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Stimulation of Microbial Activities and Polycyclic Aromatic Hydrocarbon Degradation in Marine Sediments Inhabited by *Capitella capitata*

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ABSTRACT

The effect of the polychaete Capitella capitata *on a variety of sediment microbial activities was determined for 5 months in marine microcosms with or without chronic treatment with the polycyclic aromatic hydrocarbon anthracene. Rates of microbial* $D[U\text{-}^{14}C]$*glucose assimilation and respiration as well as rates and total amounts of* $[9\text{-}^{14}C]$*anthracene mineralization were consistently greater in sediments with resident populations of* Capitella. *Anthracene treatment only affected* ^{14}C*-anthracene mineralization such that after 5 months all sediments under chronic anthracene treatment mineralized* ^{14}C*-anthracene more rapidly than sediments without anthracene or those containing only* Capitella. *Microbial incorporation of [*methyl-^{3}H*]thymidine showed no consistent pattern among treatments.*

The colonization of sediments by total numbers of meiofauna and meiofauna-sized larvae was suppressed in microcosms dosed with anthracene, in contrast to the increased numbers in Capitella*-inhabited microcosms. However, when* Capitella *was also present in anthracene-dosed microcosm sediments, the suppression of total meiofauna numbers was partially offset.*

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These results indicate that the presence of infauna may significantly stimulate certain microbial activities and meiofauna colonization in marine sediments. As a result, mitigation of the effects of organic pollutants, as well as their elimination from sediments, may be enhanced in systems with robust microbe–infauna associations.

INTRODUCTION

Interactions between sediment bacteria and eukaryotic infauna (including protozoans, meiofauna and macrofauna) are important to the trophic-dynamics of sedimentary ecosystems. Several studies have attempted to elucidate the role of sediment bacteria as a source of carbon and energy for potential microbial grazers (Fenchel, 1975; Cammen, 1980; Harper *et al.*, 1981; Fry, 1982) and have generally concluded that bacterial carbon may be assimilated directly into grazer biomass. An estimated 10% (Kemp, 1987) to 50% or more of sediment microbial production may be consumed by benthic invertebrates (Fenchel, 1975).

As the biomass and availability of microbes may be important to infaunal production, infaunal activities may directly influence bacterial numbers, activities and production in sediments. Three general hypotheses have been formulated in relation to sediment microbe–infauna interactions in order to explain the effects that such associations may have on bacterial biomass and activities. For example, it has been suggested that the bioturbating activities of sediment infauna result in an increase in the effective aerobic volume surface area of sediments and that oxygen-dependent microbial processes are enhanced accordingly (Fry, 1982; Reimers & Suess, 1983). Secondly, it has been suggested that bioturbation may indirectly enhance microbial parameters by mechanically refining detrital particles and rendering them more accessible to bacteria for colonization, degradation and mineralization (Findlay & Tenore, 1982; Reimers & Suess, 1983) possibly due to increased particle surface area. A third hypothesis predicts that in highly bioturbated and grazed sediments bacterial growth should be stimulated and an increase in cell numbers observed (Levinton, 1972; Rhoads *et al.*, 1977; Gerlach, 1978; Yingst & Rhoads, 1980; Krantzberg, 1985). This last hypothesis has been referred to as 'microbial gardening' and may be the result of grazing pressure maintaining microbial populations in a state of exponential growth coupled with increased rates of nutrient regeneration from grazing and grazer excretion processes (Johannes, 1965, 1968; Hylleberg, 1975; Riemann & Schrage, 1978; Yingst & Rhoads, 1980).

In spite of this, little direct evidence exists for the quantitative or qualitative (i.e. which specific activities) enhancement of microbial activities by infauna (Tietjen, 1980; Krantzberg, 1985). The few studies available generally indicate that enhanced microbial numbers and/or rate processes are associated with infaunal tubes, burrow-linings and burrow water (Aller *et al.*, 1983; Alongi, 1984, 1985*a*,*b*; Kristensen *et al.*, 1985). Also, general sediment microbial activities (not those associated with tubes and burrows exclusively) are enhanced in the presence of intact microbe–protozoa–infauna assemblages (Briggs *et al.*, 1979). However, we know of no studies which have attempted to examine sediment microbial organic pollutant degradation as a function of microbe–infauna associations.

In the present study estimates are provided for a suite of microbial activities, as well as colonizing meiofauna abundances, in coastal marine sediments including or excluding populations of a representative bioturbating infaunal organism (*Capitella capitata*) and a model pollutant form of organic carbon (the polycyclic aromatic hydrocarbon anthracene). Data provided here contribute toward a more comprehensive and integrated interpretation of the interactions which occur between bacteria, infauna and organic contaminants in marine sediment systems.

MATERIALS AND METHODS

The effects of *Capitella* and anthracene on sediment microbial activities were studied in flow-through microcosms. Individual microcosms consisted of 25·8 cm-diameter PVC pipe (15·0 cm height) with a square PVC base cemented on. Sediment from the intertidal mud flat at Flax Pond Salt Marsh (Long Island, NY) was collected by scraping the top 2 cm of sediment from the marsh surface. Macrobiota were removed from sediments by alternately freezing and thawing three times and sieving through a 1 mm mesh screen. The microcosms were then filled to a depth of approximately 7 cm with sediment.

Seawater (31 ppt salinity) was supplied to each microcosm by filtering incoming seawater sequentially through a sand–gravel filter followed by a 10-μm, nylon-mesh cartridge filter (Ryan-Herco; Fig. 1). A multiple-head peristaltic pump (Cole-Parmer, Chicago, IL) controlled seawater flow to the microcosms. Individual feed lines (Tygon tubing, 3/16-in internal diameter) were suspended approximately 5 cm above the water surface of the microcosms. Overlying the sediment of each microcosm were approximately 5 liters of seawater which were kept fully aerated by a small aquarium pump attached to an airstone. Seawater flow to microcosms was adjusted to 10 ml min^{-1} resulting in three complete turnovers of water each day. Water temperature in microcosms was monitored over the course of the study.

Microcosm sediments and their colonizing biota were allowed to

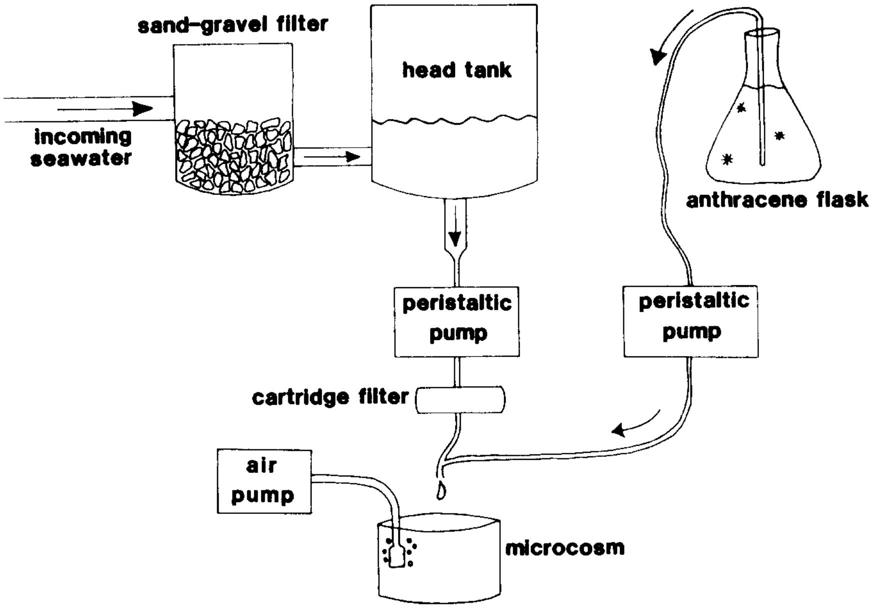

Fig. 1. Schematic diagram of the microcosm, flow-through seawater system, and anthracene-dosing system used in the present study. Refer to text for full description.

equilibrate for 4 weeks prior to use. One hundred adult *Capitella capitata* were added to 4 of the 8 microcosms during this time (equivalent to 500 animals m^{-2}). At the end of the acclimation period a preliminary assessment of microbial activities (see below) was conducted in microcosm sediments with and without *Capitella* (10 April). Immediately following this sampling (12 April) a 0·2 μm-filtered, saturated seawater solution (95 parts per 10^9) of anthracene (Kodak, Rochester, NY) was fed by separate tubing and peristaltic pump to 2 microcosms with and 2 without *Capitella*. Anthracene was used as a representative polycyclic aromatic hydrocarbon (PAH) due to its relatively low toxicity to sediment microbiota (Bauer & Capone, 1985*a*). It generally comprises a relatively small per cent of total PAH and has been found in a range of oiled sediments in concentrations of from 50 ppb to 33 ppm (Johnson & Larsen, 1985; J. E. Bauer *et al.*, unpublished data). Anthracene concentrations in microcosm water and sediments were monitored periodically over the course of the study (see below).

A schematic illustration of the experimental design is shown in Fig. 2. The three main treatments were time (three post-anthracene addition samplings), anthracene (with and without) and *Capitella* (with and without). This resulted in a total of four treatments, each replicated twice.

At each sampling, sediments to be assayed were removed randomly and without replacement so as not to re-sample in a spot where sediment had

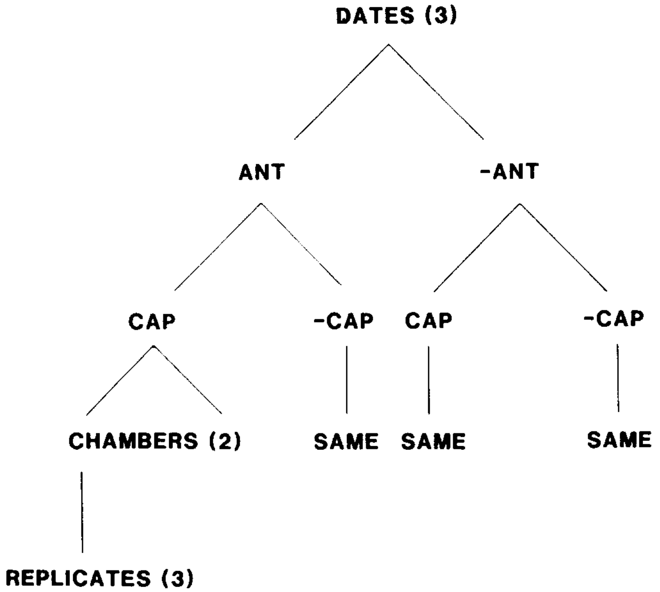

Fig. 2. Experimental treatment and sampling design employed to study the individual and interactive effects of *Capitella* and chronic anthracene dosing on sediment microbial activities. Ant = anthracene-dosed, Cap = *Capitella*-inhabited.

already been removed. A grid with sampling ports was placed over the top of each microcosm and 10 ml plastic syringe cores with tips removed (Becton-Dickinson, Rutherford, NJ) were inserted through the grid and into the sediment to the bottom of the microcosm while noting grid position for each microcosm at each sampling. Triplicate cores were taken for each parameter measured (with the exception of meiofaunal abundances, for which six cores were withdrawn from each microcosm at each sampling time) and assayed within one hour.

Microbial activities measured included assimilation of ^{14}C from D[*U*-^{14}C]glucose, respiration of D[*U*-^{14}C]glucose to $^{14}CO_2$, degradation and mineralization of [*9*-^{14}C]anthracene, incorporation of [*methyl*-^{3}H]thymidine (^{3}HTdR) into microbial cold-acid precipitable material, and reduction of $^{35}SO_4^{2-}$ by sediment anaerobes. All isotopes were obtained from Amersham, Arlington Heights, IL. The techniques used in measuring these activities have been described in Kiene & Capone (1984) and Bauer & Capone (1985*a*,*b*). The top 0–1 cm of each sediment core (diluted 1:1 with 0·2 μm-filtered seawater) was used for all activity measurements except sulfate reduction which was measured in several depth segments.

In addition to microbial activities, separate cores were taken at each sampling time for measurement of the depth of the visual redox-potential discontinuity (RPD), for determination of anthracene concentration, and for enumeration of meiofauna in microcosm sediments. Sediment sections from 0–1 cm and 4–5 cm depth were extracted and analyzed for anthracene by high-performance liquid chromatography according to the methods of Bauer & Capone (1988, in press). Anthracene detection limits were previously determined to be the equivalent of $<1\,\mathrm{ng\,ml^{-1}}$ wet sediment.

For meiofauna enumeration, six cores from each microcosm were taken to a depth of 5 cm on each sampling date. Cores were extruded into 20-ml plastic vials and preserved in 10% formalin and Rose Bengal. Vials were capped and the contents were mixed well and allowed to sit for at least 48 h at room temperature. Meiofauna were separated from sediments by the elutriation technique (Boisseau, 1957), and the extraction efficiency for all taxa was $\geq 93\%$. Meiofauna were trapped on a 63-μm mesh, and the organisms were transferred to a Petri dish and examined under a dissecting microscope. The major taxa were then identified and enumerated.

Data from the pre-anthracene treatment sampling (day 0) were analyzed by unplanned comparisons of two means (parameters measured in sediments with or without *Capitella*) and applying a least significant difference parameter (Sokal & Rohlf, 1981). Subsequent to chronic anthracene treatment all activities were subjected to three-way factorial analysis of variance with time (3 dates), *Capitella* (with and without) and anthracene (with and without) comprising the three main fixed-treatment effects.

RESULTS

The temperature of the seawater supplied to the microcosms ranged from a low of 15°C at the beginning of the experiment on 10 April, gradually increasing to a maximum of 23°C on 20 September (data not shown). Relative to outside Flax Pond water, which ranged from 9° to 25°C over the same period, microcosm water temperature was buffered and relatively constant due to the long residence time of the head tank water and its equilibration with indoor temperature.

Concentrations of anthracene in sediments of microcosms under chronic anthracene treatment (Table 1) differed significantly over both time and with sediment depth. The presence of *Capitella* had no obvious effect on the concentration of anthracene for various date–depth combinations, and microcosms not treated with anthracene never contained detectable anthracene over the course of the experiment.

Absolute concentrations of anthracene increased in both surface and subsurface sediments over time, the relative magnitude of that increase being

TABLE 1

Anthracene Concentrations in Sediments from Two Depths (0–1 cm and 3–4 cm) in Selected Microcosms Subjected to Chronic Anthracene Treatment (All values are ng anthracene ml^{-1} wet sediment. Values in parentheses represent ±1 standard deviation. Control microcosms contained no detectable anthracene at any time. The concentration of anthracene in the reservoir flask was measured at 95 (±4) ng ml^{-1} seawater on 1 June.)

Microcosm	*Date*	*0–1 cm*	*n*	*3–4 cm*	*n*
6 (+CAP)	4 May	35 (18)	2	33 (8)	2
	14 June	79 (8)	2	39 (0)	2
	20 Sept.	107 (19)	2	50 (10)	4
1 (+CAP)	4 May	54 (14)	2	41 (8)	2
	14 June	67 (2)	2	50 (7)	2
	20 Sept.	202 (2)	5	54 (7)	3
8 (−CAP)	4 May	46 (21)	4	34 (29)	3
	14 June	56 (6)	2	43 (16)	2
	20 Sept.	171 (12)	4	52 (10)	3

+CAP = with *Capitella*, −CAP = without *Capitella*.

greater in surface than in subsurface sediments. While concentrations in surface and subsurface sediments of microcosms were similar at the beginning of the experiment, the disparity in concentrations grew until, by the fifth month of treatment (September), surface concentrations were 2 to 4 times greater than those of subsurface sediments.

The depth of the redox-potential discontinuity (RPD) showed highly significant differences due to time, the presence of *Capitella* (both $P < 0{\cdot}001$) and anthracene dosing ($P < 0{\cdot}01$; Fig. 3). Prior to anthracene dosing, the presence of *Capitella* alone resulted in significantly deeper RPD. The RPD depth also gradually deepened over time in control and anthracene-treated sediments, probably due to the colonization and bioturbation of sediments by invertebrate larvae and meiofauna $<10\,\mu m$ in size. Beyond the April sampling, RPD depth in all sediments containing *Capitella* remained essentially constant at 24–28 mm.

The microbial activities measured in microcosms under the various treatments over time generally showed similar responses (Figs 4–8). Rates of sulfate reduction were low in all treatments and were not apparently different (data not shown).

Rates of ^{14}C-anthracene mineralization were significantly elevated ($P < 0{\cdot}01$, *T*-method) in the presence of *Capitella* prior to anthracene dosing (Fig. 4). After anthracene dosing commenced in May, significant treatment effects and time-dependent patterns of ^{14}C-anthracene mineralization were observed. The main treatments (time, *Capitella* and anthracene) all resulted in highly significant shifts in ^{14}C-anthracene mineralization rates ($P < 0{\cdot}01$,

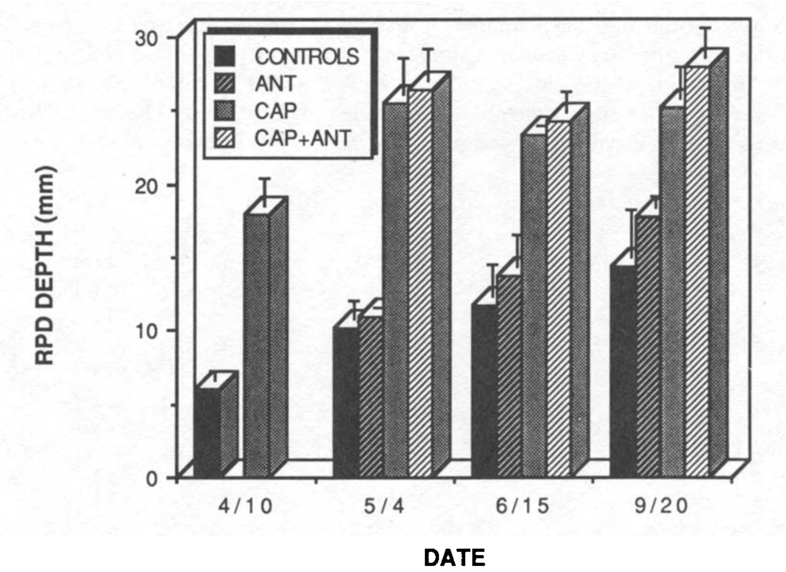

Fig. 3. Depth of the visual redox-potential discontinuity (RPD) in cores from microcosm sediments. $n = 3$ cores. Vertical bars indicate ±1 standard deviation. Ant = sediments receiving chronic anthracene dosing. Cap = sediments seeded with *Capitella capitata.*

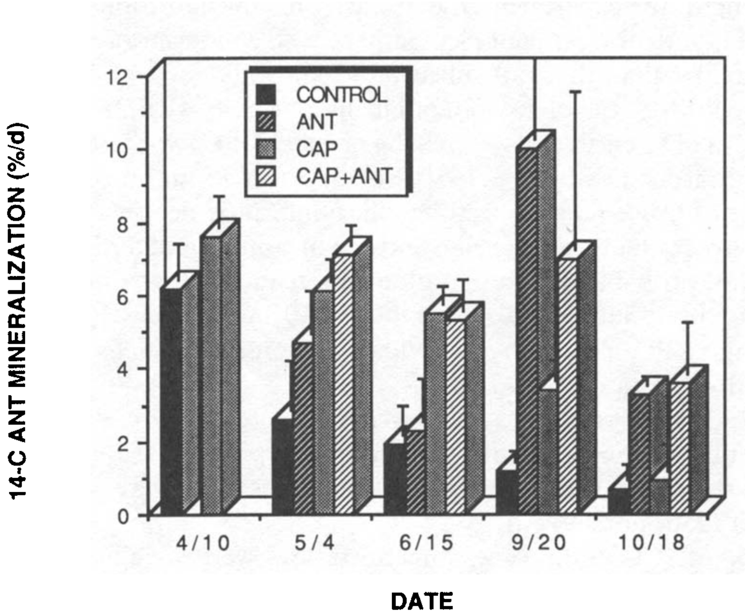

Fig. 4. Rates of mineralization of ^{14}C-anthracene in sediments from microcosms under the various treatments. $n = 3$ cores. Vertical bars indicate ±1 standard deviation. Abbreviations as in Fig. 3.

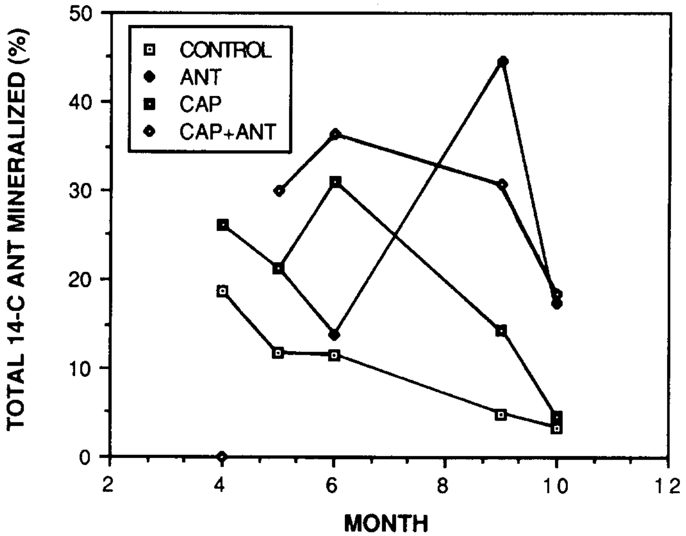

Fig. 5. Total amounts of ^{14}C-anthracene mineralized after 7 day incubations of microcosm sediments. $n = 3$ cores. Abbreviations as in Fig. 3.

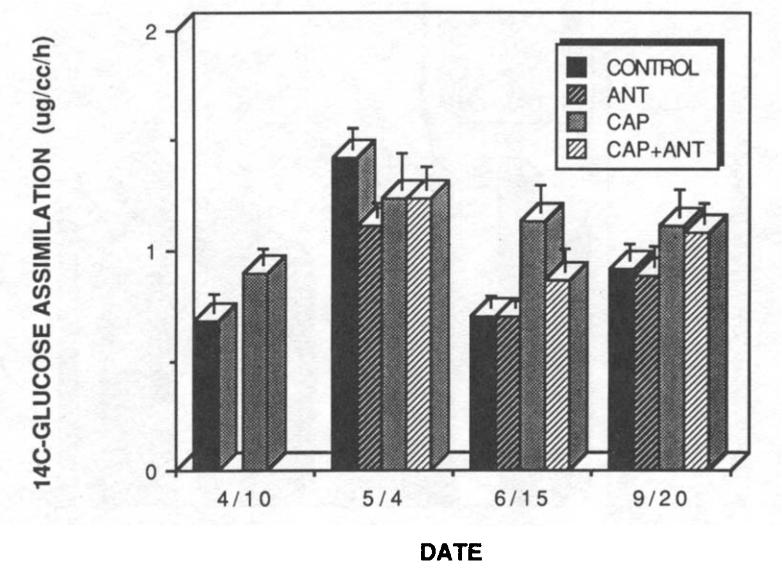

Fig. 6. Assimilation of ^{14}C-glucose by sediment bacteria from microcosms under the various treatments. $n = 3$ cores. Vertical bars indicate ±1 standard deviation. Abbreviations as in Fig. 3.

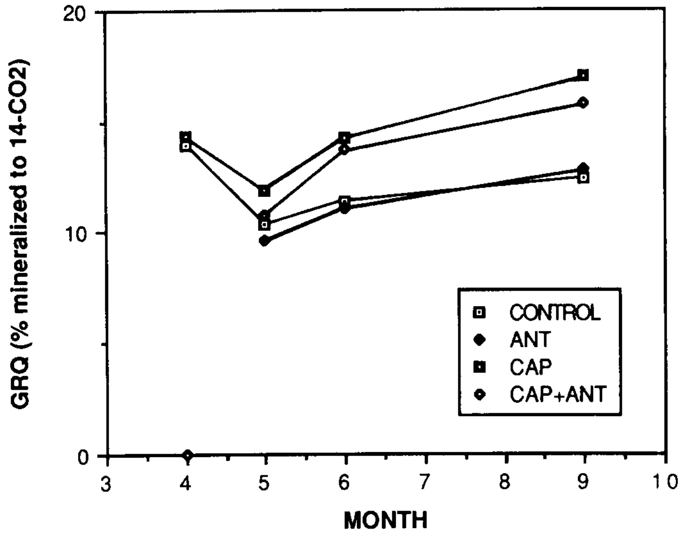

Fig. 7. Percentage of total assimilated (= biomass assimilated + respired) ^{14}C-glucose respired to $^{14}CO_2$ by sediment bacteria from microcosms under the various treatments. $n = 3$ cores. Abbreviations as in Fig. 3.

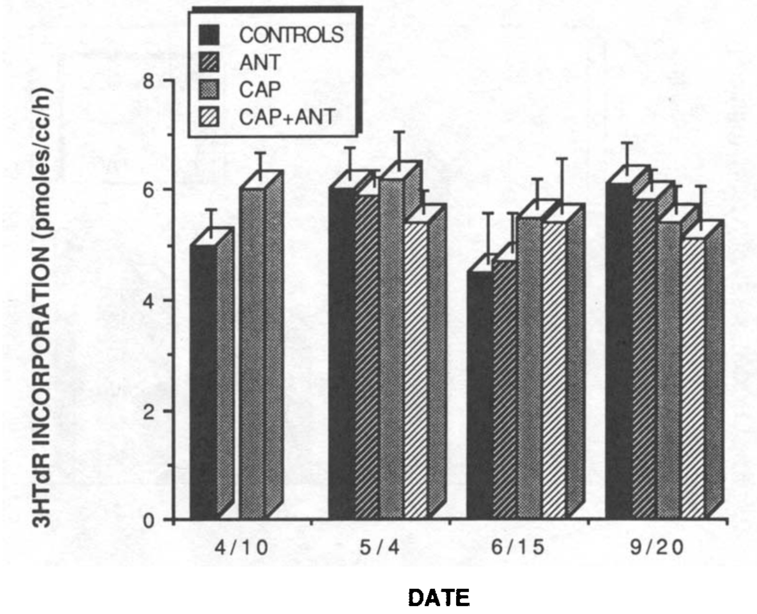

Fig. 8. Incorporation of ^{3}HTdR to cold acid-insoluble material by microcosm sediment bacteria. $n = 3$ replicates. Vertical bars indicate ± 1 standard deviation. Abbreviations as in Fig. 3.

three-way analysis of variance). However, much of this may be accounted for by significant anthracene/time, *Capitella*/time and anthracene/*Capitella* interactions (all $P < 0{\cdot}01$). An explanation for much of this interaction may be drawn from closer examination of Fig. 4. A general decrease in ^{14}C-anthracene mineralization rates occurred in all sediments over time. Up to and including the June sampling, the order of enhancement of ^{14}C-anthracene mineralization rates was controls < anthracene < *Capitella* < *Capitella* + anthracene. However, for the September and October samplings it appears that enhancement due to chronic anthracene dosing alone superseded that stimulation previously attributable exclusively to *Capitella.* By October the rate of ^{14}C-anthracene mineralization in *Capitella* sediments was identical to controls.

Similar results were obtained when the total cumulative amount of ^{14}C-anthracene mineralized by sediment bacteria (after 7-day incubations at each sampling time point) was calculated (Fig. 5). Total ^{14}C-anthracene mineralized in *Capitella*-inhabited sediments declined to control levels in October while anthracene treatment resulted in greater overall mineralization. A peak in overall mineralization paralleled a maximum in rates of mineralization (Fig. 4) in anthracene-treated microcosms.

The amount of ^{14}C-label incorporated into sediment microbial biomass from ^{14}C-glucose assimilation was greatest in all *Capitella* treatments for all but the May sampling (Fig. 6). Significantly greater amounts of ^{14}C-label were assimilated by bacteria in *Capitella*-colonized sediments at the pre-anthracene sampling time-point ($P < 0{\cdot}001$). Analysis of variance on data from the following three dates revealed significant two- and three-way interaction at all levels. When the time interaction terms were removed from the ANOVA (assuming that no time-dependent changes in the relative patterns of treatment effects existed), significant differences in ^{14}C-glucose assimilation were noted for all main treatments ($P < 0{\cdot}01$) but not for the anthracene/*Capitella* interaction.

The proportion of ^{14}C-label derived from ^{14}C-glucose and respired to $^{14}CO_2$ was similar for all treatments at the start of the experiment and diverged over time (Fig. 7). No significant differences existed in mean glucose respiratory quotients (GRQ) between control and *Capitella*-inhabited sediments prior to anthracene dosing. However, after anthracene dosing began (month 5), significantly greater percentages of glucose were respired in both *Capitella* treatments (alone and with anthracene, three-way ANOVA, $P < 0{\cdot}001$) than control or anthracene-treated sediments, which were the same. With the exception of the April sampling GRQ values for all treatments increased as a function of time ($P < 0{\cdot}001$).

Significantly greater amounts of ^{3}HTdR were incorporated into cold-acid insoluble material in *Capitella*-inhabited sediments prior to anthracene

TABLE 2

Ratios of RPD Depth and Microbial Activities in Sediments from Treatment Microcosms Relative to Control Microcosms

Parameter	*10 April*	*4 May*			*15 June*			*20 Sept.*			*18 Oct.*		
	C	*A*[a]	*C*[b]	*C + A*	*A*	*C*	*C + A*	*A*	*C*	*C + A*	*A*	*C*	*C + A*
RPD Depth	2·95	1·08	2·28	2·60	1·17	1·97	2·06	1·24	1·75	1·95	—	—	—
^{14}C-Anthracene Mineralization													
(Rate)	1·23	1·82	2·36	2·73	1·43	3·29	3·14	8·40	2·80	5·80	4·70	1·29	5·14
(Total)	1·39	1·81	1·81	2·55	1·20	2·67	3·14	9·12	2·94	6·27	5·12	1·38	5·44
^{14}C-Glucose													
Assimilation	1·32	0·80	0·89	0·89	1·00	1·61	1·24	0·97	1·21	1·17	—	—	—
GRQ	1·03	0·92	1·14	1·04	0·97	1·25	1·20	1·03	1·37	1·27	—	—	—
^{3}HTdR Incorporation	1·20	0·98	1·03	0·90	1·40	1·22	1·20	0·95	0·89	0·84	—	—	—

All values represent (treatment parameter) ÷ (control parameter).

[a] A = anthracene-dosed.

[b] C = *Capitella*-inhabited.

dosing (Fig. 8). However, subsequent to anthracene dosing, no clear pattern of ^{3}HTdR incorporation was observed with respect to the main treatments. Although *Capitella* (alone) treatments had greater rates of ^{3}HTdR incorporation than controls on 3 of the 4 sampling dates, this measure of microbial activity appeared to be subject to variables not controlled for in the system employed.

Ratios of RPD depth and microbial activity values in treated microcosms relative to those of control microcosms for all dates are summarized in Table 2. In all cases (except 4 May for ^{14}C-glucose assimilation and 20 September for ^{3}HTdR incorporation), *Capitella* and *Capitella* plus anthracene treatments resulted in ratios >1 indicating greater parameter values in treatment than in control microcosms. While chronic anthracene treatment (alone) often resulted in ratios >1 (especially for ^{14}C-anthracene mineralization, see Discussion), these ratios were much closer to unity, and hence equal to control values, than were those from either of the other two treatments.

Total numbers of meiofauna (including meiofauna-sized larvae) were generally suppressed by anthracene treatment and enhanced by the presence of *Capitella* (Table 3). Prior to anthracene dosing (10 April), the presence of *Capitella* resulted in a significant increase in total meiofauna ($P = 0{\cdot}006$ by one-way ANOVA). This significant stimulation of meiofauna numbers by *Capitella* and inhibition by anthracene continued through the June sampling. At this time, total numbers of meiofauna in *Capitella* + anthracene microcosms were lower than those in controls but intermediate between those of *Capitella* and anthracene treatments. In

TABLE 3

Mean Numbers per 10 ml Core (±1 SD) of Meiofauna-sized Animals in Microcosms Under the Various Experimental Treatments, April through September (Anthracene (Ant) dosing had not commenced on 10 April so that Ant and Cap + Ant microcosms were equivalent to control and Cap microcosms, respectively.)

Date	*Control*	*Ant*[a]	*Cap*[b]	*Cap + Ant*
10 April	12·0	11·8	39·3	33·3
	(7·8)	(3·9)	(3·1)	(27·3)
4 May	6·0	3·8	25·3	19·3
	(1·9)	(1·0)	(10·9)	(4·6)
15 June	47·2	10·6	66·5	24·5
	(19·4)	(6·8)	(34·9)	(6·5)
20 Sept.	59·4	17·0	30·2	24·5
	(27·3)	(3·4)	(15·9)	(10·1)

[a] Ant—anthracene-dosed.

[b] Cap—*Capitella*-inhabited.

September these relationships also maintained themselves except that control values of total meiofauna were significantly higher than all other treatments ($P = 0{\cdot}02$ by one-way ANOVA). Significant time/treatment interaction was noted when total meiofauna were analyzed as a function of sampling date and treatment ($P = 0{\cdot}0005$ by two-way ANOVA) indicating that treatment effects were variable over time. Treatment effects on population sizes of individual taxonomic groups of meiofauna were not noted. In general, polychaete (i.e. *Capitella*) larvae did not comprise the major component of meiofauna biomass in those microcosms inhabited by adult *Capitella.*

DISCUSSION

The present study examined alterations in selected microbial activities on an integrated level (i.e. individual burrows, pellets and structures were not examined) in sediments inhabited by populations of representative native infauna and under chronic dosing with a model petroleum hydrocarbon contaminant. No attempt was made to separate effects due to physical, chemical or biological components of these microbe–infauna interactions.

The RPD of microcosm sediments containing active *Capitella* populations was nearly twice as deep as those without the polychaete (Fig. 3). McCave (1974), Stephens (1975) and Anderson & MacFayden (1976) have also demonstrated a deepening of the RPD in the presence of active burrowing communities, most likely due to the introduction of oxygen to anoxic sediment strata. It was therefore surprising that, with greater oxygen available for anthracene degradation in sediments with *Capitella*, anthracene concentration was the same in both colonized and uncolonized microcosms (Table 1). The greatest recorded RPD depths were at 3 cm and it is possible that below this depth anthracene was accumulating slowly by diffusive and adsorptive mechanisms and was not being degraded, since oxygen is necessary for its breakdown (Hambrick *et al.*, 1980; Cerniglia, 1982; Bauer & Capone, 1985*b*). The greater rate of anthracene accumulation in surface sediments may be due to their proximity to the source of anthracene in the overlying seawater.

Anthracene has previously been found to exhibit low toxicity toward sediment bacterial ^{14}C-glucose respiration and ^{3}HTdR incorporation (Bauer & Capone, 1985*a*) even at high concentrations (100 ppm). Similar results were obtained in the present study in that values of parameters measured in microcosms receiving only anthracene dosing were close to those of control microcosms (Table 2). In addition, anthracene concentrations were similar in microcosms with or without *Capitella.* This is surprising when it is considered that anthracene mineralization is stimulated in *Capitella*-colonized sediments, especially up to and including the June sampling. It is possible that some factor(s) other than *Capitella* were controlling the distribution of sediment-bound anthracene or its metabolites.

The time-dependent differences in rates of ^{14}C-anthracene degradation in anthracene-dosed microcosms are consistent with the hypotheses that (1) anthracene degradation pathways require induction, or proliferation of anthracene-degrading populations, by prior exposure to the PAH (Heitcamp & Cerniglia, 1987) and (2) the steady increases in anthracene in anthracene-dosed microcosms represent a cumulative, adsorbed pool of the PAH. Lag periods in organic contaminant degradation have been noted in many systems and are dependent on compound concentration and time of previous exposure (Spain & Van Veld, 1983; Bauer & Capone, 1985*b*). The long lag period (at least 2 months) prior to enhanced ^{14}C-anthracene mineralization in anthracene-dosed microcosms compared to microcosms with *Capitella* suggests that significant microbial adaption to the compound alone did not occur until September. At this time the effects of enhanced degradation due to *Capitella* became diminished. Thus, for anthracene degradation, the two main factors, *Capitella* and anthracene exposure, assumed significance on different time scales, in contrast to their respective effects on other activities (see below).

Although not examined in the present study, alterations in bacterial numbers and biomass in the presence of grazing infauna have been examined by other investigators. The mud snails *Hydrobia* and *Ilyanassa* had no effect on bacterial densities in sediment microcosms (Bianchi & Levinton, 1981). Alongi & Hanson (1985) found no differences in bacterial and protozoan numbers and doubling times in sediments with or without *Capitella* at medium and high detritus rations. However, at low detritus rations, numbers were found to correlate negatively with numbers of *Capitella*. Pearson (1982) hypothesized a similar scenario for Loch Eil sediments whereby under low organic inputs macrofaunal activities should be the dominant factor influencing sediment geochemistry. Under high inputs, however, infaunal influences become muted and microbial activities are dominated by the rate of organic deposition. Microbial numbers and activities may thus remain fairly stable over a wide range of organic inputs, regardless of the presence of potential infaunal grazers (Pearson, 1982). Under such conditions a microbial population may reach an equilibrium carrying capacity as a result of saturating levels of organic material and the limitation of suitable electron acceptors. Alongi (1985*a*) did not observe higher bacterial numbers on *Capitella* tubes while Ekman (1985) demonstrated that greater rates of bacterial colonization around worm-tube mimics could be attributed entirely to structural and hydrodynamic effects.

Stimulation of microbial respiration and degradation appears to be the general rule in the presence of bioturbating infauna in both the present study (Table 2, Figs 4–7) and in previous investigations. Hargrave (1970) found normal densities of the freshwater deposit feeding amphipod, *Hyalella azteca*, to stimulate microbial oxygen consumption. *Nereis*, alone or with the ciliate *Apidisca*, stimulated sediment microbial oxidation of *Gracilaria* detritus and heterotrophic glucose uptake (Briggs *et al.*, 1979). Nematodes stimulated mineralization rates of *Gracilaria* detritus up to 300% over ambient rates (Findlay & Tenore, 1982). The presence of *Nereis* also stimulated the net decomposition of POC and PON 2·6 and 1·6 times, respectively, relative to oxygenated cores (Kristensen & Blackburn, 1987) suggesting that factors besides oxygen availability may be responsible for microbial stimulation in certain systems. The end product of organic decomposition, CO_2 has been shown to accumulate as a result of enhanced degradation rates in burrows of *Upogebia* and *Onuphis* (Aller *et al.*, 1983).

The heterotrophic activities measured in the present study, ^{14}C-glucose assimilation and respiration and ^{14}C-anthracene mineralization, were both significantly stimulated in the presence of *Capitella*. However, $^{3}HTdR$ incorporation, used as a measure of the rate of microbial DNA synthesis and growth, did not show the same patterns of enhancement consistent with main treatment effects. Hanson (1980) and Yingst & Rhoads (1980) have discussed the necessary distinction which must be drawn between microbial metabolism and growth rate since the two processes may be uncoupled. One or more of the factors resulting in such uncoupling may have been in effect in the present study. Furthermore, Yingst & Rhoads (1980) suggest that sediment bacterial growth and metabolism are enhanced more in higher-order successional stages consisting of burrowing deposit-feeders whose activities result in greater sediment homogeneity. The time-dependent heterogeneity in $^{3}HTdR$ uptake may have been a result of our system being highly heterogeneous due to a tube-building, pioneering stage species (*Capitella capitata*) dominating bioturbatory processes. Micro-scale heterogeneity in sediments in the form of biogenic structures may have more noticeable effects on such activities as microbial productivity. Such small-scale heterogeneity may be obliterated by the activities of burrowing, conveyor-belt species.

In addition to organic degradation, inorganic nutrient cycling may also be altered by the presence of infauna. Aller *et al.* (1983) and Kristensen *et al.* (1985) measured enhanced nitrification, denitrification and/or nitrate reduction in both the burrows and burrow walls of three separate tube-building deposit-feeders. The presence of polychaetes in Long Island Sound sediments in summer resulted in decreased rates of sulfate reduction down to 8 cm depth (Goldhaber *et al.*, 1977). Part of the discrepancy between the absence of alteration of microbial numbers compared to activities in the presence of infauna may be a result of labile organic matter stimulating short-term, cell-specific activity rates (Pearson, 1982).

The colonization of microcosm sediments by total meiofauna and meiofauna-sized organisms was affected by the different treatments (Table 3). Excluding September when control sediments were highest, sediments inhabited by *Capitella* were colonized by meiofauna to the greatest extent, followed by *Capitella* + anthracene, control and anthracene microcosms. In all cases, numbers in *Capitella* + anthracene microcosms were higher than those with anthracene alone but lower than those inhabited exclusively by *Capitella*, suggesting that *Capitella* may moderate the inhibitory effects of anthracene on meiofauna colonization.

It is not certain how meiofauna colonized these initially azoic sediments. However, Chandler and Fleeger (1983) found meiofauna to colonize azoic sediments through the water column rather than via migration through adjacent, colonized sediments. This suggests that suspended meiofauna, larvae and/or eggs $< 10\,\mu m$ in size (the nominal filter size) entered the microcosms through the flowing seawater. This explanation is further supported by the studies of Palmer (1986) who found tidal resuspension to be an important factor in the movement of meiofauna into the water column where they could then be transported to uncolonized sediments.

While meiofaunal colonization was impaired by the presence of anthracene, an individual component of petroleum, changes in meiofaunal abundances and colonization were not noted in response to crude oil contamination in Louisiana salt marsh sediments (Fleeger & Chandler, 1983; Decker & Fleeger, 1984). Alongi (1984, 1985*b*) observed variable responses in meiofaunal abundances in the presence of *Capitella capitata* depending upon the amount of detritus present. It is possible that meiofauna respond to many of the same infauna-controlled parameters as bacteria, resulting in an enhancement in their numbers (and possibly activities) in the presence of larger metazoans. Furthermore, total numbers of meiofauna could potentially influence, either alone or in concert with *Capitella*, the microbial activities noted here. The mechanisms for possible microbial–meiofaunal interactions are unknown in the present study, particularly in the light of the fact that while total meiofaunal abundances remained relatively stable over the course of the experiment, and control numbers actually increased, microbial activities tended to exhibit an overall decline with time. However, if meiofauna did affect microbial activities this could add to the complexity of the interactions in the system and require the need for experiments which separate effects due to exclusively to meiofauna and macrofauna.

Few studies have examined the fate of organic contaminants as a function of microbial interactions with higher trophic groups, and we know of none which have done so in sediments. However, the presence of protozoans in bacterial cultures may stimulate the microbial breakdown of crude oil (Rogerson & Berger, 1983) as well as other organic contaminants (Huang *et al.*, 1981) perhaps by supplying one or more growth factors to microbes.

The distribution of PAH in sediments, porewater and resident infauna may be largely controlled by microbial transformations of these compounds (Augenfeld *et al.*, 1982; Riley *et al.*, 1982). In fine sediments, such as those used in the microcosms, up to 70% of initially added phenanthrene remained, and the compound and its residues accumulated in resident *Abarenicola* to levels 4–6 times those in the sediments (Augenfeld *et al.*, 1982). The tissues of certain marine polychaetes (e.g. *Nereis* sp. and *Capitella capitata*) have also been found to possess hydrocarbon-degrading capabilities as a result of the presence of such mixed function oxidases as aryl hydrocarbon hydroxylase, which are able to convert PAH to polar metabolites (Lee *et al.*, 1977; Lee & Singer, 1980).

Results of the present study indicate that although a net accumulation of PAH may occur in sediments under chronic exposure, the potential of microbes in *Capitella*-inhabited sediments and those exposed to anthracene for long periods of time (>5 months) to mineralize PAH is enhanced over control sediments. The role that resident infauna play in this enhanced degradation of PAH in the natural environment due to bioturbation and their own degradative capabilities is likely complex. Gordon *et al.* (1978), in studying the relationship between the weathering rate of sediment-bound oil and the sediment-working rate of *Arenicola marina*, found that the f_1, f_2 and f_3 fractions of oil were consistently lower in the casts of the animals than in the surrounding sediments. This was attributed to a combination of enhanced hydrocarbon dissolution, stimulated microbial degradation, and uptake of oil by the worms. Such complex interactions in the biogeochemical fate of a variety of natural and xenobiotic compounds could be expected in bioturbated aquatic sediments. The result is that sediments inhabited by early successional-stage species will likely purge themselves of PAH, and organic contaminants in general, more rapidly once chronic accumulation ceases.

The general observation that sediment microbial activities are stimulated in the presence of active, bioturbating infaunal populations or intact benthic food webs leads to several plausible explanations for this phenomenon. First, sediments are largely organic-rich, electron-acceptor-poor environments compared to overlying waters. Most of the potential forms of disturbance to sediments therefore represent a perturbation of this state (i.e., a disruption in normal gradients and interfaces, as with the introduction of oxygen) and result in stimulation. That is, disturbance in sediments usually comes from above and introduces factors which are conducive to enhanced microbial activities. This, combined with the flushing of inhibitory metabolites and reduced inorganic ions (both of which may be toxic to microbes) from sediments by bioturbating activities, may account for the observed stimulation.

In conclusion, several microbial activities were stimulated, and total numbers of meiofauna were greater, in sediments inhabited by *Capitella capitata.* The chronic exposure of these sediments to the PAH anthracene had little effect on general microbial activities but stimulated ^{14}C-anthracene mineralization and resulted in lower abundances of total meiofauna. Higher levels of microbial metabolism in the presence of *Capitella* may allow such sediment systems to tolerate more effectively the effects of organic pollutant contamination. The specific mechanisms responsible for the stimulation of microbial activities and PAH degradation in the presence of infauna are still in need of identification.

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REFERENCES

Aller, R. C., Yingst, J. V. & Ullman, W. J. (1983). Comparative biogeochemistry of water in intertidal *Onuphis* (Polychaeta) and *Upogebia* (Crustacea) burrows: Temporal patterns and causes. *J. Mar. Res.*, **41**, 571–604.

Alongi, D. M. (1984). *Effects of detritus supply on trophic relationships within laboratory models of benthic food webs: Interactions among microbes, meiofauna, and the polychaete* Capitella capitata (Type I). PhD dissertation, University of Georgia.

Alongi, D. M. (1985*a*). Microbes, meiofauna, and bacterial productivity on tubes constructed by the polychaete *Capitella capitata. Mar. Ecol. Prog. Ser.*, **23**, 207–8.

Alongi, D. M. (1985*b*). Effect of physical disturbance on population dynamics and trophic interactions among microbes and meiofauna. *J. Mar. Res.*, **43**, 351–64.

Alongi, D. M. & Hanson, R. B. (1985). Effect of detritus supply on trophic relationships within experimental benthic food webs. II. Microbial responses and fate and composition of decomposing detritus. *J. Exp. Mar. Biol. Ecol.*, **88**, 167–82.

Anderson, J. M. & MacFayden, A. (1976). *The role of terrestrial and aquatic organisms in decomposition processes.* Blackwell, Oxford.

Augenfeld, J. M., Anderson, J. W., Riley, R. G. & Thomas, B. L. (1982). The fate of polyaromatic hydrocarbons in an intertidal exposure system: Bioavailability to *Macoma inquinata* (Mollusca: Pelecypoda) and *Abarenicola pacifica* (Annelida: Polychaeta). *Mar. Environ. Res.*, **7**, 31–50.

Bauer, J. E. & Capone, D. G. (1985*a*). Effects of four aromatic organic pollutants on microbial glucose metabolism and thymidine incorporation in marine sediments. *Appl. Environ. Microbiol.*, **49**, 828–35.

Bauer, J. E. & Capone, D. G. (1985*b*). Degradation and mineralization of the polycyclic aromatic hydrocarbons anthracene and naphthalene in intertidal marine sediments. *Appl. Environ. Microbiol.*, **50**, 81–90.

Bauer, J. E. & Capone, D. G. (1988). *Appl. Environ. Microbiol.* (in press.)

Bianchi, T. S. & Levinton, J. S. (1981). Nutrition and food limitation of deposit-feeders. II. Differential effects of *Hydrobia totteni* and *Ilyanassa obsoleta* on the microbial community. *J. Mar. Res.*, **39** (3), 547–56.

Boisseau, J. P. (1957). Technique pour l'etude quantitative de la faune interstitielle des sables. *C. r. Congr. Soc. Sav. Paris, Sec. Sci.*, **1957**, 117–19.

Briggs, K., Tenore, K. & Hanson, R. (1979). The role of meiofauna in detrital utilization by the polychaete, *Nereis succinea* (Frey and Leuckart). *J. Exp. Mar. Biol. Ecol.*, **36**, 225–34.

Cammen, L. M. (1980). The significance of microbial carbon in the nutrition of the deposit-feeding polychaete *Nereis succinea. Mar. Biol.*, **61**, 9–20.

Cerniglia, C. E. (1982). Aromatic hydrocarbons: Metabolism by bacteria, fungi and algae. *Rev. Biochem. Toxicol.*, **3**, 321–61.

Chandler, G. T. & Fleeger, J. W. (1983). Meiofaunal colonization of azoic estuarine sediment in Louisiana: Mechanisms of dispersal. *J. Exp. Mar. Biol. Ecol.*, **69**, 175–88.

Decker, C. J. & Fleeger, J. W. (1984). The effects of crude oil on the colonization of meiofauna into salt marsh sediments. *Hydrobiologia*, **118**, 49–58.

Ekman, J. E. (1985). Flow disruption by an animal-tube mimic affects sediment bacterial colonization. *J. Mar. Res.*, **43**, 419–35.

Fenchel, T. (1975). The quantitative importance of the benthic microfauna of an arctic tundra pond. *Hydrobiologia*, **46**, 445–64.

Findlay, S. & Tenore, K. (1982). Effect of a free-living marine nematode (*Diplolaimella chitwoodi*) on detrital carbon mineralization. *Mar. Ecol. Prog. Ser.*, **8**, 161–6.

Fleeger, J. W. & Chandler, G. T. (1983). Meiofaunal responses to an experimental oil spill in a Louisiana salt marsh. *Mar. Ecol. Prog. Ser.*, **11**, 257–64.

Fry, J. C. (1982). Interactions between bacteria and benthic invertebrates. In: *Sediment microbiology.* (Nedwell, D. B. & Brown, C. M. (Eds)), Academic Press, New York, 171–201.

Gerlach, S. A. (1978). Food-chain relationships in subtidal silty sand marine sediments and the role of meiofauna in stimulating bacterial productivity. *Oecologia*, **33**, 55–69.

Goldhaber, M. B., Aller, R. C., Cochran, J. K., Rosenfeld, J. K., Martens, C. S. & Berner, R. A. (1977). Sulfate reduction, diffusion, and bioturbation in Long Island Sound sediments: Report of the FOAM group. *Am. J. Sci.*, **277**, 193–237.

Gordon, D. C., Jr, Dale, J. and Veizer, P. D. (1978). Importance of sediment working by the deposit-feeding polychaete *Arenicola marina* on the weathering rate of sediment-bound oil. *J. Fish. Res. Board Can.*, **35**, 591–603.

Hambrick, G. A., DeLaune, R. D. & Patrick, W. H. (1980). Effect of estuarine sediment pH and oxidation-reduction potential on microbial hydrocarbon degradation. *Appl. Environ. Microbiol.*, **40**, 365–9.

Hanson, R. B. (1980). Measuring microbial activity to assess detrital decay and utilization. In: *Marine benthic dynamics*. (Tenore, K. R. & Coull, B. C. (Eds)), University of South Carolina Press, Georgetown, SC, 347–58.

Hargrave, B. T. (1970). The effect of a deposit-feeding amphipod on the metabolism of benthic microflora. *Limnol. Oceanogr.*, **15**, 21–30.

Harper, R. M., Fry, J. C. & Learner, M. A. (1981). Digestion of bacteria by *Nais variabilis* (Oligochaeta) as established by autoradiography. *Oikos*, **36**, 211–18.

Heitcamp, M. A. & Cerniglia, C. E. (1987). Effects of chemical structure and exposure on the microbial degradation of polycyclic aromatic hydrocarbons in freshwater and estuarine ecosystems. *Environ. Toxicol. Chem.*, **6**, 535–46.

Huang, T. C., Chang, M. D. & Alexander, M. (1981). Effect of protozoa on bacterial degradation of an aromatic compound. *Appl. Environ. Microbiol.*, **41**, 229–32.

Hylleberg, J. (1975). Selective feeding by *Abarenicola pacifica* with notes on *Abarenicola vagabunda* and a concept of gardening in lugworms. *Ophelia*, **14**, 113–37.

Johannes, R. E. (1965). Influence of marine protozoa on nutrient regeneration. *Limnol. Oceanogr.*, **10** (3), 434–42.

Johannes, R. E. (1968). Nutrient regeneration in lakes and oceans. In: *Advanc. Microbiol. Sea I. Academic*, 203–13.

Johnson, A. C. & Larsen, P. F. (1985). The distribution of polycyclic aromatic hydrocarbons in the surficial sediments of Penobscot Bay (Maine, USA) in relation to possible sources and to other sites worldwide. *Mar. Environ. Res.*, **15**, 1–16.

Kemp, P. F. (1987). Potential impact on bacteria of grazing by a macrofaunal deposit-feeder, and the fate of bacterial production. *Mar. Ecol. Prog. Ser.*, **36**, 151–61.

Kiene, R. P. & Capone, D. G. (1984). Effects of organic pollutants on methanogenesis, sulfate reduction and carbon dioxide evolution in salt marsh sediments. *Mar. Environ. Res.*, **13**, 141–60.

Krantzberg, G. (1985). The influence of bioturbation on physical, chemical and biological parameters in aquatic environments: A review. *Environ. Pollution* (*Ser. A*), **39**, 99–122.

Kristensen, E. & Blackburn, T. H. (1987). The fate of organic carbon and nitrogen in experimental marine sediment systems: Influence of bioturbation and anoxia. *J. Mar. Res.*, **45**, 231–57.

Kristensen, E., Jensen, M. H. & Andersen, T. (1985). The impact of polychaete (*Nereis cirens* Sars) burrows on nitrification and nitrate reduction in estuarine sediments. *J. Exp. Mar. Biol. Ecol.*, **85**, 75–91.

Lee, R. F. & Singer, S. C. (1980). Detoxification enzyme system in marine polychaetes: Increases in activity after exposure to aromatic hydrocarbons. *Rapp. P.-v Cons. Int. Explor. Mer.*, **179**, 29–32.

Lee, R. F., Furlong, E. & Singer, S. (1977). Metabolism of hydrocarbons in marine invertebrates: Aryl hydrocarbon hydroxylase from the tissues of the blue crab, *Callinectes sapidus*, and the polychaete worm, *Nereis* sp. In: *Pollutant effects on* *marine organisms.* (Giam, C. S. (Ed.)), Lexington Books, Lexington, Mass., 111–24.

Levinton, J. S. (1972). Stability and trophic structure in deposit-feeding and suspension-feeding communities. *Am. Nat.*, **106**, 472–86.

McCave, I. N. (1974). *The benthic boundary layer.* Plenum, New York.

Palmer, M. A. (1986). Hydrodynamics and structure: Interactive effects on meiofaunal dispersal. *J. Exp. Mar. Biol. Ecol.*, **104**, 53–68.

Pearson, T. H. (1982). The Loch Eil project: Assessment and synthesis with a discussion of certain biological questions arising from a study of the organic pollution of sediments. *J. Exp. Mar. Biol. Ecol.*, **57**, 93–124.

Reimers, C. E. & Suess, E. (1983). The partitioning of organic carbon fluxes and sedimentary organic matter decomposition rates in the ocean. *Mar. Chem.*, **13**, 414–68.

Rhoads, D. C., Aller, R. C. & Goldhaber, M. B. (1977). The influence of colonizing benthos on physical properties and chemical diagenesis of the estuarine seafloor. In: *Ecology of marine benthos.* (Coull, B. C. (Ed.)), Belle Baruch, University of South Carolina Press, 113–38.

Riemann, F. & Schrage, M. (1978). The mucous-trap hypothesis on feeding of aquatic nematodes and implications for biodegradation and sediment structure. *Oecologia*, **34**, 75–88.

Riley, R. G., Augenfeld, J. M., Rogers, J. E., Anderson, J. W., Thomas, B. L. & Li, S. W. (1982). *The fate of polyaromatic hydrocarbons in an intertidal sediment exposure system: Distribution and fate of polyaromatic hydrocarbons in interstitial water and sediment.* US Environmental Protection Agency.

Rogerson, A. & Berger, J. (1983). Enhancement of the microbial degradation of crude oil by the ciliate *Colpidium colpada. J. Gen. Appl. Microbiol.*, **29**, 41–50.

Sokal, R. R. & Rohlf, F. J. (1981). *Biometry.* (2nd edn). W. H. Freeman and Company, San Francisco.

Spain, J. C. & Van Veld, P. A. (1983). Adaptation of natural microbial communities to degradation of xenobiotic compounds: Effects of concentration, exposure time, inoculum, and chemical structure. *Appl. Environ. Microbiol.*, **45**, 428–35.

Stephens, G. C. (1975). Uptake of naturally occurring primary amines by marine annelids. *Biol. Bull.*, **149**, 297–407.

Tietjen, J. H. (1980). Microbial–meiofaunal interrelationships: A review. In: *Aquatic microbial ecology*, 335–8.

Yingst, J. Y. & Rhoads, D. C. (1980). The role of bioturbation in the enhancement of bacterial growth rates in marine sediment. In: *Marine benthic dynamics.* (Tenore, K. R. & Coull, B. C. (Eds)), University of South Carolina Press, Georgetown, SC, 407–22.