11	8.5	7.1	27	2.8	18	26
Benzo(a)pyrene	24	26	ND	12	8.8	22	24	23	6.2	ND	TR	5.7	TR	TR	TR	3.3	24	5.9	7.3	TR
Indeno(1,2,3-cd)pyrene	51	53	35	23	18	28	36	31	34	42	34	47	9.4	55	75	7.8	47	TR	9.6	53
Dibenzanthracene	219	325	245	195	254	221	261	543	425	306	188	248	182	288	286	142	635	168	491	453
Benzoperylene	148	87	74	71	70	72	78	113	154	168	130	188	207	267	288	127	1322	247	845	1275
Total Parent PAH	367	412	319	267	324	293	339	656	579	474	313	437	389	555	575	269	1957	415	1336	1728
Total Alkylated PAH																				
MEANS																				
Moisture, %	68%							64%				61%								65%
Total Parent PAH	246						320					291								437
Total Alkylated PAH	95						83					160								922
Total PAH	341						403					451								1359
Ratio Alk./Par.	0.39						0.26					0.55								2.11



The Department of the Interior Mission

As the Nation's principal conservation agency, the Department of the Interior has responsibility for most of our nationally owned public lands and natural resources. This includes fostering sound use of our land and water resources; protecting our fish, wildlife, and biological diversity; preserving the environmental and cultural values of our national parks and historical places; and providing for the enjoyment of life through outdoor recreation. The Department assesses our energy and mineral resources and works to ensure that their development is in the best interests of all our people by encouraging stewardship and citizen participation in their care. The Department also has a major responsibility for American Indian reservation communities and for people who live in island territories under U.S. administration.



The Minerals Management Service Mission

As a bureau of the Department of the Interior, the Minerals Management Service's (MMS) primary responsibilities are to manage the mineral resources located on the Nation's Outer Continental Shelf (OCS), collect revenue from the Federal OCS and onshore Federal and Indian lands, and distribute those revenues.

Moreover, in working to meet its responsibilities, the **Offshore Minerals Management Program** administers the OCS competitive leasing program and oversees the safe and environmentally sound exploration and production of our Nation's offshore natural gas, oil and other mineral resources. The **MMS Minerals Revenue Management** meets its responsibilities by ensuring the efficient, timely and accurate collection and disbursement of revenue from mineral leasing and production due to Indian tribes and allottees, States and the U.S. Treasury.

The MMS strives to fulfill its responsibilities through the general guiding principles of: (1) being responsive to the public's concerns and interests by maintaining a dialogue with all potentially affected parties and (2) carrying out its programs with an emphasis on working to enhance the quality of life for all Americans by lending MMS assistance and expertise to economic development and environmental protection.