



## Influence of polycyclic aromatic hydrocarbons on the meiobenthic-copepod community of a Louisiana salt marsh

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Received 16 February 1995; revised 23 June 1995; accepted 6 September 1995

### Abstract

The influence of sediment contaminated with polycyclic aromatic hydrocarbons (PAHs) on the meiobenthic copepod community of a Louisiana salt marsh was examined with microcosms of sediment containing natural faunal assemblages. Microcosms were dosed with PAH-contaminated sediment that ranged in concentration from 0.3 (controls) to 27 mg PAH/kg dry sediment, and effects were followed for 28 days. Data suggest that highest doses (High-PAH) elicited avoidance behavior by *Pseudostenhelia wellsi* (Coull and Fleeger) copepods and nauplii, particularly 12 h after contaminants were added (Day 0). Weaker avoidance behavior was indicated in non-*P. wellsi* species that was manifested as increases in nauplius/copepod ratios. Nematode/copepod ratios were calculated for each of 4 species (*P. wellsi*, *Coullana* sp., *Enhydrosoma* sp., and *Onychocampus mohammed* (Blanchard and Richard)); only nematode/*P. wellsi* ratios were significantly affected by PAH. High-PAH treatments also lead to a higher proportion of females in *P. wellsi* and *Coullana* sp. This effect was only observed from Day 7 on and is interpreted as an indication of disproportionately high mortality of males. Collectively, our data demonstrate that relatively low-level PAH contamination may cause many ecologically important impacts on copepod community structure that may not be detected at the level of higher taxon.

**Keywords:** Meiofauna; Copepods; Polycyclic aromatic hydrocarbon; Sediment; Demographics; Sex ratio

### 1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are well-recognized contaminants of coastal marine ecosystems (Kennish, 1992). In marine systems, much PAH contamination comes from petroleum that originates from spills of crude oil, discharges of refined fuels

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and byproducts of offshore production activities (Kennish, 1992). Coastal Louisiana is particularly susceptible to petroleum-hydrocarbon contaminants as the result of decades of intense offshore drilling activity (Fang, 1990). Significant toxicological effects of PAHs on benthic organisms have been observed at concentrations of  $\leq 1$  mg-PAH/kg dry sediment (e.g., Long, 1992). Petroleum hydrocarbons have been shown to affect life-history parameters (Bridges et al., 1994) and immune systems (Tahir et al., 1993) of benthic organisms, as well as the structure of entire benthic communities (Nance, 1991). Bioaccumulation of PAHs is, however, inversely correlated with percent organic carbon in sediments (Weston, 1990), and thus toxic concentrations would be expected to vary among sediment types. Field and laboratory studies have documented that the meiofaunal component of the benthos is sensitive to petroleum contaminants (Coull and Chandler, 1992), and that meiobenthic copepods are (frequently) relatively more susceptible to petroleum hydrocarbons than other meiofaunal taxa, such as nematodes (e.g., McLachlan and Harty, 1979; Elmgren et al., 1983; Stacey and Marcotte, 1987; Heip et al., 1988; Warwick et al., 1988; Sandulli and de Nicola-Giudici, 1989).

Here, we show that consideration of the effects of PAHs on copepods at the level of major taxon, or even species, can result in the failure to detect potentially ecologically important changes in the copepod community. Our data are from a microcosm study designed to examine the impact of PAH-contaminated sediment on an intact meiofaunal/microbial community from a salt marsh in coastal Louisiana. The effects of PAHs on microbial activity and abundance, meiofaunal grazing, copepod physiological condition, and higher meiofaunal taxa are presented in a separate paper (Carman et al., 1995). Here, we focus on the influences of PAHs on species, developmental stages, and sexes of the copepod community, and we discuss the ecological implications of these effects on copepod demography.

## 2. Materials and methods

Microcosm experiments were performed with a  $4 \times 5 \times 7$  factorial design, with 4 wet tables (as blocks), 7 exposure times and 5 PAH treatments as factors (Carman et al., 1995). Microcosms were constructed of 15.2 cm I.D. PVC pipe with windows covered with Nitex mesh (62  $\mu$ m) to allow exchange of water. At low tide on 28 May 1991, 140 microcosms of exposed unvegetated sediment were collected by hand from mud flats surrounded by *Spartina alterniflora* marsh. Microcosms were gently pushed into the sediment to a depth of 15 cm, mud was excavated from the outside of the microcosm, and a form-fitting base was placed on the bottom. The intact core of sediment was removed from the mud flat and transported to the Louisiana Universities Marine Consortium (LUMCON) facility at Cocodrie, LA, USA. 35 microcosms were randomly assigned to each of 4 wet tables. Wet tables were irrigated with water pumped directly from the salt marsh near LUMCON (i.e., water from the same habitat from which the microcosms were collected). Water levels were maintained at the top of the Nitex mesh to allow water to percolate through the microcosms (see, for details, Carman et al., 1995).

The PAH treatments consisted of the addition to the microcosms of sediment dosed

with 3 levels of PAH (Low, Medium and High), and 2 types of controls. In one control, no sediment was added to the microcosms; in the second control 'uncontaminated' sediment was added to the microcosms. Four replicate microcosms (1 from each wet table) of each of the 5 treatment levels (20 total microcosms) were harvested at each of 7 time intervals (0, 1, 3, 7, 14, 21 and 28 days) following a previously determined randomization schedule. Sediment cores of various sizes were collected from each microcosm for measurements of microbial activity and abundance, meiofaunal grazing, copepod physiological condition and meiofaunal abundance (Carman et al., 1995). Among the cores collected, one core (2.5 cm I.D., 12 cm long) was used for characterization of the meiofaunal community. From this core, the top 1 cm of sediment (and overlying water) was collected and preserved in borax-buffered 4% formaldehyde solution. Meiofauna were stained with rose bengal. Copepods were identified to species and further identified as male, female, gravid female or copepodite. Total nauplii, as well as *Pseudostenhelia wellsi* (Coull and Fleeger) nauplii were also enumerated. Here, we examine various aspects of the copepod community from a subset of the larger experiment (Days 0, 7, 14 and 28).

Two sediments, one a contaminated sediment collected near a Pass Fourchon 'produced-water' (contaminated water from offshore petroleum production activity) discharge site (Rabalais et al., 1991; Means and McMillin, 1993) and the other collected from a relatively clean reference area in Lake Champagne (Means and McMillin, 1993) were used for dosing microcosms with PAH-contaminated sediments. Pass Fourchon and Lake Champagne sediments are similar in physical characteristics (Means and McMillin, 1993) and the organic content is 2.3%. Both sediments were sieved through a 2 mm sieve and then homogenized on a roller mill for 1 week. High, Medium and Low doses of sediment for the microcosms were prepared by combining appropriate masses of wet contaminated and uncontaminated sediment and mixing them on a roller mill for 2 days. PAH concentrations in the added sediments were: High = 27 mg PAH/kg dry sediment (undiluted Pass Fourchon sediment); Medium = 5.4 mg PAH/kg; Low = 1.02 mg PAH/kg; Control = 0.29 (see Carman et al. (1995) for PAH composition). Microcosms were dosed with sediment sufficient to create a 1 mm-thick layer on the surface of each microcosm. Sediment treatments were administered on the evening of 29 May 1991. Day 0 samples were collected the following morning, and thus had been exposed to PAH contaminants for approximately 12 h.

Total PAH concentrations in the top 1 cm of sediment from the two types of control microcosms ranged from 0.210 to 0.445 mg PAH/kg and did not differ significantly from each other or change significantly in concentration over time (Carman et al., 1995). Final PAH concentration in the top 1 cm of High-PAH treatments was 2.87 mg PAH/kg at Day 0, and gradually decreased to a concentration of approximately 1.00 mg PAH/kg by Day 28. Further details of PAH concentrations are discussed in Carman et al. (1995).

Data were analyzed with SigmaStat statistical software as a two-factor analysis of variance (ANOVA). Although the experiment was designed as a three-factor ANOVA, we chose to simplify the analysis after repeated demonstration that 'wet-tables' (blocks) were not a significant factor (see Carman et al., 1995). Preliminary analysis also showed that the 2 controls did not differ from each other, and these data were combined into 1 control group for statistical analysis and graphical presentation. All analyses that

involved ratios were converted to proportions, or transformed by  $\log_{10}(n + 1)$  conversion to meet the assumptions of ANOVA. Abundance data were  $\log_{10}$  transformed. Normal-probability plots were examined and data were subjected to Levene's test of homogeneity of variance to determine if they were consistent with the assumptions of ANOVA. Significant ( $P < 0.05$ ) main effects were further examined with Student-Newman-Keuls a posteriori comparisons. When a significant PAH effect was detected, one-way ANOVAs were performed on individual days.

### 3. Results

Four copepod species, *Coullana* sp. (formerly referred to as *Scottolana canadensis* (Willey) in Louisiana publications), *Pseudostenhelina wellsi* (Coull and Fleeger), *Enhydrosoma* sp. and *Onychocamptus mohammed* (Blanchard and Richard) comprised an average of 94% of the copepod community. Means and standard deviations of species abundances and abundances of total copepods are presented in Table 1. In comparison to previously published values for salt marsh muds (0–325 copepods  $10 \text{ cm}^{-2}$ ; Coull, 1988), copepod abundance was exceptionally high (191–760 copepods  $10 \text{ cm}^{-2}$ ). Even the relatively low abundances of copepods on Day 28 were high in comparison to previously published values. We have no explanation for why copepod abundances were unusually high.

We considered the potential effects of PAHs on the 4 numerically dominant copepod species individually and on copepods as a group. Results of 33 two-way ANOVAs are summarized in Table 2. 12 of the ANOVAs detected a significant effect of PAH

Table 1  
Abundances of 4 copepod species and total copepods

Day	Trt	Total	<i>Coullana</i> sp.	<i>P. wellsi</i>	<i>Enhydrosoma</i>	<i>O. mohammed</i>
0	C	312 (119)	36 ( 12)	217 (118)	12 ( 12)	30 ( 20)
0	L	762 (235)	113 ( 75)	432 (118)	52 ( 43)	95 (135)
0	M	760 (299)	241 ( 94)	233 ( 88)	123 (115)	114 ( 51)
0	H	336 (189)	104 (104)	146 ( 56)	30 ( 36)	34 ( 5)
7	C	242 ( 44)	60 ( 28)	119 ( 39)	21 ( 24)	22 ( 11)
7	L	626 (120)	135 ( 40)	361 (119)	14 ( 10)	68 ( 40)
7	M	710 (112)	133 ( 53)	412 ( 47)	18 ( 13)	72 ( 55)
7	H	417 (222)	112 ( 68)	166 (149)	30 ( 7)	89 ( 68)
14	C	157 ( 51)	54 ( 20)	73 ( 41)	17 ( 25)	10 ( 7)
14	L	349 (101)	84 ( 39)	151 ( 57)	54 ( 75)	33 ( 33)
14	M	328 (130)	93 ( 46)	138 (125)	74 ( 74)	15 ( 10)
14	H	313 (238)	72 ( 34)	166 (200)	37 ( 40)	29 ( 25)
28	C	108 ( 25)	33 ( 16)	59 ( 21)	6 ( 5)	4 ( 2)
28	L	238 (112)	96 ( 49)	58 ( 38)	59 ( 48)	22 ( 9)
28	M	175 ( 60)	87 ( 5)	63 ( 55)	11 ( 8)	10 ( 5)
28	H	324 (195)	60 ( 31)	131 (176)	81 (100)	29 ( 36)

The abundances include copepodites and adults. Values are means and standard deviations ( $n = 8$  for controls,  $n = 4$  for all others) per  $10 \text{ cm}^2$ . 'Day' = day on which samples were collected. Trt, treatment level; C, combined controls; L, Low-PAH; M, Medium-PAH; H, High-PAH. See text for further explanation.

Table 2  
Two-way ANOVAs and their statistical significance

Test	$F_{3,62}$	SIGN.	A posteriori comparisons			
Total Males	6.40	<0.001	M <sup>a</sup>	L <sup>a</sup>	C <sup>a</sup>	H <sup>b</sup>
Total Females	1.17	ns				
Total Copepodites	5.50	0.002	C <sup>a</sup>	L <sup>a</sup>	H <sup>ab</sup>	M <sup>b</sup>
Total Nauplii	2.09	ns				
<i>P. wellsi</i>						
Total copepods	3.69	0.017	C <sup>a</sup>	L <sup>ab</sup>	M <sup>ab</sup>	H <sup>b</sup>
Males	3.70	0.016	L <sup>a</sup>	C <sup>a</sup>	M <sup>ab</sup>	H <sup>b</sup>
Females	0.63	ns				
Copepodites	7.47	<0.001	C <sup>a</sup>	L <sup>ab</sup>	M <sup>b</sup>	H <sup>b</sup>
Nauplii	1.221	ns				
<i>Coullana</i> sp.						
Total	2.91	0.041	M <sup>a</sup>	L <sup>ab</sup>	C <sup>ab</sup>	H <sup>b</sup>
Males	8.11	<0.001	M <sup>a</sup>	L <sup>a</sup>	C <sup>a</sup>	H <sup>b</sup>
Females	2.10	ns				
Copepodites	1.16	ns				
<i>O. mohammed</i>						
Total	0.98	ns				
Males	1.19	ns				
Females	0.77	ns				
Copepodites	2.13	ns				
<i>Enhydrosoma</i> sp.						
Total	1.62	ns				
Males	2.42	ns				
Females	1.53	ns				
Copepodites	1.63	ns				
Proportion Female						
Total	5.11	0.003	H <sup>a</sup>	M <sup>b</sup>	C <sup>b</sup>	L <sup>b</sup>
<i>P. wellsi</i>	4.69	0.005	H <sup>a</sup>	M <sup>ab</sup>	C <sup>b</sup>	L <sup>b</sup>
<i>Coullana</i> sp.	3.74	0.016	H <sup>a</sup>	M <sup>ab</sup>	L <sup>ab</sup>	C <sup>b</sup>
<i>O. mohammed</i>	0.25	ns				
<i>Enhydrosoma</i> sp.	1.03	ns				
Nauplius/ <i>P. wellsi</i>	1.91	ns				
Nauplius/copepod	4.41	0.007	H <sup>a</sup>	L <sup>ab</sup>	C <sup>b</sup>	M <sup>b</sup>
Nematode/copepod						
<i>P. wellsi</i>	4.80	0.004	H <sup>a</sup>	M <sup>ab</sup>	L <sup>ab</sup>	M <sup>b</sup>
<i>Coullana</i>	1.97	ns				
<i>O. mohammed</i>	0.11	ns				
<i>Enhydrosoma</i> sp.	0.17	ns				

Values under SIGN. are  $P$  values for main effect of PAH treatment; ns, not significant ( $P > 0.05$ ). Upper-case letters under A posteriori comparisons represent treatments: C, control; L, Low PAH; M, Medium PAH and H, High PAH. Treatments are listed in rank order of mean values (highest to lowest). Treatments with different superscripts (i.e., 'a' and 'b') differed significantly ( $P < 0.05$ ) from each other in a posteriori comparisons.

treatment. We did not correct  $p$ -values for a family-wide measure of significance, and thus our analyses are not as conservative as they could be. Nevertheless, in 11 of the significant ANOVAs, a posteriori comparisons revealed that the High-PAH treatment differed from one or more of the other treatments. In the a posteriori analysis of total copepodites, the Medium-PAH treatment differed significantly from control and Low-

PAH treatments. Other than the case of total copepodites, control, Low-PAH, and Medium-PAH treatments never differed significantly from each other.

The two-way ANOVAs also tested for the effect of Day as well as Day-treatment interactions. Only 2 significant Day-treatment interactions were detected (*O. mohammed* copepodites and total copepods); this is approximately the number of significant results that would be expected by chance from 33 tests. Significant effects of Day ( $P < 0.0001$ ) were detected for total copepods and nauplii, *P. wellsi* and nauplii, and *O. mohammed*; the general trend was one of decreasing abundance over time. For *Enhydrosoma* sp., however, total abundance ( $P = 0.461$ ), males ( $P = 0.524$ ), females ( $P = 0.458$ ) and copepodites ( $P = 0.212$ ) did not change significantly over time. Total abundance of *Coullana* sp. declined significantly over time ( $P = 0.032$ ), primarily because of the large decrease in the numbers of copepodites ( $P < 0.0001$ ). The effect of Day was marginally significant for *Coullana* sp. males ( $P = 0.0619$ ), and was not significant for females ( $P = 0.715$ ). Below, we consider in detail the effects of PAHs on the copepod community.

### 3.1. Abundance

The abundance of total (*Coullana* sp., *P. wellsi*, *Enhydrosoma* sp. and *O. mohammed* combined) copepods, females, and nauplii were not significantly influenced by PAH concentration (Table 2). Similarly, no significant overall effects of PAHs were observed on the abundances of males, females, copepodites or total abundances of *Enhydrosoma* sp. or *O. mohammed* (Table 2). The abundance of total males was significantly influenced by PAHs, and abundance in High-PAH treatments was significantly lower than in all other treatments (Table 2; Fig. 1). When considering individual days, PAHs significantly affected the abundance of total males on Day 7 ( $P = 0.038$ ), and abundances in High-PAH treatments were significantly lower than in all other treatments.

The abundance of total copepodites was significantly influenced by PAHs, and abundance in Medium-PAH treatments was significantly lower than in controls or Low-PAH treatments (Table 2; Fig. 2). Among individual days, however, copepodite abundance in High-PAH treatments was significantly lower ( $P < 0.001$ ) than all other treatments on Day 7, and significantly higher ( $P < 0.001$ ) than in all other treatments on Day 28. These convoluted results occurred partially because *Enhydrosoma* sp. copepodites generally increased over time in High-PAH treatments, while the abundance of other copepodites generally decreased (Fig. 2). Even though no significant overall effect of PAHs on *Enhydrosoma* sp. copepodites was detected, a one-way ANOVA on Day-28 data indicated that the effect of PAHs was significant ( $P = 0.0419$ ), and copepodite abundance was greatest in High-PAH treatments. A posteriori comparisons revealed that the High-PAH treatment differed significantly only from the Medium-PAH treatment.

The abundances of *P. wellsi* males and copepodites, and total *P. wellsi* (copepodites + adults) were significantly affected by PAHs, and abundances in High-PAH treatments were significantly lower than in controls (Table 2; Fig. 3). Significant effects of PAHs on total *P. wellsi* were detected on Day 0 ( $P = 0.004$ ) and Day 7 ( $P = 0.020$ ), at which times total *P. wellsi* abundances were significantly lower in High-PAH treatments than in all other treatments. PAHs significantly affected abundance of *P. wellsi* males on Day 7,

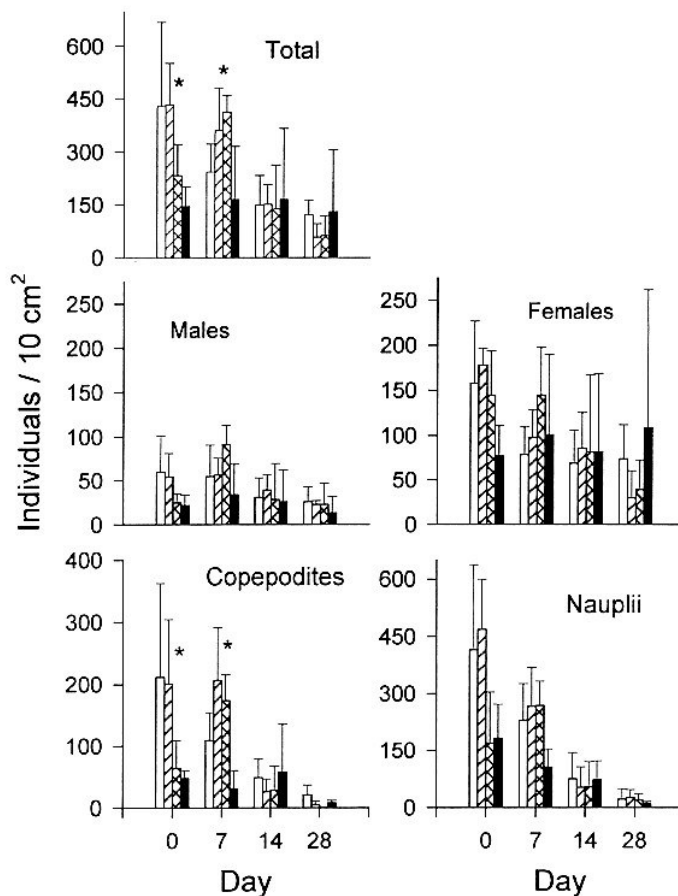


Fig. 1. Abundances of total copepod males, females and nauplii. Values are means + 1 SD (controls,  $n = 8$ ; Low-PAH, Medium-PAH and High-PAH treatments,  $n = 4$ ). Open bars, combined controls; diagonally hatched bars, Low PAH; cross-hatched bars, Medium PAH; black bars, High-PAH. Asterisks indicate individual days on which the effect of PAH was significant.

and High-PAH treatments differed significantly from Medium-PAH treatments. Significant effects of PAHs on *P. wellsi* copepodites were also detected on Day 0 ( $P = 0.017$ ) and Day 7 ( $P = 0.003$ ), and abundances were lowest in High-PAH treatments; only on Day 7, however, did a posteriori comparisons detect significant differences among treatments (High-PAH differed significantly from all other treatments).

The abundances of *Coullana* sp. males and total copepods were significantly affected by PAHs (Table 2; Fig. 4); male abundance in High-PAH treatments was significantly lower than in all other treatments, while abundance of total *Coullana* sp. in High-PAH treatments differed significantly only from controls. When considering individual days,

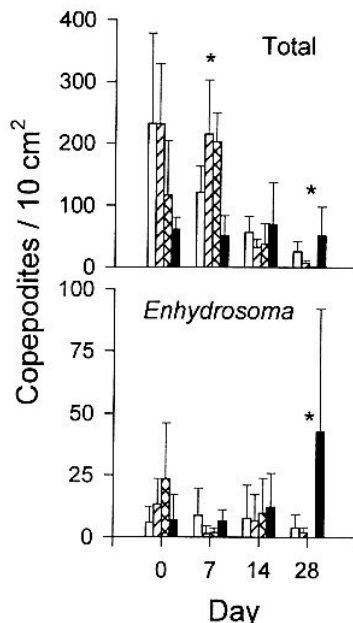


Fig. 2. Abundances of total copepodites and *Enhydrosoma* sp. copepodites. Symbols as in Fig. 1. Asterisks indicate individual days on which the effect of PAH was significant.

abundance of male *Coullana* sp. was significantly affected by PAHs only on Day 7 ( $P = 0.006$ ), at which time abundances in High-PAH treatments were significantly lower than in all other treatments. Total *Coullana* sp. in High-PAH treatments differed significantly from Medium-PAH treatments on Day 0.

### 3.2. Sex ratio

PAH concentration significantly influenced the proportion of females when considering all species collectively (Table 2; Fig. 5); the proportion of females was significantly higher in High-PAH treatments than in all other treatments. Among individual days, the effect of PAHs was significant on Day 7 ( $P = 0.034$ ), and again the proportion of females was highest in High-PAH treatments; a posteriori comparisons, however, revealed no significant differences among treatments on Day 7.

The proportion of *P. wellsi* females was significantly affected by PAHs (Table 2; Fig. 5), and proportions in High-PAH treatments were significantly higher than those in controls and the Low-PAH treatment. No significant effects of PAHs on the proportion of *P. wellsi* females were detected when examining individual days. A significant effect of PAHs on the proportion of females was also detected for *Coullana* sp. (Table 2; Fig. 5); proportions were highest in High-PAH treatments, which differed significantly from controls. The only significant single-day effect for *Coullana* sp. was on Day 0



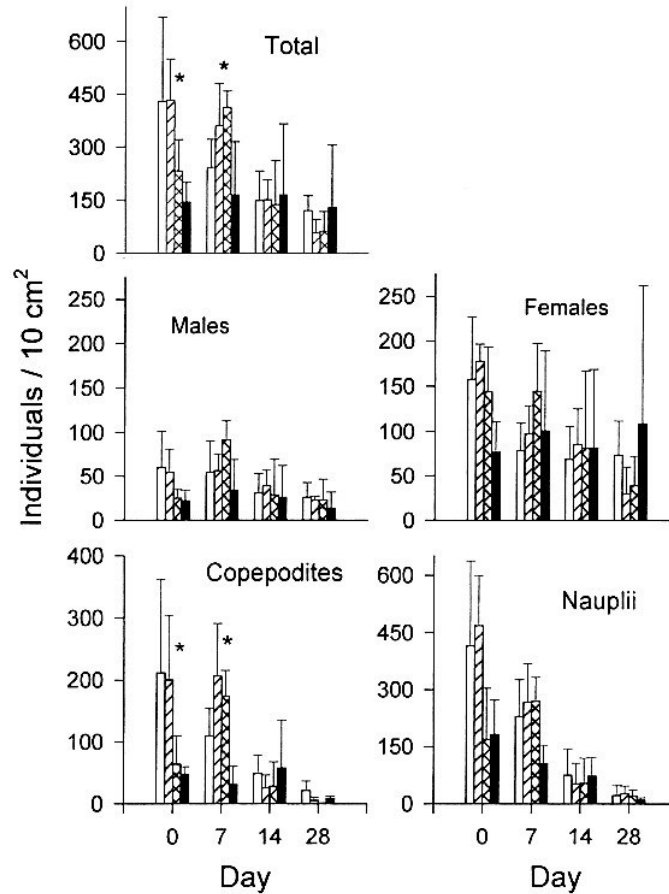


Fig. 3. Abundances of *Pseudostenhelia wellsi*: total copepods, males, females, copepodites, and nauplii. Symbols as in Fig. 1. Asterisks indicate individual days on which the effect of PAH was significant.

( $P = 0.028$ ), at which time proportions in High-PAH and Medium-PAH treatments were significantly higher than in controls; proportions in Medium-PAH were also higher than those in Low-PAH treatments.

### 3.3. Nauplius/copepod ratio

*P. wellsi* nauplii could be distinguished from other copepod nauplii in our samples because of their ellipsoid morphology (Chandler and Fleeger, 1984). Its distinctive morphology allowed us to analyze the nauplius/copepod ratio separately for *P. wellsi* nauplii and copepods (including copepodites and adults), and the nauplii and copepods of all other species combined (non-*P. wellsi*, including the few individuals not belonging

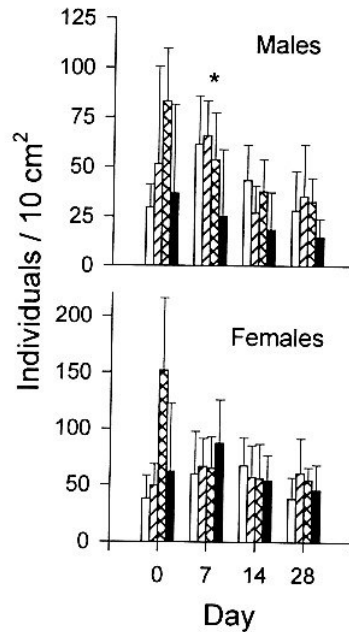


Fig. 4. Abundances of *Coullana* sp. total males and females. Symbols as in Fig. 1. Asterisks indicate individual days on which the effect of PAH was significant.

to the remaining three species explicitly considered in this paper). PAH concentration significantly affected the nauplius/copepod ratio for non-*P. wellsi* species, and the ratio in High-PAH treatments was significantly higher than in Medium-PAH treatments or controls (Table 2; Fig. 6). No significant effects of PAHs on nauplius/copepod ratios were detected on individual days.

#### 3.4. Nematode/copepod ratio

Nematode/copepod ratios were calculated for each of the 4 species considered here. Nematode data were taken from Carman et al. (1995). Overall, the nematode/*P. wellsi* ratio was significantly influenced by PAHs (Table 2; Fig. 7) and was highest in the High-PAH treatments; High-PAH treatments differed significantly only from controls. Among individual days, however, the effect of PAHs was significant only on Day 0 ( $P = 0.041$ ), at which time the ratio in High-PAH treatments was significantly higher than in controls. Although the changes were not significant, the nematode/*P. wellsi* ratio increased over time in Medium-PAH and Low-PAH treatments and showed a pattern resembling a dose-dependent response that lagged behind the High-PAH treatment (Fig. 7). Nematode/copepod ratios of the other 3 species were not significantly influenced by PAHs (Table 2; Fig. 7), although all 3 species showed highest ratios on Day 28 in

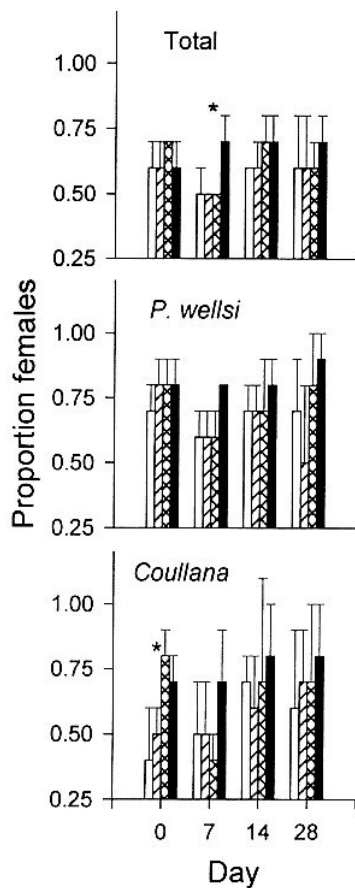


Fig. 5. Proportions of adults of total copepods, and of *P. wellsi* and *Coullana* sp. that were females. Symbols as in Fig. 1. Asterisks indicate individual days on which the effect of PAH was significant.

High-PAH treatments. When nematode/copepod ratios were considered for non-*P. wellsi* species as a group, the effect of PAHs was suggestive ( $P = 0.069$ ).

#### 4. Discussion

A major goal of our research was to test for subtle (sublethal) effects of PAHs on the meiofaunal/microbial community in this salt marsh. As such, we dosed microcosms with relatively modest (Long, 1992) concentrations of PAHs (maximum of 27 mg PAH/kg dry sediment). In a separate paper (Carman et al., 1995), we describe that we detected no significant influence of PAHs on the abundances of total copepods, nauplii, nematodes or meiofauna. Here, however, we have found that analysis of copepod

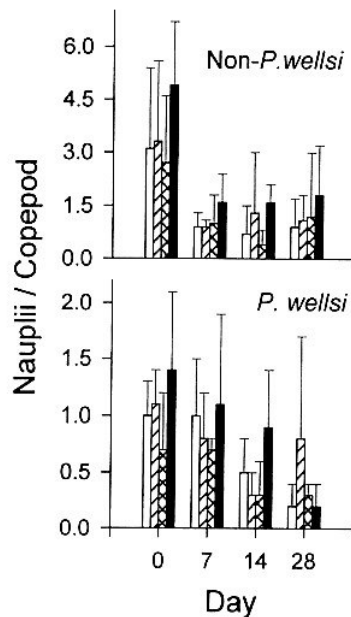


Fig. 6. The nauplius/copepod ratio for non-*P. wellsi* species and for *Pseudostenhelia wellsi*. Non-*P. wellsi* species includes all adults, copepods and nauplii other than *P. wellsi*. Symbols as in Fig. 1.

species, sexes, and developmental stages revealed several significant effects on the copepod community.

Although total copepod abundance was not influenced by PAHs, the abundance of total males (Fig. 1) and *Coullana* sp. males (Fig. 4) in High-PAH treatments was lower than in all other treatments. Further, for total copepods (not shown), *P. wellsi* (Fig. 3), and *Coullana* sp. (Fig. 4), abundance of male copepods generally declined over time in High-PAH treatments, while abundances of females remained relatively constant or increased. These trends lead to significant increases in the proportions of females for total copepods, *Coullana* sp. and *P. wellsi* (Fig. 5). We are unaware of other studies that have examined the influence of PAHs on copepod sex ratios. Di Pinto et al. (1993), however, found that LC50 concentrations of polychlorinated biphenyl (PCB) were approximately twice as high for females than for males of the benthic copepod *Microarthridion littorale*. McManus et al. (1983) observed that uptake of PCB by the planktonic copepod *Acartia tonsa* was similar for males and females, but that depuration was much faster for females because PCB was released with eggs, which are rich in lipids and provide a sink for hydrophobic contaminants (such as PCBs or PAHs). Di Pinto et al. (1993) suggested that this mode of depuration was responsible for the differential mortality between males and females that they observed, but noted that depuration via egg production ultimately leads to significantly higher mortality among nauplii. In the present study, we did not detect a reduction in total nauplii or *P. wellsi*

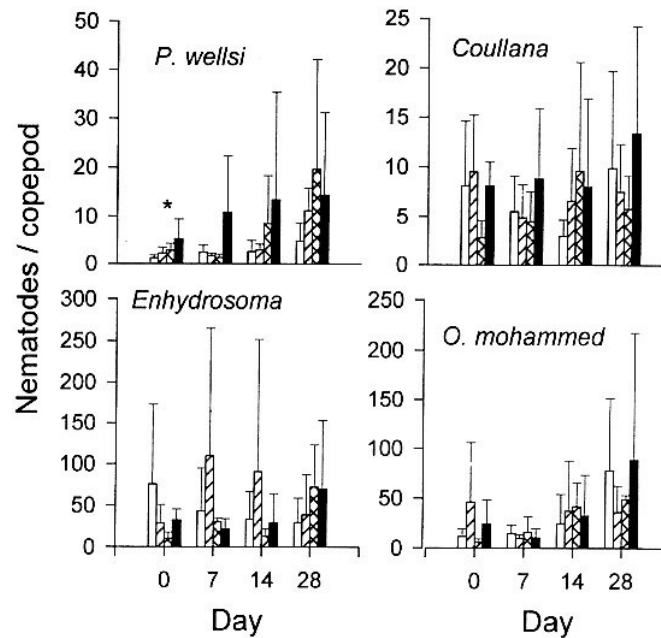


Fig. 7. The nematode/copepod ratio for each of 4 copepod species. An asterisk indicates an individual day on which the effect of PAH was significant. Symbols as in Fig. 1.

nauplii that was attributable to PAH toxicity. It is possible that other mortality factors occurring in the natural assemblages of our microcosms masked the effect of PAHs on naupliar survival that was observed by Di Pinto et al. (1993) in their single-species assays. Nevertheless, the relatively reduced abundance of males that we observed is consistent with the findings of Di Pinto et al. (1993), and suggests that direct toxicity of PAHs influenced the copepod community. Inherent behavioral differences between males and females would likely only mitigate the observed effect, as adult males of many benthic copepods (from seagrass habitats) spend more of their time in water overlying sediments than do females (Bell et al., 1988), which would minimize their exposure to sediment-bound contaminants.

A possible alternative explanation for changes in the proportion of females is that males and females responded differently to PAHs. Specifically, males may have been more likely than females to emigrate from contaminated sediments into the water resulting in less efficient sampling of copepods via coring. Such a response could be responsible for the significant increases in proportion of *Coullana* sp. females on Day 0 (Fig. 5). Day 0 samples were taken more than 12 h after application of contaminated sediment, ample time for copepods to respond behaviorally. Thus, species-specific short-term behavioral responses, in addition to direct toxic effects, may be responsible for changes in copepod sex ratios in response to PAH contamination.

It is also intriguing that the proportion of females in *O. mohammed* *Enhydrosoma* sp.

were not significantly influenced by PAHs. It is possible that subtle differences in its ecology were responsible for the different response. For example, *Coullana* lives in U-shaped burrows and *P. wellsi* lives in tubes (Chandler and Fleeger, 1987), while *O. mohammed* and *Enhydrosoma* are epibenthic. These different lifestyles might result in different exposures to contaminants. If epibenthic forms spend most of their time at the sediment-water interface their exposure to contaminants might be greater than species that live in tubes or burrows. Alternatively, if epibenthic forms frequently migrate into the overlying water, their exposure might be reduced. If depuration via egg production is the primary mechanism that causes the differences in toxicity between males and females, it is also possible that interspecific differences in seasonal reproduction could have led to the interspecific differences observed. However, the percentage of females that were ovigerous did not vary in any systematic way that would be consistent with this explanation (*Coullana* 56%; *P. wellsi* 18%; *Enhydrosoma* sp. 7%; *O. mohammed* 41%).

Among the 4 species considered, on Day 0 (after a 12 h exposure), only *P. wellsi* abundance was significantly lower in High-PAH treatments relative to other treatments; this was primarily because copepodite abundance was relatively low (Fig. 3). The abundance of *P. wellsi* nauplii was also lowest in High-PAH and Medium-PAH treatments on Day 0 (although the statistical significance was marginal;  $P = 0.07$ ; Fig. 3). Non-*P. wellsi* nauplii showed no such trend of lower abundance on Day 0, High-PAH treatments (Fig. 1). Given the relatively low levels of PAHs used in our experiment, it is doubtful that significant mortality occurred during the 12 h interval between when contaminated sediments were applied and when Day 0 samples were collected (based on  $LC_{50}$  tests on copepods exposed to PAHs; Lotufo, unpublished results). Even if such mortality had occurred, carcasses of the animals should have been present and included in abundance counts (we did not attempt to distinguish carcasses from viable animals). It is possible that a behavioral response (migration), rather than mortality, was responsible for the reduction in *P. wellsi* copepod and nauplius abundance. Since *P. wellsi* nauplii can not swim, we propose that they responded to High-PAH treatments by migrating vertically below the top 1 cm of sediment that we collected. In shallow laboratory microcosms, Chandler and Fleeger (1984) reported that *P. wellsi* nauplii, like copepodites and adults, live in tubes that penetrate down 3.9 mm into the sediment. In our microcosm samples, however, we frequently observed *P. wellsi* nauplii and copepods in tubes that were longer than 1 cm, indicating that it would have been possible for them to migrate below the 1 cm horizon. A migration to depth would only provide a temporary solution, however, because of limited oxygen availability (oxygen microelectrode measurements indicate that  $O_2$  is entirely depleted in the top 3 mm of sediment (Carman, unpublished results). In addition, contaminated field sediments would typically be distributed to depths well below 1 cm, and thus such migration might be ineffective under natural conditions. *P. wellsi* copepodites and adults are also capable of swimming, and their decreased abundance at Day 0 could have been via swimming out of sediments or migrating to below 1 cm depth. Regardless of the mechanism, we suggest that all developmental stages of *P. wellsi* responded to High-PAH treatments, especially on Day 0, by attempting to move out of the area of contamination. We plan to sample the 1–2

cm layer of sediment in future experiments in order to better understand the behavioral responses of *P. wellsi*.

Because *P. wellsi* nauplii, copepodites and adults responded similarly to contaminated sediments, the *P. wellsi* nauplius/copepod ratio was not significantly influenced by PAHs (Fig. 6). In contrast, the non-*P. wellsi* nauplius/non-*P. wellsi* copepod ratio was significantly and consistently higher in High-PAH treatments (Fig. 6). These differences probably occurred because non-*P. wellsi* nauplii were unable to vertically migrate (up or down), and their abundance in the top 1 cm of sediment did not change in response to PAH additions. Non-*P. wellsi* copepodites and adults could swim away, thus leading to the increased nauplius/copepod ratio observed (N.B.: *Coullana* sp. nauplii are unusual in that they are planktonic (Harris, 1977) and thus probably were not present when microcosms were collected at low tide in the field. *Coullana* nauplii subsequently hatching in microcosms were also probably not sampled because of their occurrence in the water).

We (Carman et al., 1995) also noted a significantly higher nauplius/copepod ratio in High-PAH treatments when considering total nauplii and copepods, and suggested that the higher ratio could have been a consequence of stunted development by copepods. In the light of the more detailed analysis presented here, it appears that a reduction in adults and copepodites of all species, but especially *P. wellsi* combined with unchanging abundance of non-*P. wellsi* nauplii, lead to the increased nauplius/copepod ratio observed (Carman et al., 1995).

Significant species-specific effects were also observed in the nematode/copepod ratio of abundance. The nematode/copepod ratio was proposed by Raffaelli and Mason (1981) as an index of pollution and is based on general observations that copepods as a group tend to be more sensitive to pollution than nematodes (see reviews by Hicks and Coull, 1983; Coull and Chandler, 1992). Shiells and Anderson (1985) and Sandulli and de Nicola-Giudici (1989) have subsequently suggested that the index is more sensitive when only interstitial copepods are used in the calculation, because non-interstitial species can emigrate from contaminated sediment and thus minimize their exposure (although we are unaware of any published results that such avoidance actually occurs). Sediment at our study site consists of a very fine-grained mud, and the copepod community does not include interstitial species. Nevertheless, a significant effect of PAHs on the nematode/copepod ratio was observed when considering all copepods combined (Carman et al., 1995). Here, we examined nematode/copepod ratios for individual species and found that the effect of PAHs was significant only for *P. wellsi* (Fig. 7). Thus, the effect of PAHs on the overall nematode/copepod ratio (Carman et al., 1995) was primarily the result of changes in the nematode/*P. wellsi* ratio. The significant effect of High-PAH on the Day 0 nematode/*P. wellsi* ratio was apparently a reflection of the vertical migration to depth or emigration from sediment by *P. wellsi* discussed above. The increasing nematode/*P. wellsi* ratio over time was apparently the result of increasing differential mortality of *P. wellsi* relative to nematodes. Although *P. wellsi* is not an interstitial species, it is an obligate sediment dweller that resides in tubes (Chandler and Fleeger, 1984) and is a poor colonist (Sun and Fleeger, 1994, but see Fleeger and Chandler, 1983). It is thus comparable to interstitial species in that it remains closely

associated with the sediment. The greater influence of PAHs on the nematode/*P. wellsi* than on nematode/non-*P. wellsi* ratios would thus appear to be consistent with previous conclusions that application of the nematode/copepod ratio as an index of contaminated habitats is best suited to interstitial species (Shiells and Anderson, 1985; Sandulli and de Nicola-Giudici, 1989).

Overall, although significant effects of PAHs were observed for *Coullana*, our results indicate that *P. wellsi* was the species that was most strongly affected by PAH contamination. Its sedentary lifestyle apparently increased its exposure to contaminants relative to other species, and we speculate that this exposure led to a strong behavioral response and ultimately relatively high mortality from PAHs. It is also possible that, for unknown reasons, *P. wellsi* is simply inherently more susceptible to PAHs than are other co-occurring species.

The apparently anomalous increase in abundance of *Enhydrosoma* sp. copepodites in High-PAH treatments on Day 28 (Fig. 2) is also worthy of comment. Decker and Fleeger (1984) and Fleeger and Chandler (1983) noted enhanced abundances of *Enhydrosoma woodini* in field sediments (from the same marsh where our microcosms were collected) 30–60 days after dosing with oil. Stacey and Marcotte (1987) detected a bloom of *E. baruchi* 6 months after addition of oil to large mesocosms. Fleeger and Chandler (1983) postulated that changes in microbial food resources may have been responsible for increases in *E. woodini*. In our study, microalgal biomass increased (though the increase was not statistically significant) in High-PAH treatments (Carman et al., 1995). Stacey and Marcotte (1987) suggested that a release from competition may stimulate growth of *E. baruchi*. Thus, while the precise mechanism for *Enhydrosoma* sp.'s delayed increases are not known, the common theme of these studies would suggest that *Enhydrosoma* sp. is uniquely suited to exploit oil-contaminated sediments after some period of weathering.

The results reported here have implications for the appropriate interpretation of the ecological consequences of anthropogenic disturbances to benthic communities. Even though relatively few effects on meiofauna were detected at the level of higher taxon (Carman et al., 1995), the relatively subtle effects of PAHs on copepod-community structure observed here could have important implications for benthic food webs. For example, various species of juvenile fish feed heavily on meiofaunal copepods (Gee, 1989; McCall and Fleeger, 1993). Behavioral responses of copepods to PAHs (or other contaminants) as they move deeper in the sediment or emigrate into the water could alter (increase or decrease) their availability to fish. Species-specific effects of contaminants (as with *P. wellsi*) could adversely affect fish that display preferences for particular copepod species (McCall, 1992). In addition, fish have shown a preference for male copepods (McCall, 1992). In our analysis, 2 of the 4 species considered experienced disproportionately high mortality among males. Significant reduction of preferred food items could lead to food limitation for juvenile fish, which could ultimately alter entire communities and ecosystems.

#### Acknowledgments

Several undergraduate and graduate students assisted with this project, including J.



Collier, C. Garrott, J. Gruner, F. Hernandez, B. Hughes, K. Lacour, G. Lotufu, M. McCall, L. Mirande and B. Sun. S. Pomarico provided superb technical assistance. E. Chesney and P. Sammarco graciously provided access to facilities at the LUMCON facility. The manuscript benefitted substantially from comments and discussions with G.T. Chandler, J. Cline, J. Fleeger, C. Gregg, M. Pace, S. Pomarico, D. Thistle and two anonymous reviewers. The research was supported by grants from the Minerals Management Service and Office of Naval Research.

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